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Thriving in the dark: a new record and new species of Annelida (Lamarck, 1802) from a Balearic anchialine cave (Western Mediterranean Sea)

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Abstract

Anchialine caves are singular aquatic environments of significant conservation interest, defined by darkness, constant temperature, and low levels of nutrients and dissolved oxygen, as well as vertically stratified water layers. Due to their challenging accessibility, these caves represent one of the few remaining unexplored frontiers on Earth, hosting short-range endemics and ecologically specialized communities highly susceptible to anthropogenic disturbances. Exploration of a cave in the northern region of Mallorca, Balearic Islands (Western Mediterranean), has revealed the presence of a new genus and species of Polynoidae, *Pollentia perezi* Capa, Pons & Jaume, 2022, with presumed deep-sea affinities. Further exploration of this cave system showed two additional polychaetes. The first is a new species of *Polycirrus* (Terebellidae), whereas the second, *Vermiliopsis labiata* (Costa, 1861) (Serpulidae), previously known to inhabit marine environments, represents the first record of this species in an achialine cave. Notably, neither the terebellid nor the serpulid exhibits apparent morphological adaptations to their cave habitat. Our study combines morphological and molecular analyses to characterise the polychaete fauna of the Cova des Bastons, Mallorca, and increases the number of taxa associated with Mediterranean anchialine environments.

Keywords: Serpulidae; Terebellidae; polychaetes; anchialine caves; marine caves.

Abbreviations

BIC: Bayesian Information Criterion. CBB-UIB: Balearic Biodiversity Centre, University of the Balearic Islands, Palma, Spain. COI: cytochrome c oxidase subunit I. CEAB-CSIC: Centre for Advanced Studies of Blanes, Spanish National Research Council, Blanes, Spain. COI: cytochrome c oxidase subunit I. Cyt b: cytochrome oxidase subunit b. HMDS: hexamethyldisilazane. MB: Methyl Blue staining. MF: main fang. MNCN: National Museum of Natural Sciences, Madrid, Spain. MT: main tooth. NCBI: National Center for Biotechnology Information. PCR: polymerase chain reaction. SEM: scanning electron microscopy.SG:: segment. UIB: University of the Balearic Islands. UL: upper lip. LL: lower lip.

Introduction

Anchialine caves, defined as caves that access coastal saline aquifers without evident connections to the open sea, represent unique environments of significant conservation importance (Stock *et al*. 1986; Illife, 2002; Romero, 2019). They generally exhibit low nutrient and dissolved oxygen levels, and strongly stratified water layers (Keith *et al.*, 2022). Their challenging accessibility has led them to remaining among the last unexplored frontiers on Earth (Iliffe, 2018; Mammola *et al.*, 2021). There is, in fact, scant knowledge about most anchialine caves around the world, and even relatively well-studied anchialine systems harbour a wealth of undiscovered biodiversity (Becking *et al.*, 2011; Worsaae *et al.*, 2018; Martínez *et al.*, 2019; Sánchez & Martínez, 2019; Capa *et al*., 2022).

Anchialine caves host animal communities comprising short-range endemic and ecologically specialized

species. These organisms often display morphological, behavioural, and physiological adaptations tailored to thrive in this particular environment (Iliffe & Kornicker, 2009). Anchialine fauna often exhibits surprising evolutionary relationships and disjunct geographical distributions (Lozano-Fernandez *et al.*, 2019; Capa *et al.,*2020), emphasizing the scientific value and potential for ecological insights within these distinct ecosystems.

The ecologically specialized anchialine fauna might be highly sensitive to local perturbations, including anthropogenic disturbances, given its natural fragmentation and limited resilience (Harmelin, 1980). Unfortunately, the specific effects of different stressors on anchialine ecosystems remain to be quantified (Becking *et al.*, 2011; Worsaae *et al.,* 2018; Martínez *et al.*, 2019; Sánchez & Martínez, 2019; Capa *et al.,* 2022; Mammola *et al.*, 2022).

The anchialine subterranean biomes, well-represented in the Caribbean, Western Australian, Indo-Pacific islands, and Mediterranean coasts (Gerovasileiou *et al.*, 2016a), notably extend to the Balearic archipelago in the Western Mediterranean (Boxshall & Jaume, 2000; Jaume *et al.*, 2008; Navarro-Barranco *et al.*, 2023). Our exploration of one of these caves in the northern region of Mallorca unveiled the presence of remarkable annelids. Among them, the scale-worm *Pollentia perezi* Capa, Pons & Jaume, 2022 (Polynoidae) exhibited deep-sea affinities and traits typically attributed to species found in cold and dark environments (Capa *et al.*, 2022). In the present study, we report two other annelid species occurring in the same cave. *Vermiliopsis labiata* (Costa, 1861) (Serpulidae) was scraped from the dark cave entrance walls, at 5-10 m depth, whereas a new species of *Polycirrus* (Terebellidae, Polycirrini) was collected further inside the cave, on sediments at 17 m depth.

Terebellids are sedentary deposit-feeding annelids bearing numerous long tentacles inserted outside of the mouth. Amongst them, *Polycirrus Grube,* 1850 is one of the most species-rich genera, characterised by lacking branchiae, bearing uncini in single rows, and often reduced chaetae and noto- and neuropodia usually present in the chaetigerous segments (Glasby & Hutchings, 2014; Hutchings *et al*, 2017; Stiller *et al.*, 2020). However, numerous species were described more than a century ago, and some of them lack many taxonomic characters considered relevant in current descriptions. In addition, types have either been lost or were never formally designated in some species. Both situations have led to erroneous attributions of cosmopolitan distributions (sensu Hutchings & Kupriyanova, 2018). This taxonomic and biogeographic uncertainty was rectified by designating a neotype for the type species, *P. medusa* Grube, 1850, redescribing all 59 species with available types or specimens from type localities, and assigning six species as *species inquirendae* (Glasby & Hutchings, 2014). Subsequent morphological and molecular investigations revealed *Polycirrus* to be paraphyletic (Stiller *et al.*, 2020). Further taxonomic work has resulted in the description of 21 new species of *Polycirrus* (Nogueira *et al.*, 2015; Cepeda & Lattig, 2016; Lavesque *et al.*, 2020, 2021; Çinar & Erdoğan-Dereli, 2023; Jimi *et al.*, 2023), bringing the total number of valid species up to 87 (Read & Fauchald, 2025).

Serpulids represent a species-rich family easily recognised by their distinctive filter-feeding radiolar crowns, calcareous tubes and, in most species, the opercula (a modified branchial radiole that seals the tube opening) (Kupriyanova *et al.*, 2006). Among them, the monophyletic genus *Vermiliopsis* Saint-Joseph, 1894 comprises 18 valid species (Kupriyanova *et al.*, 2006; Read & Fauchald, 2025). Members of the genus are characterised by an operculum as an inverse conical ampulla, with flat to conical chitinous endplate, thoracic membrane short, *Apomatus* chaetae present in the thorax (ten Hove & Kupriyanova, 2009). *Vermiliopsis labiata* (Costa, 1861) is characterised by longitudinal chambers in the tube, a toothed calcareous opercular plate, and brown parapodia (Bianchi, 1981; ten Hove & Kupriyanova, 2009). Originally described from the Mediterranean, this species is relatively uncommon and prefers dark environments, such as marine caves (Saint-Joseph, 1894; Bianchi, 1981; Gil, 2011; Rosso *et al.*, 2021).

This paper describes and illustrates a new species of *Polycirrus* and newly reports *V. labiata* from the Balearic Islands, contributing to increase of the polychaete diversity found in the anchialine caves of the archipelago. Additionally, it provides a comprehensive overview of published cave records for terebellids and *V. labiata*.

Material and Methods

Sample collection

Specimens were collected at the anchialine Cova des Bastons (also known as Cave C-11), Alcúdia, Mallorca, Balearic Islands, 39.883419º N, 3.195864º E, June 3, 2020, and July 11, 2024. The cave environment was described in Capa *et al*. (2022). The terebellid was found at 17 m depth in the bottom sediment, near the location where the holotype of *P. perezi* was collected (Capa *et al*., 2022). The serpulids were sampled closer to the entrance by gently scraping the calcareous tube aggregates from the cave wall with a diving knife. All specimens were transported alive in jars to the laboratory, where they were photographed, preserved, and stored in absolute ethanol at 4-6 °C.

Morphological analyses

Live and preserved specimens were examined under Euromex DZ.1105 stereo- and Olympus BX60 compound light microscopes. Photographs were taken with a Euromex DC.18000-PRO and a Motic 3 camera attached to the stereo and compound microscopes, respectively. Contrast of external morphological traits was enhanced by Methyl Blue staining (MB). Dissected thoracic and abdominal parapodia were mounted on holders, sputter-coated with gold (10 nm thickness) and examined under a HITACHI S-3400N scanning electron microscope

(SEM) at the University of the Balearic Islands (UIB), after dehydration in a series of mixtures of 0%, 25%, 50%, 75%, and 100% ethanol, then hexamethyldisilazane (HMDS). Vouchers and type material were deposited at the CBB-UIB, the CEAB-CSIC, and the MNCN.

DNA extraction, amplification and sequencing

A tentacle for *Polycirrus* and the posterior end for *Vermiliopsis* were dissected for DNA extraction. These tissues were incubated at 65° C in 50 µL of QuickExtract (Lucigen) for 1-5 h, followed by an incubation at 95° C for 5 min. Fragments of COI and Cyt b mitochondrial markers and the region D1-D2 of the nuclear 28S rDNA genes were amplified by PCR. The 20-µL amplification reaction contained 10 µL of Bioline MyTaqRedMix (Bioline, London, UK; with buffer, dNTPs and $MgCl_2$ incorporated into the mix), 7.4 µL H2O, 0.8 µL of each primer (10 µM) and 1 µL of DNA. The primer pairs LCO/HCO (Folmer *et al.*, 1994) and jgLCO/jgHCO (Geller *et al.*, 2013) were used for amplification of the COI gene fragment for the terebellid, and HyCOF190/HyCOR886 (Sun *et al.*, 2017) and Cyt b 424F (Boore & Brown, 2000) and cobr825 (Burnette *et al.,* 2005) were used for the serpulid for COI and Cyt b gene fragments respectively. The 28S D1-D2 fragment was amplified using the primer pairs 28SC1'/28SD2 (Lê *et al*., 1993). Primer sequences and PCR thermal profiles are available in supplementary data (Table S1, S2). The PCR products were run on a 1% agarose gel containing ethidium bromide for 30 min at 80 V and visualized under ultraviolet light. The PCR products with the expected length were cleaned using the kit microCLEAN for PCR clean-up (Microzone, Lewes, UK). Sanger cycle sequencing was performed on both strands at Macrogen DNA Sequencing Department (Madrid, Spain). Forward and reverse reads were merged into consensus sequences and edited using Geneious Prime (Kearse *et al.*, 2012). COI and Cyt b sequences were translated into amino acids in SeaView v.4.7 (Gouy *et al*., 2010) and checked for the presence of stop codons and rare nonsynonymous substitutions that could indicate the amplification of pseudogenes. Our sequences were then compared to all relevant terebellid and serpulid sequences available from public databases. Highly divergent sequences with unexpected long branches were excluded after preliminary analyses as potential misidentifications or contaminations. Also, short available sequences were eliminated to avoid the noise of missing data in phylogenetic analyses. When several sequences attributed to the same species were available, we selected the longest (suppl. data Fig. S3, S4).

Phylogenetic analyses and molecular species identification

Each single gene dataset was aligned with MAFFT v7.525 (Katoh & Standley, 2013) allowing for an automatic strategy and default parameters. Phylogenetic trees were optimized under Maximum Likelihood criterion in IQ-TREE multicore v2.0.7 (Minh *et al.*, 2020), selecting the best nucleotide substitution model using the self-implemented ModelFinder (Kalyaanamoorthy *et al.*, 2017) option. We only included the most common substitution models (--mset F81, HKY, TN, GTR) and did not apply the combination of invariant sites plus gamma-rate parameter to estimate among-site rate variation, since both are inextricably linked (Sullivan *et al.*, 1999). For the protein coding gene COI, we assessed two alternative partition schemes: 1) first codon plus second codon sites vs. third codon ones and 2) three codon partitions. Best nucleotide substitution models and partition schemes were selected based on BIC. Node support was estimated with 1000 ultrafast bootstrap replicates, the parametric aLRT test (Anisimova & Gascuel, 2006), and approximate Bayes test (Anisimova *et al.*, 2011).

The outgroup for the analyses based on the COI sequences was *Thelepus cincinnatus* (Fabricius, 1780) (Stiller *et al.*, 2020). For the more conserved 28S D1-D2 fragment, with less publicly available sequences, we selected the ampharetid *Ampharete* sp. and the sabellid *Sabella pavonina* Savigny, 1822 as outgroups. The COI and 28S terebellid phylogenetic analyses included 96 and 14 terminals, respectively, whereas the serpulid analyses included 52. The taxonomic assignment of the amplicons was assessed with BLAST at NCBI platform (Altschul *et al.*, 1990).

Results

Taxonomic account

The terebellid was identified as a *Polycirrus*, but did not match any currently known species descriptions (Glasby & Hutchings, 2014; Lavesque *et al.*, 2020; 2021). COI and 28S DNA showed sequences divergence of 15.53% (COI) and 12-13% (28S) base pairs from publicly available data. Consequently, we here describe it as a new species.

The serpulids were identified as *V. labiata*, a species with conspicuous diagnostic features inits tube, branchial crown, and chaetae (Zibrowius, 1968; Bianchi, 1981; San Martín, 2022). Therefore, we are providing a diagnosis based on the specimens collected in the Cova des Bastons. Identification was corroborated by identical 28S sequences available in NCBI from France (GenBank accession number PV928524).

Genus *Polycirrus* Grube, 1850

Type species. *Polycirrus medusa* Grube, 1850 by monotypy.

***Polycirrus burballes* sp. nov.**

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(Figs. 1-4)

Diagnosis

Terebellidae lacking branchiae, with an expanded triangular upper lip bearing two types of tentacles; an outer lower lip enlarged, resembling a ventral pad, divided in two regions, inner and outer; paired ventro-lateral pads; notopodia in segments 3-19, segment 20 achaetous; neuropodia from segment 21; capillary notochaetae with narrowly-winged limbation (anterior row) and pinnated (posterior row); neuropodial uncini of type I, with only one tooth over main fang.

Etymology

The species name *burballes*, meaning "shavings" in Mallorcan Catalan, also refers in Mallorcan cuisine to a type of spaghetti-like pasta locally produced in the small town of Porreres (www.recetasmallorquinas.es/2019/09/burballes.html). Thus, it is here used as a noun in apposition to playfully allude to the common name "spaghetti worms" frequently used for terebellids.

Holotype

CBB-UIB 101393. SPAIN – Balearic Islands • Holotype. 1 complete specimen; the Cova des Bastons (also known as Cave C-11), Alcúdia, Mallorca, Balearic Islands; 39.883419º N, 3.195864º E; 17 m depth; on calcareous silt; 11 March 2020; coll. María Capa and Joan Pérez; preserved and stored in 100% ethanol; DNA available sequences: GenBank accession numbers PV919570 (COI), PV919556 (28S rDNA).

Description

Entire specimen, 37 mm long (without tentacles) and 1.8 mm wide at thoracic level (Fig. 1A-D, H), with ca. 80 segments. Body whitish, with translucent epithelium and pale orange gut *in vivo* (Fig. 1A), turning pale beige after preservation (Fig. 1B); overall cylindrical, slightly stouter anteriorly, swollen dorsally from mid-thorax to last 40 chaetigers, with thin and fragile body wall, then uniformly cylindrical, tapering posteriorly (Fig. 1A-C).

Transverse prostomium attached to dorsal surface of base of upper lip (Fig. 1E-H). Buccal tentacles of two types: type one short uniformly cylindrical; type two long and cy-

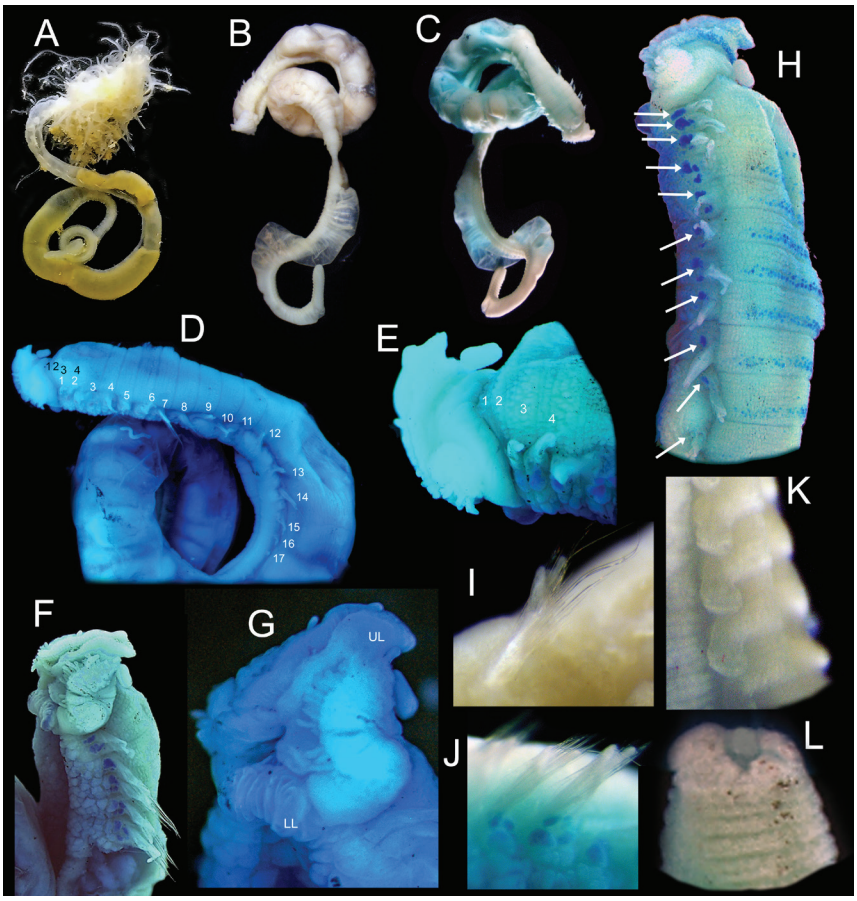

Fig. 1: *Polycirrus burballes* sp. nov. Light micrographs. Entire specimen: A. Alive. B. Preserved, ventral view. C. Preserved, stained with Methyl blue, dorsal view. Anterior end preserved, stained with Methyl blue: D. Thorax, lateral view; black numbers: first four segments; white numbers: notochaetigerous segments. E. Detail of anterior end, lateral view white numbers: first four segments. F. Thorax, ventro-lateral view. G. Detail of anterior end, ventral view; UL: upper lip; LL: lower lip. Parapodia, preserved. H. From segments 3-13 preserved, stained with Methyl blue: white arrows: nephridial papillae. I. From segment 7. J. From segment 5–6, stained with Methyl blue. K. From abdomen. L. Posterior end and pygidium.

lindrical, slightly expanding distally, and deeply grooved, with inner side of groove densely ciliated (Fig. 2A-C). Peristomium with upper and lower lips; upper lip triangular, longer than wide, hood-like, convoluted (Fig. 1G); lower lip oval, cushion-like, with eight central narrow longitudinal pads and two lateral pads 2-3 times wider (Fig. 1G).

Segment 1 dorsally triangular, with round edges, ventrally with enlarged first shield; segment 2 narrower than following segments, covered ventrally by expanded first ventral shield (Fig. 1D-F). Ventro-lateral pads on segments 1-13, first shield entire, other shields paired, separated from each other mid-ventrally by mid-ventral groove; shields of segments 11-12 progressively less developed, most reduced on 13; pads swollen, rectangular, markedly tessellated (Fig. 1F).

Notopodia elongated, bilobed, present on chaetigers 1-17 (i.e., segments 3-19, 1/4 of total body length). Notopodial lobes digitiform, distally rounded; posterior lobe twice longer than anterior lobe; first seven lobes on segments 3-9, similar in length, slightly longer than those on following notopodia (Figs 1E, F, H-I, 3A, 4A). Nephridial/genital papillae opening at the base of notopodia, at least on segments 4-7 (Fig. 1H). One achaetous segment (segment 20) between thorax and abdomen. Abdomen from segment 21 with neuropodia only, as raised flat pads, non-distinguishable in segments 21 and 22, oval and progressively wider on segments 23-29, then progressively shorter towards the pygidium (Fig. 1K, 3C, D, 4C, D).

Notochaetae very narrowly-winged in anterior and posterior rows, with wings only at tips; chaetae of anterior row ca. ⅓-¼ as long as those in posterior row (Fig. 3A-B, 4A-B). Neuropodia with Type I uncini *sensu* Glasby and Glasby (2006) throughout, with short neck; short, triangular and slightly hooked heel directed posteriorly; narrow and curved base, anteriorly pointed; dorsal button (i.e., subrostral process) as low protuberance at base of main fang; crest with single, elongate, sharp tooth above main fang (Figs 3C-F; 4C-F). Pygidium with four pads after preservation (Fig. 1L).

Ecology and distribution

The only known individual of *P. burballes* sp. nov. was found on the cave silty sediments, with no traces of tube. This suggests it may live freely, crawling over the substrate, as other polycirrids do (Hutchings *et al.*, 2017; Nogueira *et al.*, 2020). So far, the species is endemic to Cova des Bastons. Although this is the first record of a species of Terebellidae in an anchialine cave, members of the family are not rare in Mediterranean marine caves, where they probably take advantage of the abundance of deposited organic matter. Although some of the terebellid species previously reported in marine caves may need to be checked in a wider systematic context, the list currently comprises 14 species, including three *Polycirrus* - *P. aurantiacus* Grube, 1860, *P. denticulatus* Saint-Joseph, 1894, and *P. medusa* (Table 1) – from caves in Campania and Sicily, Italy (Banse, 1959; Cantone *et al*., 1979; Belloni & Bianchi, 1982; Akoumianaki & Hugues, 2004) and several unidentified specimens of the genus from a cave in Tenerife (Riera *et al.*, 2018).

Molecular analyses

BLASTn assignment of the COI sequence (650 bp) first suggested a *Polycirrus* sp. sequence from Hawai‘i (similarity=84.47%, coverage=99%, *e*=0.0), followed by other GenBank Terebelliformia sequences (similarity < 82.03%), whereas that of D1 28S sequence (657 bp) resulted in three sequences of *Polycirrus* being the most similar (similarity=87.14-86.54%, coverage=100-96%, *e*=0.0).

The 28S sequence of the specimen from the Cova des Bastons branched off at the base of a *Polycirrus* clade including all the 28S sequences available in GenBank, although with low support (BS=75, Fig. S3). The COI phylogenies inferred under maximum likelihood were topologically congruent and recovered *Polycirrus* as paraphyletic. However, the tree had poorly supported basal relationships and several unidentified Terebellidae terminals could, in fact, correspond to members of *Polycirrus*.

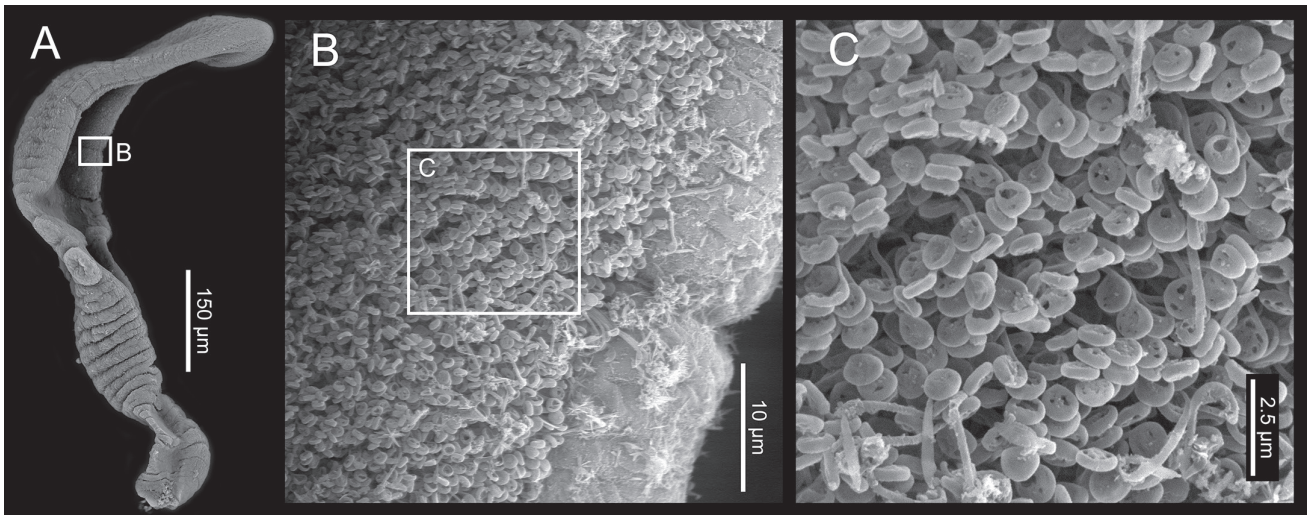

Fig. 2: *Polycirrus burballes* sp. nov. SEM micrographsof a grooved tentacle. A. Distal part of tentacle. B. Detail of the ciliated region. C. Close view of cilia with discoid tips.

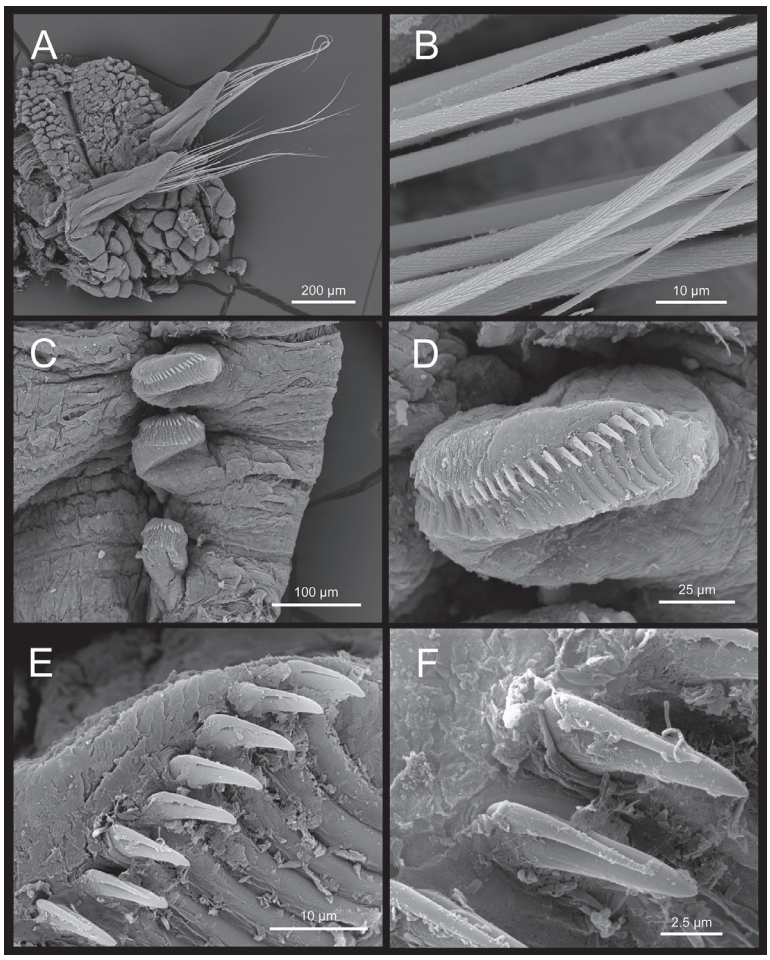

Fig. 3: *Polycirrus burballes* sp. nov. SEM micrographs. A. Thoracic parapodia from segments 5-6. B. Detail of the hirsute surface of thoracic capillaries. C. Abdominal uncinal tori. D. Detail of an abdominal uncinal torus. E. Close view of abdominal uncini. F. Detail of the uncini tips.

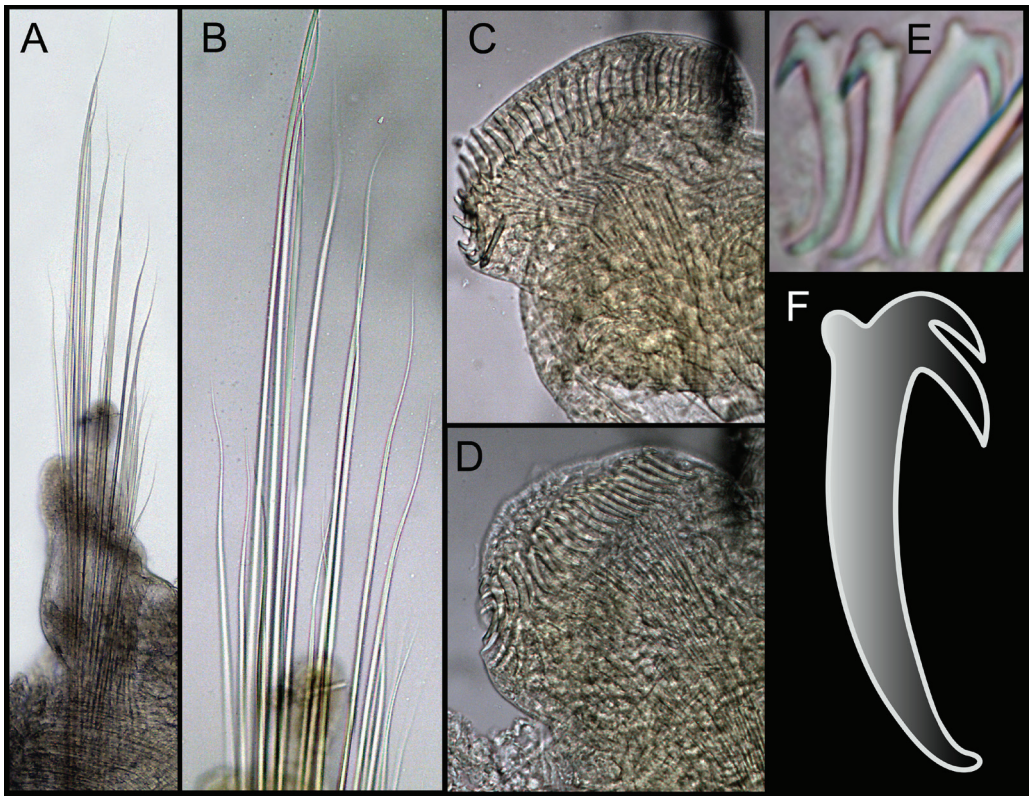

Fig. 4: *Polycirrus burballes* sp. nov. Light micrographs. A. Thoracic parapodia. B. Thoracic capillaries. C, D. Abdominal uncinal pads. E. Abdominal uncini, side view. Drawing. F. Abdominal uncinus, side view.

Table 1. Terebellids recorded in marine coastal caves (with evident connection to the open sea) in the Mediterranean Sea and North East Atlantic Ocean.

Taxon	Geographic region	Cave	References
Amphitrite sp.	North Aegean, Greece	Fara Bay Islet cave, Lesvos Island (entrance)	Gerovasileiou *et al.* (2015, 2016b)
Amphitrite variabilis (Risso, 1826)	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (semidark cave)	Banse (1959)
Amphitritides gracilis (Grube, 1860)	Sicily, Italy	Unnamed, semi-submerged cave near Catania	Cantone *et al.* (1979)
A. gracilis	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (semidark cave)	Banse (1959)
Eupolymnia nebulosa (Montagu, 1819)	Zadar, Croatia	Jama u uvali Kravljacica (entrance)	Petriccioli *et al.* (2016)
E. nebulosa	Velebit Channel, Croatia	Stražica Cape (semidark cave)	Novosel *et al.* (2002)
E. nebulosa	North Aegean, Greece	Fara Bay Islet cave, Lesvos Island (semidark cave)	Gerovasileiou *et al.* (2015)
E. nebulosa	Attica, Greece	Vouliagmeni pit, Vari-Voula-Vouliagmeni (semidark zone)	Gerovasileiou *et al.* (2015)
E. nebulosa	North Aegean, Greece	Fara Bay Islet cave, Lesvos Island (entrance)	Gerovasileiou *et al.* (2016b)
E. nebulosa	Zadar, Croatia	Y-Cave, Dugi Otok	Radolović *et al.* (2015)
Eupolymnia nesidensis (Delle Chiaje, 1828)	Campania, Italy	Grotta del Mitigliano, Massa Lubrense	Belloni & Bianchi (1982)
E. nesidensis	North Aegean, Greece	Agios Vasilios Cave, Lesvos Island (dark)	Gerovasileiou *et al.* (2016b)
E. nesidensis	North Aegean, Greece	Fara Bay Islet cave, Lesvos Island (dark)	Gerovasileiou *et al.* (2016b)
Lanice conchilega (Pallas, 1766)	Azores, Portugal	Túnel de Santa Clara (semidark cave)	Micael *et al.* (2006)
Nicolea venustula (Montagu, 1819)	Sicily, Italy	Unnamed, semi-submerged cave near Catania	Cantone *et al.* (1979)
N. venustula	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse (1959)
Pista cristata (Müller, 1776)	Sicily, Italy	Unnamed semi-submerged cave near Catania	Cantone *et al.* (1979)
Polycirrus sp.	Canary Islands, Spain	Cueva de los Cerebros, Tenerife	Riera *et al.* (2018)
Polycirrus sp.	Campania, Italy	Grotta Azzurra, Capo Palinuro (Snow hall, dark)	Akoumianaki & Hugues (2004)
Polycirrus aurantiacus Grube, 1860	Campania, Italy	Grotta del Mitigliano, Massa Lubrense	Belloni & Bianchi (1982)
P. aurantiacus	Campania, Italy	Grotta Scaletta, Massa Lubrense	Belloni & Bianchi (1982)
P. aurantiacus	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse (1959)
P. aurantiacus	Marseille, france	Niolon Cave	Bellan (1968)
Polycirrus denticulatus Saint-Joseph, 1894	Campania, Italy	Grotta del Mitigliano, Massa Lubrense	Belloni & Bianchi (1982)
Polycirrus medusa Grube, 1860	Sicily, Italy	Unnamed, semi-submerged cave near Catania	Cantone *et al.* (1979)
P. medusa	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse (1959)
Streblosoma bairdi (Malmgren, 1866)	Sicily, Italy	Unnamed, semi-submerged cave near Catania	Cantone *et al.* (1979)
Terebella lapidaria Linnaeus, 1767	Campania, Italy	Grotta Scaletta, Massa Lubrense	Belloni & Bianchi (1982)
T. lapidaria	Euskadi, Spain	Marine caves in Flysch de Igueldo, Donosti	Galán & Arrieta-Etxabe (2014)
T. lapidaria	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse (1959)
T. lapidaria	Euskadi, Spain	Cueva del Buho, Donosti	Galán & Nieto (2016)
T. lapidaria	Euskadi, Spain	Sima de acantilado de Altu o punta E de Bajo aundi	Galán *et al.* (2020)
Terebellidae	Sicily, Italy	Grotta dei Granchi, Siracusa	Sanfilippo *et al.* (2015)
Thelepus cincinnatus (Fabricius, 1780)	Campania, Italy	Grotta del Mago, Ischia	Cinelli *et al.* (1977)
T. cincinnatus	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse (1959)
Thelepus setosus (Quatrefages, 1866)	Sicily, Italy	Unnamed, semi-submerged cave near Catania	Cantone *et al.* (1979)

The cave dwelling *Polycirrus* was recovered at the base of the *Eupolymnia-Loimia* clade, although with low support (Fig. S3).

Remarks

Polycirrus burballes sp. nov. shares with other congeners the notochaetae start at segment 3, Type I uncini, and neurochaetae start at segment 18 or posterior segments (Glasby & Hutchings, 2014; Lavesque *et al.*, 2020; Nogueira *et al.*, 2020; Jimi *et al.*, 2023). These include *P. plumosus* (Wollebæk, 1912) from 95 m depth sandy bottoms at Hjeltefjorden (Norway), *P. variabilis* Hutchings & Glasby, 1986 from coral substrate, and *P. bicrinalis* Hutchings & Glasby, 1986 living amongst dead coral substrate, both on the Great Barrier Reef (Australia), and *P. mexicanus* (Rioja, 1947) from a shallow water bay in the Mexican Pacific (C. J. Glasby & Hutchings, 2014). *Polycirrus burballes* sp. nov. has the outer lower lip as a large quadrangular cushion, extending posteriorly to mid segment 3 (subconical, protruding above venter in *P. plumosus* and oblong in *P. mexicanus*). The specimen of our new species has a low protuberance at base of main fang (lacking subrostral process in *P. variabilis*). *Polycirrus burballes* sp. nov. has uncini with a single tooth above main fang (two rows of teeth in *P. bicrinalis*).

As already mentioned, 21 species were described after the taxonomic revision by Glasby and Hutchings (2014), eight of which inhabit intertidal to ~30 m deep bottoms in coral reefs, mangroves, and beaches with sand, mud and coral rubble at Lizard Island (Australia) (Nogueira *et al.*, 2015). Among them, only members of four species share with *P. burballes* sp. nov. the starting position of neuropodia after the last segment with notopodia: *P. brutus* Nogueira, Hutchings and Carrerette, 2015, *P. culcita* Nogueira, Hutchings and Carrerette, 2015, *P. cruciformis* Nogueira, Hutchings and Carrerette, 2015 and *P. oculeus* Nogueira, Hutchings and Carrerette, 2015. *Polycirrus burballes* sp. nov. differs (1) from *P. cruciformis* and *P. culcita* in the presence of finely winged and pinnate notochaetae instead of only winged, (2) from *P. brutus* in having an oval, cushion-like prostomium instead of projecting laterally as two deeply grooved, tentacular processes, and (3) from *P. oculeus* in having the neuropodia from the second segment after last notopodium (one achaetous segment) instead of third (two achaetous segments). *Polycirrus asturiensis* Cepeda and Lattig, 2016 inhabits photophilic and calcareous macroalgal substrates at 6 m depth in the Cantabric Sea (north of Iberian Peninsula) (Cepeda & Lattig, 2016) and can be easily distinguished from *P. burballes* sp. nov. in having ten notochaetigerous segments instead of seventeen, and also in having only broadly winged notochaetae instead of narrowly winged and pinnate. *Polycirrus changbunker* Nogueira, Van Deursen, Ranauro and Carrerette, 2020 inhabits shallow bottoms off Brazilian coasts, southwestern Atlantic (Nogueira *et al.*, 2020) and has a strong orange to red colour *in vivo* (instead of whitish and translucent with pale orange gut, as the holotype of *P. burballes* sp. nov.), the outer region of lower lip as a large pentagonal to circular cushion (instead of large, quadrangular in *P. burballes* sp. nov.) and two rows of teeth over the main fang (instead of a single tooth in *P. burballes* sp. nov.).

All seven species described by Lavesque *et al.* (2020) from European waters can be easily distinguished from *P. burballes* sp. nov. in having only winged notopodial chaetae (instead of winged and pinnate) and uncini with either two rows of teeth or one very long tooth with rows of shorter lateral teeth on each side (instead of a single long teeth) (Table 2). The other eleven species present in European waters differ from *P. burballes* sp. nov. in the type of uncini and the starting segment for neurochaetae (Table 2). Two other species were described from the Black Sea, *P. karadenizicus* Çinar & Erdoğan-Dereli, 2023 from muddy sands at 25 m deep and *P. rhombolabiatus* Çinar & Erdoğan-Dereli, 2023 from rocky bottoms covered by algae at 11-13 m deep, both mainly differing from *P. burballes* sp. nov. in having neuropodia from segment 15 instead of segment 21 (Table 2). Finally, three species have been recently described from 1-15 m depth in muddy sediments or inside cracks of rocks in Japan (Jimi *et al.*, 2023). *Polycirrus burballes* sp. nov. differs from all of them in having 17 segments with notopodia and neurochaetae neuropodia from the second after last notochaetigerous segment (one achaetous segment in between), while *P. aoandon* Jimi *in* Jimi *et al.* (2023) has more than 50 segments with notopodia and neurochaetae starting well before last notochaetigerous segment, *P. onibi* Jimi *in* Jimi *et al.* (2023) has 11 segments with notochaetae, and *P. ikeguchii* Jimi *in* Jimi *et al.* (2023) has the neurochaetae starting two segments before the last notochaetigerous segment (lacking achaetous segments).

Genus *Vermiliopsis* Saint-Joseph, 1894
Type species. *Vermilia multivaricosa* Mörch, 1863, new name for *Vermilia infundibulum sensu* Philippi, 1844
***Vermiliopsis labiata* (Costa, 1861)**
(Figs 5, 6)

Serpula labiata Costa, 1861
Serpula infundibulum Delle Chiaje, 1828
Hydroides (Eucarphus) infundibulum
(Delle Chiaje, 1828)
Vermiliopsis richardi Fauvel, 1909
Vermiliopsis richardi fauveli Monro, 1930

Material examined

CBB-UIB 101394. SPAIN – Balearic Islands • six specimens; the Cova des Bastons (also known as Cave C-11), Alcúdia, Mallorca, Balearic Islands; 39.884194° N, 3.195861° E; 5-10 m depth, on the walls; July 11, 2024, coll. María Capa and Joan Pérez; fixed and preserved in 100% ethanol. DNA available sequences: PV928524 (28S rDNA).

Table 2. European species of *Polycirrus*, including the main species-diagnostic characters. MF: main fang; MT: main tooth, SG: segment; α: row of small teeth (*) *species inquirenda* according to Glasby & Hutchings (2014); (**) synonymized with *P. haematodes* (Fauvel, 1927).

	Type locality	Upper lip	Lower lip	Pairs of notopodia	Start of neuropodia	Notochaetae	Uncini (type 1)	References
P. arenivorus (Caullery, 1915)	France (English Channel)	medial lobe well developed only	oblong, longer than wide	29	SG12	narrowly winged	MT absent, two rows above MF (MF:3:5)	Glasby & Hutchings (2014)
P. asturiensis Cepeda & Lattig, 2016	Spain (Cantabrian Sea)	trefoiled	cushion– shaped, wider than long	10	SG6	broadly winged	MT present, with two rows above MT (MF:1:α:α)	Cepeda & Lattig (2016)
P. aurantiacus Grube, 1860	Croatia (Adriatic Sea)	trefoiled (lateral parts blindly)	oblong, longer than wide	46	SG14	narrowly winged	MT present, with one row above MT (MF:1:0–6)	Glasby & Hutchings (2014)
P. burballes sp. nov.	Mallorca (Mediterranean Sea)	triangular, hood-like, longer than wide	swollen, divided in inner (short rectangular) and outer (large quadrangular) regions	17	SG21	narrowly-winged	Only MT present (MF:1:0)	This study
P. caliendrum Claparède, 1868 (*)	Naples (Mediterranean Sea)	–	–	75	SG6–9	–	–	Claparède (1868)
P. catalanensis Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Mediterranean Sea)	thick single medial lobe only	rectangular, longer than wide	13–15	SG15–17	narrowly winged	MT present, one crest with a very long tooth and two small lateral teeth (MF:3)	Lavesque *et al.* (2020)
P. denticulatus Saint-Joseph, 1894	France (English Channel?)	single medial lobe only	subtriangular, pointed toward mouth, longer than wide	12–13	SG12	narrowly winged, subdistally slightly expanded	MT present, at least on row of secondary teeth above MT (MF:1:α)	Glasby & Hutchings (2014)
P. elisabethae McIntosh, 1915	Scotland (North Sea)	single medial lobe only	subtriangular, pointed toward mouth, wider than long	16	SG11	winged, uniformly tapered	MT present, one row of 11–15 teeth above the MT (MF:1:11–15)	Glasby & Hutchings (2014)

Continued

Table 2 continued

	Type locality	Upper lip	Lower lip	Pairs of notopodia	Start of neuropodia	Notochaetae	Uncini (type 1)	References
P. glasbyi Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Bay of Biscay)	triangular (median lobe elongated)	oval, wider than long	18–22	SG9–11	wings subdistally	MT present, two rows above MT (MF:1:α:α)	Lavesque *et al.* (2020)
P. gujanensis Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Bay of Biscay)	trefoiled	oval, wider than long	28	SG15	narrowly winged	MT present, two rows above MT	Lavesque *et al.* (2020)
P. haematodes (Claparède, 1864) (*)	Port Vendres (Mediterranean Sea)	–	–	14–22	SG13	two types: capillary and slightly limbated	3–4 small teeth above MT	Claparède (1864), Fauvel (1927)
P. idex Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Mediterranean Sea)	single medial lobe only	oblong, slightly longer than wide	14	SG8	winged	MT present, one row above MT (MF:1:α)	Lavesque *et al.* (2020)
P. jubatus Bobretzky, 1868 (*)	Mediterranean and Black Seas			16–18	SG14			Bobretzky (1868)
P. karadenizicus Çinar & Erdoğan-Dereli, 2023	Black Sea	three foiled	semicircular, smooth	4–6	SG15	two types, winged (SG1), pinnates	MT present. (MF:2: α)	Çinar & Erdoğan-Dereli (2023)
P. latidens Eliason, 1962	Skagerrak (North Sea)	medial only, triangular	oblong, wider than long	12	SG14	winged, uniformly tapered	MT present, with 2–4 rows with one tooth above the main fang (MF:1:1:1 to MF:1:1:1:1:1)	Glasby & Hutchings (2014)
P. leoncina (Quatrefages, 1866) (*)(**)	Guetari & Donosti (Cantabrian Sea)	–	–	–	–	–	–	Quatrefages (1866)
P. medusa Grube, 1850	France (Mediterranean Sea)	trefoiled	subtriangular pointed toward mouth	12	SG15	two types, 1st row winged, 2nd row pinnate	MT present, with complete transverse series above MT (MF:1: α)	Glasby & Hutchings (2014)

Continued

Table 2 continued

	Type locality	Upper lip	Lower lip	Pairs of notopodia	Start of neuropodia	Notochaetae	Uncini (type 1)	References
P. nogueirai Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Bay of Biscay)	single elongated medial lobe	rounded, as wide as long	16–21	SG14–16	winged	MT present, two rows above MT	Lavesque *et al.* (2020)
P. norvegicus Wollebaek, 1912	Norway (North Sea)	single medial lobe only	shield-like, wider than long	14–20	SG10–12	winged, uniformly tapered	MT absent or present, one row of 1–4 above the main fang (MF:1–4)	Glasby & Hutchings (2014)
P. pallidus (Claparède, 1864) (*)	Port Vendres (Mediterranean Sea)	–	–	19	SG7	–	–	Claparède (1864)
P. pellucida (Quatrefages, 1866) (*)	Île de Bréhat (English Channel)	–	–	26–27	–	–	–	Quatrefages (1866)
P. pennarbedae Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Bay of Biscay)	triangular (median lobe elongated)	oval, wider than long	12–13	SG15–16	narrowly winged	MT present, two rows above MT (MF:1:α:α)	Lavesque *et al.* (2020)
P. plumosus (Wollebaek, 1912)	Norway (North Sea)	single medial lobe only	subconical lobe protruding above venter, longer than wide	17–19	SG18–20	two types, 1st row winged, 2nd row pinnate	Absence of MT, two rows above MF (MF:α:α)	Glasby & Hutchings (2014)
P. readi Lavesque, Hutchings, Daffe & Londoño-Mesa, 2020	France (Mediterranean Sea)	single medial lobe only, convoluted	oblong, as long as wide	13–17	SG9–10	winged	MT present, two rows above MT (MF:1:α:α)	Lavesque *et al.* (2020)
P. rhombolabiatus Çinar & Erdoğan-Dereli, 2023	Black Sea	rectangular, wider than long	rhomboidal	14–17	SG15	winged	MT present (MF:1: α:α)	Çinar & Erdoğan-Dereli (2023)

Remarks

Mallorcan cave specimens matched previous descriptions of the species (Zibrowius, 1968; Bianchi, 1981; San Martín, 2022). They showed the highly characteristic white, opaque tube with 3-7 longitudinal keels and wide peristomes (Fig 5H), as well as walls with longitudinal chambers divided by longitudinal septa (Fig. 5G, I). No other congeneric species presents tubes with longitudinal chambers, an operculum with a toothed calcareous plate, and pigmented parapodia (Bianchi, 1981; ten Hove & Kupriyanova, 2009).

The specimens from the Cova des Bastons typically showed transverse orange bands in radiolar tips (Fig. 5A, B), dark brown parapodia (Fig. 5A-E), and an operculum with a cylindrical peduncle formed from dorsalmost radiole and a short inverse conical ampulla covered by calcareous plate with coarsely toothed edge (Fig. 5A, B, D-F). They have a trilobed collar lacking tonguelets, with membranes reaching chaetiger 5 (Figs 5B, 6A) and limbate chaetae (Fig. 6A, B). Chaetae from chaetigers 2-7 are *Apomatus*-type and finely limbate (Fig. 6A, C). Uncini are saw-shaped, with 10-12 teeth above a blunt indented peg and with triangular depression (Fig. 6D-F). Abdominal segments include flat, narrow, geniculate chaetae with a crenulated edge (Fig. 6G), as well as saw-shaped uncini with blunt anterior peg (Fig. 6H-I). Long capillaries are restricted to posterior chaetigers.

Molecular analyses

COI and Cyt b fragments did not amplify for the cave serpulid, in spite we tested different PCR programs and primer pairs, whereas we amplified 778 bp for the D1 28S region. BLASTn (nucleotide level) corroborated the taxonomic assignment of the sequence to *V. labiata*, matching (similarity=100%, coverage=95%, e=0.0) a specimen from France assigned to the species (Kupriyanova *et al.*, 2009), followed by other sequence assigned to *Vermiliopsis* sp. (similarity=89.61–93%, coverage=98-86%). The 28S phylogenetic tree topology showed the sequence from the Cova des Bastons within a clade of *V. labiata*, reciprocally monophyletic to a clade including other *Vermiliopsis* sequences, always with BS > 91 (Fig. S4).

Ecology and distribution

This is the first record of *Vermiliopsis labiata* from an anchialine cave. *Vermiliopsis labiata* had been recorded (in some cases as *V. richardi*) in 17 marine caves throughout the Mediterranean and the nearby Atlantic Ocean, located in Faro (Portugal), Provence (France), Liguria, Campania, Sicily and Puglia (Italy), and Northern Aegean (Greece) (e.g., Banse, 1959; Bellan, 1965; Zibrowius, 1968, 1969; True, 1970; Monteiro-Marques, 1981; Belloni & Bianchi, 1982; Balduzzi *et al.*, 1989; Di Geronimo

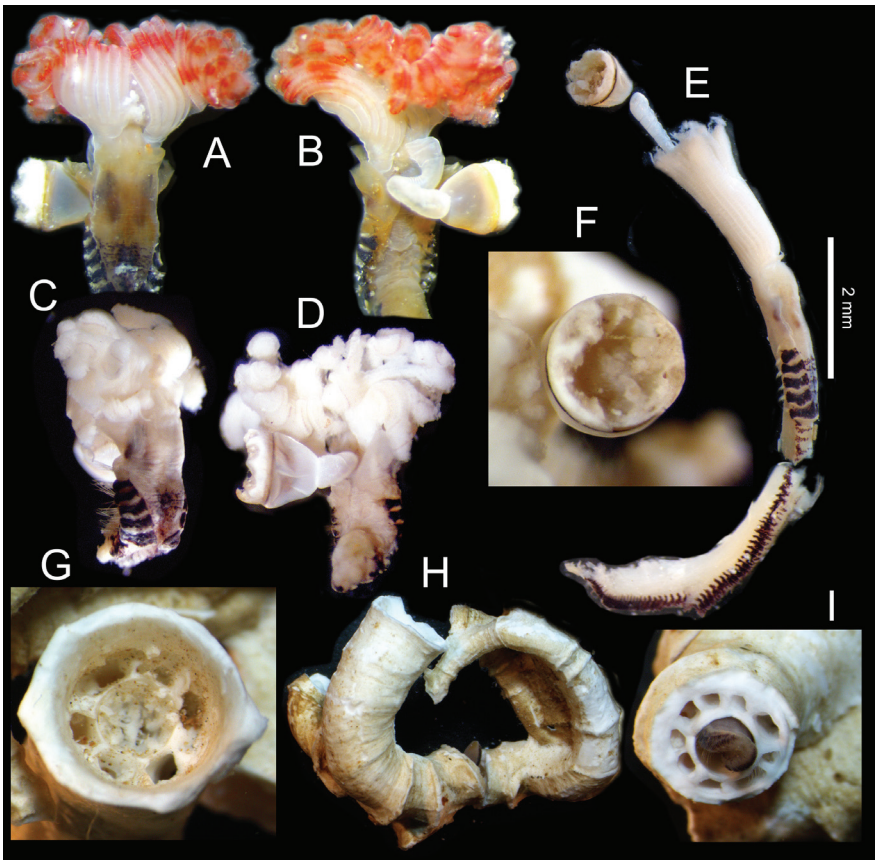

Fig. 5: *Vermiliopsis labiata.* Light micrographs. Anterior end: A. Alive, ventral view; B. Alive, dorsal view; C. Preserved, lateral view; D. Preserved, dorsal view. Entire specimen (three fragments): E. Preserved. Operculum: F. Outside the tube; G. Fitted in tube opening. Tube: H. Entire tube (in two fragments); I. Transversal section of middle tube, with the worm still inside.

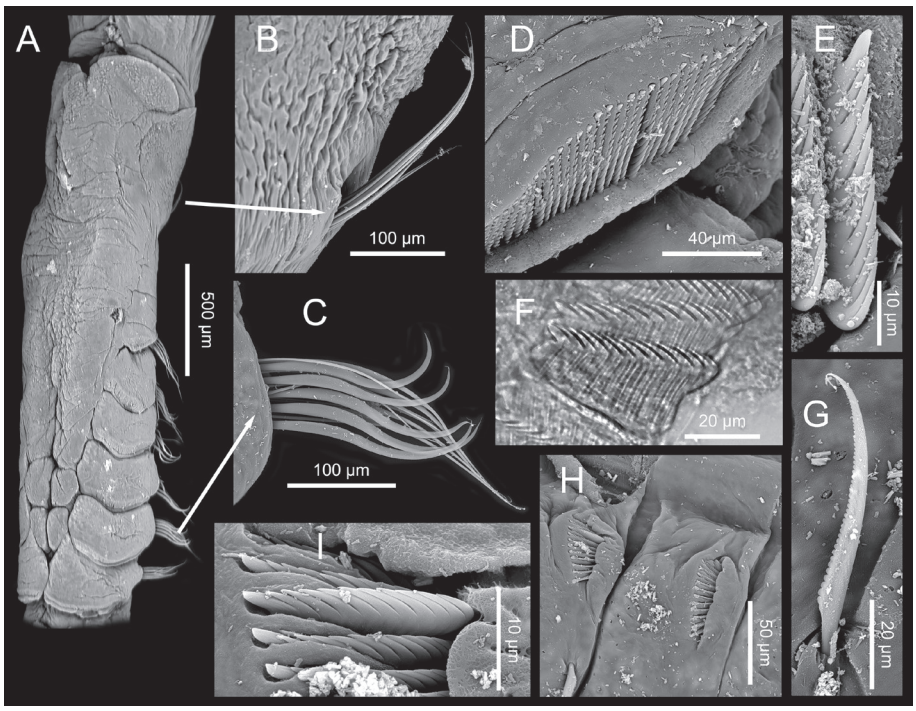

Fig. 6: *Vermiliopsis labiata.* SEM and light micrographs. Thorax: A. Anterior segments, ventro-lateral view; B. Collar capilllaries; C. *Apomatus* and finely limbate chaetae from segment 6; D. Uncinal torus; E, F. Uncini. Abdomen: G. Geniculate, crenulated chaetae; H. Two rows of uncini. I. Uncini.

et al., 1993; Taddei Ruggiero & Benigni, 1996; Sanfilippo & Mòllica, 2000; Harmelin *et al.*, 2003; Monteiro *et al.*, 2013; Gerovasileiou *et al.*, 2015; Sanfilippo *et al.*, 2015; 2017; Rosso *et al.*, 2021; Cardone *et al.*, 2022). All those caves are fully marine, lack water column stratification, and are directly connected to the ocean through relatively large entrances, allowing for the flow of particulate organic matter from the sea. Living individuals and dead tubes were recorded from the entrance zone, close to bottom of those caves (Table 3), with the generally scarce populations consisting of very few living individuals surrounded by more numerous tubes remains (Cardone *et al.*, 2022). The species was reported from open sea sciaphilous environments, such as coralligenous (Zibrowius, 1968; Bianchi, 1981; Bianchi & Morri, 2000; Serrano *et al.*, 2011; Rosso *et al.*, 2021; Cardone *et al.*, 2022; Cepeda *et al.*, 2022). The species was reported in the North-Eastern Atlantic, Indian Ocean, and Japan, although such distant, disjunct reports warrant confirmation at the species level (Gil, 2011; Cepeda *et al.*, 2022).

Discussion

Systematics and ecology

One of our major results is the description of the first terebellid species from an anchialine cave, *Polycirrus burballes* sp. nov. The species is unequivocally placed within *Polycirrus* due to the absence of branchiae, presence of an expanded, triangular, upper lip bearing two types of tentacles and an outer lower lip enlarged and resembling a ventral pad, paired ventro-lateral pads present, notopodia from segment 3, neuropodia beginning after notopodia terminates (leaving one achaetous segment), notochaetae capillaries narrowly-winged (anterior row) and pinnate (posterior row), and type I uncini (Glasby & Hutchings, 2014; Nogueira *et al.*, 2020).

Polycirrus burballes sp. nov. differs from other congeners by a unique combination of characters: triangular upper lip, 17 pairs of notopodia, followed by one achaetous segment and neuropodia starting from segment 21, uncini bearing only one tooth over main fang (Glasby & Hutchings, 2014; Lavesque *et al.*, 2020; Nogueira *et al.*, 2020; Jimi *et al.*, 2023). Interestingly, none of the characters observed in the new species can be linked to troglomorphic traits, since many *Polycirrus* lack eyes, tube, and epidermal pigmentation (Hutchings *et al.*, 2017; Nogueira *et al.*, 2020) (but see below). Unlike in other *Polycirrus* no signs of bioluminescence were observed in our specimen (Johnson & Johnson, 1959; Huber *et al.*, 1989; Kanie *et al.*, 2021; Jimi *et al.*, 2023).

Colonization and survival in anchialine environments

The Cova des Bastons is an oligotrophic, relatively isolated environment with no known opening to the sea. It is characterised by the vertical water stratification typical of anchialine habitats, with a warmer brackish layer overlaying a colder fully saline water mass (Capa *et al.*, 2022). The absence of currents, light, and primary production sustains assemblages with very low biomass and species richness (Gerovasileiou & Bianchi 2021; Capa *et al.*, 2022). To date, only three stygobites were recorded from the cave: the isopod *Metacirolana ponsi* Jaume and García, 1992, the mysid *Burrimysis palmeri* Jaume and García, 1993, and the polychaete *P. perezi* (Jaume

Table 3. Records of *Vermiliopsis labiata* (O. G. Costa, 1861) in marine coastal caves in the Mediterranean Sea and North East Atlantic Ocean.

Taxon	Region	Cave	References
Vermiliopsis labiata	Algarve, Portugal	Gruta da Nossa Senhora, Sagres	Monteiro *et al*., (2013)
V. labiata	Provence-Alpes-Côte d'Azur, France	Grotte de l'Ile de Bagaud, Toulon	Harmelin *et al.,* (2003)
V. labiata	Provence-Alpes-Côte d'Azur, France	Grotte de la Jarre, Marseille	Monteiro-Marques, (1981)
V. labiata	Provence, France	Niolon cave, Marseille	Zibrowius, (1968), True, (1970)
*V. labiat*a	Campania, Italy	Grotta del Mitigliano, Massa Lubrense	Belloni & Bianchi, (1982), Balduzzi *et al*., (1989)
V. labiata	Campania, Italy	Grotta Scaletta, Massa Lubrense	Belloni & Bianchi, (1982)
V. labiata	Campania, Italy	Grotta Tuffo Tuffo, Sorrento (dark)	Banse, (1959)
V. labiata	Liguria, Italy	Grotta marina di Bergeggi	Bianchi *et al*., (1988)
V. labiata	Puglia, Italy	Grotta Lu Lampiune, Otranto	Rosso *et al.*, (2021)
V. labiata	Puglia, Italy	Grotta Elle, Foggia	Cardone *et al*., (2022)
V. labiata	Sicily, Italy	Grotta dei Granchi, Siracusa	Sanfilippo *et al*., (2015)
V. labiata	Sicily, Italy	Grotta Gimnasium, Siracusa	Sanfilippo *et al*., (2015)
V. labiata	Sicily, Italy	Grotta di Mazzere	Sanfilippo *et al*., (2015)
V. labiata	Sicily, Italy	Grotta dell'Accademia, Ustica	Di Geronimo *et al*., (1993)
V. labiata	Sicily, Italy	Grotta IV dell'Isca, Punta Campanella	Ruggiero *et al*., (1996); Sanfilippo & Mòllica, (2000)
V. labiata	North Aegean, Greece	Fara Bay Islet cave, Lesvos Island (dark)	Sanfilippo *et al*., (2017)
V. labiata	North Aegean, Greece	Agios Vasilios Cave, Lesvos Island (semidark cave)	Gerovasileiou *et al*., (2015, 2016b); Sanfilippo *et al*., (2017)

& García, 1992, 1993; Capa *et al.*, 2022). Among them, *Pollentia perezi* cohabits with *P. burballes* sp. nov. the calcareous sediments at the further section of the cave, within the saline groundwater mass.

Despite sharing habitat, these two species belong to very divergent lineages and exhibit different lifestyles. *Pollentia perezi* is an errant, fast-moving scale-worm (Capa *et al*. 2022), likely preying on the sparse organisms or feeding on cave detritus, as most polynoids do (Plyuscheva *et al.*, 2010; Jumars *et al.*, 2015), including those inhabiting caves (Martínez *et al.*, 2016). In contrast, *P. burballes* sp. nov. is a sedentary, non-tubicolous, vagile, tentaculate surface deposit-feeder, akin to other terebellids (Jumars *et al.*, 2015). *Pollentia perezi* lacks eyes – a useless, energetically costly trait in complete darkness – but bears distinctively elongated antennae and cirri, a feature common among deep-sea and cave-dwelling species (Hartmann-Schröder, 1974; Pettibone, 1985; Gonzalez *et al.*, 2021). Conversely, none of the traits shown by *P. burballes* can be interpreted as conventional troglomorphic. Many marine terebellids, including other species of *Polycirrus*, are naturally eyeless, unpigmented, and bear large feeding appendages, which makes it difficult to distinguish cave-specific morphological adaptations within the family. A similar apparent lack of troglomorphic traits has been observed in other cave-dwelling annelids, including species of Syllidae, Acrocirridae, Fauveliopsidae, Flabelligeridae, and Nerillidae (Núñez *et al.*, 1997; Gonzalez *et al.*, 2012; Worsaae *et al.*, 2019), though notable exceptions exist (Glasby *et al.*, 2014; Núñez *et al.*, 2020; Gonzalez *et al.*, 2021; Capa *et al.*, 2022). Morphological adaptations to cave environments in annelids often differ from those observed in other groups and tend to be more conspicuous when cave colonization implies ecological shifts, such as a transition between interstitial/infaunal lifestyle to suspension feeding (Martínez *et al.*, 2013; Martínez *et al.*, 2017; Worsaae *et al.*, 2019).

Polycirrus burballes sp. nov. thus represents the first species of Terebellidae described from anchialine caves. However, whether it is a cave-exclusive species or a member of an elusive marine population surviving in this environment remains uncertain and warrants further research, particularly given our incomplete knowledge of the diversity of Terebellidae in Europe, even in relatively more accessible marine habitats (e.g., Labrune *et al.*, 2019; Lavesque *et al.*, 2021; Martin *et al.*, 2022). Regardless of its status as a cave dwelling organism, the presence of *P. burballes* sp. nov. in an anchialine cave is particularly significant for understanding the ecological dynamics of these environments within the broader con-

text of coastal aquifers. Deposit feeders like *P. burballes* sp. nov. likely play a crucial role in carbon and energy cycles, alongside often-overlooked components of cave biodiversity, such as meiofauna and microbes (Brankovits *et al.*, 2017; Brankovits *et al.*, 2022; Martínez, 2023).

Regarding *Vermiliopsis labiata*, this uncommon species is more frequently reported in caves –where it forms scattered aggregations of a few individuals surrounded by tube remains (Cinelli *et al.*, 1977)– than in open sea sciaphilous habitats (San Martín, 2022). It was reported in several Mediterranean marine caves, all of which maintain clear connections to the open sea (Zibrowius, 1968; Bianchi, 1981; San Martín, 2022). Therefore, the population found in the Cova des Bastons represents the first anchialine record for this species. However, in the Cova des Bastons, *V. labiata* did not co-occur with the polynoid and the terebellid, as it was found in a cave section relatively closer to the open sea, at 5-10 m depth within the brackish water mass. This section also hosted an unidentified sponge, along with crustaceans (authors, personal observations), suggesting periodic or ongoing water exchange with the cave's open-sea section. Such exchange likely enriches the water column with particulate organic matter (Fichez, 1991; Gerovasileiou & Bianchi 2021), which may explain the presence of a sedentary tube-dwelling annelid that never leave its calcareous tube, which are firmly attached to the substrate. While numerous serpulids have been reported from marine caves (Gerovasileiou *et al.*, 2016a), *Marifugia cavatica* Absolon & Hrabě, 1930 is the only stygobiotic species, exclusively inhabiting freshwater phreatic environments in the Dinaric karst (Kupriyanova *et al.*, 2009). Interestingly, *M. cavatica* likely colonized these subterranean environments coming from estuarine coastal habitats, rather than from marine caves (Absolon & Hrabě, 1930).

In summary, the Cova des Bastons apparently experienced multiple colonization events. Stygobites such as *P. perezi*, *B. palmeri* and *M. ponsi* are likely ancient anchialine colonisers that diverged from their marine relatives to adapt to this particular environment, reaching the cave through crevicular systems connecting the different Balearic caves. Conversely, more recent colonizers such as *V. labiata*, are morphologically and molecularly indistinguishable from those found outside the anchialine zone. Finally, the situation of *P. burballes* sp. nov. is still uncertain, because some of the supposedly stygobiotic attributes also occur in open-sea congeners, while its phylogenetic and biogeographic relationships are obscured by the scarcity of comparable information.

Overall, our findings highlight the importance of conserving the fragile anchialine environments, particularly in the Balearic Islands, amidst the accelerating climate change and increasing anthropogenic pressures (Ferguson & Gleeson, 2012). These isolated anchialine cave environments provide fragmented and oligotrophic habitats often supporting specialized, endemic species, yet their inaccessibility left their fauna poorly studied. In this context, the discovery of *P. burballes* sp. nov. alongside the uncertainties regarding its origin and distribution, underscores the need for continued exploration of these environments to fully document their biodiversity, assess whether the species are endemic or have broader distributions, and better understand the origins and evolutionary history of these unique faunas. Comprehensive studies of Cova des Bastons and similar cave systems in the Balearic Islands are therefore essential, not only for cataloguing and understanding the species present but also for implementing effective management and conservation strategies.

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Supplementary Data

The following supplementary information is available online for the article:

Fig. S1: Phylogenetic trees of the (A) nuclear 28S rDNA D1 region and (B) the mitochondrial COI Polycirrini data set. Bootstrap statistical support values are reported above tree branches. Coloured in green outgroup terminals; in orange, representatives of non-Polycirrini; in blue, sequences belonging to individuals identified as *Polycirrus* in public repositories; and in red the new species.

Fig. S2: Phylogenetic tree of the nuclear 28S rDNA data set for Serpulidae. Bootstrap statistical support values are reported above tree branches. Coloured in green outgroup terminals; in blue, sequences identified as *Vermiliopsis labiata* in public repositories; and in red the specimen from cova des Bastons.

Table S1. The primers used in study together with oligonucleotide sequence and references.

Table S2. PCR thermal profiles for each primer pair.