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The effects of depth, distance, and the Mid-Atlantic Ridge on genetic differentiation of abyssal and hadal isopods (Macrostylidae)

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ABSTRACT

The largest habitat on Earth, the abyssal oceans below 3500 m depth, is commonly assumed to represent a continuous environment due to homogeneity of environmental factors and the lack of physical barriers. Yet, the presence of bathymetric features, such as Mid-Ocean Ridges, and hadal trenches provide a discontinuation. During the Vema-TRANSIT expedition in 2014/2015 to the tropical North Atlantic, a transatlantic transect was studied following the full extent of the Vema Fracture Zone in an east-west direction and including the Puerto Rico Trench (PRT).

The aim of this study was to test whether large bathymetric features represent barriers to dispersal and may lead to differentiation and eventually speciation. In this study, these potential barriers included the Mid-Atlantic Ridge (MAR) and the transition (~ 3000 m) from the hadal PRT to the adjacent abyss. Genetic differentiation and differences in community structure (species composition) from east and west of the MAR, as well as abyssal and hadal depth zones were tested for using the poor dispersers Macrostylidae (Crustacea, Isopoda) as a model

Distribution patterns showed that certain macrostylid species have ranges extending more than 2000 km, in some cases across oceanic ridges and trench-abyss transitions. Contrastingly, there was a clear signal for geographic population structure coinciding with the east-west division of the Atlantic by the MAR as well as with the abyss-hadal zonation. These results support the hypotheses that depth gradients as well as oceanic ridges reduce dispersal even though barriers may not be absolute. Additionally, positive correlation between genetic- and geographic distances showed that the vast size of the deep sea itself is a factor responsible for creating diversity.

1. Introduction

1.1. The abyss and the hadal zone

Abyssal plains form the largest habitat on Earth and are seemingly continuous (Ramirez-Llodra et al., 2010), covering depths between 3500 m and 6500 m. Bathyal features, such as mid-ocean ridges (MOR), subdivide the abyss into basins (Watling et al., 2013). Fracture zones provide gaps amongst these ridges which form channels connecting basins across the ridges.

The hadal zone, predominantly comprised of oceanic trenches, covers only 0.24% of the Earth's surface and lies beneath the abyss. Extending from 6501 m to almost 11,000 m depth, the hadal accounts for 41% of the total depth range (Jamieson, 2015). Trenches form where oceanic crust is subducted under continental crust. As opposed to the (semi-) continuous abyss, most trenches have island character, being separated from one another by abyssal areas of often thousands of kilometres (Jamieson, 2015; Watling et al., 2013). Trench topography is asymmetric and the typical V-shape is dominated by a steep landward slope and a rather gradual seaward slope. Along these slopes, pockets and terraces are subjected to sediment accumulation which also characterizes the deep trench basins. Besides this general V-shape, a complex topography of the slopes (especially landward) results in high variability in sediment deposition and considerable proportions of rocky seafloor. Together, rocky outcrops and sediment pockets provide a greater variety of substrata than typically available in the abyss (Jamieson, 2015).

1.2. Abyssal and hadal fauna

The abyssal fauna is comprised of specialized groups of organisms, many of which are endemic to this depth zone (e.g., Brandt et al.,

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2007c; Hessler et al., 1979; Riehl et al., 2014b; Wilson, 1999). Abyssal communities have are considered highly diverse when compared to shallow-water environments (Hessler and Sanders, 1967; McClain and Schlacher, 2015; Sanders, 1968). In case of some abyssal species (at least in some mollusks), wider distributions have been observed when compared to congeners from the bathyal and shallow waters (Eilertsen and Malaquias, 2015; Etter et al., 2011; Zardus et al., 2006).

While abyssal basins are supposedly interconnected, thus supporting wide distributions of the benthos, the hadal "islands" are isolated ecosystems with largely endemic organisms that have close phylogenetic ties to abyssal relatives rather than to those in other trenches (Beliaev, 1989; Jamieson, 2015; Wolff, 1959).

1.3. Mechanisms explaining the evolution of abyssal and hadal diversity

Until now mechanisms that explain the evolution of the diverse abyssal and hadal faunas remain elusive (Ramirez-Llodra et al., 2010). Little is understood about how deep-sea taxa evolved because geographic patterns of genetic variation are poorly understood (Taylor and Roterman, 2017); and these form the essential information for inferring patterns of population differentiation and speciation (Zardus et al., 2006). Based on the assumption of a continuous and homogeneous abyss, Sanders (1968) established the stability-time hypothesis proclaiming that diversity and biogeographic patterns are determined by biological interactions. One alternative approach is the temporal-mosaic hypothesis, stating that diversity in the deep sea is determined by disturbance, that creates a mosaic of communities at different stages of succession (Rex and Etter, 2010). In general, these theories are based on contemporaneous ecological conditions, but they do not approach the question of the origination of the deep-sea diversity from the evolutionary perspective. Another explanation for the abyssal diversity is the Source-Sink hypothesis (Rex et al., 2005), or Slope–Abyss Source–Sink (SASS) hypothesis (Hardy et al., 2015). It could be seen as a somewhat extreme approach, as it rejects the potential for abyssal diversification. According to this hypothesis, abyssal plains are a mere sink of diversity originating and proliferating at the continental slopes. It assumes that larvae drift from the bathyal into the abyssal plains and settle there. As a consequence of poor energy availability, abyssal population densities are below a critical value to allow self-sustaining reproduction in the abyss. Yet, while this theory was supported by mollusc data, it does not provide a satisfying account for cases of abyssal and hadal endemism, and it also falls short in explaining high abyssal diversities of organisms without larval dispersal stage, such as asellote isopod crustaceans. For these, the wide expanse of the abyssal environment has been discussed as a potential promotor of differentiation (e.g., Wilson and Hessler, 1987), suggesting that geographic distance itself reduces gene flow across the total extend of a species' range (Isolation by Distance (Wright, 1943)). Comparing population-genetic data across invertebrate taxa a general pattern of non-neutrality emerged (Taylor and Roterman, 2017), suggesting that demographic instability or selective sweeps are common phenomena in the deep sea. Population genetic studies, however, that may provide support for one or the other hypothesis of diversification in the deep sea, are sparse and largely based on single mitochondrial markers (Taylor and Roterman, 2017).

1.4. Dispersal barriers

Although absolute barriers may not exist in the abyss (Wilson and Hessler, 1987), the subdivision of the abyssal landscape by geological features into basins seems to impact the distributions of abyssal fauna (Thistle, 2003). While apparently topographic features seem to have hardly any effect on the distribution of particularly well-dispersing abyssal organisms, such as harpacticoids and nematodes of the meiofauna (Bik et al., 2010; Lins et al., in this issue; Menzel et al., 2011), motile swimmers, such as scavenging amphipods (e.g., Havermans et al., 2013), and molluscs with larval dispersal (Zardus et al., 2006), they appear to shape biogeographic patterns found in others (see Brix and Svavarsson, 2010). Topographic features thus seem to represent dispersal barriers (Wilson and Hessler, 1987), and as such, MOR may restrict gene flow. Consequently, MOR could play a significant role in the evolution of abyssal organisms (Wilson and Hessler, 1987) through allopatric speciation.

Additionally, depth-related factors may also enhance genetic differentiation in benthic organisms (e.g., Eustace et al., 2016; Havermans et al., 2013; Held, 2003; Jennings et al., 2013; Rex and Etter, 2010). Because sediment plains at the bottom of trenches can be significantly deeper than the surrounding abyss, this depth difference may represent means of reciprocal isolation of hadal and abyssal populations and thus contribute to speciation processes.

Finally, the shear extend of the abyssal environment may contribute to genetic differentiation. A decrease in gene flow with increasing distance between potential mating partners can result in isolation by distance (Wright, 1943). Given the immense dimensions of the abyssal zone, this model of differentiation may significantly contribute to speciation there. While some asellote isopods, such as the Munnopsidae, have secondarily evolved natatory adaptations (Wilson, 1989) and are able to traverse remarkable distances (Bober et al., in this issue; Raupach et al., 2007), a strictly benthic lifestyle of many Asellota suggests a limited dispersal capability. Hence, it can be assumed that adjacent pairs of populations will be more genetically similar to each other than geographically distant populations because individuals are less likely to disperse longer distances (Wright, 1943). The consequentially limited gene flow can be assumed especially for endobenthic groups with low dispersal potential, such as the isopod crustacean family Macrostylidae Hansen (1916).

1.5. Study background and research questions

During the Vema-TRANSIT (bathymetry of the Vema-Fracture Zone and Puerto Rico TRench and Abyssal AtlaNtic BiodiverSITy study) campaign on the RV *Sonne* during northern Winter 2014–2015 benthos samples were collected at abyssal depth along an east-west transect in the tropical North Atlantic, as well as on the landward slope and the bottom trough of the Puerto Rico Trench (Brandt et al., in this issue; Devey, 2015).

The study transect followed the Vema Fracture Zone (VFZ) and crossed the Mid-Atlantic Ridge (MAR), a MOR that divides the Atlantic Ocean from North to South along its whole extend (Fig. 1). Using Macrostylidae as model organism, the following hypotheses were tested: (1) The MAR represents a barrier to the dispersal of abyssal benthic organisms leading to distinct (genetic and species) assemblages east and west; (2) the depth difference between the hadal of the Puerto Rico Trench and the adjacent abyss restricts dispersal; (3) genetic differentiation and intra-family diversity increases with geographic distance. Moreover, we asked how far macrostylid species ranges extend.

2. Material and methods

2.1. Environmental setting

The Vema-TRANSIT campaign with the German RV Sonne (expedition SO237) was conducted at the Vema Fracture Zone (VFZ) in the tropical North Atlantic, as well as the bottom and landward slope of the Puerto Rico Trench (PRT), at the boundary between Caribbean Sea and Atlantic Ocean (Fig. 1) (Brandt et al., in this issue; Devey, 2015).

The VFZ is one of the major offsets of the MAR located near 11°N and it is characterized by large transverse ridges paralleling the fracture zone. It is the northernmost of a group of large transform faults which offset the MAR left-laterally in the equatorial region between Africa and South America (Fig. 1). The trough formed by the VFZ is flat and sediment-filled, and reaches a depth of more than 5100 m. It represents a relatively level continuation of the abyssal zone between the East

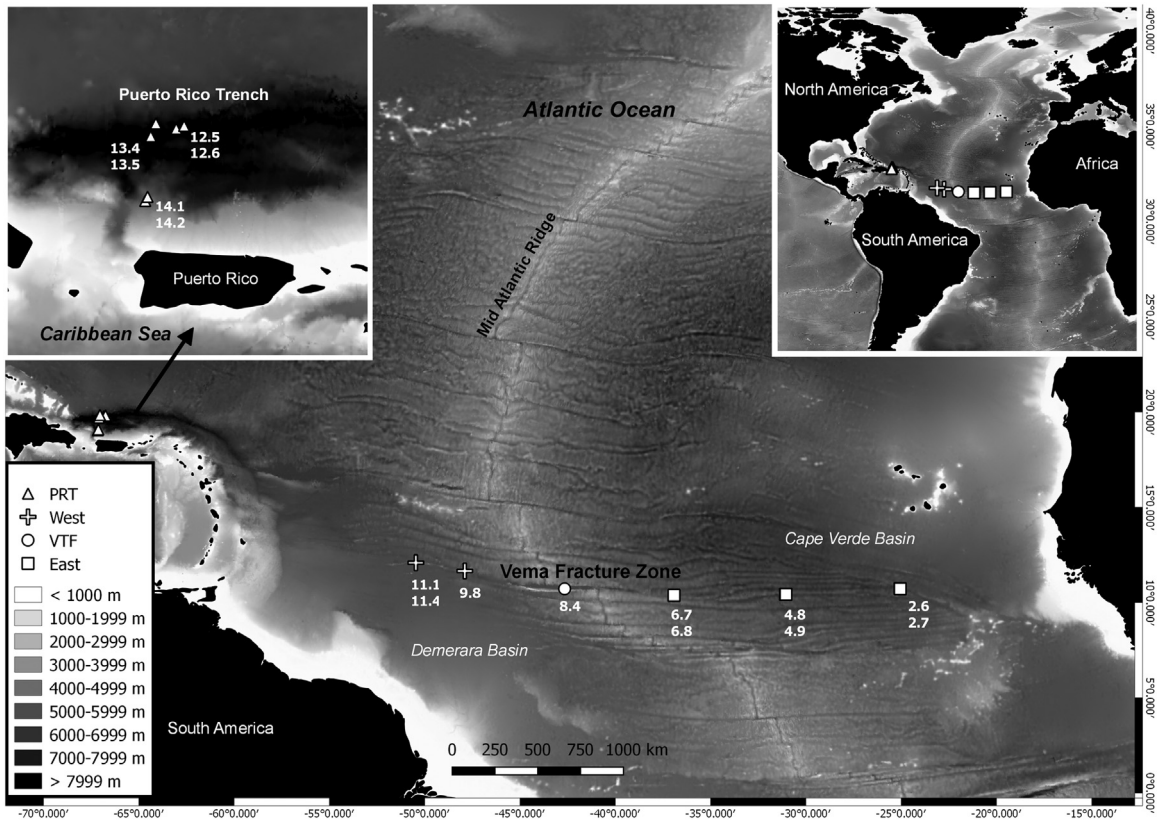

Fig. 1. Bathymetric map of the central Atlantic Ocean and the eastern Caribbean Sea showing the sampling locations (sites) of the Vema-TRANSIT campaign (SO237) in the northern winter 2014–2015. Sampling was conducted along the Vema Fracture Zone (VFZ) as well as in the hadal and bathyal of the Puerto Rico Trench (PRT). Station numbers are provided below the site symbols. Coordinates are indicated as degrees below and right of the frame of the main map. Sampling station coordinates are provided in Table 1. Map created with QGIS-Version 2.16.1-Nødebo.

Atlantic Cape Verde Basin (Gambia Abyssal Plain) and the West Atlantic Demerara Basin. The adjacent scarps of the MAR near 41°W rise approximately 3000 m above the level of the sea floor (Heezen et al., 1964; Louden et al., 1986). Moreover, Antarctic Bottom Water is flowing through the VFZ in eastward direction, reaching velocities of more than 30 cm/s (Morozov et al., 2015).

The Puerto Rico Trench (PRT) is located to the north-west of the VFZ and north of Puerto Rico and the Virgin Islands. It formed where the North American plate is subducted under the Caribbean plate which started in the Oligocene. The PRT reaches depths of more than 8300 m (Ten Brink et al., 2004) and it is the deepest part of the Atlantic Ocean. Early studies on the faunal composition and sediment analyses showed that the southern basins within the trench represent sinks for coastal and/or terrigenous organic debris and harbor abundances and diversities comparable to those at abyssal sites (George and Higgins, 1979; Tietjen et al., 1989). Contrastingly, the hadal sediments near the abyssal flanks, where the Vema-TRANSIT samples were taken, are relatively oligotrophic and support only a depauperate fauna when compared to abyssal or other hadal environments elsewhere (Richardson et al., 1995).

2.2. Collection method

Macrofauna was collected with a camera-equipped epibenthic sledge (Brandt et al., 2013) at six sites with altogether ten deployments (= stations) along the VFZ and two hadal as well as one abyssal sites with two stations respectively at the PRT (Table 1). Each gear deployment was considered a station; in order to test for differences in species composition and *p*-distance distribution by region, the sampling stations were grouped into geographic regions: all stations in the eastern VFZ are referred to as “east”, all abyssal stations in the western VFZ as “west”, the station within the MAR transform fault is further referred to as “VTF”, and samples from the hadal and abyss of the PRT were grouped as “PRT”. Because the exact coordinate of capture for each specimen cannot be inferred from the collection method, the start-trawl positions for each deployment were used. Geographic distances were calculated using the following formula:

Table 1
Coordinates and depths of the sampling stations of Vema-TRANSIT at which isopods of the family Macrostylidae Hansen (1916) were found. The coordinates provided are in the format of decimal degrees and represent the start-trawl positions recorded in the ship's log. The allocation to areas is also provided.

Station (SO237)	Latitude [dec]	Longitude [dec]	Area	Depth [m]
2–6	10.7296667	− 25.062	East	5520
2–7	10.71485	− 25.0535	East	5507
4–8	10.427	− 31.0733333	East	5725
4–9	10.4275	− 31.0496667	East	5733
6–7	10.3636667	− 36.9176667	East	5079
6–8	10.3775	− 36.9225	East	5127
8–4	10.7166667	− 42.6621667	VTF	5178
9–8	11.656	− 47.8998333	West	5001
11–1	12.0973333	− 50.4661667	West	5088
11–4	12.0805	− 50.469	West	5108
12–5	19.7808333	− 66.8336667	PRT	8338
12–6	19.8100167	− 66.7521667	PRT	8336
13–4	19.702	− 67.0965	PRT	8317
13–5	19.8353	− 67.0436167	PRT	8042
14–1	19.03495	− 67.1541167	PRT	4552
14–2	19.0778333	− 67.1295	PRT	4925

Table 2
Specimen information for Macrostylidae, collected during the Vema-TRANSIT expedition with RV Sonne (SO237) that were used for genetic analyses. (18S)

FieldID	Station	MOTU	Sex	Age	Repr. stage	GenBank Acc # 16S	GenBank Acc # 18S
VTMac017	SO237-2-7	ML08	-	manca	-	LT909174	N/A
VTMac018	SO237-2-7	ML02	-	manca	-	LT909175	N/A
VTMac019	SO237-2-7	ML02	-	manca	-	LT909176	LT960401
VTMac020	SO237-2-7	ML08	F	adult	ovigerous	LT909177	LT960405
VTMac022	SO237-2-7	ML02	-	manca	-	LT909178	N/A
VTMac023	SO237-2-7	ML08	M	Subadult		LT909179	N/A
VTMac024	SO237-2-7	ML08	F	adult		LT909180	LT960406
VTMac025	SO237-2-7	ML08	F	adult		LT909181	LT960407
VTMac026	SO237-2-7	ML08	F	adult		LT909182	N/A
VTMac027	SO237-2-7	ML08		adult		LT909183	N/A
VTMac028	SO237-2-7	ML08	F	adult	ovigerous	LT909184	N/A
VTMac029	SO237-2-7	ML03	F	subadult?		LT909185	N/A
VTMac030	SO237-2-7	ML08	F	adult	ovigerous	LT909186	N/A
VTMac031	SO237-2-7	ML08	F	adult		LT909187	N/A
VTMac032	SO237-2-7	ML08	F	adult		LT909188	LT960408
VTMac033	SO237-2-7	ML08	F	adult	ovigerous	LT909189	LT960409
VTMac034	SO237-4-8	ML08	M	Subadult		LT909190	N/A
VTMac035	SO237-2-7	ML02	-	manca	-	LT909191	N/A
VTMac036	SO237-2-7	ML08	-	manca	-	LT909192	N/A
VTMac038	SO237-2-7	ML08	M	Subadult		LT909193	N/A
VTMac039	SO237-2-7	ML08	-	manca	-	LT909194	N/A
VTMac040	SO237-2-7	ML03	F	adult	ovigerous	LT909195	LT960404
VTMac041	SO237-2-7	ML08	-	manca	-	LT909196	N/A
VTMac042	SO237-2-7	ML08	F	adult		LT909197	N/A
VTMac043	SO237-2-7	ML02	-	manca	-	LT909198	LT960402
VTMac044	SO237-2-7	ML08	F	adult		LT909199	N/A
VTMac045	SO237-2-7	ML08	F	adult		LT909200	N/A
VTMac047	SO237-2-6	ML08	F	adult		LT909201	N/A
VTMac048	SO237-2-6	ML02	F	adult		LT909202	LT960403
VTMac049	SO237-4-9	ML08	M	adult		LT909203	LT960410
VTMac050	SO237-4-8	ML08	F	subadult		LT909204	LT960411
VTMac051	SO237-4-8	ML08	F	adult		LT909205	LT960412
VTMac052	SO237-4-8	ML08	M	manca	-	LT909206	N/A
VTMac053	SO237-4-8	ML08	M	adult		LT909207	LT960413
VTMac054	SO237-4-8	ML08	F	adult		LT909208	LT960414
VTMac055	SO237-4-8	ML08	M	juvenile		LT909209	N/A
VTMac056	SO237-4-8	ML08	M	Subadult		LT909210	N/A
VTMac057	SO237-4-8	ML08	-	manca	-	LT909211	N/A
VTMac058	SO237-4-8	ML08	F	adult		LT909212	N/A
VTMac059	SO237-4-8	ML08	F	adult		LT909213	N/A
VTMac060	SO237-4-8	ML08	-	manca	-	LT909214	N/A
VTMac061	SO237-4-8	ML08	M	Subadult		LT909215	N/A
VTMac062	SO237-4-8	ML08	F	adult	ovigerous	LT909216	N/A
VTMac063	SO237-4-8	ML08	F	adult		LT909217	N/A
VTMac064	SO237-4-8	ML08	M	juvenile		LT909218	N/A
VTMac065	SO237-4-8	ML08	F	adult		LT909219	N/A
VTMac066	SO237-4-8	ML08	M	Subadult-adult		LT909220	N/A
VTMac067	SO237-4-8	ML08	-	manca?		LT909221	N/A
VTMac068	SO237-4-8	ML08	M	Subadult		LT909222	N/A
VTMac069	SO237-4-8	ML08	F	adult		LT909223	LT960415
VTMac070	SO237-4-8	ML08	F	adult		LT909224	N/A
VTMac071	SO237-4-8	ML08	F	manca	-	LT909225	N/A
VTMac072	SO237-4-8	ML08	M	adult		LT909226	N/A
VTMac073	SO237-4-8	ML08	-	manca	-	LT909227	N/A
VTMac074	SO237-4-9	ML08	F	adult		LT909228	N/A
VTMac075	SO237-4-9	ML08	M	adult		LT909229	LT960416
VTMac076	SO237-4-9	ML08	M	adult		LT909230	LT960417
VTMac077	SO237-4-9	ML08	M	adult		LT909231	LT960418
VTMac078	SO237-4-9	ML08	M	adult		LT909232	LT960419
VTMac079	SO237-4-9	ML08	M	Adult		LT909233	N/A
VTMac080	SO237-4-9	ML08	F	adult		LT909234	LT960420
VTMac081	SO237-4-9	ML08	F	adult		LT909235	LT960421
VTMac082	SO237-4-9	ML08	F	adult		LT909236	LT960422
VTMac083	SO237-4-9	ML08	F	adult		LT909237	LT960423
VTMac084	SO237-4-9	ML08	F	adult		LT909238	N/A
VTMac085	SO237-4-9	ML08	F	adult		LT909239	N/A
VTMac086	SO237-4-9	ML08	F	adult	ovigerous	LT909240	LT960424
VTMac087	SO237-4-9	ML08	F	adult		LT909241	LT960425
VTMac088	SO237-4-9	ML08	F	adult		LT909242	LT960426
VTMac089	SO237-4-9	ML08	M	juvenile		LT909243	N/A
VTMac090	SO237-4-9	ML08	F	adult		LT909244	N/A
VTMac091	SO237-4-9	ML08	F	adult		LT909245	N/A
VTMac092	SO237-4-9	ML08	M	adult		LT909246	LT960427
VTMac093	SO237-4-9	ML08	M	adult		LT909247	LT960428

(*continued on next page*)

Table 2 (*continued*)

FieldID	Station	MOTU	Sex	Age	Repr. stage	GenBank Acc # 16S	GenBank Acc # 18S
VTMac094	SO237-4-9	ML08	M	adult		LT909248	LT960429
VTMac095	SO237-4-9	ML08	M	adult		LT909249	N/A
VTMac096	SO237-4-9	ML08	F	adult		LT909250	N/A
VTMac097	SO237-4-9	ML08	-	manca	-	LT909251	N/A
VTMac098	SO237-4-9	ML08	M	juvenile		LT909252	N/A
VTMac099	SO237-4-9	ML08	F	adult	ovigerous	LT909253	N/A
VTMac100	SO237-4-9	ML08	F	adult	ovigerous	LT909254	N/A
VTMac101	SO237-4-9	ML08	F	adult		LT909255	N/A
VTMac102	SO237-4-9	ML08	M	manca	-	LT909256	N/A
VTMac103	SO237-4-9	ML08	F	adult		LT909257	LT960430
VTMac104	SO237-4-9	ML08	F	adult		LT909258	N/A
VTMac105	SO237-4-9	ML03	F	adult	ovigerous	LT909259	N/A
VTMac106	SO237-4-8	ML08	-	manca	-	LT909260	N/A
VTMac107	SO237-4-8	ML08	M	adult		LT909261	N/A
VTMac108	SO237-4-8	ML08	F	adult		LT909262	N/A
VTMac109	SO237-4-8	ML08	F	adult	ovigerous	LT909263	N/A
VTMac110	SO237-4-8	ML08	F	adult		LT909264	LT960431
VTMac111	SO237-4-8	ML08	M	adult		LT909265	N/A
VTMac112	SO237-4-8	ML08	M	adult		LT909266	N/A
VTMac113	SO237-4-8	ML08	M	adult		LT909267	N/A
VTMac114	SO237-4-8	ML08	M	juv		LT909268	N/A
VTMac115	SO237-4-8	ML08	-	manca	-	LT909269	N/A
VTMac116	SO237-4-9	ML08	M	adult		LT909270	N/A
VTMac117	SO237-4-9	ML08	F	adult	ovigerous	LT909271	N/A
VTMac118	SO237-4-9	ML08	M	manca	-	LT909272	N/A
VTMac119	SO237-4-9	ML08	M	juvenile		LT909273	N/A
VTMac120	SO237-4-8	ML08	M	adult		LT909274	N/A
VTMac121	SO237-4-8	ML08	F	adult		LT909275	N/A
VTMac122	SO237-4-8	ML08	F	adult		LT909276	N/A
VTMac123	SO237-4-8	ML08	F	adult		LT909277	N/A
VTMac125	SO237-4-8	ML08	F	adult		LT909278	N/A
VTMac126	SO237-4-8	ML08	F	adult	ovigerous	LT909279	N/A
VTMac127	SO237-4-8	ML08	F	adult		LT909280	N/A
VTMac128	SO237-4-8	ML08	M	adult		LT909281	N/A
VTMac129	SO237-4-8	ML08	F	adult		LT909282	N/A
VTMac130	SO237-4-8	ML08	M	adult		LT909283	N/A
VTMac131	SO237-4-8	ML08	M	adult		LT909284	N/A
VTMac132	SO237-4-8	ML08	M	adult		LT909285	N/A
VTMac133	SO237-6-7	ML08	M	adult		LT909286	N/A
VTMac134	SO237-6-7	Mlpap	M	adult		LT909287	LT960448
VTMac135	SO237-6-7	ML08	M	adult		LT909288	LT960432
VTMac136	SO237-6-7	ML08	M	adult		LT909289	LT960433
VTMac137	SO237-6-7	Mlpap	M	adult		LT909290	LT960449
VTMac138	SO237-6-7	ML08	F	adult		LT909291	LT960434
VTMac139	SO237-6-7	ML08	F	adult		LT909292	LT960435
VTMac140	SO237-6-7	Mlpap	F	adult		LT909293	LT960450
VTMac141	SO237-6-7	Mlpap	F	adult		LT909294	LT960451
VTMac142	SO237-6-7	ML08	F	adult		LT909295	N/A
VTMac143	SO237-6-7	ML08	F	adult		LT909296	N/A
VTMac144	SO237-6-7	ML08	M	adult		LT909297	LT960436
VTMac145	SO237-6-7	Mlpap	F	adult		LT909298	N/A
VTMac147	SO237-6-7	Mlpap	F	adult		LT909299	LT960452
VTMac148	SO237-6-7	Mlpap	F	adult		LT909300	LT960453
VTMac149	SO237-6-7	Mlpap	M	adult		LT909301	N/A
VTMac150	SO237-6-7	Mlpap	F	adult		LT909302	N/A
VTMac151	SO237-6-7	Mlpap	M	adult		LT909303	N/A
VTMac152	SO237-6-7	ML08	F	adult		LT909304	N/A
VTMac153	SO237-6-7	ML08	F	adult		LT909305	N/A
VTMac154	SO237-6-7	ML08	F	adult		LT909306	N/A
VTMac155	SO237-6-7	ML08	F	adult		LT909307	N/A
VTMac156	SO237-6-7	Mlpap	F	adult		LT909308	LT960454
VTMac157	SO237-6-7	Mlpap	F	adult		LT909309	LT960455
VTMac158	SO237-6-7	ML08	M	adult		LT909310	N/A
VTMac159	SO237-6-8	ML08	F	adult		LT909311	N/A
VTMac160	SO237-6-8	ML08	M	adult		LT909312	N/A
VTMac161	SO237-6-8	ML08	M	Subadult		LT909313	N/A
VTMac162	SO237-6-8	ML08	F	adult		LT909314	N/A
VTMac163	SO237-6-8	ML08	F	adult		LT909315	N/A
VTMac164	SO237-6-8	ML08	F	ovi	ovigerous	LT909316	N/A
VTMac165	SO237-6-8	ML08	F	ovi	ovigerous	LT909317	LT960437
VTMac166	SO237-6-7	Mlpap	F	adult		LT909318	N/A
VTMac167	SO237-6-7	Mlpap	F	adult		LT909319	LT960456
VTMac168	SO237-6-7	ML08	F	ovi	ovigerous	LT909320	LT960438
VTMac169	SO237-8-4	ML12b	F	adult		LT909321	N/A
VTMac170	SO237-8-4	ML12b	F	manca	-	LT909322	LT960440

(*continued on next page*)

Table 2 (*continued*)

FieldID	Station	MOTU	Sex	Age	Repr. stage	GenBank Acc # 16S	GenBank Acc # 18S
VTMac171	SO237-8-4	ML12b	F	manca	-	LT909323	LT960441
VTMac172	SO237-8-4	ML01	M	adult		LT909324	N/A
VTMac173	SO237-8-4	ML01	F	adult		LT909325	LT960399
VTMac174	SO237-8-4	ML01	F	adult		LT909326	N/A
VTMac175	SO237-8-4	ML01	F	ovi	ovigerous	LT909327	LT960400
VTMac176	SO237-8-4	ML01	F	ovi	ovigerous	LT909328	N/A
VTMac178	SO237-8-4	ML01	F	adult		LT909329	N/A
VTMac179	SO237-8-4	ML01	F	ovi	ovigerous	LT909330	N/A
VTMac180	SO237-9-8	ML13	F	adult		LT909331	LT960442
VTMac181	SO237-9-8	ML12	-	manca	-	LT909332	LT960439
VTMac182	SO237-9-8	Mlpap	F	ovi	ovigerous	LT909333	LT960457
VTMac183	SO237-9-8	Mlpap	F	adult		LT909334	LT960458
VTMac184	SO237-9-8	Mlpap	F	adult		LT909335	LT960459
VTMac185	SO237-9-8	ML14	F	adult		LT909336	LT960443
VTMac186	SO237-9-8	Mlpap	-	manca	-	LT909337	LT960460
VTMac187	SO237-11-1	Mlpap	M	adult		LT909338	N/A
VTMac188	SO237-11-1	ML22	-	manca	-	LT909339	N/A
VTMac189	SO237-11-1	Mlpap	F	ovi	ovigerous	LT909340	N/A
VTMac190	SO237-11-1	Mlpap	F	adult		LT909341	N/A
VTMac191	SO237-11-1	ML15	-	manca	-	LT909342	LT960444
VTMac192	SO237-9-8	Mlpap	F	ovi	ovigerous	LT909343	N/A
VTMac194	SO237-11-4	Mlpap	F	ovi	ovigerous	LT909344	LT960461
VTMac195	SO237-9-8	ML12b	F	adult		LT909345	N/A
VTMac196	SO237-11-4	ML13	-	manca	-	LT909346	N/A
VTMac197	SO237-11-4	Mlpap	F	ovi	ovigerous	LT909347	N/A
VTMac198	SO237-11-4	Mlpap	F	ovi	ovigerous	LT909348	LT960462
VTMac199	SO237-11-4	ML08	-	manca	-	LT909349	N/A
VTMac200	SO237-4-8	ML08	M	adult		LT909350	N/A
VTMac201	SO237-12-5	ML12	-	manca	-	LT909351	N/A
VTMac202	SO237-12-5	ML25	-	manca	-	LT909352	N/A
VTMac204	SO237-12-5	ML12	-	manca	-	LT909353	N/A
VTMac205	SO237-12-5	ML25	F	juvenile	-	LT909354	N/A
VTMac206	SO237-12-5	ML25	F	adult	non-ovigerous	LT909355	N/A
VTMac208	SO237-13-4	ML25	M	adult	terminal	LT909356	N/A
VTMac209	SO237-13-4	ML24	F	adult	non-ovigerous	LT909357	N/A
VTMac210	SO237-13-4	ML25	?	juvenile	-	LT909358	N/A
VTMac211	SO237-12-5	ML23	M	adult	terminal	LT909359	LT960447
VTMac212	SO237-13-4	ML25	F	adult	non-ovigerous	LT909360	N/A
VTMac213	SO237-13-4	ML25	M	adult	terminal	LT909361	N/A
VTMac215	SO237-13-4	ML25	M	adult	terminal	LT909362	N/A
VTMac216	SO237-13-4	ML25	F	adult	ovigerous	LT909363	N/A
VTMac217	SO237-13-4	ML25	F	adult	non-ovigerous	LT909364	N/A
VTMac218	SO237-13-4	ML25	F	adult	ovigerous	LT909365	N/A
VTMac220	SO237-13-4	ML25	M	adult	terminal	LT909366	N/A
VTMac221	SO237-13-4	ML25	F	adult	non-ovigerous	LT909367	N/A
VTMac223	SO237-12-5	ML22	F	adult	non-ovigerous	LT909368	LT960446
VTMac224	SO237-13-4	ML25	M	subadult	-	LT909369	N/A
VTMac227	SO237-12-6	ML25	F	adult	non-ovigerous	LT909370	N/A
VTMac227	SO237-12-6	ML25	F	adult	non-ovigerous	LT909371	N/A
VTMac228	SO237-12-6	ML25	F	adult	non-ovigerous	LT909372	N/A
VTMac229	SO237-12-6	ML25	F	adult	non-ovigerous	LT909373	N/A
VTMac232	SO237-12-5	ML12	-	manca	-	LT909374	N/A
VTMac240	SO237-12-5	ML12	-	manca	-	LT909375	N/A
VTMac243	SO237-13-4	ML25	F	adult	ovigerous	LT909376	N/A
VTMac244	SO237-13-4	ML15b	-	manca	-	LT909377	N/A
VTMac247	SO237-13-4	ML25	F	adut	ovigerous	LT909378	N/A
VTMac248	SO237-13-4	ML25	F	adult	ovigerous	LT909379	N/A
VTMac249	SO237-13-4	ML25	F	adult	non-ovigerous	LT909380	N/A
VTMac252	SO237-12-5	ML22	F	adult	non-ovigerous	LT909381	N/A
VTMac254	SO237-12-5	ML25	F	adult	non-ovigerous	LT909382	N/A
VTMac255	SO237-13-5	ML25	F	adult	non-ovigerous	LT909383	N/A
VTMac260	SO237-12-5	ML12	F	adult	non-ovigerous	LT909384	N/A
VTMac262	SO237-12-5	ML25	-	manca	-	LT909385	N/A
VTMac263	SO237-12-5	ML25	M	juvenile		LT909386	N/A
VTMac270	SO237-12-5	ML25	F	adult	non-ovigerous	LT909387	N/A
VTMac275	SO237-12-5	ML25	M	adult	terminal	LT909388	N/A
VTMac276	SO237-12-5	ML25	M	juvenile	-	LT909389	N/A
VTMac277	SO237-12-5	ML25	M	juvenile	-	LT909390	N/A
VTMac287	SO237-12-6	ML25	F	adult	non-ovigerous	LT909391	N/A
VTMac300	SO237-13-4	ML25	F	adult	non-ovigerous	LT909392	N/A
VTMac318	SO237-14-2	ML16	F			LT909393	LT960445
VTMac321	SO237-14-2	ML16	F			LT909394	N/A
VTMac322	SO237-14-2	ML16	F			LT909395	N/A

$$x = \arccos\left(\sin\left(\frac{Lat1\pi}{180}\right)\sin\left(\frac{Lat2\pi}{180}\right) + \cos\left(\frac{Lat1\pi}{180}\right)\cos\left(\frac{Lat2\pi}{180}\right)\cos\left(\left(\frac{Lon2\pi}{180}\right) - \left(\frac{Lon1\pi}{180}\right)\right)\right)6367.45\ \text{km}$$

where Lat1/Lon1 and Lat2/Lon2 stand for the start and end point coordinates (latitude and longitude) and 6367.45 km represents the approximate Earth radius in kilometres. This formula is approximating the distances, assuming absence of bathymetric features and a spherical shape of the planet (Electronic supplement 1). It is thus providing conservative values that may underestimate true distances. The dataset including detailed sampling information was published in the Mendeley repository (Riehl et al., 2017).

2.3. Study organism

Amongst the deep-sea isopods, the cosmopolitan (Riehl and Brandt, 2010) and monogeneric family Macrostylidae Hansen, 1916 with the only genus *Macrostylis* Sars, 1864, has been identified as one of the most abundant genera at several abyssal sites (De Smet et al., 2017; Elsner et al., 2015; Meyer-Löbbecke et al., 2014; Wilson, 2008). The species belonging to this genus have a highly derived (in comparison to related families) but, at the same time, strongly conserved morphology within the group (Riehl et al., 2014b), which has been interpreted as adaptation to an infaunal lifestyle (Hessler and Strömberg, 1989; Thistle and Wilson, 1987; Wägele, 1989).

2.4. Sample processing

The samples were processed according to the methods described by Riehl et al. (2014a) summed up here: once back on deck, the EBS cod ends were separated and their content sieved (300 µm) as well as fixed in a cool room at 4 °C using chilled and filtered sea water and chilled (− 20 °C) 96% ethanol. When containing additional sediment, the EBS nets were emptied on deck and the samples were washed into tubs using filtered sea water. The samples were then elutriated using the same filtered sea water and sieved (300 µm) on deck. For bulk fixation, chilled (− 20 °C) ethanol was used in a sample/ethanol ratio of approximately 1/5. Samples were stored at − 20 °C and gently rolled every 3–4 h for one day to ensure proper penetration of the ethanol into the water-soaked sediment. Subsequently, isopods as well as all other taxa were picked out and systematically sorted using stereo microscopes on board (20–200 × magnification).

Specimens were identified as members of Macrostylidae based on morphological synapomorphies: cephalothorax with dorsolateral articulations of antennulae and antennae, tagmatisation with presence of fossosoma, third pereopod with dorsolateral orientation and extended ischial lobe (Riehl et al., 2014b). Thereafter, macrostylid specimens were individually separated, received individual IDs, and were photographed as vouchers as described by Riehl et al. (2016). Finally, up to three pereopods (walking legs) per specimen were dissected for DNA extraction and transferred into extraction buffer (LGC). At all times, the specimens were kept on ice to prevent DNA degradation. The tissue samples were stored at − 20 °C and shipped on dry ice. Laboratory protocols for extraction, amplification, and sequencing followed Riehl et al. (2014a) to amplify fragments of the mitochondrial 16S rDNA gene as well as the complete nuclear 18S rDNA gene.

Generally two–three posterior pereopods were dissected from one side of the specimen and transferred into LGC PN or PVP lysis buffer and stored frozen until lysis at the LGC labs in Berlin, Germany. At LGC, total DNA extraction was performed using the default protocol for the sbx forensic kit. For PCR a mix of Mytaq Bioline polymerase and 5x Biostab PCR Optimizer Sigma-Aldrich was used. The mitochondrial 16S rDNA was amplified using the primer set 16S-SF and 16S-SR (Riehl et al., 2014a; Tsang et al., 2009). Cycle sequencing consisted of the following circles: 95 °C initial denaturation for 10 min; then 95 °C for 30 s, 48 °C for 30 s, 72 °C for 45 s repeated 36 times; final elongation was performed at 72 °C for 5 min.

Finally, for amplifying the whole nuclear 18S ribosomal DNA (SSU), three primer pairs were used (Dreyer and Wägele, 2001): 18A1 and 700R, 400 F and 1155R, as well as 1000 and 1800. Three overlapping contigs were generated in three different PCRs using the following cycling conditions: after an initial denaturation of 10 min at 95 °C, 36 cycles consisted of 94 °C (30 s), 54 °C (45 s), 72 °C (200 s), followed by a final elongation of 10 min at 72 °C.

All PCR products were cleaned up using Affymetrix™ ExoSAP-IT™ following the manufacturer's protocol in order to eliminate unincorporated primers and dNTPs. Sequencing was performed on an ABI 3730XL sequencer.

2.5. Sequence clean-up and alignment

After sanger sequencing, *.ab1 files of forward and reverse reads were imported into Geneious 9.1 (Kearse et al., 2012) and contigs were assembled using the *De Novo Assemble* function. All assemblies were checked for ambiguities and the BLAST (Johnson et al., 2008; McGinnis and Madden, 2004) algorithm on NCBI GenBank (Benson et al., 2008) was used to check for contamination. Because for only a small subset of the samples 18S could be successfully amplified and sequenced, most analyses were focussed on the 16S dataset, for which sequences could be generated from > 80% of the specimens (Table 2). To test for the potential pitfalls of mitochondrial markers (e.g., mt polymorphism, incomplete lineage sorting, introgression), 18S signal was compared to 16 S data in the population genetic study (see below). Primer regions and low-quality ends of the reads were trimmed. The consensus sequences of the assemblies were aligned using MAFFT 7 (Katoh et al., 2009, 2002; Katoh and Standley, 2013) as implemented in Geneious (Algorithm: E-INS-i; scoring matrix: 200PAM/k = 2; gap opening penalty: 1.53; offset value: 0.123). Several alignments were produced using the same method but different compositions of sequences: for the phylogeny-based species delimitation, additional 16S sequences of macrostylids and outgroup taxa were used in addition to all sequences retrieved in this study (Electronic supplement 2). These were retrieved from GenBank (Benson et al., 2008) and originate from previous studies in Macrostylidae and other closely related (Raupach et al., 2009; Wägele, 1989) Janiroidea of the families Desmosomatidae as well as Munnopsidae (Brix et al., 2015, 2014; Riehl and Brandt, 2013; Riehl and Kaiser, 2012). For the distance-based methods described below, the multiple sequence alignment included only the Vema-TRANSIT macrostylids. For population genetic analyses, alignments of each separate species were constructed.

2.6. Geographic patterns of genetic differentiation

2.6.1. Intra-family diversity pattern

The relationship between geographic distance (distance in km between sampling stations) and genetic differentiation (uncorrected p-distances) was examined based on the alignment composed of all sequenced Vema-TRANSIT macrostylids. A *p*-distance matrix based on this alignment was created using MEGA6 (Tamura et al., 2013). Thereupon, scatter plots, as well as Mantel tests were performed between the *p*-matrix and the geographic distance matrix. Analyses were conducted using the software PRIMER v6 (Anderson et al., 2008).

Based on the multivariate *p*-distance matrix (non-transformed), differences between areas (east, west, PRT, and VTF) were tested for using non-parametric permutational ANOVA (PERMANOVA) with the software PERMANOVA + for Primer (Anderson et al., 2008). The main differences were verified using a 1-fixed factor design (area) with 9999 permutations followed by pairwise pseudo *t*-tests to test for differences

between each pair (see also Electronic supplement 3).

2.6.2. Species distribution patterns

2.6.2.1. Species delimitation. The question of genetic differentiation can only be answered on the level of species and populations. For partitioning the family dataset into species, molecular operational taxonomic units (MOTU's) were defined using the Poisson tree processes (PTP) model. This approach infers putative species boundaries on a given phylogenetic input tree. As a first step, the evolutionary history of Macrostylidae was inferred based on 16S rDNA. For reconstruction of phylogenetic relationships, the more comprehensive alignment was used. A maximum likelihood (ML) approach to reconstruction of phylogeny was applied using the fast likelihood search in PHYML online with an automatic model choice (Guindon et al., 2005). During this analysis, confidence limits were evaluated using the non-parametric bootstrap method (Felsenstein, 1985) and 1000 replicates. The consensus tree was then used as input for the species delimitation, as well as for interpreting the results generated by subsequently mentioned analyses based on *p*-distances and species. The PTP method (Zhang et al., 2013) was applied for delimitation of molecular operational taxonomic units (MOTUs; in the further course of this paper also referred to as species) using the web server and the method based on maximum likelihood (http://www.exelixis-lab.org/software.html). The composition and characteristics of the species were explored using the Species Delimitation tool implemented in Geneious (Masters et al., 2011) as well as MEGA6 (Tamura et al., 2013).

2.6.2.2. Analyses of species distribution. Bray-Curtis resemblance matrices were calculated for the species (non-transformed) and differences between areas and depth zones were tested for using the same design as described above for the *p*-distances. The area VTF had to be excluded from this analysis due to the lack of replicates. PERMDISP routines were performed after the PERMANOVA results to test for homogeneity of multivariate dispersions between areas. Values were considered significant when $p < 0.05$. When the number of permutations was lower than hundred, then the *p* value of Monte Carlo (*p* (MC)) was used. CAP (Canonical Analysis of Principal Coordinates) routines were conducted to characterise the significant differences obtained from the PERMANOVA results for both *p*-distance matrix and species.

Based on the MOTU presence/absence data per sampled area, SIMPER (percentage of (dis)similarity) routines (Clarke and Gorley, 2006) were performed to identify which species contributed the most to the dissimilarities between each pair of areas.

2.6.3. Population-genetic analyses

While the previous analyses focussed on differentiation patterns within the family as a whole looking for effects of geographic distance and areas on Macrostylidae composition in general, patterns of differentiation that may lead to speciation have to be looked for within species. Four species were available in sufficient specimen numbers (N = 25–126), geographic range, and genetic diversity to allow for population-genetic test: MLpap, ML08, ML12, and ML25.

2.6.3.1. Haplotype networks. To take into account that population genealogies are often multifurcated, a networking approach was followed to analyse and illustrate population differentiation within each species. The free, open source population genetics software PopART (Population Analysis with Reticulate Trees) was employed (Leigh and Bryant, 2015). PopART was used to construct intraspecific gene genealogies (Posada and Crandall, 2001) and to draw TCS networks (Clement et al., 2000) for graphical illustration of haplotype distribution per station and region.

2.6.3.2. Population differentiation across stations and barriers. Using PopART, analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed to test hypotheses of between- and within-group differences at several hierarchical levels in a nested design: among groups (= groups of populations of an area), among populations (= conspecifics collected at one site), and within populations. While testing for the effects of potential barriers, all samples originating from each side of the barrier under investigation (MAR or depth) were treated as populations and grouped together. Testing for the effect of geographic distance, specimens were grouped according to sampling sites. Sequences shorter than the overall alignments were not removed because the datasets were already relatively small. Values were considered significant when $p < 0.05$.

To test for demographic stability and gene neutrality, Tajima's D statistics (Tajima, 1989) were calculated in PopART.

2.6.3.3. Isolation by distance. To test for the effect of geographic distance on genetic differentiation, the species with the largest representation in the dataset (126 specimens = highest number of individuals; *Macrostylis* sp. ML08) was used for the Isolation-By-Distance analysis. For the latter, the Isolation-By-Distance web service was used (Jensen et al., 2005).

3. Results

3.1. Intra-family diversity pattern

3.1.1. Alignment

In total, 16S rDNA was successfully amplified for 221 macrostylids. The MAFFT alignment was 427 base pairs (bp) long and contained 174 (40.7%) identical and 233 parsimony informative sites. Pairwise identity equalled 87%. The ungapped mean lengths of the sequences was 403.5 bp (min. 312, max. 418, SD 16.4), and the GC content 35.7%.

3.1.2. Distribution of genetic variation along the VFZ and PRT

The Mantel test revealed a significant positive correlation ($R = 0.484$, $p < 0.05$) for the comparison between geographical distance and *p*-distance matrices. A scatterplot (Fig. 2) illustrates a stepwise distribution of distances along the study transect: small p-distances of up to 3% are distributed over a maximum range of ~ 2200 km; genetic differences of up to 10% are limited to geographic ranges below ca. 2800 km, and specimens collected at stations > 3000 km apart have at least *p*-distances of 10% reaching up to ~ 35%. The distribution of these maximum values for genetic differentiation extends across the whole geographic range covered. The distribution of the *p*-distances shows a gap that is extending throughout the whole geographic range roughly between 15% and 24%.

3.1.3. Geographic patterns of genetic differentiation

From the multivariate (incl. all *Macrostylis* sequences) *p*-distance matrix, two PERMANOVA analyses were conducted to test if 1) the VTF, East, West, and PRT were significantly different from each other, and 2) if water depth was a significant factor (hadal versus abyss). Testing for differences between the areas and depth zones, all groups differed significantly (Fig. 3). Pair-wise comparisons of all areas resulted in significant differences for all pairs, except for the pair West-VTF (Tables S1 and S2 in Electronic supplement 3). PERMDISP results for the *p*-distance matrix were significant, indicating that part of the differences found were due to dispersion of the multivariate data.

3.2. Species distribution patterns

3.2.1. Species delimitation

The alignment and phylogenetic results are presented in the Electronic supplement 4. The maximum-likelihood based PTP species delimitation resulted in 19 putative species (also referred to here as molecular operational taxonomic units (MOTUs)) of which five

corresponded to previously identified species for which data was available on GenBank (see Electronic supplement 5). Only three of the 14 MOTUs collected during Vema-TRANSIT were comprised of at least 25 specimens (ML08, ML25, and MLpap), of which ML08 was by far the most abundant (126 individuals). The other twelve MOTUs comprised 1–7 specimens (Table 2, see Electronic supplement 5).

The mean intraspecific divergence per MOTU/species varied between 0.1% *p*-distance in *M. roaldi* and 4.3% in both ML01 and ML08 (Electronic supplement 6). Absolute values started at 0% and reached up to 8.1% in ML08 (see Electronic supplement 7). However, net between-group mean *p*-distances started at values as low as 5% (absolute minimum of 4.6% between MLpap and ML08) and peaked at 35.5% between ML24 and *M. scotti* (abs. max. 36.8% between ML08 and ML16; see Electronic supplement 7).

3.2.2. Geographic and bathymetric distribution of species

The species varied considerably in their distribution (Electronic supplement 5). The most frequently occurring and most abundant species, ML08, occurred at all eastern stations and one western station, and to a large extend it occurred sympatrically with the sister species MLpap. Both were distributed across the MAR but were the only ones with such a trans-Atlantic occurrence. Most species were restricted to one region. Three occurred only at hadal PRT sites (ML23–25), of which ML23 and ML24 were represented by only one specimen each. One species occurred only at the western abyssal sites, (ML13) and ML16 solely was collected at the abyssal slope of the PRT. ML01 was collected only at the VTF and ML02 occurred only on the eastern VFZ. All other species occurred across the predefined areas and depth zones: ML12, ML22, and ML23 were distributed both in the abyssal western VFZ as well as in the hadal PRT, making them the furthest distributed species geographically. However, no species was shared between the hadal and abyssal PRT sites despite their close geographical proximity. ML12b occurred both in the western VFZ as well as in the VTF. Distances between ingroup and outgroup taxa varied from 33.9% (ML12) and 43.5% (ML08) (Electronic supplements 7 and 8).

Results of the PERMANOVA test, based on the multivariate species matrix, revealed significant differences for the species composition per predefined area (Fig. 4A); and all pairwise comparisons were significant (Tables S3 and S4 in Electronic supplement 3). PERMDISP results based on the MOTU resemblance matrix were not significant.

The results of the SIMPER analysis based on presence/absence data comparing the predefined geographic areas revealed the West and the PRT to have an average dissimilarity of 96.34%. Nine MOTUS from the western abyssal sites and four in the hadal PRT contributed to this dissimilarity. Two of these MOTUs occurred in both areas (ML12 and ML22). Two MOTUs contributed the most to the total dissimilarity, together 60.79%, of which MLpap occurred only in the West while ML25 was present only in the PRT.

For the comparison between West and East, the average dissimilarity was 93.13%. While two MOTUs from the west contributed, one did so from the east. The two MOTUs that contributed the most, together 79.62%, to the total dissimilarity, were found on both sites of the MAR. ML08 alone contributed 58.43% occurring at all eastern stations with relatively high abundances and being present with only one specimen in the west. MLpap contributed 21.79% to the dissimilarity occurring with similar average abundances in both areas (Electronic supplement 3).

The east and PRT turned out to be most dissimilar, with 100% dissimilarity. Overall, 55.39% of dissimilarity was caused by only one MOTU (ML08). From the PRT and east, two and three MOTUs were found respectively, which contributed to the dissimilarity and were mutually exclusive. Besides the dominant MOTU ML08 that occurred at all eastern stations while absent at the PRT, the MOTU ML25 contributed 20.89% and had an average abundance of 6.2 at PRT stations.

Comparing the MOTU composition of the two depth zones abyss and hadal, the distance-based test revealed homogeneity of multivariate dispersions (P (perm): 0.0667; Fig. 4B).

3.3. Population-genetic analyses

3.3.1. Haplotype distribution and geographic population differentiation

Amongst all samples, four species showed a sufficient number of haplotype and geographic range to be analysed on the population level (Fig. 5). The *Macrostylis* species MLpap occurred at abyssal stations both east and west of the MAR in similar specimen numbers and thus was the only species available for comparison between areas (east and west). It was collected at three western stations with four haplotypes (#1–4) in total and at one eastern station with two haplotypes (#5–6). No haplotype was shared between east and west (see also Bober et al. (in this issue)). For this species, a strong population structure was revealed with significant differences on all levels (between stations, sites, and areas) respectively (Φ_{ST} = 0.61309, p = 0.001; Φ_{ST} = 0.72995, p = 0.001; Φ_{ST} = 0.76365, p = 0.001).

The species ML25 occurred in all of and only the hadal samples. The most common haplotype (#8) occurred at all four hadal stations and also haplotype #9 was widely distributed, occurring at three of four hadal stations. Differences between stations or sites were not significant.

The widest distribution was found for ML08 which occurred at all of the eastern stations as well as one western station. For this species sufficient 18S sequences were available to perform analyses with both, 16S and 18S datasets. The 16S haplotype network (Fig. 5) shows clustering of the sites with site SO237-2 represented by haplotypes 22–25, site SO237-4 represented by haplotypes 14–21, and site SO237-6 is represented by haplotypes 11–13. Only one specimen of this species was found on the western side of the MAR (haplotype 10) which made between-area comparison impossible. No haplotype was shared between eastern and western VFZ. Comparison between stations and sites revealed moderate (between stations) to high (between sites) population structure with significant differences respectively (Φ_{ST} = 0.43773, p < 0.001; Φ_{ST} = 0.72995, p = 0.001). The 18S data supports the presence of population structure between stations and sites (Φ_{ST} = 0.2426, p < 0.001; Φ_{ST} = 0.41833, p < 0.001).

Macrostylis sp. ML12 was found at one western abyssal VFZ site as well as three of the hadal stations (four haplotypes). No haplotype was shared between abyss and hadal depth zones. Differences between stations, sites, or areas were insignificant.

Tajima's D statistics for the species *Macrostylis* ML08 and MLpap did not provide evidence for departure from neutrality with D values of 1.06845 (p = 0.149897) and − 1.40986 (p = 0.922808) respectively (but see also the contribution of Bober et al. (in this issue)).

3.3.2. Isolation by distance (IBD)

To test whether geographic distance has an effect on the

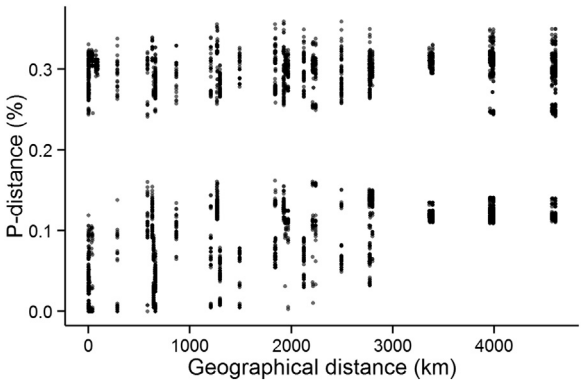

Fig. 2. Scatter plot of *p*-distance and geographic-distance matrices for the Macrostylidae (Isopoda) collected during Vema-TRANSIT in the southern North Atlantic. - Mantel test revealed a significant positive correlation.

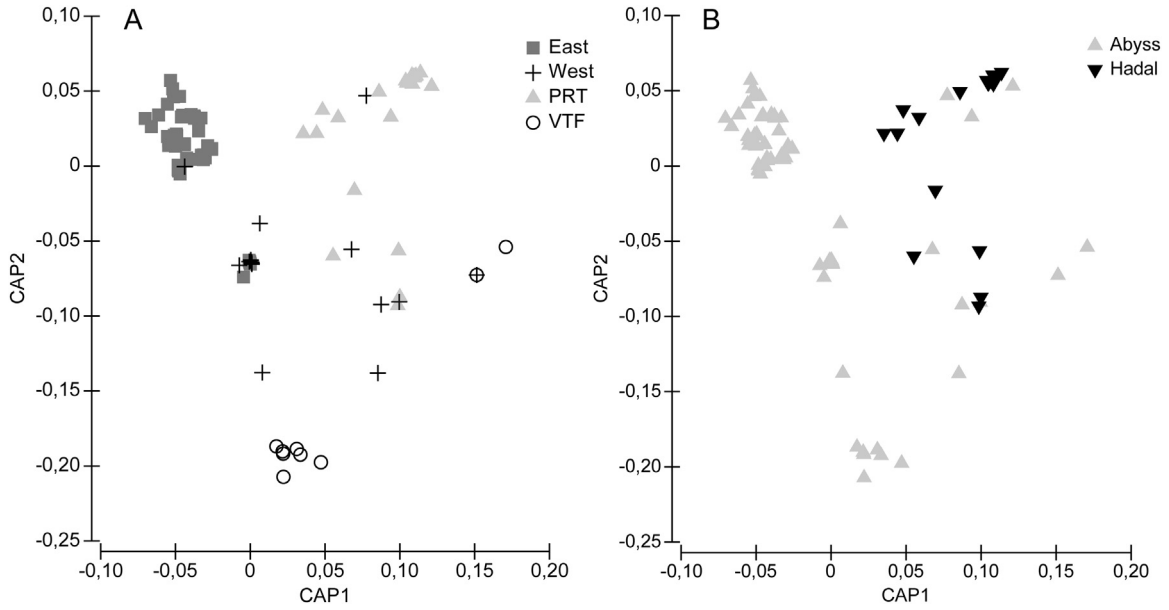

Fig. 3. Canonical analysis of principal coordinates (CAP) showing the differences between the *a priori* defined geographical areas (A: East, West, PRT, and VTF) and depth zones (B: abyss versus hadal) based on the multivariate *p*-distance matrix. All groups were significantly different from each other except for the pair West-VTF (Table S2 in Electronic supplement 3).

differentiation of a species, only the most widely distributed and at the same time most abundant MOTU of the dataset (ML08) was studied. The IBD analysis included a MANTEL test for matrix correlation between genetic distance and geographic distance (n = 7875), which resulted in a significant positive trend between geographic and genetic distances (Fig. 6), explaining 14.8% (R^2) of the observed genetic variation (Electronic supplement 10).

4. Discussion

Complex interactions of biological, physical, and historical factors cause variation in species range sizes (reviewed by McClain and Hardy (2010)). The distribution of a species is ultimately shaped by a combination of intrinsic and extrinsic factors. Intrinsic factors include taxon-specific physiological adaptations and life-history strategies that are the consequence of historical selection pressures. These determine the ability to move between habitat patches and subsequently proliferate. Extrinsic factors, however, influence taxon distribution by providing physical barriers, for instance, to dispersal. These determine the success of propagules during dispersal and settlement. Therefore, the interactions between intrinsic and extrinsic factors determine species-specific niche requirements within an environmental landscape and regulate whether or not populations can persist (McClain and Hardy, 2010).

In this paper, the spatial distribution of genetic variation, from family to population level, as well as community structure of Macrostylidae were studied across a trans-Atlantic transect. The observed patterns were used to infer the effects of potential physical, depth-related barriers, as well as geographic distance on isolation and differentiation of these isopods. Barriers were represented by the Mid-Atlantic Ridge and the hadal-abyssal depth zonation between the Puerto Rico Trench and the nearby Atlantic abyssal plains.

4.1. The MAR—a barrier to the dispersal of abyssal macrofauna?

Environmental factors, such as hydrographic conditions and seafloor topography, play an important role in limiting dispersal (McClain and Hardy, 2010). We hypothesized that the MAR reduces dispersal of abyssal benthic organisms leading to distinct (genetic and species) assemblages east and west. To test for this effect, regional differences in the distribution of haplotypes, species, *p*-distances, and the (macrostylid) community structure were studied (Figs. 3A and 4A).

The species distribution shows that most (eight out of 14) species were restricted in their distribution to one area (east or west), while only two species occurred on both sides of the MAR (Electronic supplement 5). While the scatterplot (Fig. 2) did not reveal geographic clusters of genetic variation on family level, neither genetic distances (Fig. 3A) nor species (Fig. 4A) were randomly distributed across the Atlantic. Instead, clear and significant differences between the eastern and western (and PRT) compositions of species were revealed supporting our hypothesis.

The inferred pattern of distinctness in the distribution of genetic variation shows that within both eastern and western groups, specimens were on average more similar to each other than to those of the other areas, despite overlap (Fig. 3A). This indicates that relatedness, in general, was higher within the defined areas. Nevertheless, the areas PRT, West, and VTF were relatively dispersed, while the East was closely clustered. This may have been due to the majority of the eastern specimens belonging to only two dominant species: ML08 and the closely related MLpap, both of which had occurrences on the western VFZ as well. Most dispersion of the data could be observed in the West (Fig. 3A), indicating that the macrostylid fauna there was composed of distinct rather than closely related species and lower clades (see also Electronic supplement 5).

The latter pattern could be potentially explained by environmental differences between the regions. The Demerara basin (Fig. 1) is under influence of two different water masses, the AABW and NADW, which contribute to relatively strong bottom-water dynamics and thus greater habitat heterogeneity, as has been depicted by the presence of fine and coarse sand in the sediments of this area (Devey et al., in this issue; Lins et al., in this issue) thus potentially supporting a diverse macrostylid fauna when compared to the other areas. Indications of bottom-water dynamics, however, have also been found in the eastern stations (Devey et al., in this issue). Moreover, the western area is geographically in center of the transect, increasing the potential for shared species with each of the other areas, which is exactly what was found. The macrostylid community structure (Fig. 4A) nevertheless fully supports significant differences between the areas and thus our hypothesis.

For smaller, meiofaunal organisms and macrofauna groups with dispersal stages (e.g., larvae, adult swimmers) long-range dispersal

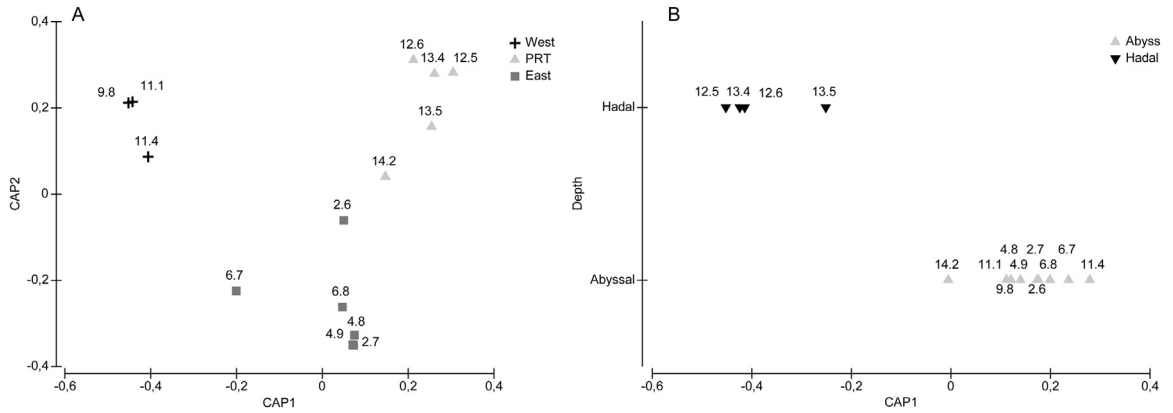

Fig. 4. CAP analysis based on the Bray-Curtis resemblance matrix for the MOTUs. A, all areas (western Vema Fracture Zone (VFZ), Eastern VFZ, and Puerto Rico Trench (PRT) differed significantly from each other. B, the depth zones abyss and hadal differed significantly and the dispersion of the data was homogeneous (P (perm): 0.0667).

across ridges has been shown (Bik et al., 2010; Menzel et al., 2011; Schüller and Hutchings, 2012; Zardus et al., 2006). Our results agree with previous findings suggesting that the predominantly benthic isopods without larvae or other dispersal stages seem to be restricted in their range by mid-ocean ridges (Bober et al., in this issue; Brix et al., 2015, in this issue; Kaiser et al., in this issue; Schnurr et al., 2014).

4.2. Unhindered exchange between hadal and adjacent abyss or hadal endemism?

Isopods are amongst the most diverse groups of multicellular organisms in trenches (Jamieson, 2015). According to Beliaev (1989) up to 63% of isopod species reported from trenches may be endemic to the hadal zone. An early study on the Puerto Rico Trench isopods suggested that most trench species have been found only in the trough and not in the adjacent abyss (Wolff, 1975). This finding suggests that the PRT is not an exception and some of the trench species may be endemic to this trench. Generally, depth zonation has been found in many benthic organisms and it has been proposed that depth related barriers promote differentiation (e.g., Jennings et al., 2013; Wilson and Hessler, 1987; Zardus et al., 2006). Wilson and Hessler (1987) suggested that due to strong gradients along the slope, the bathyal should be the region where speciation is most active in the deep sea and, although gradients are less pronounced at greater depth, this may be relevant at trench slopes as well. Accordingly, we hypothesized that the depth difference between the hadal and the abyss restricts dispersal and leads to hadal endemism.

In our study, three species occurred both at abyssal as well as hadal depth (Electronic supplement 5). Interestingly, the abyssal PRT stations had no species in common with the hadal PRT stations. Instead, the species ML12, ML15, and ML22 occurred roughly across 2000 km distance, not even considering bathymetric features between the stations, at hadal as well as abyssal sites of the western VFZ (Electronic supplement 5). Although sample size was insufficient to test for depth differences, the lack of shared haplotypes (Fig. 5) between abyssal and hadal indicates limited exchange, supporting our hypothesis.

As expected, then lack of shared species between abyss and hadal as well as *p*-distance distribution also revealed significant differences in faunal (macrostylid) community structure (Figs. 3–4; Electronic supplement 3). We did not sample along a depth transect in this study, but in other crustaceans at the Kuril-Kamchatka Trench it has been shown that assemblages gradually change with increasing depth (Kitahashi et al., 2013) until a strong difference between abyssal and hadal communities is reached at maximum depth difference. Hence, despite the close proximity to the abyssal PRT stations, the same species would not be expected to occur at both depths since the high pressure difference requires adaptations (Blankenship-Williams and Levin, 2009) which only allows specially adapted species to occur at both zones, resulting in relatively high endemism (Beliaev, 1989). Furthermore, sediment characteristics were different (Devey et al., in this issue) which may be of significance considering the burrowing macrostylid lifestyle (Hessler and Strömberg, 1989).

Until present, most data on depth differentiation (Etter and Rex, 1990) was published from bathyal depths, for instance on molluscs (Etter and Rex, 1990; Jennings et al., 2013; Zardus et al., 2006) or polychaetes (Schüller, 2010). Similarly, depth zonation seems to be common patterns also in deep-sea crustaceans. An example of depth zonation has been shown for the janiroid isopod family Haploniscidae, where some species only occurred shallower than 3000 m and others only below 3600 m (Brökeland and Raupach, 2008). Similar distribution breaks have been reported from one of the best-studied deep-sea crustaceans, the amphipod *Eurythenes gryllus* sensu lato, which is a complex of highly mobile and relatively large scavenger species. Despite the high motility and extreme (e.g., bipolar) distributions of some of its clades, depth-confined and oceanic-basin restricted clades have been observed (France and Kocher, 1996; Havermans et al., 2013). Also at hadal depths, scavenging amphipods have been found to inhabit overlapping but, nevertheless, distinct depth zones of the Kermadec Trench (Blankenship et al., 2006). Accordingly, depth-related barrier effects seem to be relevant across a wide range of crustacean feeding guilds and independent from lifestyle and depth zone.

In our study, one species was particularly abundant at all hadal stations, *Macrostylis* sp. ML25, and could not be found outside the trench (Kniesz et al., in this issue). The high abundance and dominance, in comparison to other macrostylids, may be a consequence of the accumulation of organic matter at the bottom of the trough (George and Higgins, 1979), which is common in trenches and may favor dominance of few species (Jamieson, 2001; Levin et al., 2001). However, the four hadal stations reported here were located near the northern slope of the trench, which has been characterized as rather similar to nearby abyssal sites in carbon content and faunal abundances (Richardson et al., 1995; Schmidt et al., in this issue; Tietjen et al., 1989). Hypothetically, ML25 may represent a trench-endemic species that has adapted to this environment, thus outcompeting others that may be new colonizers to the trench from nearby abyssal areas, which may be the case for the species ML12, ML15, and ML22. The ML25 haplotype network shows absence of population structure evidence for a panmictic population in the trough (Fig. 5C; Electronic supplement 9). We cannot ascertain that ML25 does not appear at abyssal depth as the density of the sampling was low. Nevertheless, the data presented here shows a clear distinction between abyssal and hadal communities suggesting that dispersal across the ~ 3000 m depth difference is not the rule.

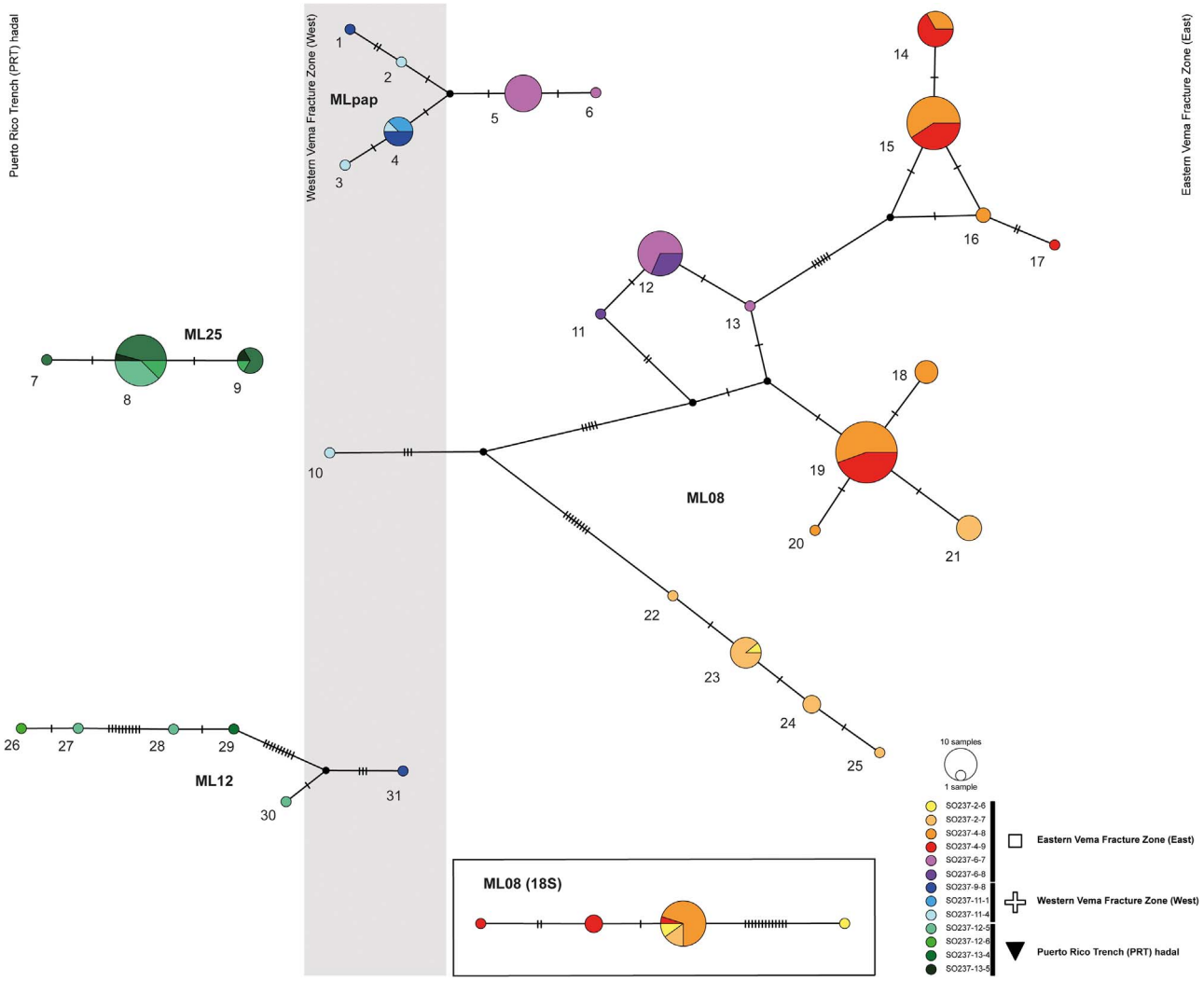

Fig. 5. Haplotype networks based on 16S rDNA data from the isopod species *Macrostylis* sp. MLpap, M. ML08, ML12, and ML25. The samples were collected at abyssal depth in the Vema Fracture Zone, east and west of the Mid Atlantic Ridge, as well as in the hadal Puerto Rico trench. The geographic origin is indicated by the vertical subdivision of the figure. Color-coded blobs indicate collection stations. The blob size stands for the number of sequences per haplotype. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. Influence of geographic distance on genetic variation

Our final hypothesis was that genetic differentiation and intra-family diversity increases with geographic distance. Generally, the effect of distance on genetic differentiation has not been often studied in inhabitants of the abyss and hadal. Given the large spatial extend of the abyssal environment as well as the likely inability of Macrostylidae to swim, it is safe to assume that due to limited dispersal, conspecific and potentially also congeneric individuals that are geographically close tend to be genetically more similar than individuals that are far apart. A previous study comparing distribution ranges of abyssal polychaetes and isopods across a ridge-free abyssal plane showed that most isopods have rather limited ranges (Janssen et al., 2015).

It has been stated that in the abyss high turnover does not necessarily preclude extensive geographical ranges if species are patchily distributed within those ranges (McClain and Hardy, 2010). However, analysing the distribution of genetic variation in relation to geographic distance, an effect of geographic differentiation could be traced throughout the hierarchic taxonomic levels studied — from populations (Fig. 5; Electronic supplement 9) through species (Fig. 6) across the whole family Macrostylidae (Fig. 2).

Geographic and genetic distances were positively associated also within the species *Macrostylis* sp. ML08 (Fig. 6). Yet, the interpretation of the Isolation-By-Distance (IBD) analysis has to be done with care because only 14.8% of the observed genetic variation could be explained by genetic distance. The distribution of the data, with widely dispersed *p*-distance values between 0 km and ~ 1300 km and only few little-dispersed points above this geographic range (Fig. 6) may have influenced this result. Consequently, besides a contribution of distance to the genetic diversification of species through restriction of gene flow (Wilson and Hessler, 1987), other factors may contribute to the evolution of these deep-sea benthic animals as well. For *Macrostylis* sp. ML pap, Bober et al. (in this issue) could not find any evidence for the effect of geographic distance. Nevertheless, the signal observed here for ML08 as well as relatively high population structure (Fig. 5) support our hypothesis of geographic distance influencing genetic differentiation and the effect of isolation by distance.

A similar approach addressing spatial geographic variation at abyssal sites in isopods (and polychaetes) dealt with connectivity in the central North Pacific abyssal nodule fields. This study identified geographic distance as the main factor explaining genetic variation and no recognizable geographic clustering could be found (Janssen et al., 2015). Geographic differentiation was reported for the isopod family Haploniscidae as well (Brix et al., 2011). And even in comparably

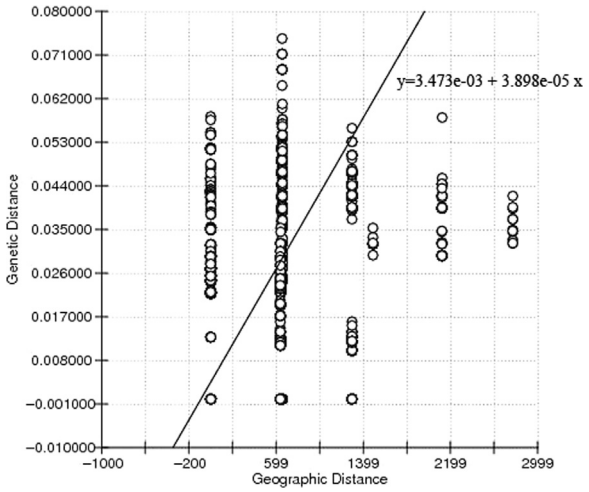

Fig. 6. The Isolation By Distance (IBD) analysis performed on the IBD web server (Bohonak, 2002).

mobile (i.e., swimming) isopods, such as the Munnopsidae, geographic clustering has been described (Raupach et al., 2007). Apparently, in deep-sea isopods in general, geographic distance contributes to diversification.

4.4. Range sizes

The supposed homogeneity of the deep-sea environment contradicts the often reported high level of local endemism (e.g., Brandt et al., 2007a; Ebbe et al., 2010; Smith et al., 2006). Generic and family-level cosmopolitanism (Wilson and Hessler, 1987), however, supports this supposition. We were interested in the ranges of macrostylid species as well as other levels of the taxonomic hierarchy in order to evaluate and understand spatial scales relevant for the evolution of this taxon.

On the family level, almost the whole range of genetic diversity (*p*-distances) was widely spread across the total geographic range studied and no geographic clustering became apparent (Fig. 2). The distribution of these maximum values extends across the whole geographic range covered above a gap in the data cloud that is parallel to the abscissa. This discontinuation of the range of genetic variation may have various reasons of historic and phylogenetic nature and indicates that the macrostylids collected belong to several distantly related clades, which is, however, not in the scope of this article. In the upper data cloud, *p*-distances ranged from roughly 24% to over 35%. Despite some fluctuation in maximum values, a plateau was evident which was spread across the whole study area (Fig. 2). This shows that at every geographic scale covered in this study, highly divergent species were co-occurring. This supports the theory of cosmopolitanism in deep-sea genera and families (Wilson and Hessler, 1987).

A conspicuous pattern that became apparent (Fig. 2) was a stepwise distribution of the lower range of *p*-distances along the study transect: small *p*-distances of up to 3% were distributed over a maximum range of ~ 2200 km. Beyond the 3% threshold, a saltatory range shift became apparent in the scatterplot (Fig. 2), with all distance pairs of up to 10% being limited to geographic ranges below ca. 2800 km.

The range of 3–10% genetic distance in 16S can be considered a "grey zone", covering variation that is often still included in the category of intraspecific variation, but considered interspecific divergence in various isopods (see, e.g., Brix et al., in this issue; Brökeland and Raupach, 2008; Held, 2003; Kaiser et al., in this issue; Raupach and Wägele, 2006; Riehl and Brandt, 2013) amongst other crustaceans (e.g., Costa et al., 2007; Havermans et al., 2013; Matzen da Silva et al., 2011). Specimens collected at stations > 3000 km apart have at least *p*-distances of 10%, reaching up to ~ 35% (Fig. 2), which is beyond the range of intraspecific variation.

In this study, net between-group mean *p*-distances started at values as low as 5% (absolute minimum of 4.6% between MLpap and ML08) and peaked at 35.5% between ML24 and *M. scotti* (abs. max. 36.8% between ML08 and ML16; see Electronic supplement 8). The mean intraspecific divergence varied between 0.1% p-distance in *M. roaldi* and 4.3% in both ML01 and ML08. Absolute values started at 0% and reached up to 8.1% in ML08 (see Electronic supplements 6 and 7). This overlap between intraspecific variation and interspecific divergence shows that working with fixed threshold values is inappropriate in Macrostylidae. Nevertheless, the distribution of genetic distances is interesting. The observed distribution of up to 3% genetic distance as a conservative measure for intraspecific variability indicates that abyssal macrostylids may have a distribution range of at least around 2200 km. This range is increased to up to 2800 km when considering intraspecific variability of up to > 8% as identified for some species here (see Supplement 7).

4.5. How can gene flow be maintained over large geographic scales without dispersal stage?

Macrostylid morphology seems to be streamlined for a fossorial (digging) and ambulatory locomotion. The typical macrostylid body is subcylindrical and elongate. Ventral spines and often slightly concave ventrolateral sternites allow for leg movement close to the body as required in a tubular or digging lifestyle. The three anterior pereonites are fused (fossosome) and their pereopods modified for digging (Riehl, 2014; Riehl et al., 2014b). None of their appendages have surface-increasing modifications that could represent natatory adaptations, such as in the Munnopsidae or several Desmosomatidae (Hessler, 1993; Hessler and Strömberg, 1989; Wilson, 1989; Wilson and Ahyong, 2015). It is thus unlikely that active swimming behavior is responsible for 2000 km-scale distributions as observed here for this taxon.

Other taxa without swimming adaptations or dispersal stages, such as harpacticoid copepods (Easton and Thistle, 2016; Menzel et al., 2011) and nematodes (Bik et al., 2010; Lins et al., 2017) of the meiofauna, have been found distributed in unexpected scales before. In these cases, erosion and passive drift with ocean-floor currents were assumed as an explanation. Erosion is less likely in macrofauna organisms and even more so in larger size classes. Nevertheless, for the benthic, shallow-water isopod *Septemserolis septemcarinata* (Miers, 1875), drifting on floating substrata such as macro algae, has been the best explanation to explain long-distance dispersal (Leese et al., 2010). While macrostylids lack strong grasping legs as much as they lack swimming legs, relatively wide geographic and depth distributions within a species have already been observed from the Antarctic shelf (Riehl and Kaiser, 2012). It is possible that macrostylids actively make use of currents by "walking" vertically into the water column. This type of emergence (not to be confused with evolutionary emergence) has been observed for deep-sea harpacticoids (Thistle et al., 2007) and cumaceans (T. Riehl, personal observation) which both represent the benthos.

Given that a relatively strong and steady bottom-water current flows eastward through the VTF (Eittreim and Ewing, 1975; Heezen et al., 1964) and that the VTF represents an abyssal continuation (Devey et al., in this issue), it may provide a potential connection between east and west of the MAR (Bober et al., in this issue; Brix et al., 2015; Guggolz et al., in this issue; Lins et al., 2017). Thus, a smaller degree of dissimilarity between the two areas than found here (> 93%; see Table S6 in the Electronic supplement 3) would be expected. In spionid and polynoid polychaetes, widely distributed, "ubiquitous" species have been identified that apparently make use of this channel to maintain gene flow between east and west (Guggolz et al., in this issue) resulting in only insignificant differences between both sides. Also previous studies on other benthic crustaceans without dispersal stage have suggested passive transport via advection, for instance through fracture

zones, as exchange routes between basins (Brix et al., 2015; Easton and Thistle, 2016; Menzel et al., 2011). This mode of dispersal may explain the trans-MAR occurrence of two macrostylid species (MLpap and ML08).

4.6. Diversification in the deep sea

At many studied abyssal sites, asellote isopods constitute one of the numerically dominating macrofaunal (0.3–10 mm) taxa (Brandt et al., 2017, 2015, 2013, 2007b, 2005; Poore and Wilson, 1993; Rex et al., 1993; Wilson, 2008). They are also the most species-rich animal group at hadal depths (Jamieson, 2015). Regularly, isopods encountered in abyssal plains have their ovaries or brood pouches filled with eggs (Elsner et al., 2013; Riehl and Brandt, 2013; Wilson, 1983) and we observed this also in the hadal species ML25 (Kniesz et al., in this issue). This indicates that isopods reproduce at abyssal as well as hadal depths contradicting the source-sink hypothesis (Rex et al., 2005). Hence, other factors should promote differentiation and eventually speciation of this group at these depths.

We showed that macrostylid species can have unexpectedly large distribution ranges. Yet, despite two species occurred on both sides of the MAR, no evidence for continuous exchange of eastern and western populations could be found. In MLpap, no haplotypes were shared between areas (Fig. 5) and significant east-west differences were found in the AMOVA. Also for ML08, no haplotype occurred on both sides of the MAR and, furthermore, this widely distributed species that occurred across thousands of kilometres at all eastern stations, was found with one single specimen in the west. The latter specimen was genetically distinct from the next two similar sequences by ten and 14 mutations respectively (Fig. 5). Accordingly, despite shared species, dispersal of macrostylids seems to be nevertheless impacted by the MAR. This is not implausible because for genetic differentiation not a complete cut of gene flow would be required but already a reduction would be sufficient (Bober et al., in this issue). Alternatively, differences in transatlantic distribution of this two group may be explained by the lack of a dispersal stage (Janssen et al., 2015). Nevertheless, it should not be overlooked that due to the scattered sampling providing these data, sampling bias (small sample size) may play a role as well and it is unclear how representative our samples were for the whole macrostylid fauna of the region.

It is noteworthy that the two species with the widest distributions (ML08 and MLpap) belong to a group of macrostylids which is characterized by extreme sexual dimorphism (Riehl et al., 2012), where adult males have elongated posterior walking legs that may potentially increase the individual range by crawling or drifting (N. Heitland and T. Riehl, unpublished data). Both species are characterized by these adaptations, while ML25, likewise belonging to this clade (Electronic supplement 5), lacks these modifications in the walking legs and was found only with a very restricted distribution (Kniesz et al., in this issue). In the trench however, ML25 haplotypes occurred well-mixed throughout the four stations sampled indicating panmixia in this trench population (Fig. 5).

Remarkably, the demographic patterns exposed by macrostylids do not match the pattern of non-neutrality observed for various vent and non-vent deep-sea taxa. Deviation from neutrality was explained with demographic bottlenecks followed by expansions (demographic instability), the prevalence of sweepstakes dispersal across patchy habitats (high variance in reproductive success) or selective sweeps (positive selection) (Taylor and Roterman, 2017). Our data suggest that suitable macrostylid habitats are persistent; complying with the assumption of generalist ecologies for these isopods, and that demographic instability does not play a role in the evolution of macrostylids.

In comparison to swimming isopods (Bober et al., in this issue) and generally to organisms with swimming capability or free larvae endobenthic and digging organisms the range size observed here may be small (Baco et al., 2016). Moreover, the connectivity-reducing effect of the MAR is likely stronger in macrostylids than in organisms with greater motility (Bober et al., in this issue; Zardus et al., 2006). The contribution of the diversification factors analysed here, amongst others, to actual speciation is this likely taxon specific.

4.7. Outlook

Most of the species collected here were represented by singletons or few individuals only making statistical analyses not viable. Additional sampling is thus required if we intend to test the applicability of the patterns observed here for few species to the whole family. Nevertheless, we found out that already on the scale of 1–2 km, significant population structure exists. With increasing geographic distance and across barriers, these differences become larger. In order to evaluate scales relevant for genetic turnover, intensive sampling across scales is needed. Using this method, also gradients, such as the depth transition between abyss and hadal, could be studied.

This study is based almost solely on a mitochondrial marker. 16S has been used for terminal clade phylogenetic inference (e.g., Brix et al., in this issue; Kaiser et al., in this issue), to infer patterns of differentiation in marine isopods (e.g., Held, 2003; Leese et al., 2008; Raupach et al., 2014; Raupach and Wägele, 2006; Riehl and Kaiser, 2012), and it has been interpreted as replacement barcoding marker in taxa where COI proved to be difficult to amplify, for instance in Asellota (Brökeland and Raupach, 2008; Raupach et al., 2007; Riehl et al., 2014a; Riehl and Kaiser, 2012). Besides the advantages that the mtDNA-specific inheritance brings for phylogeographic and phylogenetic studies (Hebert et al., 2003; Rubinoff et al., 2005), there are clear drawbacks. Amongst these, incomplete lineage sorting and introgression can cause distorted impressions of genetic structure and diversity (Galtier et al., 2009; Rubinoff et al., 2005). In order to avoid such potential pitfalls, we used nDNA (18S) for comparison with 16S. Our 18S data was limited, though, and future studies should combine the use of mtDNA with comprehensive nuclear datasets.

5. Conclusions

The relationship between physical, ecological and physiological isolating mechanisms and adaptations is reflected in the population structure of a species (Zardus et al., 2006). The abyss is regularly seen as an environment free of dispersal barriers (McClain and Hardy, 2010; Menzel et al., 2011; Rex and Etter, 2010). Yet, despite the lack of obvious isolating barriers, we identified strong genetic structure on a variety of scales in Macrostylidae. Macrostylid isopods, as endobenthic organisms (Hessler and Strömberg, 1989; Thistle and Wilson, 1996; Wägele, 1989) that have no dispersal stage, are commonly considered poor dispersers. Here we demonstrate that both statements have to be carefully evaluated and cannot be generally applied to all fauna alike.

On the one hand, the distribution of species showed that macrostylids can have distribution ranges over 2000 km and across oceanic ridges and trenches. Organisms that are apparently not adapted to active long-distance dispersal must rely on passive drifting to maintain gene flow across large distances (Easton and Thistle, 2016; Leese et al., 2010). There was, on the other hand, a clear signal for geographic structure in community composition and haplotype distribution coinciding with the east-west division of the Atlantic by the Mid Atlantic Ridge, as well as with the abyss-hadal transition. Here, between these only few species were shared and distant (across-areas) populations were divergent. This supports the hypotheses that depth gradients as well as oceanic ridges shape communities by reducing dispersal. Additionally, due to positive associations between genetic- and geographic distances within the only abundant macrostylid species ML08 as well as in the whole Macrostylidae it appears that the vast size of the deep sea itself is a factor responsible for creating diversity as hypothesized before (Wilson and Hessler, 1987). However, a wide distribution was only found for small subset of the species identified here. Most species were

restricted to single sampling sites, one area, and one depth zone. While this can be mostly explained with most species being rare in the samples and sampling being scarce, there may be species-specific patterns of distribution as well.

In conclusion, divergence could be identified across the MAR, depth contours and distance. Together, ridges, depth zonation, and distance may play a key role in the evolution of the deep-sea fauna, triggering population differentiation and ultimately leading to speciation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr2.2017.10.005.

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