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## Regular article

# *Paramytha ossicola* sp. nov. (Polychaeta, Ampharetidae) from mammal bones: Reproductive biology and population structure



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## ABSTRACT

Sunken whale carcasses, known as “whale falls”, deliver large, but relatively ephemeral pulses of organic material to the seafloor and serve as habitat for unique assemblages of deep-sea fauna that include generalist-scavenging species, chemosynthetic fauna and bone-specialist species. Despite the great deal of interest that fauna associated with whale falls have attracted, very little is known about this fauna in the deep Atlantic Ocean. Here we describe a new species of Ampharetidae that was found in an experiment using cow carcasses in the Setúbal Canyon (NE Atlantic), as a surrogate of a whale fall. Further, we analyse the size and structure of the population at two different times and use histological analyses to investigate the reproductive biology of this new species. We propose that *Paramytha ossicola* sp. nov. is a bone-specialist adapted for life in ephemeral habitats. Reproductive traits include rapid maturation, continuous and non-synchronous gametogenesis. Recruitment seems to be controlled by habitat availability and biological interactions that result in post-settlement mortality.

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## 1. Introduction

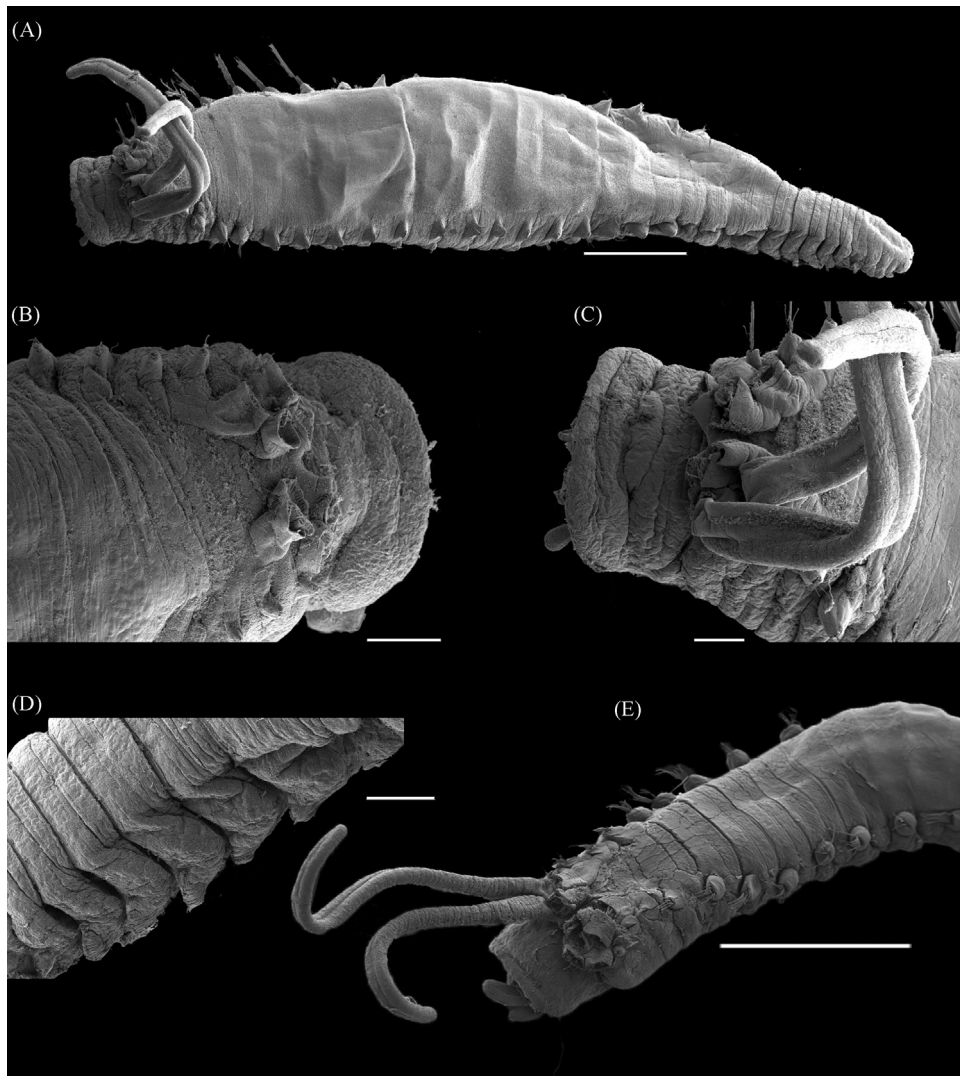
Organic falls, such as dead whales, wood and algal detritus, have long been known from the deep-sea floor, with first reports dating from *The Challenger* expedition (Wolf, 1979). But the interest in these patchy and ephemeral (at decadal scale) habitats was renewed with the discovery of chemosynthetic communities associated with whale bones, which led to the hypothesis that organic falls may serve as stepping-stones for faunal dispersal between hydrothermal vents and cold seeps (Bennet et al., 1994; Deming et al., 1997; Feldman et al., 1998; Smith et al., 1989). Since then, dozens of whale carcasses, wood and algae parcels, both natural and experimentally implanted, have been documented (e.g. Braby et al., 2007; Cunha et al., 2013; Fujiwara et al., 2007; Samadi et al., 2010; Smith and Baco, 2003) and it is currently recognized that food falls contribute to beta diversity in the deep sea by creating patches of organic enrichment and chemical or

physical disturbance (Bernardino et al., 2010, 2012; Smith, 1985; Smith et al., 1989; Snelgrove and Smith, 2002).

Whale carcasses have been investigated with greater intensity than any other type of deep-sea organic falls, but the geographical distribution of these studies shows a clear bias towards studies carried out in the Pacific Ocean. A recent experiment in the NE Atlantic using cow carcasses to simulate a whale fall resulted in the description of the macrofaunal assemblages associated to these ecosystems (Hilario et al., 2015) and in new insights on their role as stepping-stones (Génio et al., 2015) and as promoters of biodiversity (Ravara et al., 2015) in this part of the world's ocean. One interesting finding of this experiment was the presence of an ampharetid worm, initially identified as *Amphisamytha* cf. *lutzi*, occurring in large numbers, living on the exposed bones (Fig. 2E in Hilario et al. (2015)). Subsequent morphological and molecular analyses revealed that this ampharetid worm is a new species belonging to the genus *Paramytha*, which so far was only reported from hydrothermal vents in Arctic mid-ocean ridge (Kongsrud et al., 2016). Ampharetid polychaetes are well known from chemosynthetic environments (e.g. Reuscher et al., 2009; Stiller et al., 2013), and the species *Amage longibranchiata* Hartman, 1960 has previously been recorded from a whale carcass in the deep NE Pacific (Bennett et al., 1994). However, the new species described herein represents the first Atlantic record of an ampharetid living on mammal bones.

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**Fig. 1.** *Paramytha ossicola* sp. nov. Scanning Electron Microscopy (SEM micrographs): (A) habitus in dorso-lateral view; (B) anterior part of body with all branchiostyles missing, dorsal view; (C) detail of anterior part of body, dorsal view; (D) detail of abdominal chaetigers, dorso-lateral view; (E) anterior part of body, dorso-lateral view. (A–D) paratype, NHM 2016.492; (B) paratype, ZMBN 107238; (E) paratype, ZMBN 107239. Scale bars: (A, E) 0.5 mm; (B–D) 0.1 mm.

Because of the insular and ephemeral nature of deep-sea reducing environments, in general, and organic-falls, in particular, species inhabiting these habitats were predicted to have evolved r-type life history strategies (reviewed by Young (2003)). However, many reproductive traits are phylogenetically constrained rather than dictated by the environment (reviewed by Young, 2003). Studies of reproductive biology of species associated to organic-falls have focused on three taxa: wood-boring bivalves of the Xylophagidae family (Haga and Kase, 2013; Tyler et al., 2007), chemosymbiotic mytilid bivalves of the genus *Idas* (Gaudron et al., 2012; Tyler et al., 2009) and the bone-eating siboglinid polychaete *Osedax* (Rouse et al., 2004, 2008, 2009). Regarding ampharetid polychaetes from reducing environments, reproductive attributes have been reported for *Amphisamytha carldarei* (McHugh and Tunnicliffe, 1994; as *A. galapensis*) and *A. lutzi* (Blake and Van Dover, 2005; as *Amathys lutzi*). In the present study we formally describe *Paramytha ossicola* sp. nov., and investigate gametogenesis and population size and structure based on specimens recovered from cow bones at two different times, 18 and 28 months after deployment. Reproductive ecology and life history of this new species are discussed in relation to its phylogeny and habitat.

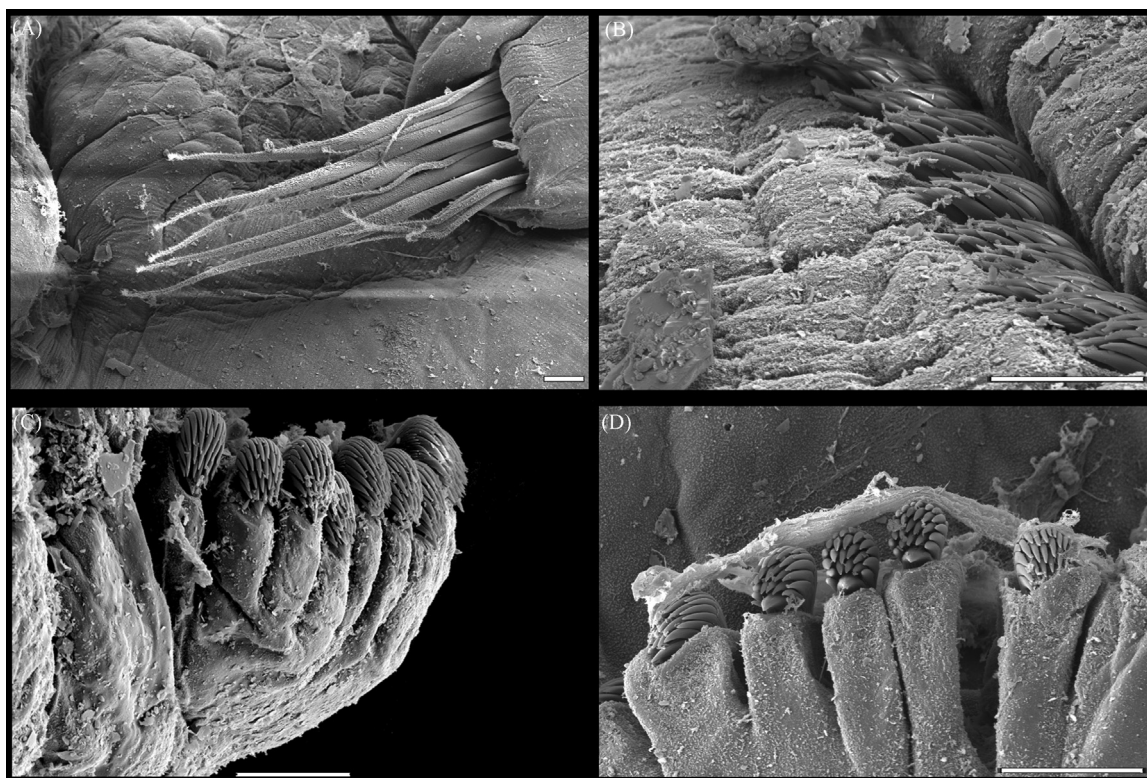
## 2. Materials and methods

### 2.1. Sample collection and morphological analyses

Ampharetid specimens were collected from bones resulting from the experimental deployment of five cow carcasses at Setúbal Canyon (38°16.850'N, 09°06.680'W; 1000 m depth), NE Atlantic. Details of the experiment are described in Hilario et al. (2015). Exposed bones were collected approximately 18 and 28 months after the deployment with the manipulator arm of ROV *Luso* and ROV *Genesis*, respectively, and brought to the ship inside closed boxes with ambient seawater. Bones were preserved individually onboard in 96% ethanol or in 4% seawater-formalin for DNA sequencing or morphologic analyses of colonizing organisms, respectively. In the laboratory, all ampharetid worms were removed from the surface and trabeculae of four bones under a stereomicroscope.

The specimens were identified using dissecting and compound microscopes. Specimens were stained in methyl blue to describe MB staining pattern and to aid in observation of morphological details. Drawings of uncini were prepared using a compound microscope with a camera lucida. A Canon 20D camera, with MP-E 65 mm and EF 100 mm macro lenses were used for digital photos of specimens. SEM





**Fig. 2.** *Paramytha ossicola* sp. nov. Scanning Electron Microscopy (SEM micrographs): (A) notochaetae; (B) thoracic uncini; (C) abdominal uncini; (D) abdominal uncini, frontal view. (A, D) paratype, ZMBN 107238; (B–C) paratype, NHM 2016.492. Scale bars: 10  $\mu$ m.

images were made using a ZEISS supra 55VP microscope at the Laboratory for Electron Microscopy, University of Bergen.

The type material of the species described herein is at the Natural History Museum, London (NHM; holotype and paratypes), the Natural History Collections, University Museum of Bergen, Norway (ZMBN; paratypes), and the Invertebrate Research Collection of the Department of Biology of the University of Aveiro (DBUA; paratypes). Material not deposited at any museum is in the collection of A. Hilário at the University of Aveiro.

## 2.2. Population structure and gametogenesis analyses

All complete specimens from the four analyzed bones were photographed using a Leica MC170HD camera attached to a stereomicroscope. The total length, from the prostomium to the pygidium, was measured through the body's median line using the open source image processing software ImageJ (<http://rsbweb.nih.gov/ij/>).

Size–frequency distributions were plotted using a 0.5 mm length-class interval chosen according to the criteria established by Kelly and Metaxas (2008): (1) most size classes must have at least five individuals, (2) the number of adjacent empty classes must be minimized, and (3) the length–class interval should be larger than the error on the length measurements. Size–frequency distributions were compared with a normal distribution using a Kolmogorov–Smirnov one-sample test. Modal decompositions were assessed using the logarithmic difference of frequencies method of Bhattacharya (1967) through a routine in FISAT (Guyanilo et al. 1996). This method provided initial estimates of the means, standard deviations, and proportions of modal components in the length–frequency distributions, which were then further refined using the MIXDIST package v.0.5–4 (MacDonald and Du, 2012) developed for the R environment (v.3.1.1) (R Core team, 2014). MIX-DIST uses maximum likelihood criteria to provide the best mathematical fit between theoretical and observed mixtures of frequency distributions.

Histological examination of 57 randomly chosen complete specimens from bone G was used to determine sex and to study gametogenesis. Each individual was dehydrated in ethanol, cleared in Histoclear™, impregnated and embedded in paraffin wax. Sections of each individual were cut at 6  $\mu$ m and stained with haematoxylin and counterstained with eosin. All sections were examined and photographed at 10 $\times$  and 40 $\times$  magnification. The sex ratio was estimated and differences from 1:1 ratio analyzed with a  $\chi^2$ -test.

To quantify oogenesis and assess synchrony, the feret diameter (diameters of hypothetical circles of area equal to the objects measured) of all oocytes sectioned through the nuclei was measured using ImageJ. Feret diameter was used to standardize variations in oocyte shape. Oocyte sizes were grouped in 10  $\mu$ m size classes and oocyte size–frequency diagrams were constructed for each individual.

The frequency of occurrence of three stages of sperm development (I–spheres of spermatogonia, II–rosettes of spermatocytes or of early spermatids, III–morulae of tailed spermatids) was determined in 5 randomly selected sections of all males. The criteria used to stage spermatogenesis were similar to those of Hutchings (1973b); Guillou and Hily (1983); McHugh and Tunnicliffe (1994) and Blake and Van Dover (2005) for other Ampharetidae.

## 3. Results

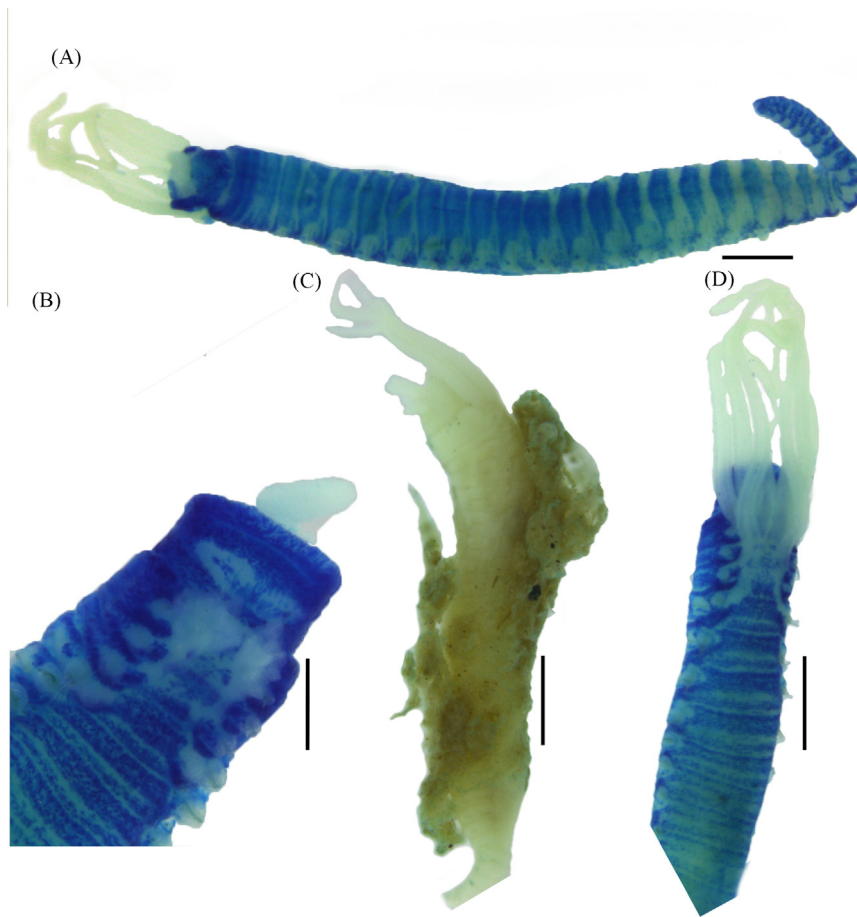
### 3.1. Systematics

**Family Ampharetidae Malmgren, 1866.**

**Subfamily Ampharetinae Malmgren, 1866.**

**Genus *Paramytha* Kongsrud et al. (2016).**

**Type species. *Paramytha schanderi* Kongsrud et al. (2016).**



**Fig. 3.** *Paramytha ossicola* sp. nov. (A) complete specimen stained in methyl blue, ventral view; (B) anterior part of body with branchiae missing, stained in methyl blue, dorsal view; (C) specimen in tube; (D) Anterior part of body, stained in methyl blue. (A–D) ZMBN 107240. Scale bars: (A, C–D) 0.5 mm; (B) 0.2 mm.

**Diagnosis.** Prostomium rectangular with thickened anterior margin, without lobes or glandular ridges. Buccal tentacles smooth. Four pairs of cirriform branchiae arranged as 2 + 1 + 1 on segments II–IV respectively; two anterior pairs in transverse row without median gap. Chaetae on segment II (paleae) absent. Number of thoracic and abdominal chaetigers interspecific variable. Modified segments absent. Abdominal neuropodia gradually increasing in size forming pinnules from about 4<sup>th</sup> abdominal chaetiger. Abdominal neuropodia with dorsal thickened pads. Thoracic and abdominal uncini of same type. Anal cirri absent.

***Paramytha ossicola* sp. nov.**

Figs. 1–4.

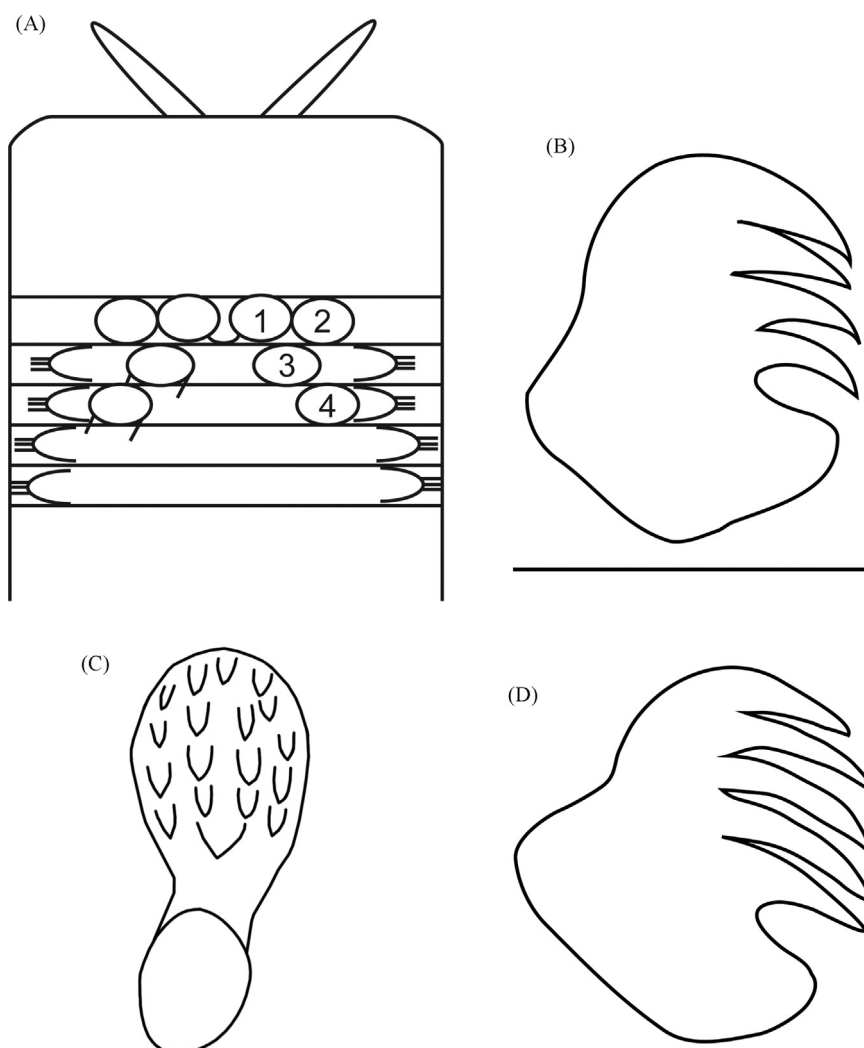
**Type locality.** Setúbal Canyon, 38°16.850'N 09°06.680'W, 1000 m depth, NE Atlantic off Portugal.

**Type material.** Type material: All from type locality. Holotype – collected in June 2013, fixed in formalin and subsequently transferred to 96% ethanol (NHM2016.491). Paratypes – collected in August 2012: 1 specimen mounted on SEM-stub (NHM2016.492), 2 specimens mounted on SEM-stub (ZMBN 107238–107239), 5 specimens, DNA-vouchers (ZMBN 107232–107236), 4 specimens in ethanol, used for staining (ZMBN 107240), 5 specimens in ethanol (ZMBN 107241); collected in June 2013: 2 specimens in ethanol (NHM2016.493–494); 8 specimens in ethanol (ZMBN 107242–107243), 12 specimens in ethanol (DBUA0001839.01).

**Diagnosis.** A *Paramytha* with 20 thoracic and up to 12 abdominal chaetigers.

**Description.** Holotype complete, with 20 thoracic and 12 abdominal chaetigers, 4.9 mm long and 0.6 mm wide in thorax. Other complete specimens are up to 7 mm long and 0.8 mm wide in thorax, with 20 thoracic and 11–12 abdominal chaetigers. Color in alcohol pale white. Prostomium and peristomium fused, not sub-divided in lobes, almost rectangular in shape with wide anterior, thickened margin (Fig. 1A–C, E). Prostomium without glandular ridges; nuchal organs and eyespots not observed. Buccal tentacles smooth, cylindrical, longitudinally grooved. Four pairs of branchiae; branchiae about 1/3–1/4 of body length, somewhat flattened, with longitudinal median furrow (Fig. 1A, C, E). Branchiostyles loosely attached to branchiophores, often lost. Branchiophores as distinct lobes firmly attached to body wall (Fig. 1A–C, E). Branchial arrangement 2 + 1 + 1 dorsally on segments II–IV (Fig. 4A); two anterior pairs arranged close together in transverse row connected by a basal membrane (Fig. 1B); 3rd pair with distinct median gap; 4th pair, in lateral positions dorsally to notopodia on segment IV (chaetigers 2). Innermost branchiae of anterior two pairs originates from segment II, outermost branchiae of anterior pairs originates from segment III, third pair originates from segment IV and posterior pair originates from segment V (Fig. 4A). Nephridial papillae not observed.

Body cylindrical, gradually tapering posteriorly, with thorax 3–4 times longer than abdomen (Fig. 1A). Segment II without chaetae (paleae). A total of 20 thoracic segments with notopodia and capillary chaetae, starting on segment III. Last 17 chaetigers of thorax with neuropodial tori with single row of uncini. Notopodia



**Fig. 4.** *Paramytha ossicola* sp. nov. (A) schematic figure indicating placement and origin of branchiae; (B) thoracic uncini, lateral view; (C) thoracic uncini, frontal view; (D) abdominal uncini, lateral view. (B–D) paratype, ZMBN 107241. Scale bars: (B–D) 10  $\mu$ m.

**Table 1**

Details of samples used in this study.

Deployment length	Bone samples	Preservation method	Bone surface area (cm <sup>2</sup> ) <sup>a</sup>	Population sample size (n)	Density (individuals · cm <sup>-2</sup> )
18 months	Bone B	Ethanol	459.06	87	0.19
	Bone C	Ethanol	259.47	46	0.18
28 months	Bone G	Formalin	485.34	1636	3.37
	Bone H	Ethanol	537.39	404	0.75

<sup>a</sup> Bone surface area from Génio et al. (2015).

as rounded lobes, about as high as wide (Fig. 1A, E), first one reduced. Anterior 2–3 notopodia in dorsal position, lateral to group of branchiae. Notopodia of chaetiger 4–6 gradually shifting to more lateral position; remaining notopodia in lateral position. All notochaetae hirsute with narrow brim (Fig. 2A), arranged in 1–2 rows with alternating long and short chaetae. Thoracic uncini with about 16 teeth arranged in 3–4 horizontal arcs above main rostrum and basal prow (Figs. 2B, 4B–C). Abdomen with up to 12 chaetigers; mid part of abdomen (about chaetigers 4–9) distinctly biannulate (Fig. 1A) with neuropodia placed on posterior annulus. Abdominal neuropodia gradually increasing in size forming pinules from about 4th abdominal chaetiger, without papillae or cirri. Abdominal neuropodia with dorsal thickened pads (Fig. 1D).

Abdominal uncini with more than 20 teeth arranged in 4–5 horizontal arcs above rostrum and basal prow (Figs. 1C–D, 4D). Anal opening terminal, surrounded with small papillae or tissue-folds; anal cirri absent. Tube flexible, with inner thin organic layer loosely incrustated with fine particular material (Fig. 3C).

Body generally deeply dyed in methyl blue; head region deeply stained except for two conspicuous spots on the posterior part (Fig. 2B); Thorax and abdomen generally with similar staining, on ventral shields and two transversal dorsal bands on each segment. Anterior three chaetigers (segments without tori) with particular strong staining laterally on segments (Fig. 2B). Branchiae, notopodia and neuropodia are not stained (Fig. 3A–B, D).



**Table 2**

Mean body length in  $\mu\text{m}$  ( $\mu$ ), standard deviations ( $\sigma$ ), and proportions ( $\pi$ ) of modal components estimated from the length–frequency distributions of *Paramytha ossicola* sp. nov. collected from bone B, G and H. Modal decomposition performed using MIXDIST. Chi-square statistic (between the observed and theoretical distributions) and degrees of freedom (no. grouping intervals – no. parameters – 1) for each distribution are also given.

Bone	$\chi^2$ (df)		Modal peaks		
			M1	M2	M3
Bone B	20.912 (7)	$\mu$	1.72	4.01	5.80
		$\sigma$	0.73	0.46	0.38
		$\pi$	0.54	0.36	0.09
Bone G	0.702 (7)	$\mu$	1.54	2.82	4.26
		$\sigma$	0.21	0.66	0.78
		$\pi$	0.37	0.58	0.20
Bone H	8.837 (6)	$\mu$	1.65	3.55	5.11
		$\sigma$	0.67	0.77	0.32
		$\pi$	0.41	0.59	0.00

**Etymology.** From Latin *ossicola*, living in/on bone; gender feminine.

**Habitat and distribution.** *Paramytha ossicola* sp. nov. is only known from the type locality, living on mammal bones.

**Remarks.** The original diagnosis of the genus *Paramytha* was based on the type species, *P. schanderi* from the Arctic Loki Castle hydrothermal vent field, as well as morphological observations on specimens of *P. ossicola* sp. nov. described herein (see Kongsrud et al., 2016). The two species are morphological similar in most respects, but differ inter alia in the number of thoracic and abdominal chaetigers. In *P. ossicola* sp. nov., 20 thoracic and up to 12 abdominal chaetigers are present compared to 15 thoracic and up to 20 abdominal chaetigers in *P. schanderi*. *P. ossicola* sp. nov. is also a smaller species and reaches only about 1/3 the body size of *P. schanderi*. The close evolutionary relationship between the two species referred to the genus *Paramytha* is supported by molecular data (Fig. 2 in Kongsrud et al., 2016, as *Paramytha* sp.).

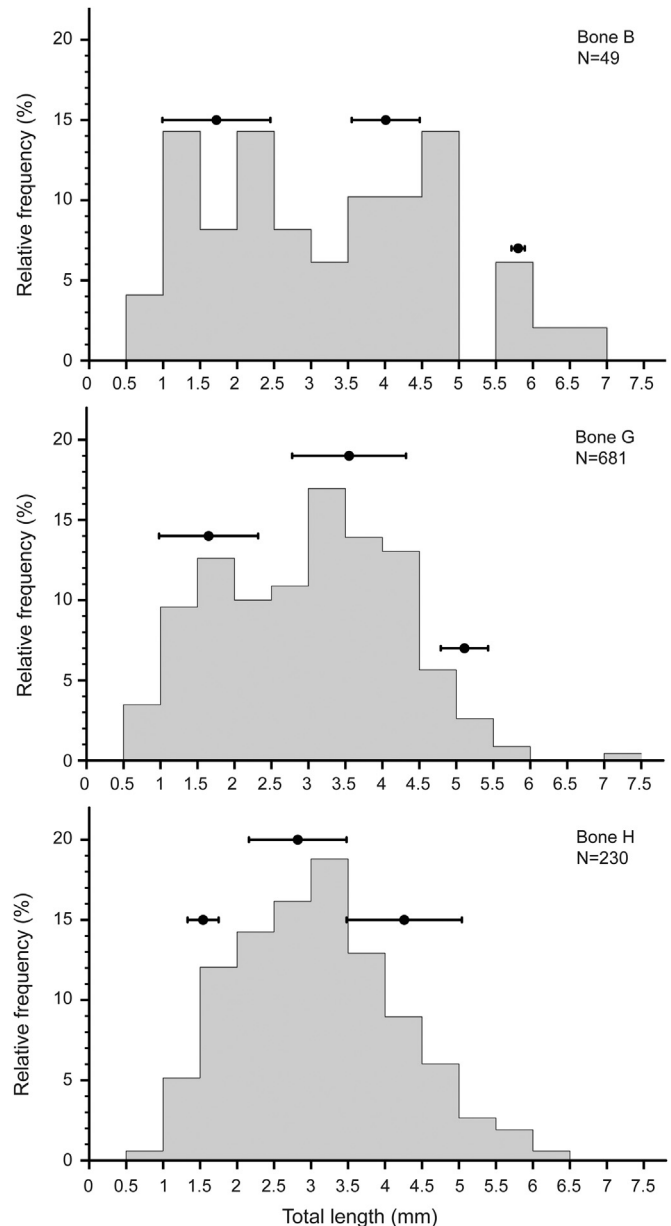
### 3.2. Population size and structure

A total of 133 and 2040 individuals were retrieved from bones collected on the first and second sampling times, respectively. Density values were higher 28 months after the deployment, with bone G presenting the highest density of *Paramytha ossicola* sp. nov. (3.37 individuals  $\text{cm}^{-2}$ ) (Table 1). The densities found in bones B, C and H are considerably smaller but still two orders of magnitude higher than those found for other species of Ampharetidae in mammal falls (Bennet et al., 1994).

Total lengths ranged from 0.79 to 7.1 mm for the bones collected after 18 months of carcass deployment, and from 0.68 to 7.1 mm for the 28-month bone samples. Length–frequency distributions were generated from measurements of the total length of 960 individuals collected from three of the sampled bones (bones B, G and H). Only 16 complete individuals could be retrieved from bone C and therefore this sample was not used for further analyses. Results of the Kolmogorov–Smirnov one-sample test show that all three distributions were not significantly different from the normal distribution ( $p < 0.0001$ ). However, modal composition analyses show a poly-modal structure in the three samples (Table 2, Fig. 5).

### 3.3. Reproductive biology

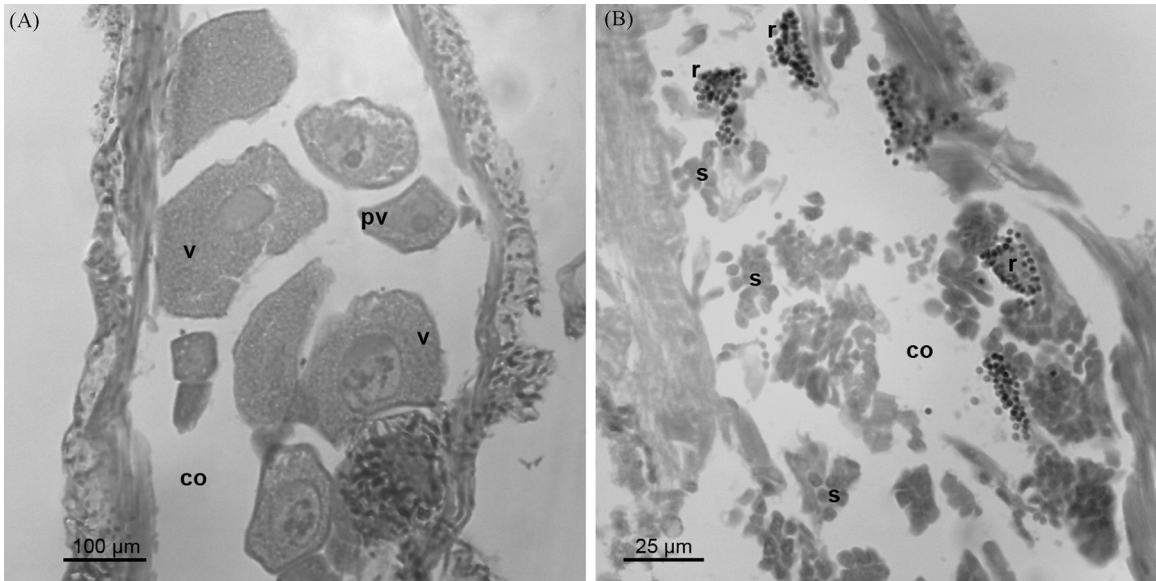
*Paramytha ossicola* sp. nov. is gonochoric and presents no sexual dimorphism. Sex could only be determined by histological examination and therefore only in individuals from bone G, the



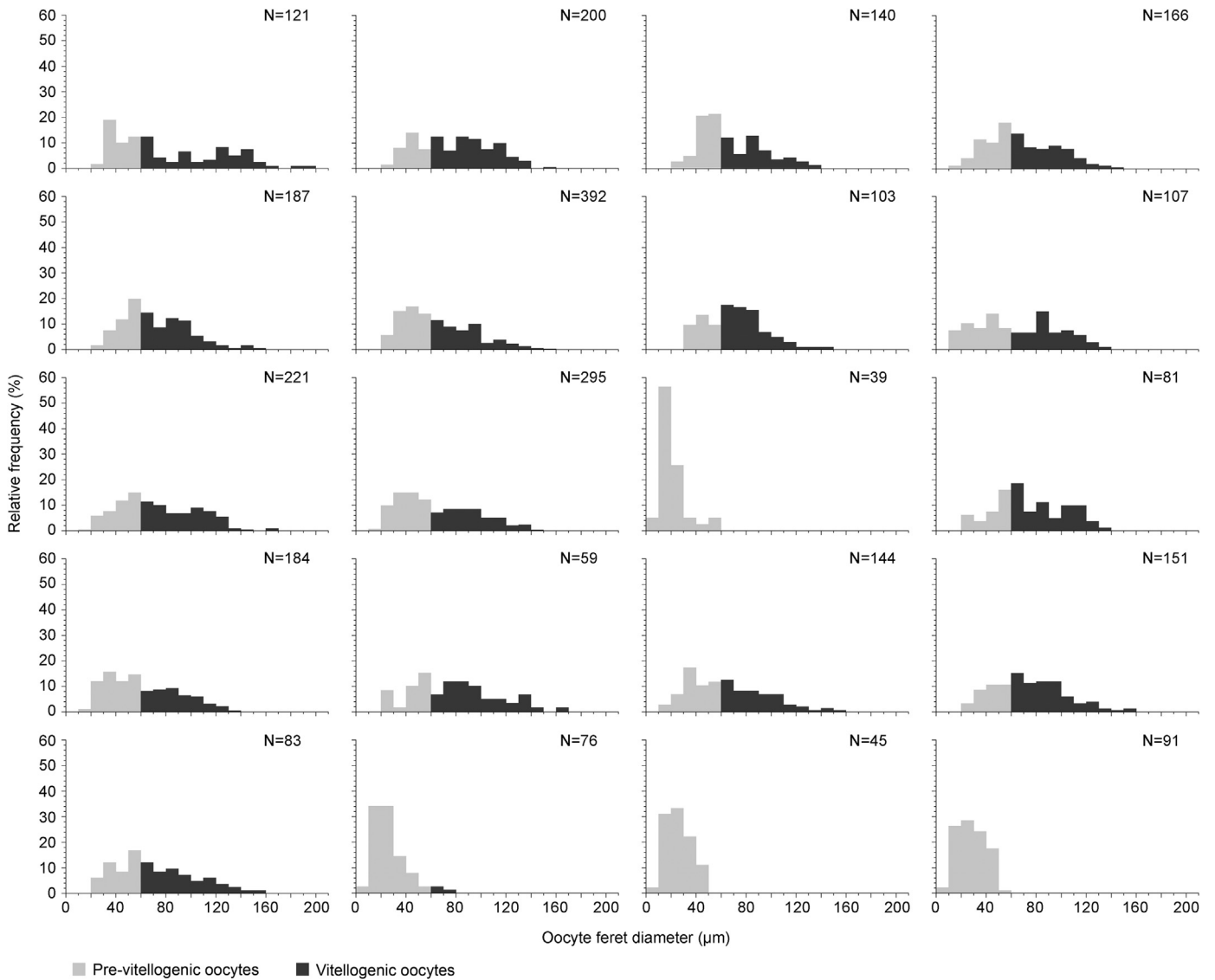
**Fig. 5.** Length–frequency distribution of *Paramytha ossicola* sp. nov. retrieved from bones B, G and H. Filled circles and error bars represent the mean lengths and standard deviation of modal components identified using MIX-DIST; *N* is the total number of individuals in the distribution.

only sample preserved in formalin, could be examined. In this bone, the sex ratio was significantly biased towards males, with 37 males and 20 females ( $\chi^2 = 3.07$ , 1 df,  $p < 0.0001$ ). Despite the absence of sexual dimorphism a significant difference ( $t$ -test,  $p = 0.007$ ) was found between the total length of males and females, the latter being larger. In both sexes, gametes are released into the anterior part of the coelomic cavity at an early stage and migrate posteriorly during maturation. Coelomic gametes were found in all females, the smallest of which was 2.2 mm, and in males larger than 2.9 mm.

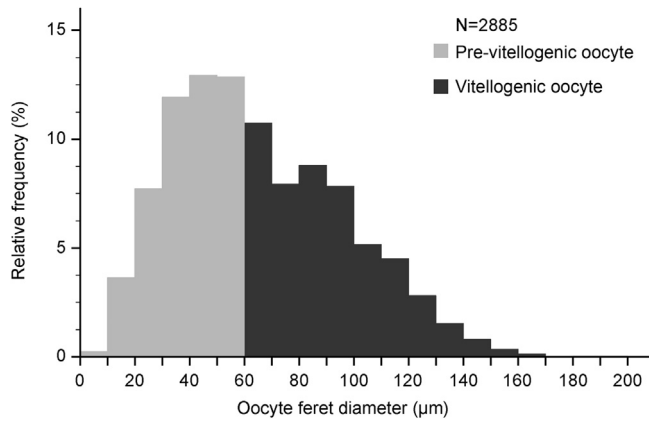
Oocytes leave the gonads when they are approximately 20  $\mu\text{m}$  and vitellogenesis begins when the oocytes measure approximately 60  $\mu\text{m}$  in diameter. Vitellogenesis was identified by the presence of yolk granules appearing in the cytoplasm. Early vitellogenic oocytes had a basophilic granular cytoplasm and mature oocytes an acidophilic cytoplasm completely granular in appearance (Fig. 6A). The observed maximum oocyte size was 190  $\mu\text{m}$ . The oocyte size–



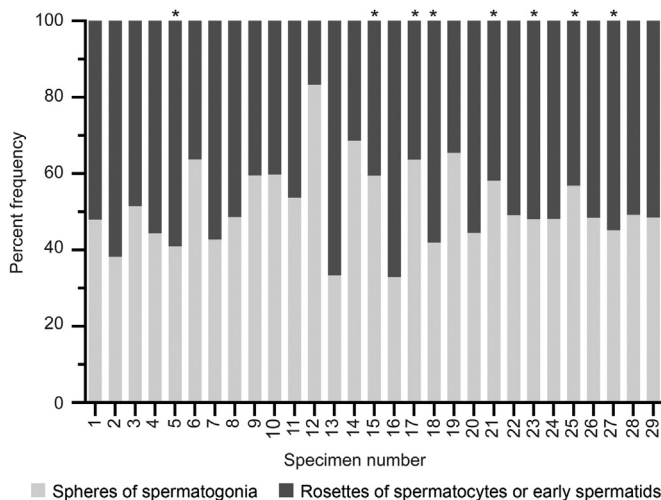
**Fig. 6.** Histological sections of (A) female and (B) male *Parsamyltha ossicola* sp. nov. co: coeloma; pv: pre-vitellogenic oocyte; r: rosettes of spermatocytes; s: spheres of spermatogonia; v: vitellogenic oocyte.



**Fig. 7.** Oocyte-size frequency distribution of each female. *N* is the total number of oocytes measured.



**Fig. 8.** Oocyte-size frequency distribution of all females. N is the total number of oocytes measured.



**Fig. 9.** Mean percent frequency of spermatogenic stages in 29 males of *Paramytha ossicola* sp. nov. Individual frequency bars are arranged from left to right according to increasing male size. \* Specimens in which tailed spermatids were observed.

frequency distributions show a wide range of oocyte sizes and vitellogenic stages (Figs. 7 and 8).

Spermatogenesis could be only analyzed in 33 male specimens, as the sections of four specimens were not good enough to quantify sperm development stages. In the smallest 4 males (total length between 1.6 and 2.5 mm) no sperm cells were visible in coelom. In the remaining 29 specimens spheres of spermatogonia ( $51.59 \pm 11.05\%$  SD) and rosettes of spermatocytes or early spermatids ( $48.41 \pm 11.05\%$  SD) were observed in the coelom (Fig. 6B); tailed spermatids could only be observed in 8 specimens but only in negligible quantities (Fig. 9).

#### 4. Discussion

Preliminary morphological analyses suggested that the ampharetid worm found living on cow bones in the Setúbal canyon (off the Portuguese coast) belonged to the species *Amphisamytha lutzi*, which is known from hydrothermal vents in the mid-Atlantic ridge. Molecular (Kongsrud et al., 2016) and morphological analyses revealed that it belongs to a new species of the genus *Paramytha*, described from Loki's Castle vent field in the Arctic mid-ocean ridge.

*Paramytha ossicola* sp. nov. is the second ampharetid worm reported to dwell in mammal bones and the first record from the Atlantic. Bone degradation processes occur progressively from the outside towards the center and are mediated mainly by anaerobic

microbial activities (Treude et al., 2009). The bone-eating worm *Osedax* can accelerate this process by eroding the bone using an acidifying mechanism (Tresguerres et al., 2013) and facilitate inner-bone colonization by microbes (Higgs et al., 2011). At this stage we have no data to support neither an active boring mechanism, nor that *P. ossicola* sp. nov. simply grows in available spaces created by other eroding processes. If, like *Osedax*, *P. ossicola* sp. nov. bores into the bone, this species, which is found in high numbers and densities, may have an equally important impact in the longevity of bones on the seafloor and on successional patterns of associated megafauna (Braby et al., 2007), and also on the marine vertebrate fossil record (Higgs et al., 2010).

Although mammal-falls have been well documented worldwide, the experimental fall in the Setúbal canyon is the only deep-sea mammal fall in the NE Atlantic that has been studied in detail (Hilario et al., 2015) and therefore it is possible that *P. ossicola* sp. nov. is a bone specialist restricted to the deep Atlantic Ocean. Despite its simultaneous occurrence with species typical from the sulphophilic phase of decomposition of mammal carcasses, fluorescent in situ hybridization analyses showed no evidence of the presence of chemoautotrophic symbionts. As most ampharetids, *P. ossicola* sp. nov. is likely a deposit- or detritus feeder exploiting the organic enrichment that results from the carcass decomposition. Further studies are required to determine if *P. ossicola* sp. nov. can use bones from other groups (e.g. fish) as substratum, which would decrease the space between suitable habitat and most likely have consequences in the species dispersal strategies.

Studies on reproductive biology and population dynamics of Ampharetidae have largely been restricted to shallow water species, which show that spawning does not occur until the worms are 2 years old and reproduce annually (Hutchings, 1973a,b; Oyenekan, 1988), and species from hydrothermal vents that suggest continuous reproduction (McHugh and Tunnicliffe, 1994; Blake and Van Dover, 2005). The reproductive anatomy of *Paramytha ossicola* sp. nov. is similar to that of almost all other ampharetid polychaetes that have been studied, including those from deep-sea hydrothermal vents, *Amphisamytha carldareii* (McHugh and Tunnicliffe, 1994) and *A. lutzi* (Blake and Van Dover, 2005): it is gonochoric, without external sexual dimorphism, with gametes that develop freely in the coelom. Although females were significantly larger than males, which can be an evidence of protandric hermaphroditism, no further evidence of hermaphroditism was found. The presence of gametes in small worms indicates that maturity is reached at a relatively early stage in the life cycle, which is a trait that has been observed in other invertebrates from organic-falls (e.g. Gaudron et al., 2012; Turner, 1973; Tyler et al., 2007; Young et al., 2013) and ampharetids from hydrothermal vents (McHugh and Tunnicliffe, 1994; Blake and Van Dover, 2005). Rapid maturation can be interpreted as an adaptation to their deep-sea ephemeral habitat.

The occurrence of a full range of oocyte sizes and different stages of oogenesis and spermatogenesis suggests that reproduction in *Paramytha ossicola* sp. nov. is continuous both on the population and individual levels. Oocyte size distributions in periodic or synchronous spawners are expected to show a single cohort (reviewed by Young et al. (2003)). The maximum oocyte diameter in *P. ossicola* sp. nov. (190 µm) is similar to that of other ampharetids that have lecithotrophic development (Blake and Van Dover, 2005; Stiller et al., 2013; Zottoli, 1983). Based on this similarity it is here hypothesized that *P. ossicola* sp. nov. produces lecithotrophic larvae. Lecithotrophic larvae tend to have lower dispersal potential than planktotrophic larvae, which could limit the ability to colonize insular habitats, such as mammal falls. However, mechanisms like the development arrest of embryos or delay metamorphosis until a suitable substratum can be found as been demonstrated in several deep-sea species, including a



terebellomorph polychaete (Pradillon et al., 2001). The need for long development arrests depends in part on the probability of encountering a suitable substratum; Smith and Baco (2003) estimated that, at any given time, there may be one whale-skeleton every 12–30 km at the deep-sea floor but, if *P. ossicola* sp. nov. is able to exploit other substrata, dispersal distances may be shorter than that.

Most studies examining population structure in deep-sea reducing environments have concluded a discontinuous recruitment pattern based on the presence of distinct peaks in size–frequency distributions (e.g. McHugh, 1989; Zal et al., 1995; Kelly et al. 2008). Although the reproductive mode of *P. ossicola* sp. nov. could suggest continuous recruitment, the populations sampled at both sampling times show polymodal structures characteristic of discontinuous recruitment. This discrepancy may be explained by a significant temporal variation in habitat availability that may result in post-settlement mortality. Hilario et al. (2015) estimated that the carcass bones were exposed for 2–16 months before the first samples were collected, but most likely neither every bone, nor all the surface of the same bone, became available at the same time. Also, depending on the ability of *P. ossicola* sp. nov. to actively degrade the bone, suitable habitat may or may not depend on abiotic and biotic factors controlling bone degradation. Further, biological interactions, both inter- and intraspecific, may also regulate *P. ossicola* sp. nov. colonization. Being a sessile deposit feeder, competition for substratum space and/or food resources could result in post-settlement mortality. However, further studies, with a high temporal resolution are needed to fully understand the factors responsible for the polymodal size–frequency distributions herein observed.

In all samples the distributions show well separated groups, and at the second sampling time, 28 months after the deployment of the carcasses, the distributions show that the size groups are almost equally spaced. This indicates that individual growth rate and the population turnover are high enough to prevent modal groups overlapping. Rapid growth, early reproduction and effective dispersal mechanisms are characteristic of opportunistic species inhabiting ephemeral habitats, as these traits are essential to colonize new habitats before their local habitat is extinguished (reviewed by Young (2003)). It is likely that *P. ossicola* sp. nov., apart from the reproductive attributes (discussed above), may also display the high growth rates necessary to employ an opportunistic life-history strategy.

## 5. Conclusions

*Paramytha ossicola* sp. nov. is a new species of the polychaete family Ampharetidae. Although ampharetids are commonly found in the periphery of whale falls, this is only the second record of an ampharetid worm found dwelling on mammal bones. *P. ossicola* sp. nov. is most likely a deposit feeder exploiting the organic enrichment that results from the carcass decomposition, but possibly also the enhanced flow conditions on the bones. Further research is necessary to understand whether *P. ossicola* sp. nov. bores into the bone and consequently the role of this species in the ecology of whale-fall communities and bone taphonomy.

The life history of *Paramytha ossicola* sp. nov. is similar to most of its deep-sea relatives and is seemingly adapted for life in ephemeral habitats. Reproductive traits include rapid maturation, continuous and non-synchronous gametogenesis, non-feeding and demersal larvae. Size–frequency analyses revealed a polymodal demographic structure typical of discontinuous recruitment. We propose that, despite continuous reproduction, recruitment is controlled by habitat availability and biological interactions that result in post-settlement mortality.

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