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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. indooceanica* 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-Petricioli *et al.* 2007).

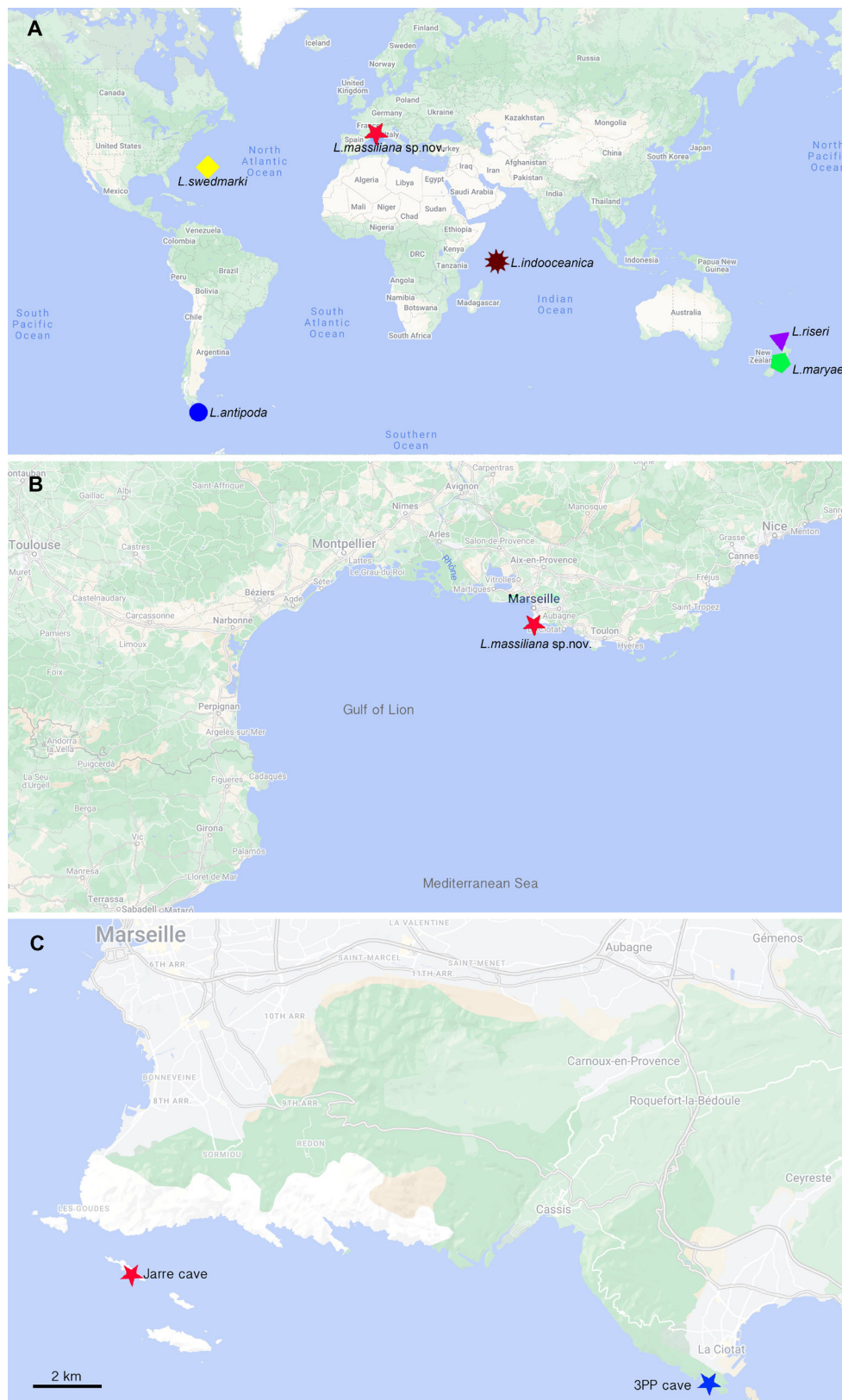**Fig. 1. A**. World map showing type locations of all species of *Laubierpholoe* Pettibone, 1992. **B**. Type location of *Laubierpholoe massiliana* Zhadan sp. nov. **C**. Locations of the two caves where *Laubierpholoe massiliana* sp. nov. was sampled.

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 floatation 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 ($MgCl_2$) 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 1 000 000. 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.

Name	18S rDNA	28S rDNA
Euthalenessa cf. *digitata*	KY657612	KY657625
Harmothoe impar	JN852829	JN852859
Laubierpholoe massiliana sp. nov. WS12216 Jarre Cave	OP035755	OP035748
Laubierpholoe massiliana sp. nov. WS12437 3PP Cave	OP035754	OP035747
Laubierpholoe massiliana sp. nov. WS12617 Jarre Cave	OP035753	OP035749
Laubierpholoe massiliana sp. nov. WS12619 Jarre Cave	OP035752	OP035746
Laubierpholoe sp. A	KY823452	–
Laubierpholoe sp. B	–	KY823466
Laubierpholoe sp. C	KY823454	KY823467
Laubierpholoe swedmarki	KY823453	–
Neoleanira tetragona	AY839570	JN852872
Pelogenia arenosa	KY823455	KY823468
Pholoe baltica	AY176301	JN852873
Pholoe longa	KY823456	KY823469
Pholoe minuta	KY823457	KY823470
Pholoe pallida	AY894302	JN852874
Pholoe sp. A	KY823458	KY823471
Pisione bulbifera	KY657613	KY657626
Pisione cf. *vestigalis*	KY657623	KY657640
Pisione remota	KY657620	KY657636
Pisionidens ixazaluohae	KX282503	KX282504
Psammolyce sp.	JN852843	JN852877
Sigalion amboinensis	AB106254	AF185187
Sthenelanella uniformis	AY894306	DQ790064

Results

Taxonomy

<div align="center">

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

</div>

Type species

Pholoe antipoda Hartman, 1967.

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.
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Figs 1–6

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

leg.; SCUBA-diving; sampling box; clayey silt; mesh size 130 µm; preservation in glutaraldehyde 2.5% in PBS, storage in ethanol 70%; field number Ma19-29-11; MNHN IA-TYPE 2058 • 2 specimens; same data as for preceding; SEM stubs; ZMMSU WS13977.

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
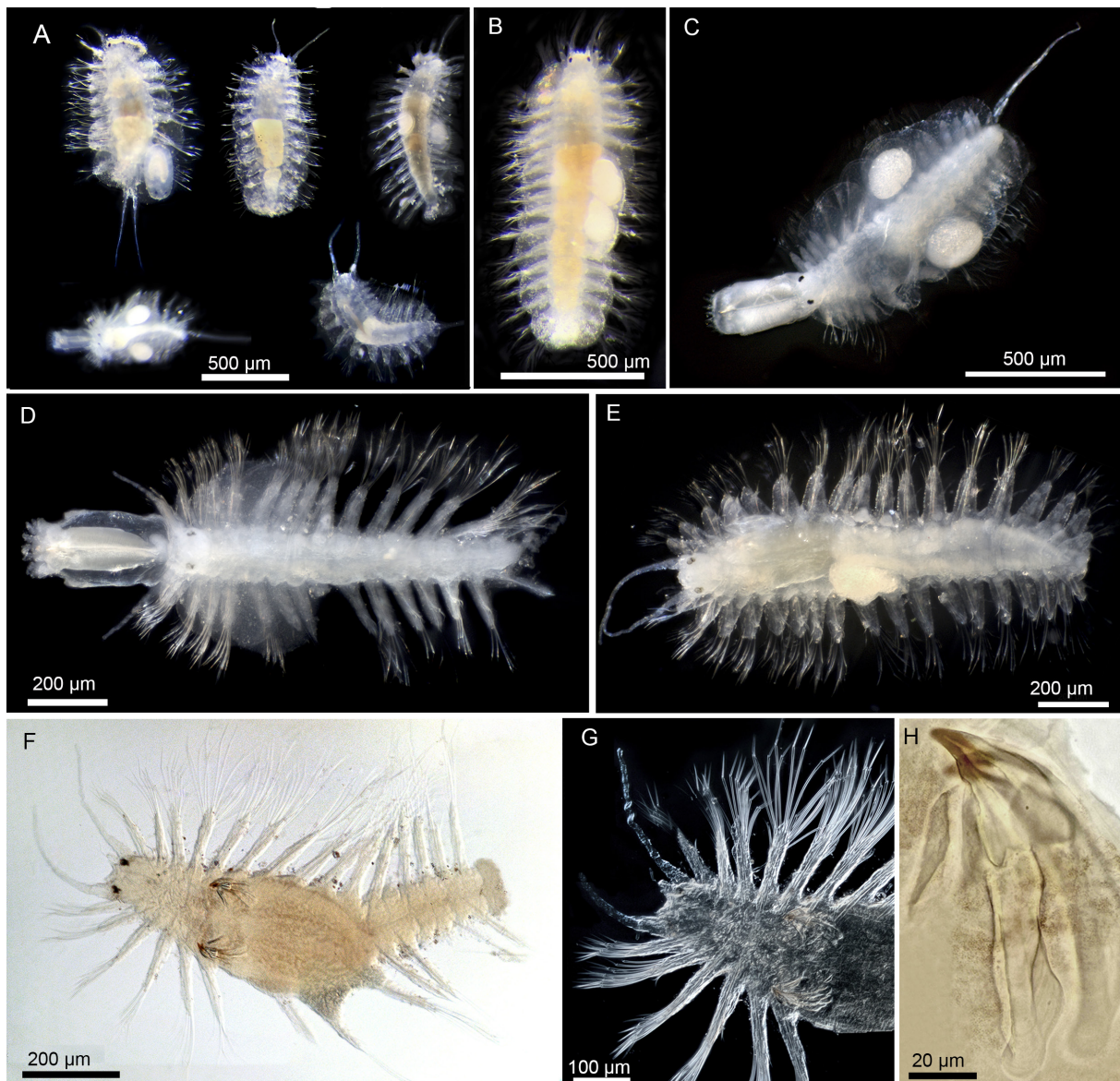

Fig. 2. *Laubierpholoe massiliana* Zhadan sp. nov., light microscopy. **A–B**. Living specimens from different samples. **C**. ZMMSU WS12418, paratype, general view with proboscis everted. **D**. ZMMSU WS16462, holotype, general view, proboscis everted. **E**. ZMMSU WS14001, paratype, general view. **F–H**. ZMMSU WS14001, paratype, compound microscope. **F**. General view. **G**. Anterior part, dark field. **H**. Right jaws.
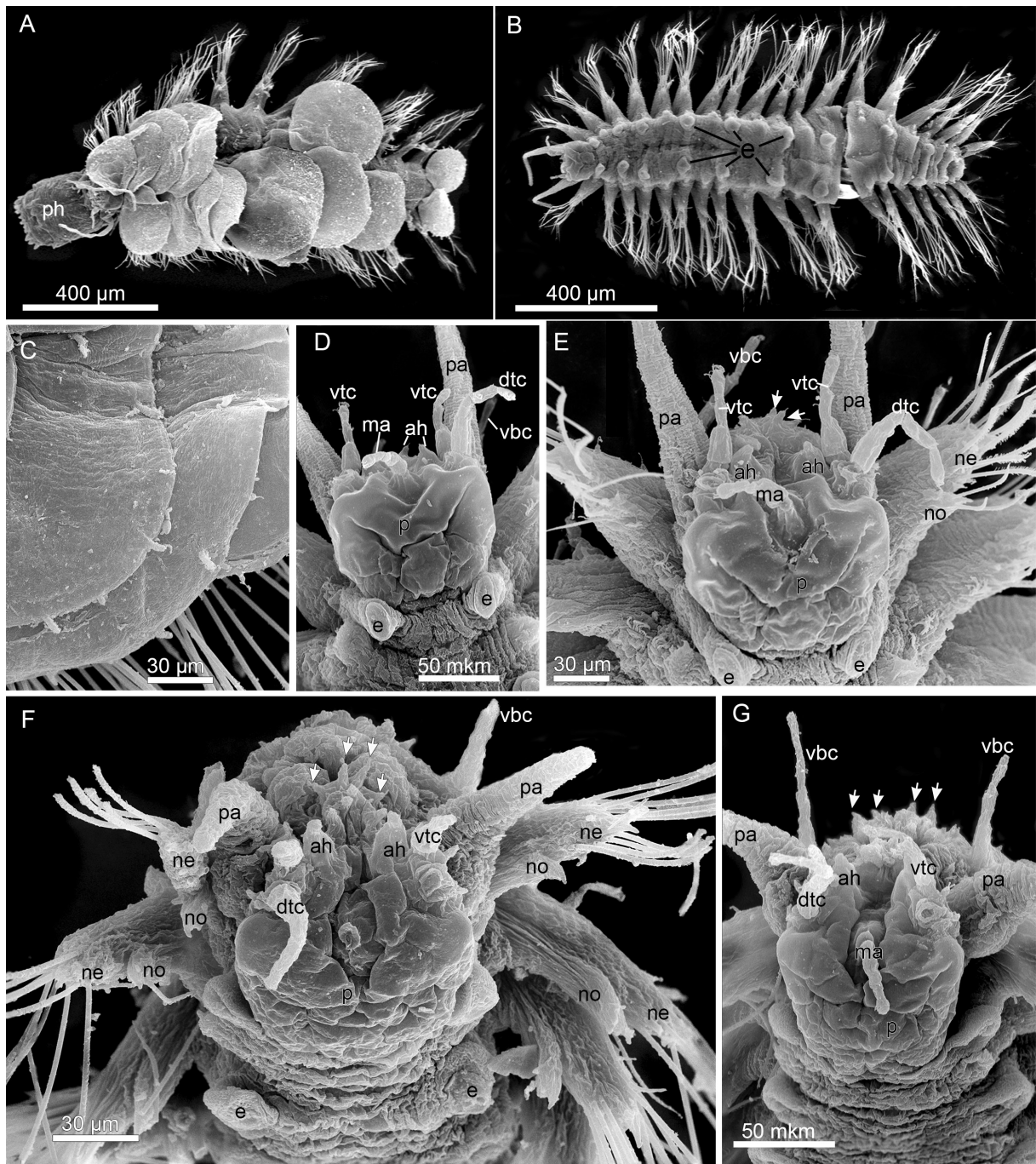
Fig. 3. *Laubierpholoe massiliana* Zhadan sp. nov., SEM. **A**. ZMMSU WS12292, general view. **B**. ZMMSU WS14001, general view, elytra omitted. **C**. ZMMSU WS13977, elytra. **D**. ZMMSU WS14001, anterior end, dorsal view. **E**. Same, dorso-anterior view. **F**. ZMMSU WS16511, anterior end, dorso-anterior 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
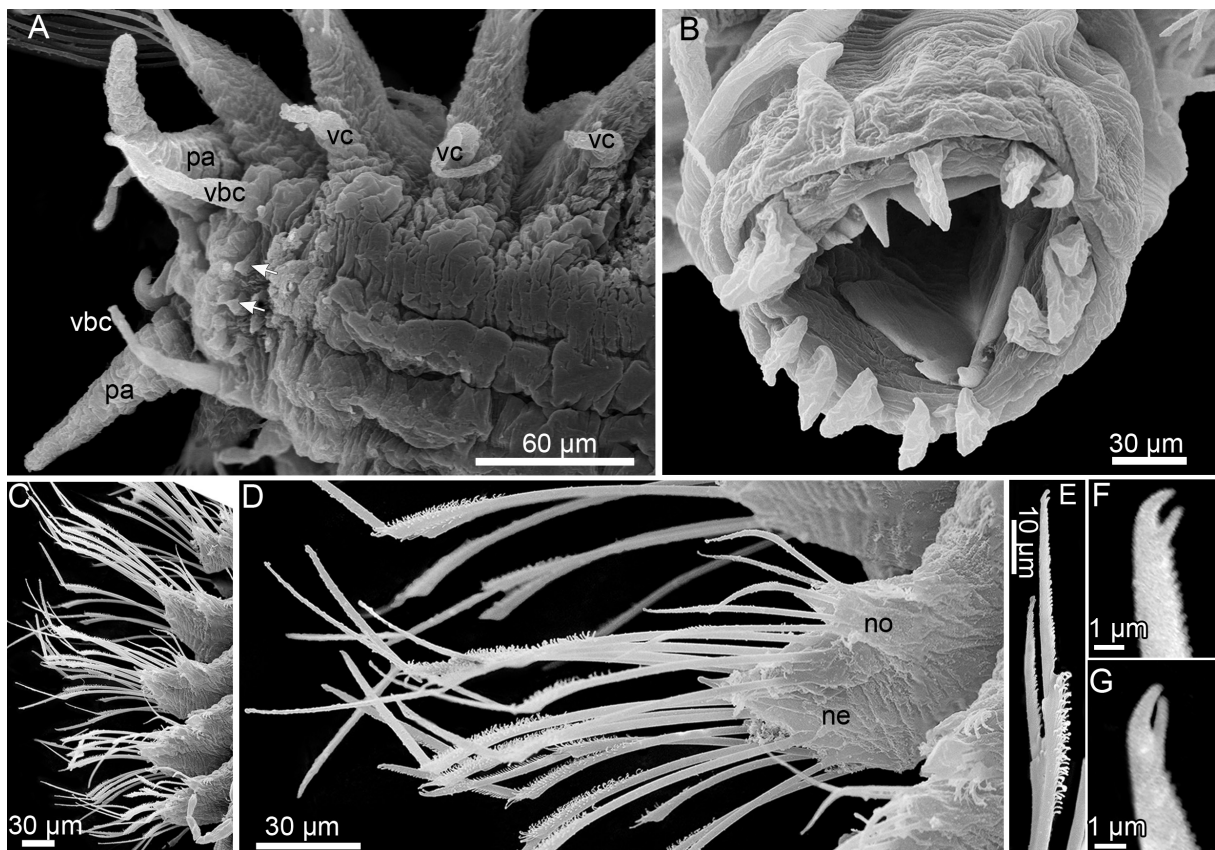

Fig. 4. *Laubierpholoe massiliana* Zhadan sp. nov., SEM. **A**. ZMMSU WS16511, ventral view. **B**. ZMMSU WS16511, pharynx, dorso-anterior view. **C**. ZMMSU WS14001, parapodia of segments I-V, dorso-anterior view. **D**. Same, parapodia of segment III, anterior view. **E**. ZMMSU WS13977, bidentate 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).
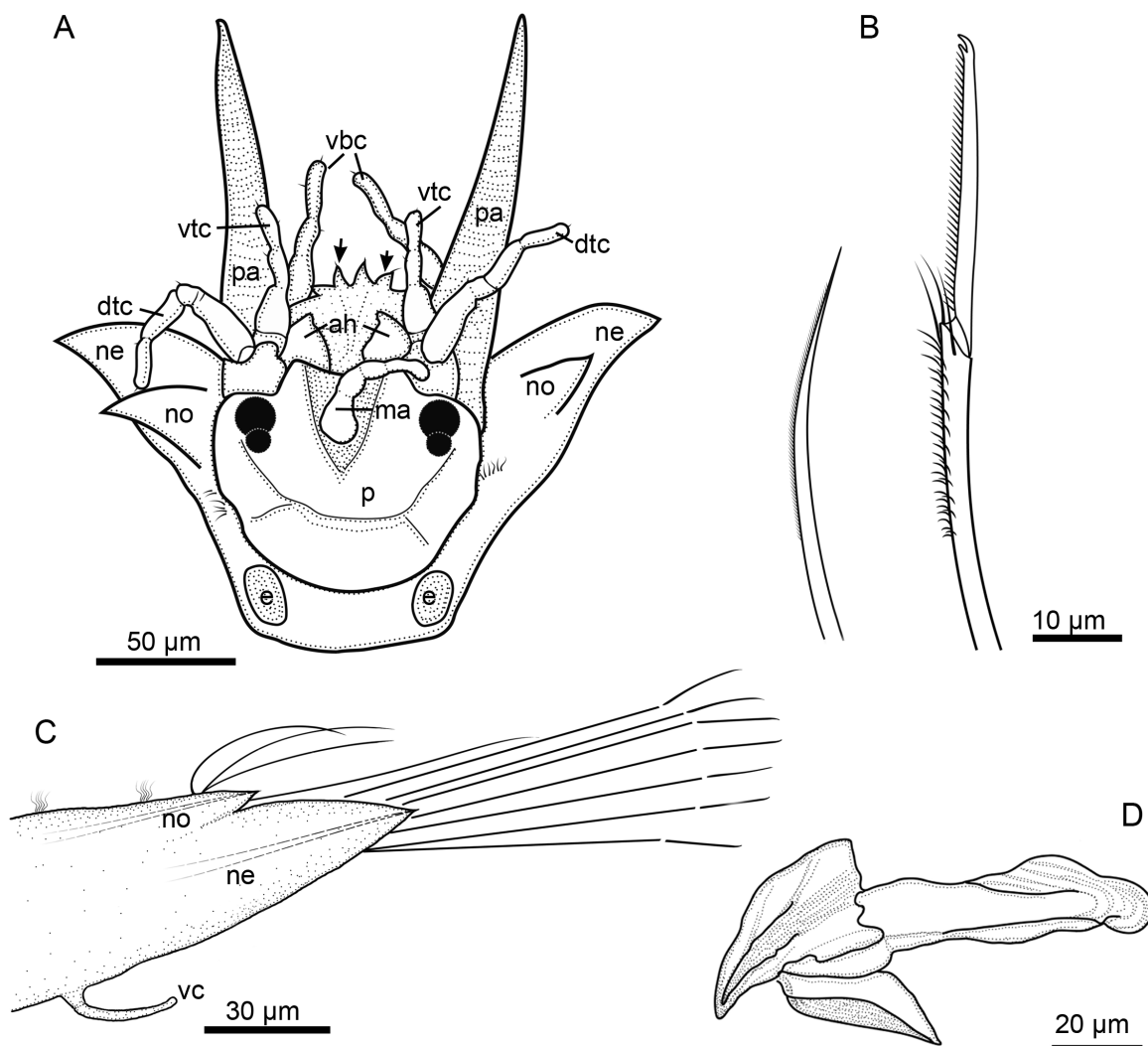
Fig. 5. *Laubierpholoe massiliana* Zhadan sp. nov., line drawings. **A**. Anterior end, dorso-anterior view. **B**. Notochaeta and bidentate neurochaeta. **C**. Parapodium, anterior view. **D**. Jaw. Abbreviations: ah = anterior horns; dtc = dorsal tentacular cirrus; ma = median antenna; ne = neuropodium; 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 (%).

| Fraction, mm	Jarre deep	Jarre middle	Jarre entrance	3PP deep	3PP middle	3PP entrance
>10	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
10–5	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
5–2	<0.1	<0.1	<0.1	<0.1	<0.1	1.2
2–1	<0.1	0.4	<0.1	<0.1	<0.1	7.9
1–0.5	3.2	4.8	2.0	1.1	0.7	34.2
0.5–0.25	21.5	9.9	3.2	1.0	1.0	18.5
0.25–0.1	29.7	17.4	12.8	1.7	1.3	20.3
0.1–0.05	19.4	22.8	22.9	4.4	3.4	7.4
0.05–0.01	13.7	30.4	37.3	49.2	61.5	4.6
0.01–0.002	6.3	6.4	10.9	28.5	20.1	1.7
0.002–0.001	2.4	2.1	0.8	3.9	4.7	<0.1
< 0.001	3.8	5.8	10.1	10.2	7.3	4.0
Interpretation	silty sand	sandy silt	silt	clayey silt	clayey silt	sand
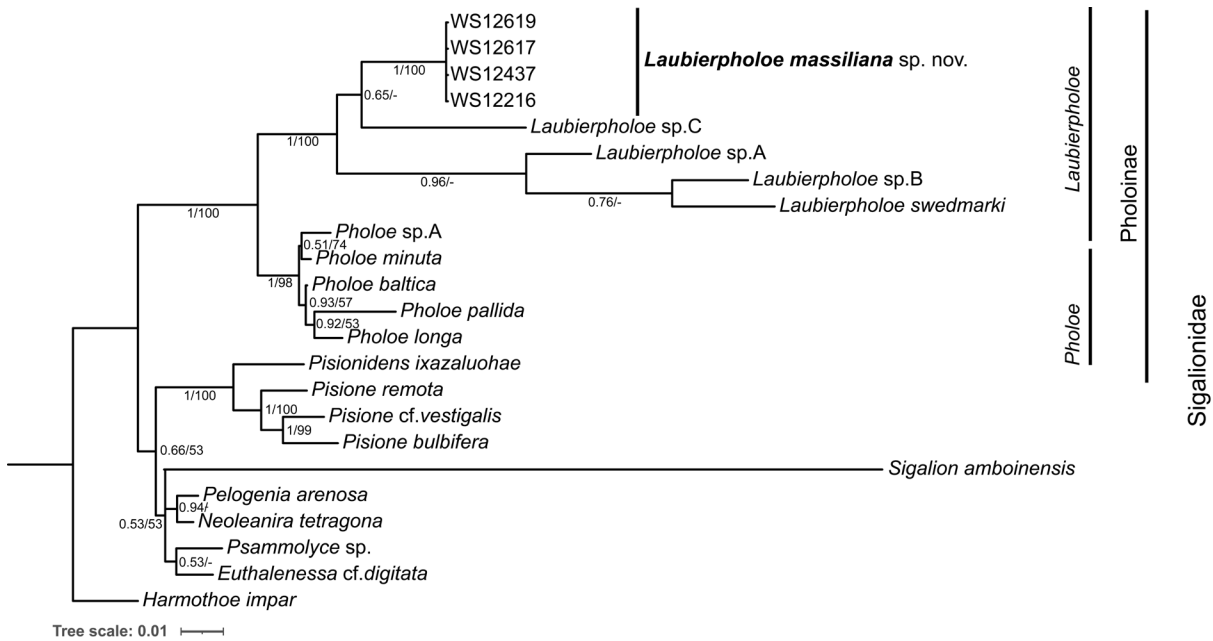
Fig. 6. Bayesian phylogenetic tree obtained with the 18S rRNA and 28S rRNA concatenated dataset showing position of *Laubierpholoe massiliana* Zhadan sp. nov. within Sigalionidae Kinberg, 1856. Posterior probabilities and bootstrap values are shown for each medium supported node.

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. indooceanica*** 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.

	Pholoe Johnston, 1839	*Laubierpholoe* Pettibone, 1992	*Taylorpholoe* Pettibone, 1992	*Imajimapholoe* Pettibone, 1992	*Metaxypsamma* Wolf, 1986
Number of segments	up to 90	up to 29	up to 19	up to 34	up to 24
Elytra	delicate, with border and surface papillae	delicate, with few short papillae on lateral border and on surface	squarish, with fringe of numerous filiform papillae on 3 sides	opaque, with papillae on lateral and posterior borders and on surface	rudimentary, as paired nodular lobes with 2–4 long filiform papillae
Elytral brooding	absent	present	present	present	absent
Median antenna	in anterior notch	in anterior notch	occipital	occipital	in anterior notch
Lateral antennae	absent or present	absent	present	absent	absent
Tentacular cirri	dorsal and ventral tentacular cirri subequal	dorsal cirrus much longer or tentacular cirri subequal	dorsal and ventral tentacular cirri subequal	with long dorsal and shorter ventral tentacular cirrus	dorsal and ventral tentacular cirri subequal
Notochaetae	two types, geniculate and slightly curved or straight	slightly curved or straight	slightly curved or straight	slightly curved or straight	absent
Neurochaetae	shafts spinose subdistally, blades short, falcate, unidentate	shafts with or without distal spines; blades falcigerous or spinigerous; unidentate or uni- and bidentate	shafts without subdistal spines, blades short, spinose, falcate, unidentate	shafts with subdistal spines, blades short, unidentate, falcate	shafts smooth; blades all similar, short, falcigerous, unidentate, with minute teeth on margin

Table 4 (continued on next page). Comparison of species of *Laubierpholoe* Pettibone, 1992.

	L. antipoda (Hartman, 1967)	*L. indooceanica* Westheide, 2001	*L. maryae* Pettibone, 1992	*L. riseri* Pettibone, 1992	*L. swedmarki* (Laubier, 1975)	*L. massiliana* Zhadan sp. nov.
Type locality, ecology, distribution	South Atlantic Ocean, off South America, Tierra del Fuego, in 57–494 m; off New Zealand, in 86–95 m	South west Indian Ocean, Seychelles, Mahé, reef flat, South India and the Seychelles; extremely clean coarse coral sand	New Zealand, South Island, Kaikoura Harbor, Rubies Reef, in 12 m, coarse broken shell; Aquarium Point, Otago Harbor, intertidal	New Zealand, North Island, Leigh, middle of Goat Island, coarse broken shell in *Amphioxus* sand, in 20 m	Northeast Atlantic Ocean, Castle Island, Bermuda, in 2–8 m; Cuba	Mediterranean Sea, Gulf of Lion, the Calanques near Marseille, marine caves, silty and sandy sediment
Body length, mm	1–3	0.6–0.875	2.8–3	1.5–1.8	1.6	0.8–1.2
Number of segments	13–27	13–15	27–29	19–26	26	16–19
Prostomium and anterior horns	oval, bilobed	bilobed	oval, bilobed, with anterior lobes projecting anteriorly	oval, bilobed, anterior lobes extended anteriorly	rounded, bilobed, with lateral horns	oval to trapezoidal, bilobed; anterior horns arising medially from ventral tentacular cirri
Median antenna	style enlarged basally, about same length as dorsal tentacular cirri	style with a distinctly narrower distal part, considerably shorter than dorsal tentacular cirri	with style about as long as prostomium, shorter than dorsal tentacular cirri	small ceratophore, style short, with filamentous tip, shorter than dorsal tentacular cirri	small ceratophore and short style, twice as short as dorsal tentacular cirri	large ceratophore and short style, shorter than dorsal tentacular cirri
Tentacular cirri	dorsal cirrus much longer than ventral	dorsal cirrus much longer than ventral	dorsal cirrus much longer than ventral	dorsal cirrus much longer than ventral	dorsal cirrus much longer than ventral	dorsal cirrus slightly longer than ventral or cirri of about same length
Eyes	2 pairs, large, separate	2 pairs, lensed, of nearly identical size	absent	2 pairs, large, anterior pair larger	2 pairs, anterior pair larger	2 pairs, very closely situated, anterior pair larger
Elytra	squarish to subreniform, smooth, with up to 7 papillae along lateral margin, sometimes few papillae on surface	irregularly oval, covering trunk and parapodia, with 3 to 4 short, capitate papillae along anterior margin and 3 to 5 on surface	large, oval, covering dorsum, delicate, with few papillae on lateral border and few on surface	large, oval, covering dorsum, with few lateral papillae; elytra on posterior segment small, enclosing pygidium	oval, not covering middorsum; transparent, with 5–8 papillae on external border and few on surface	large, round, transparent, with few oval to cirriform papillae along lateral and posterior borders and on surface
Notopodia	subconical acicular lobe, smaller and shorter than neuropodia	conical acicular lobe; shorter and smaller than neuropodia	conical acicular lobe, about as long as neuropodium	subconical acicular lobe, longer than lobe of neuropodium	small, conical acicular lobe on anterodorsal side of larger neuropodia	conical acicular lobe; shorter and smaller than neuropodia

Table 4 (continued). Comparison of species of *Laubierpholoe* Pettibone, 1992.

	L. antipoda (Hartman, 1967)	L. indooceanica Westheide, 2001	L. maryae Pettibone, 1992	L. riseri Pettibone, 1992	L. swedmarki (Laubier, 1975)	L. massiliana Zhadan sp. nov.
Neuropodia	long conical prechaetal acicular lobe and short rounded postchaetal lobe	conical lobe	conical acicular lobe	subconical acicular lobe	prechaetal conical acicular lobe and shorter rounded postchaetal lobe	conical acicular lobe
Notochaetae	numerous, slender, slightly curved, with capillary tips; upper ones shorter, more strongly curved, ventral longer	few (4–8), exclusively slender, slightly curved, with minute serrations along convex edge	numerous, slender, capillary, slightly curved	numerous, slender, capillary	few (2–4), forming fan-shaped bundle, slender, with series of small denticles	few (3–6), slender, capillary, straight or slightly curved, from smooth to finely serrated
Neurochaetae	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	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	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	shafts without distal spines; supraacicular chaetae with blades long, tapering to capillary tips; subacicular ones with blades short, falcate	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	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

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Laubierpholoe massiliana sp. nov. is up to 1.2 mm long, with 17–19 segments, larger than *L. indooceanica*, 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. indooceanica* 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, *Willeysthenelais* Pettibone, 1971, *Euthalenessa* 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 indooceanica*, 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 indooceanica* 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

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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.
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