# The occurrence of the provisional Brazilian subspecies of spiny lobster (*Panulirus argus westonii*) in Florida waters*

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The Caribbean spiny lobster, *Panulirus argus,* is distributed from Brazil throughout the Caribbean and the Gulf of Mexico to approximately North Carolina and Bermuda (Holthius, 1991). It supports major commercial fisheries in Florida, the Caribbean and Brazil. Commercially, *P. argus* is especially important to the state of Florida, where the spiny lobster fishery ranks second only to shrimp in terms of economic value. There is also a significant recreational fishery for *P. argus*, particularly in Florida. A number of studies have been initiated to gather information to manage the lobster resource carefully.

Several studies have used genetic techniques to examine population-level patterns of differentiation to delineate reproductively isolated stocks of *P. argus*. Evidence of the stock structure of spiny lobster populations could then be used to implement more effective fishery management plans. These studies have provided somewhat ambiguous results. Using allozymes, Menzies and Kerrigan (1979) and Menzies (1981) provided some evidence for genetic differences among populations, but more recent studies using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA have suggested little evidence for genetic differences among Caribbean populations of *P. argus* (Silberman et al., 1994a, 1994b). Analysis of mtDNA RFLPs have revealed suprisingly high levels of diversity among individuals, and several individuals were diverged at levels not usually seen within a species. Because previous studies did not include populations from Brazil, Sarver et al. (1998) compared *P. argus* from Caribbean locations with *P. argus* from Brazil. Using DNA sequence analysis of two mitochondrial genes, Sarver et al. (1998) found high levels of sequence divergence between Caribbean and Brazilian *P. argus*. The levels of nucleotide sequence divergence were greater than that seen between recognized species of *Panulirus*. In addition, there are striking color differences between Caribbean and Brazilian *P. argus*. As a result of these findings Sarver et al. (1998) suggested provisional recognition of two subspecies of *P. argus* (*P. argus argus* in the Caribbean and *P. argus westonii* in Brazil) until a formal taxonomic revision could be done.

The results from these studies, which suggest that *P. argus* in Brazil are genetically and taxonomically distinct from their Caribbean counterparts, have raised questions about the status of the three genetically distinct spiny lobsters reported by Silberman et al. (1994a) in their original survey of mtDNA diversity of Caribbean *P. argus*. This is significant because two of these spiny lobsters found in Silberman et al. (1994a) were caught off the coast of Miami, Florida. Our study uses DNA sequence analysis to identify the three genetically distinct spiny lobsters observed by Silberman et al. (1994a) as the Brazilian form of *P. argus* (provisionally recognized as *P. argus westonii,* in Sarver et al., 1998).

## Materials and methods

DNA samples were obtained from the samples examined by Silberman et al. (1994a). Caribbean *Panulirus argus* samples were randomly selected from the samples examined by Silberman et al. (1994a). Samples from Brazil were collected near the Rio Grande do Norte region of Brazil. Tissue samples of *Panulirus argus* from Brazil were frozen prior to DNA isolation. Standard phenol or chloroform DNA extraction techniques were used for DNA isolation (Hillis et al., 1990). A region of the mitochondrial 16S rRNA gene was amplified by the polymerase chain reaction (PCR) by using primers 16Sar and 16Sbr given in Palumbi et al.[1] PCR products were then purified and used as templates for DNA sequencing by using the ΔTaq cycle sequencing kit (U.S. Biochemical Corp., Cleveland, OH). Cycle sequencing reactions were performed by using $\gamma$-$^{33}$P-dATP end-

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[1] Palumbi, S. R., A Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski. 1991. A simple fools guide to PCR, vers. 2.0. Special publication of the University of Hawaii Department of Zoology and Kewalo Marine Laboratory, 46 p. Department of Zoology and Kewalo Marine Laboratory, Univ. Hawaii, Honolulu, HI 96822.

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labeled primers. All sequences were determined in both directions.

Aligned DNA sequence data included 441 sites. Distance and parsimony analyses of the aligned DNA sequences were done with PAUP (Swofford, 1998). Three separate distance calculations were made: uncorrected mean, Jukes-Cantor (Jukes and Cantor, 1969), and Kimura 2-parameter (Kimura, 1980) distances. Neighbor-joining trees (Saitou and Nei, 1987) were generated by using all three distance measures. Parsimony trees were produced by using branch and bound searches. Sites coded as gaps were treated either as missing, or as a fifth character state. The mitochondrial 16S rDNA sequences were aligned by using the SeqPup DNA analysis program (Gilbert[2]). Support for nodes of both parsimony and distance trees was assessed by calculating bootstrap proportion (BP) values (Felsenstein, 1985) from 1000 replicate searches by using the same tree building methods used to produce the trees. *Scyllarides nodifer* (Stimpson) (Decapoda, Scyllaridae) was used as an outgroup for phylogenetic analyses.

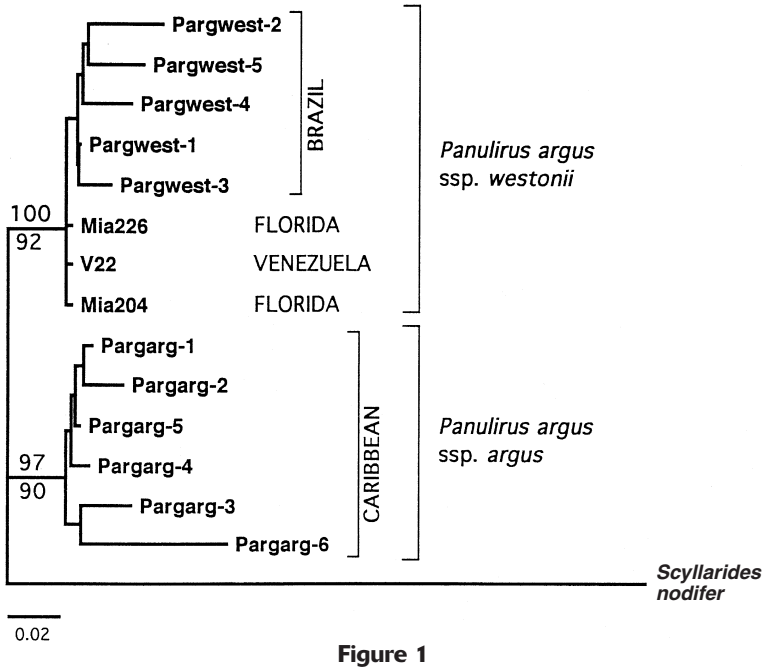


**Figure 1**

Neighbor joining tree derived from the Kimura two-parameter distances calculated from partial mitochondrial 16S rDNA data for 14 *Panulirus argus* and *Scyllarides nodifer* samples. Bootstrap values calculated from the distance analysis are shown above and bootstrap values calculated from parsimony analysis are shown below strongly supported branches.

## Results

The mitochondrial 16S rDNA region analyzed in our study included 441 aligned nucleotide positions. In addition to single base differences, the complete aligned data set included 19 presumptive insertion or deletion mutations (indels) of 1–5 bases in length. The majority of indels involved only one base (13 of 19) and only six were potentially informative.

Regardless of the type of distance calculation, there was little variation in the outcome of the different distance calculations (less than 1%). Results from an analysis with a Kimura 2-parameter model yielded distance values ranging from 5.9% to 15.1% between the Caribbean and Brazilian subspecies of *Panulirus argus* (*P. argus argus* and *P. argus westonii* respectively). For the three genetically distinct Caribbean *P. argus* from Silberman et al. (1994a), sequence divergence estimates indicated closer affinities to Brazilian *P. argus* (2.6%) than to the Caribbean *P. argus* (7.4%).

Phylogenetic analysis of Brazilian, Caribbean and the three divergent Caribbean *P. argus* indicated a tree topology that places the three divergent Caribbean samples of *P. argus* in the Brazilian clade (Fig. 1). This basic tree topology is strongly supported regardless of the method of phylogenetic analysis.

## Discussion

Traditionally, *Panulirus argus* has been thought to be distributed from North Carolina in the western Atlantic, throughout the Caribbean to Rio de Janeiro, Brazil. Sarver et al. (1998) presented data that questioned the taxonomic status of *P. argus*. DNA sequence data and morphological differences suggest that populations of *P. argus* from Brazil are genetically distinct from Caribbean populations and that the level of divergence is equivalent to the levels of divergence seen between recognized species of *Panulirus*. As a result they suggested formally recognizing two genetic forms of *P. argus* and have recommended provisional subspecific status: *P. argus argus* (Caribbean) and *P. argus westonii* (Brazil) until a formal taxonomic revision can be done. The occurrence of cryptic species in *Panulirus* is not restricted to *P. argus*. A similar situation exists in *P. longipes*, where there appears to be at least four recognizable forms of uncertain taxonomic status (George, 1972; Sekiguchi, 1991; Chan and Chu, 1996). *Panulirus homarus* has also been broken into three geographic forms that are given the rank of subspecies (Berry, 1974).

We can only speculate on the events that led to this distribution of the two *P. argus* subspecies. During the late Miocene Epoch, a *P. argus* ancestor may have occupied the North Atlantic and as Africa and South America moved apart as a result of continental drift, habitat became available for *P. argus* along the South America coast. These pop-

[2] Gilbert, D. G. 1996. SeqPup biosequence editor and analysis application, vers. 0.6f. Department of Biology, Univ. Indiana, Bloomington, IN 47405.

ulations then could have been isolated as a result of the Andes uplift during the Pliocene Epoch, which altered the pattern of the runoff and changed the course of the major rivers of the Amazon basin. Runoff from the Amazon basin continues to act as a barrier to larval migration and effectively separates Caribbean *P. argus argus* and Brazilian *P. argus westonii*. Levels of nucleotide sequence diversity observed between *P. argus argus* and *P. argus westonii* are compatible with this hypothesis.

Low numbers of the Brazilian form of *P. argus* in the Caribbean may result from rare migration events or the co-occurrence of both forms in the Caribbean. Long-range dispersal of larvae is especially well developed in *P. argus*. The complex life cycle of spiny lobsters is characterized by a protracted larval phase (the phyllosome) which can last from several months to two years and can result in prolonged transport by ocean currents (Sims and Ingle, 1967). Postlarval recruitment for some species of *Panulirus* has been associated with variation in large-scale oceanic processes (Phillips and Pearce, 1997). El niño-like events could alter the current regimes and allow some of the Brazilian forms of *P. argus* to escape into the Caribbean. Episodic recruitment events could also explain the occurrence of *Panulirus laevicauda* in the Caribbean. *Panulirus laevicauda* is abundant in Brazilian waters but is only rarely found in the Caribbean. Evidence for sporadic recruitment of *P. laevicauda* in the Caribbean was reported by Moore (1962), who noted finding a single specimen of *P. laevicauda* near Palm Beach, Florida, during 1949. Later that same year, *P. laevicauda* was reported to be nearly as abundant as *P. argus*, but in the following three years, no *P. laevicauda* were found at this location.

Occurrence of the Brazilian form of *P. argus* in Florida waters raises a number of interesting biological questions. Given the morphological differences and the high degree of genetic differentiation between Caribbean and Brazilian *P. argus,* are they capable of interbreeding? It could be possible that the existence of the Brazilian *P. argus* mtDNA haplotype in the Caribbean could be the consequence of leakage of the Brazilian *P. argus* mtDNA genome across the species boundary as a result of interbreeding. Because most of the research conducted with *P. argus* has been done with Caribbean *P. argus*, do the two forms of *P. argus* differ biologically? George (1997) considers Brazilian and Caribbean *P. argus* to be distinct ecologically and has suggested that *P. argus* is likely a complex of two species. Knowledge of the occurrence of *P. argus argus* in Brazilian waters is lacking. Taxonomic ambiguity surrounding *P. argus* is a concern because of its commercial importance. For example, Florida regulations currently prohibit the transportation or sale of imported *P. argus* during the time of year when the fishery is closed in Florida. If two species become recognized, under the present regulations, *Panulirus* from Brazil could be sold year round in Florida. If there are indeed two species of *P. argus* in Florida waters, as it appears there are, then fishery regulations will need to reflect this reclassification. Currently, fishery regulations apply to *Panulirus argus*, and all subspecies of spiny lobsters are subject to the same rules, but other species are not regulated.

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