

DIATOMS FROM THE VALPORQUERO CAVE (LEÓN, NW SPAIN), WITH THE DESCRIPTION OF *GERMAINIELLA LEGIONENSIS* SP. NOV.

María Borrego-Ramos^{1,C}, Saúl Blanco¹, and Adriana Olenici^{1,2}

Abstract

From a biological point of view, karst caves are important subterranean ecosystems, but remain to date relatively poorly-studied. The diversity in this kind of habitat is usually low due to scarce light availability with resulting lack of primary productivity. In the case of show caves, the artificial illumination allows the colonization by phototrophic microorganisms, which is known as *lampenflora*, including fungi, bacteria, cyanobacteria, chlorophytes, diatoms, mosses, etc. This study analyzes the diatom flora from Valporquero Cave (León, NW Spain), both epilithic on stalactites and moss-dwelling. In the samples observed, typical genera from moist, humid, aerophilous habitats were recorded, but some biogeographically noteworthy species, including an unknown *Germainiella* taxon described here as a new species, were found. Some of these species have already been reported from caves from different places around the world, giving an idea of the broad distribution pattern of many diatom taxa. The occurrence of diatom taxa in this cave could contribute to understanding the global diversity and distribution patterns in cave-dwelling taxa.

Introduction

Caves are peculiar environments occurring throughout the world, with rather stable climatic conditions and food supplies, except for the entrance zones. Their biological communities include mostly microorganisms, but also worms, snails, arachnids, crustaceans, millipedes, insects, fishes, and salamanders (Holsinger, 1988). In these ecosystems, microorganisms frequently grow as components of entrance biofilms (Hoffmann 2002), where diatoms (Bacillariophyta) can represent around 11% of the total. Previous analysis of biofilms in other caves revealed that these communities were formed by phototrophic microorganisms, including coccal and filamentous cyanobacteria, green unicellular eukaryotic algae, diatoms, bacteria, and fungi (Urzi et al., 2010). The distance from the cave entrance is an important factor influencing the structure of these biofilms (Cuezva et al., 2009; Abdullin, 2011; Coombes et al., 2015).

In show caves, artificial illumination allows for colonization by phototrophic microorganisms, which frequently constitute macroscopic biofilms. Cyanobacteria and Chlorophyta are the first colonizers in caves, and are able to produce exopolymeric substances (EPSs), which are suitable environments for colonization by other microorganisms (Albertano, 1993). The assemblage constituted by these microorganisms is known as *lampenflora* (Dobàt, 1963), and the diversity of *lampenflora* communities has recently been reviewed (Mulec and Kosi, 2009). However, the proliferation of *lampenflora* is currently considered as the main threat for the conservation of show caves (Grobelaar, 2000; Piano et al., 2015) and studies related to growth control methods have increased (Mulec et al., 2007), including physical (light control), chemical, and biological methods.

Aerophytic algae can survive in the environment only when the humidity is high enough (Mulec and Kosi, 2009). Diatoms have been found in many types of ecosystems including some in caves (Falasco et al., 2014). The most common genera of diatoms found in these types of environments are *Navicula* and *Diademsis* (Falasco et al., 2014), together with other aerophilic taxa.

In karst regions, the formation of caves is carried out by rainwater that becomes acidic due to the interaction with carbon dioxide in the atmosphere and soil (Niemiller and Soares, 2015). Natural caves present exceptional characteristics because of their oligotrophic conditions (Holsinger, 1988; Graening and Brown, 2003; Ortiz et al., 2014; Piano et al., 2015), as well as the air temperature, which is influenced by air circulation in the proximity of entrances and it is usually stable in the deepest zones, with minimal daily and seasonal variations (Albertano, 1993). Hydrogeologically active caves show additional particularities that depend on the hydrogeological regime (Milanovic, 2007). In these types of active caves, the presence of running waters can accelerate the growth of biofilms (Mulec and Kosi, 2009). In any case, subterranean environments present relatively homogeneous, simplified natural conditions, and therefore, represent suitable habitats to study communities in relation to several factors and to predict ecological patterns on larger scales (Falasco et al., 2014), such as the effects of climate change.

The present study was carried out in Valporquero Cave (NW Spain), a hydrogeologically active cave, internally crossed by the Valporquero river (Arrese et al., 2007), which is a subterranean stream that flows through the lower level

¹ Department of Biodiversity and Environmental Management, University of León, León, Spain The Institute of the Environment, La Serna Street No. 58 24007, León, Spain.

² Faculty of Environmental Sciences and Engineering, Babeş-Bolyai University, 30 Fântânele Street, Cluj-Napoca, 400294, Cluj-Napoca, Romania.

^CCorresponding Author: mborr@unileon.es

of the cave. We characterize for the first time the diatom flora from this cave. During the research, a novel population of an unknown naviculoid taxon was found, and is described here by means of a detailed light (LM) and scanning electron (SEM) microscopy-based description.

Materials and Methods

Study area

The Valporquero Cave (42°54'22"N 5°33'31"W, 1390 m a.s.l.) is located in NW Spain (Fig. 1), and it is considered one of the main karstic environments of the Iberian Peninsula, composed of Carboniferous Barcaliente limestones. The cave has been included under numerous environmental protection statuses, such as Natura 2000 Network (LIC Hoces de Vegacervera) or the Argüellos Biosphere Reserve.

The current appearance of the cave has been modeled by an underground hydrographic network consisting of 3524 m (known to date), of which 1300 m are modified for tourists. The annual average temperature is 7 °C and the relative humidity is around 99% (Cueva de Valporquero, 2017). From a geological perspective, this is still a dynamic cave with active speleogenesis. The Valporquero Cave has been the subject of a number of speleological and geomorphological studies (Barea et al., 1998; Arrese et al., 2007) although the inhabiting biota, apart from the entomofauna (Salgado, 1985; Bastazo et al., 1993; Petitpierre and Gómez-Zurita, 1998) remains unexplored to our knowledge.

Sampling

Two samples of epilithic algae growing on stalactites and two samples of moss-inhabiting algae in the surroundings of two artificial light lamps were collected in April 2015 and preserved in the field with 10% formaldehyde. Epilithic diatoms were extracted and analyzed following standard methods (EN 14407 2004). Moss-dwelling diatoms were prepared following Van der Werff (1955), diatoms were cleaned by adding 37% H₂O₂, and the reaction was completed by addition of saturated KMnO₄. Frustules were cleaned with distilled water three times. Permanent slides were mounted for light microscopy using Naphrax (refractive index of 1.74).

Diatoms were identified under 1000X LM with an Olympus BX60 equipped with DIC optics. LM photographs were taken with a Canon EOS400 camera. Greater than 400 individuals (valves) were counted and identified per slide using recent taxonomic references (Hofmann et al., 2011 and references therein). For scanning electron microscopy (SEM), the oxidized samples were filtered through polycarbonate membrane filters with a pore diameter of 1 or 3 µm, mounted on stubs, sputtered with gold (40 nm) with a Modular High Vacuum Coating System (BALZERS SCD 004, Liechtenstein) and studied with a JEOL JSM-6480 LV, operated at 20 Kv. Original and treated samples, together with LM slides and SEM stubs, are permanently stored at the Institute of the Environment (Diatom Lab, University of Leon, Spain, GBIF code 10031). Type material is stored in the LEB Herbarium (University of León, Spain).

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Results and Discussion

A total of 26 diatom taxa were recorded in the samples collected (Table 1). Most of the species identified are common in this type of habitat (Falasco et al., 2014). The most abundant species belong to the genera *Humidophi-*

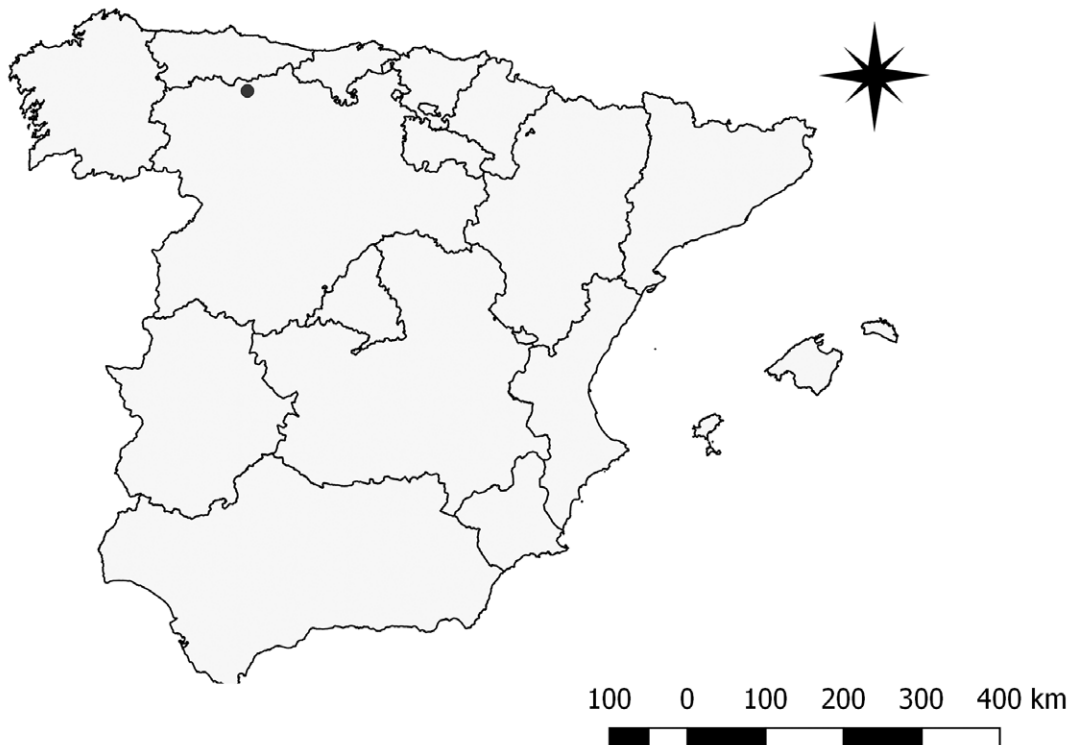


Figure 1. Geographical location of Valporquero Cave in NW Spain. (QGIS 2017).

Table 1. Checklist of the diatom species and distribution on moss-dwelling and stalactites from Valporquero Cave (León, Spain).

Diatom Species	Moss-Dwelling		Stalactites	
	Moss1	Moss2	Stal1	Stal2
<i>Achnanthydium</i> F.T. Kützing	+			
<i>Achnanthydium rivulare</i> Potapova & Ponader		+		
<i>Adlafia bryophila</i> (Petersen) Moser Lange-Bertalot & Metzeltin		+	+	
<i>Cymboplectra rupicola</i> (Grunow) Krammer var. <i>rupicola</i>				+
<i>Diadesmis gallica</i> Wm. Smith	+	+	+	+
<i>Diploneis krammeri</i> Lange-Bertalot & Reichardt		+		
<i>Diploneis oculata</i> (Brebisson in Desmazières) Cleve		+		+
<i>Encyonopsis microcephala</i> (Grunow) Krammer		+		+
<i>Encyonopsis minuta</i> Krammer & Reichardt		+		
<i>Eolimna minima</i> (Grunow) Lange-Bertalot		+		
<i>Eucoconeis laevis</i> (Oestrup) Lange-Bertalot		+		
<i>Fallacia insociabilis</i> (Krasske) D.G. Mann		+	+	+
<i>i = Germainiella legionensis</i> Blanco, Borrego-Ramos & Olenici	+	+	+	
<i>Halamphora normanii</i> (Rabenhorst) Levkov	+	+	+	+
<i>Humidophila contenta</i> (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová			+	+
<i>Humidophila dissimilis</i> (Moser, Lange-Bertalot & Metzeltin) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová		+	+	
<i>Humidophila paracontenta</i> (Lange-Bertalot & Werum) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová		+	+	
<i>Humidophila perpusilla</i> (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová		+	+	+
<i>Mayamaea cavernicola</i> Van de Vijver & Cox		+	+	+
<i>Navicula cataracta-rheni</i> Lange-Bertalot		+		
<i>Nitzschia solgensis</i> Cleve-Euler		+	+	
<i>Pinnularia</i> C.G. Ehrenberg		+	+	
<i>Pinnularia bartii</i> Metzeltin & Lange-Bertalot		+	+	
<i>Sellaphora</i> sp. C. Mereschkowsky		+		
<i>Simonsenia delognei</i> Lange-Bertalot	+			
<i>Tryblionella debilis</i> Arnott ex O'Meara	+		+	

la, *Cymboplectra*, and *Mayamaea*. With the exception of *Cymboplectra rupicola*, all these taxa are already recorded in subterranean ecosystems.

Epilithic diatom assemblages observed in the studied samples were dominated by several *Humidophila* species such as *H. paracontenta* (Lange-Bertalot and Werum) Lowe et al., *H. perpusilla* (Grunow) Lowe et al., *Humidophila dissimilis* (Moser, Lange-Bertalot and Metzeltin) Lowe et al., together with a *Diadesmis* species (D. Wm. Smith), morphologically and ecologically close to *Humidophila*. Other accompanying species such as *Mayamaea*, *Humidophila*, and *Diadesmis*, are typical genera of moist, humid, aerophilous habitats (Lowe et al., 2014). These genera exhibit the same ecological preferences, and for many years remained under the same genus *Diadesmis*, although recent molecular studies split off both taxa (Andreeva et al., 2016).

The moss-dwelling diatom assemblages presented differences regarding species diversity. One of the samples was dominated by *Mayamaea cavernicola* Van de Vijver and Cox, which had been already found in small caves on Ile de la Possession, the main island of the Crozet Archipelago (the type locality (Van de Vijver et al., 2002), and according to these authors, was recorded in a lava tube cave on the Hawaiian Islands (Rushforth et al., 1984) incorrectly identified as *Navicula seminulum* var. *hustedtii* Patrick). In contrast, the second sample was dominated by *Diadesmis gallica*, which accounted for almost the total abundance.

Many *Diadesmis gallica* were found as teratological forms, as in previous studies in caves (Falasco et al., 2015). Such morphological alterations are considered common in these conditions (Falasco et al., 2015; Lund, 1945, 1946).

Most of the species found in the communities studied belong to the genus *Humidophila*, which is considered to be adapted to low light conditions (Johansen, 2010). This genus has been already reported in other karst habitats in China (Lowe et al., 2017), Spain (Del Rosal, 2016), Canada (Lauriol et al., 2006) Slovakia (Lukešová and Nováková, 2008), and Slovenia (Mulec et al., 2007), and in limestone caves in Oregon, USA (identified as *Navicula*, (St. Clair et al., 1981), and Russia (Abdullin, 2011). It has been also found in other aerophilous habitats from Europe (e.g., Werum and Lange-Bertalot 2004) and the Antarctic region (Van de Vijver et al., 2002).

Another noteworthy species found in our samples is *Pinnularia bartii* Metzeltin and Lange-Bertalot, (Figs. 2 a–f), which had been so far recorded only in the type locality (moss samples in Río de La Plata near Colonia del Sacramento, Uruguay (Metzeltin et al., 2005), with an additional population found in Reunion Island (Metzeltin et al., 2005).

Species Description

Germainiella legionensis (Figs. 3–5)

Synonyms: ?'*Nupela* sp.' in Zimmerman et al. (2010, fig. 47:10-11), ?'*Achnanthes microcephala*' sensu Rushforth et al. (1984, Fig. 21)

Description:

Frustules rectangular in girdle view, with a short perivalvar depth (Fig. 4). Valves hyaline under LM with no conspicuous features even under DIC optics, except for the narrow, straight axial area, centrally expanded in a small, roundish central area. Outline linear-lanceolate with strongly capitate apices. Valve length 9.5–10.5 μm , width 2.0–2.5

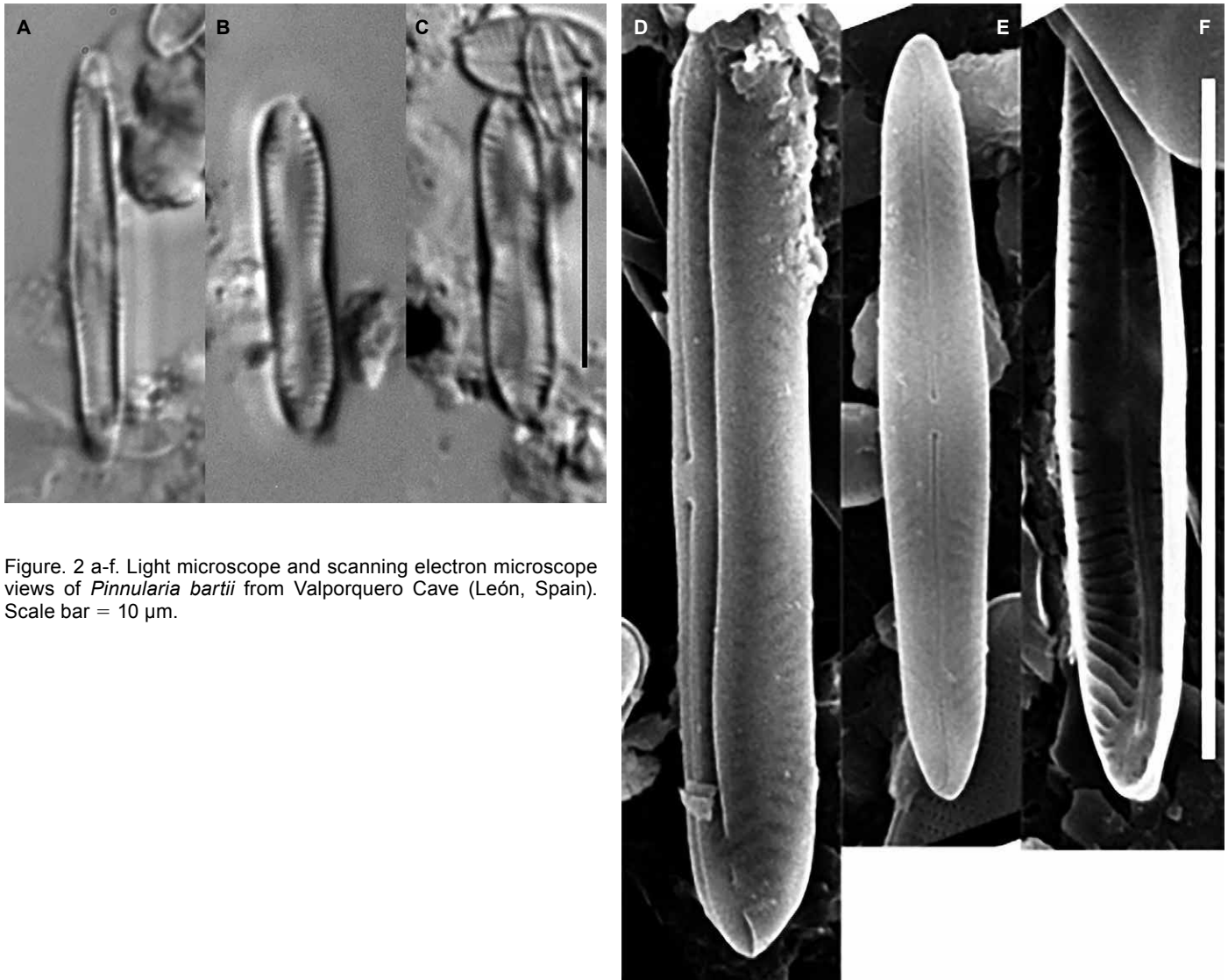


Figure. 2 a-f. Light microscope and scanning electron microscope views of *Pinnularia bartii* from Valporquero Cave (León, Spain). Scale bar = 10 μm .

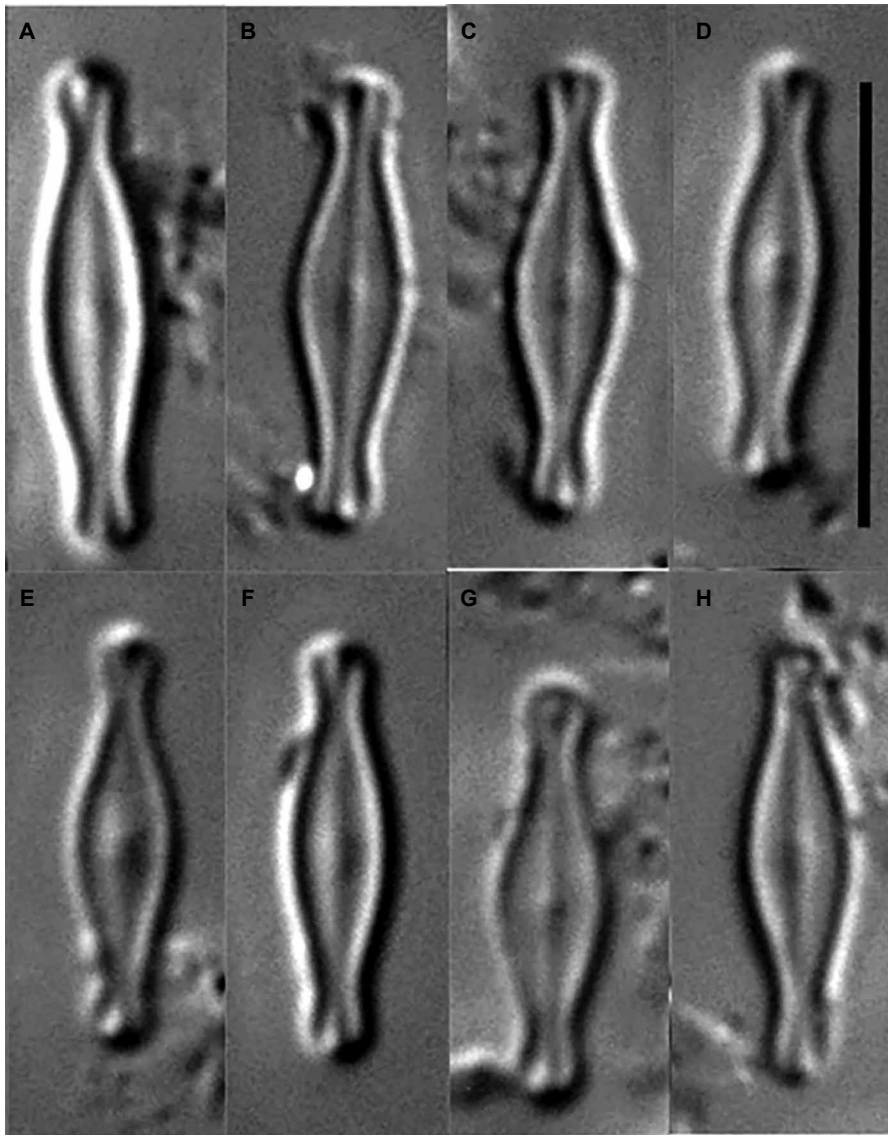


Figure 3 a-h. Light microscope images of the new diatom taxa *Germainiella legionensis* sp. nov. from Valporquero Cave (NW Spain). Individuals from the holotype specimen. Scale bar 10 μ m.

μ m, and length to width ratio 4.2–4.7. SEM: Valve covered by a velum which extends towards the mantle, so that the subconopeal space is only open to the exterior through a bilateral slit near the apices (Fig. 5a, arrows). Raphe filiform, straight, with external proximal raphe endings simple, not expanded, slightly bent towards one side of the valve. Distal external raphe endings continuing onto the valve mantle, forming sickle-shaped fissures. Striae visible only in broken valves, composed of a single, transapically expanded areola (Fig. 5b), ca. 45 in 10 μ m.

Type: Valporquero Cave (42°54'22"N 5°33'31"W), León, Spain. Samples collected on 20th March 2016.

Holotype: LEB!, microscopic slide 026. Individuals from the holotype specimen are here depicted as *Germainiella legionensis* (Figs. 3–5) Blanco, Borrego-Ramos & Olenici

Etymology: The epithet of the Species refers to the region where Valporquero Cave is located in León, Spain.

Ecology: *Germainiella legionensis* was collected in three samples of the Valporquero Cave, both on a stalactite and on mosses, near artificial light sources. In general, the samples are characterized by a low diversity and low abundance, as is typical in subterranean habitats (Holsinger, 1988; Falasco et al., 2014; Trajano et al., 2016) that could be explained by the particular ecological conditions and the absence of light. Compared to other studies (Falasco et al., 2014; 2015), the richness is relatively low but exhibit interesting species.

Distribution: Until now found in living samples in the type locality (Valporquero Cave) in León, Spain, probably also in the Thurston Lava Tube in Hawaii, and in fossil samples from Bylot Island (Canadian Arctic Archipelago), see synonyms.

Differential diagnosis: until recently, only two species had been described within *Germainiella*, the generitype *G. enigmatica* (\equiv *Navicula enigmatica* \equiv *Fallacia enigmatica*), presumed to be cosmopolitan (Metzeltin et al., 2005), and the morphologically similar *G. enigmaticoides*, restricted to the type locality (Colonia, Uruguay). In 2016, a third species, *G. clandestina*, was discovered in an artificial freshwater channel inoculated with biofilms from the Garonne River (Le Cohu et al., 2016), to date not referenced elsewhere. The new taxon described here can be easily differentiated in LM from these regarding its outline, with clearly capitated valves exhibiting a narrow neck (and not subcapitate as in *G. enigmaticoides*), and the short pervalvar depth shown in frustules seen in girdle view (compare Fig. 4 with Metzeltin et al., 2005, pl 54: figs 22–27). Moreover, the stria density is the lowest recorded within the genus (ca. 45 in 10 μ m and not greater than 50 in 10 μ m, (Le Cohu et al., 2016)). The other morphological and morphometric features correspond to those described for *G. enigmaticoides*.

Germainiella legionensis may have been earlier identified as *Achnanthes microcephala* in the samples collected in the Thurston Lava Tube, Hawaii, by Rushforth et al. (1984). According to this article, this individual occurred in a com-

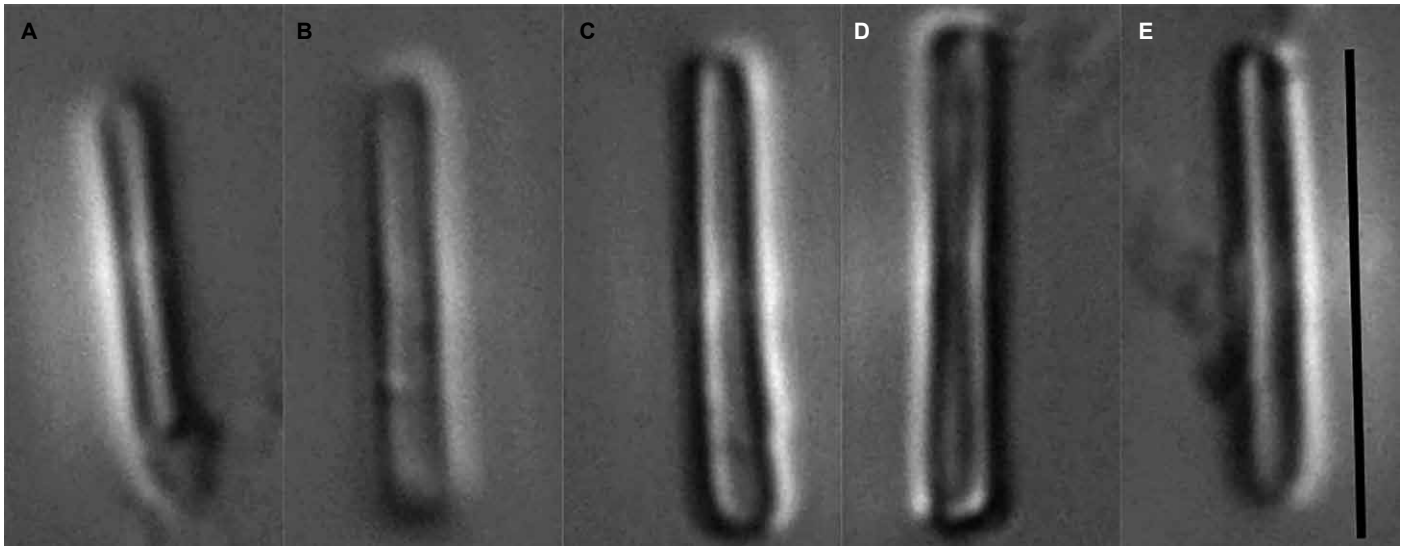


Figure. 4 a-e. Light microscope images of *Germainiella legionensis* in girdle view. Scale bar 10 μ m.

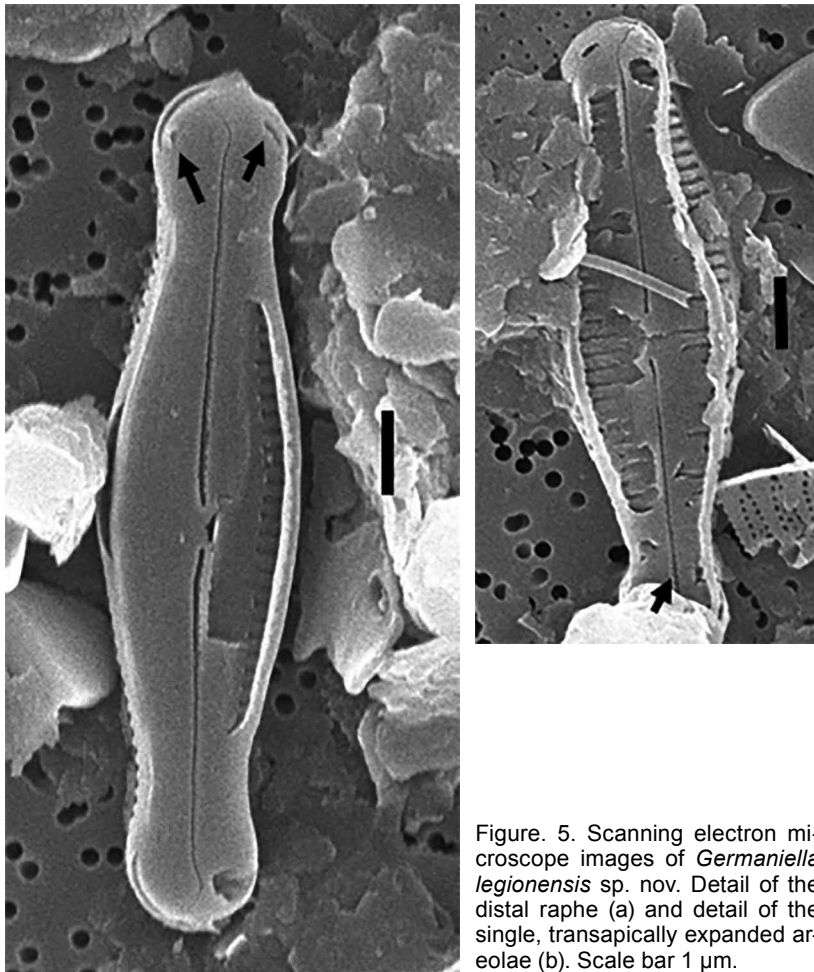


Figure. 5. Scanning electron microscope images of *Germainiella legionensis* sp. nov. Detail of the distal raphe (a) and detail of the single, transapically expanded areolae (b). Scale bar 1 μ m.

parable habitat (wet mucilage and bryophytes wall). Additionally, this species is probably present also in late Pliocene sediment samples from Bylot Island (Canadian Arctic Archipelago), under the name *Nupela* cf. *wellneri* (Lange-Bertalot) Lange-Bertalot in Rumrich 2000 (Zimmermann et al., 2010).

Taxonomical notes: the ascription of this new species to the genus *Germainiella* can be evidenced regarding the presence of all its characteristic apomorphies (Metzeltin et al., 2005), namely i) a large conopeum that covers the entire valve, including the mantle, and ii) a series of canal apertures externally on either side of the raphe (fig 5b, arrow). Additionally, *Germainiella* frustules bear girdle bands opening at valve center (Liu et al., 2017), a character not seen in our individuals. These features separate *Germainiella* taxa from the closely related genus *Pseudofallacia* (Liu et al., 2012, Le Cohu et al., 2016) but a number of other similar small naviculoid genera have been established recently, all of them bearing a single transapically elongated areola per stria (which does not correspond to the external foramen of an alveolus, as in *Oestrupia*); all of them can be discriminated by paying attention to relatively few characteristics (Table 2). Noteworthy, many of these groups also share their ecological preferences, since *Chamaepinnularia*, *Germainiella*, *Humidophila*, *Microcostatus* and others usually inhabit subaerial, humid habitats, including cave walls.

Biogeographical remarks: our observations have raised some important floristic novelties, not only for the Iberian phycoflora, but also with some additions to the cave-dwelling biota recorded to date worldwide (Falasco et al., 2014). Based on the Fenchel et al. (1997) experiment, the theory “everything is everywhere” was first proposed, stating that the microbial species found in a given habitat are a function only of habitat properties and not of historical factors

Table 2. Key to the identification of small naviculoid diatoms generally with a single external foramen per stria.

No.	Character	Genus
1	Areolae covered by a fine membrane	<i>Biremis</i> "type 1" <i>sensu</i> Witkowski et al. (2014)
1'	Otherwise	2
2	Presence of microcostae along the valve face, conopea and pseudoconopea occur frequently	<i>Microcostatus</i>
2'	Otherwise	3
3	Valves covered by a conopeum	4
3'	Otherwise	5
4	Conopeum restricted to the valve face	<i>Pseudofallacia</i>
4'	Conopeum extended to the valve mantle	<i>Germainiella</i>
5	Proximal raphe ends internally slightly deflected	6
5'	Proximal raphe ends internally straight	7
6	Hymenes depressed in internal valve surface, girdle bands perforated	<i>Microfissurata</i>
6'	Otherwise	<i>Chamaepinnularia</i>
7	Externally, distal raphe ends reach the mantle	<i>Pulchella</i>
7'	Otherwise	8
8	With stigmata	9
8'	Otherwise	<i>Humidophila</i>
9	Externally, distal raphe ends T-shaped	<i>Labellicula</i>

(Fenchel and Finlay, 2004), so that their distribution patterns are driven by many features including cell size, dispersion ability, and local or population richness (Fenchel et al., 1997). The absence of biogeographical barriers has been observed in a number of microorganisms, such as Archaea (Whitaker et al., 2003) or Foraminifera, where no genetic differences were found comparing Arctic and Antarctic populations (Darling et al., 2000), as expected in a non-barrier distribution scene. On the other hand, some recent studies have criticized the ubiquity model, e.g. in the case of diatoms, whose dispersal is considered limited in space (Vanormelingen et al., 2008; Vyverman et al., 2007), based on the evidence supporting restricted distribution in the new taxa described, as is the case of *Achnanthes* species in Hawaii, 70% of which are endemic (Lowe et al., 2009). In their study analyzing a global freshwater diatom data set, Vyverman et al. (2007) found evidence in favor of the Theory of Island Biogeography, due to the probable endemism observed in diatom floras in the Southern Hemisphere. In a recent study analyzing the distribution patterns of species within a single genus in the Antarctic Region, Kopalová et al. (2015) found that most *Humidophila* taxa showed restricted biogeographical patterns. In some cases, biogeography has been used to support taxonomic identification of diatoms inhabiting different nearby islands (Van de Vijver et al., 2008). Accordingly, several papers have demonstrated highly-restricted distributions in diatom species inhabiting these islands (Van de Vijver and Beyens, 1999; Van de Vijver et al., 2002, 2008; Van de Vijver and Cox, 2013).

In contrast, Sherwood et al. (2014) carried out a large survey of the Hawaiian Islands analyzing the non-marine algae in targeted areas, such as streams, cave walls, or terrestrial habitats, with molecular methods. These authors deepened the global distribution patterns of forty-four taxa and found only 11.4% endemic species. A similar result was reported by Chattova et al. (2014) in the Ile Amsterdam (TAFF), where only 17% of species were found endemic, and other 14% were species restricted to the sub-Antarctic. In the case of cave diatoms of the Hawaiian Islands, an increasing number of endemic new species are being described (Miscoe et al., 2016); since past studies were mainly based on European floras a few species were newly described.

In view of our results, the occurrence of populations of certain diatom taxa such as *Pinnularia bartii* or *Mayamaea cavernicola* in geographically isolated, very distant regions, but in comparable habitats, may support the ubiquity model. However, the possibility that these populations (Valporquero, Canadian Arctic, and Hawaii for *Germainiella legionensis*) are actually pseudocryptic species (Mann and Evans, 2008) cannot be discarded, although this does not seem likely since these populations share the same ecological preferences, despite the geographical isolation. Currently, new advances in molecular methods are allowing detection of pseudocryptic diversity within diatoms. In the case of cave-dwelling taxa, new studies are needed in order to understand their actual diversity and distribution patterns in these singular ecosystems.

Acknowledgements

A. Sánchez is thanked for SEM assistance. C. Wetzel (LIST, Luxembourg) and E. Falasco (Turin University) kindly helped us in taxonomic identifications.

References

- Abdullin, S.R., 2011, Effect of illumination on the distribution of phototrophic organisms in the entrance part of the Shul'gan-Tash Cave: Russian Journal of Ecology, v. 42, no. 3, p. 249–251. <https://doi.org/10.1134/S1067413611030039>
- Albertano, P., 1993, Epilithic algal communities in hypogean environments: Plant Biosystem, v. 127, no. 3, p. 386–392. <https://doi.org/10.1080/11263509309431020>
- Andreeva, S.A., Kulikovskiy, M.S., Maltsev, Y.I., Podunai, Y.A., and Gusev, E.S. 2016, Molecular genetic investigation of diatom genera *Diadesmis* and *Humidophila* (Bacillariophyceae): Botanicheskiy Zhurnal, v. 101, no. 6, p. 621–628.
- Arrese, B., Durán, J., López-Martínez, J., Altable, O., and Vázquez, J., 2007, Estudio de parámetros ambientales en una cueva hidrogeológicamente activa: la Cueva de Valporquero, in Durán, J.J., Robledo, P.A., and Vázquez, J., eds., Cuevas turísticas: aportación al desarrollo sostenible: Madrid, Publicaciones del Instituto Geológico y Minero, Hidrogeología y Aguas Subterráneas, Serie Hidrogeología y Aguas Subterráneas, p. 127–136.
- Barea, J., Durán, J.J., Heredia, N., Juliá, R., López-Martínez, J., Quintana, L., and Vallejo, M., 1998, Geomorfología del sistema kárstico de Valporquero (León, Cordillera Cantábrica): Investigaciones recientes de la Geomorfología española, p. 31–38.
- Bastazo, G., Vela, J., and Petitpierre, E., 1993, Datos faunísticos sobre Alticinae ibéricos (Col. Chrysomelidae): Boletín de la Asociación española de Entomología, v. 17, no. 1, p. 45–69.
- Chattova, B., Lebouvier, M., and Van de Vijver, B., 2014, Freshwater diatom communities from Ile Amsterdam (TAAF, southern Indian Ocean): Fottea: Journal of the Czech Phycological Society, v. 14, no. 1, p. 101–119. DOI: 10.5507/fot.2014.008
- Coombes, M.A., La Marca, E.C., Naylor, L.A., Piccini, L., De Waele, J., and Sauro, F., 2015, The influence of light attenuation on the biogeomorphology of a marine karst cave: a case study of Puerto Princesa Underground River, Palawan, the Philippines: Geomorphology, v. 229, p. 125–133. <https://doi.org/10.1016/j.geomorph.2014.10.007>
- Cueva de Valporquero, 2017, <http://www.cuevadevalporquero.es> [accessed on October 1, 2017]
- Cuezva, S., Sanchez-Moral, S., Saiz-Jimenez, C., and Cañaveras, J.C., 2009, Microbial communities and associated mineral fabrics in Altamira Cave, Spain: International Journal of Speleology, v. 38, no. 1, p. 83–92. <http://dx.doi.org/10.5038/1827-806X.38.1.9>
- Darling, K.F., Wade, C.M., Stewart, I.A., Kroon, D., Dingle, R., and Leigh Brown, A.J., 2000, Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers: Nature, v. 405, no. 6782, p. 43–47. DOI:10.1038/35011002
- Del Rosal, Y., 2016, Análisis, impacto y evolución de los biofilms fotosintéticos en espeleotemas, El caso de la Cueva de Nerja [Ph.D. thesis]: Málaga, Universidad de Málaga, 336 p.
- Dobát, K., 1963, Höhlenalgen" bedrohen die Eiszeitmalereien von Lascaux: Die Höhle, Wien, v. 14, no. 2, p. 41–45.
- Falasco, E., Bona, F., Isaia, M., Piano, E., Wetzel, C.E., Hoffmann, L., and Ector, L., 2015, *Nupela trogliphila* sp. nov., an aerophilous diatom (Bacillariophyta) from the Bossea cave (NW Italy), with notes on its ecology: Fottea, v. 15, no. 1, p. 1–9. DOI: 10.5507/fot.2015.001
- Falasco, E., Ector, L., Isaia, M., Wetzel, C.E., Hoffmann, L., and Bona, F., 2014, Diatom flora in subterranean ecosystems: a review: International Journal of Speleology, v.43, no. 3, p. 231–251. <http://dx.doi.org/10.5038/1827-806X.43.3.1>
- Fenchel, T., Esteban, G.F., and Finlay, B.J., 1997, Local versus Global Diversity of Microorganisms: Cryptic Diversity of Ciliated Protozoa: Oikos, v. 80, no. 2, p. 220–225. DOI: 10.2307/3546589
- Fenchel, T., and Finlay, B.J., 2004, The Ubiquity of Small Species: Patterns of Local and Global Diversity: BioScience, v. 54, no. 8, p. 777–784. [https://doi.org/10.1641/0006-3568\(2004\)054\[0777:TUOSSPJ\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2004)054[0777:TUOSSPJ]2.0.CO;2)
- Graening, G.O., and Brown, A.V., 2003, Ecosystem dynamics and pollution effects in an Ozark cave stream: JAWRA Journal of the American Water Resources Association, v. 39, no. 6, p. 1497–1507. <https://doi.org/10.1111/j.1752-1688.2003.tb04434.x>
- Grobbelaar, J.U., 2000, Lithophytic algae: A major threat to the karst formation of show caves: Journal of Applied Phycology, v. 12, no. 3–5, p. 309–315. <https://doi.org/10.1023/A:1008172227611>
- Hoffmann, L., 2002, Caves and other low-light environments: Aerophytic photoautotrophic microorganisms, in: Bitton, G., ed., Encyclopedia of Environmental Microbiology: New York, John Wiley & Sons, p. 835–843.
- Holsinger, J.R., 1988, Trogllobites: the evolution of cave-dwelling organisms: American Scientist, v. 76, p. 146–153.
- Johansen, J.R., 2010, Diatoms of aerial habitats, in Smol, J.P., and Stoermer, E.F., eds., The Diatoms: Applications for the Environmental and Earth Sciences: Cambridge, Cambridge University Press, p. 465–472
- Kopalová, K., Kociolek, J.P., Lowe, R.L., Zidarova, R., and Van de Vijver, B., 2015, Five new species of the genus *Humidophila* (Bacillariophyta) from the Maritime Antarctic Region: Diatom Research, v. 30, no. 2, p. 117–131. <https://doi.org/10.1080/0269249X.2014.998714>
- Lauriol, B., Prévost, C., and Lacelle, D., 2006, The distribution of diatom flora in ice caves of the northern Yukon Territory, Canada: relationship to air circulation and freezing: International Journal of Speleology, v. 35, no. 2, p. 83–92. <http://dx.doi.org/10.5038/1827-806X.35.2.4>
- Le Cohu, R., Barthès, A., Leflaive, J., and Ten-Hage, L., 2016, *Germainiella clandestina* sp. nov. (Bacillariophyta), a new species in a little known diatom genus: Fottea, Olomouc, v. 16, no. 2, p. 184–188. DOI: 10.5507/fot.2016.005
- Liu, B., Williams, D.M., and Ou, Y., 2017, *Adlafia sinensis* sp. nov. (Bacillariophyceae) from the Wuling Mountains Area, China, with reference to the structure of its girdle bands: Phytotaxa, v. 298, no. 1, p. 43–54. DOI: <http://dx.doi.org/10.11646/phytotaxa.298.1.4>
- Liu, Y., Kociolek, J.P., Fan, Y., and Wang, Q., 2012, *Pseudofallacia* gen. nov., a new freshwater diatom (Bacillariophyceae) genus based on *Navicula occulta* Krasske: Phycologia, v. 51, no. 6, p. 620–626. <https://doi.org/10.2216/11-098.1>
- Lowe, R., Kociolek, J.P., You, Q., Wang, Q., and Stepanek, J., 2017, Diversity of the diatom genus *Humidophila* in karst areas of Guizhou, China: Phytotaxa, v. 305, no. 4, p. 269–284.
- Lowe, R.L., Kociolek, P., Johansen, J.R., Van de Vijver, B., Lange-Bertalot, H., and Kopalová, K., 2014, *Humidophila* gen. nov., a new genus for a group of diatoms (Bacillariophyta) formerly within the genus *Diadesmis*: species from Hawaii, including one new species: Diatom Research, v. 29, no. 4, p. 351–360. <https://doi.org/10.1080/0269249X.2014.889039>
- Lowe, R.L., Sherwood, A.R., and Ressa, J.A., 2009, Freshwater Species of *Achnanthes* Bory from Hawaii: Diatom Research, v. 24, no. 2, p. 327–340. <https://doi.org/10.1080/0269249X.2009.9705805>
- Lukešová, A., and Nováková, A., 2008, Interactions between soil micro-flora and invertebrates in Slovak and Moravian caves, in Central European Workshop on Soil Zoology, 9th, České Budějovice.

- Lund, J.W.G., 1945, Observations on Soil Algae: Part I. *New Phytologist*, v. 44, no. 2, p. 196–219.
- Lund, J.W.G., 1946, Observations on Soil Algae: Part II. *New Phytologist*, v. 45, no. 1, p. 56–110.
- Mann, D., and Evans, K., 2008, The species concept and cryptic diversity, *in* Proceedings of the 12th International Conference on Harmful Algae, Copenhagen, International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, p. 262–268.
- Metzeltin, D., Lange-Bertalot, H., and García-Rodríguez, F., 2005, Diatoms of Uruguay. Compared with other taxa from South America and elsewhere: Koenigstein, Koeltz Scientific Books, *Iconographia Diatomologica*, v. 15, 736 p.
- Milanovic, S., 2007, Hydrogeological characteristics of some deep siphonal springs in Serbia and Montenegro karst: *Environmental Geology*, v. 51, no. 5, p. 755–759. <https://doi.org/10.1007/s00254-006-0391-1>
- Miscoe, L.H., Johansen, J.R., Kociolek, J.P., Lowe, R.L., Vaccarino, M.A., Pietrasiak, N., and Sherwood, A.R., 2016, The diatom flora and cyanobacteria from caves on Kauai, Hawaii: *Acta Botanica Hungarica*, v. 58, p. 3–4.
- Mulec, J., and Kosi, G., 2009, Lampenflora algae and methods of growth control: *Journal of Cave and Karst Studies*, v. 71, no. 2, p. 109–115.
- Mulec, J., Kosi, G., and Vrhovšek, D., 2007, Algae promote growth of stalagmites and stalactites in karst caves (Škocjanske Jame, Slovenia): *Carbonates and Evaporites*, v. 22, no. 1, p. 6–9.
- Niemiller, M.L., and Soares, D., 2015, Cave Environments, *in* Riesch, R., Tobler, M., and Plath, M., eds., *Extremophile Fishes: Heidelberg*, Springer International Publishing, p. 161–191.
- Ortiz, M., Legatzki, A., Neilson, J.W., Fryslie, B., Nelson, W.M., Wing, R.A., Soderlund, C.A., Pryor, B.M., and Maier, R.M., 2014, Making a living while starving in the dark: metagenomic insights into the energy dynamics of a carbonate cave: *The ISME Journal*, v. 8, no. 2, p. 478–491. doi: 10.1038/ismej.2013.159
- Petitpierre, E., and Gómez-Zurita, J., 1998, Los Chrysomelidae de León; NO de España (Coleoptera): *Nouvelle Revue d'Entomologie (NS)*, v. 15, no. 1, p. 13–26.
- Piano, E., Bona, F., Falasco, E., La Morgia, V., Badino, G., and Isaia, M., 2015, Environmental drivers of phototrophic biofilms in an Alpine show cave (SW-Italian Alps): *Science of The Total Environment*, v. 536, p. 1007–1018. DOI:10.1016/j.scitotenv.2015.05.089
- QGIS. 2017. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>
- Rushforth, S.R., Kaczmarek, I., and Johansen, J.R., 1984, The subaerial diatom flora of Thurston Lava Tube, Hawaii: *Bacillaria*, v. 7, p. 135–157.
- Salgado, J., 1985, Nuevos datos sobre Catopidae (Col.) capturados en cuevas: *Boletín de la Asociación española de Entomología*, v. 9, p. 271–277.
- Sherwood, A.R., Carlile, A.L., Neumann, J.M., Kociolek, J.P., Johansen, J.R., Lowe, R.L., Conklin, K.Y., and Presting, G.G., 2014, The Hawaiian freshwater algae biodiversity survey (2009–2014): systematic and biogeographic trends with an emphasis on the macroalgae: *BMC Ecology*, v. 14, no. 28. doi: 10.1186/s12898-014-0028-2
- St. Clair, L., Rushforth, S., and Allen, J., 1981, Diatoms of Oregon Caves National Monument, Oregon: *Great Basin Naturalist*, v. 41, no. 3, p. 9.
- Trajano, E., Gallão, J.E., and Bichuette, M.E., 2016, Spots of high diversity of troglobites in Brazil: the challenge of measuring subterranean diversity: *Biodiversity and Conservation*, v. 25, no. 10, p. 1805–1828. doi: 10.1007/s10531-012-0242-1
- Urzi, C., De Leo, F., Bruno, L., and Albertano, P., 2010, Microbial diversity in paleolithic caves: a study case on the phototrophic biofilms of the Cave of Bats (Zuheros, Spain): *Microbial ecology*, v. 60, no. 1, p. 116–129. doi: 10.1007/s00248-010-9710-x.
- Van de Vijver, B., and Beyens, L., 1999, Biogeography and ecology of freshwater diatoms in Subantarctica: a review: *Journal of Biogeography*, v. 26, no. 5, p. 993–1000.
- Van de Vijver, B., and Cox, E.J., 2013, New and interesting small-celled naviculoid diatoms (Bacillariophyceae) from a lava tube cave on Île Amsterdam (TAAF, Southern Indian Ocean): *Cryptogamie, Algologie*, v. 34, no. 1, p. 37–47. <https://doi.org/10.7872/crya.v34.iss1.2013.37>
- Van de Vijver, B., Frenot, Y., and Beyens, L., 2002, Freshwater Diatoms from Ile de la Possession (Crozet - Archipelago, Subantarctica): Berlin, J. Cramer in der Gebrüder. Borntraeger Verlagsbuchhandlung, *Bibliotheca Diatomologica*, v. 46, p. 412.
- Van de Vijver, B., Gremmen, N., and Smith, V., 2008, Diatom communities from the sub-Antarctic Prince Edward Islands: diversity and distribution patterns: *Polar Biology*, v. 31, no.3, p. 795–808. <https://doi.org/10.1007/s00300-008-0418-z>
- Van der Werff, A., 1955, A New Method of Concentrating and Cleaning Diatoms and Other Organism: *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*, v. 12, p. 276–277.
- Vanormelingen, P., Verleyen, E., and Vyverman, W., 2008, The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism: *Biodiversity and Conservation*, v. 17, no. 2, p. 393–405. <https://doi.org/10.1007/s10531-007-9257-4>
- Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D.A., Mann, D.G., Juggins, S., Vijver, B.V. de, Jones, V., Flower, R., Roberts, D., Chepurnov, V.A., Kilroy, C., Vanormelingen, P., and De Wever, A., 2007, Historical processes constrain patterns in global diatom diversity: *Ecology*, v. 88, no. 8, p. 1924–1931.
- Werum, M., and Lange-Bertalot, H. 2004, Diatomeen *in* Quellen unter hydrogeologischen und anthropogenen Einflüssen in Mitteleuropa und Anderen Regionen: Koenigstein, Gantner Publishing, *Iconographica Diatomologica*, v.13, 417 p.
- Whitaker, R.J., Grogan, D.W., and Taylor, J.W., 2003, Geographic Barriers Isolate Endemic Populations of Hyperthermophilic Archaea: *Science*, v. 301, no. 5635, p. 976–978. DOI:10.1126/science.1086909
- Zimmermann, C., Poulin, M., and Pienitz, R., 2010, Diatoms of North America: The Pliocene-Pleistocene freshwater flora of Bylot Island, Nunavut, Canadian High Arctic: Koenigstein, Gantner Publishing, *Iconographia Diatomologica*, v. 21, 407 p.