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Presence of two phylogenetically distinct groups in the deep-sea mussel *Acharax* (Mollusca: Bivalvia: Solemyidae)

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ABSTRACT: The family Solemyidae represents ancestral protobranch bivalves with the shallow-water genus *Solemya* and the deep-sea genus *Acharax*. All known members of this family host symbiotic sulfur-oxidizing bacteria in their gill filaments. Analysis of 18S rRNA gene sequences of *Acharax* specimens from methane-seeps off Makran (Pakistan), Java (Indonesia), the Aleutian Trench and off the Oregon, Costa Rica, and Peru margins revealed that *Solemya* spp. and *Acharax* spp. are well-separated genetically. This supports the current systematic distinction based on morphological criteria. We found 2 clearly distinct clusters within the genus *Acharax*, with specimens from the Makran, Oregon and Peru (MOP) margins in one (MOP–*Acharax*) cluster, and those from Java, the Aleutian Trench and Costa Rica (JAC) in the other (JAC–*Acharax*) cluster. The separation of MOP– and JAC–*Acharax* clusters from each other and from *Solemya* (*S. reidi* and *S. velum*) is well-supported by phylogenetic calculations employing maximum likelihood and maximum parsimony. Compared to genetic distances among other protobranch groups, distances between the MOP– and JAC–*Acharax* clusters would justify the affiliation of these clusters to separate species. This implies that species differentiation in *Acharax* based on shell morphology is likely to underestimate true species diversity within this taxon. Furthermore, our results support the hypothesis that genetic separation of *Solemya* and *Acharax* is congruent with the phylogeny of their bacterial endosymbionts.

KEY WORDS: Bivalvia · Solemyidae · *Acharax* · *Solemya* · Molecular phylogeny · 18S rRNA gene · Endosymbionts · Sulfur-oxidizing bacteria

INTRODUCTION

The family Solemyidae is a group of ancestral protobranch bivalves, and when in conjunction with the family Manzanellidae forms the superfamily Solemyoidea. As far as is known, all species of the Solemyidae live in symbiosis with chemoautotrophic sulfur-oxidizing bacteria, on which they depend to varying degrees (Fisher 1990). Some species lack an alimentary canal completely (Bernard 1980). Solemyid bivalves are cosmopolitan. The genus *Solemya* is generally found at continental-shelf and upper-slope depths (0 to 600 m), while the genus *Acharax* is found deeper, at depths from ~400 m on the continental slope to the deepest sites on the slope of the Japan Trench at 5379 m (Kuznetsov & Schileyko 1984, Métivier & von Cosel 1993, Coan et al. 2000, Okutani & Fujikura 2002).

The genus *Solemya* lives in sediments with high organic-matter content, often at reduced oxygen concentrations. Hydrogen sulfide is frequently present due to sulfate reduction coupled with organic matter degradation (Conway et al. 1992). After the discovery of the symbiotic chemoautotrophic bacteria, numerous studies of *Solemya* enlightened the physiology of the symbiosis (Cavanaugh 1983, Felbeck 1983, Powell & Somero 1986, Anderson et al. 1987, Cavanaugh et al. 1988, O'Brien & Vetter 1990, Vetter 1990, Wilmot & Vetter 1992, Lee & Childress 1994) as well as the phylogeny and transmission of the endosymbionts (Gustafson & Reid 1988, Eisen et al. 1992, Cary 1994,

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Distel et al. 1994, Krueger et al. 1996a,b, Krueger & Cavanaugh 1997).

In contrast, the genus *Acharax*, which lives buried in reducing sediments, has rarely been taken alive, and information on its phylogenetic position and diversity is lacking. Recovery or observations of the characteristic thick periostracum was often the only evidence for the presence of this genus. During this study, we recovered living specimens from methane seeps in the Indian Ocean off Pakistan (von Rad et al. 2000) and off Indonesia (Wiedicke et al. 2002), as well as in the Pacific Ocean in the Aleutian Trench (Suess et al. 1998), at Hydrate Ridge off Oregon (Sahling et al. 2002), and off Costa Rica and Peru (H. Sahling unpubl.). Hydrogen sulfide was present at these sites, reaching concentrations of up to 300 µM in the upper 25 cm at Hydrate Ridge (Sahling et al. 2002). The genus *Acharax* was also found at other Cold Seep locations (see review by Sibuet & Olu 1998) and in sediments influenced by hydrothermal venting (Juniper et al. 1992, Métivier & von Cosel 1993). Furthermore, the periostracum was frequently found in compacted clay substrata along the western North American coast (Coan et al. 2000). *Acharax* spp. apparently exploit deep-sea habitats, in which sulfide is present.

The genus *Acharax* differs morphologically from *Solemya* in having an external ligament set on a narrow nymph (Coan et al. 2000). However, at a first glance both solemyids appear so similar that specimens discovered at various deep-sea sites might have been misclassified erroneously as *Solemya* (see review by Sibuet & Olu 1998). For example, re-examination of specimens from the Aleutian Trench and from sediments off Oregon revealed *Acharax* but not *Solemya* (as reported earlier: Suess et al. 1985, Suess et al. 1998).

It was shown in a previous study with *Acharax* specimens from the same locations that this genus harbors sulfur-oxidizing bacteria in its gills (Imhoff et al. 2003). According to 16S rRNA gene-sequence analysis, these bacteria formed a new group of sulfur-oxidizing endosymbiotic Gammaproteobacteria. This group was separated from other known symbiotic bacteria, including the symbionts from *Solemya* species and from other solemyid and lucinid bivalves. The question arose as to whether the *Acharax* host, similar to its endosymbiotic bacteria, is clearly separated from its sister genus *Solemya* on a genetic basis, thereby giving support to a possible host–symbiont coevolution.

MATERIALS AND METHODS

Origin and denomination of bivalves. Specimens of *Acharax* spp. had been collected during several cruises of RV 'Sonne' from 6 different regions in the Indian and Pacific Ocean and stored at –30°C (Table 1, Fig. 1). From the shell morphology (periostracum), all specimens were identified as belonging to the same species, most probably *A. johnsoni*. This was supported by experts on bivalve taxonomy (P. V. Scott and E. V. Coan pers. comm.). A representative assortment of *Acharax* specimens, including representatives of MOP–*Acharax* and JAC–*Acharax* clusters, collected at the same sites as the individuals investigated in this study, was deposited under Accession No. 2003-039 at the Santa Barbara Museum of Natural History, USA.

DNA extraction, amplification and sequencing. Bivalve DNA was extracted from the foot (A1 Peru from the mantle) using QIAGEN® Genomic-tip 20/G (QIAGEN) with the Genomic DNA Buffer Set (QIAGEN). Primers for amplification and sequencing of bivalve 18S rDNA were designed using complete molluscan 18S rDNA sequences from the EMBL nucleotide sequence database (Stoesser et al. 2003). For detection of suitable priming regions, these sequences were aligned with the program ClustalX Version 1.81 (Thompson et al. 1997) using default parameters (gap-opening penalty= 15; gap-extension penalty > 6.66). Primer synthesis was accomplished by MWG Biotech. Polymerase chain reaction (PCR) was conducted by use of puReTaq™ Ready-To-Go™PCR Beads (Amersham Biosciences) in a Progene® thermocycler (Techne) with Primers 5′-GCCAGTAGCATATGCTTG TCTC-3′ (forward from Position 9 in the sequence of *Solemya reidi*) and 5′-CCTTGTTACGACT TTTAC-3′ (reverse from Position 1782 in the sequence of *S. reidi*). The anticipated size of the PCR product was about 1770 nucleotides (nt). Cycling conditions were 40 cycles in the following pattern: 40 s at 91°C for denaturing, 40 s at 50°C for

Table 1. *Acharax* spp. Collection data of specimens examined. Denomination: A (for *Acharax*), individual identification no., sampling region; SO: RV 'Sonne', TVG: TV-guided grab

Denomination	Cruise	Year	Position	Depth (m)	Stn
A1-Oregon	SO 143	1999	44° 34.2′ N, 125° 08.8′ W	780	TVG 71-2
A2-Oregon	SO 143	1999	44° 40.2′ N, 125° 03.3′ W	910	TVG 40-2
A8-Oregon	SO 143	1999	44° 34.2′ N, 125° 08.8′ W	780	TVG 56-1
A10-/A13-Oregon	SO 143	1999	44° 40.2′ N, 125° 03.3′ W	910	TVG 40-2
A1-/A2-/A3-Java	SO 139	1999	7° 57.5′ S, 106° 17.7′ E	2940	TVG 91
A1-/A2-Makran	SO 130	1998	24° 33.0′ N, 64° 15.6′ E	2220	TVG 330
A5-Aleutian	SO 110	1996	54° 18.2′ N, 157° 11.9′ W	4810	TVG 43
A1-Peru	SO 78	1992	ca. 9° 35′ S, ca. 80° 07′ W	ca. 3500	Dredge 163
A3-Costa Rica	SO 163	2002	9° 10.4′ N, 084° 48.2′ W	763	TVG 3

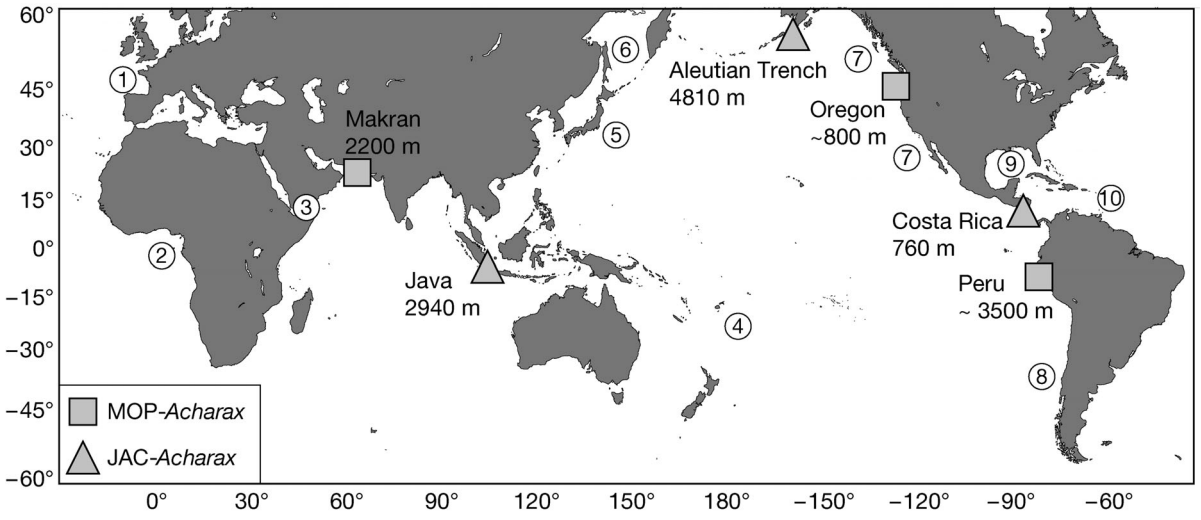

Fig. 1. *Acharax* spp. Global distribution of deep-sea solemyids. Locations and depths of *Acharax* specimens including those used in this study are indicated, as is their affiliation with the MOP (Makran, Oregon, Peru margins)–*Acharax* and JAC (Java, Aleutian Trench, Costa Rica)–*Acharax* clusters. Data from 1, 2: H. Sahling (unpubl.); 3: Kuznetsov & Schileyko (1984, *A. eremita*); 4: Métivier & von Cosel (1993, *A. alinae*); 5: Okutani & Fujikura (2002); 6: Sahling et al. (2003); 7: Coan et al. (2000); 8: Sellanes et al. (2004); 9: Carney (1994); 10: Olu et al. (1997); Makran: von Rad et al. (2000); Java: Wiedicke et al. (2002); Aleutian Trench: Suess et al. (1998); Oregon: Sahling et al. (2002); Costa Rica and Peru margins: H. Sahling (unpubl.)

annealing, and 1.5 min at 72°C for elongation; the initial step was 2 min at 91°C for denaturing, the terminal steps were 40 s at 91°C for denaturing, 1 min at 40°C for annealing, and 5 min at 72°C for elongation. PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN). Cycle sequencing from PCR products was conducted with the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit Version 2.0 (Applied Biosystems) and suitable sequencing primers designed and synthesized as described above. Capillary electrophoresis was done on an ABI PRISM® 310 Genetic analyzer (Applied Biosystems). We sequenced both strands of the 18S-rRNA gene to ensure data consistency. Sequence fragments were assembled with the program SeqMan™ II Version 4.03 (DNASTAR).

Sequence alignment. For phylogenetic analysis, the sequences of our specimens were aligned together with additional sequences of other protobranch bivalves and the sequence of the scaphopod *Antalis pilsbryi* as outgroup (Table 2). Alignment was done with the program ARB (Ludwig et al. 2004). The 18S rRNA secondary structure of *Saccharomyces cerevisiae* (Table 2) was used as guidance for manual alignment. Gap-only positions introduced by ARB and terminal parts with missing data were removed from the alignment with ClustalX, leading to a total length of the alignment of 1714 positions. The alignment is available from the corresponding author of this study.

Phylogenetic analysis. Maximum likelihood (ML) and maximum parsimony (MP) calculations were conducted with PAUP* Version 4.0 beta 10 Win (Swofford 2003). We employed the program Modeltest Version 3.7 (Posada & Crandall 1998) to determine the most appropriate model of nucleotide substitution.

Table 2. 18S rRNA gene sequences from EMBL database used for phylogenetic calculations. Sequences for scaphopod *Antalis* (*Dentalium*) *pilsbryi* and baker's yeast *Saccharomyces cerevisiae* are shown for comparison. 18S rRNA gene sequences of *Acharax* deposited under EMBL Accession No. AJ563751 to AJ563763

Taxonomy	Species	EMBL Accession No.	Sequence length
Bivalvia, Protobranchia			
Superfamily Solemyoidea			
Family Solemyidae	*Solemya reidi*	AF117737	1806
	Solemya velum	AF120524	1771
Superfamily Nuculoidea			
Family Nuculidae	*Nucula sulcata*	AF207642	1765
	Nucula proxima	AF120526	176
Superfamily Nuculanoidea			
Family Yoldiidae	*Yoldia myalis*	AF207643	176
Family Nuculanidae	*Nuculana pernula*	AY145385	181
Family Neilonellidae	*Neilonella subovata*	AF207645	1770
Scaphopoda	*Antalis pilsbryi*	AF120522	1804
Fungi, Ascomycota	*Saccharomyces cerevisiae*	V01335	1798

According to the Akaike Information Criterion (AIC) (Akaike 1974, Posada & Buckley 2004), the model TrN+I+G provided the best fit to our data. Search for the optimal tree was performed by random stepwise addition with 1000 repetitions in ML and 1 000 000 repetitions in MP (gaps treated as 'missing'), with subsequent tree-bisection-reconnection (TBR). Bootstrap analyses were accomplished with 100 replicates in ML and 10 000 replicates in MP, with 10 search repetitions per replicate in both cases.

RESULTS

Maximum likelihood (ML)

As shown in the ML tree (Fig. 2), the Solemyoidea (*genera Solemya* and *Acharax*) form a distinct sister group to the Nuculoidea (*Nucula proxima*, *N. sulcata*) and, together with the latter, a sister group to the Nuculanoidea (*Nuculana pernula*, *Yoldia myalis*, *Neilonella subovata*). The tree reveals a tripartition of the examined solemyid bivalves: (1) *Solemya* cluster: sequences of *Solemya reidi* and *S. velum;* (*2*) Makran–Oregon–Peru *Acharax* cluster (MOP–*Acharax* cluster): sequences of the specimens of *Acharax* spp. from the regions off Makran, Oregon, and from the Peru margins; (3) Java–Aleutian–Costa Rica *Acharax* cluster (JAC–*Acharax* cluster): sequences of the specimens of *Acharax* spp. from the regions off Java and Costa Rica, and from the Aleutian Trench.

Sequence differences between *Solemya reidi* and *S. velum* were very small, and the branch of *S. velum* actually collapsed to zero by the tree calculation algorithm (branch length $\leq 10^{-8}$ substitutions per alignment position). All *Acharax* specimens form a group well-separated from the *Solemya* cluster. JAC–*Acharax* in turn forms a well defined subcluster deriving from MOP–*Acharax*, showing a remarkably high difference to the other 2 solemyid clusters. The specimens from Makran exhibit considerably higher genetic differences than the other members of MOP–*Acharax* and are placed between the latter and JAC–*Acharax*.

Maximum parsimony (MP)

Results of MP calculations based upon 184 parsimony-informative characters yielded 5 equally parsimonious trees (not shown). All MP trees basically resembled the ML tree described above, as far as principle discrimination of clusters is concerned. The trees can be subdivided into 2 variants: in all trees, MOP–*Acharax* and JAC–*Acharax* are sister groups.

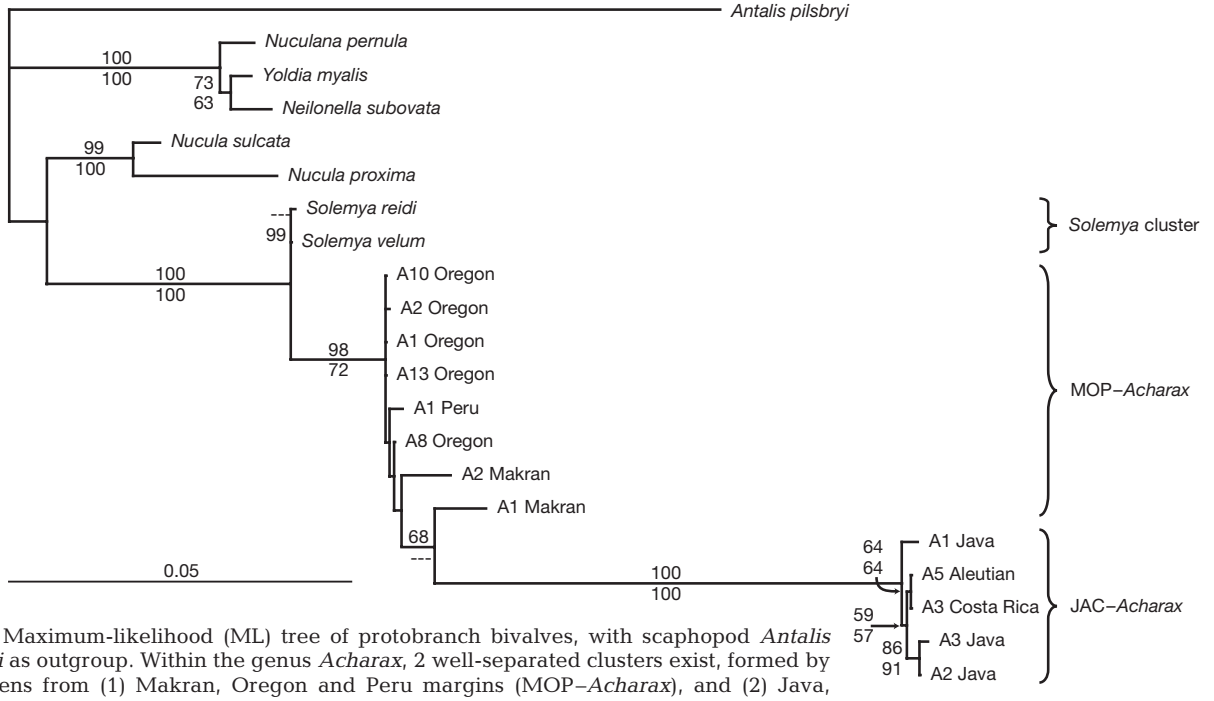

Fig. 2. Maximum-likelihood (ML) tree of protobranch bivalves, with scaphopod *Antalis pilsbryi* as outgroup. Within the genus *Acharax*, 2 well-separated clusters exist, formed by specimens from (1) Makran, Oregon and Peru margins (MOP–*Acharax*), and (2) Java, Aleutian Trench and Costa Rica (JAC–*Acharax*). Scale bar = 0.05 substitutions per alignment position. Values on topology nodes: bootstrap proportions (BP) for both calculation methods, with ML above branch and MP (maximum parsimony) below branch; nodes lacking BP values did not occur in majority-rule tree of the relevant calculation method. *Acharax* specimen denominations as in Table 1

The two variants differ in the position of A1-Makran, which clusters with JAC–*Acharax* in the second but not the first variant.

Bootstrap proportions (BP)

The BP values for the 2 phylogenetic methods are shown in Fig. 2. The clustering of taxa according to the superfamilies Nuculoidea, Nuculanoidea and Solemyoidea always shows highest agreement (99 to 100%). Also, the groups of *Solemya* and *Acharax* were well-supported by BP values between 72 and 99%. Trifurcations of *S. reidi*, *S. velum*, and *Acharax* clusters occurred in most ML bootstrap replicates because of the zero branch length of *S. velum*. Thus an explicit BP value could not be assigned to the *Solemya* cluster by PAUP* for ML calculations. Separation of JAC–*Acharax* from MOP– *Acharax* is unequivocally supported by both methods (100% each). However, the common descent of JAC– *Acharax* and A1 Makran, as suggested by the ML calculations in Fig. 2, is only poorly supported (68% in ML), or not supported at all (MP). The main reason for this is the ambiguous placement of A1 Makran in single ML and MP bootstrap trees. Lower bootstrap support likewise applies to most relations within the 2 *Acharax* clusters.

Hypervariable regions of 18S rRNA

The hypervariable regions of ribosomal nucleotide sequences are well-suited for comparison of closely related organisms, and were therefore visually inspected for signatures to establish groups and subgroups of the investigated species and specimens (Fig. 3). The comparison supported the tripartition of the examined solemyids established by sequence comparison. MOP– and JAC– *Acharax* clusters differ significantly, from each other and from *Solemya* in numerous indels (Fig. 3).

DISCUSSION

Phylogeny of Solemyidae

Comprehensive phylogenetic studies of bivalves including solemyids have been undertaken by numerous authors (Waller 1990, Steiner & Müller 1996, Adamkewicz et al. 1997, Waller 1998, Steiner & Hammer 2000, Giribet & Wheeler 2002, Giribet & Distel 2003, Campbell et al. 2004) who do not provide detailed information about the family Solemyidae. In particular, information on the genus *Acharax* is lacking. Therefore we have turned our attention to solemyids, with particular focus on *Acharax.* This genus has not been examined by means of molecular phylogeny so far, and its distinction from *Solemya* based on morphological properties is difficult.

In numerous studies, 18S rDNA has been used to analyze phylogenetic relationships of bivalves. The high degree of conservation of major parts of this gene allows the investigation of relationships between phylogenetically distant organisms, while highly variable regions of this gene also enable the distinction of closely related organisms. The results for the general phylogenetic relationships between the 3 protobranch

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                          110        120        130        140        150        160        170        180        190
                           |....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Antalis pilsbryi          UUAGAUC---AAGACCAGUGCGGGUUUUGUC--------UC------GGCAAU---CCC--------GCU---CAACUUUU
Nuculana pernula          UUAGAUC---AAGACCAA-UCGGG--GGUU---------UC-------GGCU----CCC--------G-U--CCCAU---U
Yoldia myalis             UUAGAUC---AAGACCAA-UCGGG--AGUU---------UC-------GGCU----CCC--------G-U--ACCAU---U
Neilonella subovata       UUAGAUC---AAGACCAA-UCGGG--GGCC---------UC-----GCGGCA----CCC--------G-U--GCCAU---U
Nucula sulcata            UUAGAUC---AAAACCAA-UCGGU----CCGC--------------AAGG------GCC--------GUU---CCAU---U
Nucula proxima            UUAGAAC---AAAACCAA-UCGGU---UCCGC--------------AAGG------GCC--------GCU---CAAU---U
Solemya reidi        1    UUAGAUC---AAAACCAA-UCGGU---UCUU---------UC------GGGA-----GCC--------G-U---CUGU---U
Solemya velum             UUAGAUC---AAAACCAA-UCGGU---UCUU---------UC------GGGA-----GCC--------G-U---CUGU---U
A1 Oregon                 UUAGAUC---AAAACCAA-UCGGU--GGCCUCC---UGUUC----GGGGGUUU---GCC--------GGU-CCCUGU---U
A2 Oregon                 UUAGAUC---AAAACCAA-UCGGU--GGCCUCC---UGUUC----GGGGGUUU---GCC--------GGU-CCCUGU---U
A8 Oregon                 UUAGAUC---AAAACCAA-UCGGU--GGCCCCC---UAUUC----GGGGGUUU---GCC--------GGU-CCCUGU---U
A10 Oregon           2    UUAGAUC---AAAACCAA-UCGGU--GGCCUCC---UGUUC----GGGGGUUU---GCC--------GGU-CCCUGU---U
A13 Oregon                UUAGAUC---AAAACCAA-UCGGU--GGCCUCC---UGUUC----GGGGGUUU---GCC--------GGU-CCCUGU---U
A1 Peru                   UUAGAUC---AAAACCAA-UCGGU--GGCCCGCC-UAGUUC---GGUGGGUUUU--GCC--------GGU-CCCUGU---U
A1 Makran                 UUAGAUAC--AAAACCAA-UCGGU--GGCCUUCC--UAUUC---GGGGGGUAC---GCCGA------AAU--UCAGU---U
A2 Makran                 UUAGAUC---AAAACCAA-UCGGU-GGGCCCCACC-CAUUCGGGGGGGGGUUC---GCC--------GGU-ACCUGU---U
A3 Java                   UUAGAUCGAAAAAACCAA-UCGGC-GGGCCC---------UC------GGGCCCGAUGCCGUCAAAAAAACCCCCAGU---U
A2 Java                   UUAGAUCGAAAAAACCAA-UCGGC-GGGCCC---------UC------GGGCCCGAUGCCGACAAAAAAACCCCCAGU---U
A1 Java              3    UUAGAUCGAAAAAACCAA-UCGGC-GGGCCC---------UU------GGGCCCGAUGCCGACAAAAAAACCCCCAGU---U
A5 Aleutian               UUAGAUCGAAAAAACCAA-UCGGC-GGGCUC---------UC------GGGCCCGAUGCCGACAAAAAAACCCCCAGU---U
A3 Costa Rica             UUAGAUCGAAAAAACCAA-UCGGC-GGGCUC---------UC------GGGCCCGAUGCCGACAAAAAAACCCCCAGU---U
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Fig. 3. Selected hypervariable region (Positions 115 to 189) of alignment, revealing clusters of solemyid sequences. 1: *Solemya*; 2: *Acharax* from Makran, Oregon and Peru margins (MOP–*Acharax*), 3: *Acharax* from Java, Aleutian Trench and Costa Rica (JAC–*Acharax*). *Acharax* specimen denominations as in Table 1

superfamilies (Fig. 2) are in agreement with the phylogeny of protobranch bivalves as proposed by Giribet & Wheeler (2002): the superfamily Solemyoidea represents a sister group to the superfamily Nuculoidea, both being well-separated from the superfamily Nuculanoidea. Since no 18S rDNA sequence data of Manzanellidae were available, we only considered the family Solemyidae. Within this family, the 2 genera *Solemya* and *Acharax* are clearly distinct from each other by their 18S rDNA sequence divergence. These groups and their separation are well-supported by sequence distances and the tree configuration (as demonstrated by ML and MP methods), and by high bootstrap proportions of these calculations (Fig. 2). This finding supports the current systematic distinction based on morphological criteria (Coan et al. 2000).

Because differences between closely related organisms are best resolved in the highly variable regions of ribosomal rDNA, we have included these in our considerations. Signatures of the nucleotide patterns from hypervariable regions (Fig. 3) give additional support to the separation of the groups of solemyid bivalves considered.

Two clusters of deep-sea solemyids

Within the genus *Acharax*, 2 well-separated clusters were formed by specimens from the Makran, Oregon and Peru margins (MOP–*Acharax*) on the one hand, and from Java, the Aleutian Trench and Costa Rica (JAC–*Acharax*) on the other. The partitioning of MOP– and JAC–*Acharax* are verified by both tree-building methods and bootstrap calculations. Ambiguities are evident in the placement of A1 Makran with regard to its different positioning in the MP topologies and in the ML and MP bootstrap calculations. We rate these ambiguities together with the intermediate branch lengths of A1 and A2 Makran as indications for their transient position between MOP– and JAC–*Acharax*.

The large differences in 18S rRNA gene sequences found between the *Acharax* clusters are unexpectedly high for members considered to belong to the same species. They are manifested in addition by several sequence signatures characteristic for each of the clusters (Fig. 3). Comparison with distances in other protobranch groups (Fig. 2) indicates that sequence differences would easily justify the separation of the members of MOP– and JAC–*Acharax* clusters into separate species. We therefore propose that our specimens are representatives of at least 2 different species and do not all belong to *A. johnsoni*, as assumed previously. This also implies that shell morphology does not reflect the genetic diversity of *Acharax*, and is likely to underestimate the true species diversity of this genus if used for species differentiation.

Factors that could have caused the strong separation of clusters within *Acharax* may include depth distribution, geochemical properties of the habitat and geographical separation: (1) While the genus *Solemya* (0 to 600 m) appears to be separated from *Acharax* (~400 to 5379 m) by distribution in different water depths, both *Acharax* clusters have preferences for a large range of deep-water habitats, with specimens of the JAC–*Acharax* cluster being present over the largest range of depth (Table 1, Fig. 1). (2) Phylogenetic separation of the 2 *Acharax* clusters may reflect adaptation to different geochemical environments. Such correlations were shown for the vesicomyid clams *Calyptogena pacifica* and *C. kilmeri*, 2 species whose separation was associated with the different pore-water composition of their habitats (Barry et al. 1997). Apart from the fact that all *Acharax* specimens of this study were collected at or near methane-seeps, details about their geochemical environments are not available, and an ecological explanation for the observed separation of the 2 *Acharax* clusters requires further study. (3) Members of the 2 *Acharax* clusters were not found together at any of the sampling sites, which are geographically well-separated locations (Fig. 1). This indicates that geographic distribution differentiates among groups or species of *Acharax*, although geographic isolation itself is unlikely to explain the development into 2 different clusters: the specimens from Makran, a location well-separated from other sources of deep-sea solemyids (Fig. 1), are clearly distinguishable from the other members of MOP–*Acharax* (Fig. 2). Also, species that differ in morphology from the MOP– and JAC–*Acharax* clusters — *A. eremita* in the Gulf of Aden (Kuznetsov & Schileyko 1984), and *A. alinae* found in the Lau Basin (Métivier & von Cosel 1993) — were not found at the locations of the present study (see locations 3 and 4 in Fig. 1).

The large phylogenetic distance of JAC–*Acharax* to the other solemyid clusters remains unexplained for the time being. Results from ML and MP calculations indicate that MOP– and JAC–*Acharax* have a common ancestor, and that JAC–*Acharax* is likely to have evolved from MOP–*Acharax*. Considering the sequence distances, it can be concluded that, once separated from the MOP–*Acharax* group, JAC–*Acharax* evolved much faster than the former. A possible explanation could be better adaptation of JAC–*Acharax* to the geochemical environment, faster growth and, in consequence, outcompeting of representatives of MOP–*Acharax*. Such mechanism would also explain why specimens of both clusters were not found together at the habitats investigated.

Comparison between host and symbiont phylogeny

Comparing phylogenies of the *Acharax* hosts and their symbionts provides useful insights into the evolutionary history of the symbioses. Endosymbionts of *Acharax* form a new group of sulfur-oxidizing bacteria related to, but clearly distinct from, other known symbiotic sulfur-oxidizing Gammaproteobacteria (Imhoff et al. 2003). The *Acharax* endosymbionts form a separate branch related to symbiotic sulfur bacteria of *Solemya* and other host species including lucinid bivalves and vestimentiferan tubeworms. The present study has demonstrated that the host *Acharax* is also well-separated phylogenetically from the host *Solemya*. Thus, host and endosymbiont phylogeny are in agreement in this respect. However, the large differences between MOP– and JAC–*Acharax* are not reflected in the phylogeny of their symbionts (Imhoff et al. 2003), which may have evolved at a slower rate than their host species. The large evolutionary differences among hosts as well as among their endosymbionts are in accordance with the presumed ancient history of solemyids (Coan et al. 2000): the first solemyids appeared in the lower Ordovician (~440 million yr BP), *Acharax* in the Permian or perhaps middle Devonian (225 to 280 million yr BP) and *Solemya* in the Jurassic (135 to 195 million yr BP). They are also in accordance with the hypothesis of an ancient uptake of symbionts by solemyid bivalves, as proposed by Imhoff et al. (2003).

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