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Evolution of Endosymbiosis in (xylophagous) Wood-Eating Bivalves

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Annual Report for Period:06/2004 - 06/2005**Submitted on:** 01/27/2006**Principal Investigator:** Distel, Daniel L.**Award ID:** 0129117**Organization:** University of Maine**Title:**

Evolution of Endosymbiosis in (xylotrophic) Wood-Eating Bivalves

Project Participants**Senior Personnel****Name:** Distel, Daniel**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Graduate Student****Name:** Luyten, Yvette**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Graduate student participated in lab research with support from this grant.

Name: Mamangkey, Gustaf**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Gustaf Mamangkey is a lecturer at the Tropical Marine Mollusc Programme, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, Jl. Kampus UNSRAT Bahu, Manado 95115, Indonesia. Gustaf has participated in this project as a scientific collaborator and is responsible for collecting all of the Indonesian specimens used in this study. Gustaf is an authority on the ecology and systematics of Indonesian Teridinidae. This year Gustaf has begun a Ph.D program at James Cook University Townsville, Queensland 4811 AUSTRALIA. His participation in this project will contribute to his Ph.D training.

Undergraduate Student**Name:** Burgoyne, Adam**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Undergraduate student participated in lab research with support from this grant.

Name: Willey, Jennifer**Worked for more than 160 Hours:** No**Contribution to Project:**

Undergraduate student participated in lab research with support from this grant.

Name: Chen, Tim**Worked for more than 160 Hours:** No**Contribution to Project:**

Undergraduate student participated in lab research with support from this grant.

Name: Eash, Angela**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Undergraduate student participated in lab research with support from this grant.

Name: Bosse, Ben

Worked for more than 160 Hours: No

Contribution to Project:

Undergraduate student participated in lab research with support from this grant.

Name: Amin, Mehwish

Worked for more than 160 Hours: Yes

Contribution to Project:

Technician, Programmer

Name: Spenlinhauer, Tania

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Morrill, Wendy

Worked for more than 160 Hours: Yes

Contribution to Project:

Name: Cannone, Jamie

Worked for more than 160 Hours: Yes

Contribution to Project:

Jamie Cannone is Dr. Gutell's research associate and has assisted in secondary structure inference and sequence alignment.

Other Participant

Name: Gutell, Robin

Worked for more than 160 Hours: Yes

Contribution to Project:

Dr. Gutell is a faculty member at the University of Texas and is a leading authority on RNA structure. Dr. Gutell is assisting in our research by inferring secondary structures for bivalve 18S and 28S rRNAs to assist in structural alignment of sequences.

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

Gustaf Mamangkey, Faculty of Fisheries and Marine Sciences Sam Ratulangi University, Manado, 95115 INDONESIA, collected numerous valuable specimens for this project and will serve as a consultant in evaluating the data. This year Gustaf has begun a Ph.D program at James Cook UniversityTownsville, Queensland 4811 AUSTRALIA. His participation in this project will contribute to his Ph.D training.

Dr. Robin Gutell, Faculty, University of Texas. Dr. Gutell and his research associate, Jamie Cannone were recruited as collaborators in this project to develop secondary structural models for the 18S and 28S ribosomal RNAs of specific bivalve taxa and to implement these models in creating and assessing the certainty of sequence alignments using structural features and compensatory substitutions as evidence for positional homology.

Activities and Findings

Research and Education Activities: (See PDF version submitted by PI at the end of the report)

Some Relevant History:

Funds for the current project were initially awarded to the University of Maine where I served as a faculty member until the spring of 2004. Shortly thereafter, I resigned from the University of Maine and accepted a position as Director of Ocean Genome Legacy Foundation (OGL). At this time, I requested and received a no-cost extension on this award, anticipating some inevitable disruption and delay in the progress of the project. I still retain a non-salary faculty position at the University of Maine, allowing me to continue to advise students. My graduate student has moved with me to the Ocean Genome Legacy Foundation but still retains her status as a full time student at the University of Maine, conducting her studies off campus. The Ocean Genome Legacy Foundation has requested new NSF awardee organization status and has completed all of the requirements of that request. As soon as the processing of this status change is complete, I will request transfer of this award to the Ocean Genome Legacy Foundation. In anticipation of the transfer, the project has been continued at OGL supported by existing OGL funds.

Research and Education Activities

The purpose of this investigation is to use molecular and morphological characters to explore the function, diversity, and evolution of wood boring, wood feeding, and symbiosis in wood-boring bivalves. We proposed to accomplish this by:

- 1) Producing a robust phylogeny for these morphologically diverse bivalves, and
- 2) Mapping on this phylogeny the occurrence of morphological, biochemical, and molecular traits associated with symbiosis, wood boring and wood feeding.

The taxon sampling strategy proposed includes a broad representation of the diversity of the families Teredinidae and Pholadidae and representative taxa within the parent Order Myoida. We proposed to perform phylogenetic analyses using two nuclear encoded (18S and 28S rRNA) and two mitochondrial (16S rRNA and COI) genes. The latter two biomarkers have been eliminated from the study for the following reasons: 1) we observed taxon-specific amplification failures using standard 'universal' PCR primers. These amplification failures are apparently due to variation in universal primer regions. This is supported by the observation that all of the failed amplifications fall within a single phylogenetic lineage as identified by 18S and 28S phylogenies. Unfortunately this lineage includes the two primary taxa of interest in this study, the family Teredinidae, and the pholadid subfamily Xylophaginae. We note that the same result was observed for amplification of COI for members of the Xylophaginae by other investigators (pers. comm. V. Tunnicliffe), 2) Analyses to date indicate that 18S and 28S loci provide sufficient phylogenetic resolution to address the questions proposed here.

An additional goal has been added to the project in the analysis stage. It was determined that the certainty of alignments for 28S genes, and therefore the certainty of resultant phylogenetic inferences, would be greatly improved by development of secondary structural models for bivalve 28S verified by in depth analysis of compensatory substitutions across the Class. To achieve this, a collaboration was initiated with the laboratory of Robin Gutell (University of Texas, Austin). To date we have completed a secondary structural model for *Placopecten magellanicus* 28S (the first available for a bivalve) and are in the process of producing additional structures for *Mya arenaria* 28S and 18S.

Please see attached pdf documents for a view of the 5' half of the *P. magellanicus* 2' structure). The complete structure can be viewed at <http://www.rna.icmb.utexas.edu>.

Education

To date, five undergraduate students and one graduate student have been trained in DNA sequencing, DNA cloning, sequence analysis, and phylogenetic analysis. We have also made progress toward implementing our K-12 outreach program. A collaboration has been established with the Ramey School, Ramey, Puerto Rico; a school serving the American military community in Puerto Rico. Science teacher Rex Roettger will work with me to develop research and educational materials for a high school research project to include eighteen 10th through 12th grade students. The students will conduct experiments to observe the growth of shipworms in their local waters and will record differences in infestation of wooden test panels placed in various depths and salinity regimes. The students will also contribute to and learn about this NSF funded project. They will preserve some of their specimens in ethanol for use in our NSF funded research and we will report to the students on how their efforts have contributed to the overall project.

Findings: (See PDF version submitted by PI at the end of the report)

Results

Please see attached pdf document for updated table describing sequencing progress to date.

Findings

Several important findings have been made:

Results to date provide strong support for the following:

- 1) Wood feeding (xylotrophy) has arisen only once in bivalve evolution, rather than twice in two separate families as has been previously proposed.
- 2) All wood feeding bivalves share a single common ancestor that was likely a wood boring member of the family Pholadidae
- 3) The family Teredinidae (shipworms) appears to be a sister or daughter lineage of the subfamily Xylophaginae (deep-sea wood-borers) of the family Pholadidae. Thus, the taxonomy of both families will likely require revision.

The common ancestor of all wood feeders likely had the following features:

- a) Projections for mussel attachment on the internal surface of the valves (apophyses). This feature is found in non-wood-feeding pholads and Teredinidae but was lost in the deep-sea Xylophaginae.
- b) The intestine traversed the heart. The intestine traverses the heart in all non-wood-feeding pholads. This feature is retained in Xylophaginae, but the intestine is separated from the heart in all Teredinidae except *Kuphus*.
- c) A single gill demibranch. The outer gill demibranch is retained in rock- and wood-boring pholadidae but is lost in all wood-feeding bivalves.
- d) A globular stomach.
- e) A caecum for storing wood particles during digestion.
- f) Reduced labial palps and crystalline style

g) Gill-born bacterial endosymbionts

h) Protandrous hermaphroditism

Several interesting evolutionary trends are also apparent.

In Teredinidae:

- a) The ancestral teredinid had a globular stomach. The stomach becomes progressively more elongated in more recently divergent lineages.
- b) The heart was located in an anterior position. The heart migrates toward a posterior location in more recently divergent lineages.
- c) The pallets were composed of a single unit. In more recent lineages the pallets become segmented, but segmentation is secondarily lost in the most recently diverged lineages.
- d) In the ancestral teredinid, an extension of the rectum (the anal canal) served as a secondary storage site for wood particles. Recent research demonstrates that this organ, like the caecum, contains a brush border epithelium and is likely a secondary site for wood degradation and nutrient absorption. This feature is lost in more recently diverging lineages.
- e) The ancestral teredinid was a broadcast spawner. Internal fertilization and larvipary evolved only once in the most recently divergent Teredinidae.

In xylophaginae:

- a) Apophyses are lost in all xylophaginae.

Uncertain features:

- a) The formation of a calcareous tube lining the burrow appears in all Teredinidae and at least one genus of Xylophaginae (*Xyloredo*) but is absent in all non-wood-feeding Pholadidae.
- b) Pallets are present in all Teredinidae and at least one genus of Xylophaginae (*Xylopholas*) but are absent in all non-wood-feeding Pholadidae.
- c) The separation of the heart and intestine is observed in all Teredinidae except *Kuphus*, but is absent in all Pholadidae and Xylophaginae.

The phylogenetic position of *Kuphus*, *Xyloredo*, and *Xylopholas* must be resolved before it is possible to determine which of these features is ancestral and which is derived and whether any have arisen independently in different lineages. We have obtained specimens of all three genera and are in the process of sequencing the appropriate genes.

Three manuscripts are in preparation based on the described work:

Distel, D.L., Morrill, W., Spenlinhauer, T., Luyten, Y., Cannone, J., and Gutell, R. (in preparation) A secondary structural model of the 28S rRNA from heterodont bivalves: Implications for sequence alignment and phylogeny of the bivalve order Myoida.

Distel, D.L., Mamangkey, G.F., Burgoyne, A., Morrill, W., Spenlinhauer, T., Luyten, Y., and

Amin, M. (in preparation) Phylogenetic analysis of relationships among the bivalve families Pholadidae and Teredinidae based on large and small subunit ribosomal RNA sequences: Evidence for non-monophyly of Pholadidae.

Mamangkey, G.F., Burgoyne, A., Morrill, W., Spenlinhauer, T. Luyten, Y., Amin, M. and Distel, D.L.(in preparation) Evolution of wood-boring, wood feeding and xylotrophic symbiosis in bivalvia.

Training and Development:

To date, six undergraduate students and one graduate student and one technician have been trained in DNA sequencing, DNA cloning, sequence analysis, and phylogenetic analysis. This project has also contributed to research experiences for two high school students.

Outreach Activities:

A collaboration has been established with the Ramey School, Ramey, Puerto Rico; a school serving the American military community in Puerto Rico. Science teacher Rex Roettger will work with me to develop research and educational materials for a high school research project to include eighteen 10th through 12th grade students. The students will conduct experiments to observe the growth of shipworms in their local waters and will record differences in infestation of wooden test panels placed in various depths and salinity regimes. The students will also contribute to and learn about this NSF funded project. They will preserve some of their specimens in ethanol for use in our NSF funded research and we will report to the students on how their efforts have contributed to the overall project.

We have also recently embarked on a community outreach program with the Ipswich School district. The Ocean Genome Legacy Foundation is currently in the process of developing mentoring programs for K-12 teachers in the Ipswich School District. On January 23, 2006, OGLF, in conjunction with New England Biolabs, hosted 15 representatives from the Ipswich primary, middle and high schools, including teachers and administrators, for a three-hour development workshop to formulate a working plan for educational collaboration with the Ipswich school system. OGLF also maintains one part-time intern position for students from the Ipswich High School (position currently held by Sam Wilbur, sophomore).

Journal Publications

Books or Other One-time Publications

Web/Internet Site

URL(s):

www.oglf.org

Description:

The Ocean Genome Resource and Ocean Genome Resource Database are respectively, a marine genome resource bank and an online catalog and database serving this bank. Both are under development by the Ocean Genome Legacy Foundation and are scheduled to become available to the public in the latter

half of 2006. All specimens and samples from this project are currently being accessioned into the Ocean Genome Resource and will be included in the online database which will be hosted on the OGL website (www.oglf.org). Subsamples of tissues, DNAs and amplification products from all accessioned specimens in the Ocean Genome Resource will be made available upon request for research purposes through the database via an on-line request form. Large and small subunit rRNA sequences obtained in this investigation will also be available through the Ocean Genome Resource Database via links to data files. These sequences will also be submitted to genbank where they will be connected to the samples in the OGR database via link-outs. Thus it will be possible to locate all samples or sequences from this investigation through searches of BLAST, Entrez, or the OGR database. Additionally, brief descriptions of all NSF funded research conducted at OGL will be available on the "Research" page at www.oglf.org.

Other Specific Products

Product Type:

Physical collection (samples, etc.)

Product Description:

Ocean Genome Resource Database: Samples and specimens of bivalves tissues, DNAs and voucher specimens from 110 collections supported by this award have been deposited in the Ocean Genome Resource, a public access marine genome resource collection maintained and operated by the Ocean Genome Legacy foundation. The samples are currently being accessioned.

Sharing Information:

The Ocean Genome Resource is scheduled to become available to the public in the latter half of 2006. All accessioned specimens will be included in an online database (currently under development) which will be hosted on the OGL website (www.oglf.org). Subsamples of tissues, DNAs and amplification products from all accessioned specimens can be requested via an on-line request form. Large and small subunit rRNA sequences obtained in this investigation will also be available through the Ocean Genome Resource Database via links to data files. These sequences will also be submitted to genbank where they will be connected to the samples in the OGR database via link-outs. Thus samples or sequences from this investigation can be located through searches of BLAST, Entrez, or the OGR database.

Contributions

Contributions within Discipline:

The work will provide new sequences and insights into the phylogeny of the Myoida which is currently under-represented in GenBank. We will also provide secondary structures for bivalve 18S and 28S rRNA molecules which will be widely useful within the discipline for use as sequence alignment aids. Currently, no published 28S and only one published 18S secondary structure is available for bivalves.

Contributions to Other Disciplines:

The project is demonstrating a unique and distinct progression of evolutionary events triggered by the acquisition of symbionts by a metazoan lineage. Symbiosis has been recognized as a source of evolutionary innovation, however, no well documented and detailed progression of evolutionary adaptations has been observed in a single lineage to demonstrate the types of morphological and physiological changes that may be associated with establishment of a bacterial endosymbiosis. The evolution of bacteria-animal interactions is of general interest to the study of evolutionary biology, evolutionary medicine and infectious disease, developmental biology and immunology.

Contributions to Human Resource Development:

To date, six undergraduate students and one graduate student and one technician have been trained in DNA sequencing, DNA cloning, sequence analysis, and phylogenetic analysis. The work is also contributing to the professional development of one foreign graduate student from a the developing nation of Indonesia and has contributed to research experiences for two high school students.

Contributions to Resources for Research and Education:

This project has contributed to the Ocean Genome Resource and database which will provide important physical resources (specimens), data and tools to the research and educational communities.

Contributions Beyond Science and Engineering:

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

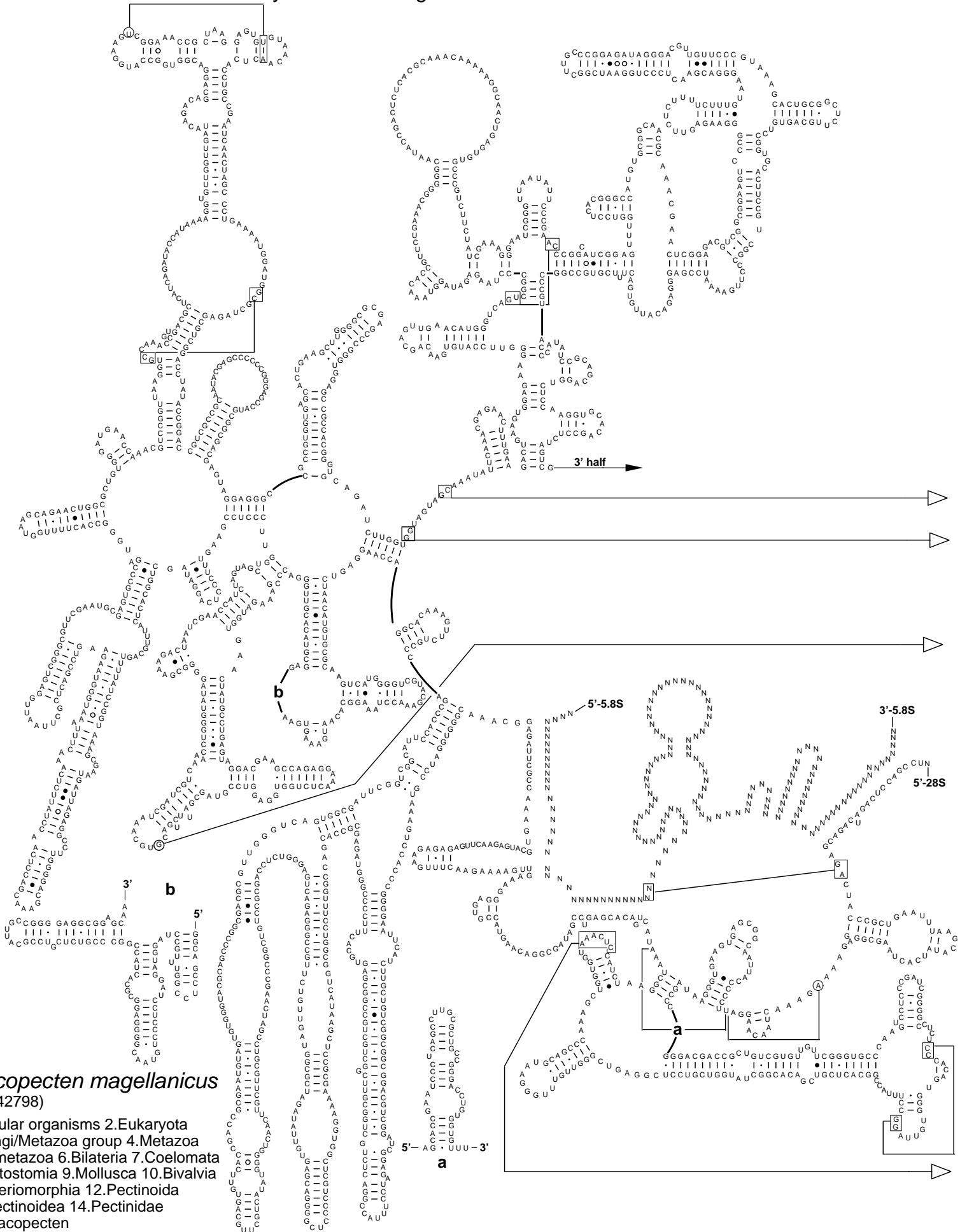
Organizational Partners

Any Journal

Any Book

Contributions: To Any Beyond Science and Engineering

Secondary Structure: large subunit ribosomal RNA - 5' half



Placopecten magellanicus
(AF342798)

1. cellular organisms
2. Eukaryota
3. Fungi/Metazoa group 4. Metazoa
5. Eumetazoa 6. Bilateria 7. Coelomata
8. Protostomia 9. Mollusca 10. Bivalvia
11. Pteriomorphia 12. Pectinoidea
13. Pectinoidea 14. Pectinidae
15. Placopecten

October 2004

Specimen number	Species name	Source	Collection site	18S bp of sequence completed	28S bp of sequence completed	Status
102	<i>Lyrodus pedicellatus</i>	Scott Gallager	Woods Hole Oceanog. Inst. MA, Culture	1782	3196	sequencing completed
106	<i>Hiatella arctica</i> (formerly <i>Petricola carditoides</i>)	R. McConaughy (SIO-UCSD)	La Jolla, CBM field station, 15m	1810	1416	sequencing completed
107	<i>Xylophaga atlantica</i>	Jack Merrill	12 miles east of Southwest Harbor, ME, 100m	1840	3143	sequencing completed
136	<i>Mya arenaria</i>	Supermarket, Bangor, ME	Maine coast	1792	3106	sequencing completed
167	<i>Cyrtopleura costata</i>	R. Mann, William and Mary, VA	Wachapreague, VA	1861	3120	sequencing completed
169	<i>Petricola pholidiformis</i>	R. Mann, William and Mary, VA	Wachapreague, VA	1799	1380	28S sequence incomplete missing first 100 bp
172	<i>Xylophaga washingtona</i>	Seth Tyler, U. of Wash.	Dredged Wood, Friday Harbor, WA	1837	1477	sequencing completed
173	Unknown shipworm	Seth Tyler, U. of Wash.	Dredged Wood, Friday Harbor, WA	n	n	no pallets, no ID
210	<i>Martesia sp.</i> (striata?)	Sherry Reed	Ft. Pierce FL., Australian pine stump	1610	n	duplicate taxon (see 281)
212	<i>Pholas dactylus</i>	Ben Totterdell,	Rocky Shore, Charmouth, Dorset, UK	1804	1454	sequencing completed
216	<i>Barnea truncata</i>	Roger Mann (VIMS)	Wachapreague, VA	1824	1506	sequencing completed
217	<i>Teredo sp.</i> Bartschi	Dan Distel	Tobago, driftwood	1828	1487	sequencing completed
218	<i>Bankia sp.</i> carinata	Dan Distel	Tobago, driftwood	n	1503	duplicate taxon (see 281)
220	<i>Teredo navalis</i>	Belfast Harbor, 9/22/00	Collection panels, Belfast pier	1830	3149	duplicate taxon (see 281)
229	<i>Bankia gouldi</i>	Battelle Lab		n	n	not yet dissected
230	<i>Teredo navalis</i>	Battelle	Station 10A	n	n	duplicate taxon
236	<i>Kuphus polythalamia</i>	Donald Dan, Ft Meyers FL	Zamboanga del Sur, Mindanao, Philippines	1795	1437	sequencing completed
258	<i>Xylophaga sp.</i>	R. Kilburn, Natal Museum, S. Africa	SE of Port Dunford, 29 02.2S: 32 19.6E, 760-800m	1777	1457	sequencing completed
259	<i>Bankia setacea</i>	Karen Ripley, WA	Brown's Bay, WA, from a rotted piling	1793	1490	sequencing completed
260	<i>Neoteredo reynii</i>	Praia Dura, Sao Paulo, Brazil	Praia Dura, Ubatuba, Brazil	1794	1462	sequencing completed
261	<i>Nausitora fusticula</i>	Praia Dura, Sao Paulo, Brazil	Praia Dura, Ubatuba, Brazil	1846	1427	sequencing completed
263	<i>Bankia carinata</i>	Dan Distel	Lac Bay, Bonaire, submerged wood	1791	1497	sequencing completed
264	<i>Spathoteredo obtusa</i>	Gustaf Mamangkey, Manado	Manado Bay, Inodesia	1769	3180	sequencing completed
266	<i>Bankia australis</i>	Gustaf Mamangkey, Manado	Manado Bay, Inodesia	1821	1497	sequencing completed
267	<i>Lyrodus massa</i>	Gustaf Mamangkey, Manado	Manado Bay, Inodesia	1816	1467	sequencing completed
268	<i>Teredothyra sp.</i> (dominicensus?)	Dan Distel	Bachelor's Beach, Bonaire, NA, sunken wood 3m	1819	1490	sequencing completed

269	Teredo sp.	Dan Distel	Bachelor's Beach, Bonaire, NA, sunken wood 3m	n	n	not yet dissected
270	Teredora sp. malleolus?	Dan Distel	Lagoen, Bonaire, NA, driftwood	n	n	not yet dissected
271	Bankia sp. zeteki?	Dan Distel	Lagoen, Bonaire, NA, driftwood	n	n	not yet dissected
273	Lyrodus sp. pedicellatus?	Dan Distel	Bachelor's Beach, Bonaire, NA, sunken wood 3m	n	n	duplicate taxon
274	Bankia australis	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	not yet dissected
275	Spathoteredo obtusa	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	duplicate taxon
276	Nausitora dunlopei	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	1818	1475	duplicate taxon
277	Lyrodus massa	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	duplicate taxon
278	Lyrodus pedicellatus	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	duplicate taxon
279	Dicyathifer manni	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	not yet dissected
280	Martesia striata	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	n	n	duplicate taxon
281	Martesia sp	Gustaf Mamangkey, Manado	West coast of Minahasa Peninsula, Inodesia	1690	1503	sequencing completed
282	Lyrodus sp.	Dan Distel	Banana River, FL, mangrove wood	1812	1173	sequencing completed
288	Xyloredos sp.	Charles Fisher	Gulf of Mexico, (27 44.75 : 91 13.31) 540m, oak	1430	1430	sequencing completed
289	Xylopholas sp.	Charles Fisher	Gulf of Mexico, (27 44.75 : 91 13.31) 540m, oak	1800	1470	sequencing completed
290	Hiatella arctica	Gonzalo Girabet, Harvard U.	Banyuls sur Mer, France, dredge, 30-60m	n	n	duplicate taxon
291	Varicorbula limulata	Gonzalo Girabet, Harvard U.	Florida, trawl FK-106	n	694	DNA extracted, but degraded
297	Panope	Taylor United, Inc	Taylor shellfish farms	1762	1412	sequencing completed
328	Mya truncata	Gulf of Maine, Inc	Cobscook Bay, Maine	1823	3096	sequencing completed