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Cold seep biogenic carbonate crust in the Levantine basin is inhabited by burrowing *Phascolosoma* aff. *turnerae*, a sipunculan worm hosting a distinctive microbiota☆

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ABSTRACT

Biogenic calcium carbonate crusts represent a cryptic habitat that is often associated with hydrocarbon seeps. Most biological observations of these crusts concern the external surfaces and the fauna inhabiting their inner cavities are generally neglected. Exposed carbonates in areas of active seepage at the 1100-m-deep base of the Palmachim slumping feature in the Levantine basin are intensively burrowed by metazoans, especially by sipunculans (peanut worms), identified by genetic and morphological markers as a potentially novel *Phascolosoma* sp., closely related to *Phascolosoma turnerae* (Rice, 1985) and named here *P.* aff. *turnerae*. Bacterial 16S-based tag encoded FLX amplicon pyrosequencing (bTEFAP) was utilized to analyze the bacterial community associated with *P.* aff. *turnerae*. We compared the bacterial community structure in *P.* aff. *turnerae* to the bacterial community structure associated with the sediment–water interface in adjacent gas seeps and in biofilm covering the carbonate crust hosting the sipunculan. A distinctive microbiota, capable of chemosynthesis and sulfide detoxification, was found in association with *P.* aff. *turnerae*.

1. Introduction

The phylum Sipuncula includes approximately 150 species that thrive across a wide range of depths and habitats in the world's oceans (Cutler, 1994). Currently there are 36 sipunculan species reported in the Mediterranean Sea (Ferrero-Vicente et al., 2012), twenty of which are known in the eastern Mediterranean Sea (Murina et al., 1999). Sipunculans have attracted the attention of the scientific community because they are a significant fishery resource in China (Ying et al., 2010) and an important part of the food chain in various marine habitats (Cutler, 1994; Kędra and Włodarska-Kowalczuk, 2008; Kohn, 1970; Levin, 2005). Their phylogenetic affinity has been considered to lie with the mollusks and annelids, but sequence analysis of various genetic markers has suggested that they are closely related to annelids (Schulze et al., 2007; Shen et al., 2009). Recent comprehensive analyses of annelid and general metazoan phylogeny has verified this hypothesis (Dunn et al., 2008; Struck et al., 2007, 2011; Weigert et al., 2014). Sipunculan taxonomy has been hindered by their conservative morphology and many species appear to have cosmopolitan distributions (Cutler, 1994; Kawauchi and Giribet, 2010). Several studies have therefore used molecular tools to explore their systematics (Kawauchi and Giribet, 2010; Kawauchi et al., 2012; Maxmen et al., 2003; Schulze et al., 2007).

Sipunculans often inhabit cryptic habitats and therefore are neglected in faunal surveys (Schulze, 2005); these habitats include wood falls (Bienhold et al., 2013; Rice, 1985), a decaying whale skull at a depth of 880 m (Gibbs, 1987) and rhizopod tests (Gooday, 1984). Several species of sipunculans are known to make burrows in calcium carbonate structures (Klein et al., 1991; Rice, 1969; Stearley and Ekdale, 1989). Specimens of *Phascolosoma turnerae*, a species usually collected from submerged wood and fibrous plant

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materials, were found within Gulf of Mexico carbonate rocks (Rice et al., 2012). Both chemical and mechanical excavation of the substrate has been suggested (Rice, 1969; Williams and Margolis, 1974).

Sipunculans have a strong resistance to extreme temperatures and some other environmental factors (Chew et al., 1994; Ying et al., 2010). It has been suggested that these characteristics may be related to the functions of coelomocytes, the immune system cells present in celom cavities of sipunculans (Ying et al., 2010). *Phascolosoma arcuatum* thrives in sulfide – rich mangrove environments and may possess sulfide detoxification mechanisms (Ip et al., 1997). Several studies describe sipunculans associated with cold seeps as well as sulfide – rich habitats (Levin et al., 2000, 2010; Levin, 2005; MacAvoy and Morgan, 2008). Isotopic studies have shown that sipunculans are heterotrophic and do not rely on endosymbiotic autotrophs, as do various siboglinids and molluscs from cold seeps (Levin, 2005; MacAvoy and Morgan, 2008).

During the November 2011 leg of the Exploration Vessel *Nautilus* cruise in the Levantine basin of the Mediterranean Sea (Coleman et al., 2011, 2012) we collected samples of biogenic calcium carbonate rock associated with a hydrocarbon seep. The rock was characterized by numerous burrows containing sipunculans resembling *P. turnerae*, named here *P.* aff. *turnerae*. To date, there is only one record of *P. turnerae* in the Mediterranean Sea, from off the Iberian Peninsula (41°07′N;0 2°25′E; depth 1100 m) (Coll et al., 2010). In this paper, we describe morphology and phylogenetic affiliation of the eastern Mediterranean *P.* aff. *turnerae* specimens and examine a distinctive microbiota associated with the sipunculans.

2. Materials and methods

2.1. Sample collection and preservation

The samples were collected during the 2011 E/V *Nautil*us field season. E/V *Nautilus* is equipped with *Hercules* and *Argus* Remotely Operated Vehicle (ROV) systems that are able to collect high-resolution video, oceanographic data, and conduct precise sampling. The samples were collected at a gas seep located at the base of the Palmachim disturbance slumping feature (32°08.6643′N 34°08.5391′E) at a depth of 1121 m (Fig. 1). The sipunculan specimens were extracted from burrows in biogenic carbonate rock. The specimens were studied onboard, two specimens were preserved in liquid nitrogen for DNA analysis, and several were preserved in 1:1 seawater: 4% formaldehyde solution. The microbial biofilm (approximately 0.5 ml) was collected from the calcium carbonate surface and flash – frozen in liquid nitrogen for further analysis. The sediment was sampled using 20 cm long, 7 cm diameter short cores at two adjacent locations: 32°09.6328′N 34°10.1081′E, at a depth of 1032 m and 32°08.9668′N 34°07.6117′E, at a depth of 1134 m. The sediments were immediately sliced into 1 cm sections and subsamples were flash-frozen in liquid nitrogen for further analysis.

2.2. Imagery and video

Microscopic observation of live specimens was performed onboard the ship immediately after recovery using a Dino-Lite

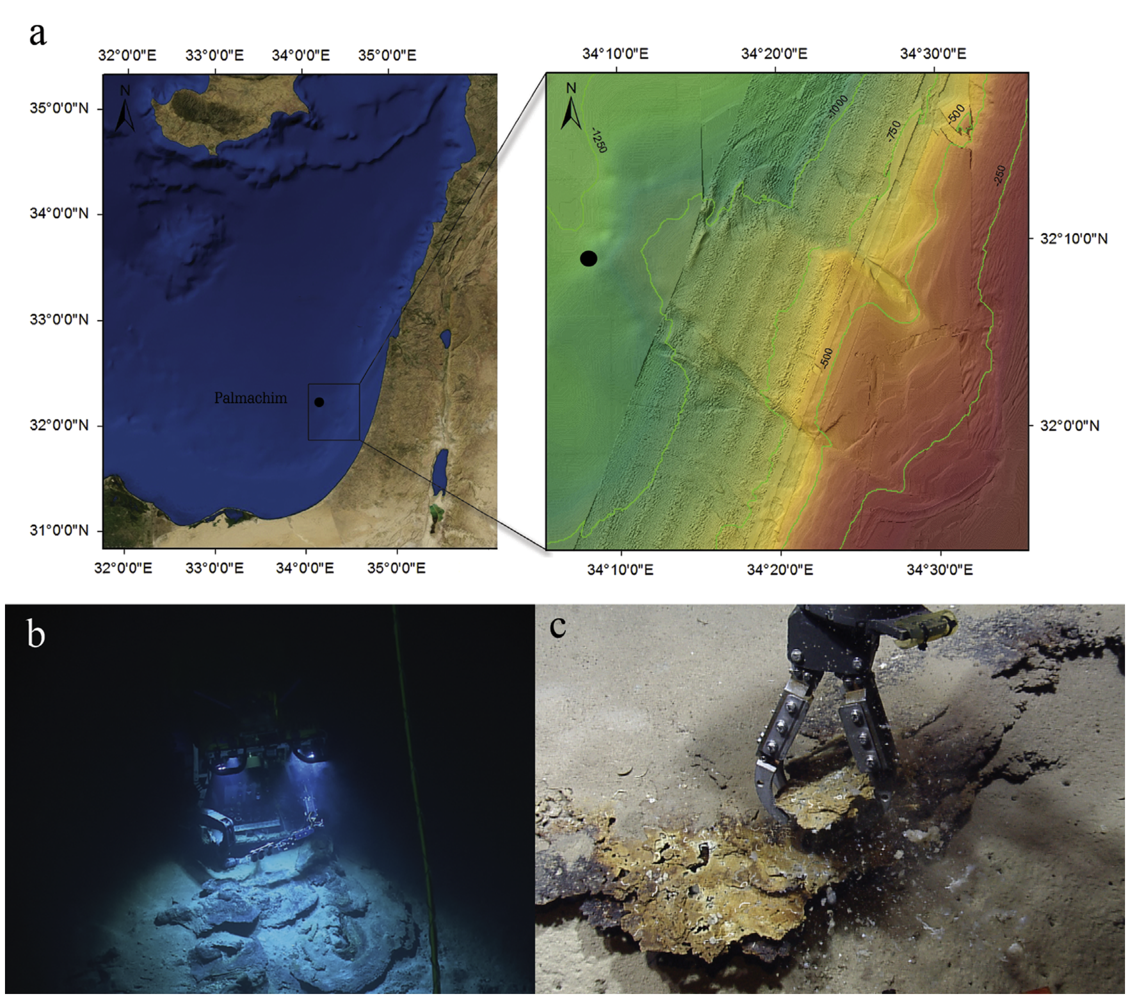

Fig. 1. The collection site for the carbonate crust inhabited by sipunculans. (a) The location of cold seep at the toe of Palmachim disturbance where the carbonate crust was collected, (b) the *Hercules* ROV hovering above the biogenic carbonate crust at the Palmachim disturbance and (c) collection of the soft carbonate crust by the ROV's arm.