



Phylogeny, biogeography, and systematics of Pacific vent, methane seep, and whalefall Parougia (Dorvilleidae, Annelida), with eight new species

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We present an updated phylogeny of the dorvilleid annelid genus *Parougia*, with the addition of new DNA sequence data from Pacific Ocean specimens from methane ‘cold’ seeps, hydrothermal vents and a whalefall. Eight new species are described, and species ranges are documented across up to 6000 km along the eastern Pacific Margin.

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Phylogeny, biogeography, and systematics of Pacific vent, methane seep, and whalefall *Parougia* (Dorvilleidae, Annelida), with eight new species

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Abstract. Dorvilleidae is a diverse group of annelids found in many marine environments and are also commonly associated with chemosynthetic habitats. One dorvilleid genus, *Parougia*, currently has 11 described species, of which two are found at vents or seeps: *Parougia wolffi* and *Parougia oregonensis*. Eight new *Parougia* species are recognized and described in this study from collections in the Pacific Ocean, all from whale-falls, hydrothermal vents, and/or methane seeps at ~600 m depth or greater. The 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 genes (18S rRNA and Histone 3). Six sympatric *Parougia* spp. were found at Hydrate Ridge, Oregon, while three of the *Parougia* species occurred at different types of chemosynthetic habitats. Two new species were found over wide geographical and bathymetric ranges. Another dorvilleid genus, *Ophryotrocha*, has previously been highlighted as diversifying in the deep-sea environment. Our results document the hitherto unknown diversity of another dorvilleid genus, *Parougia*, at various chemosynthetic environments.

<http://zoobank.org/urn:lsid:zoobank.org:pub:XXXXXXX>

Additional Key Words: chemosynthetic environments, deep sea, molecular, COI, polychaete

Running Title: Systematics of *Parougia* (Annelida)

Short Summary: We present an updated phylogeny of the dorvilleid annelid genus *Parougia*, with the addition of new DNA sequence data from Pacific Ocean specimens from methane 'cold' seeps, hydrothermal vents and a whalefall. Eight new species are described, and species ranges are documented across up to 6000 km along the eastern Pacific Margin.

Introduction

Environments such as hydrothermal vents, methane seeps, and large organic falls are nutrient-rich oases of the deep sea (Bernardino *et al.* 2012; Smith *et al.* 2015; Levin *et al.* 2016).

However, they are patchily distributed and ephemeral at decadal scales (Jørgensen and Boetius 2007; Smith *et al.* 2014; Smith *et al.* 2015; Portail *et al.* 2016). Inter-population connectivity of deep-sea invertebrates is shaped by biological and physical processes such as life-history characteristics (e.g. larval propagule dispersal-recruitment and adult behavior and motility), extrinsic factors such as deep-ocean circulation, topography of benthos, and temporal stability of ocean ridges and continental margins (Bernardino *et al.* 2012; Jang *et al.* 2016; Levin *et al.* 2016). Representatives from several annelid taxa, such as ampharetids (including alvinellins), dorvilleids, hesionids, polynoids and siboglinids (Thornhill *et al.* 2012; Levin *et al.* 2013; 2015; Rouse *et al.* 2018), have successfully diversified at these extreme habitats. Some with lecithotrophic larvae (e.g. *Alvinella pompejana*) are able to disperse great distances along Eastern Pacific hydrothermal vents (Jang *et al.* 2016). For other invertebrates, long-distance dispersal seems less probable (Oug 1990; Paavo *et al.* 2000; Mercier *et al.* 2014; Ravara *et al.* 2015). Instead, genetic structure and evolution must be heavily influenced by networks of close-range suitable habitats extending along margins, ridges, and across ocean basins through time (Bernardino *et al.* 2012).

Currently, our understanding of biodiversity in deep chemosynthetic habitats is limited by insufficient geographical sampling (Stiller *et al.* 2013; Levin *et al.* 2016). However, the rate of discovery of new species has increased (Watson *et al.* 2016), as exploring deep-sea habitats become easier. The frequency of cryptic species complexes now being discovered via molecular methods suggest significant underestimates of species richness (Nygren 2014).

Conversely, in a few instances, overestimates of interconnectivity among discrete habitats may occur as a result of phenotypic plasticity (Nygren *et al.* 2011; Stiller *et al.* 2013; Wei *et al.* 2013; Watson *et al.* 2016). However, assessing the evolution of complex life forms in extreme habitats requires robust taxonomies, that are lacking for many deep-sea taxa; Dorvilleidae Chamberlin, 1919 is a case in point.

Dorvilleidae is a globally distributed family of mostly shallow-water annelids but can also be among the dominant fauna at sites with methane seepage, hydrothermal venting, and organic falls such as wood or whales (Eibye-Jacobsen and Kristensen 1994; Goffredi *et al.* 2004; Glover *et al.* 2005; Bernardino *et al.* 2012; Thornhill *et al.* 2012; Smith *et al.* 2014; Ravara *et al.* 2015). Notably, while well known as bacteriovores (Wiklund *et al.* 2009; Taboada *et al.* 2016; Thornhill *et al.* 2012) this clade of annelids was the first animal recorded to consume Archaea as a main food source, a trophic process termed ‘archivory’ (Thurber *et al.* 2012). Dorvilleids are characterized by possessing a set of conspicuous sclerotized jaws of the ctenognath (comb-jaw) apparatus (Eibye-Jacobsen and Kristensen 1994; Kielan-Jaworowska 1966; Mierzejewski and Mierzejewska 1975; Paxton 2009) that consists of mandibular and maxillary elements. The monophyly of Dorvilleidae has been assessed with morphological data (Eibye-Jacobsen and Kristensen 1994), but sampling to date for molecular sequence data has not been comprehensive (Dahlgren *et al.* 2001; Taboada *et al.* 2015; Taboada *et al.* 2017; Thornhill *et al.* 2012; Wiklund *et al.* 2012) and many taxonomic issues remain unresolved. Dorvilleidae currently has 180 accepted species in 36 genera (WoRMS 2019) and there is DNA sequence data available for representatives from only a relatively few taxa. *Ophryotrocha* Claparède & Mecznirow, 1869 currently consists of 67 accepted species (WoRMS 2019), but it is paraphyletic, with members of *Exallopus* Jumars, 1974 and *Iphitime*

Marenzeller, 1902 and *Palpiphitime* Orensanz, 1990 nested inside (Wiklund *et al.* 2012).

Wiklund *et al.* (2012) synonymized *Palpiphitime* with *Ophryotrocha*, but left *Exallopus* and *Iphitime* as valid, pending further investigation. Multiple species within dorvilleid genera have otherwise only been sequenced for *Dorvillea* Parfitt, 1866 and *Parougia* Wolf, 1986. While the monophyly of *Dorvillea* has not been assessed in any detailed way, *Parougia* has been shown to be monophyletic (Taboada *et al.* 2015; Taboada *et al.* 2017; Wiklund *et al.* 2012), or paraphyletic (Zhang *et al.* 2017).

More than 20 species of *Ophryotrocha* have been reported, often in high abundance, on bacterial mats, vesicomyid clam beds, bushes of tube worms, and mussel beds (Blake and Hilbig 1990; Hilbig and Fiege 2001; Thornhill *et al.* 2012; Levin *et al.* 2013) in association with vents, seeps, wood, and whale falls in the Mediterranean Sea and Southern, Atlantic, and Pacific oceans (Blake 1985; Blake and Hilbig 1990; Paxton and Morineaux 2009; Wiklund *et al.* 2009; Bernardino *et al.* 2012; Thornhill *et al.* 2012; Wiklund *et al.* 2012; Taboada *et al.* 2013; Taboada *et al.* 2015; Ravara *et al.* 2015). *Parougia*, with 11 species described to date, is another taxon associated with chemosynthetic environments, although less represented than *Ophryotrocha*, with only two described taxa from vents or seeps (Blake and Hilbig 1990; Hilbig and Fiege 2001; Ravara *et al.* 2015; Taboada *et al.* 2017). *Parougia wolffi* Blake & Hilbig, 1990 was first described from Juan de Fuca hydrothermal vents, while *Parougia oregonensis* Hilbig & Fiege, 2001 was found at cold seeps off Oregon at 600-800 m depth. Since the discovery of *P. oregonensis*, it has been additionally recorded in high abundance from methane seeps at Eel River (California continental slope) (Levin *et al.* 2013). However, DNA data from Thornhill *et al.* (2012) revealed what was thought to be *P. oregonensis* as a series of cryptic species, with mitochondrial cytochrome b data grouping individuals into three

or four distinct clades. An additional *Parougia* species, *P. batia* (Jumars, 1974), was described from the northeastern Pacific (San Diego Trough) in silty mud from 1223-1229 m. Two additional *Parougia* species are known from the Southern Ocean; *Parougia furcata* (Hartman, 1953), and *Parougia diapason* Taboada, Bas & Avila, 2015 (found on a whale bones deployed at 5-15 m). However, *Parougia* is best represented from North Atlantic shallow waters: *Parougia albomaculata* (Åkesson & Rice, 1992), *Parougia bermudensis* (Åkesson & Rice), 1992, *Parougia caeca* (Webster & Benedict, 1884), *Parougia eliasoni* (Oug, 1978), *Parougia macilenta* (Oug, 1978), and *Parougia nigridentata* (Oug, 1978), though most of these have not been sequenced to date.

Taxonomic and phylogenetic studies of *Parougia* have been limited, with only 5 described taxa associated with molecular data prior to this study (Heggoy *et al.* 2007; Thornhill *et al.* 2012; Wiklund *et al.* 2012; Taboda *et al.* 2015). Here we formally describe eight new *Parougia*, including specimens of three undescribed species recovered in the Thornhill *et al.* (2012) study. Samples were collected from various Pacific deep-sea chemosynthetic habitats: **1.** hydrothermal vents at Juan de Fuca Ridge (off Oregon, USA) and the North Fiji and Lau Back-Arc Basins. **2.** methane seeps off Oregon, California, the Guaymas Basin (Mexico) and Costa Rica; **3.** a whale-fall off the coast of California. New DNA sequence data from mitochondrial and nuclear genes were analyzed, along with existing GenBank data to assess species delineation and the phylogeny and biogeography of *Parougia*.

Materials and Methods

Sample Collection and Morphology

Parougia specimens were collected during several cruises from 2005-2016. The human-occupied vehicle (HOV) *Alvin* and remotely operated vehicles (ROVs) *Jason II* and *Doc Ricketts* were used to sample material on rocks, bones, bacterial mats and sediments associated with vents, seeps, and whalefalls. Table 1 provides a description of collection locality, deposition of vouchers, and GenBank accession numbers. Generally, the posterior ends of *Parougia* specimens were used for DNA extraction and anterior ends were deposited at the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC) in La Jolla, California, USA. Paratypes of *Parougia ceruleibohnorum* sp. nov. and *Parougia theloniousblueski* sp. nov. were deposited at the Museo de Zoología, Universidad de Costa Rica (MZUCR) in San Jose, Costa Rica. Paratypes of *Parougia billiemiroae* sp. nov. and *Parougia sulleyi* sp. nov. were deposited at the Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM -ICML), Mazatlán, Mexico.

Prior to preservation, specimens were relaxed with 7% MgCl₂ in freshwater, photographed, and subsequently fixed in either 95% ethanol for DNA extraction or 10% formaldehyde in seawater for morphological studies. After a day, specimens preserved in formaldehyde were rinsed and transferred to 70% ethanol. Parapodia were dissected from the mid-body with a scalpel and mounted on microscope slides in Aqua-Mount media (Thermo Scientific). The maxillae and mandibles were separated by submerging the anterior part of the specimen in 85% lactic acid; in most cases, jaws were mounted on permanent slides. If the specimen was too delicate, it was transferred unmounted to slides and photographed. A Canon Rebel T3i digital camera mounted on Leica DMR stereomicroscope was used for light micrography. Most photographs were stacked and merged using Helicon Focus v. 4.2.7 (Helicon Soft Ltd.).

Examination of type material of *P. oregonensis* (Senckenberg Research Institute and Natural History Museum Frankfurt= SMF and Los Angeles County Museum-Allan Hancock Foundation, Los Angeles = LACM-AHF) and fresh collections (from this study) made it possible to reassign existing and new DNA data to the 'real' *P. oregonensis*, helping to mostly resolve a previous cryptic species complex found by Thornhill *et al.* (2012). We collected *Parougia* from the same type locality as *P. wolffi* (Juan de Fuca hydrothermal vents) and type material of *P. wolffi* (Canadian Museum of Nature= NMCA) was examined and compared with our *Parougia* specimens from this site. However, we determined based on morphology that these species were different, and the new species (*Parougia billiemiroae* sp. nov.) is described here from Juan de Fuca hydrothermal vents (and also Oregon and Mexican seeps). In contrast, specimens from a whalefall off San Diego, near the type locality of *P. batia*, could not be distinguished from type specimens of this species examined from LACM-AHF and we applied this name to the whalefall specimens and others from Hydrate Ridge, Oregon.

DNA Extraction, Amplification, and Sequencing

DNA was extracted using ZR Genomic DNATM-Tissue MiniPrep (Zymo Research, CA, USA) according to the manufacturer's protocols for solid tissue. Mitochondrial *Cytochrome c oxidase subunit I* (COI) DNA sequences were obtained for 74 specimens of *Parougia*. Mitochondrial 16S rRNA (16S), Cytochrome b (Cyt-b) and nuclear Histone H3 (H3) and 18S rRNA (18S) genetic markers were also sequenced from a representative of each putative species delineated via COI, and all sequences were submitted to GenBank (Table 1). Partial fragments of approximately 600 bp of COI, 450 bp of 16S, 400 bp of Cyt-b, 330 bp of H3, and 1300 bp of 18S were amplified using primers from previous studies and a newly

designed primer to amplify a shorter region of COI based on other *Parougia* sequences (Table 2). COI was amplified using one of the following PCR temperature profiles: 94°C/240s- (94°C/45s-50°C/60s-72°C/60s)*40 cycles-72°C/600s; 95°C/180s-(95°C/40s-50°C/40s-72°C/50s)*40 cycles-72°C/300s. 16S was amplified following: 95°C/180s-(95°C/40s-50°C/40s-72°C/50s)*35 cycles-72°C/300; Cyt-b: 94°C/180s-(94°C/30s-50°C/30s-68°C/60s)*38 cycles-68°C/420s; 95°C/300s-(95°C/30s-48°C/30s-72°C/30s)*40 cycles-72°C/420s; and H3: 95°C/180s-(95°C/30s-53°C/45s-72°C/45s)*40 cycles-72°C/300s; 18S: 95°C/30s-(95°C/30s-49°C/30s-72°C/90s)*40 cycles-72°C/480s; 95°C/180s-(95°C/30s-52°C/30s-72°C/90s)*40 cycles-72°C/480s. Standard PCR mixtures contained 12.5 µL of either Conquest™ PCR Master Mix 1 (Lambda Biotech) or Taq DNA Polymerase 2x Master Mix (Ampliqon), 1 µL of each primer pair (10 µM), 8.5 µL ddH₂O, and 2 µL template DNA with a total reaction volume of 25 µL. When amplification failed, 1:10 or 1:100 dilutions were made from starting template DNA, and PCR mixtures were corrected to contain 6.5 µL ddH₂O and 4 µL of diluted DNA. PCR products were then purified with ExoSAP-IT (USB Corporation, OH, USA) and Sanger sequencing was performed by Eurofins Genomics (Louisville, KY, USA) or Retrogen, Inc. (San Diego, CA, USA). Overlapping sequence fragments were assembled using Geneious version 11.1.5 (Biomatters Ltd).

Terminals, Alignment, and Species Delimitation

COI was usually obtained for each *Parougia* specimen collected for this study, as well as 16S and Cyt-b to aid in comparison with the Thornhill *et al.* (2012) data, which lacked COI. Following species delimitation, at least one terminal for each species was used with COI, Cyt-b, and 16S and nuclear H3 and 18S. These were analysed with data available for other *Parougia* and dorvilleid species on GenBank. We used sequences for only two of the many

available species of *Ophryotrocha* (Wiklund *et al.* 2012; Ravara *et al.* 2015). We used the two *Ophryotrocha* terminals from a previous study on the phylogeny of *Parougia* (Taboada *et al.* 2015). Similarly, we used terminals for *Dorvillea* and *Protodorvillea* as in Taboada *et al.* (2015). Thornhill *et al.* (2012) identified new *Parougia* taxa based on 16S and Cyt-b data and designated them as ‘*Parougia* Seep CA’ and ‘*Parougia* Seep OR’. They also identified several clades within what they referred to as *Parougia oregonensis*, Clade 1 and Clade 2, with Clade 1 showing two subclades. We used representative terminals of each of these five clades, with sequences concatenated based on the Supplementary Table in Thornhill *et al.* (2012), in our analyses. We used *Eunice pennata* Muller, 1776, was chosen based on previous studies (Wiklund *et al.* 2012; Ravara *et al.* 2015; Taboada *et al.* 2015).

Sequences were aligned using MAFFT v. 7 server (Katoh *et al.* 2002; Katoh and Toh 2008; Katoh and Standley 2013). Uncorrected pairwise distances and GTR corrected differences for COI were used to help delimit species and were generated using PAUP v. 4.0b10 (Swofford 2002). TCS haplotype networks (Templeton *et al.* 2002) were generated using PopArt (Leigh and Bryant 2015) for within species analyses where enough COI sequences were available. Owing to sequence length variations, sequences were trimmed to consistent lengths for making haplotype networks, resulting in a 383 bp region of COI for *P. batia*, 500 bp for *P. oregonensis*, 521 bp for *P. sulleyi* sp. nov., and 512 bp for *P. billiemiroae* sp. nov.

Phylogenetics

COI, 16S, Cyt-b, H3 and 18S data for one representative of each new species sequenced for this study was concatenated along with the available data from GenBank. The matrix was

analyzed using three phylogenetic methods. A maximum likelihood (ML) analysis was performed with RAxML 8 and RaxML GUI v1.5 (Silvestro and Michalak 2012; Stamatakis 2014). Each gene segment was allowed to have its own parameter estimates under the GTR+ Γ model of sequence evolution. ‘Thorough’ bootstrapping using 1000 pseudoreplicates and 10 ML runs was executed for each partition of the combined dataset. Bayesian inference (BI) analyses were run in MrBayes 3.2.2 (Ronquist *et al.* 2012) with the most appropriate evolutionary model for each gene obtained from jModelTest under the Akaike information criterion (AIC) (Darriba *et al.* 2012): GTR+I+G for COI and H3; GTR+G for 16S; HKY+I+G for Cyt-b, and trN+I+G for 18S. The analysis was performed using four independent runs of 10 million generations, sampling every 1000 generations and this was repeated three additional times. Tracer v. 1.6 (Rambaut and Drummond 2007; Rambaut *et al.* 2014) was used to check for convergence of the independent runs and the most suitable burn-in. The first million generation (= 1000 trees; 10% burn-in) were excluded and the remaining trees were combined into a majority-rule consensus tree with relative posterior probabilities. A maximum parsimony (MP) analysis was run with 1,000 random addition replicates using the TBR and heuristic search options. Clade support was assessed using 1,000 jackknife replicates (37% deletion).

Character Transformations

A habitat character was scored and mapped onto the ML *Parougia* tree topology (branch lengths included) using a Markov k-state one parameter (Mk1) model (Lewis 2001) with Mesquite 3.6 (Maddison and Maddison 2018). States for habitat were: **0.** shallower waters; **1.** seep; **2.** whale fall; **3.** vent; **4.** other enriched. Habitat preferences were characterized from

existing literature, or from this study (Table 1). *Protodorvillea kefersteini* (McIntosh, 1869), *Dorvillea rubrovittata* (Grube, 1855), and *E. pennata* are all from Atlantic shallow-water environments. *Dorvillea erucaeformis* Malmgren, 1865 has been found in slightly deeper waters approximately 180-260 m off the Norwegian west coast living on *Lophelia* coral, sand, gravel with sponges and serpulid tubes (Josefson 1975) but was also coded with '0' for this study. Species associated with whale falls include *Ophryotrocha lobifera* Oug, 1978, and *Ophryotrocha orensanzi* Taboada, Wiklund, Glover, Dahlgren, Cristobo & Avila, 2013. *Ophryotrocha lobifera* was originally described from the middle basin of Lindåspollene, western Norway on black mud with H₂S at 50 m depth (Oug 1978). Wiklund *et al.* (2009) reported *O. lobifera* on Minke whale bones from 125 m, however they noted that live specimens had eyes, which were not reported in the original description. GenBank data for *O. lobifera* used was amplified from those associated with Minke whale skeleton (Wiklund *et al.* 2009), but we coded this terminal as states '2/4'. Habitat preferences for *Parougia* included *P. eliasoni*, for which the types were recorded from mud at 28-30 m (Øresund Sweden; Oug 1978) and so was coded with state 0. *Parougia albomaculata* (polluted harbors; Åkesson and Rice 1992) and *P. bermudensis* (shallow wood-fall; Åkesson and Rice 1992) also are found in Atlantic shallow, organically enriched material. These terminals were coded as state 4 'other enriched'.

Results

Species Delimitation, Identity, and Phylogeny

Phylogenetic analysis of the concatenated dataset (Fig. 1) and the 74 new COI sequences (Suppl. Fig.1) supports the recognition of eight clades as new species-level taxa: two from West Pacific vents (*P. chutsaoi* sp. nov., *P. jessieae* sp. nov.) and six from East

Pacific vents, seeps, and a whalefall (*P. indiareinhardtiae* sp. nov., *P. billiemiroae* sp. nov., *P. theloniousblueski* sp. nov., *P. sulleyi* sp. nov., *P. zairahae* sp. nov., *P. ceruleibohnorum* sp. nov.). Uncorrected and model-corrected (GTR) distances for COI (using holotypes for the new taxa where possible) are summarized in Table 3. The previously described nominal species (*P. batia*, *P. oregonensis*, *P. diapason*, *P. albomaculata*, *P. eliasoni*, *P. bermudensis*) and the newly described species from this study were validated as being different species by a COI sequence divergence greater than 7%. The minimum uncorrected and GTR corrected pairwise distance was between *P. oregonensis* and *P. sulleyi* sp. nov. (uncorrected: 7.9%; GTR corrected: 8.6%) and *P. billiemiroae* sp. nov. and *P. theloniousblueski* sp. nov. (uncorrected: 8.1%; GTR corrected: 8.8%). Their assignment as separate species was further confirmed with morphological data and multi-locus analyses.

There were some exceptional specimens, however. COI distances among the *P. sulleyi* sp. nov. individuals ranged from 0% to 7.4%. The high values were based on two divergent haplotypes, one from Costa Rica and one from Mexico (Fig. 2, Suppl. Fig. 1). Similarly, among the *P. billiemiroae* sp. nov. individuals, a COI haplotype from Costa Rica was up to 5.8% divergent from the rest of the nominal species members (Fig. 2, Suppl. Fig. 1). We chose to refer to these divergent specimens as *P. cf. sulleyi* sp. nov. and *P. cf. billiemiroae* sp. nov., respectively (Fig. 3, Suppl. Fig. 1), because they were indistinguishable morphologically from their nominal conspecifics. The level of divergence suggests they may represent species complexes and further investigation will be needed beyond the scope of this study. *Parougia oregonensis* was previously recognized as a species complex, with three distinct lineages (Thornhill *et al.* 2012). Here we identify one of the Clade 1 subclades in Thornhill *et al.* (2012) as the ‘real’ *P. oregonensis* (Fig. 1), along with 11 individuals collected for this study

(see **Systematics** section below). The remaining Clade 1 subclade in Thornhill *et al.* (2012) is a new species (*P. sulleyi* sp. nov.), while we designate their ‘*P. oregonensis* Clade 2’ simply as *Parougia* sp., since there were no actual specimens available for this study (Fig. 1).

Specimens collected from Oregon and California that formed the sister group to *Parougia* sp., but were clearly a distinct clade, were identified as *Parougia batia*, which has a type locality in southern California (see **Systematics** section below). *Parougia* Seep CA and *Parougia* Seep OR from Thornhill *et al.* (2012) were clearly clades that were recovered in samples acquired for this study (Fig. 1) and these are described below as the new species *P. ceruleibohnorum* sp. nov. (= *Parougia* Seep OR) and *P. zairahae* sp. nov. (= *Parougia* Seep CA).

The concatenated alignment had 4008 positions and *Parougia* was well supported as a clade in all three analyses (bs 87, jk 85%, pp 1) with *P. diapason* from Deception Island, Antarctica Peninsula (Taboada *et al.* 2015) as sister to all other *Parougia*. The ML and BI tree topologies were congruent (Fig. 1) and we identified two main *Parougia* clades. Clade I formed a largely eastern Pacific group (*P. sulleyi* sp. nov., *P. oregonensis*, *P. zairahae* sp. nov., *ceruleibohnorum* sp. nov., *P. batia* and *Parougia* sp.), with one West Pacific vent species (*P. jessieae* sp. nov.). Clade II comprised a grade of shallow water Atlantic species (*P. eliasoni*, *P. albomaculata* and *P. bermudensis*), another largely eastern Pacific group (*P. indiareinhardtiae* sp. nov., *P. billiemiroae* sp. nov. and *P. theloniousblueski* sp. nov.), with one West Pacific vent species (*P. chutsaoi* sp. nov.). The MP analysis with 994 parsimony informative characters and two resulting shortest tree of length 4162 (Supp. Figs 2, 3) showed some placement differences with regards to previously sequenced terminals such as *P. eliasoni* and *P. albomaculata*. Although there was relatively strong support for some clades

across all analyses, the placement of *P. eliasoni* and *P. albomaculata* in particular was weakly supported and unstable (Fig. 1, Suppl. Figs 2, 3), possibly because these terminals only had three of the five genetic markers.

Haplotype Networks

Haplotype networks for COI were constructed for the four *Parougia* species (*P. billiemiroae* sp. nov., *P. batia*, *P. oregonensis*, *P. sulleyi* sp. nov.) where a reasonable number of specimens had been sequenced across several localities. For *P. oregonensis* (Fig. 2A), seven haplotypes were recovered from the 11 individuals sampled in this study. All haplotypes had representatives from Hydrate Ridge seeps, and six of those are represented by only one individual each (A2019, A2062, A2089, A2713, A10598, A10601). The seventh haplotype includes individuals from the San Diego whalefall, Hydrate Ridge, and Santa Monica Mounds (A2018, A2112, A2714, A6466, A6505, A2019; Fig. 2A). For *P. batia* (Fig. 2B), three haplotypes were recovered among 16 individuals, with two haplotypes represented by single individuals, both found at Hydrate Ridge (separated by two mutations; A10596, A10591). The most frequently sampled haplotype included 14 individuals: nine from the San Diego whalefall and the remaining five from Hydrate Ridge seeps (Fig. 2B).

The COI sequences showed a clade of 13 individuals that we refer to as *Parougia sulleyi* sp. nov. *sensu stricto* (Suppl. Fig. 1), recovering seven different haplotypes. All of these haplotypes were found in individuals from Guaymas Basin, with two of them shared with individuals from Hydrate Ridge (Fig. 2C). The most common haplotype included the holotype (A10621). The COI data revealed two other clades that were relatively close to *P. sulleyi* sp. nov. *sensu stricto*: one from Guaymas and the other from Costa Rica (Suppl. Fig.

1). These were more than 7% divergent from the type series and we refer to them here as *P. cf. sulleyi* sp. nov. pending further investigation. The three individuals of *P. cf. sulleyi* sp. nov. from the Costa Rica seeps shared the same haplotype (Fig. 2C). Among the 11 individuals sampled for *Parougia billiemiroyae* sp. nov. *sensu stricto*, five unique haplotypes were recovered (Fig. 2D). Four of those were represented by only one individual: two from individuals sampled at Guaymas basin (separated by eight mutations; A3350, A10611) and the third from Juan de Fuca (A6500). An additional haplotype included two individuals from Juan de Fuca (A6499, A6451). The most frequently sampled haplotype consisted of individuals from Juan de Fuca vents and the holotype from Hydrate Ridge (A6452, A6449, A6450, A6448, A6501, A2717; Fig. 2D). An exceptional twelfth individual from Costa Rica (A9678) was separated by at least 28 mutations from the other haplotypes and we refer to it as *Parougia cf. billiemiroyae* sp. nov.

3.4 Evolution of Vent/Seep *Parougia*

An ancestral 'seep' habitat for all *Parougia*, except *P. diapason*, was recovered when mapped on the ML *Parougia* topology (proportional likelihood of 0.72) (Fig. 3). For all of *Parougia*, the ancestral habitat was ambiguous. The results suggest that *P. chutsaoi* sp. nov. and *P. jessieae* sp. nov. independently colonized West Pacific vents (North Fiji Basin and Lau Back-Arc Basin, respectively), which are approximately 700 km apart. *Parougia billiemiroyae* sp. nov. was the only taxon found on both vents and seeps, and *P. batia* and *P. oregonensis* were found to occur at both seeps and whalefalls.

Discussion

Species delimitation

Molecular methods are rapidly evolving and are now considered a fast and efficient means of determining species delineations (Jörger and Schrödl 2013). The discovery of species complexes associated with chemosynthetic environments is becoming more frequent with use of this technology (Pfenninger and Schwenk 2007; Vrijenhoek 2009; Borda *et al.* 2013; Stiller *et al.* 2013). However, this form of systematics often remains incomplete, lacking the final step of formal species descriptions and contributing to the accumulation of ‘dark taxa’ on GenBank (Page 2016). This is clearly apparent in the presence of several *Parougia* species complexes on GenBank that were not formally described based on the study by Thornhill *et al.* (2012). Since *Parougia* was first erected by Wolf (1986), 11 species have been described, with only two associated with hydrothermal vents or methane seeps (Oug 1978; Wolf 1986). The present morphological and molecular analyses revealed much greater diversity of *Parougia* in the deep sea than previously recognized, increasing the total number of described species to 19 worldwide, with the first records of the clade from the western Pacific. Despite this increase, knowledge of the biodiversity and biogeography of *Parougia* is still fragmentary, as has also been argued for the much better known *Ophryotrocha* (Ravara *et al.* 2015). The sympatric occurrence of multiple dorvilleid species is becoming more recognized in deep chemosynthetic environments. Levin *et al.* (2013) identified 12 dorvilleid species present at Hydrate Ridge seep sediments (590-900 m); in this study, six *Parougia*, four new to science, were documented from Hydrate Ridge and several of these do not appear to have been recorded in Levin *et al.* (2013). Additionally, seven *Ophryotrocha* spp., one *Exallopus* sp., and one unidentified dorvilleid were found in microbial mats at Hydrate Ridge (Levin *et al.* 2013).

Based on COI sequences (Table 3), *Parougia sulleyi* sp. nov. *sensu stricto* was minimally ~8% divergent from its sister species *P. oregonensis*. Similarly, the type series of *P. billiemiroae* sp. nov. and *P. theloniousblueski* sp. nov. were around 8% divergent from each other. This level of COI divergence is similar to that between other closely related dorvilleid taxa. This include the sister species *Ophryotrocha flabella* Wiklund *et al.* 2012 and *O. globopalpata* Blake and Hilbig 1990, 6% divergent, that are both from the north-east Pacific (Wiklund *et al.* 2012) and the sister species *O. japonica* Paxton & Åkesson, 2010 *O. notoglandulata* Pfannenstiel, 1972 from Japan, which are 5% divergent (Wiklund *et al.* 2009). We sequenced some specimens that we refer to as *Parougia* cf. *sulleyi* sp. nov. and *P.* cf. *billiemiroae* sp. (Supplementary Figure 1) that were more than 7% divergent from their respective type series and so may prove to be other new species with further study. The other species pair described here, *P. zairahae* sp. nov., *ceruleibohnorum* sp. nov., were more than 11% divergent and all other *Parougia* species in this study are even more divergent from their nearest relatives than this (Table 3).

Connectivity

Mechanisms for larval dispersal and population connectivity have yet to be thoroughly examined in dorvilleids, but all dorvilleids have lecithotrophic development (Rouse 2000a, b). Relatively direct development and limited dispersal is known for many *Ophryotrocha* (Åkesson 1973; Ravara *et al.* 2015). However, for the dorvilleid *Schistomeringos rudolphi* (Delle Chiaje, 1828), pelagic larvae were observed swimming for up to a week before settling to the bottom (Richards 1967). Among known *Parougia*, laboratory cultures of *P. bermudensis* and *P. albomaculata* have exclusively obligate asexual reproduction (Åkesson and Rice 1992) while other *Parougia*, such as *P. diapason*, *P. nigridentata*, and *P. sulleyi* sp.

nov. likely have sexual reproduction, since oocytes have been noted (Taboada *et al.* 2015; Oug, 1978; this study). Oocytes were reported as being up to 180 μm in diameter in *P. nigridentata* females (Oug, 1978). However, it is not known if these are released directly into the water column or are deposited as egg masses.

Previous comparative studies have highlighted incongruence between macrofaunal communities at seeps and vents (Sibuet and Olu 1998; Wolff 2005; Portail *et al.* 2016), though there are exceptions, as we show here. Several of our new *Parougia* species are able to inhabit multiple chemosynthetic habitats, which is remarkable from a physiological standpoint (McMullin *et al.* 2000, Stiller *et al.* 2013; Portail *et al.* 2016). We found *Parougia oregonensis*, originally described from Hydrate Ridge by Hilbig and Fiege (2001), at seeps off California and a whalefall off San Diego (Fig 2A). We also found what clearly appears to be *Parougia batia*, which was originally described from silty mud off San Diego at ~1200 meters depth (Jumars 1974), on a whalefall off San Diego and also at Hydrate Ridge seeps (Fig. 2). *Parougia billiemiroae* sp. nov. was found at seeps in the Guaymas Basin (Gulf of California, Mexico) and at Hydrate Ridge, Oregon, and also hydrothermal vents at Juan de Fuca (Fig. 2D). *Parougia sulleyi* sp. nov. has a similar geographic range, from Oregon to Guaymas, Mexico, but only at seeps (Fig. 2C). In terms of distributional range, based on 16S data, *P. ceruleibohnorum* sp. nov. has the largest distribution, found from Oregon (Thornhill *et al.* 2012; Levin *et al.* 2013) to Costa Rica (this study). It is likely that there are many presently unknown seep habitats along the eastern Pacific margin of North and Central America that act as stepping stones for connectivity among the populations of these species. Further investigation and species delimitations are needed for our samples designated as *P. cf. sulleyi*

sp. nov. and *P. cf. billiemiroyae* sp. nov. from Costa Rica seeps (Figs 2C, D), which could also extend the ranges of these species much further south.

Phylogeny, habitat and biogeography

The poor support at some key nodes (Fig.1) means that drawing strong inferences from the present results about the evolution of *Parougia* would be premature. Also, the discovery of 8 new species level taxa in this study, with the possibility of further cryptic species (Suppl. Fig. 1), suggests that many more *Parougia* remain to be discovered in other oceans.

In terms of morphology, it is clear there are several different morphotypes within *Parougia*. Larger species from Clade I, such as *P. oregonensis* and *P. sulleyi* sp. nov. reach 2 cm or more in length with numerous chaetigers, while all the other members of Clade I were much smaller at 5 mm maximum, except for *P. batia* reaching 1 cm. In Clade II there were also species that reached 2 cm in length such as *P. billiemiroyae* sp. nov., *P. indiarenhardtae* sp. nov. while all the others have no known specimens longer than 1 cm. There is no clear trend though in the evolution of body size for the group. There was also variation in the structure and shapes of antennae and palps that had no obvious phylogenetic pattern (Table 4). Further taxon sampling and more DNA data are needed to give a more robust phylogeny for the understanding of morphological evolution in *Parougia*.

Keeping in mind the low support for several nodes, an ancestral 'seep' habitat was inferred here for *Parougia*, excepting *P. diapason* (Fig. 3). While there was some ambiguity it would appear that the vent-associated taxa: *P. chutsaoi* sp. nov., *P. jessieae* sp. nov. and *P. billiemiroyae* sp. nov. likely had seep-dwelling ancestors with at least two colonizations of vent habitats. *Parougia chutsaoi* sp. nov. and *P. jessieae* sp. nov. apparently independently

colonized West Pacific vents (North Fiji Basin and Lau Back-Arc Basin, respectively), which are approximately 700 km apart (Fig. 3). In the case of *P. billiemiroyae* sp. nov., which was found at Oregon seeps (Hydrate Ridge) and vents (Juan de Fuca) and Mexican seeps, flexibility with regard to habitat is apparent. This was also found for *Parougia batia*, which can live at seeps or whalefalls (Fig. 3). Unfortunately, with regards to the other major clade of dorvilleids associated with seeps, vents and whalefalls, the sampling vent *Ophryotrocha* with regards to DNA sequences has been limited to date (Zhang *et al.* 2017), so the habitat evolution of this group is also uncertain. Other annelids commonly found at seeps and vents include polynoid scaleworms, ampharetids and amphinomids. For polynoids such as *Branchipolynoe* a seep to vent switch has been inferred in the eastern Pacific clade (Lindgren *et al.* 2019). In contrast, a vent ancestry with colonization of seeps has also been inferred in other annelids such as ampharetids and amphinomids (Borda *et al.* 2013; Stiller *et al.* 2013). *Parougia billiemiroyae* sp. nov. was the only taxon found on both vents and seeps, and *P. batia* and *P. oregonensis* were found to occur at both seeps and whalefalls.

The West Pacific new vent-associated species, *P. chutsaoi* sp. nov. and *P. jessieae* sp. nov., each have sister species relationships with different East Pacific *Parougia*, which suggests various complex biogeographical scenarios and likely still undiscovered *Parougia* diversity. One possibility is connections via the Pacific Antarctic Ridge, which to date has not been studied. *Parougia furcata* and *P. diapason* were described from the Southern Ocean, although neither were associated with vents or seeps (Hartman 1953; Taboada *et al.* 2015). However, evidence of hydrothermal venting has been confirmed along the East Scotia Ridge (ESR) (Winckler *et al.* 2010; Rogers *et al.* 2012). Moreover, evidence from water column plumes suggest that vents may additionally occur along the Pacific sector of the Southern

Ocean providing potential suitable habitats for vent-associated *Parougia* spp. along the Pacific-Antarctic Ridge and the Macquarie Ridge complex (Tunnicliffe and Fowler 1996; Winckler *et al.* 2010; Rogers *et al.* 2012, Stiller *et al.* 2013). Since large parts of the global mid-ocean ridge system have not been surveyed, further exploration of these communities and associated fauna may give insight on deep trans-oceanic connections and circulation (Rogers *et al.* 2012; Stiller *et al.* 2013). In the present *Parougia* phylogeny, *P. diapason* (from experimentally deployed whale bones and enriched sediments in Antarctica) is sister to all other *Parougia* spp. Interestingly it also differs from all other *Parougia* species in having uniramous parapodia. Further molecular information, especially the inclusion of *P. furcata*, associated with silt and clay sediments, will help clarify the biogeographic history of the clade.

A potential link between Pacific and Atlantic vent and seep fauna is the Mid-Cayman spreading center. Prior to the closure of the Isthmus of Panama (~10-13 mya; Van Dover *et al.* 2002; De Baets *et al.* 2016) this ultraslow spreading ridge may have acted as a channel between Caribbean/Gulf of Mexico seeps and Eastern Pacific/Mid-Atlantic ridge vent systems (Tunnicliffe and Fowler 1996; Van Dover *et al.* 2002; Wheeler *et al.* 2013; Plouviez *et al.* 2015). Large-bodied annelids such as Hesionidae, Polynoidae, Siboglinidae, Maldanidae, Spionidae, Glyceridae, and Amphinomidae were found at the Von Damm and Beebe vent fields (Plouviez *et al.* 2015). It is possible that smaller dorvilleids may have been missed during sampling efforts. To date, no vent or seep *Parougia* have been recorded from the Atlantic, though several *Ophryotrocha* are known (Paxton and Morineauz 2009).

SYSTEMATICS

Family **DORVILLEIDAE** Chamberlin

Genus ***Parougia*** Wolf 1986

Type species: Schistomeringos nigridentata Oug, 1978: 291-294

Amended Diagnosis

Palps well-developed and stout; biarticulate, articulated, or smooth. Palps inserted ventro-laterally, typically shorter than antennae but may also be similar in length. Antennae articulated, well developed. Parapodia uniramous (*P. diapason*) or sub-biramous: notopodia present from chaetiger 2 (reduced to dorsal cirri). Subacicular compound chaetae include bidentate falcigers or spinigers. Supra-acicular chaetae include simple forms, some minutely bidentate, and furcate chaetae (when present) with long thin tines. Pygidium with single midventral cirrus and two dorsal cirri. Maxillae in four rows; inferior rows without basal plates. Inferior rows with free denticles and superior row with basal plates and many free denticles. The four rows can have additional layer of replacement denticles at various development stages.

Remarks

This diagnosis is emended to accommodate *P. diapason*. *Parougia* species may be difficult to identify with a stereomicroscope but are distinguishable via molecular results and/or a compound microscope (Table 4). Their ctenognath jaws were generally diagnosable among the species (see Taxonomic section), though features such as the oblong distally serrated free superior denticles, seen in *P. chutsaoi* sp. nov. + *P. billiemiroae* sp. nov. + *P. theloniousblueski* sp. nov.; *Parougia jessieae* sp. nov.; Figs. 8E, 6E, 12B, 10G, were scattered

across the phylogenetic tree shown here (Fig. 1). Oug (1978) reported double sets of maxillary parts at various stages of ontogeny in both *P. eliasoni* and *P. nigridentata*, suggesting that the maxillary apparatus underwent a series of molts (Bonnier 1893; Mierzejewski 1978; Paxton 2005). In several *Parougia* species from this study, additional sets of maxillary parts were found ventrolateral to the existing maxillae (Figs. 9E; 10G; 11B; 12B). In two species these were ambiguous in terms of identity, as either the inferior or a replacement row (e.g. *P. batia*, *P. ceruleibohnorum* sp. nov.; Figs. 4C; 7F). It has been suggested that in some *Parougia*, the replacement of old denticles may be a continuous process, correlated with age and size (Bonnier 1893; Oug 1978; Paxton 2004; Paxton 2006). However, in other *Parougia* (e.g. *P. albomaculata* and *P. bermudensis*), the maxillary apparatus did not show any distinct developmental stages and no molts were observed (MacNaughton *et al.* 2011). Table 4 provides a summary of the morphological features of *Parougia*.

***Parougia batia* (Jumars 1974)**

Dorvillea batia Jumars, 1974: 115-117 (Fig. 6)

(Fig. 4.)

Material Examined

Type material: Paratypes, LACM-AHF Poly 1075 and Poly 1076.

Other Material: SIO-BIC A10596 (GenBank 16S sequence MF176760); SIO-BIC A10591 (used for sequencing), A10592 (used for sequencing), A10593, A10594, A10595 (GenBank COI sequences MF176713, MF176714, MF176709, MF176710, MF176712, respectively); SIO-BIC A2718 (fixed in formaldehyde, not sequenced; slide of parapodia prepared) from USA, Oregon, Hydrate Ridge methane seeps, ROV *Jason II* dive 593, R/V *Atlantis*, 44.67° N,

125.098° W, 587 m, 6.ix.2011, collector Greg Rouse. SIO-BIC A10599 (GenBank *COI* sequence MF176716) and A10600 (GenBank *COI* sequence MF176715) collected on 4.ix.2011 with same collection data as above. SIO-BIC A6496 (used for sequencing and permanent slide preparation), A6442, A6497 (used for sequencing), A6443, A6498, A6444, A6445, A6447 (GenBank *COI* sequences MF176702, MF176707, MF176703, MF176704, MF176705, MF176706, MF176708, MF176711, respectively); SIO-BIC A6446 (fixed in formaldehyde, not sequenced) from USA, California, San Diego, Rosebud whalefall, ROV *Doc Ricketts* dive 623, R/V *Western Flyer*, 32.7716° N, 117.453° W, 850 m, 20.vi.2014, collector Greg Rouse.

Diagnosis

Parougia with body length ranging from 3-11 mm, live specimens pale. Head with articulated antennae longer than palps, slender with thin elongated palpostyle. Chaetae of two types: compound (spinigers) and simple chaetae. Mandibles L-shaped, with 2 additional locking mechanisms posteriomedially. Maxillae with fused superior basal plates; superior row with ~9 fused fang-like denticles and 10-11 free denticles, wing-like with 2 large terminal main teeth, anterior tooth longer.

Redescription

Body (Fig. 4A). Holotype reported by Jumars (1974) as 3.3 mm long and 0.34 mm wide; 44 chaetigers. Examined specimens 8-11 mm long; 0.75-1mm wide; 47-63 chaetigers. Live animal pale with posterior gut brightly yellow. Prostomium anteriorly rounded and dorsal-ventrally flattened; eyes absent, nuchal organ at level of eyes present. Dorsolateral pair of articulated antennae and distinctly shorter ventral-lateral biarticulate palps. Palpostyle short

with short tapering palpaphore. Peristomium as two rings. Pygidium with terminal anus and a pair of ventral-lateral cirri approximately 0.5-0.74 mm long; articulation of cirri not visible.

Parapodia (Figs. 4B, E). Parapodia sub-biramous; notopodia reduced to 'dorsal cirri' and supported by a thin acicula in cirrophore; neuropodia with thicker robust acicula. Notopodia present from chaetiger 2; cirrostyle swollen and bulbous narrows to conical tip; typically 0.5 times length of cirrophore. Neuropodia broaden distally but proportions of lobes vary as a result of muscle contraction during fixation. Majority with stubby conical acicular lobe; rounded truncate supra-acicular chaetal lobe; digitiform subacicular chaetal lobe and short pointed ventral cirri. All chaetae long and delicate, tapering to long, whiplike tips. Two types of chaetae present: supra-acicular chaetae simple with fine subdistal serration, generally shorter ventrally; sub-acicular chaetae spinigers; typically decrease in length ventrally; shafts and blades of varying length.

Jaws (Figs. 4C, D). Jaws situated within peristomium, seen to extend to chaetiger 3. Mandibles delicate with transparent and membranous L-shaped wings. Handles are heavily sclerotized; anterior-medial region of wings with a row of irregularly serrated small teeth. Distinctive for having an additional mid ventral locking mechanism. Superior basal plates form a V-shaped structure; 1 row composed of approximately 9 fused fanglike denticles. Superior free denticles approximately 10-11 elongated wing-like plates with 2 large terminal main teeth, anterior tooth longer.

Remarks

Parougia batia was originally described from Coronado Sea Fan region of the San Diego Trough, in silty mud at depths 1223-1229 m (Jumars 1974). Our specimens closely matched Jumars' (1974) description, and so the species also appears to inhabit a whale fall

(Rosebud; 850 m) very near the type locality off the coast of San Diego and also methane seeps at Oregon, Hydrate Ridge (587 m). Our specimens were much larger though, reaching well over 1 cm (Fig. 4A). Jumars (1974) described chaetae that lack denticulation and superior denticles with a single distal main fang in *P. batia*. In our specimens we found simple and compound chaetae with minute distal denticulations and free denticles with two thin main fangs distally (Figs 4B, E) and also in the paratypes examined. This may be due to differences in the microscopes used to study the chaetae.

Parougia batia is similar to sympatric *P. zairahae* sp. nov., sympatric *P. ceruleibohnorum* sp. nov., and western Pacific *P. jessieae* sp. nov. in having only spinigers. However, *P. batia* can be distinguished by the presence of only one type of spiniger instead of 2 distinct blade lengths that are found in the other taxa. *Parougia batia* is closely related to *P. jessieae* sp. nov. from Western Pacific vents (Fig. 1), both having features of slightly pink blood vessel loops, biarticulated palps with short palpohores and thin pointed palpostyles, and elongated articulated antennae. Closer examination of jaw morphology however reveals very different maxillae and mandibles (Figs. 4C, D; 10C, G).

Distribution

Distribution spans ~1500 km from Oregon, Hydrate Ridge to California, San Diego. Depth range 587-1229 m and present at whale-fall, methane seep, and silty mud environments.

***Parougia oregonensis* Hilbig and Fiege, 2001**

Parougia oregonensis Clade 1 (Thornhill et al. 2012)

(Fig. 5.)

Material Examined

Type Material: Holotype (SMF 8897) Northeast Pacific, USA, Oregon, Hydrate Ridge: SO 110/1a, ROPOS 339, 10.vii.1996, 44°40.2'N, 125°6.5'E, 632 m; paratype (SMF 8899), southern summit of Hydrate Ridge: SO 110/1a, TV-G 18, 13.vii.1996, 44°34.235'N, 125°08.891'W, 785 m; paratype (LACM-AHF Poly 1969), northern summit of Hydrate Ridge: SO 110/1a, ROPOS 341 suction sample 1 (SS 1), 12.vii.1996, 44°40.12'N, 125°05.80'E, 595 m.

Other Material: SIO-BIC A2018 (GenBank *COI* sequence MF176685) and A2019 (GenBank *COI* sequence MF176688) from USA, Oregon, Hydrate Ridge, HOV *Alvin* dive 4632, R/V *Atlantis*, 44.67° N, 125.097° W, 603 m, 4.viii.2010, collector Danwei Huang. SIO-BIC A2089 (GenBank *COI* sequence MF176695) from USA, Oregon, Hydrate Ridge, HOV *Alvin* dive 4633, R/V *Atlantis*, 44.45° N, 125.032° W, 643 m, 5.viii.2010, collector Danwei Huang. SIO-BIC A2112 (GenBank *COI* sequence MF176691) and A2062 (GenBank *COI* sequence MF176693; used for sequencing) from USA, Oregon, Hydrate Ridge, HOV *Alvin* dive 4635, R/V *Atlantis*, 44.5686° N, 125.1512° W, 795 m, 7.viii.2010, collector Danwei Huang. SIO-BIC A2713, A10598, A10601 (GenBank *COI* sequences MF176692, MF176694, MF176687, respectively) from USA, Oregon, Hydrate Ridge, ROV *Jason II* dive 593, R/V *Atlantis*, 44.67° N, 125.098° W, 587 m, 1.ix.2011 (for SIO-BIC A2713) or 4.ix.2011 (for SIO-BIC A10598, A10601), collector Greg Rouse. A2714 (GenBank *COI* sequence MF176686) from USA, Oregon, Hydrate Ridge, ROV *Jason II* dive 593, R/V *Atlantis*, 44.669° N, 125.098° W, 700 m, 2.ix.2011, collector Greg Rouse. SIO-BIC A6505 (GenBank *COI* sequence MF176689) from USA, California, Santa Monica Basin, ROV *Doc Ricketts* dive 476, R/V *Western Flyer*

33.843° N, 118.689° W, 650 m, 21.v.2013, collector Greg Rouse. SIO-BIC A6466 (GenBank COI sequence MF176690) from USA, California, San Diego, Rosebud whalefall, ROV *Doc Ricketts* dive 623, R/V *Western Flyer*, 32.7716° N, 117.453° W, 850 m, 20.vi.2014, collector Greg Rouse.

Diagnosis and Description

Larger *Parougia*, up to 2cm long, pair of articulated antennae, 25-30 articles. Palps much shorter, thicker, articulated, 10-15 articles. Live specimen distinctly yellow-orange (Figs 5A, B). Chaetae composed of three types: serrated capillaries (falcigers), furcate, and simple chaetae (Figs 5C, F). Mandibles L-shaped, smooth, delicate; handles sclerotized and with transparent wings (Fig. 5E). Basal plate of maxillae with row of fused small and heavily sclerotized teeth; around 16 major teeth alternating with smaller ones; 10-15 superior free denticles with large main fang and row of smaller teeth along cutting edge. Inferior denticles delicate; oval shape; distal denticles with whip-like terminal tooth (Fig. 5D).

Remarks

Parougia oregonensis is morphologically similar to its sympatric sister taxon *P. sulleyi* sp. nov. (see below) but can be distinguished by jaw composition and structure. The superior row of *P. sulleyi* sp. nov. is heavily sclerotized and each denticle has 1 main fang and an additional 1-2 teeth situated posteriorly (Fig. 9B). In *P. oregonensis*, the superior denticles have 1 main fang and many smaller teeth posteriorly and are less sclerotized (Fig. 5D). Additionally, the sympatric *P. batia*, *P. zairahae* sp. nov. and *P. ceruleibohnorum* sp. nov. tend to be much smaller, lack furcate chaetae, and have spinigers rather than falcigers. Thornhill *et al.* (2012) showed *Parougia oregonensis* as several distinct clades in their molecular results. No voucher material was deposited, but unambiguous sequence results

(Fig.1) allowed us to designate one subclade of their *P. oregonensis* Clade 1 as the real *P. oregonensis*. We describe the other one subclade of their *P. oregonensis* Clade 1 below as a new species, *P. sulleyi* sp. nov.

Distribution

Type locality: Oregon, Hydrate Ridge, 632 m. Molecular data from this study suggests a distributional range to San Diego, California and that *P. oregonensis* can inhabit both seep and whalefall habitats from 595-850 m depth (Fig 2B).

***Parougia billiemiroae* sp. nov.**

(Fig. 6.)

Material Examined

Holotype. SIO-BIC A2717 (fixed in formaldehyde, not sequenced) from USA, Oregon, Hydrate Ridge methane seeps, ROV *Jason II* dive 593, R/V *Atlantis*, 44.67° N, 125.098° W, 587 m, 5.ix.2011, collector Greg Rouse.

Paratypes. SIO-BIC A10627 (GenBank *COI* sequence MF176717), same collection data as holotype. SIO-BIC A3350 (GenBank *COI* sequence MF176727) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 387, R/V *Western Flyer*, 27.5904° N, 111.475° W, 1576 m, 14.iv.2012, collectors Greg Rouse and Sigrid Katz. SIO-BIC A10611 [to be UNAM -ICML XXXX (GenBank *COI* sequence MF176726) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North,

ROV *Doc Ricketts* dive 380, R/V *Western Flyer*, 27.5969° N, 111.487° W, 1583 m, 10.iv.2012, collectors Greg Rouse and Sigrid Katz. SIO-BIC A6499, A6500, A6448, A6449, A6450, A6501, A6451, A6452 (GenBank *COI* sequences MF176718, MF176719, MF176720, MF176721, MF176722, MF176723, MF176724, MF176725, respectively) from USA, Oregon, Juan de Fuca Ridge, Axial Seamount, ROV *Doc Ricketts* dive 876, R/V *Western Flyer*, 45.9891° N, 130.0267° W, 1550 m, 1.viii.2016, collector Greg Rouse.

Non-type material: *Parougia* cf. *billiemiroae* sp. nov. SIO-BIC A9678 (GenBank *COI* sequence) from Costa Rica, Jaco Scar methane seeps, HOV *Alvin* dive 4971, R/V *Atlantis*, 9.1174° N, 84.8396° W, 1796 m, 17.x.2018, collectors Erik Cordes and Rebecca Rutstein.

Other material examined:

Parougia wolffi Blake and Hilbig, 1990; paratypes NMCA 1989-0083 and NMCA 1989-0085.

Diagnosis

Larger *Parougia* species, 1-2 cm long, with a distinctive paired nuchal organ in prostomium. Can be phenotypically plastic, depending on locality; live specimen ranges from white to orange coloration; antennae articulated, thinner than smooth palps. Notopodia and lateral body segments (in most specimens) colored red-dark brown by large blood vessel loops and small pigment spots. Chaetae of two types: serrated capillaries (falcigers) and long simple chaetae. Mandibles boomerang-shaped, cutting edge with many irregular teeth. Basal plates of maxillae fused to horny base; superior denticles conical with semicircular cutting edges; inferior denticles elongated plates; both progressively featherlike toward anterior end.

Description

Body (Fig. 6A). Holotype body with 61 chaetigers, 14.5 mm in length, excluding antennae and palps. Elongate, thin; 1.1 mm at widest, tapering at each end. Live specimen body pale with yellowish-green gut contents visible posteriorly. Notopodia dark red from blood vessel loops. Prostomium distally rounded, without eyes but with paired nuchal organ; peristomium as two rings, approximately similar proportion as subsequent chaetigers. Pair of thinner articulated antennae, broken; thick smooth palps typically curled downward (distally). Pygidium small with terminal anus and pair of short, thin ventral-lateral cirri. Anterior ends of paratypes range from 3-7 mm with 29-59 chaetigers; live specimens yellow-orange. Paratype SIO-BIC A3350 differs from holotype; body stouter (18 mm long 1 mm wide) and live specimen dull yellow. Most notable difference is a pair of long articulated (10-15 articles) antennae that can be more than 3-4 times palp length. Paratypes from Juan de Fuca yellow-orange, external morphology resembles holotype.

Parapodia (Figs. 6D, F). Parapodia prepared from holotype. Parapodia on chaetiger 1 uniramous; from chaetiger 2 sub-biramous with long notopodia limited to 'dorsal cirri'; exceeds neuropodia length where best developed. Thin acicula in 'dorsal cirri'; cirrostyle distally rounded; paratypes conical, approximately 0.1-0.33 times of cirrophore length. Neuroacicula thick and robust. Neuropodia with digitiform ventral cirrus, truncate sub-acicular lobe, distally rounded acicular lobe, separated by 2 small notches with prominent conical post-chaetal lamellae; holotype post-chaetal lobe distinctly conical; paratypes with conical and distally rounded lobe; varies in length. Sub-acicular chaetal lobe often retracted but can be quite large. Sub-acicular chaetae with 13-18 falcigers, finely serrated blades and

subterminal sheath; falcigers decrease in length ventrally. Supra-acicular fascicle consisting of 6-10 simple chaetae all long, distally serrated with fine bidentate tips.

Jaws (Figs. 6B, C, E, G). Mandibles boomerang-shaped and anteriorly flared. Heavily sclerotized on handles and cutting edge. Cutting edge with 12-15 irregular teeth; outer 5 teeth small. Right and left base plates of maxillae fuse posteriorly, extends through chaetiger 2. Superior base plate composed of a single row of 7-9 fused denticles; denticles small at base and then steadily increases in size. Many superior free denticles; denticles in the shape of oval plates with approximately 15-19 pointed teeth along cutting edge; forms a semi-circle shape that curves toward fused posterior end; anterior denticles longer. Free denticles of inferior row delicate; elongated plates with rounded main fang cutting edge and row of many small teeth; as approach anterior end, denticles long and whip-like.

Remarks

Parougia billiemiroyae sp. nov. is widely distributed, found at Juan de Fuca hydrothermal vents, and also cold seeps off Oregon and Guaymas Basin with relatively little variation in COI among the samples across this range (Fig. 2D). We assigned one specimen with a divergent COI sequence from Costa Rica seeps as *Parougia* cf. *billiemiroyae* sp. nov. pending further sampling. Although morphologically similar to *P. wolfi* (Blake and Hilbig 1990), from Juan de Fuca Ridge, Axial Seamount, these species can be distinguished by having different jaw morphologies. *Parougia billiemiroyae* sp. nov. has only a single column of superior fused denticles on each superior row (Fig. 6E). Additionally, it has heavily sclerotized boomerang-shaped mandibles (Fig. 6G), instead of triangular mandibles with transparent lateral wings as seen in *P. wolfi*. Furthermore, *Parougia wolfi* is unique in the genus in having only furcate simple chaetae (Blake and Hilbig 1990). While *Parougia*

billiemiroae sp. nov. has simple chaetae with bidentate tips, we do not consider these as furcate chaetae. We examined two paratypes of *P. wolffi* from the National Museum of Canada (NMCA 1989-0083 and NMCA 1989-0085) to confirm the differences from *P. billiemiroae* sp. nov.

Parougia billiemiroae sp. nov. is genetically most similar to *P. theloniousblueski* sp. nov. (Fig. 1). However, *P. theloniousblueski* sp. nov. has weakly sclerotized mandibles with 7 distinct rounded teeth instead of cutting edge with 12-15 sharp irregular teeth (Figs. 6G; 12C).

Distribution

Type locality: USA, Oregon, Hydrate Ridge methane seeps at 587 m. Widely distributed, spanning over 3700 km from Oregon to Mexico, Guaymas Basin. Found at 587-1583 m depth.

Etymology

Named in honor of Billie Miro Breskin in appreciation for support of the SIO Collections from The Shifting Foundation.

***Parougia ceruleibohnorum* sp. nov.**

Parougia Seep Clade OR Thornhill *et al.* (2012)

(Fig. 7.)

Material Examined

Holotype. SIO-BIC A1401 (GenBank *16S* sequence MF176765, though this could be from A10628) from Costa Rica, Parrita Seep, HOV *Alvin* dive 4508, R/V *Atlantis*, 9.0303° N, 84.623° W, 1433 m, 1.iii.2009, collectors Greg Rouse and Danwei Huang.

Paratype. SIO-BIC A10628 (anterior end fixed in formaldehyde, prepared as slides; possibly GenBank *16S* sequence MF176765), same collection data as holotype. MZUCR 605-01 (two individuals fixed in formaldehyde and used for preparation of permanent slides) and SIO-BIC A1618 (GenBank *COI* sequence MF176697; used for sequencing) from Costa Rica, Mound 12 methane seep, HOV *Alvin* dive 4511, R/V *Atlantis*, 8.9305° N, 84.3123° W, 1001 m, 5.iii.2009, collectors Greg Rouse and Danwei Huang.

Diagnosis

Pale, translucent *Parougia*, smaller relative to most other species, less than 5 mm long. Chaetae composed of two types: spinigers, stiff and of 2 forms; one with longer blade, another short blade; simple chaetae. Smooth mandibles with more heavily sclerotized handles. Maxillae with fused superior basal plate; superior row with fused ridge of small teeth. Superior free denticles with 1 main fang and 2-4 smaller tooth posteriorly.

Description

Body (Figs. 7A, B). Anterior end of holotype 5 mm long. Live specimen pale white and relatively transparent. Prostomium broadly rounded, peristomium as two rings. Antennae and palps broken off; posterior end used for sequencing. Paratypes smaller, approximately 25-28 chaetigers with pair of short conical antennae and similar palps; unable to determine if palpostyle is present. Pygidium small with terminal anus and pair of prominent ventral-lateral articulated cirri, can be 0.2 times body length.

Parapodia (Figs. 7C, D, E). Parapodia on chaetiger 1 uniramous. Following parapodia sub-biramous; notopodia stout and distally rounded, bulbous. Holotype with conical cirrostyle; paratypes with no visible cirrostyle on mid-segment dorsal cirri; possibly due to fixation. Dorsal cirri with thin acicula; neuropodia with thick robust acicula. Supra-acicular

chaetae simple, distal end mostly broken. Sub-acicular chaetae spinigers stiff and of 2 types; one with longer blade, another short blade. Unable to determine if serrated.

Jaws (Figs. 7B, F, G). Mandibles smooth; handles more heavily sclerotized. Paratypes with scissor-like mandibles; center of wings weakly sclerotized; handles small and tear-drop shaped. Holotype maxillae with 4 rows; superior rows with basal plate of heavily sclerotized ridge of small teeth; 9 free denticles each with 1 main fang and smaller tooth posteriorly, some with additional smaller teeth around. Inferior rows elongated plate with main fang distally and serrations along posterior edge; however, this and additional fused ridged teeth on inferior row could be replacement rows; examination of more specimens is needed to resolve.

Remarks

Parougia ceruleibohnorum sp. nov. is phenotypically plastic. The holotype (SIO-BIC A1401) mandible is smooth and wing-like (Fig. 7G), while the mandibles of paratype (SIO-BIC A1446) are scissor-like with teardrop-like handles (Fig. 7B). We were unable to obtain COI for the holotype; however, the 16S sequences of SIO-BIC A1401 or A10628 (holotype or paratype from the same lot from Costa Rica), SIO-BIC A1446 (paratype from Costa Rica), and *Parougia* Seep Clade OR (Thornhill *et al.* 2012) were identical. The individuals from Costa Rica are separated by 432 m in depth; therefore, further examination is needed to determine if these may be separate species since some morphological differences were observed. Additionally, targeting *Parougia* Seep Clade OR from Hydrate Ridge (Thornhill *et al.* 2012) for further molecular and morphology work is needed to assess the relationship among ‘*Parougia* Seep Clade OR,’ ‘SIO-BIC A1446,’ and ‘SIO-BIC A1401/ A10628’. *Parougia* Seep Clade OR is separated by depth (588-880 m) giving *P. ceruleibohnorum* sp. nov. a depth range of 588-1433 m.

Paratype SIO-BIC A1446 is morphologically similar to *P. zairahae* sp. nov., the sympatric sister species of *P. ceruleibohnorum* sp. nov. Both have short, thick, conical palps and antennae that are approximately the same length, a unique feature not seen in other *Parougia*. Since we were unable to examine the jaws of *P. zairahae* sp. nov. as only a single anterior part was available, *P. zairahae* sp. nov. and *P. ceruleibohnorum* sp. nov. are generally cryptic with identification mainly based on DNA data. The maxillary apparatus is most similar to that of sympatric *P. sulleyi* sp. nov. (Fig. 11B) and *P. oregonensis* (Fig. 5D); all have basal plates with ridge of heavily sclerotized teeth.

Holotype SIO-BIC A1401 and paratype A10628 were found at 1433 m and paratype SIO-BIC 1446 at 1001 m off Costa Rica. Paratype SIO-BIC 1446 more closely resembles the external morphology of Thornhill *et al.* (2012) undescribed *Parougia* from Hydrate Ridge, Oregon (588 m), both small with short horn-like palps and antennae. Holotype SIO-BIC A1401 was slightly larger (palps and antennae broken off).

Distribution

Type locality: eastern Pacific, Costa Rica methane seep at 1433 m. Examined specimens were only from Costa Rica seeps at 1001 and 1433 m; however molecular data from Thornhill *et al.* (2012), with no voucher material, suggests a distribution range to Oregon methane seeps at Hydrate Ridge, 588-880 m.

Etymology

Named for the Bohn family, in appreciation of their support of the Scripps Institution of Oceanography Benthic Invertebrate Collection and their interest in deep-sea biodiversity.

***Parougia chutsaoi* sp. nov.**

(Fig. 8.)

Material Examined

Holotype. SIO-BIC A6453 (GenBank *COI* sequence MF176731) from West Pacific, North Fiji Back-Arc Basin, White Lady hydrothermal vents, ROV *Jason II* dive 149, R/V *Melville*, 16.9913° S, 173.9148° W, 1991 m, 27.v.2005, collector Greg Rouse.

Paratype. SIO-BIC A6502 (GenBank *COI* sequence MF176732), same collection data as holotype.

Other Material. SIO-BIC A10612 (multiple individuals fixed in formaldehyde, not sequenced), same collection data as holotype.

Diagnosis

Small *Parougia* species, less than 5 mm long; head with paired white nuchal organs on prostomium and pair of short articulated antennae; ventrolateral smooth tapering palps. Chaetae of two types: simple chaetae long with rounded distal end and distinct forked tip; finely serrated falcigers, all with short blades. Mandibles heavily sclerotized throughout; cutting edge irregular indentations. Maxillae with superior row fused; superior denticles oval-like plates with teeth along cutting edge, longer and whip-like anteriorly. Inferior free denticles elongated plates, distal end curved, many with fang and lateral small teeth.

Description

Body (Figs. 8A, B, D). Holotype small, anterior end 1.1 mm in length, excluding antennae and palps; 0.5 mm wide. Anterior end widest and tapers posteriorly; approximately 20-30 chaetigers. Live specimen and preserved specimen body pale. Prostomium broadly rounded with ventral medially incised upper lip. No eyes but with paired white nuchal organs. Pair of robust, short, articulated antennae (10 articles); ventrolateral smooth tapering palps,

antennae can be greater than 2 times the length of palps. Peristomium consisting of 2 rings as long as following chaetigers. Pygidium broken off in all specimens.

Parapodia (Figs. 8B, F, G). Parapodia of chaetiger 1 uniramous, following all sub-biramous. All parapodial structures vary in proportions due to fixation. Notopodia short with thin acicula; cirrostyle conical approximately half the length of cirrophore; longer at mid-body chaetigers. Neuropodia slender with robust neuroacicula; has long distally rounded digitiform ventral cirri, also seen to be pointed and short. Acicular lobe truncate; post-chaetal lobe elongated and digitiform. Sub-acicular chaetal lobe varies greatly in length; can extend the farthest and taper to a pointed tip. Chaetae long, those in mid-ramus may be longer than body width. Sub-acicular chaetae with 17-22 falcigers, finely serrated blades, and subterminal sheath. Blades all short, approximately 10% of longest falciger. Supra-acicular chaetae consisting of 6-8 simple chaetae; entire length of each chaeta same thickness; distal end rounded and notched.

Jaws (Figs. 8A, C, E). Jaws situated within peristomium; maxillae extend to chaetiger 2-3. Mandibles boomerang shaped, anteriorly flared; handles and cutting edge heavily sclerotized, wings slightly less. Cutting edge with 4-5 irregular indentations; can have a large rounded tooth on outer end. Maxillae with right and left basal plates jointed. Superior base plate short with 3-4 fused denticles; smallest at base. 18-23 superior free denticles in the shape of oval plates with heavily sclerotized teeth alternating short and long along cutting edge; forms semi-circle shape. Anterior superior free denticles, longer and whip-like. Row of medially situated weak sclerotized teeth on each. Inferior free denticles as elongated plates, distal end curved, many with main fang and lateral small teeth.

Remarks

Parougia chutsaoi sp. nov. is morphologically similar to closely related taxa *P. billiemiroae* sp. nov. and *P. theloniousblueski* sp. nov. However, it is limited to the West Pacific and can be distinguished by its small size and thick robust simple chaetae (Fig. 8G). *Parougia chutsaoi* sp. nov., from North Fiji Back-Arc Basin is phylogenetically distant to *P. jessieae* sp. nov. from the Lau Back-arc Basin. While both are small *Parougia* species, *P. jessieae* sp. nov. has spinigers and long and flowing capillaries (Figs. 10E, F) instead of falcigers (Figs. 8F, G).

Distribution

Type locality: known only from West Pacific, North Fiji Back-Arc Basin, White Lady hydrothermal vents at 1991 m.

Etymology

Named in honor of Chu Tsao Yen, the father of the first author.

***Parougia indiareinhardtiae* sp. nov.**

(Fig. 9.)

Material Examined

Holotype. SIO-BIC A6458 (GenBank *COI* sequence MF176737) from USA, California, Santa Monica Basin, ROV *Doc Ricketts* dive 475, R/V *Western Flyer*, 33.749° N, 119.053° W, 650 m, 21.v.2013, collector Greg Rouse.

Paratypes. SIO-BIC A6503 (GenBank *16S* sequence MF176771); SIO-BIC A6454, A6455, A6456, A6457, A6459, A6460 (GenBank *COI* sequences MF176733, MF176734, MF176735, MF176736, MF176738, MF176739, respectively); A6461 (fixed in formaldehyde and cut for parapodial slide preparations, not sequenced), same collection data as holotype.

Diagnosis

Larger *Parougia* species, 1-2 cm long; with pair of thin articulated antennae, pair of thicker smooth tapering palps. Live specimen transparent with orange gut and notopodia colored red by large blood vessel loops. Chaetae of 3 types: simple chaetae, furcate chaetae, and serrated falcigers. Mandibles wing-like more sclerotized anteromedially; denticulated cutting edge. Maxillae composed of a pair of wide basal plates with fused heavily sclerotized fangs distally. Free denticles featherlike towards anterior end.

Description

Body (Fig. 9A). Holotype incomplete (posterior end used for sequencing), anterior end 9 mm; segments crowded throughout, dorsal-ventrally flattened. Color in alcohol uniformly pale and opaque with dark red pigmentation in cirrophore. Paratypes range from 10-25 mm and with many chaetigers. Paratype SIO-BIC A6461 whole specimen anterior part same size as holotype (SIO-BIC A6458); body elongated approximately 16 mm in length and 1.2 mm wide (excluding parapodia), dorsal-ventrally flattened, with 78 chaetigers. Prostomium bluntly rounded with pair of smooth palps and pair of slightly thinner and longer articulated antennae (10-15 articles). Peristomium with two rings, the first slightly longer; the following segment and chaetigers approximately same length, slightly tapers at end. Pygidium small with terminal anus and a pair of thin ventral-lateral cirri. Live specimen relatively transparent with an orange-peel colored gut and mahogany-chestnut colored notopodia from large blood vessel loops

Parapodia (prepared from paratype SIO-BIC A6461, Figs. 9B, C, D). Parapodia on chaetiger 1 uniramous, following sub-biramous. Notopodia reduced to 'dorsal cirri'; supported by a delicate acicula extending slightly past cirrophore. Cirrostyle elongated and

conical, around 0.25-0.33 times the length of cirrophore. Notopodia notably long; exceeds neuropodia length where best developed. Neuropodia with thick robust acicula. Neuropodia broadens distally; with digitated ventral cirrus, stump-like pre-chaetal lobe, longer conical acicular lobe, and digitiform inflated supra-acicular chaetal lobe which; all lobes seen to vary greatly in length on same specimen due to preservation. Supra-acicular chaetae of 2 types: 4-6 long simple chaetae with minutely bidentate tips and subdistal serration; 0-1 shorter furcate chaetae with distally serrated shaft and asymmetric pubescent tines. Sub-acicular chaetae: 12-17 minutely serrated falcigers with elongated subterminal sheath; blades tend to decrease in length ventrally.

Jaws (Figs. 9E, F, G). Situated within peristomium; maxillae extend into chaetiger 4-5. Mandibles wing-like more sclerotized anteromedially; denticulated cutting edge. Superior maxillary rows composed of a pair of large basal plates each row with 6-10 large fused main fangs with numerous fine, smaller teeth along lateral edge. Many free denticles; anterior inferior free denticles become progressively longer and feather-like. Inferior denticles elongated and oval-shaped, dorsal margin with small teeth.

Remarks

Parougia indiareinhardtiae sp. nov. is the sister taxon to *P. bermudensis*, which is known from shallow-water wood falls in the North Atlantic (Åkesson and Rice 1992). Although found in different ocean basins, they are morphologically quite similar, though *Parougia bermudensis* lacks furcate chaetae. *Parougia bermudensis* has jaws with superior and inferior row denticles that are elongated oval- or spoon-shaped with small teeth in both rows and the rows are the same length (Åkesson and Rice 1992). The superior row is similar to this in *P. indiareinhardtiae* sp. nov. (Figs. 9E, F), but the inferior denticles do not extend

along the entire superior row. *Parougia indiareinhardtiae* sp. nov. is morphologically similar to *P. oregonensis* and *P. sulleyi* sp. nov., but differs markedly in jaw structure (Figs. 5E, D; 11B, C), with *P. indiareinhardtiae* sp. nov. as the only *Parougia* species with heavily sclerotized wide superior basal plates (Figs. 9E, F). Some specimens had well-developed replacement rows (Fig. 9E). The seven COI sequences acquired were very similar and no other morphological differences were seen.

Distribution

Type locality: known only from USA, California, Santa Monica Basin methane seep, 650 m.

Etymology

Named in honor of India Lily Reinhardt in appreciation for the support of the SIO Collections from The Shifting Foundation.

***Parougia jessiae* sp. nov.**

(Fig. 10.)

Material Examined

Holotype. SIO-BIC A6462 (GenBank COI sequence MF176700) from western Pacific, Lau Back-Arc Basin, Hine Hina, Southern Valu Fa Ridge, ROV *Jason II* dive 145, R/V *Melville*, 22.5323° S, 176.7185° W, 1821 m, 22.v.2005, collector Greg Rouse.

Paratypes. SIO-BIC A6504 (GenBank COI sequence MF176698) and A10623 (fixed in formaldehyde, not sequenced), same collection data as holotype. SIO-BIC A6463, A6464 (GenBank COI sequences MF176701, MF176699, respectively) from western Pacific, Lau

Back-Arc Basin, Hine Hina, Southern Valu Fa Ridge, ROV *Jason II* dive 146, R/V *Melville*, 22.5388° S, 176.7178° W), 1845 m, 23.v.2005, collector Greg Rouse.

Other Material. SIO-BIC A6465 (fixed in formaldehyde, not sequenced) from western Pacific, Lau Back-Arc Basin, Hine Hina, Southern Valu Fa Ridge, ROV *Jason II* dive 146, R/V *Melville*, 22.5388° S, 176.7178° W), 1845 m, 23.v.2005, collector Greg Rouse.

Diagnosis

Small *Parougia* species, less than 6 mm long. Pair of thin articulated antennae and pair of shorter, tapering biarticulate palps. Live specimen with fluorescent yellow-green gut and pink blood vessel loops in notopodia. Chaetae of two types: spinigers with 2 blade lengths; delicate simple chaetae that tapers to fine point (flared). Mandibles with heavily sclerotized handles and cutting plate with 2 ridges. Base plates fused into V shape; superior free denticles as curved spoon-like plates with minute teeth along cutting edge; most anterior denticles elongated with rounded tip; inferior row elongated delicate oval plates.

Description

Body (Fig. 10A). Holotype dorso-ventrally flattened; anterior end 0.75 mm wide and 2.8 mm long with 19 chaetigers. Paratypes 5-6 mm long with 28-43 chaetigers. Live specimens white, transparent with visible yellow gut that runs down body. Proximal end of parapodia lined with wave-like pink hue from internal blood vessel loops. Prostomium is anteriorly rounded with a pair of thin articulated antennae (~10 articles) and a pair of shorter biarticulate palps; peristomium as two rings, same as body width. Palpostyle thin and elongated. Pygidium with terminal anus and a pair of thin, articulated ventral-lateral cirri.

Parapodia (Figs. 10E, F). Parapodia on chaetiger 1 uniramous; following parapodia sub-biramous. Notopodia reduced to 'dorsal cirri' with delicate acicula in cirrophore;

distinctly shorter than neuropodia, including chaetae; cirrostyle shape differs due to muscle contraction during specimen preservation; most conical. Neuropodia with thick robust acicula; lobes also vary in proportions. Neuropodia with 4 lobes: short digitiform ventral cirrus, globular acicular lobe and post-chaetal lobe; truncate supra-acicular chaetal lobe. Examined specimens with supra-acicular chaetae consisting of 13-15 capillaries; long and flowing; sub-acicular chaetae with 2 types of spinigers (9-14); one with a long blade and another with a distinctive short blade; chaetae notably flaring out from neuropodia.

Jaws (Figs. 10B, C, D). Wings of mandibles transparent; slightly divergent sclerotized handles. Cutting edge with 2 distinct ridges; otherwise, smooth. Maxillae 4 rows, right and left base plates fused posteriorly; one row superior basal plate composed of 9-11 fused denticles, 12-14 superior free denticles; denticles elongated spoon-like plates, many needle-like teeth around cutting edge; medial denticles. Anterior superior free denticles elongated, curved, and triangular with rounded distal end. Inferior free denticles wide, semi-circular plate with small needle-like teeth. Replacement rows observed; delicate.

Remarks

Parougia jessieae sp. nov. is relatively unique among *Parougia* in the shape of its palps, which are biarticulate with thin palpostyles, otherwise only seen in *P. batia*. It is also similar to *P. batia*, with both showing (when alive) unusually obvious pink blood vessel loops in the notopodia and along the outer body located proximal to chaetigers and sub-chaetal spinigers (Fig. 10A). *Parougia jessieae* sp. nov. is sister to clade comprised of *P. batia* and *P. sp.* (Fig. 1.), reflecting an evolutionary connection between eastern and western Pacific faunas.

Distribution

Type locality: known only from West Pacific, Lau Back-Arc Basin, Hine Hina vent field at 1821-1845 m.

Etymology

Named in honor of Jessie Chou, the mother of the first author.

***Parougia sulleyi* sp. nov.**

Parougia oregonensis Clade 1 (part) Thornhill *et al.* (2012)

Possibly *Dorvillea* sp. Thurber *et al.* (2012)

(Fig. 11.)

Material Examined

Holotype. SIO-BIC A10621 (GenBank *COI* sequence MF176672) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 387, R/V *Western Flyer*, 27.5904° N, 111.475° W, 1576 m, 14.iv.2012, collectors Greg Rouse and Sigrid Katz.

Paratypes. SIO-BIC A10620 (GenBank *COI* sequence MF176677), same collection data as holotype. SIO-BIC A10604, A10605, A10606, A10607, A10608, A10609 [to be UNAM-ICML #####], A10610 (GenBank *COI* sequences MF176679, MF176675, MF176671, MF176674, MF176681, MF176680, MF176676, respectively) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 380, R/V *Western Flyer*, 27.5969° N, 111.487° W, 1583 m, 10.iv.2012, collectors Greg Rouse and Sigrid Katz. SIO-BIC A10616 (GenBank *16S* sequence MF176751), A10617 (GenBank *COI* sequence MF176669), and A10618 (GenBank *COI* sequence MF176670) from Mexico, Gulf

of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 379, R/V *Western Flyer*, 27.591° N, 111.4749° W, 1581 m, 10.iv.2012, collectors Greg Rouse and Sigrid Katz. SIO-BIC A10602 (GenBank *COI* sequence MF176678; used for sequencing and parapodial mount) and A10597 (GenBank *COI* sequence MF176673) from USA, Oregon, Hydrate Ridge, ROV *Jason II* dive 593, R/V *Atlantis*, 44.6701° N, 125.0987° W, 587 m, 4.ix.2011, collector Greg Rouse.

Other Material. The following specimens are designated as *Parougia* cf. *sulleyi* sp. nov. (see Results and below). SIO-BIC A1924 (GenBank *COI* sequence MF176684) and A1900 (GenBank *COI* sequence MF176683) from Costa Rica, Mount 12 methane seep, HOV *Alvin* dive 4588; R/V *Atlantis*, 8.9308° N, 84.3125° W, 997 m, 9.i.2010, collector Greg Rouse. SIO-BIC A1333 (GenBank *COI* sequence MF176682) from Costa Rica, Mound 12, HOV *Alvin* dive 4502, R/V *Atlantis*, 8.9285° N, 84.3131° W, 1000 m, 23.ii.2009, collectors Greg Rouse and Danwei Huang. SIO-BIC A10615 (GenBank *COI* sequence MF176667) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 379, R/V *Western Flyer*, 27.591° N, 111.4749° W, 1581 m, 10.iv.2012, collectors Greg Rouse and Sigrid Katz. SIO-BIC A10619 (GenBank *COI* sequence MF176668) from Mexico, Gulf of California, Guaymas Basin, seep at Pinkie's Vent North, ROV *Doc Ricketts* dive 387, R/V *Western Flyer*, 27.5904° N, 111.475° W, 1576 m, 14.iv.2012, collectors Greg Rouse and Sigrid Katz.

Diagnosis

Larger *Parougia* species, up to 2cm long, pair of articulated antennae, 25-30 articles. Palps much shorter, thicker, articulated, 10-15 articles. Live specimen orange; females with eggs in coelom and parapodia (Fig. 11A). With thick blood vessel loops, colored red, in

notopodia (Fig. 11D). Chaetae of 3 types: supra acicular chaetae simple and 0-2 furcate chaetae; subacicular chaetae all falcigers of 2 different blade lengths. Mandibles and maxillae heavily sclerotized, dark brown. Superior row with fused basal plates; denticles fang-like; inferior row with small and delicate free denticles.

Description

Body (Figs. 11A, D). Holotype body elongated, slender, with numerous segments; widest anteriorly and gradually tapers; anterior end 5 mm. Paratypes 12-16 mm; 86-108 chaetigers. Prostomium anteriorly rounded, dorsal-ventrally flattened and without eyes. Nuchal organ located behind antennae at posterior prostomial margin. Dorsal pair of thin articulated antennae; pair of slightly shorter and thicker articulated palps inserted ventrolaterally, both wider at proximal end; some with thin palpostyle. Peristomium as two rings. Pygidium with terminal anus and a pair of ventral-lateral articulated cirri, approximately twice most developed chaetiger length.

Parapodia (Figs. 11E, F, G). Parapodia from chaetiger 2 sub-biramous, with notopodia present; however, reduced to a 'dorsal cirri' supported by a delicate acicula in cirrophore. Cirrostyle large; 0.6 times the length of cirrophore when fully extended and narrows to conical tip. Neuropodia with thicker robust acicula approximately the same length of notoacicula. Neuropodia broadens out distally with prominent conical ventral cirrus, somewhat shorter sub-acicular lobe, short truncate acicular lobe, and short digitiform supra-acicular chaetal lobe. Supra-acicular composed of 5-9 long simple chaetae with fine subdistal serration; 0-2 distally serrated furcate chaetae with tapering pubescent tines; sub-acicular chaetae falcigers, bidentate blades of two different lengths.

Jaws (Figs. 11B, C). Jaws situated within peristomium in muscular pharynx; maxillae extend through chaetiger 4-5. Mandibles with thick, heavily sclerotized handles. Wings small, less sclerotized, vein-like transparency, and anteriorly flared; with smooth cutting edge. Maxillae right and left base plates fuse posteriorly; basal superior fused denticles ridge-like with many small teeth; heavily sclerotized. 3-4 fused denticles with 1 main fang and 1-2 smaller sharp tooth posterior to main fang. 20-23 superior free denticles of same shape, elongates anteriorly; dark brown. Inferior row of 20-23 transparent, small and delicate free denticles; conical plates lined with small teeth with curved tooth at distal end.

Remarks

Parougia sulleyi sp. nov. corresponds to one of the two Clade 1 clades of *P. oregonensis* identified in Thornhill *et al.* (2012) based on matching 16S and Cyt-b sequences (Fig. 1). No voucher material from Thornhill *et al.* (2012) is available to confirm this via morphology. Our COI sequences showed a clade of 13 individuals that we refer to as *Parougia sulleyi* sp. nov. *sensu stricto* (Suppl. Fig. 1, Fig. 2C), with seven haplotypes from Guaymas Basin, two of which are shared with the type locality of Hydrate Ridge (Fig. 2C). Five specimens from Costa Rica and Guaymas were morphologically indistinguishable from *Parougia sulleyi* sp. nov. *sensu stricto* but were all more than 7% divergent from the type series and we refer to these here as *P. cf. sulleyi* sp. nov. pending further investigation. The Costa Rican *P. cf. sulleyi* sp. nov. samples are the same taxon from Costa Rica reported in Thurber *et al.* (2012) as *Dorvillea* sp. This unusual species was found to be a consumer of Archaea while other *Parougia* taxa from California and Oregon showed no such signal in their stable isotope composition (Thurber *et al.* 2012). *Parougia sulleyi* sp. nov. can be differentiated from its sympatric sister taxon *P. oregonensis* by the structure of mandibles and

maxillae. The *Parougia sulleyi* sp. nov. jaw apparatus is heavily sclerotized; free denticles are elongated with 1 main fang and additional 1-2 smaller fang rather than small irregular teeth posterior to the main fang as seen in *P. oregonensis* (Figs. 5D, E; 11B, C). Replacement rows seen; mirrors superior row; however, no basal plate. With dark ridge of many small teeth transitioning to 20-23 inferior free denticles; also heavily sclerotized with 1 main fang and another smaller fang situated posteriorly; another row of delicate free denticles.

Distribution

Type locality: Guaymas Basin, Mexico, seep at 1576 m. Found at Oregon (Hydrate Ridge) and Mexican (Guaymas Basin) seeps, possibly to Costa Rican seeps (Mound 12); depth ~600-1600 m.

Etymology

Parougia sulleyi sp. nov. is named after the character James P. Sullivan (Sulley) from the Disney movie Monsters, Inc. *Parougia sulleyi* sp. nov. palps are shaped like Sulley's horns.

***Parougia theloniousblueski* sp. nov.**

(Fig. 12)

Material Examined

Holotype. SIO-BIC A1352 (GenBank *COI* sequence MF176730) from Costa Rica, Mound 11 methane seep, HOV *Alvin* dive 4504, R/V *Atlantis*, 8.9208° N, 84.3054° W, 1040 m, 25.ii.2009, collectors Greg Rouse and Danwei Huang.

Paratypes. SIO-BIC A1503 (GenBank *COI* sequence MF176729; used for sequencing and slide preparation) and A1337 (fixed in formaldehyde, not sequenced; used for slide

preparation) from Costa Rica, Mound 12, HOV *Alvin* dive 4502, R/V *Atlantis*, 8.9285° N, 84.3131° W, 1000 m, 23.ii.2009, collectors Greg Rouse and Danwei Huang. SIO-BIC A1614 (GenBank *COI* sequence MF176728), A1441 (fixed in formaldehyde, not sequenced), A1615 (one male fixed in glutaraldehyde, not sequenced), MZUCR 605-02 (one female fixed in glutaraldehyde, not sequenced) from Costa Rica, Mound 12 methane seep, HOV *Alvin* dive 4511, R/V *Atlantis*, 8.9305° N, 84.3123° W, 1001 m, 5.iii.2009, collectors Greg Rouse and Danwei Huang.

Diagnosis

Live specimens white-yellow in color. Prostomium transparent with prominent white paired nuchal organ at level of eyes. Thin articulated antennae; shorter thick smooth palps. Chaetae of 2 types: simple and compound chaetae; no furcate chaetae. Mandibles with 7 rounded teeth along cutting edge and wings weakly sclerotized. Maxillae superior free denticles oval and rounded; inferior denticles elongated plates, tapers at distal end.

Description

Body (Fig. 12A). Holotype anterior fragment 2.75 mm, whole specimen (from photo) approximately 10 mm; 1.1 mm thickest (excluding parapodia); body elongated, cylindrical, and dorsal-ventrally flattened; uniform width throughout body and tapers at posterior end; 51 chaetigers. Color in alcohol pink and live specimen pale-yellow with visible yellow-green gut. Paratypes approximately 8-18 mm long; 0.75-1.2 mm wide excluding parapodia; 42-52 chaetigers. Uniformly pale in ethanol; live specimens predominately white-yellowish in color. Prostomium rounded with no eyes but with prominent nuchal organ; with two peristomial achaetous segments each subequal length to subsequent chaetigers. Pair of thin articulated

antennae, 10-15 articles. Pair of smooth and thicker conical palps inserted ventral-laterally, half as long as palps.

Parapodia (Figs. 12D, E, F). Parapodia on chaetiger 1 uniramous. Subsequent parapodia sub-biramous with notopodia reduced to 'dorsal cirri'; narrows at both distal and proximal end; notopodia typically shorter than simple chaetae. Notopodia supported by a very thin acicula in cirrophore; cirrostyle approximately 0.33 times cirrophore, can be triangular or bulbous and ranges in length. Neuroacicula thick and robust. Neuropodia with distally rounded acicular lobe (sometimes notched), sub-acicular lobe that is often retracted, digitiform ventral cirrus, conical post-chaetal lamellae, and rounded supraacicular lobe. Sub-acicular chaetae with 19-28 compound bidentate falcigers; blades finely serrated. Supra-acicular chaetae simple; 10-13. Bidentate tips; distally serrated, as approach tip, serration becomes finer. Simple chaetae generally increase in length dorsally.

Jaws (Figs. 12B, C). Mandibles anteriorly flared with transparent lateral wings and heavily sclerotized handle. Cutting edge with 7 smooth rounded teeth; teeth progressively larger as approach outer ends. Maxillae base plates fuse posteriorly; Inferior base plates and maxillary carriers absent. Superior base plate comprised of 5-8 fused denticles followed by 10-12 superior free denticles; anterior ends increasingly overlap. Superior free denticles oval shaped, curves distally and lined with small irregular teeth; inferior denticles elongated thin plates, anterior end increasingly overlap. Replacement denticles weakly sclerotized semicircle with alternating large and small teeth around entire edge; delicately serrated triangular denticles overlapping first type (Fig. 12B).

Remarks

Parougia theloniousblueski sp. nov. is morphologically similar to its sister species *P. billiemiroae* sp. nov. However, they can be distinguished in that the former has distinctly shorter notopodia and a different jaw morphology. *Parougia theloniousblueski* sp. nov. is confined to methane seeps in Costa Rica, whereas *P. billiemiroae* sp. nov. has a potential distributional range from Hydrate Ridge, Oregon, to Guaymas Basin, Mexico. Large paired nuchal organs are seen in the clade that includes *P. billiemiroae* sp. nov., *P. chutsaoi* sp. nov. and *P. theloniousblueski* sp. nov. (Fig. 6A, 8D, 12A).

Distribution

Type locality: known only from eastern Pacific Margin, Costa Rica methane seeps, Mounds 11 and 12, ~1000 m depth.

Etymology

Named in honor of Thelonious Blue Breskin, in appreciation for the support of the SIO Collections from The Shifting Foundation.

Parougia zairahae sp. nov.

Parougia Seep Clade CA Thornhill *et al.* (2012)

(Fig. 13.)

Material Examined

Holotype. SIO-BIC A2067 (GenBank *COI* sequence MF176696) from USA, Oregon, Hydrate Ridge methane seeps, HOV *Alvin* dive 4629, R/V *Atlantis*, 44.57° N, 125.1548° W, 809 m, 1.viii.2010, collector Danwei Huang.

Diagnosis

Small *Parougia* species with paired conical, stubby palps and antennae. Chaetae of two types: spinigers of 2 blade lengths, 1 long and flowing, second shorter; simple chaetae.

Description

Body (Figs. 13A, E). Holotype posterior end used for sequencing, anterior end approximately 1 mm. Live whole specimen pale, body dorsal-ventrally flattened with 38 chaetigers. Prostomium broadly rounded; peristomium as two rings. Antennae short and conical; unable to determine if articulation present. Pair of conical palps; palpostyle indistinct. Pygidium small with terminal anus, ventral-lateral cirri broken off of specimen.

Parapodia (Figs. 13B, C, D). Parapodia on chaetiger 1 uniramous. Following parapodia sub-biramous with notopodia supported by delicate acicula; cirrostyle small and conical or not present due to fixation. With thick, robust neuroacicula. Typically, with short digitated ventral cirrus, irregularly rounded acicular lobe, and slightly longer conical post-chaetal lobe. Supra-acicular chaetae simple (6-8) with subdistal serration, tapers only slightly. Sub-acicular chaetae spinigers of 2 types (15-18); one that is long and flowing, second that is truncate and forms a short blade.

Jaws (Figs. 13A, E). Unable to closely examine jaws; only a single anterior part of specimen was available. Mandibles (from photo) heavily sclerotized; rectangle like wings with tear-drop shaped handles. Posterio-medially transparent.

Remarks

Parougia zairahae sp. nov. is most similar to sympatric sister taxon, *P. ceruleibohnorum* sp. nov., and is mainly distinguished by molecular data, since diagnostic features are currently lacking. They share very similar-looking antennae and palps. Only

the holotype was available for *P. zairahae* sp. nov. and we were unable to examine its jaw morphology in detail. From an external view, the *Parougia zairahae* sp. nov. mandibles most closely resemble *P. batia* and *P. ceruleibohnorum* sp. nov. (Figs. 4D, 7B, 13A). We assign the undescribed ‘*Parougia* Seep Clade CA’ samples in Thornhill *et al.* (2012) as *P. zairahae* sp. nov. based on 16S sequence data from the holotype matching theirs (Fig. 1). While the type locality for *Parougia zairahae* sp. nov. is Hydrate Ridge, Oregon, Levin *et al.* (2013) observed differential habitat preferences between *P. zairahae* sp. nov. and *P. ceruleibohnorum* sp. nov. with the latter collected from Hydrate Ridge, Oregon continental slope, the majority of individuals (89%) were found in clam bed habitat. On the other hand, their samples of *Parougia zairahae* sp. nov. were collected from Eel River, on the California continental slope, with the majority of individuals (70%) found in association with microbial mat sediments (Levin *et al.* 2013).

Distribution

Type locality: eastern Pacific, USA, Oregon, Hydrate Ridge methane seeps at 809 m. Molecular data from Thornhill *et al.* (2012) suggests a distribution to the north California continental slope offshore of the Eel River mouth (514-523 m) on methane seep habitats including vesicomyid clam aggregations and microbial mats.

Etymology

Named in honor of Zairah Lynn Shepard, daughter of first author’s friend, Shaun Ikaika Shepard.

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Conflict of Interest

The authors declare no conflicts of interest.

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Table 1. Collection data and GenBank accession numbers of specimens sequenced in this study, as well as terminals used in the phylogenetic analysis sourced from GenBank. New specimens are deposited in SIO-BIC, Scripps Institution of Oceanography Benthic Invertebrate Collection, unless otherwise noted. See online catalogue for further details: <https://sioapps.ucsd.edu/collections/bi/>

Holotypes of new species are indicated by asterisks. Habitat abbreviations: S = seep, V = vent, W= whalefall.

Species	Voucher SIO-BIC	Location	Site	Depth (m), Habitat	COI	16S	H3	CytB	18S
Dorvilleidae									
<i>Parougia batia</i> (Jumars 1974)	A6496	California	Rosebud	850, W	MF176702	MF176756	-	-	-
	A6442	California	Rosebud	850, W	MF176707	-	-	-	-
	A6497	California	Rosebud	850, W	MF176703	-	-	-	-
	A6443	California	Rosebud	850, W	MF176704	MF176757	MF279089	-	-
	A6498	California	Rosebud	850, W	MF176705	-	-	-	-
	A6444	California	Rosebud	850, W	MF176706	-	-	-	-
	A6445	California	Rosebud	850, W	MF176708	-	-	-	-
	A6447	California	Rosebud	850, W	MF176711	MF176759	-	-	-
	A10596	Oregon	Hydrate Ridge	587, S	-	MF176760	-	-	-
	A10591	Oregon	Hydrate Ridge	587, S	MF176713	-	-	-	-
	A10592	Oregon	Hydrate Ridge	587, S	MF176714	-	-	-	-
	A10593	Oregon	Hydrate Ridge	587, S	MF176709	-	-	-	-
	A10594	Oregon	Hydrate Ridge	587, S	MF176710	-	-	-	-

	A10595	Oregon	Hydrate Ridge	587, S	MF176712	MF176758	MF279080	MF198421	MF198412
	A10599	Oregon	Hydrate Ridge	587, S	MF176716	-	-	-	-
	A10600	Oregon	Hydrate Ridge	587, S	MF176715	-	-	-	-
<i>Parougia billiemiroae</i> sp. nov.	A10627	Oregon	Hydrate Ridge	587, S	MF176717	MF176767	MF279082	-	-
	A3350	Mexico	Guaymas Basin	1576, S	MF176727	MF176768	MF279083	MF198422	MF198415
	A6499	Juan De Fuca	Axial Seamount	1550, V	MF176718	-	-	-	-
	A6500	Juan De Fuca	Axial Seamount	1550, V	MF176719	-	-	-	-
	A6448	Juan De Fuca	Axial Seamount	1550, V	MF176720	-	-	-	-
	A6449	Juan De Fuca	Axial Seamount	1550, V	MF176721	-	-	-	-
	A6450	Juan De Fuca	Axial Seamount	1550, V	MF176722	-	-	-	-
	A6501	Juan De Fuca	Axial Seamount	1550, V	MF176723	-	-	-	-
	A6451	Juan De Fuca	Axial Seamount	1550, V	MF176724	-	-	-	-
	A6452	Juan De Fuca	Axial Seamount	1550, V	MF176725	-	-	-	-
	A10611, UNAM- ICMLxxx x	Mexico	Guaymas Basin	1583, S	MF176726	-	-	-	-
<i>Parougia</i> cf. <i>billiemiroae</i> sp. nov.	A9678	Costa Rica	Jaco Scar	1796, S		-	-	-	-
<i>Parougia ceruleibohorum</i> sp. nov.	A1618	Costa Rica	Mound 12	1001, S	MF176697	MF176764	-	-	-
	A1401* or A10628	Costa Rica	Parrita Seep	1433, S	-	MF176765	-	-	-
(= <i>Parougia</i> sp. Seep Clade OR in Thornhill et al. 2012)	None	Oregon	Hydrate Ridge	770, S	-	JX536709	-	JX536741	-

<i>Parougia chutsaoi</i> sp. nov.	A6453*	W. Pacific	N. Fiji	1991, V	MF176731	-	MF279076	-	-
	A6502	W. Pacific	N. Fiji	1991, V	MF176732	MF176766	MF279077	MF198429	MF198410
<i>Parougia indiareinhardtiae</i> sp. nov.	A6503	California	Santa Monica Basin	650, S	-	MF176771	-	-	-
	A6454	California	Santa Monica Basin	650, S	MF176733	-	-	-	-
	A6455	California	Santa Monica Basin	650, S	MF176734	MF176772	MF279081	-	MF198411
	A6456	California	Santa Monica Basin	650, S	MF176735	-	-	-	-
	A6457	California	Santa Monica Basin	650, S	MF176736	-	-	-	-
	A6458*	California	Santa Monica Basin	650, S	MF176737	-	-	-	-
	A6459	California	Santa Monica Basin	650, S	MF176738	-	-	-	-
	A6460	California	Santa Monica Basin	650, S	MF176739	-	-	-	-
<i>Parougia jessiae</i> sp. nov.	A6504	W. Pacific	Lau Back-Arc	1821, V	MF176698	-	-	-	-
	A6462*	W. Pacific	Lau Back-Arc	1821, V	MF176700	MF176761	MF279090	MF198427	MF198413
	A6463	W. Pacific	Lau Back-Arc	1845, V	MF176701	-	-	-	-
	A6464	W. Pacific	Lau Back-Arc	1845, V	MF176699	MF176762	-	MF198426	-
<i>Parougia oregonensis</i> Hilbig & Fiege 2001	A2018	Oregon	Hydrate Ridge	603, S	MF176685	MF176740	-	-	-
	A10598	Oregon	Hydrate Ridge	587, S	MF176694	MF176754	MF279078	MF198419	MF198418
	A10601	Oregon	Hydrate Ridge	587, S	MF176687	-	-	-	-
	A6505	California	Santa Monica Basin	650, S	MF176689	MF176746	-	-	-
	A2112	Oregon	Hydrate Ridge	795, S	MF176691	MF176741	-	-	-

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	A2089	Oregon	Hydrate Ridge	643, S	MF176695	MF176742	-	-	-
	A2713	Oregon	Hydrate Ridge	587, S	MF176692	-	-	-	-
	A2714	Oregon	Hydrate Ridge	700, S	MF176686	-	-	-	-
	A2062	Oregon	Hydrate Ridge	795, S	MF176693	MF176743	-	-	-
	A6466	California	Rosebud	850, W	MF176690	MF176753	MF279079	MF198420	-
	A2019	Oregon	Hydrate Ridge	603, S	MF176688	MF176744	-	-	-
(= part of <i>Parougia oregonensis</i> Clade 1 in Thornhill et al. 2012)	None	Oregon/California	Hydrate Ridge/ Eel River	514-880, S	-	JX536705	-	JX536730	-
<i>Parougia sulleyi</i> sp. nov.	A10616	Mexico	Guaymas Basin	1581, S	-	MF176751	-	-	-
	A10617	Mexico	Guaymas Basin	1581, S	MF176669	-	-	-	-
	A10618	Mexico	Guaymas Basin	1581, S	MF176670	-	-	-	-
	A10620	Mexico	Guaymas Basin	1576, S	MF176677	MF176747	-	-	-
	A10621*	Mexico	Guaymas Basin	1576, S	MF176672	MF176748	MF279087	MF198425	MF198417
	A10602	Oregon	Hydrate Ridge	587, S	MF176678	MF176749	-	-	-
	A10597	Oregon	Hydrate Ridge	587, S	MF176673	-	-	-	-
	A10604	Mexico	Guaymas Basin	1583, S	MF176679	MF176752	-	-	-
	A10605	Mexico	Guaymas Basin	1583, S	MF176675	MF176750	-	-	-
	A10606	Mexico	Guaymas Basin	1583, S	MF176671	-	-	-	-
	A10607	Mexico	Guaymas Basin	1583, S	MF176674	-	-	-	-
	A10608	Mexico	Guaymas Basin	1583, S	MF176681	-	-	-	-

	A10609, UNAM- ICML #xxxx	Mexico	Guaymas Basin	1583, S	MF176680	-	-	-	-
	A10610	Mexico	Guaymas Basin	1583, S	MF176676	-	-	-	-
(= part of <i>Parougia oregonensis</i> Clade 1 in Thornhill et al. 2012)	None	Oregon	Hydrate Ridge	872, S	-	JX536705	-	JX536733	-
<i>Parougia cf. sulleyi</i> sp. nov.	A1333	Costa Rica	Mound 12	1000, S	MF176682	MF176745	MF279088	MF198428	MF198416
	A1924	Costa Rica	Mound 12	997, S	MF176684	-	MF279075	-	-
	A1900	Costa Rica	Mound 12	997, S	MF176683	-	-	-	-
	A10619	Mexico	Guaymas Basin	1576, S	MF176668	MF176755	MF279086	MF198424	-
	A10615	Mexico	Guaymas Basin	1581, S	MF176667	-	-	-	-
<i>Parougia theloniousblueski</i> sp. nov.	A1614	Costa Rica	Mound 12	1001, S	MF176728	MF176769	MF279084	MF198423	MF198414
	A1352*	Costa Rica	Mound 11	1040, S	MF176730	-	-	-	-
	A1503	Costa Rica	Mound 12	1000, S	MF176729	MF176770	-	-	-
<i>Parougia zairahae</i> sp. nov.	A2067*	Oregon	Hydrate Ridge	809, S	MF176696	MF176763	MF279085	-	-
(= <i>Parougia</i> sp. Seep Clade CA in Thornhill et al. 2012)	None	California	Eel River	514, S	-	JX536708	-	JX536737	-
<i>Parougia</i> sp. (= <i>Parougia oregonensis</i> Clade 2 in Thornhill et al. 2012)	None	Oregon	Hydrate Ridge	770, S	-	JX536706	-	JX536735	-
<i>Parougia eliasoni</i> (Oug, 1978)	GenBank	Denmark	Oresund	Shallow	GQ415489	GQ41547	GQ415507	-	-
<i>Parougia albomaculata</i> (Åkesson & Rice, 1992)	GenBank	Spain	?	Shallow	EF464550	AF380115	JQ310784	-	-

<i>Parougia diapason</i> Taboada <i>et al.</i> 2012	GenBank	Antarctica	Whale bones	Shallow, W	KX555632	KX555630	KX555633	-	KX555631
<i>Parougia bermudensis</i> (Åkesson & Rice, 1992)	GenBank	Bermuda		Shallow	JQ310767	JQ310753	JQ310785	-	AF412802
<i>Dorvillea rubrovittata</i> (Grube, 1855)	GenBank	GenBank	GenBank	Shallow	JQ310754	GQ415457	GQ415490	-	-
<i>Dorvillea erucaeformis</i> (Malmgren, 1855)	GenBank	GenBank	GenBank	Shallow	AY838868	AY838827	-	-	AY176285
<i>Ophryotrocha lobifera</i> Oug, 1978	GenBank	GenBank	GenBank	Shallow	GQ415481	GQ415464	GQ415500	-	-
<i>Ophryotrocha orensanzi</i> Taboada <i>et al.</i> , 2013	GenBank	Antarctica	Whale bones	Shallow, W	KC123178	KC123176	KC123180	-	-
<i>Protodorvillea kefersteini</i> (McIntosh, 1869)	GenBank	Spain		Shallow	KF808171	DQ779634	DQ779759	-	AF412799
Outgroup <i>Eunice pennata</i> (Müller, 1776)	GenBank	?	?	Shallow	AY838870	AF321418	DQ779731	-	AY04068
									4

Table 2: PCR primers used in this study.

Primer	Sequence 5'-3'	References
16SarL	CGCCTGTTATCAAAAACAT	Palumbi (1996)
16SbrH	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan <i>et al.</i> (2000)
H3R	ATATCCTTRFFCATRATRGTGAC	Colgan <i>et al.</i> (2000)
COIF	TACAATTTATCGCCTAAACTTCAGCC	Nelson and Fisher (2000)
COIR	CATTTCAAGTTGTGTAAGCATC	Nelson and Fisher (2000)
DVCOIF	CGAGTTGAATTAGGWCAACCTGG	This Study
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> (1994)
HCO2198	TGTTGAAAYAAAATHGGRTCHCC	Folmer <i>et al.</i> (1994)
Cytb424F	GGWTAYGTWYTWCCWTGRGGWCARAT	Boore and Brown (2000)
Cytb-bp-876R	RAAWARRAAGTATCAYTCAGG	Oyarzun <i>et al.</i> (2011)
18S 1F	TACCTGGTTGATCCTGCCAGTAG	Giribet & Ribera 2000
18S 5R	CTTGGCAAATGCTTTCGC	Giribet <i>et al.</i> (1996)
18S 9R	GATCCTTCCGCAGGTTACCTAC	Giribet <i>et al.</i> (1996)
18S a2.0	ATGGTTGCAAAGCTGAAAC	Whiting <i>et al.</i> (1997), Whiting (2002)
18S 3F	GTTTCGATTCCGGAGAGGGA	Giribet <i>et al.</i> (1996)
18S bi	GAGTCTCGTTCGTTATCGGA	Whiting <i>et al.</i> (1997), Whiting (2002)

Table 3: Interspecific distances for COI: lowest, uncorrected (upper diagonal) and lowest GTR corrected pairwise distance (lower diagonal). For the new species the holotype was used where possible. Bold numbers show relatively close distances for taxon pairs.

<i>Parougia</i>	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>batia</i> (Jumars 1974)	---	0.183	0.187	0.190	0.180	0.171	0.220	0.209	0.207	0.211	0.218	0.258	0.168	0.205
2. <i>oregonensis</i> Hilbig & Fiege 2001	0.215	---	0.079	0.165	0.177	0.159	0.219	0.220	0.198	0.207	0.242	0.248	0.161	0.186
3. <i>sulleyi</i> , sp. nov.	0.224	0.086	---	0.172	0.149	0.155	0.214	0.209	0.195	0.204	0.244	0.236	0.160	0.180
4. <i>zairahae</i> , sp. nov.	0.225	0.190	0.200	---	0.115	0.173	0.220	0.208	0.206	0.209	0.215	0.253	0.185	0.215
5. <i>cerulibohnorum</i> , sp. nov.	0.211	0.209	0.171	0.128	---	0.155	0.224	0.197	0.203	0.198	0.235	0.257	0.176	0.197
6. <i>jessiae</i> , sp. nov.	0.199	0.184	0.178	0.201	0.180	---	0.209	0.217	0.189	0.212	0.239	0.264	0.186	0.192
7. <i>chutsaoi</i> , sp. nov.	0.266	0.264	0.258	0.266	0.274	0.251	---	0.193	0.181	0.242	0.249	0.263	0.206	0.211
8. <i>billiemiroae</i> , sp. nov.	0.251	0.267	0.251	0.250	0.231	0.261	0.229	---	0.081	0.215	0.239	0.268	0.204	0.197
9. <i>theloniousblueski</i> , sp. nov.	0.249	0.235	0.230	0.247	0.242	0.223	0.212	0.088	---	0.200	0.239	0.268	0.194	0.184
10. <i>indiasreihardtae</i> , sp. nov.	0.258	0.247	0.246	0.250	0.235	0.255	0.303	0.262	0.241	---	0.237	0.243	0.192	0.192
11. <i>diapason</i> Taboada <i>et al.</i> 2015	0.263	0.308	0.311	0.260	0.291	0.298	0.310	0.292	0.295	0.293	---	0.250	0.225	0.258
12. <i>albomaculata</i> (Åkesson & Rice 1992)	0.327	0.309	0.290	0.319	0.329	0.340	0.332	0.343	0.351	0.301	0.314	---	0.260	0.262
13. <i>eliasoni</i> (Oug 1978)	0.194	0.184	0.185	0.218	0.207	0.221	0.246	0.242	0.231	0.232	0.275	0.339	---	0.188
14. <i>bermudensis</i> (Åkesson & Rice 1992)	0.245	0.219	0.212	0.259	0.236	0.229	0.253	0.235	0.218	0.230	0.330	0.335	0.223	---

Table 4: Comparative list of selected characters for species of *Parougia*.

Species/taxon	Area, depth range	Habitat	Chaetigers	Antennae (Article number, if present)	Palps (Article number, if present)	Supra-acicular chaetae	Mandibles	Basal plates	Superior free denticles	Inferior free denticles
<i>P. albomaculata</i> (Akesson & Rice, 1992)	SW Mediterranean, N Atlantic, shallow water	Mud in polluted harbors	30-78	Articulated (10-18)	Biarticulated, tapering	Capillaries & furcates	3-4 teeth	Row of fused denticles	Clavate, main tooth, serrated edge	Spoon-shaped, serrated edge
<i>P. batia</i> (Jumars, 1974)	NE Pacific, 587-1,299 m	Silty mud, whale bones, methane seeps	44-63	Smooth	Biarticulated, tapering, short	Capillaries only	Bifurcate, denticulate antero-medially	Row of fused denticles	Rounded, main tooth, serrated edge	Rounded, serrated edge
<i>P. diapason</i> (Taboada, et. al. 2015)	Deception Island (Antarctica), 10 m	Whale bones	21-38	Articulated (2-3)	Articulated (2)	Capillaries only	No teeth, wing-like; 2 lobes	Row of small fused denticles	Squarish, main teeth, serrated edge	Elongate/squarish, serrated edge
<i>P. bermudensis</i> (Akesson & Rice, 1992)	Bermuda (N Atlantic), shallow water	Old pieces of wood	30-62	Articulated, (15-20)	Biarticulated, tapering	Short capillaries only	6 teeth	Row of fused denticles	Spoon-shaped, serrated edge	Spoon-shaped, serrated edge
<i>P. billiemiroae</i> sp. nov.	NE Pacific, 587-1583 m	Hydrothermal vents, cold seeps	29-61	Articulated (10-15)	Thick, smooth, tapering	Simple, distally serrated, bidentate tips	Sclerotized, 12-15 irregular teeth; wing-like	Row of 7-9 imbricated fused denticles	Rounded, many denticles, teeth along cutting edge	Elongated, serrated edge, anterior end denticles whip-like
<i>P. caeca</i> (Webster & Benedict, 1884)	N American coast (N Atlantic), 7-10 m	Dark mud with detritus	45-51	Articulated (12-15)	Biarticulated, tapering	Capillaries & furcates	Up to 5 teeth	Row of imbricated fused denticles	Squarish, main tooth, serrated edge	Oval, serrated edge
<i>P. ceruleibohorum</i> sp. nov.	NE Pacific, 588-1433 m	Methane seeps	25-28	Smooth short, conical	Smooth, tapering	Capillaries only	Smooth, handles more sclerotized, irregular cutting edge	2 rows of fused denticles, many, ridge-like	Squarish, heavy accessory dorsal tooth, secondary posterior tooth, slightly serrated edge	Conical, serrated edge, curved distal end
<i>P. chutsnoi</i> sp. nov.	W Pacific (North Fiji Back-Arc Basin), 1991 m	Hydrothermal vents	23-30	Articulated (~10)	Smooth, tapering	Simple chaetae with distal, forked tip; no furcates	Sclerotized, L-shaped	Row of 3-4 large fused denticles	Rounded, alternating short/long teeth	Elongated, anterior end denticles whip-like
<i>P. eliasoni</i> (Oug, 1978)	Norway (N Atlantic), 7-410 m	Mud and detritus	52-57	Articulated, (~25)	Biarticulated, tapering	Capillaries, short and stout & furcates	Up to 7 teeth	Row of large fused denticles	Squarish, main tooth, serrated edge	Rounded, serrated edge
<i>P. furcata</i> (Hartman, 1953)	South Georgia (Antarctica) 250-310 m	Soft mud with stones	~50	Articulated (?)	Biarticulated, tapering	Capillaries & furcates	No teeth/5-7 teeth	Row of imbricated fused denticles	Squarish, main tooth, serrated edge	Oval, serrated edge
<i>P. indiarenhardtae</i> sp. nov.	Santa Monica Basin (NE Pacific), 650 m	Methane seeps	16-78	Articulated (10-15)	Smooth, tapering	Simple bidentate with subdistal serration, 0-1 short furcates	Sclerotized, denticulated cutting edge	2 rows of ~10 fused denticles on large plate, sclerotized main fangs	Squarish, heavy accessory main tooth, serrated edge	Elongated, oval, feather-like
<i>P. jessiae</i> sp. nov.	W Pacific (Lau Back-Arc Basin)	Hydrothermal vents	19-43	Articulated (~10)	Biarticulated, tapering, short	Capillaries only	Smooth, 2 ridges on cutting edge, wing-like	2 rows of 9-11 fused small denticles	Rounded, some elongated, serrated edge	Elongated, oval, serrated edge
<i>P. macilenta</i> (Oug, 1978)	Lindaspollene (N Atlantic), 30-35 m	Sandy mud	30-22	Articulated (14-17)	Biarticulated	Capillaries & furcates	No teeth, partially subdivided	Row of imbricated fused denticles	Squarish, 1-2 large teeth, serrated edge	Triangular, serrated edge
<i>P. nigrindentata</i> (Oug, 1978)	Lindaspollene (N Atlantic), 20-70m	Black mud with SH2	48-57	Articulated (10-15)	Biarticulated	Capillaries & furcates	Up to 8 teeth, chitinized	Row of imbricated fused denticles	Elongate, main tooth, coarsely serrated edge	Oval, serrated edge
<i>P. oregonensis</i> (Hilbig and Fiege, 2001)	NE Pacific, 595-850 m	Cold seeps, whalefall	47-80	Articulated (25-30)	Articulated (10-15)	Capillaries & furcates	No teeth, wing-like	Row of small fused denticles	Squarish, heavy accessory dorsal tooth	Rounded, serrated edge, some with whip-like tooth
<i>P. sulleyi</i> sp. nov.	NE Pacific, 600-1600 m	Cold seeps	86-108	Articulated (25-30)	Articulated (10-15)	Capillaries, distally serrated & furcates	Smooth, large sclerotized handles	2 rows of fused denticles, many, ridge-like	Rectangle, heavy accessory dorsal tooth, secondary posterior tooth	Conical, serrated edge, curved distal end
<i>P. theloniousblueski</i> sp. nov.	Eastern Pacific Margin, Costa Rica, ~1000 m	Methane seeps	42-52	Articulated (10-15)	Thick, smooth, tapering	Simple bidentate, with subdistal serration	7 smooth teeth along cutting edge, wing-like	Row of rounded fused denticles	Rounded, serrated edge	Elongated, oval
<i>P. wolffi</i> Blake and Hilbig 1990	NE Pacific, 1545-2200 m	Hydrothermal vents	37-56	Unknown	Smooth, tapering	Simple bidentate	5 large, 5 small teeth, chitinized	2 rows of imbricated fused denticles	Elongate, whip-like tooth, slanted edge	Elongate, some slanted
<i>P. zairahae</i> sp. nov.	NE Pacific, 514-809 m	Methane seeps	38	Smooth short, conical	Smooth, tapering	Simple, subdistal serration	Rectangular wings, heavily sclerotized	Not observed	Not observed	Not observed

Figure Legends

Fig. 1. Maximum likelihood tree from concatenated 5-gene (COI, 16S, Cyt-b, H3, and 18S) dataset. ML bootstrap scores (BS) are followed by maximum parsimony (MP) jackknife consensus tree values, and BI posterior probability (PP). A hyphen (-) denotes that the node was not recovered in the MP.

Fig. 2. COI haplotype networks for two new (*P. sulleyi* sp. nov. and *P. billiemiroyae* sp. nov.) and two previously described *Parougia* species. **A.** *P. oregonensis*; **B.** *P. batia*; **C.** *P. sulleyi* sp. nov.; **D.** *P. billiemiroyae* sp. nov.

Fig. 3. Ancestral state reconstruction for habitat using the Mk1 model on the maximum likelihood tree topology. Likelihood values of greater than 50% for a given state recovered are denoted near nodes. An asterisk (*) indicates a node with values of 100%. Scores with the same % are separated by (/).

Fig. 4. *Parougia batia*. **A.** Live animals (SIO-BIC A2718), ventro-lateral palps (*pp*), antennae (*ant*); **B.** Mid-parapodium: dorsal cirrus (*dc*), supra-acicular chaetal lobe (*spl*), acicular lobe (*al*), sub-acicular chaetal lobe (*sbl*) ventral cirrus (*vc*); **C.** Partial maxillae: superior free denticles (*sfd*), fused superior denticles on basal plate (*bp*), and possible replacement denticles (*rd*); **D.** Mandibles; **E.** Sub-acicular spinigers. Scale bars= 3 mm (A), 100 µm (B), 20 µm (C, D), 30 µm (E).

Fig. 5. *Parougia oregonensis*. **A.** Anterior of live animal (SIO-BIC A2089), ventro-lateral palps (*pp*), antennae (*ant*); **B.** Whole live specimen (SIO-BIC A2713); **C.** Mid-parapodium:

dorsal cirrus (*dc*), supra-acicular chaetal lobe (*spl*), acicular lobe (*al*), post-chaetal lobe (*pocl*) ventral cirrus (*vc*).; **D.** Partial maxillae: superior free denticles (*sfd*), inferior free denticles (*ifd*), fused superior denticles on base plate (*bp*). **E.** Mandibles. **F.** Furcate chaetae. Scale bars= 2.5 mm (A, B), 100 μ m (C), 45 μ m (D), 75 μ m (E), 30 μ m (F).

Fig. 6. *Parougia billiemiroae* sp. nov. **A.** Whole live specimen (SIO-BIC A2717), ventro-lateral palps (*pp*), antennae (*ant*), nuchal organ (*n*); **B.** Mid-section free denticles: superior free denticles (*sfd*), inferior free denticles (*ifd*); **D.** Anterior free denticles; **E.** Maxillae; **F.** Mid-parapodium: dorsal cirrus (*dc*), acicular lobe (*al*), post-chaetal lobe (*pocl*); sub-acicular chaetal lobe (*sbl*), ventral cirrus (*vc*); **G.** Mandibles. Scale bars=3mm (A), 22 μ m (B), 15 μ m (C), 45 μ m (D), 7 μ m (E), 100 μ m (F), 50 μ m (G).

Fig. 7. *Parougia ceruleibohnorum* sp. nov. **A.** Anterior of preserved specimen (SIO-BIC A1401); **B.** Live animal paratypes (SIO-BIC A1446), ventro-lateral palps (*pp*), antennae (*ant*), mandibles (*man*); **C.** Sub-acicular chaetae: spinigers of two blade lengths; **D.** Closeup of parapodia (SIO-BIC A10628), dorsal cirrus (*dc*), ventral cirrus (*vc*); **E.** Mid-parapodium; **F.** Maxillae (SIO-BIC A10628), superior free denticles (*sfd*), superior fused denticles (*bp*), inferior free denticles (*ifd*). Scale bars= 2 mm (A), 1 mm (B), 20 μ m (C), 1 mm (D), 45 μ m (E), 35 μ m (F), 20 μ m (G).

Fig. 8. *Parougia chutsaoi* sp. nov. **A.** Anterior (ventral) of preserved specimen (SIO-BIC A6453), ventro-lateral palps (*pp*), antennae (*ant*), mandibles (*man*); **B.** Anterior (dorsal) of preserved specimen, maxillae (*max*); **C.** Mandibles (SIO-BIC A6502); **D.** Anterior live

specimen (SIO-BIC A10612); **E.** Partial maxillae: superior free denticles (*sfd*), base plate (*bp*), inferior free denticles (*ifd*); **F.** Mid-parapodium: dorsal cirrus (*dc*), acicular lobe (*al*), post-chaetal lobe (*pocl*); sub-acicular chaetal lobe (*sbl*), ventral cirrus (*vc*); **G.** Supra-acicular simple robust chaetae, distally notched. Scale bars= 1 mm (A, B), 25 μ m (C), 30 μ m (E), 40 μ m (F), 5 μ m

Fig. 9. *Parougia indiareinhardtiae* sp. nov. **A.** Live specimen (SIO-BIC A6461), ventro-lateral palps (*pp*), antennae (*ant*); **B.** Mid-parapodium: dorsal cirrus (*dc*), supra-acicular chaetal lobe (*spl*), acicular lobe (*al*), pre-chaetal lobe (*prcl*), ventral cirrus (*vc*); **C.** Supra-acicular furcate chaeta; **D.** Sub-acicular chaetae, falcigers; **E.** Maxillae: superior free denticles (*sfd*), superior fused denticles on base plate (*bp*), inferior free denticles (*ifd*), replacement denticles (*rd*); **F.** Mandibles; **G.** Maxillae without replacement denticles. Scale bars=2 mm (A), 70 μ m (B), 20 μ m (C, D), 30 μ m (E), 45 μ m (F), 40 μ m (G).

Fig. 10. *Parougia jessieae* sp. nov. **A.** Live whole specimen (SIO-BIC A6465), ventro-lateral palps (*pp*), antennae (*ant*); **B.** Mandibles; **C.** Left mandible; **D.** Anterior of preserved specimen (SIO-BIC A10623); **E.** Sub-acicular spinigers of two blade lengths; **F.** Mid-parapodium; dorsal cirrus (*dc*), supra-acicular chaetal lobe (*spl*), acicular lobe (*al*), post-chaetal lobe (*pocl*), ventral cirrus (*vc*); **G.** Partial maxillae: superior free denticles (*sfd*), fused denticles on base plate (*bp*), inferior free denticles (*ifd*), replacement denticles (*rd*). Scale bars= 1 mm (A), 20 μ m (B, G), 15 μ m (C), 1 mm (D), 30 μ m (E), 70 μ m (F).

Fig. 11. *Parougia sulleyi* sp. nov. **A.** Live animal (SIO-BIC A10616), female with eggs in coelom; **B.** Partial maxillae: superior free denticles (*sfd*), superior fused denticles on base plate (*bp*), inferior free denticles (*ifd*), replacement denticles (*rd*); **C.** Left mandible; **D.** Anterior of live specimen (SIO-BIC A1333, *Parougia* cf. *sulleyi* sp. nov.), ventro-lateral palps (*pp*), antennae (*ant*), blood vessel loops in dorsal cirri (notopodia); **E.** Mid-parapodium: supra-acicular chaetal lobe (*spl*), acicular lobe (*al*), sub-acicular chaetal lobe (*sbl*), ventral cirrus (*vc*); **F.** Supra-acicular furcate chaeta; **G.** Mid-parapodium: dorsal cirrus (*dc*). Scale bars= 1 mm (A), 30 μ m (B), 40 μ m (C), 0.5 mm (D) 70 μ m (E, G), 25 μ m (F).

Fig. 12. *Parougia theloniousblueski* sp. nov. **A.** Live animals (SIO-BIC A1441), ventro-lateral palps (*pp*), antennae (*ant*); **B.** Partial maxillae: superior free denticles (*sfd*), superior fused denticles (*sfsd*), inferior free denticles (*ifd*), replacement denticles (*rd*); **C.** Mandibles; **D.** Mid-parapodium: dorsal cirrus (*dc*); **E.** Supra acicular simple chaetae, notched; **F.** Mid-parapodium: acicular lobe (*al*), post-chaetal lobe (*pocl*), sub-acicular chaetal lobe (*sbl*), ventral cirrus (*vc*). Scale bars=4 mm (A), 25 μ m (B), 30 μ m (C), 15 μ m (D), 10 μ m (E), 50 μ m (F).

Fig. 13. *Parougia zairahae* sp. nov. **A.** Live animal (ventral) (SIO-BIC A2067), mandibles (*man*); **B.** Mid-parapodium: dorsal cirrus (*dc*), post-chaetal lobe (*pocl*), acicular lobe (*al*), ventral cirrus (*vc*); **C.** Sub-acicular spinigers, long and flowing; **D.** Sub acicular spinigers, short and blunt; **E.** Dorsal view of preserved specimen (SIO-BIC A2067), ventro-lateral palps (*pp*), antennae (*ant*). Scale bars= 1 mm (A), 50 μ m (B), 20 μ m (C), 15 μ m (D), 70 μ m (E).

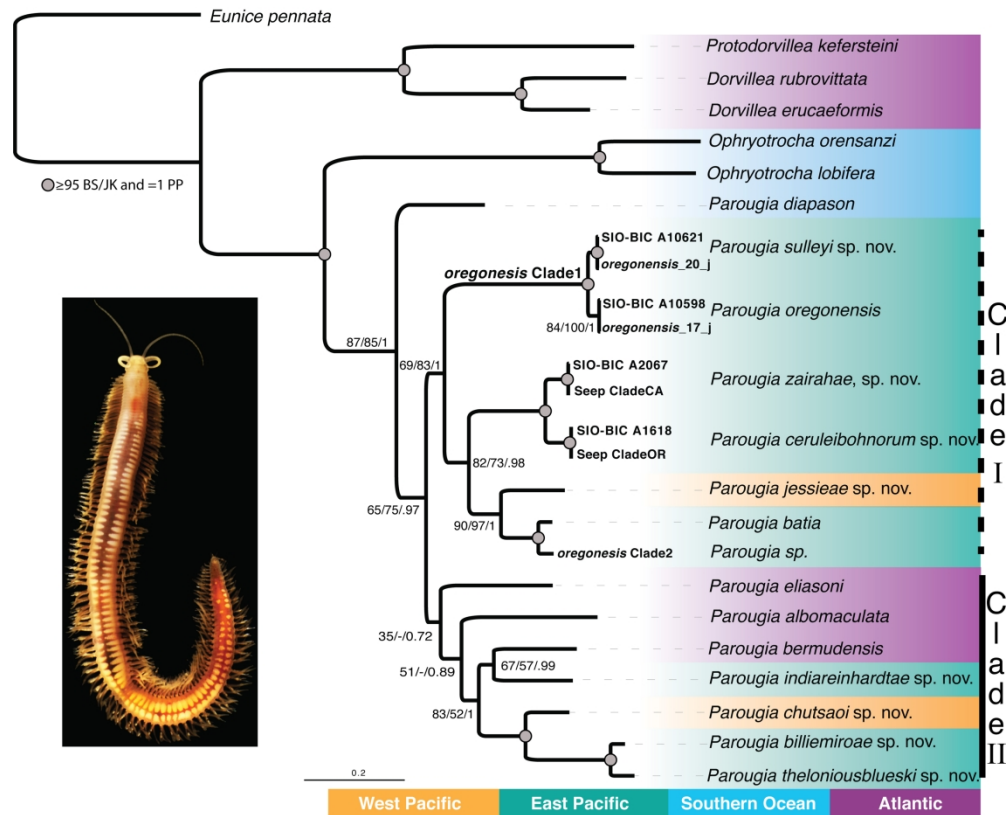


Fig. 1. Maximum likelihood tree from concatenated 5-gene (COI, 16S, Cyt-b, H3, and 18S) dataset. ML bootstrap scores (BS) are followed by maximum parsimony (MP) jackknife consensus tree values, and BI posterior probability (PP). A hyphen (-) denotes that the node was not recovered in the MP.

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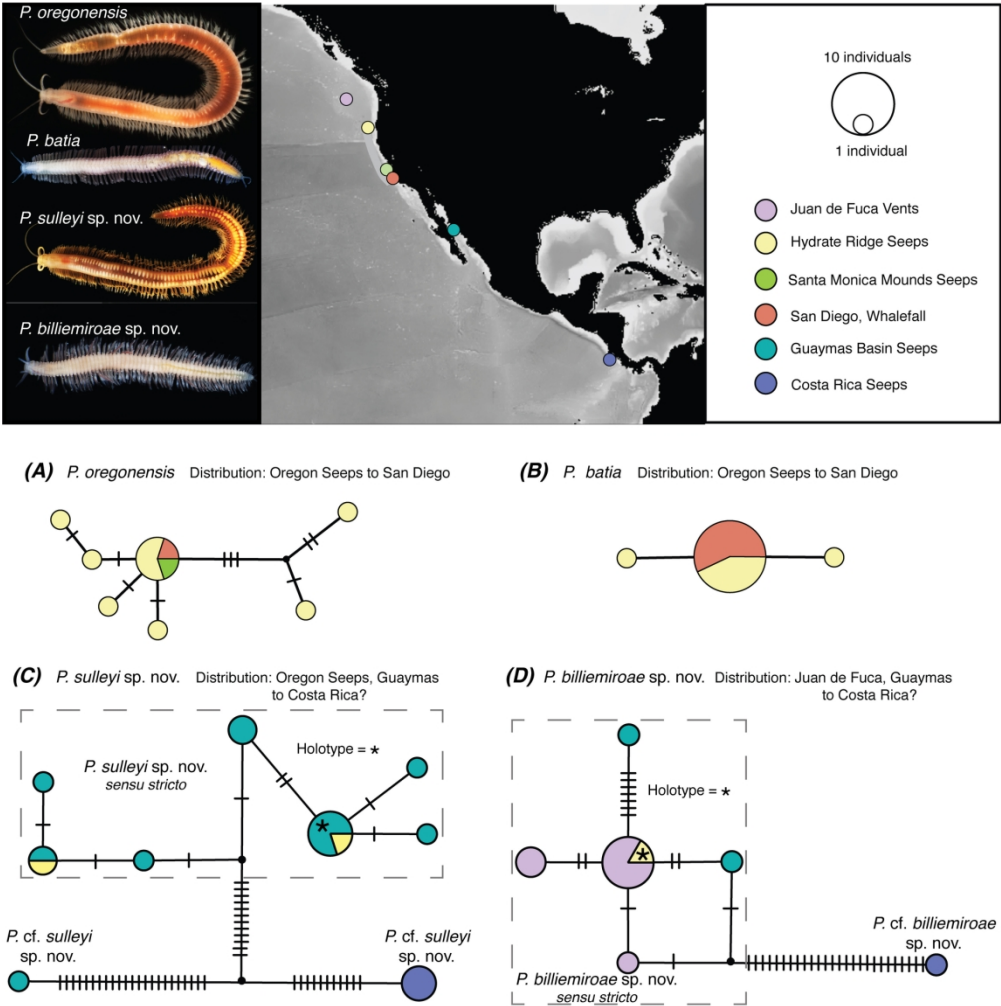


Fig. 2. COI haplotype networks for two new (*P. sulleyi* sp. nov. and *P. billiemiroyae* sp. nov.) and two previously described *Parougia* species. A. *P. oregonensis*; B. *P. batia*; C. *P. sulleyi* sp. nov.; D. *P. billiemiroyae* sp. nov.

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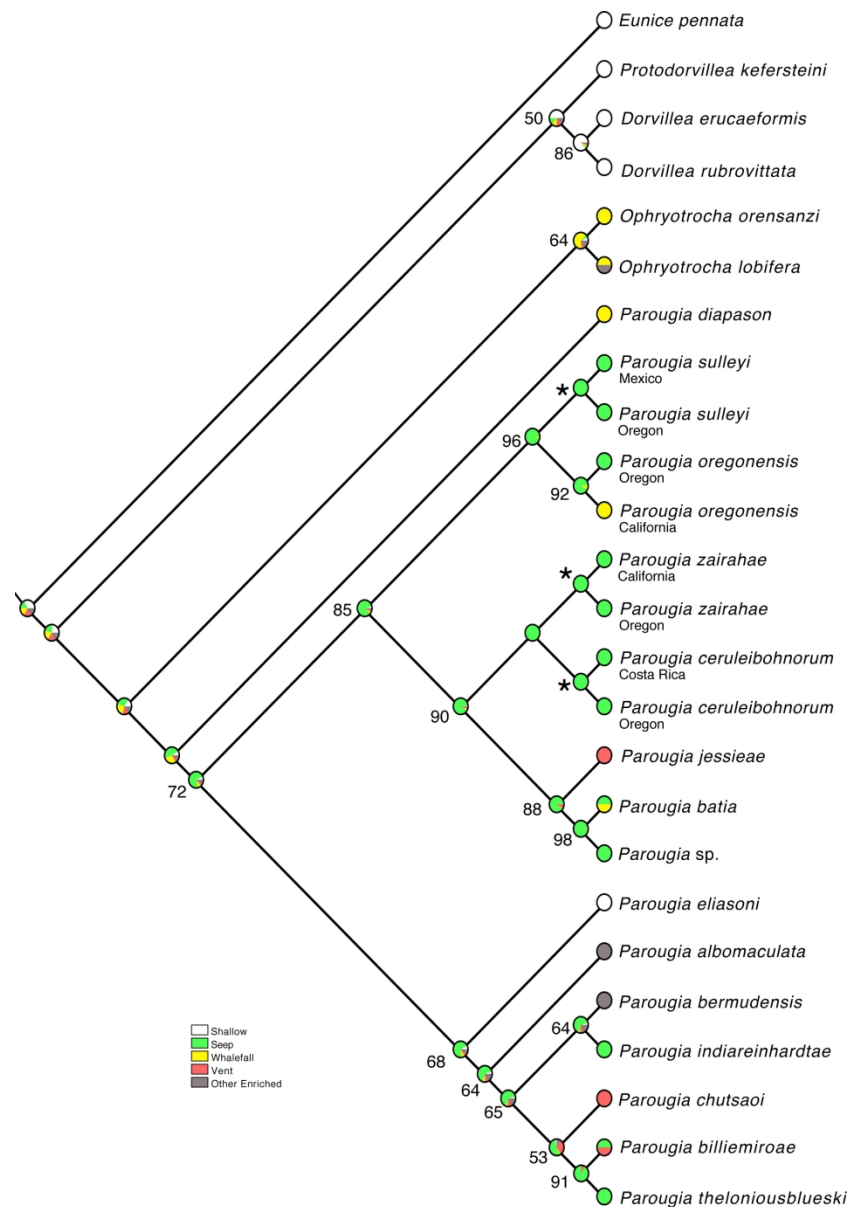


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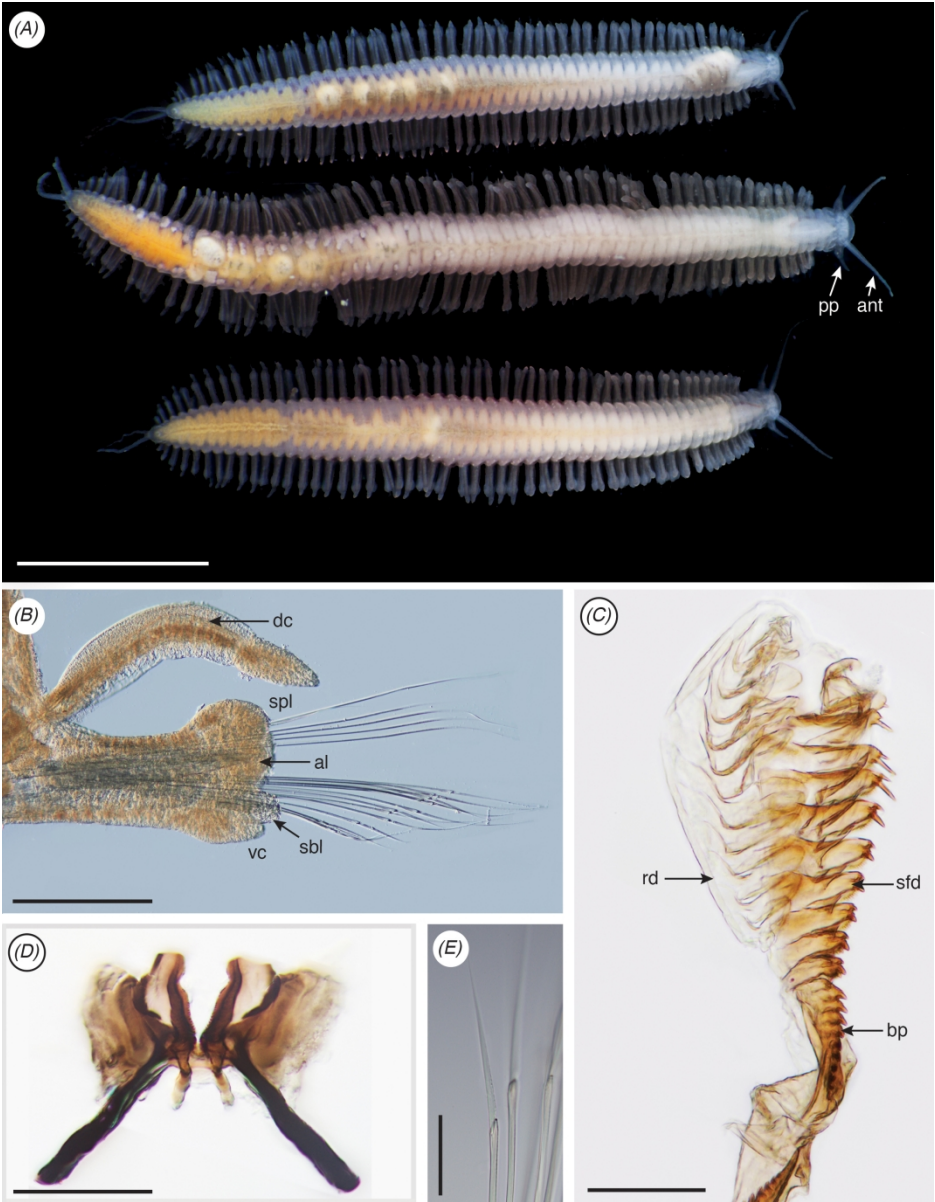


Fig. 4. *Parougia batia*. A. Live animals (SIO-BIC A2718), ventro-lateral palps (pp), antennae (ant); B. Mid-parapodium: dorsal cirrus (dc), supra-acicular chaetal lobe (spl), acicular lobe (al), sub-acicular chaetal lobe (sbl) ventral cirrus (vc); C. Partial maxillae: superior free denticles (sfd), fused superior denticles on basal plate (bp), and possible replacement denticles (rd); D. Mandibles; E. Sub-acicular spinigers. Scale bars= 3 mm (A), 100 µm (B), 20 µm (C, D), 30 µm (E).

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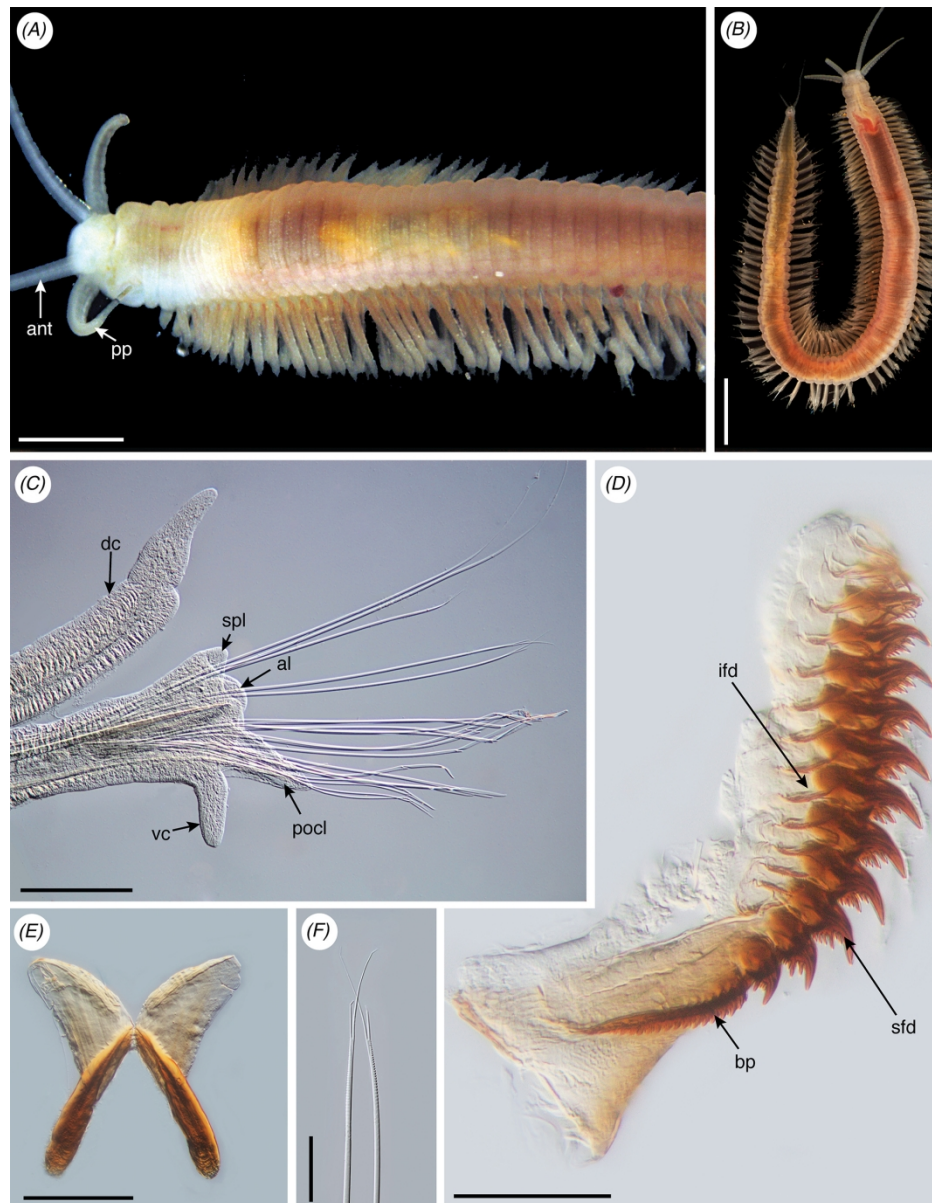


Fig. 5. *Parougia oregonensis*. A. Anterior of live animal (SIO-BIC A2089), ventro-lateral palps (pp), antennae (ant); B. Whole live specimen (SIO-BIC A2713); C. Mid-parapodium: dorsal cirrus (dc), supra-acicular chaetal lobe (spl), acicular lobe (al), post-chaetal lobe (pocl) ventral cirrus (vc).; D. Partial maxillae: superior free denticles (sfd), inferior free denticles (ifd), fused superior denticles on base plate (bp). E. Mandibles. F. Furcate chaetae. Scale bars= 2.5 mm (A, B), 100 μ m (C), 45 μ m (D), 75 μ m (E), 30 μ m (F).

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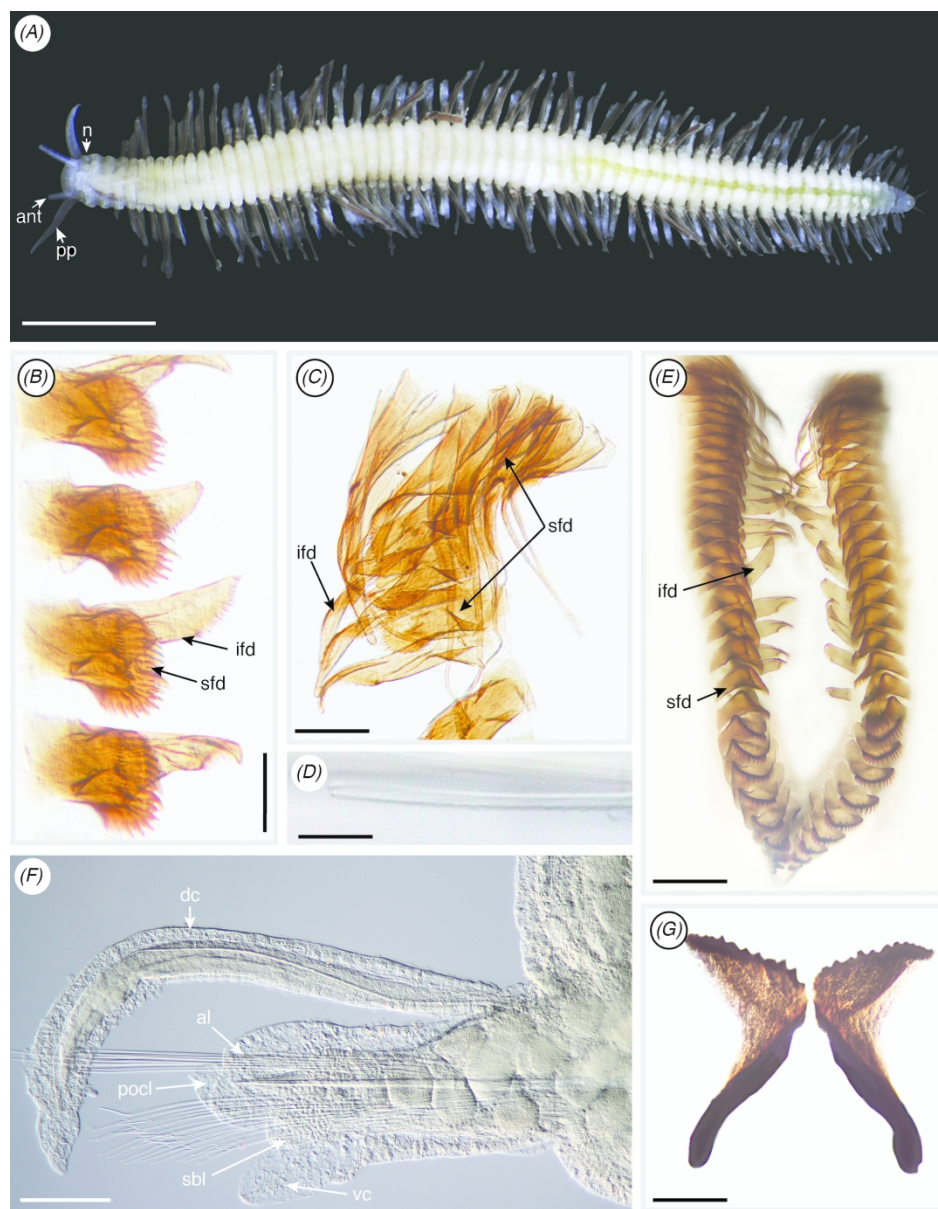


Fig. 6. *Parougia billiemiroae* sp. nov. A. Whole live specimen (SIO-BIC A2717), ventro-lateral palps (pp), antennae (ant), nuchal organ (n); B. Mid-section free denticles: superior free denticles (sfd), inferior free denticles (ifd); D. Anterior free denticles; E. Maxillae; F. Mid-parapodium: dorsal cirrus (dc), acicular lobe (al), post-chaetal lobe (pocl); sub-acicular chaetal lobe (sbl), ventral cirrus (vc); G. Mandibles. Scale bars=3mm (A), 22 μ m (B), 15 μ m (C), 45 μ m (D), 7 μ m (E), 100 μ m (F), 50 μ m (G).

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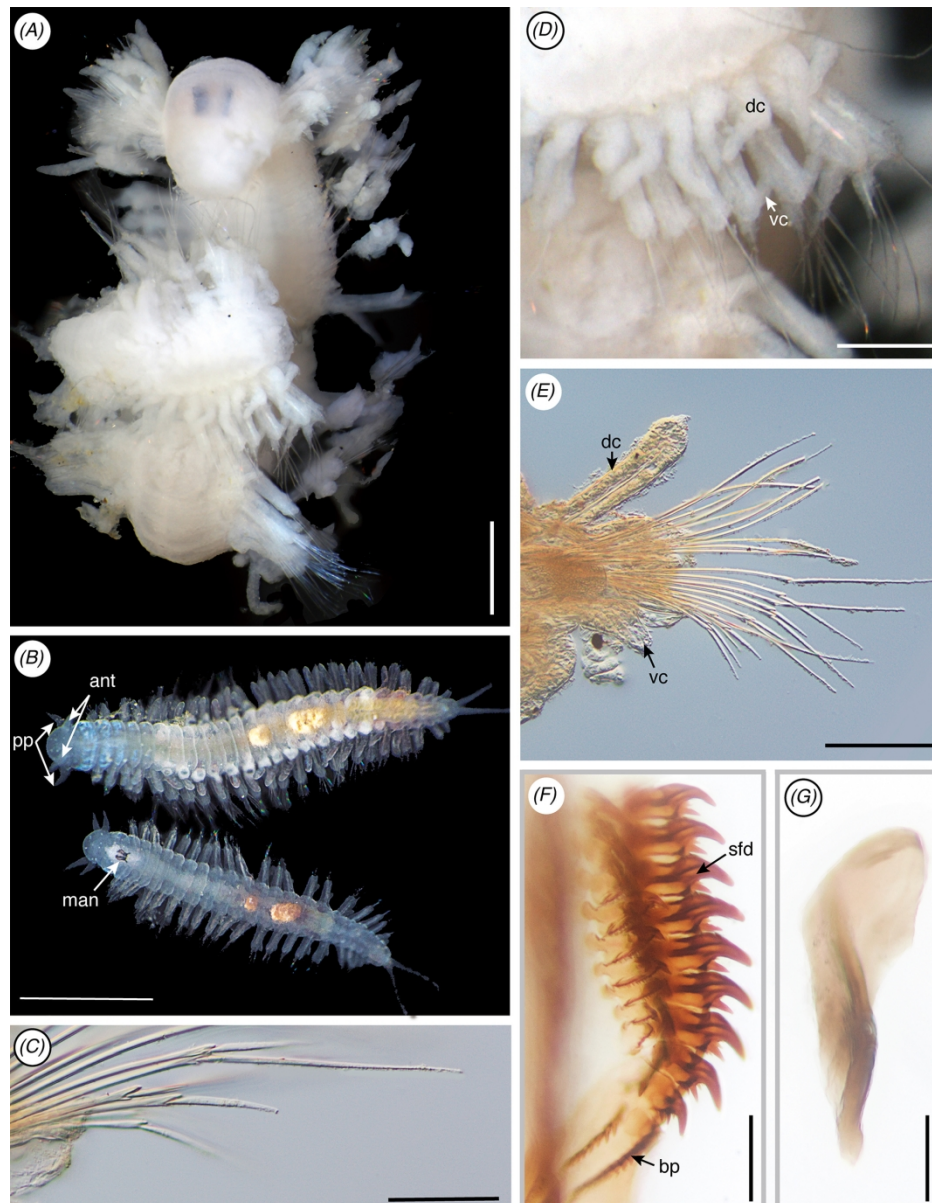


Fig. 7. *Parougia ceruleibohnorum* sp. nov. A. Anterior of preserved specimen (SIO-BIC A1401); B. Live animal paratypes (SIO-BIC A1446), ventro-lateral palps (pp), antennae (ant), mandibles (man); C. Sub-acicular chaetae: spinigers of two blade lengths; D. Closeup of parapodia (SIO-BIC A10628), dorsal cirrus (dc), ventral cirrus (vc); E. Mid-parapodium; F. Maxillae (SIO-BIC A10628), superior free denticles (sfd), superior fused denticles (bp), inferior free denticles (ifd). Scale bars= 2 mm (A), 1 mm (B), 20 µm (C), 1 mm (D), 45 µm (E), 35 µm (F), 20 µm (G).

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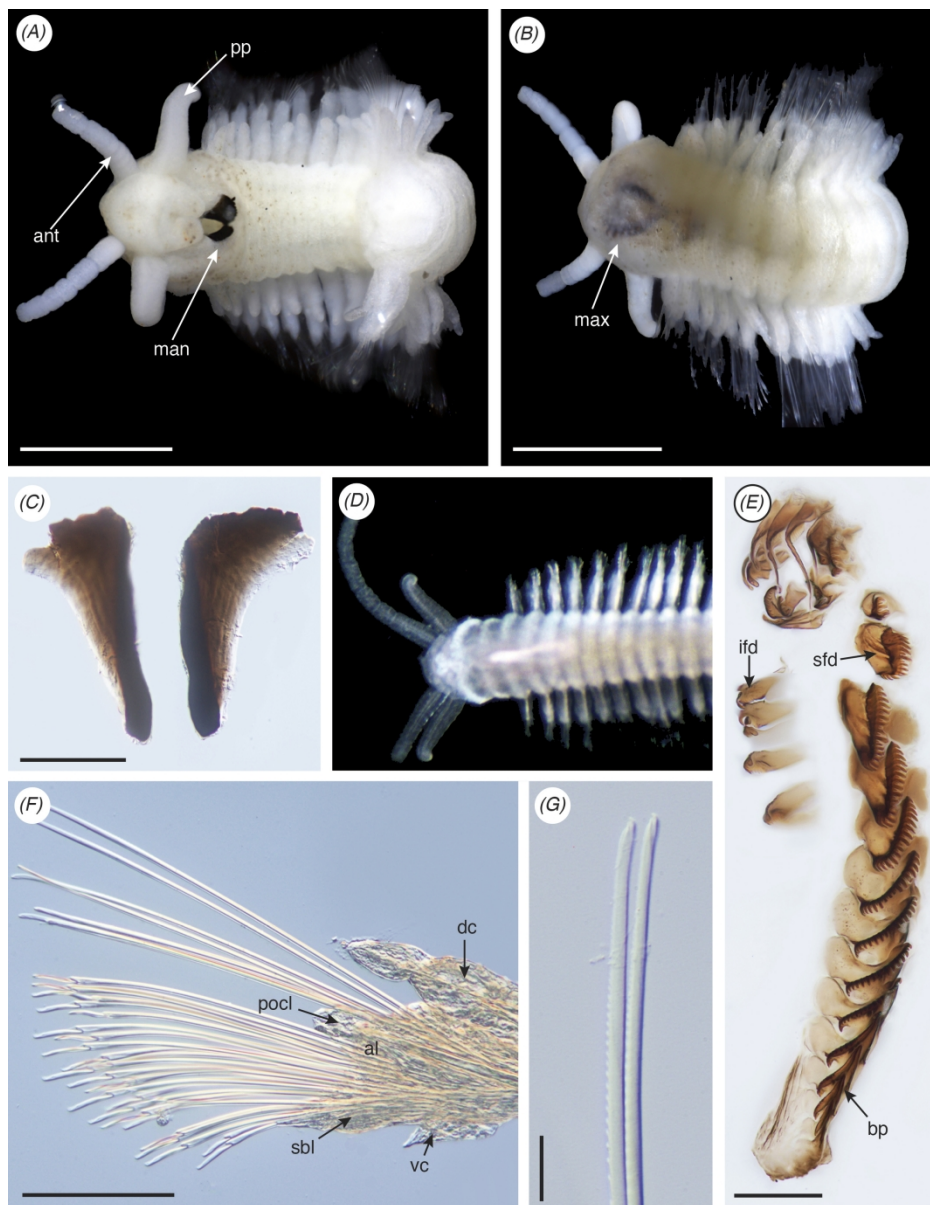


Fig. 8. *Parougia chutsaoi* sp. nov. A. Anterior (ventral) of preserved specimen (SIO-BIC A6453), ventro-lateral palps (pp), antennae (ant), mandibles (man); B. Anterior (dorsal) of preserved specimen, maxillae (max); C. Mandibles (SIO-BIC A6502); D. Anterior live specimen (SIO-BIC A10612); E. Partial maxillae: superior free denticles (sfd), base plate (bp), inferior free denticles (ifd); F. Mid-parapodium: dorsal cirrus (dc), acicular lobe (al), post-chaetal lobe (pocl); sub-acicular chaetal lobe (sbl), ventral cirrus (vc); G. Supra-acicular simple robust chaetae, distally notched. Scale bars= 1 mm (A, B), 25 μ m (C), 30 μ m (E), 40 μ m (F), 5 μ m

215x277mm (300 x 300 DPI)

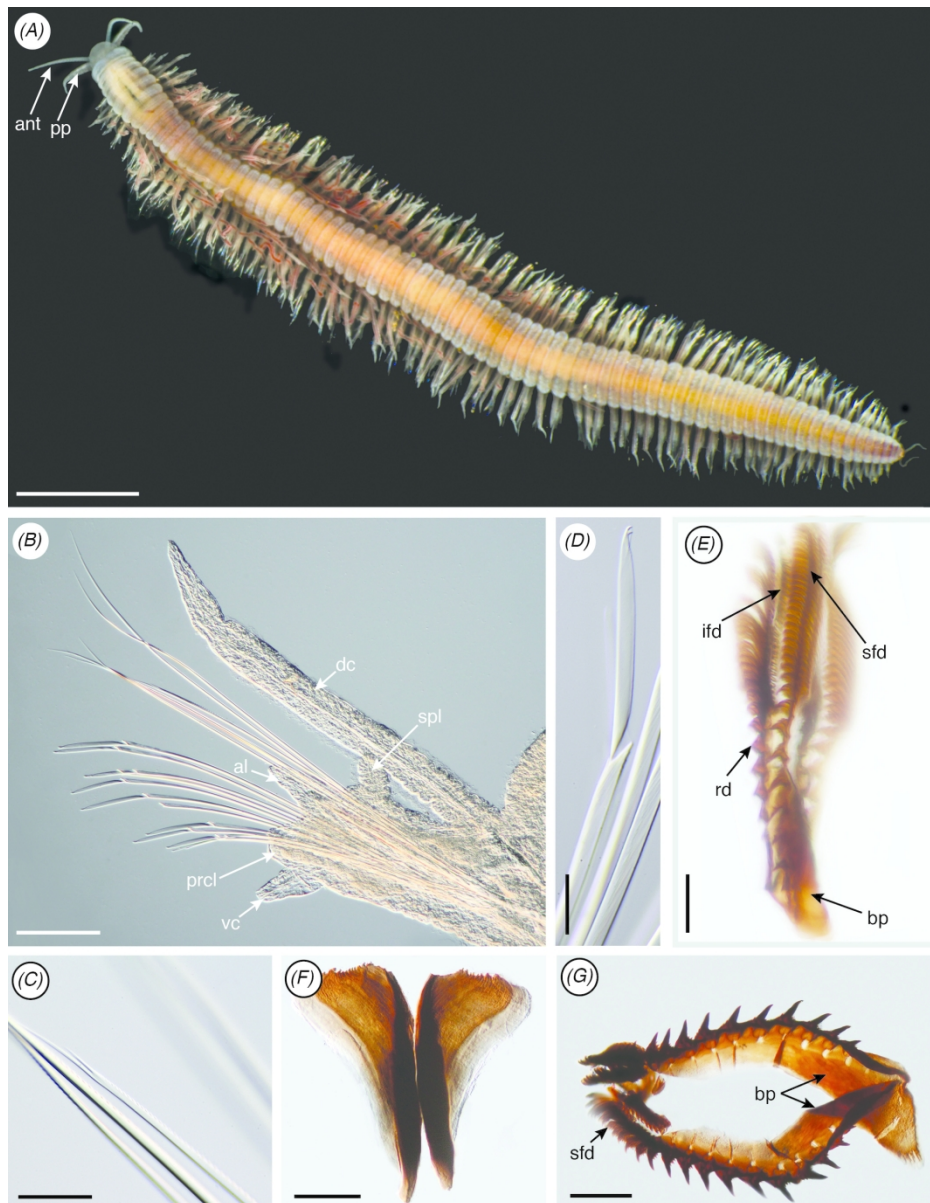


Fig. 9. *Parougia indiareinhardtiae* sp. nov. A. Live specimen (SIO-BIC A6461), ventro-lateral palps (pp), antennae (ant); B. Mid-parapodium: dorsal cirrus (dc), supra-acicular chaetal lobe (spl), acicular lobe (al), pre-chaetal lobe (prcl), ventral cirrus (vc); C. Supra-acicular furcate chaeta; D. Sub-acicular chaetae, falcigers; E. Maxillae: superior free denticles (sfd), superior fused denticles on base plate (bp), inferior free denticles (ifd), replacement denticles (rd); F. Mandibles; G. Maxillae without replacement denticles. Scale bars=2 mm (A), 70 μ m (B), 20 μ m (C, D), 30 μ m (E), 45 μ m (F), 40 μ m (G).

175x224mm (300 x 300 DPI)

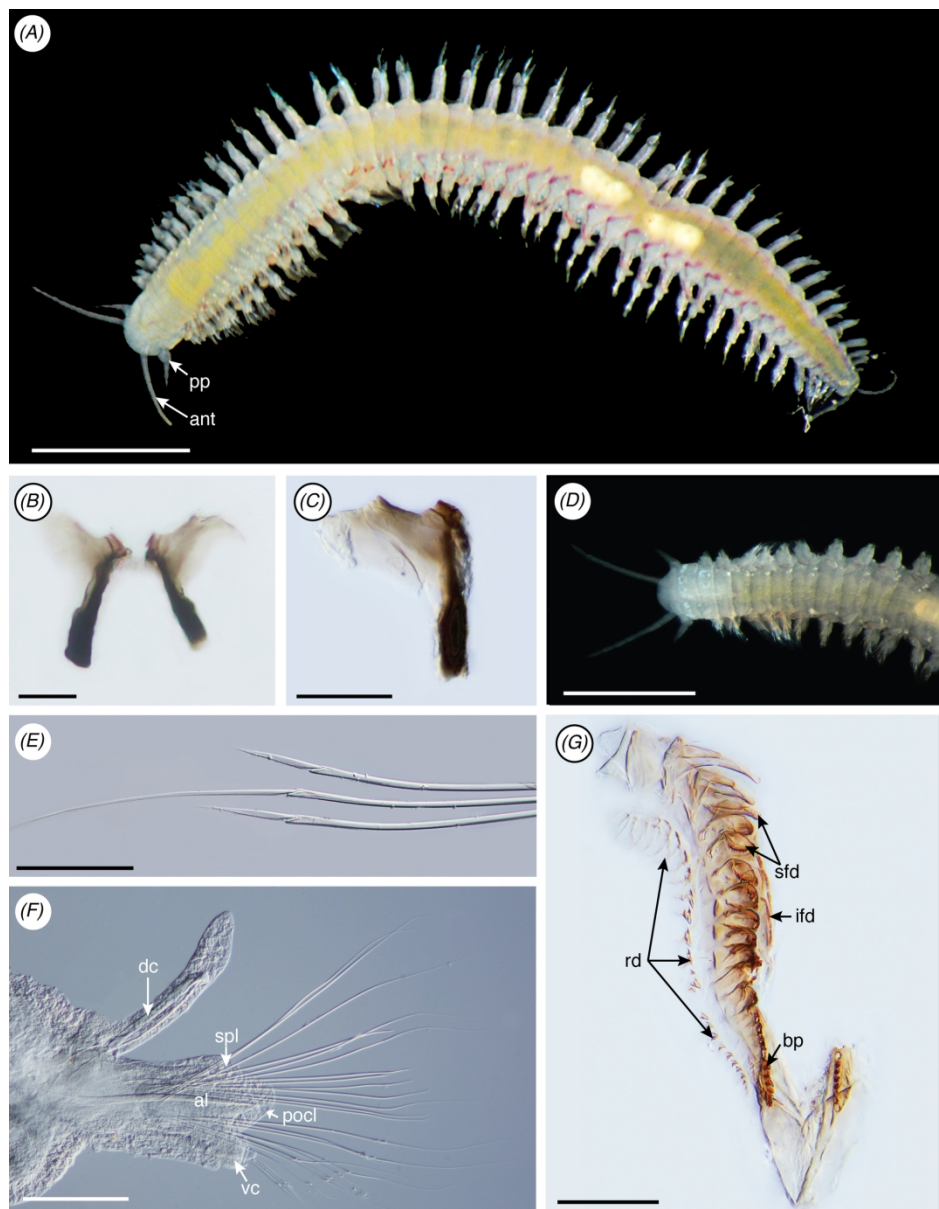


Fig. 10. *Parourgia jessieae* sp. nov. A. Live whole specimen (SIO-BIC A6465), ventro-lateral palps (pp), antennae (ant); B. Mandibles; C. Left mandible; D. Anterior of preserved specimen (SIO-BIC A10623); E. Sub-acicular spinigers of two blade lengths; F. Mid-parapodium; dorsal cirrus (dc), supra-acicular chaetal lobe (spl), acicular lobe (al), post-chaetal lobe (pocl), ventral cirrus (vc); G. Partial maxillae: superior free denticles (sfd), fused denticles on base plate (bp), inferior free denticles (ifd), replacement denticles (rd). Scale bars= 1 mm (A), 20 μ m (B, G), 15 μ m (C), 1 mm (D), 30 μ m (E), 70 μ m (F).

174x224mm (300 x 300 DPI)

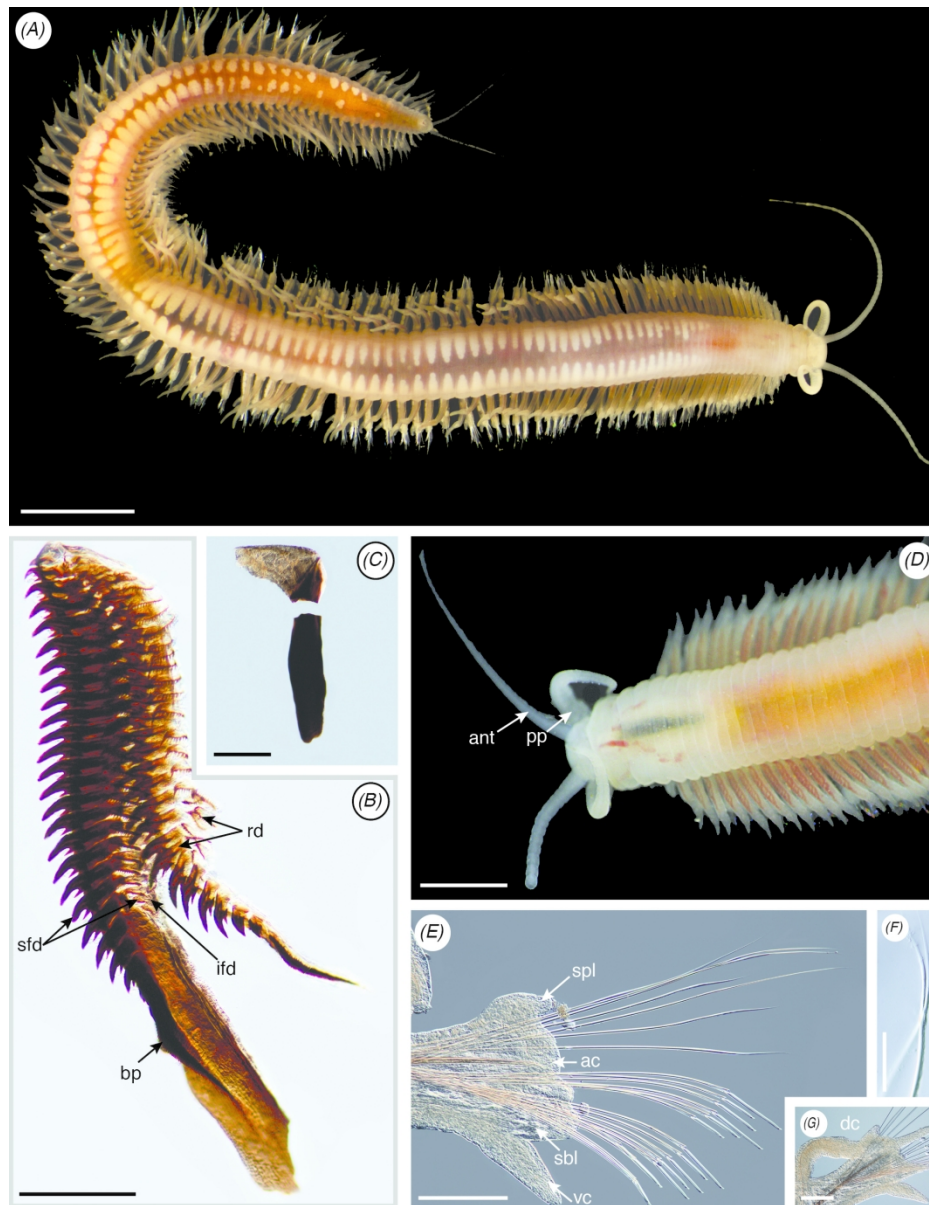


Fig. 11. *Parougia sulleyi* sp. nov. A. Live animal (SIO-BIC A10616), female with eggs in coelom; B. Partial maxillae: superior free denticles (sfd), superior fused denticles on base plate (bp, inferior free denticles (ifd), replacement denticles (rd); C. Left mandible; D. Anterior of live specimen (SIO-BIC A1333, *Parougia* cf. *sulleyi* sp. nov.), ventro-lateral palps (pp), antennae (ant), blood vessel loops in dorsal cirri (notopodia); E. Mid-parapodium: supra-acicular chaetal lobe (spl), acicular lobe (al), sub-acicular chaetal lobe (sbl), ventral cirrus (vc); F. Supra-acicular furcate chaeta; G. Mid-parapodium: dorsal cirrus (dc). Scale bars= 1 mm (A), 30 μ m (B), 40 μ m (C), 0.5 mm (D) 70 μ m (E, G), 25 μ m (F).

174x224mm (300 x 300 DPI)

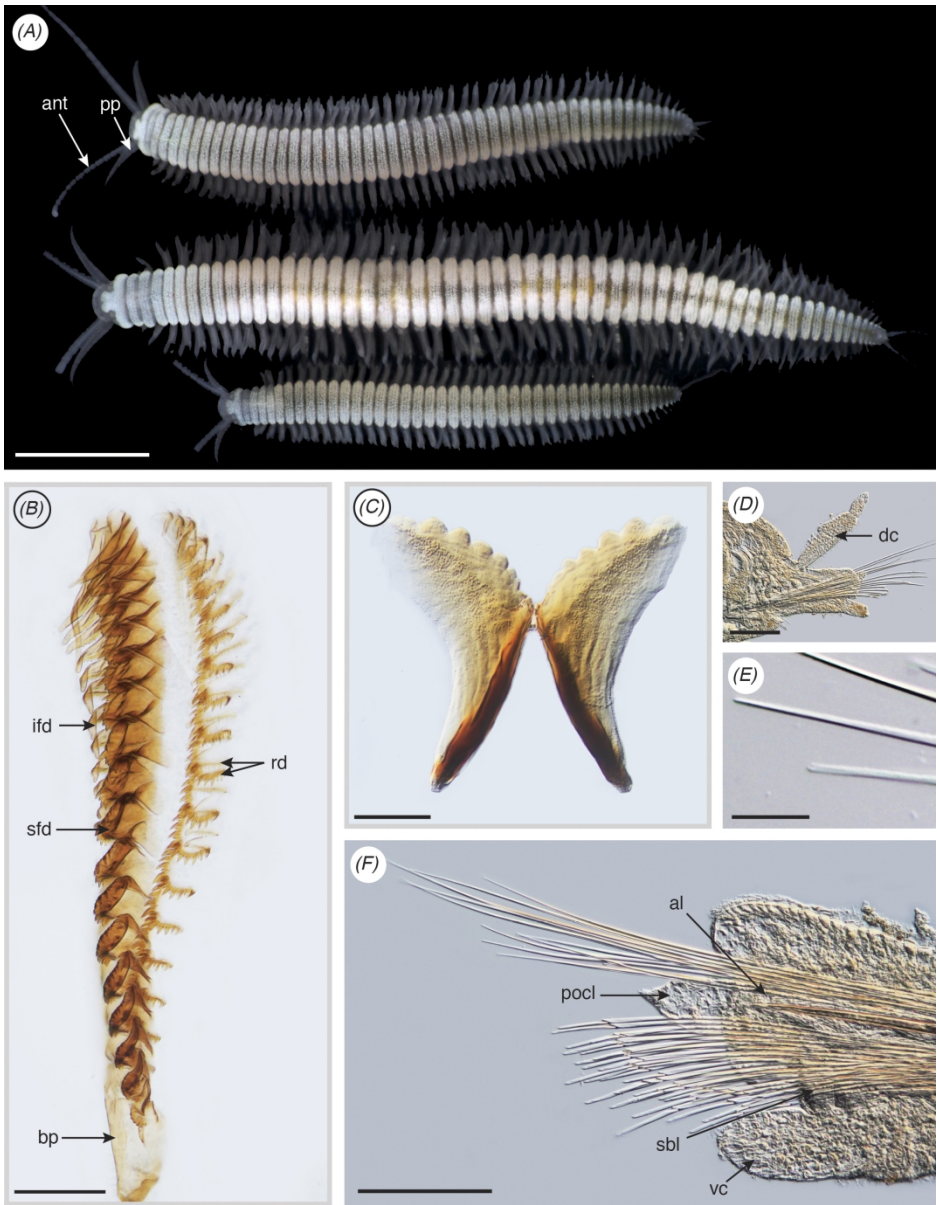


Fig. 12. *Parougia theloniousblueski* sp. nov. A. Live animals (SIO-BIC A1441), ventro-lateral palps (pp), antennae (ant); B. Partial maxillae: superior free denticles (sfd), superior fused denticles (sfsd), inferior free denticles (ifd), replacement denticles (rd); C. Mandibles; D. Mid-parapodium: dorsal cirrus (dc); E. Supra acicular simple chaetae, notched; F. Mid-parapodium: acicular lobe (al), post-chaetal lobe (pocl), sub-acicular chaetal lobe (sbl), ventral cirrus (vc). Scale bars=4 mm (A), 25 μ m (B), 30 μ m (C), 15 μ m (D), 10 μ m (E), 50 μ m (F).

215x277mm (300 x 300 DPI)

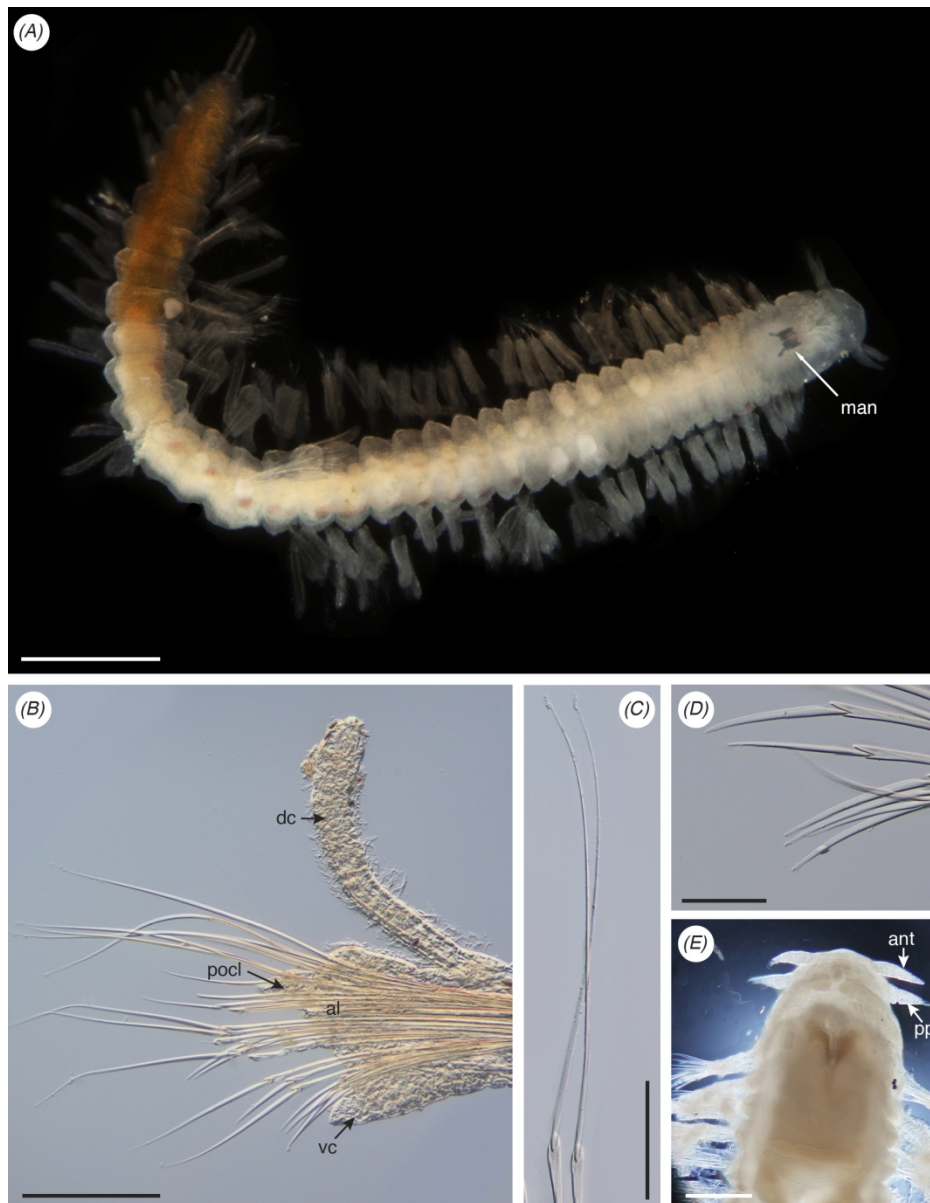
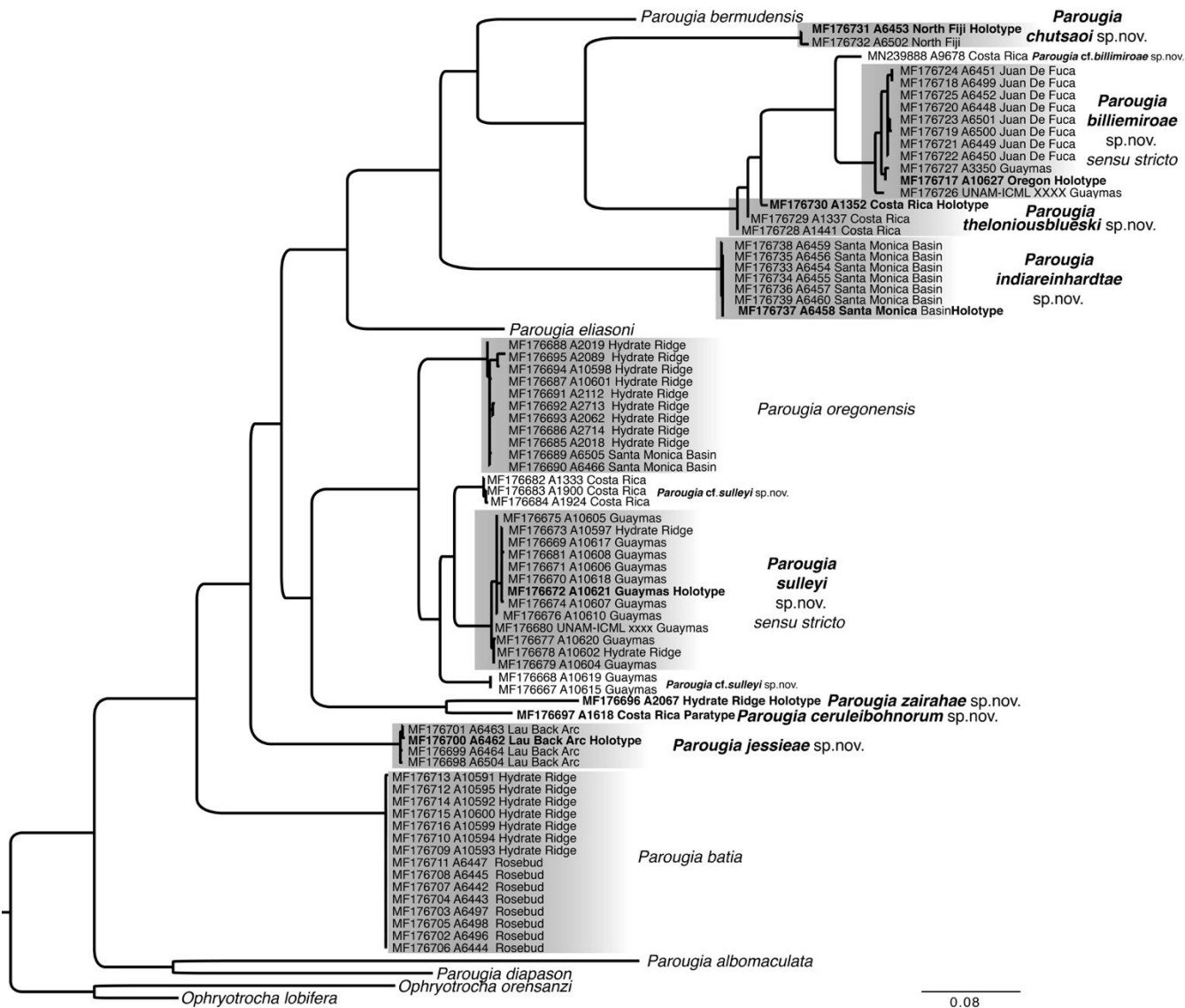


Fig. 13. *Parougia zairahae* sp. nov. A. Live animal (ventral) (SIO-BIC A2067), mandibles (man); B. Mid-parapodium: dorsal cirrus (dc), post-chaetal lobe (pocl), acicular lobe (al), ventral cirrus (vc); C. Sub-acicular spinigers, long and flowing; D. Sub acicular spinigers, short and blunt; E. Dorsal view of preserved specimen (SIO-BIC A2067), ventro-lateral palps (pp), antennae (ant). Scale bars= 1 mm (A), 50 μ m (B), 20 μ m (C), 15 μ m (D), 70 μ m (E).

175x224mm (300 x 300 DPI)

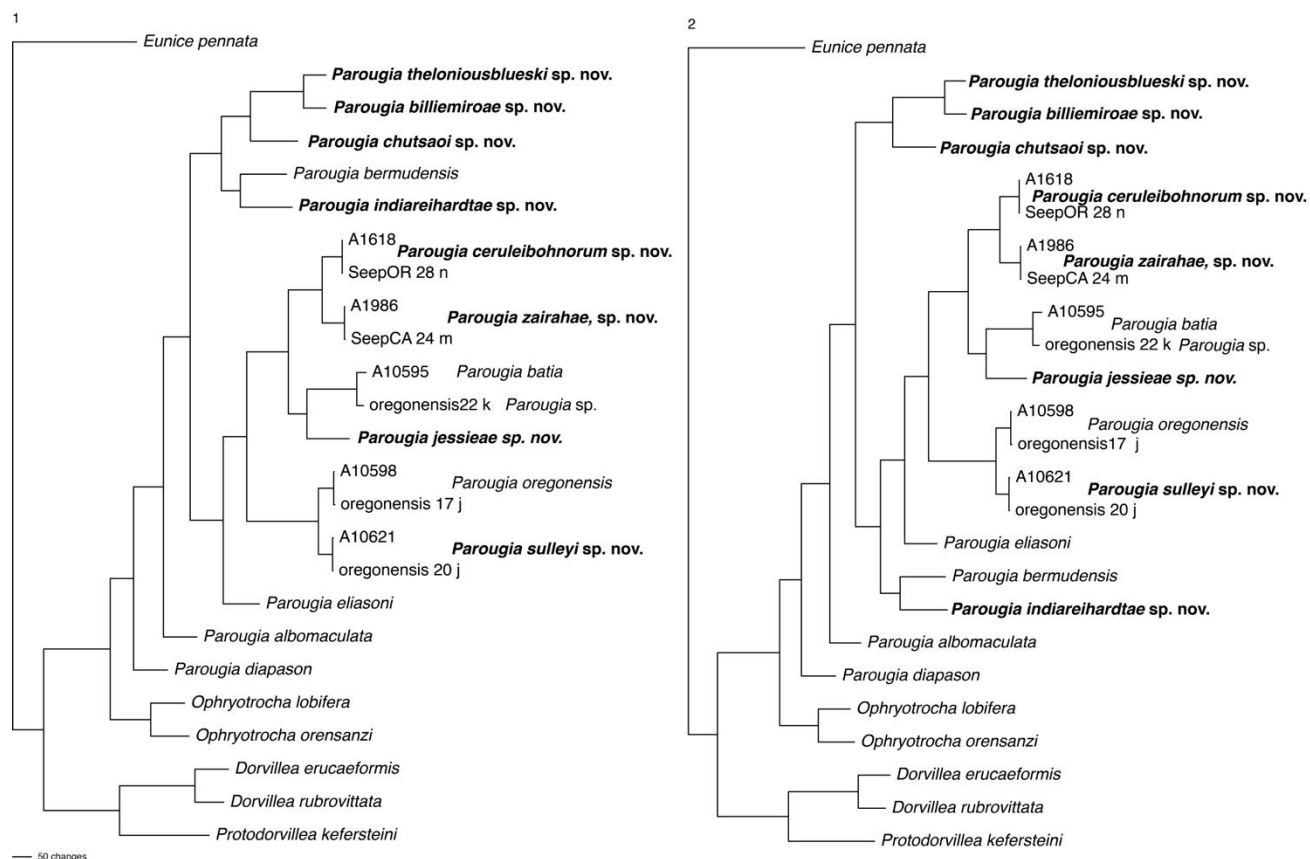
Supplementary Figures
Supplementary Figure 1

Maximum likelihood tree from COI data for all terminals used in this study. This topology was used to aid in 'species' taxon determination.



Supplementary Figure 2

Two shortest tree of length 4162 generated under maximum parsimony in PAUP* from 994 parsimony informative characters in the concatenated 5-gene (COI, 16S, Cyt-b, H3, and 18S) dataset.



Supplementary Figure 3

Majority rule consensus tree of 100 bootstrap replicates generated under maximum parsimony in PAUP* from 994 parsimony informative characters in the concatenated 5-gene (COI, 16S, Cyt-b, H3, and 18S) dataset.

Bootstrap consensus tree

