New record and new species of Laubierpholoe Pettibone, 1992 (Annelida, Sigalionidae) from the soft bottom of submarine caves near Marseille (Mediterranean Sea) with discussion on phylogeny and ecology of the genus

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Abstract. A new species of Laubierpholoe Pettibone, 1992 (Annelida, Sigalionidae), Laubierpholoe massiliana Zhadan sp. nov., was found in two submarine caves near Marseille (France). This is the first record of the genus in the Mediterranean Sea. The new species differs from congeners by inhabiting soft sediments instead of having an interstitial lifestyle and by several morphological characters: the ventral tentacular cirri slightly shorter or of similar length to the dorsal tentacular cirri, the presence of bidentate neurochaetae, the body length, and the number of segments. Molecular phylogenetic analysis using 18S rRNA and 28S rRNA sequences confirmed that the new species belongs to the genus Laubierpholoe, as well as the monophyly of the genus. The ecology of the new species and its adaptation to the cave-dwelling lifestyle are discussed. An identification key for all known species of Laubierpholoe is provided.

Keywords. Polychaeta, Pholoinae, identification key, marine caves, The Calanques.

Introduction

Pholoinae Kinberg, 1858 is a group of scale-worms which are small (up to 2 cm long, up to 90 segments) and common in all kinds of marine intertidal and subtidal habitats. They have been treated as a separate family or as a part of Sigalionidae Kinberg, 1856 (Barnich & Fiege 2003; Wiklund et al. 2005; Norlinder et al. 2012). According to recent phylogenetic analysis of Aphroditiformia Levinsen, 1883 using four molecular markers and 87 morphological characters, Pholoinae is nested within Sigalionidae and considered to be a subfamily (Gonzalez et al. 2018a). The diagnostic characters of Pholoinae are: body typically elongate and narrow with as few as 15 segments; notochaetae geniculate and finely tapered; simple neurochaetae absent; neuropodial stylodes possibly present; elytral brooding present in some genera (Gonzalez et al. 2018a).

Within Pholoinae, the genus *Laubierpholoe* Pettibone, 1992 includes five meiobenthic species. The first species of the genus was described as *Pholoe antipoda* (Hartman, 1967) from Tierra del Fuego (South Atlantic Ocean) (Fig. 1A). Its main differences from other species of *Pholoe* Johnston, 1839 were a much smaller size, a reduced number of segments and details of the elytra and neuropodial falcigers. The second species, *Pholoe swedmarki* (Laubier, 1975) was later described from Bermuda (Northeast Atlantic Ocean) (Fig. 1A) from 2–8 m depth. The author was unaware of the previous description of *P. antipoda* and compared *P. swedmarki* with other species of *Pholoe*. Laubier (1975) noticed its much smaller size (maximum length 1.6 mm, width 400 μm), smaller number of notochaetae, elongated palps but reduced ventral cirri, and smooth elytra with few papillae. He also described developing embryos inside the elytra, postulated internal fertilization and considered these characters as adaptations to interstitial life style (Laubier 1975).

Pettibone (1992) performed the revision of Pholoidae Kinberg, 1858 and provided a diagnosis of this family including diagnoses of all genera and species as well as identification keys to all taxa. She erected the new genus *Laubierpholoe* for the two above-mentioned previously described species and for two new species: *L. maryae* Pettibone, 1992 and *L. riseri* Pettibone, 1992, both from New Zealand (Fig. 1A). The new genus was distinguished by the following main characters: achaetous tentaculophores lateral to prostomium, each with a long dorsal and a very short ventral tentacular cirrus; stout, very long palps; notochaetae of a single type, slightly curved or straight; and up to 29 segments.

The last species of *Laubierpholoe*, *L. indooceaneica* Westheide, 2001, was described from coral reef flats of Krusadai Island and the Seychelles (Indian Ocean) (Fig. 1A). It is the smallest (less than 1 mm long) of all species of the genus and differs by the presence of hook-like blades in some of the compound neurochaetae. Phylogenetic analysis of Aphroditiformia confirmed the position of *Laubierpholoe* within Pholoinae and the clade was well supported by molecular data and by the apomorphy presence of elytral brooding (Gonzalez et al. 2018a). The other interstitial pholoin genera *Imajimapholoe* Pettibone, 1992 and *Taylorpholoe* Pettibone, 1992 also have elytral brooding and direct development. In contrast, the interstitial *Metaxypsamma uebelackerae* Wolf, 1986 lacks elytra. No molecular data are available for these genera at the moment.

Potential reservoirs of such easily overlooked meiobenthic taxa exist very close to the shore. In the Mediterranean, one of the best-studied areas in the world, underwater marine caves have been shown to be hotspots of unknown diversity because of their more difficult access and peculiar environmental conditions. Caves are numerous near Marseille (SE France) thanks to the karstic nature of the seashore (Harmelin et al. 1985). Darkness and poor water circulation create oligotrophic environmental conditions similar to the deep sea and it is remarkable that many organisms found in marine caves belong to deep water taxa (e.g., Calado et al. 2004; Janssen et al. 2013; Chevaldonné et al. 2015; Cárdenas et al. 2018; Chevaldonné & Pretus 2021). In addition, some caves with particular geomorphologies provide a cold thermal regime (mean ca 13–15°C) similar to that of the Mediterranean deep sea (Vacelet et al. 1994; Bakran-Petričioli et al. 2007).
Fig. 1. A. World map showing type locations of all species of *Laubierpholoe* Pettibone, 1992. B. Type location of *Laubierpholoe massiliana* Zhadan sp. nov. C. Locations of the two caves where *Laubierpholoe massiliana* sp. nov. was sampled.
Only a few studies have focused on cave sediment fauna, often with an emphasis on targeted taxonomic groups (e.g., Janssen et al. 2013; Zeppilli et al. 2018). In the 3PP and Jarre Caves in the Calanques National Park near Marseille (Fig. 1B–C), it has been shown that sea water remained colder than the outside environment or other caves throughout the year (Bakran-Petricioli et al. 2007). For instance, though it is only 25–30 m deep, the 3PP Cave has been shown to display meiofaunal organisms usually found at abyssal sites (Janssen et al. 2013). These are also large caves with extended areas of silty bottom favourable for the study of cave sediment fauna. Therefore, they have been the focus of recent investigations of macro- and meiofauna, especially of presently poorly-studied groups (Vortsepneva et al. 2021). A diverse annelid fauna was discovered there including a new species of *Laubierpholoe* described here with discussion of its taxonomy, phylogeny and the ecology of the group.

**Material and methods**

**Sampling and preservation**

All material was sampled in the Calanques National Park near Marseille (Fig. 1B–C) in 2018–2020. Samples were taken close to the entrance, in the middle (40 m from entrance) and the deep (60–70 m from entrance) parts of Jarre and 3PP Caves at 18–25 m depth. The coordinates of the entrance of Jarre Cave are 43.19556° N, 5.3658333° E and 43.16306° N, 5.6° E for 3PP Cave. Bottom sediment was collected by SCUBA diving with 20 cm-wide sampling boxes at about one cm sediment depth and a length of 120 cm. Samples were washed using the flotation method and a sieve with mesh size 130 μm. In total, about 50 specimens of *Laubierpholoe* were collected. They were relaxed using a magnesium chloride (MgCl₂) solution isotonic to seawater for 30 min and preserved in 96% ethanol or in 2.5% glutaraldehyde (Electron Microscopy Supplies, EMS, Pennsylvania, USA) buffered with 0.1 mol phosphate buffer and transferred to 70% ethanol after washing with the same buffer.

**Granulometric analysis**

Subsamples of sediment were taken in 3PP and Jarre Caves in the deep, middle and near entrance parts and granulometric analysis were performed in Limited Liability Company “Laboratory” (Sankt-Petersburg, Russia).

**Morphological analysis**

Whole specimens were photographed via stereo microscope and compound microscope using an iPhone 6 with a Labcam (iDu Optics, Detroit, Michigan, USA) adapter for living specimens and a Leica microscope (Leica Microsystems GmbH, Germany) with a Leica adapter for preserved ones. For SEM, specimens were dehydrated in a graded ethanol series (20–25 min per step), then transferred to acetone and critical point dried. Whole specimens and their fragments were coated with platinum-palladium and examined using a Camscan S-2 (Cambridge Instruments, London, United Kingdom) scanning electron microscope at the Laboratory of Electron Microscopy at the Biological Faculty of Moscow University.

**DNA amplification and sequencing**

The Promega Wizard SV Genomic DNA Puriﬁcation Kit and protocol (Promega Corporation, Madison, USA) were used for tissue lysis and DNA puriﬁcation. Polymerase chain reaction (PCR) amplification of nuclear 18S rRNA and fragments of 28S rRNA was accomplished with the standard primers. The 18S rRNA gene was PCR ampliﬁed in three overlapping fragments using primer pairs 1 F-5R, 3 F-18Sbi and 18Sa2.0–9 R (Giribet et al. 1996, 1999). For the 28S rRNA gene we used C1’ and C2 primers (Le et al. 1993). The universal primers 16Sar-L and 16Sbr-H (Palumbi & Kessing 1991) did not yield products that could be sequenced, therefore for the 16S rRNA gene we tried to use the primer pair 16SAnnF-16-SAnnR (Sjölin et al. 2005), unfortunately with the same result. We were also unable to amplify the CO1 gene fragment (Folmer fragment) using the primers jgLCO1490 and jgHCO2198 (Geller et al. 2013). All loci
were amplified using the Encyclo PCR kit (Evrogen JointStock Company, Russia). We amplified a 25 μl reaction mix containing 1x PCR buffer, 1 μl of 10 μM of primer pair mix, 1 μl of template, 0.2 mM of each dNTP and 0.5 units Taq polymerase. Reaction mixtures were heated on Veriti® Thermal Cycler to 95°C for 300 s, followed by 35 cycles of 15 s at 95°C, 20 s at a specific annealing temperature, and 45–60 s at 72°C, depending on the length of fragment, and then a final extension of 7 min at 72°C. Annealing temperature was set to 49°C for the 18S primer pairs 1 F-5R and 18Sa2.0–9 R, 52°C for the 18S primer pair 3 F-18Sbi and for the 28S primer pair C1’–C2 (Rousset et al. 2007). We used the Promega PCR Purification Kit and protocol (Promega) to purify our amplification products which were sequenced in both directions. Each sequencing reaction mixture included 1 μl of BigDye (Applied Biosystems, PerkinElmer Corporation, Foster City, CA), 1 μl of 1 μM primer and 1 μl of DNA template and was processed for 40 cycles of 96°C (15 s), 50°C (30 s) and 60°C (4 min). Samples were purified prior to sequencing by ethanol precipitation to remove unincorporated primers and dyes. Products were re-suspended in 12 μl formamide and electrophoresed in an ABI Prism 3500 sequencer (Applied Biosystems). All new DNA sequences have been submitted to the NCBI GenBank repository.

**Data analysis**

Nucleotide sequences were edited using the software CodonCode Aligner ver. 5.0.2 (CodonCode Corporation) and checked for identity against the nuclear redundant (default) database of GenBank using BLASTn (Altschul et al. 1990). Data analysis included 8 sequences from this study (four specimens of *Laubierpholoe massiliana* Zhadan sp. nov., 18S rRNA and 28S rRNA) and sequences of 18S rRNA and 28S rRNA obtained from GenBank for species of Sigalionidae including Pelogeniinae Chamberlin, 1919, Pholoinae, Pisioninae Ehlers, 1901, and Sigalioninae Kinberg, 1856. *Harmothoe impar* (Johnston, 1839), species of Polynoidae Kinberg, 1856, was included as an outgroup species. GenBank accession numbers for sequences used in the present study are provided in Table 1. The sequences were aligned with the MAFFT multiple alignment tool (Katoh & Standley 2013) with default parameters: MAFFT flavour: auto; gap extension penalty: 0.123; gap opening penalty: 1.53; direction of nucleotide sequences: do not adjust direction; matrix selection: no matrix. Then sequences were curated with Gblocks (Castresana 2000) with default parameters: minimum number of sequences for a conserved position (b1): 50% of the number of sequences +1; minimum number of sequences for a flank position (b2): 85% of the number of sequences; maximum number of contiguous non-conserved positions (b3): 8; minimum length of a block (b4): 10; allowed gap positions (b5): none. The final lengths for individual alignments were 1407 and 675 bp for the 18S and 28S respectively. The 18S rDNA and 28S rDNA alignments were concatenated using MEGA X software (Kumar et al. 2018). We performed phylogenetic reconstruction for concatenated 18S+28S alignment using Bayesian inference (BI) and maximum likelihood (ML) analyses. For BI the best fit model selection was performed using MEGA X software the GTR+G+I model was selected. The BI analysis was run in MrBayes ver. 3.2.7 (Huelsenbeck & Ronquist 2001). We made one run, four chains were ran simultaneously, three heated and one cold. The number of generations was set to 1000000. Chains were sampled every 500th generation and 0.25 of the samples was discarded as burnin. The ML analysis was performed in PhyML (Guindon et al. 2010) with SMS (Smart Model Selection) (Lefort et al. 2017); statistical criterion to select the model was AIC (Akaike information criterion), selected model was GTR+G+I; tree topologies were searched using SPR (Subtree pruning and regrafting); node support was assessed with 200 bootstrap replicates. All analyses were performed using NGPhylogeny.fr server (Lemoine et al. 2019). Phylogenetic trees were visualized and processed in ITOL v5 (Letunic & Bork 2021). The trees were rooted using *Harmothoe impar* as an outgroup. Posterior probability (PP) and bootstrap support (B) were used for nodal support in BI and ML analyses respectively.

**Abbreviations used for museums**

| ZMMSU | Zoological Museum of Moscow State University, Russia; WS numbers refer to the White Sea branch of the museum |
| MNHN | Muséum national d’histoire naturelle, Paris, France |
Table 1. GenBank accession numbers of sequences used for the analysis.

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Results

Taxonomy

Class Polychaeta Grube, 1850
Subclass Errantia Audouin & H. Milne Edwards, 1832
Order Phyllodocida Dales, 1962
Suborder Aphroditiformia Levinsen, 1883
Family Sigalionidae Kinberg, 1856
Subfamily Pholoinae Kinberg, 1858

Genus *Laubierpholoe* Pettibone, 1992

Type species


Diagnosis (after Pettibone 1992), emended (changes in bold)

Body small, linear, with relatively few segments (up to 29). Elytra and elytrophores on segments 2, 4, 5, 7, continuing on alternate segments to 23, then on every segment to end of body. Dorsal tubercles on segments lacking elytra. Elytra delicate, with few short papillae on lateral border and on surface. Without
dorsal cirri or branchiae. Prostomium and first or tentacular segment fused, ventrally forming anterior lip of mouth, without facial tubercle, with or without papillae. Prostomium rounded, bilobed; median antenna with ceratophore in anterior notch of prostomium; lateral antennae absent; with or without 2 pairs of eyes. Tentaculophores lateral to prostomium, achaetous, each with long dorsal and much shorter ventral tentacular cirrus or tentacular cirri of about same length; palps stout, very long, emerging ventral and lateral to tentaculophores, rugose. Second or buccal segment with first pair of large elytrophores and elytra, biramous parapodia, long ventral buccal cirri, and forming lateral and posterior lips of mouth. Muscular pharynx with 9 dorsal and 9 ventral border papillae and 2 pairs of jaws. Parapodia biramous; notopodial conical acicular lobe without subdistal bract; neuropodial conical acicular lobe without distal papillae. Notochaetae simple, slender, capillary, slightly curved and straight. Neurochaetae stouter than notochaetae, compound, falcigerous or spinigerous; shafts with or without distal spinules; blades capillary or falcate with unidentate or uni- and bidentate tips. Ventral cirri short, tapering, on all segments. Pygidium with pair of anal cirri. Development characterised by reduction of egg number and development of embryos and juveniles within elytra (elytral brooding).

Laubierpholoe massiliana Zhadan sp. nov.

Diagnosis
Body with 16–19 segments, 8–10 pairs of elytra; two pairs of closely arranged eyes; dorsal tentacular cirri of same length or slightly longer than ventral ones; notopodia smaller and shorter than neuropodia; notochaetae few (3–6), some neurochaetae with bidentate tips.

Etymology
The species name refers to the type locality (Massilia – the old Roman name for Marseille).

Material examined
All material investigated was collected in the Mediterranean Sea, Gulf of Lion, The Calanques, near Marseille (Fig. 1B–C). The coordinates of the entrance of the Jarre Cave: 43.19556° N, 5.3658333° E; for 3PP Cave: 43.16306° N, 5.6° E.

Holotype
FRANCE • 1 specimen (pharynx everted, anal cirri damaged; 17 segments; body length without pharynx and anal cirri 0.925 mm, including pharynx 1.31 mm, body width 150 μm, including elytra 490 μm, including chaetae 720 μm); Mediterranean Sea, Gulf of Lion, The Calanques, near Marseille, Jarre Cave, deep part; 43.19556° N; 5.3658333° E; depth 18 m; 24 Oct 2019; P. Chevaldonné leg.; SCUBA-diving; sampling box; silty sand; mesh size 130 μm; preservation in glutaraldehyde 2.5% in PBS, storage in ethanol 70%; field number Ma19-28-03; ZMMSU WS16462.

Paratypes
FRANCE • 15 specimens; same data as for holotype; preservation glutaraldehyde 2.5% in PBS, storage in ethanol 70% (7 specimens), SEM stubs (3 specimens), permanent slides (5 specimens); field number Ma19-28-03; ZMMSU WS14001 • 6 specimens; same locality as for holotype; 12 Mar. 2020; P. Chevaldonné leg.; SCUBA-diving; sampling box; mesh size 130 μm; field number Ma20-03-01; preservation in glutaraldehyde 2.5% in PBS, SEM stubs; ZMMSU WS16511 • 3 specimens; Jarre Cave, middle part; 10 May 2019; depth 19 m; P. Chevaldonné leg.; SCUBA-diving; sampling box; sandy silt; mesh size 130 μm; preservation in EtOH 96%; field number Ma19-24-01; ZMMSU WS12418 • 3 specimens; 3PP Cave, middle part; 43.16306° N, 5.6° E; 28 Oct. 2019; depth 25 m; P. Chevaldonné
Other material
FRANCE • 6 specimens; 3PP Cave, middle part; 43.16306° N, 5.6° E; 10 May 2019; depth 25 m; P. Chevaldonné leg.; SCUBA-diving; sampling box; clayey silt; EtOH 96% (4 specimens), SEM stubs (2 specimens); field number MA19-20-01; ZMMSU WS12292 • 1 specimen; 3PP Cave, middle part; 43.16306° N, 5.6° E; depth 25 m; 7 May 2019; P. Chevaldonné leg.; SCUBA-diving; hand corer; clayey

Fig. 3. *Laubierpholoe massiliana* Zhadan sp. nov., SEM. A. ZMMSU WS12292, general view. B. ZMMSU WS14001, general view, elytra omitted. C. ZMMSU WS13977, elytra. D. ZMMSU WS14001, anterior end, dorsal view. E. Same, dorso-anterior view. F. ZMMSU WS16511, anterior end, dorso-anterior view, median antenna broken. G. ZMMSU WS16511, dorso-anterior view. Abbreviations: ah = anterior horns; dtc = dorsal tentacular cirrus; e = elytrophores; ma = median antenna; ne = neuropodium; no = notopodium; p = prostomium; pa = palp; ph = pharynx; vbc = ventral buccal cirrus; vtc = ventral tentacular cirrus. Arrows indicate papillae.
silt; mesh size 130 μm; DNA; field number Ma19-13-01; ZMMSU WS12216; • 1 specimen; same locality as for preceding; 10 May 2019; P. Chevaldonné leg.; SCUBA-diving; sampling box; clayey silt; mesh size 130 μm; DNA; field number Ma19-20-01; ZMMSU WS12619 • 1 specimen; 10 May 2019; 3PP Cave, deep part; 43.16306° N; 5.6° E; depth 25 m; P. Chevaldonné leg.; SCUBA-diving; sampling box; clayey silt; mesh size 130 μm; DNA; field number Ma19-19-12; ZMMSU WS12617 • 1 specimen; Jarre Cave, middle; 43.19556° N; 5.36583° E; depth 19 m; 10 May 2019; P. Chevaldonné leg.; SCUBA-diving; sampling box; sandy silt; mesh size 130 μm; DNA; field number Ma19-24-01; ZMMSU WS12437.

**Description** (based on all specimens investigated)

**Body.** Short, up to 1.2 mm long (without appendages), up to 0.25 mm wide (without elytra), 0.65 mm (with elytra), 0.8 mm (with chaetae), 16–19 segments, body surface smooth. Living worms whitish, semi-transparent, often with transverse white stripe along anterior border of prostomium, intestine content yellowish to brown (Fig. 2A–B). Elytra 8–10 pairs, round, transparent, with few oval to cirriform papillae on surface and along lateral and posterior borders; some enclosing developing embryos (Figs 2A–C, E, 3A, C). Prostomium and tentacular segment fused; prostomium oval to trapezoidal, bilobed; lobes rounded with notches above tentaculophores (Figs 3D–G, 5A). Two pairs of very closely situated, almost fused eyes near anterolateral border, anterior pair larger than posterior (Figs 2A–F, 5A). Median antenna with large ceratophore and short style, dorsal in anterior notch of prostomium (Figs 3D–G, 5A). Tentacular

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**Fig. 4.** _Laubierpholoe massiliana_ Zhadan sp. nov., SEM. A. ZMMSU WS16511, ventral view. B. ZMMSU WS16511, pharynx, dorso-anterior view. C. ZMMSU WS14001, parapodia of segments I-V, dorso-anterior view. D. Same, parapodia of segment III, anterior view. E. ZMMSU WS13977, bidentate neurochaetae. F–G. ZMMSU WS12292, tips of bidentate neurochaetae. Abbreviations: ne = neuropodium; no = notopodium; pa = palp; vbc = ventral buccal cirrus; vc = ventral cirrus. Arrows indicate papillae.
segment bearing medial anterior horns and lateral tentaculophores with dorsal and ventral tentacular cirri; tentacular cirri longer than median antenna, ventral tentacular cirrus equal or subequal to dorsal cirrus (Figs 3D–G, 5A). Anterior horns conical, arising medially from ventral tentacular cirri (Figs 3D–G, 5A). Upper and lower lips bearing conical papillae with a single sensory cilium distally (Figs 3E–G, 4A). Palps very long, up to 250 μm, stout, tapering, ventrolateral to tentaculophores (Figs 2A, D–G, 3D–E, G, 5A). Segment 2 with first pair of bulbous elytrophores, biramous parapodia, ventral buccal cirri lateral to mouth, longer than dorsal tentacular cirri (Figs 3D–G, 5A), ventral cirri from segment 3 onwards smaller than buccal cirri (Fig. 4A). Pharynx reaching segment 7 when inverted, with two pairs of jaws, 9 dorsal and 9 similar-sized ventral border papillae or three median papillae slightly smaller than lateral (Figs 2C–D, F–H, 4B, 5D).

**Fig. 5.** *Laubierpholoe massiliana* Zhadan sp. nov., line drawings. A. Anterior end, dorso-anterior view. B. Notochaeta and bidentate neurochaeta. C. Parapodium, anterior view. D. Jaw. Abbreviations: ah = anterior horns; dtc = dorsal tentacular cirrus; ma = median antenna; ne = neuropodium; no = notopodium; p = prostomium; pa = palp; vbc = ventral buccal cirrus; vc = ventral cirrus; vtc = ventral tentacular cirrus. Arrows indicate papillae.
PARAPODIA. Long, notopodia smaller and shorter than neuropodia, both with conical acicular lobes with projecting acicula; notopodia with two ciliated strips on dorsal side, one near base, the second subdistally (Figs 4C–D, 5C). Notochaetae few (3–6), slender, tapering to fine tips, straight or slightly curved, with series of small denticles (Figs 4C–D, 5B–C). Neurochaetae longer and stouter than notochaetae, compound (Figs 2D–G, 3A–B); shafts with long distal spines; upper blades slightly longer than lower; blades serrated, straight, falcate and of two types: supraacicular blades unidentate and subacicular blades uni- and bidentate (bidentate tips distinct at high magnification, Figs 4C–G, 5B). Ventral cirri at neuropodial bases, thin, short, with round tips (Figs 4A, 5C). Pygidium with pair of very long anal cirri, up to 550 μm (Fig. 2A, C), easily lost; anus terminal.

**Type locality**
The Calanques, near Marseille, Jarre Cave (Fig. 1C).

**Distribution**
The Calanques, near Marseille, Jarre and 3PP marine caves.

**Ecology**
Inhabits the upper layer of soft sediments in the middle and deep parts of marine caves at a depth of 19–25 m. The sediment type in Jarre Cave was defined as silty sand in the deep part and sandy silt in the middle part, and in 3PP Cave as clayey silt in both deep and middle parts (Table 2).

**Molecular data**
The trees obtained with the 18S rRNA and 28S rRNA concatenated dataset with BI (Fig. 6) and ML analyses (Supp. file 1) have similar topology. Analysed specimens of *Laubierpholoe massiliana* sp. nov. form a highly supported clade within *Laubierpholoe* (PP = 1, B = 100); their sequences are identical despite their origin from two different caves. *Laubierpholoe* (PP = 1, B = 97), *Pholoe* (PP = 1, B = 98) and Pholoinae (PP = 1, B = 100) are well-supported groups within Sigalionidae. *Laubierpholoe* includes *L. massiliana* sp. nov. and all other species present in GenBank (*L. swedmarki* and undescribed species A, B and C); it forms a sister group with *Pholoe*. The relationships of species of *Laubierpholoe* are different in BI and ML analyses and the subclades have low support. In the BI tree, *L. massiliana* sp. nov. is in a sister group with *Laubierpholoe* sp. C, and together they form a sister group with *Laubierpholoe* sp. A, *Laubierpholoe* sp. B and *L. swedmarki*; *Laubierpholoe* sp. B and *L. swedmarki* form a clade which is a sister group with *Laubierpholoe* sp. A. In the ML tree, *L. massiliana* sp. nov. is in a sister group with the clade comprising *Laubierpholoe* sp. B and *Laubierpholoe* sp. C, and together they form a sister group with the clade *L. swedmarki – Laubierpholoe* sp. A.
Table 2. Results of granulometry for six sampling sites. Numbers mean content of each fraction (%).

<table>
<thead>
<tr>
<th>Fraction, mm</th>
<th>Jarre deep</th>
<th>Jarre middle</th>
<th>Jarre entrance</th>
<th>3PP deep</th>
<th>3PP middle</th>
<th>3PP entrance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 10</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>10–5</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>5–2</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>2–1</td>
<td>&lt;0.1</td>
<td>0.4</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>7.9</td>
</tr>
<tr>
<td>1–0.5</td>
<td>3.2</td>
<td>4.8</td>
<td>2.0</td>
<td>1.1</td>
<td>0.7</td>
<td>34.2</td>
</tr>
<tr>
<td>0.5–0.25</td>
<td>21.5</td>
<td>9.9</td>
<td>3.2</td>
<td>1.0</td>
<td>1.0</td>
<td>18.5</td>
</tr>
<tr>
<td>0.25–0.1</td>
<td>29.7</td>
<td>17.4</td>
<td>12.8</td>
<td>1.7</td>
<td>1.3</td>
<td>20.3</td>
</tr>
<tr>
<td>0.1–0.05</td>
<td>19.4</td>
<td>22.8</td>
<td>22.9</td>
<td>4.4</td>
<td>3.4</td>
<td>7.4</td>
</tr>
<tr>
<td>0.05–0.01</td>
<td>13.7</td>
<td>30.4</td>
<td>37.3</td>
<td>49.2</td>
<td>61.5</td>
<td>4.6</td>
</tr>
<tr>
<td>0.002–0.001</td>
<td>2.4</td>
<td>2.1</td>
<td>0.8</td>
<td>3.9</td>
<td>4.7</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>&lt; 0.001</td>
<td>3.8</td>
<td>5.8</td>
<td>10.1</td>
<td>10.2</td>
<td>7.3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Interpretation: silty sand, sandy silt, silt, clayey silt, clayey silt, sand.

Fig. 6. Bayesian phylogenetic tree obtained with the 18S rRNA and 28S rRNA concatenated dataset showing position of *Laubierpholoe massiliana* Zhadan sp. nov. within Sigalionidae Kinberg, 1856. Posterior probabilities and bootstrap values are shown for each medium supported node.
Key to species of Laubierpholoe

1. Eyes absent; notopodium about as long as neuropodium .......................... L. maryae Pettibone, 1992
   - Eyes present ............................................................................................................... 2

2. Dorsal tentacular cirri of same length or slightly longer than ventral; some neurochaetae with bidentate tips (look under high magnification) ........................................... L. massiliana Zhadan sp. nov.
   - Dorsal tentacular cirri much longer than ventral, all neurochaetae unidentate .......................... 3

3. All neurochaetal blades similar, short ........................................................................................................................... 4
   - Neurochaetal blades of different size and shape, can include spinigers, or smooth and serrate, or hook-like types .................................................................................................................................. 5

4. Dorsal tentacular cirrus of about same length as median antenna, eyes separate, notochaetae numerous (20–30) .......................................................................................... L. antipoda (Hartman, 1967)
   - Dorsal tentacular cirrus twice as long as median antenna, eyes closely arranged, notochaetae few (2–4) ................................................................. L. swedmarki (Laubier, 1975)

5. Notopodium longer than neuropodium, notochaetae numerous; neurochaetae of two types: supraacicular with blades long, tapering to capillary tips; subacicular with blades short ...................... L. riseri Pettibone, 1992
   - Notopodium shorter and smaller than neuropodium, 4–8 notochaetae; neurochaetae starting from CH4 have serrated and smooth blades, posteriormost five segments also with hook-like neurochaetal blades ................................................................................. L. indoceanica Westheide, 2001.

Discussion

Emendation of generic diagnosis of Laubierpholoe and its consequences

A very short ventral tentacular cirrus has been considered a diagnostic character for Laubierpholoe, in contrast to Pholoe, Taylorpholoe and Metaxypsamma Wolf, 1986 where tentacular cirri are subequal (Pettibone 1992). Since ventral tentacular cirri in L. massiliana sp. nov. are as long as or slightly shorter than the dorsal ones, this character lost its importance, leading to a modification of the generic diagnosis to: “Tentaculophores … each with long dorsal and much shorter ventral tentacular cirrus or tentacular cirri of about same length”. This emendation leads to a problem in distinguishing genera of Pholoinae although other characters remain useful. Laubierpholoe and Pholoe can be discerned by the number of segments (up to 90 in Pholoe, up to 29 in Laubierpholoe), the elytral brooding of embryos in Laubierpholoe and the presence of two types of notochaetae in Pholoe (shorter, strongly bent (i.e., geniculate), and longer, slightly curved or straight) whereas all notochaetae in species of Laubierpholoe are similar, slightly curved or straight. The genus Laubierpholoe has common characters with other interstitial pholoin genera such as Taylorpholoe and Imajimapholoe. They also have a small size, elytral brooding and a single type of notochaetae. Laubierpholoe is distinguished by a medial antenna inserted in the anterior notch while in Taylorpholoe and Imajimapholoe the median antenna is situated occipitally at the posterior border of the prostomium. Another interstitial pholoin genus, Metaxypsamma, is easily recognized by its uniramous parapodia and rudimental elytra, transformed to filiform papillae. The other character of L. massiliana sp. nov. leading to an emendation of the generic diagnosis is the presence of bidentate neurochaetae. It is unique for Pholoinae and discussed below. The comparison of Pholoinae genera is summarized in Table 3.

Comparison with other species of Laubierpholoe (Table 4)

Laubierpholoe massiliana sp. nov. has the typical characters of the genus: small size, few segments and elytra, median antenna in anterior notch of prostomium, one type of notochaetae, and elytral embryo brooding.
Table 3. Comparison of genera of Pholoinae Kinberg, 1858.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Number of segments</td>
<td>up to 90</td>
<td>up to 29</td>
<td>up to 19</td>
<td>up to 34</td>
<td>up to 24</td>
</tr>
<tr>
<td>Elytra</td>
<td>delicate, with border and surface papillae</td>
<td>delicate, with few short papillae on lateral border and on surface</td>
<td>squarish, with fringe of numerous filiform papillae on 3 sides</td>
<td>opaque, with papillae on lateral and posterior borders and on surface</td>
<td>rudimentary, as paired nodular lobes with 2–4 long filiform papillae</td>
</tr>
<tr>
<td>Elytral brooding</td>
<td>absent</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Median antenna</td>
<td>in anterior notch</td>
<td>in anterior notch</td>
<td>occipital</td>
<td>occipital</td>
<td>in anterior notch</td>
</tr>
<tr>
<td>Lateral antennae</td>
<td>absent or present</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Tentacular cirri</td>
<td>dorsal and ventral tentacular cirri subequal</td>
<td>dorsal cirrus much longer or tentacular cirri subequal</td>
<td>dorsal and ventral tentacular cirri subequal</td>
<td>with long dorsal and shorter ventral tentacular cirrus</td>
<td>dorsal and ventral tentacular cirri subequal</td>
</tr>
<tr>
<td>Notochaetae</td>
<td>two types, geniculate and slightly curved or straight</td>
<td>slightly curved or straight</td>
<td>slightly curved or straight</td>
<td>slightly curved or straight</td>
<td>absent</td>
</tr>
<tr>
<td>Neurochaetae</td>
<td>shafts spinose subdistally, blades short, falcate, unidentate</td>
<td>shafts with or without distal spines; blades falcigerous or spinigerous; unidentate or unidentate and bidentate</td>
<td>shafts without subdistal spines, blades short, spinose, falcate, unidentate</td>
<td>shafts with subdistal spines, blades short, unidentate, falcate</td>
<td>shafts smooth; blades all similar, short, falcigerous, unidentate, with minute teeth on margin</td>
</tr>
</tbody>
</table>
Table 4 (continued on next page). Comparison of species of *Laubierpholoe* Pettibone, 1992.

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</thead>
<tbody>
<tr>
<td><strong>Type locality, ecology, distribution</strong></td>
<td>South Atlantic Ocean, off South America, Tierra del Fuego, in 57–494 m; off New Zealand, in 86–95 m</td>
<td>South west Indian Ocean, Seychelles, Mahé, reef flat, South India and the Seychelles; extremely clean coarse coral sand</td>
<td>New Zealand, South Island, Kaikoura Harbor, Rubies Reef, in 12 m, coarse broken shell; Aquarium Point, Otago Harbor, intertidal</td>
<td>New Zealand, North Island, Leigh, middle of Goat Island, coarse broken shell in Amphiprion sand, in 20 m</td>
<td>Northeast Atlantic Ocean, Castle Island, Bermuda, in 2–8 m; Cuba</td>
<td>Mediterranean Sea, Gulf of Lion, the Calanques near Marseille, marine caves, silty and sandy sediment</td>
</tr>
<tr>
<td><strong>Body length, mm</strong></td>
<td>1–3</td>
<td>0.6–0.875</td>
<td>2.8–3</td>
<td>1.5–1.8</td>
<td>1.6</td>
<td>0.8–1.2</td>
</tr>
<tr>
<td><strong>Number of segments</strong></td>
<td>13–27</td>
<td>13–15</td>
<td>27–29</td>
<td>19–26</td>
<td>26</td>
<td>16–19</td>
</tr>
<tr>
<td><strong>Prostomium and anterior horns</strong></td>
<td>oval, bilobed</td>
<td>style with a distinctly narrower distal part, considerably shorter than dorsal tentacular cirri</td>
<td>oval, bilobed, with anterior lobes projecting anteriorly</td>
<td>oval, bilobed, anterior lobes extended anteriorly</td>
<td>rounded, bilobed, with lateral horns</td>
<td>oval to trapezoidal, bilobed; anterior horns arising medially from ventral tentacular cirri</td>
</tr>
<tr>
<td><strong>Median antenna</strong></td>
<td>style enlarged basally, about same length as dorsal tentacular cirri</td>
<td>with style about as long as prostomium, shorter than dorsal tentacular cirri</td>
<td>small ceratophore, style short, with filamentous tip, shorter than dorsal tentacular cirri</td>
<td>small ceratophore and short style, twice as short as dorsal tentacular cirri</td>
<td>large ceratophore and short style, shorter than dorsal tentacular cirri</td>
<td></td>
</tr>
<tr>
<td><strong>Tentacular cirri</strong></td>
<td>dorsal cirrus much longer than ventral</td>
<td>dorsal cirrus much longer than ventral</td>
<td>dorsal cirrus much longer than ventral</td>
<td>dorsal cirrus much longer than ventral</td>
<td>dorsal cirrus slightly longer than ventral or cirri of about same length</td>
<td></td>
</tr>
<tr>
<td><strong>Eyes</strong></td>
<td>2 pairs, large, separate</td>
<td>2 pairs, lensed, of nearly identical size</td>
<td>absent</td>
<td>2 pairs, large, anterior pair larger</td>
<td>2 pairs, anterior pair larger</td>
<td>2 pairs, very closely situated, anterior pair larger</td>
</tr>
<tr>
<td><strong>Elytra</strong></td>
<td>squarish to subreniform, smooth, with up to 7 papillae along lateral margin, sometimes few papillae on surface</td>
<td>irregularly oval, covering trunk and panopodia, with 3 to 4 short, capitate papillae along anterior margin and 3 to 5 on surface</td>
<td>large, oval, covering dorsum, delicate, with few papillae on lateral border and few on surface</td>
<td>large, oval, covering dorsum, with few lateral papillae; elytra on posterior segment small, enclosing pygidium</td>
<td>oval, not covering mid dorsum; transparent, with 5–8 papillae on external border and few on surface</td>
<td>large, round, transparent, with few oval to cirriform papillae along lateral and posterior borders and on surface</td>
</tr>
<tr>
<td><strong>Notopodia</strong></td>
<td>subconical acicular lobe, smaller and shorter than neuropodia</td>
<td>conical acicular lobe; shorter and smaller than neuropodia</td>
<td>conical acicular lobe, about as long as neuropodium</td>
<td>subconical acicular lobe, longer than lobe of neuropodium</td>
<td>small, conical acicular lobe on anterodorsal side of larger neuropodium</td>
<td>conical acicular lobe; shorter and smaller than neuropodia</td>
</tr>
</tbody>
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<tbody>
<tr>
<td><strong>Neuropodia</strong></td>
<td>long conical prechaetal acicular lobe and short rounded postchaetal lobe</td>
<td>conical lobe</td>
<td>conical acicular lobe</td>
<td>subconical acicular lobe</td>
<td>prechaetal conical acicular lobe and shorter rounded postchaetal lobe</td>
<td>conical acicular lobe</td>
</tr>
<tr>
<td><strong>Notochaetae</strong></td>
<td>numerous, slender, slightly curved, with capillary tips; upper ones shorter, more strongly curved, ventral longer</td>
<td>few (4–8), exclusively slender, slightly curved, with minute serrations along convex edge</td>
<td>numerous, slender, capillary, slightly curved</td>
<td>numerous, slender, capillary</td>
<td>few (2–4), forming fan-shaped bundle, slender, with series of small denticles</td>
<td>few (3–6), slender, capillary, straight or slightly curved, from smooth to finely serrated</td>
</tr>
<tr>
<td><strong>Neurochaetae</strong></td>
<td>shafts with row of subdistal denticles; blades short, with row of minute denticles and entire, falcate tips; upper ones with blades slightly longer than lower ones</td>
<td>shafts distally minutely spinose; in CH1 all blades minutely serrated, from CH2 two uppermost supraacicular chaetae with blades long, serrated; other supraacicular ones with blades long, smooth, subacicular ones with blades short, smooth; hook-like blades in five posteriormost segments</td>
<td>shafts without distal spines; few supraacicular chaetae with blades long, minutely spinose and blunt tip, other supraacicular ones shorter, coarsely serrated, tapering to sharp tip; subacicular with blades shorter, falcate, minutely spinose</td>
<td>shafts without distal spines; supraacicular chaetae with blades long, tapering to capillary tips; subacicular ones with shorter, falcate</td>
<td>shafts with numerous distal and subdistal spines; blades short and falcate, with entire tips, upper ones longer, with more numerous spines, lower ones shorter with fewer, minute spines</td>
<td>shafts with numerous subdistal and long distal spines; upper blades slightly longer than lower ones; blades serrated, straight, falcate and of 2 types: supraacicular ones unidentate, subacicular ones uni- and bidentate</td>
</tr>
</tbody>
</table>
Laubierpholoe massiliana sp. nov. is up to 1.2 mm long, with 17–19 segments, larger than L. indoceanica, which is shorter than 1 mm with 13–15 segments, but smaller than other species which can reach up to 1.6 mm with 26 segments (L. swedmarki) and up to 3 mm and 26–29 segments in L. antipoda, L. riseri and L. maryae.

According to Gonzalez et al. (2018a), the genus Laubierpholoe has prostomial “cephalic” peaks. These structures, similar to those in polynoids, are illustrated by Pettibone (1992) and Westheide (2001). There the prostomium looks bilobed with the anterior lobes forming acute horns or peaks. Conversely, in L. swedmarki the prostomium is described as trapezoidal, with two lateral horns situated more ventrally. These horns, projected forward, are below the prostomium in fig.1 (Laubier 1975). The shape of the prostomium was used to distinguish L. swedmarki from other species of Laubierpholoe: “bilobed prostomium rounded anteriorly, with small laterally projecting horns” versus “bilobed prostomium with anterior lobes projecting forward” (Pettibone 1992). SEM investigation showed that in L. massiliana sp. nov. the prostomium has an anterior depression where the medial antenna rises, but its anterior lobes are rounded, with notches above the tentaculophores. Two conical horns are situated more ventrally on the level of the ventral tentacular cirri, most probably originating from the tentacular segment. Therefore, they are not homologous with polynoid cephalic peaks. Other species of Laubierpholoe should be reinvestigated to establish the shape of their prostomium and position anterior of horns.

Unlike other species of Laubierpholoe, in L. massiliana sp. nov. ventral tentacular cirri are as long as the dorsal ones or slightly shorter. This led us to modify the generic diagnosis (discussed above) and allows to distinguish L. massiliana sp. nov. from its congeners.

One of the distinguishing characters for the species of Laubierpholoe is the number of notochaetae. It varies from 2–4 in L. swedmarki to 4–8 in L. indoceanica to numerous in L. antipoda, L. riseri and L. maryae; L. massiliana sp. nov. has a few (3–6) notochaetae, which is slightly more than in L. swedmarki but less than in other species. They can be straight or slightly curved which is typical for Laubierpholoe.

Laubierpholoe massiliana sp. nov. differs from all congeners by the shape of its neurochaeta. They are unusual not just for the genus Laubierpholoe but for the whole Pholoinae subfamily, as the blades of at least some of the neurochaetae are long and bidentate. Bidentate, sometimes deeply split neurochaetal blades are present in other sigalionids, e.g., Pelogeniinae subfamily, genera Sthenelais Kinberg, 1856, Fimbriosthenelais Pettibone, 1971, Willeystenelais Pettibone, 1971, Euhaltenessa Darboux, 1899 (Pettibone 1970, 1971, 1997) but have not been previously described in Pholoinae. Pettibone (1992) diagnosed the former family ‘Pholoidae’ as having “blades short, falcate, and unidentate”. Laubierpholoe indoceanica, described after this revision, has several types of neurochaetal blades, some comparatively long and hook-like (Westheide 2001: fig. 2J–K, N). Bidentate tips in L. massiliana sp. nov. are seen only with high magnification or with SEM and might have been overlooked in other species. We changed the diagnosis of the genus in that point to: “…blades unidentate or uni- and bidentate”.

Ecology

All previously described species of Laubierpholoe inhabit interstitial biotopes like coarse sand, gravel, broken shells, or carpets of benthic diatoms (Table 4). They share adaptations to interstitial lifestyle like small size, very long palps, reduction of head appendages, small number of notochaetae, smooth elytra with few papillae, and intra-elytral brooding of embryos. Laubierpholoe massiliana sp. nov. is unique within the genus for living in soft sediment habitats, such as silty sand and clayey silt (Tables 2, 4). It is otherwise very similar to other species in size, morphology, and reproduction biology. Since no adaptations to digging in soft sediment were found, we suppose that L. massiliana sp. nov. moves on or just below the sediment surface. This should be confirmed by direct observation of living worms. We have not found L. massiliana sp. nov. in samples collected outside the caves in different sediments.
Adaptation to cave dwelling
Polynoidae inhabiting marine caves have long sensory parapodial cirri and no eyes or pigmentation, unlike their non-cave dwelling relatives (eyes were plausibly lost in correlation with specialization and colonization of deep-sea habitats) (Gonzalez et al. 2018b; Capa et al. 2022). Among Sigalionidae, only the genus *Laubierpholoe* includes cave dwelling representatives.

*Laubierpholoe* sp. is reported from several anchialine caves in the Canary Islands. This undescribed species was found in the Corona lava tube (Lanzarote Island) in a carpet of benthic diatoms and in the sandy bottom of the Tenerife littoral (Brito et al. 2009; Martínez García et al. 2009). Riera et al. (2018) recorded the same species also from Los Cerebros Cave (Tenerife Island) in sand or gravel sediments. One specimen of this undescribed species from the Tenerife cave was used for phylogenetic analysis and represented as *Laubierpholoe* sp. C. (Gonzalez et al. 2018a). This species lacks eyes, but has short parapodial cirri; pigmentation information is absent.

Another species of *Laubierpholoe* lacking eyes is *L. maryae*, collected on coarse sediment in the intertidal and shallow subtidal zone of New Zealand (Pettibone 1992). The reason for this absence is not clear as interstitial lifestyle alone does not lead to eye loss in other species of Pholoinae.

*Laubierpholoe massiliana* sp. nov. has developed eyes and short parapodial cirri. Its elytra are transparent and lack pigment and its body is whitish and semitransparent. Living specimens have very long anal cirri (longer than palps, half of the body length), easily lost during sample treatment and preservation. They may play the same role as elongated parapodial cirri in cave polynoids. This hypothesis needs confirmation through comparison with species of *Laubierpholoe* of other marine biotopes. Little information exists on body coloration and length of anal cirri within the genus’s interstitial non-cave dwelling species. *Laubierpholoe indoocceanica* are whitish-brown to colourless, almost transparent, some with yellowish-brown spots on the elytra and long pygidial cirri, much shorter than the palps (Westheide 2001). *Laubierpholoe swedmarki* is of white colour and without pygidial cirri (most probably lost) (Laubier 1975). *Laubierpholoe riseri* has long anal cirri, also shorter than the palps (Pettibone 1992). The body colour and pygidial cirri presence in other species of *Laubierpholoe* are unknown. The pigmentation loss and longer anal cirri displayed by *Laubierpholoe massiliana* sp. nov. have to remain unexplained for the time being. They could be regarded as adaptations to the cave dwelling lifestyle or they could be inherited from their ancestors.

Geography
Other species of *Laubierpholoe* were recorded in the Atlantic (off South America, Bermuda, Cuba), Indian (Seychelles) and Pacific (New Zealand) Oceans (Fig. 1A, Table 4). Our finding is the first record of the genus in the Mediterranean Sea. The presence of *L. massiliana* sp. nov. is currently confirmed only for the two marine caves in the Calanques, but the species is likely to be found in other caves with soft sediments in the Mediterranean Sea. However, we have not found *L. massiliana* sp. nov. in samples collected outside the caves.

Phylogeny
Our results inferred both from BI and ML confirmed that *Laubierpholoe massiliana* sp. nov. indeed belongs to the genus *Laubierpholoe*, as well as the genus monophyly. It matches the previous results by Gonzalez et al. (2018a). The weak support and discordance with ML analysis of the clades within *Laubierpholoe* can be explained by data deficiency for other species of *Laubierpholoe* (Table 1).

Gonzalez et al. (2021) studied the mitochondrial genomes of Polynoidae with different lifestyles and found similarity in cave and pelagic polynoids. Exciting future research could investigate the mitochondrial genome of cave dwelling Sigalionidae in comparison with other representatives of the family.
Acknowledgements

This work has been supported by the grant of the Russian Science Foundation, RSF 21-74-20028. We thank Laurent Vanbostal and Alexander Ereskovsky (IMBE, CNRS, Aix Marseille University) for their support in organizing the visit of Russian authors to Marseille and their various help with collecting and processing the samples. Logistics and field assistance were provided by IMBE and OSU Institut Pythéas in Marseille, France, through the diving and laboratory facilities at Station Marine d’Endoume, its research vessels Antedon 2 and Astroides, and their pilots and crew. Thanks to Glaﬁra Kolbasova for her help with sample processing, to Alexandra Bezmennova for the consultations on molecular phylogenetic methods and to Anna Sokolova for language editing. We are grateful to Ruth Barnich (Senckenberg Research Institute) and an anonymous reviewer for their very helpful comments.

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ZHADAN A. et al., New species of Laubierpholoe from Marseille caves


Manuscript received: 22 July 2022
Manuscript accepted: 10 March 2023
Published on: 16 June 2023
Topic editor: Tony Robillard
Desk editor: Radka Rosenbaumová

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**Supplementary files**

Supp. file 1. Maximum likelihood tree obtained with the 18S rRNA and 28S rRNA concatenated dataset showing position of *Laubierpholoe massiliana* Zhadan sp. nov. within Sigalionidae Kinberg, 1856. Bootstrap support values are shown for each medium supported node. https://doi.org/10.5852/ejt.2023.875.2139.9073