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MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF THE GENUS *PSEUDOMMA* (PERACARIDA: MYSIDA)

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A B S T R A C T

We used DNA sequences from 18S rDNA (808 bp) and COI mtDNA (599 bp) to infer evolutionary history of northern groups of the deep-sea mysid genus *Pseudomma*. The V4–V7 regions of 18S show an average of 1.31% sequence divergence between species. A secondary structure model is constructed and used in phylogenetic analyses to allow for different evolutionary rates in paired and unpaired nucleotide partitions. COI is observed as highly variable with uncorrected p-distance averaging 33%. Phylogenies for these sequences were estimated by maximum-likelihood, Bayesian, and maximum-parsimony analyses. More or less similar tree topologies were obtained for each gene with these methods. *Pseudomma longisquamosum* was placed in a basal clade, using *Parapseudomma* and *Amblyops* as outgroups, but the exact relationship of other basal taxa is less clear when results from the two genes are compared. An ancient presence of *Pseudomma* in the Tethys Sea is suggested by phylogenetic structure, molecular clock considerations, and present distributions. A well-supported Atlantic clade may have diverged from Indo-Pacific groups in the Miocene because of the closure of the Gibraltar Strait. More recent speciation events are proposed in the Norwegian Sea, and an Arctic intrusion from the North Pacific across the Bering Strait is suggested for the circumpolar species *Pseudomma truncatum*.

Mysids of the genus *Pseudomma* (Peracarida: Mysida) constitute a diverse and abundant benthic group containing 38 species found in deep-sea waters around the world (Meland, 2004). Recent studies on deep-sea assemblages from previously unexplored regions are constantly confirming the widespread distribution of this genus by a continuing discovery of species new to science (Murano and Mauchline, 1999; Meland, 2004). Records of distribution show that *Pseudomma* species are mostly endemic in well-established biogeographical regions. Areas include Antarctic waters (9 species) and southern Australia (1 species), the Gulf of Mexico (1 species), the East Pacific (2 species), Northwest Pacific and Japan Sea (17 species), and the North Atlantic (6 species). In addition to these apparent endemic species, *Pseudomma truncatum* S. I. Smith, 1879, has a circumpolar boreal distribution. A recent discovery of *Pseudomma kruppi* W. Tattersall, 1909, in Sagami Bay (Meland, 2004) has extended its distribution from the Bay of Biscay and Mediterranean Sea to also include the West Pacific.

The global distribution of *Pseudomma* suggests that these mysids are common components of the deep-sea fauna. Much of what we know about dispersal and speciation in deep-sea organisms has come from studies of hydrothermal vent species. In a phylogenetic study concerning species diversity of vent associated bresiliid shrimps, Shank *et al*. (1999) found that genetic divergence suggested a radiation dating to the Miocene (< 20 mya). Additionally, genetic distance was in no way correlated to geographical distance between previously assumed closely related species. These findings indicate that some vent organisms must be derived from more remote deep-sea areas, or possibly from shallow-water ancestors. Considering a hypothesis of deep-sea recruitment from cold polar regions, Held (2000) presented phylogenetic evidence for several invasions of serolid isopods from the Antarctic shelf and also colonization from South American shallow water into the deep sea. The idea of a widely distributed fauna in the ocean basins, resulting from the absence of barriers and a confluence of the ocean floor is highly recognized (Gage and Tyler, 1991). In the gammarid amphipod *Eurythenes gryllus* Lichtenstein, 1822, genetic divergence in 16S rDNA has shown an overall low level of divergence among abyssal populations, which was interpreted as historically

high levels of gene flow between apparently homogeneous environments on the ocean floor (France and Kocher, 1996). In contrast, bathyal populations of *E. gryllus* were shown to have a higher degree of divergence indicating multiple divergence events on continental slopes due to isolation between ocean basins. These observations suggest that ecological and physical conditions are important isolating mechanisms that may lead to speciation. Therefore, as stated by France and Kocher (1996), "A major challenge to deep sea biologists is to identify the barriers to gene flow that may isolate gene pools and lead to reproductive isolation in the absence of absolute spatial isolation."

In a recent morphology-based analysis concerning *Pseudomma* phylogeny, a considerable amount of homoplastic similarity was revealed and a meaningful interpretation on origin and radiation in early lineages of *Pseudomma* proved to be difficult (Meland, 2004). These results conformed to the idea that low phenotypic variation within *Pseudomma* could be an effect of stabilizing selection on organisms that are highly adapted to a homogeneous environment. Meland (2004) also obtained robust branching patterns of several shallower nodes, emerging in three consistent lineages. Following the notion that geographical distribution reflects evolutionary history, distribution records and phylogeny were used to define species groups within three major geographic areas, North Atlantic, North West Pacific, and Antarctic.

Where morphology has in some cases been found inadequate to resolve phylogenetic relationships between closely related and morphologically similar crustacean species, a steady increase of molecular studies has revealed genetic differentiation that has given new insights and better understanding of taxonomic relationships and evolutionary history (*Penaeus:* Baldwin *et al*. (1998); *Gammarus:* Meyran *et al*. (1997); *Cancer:* Harrison and Crespi (1999); Panopeidae: Schubart *et al*. (2000)).

In this paper, we will examine a segment of the mtDNA gene cytochrome c oxidase subunit I (COI) and the V4–V7 region of the nuclear small subunit rDNA (18S) for phylogenetic signal in *Pseudomma*. Resulting phylogenies are compared to the morphology-based phylogenies in Meland (2004) and used to investigate how morphological character evolution is reflected in the phylogeny derived from molecules. We shall also attempt to address questions on how major historical events may have influenced dispersal and colonization of *Pseudomma* species in the Northern Hemisphere through direct comparisons of phylogeny with geological history.

Materials and Methods

Sampling and Identification

Our analyses included 16 of the 38 recognized *Pseudomma* species. The remaining species were not represented because of difficulties in obtaining properly preserved material for DNA extraction and sequencing. Ten species in this study are representatives of the Northwest Pacific fauna, and four species are from the North Atlantic, constituting 59% and 67% of *Pseudomma* found in these two regions, respectively. We also include the more widely distributed species *Pseudomma truncatum* and *P. kruppi*, giving an overall representation of 64% for *Pseudomma* species that are described from the Northern Hemisphere.

Specimens used in this study are archived as ethanol preserved vouchers. Sampling areas, museum catalogue numbers, and GenBank accession numbers are listed in Table 1. For most species, DNA from more than one specimen was sequenced in order to confirm the results obtained and also to test for intraspecific variation. *Pseudomma* species from the North Pacific were collected in Sagami Bay and Tateyama Bay on two sampling cruises with the R/V "Seiyo Maru" in the spring of 1999. North Atlantic *Pseudomma* species were sampled and sorted by Dr. T. Brattegard during two BIOICE cruises with R/V's "Håkon Mosby" and "Bjarni Sæmundsson" in the summer of 2000 and 2001. *Pseudomma truncatum* was sampled by Dr. T. Brattegard with the R/V "Jan Mayen" in the surrounding waters of Svalbard in 2001. Dr. R.Väinölä kindly donated *P. truncatum* samples from the White Sea. Additional specimens were also collected on local sampling cruises onboard the R/V "Hans Brattstrøm" from local fjords outside Bergen. All mysid material was sorted by hand directly from an epibenthic- or RP-sledge and preserved directly in 96% ethanol.

Species were identified by KM. *Pseudomma* sp. M (= *P. sarsi* in Murano, 1974; Meland, 2004), *Pseudomma* sp. M&M (Murano and Mauchline, 1999; Meland, 2004), and a recently discovered species that shares similarities with *P. marumoi* Murano, 1974, have yet to be formally described. As suggested in Meland (2004), *P. affine* G. O. Sars, 1870, has differentiated into two morphotypes, a northern North Atlantic form (type I) and a smaller southern North Atlantic form (type II).

Both *Parapseudomma* Nouvel and Lagardère, 1976, and *Amblyops* G. O. Sars, 1872, are assumed close relatives of the genus *Pseudomma* and were chosen as outgroups. Originally placed within *Pseudomma*, a close relationship with *Parapseudomma calloplura* (Holt and Tattersall, 1905) seems justified. This monotypic genus has been reported from the Bay of Biscay, Mediterranean Sea, and ocean areas south of Japan. A sister group relationship with *Amblyops* is suggested due to the reduction of the compound eye into two flattened ocular plates, possibly an intermediate stage before the complete fusion of the eyeplate seen in *Pseudomma* and *Parapseudomma*.

Extraction and Sequencing

PCR.—DNA was extracted from abdominal muscle tissue using one of the two methods mentioned below. When the need for maximum DNA yield was crucial because of limited availability of specimens, a chloroform/phenol

Table 1. Mysida species included in the present study indicating sampling locations, University of Bergen, Zoological Museum catalogue number, and GenBank accession numbers.

	Sampling location	Catalogue no. ZMBN	GenBank no.	
			18S	COI
Pseudomma affine type I	Iceland Basin, Iceland	68252	AY624283	AY624266
Pseudomma affine type II	Byfjorden, Bergen, Norway	68253	AY624284	
Pseudomma affine type II G. O. Sars, 1870	Byfjorden, Bergen, Norway	68254	AY624285	AY624267
Pseudomma brevisquamosum Murano, 1974	E. Sagami Bay, Japan	68255	AY624286	AY624268
Pseudomma crassidentatum Murano, 1974	E. Sagami Bay, Japan	68256	AY624287	AY624269
Pseudomma frigidum Hansen, 1908	Jan Mayen	68257	AY624288	AY624270
Pseudomma izuensis Murano, 1966	W. Sagami Bay, Japan	68258	AY624289	
Pseudomma kruppi W. Tattersall, 1909	Tateyama Bay, Japan	68259	AY624290	AY624271
Pseudomma lamellicaudum Murano, 1974	N. Sagami Bay, Japan	68260	AY624291	AY624272
Pseudomma latiphthalmum Murano, 1974	Tateyama Bay, Japan	68261	AY624292	AY624273
Pseudomma longisquamosum Murano, 1974	E. Sagami Bay, Japan	68262	AY624293	AY624274
Pseudomma marumoi Murano, 1974	Tateyama Bay, Japan	68263	AY624294	AY624275
Pseudomma roseum G. O. Sars, 1870	Trondheimsfjorden, Norway	682264	AY624295	AY624276
Pseudomma surugae Murano, 1974	Tateyama Bay, Japan	68265	AY624296	
Pseudomma truncatum S. I. Smith, 1879 (Svalbard)	Hinlopen, Svalbard	68266	AY624297	AY624277
Pseudomma truncatum (White Sea)	Chupa Inlet, White Sea	68267	AY624298	AY624278
Pseudomma sp. (near *P. marumoi*) Meland	Tateyama Bay, Japan	68268	AY624299	AY624279
Pseudomma sp. M (= *P. sarsi* in Murano, 1974)	E. Sagami Bay, Japan	68269	AY624300	AY624280
Pseudomma sp. M&M (of Mauchline and Murano, 1999)	Iceland Basin, Iceland	68270	AY624301	AY624281
Parapseudomma calloplura (Holt and Tattersall, 1905)	E. Sagami Bay, Japan	68271	AY624302	
Amblyops abbreviata (M. Sars, 1869)	Trondheimsfjorden, Norway	68272	AY624303	AY624282

extraction protocol was slightly modified by using vacutainer tubes for separating phases (Dahle *et al*., 1997). Alternatively, spin column extractions were performed with a Qiagen© extraction kit following the recommendations of the manufacturer. To improve DNA yield, complete removal of ethanol was achieved by diluting preserved tissue in sterile H_2O for 24 hours prior to digesting. Additionally, lengthening digestion time to 24 hours significantly increased the amount of DNA extract. Extracted DNA was diluted 1:9 with sterile H_2O because pure extract resulted in unsuccessful PCR runs.

The PCR amplifications were carried out in 50 μL reactions containing 4 μL DNA template, 2 units of Taq DNA polymerase, and 4 μL 10× PCR buffer (Promega©), 2 μL of each primer (10 μM), 4 μL dNTP mix (1.25 mM for each nucleotide), and 36.8 μL sterile H_2O. All amplification reactions were carried out in a Peltier Thermal Cycler 2000 (MJ Research, Inc.).

Nuclear Small Subunit Ribosomal DNA (18S).—A ~800 bp sequence covering the V4 and V7 regions of the 18S rDNA was PCR amplified using primers developed by the first author: 18sv4f (5′-ATTCCAGCTCCAATAGCG-3′) and 18sv7r (5′-CTGTTATTGCTCAATCTCGT-3′). Cycle conditions were 3 min at 94°C for initial denaturation; 94°C, 30 s; 50°C, 1 min; 72°C, 2 min for 40 cycles, followed by 72°C, 10 min for final extension.

Mitochondrial Cytochrome C Oxidase Subunit I (COI).—A ~600 bp region of COI mtDNA was amplified using the universal primers described by Folmer *et al*. (1994): LCOI490 (5′-GGTCAACAAATCATAAAGATATTGG-3′), HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). Cycle conditions were 3 min at 95°C for initial denaturation; 95°C, 1 min; 40°C, 1 min; 72°C, 1.5 min for 35 cycles, followed by 72°C, 7 min for final extension.

DNA Sequencing.—The PCR products were electrophoresed through an ethidium bromide stained 1% agarose gel and examined under ultraviolet illumination. In the case of low DNA concentration and/or more than one amplification product, the band conforming to the expected sequence length was excised directly from the gel and used in a second PCR run with 2 μL template. Final DNA concentration was estimated by comparing band intensity with a DNA marker. Products were purified using commercially available spin columns (Qiagen©).

Both strands were cycle sequenced in 10 μL reaction using 0.5–2.0 μL purified PCR product, 2.2 μL ABI Big Dye terminators, and 0.5 μL of the same primers used for

amplification. Cycle conditions were 5 min at 95°C for initial denaturation; 96°C, 10 sec; 50°C, 5 sec; 60°C, 4 min for 25 cycles. Products were separated and analysed with an ABI 3700 PE automated sequencer.

Sequence Alignment and Phylogenetic Analyses

Nucleotide sequences were assembled using the software package Vector NTI 6. Multiple sequence alignments for 18S and COI were carried out with the editing program BioEdit 5.09 (Hall, 1999), using an implemented version of ClustalW (Thompson *et al*., 1994). Secondary structure of *Pseudomma* 18S was inferred by comparing the data with Malacostraca sequences downloaded from the European Ribosomal RNA Database (http://oberon.fvms.ugent.be:8080/rRNA/). We used the program DCSE (De Rijk and De Wachter, 1993) for this purpose and subsequently used secondary structure to partition the aligned data into paired (helix) and nonpaired (loop) partitions. Secondary structure was drawn with RNAVIZ 2.0 (De Rijk and De Wachter, 1997). To test for significance of convergence between helix and loop partitions in 18S, the Incongruence Length Difference (ILD) test (Farris *et al*., 1994) was applied using the computer program WinClada (Nixon, 2002) with NONA (Goloboff, 1999). The COI nucleotides were translated to amino acids based on the *Drosophila* mitochondrial code in MEGA 2.1 (Kumar *et al*., 2001) and MacClade 4.0 (Maddison and Maddison, 1997). The COI sequences were verified by subjecting translated sequences to Blast searches in the SwissProt database.

To evaluate the properties of 18S and COI for reconstructing *Pseudomma* phylogeny, nucleotide composition and sequence divergence were calculated in MEGA 2.1 (Kumar *et al*., 2001). Substitution patterns were inferred using pairwise sequence comparisons of transition/transversion rates on p-distance estimates. Pairwise comparisons of substitution in first, second, and third codon positions of COI were investigated for transition/transversion bias and possible saturation.

Maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Trees were found by 100 replicate heuristic searches using the tree-bisection-reconnection (TBR) branch swapping algorithm with starting trees obtained by random stepwise addition. Robustness of resulting nodes in the MP analysis was evaluated by parametric bootstrapping (Felsenstein, 1985) of 1000 pseudoreplicate heuristic TBR searches. Maximum likelihood (ML) searches were conducted using DNA evolution models that were determined using the likelihood-ratio tests in Modeltest 3.06 (Posada and Crandall, 1998). ML analyses were done with PAUP. Bayesian phylogeny estimations were performed with MrBayes 2.01 (Huelsenbeck and Ronquist, 2001), which, like the more recent MrBayes 3.0 (Ronquist and Huelsenbeck 2003), allows for estimation of separate substitution rate parameters on partitioned data (albeit with less refinement of options). The program was run with eight Markov Monte Carlo chains for 10^6 generations with parameter sampling every 100 and 200 generations in 18S and COI, respectively. MrBayes was also used to compute posterior probabilities from all compatible clades among the set of trees that were generated following stationarity of log-likelihood estimates.

The log-likelihood ratio test (Huelsenbeck and Crandall, 1997) was performed with PAUP* to test the hypothesis of constant evolutionary rates in COI and 18S. Twice the difference in log-likelihoods (δ) with (L_o) and without (L_1) a molecular clock enforced was used as a test statistic in a χ^2 distribution with *n taxa*–2 degrees of freedom. Because there are no fossil records or vicariance events against which a molecular clock for *Pseudomma* can be calibrated with confidence, we tentatively dated the clades with earlier estimates of evolutionary rates. In the case when the null hypothesis could not be rejected, we used PAUP* to re-estimate branch lengths with a molecular clock enforced. Lineage divergence times were computed from this ultrametric tree, based on empirical evolutionary rates (Moran *et al*., 1993; Escalante and Ayala, 1995).

Tree topologies were compared to results from an analysis based on morphological characters (Meland, 2004) by direct identification of matching branching patterns. Exploration of morphological character evolution was done in MacClade by mapping state changes onto the molecular based phylogenies.

Results

Analysis of the Sequence Data

18S.—The 808 bp segment of the 18S gene showed no insertions or deletions across the mysid taxa and proved to be highly conservative. Secondary structure models from a homologous region in other Malacostraca (GenBank accession nos. M34360, M34361, M34362, M91060, U19182) were used to align and evaluate positioning of 46 variable nucleotide sites in our mysid data by constructing secondary structure models for the V4 to V7 regions in *Pseudomma* (Fig. 1). We basically adopted the framework of stems and loops suggested in these data base models. However, as opposed to these reference models, we only considered standard RNA base pairs (G-U, A-U, G-C) and in a few cases U-U pairings for the mysid models. Unlike Isopoda (Held, 2000; Dreyer and Wägele, 2002) and Amphipoda (Dreyer and Wägele, 2002), Mysida are missing long expansion segments in the V4 and V7 regions. Our sequences therefore aligned reasonably well to Decapoda sequences. In general, we also found that the secondary structure suggested for decapods could be applied, but that some bulges in decapod helices could be closed in *Pseudomma* by considering canonical base pairings.

Overall mean p-distance (uncorrected) between *Pseudomma* sequences was 1.31% ± 0.21 SE. Within *Pseudomma*, a maximum distance of 2.2% was found between *P. longisquamosum* and *P. marumoi*. *Pseudomma roseum* and *P. frigidum* proved to have identical sequences. Just one of these sequences was used in the 18S analyses. We included both taxa as undifferentiated sister groups in the resulting trees *a posteriori*. Maximum distance between *Pseudomma* and the outgroups did not exceed 3%. Only 32 sites were parsimony informative.

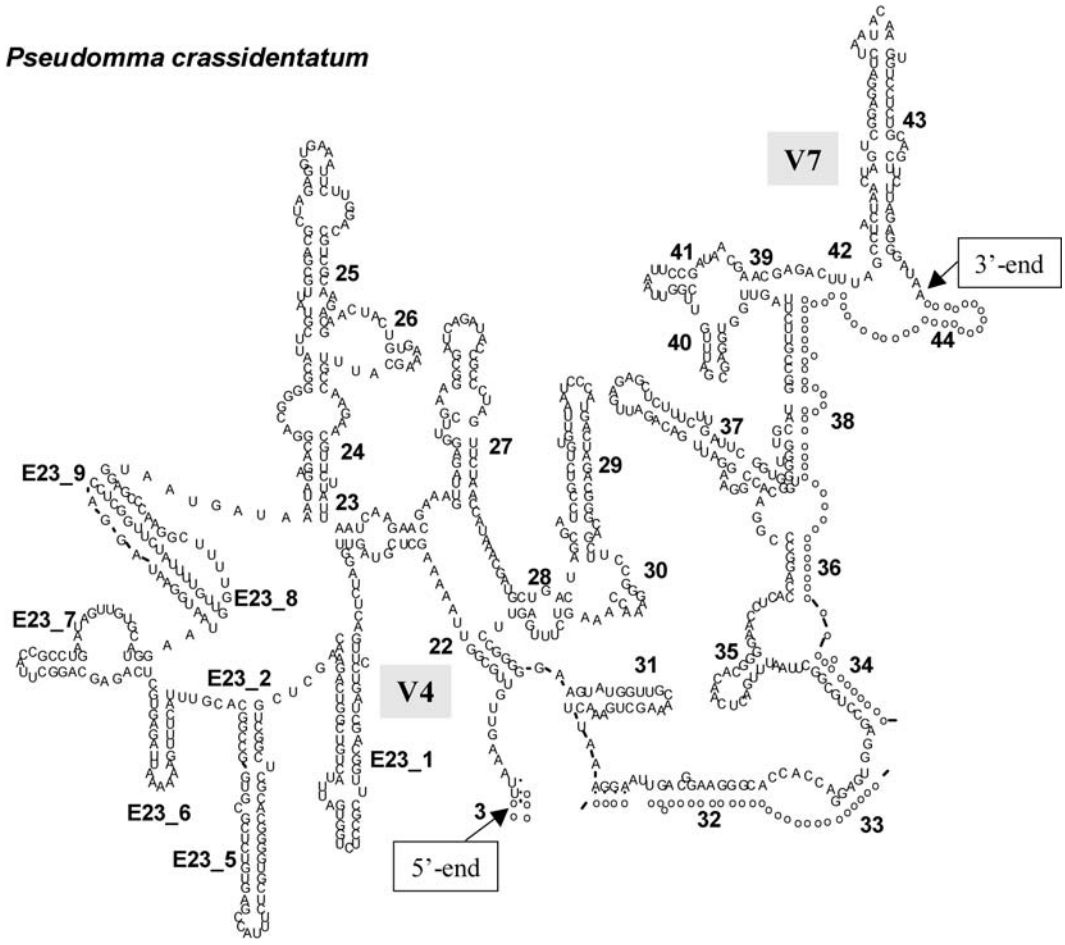

Fig. 1. Putative secondary structure of the V4–V7 region in SSU rDNA for *Pseudomma crassidentatum* Murano, 1974. Helix numbering following Wuyts *et al*. (2002).

Base frequencies were slightly biased towards T (26%) and G (28%) in the total sequence. When putative paired bases (in helices) and unpaired bases (in loops and bulges) were considered as separate data partitions, a biased base composition was more accentuated (Table 2), certainly reflecting the strong contribution of guanine to the formation of secondary structure stems. With pairwise comparisons, transitions were approximated to be twice as common as transversions, with C-T transitions being the most common.

MrBayes was set up to estimate phylogeny under a general time reversible model with site specific rates in loops and helices. (This is equivalent to a GTR model with linked partitions in MrBayes 3.0.) Log-likelihood values converged towards stationarity after about 190 samplings. The initial 200 trees and parameter estimates were discarded, and posterior probabilities were computed on all compatible clades from the remaining trees (Fig. 2a). Mean lnL for the trees was −1684.05 with variance 29.47. A predominance of CT mutations contributed to a mean transition/transversion ratio of 2.03. Substitutions were estimated to be 1.37 times more frequent in

Table 2. Nucleotide composition (percent) in the 18S rDNA data set of Mysida.

	Secondary structure partitions		
Nucleotide composition (%)	Paired (463 bp)	Unpaired (345 bp)	All (808 bp)
A	16.3	37.0	25.1
T	24.9	26.6	25.7
C	23.8	17.5	21.1
G	35.0	18.8	28.1

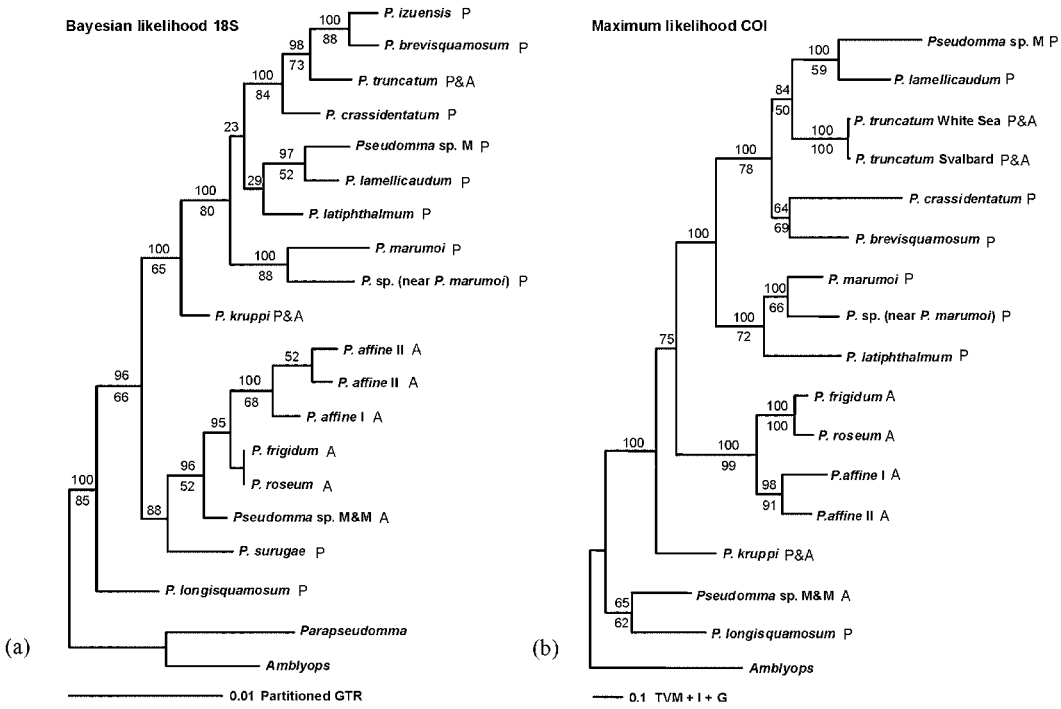

Fig. 2. Phylogenetic trees based on the V4–V7 region of the nucleotide small subunit rDNA (18S), and a 599 bp segment of the mitochondrial cytochrome oxidase subunit I mtDNA (COI). (a) Bayesian consensus phylogram from estimates on 18S data assuming a GTR model with separate rates in helices and loops. Bayesian posterior probabilities computed from 9800 optimal trees with mean $-\ln L = 1684.05$ are indicated above branches and maximum parsimony boostrap values >50% are shown below branches. (b) Maximum likelihood phylogram from COI data using a transversional model (Posada and Crandall, 1998) with unequal base frequencies, unequal transition and transversion rates, gamma correction with four rate categories, and accounting for invariable sites (TVM+I+G) species, respectively. Bayesian posterior probabilities from 4000 optimal trees with mean $-\ln L = 6436.43$ are indicated above branches and maximum parsimony bootstrap values >50% are shown below branches. P and A denote Pacific and Atlantic species, respectively.

base paired sites. However, this is very similar to the ratio of paired and unpaired positions (Table 2), so the substitution rate per site was approximately the same in helices and loops. The ILD test allowed for the null hypothesis of consistency in phylogenetic signal between helices and loops to be accepted (P value = 0.27).

The ML searches on nonpartitioned data were conducted in PAUP using the Tamura and Nei model (Tamura and Nei, 1993) with equal base frequencies, gamma correction, and invariable sites. The following parameter estimates, suggested by Modeltest, were used in this search: ($\alpha 1_{(A-C)} = 1.0000$, $\alpha 2_{(A-G)} = 1.9458$, $\alpha 3_{(A-T)} = 1.0000$, $\alpha 4_{(C-G)} = 1.0000$, $\alpha 5_{(C-T)} = 5.2313$, $\alpha_{gamma} = 0.6060$, $p_{invar} = 0.8189$). The resultant tree ($-\ln L = 1623.8746$) was in close agreement with the topology found in Bayesian searches on partitioned data (Fig. 2a), with the exception of *P. surugae* and *P. longisquamosum* having a basal position to *P. kruppi* and the remaining North Pacific clades.

The MP searches on nonpartitioned data resulted in 30 equally parsimonious trees (Length: 74 steps, CI: 0.69, RI: 0.77). Although less resolved, a strict consensus of the MP trees was found to be congruent with the topology displayed in the Bayesian tree (Fig. 2a).

COI.—Sequenced COI segments consisted of 599 bp that were aligned for 17 taxa. Because of insufficient PCR products, *Pseudomma izuensis*, *P. surugae*, and *Parapseudomma calloplura* were not included in the COI analyses. The sequenced COI fragments were confirmed by alignment with two transmembrane respiratory domains in COI (nucleotide positions 96–693) from *Drosophila yakuba* (SwissProt: P00400).

The COI section contained 362 variable sites resulting in a high degree of sequence divergence between taxa (avg. 33%). Fifty four

percent of the variable sites occurred in the third codon position, and 29% and 17% occurred in the first and second positions, respectively. Partly because of a substantial nucleotide bias towards adenine and thymine in the third position, an average AT content of 60% was observed in the sequenced COI fragment. A similar nucleotide bias in favour of thymine was also observed in the second position, but biased against adenine. Nucleotide composition and substitution patterns are summarized in Table 3. Numbers of transitions *versus* numbers of transversions in all pairwise comparisons of COI were plotted for each codon position (Fig. 3). Saturation was clearly indicated in third codon positions. Most informative substitutions were synonymous third position changes. Almost all substitutions (58) in the second position were nonsynonymous, and about half of the 105 variable first position sites resulted in amino acid change. Compared to similar studies on crustacean COI, the level of amino acid substitution is very high, with pairwise amino acid distances between *Pseudomma* species averaging 25%. Scoring across all taxa, 94 out of the 362 variable nucleotide sites resulted in changes in amino acid sequence, but only 82 were parsimony informative. Overall patterns of base composition and substitutions found in *Pseudomma* are comparable to those found in other surveys of arthropod mtDNA (Spicer, 1995; Meyran *et al*., 1997; Baldwin *et al*., 1998; Shank *et al*., 1999; Wetzer, 2001) and provide some assurance that the sequences used in this study are not nuclear pseudogenes (Sunnucks and Hales, 1996; Benasson *et al*., 2001).

Taking into account unequal base frequencies and unequal transition and transversion rates, a tranversional model (TVM+I+G) was chosen to represent the evolution of COI in the ML analyses. The model was run with PAUP using gamma correction with four rate categories and parameter values suggested by Modeltest ($A\pi = 0.2798$, $C\pi = 0.1788$, $G\pi = 0.1610$, $T\pi = 0.3803$; $\alpha 1_{(A-C)} = 0.4762$, $\alpha 2_{(A-G)} = 6.5276$, $\alpha 3_{(A-T)} = 1.3164$, $\alpha 4_{(C-G)} = 2.9216$, $\alpha 5_{(C-T)} = 6.5276$; $\alpha = 0.4798$; $p_{invar} = 0.2422$). The resulting tree ($-\ln L = 6581.2290$) is shown in Fig. 2b. The TVM+I+G model is not implemented in MrBayes, but we obtained the same topology using gamma corrected separate rates for each codon position. Likelihood values converged towards stationarity after about 200 samplings. Posterior probabilties computed on all compatible clades returned low support on basal nodes, particularly indicating an unresolved relationship between *Pseudomma longisquamosum* and *Pseudomma* sp. M&M. Mean lnL for these trees was –6436.43, and the variance was 30.66.

Table 3. Nucleotide composition and substitution data for COI in Mysida.

	COI Codon Position			
	1st (200 bp)	2nd (200 bp)	3rd (199 bp)	All (599 bp)
Base frequency:				
A	27.8	15.0	30.2	24.3
T	24.3	43.2	41.5	36.3
C	20.0	24.6	15.3	20.0
G	28.0	17.2	13.0	19.4
Substitutions:				
Transitions	24	11	49	84
Transversions	23	15	63	101
Transitions/transversions	1.1	0.7	0.8	0.8
Properties:				
Identical sites	95	138	4	237
Variable sites	105	62	195	362
Nonsynonymous sites	48	58	0	106
Informative sites	87	48	193	328

Maximum Parsimony analyses (MP) on COI sequences resulted in a single most parsimonious tree (Length: 1620 steps, CI: 0.44, RI: 0.43). This tree was comparable to the topology from Bayesian and maximum likelihood analyses (Fig. 2b), but disagreement in the MP tree was seen in the position of the *Pseudomma* sp. M&M – *P. longisquamosum*, *P. kruppi* clade having a basal placement to the Atlantic clade. The ambiguous placement of these species was also indicated by low statistical confidence on basal nodes in all COI analyses.

Phylogenies inferred with different methods from 18S and COI sequences were clearly comparable. Two major lineages representing North Pacific and North Atlantic species were found to be consistent and supported by both genes. The latter includes *P. roseum*, *P. frigidum*, and *P. affine* I and II with high probability, while the results are somewhat diverging with respect to *Pseudomma* sp. M&M. Weak support was mostly observed in basal nodes and partly due to the ambiguous placement of *P. kruppi*. From 18S, *P. kruppi* is suggested to be closely related to the North Pacific *Pseudomma* species, whereas COI suggests a closer affinity to North Atlantic species. All phylogenies indicate that North Atlantic populations of the circumpolar species *P. truncatum* are closely related to *Pseudomma*

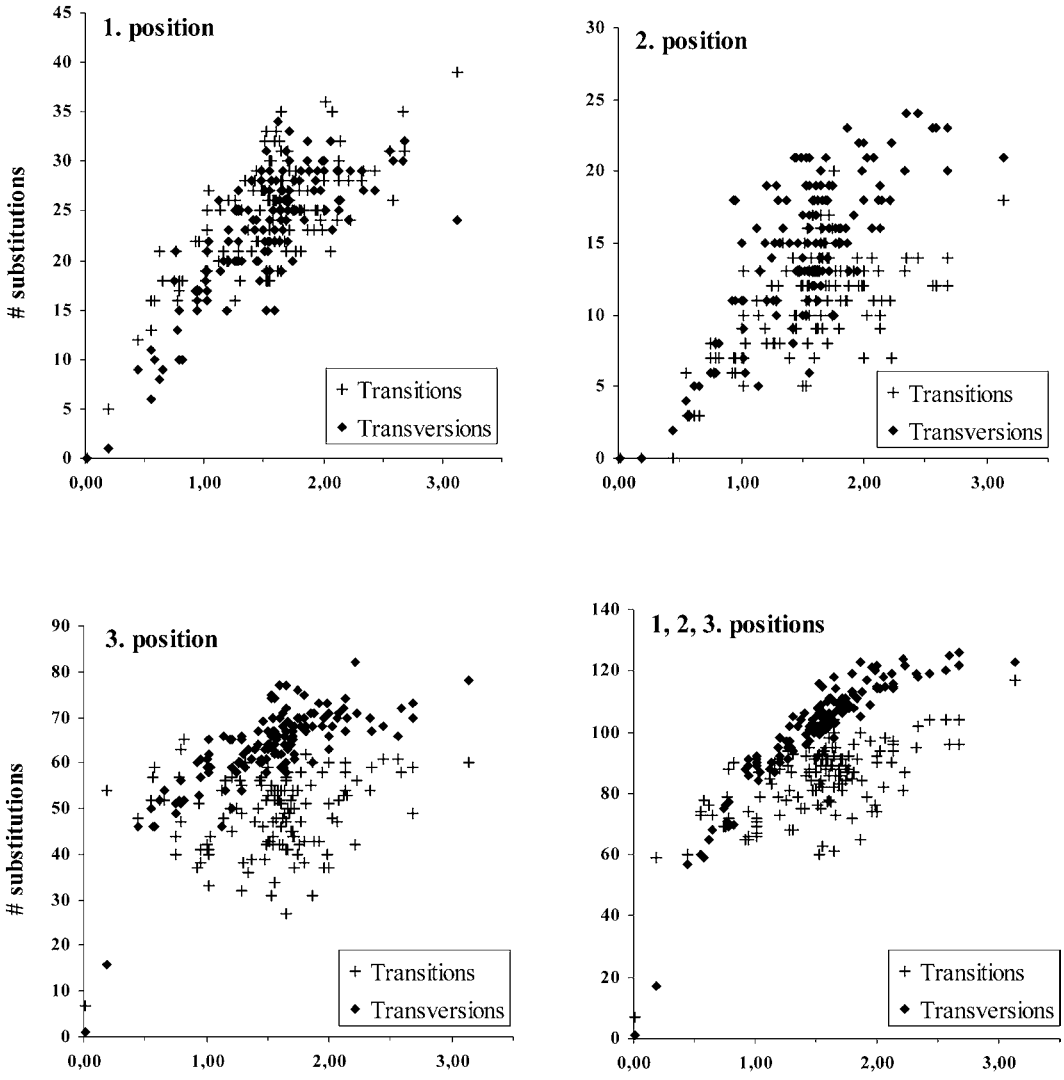

Fig. 3. Saturation plots by codon positions of transitions and transversions in cytochrome oxidase subunit I (COI) between studied Mysida species. Evolutionary distances were estimated using the TVM+I+G model for sequence evolution selected by Modeltest 3.06 (Posada and Crandall, 1998).

species from the North Pacific. Also, with exception of the COI ML analysis, *Pseudomma* sp. M&M from the Iceland Basin is suggested having an ancestral position to the North Atlantic group.

In comparing the molecular with morphological based phylogenies (Meland, 2004), congruent topology was recognized in the North Atlantic clade. There were also shared affinities between the molecular clades containing *Pseudomma* sp. M, *P. truncatum*, *P. crassidentatum*, and *P. lamellicaudum* that were also placed within a similar morphological clade (Meland, 2004: fig. 3). Morphological character mapping on the molecular topologies revealed a considerable amount of homoplasy in terms of morphological development. Characters containing limited phylogenetic information were mainly observed in the eyeplate, feeding appendages, female pleopods, uropods, and telson. On the other hand, morphology of the antennal scale, the third to fourth pleopods of males, and male genital organs defined several clades on the molecular phylogenies allowing for inference on the evolutionary history of these characters (Fig. 4).

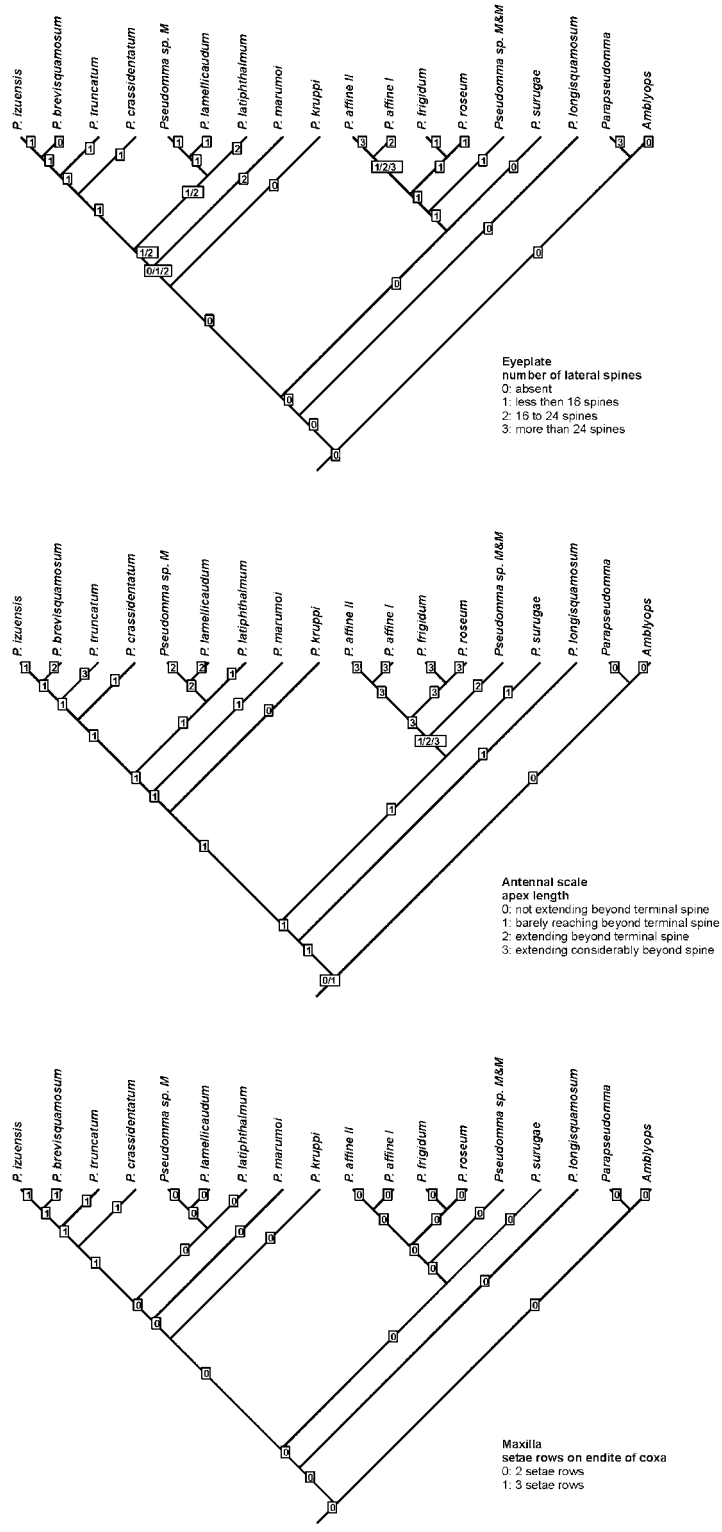

Fig. 4. Cladograms showing morphological characters from Meland (2004) optimized onto an 18S rDNA-based phylogeny.

How Old is *Pseudomma*?

The rate of COI evolution in the examined *Pseudomma* species was shown to be largely heterogeneous. Pairwise relative rate tests (Tajima, 1993) performed with MEGA showed noticeable divergence in substitution rates when the basal taxa in the COI tree (*Pseudomma* sp. M&M, *P. longisquamosum*, and *P. kruppi*) were compared with the remaining taxa. The maximum likelihood tree produced with the TVM+G+I model (Fig. 2b) was used to estimate likelihoods, giving –6610.27024 with and –6579.30120 without a molecular clock enforced, resulting in $\delta = 61.93808$ with 15 degrees of freedom. The obtained P-value < 0.001 rejected a null hypothesis of a homogeneous rate of evolution. Failing to meet the constant rate assumption of a molecular clock, estimation of divergence time between species could not be applied to the COI data.

The log-likelihood ratio test was performed on the Bayesian consensus/MP tree derived from the 18S data (Fig. 3a). Because the tree obtained with maximum likelihood had a slightly different topology, model parameters were recalculated in PAUP* with a GTR+I model (Rodríguez *et al*., 1990) ($A\pi = 0.24994$, $C\pi = 0.21367$, $G\pi = 0.27928$, $T\pi = 0.25711$; $\alpha 1_{(A–C)} = 0.31476$, $\alpha 2_{(A–G)} = 1.83636$, $\alpha 3_{(A–T)} = 2.10946$, $\alpha 4_{(C–G)} = 0.07082$, $\alpha 5_{(C–T)} = 4.84935$; $p_{invar} = 0.90331$). Estimated log-likelihoods of –1626.73782 with a molecular clock enforced, and –1613.80977 without this constraint gave a test statistic $\delta = 25.85614$ with 17 degrees of freedom. The obtained P-value $0.1 > P > 0.05$ allowed for the null hypothesis of clock-like evolution in 18S to be accepted. A general substitution rate of 0.8% per 100 my (Escalante and Ayala, 1995) suggests that *Pseudomma* diverged from the outgroups about 96 mya. However, taking into account that substitution rates are highly variable in different sections of this molecule (van de Peer *et al*., 2000) and that our data include the E23 and 43 helices, which are considered to be hypervariable in a broader taxon context, rates of 2–4% per 100 my (Moran *et al*., 1993) might be more appropriate. In an attempt to recognize a concordance in the evolutionary history of *Pseudomma* with major geological events, a substitution rate of 3% per 100 million years is particularly interesting because it dates the divergence between *P. surugae* and its North Atlantic sister clade to 21 million years ago (Fig. 5). Thus, the closure of the Tethys Sea in the Mediterranean region, about 20 mya, is a candidate event for Atlantic–Pacific vicariance in the deeper nodes of *Pseudomma*. Accepting a provisional clock rate of 3% per 100 million years for the 18S sequences means that our basal *Pseudomma* node is approximately 32 million years old.

Discussion

Properties of COI

Despite independent modes of evolution, COI and 18S proved to share comparable phylogenetic structure. Maximum parsimony, maximum likelihood, and Bayesian inference resulted in similar topologies, and increased the support for both genes reflecting the phylogenetic history of the studied *Pseudomma* species.

Observed distances in COI between closely related *Pseudomma* species was unexpectedly large. Comparable observations of sequence divergence (p-distance) in other crustacean taxa have been shown in penaeid shrimp: 19% (Baldwin *et al*., 1998); cirolanid isopods: 34% (Wetzer, 2001); bresiliid vent shrimp: 20% (Shank *et al*., 1999); cancer crabs: 17% (Harrison and Crespi, 1999); and gammarid amphipods: 25% (Meyran *et al*., 1997). In terms of interspecific sequence divergence, the observed average genetic distance of 30% in mysids is considered very high. The observed A-T bias, substitution rate variation, and third position saturation found in the mysid COI sequences are all characteristic properties of arthropod mitochondrial genes (Simon *et al*., 1994; Blouin *et al*., 1998). The most striking feature was seen in the high number of variable sites in the second position and indications of transitional saturation. Nonetheless, translating to amino acids did not reveal stop codons and also conformed to the general domains that are expected in this portion of sequenced COI. To address the possibility of misleading results because of the potential problem of saturation, a separate data partition excluding third position was constructed and run under maximum parsimony. This analysis gave a less resolved tree but was concordant with the other analyses.

Tracing Morphology

Mainly due to low variation and apparent convergence of morphological traits in several lineages, a morphology-based analysis has

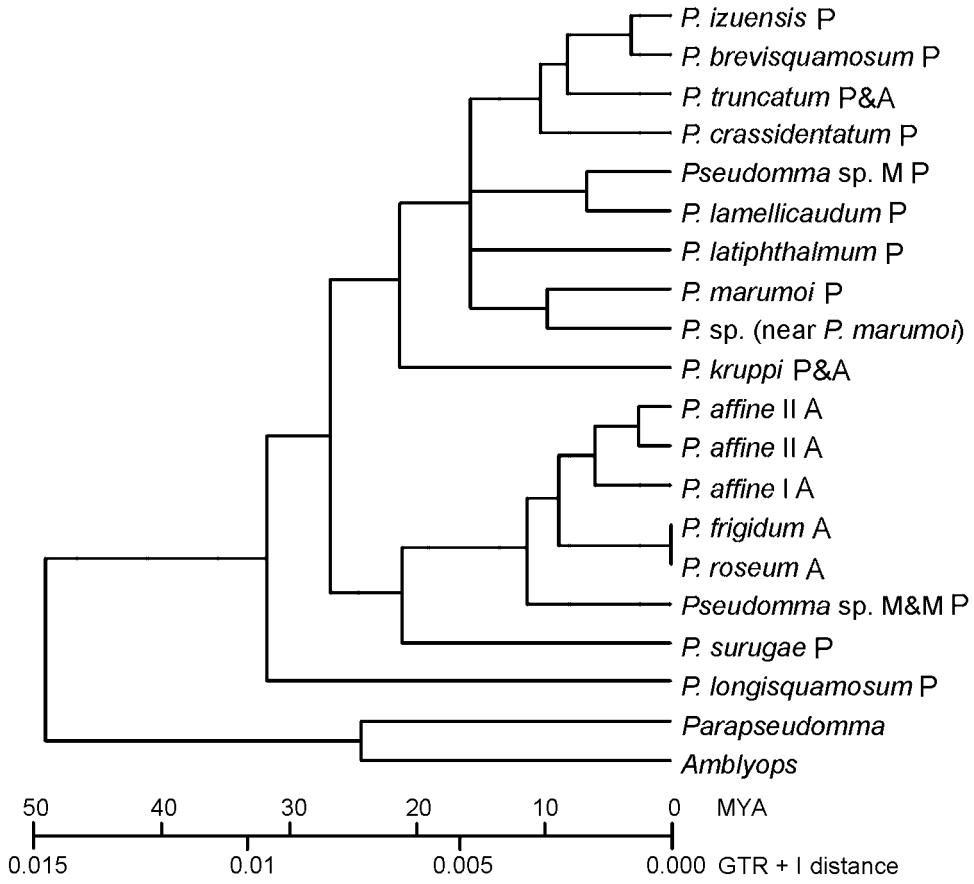

Fig. 5. Tree from 18S data (see Fig. 2a and text) constrained to a molecular clock. Model parameters and branch lengths re-estimated in PAUP* 4.0b10 (Swofford, 2002). Nodes provisionally dated assuming an evolutionary rate of 3% per 100 million years (see text). P and A denote Pacific and Atlantic species, respectively.

given somewhat ambiguous results on the evolutionary history of *Pseudomma* (Meland, 2004). In comparing these morphological phylogenies with our results in this study, considerable agreement between COI and 18S trees suggest that the relative age of *Pseudomma* lineages may be better reflected by molecules than by morphology. Large genetic distance between morphologically similar taxa may either be due to elevated mutation rates or could be reflecting long divergence times. We suggest that the latter is true for *Pseudomma* and that morphological similarity between species is masking a long history of evolution maintained by stabilizing selection, which has lead to phenotypic stasis (Palumbi and Benzie, 1991). Assuming that the species relationships proposed in this study are more accurate estimates of *Pseudomma* evolution, the COI and 18S trees were used to investigate to what extent the presented phylogenies were supported by morphological characters.

The compound eye seems to be a directed reduction from nonserrated separate eye plates that have gradually fused. Increasing fusion is correlated with increasing spine numbers and protrusion of the anterior region of the fused plate. The molecular phylogeny suggests such modifications of the eye plates to be reoccurring events within separate clades (Fig. 4).

There seems to be little phylogenetic information in the morphology of the feeding appendages, maxilla, maxillule, and first and second maxillipeds. Character states are spread across species making it difficult to identify a coherent pattern for inference of evolutionary history. An example is seen in a set of well-developed teeth on the inner lateral margin of the maxillule basis (Meland, 2004: fig. 1I). From the COI tree, this peculiar character has

evolved in a *Pseudomma truncatum* ancestor, is retained in *P. lamellicaudum*, but lost in *Pseudomma* sp. M. Reconstructed on the 18S tree, the character state evolved twice in independent lineages. This character is also seen in the Japan Sea endemic species *P. okiyamai*. Although specimens of *P. okiyamai* could not be obtained for this sequence study, the morphological similarities suggest a relationship with *P. truncatum*.

The molecular phylogeny implies a gradual reduction in annulation of exopods and endopods on male pleopods. The evolution of sexual characters seen in the modified setae on the third and fourth pleopods have been shown to define deeper nodes in the phylogeny, and a similar evolutionary history is supported by this study. As for the female pleopod characters, the molecular phylogeny reveals large amounts of homoplasy in morphological features. There is no consistent pattern of evolution in the uropods nor the telson. As suggested for the armature of the mandibular palp and female pleopod morphology (Meland, 2004), the lengthening of the uropod exopod is possibly correlated to species size. Prone to homoplasy, the phylogenetic information of such characters is therefore very limited.

Several studies on peracarid taxa have shown an increase in body size with increasing depth (Wilson, 1983; France and Kocher, 1996; Yampolsky and Scheiner, 1996; Svavarsson *et al*., 2001), and the observed patterns of size and temperature correlation are most likely an effect of depth distributions.

Both molecular datasets provide a clade consisting of *Pseudomma affine* I, *P. affine* II, *P. roseum*, and *P. frigidum*. In these species, the antennal scale has an elongated apex that is armed with more than 14 setae and forms a synapomorphy for the Atlantic species reported from the Norwegian Sea (Fig. 4). A similar elongation is seen in *P. truncatum*, but then not associated with an increased number of setae. Two sexual characters forming potential synapomorphies for North Atlantic species are also observed in the lengthened male genital organ and modified setae on the endopod of the third male pleopod. Both of these characters are found in the sub-Antarctic species *P. calmani*, and the modified setae on the third endopod are also shared with the Atlantic species *P. jasi*. The 18S data suggest a Pacific clade comprising *P. izuensis*, *P. brevisquamosum*, *P. truncatum*, and *P. crassidentatum*. In these species the endite of the maxillar coxa has three rows of setae (Meland, 2004: fig. 1J). As in the aforementioned characters defining the North Atlantic clade, this apparent synapomorphy is also found in *P. armatum*, *P. belgicae*, *P. longicaudum*, *P. magellanensis*, and *P. schollaertensis*, which are endemic to Antarctic waters. Because of the absence of Antarctic species in the current study, we were unable to provide molecular evidence for the suggested affiliation between northern and southern endemics. Additional support for a close bipolar relationship in *Pseudomma* species remains to be found when Antarctic material becomes available for molecular analyses.

Given the high degree of morphological convergence among allopatric species of *Pseudomma* in the Atlantic and Pacific oceans, we believe that molecular data provide a more accurate guide to the genealogical relationships of this genus than the trees inferred from morphology (Meland, 2003). Support for this presumption is provided by the apparent conservative evolution of *Pseudomma* morphology.

Biogeography

Species of the genus *Pseudomma* are globally distributed throughout all deep-sea provinces, and the absence of *Pseudomma* records from the Indian Ocean is most likely due to low sampling effort. In this study, we included species from the Northwest Pacific, North Atlantic, Norwegian Sea, and Arctic regions. The basal phylogenetic placement of *P. longisquamosum* (West Pacific) is also indicated by morphology, which additionally suggests that the Antarctic species group may be even older (Meland, 2004) than the species examined in the present paper. Accepting a relatively high constant mutation rate for 18S rDNA (0.3% per 10 million years) means that the radiation of North Atlantic and North Pacific *Pseudomma* clades may have started 26 million years ago. In effect, current species-distribution and molecular-clock considerations indicate that the oldest Pacific and Atlantic lineages may be remnants of a Tethys Sea fauna dating back to at least late Oligocene. The phylogenetic placement and present distribution of *P. kruppi* in both the Atlantic and Pacific oceans also indicate an early history of *Pseudomma* prior to the isolation of modern day oceans in early Miocene (Kennett, 1982). Additional support for the presence of highly developed mysids in late Paleogene is found in the mysid fossil

records, where the extant genus *Siriella*, bearing close resemblance to modern species, is reported from Jurassic strata in France (Secretan and Riou, 1986; Taylor *et al*., 2001). It is also fairly well documented that the peracarids were already a diverse and important taxon in late Paleozoic (Schram, 1974).

Although 18S and COI suggest different placements of *Pseudomma* sp. M&M and *P. kruppi*, the present analysis gives good support for the existence of two principal lines of descent in Atlantic and Pacific clades. Following an idea of a southern temperate distribution dating back to the Oligocene, a possible explanation for the separation of these two lines would be the closing of the Tethys Sea between Eurasia and Africa in the Miocene (20 mya) (Kennett, 1982). This would suggest that ancestral lineages including *Pseudomma* sp. M&M, *P. longisquamosum*, and *P. kruppi* persisted more or less unchanged during the destruction of Tethys seaways. One could also envision the splitting of these two major lines as the result of the formation of the Panamanian Isthmus during the Miocene–Pliocene (Knowlton and Weigt, 1998). However, both 18S and COI divergence suggest a much older age for these clades than the accepted separation of the Caribbean and Eastern Pacific about 3 mya. Additional support is also seen in the distribution of extant *Pseudomma* species being highly concentrated in the Eastern Atlantic and Western Pacific. Although the Gulf of Mexico and East Pacific species of *Pseudomma* are not included in this study, it is interesting to note their closer morphological resemblance with sub-Antarctic species compared to the included northern species in the Pacific and Atlantic (Meland, 2004). After the opening of the Drake Passage in early Miocene, the subsequent formation of the circumpolar current resulted in the establishment of an effective barrier between the South American and Antarctic faunas (Crame, 1999). An ancestral line of *Pseudomma* may have persisted in the Antarctic while South American species radiated northward along the eastern Pacific. It is therefore not unlikely that East Pacific and Gulf of Mexico species are descendants of a South American lineage originating in the Magellan province. Additional sequence data from these regions are necessary to test such a scenario.

Sequence divergence between the North Atlantic species was shown to be relatively low in both 18S and COI. The 18S data suggest the onset of speciation and radiation in the North Atlantic to have taken place approximately 10 million years ago. The present distribution of *P. roseum* and *P. affine* II in both the East and West Atlantic indicates that *Pseudomma* species could have been distributed throughout the northern Atlantic region prior to the submergence of the Greenland-Faeroe ridge in mid-Miocene (Thiede *et al*., 1990). Recent sampling in the Iceland Basin south of Iceland has revealed both described and new species of mysids. The presence of *P. affine* I, *P. roseum*, and *Pseudomma* sp. M&M in that material suggests that this faunal assemblage could be indigenous to an Amphi-Atlantic mysid fauna dating back to Miocene. Present depth distribution and temperature preferences (see Brattegard and Meland, 1997) suggest that the *P. frigidum*–*P. roseum* line and *P. affine* I have a cold deep-water distribution, whereas *P. affine* II has evolved a tolerance for warmer temperatures, giving the opportunity to extend its range onto the continental shelf. During Pliocene, the formation of the Norwegian Sea was completed and intrusion of deep Atlantic water west and east of the Faeroe Islands allowed for a northward migration (Thiede *et al*., 1990).

Colonization of the Norwegian Sea was probably disrupted by the onset of Quaternary glacial events. During periods of glaciation, deep-sea organisms such as *Peudomma frigidum*, and possibly *P. affine* I might have found refuge in the deep-water basins of the Norwegian and Greenland Seas, while continental shelf and warmer water species probably experienced extinction or were forced further south (Vermeij, 1989). A recolonization by *P. affine* I into the Norwegian Sea and the radiation of *P. affine* II and *P. roseum* into Norwegian fjords must have taken place after the final retreat of glacial ice 15–10k years ago. A second colonization of the Norwegian Sea by *P. affine* populations is understood by their early splitting into cold- (*P. affine* I: $< 6°C$) and warm-temperature (*P. affine* II: $> 6°C$) forms in Atlantic waters, allowing for post-glacial migration into both boreal cold, deep water and warmer shelf and fjord habitats as these were made available. The origins of *P. frigidum* and *P. roseum* is less understood. Identical 18S and low COI distance indicates a recent speciation and is possibly a result of a biotic interchange involving *P. roseum* descending into the deep basins of the Norwegian and Greenland Sea. Opposed to the restricted distribution of

P. frigidum in the cold water masses (< 0°C) of the Norwegian and Greenland Sea, *P. roseum* is known from shelf areas and fjords in both the East and West Atlantic, and its preference for water temperatures > 0°C support this idea. Until we are able to compare our data to *P. roseum* species from the West Atlantic, we cannot establish when the first colonization of *P. roseum* into the Northern Seas took place.

With the exception of *P. truncatum*, all species in the North Pacific clade are endemic to Suruga and Sagami Bay, Japan. After the closure of the Tethys Sea, the Pacific line must have experienced a rapid radiation in northern hemisphere mysids. Approximately 50 percent of described *Pseudomma* are endemic to the West Pacific, generally found in the warm-temperate Japan region. This high proportion of the global species diversity is certainly biased to some extent by more intensive sampling in these waters (Murano, 1974, 1975, 1976, 1977, 1981) than in the Indian Ocean and Southern Atlantic. However, Antarctic and North Atlantic benthic communities are equally well documented. The observation that 20% of the described *Pseudomma* species are endemic to the Antarctic and only 10% are found endemic to the North Atlantic thus probably reflects higher speciation rates in the West Pacific. The actual evolutionary history and mechanisms leading to higher speciation rates is not understood. It is possible that the species described from Sagami Bay have either previously had or still have a much wider distribution and that they also occur in the deep sea outside coastal Japan. Support for a southern origin is indicated by the shelf fauna of southern Japan, which has been referred to as a tropical or subtropical assemblage (Briggs, 1974). The observed species diversity in Sagami Bay could then be the result of several dispersal routes that are accommodated by southern deep-water currents and the northern Oyashio Water flowing southeastward through the Oshima East Channel along the Bosa Peninsula (Tomoharu *et al*., 1998). If this could be demonstrated, a general accumulation of hyperbenthic deep-sea organisms, including *Pseudomma*, would be expected, and Sagami Bay would be a diversity "sink-hole" rather than a speciation center.

Unlike the restricted range observed in most species of *Pseudomma*, *P. truncatum* is widespread with a circumpolar boreal distribution. Variation in morphology between populations from separate biogeographical subregions has lead to questions concerning the taxonomic status of populations (Kathman *et al*., 1986; Meland and Brattegard, 1995; Meland, 2004). Ranging from British Columbia and the Okhotsk Sea into the Arctic Sea and Polar Regions of the Norwegian Sea, *P. truncatum* is considered a true cold-water species. In this study, we used *P. truncatum* specimens from Svalbard and the White Sea. The molecular phylogenies gave strong support for *P. truncatum* as a recently derived species originating from North Pacific ancestors. A circumpolar distribution implies that *P. truncatum* must have at some point in history transversed the Bering Strait. During the late Cenozoic several episodes of Beringian submergence allowed cold-water marine species to move between North Pacific and Arctic-Atlantic waters, effectively ending a 100 million year separation that had persisted since the middle Cretaceous (Marincovich *et al*., 1990; Vermeij, 1991). A possible interpretation from the 18S data is that speciation was initialized by a migration of a Pacific most recent ancestor into the Arctic oceans during submergence of the Bering Strait in late Miocene approximately 10 mya. An estimated re-closing of the Bering Strait 8 mya effectively isolated these immigrants from the North Pacific subsequently leading to *P. truncatum* in Arctic waters. Further dispersal into the Norwegian Sea and Northwest Atlantic can be interpreted as independent western and eastern Arctic migration events. A second submergence of the Bering Strait in early Pliocene between 4.8 and 7.3–7.4 mya (Marincovich and Gladenkov, 2001) would then have allowed for the Arctic *P. truncatum* to colonize the North Pacific. It could be argued that deep-water species such as the *Pseudomma* could not have transcended the relatively shallow Bering Strait. However, the occurrence of *P. truncatum* populations in shallow depths of both the Bering Sea and the Kara Sea imply that they could invade across shallow water sills. Preferred depths seem to depend on temperature and other oceanographical conditions that undoubtedly have varied substantially through the Miocene and Pliocene.

This interpretation contrasts with most estimates that suggest an age no earlier than 7.4 million years for the initial opening of the Bering Strait (see Marincovich and Gladenkov, 2001) and the tentative nature of our time estimates should not be forgotten. An equally parsimonious explanation would be to suggest that *Pseudomma truncatum* originated somewhere else in the Pacific and invaded the Arctic

at the earliest opening of a Beringian seaway. Nonetheless, other DNA-based studies have also suggested an early date for Trans-Arctic dispersal. The genus *Cancer* has been proposed to have invaded the Atlantic Ocean from the North Pacific 6–12 mya (Harrison and Crespi, 1999); likewise, Collins *et al*. (1996) proposed that *Nucella* invaded the Atlantic Ocean 7–8 mya. Our analyses of 18S suggest slightly higher mutation rates in North Pacific lineages as is indicated by comparably longer branch lengths than in North Atlantic clades (Fig. 2a). Consequently, the estimation of a first opening of the Bering Strait may be biased to be older due to higher mutation rates in this lineage. If nothing else, our results certainly indicate a close connection between *Pseudomma* in the Arctic and North Pacific. Until molecular data from East Pacific and West Atlantic *P. truncatum* populations are obtained, more accurate estimates of the timing and geography of dispersal of *Pseudomma* species between the Atlantic and Pacific oceans in Arctic waters can not be established.

This study suggests that both dispersal and vicariance have played an important role in evolutionary history of species within the genus *Pseudomma*. Major vicariance events such as the closing of the Tethys Sea through collision of Africa and Europe and formation of the Panamanian Isthmus lead to abrupt limitations in dispersal. Formation of the Norwegian Sea and opening of the Bering Strait played equally important roles by introducing new dispersal routes into new geographical regions. Presence of *Pseudomma* during these events imply an initially widespread distribution throughout the Tethys Sea dating back to mid-Oligocene. In the absence of Antarctic species for molecular analyses, it remains to be seen how *Pseudomma* from southern waters are related to northern lineages.

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