Hurricane Mitch: Population Genetic Structure of Pacific White Shrimp from the Gulf of Fonseca, Honduras

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Tables

Table 1. Sample sizes before and after the exclusion of genetic outliers for collection sites by estuary and site within estuary.

Table 2. Analysis of Molecular Variance (AMOVA), based on 87 AFLP markers, for Litopenaeus vannamei populations collected from multiple estuaries adjoining the Gulf of Fonseca, Honduras: a) Nested AMOVA for complete data set consisting of 189 postlarval shrimp from 16 estuaries and 19 collection sites. b) Results from reduced data set (N = 144) consisting of only the 18 collection sites with a core group of three or more genetically similar postlarvae.

Table 3. Matrix of pairwise fixation indexes and their probablities for Litopenaeus vannamei compared among estuaries (N = 18) adjoining the Gulf of Fonseca, Honduras, following the exclusion of genetic outliers. F-statistics are given below the diagonal; p-values, above the diagonal.

Figures

Fig. 1. Map of the the Gulf of Fonseca, Honduras, showing the 19 collection sites for Litopenaeus vannamei postlarvae for which data are reported. Colored (non-black) ovals encircle 4 distinct phenetic clusters, three of which display disjunct distributions spanning multiple lobes of the Gulf (blow-up boxes). Black ovals encircle collection sites that were genetically distinct from all others. Shrimp farms are represented by blue-filled boxes. All collections were conducted within a tetrahedral area with intersections at the following coordinates (proceeding clockwise from the southwestern extreme): north latitude (N) 13º 22' 5.3", west longitude (W) 87º 43' 1.6"; N 13º 27' 0.2", W 87º 39' 45.1"; N 13º 28' 3.2", W 87º 34' 20.2"; and N 13º 1' 28.6", W 87º 17' 44.6".

Fig. 2. UPGMA cluster analysis of similarity coefficients (Lynch 1990) calculated on the basis of 87 AFLP markers from 10 postlarvae of Litopenaeus vannamei collected from la Boca de Conchalitos, Gulf of Fonseca, Honduras. Diagonal slashes represent a breakpoint between a core group of 7 genetically similar individuals and 3 outliers.

Fig. 3. UPGMA cluster analysis of similarity coefficients (Lynch 1990) calculated from 18 composite haplotypes of Litopenaeus vannamei postlarvae, each consisting of 87 AFLP markers, and representing estuaries adjoining the Gulf of Fonseca, Honduras. The diagonal slashes represent statistically supported breakpoints, consistent with the overall analysis of 144 individuals, between 10 distinct branches separating groups of from 1 to 5 collection sites each.

Background

Marine aquaculture (including shellfish) currently accounts for >25% of all fish consumed by humans, and is believed to cause severe damage to worldwide coastal environments through habitat destruction, waste disposal, disease transmission, and biological pollution of native populations with exotic genotypes (Naylor et al. 1998, 2000). While scientific documentation of the latter has been provided for some vertebrate species, such as the well-known case of Atlantic salmon (see Gross 1998), relatively little attention has been paid to invertebrates such as shrimp, which are now farmed over hundreds of thousands of hectares of former mangrove forests, particularly in southeast Asia and Latin America. Cultured shrimp are subjected to overcrowding and self-pollution and are thus more susceptible to disease outbreaks [(Kautsky et al. 2000; Leung and Tran 2000) although attempts to culture disease-resistant shrimp are currently underway (Clifford 1998)]. In addition, cultured shrimp are often intentionally inbred in an effort to enhance overall size and growth potential (Dumas and Ramos 1999; Ibarra 1999; De Beausset et al. 2001; De Donato et al. 2001), thereby reducing their genetic diversity, i.e. adaptive potential, relative to their wild counterparts (Sbordoni et al. 1986; Harris et al. 1990; Sunden and Davis 1991; Wolfus et al. 1997; Xu et al. 2001, Zhuang et al. 2001). Thus, the inadvertent release of captive shrimp into tropical estuaries and their subsequent assimilation by native populations may serve to both enhance disease transmission and reduce long-term population viability.

In Honduras, the aquaculture industry is most heavily concentrated along the Pacific Coast surrounding the Gulf of Fonseca. Postlarval shrimp used for stocking purposes are acquired as highly inbred lines from foreign sources in Panama, Ecuador, and the United

States (A. Oviedo, personal communication), and to a lesser extent from the local estuaries where they are harvested by local fishermen (DeWalt et al. 1996a). Thus, it is not surprising that several extremely virulent diseases, including white spot virus (Lightner et al. 1998; Wang et al. 1999; Soto and Lotz 2001), first appeared in the population of Pacific white shrimp (Litopenaeus vannamei) native to the Gulf of Fonseca immediately following the flushing of numerous aquaculture ponds as a result of Hurricane Mitch in October of 1998.

In the current investigation, we tested the hypothesis that the mixing of captive and native stocks of L. vannamei in the Gulf of Fonseca as a result of the 1998 hurricane, possibly coupled with a chronic history of smaller scale releases, could be detected in the form of multiple genetically distinct subpopulations. Since it is well established that adult L. vannamei mate off-shore where their planktonic larvae are subject to the randomizing effects of winds and tides (Benzie 2000), we would expect their postlarvae, which occupy shallow areas near shore, to form a genetically homogeneous, or panmictic, population within the approximately 5,000 km^2 area of the Gulf of Fonseca (Lester 1979). Numerous studies utilizing genetic markers have assessed genetic heterogeneity in penaeid shrimps, although these comparison have generally been concucted over distances of hundreds or even thousands of kilometers (Benzie et al. 1993; Bouchon et al. 1994; Tassanakajon et al.1998a, 1998b; Brooker et al. 2000; and Xu et al.2001, Zhuang et al. 2001). In spite of this, levels of genetic differentiation have generally been found to be low except where major biogeographic boundaries act to disrupt gene flow (Benzie 2000). We reasoned that genetic differentiation, should it occur within the relatively small overall area encompassed by the Gulf of Fonseca as a

result of inadvertent shrimp farm releases, would be relatively easy to detect, given the results of a study by France et al. (1999) which showed extensive genetic variation among populations of hatchery-raised shrimp.

Methodology

Postlarval shrimp (L. vannamei) were collected from 33 sites occupying estuaries surrounding the Gulf of Fonseca, Honduras (Fig. 1) during January and April of 2001. Collection sites were chosen opportunistically over the entire geographic range of the gulf as it borders the country of Honduras, including sites in the southern lobe as far south as Estero San Bernardo, and sites in Bahia San Lorenzo and Bahia Chismuyo as far west as Estero el Capulin. In order to detect the possible presence of localized genetic structure within estuaries, some estuaries were sampled at multiple points. All estuaries were bordered by red mangrove (Rhizophora mangle)-dominated intertidal swamps, although in many areas a narrow mangrove fringe was all that separated commercial shrimp ponds from the open waters of the estuary (Fig. 1).

Collections were performed in shallow shoreline waters using a hand-held seine with a fine mesh sufficient to capture postlarval shrimp $3 mm in length. Postlarval shrimp were hand-sorted from fish fry and other invertebrates into appropriately labeled plastic screw-cap tubes filled with absolute ethanol, and shipped to the US at ambient temperatures. In the laboratory, ethanol was rinsed from the specimens in deionized water for 15 minutes prior to DNA extraction.

DNA was extracted from whole postlarvae up to ≈ 2 cm in length according to the procedure of Coen et al. (1982) (tissue was sectioned from postlarvae > 2 cm to achieve a final volume approximately equivalent to a 2 cm-long individual). Extractions were

performed on 8-50 individuals per collection site, with the precise number depending on the abundance of postlarvae in a particular sample.

A genetic profile was developed for each individual postlarva from amplified fragment length polymorphisms (AFLP) according to the methods of Travis et al. (1996), with the following modifications. The procedure was performed on an initial quantity of DNA equaling 50 ng per sample. A single primer combination was used, consisting of the selective nucleotides ACG and AGT attached to the 3' end of the EcoRI- and MseI-primer, respectively. Electrophoresis of amplified fragments was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). This required the use of a fluorescently-labeled EcoRI-primer during the final selective restriction fragment amplification, which was performed on a 2-fold dilution (in TE, pH 8.0) of the preamplification product. A 10-fold dilution of the final reaction product was performed in deionized formamide prior to electrophoresis.

The presence or absence of amplified fragments was tabulated on a per individual basis using Genographer Version 1.6 (Benham 2001). Although several hundred fragments were identifiable over the entire sample with the use of this software, we adopted a highly conservative approach to fragment selection in order to avoid problems of low repeatability associated with certain loci. We accomplished this by setting the illumination intensity of the software within a narrow range (ca. 5 units) of its lowest possible value, and then selecting only those fragments and individuals for which $\geq$ 90% of the data could be unambiguously scored, i.e. only those fragments for which all fluorescence peaks fell within a narrow range of intensity values. In addition, in order to avoid introducing an upward bias into our analyses of population structure, all loci for

which the frequency of the dominant haplotype (x) was > 0.65 were omitted according to the $x < 3/N$ rule of Lynch and Milligan (1994), where N was set at a lower bound of 8 individuals per collection site.

Population structure was assessed using statistical methods specifically designed to overcome the shortcomings inherent in dominant marker data such as AFLP's (foremost among them the necessity of assuming Hardy-Weinberg equilibrium frequencies of alleles). First, an Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) was used to hierarchically partition the total genetic variance within and among estuaries surrounding the Gulf of Fonseca, using Arlequin Version 2.0 (Schneider et al. 2000). Second, traditional F-statistics were computed using a newly-developed Bayesian method which requires no prior knowledge of the level of inbreeding occurring within populations (Holsinger et al. 2002). Third, pairwise F-statistics were computed among all possible comparisons of collection sites using the F_{ST}-analog, F', based on the mean similarity coefficient, S, of Lynch (1990), where $F' = (1-S_{ij}) / \{2 - S_{ij} - [(S_i + S_j) / 2]\}$ for two populations i and j. The significance of pairwise F-statistics was evaluated on the basis of a permutation procedure which randomized individuals over each pair of populations under comparison, with the use of Arlequin Version 2.0 (Schneider et al. 2000). A total of 1,023 permutations were used to obtain each null distribution for the purpose of hypothesis testing under the null hypothesis of no differentiation, with alpha set at 0.025. A multivariate (UPGMA) cluster analysis was used to provide a visual representation of population structure with the aid of NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) Version 2.1 (Rohlf 2000). Clusters were formed on the basis of pairwise similarity coefficients calculated according to Lynch (1990).

Results

Although sample sizes were initially set at a minimum of 25 per collection site, a variety of unanticipated factors acted to severely limit these numbers, including the scarcity and unusually small sizes of postlarvae in several overharvested estuaries, the occasional contamination of samples with postlarvae of closely-related species, and difficulties in obtaining shipping materials necessary for adequate tissue preservation. As a result, the number of postlarvae of L. vannamei for which AFLP profiles were successfully constructed ranged from 2 to 22 individuals per collection site. In an effort to overcome statistical issues associated with small sample sizes, we therefore eliminated all samples with less than 8 individuals, leaving 19 collection sites in the overall sample, for a total N of 189 individuals (mean of 9.95 individuals per site; Table 1). We reasoned that any further statistical issues related to small sample sizes would only serve to reduce the power of our tests to detect significant patterns of differentiation within the Gulf of Fonseca, thereby rendering them more conservative.

Genetic structure was characterized from a total of 87 polymorphic molecular markers. Structure on a local scale was explored by AMOVA, with 16 groups formed from the 19 collection sites included in the statistical comparisons. Each group represented a single estuary: 13 estuaries were represented by a single collection site, while 3 of the larger estuaries were represented by 2 collection sites. The results of this AMOVA are shown in Table 2a, and demonstrate that differentiation among estuaries contributes 17.06% of the genetic variation within and among shrimp populations occupying the Gulf of Fonseca ($df = 15$, $p = 0.0577$). Within group differences among the collections sites also contributed a substantial portion of the higher order genetic

partitioning within the Gulf, accounting for an additional 15.48% of overall genetic variation (df =3, $p < 5 \times 10^{-6}$). Overall, the level of differentiation among collection sites represented 33% of total genetic variation among L. vannamei in the Gulf of Fonseca (df = 18, $p < 5 \times 10^{-6}$). Holsinger's Bayesian estimator (Holsinger et al. 2002) corroborated these results, with an overall F_{ST}-value of 0.25 " 0.01 (s.d.). Taken together, these findings indicate that the multiple collection sites within some of the larger estuaries are only marginally more similar to one another than they are to samples collected from other estuaries.

A UPGMA cluster analysis, performed on the entire sample of 189 postlarvae, failed to show a hierarchical pattern of relationships within and among estuaries of the Gulf of Fonseca, and will therefore not be presented. However, when the clustering algorithm was applied to each collection site individually, a very clear pattern was detected for 18 of the 19 collection sites (the sample from Estero el Pedregal represented the sole exception). For each of these sites, there existed a core group of genetically homogeneous shrimp, each sharing > 45% of their molecular markers in common with all others. For 13 of these sites, there were also several outliers which were highly distinct from all other postlarvae in the sample. This pattern is illustrated in Fig. 2 for the Boca de Conchalitos site. Once this pattern was detected, all outliers were omitted, and the cluster analysis repeated for these 18 collection sites. For the sake of illustration, a dendogram is presented in Fig. 3, which was constructed from the composite AFLP profile of each core group (composite profiles were constructed by assigning the presence of a marker only when it was detected in > 50% of the individuals sampled from a site).

A second fixation index was calculated on the basis of Holsinger's Bayesian estimator (Holsinger et al. 2002) , using only the reduced set of 144 postlarvae representing the 18 collection sites for which a genetically homogenous core group of individuals could be identified (see Table 1 for a list of reduced sample sizes by site). The overall F_{ST} calculated from these data was striking at 0.35 " 0.01 (s.d.).

Likewise, the pairwise F-statistics calculated from the reduced data set (Table 3) were strikingly large for many of the comparisons among collection sites. On the basis of this analysis, a total of 10 statistically independent phenetic clusters were apparent, each consisting of the postlarvae collected from 1-5 distinct locations. The breakpoints for each cluster are shown in Fig. 2 as diagonal slashes. Differentiation among collection sites within statistically supported clusters averaged just 0.0332 " 0.0476 (s.d.), while differentiation among pairs of collection sites from separate clusters averaged well over an order of magnitude larger at 0.4785 " 0.1266.

The results of a second AMOVA, with the higher-order groups defined on the basis of the significance of the pairwise F-statistics, are presented in Table 2b. This analysis yielded a strikingly large component of genetic variance attributable to differentiation among groups, accounting for 49% of the total genetic variation (df = 5, $p < 5 \times 10^{-6}$), which reflects the high level of coherence within phenetic clusters. Based on this analysis, partitioning among collection sites within groups was statistically in spite of accounting for < 1.5% of overall variation (df = 8, , $p < 5 \times 10^{-6}$).

Discussion

The results of this study indicate pronounced genetic differentiation among the estuaries surrounding the Gulf of Fonseca in Honduras, with the among-site component

of genetic variance accounting for >30% of the overall level of genetic variation and an F_{ST} of 0.25. Following the identification of phenetic clusters formed from samples representing multiple estuaries, this value climbed to nearly 50%. This level of differentiation is quite remarkable when considered in light of what is known of the life history of L. vannamei, and the results of similar studies. We found pairwise F_{ST}-values ranging up to a maximum of 0.70 when comparing populations representing distinct phenetic clusters. This is substantially greater than the values reported from other studies of penaeid shrimps which have relied on neutral molecular markers. For example, based on 35 RAPD markers, Zhuang et al. (2001) conducted a comparison of P. chinensis populations between the Bohai and Yellow Seas off the coast of southern China, and found values of G_{ST} averaging approximately 0.30. Several additional studies employing RAPD markers have compared populations in terms of Lynch's (1990) measure of interpopulational dissimilarity (simply the converse of the average between-population similarity corrected for within-population similarity). For example, Tassanakajon et al. (1998a) found maximum dissimilarity values of 0.245 in a comparison of Penaeus monodon populations from opposite sides of the Malaysian peninsula based on 58 RAPD markers, while Klinbunga et al. (2001) found much lower values ranging from – 0.002 – 0.037 when the same species was compared over smaller spatial scales on the basis of 53 RAPD markers. The former study sampled from sites separated by up to 1,000 km, while the latter focused on populations within 650 km. For the purposes of comparison, we recalculated our estimates of differentiation according to the same methods, and found that they ranged up to a maximum of 0.407. Interestingly, Garcia et al. (1994) found a maximum dissimilarity of 0.25 based on 65 RAPD markers during a comparison of four

captive families of L. vannamei originating in Mexico and Ecuador, although it is unclear whether these values were corrected for within-population similarity.

Compelling evidence for the frequent escape of L. vannamei postlarvae from aquaculture ponds into the nearby estuaries of the Gulf of Fonseca is provided not only by the unexpectedly high levels of genetic differentiation among estuaries, but also by large average coefficients of inbreeding. Unfortunately, the high concentration of shrimp farms surrounding the entire gulf (see Fig. 1) was such that we were unable to obtain samples from truly pristine estuaries that might have been used to rule out the possibility that the levels of genetic differentiation we observed were actually the result of some heretofore unidentified natural process. However, we were able to bolster our conclusions by estimating inbreeding coefficients using the same Bayesian method used to generate estimates of F_{ST}, although considerable caution must be taken in the interpretation of these values because they are much less constrained by the Bayesian model (Holsinger et al. 2002). The sheer magnitude of the inbreeding estimates derived from the current data set, which were in excess of 0.99, is again consistent with the escape of highly inbred lines of shrimp commonly raised by the aquaculture industry throughout the world (Sbordoni et al. 1986; Harris et al. 1990; Sunden and Davis 1991; Wolfus et al. 1997; Xu et al. 2001). Evidence of the mixing of wild and hatchery-raised shrimp has also been reported for P. monodon from a wild population in the Philippines, in the form of deviations from expected Hardy-Weinberg equilibrium frequencies among the alleles of 6 microsatellite loci (Xu et al. 2001). In addition, Klinbunga et al. (2001) reported contradictory results between nuclear (RAPD) and mtDNA markers in their assessment of genetic differentiation of P. monodon populations across the Malaysian

peninsula which they hypothesized could have been the result of localized wild stock displacement by aquaculture activity.

Perhaps the most telling result in support of the escape of captive shrimp into the Gulf of Fonseca is provided by our finding that several phenetic clusters, comprised of individuals from multiple collections sites around the Gulf, were not limited to a single geographically isolated area. Figure 1 displays a graphic representation of the disjunct distribution of these clusters, with each colored (non-black) oval in the figure representing a cluster with representatives from multiple collection sites. In fact, three of the four clusters comprised of samples from more than a single collection site could be localized to 2 separate areas, in each case occupying two distinct lobes of the Gulf. It is difficult to envision how such a pattern could have arisen through either the passive movement of planktonic larvae, or the active movement of adults or postlarvae. Tassanakajon et al. (1998a) uncovered a similar pattern of population clustering, explicable only in terms of shrimp farm releases, in P. monodon from the waters surrounding the Malaysian peninsula.

Several possible alternative explanations for the observed results, namely that the behavioral attributes or ecological requirements of the postlarvae caused them to be recruited to their natal estuaries, or that a small number of family cohorts were overrepresented in the data, deserve consideration. Recent evidence from a Caribbean reef fish (Elactinus evelynae) shows that, in spite of producing larvae that remain pelagic for about 3 weeks, reefs lying within as little as 23 km can be highly differentiated (Taylor and Hellberg 2003). However, the average distance among collection sites found to be highly differentiated in our study of L. Vannamei were at times separated by < 3

km, and, unlike the reef fish studied, we were focusing on an organism whose adults are pelagic and do not release eggs in the vicinity of their natal estuary. The other alternative, that a so-called "sweepstakes effect" was responsible for distributing the postlarval cohorts of a limited number of highly fecund females among a disjunct distribution of estuaries, also seems unlikely given the sheer number of eggs that a single female would have to produce to create such an effect, as well as the fact that previous studies utilizing detailed temporal sampling of marine species with life histories optimally suited to producing such an effect have yielded negative results (Flowers et al. 2002).

The consequences of these findings to the natural populations of the Gulf of Fonseca, as well as the shrimp aquaculture industry, are mixed. The core group data, in particular, suggest that postlarvae escaping from aquaculture ponds into the adjacent estuaries remain quite isolated for at least several months before presumably making their way into the open water of the Gulf for the purpose of mating. Given the highly limited time frame under which epizootics like white spot spread through localized populations, this sort of gradual genetic assimilation of the aquaculture escapees into the overall population should preclude the occurrence of a catrastrophic disease event. Such an event would not only impact local fisherman who rely on shrimp for their subsistence, but would also eliminate an important source of postlarvae for the aquaculture industry, since many commercial operations purchase at least a portion of their postlarval stocks (> 60% in 1993; DeWalt et al. 1996a) from local sources. On the other hand, the mixing of wild and hatchery-raised stocks could bring about a reduction in overall fitness levels of shrimp occupying the Gulf of Fonseca if the escapees are poorly adapted to the local

environment through differences in historical selection pressures, random drift, or the negative effects of inbreeding that could accompany an artificial bottleneck (see Zhuang et al. 2001 for a similar discussion related to P. Chinensis). This could lead to a further decline of the L. vannamei population in the Gulf, which is already under stress from overharvesting of postlarvae, destruction of nursery habitat, and deteriorating water quality (DeWalt et al. 1996a, 1996b). Similarly, Klinbunga et al. (2001) conclude that, due to potential adaptive differences between natural P. Monodon populations and hatchery-reared larvae in Thailand, the pollution of locally-adapted gene pools is a matter of national concern. In the Gulf of Fonseca, the genetic integrity of the native L. vannamei population has been severely compromised, resulting in the potential loss of a valuable genetic resource for future farming practices (Benzie 2000).

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Unpublished Materials

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Table 1. Sample sizes before and after the exclusion of genetic outliers for collection sites by estuary and site within estuary.

Estuary	Site Within Estuary	Sample Size (N)	No. In Core Group
Estero el Pedregal		10	0
Rio Choluteca		9	8
Estero Purgatorio		22	16
Estero Guipo	Left Branch	8	8
Estero Guipo	Right Branch	10	10
El Cubo		9	9
Guapinol		10	8
Estero Guapinol	Left Branch	9	9
Estero Guapinol	Right Branch	13	10
Boca de Conchalitos		10	7
Paso la Oscurana		9	8
Paso Playa Salada		9	6
Estero Playa Salada		9	4
Esteron		9	6
Estero el Apintal		9	5
Boca de la Brea	Lower	10	10
Boca de la Brea	Upper	8	5
Estero el Cubolero		8	8
Estero el Carrizo		8	7

Table 2. Analysis of Molecular Variance (AMOVA), based on 87 AFLP markers, for Litopenaeus vannamei populations collected from multiple estuaries adjoining the Gulf of Fonseca, Honduras: a) Nested AMOVA for complete data set consisting of 189 postlarval shrimp from 16 estuaries and 19 collection sites. b) Results from reduced data set (N = 144) consisting of only the 18 collection sites with a core group of three or more genetically similar postlarvae.

	Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	F_{ST}
a)						
	Among Groups	15	586.25	1.58	17.06	--
	Among Collection Sites Within Groups	3	59.60	1.44	15.48	--
	Within Collection Sites	170	1,064.06	6.26	67.46	--
	Total	188	1,709.92	9.28	--	0.25
b)						
	Among Collection Sites	9	648.34	4.93	49.05	--
	Among Collection Sites Within Groups	8	48.93	0.15	1.44	--
	Within Collection Sites	126	626.59	4.97	49.51	--
	Total	143	1,323.86	10.04	--	0.35

Table 3. Matrix of pairwise fixation indexes and their probablities for Litopenaeus vannamei compared among estuaries (N = 18) adjoining the Gulf of Fonseca, Honduras, following the exclusion of genetic outliers. F-statistics are given below the diagonal; p-values, above the diagonal.

	RC	EP	LBEG	RBEG	EC	G	LBEGl	RBEGl	BDC
Rio Choluteca	--	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Estero Purgatorio	0.4134	--	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Left Branch Estero Guipo	0.6195	0.5284	--	0.1504	0.2812	< 0.001	0.5840	< 0.001	< 0.001
Right Branch Estero Guipo	0.6018	0.5268	0.0289	--	0.6289	< 0.001	0.2070	< 0.001	< 0.001
El Cubo	0.5639	0.4831	0.0364	-0.0133	--	< 0.001	0.2246	< 0.001	< 0.001
Guapinol	0.5457	0.4798	0.4842	0.5277	0.4269	--	0.0010	0.6807	< 0.001
Left Branch Estero Guapinol	0.5779	0.4926	-0.0350	0.0042	0.0151	0.3807	--	0.0010	< 0.001
Right Branch Estero Guapinol	0.5549	0.4729	0.3959	0.4501	0.3396	-0.0183	0.2911	--	< 0.001
Boca de Conchalitos	0.4914	0.4095	0.3657	0.3600	0.3010	0.4797	0.3227	0.4414	--
Paso la Oscurana	0.4691	0.4347	0.3276	0.3294	0.2861	0.5117	0.3145	0.4689	0.0951
Paso Playa Salada	0.5167	0.4594	0.5791	0.4882	0.4526	0.5489	0.5074	0.5346	0.4576
Estero Playa Salada	0.4828	0.0472	0.6384	0.6248	0.5860	0.5574	0.5916	0.5614	0.5222
Esteron	0.4198	0.0830	0.5907	0.5709	0.5297	0.5228	0.5458	0.5266	0.4632
Estero el Apintal	0.5918	0.5555	0.5741	0.5529	0.4890	0.5984	0.5232	0.5751	0.2704
Lower Boca de la Brea	0.6012	0.4411	0.2611	0.2050	0.2019	0.6076	0.2511	0.5488	0.3665
Upper Boca de la Brea	0.4816	0.4533	0.4668	0.4532	0.4022	0.5352	0.4436	0.5081	0.1723
Estero el Cubolero	0.6475	0.5725	0.0408	0.0392	0.0937	0.6003	0.0839	0.5351	0.3768
Estero el Carrizo	0.5551	0.4747	0.6107	0.6414	0.5632	0.1146	0.5486	0.2316	0.5500

Table 3. (Continued)

	PLO	PPS	EPS	E	EEA	LBDLB	UBDLB	EEC	EECz
Rio Choluteca	< 0.001	< 0.001	0.0020	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Estero Purgatorio	< 0.001	< 0.001	0.4150	0.02540	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Left Branch Estero Guipo	< 0.001	< 0.001	0.0020	< 0.001	< 0.001	< 0.001	0.0010	0.1300	< 0.001
Right Branch Estero Guipo	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0010	0.1436	< 0.001
El Cubo	< 0.001	0.0010	0.0010	0.0010	< 0.001	< 0.001	< 0.001	0.1182	< 0.001
Guapinol	< 0.001	0.0010	0.0010	< 0.001	0.0020	< 0.001	< 0.001	< 0.001	0.0029
Left Branch Estero Guapinol	< 0.001	< 0.001	0.0010	< 0.001	0.0010	< 0.001	0.0010	0.0654	< 0.001
Right Branch Estero Guapinol	< 0.001	< 0.001	0.0010	0.0010	0.0010	< 0.001	< 0.001	< 0.001	< 0.001
Boca de Conchalitos	< 0.001	< 0.001	0.0029	0.0010	0.0020	< 0.001	0.0166	< 0.001	< 0.001
Paso la Oscurana	--	< 0.001	0.0010	< 0.001	< 0.001	< 0.001	0.0068	< 0.001	< 0.001
Paso Playa Salada	0.4863	--	0.0068	0.0029	0.0020	< 0.001	0.0020	< 0.001	< 0.001
Estero Playa Salada	0.5306	0.5289	--	0.5664	0.0068	0.0010	0.0078	0.0020	0.0039
Esteron	0.4680	0.4524	-0.0308	--	0.0020	< 0.001	0.0039	< 0.001	0.0020
Estero el Apintal	0.2642	0.5482	0.6261	0.5438	--	< 0.001	0.0918	< 0.001	0.0020
Lower Boca de la Brea	0.3399	0.5816	0.5483	0.5069	0.5836	--	0.0010	< 0.001	< 0.001
Upper Boca de la Brea	0.1461	0.4796	0.5431	0.4426	0.1225	0.4685	--	< 0.001	0.0010
Estero el Cubolero	0.3482	0.6036	0.6697	0.6266	0.5971	0.2020	0.4998	--	< 0.001
Estero el Carrizo	0.5566	0.5699	0.5403	0.5169	0.6501	0.6821	0.5777	0.7022	--

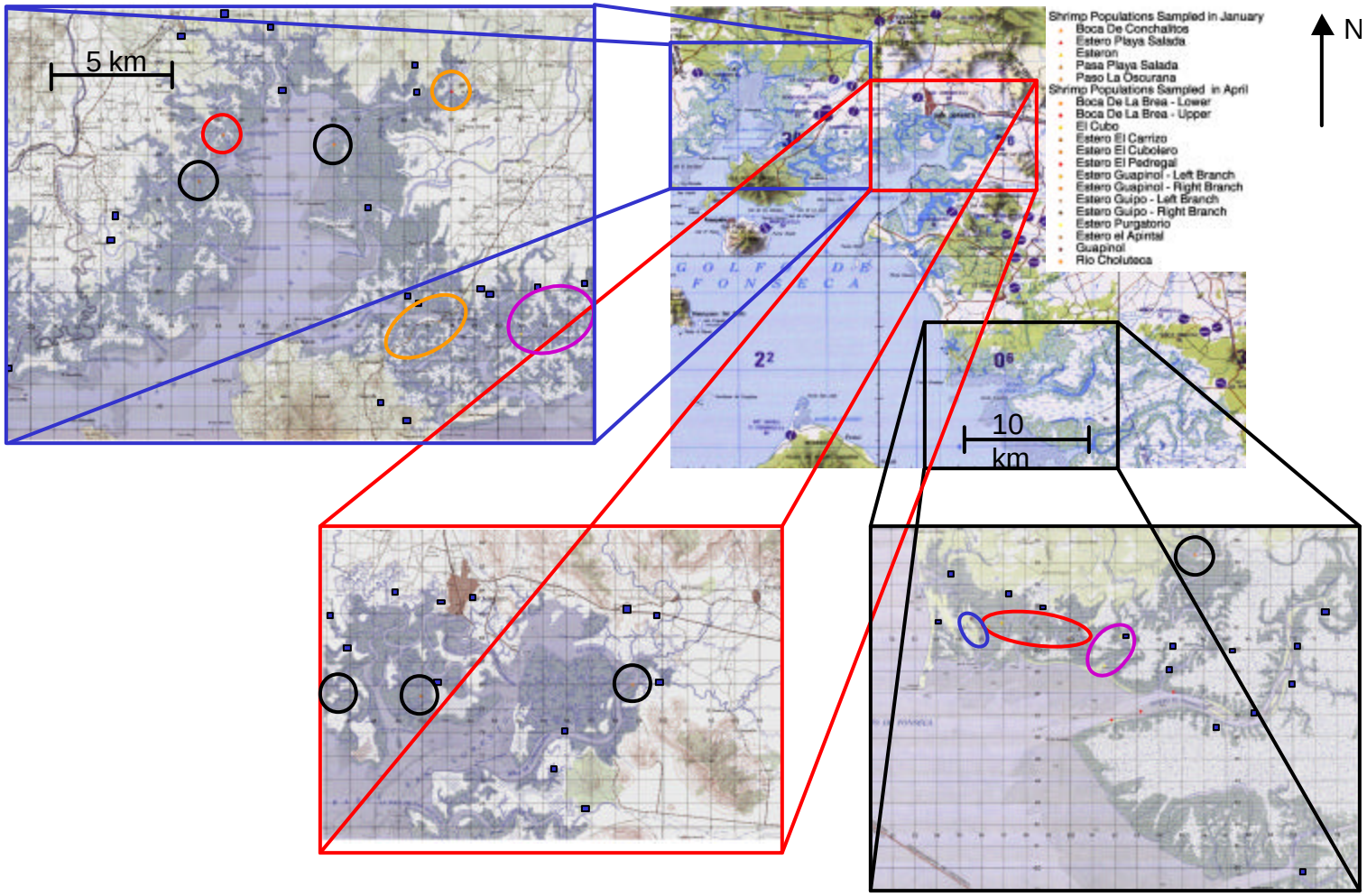

Fig. 1. Map of the the Gulf of Fonseca, Honduras, showing the 19 collection sites for Litopenaeus vanname*i* postlarvae for which data are reported. Colored (non-black) ovals encircle 4 distinct phenetic clusters, three of which display disjunct distributions spanning multiple lobes of the Gulf (blow-up boxes). Black ovals encircle collection sites that were genetically distinct from all others. Shrimp farms are represented by blue-filled boxes. All collections were conducted within a tetrahedral area with intersections at the following coordinates (proceeding clockwise from the southwestern extreme): north latitude (N) 13º 22' 5.3", west longitude (W) 87º 43' 1.6"; N 13º 27' 0.2", W 87º 39' 45.1"; N 13º 28' 3.2", W 87º 34' 20.2"; and N 13º 1' 28.6", W 87º 17' 44.6".

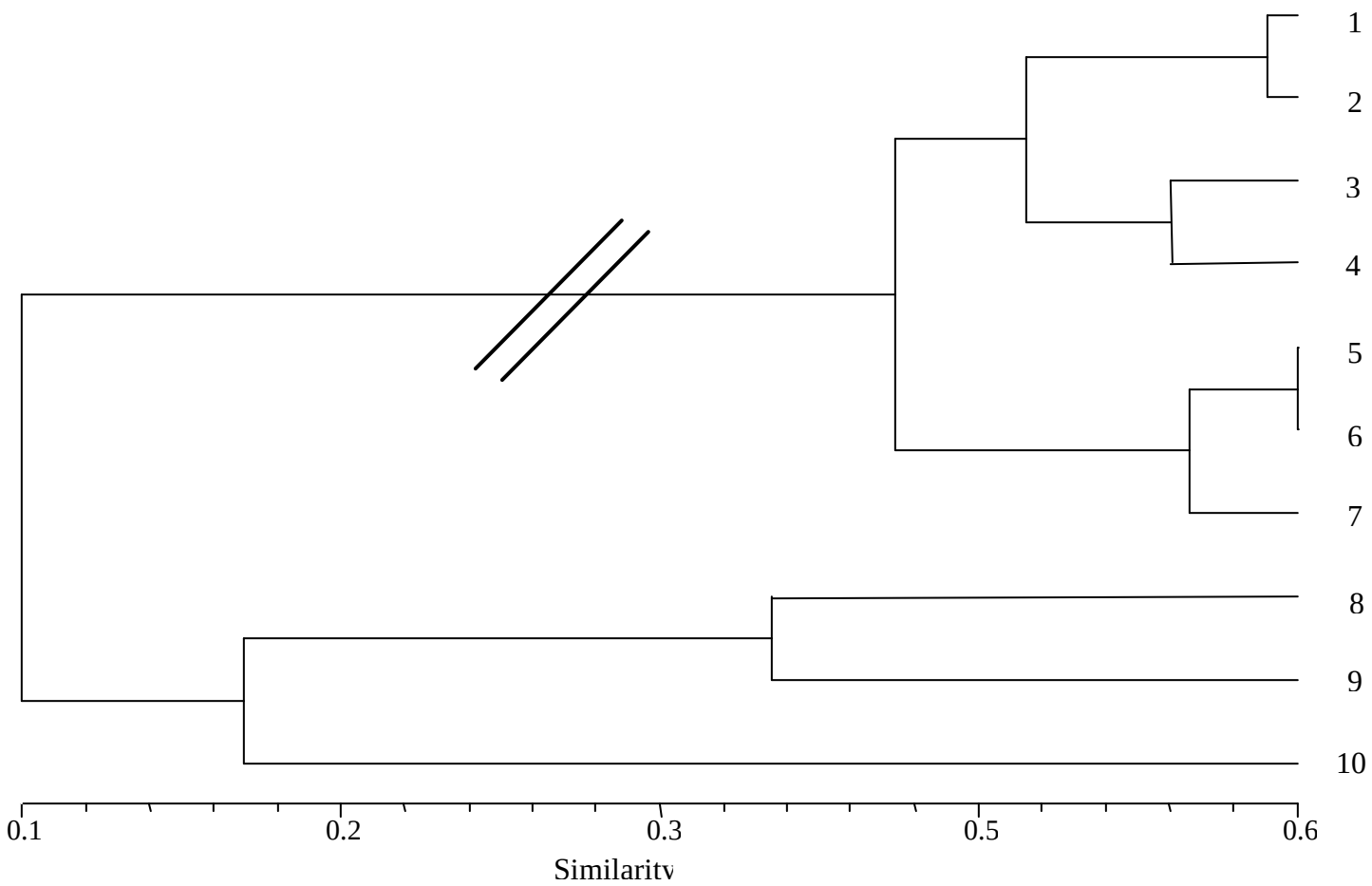

Fig. 2. UPGMA cluster analysis of similarity coefficients (Lynch 1990) calculated on the basis of 87 AFLP markers from 10 postlarvae of Litopenaeus vannamei collected from la Boca de Conchalitos, Gulf of Fonseca, Honduras. Diagonal slashes represent a breakpoint between a core group of 7 genetically similar individuals and 3 outliers.

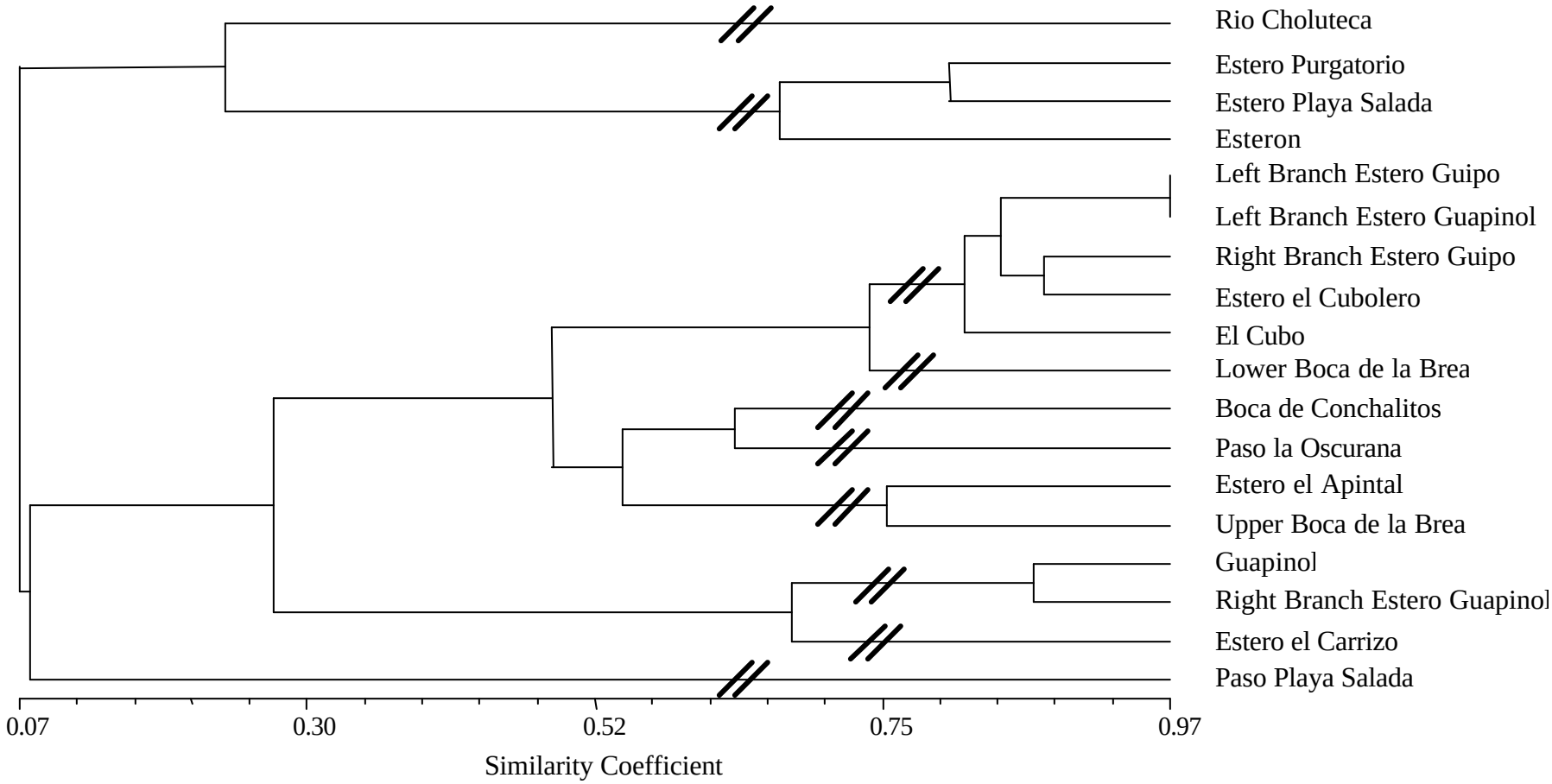

Fig. 3. UPGMA cluster analysis of similarity coefficients (Lynch 1990) calculated from 18 composite haplotypes of Litopenaeus vannamei postlarvae, each consisting of 87 AFLP markers, and representing estuaries adjoining the Gulf of Fonseca, Honduras. The diagonal slashes represent statistically supported breakpoints, consistent with the overall analysis of 144 individuals, between 10 distinct branches separating groups of from 1 to 5 collection sites each.