

## Research article

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***Pseudopolydora* (Annelida: Spionidae) from the Arabian Gulf, Kuwait**Vasily I. RADASHEVSKY <sup>1,\*</sup>, Manal AL-KANDARI <sup>2</sup>,  
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**Abstract.** Seven species of *Pseudopolydora* are described and illustrated from the Arabian Gulf, Kuwait: *P. achaeta* Radashevsky & Hsieh, 2000, *P. antennata* (Claparède, 1868), *P. arabica* Radashevsky & Al-Kandari, 2020, *P. auha* sp. nov., *P. kuwaiti* sp. nov., *P. melanopalpa* sp. nov., and *P. multispinosa* sp. nov. The morphology of the developed planktonic larvae is described for *P. antennata* and *P. kuwaiti* sp. nov. Adults of all species live in tubes in soft sediments, while adults of *P. kuwaiti* sp. nov. also bore in shells of gastropods and dead corals encrusted by coralline algae. *Pseudopolydora antennata* and *P. arabica* form dense settlements up to 50 000 individuals per 1 m<sup>2</sup>, while other species are comparatively rare. The phylogenetic relationships between the examined species and other *Pseudopolydora* (18 species in total) were assessed in an analysis of sequence data of four gene fragments: mitochondrial *16S* rDNA, nuclear *18S* rDNA and *28S* rDNA, and *Histone 3* (2473 bp in total). Three species complexes are distinguished involving *P. antennata*, *P. diopatra* Hsieh, 1992 and *P. paucibranchiata* (Okuda, 1937). Sets of adult morphological features shared by species of each complex and an identification key to *Pseudopolydora* species from the Arabian Gulf are provided.

**Keywords.** Polychaete, systematics, reproduction, larval development, molecular analysis.

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**Introduction**

Comprehensive surveys since 2004 seeking to document the distribution and abundance of macrofauna in the Arabian Gulf, Kuwait discovered high biodiversity in the region (Al-Yamani *et al.* 2009, 2012,

2019; Al-Rifaie *et al.* 2012; Al-Kandari *et al.* 2019). Annelids, molluscs and crustaceans prevailed both in the intertidal and in shallow waters. Of polychaetous annelids, 172 species from 46 families were distinguished (Al-Kandari *et al.* 2019). The most diverse and abundant species belonged to the family Spionidae Grube, 1850, followed by Nereididae Blainville, 1818 and Serpulidae Rafinesque, 1815. *Pseudopolydora* Czerniavsky, 1881 were among the most common spionids, but their specific identity remained uncertain. Genetic comparison of the Arabian *Pseudopolydora* and similar worms from other regions clarified some issues (Radashevsky *et al.* 2020), and one new species, *P. arabica* Radashevsky & Al-Kandari, 2020, was described from the Gulf. The purpose of the present study is to describe other *Pseudopolydora* from Kuwait, compare them genetically with similar species, and provide an identification key to the species from the Arabian Gulf.

## Material and methods

### Field sampling

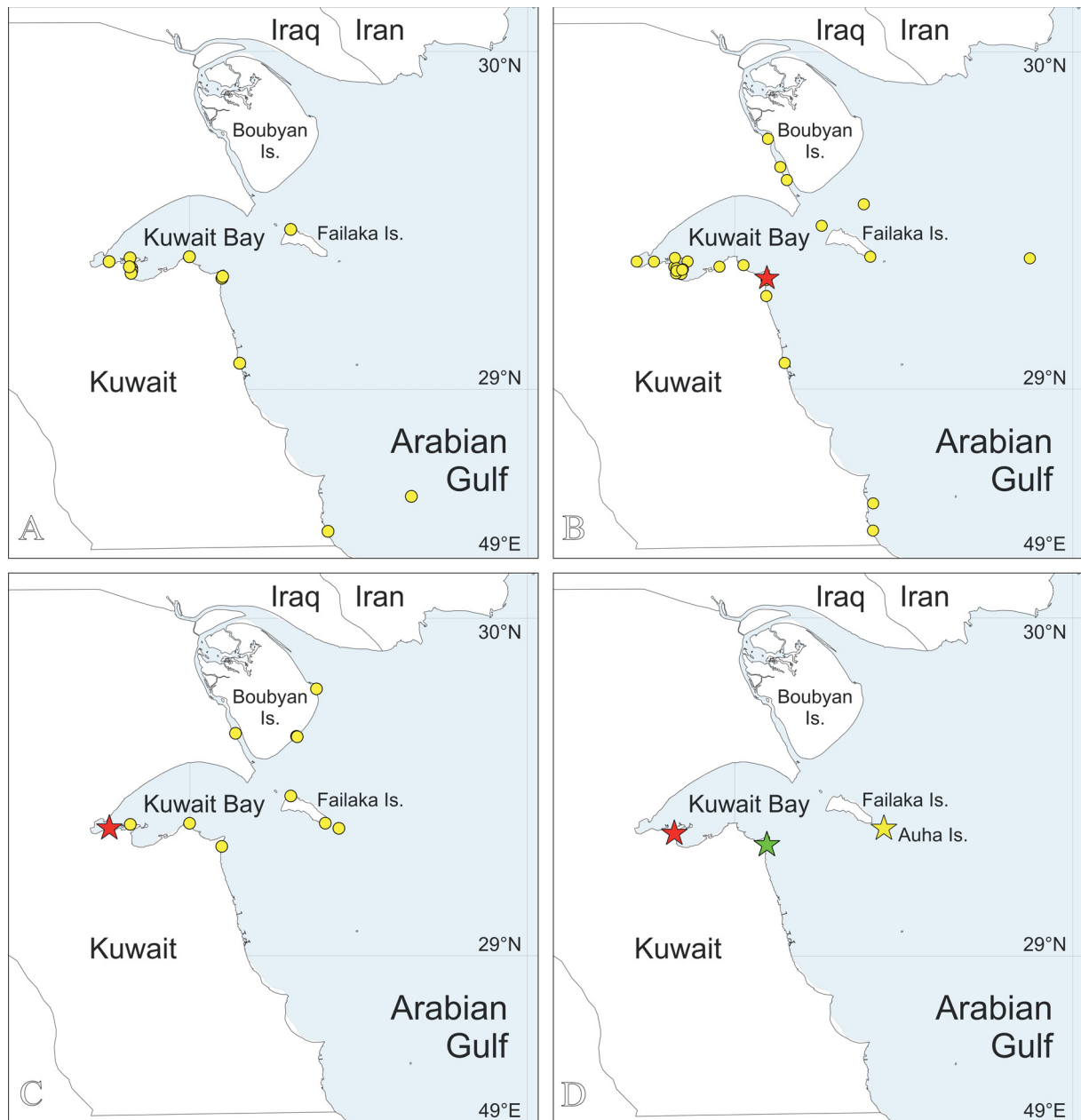
Collections were made from intertidal and shallow water habitats in Kuwait in 2014–2020 (Fig. 1). Sediments collected for this study were washed in the field on a 500 µm mesh sieve, and *Pseudopolydora* worms retained in the residues were removed. Additional details of sampling methods were described by Al-Kandari *et al.* (2019). We also collected a range of potential substrata, including mollusks, dead corals, coralline algae and the above-ground tube-caps of the onuphid polychaetes. Polychaetes were gently removed after cracking infested shells and coralline algae with a hammer and pliers. Plankton tows were made in March 2016 in Kuwait Bay, in surface waters (to a depth of 10 meters) with a 100 µm mesh plankton net.

### Laboratory study and museum deposits

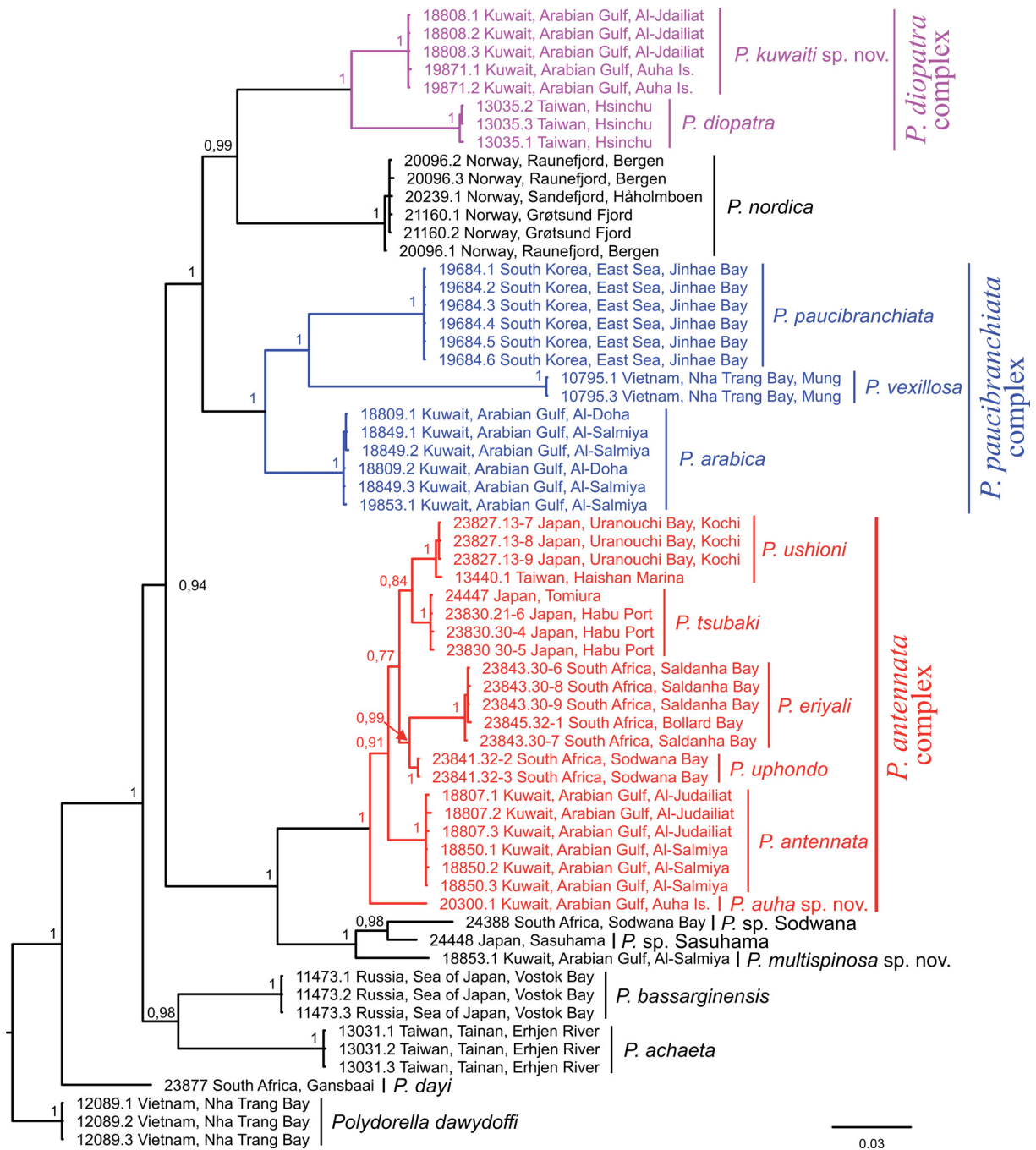
Live plankton samples were sorted and *Pseudopolydora* larvae were removed and maintained in sea water in Petri dishes in the laboratory. Live adults and the larvae were relaxed in isotonic magnesium chloride and examined and photographed using a Leica DM2500 compound microscope and a Leica M125 stereo microscope equipped with Leica DFC450 digital cameras. Earlier collected formalin-fixed specimens were stained with an alcohol solution of methylene green (MG), examined whole or dissected to observe particular structures, and then photographed using light microscopes equipped with digital cameras. Images of multiple focal layers were stacked using Zerene Stacker ver. 1.04 software. Images of parts of worms were stitched into panoramas using Kolor Autopano Pro ver. 3.7.0 and PTGui ver. 11.31 software. Relations between morphometric variables were explored using Statistica ver. 6.0 software. After examination, worms were fixed in 10% formalin solution, rinsed in fresh water, transferred to 70% ethanol and then deposited in the polychaete collections of the MIMB and NHMUK (see abbreviations, below). Some specimens were preserved in 95% ethanol and genetically examined by Radashevsky *et al.* (2020) and in the present study.

We additionally examined *Pseudopolydora* specimens from the Arabian Gulf reported by Mohammad (1971) and deposited in the NHMUK. Complete information about examined material is given in Supp. Table ESM1. Information about type specimens is also given in the Results preceding the description of the new species. To link sequences used in the corresponding molecular analysis by Radashevsky *et al.* (2020) with the material described herein, unique numbers (VIR ID) from the author's database are given to samples in Supp. Table ESM1. These numbers without letters precede collecting location names on the phylogenetic tree based on genetic analysis (Fig. 2). When more than one individual of the same species were used in the genetic analysis, individual numbers follow the VIR ID numbers separated by a dot.

Sampling locations reported in the present study are plotted on maps using QGIS ver. 3.18.0 software and the geodata provided by the OpenStreetMap Project (<https://osmdata.openstreetmap.de>). Final maps and the figures were prepared using CorelDRAW®2019 software.



**Fig. 1.** Maps showing records (yellow circles) and type localities (stars) of *Pseudopolydora* spp. from the Arabian Gulf, Kuwait (see Supp. Table ESM1). **A.** *P. antennata* (Claparède, 1868). **B.** *P. arabica* Radashevsky & Al-Kandari, 2020 (red star = type locality: Al-Salmiya, Kuwait). **C.** *P. kuwaiti* sp. nov. (red star = type locality: Al-Judailiat). **D.** Yellow star = type locality of *Pseudopolydora auha* sp. nov.: Auha Is.; red star = type locality of *P. melanopalpa* sp. nov.: Sulaibikhat Bay; green star = type locality of *Pseudopolydora multispinosa* sp. nov.: Al-Salmiya, Kuwait.



**Fig. 2.** Majority rule consensus tree of the Bayesian inference analysis of the combined *16S* (234 bp), *18S* (1639 bp), *28S* (294 bp), and *Histone 3* (306 bp) sequences (2473 bp in total) of *Pseudopolydora* spp. rooted with sequences of *Polydorella dawydoffi* Radashevsky, 1996. Posterior probabilities are shown on the branches. The numbers without letters preceding collecting locations are unique numbers from the VIR database linking the individuals on the tree with the sampling data in Table 1 and Supp. Table ESM1. *Pseudopolydora* complexes are defined by unique sets of characters shared by the species.

### DNA extraction, amplification, and sequencing

We used the ReliaPrep gDNA Tissue Miniprep System (Promega Corporation, Madison, WI, USA) for DNA extraction and purification with standard protocol for animal tissue. Polymerase chain reaction (PCR) amplification of nuclear 18S rDNA, D1 region of 28S rDNA and Histone 3, and mitochondrial 16S rDNA gene fragments was accomplished with the primers and conditions described by Radashevsky *et al.* (2014, 2016). Purified PCR products were sequenced in both directions on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems) using the BrilliantDye Terminator ver. 1.1 Cycle Sequencing Kit (NimaGen) and the same primers as for PCR. Sequence editing and contig assembly were performed using SeqScape ver. 2.5 (Applied Biosystems). GenBank accession numbers of the obtained sequences are shown in Table 1.

### Data analysis

We aligned DNA sequences using the MAFFT ver. 7.475 software with the default settings (automatically chosen algorithms) (Kato *et al.* 2002; Kato & Standley 2013). Ambiguous positions and gaps were excluded from subsequent analysis using trimAl ver. 1.2 (Capella-Gutiérrez *et al.* 2009) with an automated heuristic approach. As the obtained sequences were closely related, we chose to employ uncorrected values of sequence divergence (pairwise distances,  $p$ , see Nei & Kumar 2000) instead of complex distance measures (i.e., corrected by best-fit evolutionary model). Distances both within and between groups were calculated in MEGA ver. 5.1 (Tamura *et al.* 2011). We concatenated DNA data partitions using SequenceMatrix (Vaidya *et al.* 2011) and specified substitution models for each partition individually. The best-fitting nucleotide substitution models for Bayesian analysis were selected in MrModeltest ver. 2.3 (Nylander 2004) using Akaike Information Criterion (AIC): SYM+G for each data partition.

We used MrBayes ver. 3.2.7 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) via the CIPRES web portal (Miller *et al.* 2010) for the Bayesian analysis of 40 000 000 generations, four parallel chains and sample frequencies set to 1000, in two separate runs. Based on the convergence of likelihood scores, 25% of sampled trees were discarded as burn-in.

In the analysis, we also included sequences of *Pseudopolydora* spp. from Japan and South Africa obtained by Abe *et al.* (2016), Williams *et al.* (2017), Simon *et al.* (2019), and Abe & Sato-Okoshi (2021), and our newly obtained sequences of *Pseudopolydora ushioni* Simon, Sato-Okoshi & Abe, 2017 from Taiwan. The phylogenetic tree was rooted using the sequences of *Polydorella dawydoffi* Radashevsky, 1996 according to a preliminary phylogenetic analysis of molecular data for spionid polychaetes, where *Polydorella* Augener, 1914 appeared sister to *Pseudopolydora* Czerniavsky, 1881 (Radashevsky *et al.* unpubl. data).

### Abbreviations and Museum acronyms

ESM	=	electronic supplementary material
MG	=	methylene green
MIMB	=	Museum of the National Scientific Center of Marine Biology, Vladivostok, Russia
NHMUK	=	Natural History Museum, London, UK
VIR	=	Vasily I. Radashevsky personal database

## Results

### Molecular analysis

The combined aligned sequences of *Polydorella* and *Pseudopolydora*, with gaps excluded, comprised in total 2473 bp, including 234 bp (91.7% of original sequences) for 16S rDNA, 1639 bp (96.8% of original sequences) for 18S rDNA, 294 bp (98.6% of original sequences) for 28S rDNA and 306 bp for *Histone 3*.

**Table 1** (continued on next page). Taxa, sampling locations, museum registration numbers of voucher specimens and GenBank accession numbers of sequences used in the analysis. New sequences are given in bold. <sup>1</sup>Abe *et al.* 2016; <sup>2</sup>Williams *et al.* 2017; <sup>3</sup>Simon *et al.* 2019; <sup>4</sup>Radashevsky *et al.* 2020; <sup>5</sup>Abe & Sato-Okoshi 2021.

Species	Location	Date	Voucher MIMB	GenBank accession number*			Histone 3
				16S	18S	28S	
1. <i>Polydorella dawydoffi</i> Radashevsky, 1996	Vietnam, Nha Trang Bay <sup>4</sup>	10 Jun. 2006	MIMB 40922; VIR 12089	<b>MW904113-15</b>	MG460975-77	MG460831-33	
2. <i>Pseudopolydora achaeta</i> Radashevsky & Hsieh, 2000	Taiwan, Tainan, Erhjen River <sup>4</sup>	18 Apr. 2007	MIMB 40923; VIR 13031	<b>MW904119-21</b>	MG460978-83	MG460834-36	
3. <i>P. antennata</i> Claparède, 1868	Kuwait, Arabian Gulf, Al-Judailiat	4 Feb. 2015	MIMB 40895; VIR 18807	<b>MW904100-02</b>	–	<b>MW904153-55</b> <b>MW923736</b>	
4. <i>P. antennata</i> Claparède, 1868	Kuwait, Arabian Gulf, Al-Salmiya	5 Mar. 2015	MIMB 40896; VIR 18850	<b>MW904097-99</b>	<b>MW911607-09</b>	<b>MW923733-35</b>	
5. <i>P. arabica</i> Radashevsky & Al-Kandari, 2020	Kuwait, Arabian Gulf, Al-Doha <sup>4</sup>	5 Feb. 2015	MIMB 39111; VIR 18809	MG460944-45	<b>MW904126-27</b>	MG461024-25 MG460869-70	
6. <i>P. arabica</i> Radashevsky & Al-Kandari, 2020	Kuwait, Arabian Gulf, Al-Salmiya <sup>4</sup>	5 Mar. 2015	MIMB 39112; VIR 18849	MG460946-48	<b>MW904123-25</b>	MG461026-28 MG460871-73	
7. <i>P. arabica</i> Radashevsky & Al-Kandari, 2020	Kuwait, Arabian Gulf, Al-Salmiya <sup>4</sup>	8 Feb. 2016	MIMB 39056; VIR 19853	MG460957	<b>MW904122</b>	MG461037 MG460882	
8. <i>P. auha</i> sp. nov.	Kuwait, Arabian Gulf, Auha Is.	29 Jan. 2017	MIMB 40907; VIR 20300	<b>MW904103</b>	<b>MW904112</b>	<b>MW904156</b> <b>MW923741</b>	
9. <i>P. bassarginensis</i> (Zachs, 1933)	Russia, Sea of Japan, Vostok Bay <sup>4</sup>	8 Oct. 2006	MIMB 40924; VIR 11473	MG460894-96	<b>MW904116-18</b>	MG460970-72 MG460825-27	
10. <i>P. dayi</i> Simon, 2009	South Africa, Gansbaai <sup>2</sup>	–	VIR 23877	–	KY677907	–	
11. <i>P. diopatra</i> Hsieh, 1992	Taiwan, Hsinchu <sup>4</sup>	26 Apr. 2007	MIMB 33069; VIR 13035	MG460906-08	<b>MW904139-41</b>	MG460984-86 MG460837-39	
12. <i>P. eriyali</i> Simon, Sato-Okoshi & Abe, 2017	South Africa, Bolland Bay <sup>3</sup>	21 Jan. 2015	VIR 23845	LC107864	LC107846	LC107850	
13. <i>P. eriyali</i> Simon, Sato-Okoshi & Abe, 2017	South Africa, Saldanha Bay <sup>3</sup>	Sep. 2010	VIR 23843	LC107860-63	AB973930-33	AB973940-43	
14. <i>P. kuwaitii</i> sp. nov.	Kuwait, Arabian Gulf, Al-Jdailiat <sup>4</sup>	4 Feb. 2015	MIMB 40713; VIR 18808	MG460941-43	<b>MW904136-38</b>	MG461021-23 MG460866-68	
15. <i>P. kuwaitii</i> sp. nov.	Kuwait, Arabian Gulf, Auha Is.	10 Feb. 2016	VIR 19871	<b>MW904106-07</b>	<b>MW904134-35</b>	<b>MW904150-51</b> <b>MW923731-32</b>	
16. <i>P. multispinosa</i> sp. nov.	Kuwait, Arabian Gulf, Al-Salmiya	5 Mar. 2015	MIMB 40906; VIR 18853	<b>MW904095</b>	–	<b>MW904157</b> <b>MW923738</b>	

**Table 1** (continued). Taxa, sampling locations, museum registration numbers of voucher specimens and GenBank accession numbers of sequences used in the analysis. New sequences are given in bold. <sup>1</sup>Abe *et al.* 2016; <sup>2</sup>Williams *et al.* 2017; <sup>3</sup>Simon *et al.* 2017; <sup>4</sup>Radashevsky *et al.* 2020; <sup>5</sup>Abe & Sato-Okoshi 2021.

Species	Location	Date	Voucher MIMB	GenBank accession number*		
				I6S	I8S	28S
17. <i>P. nordica</i> Radashevsky, 2021	Norway, North Sea, Raunefjord, Bergen <sup>1</sup>	25 Jul. 2014	MIMB 40380; VIR 20096	MG460960–61	<b>MW904144–45</b>	MG461040–41 MG460885–86
18. <i>P. nordica</i> Radashevsky, 2021	Norway, Skagerrak, Sandefjord, Håholmboen <sup>4</sup>	29 May. 2011	VIR 20239	MG460962	–	MG461042
19. <i>P. nordica</i> Radashevsky, 2021	Norway, Grottsund Fjord	12 Sep. 2018	MIMB 39054; VIR 21160	<b>MW904104–05</b>	<b>MW904142–43</b>	<b>MW904148–49</b> <b>MW923739–40</b>
20. <i>P. paucibranchiata</i> (Okuda, 1937)	South Korea, East Sea, Jimhae Bay <sup>4</sup>	14 Oct. 2015	MIMB 33602; VIR 19684	MG460949–54	<b>MW904128–33</b>	MG461029–34 MG460874–79
21. <i>P. tsubaki</i> Simon, Sato-Okoshi & Abe, 2017	Japan, Habu Port <sup>3,5</sup>	21 Apr. 2016	VIR 23830	LC107857–59, LC595762	AB973929, LC545920	AB973937–39
22. <i>P. tsubaki</i>	Japan, Tomiura <sup>5</sup>	Jun. 2016	VIR 24447	LC595762	LC545920	–
23. <i>P. ushioni</i> Simon, Sato-Okoshi & Abe, 2017	Japan, Uranouchi Bay, Kochi <sup>1,3</sup>	20 Sep. 2011	VIR 23827	LC107854–56	AB973926–28	AB973934–36
24. <i>P. ushioni</i> Simon, Sato-Okoshi & Abe, 2017	Taiwan, Haishan Marina	13 Jun. 2008	MIMB 40925; VIR 13440	<b>MW904096</b>	<b>MW904108</b>	<b>MW904152</b> <b>MW923737</b>
25. <i>P. uphondo</i> Simon, Sato-Okoshi & Abe, 2017	South Africa, Sodwana Bay <sup>3</sup>	Dec 2014	VIR 23841	LC107865–66	LC107847–48	LC107851–52
26. <i>P. vexillosa</i> Radashevsky & Hsieh, 2000	Vietnam, Nha Trang Bay, Mung Is. <sup>4</sup>	27 Jun 2006	MIMB 40926; VIR 10795	MG460890–91	<b>MW904146–47</b>	MG460966–67 MG460821–22
27. <i>Pseudopolydora</i> sp. Sasuhama	Japan, Sasuhama <sup>5</sup>		VIR 24448	LC595763	LC545921	–
28. <i>Pseudopolydora</i> sp. Sodwana	South Africa, Sodwana Bay <sup>3</sup>	2014	VIR 24388	LC107867	LC107849	LC107853

\* Two last digits are shown for the second and other numbers in a successive series.

The combined concatenated dataset contained at least 480 variable sites, 444 of which were parsimony informative; the frequencies of variable and informative sites were 19.4% and 92.5%, respectively. The frequency of variable sites in the aligned sequences of mitochondrial *16S* rDNA (46.1%) was greater than those in sequences of the nuclear *28S* rDNA (18.7%), *18S* rDNA (13.7%) and *Histone 3* (30%).

The lowest interspecies *p*-distances obtained in the analysis were between *P. uphondo* Simon, Sato-Okoshi & Abe, 2017/*P. tsubaki* Simon, Sato-Okoshi & Abe, 2017 and *P. uphondo*/*P. antennata* taxon pairs: 0.06% for *18S* rDNA. The highest interspecies *p*-distances were between *P. eriyali* Simon, Sato-Okoshi & Abe, 2017 and *P. vexillosa* Radashevsky & Hsieh, 2000: 28.12% for *16S* rDNA (Supp. Table ESM2).

The Bayesian analysis resulted in a fully resolved consensus tree and revealed the most basal position of *P. dayi* Simon, 2009 within all species of *Pseudopolydora* (Fig. 2).

### Morphology and biology

Phylum Annelida Lamarck, 1809  
Family Spionidae Grube, 1850

Genus *Pseudopolydora* Czerniavsky, 1881

*Pseudopolydora* Czerniavsky, 1881: 362.

*Carazzia* Mesnil, 1896: 227.

*Polydora* (*Carazzia*) – Fauvel 1927: 48; 1953: 316.

*Polydora* (*Pseudopolydora*) – Hartmann-Schröder 1971: 317; 1996: 322.

*Pseudopolydora* – Fauchald 1977: 25. — Blake & Kudenov 1978: 267. — Blake 1996: 202. — Blake *et al.* 2020: 79–80.

### List of species of *Pseudopolydora* from the Arabian Gulf and their type localities

*Pseudopolydora achaeta* Radashevsky & Hsieh, 2000, South China Sea, Taiwan.

*Pseudopolydora antennata* (Claparède, 1868). Tyrrhenian Sea, Gulf of Naples, Italy.

*Pseudopolydora arabica* Radashevsky & Al-Kandari, 2020. Arabian Gulf, Al-Salmiya, Kuwait.

*Pseudopolydora auha* sp. nov. Arabian Gulf, Auha Is., Kuwait.

*Pseudopolydora kuwaiti* sp. nov. Arabian Gulf, Al-Judailiat, Kuwait.

*Pseudopolydora melanopalpa* sp. nov. Arabian Gulf, Sulaibikhat Bay, Kuwait.

*Pseudopolydora multispinosa* sp. nov. Arabian Gulf, Al-Salmiya, Kuwait.

### Identification key to *Pseudopolydora* from the Arabian Gulf

1. Chaetiger 5 distinctly larger than chaetiger 4. Chaetiger 5 spines arranged in a curved diagonal double row. Occipital antenna absent. Yellow bands regularly arranged on palps (invisible after fixation). Caruncle to end of chaetiger 2. Tube-dwelling or shell-boring ..... *P. kuwaiti* sp. nov.
  - Chaetiger 5 same in size as chaetiger 4. Chaetiger 5 spines arranged in a slightly curved, J- or U-shaped vertical double row. Occipital antenna present. Yellowish chromatophores present or absent on palps in live. Caruncle to end of chaetiger 2 or longer. Tube-dwelling ..... 2
2. Chaetiger 5 spines arranged in a straight or slightly curved vertical double row. Caruncle to end of chaetiger 2. Black pigment present on dorsal side of 10–15 anterior chaetigers ..... *P. achaeta* Radashevsky & Hsieh, 2000
  - Chaetiger 5 spines arranged in a J- or U-shaped vertical double row. Caruncle extending beyond chaetiger 2. Black pigment absent on dorsal side of anterior chaetigers ..... 3



3. Prostomium anteriorly entire, rounded. Chaetiger 5 spines arranged in a J-shaped double row. Yellowish chromatophores regularly arranged on palps (invisible after fixation). Caruncle to end of chaetiger 4. Pygidium disc-like to cup-shaped with only dorsal gap .....  
..... *P. arabica* Radashevsky & Al-Kandari, 2020
- Prostomium anteriorly incised to bifurcated. Chaetiger 5 spines arranged in a U-shaped double row. Yellowish chromatophores absent on palps. Caruncle to end of chaetiger 4 or longer. Pygidium bilobed with dorsal and ventral clefts ..... 4
4. Fine black pigment scattered on distal end of palps. Prostomium anteriorly incised, with two rounded lobes ..... *P. melanopalpa* sp. nov.
- No pigmentation on palps. Prostomium with two rounded lobes or long pointed antero-lateral processes ..... 5
5. Chaetiger 5 with up to 32 spines in the outer (anterior) row of notochaetae, and 27 spines in the inner (posterior) row of notochaetae; notopodial postchaetal lamellae same as on chaetiger 4 or 6. MG staining of formalin-fixed specimens drastically intense on ventral side from chaetiger 8 onwards. Notopodial anterior-row spines of chaetiger 5 with distal part of stem curved, gradually narrowing, with wide, distally pointed limbation ..... *P. multispinosa* sp. nov.
- Chaetiger 5 with fewer spines in notopodia; notopodial postchaetal lamellae reduced. MG staining on ventral side of formalin-fixed specimens comparatively weak, gradually changing along body. Notopodial anterior-row spines of chaetiger 5 with distal part of stem enlarged, with concavity on top and large triangular tooth on its side directed upwards; fine bristles arising from concavity forming long flag-like pointed geniculate transparent tip ..... 6
6. Caruncle to end of chaetiger 4 ..... *P. antennata* (Claparède, 1868)
- Caruncle to end of chaetiger 6 ..... *P. auha* sp. nov.

*Pseudopolydora achaeta* Radashevsky & Hsieh, 2000

Fig. 3

*Pseudopolydora achaeta* Radashevsky & Hsieh, 2000: 223–226, figs 4–5, 11a.

*Pseudopolydora achaeta* – Lana *et al.* 2006: 50. — Zvyagintsev *et al.* 2011: 53. — Abe *et al.* 2014: 3–5; 2016: 654–656, fig. 3; 2019: 6–11. — Bogantes *et al.* 2021: 581, fig. 2a–b.

*Pseudopolydora* aff. *achaeta* – Abe & Sato-Okoshi 2021: 56–57, fig. 9a–b (larval morphology).

*Pseudopolydora* sp. A – Radashevsky & Migotto 2006: fig. 1c.

### Description

Two specimens were found in Sulaibikhat Bay, Kuwait, comprising 20-chaetiger anterior fragment of a small juvenile and a 70-chaetiger complete female about 16 mm long and 0.8 mm wide (MIMB 40934; Fig. 3A–B). Transverse bands of diffused black pigment present on dorsal side of up to 15 anterior chaetigers; small middorsal melanophores present from chaetigers 4–6 to chaetigers 10–12. Prostomium anteriorly weakly incised, notched or almost blunt, posteriorly extending to end of chaetiger 2 as a low caruncle. Occipital antenna present. Chaetiger 1 reduced, weakly separated from peristomium, with small notopodial and well developed neuropodial lamellae; notochaetae absent; neurochaetae comprising 1–5 very fine, hair-like capillaries. Chaetiger 5 same in size as chaetigers 4 or 6, with dorsal superior and ventral capillaries same in shape and number as those chaetae on chaetigers 4 or 6; two kinds of heavy spines arranged in a vertical slightly curved double row; noto- and neuropodial postchaetal lamellae present (Fig. 3C–E). Anterior-row spines pennoned, with curved pointed tip, without subdistal constriction (Fig. 3F), up to 22 in a series; posterior-row spines simple falcate (Fig. 3G), up to 19 in

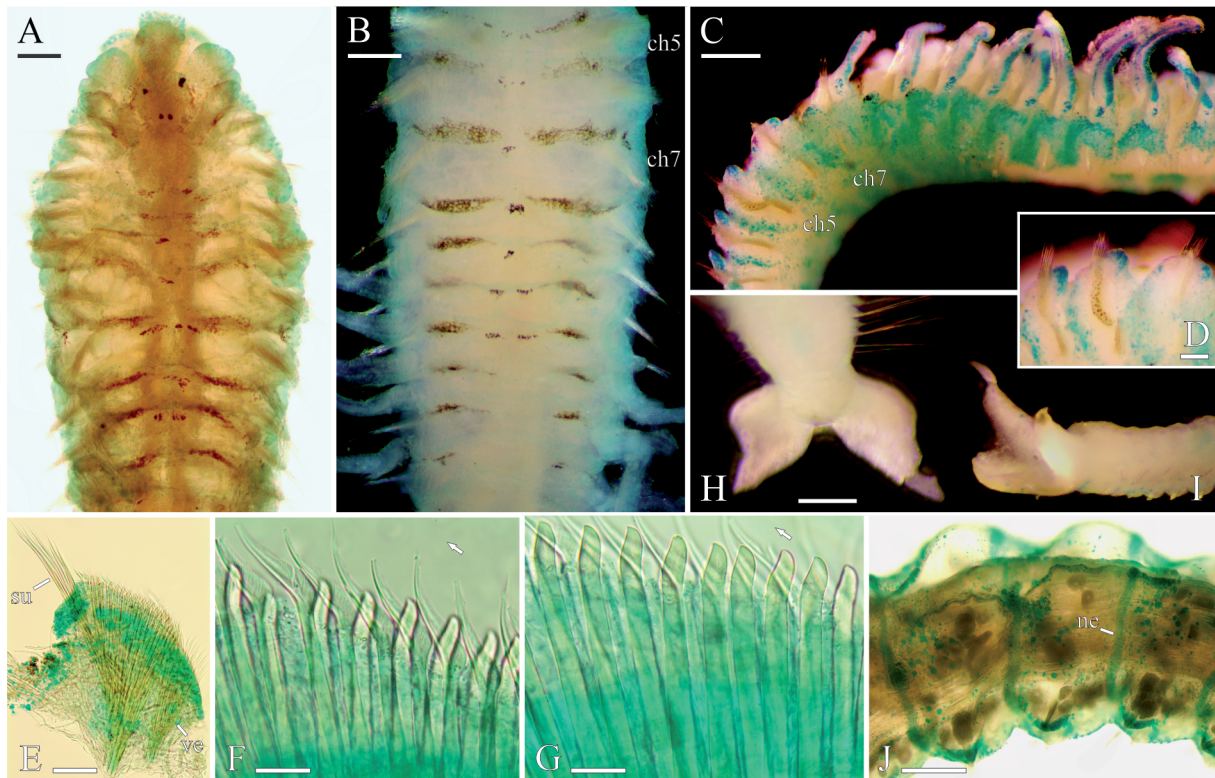
a series. Bidentate hooded hooks in neuropodia from chaetiger 8, up to 15 in a series. Branchiae from chaetiger 7 to chaetiger 15. Pygidium flaring disc with wide dorsal gap and dorso-lateral processes (Fig. 3H–I). Glandular pouches in neuropodia from chaetiger 1, largest and paired in each neuropodium in chaetigers 6 and 7, single in other neuropodia.

### MG staining

Intensely stained ventral and lateral sides and notopodial postchaetal lamellae of 15–16 anterior chaetigers, outer edges of branchiae (Fig. 3C); narrow transverse bands on dorsal side of branchiate chaetigers.

### Remarks

*Pseudopolydora achaeta* was originally described from the South China Sea, Taiwan, as a common polychaete inhabiting tubes in soft sediments in brackish-water environments (Radashevsky & Hsieh 2000). Since then, the species was reported from Paraná and São Paulo (Brazil) (Lana *et al.* 2006; Radashevsky & Migotto 2006), Sea of Japan (Russia) (Zvyagintsev *et al.* 2011), Pacific side of Honshu



**Fig. 3.** Adult morphology of *Pseudopolydora achaeta* Radashevsky & Hsieh, 2000 (formalin-fixed and stained with MG specimens, MIMB 40934). **A.** Anterior end of a small juvenile, dorsal view, palps missing. **B.** Chaetigers 5–14 of a 70-chaetiger female, dorsal view. **C.** Chaetigers 3–15 of a 70-chaetiger female, left lateral view. **D.** Chaetigers 4–6, left lateral view, showing vertical arrangement of heavy spines in notopodium of chaetiger 5. **E.** Parapodia of chaetiger 5. **F.** Heavy spines from notopodium of chaetiger 5, focused on anterior-row spines with a pennoned distal end. **G.** Same, focused on posterior-row falcate spines. **H.** Posterior end, dorsal view. **I.** Same, right lateral view. **J.** Middle female chaetigers, left lateral view. Abbreviations: arrow showing the direction towards the posterior end of the body; *ch5* = *ch7* = chaetigers 5 and 7; *ne* = nephridium; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A, D–E = 100  $\mu$ m; B–C, H–J = 200  $\mu$ m; F–G = 20  $\mu$ m.

Island (Japan) (Abe *et al.* 2014, 2016, 2019; Abe & Sato-Okoshi 2021), and from Florida (USA) (Bogantes *et al.* 2021). Here, for the first time, we report it for the Arabian Gulf (Kuwait). The only female had small oocytes up to 65 µm in diameter developing from chaetiger 15 onwards (Fig. 3J). The morphology of the specimens fits the diagnostic characters of *P. achaeta*. The same pattern of methylene green staining was observed in the type specimens of *P. achaeta* from Taiwan (MIMB 3401). The wide distribution of this species outside of its native area in the Northwest Pacific can be explained by unintentional human-mediated transportations of larvae with ballast water of ships, followed by successful invasions.

### Distribution

South China Sea: Taiwan; Japan: Pacific side of Honshu Island; Brazil: Paraná, São Paulo; Atlantic USA: Florida; Arabian Gulf: Kuwait.

### *Pseudopolydora antennata* (Claparède, 1868)

Figs 1A, 4, 5A–C, 6–9

*Polydora antennata* Claparède, 1868: 320–321, pl. XXI fig. 3, 3a–c.

*Polydora antennata* – Claparède 1869: 60–61, pl. XXI fig. 3, 3a–c; 1870: 60–61, pl. XXI fig. 3, 3a–c. — Carus 1885: 257. — Carazzi 1893: 25–26, pl. 2 figs 11–12. — Lo Bianco 1893: 30. — Bhaud 1972: 125.

*Pseudopolydora antennata* – Czerniavsky 1881: 362. — Guille & Laubier 1966: 271. — Mohammad 1971: 296. — Lardicci 1989: 137–138. — Simboura & Nicolaidou 2001: 78. — García-Arberas & Rallo 2002: 50. — Dauvin *et al.* 2003: 84. — Castelli *et al.* 2008: 356. — Mikac 2015: 121. — Radashevsky 2021: 2–7, figs 1–2.

*Pseudopolydora* sp. A – Al-Kandari *et al.* 2019: 9. — Radashevsky *et al.* 2020: table 1, fig. 1.

### Description

Adults up to 16 mm long, 1 mm wide with 86 chaetigers. No pigmentation on body and palps; fine black pigment scattered on dorsal side of anterior chaetigers without particular pattern in some individuals. Prostomium anteriorly bifurcated, with two long, distally narrowing lobes (Fig. 4A–D), posteriorly extending to end of chaetiger 4 as a low caruncle, shorter in small individuals. Long cirriform occipital antenna present on caruncle between palps (Fig. 4C). Two pairs of black eyes arranged trapezoidally, comprising one pair of median eyes, and one pair of lateral eyes situated anteriorly and set wider apart. Palps as long as 15–25 chaetigers, with frontal longitudinal groove lined with fine cilia, and short compound non-motile cilia arising directly from palp surface sparsely arranged in line on sides of groove and sparsely scattered on lateral and abfrontal palp surfaces.

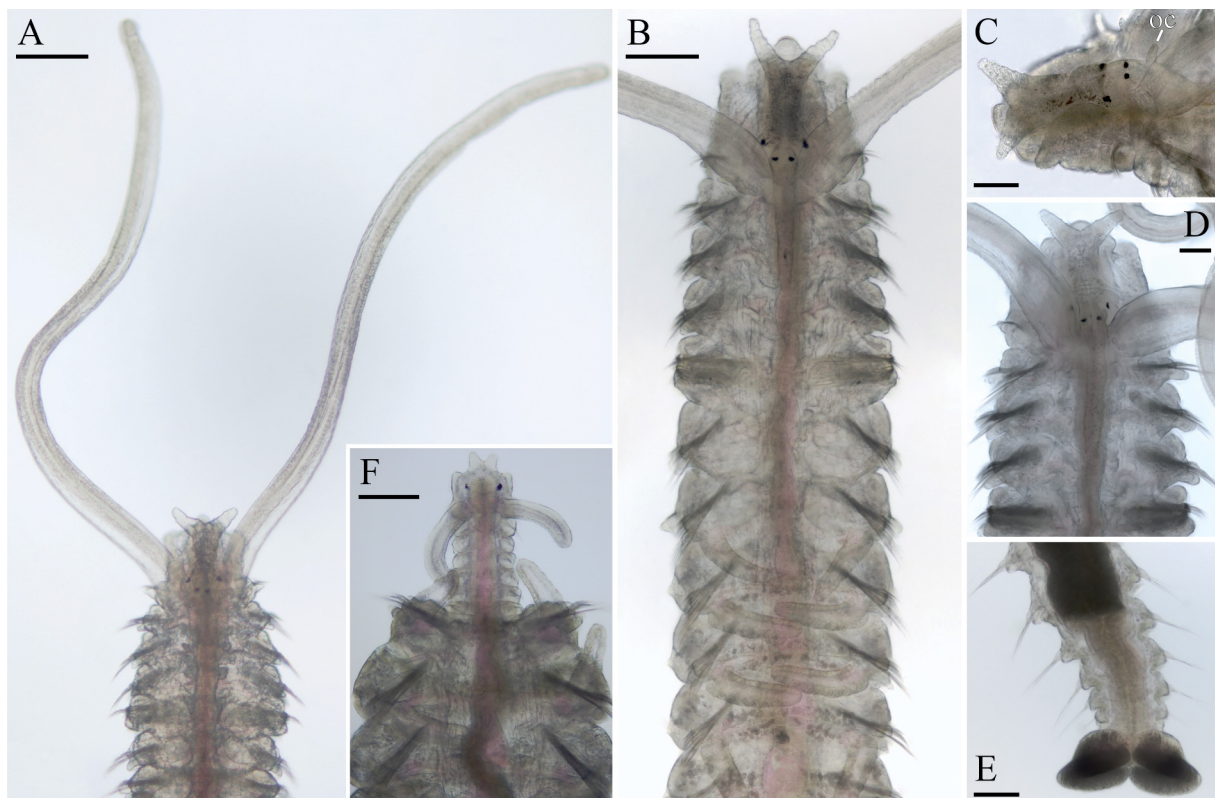
Chaetiger 1 with short capillaries in neuropodia, short notopodial lamellae and well-developed neuropodial postchaetal lamellae; notochaetae absent. Chaetiger 2 notochaetae all slender capillaries. Anterior-row notopodial capillaries on chaetiger 3 with slightly enlarged wing; capillaries on chaetiger 4 with wing slightly larger than on chaetigers 3. Anterior-row capillaries on chaetigers 3, 4, 6 and 7 arranged in J-shaped series; posterior-row capillaries on these chaetigers arranged in vertical series (Fig. 6A). Posterior notopodia with a few long alimbate capillary chaetae arising from elongated fleshy notopodial lobes. Posterior neuropodia with elongated fleshy lobes, with hooks arranged in line on top of lobes.

Chaetiger 5 same in size as chaetiger 4 or 6, with dorsal superior capillaries, two kinds of notopodial spines arranged in a double U-shaped row, and ventral capillaries; notopodial postchaetal lamellae reduced, but neuropodial lamellae same as on chaetigers 4 and 6 (Fig. 6B). Dorsal superior capillaries

shorter and fewer than those capillaries on chaetigers 4 and 6. Ventral capillaries same in size, number and arrangement (in three groups) as those on chaetiger 4 (Fig. 6B). Upper posterior part of double U-shaped row of spines equal to or slightly lower than upper anterior part. Newly developed spines in posterior upper part of U-shaped row slightly larger than older spines in anterior upper part of row. Outer (anterior-row) notopodial spines up to 11 in a series, with distal part of stem enlarged, with concavity on top and large triangular tooth on its side directed upwards and facing towards the inside of the U-shaped row of spines; fine bristles arising from concavity forming long flag-like pointed transparent tip which usually broken in worn old spines in anterior upper part of row (Fig. 6B–C). Inner (posterior-row) notopodial spines up to eight in a series, falcate, with short rounded distal part geniculate, with subdistal bulbous swelling bearing very short fine bristles and facing towards the inside of the U-shaped row of spines (Fig. 6B–C).

Hooks in neuropodia from chaetiger 8, up to 30 in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang; upper part of shaft with constriction; lower part of shaft bent at right angle in hooks in posterior neuropodia (Fig. 6D–E).

Branchiae up to 42 pairs from chaetiger 7 to chaetiger 48, fewer in small individuals (Fig. 5A), present beyond midbody in individuals with more than 30 chaetigers (Fig. 5C). Branchiae full-sized from chaetigers 10–11, free from notopodial postchaetal lamellae, flattened, with surfaces oriented



**Fig. 4.** Adult morphology of *Pseudopolydora antennata* (Claparède, 1868) (live individuals). **A.** MIMB 40895. **B–C, E.** MIMB 40897. **D.** MIMB 40899. **F.** MIMB 40896. **A–B, D.** Anterior ends, dorsal view. **C.** Anterior end, left lateral view. **E.** Posterior end, dorsal view. **F.** Middle stalk chaetigers regenerating seven anterior chaetigers, dorsal view. Abbreviations: *oc* = occipital antenna. Scale bars: A–B, F = 200  $\mu$ m; C–E = 10  $\mu$ m.

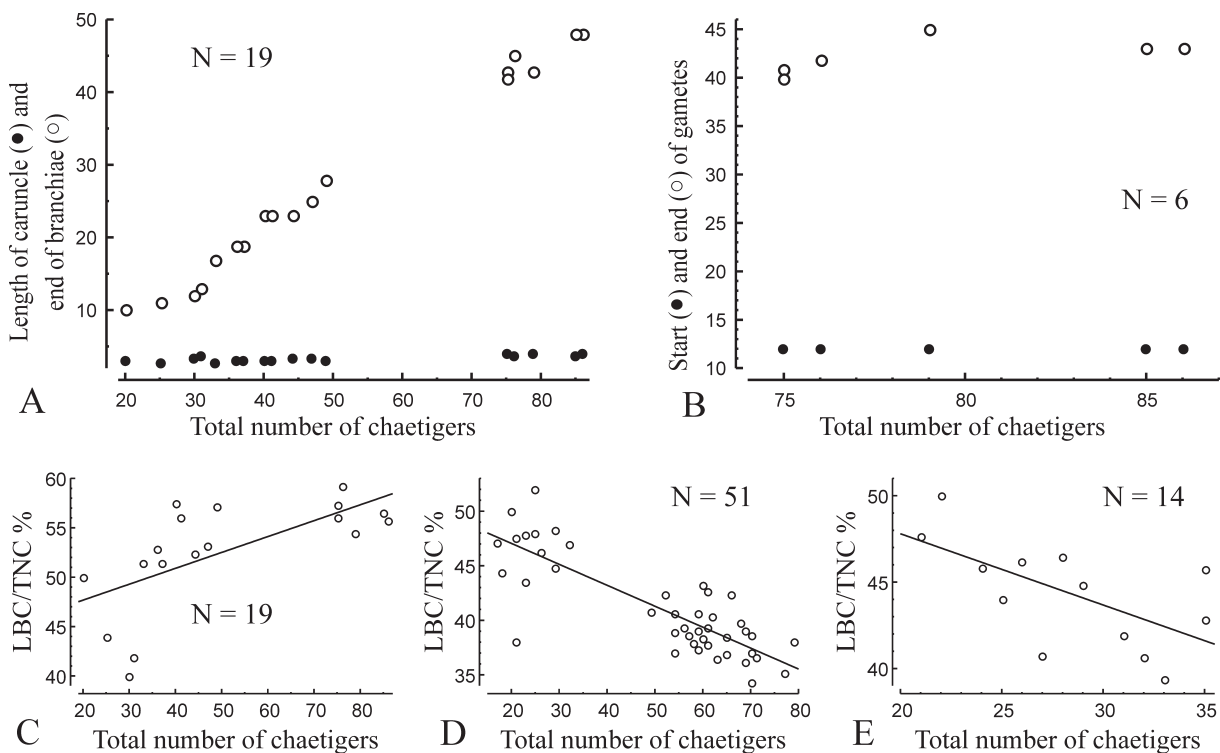
perpendicular to body axis, with longitudinal ciliation (extension of nototroch) running on inner anterior edge.

Nototrochs usually from chaetiger 7 onwards, occasionally from chaetiger 6 or 8, composed of single rows of cilia in both sexes. On branchiate chaetigers, nototroch cilia long, arranged in transverse lines and extending onto branchiae; on posterior abranchiate chaetigers, cilia arranged in U-shaped bands, with arms directed posteriorly. Additional ciliation on chaetigers absent.

Pygidium bilobed, with two semi-oval lateral lobes (Fig. 4E), white due to great number of spindle-shaped glandular cells with striated content.

Subspherical and of irregular shape glandular cells with striated content present on dorsal side of chaetigers. Cells few on anterior and posterior chaetigers, forming distinct paired gatherings from chaetigers 8–11 through two thirds of body, making this part of dorsum whitish in life (Figs 4B, 7C).

Glandular pouches in neuropodia from chaetiger 1, largest and paired in each neuropodium in chaetigers 6 and 7, single in other neuropodia (Fig. 7A–B).

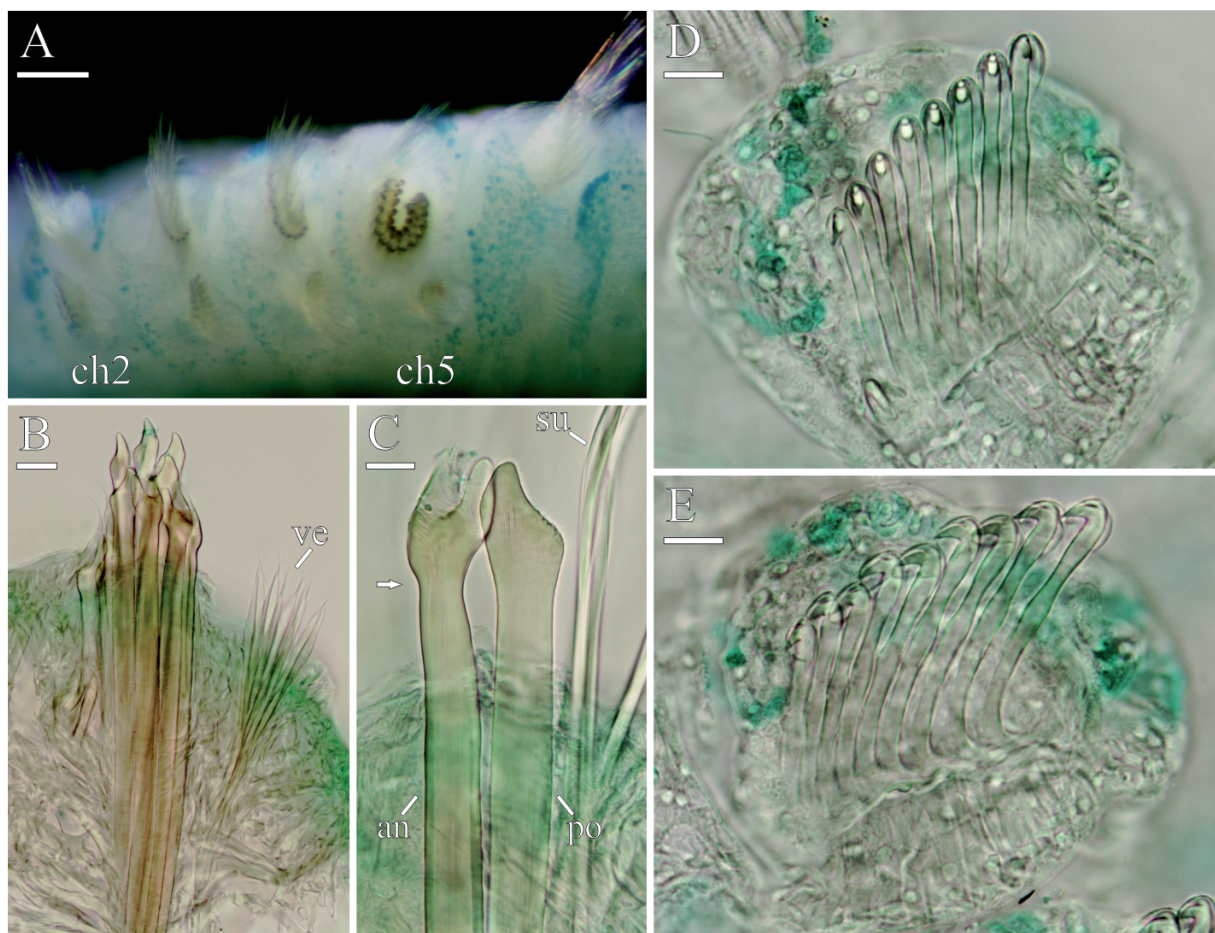


**Fig. 5.** Adult characteristics of *Pseudopolydora* spp. from the Arabian Gulf, Kuwait. **A–C.** *Pseudopolydora antennata* (Claparède, 1868). **D.** *P. arabica* Radashevsky & Al-Kandari, 2020. **E.** *P. kuwaiti* sp. nov. **A.** Relationships between caruncle length (in chaetiger numbers) and total number of chaetigers, and between distribution of branchiae (referring to number of the last branchiate chaetiger) and total number of chaetigers. **B.** Relationships between distribution of gametes (referring to number of the first and the last chaetigers with gametes) and total number of chaetigers. **C–E.** Relationships between distribution of branchiae (referring to number of the last branchiate chaetiger LBC divided by the total number of chaetigers TNC) and total number of chaetigers.

Digestive tract without ventral buccal bulb and gizzard-like structure, not pigmented.

Main dorsal blood vessel transformed into gut sinus in anterior part of midgut. Heart body absent. Blood red, without globules or other elements.

Nephridia from chaetiger 4 onwards, very narrow in chaetigers 4–6, well-developed from chaetiger 7. In female fertile chaetigers, paired nephridia on each chaetiger opening to exterior via common middorsal nephridiopore situated anterior to nototroch; walls of distal parts of nephridia containing glandular cells marking nephridia in live individuals (Fig. 7D–F) and also in fixed specimens stained with MG (Fig. 8C).



**Fig. 6.** Chaetal morphology of *Pseudopolydora antennata* (Claparède, 1868) (formalin-fixed and stained with MG specimens, MIMB 40895). **A.** Chaetigers 2–6, left lateral view, showing mirror reflected J-shaped arrangement of the anterior-row notochaetae in notopodia of chaetigers 3 and 4, and U-shaped arrangement of spines in notopodium of chaetiger 5. **B.** Parapodium of chaetiger 5, dorsal superior capillaries missing. **C.** Notochaetae of chaetiger 5. **D–E.** Series of bidentate hooded hooks from middle neuropodia, frontal (D) and lateral (E) view. Abbreviations: *arrow* showing the direction towards the inside of the U-shaped row of chaetiger 5 spines; *an* = anterior-row spine from notopodium of chaetiger 5; *ch2*, *ch5* = chaetigers 2 and 5; *po* = posterior-row spine from notopodium of chaetiger 5; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A = 100  $\mu$ m; B = 20  $\mu$ m; C–E = 10  $\mu$ m.

### MG staining

Intensely stained dorsal sides of the prostomium and peristomium; most intensely stained blotches on the dorsal and lateral sides from chaetigers 7–9 onwards; wide transverse bands on the ventral side from chaetigers 7–8 to chaetigers 10–11 (Fig. 8A–F).

### Regeneration

One broken individual regenerated 7 anterior chaetigers (Fig. 4F).

### Habitat

Adult *P. antennata* inhabit silty tubes in muddy sand intertidally and in shallow waters. The population density in some local places can reach a maximum of 50 000 individuals per 1 m<sup>2</sup>.



**Fig. 7.** Adult morphology of *Pseudopolydora antennata* (Claparède, 1868) (live individuals). **A–B.** MIMB 40895. **C–D.** MIMB 40897. **E–F.** MIMB 40899. **A.** Anterior end, semi-preserved, dorsal view, showing arrangement of glandular pouches in neuropodia. **B.** Chaetigers 6–7, semi-squashed, dorsal view, showing double glandular pouches in each neuropodium. **C.** Middle chaetigers, dorsal view, showing paired gatherings of glandular cells on the dorsum. **D–F.** Female fertile chaetigers, dorsal view, showing distal parts of paired nephridia opening to a common middorsal nephridiopore on each chaetiger. Abbreviations: *ga* = gatherings of glandular cells; *gl* = glandular pouches in neuropodia; *ne* = nephridia in female fertile chaetigers; *oo* = oocytes; *po* = nephridiopore. Scale bars: A = 200 µm; B–F = 100 µm.

## Reproduction

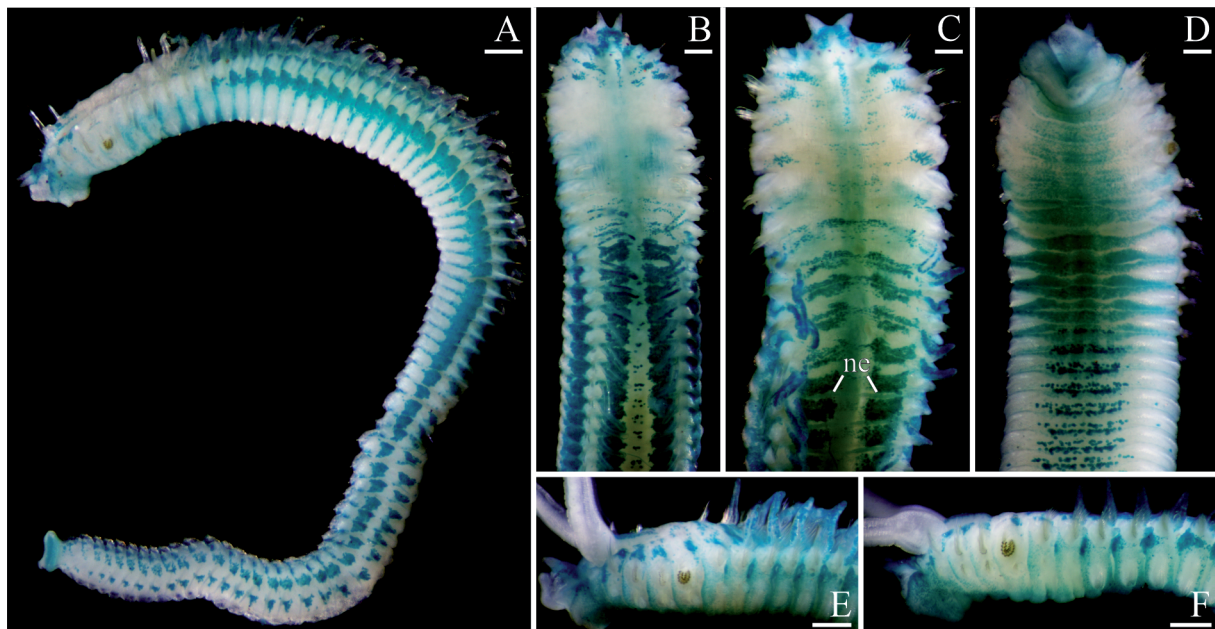
*Pseudopolydora antennata* is gonochoristic. Both females and males become mature after growing to about 10 mm long with 70 chaetigers. Of 21 examined mature individuals, 14 were females and seven were males. The gametes developed from chaetiger 12 to chaetigers 40–45 (Fig. 5B). Paired testes or ovaries were attached to segmental blood vessels in fertile chaetigers.

Oogenesis was partly intraovarian. Developed coelomic oocytes were spherical, about 130  $\mu\text{m}$  in diameter, with smooth envelope less than 1  $\mu\text{m}$  thick (Fig. 7E–F).

Spermatogonia proliferated in testes; spermatogenesis occurred in the coelomic cavity. Spermatids, each about 4  $\mu\text{m}$  in diameter, were joined in tetrads. Spermatozoa were introsperm with pointed acrosome about 1  $\mu\text{m}$  long, straight elongated nucleus about 1.5  $\mu\text{m}$  in diameter and 6  $\mu\text{m}$  long, midpiece 33  $\mu\text{m}$  (head + midpiece about 40  $\mu\text{m}$  long), and a flagellum about 40  $\mu\text{m}$  long (the precision of measurement of spermatids and parts of head of spermatozoa were at maximum to the nearest 0.5  $\mu\text{m}$  and flagellum to the nearest 2  $\mu\text{m}$ ).

## Larval development in the plankton

The spermatophores and egg capsules, typically produced by *Pseudopolydora*, were not observed in *P. antennata* from the Arabian Gulf. The 13–15-chaetiger larvae of the species were collected in plankton in March 2016. The larvae were identified by the morphology of the heavy spines in the notopodia of chaetiger 5. Although similar spines are also present in worms of two other species described below, those species were quite rare and not found in the area where numerous larvae were collected; *P. antennata* worms were common in that area and the larvae are therefore referred to this species. The larvae were maintained in Petri dishes in the laboratory but did not settle during the study.

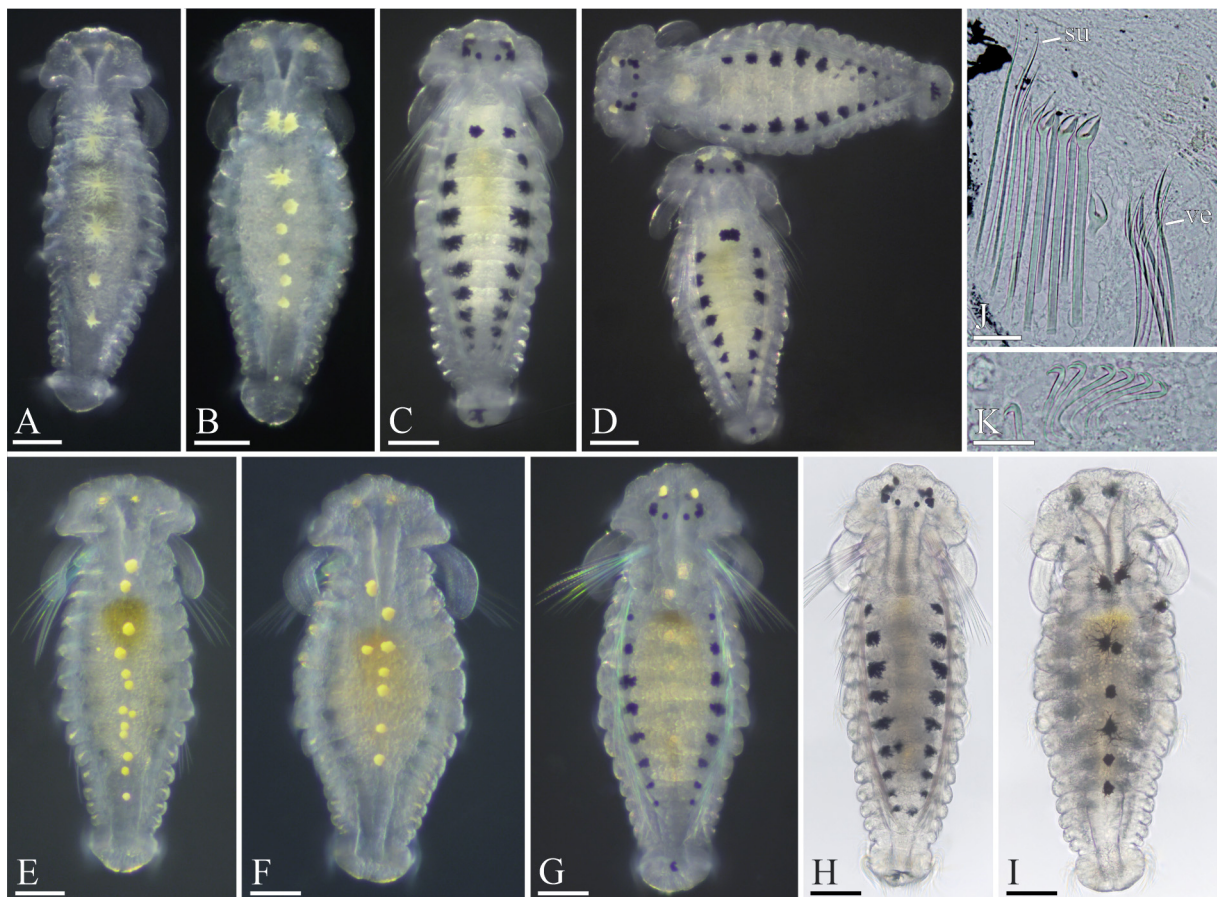


**Fig. 8.** MG staining of *Pseudopolydora antennata* (Claparède, 1868) (formalin-fixed specimens, MIMB 40895). **A.** Left lateral general view, palps missing. **B–D.** Anterior ends, dorsal (B–C) and ventral (D) view, palps missing. **E–F,** Anterior ends, left lateral view. Abbreviations: *ne* = nephridia in female fertile chaetiger. Scale bars: A, E–F = 200  $\mu\text{m}$ ; B–D = 100  $\mu\text{m}$ .



### 13–14-chaetiger larvae

Larvae 750–850  $\mu\text{m}$  long and up to 300  $\mu\text{m}$  wide in middle part, intensely pigmented with large melanophores and chromatophores (Fig. 9A–I). Distinct black spots present between median and lateral eyes, and small ramified melanophores of variable shape situated anterior to each pair of lateral eyes. Of 29 examined larvae, 13 (45%) larvae with one pair of large ramified dorsal melanophores on chaetiger 3 (Fig. 9C–D); 3 (10%) larvae with only one of these melanophores (either left or right) present; and 13 (45%) larvae without dorsal melanophores on chaetiger 3 (Fig. 9D, G–H). Large ramified dorso-lateral melanophores invariably present from chaetiger 4 onwards. Small to large ramified middorsal melanophore present on pygidium, situated posterior to telotroch. Melanophore in lateral peristomial lips, middorsal and lateral melanophores on chaetigers absent. One, rarely two pairs of subspherical yellow chromatophores present in front of lateral eyes, near melanophores in anterior part of prostomium. Larger subspherical yellow midventral chromatophores arranged from chaetiger 2 to chaetigers 7–12; shape and arrangement of these chromatophores greatly variable (Fig. 9A–B, E–F, I). They are usually single, occasionally double per chaetiger, rarely missing on chaetiger 6 or 7 or both. Chromatophore of chaetiger 3 often expanded onto posterior part of chaetiger 2. Yellow pigment diffused in anterior part of prostomium and in pygidium.



**Fig. 9.** Larval morphology of *Pseudopolydora antennata* (Claparède, 1868). Live larvae in reflected (A–G) and transmitted (H–K) light. **A.** 13-chaetiger larva, ventral view. **B.** 14-chaetiger larva, ventral view. **C.** The same, dorsal view. **D.** 14-chaetiger larvae, dorsal view, showing variation in pigmentation. **E.** 14-chaetiger larva, ventral view. **F.** 15-chaetiger larva, ventral view. **G.** The same, dorsal view. **H.** 14-chaetiger larva, dorsal view. **I.** The same, ventral view. **J.** Chaetae of chaetiger 5 of a 15-chaetiger larva. **K.** Hooded hooks from neuropodium of chaetiger 10 of a 15-chaetiger larva. Abbreviations: *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A–I = 100  $\mu\text{m}$ ; J–K = 20  $\mu\text{m}$ .

Chromatophores absent in lateral peristomial lips and on ventral side of pygidium. Both melanophores and chromatophores changing their shape and size according to light intensity, thus appearing small and compact (Fig. 9E–F) or greatly expanded and ramified (Fig. 9A, C).

Prostomium wide and rounded anteriorly, posteriorly narrow, extending to end of chaetiger 1 as a low caruncle. Occipital antenna absent. Three pairs of black eyes arranged almost in a transverse line, comprising one pair of small rounded median eyes and two pairs of lateral eyes; lateral eyes on each side situated close to each other and appearing as one comma-shaped eye. Black pigment spot situated between lateral and median eyes on each side. One pair of spherical unpigmented ocelli, each about 20 µm in diameter, situated in front of pigmented eyes. Palps arising from posterior dorso-lateral edges of peristomium, posterior to prototroch; each palps as long as about three chaetigers. Ciliation on palps not yet developed.

Prototroch and telotroch well developed. Nototrochs and grasping cilia from chaetiger 3 onwards. Triangular neurotroch extending from ventral peristomial lip to end of chaetiger 1. Two small ciliated cells situated on each side of neurotroch. Ventral ciliary pit absent. Gastrotroch on chaetiger 3 composed of two small cells with short cilia; those on chaetigers 5 and 7 each composed of 8–10 large cells with long numerous cilia; additional gastrotroch usually present on chaetiger 9, and in some larvae also on chaetiger 10 and/or chaetiger 11, each composed of one to two pairs of small cells with short cilia.

Larval serrated bristles present in all notopodia including chaetiger 5; those in chaetiger 1 longest, up to 250 µm long; bristles in chaetigers 2 and 3 175–200 µm long, then gradually becoming shorter in succeeding chaetiger. Few short adult capillaries present in notopodia from chaetiger 2, and in neuropodia in chaetigers 1–7.

Chaetiger 5 with two kinds of heavy spines in notopodia in addition to short adult capillaries and long provisional serrated bristles. Spines comprising 2–4 anterior-row spines with enlarged distal end of stem and pointed tip, and 1–2 posterior-row simple falcate spines (Fig. 9J).

Hooks in neuropodia from chaetiger 8 onwards, up to seven in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang, with outer hood; lower part of shaft bent almost at right angle (Fig. 9K).

Branchiae short, on chaetigers 7–9. Pygidium semispherical.

Lateral sensory organs each 5–6 µm in diameter with thin non-motile cilia up to 20 µm long situated between noto- and neuropodia from chaetiger 1 onwards, including chaetigers 3–5.

Glandular pouches in neuropodia from chaetigers 2–3, very small in first chaetigers, large in chaetigers 6 and 7.

Large lateral lips and small ventral lip of peristomium forming voluminous vestibulum. Narrow oesophagus extending through 2–3 chaetigers. Gizzard-like structure absent in digestive tract. Wall of voluminous midgut with numerous lipid globules, each up to 15 µm in diameter.

Circulatory system developed and functional; blood transparent, without pigment.

Two pairs of protonephridia in chaetigers 1 and 2. Metanephridia not yet developed.

#### **14–15-chaetiger larvae**

Larvae 850–900 µm long, with glandular pouches in neuropodia from chaetiger 1, protonephridia in chaetigers 1 and 2, and metanephridia from chaetiger 4 onwards.

## Remarks

Adult *Pseudopolydora* from the Arabian Gulf described above appear identical to *P. antennata* from Italy recently re-described by Radashevsky (2021), and are consequently referred to this species. They have the prostomia anteriorly bifurcated, with two long, distally narrowing lobes, caruncles extending to the end of chaetiger 4, comparatively short occipital antennae, chaetiger 5 spines of the same morphology, bilobed pygidia with two fleshy semi-oval lateral lobes, and gametes developing from chaetiger 12.

The exact dimensions of the gametes of *P. antennata* from the Mediterranean remain unknown but worms from the Arabian Gulf are unusual among *Pseudopolydora* in having spermatids joined in tetrads instead of octads, and the spermatozoa with an extremely long midpiece, about 33  $\mu\text{m}$  instead of 3.2–4.6  $\mu\text{m}$  long as in other examined species (see review by Blake & Arnofsky 1999: table 2).

The morphology of genuine *P. antennata* larvae from the Mediterranean has never been described or illustrated (see review by Radashevsky 2021). Hannerz (1956) described and illustrated pelagic, ready to metamorphose 11- and 13–14-chaetiger *Pseudopolydora* larvae, and a 19-chaetiger juvenile from Gullmar Fjord, Sweden. He referred them to *P. antennata* and reported that the larvae occurred in the Fjord from July through November but were relatively scarce. The Arabic larvae herein referred to *P. antennata* are similar to those described by Hannerz (1956) only in having dorso-lateral ramified melanophores from chaetiger 4 onwards, but differ significantly by having 1–2 pairs of subspherical yellow chromatophores in front of lateral eyes, large yellow melanophores on the ventral side of chaetigers, main gastrotrachs on chaetigers 3, 5, 7, and small additional gastrotrachs on chaetigers 9, 10 and/or chaetiger 11, instead of gastrotrachs on chaetigers 5 and 7 only. Moreover, the Arabic larvae are unique among *Pseudopolydora* larvae in the absence of large ramified middorsal melanophore on chaetiger 1. Rasmussen (1973: 113) described and perfectly illustrated egg capsules laid by *Pseudopolydora* in Isefjord, Denmark, and noted that “The hatched larvae from the Isefjord agreed closely with the description [of *P. antennata*] given by Hannerz (1956, pp 126–130)”. Following Hannerz (1956), Rasmussen (1973) referred Danish worms to *P. antennata*. Obviously, larvae from the Arabian Gulf referred to *P. antennata* in the present study are not conspecific with those from Gullmar Fjord described by Hannerz (1956). Which of them belong to *P. antennata* remains to be clarified.

The larvae from the Arabian Gulf referred to *P. antennata* are characterised by the following features: one pair of large ramified dorsal melanophores on chaetiger 3 (one or both of them can be absent), large ramified dorso-lateral melanophores from chaetiger 4 onwards, middorsal melanophore on the pygidium, paired yellow chromatophores in the anterior part of prostomium, and large median, usually unpaired, yellow chromatophores on the ventral side from chaetiger 2 onwards. Melanophores in lateral peristomial lips, middorsal and lateral melanophores on chaetigers, and chromatophores on the ventral side of pygidium, characteristic for larvae of other species of *Pseudopolydora*, are absent.

## Distribution

Mediterranean Sea; Arabian Gulf (Fig. 1A). See comments by Radashevsky (2021) about other records of this species.

*Pseudopolydora arabica* Radashevsky & Al-Kandari, 2020  
Figs 1B, 5D, 10

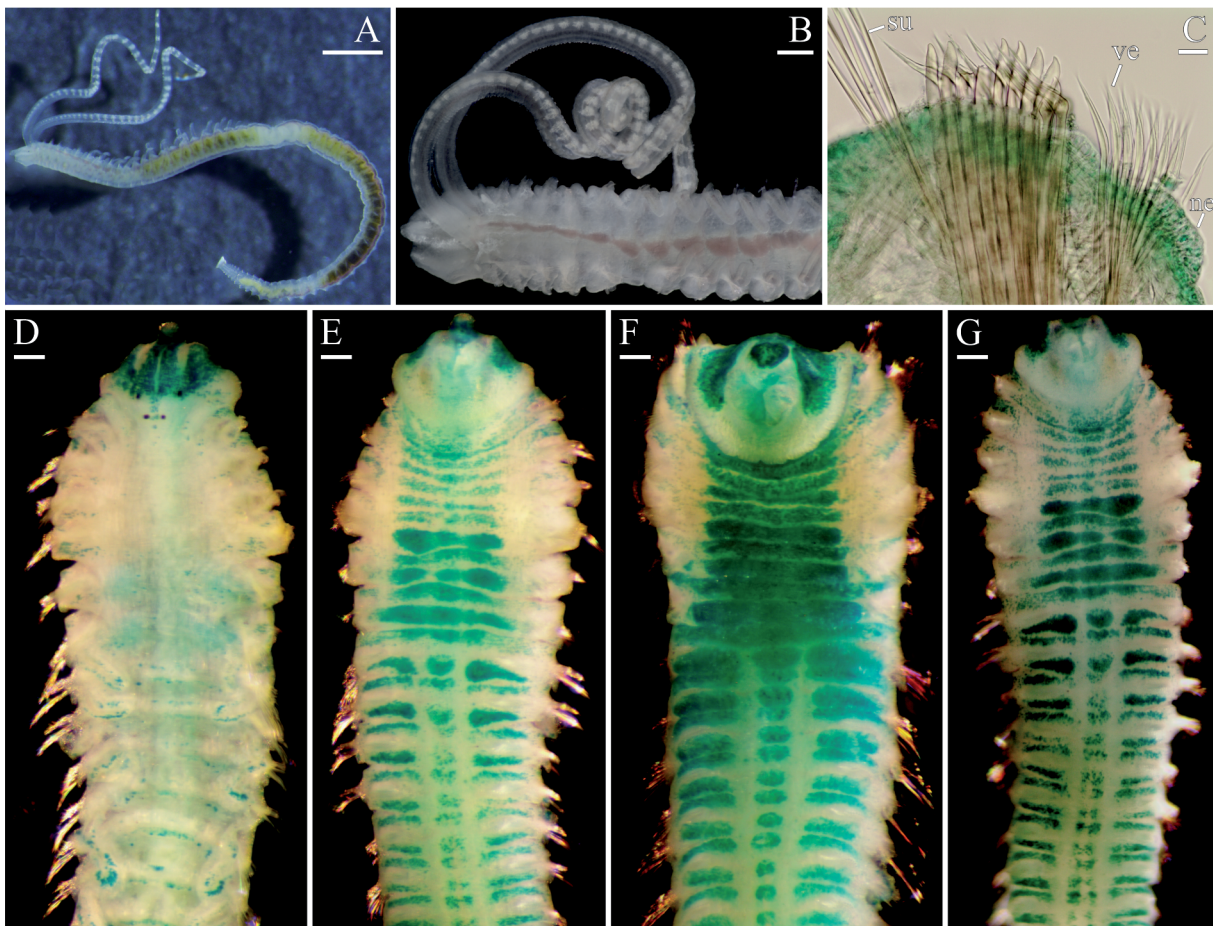
*Pseudopolydora arabica* Radashevsky & Al-Kandari, 2020: 2–10, figs 2–7.

*Pseudopolydora* sp. B – Al-Kandari *et al.* 2019: 9. — Radashevsky *et al.* 2020: table 1, fig. 1.

*Pseudopolydora paucibranchiata* – Swaleh & Mustaqim 1993: 204, fig. 2. — Not Okuda 1937.

**Adult diagnostic features**

Adults up to 20 mm long, 1 mm wide with 80 chaetigers (Fig. 10A). Live individuals with yellowish-white pigment on lateral sides of prostomium and dorsal side of 3–5 anterior chaetigers (most intense on chaetigers 1–2), and up to 50 yellowish-white ramified chromatophores on each palp in (Fig. 10A–B); chromatophores not visible after fixation. Prostomium anteriorly narrow and rounded (Fig. 10B). Caruncle extending to end of chaetiger 4. Occipital antenna present. Chaetiger 1 with short capillaries in neuropodia; notochaetae absent. Anterior-row capillaries in notopodia from chaetiger 7 to chaetigers 10–20 with wide subtriangular, pennoned limbation. Chaetiger 5 almost as same in size as chaetigers 4 and 6 (Fig. 10B); dorsal superior capillaries slightly shorter and fewer than those capillaries on chaetigers 4 and 6; ventral capillaries as same as those on chaetiger 4; two kinds of notopodial spines arranged in a double J-shaped row; outer (anterior-row) notopodial spines with geniculate distal tip with wide limbation; inner (posterior-row) notopodial spines simple falcate (Fig. 10C). Bidentate hooded hooks in neuropodia from chaetiger 8. Branchiae from chaetiger 7 to chaetiger 30, up to 24 pairs. Pygidium small,



**Fig. 10.** Adult morphology of *Pseudopolydora arabica* Radashevsky & Al-Kandari, 2020 live individuals (A–B) and formalin-fixed specimens stained with MG (C–G). A–C. MIMB 39111. D–G. MIMB 40911. A. Left lateral general view, in reflected light, showing yellowish-white chromatophores on palps. B. Anterior end, dorsal view, in reflected light. C. Parapodia of chaetiger 5. D. Anterior end, dorsal view. E–F. Anterior ends, ventral view. Abbreviations: *ne* = neuropodial postchaetal lamella; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A = 1 mm; B = 200 µm; C = 20 µm; D–G = 100 µm.

cup-shaped, with dorsal gap. Glandular pouches in neuropodia from chaetiger 1, largest in chaetigers 6 and 7, single pouch in each neuropodium.

#### **MG staining**

Intensely stained dorsal side of the prostomium in front of eyes, dorsal side of the peristomium, and ventral side of chaetigers: complete transverse paired bands moderate intense on chaetigers 1–4, very intense complete transverse paired bands on chaetigers 5–7, and paired transverse bands split into three parts from chaetiger 8 onwards (Fig. 10D–G). Remarkably, the three-parts banding was associated with the start of the hooded hooks in neuropodia and was invariable in all examined specimens.

#### **Remarks**

*Pseudopolydora arabica* Radashevsky & Al-Kandari, 2020 was originally described from the Arabian Gulf as one of the common polychaetes inhabiting soft sediments. The population density of the species in some places reaches 50 000 individuals per 1 m<sup>2</sup>.

#### **Distribution**

Arabian Gulf: Kuwait (Fig. 1B).

#### *Pseudopolydora auha* sp. nov.

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Figs 1D, 11

#### **Diagnosis**

Caruncle to end of chaetiger 6. Branchiae present beyond the middle of the body. Pygidium bilobed.

#### **Etymology**

The name refers to the type locality of the species in the intertidal of Auha Island, Arabian Gulf.

#### **Material examined**

##### **Holotype**

KUWAIT • 1 spec.; Arabian Gulf, Auha Is.; 29.3764° N, 48.4391° E; 29 Jan. 2017; Vasily I. Radashevsky leg.; low intertidal; from shell limestone encrusted by coralline alga; MIMB 40907.

##### **Paratype**

KUWAIT • 1 spec.; same collection data as for holotype; GenBank 16S gene MW904103, GenBank 18S gene MW904112, GenBank 28S gene MW904156, GenBank Histone 3 gene MW923741; used entirely for molecular analysis.

#### **Description**

Two complete individuals in good condition with palps were found together in a shell limestone encrusted by coralline alga. The 22-chaetiger individual is designated as the holotype. The 42-chaetiger paratype was examined and then used entirely for molecular analysis.

Holotype about 2 mm long, 0.37 mm wide with 22 chaetigers (Fig. 11A). Paratype about 5 mm long, with 42 chaetigers. Pigmentation absent on body and palps. Prostomium anteriorly bifid, with two short triangular lobes (Fig. 11A), posteriorly extending to end of chaetiger 6 as a low caruncle in both types. Occipital antenna present on caruncle between palps. Two pairs of black eyes arranged trapezoidally, comprising one pair of median eyes, and one pair of lateral eyes situated anteriorly and set wider

apart. Palps as long as 15–20 chaetigers, with frontal longitudinal groove lined with fine cilia, and short compound non-motile cilia arising directly from palp surface sparsely arranged in line on sides of groove and sparsely scattered on lateral and abfrontal palp surfaces.

Chaetiger 1 with short capillaries in neuropodia, and small lamellae in both rami; notochaetae absent. Chaetiger 2 notochaetae all slender capillaries with narrow limbation. Anterior-row notopodial capillaries on chaetiger 3 with slightly enlarged wing; capillaries on chaetiger 4 with wing slightly larger than on chaetigers 3. Anterior-row capillaries on chaetigers 3, 4, 6 and 7 arranged in J-shaped series; posterior-row capillaries on these chaetigers arranged in vertical series. Posterior notopodia with a few long alimbate capillary chaetae.

Chaetiger 5 slightly larger than chaetigers 4 or 6, with dorsal superior capillaries, two kinds of notopodial spines arranged in a double U-shaped row, and ventral capillaries; noto- and neuropodial postchaetal lamellae same as on chaetiger 4 (Fig. 11D). Dorsal superior capillaries slightly shorter and fewer than those capillaries on chaetiger 4. Ventral capillaries same in size, number and arrangement (in three groups) as those on chaetiger 4. Newly developed spines in posterior upper part of U-shaped row slightly larger than older spines in anterior upper part of row. Outer (anterior-row) notopodial spines 4–6 in a series, with distal part of stem enlarged, with concavity on top and large triangular tooth on its side directed upwards and facing towards the inside of the U-shaped row of spines; fine bristles arising from tooth and concavity forming a long flag-like pointed transparent tip which is usually broken in worn old spines in the anterior upper part of row (Fig. 11E). Inner (posterior-row) notopodial spines 4–5 in a series, falcate, with short rounded distal part geniculate, with subdistal bulbous swelling bearing very short fine bristles and facing towards the inside of the U-shaped row of spines (Fig. 11E).

Hooks in neuropodia from chaetiger 8, up to 20 in a series, not accompanied by capillaries, in posterior chaetigers situated on top of prominent fleshy neuropodial lobes. Hooks bidentate, with upper tooth closely applied to main fang; upper part of shaft with constriction; lower part of shaft bent at right angle (Fig. 11F).

Branchiae from chaetiger 7 to chaetiger 15 in holotype, and to chaetiger 24 in paratype, on chaetiger 7 slightly shorter than those on chaetiger 8, full-sized from chaetigers 9–10, free from notopodial postchaetal lamellae, flattened, with surfaces oriented perpendicular to body axis, with longitudinal ciliation (extension of nototroch) running on inner edge.

Pygidium bilobed, with two semi-oval lateral lobes, white due to many spindle-shaped glandular cells with striated content.

Glandular pouches in neuropodia from chaetiger 1, largest and paired in each neuropodium in chaetigers 6 and 7, single in other neuropodia.

Digestive tract without ventral buccal bulb and gizzard-like structure, without pigmentation.

Nephridia from chaetiger 4 onwards.

### **MG staining**

Intensely stained anterior part of the prostomium in front of eyes, and dorso-lateral sides of the peristomium. Weakly stained lateral sides of chaetigers and scattered glandular cells on the ventral side of chaetigers.

### Habitat

The two types of *P. auha* sp. nov. were found together in a shell limestone encrusted by a coralline alga. Whether the worms made the burrows themselves or occupied empty holes made by other organisms remains uncertain.

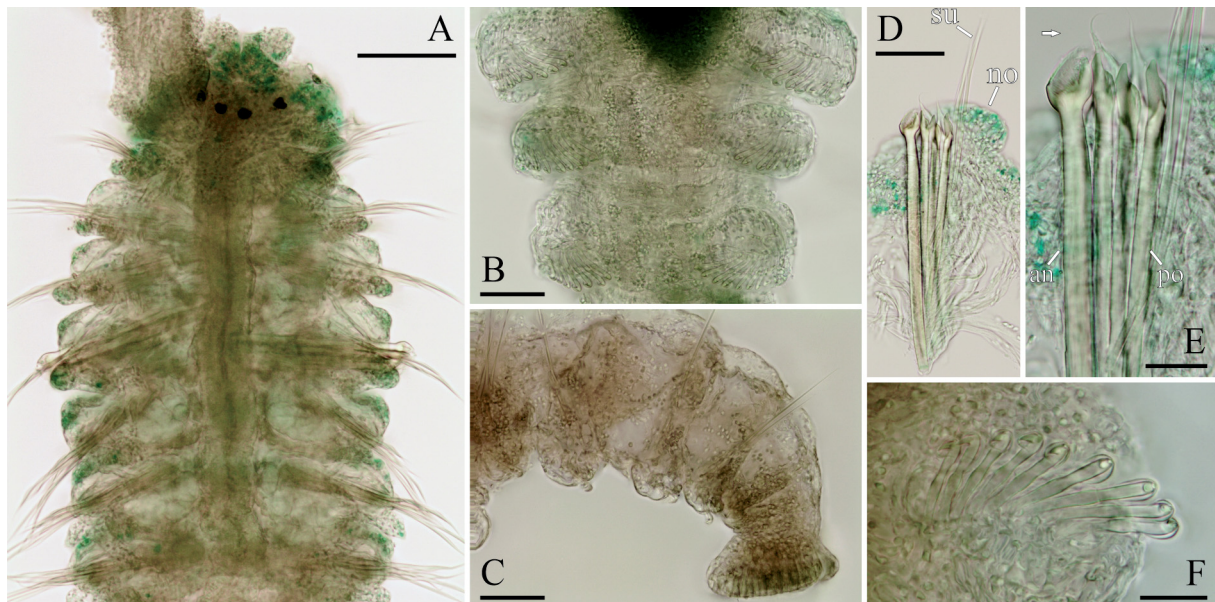
### Reproduction

Both examined individuals of *P. auha* sp. nov. were juveniles.

### Remarks

In the Arabian Gulf, adults of *P. auha* sp. nov. appear very similar to those of *P. antennata* and *P. melanopalpa* sp. nov. (see below). They all have heavy spines in the notopodia of chaetiger 5 of the same morphology, branchiae beyond the middle of the body, and the bilobed pygidia with dorsal and ventral clefts (characters shared by all members of the *P. antennata*-complex, see below in the Discussion). However, they can be distinguished by the prostomium shape, length of the caruncle, and palp pigmentation. In *P. antennata*, the prostomium is anteriorly bifurcated, with two long pointed antero-lateral processes (to which the name of the species was referred), whereas in *P. auha* sp. nov. and *P. melanopalpa* sp. nov. the prostomia are incised, with two short lobes. In *P. antennata*, the caruncle extends posteriorly maximum to the end of chaetiger 4, whereas in *P. melanopalpa* sp. nov. the caruncle extends to the end of chaetiger 5, and in *P. auha* sp. nov. it extends to the end of chaetiger 6.

Adults of *P. auha* sp. nov. appear very similar to those of *P. uphondo* Simon, Sato-Okoshi & Abe, 2017 and *P. eriyali* Simon, Sato-Okoshi & Abe, 2017 both of which were described from South Africa by Simon *et al.* (2019). They share the characters of the *P. antennata*-complex (see below in the Discussion);



**Fig. 11.** Adult morphology of *Pseudopolydora auha* sp. nov., holotype (MIMB 40907), formalin-fixed and stained with MG). **A.** Anterior end, dorsal view. **B.** Posterior chaetigers, ventral view. **C.** Posterior end, left lateral view. **D.** Notopodium of chaetiger 5. **E.** Notochaetae of chaetiger 5. **F.** A series of hooded hooks from neuropodium of chaetiger 12. Abbreviations: *arrow* showing the direction towards the inside of the U-shaped row of spines of chaetiger 5; *an* = anterior-row spine from notopodium of chaetiger 5; *no* = notopodial postchaetal lamella; *po* = posterior-row spine from notopodium of chaetiger 5; *su* = dorsal superior capillary chaetae. Scale bars: A = 100 µm; B–D = 50 µm; E–F = 20 µm.

the morphological differences between the three species are very subtle (see Simon *et al.* 2019: table 1) although genetically they are well separated (Fig. 2). Adult *P. eriyali* and *P. auha* sp. nov. have caruncles extending to the end of chaetiger 6, while in *P. uphondo* the caruncle extends to the end of chaetiger 5. Additional specimens of *P. auha* sp. nov. should be examined to understand the morphological variability of this species and the differences between this species and *P. eriyali* and *P. uphondo*.

### Distribution

Arabian Gulf: Kuwait (Fig. 1D).

#### *Pseudopolydora kuwaiti* sp. nov.

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Figs 1C, 5E, 12–16

*Pseudopolydora* sp. C – Al-Kandari *et al.* 2019: 9. — Radashevsky *et al.* 2020: table 1, fig. 1.

### Diagnosis

Prostomium anteriorly notched to weakly incised, with two rounded lobes. Palps with up to 11 yellow bands. Occipital antenna absent. Anterior-row capillaries in notopodia from chaetiger 7 to chaetigers 9–14 with wide subtriangular, pennoned limbation. Chaetiger 5 larger than chaetigers 4 and 6, dorsally overlapping anterior part of chaetiger 6; lower (anterior-row) notopodial spines, with distal part of stem enlarged, cup-shaped, with a concavity on top and long pennoned, pointed tip. Pygidium small and fleshy, disc-like to cup-shaped, with dorsal gap to incision.

### Etymology

The name refers to the type locality of the species in Kuwaiti waters.

### Material examined

#### Holotype

KUWAIT • 1 spec.; Arabian Gulf, Al-Judailiat; 29.377° N, 47.759° E; 4 Feb. 2015; Vasily I. Radashevsky leg.; low intertidal; fine sand; MIMB 40853.

#### Paratypes

KUWAIT • 8 specs; Arabian Gulf, Khor Al-Subiya (Al-Shumaima); 29.658° N, 48.1327° E; 25 Nov. 2014; low intertidal; muddy sand + gravel around oyster aggregations; MIMB 40708 • 2 specs; northwest of Failaka Is.; 29.4722° N, 48.2965° E; 22 Dec. 2014; middle intertidal; on stone; MIMB 40709 • 2 specs; south of Boubyan Is.; 29.6478° N, 48.3156° E; 23 Dec. 2014; low intertidal; on stone; MIMB 40710 • 45 specs; Boubyan Is.; 29.649° N, 48.3136° E; 24 Jan. 2015; low intertidal; on empty shells and stones; MIMB 40711 • 1 spec.; Boubyan Is.; 29.7897° N, 48.3727° E; 25 Jan. 2015; upper intertidal; on stone; MIMB 40712 • 17 specs; same collection data as for holotype; MIMB 40713 • 2 specs; Al-Doha (Aushairij); 29.388° N, 47.821° E; 5 Feb. 2015; low intertidal; shells, sand, gravel, stone; MIMB 40854 • 1 spec.; Al-Bedaa; 29.3228° N, 48.092° E; 2 Mar. 2016; low intertidal; sand; MIMB 40855 • 3 specs; Auha Is.; 29.3764° N, 48.4391° E; 29 Jan. 2017; low intertidal; from shell limestone encrusted by coralline alga; MIMB 40856 • 1 spec.; Ras Ajuza, Kuwait City; 29.3913° N, 47.9967° E; 29 Apr. 2017; low intertidal; sandy tube attached to the rock; MIMB 40857.

#### Other material

KUWAIT • 2 specs; Auha Is.; 29.3764° N, 48.4391° E; 29 Jan. 2017; low intertidal; from dead coral encrusted by coralline algae and from shell of the gastropod *Indothais scalaris* (Shubert & J.A. Wagner, 1829); used in molecular analysis; VIR 19871 • 1 spec.; east of Failaka Is.; 29.3916° N, 48.3989° E;



14 Feb. 2017; low intertidal; from gastropod shell occupied by hermit crab; examined but not preserved; VIR 20287.

### **Description**

Adults up to 6 mm long, 0.4 mm wide with 35 chaetigers (Fig. 12A). Fine black pigment usually scattered on dorsal and ventral sides of head and 10–13 anterior chaetigers (Fig. 13A–C). Up to 11 yellow bands (branched chromatophores) present on each palp when alive (Figs 12A–C, E, 13B, 14A), not visible after fixation. Prostomium anteriorly usually notched or weakly incised, with two rounded lobes (Figs 12B, 13D), occasionally blunt (Fig. 13A), posteriorly extending to end of chaetiger 2 as a low caruncle (Fig. 14C). Usually two pairs of black eyes present. Occipital antenna absent (Fig. 12D). Palps as long as 15–20 chaetigers, with longitudinal frontal groove lined with fine cilia, short motile compound cilia regularly arranged in one row along ciliated groove, and non-motile cilia arising from palp surface and scattered on lateral and abfrontal surfaces.

Chaetiger 1 with short capillaries in neuropodia, short notopodial postchaetal lamellae and well-developed neuropodial lamellae; notochoetae absent. Notochoetae of chaetigers 2–4 and 6, and neurochaetae of chaetigers 1–7 slender capillaries with narrow wing. Anterior-row capillaries in notopodia from chaetiger 7 to chaetigers 9–14 (chaetiger 12 in holotype) with wide subtriangular, pennoned limbation (Figs 14C, 15A–B), up to five in a series; superior and posterior-row notochoetae in these chaetigers slender capillaries with narrow wing; superior capillaries longer than posterior-row capillaries, with long narrow wing. Posterior notopodia with a few long alimbate capillary chaetae.

Chaetiger 5 larger than chaetigers 4 and 6, dorsally overlapping anterior part of chaetiger 6, with up to four dorsal superior capillaries, two kinds of notopodial spines arranged in a curved diagonal or almost horizontal double row, and ten ventral capillaries; notopodial lamellae lacking but neuropodial postchaetal lamellae present, same as on chaetigers 4 and 6 (Figs 12B, F, 13A). Dorsal superior capillaries shorter and fewer than those capillaries on chaetigers 4 and 6. Ventral capillaries same in size, number and arrangement (in three groups) as those on chaetiger 4. Lower (anterior-row) notopodial spines, with distal part of stem enlarged, cup-shaped, with concavity on top and long pennoned, pointed distal tip (Fig. 15C), up to ten in a series. Upper (posterior-row) notopodial spines simple falcate (Fig. 15C), up to seven in a series. Newly developed spines in posterior part of curved series slightly larger than older spines in anterior part of series (Fig. 12F).

Hooks in neuropodia from chaetiger 8, up to 11 in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang; upper part of shaft with constriction; lower part of shaft bent at right angle in hooks in posterior neuropodia (Fig. 15D–E).

Branchiae from chaetiger 7 to chaetiger 16 (chaetiger 16 in holotype), up to ten pairs, fewer in small individuals (Figs 5E, 14B), free from notopodial postchaetal lamellae, flattened, with surfaces oriented perpendicular to body axis, with longitudinal ciliation (extension of nototroch) on inner anterior edge.

In immature individuals and females, short nototroch present on chaetiger 3 and longer nototrochs with longer cilia from chaetiger 7 onwards, each composed of single row of ciliated cells extending onto branchiae on branchiate chaetigers. In males, nototrochs present from chaetiger 2 onwards, each composed of two rows of ciliated cells (anterior row extending onto branchiae; posterior row running only across chaetiger), and additional intersegmental transverse ciliary rows present from chaetiger 3, situated on anterior edge of chaetigers; cilia of nototrochs longer than those of intersegmental rows. In both sexes, nototrochs on branchiate chaetigers as transverse ciliary bands, on postbranchiate chaetigers gradually becoming U-shaped, with arms directed posteriorly. One or two pairs of short compound motile cilia usually present on dorsal side of each of chaetigers 4 and 5.

Pygidium small and fleshy, disc-like to cup-shaped, with dorsal gap to incision (Fig. 13E–F), white due to numerous fusiform glandular cells with striated content.

Glandular pouches in neuropodia from chaetiger 1, largest in chaetigers 6 and 7, single pouch in each neuropodium (Fig. 15D).

Digestive tract without ventral buccal bulb and gizzard-like structure. Oesophagus narrow, extending through 7–8 anterior chaetigers.

Main dorsal blood vessel transformed into gut sinus in anterior part of midgut. Heart body absent. Blood red, without globules or other elements.



**Fig. 12.** Adult morphology of *Pseudopolydora kuwaiti* sp. nov. (live individuals, MIMB 40713). **A.** Left lateral general view, female, in reflected light, showing white chromatophores on palps. **B–D.** Anterior ends, in transmitted light, dorsal (B) and left lateral (C–D) view. **E.** Fragment of palp with chromatophore and inner blood vessel. **F.** Chaetigers 4–6, left lateral view, showing curved diagonal row of heavy spines in notopodium of chaetiger 5. Abbreviations: *ho* = hooded hooks; *no* = notopodial postchaetal lamella; *pe* = pennoned capillary notochaetae; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A = 200  $\mu$ m; B–D = 50  $\mu$ m; E = 20  $\mu$ m.

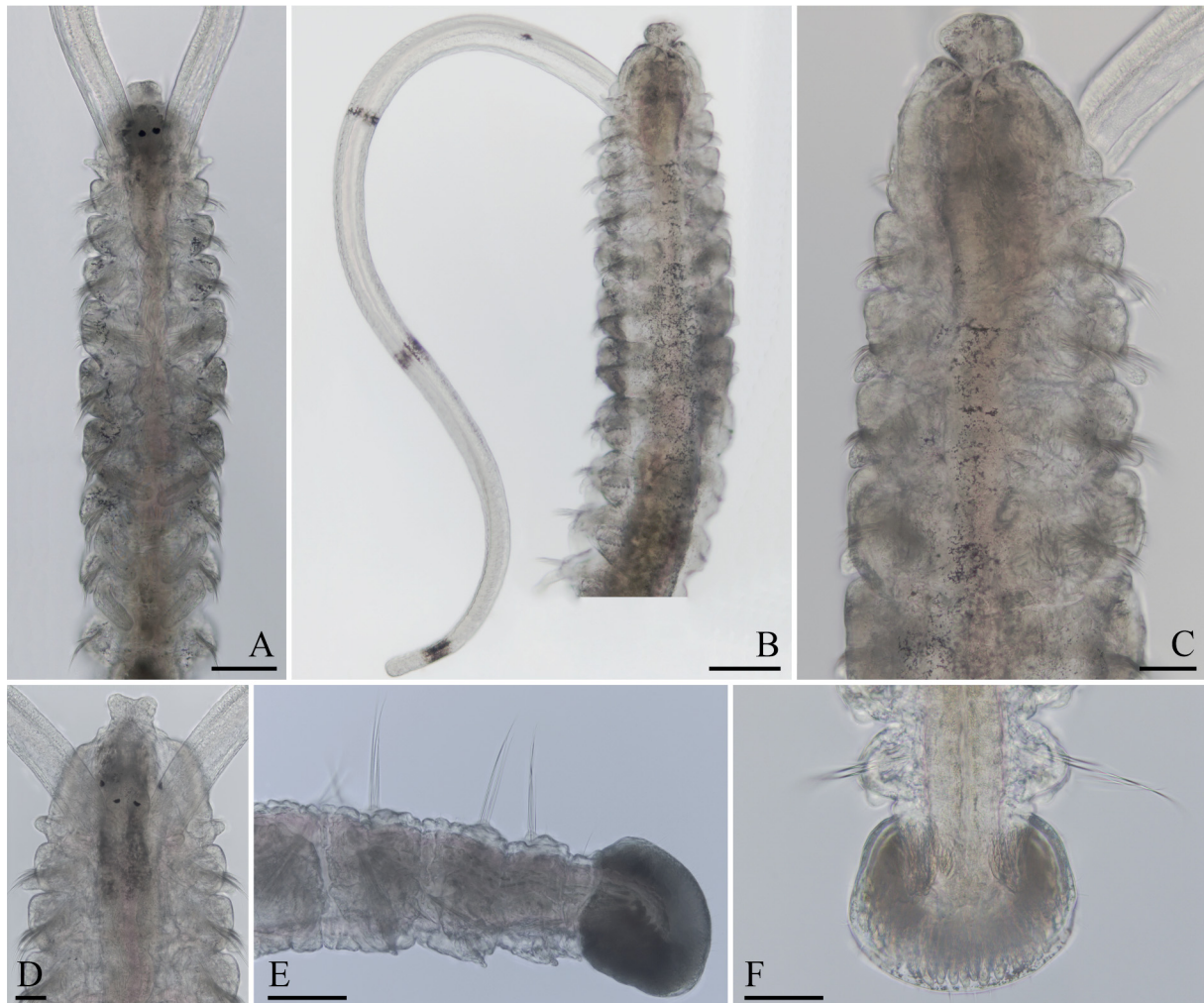
Nephridia from chaetiger 4 onwards. In female fertile chaetigers, paired nephridia on each chaetiger opening to exterior via common middorsal nephridiopore anteriorly to nototroch.

### Habitat

Adult *P. kuwaiti* sp. nov. inhabit silty tubes in muddy sand intertidally and in shallow waters and also bore in live mollusc shells, dead corals and shell limestone encrusted by coralline algae. While the species is comparatively rare, the population density in some local places can reach a maximum of one thousand individuals per 1 m<sup>2</sup>. In hard substrata, worms occasionally form aggregations of 1–3 individuals per 1 cm<sup>2</sup>.

### Reproduction

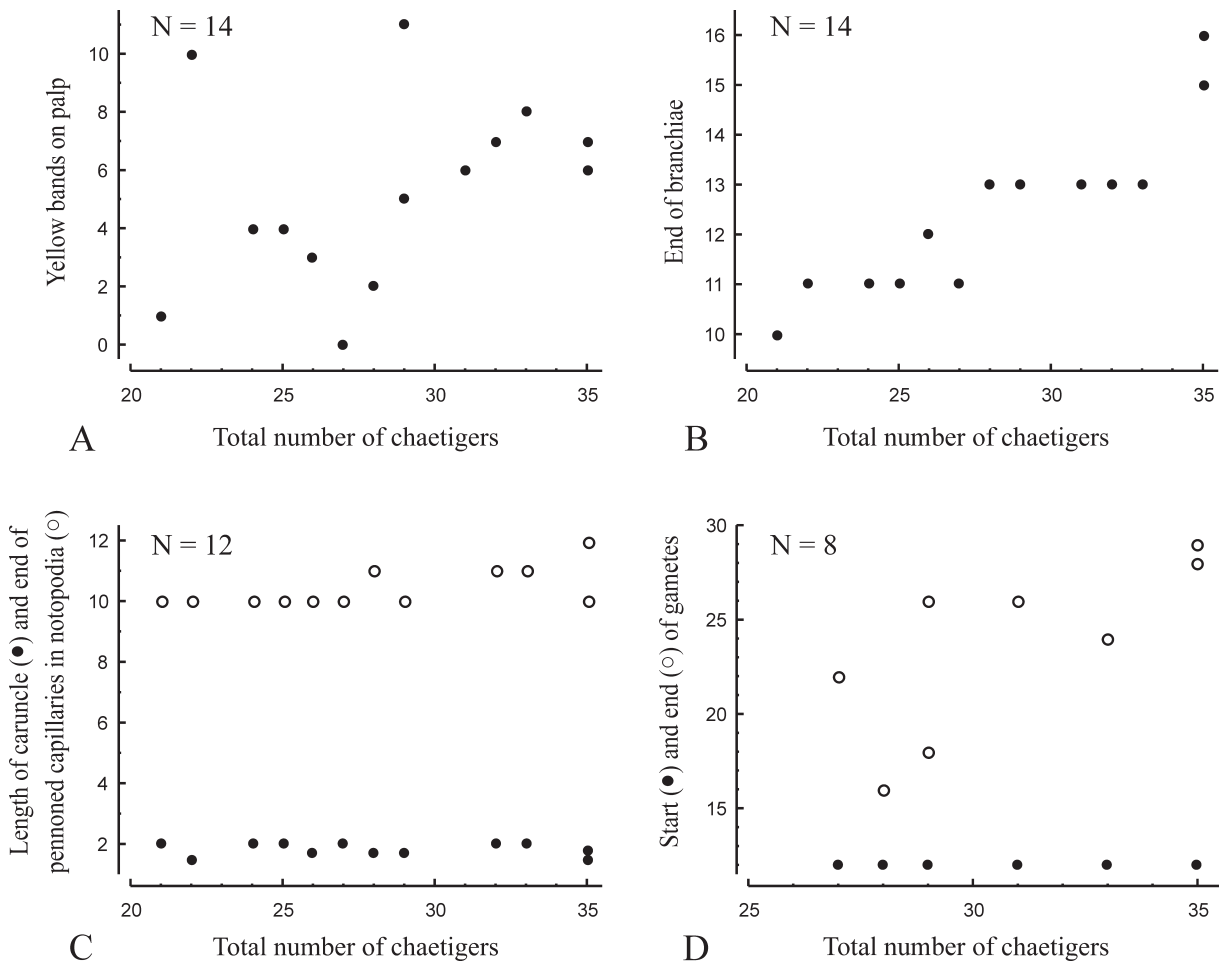
*Pseudopolydora kuwaiti* sp. nov. is gonochoristic. Both in females and males, gametes developed from chaetiger 12 to chaetigers 16–29 (Fig. 14D). Paired testes or ovaries were attached to segmental blood vessels in fertile chaetigers.



**Fig. 13.** Adult morphology of *Pseudopolydora kuwaiti* sp. nov. (live individuals, MIMB 40713). A–D. Anterior ends, dorsal (A, D) and ventral (B–C) view. E–F. Posterior ends, left lateral (E) and dorsal (F) view. Scale bars: A = 500 µm; B–C = 200 µm; D = 100 µm; E = 50 µm.

Oogenesis was partly intraovarian (Fig. 15F). Developed coelomic oocytes were spherical, about 100  $\mu\text{m}$  in diameter. Oocyte envelope was smooth, less than 1  $\mu\text{m}$  thick.

Spermatogonia proliferated in testes; spermatogenesis occurred in the coelomic cavity. Spermatids, each 3–4  $\mu\text{m}$  in diameter, were joined in octads. Spermatozoa were introsperm with pointed acrosome about 1.5  $\mu\text{m}$  long, straight elongated nucleus about 1.5  $\mu\text{m}$  in diameter and 6  $\mu\text{m}$  long, midpiece 3.5  $\mu\text{m}$  (head + midpiece about 11  $\mu\text{m}$  long), and a flagellum about 32  $\mu\text{m}$  long (the precision of measurement of spermatids and parts of head of spermatozoa were at maximum to the nearest 0.5  $\mu\text{m}$  and flagellum to the nearest 2  $\mu\text{m}$ ).



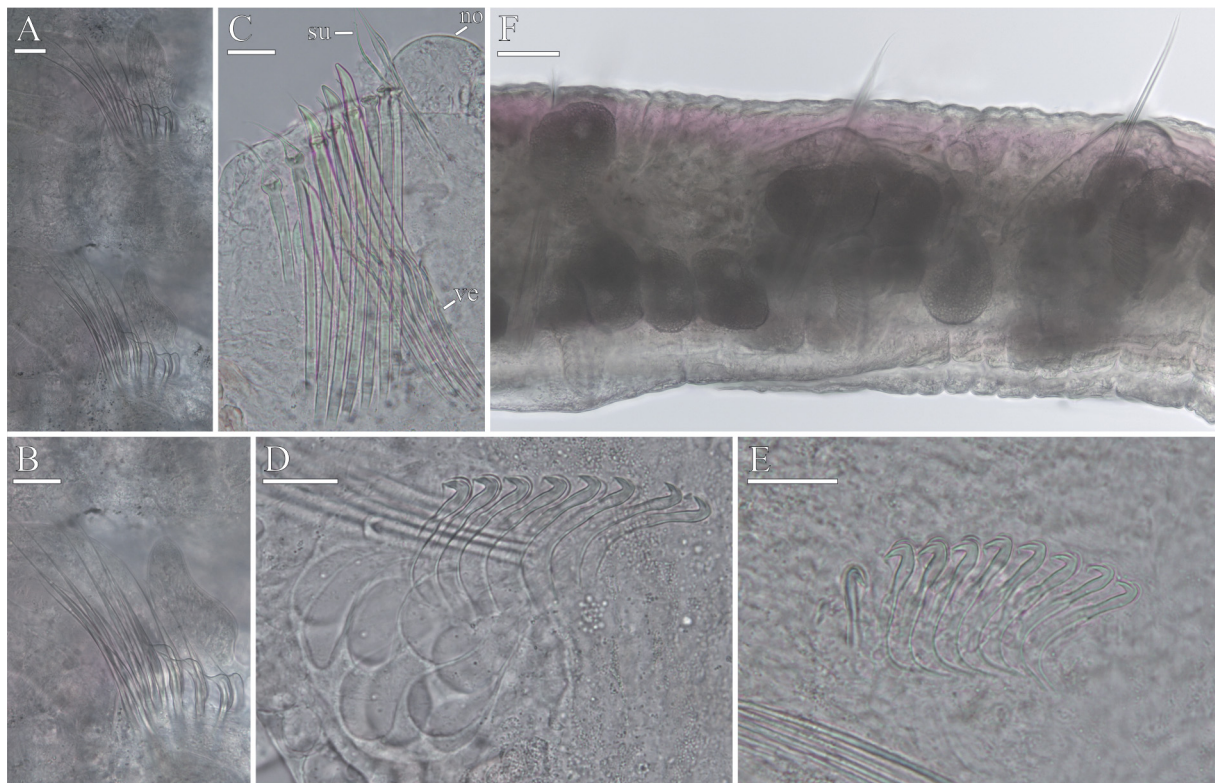
**Fig. 14.** Adult characteristics of *Pseudopolydora kuwaiti* sp. nov. **A.** Relationships between number of yellow bands on palp and total number of chaetigers. **B.** Relationships between distribution of branchiae (referring to number of the last branchiate chaetiger) and total number of chaetigers. **C.** Relationships between caruncle length (in chaetiger numbers) and total number of chaetigers, and distribution of pennoned capillaries in notopodia (referring to number of the last chaetiger with pennoned capillaries) and total number of chaetigers. **D.** Relationships between distribution of gametes (referring to number of the first and the last chaetigers with gametes) and total number of chaetigers.

### Larval development in the plankton

The spermatophores and egg capsules, typically produced by *Pseudopolydora*, were not observed in *Pseudopolydora kuwaiti* sp. nov. The 10–14-chaetiger larvae of this species were collected in plankton in March 2016. The 14-chaetiger larvae were easily identified by the specific heavy spines in the notopodia of chaetiger 5. Small larvae, with heavy spines not yet developed, were identified by the specific pigmentation, revealed in larger larvae.

#### 10-chaetiger larvae

Larvae 500–550  $\mu\text{m}$  long. Posterior middle part of prostomium slightly inflated as a caruncle but not extending over chaetiger 1. Nuchal ciliary patches on sides of caruncle at level of prototroch. Palps as long as about half of chaetiger 1. Notopodia with only provisional serrated bristles. Short adult capillaries in neuropodia of chaetigers 2–7. Heavy spines in chaetiger 5 and branchiae not yet developed. Hooks in neuropodia from chaetiger 8. Pygidium semispherical. Gastrotrochs on chaetigers 3, 5 and 7. Glandular pouches absent. One pair of protonephridia in chaetiger 1.



**Fig. 15.** Chaetal and gamete morphology of *Pseudopolydora kuwaiti* sp. nov. (live individuals, MIMB 40713). **A.** Notopodia of chaetigers 8 and 9, showing pennoned capillaries in the anterior row of notochaetae. **B.** Notopodium of chaetiger 10, showing pennoned capillaries in the anterior row of notochaetae. **C.** Chaetae of chaetiger 5. **D.** A series of hooded hooks and glandular pouch from neuropodium of chaetiger 10. **E.** A series of hooded hooks from a posterior neuropodium. **F.** Female fertile chaetigers, left lateral view, showing vitellogenic oocytes in ovaries. Abbreviations: *no* = notopodial postchaetal lamella; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A–E = 20  $\mu\text{m}$ ; F = 50  $\mu\text{m}$ .

**11-chaetiger larvae**

Larvae 550–600 µm long, with palps as long as 1–2 chaetigers. Notopodia with only provisional serrated bristles. Short adult capillaries in neuropodia of chaetigers 1–7; those in chaetiger 1 shorter and fewer than in succeeding chaetigers, 1–2 in a tuft. Notopodia of chaetiger 5 with 1–2 anterior-row spines with pointed distal tip, and one posterior-row heavy falcate spine among provisional serrated bristles. Branchiae not yet developed. Hooks in neuropodia from chaetiger 8. Glandular pouches in neuropodia from chaetiger 3 onwards; pouches in chaetigers 3–5 each composed of one small glandular cell; from chaetiger 6 onwards, each pouch composed of two cells. One pair of protonephridia in chaetiger 1.

**12–13-chaetiger larvae**

Larvae 650–700 µm long, with palps as long as 2–3 chaetigers (Fig. 16A). Short adult capillaries in neuropodia of chaetigers 1–7. Chaetiger 5 with two kinds of heavy spines in notopodia, comprising three anterior-row spines and two posterior-row falcate spines. Single capillary chaetae with pennoned limbation in notopodia of chaetigers 7 and 8. Short branchial buds on chaetiger 7. Gastrotrochs on chaetigers 3, 5, 7 and 11. Glandular pouches in neuropodia from chaetigers 2–3. Protonephridia in chaetigers 1 and 2; metanephridia from chaetiger 4 onwards.

**14-chaetiger larvae**

Larvae 700–750 µm long and up to 200 µm wide in middle part (Fig. 16B–E). Intense pigmentation comprising small melanophores and fine yellowish pigment scattered all along body, in anterior part of prostomium, in pharynx and in pygidium; distinct chromatophores absent. A small black spot present between median and lateral eyes on each side of prostomium. Small middorsal melanophores in anterior part of chaetiger 1 and on pygidium at level of telotroch; chaetiger 1 melanophore occasionally heavily ramified and hardly discernible. Small paired dorsal melanophores from chaetiger 3 onwards. Small dorso-lateral melanophores from chaetigers 3–4 onwards, usually indistinct, difficult to recognize. Single small lateral melanophores on either side on anterior edge of chaetiger 2; in some larvae, lateral melanophores appearing as indistinct spots of diffused black pigment.

Prostomium anteriorly wide and rounded, posteriorly extending to middle of chaetiger 1 as a low narrow caruncle. Nuchal organs large round ciliary patches on sides of caruncle on anterior half of chaetiger 1. Occipital antenna absent. Three pairs of black eyes arranged almost in a transverse line, comprising one pair of small rounded median eyes and two pairs of lateral eyes; lateral eyes on each side situated close to each other and appearing as one comma-shaped eye. One pair of spherical unpigmented ocelli, each about 17 µm in diameter, present in front of pigmented eyes. One pair of compound motile cilia, each about 20 µm long, situated near median eyes, and one pair of compound cells, each about 75 µm long, situated near lateral eyes. One pair of palps arising from posterior dorso-lateral edges of peristomium, posterior to prototroch; each palp as long as about four chaetigers. Shallow frontal longitudinal groove on palps densely ciliated.

Prototroch and telotroch well developed. Nototrochs and grasping cilia from chaetiger 3 onwards. Triangular neurotroch extending over ventral peristomial lip from mouth to end of chaetiger 1. Two small ciliated cells present on each side of neurotroch. Ventral ciliated pit absent. Gastrotrochs on chaetigers 5, 7 and 11, each composed of six large cells with numerous long cilia.

Provisional serrated bristles in all notopodia including chaetiger 5; those in anterior chaetigers up to 175 µm long, gradually becoming shorted in succeeding chaetiger. Short adult capillaries in notopodia from chaetiger 2, and in neuropodia in chaetigers 1–7. Single capillary chaetae with flag-like limbation in notopodia of chaetigers 7 and 8.

Chaetiger 5 with two kinds of heavy spines in notopodia in addition to short adult capillaries and long provisional bristles. Spines comprising 3–4 anterior-row spines with wide cup-shaped distal end of stem bearing long transparent pointed tip, and 2–3 posterior-row simple falcate spines.

Hooks in neuropodia from chaetiger 8 onwards, up to five in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang, with outer hood; lower part of shaft bent almost at right angle.

Branchiae short, on chaetigers 7 and 8. Pygidium semispherical, divided into two rounded lobes by vertical median furrow and bearing four short papillae.

Lateral sensory organs, each 4–5  $\mu\text{m}$  in diameter, with thin non-motile cilia up to 20  $\mu\text{m}$  long situated between noto- and neuropodia from chaetiger 1 onwards, including chaetigers 3–5.

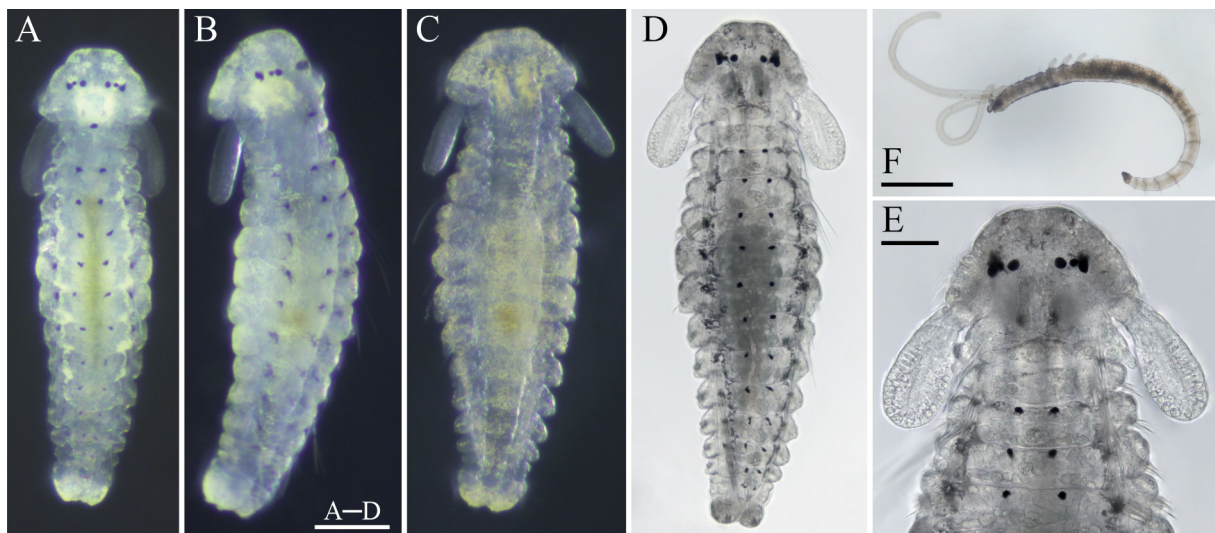
Numerous oval to spherical glandular cells with striated content present in anterior part of prostomium, on dorsal side of chaetigers, in pygidium and palps (Fig. 16E).

Glandular pouches in neuropodia from chaetigers 1–2, very small in first chaetigers, large in chaetigers 6 and 7, gradually diminishing in size in succeeding chaetigers.

Large lateral lips and small ventral lip of peristomium lined with numerous short cilia and forming voluminous vestibulum. Compound motile cilia up to 65  $\mu\text{m}$  long present on lateral and ventral sides of lateral peristomial lips. Narrow oesophagus extending through three chaetigers. Gizzard-like structure absent in digestive tract. Numerous lipid globules up to 10  $\mu\text{m}$  in diameter present in wall of voluminous midgut.

Circulatory system developed and functional; blood transparent, without pigment.

Two pairs of protonephridia in chaetigers 1 and 2. Metanephridia from chaetiger 4 onwards.



**Fig. 16.** Larval and juvenile morphology of *Pseudopolydora kuwaiti* sp. nov. Live larvae in reflected (A–C) and transmitted (D–E) light. **A.** 13-chaetiger larva, dorsal view. **B.** 14-chaetiger larva, left lateral view. **C.** The same, ventral view. **D.** The same, dorsal view. **E.** The same, anterior end. **F.** 21-chaetiger juvenile, left lateral view. Scale bars: A–D = 100  $\mu\text{m}$ ; E = 50  $\mu\text{m}$ ; F = 500  $\mu\text{m}$ .

## Settlement and metamorphosis

### 14-chaetiger larvae

Larvae about 700  $\mu\text{m}$  long easily settled and underwent gradual metamorphosis in Petri dishes. They started crawling and in the absence of sediment built transparent mucous tubes. The metamorphosis comprised loss of larval features, such as provisional bristles in notopodia, proto-, telo-, neuro-, gastrotrochs and grasping cilia, nototrochs on chaetigers 3–5, and also the transformation of the head and rapid elongation of the palps. The prostomium became distinctly separated from the peristomium. Nuchal organs transformed from oval ciliary patches to longitudinal narrow ciliary bands on the posterior sides of the prostomium. Lateral peristomial lips became reduced and transformed into dorso-lateral ciliary folds of the foregut. The ventral peristomial lip enlarged and formed the adult peristomium and basement for the palps. Unpigmented ocelli in the prostomium and larval protonephridia in chaetigers 1 and 2 became reduced and disintegrated.

### 20–21-chaetiger juveniles

Juveniles about 2 mm long, with remains of yellowish pigment scattered on head and pygidium (Fig. 16F). Prostomium anteriorly narrow and rounded, posteriorly extending over 1–2 chaetigers. Occipital antenna absent. Palps as long as 12–15 chaetigers, without yellowish chromatophores or one chromatophore present on each palp. Capillary chaetae with pennoned limbation in notopodia of chaetigers 7–10. Branchiae on chaetigers 7–10. Glandular pouches in neuropodia from chaetiger 1. Metanephridia from chaetiger 4 onwards.

## Remarks

Adult *P. kuwaiti* sp. nov. appear similar to the Asian Pacific *Pseudopolydora diopatras* Hsieh, 1992. Both have yellow ramified chromatophores on palps, caruncles without occipital antenna extending to the end of chaetiger 2, and small cup-shaped pygidia (Hsieh 1992). They differ from other *Pseudopolydora* by having greatly enlarged chaetiger 5 (instead of chaetiger 5 being similar in size to chaetigers 4 and 6) with the notopodial heavy spines arranged in a diagonal instead of a vertical double row, and also by the unique morphology of the pennoned spines, which have a stem with a cup-shaped distal end and a long pointed tip which is easily broken at the base. The two species differ from each other genetically but morphologically are very similar and can only be distinguished by small paired melanophores on the ventral side of the anterior chaetigers which are present in *P. diopatras* from the Asian Pacific and absent in *P. kuwaiti* sp. nov. from the Arabian Gulf.

Larvae of *P. kuwaiti* sp. nov. appear similar to those of *P. arabica* (see Radashevsky & Al-Kandari 2020). Both occur in the plankton at the same time, have gastrotrochs on chaetigers 3, 5, 7 and 11, and are intensely pigmented with yellow and black pigments. They differ in that the larvae of *P. arabica* may postpone metamorphosis in the absence of substratum and attain 1250  $\mu\text{m}$  long with 23 chaetigers, whereas the largest larvae of *P. kuwaiti* sp. nov. caught from the plankton were 750  $\mu\text{m}$  long with 14 chaetigers. The developed 15–17-chaetiger larvae of *P. arabica* have one to three yellow ramified chromatophores on each palp, paired dorsal melanophores from chaetigers 6–7, dorso-lateral melanophores from chaetiger 4 to chaetigers 6–10, and one pair of small melanophores in lateral peristomial lips. In *P. kuwaiti* sp. nov., chromatophores on palps appear in juveniles; the developed larvae have paired dorsal melanophores from chaetiger 3 onwards, and dorso-lateral melanophores from chaetigers 3–4 onwards; melanophores in lateral peristomial lips are absent.

*Pseudopolydora kuwaiti* sp. nov. is the only species of the genus inhabiting soft sediments as well as boring into mollusc shells and dead corals encrusted by coralline algae. Our genetic analysis confirmed the conspecificity of the tube-dwelling and shell-boring individuals (Fig. 2).

## Distribution

Arabian Gulf: Kuwait (Fig. 1C).



*Pseudopolydora melanopalpa* sp. nov.

urn:lsid:zoobank.org:act:DA98F3F8-54B1-4CCC-BC82-16C3ABF0DC34

Figs 1D, 17–19

**Diagnosis**

Prostomium anteriorly incised, with two short rounded lobes. Caruncle to end of chaetiger 5. Fine black pigment scattered on distal part of palps.

**Etymology**

The species name refers to fine black pigment scattered on the distal part of palps in adults.

**Material examined**

**Holotype**

KUWAIT • 1 spec.; Arabian Gulf, Sulaibikhat Bay, desalinization station outfall area; 29.36157° N, 47.81915° E; 6 Feb. 2019; Vladimir A. Grintsov leg.; low intertidal; mud; MIMB 40908.

**Paratypes**

KUWAIT • 5 specs; same collection data as for holotype; MIMB 40909.

**Description**

All types: anterior fragments of females in good condition with palps; one 25-chaetiger fragment with a few small newly regenerated posterior achaetous segments with a small bilobed pygidium. Holotype: 39-chaetiger anterior fragment about 10 mm long, broken in the middle (Fig. 17A). Specimens up to 10 mm long, 1 mm wide with 45 chaetigers; when complete, worms apparently up to 15 mm long with at least 60 chaetigers. No pigmentation on body; fine black pigment scattered on distal part of palps (Figs 17A, C, E, 18C). Prostomium anteriorly incised, with two short rounded lobes (Figs 17C–E, 18A–B), posteriorly extending to end of chaetiger 5 (end of chaetiger 4 in holotype) as a low caruncle. Peristomium with two lateral lips as thick folds in front of large ventral lip (Fig. 17A). Short occipital antenna present on caruncle between palps (Fig. 18C). Two pairs of black eyes arranged trapezoidally, comprising one pair of median eyes, and one pair of slightly larger lateral eyes situated anteriorly and set wider apart. Palps as long as 10–15 chaetigers, with frontal longitudinal groove lined with fine cilia, and short compound non-motile cilia arising directly from palp surface sparsely arranged in line on sides of groove and sparsely scattered on lateral and abfrontal palp surfaces.

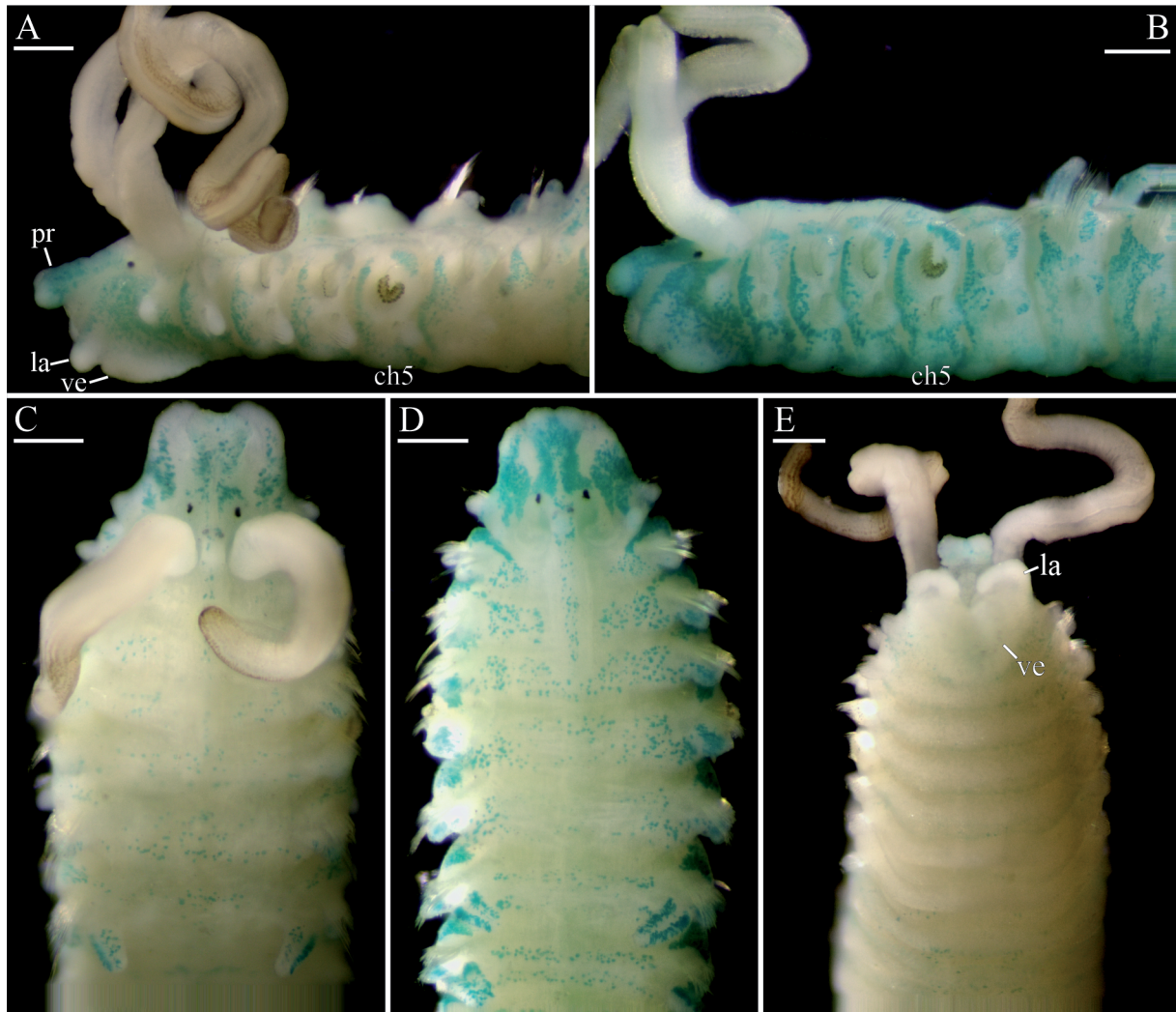
Chaetiger 1 with short capillaries in neuropodia, small notopodial lamellae and well-developed neuropodial postchaetal lamellae; notochaetae absent. Chaetiger 2 notochaetae all slender capillaries with narrow limbation. Anterior-row notopodial capillaries on chaetiger 3 with slightly enlarged wing; capillaries on chaetiger 4 with wing slightly larger than on chaetigers 3. Anterior-row capillaries on chaetigers 3, 4, 6 and 7 arranged in J-shaped series; posterior-row capillaries on these chaetigers arranged in vertical series (Figs 17A–B, 18C). Posterior notopodia with a few long alimbate capillary chaetae.

Chaetiger 5 same in size as chaetigers 4 or 6, with dorsal superior capillaries, two kinds of notopodial spines arranged in a double U-shaped row, and ventral capillaries; notopodial postchaetal lamellae absent; neuropodial lamellae same as on chaetigers 4 and 6 (Figs 17A–B, 18C). Dorsal superior capillaries shorter and fewer than those capillaries on chaetigers 4 and 6. Ventral capillaries same in size, number and arrangement (in three groups) as those on chaetigers 4 and 6. Upper posterior part of double U-shaped row of spines slightly lower than upper anterior part. Newly developed spines in posterior upper part of U-shaped row slightly larger than older spines in anterior upper part of row. Outer (anterior-row) notopodial spines up to 12 in a series, with distal part of stem enlarged, with concavity on

top and large triangular tooth on its side directed upwards and facing towards the inside of the U-shaped row of spines; fine bristles arising from concavity forming long flag-like pointed transparent tip which usually broken in worn old spines in anterior upper part of row (Fig. 19A–B, D). Inner (posterior-row) notopodial spines up to nine in a series, falcate, with short rounded distal part geniculate, with subdistal bulbous swelling bearing very short fine bristles and facing towards the inside of the U-shaped row of spines (Fig. 19A, C–D).

Hooks in neuropodia from chaetiger 8, up to 30 in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang; upper part of shaft with constriction; lower part of shaft bent at right angle (Fig. 19E–F).

Branchiae from chaetiger 7 to chaetiger 42, up to 36 pairs, on chaetiger 7 1.5–2 times as short as those on chaetiger 8, full-sized from chaetigers 10–11, fewer in small individuals, free from notopodial



**Fig. 17.** Adult morphology of *Pseudopolydora melanopalpa* sp. nov. (formalin-fixed and stained with MG specimens). **A.** Holotype (MIMB 40908). **B–E.** Paratypes (MIMB 40909). **A–E.** Anterior ends, left lateral (A–B), dorsal (C–D), and ventral (E) view. Abbreviations: *ch5* = chaetiger 5; *la* = lateral peristomial lip; *pr* = prostomium; *ve* = ventral peristomial lip. Scale bars = 200  $\mu$ m.

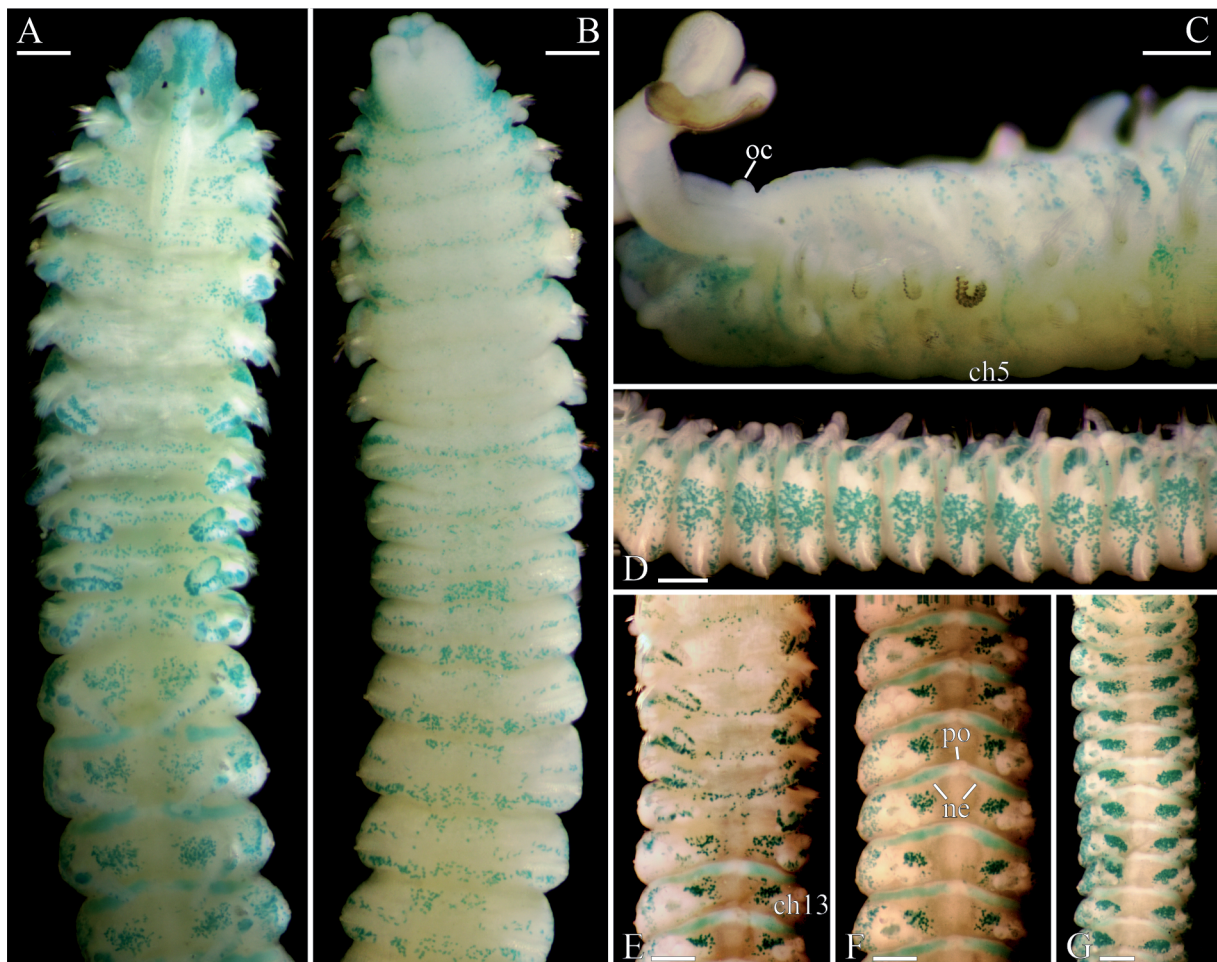
postchaetal lamellae, flattened, with surfaces oriented perpendicular to body axis, with longitudinal ciliation (extension of nototroch) on inner edge.

Pygidium bilobed, with two semi-oval lateral lobes, white due to great number of spindle-shaped glandular cells with striated content.

Subspherical to irregular shape glandular cells with striated content present on dorsal side of chaetigers. Cells few on anterior and posterior chaetigers, forming distinct paired gatherings from chaetiger 12 to chaetigers 30–35 (Figs 17D, 18A).

Glandular pouches in neuropodia from chaetiger 1, largest and paired in each neuropodium in chaetigers 6 and 7, single in other neuropodia (Fig. 19E).

Digestive tract without ventral buccal bulb and gizzard-like structure, without pigmentation.



**Fig. 18.** Adult morphology of *Pseudopolydora melanopalpa* sp. nov. (formalin-fixed and stained with MG specimens, MIMB 40909). A–C. Anterior ends, dorsal (A), ventral (B), and left lateral (C) view. D. Middle chaetigers, left lateral view. E. Chaetigers 7–14, dorsal view. F–G. Middle chaetigers, dorsal view. Abbreviations: *ch5*, *ch13* = chaetigers 5 and 13; *ne* = nephridia in female fertile chaetigers; *oc* = occipital antenna; *po* = nephridiopore. Scale bars = 200  $\mu$ m.

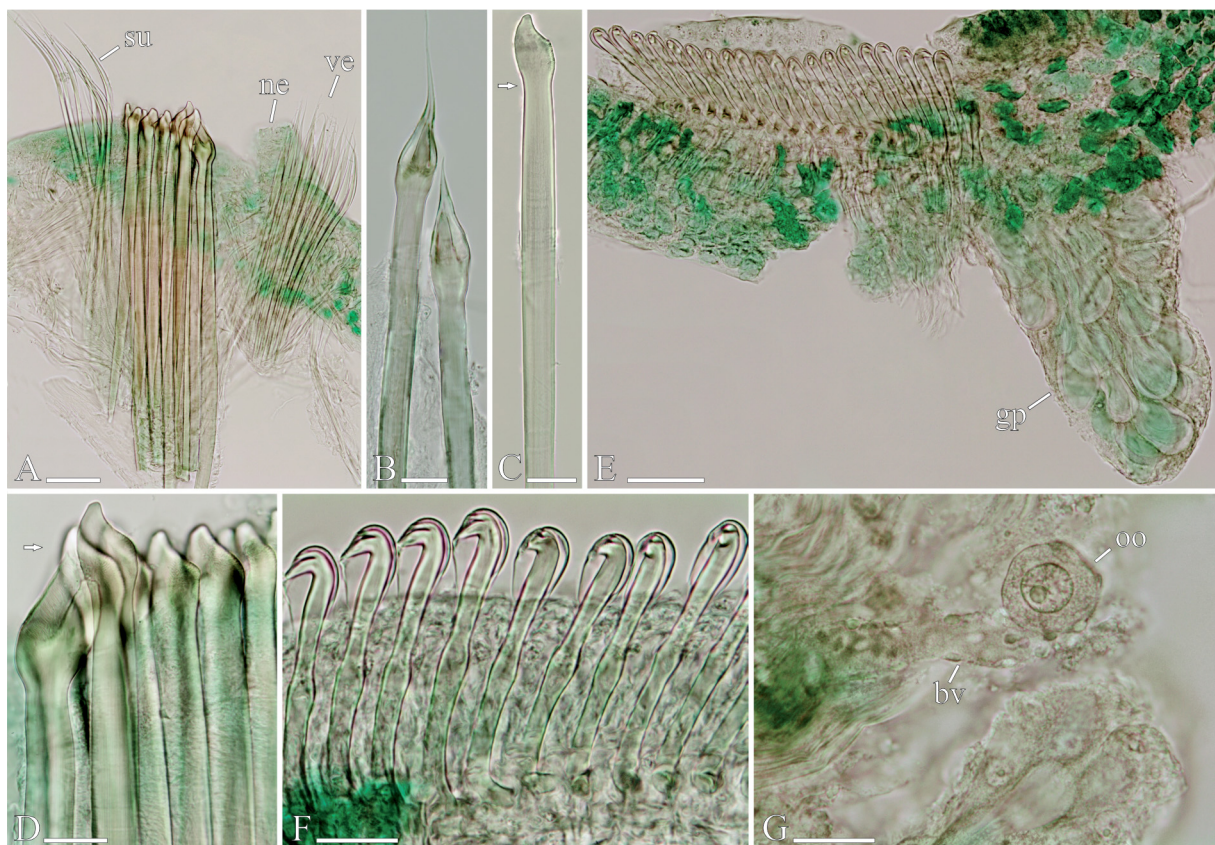
Nephridia from chaetiger 4 onwards. Distal parts of nephridia on female fertile chaetigers enlarged, containing glandular cells which absorbing MG and therefore well seen in fixed specimens from chaetiger 13 (Fig. 18A, E–F). Nephridia of chaetiger 13 used to release gametes from chaetiger 12. In female fertile chaetigers, paired nephridia on each chaetiger opening to exterior via common middorsal nephridiopore anteriorly to nototroch (Fig. 18F).

### MG staining

Intensely stained anterior part of prostomium in front of eyes, dorso-lateral sides of peristomium, scattered glandular cells on dorsal side of chaetigers 7–11, and paired gatherings of glandular cells on dorsal side from chaetiger 12 to chaetigers 30–35. Weakly stained lateral sides of chaetigers; scattered glandular cells on ventral side of chaetigers (Figs 17–18).

### Habitat

Adult *P. melanopalpa* sp. nov. inhabit silty tubes in the muddy intertidal.



**Fig. 19.** Chaetal and gamete morphology of *Pseudopolydora melanopalpa* sp. nov. (formalin-fixed and stained with MG specimens, MIMB 40909). **A.** Parapodia of chaetiger 5. **B.** Anterior-row spines of chaetiger 5, view from the outside of the U-shaped spine row. **C.** Posterior-row falcate spine of chaetiger 5, lateral view. **D.** Notopodial spines of chaetiger 5, lateral view. **E.** Neuropodium of chaetiger 10, showing glandular pouch associated with a series of hooded hooks. **F.** Hooded hooks from middle neuropodium. **G.** Small previtellogenic oocyte on a genital blood vessel. Abbreviations: *arrows* showing the direction towards the inside of the U-shaped row of spines; *bv* = genital blood vessel; *gp* = glandular pouch; *ne* = neuropodial postchaetal lamella; *oo* = oocyte; *su* = dorsal superior capillary chaetae; *ve* = ventral superior capillary chaetae. Scale bars: A, E = 50  $\mu$ m; B–D, F–G = 20  $\mu$ m.

### Reproduction

All six type specimens of *P. melanopalpa* sp. nov. are females with oocytes from chaetiger 12 to chaetigers 32–40. Paired ovaries are attached to the segmental blood vessels in fertile chaetigers (Fig. 19G). The developed coelomic oocytes are about 120 µm in diameter, with smooth thin envelope less than 1 µm thick.

### Remarks

Adult *P. melanopalpa* sp. nov. share a series of characters with members of the *P. antennata*-complex (see below in the Discussion). They differ, however, from other species of this complex by having black pigment on the distal part of palps, and the prostomium with two rounded lobed instead of pointed fronto-lateral extensions (see Simon *et al.* 2019: table 1).

### Distribution

Arabian Gulf: Kuwait (Fig. 1D).

#### *Pseudopolydora multispinosa* sp. nov.

urn:lsid:zoobank.org:act:6079E358-FCE7-444E-861D-7B3D7FE93A4F

Figs 1D, 20–21

*Pseudopolydora* cf. *corniculata* – Al-Kandari *et al.* 2019: 9. — Not Radashevsky & Hsieh 2000.

### Diagnosis

Prostomium with two long, pointed fronto-lateral extensions. Caruncle to end of chaetiger 6. Occipital antenna long, cirriform. Chaetiger 5 with numerous spines in notopodia – up to 32 in the anterior row and 27 in the posterior row, and well developed notopodial postchaetal lamellae. Branchiae posteriorly present beyond the middle of the body. Formalin-fixed specimens intensely stained with MG on the ventral side of body from chaetiger 8 onwards.

### Etymology

The species name refers to the large number of heavy spines in the notopodia of chaetiger 5.

### Material examined

#### Holotype

KUWAIT • 1 spec.; Arabian Gulf, Al-Salmiya, cove near Lebanese Restaurant; 29.32778° N, 48.09299° E; 5 Mar. 2015; Vasily I. Radashevsky leg.; middle intertidal; fine sand; MIMB 40906.

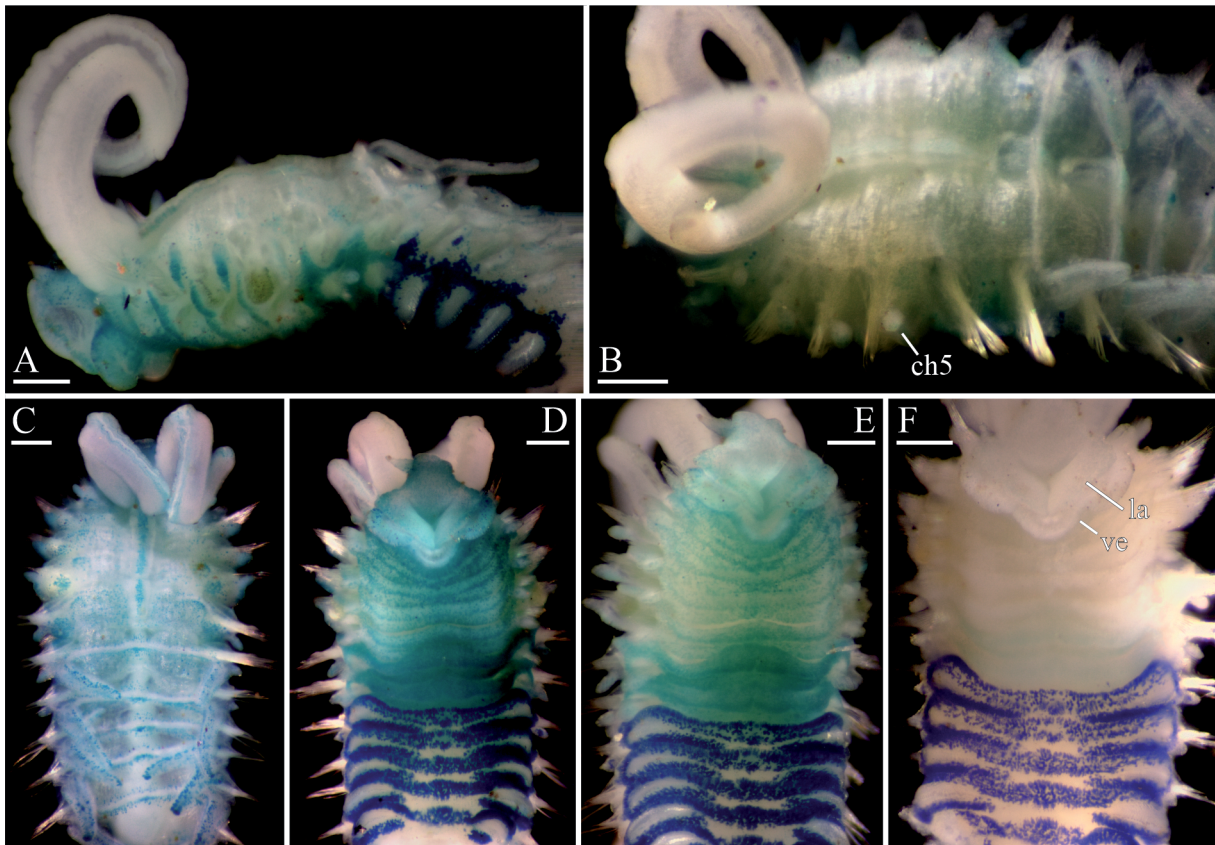
### Description

#### Holotype

About 10 mm long, 1 mm wide for about 40 chaetigers with small regenerating posterior chaetigers and pygidium. Pigmentation absent on palps and body. Prostomium anteriorly bifurcated, with two long, pointed fronto-lateral processes (left process broken) (Fig. 20D–E), posteriorly extending to end of chaetiger 6 as a low caruncle (Fig. 20B–C). Long cirriform occipital antenna present on caruncle between palps. Two pairs of black eyes arranged trapezoidally. In life, palps longer than body, flexible, with frontal longitudinal groove lined with fine cilia, and short compound non-motile cilia arising directly from palp surface arranged on sides of groove and sparsely scattered on lateral and abfrontal palp surfaces.

Chaetiger 1 well separated from peristomium, with short winged capillaries in neuropodia and well developed cirriform noto- and neuropodial postchaetal lamellae; notochaetae absent. Chaetiger 2 notochaetae all slender capillaries with narrow limbation. Anterior-row notopodial capillaries on chaetiger 3 with slightly enlarged wing; capillaries on chaetiger 4 with wing slightly wider than on chaetigers 3, intermediate between those on chaetigers 3 and 5. Anterior-row capillaries on chaetigers 3, 4, 6 and 7 arranged in J-shaped series; posterior-row capillaries on these chaetigers arranged in vertical series (Fig. 20A). Posterior notopodia with a few long alimbate capillaries arising from elongated fleshy notopodial lobes. Posterior neuropodial lobes also elongated and fleshy, each with a series of hooks on top.

Chaetiger 5 as same in size as chaetigers 4 and 6, with dorsal superior capillaries, two kinds of dorsal spines arranged in a double U-shaped row, and ventral capillaries; postchaetal lamellae well developed in both rami, same as on chaetigers 4 and 6 (Figs 20A–B, 21A). Dorsal superior and ventral capillaries similar in size, number and arrangement to those on chaetigers 4 and 6 (Fig. 21A). Outer (anterior-row) notopodial spines with distal part of stem curved, gradually narrowing, with wide, distally pointed limbation (Fig. 21B–C), 32 in a series. Inner (posterior-row) notopodial spines simple falcate (Fig. 21B–C), 27 in a series. Posterior upper part of U-shaped series of spines at same level as anterior upper part. Newly developed spines in posterior upper part of U-shaped series slightly larger than older spines in anterior upper part of series.



**Fig. 20.** Adult morphology of *Pseudopolydora multispinosa* sp. nov., holotype (MIMB 40906, formalin-fixed and stained with MG). **A–C.** Anterior end, left lateral (A) and dorsal (B–C) view. **D–F.** Anterior end, ventral view, showing changing of MG staining in time after return of the specimen to clean ethanol: after 1 minute (D), after 15 minutes (E), after 1 hour (F). Abbreviations: *ch5* = chaetiger 5; *la* = lateral peristomial lip; *ve* = ventral peristomial lip. Scale bars = 200  $\mu$ m.

Hooks in neuropodia from chaetiger 8, up to 30 in a series, not accompanied by capillaries. Hooks bidentate, with upper tooth closely applied to main fang; shaft with constriction on upper part and lower part bent at right angle (Fig. 21D–E).

Branchiae from chaetiger 7 to chaetiger 29, flattened, with surfaces oriented perpendicular to body axis, free from notopodial postchaetal lamellae.

Nototrochs from chaetiger 5 onwards, composed of single rows of cilia. On branchiate chaetigers, nototroch cilia long, arranged in transverse lines and extending onto branchiae; on posterior abranchiate chaetigers, cilia arranged in U-shaped bands, with arms directed posteriorly. Nototrochs on chaetigers 5 and 6 interrupted in the middle by caruncle; nototrochs on succeeding chaetigers complete. Intersegmental ciliation absent.

Pygidium bilobed, with two semi-oval lateral lobes, white due to numerous spindle-shaped glandular cells with striated contents.

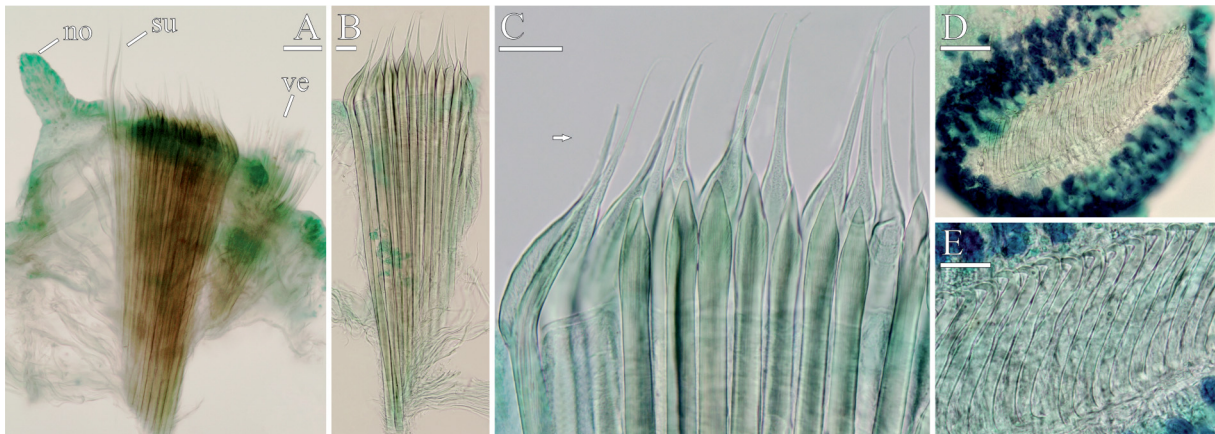
Glandular pouches in neuropodia from chaetiger 1, largest and paired in chaetigers 6 and 7, single in other neuropodia.

Digestive tract without gizzard-like structure and pigmentation.

Nephridia from chaetiger 4 onwards, very narrow in chaetigers 4–6, prominent, greenish from chaetiger 7, in female fertile chaetigers opening to exterior via single middorsal nephridiopore.

### MG staining

Weakly stained prostomium, caruncle, dorsal side of peristomium and ventral side of seven anterior chaetigers. Intensely stained ventral side from chaetiger 8 (Fig. 20A, E–F). As usual for other species, pale greenish staining on the anterior chaetigers and on the dorsum disappeared soon after placing the specimen into clean ethanol; intense violet staining on the ventral side from chaetiger 8, on the contrary, remained for hours (Fig. 20F).



**Fig. 21.** Chaetal morphology of *Pseudopolydora multispinosa* sp. nov., holotype (MIMB 40906, formalin-fixed and stained with MG). **A.** Parapodia of chaetiger 5. **B–C.** Notopodial spines of chaetiger 5. **D.** Neuropodium of chaetiger 12, showing glandular cells intensely stained with MG around a series of hooded hooks. **E.** Hooded hooks from neuropodium of chaetiger 12. Abbreviations: *arrow* showing the direction towards the inside of the U-shaped row of spines; *no* = notopodial postchaetal lamella; *su* = dorsal superior capillary chaetae; *ve* = ventral capillary chaetae. Scale bars: A = 50  $\mu$ m; B–E = 20  $\mu$ m.

### Habitat

The only individual of *P. multispinosa* sp. nov. was found in a silty tube in a muddy sand intertidal habitat.

### Reproduction

*Pseudopolydora multispinosa* sp. nov. is probably gonochoristic. The holotype is a female without oocytes but with glandular cells in the wall of the distal part of enlarged nephridia typical for female fertile chaetigers in *Pseudopolydora* worms. The enlarged nephridia open to the exterior via single middorsal nephridiopore, appearing first on chaetiger 13. The oocytes may thus develop from chaetiger 12 onwards.

### Remarks

*Pseudopolydora multispinosa* sp. nov. is unusual among *Pseudopolydora* in having numerous spines in the notopodia of chaetiger 5 (up to 32 in the anterior row and 27 in the posterior row), well developed notopodial postchaetal lamellae on chaetiger 5 (as same as on chaetigers 4 and 6), and branchiae posteriorly arranged beyond the middle of the body. The majority of *Pseudopolydora* adults have notopodial postchaetal lamellae on chaetiger 5 totally lacking or greatly reduced, and branchiae limited to the anterior half of body. The only known individual of *P. multispinosa* sp. nov. has a unique pattern of MG staining: an intensely stained ventral side of the body from chaetiger 8 onwards, which remained for hours after placing the specimen into clean ethanol.

*Pseudopolydora multispinosa* sp. nov. appears similar to *P. corniculata* Radashevsky & Hsieh, 2000 from Taiwan described by Radashevsky & Hsieh (2000). They have prostomia with long pointed frontolateral horns, caruncles extending beyond chaetiger 5, chaetiger 1 with long cirri-form postchaetal lamellae in both rami, chaetiger 5 with postchaetal lamellae well developed in both rami, notopodial spines of chaetiger 5 of similar morphology, and bilobed pygidia. *Pseudopolydora multispinosa* sp. nov. differs, however, by the large number of spines in the notopodia of chaetiger 5.

### Distribution

Arabian Gulf: Kuwait (Fig. 1D).

### Discussion

#### *Pseudopolydora* in the Arabian Gulf

Only two species of *Pseudopolydora*: *P. antennata* and *P. paucibranchiata* were reported from the Arabian Gulf and the Arabian Sea until recently (see review by Wehe & Fiege 2002; Joydas *et al.* 2015). Our previous work (Al-Kandari *et al.* 2019; Radashevsky *et al.* 2020; Radashevsky & Al-Kandari 2020) and the present study discovered seven species occurring in the intertidal and shallow waters of Kuwait. *Pseudopolydora achaeta* is reported for the Arabian Gulf for the first time. The records of *P. antennata*, originally described from the Tyrrhenian Sea, Gulf of Napoli, Italy, is herein confirmed for Kuwait (see comments below). The records of *P. paucibranchiata* were not confirmed but a new species, *P. arabica*, morphologically very similar but genetically different from *P. paucibranchiata*, was described by Radashevsky & Al-Kandari (2020) instead. Four new species: *P. auha* sp. nov., *P. kuwaiti* sp. nov., *P. melanopalpa* sp. nov., and *P. multispinosa* sp. nov. are described in the present study. The distribution of the new species should further be investigated to understand whether they are endemics of the Gulf or occur over a wider range in the Indian Ocean or elsewhere.

#### *Pseudopolydora antennata*

Taxonomic confusions resulted from the opaque concepts of the identity of *P. antennata*. Morphological characteristics of this species were discussed in details by Simon *et al.* (2019) and Radashevsky (2021).



The latter study for the first time raised a question about the origin of *Pseudopolydora* and suggested that early members of the genus might have “originated and diverged in the Indo-West Pacific and later either naturally arrived in the Atlantic Ocean and the Mediterranean (or vice-versa) or were transported to these regions by human activities (as was *P. paucibranchiata*)” (Radashevsky 2021: 19). *Pseudopolydora antennata*, which was first described outside of its native range, was suggested as one of those invaders in the Mediterranean. *Pseudopolydora* worms from the Arabian Gulf fit the morphology of *P. antennata*, but, in light of a series of sibling and cryptic species discovered within the genus, the ultimate conclusion about the conspecificity of the Mediterranean and Arabian populations should be inferred from a genetic comparison of the corresponding individuals. No sequences of *P. antennata* from the Tyrrhenian Sea or any other part of the Mediterranean are yet available.

### Phylogenetic relationships and complexes of species of *Pseudopolydora*

The results of the Bayesian analysis of the combined dataset of four genetic markers are congruent with the morphological characteristics of the species. The analysis showed that the worms sharing diagnostic features of *P. antennata*, *P. diopatra* and *P. paucibranchiata* form three monophyletic groups which we refer to as species complexes (Fig. 2). Members of the *P. antennata* complex share the following characters: 1) palps without chromatophores, 2) the prostomium anteriorly bifurcated, 3) occipital antenna present, 4) chaetiger 5 similar in size to chaetiger 4, dorsally not overlapping anterior part of chaetiger 6, 5) chaetiger 5 spines arranged in a U-shaped double row, 6) chaetiger 5 anterior-row notopodial spines with enlarged distal end having concavity on top and large triangular tooth on its side directed upwards, and fine bristles arising from concavity and forming long flag-like pointed geniculate transparent tip, 7) chaetiger 5 posterior-row notopodial spines falcate, with short rounded distal part geniculate, with subdistal bulbous swelling bearing very short fine bristles and facing towards the inside of the U-shaped row of spines, 8) the pygidium bilobed, with two semi-oval lateral lobes, and 9) glandular pouches paired in each neuropodium in chaetigers 6 and 7.

Members of the *P. diopatra* complex share the following characters: 1) palps with yellowish-white bands, 2) the prostomium anteriorly weakly incised to blunt, 3) occipital antenna absent, 4) chaetiger 5 larger than chaetiger 4, dorsally overlapping anterior part of chaetiger 6, 5) chaetiger 5 spines arranged in a curved diagonal or almost horizontal double row, 6) chaetiger 5 anterior-row notopodial spines with distal part of stem enlarged, cup-shaped, with concavity on top and long pennoned, pointed distal tip, 7) chaetiger 5 posterior-row notopodial spines simple falcate, 8) the pygidium disc-like to cup-shaped, with dorsal gap to incision, and 9) glandular pouches single in each neuropodium in chaetigers 6 and 7.

Members of the *P. paucibranchiata* complex share the following characters: 1) palps with yellowish-white chromatophores, 2) the prostomium anteriorly entire, rounded, 3) occipital antenna present, 4) chaetiger 5 similar in size to chaetiger 4, dorsally not overlapping anterior part of chaetiger 6, 5) chaetiger 5 spines arranged in a J-shaped double row, 6) chaetiger 5 anterior-row notopodial spines with geniculate distal tip with wide limbation, 7) chaetiger 5 posterior-row notopodial spines simple falcate, 8) the pygidium disc-like to cup-shaped, with dorsal gap to incision, and 9) glandular pouches single in each neuropodium in chaetigers 6 and 7.

The specified complexes do not include all the species of *Pseudopolydora* described up to now. The characters annotated above are not apomorphies (or, at least, not all of them) but merely sets of features shared by members of each complex. The phylogenetic relationships between species of *Pseudopolydora* and the evolutionary transformation of characters within this genus will be investigated in a future analysis of their morphological, reproductive and ecological characteristics.

### Branchial distribution

Simon *et al.* (2019) for the first time used the proportion of branchiate chaetigers as one of the fixed diagnostic characteristics to delineate species of *Pseudopolydora*. However, the analysis of the distribution of branchiae in three species examined in the present study showed that this proportion changes during ontogenetic growth, and two different kinds of correlations can be distinguished (Fig. 5C–E). In *P. antennata*, new pairs of branchiae develop faster than new chaetigers appear in the prepygidial growth zone. Consequently, the proportion of branchiate chaetigers increases during ontogeny. In individuals with more than 30 chaetigers, branchiate chaetigers exceed the middle of the body (Fig. 5C). On the contrary, in *P. arabica* and *P. kuwaiti* sp. nov., new pairs of branchiae develop slower than new chaetigers appear in the prepygidial growth zone. Small individuals of these species have branchiae occurring until the middle of the body, while large individuals have branchiae on only the first  $\frac{1}{3}$ – $\frac{2}{5}$  part of the body (Fig. 5D–E). It seems likely that the former kind of positive correlation is characteristic for the species of the *P. antennata* complex, while the latter kind of negative correlation is characteristic for the species of the *P. diopatra* and *P. paucibranchiata* complexes. This assumption should be verified in further studies.

### MG staining

Simon *et al.* (2019) for the first time successfully used methylene green staining to delineate species of *Pseudopolydora*. They showed that staining patterns anterior to chaetiger 6 supported the division of the examined specimens into five species, although “all species had similar patterns in dorsal staining posterior to chaetiger 6” (Simon *et al.* 2019: 19). In the present study, we found unique, species specific patterns of MG staining posterior to chaetiger 6 in *P. arabica* and *P. multispinosa* sp. nov. The MG staining in other species was either weak or similar to patterns revealed by Simon *et al.* (2019). No stain was applied to *P. kuwaiti* sp. nov.

### Habitat

All previously studied species of *Pseudopolydora* live in sandy or silty tubes in soft sediments or attached to hard substrata. Adult *P. kuwaiti* sp. nov. inhabit silty tubes in muddy sand intertidally and in shallow waters and bore in live mollusc shells, dead corals and shell limestone encrusted by coralline algae. No apparent morphological differences were found between the tube-building and shell-boring worms and the genetic analysis confirmed their conspecificity (Fig. 2; Table 2). *Pseudopolydora auha* sp. nov. was found in burrows in shell limestone encrusted by coralline alga but it was not certain if the worms made the burrows themselves or secondarily occupied empty holes made by other organisms.

Among spionids, species able to bore in hard substrata are present only among some genera of the tribe Polydorini Benham, 1896. Because (1) Polydorini is an advanced clade in the phylogeny of Spionidae, (2) all the other spionids are either free crawlers or tube dwellers (TD), and (3) the shell borers (SB) occur in every polydorin genus, Radashevsky & Pankova (2013) suggested that the ability to bore evolved more than once within Polydorini. The unique example of shell-boring *P. kuwaiti* sp. nov. among predominantly tube-dwelling species of *Pseudopolydora* supports this suggestion.

Remarkably, all shell-boring polydorins are innately able to build tubes. Settled larvae of those species first make silty tubes and then start boring into the substratum. Adults make pairs of tubes that extend from two joined apertures of their burrow. Nevertheless, the majority of polydorins are strictly constrained to only one mode of life, which attribute can be used as an additional character to facilitate identification of species. Conspecific SB and TD individuals were reported in several species of Polydorini based on their morphological characteristics (see Radashevsky & Pankova 2013). Genetic support for their conspecificity is available to date only for *Dipolydora carunculata* (Radashevsky, 1993), *Polydora*

*lingshuiensis* Ye *et al.*, 2015, and *Boccardia proboscidea* Hartman, 1940 (see Radashevsky & Pankova 2013; Ye *et al.* 2015; Radashevsky *et al.* 2019, respectively).

### Reproductive biology

All studied species of *Pseudopolydora* are gonochorists and do not reproduce asexually. Remarkably, some other spionids now living in the same habitats and often co-occurring with *Pseudopolydora* have evolved different reproductive strategies. For example, species of *Pygospio* Claparède, 1863 reproduce asexually by architomy (Blake *et al.* 2020), while all examined species of *Rhynchospio* Hartman, 1936 are simultaneous hermaphrodites (Radashevsky & Choi 2021). These examples show strong phylogenetic bias in the evolution of reproductive characteristics within these genera and at the same time a great evolutionary plasticity of reproductive characteristics within the family Spionidae.

Females of the examined species of the *P. diopatra* and *P. paucibranchiata* complexes lay numerous small eggs about 100 µm in diameter which develop into 3-chaetiger larvae inside the capsules. Hatched larvae are planktotrophic and settle and metamorphose after the development of 13–17 chaetigers. This type of development was observed in *P. diopatra* and *P. nordica*, and probably is also characteristic for *P. kuwaiti* sp. nov. Following Radashevsky & Al-Kandari (2020), we suggest that this type of larval development is basal in Polydorini and possibly in the entire subfamily Spioninae Söderström, 1920. Subsequently, in the course of the evolution of Spioninae, this type of larval development was modified in different ways in different genera.

The sexuality and the mode of larval development of *P. melanopalpa* sp. nov. remain uncertain. All six type specimens of this species are females with the developed coelomic oocytes about 120 µm in diameter.

### Biogeography

In a review of records of polychaetous annelids from the seas surrounding the Arabian Peninsula, Wehe & Fiege (2002) noted that of the 807 taxa (species, subspecies and species groups), 161 (20%) could be considered endemic to the Arabian region, of which 35 (22%) were endemic to the Arabian Gulf. The species diversity was highest in the Red Sea (567), followed by the Arabian Gulf (231). Among Spionidae, 22% (8 of 36 in total) of species were considered endemic to the Arabian region. Overall, there are few revisions of most polychaete families, accompanied by molecular studies, in the region, and thus the origin and the biogeography of the Arabian worm fauna are still poorly understood. Of the 44 species of Polynoidae from the seas surrounding the Arabian Peninsula, 5 (11%) were in common with the Mediterranean Sea, at least 30 (68%) were in common with the Indo-West Pacific, and 10 (23%) species were considered endemic to the region (Wehe 2006). Of the 19 species of Sigalionidae from the seas surrounding the Arabian Peninsula, 4 (21%) were in common with the Mediterranean Sea, at least 8 (47%) were in common with the Indo-West Pacific, and 9 (48%) species were considered to be “endemics” to the region (Wehe 2007). Some of these numbers (and percentages) would be expected to change with more molecular analyses, potentially revealing a lack of conspecificity with, for example, certain Mediterranean or far-flung Indo-West Pacific taxa.

The review by Wehe & Fiege (2002) and studies by Wehe (2006, 2007) showed the high faunal affinities between the whole Arabian region and the Indo-West Pacific. As to the endemism in the Arabian region, Wehe (2006: 179) assumed that “the rate of endemics will decrease with further sampling activities around the Indo-West Pacific.” In contrast, molecular studies may reveal, as suggested above, that regional endemics have been long overlooked, and thus the degree of endemism may increase. Complicating our understanding of regional biodiversity and biogeography will be the continued introduction of alien species resulting from ever-increasing shipping activity (Subba Rao 2005), and thus raising the inevitable question about the potential risk to endemic biota.

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## Supplementary material

**Supp. file.** Additional data. <https://doi.org/10.5852/ejt.2021.773.1519.5149>

**Table ESM1.** Sampling location data and museum registration numbers of *Pseudopolydora* spp. from the Arabian Gulf, Kuwait.

**Table ESM2.** Uncorrected pairwise average distances ( $p$ , in %) between specific clades for 2473 bp total of *16S* (234 bp), *18S* (1639 bp), *28S* (294 bp) and *Histone 3* (306 bp) sequences used in the analysis. The numbers of corresponding samples from Table 1 are given in parentheses after the names of the species and sampling locations. Average distances between conspecific individuals from one clade in *italics*; maximal values of average distances between conspecific individuals from one clade in *bold italics*; minimal values of average distances between species underlined; maximal values of average distances between species in bold.