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urn:lsid:zoobank.org:pub:214BF6A7-233B-47F6-8819-A1284093B0DB

Cutting the ribbon: bathyal Nemertea from seeps along the Costa Rica margin, with descriptions of 2 new genera and 9 new species

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Abstract. The taxonomy of ribbon worms (Nemertea) is particularly challenging due to the sparsity of distinct morphological characters, causing a significant underestimation of the group's true diversity. The number of named deep-sea species is very limited and there is a vast number of undescribed deep-sea nemerteans still to be discovered. In this paper we figuratively 'cut the ribbon' and name seven new species of monostiliferous hoplonemerteans from seeps and seamounts along the Costa Rican margin, one from seeps along the Oregon margin, and one from vents of the Juan de Fuca Ridge, USA. The species *Chernyshevia escarpiaphila* gen. et sp. nov. and five species of the genus *Alvinonemertes* gen. nov. (*Alvinonemertes dariae* gen. et sp. nov., *Alvinonemertes dagmarae* gen. et sp. nov., *Alvinonemertes christianeae* gen. et sp. nov., *Alvinonemertes claudiae* gen. et sp. nov., *Alvinonemertes tatjanae* gen. et sp. nov.) represent Oerstediina, whereas the three species *Tetrastemma sundbergi* sp. nov., *Tetrastemma polyakovae* sp. nov., and *Tetrastemma strandae* sp. nov. represent Amphiporina. One species of tubulanid palaeonemerteans is described but not provided with a species name due to lacking sequence data for comparison. Additionally, we provide sequence data for one lineid heteronemertean, one reptant hoplonemertean, and two further eumonostiliferan hoplonemertean species. We use an integrative, turbotaxonomic approach combining DNA sequence data with concise morphological descriptions and fully digitized serial histological sections made available as cybertypes.

Keywords. Deep sea, ribbon worms, cold seeps, integrative taxonomy, turbo taxonomy.

Introduction

Nemertea (ribbon worms) are predatory, vermiform, mostly marine animals with a cosmopolitan distribution (Gibson 1995). Most of the approximately 1300 named species inhabit the littoral and sublittoral zones of the world's oceans, whereas only a small number of species is described from the deep sea (Gibson 1995; Kajihara *et al.* 2008). However, an undiscovered high species diversity is assumed beneath 3000 m depth (Chernyshev 2020). The largest fraction of deep-sea nemerteans belongs to a pelagic group of polystiliferous hoplonemerteans, called Pelagica Brinkmann, 1917, that is most abundant between 600–3000 m depth (Roe & Norenburg 1999; Maslakova & Norenburg 2001; Kajihara & Lindsay 2010). Despite the large number of described pelagic polystiliferan species, this group is still poorly studied and half of all species descriptions are based on single specimens (Chernyshev & Polyakova 2018a). Even less information is available on deep-sea benthic species. Until 2013, only 14 benthic species were known to occur in depths exceeding 1000 m, both in Atlantic and Pacific waters (Chernyshev 2013). Half of these species were described from few specimens in the late 19th, early 20th century, with no molecular data, often only insufficiently characterized and are therefore likely to be error-prone (Table 1). Within the last decade, there has been an increasing effort to describe benthic deep-sea nemerteans from Japanese and Russian waters (reviewed in Chernyshev 2014, 2020). This led to the description of 10 additional species and an increase of sequence data, not yet attached to a name (Chernyshev 2013; Chernyshev *et al.* 2015; Chernyshev & Polyakova 2018a, 2018b, 2019; Hookabe *et al.* 2020b). Described benthic deep-sea nemerteans predominantly belong to monostiliferous hoplonemerteans as well as heteronemerteans (Chernyshev 2013, 2020; Chernyshev *et al.* 2015). To date, only two palaeonemerteans have been described from depths beneath 1000 m (Hubrecht 1887; Chernyshev 2013).

So far, the only deep-sea nemertean species described from the Northeastern Pacific is *Thermanemertes valens* Rogers, Gibson & Tunnicliffe, 1996, a species that can be found in association with hydrothermal vents in the Juan de Fuca Ridge (Rogers *et al.* 1996). However, several collection trips to the Costa Rica margin in the East Pacific during the last 10 years yielded numerous nemertean specimens. This area is characterized by the presence of methane seeps of which more than 40 were discovered at various depths since 2008 (Sahling *et al.* 2008; Levin *et al.* 2012; Zapata-Hernández *et al.* 2014). In the sampled segment, the Costa Rica margin comprises diverse seep structures related to mounds, landslide scars, or other causes (Sahling *et al.* 2008). Jaco Scar, formed by a subducting seamount, may even represent an intermediate between hydrothermal vent and methane seep characteristics ("hydrothermal seep") (Levin *et al.* 2012). Overall, the area provides a variety of habitats, and a rich hub of biodiversity for numerous deep-sea species associated with chemosymbiotic organisms (van Dover *et al.* 2002; Vrijenhoek 2010; Levin *et al.* 2015; Goffredi *et al.* 2020). In the last couple of years, different taxa have been recorded or described from Costa Rican seep sites, including various annelids, e.g., *Lamellibrachia barhami* Webb, 1969 (McCowin & Rouse 2018), *Amphisamytha fauchaldi* Solís-Weiss & Hernández-Alcántara, 1994 (Stiller *et al.* 2013), and *Archinome levinae* Borda, Kudenov, Chevaldonné, Blake, Desbruyères, Fabri, Hourdez, Pleijel, Shank, Wilson, Schulze & Rouse, 2013 (Borda *et al.* 2013), *Laminatubus* spp. (Rouse & Kupriyanova 2021), and Hesionidae Grube, 1850 (Rouse *et al.* 2018a), bivalves, e.g., *Archivesica gigas* (Dall, 1896) (Breusing *et al.* 2019), *Pliocardia krylovata* A.M. Martin & Goffredi, 2012 (Martin & Goffredi 2012), and *Bathymodiolus* spp. (McCowin *et al.* 2020), and crustaceans, e.g., *Alvinocaris costaricensis* Martin, Wall, Shank, Cha, Seid & Rouse, 2018 (Martin *et al.* 2018).

Nemertean taxonomy and species descriptions have been hampered by the scarcity of distinct external characters and the necessity of laborious anatomical studies (Sundberg *et al.* 2016a). This has led to a disproportionately high taxonomic impediment for the clade. In fact, in most ecological surveys nemerteans hardly get diagnosed beyond 'Nemertea sp.' (e.g., León-Morales & Vargas 1998; Schander & Willassen 2005; Levin *et al.* 2017), not reflecting the true diversity of the group. Naming these species is crucial for communication among scientists, but also for the proper documentation of biodiversity, which directly affects conservation efforts (Delić *et al.* 2017).

Table 1 (continued on next page). Known species of benthic nemerteans exceeding 1000 m. Sampling depth, locality, number of collected specimens (#), reference and presence of genetic data (Gd) are given. In some cases, the exact number of specimens cannot be determined (s = several).

Species	Depth (m)	Locality	#	Reference	Gd
Palaeonemertea					
Carinina grata	2267–2450	NW Atlantic (b/w Bermuda & Halifax)	2	Hubrecht 1887	/
Cephalothrix iwatai	1494–3334	NW Pacific (Sea of Japan)	200	Chernyshev 2013	1
Heteronemertea					
Baseodiscus australis	1280	SW Pacific (New Zealand)	1	Hubrecht 1887	/
Baseodiscus abyssorum	1353	NE Atlantic (France)	1	Joubin 1902	/
Baseodiscus giardii	548–1280	SW Pacific (New Zealand)	1	Hubrecht 1887	/
Cerebratulus angusticeps	20–2010	SW Pacific (New Zealand)	2	Hubrecht 1887	/
Cerebratulus fuscus	20–1460	Arctic waters, Mediterranean, NE Atlantic	s	Bürger 1904	19
Cerebratulus macroren	1280–1290	W Pacific (Japan & New Zealand)	2	Hubrecht 1887	/
Micrura bathyalis	2670–3426	NW Pacific (Sea of Japan)	9	Chernyshev 2013	1
Parborlasia corrugata	0–3590	Antarctic waters	s	Gibson 1983	s
Sonnenemertes cantelli	3306–4869	NW Pacific (Sea of Okhotsk & KKT)	6	Chernyshev *et al.* 2015	6
Hoplonemertea					
Monostilifera					
Abyssonemertes kajiharai	5510–5514	NW Atlantic (Vema Fracture Zone)	1	Chernyshev & Polyakova 2018b	1
Emplectonema giganteum	160–1560	NW Atlantic (Maine)	>6	Verrill 1892	/
Galathenemertes giribeti	7256	NW Pacific (KKT)	5	Chernyshev & Polyakova 2019	2
Nemertovema hadalis	8336	NW Atlantic (Puerto Rico Trench)	1	Chernyshev & Polyakova 2018b	1
Nemertovema norenburgi	8221–8282	NW Pacific (KKT)	2	Chernyshev & Polyakova 2019	2
Ovicides davidi	2595–2650	SE Pacific (Pagodes vent site)	36	Shields & Segonzac 2007	/
Ovicides jasoni	1891–2719	SW Pacific (North Fiji Basin & Lau Basin)	juv	Shields & Segonzac 2007	/
Ovicides jonesi	2204	SE Pacific (Sebastian's Steamer vent site)	?	Shields & Segonzac 2007	/

Table 1 (continued). Known species of benthic nemerteans exceeding 1000 m. Sampling depth, locality, number of collected specimens (#), reference and presence of genetic data (Gd) are given. In some cases, the exact number of specimens cannot be determined (s = several).

Species	Depth (m)	Locality	#	Reference	Gd
Monostilifera					
Proamphiporus crandalli	5495	NW Pacific (KKT)	1	Chernyshev & Polyakova 2019	1
Proamphiporus kaimeiae	262	NW Pacific (Japan)	1	Hookabe *et al.* 2020b	1
Thermanemertes valens	2280	NE Pacific (Juan de Fuca Ridge)	14	Rogers *et al.* 1996	/
Polystilifera?					
Uniporus alisae	3301	NW Pacific (Sea of Okhotsk)	1	Chernyshev & Polyakova 2018a	1
Uniporus hyalinus	1000–1200	NE Atlantic (Norwegian Sea)	2	Brinkmann 1914–1915	/

Traditionally, species descriptions of nemerteans were strongly based on morphological data, especially in the form of histological sectioning. Nevertheless, it has been shown that only one third of the commonly investigated characteristics are actually informative when it comes to differentiation between species and genera (Strand *et al.* 2014). In contrast to this, it has been found that in nemertean systematics a description of external morphology linked with a genetic barcode is sufficient for species descriptions (Sundberg & Strand 2010; Strand & Sundberg 2011; Strand *et al.* 2014; Sundberg *et al.* 2016a). Therefore, it has been proposed by Sundberg *et al.* (2016a) that the description of new nemertean species is regarded as valid if it includes accurate information on type locality and deposition of type material, a description of external morphology, and at least a DNA COI barcode. Since then, several new nemertean species have been described in this way (e.g., Strand & Sundberg 2011; Kajihara 2015; Kajihara *et al.* 2018; Chernyshev *et al.* 2020). This goes hand in hand with the turbo-taxonomic approach where short diagnoses and descriptions are linked with appropriate DNA sequence data. Turbotaxonomy was first used to describe 179 wasp species (Butcher *et al.* 2012) and has been applied in many cases since (e.g., Riedel *et al.* 2013; Summers *et al.* 2014; Rouse *et al.* 2018b; Sharkey *et al.* 2021).

For this paper, we follow the guidelines proposed by Sundberg *et al.* (2016a) and base the erection and description of new species and genera on molecular data in combination with a short description of external morphology. Nevertheless, we think that providing as much information as possible on the newly described species is crucial for future efforts in resolving nemertean taxonomy. Therefore, we not only provide a COI barcode, but also include further genetic markers (16S, H3, 18S) to facilitate phylogenetic placement of the species described in this study. Moreover – whenever enough specimen material was present – we histologically sectioned specimens of each newly described species and provide short descriptions of significant internal characteristics that will allow to conduct further research on the newly described species in the future.

Along the Costa Rica margin, around 80 nemertean specimens representing 12 species were collected at depths between 900 and 2200 meters. In this paper we describe and name 7 new species and 2 new genera and provide genetic data for all 12 species. Two more nemertean species from from the Hydrate

Ridge seep off the Oregon margin and hydrothermal vents at the Juan de Fuca Ridge are described as they are closely related to some of the Costa Rican species. Furthermore, we have made all of our digitized histological sections available as cybertypes, in order to aid future taxonomic work on deep-sea nemerteans.

Material and methods

Specimen collection

Specimens were collected during seven deep-sea expeditions along the Costa Rica margin (Fig. 1A), the Oregon margin and the Juan de Fuca Ridge between 2009 and 2019 using RV *Atlantis* and HOV *Alvin* (AT15-44, AT15-59, AT15-68, AT37-13, AT42-03), RV *Falkor* and ROV *SuBastian* (FK190106), and RV *Western Flyer* and ROV *Doc Ricketts* (Northern 2016 expedition). Specimen collection in Costa Rica was authorized by permits issued by CONAGEBIO (Comisión Nacional para la Gestión de la Biodiversidad), ACMC (Área de Conservación Marina Coco), INCOPESCA (Instituto Costarricense de Pesca y Acuicultura), and SINAC (Sistema Nacional de Áreas de Conservación) under MINAE (Ministerio de Ambiente y Energía), Government of Costa Rica: INCOPESCA-CPI-003-12-2018, R-070-2018-OT-CONAGEBIO, SINAC-CUSBSE-PI-R-032-2018, SINAC-SE-CUS-PI-R-035-2017, and the Contract for the Grant of Prior Informed Consent between MINAE-SINAC-ACMC and Jorge Cortés Nuñez for the Basic Research Project: "FK190106 - Cuantificación de los vínculos biológicos, químicos y físicos entre las comunidades quimiosintéticas con el mar profundo circundante". Information on collected specimens is provided in Table 2.

Specimens were photographed alive under Leica MZ8 or MZ9.5 stereo microscopes with a camera attachment (e.g., for Canon PowerShot G9 or Canon EOS Rebel T6i or T6s). Whenever the animals were large enough posterior ends were subsampled and preserved in 95% ethanol for DNA extraction. Small specimens were preserved only in ethanol.

Histology

Specimens used for histology were first relaxed using an isotonic mix of equal volumes of 7% $MgCl_2$ and seawater and fixed in 10% formaldehyde in seawater or 4% paraformaldehyde in Sorenson's phosphate buffer pH 7.4, rinsed in fresh water and transferred to 70% ethanol. Prior to further processing they were postfixed with Bouin's solution (modified after Dubosque–Basil, containing 60% ethanol). The specimens were then dehydrated completely in an ascending ethanol series, followed by an incubation in methylbenzoate and subsequently in butanol. Before the animals were embedded in Paraplast (McCormick Scientific, Richmond, USA), they were preincubated in Histoplast (Thermo Scientific, Dreieich, Germany) at 60°C for several days with multiple medium changes. Serial sections of 5 µm thickness were prepared using an Autocut 2050 microtome (Reichert-Jung, Leica, Wetzlar, Germany) and transferred on to glass slides coated with albumen-glycerin. Sections were stained following the AZAN trichrome staining protocol. Coverslips were mounted using Malinol. Sections were photographed with an Olympus BX-51 microscope equipped with an Olympus cc12 camera and a slide 2.2 system (Olympus, Hamburg, Germany). Digitalized thin sections were aligned with Imod (Kremer *et al.* 1996) and Imod align (http://www.q-terra.de/).

All image series are deposited in Zenodo (https://zenodo.org). Complete image stacks can be downloaded using Zenodo_get (Völgyes & Lupton 2020).

DNA extraction, PCR amplification and sequencing

DNA of 22 Costa Rican and 2 US American specimens was extracted using the DNeasy Blood and Tissue Kit (Qiagen) and the Quick-DNA™ Microprep Plus Kit (Zymo Research) following the manufacturers' protocols. Four gene fragments were amplified from the genomic DNA: partial

mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA and nuclear histone H3 and 18S rRNA. Amplification of these gene fragments was accomplished using the primers given in Supp. file 1: Table 1. Polymerase chain reactions (PCR) were performed using FastGene® Taq ReadyMix (Nippon Genetics).Thermal cycling was initiated with 2 min at 94°C, followed by 35 (H3, 18S) or 40 cycles (COI, 16S) of denaturation (30 s at 94°C), annealing (60 s at 48°C for COI, at 51°C for 16S, and
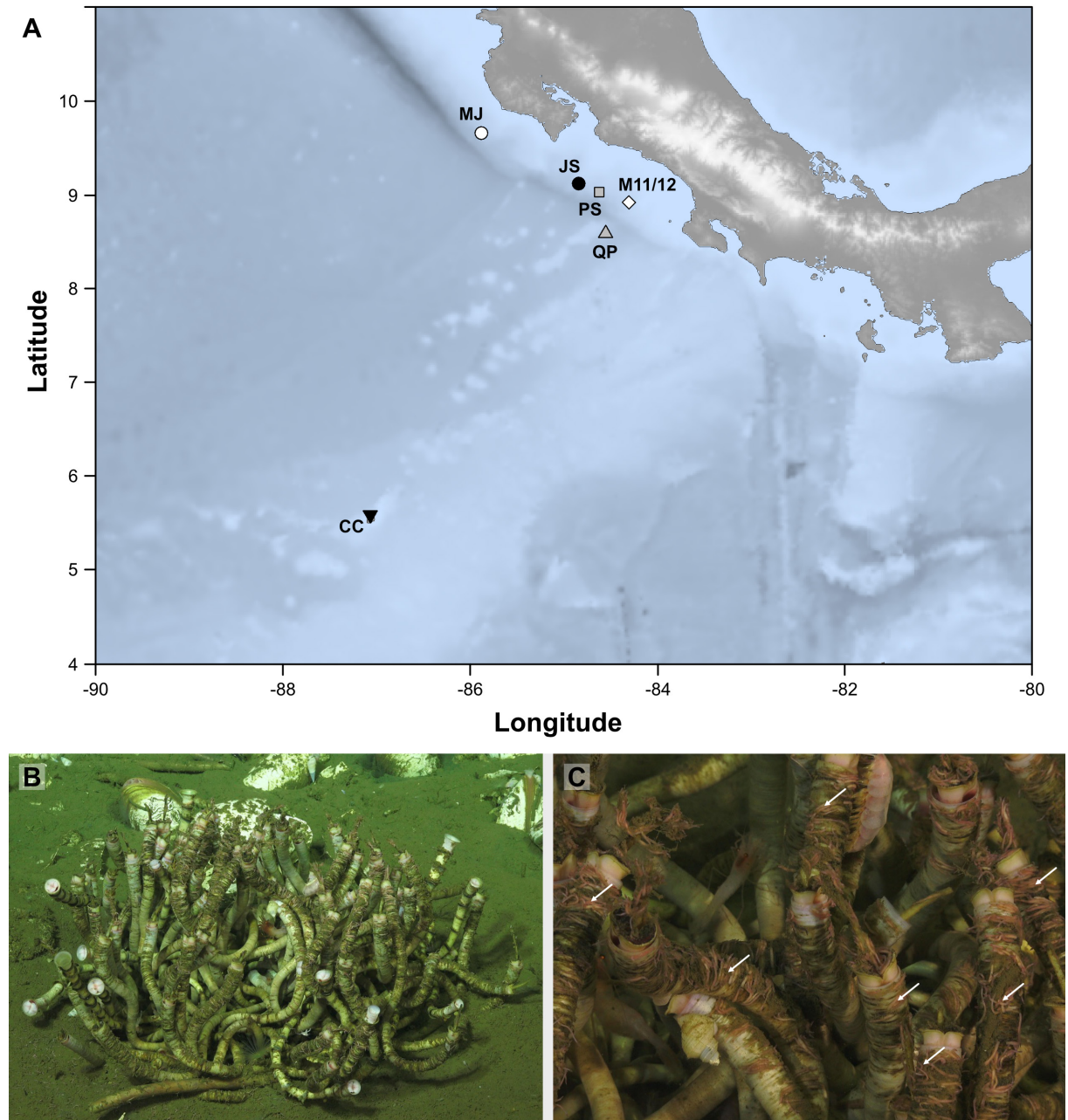

Fig. 1. Sampling sites along the Costa Rica margin and exemplary habitat pictures. **A**. Map of the seven sampling sites along the Costa Rica margin: Cocos Canyon (CC), Jaco Scar (JS), Mound 11 (M11), Mound 12 (M12), Mound Jaguar (MJ), Parrita Seep (PS), and Quepos Plateau (QP). **B**. Aggregation of the vestamentiferan *Escarpia spicata* Jones, 1985. **C**. Nemerteans of the species *Chernyshevia escarpiaphila* sp. nov. (arrows) found on *Escarpia spicata*. Photo credit: ROV *SuBastian*/Schmidt Ocean Institute.

Table 2 (continued on next page). List of the species, location data (seep habitat unless otherwise specified), and GenBank accession numbers of sequences obtained in the present study. Specimen IDs are from the Benthic Invertebrate Collection of Scripps Institution of Oceanography (SIO-BIC) or the Nemertea Collection of the Museum of Zoology, University of Costa Rica (MZUCR Nemertea Collection). Letter prefixes for dives are "D" for ROV *Doc Ricketts* "A" for HOV *Alvin* and "S" for ROV *Subastian*.

Taxonomy	Specimen ID	Type	Dive	Locality	Coordinates	Depth (m)	18S	16S	COI	H3
PALAEONEMERTEA										
Tubulanus cf. *lutescens*	SIO-BIC N107	–	A4505	Mound 11	8.920° N, 84.306° W	1019–1045	ON186261	ON036062	ON021852	ON182043
	SIO-BIC N120	–	A4586	Mound 12	8.931° N, 84.313° W	982–1000	ON186262	ON036063	ON021854	ON182044
	SIO-BIC N233	–	A4907	Mound 12	8.930°N, 84.313°W	999	ON186263	ON036061	ON021853	ON182046
	SIO-BIC N257	–	A4974	Mound 12	8.93037°N, 84.31319°W	996	ON186264	ON036060	ON021851	ON182045
HETERONEMERTEA										
Lineidae	SIO-BIC N254	–	A4976	Jaco Scar	9.11507°N, 84.83978°W	1887	–	ON036059	ON021872	ON182047
HOPLONEMERTEA – POLYSTILIFERA										
Reptantia	SIO-BIC N263	–	S0226 S2	Cocos Canyon (seamount)	5.58451°N, 87.06755°W	950	ON186260	ON036058	ON02170	ON182053
HOPLONEMERTEA – MONOSTILIFERA										
Chernyshevia escarpiaphila sp. nov.	SIO-BIC N112	paratype	A4509	Jaco Scar	9.117°N, 84.843°W	974–1866	ON186250	–	ON021859	ON182035
	SIO-BIC N235	paratype	A4916	Jaco Scar	9.119°N, 84.843°W	1604–1854	ON186253	ON036046	ON021863	ON182036
	SIO-BIC N236	paratype	A4916	Jaco Scar	9.119°N, 84.843°W	1604–1854	–	ON036045	–	ON182037
	SIO-BIC N237	paratype	A4916	Jaco Scar	9.119°N, 84.843°W	1604–1854	–	–	ON021862	ON182038
	SIO-BIC N264	paratype	S0230 S2	Mound Jaguar	9.65556°N, 85.88046° W	1909	–	ON036044	ON021861	ON182039
	SIO-BIC N266	paratype	A4591	Jaco Scar	9.118° N, 84.839° W	1752–1802	ON186251	ON036043	ON021860	ON182040
Alvinonemertes dariae sp. nov.	SIO-BIC N262	paratype	A4990	Parrita Seep	9.03268° N, 84.62127° W	1407	ON186252	ON036048	ON021865	–
Alvinonemertes dagmarae sp. nov.	SIO-BIC N260	holotype	A4981	Quepos Plateau Hills (seamount)	8.5323° N, 84.79072° W	1410	ON186254	ON036049	ON021866	ON182034

Table 2 (continued on next page). List of the species, location data (seep habitat unless otherwise specified), and GenBank accession numbers of sequences obtained in the present study. Specimen IDs are from the Benthic Invertebrate Collection of Scripps Institution of Oceanography (SIO-BIC) or the Nemertea Collection of the Museum of Zoology, University of Costa Rica (MZUCR Nemertea Collection). Letter prefixes for dives are "D" for ROV *Doc Ricketts* "A" for HOV *Alvin* and "S" for ROV *Subastian*.

Taxonomy	Specimen ID	Type	Dive	Locality	Coordinates	Depth (m)	18S	16S	COI	H3
Alvinonemertes christianeae sp. nov.	SIO-BIC N253	holotype	A4976	Jaco Scar	9.11507° N, 84.83978° W	1887	–	ON036052	ON021867	–
	MZUCR-102-01	paratype	A4980	Quepos Plateau Hills (seamount)	8.58548° N, 84.54836° W	2184	–	ON036051	–	–
Alvinonemertes claudiae sp. nov.	SIO-BIC N231	paratype	D875	Juan de Fuca Ridge (vent)	44.659° N, 130.364° W	2250	–	ON036050	ON021868	–
Alvinonemertes tatjanae sp. nov.	SIO-BIC N119	holotype	A4631	Hydrate Ridge	44.670° N, 125.098° W	618	ON186256	ON036047	ON021864	ON182041
Tetrastemma sundbergi sp. nov.	SIO-BIC N256	holotype	A4978	Mound 12	8.93°N, 84.31°W	996–999	ON186255	ON036054	ON021855	ON182052
Tetrastemma polyakovae sp. nov.	MZUCR-101-01	paratype	A4978	Mound 12	8.93°N, 84.31°W	996–999	ON186259	ON036057	ON021858	ON182050
	SIO-BIC N258	holotype	A4978	Mound 12	8.93030°N, 84.31260°W	997	ON186258	ON036056	ON021857	ON182051
Tetrastemma strandaae sp. nov.	MZUCR-100-01	paratype	A4913	Jaco Scar	9.12°N, 84.84°W	1798–1908	–	ON036055	ON021856	ON182049
Eumonostilifera	SIO-BIC N259	–	A4980	Quepos Plateau Hills (seamount)	8.59°N, 84.56°W	1872–2020	ON186269	ON036053	ON021869	ON182042
Eumonostilifera	SIO-BIC N109	–	A4508	Parrita Seep	9.030° N, 84.623° W	1401–1433	ON186257	–	ON021871	ON182048

at 54°C for H3, 30 s at 52°C for 18S), and elongation (60 s at 72°C), and terminated with a 2 min (COI, 16S)/10 min (H3, 18S) final elongation at 72°C. Amplified products were purified using illustra ExoProStar 1-Step (GE Healthcare) following the manufacturer's instructions. Sanger-sequencing of the purified PCR products was performed by LGC Genomics (Berlin, Germany). GenBank accession numbers of sequences obtained in the present study are given in Table 2.

Sequence analysis and phylogenetic analyses

The obtained sequences for all markers were aligned in Geneious Prime® ver. 2020.0.5 (Biomatters) using the implemented MAFFT ver. 7 (Katoh *et al.* 2002, 2019; Katoh & Standley 2013) with default parameters. All ambiguous positions were excluded with Gblocks ver. 0.91b using default parameters (Castresana 2000). BLAST searches as implemented in NCBI website were conducted for all sequences. All four markers were concatenated using SequenceMatrix (Vaidya *et al.* 2011). Sequences represented all three large nemertean taxa (Palaeonemertea Hubrecht, 1879, Heteronemertea Bürger, 1892, and Hoplonemertea Hubrecht, 1879). Accordingly, four different supermatrices were formed with a total length of approximately 3000 bp (Hoplonemertea was separated into Polystilifera and Monostilifera). Selection of the optimal nucleotide substitution models for the four supermatrices and of the partition schemes were performed using PartitionFinder with implementation of the "greedy" search scheme (Lanfear *et al.* 2017). All four supermatrices were combined with relevant species included in previous phylogenetic analyses, with special emphasis on deep-sea species (Thollesson & Norenburg 2003; Andrade *et al.* 2012; Kvist *et al.* 2014, 2015; Chernyshev & Polyakova 2018a, 2018b, 2019). Information on included specimens are provided in Supp. file 1: Table 2. The best-fitting substitution models for the four supermatrices separated by the four gene fragments as calculated by PartitionFinder are given in Supp. file 1: Table 3.

In order to unravel the systematic position of the newly described deep-sea nemertean species, both maximum likelihood (ML) and Bayesian inferences (BI) approaches were applied. *Sonnenemertes cantelli* Chernyshev, Abukawa & Kajihara, 2015 and *Micrura callima* Sundberg & Gibson, 1995 were used as outgroup for the palaeonemertean and both hoplonemertean datasets, whereas *Tubulanus polymorphus* Renier, 1804 and *Carinoma mutabilis* Griffin, 1898 were selected as outgroup for the heteronemertean dataset.

The first tree using ML as optimality criterion was calculated in iqtree ver. 1.6.12 (Nguyen *et al.* 2015; Chernomor *et al.* 2016) under optimal nucleotide substitution models and partitioning schemes as suggested by PartitionFinder. Branch support was estimated using ultrafast bootstrap (Hoang *et al.* 2018) analysis with 1000 bootstrap replicates.

The second tree using ML as optimality criterion was calculated using RAxML ver. 8.2.11 (Stamatakis 2014) as implemented in Geneious. For all supermatrices GTR+G+I was applied as best-fitting substitution model. Branch support was estimated using 1000 bootstrap replicates.

The third tree based on BI as optimality criterion was conducted using MrBayes ver. 3.2.7a (Ronquist *et al.* 2012) on the CIPRES server based on the optimal substitution models and partition schemes suggested by PartitionFinder. Two parallel runs with four Markov chains in each run during 2 500 000 generations were launched with 25% of the sampled trees discarded as burn-in. Branch support was estimated using posterior probabilities with probabilities less than 50% in the consensus tree being collapsed.

Additionally, pairwise distances between the new species and relevant related species were calculated in MEGA ver. 6.06 (Tamura *et al.* 2013) using uncorrected p-distances. A TCS haplotype network (Clement *et al.* 2000) based on statistical parsimony was calculated in PopART (Leigh & Bryant 2015) for the newly described species of *Tubulanus* Renier, 1804.

Repositories

MZUCR = Nemertea Collection, Museum of Zoology of the University of Costa Rica, San José, Costa Rica

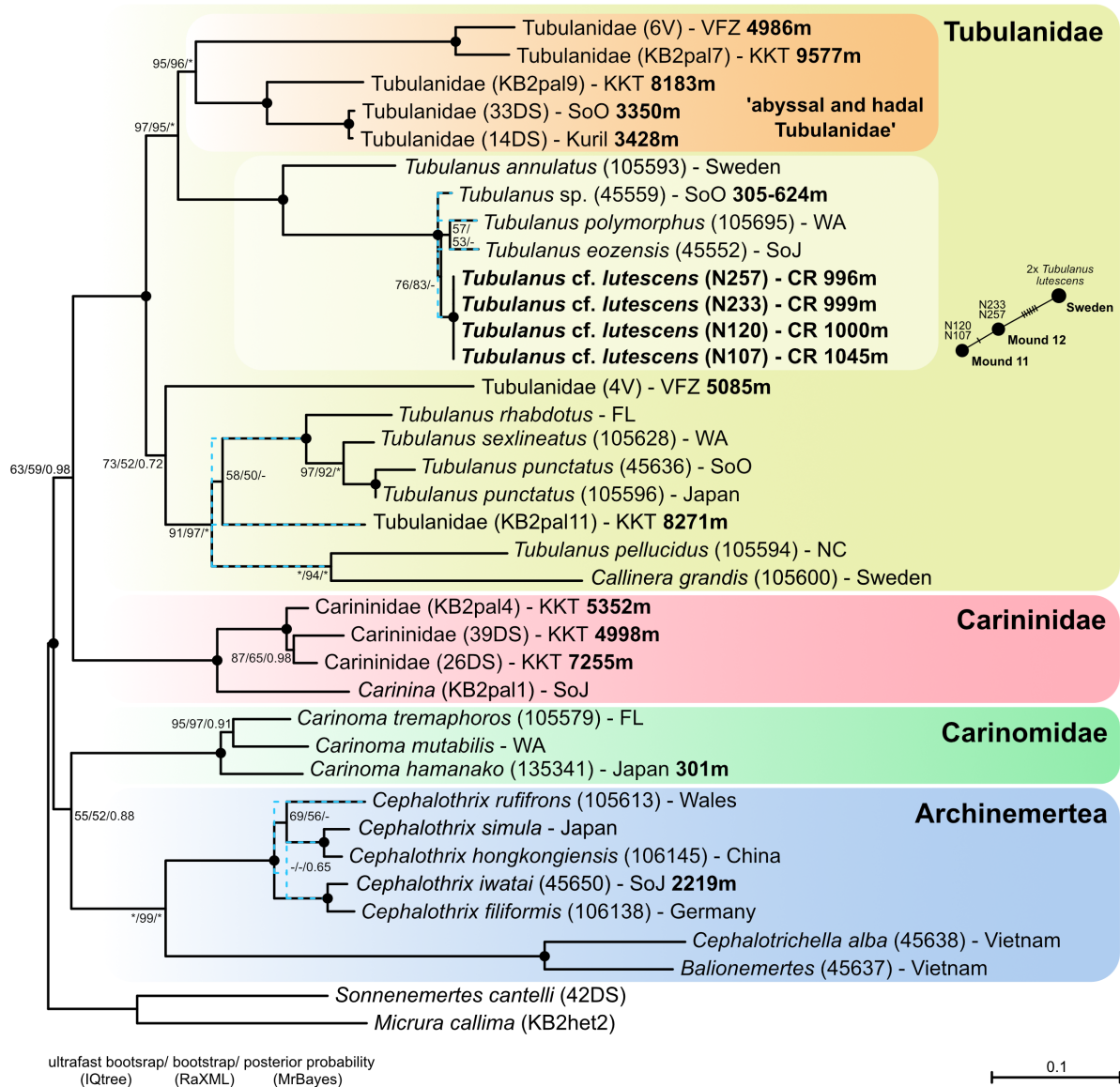SIO-BIC = Scripps Institution of Oceanography Benthic Invertebrate Collection, La Jolla, California, USA

Fig. 2. Maximum likelihood (ML) tree for the palaeonemertean supermatrix based on the concatenated four gene dataset (16S, COI, 18S, H3). Numbers beside nodes indicate support values. Black circles indicate nodal support of 100/100/1.0; asterisks indicate nodal support of either 100/ or /1.0. Alternative branches found in the BI analysis are given as blue dotted lines. *Sonnenemertes cantelli* Chernyshev, Abukawa & Kajihara, 2015 and *Micrura callima* Sundberg & Gibson, 1995 were used for outgroup rooting. Specimens belonging to the new species *Tubulanus* cf. *lutescens* are highlighted in bold. When available a specimen-ID, sampling locality, and depth (for deep-sea species) are provided. A statistical parsimony haplotype network based on the COI gene fragment reveals close relationship between *Tubulanus* cf. *lutescens* and *Tubulanus lutescens*.

Results

Twelve distinct species were identified in our phylogenetic analyses of sequence data (COI, 16S, H3, 18S) from approximately 80 collected specimens. These include one species of tubulanid Palaeonemertea, one species of lineid Heteronemertea, one species of polystiliferous Hoplonemertea, and nine species of monostiliferous Hoplonemertea.

<div align="center">

Phylum Nemertea
Class Palaeonemertea Hubrecht, 1879

Family **Tubulanidae** Bürger, 1904

</div>

Tubulanidae is the only palaeonemertean family that was represented in the Costa Rican samples. In total, six tubulanid specimens were collected at depths around 1000 m from two nearby localities (Mound 11 & Mound 12). A DNA analysis of four specimens collected over different years showed that all specimens belong to the same species that is firmly nested within Tubulanidae (Fig. 2).

<div align="center">

Genus *Tubulanus* Renier, 1804

***Tubulanus* cf. *lutescens* Cantell, 2001**
Figs 3A–B, 4

</div>

Diagnosis

Tubulanus cf. *lutescens* can be clearly identified as a member of the genus *Tubulanus* based on the amount of body wall, proboscis, and rhynchocoel muscle layers, the position of the nervous system, the composition of the cerebral organs, and several other internal characteristics (Gibson 1982; Sundberg & Hylbom 1994; Gibson & Sundberg 1999). As in other members of the genus, the body wall is composed of three muscle layers (outer circular, median longitudinal, and inner circular musculature), whereas the proboscis is composed of two layers (outer longitudinal, inner circular) and the rhynchocoel wall of only one circular muscle layer. The nervous system is located between the epidermal basement membrane and the outer circular body wall musculature and the cerebral organs consist of ciliated canals in the epidermis. Moreover, a mid-dorsal blood vessel and eyes are absent. The collected specimens are of bright red coloration and lacks a conspicuous colour pattern. A lower dorsal nerve is absent.

Material examined

COSTA RICA • 1 spec. (fixed in paraformaldehyde and prepared as 67 slides with transverse sections); methane seep Mound 12; 8.930° N, 84.313° W; depth 990–999 m; 22 May 2017; Lisa Levin and Charlotte Seid leg.; collected by HOV *Alvin*, Dive 4907; SIO-BIC N277 (ex SIO-BIC N233) (image series: https://doi.org/10.5281/zenodo.6368041) • 1 spec. (anterior fixed in paraformaldehyde, posterior fixed in ethanol); same collection data as for preceding; GenBank: ON186263, ON036061, ON021853, ON182046; SIO-BIC N233 • 1 spec. (fixed in paraformaldehyde); same collection data as for preceding; MZUCR Nemertea Collection MZUCR-103-01 (ex SIO-BIC N233) • 1 spec. (piece in ethanol); methane seep Mound 11; 8.920° N, 84.306° W; depth 1019–1045 m; 26 Feb. 2009; Greg Rouse and Mike Skowronski leg.; collected by HOV *Alvin*, Dive 4505; GenBank: ON186261, ON036062, ON021852, ON182043; SIO-BIC N107 • 1 spec. (piece in ethanol); methane seep Mound 12; 8.931° N, 84.313° W; depth 982–1000 m; 7 Jan. 2010; Lisa Levin and Graham Nash leg.; collected by HOV *Alvin*, Dive 4586; GenBank: ON186262, ON036063, ON021854, ON182044; SIO-BIC N120 • 1 spec. (ethanol); methane seep Mound 12; 8.93037° N, 84.31319° W; depth 996 m; 20 Oct. 2018; Lisa Levin and Kyle Metcalfe leg.; collected by HOV *Alvin*, Dive 4974; GenBank: ON186264, ON036060, ON021851, ON182045; SIO-BIC N257.

Description

Specimens up to 20 cm in length, up to 4 mm in diameter. Body more or less cylindrical. Tapered head slightly wider than rest of body (Fig. 3A). One pair of dorsal, V-shaped cephalic furrows in front of brain lobes (Fig. 3B). Body bright red, trunk slightly translucent. Anterior tip brighter than rest of head (Fig. 3A–B). Epidermal constrictions along whole body length. Gut and gonads visible through body wall (Fig 3A).

Epidermis in foregut region 150–200 μm thick. Body wall composed of outer circular, middle longitudinal and inner circular musculature (Fig. 4E). Rhynchocoel wall only with circular muscle layer (Fig. 4E). Proboscis with two layers of musculature (outer longitudinal, inner circular), with two nerves, no proboscis armature (Fig. 4C). Proboscis pore subterminal, ventral (Fig. 4A); mouth opening separate, behind cerebral ganglia (Fig. 4B). Cerebral ganglia with only outer neurilemma, neurochord cells absent. Dorsal and ventral ganglia fused, located in ventral part of head (Fig. 4D). Lateral nerve cords without accessory nerves, myofibrillae absent (Fig. 4F). Precerebral nerves numerous, not grouped (Fig. 4A); buccal nerves paired. Upper dorsal nerve present (Fig. 4E). Nervous system between epidermal basement membrane and outer circular body wall musculature (Fig. 4D, F). Eyes lacking. Cerebral organs as simple ciliated canals in epidermis, posterior to ventral ganglia (Fig. 4D). Apical organ as depression frontal of rhynchopore, cephalic glands in anterior half of head, scattered between muscle fibres of head. Blood vascular system consisting of cephalic loop giving rise to two paired postcerebral, lateral
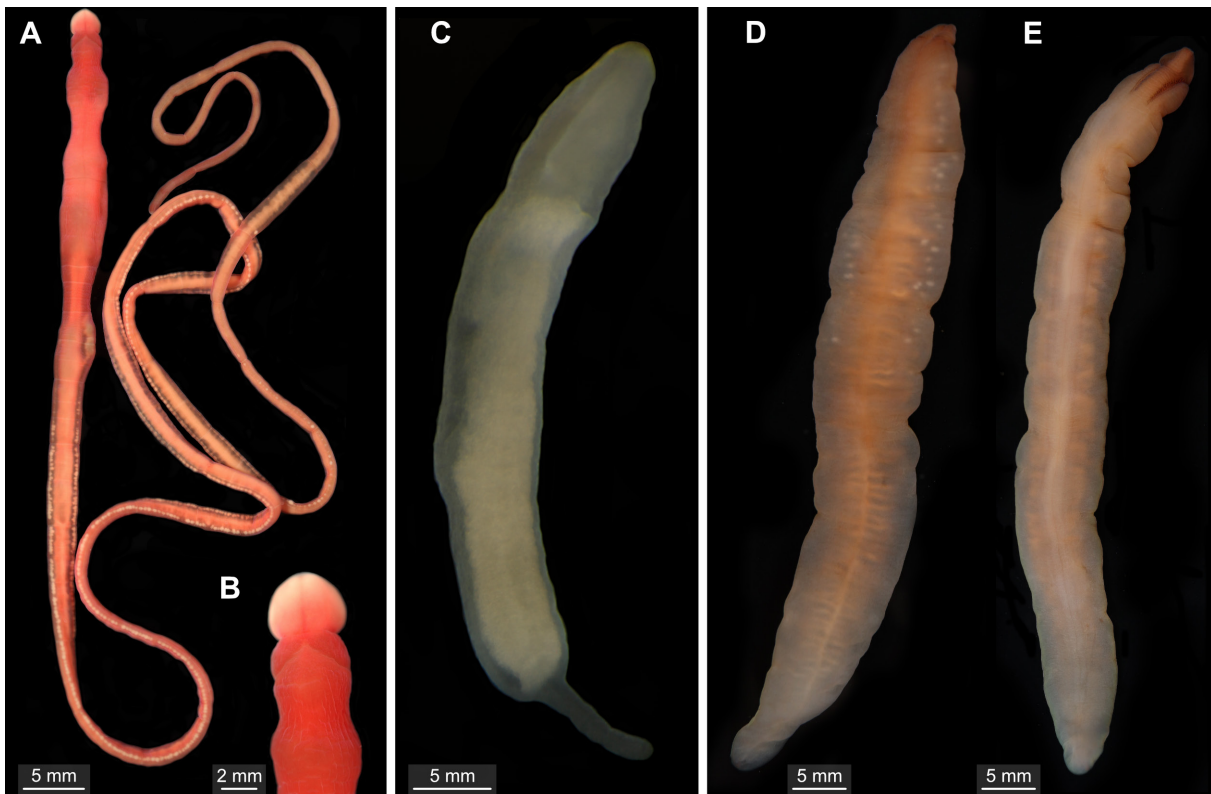

Fig. 3. Live images of palaeonemerteans, heteronemerteans, and polystiliferous hoplonemerteans collected along the Costa Rica margin. **A**. *Tubulanus* cf. *lutescens* (SIO-BIC N107) (Palaeonemertea). **B**. Dorsal surface of head region of *Tubulanus* cf. *lutescens* (SIO-BIC N233). **C**. Lineidae (SIO-BIC N254) (Heteronemertea). **D–E**. Reptantia (SIO-BIC N263) (Polystilifera) dorsal (D) and ventral view (E).

vessels (Fig. 4A–B). Mid-dorsal blood vessel absent, smaller vessels splitting from lateral blood vessels in foregut region.

Remarks

The Costa Rican specimens are attributed to the genus *Tubulanus*, based on the phylogenetic analyses and the similarity of external and internal characteristics. In the phylogenetic analysis, this species belongs to a highly supported clade containing *Tubulanus eozensis* Yamaoka, 1940, *Tubulanus polymorphus*, and an undescribed tubulanid from the Sea of Okhotsk (Fig. 3). A clade of undescribed abyssal and hadal tubulanids is sister to that clade. The pairwise distances of the COI gene between the four deep-sea specimens are below 1%. Interestingly, pairwise distances between *Tubulanus* cf. *lutescens* and two sequences of *Tubulanus lutescens*, a shallow water species collected in Sweden, are only 1–1.3%. In a TCS haplotype network analysis of the mitochondrial COI gene *Tubulanus lutescens* is only separated from our specimens by six nucleotide substitutions (Fig. 3). However, the two species differ by body coloration as *Tubulanus* cf. *lutescens* is bright red, whereas *Tubulanus lutescens* is yellow. Moreover, *Tubulanus* cf. *lutescens* lacks a lower dorsal nerve which is present in *Tubulanus lutescens*. The markedly dissimilar habitats of both species (*T. lutescens* intertidal from Northern Europe vs *T.* cf. *lutescens* from
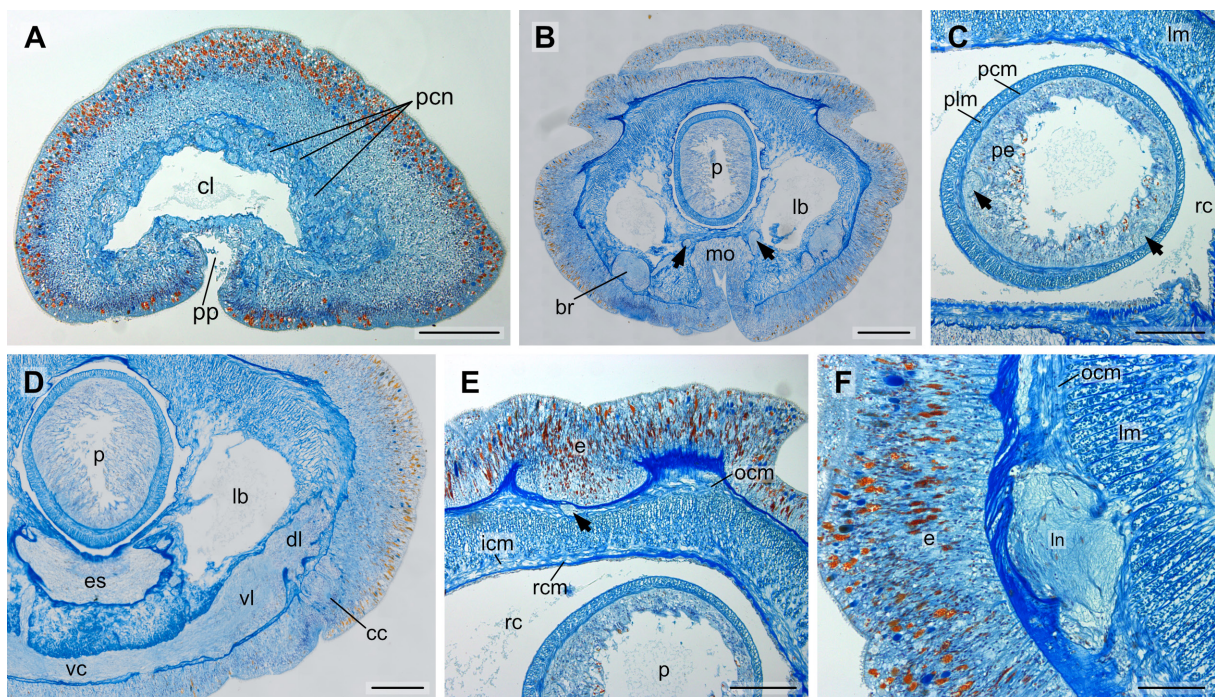

Fig. 4. *Tubulanus* cf. *lutescens* Cantell, 2001 (SIO-BIC N277), transverse histological sections. **A**. Anterior region of head with proboscis pore. **B**. Posterior brain region with mouth opening and buccal nerves (arrowheads). **C**. Proboscis composition and the two proboscis nerves (arrowheads) in the foregut region. **D**. Brain region showing the cerebral canal. **E**. Body wall composition in the foregut region, arrowhead indicates upper dorsal nerve. **F**. Lateral nerve cord in the foregut region. Abbreviations: br = brain; cc = cerebral canal; cl = cephalic loop; dl = dorsal brain lobe; e = epidermis; es = esophagus; icm = inner circular muscle layer; lb = lateral blood vessel; lm = longitudinal muscle layer; ln = lateral nerve cord; mo = mouth opening; ocm = outer circular muscle layer; p = proboscis; pcm = circular musculature of proboscis; pcn = precerebral nerves; pe = proboscis epithelium; plm = longitudinal musculature of proboscis; pp = proboscis pore; rc = rhynchocoel; rcm = circular musculature of rhynchocoel; vc = ventral commissure; vl = ventral brain lobe. Scale bars: A–C, E = 200 μm; D, F = 100 μm.
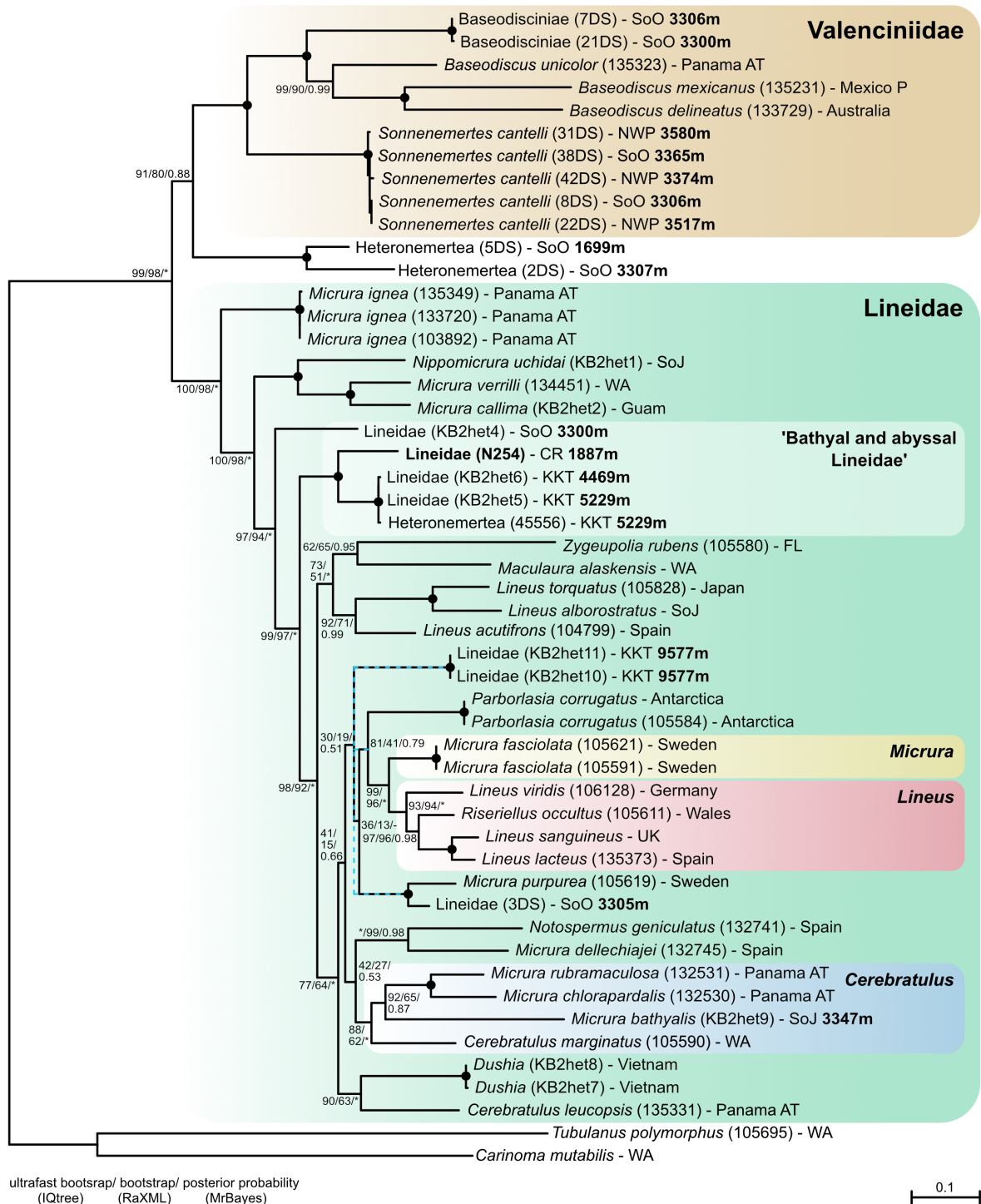
Fig. 5. Maximum likelihood (ML) tree for the heteronemertean supermatrix based on the concatenated four gene dataset (16S, COI, 18S, H3). Numbers beside nodes indicate support values. Black circles indicate nodal support of 100/100/1.0; asterisks indicate nodal support of either 100/ or /1.0. Alternative branches found in the BI analysis are given as blue dotted lines. *Tubulanus polymorphus* Renier, 1804 and *Carinoma mutabilis* Griffin, 1898 were used for outgroup rooting. The specimen found at the Costa Rica margin is given in bold. Specimen-ID, sampling locality, and depth (for deep-sea species) are provided.

ca 1000 m depth in Eastern Pacific) might indicate that they represent different species. Future analyses of nuclear sequences of *T. lutescens* are warranted to determine whether *Tubulanus* cf. *lutescens* and *T. lutescens* are indeed two different species.

Order **Heteronemertea** Bürger, 1892

One single heteronemertean specimen (SIO-BIC N254), representing a member of Lineidae McIntosh, 1873, was collected in associated with experimentally deployed wood at 1887 m depth at the Jaco Scar methane seep. This specimen is 45 mm long and has a diameter of 5 mm. The body is translucent and of pale whitish coloration. It possesses a long caudal cirrus (Fig. 3C). It forms a clade with three undescribed lineids collected in the abyssal vicinity of the Kuril-Kamchatka Trench (Fig. 5). Based on the concatenated analysis, this clade cannot be attributed to any of the known heteronemertean genera. Pairwise distances of the COI gene between Lineidae sp. SIO-BIC N254 and those three heteronemerteans
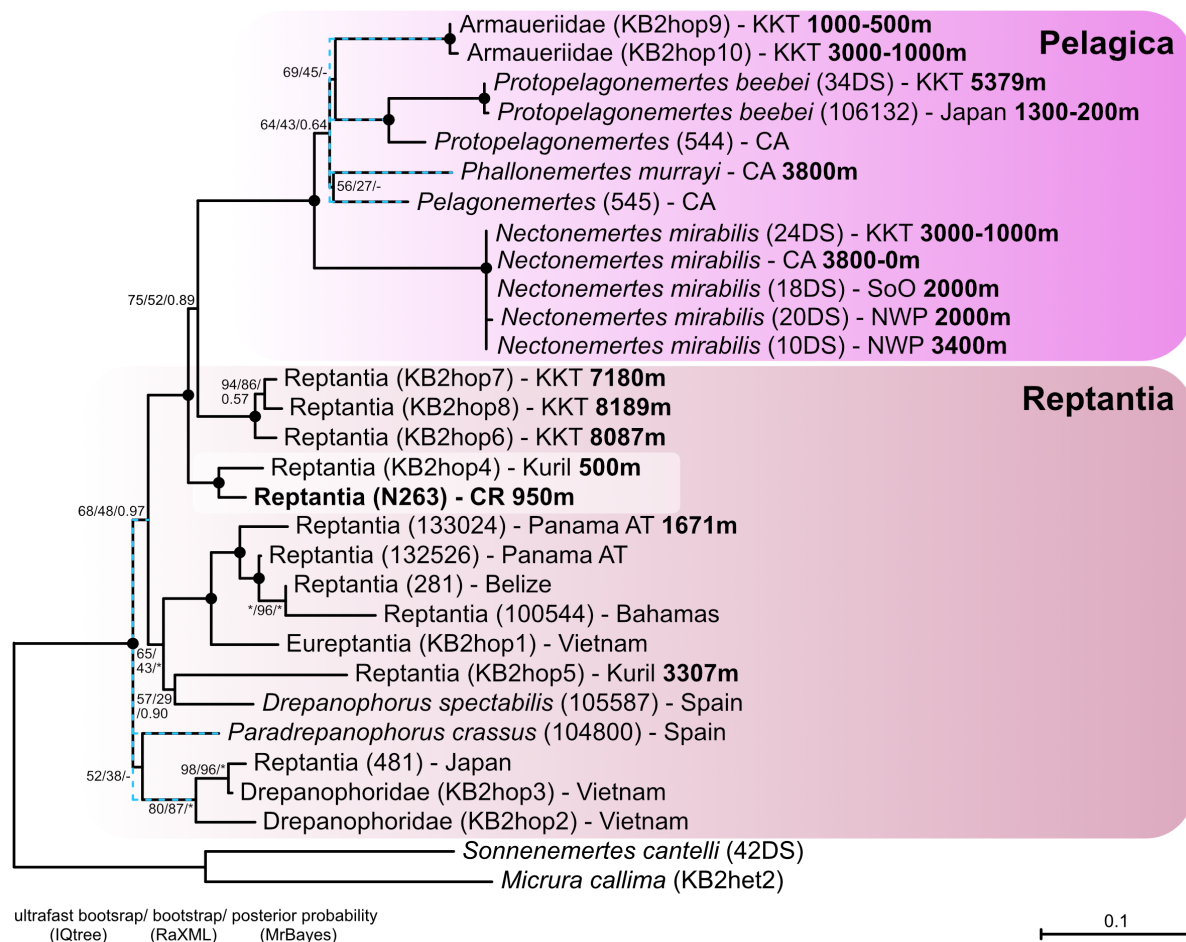

Fig. 6. Maximum likelihood (ML) tree for the polystiliferan supermatrix based on the concatenated four gene dataset (16S, COI, 18S, H3). Black circles indicate nodal support of 100/100/1.0; asterisks indicate nodal support of either 100/ or /1.0. Alternative branches found in the BI analysis are given as blue dotted lines. *Sonnenemertes cantelli* Chernyshev, Abukawa & Kajihara, 2015 and *Micrura callima* Sundberg & Gibson, 1995 were used for outgroup rooting. The reptant hoplonemertean specimen collected at the Costa Rica margin is given in bold. Specimen-ID, sampling locality, and depth (for deep-sea species) are provided.

are around 14%. Since no material for histological sectioning is left, we do not describe this species herein, also due to the convoluted taxonomy of Lineidae, that needs extensive revision and goes beyond the scope of our study. However, the sequence data is published in order to facilitate future studies on deep-sea Lineidae.

Class Hoplonemertea Hubrecht, 1879

Order **Polystilifera** Brinkmann, 1917

One polystiliferan nemertean was collected at 950 m depth from non-seep seamount habitat at Cocos Canyon (SIO-BIC N263). The specimen is 65 mm long and dorso-ventrally flattened. The body is translucent and coloration is pale pinkish (Fig. 3D–E). This reptant hoplonemertean is sister to another undescribed Reptantia Brinkmann, 1917 from the Kuril Islands (500 m depth). Together with three further unidentified hadal reptant hoplonemerteans, this clade is sister to Pelagica Brinkmann, 1917 (Fig. 6). Again, we choose not to name this species as a thorough taxonomic revision of Reptantia is warranted. The histological data set is published herein: https://doi.org/10.5281/zenodo.6375286.

Order Monostilifera Brinkmann, 1917

Suborder **Eumonostilifera** Chernyshev, 2003

Most of the collected nemertean specimens belong to eumonostiliferous hoplonemerteans. Nine species in three genera are here described as new to science, including two species from the northeastern Pacific outside Costa Rica. In addition, sequences were obtained for two more species that are not described here due to lacking histological material. Five Costa Rican and the two NE Pacific species can be attributed to the clade Oerstediina, whereas the remaining four species group with the Amphiporina clade (Fig. 7).

The first undescribed species is represented by six small (6–8 mm long), bright orange to red specimens (SIO-BIC N259) that have one pair of dorsal, V-shaped cephalic furrows (Fig. 8I). This species is sister to two unidentified eumonostiliferans from the depths of the Vema Fracture Zone (ca 5000 m). Together with *Abyssonemertes kajiharai* Chernyshev & Polyakova, 2017 they form a well-supported clade (Fig. 7). Interspecific p-distances within this clade based on the COI gene vary between 11% and 14%. The second undescribed species (SIO-BIC N109) is represented by one large specimen (Fig. 8O) that shares a clade with several species of *Paranemertes* Coe, 1901, *Tortus tokmakovae* Chernyshev, 1991, and two species of *Amphiporus* Ehrenberg, 1831 (Fig. 7). The exact position within this clade cannot be fully resolved. Cratenemertea Chernyshev, 2003 were not represented in the samples.

Infraorder Oerstediina Chernyshev & Polyakova, 2019

Genus *Chernyshevia* gen. nov.
urn:lsid:zoobank.org:act:BFBBBA9E-80F4-4F57-9A81-B85FE723A7A6

Type species

Chernyshevia escarpiaphila gen. et sp. nov.

Diagnosis

Monostiliferan nemertean lacking eyes, with one pair of ventro-lateral cephalic furrows continuing as unforked ciliated canal; cerebral organs close in front of brain; rhynchocoel extending to posterior end of body; apical organ present; cephalic glands extending to brain; lateral nerve cords with small accessory nerves; mid-dorsal blood vessel without vascular plug; intestinal cecum with anterior diverticula; sexes separate.
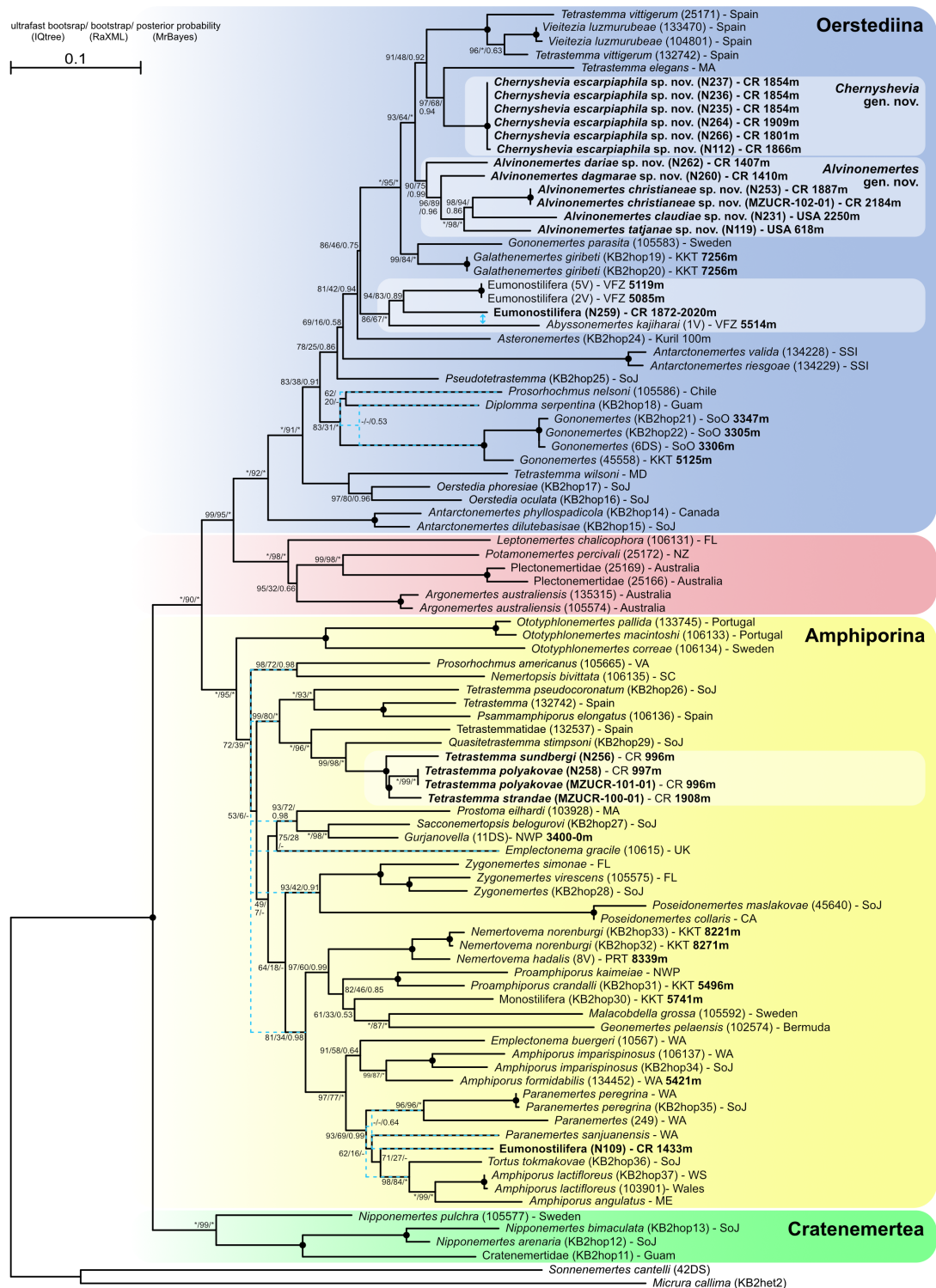
Fig. 7. Maximum likelihood (ML) tree for the monostiliferan supermatrix based on the concatenated four gene dataset (16S, COI, 18S, H3). Black circles indicate nodal support of 100/100/1.0; asterisks indicate nodal support of either 100/ or /1.0. Alternative branches found in the BI analysis are given as blue dotted lines. *Sonnenemertes cantelli* Chernyshev, Abukawa & Kajihara, 2015 and *Micrura callima* Sundberg & Gibson, 1995 were used for outgroup rooting. Specimens belonging to new species are given in bold. Specimen-ID, sampling locality, and depth (for deep-sea species) are provided.

Etymology

The genus is named after Dr Alexei Chernyshev to acknowledge his significant contributions to the study of deep-sea nemerteans.

Phylogenetic remarks

The genus is sister to the represented specimen of *Tetrastemma elegans* (Girard, 1852) (Fig. 7) that is in need of revision. This clade is sister to the monotypic genus *Vieitezia* Junoy, Andrade & Giribet, 2010, including specimens of the type species *Vieitezia luzmurubeae* Junoy, Andrade & Giribet, 2010, and specimens of *Tetrastemma vittigerum* (Bürger, 1904). The genus is nested within a clade that also includes the new genus *Alvinonemertes* gen. nov., as well as the represented specimens of the genera *Gononemertes* Bergendal, 1900 and *Galathenemertes* Chernyshev & Polyakova, 2019 (Fig. 7).

Remarks

The genus *Chernyshevia* gen. nov. is erected as new genus based on its phylogenetic position and the questionable position of different species of '*Tetrastemma*' (*T. elegans* and *T. vittigerum*) that are nested within the same clade as *Chernyshevia*. Moreover, some morphological differences between specimens of the genera *Vieitezia* and *Chernyshevia*, such as the absence or presence of an accessory nerve, the dimension of the cephalic glands, and the presence of separate sexes, might also support the presence of a new genus.

Species included

Only the type species *Chernyshevia escarpiaphila* gen. et. sp. nov.

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Chernyshevia escarpiaphila gen. et sp. nov.

urn:lsid:zoobank.org:act:11DFF601-BBBA-409F-B072-5A93BA8C349F

Figs 1B–C, 8A–B, 9

</div>

Diagnosis

As for the genus; body pale white to pinkish, translucent; up to 10 mm in length.

Etymology

Named after vestimentiferan *Escarpia spicata* Jones, 1985, on which this species occurs with high abundance.

Material examined

Holotype

COSTA RICA • ♂ (fixed in formaldehyde and prepared as 12 slides with transverse sections, see cybertype); methane seep Mound Jaguar; 9.65556° N, 85.88046° W; depth 1909 m; 25 Jan. 2019; Greg Rouse and Avery Hiley leg.; collected by ROV *SuBastian*, Dive S0230 S2; found on *Escarpia spicata*; SIO-BIC N279 (ex SIO-BIC N264).

Paratypes

COSTA RICA • 1 spec. (fixed in formaldehyde and prepared as 7 slides with transverse sections, see cybertype); same collection data as for holotype; MZUCR Nemertea Collection MZUCR-104-01 (ex SIO-BIC N264) • 13 specs (8 fixed in formaldehyde, 5 fixed in ethanol); same collection data as for holotype; GenBank: ON036044, ON021861, ON182039; SIO-BIC N264 • 3 specs (ethanol); methane seep Jaco Scar; 9.119° N, 84.843° W; depth 1604–1854 m; 31 May 2017; Erik Cordes and April Stabbins leg.; collected by HOV *Alvin*, Dive 4916; GenBank: ON021862, ON182038; SIO-BIC N237 • 2 specs (ethanol); same collection data as for preceding; GenBank: ON036045, ON182037; SIO-BIC N236 • 6 specs (ethanol); same collection data as for preceding; GenBank; ON186253, ON036046, ON021863,
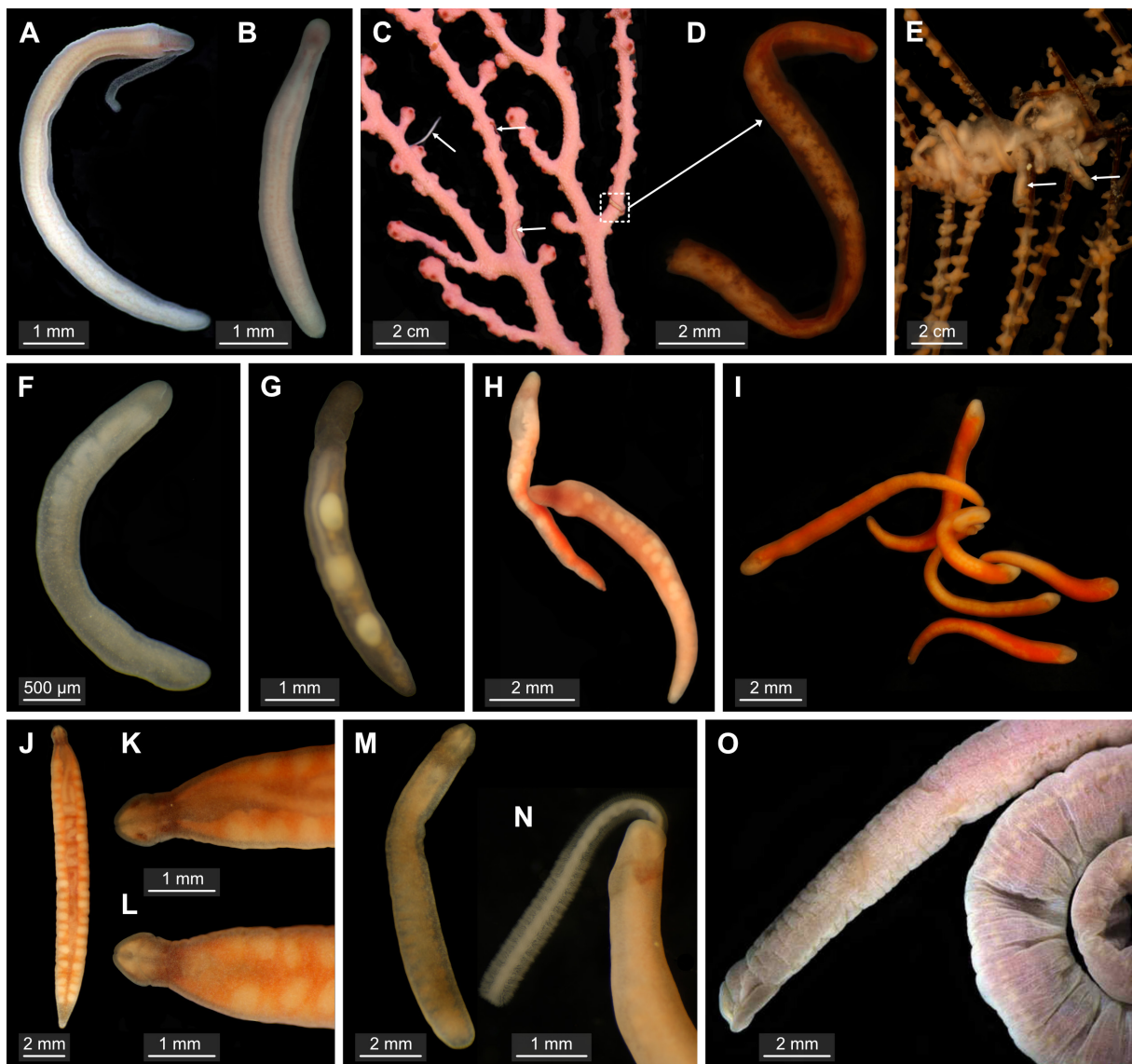
Fig. 8. Live images of eumonostiliferous hoplonemerteans collected along the Costa Rica margin and the NE Pacific. **A**. *Chernyshevia escarpiaphila* sp. nov., paratype (SIO-BIC N112). **B**. *Chernyshevia escarpiaphila* sp. nov., paratype (SIO-BIC N266). **C**. Octocoral *Paragorgia* sp. (SIO-BIC Co3054) with several specimens of *Alvinonemertes dariae* sp. nov., holotype (arrows; ex SIO-BIC N262). **D**. *Alvinonemertes dariae* sp. nov., holotype (ex SIO-BIC N262). **E**. Antipatharian coral (SIO-BIC Co3031) with specimens of *Alvinonemertes dagmarae* sp. nov., holotype (SIO-BIC N260). **F**. *Alvinonemertes christianeae* sp. nov., holotype (SIO-BIC N253). **G**. *Alvinonemertes claudiae* sp. nov., holotype (ex SIO-BIC N231). **H**. *Alvinonemertes tatjanae* sp. nov., holotype (SIO-BIC N119). **I**. Eumonostilifera (SIO-BIC N259). **J–L**. *Tetrastemma sundbergi* sp. nov., holotype (SIO-BIC N256) whole animal (J), dorsal view of head (K), and ventral view of head (L). **M**. *Tetrastemma polyakovae* sp. nov. (MZUCR Nemertea Collection MZUCR-101-01). **N**. *Tetrastemma polyakovae* sp. nov., holotype (SIO-BIC N258), with everted proboscis. **O**. Eumonostilifera (SIO-BIC N109).

ON182036; SIO-BIC N235 • 6 specs (ethanol); methane seep Jaco Scar; 9.118° N, 84.839° W; depth 1752–1802 m; 12 Jan. 2010; Elena Perez and Geoff Cook leg.; collected by HOV *Alvin*, Dive 4591; SIO-BIC N266 • 2 specs (ethanol); methane seep Jaco Scar; 9.117° N, 84.843° W; depth 974–1866 m; 3 Mar. 2009; Elena Perez and Jake Bailey leg.; collected by HOV *Alvin*, Dive 4509; GenBank: ON186250, ON021859, ON182035; SIO-BIC N112.

Cybertypes
Images of serial sections of holotype SIO-BIC N279 uploaded as N264a:
https://doi.org/10.5281/zenodo.5346245

Images of serial sections of paratype MZUCR Nemertea Collection MZUCR-104-01 uploaded as N264b: https://doi.org/10.5281/zenodo.5346245

Description
Specimens 6–10 mm long and 0.5 mm in diameter (Fig. 8A–B). Body cylindrical. One pair of ventro-lateral cephalic furrows. Head rounded, not demarcated from rest of the body. Body coloration pale whitish to pinkish, body translucent (Fig. 8A). Cerebral ganglia and internal organs visible through body wall (Fig. 8B).
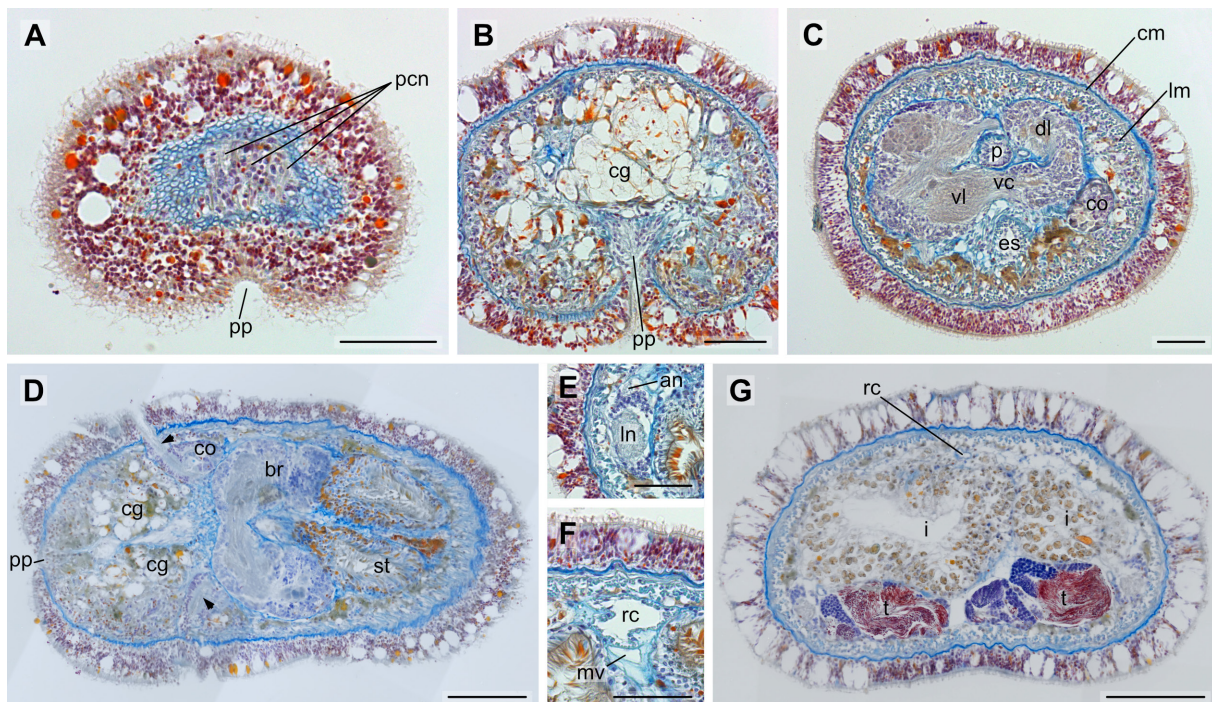

Fig. 9. *Chernyshevia escarpiaphila* gen. et sp. nov. **A–C.** Paratype (MZUCR Nemertea Collection MZUCR-104-01), transverse histological sections. **D–G.** Holotype (SIO-BIC N279), histological sections. **A.** Anterior tip of head with proboscis pore. **B.** Region anterior to cerebral ganglia with well-visible cephalic glands. **C.** Brain region depicting ventral commissure. **D.** Longitudinal section through brain region showing the cerebral canal (arrowheads). **E.** Lateral nerve cord with accessory nerve in the stomach region. **F.** Rhynchocoel in stomach region with well-visible mid-dorsal vessel. **G.** Intestinal region with testes. Abbreviations: an = accessory nerve; br = brain; cg = cephalic gland; cm = circular muscle layer; co = cerebral organ; dl = dorsal brain lobe; es = esophagus; i = intestine; lm = longitudinal muscle layer; ln = lateral nerve cord; mv = mid-dorsal vessel; p = proboscis; pcn = precerebral nerves; pp = proboscis pore; rc = rhynchocoel; st = stomach; t = testis; vc = ventral commissure; vl = ventral brain lobe. Scale bars = 100 μm.

Epidermis in foregut region 50 µm thick. Body wall composed of two muscle layers (outer circular, inner longitudinal; Fig. 9C). Combined proboscis and esophagus opening subterminal, ventral (Fig. 9B, D); stomach with pouches, surrounded by thin layer of longitudinal musculature; stomach (Fig. 9D) longer than pylorus; intestinal cecum with unpaired anterior ventral and paired anterior lateral pouches located alongside stomach; intestine with lateral pouches. Precerebral septum closed. Rhynchocoel wall with two very thin muscle layers (outer circular, inner longitudinal), rhynchocoel (Fig. 9G) almost extending to posterior end of body. Proboscis with outer circular, middle longitudinal, and inner circular muscle layers. Number of proboscis nerves indiscernible and proboscis armature not visible. Proboscis pore subterminal, ventral (Fig. 9A–B). Nervous system with outer neurilemma only, without neurochord cells; short dorsal commissure, broad ventral commissure (Fig. 9C). Precerebral nerves forming two dorso-lateral groups. Lateral nerve cords with small accessory nerve, no myofibrillae visible (Fig. 9E). Cerebral ganglia and lateral nerve cords located in longitudinal muscle layer (Fig. 9C–D). Eyes absent. Cerebral organs half the size of brain lobes, close in front of brain, surrounded by cephalic glands (Fig. 9C). Paired cerebral canal simple, unforked, opens ventro-laterally into cephalic furrows (Fig. 9D). Apical organ as depression at anterior tip of head, unpaired, cephalic gland extending to brain, distributed between muscle fibres of head (Fig. 9B, D). Circulatory system consisting of cephalic loop and three postcerebral vessels, mid-dorsal vessel not forming vascular plug (Fig. 9F). Testes sac like, located ventrally, close to lateral nerve cord (Fig. 9G).

Ecology

This species is the most abundant nemertean species collected along the Costa Rica margin. It occurs in close connection to *Escarpia spicata* with numerous specimens present on the tubes of this vestimentiferan (Fig. 1B–C). The whole epifauna found on the tubes of *Escarpia spicata* was extracted with a suction sampler.

Remarks

Chernyshevia escarpiaphila sp. nov. is attributed to the new genus *Chernyshevia* gen. nov. based on the executed phylogenetic analysis. In the phylogenetic analysis, it is sister to *Tetrastemma elegans*. Both species belong to a well-supported clade containing *Vieitezia luzmurubeae*, the type species of the genus, and *Tetrastemma vittigerum* (Fig. 7). A clade containing the five newly described species of the genus *Alvinonemertes* gen. nov. is sister to this clade. Intraspecific p-distances of the COI gene fragment vary between 2% and 4%.

Genus ***Alvinonemertes*** gen. nov.
urn:lsid:zoobank.org:act:CFDEAFD6-7280-41BB-92E2-E6B4C66E28F1

Type species

Alvinonemertes dariae gen. et sp. nov.

Diagnosis

Small monostiliferous hoplonemerteans less than 20 mm long, usually lacking distinct coloration. Eyes absent, one pair of cephalic furrows. Body wall consisting of outer circular and inner longitudinal muscle layers, proboscis with three muscle layers (outer circular, middle longitudinal, and inner circular). Proboscis with two accessory stylet pouches bearing two to five accessory stylets. Cerebral ganglia and lateral nerves in body wall longitudinal musculature. Lateral nerve cords with accessory nerves. Mid-dorsal blood vessel present.

Etymology

Named after the HOV *Alvin* that was used to collect the type species and three of the other species of the here described nemerteans.

Phylogenetic remarks

The genus is sister to the clade comprising specimens of the monotypic genera *Vieitezia* and *Chernyshevia* gen. nov., as well as specimens of *Tetrastemma vittigerum* and *T. elegans*. The genus is nested within a clade that also includes the represented specimens of the genera *Gononemertes* and *Galathenemertes* (Fig. 7).

Remarks

The genus *Alvinonemertes* gen. nov. is erected as new genus based on its phylogenetic position as sister to the aforementioned clade comprising *Chernyshevia* gen. nov. and *Vieitezia*. Interspecific pairwise p-distances of COI vary between 10% and 14% within the genus.

Species included

Alvinonemertes dariae gen. et sp. nov., *Alvinonemertes dagmarae* gen. et sp. nov., *Alvinonemertes christianeae* gen. et sp. nov., *Alvinonemertes claudiae* gen. et sp. nov., *Alvinonemertes tatjanae* gen. et sp. nov.

Alvinonemertes dariae gen. et sp. nov.
urn:lsid:zoobank.org:act:D94F4501-3FB4-4F11-8D9E-EB75E20EEB34
Figs 8C–D, 10A–G

Diagnosis

Alvinonemertes dariae gen. et sp. nov. is identified as member of the genus *Alvinonemertes* gen. nov. based on internal morphology and molecular data. It differs from all other species in the genus by its body coloration and pigmentation. It can be differentiated from *A. claudiae* gen. et sp. nov. (the only other species with internal data) by the length of the rhynchocoel (⅓ of body length vs whole body), the number of accessory stylets per pouch (2 vs 4-5) and the position of the cerebral organs (alongside brain vs in front of brain).

Etymology

Named for Dr Daria Krämer, acknowledging her contributions to nemertean taxonomy.

Material examined

Holotype
COSTA RICA • ♂ (fixed in formaldehyde and prepared as 23 slides with transverse sections, see cybertype); methane seep Parrita Seep; 9.03268° N, 84.62127° W; depth 1407 m; 5 Nov. 2018; Jorge Cortés and Drew Bewley leg.; collected by HOV *Alvin*, Dive 4990; associated with an octocoral identified as *Paragorgia* Milne Edwards, 1857 (SIO-BIC Co3054); SIO-BIC N281 (ex SIO-BIC N262).

Paratypes
COSTA RICA • 3 specs (fixed in ethanol); same collection data as for holotype; GenBank: ON186252, ON036048, ON021865; SIO-BIC N262 • 1 spec. (fixed in ethanol); same collection data as for holotype; MZUCR Nemertea Collection MZUCR-105-01 (ex SIO-BIC N262).

Cybertype
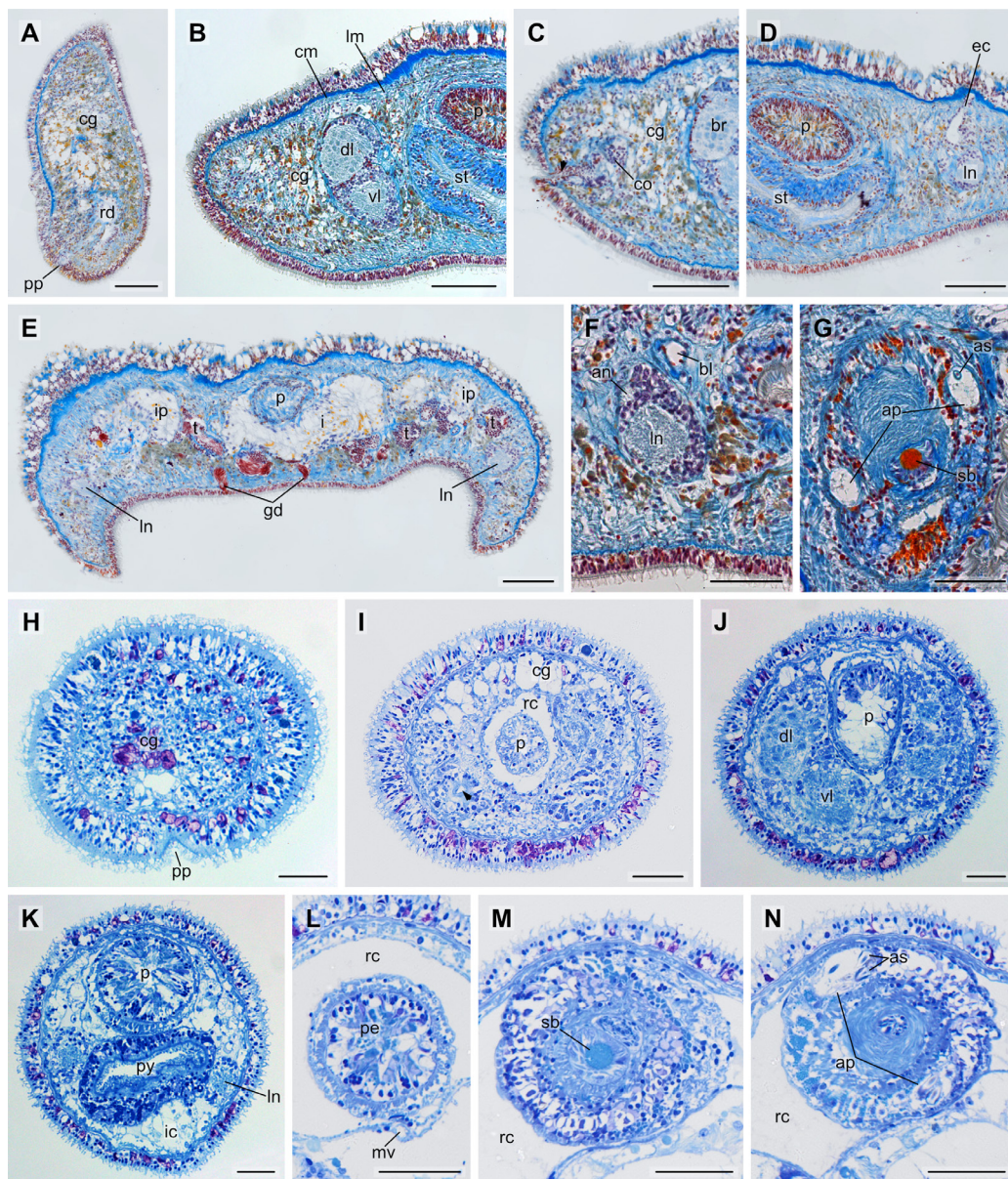Images of serial sections of holotype: https://doi.org/10.5281/zenodo.5346500.

Fig. 10. A–G. *Alvinonemertes dariae* gen. et sp. nov., holotype (SIO-BIC N281), transverse histological sections. **H–N.** *Alvinonemertes claudiae* gen. et sp. nov., holotype (SIO-BIC N283), transverse histological sections. **A.** Anterior tip of head with proboscis pore. **B.** Brain region. **C.** Anterior brain region with cerebral canal (arrowhead). **D.** Stomach region with excretory canal. **E.** Posterior body region with testes. **F.** Lateral nerve cord with accessory nerve in stomach region. **G.** Proboscis armature, accessory stylet pouches with accessory stylets. **H.** Anterior tip of head with proboscis pore. **I.** Region anterior to cerebral ganglia with well-visible cerebral canal (arrowhead). **J.** Anterior brain region. **K.** Pyloric region. **L.** Proboscis and mid-dorsal vessel in stomach region. **M.** Proboscis armature, circular basis of central stylet. **N.** Proboscis armature, accessory stylet pouches with accessory stylets. Abbreviations: an = accessory nerve; ap = accessory stylet pouch; as = accessory stylet; bl = blood vessel; br = brain; cg = cephalic glands; cm = circular muscle layer; co = cerebral organ; dl = dorsal brain lobe; ec = excretory canal; gd = gonoduct; i = intestine; ic = intestinal cecum; ip = intestinal pouches; lm = longitudinal muscle layer; ln = lateral nerve cord; mv = mid-dorsal vessel; p = proboscis; pe = proboscis epithelium; pp = proboscis pore; py = pylorus; rc = rhynchocoel; rd = rhynchodaeum; sb = stylet basis; st = stomach; t = testis; vl = ventral brain lobe. Scale bars: A–G = 100 µm; H–N = 30 µm.

Description

Specimens 15 mm long and 0.7 mm in diameter. Body flattened. One pair of cephalic furrows. Head not demarcated from rest of body, rounded. Body coloration orange with several red pigment spots distributed over body surface. Internal organs well visible through body wall (Fig. 8D).

Epidermis in foregut region 30 μm thick. Body wall composed of two muscle layers (outer circular, inner longitudinal; Fig. 10B). Esophagus opening subterminally; stomach without pouches (Fig. 10B, D); pylorus shorter than stomach; intestinal cecum with unpaired ventral process under stomach; intestine with several lateral pouches (Fig. 10E). Excretory system confined to foregut region, canals outside musculature (Fig. 10D). Precerebral septum closed. Rhynchocoel wall with two very thin muscle layers (outer circular, inner longitudinal), rhynchocoel approximately ⅓ of body length. Proboscis with outer circular, middle longitudinal, and inner circular muscle layers. Proboscis with central and accessory stylets. Stylet basis circular in cross section, two accessory stylet pouches with two stylets per pouch (Fig. 10G). Proboscis nerves not visible. Proboscis pore subterminal, ventral (Fig. 10A). Nervous system with thin outer neurilemma, without neurochord cells (Fig. 10B–C); dorsal and ventral commissures approximately same size. Lateral nerve cords with accessory nerve (Fig. 10F). Cerebral ganglia and lateral nerves in longitudinal muscle layer (Fig. 10B). Eyes absent. Cerebral organs less than half the size of brain lobes, alongside brain (Fig. 10C). Paired cerebral canal simple, unforked, opens laterally into cephalic furrows (Fig. 10C). Apical organ present, unpaired; cephalic glands extending to brain (Fig. 10A–C). Blood system consisting of cephalic loop, mid-dorsal vessel not forming vascular plug. Testes sac like, alternating with intestinal diverticula, ventral (Fig. 10E). Females unknown.

Ecology

The species seems to occur in close association with octocoral *Paragorgia* sp. as several nemertean specimens were sampled from one colony (Fig. 8C).

Remarks

Alvinonemertes dariae gen. et sp. nov. is attributed to the genus *Alvinonemertes* gen. nov. based on the executed phylogenetic analyses. In the concatenated analysis, this species is sister to the other four newly described species attributed to the genus *Alvinonemertes* (Fig. 7).

Alvinonemertes dagmarae gen. et sp. nov.
urn:lsid:zoobank.org:act:09E39C02-B087-4A95-A753-5D3C2F930632
Fig. 8E

Diagnosis

Alvinonemertes dagmarae gen. et sp. nov. can be attributed to the genus *Alvinonemertes* gen. nov. based on the strong support for a sister group relationship between the five species of this genus.

Etymology

For Dagmar Wenzel, lab technician at the Institute of Evolutionary Biology, University of Bonn. Acknowledging her support in the molecular lab, which included DNA extractions and PCRs for some of the specimens used in this study.

Material examined

Holotype
COSTA RICA • spec. (ethanol); non-seep seamount Quepos Plateau Hills; 8.5323° N, 84.79072° W; depth 1410 m; 27 Oct. 2018; Charlotte Seid and Juan José Alvarado leg.; collected by HOV *Alvin*, Dive

4981; associated with a colony of *Antipatharia* Milne Edwards, 1857 (SIO-BIC Co3031); GenBank: ON186254, ON036049, ON021866, ON182034; SIO-BIC N260.

Description

Specimen 5 cm long and 1 mm in diameter. One pair of cephalic furrows. Head rounded, not demarcated from rest of body. Body coloration white to yellowish, translucent. Internal organs visible through body wall (Fig. 8E).

Ecology

This species seems to be closely associated with antipatharian corals as several specimens were collected wrapped around one colony (Fig. 8E).

Remarks

The new species is attributed to the genus *Alvinonemertes* gen. nov. based on the executed phylogenetic analyses. In the concatenated analysis, *A. dagmarae* gen. et sp. nov. is sister to a clade comprising the three species *A. christianeae* gen. et sp. nov., *A. claudiae* gen. et sp. nov., and *A. tatjanae* gen. et sp. nov. and belongs to the well supported clade representing the genus (Fig. 7).

Alvinonemertes christianeae gen. et sp. nov.
urn:lsid:zoobank.org:act:B7C6217E-4889-4C9B-AEB8-B306A24FD93B
Fig. 8F

Diagnosis

Alvinonemertes christianeae gen. et sp. nov. can be attributed to the genus *Alvinonemertes* gen. nov. based on the strong support for a sister group relationship between the five species of this genus.

Etymology

For Christiane Wallnisch, lab technician at the Institute of Evolutionary Biology, University of Bonn. Acknowledging her support in the histology lab over many years. Christiane is a masterful histologist and has not only trained the first and the last author in histological sample preparation but has also sectioned most of the nemerteans included in this study.

Material examined

Holotype
COSTA RICA • spec. (ethanol; entirely used up in DNA extraction; only image and DNA available); methane seep Jaco Scar; 9.11507° N, 84.83978° W; depth 1887 m; 22 Oct. 2018; Shana Goffredi and Drew Bewley leg.; collected by HOV *Alvin*, Dive 4976; associated with experimentally deployed wood; SIO-BIC N253.

Paratype
COSTA RICA • 1 spec. (ethanol; entirely used up in DNA extraction; only image and DNA available); non-seep seamount Quepos Plateau; 8.58548° N, 84.54836° W; depth 2184 m; 26 Oct. 2018; Lisa Levin and Todd Litke leg.; collected by HOV *Alvin*, Dive 4980; associated with a naturally occurring wood fall; SIO-BIC N261.

Description

Specimens 4–5 mm long and 0.5 mm in diameter. Body rounded. One pair of cephalic furrows. Head not demarcated from rest of body, rounded. Body coloration translucent white. Internal organs well visible through body wall (Fig. 8F).

Ecology

This species was collected on naturally occurring and experimentally deployed wood (Pereira *et al*. 2022), including the same deployment associated with a paratype of the scaleworm *Peinaleopolynoe elvisi* Hatch & Rouse in Hatch, Liew, Hourdez & Rouse, 2020 (MZUCR 1000-01 ex SIO-BIC A9752).

Remarks

The new species is attributed to the genus *Alvinonemertes* gen. nov. based on the executed phylogenetic analysis. In the concatenated analysis, *A. christianeae* gen. et sp. nov. is sister to the newly described species *A. claudiae* gen. et sp. nov. from the North western Pacific. Together, both species are sister to *A. tatjanae* gen. et sp. nov. from the North western Pacific. This well-supported clade is firmly nested within the new genus *Alvinonemertes* (Fig. 7).

Alvinonemertes claudiae gen. et sp. nov.
urn:lsid:zoobank.org:act:1BA9AE85-AB72-4736-9132-79796011C259
Figs 8G, 10H–N

Diagnosis

Alvinonemertes claudiae gen. et sp. nov. can be attributed to the genus *Alvinonemertes* gen. nov. based on molecular data. It differs from all other species in the genus by its very translucent body that is pale yellowish. It can be differentiated from *A. dariae* gen. et sp. nov. by the characteristics given in the diagnosis of *A. dariae*.

Etymology

For Claudia Müller, lab technician at the Institute of Evolutionary Biology, University of Bonn. Acknowledging her support in the immunohistology lab over many years and also in recognition of her devotion to the care of our live annelid and nemertean worm cultures.

Material examined

Holotype
NORTHEASTERN PACIFIC OCEAN • ♂ (fixed in formaldehyde and prepared as 15 slides with transverse sections, see cybertype); hydrothermal vents at South Cleft, Juan de Fuca Ridge; 44.659° N, 130.364° W; depth ca 2250 m; 31 Jul. 2016; Greg Rouse leg.; collected by ROV *Doc Ricketts*, Dive 875; SIO-BIC N283 (ex SIO-BIC N231).

Paratypes
NORTHEASTERN PACIFIC OCEAN • 1 spec. (fixed in formaldehyde); same collection data as for holotype; SIO-BIC N284 (ex SIO-BIC N231) • 3 specs (ethanol); same collection data as for holotype; GenBank: ON036050, ON021868; SIO-BIC N231.

Cybertype
Images of serial sections of holotype: https://doi.org/10.5281/zenodo.5346520.

Description

Specimens 6 mm long and 0.7 mm in diameter. Body cylindrical. One pair of cephalic furrows, forming the shape of an anteriorly open V. Head not demarcated from rest of body, rounded. Body translucent. Internal organs, such as gut and gonads, well visible through body wall (Fig. 8G).

Epidermis in foregut region 10 μm thick. Body wall composed of two muscle layers (outer circular, inner longitudinal). Esophagus opening subterminal, ventral; stomach without pouches; pylorus longer

than stomach; intestinal cecum with ventral unpaired anterior pouch under stomach and one pair of lateral pouches (Fig. 10K); intestine with lateral diverticula. Precerebral septum closed. Rhynchocoel wall with very thin outer circular and inner longitudinal muscle layers, rhynchocoel almost extending to posterior end of body. Proboscis with outer circular, middle longitudinal, and inner circular muscle layers. Proboscis with 10 proboscis nerves; central stylet and two accessory stylet pouches present, four to five accessory stylets per pouch (Fig. 10M–N). Stylet basis circular in cross section. Proboscis pore subterminal, ventral (Fig. 10H). Nervous system with thin outer neurilemma, without neurochord cells (Fig. 10J); dorsal commissure short, ventral commissure broad. Lateral nerve cords without accessory nerve (Fig. 10K). Cerebral ganglia and lateral nerves in longitudinal muscle layer (Fig. 10J–K). Eyes absent. Cerebral organs less than half the size of brain lobes, close in front of brain. Paired cerebral canal simple, unforked, opens ventro-laterally into cephalic furrows (Fig. 10I). Apical organ present, unpaired; cephalic gland extending to brain (Fig. 10H, J). Circulatory system consisting of cephalic loop, mid-dorsal vessel not forming vascular plug ((Fig. 10L). Testes sac like, alternating with intestinal diverticula, situated ventrally.

Ecology

Associated with hydrothermal vents, alongside other vent fauna such as the annelids *Branchinotogluma tunnicliffeae* (Pettibone, 1988) (e.g., SIO-BIC A7716), *Paralvinella sulfincola* Desbruyères & Laubier, 1993 (e.g., SIO-BIC A7724) and *Ridgeia piscesae* Jones, 1985 (e.g., SIO-BIC A7719).

Remarks

The new species is attributed to the genus *Alvinonemertes* gen. nov. based on the executed phylogenetic analyses. In the concatenated analysis, *A. claudiae* gen. et sp. nov. is sister to *A. christianeae* gen. et sp. nov. (Fig. 7).

Alvinonemertes tatjanae gen. et sp. nov.
urn:lsid:zoobank.org:act:B3C2ABC6-34E6-497C-9A66-023A5751DC78
Fig. 8H

Diagnosis

Alvinonemertes tatjanae gen. et sp. nov. can be attributed to the genus *Alvinonemertes* gen. nov. based on the strong support for a sister group relationship between the five species of this genus.

Etymology

For Tatjana Bartz, lab technician at the Institute of Evolutionary Biology, University of Bonn. Acknowledging her support in the electron microscopy lab over many years. Tatjana has trained the last author in specimen preparation for transmission electron microscopy, for which he will ever be grateful.

Material examined

Holotype
USA • spec. (ethanol); Hydrate Ridge, Oregon margin; 44.670° N, 125.098° W; depth ca 618 m; 3 Aug. 2010; Victoria Orphan and José Patterson leg.; collected by HOV *Alvin*, Dive 4631; GenBank: ON186256, ON036047, ON021864, ON182041; SIO-BIC N119.

Description

Specimens 7–9 mm long and 0.7 mm in diameter. Body rounded. One pair of cephalic furrows. Head not demarcated from rest of body, rounded. Body pale red to pinkish with several red pigment spots distributed over body surface. Gonads and gut well visible through body wall (Fig. 8H).

Remarks

Alvinonemertes tatjanae gen. et sp. nov. is attributed to the genus *Alvinonemertes* gen. nov. based on the executed phylogenetic analyses. In the concatenated analysis, it is sister to the clade comprising *A. christianeae* gen. et sp. nov. and *A. claudiae* gen. et sp. nov. (Fig. 7).

Infraorder Amphiporina Chernyshev & Polyakova, 2019
Family Tetrastemmatidae Hubrecht, 1879
Genus *Tetrastemma* Ehrenberg, 1831

Tetrastemma sundbergi sp. nov.
urn:lsid:zoobank.org:act:10E0A1CA-5CC4-40D6-879D-2861A91821C9
Figs 8J–L, 11A–G

Diagnosis

As for the genus (compare Sundberg & Gibson 1995). *Tetrastemma sundbergi* sp. nov. has a bright orange yet translucent body. The head is clearly demarcated from the rest of the body and is slenderer than the rest of the body. On dorsal surface with two red spots at lateral sides of the head. Dorsal and ventral ganglia are fused. Cerebral organ alongside brain.

Etymology

For Dr Per Sundberg in recognition of his contributions to nemertean taxonomy.

Material examined

Holotype
COSTA RICA • ♀ (fixed in formaldehyde and prepared as 33 slides with transverse sections, see cybertype); methane seep Mound 12; 8.93° N, 84.31° W; depth 996–999 m; 24 Oct. 2018; Jorge Cortés and Oliver Ashford leg.; collected by HOV *Alvin*, Dive 4978; GenBank: ON186255, ON036054, ON021855, ON182052; SIO-BIC N256.

Cybertype
Images of serial sections of holotype: https://doi.org/10.5281/zenodo.5346538.

Description

Specimens 15 mm long and 0.7 mm in diameter. Body flattened (Fig. 8J). Two pairs of cephalic furrows. Paired anterior cephalic furrows V-shaped, dorsally pointing posteriorly (Fig. 8K). Posterior furrows U-shaped, located ventrally, pointing anteriorly (Fig. 8L). Head clearly demarcated from rest of body, not wider than trunk, tapered. Body coloration orange. Two dark brown spots on the lateral sides of the head in place of eyes. Cerebral ganglia, gut and gonads well visible through body wall (Fig. 8J).

Epidermis in foregut region 30 μm thick. Body wall composed of outer circular and inner longitudinal muscle layers (Fig. 11B). Esophagus opening subterminal, ventral; stomach with lateral pouches; pylorus shorter than stomach; intestinal cecum with paired lateral, anterior pouches along brain lobes (Fig. 11D); intestine with deep lateral pouches. Excretory system confined to foregut region, canals inside musculature (Fig. 11F). Precerebral septum closed. Rhynchocoel wall with two very thin muscle layers (outer circular, inner longitudinal), rhynchocoel almost extending to posterior end of body. Proboscis with outer circular, middle longitudinal, and inner circular muscle layers (Fig. 11C). Proboscis with 10 nerves (Fig. 11C); central stylet plus 2 accessory stylet pouches with 2 stylets each; stylet basis circular in cross section. Proboscis pore subterminal, ventral (Fig. 11A). Nervous system with thin outer neurilemma only, without neurochord cells; dorsal and ventral ganglia fused (Fig. 11B); broad ventral
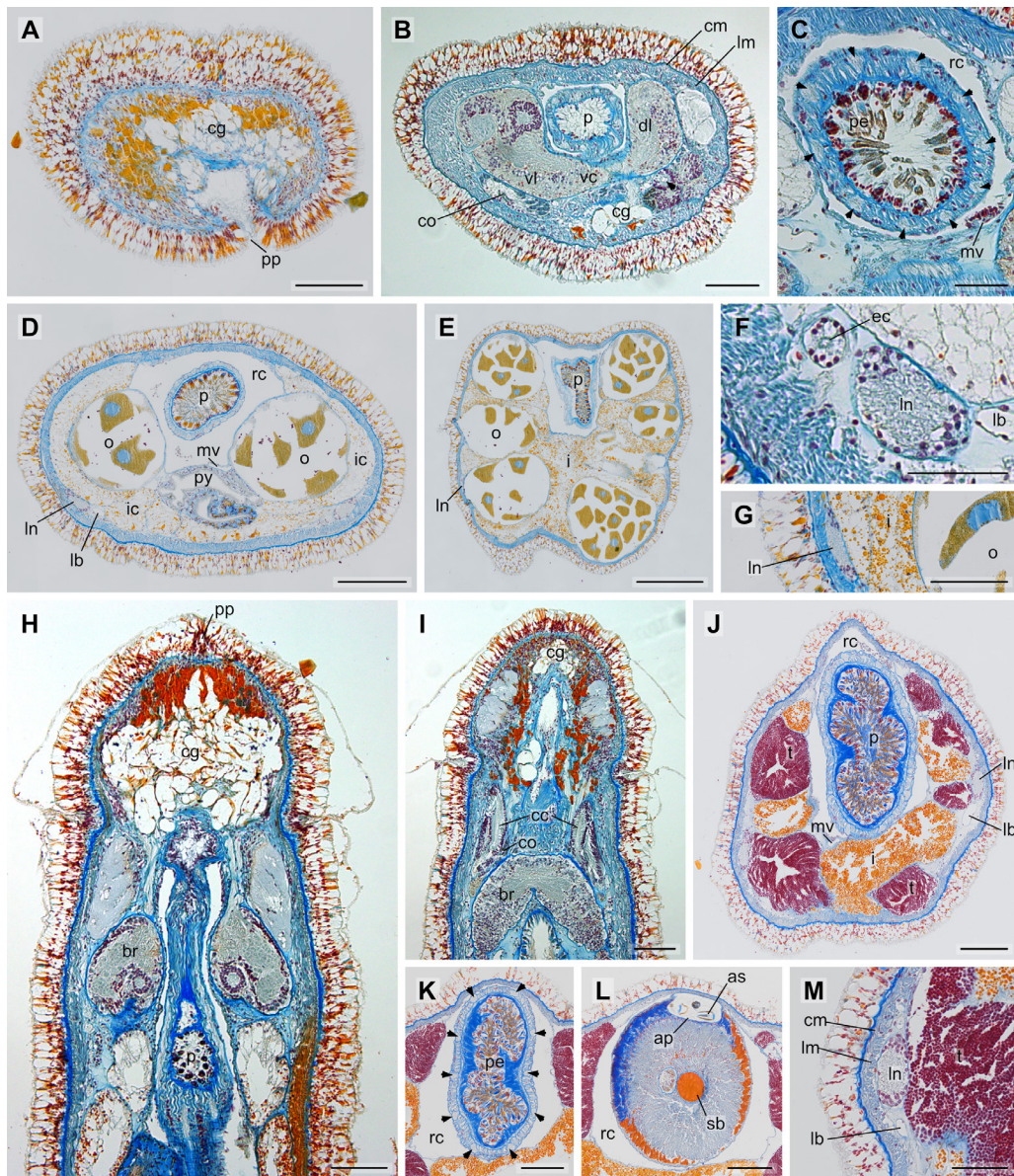
Fig. 11. A–G. *Tetrastemma sundbergi* sp. nov., holotype (SIO-BIC N256), transverse histological sections. **H–M**. *Tetrastemma strandae* sp. nov., holotype (SIO-BIC N285), horizontal histological sections (H–I) and transverse histological sections (J–M). **A**. Anterior tip of head with proboscis pore. **B**. Brain region with well-visible cerebral canal (arrowhead). **C**. Proboscis with 10 proboscis nerves (arrowheads) in stomach region. **D**. Pyloric region. **E**. Intestinal region with ovaries. **F**. Anterior part of lateral nerve cord. **G**. Posterior part of lateral nerve cord located in body wall musculature. **H**. Longitudinal section through anterior tip with proboscis pore. **I**. Longitudinal section through the ventral part of the anterior tip. **J**. Intestinal region with testes. **K**. Proboscis in stomach region with 10 proboscis nerves (arrowheads). **L**. Proboscis armature. **M**. Lateral nerve cord in intestinal region. Abbreviations: ap = accessory stylet pouch; as = accessory stylet; br = brain; cc = cerebral canal; cg = cephalic glands; cm = circular muscle layer; co = cerebral organ; dl = dorsal brain lobe; ec = excretory canal; i = intestine; ic = intestinal cecum; lb = lateral blood vessel; lm = longitudinal muscle layer; ln = lateral nerve cord; mv = mid-dorsal-vessel; o = ovary; p = proboscis; pe = proboscis epithelium; pp = proboscis pore; py = pylorus; rc = rhynchocoel; sb = stylet basis; t = testis; vc = ventral commissure; vl = ventral brain lobe. Scale: 100 µm.

commissure, narrow dorsal commissure. Precerebral nerves in two groups with 3–4 nerves each. Lateral nerve cords without accessory nerve (Fig. 11F–G). Cerebral ganglia and anterior part of lateral nerve cords internal to longitudinal muscle layer (Fig. 11B, F); posteriorly, lateral nerve cords within body wall musculature (Fig. 11G). Eyes absent. Cerebral organs less than half the size of brain lobes, alongside brain. Paired cerebral canal simple, unforked, opens ventro-laterally into cephalic furrows (Fig. 11B). Apical organ present, unpaired; cephalic gland extending to brain, scattered between muscle fibres in anterior part of head, forming distinct lobules further back (Fig. 11A–B). Blood system consisting of cephalic loop, mid dorsal vessel with vascular plug (Fig. 11C–D). Ovaries lying above each other, alternating with intestinal diverticula (Fig. 11D–E).

Remarks

Tetrastemma sundbergi sp. nov. is attributed to the genus *Tetrastemma* based on the executed phylogenetic analyses. Furthermore, this assignment is supported by morphological characteristics such as the presence of a vascular plug, small cerebral organs, and the amount of muscle layers. *Tetrastemma sundbergi* is sister to a clade formed by the two other newly described species of *Tetrastemma*. These three species are sister to three yet undescribed specimens from the Kuril Islands. Together, this clade is sister to the two former species of *Quasitetrastemma* Chernyshev, 2004, *Tetrastemma stimpsoni* Chernyshev, 1992 and *Tetrastemma nigrifrons* (Coe, 1904). This clade is firmly nested within the genus *Tetrastemma* (Fig. 7). The three species differ morphologically from the two closely related, former species of *Quasitetrastemma* by the absence of lateral accessory nerves.

Tetrastemma polyakovae sp. nov.
urn:lsid:zoobank.org:act:0A69EED8-5057-4882-A12C-AB4FC69B7B63
Fig. 8M–N

Diagnosis

Tetrastemma polyakovae sp. nov. can be attributed to the genus *Tetrastemma* based on the strong support for a sister group relationship between the three species of this genus included in the analysis.

Etymology

For Dr Neonila E. Polyakova, in recognition of her contributions to the study of deep-sea nemerteans.

Material examined

Holotype
COSTA RICA • spec. (ethanol); methane seep Mound 12; 8.93030° N, 84.31260° W; depth 997 m; 24 Oct. 2018; Jorge Cortés and Oliver Ashford leg.; collected by HOV *Alvin*, Dive 4978; GenBank: ON186259, ON036056, ON021857, ON182051; SIO-BIC N258.

Paratype
COSTA RICA • 1 spec. (ethanol; entirely used up in DNA extraction; only image and DNA available); same collection data as for holotype; 8.93° N, 84.31° W; depth 996–999 m; GenBank: ON036057, ON021858, ON182050; MZUCR Nemertea Collection MZUCR-101-01 (ex SIO-BIC N255).

Description

Specimens 15 mm long and 1 mm in diameter. Body rounded. Two pairs of cephalic furrows. Head not demarcated from rest of body, rounded. Body coloration pale orange to brownish. Internal organs well visible through body wall (Fig. 8M–N).

Remarks

The new species is attributed to the genus *Tetrastemma* based on the executed phylogenetic analyses. This species is sister to *Tetrastemma strandae* sp. nov. (Fig. 7). Intraspecific pairwise p-distances are 2%, whereas interspecific distances between *T. polyakovae* sp. nov. and the two newly described species vary between 7% and 8%. Distance between this species and *Tetrastemma stimpsoni* is 12.5%.

Tetrastemma strandae sp. nov.

urn:lsid:zoobank.org:act:7D6FE621-D556-4FE5-8771-C5DB96C39CD1

Fig. 11H–M

Diagnosis

As for the genus. *Tetrastemma strandae* sp. nov. can be identified based on the executed phylogenetic analyses.

Etymology

For Dr Malin Strand, in recognition of her contributions to nemertean taxonomy and systematics.

Material examined

Holotype
COSTA RICA • ♂ (fixed in formaldehyde and prepared as 26 slides with transverse sections, see cybertype); methane seep Jaco Scar; 9.12° N, 84.84° W; depth 1798–1908 m; 28 May 2017; Greg Rouse and Jorge Cortés leg.; collected by HOV *Alvin*, Dive 4913; SIO-BIC N285 (ex SIO-BIC N234).

Paratype
COSTA RICA • 1 spec. (ethanol); same collection data as for holotype; GenBank: ON186258, ON036055, ON021856, ON182049; MZUCR Nemertea Collection MZUCR-100-01 (ex SIO-BIC N234).

Cybertype
Images of serial sections of holotype: https://doi.org/10.5281/zenodo.6372372.

Description

Epidermis in foregut region 30 μm thick. Body wall composed of two muscle layers (outer circular, inner longitudinal). Esophagus opening subterminal into opening of proboscis pore, ventral; stomach with lateral pouches, pylorus longer than stomach; intestinal cecum with anterior, lateral pouches; intestine with lateral pouches. Excretory system confined to foregut region, canals outside musculature. Precerebral septum closed. Rhynchocoel wall with two very thin muscle layers (outer circular, inner longitudinal), rhynchocoel almost extending to posterior end of body. Proboscis with outer circular, middle longitudinal, and inner circular muscle layers (Fig. 11J). Proboscis with 10 nerves (Fig. 11K); central and accessory stylets present; two accessory stylet pouches with two stylets per pouch; stylet basis circular in cross section (Fig. 11L). Proboscis pore subterminal, ventral (Fig. 11H). Nervous system with thin outer neurilemma, without neurochord cells (Fig. 11H–I). Two groups of precerebral nerves with three to four nerves each. Lateral nerve cords without accessory nerve (Fig. 11M). Cerebral ganglia and anterior part of lateral nerves internal to longitudinal muscle layer (Fig. 11H–I, M); posteriorly, lateral nerve cords within body wall musculature. Eyes absent. Cerebral organs less than half the size of brain lobes, close in front of brain. Paired cerebral canal simple, unforked, opens ventro-laterally (Fig. 11I). Apical organ present, unpaired; cephalic gland extending to brain, scattered between muscle fibres in anterior part of head, forming distinct lobules further back (Fig. 11H–I). Circulatory system consisting of cephalic loop, mid dorsal vessel with vascular plug (Fig. 11J). Testes sac like, alternating with intestinal diverticula (Fig 11J). Females not investigated.

Remarks

The new species is attributed to the genus *Tetrastemma* based on the executed phylogenetic analysis and internal morphological characters (as for *T. sundbergi* sp. nov.). In the concatenated analysis, *Tetrastemma strandae* sp. nov. is sister to *T. polyakovae* sp. nov. (Fig. 8).

Discussion

So far, only little is known about the nemertean fauna of Costa Rica (Sibaja-Cordero 2018; Sibaja-Cordero *et al.* 2019). General biodiversity monitoring programs along the Costa Rican Pacific coast yielded only few nemertean specimens. None of these specimens were identified any further than "Nemertea sp." (e.g., Dexter 1974; León-Morales & Vargas 1998) except for *Carcinonemertes* Coe, 1902 that has been collected in the Gulf of Nicoya (Okazaki & Wehrtmann 2014). Before this study, even less was known on the deep-sea nemerteans of the Costa Rican Pacific coast. Over several deep-sea expeditions, numerous benthic nemertean specimens were sampled in the last decade in the bathyal zone of the Costa Rica margin. Here, we have named nine new species out of these specimens and others from habitats of the northeastern Pacific significantly broadening our knowledge of northern Pacific benthic deep-sea nemertean fauna. In contrast to pelagic groups, benthic nemerteans occurring beyond 1000 m depths mostly belong to families that comprise numerous shallow-water species (Chernyshev 2013; Chernyshev & Polyakova 2019). The bathyal zone of the Costa Rica margin at a depth between 950 m and 2200 m is characterized by the presence of seamounts, landslide scars and diverse seep structures, including a so-called 'hydrothermal seeps' that may give rise to several well-adapted nemertean species (Sahling *et al.* 2008; Levin *et al.* 2012). So far, there are only four deep-sea nemertean species that are known to occur in association with hydrothermal vents, three of which belong to the genus *Ovicides* Shields, 2001 and are hydrothermal vent crab symbionts (Shields & Segonzac 2007), and the free-living hydrothermal vent species, *Thermanemertes valens* Rogers, Gibson & Tunnicliffe, 1996 (Rogers *et al.* 1996). Nemerteans from methane seeps have so far not been identified to species level (e.g., Dando *et al.* 1991; Levin *et al.* 2003, 2006, 2017) and species from hydrothermal seeps were unknown. Thus, we provide the first species descriptions of nemerteans both from methane seeps and a hydrothermal seep.

Overall, our findings concerning the composition of the sampled deep-sea fauna are in accordance with that already reviewed in Chernyshev (2020). In all samples of the deep-sea expeditions to the Kuril-Kamchatka Trench, the Vema Fracture Zone, and the Costa Rica margin, eumonostiliferous hoplonemerteans and palaeonemerteans comprise the highest number of sampled individuals (Chernyshev & Polyakova 2018b, 2018a, 2019). Most collected deep-sea specimens represent species new to science that are in many cases placed into new genera (Chernyshev 2020). The vast majority of collected specimens can be attributed to tubulanid palaeonemerteans, heteronemerteans, and eumonostiliferans (Chernyshev & Polyakova 2018b). This was also true for the specimens sampled along the Costa Rica margin. Typically, deep-sea nemerteans lack a distinct color pattern being uniformly colored in white, yellowish, or lightly reddish, and are mostly small in size (Chernyshev *et al.* 2015; Chernyshev 2020).

Palaeonemertea Hubrecht, 1879

Deep-sea palaeonemerteans mainly belong to either the genus *Tubulanus* or *Carinina* Hubrecht, 1885, usually representing species new to science (Chernyshev 2020). Except for *Cephalothrix iwatai* Chernyshev, 2013, no other deep-sea archinemerteans are known (Chernyshev 2013; Chernyshev & Polyakova 2019). Representatives of the palaeonemertean genus *Carinoma* Oudemans, 1885 have never been collected (Chernyshev & Polyakova 2019). In contrast to previous deep-sea expeditions, only few tubulanid specimens were collected at the Costa Rica margin and all tubulanid specimens belong to the same species. In this study, we describe these specimens that are firmly nested within the genus. Both molecular and morphological investigations are in clear accordance with descriptions of this genus (Gibson 1982; Sundberg & Hylbom 1994; Gibson & Sundberg 1999). Most deep-sea tubulanids

sampled in the vicinity of the Kuril-Kamchatka Trench or the Vema Fracture Zone are closely related species with a depth range between 3000 and 9500 m that form a well-supported clade with specimens of *Tubulanus izuensis* Hookabe, Asai, Nakano, Kimura & Kajihara, 2020 sampled at depths of 244–436 m in the Sea of Kumano (Chernyshev & Polyakova 2019; Hookabe *et al.* 2020a). Interestingly, *Tubulanus* cf. *lutescens* is not part of this mostly abyssal and hadal clade, but is more closely related to different shallow water species. Based on the COI gene fragment alone, the new species is closely related to a Swedish shallow-water species, *Tubulanus lutescens* (Sundberg *et al.* 2009, 2016b). In order to fully clarify this relationship however, more genes need to be sequenced for *Tubulanus lutescens*. The Costa Rican deep-sea specimens differ both slightly in external and in internal morphology from the original description of *Tubulanus lutescens* (Cantell 2001). *Tubulanus* cf. *lutescens* is bright red, whereas *T. lutescens* has a yellow body coloration. Furthermore, *T.* cf. *lutescens* has less well-developed cerebral sense organs and lacks a lower dorsal nerve that is present in *Tubulanus lutescens* (Cantell 2001). Due to the low p-distance and the only slight differences in morphology, it cannot be terminally determined whether both belong to the same species or actually represent two different species. To answer this questions, a thorough investigation of the original *T. lutescens* description combined with additional molecular markers will be necessary.

Heteronemertea Bürger, 1892

In contrast to Palaeonemertea, deep-sea hetero- and hoplonemerteans are predominantly assigned to new genera (Chernyshev 2020). Deep-sea heteronemerteans either belong to the families Lineidae or Valenciniidae Hubrecht, 1879 (Chernyshev & Polyakova 2018a). The only sampled Costa Rican heteronemertean specimen clearly belongs to the family Lineidae and is part of a well-supported clade that comprises three other unidentified lineid specimens sampled between 4500 and 5200 m in the Kuril-Kamchatka Trench (Chernyshev & Polyakova 2019). Based on comparably low interspecific pairwise p-distances (14%) all four specimens are most likely members of the same genus. Within Lineidae, only the two deep-sea benthic species *Cerebratulus macroren* Hubrecht, 1887 and *Micrura bathyalis* Chernyshev, 2013 have been collected in the North Pacific (Table 1). The former species has been described by Hubrecht in 1887 based on two specimens and has not been collected since. Thus, molecular sequence data is missing for this species and the morphological description does not allow for a proper comparison to the Costa Rican specimen, especially since distinctive external characters are often lacking in deep-sea nemertean species (Chernyshev *et al.* 2015). In contrast to this, *Micrura bathyalis* that has been described in 2013 can be clearly distinguished from the Costa Rican specimen based on molecular data (Chernyshev 2013). In our phylogenetic analysis, *M. bathyalis* is firmly nested within a clade comprising the type species of the genus *Cerebratulus* Renier, 1804. As no material from the Costa Rican specimen was left for histological sectioning, naming the species and genus is difficult. The family Lineidae comprises three large genera that are, based on phylogenetic analyses, not monophyletic (*Micrura* Ehrenberg, 1828, *Lineus* Sowerby, 1806, *Cerebratulus*) and, as a result, numerous small monotypic genera have been established in the recent past (Strand & Sundberg 2005a; Andrade *et al.* 2012; Kvist *et al.* 2014, 2015; Sundberg 2015). Thus, assigning a new species to any of these genera presents some difficulties.

Hoplonemertea Hubrecht, 1879

Among benthic hoplonemerteans, monostiliferan species are found far more often than polystiliferan species. Reptant hoplonemerteans have already been collected in depths exceeding 1000 m, but no species has been described so far (Chernyshev & Polyakova 2019). Most likely deep-sea specimens represent new species in new genera, but unfortunately only very little comparable genetic data is present for this group rendering species delimitation difficult. Based on our findings and the findings of Chernyshev & Polyakova (2019), reptant hoplonemerteans cannot be regarded as monophyletic group as one hadal and one bathyal clade of reptant Polystilifera is closer related to Pelagica than to the remaining Reptantia.

According to Chernyshev & Polyakova (2019), this may suggest that pelagic polystiliferan nemerteans descended from deep-sea reptant polystiliferan ancestors. In this work, a single reptant nemertean was collected along the Cocos Ridge. This specimen is sister to a specimen sampled at 500 m depths in the Kuril-Kamchatka Trench. Within Reptantia, the genus *Siboganemertes* Stiasny-Wijnhoff, 1923 is the only genus exclusively found in the bathyal zone, although this monotypic genus is only known from one specimen found in the Indian Ocean (Stiasny-Wijnhoff 1923). Reptant hoplonemerteans comprise around 50 described species of which many were described from single specimens at the beginning of the last century. Thus, descriptions are often incomplete and no molecular data is available for comparison. Therefore, we decided to postpone the descriptions of the Costa Rican polystiliferan specimen, as well of the lineid specimen, until additional descriptive and comparative material should become available.

In contrast to Reptantia, numerous eumonostiliferous hoplonemerteans have been sampled from the deep sea, including several species described in the last 20 years (compare Chernyshev 2020). Eumonostiliferan nemerteans are the most abundant group in deep-sea samples (Chernyshev & Polyakova 2018b, 2018a, 2019). Interestingly, the eumonostiliferan nemerteans in this study mainly belong to three genera: *Chernyshevia* gen. nov., *Alvinonemertes* gen. nov., and *Tetrastemma* that seemed to be fairly abundant at the Costa Rica margin. The first two genera belong to the clade Oerstediina, whereas the latter belongs to the clade Amphiporina (Chernyshev & Polyakova 2019; Kajihara 2021). The genus *Alvinonemertes* not only comprises Costa Rican specimens, but also two species that were collected during deep-sea expeditions off Oregon and the Juan de Fuca Ridge. This indicates that this genus might be widespread in the northeastern Pacific. So far, this genus comprises five species and appears to be truly bathyal. Interestingly, two of the newly described species in this genus occur in close relationship with deep-sea corals and might be symbiotic. Among deep-sea monostiliferan nemerteans, several symbiotic species have been recovered: *Galathenemertes giribeti* Chernyshev & Polyakova, 2019 is commonly found in the tube of the anemone *Galatheanthemum* Carlgren, 1956 (Chernyshev & Polyakova 2019), the three species of *Ovicides* occur in symbiosis with different hydrothermal vent crab species (Shields & Segonzac 2007), and four unidentified species of *Gononemertes* occur in the peribranchial cavity of deep-sea *Culeolus* Herdman, 1881 ascidians (Chernyshev *et al.* 2015; Chernyshev & Polyakova 2018a; Sanamyan *et al.* 2018).

The species *Chernyshevia escarpiaphila* gen. et sp. nov. is interesting as it appeared to be the most abundant species collected at the Costa Rica margin: around 50 specimens of this species were collected during four deep-sea expeditions between 2009 and 2019. This species appears to be quite common in depths around 1800 m as it can be found in large numbers on *Escarpia spicata* (Fig. 1B–C). Besides *Cephalothrix iwatai*, this is the only bathyal species known from roughly 50 specimens (Chernyshev 2013). Along the Costa Rica margin, this species was collected at two seep localities that are ca 130 km apart (the Jaco Scar hydrothermal seep site and Mound Jaguar). *Escarpia spicata* occurs from California to northern Chile (Kobayashi & Araya 2018) and has been reported from seeps, sedimented vents, and a whale fall (Karaseva *et al.* 2016), so further sampling of *E. spicata* epifauna may reveal whether *Chernyshevia escarpiaphila* sp. nov. co-occurs with its host across this latitudinal and habitat range. Based on the phylogenetic analysis, this species is closely related to *Tetrastemma vittigerum* and *Vieitezia luzmurubeae*. These two Mediterranean species share a very similar morphology and are often confused with each other (Junoy *et al.* 2010). Like the aforementioned heteronemertean "hold-all" genera, the genus *Tetrastemma* has been regarded as paraphyletic and lacks distinct morphological synapomorphies (Chernyshev 2004; Strand & Sundberg 2005a, 2005b). A recent phylogenetic analysis revealed that the major group of *Tetrastemma* is indeed monophyletic, although several species previously assigned to this genus (e.g., *Tetrastemma vittigerum*) actually represent different genera (Chernyshev *et al.* 2021). Therefore, based on some morphological differences between *Vieitezia luzmurubeae* and *Chernyshevia escarpiaphila* gen. et sp. nov. (e.g., accessory nerve absent vs present, coloration), we decided to erect the new genus *Chernyshevia* gen. nov.

In contrast to all abyssal and hadal Amphiporina described so far, the newly described species of *Tetrastemma* do not belong to the same subclade including the genera *Nemertovema* Chernyshev & Polyakova, 2018 and *Proamphiporus* Chernyshev & Polyakova, 2019 (Chernyshev & Polyakova 2019). Instead, they are part of a subclade comprising the majority of species of *Tetrastemma* (Chernyshev *et al.* 2021). The Costa Rican specimens are closely related to the two species *Tetrastemma nigrifrons* and *T. stimpsoni* that previously belonged to the now-rejected genus *Quasitetrastemma* (Chernyshev *et al.* 2021).

Two species remain undescribed as their position on the tree cannot be fully resolved and insufficient material was available for histology. One species might belong to the genus *Abyssonemertes* Chernyshev & Polyakova, 2017 that – so far – only comprises one species collected in the Vema Fracture Zone in abyssal waters (Chernyshev & Polyakova 2018b). In order to answer the question whether *Abyssonemertes* can be found both in Atlantic and Pacific deep-sea zones like the genus *Nemertovema*, additional specimens of the Costa Rican species would be needed to compare internal morphology.

Conclusions

Overall, our data illustrate the taxonomic diversity of bathyal nemerteans as all major groups were represented. As has been shown previously, palaeonemerteans and especially eumonostiliferous hoplonemerteans are most abundant in depths below 1000 meters. In many cases, Costa Rican and Northwest Pacific species do not belong to the same subclades indicating that the distribution range of deep-sea nemertean species might be limited. We therefore suspect that in the zones below 1000 m numerous nemertean species and genera new to science remain to be discovered. Naming these species is pivotal for proper diversity estimations and communication among scientists (Godfray *et al.* 2004; Dayrat 2005) and should not be deferred due to the limitations of in depth morphological study (Sundberg *et al.* 2016a). Here, we figuratively 'cut the ribbon', and hope that our integrative, turbotaxonomic approach with histological data provided as cybertypes will be a door opener for the taxonomy of deep-sea nemerteans.

Acknowledgements

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Supplementary material

Supp. file 1. Additional information. https://doi.org/10.5852/ejt.2022.845.1959.7937

> **Table 1.** Amplified gene fragments and primers used in the present study.

> **Table 2.** Information on additional sequences taken from GenBank. Species name, specimen ID, sampling locality, sampling depth, GenBank accession numbers (for 18S rRNA, 16S rRNA, COI, and H3), and reference are provided.

> **Table 3.** Best-fitting substitution models for all included genes and the three codon positions (for COI and H3) as calculated by PartitionFinder. Models are presented for each of the four super matrices.