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Phylogeny of Ophryotrocha (Annelida Dorvilleidae) revisited, with description of six new species from eastern Pacific seeps and whalefalls

Author

Pruitt, Jessica

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Phylogeny of *Ophryotrocha* (Annelida Dorvilleidae) revisited, with description of six new species from eastern Pacific seeps and whalefalls

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Marine Biology

by

Jessica Pruitt

Committee in charge

Professor Greg Rouse, Chair
Professor Ryan Hechinger
Professor Lisa Levin

2021

The thesis of Jessica Pruitt is approved and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

Phylogeny of *Ophryotrocha* (Annelida Dorvilleidae) revisited, with description of six new species from eastern Pacific seeps and whalefalls

by

Jessica Pruitt

Master of Science in Marine Biology

University of California San Diego 2021

Professor Greg Rouse, Chair

Dorvilleidae is a diverse group of annelids, well known from deep sea chemosynthetic environments. Of the dorvilleids, *Ophryotrocha* is the most speciose genus with over 75 accepted species, but also contains several cryptic species and has been shown to be paraphyletic in many studies. Twenty-one new species of *Ophryotrocha* are recognized in this study from deep sea methane seep and whale fall environments in the northeast Pacific Ocean, six of which are being formally described. Eight previously known-to-science species were also recovered. The *Ophryotrocha* specimens were studied using morphology and phylogenetic analyses of DNA sequences from mitochondrial (cytochrome c oxidase subunit I, 16S rRNA, and cytochrome b) and nuclear (18S rRNA and histone 3) genes. The current *Ophryotrocha* phylogeny is examined and the issue of its paraphyly is addressed, but cannot be resolved in this study.

INTRODUCTION

Hydrothermal vents, methane seeps, and organic falls (ie. wood, whale carcasses, etc.) are nutrient-rich oases in an otherwise nutrient-deprived deep ocean (Lundsten et al. 2010; Bernardino et al. 2012; Smith et al. 2015; Levin et al. 2016). While these environments are patchily distributed, it has been theorized that these sites act as “stepping stones,” allowing metazoans and larvae with otherwise short dispersal capabilities to travel greater distances (Smith and Baco 2003; Smith et al. 2015). These chemosynthetic environments typically have elevated hydrogen sulfide levels, which plays an important role in structuring the faunal communities found at these sites (Levin et al. 2003; Levin et al. 2013). Despite sulfide being toxic to most Metazoa, a relatively diverse community of animals can still be found at vents, seeps, and organic falls compared to background communities (Levin et al. 2003; Smith and Baco 2003; Levin 2005; Bernardino et al. 2012; Zapata-Hernandez et al. 2014; Smith et al. 2015). For instance, vesicomyid clams, bathymodiolin mussels, as well as various annelid taxa, such as dorvilleids, are capable of tolerating the sulfide levels found in these reducing environments (Levin et al. 2003; Smith and Baco 2003; Thornhill et al. 2012; Levin et al, 2013; Smith et al. 2015) .

Dorvilleidae Chamberlin, 1919 is a diverse family within the clade Eunicida, with some 32 genera and over 200 named species (Read and Fauchald, 2021). Dorvilleidae are globally distributed but can be surprisingly common in hostile environments. Historically, they have been found in shallow nutrient-rich waters, like harbors and beneath fish farms, as well as in aquaria. With continued exploration of the deep sea, dorvilleids have been found to establish diverse assemblages at hydrothermal vents, methane seeps, and organic falls (Jumars 1974; Paxton and Morineaux 2009; Thornhill et al. 2012; Wiklund et al. 2012; Ravara et al. 2015; Zhang et al. in prep). While most dorvilleids are well known bacterivores (Thornhill et al. 2009), there have also been cases of parasitism (Gaston and Benner 1981; Rossi 1984) and the only known animal to graze on archaea (Thurber et al. 2012). Of the dorvilleid genera, *Ophryotrocha* Claparede and Mecznikow, 1869 is the most speciose, currently with over 76 accepted species (Read and Fauchald, 2021).

Many *Ophryotrocha* species have been described, though the morphological differences between them can be subtle (Hoisaeter and Samuelsen 2006; Paxton and Åkesson 2007; Wiklund et al. 2009; Wiklund et al. 2012; Nygren 2014; Taboada et al. 2017). As a stark example, Paxton and Åkesson (2007) redescribed the type species *Ophryotrocha puerilis* Claparede and Mecznikow, 1869, after discovering that the original description seemed to have been derived from two different worms, *Ophryotrocha labronica* La Greca and Bacci, 1962 and *Ophryotrocha puerilis*, both of which are found in the shallow water Mediterranean. Unsurprisingly, the advent of DNA sequencing has revealed several cryptic species complexes among *Ophryotrocha* (Nygren 2014; Taboada et al. 2017).

An early phylogenetic study of *Ophryotrocha* included chromosome counts and morphology of 20 *Ophryotrocha* (Pleijel and Eide 1996). Dahlgren et al. (2001) then used mitochondrial 16S rRNA for 18 *Ophryotrocha* and divided *Ophryotrocha* into three informal groups, also based upon life history and reproductive strategies. Heggoy et al. (2007) also used 16S and mitochondrial cytochrome c oxidase I (COI) for 14 *Ophryotrocha* species, as well as one *Iphitime* Marenzeller, 1902. They concluded that

Iphitime fell within *Ophryotrocha*. Subsequent molecular studies have been done with COI, 16S, and nuclear Histone H3 and have shown that not only *Iphitime*, but at least five other dorvilleid genera may fall within *Ophryotrocha* (Wiklund et al. 2009; Thornhill et al. 2012; Wiklund et al. 2012).

Despite this paraphyly, *Ophryotrocha* continue to be discovered, especially with the increased exploration of the deep sea. However, many of these worms are being left undescribed, with some even unsequenced (Thornhill et al. 2012; Amon et al. 2013; Levin et al. 2013; Smith et al. 2015; da Rocha Miranda et al. 2020). This rise in "dark taxa" (Page, 2016) is becoming more common as many deep-sea studies try to tackle overall biodiversity counts in certain regions with less focus on species descriptions (Danovaro et al. 2010; Thornhill et al. 2012; Smith et al. 2015; Saeedi et al. 2019). One such example of this is the study of Thornhill et al. (2012) on methane seep dorvilleids, which found several sympatric dorvilleid genera living at seeps in Oregon and California, USA. Cytochrome-b (CytB) and 16S DNA data found 5 apparently new-to-science species of *Ophryotrocha*, as well as undescribed *Exallopus* Jumars, 1974, *Parougia* Wolf, 1986, *Pinniphitime* Orensanz, 1990, and *Pseudophryotrocha* Hilbig and Blake, 1991 taxa. With the exception of several *Parougia* having now been named (Yen and Rouse, 2020), the others remain undescribed to this date.

Here I update the phylogeny of *Ophryotrocha* and describe six new species from deep-sea organic falls and methane seeps in the Northeast Pacific, ranging from Oregon, USA to Costa Rica. Three of the undescribed *Ophryotrocha* species from Thornhill et al. (2012) are named as a result. Sixteen other new species of *Ophryotrocha* are also recognized, but not named in this study.

MATERIALS AND METHODS

Sample Collection

A series of *Ophryotrocha* specimens were collected on various cruises in the eastern Pacific Ocean from 2006 to 2020 using the human-occupied vehicle (HOV) *Alvin* and remotely-operated vehicles (ROVs) *Jason II*, *Doc Ricketts*, *SuBastian*, *Tiburon*, and *Hercules* at methane seep and mammal fall environments between Oregon, USA and Costa Rica (Table 1, Figure 1), ranging from 500 – 2,000 meters depth. Animals were relaxed with 7% magnesium chloride in freshwater and live photographs were taken shipboard using a Lecia MZ9.5 stereomicroscope and a Canon Rebel SLR camera. Specimens were then put in 95% ethanol for DNA extraction or 10% formalin and seawater for morphology and voucher material. Some specimens were cut in two and placed in ethanol (posterior) and formalin (anterior). Formalin-fixed material was rinsed in freshwater and transferred to 70% ethanol. All type and paratype material is stored at Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC) in La Jolla, California, USA.

Morphological Analysis

Jaw Analysis

Anterior ends of *Ophryotrocha* were dissolved in a pancreatin solution for jaw analysis (following Alvarez-Padilla and Hormiga (2007) and Macnaughton et al. (2010)). A stock solution of saturated borax/H2O was made by heating water to 60°C and stirring in household borax until dissolved. The solution was left to cool to room temperature, 20°C. 0.25 grams of pancreatin USP grade enzyme complex was dissolved in 17.5 mL of water and stirred until the pancreatin was dissolved. The borax aqueous solution was added to the pancreatin solution and mixed well. The solution was then filtered with cotton into a clean vial and stored at -20°C until use. Preserved animals were rinsed in freshwater and anterior ends were left in a drop of pancreatin solution for several hours to overnight at room temperature. The jaws and any remaining tissue were rinsed several times with molecular grade pure water on a round coverslip. Once the water evaporated, the jaws were left dried to the coverslip and this was mounted to a carbon disc on a SEM stub before coating with gold/palladium and examined with a Zeiss Evo10 SEM.

Scanning Electron Microscopy (SEM) Protocol

Segments and parapodia were isolated from the midbody via transverse section with a scalpel and placed in a 4% osmium tetroxide ($OsO4$) freshwater solution for several hours. The tissue samples were rinsed with DI water three times and placed in 70% ethanol. Parapodia were rinsed in 90% ethanol twice, 100% ethanol three times, then a 50/50 ethanol/hexamethyldisilane (HMDS) solution, a 25/75 ethanol/HMDS solution, and finally rinsed three times in 100% HMDS, following Murtey and Ramasamy (2016). Sections were left to dry overnight and transferred to an SEM stub covered with carbon tabs and aluminum tape. Stubs were coated with gold/palladium and examined with a Zeiss EVO-10 SEM. Some full bodies of *Ophryotrocha* were also prepared for SEM using this method.

Light Micrography Protocol

Parapodia were dissected from the midbody with a scalpel and permanently mounted on slides with Aqua-Mount (Polysciences). A Lecia DMR compound microscope with interference contrast was used for light micrography with a Canon Rebel T3i digital camera. Some photographs were stacked and merged using Helicon (v 7.5.5, Helicon Soft, Ltd.).

DNA Analysis

DNA extraction, amplification, and sequencing

Specimen DNA was extracted from 76 *Ophryotrocha* specimens using Zymo DNA extraction Microkit Plus (Zymo Research, California, USA) following the solid tissues protocol. Regions of three mitochondrial genes: cytochrome oxidase subunit I (COI, ~600 bp), cytochrome b (CytB, ~400 bp), and 16S rRNA (16S, ~450 bp) and two nuclear genes: Histone H3 (H3 ~330 bp) and 18S rRNA (18S ~1300 bp) were targeted and amplified with primers listed in Table 2. Most specimens were sequenced for COI. In the case of COI not being amplified, 16S was used. One representative from each “species” with two or more very similar COI sequences was then selected for the remaining genes. For amplification, the following PCR temperature profiles were used: for COI [94°C/ 3 min- (94°C/ 30 s - 47°C/ 45 s - 72°C/ 1 min) x 5 cycles - (94°C/ 30 s - 52°C/ 45 s - 72°C/ 1 min) x 30 cycles - 72°C/ 5 min]; for CytB one of the following was used [94°C/2 min - (94°C/ 30 s - 45°C/ 1 min - 68°C/ 1 min) x 35 cycles - 68°C/ 7 min] or [95°C/ 5 min - (95°C/ 30 s - 48°C/ 30 s - 72°C/ 30 s) x 40 cycles - 72°C/ 7 min]; for 16S

[95°C4/ 3 min - (95°C/ 40 s - 50°C/ 40 s - 72°C/ 50 s) x 40 cycles - 72°C/ 5 min]; for H3 [95°C/3 min - (95°C/ 30 s - 53°C/ 45 s - 72°C/ 45 s) x 40 cycles - 72°C/ 5 min]; for 18S [95°C/ 3 min - (95°C/30 s - 50°C/ 30 s - 72°C/ 1.5 min) x 40 cycles - 72°C/ 8 min] and [95°C/ 3 min - [95°C/30 s - 52°C/ 30 s - 72°C/ 1.5 min) x 40 cycles - 72°C/ 8 min]. Standard PCR mixtures contained 12.5 µL of either Apex 2.0x Taq Red DNA Polymerase Master Mix (Genessee Scientific) or Conquest PCR 2.0x Master Mix 1 (Lamda Biotech), 8.5 µL of ddH2O, 1 µL of each primer pair, and 2 µL of template DNA for a total of 25 µL. After amplification, PCR products were purified with ExoSAP-IT (USB Affimetrix, Ohio, USA). Sanger sequencing was performed by Eurofins Genomics in Louisville, Kentucky, USA. Overlapping sequence fragments were assembled via de novo assembly using Geneious (v11.0.5, Biomatters, Ltd.) to form consensus sequences.

Phylogeny and Haplotype Networks

COI sequences were collected from most specimens in this study to aid in species delineation. 18S, 16S, CytB, and H3 sequences were then also generated for a subset of specimens. These genes were chosen to allow comparisons with previous studies, in particular Thornhill et al. (2012) and Wiklund et al. (2012), both of which included dorvilleids from the northeast Pacific. The 18S rRNA gene sequences were used to infer a more robust overall phylogeny, though very few 18S sequences of *Ophryotrocha* have been published to date. In addition to available *Ophryotrocha* data, *Iphitime*, *Exallopus*, *Pseudophryotrocha*, and *Pinniphitime* data from GenBank (Table 3) were included in this analysis, as these genera have been shown to lie within

Ophryotrocha in previous studies (Heggoy et al. 2007; Wiklund et al. 2009; Thornhill et al. 2012; Wiklund et al. 2012). The Thornhill et al. (2012) study lists their sequences based on numbered and lettered haplotypes from each locale. Using the supplemental material from their paper, relevant concatenated haplotypes that represented an individual for each of their nominal species were chosen to be included in this study. Sequence data from new hydrothermal vent *Ophryotrocha* species were also included in this analysis and are listed as *Ophryotrocha* sp. vent 1 through 4 (Zhang et al. in prep).

Newly generated sequences for each gene were combined with the available GenBank data in Mesquite (v9.6 build 917, Maddison and Maddison 2019) and aligned with the MAFFT plugin. For COI, uncorrected pairwise distances were used to help delineate species and generated using PAUP (v4.0a build 168, Swofford 2002). A maximum likelihood analysis using RAxML 8 (Stamatakis 2014) via RAxMLGUI (v1.5, Silvestro and Michalak 2012) and the GTR+G mode was performed on each gene alignment. Each gene was imported into Sequence Matrix (v1.8, Vaidya et al. 2011) to create a concatenated matrix.

Dorvillea erucaeformis (Malmgren, 1865) and *Dorvillea rubrovittata* (Grube, 1855) were used as outgroups based on Wiklund et al. (2012). Maximum likelihood (ML) and Bayesian inference (BI) analyses were run on the concatenated data. The maximum likelihood analyses were run with RAxMLGUI using GTR+G for each partition. The thorough bootstrap option with 100 replicates was used to assess tree support. Bayesian inference was run with MrBayes 3.2 (Ronquist et al. 2012). jModeltest 2.1.10 (v20160303, Guindon and Gascuel 2003; Darriba et al. 2012) was used to determine the appropriate best-fit model using the Bayesian Inference Criterion (BIC). COI, 16S, CytB, and H3 were assigned the SYM+I+G model and 18S was assigned the SYM+G model. Analyses were performed using four independent runs of 10 million generations, sampling a tree every 1000 generations. Tracer 1.7 (Rambaut et al. 2018) was used to check convergence of the runs and for setting the burn-in, which was set to 10%. The remaining were combined into a majority-rule consensus tree with the relative posterior probabilities. Final trees were examined with FigTree (v1.4.4, Rambaut 2018) and edited with Adobe Illustrator (v24.2.1, Adobe Inc. 2019).

TCS haplotype networks (Clement et al. 2002) were generated using PopART (Leigh and Bryant 2015) for each of the six new species being described in this study, as well as some of the other undescribed species and species previously known to science. COI alignments were trimmed for each species as followed: *Ophryotrocha tilici* n. sp. 596 bp; *Ophryotrocha scoobyi* n. sp. 579 bp; *Ophryotrocha haleyae* n. sp. 510 bp; *Ophryotrocha averysea* n. sp. 377 bp; *Ophryotrocha amyae* n. sp. 598 bp; *Ophryotrocha manhellafinae* n. sp. 579 bp; *Ophryotrocha* n. sp. 9 543 bp; *Ophryotrocha* n. sp. 11 572 bp; *Ophryotrocha platykephale* 540 bp; "*Exallopus* cf. *jumarsi*" 428 bp; *Ophryotrocha* cf. *flabella* 572 bp; *Ophryotrocha longicollaris* 572 bp; *Ophryotrocha* cf. *craigsmithi* 572 bp; *Ophryotrocha langstrumpae* 572 bp; *Ophryotrocha* cf. *nauarchus* 549 bp. Corresponding maps were generated with RStudio (v1.1.463, RStudio Team, 2020).

RESULTS

Phylogenetic

COI sequences were obtained for 70 specimens collected for this study. When combined with the available data on GenBank (Table 3), the new sequences grouped into 24 distinct lineages (Figure 2). Four of these lineages were less than 1% divergent from named sequences on GenBank and generally matched them in morphology, so the relevant names are applied to these taxa: *Ophryotrocha langstrumpae* Wiklund et al., 2012, *Ophryotrocha longicollaris* Wiklund et al., 2012, and “*Exallopus* cf. *jumarsi*” Blake, 1985. We also obtained sequences for the first time of the previously described *Ophryotrocha platykephale* Blake, 1985. Other sequences were less than 5% divergent from previously published sequences so these are referred to these taxa, though further investigation is required: *Ophryotrocha* cf. *craigsmithi* Wiklund et al., 2009, *Ophryotrocha* cf. *flabella* Wiklund et al., 2012, *Ophryotrocha* cf. *nauarchus* Wiklund et al., 2012. For all other species delineations, the COI sequence differences were much greater than this 5% difference and the morphological differences were clear. This left 17 lineages for which there were no available names. For six of these lineages, multiple COI sequences were obtained, and several specimens were available for morphological study and these were chosen for full taxonomic descriptions as new species. For the other 11, there was only a singleton or very few specimens to sequence (Figure 2) and so formal descriptions were not considered wise at this time. These undescribed species are shown as *Ophryotrocha* n. sp. 1 – 11 in Figure 2 and Table 3. Uncorrected COI distances for the six newly described species and their closest relatives are summarized in Table 4.

COI could not be obtained for all specimens that were collected for this study. However, 16S was sequenced for these additional seven specimens and they were included in the concatenated analyses of five genes (Figure 3). This revealed a further four likely new species that are not described here but are referred to as *Ophryotrocha* n. sp. 12 – 15. Also, *Ophryotrocha* cf. *batillus* Wiklund et al., 2012, for which no COI was available, was also shown to be present in our samples based on 16S and other genes (Figure 3). Overall, the results suggest that there were 29 *Ophryotrocha* species found in this study, eight previously known species and 21 new species, with six new species being formally described below. Notes and images of examined material for the new undescribed species and the already known species of *Ophryotrocha* is also found below.

The concatenated alignment of COI, 16S, CytB, 18S, and H3 had 4,061 positions and included one or two terminals for each of the 29 species found in this study as well as available data from GenBank. Figure 3 shows the concatenated maximum likelihood (ML) phylogeny with bootstrap support for nodes above 50% and posterior probabilities for the Bayesian Inference analysis (BI) which was congruent in topology. Support scores less than 50% or 0.5 PP are shown with a blank. The analyses were largely congruent for the well supported nodes. *Ophryotrocha* fell into two clades, referred to here as Clade A and Clade B.

Clade A had 96/0.99 (ML/BI) support and contained terminals from three other currently accepted genera (*Exallopus*, *Pinniphitime*, and *Pseudophryotrocha*). This clade consisted of all deep-sea taxa from reducing environments with the exception of *Ophryotrocha longidentata* Josefson, 1975, which is from a non-enriched shallow water site. Fourteen of the new species fell in this clade, five of which are being formally described (Figures 2 - 3): *O. manhellafinae* n. sp., *O. scoobyi* n. sp., *O. averysea* n. sp., *O. haleyae* n. sp., and *O. tilici* n. sp. *Ophryotrocha averysea* n. sp. was found to group with sequences referred to previously as "*Ophryotrocha* Seep 4" in Thornhill et al. (2012). No COI sequences had previously been obtained for *Ophryotrocha* Seep 4 and the inclusion of 16S and CytB data allowed for this inference. The identities of two other of the undescribed species from Thornhill et al. (2012) were also established, owing to the further sequencing of terminals used in this study. *Exallopus* sp. Seep was very close to what was published as *Exallopus jumarsi* by Wiklund et al. (2012) and a specimen of this taxon of this species was also recovered in this study (but see below about the identity as *Exallopus*). *Ophryotrocha* sp. Seep 3 from Thornhill et al. (2012) grouped close to *Ophryotrocha flabella*, and three specimens collected for this study were close enough to be referred to as *O.* cf. *flabella*. Several other specimens collected for this study were placed close to previously published Clade A sequences: *O. longicollaris* and *O. nauarchus*. The deep-sea vent/seep taxon *O. platykephale*, which had not previously been sequenced, was also found in Clade A, as sister group to two of the new species, *O. manhellafinae* n. sp. and *O. haleyae* n. sp. (Figures 2 - 3).

Clade B had good support (85/0.99 ML/BI) and contained the type species for *Ophryotrocha, O. puerilis*. This clade contained a mixture of shallow water, deep water, and symbiotic *Ophryotrocha*. Several subclades that had been given informal names by previous authors were recovered and are labelled on Figure 3. These are the “gracilis/hartmanni,” “labronica,” and “lobifera” clades (Wiklund et al. 2012; Taboada et al. 2013; Salvo et al. 2014; Taboada et al. 2017). From within Clade B, our sampling recovered one specimen close to *O. batillus*, one specimen matching *O. langstrumpae*, and one specimen relatively close to *O. craigsmithi*. These formed a clade that was the well supported clade to five species of vent *Ophryotrocha* and *O. clava* Taboada et al., 2013. Seven of the new species from this study fell in this clade, with *Ophryotrocha amyae* n. sp. the only one being formally described. Clade B also contained three members of one other genus, *Iphitime*, further rendering the genus paraphyletic (Figures 2 - 3).

Haplotype Networks

New Species

Haplotype networks based on COI were constructed for the six newly described species: *Ophryotrocha amyae* n. sp., *O. averysea* n. sp., *O. haleyae* n. sp., *O. manhellafinae* n. sp., *O. scoobyi* n. sp., and *O. tilici* n. sp. (Figure 4). Networks were also constructed for the undescribed new species where more than one individual had been sequenced: *Ophryotrocha* n. sp. 9 and *O.* n. sp. 11 (Figure 5 I and G) Haplotype networks were also made for previously known species with available data for which new data was generated here. In three cases the specimens from this study were relatively distant and so are designated as *O.* cf. *flabella*, *O.* cf. *nauarchus*, and *O.* cf. *craigsmithi* respectively (Figure 5 H, C, and D). Previously known taxa for which we added new data were *Exallopus jumarsi* (but see below regarding doubts about this identity), *O. longicollaris*, *O. platykephale*, and *O. langstrumpae* (Figure 5 A, B, E, and F).

Unique haplotypes were recovered from each of the four individuals sampled for *O. haleyae* sp. nov. (Figure 4A): A2726 from Hydrate Ridge, A12021 from Rosebud whalefall, A2722 from Hydrate Ridge, and the holotype A10052 from Costa Rica. This species ranges across more than 6,000 km. Eleven individuals were sampled for *O. averysea* with nine haplotypes recovered (Figure 4B). One haplotype was found for an individual from Monterey Bay (A12028) and one from the Rosebud whalefall (A12363). Another haplotype was found for two individuals from the Rosebud whalefall (A12355 and A12026). There were six haplotypes from the six individuals from the Rosebud whalefall (A12361, A12022, A10473), one from Monterey Bay (A12025), and two from Hydrate Ridge (A2724, A2044). There was also a haplotype for the holotype from the Pedro whalefall (A12023). *Ophryotrocha tilici* sp. nov. (Figure 4C) showed six haplotypes from the eight individuals sampled in this study. The two individuals from the Rosebud whalefall (A12011, A12012) had different haplotypes as were the single individuals sequenced from Hydrate Ridge (A2723) and Fossil Hill (A12014). Costa Rica showed two haplotypes for the four individuals sequenced, including sampled the holotype (A10113, A1425, A1411, A10084). This species also ranges across more than 6,000 km. For *O. scoobyi* sp. nov. (Figure 4D), six haplotypes were recovered from the seven individuals sampled, all from the Rosebud whalefall. Five of these were represented by a singleton, including the holotype (A12356, A12359, A12017, A12018, A12019) and the sixth haplotype was represented by two individuals (A12360 and

A12358). *O. manhellafinae* sp. nov. showed two haplotypes from the two individuals sampled (Figure 4E), the holotype from Santa Monica Mound (A12334) and one from Costa Rica (A10114). Two haplotypes were recovered from the three individuals sampled for *O. amyae* sp. nov. (Figure 4F). One haplotype represented by the holotype from Hydrate Ridge (A2720) and the second haplotype consisted of two individuals, both from Monterey Bay (A12029 and A12030).

Ophryotrocha n. sp. 9 showed four haplotypes from the four individuals sequenced (Figure 5I). Each haplotype is represented by a single individual: A12007 and A12010 from Rosebud whalefall and A12009 and A12008 from Del Mar Seeps. *Ophryotrocha* n. sp. 11 recovered two haplotypes from the two individuals sampled (Figure 5G), A12597 and A12598 from Monterey Bay.

Previously Known Species

Exallopus jumarsi was originally described from sedimented hydrothermal mounds in the Guaymas Basin, Mexico (Blake, 1985). Here we found one specimen from Hydrate Ridge that was only a few bases different from a sequence published as *E. jumarsi* by Wiklund et al. (2012) from a California whalefall (Figure 5A). *Exallopus* sp. Seep from Thornhill et al. (2012) also fell with these sequences in the concatenated analysis (Figure 3), but no COI sequence was available to include it with the haplotype analysis. Examination of the voucher specimen of the Hydrate Ridge specimen (SIO-BIC A2663) showed that it lacked the distinctive anterior chaetae for *Exallopus*. This, in addition to the distance and difference in habitat from the type locality, raises doubts about the identification by Wiklund et al. (2012).

Ophryotrocha longicollaris was originally described from a whalefall in the San Clemente Basin, California 1960 m depth and a single COI sequence was published (Wiklund et al. 2012). Two specimens of *O. longicollaris* were collected here from two other California whalefalls at much shallower depths. Two haplotypes were recovered from these individuals, one from Monterey Bay and the other from the whalefall off San Diego (Rosebud) and they were at most two bases divergent from the Wiklund et al. (2012) sequence (Figure 5B).

Ophryotrocha nauarchus was described from a whalefall in the Santa Catalina Basin, California at 1240 m depth with additional accounts at various whalefalls along Southern California (Wiklund et al. 2012). One specimen was found from the Rosebud whalefall in Southern California (A12031) with a COI sequence that was 5% (29 bases) divergent from the *O. nauarchus* sequence published by Wiklund et al. (2012). Given this level of divergence, the new specimen is referred to as *O.* cf. *nauarchus* (Figure 5C).

Ophryotrocha craigsmithi was described from whale bones in the North Atlantic at 125 m depth (Wiklund et al. 2009). A specimen from a deep water whalefall in Monterey Bay, CA (A12033) showed a 3% (21 bases) COI sequence difference from the Wiklund et al. (2009) *O. craigsmithi* sequence (Figure 5D) and is referred to here as *O.* cf. *craigsmithi*.

Ophryotrocha platykephale was originally described from sedimented hydrothermal vents in the Guaymas Basin, Mexico, at 2,000 m depth (Blake, 1985). There have been no previously published sequences for *O. platykephale*. Two specimens with similar morphology to Blake's *O. platykephale* were collected at

Guaymas methane seeps, only 68 km from the type locality, and other individuals were collected from methane seeps in Costa Rica and California. Two haplotypes were recovered from the three individuals sequenced (Figure 5E). One haplotype was represented by a single individual from Costa Rica (A9878). The second haplotype is represented by two individuals, one from Del Mar Seeps (A12020) and one from Guaymas seeps (A3260).

Ophryotrocha langstrumpae was originally described from a wood parcel deployed in the Santa Cruz Basin, California at 1,672 m depth (Wiklund et al. 2012). One specimen of *O. langstrumpae* was found at the Hydrate Ridge seeps (A2728) at a much shallower depth. There are two mutations between *O. langstrumpae* from Wiklund et al. (2012) and the one in this study (Figure 5F).

Ophryotrocha flabella was originally described from a whalefall in the Santa Cruz Basin, California at 1,675 m depth (Wiklund et al. 2012). Three specimens were found from methane seeps in Costa Rica that were 4.3% divergent (23 bases) from *O. flabella*, and there are referred to here as *O.* cf. *flabella*. Two haplotypes were recovered from the three individuals sampled in this study (Figure 5H) from Costa Rica.

DISCUSSION

Biogeography and Sympatric Species

Dorvilleidae has been propounded as a key annelid group, able to exploit extreme, yet energy-rich, habitats in the deep sea (Thornhill et al. 2012; Levin et al. 2013; Smith et al. 2015). *Ophryotrocha* are also well-known for their cryptic species complexes that have begun to be resolved in recent years (Hoisaeter and Samuelsen 2006; Paxton and Åkesson 2007; Wiklund et al. 2009; Wiklund et al. 2012; Nygren et al. 2014; Taboada et al. 2017). Historically, reproductive studies and morphology have been used to distinguish among *Ophryotrocha* species (Åkesson and Paxton 2005; Paxton and Åkesson 2007; Paxton and Åkesson 2010). With the emergence of DNA sequencing, it has become easier to accurately delineate species.

The results from this study provide further support for both these points and have shown that the diversity in the eastern Pacific is actually even greater than previously reported (Thornhill et al. 2012; Wiklund et al. 2012). As shown by for seeps of California and Oregon by Thornhill et al. (2012), multiple *Ophryotrocha* species can simultaneously inhabit the same site. For example, four of the six newly described species in this study inhabit the Rosebud whalefall in Southern California, as well as five of the 15 undescribed species and one previously known species, for a total of 10 *Ophryotrocha* species sharing one sitet. The sampling was however over a period of some years. It has also been suggested that *Ophryotrocha* are early successional species and are competitively displaced over time (Fournier and Conlan 1994; Mullineaux et al. 2003; Conlan and Kvitek 2005; Lee et al. 2006). There is also evidence of “seasonality” among *Ophryotrocha*, with species exhibiting different abundances throughout the year (Prevedilli et al. 2005). However, some species, such as *O.* "*platykephale*", can be seen at stable populations year-round (Levin et al. 2003). It is possible that the sympatric *Ophryotrocha* species discovered in this study could have shown succession. The sporadic and haphazard sampling does not allow for further assessment of this.

There were several examples documented here of *Ophryotrocha* species spanning large distances, with *O. haleyae* sp. nov. and *O. tilici* sp. nov. found from Oregon, USA to Costa Rica – over 6,000 km and inhabiting both seeps and whalefalls. A series of other taxa in this study also were found at both whalefalls and seeps (Figures 4 - 5). *Ophryotrocha platykephale* was originally described from hydrothermal vents of the Guaymas Basin, Mexico (Blake, 1985), however in this study it was found at Guaymas methane seeps, just 68 km from the type locality. It was also recorded at methane seeps of Costa Rica and California (Figure 5). This data confirms *O. platykephale* as one of the few animals that can live at both methane seeps and hydrothermal vents. It has been proposed that whalefalls act as habitat islands, or "stepping-stones," among methane seeps and hydrothermal vents, due to these environments sharing several of the same species (Smith and Baco 2003; Smith et al. 2015). This study provides further evidence of the stepping-stone phenomenon, as seen in annelids such as *Amphisamytha* and *Archinome* in the eastern Pacific (Borda et al. 2013; Stiller et al. 2013).

Phylogeny and Paraphyly

The phylogenetic results of this study (Figure 3) recovered two major well-supported clades within *Ophryotrocha*, referred to here as clades A and B. Both these clades contained terminals that are listed on GenBank under other genus names, *Exallopus, Iphitime, Pinniphitime*, and *Pseudophryotrocha* (Figures 2 - 3), thus making *Ophryotrocha* paraphyletic. Wiklund et al. (2009) previously made *Palpiphitime* Orensanz, 1990 a junior synonym of *Ophryotrocha* based on having sequence data for the type species *Palpiphitime lobifera* and finding it nested within *Ophryotrocha*. The paraphyly of *Ophryotrocha* shown here has also been found in previous studies (Thornhill et al. 2012; Wiklund et al. 2012; Taboada et al. 2017), but DNA sequences for the type species for the various genera involved in the situation cannot be convincingly resolved. Also, the identity of sequences referred to as *Exallopus* (Thornhill et al. 2012; Wiklund et al. 2012) that match closely to those from a specimen in this study that does not appear to be *Exallopus* (Figure 23D). This suggests that care must be taken with careful examination of voucher material before decisions about synonymy are made.

Ophryotrocha puerilis has a sub-species associated with it, *Ophryotrocha puerilis siberti* (McIntosh, 1885). This was proposed by Bacci and La Greca (1953), who found *Ophryotrocha* from two sites in European waters, off Naples and Plymouth, that looked similar. The Plymouth worms seemed to match the description of *Staurocephalus siberti* McIntosh, 1885, while also seemingly being closely related to *Ophryotrocha puerilis*, both having the same number of chromosomes. While it was recognized that these two were separate taxa, they proposed a trinomial nomenclature, and this has been commonly used. From the results here (Figures 2 - 3) and previously Taboada et al.

(2017), it is evident that *O. puerilis* and *O. puerilis siberiti* can be regarded as different species, with COI sequence difference between the two taxa at ~20%. This is well above the species delineation distance seen for other *Ophryotrocha*. They therefore should be treated as *Ophryotrocha puerilis* and *Ophryotrocha siberti* respectively.

SYSTEMATICS

Ophryotrocha tilici sp. nov.

Figures 6 - 8

Material Examined

Holotype**:** SIO-BIC A1425 (GenBank COI Sequence MT435563) from Jaco Summit, Costa Rica (9.1723°N, 84.7987°W), HOV *Alvin* Dive 4510, 745 m depth, 4 March 2009.

Paratypes: SIO-BIC A10084 (GenBank COI Sequence MT435566) from The Thumb, Costa Rica (9.0489°N, 84.3938°W), ROV *SuBastian* Dive 217, 1072 m depth, 10 January 2019. SIO-BIC A1411 (GenBank COI Sequence MT435564) from Quepos Plateau, Costa Rica (9.1172°N, 84.8417°W), HOV *Alvin* Dive 4509,1866 m depth, 3 March 2009. SIO-BIC A10113 (GenBank COI Sequence MT435562) from Rio Bongo Scar, Costa Rica (9.2939°N, 85.287°W), ROV *SuBastian* Dive 219, 609 m depth, 13 January 2019. SIO-BIC A12011 (GenBank COI Sequence MT435559) and SIO-BIC A12012 (GenBank COI Sequence MT435560) from Rosebud whale-fall, California, USA (32.7768°N, 117.4883°W), ROV *Doc Ricketts* Dive 623, 850 m depth, 20 June 2014. SIO-BIC A12014 (GenBank COI Sequence MT435565) from Fossil Hill, Santa Monica Basin, California, USA (33.749°N, 119.053°W), ROV *Doc Ricketts* Dive 628, 650 m depth, 23 June 2014. SIO-BIC A2723 (GenBank COI Sequence MT435561) from Hydrate Ridge, Oregon, USA (44.6695°N, 125.0981°W) ROV *Jason II* Dive 593, 587-610 m depth, 3 September 2011.

Description

Body widest anterior, tapering gradually until posterior (Fig 6A). Yellow - orange in life (Fig 6A, 7A-D, 8). Eyes absent. Fusiform antennae inserted dorsally (Fig 6A, 6C). Without conspicuous palps (Fig 6C). Segmental ciliation (Fig 6C). Jaws remarkably small. Mandible anterior cutting edge with uniform teeth (Fig 6F). Cutting plate sub-triangular (Fig 6F). Maxillae L-shaped (Fig 6F). Parapodia uniramous (Fig 6B). Simple supra- and sub-acicular chaetae (Fig 6B, 6D, 6E). Pygidium with terminal anus. One pair of short pygidial cirri, also with a longer unpaired appendage inserted ventrally (Fig 6A, 7A).

Distribution and Ecology

This species occurs from Hydrate Ridge, Oregon, USA to Costa Rica. It is found at organic falls and methane seeps.

Etymology

Ophryotrocha tilici n. sp. is named for a previous Rouse Lab post-doc, Ekin Tilic, in recognition of his salient work with annelids.

Remarks

Ophryotrocha tilici sp. nov. was most closely related to *Ophryotrocha* new species 8 from this study. It also fell in a clade with *Ophryotrocha* n. sp. 9 of this study, and the genera *Pinniphitime* and *Pseudophryotrocha*. *Ophryotrocha tilici* sp. nov.

possesses characteristics similar to both *Pinniphitime* and *Pseudophryotrocha*, including inconspicuous palps, reduced jaws, and segmental ciliation. *O. tilici* n. sp. has remarkably small jaws. Of the nominal new species, *O. tilici* n. sp. is the only one to have fusiform antennae and lacks palps. *Ophryotrocha tilici* n. sp. has an unpaired pygidial appendage, which can also be seen in *Ophryotrocha eutrophila* Wiklund, 2009 and *Ophryotrocha flabella* Wiklund 2012, however, the appendage for *O. tilici* n. sp. is longer than the paired pygidial cirri, whereas for *O. eutrophila* and *O. flabella* it is shorter. Unlike most *Ophryotrocha* spp., both supra- and sub-acicular chaetae are simple. *Ophryotrocha tilici* n. sp. was found in dense aggregations at methane seeps (Fig 8).

Ophryotrocha tilici n. sp. fell in a clade with *Ophryotrocha* n. sp. 8, *Ophryotrocha* n. sp. 9, and *Pinniphitime* sp. Seep (Thornhill et al. 2012). *Pinniphitime* was erectedby Orensanz (1990) and to this date only contains one nominal species, *P. pinnognatha* Orensanz, 1990, from shallow waters in Antarctica. The original diagnosis of *Pinniphitime* from (Orensanz 1990) includes "prostomium without conspicuous appendages." The three new species mentioned above also lack conspicuous palps and antennae. *Pseudophryotrocha* sp. Seep (Thornhill et al. 2012) is the next closest relative to this clade. *Pseudophryotrocha* was erected in 1991 by Hilbig & Blake and to this date only contains the three species from that study of the deep water US Atlantic. The diagnosis for *Pseudophryotrocha* (Blake and Hilbig 1990) includes "(...) segmental ciliation. (...)prostomium with or without two short clavate antennae; palps lacking; jaw apparatus greatly reduced." The three new species above also possess some, if not all, of these characteristics. *Ophryotrocha tilici* sp. nov. does not clearly fall into either of these genera. It is difficult to accurately place *O. tilici* sp. nov. without observation of the type species for *Pseudophryotrocha* and *Pinniphitime* and these also lack DNA data.

Ophryotrocha scoobyi sp. nov.

Figures 9 - 10

Material Examined

Holotype: SIO-BIC A12017 (GenBank COI Sequence MT435571) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Doc Ricketts* Dive 471, 850 m depth, 18 May 2013.

Paratypes: SIO-BIC A12016, SIO-BIC A12018 (GenBank COI Sequence MT435572), and SIO-BIC A12019 (GenBank COI Sequence MT435573) from the same location and dive as holotype. SIO-BIC A12356 (GenBank COI Sequence MT435567), SIO-BIC A12359(GenBank COI Sequence MT435568), SIO-BIC A12360 (GenBank COI Sequence MT435569), and SIO-BIC A12358 (GenBank COI Sequence MT435570) from the same location as holotype, ROV *Doc Ricketts* Dive 1253, 850 m depth, 9 February 2020.

Description

Body widest anterior tapering to end (Fig 9A). Blue - green markings just below head in life (Fig 9A, 10A-B). Eyes absent. Simple digitiform antennae inserted dorsally (Fig 9A). Biarticulate palps inserted laterally, palpophores globular, palpostyles digitiform (Fig 9A). Maxillae P-type, forceps with more than 20 irregular teeth and larger fang on anterior end (Fig 9F). Seven pairs of free denticles. D1-D5 longer than wide, D6-D7 wider sub-equal with long (Fig 9F). Parapodia uniramous with stub-like dorsal cirri (Fig 9B). Simple supra-acicular chaetae (Fig 9C, 9D). Compound sub-acicular chaetae (Fig 9C - E). Small dorsal lateral lobes on chaetigers (Fig 9A). Bulbous pygidium with terminal anus (Fig 9A). One pair pygidial cirri, twice as long as antennae (Fig 9A).

Distribution and Ecology

This species is only known from the Rosebud whale-fall in Southern California.

Etymology

Ophryotrocha scoobyi sp. nov. is named for the fictional cartoon character Scooby-Doo, as the blue markings behind its head are reminiscent of Scooby’s blue collar.

Remarks

Ophryotrocha scoobyi n. sp. was most closely related to *Ophryotrocha* new species 2 from this study. Both of these species were only found at whale-falls and have similar morphology though there is distinctive blue-green markings below the head on *O. scoobyi* sp. nov.

Ophryotrocha haleyae sp. nov.

Figures 11 - 12

Material Examined

Holotype: SIO-BIC A10052 (GenBank COI Sequence MT435579) from Jaco Summit, Costa Rica (9.1734°N, 84.8038°W), ROV *SuBastian* Dive 213, 730-820 m depth, 6 January 2019.

Paratypes: SIO-BIC A2726 (GenBank COI Sequence MT435574) from Hydrate Ridge, Oregon, USA (44.67°N, 125.098°W), ROV *Jason II* Dive 593, 587-610 m depth, 5 September 2011. SIO-BIC A2722 (GenBank COI Sequence MT435577) from Hydrate Ridge, Oregon, USA (44.67°N, 84.8038°W), ROV *Jason II* Dive 593, 587-610 m depth, 3 September 2011. SIO-BIC A12021 (GenBank COI Sequence MT435576) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Hercules* Cruise NA066, 850 m depth, 1 August 2015.

Description

Body uniform until posterior, gradually tapering to end (Fig 11A). Yellow in life (Fig 11A). Eyes absent. Digitiform antennae inserted dorsally (Fig 12A). Bi-articulate palps inserted laterally, palpophores globular, palpostyles digitiform (Fig 12A). Segmental ciliation (Fig 12B). Mandibles rod like with smooth anterior cutting edge (Fig 12C). Maxillae K-type with long smooth forceps (Fig 12C). Seven pairs of free denticles, D1-D3 longer than wide, D4-D7 wider sub-equal with long with short comb-like teeth (Fig12D, 12E). Small dorsal lateral lobes (Fig 11A, 11D, 12A, 12B). Uniramous parapodium with long digitiform dorsal cirri (Fig 11D). Simple supra-acicular chaetae (Fig 11C, 11F, 11G). Compound sub-acicular chaetae (Fig 11C, 11E). Bulbous pygidium with terminal anus (Fig 11A). One pair pygidial cirri, half as long as antennae (Fig 11A).

Distribution and Ecology

This species occurs from Hydrate Ridge, Oregon, USA to Costa Rica. It is found at methane seeps and organic falls.

Etymology

This species is named for Haley Hoek, the granddaughter of long-time collaborator Bob Vrijenhoek.

Remarks

Ophryotrocha haleyae n. sp. is most closely related to *Ophryotrocha manhellafinae* n. sp. The two have similar morphology with the exceptions of the dorsal parapodial projections being shorter in *O. haleyae* n. sp. and the jaw forceps of *O. haleyae* n. sp. are smooth with no fangs. The numerous chaetae per parapodium gives *O. haleyae* n. sp. a “hair-like” appearance. This species also fell in a clade with *O. platykephale*, but shows some morphological differences. The body length of *O haleyae* n. sp. is much shorter, the antennae and palps are longer. It seems that the three *Ophryotrocha* spp. in this clade all possess a dorsal parapodial projection, giving a “thumbs up” appearance.

Ophryotrocha averysea sp. nov.

Figures 13 - 14

Material Examined

Holotype: SIO-BIC A12023 (GenBank COI Sequence MT435584) from Pedro whale-fall near Channel Islands, California, USA (33.7704°N, 119.5241°W), ROV *Doc Ricketts* Dive 474, 1893 m depth, 20 May 2013.

Paratypes: SIO-BIC A12361 (GenBank COI Sequence MT435580), SIO-BIC A12355 (GenBank COI Sequence MT435581), SIO-BIC A12363 (GenBank COI Sequence MT435582) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Doc Ricketts* Dive 1253, 850 m depth, 9 February 2020. SIO-BIC A12022 (GenBank COI Sequence MT435583) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Doc Ricketts* Dive 623, 850 m depth, 20 June 2014. SIO-BIC A12025 (GenBank COI Sequence MT435586) from LuSeal seal fall, Monterey Bay, California, USA (36.7721°N, 122.0831°W), ROV *Doc Ricketts* Dive 93, 600 m depth, 16 November 2009. SIO-BIC A12026 (GenBank COI Sequence MT435587) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Hercules* Cruise NA066, 850 m depth, 1 August 2015. SIO-BIC A12028 (GenBank COI Sequence MT435588) from Pebbles whale-fall, Monterey Submarine Canyon, Monterey Bay, California, USA (36.803°N, 121.9946°W), ROV *Doc Ricketts* Dive 205, 633 m depth, 26 October 2010. (SIO-BIC A2044) from Hydrate Ridge, Oregon, USA (44.6698°N, 125.099°W), HOV *Alvin* Dive 4634, 603 m depth, 6 August 2010. SIO-BIC A2724

(GenBank COI Sequence MT435591) from Hydrate Ridge, Oregon, USA (44.67°N, 125.098°W), ROV *Jason II* Dive 593, 587 - 610 m depth, 5 September 2011. SIO-BIC A10473 (GenBank COI Sequence MT435590) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *SuBastian* Dive 176, 850 m depth, 19 October 2018.

Description

Body uniform with rounded posterior (Fig 13A). White - blue in life (Fig 13A). Eyes absent. Digitiform antennae inserted dorsally (Fig 14A). Biarticulate palps inserted laterally, palpophores globular, palpostyles digitiform (Fig 14A). Maxillae with seven pairs of free denticles (Fig 13F). Forceps with more than 30 irregular teeth with intermittent fangs. D1-D4 longer than wide with periodic large fangs. D5-D7, wider than long (Fig 13F). Parapodium uniramous with stub-like dorsal cirri (Fig 13B, 13C). Simple supra-acicular chaetae with rounded oar-like tip (Fig 13D). Compound sub-acicular chaetae with pointed tip (Fig 13E). Small dorsal lateral lobes on chaetigers (Fig 14A). Pygidium with terminal anus. One pair pygidial cirri, slightly shorter than antennae (Fig 13A).

Distribution and Ecology

This species occurs from Hydrate Ridge, Oregon, USA to the Rosebud whale-fall in Southern California, USA. It is found at methane seeps and organic falls.

Etymology

This species is named after Avery Hatch in recognition of all her hard work for the Rouse Lab.

Remarks

Ophryotrocha averysea n. sp. is most closely related to *Ophryotrocha* sp. vent 5 from Zhang et al. (in prep). The DNA sequences from this species matched those from *Ophryotrocha* sp. Seep 4 from Thornhill et al. 2012. *Ophryotrocha averysea* n. sp. has a unique blue-white hue in live specimens. The body of *O. averysea* n. sp. is uniform laterally, giving it a “straight-line” and “neat” appearance. The jaws of this species are unique with the irregular placement of fangs on the forceps.

Ophryotrocha amyae sp. nov.

Figures 15 - 16

Material Examined

Holotype: SIO-BIC A2720 (GenBank COI Sequence MT435592) from Hydrate Ridge, Oregon, USA (44.669°N, 125.098°W), ROV *Jason II* Dive 593, 700 m depth, 2 September 2011.

Paratypes: SIO-BIC A12029 (GenBank COI Sequence MT435593) from LuSeal seal fall, Monterey Bay, California, USA (36.7721°N, 122.0831°W), ROV *Doc Ricketts* Dive 93, 600 m depth, 16 November 2009. SIO-BIC A12030 (GenBank COI Sequence MT435594) from Pebbles whale-fall, Monterey Submarine Canyon, Monterey Bay, California, USA (36.803°N, 121.9946°W), ROV *Doc Ricketts* Dive 205, 633 m depth, 26 October 2010. SIO-BIC A12600 from Francisco whale, Monterey Submarine Canyon, Monterey Bay, California, USA (36.7721°N, 122.0831°W), ROV *Doc Ricketts* Dive 95, 1018 m depth, 17 November 2009.

Description

Body widest in middle (Fig 15A). Red anterior and yellow posterior in life (Fig 16A). Eyes absent. Biarticulate antennae inserted dorsally (Fig 16B). Biarticulate palps inserted laterally, palpophores globular, palpostyles digitiform (Fig16B). Mandible rod-like, anterior cutting edge with more than 15 uniform teeth (Fig 15D, 15G, 15H). Large wide fang inside (Fig 15H). Maxillae K-type with long smooth forceps (Fig 15I). Seven free denticles attached by ligament strut to forceps (Fig 15I). Denticles wider than long with alternating small and large fangs (Fig 15I). Parapodium biramous with dorsal and ventral cirri and acicular lobe (Fig15C, 16D, 16E). Simple supra-acicular chaetae (Fig 15E). Compound sub-acicular chaetae with rounded blunt tip (Fig 15F, 16F). Prominent dorsal and ventral lateral lobes on chaetigers (Fig16A, 16B, 16D, 16E). Bulbous pygidium with terminal anus (Fig 16A). One pair pygidial cirri, twice as long as antennae (Fig 16A).

Distribution and Ecology

This species occurred from Hydrate Ridge, Oregon to central California, USA. It was found at methane seeps and organic falls

Etymology

This species is named after Amy Halseth.

Remarks

Ophryotrocha amyae n. sp. is the only new nominal species that falls in Clade B, being most closely related to a clade containing *Ophryotrocha shieldsi* Paxton and Davey, 2010 and *Ophryotrocha lipovskyae* (Paxton, 2009), both from shallow water organic falls beneath salmon-farm cages in Australia and Canada respectively. These three *Ophryotrocha* taxa possess conspicuous dorsal and ventral lateral lobes on the chaetigers and dorsal and ventral cirri and acicular lobes on the parapodia giving them the appearance of having hands or claws, as well as highly sclerotized mandibles. *Ophryotrocha amyae* n. sp. fell in the “lobifera” clade. Members of this clade all share the dorsal and ventral lateral lobes on the chaetigers and possess dorsal and ventral cirri and acicular lobes on the parapodia. *Ophryotrocha amyae* n. sp. has a unique red-yellow hue in life that makes it stand out from other *Ophryotrocha* spp. *Ophryotrocha amyae* n. sp. also has well defined jaws, easily seen in the live specimens (Fig 16C). The sub-acicular compound chaetae of *O. amyae* n. sp. differs from the other new nominal species in that the tips are more rounded as opposed to a sharp point.

Ophryotrocha manhellafinae sp. nov.

Figures 17 – 18

Material Examined

Holotype: SIO-BIC A12334 (GenBank COI Sequence MT435595) from Santa Monica Mound, California, USA (33.7994°N, 118.6463°W), ROV *Doc Ricketts* Dive 1252, 789 - 807 m depth, 8 February 2020.

Paratype: SIO-BIC A10114 (GenBank COI Sequence MT435596) from Rio Bongo Scar, Costa Rica (9.2862°N, 85.2757°W), ROV *SuBastian* Dive 219, 480 - 650 m depth, 13 January 2019.

Description

Body uniform until posterior, tapering slightly (Fig 17A, 17B). Pink - red in life (Fig 17A, 17B). Eyes absent. Digitiform antennae inserted dorsally (Fig 18A). Biarticulate palps inserted laterally, same length as antennae, palpophores globular, palpostyles digitiform (Fig 18C). Segmental ciliation (Fig18A, 18C). Maxillae with seven free denticles (Fig18B). Forceps with patterned small teeth and larger fang (Fig 18B). Denticles longer than wide (Fig 18B). Long dorsal and ventral digitiform lateral lobes (Fig 17A, 17B, 17E, 18A, 18C, 18E). Slight biramous parapodium with long digitiform dorsal cirri (Fig 17E, 17F, 18D). Simple supra-acicular chaetae (Fig 17C). Compound sub-acicular chaetae (Fig 17D). Bulbous pygidium with terminal anus (Fig 18E). One pair pygidial cirri, same length as antennae (Fig 18A).

Distribution and Ecology

This species occurred from Southern California, USA to Costa Rica. It was only found from methane seeps.

Etymology

This species is named after Manuela Vazquez, using a play-on-words with a nickname “manhella fine.”

Remarks

The closest relative of *Ophryotrocha manhellafinae* n. sp. was *Ophryotrocha haleyae* n. sp. The morphology is similar with the exception of the fingerlike dorsal parapodial projections, which are longer than those of *O. haleyae* and the irregular fangs on the jaw forceps, unlike those of *O. haleyae* n. sp., which are smooth. The jaws of *O. manhellafinae* n. sp. are also unique, with patterned fangs. *Ophryotrocha manhellafinae* also fell in a clade with *O. platykephale* and has some morphological differences. The length of *O. manhellafinae* n. sp. is much shorter, while the antennae and palps are longer. However, *O. manhellafinae* n. sp. and *O. platykephale* have similar dorsal and ventral lobes and parapodial projections. It seems that the three *Ophryotrocha* spp. in this clade all possess a dorsal parapodial projection, giving a “thumbs up” appearance.

Appendix

Other Material Examined

Ophryotrocha n. sp. 1

Figure 19A

Material examined: (SIO-BIC A10320) from Point Dume, California, USA (33.9417°N, 118.8445°W) ROV *SuBastian* Dive 163, 729 m depth, 8 October 2018. (SIO-BIC A10411) from the same location as previous, ROV *SuBastian* Dive 164, 709 - 719 m depth, 9 October 2018.

Ophryotrocha n. sp. 2

Figure 19B

Material examined: (SIO-BIC A12015) from Pebbles whale-fall, Monterey Submarine Canyon, Monterey Bay, California, USA (36.803°N, 121.9946°W), ROV *Doc Ricketts* Dive 207, 633 m depth, 27 October 2010.

Ophryotrocha n. sp. 3

Figure 19C

Material examined: (SIO-BIC A12599) from Francisco whale-fall, Monterey Submarine Canyon, Monterey Bay, California, USA (36.772°N, 121.995°W), 1000 m depth.

Ophryotrocha n. sp. 4

Material examined: (SIO-BIC A10334) from Point Dume, California, USA (33.941°N, 118.8448°W), ROV *SuBastian* Dive 164, 725 m depth, 9 October 2018.

Ophryotrocha n. sp. 5

Figure 19D

Material examined: (SIO-BIC A12297) from Del Mar Seeps, California, USA (32.9047°N, 117.7819°W) ROV *Doc Ricketts* Dive 1246, 1018 - 1023 m depth, 5 February 2020.

Ophryotrocha n. sp. 6

Material examined: (SIO-BIC A9800) from Mound 12, Costa Rica (8.9304°N, 84.3126°W), HOV *Alvin* Dive 4978, 999 m depth, 24 October 2018.

Ophryotrocha n. sp. 7

Figure 20A

Material examined: (SIO-BIC A2719) from Hydrate Ridge, Oregon, USA (44.67°N, 125.098°W), ROV *Jason II* Dive 593, 587 - 610 m depth, 1 September 2011.

Ophryotrocha n. sp. 8

Figure 20B

Material examined: (SIO-BIC A12340) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Doc Ricketts* Dive 1253, 850 m depth, 9 February 2020.

Ophryotrocha n. sp. 9

Figure 20C

Material examined: (SIO-BIC A12007 and SIO-BIC A12010) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W) ROV *Doc Ricketts* Dive 471, 850 m depth, 18 May 2013. (SIO-BIC A12008 and SIO-BIC A12009) from Del Mar Seeps, California, USA (32.9042°N, 117.7823°W), ROV *Doc Ricketts* Dive 472, 1020 - 1036 m depth, 19 May 2013. (SIO-BIC A12024) from Del Mar Seeps, California, USA (32.9042°N, 117.7823°W), ROV *Doc Ricketts* Dive 472, 1020 - 1036 m depth, 19 May 2013.

Ophryotrocha n. sp. 10

Material examined: (SIO-BIC A9823) from Quepos Plateau, Costa Rica (8.5855°N, 84.5484°W), HOV *Alvin* Dive 4980, 2184 m depth, 26 October 2018.

Ophryotrocha n. sp. 11

Material examined: (SIO-BIC A12597 and SIO-BIC A12598) from a whale-fall, Davidson Seamount, Monterey Bay, California, USA (35.7°N, 122.7°W) ROV *Hercules* Cruise NA117, 3240 m depth, 16 October 2019.

Ophryotrocha n. sp. 12

Figure 20D

Material examined: (SIO-BIC A12296) from Del Mar Seeps, California, USA (32.9047°N, 117.7819°W), ROV *Doc Ricketts* Dive 1246, 1018 - 1023 m depth, 5 February 2020. (SIO-BIC A2727) from Hydrate Ridge, Oregon, USA (44.67°N, 125.098°W), ROV *Jason II* Dive 593, 587-610 m depth, 5 September 2011.

Ophryotrocha n. sp. 13

Material examined: (SIO-BIC A10482) from Rosebud whale-fall, California, USA (32.7769*N, 117.4881°W), ROV *SuBastian* Dive 176, 850 m depth, 19 October 2018.

Ophryotrocha n. sp. 14

Material examined: (SIO-BIC A10468, SIO-BIC A10475, SIO-BIC A10479, and SIO-BIC A10480) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *SuBastian* Dive 176, 850 m depth, 19 October 2018.

Ophryotrocha n. sp. 15

Material examined: (SIO-BIC A12601) from LuSeal seal fall, Monterey Bay, California, USA (36.7721°N, 122.0831°W), ROV *Doc Ricketts* Dive 93, 600 m depth, 16 November 2009.

Ophryotrocha platykephale Blake, 1985:

Figure 21

Material examined: (SIO-BIC A9878) from Parrita Seep, Costa Rica (9.03°N, 84.62°W), HOV *Alvin* Dive 4990, 1435 m depth, 5 November 2018. (SIO-BIC A3262) from Pinkie's "Vent", Guaymas Basin, Mexico (27.5969°N, 111.487°W), ROV *Doc Ricketts* Dive 380, 1572 - 1583 m depth, 10 April 2012. (SIO-BIC A12020) from Del Mar Seeps, California, USA (32.9042°N, 117.7823°W), ROV *Doc Ricketts* Dive 472, 1020 - 1036 m depth, 19 May 2013. (SIO-BIC A3260) from "Pinkie's Vent", Guaymas Basin, Mexico (27.591°N, 111.4749°W), ROV *Doc Ricketts* Dive 379, 1581 m depth, 10 April 2012.

Ophryotrocha longicollaris Wiklund, 2012:

Figure 22B

Material examined: (SIO-BIC A12032) from Pebbles whale-fall, Monterey Submarine Canyon, Monterey Bay, California, USA (36.803°N, 121.9946°W), ROV *Doc Ricketts* Dive 207, 633 m depth, 27 October 2010. (SIO-BIC A10483) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *SuBastian* Dive 176, 850 m depth, 19 October 2018.

Ophryotrocha langstrumpae Wiklund, 2012:

Figure 22A

Material examined: (SIO-BIC A2728) from Hydrate Ridge, Oregon, USA (44.6701°N, 125.0987°W), ROV *Jason II* Dive 593, 587 - 610 m depth, 4 September 2011.

Ophryotrocha cf. *flabella* Wiklund, 2012:

Figure 23A

Material examined: (SIO-BIC A1410) from Jaco Scar, Costa Rica (9.1172°N, 84.8417°W), HOV *Alvin* Dive 4509, 1866 m depth, 3 March 2009. (SIO-BIC A9928 and SIO-BIC A9929) from Mound 11, Costa Rica (8.9221°N, 84.3045°W), HOV *Alvin* Dive 4988, 1010 m depth, 3 November 2018.

Ophryotrocha cf. *nauarchus* Wiklund, 2012:

Figure 23C

Material examined: (SIO-BIC A12031) from Rosebud whale-fall, California, USA (32.7769°N, 117.4881°W), ROV *Doc Ricketts* Dive 623, 850 m depth, 20 June 2014.

Ophryotrocha cf. *batillus* Wiklund, 2012:

Figure 23B

Material examined: (SIO-BIC A9610) from Mound 12, Costa Rica (8.93°N, 84.3117°W), HOV *Alvin* Dive 4972, 992 m depth, 20 October 2018.

Exallopus cf. *jumarsi* Blake, 1985:

Figure 23D

Material examined: (SIO-BIC A2663) from Hydrate Ridge, Oregon, USA (44.67°N, 125.098°W), ROV *Jason II* Dive 593, 587 - 610 m depth, 1 September 2011.

Ophryotrocha cf. *craigsmithi* Wiklund, 2009:

Figure 22C

Material examined: (SIO-BIC A12033) from Patrick whale-fall, Monterey Bay, California, USA (36.7078°N, 122.106°W) ROV *Tiburon* Dive 1048, 1844 m depth, 20 October 2006.

Table 1: Sampling site locales from deep-sea cruises in this study.

Locale	Country	Environment	Latitude	Longitude
Hydrate Ridge	Oregon, USA	Seep	44.6695	-125.0981
Hydrate Ridge	Oregon, USA	Seep	44.4500	-125.032
Hydrate Ridge	Oregon, USA	Seep	44.6701	-125.0987
Monterey Bay (Davidson Seamount)	California, USA	Whale	35.7000	-122.7000
Monterey Bay ("Patrick")	California, USA	Whale	36.7078	-122.1060
Monterey Bay ("LuSeal")	California, USA	Seal	36.7721	-122.0831
Monterey Bay ("Pebbles")	California, USA	Whale	36.8030	-121.9946
Monterey Bay ("Francisco")	California, USA	Whale	36.7720	-121.9950
Channel Islands ("Pedro")	California, USA	Whale	33.7704	-119.5241
Fossil Hill	California, USA	Seep	33.7490	-119.0530
Santa Monica Mound	California, USA	Seep	33.7794	-118.6463
Point Dume	California, USA	Seep	33.9417	-118.8445
Del Mar Seeps	California, USA	Seep	32.9042	-117.7823
San Diego ("Rosebud")	California, USA	Whale	32.7768	-117.4883
Guaymas Basin	Mexico	Seep	27.5969	-111.4870
Rio Bongo	Costa Rica	Seep	9.2939	-85.2870
Rio Bongo	Costa Rica	Seep	9.2862	-85.2757
Jaco Summit	Costa Rica	Seep	9.1723	-84.7987
Jaco Summit	Costa Rica	Seep	9.1734	-84.8083
Jaco Scar	Costa Rica	Seep	9.1172	-84.8417
The Thumb	Costa Rica	Seep	9.0489	-84.3938
Quepos Plateau	Costa Rica	Seep	8.5855	-84.5484
Mound 11	Costa Rica	Seep	8.9221	-84.3045
Mound 12	Costa Rica	Seep	8.9300	-84.3117
Parrita Seep	Costa Rica	Seep	9.0300	-84.6200

Table 2: Primer sets used in PCR analysis in this study.

Primer	Sequence 5' – 3'	Reference
LCO 1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO 2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
CytB 424F	GGWTAYGTWYTWCCWTGRGGWCARAT	Boore and Brown (2000)
CytB-bp-876R	RAAWARRAAGTATCAYTCAGG	Oyarzun et al. (2011)
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (2000)
H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (2000)
16SarL	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACG	Palumbi (1996)
18S 1F	TACCTGGTTGATCCTGCCAGTAG	Giribet et al. (1996)
18S 5R	CTTGGCAAATGCTTTCGC	Giribet et al. (1996)
18S 9R	GATCCTTCCGCAGGTTCACCTAC	Giribet et al. (1996)
18S A2.0	ATGGTTGCAAAGCTGAAAC	Whitting et al. (1997); Whiting (2002)
18S 3F	GTTCGATTCCGGAGAGGGA	Giribet et al. (1996)
18S Bi	GAGTCTCGTTCGTTATCGGA	Whiting et al. (1997); Whiting (2002)

Table 3: Collection data and GenBank accession numbers of specimens for this study as well as terminals used in phylogenetic analysis.

Species Name	**Voucher (SIO-BIC)**	**COI**	**16S**	**H3**	**CytB**	**18S**
Dorvillea erucaeformis		AY838868	GQ478122			DQ779647
Dorvillea rubrovittata		JQ310754	GQ415457	GQ415490		
Exallopus jumarsi		JQ310755	JQ310744			
“ *E.* cf. *jumarsi*”	A2663	MT435624				
Exallopus sp. Seep haplotype 39g			JX536712		JX536752	
Iphitime cuenoti		KR004718				
Iphitime hartmanae		GQ415472	GQ415458	GQ415491		
Iphitime paguri		EF464549				
Ophryotrocha adherens		JQ310756	AF321421	JQ310768		
O. alborana		GQ415473	AF321422	GQ415492		
O. amyae sp. nov.	A2720	MT435592	MT443982	MT673725	MT673738	
	A12029	MT435593				
	A12030	MT435594				

Table 3 continued

O. averysea sp. nov.	A12361	MT435580				
	A12355	MT435581				
	A12363	MT435582				
	A12022	MT435583				
	A12023	MT435585	MT443980	MT673724		MT668980
	A12025	MT435586				
	A12026	MT435587				
	A12028	MT435588				
	A2044	MT435589				
	A10473	MT435590				
	A2724	MT435591				
O. batillus			JQ310745	JQ310769		
O. cf. *batilus*	A9610		MT444001			
O. clava		KC123177	KC123175	KC123179		

Table 3 continued

O. costlowi		JQ310757	JQ310746	JQ310770		
O. craigsmithi		GQ415474	GQ415459	GQ415493		
O. cf. *craigsmithi*	A12033	MT435627				
O. cyclops		KM979519	KM979517	KM979518		
O. diadema		JQ310758	AF321425	JQ310771		
O. eutrophila		GQ415475	GQ415460	GQ415494		
O. flabella		JQ310759	JQ310747	JQ310772		
O. cf. *flabella*	A1410	MT435616	MT443998			
	A9929	MT435617		MT673731		MT668985
	A9928	MT435618				
O. geryonicola		GQ415476	GQ415461	GQ415495		
O. globopalpata		GQ415477	GQ415462	GQ415496		
O. gracilis		EF464545	AF321424	GQ415497		

Table 3 continued

O. haleyae sp. nov.	A2726	MT435574				
	A12021	MT435576			MT673737	
	A2722	MT435577				
	A10052	MT435579		MT673723		
O. hartmanni		EF464546	AF321419	JQ310773		
O. japonica		GQ415478	GQ415463	GQ415498		
O. jiaolongi		KY906965	MF398967	MF398972		
O. labronica		GQ415479	AF321429	GQ415499		AY838855
O. langstrumpae		JQ310760	JQ310748	JQ310774		
	A2728	MT435623				
O. lipovskyae		GQ415480				
O. lobifera		GQ415481	GQ415464		GQ415500	

Table 3 continued

O. longicollaris		JQ310761	JQ310749	JQ310775		
	A12032	MT435625				
	A10483	MT435626				
O. longidentata		GQ415482	GQ415471	GQ415501		
O. lusa				KP731520		
O. macrovifera		JQ310762	AF321460	JQ310776		
O. maculate		GQ415483	GQ415465	JQ310777		
O. magnadentata		JQ310763	JQ310750	JQ310778		
O. mammilata				KP731527		
O. manhellafinae sp. nov.	A12334	MT435595	MT443983	MT673726	MT673739	MT668981
	A10114	MT435596		MT673727		

Table 3 continued

O. mediterranea		KR004790				
Ophryotrocha n. sp. 1	A10320	MT435603				MT668984
	A10411	MT435604	MT443989	MT673730		
O. n sp. 2	A12015	MT435601		MT673729		MT668983
O. n. sp. 3	A12599	MT435610				
O. n. sp. 4	A10334	MT435609	MT443994	MT673734		
O. n. sp. 5	A12297	MT435608				
O. n. sp. 6	A9800	MT435612				
O. n. sp. 7	A2719	MT435611				
O. n. sp. 8	A12340	MT435607				

Table 3 continued

O. n. sp. 9	A12007	MT435597	MT443984	MT673728		MT668982
	A12009	MT435598	MT443985			
	A12010	MT435599	MT443986			
	A12008	MT435600	MT443987			
O. n. sp. 10	A9823	MT435615				
O. n. sp. 11	A12597	MT435605				
	A12598	MT435606				
O. n. sp. 12	A12296		MT443996			
O. n. sp. 13	A10482		MT443997			

Table 3 continued

O. n. sp. 14	A10468		MT443990			
	A10479		MT443991			
	A10480		MT443992			
	A10475		MT443993			
O. n. sp. 15	A12601	MT435614				
O. nauarchus		JQ310764	JQ310751	JQ310779		
O. cf. *nauarchus*	A12031	MT435619				
O. notoglandulata		EF464542	AF321431	JQ310780		
O. obscura			AF321435			
O. orensanzi		KC123178	KC123176	KC123180		
O. permanni		GQ415484	AF321432	GQ415502		

Table 3 continued

O. platykephale	A9878	MT435620				
	A3262	MT435621	MT443999	MT673732		
	A12020	MT435622	MT44400	MT673733	MT673736	MT668986
O. puerilis		EF464544	AF321423			
O. puerilis siberti		GQ415486	GQ415467	GQ415505		
O. robusta		EF464547	AF321433	JQ310781		
O. rubra		GQ415487	GQ415468	GQ415505		
O. sadina				KP731543		

Table 3 continued

O. scoobyi sp. nov.	A12356	MT435567				
	A12359	MT435568				
	A12360	MT435569				
	A12358	MT435570				
	A12017	MT435571	MT443977	MT673722		MT668979
	A12018	MT435572				
	A12019	MT435573	MT443978			
O. scutellus		GQ415488	GQ415469	GQ415506		
O. shieldsi		HM181931	HM181932	JQ310782		
O. socialis		JQ310765	AF321420	JQ310783		

Table 3 continued

Ophryotrocha sp. Seep 1 haplotype 1a			JX536696		JX536714	
O. sp. Seep 1 haplotype 4c			JX536698		JX536717	
O. sp. Seep 2 haplotype 7d			JX536699		JX536720	
O. sp. Seep 2 haplotype 5e			JX536700		JX536718	
O. sp. Seep 3 haplotype 11f			JX536701		JX536724	
O. sp. Seep 4 haplotype 13g			JX536702		JX536726	
O. sp. Seep 4 haplotype 14h			JX536703		JX536727	
O. sp. Seep 4 haplotype 15i			JX536704		JX536728	
O. sp. Seep 5 haplotype 37p			JX536711		JX536750	
Ophryotrocha sp. vent 1	S25148					

Table 3 continued

O. sp. vent 2	S25207					
O. sp. vent 3	D00204097					
O. sp. vent 4	D00194094					
O. sp. vent 5	J2146					
O. tilici sp. nov.	A12011	MT435559	MT443971		MT673735	
	A12012	MT435560	MT443972			
	A2723	MT435561	MT443973			
	A10113	MT435562				
	A1425	MT435563	MT443974			
	A1411	MT435564				
	A12014	MT435565	MT443975			
	A10084	MT435566	MT443976	MY673721		MT668978

Table 3 continued

O. urbis		LC504277				
O. vivipara		JQ310766	JQ310752			
Pinniphitime sp. Seep haplotype 41					JX536754	
Pseudophryotrocha sp. Seep haplotype r			JX536713			

Table 4: Uncorrected interspecific COI distances for new nominal species and their closest relatives.

	O. craigsmithi	*O.lipovskyae*	*O.* n. sp. 9	*O.tilici* sp. nov.	*O.* n. sp. 2	*O. scoobyi* sp.nov.	*O. platykephale*	*O. haleyae* sp. nov.	*O. averysea* sp. nov.	*O. amyae* sp. nov.	*O. manhellafinae* sp. nov.
O.craigsmithi	-										
O. lipovskyae	0.19	-									
O. n. sp. 9	0.23	0.20	-								
O. tilici sp. nov.	0.23	0.20	0.12	-							
O. n. sp. 2	0.27	0.22	0.20	0.18	-						
O.a scoobyi sp. nov.	0.27	0.22	0.19	0.19	0.10	-					
O.platykephale	0.25	0.23	0.20	0.20	0.18	0.18	-				
O. haleyae sp. nov.	0.26	0.23	0.20	0.20	0.19	0.18	0.12	-			
O. averysea sp. nov.	0.29	0.24	0.22	0.21	0.18	0.18	0.17	0.16	-		
O. amyae sp. nov.	0.18	0.15	0.19	0.20	0.22	0.21	0.23	0.23	0.25	-	
O. manhellafinae sp. nov.	0.26	0.24	0.21	0.22	0.18	0.19	0.13	0.11	0.19	0.23	-
O. n. sp. 8	0.26	0.22	0.14	0.16	0.22	0.21	0.21	0.23	0.24	0.22	0.23

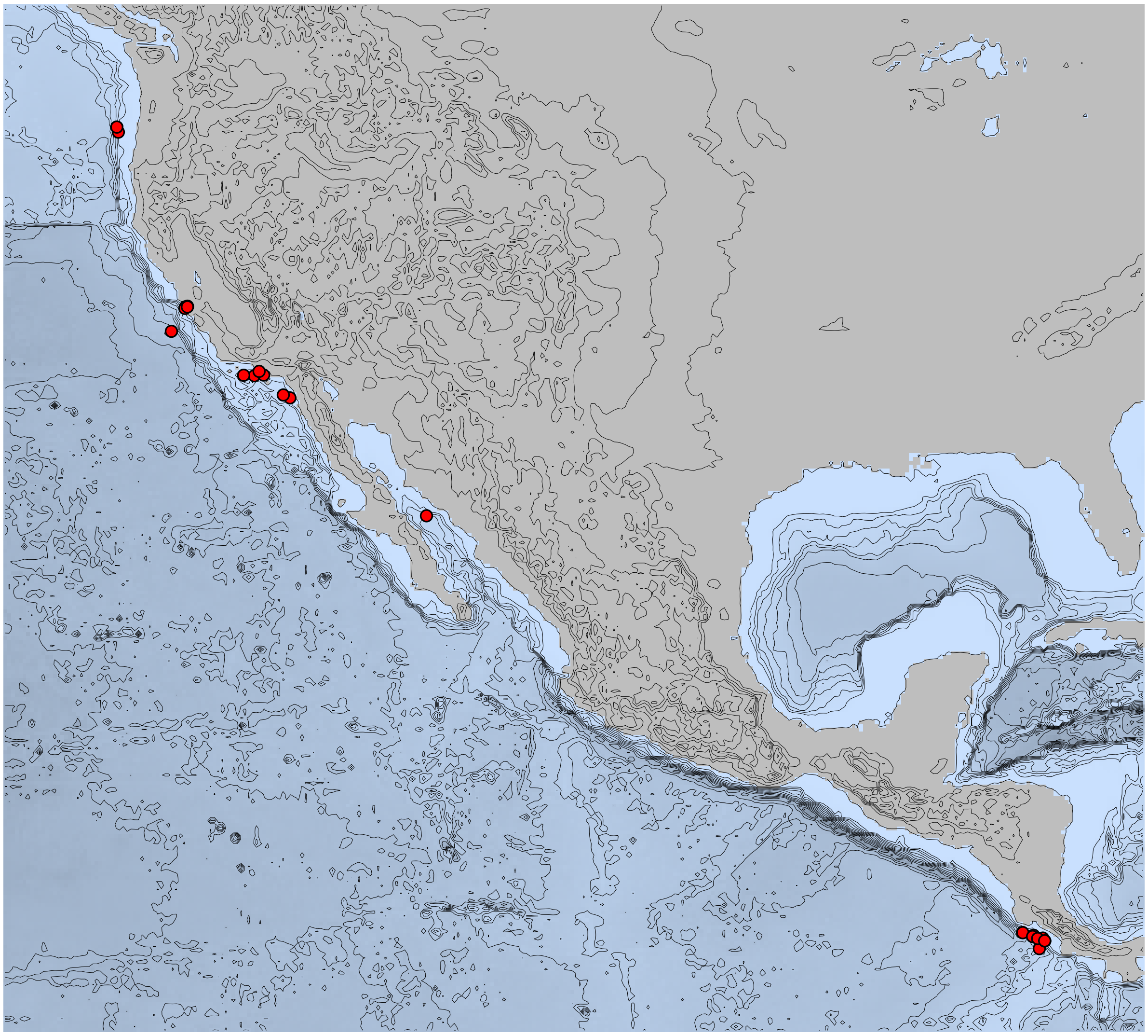


Figure 1: A map of sample sites from this study.

Figure 2: Maximum likelihood phylogenetic tree from COI gene dataset. Names in bold were sequenced in this study.

Figure 3: Phylogenetic analysis from concatenated 5-gene (COI, 16S, HH3, Cyt-B, 18S) dataset. Maximum likelihood tree with bootstrap/posterior probability values from the ML/MrBayes respectively. “*” indicates support value of 100 and a blank indicates a support value of less than 50. The two main clades are denoted and the “gracilis/hartmanni”, “lobifera”, and “labronica” clades from previous studies are shown. Names in bold are from this study.


Exallopus sp. Seep (39q)
Exallopus jumarsi
A2663 'Exallopus jumarsi'
Ophryotrocha globopalpata
Ophryotrocha sp. Seep 3 (11f)
Ophryotrocha flabella
Ophryotrocha cf. flabella
Ophryotrocha haleyae sp. nov.
Ophryotrocha manhellafinae sp. nov.
Ophryotrocha platykephale
Ophryotrocha sp. vent 5
Ophryotrocha sp. Seep 4 (13g)
Ophryotrocha sp. Seep 4 (14h)
Ophryotrocha averysea sp. nov.
Ophryotrocha sp. Seep 4 (15i)
Ophryotrocha n. sp. 2
Ophryotrocha scoobyi sp. nov.
Ophryotrocha sp. Seep 5 (37p)
Ophryotrocha n. sp. 1
Ophryotrocha longidentata
Ophryotrocha n. sp. 5
Ophryotrocha n. sp. 4
Ophryotrocha n. sp. 3
Ophryotrocha n. sp. 7
Ophryotrocha n. sp. 6
Ophryotrocha cf. nauarchus
Ophryotrocha nauarchus
Ophryotrocha magnadentata
Ophryotrocha longicollaris
A12032 Ophryotrocha longicollaris
Pseudophryotrocha sp. Seep (r)
Pinniphitime sp. Seep (41)
Ophryotrocha n. sp. 9
Ophryotrocha n. sp. 8
Ophryotrocha tilici sp. nov.
Clade A
96/0.99
/
Ophryotrocha puerilis
Ophryotrocha siberti
Ophryotrocha eutrophila
Ophryotrocha langstrumpae
A2778 Ophryotrocha langstrumpae
Ophryotrocha sadina
Ophryotrocha n. sp. 12
Ophryotrocha scutellus
Ophryotrocha n. sp. 13
Ophryotrocha n. sp. 14
Ophryotrocha lusa
Ophryotrocha batillus
A9610 Ophryotrocha batillus
Ophryotrocha n. sp. 11
Ophryotrocha n. sp. 10
Ophryotrocha gracillis
Ophryotrocha sp. Seep 2 (7d)
Ophryotrocha sp. Seep 2 (5e)
Ophryotrocha hartmanni
Ophryotrocha maculata
'gracilis'/'hartmanni' clade
Iphitime paguri
Iphitime hartmanae
Iphitime cuenoti
Ophryotrocha sp. vent 4
Ophryotrocha sp. vent 3
Ophryotrocha sp. vent 1
Ophryotrocha jiaolongi
Ophryotrocha sp. vent 2
Ophryotrocha clava
Clade B
85/0.99
Ophryotrocha socialis
Ophryotrocha sp. Seep 1 (1a)
Ophryotrocha sp. Seep 1 (4c)
Ophryotrocha adherens
Ophryotrocha vivipara
Ophryotrocha geryonicola
Ophryotrocha mediterranea
Ophryotrocha urbis
Ophryotrocha cyclops
Ophryotrocha n. sp. 15
A12033 Ophryotrocha cf. craigsmithi
Ophryotrocha craigsmithi
Ophryotrocha amyae sp. nov.
Ophryotrocha shieldsi
Ophryotrocha lipovskyae
Ophryotrocha lobifera
Ophryotrocha orensanzi
'lobifera' clade
Ophryotrocha robusta
Ophryotrocha rubra
Ophryotrocha japonica
Ophryotrocha notoglandulata
Ophryotrocha macrovifera
Ophryotrocha labronica
Ophryotrocha obscura
Ophryotrocha permanni
Ophryotrocha costlowi
'labronica' clade
Ophryotrocha mammilata
Ophryotrocha alborana
Ophryotrocha diadema
Dorvillea erucaeformis
Dorvillea rubrovittata
0.2

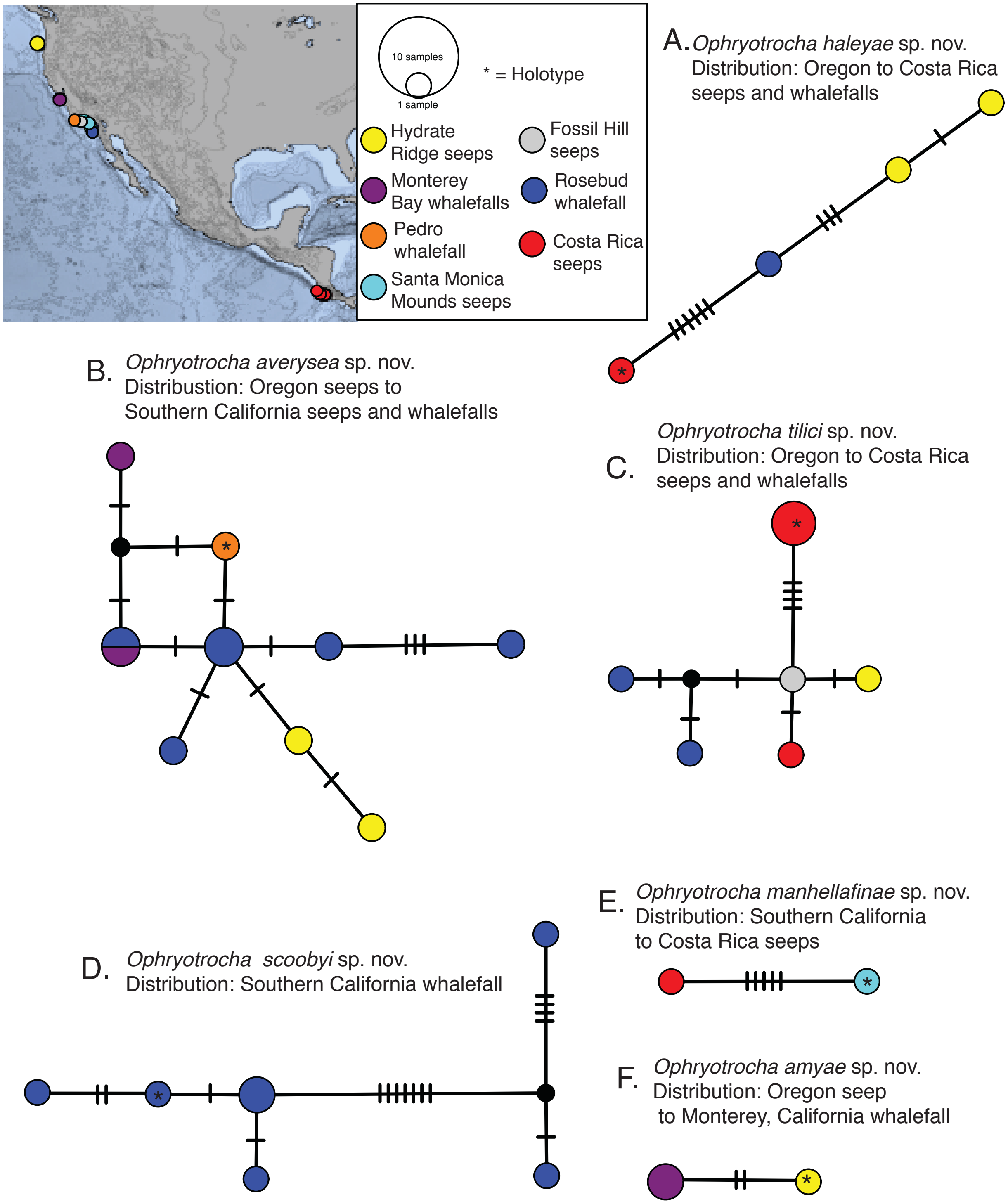


Figure 4: COI Haplotype networks for six new *Ophryotrocha* species. (A.) *O. haleyae;* (B.) *O. averysea;* (C.) *O. tilici;* (D.) *O. scoobyi;* (E.) *O. manhellafinae;* (F.) *O. amyae*

Figure 5: COI Haplotype Networks for seven previously described species and two new *Ophryotrocha* species. (A.) *"Exallopus jumarsi"*; (B.) *Ophryotrocha longicollaris*; (C.) *Ophryotrocha nauarchus*; (D.) *Ophryotrocha craigsmithi*; (E.) *Ophryotrocha platykephale*; (F.) *Ophryotrocha langstrumpae*; (G.) *Ophryotrocha* n. sp. 11; (H). *Ophryotrocha flabella*; (I.) *Ophryotrocha* n. sp. 9

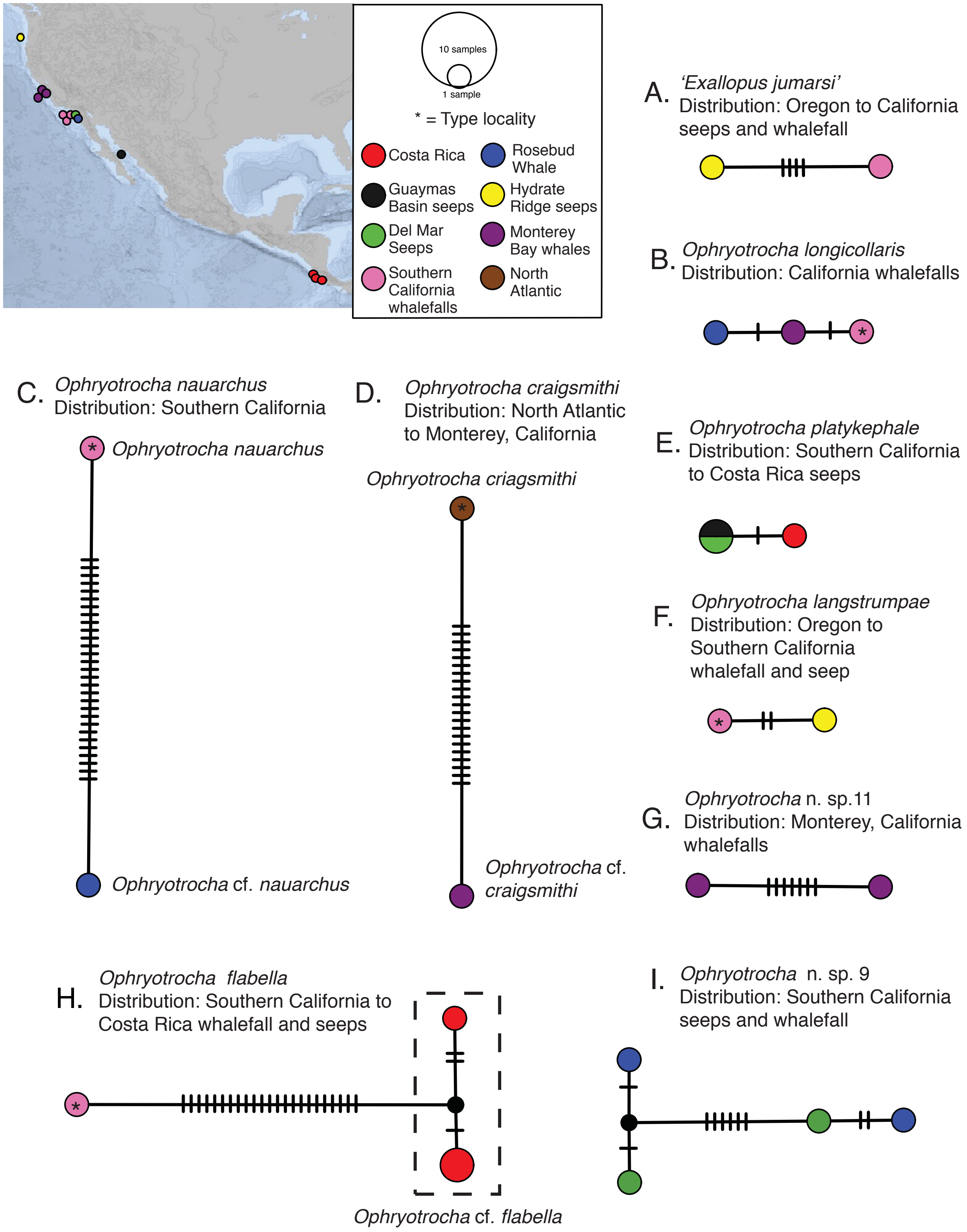

10 samples
1 sample
* = Type locality
Costa Rica
Rosebud Whale
Guaymas Basin seeps
Hydrate Ridge seeps
Del Mar Seeps
Monterey Bay whales
Southern California whalefalls
North Atlantic
A. ‘Exallopus jumarsi’
Distribution: Oregon to California seeps and whalefall
B. Ophryotrocha longicollaris
Distribution: California whalefalls
C. Ophryotrocha nauarchus
Distribution: Southern California
Ophryotrocha nauarchus
Ophryotrocha cf. nauarchus
D. Ophryotrocha craigsmithi
Distribution: North Atlantic to Monterey, California
Ophryotrocha criagsmithi
Ophryotrocha cf. craigsmithi
E. Ophryotrocha platykephale
Distribution: Southern California to Costa Rica seeps
F. Ophryotrocha langstrumpae
Distribution: Oregon to Southern California whalefall and seep
G. Ophryotrocha n. sp.11
Distribution: Monterey, California whalefalls
H. Ophryotrocha flabella
Distribution: Southern California to Costa Rica whalefall and seeps
Ophryotrocha cf. flabella
I. Ophryotrocha n. sp. 9
Distribution: Southern California seeps and whalefall

Figure 6: *Ophryotrocha tilici* sp. nov.: (A.) full body dorsal view (SIO-BIC A12011); (B.) light micrograph of mid-body parapodium (SIO-BIC A1425); (C.) SEM of head (SIO-BIC A12012); (D.) light micrograph of simple sub-acicular chaetae (SIO-BIC A12014); (E.) SEM of simple supra-acicular chaetae (SIO-BIC A1425); (F.) light micrograph of jaws (SIO-BIC A1425). Scale bars: (B-C) 100 μm; (D) 50 μm; (E-F) 10 μm

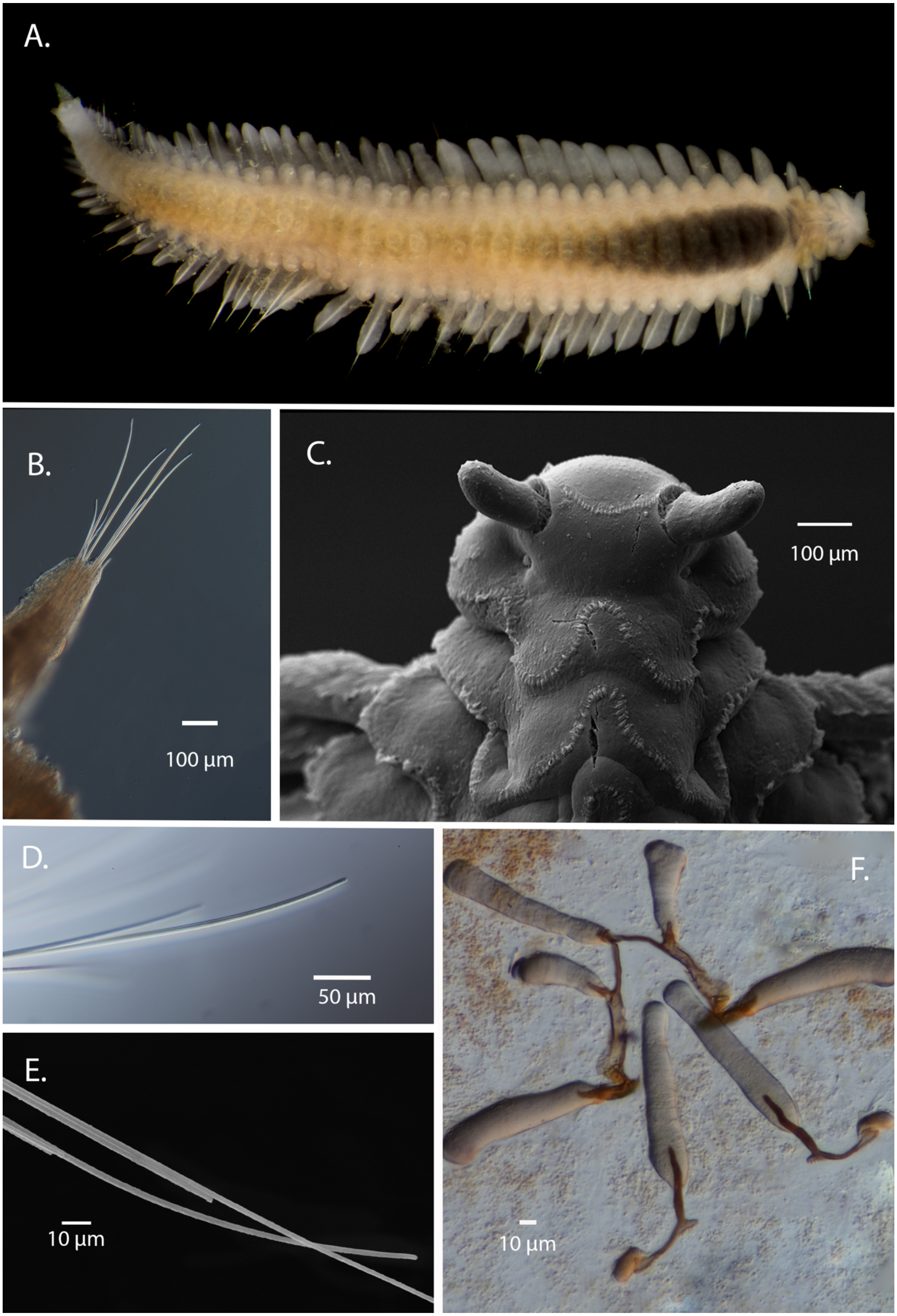
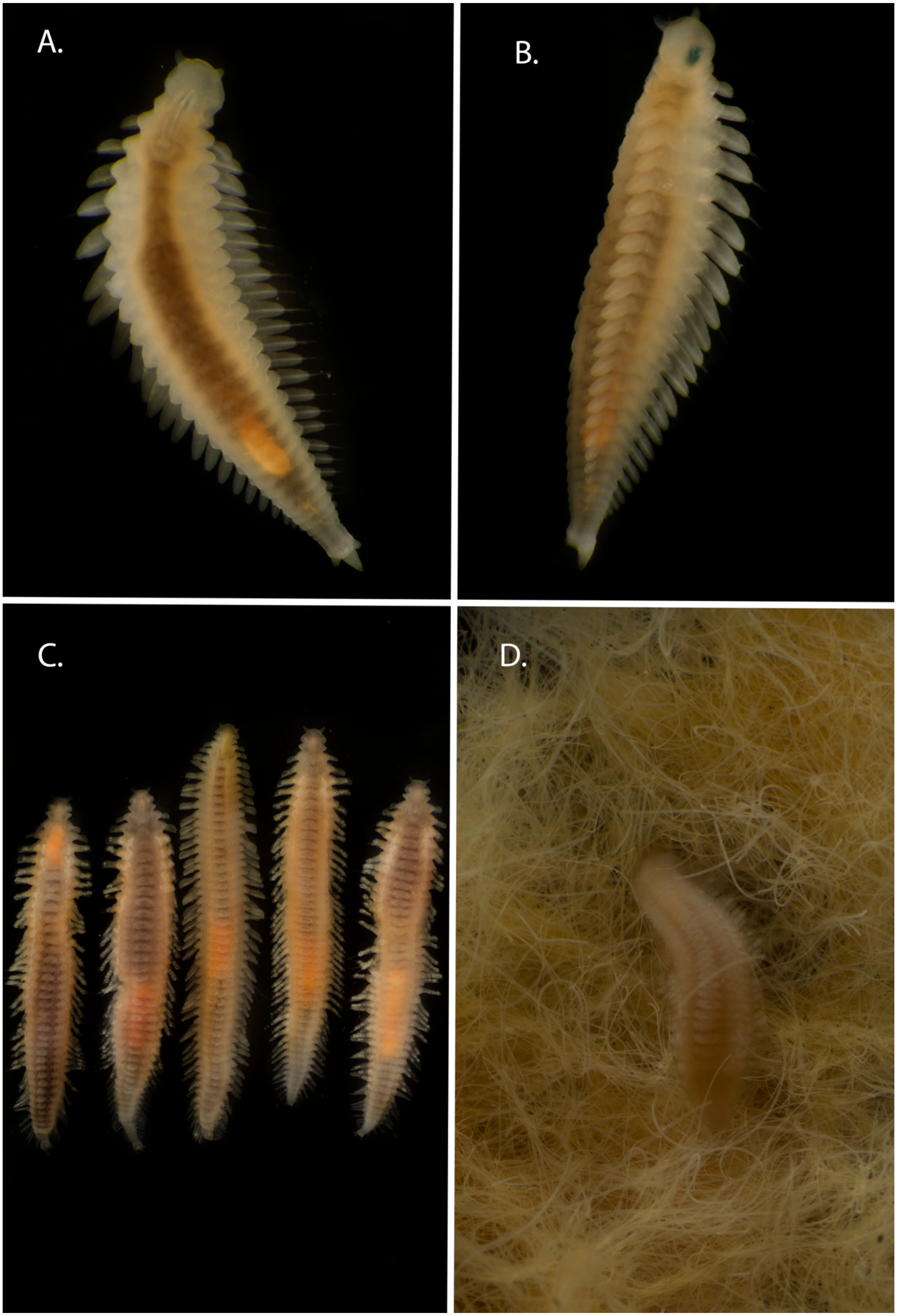

A.
B.
100 µm
C.
100 µm
D.
50 µm
E.
10 µm
F.
10 µm

Figure 7: *Ophryotrocha tilici* sp. nov.: (A.) full body dorsal view (SIO-BIC A10084); (B.) full body ventral view (SIO-BIC A10084); (C.) live animals (SIO-BIC A12014); (D.) live animal on bacterial mat (SIO-BIC A12014)


A.
B.
C.
D.

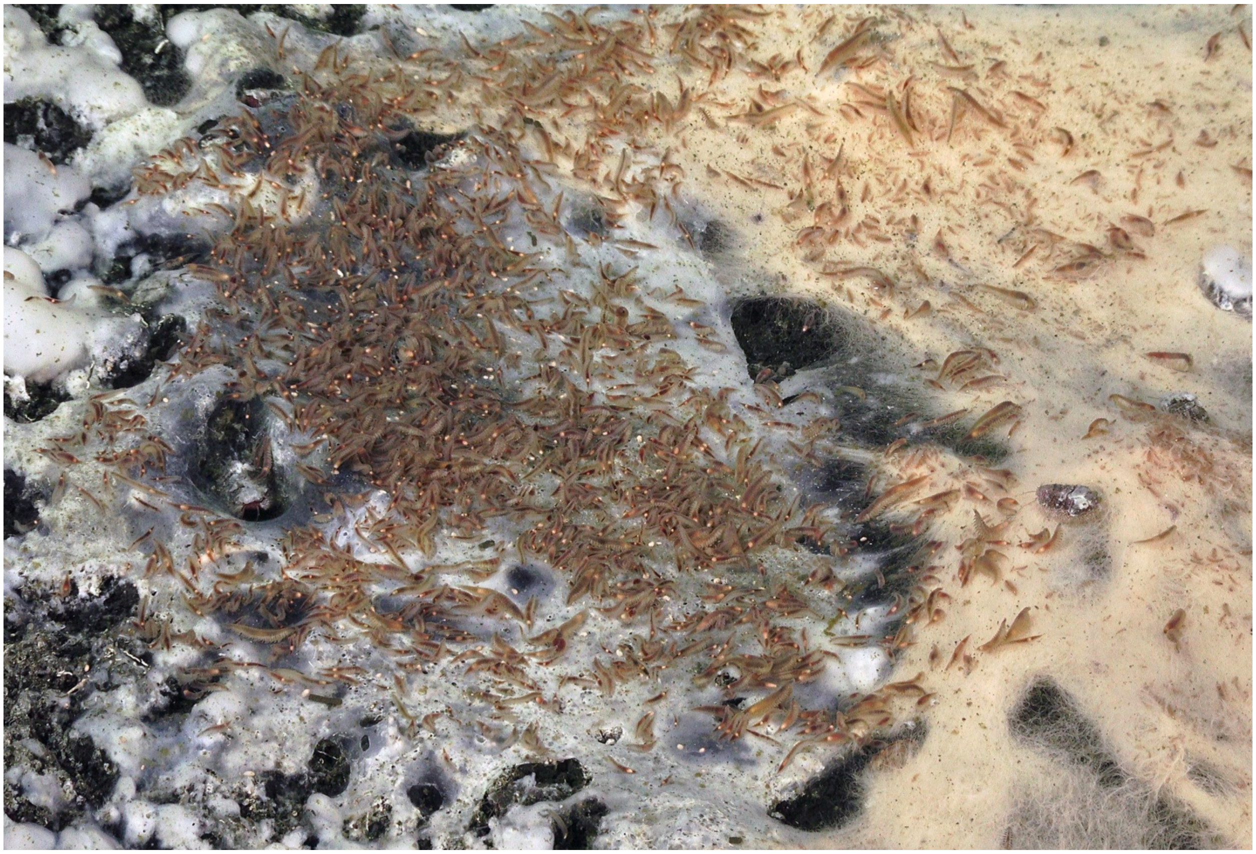


Figure 8: *Ophryotrocha tilici* sp. nov.*:* in-situ shot of dense aggregation on bacterial mat from “The Thumb” seep location, Costa Rica. (SIO-BIC A10084)

Figure 9: *Ophryotrocha scoobyi* sp. nov.: (A.) full body dorsal view (SIO-BIC A12019); (B.) light micrograph of parapodium (SIO-BIC A12359); (C.) light micrograph of parapodium (SIO-BIC A12359); (D.) light micrograph of simple supra-acicular and compound sub-acicular chaetae (SIO-BIC A12017); (E.) SEM of compound sub-acicular chaetae (SIO-BIC A12017); (F.) SEM of jaws (SIO-BIC A12360); Scale bars: (B) 100 µm; (C-D) 50 µm; (E) 10 µm; (F) 20 µm

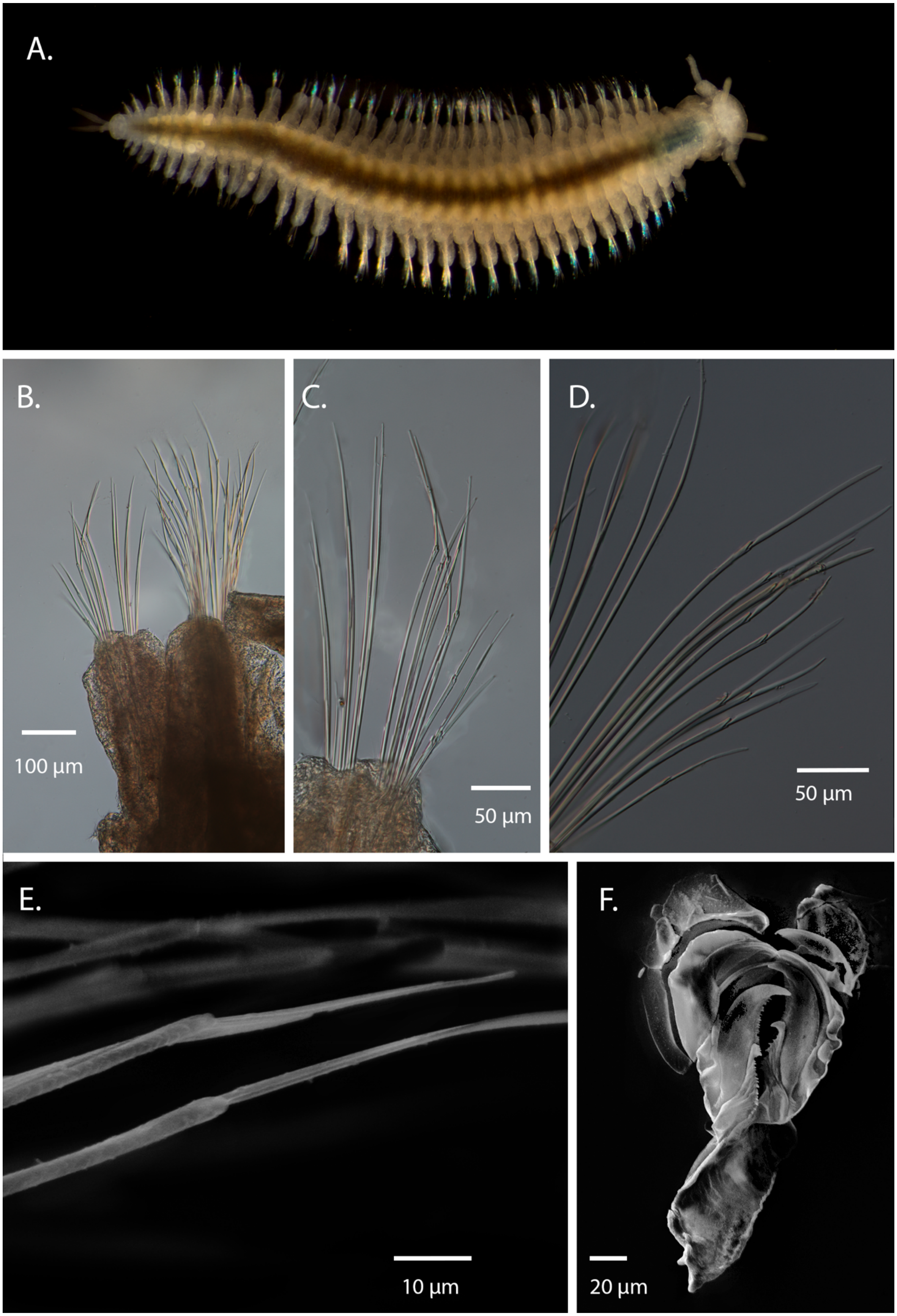

A.
B.
100 µm
C.
50 µm
D.
50 µm
E.
10 µm
F.
20 µm

Figure 10: *Ophryotrocha scoobyi* sp. nov.: (A.) full body dorsal view (SIO-BIC A12360); (B.) full body ventral view (SIO-BIC A12360)


A.
B.

Figure 11: *Ophryotrocha haleyae* sp. nov.: (A.) full body dorsal view (SIO-BIC A10052); (B.) SEM of parapodium (SIO-BIC A10052); (C.) light micrograph of parapodium (SIO-BIC A2722); (D.) light micrograph of parapodium (SIO-BIC A2722); (E.) light micrograph of compound sub-acicular chaetae (SIO-BIC A2722); (F.) SEM of simple supra-acicular (SIO-BIC A10052); (G.) light micrograph of simple supra-acicular (SIO-BIC A2722); Scale bars: (B, D) 100 µm; (C) 150 µm; (E, G) 50 µm; (F) 20 µm

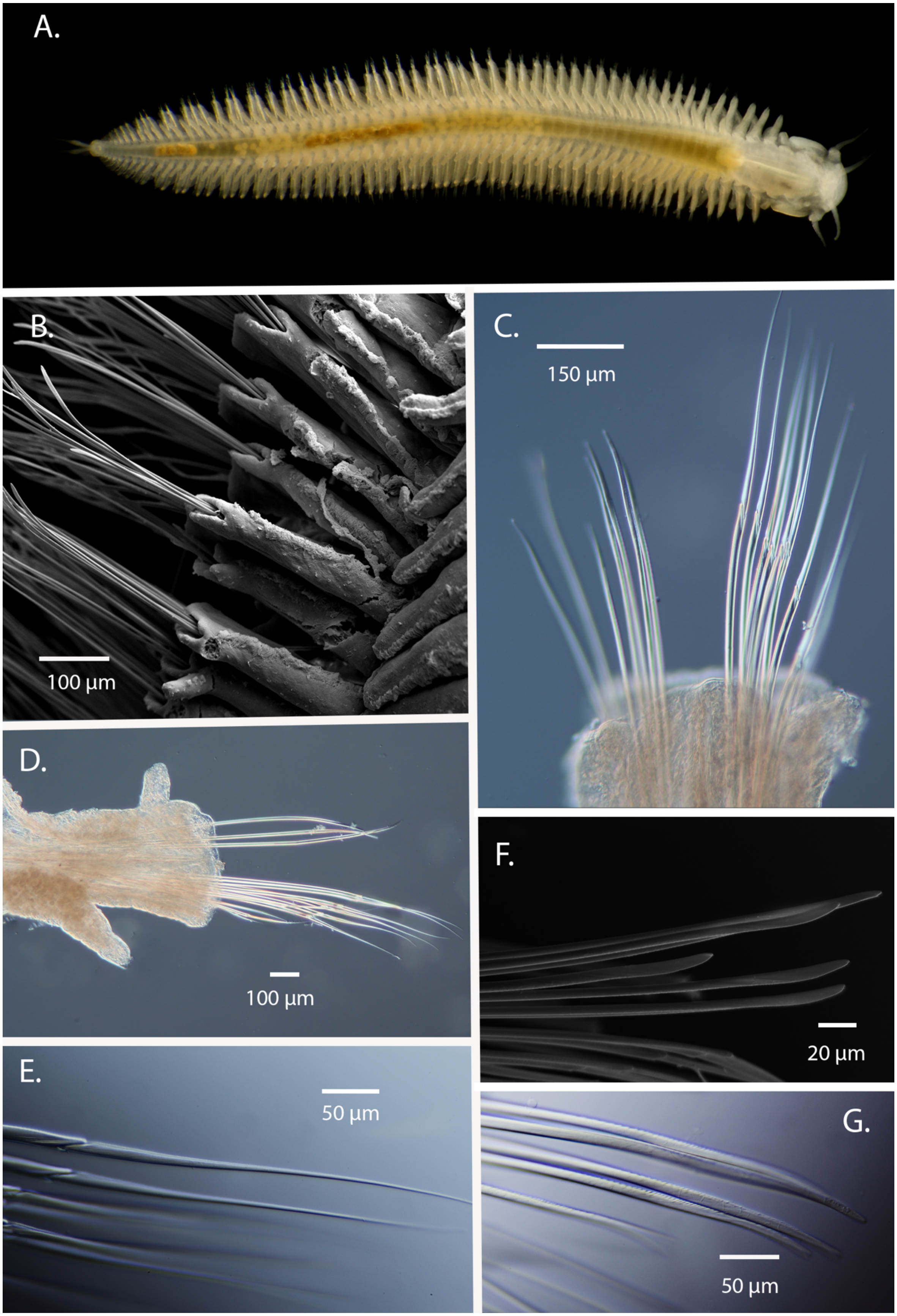

A.
B.
100 µm
C.
150 µm
D.
100 µm
E.
50 µm
F.
20 µm
G.
50 µm

Figure 12: *Ophryotrocha haleyae* sp. nov.: (A.) anterior dorsal view (SIO-BIC A2726); (B.) SEM of anterior dorsal view (SIO-BIC A10052); (C.) SEM of jaw maxillae (SIO-BIC A2726); (D.) SEM of jaw denticles (SIO-BIC A2726); (E.) SEM of jaw fangs (SIO-BIC A2726); Scale bars: (B, D) 20 μm; (C, E) 100 μm

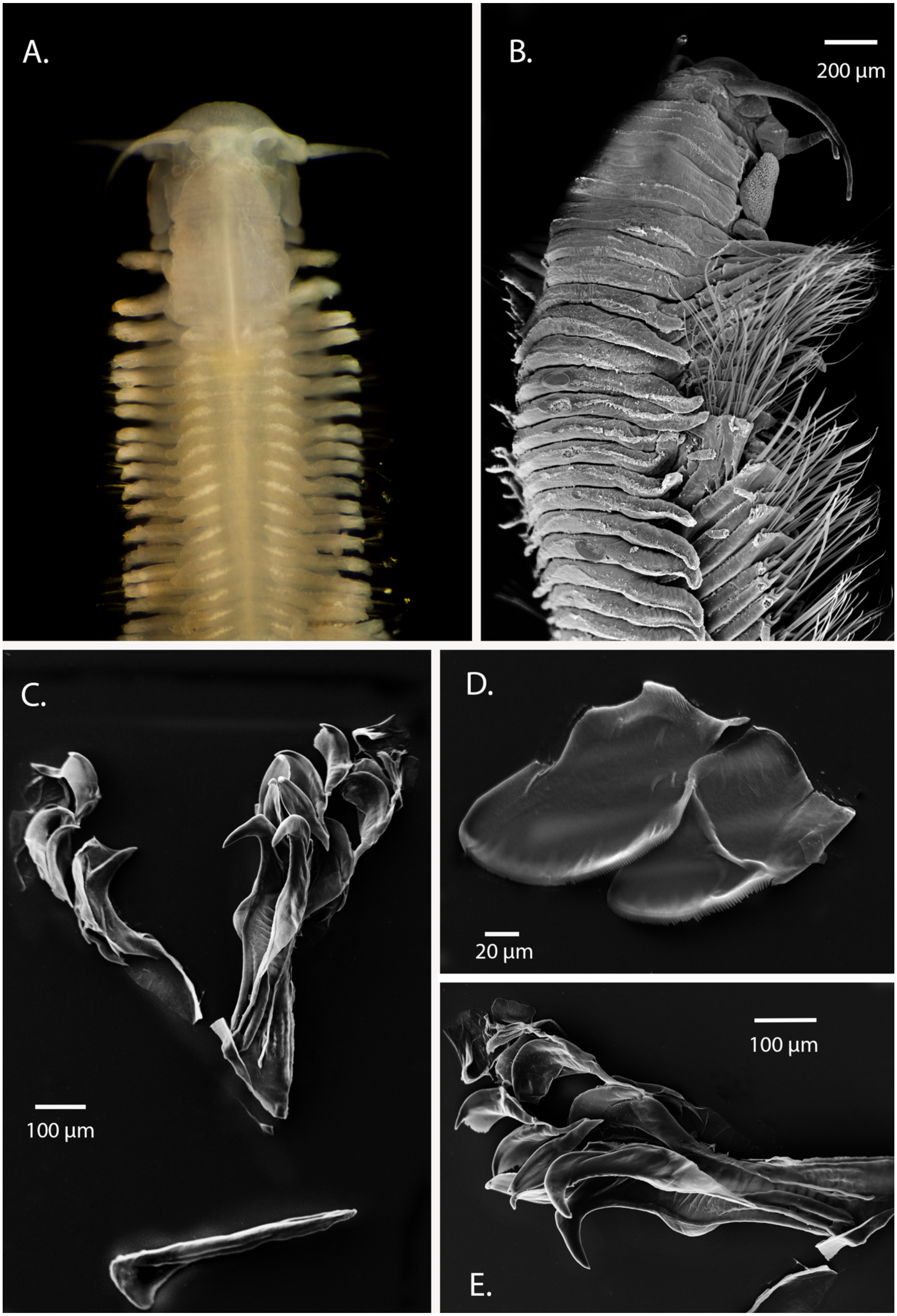

A.
B.
200 µm
C.
100 µm
D.
20 µm
100 µm
E.

Figure 13: *Ophryotrocha averysea* sp. nov.: (A.) full body dorsal view (SIO-BIC A2724); (B.) light micrograph of parapodium (SIO-BIC A12361); (C.) SEM of parapodium (SIO-BIC A12023); (D.) SEM of simple supra acicular chaetae (SIO-BIC A12363); (E.) SEM of compound sub-acicular chaetae (SIO-BIC A12023); (F.) SEM of jaw maxillae (SIO-BIC A12363); Scale bars (B) 100 µm; (C) 50 µm; (D-E) 20 µm; (F) 40 µm

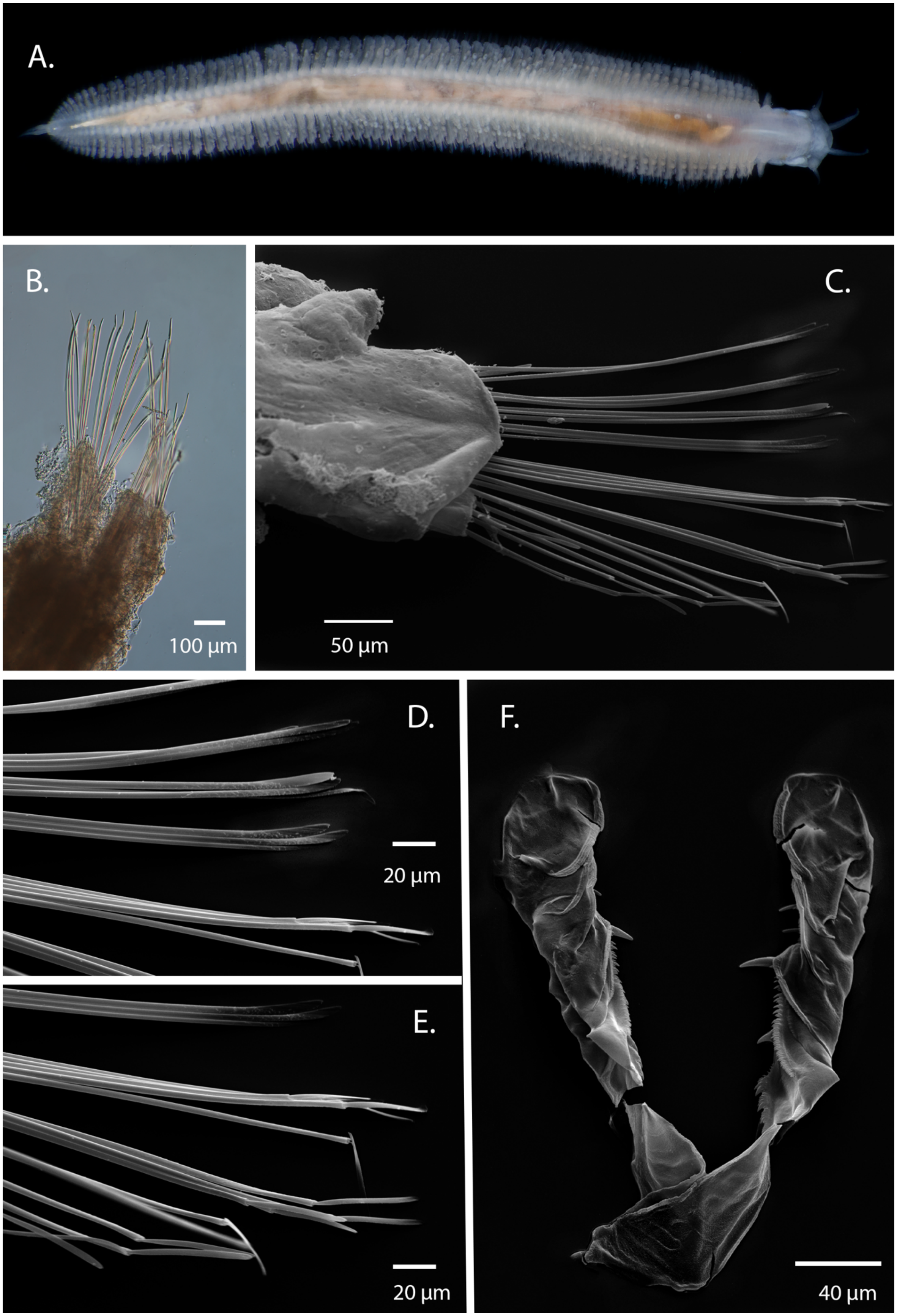

A.
B.
100 µm
C.
50 µm
D.
20 µm
E.
20 µm
F.
40 µm

Figure 14: *Ophryotrocha averysea* sp. nov.: (A.) anterior dorsal view (SIO-BIC A2724); (B.) anterior ventral view (SIO-BIC A2724)

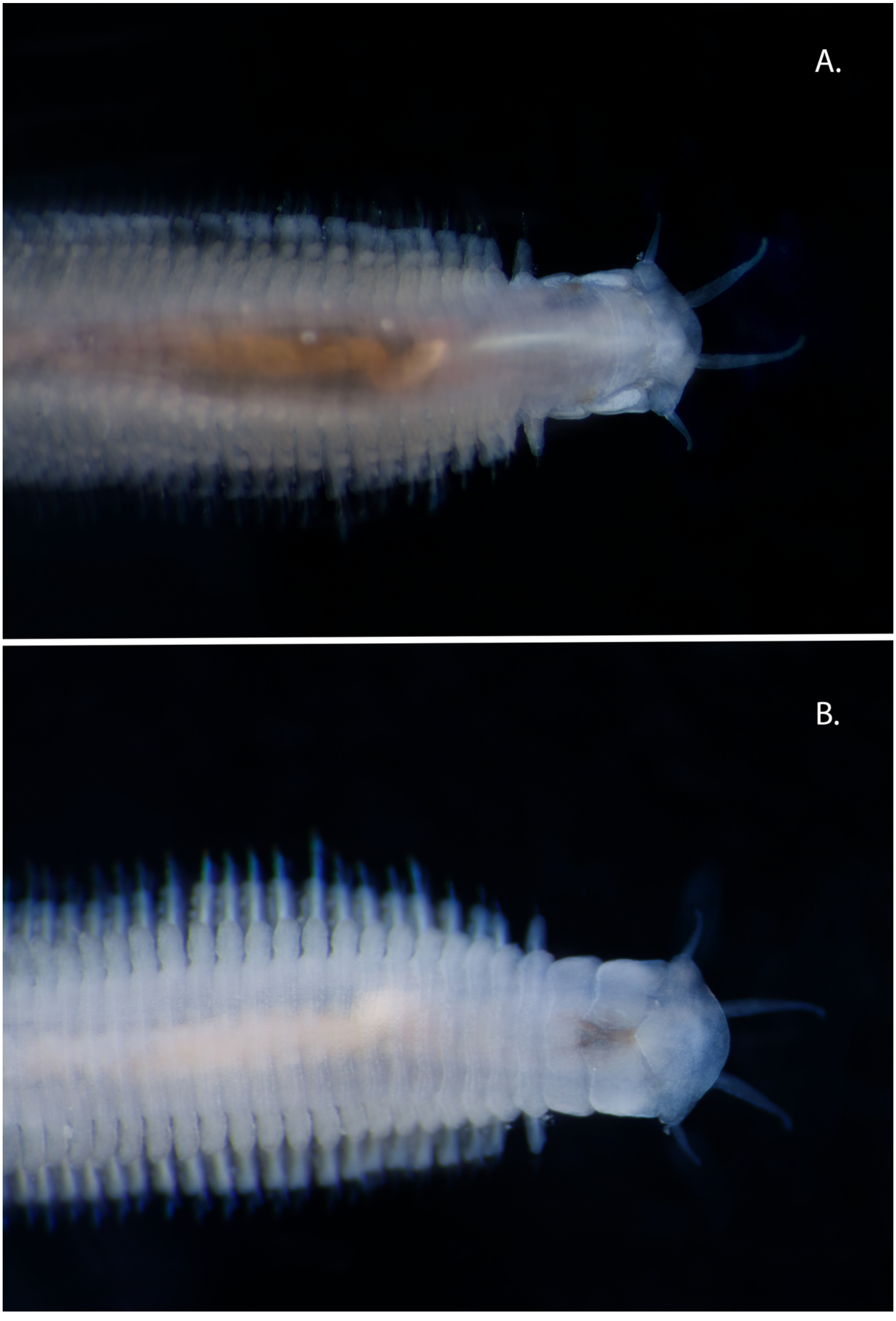

A.
B.

Figure 15: *Ophryotrocha amyae* sp. nov.: (A.) full body dorsal view(SIO-BIC A2720); (B.) light micrograph of parapodium (SIO-BIC A2720); (C.) SEM of parapodium (SIO-BIC A12030); (D.) SEM of jaws (SIO-BIC A12030); (E.) SEM of simple supra-acicular and compound sub-acicular chaetae (SIO-BIC A12030); (F.) SEM of compound sub-acicular chaetae (SIO-BIC A12030); (G.) SEM of jaw mandible (SIO-BIC A12030); (H.) SEM of jaw mandible cutting edge and denticles (SIO-BIC A12030); (I.) SEM of fang and denticles (SIO-BIC A12030) Scale bars: (B, D, G, I) 100 µm; (C) 20 µm; (E-F) 10 µm; (H) 40 µm

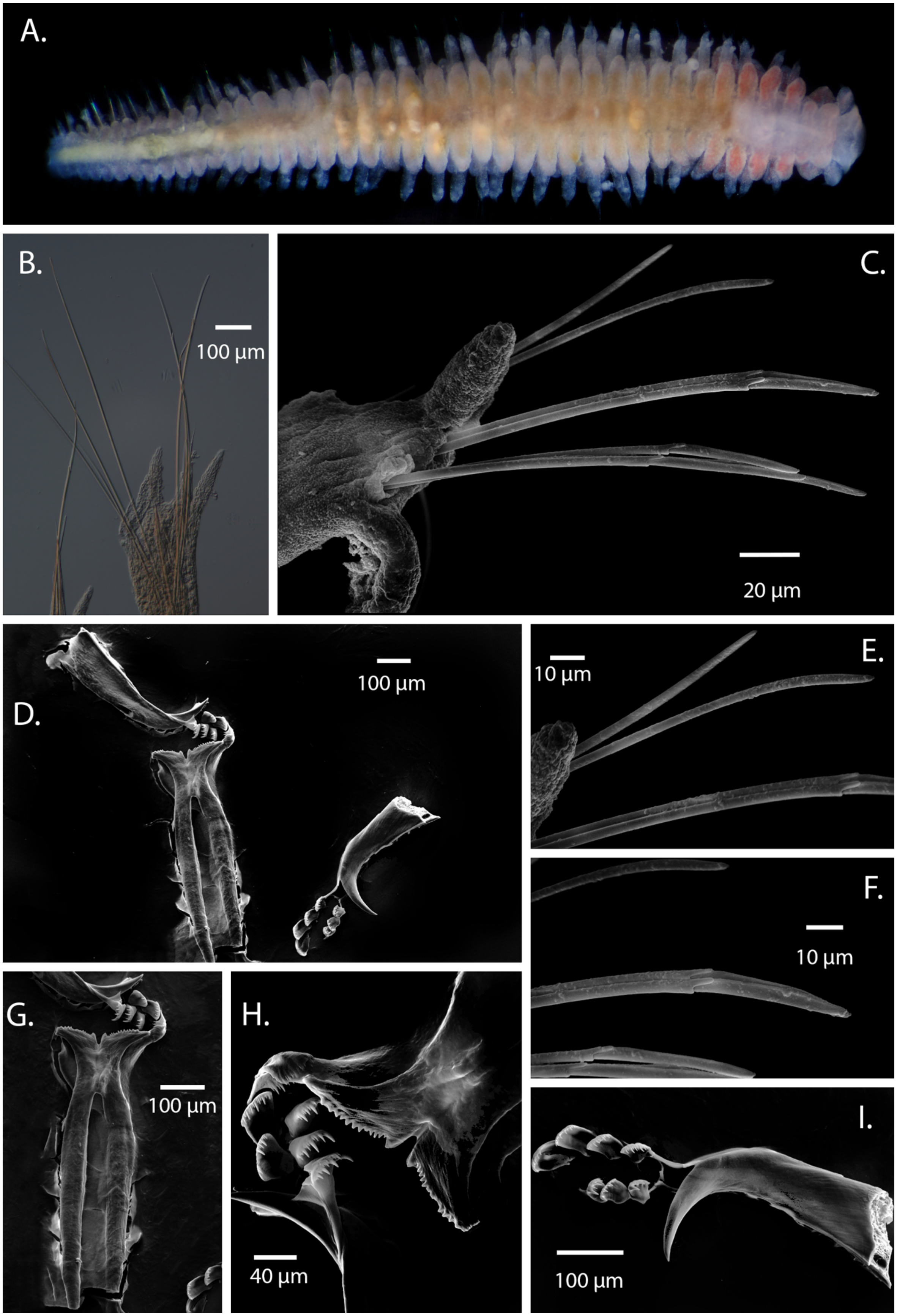

A.
B.
100 µm
C.
20 µm
D.
100 µm
E.
10 µm
F.
10 µm
G.
100 µm
H.
40 µm
I.
100 µm

Figure 16: *Ophryotrocha amyae* sp. nov.: (A.) fully body dorsal view (SIO-BIC A12029); (B.) anterior dorsal view (SIO-BIC A12029); (C.) anterior ventral view (SIO-BIC A12029); (D.) light micrograph of parapodium (SIO-BIC A12029); (E.) light micrograph of parapodium (SIO-BIC A12029); (F.) light micrograph of compound sub-acicular chaetae (SIO-BIC A12029) Scale bars: (D, F) 100 µm; (E) 200 µm

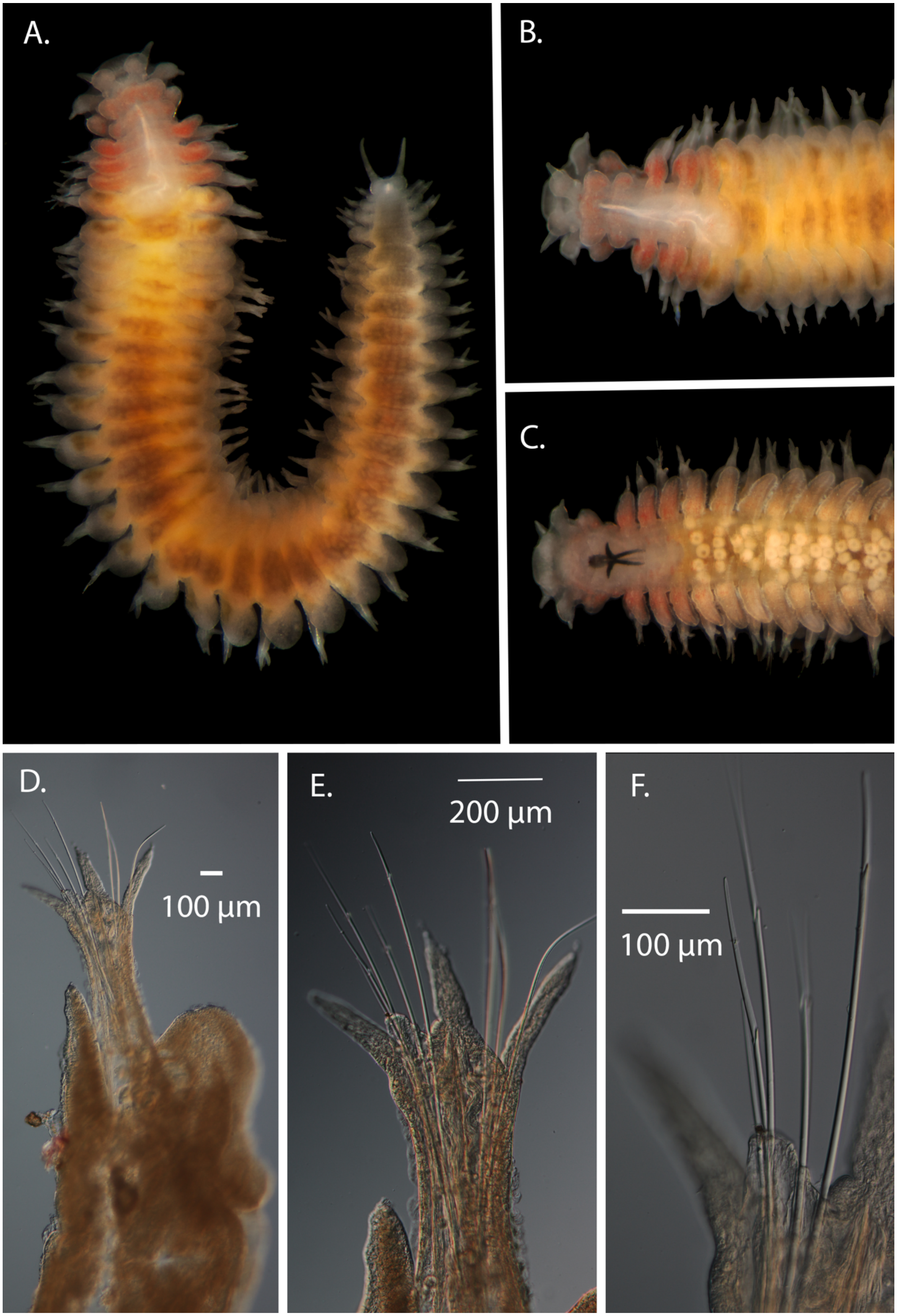

A.
B.
C.
D.
100 µm
E.
200 µm
F.
100 µm

Figure 17: *Ophryotrocha manhellafinae* sp. nov.: (A.) full body dorsal view (SIO-BIC A12334); (B.) full body ventral view (SIO-BIC A12334); (C.) light micrograph of simple supra-acicular chaetae (SIO-BIC A12334); (D.) light micrograph of compound sub-acicular chaetae (SIO-BIC A12334); (E.) light micrograph of parapodium (SIO-BIC A12334); (F.) light micrograph of parapodium (SIO-BIC A12334); Scale bars: (C) 50 µm; (D) 20 µm; (E) 200 µm; (F) 100 µm

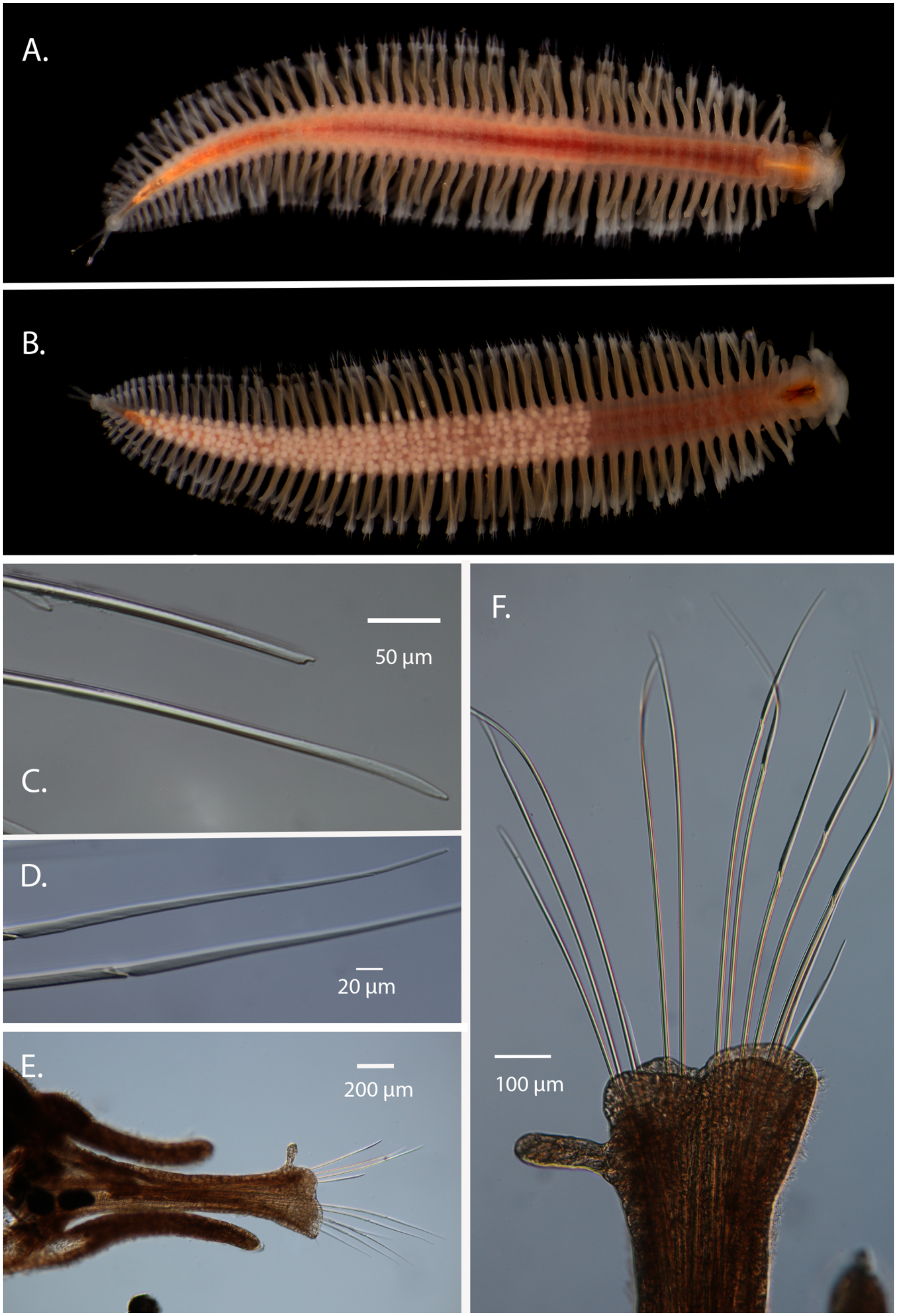

A.
B.
C.
50 µm
D.
20 µm
E.
200 µm
F.
100 µm

Figure 18: *Ophryotrocha manhellafinae* sp. nov.: (A.) SEM of anterior dorsal view 100 µm (SIO-BIC A12334); (B.) SEM of jaw maxillae (SIO-BIC A12334); (C.) SEM of dorsal view (SIO-BIC A12334); (D.) SEM of parapodium (SIO-BIC A12334); (E.) SEM of posterior dorsal view (SIO-BIC A12334); Scale bars: (A-B, D-E) 100 µm; (C) 300 µm

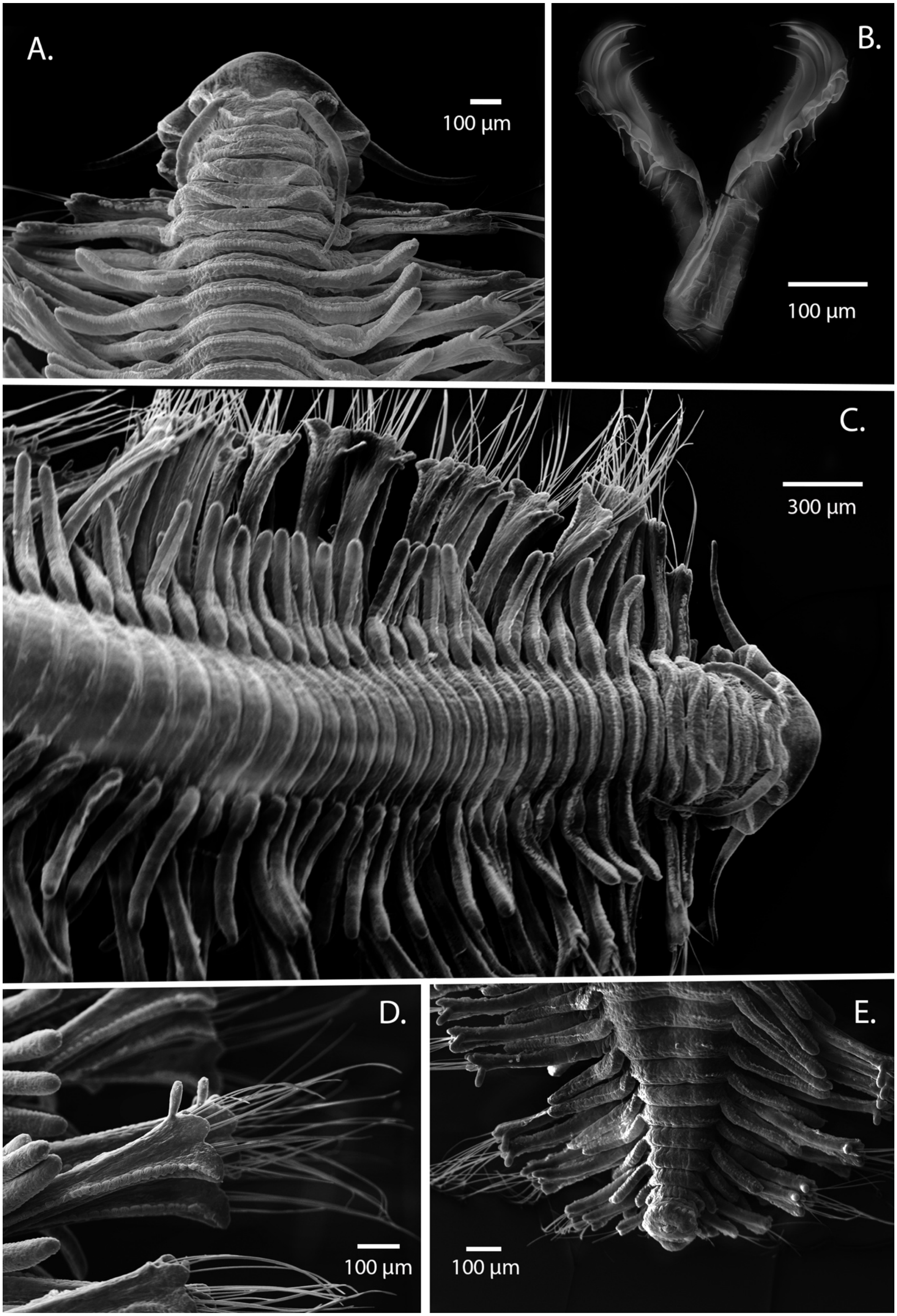

A.
100 µm
B.
100 µm
C.
300 µm
D.
100 µm
E.
100 µm

Figure 19: Unknown *Ophryotrocha* species: (A.) *Ophryotrocha* n. sp. 1 (SIO-BIC A10411); (B.) *Ophryotrocha* n. sp. 2 (SIO-BIC A12015); (C.) *Ophryotrocha* n. sp. 3 (SIO-BIC A12599); (D.) *Ophryotrocha* n. sp. 5 (SIO-BIC A12297)

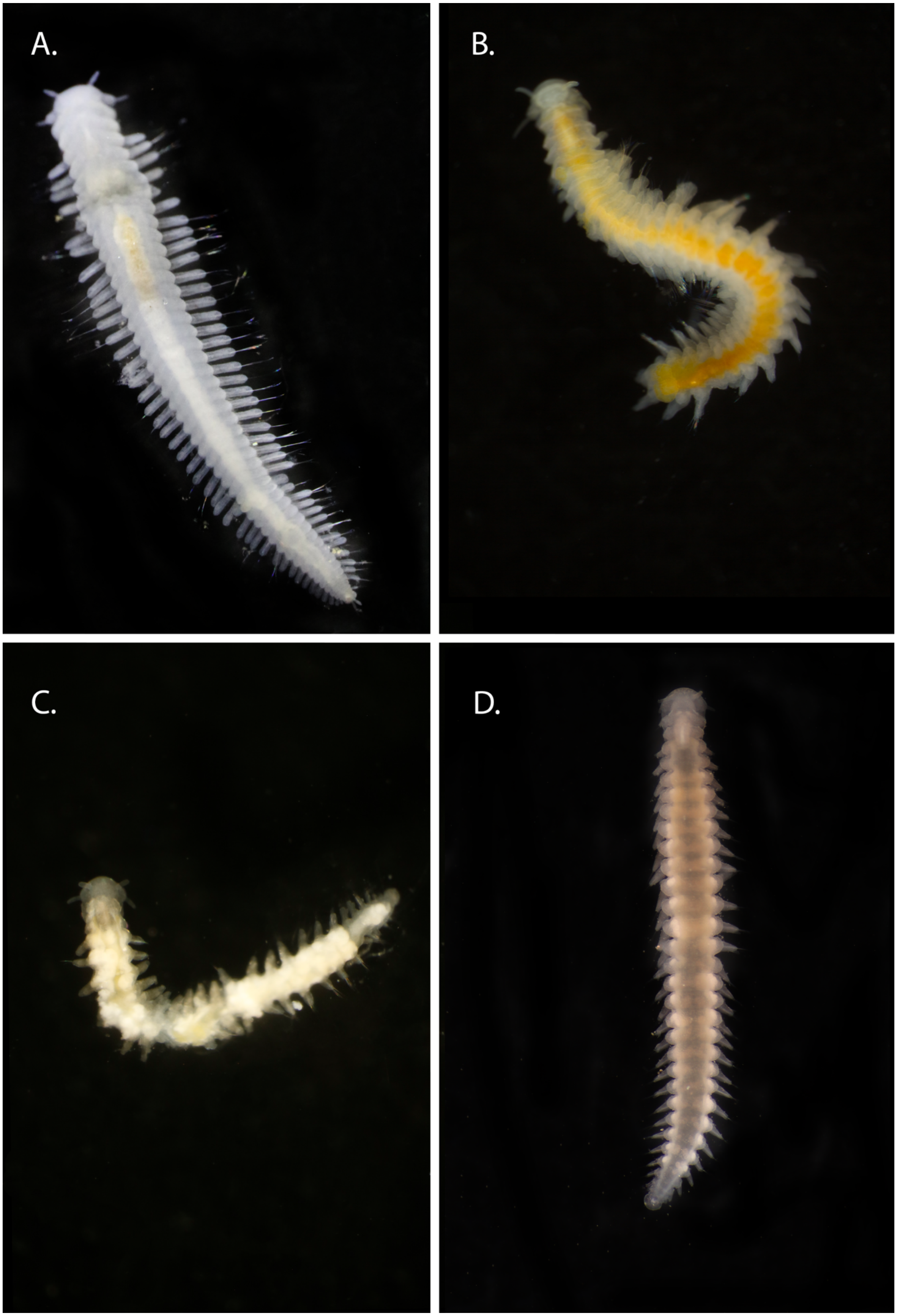

A.
B.
C.
D.

Figure 20: Unknown *Ophryotrocha* species: (A.) *Ophryotrocha* n. sp. 7 (SIO-BIC A2719); (B.) *Ophryotrocha* n. sp. 8 (SIO-BIC A12340); (C.) *Ophryotrocha* n. sp. 9 (SIO-BIC A12007); (D.) *Ophryotrocha* n. sp. 12 (SIO-BIC A12296)

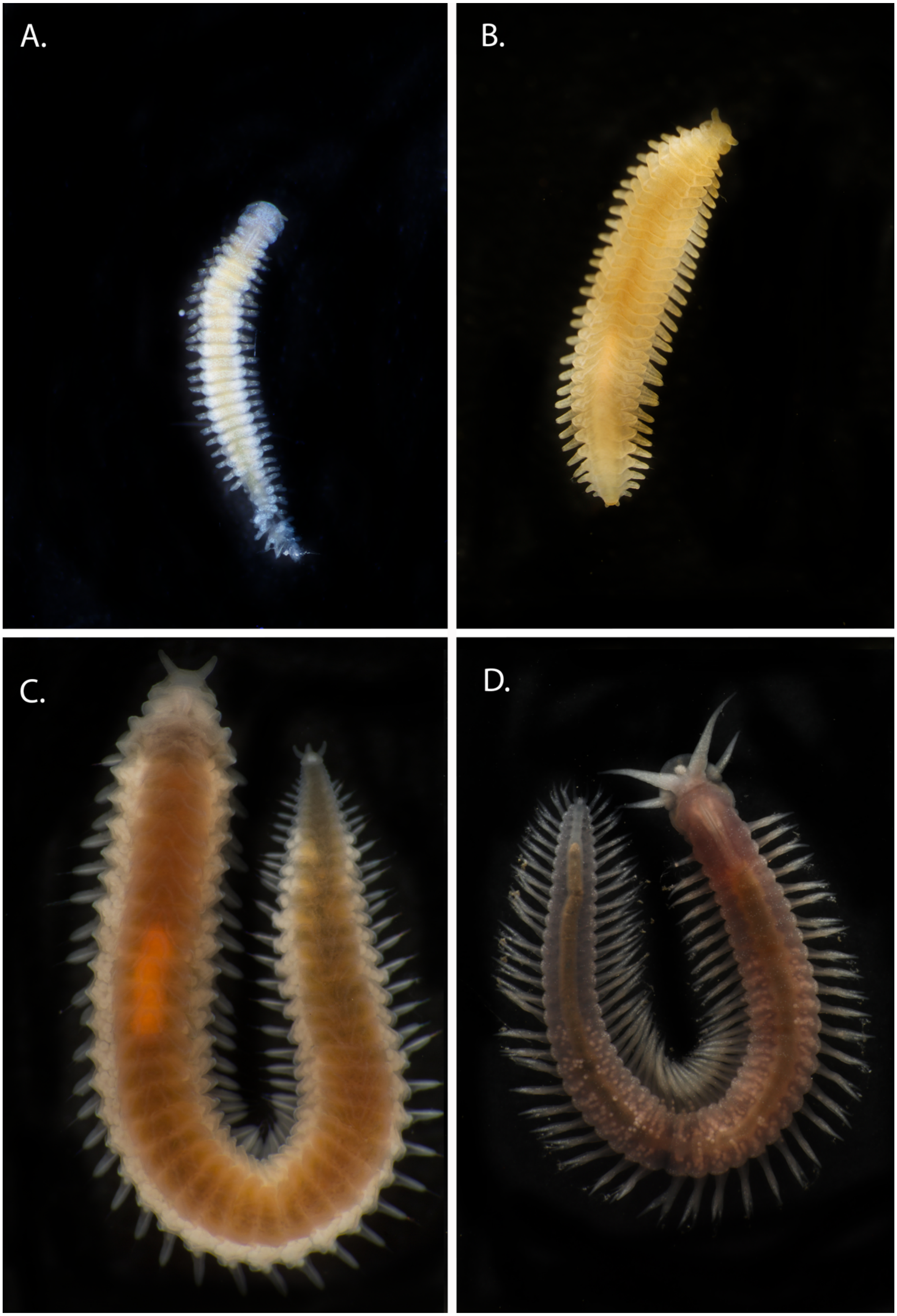

A.
B.
C.
D.

Figure 21: *Ophryotrocha platykephale*: (A.) full body view (SIO-BIC A3262); (B.) ventral anterior view (SIO-BIC A9878); (C.) full body side view (SIO-BIC A3260); (D.) full body dorsal view (SIO-BIC A12020); (E.) anterior dorsal view (SIO-BIC A12020); (F.) ventral anterior view (SIO-BIC A12020)

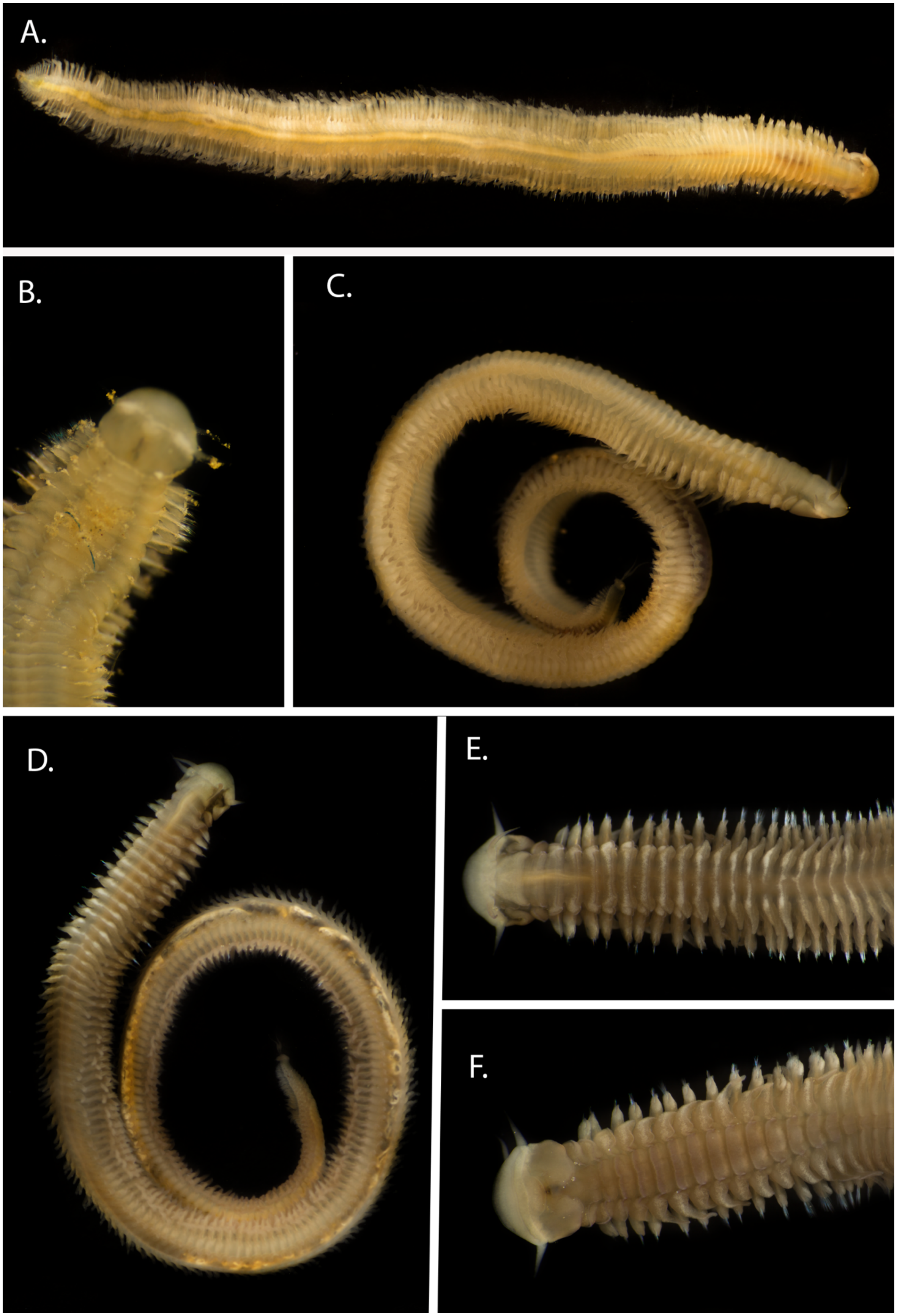

A.
B.
C.
D.
E.
F.

Figure 22: Previously known *Ophryotrocha* species recovered in this study: (A.) *O. langstrumpae* (SIO-BIC A2728); (B.) *O. longicollaris* (SIO-BIC A12032); (C.) *O.* cf. *craigsmithi* (SIO-BIC A12033)

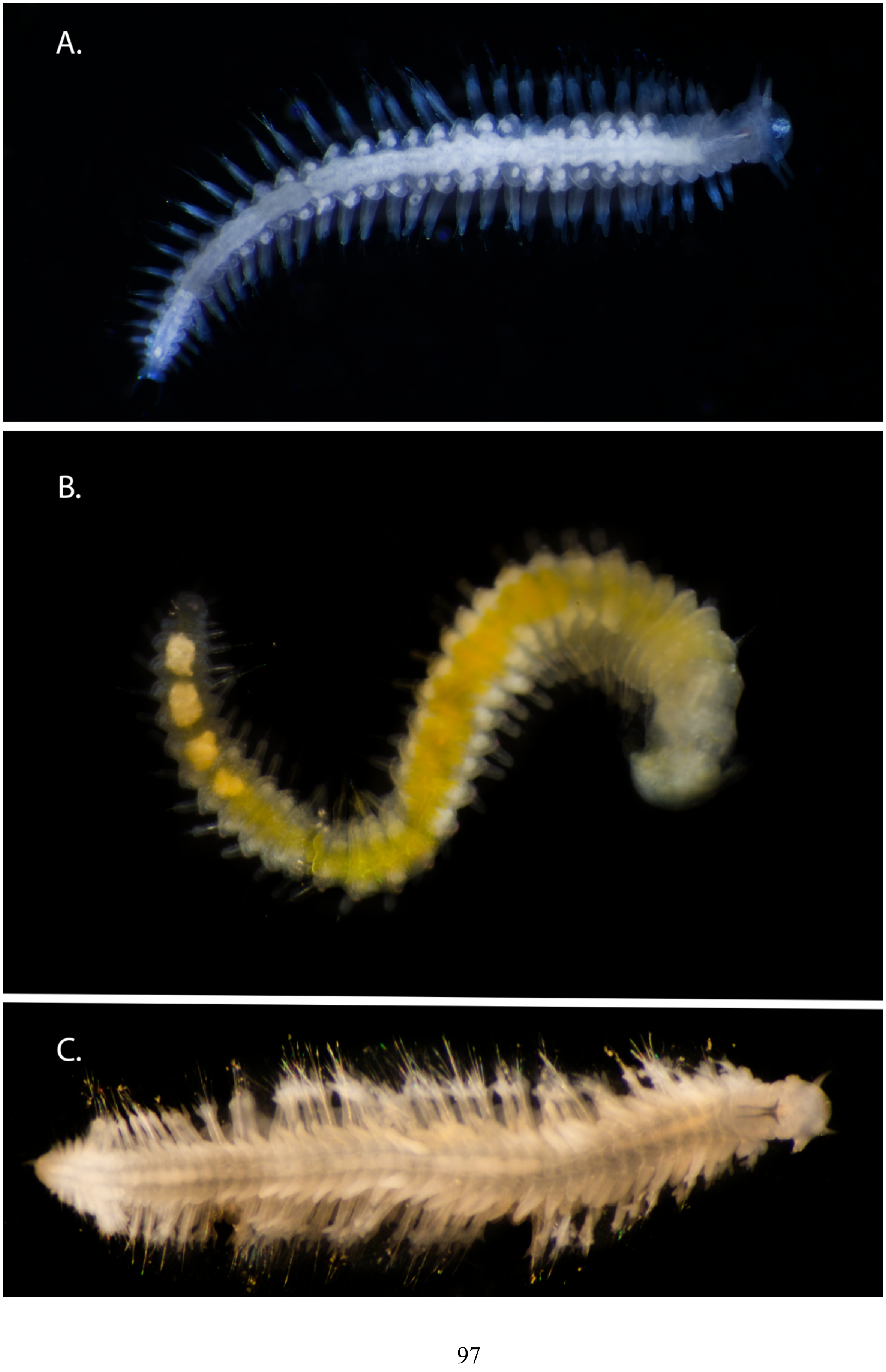
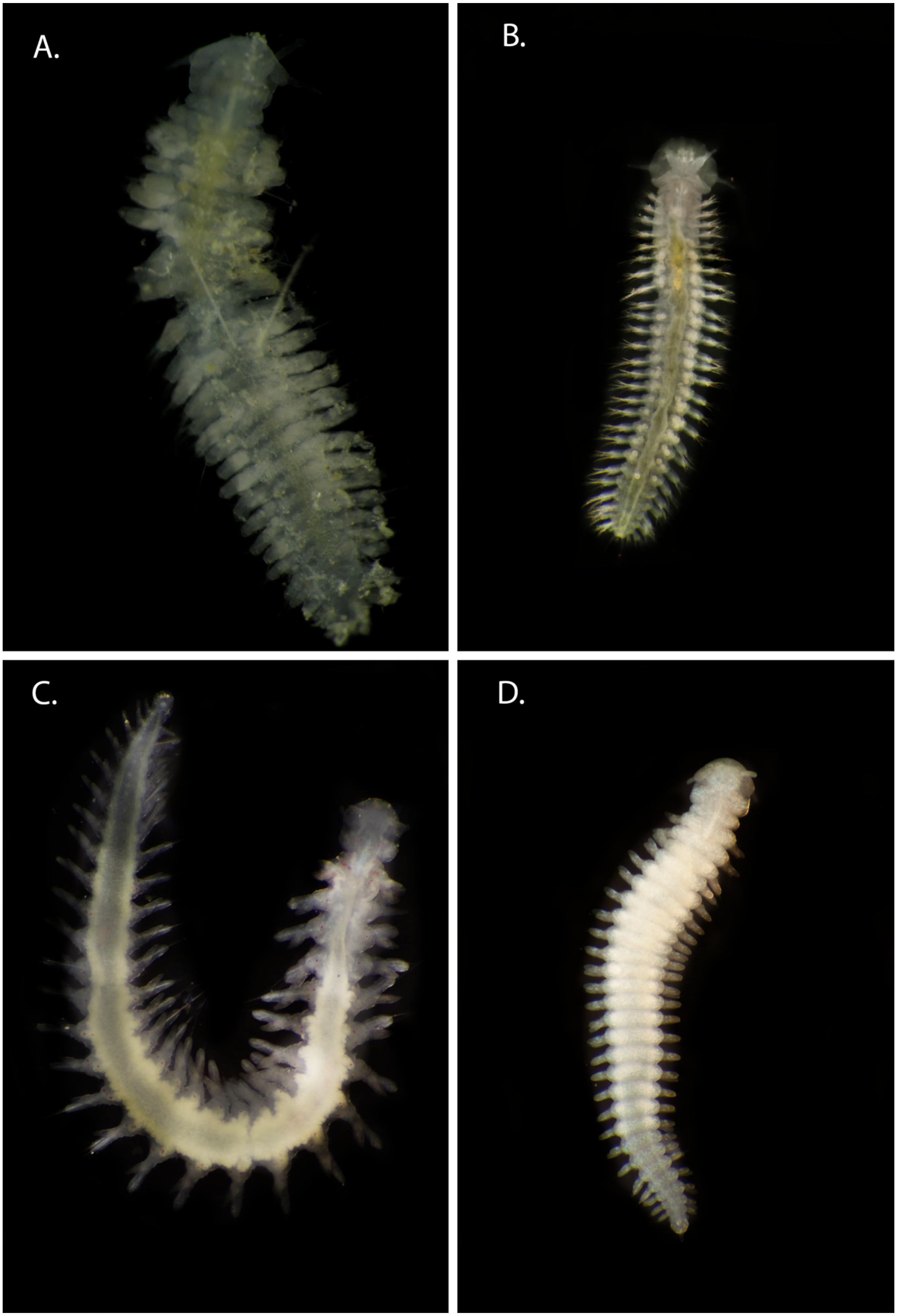

A.
B.
C.

Figure 23: Previously known *Ophryotrocha* species recovered in this study: (A.) *O.* cf. *flabella* (SIO-BIC A1410); (B.) *O.* cf. *batilus* (SIO-BIC A9610); (C.) *O.* cf. *nauarchus* (SIO-BIC A12031); (D.) "*Exallopus jumarsi*" (SIO-BIC A2663)


A.
B.
C.
D.

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REFERENCES

Åkesson, B.; Paxton, H. Biogeography and incipient speciation in *Ophryotrocha labronica* (Polychaeta, Dorvilleidae). *Mar. Biol. Res.* 2005, *1*, 127–139.

Alvarez-Padilla, F.; Hormiga, G. A Protocol For Digesting Internal Soft Tissues And Mounting Spiders For Scanning Electron Microscopy. *J. Arachnol.* 2007, *35*, 538–542.

Amon, D.J.; Glover, A.G.; Wiklund, H.; Marsh, L.; Linse, K.; Rogers, A.D.; Copley, J.T. The discovery of a natural whale fall in the Antarctic deep sea. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* 2013, *92*, 87–96.

Bacci, G.; La Greca, M. Genetic and Morphological Evidence for Subspecific Differences between Naples and Plymouth Populations of Ophryotrocha puerilis. *Nature* 1953, *171*, 1115–1115.

Bernardino, A.F.; Levin, L.A.; Thurber, A.R.; Smith, C.R. Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. *PLoS One* 2012, *7*, e33515.

Blake, J.A. Polychaeta from the vicinity of deep-sea geothermal vents in the eastern Pacific. I. Euphrosinidae, Phyllodocidae, Hesionidae, Nereididae, Glyceridae, Dorvilleidae, Orbiniidae, and Maldanidae. *Bull. Biol. Soc. Wash.* 1985, *6*, 67–101.

Blake, J.A.; Hilbig, B. Polychaeta from the vicinity of deep-sea hydrothermal vents in the eastern Pacific. II. New species and records from the Juan de Fuca and Explorer Ridge systems. 1990.

Boore, J.L.; Brown, W.M. Mitochondrial Genomes of Galathealinum, Helobdella, and Platynereis: Sequence and Gene Arrangement Comparisons Indicate that Pogonophora Is Not a Phylum and Annelida and Arthropoda Are Not Sister Taxa. *Molecular Biology and Evolution* 2000, *17*, 87–106.

Borda, E.; Kudenov, J.D.; Chevaldonné, P.; Blake, J.A.; Desbruyères, D.; Fabri, M.-C.; Hourdez, S.; Pleijel, F.; Shank, T.M.; Wilson, N.G.; Schulze, A.; Rouse, G.W. Cryptic species of Archinome (Annelida: Amphinomida) from vents and seeps. *Proc. Biol. Sci.* 2013, *280*, 20131876.

Chamberlin, R.V. *The Annelida Polychaeta [Albatross Expeditions] Memoirs of the Museum of Comaprative Zoology at Harvard College*; 1919; p. 48.

Claparède, É.; Mecznikow, E. Beiträge zur Kenntnis der Entwicklungsgeschichte der Chaetopoden. In *Zeitschrift für wissenschaftliche Zoologie.*; 1869; pp. 163–205.

Clement, M.; Snell, Q.; Walker, P.; Posada, D.; Crandall, K. TCS: estimating gene genealogies. In Proceedings of the Parallel and Distributed Processing Symposium, International; 2002; Vol. 2, pp. 0184–0184.

Colgan, D.J.; Ponder, W.F.; Eggler, P.E. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zool. Scr.* 2000, *29*, 29–63.

Conlan, K.E.; Kvitek, R.G. Recolonization of soft-sediment ice scours on an exposed Arctic coast. *Mar. Ecol. Prog. Ser.* 2005, *286*, 21–42.

da Rocha Miranda, V.; Rodrigues, A.R.; dos Santos Brasil, A.C. A new species of Ophryotrocha (Annelida: Dorvilleidae) associated with the coral Lophelia pertusa (Anthozoa: Caryophylliidae)., doi:10.11606/1807-0205/2020.60.13.

Dahlgren, T.G.; Akesson, B.; Schander, C.; Halanych, K.M.; Sundberg, P. Molecular phylogeny of the model annelid *Ophryotrocha*. *Biol. Bull.* 2001, *201*, 193–203.

Danovaro, R.; Company, J.B.; Corinaldesi, C.; D'Onghia, G.; Galil, B.; Gambi, C.; Gooday, A.J.; Lampadariou, N.; Luna, G.M.; Morigi, C.; Olu, K.; Polymenakou, P.; Ramirez-Llodra, E.; Sabbatini, A.; Sarda, F.; Sibuet, M.; Tselepides, A. Deep-sea biodiversity in the Mediterranean Sea: the known, the unknown, and the unknowable. *PLoS One* 2010, *5*, e11832.

Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 2012, *9*, 772.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 1994, *3*, 294–299.

Fournier, J.A.; Conlan, K.E. A new species of Ophryotrocha (Polychaeta, Dorvilleidae) associated with ice scours in the Canadian Arctic Archipelago. *Mémoires du Muséum national d'histoire naturelle (1993)* 1994, *162*, 185–190.

Gaston, G.R.; Benner, D.A. On Dorvilleidae and Iphitimidae (Annelida: Polychaeta) with a redescription of *Eteonopsis geryonicola* and a new host record. *Proceedings of the Biological Society of Washington* 1981, *94*, 76–87.

Giribet, G.; Carranza, S.; Baguna, J.; Riutort, M.; Ribera, C. First molecular evidence for the existence of a Tardigrada+ Arthropoda clade. *Mol. Biol. Evol.* 1996, *13*, 76–84.

Grube, E. Beschreibung neuer oder wenig bekannter Anneliden. *Archiv für Naturgeschichte.* 1855, *21*, 81–136.

Guindon, S.; Gascuel, O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 2003, *52*, 696–704.
Heggøy, K.; Schander, C.; Åkesson, B. The phylogeny of the annelid genus *Ophryotrocha* (Dorvilleidae). *Mar. Biol. Res.* 2007, *3*, 412–420.

Hilbig, B.; Blake, J.A. Dorvilleidae (Annelida:Polychaeta) from the U.S. Atlantic slope and rise. Description of two new genera and 14 new species, with a generic revision of Ophryotrocha. *Zool. Scr.* 1991, *20*, 147–183.

Høisæter, T.; Samuelsen, T.J. Taxonomic and biological notes on a species of Iphitime (Polychaeta, Eunicida) associated with Pagurus prideaux from western Norway. *Mar. Biol. Res.* 2006, *2*, 333–354.

Josefson, A. Ophryotrocha longidentata sp.n. and Dorvillea erucaeformis (Malmgren) (Polychaeta, Dorvilleidae) from the West Coast of Scandinavia. *Zool. Scr.* 1975, *4*, 49–54.

Jumars, P.A. A generic revision of the Dorvilleidae (Polychaeta), with six new species from the deep North Pacific. *Zool. J. Linn. Soc.* 1974, *54*, 101–135.

La Greca, M.; Bacci, G. Una nuova specie diOphryotrochadelle coste tirreniche (Annelida, Polychaeta). *Bolletino di zoologia* 1962, *29*, 7–18.

Lee, H.W.; Bailey-Brock, J.H.; McGurr, M.M. Temporal changes in the polychaete infaunal community surrounding a Hawaiian mariculture operation. *Mar. Ecol. Prog. Ser.* 2006, *307*, 175–185.

Leigh, J.W.; Bryant, D. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 2015, *6*, 1110–1116.

Levin, L.A. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. In *Oceanography and Marine Biology*; CRC Press, 2005; pp. 11–56.

Levin, L.A.; Baco, A.R.; Bowden, D.A.; Colaco, A.; Cordes, E.E.; Cunha, M.R.; Demopoulos, A.W.J.; Gobin, J.; Grupe, B.M.; Le, J.; Metaxas, A.; Netburn, A. N.; Rouse, G.W.; Thurber, A. R.; Tunnicliffe, V.; Van Dover, C.L.; Vanreusel, A.; Watling, L. Hydrothermal Vents and Methane Seeps: Rethinking the Sphere of Influence. *Frontiers in Marine Science* 2016, *3*, 72.

Levin, L.A.; Ziebis, W.; F. Mendoza, G.; Bertics, V.J.; Washington, T.; Gonzalez, J.; Thurber, A.R.; Ebbe, B.; Lee, R.W. Ecological release and niche partitioning under stress: Lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. *Deep Sea Res. Part 2 Top. Stud. Oceanogr.* 2013, *92*, 214–233.

Levin, L.A.; Ziebis, W.; Mendoza, G.F.; Growney, V.A.; Tryon, M.D.; Brown, K.M.; Mahn, C.; Gieskes, J.M.; Rathburn, A.E. Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. *Mar. Ecol. Prog. Ser.* 2003, *265*, 123–139.

Lundsten, L.; Schlining, K.L.; Frasier, K.; Johnson, S.B.; Kuhnz, L.A.; Harvey, J.B.J.; Clague, G.; Vrijenhoek, R.C. Time-series analysis of six whale-fall communities in Monterey Canyon, California, USA. *Deep Sea Res. Part I* 2010, *57*, 1573–1584.

Macnaughton, M.O.; Worsaae, K.; Eibye-Jacobsen, D. Jaw morphology and ontogeny in five species of *Ophryotrocha*. *J. Morphol.* 2010, *271*, 324–339.

Maddison, W.P.; Maddison, D.R. Mesquite: a modular system for evolutionary analysis. Version 3.61. *http://mesquiteproject.org* 2019.

Malmgren, A. Nordiska Hafs-Annulater. Oefv. K. *Vetensk. Akad. Stockholm, Fork* 1865, *21*.

Marenzeller, E. Südjapanische Anneliden, III, Denkschr, Math. *Nat. Cl. , Akad. Wiss. , Wien* 1902, *72*, 563–582.

McIntosh, W.C. Report on the Annelida Polychaeta collected by HMS“ Challenger” during the years 1873-76. Reports on the Scientific Results of the Voyage of HMS“ Challenger.” *Zoology* 1885, *12*, 1–554.

Mullineaux, L.S.; Peterson, C.H.; Micheli, F.; Mills, S.W. Successional mechanism varies along a gradient in hydrothermal fluid flux at deep-sea vents. *Ecol. Monogr.* 2003, *73*, 523–542.

Murtey, M.D.; Ramasamy, P. Sample Preparations for Scanning Electron Microscopy – Life Sciences. In *Modern Electron Microscopy in Physical and Life Sciences*; Janecek, M., Kral, R., Eds.; InTech, 2016 ISBN 9789535122524.

Nygren, A. Cryptic polychaete diversity: a review. *Zool. Scr.* 2014, *43*, 172–183.

Orensanz, J.M. The eunicemorph polychaete annelids from Antarctic and Subantarctic seas: With addenda to the eunicemorpha of Argentina, Chile, New Zealand, Australia, and the southern Indian Ocean. In *Biology of the Antarctic Seas XXI*; Kornicker, L.S., Ed.; Antarctic Research Series; American Geophysical Union: Washington, D. C., 1990; Vol. 52, pp. 1–183 ISBN 9780875907611.

Oyarzun, F.X.; Mahon, A.R.; Swalla, B.J.; Halanych, K.M. Phylogeography and reproductive variation of the poecilogonous polychaete Boccardia proboscidea (Annelida: Spionidae) along the West Coast of North America. *Evol. Dev.* 2011, *13*, 489–503.

Page, R.D.M. DNA barcoding and taxonomy: dark taxa and dark texts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2016, *371*, doi:10.1098/rstb.2015.0334.

Palumbi; Sr Nucleic acids II : the polymerase chain reaction. *Molecular systematics* 1996, 205–247.

Paxton, H. A New Species of *Palpiphitime* (Annelida: Dorvilleidae) from Western Canada. *Proceedings of the Biological Society of Washington* 2009, *122*, 26–31.

Paxton, H.; Åkesson, B. Redescription of *Ophryotrocha puerilis* and *O. labronica* (Annelida, Dorvilleidae). *Mar. Biol. Res.* 2007, *3*, 3–19.

Paxton, H.; Åkesson, B. The *Ophryotrocha labronica* group (Annelida: Dorvilleidae)—with the description of seven new species. *Zootaxa* 2010, *2713*, 1–24.

Paxton, H.; Morineaux, M. Three Species of Dorvilleidae (Annelida: Polychaeta) Associated with Atlantic Deep-Sea Reducing Habitats, With The Description of *Ophryotrocha fabriae*, New Species. *Proceedings of the Biological Society of Washington* 2009, *122*, 14–25.

Pleijel, F.; Eide, R. The phylogeny of *Ophryotrocha* (Dorvilleidae: Eunicida: Polychaeta). *J. Nat. Hist.* 1996, *30*, 647–659.

Prevedelli, D.; Massamba N'Siala, G.; Simonini, R. The seasonal dynamics of six species of Dorvilleidae (Polychaeta) in the harbour of La Spezia (Italy). *Mar. Ecol.* 2005, *26*, 286–293.

Rambaut, A. FigTree v1. 4.4, a graphical viewer of phylogenetic trees 2018.

Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* 2018, *67*, 901–904.

Ravara, A.; Marçal, A.R.; Wiklund, H.; Hilário, A. First account on the diversity of *Ophryotrocha* (Annelida, Dorvilleidae) from a mammal-fall in the deep-Atlantic Ocean with the description of three new species. *System. Biodivers.* 2015, *13*, 555–570.

Read, G., Fauchald, K., 2021. World Polychaeta database: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=914.

Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 2012, *61*, 539–542.

Rossi, M.M. A New Genus and Species of Iphitimid Parasitic in an Aphroditid (Polychaeta), with an Emendation of the Family Iphitimidae. *Bulletin of the Southern California Academy of Sciences* 1984, *83*, 163–166.

Saeedi, H.; Bernardino, A.F.; Shimabukuro, M.; Falchetto, G.; Sumida, P.Y.G. Macrofaunal community structure and biodiversity patterns based on a wood-fall experiment in the deep South-west Atlantic. *Deep Sea Res. Part I* 2019, *145*, 73–82.

Salvo, F.; Wiklund, H.; Dufour, S.C.; Hamoutene, D.; Pohle, G.; Worsaae, K. A new annelid species from whalebones in Greenland and aquaculture sites in Newfoundland: Ophryotrocha cyclops, sp. nov. (Eunicida: Dorvilleidae). *Zootaxa* 2014, *3887*, 555–568.

Silvestro, D.; Michalak, I. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* 2012, *12*, 335–337.

Smith, C.R.; Baco, A.R. Ecology of whale falls at the deep-sea floor. *Oceanogr. Mar. Biol. Annu. Rev.* 2003, *41*, 311–354.

Smith, C.R.; Glover, A.G.; Treude, T.; Higgs, N.D.; Amon, D.J. Whale-fall ecosystems: recent insights into ecology, paleoecology, and evolution. *Ann. Rev. Mar. Sci.* 2015, *7*, 571–596.

Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014, *30*, 1312–1313.

Stiller, J.; Rousset, V.; Pleijel, F.; Chevaldonné, P.; Vrijenhoek, R.C.; Rouse, G.W. Phylogeny, biogeography and systematics of hydrothermal vent and methane seep Amphisamytha (Ampharetidae, Annelida), with descriptions of three new species. *System. Biodivers.* 2013, *11*, 35–65.

Swofford, D.L.; Others Phylogenetic analysis using parsimony (* and other methods) 2002.

Taboada, S.; Leiva, C.; Bas, M.; Schult, N.; McHugh, D. Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zool. Scr.* 2017, *46*, 611–624.

Thornhill, D.J.; Dahlgren, T.G.; Halanych, K.M. Evolution and Ecology of Ophryotrocha (Dorvilleidae, Eunicida). *Annelids in modern biology* 2009, 242–256.

Thornhill, D.J.; Struck, T.H.; Ebbe, B.; Lee, R.W.; Mendoza, G.F.; Levin, L.A.; Halanych, K.M. Adaptive radiation in extremophilic Dorvilleidae (Annelida): diversification of a single colonizer or multiple independent lineages? *Ecol. Evol.* 2012, *2*, 1958–1970.
Thurber, A.R.; Levin, L.A.; Orphan, V.J.; Marlow, J.J. Archaea in metazoan diets: implications for food webs and biogeochemical cycling. *ISME J.* 2012, *6*, 1602–1612.

Vaidya, G.; Lohman, D.J.; Meier, R. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 2011, *27*, 171–180.

Whiting, M.F. Phylogeny of the holometabolous insect orders: molecular evidence. *Zool. Scr.* 2002, *31*, 3–15.

Whiting, M.F.; Carpenter, J.C.; Wheeler, Q.D.; Wheeler, W.C. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* 1997, *46*, 1–68.

Wiklund, H.; Altamira, I.V.; Glover, A.G.; Smith, C.R.; Baco, A.R.; Dahlgren, T.G. Systematics and biodiversity of *Ophryotrocha* (Annelida, Dorvilleidae) with descriptions of six new species from deep-sea whale-fall and wood-fall habitats in the north-east Pacific. *System. Biodivers.* 2012, *10*, 243–259.

Wiklund, H.; Glover, A.G.; Dahlgren, T.G. Three new species of *Ophryotrocha* (Annelida: Dorvilleidae) from a whale-fall in the North-East Atlantic. *Zootaxa* 2009, *2228*, 43–56.

Wolf, P.S. Three new species of Dorvilleidae (Annelida: Polychaeta) from Puerto Rico and Florida and a new genus for dorvilleids from Scandinavia and North America. *Proceedings of the Biological Society of Washington* 1986, *99*, 627–638.

Yen, N.K.; Rouse, G.W. Phylogeny, biogeography and systematics of Pacific vent, methane seep, and whale-fall Parougia (Dorvilleidae : Annelida), with eight new species. *Invertebr. Syst.* 2020, *34*, 200–233.

Zapata-Hernández, G.; Sellanes, J.; Thurber, A.R.; Levin, L.A.; Chazalon, F.; Linke, P. New insights on the trophic ecology of bathyal communities from the methane seep area off Concepción, Chile (~36° S). *Mar. Ecol.* 2014, *35*, 1–21.