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# An Investigation of the Evolution of Autotrophic Endosymbioses in Bivalves By Comparative Molecular Phylogenetic Analysis of Host and Symbiont rRNA Sequences

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**Principal Investigator:** Distel, Daniel L.

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**Organization:** University of Maine

An Investigation of the Evolution of Autotrophic  
Host and Symbiont rRNA Sequences

Endosymbioses in Bivalves By Comparative Molecular

Phylogenetic Analysis of

### Project Participants

#### Senior Personnel

**Name:** Distel, Daniel

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

#### Post-doc

#### Graduate Student

**Name:** Beaudoin, David

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

**Name:** Xu, Pei-Ning

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

#### Undergraduate Student

**Name:** Gyurko, Lisa

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Coro, Shannon

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Huard, Jennifer

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Draheim, Roger

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Farquhar, John

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Kilburn, Elizabeth

**Worked for more than 160 Hours:** No

**Contribution to Project:**

**Name:** Toussaint, Noelle  
**Worked for more than 160 Hours:** No  
**Contribution to Project:**

**Name:** Morrill, Wendy  
**Worked for more than 160 Hours:** Yes  
**Contribution to Project:**

Wendy began on this project as an undergraduate doing her senior research and continued after graduation as a lab technician.

### Organizational Partners

### Other Collaborators or Contacts

Dr. Ellen Kenchington, Dr. Marco Abbiati, Dr.s Allan and Eve Southward, Dr. Gustav Paulay, Dr. Robert Feldman, Dr. Robert Vrijenhoek, Dr. Charles Fisher, Dr. Brian Beal, Ryan Carnegie and Gordon King provided specimens, Dr. Thomas Waller and Dr. Arthur Bogan assisted with species identifications, Dr. Ellen Kenchington also provided the use of sequence data prior to their publication and Dr. Martin Polz, Dr. Andrew Peek and Dr. John Huelsenbeck provided thoughtful discussion of manuscripts and analyses.

### Activities and Findings

#### **Project Activities and Findings: (See PDF version submitted by PI at the end of the report)**

My major research activities were to select taxa for analysis, arrange collection, prepare specimens, amplify and sequence gene fragments and to perform phylogenetic analyses on the resulting data, to publish results and to oversee the work of 2 graduate students and 1 lab technician that contributed to the project. In addition I advised numerous undergraduate students who contributed to the project either as volunteers or during the course of senior research projects or undergraduate work-study jobs. A portion of my efforts during the funding period also contributed to the creation and maintenance of the Biological Computing Facility which I established at the University of Maine (with support from the BMMB department, U. Maine) and which serves the U. Maine research community. Teaching related to this research also extended to a summer Marine Molecular Biology Workshop described below.

#### **Project Training and Development: (See PDF version submitted by PI at the end of the report)**

Since the beginning of this funding period, *ssrRNA* sequences have been fully or partially determined for hosts and symbionts from 31 new symbiotic associations and 17 non-symbiotic bivalves including 14 previously unexamined superfamilies (6 of which bear symbionts)[see PDF file attached to previous section for table]. This makes possible the first comprehensive phylogeny for bivalves based on nearly complete *ssrRNA* sequences and considerably expands our knowledge of the phylogeny and diversity of symbiotic bivalves. Gill-borne bacterial endosymbioses were described for the first time in 2 new bivalve families. The first comprehensive phylogeny for the family Mytilidae based on 18S rRNA sequences was completed.

and published. The first molecular phylogenetic evidence for the proposed mytilid subfamily Bathmodiolinae was published and it was demonstrated that the deep-sea wood- and bone-associated mussels of the genera *Idas*, *Myrina*, *Adipicola*, and *Benthomodiolus* form a clade that also contains hydrothermal vent and seep mussels of the subfamily Bathymodiolinae. It was proposed that these genera also be included in this subfamily. It was also proposed that wood and bone deposits may have served as evolutionary stepping stones for the introduction of symbiont-containing mussels to hydrothermal vents and cold seeps [see attached PDF file for tree]. The anticipated congruent evolution of hosts and symbionts was not observed in either of the two bivalve families examined. In the Mytilidae resolution of the host phylogeny was insufficient to conclude whether congruence could be observed. Continuing investigations with additional genes (mitochondrial 16S, COI) continues without an identified funding source. In the second family, Lucinidae, significant congruence between host and symbiont phylogeny has not been observed. Publication of data awaits results from one additional very rare Genus (*Anodontia*), specimens for which have only recently been located and will be collected shortly. Results from host phylogenetic analyses indicate however that Lucinaceans are among the deepest branching eulamellibranchs.

#### **Research Training:**

This project contributed to the training of two graduate students who received Masters Degrees during the funding period. One undergraduate student trained under the project went on to become a laboratory technician in my lab. Several undergraduate students completed the research requirements for their BS degrees by contributing to this project. Three undergraduate students received Work Merit Scholarships, awarded by the University of Maine, for their contributions to this research project. In addition two high school students worked on summer projects

extended from this project in the summer just after the close of the funding period, one through the Maine Upward Bound Program and the other through the River Project. Both students received honors for their efforts. Additionally, this project contributed to SMS 587 Marine Molecular Biology Training Course. In each year of this project ten students in this graduate course received molecular biology training while learning to clone, amplify, sequence and analyse 18S rRNA sequences. These students also contributed valuable data to this project.

#### **Outreach Activities:**

The previously described summer course SMS Marine Molecular Biology Training Course, in which students have contributed to this research while learning to amplify, clone, sequence and analyse 18S rRNA genes, has a strong outreach component. Students have included faculty members from College of the Atlantic, ME, Auburn University, AL, and the University of Maine and graduate students from Boston University, Boston, MA, U. Maine, Orono and Machias, ME, College of the Atlantic, Bar Harbor, ME and the University of Muenchen, Germany.

#### **Journal Publications**

Distel, D.L., "Evolution of chemoautotrophic endosymbiosis in bivalves", *Bioscience*, p. 277-286, vol. 48, (1998). ) Published

Distel, D.L., "Phylogenetic relationships among Mytilidae (Bivalvia): 18S rRNA data suggest convergence in mytilid body plans.", *Molecular Phylogenetics and Evolution*, p. , vol. , (). ) Accepted

Distel, D.L., A. Baco, E. Chuang, W. Morrill, C.M. Cavanaugh and C. Smith, "Wooden steps for vent invasion: Mussels from deep-sea wood and bone deposits are closely related to hydrothermal vent species.", *Nature*, p. , vol. , (2000). ) Accepted

#### **Books or Other One-time Publications**

#### **Web/Internet Sites**

URL(s):

Description:

#### **Other Specific Products**

#### **Contributions**

##### **Contributions within Discipline:**

Products: As a result of this project the number of nearly complete bivalve 18S rRNA gene sequences submitted to Genbank will be doubled. The same is true for 16S rRNA sequences for bacterial endosymbionts of bivalves. These sequences will be available for use by other researchers.

Findings: We have shown that two traditional mytilid subfamilies are not monophyletic as currently described. Additionally we have shown evidence for convergence in body plans within both modioliform and mytiliform mussels. We have demonstrated that hydrothermal vent/cold seep mussels represent a clade distinct from other mytilid subfamilies and in so doing support the subfamily Bathymodiolinae proposed for these mussels. We have also demonstrated that this clade includes other non-vent/seep mussels of previously undetermined phylogenetic affiliation. These mussels are typically associated with wood and bone deposits in the deep sea and at least one species has been shown to contain chemoautotrophic symbionts. These results support and extend the 'stepping stone hypothesis proposed by Smith et al. 1989 Nature 341:27-28, by demonstrating that mussel species associated with whale bones and wood are plausible progenitors of symbiont-containing hydrothermal vent mussels. Work in progress will demonstrate that Lucinid clams include the deepest branching eulamellibranchs known, supporting the proposed antiquity of this group and the relationship of modern Lucinids to the fossil Babinkacea. We will also show that phylogenetic trees inferred for symbiotic bacteria harbored by Lucinaceans are not congruent with trees inferred for the hosts based on 16/18S rRNA sequences.

##### **Contributions to Other Disciplines:**

##### **Contributions to Human Resource Development:**

Two graduate students and one undergraduate student whose research were supported by this grant have gone on to professional careers in science. The undergraduate student became a research assistant in my lab and has worked for three other laboratories at the University of Maine. The graduate students are currently working as research assistants at Woods Hole Oceanographic Institution and a Ph.D student at

Johns Hopkins School of Medicine.

**Contributions to Science and Technology Infrastructure:**

**Beyond Science and Engineering:**

**Categories for which nothing is reported:**

Organizational Partners

Any Book

Any Product

Contributions: To Any Other Disciplines

Contributions: To Any Science or Technology Infrastructure

Contributions: Beyond Science or Engineering

Table:1 Species Used In This Study	Project Status		Collection Site	Habitat (Depth in m)
	18S	16S		
<b>Phylum Mollusca</b>				
Class Bivalvia				
Protobranchia				
Superfamily Nuculidae				
Family Solemyidae				
<i>Solemya occidentalis</i> *	p	p	Carrie Bow Bay, Belize	reducing sediment
<i>Solemya velum</i> *	rc	rc	Cape Cod, MA	eel grass bed
<i>Solemya reidi</i> *	rc	rc	Santa Monica Bay, CA	sewage outfall (150)
Family Nuculanidae				
<i>Nuculana grasslei</i>	c	t	Guaymas Basin	hydrothermal vent
<i>Nucula sp.</i>	c	-	Cape Cod, MA	
<i>Yoldia sp.</i>	c	-	Cape Cod, MA	
Filibranchia				
Family Mytilidae				
Subfamily Crenellinae				
<i>Musculus senhousi</i>	c	-		
Subfamily Lithophaginae				
<i>Lithophaga lithophaga</i>	c	-	Rivorno, Italy	rocky shore
<i>Lithophaga nigra</i>	c	-	Sella Bay, Guam	dead coral
Subfamily Modiolinae				
<i>Modiolus modiolus</i>	c	-		
<i>Modiolus auriculatus</i>	c	-	Guam	Agana reef flat
<i>Louisiana mussel (mussel III)</i> *	c	t	Louisiana Slope	cold-water seep (600)
<b>Mid-Atlantic Ridge (MAR) mussel*</b>	p	p	Mid-Atlantic Ridge	hydrothermal vent (3500)
<i>Louisiana (LA) mussel (mussel Ia)</i> *	p	p	Louisiana Slope	cold-water seep (600)
<i>Idasola washingtona</i> *	c	t	Santa Catalina Basin, CA	whale carcass (1240)
<i>Florida escarpment (FL) mussel</i> *	p	p	W. Florida Escarpment	cold-water seep (3000)
Family Ostreoidae				
<i>Ostrea edulis</i>	c	-	Damariscotta River, ME	intertidal
Lamellibranchia				
Family Myidae				
<i>Mya arenaria</i>	c	-	Salisbury Cove, ME	intertidal
Family Vesicomidae				
<i>Vesicomya cordata</i> *	p	p	Gulf of Mexico	reducing sediment (600)
<i>Calyptogena magnifica</i> *	rc	rc	Galapagos Rift	hydrothermal vent (2300)
<i>Calyptogena sp. Japan Trench</i> *	p	p	Kaiko Trench, Japan	reducing sediment (2000)
<i>Calyptogena sp. Monterey</i> *	rc	rc	Monterey Canyon, CA	reducing sediment (3400)
Family terebridae				
<i>Lyrodes pedicellatus</i> *	c	c		submerged wood
Family Pholadidae				
<i>Xylophaga atlantica</i> *	c	c	SW Harbor, ME	submerged wood (100)
<i>Xylophaga washingtona</i> *	c	c	Monterey Bay, CA	submerged wood (61)
Superfamily Lucinacea				
Family Lucinidae				
<i>Lucinoma annulata</i> *	p	p	Santa monica, CA	reducing sediment (70)
<i>Lucinoma borealis</i> *	c	c	Plymouth Sound, UK	reducing sediment
<i>Lucinoma aequizonata</i> *	c	c	Santa Barbara Basin, CA	reducing sediment (500)
<i>Codakia orbicularis</i> *			Nassau, Bahamas	intertidal
<i>Codakia costata</i> *	p	p	Whalebone Bay, Bermuda	eelgrass bed (subtidal)
<i>Anodontia philippiana</i> *	c	c	Ferry Reach, Bermuda	mangrove swamp
<i>Loripes lucinalis</i> *	c	c	Plymouth Sound, UK	reducing sediment
<i>Lucina pectinata</i> *	c	i	Puerto Rico	
<i>Lucina floridana</i> *	p	p	St. Joseph Bay, FL	eelgrass bed (subtidal)
<i>Parvalucina tenuisculpta</i> *	c	c	Port Alberni, BC	reducing sediment (70)
<i>Parvalucina tenuisculpta</i> *	c	c	San Diego, CA	reducing sediment (60)
<i>Ctena bella</i> *	i	i	Mangilao, Guam	
<i>Unidentified lucinid</i> *	t	t	Port Alberni, BC	
Family Thyasiridae				
<i>Thyasira sarsi</i> *	c	?	Sweden	reducing sediment (15)
<i>Thyasira flexuosa</i> *	c	c	Plymouth Sound, UK	reducing sediment (15)
<i>Thyasira gouldi</i> *	c	c	Port Alberni, BC	reducing sediment
<i>Axinopsida serricata</i>	c	?	Port Alberni, BC	reducing sediment
<i>Axinus grandis</i>	c	?	Juan de Fuca straight	sedimented vent site
Family Fimbridae				
<i>Fimbria fimbriata</i> *	c	c	Mangilao, Guam	tidal flat
Family Ungulinidae				
<i>Diplodonta sp.</i>			Located; not yet collected	
Family Carditidae				
<i>Cardita sp.</i>	c	-	Cape Cod, MA	
<i>Laevicardium sp.</i>	c	-	Cape Cod, MA	
Family Cultellidae				
<i>Ensis sp.</i>	c	-	Cape Cod, MA	
Family Astartidae				
<i>Astarte sp.</i>	c	-	Cape Cod, MA	
Family Veneridae				
<i>Gemma sp.</i>	i	-	Cape Cod, MA	
Family Pholadidae				
<i>Cyrtopleura costata</i>	t	?	Perrymore Island, VA	intertidal
<i>Barnae truncata</i>	t	?	Perrymore Island, VA	intertidal
Family Petricolidae				
<i>Petricola carditoides</i>	c	-	La Jolla, CA	submerged concrete
<i>Petricola pholididormis</i>	t	-	Perrymore Island, VA	intertidal
Family Dreissenidae				
<i>Dreissena polymorpha</i>	t	-	Perrymore Island, VA	intertidal
Class Gastropoda				
<i>Alvinocoencha hessleri</i> *	t	t	Marianas back arc basin	hydrothermal vent (4000)
* = known or suspected symbiont-bearing species.				
c = complete double strand sequence (~1500bp / 16S rRNA, 1800bp / 18S rRNA)				
i = work in progress				
r = redone this study				
p = partial and/or single strand sequence				
t = to be examined				
? = suspected symbiotic association				
- = no known symbiotic association				

