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PHYLOGENETIC RELATIONSHIPS AMONG NINE SCALLOP SPECIES (BIVALVIA: PECTINIDAE) INFERRED FROM NUCLEOTIDE SEQUENCES OF ONE MITOCHONDRIAL AND THREE NUCLEAR GENE REGIONS

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ABSTRACT Current knowledge of the evolutionary relationships among scallop species (Mollusca: Bivalvia: Pectinidae) in the Indo-Pacific region is rather scanty. To enhance the understanding of the relationships within this group, phylogenies of nine species of scallops with the majority from coastal regions of Thailand, were reconstructed by maximum parsimony, maximum likelihood, and Bayesian methods using sequences of the 16S rRNA of the mitochondrial genome, and a fragment containing the ITS1, 5.8S and ITS2 genes of the nuclear DNA. The trees that resulted from the three methods of analysis were topologically identical, however, gained different levels of support at some nodes. Nine species were clustered into two major clades, corresponding to two subfamilies (Pectininae and Chlamydinae) of the three currently recognized subfamilies within Pectinidae. Overall, the relationships reported herein are mostly in accordance with the previous molecular studies that used sequences of the mtDNA cytochrome oxidase subunit I, and the classification system based on microsculpture of shell features and morphological characteristics of juveniles. Levels of divergences were different among genes (i.e., the 5.8S gene showed the lowest levels of nucleotide divergence at all levels, whereas the 16S rRNA showed the highest level of variation within species, and ITS2 gene revealed the highest level of divergence at higher levels).

KEY WORDS: Pectinidae, scallops, phylogeny, nucleotide sequences

INTRODUCTION

Bivalves of the family Pectinidae, often referred to as scallops, are among the better-known shellfishes. Scallops are distributed worldwide and inhabit a wide variety of environments in all seas from polar regions to the tropics (Brand 2006). Scallops are also well known because of their commercial importance and contribute significantly to commercial fisheries as well as aquaculture production. Many species are currently cultured, and the average annual aquaculture production of scallops in the period 2000–2004 was about 1.17 million t, valued at $63.6 million USD, accounting for about 61.2% global scallop production (FAO 2006). Given their importance, scallops have been the subject of much research (Shumway & Parsons 2006).

Currently about 400 living scallop species are recognized and are reputed to have a very complex taxonomy. A number of classification systems have been proposed based on morphological characters (e.g., Hertlein 1969, Korobkov 1960, Waller 1991, 1993, 2006). The current consensus and well-accepted system is that of Waller, who classified Pectinidae, based on microsculpture of shell features and morphological characteristics of juveniles. Waller (1991, 1993) suggested the division of Pectinidae into three subfamilies, comprising of Camptonectinae, Chlamydinae, and Pectininae. The subfamily Chlamydinae was further divided into four tribes: Chlamydini, Crassadomini, Mimachlamydini and Aequipectini, and Pectininae into three tribes: Palliolini, Decatopectini, and Pectinini. In a recent revision, Waller (2006) suggested the additional tribe Amusiini in Pectininae.

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With the recent advances in the field of molecular phylogenetics, several attempts have been made to reconstruct the phylogeny of Pectinidae using molecular data. Sequences from a number of gene regions have been used to infer scallop phylogenies, such as the 16S rRNA and 12S rRNA (Capana et al. 1999, 2000b, Capana et al. 2000a) and the cytochrome oxidase subunit I (COI) (Giribet & Wheeler 2002, Matsumoto & Hayami 2000) of the mitochondrial genome, and 18S rRNA (Capana et al. 1999, Frischer et al. 1998, Giribet & Carranza 1999, Giribet & Wheeler 2002, Winnepenninckx et al. 1996) and the internal transcribed spacer I (ITS1) to internal transcribed spacer II (ITS2) (Insua et al. 2003) of the nuclear DNA. In general, phylogenies recovered from molecular DNA sequences support the classification system proposed by Waller (1991, 1993) (Barucca et al. 2004, Matsumoto & Hayami 2000). However, most studies used scallop samples from the north Atlantic and the north Pacific regions, and little attention has been paid to species from the Indo-Pacific. The only study to date on scallop systematics in this region was that of Matsumoto and Hayami (2000), but was confined to Japan.

The Indo-Pacific coastal region harbors a rich scallop fauna, with about 185 known species belonging to 38 genera currently listed in the Ocean Biogeographic Information System (OBIS) Indo-Pacific Molluscan Database (http://data.acnatsci.org/obis/). Many scallop species found in the Indo-Pacific region are of commercial importance and some are being cultured, such as for example *Chlamys farreri* and *Mimachlamys nobilis* in China (Guo & Luo 2006), *Patinopecten yessoensis* in Japan (Kosaka & Ito 2006), and *Pecten* spp. in Australia (Saavedra & Peña 2004). The Asian region has been leading cultured scallop production over the last many decades, contributing about

97.9% to that of the world (FAO 2006). In Thailand, as in most of SE Asia, many wild populations of scallops have been overexploited and efforts have been made, although still at a very early stage, in filling the gap between demand and supply through aquaculture. In this regard for example, hatchery production techniques have been developed for species such as *Mimachlamys senatoria* (Nugranad & Promjinda 1997).

The objective of this study is to undertake a phylogenetic analysis of sequences from four gene regions, including partial 16S rRNA gene region of the mitochondrial DNA, and the complete sequences of three nuclear genes (i.e., ITS1, 5.8S, and ITS2) to investigate phylogenetic relationships among nine scallop species in the Indo-West Pacific region with samples mainly obtained from Thailand coastal areas.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

A total of 29 individuals of scallops, believed to be from nine species were collected from 2004–2006 along the coast of Thailand. Upon capture, a small portion (approximately 150 mg) of adductor muscle was preserved in 95% ethanol. Voucher specimens comprising of preserved tissues and shells were held at the Kasetsart University Museum of Fisheries, Bangkok, Thailand. For comparative purposes, three individuals of *Decatopecten radula* from Lampung, Indonesia; three and two individuals of *Mimachlamys nobilis* were also obtained from Hainan, China and Kochi Prefecture, Japan, respectively. Details on localities and sample sizes are given in Table 1 and Figure 1.

Total genomic DNA was extracted using phenol/chloroform standard method as described by Taggart et al. (1992) with a slight modification. The individual DNA was resuspended in TE buffer (10 mM Tris-HCl pH 7.2; 1 mM EDTA pH 8.0) and stored at –20°C until required.

TABLE 1.

Details of specimens of Pectinid species used in the present study. Most of the samples were from Thailand otherwise indicated.

Species	Sample Code	Sample Size	Locality
Amusium pleuronectes	Ap-T	2	Trat
	Ap-NT	1	Narathiwas Province
Annachlamys macassarensis	Am-NT	4	Narathiwas Province
Decatopecten radula	Dr-IN	3	Lampung, INDONESIA
Decatopecten plica	Dp-PJ	4	Bangsapan, Prajuabkirikhan
Mimachlamys nobilis	Mn-BC	3	Bangsare, Chonburi
	Mn-SC	3	Samaesan, Chonburi
	Mn-CN	3	Hainan, CHINA
	Mn-JP	2	Kochi Prefecture, JAPAN
	Mn-GB		GenBank Assession Number AJ571620[1] and AY690599[2]
Mimachlamys senatoria	Ms-BC	1	Bangsare, Chonburi Province
	Ms-SC	2	Samaesan, Chonburi Province
	Ms-PB	1	Cha-am, Phetchaburi Province
	Ms-PJ	1	Bangsapan, Prajuabkirikhan
	Ms-PK	2	Phuket
Mimachlamys spp.	M?-KB1	2	Lanta Noi Island, Krabi Province
Minnivola pyxidata	Mp-KB	3	Lanta Noi Island, Krabi Province
Mimachlamys varia	Mv-GB		GenBank Assession Number AJ243575[3] and AJ534978[4]
Semipallium fulvicostatum	Sf-CP	2	Cha-am, Phetburi Province

[1] Barucca et al. (2004)
[2] Bao et al. (unpublished)
[3] Capana et al. (2000b)
[4] Insua et al. (2003)

PCR Amplification and Sequencing

A fragment of the large ribosomal mitochondrial gene (16Sr RNA) was amplified using primers 16Sar (5′ CGC CTG TTT AAC AAA AAC AT-3′) and 16Sbr (5′-CCG GTC TGA ACT CAG ATC ATG T-3′) (Palumbi et al. 1991). The transcribed spacer (ITS) region, comprising of ITS1, the 5.8 rRNA gene, and ITS2, was amplified using primers designed by Heath et al. (1995), which anneal to the 3′-end of the 18S gene and to the 5′-end of the 28S gene (forward - 5′GTT TCC GTA GGT GAA CCT G 3′, reverse - 5′ CTC GTC TGA TCT GAG GTC G 3′). PCR was performed in a total volume of 30 µl containing approximately 50 ng template DNA, 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, and 1 unit Taq Polymerase (Promega). PCR conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 5 min.

The majority of samples were analyzed at the Laboratory of Population Genetic Informatics, Tohoku University, Japan where PCR products were purified with the ExoSAP-IT (usb) and sequenced using an ABI Prism 377 DNA Sequencer (Applied Biosystems) by using the BigDye Terminator (Version 3) Cycle Sequencing Ready Reaction Kit. Remaining samples were sent to Macrogen Inc., Korea for purification and sequencing.

Data Analysis

The data set included a total of 39 sequences of nine scallop species. In addition, sequences from the GenBank for *M. nobilis* ([16S rRNA: AJ571620, Barucca et al. [2004]; ITS1, 5.8S and ITS2: AY690599; Bao et al. [unpublished]), and *M. varia* (16S rRNA: AJ243575, Capana et al. [2000b]; ITS1, 5.8S and ITS2: AJ534978, Insua et al. [2003]) were also included for comparative purposes. Sequences of *Ostrea edulis* (Genbank Accession No. DQ280032 [Giribet et al. 2006] for 16S rRNA, and U88709 [Carnegie, unpublished] for other gene regions) were used as an outgroup.

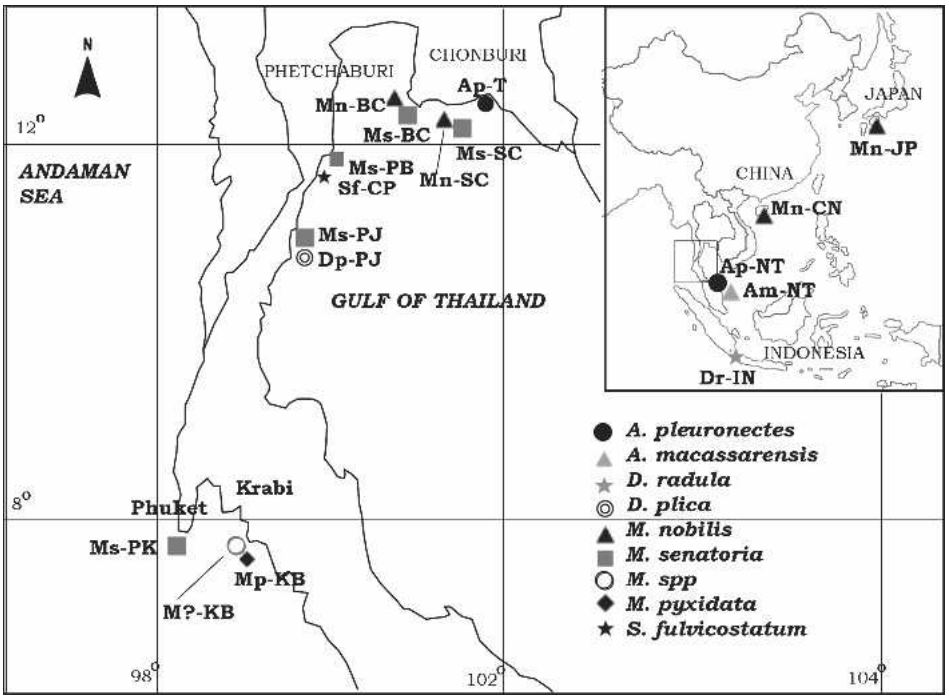

Figure 1. Sampling localities of nine scallop species in the present study.

The 5.8S gene fragment was aligned by eyes and the amino acid sequences were translated with reference to *Pecten maximus* (GenBank Accession No. AJ428410) as a test of the presence of nuclear paralogues. Alignment of the 16S rRNA, ITS1 and ITS2 gene regions was undertaken using the program SOAP (Löytynoja & Milinkovitch 2001). This program generates and compares alignments corresponding to 30 different sets of alignment parameters (gap extension penalty from 12–17 in steps of 1; gap opening penalty from 6–8 in steps of 0.5). A strict consensus of all of these alignment combinations was then used for further analysis.

Significant differences in base composition were tested for each data partition using homogeneity χ^2 analysis as implemented in PAUP* 4.0b10 (Swofford 2001). The presence of significant heterogeneity between partitions was assessed using partition homogeneity test (Farris et al. 1995) as implemented in PAUP* 4.0b10 (Swofford 2001).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian approaches were used to estimate phylogenetic relationships among the subject taxa. MP and ML phylogenetic analyses were executed in PAUP*4.0b10 (Swofford 2001). For the MP analysis, trees were generated using the heuristic search option with TBR branch swapping using 1,000 random taxon additions and gaps were treated as missing data. The best fit substitution model was estimated using MODELTEST version 3.07 (Posada & Crandall 1998). The best-fit maximum likelihood score was chosen using Akaike's information criterion (AIC), because this reduced the amount of unnecessary parameters that contribute little to describing the data by penalizing more complex models (Bernham & Anderson, 2002; Nylander et al., 2004). For the ML analyses heuristic searches with TBR branch swapping and 100 random additions of taxa were also performed.

Uncorrected ("p") sequence divergence values were calculated between samples. Phylogenetic confidence in the nodes recovered from parsimony was estimated by nonparametric bootstrapping (Felsenstein 1985), analyzing 1,000 pseudoreplicates of data sets, whereas because of computational constraints only 200 pseudo-replicates were performed for ML. Bayesian inferences were used to investigate optimal tree space using the program MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). For each analysis, four Markov chains were run, with each chain starting from a random tree and three million generations generated. Sampling from the chain occurred every 500th tree for each of the four partitions (16S rRNA, ITS1, 5.8S, ITS2), followed by the total evidence. In these combined analyses, genes were partitioned according to substitution models selected using MODELTEST, using unlinked parameters. A fifty percent majority rule consensus tree was generated from the trees retained, with posterior probabilities for each node estimated by the percentage of time the node was recovered. For the Bayesian analyses, data sets were run a minimum of four times to test whether they converge on the same topology.

We used Bayesian analysis (MrBayes 3.0b4) to estimate base frequencies, transition matrices, proportion of invariant sites and Γ-shapes for each partition by unlinking estimates of these for each partition. For all runs, stationarity as reached after about 2.2×10^6 generations and parameter estimates were based on the last 1,000 trees (i.e., last half million generations).

RESULTS

A total of 39 sequences for each gene (16S rRNA, ITS1, 5.8S, and ITS2) were obtained from the nine scallop species used in the present study. All sequences were deposited in GenBank (GenBank Assession Numbers DQ873890-DQ873916 for the

regions from ITS1 to ITS2, DR873917-DQ873942 for 16S rRNA). Alignment of 16S rRNA resulted in 606 base pairs (bp), of which four regions consisting of 158 bp (between bases 220–239, 389–309, 550–565, and 597–603 in the original alignment) were deemed unstable and removed from further analysis. Similarly, of 320 bp obtained from the ITS1 region, six regions with a total of 77 bp (between bases 22–29, 95–108, 124–135, 198–203, 248–255, and 297–330 in the original alignment) were inconsistent between alignments and therefore excluded from further analysis. Four regions of the ITS2 gene, consisting of 78 bp (between bases 123–139, 224–242, 247–283, and 248–352) were found unstable and removed. Only 14 bp at the 3′-end of the 18S rRNA gene was obtained, and these were invariable among samples examined and as such also excluded from further analyses.

Chi-square tests in base composition among taxa indicated no significant differences for any of the genes ($df = 111$, $P = 0.99 - 1.00$, Table 2). No significant heterogeneity between data partitions was detected under the partition homogeneity test ($P = 0.09$), and as such all data partitions were combined for further analysis.

In a total of 1142 bp of the combined data set, 771 sites were variable, of which 447 sites were parsimony informative. Bayesian estimates for GTR substitution matrices, proportions of invariant sites, and Γ-shapes are given in Table 2. These data indicated very different evolutionary dynamics for each of the partitions, in particular Γ-shape values varied among the partitions and extremely high in the ITS1 and ITS2 gene regions. Levels of divergence between species estimated as uncorrected "p" distances are presented in Table 3. Overall, low levels of intraspecific variation were observed, ranging from 0.000 (*D. radula*) to 0.003 (*M. senatoria*). The lowest level of between species differentiation (0.073) was observed between *M. nobilis* and *M. senatoria*. All species showed high levels of variation to the outgroup, *O. edulis* with an average genetic distance of 0.528.

A comparison among gene partitions on level of divergence is shown in Figure 2. Among the partitions, the 5.8S gene showed the lowest variation at all levels (0.000 within species, 0.011 between species, 0.018 between genera and 0.021 between subfamilies), whereas the ITS2 gene fragment showed the highest level of divergence at species level or higher (0.150 between species, 0.317 between genera, and 0.471 between subfamilies). The 16S rRNA gene region showed the highest level of intraspecific variation (0.005), however, less nucleotide divergence was found at higher levels compared with the two ITS genes.

The maximum parsimony analysis for the combined data set with all sites weighted equally gave three most parsimonious trees at the length of 1547. A strict consensus of these trees recovered identical topology to the tree obtained from Bayesian analysis (Fig. 3). The MP tree was supported by high bootstrap values (79% to 100%) at major nodes, but support from ML analysis was lower than 50% at some nodes, and Bayesian posterior probabilities ranged from moderate to high (0.54–1.00).

The tree was bifurcated into two major clades. The first clade (Pectininae) represented tribes Amusiini, Decatopectini, and Pectinini, in which Decatopectinini was found to be more closely related to Pectinini with high level of support (60% to 80% bootstrap, 0.82 posterior probability). Except for the three samples of *D. radula*, which were monomorphic, all others in this clade were polymorphic at least in one gene region. Three haplotypes were observed among Thai samples of *A. pleuronectes*, which showed an average of 0.003 divergence to the sequence of the same species obtained from GenBank.

The remaining samples were clustered into the second group, comprising of Chlamydinae (Chlamidini and Mimachlamydini) with low to moderate support (posterior probability of 0.54, bootstrap 79% for MP and <50 for ML) and a sample of Pectininae (*Minnivola pyxidata*). Within Mimachlamydini, a strong correlation between patterns of genetic variation with

TABLE 2.

Base frequencies of each gene partition and chi-square tests of bias among taxa, and substitution rate matrices, proportion of invariant sites and Γ-shapes (α), estimated using Bayesian analyses of 16S rRNA, ITS1, 5.S and ITS2 partitions, and the combined data set (estimates are based on means of 1,000 saved trees representing generations 2.5–3 × 10^6 in the MCMC analysis).

	Base Frequencies						Substitution Rate Matrices					
Gene Partitions	A	C	G	T	χ^2	*P*		C	G	T	*p*(inv)	α
16S rRNA	0.23	0.19	0.29	0.29	37.16	1.00	A	0.033	0.383	0.120	0.155	0.36
							C		0.012	0.398		
							G			0.054		
ITS1	0.33	0.27	0.20	0.20	65.97	0.99	A	0.190	0.150	0.126	0.294	105.24
							C		0.134	0.204		
							G			0.195		
5.8S	0.24	0.26	0.28	0.21	5.36	1.00	A	0.122	0.223	0.229	0.107	2.63
							C		0.056	0.260		
							G			0.110		
ITS2	0.27	0.24	0.25	0.24	71.54	0.99	A	0.130	0.169	0.166	0.0147	119.37
							C		0.132	0.255		
							G			0.148		
Combined data	0.26	0.23	0.26	0.25	36.49	1.00	A	0.149	0.158	0.209	0.122	68.56
							C		0.087	0.238		
							G			0.159		

TABLE 3.

Mean uncorrected "p" genetic distances between species examined in the present study based on the combined data set. The numbers on the diagonal are within species variation, dash (—) indicates only one sample was sequenced.

	Species	1	2	3	4	5	6	7	7	9	10	11
1.	*Amusium pleuronectes*	0.001										
2.	*Annachlamys macassarensis*	0.226	0.007									
3.	*Decatopecten radula*	0.117	0.145	0.000								
4.	*Decatopecten plica*	0.186	0.150	0.100	0.001							
5.	*Mimachlamys nobilis*	0.232	0.243	0.242	0.235	0.002						
6.	*Mimachlamys senatoria*	0.242	0.236	0.242	0.237	0.073	0.003					
7.	*Mimachlamys spp.*[1]	0.191	0.232	0.236	0.238	0.121	0.115	0.001				
8.	*Minnivola pyxidata*	0.213	0.230	0.235	0.231	0.134	0.123	0.104	—			
9.	*Mimachlamys varia*[1]	0.255	0.246	0.250	0.261	0.184	0.172	0.169	0.166	—		
10.	*Semipallium fulvicostatum*	0.226	0.225	0.246	0.241	0.181	0.168	0.178	0.176	0.204	—	
11.	*Ostrea edulis*[2]	0.516	0.215	0.520	0.535	0.536	0.538	0.530	0.528	0.539	0.527	—

[1] Capana et al. (2000b) and Insua et al. (2003) for 16S rRNA and ITS1, 5.8S and ITS2, respectively
[2] Giribet et al. (2006) and Carnegie (unpublished) for 16S rRNA and ITS1, 5.8S and ITS2, respectively

geographical distribution was observed. *Mimachlamys varia*, a species that is distributed through out Mediterranean and extending to the North Sea, and was genetically differentiated (0.178 nucleotide divergence) from its congeners occurring in the Indo-West Pacific region.

DISCUSSION

Phylogenetic Relationships

The phylogenetic analysis of a total of 1,142 bp comprising of mitochondrial and nuclear genes indicates that scallops species included in the present study constitute clearly distinct lineages. Nine scallop species from Thailand were well grouped into two subfamilies of the three currently recognized subfamilies of Pectinidae, corresponding to Pectininae and Chlamydinae of Waller (1991, 2006). The subfamily Pectininae appears to be paraphyletic considering the position of *M. pyxidata*, a species that has not been examined by Waller (1991, 2006) and or subjected to other molecular studies (Capana et al. 2000b, Giribet et al. 2006, Matsumoto & Hayami 2000).

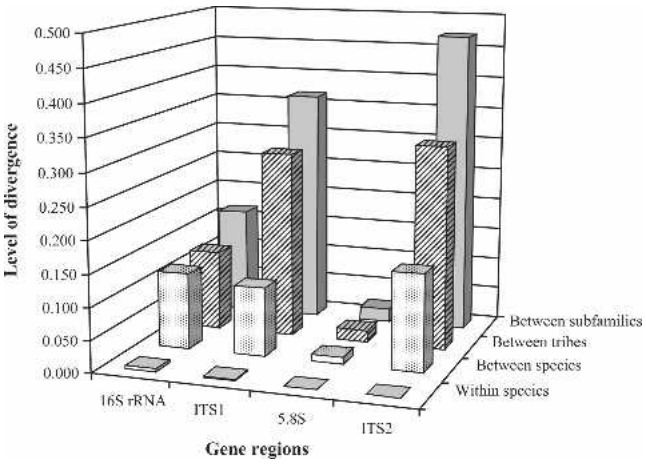

Figure 2. Comparison of levels of nucleotide divergences between four different gene fragments used in the present study.

Overall, the phylogenetic relationships presented herein are complimentary to those suggested by Waller (2006), as slightly modified from Waller (1991, 1993). With the addition of nine species from the Indo-Pacific region used in the present analysis, the findings conform to Waller's system not only in the constituent genera but also in the ranking of subfamilies and tribes. This observation is also supported by the results of Matsumoto and Hayami (2000), who inferred the relationships among seven Japanese scallop species using sequences of the COI mtDNA gene region. As suggested by Matsumoto and Hayami (2000), molecular data seem to support Waller's classification system probably because Waller used a cladistic approach based on microscopic characters appearing in the early dissoconchs, such as shell microsculture, patterns of radical ribs, and dentition. These characters are believed to be less influenced by changes in life history, unlike in the case of adult shells (Hertlein 1969, Korobkov 1960, Thiele 1935).

In a recent revision, Waller (2006) suggested that genus *Amusium* be removed from the tribe Pectinini, and included in a new tribe (Amusiini) together with the other two genera. Our data support this view considering the position of *A. pleuronectes* in association with Decatopectinini and Pectinini species. In addition, the mean genetic distance between *A. pleuronectes* and Decatopectinini (0.170) is higher than that between Decatopectinini and Pectinini (0.148), justifying the recognition of Amusiini as a distinct tribe. It is also noted that this observation is similar to that of Matsumoto and Hayami (2000), but these authors, however, suggested that a subfamilial status for *Amusium* was unwarranted, and did not discuss about its tribal status.

The phylogenetic tree recovered from our data is not in conformity to that of Waller (2006) at one occasion. Although limited number of taxa were examined in the present study, our findings and that of Matsumoto and Hayami (2000) indicate the close relationship between Decatopectinini and Pectinini species (posterior probability of 0.82, and bootstrap supports resulted from MP and ML analysis are 82 and 60, respectively). Waller (2006) using morphological data and fossil records,

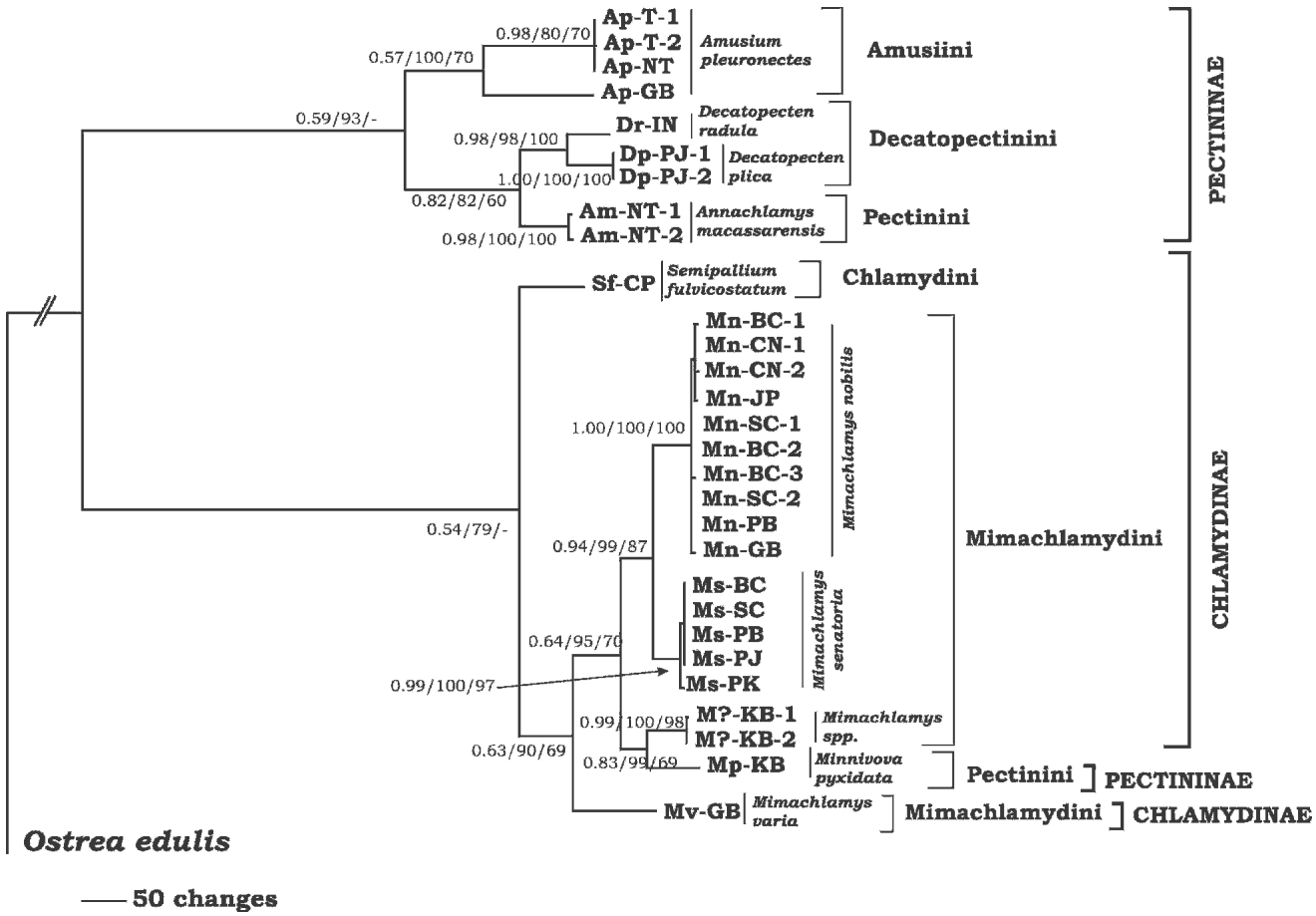

Figure 3. Bayesian estimation of the phylogenetic relationships among 10 scallops species, and the outgroup using combined data of four gene fragments (16S rRNA of the mitochondrial genome, and ITS1, 5.8S and ITS2 of the nuclear DNA). Tree produced from 3 × 10^6 generations using the GTR + I+Γ model of sequence evolution unlinked across all partitions. Numbers at each node represent posterior probabilities, bootstrap support for MP and bootstrap support for ML, respectively.

however, suggested that Decatopectinini is a sister group of the (Pectinini + Amusiini). Further investigations with broader taxon sampling (i.e., more species representing each tribe would be useful in resolving this uncertainty).

It is difficult to compare the phylogenetic relationships recovered from our data to that of Winnepenninckx et al. (1996) and Frischer et al. (1998) because of differences in taxon samplings. However, it is noted that the tree recovered by Frischer et al. (1998) using 18S rRNA gene sequences of seven scallop species from the north Atlantic and northeast Pacific were interpreted as partially inconsistent with Waller's system, although bootstrap supports were low (<50%) at some nodes. For example, species that represents the subfamily Pectininae (*Placopecten magellanicus*) appeared to constitute Chlamydinae. Similar branching patterns were also observed by Winnepenninckx et al. (1996). The different observations among molecular studies indicate the need for an evaluation of the utility of different gene regions for Pectinidae systematic studies with additional species.

The placement of *M. minnivola* (Pectinini) (Dijkstra 1998) within the Chlamidinae group raises an intriguing question relating to paraphyletic status of Pectinini. This finding indicates that there are problems with shell morphological based taxonomy. It is noted that members of genus *Minnivola* were not examined by Waller (1991, 1993, 1996) or any other molecular studies and as such its position is based solely on morphological features of adult shells. There could be a possibility that the gene trees generated in this study do not reflect the true phylogenetic relationship among taxa or there is possible presence of molecular convergence. However, the use of combination of one mitochondrial gene region and other three nuclear genes would be sufficient to eliminate these doubts.

Levels of Divergence

Although sequences of the two gene fragments used in the present study for phylogenetic inference have been examined previously (Barucca et al. 2004, Capana et al. 2000b, Insua et al. 2003), this is the first study that used a combination of these genes. In addition to COI sequences obtained by Matsumoto and Hayami (2000), data presented herein provide useful insight into the phylogeny of Pectinidae and the utility of a particular gene in systematic studies of this group. Overall, the fragment from 5.8S ribosomal coding gene showed the least variation, and the fragments of ITS genes showed considerably greater levels of divergence between major groups. The 16S rRNA gene of the mitochondrial genome although showed the highest level of intraspecific variation, it reveals lower a level

of variation at higher levels compared with the two nuclear ITS genes.

The levels of divergence at intraspecific level observed in our study are lower than those previously reported. For example, the highest level of divergence was observed within *M. nobilis* in the 16S rRNA gene (0.006), is much lower than that found in European species (i.e., *Pecten jacobaeus* [0.200]) and *Pecten maximus* (0.420), and Australian king scallops, *Pecten novaezelandiae* (0.300) (Saavedra & Peña 2004). These differences could be because of a number of factors, including differences in taxon sampling, sample size, the models used for estimating genetic distances, and probably the exclusion of a number of sites in our data set after alignment in the data set.

Levels of interspecific variation of the 16S rRNA gene region observed in our study are, however, very similar to that reported by Saavedra and Peña (2004). The latter study reported average values for interspecific comparisons among two European species and two Australasian species and ranged from 0.042–0.160, whereas our results ranged from 0.072–0.166. Frischer et al. (1998) reported only 0.095 at the maximum divergence observed among seven pectinids for the 18S rRNA gene, and according to Matsumoto and Hayami (2000) this level of variation indicates 18S rRNA gene is too conservative compared with COI gene, which has 30% amino acid variable sites among 17 pectinids. However, the level of divergence observed herein for the 5.8S gene sequences are even lower than that of the 18S rRNA gene reported by Frischer et al. (1998). As for the ITS genes, level of variation observed in the present study are commensurate with that determined by Insua et al. (2003).

In conclusion, scallops species analyzed in this study belong to two subfamilies, Pectininae and Chlamydinae of Pectnidae. Analysis of both mitochondrial and nuclear genes of these samples has resulted in a phylogeny that is largely consistent with those previously described based on nonadaptive morphological characters of scallop species. Our sampling was mainly confined to a relatively small geographic region, and an extension to this study with additional number of species in the Indo-Pacific region is warranted to understand better the evolutionary relationships within this group.

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