Phylogeny of Hesionidae (Aciculata, Annelida), with four new species from deep-sea eastern Pacific methane seeps, and resolution of the affinity of *Hesiolyra*

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Abstract.   Hesionidae Grube, 1850 currently comprises over 175 species in 28 genera, placed in several subfamilies. Discoveries in recent years have largely been of deep-sea taxa. Here we describe a further four new hesionid species, mainly from methane 'cold' seeps at around 1000–1800 m depths off the Pacific coast of Costa Rica and new record of another species. Several of these taxa also occur at methane seeps in the Guaymas Basis (Mexico) and off the USA west coast (California and Oregon). The phylogenetic relationships within Hesionidae are reassessed via maximum parsimony and maximum likelihood analyses of DNA sequences from nuclear (18S rRNA and 28SrRNA) and mitochondrial (16SrRNA and Cytochrome c oxidase I) loci for the new samples. On the basis of these results, we refer one of the new species to *Gyptis* Marion & Bobretzky in Marion, 1874, one to *Neogyptis* Pleijel, Rouse, Sundkvist & Nygren, 2012, and two to *Sirsoe* Pleijel, 1998. The new species *Gyptis robertscrippsi* n. sp., *Neogyptis jeffruoccoi* n. sp., *Sirsoe dalailamai* n. sp. and *Sirsoe munki* n. sp. We refer to a collection of individuals from seeps ranging from Oregon to Costa Rica as *Amphiduropsis* cf. *axialensis* (Blake & Hilbig, 1990), even though this species was described from hydrothermal vents off Oregon. *Neogyptis jeffruoccoi* n. sp. was generally found living inside the solemyid clam *Acharax johnsoni* (Dall, 1891). The position of *Hesiolyra bergi* Blake, 1985 is resolved on the basis of newly-collected specimens from near the type locality and, as a result, Hesiolyrinae Pleijel, 1998 is synonymized with Gyptini Pleijel, 1998 (and Gyptinae Pleijel, 1998).

http://zoobank.org/urn:lsid:zoobank.org:pub:9C0E88EE-34F8-4F25-9EC8-91797618AC86

Additional keywords: polychaete, biogeography, connectivity, species ranges.

Introduction

Hesionid annelids have proven to be common on deep-sea hydrothermal vents, cold seeps and whale falls (e.g. Blake 1985; Desbruyères and Toulmond 1998; Pleijel *et al*. 2008; Summers *et al*. 2015), with the majority described from hydrothermal vents or whale falls (see Summers *et al*. 2015). To date, the only seep-associated hesionid that has been formally named is the spectacular iceworm, *Sirsoe methanicola* (Desbruyères and Toulmond 1998), from methane hydrates in the Gulf of Mexico (Fisher *et al*. 2000), which was originally placed in *Hesiocaeca* Hartman, 1965. An undescribed species of *Vrijenhoekia* Pleijel, Rouse, Ruta, Wiklund & Nygren, 2008, collected from seeps in the Guaymas Basin (Gulf of California) in Mexico (as *Vrijenhoekia* sp. A), was included in the analyses of Summers *et al*. (2015) and hesionids have been recorded from methane seeps in various ecological studies (e.g. Grupe *et al*. 2015; Portail *et al*. 2015; Levin *et al*. 2017). We here provide descriptions of four new hesionid species. One of the

new species belongs in *Gyptis* Marion & Bobretzky in Marion, 1874, one in *Neogyptis* Pleijel, Rouse, Sundkvist & Nygren, 2012, and two in *Sirsoe* Pleijel, 1998. Their type localities are methane seeps from off the Pacific coast of Costa Rica. These seeps are present at several sites ranging in depth from 1000 to 1800 m. Details of the locality and ecosystem have been outlined previously by Levin *et al*. (2012, 2015). Several of the species were also found at methane seeps in the Guaymas Basin (Mexico) and off the USA west coast (California and Oregon). Details on the ecological setting and faunal assemblages of these sites are also available (Boetius and Suess 2004; Portail *et al*. 2015; Grupe *et al*. 2015; Levin *et al*. 2017).

Apart from morphology, the affinities of the new species were analysed according to 18S rDNA, 16S rDNA, 28S rDNA and COI nucleotide sequences. Also, since we recently were able to collect new specimens of *Hesiolyra bergi* Blake, 1985 from hydrothermal vents close to the type locality, we decided to include their sequences in the phylogenetic analyses in order to

resolve the position of this taxon, which has been regarded as uncertain (Summers *et al.* 2015). We also refer to a collection of individuals from seeps ranging from Oregon to Costa Rica as *Amphiduropsis* cf. *axialensis* (Blake & Hilbig, 1990). This species was described from hydrothermal vents, with the type locality being Axial Seamount (Juan de Fuca Ridge) off Oregon (Blake and Hilbig 1990). However, we could not distinguish our specimens morphologically from this taxon and no DNA sequences are available from the type locality.

Vrijenhoekia is the sister group to *Sirsoe* and was erected by Pleijel *et al.* (2008) for *Vrijenhoekia balaenophilia* Pleijel, Rouse, Ruta, Wiklund & Nygren, 2008, a species of hesionid found in ~3000 m depth associated with a whale fall. *Vrijenhoekia* was distinguished from *Sirsoe* by, among other features, the lack of a median antenna in the former, but several other *Vrijenhoekia* species have been shown to have this structure (Summers *et al.* 2015). Notably, no sequences have yet been obtained for the type species of *Sirsoe*, *Sirsoe grasslei* (Blake, 1991) but, to date, *Sirsoe* and *Virjenhoekia* appear to be reciprocally monophyletic (Summers *et al.* 2015). Pleijel *et al.* (2012) carried out a partial revision of *Gyptis*, which proved to be non-monophyletic. Thus, they erected *Neogyptis* Pleijel, Rouse, Sundkvist & Nygren, 2012 for those species that formed a clade sister to *Amphiduros*. Summers *et al.* (2015) recently described a series of new deep-water hesionid species from the east Pacific, which included members of *Gyptis*, *Neogyptis*, *Sirsoe* and *Vrijenhoekia*. All these new species were from whale falls or benthic habitats with very low oxygen, rather than from methane seeps.

Material and methods

Terminals

The most recent detailed molecular phylogeny of Hesionidae was done by Summers *et al.* (2015) with 42 terminals. In the present study we included the terminals from the dataset of Summers *et al.* and added data covering the five new species described here, as well as sequence data generated from a more restricted hesionid analysis (Martin *et al.* 2015). We also added newly generated sequence data for *Hesiolyra bergi* from near the type locality. The new data for 18S and COI for *Hesiolyra bergi* were virtually identical to those already in GenBank, and the addition of 16S data allowed for a more stable and well supported placement for the taxon (see below).

Specimen collection and preparation

Costa Rican and Hydrate Ridge (Oregon) specimens were collected via the RV *Atlantis* using the submersible *Alvin* or the remote-operated vehicle (ROV) *Jason II* in 2009, 2010, 2011 and 2017 (see Levin *et al.* 2012, 2015 for details about Costa Rican sites and Levin *et al.* 2017 for Hydrate Ridge). Specimens from Guaymas Basin (Mexico) and Del Mar (California) seeps were collected via the RV *Western Flyer* and ROV *Doc Ricketts* on expeditions in 2012 and 2013 led by Bob Vrijenhoek (Monterey Bay Aquarium and Research Institute, MBARI). Specimens were sorted alive and micrographs were taken using a Leica S8Apo or MZ9.5 stereo microscope (LM). Specimens were relaxed in 7% MgCl in distilled water before study, photography and fixation. Those fixed in

formaldehyde (10% in seawater) were subsequently cleaned in distilled water and stored in 50% ethanol. Specimens fixed for scanning electron microscopy (SEM) were relaxed, fixed in 1% osmium tetroxide in sea water for 1 h, rinsed repeatedly in distilled water and stored in 70% ethanol. They were then dehydrated and critical point dried, sputter-coated with gold, and examined in a FEI Quanta 600 Scanning Electron Microscope. Parapodia and chaetae were studied using a Leica DMR compound microscope (LM). All specimens are deposited at Scripps Institution of Oceanography, Benthic Invertebrate Collection, La Jolla, California, USA (= SIO-BIC), or the Museo de Zoología (Universidad de Costa Rica), San José, Costa Rica (= MZUCR).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from specimens fixed in 95% ethanol with a Qiagen DNeasy Blood and Tissue kit (Germantown, MD) following manufacturer protocols. Sequences of two mitochondrial markers: 16S rRNA (16S) and Cytochrome oxidase subunit 1 (COI) and two nuclear markers: 28S rRNA (28S) and 18S rRNA (18S) were generally sequenced for one specimen of each new species (Table 1), as well as for *Amphiduropsis* cf. *axialensis* and *Hesiolyra bergi* (though not 28S for the latter). Additional COI sequences were also obtained for several other specimens for each of the new species (see *Material Examined* for their GenBank numbers). The newly generated data were combined with the datasets found in Summers *et al.* (2015) and Martin *et al.* (2015).

For all markers, either Apex Taq RED Master Mix (Genesee Scientific) or Lambda Conquest PCR mastermix (Lambda Biotech) following the manufacturer's protocol was used and DNA was amplified using the primers and profiles specified in Summers *et al.* (2015). PCR products were purified with ExoSAP-IT (GE Healthcare, Uppsala, Sweden) and sequenced by Eurofins Inc. using Applied Biosystems 3730xl DNA analysers. Overlapping sequence fragments were assembled using Geneious R8 (Biomatters). All sequences have been deposited in GenBank (Table 1).

Molecular analyses

The rDNA sequences (18S, 28S and 16S) were separately aligned using MAFFT (Katoh *et al.* 2002), with the iterative refinement method Q-INS-i, and default gap open and extension values. 18S, 28S and 16S were then assessed for ambiguous areas of alignment using Gblocks (Castresana 2000) with least stringent settings. COI sequences were aligned using MUSCLE (Edgar 2004). The sequence partitions were concatenated and analysed using Maximum Parsimony (MP) in PAUP* (Swofford 2002), Maximum Likelihood (ML) with RAxML 8 (Stamatakis 2014) using the same methods as performed in Summers *et al.* (2015). Analyses were run on concatenated datasets either using Gblocked rDNA partitions or as a 'complete' dataset. PopART 1.1 (Leigh and Bryant 2015) was used to generate COI haplotype networks (median-joining; 95% confidence interval, epsilon set to zero) for four of the new species that occurred across multiple locations and/or depths. Localities ranged from Costa Rica to Oregon (USA), a distance of over 6000 km. For *Gyptis robertscrippsi* sp. nov., there were eight

Table 1.   GenBank numbers and voucher information for taxa included in this study

New sequences are shown in bold

Specimen	Origin	Voucher	18S	16S	28S	COI
Dysponetus caecus	Sweden	–	AY839568	EU555047	EU555028	AF221568
Nereis pelagica	Sweden	SMNH83519	AY340438	AY340470	AY340407	–
Amphiduros fuscescens	France	SMNH	DQ442584	DQ442569	DQ442598	DQ442561
Amphiduropsis* cf. *axialensis	**Costa Rica**	**SIO-BIC A1758**	**MG649239**	**MG523356**	**MG649243**	**MG517505**
Amphiduros pacificus	California	SIO-BIC A2514-15	JN631334	JN631324	JN631345	JN631312
Gyptis brunnea	California	SIO-BIC XXX	JN631335	JN631323	JN631346	JN631313
Gyptis golikovi	Svalbard	SIO-BIC A2509	JN631336	JN631321	JN631347	–
Gyptis hians	California	SIO-BIC A2338	JN571891	JN571880	JN571900	JN571824
Gyptis pacifica	Japan	SIO-BIC A2516-17	JN631337	JN631322	JN631348	JN631314
Gyptis paucilineata	Australia	SAM E3687	–	–	–	EU498243
Gyptis polymorpha	Australia	SIO-BIC A1046	–	–	–	EU498247
Gyptis propinqua	France, Sweden	SMNH83513, 15	–	DQ442573	DQ442602	EU498229
***Gyptis robertscrippsi* sp. nov.**	**Costa Rica**	**SIO-BIC A1754**	**MG649238**	**MG523360**	**MG649247**	**MG517513**
Gyptis simpsonorum	Australia	SAM E3686	–	–	–	EU498237
Gyptis shannonae	California	SIO-BIC A3250	KP745537	KP745534	KP745540	–
Hesiolyra bergi	East Pacific Rise	–	AM159577	–	–	GQ474434
Hesiolyra bergi	**East Pacific Rise**	**SIO-BIC A6314**	**MG649237**	**MG523359**	–	**MG517521**
Hesione sp.	New Caledonia	SMNH81620	DQ442617	DQ442615	DQ442619	–
Hesiospina aurantiaca	Sweden	SIO-BIC A2513	JN631329	JN631319	JN631342	–
Hesiospina vestimentifera	Fiji	SIO-BIC A2510	JN631330	JN631320	JN631343	JN631310
Heteropodarke formalis	Belize	GNM 86187	–	KJ855065	KJ855076	–
Heteropodarke pleijeli	PNG	GNM 86188	–	KJ855066	KJ855077	–
Leocrates chinensis	New Caledonia	SMNH83510	DQ442589	DQ442575	DQ442605	DQ442565
Micropodarke dubia	New Caledonia	SMNH83521	JN571888	DQ442576	JN571899	JN571825
Neogyptis carriebowcayi	Belize	SIO-BIC A2468	JN631338	JN631325	JN631349	JN631315
Neogyptis fauchaldi	Belize	SIO-BIC A2485	JN631339	JN631326	–	JN631316
Neogyptis hinehina	Off Fiji	SIO-BIC A2493	JN631340	JN631328	JN631350	JN631317
***Neogyptis jeffruoccoi* sp. nov.**	**Costa Rica**	**SIO-BIC A1448**	**MG649242**	–	**MG649244**	**MG517514**
Neogyptis mediterranea	France	SMNH4398	–	DQ442572	DQ442601	DQ442563
Neogyptis rosea	Norway	SMNH8351	JN571890	DQ442574	DQ442603	JN571826
Neogyptis julii	California	SIO-BIC A3249	KP745538	KP745535	KP745541	KP745532
Neogyptis sp. A	Florida	SIO-BIC A2505	JN631341	JN631327	JN631351	JN631318
Nereimyra aphroditoides	Greenland	SIO-BIC 2285	–	JF317211	JF317204	JF317198
Nereimyra punctata	Sweden	SMNH76989	DQ442591	DQ442577	DQ442606	DQ442566
Nereimyra woodsholea	Norway	MNH76986	–	–	JF317207	AY644802
Oxydromus fauveli	Japan	–	–	–	KJ855078	KJ855071
Oxydromus flexuosus	Sweden	SMNH83508	DQ442592	DQ442578	DQ442607	DQ442567
Oxydromus humesi	Spain	GNM 86193	KJ855075	KJ855070	KJ855082	–
Oxydromus microantennatus	Australia	GNM 86192	–	KJ855067	KJ855079	KJ855072
Oxydromus obscurus	North Carolina	GNM 86189	–	KJ855068	KJ855080	KJ855073
Oxydromus pallidus	Sweden	SMNH83518	DQ442593	DQ442579	DQ442608	–
Oxydromus pugettensis	California	GNM 86190	DQ790086	KJ855069	KJ855081	KJ855074
Podarkeopsis arenicolus	France	SMNH83509	JN571889	JN571879	DQ442609	JN571827
Podarkeopsis perksinsi	California	SIO-BIC A2339	JN571892	JN571881	JN571901	JN571828
Podarkeopsis helgolandica	Sweden	SIO-BIC 2511-12	JN631331	–	JN631344	JN631311
Psamathe fusca	Sweden	SMNH83516	DQ442595	DQ442581	DQ442610	DQ513294
***Sirsoe dalailamai* sp. nov.**	**Costa Rica**	**SIO-BIC A1767**	**MG649240**	**MG523357**	**MG649245**	**MG517498**
Sirsoe methanicola	Gulf of Mexico	–	JN631332	DQ442582	DQ442611	DQ513295
***Sirsoe munki* sp. nov.**	**Costa Rica**	**SIO-BIC A1409**	**MG649241**	**MG523358**	**MG649246**	**MG517510**
Sirsoe sirikos	California	SIO-BIC A2323	JN571893	JN571882	JN571902	JN571829
Syllidia armata	Sweden	SMNH83514	DQ442596	DQ442583	DQ442612	DQ442568
Vrijenhoekia balaenophila 1	California	SMNH6305	JN631333	DQ513301	DQ513306	DQ513296
Vrijenhoekia balaenophila 2	California	SMNH6307	–	DQ513302	DQ513307	DQ513297
Vrijenhoekia ahabi	California	SIO-BIC A2327	JN571898	JN571887	JN571907	JN571876
Vrijenhoekia falenothiras	California	SIO-BIC A2345	JN571897	JN571886	JN571906	JN571875
Vrijenhoekia ketea	California	SIO-BIC A2341	JN571896	JN571885	JN571905	JN571838
Vrijenhoekia sp. A	Mexico	SIO-BIC A3255	KP745539	KP745536	KP745542	KP745533

COI sequences, for *Neogyptis jeffruoccoi* sp. nov. seven, for *Amphiduropsis* cf. *axialensis* 10 and, for *Sirsoe dalailamai* sp. nov. there were nine COI sequences.

Note on hesionid nomenclature

Costa and Christoffersen (2017), referring to the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999), suggested a series of replacement names for the following hesionid group names: Ophiodrominae Pleijel, 1998, Ophiodromini Pleijel, 1998, Psamathinae Pleijel, 1998, and Amphidurini Pleijel, Rouse, Sundkvist & Nygren, 2012. The first two were justified by the recent synonymy of the type genus *Ophiodromus* Delle Chiaje, 1827 with *Oxydromus* Grube, 1855 (Villalobos-Guerrero and Harris 2012). For the last two names they argued for replacement based on the oldest available generic names in the clades. However, both these suggestions are based on misinterpretations of the Code, Articles 40 (Synonymy of the type genus) and 64 (Choice of type genus), and the names introduced by Costa and Christoffersen (2017) should be treated as *nomina nuda*.

Results and discussion

Overall our results were generally congruent with those of Summers *et al*. (2015). The restricted versus full datasets analysed under MP and ML were largely congruent and only the results of the first one are reported here (Fig. 1). Inconsistencies were found relating to the positions of *Hesiospina vestimentifera* Blake, 1985 and *Psamathe* Johnston, 1836. Either *H. vestimentifera* appears in a grade with *H. aurantiaca* (Sars, 1862) and *Psamathe*, or *H. vestimentifera* is sister group to *Psamathe*. *Hesiospina* did not form a clade in either of the analyses. Also, in both the MP analyses, *Neogyptis* was paraphyletic, with *Amphiduros+Amphiduropsis* nested well inside the group, a result seen in Summers *et al*. (2015) for *Amphiduros*. In the ML analyses, *Neogyptis* was a well supported clade that formed the sister group to *Amphiduros +Amphiduropsis* (Fig. 1) and this is accepted here for taxonomic purposes.

Gyptis (and Gyptini Pleijel 1998) formed a well supported clade that, together with *Hesiolyra*, was sister group to Ophiodromini, and the new species *G. robertscrippsi* sp. nov. was the sister group to all remaining *Gyptis* taxa. *Hesiolyra bergi* was consistently the sister group to *Gyptis* in all analyses, though with less support in the MP than in the ML results (Fig. 1). In contrast, *Hesiolyra* fell outside Hesionidae as sister group to *Nereis*, one of the outgroups in the MP results in Summers *et al*. (2015). The addition of more data (16S) presumably helped stabilise its position within Hesionidae. The consistent placement of *Hesiolyra* as sister group to *Gyptis* allows for the argument that it may be considered as part of Gyptini, since otherwise this tribe contains only *Gyptis*, and we here synonymise Hesiolyrini (and Hesiolyrinae) with Gyptini.

Neogyptis jeffruoccoi sp. nov., was consistently recovered with strong support as the sister group to the recently described species, *Neogyptis hinehina* Pleijel, Rouse, Sundkvist, & Nygren, 2012, from hydrothermal vents at 1800 m depth off Fiji. In all analyses, *Amphiduropsis* cf. *axialensis* was sister group to the other two included species of *Amphiduros*. *Sirsoe dalailamai* sp. nov. and *S. munki* sp. nov. were recovered as a clade inside *Sirsoe*, with the former as the sister group to *S. methanicola* from the Gulf of Mexico. Apart from the much smaller size of *S. munki* sp. nov., there are no obvious morphological differences between the two new species, or between these and *S. methanicola*.

The median-joining haplotype networks generated from COI data obtained for the four new species that were sampled from multiple methane seep locations and/or depths of 1000–1800 m in Costa Rica are shown in Fig. 2. *G. robertscrippsi* sp. nov. was found only in Costa Rica, but at both 1000 and 1800 m depths (Fig. 2A). There was intraspecific variation (uncorrected) of up to 5.7% for *G. robertscrippsi* sp. nov. This distance was found from the holotype (SIO BIC A1314, * in Fig. 2A) and a paratype (SIO BIC A8105), both from 1000 m, to the haplotype for two specimens from 1800 m. There was also nearly 5% uncorrected distance among the specimens from 1000 m depth (Fig. 2A). Pending further data, we regard all these specimens as part of *G. robertscrippsi* sp. nov., though this is a relatively high level of intraspecific variability of COI relative to other annelids (Nygren 2014). *Neogyptis jeffruoccoi* sp. nov. was arguably found from Costa Rica to southern California. The intraspecific variation was maximally 3.3% (uncorrected), with a clear break between the Costa Rican and the Mexican/ Californian specimens (Fig. 2B). We regard this amount of variation to be insufficient to separate these as different taxa, though further investigation is warranted. The widest-ranging species was *Amphiduropsis* cf. *axialensis* (Fig. 2C), found from Costa Rica, Mexico, California and Oregon, with a maximum pairwise distance of 2.2% (uncorrected). Unfortunately we lacked material of this taxon from its type locality. *Sirsoe dalailamai* sp. nov. was recorded from Costa Rica from 1000 and 1800 m and also from the Guaymas Basin in Mexico, with a maximum difference of three bases only (Fig. 2D).

Taxonomy

GYPTINI Pleijel, 1998

Gyptis Marion & Bobretzky in Marion, 1874

Gyptis robertscrippsi, sp. nov.
(Figs 3–5)

Material examined

Holotype. Anterior part with 27 segments fixed in formaldehyde (SIO-BIC A1314), posterior part in 95% ethanol (SIO-BIC A1479) and destroyed for DNA sequencing (COI = MG517511), 3 slides with mounted parapodia from segments 17–19 (right side) (SIO-BIC A1314 1), Alvin Dive 4501, Costa Rica, 08°55.800′N, 84°18.810′W, depth 1008 m, coll. GWR, 22.ii.2009.

Paratypes. 2 spms fixed in formaldehyde (MZUCR 398-02), 1 posterior end in 95% ethanol (SIO-BIC A1480), Costa Rica, same collection data as holotype; 1 spm fixed in formaldehyde (SIO-BIC A1367), *Alvin* Dive 4505, Costa Rica, 08°55.19′N, 84°18.33′W, depth 1045 m, coll. GWR, 26.ii.2009; 5 spms, 2 fixed in formaldehyde (SIO-BIC A1748) and 3 in ethanol (SIO-BIC A1749; COI = MG517512), *Alvin* Dive 4586, Costa Rica, 08°55.85′N,
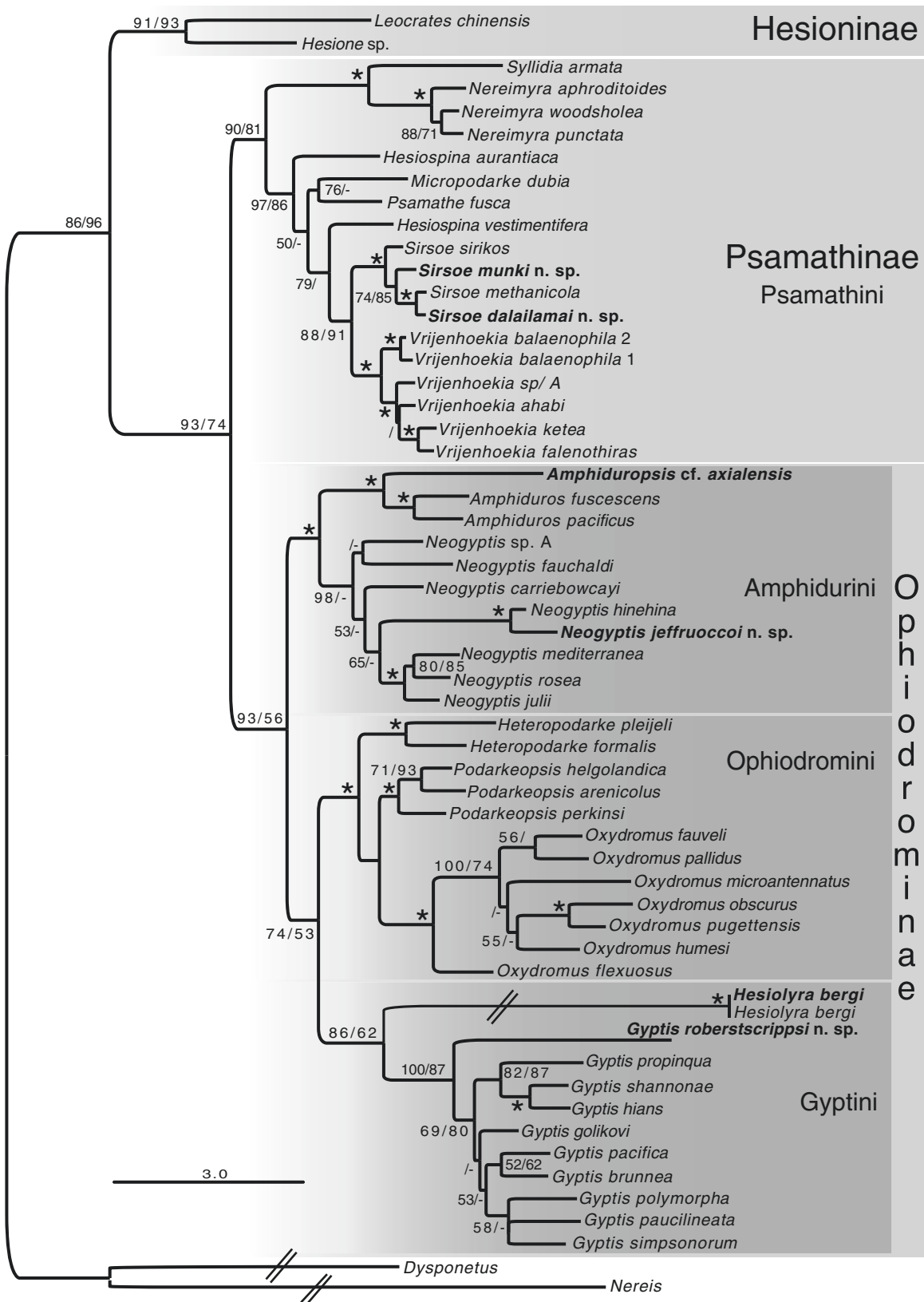
Fig. 1. Maximum likelihood (ML) tree of concatenated dataset (COI, 18S, 16S, 28S); asterisks indicate supports with bootstrap values greater than 95% in both ML and maximum parsimony (MP) analyses. Blank, <50% support; –, node absent in MP. Terminals not previously used in hesionid phylogenetics (the new species, *Amphiduropsis* cf. *axialensis* and newly sequenced *Hesiolyra bergi*) are shown in bold.
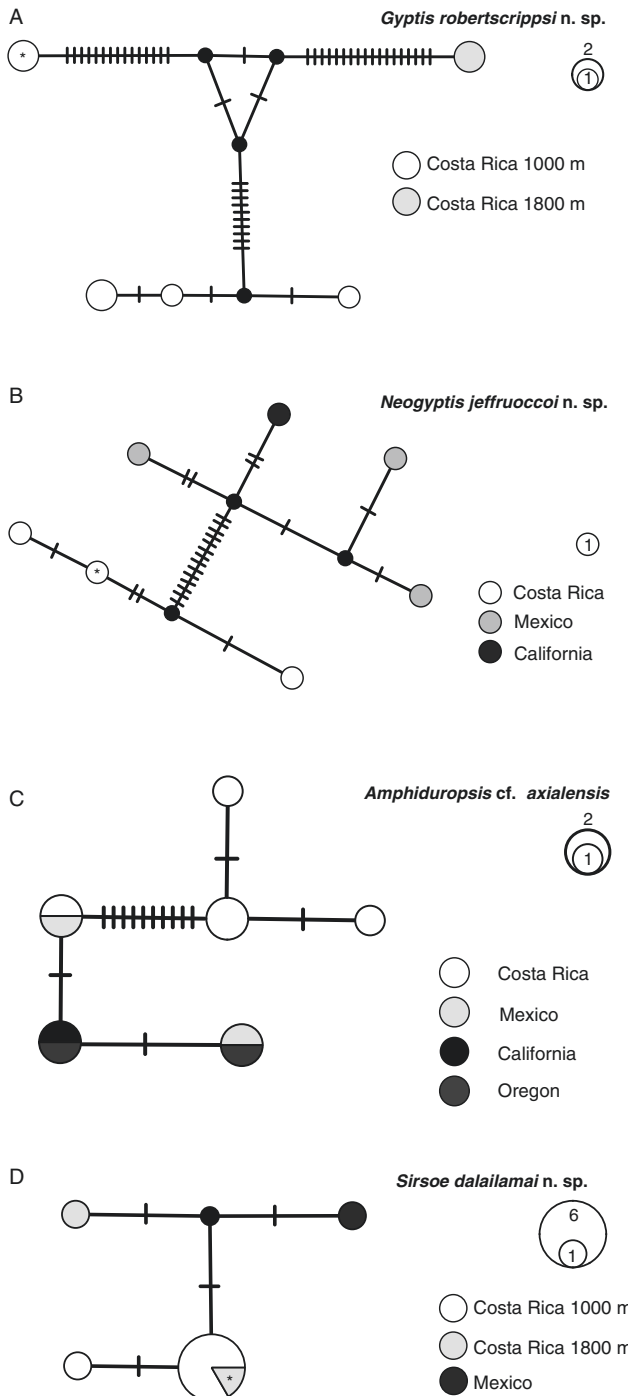
Fig. 2. Median-joining haplotype networks. Slashes indicate nucleotide changes, i.e. missing haplotype. Small black circles denote missing haplotypes. An asterisk indicates the holotype's haplotype for each species. *A*, *Gyptis robertscrippsi* sp. nov.; *B*, *Neogyptis jeffruoccoi* sp. nov.; *C*, *Amphiduropsis* cf. *axialensis*; *D*, *Sirsoe dalailamai* sp. nov.

84°18.78′W, depth 1000 m, coll. GWR, 7.i.2010; 4 spms fixed in osmium and mounted for SEM (SIO-BIC A1750), *Alvin* Dive 4586 (Costa Rica, as above); 1 spm fixed in formaldehyde, posterior fixed in 95% ethanol (SIO-BIC A1751), *Alvin* Dive 4586 (Costa Rica, as above); 1 spm fixed in formaldehyde (SIO-BIC A1752), *Alvin* Dive 4586 (Costa Rica, as above); 4 spms fixed in formaldehyde

(SIO-BIC A1753), *Alvin* Dive 4586 (Costa Rica, as above); 2 spms fixed in formaldehyde (SIO-BIC A1754), 1 posterior end fixed in 95% ethanol (SIO-BIC A1755 destroyed; COI = MG517513), *Alvin* Dive 4588, Costa Rica, 08°55.85′N, 84°18.75′W, depth 997 m, coll. GWR, 9.i.2010; 2 spms fixed in formaldehyde (SIO-BIC A1756), Alvin Dive 4588 (Costa Rica, as above); 1 spm fixed in formaldehyde (SIO-BIC A8100; COI = MG640327), *Alvin* Dive 4906, Costa Rica, 8.93°N, 84.31°W, depth 1001 m, coll. Erik Cordes, 21. v.2017; 6 spms fixed in formaldehyde, posteriors in ethanol (SIO-BIC A8105, 8108; COI = MG640328, MG640329), *Alvin* Dive 4910, Costa Rica, 8.93°N, 84.31°W, depth 1004 m, coll. GWR, 25.v.2017; 5 spms fixed in ethanol (SIO-BIC A8112; COI = MG640330–31), *Alvin* Dive 4914, Costa Rica, 9.12°N, 84.84°W, depth 1886 m, coll. GWR, 29.v.2017.

Measurements

Entire, non-regenerating specimens (n = 7) 9.5–12 mm long for 26–33 segments.

Description

Live specimens transparent with red dorsal and ventral blood vessels and parapodia, gut yellow, prostomium with distinct rust-red sickle-shaped markings (nuchal organs?) near posterior corners (Fig. 3). Fixed specimens whitish with weakly pink proboscis region. Possible nuchal organs (sickle-shaped markings) yellowish green. Body outline elliptical in dorsal view, ventrally flattened. Prostomium forming a trapezoid with shorter posterior side, slightly wider than long, with small median posterior incision. Palpophores cylindrical, weakly tapering, palpostyles slightly longer than palpophores, of even width with rounded tips (Fig. 4A). Paired antennae long, thin, tapering to rounded tips, slightly shorter than palps. Median antenna inserted slightly anterior to middle of prostomium, half length of paired antennae, with fine, pointed tip (Fig. 4A). Eyes absent. Nuchal organs small, along lateral sides of prostomium. Lip pads absent. Proboscis with ~30 elongated, pointed terminal papillae. Non-everted proboscis internally reaching segment 10. Dorsal cirri and cirrophores of segments 1–5 stouter than on following segments (Fig. 4B), with dorsal cirri of segment 1 reaching segment 13–14, dorsal cirri of segment 2 reaching segment 15–17, dorsal cirri of segment 3 reaching segment 11–12, dorsal cirri of segment 4 reaching segment 14–16, and dorsal cirri of segment 5 reaching segment 15–16. Ventral cirri of segments 1–4 with well delineated cirrophores and longer and stouter cirrostyles than on following segments, with ventral cirri of segment 1 reaching segment 8–9, ventral cirri of segment 2 reaching segment 7–8, ventral cirri of segment 3 reaching segment 6, and ventral cirri of segment 4 reaching segment 8–9. Segment 5 with neuropodial lobes and neurochaetae only, notopodial lobes and notochaetae from segment 6 (Fig. 4B). Elevated and slightly stouter dorsal cirri on segments 8, 10, 12, 15, 17, 19, 21, 23, 25, and 27. Dorsal cirri varying from slightly shorter than body width to several times longer. Notopodial acicular lobes triangular (Fig. 5A). Two kinds of notochaetae, ~30 straight chaetae with two alternating rows of teeth, and, more ventral in chaetal bundle, 10–20 distally curved chaetae with convex side serrated and fine, elongated tips (Fig. 5B). All notochaetae chambered, with distinct diaphragms but without visible longitudinal canals in LM. Prechaetal neuropodial acicular lobes triangular, postchaetal lobes shorter and rounded. About 50 neurochaetae, all compound with distinctly internally chambered

Fig. 3. *Gyptis robertscrippsi* sp. nov. Micrograph of relaxed, living paratypes (SIO-BIC A1750), dorsal view. Scale bar, 2 mm.

shafts (Fig. 5*C*), blades with fine serration, ending in a rounded tip, dorsal and median blades up to 3 times longer than ventral ones (Fig. 5*A*). Noto- and neuroaciculae internally chambered, with accessory, much smaller second acicula. Ventral cirri subdistally inserted on underside of neuropodium, reaching past neuropodial lobes, evenly tapered with rounded tips, distal part annulated, without distinct cirrophores (Fig. 4*C*). Pygidium with pair of annulated cirri (broken in all observed specimens) and large papilla (Fig. 4*D*).

Distribution

Known only from the Costa Rica cold seeps at ~1000 to ~1800 m depth.

Remarks

Even though the COI variation among the specimens of *Gyptis robertscrippsi* sp. nov. was up to nearly 6% (see Fig. 2*A*), we did not detect any morphological variation and consider the specimens studied here to all be the same taxon pending further study. *Gyptis* previously included 17 described species (Pleijel 1998), with four more recent additions (Averincev 1990; Pleijel *et al*. 2009). However, based on analyses of molecular data, Pleijel *et al*. (2012) showed that *Gyptis* was paraphyletic and erected *Neogyptis* for part of the group. Following this revision *Gyptis sensu stricto* came to include 17 species and *Neogyptis* nine species (five new *Neogyptis* species were also introduced in that study). Morphologically, the two groups can be distinguished by the subdistally inserted ventral cirri in

Gyptis, and the distally inserted ventral cirri in *Neogyptis*. *Gyptis robertscrippsi* sp. nov. also has subdistally inserted ventral cirri and belongs to *Gyptis*, as evidenced both by morphology and our new analyses (Fig. 1). Among these 17 species of *Gyptis*, only *G. bruneli* (Pettibone, 1961) from St Lawrence in Canada, and *G. hians* Fauchald & Hancock, 1981, from deep water off Oregon, are similar to *G. robertscrippsi* sp. nov. in lacking eyes. Examination of the type material of these species shows that *G. robertscrippsi* sp. nov. differs from *G. bruneli* in having much longer palpophores, palpostyles, and dorsal cirri, and from *G. hians* in having a much smaller number of papillae in the terminal proboscis ring (30 versus >100), a more elongated body shape and much shorter acicular lobes in both noto- and neuropodia.

Etymology

This species is named in memory of Robert P. Scripps.

Hesiolyra Blake, 1985

Hesiolyra bergi Blake, 1985

Material examined

24 spms, (SIO-BIC A6314; COI = MG517521), *Doc Ricketts* Dive 752, Alarcon Rise, Gulf of California, Mexico, 23.36°N, 108.53°W, depth 2299 m, coll. GWR, 20 April 2015; 1 spm (SIO-BIC A6175), *Doc Ricketts* Dive 753, Alarcon Rise, Gulf of California, Mexico, 23.36°N, 108.53°W, depth 2300 m, coll. GWR, 21 April 2015.
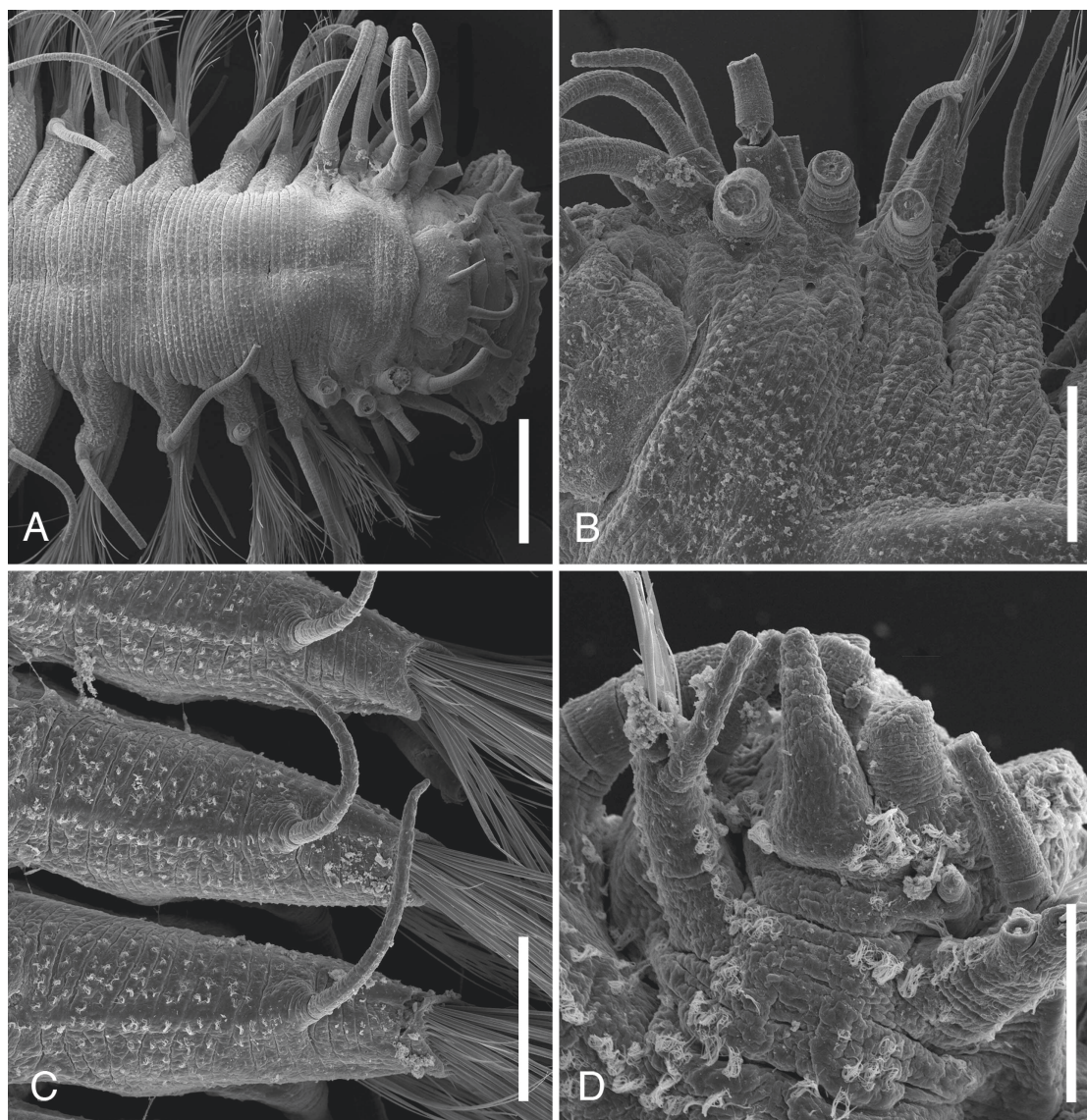
Fig. 4. *Gyptis robertscrippsi* sp. nov. SEM micrographs of paratypes (SIO-BIC A1750). *A*, anterior end, dorsal view; *B*, anterior end, dorsal view, right side; *C*, ventral cirri, median segments; *D*, Posterior end, ventral view. Scale bars: *A*, 500 µm; *B*, *C*, 250 µm; *D*, 100 µm.

Remarks

Blake's (1985) original specimens were all fixed in glutaraldehyde or formaldehyde and unsuitable for sequencing. In two previous studies specimens were sequenced for 18S rDNA (Pradillon *et al.* 2007) and COI (Plouviez *et al.* 2009) and these sequences were included in the phylogenetic analyses by Summers *et al.* (2015). Nevertheless, the identity could not be confirmed, as there was no voucher material for either study. Also, *H. bergi* showed a very long branch relative to all other hesionids in the analyses of Summers *et al.* (2015) and did not fall inside Hesionidae in one analysis. These results raised doubts about the validity of either, or both, sequences. The present inclusion of new sequences (COI, 16S, 18S and 28S) from a specimen collected from near the type area shows morphology in agreement with the original type material. The analysis of the newly generated data showed that the GenBank data (Pradillon *et al.* 2007; Plouviez *et al.* 2009) were

indeed correct and that *Hesiolyra* does have a long branch relative to other hesionids (Fig. 1), but also that it is deeply nested within Ophiodrominae.

AMPHIDURINI Pleijel, Rouse, Sundkvist & Nygren 2012

Neogyptis Pleijel, Rouse, Sundkvist & Nygren, 2012

Neogyptis jeffruoccoi, new species

(Figs 6–8)

Material examined

Holotype. Complete spm, anterior part fixed in formaldehyde (SIO-BIC A1448), posterior end in 95% ethanol (SIO-BIC A1620 destroyed;
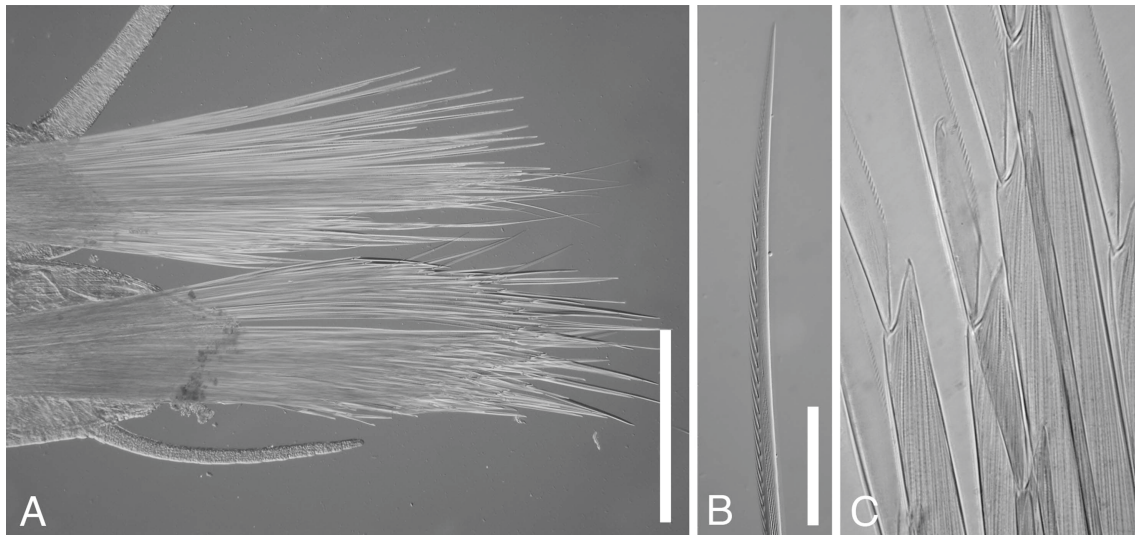
Fig. 5. *Gyptis robertscrippsi* sp. nov. LM micrographs of holotype (SIO-BIC A1314). *A*, median parapodium, anterior view; *B*, notochaeta median parapodium with serrated convex side; *C*, neurochaetae median parapodium. Scale bars: *A*, 200 μm; *B*, 30 μm; *C* same scale as *B*.
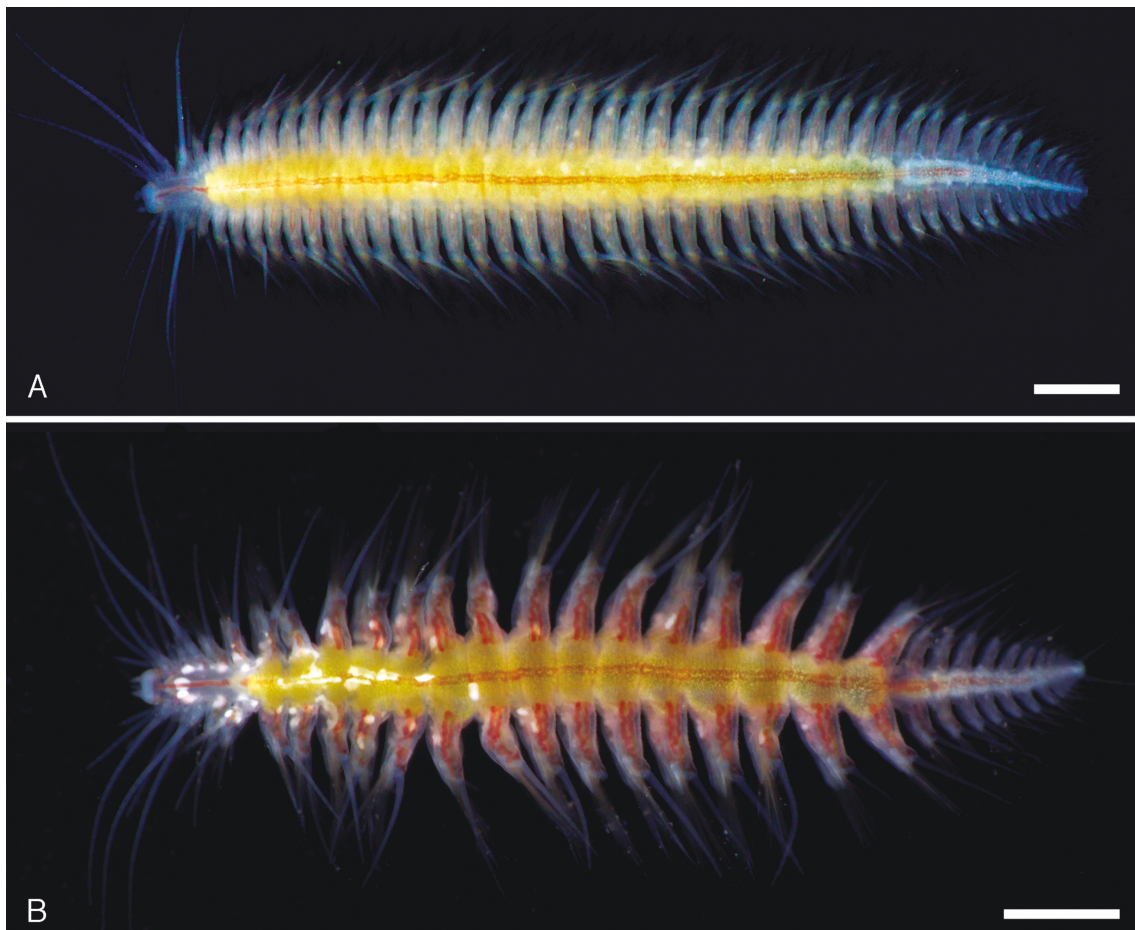

Fig. 6. *Neogyptis jeffruoccoi* sp. nov. Micrograph of relaxed, live specimens in dorsal view. *A*, male, holotype (SIO-BIC A1448); *B*, female, paratype (SIO-BIC A1348). Scale bars, 1 mm.
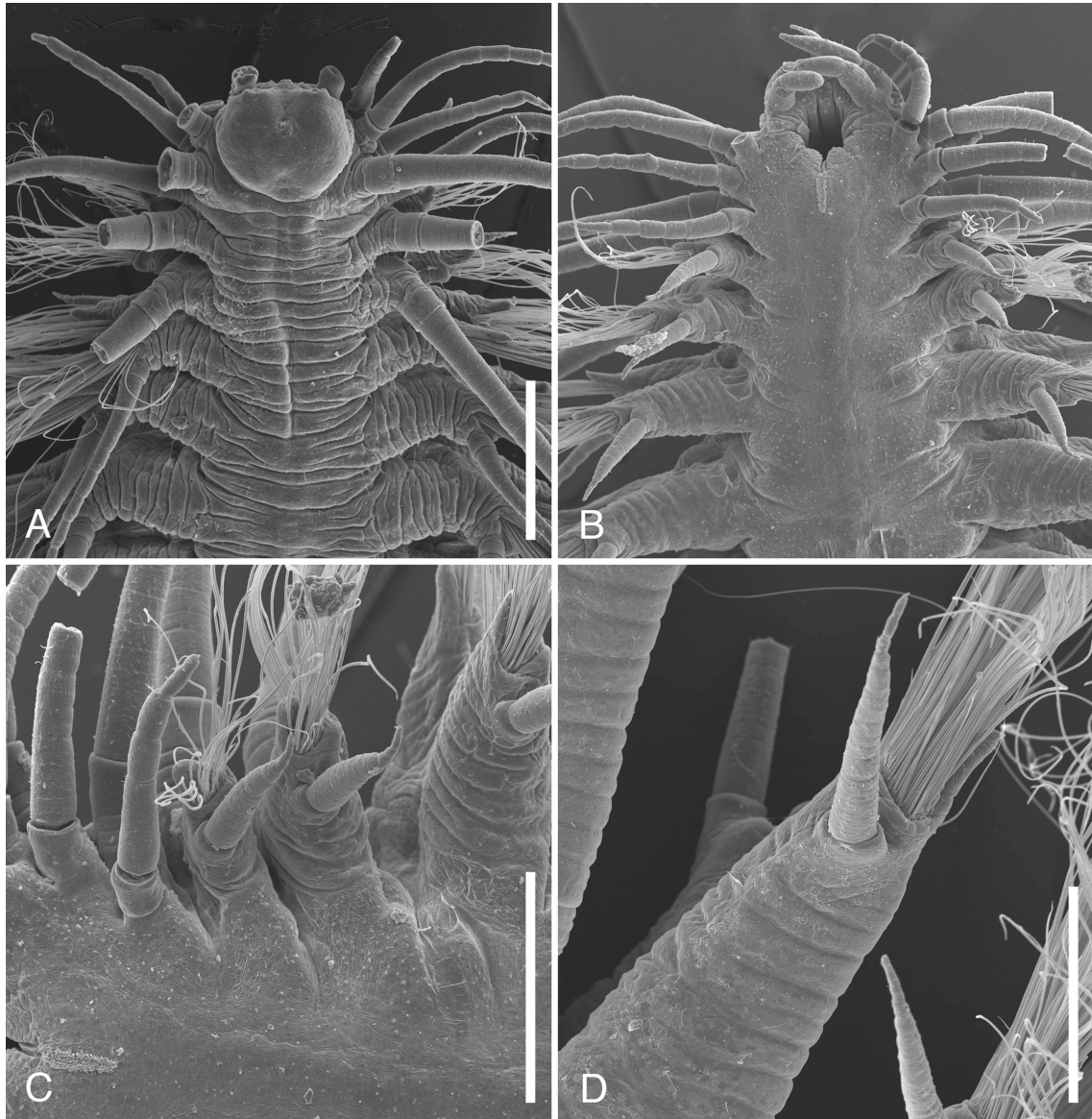
Fig. 7. *Neogyptis jeffruoccoi* sp. nov. SEM micrographs of paratypes (SIO-BIC A1534, A8210). *A*, anterior end, dorsal view; *B*, anterior end, ventral view; *C*, anteriormost segments, left side, ventral view; *D*, ventral cirri, median segments. Scale bars: *A*, 250 μm; *C*, *D* 200 μm; *B* same scale as *A*.

COI = MG517514), *Alvin* Dive 4511, Costa Rica, 08°55.83′N, 84°18.74′W, depth 1001 m, coll. GWR, 5.iii.2009.

Paratypes. 1 spm, anterior end fixed in formaldehyde (MZUCR 399-01), posterior end in 95% ethanol (SIO-BIC A1519 destroyed; COI = MG517516), *Alvin* Dive 4503, Costa Rica, 08°55.86′N, 84°18.43′W, depth 1005 m, coll. GWR, 24.ii.2009; 1 male fixed in formalin and osmium (SIO-BIC A1359), *Alvin* Dive 4505, Costa Rica, 08°55.19′N, 84°18.33′W, depth 1045 m, coll. GWR, 26.ii.2009; 1 female fixed in formaldehyde (SIO-BIC AA1757), posterior end in 95% ethanol (SIO-BIC A1532 destroyed; COI = MG517515), *Alvin* Dive 4505 (Costa Rica, as above); 1 male fixed in glutaraldehyde and osmium for TEM (SIO-BIC A1533), *Alvin* Dive 4505 (Costa Rica, as above); 1 female fixed in formalin (SIO-BIC A1772), *Alvin* Dive 4505 (Costa Rica, as above); 1 male fixed in osmium tetroxide and mounted for SEM (SIO-BIC A1534), *Alvin* Dive 4505 (Costa Rica, as above); 1 spm fixed in osmium tetroxide and mounted for SEM (SIO-BIC A8210), same collection data as holotype; 2 spms fixed in osmium tetroxide and mounted for SEM (SIO-BIC

A8211), *Alvin* Dive 4588, Costa Rica, 08°55.85′N, 84°18.75′W, depth 997 m, coll. GWR, 9.i.2010; 2 spms fixed in 95% ethanol (SIO-BIC A3251; COI = MG517519–20), *Doc Ricketts* Dive 380, Guaymas Basin, Mexico, 27.60°N, 111.49°W, depth 1572 m, coll. GWR, 10.iv.2012; 2 spms fixed in 95% ethanol (SIO-BIC A3252; COI = MG517517), Doc Ricketts Dive 387, Guaymas Basin, Mexico, 27.59°N, 111.48°W, depth 1576 m, coll. GWR, 14.iv.2012.

Other material. 1 spm (destroyed for DNA sequencing), *Doc Ricketts* Dive 472, Del Mar Seep, California, 32.90°N, 117.78°W, depth 1020 m, coll. GWR, 19.v.2013.

Measurements

Holotype 11.5 mm long for 45 segments (measured live, relaxed). Paratype SIO-BIC A1317, 8 mm long for 31 segments (measured live, relaxed). Paratype male SIO-BIC A1359 12 mm long for 37 segments (measured fixed).
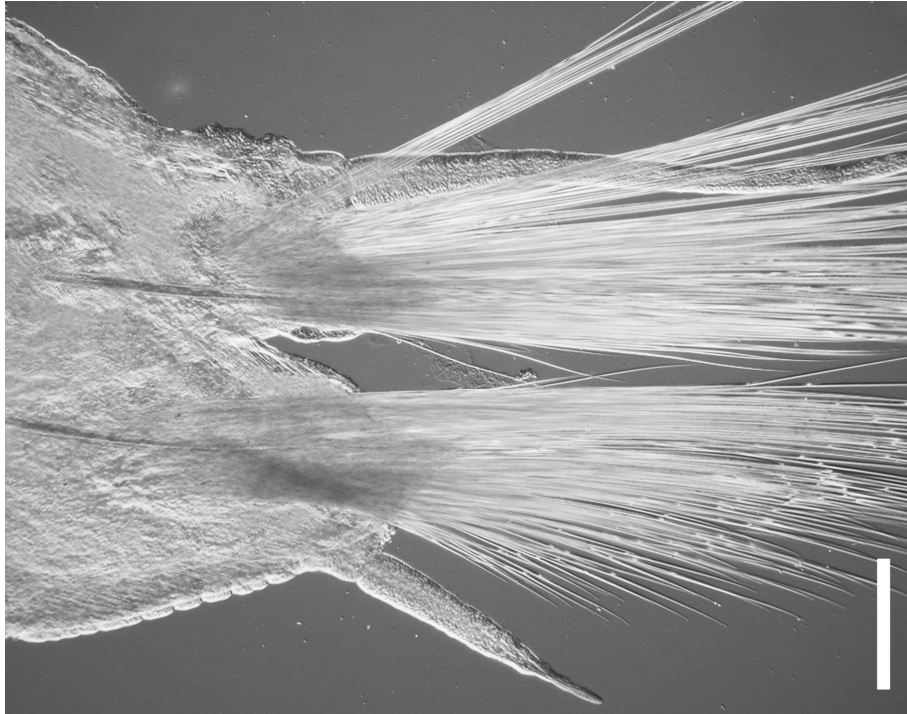
Fig. 8. *Neogyptis jeffruoccoi* sp. nov. LM micrograph of median parapodium from holotype (SIO-BIC A1448), posterior view. Scale bar, 100 µm.

Description

Live specimens transparent with more or less distinct red dorsal and ventral blood vessels and parapodia, gut yellow; eggs white. Fixed specimens whitish. Body outline elliptical in dorsal view, dorso-ventrally distinctly flattened (Fig. 6*A*, *B*). Prostomium rounded (Fig. 7*A*), slightly wider than long, weakly incised frontally between insertion of paired antennae. Palpophores and palpostyles of equal length, palpophores cylindrical, palpostyles tapering (Fig. 7*B*). Paired antennae of same length as palps, cylindrical with well delineated fine, elongated tips. Median antenna similar to paired antennae but thinner. Eyes absent. Nuchal organs small, bordering lateral sides of prostomium. Lip pads absent. Proboscis with terminal ring of 10 small, pointed papillae. Non-everted proboscis internally reaching segment 7. Dorsal cirri and cirrophores of segments 1–5 slightly stouter than on following segments, with dorsal cirri of segment 1 reaching segment 9, dorsal cirri of segment 2 reaching segment 13, dorsal cirri of segment 3 reaching segment 11–12, and dorsal cirri of segments 4 and 5 reaching segment 13. Ventral cirri of segment 1–3 with well delineated cirrophores and longer and stouter cirrostyles than on following segments, with ventral cirri of segment 1 reaching segment 8, ventral cirri of segment 2 reaching segment 7–8, and ventral cirri of segment 3 reaching segment 6–7. Ventral cirri of segment 4 similar to those of following segments (Fig. 7*C*). Segments 4 and 5 with neuropodial lobes and neurochaetae only, notopodial lobes and notochaetae from segment 6 (Fig. 7*C*). Slightly stouter and more elevated dorsal cirri on segments 5, 8, 10, 12, 15, 17 and 19, thereafter not visible on available material. Dorsal cirri distinctly annulated, reaching as far or slightly beyond

chaetae. Notopodial acicular lobes triangular. Notochaetae ~50, of single kind, capillaries with alternating rows of teeth and very fine, elongated tips. Notochaetae internally chambered, with distinct diaphragms but without visible longitudinal canals in LM. Prechaetal neuropodial acicular lobes triangular, postchaetal lobes poorly developed. About 50 neurochaetae, all compound with distinctly internally chambered shafts, blades with very fine serration, ending in a rounded tip, dorsal and median blades up to 4 times longer than ventral ones. Noto- and neuroaciculae internally chambered, with accessory, much smaller second acicula. Ventral cirri reaching well beyond neuropodial lobes, long, thin, with distinctly demarcated and annulated tips, distally inserted on underside of neuropodium, inserted on indistinct cirrophores (Figs 7*D*, 8). Pygidium with pair of annulated cirri, similar to dorsal cirri, pygidial papilla absent.

Distribution

Known from cold seeps off the Pacific coast of Costa Rica (Mound 12) at ~1000 m, Mexico (Guaymas Basin) at ~1500 m, and California (Del Mar) at ~1000 m.

Remarks

With the exception of the specimen from California, all *Neogyptis jeffruoccoi* sp. nov. specimens (Costa Rica and Guaymas) were collected from within the mantle cavity of the clam *Acharax johnsoni* (Dall, 1891). The *Acharax* either hosted a solitary specimen, or sometimes a pair of each sex, as in Fig. 6. The same *Acharax* specimens also generally hosted

the chrysopetalid parasite *Natsushima sashai* Aguado & Rouse, 2011. The specimen collected from the Del Mar seep was not associated with *A. johnsoni*, though these clams do occur at this site (Grupe *et al*. 2015). While several hesionids are known to be symbionts with other animals (Martin and Britayev 1998), only two others are known to be symbiotic with bivalve molluscs. *Oxydromus okapa* Martin, Meca & Gil, in Martin *et al*. (2017) and *Oxydromus humesi* (Pettibone, 1961), which are not closely related to *N. jeffruoccoi* sp. nov., are obligate symbionts of tellinoid bivalves, living inside the mantle cavity (Martin *et al*. 2012, 2015). They are both inferred to be likely parasites of the bivalves (Martin *et al*. 2017), and we suggest that *N. jeffruoccoi* sp. nov. may also be parasitic on *A. johnsoni*.

As outlined above, the intraspecific variation across the seven COI sequences for *N. jeffruoccoi* sp. nov. was maximally 3.3% (uncorrected), with a clear break between the Costa Rican specimens and the Mexican/Californian specimens (Fig. 2B). Given that the Guaymas specimens were also found living inside *A. johnsoni*, and there was no obvious morphological variation among the *N. jeffruoccoi* sp. nov. specimens, we consider them all to be the same species. The distribution of *N. jeffruoccoi* sp. nov. from Costa Rica to California is similar to the known distribution of the chrysopetalid *Micospina aurobohnorum* Watson, Carvajal, Pleijel, Sergeeva & Rouse, 2016, though the latter was found on a whale fall in California rather than at a seep (Watson *et al*. 2016).

Neogyptis previously included *N. carriebowcayi* Pleijel, Rouse, Sundkvist & Nygren, 2012, *N. crypta* (Pleijel, 1993), *N. fauchaldi* Pleijel, Rouse, Sundkvist & Nygren, 2012, *N. julii* Summers, Pleijel & Rouse, 2015, *N. hinehina*, *N. hongkongensis* Pleijel, Rouse, Sundkvist & Nygren, 2012, *N. mediterranea* (Pleijel, 1993), *N. nonatoi* Rizzo & Salazar-Vallejo, 2014, *N. plurisetis* (Hilbig, 1992), *N. rosea* (Malm, 1874), and *N. vostokensis* Pleijel, Rouse, Sundkvist & Nygren, 2012. Apart from the molecular data, the distal insertion of the ventral cirri indicates that our new species belongs to *Neogyptis*, rather than *Gyptis*, and it is distinct from *Amphiduros* in having a terminal ring of papillae on the proboscis. It shares the lack of eyes with *N. hinehina*. Within the *Neogyptis* clade, it is unique in having neurochaetae from segment 4, rather than from segment 5, as in other members of *Neogyptis*.

Etymology

This species is named in memory of Jeff Ruocco.

Amphiduropsis Pleijel, 2001

Amphiduropsis cf. *axialensis* (Blake & Hilbig 1990)

(Figs 9–11)

Material examined

Complete spm, anterior part fixed in formaldehyde (SIO-BIC A1758), posterior end in 95% ethanol and destroyed for DNA sequencing; COI = MG517505, *Alvin* Dive 4589, Costa Rica, 08°55.79′N, 84°18.72′W, depth 998 m, coll. GWR, 10.i.2010. 1 spm fixed in formaldehyde (MZUCR 398-01), *Alvin* Dive 4501, Costa Rica, 08°55.80′N, 84°18.81′W, depth 1008 m, coll. GWR, 22.ii.2009; 1 spm fixed in formaldehyde (SIO-BIC A1759), posterior end fixed in 95% ethanol destroyed (COI = MG517503) *Alvin* Dive

4501 (as above); 4 spms fixed in formaldehyde (SIO-BIC A1760), 1 posterior end in 95% ethanol (SIO-BIC A1770 destroyed; COI = MG517502), same collection data as holotype; 1 anterior end fixed in formaldehyde (SIO-BIC A1317), *Alvin* Dive 4501 (Costa Rica, as above); 1 spm fixed in formaldehyde (SIO-BIC A1761), posterior end fixed in 95% ethanol (SIO-BIC A1771), *Alvin* Dive 4586, Costa Rica, 08°55.85′N, 84°18.78′W, depth 1000 m, coll. GWR, 7.i.2010; 2 spms fixed in formaldehyde (SIO-BIC A1762), 1 spm in 95% ethanol (SIO-BIC A1763; COI = MG517504), *Alvin* Dive 4588, Costa Rica, 08°55.85′N, 84°18.75′W, depth 997 m, coll. GWR, 9.i.2010; 1 spm fixed in formaldehyde, posterior end in ethanol, destroyed for DNA sequencing (SIO-BIC A8110; COI = MG640338), *Alvin* Dive 4910, Costa Rica, 8.93°N, 84.31°W, depth 1004 m, coll. GWR, 25.v.2017; 1 spm anterior end fixed in formaldehyde (SIO-BIC A2063), posterior end fixed in 95% ethanol (SIO-BIC A1998; COI = MG517499), Hydrate Ridge, Oregon, *Alvin* Dive 4629, 44.57°N, 125.1548°W, depth 809 m, coll. Danwei Huang, 1. viii.2010; 1 spm anterior end fixed in formaldehyde (SIO-BIC A2731), posterior end fixed in 95% ethanol (destroyed; COI = MG517500), Hydrate Ridge, Oregon, *Jason II* Dive 593, 44.67°N, 125.0987°W, depth 587 m, coll. GWR, 4.ix.2011; 2 spms fixed in 95% ethanol (SIO-BIC A3254 and A3256; COI = MG517506, MG517507), Guaymas Basin, Mexico, *Doc Ricketts* Dive 387, 27.59°N 111.47°W, depth 1576 m, coll. GWR, 14.iv.2012. 1 spm (destroyed for DNA sequencing, COI = MG517501), *Doc Ricketts* Dive 472, Del Mar Seep, California, 32.90°N, 117.78°W, depth 1020 m, coll. GWR, 19.v.2013.

Measurements

SIO-BIC A1313 3.5 mm long for 24 segments, SIO-BIC A1761 21 mm long for 35 segments (last segments missing), SIO-BIC A1760 18.5 mm long for 27 segments (posterior end regenerating),SIO-BIC A1760 24 mm long for 29 segments (last segments missing).

Description

Live specimens transparent with yellow to brown gut with red dorsal and ventral blood vessels, 1 specimen lemon green. Fixed specimens yellowish to white. Body outline in dorsal view anteriorly truncated and posteriorly tapered (Fig. 9). Prostomium rounded rectangular, slightly wider than long, with small median posterior incision. Palpophores cylindrical, palpostyles 4–5 times longer and much thinner, with rounded tips. Paired antennae similar to palpostyles, as long as palps (Fig. 10*A*). Median antenna inserted medially on prostomium, much shorter than paired antennae and evenly tapering. Eyes absent. Nuchal organs follow postero-dorsal corners of prostomium. Lip pads absent. Proboscis with ciliated ring but without terminal papillae (Fig. 10*B*). Non-everted proboscis long, internally reaching up to segment 12–15 depending on specimen size. Dorsal cirri and cirrophores of segments 1–5 stouter and longer than on following segments, with dorsal cirri of segment 1 reaching segment 8, dorsal cirri of segment 2 not observed entire, and dorsal cirri of segments 3–5 reaching about segment 11. Ventral cirri of segments 1–4 with well delineated cirrophores and stouter and longer cirri than on following segments, with ventral cirri of segments 1 and 2 reaching segment 7, and ventral cirri of segments 3 and 4 reaching segment 8. Segment 5 with neuropodial lobes and neurochaetae only, notopodial lobes and notochaetae from segment 6. Alternation of dorsal cirri not observed. Dorsal cirri highly variable in length, from slightly shorter than body width to many times longer, thin, annulated. Notopodial lobes elongated conical (Fig. 11*A*).

Fig. 9.   *Amphiduropsis* cf. *axialensis* Micrograph of relaxed, live specimens, upper specimen paratype (SIO-BIC A1317), lower specimen destroyed for DNA sequencing. Scale bar, 2 mm.

Two kinds of notochaetae, 50+ straight chaetae with alternating rows of teeth and very fine, elongated tips, and, more ventral in chaetal bundle, ~10 distally curved chaetae with convex side serrated and fine, elongated tips (Fig. 11*B*). All notochaetae chambered, with distinct diaphragms but without visible longitudinal canals in LM. Prechaetal neuropodial aciculary lobes triangular, postchaetal lobes shorter and rounded. Neurochaetae 50+, all compound with distinctly internally chambered shafts, blades without visible serration in LM, ending in a rounded tip, dorsal and median blades up to 3 times longer than ventral ones (Fig. 11*C*). Single noto- and neuroaciculae, internally chambered. Ventral cirri evenly tapering and pointed, not annulated, distally inserted on underside of neuropodium, reaching well beyond tips neuropodial lobes, inserted on small cirrophores. Pygidial cirri similar to longest dorsal cirri, median pygidial papilla unknown.

Distribution

These specimens were collected from cold seeps off the Pacific coast of Costa Rica (Mound 12) at ~ 1000 m, Mexico (Guaymas Basin) at ~1500 m, California (Del Mar) at ~1000 m, and Oregon (Hydrate Ridge) at ~800 m. The type locality of *Amphiduropsis axialensis* is hydrothermal vents at 1,545 meters depth on Axial Seamount (Juan de Fuca ridge) (Blake and Hilbig 1990), about 400 km to the northwest of Hydrate Ridge.

Remarks

Amphiduropsis is monotypic, containing only *A. axialensis*. *Amphiduropsis* was erected by Pleijel (2001) who argued that *Amphiduros* had a clear set of apomorphies if *A. axialensis* was excluded from the genus. *Amphiduropsis axialensis* differs from *Amphiduros* in having long and thin paired antennae and palpostyles, in lacking eyes and in having very short palpophores. It does show the absence of a terminal ring with papillae on the proboscis, which is typical of *Amphiduros* but also occurs in other hesionids, such as *Oxydromus* Grube, 1855. The proboscis is unusual in being very long, and in the non-everted state it reaches segment 12–15 internally. Our specimens matched the previous descriptions of *Amphiduropsis axialensis* by (Blake and Hilbig 1990, Pleijel 2001) and we found no features that could distinguish it as another species of *Amphiduropsis*. We don't refer to the specimens unequivocally as *Amphiduropsis axialensis* since no DNA sequences have been obtained for specimens from the type locality. *Amphiduropsis* cf. *axialensis* was the widest-ranging species in this study, having been found over a range of 6000 km from off Costa Rica, Mexico, California and Oregon. A similar range, based on molecular data, has been reported for the ampharetid annelid *Amphisamytha fauchaldi* Solís-Weiss & Hernández-Alcántara, 1994 by Stiller *et al.* (2013).
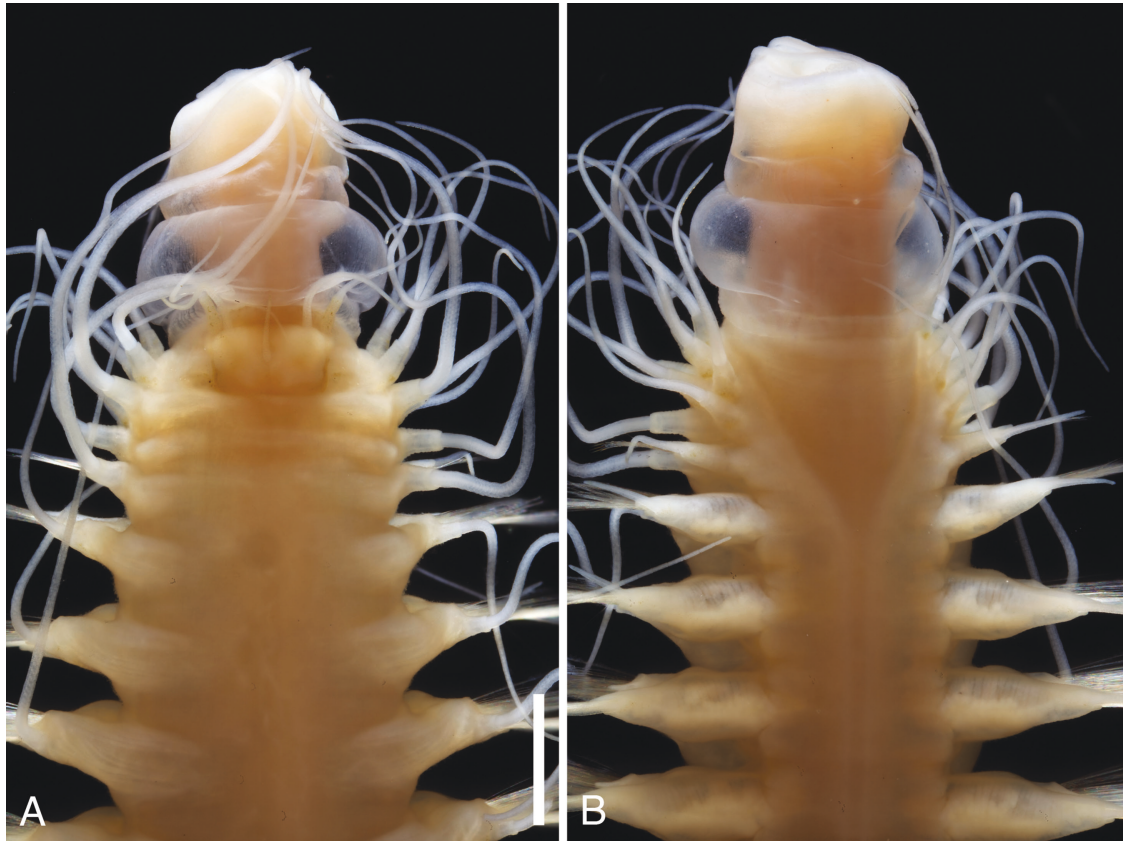
Fig. 10. *Amphiduropsis* cf. *axialensis* LM micrographs of fixed holotype (SIO-BIC A1758). *A*, anterior end, dorsal view; *B*, anterior end, ventral view. Scale bar: *A*, 100 μm; *B* same scale as *A*.
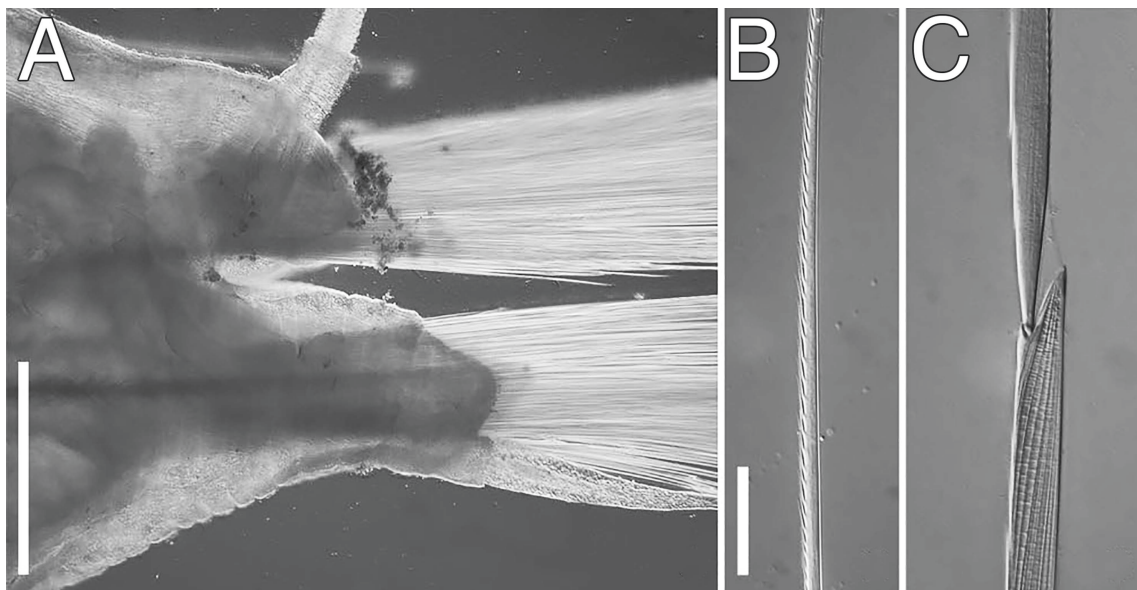

Fig. 11. *Amphiduropsis* cf. *axialensis* LM micrographs of holotype (SIO-BIC A1758). *A*, median parapodium, posterior view; *B*, notochaeta from median parapodium with serrated convex side; *C*, compound chaeta. Scale bars: *A*, 200 μm; *B*, 10 μm; *C* same scale as *B*.

PSAMATHINI Pleijel, 1998

Sirsoe Pleijel, 1998

Sirsoe dalailamai, sp. nov.

(Figs 12–13)

Material examined

Holotype. Complete spm fixed in formaldehyde (SIO-BIC A1766), *Alvin* Dive 4590, Costa Rica, 09°07.05′N, 84°50.37′W, depth 1800 m, coll. GWR, 11.i.2010.

Paratypes. 3 spms fixed in formaldehyde (MZUCR 401-01), posterior end fixed in 95% ethanol and destroyed for DNA sequencing (COI = MG517498), *Alvin* Dive 4588, Costa Rica, 08°55.85′N, 84°18.75′W, depth 997 m, coll. GWR, 9.i.2010; 1 spm fixed in formaldehyde (SIO-BIC A1405), posterior end in 95% ethanol and destroyed for DNA sequencing, *Alvin* Dive 4508, Costa Rica, 09°01.82′N, 84°37.38′W, depth 1433 m, coll. GWR, 1.iii.2009; 5 spms fixed in formaldehyde (SIO-BIC A1768), 1 entire spm and 2 posterior ends in 95% ethanol (SIO-BIC A1769; COI = MG517496), *Alvin* Dive 4590 (Costa Rica, as for holotype above); 6 spms fixed in formaldehyde, posterior end in ethanol, destroyed for DNA sequencing (SIO-BIC A8102–8109; COI = MG640333–MG640337), *Alvin* Dive 4910, Costa Rica, 8.93°N, 84.31°W, depth 1004 m, coll. GWR, 25.v.2017; 12 spms, 1 fixed in formaldehyde, 11 in ethanol (SIO-BIC A81111; COI = MG640338), *Alvin* Dive 4914, Costa Rica, 9.12°N, 84.84°W, depth 1886 m, coll. Chris Roman, 29.v.2017; 1 spm fixed in formaldehyde, posterior end in 95% ethanol and destroyed for DNA sequencing (SIO-BIC A3253; COI = MG517497), *Doc Ricketts* Dive 387, Guaymas Basin, Mexico, 27.59°N, 111.48°W, depth 1576 m, coll. GWR, 14.iv.2012.

Measurements

Holotype 16 mm long for 35 segments. Paratype SIO-BIC A1767 13.5 mm for 39 segments. Paratypes SIO-BIC A1768 23 mm long for 40 segments and 15 mm long for 40 segments.

Description

Live specimens brown-red to yellowish, gut yellow, mid-dorsal red blood vessel distinct, smaller specimens more transparent with yellow gut (Fig. 12). Fixed specimens white to yellow brown. Body outline anteriorly truncate and posteriorly tapered in dorsal view, venter flattened. Prostomium rounded octogonal, slightly wider than long, posteriorly poorly delineated and fused to segment 1. Palpophores and palpostyles together evenly tapering, palpostyles slightly longer than palpophores (Fig. 13A). Paired antennae much thinner than palps, tapering to fine points, shorter than palps (Fig. 13A). Median antenna inserted near posterior border of prostomium (Fig. 13A), very small and pointed, not distinct in all examined specimens (possibly retractile). Eyes absent. Nuchal organs forming two short but distinct slits postero-laterally on prostomium. Lip pads absent (Fig. 13B). Proboscis with 10 tapering papillae situated on dorsal half of the proboscis opening (Fig. 13C); papillae elongated, tapering, decreasing in size towards the ventral side. Proboscis opening midventrally with incision and small rounded knob, also visible on specimens with non-everted proboscis (Fig. 13B, C). Non-everted proboscis reaching segment 9 (Fig. 13A). Anterior dorsal cirri and cirrophores not obviously differing from following ones. Anterior ventral cirri of segments 1–3 with well delineated cirrophores and longer and stouter cirri than on following segments, with ventral cirri of segment 1 reaching segment 5, ventral cirri of segment 2 reaching segment 6, and ventral cirri of segment 3 reaching segments 5–6. Segment 3 with small triangular (neuropodial?) lobes dorsally to insertion of ventral cirri. Segment 4 with neurochaetae and fully developed neuropodial lobes (Fig. 13B). Notochaetae absent from all segments. Elevated and slightly stouter dorsal cirri on segments 5, 8, 12, 15, 17, 20, 23, 26, 28, and 30 (Fig. 12). Dorsal cirri reaching well beyond chaetae, about equal in length to body width, proportionally longer in smaller specimens. Dorsal cirri weakly annulated with very elongated sections. Neuropodial prechaetal lobes triangular (Fig. 13D), postchaetal lobes slightly shorter and rounded. Neurochaetae 30–50, all compound with distinctly internally chambered shafts, ending in rounded tip, dorsal and median blades up to 3 times longer than ventral ones. Most blades with fine serration but some with elongated teeth (Fig. 13E), situated medially in chaetal bundle. Noto- and neuroaciculae internally chambered, 3 notoaciculae and 2 neuroaciculae in median parapodia. Aciculae of equal size in each parapodial lobe.

Fig. 12. *Sirsoe dalailamai* sp. nov. Micrograph of living, relaxed paratype (SIO-BIC A1767), dorsal view. Scale bar, 3 mm.
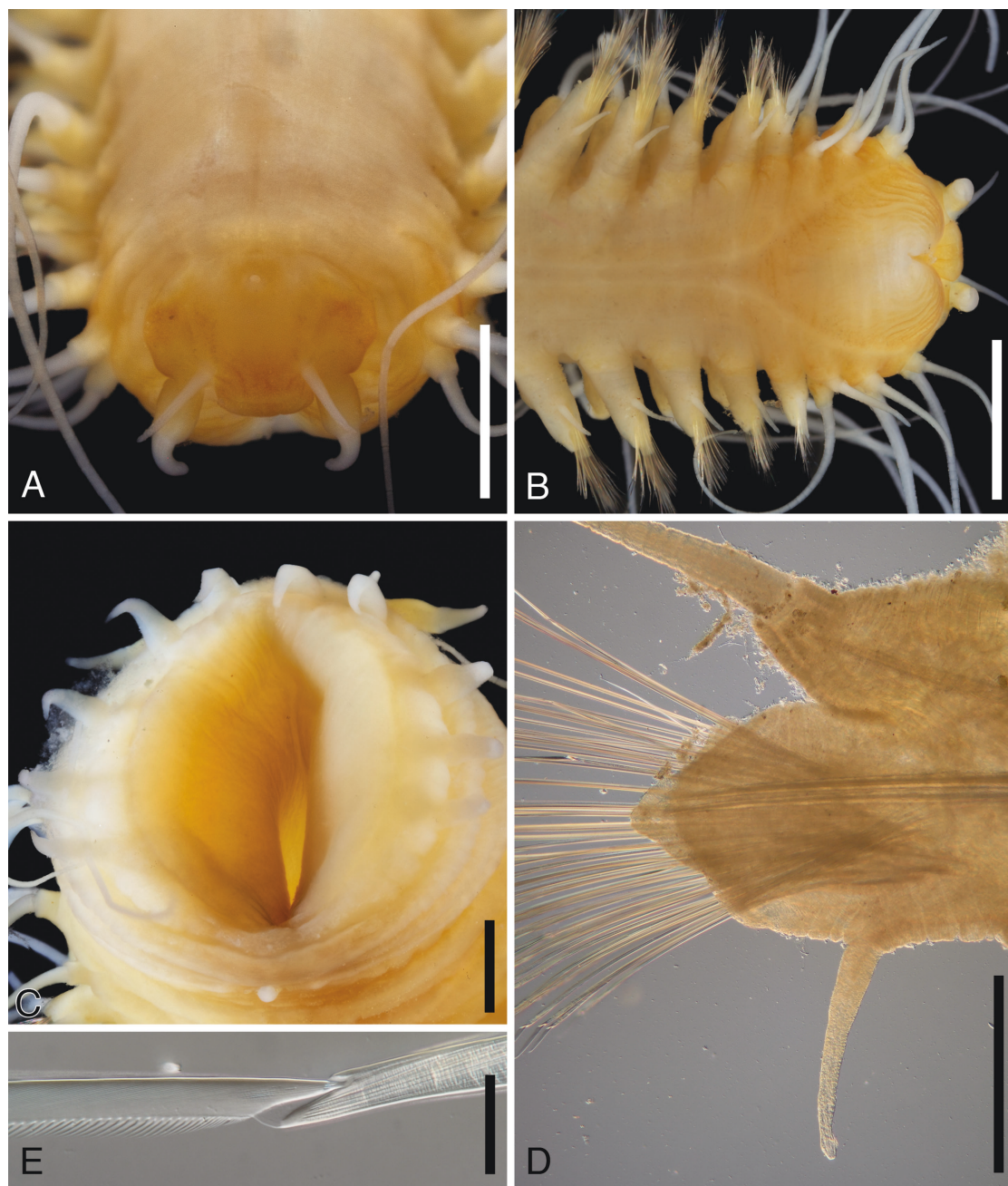
Fig. 13. *Sirsoe dalailamai* sp. nov. Micrograph of fixed holotype (SIO-BIC A1766) (*A*, *B*, *D*, *E*) and paratype (SIO-BIC A1768) (*C*). *A*, anterior end, antero-dorsal view; *B*, anterior end, ventral view; *C*, proboscis opening, antero-ventral view; *D*, median parapodium; *E*, chaeta from median segment. Scale bars: *A*–*C*, 2.5 mm; *D*, 100 μm; *E*, 50 μm.

Ventral cirri reaching about as far as tips of neuropodial lobes, evenly tapering, subdistally inserted on underside of neuropodium, without distinct cirrophores (Fig. 13*E*). Pygidial cirri similar to dorsal cirri (entire ones not observed); median pygidial papilla absent.

Distribution

Known from cold seeps off the Pacific coast of Costa Rica at ~1000–1800 m, and Mexico (Guaymas Basin) at ~1500 m.

Remarks

Prior to this study, *Sirsoe* included *S. grasslei*, *S. methanicola*, and *S. sirikos* Summers, Pleijel & Rouse, 2015. *Sirsoe dalailamai* sp. nov. is the sister species to *S. methanicola* (Fig. 1) and is similar to this species in having a tiny knob-like median antenna on the posterior dorsal part of the prostomium and neurochaetae appearing from segment 3. Morphologically, we cannot actually provide any obvious differences between these two species and the erection of the new species is justified

solely by molecular data. However, *S. methanicola* is from the Gulf of Mexico and *S. dalailamai* sp. nov. from off the Pacific coast of Costa Rica and they are separated from each other by COI with a minimum uncorrected pairwise distance of 10.5%. The distance between the *S. dalailamai*–*S. methanicola* clade and *S. munki* is 13.5%. *Sirsoe methanicola* (commonly known as the 'methane ice worm') is notable for being associated with methane hydrates in the Gulf of Mexico at ~540 m depth and feeds on chemoautotrophic bacteria (Fisher *et al.* 2000). *Sirsoe dalailamai* sp. nov. was associated with vestimentiferan and mussel assemblages in areas of active methane seepage, but there were no methane hydrates present.

Etymology

This species is named in honour of His Holiness the 14th Dalai Lama (Tenzin Gyatso).

Sirsoe munki, new species
(Figs 14–15)

Material examined

Holotype. Initially complete spm, anterior part fixed in formaldehyde (SIO-BIC A1409), posterior end in 95% ethanol and mostly destroyed for DNA sequencing (COI = MG517510), *Alvin* Dive 4509, Costa Rica, 9.12°N, 84.84°W, depth 1866 m, coll. GWR, 3.iii.2009.

Paratypes. 2 spms (SIO-BIC A1765, MZUCR 400-01; COI = MG517508, MG517509), fixed in 95% ethanol, posterior ends destroyed for sequencing, *Alvin* Dive 4590, Costa Rica, 9.12°N, 84.84°W, depth 1800 m, coll. GWR, 11.i.2010.

Measurements

Holotype (complete) 4.25 mm long for 23 segments.

Description

Live specimens semitransparent with yellow gut. Fixed specimens white to yellowish. Body outline in dorsal view anteriorly truncated and posteriorly tapered (Fig. 14). Prostomium rounded rectangular, slightly wider than long. Palpophores and palpostyles evenly tapered, palpophores short, palpostyles longer. Paired antennae thinner and shorter than palps. Median antenna tiny, knob-like, positioned mid-dorsally on prostomium. Eyes absent. Nuchal organs not observed. Everted proboscis not observed, non-everted proboscis short, internally reaching segment 6. Dorsal cirri very long, anteriormost cirri and cirrophores apparently similar to those on following segments. Ventral cirri of segments 1–2 longer than ventral cirri of following and median segments, segment 3 with parapodial lobes, neurochaetae and ventral cirri similar to following ones. Multiple aciculae in noto- and neuropodia (Fig. 15*A*). Notochaetae absent. About 30 neurochaetae, dorsal and ventral with shorter blades, median ones longer (Fig. 15*B*). Blades finely serrated. Alternation of dorsal cirri not observed. Ventral cirri not reaching tips of neuropodial lobes, evenly tapered, subdistally inserted on underside of neuropodium, without distinct cirrophores (Fig. 15*A*). Pygidial cirri not observed.

Distribution

Known only from the Costa Rica cold seeps at ~1800 m.

Fig. 14. *Sirsoe munki* sp. nov. Micrograph of living, relaxed holotype (SIO-BIC A 1409). Scale bar, 1 mm.
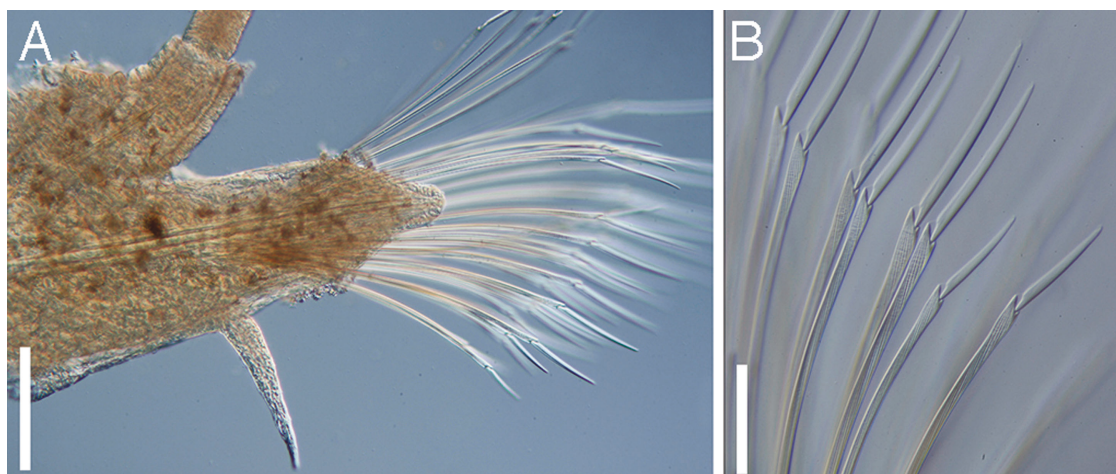
Fig. 15. *Sirsoe munki* sp. nov. LM micrographs of fixed holotype (SIO-BIC A 1409). *A*, median parapodium, anterior view; *B*, chaetae from median segment. Scale bars: *A*, 100 µm; *B*, 50 µm.

Remarks

Sirsoe munki sp. nov. is referred to *Sirsoe* and as a new species mainly based on molecular data. Due to the small size of the specimens and the absence of mature ones, we cannot at present exclude that the collected specimens were subadults. The minimum uncorrected pairwise distance of CO1 between *S. munki* sp. nov. and the sister group, *S. dalailamai*–*S. methanicola*, is 13.5%.

Etymology

This species is named in honour of SIO Research Professor of Geophysics, Emeritus, Walter Munk.

Conflicts of interest

The authors declare no conflicts of interest.

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