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Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence

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Abstract Mud crabs of the family Panopeidae are common organisms in coastal soft-bottom, vegetated, rubble, and oyster-bed communities along the temperate and tropical coastlines of the American continent. Similar morphology among many species renders their distinction and classification difficult. Here, we present phylogenies of western Atlantic Panopeidae based on DNA sequences of the mitochondrial large subunit rRNA (16S; 529 basepairs) and cytochrome oxidase I (COI; 640 basepairs) genes. Results suggest that the speciose genera *Panopeus* and *Eurypanopeus* are not monophyletic and that their taxonomy does not accurately reflect evolutionary partitions. In two cases (*P. herbstii* complex and *E. depressus* and allies), the molecular findings strongly support sister-species relationships that differ from previous morphology-based assumptions. We suggest that convergence or morphological stasis are responsible for the phenotypic similarities between divergent evolutionary lineages.

Introduction

Crabs of the family Panopeidae Ortmann, 1893 are among the most abundant and conspicuous invertebrates of marine intertidal and shallow subtidal habitats

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of the northwestern Atlantic. They construct burrow systems in the soft sediment of grassbeds, marshes, and mangroves, or live in oyster beds and among rock or shell rubble. Different species can be encountered in hypersaline lagoons, oligohaline estuaries, and freshwater habitats. Several panopeid crabs are important predators of barnacles, clams, oysters, and their boring parasites (Guida 1976), thereby playing an obvious role in shaping the structure of shallow marine communities. Nevertheless, the biology of most of the species is poorly known (but see McDonald 1982), and in many ecological studies they are often lumped into categories such as “Xanthidae”, “Panopeidae”, or “*Panopeus* sp.”. This is attributable to the “extreme morphological similarity” (Martin and Abele 1986: p. 182) that exists among many of the species.

In ecological or taxonomic studies, panopeid crabs are often included within the family Xanthidae Macleay, 1838 (sometimes as the subfamily Panopeinae). Guinot (1978) studied several morphological characters of the Xanthidae sensu lato, and concluded that eight different families should be recognized, which she placed within the newly erected superfamily Xanthoidea. This taxonomy has been adopted by some crustacean workers, and is gaining acceptance (Martin and Davis unpublished MS). Recent molecular work suggests that the xanthoid crabs are polyphyletic (Schubart et al. 2000). Therefore Guinot’s taxonomy, and the recognition of Panopeidae and other families, seems preferable to the traditional inclusion of all xanthoid crabs within a single family.

Martin and Abele (1986) pointed out that there is a lack of established and consistent criteria upon which generic distinctions of xanthoid crabs are based. Differences in carapace and cheliped morphology are often vague. Furthermore, they may reflect convergent adaptations to particular habitats and food sources. The result of these uncertainties has been a repeated shifting of some species among several genera. Martin and Abele argued for the increased emphasis on male pleopod morphology to establish phylogenetic relationships

among xanthoid crabs. They concluded (p. 195) that “if pleopod morphology is accepted as a strong generic character within the Panopeidae, then several genera within the panopeid group should be synonymized with genera acknowledged at present”. This is true not only for the genera *Eurypanopeus*, *Eurytium* and *Neopanope*, but also for some species presently placed within the Goneplacidae that could be placed in the genus *Panopeus* if pleopod morphology only were to be considered (Martin and Abele 1986).

Larval and postlarval stages provide additional morphological characters for resolving phylogenetic relationships (Rice 1980; Martin 1988). Unfortunately, descriptions of these stages are available for only a few xanthoid species. The morphology of the antennal exopod and larval mouthparts was used to split the Xanthoidea into 4 to 6 groups (Rice 1980; Martin 1984; Martin et al. 1985). However, within the Panopeidae, larval morphology varies little (with a few exceptions such as *Rhithropanopeus* or *Panopeus bermudensis*), and often does not allow ready separation of larvae within or among genera (e.g. *Panopeus* and *Eurytium*: Martin et al. 1985, 1998; Rodríguez and Paula 1993).

Apparently, morphological convergences in response to similar life forms, as well as possible morphological stasis in key characters (pleopods and larvae), have inhibited the resolution of phylogenetic relationships within the Panopeidae. In the present study, we used mitochondrial, large-subunit rRNA (16S) and cyto-

chrome oxidase I (COI) gene-sequences as molecular characters for inference of phylogenetic relationships among northwestern Atlantic Panopeidae. Our results provide some support for the current generic subdivision, but also indicate that major revisions are necessary to the taxonomy of the genera *Panopeus* and *Eurypanopeus*, changes that are in part concordant with recent morphological findings.

Materials and methods

All panopeid genera (excluding *Micropanope*) from the northwestern Atlantic were collected and identified by D. Felder and C. Schubart between 1997 and 1999 along the North American coast between South Carolina and Texas. In addition, ethanol-preserved material of *Eurypanopeus crenatus* collected in Peru in 1935 was obtained from the Natural History Museum of Los Angeles County. This species was included in our analysis, because it represents the type species of the genus *Eurypanopeus*. All specimens from which DNA for this study was obtained (Table 1) are archived as ethanol-preserved vouchers. For most species, DNA of more than one specimen has been sequenced in order to confirm the results obtained and to test for population differences. For this phylogenetic study, however, only single haplotypes were used. Previously unpublished sequences were submitted to the European Molecular Biology Laboratory (EMBL) database (Accession Nos.: AJ274680 to AJ274704).

Specimens used for DNA analysis were preserved in 75 to 90% ethanol. For extraction of DNA, amplification and sequencing of the 16S_r-br region, the protocol of Schubart et al. (2000) was followed. In two cases (*Dyspanopeus texanus* and *Eurypanopeus*

Table 1 Xanthoid crab species (not including outgroups) used for phylogeny reconstructions, with locality and date of collection, museum catalog number, and genetic database (EMBL) accession

numbers (LACM Natural History Museum of Los Angeles County, Los Angeles; ULLZ University of Louisiana Lafayette Zoological Collection, Lafayette)

Species	Collection site, date	Catalogue No.	EMBL # (16S & COI)
Panopeidae Ortmann, 1893			
<i>Dyspanopeus sayi</i> (Smith, 1869)	Florida: Fort Pierce, 1998	ULLZ 3752	AJ274694
<i>Dyspanopeus texanus</i> (Stimpson, 1859)	Texas: Mustang Island, 1997	ULLZ 3871	AJ274695
<i>Eurypanopeus crenatus</i> (H. Milne Edw. & Lucas, 1844)	Peru: Independence Bay, 1935	LACM CR 1935023313	AJ274685
<i>Eurypanopeus abbreviatus</i> (Stimpson, 1860)	Florida: Fort Pierce, 1998	ULLZ 3753	AJ274686 & AJ274700
<i>Eurypanopeus abbreviatus ater</i> Rathbun, 1930	Texas: South Padre Island, 1998	ULLZ 3892	AJ274687 & AJ274701
<i>Eurypanopeus depressus</i> (Smith, 1869)	Louisiana: Grande Isle, 1997	ULLZ 3785	AJ274688 & AJ274702
<i>Eurypanopeus dissimilis</i> (Benedict & Rathbun, 1891)	Florida: Fort Pierce, 1997	ULLZ 3821	AJ274689 & AJ274703
<i>Eurypanopeus turgidus</i> (Rathbun, 1930)	Texas: Mustang Island, 1997	ULLZ 3872	AJ274690 & AJ274704
<i>Eurytium limosum</i> (Say, 1818)	Louisiana: Grande Isle, 1997	ULLZ 3781	AJ274696
<i>Hexapanopeus angustifrons</i> (Benedict & Rathbun, 1891)	Florida: Capron Shoals, 1998	ULLZ 3771	AJ274691
<i>Hexapanopeus paulensis</i> Rathbun, 1930	Texas: South Padre Island, 1998	ULLZ 3891	AJ274692
<i>Neopanope packardii</i> (Kingsley, 1879)	Florida: Fort Pierce, 1998	ULLZ 3772	AJ274693
<i>Panopeus americanus</i> de Saussure, 1857	Florida: Fort Pierce, 1998	ULLZ 3743	AJ274683
<i>Panopeus bermudensis</i> Benedict & Rathbun, 1891	Florida: Fort Pierce, 1998	ULLZ 3751	AJ274684
<i>Panopeus lacustris</i> Desbonne, 1867	Florida: Fort Pierce, 1997	ULLZ 3817	AJ274681
<i>Panopeus herbstii</i> H. Milne Edwards, 1834	South Carolina: Charleston, 1997	ULLZ 3778	AJ130815 & AJ274699
<i>Panopeus obesus</i> Smith, 1869	Texas: Port Lavaca, 1997	ULLZ 3880	AJ274680
<i>Panopeus occidentalis</i> de Saussure, 1857	Florida: Fort Pierce, 1997	ULLZ 3820	AJ274682
<i>Panopeus simpsoni</i> Rathbun, 1930	Louisiana: Grande Isle, 1997	ULLZ 3783	AJ274679
<i>Rhithropanopeus harrisi</i> (Gould, 1841)	Delaware: Woodland Beach, 1994	ULLZ 3836	AJ274697
Xanthidae Dana, 1851			
<i>Cataleptodius floridanus</i> (Gibbes, 1850)	Florida: Fort Pierce, 1998	ULLZ 3744	AJ274698
<i>Xantho poressa</i> (Olivier, 1792)	Spain: Cádiz, 1998	ULLZ 3808	AJ130814

crenatus), internal primers originally designed for grapsoid crabs, 5'-GACGATAAGACCCTATAAAGCTT-3' and 5'-TTATCRC-CCCAATAAAATA-3' (16L15 and 16H16, respectively) were used to obtain the complete 16Sar-br sequence. For the COI gene, the primer combination COIa and COIf (see Schubart et al. 1998) was applied in combination with the following PCR profile: 38 cycles with 1 min at 94 °C for denaturing, 1 min at 52 to 55 °C for annealing, and 2 min at 72 °C for extension. All sequences used for this analysis were confirmed by sequencing of both strands. Sequences were aligned manually with the multisequence editing program ESEE (Cabot and Beckenbach 1989), with special consideration of the secondary structure of the gene (Schneider-Broussard and Neigel 1997). For phylogenetic analyses, the complete 16Sar-br and COIa-f regions were used. For the latter gene, however, only a few species could be compared, since the primers did not work consistently. The following 16S sequences from molecular databases were included (Accession Nos. in parentheses): *Panopeus herbstii* (AJ130815), *Xantho poressa* (AJ130814), *Trapezia cymodoce* (AJ130816), and *Menippe mercenaria* (U20737), the latter two sequences being used as outgroups. Here it has to be noted that the database sequence U75270 originally assigned to *P. herbstii* from North Carolina was found to correspond closely to our sequence of *Dyspanopeus sayi*, and does not resemble any other known *Panopeus* sequence. We therefore assume an identification mistake (both species occur in North Carolina). Wherever in Geller et al. (1997) reference is made to *P. herbstii*, we suggest it should read *D. sayi*. The program MEGA (Kumar et al. 1993) was used to calculate Kimura two-parameter distance-estimates of sequence divergence and to infer phylogenetic relationships by neighbor-joining (NJ) (Saitou and Nei 1987). Statistical significance of groups within inferred trees was evaluated by the interior branch method (Rzhetsky and Nei 1992) and by bootstrapping the maximum parsimony (MP) analysis with 2000 replicates using the program PAUP (heuristic search with random sequence addition and tree-bisection and reconnection as branch-swapping option) (Swofford 1993). For the MP analysis, transversions were weighted three times greater than transitions and gaps were omitted. Molecular clock estimates for *Eurypanopeus* were based on the rates calculated for grapsoid crabs in Schubart et al. (1998).

Results

The complete alignment of the 16Sar-br region for 25 xanthoid species consisted of 529 positions (not including the primer regions), while the COIa-f region comprised 640 nucleotides, but was used for only six species. Of all the positions, 193 were variable and 119 parsimony-informative for 16S; 140 were variable and 92 parsimony-informative for COI.

For the 16S phylogeny, neighbor joining (NJ) and maximum parsimony (MP) methods rendered similar tree topologies, that differed only in the number of unresolved nodes and in one split within the main *Panopeus* group (see first subsection of "Discussion"). The consensus topology for the two methods (Fig. 1) shows confidence values (only > 50%) of the interior branch method in the NJ analysis and bootstrap values (only > 50%) of a branch and bound heuristic search with MP. With the latter method, we obtained one most parsimonious consensus tree of the length 894, with consistency index (CI) = 0.554 and retention index (RI) = 0.464. Several sister-group relationships and monophyletic relationships are strongly supported (90 to 100% confidence level) by DNA sequences of this mitochondrial gene: (1) all Panopeidae and Xanthidae in

comparison to the xanthoid outgroups *Trapezia cymodoce* and *Menippe mercenaria*; (2) all Panopeidae except *Panopeus bermudensis* (only NJ); (3) *P. herbstii*, *P. simpsoni*, and *P. obesus*; (4) the latter three plus *P. occidentalis* and *P. lacustris*; (5) *Hexapanopeus angustifrons* and *H. paulensis*; (6) *Eurypanopeus abbreviatus* and *E. abbreviatus ater*; (7) *Dyspanopeus sayi* and *D. texanus*; (8) the latter two plus *Neopanopeus packardii*; (9) *E. depressus* and *E. turgidus*.

Phylogenetic relationships inferred from COI sequence data within the genus *Eurypanopeus* (Fig. 2) were consistent with those inferred by 16S sequence data. The tree represents the consensus topology for the two methods and shows confidence values of the interior branch method in the NJ analysis and bootstrap values of a branch-and-bound heuristic search with MP. With the latter method, we obtained one most parsimonious consensus tree of the length 254, with CI = 0.878 and RI = 0.828. Sister-group relationships between *E. abbreviatus* and *E. abbreviatus ater*, as well as for *E. depressus* and *E. turgidus*, were thereby strongly supported.

Discussion

The intention of this study was to help resolve phylogenetic relationships within the Panopeidae as well as to examine the possible role of convergence and morphological stasis in systematic groups that have been defined on the basis of a few adult morphological characters. We address biogeographic questions by testing for several species (or subspecies) the likelihood and degree of endemism within the Gulf of Mexico. These topics are considered within the framework of a taxonomic revision of some of the more important panopeid groupings from the northwestern Atlantic.

Genus *Panopeus* H. Milne Edwards, 1834

The type of this genus, *Panopeus herbstii*, was subdivided by Rathbun (1930) into four forms (forma *typica*, *simpsoni*, *obesa*, and *crassa*) with partly overlapping geographical distributions. Turner and Lyerla (1980) noted allozymic differences between the forma *simpsoni* and forma *typica* from South Carolina. Based on morphometric, ecological, and hemocyanin electrophoretic differences (Reames and Williams 1984; Sullivan et al. 1984), Williams (1984) raised these forms to full species (*P. herbstii*, *P. simpsoni*, *P. obesus*, and *P. lacustris*) and described two additional species from the southwestern Atlantic as belonging to this complex (*P. austrobesus*, *P. meridionalis*). He considered *P. simpsoni* to be endemic to the Gulf of Mexico and, based on claw morphology, placed *P. herbstii* and *P. simpsoni* into one group and *P. obesus* and *P. lacustris* into another. This clearly corresponds to the observed color pattern of

Fig. 1 Phylogenetic relationships among panopeid (and xanthid) crab species from the northwestern Atlantic, inferred from 529 basepairs of 16S rRNA gene. Tree topology represents consensus of neighbor-joining and maximum-parsimony analyses. Confidence values are from internal node test (*upper values*) and 2000 bootstrap maximum-parsimony analysis (*lower italicized values*). Only values above 50 are presented (*T* type species of all panopeid genera)

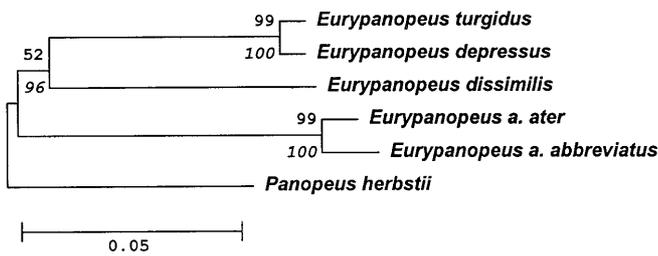
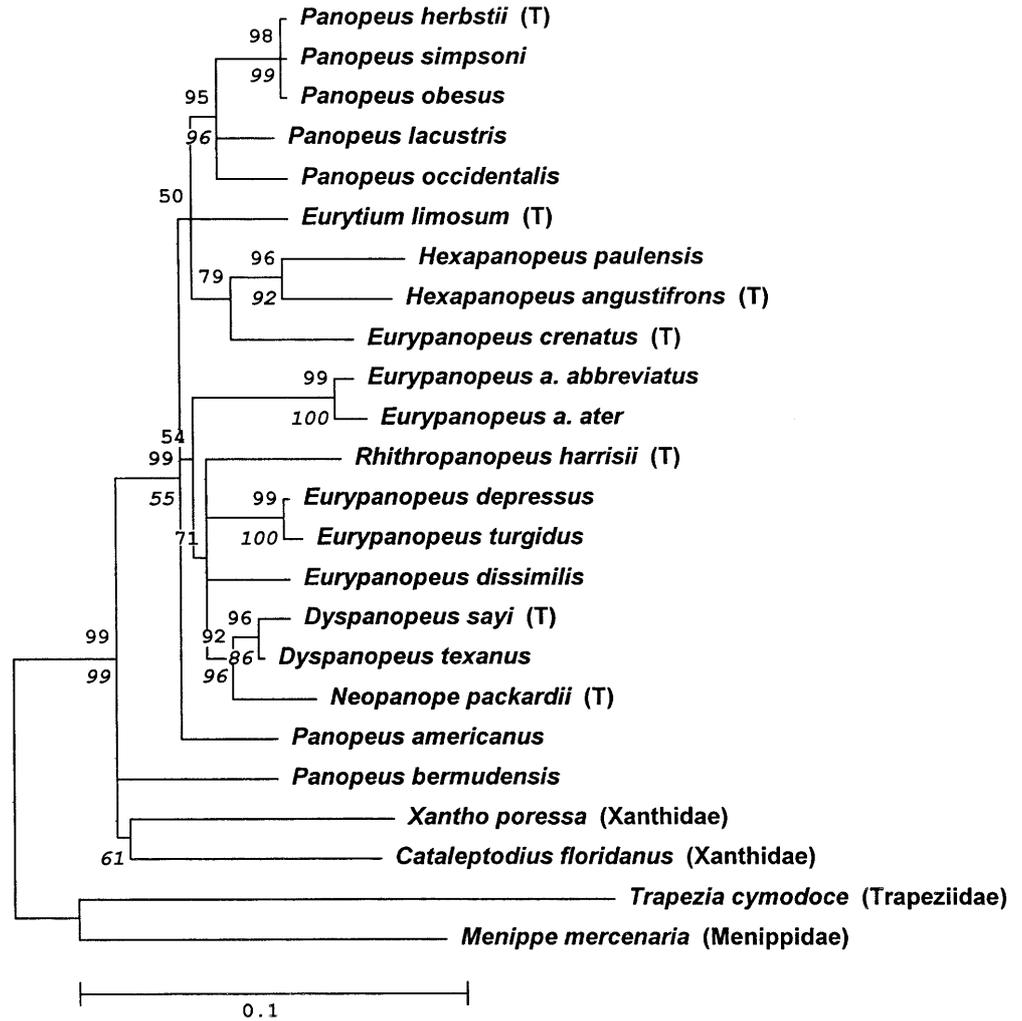


Fig. 2 *Eurypanopeus* spp. Phylogenetic relationships among several species from northwestern Atlantic, inferred from 640 basepairs of COI gene. Tree topology represents consensus of neighbor-joining and maximum-parsimony analyses. Confidence values are from internal node test (*upper values*) and 2000 bootstrap maximum-parsimony analysis (*lower italicized values*)

carapace and chelae with a cream to light gray color predominating in *P. herbstii* and *P. simpsoni*, while *P. obesus* and *P. lacustris* are dark purple to russet (Williams 1984; Reames and Williams 1984; Schubart et al. own personal observations). However, our molecular phylogeny clearly suggests that the three temperate species *P. herbstii*, *P. simpsoni*, and *P. obesus*

are very closely related to each other, while the tropical *P. lacustris* represents an outgroup to all of them (Fig. 1). The observed 16S sequence divergence between the first three species does not allow distinction of *P. herbstii* from *P. simpsoni*, while *P. obesus* shows only one diagnostic difference from the latter two in a comparison of sequences from 14 individuals (Schubart et al. unpublished data). On the other hand, *P. lacustris* differs in 16 diagnostic positions (in a comparison of 8 individuals) from *P. obesus*. There is indirect support for this phylogenetic relationship provided by the COI gene, in so far as the primers easily amplified the three temperate species, but failed to do so for several specimens of *P. lacustris*. Are similarities in color and claw morphology between *P. obesus* and *P. lacustris* thus based on convergence? This is at least a possibility, when we consider that both species often live in dark muddy to coarse substrates between marsh or mangrove roots or under adjacent rubble, and probably feed on the available motile prey (i.e. smaller crustaceans and snails). In contrast, *P. herbstii* and *P. simpsoni* are common among lightly colored oysters and their claws are probably adapted to this sessile hard-shelled food source. In

feeding experiments, Reames and Williams (1984) showed that *P. obesus* was able to chip *Littorina* snails open, while *P. simpsoni* was unable to do so. It is also possible that the generalists *P. obesus* and *P. lacustris* represent the conserved morph, while the specialized *P. herbstii* and *P. simpsoni* (whether one or two species) exhibit derived conditions.

The molecular phylogeny also places the tropical species *Panopeus occidentalis* very close (genetic distance, $d = 0.022$ to 0.026 , see Table 3) to the *P. herbstii* complex, and the five species form a strongly supported monophyletic unit. This was the only case in which the NJ and MP topologies differed (Fig. 3): NJ places *P. occidentalis* closer to *P. herbstii*, *P. simpsoni*, and *P. obesus* (89%), while MP suggests that *P. lacustris* is the sister group of the latter (58%). The most obvious morphological difference of *P. occidentalis* from the remaining species is a consistently deep distal groove in the chelar carpus.

Two other analyzed species of *Panopeus* were genetically more distinct. While the position of *P. americanus* on the tree remains unresolved, there is obvious indication that *P. bermudensis* not only differs from other members of the genus *Panopeus*, but constitutes an outgroup to all studied Panopeidae (Fig. 1). This confirms results of previous studies that described divergent larval (Martin et al. 1985) and adult (Felder and Manning unpublished data) morphology, justifying placement of this species within a new genus.

Genera *Neopanope* A. Milne Edwards, 1880
and *Dyspanopeus* Martin and Abele, 1986

Martin and Abele (1986) erected the genus *Dyspanopeus* to separate the species *D. sayi* and the Gulf of Mexico endemic *D. texanus* (subspecies previous to separation by Abele 1972) from *N. packardii*. This seemed necessary because of the very distinct morphology of the male pleopods. While the pleopod of *N. packardii* shows little variation from the typical panopeid form, and in a “pleopod taxonomy” would argue for a placement of the species within *Panopeus* (see Martin and Abele 1986), the pleopods of *Dyspanopeus* are quite distinct and unique within the Panopeidae. However, the molecular phylogeny places these two genera closely together, corroborating the taxonomic grouping previous to study of the gonopods (Rathbun 1898).

Genus *Eurypanopeus* A. Milne Edwards, 1880

Morphologically, the genus *Eurypanopeus* usually differs from *Panopeus* by its broader, more depressed, and smoother carapace, with a less advanced front (Rathbun 1930). However, these vague definitions have caused repeated confusion in the literature: Benedict and Rathbun (1891) did not accept the genus *Eurypanopeus* (nor *Eurytium*), and included all representatives within

the genus *Panopeus*. Subsequent to Rathbun’s (1897) revalidation of *Eurypanopeus*, the generic name has been in use, but uncertainty remained about the species to be included (Martin and Abele 1986). The type species of the genus is the southeastern Pacific *E. crenatus*. We included this species in our molecular comparison, only to discover that among the northwestern Atlantic Panopeidae it seems to be closest to two *Hexapanopeus* species and not to any of the species of *Eurypanopeus* (Fig. 1). If this result is corroborated in the future, the logical taxonomic consequence would be the reclassification of all northwestern Atlantic *Eurypanopeus* into other genera.

For any upcoming classification, we suggest that *Eurypanopeus depressus* and *E. turgidus* should be considered congeneric on the basis of their striking genetic similarity (Figs. 1 and 2). On the basis of adult morphological and ecological similarity, *E. depressus* was always thought to be the sister-species of *E. dissimilis* (see Rathbun 1930). On the other hand, *E. turgidus*, an endemic species of the western Gulf of Mexico, is most often considered a member of the genus *Panopeus*, despite the fact that gonopodal morphology suggests close alliance to *Eurypanopeus* (see: Powers 1977; Martin and Abele 1986; Abele and Kim 1986). The present results from two mitochondrial genes (Table 2) support a very recent divergence [0.6 to 0.7 million years ago (mya)] between *E. turgidus* and the widely distributed *E. depressus*, while they distance *E. dissimilis* from both of these species (5.5 to 7.7 mya divergence). Are morphological similarities in form and color between *E. dissimilis* and *E. depressus* thus based on convergence? Both species occur in the same habitat, the interstices within oyster clumps. In tropical southern Florida they are usually found sympatrically, but there is as yet no evidence for ecological separation that allows them to coexist (see Blyler 1987 for larvae). The alternative to a convergence hypothesis would be one of morphological stasis that could have characterized *E. dissimilis* and *E. depressus* over the last few million years and that can be explained by the stable environment provided by oyster clumps. In contrast, the sister species *E. turgidus* and *E. depressus* appear to constitute an interesting example of rapid morphological change following recent speciation.

Table 2 *Eurypanopeus* spp. Kimura two-parameter distances (upper values) and estimated million years since separation (lower values) between three panopeid species of the genus *Eurypanopeus*, based on sequences of two mitochondrial DNA genes: 16S rRNA (above diagonal) and COI (below diagonal)

	<i>E. turgidus</i>	<i>E. depressus</i>	<i>E. dissimilis</i>
<i>E. turgidus</i>		0.004 0.61	0.041 6.23
<i>E. depressus</i>	0.011 0.66		0.036 5.45
<i>E. dissimilis</i>	0.121 7.29	0.127 7.65	

Table 3 Pairwise Kimura two-parameter distance between 20 species of Panopeidae, two Xanthidae and two outgroups (1: *Panopeus herbstii*; 2: *Panopeus simpsoni*; 3: *Panopeus oboeus*; 4: *Panopeus lacustris*; 5: *Panopeus occidentalis*; 6: *Panopeus americanus*; 7: *Eurypanopeus abbreviatus*; 8: *Eurypanopeus abbreviatus abbreviatus*; 9: *Eurypanopeus abbreviatus ater*; 10: *Eurytium limosum*; 11: *Hexapanopeus angustifrons*; 12: *Hexapanopeus paulensis*; 13: *Rhithropanopeus harrisi*; 14: *Eurypanopeus turgidus*; 15: *Eurypanopeus depressus*; 16: *Eurypanopeus dissimilis*; 17: *Neopanope packardii*; 18: *Dyspanopeus texanus*; 19: *Dyspanopeus sayi*; 20: *Panopeus bermudensis*; 21: *Cataleptodius floridanus*; 22: *Xantho porressa*; 23: *Trapezia cymodoec*; 24: *Menippe mercenaria*)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1	0.000																								
2		0.002																							
3			0.002																						
4				0.034																					
5					0.026																				
6						0.055																			
7							0.053																		
8								0.086																	
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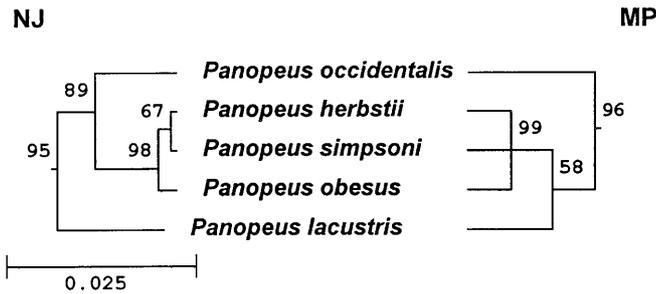


Fig. 3 *Panopeus* spp. phylogenetic relationships among five species from northwestern Atlantic, inferred from 529 basepairs of 16S rRNA gene and differences found between neighbor-joining (NJ) and maximum-parsimony (MP) analyses. Confidence values are from internal node test (NJ) and from 2000 bootstrap maximum-parsimony analysis (MP)

Another case of recent divergence and endemism in the Gulf of Mexico is shown by the two subspecies of the tropical crab *Eurypanopeus abbreviatus* (*E. abbreviatus abbreviatus* and *E. abbreviatus ater*). These subspecies are isolated by the temperate northern Gulf of Mexico, and clear genetic differences ($d = 0.01$ for 16S and $d = 0.021$ for COI) were present for both mitochondrial genes. Genetic distances between these subspecies are greater than those between *Dyspanopeus texanus* and *D. sayi* or between *E. turgidus* and *E. depressus* (Table 3). Since morphological differences have also been reported (Rathbun 1930; Felder unpublished data), *E. ater* should probably be considered a valid endemic species of the Gulf of Mexico.

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