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Molecular Phylogeny of Western Atlantic *Farfantepenaeus* and *Litopenaeus* Shrimp Based on Mitochondrial 16S Partial Sequences

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Partial sequences for the 16S rRNA mitochondrial gene were obtained from 10 penaeid shrimp species: *Farfantepenaeus paulensis, F. brasiliensis, F. subtilis, F. duorarum, F. aztecus, Litopenaeus schmitti, L. setiferus,* and *Xiphopenaeus kroyeri* from the western Atlantic and *L. vannamei* and *L. stylirostris* from the eastern Pacific. Sequences were also obtained from an undescribed morphotype of pink shrimp (morphotype II) usually identified as *F. subtilis.* The phylogeny resulting from the 16S partial sequences showed that these species form two well-supported monophyletic clades consistent with the two genera proposed in a recent systematic review of the suborder Dendrobranchiata. This contrasted with conclusions drawn from recent molecular phylogenetic work on penaeid shrimps based on partial sequences of the mitochondrial COI region that failed to support recent revisions of the Dendrobranchiata based on morphological analysis. Consistent differences observed in the sequences for morphotype II, coupled with previous allozyme data, support the conclusion that this is a previously undescribed species of *Farfantepenaeus.*

INTRODUCTION

The shrimps of the Family Penaeidae are known around the world as valuable resources for fisheries and aquaculture in tropical and subtropical regions (Neal and Maris, 1985; Provenzano, 1985). Despite their commercial importance, many aspects of the biology of the Penaeidae are poorly understood and there is still much debate about fundamental issues, such as systematics and population dynamics (Dall *et al.*, 1990). The systematics of the group, in particular, has not yet been fully resolved, even for the heavily exploited and widely studied species of the genus *Penaeus* (*sensu* Perez-Farfante, 1969, used throughout the present paper). Perez-Farfante and Kensley (1997) have recently reviewed the systematics of the suborder Dendrobranchiata and have proposed that the five subgenera of the genus *Penaeus* be raised to generic status.

Biochemical approaches to the study of the systematics and population genetics of the Penaeidae, using allozymes, has previously led to the conclusion that the morphological similarity of the group is mirrored by a low level of genetic polymorphism (e.g., Lester, 1979; Redfield *et al.*, 1980; Mulley and Latter, 1980; Sunden and Davis, 1991; Benzie *et al.*, 1992; Tam and Chu, 1993). However, Palumbi and Benzie (1991) discovered high levels of molecular divergence between *Penaeus stylirostris, P. vannamei, P. esculentus,* and *Metapenaeus endaevori,* as revealed by partial sequencing of 12S and COI mtDNA regions. The discovery of such variability in DNA sequences and the recent availability of more efficient DNA-based techniques have generated a surge of new molecular work on *Penaeus* (e.g., Garcia *et al.*, 1996; Ball *et al.*, 1998; Tassanakajon *et al.*, 1998; Moore *et al.*, 1999; Xu *et al.*, 1999).

Among the most recent work, Baldwin *et al.* (1998) have approached the phylogeny and biogeography of *Penaeus* shrimps through the partial sequencing of the mitochondrial COI gene. They concluded that molecular data from this gene does not support the systematic revisions of Perez-Farfante and Kensley (1997) based on thelycum condition. The thelycum is the female external reproductive structure and its morphology is crucially involved in reproductive strategy. When a species has a closed thelycum, spermatophores can be implanted only after molting when the exoskeleton is still soft. When the thelycum is open this is not the case and mating usually occurs toward the end of the molt cycle before ecdysis (Dall *et al.*, 1990). The function of this external reproductive structure and its ecological relevance give the thelycum great importance as a taxonomic character. Perez-Farfante (1969) proposed the subdivision of the genus *Penaeus* into four subgenera, including *Litopenaeus,* which was diagnosed by an

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TABLE 1

Sample Sizes and Locations for the Brazilian Shrimp Species *Farfantepenaeus paulensis, F. subtilis, F. brasiliensis, Litopenaeus schmitti,* and *Xiphopenaeus kroyeri*

	F. paul.	morph. II	*F. subt.*	*F. bras.*	*L. schmi*	*X. kroy.*
Rio Grande (32°S)	2					
Florianópolis (28°S)	2					
Guaratuba (25°S)					2	2
Santos (24°)				2		
Vitória (20°S)		2				
Recife (8°S)		2			2	
Fortaleza (4°S)		4	4		4	
São Luís (2°S)			4			2
Total	4	8	8	2	8	4

open thelycum. The fact that the molecular data gathered by Baldwin *et al.* (1998) failed to support the current proposed taxonomy (Perez-Farfante and Kensley, 1997) was surprising and raises an interesting phylogenetic question. It is apparent that additional sequence data is required to resolve contrasting conclusions of morphological studies and those based on the mitochondrial COI gene.

In the present paper, the phylogeny of western Atlantic prawns, from the genera *Farfantepenaeus* and *Litopenaeus,* is reconstructed through partial sequencing of the 16S mitochondrial region. The status of a morphotype of pink shrimp from the northeastern Brazilian coast usually identified as *F. subtilis* is also investigated. Morphological variation within *F. subtilis,* previously recorded by Perez-Farfante (1969), along with more recent biochemical genetic evidence indicates that this sympatric morphotype is in fact reproductively isolated from *F. subtilis* (D'Incao *et al.*, 1998).

MATERIAL AND METHODS

Sampling

Ten species were screened in the present study, 5 *Farfantepenaeus,* 4 *Litopenaeus,* and the species *Xiphopenaeus kroyeri,* which was used as an outgroup for the phylogenetic analysis (see Tables 1 and 2). In addition to these species, we studied a previously undescribed morphotype of pink shrimp, usually identified as *F. subtilis,* which will be simply referred to as "morphotype II."

All samples consisted of a piece of tail muscle preserved either in 99% ethanol or in 6 M urea, 1% sarcosyl, 10 mM $NaPO_4$, pH 6.8. *F. paulensis, F. brasiliensis, F. subtilis, L. schmitti,* and *X. kroyeri* were acquired from fishermen or from fresh fish markets at eight sampling sites along the Brazilian coast between 33°S and 2°S (Table 1; see also Fig. 1), during June 1999. For the most southern sampling site *F. paulensis* was supplied by the Carcinology Laboratory of the University of Rio Grande (Rio Grande, Brazil). *F. duorarum, F. aztecus,* and *L. setiferus* were caught in Charleston Harbor (33°N) by researchers of the South Carolina Department of Natural Resources in May 1999. In addition to western Atlantic species, *L. vannamei* and *L. stylirostris* from the eastern Pacific were also sampled. *L. vannamei* was obtained from the experimental culture ponds of the Marine Shrimp Laboratory of the Federal University of Santa Catarina (Florianopolis, Brazil), and *L. stylirostris* was obtained from fisherman off the coast of Ensenada, Baja California (32°N). Finally, four samples of morphotype II were the same specimens used in a previous allozyme study (D'Incao *et al.,* 1998), and they were first sampled at Fortaleza (4°S, Ceará, Brazil) along with *F. subtilis* (see Fig. 1). In this case whole individuals have been preserved for 7 years in 70% ethanol.

PCR and Sequencing

DNA was extracted through digestion of a small piece of muscle in 100 mM Tris–HCL, pH 8.0, 1.25% SDS, and 390 ng/μl proteinase K (approx. 0.012 units/μl of Boehringer Mannheim, Cat. No. 1373-196). The 400-μl preparations were incubated for 3–4 h at 55°C in a water bath and then a standard phenol/chloroform–isoamyl alcohol extraction was carried out with precipitation of DNA by 2:1 ice-cold 100% ethanol plus 1:10 3 M sodium acetate (600 mM final concentration). The 20-μl PCRs were performed in 0.5-ml tubes, using ultrapure PCR water, and contained 200 μM each dNTP, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 1 μM each primer, 1 unit *Taq,* and 10 to 50 ng template DNA. The primers used to amplify the 3′ end of the 16S rRNA mitochondrial gene were the 16Sar (CGC CTG TTT ATC AAA AAC AT) and 16Sbr (CCG GTC TGA ACT CAG ATC ACG T); their positions in the human genome are 2510 and 3080, respectively (Simon *et al.,* 1994; Palumbi 1996). PCR was performed either on a Perkin–Elmer 480 or on a Hybaid PCR-

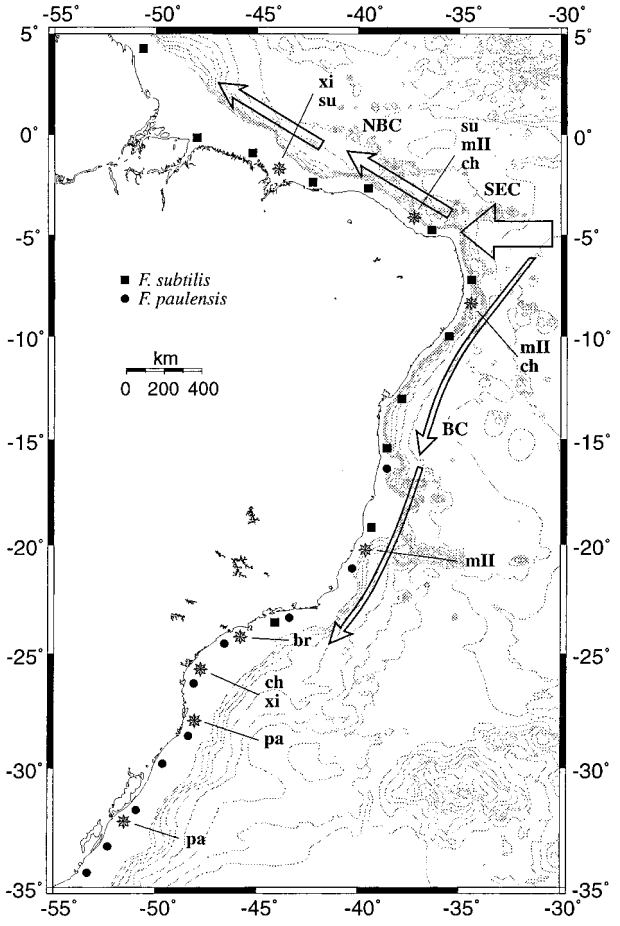

FIG. 1. Sampling sites for the Brazilian coast prawns used in the present study. Codes as follows: su, *Farfantepenaeus subtilis;* pa, *F. paulensis;* mII, *morphotype II;* br, *F. brasiliensis;* ch, *Litopenaeus schmitti;* xi, *Xiphopenaeus kroyeri.* Also shown is the distribution of *F. subtilis* and *F. paulensis,* according to Perez-Farante (1969) (*F. paulensis* distribution includes modifications by D'Incao, 1995), bathymetry, and circulation pattern (according to Johns *et al.,* 1990; SEC, South Equatorial Current; NBC, North Brazil Current; BC, Brazil Current).

Express thermocycler and comprised a 94°C/4 min initial denaturing step followed by 30 cycles of 94°C/1 min, 52°C/1 min, and 72°C/1 min. A final elongation step of 72°C/10 min was used. Oil overlay or hot lid worked equally well. PCR products were purified with Qiagen Qiaquick PCR Purification columns (Cat. No. 28106) according to manufacturers guidelines. The 10-μl cycle-sequencing reactions were prepared using Perkin–Elmer BigDye Terminator Ready Reaction mixes (Cat. No. 4303152), using the concentrations, amounts, and thermocycling conditions recommended by the manufacturer. The products of cycle-sequencing were purified through Qiagen DyeEx Spin kits (Cat. No. 63104) and screened in a Perkin–Elmer ABI 377 automated sequencer. All samples were double-checked by reverse sequencing.

Phylogenetic Analysis

Sequences were aligned using Clustal X (Thompson *et al.,* 1997) and double-checked by eye. A consensus sequence for each species and morphotype II was drawn from the initial set. The consensus sequences retain the variable sites, which were very small in number in this study. Reported sequences for *F. notialis* (detailed in Machado *et al.,* 1993) and *P. monodon* (K. H. Chu, J. G. Tong, and T. Y. Chan, unpublished; GenBank Accession No. AF105039) were also included in the analysis. All phylogenetic analyses, as well as basic statistics, were performed using PAUP* 4.0 beta 4a (Swofford, 1998). Three methods of tree building were used: maximum-likelihood, maximum-parsimony, and neighbor-joining. For all methods, tree topology was evaluated by bootstrapping of the original data set. For maximum-likelihood and maximum-parsimony, a heuristic search was employed and starting trees were always obtained by random sequence addition. Tree visualization and drawing were carried out using TreeView version 1.5 (Page, 1996).

Maximum-likelihood analysis was performed under the General Time-Reversible (GTR) model of base substitution. The Modeltest 3.0 (Posada and Crandall, 1998) algorithm was used to evaluate the choice of GTR model, which produced the most significant log-likelihood values, among various models tested. Site-specific substitution rates (SSR) and discrete approximation of the gamma (Γ) distribution were used in two separate analyses to correct for the among-site variation in the substitution rates. On GTR + SSR analysis, substitution rates were calculated considering the structural analysis by Machado *et al.* (1993), which divided the 3′ end of the 16S gene into two domains: domain A, with higher substitution rates, corresponded to sites 209–371 in our sequences. Base frequencies, substitution rates for the six different substitution types, and relative rates for the two domains considered, or the shape parameter for the Γ distribution (α), were estimated from the data set through maximum-likelihood, considering the topology of an initial neighbor-joining tree. The estimated values were used in searches with 1000 sequence addition replicates and in the subsequent bootstrappings, which consisted of 100 replicates with 100 sequence additions per replicate.

On maximum-parsimony analysis, gaps were considered as meaningful characters and multistate sites on the consensus sequences were considered polymorphic. The tree presented was found through a search with 1000 sequence additions. Bootstrapping of the maximum-parsimony tree consisted of 1000 bootstrap replicates, each with 100 sequence addition replicates. Finally, a neighbor-joining tree was constructed from the distances calculated under the GTR + SSR model with the same parameters used for the maximum-

TABLE 2

Penaeidae: Pairwise Distances Matrix[a]

	F. sub.	m II	*F. paul.*	*F. azt.*	*F. duo.*	*F. not.*	*F. bras.*	*L. van.*	*L. sty.*	*L. sch.*	*L. set.*	*P. mon.*	*K. kro.*
F. subtilis (8)	0.002												
Morphotype II (8)	0.040	0.004											
F. paulensis (4)	0.043	0.051	0.000										
F. aztecus (3)	0.044	0.056	0.043	0.000									
F. duorarum (3)	0.054	0.062	0.057	0.053	0.000								
F. notialis[b]	0.062	0.068	0.067	0.062	0.004	—							
F. brasiliensis (2)	0.065	0.072	0.059	0.050	0.055	0.062	0.000						
L. vannamei (3)	0.099	0.081	0.104	0.091	0.095	0.109	0.098	0.000					
L. stylirostris (4)	0.085	0.080	0.099	0.087	0.085	0.100	0.084	0.038	0.001				
L. schmitti (8)	0.121	0.113	0.132	0.113	0.113	0.121	0.110	0.071	0.067	0.001			
L. setiferus (3)	0.114	0.107	0.125	0.106	0.106	0.118	0.103	0.066	0.065	0.009	0.000		
P. monodon[c]	0.131	0.130	0.141	0.131	0.122	0.126	0.111	0.125	0.113	0.134	0.135	—	
X. kroyeri (4)	0.183	0.182	0.183	0.178	0.166	0.163	0.173	0.163	0.165	0.190	0.178	0.161	0.000

[a] Average pairwise GTR + SSR distances (sample sizes in parentheses).
[b] Distances calculated between consensus sequences and 16S sequence from Machado *et al.* (1993).
[c] Distances calculated between consensus sequences and 16S GenBank Accession No. AF105039.

likelihood analysis. The topology of the neighbor-joining tree was evaluated by 1000 bootstraps.

RESULTS

Variability of Sequences

The partial 16S rRNA mitochondrial gene sequences obtained comprised fragments of 485 to 488 bp of the 3′ end of the gene. All the original sequences were deposited in GenBank under the Accession Nos. AF192051 to AF192093 and AF255054 to AF255057. Average composition of the fragments studied were A = 32.6%, C = 13.2%, G = 21.6%, and T = 32.6% (when *F. notialis* and *P. monodon* sequences are included: A = 32.9%, C = 13.0%, G = 21.3%, and T = 32.8%). Significant differences in base composition across all taxa were not detected ($\chi^2 = 6.80$; $df = 36$; $P > 0.999$). Nevertheless, there was a bias in segment composition toward A + T that has been observed in other arthropodan mitochondrial gene sequences (Simon *et al.*, 1994). The domain A of Machado *et al.* (1993) concentrates on average almost two-thirds of the absolute number of substitutions in a one-third stretch of the sequence. On the other hand, loops and stems presented on average 42 and 58% of the pairwise substitutions from a total of 234 and 259 sites, respectively. On stems, compensatory substitutions are observed in a number of sites, as observed by Machado *et al.* (1993). The secondary structure of loops and stems have been tentatively defined by following Palmero *et al.* (1988) and Machado *et al.* (1993).

Table 2 presents the average pairwise GTR + SSR distances matrix for the studied 16S sequences. Morphotype II shows a degree of genetic divergence from *F. subtilis* comparable to the pairwise distances between *F. subtilis, F. aztecus,* and *F. paulensis* and much higher than those between *F. duorarum* and *F. notialis* and between *L. schmitti* and *L. setiferus* (Table 2). In addition, an insertion of three T's was observed near the 5′ end of the sequence (sites 29–31), in a region otherwise conserved among the species studied. According to the model for *Artemia,* this region corresponds to a loop in the secondary structure of the 16S rRNA (Palmero *et al.*, 1988).

Phylogenetic Analyses

Two basic topologies resulted from the tree search methods described (Fig. 2). They showed considerable agreement, the only major difference among them being the internal branching of the genus *Litopenaeus.* Figure 2A presents the strict consensus topology resulting from the maximum-parsimony analysis, on which two equally parsimonious trees were produced. Bootstrap values for this tree were high among different genera but often low within genera.

The majority rule consensus tree resulting from neighbor-joining was identical to the maximum-parsimony tree. Maximum-likelihood produced an alternative branching for *Litopenaeus* (Fig. 2B). Branch support followed a pattern similar to that of maximum-parsimony, with good support for *Farfantepenaeus* and *Litopenaeus* but poor resolution among species within the genera. However, GTR + Γ maximum-likelihood bootstrap shows poor support for *Litopenaeus.* We observed that an increase in bootstrap replicates and in the sequence additions per replicate increased the branch support for this model, but significant improvement in this testing would require a large amount of computer power, not available to us at the present time.

Summarizing the information on Fig. 2, there is strong support for *Farfantepenaeus* and for *Litope-*

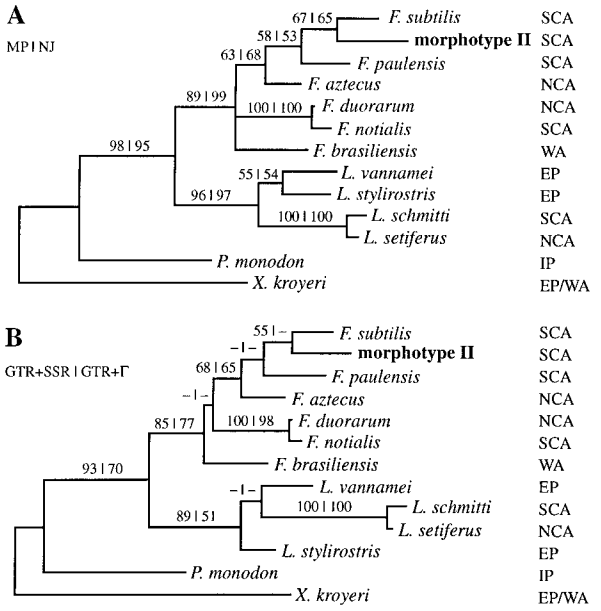

FIG. 2. Topologies resulting from the phylogenetic methods used. (A) Strict consensus tree resulting from the two most-parsimonious trees obtained through heuristic search with 1000 sequence addition replicates (L = 240; CI = 0.733; RI = 0.668; RC = 0.490). Bootstrap support shown for maximum-parsimony (MP) and neighbor-joining (NJ). Branch lengths correspond to maximum-parsimony analysis. (B) Maximum-likelihood tree obtained after an heuristic search with 1000 sequence addition replicates under the GTR + SSR model. Bootstrap branch support shown for two models of sequence substitution. Distribution codes: SCA, Caribbean and/or South America; NCA, Gulf of Mexico and North America; WA, North to South America; EP, Eastern Pacific; IP, Indo–Western Pacific.

naeus. Within the genus *Farfantepenaeus,* a lack of resolution resulted in a polytomy among *F. brasiliensis, F. dourarum–F. notialis,* and the remaining species. However, the group formed by *F. aztecus, F. paulensis, F. subtilis,* and morphotype II was supported. Within the genus *Litopenaeus,* the data did not provide enough information to resolve the relationships between the Pacific and the Atlantic species, but *L. schimitti* and *L. setiferus* clearly form a monophyletic group.

DISCUSSION

Morphotype II

In the western Atlantic, six species of *Farfantepenaeus* and two species of *Litopenaeus* have been described. There are an additional two and three species, respectively, in the eastern Pacific. Both *Farfantepenaeus* and *Litopenaeus* are endemic to the Americas, except for *F. notialis,* which occurs on the Atlantic coast of Africa, and no species occur on both sides of the Americas (Dall *et al.,* 1990; Perez-Farfante and Kensley, 1997). During the present study the genetic relationships among all western Atlantic species, including morphotype II, were evaluated. An allozyme study was previously carried out on *F. paulensis, F. subtilis,* and morphotype II from Brazil by D'Incao *et al.* (1998). Forty samples of pink shrimp consisting of 21 genuine *F. subtilis* and 19 of a second, reproductively isolated group designated as morphotype II were analyzed from Fortaleza (4°S, Ceará, Brazil). Morphotype II possessed adrostral and dorsolateral sulci that resembled *F. paulensis* (F. D'Incao, unpublished observations). Across the 18 allozyme loci studied, morphotype II was found to be genetically more similar to *F. paulensis* (Nei's 1978 genetic identity, I = 0.985) than to *F. subtilis* (I = 0.947). In addition, morphotype II shared no alleles with *F. subtilis* at a polymorphic phosphogluconate dehydrogenase (enzyme number 1.1.1.44, IUB, 1984) locus (D'Incao *et al.,* 1998). The presence of a diagnostic locus in sympatric populations is a strong indication of reproductive isolation.

The 16S mitochondrial sequence data in the present study also support the specific status of morphotype II. This putative species showed little sequence diversity across a wide geographical range among samples from Vitória (20°S, Espírito Santo, Brazil), Recife (8°S, Pernambuco, Brazil), and Fortaleza (4°S; refer to Fig. 1), as well as fixed sequence differences from the remaining species of *Farfantepenaeus* in the present study. Morphotype II presents a level of genetic divergence from the most closely related species (0.04–0.06) which is comparable or higher than that between other well-characterized species in this group (Table 2). The 16S mitochondrial gene is considered to be relatively conserved and estimates of divergence of 4–6% have been recorded among species of the same genera for other arthropoda (Simon *et al.,* 1994). However, in contrast to the previous findings with allozyme studies (D'Incao *et al.,* 1998), the 16S sequences of morphotype II are more similar to *F. subtilis* than to *F. paulensis.* The sympatric distribution and level of genetic divergence between morphotype II and both *F. subtilis* and *F. paulensis* indicate that morphotype II is reproductively isolated from these species. Furthermore, the genetic divergence of morphotype II from the remaining western Atlantic species suggests that this animal is an undescribed species of *Farfantepenaeus.*

Alternatively, this organism could be (1) an occasional hybrid or (2) an introduced exotic species. If it is an occasional hybrid, it would have to be assumed that an extensive and continuous hybridization process was taking place between species (hybrid swarms—see Gardner, 1997). This is not supported by molecular evidence, as maternal inheritance of mitochondrial DNA would produce sequence agreement with other *Farfantepenaeus* species. As for the second hypothesis, there are no records of any attempt to culture exotic *Farfantepenaeus* in northeastern Brazil, but the introduction of exotic larvae in tanker ballast water cannot be ruled out (e.g., Carlton, 1985). Finally, Perez-

Farfante (1967, 1969) had already recorded the presence of a different morphotype of *P. subtilis* across its range of distribution, but attributed this to environmental influences producing phenotypic variation.

Phylogenetic Reconstruction

According to the review by Dall *et al.* (1990), the Family Penaeidae is assumed to have had an Indo–West Pacific origin during the late Triassic, with the earliest fossil record of *Penaeus* from the Jurassic, making this group the oldest taxon among the Penaeidae. Schram (1982) also supports a Triassic origin of family Penaeidae. Together, these studies suggest that the extant Penaeidae form a monophyletic group, with a *Penaeus* shrimp as a common ancestor. Other groups may also be considered as ancestral to the Penaeidae but there is no fossil evidence to support this (Burkenroad, 1983). However, it must be noted that the fossil record for crustaceans is poor, especially for those with poorly calcified, soft exoskeletons (Schram, 1982).

Based on allozyme and zoogeographic data, Dall *et al.* (1990) suggest that most penaeid genera must have arisen in the last 20 My and that a large number of *Penaeus* species may have originated less than 2 My BP. Sequence data from the COI gene support the hypothesis of Dall *et al.* (1990) that the genus *Penaeus* arose in the Indo Pacific, based on taxa from this area showing the deepest mitochondrial DNA lineages and the highest mitochondrial DNA diversity (Baldwin *et al.*, 1998). COI data also support the radiation of *Penaeus* westward into the eastern Atlantic and eastward into the eastern Pacific and western Atlantic. The final elevation of the Central America isthmus, dated to between 3.1 and 3.5 My BP (Kennett, 1982) created a barrier to gene flow between eastern Pacific and western Atlantic populations and probably led to speciation.

Phylogenetic reconstruction by Baldwin *et al.* (1998), failed to support the revision of the genus *Penaeus* by Perez-Farfante and Kensley (1997). This was largely because the genera *Farfantepenaeus* and *Litopenaeus*, separated on the basis of thelycum condition, were not supported as monophyletic groups by the phylogeny based on COI sequences. However, the monophyletic status of these genera is well supported in the present study by the 16S mitochondrial DNA sequence data from the western Atlantic and the two eastern Pacific species. The phylogenetic reconstructions based on 16S sequences (Fig. 2) clearly agree on this point and support the revision of the genus *Penaeus* by Perez-Farfante and Kensley (1997). They also agree in other ways with this morphological classification. For example, western Pacific *P. monodon* fall outside the group formed by *Farfantepenaeus* and *Litopenaeus* (Fig. 2). Such agreement seems to extend to the level of specific relationships: Perez-Farfante (1969) points to close morphological similarity between *L. setiferus* and *L. schmitti* (considered a single species until Burkenroad, 1936), between *F. duorarum* and *F. notialis*, and among *F. aztecus*, *F. subtilis*, and *F. paulensis* (refer to Fig. 2). Mulley and Latter (1980) and Tam and Chu (1993) have previously presented allozyme data that are in agreement with other subdivisions of *Penaeus* proposed by Perez-Farfante and Kensley (1997).

Relationships within the genus *Farfantepenaeus* are neither clear nor well supported. Our findings agree with those of Baldwin *et al.* (1998) that *F. paulensis* appears to have arisen after divergence of *F. brasiliensis* and *F. duorarum*, but show a lack of resolution regarding *F. brasiliensis* and *F. duorarum–F. notialis*. The monophyly of *F. duorarum* and *F. notialis* is strongly supported. The similarity among the sequences of these two species is so high that specific status could not be supported solely on the basis of the 16S gene. Finally, it appears that *F. aztecus*, *F. paulensis*, *F. subtilis*, and morphotype II have some support as a monophyletic group. However, the poor bootstrap support may suggest a recent radiation involving these species.

Evolution of Brazilian Farfantepenaeus

The amplitude and frequency of climatic change increased from the late Pliocene, reaching a maximum throughout the Quaternary, when many glacial events caused large sea level oscillations (e.g., Kennett, 1982). Therefore, the assumption made by Dall *et al.* (1990), that lowered sea level must have had a significant effect in increasing separation among populations of the shallow-water penaeids, seems plausible. Analyzing Caribbean fossil coral reefs, Fairbanks (1989) demonstrated that around 18,000 years BP, during the last glacial maximum, the sea level in the western Atlantic was approximately 120 m below current levels. On the Brazilian coast the narrow and steeply sloping northeastern platform is likely to have been largely above sea level during much of the Quaternary. This may have intensified the isolation of the coastal populations between the north and the south coasts of Brazil (refer to Fig. 1). In addition, in this coastal region the South Equatorial Current splits into two arms, one flowing northward, forming the North Brazil Current, and another flowing southward, forming the Brazilian Current (Pickard and Emery, 1982; see Fig. 1). This pattern was probably already established in the Miocene (Kennett, 1982; Hodell and Kennett, 1985). Sea level variations coupled with the ocean circulation pattern may be sufficient to explain the isolation and speciation of the southern *F. paulensis*. *F. paulensis* is a species adapted to colder water and to lower levels of salinity (Perez-Farfante, 1969) and therefore it may have originated as an isolated southern population during the late Pliocene. Radiation in western Atlantic

Farfantepenaeus, as suggested by 16S phylogeny, could have been a consequence of periodic habitat fragmentation in the last 2–3 million years.

CONCLUSIONS

Phylogenetic conclusions based on one or even a few mitochondrial genes may be misleading (Cummings *et al.*, 1995). In contrast with COI data presented by Baldwin *et al.* (1998), phylogenetic reconstruction based on 16S partial sequencing in the present paper supports the monophyletic status of the genera *Farfantepenaeus* and *Litopenaeus*, as described by Perez-Farfante and Kensley (1997), at least among western Atlantic species. The revision of the taxonomy of the genus *Penaeus* by Perez-Farfante and Kensley (1997) is also supported in other ways by 16S data. However, agreement between 16S and COI data is clear in some cases, such as where both genes suggest a more recent origin of *F. paulensis* than *F. duorarum* and *F. brasiliensis*. Finally, a previously undescribed species of the genus *Farfantepenaeus* has been identified.

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