Cah. Biol. Mar. (2010) 51 : 475-478

Presence of Abyssoanthus sp. (Anthozoa: Zoantharia) in the Mediterranean Sea: an indication of non-dependence of *Abyssoanthus* to chemosynthetic-based ecosystems?

Frederic SINNIGER[1,2], Kevin A. ZELNIO[3], Marco TAVIANI[4] and James Davis REIMER[5,6]
(1) *Departement of Marine Science, University of the Ryukyus, 1 Senbaru, Nishihara, 903-0213 Okinawa, Japan*
(2) *Department of Oceanography, Florida State University, OSB, 117 North Woodward St., Tallahassee, FL 32306, USA*
Tel: +1 850 644 6700, Fax: +1 850 644 2581. Email: fredsinniger@hotmail.com
(3) *Duke University, Duke University Marine Lab, 135 Duke Marine Lab Rd., Beaufort, NC 28516, USA*
(4) *ISMAR, Consiglio Nazionale delle Ricerche, via Gobetti 101, Bologna, 40129, Italy*
(5) *Marine Biodiversity Research Program, Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, Japan*
(6) *Molecular Invertebrate Systematics and Ecology Laboratory, Rising Star Program, Transdisciplinary Research Organization for Subtropical Island Studies (TRO-SIS), University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

Abstract: Zoanthids are present in most marine environments, especially where there are hard substrates available. A new species, genus and family (*Abyssoanthus nankainensis* Reimer and Fujiwara 2007, family Abyssoanthidae) have been recently described from a cold seep setting at a depth > 3000 m in the Nankai Trench, Pacific Ocean off Japan. A molecularly and morphologically similar zoanthid was also found in the Japan Trench and morphologically similar zoanthids were found associated with cold seep environments in the Gulf of Mexico (Atlantic Ocean). Some minute zoanthid polyps and tissues fragments were collected in 2004 in the bathyal plain of the western Mediterranean Sea, in an environment not known to be chemosynthetically active. Their minute size (< 2 mm in diameter) and sand coating made it impossible to identify these specimens morphologically so they were submitted to biomolecular analyses. DNA was extracted and the large mitochondrial ribosomal subunit gene (mt 16S rDNA) was amplified and sequenced. Surprisingly, the sequences obtained showed clear similarities between the deep Mediterranean sea zoanthid and the putative cold-seep zoanthid *A. nankaiensis*. Beside documenting an almost cosmopolitan distribution of *Abyssoanthus* these results could indicate (1) the previously uncharted presence of a bathyal cold seep site in the western Mediterranean Sea or (2) alternatively that this genus is not strictly dependent upon chemosynthetic environments. We favour the second hypothesis because the possibility of a cold seep setting at this specific Mediterranean site does not seem substantiated by any morphological, physical or biological evidence.

Keywords: Zooanthid ● Deep-sea ● DNA barcoding● Cold seep

Reçu le 16 février 2010 ; accepté après révision le 28 juin 2010.
Received 16 February 2010; accepted in revised form 28 June 2010.

Introduction

Zoanthids (order Zoantharia) are colonial anthozoans almost ubiquitous in the marine environment (Sinniger et al., 2010). In spite of their relative abundance, zoanthids have been overlooked by scholars because of the intrinsic difficulty in establishing a sound taxonomy based on external morphologic criteria. However, the advances in zoanthid molecular phylogeny and DNA taxonomy (Reimer et al., 2007, Sinniger et al., 2008, Sinniger & Häusserman, 2009) have clarified the relationship between the different groups within the order and contributed to emphasize the importance of the ecological characterisitcs in zoanthid taxonomy (Sinniger et al., 2010). The most common and diverse deep-sea zoanthids are members of the genus *Epizoanthus* which are associated with pagurid shells (Ryland et al., 2000) or hexactinellid stalks (Beaulieu, 2001) and are distributed from a few hundred to thousands of meters of depth. It is only recently that a new zoanthid species, genus and family (*Abyssoanthus nankaiensis* Reimer & Fujiwara 2007, family Abyssoanthidae) were described from a cold seep off Japan (Reimer et al., 2007). The family description of Abyssoanthidae refers to the cold seep environment where this zoanthid was found. Another molecularly and morphologically similar, although slightly larger, zoanthid was also found in a cold seep environment in the Japan Trench. Chemosynthesis-based environments have often shown highly adapted biological communities with surprising biomass considering the extreme ecological conditions (Laubier, 1993). DNA evidence shows that a specimen collected in the Mediterranean Sea, at a location without known chemosynthetic activity, is closely related to *A. nankaiensis*. Here we present the preliminary results of our investigation of the relationship between this Mediterranean zoanthid and the different specimens found in chemosynthetic environments elsewhere in the world.

Materials and Methods

Sampling

The Mediterranean zoanthid was collected ≈ 2000 m depth using an Agassiz trawl during the COBAS cruise (RV *Urania,* Station 38, 36°56.0135’N-01°21.7587’W, 2193-1823 m: picked by H. Zibrowius). Japanese *Abyssoanthus* samples were collected using the Shinkai 6500 submersible (RV *Yokosuka*, dives #884, 32°34.945’N-134°41.545’E, 3259 m, and dives #1038 and #1041, 39°06.50’N-143°53.4’E, 5347-5360 m). Cold seep and *Lophelia*-reef associated zoanthids in the Gulf of Mexico were collected on the Upper Louisiana Slope in 2006 during the Expedition to the Deep Slope (from 26°10.8’N-94°37.4’W to 28°21.4’N-88°47.5’W, 2744-1399 m). Zoanthid specimens were fixed and preserved in ethanol (minimum 70%) after collection.

DNA extraction and sequencing

DNA was extracted from ethanol-preserved samples using the DNeasy Plant Minikit (QIAGEN) or a guanidium thiocyanate extraction protocol (Sinniger et al., 2010). Specimens were then amplified for partial mt 16S rDNA using standard Taq polymerase and primers described in Sinniger et al. (2005). Sequencing was carried out using a BigDye Terminator Cycle Sequencing Ready Reaction Kit following the manufacturer instructions (Applied Biosystems) for both strands of each marker. Sequences were run on an ABI-3100 Avant automatic sequencer and are publicly available (GenBank accession numbers HM588669-HM588674).

Sequence analyses

Sequences were inserted in the alignment previously used in Sinniger et al. (2010) and manually aligned using BioEdit v.5.0.9 (Hall, 1999). Alignments were analysed with the Maximum Likelihood (ML) method using PhyML v 3.0 (Guindon & Gascuel, 2003). Analyses were performed with GTR nucleotide substitution matrix, a gamma 1 invariant model with six categories, estimated parameter and estimated frequencies of amino acids. Species belonging to the macrocnemic family Epizoanthidae were used as outgroups.

Results

Due to the minute size of the Mediterranean specimen and the presence of sediment incrustation on the column of the zoanthid, no histological or detailed morphological examinations could be performed. Partial mt 16S rDNA sequences from both Japanese specimens (*A. nankaiensis* and Japan Trench zoanthid) show only one variable site, while the Mediterranean sample displays a few more diverging sites, mainly located in polyC and polyG regions. Two other zoanthids found on tubeworms and *Lophelia* in chemosynthetic environments in Gulf of Mexico show clearly distinct sequences. Phylogenetic analyses place the Mediterranean zoanthid closely related to *A. nankaiensis* and the Japan Trench specimen, while the two Gulf of Mexico zoanthids appear to be related to Epizoanthidae and Parazoanthidae, respectively (fig. 1).

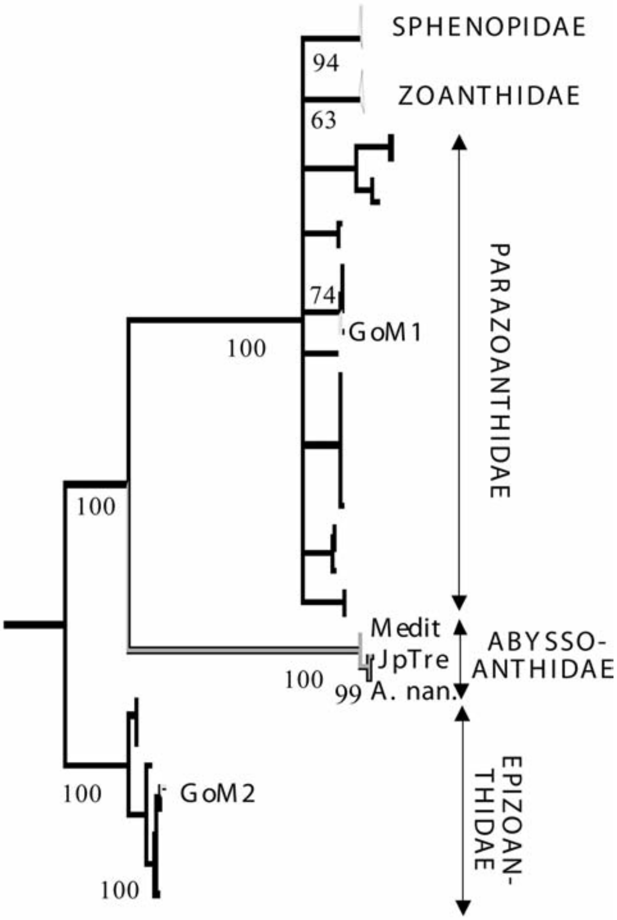

Figure 1. 16S Maximum Likelihood phylogenetic tree indicating the position of the Mediterranean zoanthid and the other "chemosynthetic" specimens among different zoanthid families. GoM1 and 2 indicate Gulf of Mexico specimens, Medit indicate the Mediterranean specimen, JpTre indicate the Japan Trench specimen and A. nan. indicates *Abyssoanthus nankaiensis*.

Figure 1. Arbre phylogénétique construit sur le principe du maximum de vraisemblance avec le gène 16S montrant la position du zoanthaire méditerranéen et d'autres spécimens "chimiosynthétiques" parmi différentes familles de zoanthaires. GoM1 et 2 désignent les spécimens du Golfe du Mexique, Medit le spécimen de Méditerranée, JpTre celui de la fosse du Japon et A. nan. désigne *Abyssoanthus nankaiensis*.

Discussion

Until recently, the only known deep sea zoanthids were a few epizoic species associated with hexactinellid stalks or pagurid shells, which belong to known families such as Parazoanthidae or Epizoanthidae. The recent discovery of a very divergent group of zoanthids presumably associated with different chemosynthetic environments has led to the hypothesis that some zoanthids may have evolved in association with chemosynthetic environments (Reimer et al., 2007), as documented for many other vent/seep type taxa (i.e. Kojima et al., 2001). These environments were also proposed as a potential character for the family Abyssoanthidae (Reimer et al., 2007). However, the demonstration that the communities composition and trophic pattern of deep coral communities adjacent to seepage are distinct from seep communities (Cordes et al., 2008; Becker et al., 2009) and the discovery of an *Abyssoanthus*-related zoanthid within a non-chemosynthetic environment led to a reconsideration of the initial hypothesis. The taxonomic position of the zoanthids collected in the Gulf of Mexico seems to confirm the independence of deep-coral-associated zoanthid and chemosynthetic communities. While the potential for chemosynthetic activity at such western Mediterranean Sea cannot be completely discard, by examining the fauna at the sampling location and additional zoanthids from chemosynthetic environments a picture emerges that members of this group may be seep colonists as defined in Carney (1994). The occurrence of zoanthids in chemosynthetic environments may simply be related to the presence of hard substrates suitable for colonisation combined with high localised primary production. Apparently similar zoanthids to the one found in the western Mediterranean and in Japan were found associated with a chemosynthetic environment in eastern Mediterranean Sea (identified as *Isozoanthus* sp. in Ritt et al., 2010) and in the Eastern Lau Spreading Center (Zelnio et al., 2009). Obtaining DNA from this zoanthid and the Lau Basin specimen will help to clarify the relationship between different zoanthids and evaluate the chemosynthetic environment association hypothesis. Stable isotope analyses such as those performed in Becker et al. (2009) could also help understanding the relationship of these zoanthids to chemosynthesis. Additionally, the lack of records for *Abyssoanthus*-like zoanthids in non-chemosynthetic environments may be due to the bias of exploration towards geologically active deep sea areas and further sampling in non chemosynthetic environment will be essential to determine the endemic or colonist status of organisms found in chemosynthetic environments as suggested in Carney (1994).

Acknowledgements

The authors wish to thank the respective captains and crews of the research vessels involved in the sampling/ observations of the deep sea zoanthids discussed here. Special acknowledgement to Dr. Helmut Zibrowius who found the Mediterranean zoanthid and identified it as a zoanthid during the COBAS cruise, and to Dr. Tom Schultz for the use of materials and facilities at the Duke Marine

Laboratory. F. Sinniger was funded by the JSPS post-doctoral fellowship and grant in aid, K. Zelnio by a TAWNI award (ChEss) and the Marine Conservation Molecular Facility at Duke Marine Lab, and J.D. Reimer by the Rising Star Program at the University of the Ryukyus. Zoanthids collected from the Gulf of Mexico was supported through grants and subcontracts to C.R. Fisher, E.E. Cordes, and TDI Brooks Prime from the Mineral Management Service and NOAA Ocean Exploration. This is a contribution to EU Hermione programme (contract number 226354) and ISMAR-Bologna scientific contribution n. 1671.

References

Beaulieu S.E. 2001. Life on glass houses: sponge stalk communities in the deep-sea. *Marine Biology,* **138**: 803-817.

Becker E.L., Cordes E.E., Macko S.A. & Fisher C.R. 2009. Importance of seep primary production to *Lophelia pertusa* and associated fauna in the Gulf of Mexico. *Deep-Sea Research I,* **56**: 786-800.

Carney R. 1994. Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. *Geo-Marine Letters,* **14:** 149–159.

Cordes E.E., McGinley M.P., Podowski E.L., Becker E.L., Lessard-Pilon S., Viada S.T. & Fisher C.R. 2008. Coral communities of the deep Gulf of Mexico. *Deep-Sea Research I,* **55**: 777-787.

Guindon S. & Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology,* **52**: 696-704.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**: 95-98.

Kojima S., Ohta S., Yamamoto T., Miura T., Fujiwara Y. & Hashimoto J. 2001. Molecular taxonomy of vestimentiferans of the western Pacific and their phylogenetic relationship to species of the eastern Pacific. I. Family Lamellibrachiidae. *Marine Biology,* **139**: 211-219.

Laubier L. 1993. Les oasis éphémères des abysses: la fin d'un paradigme. *La Recherche,* **24**: 855-862.

Reimer J.D., Hirano S., Fujiwara Y., Sinniger F. & Maruyama T. 2007. Morphological and molecular characterization of *Abyssoanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a northwest Pacific methane cold seep. *Invertebrate Systematics,* **21**: 255-262.

Ritt B., Sarrazin J., Caprais J-C., Noël P., Gauthier O., Pierre C., Henry P. & Desbruyères D. 2010. First insights into the structure and environmental setting of cold-seep communities in the Marmara Sea. *Deep-Sea Research I,* **57**: 1120-1136.

Ryland J.S., de Putron S., Scheltema R.S., Chimonides P.J. & Zhadan D.G. 2000. Semper's (zoanthid) larvae: pelagic life, parentage and other problems. *Hydrobiologia,* **440**: 191-198.

Sinniger F., Montoya-Burgoss J.I., Chevaldonne P. & Pawlowski J. 2005. Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on mitochondrial ribosomal genes. *Marine Biology,* **147**: 1121-1128.

Sinniger F., Reimer J.D. & Pawlowski J. 2008. Potential of DNA Sequences to identify zoanthids (Cnidaria: Zoantharia). *Zoological Science,* **25**: 1253-1260.

Sinniger F. & Häussermann V. 2009. Zoanthids (Cnidaria: Hexacorallia: Zoantharia) from shallow waters of the southern Chilean fjord region, with descriptions of a new genus and two new species. *Organisms Diversity and Evolution,* **9**: 23-36.

Sinniger F., Reimer J.D. & Pawlowski J. 2010. The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: Description of two new genera. *Marine Biodiversity,* **40**: 57-70.

Zelnio K.A., Rodriguez E. & Daly M. 2009. Hexacorals (Anthozoa: Actiniaria, Zoanthidea) from hydrothermal vents in the south-western Pacific. *Marine Biology Research,* **5**: 547-571.