ELSEVIER

Journal of Experimental Marine Biology and Ecology, 203 (1996) 117–131

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Molecular data and biogeography: resolution of a controversy over evolutionary history of a pan-tropical group of invertebrates[1]

Jeffrey Levinton[a,*], Christian Sturmbauer[b], John Christy[c]

[a]*Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, USA*
[b]*Institute of Zoology, University of Innsbruck, A-6020 Innsbruck, Austria*
[c]*Smithsonian Tropical Research Institute, APO , Miami, FL 34002, USA*

Received 14 December 1995; accepted 27 February 1996

Abstract

Phylogenetic analyses of sequences of 16S rDNA suggest that the current hypothesis of the evolution of the pan-tropical fiddler crab genus *Uca* (Decapoda, Brachyura, Ocypodidae) is false. This hypothesis rests of the assumption that the increase of complexity of reproductive behavior, together with a tendency of a habitat shift towards the higher intertidal, arose only once in the history of the fiddler crabs. The origin of fiddler crabs was placed in the IndoPacific, which is widely thought to be a center of origin for several marine groups. There, relatively lower intertidal crabs were thought to give rise to a radiation in the Americas in which higher intertidal forms with more complex reproductive behavior evolved, and finally, derived crabs were thought to disperse back to the IndoPacific from the Americas. In contrast our phylogeny suggests that the ancestral group, which shows complex reproductive behavior, now resides wholly in the American-Atlantic region, as opposed to the postulated Indo-west-Pacific. Behavioral and ecological complexity must have arisen independently in the American and IndoPacific faunal regions. The pan-tropical subgenus *Celuca* seems to be polyphyletic, which suggests that the evolution of morphology, ecology and behavior involves convergence in geographically separated locales. This study highlights the dangers of postulating evolution from a center of origin, even if it fits data that can be assigned to an evolutionary trend.

Keywords: Biogeography; Phylogeny; Ocypodidae; Crustacea; Evolution; Behavior

*Corresponding author.

[1]Contribution number 970 from the Program in Ecology and Evolution, State University of New York at Stony Brook.

0022-0981/96/$15.00 Copyright © 1996 Elsevier Science B.V. All rights reserved
PII S0022-0981(96)02573-7

1. Introduction

Molecular data have been important in reviving the field of evolutionary biogeography, which is so filled with exciting unsolved problems, especially in the marine realm. The revival was inspired by the renaissance of phylogenetic reconstruction afforded by DNA sequencing and sophisticated algorithms of tree construction. In addition, it has been enabled by pioneering thinking relating systematics to biogeography (Rosen, 1975; Nelson and Platnick, 1981) and recent work that established an interface between molecular studies of geographic speciation and intraspecific–specific differentiation near coastal biogeographic barriers (Avise et al., 1987; Avise, 1992; Burton and Lee, 1994).

In the 19th century, the ichthyologist John Richardson recognized the Indo-Pacific as a distinct region (Kay, 1984), but Ekman (1953) was instrumental in defining it as a major biogeographic province, with many species widespread in their distribution from eastern Africa to Hawaii, and even the Pacific coast of Panama. The number of species of many groups reached a maximum in the western Pacific and this area was thought to be a center of origin, from which species originated and spread in all directions through the entire IndoPacific-Pacific realm (Darlington, 1957; den Hartog, 1970; Briggs, 1974; Mukai, 1993). Declines of species richness along current directions from the putative center favor a center of origin for seagrasses in the Indo-Malayan region (Mukai, 1993). Stehli and Wells (1971) found that coral genera in the outer reaches of the IndoPacific-Pacific realm were geologically older, whereas the so-called center of diversity contained these older genera as well as geologically much younger genera that added to the species richness. These ideas and data fit well with Darlington's (Darlington, 1957) notion of a center of origin for tropical terrestrial biotas in low latitude continental areas.

The center of origin notion implies a center where species and more inclusive taxonomic groups arise and then spread, perhaps aided by larval dispersal, across wide expanses of ocean. Presumably, more time would permit more dispersal and establishment of species. Without extinction at the periphery, or heightened speciation at the center, the diversity gradient could not be maintained. In the Pacific, periodically lowered stands of sea level during the Pleistocene may have allowed a much large number of steppingstone islands in the Pacific than are now present at a relatively high stand of sea level. Furthermore, the Pleistocene history in the IndoPacific of sea-level rises and falls may have created a great deal of instability, causing contractions, extinctions of species with small ranges, and expansions of surviving species' ranges and a reduction of geographic differentiation. This may explain in part the widespread species distributions that are commonly observed (Potts, 1983).

In recent years the center of origin theory has come under heavy criticism, principally by phylogenetic systematists, who argue that patterns of phylogenetic splitting have a geographic context. Under this argument, dispersal from a center of origin is less likely than a geographic structure where the phylogenetic splitting of a clade corresponds best to the establishment of geographic barriers, across which isolation and speciation occurs (McCoy and Heck, 1976; Kay, 1984). A cladistic analysis of the coral genera *Symphyllia* and *Coscinaracea* suggests that geographic structuring is far more prominent than previously supposed. Derived species showing strong endemism exist at the

periphery of IndoPacific-Pacific coral reef distributions, which argues against the center of origin–dispersalist hypothesis (Pandolfi, 1992). Indeed, it has been suggested that the relatively high species richness found in the western Pacific may relate to its location at the intersection of two species-rich biogeographic realms in the Indian Ocean and the Pacific proper (Kay, 1984), although the broad longitudinal range of many IndoPacific-Pacific species might argue against this. Ekman (1953) argued that the Indo-Malayan maximum of diversity might be a relict of reduced extinction in this area, relative to more peripheral areas where climatic deterioration occurred.

In order to understand the origin of such large-scale biogeographic patterns, we must understand the evolutionary history of groups with pan-tropical distribution. A phylogenetic analysis, combined with a biogeographic analysis and the analysis of behavioral and morphological characters, might address the question which abiotic and which biotic factors have triggered the spread of a group in question.

The crustacean family Ocypodidae, and in particular the fiddler crabs (genus *Uca*) represent such an ideal model case to test major phylogeographic trends. We take a molecular approach using associations among DNA sequences to address an evolutionary and biogeographic hypothesis (Crane, 1975) that suggests an evolutionary trend from primitive, behaviorally simple, and lower intertidal forms to more advanced, behaviorally complex and higher intertidal forms. Moreover, the ancestral fiddler crabs were believed to originate in the Indo-Malayan region, which is favored as an evolutionary center of origin for many groups of marine organisms.

2. Fiddler crabs and biogeographic hypotheses

Our knowledge of fiddler crab systematics and biogeography relies mainly on the monumental work of Crane (1975), who over several decades studied their behavior, morphology, and biogeography. *Uca* belongs to the pan-tropical family Ocypodidae, which includes both deposit-feeding and carnivorous intertidal species (Crane, 1975; Zimmer-Faust, 1987). All species of *Uca* are characterized by a strong sexual dimorphism; males have one extraordinarily large major cheliped that comprises nearly half the body weight. The claw is employed in combat, threats, and courtship (Crane, 1975; Christy and Salmon, 1984). Males wave the major cheliped rhythmically, which attracts females and some species also use this appendage to produce seismic signals during courtship. The minor cheliped is small and is similar in size and morphology to both claws of a like-sized female; it is used exclusively in feeding upon sediment.

Uca species (over 80 are now described; Salmon and Zucker, 1987) occur throughout the tropics although surely their greatest diversity is developed in the New World, on both the Pacific and Atlantic sides of Central America (Crane, 1975). At least 30 species are endemic to the Pacific Coasts of Central and northern South America. A considerable number of species are also found throughout the IndoPacific-Pacific region, but only one species is found in the eastern Atlantic. *U. tangeri* occurs on the west coast of Africa, from Angola to Morocco and also in Spain and Portugal.

Crane (1975) recognized 9 subgenera of fiddler crabs, but noted a dichotomy between so-called broad-front species (referring to the distance between the eye stalks) and

narrow-front species. Narrow-front species occur in the IndoPacific-Pacific, and to some degree in the Americas whereas broad-front species, belonging to the subgenera *Celuca* and *Minuca*, occur in the Americas. An important exception is the subgenus *Celuca*, a subgenus of broad-fronted species that is found both in the Americas and in the IndoPacific-Pacific region.

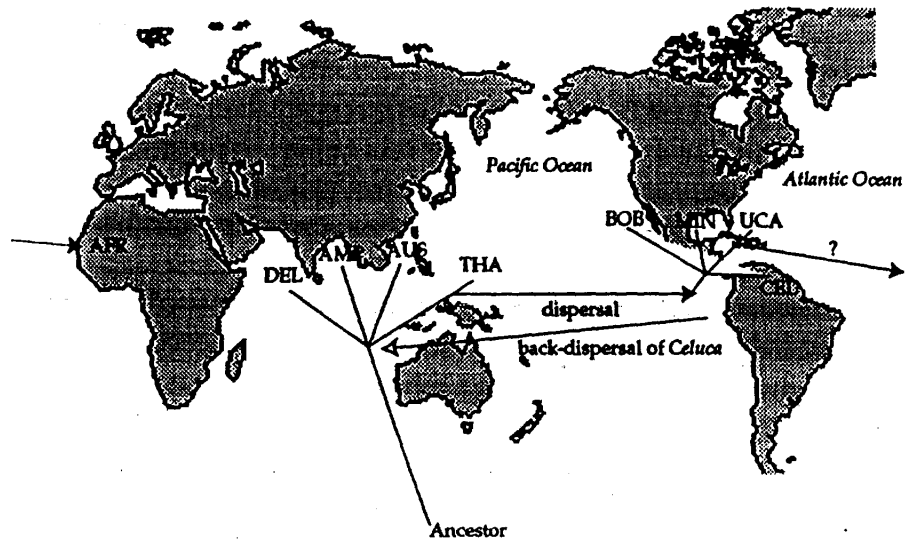
Crane's hypothesis explaining the evolution and biogeographic distribution of *Uca* depended heavily on her conception of a general dichotomy in behavior and morphology between IndoPacific and American species. With the exception of the subgenus *Celuca*, the IndoPacific species were thought to be ancestral, that is more allied with a putative ancestor, that was more marine (lower intertidal), less complex in reproductive behavior, and having more ancestral morphological features. Specifically, the IndoPacific species have a relatively simple vertical waving display that is used only in basic territoriality and inter-male relationships and little or not at all in courtship. The period spent waving is very limited relative to American species. Copulation was on the surface, or males might follow females down burrows (Murai et al., 1995). In most cases, the waving display is not associated with the approach of females to males.

By contrast, most American species have more complex waving patterns, and display and combat by males is closely associated with the defense of a burrow, within which copulation takes place. Following a waving display, the female approaches the male and may follow him into his burrow, where copulation takes place. Copulation is generally preceded by more complex mate recognition behavior and waving displays (Crane, 1975). In several species studied, after copulation the female ovulates and incubates in the male's burrow until she emerges to release her larvae. Waving displays in American species are often in dense aggregations of males, and in some species it has been demonstrated females appear to be more attracted to groups of males than to those waving singly (Zucker, 1983, 1984). Overall, reproductive behavior is thus more complex in American species, especially in the subgenera *Uca*, *Minuca*, and *Celuca*. Crane argued that the rise of this increased complexity was associated with adaptation to higher intertidal habitats, although American species with such reproductive traits occupy a broad spectrum of the intertidal. Morphologically, the American group is more complex, as the subgenus *Uca* has species with narrow fronts.

Crane's data and hypothesis of evolutionary direction and biogeography are important because they concern a pan-tropical genus whose history might be relevant to the understanding of the history of the entire tropical marine biota. Ekman (1953) argued that extant pan-tropical invertebrate groups probably arose during the time that the Tethyan sea connected both the Atlantic and Pacific tropical realms in the late Mesozoic and early Cenozoic. In contrast, Crane's hypothesis associated separate episodes of evolution with the apparent isolation afforded; by the broad expanse of the Pacific and the Atlantic. Crane thought that the west African *Uca tangeri* was a close associate of members of the American subgenus *Uca*, and therefore we might argue that *U. tangeri* arose from a chance dispersal event across the Atlantic.

Fig. 1 shows Crane's hypothesis as a phylogeographic history and an interpretation of the hypothesis in the form of a cladogram. Most importantly, the IndoPacific groups are ancestral. Crane (1975) argued that a member of the subgenus *Thalassuca* dispersed across the Pacific and was ancestor of the American species. Presumably, the invasion of

(a)



(b)

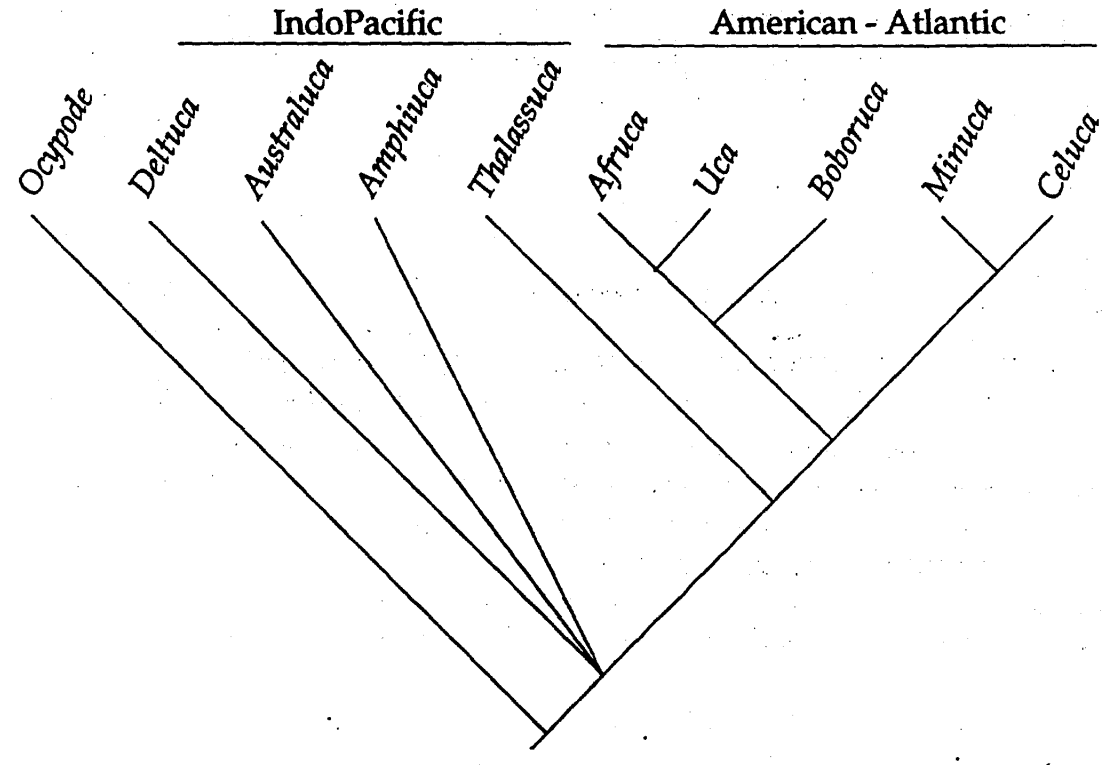


Fig. 1. (a) Geographic depiction of Crane's hypothesis of the evolution and biogeography of fiddler crabs, showing an origin in the IndoPacific, spread of a member of the subgenus *Thalassuca* to the Americas, radiation of American subgenera, and a return dispersal event of a member of the genus *Celuca* to the IndoPacific; (b) Cladogram representation of relationships implicit in this hypothesis. AFR = subgenus *Afruca*; BOB = *Boboruca*; CEL = *Celuca*; MIN = *Minuca*, UCA = *Uca*; DEL = *Deltuca*; AMP = *Amphiuca*; AUS = *Australuca*; THA = *Thalassuca*.

the high intertidal in the Americas allowed a radiation that surpassed the IndoPacific ancestral radiation in diversity.

This hypothesis is intuitively satisfying because it conforms to the IndoPacific center of origin notion, also championed by Briggs (1974). The hypothesis also postulates an internally consistent picture of evolution from a 'primitive' state of relatively simple reproduction to a more 'advanced' state of complex reproductive behavior, with many features not seen in the IndoPacific. Furthermore, 12 of the 14 genera in the family Ocypodidae are restricted to the IndoPacific. Many of these subgenera include diminutive, monomorphic, deposit-feeding species whose traits are plausible as ancestors to the genus *Uca*. All that is required to make the hypothesis of IndoPacific origins work is to have a mechanism of trans-Pacific dispersal to explain the rise of the American clade and one case of back-dispersal, which would explain the presence of species of the subgenus *Celuca* in the IndoPacific. Crane (1975) thought that the Bering Sea land bridge might have been a route of access during times of warmer climate (being subtropical to tropical, no species lives in such cold water in the northern Pacific Ocean today), but also suggested the possibility of island hopping across the tropical Pacific. This hypothesis was criticized by Salmon and Zucker (1987), who noted the improbability of a Bering Sea dispersal event and argued that the American and IndoPacific groups might have been separate sister clades that diverged long ago. Thus, there would be no implication of 'advancement' in the American style of increased complexity of reproductive behavior. Salmon and Zucker's hypothesis implies that the subgenus *Celuca* is polyphyletic, as species of this subgenus occur both in the Americas and in the IndoPacific.

3. Molecular and phylogenetic methods

Sequences of 16S ribosomal RNA of up to 491 base pairs were obtained for 24 species of the genus *Uca*, representing all nine subgenera (Table 1). Samples were obtained from the Pacific and Atlantic coasts of Panama, Tasmania, the east coast of South Africa, Taiwan, Portugal, and the East coast of North America. To place the genus *Uca* in a larger phylogenetic framework species of other Ocypodids were obtained from the east coast of North America, Caribbean (*Ocypode quadrata*), and Tasmania (*Scopimera inflata, Macrophthalmus latifrons, Heloecius cordiformis*). We also sequenced the gecarcinid *Cardisoma guanhumi* (St. John's, US Virgin Islands), and the grapsid *Chasmagnathus granulosus* (Argentina) as outgroups.

DNA was extracted from leg muscle tissues of frozen or ethanol-preserved specimens using standard methods (Sambrook et al., 1989). Two amplifications (one symmetric and one asymmetric; (Gyllenstein and Erlich, 1988) via the polymerase chain reaction (PCR) (Saiki et al., 1989) were performed as described elsewhere (Sturmbauer and Meyer, 1993) using universal primers (16sar and 16sbr; Palumbi et al., 1991). The amplification products were ultrafiltrated, directly sequenced by means of the chain termination method (Sanger et al., 1977), and electrophoresed on 6% acrylamide–bisacrylamide–urea gels. For each specimen studied, sequence was obtained from both sense and antisense strands, electrophoresed with sufficient overlap. Sequences were aligned by

Table 1
List of species employed in the study, with locations of collections.

Genus	Subgenus (if *Uca*)	Species	Location
Chasmognathus		*granulosus*	Argentina
Cardisoma		*guanhumi*	St. Johns, US Virgin Islands
Scopimera		*inflata*	North Stradbroke Island, Queensland, Australia
Heloecius		*cordiformis*	Tasmania
Macrophthalmus		*latifrons*	Tasmania
Ocypode		*quadrata*	North Carolina, USA; St. Johns, US Virgin Islands
Uca	*Deltuca*	*arcuata*	Taiwan
	Deltuca	*coarctata urvillei*	Natal, South Africa
	Australuca	*polita*	Taiwan
	Australuca	*seismella*	Taiwan
	Australuca	*signata*	Taiwan
	Thalassuca	*vocans hesperiae*	Natal, South Africa
	Thalassuca	*vocans borealis*	Taiwan
	Celuca	*lactea lactea*	Taiwan
	Celuca	*lactea annulipes*	Natal, South Africa
	Amphiuca	*chloropthalmus*	Natal, South Africa
	Minuca	*panamensis*	Panama (Pacific Coast)
	Minuca	*pugnax*	New York, USA
	Minuca	*minax*	North Carolina, USA
	Minuca	*vocator*	Panama (Caribbean coast)
	Minuca	*vocator*	Panama (Pacific coast)
	Minuca	*rapax*	Panama (Caribbean coast)
	Minuca	*galapagensis*	Panama (Pacific coast)
	Celuca	*argillicola*	Panama (Pacific coast)
	Celuca	*pugilator*	New York, USA
	Celuca	*panacea*	Florida, Gulf Coast, USA
	Boboruca	*thayeri*	Panama (Caribbean coast)
	Uca	*intermedia*	Panama (Pacific coast)
	Uca	*stylifera*	Panama (Pacific coast)
	Uca	*heteropleura*	Panama (Pacific coast)
	Afruca	*tangeri*	Portugal

eye and the alignment was improved iteratively utilizing the tree length of the most parsimonious trees from a parsimony search as optimization criterion. The alignment alternative resulting in shorter trees was further optimized.

Regions of high and low variability were identified in a sliding-window analysis (window width nine base pairs, three base pairs overlap; not shown). Regions of high variability were defined as having more than 20% base substitution in the window. Then the relative frequencies of transitions (TS) and transversions (TV) were identified for both, the regions of low variability and the regions of high variability from all pairwise comparisons with less than 5% uncorrected differences (511 pairwise comparisons in the region of low variability; 19 pairwise comparisons in the regions of high variability; see also Sturmbauer and Meyer, 1992). The deviation of the TS–TV ratios from the theoretically expected ratio of two TV per one TS was then used as weighting scale in the phylogenetic analyses.

The sequence data were analyzed by the parsimony method using PAUP (Version 3.1.1,

Swofford and Begle, 1993). The large number of taxa analyzed made the application of heuristic search procedures necessary. In order to make the heuristic searches more reliable (Swofford, personal communication), the option 'random addition of taxa' with 20 replications was chosen in PAUP.

Neighbor-joining analyses (Saitou and Nei, 1987) were performed with NJBOOT2 (Tamura and Nei, 1992) using Kimura distances. Statistical analyses were done only for parsimony and neighbor-joining by means of the bootstrap method (Felsenstein, 1985; 500 replications, heuristic search, PAUP-option 'simple addition of taxa').

The phylogenetic analysis was performed in three steps. A first analysis was performed in which only TV and insertion-deletion events (indels) were used, to minimize the effect of multiple base substitutions. This analysis included 28 taxa of fiddler crabs, and four additional species of the family Ocypodidae (Table 1). *Chasmognathus granulosus* of the family Grapsidae was chosen as outgroup.

A second analysis was performed to determine the branching order of the deep branches within the fiddler crabs. Here, transversions were weighted over transitions according to their deviation from the expected frequency in the regions of low and high variability. 28 individuals out of the total of 35 sequenced individuals were included in the analysis, so that each sub-species was represented. *Ocypode quadrata* was used as outgroup based on the first analysis.

In a third analysis all distantly related taxa (*Uca* and *Afruca*) were omitted, to analyze the relationships of the more recent phylogenetic splits. Due to the relative recency of these splits, all observed base substitutions and indels were weighted equally. Based on the previous analysis the well-supported IndoPacific clade was declared as outgroup.

4. Results

4.1. Transition–transversion ratios

In the regions of low variability transitions (TS) were 2.6 times more frequent than transversions (TV). Because transversions were five times less frequent than theoretically expected, they were given a weight of five over transitions in the second phylogenetic analysis. In the highly variable region TS were five times more frequent than transversions, and consequently TV were weighted ten times over TS.

4.2. Phylogenetic analysis

The first analysis, based on TV and indels only, resulted in 12 most parsimonious trees of a length of 462 steps (unweighted tree lengths 1079 to 1083 steps; consistency index excluding uninformative characters, 0.36 (Kluge and Farris, 1969). A strict consensus of those 12 most parsimonious trees and the neighbor-joining tree is shown in Fig. 2. While the remaining ocypodids are more distantly related to fiddler crabs (the Caribbean land crab *Cardisoma*, for example, clusters closely with the Australian sand bubbler ocypodid *Scopimera*), the ghost crab *Ocypode quadrata* seems strongly associated with members of the subgenus *Uca* and the subgenus *Afruca*. This clade represents the most ancestral

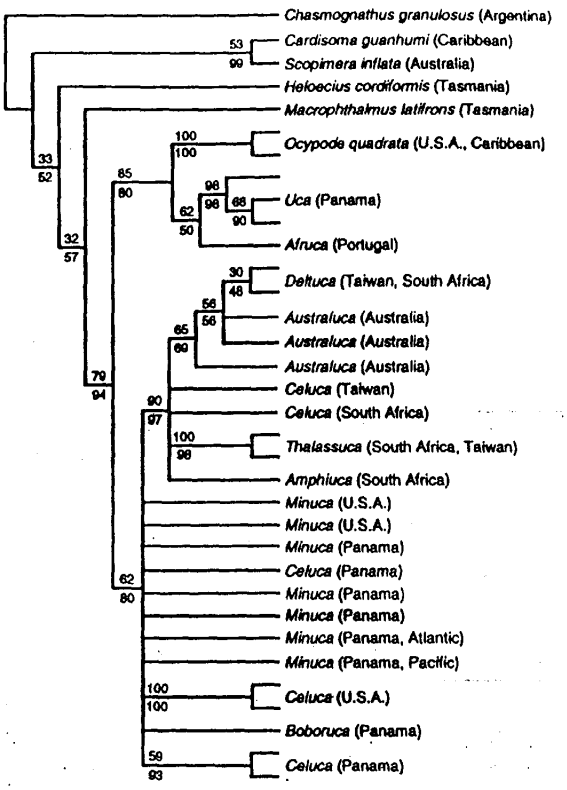


Fig. 2. Tree of relationships for members of the family Ocypodidae, including fiddler crabs (*Uca*), ghost crab *Ocypode quadrata*, the ocypodids Scopimera *inflata*, Heloecius *cordiformis* and *Macrophthalmus latifrons*, the land crab *Cardisoma guanhumi*, and the grapsid *Chasmognathus granulosus*. Numbers above stems indicate bootstrap values for neighbor joining tree while those below indicate bootstrap values for parsimony tree.

clade of the fiddler crabs, suggesting that *Ocypode quadrata* may actually belong to the fiddler crabs.

Ocypode was thus used as outgroup for the second phylogenetic analysis which resulted in a single most parsimonious tree of a weighted tree length of 3689 steps (unweighted tree length 771 mutations, consistency index excluding uninformative characters 0.42). In the third analysis, a parsimony search resulted in three most parsimonious trees (tree length 581 mutations; consistency index excluding uninformative characters, 0.47). The cladogram presented in Fig. 3 summarizes the second and third phylogenetic analysis.

Our analysis suggests clearly that the subgenera *Uca* and *Afruca* represent the most ancestral clade of fiddler crabs. *Uca* resides wholly in the Americas while *Uca Afruca tangeri* is distributed at the eastern coasts of the Atlantic from West Africa to Portugal. All of the other fiddler crabs comprise the sister group of this ancestral *Uca–Afruca* grouping. The IndoPacific fiddlers (members of the subgenera *Deltuca, Thalassuca, Amphiuca*, and *Celuca*) are strongly supported (85 percent bootstrap value) as a monophyletic group, while the relationships within the American clade are less clear, due to short branches. Most importantly, however, members of the subgenus *Celuca* (two apparently distinct and geographically distant samples—South Africa and Taiwan—of *U. lactea*) are found within the well-supported IndoPacific group and also in

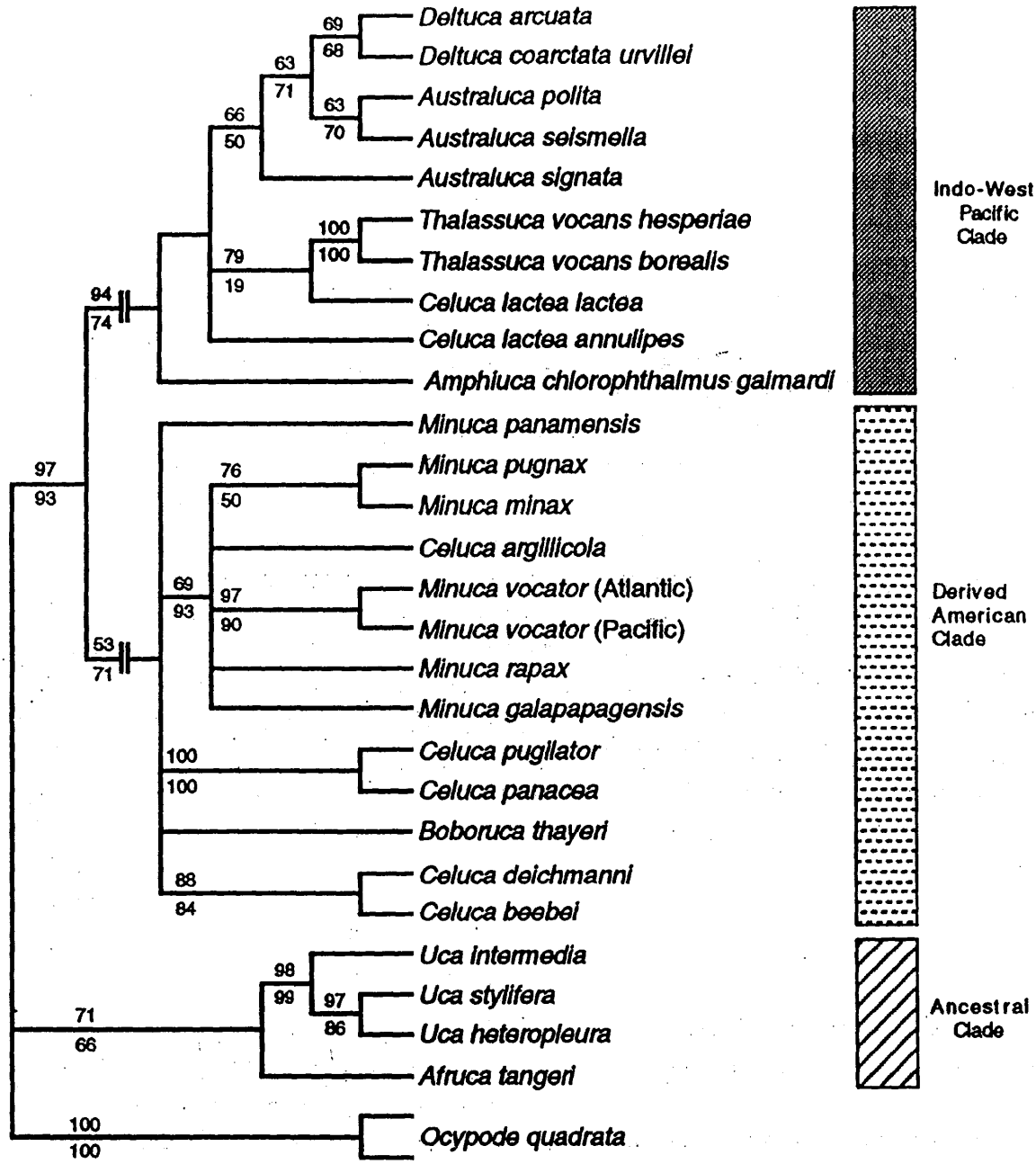


Fig. 3. Phylogenetic relationships of the fiddler crabs. The cladogram shown is a strict consensus tree of the most parsimonious tree(s) and neighbor-joining tree. Numbers above the branches are bootstrap values obtained by parsimony, those below the branches are from neighbor-joining.

the American sister groups (*U. deichmani, U. beebei, U. pugilator, U. panacea, U. argillicola*), suggesting strongly that *Celuca* is a polyphyletic subgenus (Fig. 3). The third phylogenetic analysis addressed the branching order within the 'derived' American clade of fiddler crabs. The strongly supported Indo-Pacific clade was declared as outgroup and all substitutions and indels were now weighted equally. Most importantly, even the American species of the subgenus *Celuca* appear to be polyphyletic since *Celuca argillicola* is resolved with several species of *Minuca*

5. Discussion

Our results suggest that the hypothesis of IndoPacific origins of *Uca* cannot be supported. The ancestral part of the *Uca* clade is apparently to be found within the subgenus *Uca*, combined with *Afruca*. If the present distributions of these two subgenera also reflect their past, this places the ancestral stock of *Uca* in the Atlantic-American region. It appears that the remaining species of *Uca* derives from a species with characters of this ancestral group, but that there is a split between the remaining American and IndoPacific-Pacific species. Thus, the subgenus *Celuca*, formerly hypothesized to have originated in the Americas but migrated back to the IndoPacific, is polyphyletic. If the molecular data and analysis are correct, it must follow that morphological features used to characterize the subgenus *Celuca* represent evolutionary convergence. Fig. 4 shows in simplified form our working hypothesis of the relationships among *Uca* and related species.

Our data do not support the center of origin theory in the IndoPacific. Indeed, our results suggest that there is no evolutionary trend from a so-called primitive state of lower intertidal existence and simple reproductive behavior to an American style of higher intertidal life and more complex reproductive behavior. If anything, the subgenera *Afruca* and *Uca* bear many of the so-called advanced features of reproductive behavior, even though they also have some so-called ancestral features, such as narrow fronts. We would rather suggest that reproductive complexity has evolved and perhaps has been lost in evolution more than once. This is exemplified by the presence of '*Celuca*' types in both the American and IndoPacific theaters.

Furthermore, one aspect of the so-called derived reproductive behavior, namely females following males into burrows, has been recently recognized in the Indo-west-Pacific *Uca tetragonon* (Murai et al., 1995). This further emphasizes the likelihood of

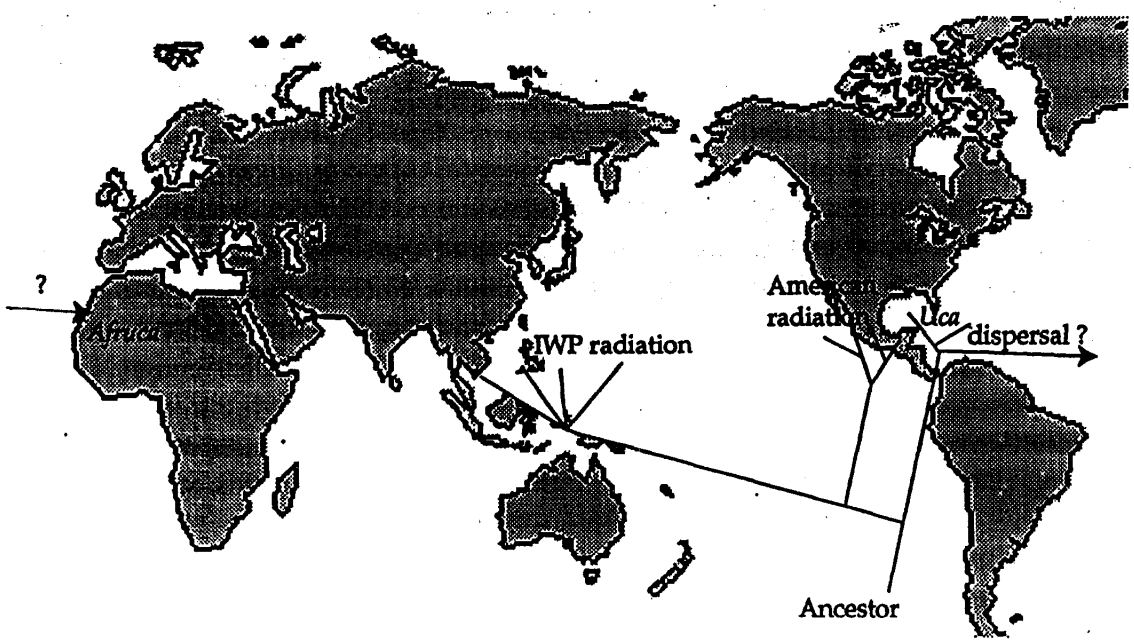


Fig. 4. Revised hypothesis of evolution and biogeography of the fiddler crabs.

multiple independent evolution of behavioral traits. The heterogeneity of reproductive behavior among species of fiddler crabs has been underemphasized and more ecological work is needed to understand the selective forces behind this variation (Zucker, 1983).

Even if the ancestral subgenera *Uca* and *Afruca* are now found only in the Americas, we cannot be certain that the genus *Uca* arose in the New World. It is certainly possible that many distributions found today are relicts of past rearrangements of climate and of extinctions (Ekman, 1953). It is interesting to note, however, that the oldest fossils of fiddler crabs, identified as *Uca maracoani*, belong to the putative ancestral subgenus and were found in the lower Miocene of Brazil (Brito, 1993). The fossil record of fiddler crabs, however, is quite poor, so this encouraging result must be greeted with some degree of scepticism. The center of origin notion would require that the IndoPacific species occupy an ancestral position in the tree; they do not. Thus, our results minimally falsify the hypothesis of IndoPacific origins and the notion that evolution proceeded from the simple to the complex, with regard to reproductive behavior.

It is fair to say that Crane's hypothesis of IndoPacific origins was based upon the expectation that behavioral evolution should proceed from the simple to the complex and that the Indo-Malayan region was the wellspring of diversity, leading to dispersal of forms outward to the eastern Pacific (Briggs, 1974). Although the greater eastern Pacific is a strong barrier to dispersal (Ekman, 1953), as is the region between the continental western Pacific and the central Pacific (Vermeij, 1987), there is some evidence supporting current systems as transporters of propagules and determinants of diversity gradients in seagrasses in the southwest Pacific (Mukai, 1993). Nevertheless, our data support the hypothesis that the eastern Pacific is an important barrier that has kept separate two sister clades of fiddler crabs. To this degree, our data support the hypothesis of (Salmon and Zucker, 1987), with the exception of the ancestral status of the subgenera *Uca* and *Afruca* Others have also argued that geographic barriers within the Indian and Pacific Oceans have been more influential on diversification than diffusion from a southwest Pacific center of diversity (McCoy and Heck, 1976; Kay, 1984). Such barriers may be imprinted over a center of origin influence. The data of (Mukai, 1993) on seagrasses, for example, very much support the center of origin notion.

The geography and evolutionary position of *U. Afruca tangeri* pose the most important and fascinating questions of all. Despite the fact that the genus *Uca* is by far the most diverse in the Americas, *U. tangeri* is the only species found on the east side of the Atlantic Ocean, ranging from Angola to Portugal. There is no strong regional differentiation in color over its range, which is the largest latitudinal range of any species of *Uca* (Crane, 1975). Two hypotheses might explain this distribution. First, *U. tangeri* might be a relatively recent arrival across the Atlantic, deriving from some other member of the subgenus *Uca*. Alternatively, the presence of this species might reflect an ancient presence of the subgenus *Uca* in the Atlantic region. This would beg the question of why there has been no subdivision of this species over such a broad latitudinal extent and for such a long period of time.

Overall, our data suggest that *Uca* and its close ally *Afruca* arose within a proto-Atlantic realm, perhaps before the differentiation of the great Tethyan Sea that formerly connected tropical marine biotas from the Pacific to the Caribbean. The split between

Indo-West Pacific subgenera (*Thalassuca, Deltuca, Australuca, Amphiuca*, IndoPacific members of *Celuca*) and the American–Atlantic fiddler crab subgenera (*Minuca, Boboruca*, American species of *Celuca*) other than *Afruca* and *Uca*, must have been caused by the separation of the Tethyan realm between Pacific and Atlantic–Caribbean geographic regions. Before the establishment of the Isthmus of Panama in the Pliocene there was free connection between the Caribbean and the Pacific. Currently, the presence of the isthmus is reflected only in subspecific differentiation of a few species of fiddler crabs (Crane, 1975).

The close relationship of *Uca* and *Ocypode* has been suggested before (Crane, 1975) and it suggests a pattern of evolutionary direction different from the marine–primitive to the high intertidal–advanced scheme mentioned above. Like *Ocypode* and other ecologically similar crabs, *Uca* has vision that is strongly adapted to terrestrial life (Zeil et al., 1986). Image detection is far more acute in the vertical direction and ommatidia are concentrated at the eye's equator, suggesting that the crab is particularly adept at detecting vertical motion at the horizon (Langdon and Hernkind, 1985; Zeil et al., 1986). In *Uca pugilator*, there are distinct visual reactions to other crabs and to predators, whose images break the horizon (Land and Layne, 1995b,a). The vertical waving of males, however, penetrates above the horizon, so females may initially perceive the wave as a predatory threat and thus take strong notice (Land and Layne, 1995a). This hypothesis would suggest that the origin of *Uca*'s complex mating system lies in adaptation to high intertidal life and could only have followed the evolution of its visual system in response to predation. This would suggest that the visual system to detect predators was co-opted into the complex reproductive system, which is also strongly dependent upon visual cues (Christy and Salmon, 1991; Christy, 1995). Such an evolutionary change would occur most likely in a strongly terrestrial setting. The long, close-set eyes (i.e., narrow fronts), the vertical pseudopupils, and the concentration of ommatidia near the equatorial plane of the eye would suggest that the reproductive system of *Uca* arose on open tidal flats (Zeil et al., 1986).

In conclusion, our data apparently suggest that the IndoPacific center of origin hypothesis does not apply to the pan-tropical genus *Uca*, nor does an evolutionary trend from apparently simple to more complex reproductive behavior. The novel reproductive behavior and dimorphism of *Uca* represents an evolutionary change that is clearly related to semiterrestrial life, but its many variations seen among the species probably represent a variety of convergent evolutionary events. The apparent separation of IndoPacific and American crabs aside from those in the ancestral clade may reflect a vicariant event that isolated Atlantic-eastern Pacific and western Pacific groups.

Acknowledgments

We are very grateful to the following individuals who provided us with crabs: A. Richardson (Tasmania), D. Gerhart and D. Ritchoff (North Carolina), I.-Jiunn Cheng (Taiwan), P. Backwell (Natal), D. Fielder (Queensland, Australia), J. Paula (Portugal). This paper was partially supported by grants from the National Science Foundation and from the Hudson River Foundation.

References

Avise, J.C., 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation. *Oikos*, Vol. 63, pp. 62–76.

Avise, J.C., J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J.E. Neigel, C.A. Reeb and N.C. Saunders, 1987. Intraspecific phylogeography: The mitochondrial bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.*, Vol. 18, pp. 489–522.

Briggs, J.C., 1974. *Marine Zoogeography*. McGraw-Hill, New York, 475 pp.

Brito, I.M., 1993. O aspecto moderno da carcinofauna da Formacao Pirabas Oligoceno–Mioceno do Estado do Para. *An. Academ. Brasileira Cienc.*, Vol. 65, pp. 57–61.

Burton, R.S., and B.-N. Lee, 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proc. Nat. Acad. Sci. USA*, Vol. 91, pp. 5197–5201.

Christy, J.H., 1995. Mimicry, mate choice, and the sensory trap hypothesis. *Am. Nat.*, Vol. 146, pp. 171–181.

Christy, J.H. and M. Salmon, 1984. Ecology and evolution of mating systems of fiddler crabs (Genus *Uca*). *Biological Reviews Cambridge*, Vol. 59, pp. 483–509.

Christy, J.H. and M. Salmon, 1991. Comparative studies of reproductive behavior in mantis shrimps and fiddler crabs. *Amer. Zool.*, Vol. 31, pp. 329–337.

Crane, J., 1975. *Fiddler crabs of the world*. Princeton University Press, Princeton, NJ. 736 pp.

Darlington, P.D., 1957. *Zooeography: The geographical distribution of animals*. John Wiley, New York.

Den Hartog, C., 1970. *The seagrasses of the world*. North Holland, Amsterdam.

Ekman, S., 1953. *Zoogeography of the sea*. Sidgwick and Jackson, London, 417 pp.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, Vol. 39, pp. 783–791.

Gyllenstein, U.B. and H.A. Erlich, 1988. Generation of single stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA–DQA locus. *Proc. Nat. Acad. Sci. USA*, Vol. 85.

Kay, E.A., 1984. Patterns of speciation. In, *Biogeography of the tropical pacific: a symposium*, edited by Radovsky, F.J., P.H. Raven and S.H. Sohmer: Association of Systematics Collections and the Bernice P. Bishop Museum, Lawrence KA, pp. 15–31.

Kluge, A.G. and J.S. Farris, 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.*, Vol. 18, pp. 1–32.

Land, M. and J. Layne, 1995a. The visual control of behaviour in fiddler crabs. I. Resolution, thresholds and the role of the horizon. *J. Comp. Physiol. A*, Vol. 177, pp. 81–90.

Land, M. and J. Layne, 1995b. The visual control of behaviour in fiddler crabs. II. Tracking control systems in courtship and defence. *J. Comp. Physiol. A*, Vol. 177, pp. 91–103.

Langdon, J.W. and W.F. Hernkind, 1985. Visual shape discrimination in the fiddler crab *Uca pugilator*. *Mar. Behav. Physiol.*, Vol. 11, pp. 315–325.

McCoy, E.D. and J.K.L. Heck, 1976. Biogeography of corals, seagrasses, and mangroves: an alternative to the center of origin concept. *Syst. Zool.*, Vol. 25, pp. 201–210.

Mukai, H., 1993. Biogeography of the tropical seagrasses in the western Pacific. *Austr. J. Mar. Freshw. Res.*, Vol. 44, pp. 1–17.

Murai, M., T. Koga, S. Goshima and S. Poovachiranon, 1995. Courtship and the evolution of underground mating in *Uca tetragonon* (Decapoda: Ocypodidae). *J. Crust. Biol.*, Vol. 15, pp. 655–658.

Nelson, G. and N. Platnick, 1981. *Systematics and biogegraphy: Cladistics and vicariance*. Columbia University Press, New York.

Palumbi, S.R., A. Martin, S. Romano, W.O. McMillan, L. Stice, and G. Grabowski, 1991. *Simple Fool's Guide to PCR*. University of Hawaii, Honolulu.

Pandolfi, J.M., 1992. Successive isolation rather than evolutionary centres for the origination of Indo-Pacific reef corals. *J. Biogeogr.*, vol. 19, pp. 593–609.

Potts, D.C., 1983. Evolutionary disequilibrium among Indo-Pacific corals. *Bull. Mar. Sci.* Vol. 33, pp. 619–632.

Rosen, D.E., 1975. The vicariance model of Caribbean biogeography. *Syst. Zool.*, Vol. 24, pp. 431–464.

Saiki, R.K., D.H. Gelfland, S. Stoffel, S. Scharf, R. Higuchi, R.Horn, K.B. Mullis and H.A. Erlich, 1989. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, Vol. 239, pp. 487–491.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, Vol. 4, pp. 406–425.

Salmon, M. and N. Zucker, 1987. Interpreting differences in the reproductive behaviour of fiddler crabs (Genus *Uca*). In, *NATO Advanced Research Workshop on Behavioural Adaptations to Intertidal Life*, edited by Chelazzi, G. and M. Vannini. NATO ASI Series A: Plenum Press, New York, pp. 387–408.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.

Sanger, F., S. Nicklen and A. Coulson, 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Nat. Acad. Sci. USA*, Vol. 74, pp. 5463–5467.

Stehli, F.G. and J.W. Wells, 1971. Diversity and age patterns in hermatypic corals. *Syst. Zool.*, Vol. 20, pp. 115–126.

Surmbauer, C. and A. Meyer, 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature*, Vol. 359, pp. 578–581.

Sturmbauer, C. and A. Meyer, 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes of Lake Tanganyika, East Africa. *Mol. Biol. Evol.*, Vol. 10, pp. 751–768.

Swofford, D.L. and D.P. Begle, 1993. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1*. Illinois Natural History Survey, Champaign-Urbana.

Tamura, K. and M. Nei, 1992. *NJBOOT2 version 1.03 computer program*. Pennsylvania State University, State College PA.

Vermeij, G.J., 1987. The dispersal barrier in the tropical Pacific: Implications for molluscan speciation and extinction. *Evolution*, Vol. 41, pp. 1046–1058.

Zeil, J., G. Nalbach and H.-O. Nalbach, 1986. Eyes, eye stalks and the visual world of semi-terrestrial crabs. *J. Comp. Physiol. A*, Vol. 159, pp. 801–811.

Zimmer-Faust, R.K., 1987. Substrate selection and use by a deposit-feeding crab. *Ecology*, Vol. 68, pp. 955–970.

Zucker, N., 1983. Courtship variation in the neo-tropical fiddler crab *Uca deichmanni*: Another example of female incitation to male competition? *Mar. Behav. Physiol.*, Vol. 10, pp. 57–79.

Zucker, N., 1984. Delayed courtship in the fiddler crab *Uca musica terpsichores*. *Animal Behav.*, Vol. 3, pp. 735–742.