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The occurrence of *Natsushima bifurcata* (Polychaeta: Nautiliniellidae) in *Acharax* hosts from mud volcanoes in the Gulf of Cadiz (south Iberian and north Moroccan Margins)

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SUMMARY: Fifteen specimens of nautiliniellid polychaetes were found in the mantle cavity of bivalves of the genus *Acharax* (Solemyidae) collected in several mud volcanoes from the Gulf of Cadiz. They were identified as *Natsushima bifurcata* Miura and Laubier (1990), a species previously known only from Sagami Bay (Japan). The morphological comparison of these specimens with the holotype suggests that they belong to the same morphological species, although further studies should consider whether they could be genetically different. The ecology of the species is discussed in relation to the available information on the family. The occurrence of *Natsushima bifurcata* in the Gulf of Cadiz is the first record of the species in the North Atlantic.

Keywords: *Natsushima bifurcata*, Gulf of Cadiz, mud volcanoes, Polychaeta.

RESUMEN: APARICIÓN DE *NATSUSHIMA BIFURCATA* (POLYCHAETA: NAUTILINIELLIDAE) EN HUÉSPEDES DEL GÉNERO *ACHARAX* DE VOLCANES DE FANGO EN EL GOLFO DE CÁDIZ (MÁRGENES SUR DE LA PENÍNSULA IBÉRICA Y NORTE DE MARRUECOS). – Quince ejemplares de poliquetos pertenecientes a la familia Nautiliniellidae se encontraron en la cavidad del manto de bivalvos del género *Acharax* (Solemyidae) procedentes de varios volcanes de fango del Golfo de Cádiz. Los ejemplares se identificaron como *Natsushima bifurcata* Miura y Laubier (1990), una especie citada únicamente de la bahía de Sagami (Japón). El presente trabajo analiza la morfología de los ejemplares de Cádiz en comparación con la del holotipo y sugiere que ambas poblaciones pertenecen al mismo morfotipo, sin que no por ello se pueda descartar la existencia de diferencias en el componente genético. Asimismo, se discuten aspectos ecológicos basados en la información disponible sobre la familia. La aparición de *Natsushima bifurcata* en el Golfo de Cádiz representa la primera cita para el Atlántico Norte.

Palabras clave: *Natsushima bifurcata*, Golfo de Cádiz, volcanes de fango, Polychaeta.

INTRODUCTION

Miura and Laubier (1990) used the names Nautiliniellidae and *Nautiliniella* to replace the homonyms Nautilinidae and *Nautilina* (Miura and Laubier, 1989), because the genus name had already been occupied by a cephalopod mollusc. At present, this small family of uncommon polychaetes includes fifteen species ascribed to eleven genera found in the

mantle cavity of deep-sea bivalve molluscs of the families Solemyidae, Mytilidae, Thyasiridae and Vesicomidae (Miura and Hashimoto, 1996; Dreyer *et al.*, 2004). Nautiliniellids are mainly restricted to Pacific hydrothermal vents and cold seeps (Blake, 1993; Miura and Hashimoto, 1993, 1996; Miura and Laubier, 1989, 1990; Miura and Ohta, 1991). Up to now, only two species, *Petrecca thyasira* Blake, 1990, found in the gill filaments of *Thyasira insignis*

specimens from the Laurentian Fan, and *Vesicomycicola trifurcatus* Dreyer, Miura and Van Dover, 2004, found in the mantle cavity of the vesicomycid clams from the Blake Ridge cold seep off the coast of South Carolina, are known to occur in the Atlantic Ocean (Blake, 1990; Dreyer *et al.*, 2004). Nautiliniellids are commonly quoted as commensals or parasites (Blake, 1990; Miura and Laubier, 1990) but the nature of the association between the polychaetes and their bivalve hosts is still unclear.

Communities of benthic animals associated with cold seeps are known from several locations on active and passive continental margins of the Pacific and have recently been discovered also in the Atlantic Ocean (Olu-Le Roy *et al.*, 2004). The occurrence of mud volcanism, cold seepage, hydrocarbon venting and gas hydrates in the Gulf of Cadiz has been intensively investigated since 1996 (e.g. Pinheiro *et al.*, 2003; Van Rensbergen *et al.*, 2005). Within the framework of the UNESCO/IOC Training Through Research Programme, seven research cruises were conducted for this specific purpose: TTR9 (1999), TTR10 (2000), TTR11 (2001), TTR12 (2002), TTR14 (2004), TTR15 (2005) and TTR16 (2006).

The solemyid bivalve *Acharax* sp. is one of the most common species of the chemoautosynthesis-based assemblage of the mud volcanoes from the Gulf of Cadiz (Rodrigues and Cunha, 2005). In three of these mud volcanoes some of the bivalve specimens were found to host in their mantle cavity nautiliniellid polychaetes identified as *Natsushima bifurcata* Miura and Laubier (1990), previously known only from Sagami Bay (Japan). These recently collected specimens verify the original concept of the species and provide the first record of *N. bifurcata* for the North Atlantic.

MATERIAL AND METHODS

Study site

The Gulf of Cadiz is located at the crossroads of the European and African Atlantic margins and the Mediterranean. The compression between the Eurasian and African tectonic plates creates an interesting geophysical template shaped by volcanic activity and by the interaction between the topography and the circulation of the Atlantic and Mediterranean Ocean Waters. Geologically, the set-

ting of the Gulf of Cadiz is extremely complex and still under debate but one of the most important structures is a large olistostrome complex emplaced in an accretionary wedge-type environment. (Sartori *et al.*, 1994; Maldonado *et al.*, 1999; Gutscher *et al.*, 2002). Since the discovery of the first mud volcano in 1999, about 30 other sites at depths ranging from 200 to 4000 m, with varying degrees of hydrocarbon-rich gas seepage activity, have been located and sampled (Pinheiro *et al.*, 2003; Van Rensbergen *et al.*, 2005).

Collection of samples

Samples were collected during the TTR cruises (Training Through Research Programme, IOC-UNESCO) onboard the RV Prof. Logachev. A TV-assisted grab was used to locate interesting sampling sites in the target mud volcanoes. Whenever *Acharax* specimens were collected, usually from sites within the crater of active mud volcanoes, they were opened and examined onboard for the presence of nautiliniellid polychaetes. The biological material was preserved in 70 or 96% ethanol (the latter will be used for future molecular analysis).

SYSTEMATICS

Family NAUTILINIELLIDAE Miura and Laubier, 1989
Natsushima Miura and Laubier, 1990

Natsushima bifurcata Miura and Laubier, 1990
(Fig. 1)

Natsushima bifurcata Miura and Laubier, 1990: 322-323, Fig. 2 a-f; Miura and Hashimoto, 1996: 265-266.

Type material. Holotype (NSMT-Pol. H-294), complete; Pacific Ocean, off Hatsushima, Sagami Bay, 34°00.0'N, 139°13.8'E, 1170 m, 19 November 1987, deep-sea submersible "Shinkai-2000" Dive 315; collected from the mantle cavity of *Acharax* sp.

Other material. 1 complete specimen (USNM 172135); Pacific Ocean, off Hatsushima, Sagami Bay, 34°58.12'N, 139°31.24'E, 1114 m, 17 May 1989, Dolphin 3000, collected from the mantle cavity of *Acharax johnsoni*. 3 complete specimens (DBUA 00711), Atlantic Ocean, Gulf of Cadiz, Jesus Baraza mud volcano, 35°35.439'N, 7°12.264'W, 1105 m, TTR12 station AT391GR, 9 July 2002; 1 damaged specimen (DBUA 00765), same locality, Yuma mud volcano, 35°24.973'N, 7°05.461'W, 960 m, TTR14 station AT524GR, 2 August 2004; 4 complete specimens (DBUA 00791-01), same locality, same mud volcano, 35°25.820'N, 7°06.330'W, 1030 m, TTR16 station AT604GR, 29 May 2006; 4 complete and 1 incomplete specimens (DBUA 00791-02), same locality, same mud volcano, 35°25.046'N, 7°05.450'W, 975 m, TTR16, AT605GR, 29 May 2006; 2 complete specimens (DBUA 00791-03) same locality, Ginsburg mud volcano, 35°22.677'N, 7°04.979'W, 920 m, TTR16 station AT607GR, 29 May 2006. All

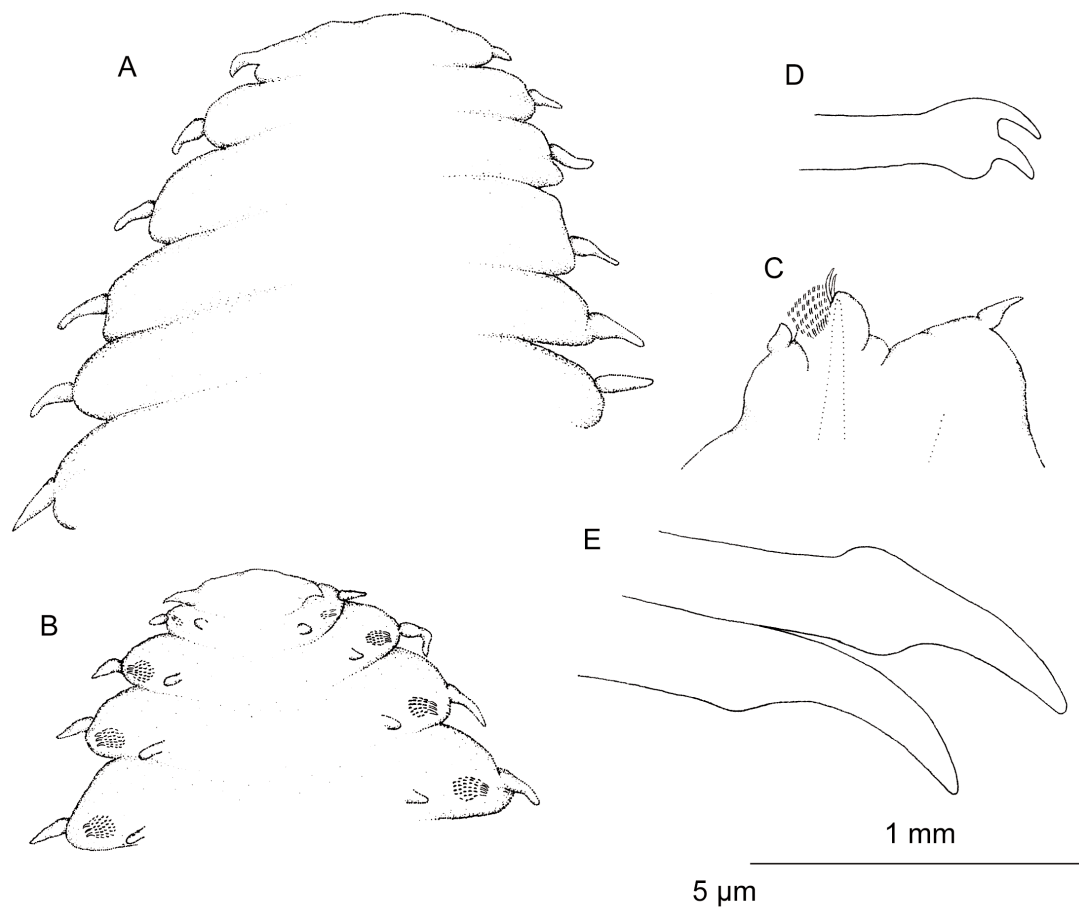


FIG. 1. – *Natsushima bifurcata* DBUA 00711: A, anterior region, dorsal view; B, anterior region, ventral view; C, 29th parapode, antero-posterior view; D, neuropodial bifurcate setae; E, neuropodial hooks.

specimens from Gulf of Cadiz were collected onboard the RV *Prof. Logachev* with a TV-assisted grab, and were found inside the mantle cavity of *Acharax* sp.

Diagnosis. Body vermiform, flattened ventrally and arched dorsally, with fairly uniform width throughout. Short prostomium, much wider than long, slightly incised anteriorly, with only a pair of lateral antennae and without eyes. Tentacular segment partially fused to prostomium, with dorsal and ventral cirri, neuroaciculae, and few neurosetae. Parapodia sub-biramous, with well-developed dorsal and ventral cirri. Notopodia short conical, similar throughout the body, with slender notoacicula and without setae. Neuropodia cylindrical, with neuroacicula and numerous setae of two kinds – up to 4 (usually 3) stout slightly curved hooks placed more dorsally and more than 100 smaller bifurcate setae placed ventrally.

Supplementary description. Largest specimens measure 17 mm in length and ~3 mm in width, for 72 setigers. Colour in life pink, and after preservation white.

Remarks. The original description of the holotype mentions that the specimens were found inside *Solemya* sp. bivalves (Miura and Laubier, 1989: 322) but the identification of the bivalve was corrected to *Acharax johnsoni* in a subsequent work by Ohta (1990, cited in Miura and Hashimoto, 1996: 266).

The specimens from the Gulf of Cadiz are larger than the ones from the type locality (Table 1) and in some neuropodia (Fig. 2) there are 4 hooks (up to 3 in the original description). The diagnosis of the species was updated accordingly.

Ecology. The host bivalve, *Acharax* sp. has a wide distribution in the Gulf of Cadiz: it has been recorded from ten mud volcanoes in the Moroccan and Portuguese margins at depths varying from 358 to 3902 m. The nautiliniellid polychaetes were found in the mantle cavity, near the gill filaments, of *Acharax* specimens collected from the Jesús Baraza, Yuma and Ginsburg mud volcanoes, all located in the Western Moroccan field within a bathymetric range restricted to 920 and 1105 m (Fig. 3). At these

TABLE 1. – Measurements of the specimens examined from the Gulf of Cadiz (GoC) and Sagami Bay (SB). JB, Jesús Baraza mud volcano; Y, Yuma mud volcano; G, Ginsburg mud volcano. Width includes parapodia.

Specimens	N° of setigers	Total length (mm)	Width (mm)	Length of dorsal cirri(mm)
GoC-JB1	72	17.0	3.0	0.15
GoC-JB2	67	17.0	3.0	0.15
GoC-JB3	75	12.0	2.1	0.15
GoC-Y1	>60	~9.6	1.4	0.15
GoC-Y2	71	8.2	1.6	0.14
GoC-Y3	99	18.0	2.1	0.17
GoC-Y4	79	16.0	1.8	0.17
GoC-Y5	40	9.3	1.6	0.17
GoC -Y6	50	8.6	1.4	0.17
GoC-Y7	72	13.9	1.4	0.12
GoC-Y8	71	17.0	2.1	0.17
GoC-Y9	55	8.8	1.4	0.15
GoC-Y10	48	7.7	1.4	0.15
GoC-G1	80	14.9	1.3	0.12
GoC-G2	88	21.4	3.2	0.17
SB	70	15.0	1.5	0.14
SB-holotype	47	5.0	0.6	0.07

three mud volcanoes, observed infestation rates varied from 12.5 to 75% and the number of nautilinielid individuals found in each bivalve varied from 1 to 3 (Table 2). The infested *Acharax* specimens measured between 3.2 and 6.9 cm (total length).

Distribution. East Pacific, Sagami Bay: Hatushima and Okino-yama cold seeps, 1114-1170 m,

inside the mantle cavity of *Acharax johnsoni* Dall, 1891. North Atlantic, Gulf of Cadiz: Jesús Baraza, Yuma and Ginsburg mud volcanoes, 920-1105 m, inside the mantle cavity of an undetermined *Acharax* species.

DISCUSSION

The genus *Natsushima* comprises at present two described species, *N. bifurcata* Miura and Laubier, 1990 and *N. graciliceps* Miura and Hashimoto, 1996. The two species differ by the presence of respectively short conical and elongated notopodia in the middle segments, by the presence of a fine embedded notoacacula in the former and by the different size of dorsal and ventral cirri, which are smaller in the latter.

Both Sagami Bay specimens of *N. bifurcata* occur in cold seeps associated with Solemyid bivalves at depths of around 1000 m, and the specimens recently collected in the Gulf of Cadiz corroborate this information. In fact, it is noteworthy that despite the wider bathymetric range of *Acharax* in the Gulf of Cadiz (358-3902 m), up to now only the populations from the mud volcanoes of the Western Moroccan field, located at depths of 920-1105 m, were found to be infested by nautilini-

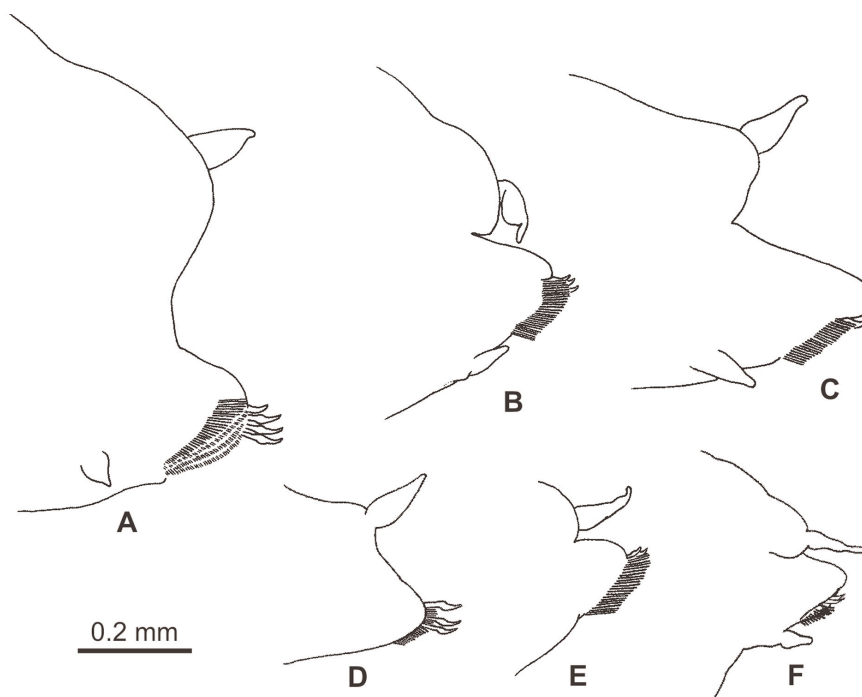


FIG. 2. – Median parapodes: specimens A, B and C collected in the Gulf of Cadiz, in July 2002, DBUA 00711; specimen D from Sagami Bay, USNM 172135; specimen E collected in Gulf of Cadiz, in August 2004, DBUA 00765; Hototype F, from Sagami Bay, NSMT-Pol. H-294, re-illustrated from Miura and Laubier (1990).

TABLE 2. – Infestation rates; number of specimens of *Natsushima bifurcata* and *Acharax* sp. collected in each mud volcano (Number of *N. bifurcata* in each host *Acharax* in brackets).

Mud volcanoes	Depth (m)	<i>N. bifurcata</i> n. of spec.	<i>Acharax</i> n. of spec.	n. infested <i>Acharax</i>	Infestation rate (%)
Mercator	358	0	15	0	0
Fiuza	414	0	2	0	0
Kidd	489	0	40	0	0
Meknes	701	0	10	0	0
Ginsburg	920	2	8	1 (2)	12.5
Yuma	960	10	13	5 (1/2/2/2/3)	38.5
Jesús Baraza	1105	3	4	3 (1/1/1)	75.0
Darwin	1115	0	2	0	0
Carlos Ribeiro	2230	0	1	0	0
Porto	3902	0	1	0	0

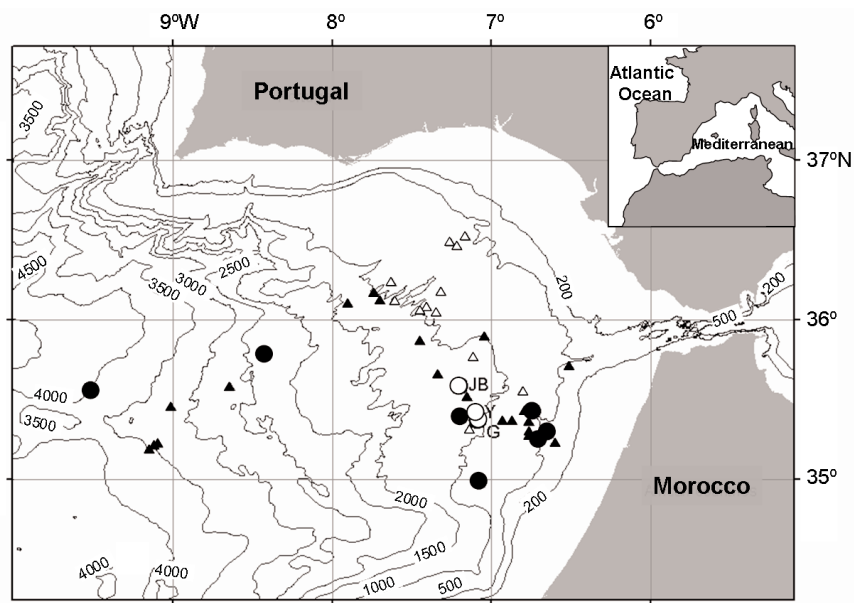


FIG. 3. – Map of the study area: full triangles - sampled sites; empty triangles – other known mud volcanoes; full circles – sites with *Acharax* populations; empty circles – sites with infested *Acharax* populations. JB, Y and G – Jesús Baraza, Yuma and Ginsburg mud volcanoes.

ieillids (up to 75% infestation rate). The majority (69.8%) of the 96 *Acharax* specimens were collected at the four shallowest mud volcanoes (358-701 m) but none of these specimens were infested. The populations from the three deepest mud volcanoes (1115-3902 m) were also not infested but the number of specimens collected was much lower (only 4.2% of the total).

According to Neulinger *et al.* (2006), *Acharax* species differentiation based on shell morphology is likely to underestimate true species diversity within this taxon. A phylogenetic study based on some *Acharax* specimens from localities of the Pacific and Indian oceans (Aleutian Trench, off Oregon, Costa Rica, and Peru margins, and off Makran, and Java) resulted in two clusters that group populations located far apart (Makran, Oregon, and Peru in one cluster and Java, Aleutian Trench and Costa Rica in the other). These authors

propose that the specimens are representatives of at least 2 different species and do not all belong to *A. johnsoni*, as assumed previously.

These results emphasize the need for further morphological and probably also genetic studies to solve taxonomic affinities within *Acharax* populations. Until then, we cannot assume that the Gulf of Cadiz and Sagami Bay specimens belong to the same species but nor can we discard this possibility. The same applies to the *Natsushima* specimens. We could not find morphological evidence to support the establishment of a new species. The comparison with the holotype only revealed differences in the proportions of the specimens (Fig. 2; Table 1). The largest specimens from the Gulf of Cadiz are 2 to 3 times larger than the holotype, the setigers are broader and the parapodia, including cirri, are thicker. The holotype seems to have proportionally longer and thinner dorsal cirri and antennae, and it is

also smaller. This different appearance is due to the more corpulent body of the larger specimens, resulting in proportionally broader and shorter cirri. In support of this, medium-sized specimens, such as the DBUA 00765 from the Gulf of Cadiz, and the USNM 172135 from Sagami Bay, show “intermediate” morphological features between the larger, more corpulent specimens and the holotype. It is possible that *N. bifurcata* from Sagami Bay and the specimens from Gulf of Cadiz are cryptic species but this question can only be answered with more knowledge on reproductive patterns and by the analysis of DNA sequences. As the preservation of the Japanese specimens does not allow DNA analysis, we must consider that both the Cadiz and the Sagami specimens belong to the same morphological species.

There are several cases of specimens from distant locations of other deep sea species that are ascribed to the same morphological species. An example is the commensal polychaete *Branchipolynoe seepensis* Pettibone, 1986 that was recorded in several bivalve host species both in the Gulf of Mexico and in the mid-Atlantic Ridge. In this case, subsequent studies carried out by Chevaldonné *et al.* (1998) determined the genetic divergence for the COI and 16S rDNA genes and showed that the Atlantic and Pacific populations should be considered as two isolated phylogenetic species.

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