Lamellibrachia anaximandri n. sp., a new vestimentiferan tubeworm (Annelida) from the Mediterranean, with notes on frenulate tubeworms from the same habitat

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ABSTRACT

A new species of lamellibrachiid vestimentiferan, *Lamellibrachia anaximandri* n. sp., has been found in the Eastern Mediterranean, close to cold seeps of fluid carrying dissolved methane and sources of sulfide in superficial sediments. It occurs at about 1100 to 2100 m depth, on some of the mud volcanoes on the Anaximander Mountains, south of Turkey, on the Mediterranean Ridge, south of Crete, and on the Nile deep-sea fan. In addition, it has been obtained from rotting paper inside a sunken ship, torpedoed in 1915 and lying at 2800 m depth, southeast of Crete. Some frenulate pogonophores also occur on the mud volcanoes (including a species of *Siboglinum* resembling *S. carpinei* and tubes of other unidentified genera). The new *Lamellibrachia* is the first vestimentiferan species to be described from the Mediterranean. It differs from *L. luymesi* taken from the Gulf of Mexico population in the very weak development of collars on its tube and in having a smaller number of pairs of branchial lamellae in the branchial plume. Sequencing of the COI and the mt16S genes confirms a difference at the species level between the new species and *L. luymesi*, and a

KEY WORDS
Annelida,
Pogonophora,
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new species.

difference between these two species and four described species of *Lamellibrachia* from the Pacific Ocean. The largest individuals of *L. anaximandri* n. sp. may be many years old, but there are numerous young individuals at some sites, showing that favourable conditions are available for settlement and early growth. The development of the branchial plume in a series of young stages reveals that the sheath lamellae, which are characteristic of the genus *Lamellibrachia*, begin to form only after the establishment of several pairs of branchial lamellae. Examination of the adult trophosome by transmission electron microscopy shows Gram-negative bacteria without internal stacked membranes, indicating that the symbionts are most probably sulfide oxidizing.

RÉSUMÉ

Lamellibrachia anaximandri *n. sp., un nouveau ver vestimentifère (Annelida) de Méditerranée, avec des remarques sur les vers frénulés du même habitat.*
Une nouvelle espèce de vestimentifère lamellibrachiidé, *Lamellibrachia anaximandri* n. sp. a été trouvée en Méditerranée orientale à proximité de suintements froids riches en méthane et de sources de sulfures dissous dans les sédiments superficiels. Elle vit à une profondeur d'environ 1100 à 2100 m, sur certains volcans de boue de la chaîne d'Anaximandre au sud de la Turquie, sur la ride méditerranéenne au sud de la Crète, et dans l'estuaire profond du Nil. Elle a d'autre part été trouvée dans du papier putréfié à l'intérieur d'un bateau coulé, torpillé en 1915 et gisant par 2800 m de fond au sud-est de la Crète. Quelques pogonophores frenulés sont aussi présents au niveau des volcans de boue (dont une espèce de *Siboglinum* ressemblant à *S. carpinei* ainsi que des tubes d'autres genres non identifiés). Le nouveau *Lamellibrachia* est la première espèce de vestimentifère décrite de Méditerranée. Il diffère de *L. luymesi* de la population du Golfe du Mexique par le très faible développement des annulations de son tube et par un plus petit nombre de paires de lamelles branchiales de son panache branchial. Le séquençage des gènes COI et mt16S confirme la différence spécifique entre cette nouvelle espèce et *L. luymesi*, ainsi qu'une différence entre ces deux espèces et les quatre espèces de *Lamellibrachia* décrites de l'océan Pacifique. Les plus grands individus de *L. anaximandri* n. sp. sont peut-être très vieux, mais la présence de nombreux jeunes individus sur certains sites montre que les conditions actuelles sont favorables à son recrutement et à sa croissance initiale. Le développement du panache branchial dans une série de jeunes stades révèle que les gaines lamellaires enveloppantes, qui sont caractéristiques du genre *Lamellibrachia*, commencent à se former seulement après le déploiement de plusieurs paires de lamelles branchiales. L'examen du trophosome de l'adulte en microscopie électronique à transmission montre des bactéries Gram-négatives sans membranes internes empilées, indiquant que les symbiontes sont probablement sulfo-oxydants.

MOTS CLÉS
Annelida,
Pogonophora,
Siboglinidae,
Lamellibrachia,
suintement froid,
volcan de boue,
assemblages chimiosynthétiques,
sulfure,
méthane,
Méditerranée,
espèce nouvelle.

INTRODUCTION

Vestimentiferans were discovered in the Mediterranean on a mud volcano of the Anaximander mountains (Fig. 1) in 1996 (Woodside *et al.* 1997) and more specimens were collected at similar sites in 1998, 2003 and 2007 (Olu-Le Roy *et al.* 2004; Bayon *et al.* 2009). These tubeworms live at a normal Mediterranean deep-water temperature of about 13°C, in situations where mud and fluid are expelled

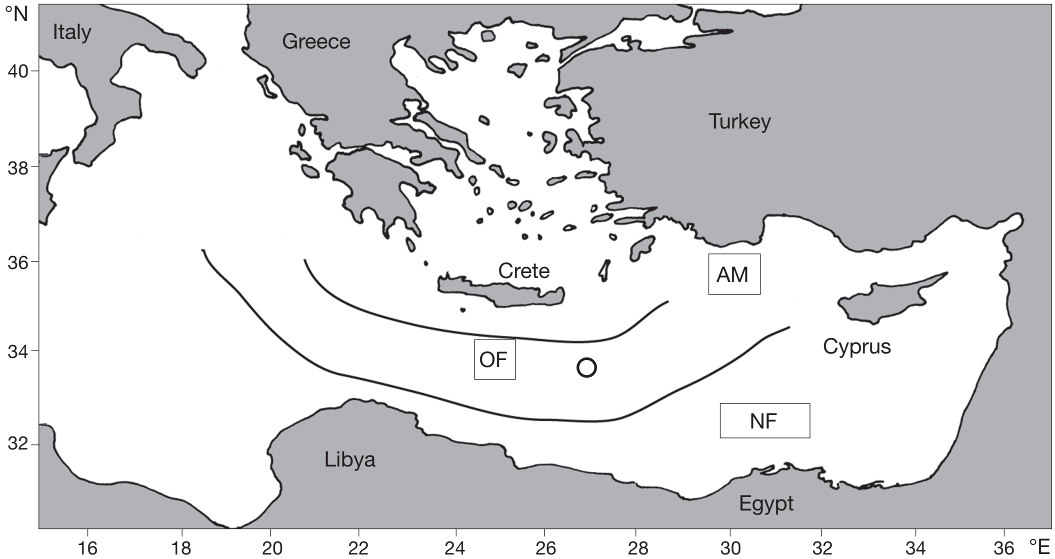


FIG. 1. — Map of the eastern Mediterranean Basin, showing the position of the Mediterranean Ridge (black lines), the three areas studied (**AM**, Anaximander Mountains; **NF**, Nile fan; **OF**, Olimpi Field) and the approximate position of S.S. *Persia* shipwreck (○).

from the seafloor. Tubeworms of various sizes have been found, including many young specimens, the latter usually attached to solid surfaces but occasionally on older tubes of their own species. In 2003, vestimentiferans were found inside the wreck of the S.S. *Persia* about 110 km southeast of Crete, lying at 2800 m depth (Hughes & Crawford 2006).

Although some well-known vestimentiferans live in warm sulfidic environments near deep-sea hydrothermal vents, for example *Riftia pachyptila* Jones, 1981, members of genera such as *Lamellibrachia* and *Escarpia* live where methane-rich cool fluid is expelled from the underlying rocks or sediments, resulting in high sulfide levels in the superficial sediments, but lower concentrations of dissolved sulfide in seawater than in hot vent fluids (Hecker 1985; Jones 1985; Suess *et al.* 1985; Julian *et al.* 1999; Bergquist *et al.* 2002). In both situations the vestimentiferans rely for nutrition on internal chemoautotrophic bacteria that can oxidize sulfide and fix carbon dioxide.

In the Pacific, *Lamellibrachia barhami* Webb, 1969 lives at cold seeps along the continental margin of North America, from British Columbia to Costa Rica (Webb 1969, 1977; McMullin *et al.* 2003; Mau *et al.* 2006) and also in cool conditions at a sediment covered hydrothermal site, Middle Valley, on the Juan de Fuca Ridge (Juniper *et al.* 1992, fig. 7A), while *L. columna* Southward, 1991 lives in cool conditions near hydrothermal vents in the Lau Basin in the southwest Pacific. *Lamellibrachia satsuma* Miura, Tsukahara & Hashimoto, 1997 occurs off southern Japan and at other hydrothermal sites as far south as the Mariana Trough (Fig. 2). It lives at 82 to 430 m depth, in warm conditions, at 12 to 16°C (Miake *et al.* 2006). *Lamellibrachia juni* Miura & Kojima, 2006 was described recently from a hydrothermal site, at Brothers Caldera in the Kermadec Arc, north of New Zealand. Material of apparently the same species has also been collected from the Manus Basin and the South Mariana Volcanic Arc (Kojima *et al.* 2006; Fujikura *et al.* 2008). There is also a report of a *Lamellibrachia* species at cold seeps east of New Zealand (Baco *et al.* 2010). Analysis of the COI gene of Pacific *Lamellibrachia* specimens has been used to distinguish *L. barhami*, *L. columna*, *L. satsuma* and *L. juni* from one another (Kojima *et al.* 2001; McMullin *et al.* 2003) and to indicate that there are at least two undescribed species of *Lamellibrachia* in the western Pacific (Fig. 2).

FIG. 2. — Geographical distribution of *Lamellibrachia* species: *L. anaximandri* n. sp. (○), *L. barhami* Webb, 1969 (△), *L. columna* Southward, 1991 (□), *L. juni* Miura & Kojima, 2006 (■), *L. luymesi* Van der Land & Nørrevang, 1975 (●), *L. satsuma* Miura, Tsukahara & Hashimoto, 1997 (▲), *L. victori* Mañé-Garzón & Montero, 1985 (☆) and unnamed species (★).

In the Atlantic Ocean, records of *Lamellibrachia* are fewer. *Lamellibrachia luymesi* Van der Land & Nørrevang, 1975 was described from a single specimen trawled at 500 m depth off Guyana (Van der Land & Nørrevang 1975, 1977) and *L. victori* was described from two specimens dredged at 300 m depth off Uruguay (Mañé-Garzón & Montero 1985). A large population of vestimentiferans occurs at hydrocarbon seeps in 550 to 650 m depth in the northern Gulf of Mexico (MacDonald *et al.* 1989; Bergquist *et al.* 2002; Cordes *et al.* 2006) (Fig. 2). Two species are present: one is an escarpiid named *Seepiophila jonesi* Gardiner, McMullin & Fisher, 2001 (Gardiner *et al.* 2001), the other, referred to as "*Lamellibrachia* sp." in many publications, has now been identified as the Guyana species, *L. luymesi*, by Gardiner & Hourdez (2003). Two unnamed species, referred to as Gulf of Mexico *L.* sp. 1 and sp. 2 have been found on the lower Louisiana slope at depths greater than about 950 m (Cordes *et al.* 2007; Miglietta *et al.* 2010). The status of *L. victori* is uncertain and genetic investigation is needed to confirm its distinctiveness from *L. luymesi*.

There is only one record of *Lamellibrachia* from the eastern margin of the Atlantic, off northern Spain (Fig. 2). Vestimentiferan tubes were recovered in 1991 from the rotting organic cargo of a ship named the *François Vieljeux*, which sank in 1979 and lies in 1160 m depth (Dando *et al.* 1992). The tubes contained decayed worm tissue, from which sufficient DNA was extracted to allow the animal to be identified as a member of the genus *Lamellibrachia* (Williams *et al.* 1993).

The new species of *Lamellibrachia* described here comes from living adult samples collected at depths ranging from 1148 to 2132 m. Juveniles have also been found at 3014 m depth (Table 1). It has been found on mud volcanoes of the Mediterranean Ridge and the Anaximander Mountains, the latter being detached continental blocks located between the coast of Turkey and the eastern end of the Mediterranean Ridge (Fig. 1). This ridge is an accretionary prism of compressed seafloor sediments, produced during the subduction of the African tectonic plate under the European plate. Gas-rich mud and breccia are expelled under pressure to form mounds termed mud volcanoes (Limonov *et al.* 1996; Charlou *et al.* 2003; Dmitrov & Woodside 2003; Zitter *et al.* 2005).

The ANAXIPROBE expedition in 1996 (Woodside *et al.* 1997, 1998) found a large piece of perforated carbonate concretion, with white tubes more than 20 cm long projecting from it, dredged in a region of active gas venting (Station 209D-8 of the ANAXIPROBE expedition). The sample was taken in a dredge hauled up-slope from 1854 to 1165 m depth. Part of a worm, in a fragment of tube, was identified as a lamellibrachiid vestimentiferan, the first to be collected in the Mediterranean (Woodside *et al.* 1997). In 1998 both the Anaximander region and the Olimpi Mud Field, south of Crete, were studied by the participants in the MEDINAUT expedition (Pancost *et al.* 2000; Huguen *et al.* 2001). This expedition used the research submersible *Nautile* to photograph and collect the benthic fauna from mud volcanoes and a fault escarpment on the Anaximander Mountains, and also from the Olimpi mud volcano field south of Crete, at depths of 1165 to 2030 m (Fig. 1; Table 1). Chemosynthetic communities dominated by small bivalves were found on six mud volcanoes and a fault scarp in the Anaximander and Olimpi regions (Olu-Le Roy *et al.* 2004). *In situ* photographs show vestimentiferan tubes emerging from cracks and crevices among tumbled rock fragments (Fig. 3). Some sediment samples contained frenulate pogonophores. In 2003, mud volcanoes and seeps on the Nile Deep Sea Fan were explored by the NAUTINIL expedition (Foucher *et al.* 2004). Here, the submersible *Nautile* collected vestimentiferans from depths of 1148 to 2130 m. The MEDECO Expedition in 2007 visited the Olimpi Field, the Anaximander Mountains and the Nile deep-sea fan (Bayon *et al.* 2009). More *Lamellibrachia* were collected in all three areas, and juveniles were found on carbonate concretions collected at about 3014 m in the Nile deep-sea fan's western province around Cheops mud volcano (Huguen *et al.* 2009), where no adults were visible.

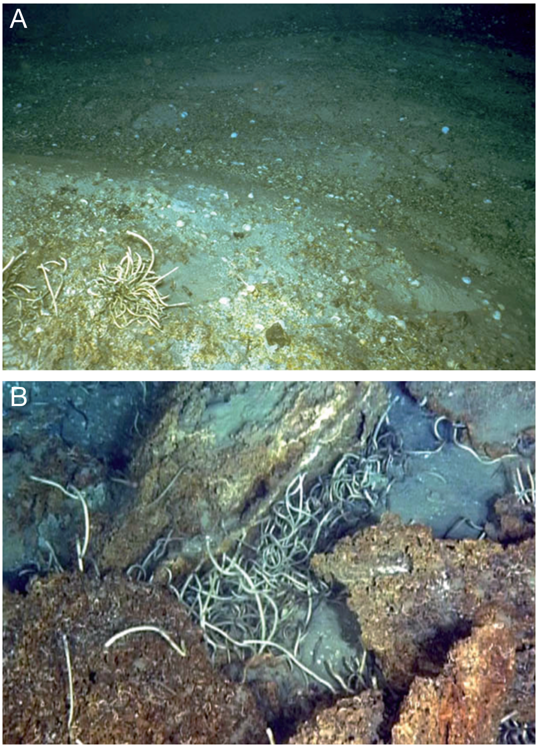


Fig. 3. — *In situ* photographs of *Lamellibrachia anaximandri* n. sp. in the Anaximander Mountain region (courtesy of IFREMER): **A**, at Kazan mud volcano, a gravel and mud situation, with bivalve shells scattered on the surface; the cluster of tubes appears to be growing upward from the sediment; **B**, at Amsterdam mud volcano, the tubes emerge from crevices between broken rocks.

In 2003, vestimentiferans were found inside the wreck of the S.S. *Persia*, a passenger ship torpedoed in 1915, during the First World War. The salvage operations were carried out by Alec and Moya Crawford, who had earlier salvaged the cargo of the *François Vieljeux*. They found the *Persia* about 110 km southeast of Crete, at 2800 m depth (Fig. 1). The vestimentiferans were collected during salvage operations (Hughes & Crawford 2006).

For the present paper we have examined specimens from all these sites, we describe their morphology and use a COI and 16S mitochondrial phylogeny to assess the position of the Mediterranean *Lamellibrachia* species among others of this worldwide genus.

TABLE 1. — Samples of Mediterranean Pogonophora from cruises ANAXIPROBE-1996, MEDINAUT-1998, NAUTINIL-2003 and MEDECO-2007.

Site name/ mud volcano	Cruise	Year	Latitude N	Longitude E	Depth (m)	Dive No.	Sample	Vestimentifera	Frenulata
Anaximander Mountains									
Amsterdam MV	MEDINAUT	1998	35°20’	30°16’30	2030	MN8	BT1	*Lamellibrachia anaximandri* n. sp.	–
							BT4	*L. anaximandri*	*Siboglinum* cf. *carpinei*
	MEDECO	2007	35°20.0902	30°16.2974	2025	MD334-13	CL9	*L. anaximandri*	–
			35°20.0928	30°16.2989	2023	MD334-13	CL10	*L. anaximandri*	–
Faulted Ridge	MEDINAUT	1998	35°26.5 to 35°27.3	30°24.7 to 30°24.5	1650-1300	MN9	BT7	*L. anaximandri*	–
							BT8	*L. anaximandri*	–
							Stat2	*L. anaximandri*	–
								L. anaximandri	–
	ANAXIPROBE	1996	35°25.9 to 35°27.1	30°24.55 to 30°24.61’	1854-1165	—	209D	*L. anaximandri*	–
Kazan MV	MEDINAUT	1998	35°26’	30°33’30	1700	MN10	BT1	*L. anaximandri*	*Siboglinum* cf. *carpinei*
			35°26’	30°33’30	1700	MN12	BT2	*L. anaximandri*	–
							BT9/10A	*L. anaximandri*	–
Kula MV	MEDINAUT	1998	35°44’	30°27’30	1630	MN11	BT	*L. anaximandri*	–
Olimpi Field									
Napoli MV	MEDINAUT	1998	33°43’30	24°41’	1945	MN4	BT	*L. anaximandri*	–
			33°43’30	24°41’	1945	MN6	BT2	*L. anaximandri*	–
							CT3	–	*Siboglinum* sp.
	NAUTINIL	2003	33 °5.71	24°31.29	1948	NL21	BIO1	*L. anaximandri*	–
			33°35.71	24°31.29	1948	NL21	BIO2	*L. anaximandri*	–
			33°43.580	24°41.086	1939	NL22	CC1	*L. anaximandri*	–
			33°43.608	24°41.075	1939	NL22	CC2	*L. anaximandri*	–
	MEDECO	2007	33°43.7496	24°40.9425	1943	MD 331-10	CL4	*L. anaximandri*	–
Milano MV	MEDINAUT	1998	33°44’	24°47	1955	MN3	BC	–	*Siboglinum* cf. *carpinei*
							BT2	*L. anaximandri*	–
			33°44’	24°47	1955	MN5	BT4	*L. anaximandri*	*Siboglinum* cf. *carpinei* and *Siboglinum* sp.
							CB2	*L. anaximandri*	–
Nile deep-sea fan									
Central pockmark area	NAUTINIL	2003	32°38.389	29°54.875	2132	NL6	CC1	*L. anaximandri*	–
			32°38.499	29°55.367	2129	NL6	BIO1	*L. anaximandri*	–
			32°31.603	30°20.657	1692	NL7	CC1	*L. anaximandri*	–
			32°31.603	30°20.657	1692	NL7	BIO1	*L. anaximandri*	–
			32°38.442	29°54.990	2129	NL14	BIO1	*L. anaximandri*	–
			32°38.307	29°54.952	2130	NL14	BIO2	*L. anaximandri*	–
	MEDECO	2007	32°30.0305	30°15.6046	1686	MD-336-15	CC1	*L. anaximandri*	–
			32°32.0574	30°21.3645	1694	MD 337-16	CC1	*L. anaximandri*	–
			32°32.1554	30°21.1925	1699	MD 339-18	Biobox 4	*L. anaximandri*	–
			32°32.1012	30°21.0738	1701	MD 339-18	Biobox	*L. anaximandri*	–
Western province Cheops MV	MEDECO	2007	32°08.4823	28°09.7025	3014	MD 343-22	Biobox	*L. anaximandri* juveniles	–
East delta	NAUTINIL	2003	32°21.905	31°42.654	1163	NL11	BIO1	*L. anaximandri*	–
			32°22.074	31°42.167	1148	NL11	BIO2	*L. anaximandri*	–

MATERIAL AND METHODS

The ANAXIPROBE project was combined with the "International Training through Research" program (TTR-6) aboard the Russian research vessel *Gelendzhik* in 1996, under Dutch-Russian supervision (Woodside *et al.* 1997). MEDINAUT was part of a Dutch-French program, aboard the French Research Vessel *Nadir*, using the research submersible *Nautile*. The geological setting, sampling methods and ecology were described by Olu-Le Roy *et al.* (2004) and Zitter *et al.* (2005). About 60 vestimentiferan specimens and numerous tube fragments were examined, as well as 5 samples of small frenulates (Table 1).

The NAUTINIL expedition was part of a European Science Foundation program named MEDIFLUX (part of EUROMARGIN). It took place in 2003 on the French Research Vessel *L'Atalante* (Foucher *et al.* 2004). Loncke *et al.* (2004) have described the geological and geophysical background of the seeps on the Nile margin. Vestimentiferans were obtained from 4 sites on the Nile Fan and some also from Napoli and Milano mud volcanoes in the Olimpi region (Table 1). Nine animals from the Nile fan have been examined.

The MEDECO Expedition took place in 2007 as part of the European program named HERMES (Hotspot Ecosystem Research on Margins of European Seas). A remotely operated vehicle (ROV) was used to investigate the mud volcanoes Napoli and Amsterdam, and two areas of the Nile Fan. Samples, photographs and measurements of living vestimentiferans were obtained from all areas.

Some of the contents of the S.S. *Persia* mail-room were salvaged by Alec and Moya Crawford, of the salvage company "Deep Tek", using a remote sensing grab (Hughes & Crawford 2006). Specimens of the vestimentiferans were loaned by Dr David Hughes. Parts of three animals and several tube fragments have been examined.

Most specimens from the various sites were preserved in 3.7% formalin and transferred later to 70% ethanol. The specimens sent to Plymouth for identification were collected at the sites listed in Table 1. The tubes were measured and photographed, then cut open. The animals were examined and measured under a dissecting microscope. Photographs were taken with a digital camera. Some branchial filaments, pieces of epidermis with cuticular plaques, parts of the oviducts, sperm ducts, and the trophosome were placed on slides, in 50% glycerol, under coverslips, for examination at higher magnifications. Measurements of the sizes of epidermal plaques, bacteria and spermatozoa were made using a micrometer eyepiece. Specimens from the NAUTINIL expedition were measured using a vernier calliper, photographs were taken with a digital camera.

TRANSMISSION ELECTRON MICROSCOPY

Trophosomal tissue was dissected and fixed in glutaraldehyde during the NAUTINIL cruise from tubeworms taken in the centre of the Nile Deep-Sea Fan (NL6). The fixed samples were postfixed at Roscoff in 1% osmium tetroxide in cacodylate buffer 0.2M pH 7.4, embedded in Epon 812 resin, sectioned at 60 nm, and contrasted as previously described (Andersen *et al.* 2002). Sections of the trophosomal bacteriocytes of two individuals from the NL6 dive in the centre of the Nile deep-sea fan (32°30.42N, 29°46.05E, 1600 m depth) were observed with a JEOL SX 1200 transmission electron microscope (TEM).

TYPE SPECIMENS

These have been deposited in the Muséum national d'Histoire naturelle, Paris and the Natural History Museum, London.

COMPARATIVE MATERIAL

One specimen of *Lamellibrachia luymesi* from 450 m depth at Green Canyon in the Gulf of Mexico was provided by Dr S. L. Gardiner. Tubes of *Lamellibrachia* sp. from the *François Vieljeux* wreck, collected during salvage operations by Moya and Alec Crawford, were provided by Dr David Dixon.

MOLECULAR METHODS

The animals used for molecular studies were collected during various dives at three different locations in the Mediterranean (Table 2). Pieces of tissue were frozen or preserved on board in 95% ethanol. After a quick drying of the tissue by pressing between

TABLE 2. — Specimens of *Lamellibrachia anaximandri* n. sp. used for molecular studies, collection sites and depths.

Site name/mud volcano	Cruise	Year	Latitude N	Longitude E	Depth (m)	Dive No.	Number of individuals	Gene	Accession number
Anaximander Mountains									
Amsterdam MV	MEDECO	2007	35°20,090’	30°16.297’	2025	Victor 334	2	mt16S	HM746775-76
Olimpi field									
Napoli MV	MEDECO	2007	33°43.750’	24°40.942’	1943	Victor330	5	mt16S	HM746777-81
Nile deep-sea fan									
Central pockmark area	NAUTINIL	1993	32°38.442	29°54.990	2129	NL14	1	mtCOI	EU0466616
	MEDECO	2007	32°30.031’	30°15.605’	1686	Victor 336	3	mt16S	HM746782-84

two layers of paper towel, the samples (*c.* 50 mg) were digested in CTAB-PVP buffer (Cullings 1992; Doyle & Doyle 1987). The tissues were digested overnight at 55°C in this buffer and the product was centrifuged 5 min at 5000 g to remove large debris. DNA was then separated from the digestion product following Doyle & Doyle 1987’s protocol.

A portion of the mitochondrial gene coding for the cytochrome oxidase subunit I (COI) was amplified using the “universal” primers HCOI and LCOI designed by Folmer *et al.* (1994). As this amplification proved difficult, we also amplified a 524 bp fragment of the mitochondrial ribosomal 16S gene using primers 16Sar and 16Sbr (Palumbi *et al.* 1991).

The optimal PCR cycling parameters were 1 cycle: 3 min/96°C; 35 cycles: 1.15 min/50°C, 1 min/72°C, 1 min/96°C; 1 cycle: 2 min/50°C, 10 min/72°C. For the COI fragment, the product was cloned using the TOPO-TA cloning for Sequencing kit (Invitrogen) according to the manufacturer’s directions, and both strands of the insert sequenced using primers on the vector. For the 16S fragment, the amplification product was treated with EXOSAP-IT (USB) and then both strands directly sequenced with the primers used for the initial amplification. Sequencing was performed with the Big Dye® Terminator V3.1 Cycle Sequencing kit (Applied Biosystems). Reactions were subsequently run on a 16-capillary 3130 Applied Biosystems sequencer.

The two sequence strands for each individual were assembled and proof-read in CodonCode Aligner to generate a continuous fragment. Sequences from representative species that inhabit cold seeps have been chosen and, when possible, animals from different locations spanning the whole distribution range of the species were selected. *Riftia pachyptila*, a hydrothermal vent vestimentiferan was chosen as an outgroup. The sequences were aligned manually with previously published sequences (for accession numbers, see Figures 14 and 15). After removal of the primers and trimming of the 5’ and 3’ ends to accommodate the shorter sequences from the databases, the final alignment was 536 bp long for the mitochondrial COI sequences, and 461 bp for the mitochondrial 16S sequences.

PHYLOGENETIC METHODS

Two different approaches were used to produce phylogenetic trees. In the first method, the tree was constructed by Neighbour Joining, on Kimura-2-Parameter distances. Bootstrap values were calculated on 1000 re-sampling replicates. To possibly improve confidence on the branching pattern, we determined the best nucleotide substitution model with jModelTest (Posada 2008). The best model based on maximum likelihood (BIC criterion) was determined to be HKY+I+G for mtCOI (HKY method, with a portion of invariant sites and the mutation rate among sites following a gamma distribution), and TIM2+G for mt16S (TIM2 model with the mutation rate among sites following a gamma distribution). These parameters were used as priors for a Bayesian determination of the phylogenies with MrBayes V3.1.2 (Ronquist & Huelsenbeck 2003). Iterations were run for 200 000 generations (sufficient to reach convergence of the two parallel runs), with a burn-in of 1000 generations. Sampling of the tree every 1000 generations allowed us to determine a consensus tree and to determine posterior probabilities for each branch.

Abbreviations

Repositories

MNHN	Muséum national d'Histoire naturelle, Paris;
NHM	Natural History Museum, London.

Cruise names (see station details in Table 1)

MD	MEDECO;
MN	MEDINAUT;
NL	NAUTINIL.

Other abbreviations

MV	mud volcano;
SMBA	Scottish Marine Biological Association, Oban.

Nomenclature

The inclusion of pogonophorans in the phylum Annelida is now supported by molecular and morphological evidence (e.g., McHugh 1997; Rouse & Fauchald 1997; Halanych *et al.* 2001; Halanych 2005), though there are objections to this hypothesis (Malakhov *et al.* 1996; Salvini-Plawen 2000). The existing name of one frenulate family, the Siboglinidae (Caullery 1914), has been applied to the whole pogonophore clade and ranked as a family of polychaetes by McHugh (1997) and Rouse (2001). Analysis of an extensive molecular database for more than 200 "annelids" by Rousset *et al.* (2007) found that the rooting of the annelid phylogenetic tree remains a major problem and that the sister group of the monophyletic "Siboglinidae" clade could not be inferred convincingly. This taxon is usually divided into at least two subsidiary taxa, currently under the names Frenulata and Vestimentifera, and these can still be considered to contain several families each (Southward *et al.* 2005). Therefore the older name Pogonophora is used here, divided into Frenulata and Vestimentifera (including Monilifera), and the older classification and family names are used (Ivanov 1963; Southward *et al.* 2002, 2005; note comments by Bartolomaeus *et al.* 2005). Caullery's original Siboglinidae and the genus *Siboglinum* were named in 1914 but without defining either taxon. Caullery named the type species *Siboglinum weberi* in 1944. His type material, from the Siboga Expedition (Caullery 1914, 1944), when examined in the light of later discoveries, was found to contain at least 16 species of pogonophore, belonging to 4 or more genera (all frenulates). A lectotype of *S. weberi* was therefore selected (Southward 1961) as a species common in the collection, having all the characters of *Siboglinum* as described by Caullery (1914, 1944) and providing sufficient material for a complete redescription. Ivanov, in 1963, accepted the redescribed *Siboglinum weberi* as the type species of the genus. He redefined the family Siboglinidae to include two genera, *Siboglinum* (1 tentacle) and *Siboglinoides* (2 tentacles). About 70 species of *Siboglinum* have now been described and the genus is in need of revision.

SYSTEMATICS

Phylum ANNELIDA Lamarck, 1809
POGONOPHORA, VESTIMENTIFERA
Webb, 1969
(= Polychaeta, Siboglinidae, vestimentiferans
Rouse, 2001)
Family LAMELLIBRACHIIDAE Webb, 1969
Genus *Lamellibrachia* Webb, 1969

TYPE SPECIES. — *Lamellibrachia barhami* Webb, 1969.

Remarks

The genus *Lamellibrachia* is characterized by a smooth-surfaced obturaculum without any axial secreted structures, surrounded by two series of axially orientated lamellae, the inner series composed of pinnulate branchial filaments; the outer series composed of filaments without pinnules, that adhere together side by side for most of their length; a single dorsal excretory pore; posterior margin of the vestimental folds discontinuous. The tube may or may not have an anterior series of collars and terminal funnel.

Lamellibrachia anaximandri n. sp.
(Figs 3-13)

Lamellibrachia sp. – Woodside *et al.* 1997: 127. — Olu-Le Roy *et al.* 2004. — Hughes & Crawford 2006: 1. — Duperron *et al.* 2009: 395-406.

HOLOTYPE. — **Anaximander Mountains, eastern Mediterranean**. MEDINAUT, Kazan MV, dive MN12, sample BT2, 35°26'N, 30°33'30E, 1700 m, 5.XII.1998, ♂ (MNHN POLY TYPE 1512).

Paratypes. — **Anaximander Mountains, eastern Mediterranean**. MEDINAUT, Kazan MV, dive MN12, sample BT2, 35°26'N, 30°33'30E, 1700 m, 5.XII.1998, 2 specimens (MNHN POLY TYPE 1513; MNHN POLY TYPE 1514). — Kazan MV, dive MN12, sample BT9/10B, 35°26'N, 30°33'30E, 1700 m, 5.XII.1998, 1 specimen (MNHN POLY TYPE 1515). — Faulted Ridge, dive MN9, sample stn 2, 35°27.3'N, 30°24.5'E, 1300 m, 30.XI.1998, 1 ♂ (MNHN POLY TYPE 1516). — Faulted Ridge, dive MN9, sample BT8, 35°27.3'N, 30°24.5'E, 1300 m, 30.XI.1998, 1 ♂ (MNHN POLY TYPE 1517). — Amsterdam MV, dive MN 8, sample BT 1, 35°20'N, 30°16.30'E, 2030 m, 29.XI.1998, 1 ♀ (MNHN POLY TYPE 1518). — Kazan MV, dive MN12, sample BT2, 1 specimen (NHM 2010.233). — Kula MV, dive MN11, sample BT, 35°44'N, 30°27 30'E, 1630 m, 2.XII.1998, 1 specimen (NHM 2010.232). — Faulted Ridge, dive MN9, sample BT7, 35°27.3'N, 30°24.5'E, 1300 m, 30.XI.1998, 1 ♂ (NHM 2010.228). — Amsterdam MV, dive MN8, sample BT1, 35°20'N, 30°16.30'E, 2030 m, 29.XI.1998, 1 specimen (NHM 2010.231).
Olimpi Field. Eastern Mediterranean, MEDINAUT, Milano MV, dive MN 5, sample BT4, 33°44'N, 24°47'E, 1955 m, 21.XI.1998, part of 1 mature ♀ (MNHN POLY TYPE 1519). — Milano MV, dive MN 5, sample BT4 33°44'N, 24°47'E, 1955 m, 21.XI.1998, 1 specimen (NHM 2010.230). — Napoli MV, dive MN4 33°43'N, 24°41'E, 1945 m, 22.XI.1998, sample BT, 1 ♂ (NHM 2010.229).

Empty tubes. — **Anaximander Mountains, eastern Mediterranean**. Kazan MV, dive MN12, sample BT9/10B, 35°26'N, 30°33'30E, 1700 m, 5.XII.1998, 2 large empty tubes (MNHN add type 1512(BT9); add type(10B)). Other MEDECO specimens at present being investigated by Eve Southward at Plymouth and Ann Andersen at Roscoff will be returned to CENTOB, IFREMER, Centre de Brest, Plouzané, France.

Other material examined. — **Nile deep-sea fan**. Vestimentiferans collected by the NAUTINIL Expedition during three dives in the Central Province and one dive at Aton East Delta, 9 individuals have been measured (for localities and depths, see Table 1 and Fig. 1), these and other Nile fan specimens are to be retained for study at Roscoff. Wreck of S.S. *Persia*: 110 km SE of Crete, exact co-ordinates not available, depth 2800 m. several dry tube fragments and parts of 3 animals have been examined, in the possession of David Hughes, SMBA, Oban. Anaximander Mountains and the Nile fan: 2007 MEDECO Expedition, Olimpi Field, living specimens were studied (Table 1).

Etymology. — The specific name *anaximandri* is derived from the region of the first discovery, the Anaximander Mountains, which were named after a Greek geographer of the 6th century B.C.

Description

Tubes

The full-grown tubes from the Anaximander and Olimpi regions have a thick, hard wall and a generally smooth exterior (Fig. 4A, B). They are grayish or brownish white in colour. The maximum diameter measured is 9 mm, but most tubes have a maximum diameter of between 3 and 6 mm, at the anterior end (Fig. 4C-F). The middle part of the tube is about 3 to 3.5 mm in diameter, while the posterior part tapers to 2 mm or less, ending in a long, dark brown "root" about 1 mm diameter. The anterior 30 to 50 mm of the tube has a slightly rough surface and transversely striped appearance, produced by short overlaps of the outer layers (Fig. 4D-F), but there are no projecting collars and no anterior funnel (Fig. 4B). Behind this region the surface becomes smoother. A total length of 80 cm was reported by Olu-Le Roy *et al.* (2004), but during the MEDECO cruise, the longest tube measured was 153 cm. However, most of the available tubes are broken. The anterior ends of some tubes have clearly been renewed during life, after suffering damage.

Animals

Live specimens from the Nile Fan and Olimpi region are shown in Figure 5.

Eighteen fixed animals inhabiting the full-grown tubes from the Anaximander and Olimpi regions described above are referred to here as the "type series" and correspond to fixed specimens illustrated in Figure 6. The obturaculum diameter ranges in these specimens from 2.7 to 5 mm (mean = 3.5 mm, n = 13) and the length from 5.5 to 12 mm (mean = 8.9, n = 15) (Figs 6A, B; 7B; Table 3). The two halves of the obturaculum form a funnel at the top, extending for about half the length of the plume (Fig. 6B). The inner and outer surfaces are smooth. The narrow stalk is elliptical in cross section. The plume has from 3 to 9 pairs of outer sheath lamellae and 8 to 17 pairs of branchial lamellae. The filaments of the sheath lamellae adhere closely together except at their extreme tips and they have no pinnules or bands of cilia. The branchial filaments have two rows of short pinnules and two longitudinal bands of cilia near their tips (Fig. 6C), but the proximal region lacks pinnules and cilia.

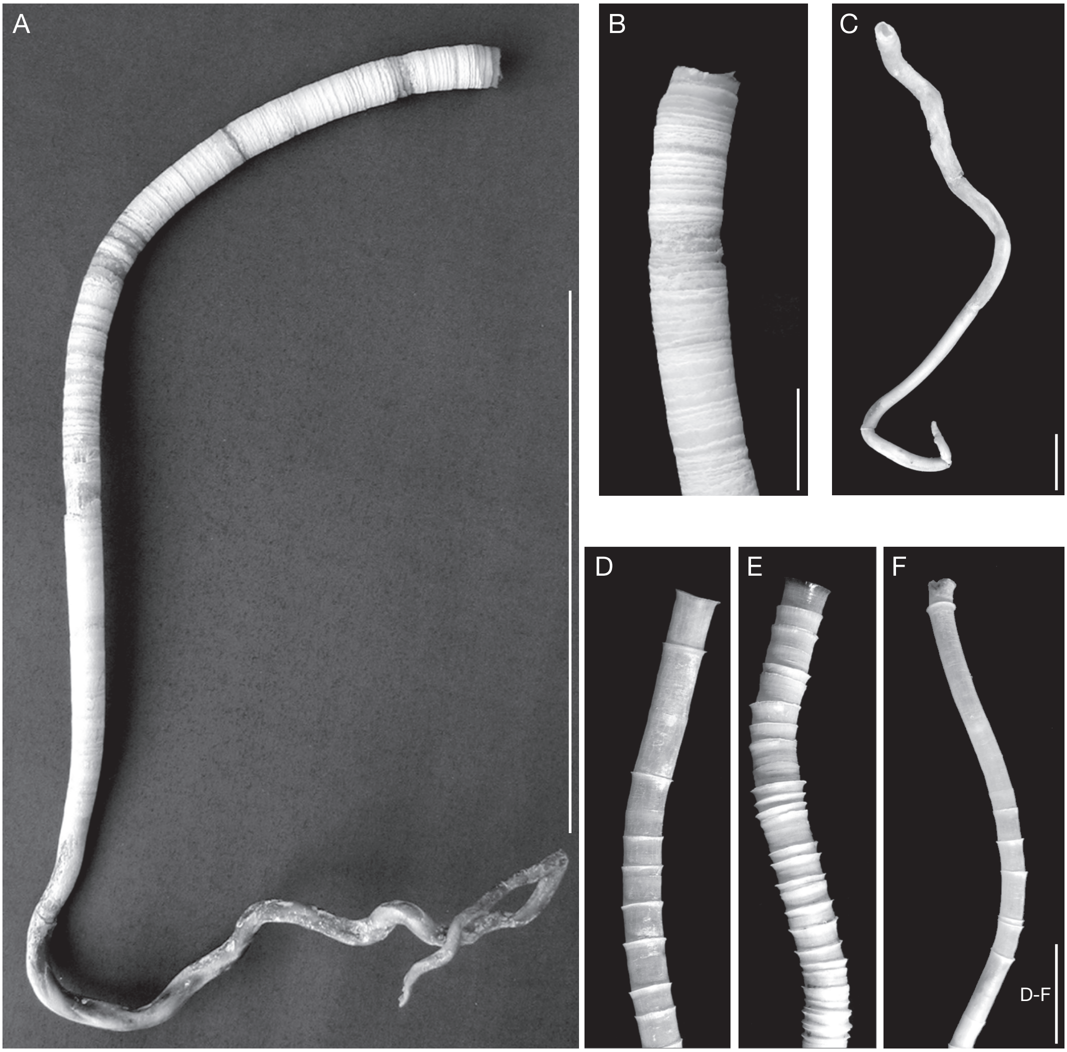


Fig. 4. — Tubes of *Lamellibrachia anaximandri* n. sp. from Anaximander Mountains: **A**, full-grown tube (dive MN12, Kazan mud volcano); **B**, anterior end of same tube; **C**, young tube with soft wall (type C) (dive MN12 on Kazan mud volcano); **D**-**F**, young tubes with small collars (type D) (dive MN9 on Faulty Ridge). Scale bars: A, 100 mm; B, 10 mm; C-F, 5 mm.

The pinnulate region may be damaged or even missing in larger specimens. The obturaculum is stiff and retains its proportions after fixation, but the muscular vestimental region contracts variably. After fixation inside the tube, the diameter of the vestimental region ranges from 2.4 to 4.9 mm (mean = 3.35 mm; n = 17) and the length ranges from 14 to 55 mm (mean = 31.5 mm; n =16). The photographs of living animals from the Nile fan and Napoli MV in Figure 5 confirm the funnel shape and proportions of the obturaculum (Fig. 5A-D) and show the length and position of the branchial and sheath lamellae. In fixed specimens the ratio of vestimental length to obturacular length (VL/OL) varies from 1:1 to 4.9:1 (mean = 3.7; n = 15). (Fig. 7B, E). The anterior ends of the vestimental folds form a collar about 1 mm high at the base of the plume (Fig. 6A, D). The posterior ends of the

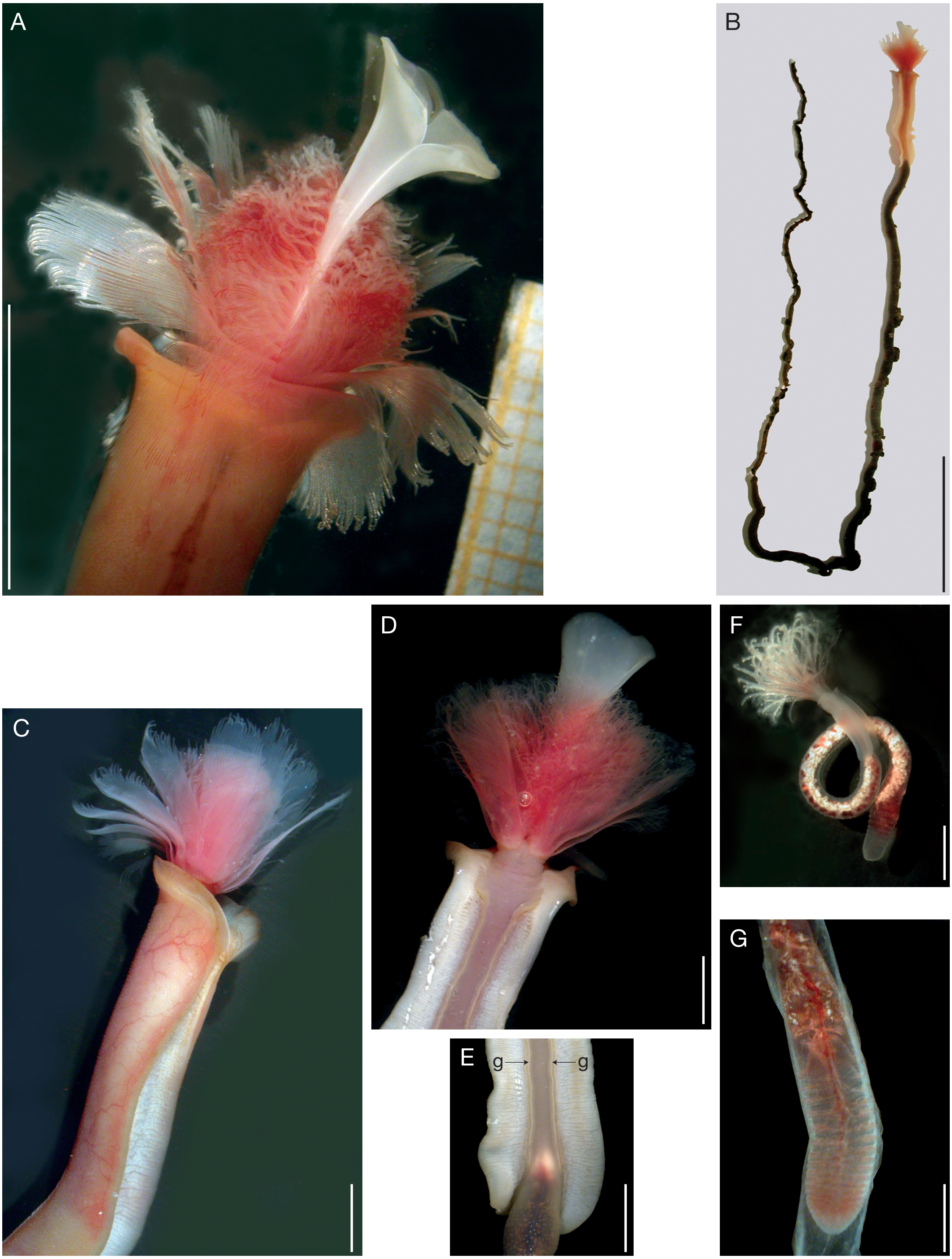
TABLE 3. — Comparison of body size measurements in *Lamellibrachia* Webb, 1969 species. Abbreviations and notes: *, *L. anaximandri* n. sp. type series from Anaximander and Olimpi regions; **, approximate measurements, taken from illustrations; ***, *L. barhami* Webb, 1969: **1**, California; **2**, California; **3**, Middle Valley (Juan da Fuca Ridge); **N**, number of specimens measured (approx.); **D**, diameter; **L**, length; **VL/OL**, vestimentum length/obturaculum length.

		Tube aperture (mm)	**Obturaculum (mm)**		**Vestimentum (mm)**		**VL/OL**	**Pairs**	**Pairs**	**Vestimental (mm)**	**Trunk (mm)**
	N	D	L	D	L	D		sheath lamellae	branchial lamellae	plaque D	plaque D
L. anaximandri n. sp.											
1. Type series*	17	4 to 9	5.5 to 12	2.8 to 5	14 to 55	2.4 to 5	2 to 4.9	3 to 9	8 to 12	55 to 60	60 to 80
2. Nile fan sample	9	3.6 to 6	6 to 17	1.8 to 6	12 to 40	2.2 to 4	1.7 to 4	5 to 7	8 to 19	60 to 70	70 to 95
L. luymesi Van der Land & Nørrevang, 1975 Van der Land & Nørrevang 1975, 1977											
	1	10	13	9	63	?	4.8	6	19	?	?
L. luymesi											
1. Gardiner & Hourdez 2003	40	3.4 to 9.7	6.6 to 16.3	3.5 to 5	26.9 to 61.6	3.7 to 5.2	2.6 to 6.7	4 to 8	15 to 22	?	?
2. ECS unpubl.	1		9.75	4.5	42	4.8	4.3	5	?	55 to 60	75 to 85
*L. barhami****											
1. Webb 1969	3	7.3 to 9	4.5 to 9	5**	25 to 35	4.7 to 6	2.8 to 7.8	2	25		
2. Jones 1985	5	7.5 to 10	8 to 12	4.5 to 10	23 to 37	4.5 to 8	2.5 to 4.6	max 4	max 25		
3. ECS unpubl.	5	8 to 12	10 to 16	6 to 12	32 to 60	6 to 8	2.1 to 2.7	5	19	60 to 150	115 to 160
L. victori Mañé-Garzón & Montero, 1985 Mañé-Garzón & Montero 1985											
	2	15	13	13**	65	13**	5	7	18**	?	?
L. columna Southward, 1991 Southward 1991											
	13	15 to 20	15 to 42	8 to 13	60 to 120	8 to 13	2.2 to 4.5	8 to 16	21	65 to 90	70 to 120
L. satsuma Miura, Tsukahara & Hashimoto, 1997 Miura *et. al.* 1997											
	64	2.5 to 8.7	1.8 to 9.8	1 to 5.6	7.2 to 24		2.1 to 8.3	0 to 4	max 19	35 to 63	51 to 82
L. juni Miura & Kojima, 2006 Miura & Kojima 2006											
	8	8.2 to 12.8	6.6 to 12.9		21.9 to 43.1		2.7 to 3.9	max 4	max 35		

folds are separated by a wide ventral gap (Fig. 6E). The ventral ciliated field is 1 to 2 mm wide.

Males have a pair of dorsal genital grooves (Fig. 5D, E, live male from Napoli MV) flanked by very narrow epidermal folds, extending from paired gonopores near the hind end of the vestimental region, forward to the collar region. The spermatozoa are elongated, like those of other vestimentiferans and their spiral heads are about 30 µm long. Immature females have no obvious gonopores or grooves, but in a fixed mature female specimen from Napoli MV, lacking a plume and the anterior part of the vestimental region, two very small gonopores are visible on the dorsal side, about 8 mm anterior to the posterior end of the vestimental region. The right pore is a little anterior to the left (Fig. 6F, arrows). Dorsally, the vestimental folds of this mature female are covered by wide bands of soft epidermis, 250-400 µm in thickness, separated

FIG. 5. — Living specimens of *Lamellibrachia anaximandri* n. sp. after removal from their tubes, MEDECO Expedition, 2007: **A**, specimen from the Nile Fan, Central province, dive 339, ventral view of plume and collar region, note pale sheath lamellae, red branchial filaments and white obturaculum; **B**, specimen from Olimpi field, dive 330, Napoli mud volcano; animal, minus opisthosoma; note red plume, paler vestimental region and dark trunk; **C**, specimen from Nile fan, Central province, dive 339 lateral view of plume and anterior vestimental region; **D**, male specimen from Olimpi field, dive 330, Napoli mud volcano, dorsal view of plume and anterior end of vestimental region, showing anterior ends of genital grooves; **E**, same male specimen, dorsal view of posterior end of vestimental region showing posterior parts of genital grooves (arrows, g); **F**, complete juvenile about 17 mm long from the Eastern province of the Nile fan, on the south fringe of Cheops mud volcano, dive 343; note plume of branchial filaments without sheath lamellae; **G**, opisthosoma



of another small specimen from the same area as Figure 5F, dive 343, still inside thin-walled tube. Colour photos: Ann Andersen and Matthieu Bruneaux. Scale bars: A, 10 mm; B, 4 cm; C-E, 5 mm; F, G, 1 mm.

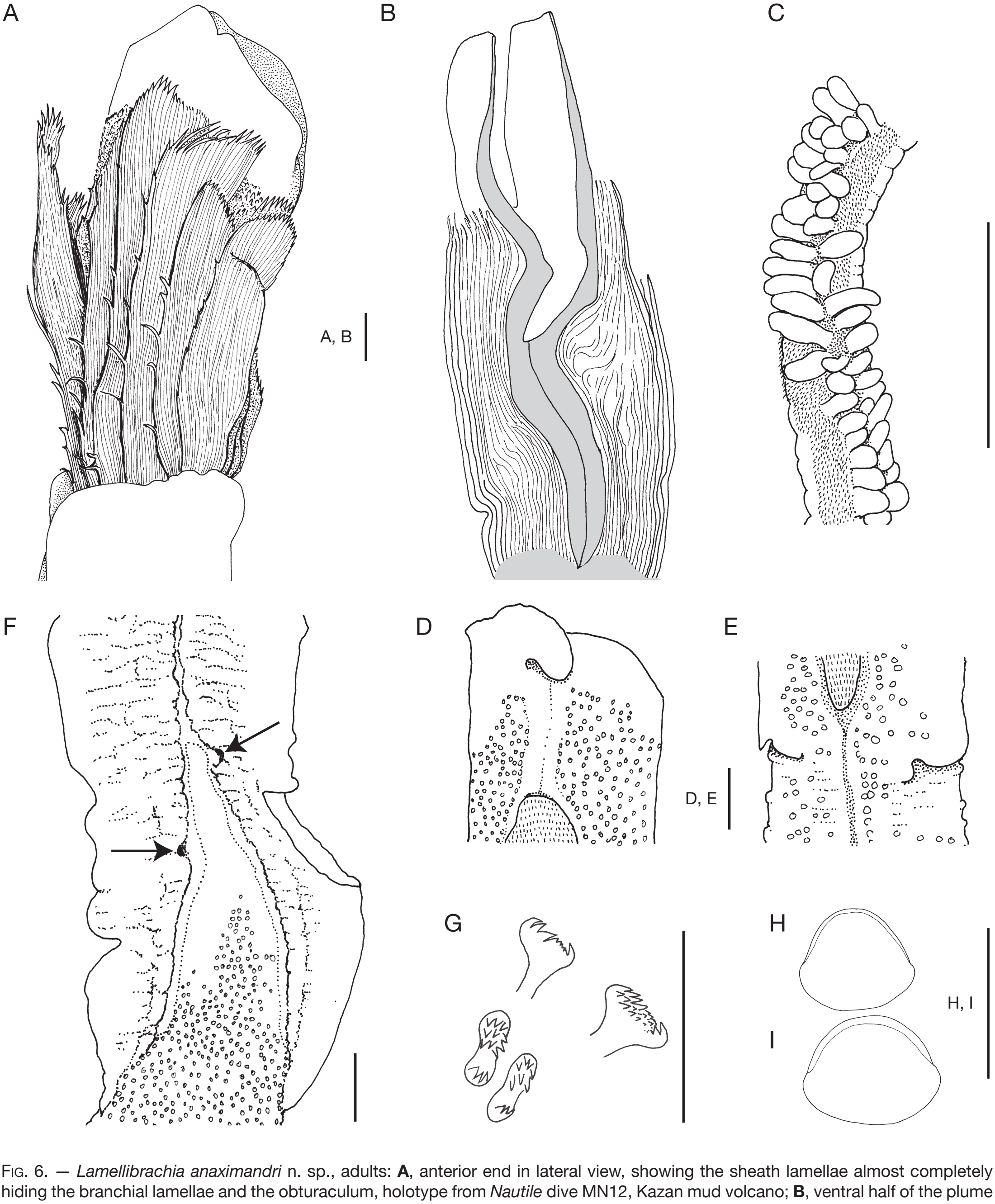


Fig. 6. — *Lamellibrachia anaximandri* n. sp., adults: **A**, anterior end in lateral view, showing the sheath lamellae almost completely hiding the branchial lamellae and the obturaculum, holotype from *Nautile* dive MN12, Kazan mud volcano; **B**, ventral half of the plume and obturaculum of another specimen, cut longitudinally to show the depth of the funnel of a specimen from unlabelled MEDINAUT sample; **C**, part of a branchial filament with pinnules and two bands of cilia, specimen from *Nautile* dive MN8 Amsterdam mud volcano; **D**, **E**, anterior and posterior ends of vestimental region in ventral view, showing the vestimental folds and the two ends of the ciliated field of a specimen from *Nautile* dive MN8, Amsterdam mud volcano; **F**, dorsal surface of posterior vestimental region of large female, with vestimental folds spread outward, two arrows mark the gonopores, from *Nautile* dive MN5 Napoli mud volcano; **G**, chaetae, in face and side view, from an opisthosome with 43 chaetigers (sample MN10 BT1); **H**, **I**, vestimental and abdominal plaques, respectively. Scale bars: A, B, D-F, 1 mm; C, 0.1 mm; G, 10 µm; H, I, 100 µm.

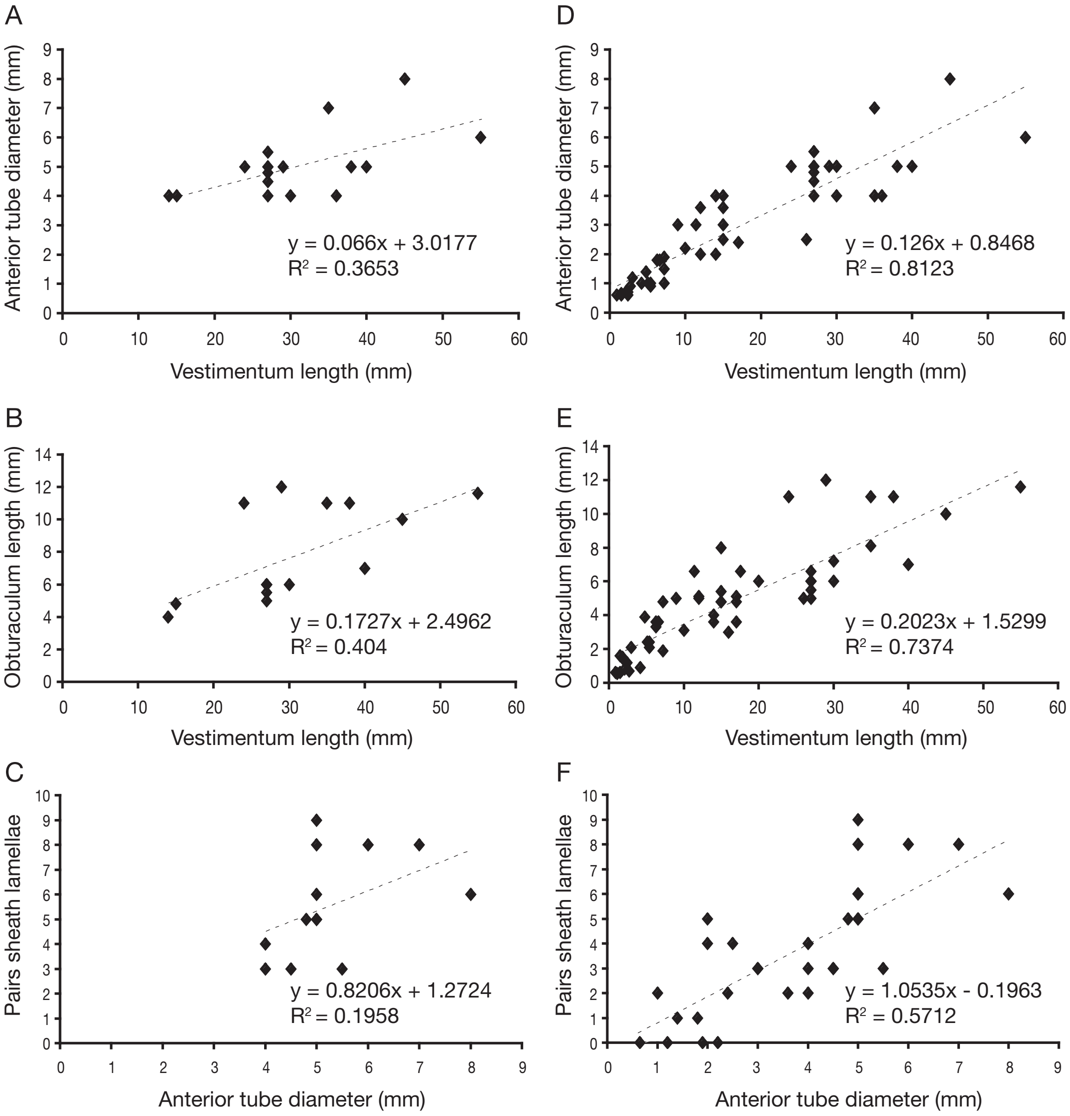


FIG. 7. — Changing dimensions with growth in *Lamellibrachia anaximandri* n. sp. from Olimpi and Anaximander regions: **A**-**C**, type series (individuals having anterior tube diameter >3.5 mm); **D**-**F**, individuals of all sizes including type series. **A** and **D**, anterior tube diameter related to length of vestimentum; **B** and **E**, obturaculum length related to vestimentum length; **C** and **F**, number of pairs of sheath lamellae related to anterior tube diameter.

by a mid-dorsal strip of thinner, smooth-surfaced epidermis, which divides into two, anterior to the gonopores. The gonopores open onto these paired strips, which are not ciliated. A few oocytes (100 µm diameter) have stuck to the strips, near the gonopores, indicating recent expulsion from the gonoducts.

Cuticular plaques are present on the vestimental and the trunk papillae. The maximum diameter of the oval plaques on the vestimental region ranges from 50 to 70 µm, while those on the anterior part of the trunk are slightly larger, ranging from 60 to 80 µm (Fig. 6H, I). The trunk is probably as much as 500 mm long

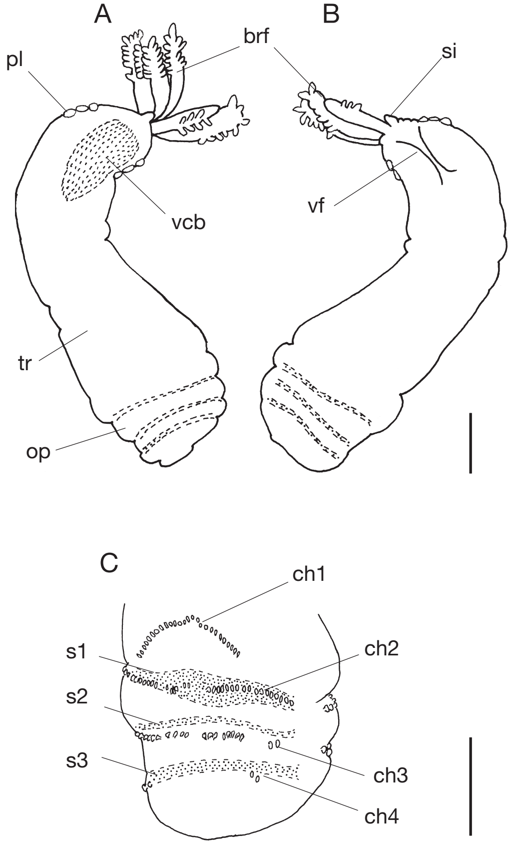


FIG. 8. — The smallest juvenile of *Lamellibrachia anaximandri* n. sp., 1 mm long, from Kazan MV, dive MN10 BT1: **A**, **B**, whole animal, ventral and dorsal; **C**, opisthosome. Abbreviations: **brf**, branchial filaments; **ch1** to **ch4**, rows of chaetae; **op**, opisthosome; **pl**, plaques; **s1**, **s2**, **s3**, first three septa; **si**, siphon; **tr**, trunk; **vcb**, ventral ciliated band; **vf**, vestimental folds. Note that the first row of chaetae is anterior to the first septum. Scale bars: 100 µm.

in large individuals, but the only complete animal in the type series has a shorter trunk (80 mm) and a regenerating opisthosome 2.5 mm long and 2 mm wide, with 11 chaetigerous segments. Long fragments of trunk have been found inside broken tubes. Several isolated opisthosomes have been found in the samples, the largest opisthosome being 7 mm long by 2 mm wide, with about 50 chaetigerous segments. The chaetae are arranged in irregular single to double rows. Their heads are 5 to 7 µm long by 2 µm wide, with an anterior group of 4 to 6 teeth and a posterior group of 4 rows of about 4 teeth each (Fig. 6G).

Small specimens

Tubes less than 3 mm in anterior diameter, collected at three sites in the Anaximander region (Amsterdam MV, Kazan MV and Faulted Ridge; Table 1) were examined and the animals inhabiting them were measured. An additional small specimen was collected in the Olimpi Region, at Milano MV. The relationships between various dimensions of small and large specimens can be compared (Fig. 7). There is greater variability in the older population than in the younger. The correlation coefficients R^2 are greatly increased by the inclusion of small specimens; compare Figure 7A and D, B and E, C and F.

Juveniles. These are defined here as individuals inhabiting tubes less than 1 mm in diameter and less than 40 mm long. Numerous such young tubes, established on pieces of calcareous crust, were collected at Kazan MV (MN10 BT1); four others were found among a sample of larger tubes from Faulted Ridge (MN9 BT8). Most of them adhere to the substratum for at least 15 mm of their length and have a sealed posterior bulb. The anterior part of the tube lifts away from the rock. There are no collars.

The smallest juvenile is 0.93 mm long (Figs 8A, B; 9A). It has five branchial filaments 0.13 mm long with two rows of pinnules each. It has no obturaculum, but there is a small remnant of the larval feeding organ (Fig. 8B, si), the siphon or medial process described for young *Ridgeia piscesae* Jones, 1985 (Southward 1988; Jones & Gardiner 1988) and *Riftia pachyptila* Jones, 1981 (Jones & Gardiner 1988). The next juvenile stage is represented by three individuals about 6 mm long (Fig. 9B). They have several pinnulate branchial filaments, arranged in two or more pairs of lamellae. A small obturaculum (diam. 0.4 mm. length 0.5 to 0.8 mm) is present centrally among the branchial filaments. The opisthosoma has about 6 chaetigerous segments. The third juvenile stage, represented by four specimens about 10 mm long (Fig. 9C, D), has more branchial filaments, a considerably longer trunk and a wider vestimental region, but the vestimental and opisthosomal lengths have scarcely increased. There are no sheath lamellae.

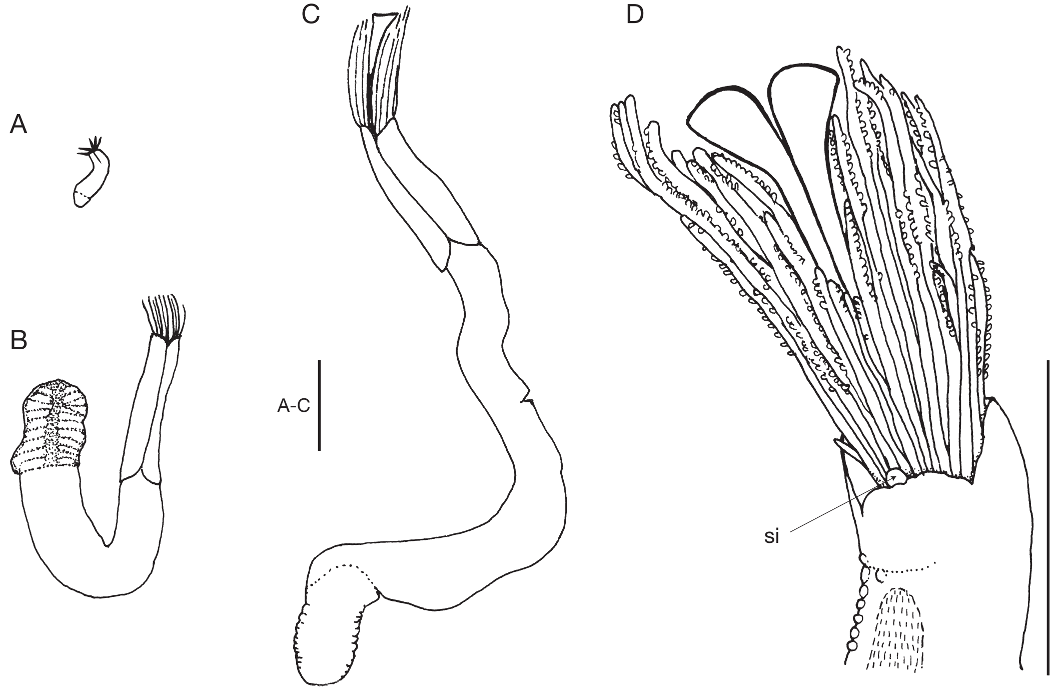


FIG. 9. — Stages in the growth of young *Lamellibrachia anaximandri* n. sp., from dive MN10: **A**-**C**, dorsal views of 1.0, 6.0 and 10.5 mm animals; **D**, ventral view of anterior end of C in more detail. Abbreviation: **si**, siphon. Scale bars: 1 mm.

A remnant of the siphon is still present (Fig. 9D, si) and the obturaculum is slightly longer than in the 6 mm specimens. The opisthosome has from 7 to 10 chaetigerous segments. A juvenile taken from the carbonate crust of the north flank of Cheops MV in the western province of the Nile fan, at about 3000 m depth during the MEDECO cruise, is shown in Figure 5F. It has no sheath lamellae. The opisthosome of another juvenile from the same site has at least 10 chaetigerous segments (Fig. 5G).

Young specimens in thin-walled soft tubes more than 40 mm long. Amsterdam MV (MN8 BT4'). Thirteen tubes of diameter 0.6 to 1.5 mm and up to 160 mm long have soft, limp walls and no collars or segmentation. The posterior ends are flattened or broken. These tubes were stuck together, with some sand grains and tube debris attached. Thirteen animals were measured, eight of these were complete. The obturaculum diameter is 1 mm or less; and the maximum obturaculum length is 3.9 mm; the vestimentum length is 0.2 to 7.8 mm and the trunk length 5.4 to 27 mm. A greater trunk length of 35 mm was measured in an incomplete specimen. Where present, the opisthosoma is 0.8 to 1.8 mm long.

Young specimens in sinuous, thick-walled tubes. Kazan MV (MN12 BT 9/10A). Thirty sinuous tubes with thick, "cartilaginous" walls and an irregular surface have a diameter of 2 to 3 mm and are up to 160 mm long. Their posterior ends are missing. Some have an anterior smooth, thin-walled, cylindrical, region a few mm long (Fig. 4C). Twelve animals were measured, all incomplete posteriorly. The obturaculum diameter is 1.4 to 3.0 mm and the length is 3.0 to 7.2 mm. The vestimentum length is 12 to 30 mm and the incomplete trunk up to 100 mm long. No opisthosomes were found.

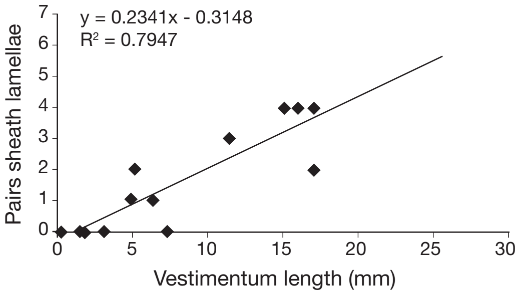


FIG. 10. — Number of pairs of sheath lamellae in young *Lamellibrachia anaximandri* n. sp. in relation to length of vestimentum.

Young specimens in straight tubes with stiff walls and collars. Faulted Ridge (MN9 BT 8). Seventeen straight tubes with thin walls, short segments and narrow collars (Fig. 4D-F). The walls are colourless to pale yellow and semi-transparent. The anterior diameter ranges from 1.8 to 4.8 mm, the maximum length is 140 mm but the posterior end is missing. Eight animals were measured, all incomplete posteriorly. The obturaculum diameter is 1.2 to 3.5 mm, the obturaculum length 3.3 to 7.5 mm. The vestimentum length is 6.3 to 27 mm and the incomplete trunk up to 35 mm. No opisthosomes were found.

Tube development

The secretion of the tube begins at the time of settlement with the formation of a narrow tube with a sealed posterior end and open anterior end, attached to a hard surface (type A). As the animal grows, the open end of the tube is extended and lifts from the substratum, remaining narrow and thin-walled (type B). In some circumstances, secretion of more copious tube material produces a thicker-walled, softer tube (type C). Both the B and C types then grow episodically, adding segments with narrow collars to the open end (type D). The anterior diameter increases gradually, to at least 3 mm and the new segments become shorter, eventually being visible as slight overlaps (see Fig. 4B, E). The tube wall is thickened internally by more layers of secretion and the external collars or overlaps become eroded to leave a fairly smooth tube surface, culminating in the typical hard, tough, tube described above (Fig. 4A). Extension also takes place at the posterior end of the tube, giving rise to the narrow tapering "root" that may extend deep into crevices or soft sediment, carried by the burrowing activity of the opisthosome and filled by the continuously growing trunk region.

Animal development

After settlement and the first formation of a tube the development of the young animal continues with the differentiation and growth of the vestimental, trunk and opisthosomal regions (Fig. 8). A small vestige of the larval feeding organ (siphon) is retained while the earliest (pinnulate) branchial filaments are being formed (Fig. 8B, si). The obturaculum begins to develop in the centre of the plume of filaments, enclosed between the first few pairs of branchial lamellae. This resembles the development of the plume and obturaculum in *Ridgeia piscesae* and *Riftia pachyptila* (Southward 1988; Jones & Gardiner 1988). Figure 5F shows a living juvenile about 17 mm total length, without sheath lamellae. The characteristic sheath lamellae of *Lamellibrachia* begin to develop after this, when the vestimental region has reached about 5 mm in length (Fig. 10). The first two sheath lamellae are each composed of a few smooth filaments in a row, and are based on either side of the mid-ventral line, posterior to the branchial lamellae. More filaments are produced at the two margins, so that the lamellae are gradually extended around the sides of the body, towards the dorsal side. The next two sheath lamellae originate behind the first two, again close to the mid-ventral line, and more pairs behind them, so that as the animal grows in diameter, it develops left and right series of sheath lamellae. The filaments grow in length to enclose the branchial lamellae when the animal is retracted inside its tube (Fig. 6A, B). This suggests a protective and possibly a sensory function. Photographs and videos of living *Lamellibrachia*, *in situ*, taken on the MEDECO expedition show that when the plume is extended the sheath lamellae open like pink petals around the mass of red branchial lamellae. The plumes of living animals, after removal from their tubes are shown in Figure 5. Any respiratory function of the sheath filaments is likely to be poorer than that of the branchial filaments, because the sheath filaments have a much thicker cuticle, they lack pinnules, and the afferent and efferent blood vessels are narrower than those of the branchial filaments (Van der Land & Nørrevang 1977: pl. 23, fig.109).

Size at maturity
Young specimens lack external sexual characters. In the fixed material, male genital grooves have been noted in ten specimens ranging from 14 to 35 mm vestimental length, in tubes 2 to 7 mm anterior tube diameter. The female gonopores have been seen in 3 specimens, 26, 36 and 45 mm vestimental length, in addition to the damaged specimen described earlier (Fig. 6F). These pores are apparently always very small. The development of the gonads has not yet been investigated.

NILE FAN SPECIMENS
(FIGS 5A, C, F, G; 11; 12; TABLE 1)
Figure 11A shows numerous occupied tubes emerging from a small scarp or fault in the carbonate crust. Figure 11B shows a cluster of younger individuals with red branchial plumes extended, in a sea-floor crevice in the carbonate crust. The younger tubes in both populations have narrow collars at the anterior end (Fig. 11C). The specimens collected in 2003 (Table 1) have tube diameters in the range 3.5 to 6 mm. They are fairly smooth-walled but some have flared anterior ends with slight collars (Fig. 11D). The tube colour is grayish. Specimens that were fixed outside their tubes (Fig. 12A, B, D) show the obturaculum and plume clearly. Measurements of 6 specimens and photographs of 3 others show that the obturaculum diameter ranges from 1.8 to 6 mm; the obturaculum length from 6 to 17 mm, the vestimentum diameter from 2.2 to 4 mm and the vestimentum length from 12 to 40 mm. There are from 5 to 7 pairs of sheath lamellae and from 8 to 19 pairs of branchial lamellae (Fig. 12A). The epidermal plaques on the vestimentum measure 60-70 µm and those on the anterior trunk 70 to 96 µm (measured in one specimen). The dimensions of the animal and its plaques are within the range found in the type material of *L. anaximandri* n. sp., apart from a slight increase in the maximum number of branchial lamellae, so based on the morphological characteristics it is probable that the Nile fan specimens belong to this species, but the genetic results are necessary to confirm this.

Sections of the trophosome of specimens of *Lamellibrachia* collected in the centre of the Nile deep-sea fan (NL6) show endosymbiotic bacteria of coccoid shape ranging from 1 to 7 µm diameter (mean = 3.12 ± 1.22 µm, n = 262) (Fig. 12C). The bacteria lack the internal membranes that are typical of methanotrophs, but show a clear periplasm between an inner cytoplasmic membrane and an outer cell wall, which is typical of Gram-negative bacteria. They also contain small spherical membrane-bound bodies similar to the sulfur globules of some thiotrophic bacteria. Based on these morphological characteristics we conclude that the endosymbiotic bacteria are probably thiotrophic.

S.S. *PERSIA* SPECIMENS (FIG. 13)
Hughes & Crawford (2006) reported that hundreds of *Lamellibrachia* tubes and fragments were found in the mailroom of the *Persia*, ranging up to 105 cm in length and 9 mm in diameter. Most of the tubes were empty. In the eleven dry fragments of tube examined in the present study, the diameter varies from 3.5 to 9 mm. The colour is dark brown and the surface is generally worn, with slight overlaps remaining. The anterior end of one tube is present (Fig. 13B). The top 20 mm has shrunk during drying, but it appears to have been smooth and thin-walled. The next part consists a series of about ten closely overlapping layers, followed by a smooth section about 30 mm long and then another series of overlapping layers (Fig. 13A).

Parts of three animals have been examined. One has a complete obturaculum, branchial plume and vestimental region (Fig. 13C). The obturaculum measures about 8 mm long by 4 mm in diameter, the vestimental region 17 by 3 mm, and the incomplete trunk 55 by 3 mm. There are 3 or 4 pairs of sheath lamellae and at least 15 pairs of branchial lamellae. The distal parts of the branchial filaments have deteriorated and the pinnules have been lost, but some cilia are visible. The filaments of the sheath lamellae have also deteriorated and are separated

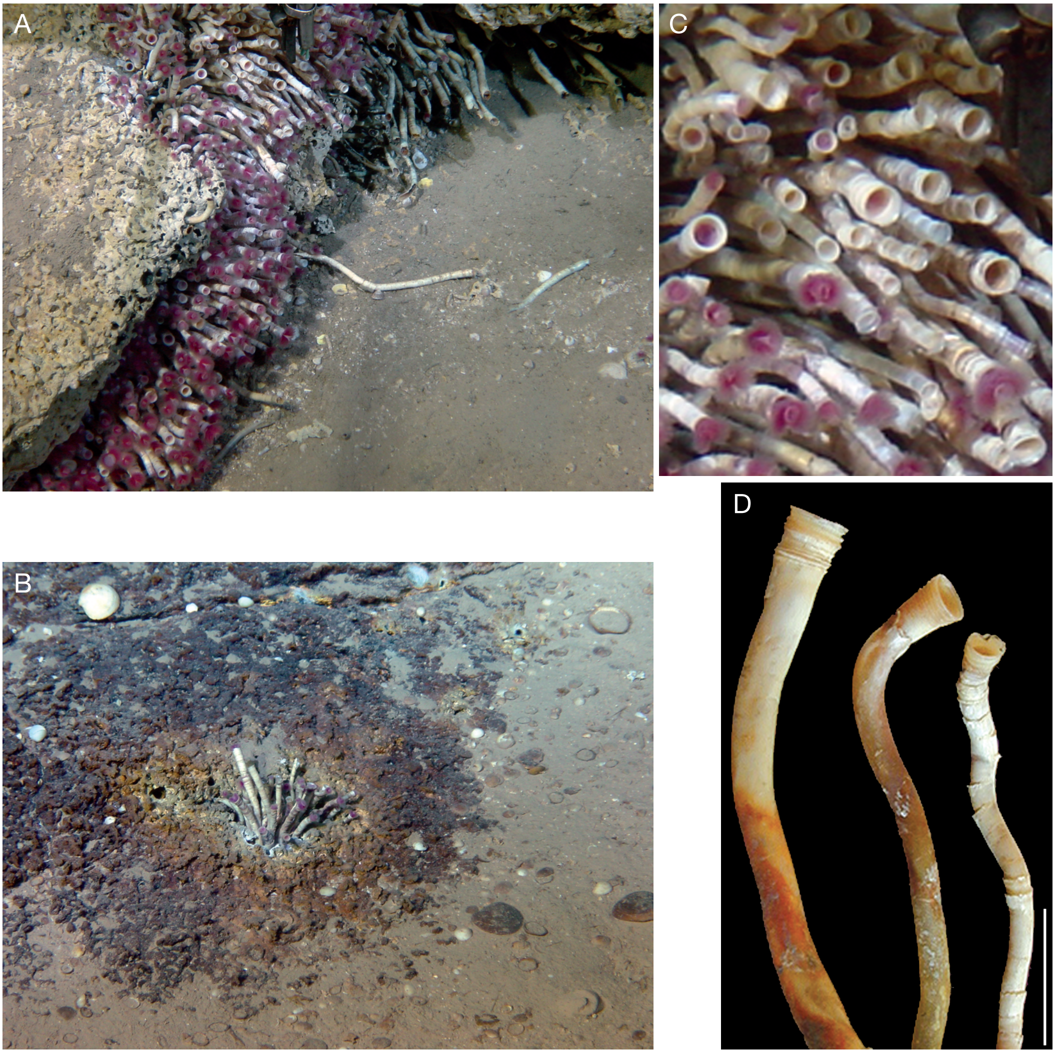


FIG. 11. — *Lamellibrachia anaximandri* n. sp. from the Central Area of the Nile deep-sea fan: **A**, *in situ* photograph of abundant live animals, in scarp situation (dive MD 336, 1686 m depth) (courtesy of IFREMER); **B**, *in situ* photograph of small patch of live animals emerging from hole in calcareous crust (dive MD 338, 1701 m depth) (courtesy of IFREMER); **C**, detail from Figure 11A, showing the variety of tube diameters and collar arrangements present in this population; **D**, anterior ends of tubes (dive NL6). Scale bar: 2 cm.

quite widely distally, though they adhere together proximally. The vestimental plaques range from 60 to 70 µm in diameter and the trunk plaques range from 70 to 95 µm, measured on one specimen. The dimensions of the animal and its plaques are within the range found in the type material of *L. anaximandri* n. sp., and it is probable that the *Persia* specimens belong to this species.

MOLECULAR RESULTS

The phylogenetic trees based on the mitochondrial 16S and COI data exhibit a similar topology (Figs 14; 15). In both trees, there is a very strong support for a lamellibrachiid clade and a distinct escarpiid clade. The COI tree (Fig. 14) indicates that *Lamellibrachia juni*, from the Kermadec Arc (South West Pacific), is clearly very different from

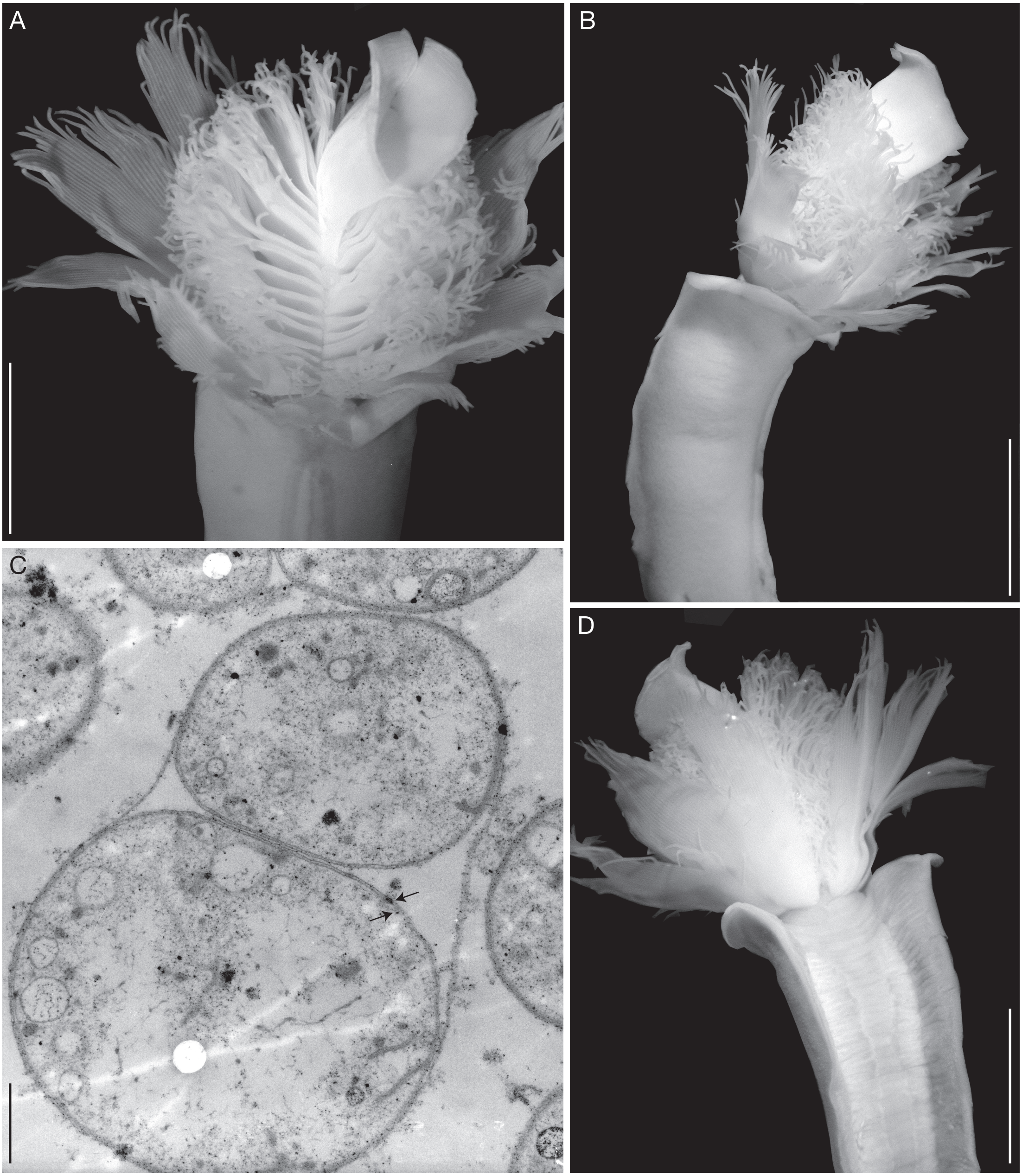


FIG. 12. — *Lamellibrachia anaximandri* n. sp., specimens from the Nile deep-sea fan (*Nautile* dive NL14): **A**, **B**, **D**, anterior ends in ventral, right side and dorsal view; **C**, TEM of bacteria in trophosome; the inner arrow shows the inner cytoplasmic membrane, the outer arrow shows the outer cell wall. Scale bars: A, B, D, 10 mm; C, 1 µm.

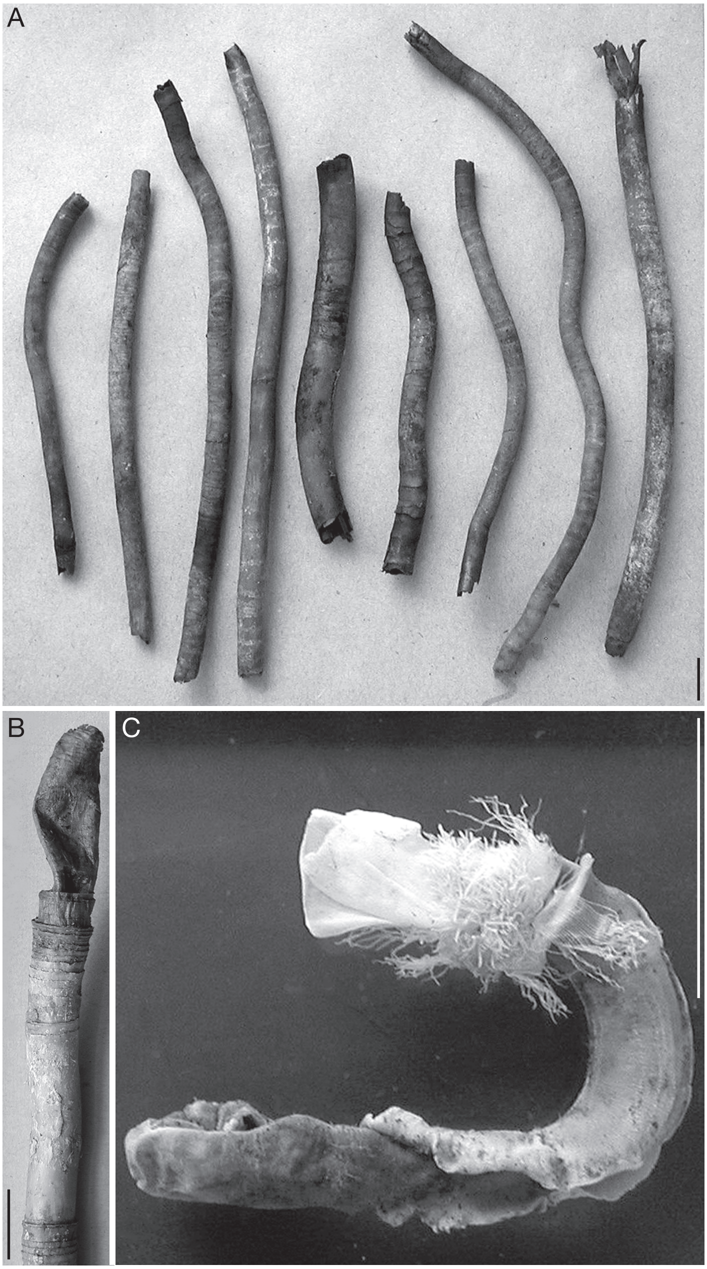


FIG. 13. — *Lamellibrachia anaximandri* n. sp. from S.S. *Persia* shipwreck: **A**, various fragments of tube; **B**, soft anterior region of tube and narrow overlaps; **C**, anterior end of animal, lateral view. Scale bars: 10 mm.

all other species of *Lamellibrachia*. No data are currently available in the databases for the mt16S gene for this species. The COI tree also shows that all the other species of *Lamellibrachia* form a very well supported clade that comprises species from the Atlantic side (Mediterranean and Gulf of Mexico),

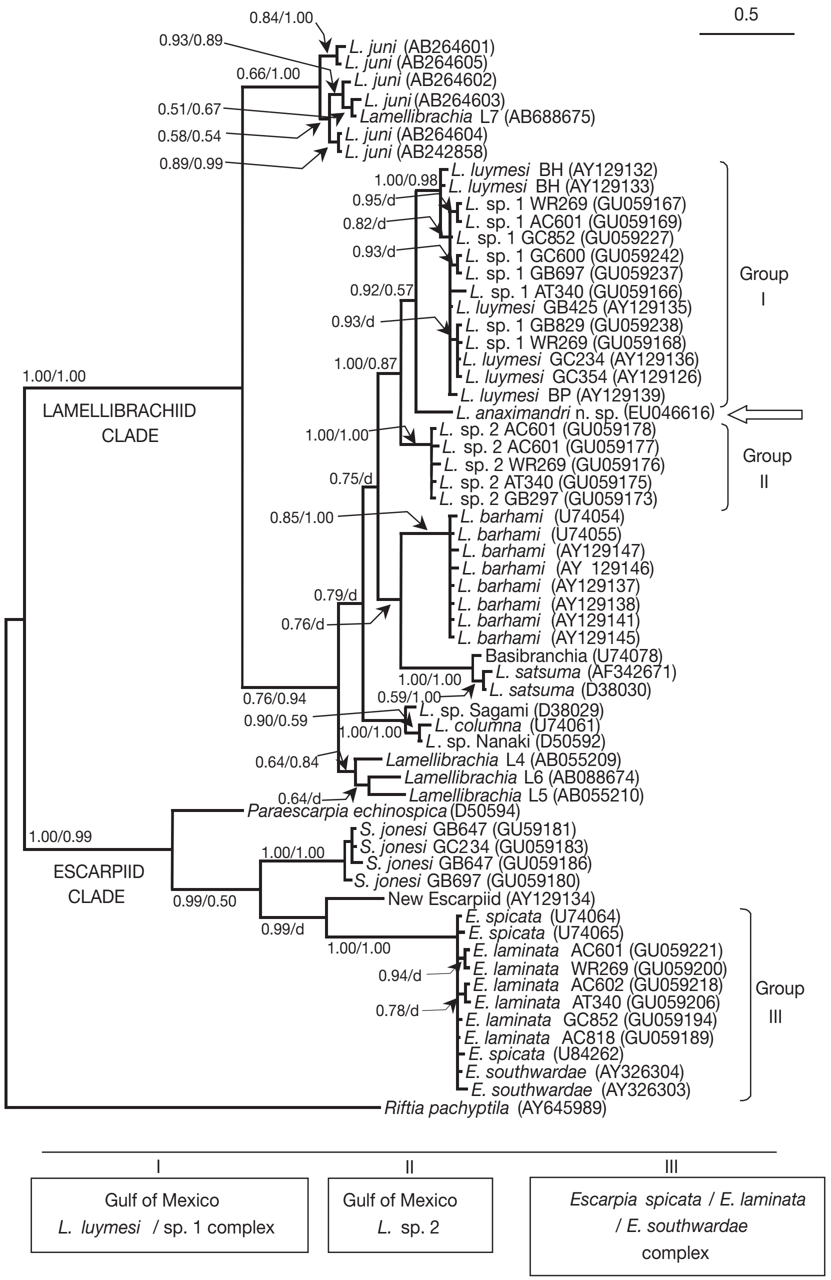


FIG. 14. — Phylogeny of cold seep vestimentiferan species. Tree constructed by a Bayesian approach based on a 536-bp alignment of the partial mitochondrial COI sequence. For each branch, bootstrap values for 1000 replicates for the Neighbour-Joining method with Kimura-2-parameter distances are indicated (number after the "/" sign), along with Bayesian posterior probability values (number before the "/" sign). *Riftia pachyptila* was used as an outgroup. Only values greater than 50% bootstrap or 0.50 posterior Bayesian probability are shown. "d" indicates a different branching pattern for the Neighbor Joining method. Sampling locations are given after the species name for each individual sequence when possible and accession numbers in GenBank are given for each sequence (between parentheses). The large arrow indicates the position of *Lamellibrachia anaximandri* n. sp.

and the Pacific side (Western and Eastern). In this phylogeny, *L. anaximandri* n. sp. forms a cluster with *L. luymesi* and *L.* sp. 1 from the deep Gulf of Mexico, with a fairly good confidence (group I in Fig. 14, 0.92 posterior probability and 57% bootstrap value). Within this cluster, however, *L. anaximandri* n. sp. is clearly apart from the *L. luymesi* cluster (supported by a 98% bootstrap value and a 1.00 posterior probability). The second deep Gulf of Mexico species (*L.* sp. 2) also forms a monophyletic cluster (group II in Fig. 14) that is a sister-group to *L. luymesi*/*L.* sp. 1. The relationship of the *L. luymesi*/*L. anaximandri* n. sp. cluster with other *Lamellibrachia* remains unclear because of the short internal branches and both tree construction methods do not yield the same branching pattern. The mt16S tree tends to show the same pattern (groups I and II), but the grouping of *L. anaximandri* n. sp. with either one of these two groups remains unclear (Fig. 15). All mt16S sequences for *L. anaximandri* n. sp. form a monophyletic group (98% bootstrap value but posterior probability below 0.50), regardless of the area of sampling in the Mediterranean (Nile fan central pockmark, Anaximander Mountains, and Napoli mud volcano in the Olimpi field).

COMPARISON WITH OTHER SPECIES

Some characteristics of *Lamellibrachia anaximandri* n. sp. can be compared with those of seven other species of *Lamellibrachia* (Table 3). Morphology and dimensions alone do not distinguish all the species from one another very satisfactorily. However, the molecular sequence data (Fig. 14) clearly separate *L. anaximandri* n. sp. from *L. luymesi*.

Lamellibrachia columna is the largest of the seven species, having tubes up to 20 mm in diameter, whereas the tubes of the others are generally less than 15 mm wide at the anterior end. The full-grown tube of *L. anaximandri* n. sp. resembles those of *L. columna* and the holotype (Fig. 16C) of *L. luymesi* (Van der Land & Nørrevang 1977: fig. 124), because it lacks the wide collars seen in *L. barhami*, *L. satsuma*, *L. victori* and the Gulf of Mexico population of *L. luymesi*. *Lamellibrachia juni*, a recently described species from the Kermadec Arc, has an unusually thin tube wall, with small collars and a maximum tube diameter of 8 to 12.8 mm.

The diameter and proportions of the various parts of the animals change as they grow: in *Lamellibrachia anaximandri* n. sp., the diameter of the vestimental region is slightly less than that of the anterior end of the tube, and the diameter of the obturaculum is about the same as that of the vestimental region. The length of the vestimental region is about 5 to 7 times the tube diameter (Fig. 7A, D) in both large and small *Lamellibrachia anaximandri* n. sp. The number of sheath lamellae increases as the vestimental diameter increases, but there is considerable variation among both small and large animals (Fig. 7C, F). The range for the species (3 to 9 pairs) is about the same as in *Lamellibrachia luymesi*, which has a maximum of 8 pairs, but *L. satsuma*, *L. barhami* and *L. juni* have only 4 or 5. *Lamellibrachia columna* has 8 to 16 pairs of sheath lamellae. The branchial lamellae are often difficult to count accurately because they stick together, but *Lamellibrachia anaximandri* n. sp. has probably the fewest (8 to 17 pairs; 19 in a Nile fan specimen). Maxima for other species range from 19 to 25 pairs. *Lamellibrachia juni* has an exceptionally large number, up to 35 pairs. The ratio of vestimentum length to obturaculum length (VL/OL) has been used in the past to indicate differences between some genera and species of vestimentiferans, (Jones 1985; Southward *et al.* 1995; Miura *et al.* 1997). In *Riftia*, the obturaculum is about twice the length of the vestimentum, in *Ridgeia*, OL and VL are more or less equal, in other genera, VL is longer than OL. Table 3 shows the wide variation in this ratio for each species of *Lamellibrachia* and indicates that it is not very useful in separating species of *Lamellibrachia.* The size of plaques on the vestimental and trunk papillae (Fig. 6H,I; Table 3) can be a useful character. However, because the plaques are collagenous, not chitinous, they shrink considerably when dehydrated. Measurements from electron micrographs are smaller than those made from specimens in aqueous media and are not directly comparable. In the present case, the plaques of *Lamellibrachia anaximandri* n. sp., *L. barhami*, *L. columna* and *L. luymesi* from the Gulf of Mexico have all been examined in aqueous media (see Materials and methods). *Lamellibrachia anaximandri* n. sp. has the smallest plaques (55 to

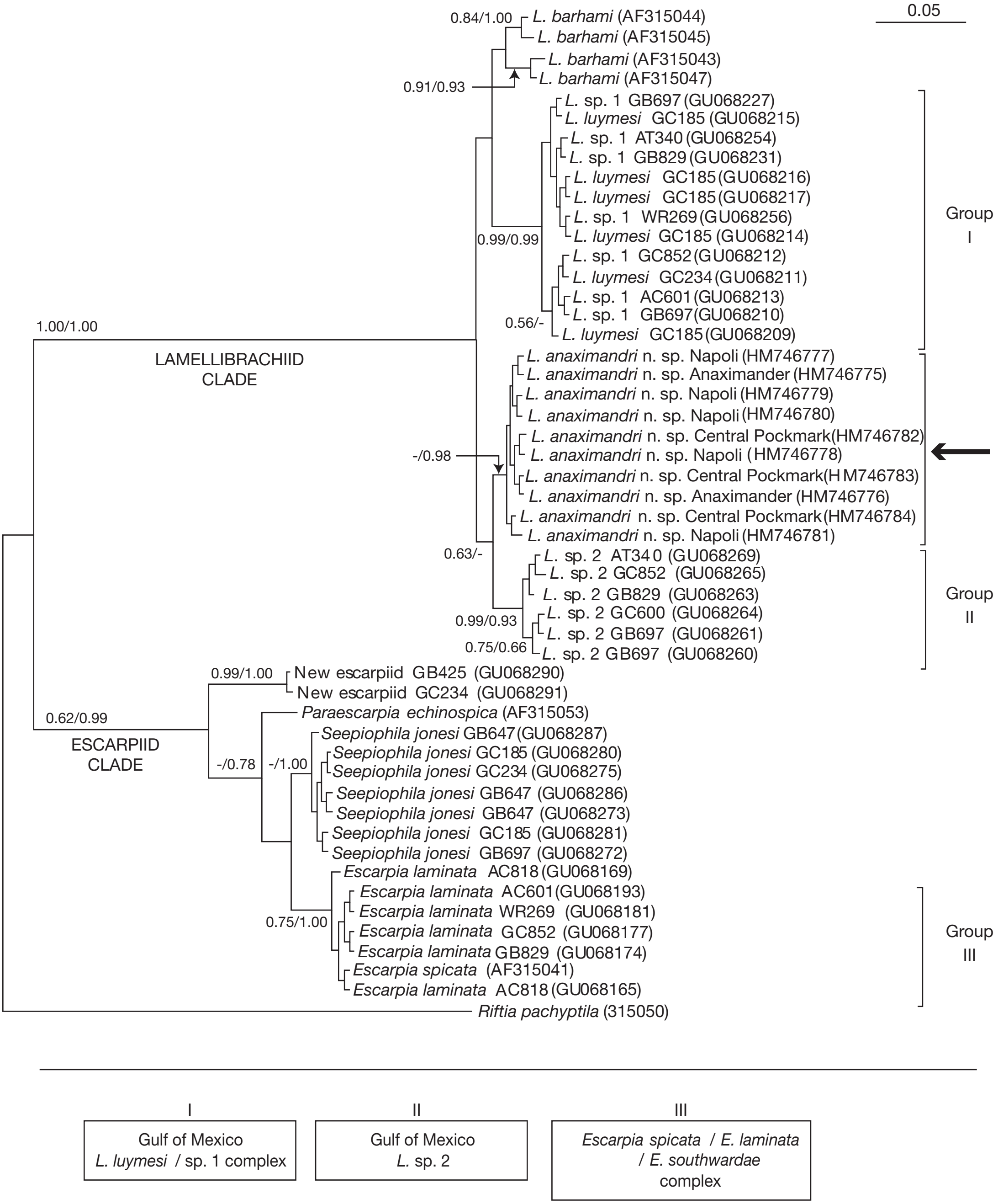


FIG. 15. — Phylogeny of cold seep vestimentiferan species. Tree constructed by a Bayesian approach based on a 461-bp alignment of the partial mitochondrial 16S sequence. For each branch, bootstrap values for 1000 replicates for the Neighbour-Joining method with Kimura-2-parameter distances are indicated (number after the "/" sign), along with Bayesian posterior probability values (number before the "/" sign). *Riftia pachyptila* was used as an outgroup. Only values greater than 50% bootstrap or 0.50 posterior Bayesian probability are shown. "-" indicates values smaller than 0.50. Sampling locations are given after the species name for each individual sequence when possible and accession numbers in GenBank are given for each sequence (between parentheses). The large arrow indicates the position of *Lamellibrachia anaximandri* n. sp. group.

80 µm) and the size is consistent among 12 large specimens. *Lamellibrachia luymesi* from the Gulf of Mexico has vestimental plaques 55 to 60 µm in diameter and trunk plaques 75 to 85 µm, very similar to those of *L. anaximandri* n. sp. *Lamellibrachia columna* has slightly larger plaques, particularly on the trunk. *Lamellibrachia barhami* has the largest plaques of these three (up to 160 µm on the trunk). In conclusion, the new species is closest morphologically to *Lamellibrachia luymesi*, but its tube lacks the wide and obvious collars of the Gulf of Mexico specimens. However, the tube of the holotype of *L. luymesi* from Guyana (Fig. 16C) is similar to that of *L. anaximandri* n. sp. Differences between the animals are the slightly shorter vestimental region and the fewer branchial lamellae of *L. anaximandri* n. sp. The morphological closeness to *L. luymesi* concurs with the phylogenetic analysis that places *L. anaximandri* n. sp. with *L. luymesi*, *L.* sp. 1, and *L.* sp. 2 from the deep Gulf of Mexico (Figs 14; 15). The observed sequence differences between *L. anaximandri* n. sp. and the other species, however, are greater than typical within-species distances (see Miglietta *et al.* 2010) and support the new species status of *L. anaximandri* n. sp. All mt16S sequences for *L. anaximandri* n. sp. from the three locations sampled in the Mediterranean form a monophyletic cluster, with structure that reflects the geography. There is therefore no molecular evidence that animals from these different areas belong to distinct species. The possibility remains though, that the areas are inhabited by different species that do not differ in either mtCOI or mt16S sequences. This was observed for *L. luymesi* and *L.* sp. 1 that inhabit different depth ranges in the Gulf of Mexico (group I in Figs 14 and 15) and differ morphologically (Miglietta *et al.* 2010). This situation was also observed for the genus *Escarpia* (group III in Figs 14 and 15). However, co-occurring species such as *L.* sp. 1 and *L.* sp. 2 in the deep Gulf of Mexico usually differ genetically for the molecular markers we sequenced. Interestingly, all the Gulf of Mexico and the Mediterranean species tend to group together. Cordes *et al.* (2007) have outlined the potential for larval dispersal from east to west and west to east in the equatorial region between the Gulf of Mexico, Caribbean and West African seeps. Olu-Le Roy *et al.* (2007) similarly showed that there are two amphi-atlantic complexes of species in the genus *Bathymodiolus*, based on both morphological and molecular data. Unfortunately, only a portion of the nuclear 28S gene (less variable than mt16S and mtCOI) has been sequenced for the Vigo worm (Williams *et al.* 1993), preventing any comparisons with our samples.

In the overall phylogeny, *L. juni* clearly forms a separate cluster from all other species of *Lamellibrachia*, as reported earlier by Miura & Kojima (2006). The relationship of the Atlantic cluster (*L. luymesi, L.* sp. 1, *L.* sp 2, and *L. anaximandri* n. sp.) with the other species of *Lamellibrachia* remains unclear because of the short branch lengths in the cluster excluding *L. juni*. These short branches are not due to the saturation of the phylogenetic signal (COI is used to study the phylogeny of all the vestimentiferan tubeworms; see McMullin *et al.* 2003) and could indicate either a slow-down of evolutionary rates around that time or a rapid radiation of the populations to form the current genetic species. A slow-down of the evolutionary rate could be attributed to the extreme longevity of *Lamellibrachia* (Bergquist *et al.*, 2000) and explain the lack of genetic differences between *L.* sp. 1 and *L. luymesi*. Although the deep branches are not well supported, the fact that *L. luymesi* and *L. anaximandri* n. sp. form a monophyletic cluster, with all other species more basal, suggests that the genus *Lamellibrachia* most likely originated in the Pacific and later colonized the Atlantic region. Based on these data, however, the actual path from the Pacific to the Atlantic remains unclear. Collections from additional locations such as the Indian Ocean would be necessary to address this question.

NOTES ON FRENULATE POGONOPHORES

Frenulate pogonophores are scarcely known from the Mediterranean and may be unfamiliar to biologists working there. Ivanov (1970) described the hair-like *Siboglinum carpinei* and noted the presence of other species near Corsica. IFREMER has collected some specimens from just east of the Gibraltar Strait

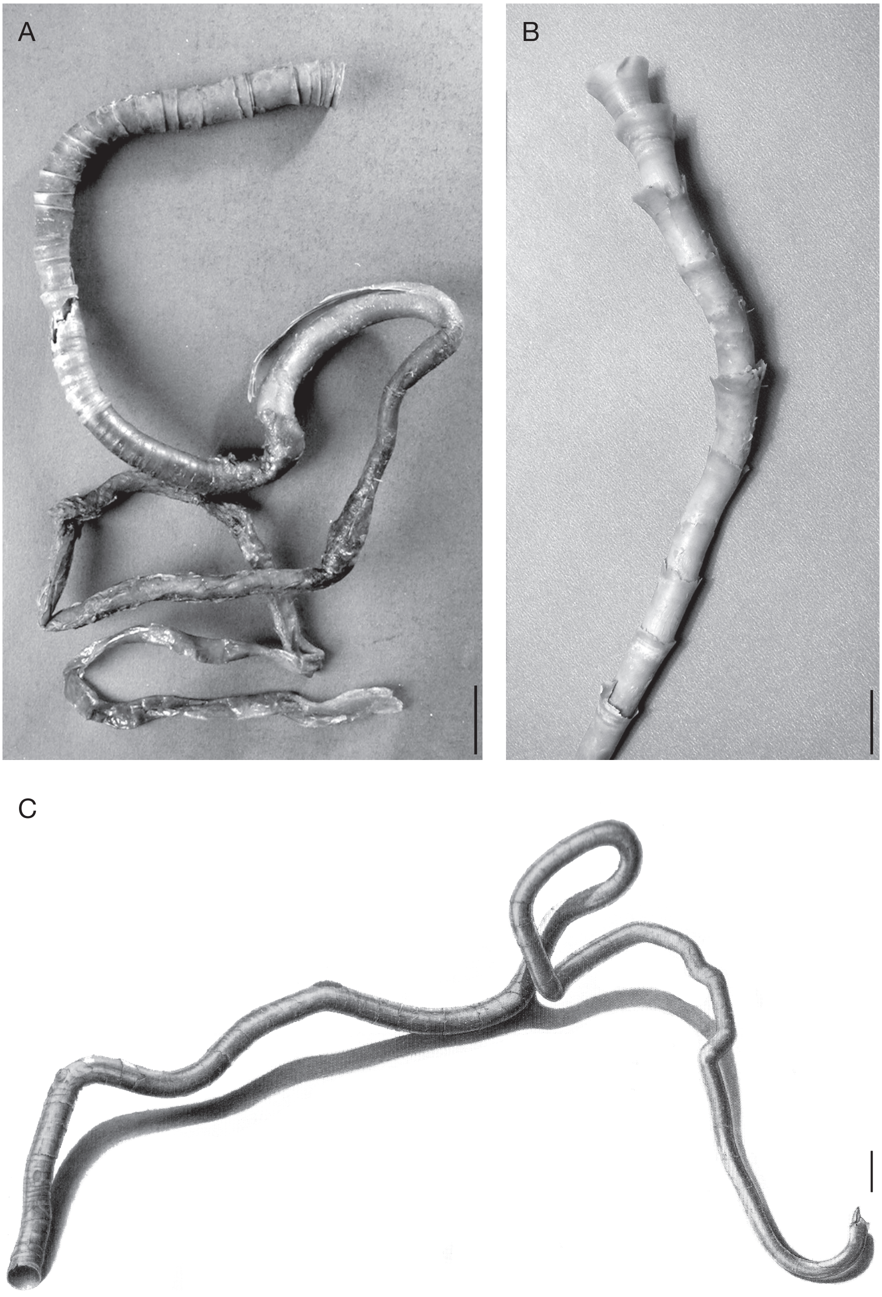


FIG. 16. — **A**, tube of the *Lamellibrachia* sp. (Vigoworm) from the wreck of the “François Vieljeux”; **B**, tube of *Lamellibrachia luymesi* Van der Land & Nørrevang, 1975, from Green Canyon 234, Gulf of Mexico, 540 m (Gardiner & Hourdez 2003; specimen #15 provided by Dr S. L. Gardiner); **C**, tube of the holotype of *Lamellibrachia luymesi* from Guyana (fig. 124 from Van der Land & Nørrevang 1977, with permission from the Royal Danish Academy of Sciences and Letters). Scale bars: 10 mm.

(BALGIM 1984, ECS, unpublished). Outside the Mediterranean, on the European Atlantic continental slope, there are about 20 species in the Bay of Biscay (Southward 1979) and at least 15 in the Gulf of Cadiz (Hilário *et al.* 2010).

The tubes of frenulates are unbranched and almost cylindrical, tapering slightly toward the posterior end. The tube diameter of most species is between 0.1 and 1 mm; the maximum is about 2.5 mm. The adult length ranges from about 100 mm for the smallest to more than 1 m for the largest. Many species have flexible tubes but some are rigid. The animals can move up and down as they secrete their tube, laying down layers from the inside. The tube wall material is fibrous chitin in a protein matrix, providing strength and elasticity. The wall is often composed of narrow dense rings separated by transparent interspaces (Fig. 17 A-C). In some species, short segments are formed as the tube is added to at the anterior end and sometimes these overlap like nested funnels. Tubes may be transparent and colourless or show various shades of yellow, red-brown or even black. The tube characters, including the diameter, are remarkably specific, though some changes occur as they grow and age (Ivanov 1963; Southward 1969; Southward *et al.* 2005).

Various fragments of brown tubes, found in sediment samples collected during MEDINAUT dives on mud volcanoes, have been identified as frenulate pogonophores. Some contain animals. These MEDECO specimens at present being investigated by Eve Southward at Plymouth will be returned to CENTOB, IFREMER, Centre de Brest, Plouzané, France.

POGONOPHORA, FRENULATA Webb, 1969
(= Polychaeta, Siboglinidae, frenulates Rouse, 2001)
Family SIBOGLINIDAE Caullery, 1914 (revised Ivanov 1963)
Genus *Siboglinum* Caullery, 1914 (revised Ivanov 1963)

TYPE SPECIES. — *Siboglinum weberi* Caullery, 1944. Redescribed by Southward (1961).

REMARKS

The genus *Siboglinum* is characterized by a single anterior tentacle, with or without pinnules and without epidermal glands; the anterior part of the trunk carries two rows of papillae, containing pyriform glands, almost always without adhesive plaques; there are two or three girdles (annuli) of chaetae; the postannular part of the trunk carries ventral papillae, singly or in small groups, opposed by large dorsal glandular shields; the spermatophores are spindle-shaped; the tube is usually ringed, and sometimes also segmented.

Siboglinum* cf. *carpinei

Specimens resembling *Siboglinum carpinei* Ivanov, 1970 (type locality near Corsica) were collected from Milano mud volcano (Table 1, dive MN5). The tubes are 0.10 to 0.12 mm in diameter, with regular, narrow, reddish-brown rings separated by colourless interspaces (Fig. 17A, B). The anterior part of the tube is divided into segments with 7 to 9 rings, demarcated by slight constrictions of the tube wall (Fig. 17A, C). The animals have a single pinnulate tentacle, a forepart about 0.5 mm long and three girdles (annuli) of chaetae. Some tube fragments from Amsterdam MV (Table 1, dive MN8) are similar in size and pattern. Fragments of wider tubes (0.27 to 0.3 mm diameter) also found at Milano Mud Volcano (Table 1, dive MN5), are uniformly dark brown and lack any trace of rings or segments. The difference in size and colour from the tubes of the suspected *Siboglinum carpinei* suggests that they belong to a second frenulate species, perhaps of another genus. It is not possible to make a more definite identification, based on these tubes alone.

Genus 2, unidentified

One 14 cm long piece of straight tube, 1.14 to 2.0 mm in diameter, was found in an unlabelled MEDINAUT sample of *Lamellibrachia anaximandri* n. sp. The tube wall is brown, uniformly smooth-surfaced and without funnels or segments. In size and general appearance it resembles the tubes of both *Lamellisabella denticulata* Southward, 1978 and *Spirobrachia tripeira* Hilário &

Cunha, 2008, two multitentaculate frenulate species found recently in the Gulf of Cadiz, just outside the Mediterranean, at about 3000 m depth on a mud volcano (Hilario & Cunha 2008). It is not possible to make a definite identification based on this tube alone, but it does indicate that one of the larger frenulate tubeworms exists in the eastern Mediterranean.

DISCUSSION

Ecology

Lamellibrachia anaximandri n. sp. lives in environments fairly rich in dissolved methane (Werne *et al.* 2004). However, its dependence on sulfide-oxidation is supported by our TEM observations and the stable carbon and nitrogen isotope ratios (δ^{13}C -26.6 to -23.6‰, δ^{15}N -0.8 to 0.2‰), reported by Fiala-Medioni *et al.* (2001) for specimens from the Anaximander region. Duperron *et al.* (2009), investigating *Lamellibrachia* from the Nile fan region, have found that the principal endosymbiont is a gammaproteobacterium closely related to sulfide-oxidising symbionts in other vestimentiferans.

The tubes of *L. anaximandri* n. sp. have been seen on various substrates, always in close association with carbonate crusts, sometimes in tubular channels in these crusts (Olu-Le Roy *et al.* 2004). Young tubes are attached to crusts, larger tubes are more scattered and their posterior ends extend some distance into sediment. Clusters of hundreds of individuals were observed on the Amsterdam and Kazan mud volcanoes. The populations were densest on Amsterdam MV, which emits the greatest flux of methane of the investigated mud volcanoes south of Crete and Turkey. This species was also observed on the Kula MV and along fractures of Faulted Ridge in the Anaximander area and on the Milano and Napoli mud volcanoes in the Olimpi area (Olu-Le Roy *et al.* 2004). The other fauna includes chemosynthetic bivalves, *Lucinoma kazani* (up to 40 mm long) and four smaller species: *Idas modiolaeformis*, *Myrtea amorpha*, *Thyasira striata* and *Isorropodon perplexum*, all less than 20 mm long, as well as small frenulate pogonophores. All these species live in muddy sediments and contain sulfur-oxidizing symbionts (Olu-Le Roy *et al.* 2004). *Idas modiolaeformis* contains both thiotrophic and methanotrophic symbionts (Duperron *et al.* 2008). The tubes of *Lamellibrachia anaximandri* n. sp. are smooth-surfaced and lack epifauna (Figs 3; 11). A few species of mobile gastropod are present on crusts around the tubes and a limpet (*Lepetella* sp.) has been found on the tubes (Olu-Le Roy *et al.* 2004), but the associated fauna is very impoverished compared with the diverse and abundant communities associated with the vestimentiferan clusters of the shallow and deep seeps of the continental slope in the Gulf of Mexico (Cordes *et al.* 2007, 2010).

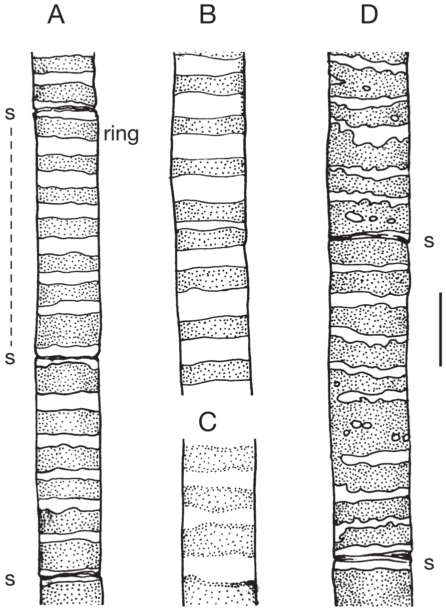


Fig 17. — *Siboglinum* cf. *carpinei* tubes from dive MN5 (Milano MV): **A**-**C**, anterior, middle and posterior regions of one tube, 0.10 mm diameter; **D**, anterior region of a second tube, 0.12 mm diam. The doted line represents the segment length. Abbreviation: **s**, segment boundary. Scale bar: 0.1 mm.

The lifespan of the vestimentiferans in the Mediterranean is unknown, but could be long. It is known that *Lamellibrachia luymesi* grows very slowly in the Gulf of Mexico and that its growth rate varies considerably according to the availability of sulfide and the age of the individual (Fisher *et al.* 1997; Bergquist *et al.* 2002). A lifespan of 100 to 150 years has been estimated for the longest individuals of *L. luymesi*, so a lifespan of the same order would not be surprising in *L. anaximandri* n. sp.

DISTRIBUTION

In a review of the biogeography and phylogeny of vestimentiferans, McMullin *et al.* (2003) have shown that *Lamellibrachia* is the most widely distributed of the genera; it also has a significantly slower rate of COI evolution than the vent vestimentiferans and *Escarpia*. However, there are many large blank areas in the distribution maps of vestimentiferans because the discovery and exploration of seep habitats is difficult and relies mainly on geological and economic interests. The Mediterranean is now receiving attention. All our specimens of *Lamellibrachia anaximandri* n. sp. have come from the eastern basin of the Mediterranean, from depths of 1100 to 3000 m. However, vestimentiferans are known to occur in the Tyrrhenian Sea (video-recording by Teschfilm, Germany, courtesy of Christian Lott). They were seen on a submarine volcano north of Sicily. The videos show clumps of tubeworms with red plumes and white obturacula settled on rock, close to white patches of bacterial filaments. More recently, a shipwreck colonized by tubeworms has been found in the southern Tyrrhenian Sea, south of Italy, and COI analyses show a close similarity to *L. anaximandri* n. sp. (Gambi *et al.* 2011).

During the Messinian salinity crisis, the Mediterranean more or less dried out and the earlier deep-water fauna was lost (Hsu *et al.* 1977). Atlantic deep-water species recolonized the deep basins about 5 million years ago. The present deep-water benthic fauna remains an impoverished version of the Atlantic fauna with apparently very few recently evolved Mediterranean species (Fredj & Laubier 1985; Bouchet & Taviani 1992; Krönke *et al.* 2003). Conditions are oligotrophic and warm (13-14°C) compared with the Atlantic Ocean at the same depths. Supposing that a *Lamellibrachia* species entered the Mediterranean about 5 million years B.P., has it given rise to the new species *L. anaximandri* n. sp. since then, or did *L. anaximandri* n. sp. already exist in the eastern Atlantic?

The only record of *Lamellibrachia* on the eastern side of the Atlantic is from the wreck of the *François Vieljeux*, a ship that sank off the north-west coast of Spain, near Vigo in 1979 and was salvaged in 1991 (Dando *et al.* 1992). One of the Vigo worm tubes is illustrated in Figure 16A. The tubes are more than one metre long and have a maximum anterior diameter of 12 mm. They must have grown to this large size in about 10 years, presumably having found sufficient dissolved sulfide in the decaying sisal, beans and sunflower seeds packed on top of the copper bars in the cargo. They have conspicuous anterior funnels with collars (Fig. 16A), unlike *L. anaximandri* n. sp., but more like *L. luymesi* from the Gulf of Mexico (Fig. 16B). The analysis by Williams *et al.* (1993) of 28S rDNA from decaying tissue in the Vigo tubes indicated a small difference between the Vigo worm and a Gulf of Mexico *Lamellibrachia* specimen collected from 600 m on the Louisiana slope.

The equatorial dispersal of seep mussels across the Atlantic is a possibility considered by Cordes *et al.* (2007), since mussel larvae can have a long planktonic life. However, a larval survival of a few weeks is known for vestimentiferans (Marsh *et al.* 2001) i.e., the energy reserves of a non-feeding larva of *Riftia pachyptila* suffice for only about 38 days, making this transatlantic route seem improbable for *Lamellibrachia* species.

The subsurface "Mediterranean water" flowing from the Gibraltar Strait, through the Gulf of Cadiz and around the continental margin of Spain and Portugal (Zenk & Armi 1990) extends over the position of the wreck of the *Francois Vieljeux*, but it is not likely that the larval colonists of the wreck could have been carried 1000 km directly from the Mediterranean. It is possible that *Lamellibrachia anaximandri* n. sp. may be found in suitable habitats within the influence of this current outside the Mediterranean. For example, 50 mud volcanoes have been found in the Gulf of Cadiz, in depths ranging from 200 to 4000 m (Van Rensbergen *et al.* 2005; Van Roij *et al.* 2005). Recent DNA analysis of samples of frenulates from 13 of these mud volcanoes indicates that 15 taxa are present (Hilário *et al.* 2010).

To the south, on the Mauritanian slope, off Banc Arguin at 18°40N, 16°45W a diverse fauna has been sampled, including a seep mussel, *Bathymodiolus mauritanicus* Cosel, 2002, indicating the presence of active seeps at about 1200 m depth (Cosel 2002). Off the Nigerian coast at depths of 1700 to 2200 m, seeps with *Bathymodiolus* species exist at the coordinates 4°59N, 4°08E (Cordes *et al.* 2007). In the area of the

Congo River Canyon, several pockmarks with active seepage are known, such as REGAB (Olu-Le Roy *et al.* 2007) at 5°47S and 9°42E at 3148 m depth, housing the vestimentiferan tubeworm *Escarpia southwardae* Andersen, Hourdez, Marie, Jollivet, Lallier & Sibuet, 2004 (Andersen *et al.* 2004). Closer to the Congo river fan at a depth of about 3100 m (4°48S, 9°52-56E) three pockmarks (Wormhole, Black Hole and Hydrate Hole) have been surveyed and big vestimentiferan tubeworms are present, probably *Escarpia southwardae* (Sahling *et al.* 2008).

We would like to know whether the new Mediterranean species, *Lamellibrachia anaximandri* n. sp., lives outside the Mediterranean in the eastern Atlantic Ocean and the Lusitanean and West African margins are certainly promising regions to look for it.

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