Diplura in caves: diversity, ecology, evolution and biogeography

Alberto Sendra[1], Ferran Palero[2,3], Alberto Jiménez-Valverde[4] & Ana Sofia P. S. Reboleira[5,6*]

[1]Colecciones Entomológicas Torres-Sala, Servei de Patrimoni Històric, Ajuntament de València, València, Spain
[2]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de Valencia, C/Catedrático Jose Beltran 2, 46980 Paterna, Spain
[3]Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
[4]Research Team on Soil Biology and Subterranean Ecosystems. Department of Life Sciences. Faculty of Science. University of Alcalá (UAH). A.P. 20. Campus Universitario. E-28805, Alcalá de Henares, Madrid, Spain
[5]Natural History Museum of Denmark, University of Copenhagen, 2100 Copenhagen, Denmark
[6]Centre for Ecology, Evolution and Environmental Changes (cE3c), and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
[*]Corresponding author. E-mail: asreboleira@fc.ul.pt

Abstract

Diplurans (Hexapoda) are considered the “ideal cavernicolous animal” having one of the highest ratios of cave-adapted vs. non-cave-adapted species. They are successful colonizers of subterranean habitats, thriving in all cryptic dark terrestrial environments. Diplurans play an important role in the decomposition of organic matter below the ground and are very sensitive to anthropogenic pressures. We present the first comprehensive review about cave Diplura diversity, ecology, evolution, distribution and biogeography. We provide a roadmap for research questions regarding the ecology, aimed at stimulating the pursuit of new studies on this fascinating group. Filling these current knowledge gaps will contribute to conservation efforts for cave ecosystems.

Keywords: Entognatha, Hexapoda, subterranean ecosystems, troglobiont, two-pronged bristletails.

Introduction

Diplura is one of the most ubiquitous groups of cave-dwelling animals worldwide (Sendra *et al.*, 2020a). Diplurans are basal hexapods, that are considered a sister group to insects, and presumably represent the transition from the highly-specialized aquatic Remipedia to a terrestrial lifestyle (Beutel *et al.*, 2017; Lozano-Fernandez *et al.*, 2019). They became terrestrial most likely in the Early Ordovician (Misof *et al.*, 2014), after acquiring a tracheal system, indirect sperm transference by spermatophores and Malpighian tubules – although reduced or absent in some families (Beutel *et al.*, 2017; Nasonov, 1887). Diplura might date back to the Upper Carboniferous (japygid-like fossil: Kukalová-Peck, 1987), although the first fossil recognized as a true dipluran is known from the Lower Cretaceous of Brazil (Wilson & Martill, 2001).

Diplurans are usually called “two-pronged bristletails” because of their two cerci at the hind end of the abdomen. They comprise more than 1000 extant species, arranged into ten families, with Campodeidae and Japygidae having the lion’s share and comprising 84% of all the order’s diversity (Condé, 1956; Paclt, 1957; Pagés, 1959, 1989; Rusek, 1982, Sendra, 2015, Sendra *et al.*, 2020a). All diplurans are terrestrial with great affinity to moist environments, and found mainly in soil and cave ecosystems (Denis, 1949; Condé, 1956; Koch, 2009). Monophyly of Diplura has been supported by morphology and phylogenetic analyses (Chen *et al.*, 2014), but molecular evidence is limited to a few representative families (Figure 1) and public databases still include poor identifications and strong biases towards one or two taxa. For example, 81% of the Diplura sequences available in Genbank (46501 out of 57545 entries) belong to an unidentified *Megajapyx* species and were produced in a single phylogenomic study focused primarily on apterous insects. Evolutionary relationships among dipluran taxa are yet to be clarified using molecular methods because some groups are rare and/or difficult to sample.

All diplurans are successful colonizers of subterranean or hypogean habitats (Figure 2; Table S1), thriving in dark and cryptic environments below the Earth's surface (Racovitză, 1907; Condé 1956; Sendra, 2015). They lack eyes but have lateral sensory organs below the integument that presumably have light-perceptive function (George 1963). Diplurans are very fragile, mostly covered with a thin cuticle. Furthermore, diplurans are almost completely depigmented. Their traits match the hypogean life-style either in the soil or deeper in caves. One in seven Diplura species are cave-adapted, and although other zoological groups have a higher number of cave-adapted species (e.g., Coleoptera: 2500; Collembola: 500; Orthoptera: 250; Araneae: 1000; Diplopoda: >200) (Culver & Shear 2012; Mammola & Isaia, 2017; Deharveng & Bedos, 2018), diplurans have one of the highest ratios of cave-adapted vs. non-cave-adapted species (153/854) compared with other taxonomical orders. Pagés (1964) and Bareth & Pagés (1994) briefly summarized the previous existing knowledge on cave diplurans.

Here we present a critical overview of the current knowledge on the diversity, evolution, ecology, distribution and biogeography of cave-adapted diplurans, also providing an updated worldwide checklist. This constitutes a fundamental starting point for understanding major biases in knowledge, to tailor future studies, and to definitely contribute to the protection of cave ecosystems and their associated fauna.

Dipluran habitats

Diplurans live below the surface, in the so-called subterranean or hypogean habitats. They are distributed from the soil or edaphic habitat, i.e., the most superficial and non-consolidated mix of organic matter with rock debris layer, to cave habitats which extend through the network of spaces present in the consolidated rock (bedrock) (Figure 2). There are four main layers or horizons (Figure 2) in mature and well-developed soils (Eisenbeis & Wichard, 1987). Species inhabiting the soil, especially the O, A and B horizons, have small bodies adapted to dwell in small voids.

Cave habitats refer to caves itself and also the network of cracks and voids in consolidated rock, usually in karst or volcanic areas (Moldovan *et al.*, 2018). These interstitial spaces follow the hydraulic concept of caves regardless of whether they are filled with air or water (Lauritzen, 2018), and they span from less than 5 mm to hundreds of meters in width. Species dwelling caves are called cave-adapted, cavernicolous, troglobionts or troglobites. These species live permanently in caves and exhibit similar convergent evolutionary traits, such as depigmented and elongated bodies and appendages, and hypertrophy of sensorial organs (Camacho 1992; Juberthie & Decu, 1994; Sket, 2008). As discussed below, these characters are ostensible in–cave-adapted diplurans. The C horizon of the soil, also known as the mesovoid shallow substratum (MSS) from a biological point of view, is considered an ecotonal habitat where soil-adapted and cave-adapted species can coexist (Bareth, 1983; Juberthie *et al.*, 1980, 1982; Moseley, 2010; Ortuño *et al.*, 2013).

Historical account of research

Cave-adapted diplurans have long attracted scientists' attention for their remarkable slender bodies and elongated appendages. During the late 19th century, several naturalists and zoologists such as Alpheus Spring Packard (North-America, 1839-1905) and Armand Viré (France, 1869-1951) described dipluran species based on the length of antennae, legs and cerci. Detailed modern descriptions of cave-adapted diplurans were made by–the French entomologist Jean Robert Denis (1893-1969): with *Litocampa sollaudi* from a cave in the French Jura and *Plusiocampa sollaudi* from eastern Pyrenees (Denis, 1930). Meanwhile, Filippo Silvestri (1873-1949), the prolific Italian entomologist, described nine cave-adapted campodeids collected around the world and established the genera *Plusiocampa* and *Tachycampa* (Silvestri, 1936, 1949, among others). The German Petr Wygodzinsky (1916-1987) published two genera and four cave-adapted species from Mexico (*Paratachycampa* and *Juxtlacampa*) while escaping Nazi persecution (Wygodzinsky, 1944) (Figure 3).

It was during the second half of the last century that most cave dipluran taxa were described. The greatest impulse to the systematics of cave dipluran was given by Bruno Condé (France, 1920-2004), a French entomologist that described 76 new cave-adapted species in many genera (i.e. *Anisocampa, Campodea, Cestocampa, Hystrichocampa, Juxtlacampa, Leletocampa*, *Litocampa, Oncinocampa, Paratachycampa, Patrizicampa, Plusiocampa*, *Podocampa* and *Simlacampa*) (Condé, 1956). This great effort was followed by a handful of contemporary entomologists such as Jean Pagés (1925-2009), Boris Pimenovitch Chevrizov (1951-1993), Mark Alan Muegge (1956-2015), Lynn Ferguson and Camille Bareth (active) (Figure 3). To date, 153 cave-adapted species and 16 subspecies of cave-diplurans have been described (Table S1).

Diversity

There is a strong unequal representation of cave-adapted taxa within phyletic lines (families, subfamilies and genera) in arthropod orders present in cave ecosystems (i.e. Coleoptera, Collembola) (Deharveng & Bedos, 2018). Diplura is no exception; out of ten dipluran families, only the Campodeidae and Japygidae include cave-adapted taxa (Figs. 4-5). Campodeidae is the most diverse family and includes 95% of all cave-adapted diplurans (Sendra *et al.*, 2020a). About 30% (146 spp.) of Campodeidae are found in caves and subfamilies have an unequal presence in these habitats (Figure 5; Table S1). Plusiocampinae includes 75 cave-adapted species (82% of its diversity) and it is the dominating group in caves despite being the second most diversified subfamily (Sendra *et al.*, 2020a). Lepidocampinae only includes three cave-adapted taxa, corresponding to about 16% of its diversity. The most diverse subfamily, Campodeinae, includes 52 true cave-dwelling taxa (15% of its diversity) (Sendra, 2015). Finally, the *incertae sedis* tachycampoid group deserves a special mention as 16 of 17 total species are cave-adapted (Sendra *et al.*, 2019, 2020a) (Figure 5, Table S1).

Japygidae is the second most diverse family of diplurans but with only eight cave-adapted species, corresponding to 2% of its diversity (Bareth & Pagés, 1994; Muegge, 1992; Sendra *et al.*, 2006).

At the generic level cave-adapted diplurans are represented by 25 campodeid and 7 japygid genera. Cave-adapted taxa mainly belong to the three most speciose campodeid genera: *Plusiocampa* with 62 out of 71 (87%), *Litocampa* with 24 out of 32 (75%), and *Campodea* with 14 species out of 185 (8%) (Figure 5; Table S1). It is clear the relictual condition of some cave-adapted Diplura, e.g. 40% (13 out of 31) of these genera are monotypic and the other eight genera (30% of all) are almost only represented by cave-dwelling species.

Convergent evolutionary traits

The strong selective pressures that the cave environment imposes on organisms have shaped the morphology of diplurans convergently (Condé, 1956; Sendra *et al.*, 2017b). Cave diplurans have been considered by Emil Racovitză (1907), the so-called father of Biospeleology, as the ideal cavernicolous animals ("le Cavernicole idéal"), because of their depigmented soft body, lack of eyes and long and slender appendages (Figure 4).

Morphological adaptations

Body. Cave-adapted taxa are distinguished at first sight from their soil-dwelling counterparts by their larger size, slender and elongated body and longer appendages (Figure 4). This is particularly evident in cave-adapted campodeids, which have remarkably large bodies (up to 10 mm, instead of the usually less than 4 mm in soil dwelling species) and is less evident in cave-adapted japygids.

Antennae. Cave-adapted diplurans show antennae up to twice as long as their body. The elongation of antennae results either from antennomere elongation (i.e. *Paratachycampa* and *Pacificampa*) or from an increase in the number of antennomeres (i.e. *Lepidocampa*). Among japygids, *Gollumjapyx smeagol* holds the record for the family with 55 antennomeres and no apparent antennomere elongation (Sendra *et al.*, 2006). The number of antennomeres can range from up to 30 in soil species to up to 84 in cave-adapted taxa (e.g. *Lepidocampa beltrani*) (Sendra *et al.*, 2017b).

Cerci. Cave-adapted campodeids show longer cerci than soil-dwelling species (Condé, 1956; Sendra *et al.* 2006, 2017b). Cerci can be up to four times longer than the body length, as in *Paratachycampa hispanica*, whereas cerci from soil-dwelling species are usually shorter than the body Similarly, the grasping forceps of cave-adapted japygids are longer and slender than those in soil-adapted japygids (Muegge, 1992; Pagés, 1964). In general, cave-adapted species tend to have longer cerci than what would be expected according to their body size (Sendra *et al.* 2017b).

Interestingly, two soil species (*Campodea (Dicampa) catalana* Denis, 1930 and *Campodea (Campodea) grassii* Silvestri, 1912) present a noticeable elongation of appendages in populations from the C horizon compared to populations living in the upper layers of the soils (Sendra *et al.*, 2017a).

Sensorial equipment. All kind of sensilla increase in number in the body and appendages (e.g. antennomeres and cerci become longer and larger) of cave-adapted species, improving sensorial perception in cave habitats. Mechanoreceptors are particularly relevant in some cave-adapted taxa such as *Whittencampa*, *Plusiocampa* (*Stygiocampa*), *Paratachycampa* and *Juxtlacampa*, which present more clothing setae and macrosetae in their ventral side.

Cupuliform organ. Olfactory receptors in Campodeidae occupy a cuticular invagination at the end of the last antennomere, the so-called cupuliform organ (Condé, 1956; Juberthie-Jupeau & Bareth, 1980). Each olfactory receptor is made up of a multiperforated cuticular layer, but they present remarkable differences between cave and soil campodeids (Figure 6). Soil species have 4 to 6 spheroidal receptors with one simple fold (Figure 6A), while cave-adapted species have two or three folds and up to 28 receptors as it is the case in *Jeannelicampa stygia* (Condé, 1952, 1956; Juberthie-Jupeau & Bareth, 1980; Sendra *et al.*, 2020a). Folds are extremely heterogeneous in cave-adapted taxa, from concentric (Figure 6B) or radial (Figure 6C) to a network-shaped structure (Figure 6D), or oviform and tree-shaped (Figure 6E) (Sendra *et al.*, 2017c, 2018). The recent discovery of *Remycampa herbanica* from a lava tube in the Fuerteventura Island revealed the presence of a new type of finger-like receptors (Figure 6F) (Sendra *et al.*, 2020b).

Gouge sensilla. Another antennal structure clearly affected by the cave lifestyle in campodeids is represented by the gouge sensilla, with unknown specific function. It is a setiform structure multiparous on the external side, located as a whorl on the distal part of antennomeres. First remarked by Bareth & Condé (1981) in two cave-adapted *Paratachycampa* from the Iberian Peninsula, these sensilla are also found in soil taxa (Sendra *et al.*, 2010) although they are less porous, less abundant and usually shorter than in cave-adapted species (Sendra et. 2020a,b).

Placoid sensilla. In japygids, plaicoid sensilla, which are located on the surface of the latest antennomeres and have an unknown function, are more abundant in cave-adapted species than soil-adapted species (Pagés, 1951).

Pretarsal adaptations. The pretarsal structures, which have two claws and two external lateral processes, are identical among japygid species, but in campodeids they show an astonishing variety in types of claws and lateral processes (Figure 7). Among Plusiocampinae genera, there seems to be an apparent relationship between the form of the pretarsal structures and type of habitat. Cave-adapted species of *Plusiocampa* have larger claws and bigger lateral crests than their soil relatives (Condé, 1956; Sendra *et al.* 2020a). The two pretarsus claws of some *Plusiocampa* tend to be unequal in size (Figure 7), in an analogous way to what has been observed in cave-dwelling Collembola, and which may be an adaptation for walking on clay sediments (Christiansen, 1965, 2012). Several highly cave-adapted genera such as *Juxtlacampa*, *Paratachycampa* or *Whittencampa*, and some species of *Cestocampa* and *Lepidocampa*, present cuticular expansions or foliate barbs on the lateral process of the pretarsus (Sendra *et al.*, 2016; Sendra & Deharveng, 2020). These foliate barbs seem to have sticky properties allowing those diplurans to walk on smooth surfaces such as, for instance, speleothems (Sendra et al, 2017b).

Physiological adaptations

Little is known about physiological adaptations of diplurans in cave ecosystems. Histological studies suggested that there is an absence of a seasonal reproductive cycle in males of *Campodea majorica valentina* and *Paratachycampa hispanica*, which keep their testis and glandular cells active all year round (Sendra *et al.*, 2017b). Testis and glandular cells persist in some soil campodeids during the cold season, when glandular setae vanish to reappear months later (Bareth, 1968).

Ecology

Habitat. Diplurans can be found in subsurface terrestrial habitats (Figure 2). Soil diplurans live among the leaf litter and decaying organic matter in the O horizon, penetrating into the more mineralized A and B horizons through voids and burrows made by animals or plant roots. They are well adapted to different soil habitats in temperate, subtropical and tropical climates, spanning from desert areas to cold and high mountains, although they avoid dry or frozen soils (Condé, 1956; Sendra *et al.*, 2019). Japygids are able to dig into the substrate (Pagés, 1967), a behaviour rarely shown in campodeids (Bareth, 1986). All japygids and many campodeids are adapted to dwell in small soil voids, usually in the B horizon, and always in close contact with the substratum (Gyger, 1960; Pagés. 1967). Cave-adapted diplurans can only reach the MSS if there is a physical continuum between this habitat and the network of voids of the bedrock (Sendra *et al.*, 2017a). Cave-adapted diplurans have been observed in some of the world's deepest caves down to 1000 m depth, such as *Plusiocampa hoelzeli* in Renejevo brezno (Kanin Mountain, Slovenia) and *Plusiocampa (Stygiocampa)* sp. from Lukina Jama (Trojama, Croatia) (Sendra al., 2020a).

Syntopy. Up to four species of campodeids and one japygid have been reported from the 'Avenc d'En Serenge' cave, where cave-adapted (*Paratachycampa hispanica*, *Gollumjapyx smeagol*, *Campodea* aff. *egena* and *Litocampa vandeli*) and edaphic species (*Campodea pieltaini* Silvestri, 1932) co-occur in the deepest part of the cave (Bareth & Condé 1981; Sendra *et al.*, 2006). Syntopy in caves is frequent but detailed data on micro-spatial distribution of Diplura within caves is scarce (Condé, 1956). Two cave-adapted campodeids (*Plusiocampa (Stygiocampa) nivea* and *Plusiocampa (Plusiocampa) ternovensis*) seem to be spatially segregated and to occupy different cave depths in Slovenian caves (i.e. in Velika ledena jama v Paradani and Bela Griža Caves) (Sendra *et al.*, 2020a). This suggests a complex relationship between syntopic species with different within-cave microhabitat preferences.

Food preferences. Campodeids feed directly on organic matter that percolates through the underground spaces, and they usually act as scavengers (Condé, 1956). Opportunistic predatory behavior has also been observed, and broken appendages or even complete microarthropods (e.g. mites) have be found in the gut contents of campodeids (Sendra *et al.*, 2020a). Japygids show an active predatory and maternal care behavior (Kasaroff, 1935; Gyger, 1960; Pagés, 1967). Cave-adapted japygids most likely prey on campodeids, as it is the case in soil habitats (Muegge & Carlton, 1998; Sendra *et al.*, 2006). The lower abundance of predatory diplurans in caves may be linked to the oligotrophic conditions of most caves, and also to the maternal care behavior which is presumably very demanding in terms of energy.

Parasitism. Very little is known about diplurans' parasites despite an increasing recent interest in parasitism in caves (Jensen *et al.*, 2019, Reboleira *et al.*, 2015). A few observations are limited to the presence of 'Amphoromoph' fungi in the cuticle of campodeids, similar to those observed in millipedes and spiders (Enghoff & Reboleira, 2017, Henriksen *et al.* 2018). Also, cysts and larvae of Gordiidae nematomorphs (Bareth, 1974), as well as nematode larvae (Condé, 1955), have been observed on diplurans.

Current and historical distribution limits

Cave-adapted diplurans occur in karst areas where caves are formed by the dissolution of soluble rocks, and in volcanic caves formed by lava movements (Condé, 1956; Ferguson, 1991a; Sendra *et al.* 2016, 2017b, 2020a,b; Sendra & Dehanverg 2020). Therefore, the potential distribution of the group is large since karst areas cover approximately 15% of the Earth surface (Ford & Williams, 2007) and volcanic caves (e.g., lava tubes) are sprinkled all across the globe. The lack of an homogeneous and substantial sampling effort in caves around the world is the most important factor shaping our current understanding of cave-adapted diplurans' distribution (Figure 8A). Europe and North-America are the best characterized areas and, although the American caves have been well sampled, many taxa remain unknown. In fact, Ferguson (1982, 1986, 1991a, 1991b) has quoted dozens of North-America localities with still undescribed cave species of *Haplocampa*, *Litocampa* and *Eumesocampa*. Only a handful of cave-adapted diplurans are known from other karst and volcanic areas of the world, and vast extensions in the Horn of Africa, Andes or China have great potential for the discovery of new dipluran taxa (Figure 8A).

Historical events are known to shape the current distribution of cave-adapted diplurans (Culver *et al.*, 2006). One of the best-studied examples is the ice sheet and permafrost extension during the Pleistocene glaciations (Belles, 1987), which wiped out terrestrial subterranean fauna (including diplurans) from high latitudes and altitudes. This is clearly recognizable by the absence of cave-adapted taxa north of the previous ice front (Figure 8). *Haplocampa wagnelli* is one interesting exception, found in some caves in Vancouver that were under the Canadian ice sheet (Sendra & Wagnell, 2019) (Figure 8A). Current distribution limits of cave-adapted diplurans are also imposed by contemporary extreme climates (i.e. frozen or very dry conditions) (Figure 8). Low primary production at the surface in glacial or desert regions reduces organic matter percolation towards subsurface habitats, so terrestrial cave fauna is very scarce (Culver *et al.* 2006). Exceptional cave ecosystems maintained by chemolithotrophs, such as the Movile Cave (Romania), can sustain large biological communities and include cave-adapted diplurans (Condé, 1996; Sarbu *et al.*, 2000). Impermeable geological layers may also limit the distribution of terrestrial cave fauna (Sendra *et al.*, 2014). Hypogenic caves are likely to be colonized after the removal of the confining layers (Jiménez-Valverde *et al.*, 2017). Isolation by impermeable layers explain why many long caves located in hypogenic karst areas (Lechuguilla Cave, New Mexico, USA; Jewel Cave, South Dakota, USA; Optimisticheskajan and Zolushka, Ukraine) lack cave-adapted fauna (Klimchouk, 2007).

Biogeographical patterns

Despite the lack of sampling effort for cave-adapted campodeids in many areas, some biogeographical patterns arise within different phyletic lines (e.g. subfamilies and genera), at least for the most diversified and well-known groups. The subfamily Lepidocampinae has a pantropical distribution, and their three cave-adapted species are found in Papua New Guinea, Indonesia and Reunion Island (Condé, 1956; Sendra *et al.*, 2017b). Plusiocampinae has a Euro-Asiatic distribution, with Southeast Asia emerging as a potential centre of origin (Sendra & Deharveng, 2020), but most taxa are known from the Euro-Mediterranean area (Sendra *et al.*, 2020a). Only the monotypic *Condeicampa* from North-America, can be found outside these regions (Ferguson, 1996). Campodeinae occupy the Holarctic region from North-America (*Haplocampa, Eumesocampa*) to Eastern Asia (*Pacificampa*), although the highest diversification occurs in the Menditerranean basin (*Campodea*, *Podocampa* and *Litocampa*). Two species of Campodeidae occupy south-hemispheric regions: *Cocytocampa humphreysi* is found in caves of Cape Range peninsula, NW Australia, and can be considered an early-stage cave-adapted species (Condé, 1998); *Anisocampa leleupi*, from South-Africa, is a highly cave-adapted species which shares several taxonomical traits with Plusiocampinae (Condé, 1952).

The biogeographical data currently available (Table S1) points out the importance of plate tectonics in the current distribution of cave-adapted species of diplurans. Cave-adapted Campodeinae (*Campodea*, *Litocampa* and *Podocampa*) have an amphi-Atlantic distribution and are abundant both in Western-Europe and Eastern North-America (Condé, 1956; Wygodzinsky, 1944). Similarly, the tachycampoid phyletic line is present in restricted cave areas in Mexico-Guatemala-Brazil and Spain-Sardinia-Algeria-Morocco, suggesting a former joint distribution area before the opening of the Atlantic Ocean (Bareth & Condé, 1981; Sendra *et al.*, 2020a). Some genera within the tachycampoid group such as *Oncinocampa*, *Tachycampa* and *Paratachycampa*, and the Campodeinae *Litocampa* and *Podocampa*, all include species from both sides of the Atlantic (Bareth & Condé, 1981; Sendra *et al.*, 2020a). Furthermore, the fragmentation of microplates could explain the distribution of *Plusiocampa* in eastern Mediterranean islands and its arrival to the Kabylie region in Algeria (Sendra *et al.*, 2019; Sendra *et al.*, 2020a). Finally, only eight unrelated cave-adapted japygids are scattered throughout a few caves in Eurasia, Africa and North-America, which prevents us from defining any biogeographical pattern.

Future perspectives

The main cause for the general lack of knowledge on the distribution, biology and ecology of cave-adapted species is the inaccessibility of most cave ecosystems to humans (Mammola et al. 2019). The so-called Racovitzan impediment (Ficetola *et al.*, 2019) refers to this knowledge shortfall, and it hinders our understanding of the sensitivity of cave habitats to anthropogenic pressures (Castaño-Sánchez *et al.*, 2020a). Dipluran studies on cave-adapted taxa continue to gain momentum, and further discoveries of new taxa are expected as suggested by the sustained accumulation of new species since the beginning of the twentieth century (Figure 5, Table S1). Recent explorations in tropical and subtropical karst regions revealed a high diversity of cave-adapted diplurans (Sendra *et al.* 2016, 2017b; Sendra & Deharveng, 2020).

A simplified roadmap can be drawn to highlight the key steps needed to better understand cave Diplura diversity and improve their conservation. First of all, we need to increase our current knowledge on species distribution. A world updated checklist of all cave-adapted dipluran species with their distribution is provided here for the first time (Table S1). A strong bias to the detriment of cave regions outside Europe and North America is clearly identified (Figure 8). Well-designed intensive surveys are urgently needed to cover unstudied areas; for instance, extensive karst areas located in the Horn of Africa, the Andes or China have a great potential for the discovery of new taxa. Second, further studies are needed to better understand the evolution of dipluran's adaptation to caves. The strong morphological convergence as result of the adaptation to the similar environmental pressures of caves often masks the evolutionary relationships among cave taxa, so it is urgent to establish a molecular phylogeny to analyze the diversification patterns, as well as major evolutionary events triggering cave colonization. Several cave-adapted dipluran species are considered 'relicts' and may provide relevant information about the impact of past events on current biogeographical patterns. A sound phylogenetic tree will provide the evolutionary framework needed to improve our understanding of key biological features of cave-adapted diplurans such as the functioning of sensorial and glandular structures, life-cycles, reproductive traits, and food preferences. Third, the ecology and behavior of cave-adapted diplurans remain poorly known (Condé, 1956; Bareth, 1974; Turquín & Bouvet, 1983), therefore a combination of *in situ* and lab studies are fundamental for understanding their life-cycle and physiology. Moreover, cave animals are typically exposed to small thermal variation in cave ecosystems, so the temperature rise in subterranean ecosystems may pose a potential risk to their survival (Mammola *et al.*, 2019; Castaño-Sánchez *et al.*, 2020b). Similarly, no data is currently available on their response to contaminants and temperature tolerance, which is a key evidence line to environmental risk assessment for cave ecosystems (Castaño-Sánchez *et al.*, 2020).

Many research questions regarding the ecology, evolution and conservation of subterranean diplurans remain unknown and conservation efforts are doomed to fail without filling these gaps. Our updated contribution on the state-of-the-art of cave Diplura research will stimulate the pursuit of new studies on this fascinating group.

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References

Bareth C. 1968. Biologie sexuelle et formations endocrines de *Campodea remyi* Denis (Diploures Campodéidés), *Revue d'Écologie et de Biologie du Sol* 5 (3): 303–426.

Bareth C. 1974. Présence de Kystes et de Larves de Gordiacés chez plusieurs espèces de Campodés cavernicoles (Diploures Campodéidés), *Annales de Spéléologie* 29 (4): 657–662.

Bareth C. 1983. Diploures Campodéidés du milieu souterrain superficiel de la Region Ariegeoise. *Mémoires de Biospéologie,* 10: 67–71.

Bareth C. 1986. Acquisitions récentes sur l'écologie et la biologie des Diploures Campodéidés (Insectes Apterygota), *2nd International Séminar on Apterygota*, Siena, Italy 1986: 99–103.

Bareth C, Condé B. 1981. Nouveaux Campodéidés de grottes d'Espagne, *Revue suisse de Zoologie* 88 (3): 775–786.

Bareth C, Pagés J. 1994. Diplura. In: Juberthie C, Decu V, eds. *Encyclopaedia Biospeologica I*, Sociétè Biospéologie. Moulis-Bucarest. 277–283.

Bellés X. 1987. *Fauna Cavernícola i Intersticial de la península Ibèrica i les Illes Balears*. CSIC. Ed. Moll.

Beutel RG, Yavorskaya MI, Mashimo Y, Fukui M, Meusemann K. 2017. The Phylogeny of Hexapoda (Arthropoda) and the Evolution of Megadiversit, *Proceedings of Arthropododan Embryological Society of Japan* 51: 1–15.

Camacho AI. 1992. A classification of the aquatic and terrestrial subterranean environment and their associated fauna. In: Camacho AI, ed. *The Natural History of Biospeleology*. Museo Nacional de Ciencias Naturales, CSIC, 57–103.

Castaño-Sánchez A, Hose GC, Reboleira ASPS. 2020a. Ecotoxicological effects of anthropogenic stressors in subterranean organisms: A review, *Chemosphere* 244: 125422.

Castaño-Sánchez A, Hose GC, Reboleira ASPS. 2020b. Salinity and temperature increase impact groundwater crustaceans. *Scientific Reports* 10: 12328.

Chen Z, Auler A, Bakalowicz M, Drew D, Griger F, Hartmann J, Jiang G, Moosdorf N, Richts A, Stevanovic Z, Veni G, Goldscheider N. 2017. The World Karst Aquifer Mapping project: concept, mapping procedure and map of Europe, *Hydrogeology Journal,* 25: 771–785.

Chen WJ, Koch M, Mallatt JM, Luan YX. 2014. Comparative analysis of mitochondrial genomes in Diplura (Hexapoda, Arthropoda): taxon sampling is crucial for phylogenetic inferences, *Genome Biology and Evolution* 6 (1): 105–120.

Christiansen KA. 1965. Behaviour and form in the evolution of cave collembola, *Evolution*, 19 (4): 529–537.

Christiansen KA. 2012. Morphological adaptations. In: White WB, Culver DC, eds. *Encyclopedia of caves*. Academic Press, 517–528.

Condé B. 1952. Campodéidés cavernicoles d'Afrique septentrionale (note préliminaire). *Notes Biospéologiques* 7: 61–67.

Condé B. 1955. Mission Henri Coiffait au Liban (1951). 4. Protoures et Diploures Campodéidés. *Biospeologica* 75: 397–412.

Condé B. 1956. Matériaux pour une Monographie des Diploures Campodéidés. *Mémoires du Muséum National d'Histoire naturelle, Série A. Zoologie* XII: 1−202.

Condé B. 1964. Découverte d'un Campodéidé troglobie en Afrique Australe, *Third International Congress of Speleology* II: 51−59.

Condé B. 1996. Diploures Campodéidés de la Pestera de la Movile (Movile Cave), Dobroudja méridionales (Roumanie). *Revue suisse de Zoologie* 103 (1): 101−114.

Condé B. 1998. First record of campodeids (Diplura: Campodeidae) from caves in Australia, *Records of the Western Australian Museum* 19: 145−152.

Culver DC, Deharveng L, Bedos A, Lewis JJ, Madden M, Redell JR, Sket B, Trontelj P, White D. 2006. The mid-latitude biodiversity ridge in terrestrial cave fauna, *Ecography* 29: 120−128.

Culver DC, Shear. 2012. *Myriapods*. In: White BW, Culver DC, eds. *Encyclopedia of Caves*. 2nd edition. 538−542.

Deharveng L, Bedos A. 2018. Diversity of Terrestrial Invertebrates in Subterranean Habitats. In: Moldovan OT, Kováč L, Halse S, eds. *Cave Ecology*. Switzerland: Springer, 107−172.

Denis JR. 1949. Ordre des Diploures. In: Grassé PP, ed. *Traité de Zoologie* IX. Paris: Masson, 160−185

Denis JR. 1930. Sur la faune française des Aptérygotes XIe note: Diploures avec tableau de détermination des espèces françaises, *Bulletin de la Société Zoologique de France* 55: 19–41.

Ehlers J, Gibbard PL, Hughes PD. 2011. *Quaternary glaciations - extent and chronology*. Elsevier.

Eisenbeis G, Wichard W. 1987. *Atlas on the Biology of Soil Arthropods*. Springer-Verlag.

Enghoff H. & Reboleira ASPS. 2017. Diversity of non-Laboulbenialean fungi on millipedes, *Studies in Fungi* 2 (1): 130–137.

Ferguson LM. 1982. Cave Diplura of the United States, *Proceedings of the 8th International Congress Speleology, Kentucky* 11−12.

Ferguson LM. 1986. Cavernicolous Campodeids (Insecta: Diplura) of Mexico. *9º Congreso Internacional de Espeleologia, Barcelona* 2: 91−93.

Ferguson LM. 1991a. Diplura of Lava Tube Caves. *Proceedings of the 6th International Symposium Vulcacospeleology Hilo Hawaii* 281−284.

Ferguson LM. 1991b. *Campodeicampa langei*, new genus and species of Dipluran (Diplura: Campodeidae) from Whipple Cave, Nevada, USA, *Mémoires de Biospéologie* XXIII: 133−141.

Ferguson LM. 1996. *Condeicampa langei*, new genus and species of Dipluran (Diplura: Campodeidae) from Whipple Cave, Nevada, USA, *Mémoires de Biospéologie* XXIII: 133−141.

Ficetola GF, Canedoli C, Stoch F. 2019. The Racovitzan impediment and the hidden biodiversity of unexplored environments, *Conservation Biology* 33: 214–216.

Ford DC, Williams PW. 2007. *Karst Hydrology and Geomorphology*. London, Wiley Chichester, 2nd ed.

George M. 1963. Studies on *Campodea* (Diplura) the anatomy of the glands and sense-organs of the head. *Quarterly Journal of Microscopical Science* 104: 1−21.

Gibert J. Deharveng L. 2002. Subterranean Ecosystems: A truncated functional biodiversity, *BioScience* 52 (6): 473−481.

Ginet R, Decu V. 1977. Initiation à la Biologie et a l'Écologie Souterraines. Ed. Universitaires, Jean-Pierre Delarge.

Gyger H. 1960. Untersuchungen zur postembtyonalen Entwicklung von *Dipljapyx humberti* (Grassi), *Verhandlungen der Naturforschenden Gesellschaft in Basel* 71 (1): 29–95.

Henriksen CB, Reboleira ASPS, Scharff N, Enghoff H. 2018. First record of a *Basidiobolus*/Amphoromorpha fungus from a spider, *African Journal of Ecology* 56 (1): 153−156.

Howarth FG, Moldovan OT. 2018. Where Cave Animals Live. In: Moldovan OT, Kováč, L, Halse S, eds. *Cave Ecology*. Switzerland: Springer, 23−37.

Jeannel RG. 1943. Fossiles vivants des cavernes. Paris: Ed Gallimard.

Jensen KM, Rodrigues L, Pape T, Garm A, Santamaria S, Reboleira ASPS. 2019. Hyperparasitism in caves: bats, bat flies and ectoparasitic fungus interaction, *Journal of Invertebrate Pathology* 166: 107206.

Jiménez-Valverde A, Sendra A, Garay P, Reboleira ASPS. 2017. Energy and speleogenesis: Key determinants of terrestrial species richness in caves, *Ecology and Evolution* 2017: 10207.

Juberthie-Jupeau L, Bareth C. 1980. Ultrastructure des sensilles de l'organe cupuliforme de l'antenne des Campodes (Insecta: Diplura), *International Journal Insect Morphology and Embryology* 9: 255–268.

Juberthie C, Buillon M, Delay B. 1982. Sur l’existence du milieu souterrain en zone calcaire, *Mémoires de Biospéologie* 8: 77–93.

Juberthie C, Decu V. 1994. Structure et diversite du domaine souterrain particularites des habitats et adaptations des espèces. In: Juberthie C. Decu V. eds. *Encyclopaedia Biospeologica*, Moulis-Bucarest: Société de Biospéologie, 5–22.

Juberthie C, Delay B, Buillon M. 1980. Extension du milieu souterrain en zone non-calcaire: descrption d’un nouveau milieu et de son peuplement par les coléoptères troglobies. *Mémoires de Biospéologie* 7: 19–52.

Koch M. 2009. Diplura. In: Resh VH, Cardé RT. *Encyclopedia of Insects* 2nd edition, Elsevier, 281–283.

Kosaroff G. 1935. Beobachtungen über die Ernährung der Japygiden, *Bulletin des Institutions Royales d'Histoire Naturelle a Sophia-Bulgarie* VIII: 181−185.

Kukalová-Peck J. 1987. New Carboniferous Diplura, Monura, and Thysanura, the hexapod ground plan and the role of thoracic side lobes in the origin of wings (Insecta), *Canadian Journal Zoology* 65: 2327−2345.

Klimchouk AB. 2007. Hypogene Speleogenesis: Hydrogeological and Morphogenetic Perspective. Special Paper nº 1, National Cave Karst Research Institute, Carlsbad, NM.

Lauritzen SE. 2018. Physiography of the Caves. In: Moldovan OT, Kováč L, Halse S, eds. *Cave Ecology.* Switzerland: Springer, 7−21.

Lozano-Fernandez J, Giacomelli M, Fleming J, Chen A, Vinther J, Thomsen PF, Glenner H, Palero F, Legg DA, Iliffe M, Pisani D, Olesen J. 2019. Pancrustacean evolution illuminated by taxon-rich genomic-scale data sets with an expanded remipede sampling, *Genome Biology and Evolution* 11 (8): 2055–2070.

Mammola S, Isaia M. 2017. Spiders in caves, *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 284.

Mammola S, Cardoso P, Culver DC, Deharveng L, Ferreira RL, Fišer C, Galassi DPM, Griebler C, Halse S, Humphreys WF, Isaia M, Malard F, Martinez A, Moldovan OT, Niemiller ML, Pavlek M, Reboleira ASPS, Souza-Silva M, Teeling EC, Wynne JJ, Zagmajster M. 2019. Scientists’ warning on the conservation of subterranean ecosystems, *BioScience* 69(8): 641–650.

Misof B *et al.* 2014. Phylogenomics resolves the timing and pattern of insect evolution, *Science*, 346: 763−767.

Moldovan OT, Kováč L, Halse, S. 2018. Preamble. In: Moldovan OT, Kováč L, Halse S. eds. *Cave Ecology.* Switzerland: Springer, 1−4.

Moseley M. 2010. Are all caves ecotones? *Cave and karst science* 3: 53−58.

Muegge MA. 1992. New Species of Cavernicolous Japygid (Diplura: Japygidae) from Texas. *Annals of the Entomological Society of America* 85 (4): 406−412.

Muegge MA, Carlton CE. 1998. New records of Japygoidea (Hexapoda: Diplura) from Louisiana, with notes on behavior. *Entomological News* 109 (3): 177−182.

Nasonov N. 1887. K morfologiji nissich (recte: nizsich) nasekomych. *Lepisma, Campodea* i *Lipura*. Izvest. Imp, *Obsc. Ljubit. Jestestvozn* 52 (I): 15−86.

Olson D, Dinerstein E. 2002. The Global 200: Priority Ecoregions for Global Conservation, *Annals of the Missouri Botanical Garden* 89: 199−224.

Ortuño VM, Gilgado JD, Jiménez-Valverde A, Sendra A, Pérez Suárez G, Herrero-Borgoñón JJ. 2013. The ‘‘Alluvial Mesovoid Shallow Substratum’’, a new subterranean habitat, PLoS ONE 8: e76311.

Paclt J. 1957. Diplura. in Genera Insectorum. P. Wytsman, 212º fasc: 1−123.

Pagés J. 1951. Contribution à la connaissance des Diploures, *Suplement du Bulletin Scientifique de Bourgogne* 9: 1−97.

Pagés J. 1959. Remarques sur la classification des diploures, *Travaux du Laboratoire de Zoologie et de la Station Aquicole Grimaldi de la Faculte des Sciences de Dijon* 26: 1−25.

Pagés J. 1964. Remarques sur les Japygidae signales dans le domaine souterrain, *International Journal of Speleology* 1(1,2): 191−201.

Pagés J. 1967. Données sur la Biologie de *Dipljapyx humberti* (Grassi), *Revue Ecologie et Biologie du Sol* 4 (2): 187−281.

Pagés, J. 1989. Sclérites et appendices de l'abdomen des Diploures (Insecta, Apterygota), *Archives des Sciences Genève* 42 (3): 509−551.

Racovitză EG. 1907. Essai sur les problèmes biospéologiques, *Archives de Zoologie Expérimentale et Générale* 6: 371−488.

Reboleira ASPS, Malekhosseini MJ, Sadeghi S, Enghoff H. 2015. Highly disjunct and highly infected millipedes – a new cave-dwelling species of *Chiraziulus* (Diplopoda: Spirostreptida: Cambalidae) from Iran and notes on Laboulbeniales ectoparasites, *European Journal of Taxonomy* 146: 1–18.

Rusek J. 1982. *Octostigma herbivora* n. gen. & sp. (Diplura: Projapygidae: Octostigmatidae n. fam.) injuring plant roots in the Tonga Islands, *New Zealand Journal Zoology* 9: 25−32.

Sarbu SM, Galdenzi S, Menichetti M, Gentile G. 2000. Geology and biology of the Frasassi caves in central Italy: an ecological multi-disciplinary study of a hypogenic underground karst system. In: Wilkens H, Culver DC, Humphreys WF, eds. *Subterranean Ecosystems*. Elsevier, 359−378.

Sendra A. 2015. Clase Entognatha. Orden Diplura, *Revista IDE@* 35: 1−11.

Sendra A, Reboleira ASPS. 2014. La extensión y los límites de la fauna en los hábitats subterráneos, *Boletín Asociación española de Entomología* 38 (3−4): 203−224.

Sendra A, Garay P, Ortuño VM, Gilgado JD, Teruel S, Reboleira ASPS. 2014. Hypogenic versus epigenic subterranean ecosystem: lessons from eastern Iberian Peninsula. *International Journal of Speleology* 43 (3): 253−264.

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

Sendra A, Teruel S, Satar A, Tusun S, Özbay C. 2010. New species, new records, and distribution of Campodeidae (Diplura) in Anatolia, *Zootaxa* 2639: 40−52.

Sendra AJ, Palacios J, García A, Montejo M. 2016. New Species of Campodeidae (Diplura) from Mexican caves, *Zootaxa* 4072 (5): 540–558.

Sendra A, Jiménez-Valverde A, Gilgado JD, Ledesma E, Baquero E, Pérez-Suarez G. Cuesta E, Herrero-Borgoñon JJ, Jordana R, Tinaut R, Barranco P, Ortuño VM. 2017a.

Diplurans of subsurface terrestrial habitats in the Iberian Peninsula, with a new species description (Diplura: Campodeidae), *Zootaxa* 4291 (1): 61−80.

Sendra A, Jiménez-Valverde A, Rochatc J, Legrosc V, Gasnierc S, Cazanove G. 2017b. A new and remarkable troglobitic *Lepidocampa* Oudemans, 1890 species from La Réunion Island, with a discussion on troglobiomorphic adaptations in campodeids (Diplura), *Zoologischer Anzeiger* 266: 95–104.

Sendra A, Sket B, Stoev P. 2017c. A striking new genus and species of troglobitic Campodeidae (Diplura) from Central Asia, *Subterranean Biology* 23: 47−68.

Sendra A, Yoshizawa K, Ferreira RL. 2018. New oversize troglobitic species of Campodeidae in Japan (Diplura), *Subterranean Biology* 27: 53–7.

Sendra A, Wagnell C. 2019. The cave-dwelling dipluran (Diplura, Campodeidae) on the edge of the Last Glacial Maximum in Vancouver Island caves, North America (Canada), *Subterranean Biology* 29: 59−77.

Sendra A, Nicolosi G, Amore E. 2019. Subterranean Campodeidae fauna from Sicily Island (Diplura); its biogeographical interest with the description of a new species of *Plusiocampa*, *Zootaxa* 4679 (2): 297–317.

Sendra A, Antić D, Barranco P, Borko Š, Christian E, Delić T, Fadrique F, Faille A, Galli L, Gasparo F, Georgiev D, Giachino PM, Kováč L, Lukić M, Marcia P, Miculinić K, Nicolosi G, Palero F, Paragamian K, Pérez T, Polak S, Prieto C, Turbanov I, Vailati D, Reboleira ASPS. 2020a. Flourishing in subterranean ecosystems: Euro-Mediterranean Plusiocampinae and tachycampoids (Diplura, Campodeidae), *European Journal of Taxonomy* 591: 1−138.

Sendra A, Deharveng L. 2020. *Whittencampa troglobia*, a new genus and species of troglomorphic Plusiocampinae from China (Diplura: Campodeidae), *The Raffles Bulletin of Zoology, supplement* 35: 68−77.

Sendra A, López H, Selfa J, Oromí P. 2020b. Two new dipluran species unearthed from subterranean habitats of the Canary Islands (Arthropoda, Hexapoda, Entognatha), *Subterranean Biology* 34: 39–59.

Sendra A, Nikoloudakis I, Gavalas I, Selfa J, Paragamian K. 2020c. A surprising new genus and species of cave-adapted Plusiocampinae *Cycladiacampa irakleiae* (Diplura, Campodeidae) from Irakleia Island, Cyclades Islands in the Aegean Archipelago (Greece). *Subterranean Biology* 35: 15–32.

Silvestri F. 1936. Una nuova specie di Campodeidae (Dicillura) rappresentante di un nuovo genere, di grotta del Marrocco, *Bulletin de la Société des Sciences naturelles du Marroc* 16: 86−89.

Silvestri F. 1949. Descrizione di due specie nuove cavernícole di Campodeidae (Insecta Diplura) della regione del Monte Tauro, *Bolletino del Laboratorio d'Entomologia agraria in Portici* IX: 27−31.

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

Turquín MJ, Bouvet Y. 1983. La répartition spatiale de *Plusiocampa sollaudi* (Insecta Diploure) dans le milieu souterrain, *Memoires de Biospéologie* 10: 159−162.

Vandel A. 1964. *Biospéléologie: La Biologie des Animaux Cavernicoles*. Paris: Gauthier-Villars.

Wilson HM, Martill DM. 2001 A new japygid dipluran from the lower Cretaceous of Brasil, *Palaeontology* 44: 1025−1031.

Wygodzinsky P. 1944. Contribuição ao conhecimento da familia Campodeidae (Entotrophi, Insecta) do Mexico, *Annales de la Escuela Nacional de Ciencias Biológicas* 3: 367−404.

Figures caption

Figure 1. Maximum likelihood (ML) evolutionary trees of Diplura. A) ML tree obtained from 18S rDNA data available in Genbank. B) ML tree obtained from COX1 data. Only bootstrap support values above 70 are shown.

Figure 2. Conceptual model of the major compartments of the subterranean habitats and corresponding dipluran habitus. Soil horizons: O is mainly formed by leaf litter, whereas the A and B horizons have gradual increase in mineral fraction and decrease in voids' size, the C horizon is formed by unconsolidated, mid-size clasts with large voids; this horizon has the mesovoid shallow substratum (MSS).

Figure 3. Cave-adapted dipluran species described from 1871 to 2020; photographs of the authors arranged from right to left and from top to bottom: Alpheus Spring Packard, Armand Viré, Filippo Silvestri, Jean Robert Denis, Petr Wygodzinsky, Boris Pimenovitch Chevrizov, Bruno Condé, Jean Pagés and Mark Alan Muegge. Courtesy of Bernd Hauser, Sergei Golovatch and Ernest C. Bernard.

Figures 4. Two cave-adapted Diplura: A) *Plusiocampa hoffmanni* Sendra & Paragamian, 2020 from Spilaio Sfento Trypa Cave, Crete, Greece (author: Kaloust Paragamian); B) *Gollumjapyx smeagol* Sendra & Ortuño, 2006 from Avenc d'En Serenge, Cabanes, Castellón, Spain. Courtesy of José María Azkárraga.

Figure 5. Bar chart showing the relative number of soil (orange) and cave-adapted (blue) dipluran species per family, subfamily and genus.

Figure 6. Olfactory chemoreceptor of the last antennomere in soil-adapted species: A) *Campodea (Paurocampa) suensoni* Tuxen, 1930 from Dos Aguas, Valencia, Spain; and cave-adapted species: B) *Cycladiacampa irakleiae* Sendra, 2020 from Spilaio Ioanni cave, Irakleia Island, Greece; C) *Pacificampa daidarabotchi* Sendra, 2018 from Mejito-do Cave, Kyushu Island, Japan; D) *undescribed* Plusiocampinae from Huitième Ciel Cave. Banqiao, Hubei, China; E) *Turkmenocampa mirabilis* Sendra & Stoev, 2017 from Kaptarhana Cave, Koytendog District, Lebap, Turkmenistan; and F) *Remycampa herbanica* Sendra & Oromí, 2020 from Montaña Blanca Cave, Fuerteventura Island, Spain.

Figure 7. Pretarsal adaptations in cave-adapted campodeid species: A) *Lepidocampa beltrani* Sendra, 2017 from Caverna Batu, La Reúnion Island, France; B) *Turkmenocampa mirabilis* Sendra & Stoev, 2017 from Kaptarhana cave, Koytendog District, Lebap, Turkmenistan; C) *Anisuracampa* sp. from Win Twin Cave, Ywangan, Shan State, Myanmar; and D). *Patrizicampa sardoa* Condé, 1962 from Grotta di Mesu'e Monte, Baunei, Sardinia, Italy.

Figures 8. Distribution of cave-adapted diplurans: (a) worldwide, and (b) Euro-Mediterranean region. In yellow: karst areas (source: Chen *et al.*, 2017). In orange: deserts (source: Olson & Dinerstein, 2002). In blue: ice cover during the Last Glacial Maximum (source: Ehlers *et al.*, 2011). In black: hypogenic karst areas (source: Klimchouk, 2007).