Siboglinidae: a model system for the understanding of evolution, adaptive radiation, microbial symbioses and ecology at extreme environments

Report on the ChEss workshop held

27 – 31 October 2008

at

East-West Centre, Honolulu, Hawaii

Workshop participants, back row left to right: *Yoshi Fujiwara, Irmgard Eichinger, Caroline Verna, Cris Little, Dominique Cowart, Monika Bright, Dan Thornhill*

Second row from back left to right: Florence Pradillon, *Craig Young, Maria Pia Miglietta, Sigrid Katz, Helena Wiklund, Nadya Rimskaya-Korsakova*

Third row from back left to right: *Ken Halanych, Andrea Nussbaumer, Clara Rodrigues, Chuck Fisher, Ann Andersen, Maria Capa, Iris Altamira, Nadezhda Karaseva*

Front row left to right: *Adrian Glover, Ana Hilário, Thomas Dahlgren, Craig Smith, Nick Higgs*

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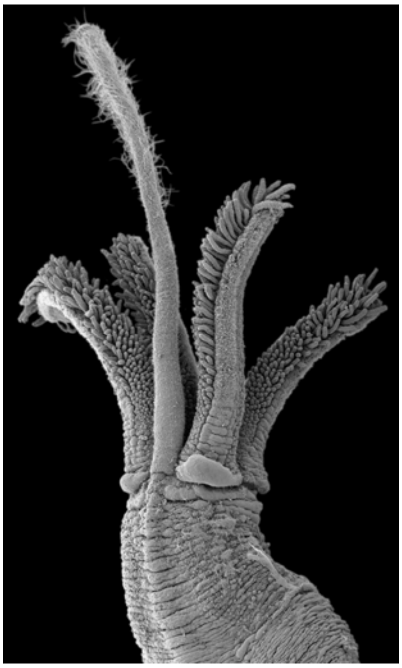
1. Aims and objectives

Recent years have seen a revolution in our understanding of Siboglinidae – an enigmatic clade of deep-sea worms known from extreme deep-sea environments such as hydrothermal vents, cold hydrocarbon seeps and the carcasses of dead whales. Molecular systematic methods now place the former phyla Pogonophora and Vestimentifera within the highly derived polychaete clade Siboglinidae. Within the last 4 years, an entirely new radiation of siboglinids, *Osedax*, has been discovered living on whale-carcasses. New advances in our knowledge of siboglinid anatomy coupled with molecular characterization of microbial symbiont communities is revolutionizing our knowledge of host-symbiont relationships in the metazoa. The unique evolutionary association of siboglinids with both geology, in the formation of spreading centres and seeps, and biology with the evolution of large whales, offers opportunities for studies of vicariant evolution and the calibration of molecular clocks.



Osedax mucofloris, scanning electron micrograph (Photo: A Glover)

Despite these advances, there has been a lack of synthesis between research teams working on different aspects of Siboglinidae. The mud-dwelling frenulates remain poorly studied. Vent and seep siboglinid research has largely been separated from that going on within the polychaete community, and the recent discoveries from whale-falls need to be integrated with that from vents and seeps. The goals of our workshop were to:

- Present and discuss the latest research on siboglinids within a small, informal workshop/discursive environment
- Inspire and train young researchers in the evolution and ecology of siboglinids from extreme environments

- Highlight taxonomic gaps and develop new break-out teams with future taxonomic exchange proposals to CoML
- Integrate systematic, ecological and microbial research using Siboglinidae as a model system
- Develop an outline and writing-tasks for a large multi-author review paper for *PLOSOne* to coincide with CoML 2009 events

2. Organising committee

Dr Adrian Glover, The Natural History Museum, London; **Dr Ana Hilário**, University of Aveiro; **Dr Thomas Dahlgren**, University of Göteborg; **Dr Maria Baker**, University of Southampton; **Dr Eva Ramirez Llodra**, Institute of Marine Sciences, Barcelona; **Dr Craig Smith**, University of Hawaii

3. Summary of presentation sessions

The workshop was attended by 27 people, from 10 different countries (USA, Sweden, Australia, France, Japan, Germany, UK, Russia, Portugal, Austria) and 17 different institutions. During the course of the workshop, additional scientists from the local Kewalo Marine Lab, University of Hawaii Zoology Department and the Oceanography Department also attended sessions. The four day workshop was structured into both presentation and more general discussion sessions, and the meeting began on the morning of Tuesday 28 October, after a mixer social event the evening before at the Kaimana Hotel, where the majority of participants were staying.

Session 1 – taxonomy. The first session was designed to outline some of the very recent discoveries of novel species and habitats. Yoshi Fujiwara (JAMSTEC) outlined recent discoveries from studies of whale-fall ecosystems off the coast of Japan. Maria Pia Miglietta (PSU) outlined discoveries from the Gulf of Mexico, and showed how molecular evidence was providing conflicting evidence for understanding the broad biogeographic trends in species such as *Lamellibrachia* and *Escarpia*. Adrian Glover (Natural History Museum) presented a paper on a new species *Osedax* from Southern Californian whale-falls, as well as summarizing our taxonomic knowledge to date for the group. Ana Hilário (University of Aveiro) talked about recent taxonomic discoveries, including a new genus, from the mud volcanoes of the Gulf of Cadiz.

Session 2 – evolution. The second session was designed to highlight recent studies in the field of phylogenetics and fossil evidence. Cris Little (University of Leeds) opened the session with an overview of the fossil record showing that there were likely siboglinids at Mesozoic seeps and vents and that similar fossils from these environments in the Palaeozoic hint at an even older origin for the group. Maria Capa (University of Sydney) then provided an overview of the phylogenetic position of Sibgolinidae including considerable weight of evidence from the closely-related Sabellidae polychaete clade. Ken Halanych (Auburn University) finally provided an overview of the latest molecular evidence on the phylogeny of siboglinids.

Session 3 – anatomy and physiology. Owing to the great interest in siboglinids in terms of their nutrition, habitat and ecology, considerable work has been done on their anatomy and physiology, in fact more than most other polychaetes. This session was designed to showcase the latest results in this area. Chuck Fisher (PSU) demonstrated the physiological diversity of the group, outlining the latest data from both seep species (e.g quantifying the process of sulphide uptake in *Lamellibrachia*) and showing how new data on the multiple growth forms of some well known vestimentiferans, as well as new very small vestimentiferans, will alter our understanding of the evolution of novel physiology in the group. Ann Andersen (University of Paris VI – Station Biologique of Roscoff) reviewed our knowledge of hemoglobins in siboglinids within a phylogenetic framework. Nadya Karaseva (Shirshov Institute) reviewed some of the ultra-structural characters in *Oasisia* and *Riftia* and outlined some of the changes in the ideas concerning homology in the group since the acceptance of the annelid ancestry in the group. Monika Bright (University of Vienna) reviewed our knowledge of the evolution of the trophosome, and proposed several evolutionary models for the last common stem species of siboglinids. Irmgard Eichinger (University of Vienna) discussed new data concerning the origin and evolution of the trophosome in Sclerolinum, suggesting it was homologous with the trophosome of vestimentiferans. Sigrid Katz (University of Vienna) showed some of the first investigations of the large and complex trophosome of the recently discovered *Osedax* clade. Nadya Rimskaya-Korsakova showed some of the latest information on the microscopic anatomy of the well known species *Riftia pachyptila*.

Session 4 – symbiosis. The presence of microbial symbioses and in most cases, chemoautotrophy, is a key feature of siboglinids. The recent discovery of a putative heterotrophic symbiont in the trophosome (roots) of *Osedax* has recently spurred new investigations into symbiosis in siboglinids. Andrea Nussbaumer (University of Vienna) opened the session with a discussion of recognition processes between Vestimentifera and their symbionts. Dan Thornhill (Auburn University) showed new data on symbiont diversity from a phylogenetic point of view in the less well known frenulate group *Siboglinum*. Caroline Verna (MPI, Bremen) summarised the latest findings with regards the symbionts in *Osedax*, where a high diversity of symbionts has been identified. Clara Rodrigues (University of Aveiro) presented results from a study of the poorly-known symbionts from deep-water frenulates on the mud volcanoes of the Gulf of Cadiz. Ana Hilário presented a second paper on behalf of Eve Southward (who was unable to attend) on frenulate nutrition, in particular the idea that much of the nutrition for these animals may still come from uptake across the body walls and that the animals exhibit mixotrophy.

Session 5 – reproductive biology and ecology. The final session dealt with recent developments in reproductive biology and ecology. Craig Young (University of Oregon) outlined some of the latest results on the embryonic and larval development of *Lamellibrachia*, *Riftia* and two new species of *Osedax*. Craig Smith (University of Hawaii) outlined the distribution and habitat conditions for *Osedax* on a variety of whale-falls from Southern California showing that local ecological conditions may be very important in determining the role *Osedax* plays in whalebone decomposition.

Florence Pradillon (JAMSTEC) discussed new data from Japanese whale-falls, where eight different species of *Osedax* have now been recovered. Thomas Dahlgren (Göteborg University) presented the final paper of the meeting, on a new population of *Osedax* identified from bones recovered in a shrimp trawl in the North Sea, highlighting the population genetic evidence for a large *Osedax* population in the region.

4. Summary of discussion sessions

The discussion sessions were formed around smaller groups of 5-7 researchers who were organized into thematic groups, with the overall goals to:

- identify NEW collaborative projects
- identify NEW collaborative publications
- contribute sections to a joint review paper (PLOS one)

These sessions formed as follows:

- What are the **key questions?** (All participants)
- **Evolution** – H Wiklund, D Cowart, M Capa, M Bright, MP Miglietta, N Karaseva, N Rimskaya-Korsakova
- **Symbiosis** – A Nussbaumer, Y Fujiwara, D Thornhill, C Verna, C Rodrigues
- **Ecology** – A Glover, N Higgs, A Andersen, C Fisher, C Smith, K Halanych, S Katz
- **Dispersal & Biogeography** – C Young, A Hilário, F Pradillon, T Dahlgren, C Little, I Eichinger

Some of the key discussion points for the **evolution session** were:

- what is the relationship from population to species level
- what is the sister taxon to siboglinids?
- which genes and primers should be used? – lack of agreement
- poor knowledge of morphology, e.g. frenulates, sibog nervous systems, excretory systems - integration of these data informative
- what are the larvae like? - running out of methods of studying siboglinid larvae
- characters - can you track character evolution? can some characters be linked to symbiosis?
- new characters need to be assessed - e.g hemoglobin
- genera that are species rich are those with the least larval dispersal characteristics? OR is this speciation the result of niche separation?
- branch length and molecular clocks - e.g length of *Osedax* branches

Some of the key discussion points for the **symbiosis session** were:

- what are the symbionts doing?

- identify gene pathways for acquisition and maintanence of symbiosis
- symbiont diversity
- standardise methods between groups
- diversity of symbiosis types (host morphology, and the entire symbiont system)

Some of the key discussion points for the **ecology session** were:

- evolution of habitat use
- what is the current habitat of siboglinids? depth?
- why are vestimentifera not present in some habitats which appear suited chemistry-wise
- how has ecology driven speciation - what creates niche separation?
- spatial distributions on 1-100km scale?
- where do they fit in the food chain?
- competition with bacterial mat - succession (Tevnia->Riftia?) Not well understood.
- acquisition of symbionts - ecological reasons?

Some of the key discussion points for the **biogeography session** were:

- no vestimentiferans on MAR
- no seep vestimentiferans on some Atlantic seeps (Gulf of Cadiz) – the Vigo shipwreck paradox.
- larval dispersal must have a big control, no evidence for long-term planktotrophic dispersal in ANY siboglinid, the few species we know about have short dispersal times
- if lecithotrophic, how to explain the species-rich groups e.g *Osedax* and frenulates? The frenulates mostly brood (based on 2 species) - drive species diversity?
- why is *Osedax* so speciose? How often can you find a habitat that is suitable?
- vent sites are linear, whale-falls are how common along migration routes? *Osedax* may not last a long time on exposed whale bones
- temperature control of larval distribution - is there a lower level as well as an upper level? why can't larvae adapt?
- How does *Osedax* or other sibogs maintain an adequate larval pool? Larvae can be cultured easily in the lab

5. Outputs and recommendations

This was the first workshop ever held on Siboglinidae. Whilst many of the participants had met each other at meetings more habitat-focussed (such as the vent and seep meetings) or taxon-focussed (such as the polychaete meeting) this particular group had never met. This networking is vital to developing new collaborations and synthesising results across discipline boundaries. Discussions ranged from microbial biology to whale biology, evolution to ecology and both traditional and cutting-edge techniques were shown to be vital to the understanding of this group. Alongside the new collaborations that have developed, our initial output will be a major review paper to be submitted to PLOSone. If future funding is made available, we anticipate that we will aim for a follow-up workshop or smaller visits within the working groups developed during this proposal (for example, taxonomic exchange visits).

6. Appendix: abstracts from presentation sessions

Siboglinids from vents and seeps: comparison of their hemoglobins in relation with their habitats.

Ann. C. Andersen, Matthieu Bruneaux, Carolina Giraldo, Cédric Meunier, Stéphane Hourdez, Morgane Rousselot, Franck Zal

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The Siboglinid tubeworms vestimentifera and pogonophora from vent and seeps live in contrasted habitats with respect to substratum, oxygen and sulfide concentrations. Extracellular hemoglobins dissolved in the tubeworm's blood and coelomic fluid bind both oxygen and sulfide from the environment for transport to the endosymbiotic bacteria that use the chemical energy to synthesize organic compounds for the benefit of their host. Because hemoglobin is essential in the symbiosis, we compare these hemoglobin's structure and functional properties in relation with the external life conditions in two pogonophora- and three vestimentifera-species. The seep species are: the pogonophora, *Sclerolinum contortum,* Brattegaard (1966) and *Oligobrachia haakonmosbiensis (webbi*) Smirnov (2000) from the Norwegian margin; the seep vestimentifera are *Escarpia southwardae,* Andersen et al. (2004) from the west African pockmarks and *Lamellibrachia n. sp*. (Southward, Andersen & Hourdez, description in preparation) from the Mediterranean carbonate crusts. The hydrothermal vent vestimentifera is *Riftia pachyptila*, Jones 1981, from the East Pacific Ridge. This synthesis aims to enlighten the possible links between hemoglobins, as oxygen- and sulphide- transporters, and the habitats conditions of these tubeworms, as it also compares pogonophora and vestimentifera in a phylogenetic point of view, based on hemoglobins.

Tropholution – Evolution of trophosomes in Siboglinidae

Monika Bright, Irmgard Eichinger, Sigrid Katz and Andrea D. Nussbaumer

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The monophyletic family of Siboglinidae (Polychaeta), existing in different deep-sea habitats from hydrothermal vents, over cold seeps to whale and wood falls, is one of the most exciting example of marine microbial symbiosis. In contrast to other non-symbiotic polychaete relatives, siboglinids formed bacterial symbioses allowing them to survive and prosper in these highly toxic, sulfide-rich environments. Apparently the evolution of this taxon involved the capability of the hosts to establish endosymbiosis in a variety of tissues leading to tightly nutritional associations, the loss of a functioning digestive system, and the transformation of host morphology to serve this symbiosis. However, several pathways must have led to this successful adaptation as reflected in the astonishing variety of siboglinid body plans.

We are proposing that in the last common stem species of siboglinids a microbial association was established. Transmission was horizontal and via the skin. The infection process was not yet limited to a certain life history stage as in extant sibloginids and the establishment of the microbes was not yet restricted to certain tissues. Also a fully functioning nutritional association was not yet developed and accordingly the digestive tract was not reduced. In each stem species of the taxa frenulates, vestimentiferans, *Osedax*, and *Sclerolinum* the transformation occurred independently from a feeding animal being randomly infected during its life and containing microbes widespread in a variety of tissues to a symbiotic entity with infection being restricted to early sessile ontogenetic stages, the establishment of the symbiont-housing organ in a specific tissue and body region and accordingly the reduction of the digestive tract.

While the origin of the trophosomes in frenulates and in vestimentiferans has been studied in detail (Nussbaumer et al.; this workshop), not much is known in *Osedax* and *Sclerolinum*. The ongoing project TROPHOLUTION investigates the origin, organization and cell cycle dynamics of the trophosomes in *Sclerolinum* from the hydrocarbon seeps of the Gulf of Mexico (see Eichinger et al., this workshop) and the recently discovered *Osedax* from whale falls off the coast of California (see Katz et al., this workshop) using ultrastructural, molecular, and immunohistochemical techniques. Our aim is to test the above outlined hypothesis but also to extend our knowledge on microbial symbiosis from beneficial to pathogen associations.

The phylogenetic position of Siboglinidae from morphological and molecular data

Maria Capa

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Although some authors still consider members of Siboglinidae as the phylum Pogonophora, most researches agree that they belong to the Annelida. What is still not resolved is the position within the annelid topology.

In a project undertook with the aim of investigate the monophyly of the Order Sabellida and the composition of the clade, members previously included within it were considered as the ingroup (Sabellidae, Serpulidae, Sabellariidae, Siboglinidae, Oweniidae) and Spionidae, Cirratulidae and Terebellidae were considered as the outgroup. The maximum parsimony analyses using both morphological and molecular data (partial 18s, 28s and 16s) resulted in Sabellida being paraphyletic with Spionidae as the sistergroup of Sabellariidae and also Siboglinidae being the sistergroup of Oweniidae, although not with a high support value.

Briefly, new material of "Sabellida" will be collected and other loci will be sequenced with the aim of throwing more light to the position of Siboglinidae among annelids and asses the phylogenetic relationships with other families.

Barcoding bones – a second population of Osedax mucofloris identified by DNA from bones found in a shrimp trawl

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A barcode approach was used to investigate four large whale vertebras collected in a shrimp trawl in the North East Atlantic. Sequencing of bone tissue showed that the bones were from a sperm whale, the third time the species is recorded in Swedish waters. A survey of the bone surfaces and collection of DNA samples from any cavities found disclosed four individuals of *Osedax mucofloris* representing the only known naturally occurring population of that species. Population genetic analysis of the data indicates a close connection to the previously known populations at experimentally sunk whale falls, deep divergence of *Osedax mucofloris* haplotypes and a very high effective population size in the Skagerrak and the surrounding shallow seas.

Frenulate nutrition: how important is mixotrophy?

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Frenulate pogonophores (Annelida: Pogonophora a.k.a. Siboglinidae) typically inhabit muddy sediments on the continental slope, although an increasing number of species are now being discovered in sedimentary settings around hydrothermal vents and cold seeps. Early studies on their nutrition concentrated on the uptake of dissolved organic matter (DOM) but were not able to demonstrate that any species could completely satisfy the demands of respiration and growth by uptake of DOM at concentrations found in the habitat. Subsequent measurements of $\delta^{13}C$ in frenulate tissues and the finding of endosymbiotic bacteria in the post-annular region, suggested that the frenulates derived their nutrition from chemosynthetic bacteria. The contribution of chemoautotrophy to nutrition was re-enforced by the low ^{13}C-content of the tissues, typically lower than - 40‰$\delta^{13}C$. We review the data on the concentrations of DOM and reduced inorganics and methane in the sediment of frenulate habitats and suggest that some frenulates are mixotrophic, deriving their nutrition from a both DOM and from endosymbiotic sulphur-oxidising bacteria.

Origin and organization of the trophosome in a new species of *Sclerolinum* from the Gulf of Mexico

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The small genus Sclerolinum belongs to the unique monophyletic polychaete family Siboglinidae that consists of four taxa, the Vestimentifera, Frenulata, Sclerolinum, and Osedax. All representatives develop a symbiont-housing organ, the trophosome, and loose a functioning digestive system during their ontogenesis. The Vestimentiferan´s trophosome - a complex lobate organ with a mainly apolar tissue of bacteriocytes - develops from the visceral mesoderm between the dorsal blood vessel and the gut. In Sclerolinum, the sister taxon to Vestimentifera, the trophosome has been described as a simple two-layered cylinder. The inner layer – a solid core of bacteriocytes - is thought to originate from the endodermal midgut. We have been reinvestigating the origin and ultrastructure of this symbiont-bearing organ in smaller and lager specimens of a recently discovered new species of Sclerolinum from the Gulf of Mexico. By sequencing the mitochondrial 16S gene the new species we found is most closely related to S. brattstromi and the description is on its way.

A ventrally located duct we interpret as part of the digestive system lacked symbionts at all stages and consisted of a ciliated endothelium surrounded by a myoepithelium in smaller specimens, but was reduced to some ciliated cells in lager ones. In smaller specimens the trophosome was found to be restricted to a few bacteriocytes located next to the dorsal blood vessel and was embedded within non-symbiotic mesenchyme. In lager individuals, the trophosome was invading the whole body cavity of the trunk, cylindral in shape, and was penetrated by intercellular blood spaces. The trophosome was composed of an apolar tissue of bacteriocytes containing pleomorphic, endosymbiotic bacteria lying free in the cytoplasm of the host cells and a non-symbiotic, visceral peritoneum surrounding the bacteriocytes and lining the coelomic cavity of the trunk. Therefore, we suggest that the trophosomes of the two sister taxa Sclerolinum and vestimentiferans is a homologous structure having evolved from the visceral mesoderm in the last common stem species of the taxon Sclerolinum + Vestimentifera.

Physiological ecology of the Vestimentiferan Siboglinids

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All known siboglinids in the vestimentiferan clade have numerous features in common, including many of the unique aspects of their gross anatomy, similar hemoglobins with similar properties, and intracellular sulfide-oxidizing symbionts harbored in cells in an internal organ, the trophosome. All must transport sulfide, oxygen, and inorganic carbon from the external environment to their internal symbionts and transport the waste products of sulfide oxidation back to the external environment. However, there is a surprising degree of variation in the physiological ecology of different species. The giant hydrothermal vent tubeworm, Riftia pachyptila, from the East Pacific Rise, uses its well vascularized gill-like plume as the primary site for exchange of dissolved gases including sulfide and oxygen, which are both bound by vestimentiferan hemoglobin with high affinity and capacity. Another vestimentiferan, Lamellibrachia luymesi, is found associated with hydrocarbon seeps in the Gulf of Mexico. Unlike its hydrothermal vent relatives, this cold seep species is very long-lived and sulfide is rarely detectable around the plumes of adult aggregations. Laboratory experiments demonstrate that L. luymesi can take up sufficient sulfide across posterior portions of the animal, nicknamed roots, to fuel net DIC uptake and autotrophy. These roots can extend meters into the sulfide rich sediments and create a rhizosphere strongly influenced by the tubeworms. However, models based on data from a combination of laboratory and field data indicated that seepage alone could not provide sufficient sulfide to support even moderate sized assemblages over their centuries-long lifespans. The models suggested that if the tubeworms released the waste products of sulfide oxidation by their symbionts (sulfate and hydrogen ions), directly into sediments containing high levels of organic material then microbial consortia capable of producing sulfide from dissimilatory sulfate reduction could "recharge" the rhizosphere with sulfide. Recent additional experiments with live animals, enzyme assays, in situ measurements, and models confirm that these tubeworms release sulfate and hydrogen ions across their roots and can live for centuries as a result of symbioses with internal chemoautotrophic bacteria and external consortia of free-living microbes.

Two other species of vestimentiferan, are not as well studied but have characteristics of both the vent and seep species described above suggesting further study of these species will provide additional insights into the physiological diversity of the group. Ridgeia pisceseae from North East Pacific hydrothermal vents has multiple growth forms, and will apparently grow posterior extensions into cracks in the basalt when required by environmental conditions. Another species recently discovered on the Eastern Lau Spreading center is very small, with about half of its 1-2 cm total length normally embedded in the chimney walls it inhabits.

Siboglinids in Japanese waters – A new insight through *in situ* whale-fall experiments

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Siboglinid polychaetes occur over a wide range of depths (from littoral to hadal) and habitats (hydrothermal vents, seeps and whale falls) in Japanese waters. Nine potential species of vestimentiferans, nine of *Osedax*, and several frenulates have been recorded to date but no moniliferan siboglinids in this region. The vestimentiferan tubeworm *Lamellibrachia satsuma* has been discovered from seeps at a depth of 100 m in Kagoshima Bay, which is the shallowest habitat for vestimentiferans yet discovered. This species inhabits not only these shallow-water seeps but also hydrothermal vents at a depth of 450 m on the Nikko Seamount that is 1,500 km distance from Kagoshima Bay. An in situ bone deployment experiment was conducted in Kagoshima Bay. Six vertebrae of a Baird's beaked whale were deployed beside a *L. satsuma* clump in July 2005. Bone retrieval was conducted in October 2006, June 2007 and July 2008. More than 330 individuals of *L. satsuma* settled on a vertebra in 2008 and the maximum length of the tube was 87 cm. Molecular phylogenetic analyses showed two distinct phylotypes of thioautotrophic symbionts from the whale-fall *L. satsuma*, which was differ from the "original" *L. satsuma* symbiont. This is the first record to show a single species of vestimentifera inhabiting at vents, seeps, and whale falls in Japanese waters. This plasticity of symbiont selection might explain such a wide habitat range in this species.

New species and records of *Osedax* from Southern California, with further observations on the anatomy, life history and feeding ecology of the genus

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A series of unidentified whale-fall siboglinids from Alvin Dives in the Southern California basins area carried out in 1995, 1998, 2000, 2002 and 2004 were examined. These species corresponded to the more recently described Osedax frankpressi Rouse et al., 2004 and a new species of Osedax as yet undescribed. We present a description of the new species using light and electron microscopy, and present the new species within a phylogenetic context using 18S and COI sequences. Initial evidence points to a monophyletic Osedax clade of at least 10 different species, some awaiting formal descriptions. We also present new data on the anatomy, life history and feeding ecology of Osedax mucofloris Glover et al., 2005.

Siboglinid evolutionary history and questions for future research

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Compared to most other annelids, considerable work has been done on the evolutionary history of siboglinids. Whereas much of the early debate focused on whether siboglinids are in fact annelids, more recent work has examined relationships within the group. We now understand that siboglinids are comprised of four distinct lineages, vestimentiferans, frenulates, Osedax, and Sclerolinum. Of these, vestimentiferans have received the most attention because of their prominent occurrence at hydrothermal vents and hydrocarbon seeps. In contrast, frenulates, which are by far the most diverse clade and are found throughout the deep sea, have been very poorly sampled for phylogenetic analyses. This report will provide an up-to-date summary of the current status of siboglinid evolution, thereby providing a comparative framework for the group. Based on this phylogeny, we will outline issues of interest that deserve more exploration. Additionally, we will underscore the need for a community-based approach if we are to achieve a thorough understanding of siboglinid evolutionary history.

Siboglinids from mud volcanoes in the Gulf of Cadiz

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Biological research on mud volcanoes in the Gulf of Cadiz started in 2000; hundreds of samples were collected and more than 500 species were identified. Studies of faunal assemblages have shown that chemosynthetic communities in most of the mud volcanoes are dominated by frenulate Siboglinids, presenting an excellent opportunity to extend our knowledge on this understudied group. Molecular and morphological analysis of specimens collected from several mud volcanoes showed that there are at least 20 different species of 7 different genera. At least two genera and four of the species found in the Gulf of Cadiz have never been found in NE Atlantic calling into question their endemicity. The high diversity of frenulates found in the Gulf of Cadiz is likely to be supported by the environmental heterogeneity generated by the geological and physical settings of each mud volcano.

Comparison of Vestimentifera *Oasisia alvinae* and *Ridgea phaeophiale* with polychaeta (other Sabellidae) at anatomical and histological levels

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Vestimentifera is recently established and very peculiar group of gutless worms. The first representative of this group, *Lamellibrachia luymesi*, was described from the North Atlantic 40 years ago. The microscopic anatomy of vestimentiferans has been studied in detail only for three species. Detailed anatomy of *Lamellibrachia luymesi* Land et Nørrevang, 1977 was given with the species description. Jones (1981) presented the detailed anatomy of *Riftia pachyptila* complemented later by details of ultrastructure (Gardiner and Jones, 1993). Microscopic anatomy of *Ridgeia phaeophiale* (= Ridgeia piscesae) Jones, 1985 was studied and presented in a series of publications discussed by Malakhov and Galkin (1998).

Phylogenetic relationships of the group have been debated since vestimentiferans were first described. As a result, their taxonomical status changed significantly several times and over many years it was a subject of hard debates among researchers. Johnes (1985) considered vestimentiferans as a separate phylum. Later Vestimentifera were moved in one phylum with Pogonophora. At present this group is usually considered as the subfamily Siboglinidae in the family Sabellidae.

Modern morphological and molecular data indicate that Vestimentifera are nested within Pogonophora. The idea that both these groups are "polychaetes" has been supported by several morphological studies and by a number of molecular analyses. While the placement of siboglinids within the polychaetes is now generally accepted, their more precise taxonomical positioning was not rigorously assessed.

We conducted anatomical and histological analysis of all parts of the body and made reconstructions of all main systems of organs of *Oasisia alvinae* Jones, 1985.

The aim of present research is a comparison at anatomical and histological levels of two species of Vestimentifera, *Oasisia alvinae*, with other sabellid polychaetes.

Although Siboglinidae are with no doubt a part of Annelida, more morphological and anatomical evidence is needed in discussion of including Vestimentifera in the family Sabellidae.

Origin and organization of the trophosome in *Osedax* from the Monterey Canyon

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The most recently discovered taxon within the family Siboglinidae is the whale fall specialist Osedax. A unique symbiont housing organ, we name trophosome, has functionally replaced the gut in Osedax as well as in other siboglinids. In frenulates, Sclerolinum and Vestimentifera the trophosome is an elongate organ taking up the majority of the trunk region housing autotrophic sulfide- or methane-oxidizing bacteria. While in frenulates and Sclerolinum it has been described as a simple, two-layer cylinder developing from the endodermal midgut, the trophosome in Vestimentifera is a complex lobate, apolar tissue developing from the visceral mesoderm between the dorsal blood vessel and the gut. However, even among siboglinids Osedax is exceptional, as the trophosome in these marine worms houses heterotrophic bacterial symbionts and is situated in their complexly branching root-like structures that invade and extract organic molecules from sunken bones at a wide range of depths in the world's oceans.

To clarify the origin and ultrastructure of the trophosome, we have been investigating mature Osedax specimens of different species from the Monterey Bay, California. The trophosome was a massive apolar tissue took up most of the body cavity and was located basal to the somatic musculature in the posteriormost body region, called roots. It was composed of bacteriocytes in which the symbionts were housed in vacuoles and of non-symbiotic cells with abundant endoplasmic reticulum. Therefore, we suggest that the trophosome stems from somatic mesoderm. The trophosome itself showed a bottom-up organization, with bacteriocytes housing intact and dividing bacteria at the tips of the roots and bacteriocytes with degraded bacteria as well as massive degradation of non-symbiotic cells further away from the tips, surrounding the ovisac. Concurrent with these changes we saw changes in the ultrastructure of the epidermal cells of the root structures.

In addition to the trophosomal symbionts we found bacteria associated with gland cells in the trunk region anterior of the ovisac. These glands take up a large proportion of the trunk and are proposed to be responsible for the production of the gelatinous tubes of the worms (Rouse et al. 2008). Bacteria were found extracellular in these glands.

Rouse GW, Worsaae K, Johnson S, Jones WJ, and RC Vrijenhoek. 2008 Acquisition of dwarf male "harems" by recently settled females of *Osedax roseus* n. sp. (Siboglinidae; Annelida). The Biological Bulletin 214: 67-82.

Tubes and taphonomy: the siboglinid fossil record

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This presentation will discuss how vestimentiferan tubes differ morphologically from other tubicolous polychaetes present at chemosynthetic sites, such as frenulates, chaetopterids and serpulids, and investigate how they become mineralized at modern hydrothermal vents and hydrocarbon seeps. Evidence will be presented that tubes morphologically similar to vestimentiferan tubes have a fossil record at seeps and vents that goes back to the Palaeozoic (~430 million years for vents, ~395 million years for seeps), significantly before the divergence estimate of this group from the moniliferans (Sclerolinum) at 115-138 million years, based on mtCOI. At present there is no fossil record for Osedax at whale falls, but strategies for finding fossil borings made by this animal are being developed.

Species boundaries of Gulf of Mexico vestimentiferans inferred from nuclear and mitochondrial genes

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Vestimentiferan tubeworms are abundant in sulfidic deep-sea hydrothermal vents and cold seeps. There are at least 5 species associated with cold seeps in the Gulf of Mexico. The physiology and ecology of two of the species previously known from the upper Louisiana slope (*Lamellibrachia luymesi* and *Seepiophila jonesi*), from depths above about 1,000 m, are relatively well understood. A rare species from the upper slope (sp. nov. 1) and the two species found at greater depths in the Gulf of Mexico (*Lamellibrachia* sp. nov. and *Escarpia laminata*) are not yet as well studied. Here we report the results of a study using a multi-gene phylogenetic framework to examine the distribution of vestimentiferans in the Gulf of Mexico, their species boundaries and role of depth, distance or large-scale currents as possible genetic break points. We sequenced fragments of the mitochondrial large ribosomal subunit rDNA gene (16S) and mitochondrial cytochrome oxidase 1 (COI) of nearly 200 vestimentiferans collected from 10 sites greater than 1000m depth in Gulf of Mexico during 2006 and 2007 as well as samples collected from shallower sites and elsewhere in the world. The species-level implications of our phylogenetic hypotheses are clear in our current data set, and some consistent genetic structure within species suggests further hypotheses with respect to intraspecific variation structured by depth and or longitudinal genetic break points. The addition of sequences of the nuclear gene Elongation Factor 1α, and a globin gene will further help us to address the presence of depth, currents and/or longitudinal breaks that may account for intraspecific genetic diversity within the Gulf of Mexico.

Finding the partner for life – a new project on the recognition processes between Vestimentifera and their symbionts

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Diversity and succession of *Osedax* species on sperm whale carcasses around Japan

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Since the discovery of the *Osedax* genus, 10 species have been reported, all associated to decaying bones, but in a wide range of environmental condition. The success of *Osedax* in colonizing such a patchy habitat over broad geographic and bathymetric ranges implies efficient dispersal and colonization strategies.

We analyzed colonization patterns of *Osedax* species on a sperm whale carcass implanted at 925 m depth in Sagami Bay (Japan). Eight different species successively colonizing the bones were identified using molecular techniques. *O. japonicus* that colonizes a close but shallow (225 m) sperm whale fall site was not found, whereas 2 of the early colonists in Sagami Bay are also known from distant sites in the western Pacific (off California). This suggests very efficient dispersal possibly limited by depth related environmental parameters. In addition, I investigated embryonic and larval development in order to understand the mechanisms underlying dispersal and colonization efficiency. In a Sagami bay species, embryonic development exhibits remarkably rapid cleavage rates, even at the low temperature (3-4°C) that appear to be suitable for embryos. This feature is usually associated with short dispersal range, suggesting that spatial and temporal frequency of suitable bones may be essential for maintaining *Osedax* populations.

Microscopic anatomy of hydrothermal vent tubeworm *Riftia pachyptila*, Jones 1981. Based on findings from 21ºN of East-Pacific Rise.

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Vestimentifera is a group of very peculiar gutless worms. The microscopic anatomy of vestimentiferans has been studied in detail only for three species: 1) *Lamellibrachia lumesi* (Van der Land and Nørrevang, 1977); 2) *Riftia pachyptila* (Jones, 1981); 3) *Ridgeia piscesae* Jones, 1985 (Malakhov and Galkin, 1996). Fine details of anatomy of *Riftia pachyptila*, the largest vestimentiferans, described from the first discovered hydrothermal vent field off Galapagos Islands, remain poorly known, in part because of the size of this organism (adults reach 2 m in length). We intend to study details of microscopic anatomy of *Riftia pachyptila* to get a better understanding of positioning of the genus *Riftia* within the system of Vestimentifera and compare at morphological and anatomical levels this species with other species of *Riftia* from different sites.

Jones, ML 1981. *Riftia pachyptila*, new genus, new species, the vestimentiferan worm from the Galapagos Rift geothermal vents (Pogonophora). Proc. Biol. Soc. Wash. 93: 1295 - 1313.

Malakhov VV, Popelyaev IS, and SV Galkin. 1996. Microscopic anatomy of *Ridgeia phaeophiale* Jones, 1985 (Pogonophora, Vestimentifera) and the problem of the position of Vestimentifera in the system of the animal kingdom. Russ J Mar Biol.

van der Land, JL, and A Norrevang. 1977. Structure and relationships of *Lamellibrachia* (Annelida, Vestimentifera). Danske Vidensk. Selsk. Biol. Skr. 21(3): 1-102.

The bacteria inside Frenulata (Siboglinidae, Polychaeta) species from mud volcanoes in the Gulf of Cadiz

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Symbiosis involving chemoautotrophic bacteria allow frenulate tubeworms to thrive in reducing marine environments where the endosymbiont bacteria oxidize the reduced chemicals, fueling their own energetic and biosynthetic needs in addition to those of their hosts.

Under the framework of the TTR programme and the EU-funded project HERMES several chemosynthetic frenulata species have been discovered in the mud volcanoes from Gulf of Cadiz. These include the widespread genus *Siboglinum* but also the genera *Lamellisabella*, *Polybrachia*, *Spirobrachia*, and the new genus *Bobmarleya*. On this study we used PCR-DGGE analysis of 16S rRNA genes and analysis of bacterial clone libraries to investigate the genetic diversity of symbiotic bacteria associated with nine species of frenulates from several mud volcanoes in the Gulf of Cadiz.

Multiple bands in the DGGE profile indicate the presence of several bacterial phylotyes in the trunk of each frenulate specimen, with one major band in each lane suggesting that each worm harbours predominantly one phylotype of endosymbiont. The same phylotype appears on different species from different mud volcanoes. *Siboglinum* sp. specimen from Captain Arutyunov was the only worm to give a positive result for the pmoA primers tested and our results provide the first molecular evidence of Siboglinum sp. harboring methanotrophic symbionts.

Future studies are essential to understand the role of symbionts in frenulate species from the Gulf of Cadiz, namely to assess their metabolic potential by sequencing target functional genes encoding proteins involved in the sulphide oxidation pathway and in carbon fixation.

Distribution and habitat conditions of siboglinids on southern California whale falls

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Sibliginid polychaetes, including two species of *Osedax* and *Escarpia spicata*, have been collected from implanted (3) and natural (1) whale falls at four bathyal sites off southern California from 1995 and 2004. While *Osedax* spp. occurs in abundance on some lipid-rich southern California skeletons, on others they appear to be rare. Habitat conditions, e.g., low bottom-water oxygen, do not explain these variations in *Osedax* abundance because Osedax spp. can be rare on skeletons in oxygen replete waters such as in the San Diego Trough. We postulate that abundance and population connectivity varies along the known range of *Osedax* in the eastern North Pacific, potentially resulting in substantial regional differences in the role *Osedax* plays in whale-skeleton decomposition.

Siboglinid endosymbiont diversity: identifying mechanisms of tubeworm/ bacterial interactions

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Four major groups of siboglinids are known, including vestimentiferans, moniliferans, Osedax spp. and frenulates. Although endosymbionts of vestimentiferans and *Osedax* spp. have been previously characterized, little is currently known about endosymbiotic bacteria associated with frenulate and moniliferan siboglinids. This is particularly surprising given that frenulates are the most diverse and widely-distributed group of siboglinids. Here, we review recent advances in the molecular characterization of endosymbiotic bacteria associated with the frenulate and monoliferan siboglinids. Phylogenetic analysis indicates that at least three major clades of endosymbiotic γ-proteobacteria associate with siboglinid Annelids, with each clade corresponding to a major siboglinid group. Frenulate endosymbionts are a group of γ–proteobacteria that are divergent from bacteria associated with vestimentiferan or *Osedax* hosts. We also discuss a new technique, laser-capture microdissection (LCM), which allows for the precise excision of tissue regions of interest. This method, when used in concert with molecular and genomic techniques such as expressed sequence tag surveys using 454 FLX pyrosequencing technology, may enable investigations into physiological processes and mechanisms in this symbiosis. Furthermore, adopting a comparative approach using different siboglinid groups, such as worms harboring thiotrophic vs. methanotrophic endosymbionts, may yield considerable insight into the evolution of this lineage.

Symbionts diversity of *Osedax mucofloris* in North Atlantic whale falls

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Long after they die and fall to the ocean floor the skeletal remains of large whales constitute the energy source for a fauna of highly specialized species. Osedax, one of these specialists, is a siboglinid polychaete that forms a sister group relationship to the siboglinid tubeworms from hydrothermal vents and cold seeps. Osedax species lack a gut as do the tubeworms, but they do not house their symbionts in the trophosome. Instead their posterior end has evolved into a "root" structure that extends into the bone tissues and is filled with symbiotic bacteria. These symbionts are hypothesized to degrade the lipids and collagen in the whale bone to provide their host with nutrition. We are currently describing the symbiotic community of Osedax mucofloris from North-Atlantic whale falls. Our main objective is to characterize this symbiosis using comparative 16S rRNA sequence analysis and fluorescence in situ analysis (FISH) to better understand the role of biogeography and host specificity in the establishment of these symbioses. We have identified at least six major endosymbiotic 16S rRNA phylotypes in O. mucofloris. Based on our clone library analyses, some of these symbiont phylotypes co-occur within the same host individual and we are currently using FISH to examine the distribution of symbiont phylotypes in individual worms. A comparison of host COI haplotypes with symbiont 16S rRNA phylotypes showed no congruence in the 24 individuals examined. This indicates that the variability of symbiont phylotypes within the O. mucofloris population is not due to host selection. We now plan to examine if the distribution of symbiont phylotypes in the host population reflects their distribution in the surrounding seawater.

Developmental constraints in siboglinids with widely divergent adult life-history traits

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Deterministic life-history models such as r-K selection theory make useful, though often simplistic, predictions of traits that animals should evolve under different selective regimes. In former times, when the deep sea was regarded as a spatially homogeneous and temporally stable environment, it was predicted that deep-sea organisms should have suites of “K-selected” traits that include continuous breeding, low fecundity, limited dispersal, direct development, slow growth, long life, and parental protection. Ruth Turner’s discovery of opportunistic wood-boring bivalves with classic r-selected life-history traits in the 1970's provided the first major exception to the traditional paradigm. These xylophagid bivalves, which from an ecological perspective have much in common with *Osedax*, demonstrate the classic "r-selected" life-history traits predicted for weedy opportunists. Siboglinids provide both affirmations of and exceptions to these rules. Adult tubeworms from temporally stable seeps are long-lived and slow growing, whereas tubeworms from ephemeral vents are fast-growing and short-lived. Thus, on the basis of adult traits, these animals are textbook examples of r-K selection. However, developmental traits of seep, vent and whale-fall tubeworms are virtually identical, suggesting strong phylogenetic constraints in the early early stages. In this presentation, I will review the embryonic and larval development of *Lamellibrachia, Riftia* and two species of *Osedax* in the context of life-history theory and will present some unpublished data on the physiological tolerances and dispersal potential of tubeworm larvae.

7. Appendix: workshop participants and contact details

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