



High diversity and pan-oceanic distribution of deep-sea polychaetes: *Prionospio* and *Aurospio* (Annelida: Spionidae) in the Atlantic and Pacific Ocean

Theresa Guggolz¹ · Karin Meißner² · Martin Schwentner^{1,3} · Thomas G. Dahlgren^{4,5,6} · Helena Wiklund⁷ · Paulo Bonifácio⁸ · Angelika Brandt^{9,10}

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Abstract

Prionospio Malmgren 1867 and *Aurospio* Maciolek 1981 (Annelida: Spionidae) are polychaete genera commonly found in the deep sea. Both genera belong to the *Prionospio* complex, whose members are known to have limited distinguishing characters. Morphological identification of specimens from the deep sea is challenging, as fragmentation and other damages are common during sampling. These issues impede investigations into the distribution patterns of these genera in the deep sea. In this study, we employ two molecular markers (16S rRNA and 18S) to study the diversity and the distribution patterns of *Prionospio* and *Aurospio* from the tropical North Atlantic, the Puerto Rico Trench and the central Pacific. Based on different molecular analyses (Automated Barcode Gap Discovery, GMYC, pairwise genetic distances, phylogenetics, haplotype networks), we were able to identify and differentiate 21 lineages (three lineages composed solely of GenBank entries) that represent putative species. Seven of these lineages exhibited pan-oceanic distributions (occurring in the Atlantic as well as the Pacific) in some cases even sharing identical 16S rRNA haplotypes in both oceans. Even the lineages found to be restricted to one of the oceans were distributed over large regional scales as for example across the Mid-Atlantic Ridge from the Caribbean to the eastern Atlantic (> 3389 km). Our results suggest that members of *Prionospio* and *Aurospio* may have the potential to disperse across large geographic distances, largely unaffected by topographic barriers and possibly even between oceans. Their high dispersal capacities are probably explained by their free-swimming long-lived planktonic larvae.

Keywords Distribution patterns · Haplotype networks · Vema-Fracture-Zone · Clarion-Clipperton Fracture Zone · 16S rRNA · 18S

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✉ Theresa Guggolz
Theresa.Guggolz@uni-hamburg.de

¹ Zoological Museum Hamburg, Center of Natural History, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

² German Centre for Marine Biodiversity Research, Senckenberg am Meer, c/o Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

³ 3rd Zoological Department, Natural History Museum Vienna, Burgring 7, 1010 Vienna, Austria

⁴ Department of Marine Sciences, University of Gothenburg, PO Box 461, SE-405 30 Gothenburg, Sweden

⁵ Gothenburg Global Biodiversity Centre, PO Box 461, SE-405 30 Gothenburg, Sweden

⁶ NORCE Norwegian Research Centre, Postboks 22 Nygårdstangen, 5838 Bergen, Norway

⁷ Life Sciences Department, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

⁸ Ifremer, Centre Bretagne, REM EEP, Laboratoire Environnement Profond, ZI de la Pointe du Diable, CS 10070, 29280 Plouzane, France

⁹ Senckenberg Research Institute and Natural History Museum, Senckenberganlage 25, 60325 Frankfurt, Germany

¹⁰ Biozentrum, Campus Riedberg, Goethe-University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

Introduction

The genus *Prionospio* Malmgren 1867 is one of the most diverse and speciose taxon within the Spionidae (Guggolz et al. 2018; Paterson et al. 2016). *Prionospio* is morphologically not well defined, even after several revisions and the erection of closely related subgenera and new genera in a *Prionospio* complex (Foster 1971; Maciolek 1985; Sigvaldadottir 1998; Wilson 1990). Currently, the *Prionospio* complex comprises the genera *Aurospio* Maciolek 1981, *Laubieriellus* Maciolek 1981a, *Orthoprionospio* Blake and Kudenov 1978, *Prionospio* Malmgren 1867, *Streblospio* Webster 1879, and *Paraprionospio* Caullery 1914. Most of these revisions were mainly based on shallow-water species, but *Prionospio* can also be regarded a typical deep-sea genus, often found in high abundances (Blake et al. 2018; Guggolz et al. 2018; Guggolz and Meißner pers. observations).

Despite the typical occurrence of *Prionospio* in deep-sea samples, the number of reported species is rather limited (Glover and Fauchald 2018: 29 species—<http://www.marinespecies.org/deepsea/>). Only a few generic characters are available to distinguish between the genera of the *Prionospio* complex; often, only the arrangement of the branchiae is important for the characterization of different subgenera and genera (Paterson et al. 2016). These characters seem to be sufficient to identify specimens in appropriate conditions, but the morphological identification of these soft-bodied annelids from deep-sea samples is often difficult. Due to their fragility, the majority of specimens from these depths are incomplete or damaged (Bogantes et al. 2018; Guggolz et al. 2018, 2019). For example, the genus *Aurospio* is mainly distinguished from *Prionospio* by the number of the branchiae and on which segment they are beginning (Sigvaldadóttir and Mackie 1993), but these appendages are often lost or damaged during sampling procedures.

Species of both genera, *Prionospio* and *Aurospio*, are reported to be widespread (Paterson et al. 2016) or even cosmopolitan (Mincks et al. 2009) (e.g., *Aurospio dibranchiata* Maciolek 1981). A wide dispersal potential in the abyssal has been reported for many benthic invertebrates (Etter et al. 2011; Linse and Schwabe 2018; Schüller and Ebbe 2007); however, these distribution patterns, based solely on morphological taxonomic identification, have to be treated with caution. Recent studies, employing molecular tools, often indicated a more complex scenario. Several of these presumably widespread species were found to be composed of a number geographically restricted and morphologically cryptic species (Bickford et al. 2007; Vrijenhoek 2009) or simply misidentified (Álvarez-Campos et al. 2017; Nygren et al. 2018; Sun et al. 2016). Hence, hypothesizing distribution patterns in the deep sea is still challenging, and integrative approaches, which combine morphological and molecular

techniques, are essential to identify and delimit species (Glover et al. 2016; Hutchings and Kupriyanova 2018). One important aspect of the present study is to examine the diversity and the dispersal capacity of *Prionospio* and *Aurospio* in the Vema-Fracture-Zone (VFZ). Both genera, *Prionospio* and *Aurospio*, are supposed to have planktonic larvae and thus a potential for a widespread geographic distribution (Wilson 1991; Young 2003). We investigate the dispersal along the VFZ and test for barrier effect of the Mid-Atlantic Ridge (MAR) as this underwater mountain ridge is often postulated to represent a topographic barrier for distribution of benthic invertebrates (Bober et al. 2018; McClain et al. 2009; Priede et al. 2013). However, a barrier effect of the MAR on the spionid *Laonice* Malmgren 1867 has recently been rejected (Guggolz et al. 2019). The herein studied *Prionospio* and *Aurospio* will be another important step towards understanding the distribution patterns of species in the deep sea along the MAR. In addition, a potential pan-oceanic distribution is analyzed, by comparing DNA sequences of specimens from the VFZ (tropical Atlantic) with those of the Clarion-Clipperton Fracture Zone (CCZ) from the central Pacific.

Material and methods

Collection and identification of specimens

During the VEMA-Transit expedition in December 2014 to January 2015, 197 of the 332 analyzed specimens were collected from the tropical North Atlantic and the Puerto Rico Trench (Fig. 1: VFZ and PRT). Detailed information about sample treatment and sampling localities are described in Guggolz et al. (2018) and Devey (2015). Four areas were defined for samples from the Atlantic according to the geographical position as following: the eastern part of the Vema-Fracture Zone (eVFZ), extending eastwards from the MAR in the Cape Verde Basin; the western part of the Vema Fracture Zone (wVFZ), extending westwards from the MAR in the Demerara Basin; the Vema Transform Fault (VTF), located between these two areas in the MAR; the Puerto Rico Trench (PRT), located in the shallower part of the trench near Puerto Rico. Maximum distances within areas varied between 276 km (wVFZ) and 1298 km (eVFZ). The eastern-most and western-most studied sites were separated by 4610 km.

The Clarion-Clipperton Fracture Zone (CCZ) is a vast area (about 6 million km²) in the Equatorial Pacific Ocean with high commercial interest because of the presence of polymetallic nodules in the seabed between 4000 and 5000 m depth. The International Seabed Authority (ISA) is in charge of management of deep-sea mineral resources and of protection of marine environment in areas beyond national jurisdiction (Lodge et al. 2014). The ISA provides licenses to the contractors that intend to explore mineral deposits in,

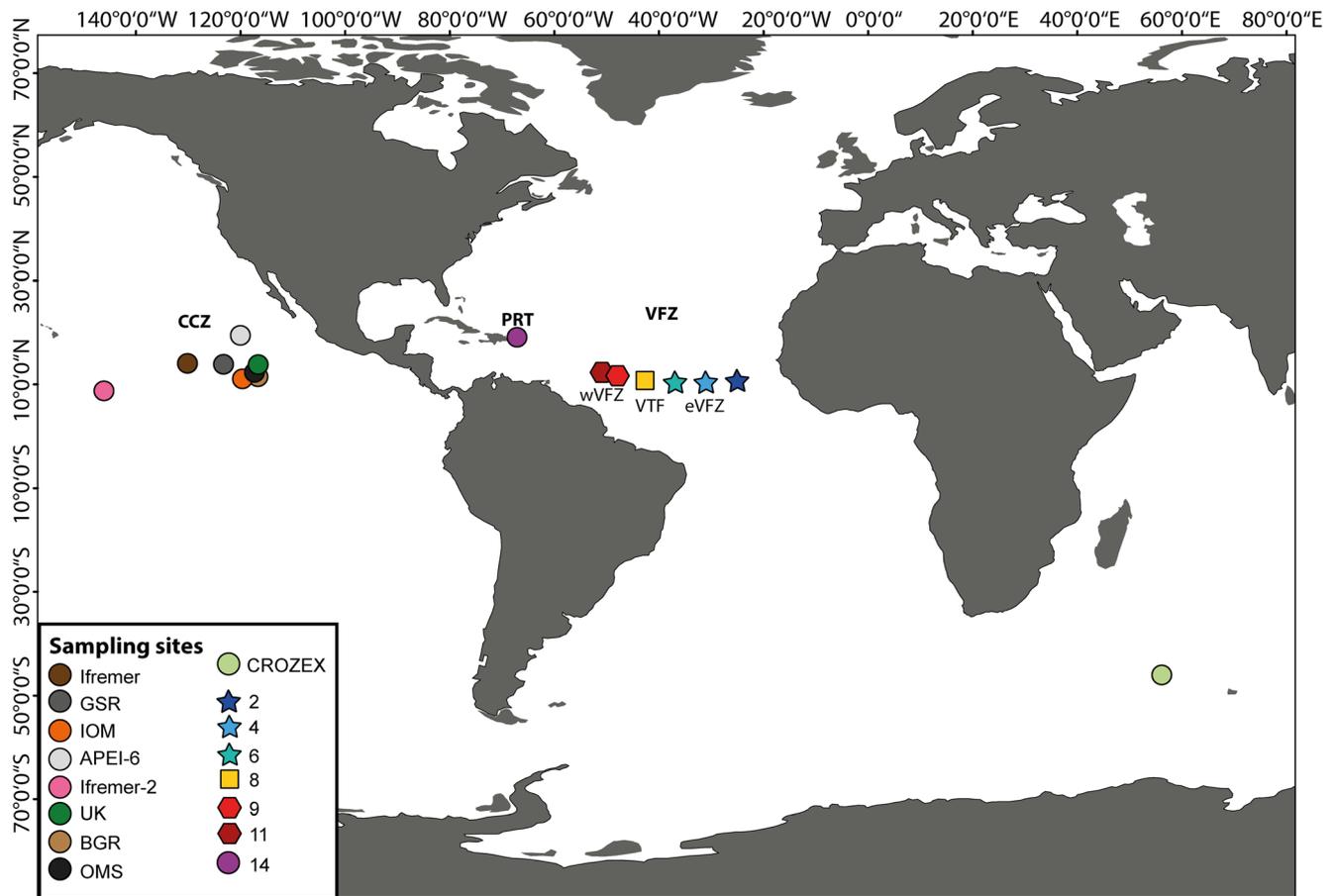


Fig. 1 Map of the worldwide sampling localities. The Clarion Clipperton Fracture Zone (CCZ) in the Pacific, the eastern Vema Fracture zone (eVFZ–stars), the Vema Transform Fault (VTF–rectangular), the western Vema Fracture Zone (wVFZ–hexagon), and the Puerto Rico Trench (PRT)

e.g., the CCZ. To get and keep an exploration contract for an area, the contractor is required to carry out surveys and fauna inventories (Lodge et al. 2014). Furthermore, the ISA administers the regional environmental management plan across the CCZ, so-called Areas of Particular Environmental Interest (APEI). Sequences of 122 specimens morphologically assigned to *Prionospio* and *Aurospio* from eight exploration contract areas and one APEI were included (Fig. 1: the German exploration contract area ‘BGR’, Russia and Poland among other countries ‘IOM’, Belgium ‘GSR’, French ‘Ifremer’ & ‘Ifremer-2’, Singapore ‘OMS’, Britain UK-1 and the APEI-6). Of these, 53 specimens were collected on the two United Kingdom Seabed Resources Ltd. (UKSR) cruises AB01 and AB02 to the UK-1 exploration contract area stratum A and stratum B, the OMS contract area, and the APEI-6. Details on sampling methods are given in Glover et al. (2016). Maps and metadata from UK-1 stratum A have been published in earlier taxonomical work on macrofaunal material from this cruise (Dahlgren et al. 2016; A. Glover et al. 2016; Wiklund et al. submitted, 2017). In addition, 69 specimens morphologically assigned to *Prionospio* and *Aurospio* from BGR, IOM, GSR, and Ifremer were sampled using box-corers (0.25 m²) or epibenthic sledge during EcoResponse

SO239 cruise on board of the RV *Sonne* in March/April 2015 funded by JPI Oceans framework (Martínez Arbizu and Haeckel 2015).

All specimens were sorted and identified at least to genus level (*Prionospio* or *Aurospio*) using dissecting and compound microscopes. Specimens have been deposited in the collection of the Center of Natural History (Universität Hamburg, Germany), Ifremer (France), and Natural History Museum London (Supplement 1).

The map of the sampling areas (Fig. 1) was created using ArcGIS 10.4.1 (www.esri.com).

DNA extraction, PCR amplification, sequencing, and alignment

For the VFZ specimens, one or two parapodia were dissected and transferred into 30 µl of 10% Chelex 100 solution in purified water, incubated for 30 min at 56 °C and 10 min at 99 °C. Polymerase chain reactions (PCR) were performed with a total volume of 15 µl consisting of 1.5 µl DNA extract, 7.5 µl AccuStart II PCR ToughMix (Quanta Bio, Germany), 0.6 µl of each primer (10 mmol), 0.3 µl of GelTrack loading dye (QuantaBio, Germany), and 4.8 µl Millipore H₂O.

Fragments of mitochondrial (16S) and nuclear (18S) rRNA genes were amplified (see Table 1 for list of primers) with initial denaturation step of 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C, 45 s at 43 °C, and 45 s at 72 °C, followed by a final elongation step for 5 min at 72 °C. Success of amplification was determined via gel electrophoresis on 1% agarose/TAE gel. For sequencing, 8 µl of the PCR products was purified using FastAP (1.6 µl; 1 U/µl) and Exonuclease I (0.8 µl; 20 U/µl) (Thermo Fisher Scientific, Germany) with an incubation time of 37 °C for 15 min followed by 15 min at 85 °C. For some of the specimens from the CCZ (BGR, IOM, GSR and Ifremer), DNA extractions were realized with NucleoSpin Tissue (Macherey-Nagel) kit and PCR amplifications as following into 25-µl mixtures, including 5 µl of Green GoTaq® Flexi Buffer (final concentration of 1×), 2.5 µl of MgCl₂ solution (final concentration of 2.5 mM), 0.5 µl of PCR nucleotide mix (final concentration of 0.2 mM each dNTP), 9.875 µl of nuclease-free water, 2.5 µl of each primer (final concentration of 1 µM), 2 µl template DNA, and 0.125 U of GoTaq® G2 Flexi DNA Polymerase (Promega). The temperature profile was 95 °C/240 s, 94 °C/30 s, 52 °C/60 s, 72 °C/75 s *35 cycles, 72 °C/480 s, 4 °C. Purified PCR products were sent to Macrogen Europe, Inc. (Amsterdam-Zuidoost, Netherlands) for sequencing. The remaining specimens from the CCZ (APEI-6, OMS, UK-1) were extracted with DNeasy Blood and Tissue Kit (Qiagen) using a Hamilton Microlab STAR Robotic Workstation. PCR mixtures contained 1 µl of each primer (10 µM), 2 µl template DNA, and 21 µl of Red Taq DNA Polymerase 1.1× MasterMix (VWR) in a mixture of total 25 µl. The PCR amplification profile consisted of initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were purified using Millipore Multiscreen 96-well PCR Purification System, and sequencing was performed on an ABI 3730XL DNA Analyser (Applied Biosystems) at Natural History

Museum Sequencing Facility, using the same primers as in the PCR reactions.

In total, 331 specimens were successfully sequenced for 16S rRNA and 63 specimens for 18S. Additionally, the mitochondrial COI gene and the nuclear 28S and ITS genes were tested with considerably worse success and therefore deliberately omitted. 18S is known to show some degree of divergence, even at species level in spionids, and thus successfully used for supporting mitochondrial markers (Guggolz et al. 2019; Meißner et al. 2014; Meißner and Blank 2009). Sequences were assembled and corrected with Geneious 6.1.8 (Kearse et al. 2012; <http://www.geneious.com>) and deposited in GenBank (for accession numbers, see Supplement Table 1). The obtained sequences of the different gene fragments were aligned separately using MAFFT (Katoh and Standley 2013; V 7.402) implemented with CIPRES Science Gateway V.3.3 (Miller et al. 2010; www.phylo.org).

Initial identification of species, phylogenetic analyses, and haplotype networks

To obtain a first estimation of the number of species present in our data set, the Automated Barcode Gap Discovery (Puillandre et al. 2012; ABGD) was conducted with 16S rRNA. The ABGD identifies potential barcoding gaps separating hypothetical species, which is based on the assumption that interspecific genetic distances are larger than intraspecific distances. The ABGD analysis was run on the web-based version of the software (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>). Pairwise uncorrected *p*-distances were used for the analyses (Table 2), which were calculated with MEGA7 (Kumar et al. 2016), including all available sequences for each gene, respectively. Standard settings for ABGD were kept, except for *P*_{min} (0.005), the numbers of steps (100), and the relative gap width (*X* = 0.5). In the following, we will use the term lineages rather than species for the units delimited by ABGD, as not all of these might correspond to actual species. Additionally, the single locus tree-based

Table 1 Primers used in this study

Gene	Primer	Primer sequence 5'-3'	Authors
16S	16Sar	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
	16Sbr	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
	16Sb-L	CCGGTCTGAACTCAGATCACGT	Palumbi et al. (1991)
	Ann16SF	GCGGTATCCTGACC GTRCWAAGGTA	Sjölin et al. (2005)
18S	Uni 18S F	GCTTGCTCAGAGATTAAGCC	Dzikowski et al. (2004)
	HET 18S R	ACGGAAACCTTGTTACGA	Dzikowski et al. (2004)
	18SA	AYCTGGTTGATCCTGCCAGT	Medlin et al. (1988)
	18SB	ACCTTGTTACGACTTTTACTTCCTC	Nygren and Sundberg (2003)
	620F	TAAAGYTGTYGCAGTTAAA	Nygren and Sundberg (2003)
	1324R	CGGCCATGCACCACC	Cohen et al. (1998)

Table 2 Percentage of uncorrected p-distances within (diagonal panels) and among lineages (upper panels) for 16S (bold) and 18S (regular). “X” means no or only one sequence available

	<i>Prionospio</i> sp. A	<i>Prionospio</i> sp. B	<i>Prionospio</i> sp. C	<i>Prionospio</i> sp. D	<i>Aurospio</i> cf. <i>dibranchiata</i>	<i>Prionospio</i> sp. E
<i>Prionospio</i> sp. A	0.7 - 2.0 X	20.2 - 22.2 X	21.6 - 22.4 X	20.6 - 22.7 X	23.1 - 27.6 X	21.0 - 24.8 X
<i>Prionospio</i> sp. B		0 - 1.2 0	8.4 - 10.3 0	9.5 - 15.9 0	18.6 - 22.1 0.3	17.6 - 22.5 X
<i>Prionospio</i> sp. C			0 - 0.2 0	5.1 - 6.2 0	18.3 - 22.5 0.3	18.6 - 22.4 X
<i>Prionospio</i> sp. D				0 - 0.5 X	16.8 - 23.9 0.3	15.9 - 22.7 X
<i>Aurospio</i> cf. <i>dibranchiata</i>					0 - 2.9 0	20.4 - 26.5 X
<i>Prionospio</i> sp. E						0 - 3.3 X
<i>Prionospio</i> sp. F						
<i>Aurospio</i> <i>foodbancsia</i>						
<i>Prionospio</i> sp. G						
<i>Prionospio</i> sp. H						
<i>Prionospio</i> sp. I						
<i>Prionospio</i> sp. K						
<i>Prionospio</i> sp. L						
<i>Prionospio</i> sp. M						
<i>Prionospio</i> sp. N						
<i>Prionospio</i> sp. O						
<i>Prionospio</i> sp. P						
<i>Prionospio</i> sp. Q						
<i>Aurospio</i> sp. R						
<i>Aurospio</i> sp. S						
<i>Aurospio</i> sp. T	16S 18S					

species delimitation analysis GMYC (general mixed Yule coalescent; Pons et al. 2006) was used to support the results of the ABGD. For the GMYC analyses, an ultrametric tree was calculated with BEAST 2.4.6 (Bouckaert et al. 2014) employing the YULE coalescence model for 50×10^6 generations, retaining every 5000th tree. In this analysis, each haplotype was included only once. Convergence was assessed with Tracer v1.6 and TreeAnnotator v2.4.6 was used to obtain the final tree. GMYC was run in R, version 3.6.1 (R Core Team 2018) using standard settings for the single threshold analysis.

To assess the phylogenetic relationships among the studied specimens and to assess whether the lineages suggested by ABGD are monophyletic, phylogenetic analyses were performed with Bayesian inference and maximum likelihood. Both gene fragments were analyzed separately, as well as concatenated with MrBayes (Ronquist and Huelsenbeck 2003; version 3.2). The maximum likelihood was performed using Randomized Axelerated Maximum Likelihood (Stamatakis 2014; RAxML v.8.2.10) on XSEDE with rapid bootstrapping (1000 iterations) via the CIPRES Science Gateway V.3.3 (Miller et al. 2010; www.phylo.org).

Whether a given phylogenetic hypothesis can be rejected by the data is often not obvious from the tree topology, especially in cases where some branches are not significantly supported. We used the approximately unbiased test (Shimodaira 2002) as implemented in the program CONSEL (Shimodaira and Hasegawa 2001) to test the statistical significance of likelihood differences between the best ML topology and the ML topology, in which the genus *Aurospio* was constrained to be monophyletic. The constrained ML tree and the site-wise likelihoods associated with each topology (necessary for conducting the approximately unbiased test) were calculated with GARLI 2.0 (Zwickl 2006). Due to missing sequences of the 18S genes for a large part of the analyzed specimens and the very few mutations, only the phylogenetic analysis of the 16S rRNA gene is included. For the 16S rRNA analysis, different spionids that are not assigned to the *Prionospio* complex were chosen as outgroups (Supplement 1). Additionally, available 16S rRNA sequences of *Prionospio* and *Aurospio* from GenBank were included in the analyses (Supplement 1). Four chains were run for 5×10^7 generations, with sampling every 1200th generation, and discarding the first 25% as

Table 2 (Continued)

<i>Prionospio</i> sp. F	<i>Aurospio foodbancsia</i>	<i>Prionospio</i> sp. G	<i>Prionospio</i> sp. H	<i>Prionospio</i> sp. I	<i>Prionospio</i> sp. K	<i>Prionospio</i> sp. L	<i>Prionospio</i> sp. M
20.4 - 21.6 X	24.6 - 25.8 X	20.5 - 21.0 X	22.8 - 28.7 X	20.0 - 20.5 X	23.8 - 25.0 X	24.0 - 26.6 X	25.0 - 25.7 X
13.6 - 15.3 X	18.6 - 21.9 0.7	10.0 - 16.0 0.1	18.8 - 24.1 0.8 - 0.9	17.0 - 18.7 0.8	19.8 - 21.7 X	18.9 - 22.2 0.7	21.4 - 25.5 0.7
14.6 - 15.1 X	19.3 - 20.5 0.7	13.7 - 14.4 0.1	18.7 - 22.9 0.8 - 0.9	18.5 - 18.6 0.8	18.3 - 18.8 X	14.2 - 21.6 0.7	22.1 - 23.1 0.7
14.8 - 16.1 X	19.6 - 21.3 0.7	15.4 - 16.5 0.1	18.0 - 24.3 0.8 - 0.9	18.3 - 21.0 0.8	19.5 - 21.0 X	15.8 - 22.4 0.7	18.9 - 24.3 0.7
16.2 - 18.7 X	22.2 - 28.1 0.5	16.7 - 21.1 0.4	19.6 - 21.7 0.7 - 0.8	18.9 - 21.7 0.7	22.8 - 27.4 X	22.9 - 27.8 0.5	22.4 - 28.2 0.5
18.6 - 20.9 X	21.5 - 25.7 1.3	15.6 - 21.2 1.9	21.6 - 32.2 1.4 - 1.5	18.4 - 22.0 1.4	23.9 - 27.8 X	22.7 - 29.1 1.3	23.4 - 28.7 1.3
X X	20.5 - 21.2 X	14.5 - 14.8 X	17.4 - 21.3 X	17.5 X	20.6 X	19.4 - 20.7 X	21.3 - 22.3 X
	0 - 1.2 X	19.7 - 20.7 0.8	21.5 - 27.3 0.5 - 0.7	20.3 - 20.7 0.5	16.3 - 17.0 X	12.8 - 13.8 0	15.1 - 16.3 0
		0 - 2.5 0	18.3 - 24.3 0.9 - 1.0	16.4 - 16.7 0.9	20.4 - 20.7 X	18.4 - 20.3 0.8	18.9 - 20.2 0.8
			0 - 4.8 0 - 0.1	11.6 - 16.6 0	20.1 - 25.9 X	20.9 - 26.9 0.3 - 0.4	22.7 - 30.1 0.3 - 0.4
				X X	21.2 X	22.0 - 23.0 0.3	23.4 - 24.2 0.3
					X X	13.9 - 14.0 X	17.8 - 18.0 X
						0 - 4.9 0	15.2 - 16.9 0
							0 - 1.7 0

burn-in. Thus, the convergence chain runs were validated using TRACER v.1.7.1 (Rambaut et al. 2018). The GTR + I + G substitution model was identified by MEGA7 as the best fitting model under the AIC criterion.

To better visualize the geographic distribution of the genetic diversity, median-joining haplotype networks were generated with Network 5.0.0.3 (<http://fluxus-engineering.com/>) and popART 1.7 (Bandelt et al. 1999) for each gene fragment. In 16S rRNA, networks were calculated separately for each lineage. The generated haplotype networks were redrawn with Adobe Illustrator CS6.

Analyses of population differentiation were performed with Arlequin 3.5 (Excoffier and Lischer 2010) for lineages with sufficiently large specimen numbers (at least four specimens per site). Pairwise Φ_{st} was calculated different lineages identified as *Aurospio* cf. *dibranchiata*, *Aurospio* sp. S, and *Prionospio* sp. B, sp.H (16S rRNA). For *Aurospio* cf. *dibranchiata*, the areas eVFZ, wVFZ, and CCZ, for *Aurospio* sp. S the areas eVFZ and CCZ and for *Prionospio* sp. B the areas eVFZ, VTF and CCZ and for *Prionospio* sp. G the sites 2 and 4 were compared (Tables 3 and 4).

Results

Alignment

The alignment of the 16S rRNA fragment included a total of 331 sequences with a minimum length of 442 base pairs (bp), of which 237 bp were variable and 223 bp were parsimony

informative. The alignment of the 18S fragment consisted of 59 sequences with 845 bp minimum length, of which 25 bp were variable and 20 bp were parsimony informative.

Species delimitation and diversity

The ABGD analysis of the 16S rRNA dataset retrieved 21 lineages when a barcode gap threshold of 1.49% was employed. The 21 lineages were designated to *Prionospio* sp. A to sp. P, *Aurospio* sp. Q to sp. T, as well as *Aurospio* cf. *dibranchiata* and *Aurospio foodbancsia* Mincks et al. 2009. The lineage *Aurospio* cf. *dibranchiata* is named according to corresponding GenBank records assigned in the analyses (Supplement 1), but with reservation, as no genetic data is available for the holotype or from the type locality. With higher barcode threshold values, a few lineages collapsed (5.1–5.2% = 20 lineages: lineages *Prionospio* sp. C and sp. D collapse; 5.4–8.5% = 19 lineages: lineages *Prionospio* sp. N and sp. O collapse). The GMYC analyses recovered also 21 lineages with slight differences to the results of the ABGD analysis. The lineages *Prionospio* sp. C and sp. D were grouped together into one lineage and lineage *Prionospio* sp. H was separated in two lineages.

Pairwise genetic distances (uncorrected p -distances) between the 21 lineages varied for 16S rRNA from 5.1–32.0% (based on the 21 lineages derived from 16S) (Table 2). The lowest pairwise distances were found between lineages *Prionospio* sp. C and sp. D (16S: 5.1–6.2%), as well as between lineages *Prionospio* sp. N and sp. O (16S: 5.2–5.9%; Table 2). All other inter-lineage distances exceeded 8.5% for

Table 2 (Continued)

<i>Prionospio</i> sp. N	<i>Prionospio</i> sp. O	<i>Prionospio</i> sp. P	<i>Prionospio</i> sp. Q	<i>Aurospio</i> sp. R	<i>Aurospio</i> sp. S	<i>Aurospio</i> sp. T
24.0 - 26.8 X	25.2 - 26.4 X	23.0 - 25.5 X	28.8 - 29.0 X	23.3 - 23.0 X	20.9 - 22.8 X	24.0 - 26.7 X
18.2 - 22.6 0.7	20.0 - 22.4 0.9	20.1 - 22.2 0.5	22.6 - 23.7 X	20.5 - 21.7 X	17.8 - 21.5 0.3	18.2 - 21.8 X
18.2 - 23.2 0.7	19.7 - 22.8 0.9	20.4 - 22.4 0.5	22.9 - 23.3 X	20.5 X	18.8 - 21.5 0.3	18.2 - 22.4 X
16.5 - 24.4 0.7	21.0 - 24.1 0.9	19.7 - 23.2 0.4	21.9 - 23.9 X	21.2 - 23.0 X	15.6 - 22.0 0.3	15.9 - 23.5 X
12.8 - 27.8 0.5	22.7 - 27.2 0.5	19.0 - 27.0 1.1	25.9 - 28.2 X	24.0 - 27.5 X	13.2 - 16.9 0	12.3 - 27.1 X
21.4 - 28.4 1.3	25.0 - 28.0 1.5	20.7 - 24.1 X	25.1 - 27.9 X	25.6 - 28.8 X	19.8 - 24.1 1.6	21.4 - 27.4 X
17.6 - 22.3 X	22.5 - 22.8 X	18.7 - 20.1 0.1	22.3 X	22.6 X	17.7 - 17.9 X	17.3 - 21.3 X
18.2 - 21.5 0	18.5 - 20.0 0.2	21.0 - 22.5 0.7	24.7 - 25.7 X	19.5 - 20.0 X	21.3 - 24.1 0.5	18.1 - 23.4 X
15.9 - 24.4 0.8	23.7 - 24.4 0.1	21.8 - 23.5 0.2 - 0.3	23.7 - 23.9 X	22.2 - 22.5 X	15.3 - 17.1 0.4	15.6 - 23.5 X
17.8 - 27.1 0.3 - 0.4	23.9 - 27.2 0.5 - 0.7	21.3 - 28.5 0.2	26.6 - 32.0 X	24.5 - 28.8 X	20.1 - 26.2 0.7 - 0.8	18.0 - 26.7 X
18.4 - 23.7 0.3	24.2 - 24.4 0.5	21.6 - 22.9 X	24.9 X	23.6 X	19.4 - 21.9 0.7	18.2 - 23.4 X
14.7 - 21.0 X	15.5 - 15.6 X	22.7 - 23.7 0.2	25.6 X	16.4 X	22.7 - 24.7 X	23.1 X
16.8 - 21.4 0	17.8 - 19.3 0.2	23.6 - 25.8 0.2	25.2 - 25.5 X	18.4 - 19.5 X	21.5 - 25.3 0.5	16.2 - 24.2 X
21.5 - 22.9 0	21.8 - 22.1 0.2	23.2 - 24.2 0.2	28.6 - 29.2 X	19.4 - 19.7 X	22.0 - 25.3 0.5	21.5 - 25.5 X
0 - 5.8 X	5.2 - 5.9 0.2	21.1 - 22.6 0.2	24.8 - 25.3 X	14.9 X	23.1 - 23.7 0.5	23.1 - 25.9 X
	0 - 0.8 X	21.0 - 22.0 0.3	25.3 - 25.5 X	14.4 - 14.7 x	22.7 - 23.7 0.8	22.4 - 25.5 X
		0 - 0.8 X	24.0 - 26.0 X	24.0 - 24.7 X	21.5 - 23.7 0.4	21.8 - 26.2 X
			X X	24.7 X	27.4 - 28.1 X	26.5 - 29.0 X
				X X	24.9 X	24.9 - 28.0 X
					0 - 0.7 0	8.0 - 10.3 X
						0 - 1.4 X

16S rRNA. The highest observed intra-lineage distances were found in lineage *Prionospio* sp. H with 4.8% for 16S rRNA (Table 2). Pairwise distances between the single lineages found for 18S varied from 0 to 1.9%, with highest distances between lineage *Prionospio* sp. E and sp. G and the highest intra-lineage pairwise distances were found for lineage *Prionospio* sp. E with 0.1% (Table 2).

The phylogenetic analyses of 16S recovered all 21 lineages as reciprocal monophyletic with full support each (Fig. 2; supplement 2). The two genera were not recovered as monophyletic as *Aurospio foodbancsia*, *Aurospio* sp. R and *Aurospio* sp. Q were nested among the *Prionospio* lineages (Fig. 2) and *Aurospio* sp. S, sp. T and *Aurospio* cf. *dibranchiata* formed a monophyletic clade, but also, this clade was nested within *Prionospio* lineages (Fig. 2). The approximately unbiased test rejected the ML topology, in which the genus *Aurospio* was

constrained to be monophyletic, as being significantly worse than the unconstrained ML topology ($p = 1.0 \times 10^{-81}$).

In the haplotype networks of 16S rRNA (Fig. 3), a total of 128 haplotypes (h1-16S h128-16S) were found with a maximum of 28 haplotypes in one lineage (*Prionospio* sp. H, h31-16S h58-16S; Fig. 3). Notably, *Prionospio* H and *Aurospio* cf. *dibranchiata* had the highest intra-lineage 16S distances with up to 4.8%, and in both cases, these high distances were observed among individuals from Atlantic and Pacific oceans. However, other individuals from these regions either shared 16S rRNA haplotypes or featured haplotypes separated by only 1.3% (Fig. 3; Table 2).

The 18S network showed a total of 18 genotypes (h1-18S h18-18S; Fig. 4). The lineages *Prionospio* sp. D, sp. E, sp. G, sp. O and sp. P are well differentiated from each other, while other lineages shared genotypes and cluster together with no

Table 3 Population indices for 16S of selected *Prionospio* or *Aurospio* species among geographic areas and oceans. Nucleotide diversity, Tajima's D and Fu's Fs are reported only for the areas, not the individual areas

	Area	No. of ind.	No. of haplotypes	Nucleotide diversity \pm SD	Tajima's D (<i>p</i> value)	Fu's F_s (<i>p</i> value)
<i>Prionospio</i> sp. B						
Atlantic	eVFZ	15	8	0.0039 \pm 0.002	-0.897 (0.18)	-1.999 (0.06)
	VTF	4	2	0.0015 \pm 0.0019		
Pacific	CCZ	12	4	0.0056 \pm 0.0039	0.000 (1.00)	2.076 (0.86)
<i>Prionospio</i> sp. H						
Atlantic	eVFZ	38	22	0.0101 \pm 0.0060	-1.730 (0.03)	-14.396 (0.00)
Pacific	CCZ	9	8	0.0091 \pm 0.0062	-1.166 (0.13)	-5.062 (0.00)
<i>Aurospio</i> sp. S						
Atlantic	eVFZ	11	4	0.0026 \pm 0.0022	-0.384 (0.32)	-0.939 (0.12)
Pacific	CCZ	4	3	0.0029 \pm 0.0029	-0.709 (0.28)	-0.887 (0.10)
<i>Aurospio</i> cf. <i>dibranchiata</i>						
Atlantic	eVFZ	36	12	0.0052 \pm 0.0034	-1.471 (0.05)	-5.703 (0.00)
	wVFZ	7	5	0.0053 \pm 0.0039		
Pacific	CCZ	24	8	0.0074 \pm 0.0046	0.112 (0.59)	-0.208 (0.50)

eVFZ eastern Vema Fracture Zone, wVFZ western Vema-Fracture Zone, VTF Vema Transform Fault, CCZ Clarion-Clipperton Fracture Zone

or one mutational step (*Prionospio* sp. L, sp. M, sp. N and *Aurospio foodbancsia*; *Prionospio* sp. H and sp. I; *Prionospio* sp. B and sp. C; *Aurospio* cf. *dibranchiata* and *Aurospio* sp. S; Fig. 4).

For several *Prionospio* and *Aurospio* lineages, pan-oceanic distributions with occurrences in the Atlantic as well as Pacific were recorded. *Prionospio* sp. A, sp. B, sp. E, sp. H, sp. L, and

Table 4 Pairwise Φ_{st} values among different sites for 16S of selected *Prionospio* or *Aurospio* species among areas. Asterisk indicating significant *p* values

<i>Prionospio</i> sp. B	Atlantic		Pacific	
	eVFZ	VTF	CCZ	
eVFZ	0.00			
VTF	0.00	0.00		
CCZ	0.31*	0.20	0.00	
<i>Prionospio</i> sp. H	Atlantic		Pacific	
	eVFZ	CCZ		
eVFZ	0.00			
CCZ	0.31*	0.00		
<i>Aurospio</i> sp. S	Atlantic		Pacific	
	eVFZ	CCZ		
eVFZ	0.00			
CCZ	0.23*	0.00		
<i>Aurospio</i> cf. <i>dibranchiata</i>	Atlantic		Pacific	
	eVFZ	VTF	CCZ	
	eVFZ	0.00		
	wVFZ	0.15*	0.00	
CCZ	0.58*	0.54*	0.00	

eVFZ eastern Vema Fracture Zone, wVFZ western Vema-Fracture Zone, VTF Vema Transform Fault, CCZ Clarion-Clipperton Fracture Zone

sp. N, as well as *Aurospio* sp. S and *Aurospio* cf. *dibranchiata*, were recorded from the VFZ in the Atlantic and the CCZ in the Pacific whereas *Aurospio* cf. *dibranchiata* and *Prionospio* sp. E occurred furthermore in the Southern Ocean (Fig. 2; Supplement 2). However, not only lineages, but also five 16S rRNA haplotypes were shared between specimens from the Pacific and the Atlantic (*Prionospio* sp. B, h9-16S; *Prionospio* sp. H, h35-16S; *Prionospio* sp. L, h62-16S; *Aurospio* sp. S, h94-16S; *Aurospio* cf. *dibranchiata*, h111-16S; Fig. 3). The majority of the pan-oceanic distributed species show one haplotype per species shared between the Atlantic and the Pacific and a separation between both oceans is observable, though often by only few mutational steps. In *Prionospio* sp. A and sp. N specimens of each ocean do not share haplotypes and Atlantic and Pacific haplotypes are separated by three and six mutations, respectively (Fig. 3). Also for *Prionospio* sp. E, the haplotypes are not shared between oceans, but there is also no clear separation between Pacific and Atlantic as the haplotypes of the Atlantic intermingle with haplotypes from the Pacific (and to a lesser degree with those from Antarctica) (Fig. 3). The single specimen from the Pacific assigned to *Prionospio* sp. L shares a haplotype with specimens from the Atlantic (Fig. 3; Supplement 1; h62-16S: 1 Pacific specimen and 6 Atlantic specimens). *Prionospio* sp. B show a pan-oceanic shared haplotype (Fig. 3; Supplement 1; h9-16S: three Atlantic specimens and nine Pacific specimens) and no obvious separation of the oceans. Population genetics of *Prionospio* sp. B showed significant difference between the population from the eVFZ and the population from the Pacific, but no significant differences between the specimens from the VTF and the Pacific were found (Table 4). Also, *Prionospio* sp. H and *Aurospio* cf. *dibranchiata*

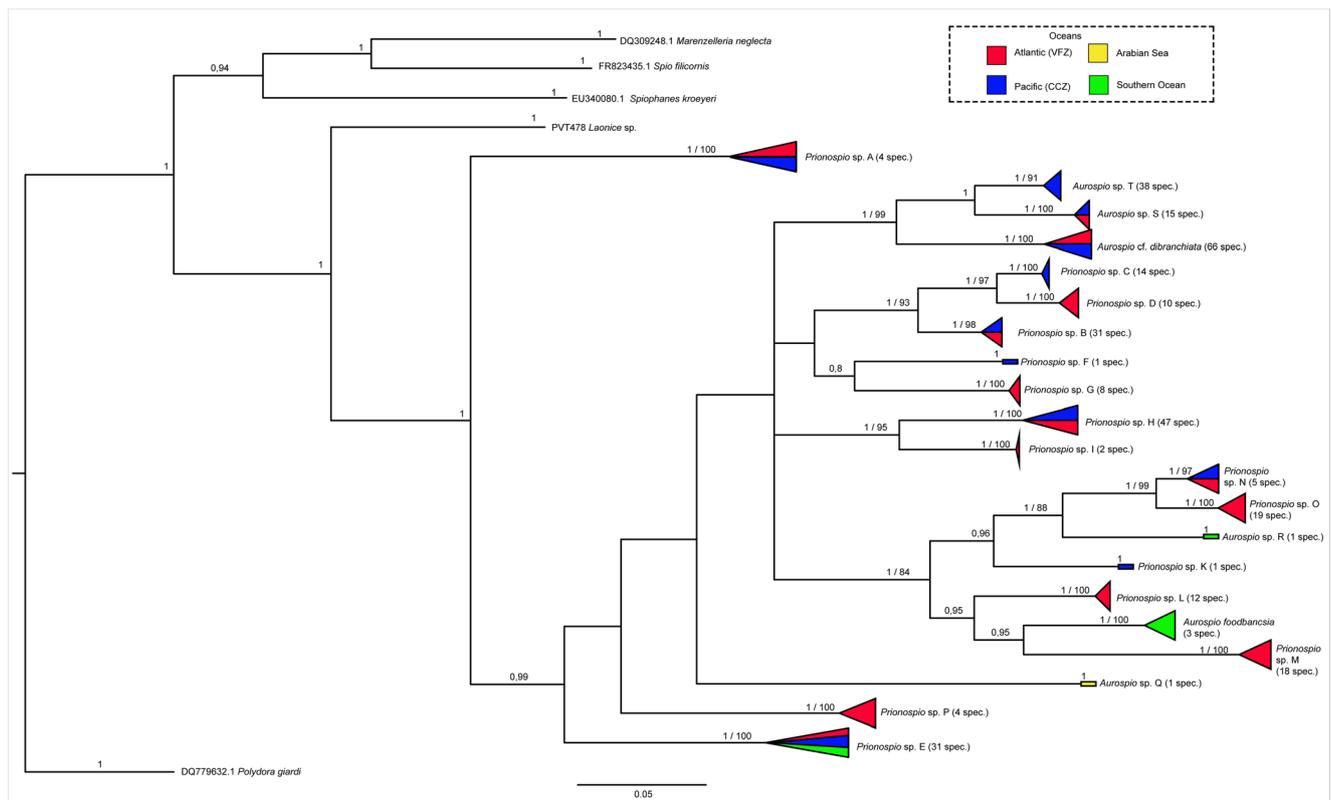


Fig. 2 Phylogenetic tree of *Prionospio* and *Aurospio* species obtained in the study based on mitochondrial 16S gene fragments. Individual specimens can be found in Supplement 2. Posterior probabilities shown next to the nodes (values below 0.8 are not shown). Bootstrap values are

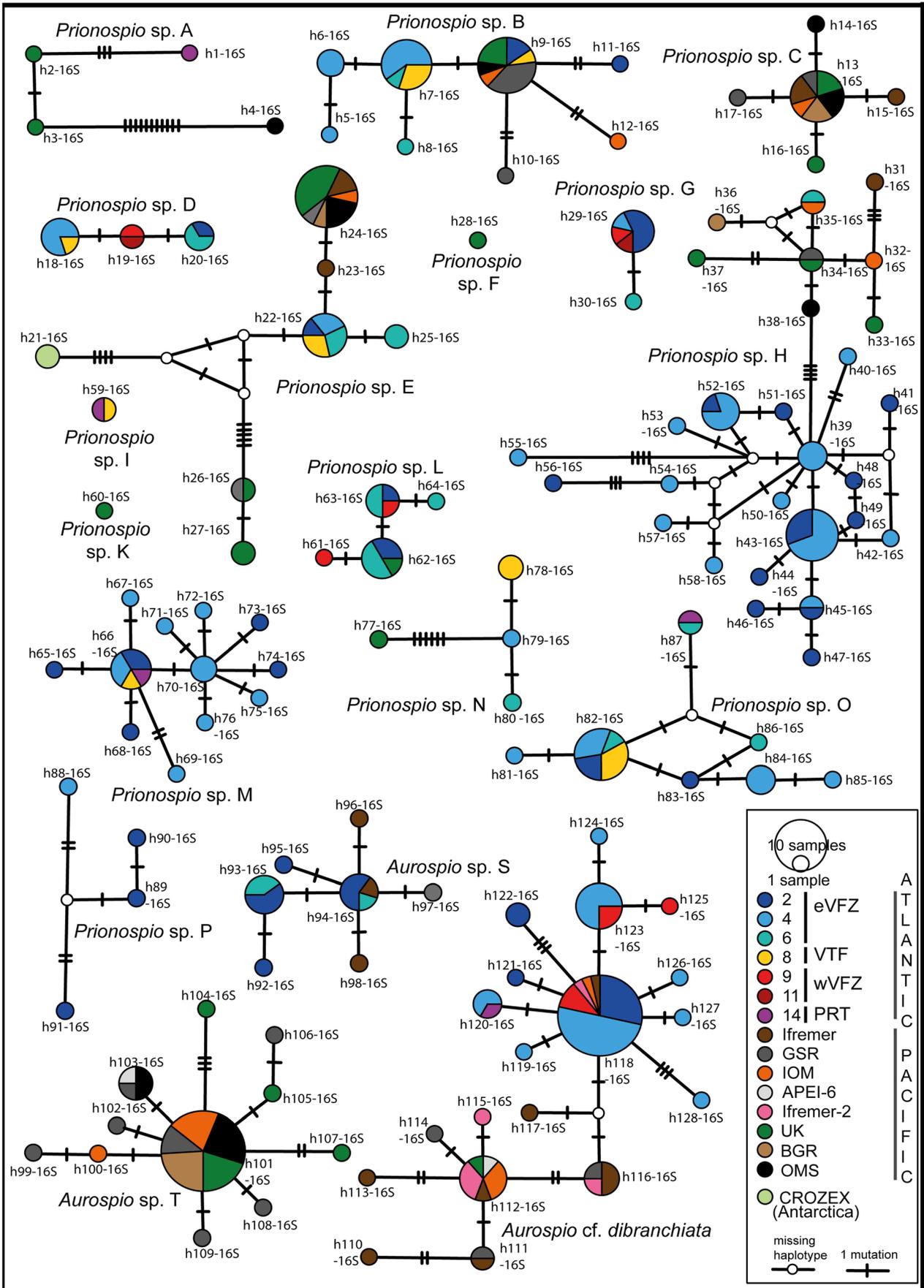
shown after slashes (values below 80 are not shown). Numbers of specimens are given in brackets. Individual occurrence in different oceans is color coded non proportional (see legend in the right upper corner)

each have one haplotype distributed across the Atlantic and the Pacific (Fig. 3; Supplement 1; h35-16S: one Pacific and one Atlantic specimen; h118-16S: one Pacific and 23 Atlantic specimens), and a separation of both oceans can be seen in the networks with a minimum of four mutations in *Prionospio* sp. H and two mutations in *Aurospio* cf. *dibranchiata* between the other haplotypes. Population differentiation was significant between the Atlantic and Pacific specimens for *Prionospio* sp. H (Table 4). For *Aurospio* cf. *dibranchiata*, significant differences were also found within the Atlantic population (eVFZ and VTF; Table 4). Tajima's D and Fu's F_s reveal significant negative values for *Prionospio* sp. H (Table 4). *Aurospio* cf. *dibranchiata* showed also negative significant values, but only for the Atlantic population (Table 3: Fu's F_s). *Aurospio* sp. S showed one shared haplotype (Fig. 3; Supplement 1; h94-16S: one Pacific and four Atlantic specimens), but with no clear separation of both oceans, as most other haplotypes are separated by only one mutational step from the shared haplotype. Nevertheless, population differentiation was significant between the Atlantic and Pacific specimens for lineage *Aurospio* sp. S (Table 4).

Thirteen of the identified lineages were restricted to one of the sampled oceans: *Prionospio* sp. D, sp. G, sp. I, sp. M, sp. O and sp. P to the Atlantic; *Prionospio* sp. C, sp. F, sp. K and *Aurospio* sp. T to the Pacific; *Aurospio foodbancsia* and *Aurospio* sp. R to the Southern Ocean; and *Aurospio* sp. Q to the Arabian Sea (Fig. 2). Moreover, some of these lineages were distributed over larger regional scales within these oceans and sea. For instance, *Prionospio* sp. G had one haplotype recorded from the eVFZ and the wVFZ (Fig. 3: h29-16S) and *Prionospio* sp. L even shared a haplotype between the eVFZ and the PRT (Fig. 3: h61-16S).

Discussion

In the present study based on the mitochondrial marker 16S rRNA gene, 21 lineages were identified consistently and well supported for *Prionospio* and *Aurospio* from the deep Atlantic and Pacific. But limits of mitochondrial markers for species delimitation are well known, as mitochondrial DNA poses potential risks like the retention of ancestral polymorphism, male-biased gene flow, hybrid introgression, and paralogy (Galtier et al. 2009; Moritz and Cicero 2004). Species delimitation with GMYC is largely corresponding to the lineages



◀ **Fig. 3** Haplotype networks of *Prionospio* and *Aurospio* species from the different localities of 16S gene fragments. Sampling localities are color coded

identified with ABGD, except the collapse of lineages *Prionospio* sp. C and sp. D to one lineage and the separation of lineage *Prionospio* sp. H into two lineages. However, taking into account the results for the nuclear 18S gene, the lineages *Prionospio* sp. D, sp. E, sp. G, sp. O and sp. P can be easily differentiated. Other lineages share 18S genotypes (*Prionospio* sp. B and sp. C; *Prionospio* sp. H and sp. I; *Prionospio* sp. L, sp. M, sp. N and *Aurospio foodbanesia*; *Aurospio* cf. *dibranchiata* and *Aurospio* sp. S), but lacking differentiation in 18S analyses is not surprising, as the gene is known for the lower discriminating power at species level for metazoans (Halanych and Janosik 2006; Hillis and Dixon

1991). The lack of differentiation for some lineages in 18S is probably not the result of the lack of reproductive isolation among these species, but rather caused by a combination of the lower substitution rate in this marker and incomplete lineage sorting (Funk and Omland 2003). All lineages, which share an identical 18S genotype, had uncorrected pairwise distances exceeding 8.4% in 16S rRNA (lineages *Prionospio* sp. B and sp. C: 8.4–10.3%; lineages *Prionospio* sp. H and sp. I: 11.6–16.6%; lineages *Prionospio* sp. L, sp. M, sp. N and *Aurospio foodbanesia*: 12.8–22.9%; lineages *Aurospio* cf. *dibranchiata* and sp. S: 13.2–16.9%) and the intraspecific uncorrected distances were always lower within these lineages (16S: 0–5.8%). The levels of interspecific differentiation in 16S rRNA are comparable to those observed among other polychaete species, which usually exceeded 5% for the mitochondrial marker 16S (Álvarez-Campos et al. 2017; Brasier

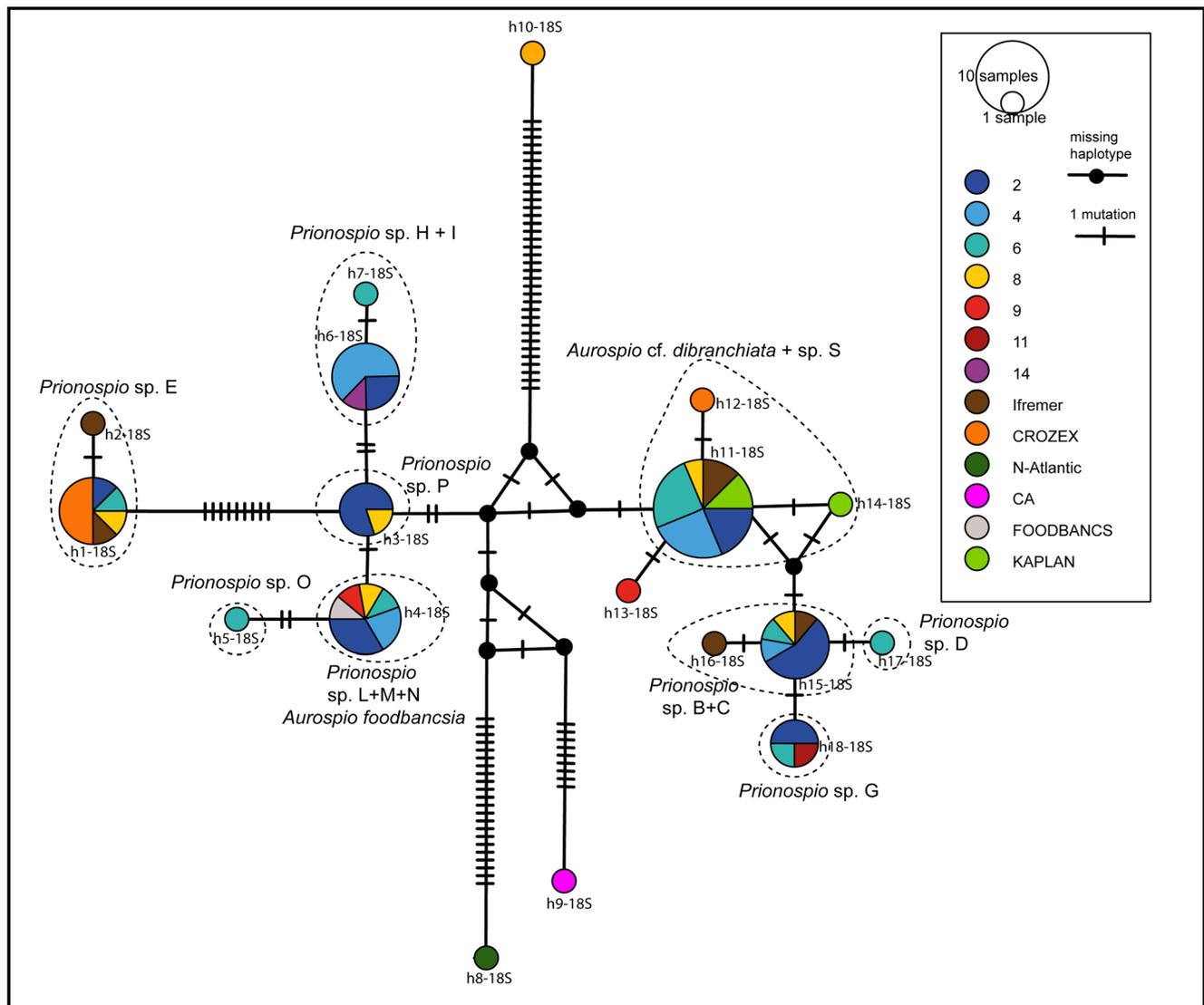


Fig. 4 Genotype networks of *Prionospio* and *Aurospio* species from the different localities of 18S gene fragments. Sampling localities are color coded. Different lineages are circled

et al. 2016; Meißner et al. 2016; Wiklund et al. 2009). We imply thresholds about 5–6% for 16S rRNA to distinguish between *Prionospio* and *Aurospio* species. In a recent study, Guggolz et al. (2019) found barcoding gaps of ~2–2.8% in 16S rRNA between *Laonice* species, another spionid genus, from the tropical Atlantic. The fact that we found higher intraspecific distances, in particular within *Prionospio* sp. H and *Aurospio* cf. *dibranchiata*, might be explained by the larger geographic sampling in our study. It appears likely that the overall intraspecific diversity would be higher if additional regions and populations were studied. Based on the higher pairwise distances for 16S rRNA between lineages C and D (5.1–6.2%) compared to the distances within lineage *Prionospio* sp. H (4.8%), we follow the species delimitation of ABGD instead of GMYC. Based on the available data, we postulate that each one of the 21 lineages, identified with ABGD, represents a separate species within the investigated dataset with reservations for lineages *Prionospio* sp. C, sp. D and sp. H. One has to keep in mind that a higher number of species might be present within the dataset (e.g., *Prionospio* sp. E and sp. A might be further split) using a less conservative threshold for delimitating species, but this additional splitting and the differences found between ABGD and GMYC analyses would not change the main finding, concerning the dispersal potential of *Prionospio* and *Aurospio* (see below).

Unfortunately, our previous morphology-based species identification of the *Prionospio* and *Aurospio* specimens from the tropical Atlantic (Guggolz et al. 2018) showed high inconsistencies with the results presented here regarding the number of species. The assignment to genera showed consistency with the molecular results, but the number of species had been underestimated with morphological identification (Guggolz et al. 2018: eight *Prionospio* species, one *Aurospio* species). As all of the studied specimens were damaged and important species discriminating characters were often missing, morphological identification was limited, which could explain this inconsistency. The problem of morphological identification is known for deep-sea polychaetes, due to their soft bodies resulting in easy fragmentation and the way of sampling in-fauna from deep-sea sediments, where extreme care must be taken regarding sieving techniques to preserve morphology (Bogantes et al. 2018; Glover et al. 2016; Guggolz et al. 2019). Furthermore, *Prionospio* and *Aurospio* are classified within the *Prionospio* complex, in which the generic characters are limited and still under debate (Paterson et al. 2016; Sigvaldadottir 1998; Wilson 1990; Yokoyama 2007). Especially, the taxonomic boundaries of *Prionospio* and *Aurospio* are problematic, as the main distinguishing feature is the beginning and shape of the branchiae, which has been suggested to be insufficient to discriminate the genera (Paterson et al. 2016; Sigvaldadottir 1998; Wilson 1990). The 16S rRNA phylogeny of the present work highlights this difficulty as monophyly for both genera, *Prionospio* and

Aurospio, was not supported. Of the 21 species identified within the study, *Aurospio foodbanesia*, *Aurospio*, sp. Q and sp. R were studied solely on the basis of sequence data obtained from GenBank. For *Aurospio* sp. Q and sp. R, only single sequences each were available from GenBank without further taxonomic information and these could have been misidentified, a well-known problem (Collins and Cruickshank 2012; Kvist et al. 2010). In contrast, the available sequences for *Aurospio foodbanesia* are based on the original description of the type specimen (Mincks et al. 2009). However, even if a misidentification of *Aurospio* sp. R and sp. Q was assumed, the relationship of *Aurospio foodbanesia* does not support monophyly of *Aurospio*. *Aurospio* cf. *dibranchiata*, A. sp. S and sp. T cluster within *Prionospio* in the 16S rRNA analyses and shared a 18S genotype with *Prionospio* species also the monophyly of *Prionospio* is not supported. Although we could not find support for the monophyly of the two genera, conclusions about the paraphyly of both genera should be treated with caution, as the available 16S rRNA and 18S data might not sufficiently reflect relationships. To resolve these problems, there is a need for broad taxon sampling and a taxonomic revision including morphological and genetic data including more genes.

Irrespective of the taxonomic status of *Prionospio* and *Aurospio*, the dataset presented here provides profound insights into the diversity of these genera in the deep sea. In the samples for the tropical Atlantic studied herein, spionids were a dominant faunal component with more than 73% of all sampled polychaetes (Guggolz et al. 2018). The potential 19 deep-sea species (excluding the two GenBank entries *Aurospio* sp. Q and R) make up around 17% of the worldwide described *Prionospio* and *Aurospio* species (around 108 species listed in WoRMS <http://www.marinespecies.org/>, Read and Fauchald 2018), keeping in mind that we cannot say for sure whether they represent species new to science or already described species. Our findings support previous reports that both genera are abundant in the deep sea, and presumably the number of species is still underestimated (Paterson et al. 2016).

One of the main aims of this study was to assess the distribution and dispersal potential of the studied species. Some species were found to be restricted to one of the investigated oceans (*Prionospio* sp. C, D, F, G, I, K, L, M, O, P and *Aurospio* sp. T; Figs. 2 and 3), but there are also several species found to have a pan-oceanic distribution (*Prionospio* sp. A, B, E, H, N and *Aurospio* cf. *dibranchiata*, sp. S; Figs. 2 and 3). Most of these species found in the Atlantic, as well as in the Pacific, show comparable patterns regarding the haplotype networks and thus of the distribution of their intraspecific genetic diversity. Identical haplotypes are shared between populations inhabiting both oceans (i.e., h9-16S, h35-16S, h95-16S, h119-16S; Fig. 3), and other haplotypes differ only on one or a few mutations in 16S rRNA between oceans. There are three possible scenarios explaining this remarkable

finding: (1) shared haplotypes were already present in the ancestral population > 3 million years ago (O’Dea et al. 2016) before Atlantic and Pacific populations were separated by the Isthmus of Panama (vicariance), (2) historic gene flow between Atlantic and Pacific populations, and (3) recent and/or continuous gene flow between Atlantic and Pacific populations. In scenario (1), the observed distribution of haplotypes would be due to variance rather than dispersal and gene flow. Identical haplotypes would be ancestral and retained unchanged in both populations over 3 million years. This would imply very low substitution and speciation rates for these species as we have no reason to believe that the Atlantic and Pacific populations in question represent different species. In turn, this would imply very old ages for the studied genera and all other studied species pairs, as these species were separated by 5.1–32.0% uncorrected *p*-distance in 16S rRNA, which would require hundreds of millions of years to accumulate at this rate. We deem this rather unlikely and therefore favor scenarios (2) and (3).

Scenarios (2) and (3) both assume gene flow between Atlantic and Pacific populations of several species much later than the closure of the Isthmus of Panama. With the available data, it is not possible to assess the timing and frequency of gene flow events within each species. However, dispersal and gene flow rates are apparently too low to lead to population admixture between oceans. In several of the studied species, dispersal might have occurred only once, resulting in the colonization of the respectively other ocean, though repeated and on-going dispersal and gene flow is possible as well.

It is widely accepted that one of the main driving factors for such wide dispersal capacities of marine invertebrates are long-lived planktonic larval stages (Eckman 1996; Rex et al. 2005; Scheltema 1972; Schüller and Ebbe 2007; Yearsley and Sigwart 2011). Even if the specific type of larvae of the herein studied species is unknown, other species of *Prionospio* and *Aurospio* are reported to develop via planktonic larvae like other species of these genera (Blake et al. 2018; Mincks et al. 2009; Young 2003). The planktonic larval duration and dispersal distances are potentially higher in the deep sea, as the cold temperature and consequently reduced metabolic rates are identified as one of the main driving factors for extended larval stages (McClain and Hardy 2010; O’Connor et al. 2007). Considering the enormous distances between the CCZ and the VFZ, it seems highly unlikely that larvae drift all the way between the populations. It rather appears plausible that the distribution over such large geographical distances is linked to ocean currents, connecting different suitable habitat patches in a stepwise fashion (McClain and Hardy 2010; Rex and Etter 2010; Young et al. 2008). The direction of dispersal of *Aurospio* and *Prionospio* larvae remains unknown, as well as whether the species originated from the Pacific or the Atlantic. Probably, additional populations occur in the un-sampled regions between our study sites, which

might have acted as stepping stones. Alternatively, some of the studied species might have a continuous distribution linking the Atlantic and Pacific populations. Further comparison to populations from other localities would help to clarify these issues. The potential to find conspecific specimens in other deep-sea areas and another indication for the enormous distribution potential of some spionids is supported by specimens from the Crozet Islands (Antarctica) that were assigned to *Prionospio* sp. E. The Southern Ocean could also connect the Atlantic and the Pacific most likely with the eastwards flowing deep-water currents (Rahmstorf 2002; Stow et al. 2002).

A pan-oceanic distribution found for *Aurospio* and *Prionospio* species as evidenced from molecular data is so far unique for abyssal annelids, even if wide distribution ranges of polychaete species are not unusual (Böggemann 2016; Eilertsen et al. 2018; Georgieva et al. 2015; Guggolz et al. 2019; Meißner et al. 2016; Schüller and Hutchings 2012). However, Hutchings and Kupriyanova (2018) are particularly highlighting that reported “cosmopolitan” species should be treated with caution. For some species, wide distribution ranges have been based on misidentification or cryptic species and subsequently rejected using molecular marker (Álvarez-Campos et al. 2017; Nygren 2014; Nygren et al. 2018; Sun et al. 2016). The assumption that planktonic larvae in the deep sea are staying longer in the water column and transported via currents successfully over long distances raises the question of what the potential dispersal barrier restricting the distribution are. Life-history traits like larval behavior, larval mortality, and physiological tolerances for vertical movement and settlement in different habitats are probably important (Glover et al. 2001; McClain and Hardy 2010; Virgilio et al. 2009). It has been suggested that connectivity in the deep sea has often been associated with a common bathymetry rather than spatial vicinity (Glover et al. 2001). Thus, topographic barriers, like ridges, canyons, and rises, are supposed to influence distribution patterns (Eckman 1996; Guggolz et al. 2018; McClain and Hardy 2010; Won et al. 2003). Such a potential barrier is the MAR, dividing the Atlantic in eastern and western basins (Riehl et al. 2018). Some of the herein studied species (*Prionospio* sp. G, sp. L, sp. M and *Aurospio* cf. *dibranchiata*) occurring in the tropical Atlantic were found to be distributed across the MAR with wide distribution ranges of up to > 4000 km (Figs. 1 and 3) with no or only little genetic differentiation between populations. Several haplotypes were found to be identical (h29-16S, h59-16S, h61-16S, h69-16S, h119-16S, h121-16S; Fig. 3), indicating gene flow across the MAR. Even for species restricted to one side of the MAR (eVFZ: *Prionospio* sp. H, sp. P and *Aurospio* sp. S) or at least to the VTF (*Prionospio* sp. B, sp. E, sp. N, sp. O), shared haplotypes were found across hundreds of kilometers (47-16S, h9-16S, h22-16S, h87-16S; Figs. 1 and 3). These results are strongly refuting a

barrier effect of the MAR for these *Prionospio* and *Aurospio* species. The dispersal potential across topographic barriers like the MAR and over large geographic distances was also reported recently for species of the spionid genus *Laonice* (Guggolz et al. 2019). The importance of dispersal via larvae is emphasized by distribution patterns found for brooding taxa like isopods, which exhibited very limited or no gene flow across the MAR (Bober et al. 2018; Brix et al. 2018).

In summary, the results of this study are expanding our knowledge of the diversity and distribution patterns of *Aurospio* and *Prionospio* in the deep sea. We could identify 21 lineages with some assigned to already described species (*Aurospio foodbancsia*, *Aurospio* cf. *dibranchiata*) and others remaining unknown at present. These other molecularly delineated potential species cannot be differentiated from described species, due to lack of sufficient morphological characters as most individuals were damaged and incomplete and because genetic data for most of the described species is lacking. The data do not support the monophyly for the two genera, which is highlighting the importance of molecular analyses additionally to morphological examinations to revise the *Prionospio* complex. A remarkable degree of pan-oceanic distribution in some of the species was shown, especially in the tropical Atlantic. The pan-oceanic distribution is indicating the potential of widespread distribution for *Prionospio* and *Aurospio* species, even over topographic barriers and should be kept in mind for further studies on distribution patterns in deep-sea polychaetes.

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Data availability The datasets generated during and/or analyzed during the current study are available in the GenBank repository (see corresponding GenBank numbers in Supplementary file 2).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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