



New species of Ampharetidae (Annelida: Polychaeta) from the Arctic Loki Castle vent field



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ABSTRACT

Ampharetid polychaetes adapted to live in chemosynthetic environments are well known from the deep Pacific and Atlantic Oceans, but to date no such species have been reported from the Arctic Ocean. Here, we describe two new species, *Paramytha schanderi* gen. et sp. nov. and *Pavelius smileyi* sp. nov., from the Arctic Loki's Castle vent field on the Knipovich Ridge north-east of the island of Jan Mayen. The new species are both tube-builders, and are found in a sedimentary area at the NE flank of the vent field, characterized by low-temperature venting and barite chimneys. The new genus, *Paramytha*, is characterized by a prostomium without lobes or glandular ridges, smooth buccal tentacles, four pairs of cirriform branchiae arranged as 2+1+1 without median gap dorsally on segments II–IV, absence of chaetae (paleae) on segment II, and absence of modified segments. *P. smileyi* sp. nov. is placed in the previously monotypic genus *Pavelius*, primarily based on the presence of a rounded prostomium without lobes and four pairs of branchiae arranged in a single transverse row without median gap dorsally on segment III. *Pavelius smileyi* sp. nov. differs from the type species, *Pavelius uschakovi*, in the number of thoracic and abdominal chaetigers, and the absence of chaetae (paleae) on segment II. The phylogenetic position of the two new species from Loki's Castle is further explored by use of molecular data. New sequences of mitochondrial (16S rDNA and cytochrome c oxidase subunit 1, COI) and nuclear (18S rDNA) markers have been produced for both species from Loki's Castle, as well as for specimens identified as *Paramytha* sp. from Setúbal Canyon off Portugal, and for the following species: *Pavelius uschakovi*, *Grassleia* cf. *hydrothermalis*, *Sosane wireni*, *Amphicteis ninonae* and *Samythella neglecta*. Results from phylogenetic analysis, including 22 species and 12 genera of Ampharetidae, recovered *Paramytha* gen. nov. as monophyletic with maximum support, and a close relationship between the genera *Pavelius* and *Grassleia* which together form a well supported monophyletic clade.

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1. Introduction

The family Ampharetidae is the second largest family within the order Terebellida with more than 300 species and 100 genera described (Jirkov, 2011). The family has a world-wide distribution and is well represented in deep-sea environments, often as one of the more dominant families of polychaetes in soft bottom habitats (Rouse and Pleijel, 2001). Ampharetid polychaetes are also well known from chemosynthetic environments such as hydrothermal vents and cold seeps (Reuscher et al., 2009; Stiller et al., 2013; Thurber et al., 2013), as well as from organic falls (Zottoli, 1982;

Bennet et al., 1994; Queiros et al., 2017). To date, there are no records of ampharetids considered as obligate to chemosynthetic environments from the Arctic or the Antarctic. However, recent identification of fauna samples from the Arctic Loki's Castle hydrothermal vent field at 2350 m depth on the Mohn–Knipovich ridge north-east of Jan Mayen has documented a total of 14 species of polychaetes, including two ampharetids. Unlike the more shallow water hydrothermal vent sites in the Arctic (Fricke et al., 1989; Schander et al., 2010), the fauna at Loki's Castle has been shown to be endemic and highly adapted to the chemosynthetic environment (Pedersen et al., 2010; Tandberg et al., 2012). Until now, only the two dominating polychaetes, the siboglinid *Sclerolinum contortum* Smirnov, 2000 and the maldanid *Nicomache lokii* Kongsrud and Rapp, 2012 have been reported (Pedersen

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Table 1
PCR and sequencing primers.

Marker	Primer name	Sequence 5'-3'	Direction	Source
COI	LCO1490	GGTCAACAATCATAAAGATATTGG	Forward	Folmer et al. (1994)
	HCO2198	TAAACTTCAGGTGACCAAAAATCA	Reverse	–
16S	16Sar-L	CGCCTGTTATCAAAAACAT	Forward	Palumbi et al. (1991)
	16Sbr-H	CCGGTCTGAAGCTCAGATCACGT	Reverse	–
18S	18e	CTGGTTGATCCTGCCAGT	Forward	Hillis and Dixon (1991)
	18L	GAATTACCGCGCTGCTGGCACC	Reverse	Halanych et al. (1995)
	18F509	CCCCGTAATTGGAATGAGTACA	Forward	Struck et al. (2002)
	18R	GTCCCTCCGCAATTTCITTAAG	Reverse	Passamaneck et al. (2004)
	18F997	TTCGAAGACGATCAGATACCG	Forward	Struck et al. (2002)
	18R1843	GGATCCAAGCTTGATCCTTCTGCAGGTTACCTAC	Reverse	Struck et al. (2005), modified from Cohen et al. (1998)

et al., 2010; Kongsrud and Rapp, 2012), and several of the remaining species are considered new to science.

In the present study, we formally describe two new species of Ampharetidae from the Loki's Castle vent field. The most abundant one belongs to the subfamily Ampharetinae, but based on morphological characteristics the species could not be further identified to any hitherto described genera, and consequently a new genus has been proposed. The other ampharetid species found at Loki's Castle is described as a new species of *Pavelius* Kuznetsov and Levenstein, 1988, a genus originally described from cold seeps in the Sea of Okothsk, NW Pacific. The genus *Pavelius* was considered a junior synonym to *Phyllocomus* Grube, 1877 by Jirkov (2011), but is here recognized as a valid genus, now containing two species. An emended diagnosis of the genus *Pavelius* is provided.

The phylogenetic relationships of the new species from Loki Castle with other ampharetids have been further explored by use of molecular data. DNA sequences of mitochondrial (16S rDNA and cytochrome c oxidase subunit 1, COI) and nuclear (18S rDNA) markers were produced for the two new species described herein, as well as for six other species, including specimens identified as *Paramytha* sp. from Setúbal Canyon, Portugal (see Queiros et al. (2017)), and *Pavelius uschakovi* Kuznetsov and Levenstein, 1988. A concatenated phylogenetic analysis, including additional data from GenBank for 14 ampharetids, is presented.

2. Material and methods

2.1. Sample collection and morphological analysis

All samples were collected from the Loki's Castle vent field (Fig. 1) using the "Bathysaurus" XL remotely operated vehicle (ROV) provided by Argus Remote Systems during cruises with the R/V *G. O. Sars* in July 2008, August 2009, and July 2010. The fauna samples were sorted into main groups on board and fixed in either 96% ethanol or 6% buffered formaldehyde.

In the laboratory, specimens were examined by use of a Leica MZ Stereomicroscope and a Leica DM 6000 B compound microscope. A Leica M205C stereo microscope was used to make digital photos of specimens. The Leica LAS software was used to make compound images with the 'Z-stack' option. SEM micrographs were taken using a ZEISS Supra 55VP SEM on dried and gold/palladium coated material in the Laboratory for Electron Microscopy, University of Bergen. Final editing of plates and drawings were prepared in Adobe Photoshop and Illustrator version CS5. All examined specimens, including types, have been deposited in the Department of Natural History, University Museum of Bergen, Norway (ZMBN).

2.2. Taxon sampling for the molecular phylogenetic analysis

New DNA-sequences were produced for four specimens of *Pavelius smileyi* sp. nov. and three specimens of *Paramytha schanderi* gen. et sp. nov. in addition to four specimens identified as *Paramytha* sp. collected from mammal bones in the Setúbal Canyon off Portugal (see Queiros et al. (2017)), and for one specimen of each of the following species: *Pavelius uschakovi* Kuznetsov and Levenstein, 1988, *Grassleia* cf. *hydrothermalis* Solis-Weiss, 1993, *Samytheta neglecta* Wollebaek, 1912, *Amphicteis ninonae* Jirkov, 1985 and *Sosane wireni* (Hessle, 1917) (Table 2). DNA voucher specimens are located at the Department of Natural History, University Museum of Bergen, apart from the *Grassleia* specimen, which is housed at the Scripps Oceanography Benthic Invertebrate Collection (SIO-BIC). Available sequences of *Amphisamytha* spp. and other ampharetids from non-chemosynthetic habitats were downloaded from GenBank, as well as species from Alvinellidae and Terebellidae as outgroups. In total 33 terminals, representing 22 species and 12 genera of ampharetids were included in the analysis.

2.3. DNA extraction, amplification and sequencing

The mitochondrial genetic markers cytochrome c oxidase subunit I (COI) and 16S rRNA (two primers each, see Table 1), and the nuclear marker 18S rRNA (six primers in three pairs, see Table 1) were chosen for the phylogenetic analysis.

DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit, following the manufacturer's protocol (spin-column protocol). The PCR reaction contained 2.5 µL CoralLoad buffer from QIAGEN, 1 µL MgCl (QIAGEN, 25 mM), 2 µL dNTP (TaKaRa, 2.5 mM of each dNTP), 1 µL of each of the primers (10 µM solution), 0.15 mL TaKaRa HS Taq, 1 or 2 µL DNA extract and ddH₂O to make the total reaction volume 25 µL. PCR cycling profiles were as follows: COI – 5 min at 95 °C, 5 cycles with 45 s at 95 °C, 45 s at 45 °C, and 1 min at 72 °C, followed by 35 cycles of 45 s at 95 °C, 45 s at 51 °C, and 1 min at 72 °C, and finally 10 min at 72 °C. 16S – 5 min at 95 °C, 35 cycles with 30 s at 95 °C, 30 s at 50 °C, and 1.5 min at 72 °C, and finally 10 min at 72 °C. 18S – 3 min at 94 °C, 35 cycles with 1 min at 94 °C, 1.5 min at 42 °C, and 2 min at 72 °C, and finally 7 min at 72 °C.

Quality and quantity of PCR products was assessed by gel electrophoresis imaging using a FastRuler DNA Ladder (Life Technologies) and GeneSnap and GeneTools (SynGene) for image capture and band quantification. Successful PCRs were purified using Exonuclease 1 (EXO, 10 U mL⁻¹) and Shrimp Alkaline Phosphatase (SAP, 10 U mL⁻¹, USB Europe, Germany) in 10 µL reactions (0.1 mL EXO, 1 µL SAP, 0.9 µL ddH₂O, and 8 µL PCR product). Samples were incubated at 37 °C for 15 min followed by an inactivation step at 80 °C for 15 min. The purified PCR products were sequenced using BigDye v3.1 (Life Technologies) and run on

Table 2
Specimens used for phylogenetic analyses with museum voucher number, sampling location and GenBank accession numbers of sequences included in present study.

Species	Voucher	Location	COI	16S	18S
Terebellidae					
<i>Polycirrus carolinensis</i> Day, 1973	SIO-BIC A1101	Curlew Bank, Belize	JX423769	JX423681	JX423651
<i>Terebella lapidaria</i> Linnaeus, 1767	SIO-BIC A1102	Plymouth, UK	JX423771	JX423683	JX423653
Alvinellidae					
<i>Alvinella caudata</i> Desbruyères and Laubier, 1986	SIO-BIC A1092	German Flats, E.P.R.	JX423737	JX423669	JX423641
Ampharetidae, Mellininae					
<i>Mellinna albicincta</i> Mackie and Pleijel, 1995	SIO-BIC A1113	Trondheimsfjord, Norway	JX423767	JX423679	JX423649
Ampharetidae, Ampharetinae					
<i>Ampharete finmarchica</i> (Sars, 1865)	SIO-BIC A1100	Hornsunddjupet, Svalbard	JX423738	JX423670	JX423642
<i>Ampharete octocirrata</i> (Sars, 1835)	SIO-BIC A1109	Trondheimsfjord, Norway	JX423770	JX423682	JX423652
<i>Amphicteis ninona</i> Jirkov, 1985	ZMBN 95441	Norwegian Sea	KX497038	KX513562	–
<i>Amphisamytha julianae</i> Stiller et al., 2013	–	North Fiji Basin, W. Pacific	JX423763	JX423676	JX423647
<i>Amphisamytha bioculata</i> (Moore, 1906)	SIO-BIC A2524	San Nicholas Is., CA, USA	JX423685	JX423654	JX423634
<i>Amphisamytha caldareii</i> Stiller et al., 2013	SIO-BIC A2576-7	South Cleft, Juan de Fuca	JX423726	JX423664	JX423638
<i>Amphisamytha fauchaldi</i> Solís-Weiss and Hernández-Alcántara, 1994	SIO-BIC A2563	Hydrate Ridge, OR, USA	JX423699	JX423658	JX423636
<i>Amphisamytha galapagensis</i> Zottoli, 1983	–	German Flats, E.P.R.	JX423711	JX423662	JX423637
<i>Amphisamytha jacksoni</i> Stiller et al., 2013	–	South Cleft, Juan de Fuca	JX423758	JX423675	JX423646
<i>Amphisamytha lutzii</i> (Desbruyères and Laubier, 1996)	SIO-BIC A2530	Rainbow, Mid-Atlantic Ridge	JX423736	JX423667	JX423639
<i>Amphisamytha vanuatuensis</i> Reuscher et al., 2009	–	Lau Back-Arc Basin, W. Pacific	JX423741	JX423673	JX423645
<i>Anobothrus gracilis</i> Malmgren, 1866	SIO-BIC A1106	Trondheimsfjord, Norway	JX423739	JX423671	JX423643
<i>Eclysippe vanelli</i> (Fauvel, 1936)	SIO-BIC A1108	Trondheimsfjord, Norway	JX423766	JX423678	JX423648
<i>Grassleia cf. hydrothermalis</i> Solís-Weiss, 1993	SIO-BIC A6137	Pinkie's Vent, Gulf of California	KX497032	KX513552	KX513568
<i>Paramytha schanderi</i> gen. et sp. nov.	ZMBN 87801	Loki's Castle vent field	–	KX513556	KX513572
<i>Paramytha schanderi</i> gen. et sp. nov.	ZMBN 87820	Loki's Castle vent field	KX497035	KX513555	KX513571
<i>Paramytha schanderi</i> gen. et sp. nov.	ZMBN 87821	Loki's Castle vent field	–	KX513559	KX513575
<i>Paramytha</i> sp.	ZMBN 107232	Setúbal Canyon, Portugal	–	KX513547	KX513563
<i>Paramytha</i> sp.	ZMBN 207233	Setúbal Canyon, Portugal	–	KX513548	KX513564
<i>Paramytha</i> sp.	ZMBN 107234	Setúbal Canyon, Portugal	–	KX513549	KX513565
<i>Paramytha</i> sp.	ZMBN 107236	Setúbal Canyon, Portugal	–	KX513550	KX513566
<i>Pavelius smileyi</i> sp. nov.	ZMBN 87807	Loki's Castle vent field	KX497034	KX513554	KX513570
<i>Pavelius smileyi</i> sp. nov.	ZMBN 87809	Loki's Castle vent field	–	KX513557	KX513573
<i>Pavelius smileyi</i> sp. nov.	ZMBN 87810	Loki's Castle vent field	KX497036	KX513558	KX513574
<i>Pavelius smileyi</i> sp. nov.	ZMBN 87825	Loki's Castle vent field	KX497037	KX513560	KX513576
<i>Pavelius uschakovi</i> Kuznetsov and Levenstein, 1988	ZMBN 108241	Okhotsk Sea, Russia	KX497033	KX513553	KX513569
<i>Samythella neglecta</i> Wollbaek, 1912	ZMBN 99276	Norwegian Sea	–	KX513551	KX513567
<i>Sosane wahrbergi</i> (Eliason, 1955)	SIO-BIC A1118	Gullmarsfjorden, Sweden	JX423768	JX423680	JX423650
<i>Sosane wireni</i> (Hessle, 1917)	ZMBN 95447	Lysefjorden, Norway	KX497039	KX513561	KX513577

an Automatic Sequencer 3730XL at the sequencing facility of the Institute of Molecular Biology, University of Bergen.

2.4. Alignments and phylogenetic analysis

Sequences were assembled using Geneious (Biomatters Ltd.), checked for potential contamination using BLAST (Altschul et al., 1990) and have been deposited in GenBank (Table 2).

COI sequences were aligned using MUSCLE (Edgar, 2004), and 16S and 18S sequences were aligned using MAFFT (Katoh and Standley, 2013) with the Q-INS-i method. Blocks of ambiguous data were identified and excluded from the 16S and 18S alignments using Gblocks with relaxed settings (Kück et al., 2010; Talavera and Castresana, 2007; for settings see Table 3). Saturation was tested for the first, second and third codon positions of the COI gene by plotting pairwise uncorrected p-distances against total substitutions (transitions+transversions), but no saturation was detected. Pairwise genetic distances for COI and 16S were calculated in Geneious (Biomatters Ltd). For 16S distances were calculated on the alignment after trimming with Gblocks. The best-fitting model of evolution for each gene was found using JModelTest 2.1.4 (Darriba et al., 2012; Guindon and Gascuel, 2003). For all genes the GTR+I+G model was considered the best fit according to the Akaike Information Criterion, but due to statistical concerns regarding the coestimation of the

Table 3
Settings for Gblocks analysis of 16S and 18S alignment.

	16S	18S
Minimum number of sequences for conserved positions	17	17
Minimum number of sequences for flank positions	17 (28)	17 (27)
Maximum number of contigs at non-conserved positions	10 (8)	10 (8)
Minimum length of block	5 (10)	5 (10)
Allowed gap positions	all (none)	all (none)
Original number of positions	880	1969
Number of positions in Gblocks alignment	622	1842

gamma and invariant-site parameters (discussed in the RAxML manual; Stamatakis, 2008) the GTR+G model was chosen instead.

Single gene and concatenated datasets (with missing data coded as "?") were analyzed in MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with two parallel runs of 5 million generations for the single gene datasets and 10 million generations for the concatenated dataset. Convergence of runs was checked using Tracer v1.5 (Rambaut and Drummond, 2009) and the burn-in was set to 10%. Consensus phylogenies were generated in MrBayes, annotated and converted to graphics in Figtree 1.3.1 (Rambaut, 2012), and final adjustments were made in Adobe Illustrator CS6.

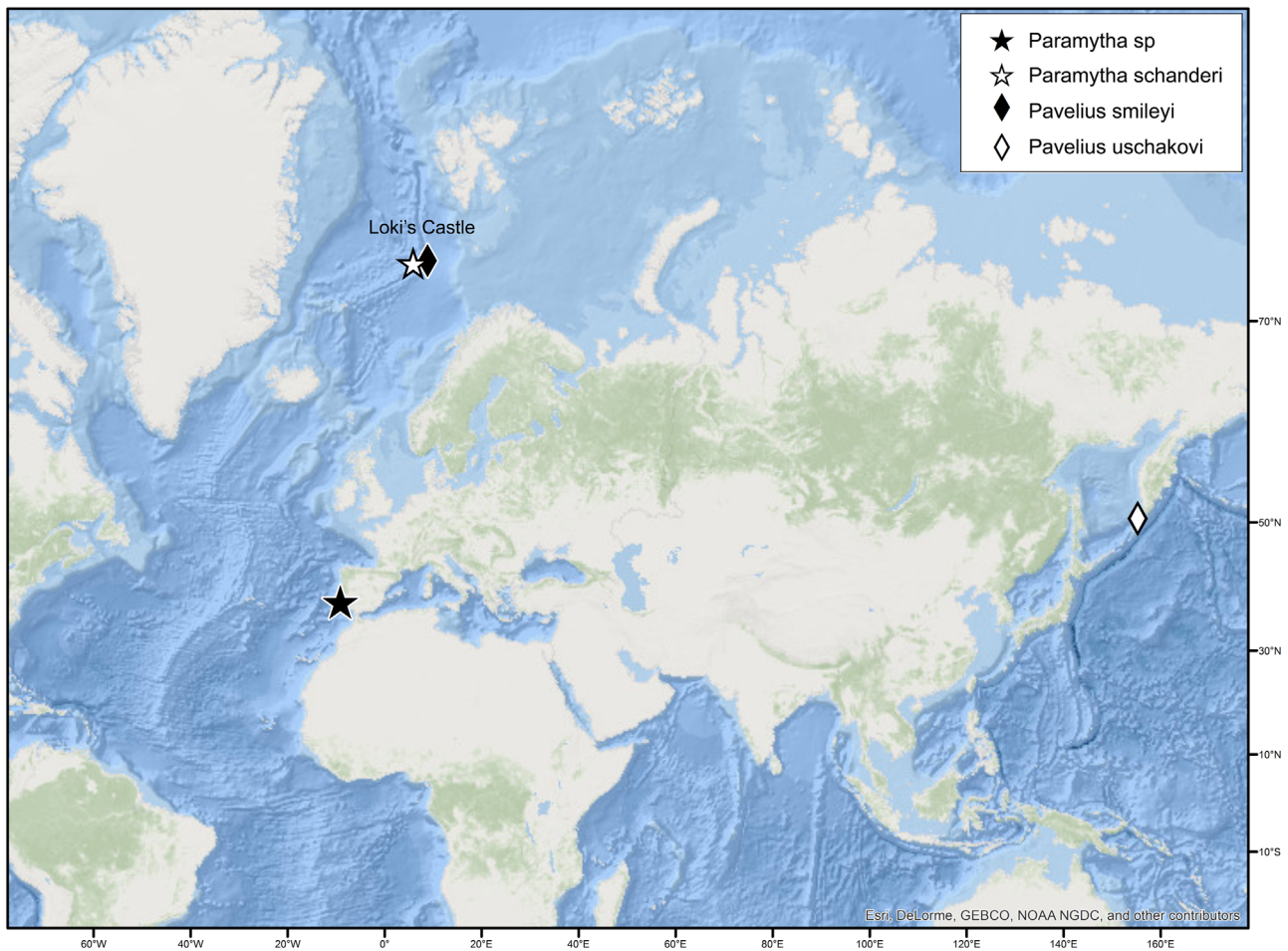


Fig. 1. Map showing records of species referred to *Paramytha* gen. nov. and *Pavelius* Kuznetsov and Levenstein, 1988. Exact localities: Loki's Castle hydrothermal vent field, 73°33'N 08°09'E, 2350 m depth; Off Island Paramushir (Kuril islands), south-eastern part of the Sea of Okhotsk, 50°30.88'N 155°18.14'E, 800 m depth, cold seeps; Setúbal Canyon, NE Atlantic off Portugal, 38°16.850'N 09°06.680'W, 1000 m depth, mammal bones.

3. Results

3.1. Molecular phylogenetic analyses

We were not able to amplify COI for all species (see Table 2), but 16S and 18S was successfully sequenced for all specimens except *Amphitecis ninonae*, for which amplification of 18S failed. The Gblocks analysis excluded 258 positions from the 16S alignment and 127 positions from the 18S alignment (Table 3).

COI intraspecific genetic distances for *Pavelius smileyi* sp. nov. was <0.3%, while the closest related species, *Grassleia* cf. *hydrothermalis*, differed by 13.1%. The single COI sequence of *Paramytha schanderi* gen. et sp. nov. was 14.6% different from the closest species, *Ampharete octocirrata*. For the entire COI dataset, the lowest interspecific distance was 12.6% between *Amphisamytha fauchaldi* and *Amphisamytha lutzi*. For 16S the sequences of *Paramytha schanderi* gen. et sp. nov. diverged by 0.4–1.1%, while the distance to the closest species (*Paramytha* sp.) ranged between 17.6% and 19.4%. The sequences of *Paramytha* sp. diverged by 0–0.4%. The 16S sequences of *Pavelius smileyi* sp. nov. diverged by 0–0.4%, and the distance to the closest species, *Pavelius uschakovi*, was 15%. In the entire 16S dataset, the closest interspecific distance was 9.7% between *Amphisamytha lutzi* and *Amphisamytha caldareii*.

The single gene trees and the combined tree all recovered *Pavelius smileyi* sp. nov., *Paramytha schanderi* gen. et sp. nov. and *Paramytha* sp. as monophyletic with maximum support, and *Paramytha schanderi* gen. et sp. nov. and *Paramytha* sp. as sister species

(Fig. 2; Supplementary Material, Figs S1–S3). The concatenated tree recovers Ampharetidae as paraphyletic with high support, with *Melinna albicincta* (Ampharetidae, Melinninae) as sister to *Alvinella caudata* (Alvinellidae)+Ampharetinae, and with both *Paramytha* gen. nov. and *Pavelius* recovered well within the subfamily Ampharetinae. *Paramytha* gen. nov. shows no close connection to any of the other genera included in the analysis. In the combined tree *Pavelius smileyi* sp. nov. is recovered in a well-supported clade together with *Pavelius uschakovi* and *Grassleia* cf. *hydrothermalis*., but the internal relationships between these tree species are not resolved. It is interesting to note that *Ampharete finnarchica* and *Ampharete octocirrata* are not recovered together, and neither are *Sosane wireni* and *Sosane wahrbergi*.

3.2. Systematics

Family Ampharetidae Malmgren, 1866.
Subfamily Ampharetinae Malmgren, 1866.

3.2.1. Genus *Paramytha* gen. nov

Type species: *P. schanderi* sp. nov.

Additional species: *Paramytha* sp. (Queiros et al., 2017).

3.2.1.1. Diagnosis. Prostomium rectangular with thickened anterior margin, without lobes or glandular ridges. Buccal tentacles smooth. Four pairs of cirriform branchiae arranged as 2+1+1 on segments II–IV respectively; two anterior pairs in transverse row

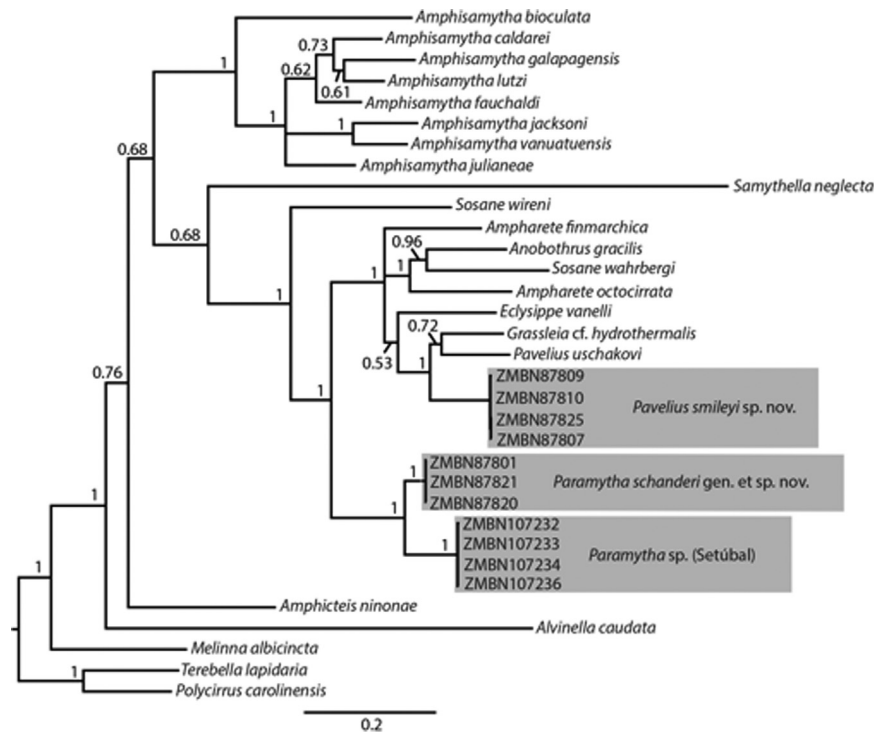


Fig. 2. Phylogenetic tree inferred from analysis of combined dataset (COI, 16S and 18S) in MrBayes. Node labels are posterior probabilities (PP) and nodes with less than 0.5 PP have been collapsed.

without median gap. Chaetae on segment II (paleae) absent. Number of thoracic and abdominal chaetigers interspecifically variable. Modified segments absent. Abdominal neuropodia gradually increasing in size forming pinnules from about 4th abdominal chaetiger. Anal cirri absent.

3.2.1.2. Etymology. The generic name is based on the stem “amytha” as commonly used in ampharetid nomenclature. Gender female.

3.2.1.3. Remarks. The generic diagnosis is based on the type species and on specimens identified as *Paramytha* sp. collected from the Setúbal Canyon off the coast of Portugal in 1000 m depth, dwelling on mammal bones (Queiros et al., 2017). *Paramytha* sp. is morphologically similar to *P. schanderi* gen. et sp. nov. in most respects, but differs most noticeably in the number of thoracic and abdominal chaetigers. In *P. schanderi* gen. et sp. nov., 15 thoracic and up to 20 abdominal chaetigers are present compared to 20 thoracic and up to 12 abdominal chaetigers in specimens identified as *Paramytha* sp. from Setúbal Canyon. The inclusion of the specimens from Setúbal Canyon as a separate species in *Paramytha* is supported by molecular data (see Section 3.1).

Paramytha gen. nov. appears to be related to *Phyllocomus* Grube, 1877 and *Orochi* Reuscher et al., 2015, and these genera share the presence of a prostomium without lobes and glandular ridges, four pairs of branchiae, absence of chaetae on segment II (paleae), and absence of modified segments. However, the shape of the prostomium here described for *Paramytha* gen. nov., being rectangular with a thickened anterior margin is distinctly different from the spade-like prostomium described for *Orochi* and *Phyllocomus* (Reuscher et al., 2015). *Orochi* and *Phyllocomus* differ further from *Paramytha* gen. nov. by the presence of a high membrane connecting the branchiae. *Phyllocomus* differ from *Paramytha* gen. nov. and *Orochi* in the presence of strongly modified branchiae, and *Orochi* differs from *Paramytha* gen. nov., and all other ampharetids, in that the neuropodia of the last thoracic chaetiger

are of the same shape as abdominal pinnules (Reuscher et al., 2015). The segmental arrangement of the four pairs of branchiae in *Paramytha* as 2+1+1 on segment II–IV is characteristic, and differs from the more common arrangement in the ampharetids, including *Orochi* and *Phyllocomus*, where the branchiae are located on only 1 or 2 segments (see e.g. Holthe (1986), Reuscher et al. (2009, 2015)). Within Ampharetinae, *Decemunciger* Zottoli, 1982 seems to be the only other genus with four pairs of cirriform branchiae arranged segmentally as 2+1+1, and with only a small median gap between the two groups of branchiae (Zottoli, 1982). Segmental arrangement of branchiae is also seen in some species referred to *Amage* Malmgren, 1866 and *Grubianella* McIntosh, 1885, but in these genera the two groups of branchiae are well separated by a wide median gap (e.g. Holthe, 1986; Schüller and Jirkov, 2013). *Decemunciger* is also similar to *Paramytha* gen. nov. by the lack of chaetae on segment II (paleae) and presence of smooth buccal tentacles (Zottoli, 1982). However, *Decemunciger* differs from *Paramytha* gen. nov. by the presence of a lobed prostomium (Zottoli, 1982).

Based on the morphological characteristics we conclude that *P. schanderi* gen. et sp. nov. and the related species from Setúbal Canyon off Portugal cannot be placed in any previously described genus, hence a new genus is proposed.

3.2.2. *Paramytha schanderi* sp. nov.

Figs. 3–5 and 9.

3.2.2.1. Type locality. Loki Castle vent field, 73°33'N 08°09'E, 2350 m depth.

3.2.2.2. Type material. Type locality, from sedimentary area with low-temperature diffuse venting with barite chimneys, R/V “G.O. Sars” H2DEEP cruise 2009 sample ROV-8, 07 August 2009, fixed in 96% ethanol, holotype (ZMBN 87798), 7 paratypes in 96% ethanol (ZMBN 87800, 87802, 87803, 87815, 87821, 87823, 87824) and 1 paratype mounted for SEM (ZMBN 87799).



Fig. 3. *Paramytha schanderi* gen. et sp. nov. (A) holotype (ZMBN 87798), lateral view; (B) paratype (ZMBN 87800), partly in tube; (C) paratype (ZMBN 87799-1), stained in methyl blue, ventral and partly lateral view; (D) Same, dorsal view. Scale bars: 1.0 mm.

3.2.2.3. Additional material. Type locality: R/V “G.O. Sars” BIODEEP cruise 2008, sample ROV-11, 14 July 2008, fixed in 96% ethanol: 4 spms (ZMBN 87817–87820). R/V “G.O. Sars” CGB DEEP cruise 2010: Sample ROV-05, 16 July 2010, fixed in 96% ethanol: 2 specimens, both partly damaged (ZMBN 87814), 1 complete specimen (ZMBN 87827); Sample ROV-09, 18 July 2010, fixed in 96% ethanol: 6 spms (ZMBN 87797, 87801, 87804–87806, 87816).

3.2.2.4. Diagnosis. A *Paramytha* with 15 thoracic and up to 20 abdominal chaetigers.

3.2.2.5. Description. Holotype, complete female with 15 thoracic and 19 abdominal chaetigers, 10 mm long and 1.5 mm wide in thorax (Fig. 3A). Other complete specimens are up to 18 mm long and 2.2 mm wide in thorax, with 15 thoracic and 18–20 abdominal chaetigers. Color in ethanol pale. All specimens examined with buccal tentacles partly or fully extended. Prostomium and peristomium

fused, not sub-divided in lobes, almost rectangular in shape with wide anterior, thickened margin (Fig. 4A–D). Prostomium without glandular ridges; possible nuchal organs as small depressions dorsally on posterior part of prostomium. Eyespots absent. Buccal tentacles smooth, cylindrical, longitudinally grooved, some with swollen base (may be related to fixation) (Fig. 3A); buccal tentacles inserted on large tentacular membrane (Fig. 4B). Four pairs of branchiae; branchiae about 1/3–1/4 of body length, cylindrical (Fig. 3A). Branchiostyles loosely attached to branchiophores, often lost. Branchiophores as distinct lobes firmly attached to body wall (Fig. 4A–D). Branchial arrangement 2+1+1 dorsally on segments II–IV, respectively (Figs. 4A–D, 9A). Two anterior pairs arranged closely together in transverse row without median gap; 3rd pair with distinct median gap; 4th pair, in lateral position dorsally to notopodia on segment IV (chaetiger 2). Innermost branchiae of anterior pairs originating from segment II, outermost branchiae of anterior pairs originating from segment III. Third pair originating from segment IV and posterior pair

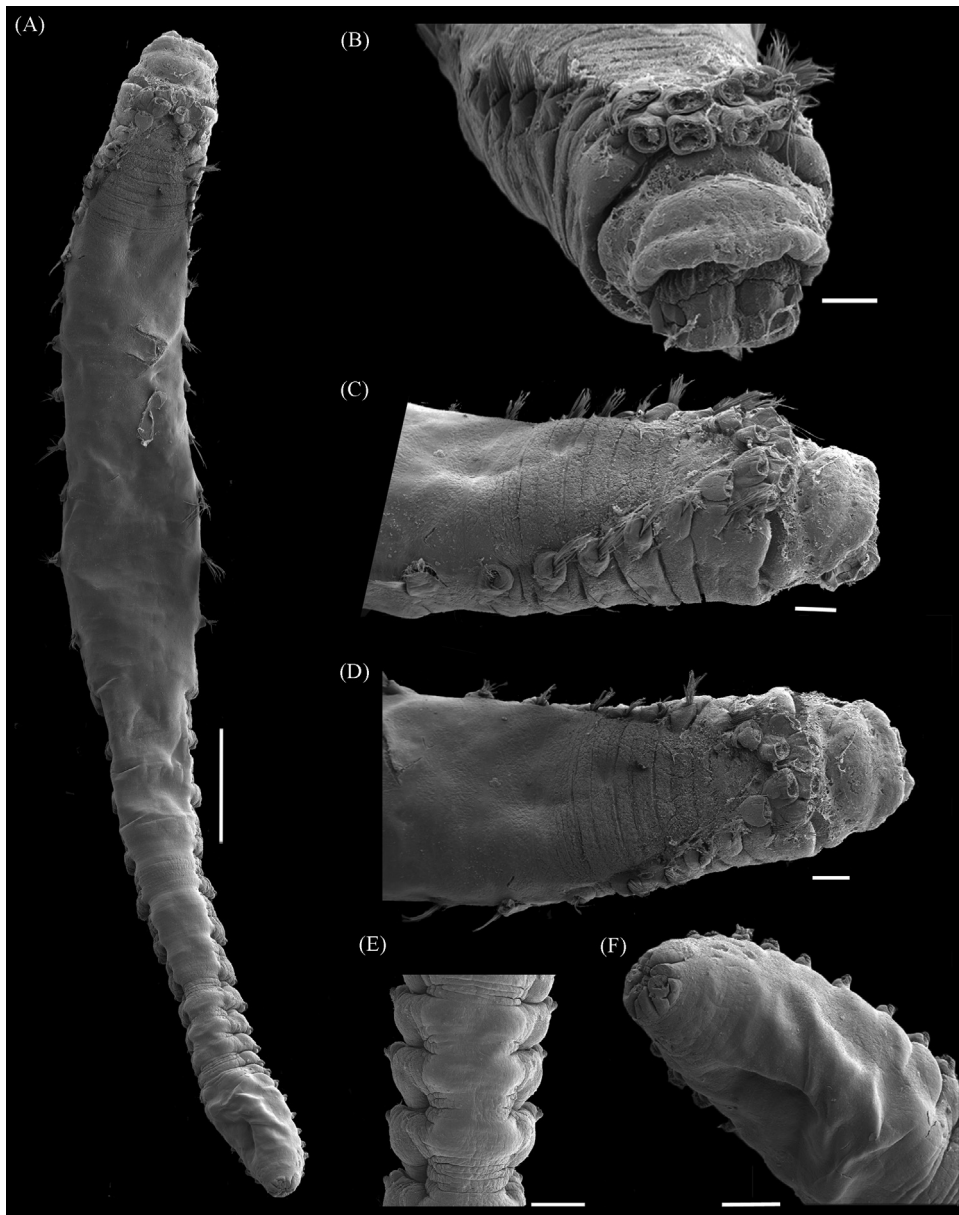


Fig. 4. *Paramytha schanderi* gen. et sp. nov. SEM micrographs of paratype (ZMBN 87799-2), branchiae and buccal tentacles lost: (A) complete specimen, dorsal view; (B) head and anterior part of body, frontal view; (C) same, dorso-lateral view; (D) same, dorsal view; (E) abdominal chaetigers 7–10, dorsal view. (F) posterior part of body and pygidium, dorsal view. Scale bars: (A) 1.0 mm; (B–F) 0.2 mm.

originating from segment V (Fig. 9A). Nephridial papillae not observed. Body cylindrical with thorax and abdomen of similar length (Figs. 3C–D, 4A). Segments II–IV appear as fused (Fig. 4C), but all three segments discernible when stained in methyl blue (Fig. 3C); segmentation indistinct dorsally in mid-body segments (Fig. 4A). Segment II without chaetae (paleae). A total of 15 thoracic segments with notopodia and capillary chaetae, starting on segment III (Fig. 4A); last 12 chaetigers of thorax with neuropodial tori bearing single row of uncini. Notopodia as rounded lobes, anterior 7 distinctly set off from body, remaining notopodia less developed and close to body wall (Fig. 3A). Notopodia of anterior two chaetigers less developed than notopodia in chaetigers 3–7 (Fig. 4C). Anterior 2–3 notopodia in dorsal position, lateral to group of branchiae; notopodia of chaetiger 4–7 gradually shifting to more lateral position; remaining notopodia in lateral position (Figs. 3A, 4A, C). Notochaetae arranged in vertical rows with alternating short and long chaetae; all notochaetae hirsute, with narrow brim (Fig. 5A–B). Neuropodial tori oval in shape in

anterior uncingerous segments, becoming smaller and more rounded in posterior part of thorax. Thoracic uncini with 15–20 teeth arranged in 3–4 horizontal arcs above main rostrum and basal prow (Fig. 5C). Abdomen muscular with distinct ventral longitudinal groove, interrupted with small transverse segmental ridges (Fig. 3C). Abdominal neuropodia gradually increase in size forming pinnules from about 4th abdominal chaetiger, without papillae or cirri. Abdominal neuropodia with dorsal thickened ridge (Fig. 4E). Abdominal uncini with numerous teeth arranged in 5 horizontal arcs above rostrum and basal prow (Fig. 5D–E). Anal opening terminal, surrounded with small papillae or tissue-folds (Fig. 4F); anal cirri absent. Tube flexible, up to about 50 mm in length, with inner thin transparent organic layer, incrustated with fine-particulate material, pieces of polychaete tubes and small shell fragments (Fig. 3B). Head and thorax generally deeply dyed in methyl blue except branchial region, parapods and nuchal organs (Fig. 3C–D). Posterior part of body without distinct staining pattern.

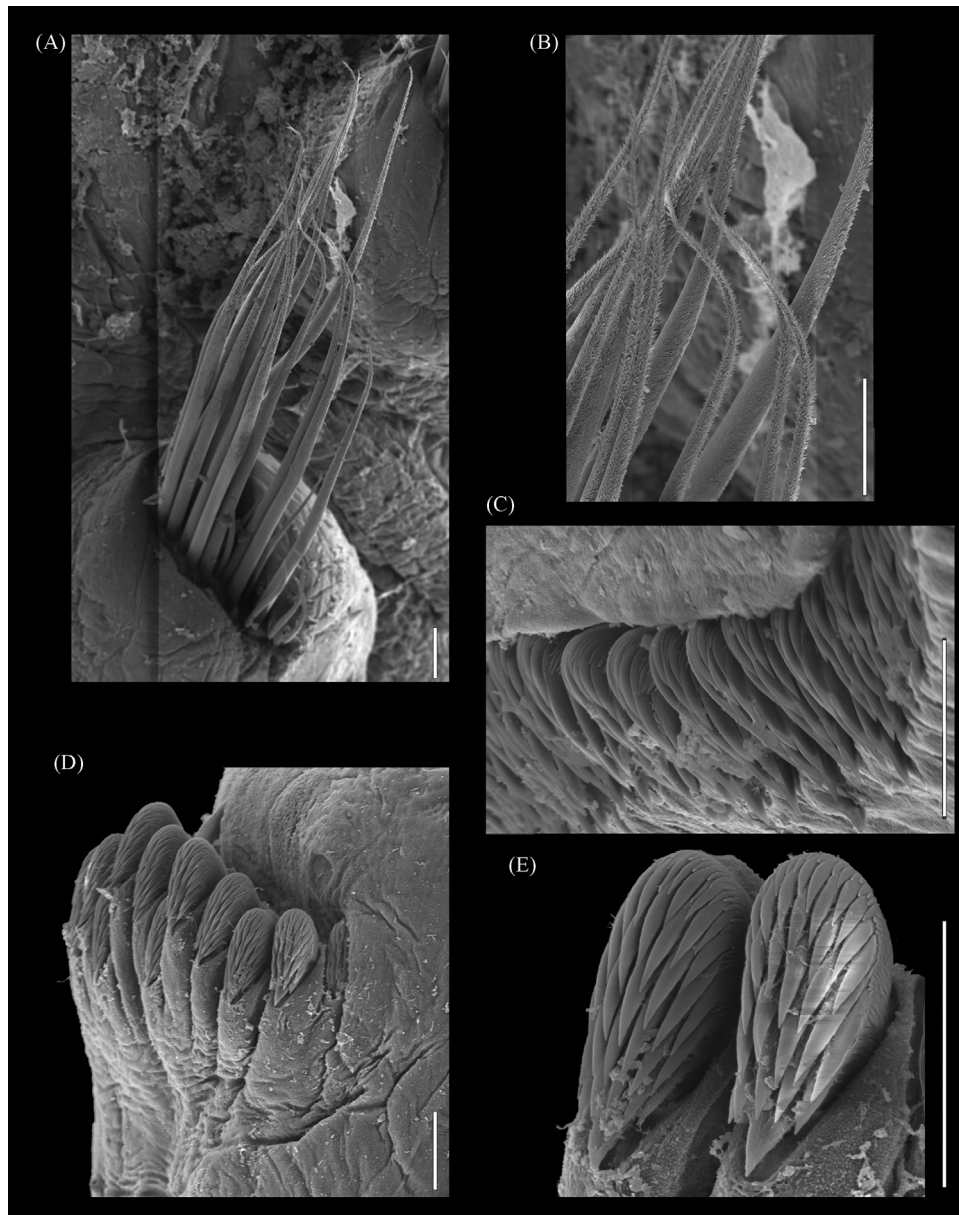


Fig. 5. *Paramytha schanderi* gen. et sp. nov. SEM micrographs of paratype (ZMBN 87799-2). (A) capillary chaetae; (B) same, close up of distal ends; (C) thoracic uncini; (D) abdominal uncini; (E) same, close up. Scale bars: (A–B, D) 20 μm ; (C, E) 10 μm .

3.2.2.6. Reproduction. Gonochoric, without sexual dimorphism. Females with oocytes in thoracic and anterior abdominal chaetigers, visible through body wall; oocytes of different sizes, up to about 20 μm in diameter. One female with oocytes in tube (ZMBN 87827). Several males observed with clusters of sperm in anterior part of body.

3.2.2.7. Etymology. The species is named in honor of our late colleague and dear friend Professor Christoffer Schander.

3.2.3. Genus *Pavelius* Kuznetsov and Levenstein, 1988, emended

Type species: *Pavelius uschakovi* Kuznetsov and Levenstein, 1988: 819–824.

3.2.3.1. Diagnosis, emended. Prostomium rounded, without lobes or glandular ridges. Buccal tentacles smooth. Chaetae on segment II (paleae) present or absent, if present, similar to notochaeta, but smaller. Four pairs of branchiae, arranged in a single transverse

row on segment III. Males with large nephridial papillae on chaetiger 4. Number of thoracic and abdominal chaetigers interspecifically variable, 14–15 thoracic and up to 24 abdominal chaetigers. Modified segments absent. Neuropodia enlarged as pinnules from abdominal chaetiger 2 or 3. Anal cirri absent.

3.2.3.2. Remarks. The generic diagnosis has been emended to include the new species described herein, specifically related to the number of thoracic chaetigers, presence/absence of chaetae on segment II (paleae) and the presence of two types of neuropodia, tori and pinnules. In addition, new information about the type species, *P. uschakovi*, has been provided by Jirkov (2011, pers. comm.), based on re-examination of specimens from type locality: The prostomium is without lobes, nephridial papillae on chaetigers 4 are only present in males and thus represent a dimorphism, the abdominal region have up to 24 chaetigers, and the neuropodia are enlarged as pinnules from the 3rd abdominal chaetiger.



Fig. 6. *Paveilius smileyi* sp. nov. (A) paratype (ZMBN 87810), complete specimen stained in methyl blue, lateral view; (B) holotype (ZMBN 87807), lateral view; (C) paratype (ZMBN 87810), head region in frontal view; (D) paratype (ZMBN 87812), with posterior part of body in tube. Scale bars: 2.0 mm.

Grassleia hydrothermalis Solis-Weiss, 1993, described from chemosynthetic environments in the deep E Pacific, also have a rounded prostomium without lobes and glandular ridges, and four pairs of branchiae arranged in a single transverse row without median gap. *G. hydrothermalis*, however, differs from the species of *Paveilius* by the absence of neurochaetae on the 5th chaetiger (segment 6), probably unique within the Ampharetidae, as well as the presence of a very short abdomen with only 7 chaetigers compared to more than 20 in species of *Paveilius* (see Solis-Weiss (1993)). We consider these genera to be closely allied, which is supported by the molecular analysis (Fig. 2).

3.2.4. *Paveilius smileyi* sp. nov Figs. 6–9.

3.2.4.1. Type locality. Loki Castle vent field, Arctic mid-ocean ridge, 73°33'N 08°09'E, 2350 m depth.

3.2.4.2. Type material. Type locality from sedimentary area with low-temperature diffuse venting with barite chimneys, R/V “G.O. Sars” H2DEEP cruise 2009 sample ROV-8, 07 August 2009, fixed in 96% ethanol, holotype (ZMBN 87807) and 1 paratype (ZMBN 87809). R/V “G.O. Sars” CGB DEEP cruise 2010: Sample ROV-04, 15 July 2010, fixed in 6% formaldehyde and preserved in 80% ethanol: 1 paratype (ZMBN 87808-1), 2 paratypes (ZMBN 87812), 1 paratype mounted for SEM (ZMBN 87808-2), 1 paratype fixed in 96% ethanol (ZMBN 87826); sample ROV-05, 16 July 2010, fixed in 96% ethanol: 1 paratype (ZMBN 87810); sample ROV-06, July 2010, fixed in 96% ethanol: 1 paratype (ZMBN 87825).

3.2.4.3. Diagnosis. A *Paveilius* with 14 thoracic and up to 21 abdominal chaetigers; chaetae on segment II (paleae) absent.

3.2.4.4. Description. Holotype, complete male, with 14 thoracic and 20 abdominal chaetigers, 26 mm long and 3.0 mm wide in thorax (Fig. 6B). Other complete specimens are up to 28 mm in length and



Fig. 7. *Pavelius smileyi* sp. nov. SEM micrographs: (A) complete specimen, lateral view; (B) head and anterior part of body, frontal view; (C) same, lateral view; (D) same, dorsal view; (E) details of branchiae; (F) posterior part of body and pygidium, dorsal view. (A–D) paratype, ZMBN 87808; (E–F) paratype, ZMBN 87811. Scale bars: (A) 1.0 mm; (B–F) 0.5 mm.

3.1 mm wide in thorax, with 14 thoracic and 20–21 abdominal chaetigers. Color in ethanol pale to brownish (Fig. 6B–D). Examined specimens with buccal tentacles withdrawn, or only partly extended. Prostomium broadly rounded, fused with peristomium dorsally, without lobes and glandular ridges (Fig. 7A–D). Paired nuchal organs as short, ciliated slits, centrally placed on prostomium (Fig. 7B). Eyes absent. Buccal tentacles smooth, cylindrical, longitudinally grooved. Segment I with distinct segmental borders (Fig. 7C–D). Four pairs of branchiae arranged close together in transverse row without median gap, dorsally on segment III (chaetiger 1) (Fig. 7A–D); branchiostyles relatively short, less than 1/5 of body length, tapering distally (Fig. 6A–D). Branchiophores as distinct lobes, fused at base, firmly attached to body wall (Fig. 7B–D). Second outermost branchiae originating from segment II, outermost branchiae originating from segment III, innermost branchiae originating from segment IV, second innermost branchiae originating from segment V (Fig. 9B). Distinct oval-shaped

patch posterior to row of branchiae on segment 4 (chaetiger 2), covering half width of segment, with distinct anterior papillae arising slightly posterior and between the two branchial groups (Fig. 7D). Males with nephridial papillae as short lobes on chaetiger 4, posterior to notopodia. Body cylindrical, tapering posteriorly, with thorax and abdomen of similar length (Figs. 6A–B; 7A). Segment II without chaetae (paleae). A total of 14 thoracic segments with notopodia and capillary chaetae, starting on segment III (Fig. 7A); last 11 with neuropodial tori bearing single row of uncini. Notopodia as rounded lobes, up to three times longer than wide, gradually increasing in size from 1st to 3rd chaetigers (Fig. 7A, C). Notochaeta as hirsute capillaries (Fig. 8A–B), arranged in vertical rows; capillaries from anterior row generally thinner and shorter than from more posterior rows (Fig. 8A). Thoracic neuropodial tori oval in shape (Fig. 7A; 8A). Thoracic uncini with about 8 teeth arranged in 2–3 vertical rows above main rostrum and basal prow (Fig. 8C). First abdominal segment with

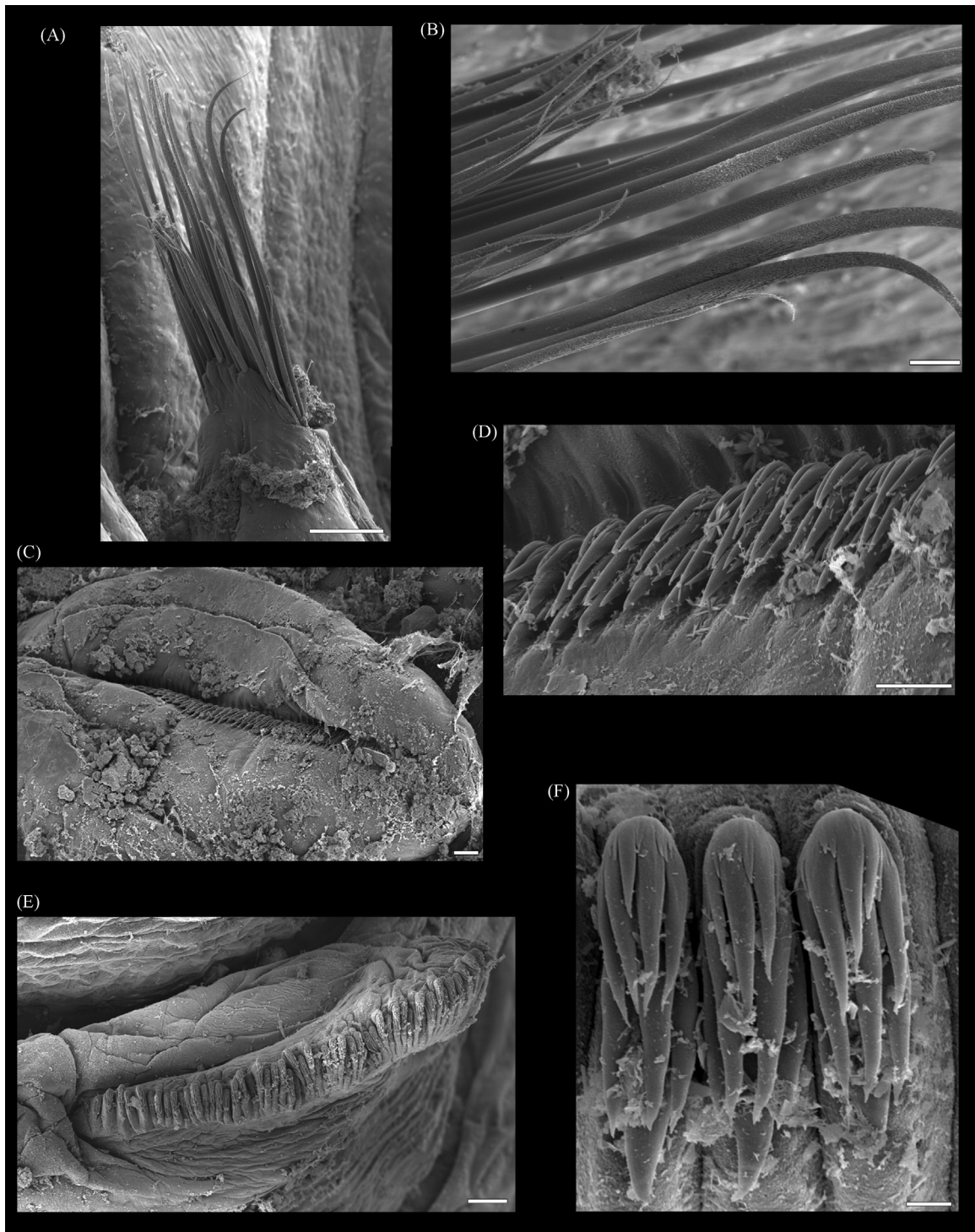


Fig. 8. *Pavelius smileyi* sp. nov. SEM micrographs of paratype (ZMBN 87808): (A) capillary chaetae; (B) details of capillary chaetae; (C) thoracic tori with uncini; (D) details of thoracic uncini; (E) abdominal neuropodia with uncini; (F) details of abdominal uncini. Scale bars: (A) 0.1 mm; (B–C, E) 20 μ m; (D) 10 μ m; (F) 2 μ m.

neuropodia as thoracic type (tori); remaining abdominal neuropodia as weakly developed pinnules (Fig. 8E), without papillae or cirri. Abdominal uncini with up to 12–15 teeth above main rostrum, alternating in 4 vertical rows (Fig. 8F). Anal opening terminal, surrounded by small papillae or tissue-folds (Fig. 7F); anal cirri absent. Tube with thin organic layer incrustated with thick layer of fine mud (Fig. 6D). Head region (except nuchal organs), thoracic ventral glandular pads and basal part of notopodia deeply stained in methyl blue (Fig. 6A).

3.2.4.5. Reproduction: gonochoric. Females with oocytes and males with clusters of sperm in anterior part of body, observed by dissection. Large nephridial papilla on chaetiger 4 present in males.

3.2.4.6. Etymology. The species name refers to the “happy” appearance of the worm.

3.2.4.7. Remarks. The genus *Pavelius* includes at present two species, *P. uschakovi* and *Pavelius smileyi* sp. nov., both described from

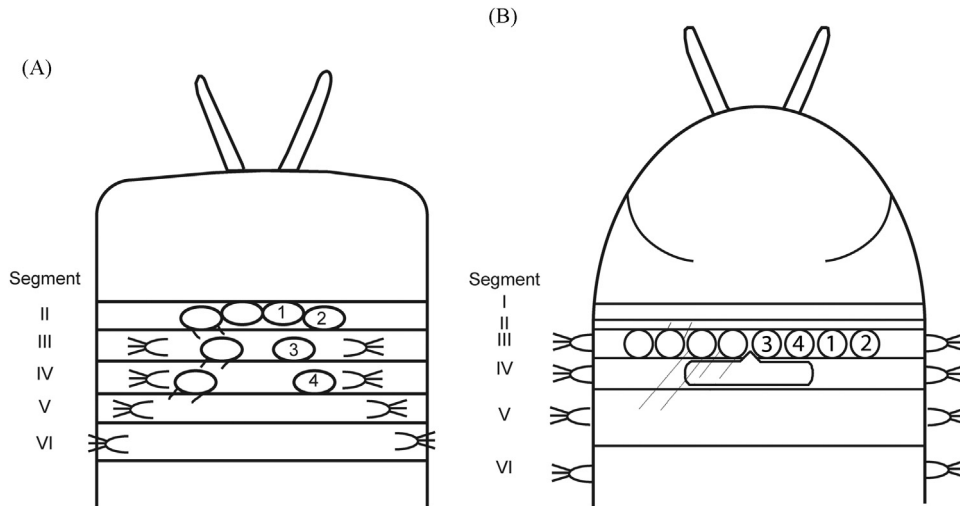


Fig. 9. Schematic illustrations of important taxonomical characters related to the anterior part of the body. (A) *Paramytha schanderi* gen. et sp. nov.; (B) *Pavelius smileyi* sp. nov.

chemosynthetic environments. *Pavelius smileyi* differs from *P. uschakovi* in the presence of 14 thoracic and up to 21 abdominal chaetigers rather than 15 thoracic and up to 24 abdominal chaetigers (Kuznetsov and Levenstein 1988; Jirkov, pers. comm.). *P. uschakovi* also have, in contrast to *Pavelius smileyi*, a few, small and thin chaetae (paleae) on segment II.

4. Discussion

The taxonomy of the family Ampharetidae is complex, with a high number of genera of which many are poorly characterized (see Reuscher et al. (2009), Jirkov (2011)). Traditionally, number of thoracic chaetigers and presence or absence of chaetae (paleae) on segment II, have been considered as important characters to define genera in the family (Holthe, 1986). However, in a number of genera, e.g. *Ampharete* Malmgren, 1866, *Anobothrus* Levensen, 1884, *Amage* Malmgren, 1866 and *Amphisamytha* Hessle, 1917, some variation in these characters has been described (Jirkov, 2009; Imajima et al., 2012; Schüller and Jirkov, 2013; Stiller et al., 2013; Reuscher et al., 2015). In the present study, we document interspecific variation in number of thoracic chaetigers in both *Paramytha* gen. nov. and *Pavelius*, and the presence or absence of chaetae (paleae) on segment II in *Pavelius*, thus supporting that number of thoracic chaetigers and the presence or absence of chaetae on segment II are of limited value in defining genera of Ampharetidae.

Jirkov (2009, 2011) emphasized the shape of the prostomium as an important character to delimitate genera in the subfamily Ampharetinae. The prostomium in both *Paramytha* gen. nov. and *Pavelius* may be described as unilobed without glandular ridges, as in a number of other genera in the subfamily Ampharetinae (see Jirkov (2011), Reuscher et al. (2015)). We are not able to assign the characteristic prostomium in *Paramytha* gen. nov., being rectangular in shape with a thickened anterior margin, to any of the “typical” prostomial types in the subfamily as described by Jirkov (2011). The presence of a wide, rounded prostomium with distinct nuchal organs seems to be characteristic for the genera *Pavelius* and *Grassleia*. However, the use of prostomial shape to delimitate genera of ampharetids might be problematic as the shape to some degree will depend on whether the buccal tentacles are withdrawn or extended (see Day, 1964). At present, we consider the characteristic and unusual arrangement of the branchiae to be a key character defining *Paramytha* (see Section 3.2.1.3) and *Pavelius* (see Section 3.2.3.2).

Molecular data is presently only available for a selection of species (and genera) of ampharetids and thus the molecular phylogeny presented here provides limited information about relationships among the currently recognized genera of the family. However, the molecular data clearly support the inclusion of *Pavelius smileyi* sp. nov. in *Pavelius*, and also the expected relationship between *Pavelius* and *Grassleia* (see Section 3.2.3.2). *Paramytha* gen. nov. forms a well supported monophyletic group within the subfamily Ampharetinae, but no clear sister relationship with other genera were identified. Based on morphological data, *Paramytha* gen. nov. is here considered to be related to the genera *Phyllocomus* and *Orochi*, and perhaps *Decemunciger* (see Section 3.2.1.3). At present, molecular data is not available to test this hypothesis.

Ampharetid polychaetes are among the more common families recorded from hydrothermal vents and cold seeps with 17 species representing 8 different genera considered as exclusively adapted to live in these chemosynthetic environments (Kuznetsov and Levenstein, 1988; Solis-Weiss, 1993; Reuscher et al., 2009, 2012; Stiller et al., 2013; present study). The genera *Amage* (with about 25 species), *Glyphanostomum* (five species) and *Anobothrus* (about 20 species) are each only represented by a single species adapted to chemosynthetic environments, and most species in these genera are found in other marine environments. The genus *Amphisamytha* includes seven species adapted to vent and seep habitats and two additional species known from shallow waters in the Pacific. The genera *Pavelius* (two species), *Grassleia* (one species) and *Paramytha* gen. nov. (two species) are only known from chemosynthetic environments. Morphological and molecular data (see Fig. 2) indicate that adaptation to live in chemosynthetic environment has evolved several times within the ampharetids.

In the initial exploration of the fauna from the Loki’s Castle vent field it has been speculated that the fauna has more in common with the North Pacific than with the fauna in the Atlantic south of the Faroe-Iceland-Greenland ridge (Pedersen et al., 2010, Kongsrud and Rapp, 2012). The close relationship of *Pavelius smileyi* sp. nov. with *P. uschakovi* from the NW Pacific, and also *Grassleia* cf. *hydrothermalis* from the NE Pacific (see Section 3.2.3.2) supports the connection between the Arctic and Pacific deep-sea chemosynthetic faunas. *P. schanderi* gen. et sp. nov., on the other hand, is related to a bone-living species of *Paramytha* from off the Coast of Portugal at 1000 m depth (Queiros et al., 2017). The recently recorded maldanid *Nicomache* sp. from the mid-Cayman Ridge in the Caribbean (Plouviez et al., 2015) is very similar to *Nicomache*

lokii (Kongsrud and Rapp, 2012) in the mitochondrial marker COI (< 1.5%, Genbank accession numbers: *Nicomache* sp: KJ566962; *N. lokii*: FR877579, FR877578), and clearly demonstrates a connection between Atlantic and Arctic chemosynthetic faunas. A similar case has been demonstrated for the siboglinid *Sclerolinum contortum* Smirnov, 2000, which based on molecular data has been shown to be widespread in chemosynthetic environments both in the Arctic (including Loki's Castle), the Gulf of Mexico and in the Antarctic (Georgieva et al., 2015). These highly contrasting links to other known vent and seep faunas, from both the Atlantic and Pacific Oceans, call for a more comprehensive study aiming to investigate the genetic connectivity and phylogeographic history of polychaetes inhabiting chemosynthetic habitats at a large geographic scale.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dsr2.2016.08.015>.

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