Article

A new species and phylogenetic insights in *Hesiospina* (Annelida, Hesionidae)

PAULO BONIFÁCIO[1,3], YANN LELIÈVRE[1,2] & EMMANUELLE OMNES[1]

[1]*Ifremer, Centre de Bretagne, REM/EEP, Laboratoire Environnement Profond, ZI de la Pointe du Diable, CS 10070, F-29280 Plouzané, France. E-mail: bonif@me.com, Emmanuelle.omnes@ifremer.fr*

[2]*Département de sciences biologiques, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, Québec H3C 3J7, Canada. E-mail: yann.lelievre@ifremer.fr*

[3]*Corresponding author: Paulo Bonifácio. E-mail: bonif@me.com*

Abstract

Hesionids are a very speciose group of polychaetes. In the deep sea, they occur in different environments such as hydrothermal vents, cold seeps, abyssal depths or whale falls. In the present study, a new species of Hesionidae, *Hesiospina legendrei* **sp. nov.** has been identified based on morphological and molecular (16S and COI genes) data from hydrothermal vents located in Juan de Fuca Ridge (NE Pacific Ocean). This new species is characterized by trapezoid prostomium; proboscis with high number of distal papillae (20–27), a pair of sac-like structures inserted ventro-laterally in proboscis; notopodia lobe reduced with multiple, slender aciculae on segments 1–5; and neuropodia developed with single, simple chaeta, and numerous, heterogomph falcigers, with 1–2 inferiormost having elongated hood. *Hesiospina legendrei* **sp. nov.** is the third described species in the genus. Sequences from the two previously described *Hesiospina* species are included in the molecular analyses, and although the genes used in this study are not sufficient to resolve the relationships on genus level, the result raises questions about the cosmopolitan aspect of *H. vestimentifera*.

Key words: Juan de Fuca Ridge; hydrothermal vent; morphology, molecular; taxonomy, deep-sea, Hesionidae

Introduction

The genus *Hesiospina* Imajima & Hartman 1964 belongs to the family Hesionidae which has 22 genera listing about 150 valid species (Wilson 2000). Hesionids are benthic polychaetes typically having long tentacular and dorsal cirri (often broken in collected material) that occur from the subtidal to the deep-sea (Wilson 2000), with about 80% of the species occurring in shallow waters above 500 m depth (Glover *et al.* 2018). In the deep sea, they can be found living in hydrothermal vents (Blake 1985; Blake & Hilbig 1990; Tsurumi & Tunnicliffe 2001, 2003; Bergquist *et al*. 2007; Marcus *et al*. 2009), cold seeps (Desbruyères & Toulmond 1998), abyssal depths (Hartman 1965) or, even, whale falls (Pleijel *et al*. 2008; Summers *et al.* 2015). Among Hesionidae genera, only *Hesiospina* present a subbiramous parapodia with stout protruding notopodial hook as an apomorphy (Pleijel 1998, 2004). Based on morphological phylogenetic analysis, Pleijel (1998) was able to identify this apomorphy and recovered *Hesiospina* as monophyletic. Phylogenetic studies based only on molecular data (Pleijel *et al*. 2012; Summers *et al.* 2015) have shown *Hesiospina* as not monophyletic. The common agreement in all these studies is that *Hesiospina* is closely related to *Psamathe* Johnston 1836, *Micropodarke* Okuda 1938, *Sirsoe* Pleijel 1998 and *Vrijenhoekia* Pleijel *et al*. 2008, within the subfamily Psamathinae Pleijel 1998.

Hesiospina similis (Hessle 1925), originally described as *Kefersteinia similis*, was described from Japanese material. Later, Pleijel (2004) reviewed *Hesiospina* species and synonymised *H. similis* with *Castalia longicornis* Sars 1862 and *Hesiospina aurantiaca* (Sars 1862) both from western Norway, giving to this species a very wide distribution. Still according to Pleijel (2004), only two species are valid for this genus: *H. aurantiaca* and *Hesiospina vestimentifera* Blake 1985. *Hesiospina aurantiaca* is mostly distributed in shallow waters (1–545 m depth) from West and East sides of the Atlantic Ocean, western Pacific Ocean and Mediterranean Sea while *H. vestimentifera* is associated with hydrothermal vents located mostly in the eastern Pacific Ocean (Blake 1985;

Tunnicliffe *et al.* 1998; Pleijel 2004) but as well in south-western Pacific (Pleijel *et al.* 2012). Furthermore, *H. vestimentifera* was described as the most common epifaunal hesionid and was frequently observed in association to siboglinids: *Riftia pachyptila* Jones 1981 at Galápagos Rift and at East Pacific Rise (Blake 1985); and *Ridgeia piscesae* Jones 1985 at Juan de Fuca Ridge (Tsurumi & Tunnicliffe 2001, 2003; Bergquist *et al.* 2007; Marcus *et al.* 2009). Although only two species of *Hesiospina* are currently accepted, Goffredi *et al.* (2017) suggested the presence of four new species of *Hesiospina* in recently discovered hydrothermal vents in the Alarcón Rise (southern Gulf of California). This data suggests that the species diversity of this genus has been undersampled and underestimated, and their cosmopolitanism should be investigated.

Hesiospina specimens were collected during sampling of benthic invertebrates at the Juan de Fuca Ridge. This ridge is an intermediate spreading-rate ridge (about 500 km) between the Pacific and the Juan de Fuca plates in the north-eastern Pacific Ocean (Kelley *et al.* 2012). A submarine volcano, numerous chimneys and hydrothermal vents are distributed on this ridge. For instance, the Endeavour segment (about 90 km), in the northern part, has approximately 800 individual extinct and active chimneys distributed on five major hydrothermal fields (Kelley *et al.* 2012). This area has been extensively studied in many scientific domains since 1982 with many records of numerous taxa (Sarrazin *et al.* 1997, 1999; Tsurumi & Tunnicliffe 2001, 2003; Bergquist *et al.* 2007; Marcus *et al.* 2009). Benthic communities associated with hydrothermal vent ecosystems are based on local chemosynthetic microbial production (Childress & Fisher 1992) and generally characterised by low diversity, large biomass and high levels of endemic species (Tunnicliffe 1991). Vent sites of the north-eastern Pacific Ocean are colonized by dense populations of siboglinid *Ridgeia piscesae* tubeworms (Sarrazin *et al.* 1997; Bergquist *et al.* 2007). *Ridgeia piscesae* tubeworms inhabit a wide range of habitats, ranging from diffuse flow to high temperature fluid emissions (Sarrazin *et al.* 1997; Urcuyo *et al.* 2007). Physical structure of *R. piscesae* assemblages contributes to increase local diversity by providing microhabitats that can be colonized by dense meio- and macrofaunal communities (Tsurumi & Tunnicliffe 2001, 2003; Govenar *et al.* 2002; Bergquist *et al.* 2007; Marcus *et al.* 2009). Among the numerous symbionts observed, *Hesiospina vestimentifera* have often been recorded in low abundances along Juan de Fuca Ridge (Blake & Hilbig 1990; Tsurumi & Tunnicliffe 2001, 2003; Bergquist *et al.* 2007; Marcus *et al.* 2009). Unlike other sites of the Endeavour segment, few samplings have been carried out on the Grotto edifice until a wide range of instrumental and observational instruments were deployed by the *Ocean Networks Canada* cabled observatory (University of Victoria, British Columbia, Canada), in order to reach a comprehensive understanding of hydrothermal ecosystem dynamics and functioning (Barnes *et al.* 2007; Taylor 2009). From this survey, a new species of *Hesiospina* from the Juan de Fuca Ridge is described based on morphological characters and supplemented by molecular data. Furthermore, insights about the *Hesiospina* phylogeny are discussed.

Material and methods

Study site and sampling design. The 90 km Endeavour Segment is located in the northern part of the Juan de Fuca Ridge, an intermediate spreading centre between the Pacific Plate and the Juan de Fuca Plate (Kelley *et al.* 2012). The axial valley of Endeavour hosts five major hydrothermal vent fields separated by 2–3 km. Within the Main Endeavour vent field (MEF), Grotto (Fig. 1; 47°56.958'N, 129°5.899'W) is an active hydrothermal sulphide vent cluster located at 2196 m depth that covers 450 m^2 of surface is 10 m high (Xu *et al.* 2014). Like many other structures in the MEF, the local fauna is composed mostly of the siboglinid *Ridgeia piscesae* tubeworm assemblages and their associated fauna (Sarrazin *et al.* 1997; Bergquist *et al.* 2007).

Six samples of *Ridgeia piscesae* tubeworm assemblages with their associated fauna were collected during the *Ocean Networks Canada Expedition 2015: Wiring the Abyss* cruise, on-board the *RV Thomas G. Thompson* (25 August—14 September 2015; Dive J0831; Lelièvre *et al.* 2018). For each sample, a first suction was taken to recover the mobile fauna, followed by the collection of tubeworms in a collect box and their associated fauna using the *Remotely Operated Vehicle* (ROV) Jason. A last suction sample on the bare surface was performed to recover the remaining fauna. Once brought on board, faunal samples were washed, sieved (250 μm, 63 μm and 20 μm mesh sizes) and sorted. Macrofaunal specimens were fixed/preserved in 96% ethanol. Specimens of the new *Hesiospina* were present in 3 of the samples (S1, S2 and S3).

Hesiospina **observation.** The worms were examined under a Leica M125 stereomicroscope and a Nikon Eclipse E400 microscope, and photographed with a Nikon DS-Ri 2 camera. The software Leica LAS Interactive

Measurements was used to measure the length and the width (5th segment, without parapodia). Drawings using stacked pictures were done using an iPad Pro with Apple pencil and Graphic App, and then processed using Adobe Illustrator and Photoshop. The specimen analysed with scanning electron microscopy (SEM) was prepared by dehydration in three immersions in 96 % ethanol for 15 minutes each, followed by critical-point drying, then covered with gold and photographed using the Quanta200 FEI (Ifremer). As hesionids have the segments 1–3 or 1–5 fused without chaetae we given the correspondent number of the chaetiger in the text as well (Rizzo & Salazar-Vallejo 2014). Type specimens were deposited at the Muséum National d'Histoire Naturelle de Paris (MNHN, France). This article is registered in ZooBank under http://zoobank.org/urn:lsid:zoobank.org:pub:520D1DA8-2250-4318-8078-9C0E197A9722. *Hesiospina legendrei* **sp. nov.** is registered in ZooBank under LSID urn:lsid:zoobank.org:act:DC771CF6-CD76-4A76-9CE9-6BB5F3A258AB

DNA extraction, amplification and sequencing. Extraction of DNA was done with NucleoSpin Tissue (Macherey-Nagel) kit following protocol supplied by the manufacturers. About 420 bp (16S) and 690 bp (cytochrome c oxidase subunit I, COI) were amplified using primers: Ann16SF and 16SbrH for 16S (Palumbi 1996; Sjölin *et al.* 2005); and polyLCO, polyHCO, LCO1490 and HCO2198 for COI (Folmer *et al.* 1994; Carr *et al.* 2011).

The Polymerase Chain Reaction (PCR), with 25 µL mixtures, contained: 5 µL of Green GoTaq® Flexi Buffer (final concentration of 1X), 2.5 µL of MgCl2 solution (final concentration of 2.5 mM), 0.5 µL of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 9.875 µL of nuclease-free water, 2.5 µL of each primer (final concentration of 1 µM), 2 µL template DNA and 0.125 U of GoTaq® G2 Flexi DNA Polymerase (Promega). The temperature profile was: 95ºC/240s—(94ºC/30s-52ºC/60s-72ºC/75s *35 cycles for 16S or *40 for COI—72ºC/480s—4ºC. PCR products, which produced bright bands after electrophoresis on a 1% agarose gel, were sent to the MacroGen Europe Laboratory in Amsterdam (Netherlands) to obtain sequences, using the same set of primers as used for the PCR.

Overlapping sequence (forward and reverse) were assembled using Geneious Pro 8.1.7 2005–2015 (Biomatters Ltd.) and then aligned using MAFFT plugin (Katoh *et al.* 2002) for 16S gene and with MUSCLE (Edgar 2004) for COI using Geneious Pro 8.1.7 2005–2015 (Biomatters Ltd.). All sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The GenBank accession numbers are included in the section type-material.
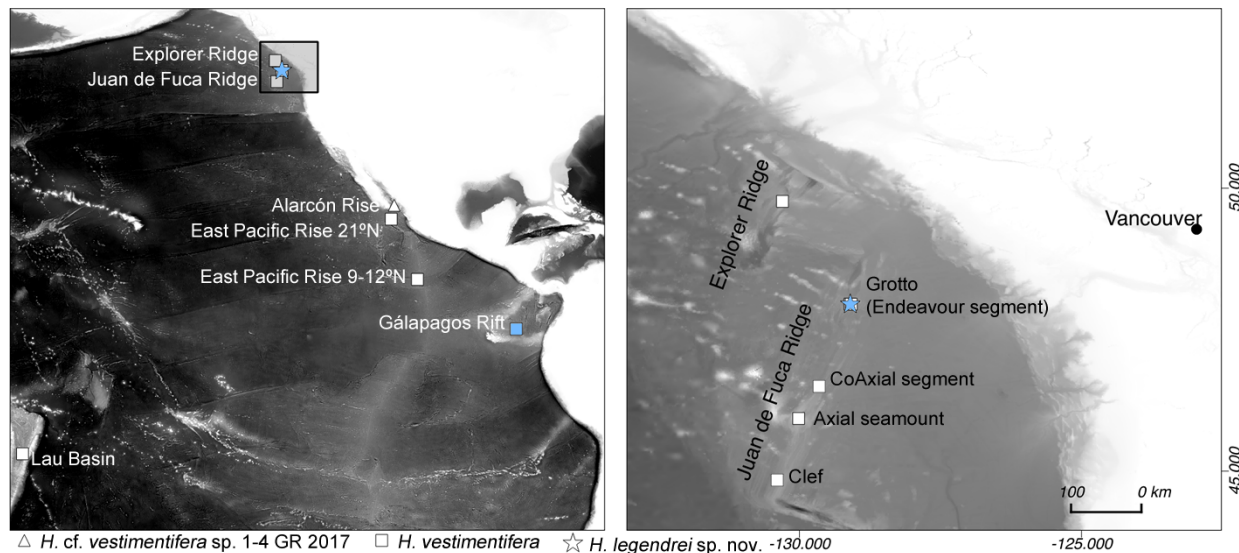

FIGURE 1. Pacific Ocean showing the general distribution of *Hesiospina vestimentifera*, *H.* cf. *vestimentifera* sp. 1-4 GR 2017 and *H. legendrei* **sp. nov.**, and the sampling site (Grotto) in Juan de Fuca Ridge. Filled blue symbols indicating the type locality for the correspondent species.

Phylogenetic analyses and genetic divergence. For the purposes of the present study, sequences of 16S, 18S, 28S and COI genes available in GenBank from one chrysopetalid and one hesionid (subfamily Hesioninae) were included as outgroups (Table 1). Available sequences from all species belonging to the subfamily Psamathinae were included as the ingroup (Table 1). Each gene set (i.e., 16S, 18S, 28S and COI) was concatenated in one partitioned genetic data set using SequenceMatrix (Vaidya *et al.* 2011). The evolutional model used was GTR + G for all genes. The Bayesian phylogenetic analysis was executed in MrBayes 3.2.6 (Ronquist *et al.* 2012) with

10,000,000 generations. At every 1000 generations, one chain was sampled, at the end 25% were discarded as burnin. The node support corresponds to the Posterior Probability (PP). The Maximum Likelihood was computed using Randomized Axelerated Maximum Likelihood (RAxML 8.2.10; Stamatakis 2014) on XSEDE with rapid-bootstrapping (1000 iterations). The support of nodes is indicated in Maximum Likelihood Bootstrap (MLB). MrBayes and RAxML were performed in CIPRES Science Gateway (Miller *et al.* 2010). The tree files were interpreted/edited with FigTree v1.4.2 (available in http://tree.bio.ed.ac.uk/software/figtree/). The COI sequence of *Hesiospina* cf. *aurantiaca* MB-2010 was not included in phylogenetic analysis because it does not seem to belong to a *Hesiospina* species (see Remarks and Table 1 for detailed information).

TABLE 1. GenBank accession number of taxa included in phylogenetic analysis. * not from type specimen, from Lau Basin. ** not included in phylogenetic analysis, see Remarks section for detailed information.

Taxa	16S	18S	28S	COI
Chrysopetalidae Ehlers 1864 Dysponetinae Aguado, Nygren & Rouse 2013 *Dysponetus caecus* (Langerhans 1880)	EU555047	AY839568	EU555028	AF221568
Hesionidae Grube 1850 Hesioninae Grube 1850 *Leocrates chinensis* Kinberg 1866	DQ442575	DQ442589	DQ442605	DQ442565
Psamathinae Pleijel 1998 *Hesiospina aurantiaca* (Sars 1862)	JN631319	JN631329	JN631342	JF317197
Hesiospina cf. *aurantiaca* MB-2010	-	GQ426577	-	GQ426676**
Hesiospina cf. *vestimentifera* sp. 1 GR 2017	-	-	-	KY684712
Hesiospina cf. *vestimentifera* sp. 2 GR 2017	-	-	-	KY684718
Hesiospina cf. *vestimentifera* sp. 3 GR 2017	-	-	-	KY684719
Hesiospina cf. *vestimentifera* sp. 4 GR 2017	-	-	-	KY684720
Hesiospina legendrei **sp. nov.**	MH349480	-	-	MH349483
Hesiospina sp. BC-2003	AY340464	AY340435	AY340401	-
Hesiospina vestimentifera Blake 1985 *	JN631320	JN631330	JN631343	JN631310
Micropodarke dubia (Hessle 1925)	DQ442576	JN571888	JN571899	JN571825
Nereimyra aphroditoides (O. Fabricius 1780)	JF317211	-	JF317204	JF317198
Nereimyra punctata (Müller 1788)	DQ442577	DQ442591	DQ442606	DQ442566
Nereimyra woodsholea (Hartman 1965)	JF317215	-	JF317207	AY644802
Psamathe fusca Johnston 1836	DQ442581	DQ442595	DQ442610	DQ513294
Sirsoe methanicola (Desbruyères & Toulmond 1998)	DQ442582	JN631332	DQ442611	DQ513295
Sirsoe sirikos Summers, Pleijel & Rouse 2015	JN571882	JN571893	JN571902	JN571829
Syllidia armata Quatrefages 1866	DQ442583	DQ442596	-	DQ442568
Vrijenhoekia ahabi Summers, Pleijel & Rouse 2015	JN571887	JN571898	JN571907	JN571876
Vrijenhoekia balaenophila Pleijel, Rouse, Ruta, Wiklund & Nygren 2008	DQ513301	JN631333	DQ513306	DQ513296
Vrijenhoekia falenothiras Summers, Pleijel & Rouse 2015	JN571886	JN571897	JN571906	JN571875
Vrijenhoekia ketea Summers, Pleijel & Rouse 2015	JN571885	JN571896	JN571905	JN571838
Vrijenhoekia sp. A	KP745536	KP745539	KP745542	KP745533

The genetic divergence over sequence pairs was calculated within species (intraspecific variation) and between species (interspecific variation) using Kimura 2-parameter (K2P; Kimura 1980) model and p-distance in MEGA7 (Kumar *et al.* 2016).

Results

Systematics

Family Hesionidae Grube 1850.

Subfamily Psamathinae Pleijel 1998.

Genus *Hesiospina* Imajima & Hartman 1964.

Type species: Kefersteinia similis Hessle 1925.

Diagnosis *(based on Pleijel 1998, 2004):* Number of segments variable. Prostomium about rectangular. Facial tubercle present or absent. Palps biarticuled; palpophores well developed; palpostyles ovoid or tapered. Median antenna absent; paired antenna present. Two pairs of eyes present or absent. Nuchal organs middorsally coalescing. Lip glands absent. Terminal ring of proboscis with more than 10 papillae; ventral incision absent; dorsal and ventral jaws plates present or absent. Enlarged dorsal cirri on segments 1–5, from segment 6 (chaetiger 1) dorsal cirri long; enlarged ventral cirri on segments 1–4. Protruding notopodial hooks present from median segments; notochaetae absent. Neuropodial lobe and neurochaetae present from segment 5 (chaetiger 1); blades of compound neurochaetae with uni- or bidentate tips. Ventral cirri inserted subdistally. Pygidium as a simple ring or cone with pair of anal cirri.

Hesiospina legendrei sp. nov.
(Figs. 2–4)

Type material*:* NE Pacific Ocean, Endeavour Segment (Juan de Fuca Ridge), Grotto site (47°56.958'N, 129°5.899'W), 2196 m depth, collected by ROV Jason 6–7 September 2015, Dive J0831: Holotype, MNHN-IA-TYPE 1845, J0831-S1 incomplete specimen, length 13.27 mm, width 1.72 mm, 37 segments, accession GenBank MH349482 for 16S and MH349485 for COI; Paratype 1, MNHN-IA-TYPE 1846, J0831-S3 incomplete specimen, length 11.51 mm, width 1.73 mm, 33 segments, accession GenBank MH349481 for 16S and MH349484 for COI; Paratype 2, MNHN-IA-TYPE 1847, J0831-S1 complete specimen, length 11.17 mm, width 1.05 mm, 43 segments, accession GenBank MH349480 for 16S and MH349483 for COI; Paratype 3, MNHN-IA-TYPE 1848, J0831-S3 complete specimen, length 13.94 mm, width 1.20 mm, 50 segments; Paratype 4, MNHN-IA-TYPE 1849, J0831-S3 complete specimen, length 12.18 mm, width 1.35 mm, 42 segments.

Additional material*:* NE Pacific Ocean, Endeavour Segment (Juan de Fuca Ridge), Grotto site (47°56.958'N, 129°5.899'W), 2196 m depth, collected by ROV Jason 6–7 September 2015, Dive J0831: Additional material 1 MNHN-IA-PNT 82, J0831-S3 incomplete specimen, length 12.99 mm, width 1.39 mm, 30 segments; Additional material 2 MNHN-IA-PNT 83, J0831-S3 incomplete specimen, length 11.33 mm, width 1.20 mm, 38 segments; Additional material 3 MNHN-IA-PNT 84, J0831-S3 incomplete specimen, length 11.46 mm, width 1.03 mm, 40 segments; Additional material 4 MNHN-IA-PNT 85, J0831-S2 incomplete specimen, length 13.65 mm, width 1.19 mm, 38 segments; Additional material 5 MNHN-IA-PNT 86, J0831-S2 incomplete specimen, length 12.28 mm, width 1.06 mm, 34 segments; Additional material 6 MNHN-IA-PNT 87, J0831-S2 incomplete specimen used to SEM. NE Pacific Ocean, Endeavour Segment (Juan de Fuca Ridge), Grotto site (47°56.958'N, 129°5.899'W), 2196 m depth, collected by ROV ROPOS 19 May 2014: Additional material 7 MNHN-IA-PNT 88, ONC 2014 R1689 incomplete specimen, length 11.36 mm, width 1.30 mm, 29 segments; Additional material 8 MNHN-IA-PNT 89, ONC 2014 R1689 complete specimen, length 19.55 mm, width 1.21 mm, 47 segments

Description *(based on holotype and paratypes):* Moderate-sized species, up to 19.55 mm long, up to 50 segments; dorsoventrally cylindrical anteriorly and posteriorly slightly flattened; live specimen not observed; ethanol preserved pale yellow to white; from segment 4–5, ventral-posterior part of neuropodia slightly inflated, with slight to dark brownish pigmentation.

Prostomium wider than long, trapezoid, anteriorly wide, slightly notched posteriorly (Fig. 2A, 4A). Two antennae, smooth, thin, slightly tapering, long (as long as palpophores and palpostyle) with rounded tips, inserted on anterior margin close to palpophores; without median antenna. Palpophores cylindrical, slightly annulated, long (shorter than prostomium length) with palpostyle ovoid, thinner, shorter (about 1/3 of palphophores; Fig. 2A). Eyes absent. Nuchal organs as a patch of pores located laterally to prostomium (Fig. 4A). Everted proboscis large, thick, bulbous (as long as the first 6 segments when everted; Fig. 2A); basally encircled with numerous, small, conical, pointed papillae

(increasing in size distally), ranged in about 21–27 poorly defined vertical rows with 2–5 papillae by row; medially with a pair of large, sac-like structures, inflated, distally with 7–13 digitiform extensions, inserted ventro-laterally; distally with 20–27 elongate, thin, similar-sized papillae; not ciliated (Fig. 2A, B); two pairs of small, delicate jaws, both pairs basally jointed, dorsal pair anteriorly rounded, ventral pair anteriorly slightly pointed (Fig. 2C, D).
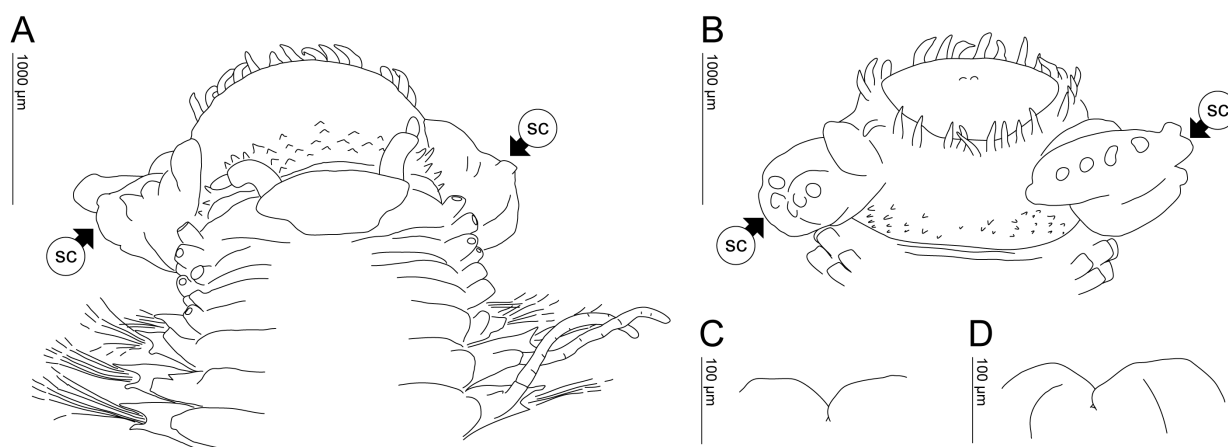

FIGURE 2. *Hesiospina legendrei* **sp. nov.**, paratype 1 MNHN-IA-TYPE 1846 Figures A–B, paratype 4 MNHN-IA-TYPE 1849 Figures C–D. A, anterior end with everted proboscis with sac-like structure (sc), dorsal view, paired antennae missing. B, anterior end with everted proboscis with sac-like structure (sc), ventral view. C, dissected dorsal pair of jaws. D, dissected ventral pair of jaws.

Dorsal cirri articulated, in segments 1–4 as tentacular cirri, very long (reaching 9 subsequent segments) with well-developed, cylindrical cirrophores; in subsequent segments, style shorter, long (reaching 2–5 subsequent segments) with smaller cirrophores.

Ventral cirri in segments 1–4 slightly articulated, long (reaching 4 subsequent segments) with well-developed, cylindrical cirrophores; in subsequent segments, style not articulated, slightly thinner, short (reaching distal neuropodia margin) with indistinct cirrophores, inserted subdistally (Fig. 3A).

Parapodia subbiramous (Fig. 3A). Notopodia reduced to small lobe; in segments 1–5 with 5–6 internal, slender aciculae; from segment 6 (chaetiger 2) to last segments with single internal, stouter acicula; from segments 7–8 (chaetigers 3–4) to last segments with single stout protruding hook (plate-like striae), very gently curved distally, with blunt to slightly pointed tips, emerging from small lobe located anteriorly to dorsal cirrophore (Fig. 3A–B, 4B).

Neuropodia well-developed, subtriangular, from segment 5 (chaetiger 1) to last segments; pre-chaetal lobe developed, triangular with pointed, prominent tips; post-chaetal not so developed, triangular, shorter, prominent (Fig. 3A). Neurochaeta starting from segment 5 (chaetiger 1) to last segments of 2 types: (a) single stout, simple, long, slightly curved chaeta, with blunt tips, located closer to the neuroacicula (Fig. 3F, 4C); and (b) numerous (about 30–36 observed), slender, composite heterogomph falcigers with faintly serrated (Fig. 3C, E, 4G), tapering blades, with prolonged subdistal tooth and unidentate tips (Fig. 3C, E, 4F, H); medial blades at least 4 times longer than the inferiormost chaetae on fascicle, some with distinct, bigger or prolonged teeth series near base of blade (Fig. 3E, 4D–E); few (1–2 observed) inferiormost neurochaeta with elongated hood (Fig. 3C–D, 4I). All chaetae shafts, aciculae and hooks internally camerated (not drawn).

Pygidium as simple ring, bulbous, termino-dorsally located; last 2 segments very reduced.

Morphological variation: Few morphological variations were observed, for instance protruding hook appearing from segment 7 or 8 (chaetigers 3–4), occurred each case in half of analysed specimens. The number of distal papillae in the proboscis varied from 20 to 27 and seems to be linked to age-size (2 worms with everted proboscis). Few specimens in bad conditions presented the protruding hook accompanied externally by the internal acicula appearing to have "2 protruding hooks". The holotype presented one segment with pre-chaetal lobe having slightly bifurcate tips in anterior region while the additional material 7 MNHN-IA-PNT 88 presented pre-chaetal lobe with bifurcate tips in few anterior segments. Furthermore, some specimens presented crenulated post-chaetal lobes without defined pattern, probably linked to an artefact of fixation.

Etymology: The new species is named in honour of Dr. Pierre Legendre (Université de Montréal) for his many contributions to marine ecology and deep-sea research.

Genetic data: DNA sequencing, for holotype and paratypes 1 and 2, was successful for 16S (404 to 421 bp length) and COI (504 to 687 bp length). Intraspecific variation for COI divergence was of 0.0% (Table 2).

TABLE 2. Cytochrome oxidase I evolutionary distances for species of the genera *Hesiospina*, *Sirsoe* and *Vrijenhoekia*. Analyses were conducted using the Kimura 2-parameter model (above diagonal) and p-distance (below diagonal) in MEGA7.

	Hesiospina aurantiaca	*Hesiospina cf. aurantiaca* MB-2010	*Hesiospina cf. aurantiaca* MB-2010	*Hesiospina cf. vestimentifera* sp. 1 GR-2017	*Hesiospina cf. vestimentifera* sp. 2 GR-2017	*Hesiospina cf. vestimentifera* sp. 3 GR-2017
Hesiospina aurantiaca		0.755	0.755	0.198	0.289	0.217
Hesiospina cf. aurantiaca MB-2010	0.472		0.000	0.685	0.719	0.685
Hesiospina cf. aurantiaca MB-2010	0.472	0.000		0.685	0.719	0.685
Hesiospina cf. vestimentifera sp. 1 GR-2017	0.173	0.444	0.444		0.173	0.074
Hesiospina cf. vestimentifera sp. 2 GR-2017	0.236	0.458	0.458	0.152		0.173
Hesiospina cf. vestimentifera sp. 3 GR-2017	0.187	0.444	0.444	0.069	0.152	
Hesiospina cf. vestimentifera sp. 4 GR-2017	0.215	0.472	0.472	0.138	0.166	0.083
Hesiospina legendrei sp. nov.	0.180	0.444	0.444	0.125	0.138	0.118
Hesiospina legendrei sp. nov.	0.180	0.444	0.444	0.125	0.138	0.118
Hesiospina legendrei sp. nov.	0.180	0.444	0.444	0.125	0.138	0.118
Hesiospina vestimentifera	0.222	0.493	0.493	0.201	0.173	0.208
Sirsoe methanicola	0.256	0.493	0.493	0.187	0.215	0.215
Sirsoe sirikos	0.243	0.451	0.451	0.208	0.201	0.208
Vrijenhoekia ahabi	0.229	0.423	0.423	0.180	0.152	0.173
Vrijenhoekia balaenophila	0.215	0.430	0.430	0.173	0.187	0.159
Vrijenhoekia falenothiras	0.229	0.479	0.479	0.159	0.145	0.152
Vrijenhoekia ketea	0.222	0.479	0.479	0.180	0.208	0.173
Vrijenhoekia sp. A	0.263	0.458	0.458	0.229	0.229	0.250

TABLE 2. (Continued)

	Hesiospina cf. vestimentifera sp. 4 GR-2017	Hesiospina legendrei sp. nov.	Hesiospina legendrei sp. nov.	Hesiospina legendrei sp. nov.	Hesiospina vestimentifera	Sirsoe methanicola
Hesiospina aurantiaca	0.256	0.207	0.207	0.207	0.266	0.317
Hesiospina cf. aurantiaca MB-2010	0.758	0.689	0.689	0.689	0.828	0.869
Hesiospina cf. aurantiaca MB-2010	0.758	0.689	0.689	0.689	0.828	0.869
Hesiospina cf. vestimentifera sp. 1 GR-2017	0.156	0.137	0.137	0.137	0.237	0.217
Hesiospina cf. vestimentifera sp. 2 GR-2017	0.190	0.154	0.154	0.154	0.199	0.256
Hesiospina cf. vestimentifera sp. 3 GR-2017	0.089	0.129	0.129	0.129	0.247	0.258
Hesiospina cf. vestimentifera sp. 4 GR-2017		0.208	0.208	0.208	0.321	0.312
Hesiospina legendrei sp. nov.	0.180		0.000	0.000	0.220	0.238
Hesiospina legendrei sp. nov.	0.180	0.000		0.000	0.220	0.238
Hesiospina legendrei sp. nov.	0.180	0.000	0.000		0.220	0.238
Hesiospina vestimentifera	0.256	0.187	0.187	0.187		0.230
Sirsoe methanicola	0.250	0.201	0.201	0.201	0.194	
Sirsoe sirikos	0.201	0.187	0.187	0.187	0.166	0.180
Vrijenhoekia ahabi	0.229	0.159	0.159	0.159	0.180	0.222
Vrijenhoekia balaenophila	0.208	0.152	0.152	0.152	0.173	0.222
Vrijenhoekia falenothiras	0.159	0.152	0.152	0.152	0.201	0.208
Vrijenhoekia ketea	0.166	0.194	0.194	0.194	0.236	0.208
Vrijenhoekia sp. A	0.270	0.215	0.215	0.215	0.229	0.215

TABLE 2. (Continued)

|	Sirsoe sirikos	Vrijenhoekia ahabi	Vrijenhoekia balaenophila	Vrijenhoekia falenothiras	Vrijenhoekia ketea	Vrijenhoekia sp. A
Hesiospina aurantiaca	0.300	0.276	0.258	0.274	0.264	0.329
Hesiospina cf. aurantiaca MB-2010	0.713	0.626	0.642	0.773	0.777	0.716
Hesiospina cf. aurantiaca MB-2010	0.713	0.626	0.642	0.773	0.777	0.716
Hesiospina cf. vestimentifera sp. 1 GR-2017	0.248	0.210	0.199	0.180	0.208	0.277
Hesiospina cf. vestimentifera sp. 2 GR-2017	0.236	0.172	0.217	0.262	0.246	0.274
Hesiospina cf. vestimentifera sp. 3 GR-2017	0.248	0.200	0.180	0.171	0.198	0.308
Hesiospina cf. vestimentifera sp. 4 GR-2017	0.239	0.279	0.245	0.180	0.190	0.341
Hesiospina legendrei sp. nov.	0.219	0.180	0.172	0.171	0.226	0.255
Hesiospina legendrei sp. nov.	0.219	0.180	0.172	0.171	0.226	0.255
Hesiospina legendrei sp. nov.	0.219	0.180	0.172	0.171	0.226	0.255
Hesiospina vestimentifera	0.191	0.211	0.200	0.238	0.290	0.278
Sirsoe methanicola	0.213	0.271	0.266	0.246	0.246	0.257
Sirsoe sirikos		0.220	0.227	0.190	0.262	0.208
Vrijenhoekia ahabi	0.187		0.181	0.193	0.243	0.227
Vrijenhoekia balaenophila	0.194	0.159		0.198	0.208	0.266
Vrijenhoekia falenothiras	0.166	0.166	0.173		0.148	0.218
Vrijenhoekia ketea	0.215	0.201	0.180	0.131		0.290
Vrijenhoekia sp. A	0.180	0.194	0.222	0.187	0.236	
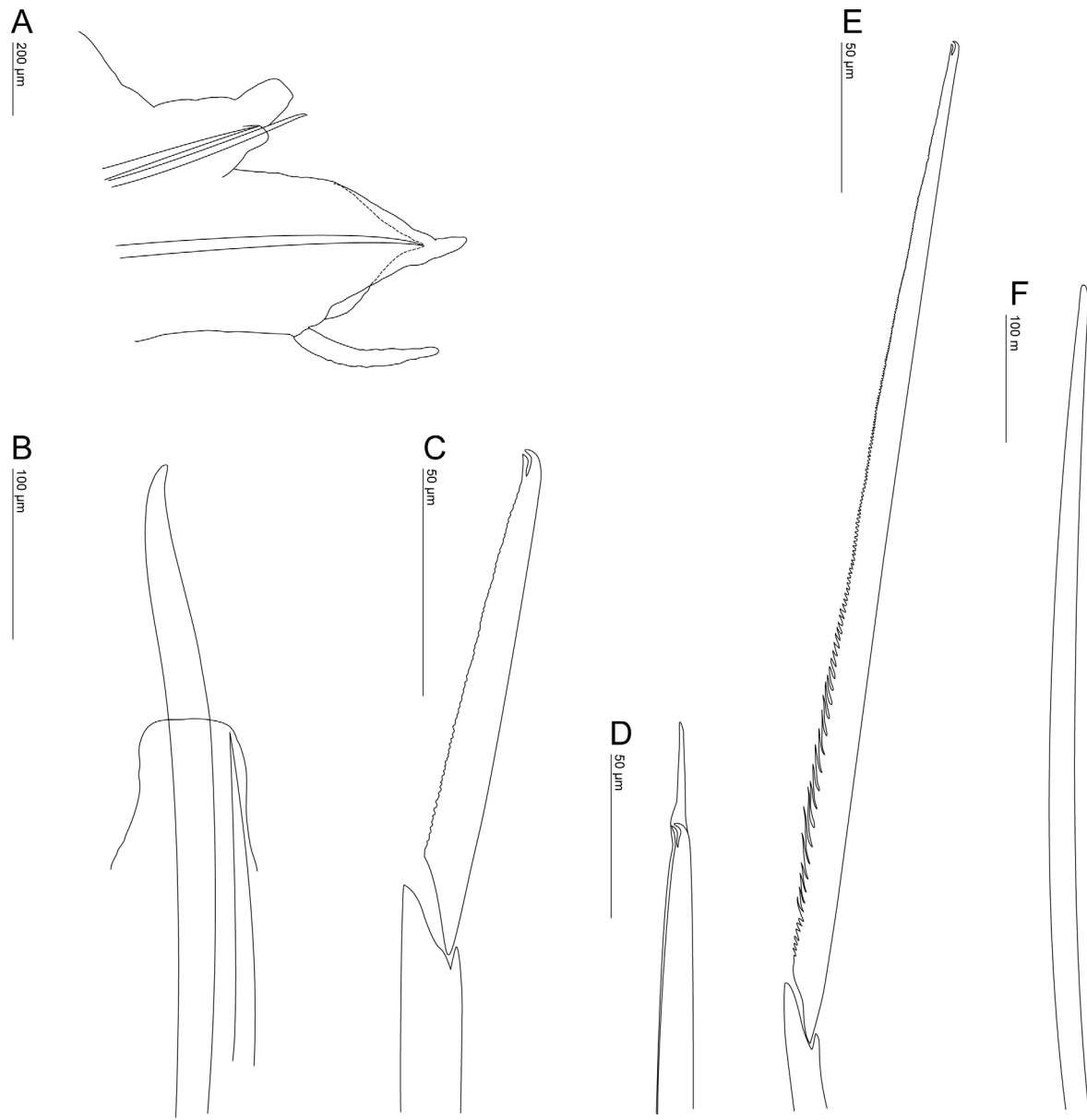
FIGURE 3. *Hesiospina legendrei* **sp. nov.**, holotype MNHN-IA-TYPE 1845 Figures A–F. A, left parapodia, segment 16 (chaetiger 12), anterior view, neurochaetae omitted. B, left notopodia, segment 13 (chaetiger 9), with protruding hook and notoacicula. C, composite lower neurochaeta, segment 16 (chaetiger 12). D, detail of composite lower neurochaeta with elongate hood, segment 17 (chaetiger 13). E, composite middle neurochaeta, segment 16 (chaetiger 12). F, simple neurochaeta, segment 31 (chaetiger 27).

Distribution*:* Grotto edifice within Main Endeavour vent field (Juan de Fuca Ridge; 2196 m depth).

Ecological notes*:* Lelièvre *et al.* (2018) provided the first characterization of the biodiversity, community structure and trophic ecology in Grotto hydrothermal edifice (Main Endeavour, Juan de Fuca Ridge) which showed a site dominated by dense populations of tubeworms *Ridgeia piscesae.* Associated to this species, 35 other taxa can be found as well, mainly represented by gastropods and polychaetes, along the 6 sampled stations in this study. Among the 19 species of polychaetes observed associated to *Ridgeia piscesae*, *Hesiospina legendrei* **sp. nov.** was found in 3 out of 6 sampled stations with abundance varying between 9.8 and 14.3 ind.m^{-2}. The same study found highest δ^{15}N values and suggested the new species *H. legendrei* **sp. nov.** being among the top predators in the benthic food web.
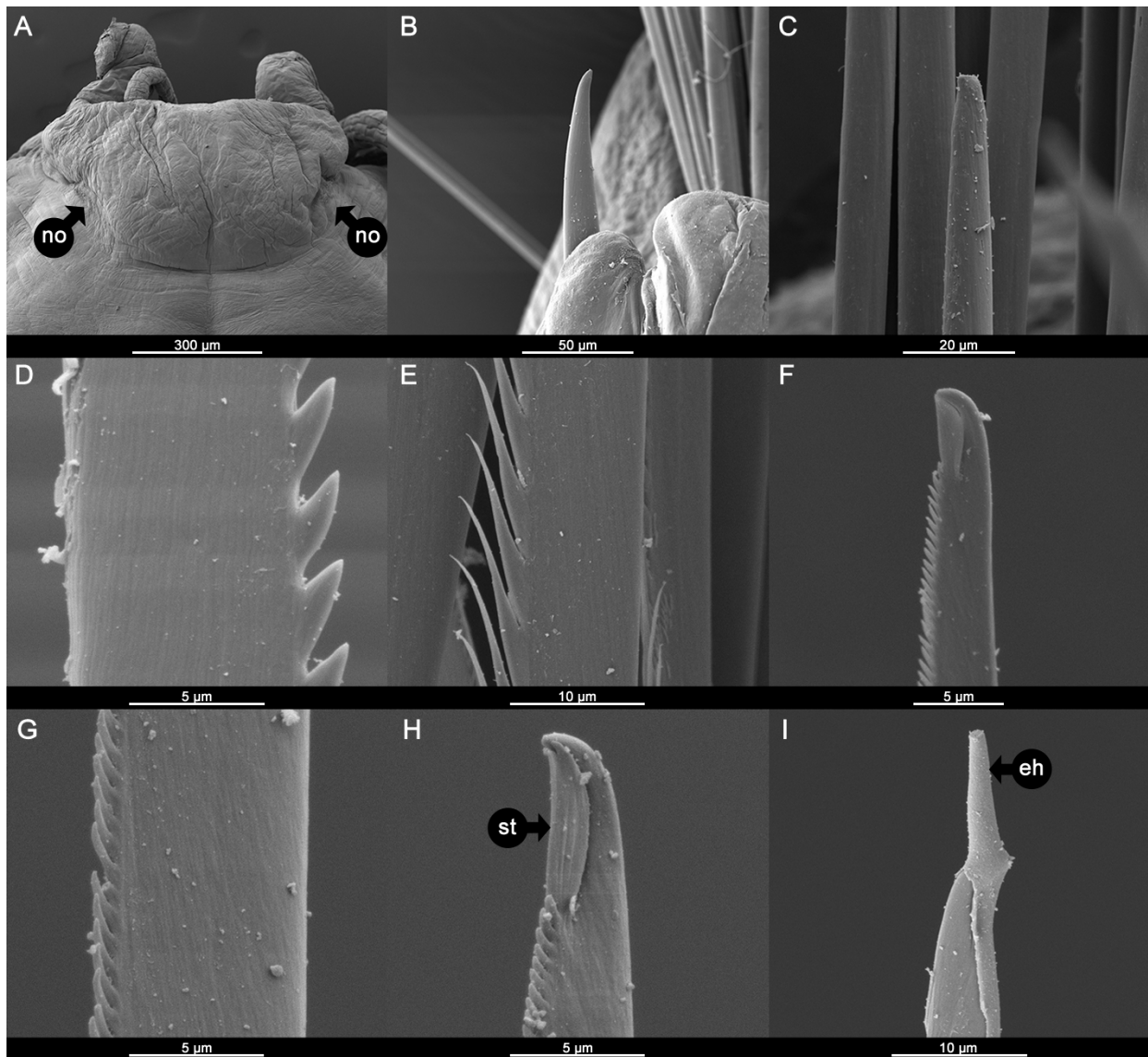
FIGURE 4. SEM of *Hesiospina legendrei* **sp. nov.**, additional material 6 MNHN-IA-PNT 87. A, prostomium showing nuchal organs (no), dorsal view. B, single hook from notopodia. C, simple neurochaeta. D, detail of serration from middle composite neurochaeta with bigger teeth, near base of blade. E, detail of serration from middle composite neurochaeta with prolonged teeth, near base of blade. F, detail of tip from middle composite neurochaeta. G, detail of serration from lower composite neurochaeta with typical teeth, near base of blade. H, detail of prolonged subdistal tooth (st) from lower composite neurochaeta. I, detail of tip from lower composite neurochaeta with elongate hood (eh).

Remarks*:* Together with *Hesiospina aurantiaca* and *H. vestimentifera*, *H. legendrei* **sp. nov.** is the third species described in the genus. *Hesiospina aurantiaca*—which lives mostly in shallow waters of the Atlantic, Caribbean, and western Pacific—can be easily differentiated from the hydrothermal vent taxa (*H. vestimentifera* and *H. legendrei* **sp. nov.**) by the presence of a protruding hook distally curved, elongate palpostyles, eyes present, proboscis unarmed and absence of papillae in basal part of proboscis (Pleijel 2004). *Hesiospina vestimentifera* and *H. legendrei* **sp. nov.** are both found in hydrothermal vent environments and share similarities such as: protruding hook slightly curved, ovoid palpostyle, eyes absent, proboscis with jaws and presence of papillae in basal part of proboscis. However, *H. legendrei* **sp. nov.** can be differentiated from *H. vestimentifera*, by having a sac-like structure (Fig. 2A–B) present ventro-laterally in proboscis (absent in *H. vestimentifera*). Furthermore, *H. legendrei* **sp. nov.** differs from *H. vestimentifera*, in having a greater number of distal papillae (about 20–27 observed) versus 13–14 (Blake 1985) or 10–15 (Pleijel 2004), notopodial hook from segment 7–8 (chaetigers 3–4) instead of segment 8–9 (chaetigers 4–5), and notopodia lobes with multiple slender aciculae in only the first 5 segments instead of the first 4 segments in *H. vestimentifera*. The elongate hood present in very few composite neurochaetae in *H.*

legendrei **sp. nov.** seems to be a unique character among this genus but it should be investigated in the other species because it was rarely observed in our specimens. Moreover, genetic divergences support the new species with high Kimura 2-parameter distance (K2P) and p-distance for COI gene (Table 2) varying respectively from 12.9 % and 11.8% between *H. legendrei* **sp. nov.** and *H.* cf. *vestimentifera* sp. 3 GR-2017 to 22.0% and 18.8% between *H. legendrei* **sp. nov.** and *H. vestimentifera*. It needs to be highlighted here two observations: (a) the type locality of *H. vestimentifera* is Gálapagos Rift (Blake 1985) but the only available DNA sequence was provided from one specimen sampled in Tui Malila, Lau Basin (about 1900 m depth; Pleijel *et al.* 2012); and (b) extreme values were observed between the sequence identified as *H.* cf. *aurantiaca* MB-2010 and all others resulting in the exclusion of the COI sequence from this species of phylogenetic analysis.

Discussion

Hessle (1925) described the first *Hesiospina* species, as *Kefersteinia similis*, from material sampled in Japan. Later, Imajima & Hartman (1964) erected the genus *Hesiospina* for this species based on the presence of notopodial hooks and simple acicular neurochaetae as major characters differentiating *Hesiospina* from *Kefersteinia* Quatrefages 1865–66 (currently unaccepted). Furthermore, Pleijel (2004), in his morphological based revision of *Hesiospina*, considered *H. similis* and *Castalia longicornis* Sars 1862 as synonyms of *H. aurantiaca* originally described by Sars (1862) from Norway. For this species, many records around the world (e.g. Japan, Italy, France, Norway, Australia, Mexico and Papua New Guinea) were also considered as synonymy by Pleijel (2004) who suggested that *H. aurantiaca* is abundant in shallow waters in temperate and tropical regions. However, he has highlighted morphological differences (without clear pattern) from specimens around the world which could suggest the presence of different species: absence of bidentate compounds in specimens from Gulf of Mexico; and larger sizer and higher number of proboscis papillae in specimens from western Mediterranean. The current idea of *H. aurantiaca* being a cosmopolitan species should be investigated. On the other hand, *H. vestimentifera* was originally described by Blake (1985) from deep-sea hydrothermal vents as the most common epifaunal hesionid from the Gálapagos Rift and East Pacific Rise vent sites (Fig. 1). Pleijel (2004, 2012) considered all records along the east (Gálapagos Rift and East Pacific Rise 9–21°N) and north-east (Juan de Fuca Ridge and Explorer Ridge) coast of North America and from Lau Basin as *H. vestimentifera* (Fig. 1). He intended in his revision "to simply apply species name for the smallest monophyletic groups" using the two morphotypes that he observed (i.e. *H. aurantiaca* and *H. vestimentifera*). Establishing cosmopolitan species can result in: (a) underestimation of species-level diversity and (b) a large range of morphological variation for characters within a species. Many other polychaetes have cosmopolitan distributions mainly due to short descriptions without detailed illustration (Hutchings & Kupriyanova 2017). In this way, several species were supposed to be widely distributed when, actually, in most of cases they were represented by two or more species with limited geographical distribution (Stiller *et al.* 2013; Parapar & Hutchings 2015; Parapar *et al.* 2016; Hutchings & Kupriyanova 2017). Likewise, Carr *et al.* (2011) studying cryptic species from three oceans (Arctic, Pacific and Atlantic) showed that morphological analysis alone tended to underestimate the number of species. Recently, Goffredi *et al.* (2017) noticed the presence of four new species of *Hesiospina* from southern Gulf of California, this suggests that *H. vestimentifera* should not be considered as "cosmopolitan" species and more investigation in this group should be done. As observed in this study, molecular analyses are important tools that should be used to separate species based on sequence divergence in combination with taxonomic studies. The COI divergence found in the present study agrees with the variation observed by Summers *et al.* (2017) for *Sirsoe* and *Vrijenhoekia* species (Table 2).

Juan de Fuca Ridge has about 500 km of length (Kelley *et al.* 2012), in northern part is located the Endeavour segment with 90 km hosting five major hydrothermal vent fields (Fig. 1). In the southern part is located an active volcano, the Axial seamount, with the summit at 1500 m depth and having a three-sided caldera with three vent fields (Fig. 1; Tsurumi & Tunnicliffe 2003). The CoAxial segment is located northern to Axial seamount while the Clef is the southernmost discrete segment (Fig. 1; Tsurumi & Tunnicliffe 2003). Although *Hesiospina vestimentifera* had been recorded for along all these areas by previous studies (Fig. 1; Blake & Hilbig 1990; Tunnicliffe *et al.* 1998; Tsurumi & Tunnicliffe 2001, 2003; Pleijel 2004; Bergquist *et al.* 2007; Marcus *et al.* 2009), in the present study this species was not observed in Grotto hydrothermal edifice located in Endeavour segment. Due to the high similarities between *H. vestimentifera* and *H. legendrei* **sp. nov.** it is not surprising if specimens of *H. legendrei* **sp. nov.** could be incorrectly identified as *H. vestimentifera* in Juan de Fuca Ridge. We suggest that

the specimens from the area, especially from Endeavour segment, should be re-examined to verify misidentifications. A careful morphological observation should be paid to these species, especially concerning pharynx which could easily differentiate these species but is often overlooked. Also, it is important to stress here the essential role of taxonomists doing detailed descriptions supplemented by morphological and molecular data. It is fundamental to compare specimens with type material or, at least to type description, and to deposit vouchers in a museum. In the present study, the type material of *H. vestimentifera* was not required because the distinct characters presented by *H. legendrei* **sp. nov.** were clearly diagnostic of a new species.

However, there is an urgent need to review the specimens of *H. vestimentifera* from different localities in order to define, based in morphology and molecular tools, if they belong to the same species. The most complete study of hesionid phylogeny based on morphological data was done by Pleijel (1998) who showed that *Hesiospina aurantiaca* and *H. vestimentifera* were a monophyletic group supported by the presence of a notopodial hook as an apomorphy. Subsequent studies based only on molecular data instead showed *Hesiospina* as paraphyletic. Pleijel *et al*. (2012) showed *H. vestimentifera* (not type specimen, from Lau Basin) as sister to a clade including *Sirsoe* and *Vrijenhoekia,* and *H. aurantiaca* as a sister to a clade including these taxa and *Psamathe* and *Micropodarke* with high support (PP = 1; MLB = 92). Summers *et al*. (2015) also found *H. aurantiaca* and *H. vestimentifera* to be paraphyletic, within a clade including *Sirsoe* and *Vrijenhoekia* but with low support (MLB = 70). The phylogenetic analyses presented here (Fig. 5A–B) based on concatenated sequences of 16S, 18S, 28S and COI genes and including all *Hesiospina* sequences available (except COI for *H*. cf. *aurantiaca* MB-2010) were unclear. Indeed, for Bayesian and Maximum Likelihood analyses the species closely related to *H. vestimentifera* composed a group (PP = 0.81; MLB = 79). However, while for Bayesian inference the species closely related to *H. aurantiaca* remained unsolved, for Maximum Likelihood inference that species composed a sister-group of *H. vestimentifera* grouping with low support (MLB = 28). The species *H*. cf. *aurantiaca* MB-2010, in this case 18S gene, remained unsolved in Bayesian inference and belonged to *Sirsoe* genus in Maximum Likelihood inference indicating maybe a problem with the sequences for this species. On the other hand, *H. vestimentifera* was observed as sister-group of the cluster *H. legendrei* **sp. nov.** and Goffredi's *Hesiospina* species. This grouping of species based on locality could indicate a different regional speciation forcing in *Hesiospina* species from East Pacific (*H. legendrei* **sp. nov.** and *H*. cf. *vestimentifera* spp) versus species from West Pacific (*H. vestimentifera* from Lau Basin). Likewise, Stiller *et al*. (2013) has showed that the ampharetid *Amphisamytha galapagensis* Zottoli 1983, once considered the most widespread (East and West Pacific; Tunnicliffe *et al.* 1998) hydrothermal vent invertebrate, is actually a cryptic species with speciation clearly based on biogeographic patterns. They showed: (a) two species located western Pacific (Lau Basin), one restricted to cold seeps et the other to hydrothermal vents; and (b) four species located eastern Pacific, one restricted to cold seeps, one restricted to north-east Pacific and only two being sympatric. This illustrates how the biogeographical patterns could being affecting *Hesiospina* species. Unfortunately, the current sequences associated to *H. vestimentifera* do not belong to type specimen or, even, type locality (Galápagos Rift) giving to this sequence some uncertainties concerning its real identity. *Hesiospina legendrei* **sp. nov.** increases the diversity within *Hesiospina* genus and allows to question the cosmopolitan aspect of *Hesiospina* species. For the future, three main ideas should remain in the mind: (a) to revisit specimens recently/ previously identified as *H. aurantiaca* and *H. vestimentifera* and to compare them to type descriptions; (b) to possibly describe the new species based on morphological and molecular data with detailed illustrations; and (c) the urgent need of DNA sequences from *H. vestimentifera* from type locality.
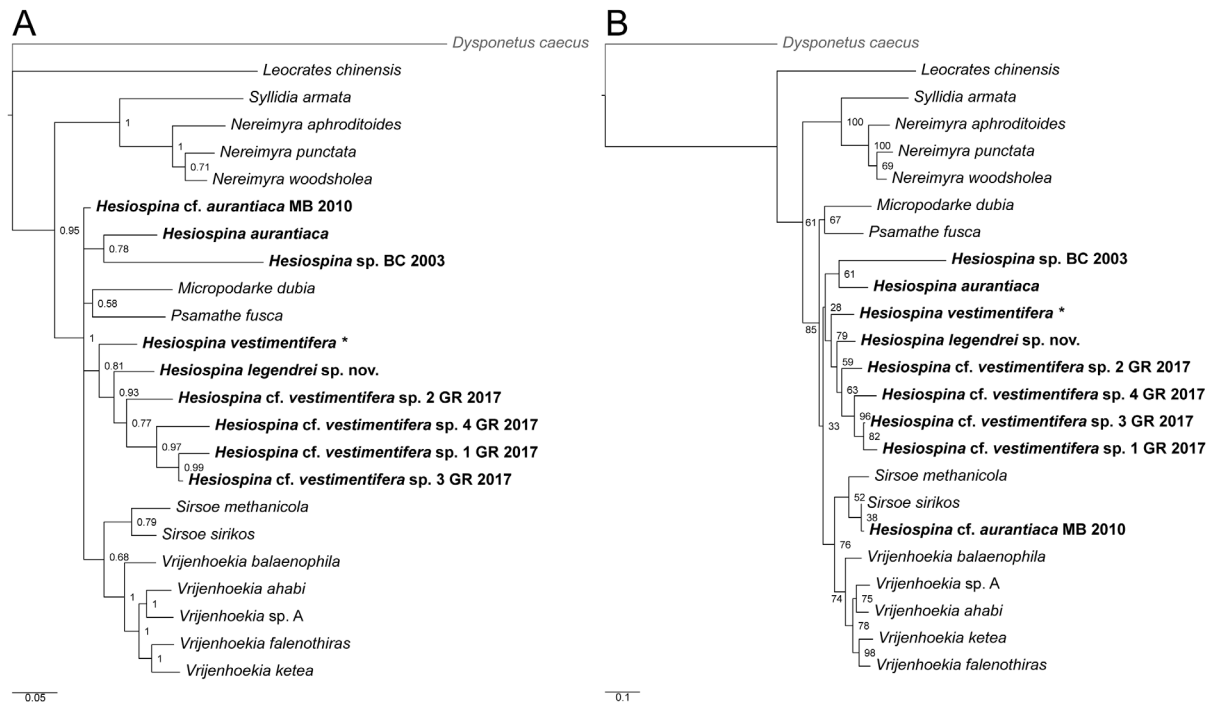
FIGURE 5. Molecular phylogenetic relationship within Psamathinae subfamily (black line) based on concatenated genes (16S, 18S, 28S and COI). A, Bayesian phylogenetic inference with node values indicating the Posterior Probabilities (PP). B, Maximum likelihood phylogenetic inference with node values indicating the Maximum Likelihood Bootstrap (MLB). * not type specimen, from Lau Basin.

Acknowledgments

References

Aguado, M.T., Nygren, A. & Rouse, G.W. (2013) Two apparently unrelated groups of symbiotic annelids, Nautiliniellidae and Calamyzidae (Phyllodocida, Annelida), are a clade of derived chrysopetalid polychaetes. *Cladistics*, 29, 610–628.
https://doi.org/10.1111/cla.12011

Barnes, C.R., Best, M.M.R., Bornhold, B.D., Juniper, S.K., Pirenne, B. & Phibbs, P. (2007) The NEPTUNE Project—A cabled ocean observatory in the NE Pacific: Overview, challenges and scientific objectives for the installation and operation of Stage I in Canadian waters. *2007 Symposium on Underwater Technology and Workshop on Scientific Use of Submarine Cables and Related Technologies*, 2007, 308–313.
https://doi.org/10.1109/UT.2007.370809

Bergquist, D.C., Eckner, J.T., Urcuyo, I.A., Cordes, E.E., Hourdez, S., Macko, S.A. & Fisher, C.R. (2007) Using stable isotopes and quantitative community characteristics to determine a local hydrothermal vent food web. *Marine Ecology Progress Series*, 330, 49–65.

https://doi.org/10.3354/meps330049

Blainville, H. (1828) Dictionnaire des Sciences naturelles, dans lequel on traite méthodiquement des différens êtres de la nature, considérés soit en eux-mêmes, d'après l'état actuel de nos connoissances, soit relativement a l'utilité qu'en peuvent retirer la médecine, l'agriculture, le commerce et les arts. *In*: *Suivi d'une biographie des plus célèbres naturalistes. Vol. 57.* F.G. Levrault, Strasbourg & Paris, 628 pp.

Blake, J.A. (1985) Polychaeta from the vicinity of deep-sea geothermal vents in the Eastern Pacific. I: Euphrosinidae, Phyllodocidae, Hesionidae, Nereididae, Glyceridae, Dorvilleidae, Orbiniidae, and Maldanidae. *Bulletin of the Biological Society of Washington*, 6, 67–101.

Blake, J.A. & Hilbig, B. (1990) Polychaeta from the vicinity of deep-sea hydrothermal vents in the eastern Pacific. II. New species and records from the Juan de Fuca and Explorer Ridge. *Pacific Science*, 44, 219–253.

Carr, C.M., Hardy, S.M., Brown, T.M., MacDonald, T. & Herbert, P.D.N. (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE*, e22232. https://doi.org/10.1371/journal.pone.0022232

Childress, J.J. & Fisher, C.R. (1992) The biology of hydrothermal vent animals: physiology, biochemistry, and autotrophic symbioses. *Oceanography and Marine Biology—An Annual Review*, 30, 337–441.

Desbruyères, D., Segonzac, M. & Bright, M. (2006) Handbook of deep-sea hydrothermal vent fauna. *Denisia*, 18, 1–544.

Desbruyères, D. & Toulmond, A. (1998) A new species of hesionid worm, *Hesiocaeca methanicola* sp. nov. (Polychaeta: Hesionidae), living in ice-like methane hydrates in the deep Gulf of Mexico. *Cahiers de Biologie Marine*, 39, 93–98.

Edgar, R.C. (2004) Muscle: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797. https://doi.org/10.1093/nar/gkh340

Folmer, O., Black, M., Hueh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit i from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Glover, A.G., Higgs, N. & Horton, T. (2018). World Register of Deep-Sea species (WoRDSS). Available from: http://www.marinespecies.org/deepsea (accessed 4 April 2018)

Goffredi, S.K., Johnson, S., Tunnicliffe, V., Caress, D., Clague, D., Escobar, E., Lundsten, L., Paduan, J.B., Rouse, G., Salcedo, D.L., Soto, L.A., Spelz-Madero, R., Zierenberg, R. & Vrijenhoek, R. (2017) Hydrothermal vent fields discovered in the southern Gulf of California clarify role of habitat in augmenting regional diversity. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20170817. https://doi.org/10.1098/rspb.2017.0817

Govenar, B.W., Bergquist, D.C., Urcuyo, I.A., Eckner, J.T. & Fisher, C.R. (2002) Three *Ridgeia piscesae* assemblages from a single Juan de Fuca Ridge sulphide edifice: structurally different and functionally similar. *Cahiers de Biologie Marine*, 43, 247–252.

Grube, A.E. (1850) Die Familien der Anneliden. *Archiv für Naturgeschichte*, 16, 249–364.

Hartman, O. (1965) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. *Allan Hancock Foundation Publications: Occasional Papers*, 28, 1–384.

Hessle, C. (1925) Einiges über die Hesioniden und die Stellung der Gattung *Ancistrosyllis*. *Arkiv för Zoologi*, 17, 1–36.

Hutchings, P. & Kupriyanova, E. (2017) Cosmopolitan polychaetes—fact or fiction? Personal and historical perspectives. *Invertebrate Systematics*, 32 (1), 1–9. http://dx.doi.org/10.1071/IS17035

Imajima, M. & Hartman, O. (1964) The polychaetous annelids of Japan. *Allan Hancock Foundation Publications: Occasional Papers*, 26, 1–452.

Jones, M.L. (1981) *Riftia pachyptila*, new genus, new species, the vestimentiferan worm from the Galápagos Rift geothermal vents (Pogonophora). *Proceedings of the Biological Society of Washington*, 93, 1295–1313.

Jones, M.L. (1985) On the Vestimentifera, new phylum: six new species, and other taxa, from hydrothermal vents and elsewhere. *Bulletin of the Biological Society of Washington*, 6, 117–158.

Johnston, G. (1836) Art. IV. Illustrations in British Zoology. Cl. Annelides, Ord. Errantes, Fam Nereides. Genus *Psamathe*. *Magazine of natural history and journal of zoology, botany, mineralogy, geology and meteorology*, 9, 14–17.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) Mafft: A novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acids Research*, 30, 3059–3066. https://doi.org/10.1093/nar/gkf436

Kelley, D.S., Carbotte, S.M., Caress, D.W., Clague, D.A., Delaney, J.R., Gill, J.B., Hadaway, H., Holden, J.F. Hooft, E.E.E., Kellogg, J.P., Lilley, M.D., Stoermer, M., Toomey, D., Weekly, R. & Wilcock, W.S.D. (2012) Endeavour Segment of the Juan de Fuca Ridge: one of the most remarkable places on Earth. *Oceanography*, 25, 44–61. https://doi.org/10.5670/oceanog.2012.03

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16 (2), 111–120. https://doi.org/10.1007/BF01731581

Kinberg, J.G.H. (1866) Annulata nova. [Continuatio, Acad. propos. die 18 Aprilis 1866.]. *Öfversigt af Königlich Vetenskapsakademiens förhandlingar, Stockholm*, 23, 97–103.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874.
https://doi.org/10.1093/molbev/msw054

Langerhans, P. (1880) Die wurmfauna Madeiras. II. *Zeitschrift für wissenschaftliche Zoologie*, 33, 271–316.

Lelièvre, Y., Sarrazin, J., Marticorena, J., Schaal, G., Day, T., Legendre, P., Hourdez, S. & Matabos, M. (2018) Biodiversity and trophic ecology of hydrothermal vent fauna associated with tubeworm assemblages on the Juan de Fuca Ridge. *Biogeosciences*, 15, 2629–2647.
https://doi.org/10.5194/bg-15-2629-2018

Marcus, J., Tunnicliffe, V. & Butterfield, D.A. (2009) Post-eruption succession of macrofaunal communities at diffuse flow hydrothermal vents on Axial Volcano, Juan de Fuca Ridge, Northeast Pacific. *Deep-sea research. Part II, Topical studies in oceanography*, 56, 1586–1598.
https://doi.org/10.1016/j.dsr2.2009.05.004

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 2010, 1–8.
https://doi.org/10.1109/GCE.2010.5676129

Müller, O.F. (1788) *Zoologia Danica seu animalium Daniae et Norvegiae rariorum ac minus notorum descriptiones et historia. Volumen secundum.* Havniae, Copenhague, 56 pp.

O. Fabricius. (1780) *Fauna Groenlandica, systematice sistens, Animalia Groenlandiae occidentalis hactenus indagata, quoad nomen specificum, triviale, vernaculumque synonyma auctorum plurium, descriptionem, locum, victum, generationem, mores, usum, capturamque singuli prout detegendi occasio fuit, maximaque parte secundum proprias observationes.* Impensis Ioannis Gottlob Rothe Hafniae, Copenhagen, 450 pp.

Okuda, S. (1938) Polychaetous annelids from the vicinity of the Mitsui Institute of Marine Biology. *Japanese Journal of Zoology*, 8, 75–105.

Palumbi, S.R. (1996) The polymerase chain reaction. *In*: Hillis, D.M. & Moritz, G. (Eds.), *Molecular systematics*. Sinauer Associates, Sunderland, pp. 205–247.

Parapar, J. & Hutchings, P. (2015) Redescription of *Terebellides stroemii* (Polychaeta, Trichobranchidae) and designation of a neotype. *Journal of the Marine Biological Association of the United Kingdom*, 95, 132–141.
https://doi.org/10.1017/S0025315414000903

Parapar, J., Moreira, J. & Martin, D. (2016) On the diversity of the SE Indo-Pacific species of *Terebellides* (Annelida, Trichobranchidae), with the description of a new species. *Peerj*, 4, e2313.

Pleijel, F. (1998) Phylogeny and classification of Hesionidae (Polychaeta). *Zoologica Scripta*, 27, 89–163.
https://doi.org/10.1111/j.1463-6409.1998.tb00433.x

Pleijel, F. (2001) Revision of *Amphiduros* Hartman, 1959 (Polychaeta, Hesionidae, Gyptini). *Ophelia*, 54, 15–28.
https://doi.org/10.1080/00785326.2001.10409453

Pleijel, F. (2004) A revision of *Hesiospina* (Psamathini, Hesionidae, Polychaeta). *Journal of Natural History*, 38, 2547–2566.
https://doi.org/10.1080/00222930310001647433

Pleijel, F., Rouse, G.W., Ruta, C. Wiklund, H. & Nygren, A. (2008) *Vrijenhoekia balaenophila*, a new hesionid polychaete from a whale fall off California. *Zoological Journal of the Linnean Society*, 125, 625–634.
https://doi.org/10.1111/j.1096-3642.2007.00360.x

Pleijel, F., Rouse, G.W., Sundkvist, T. & Nygren, A. (2012) A partial revision of *Gyptis* (Gyptini, Ophiodrominae, Hesionidae, Aciculata, Annelida), with descriptions of a new tribe, a new genus and five new species. *Zoological Journal of the Linnean Society*, 165, 471–494.
https://doi.org/10.1111/j.1096-3642.2012.00819.x

Quatrefages, A. (1866 [imprint date 1865]) *Histoire naturelle des Annelés marins et d'eau douce. Annélides et Géphyriens. Vol. 1*. Librarie Encyclopédique de Roret, Paris, 588 pp.

Rizzo, A.E. & Salazar-Vallejo, S.I. (2014) Hesionidae Grube, 1850 (Annelida: Polychaeta) from South-Southeastern Brazil, with descriptions of four new species. *Zootaxa*, 3856 (2), 267–291.
https://doi.org/10.11646/zootaxa.3856.2.7

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
https://doi.org/10.1093/sysbio/sys029

Sarrazin, J., Juniper, S.K., Massoth, G. & Legendre, P. (1999) Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. *Marine Ecology Progree Series*, 190, 89–112.
https://doi.org/10.3354/meps190089

Sarrazin, J., Robigou, V., Juniper, S.K. & Delaney, J.R. (1997) Biological and geological dynamics over four years on a high-temperature sulfide structure at the Juan de Fuca Ridge hydrothermal observatory. *Marine Ecology Progree Series*, 153, 5–24.
https://doi.org/10.3354/meps153005

Sars, M. (1862) Uddrag af en af detaillerade Afbildningar ledsaget udførlig Beskrivelse over følgende Norske Annelider. *Forhandlinger i Videnskabsselskabet i Kristiania*, 1861, 87–95.

Sjölin, E., Erséus, C. & Källersjö, M. (2005) Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution*, 35, 431–441.
https://doi.org/10.1016/j.ympev.2004.12.018

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenie. *Bioinformatics*, 30, 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Stiller, J., Rousset, V., Pleijel, F., Chevaldonné, P., Vrijenhoek, R.C. & Rouse, G.W. (2013) Phylogeny, biogeography and systematics of hydrothermal vent and methane seep *Amphisamytha* (Ampharetidae, Annelida), with descriptions of three new species. *Systematics and Biodiversity*, 11, 35–65.
https://doi.org/10.1080/14772000.2013.772925

Summers, M., Pleijel, F. & Rouse, G.W. (2015) Whale falls, multiple colonisations of the deep, and the phylogeny of Hesionidae (Annelida). *Invertebrates Systematics*, 29, 105–123.
https://doi.org/10.1071/IS14055

Taylor, S.M. (2009) Transformative ocean science through the VENUS and NEPTUNE Canada ocean observing systems. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, 602, 63–67.
https://doi.org/10.1016/j.nima.2008.12.019

Tsurumi, M. & Tunnicliffe, V. (2001) Characteristics of a hydrothermal vent assemblage on a volcanically active segment of Juan de Fuca Ridge, northeast Pacific. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 530–542.
https://doi.org/10.1139/f01-005

Tsurumi, M. & Tunnicliffe, V. (2003) Tubeworm-associated communities at hydrothermal vents on the Juan de Fuca Ridge, northeast Pacific. *Deep-Sea Research Part I: Oceanographic Research Papers*, 50, 611–629.
https://doi.org/10.1016/S0967-0637(03)00039-6

Tunnicliffe, V. (1988) Biogeography and evolution of hydrothermal-vent fauna in the eastern Pacific Ocean, *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 233, 347–366.

Tunnicliffe, V. (1991) The biology of hydrothermal vents: ecology and evolution. *Oceanography and Marine Biology—An Annual Review*, 29, 319–407.

Tunnicliffe, V., McArthur, A.G. & McHugh, D. (1998) A biogeographical perspective of the deep-sea hydrothermal vent fauna. *Advances in Marine Biology*, 34, 353–442.
https://doi.org/10.1016/S0065-2881(08)60213-8

Urcuyo, I., Bergquist, D., MacDonald, I., VanHorn, M. & Fisher, C.R. (2007) Growth and longevity of the tubeworm *Ridgeia piscesae* in the variable diffuse flow habitats of the Juan de Fuca Ridge. *Marine Ecology Progress Series*, 344, 143–157.
https://doi.org/10.3354/meps06710

Vaidya, G., Lohman, D.J. & Meier, R. (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, 27, 171–180.
https://doi.org/10.1111/j.1096-0031.2010.00329.x

Wilson, R.S. (2000) Family Hesionidae. *In*: Beesley, P.L., Ross, G.J.B. & Glasby, C.J. (Eds.), *Polychaetes & Allies: The Southern Synthesis. Fauna of Australia. Vol. 4a. Polychaeta, Myszotomida, Pogonophora, Echiura, Sipuncula*. CSiRO Publishing, Melbourne, pp. 131–133.

Xu, G., Jackson, D.R., Bemis, K.G. & Rona, P.A. (2014) Time-series measurement of hydrothermal heat flux at the Grotto mound, Endeavour Segment, Juan de Fuca Ridge. *Earth and Planetary Science Letters*, 404, 220–231.
https://doi.org/10.1016/j.epsl.2014.07.040

Zottoli, R.A. (1983) *Amphisamytha galapagensis*, a new species of ampharetid polychaete from the vicinity of abyssal hydrothermal vents in the Galapagos Rift, and the role of this species in rift ecosystems. *Proceedings of the Biological Society of Washington*, 96, 379–391.