2009

What's up with Fiddler Crabs: Does Larval Dispersal Render Genetic Similarity in the Disjunct Distribution of *Uca minax* (*Uca*: Ocypodidae)?

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WHAT'S UP WITH FIDDLER CRABS:

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IN THE DISJUNCT DISTRIBUTION OF *UCA MINAX* (*UCA*: OCYPODIDAE)?

A Thesis

Submitted

in Partial Fulfillment

of the Requirements for the Designation

University Honors

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May 2009

This Study by: Alexa Warwick

Entitled: What's up with fiddler crabs: does larval dispersal render genetic similarity in the disjunct distribution of *Uca minax* (*Uca*: Ocypodidae)?

has been approved as meeting the thesis requirement for the Designation

University Honors

5/4/09
Date — Dr. Peter Berendzen, Honors Thesis Advisor, Biology

5/5/09
Date — Dr. Carl Thurman, Honors Thesis Advisor, Biology

5/8/09
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ABSTRACT

The red-jointed fiddler crab, *Uca minax,* has a disjunct distribution along the Atlantic and Gulf coasts in North America. Previous studies have not found any significant discrepancies in morphology, physiology, or allozymes between the two groups of *U.minax.* The primary objective of this study was to use DNA sequence data to test whether genetic variation is consistent with patterns of morphological, physiological, and allozyme variation of the disjunct populations. We collected mitochondrial cytochrome oxidase subunit I and nuclear internal transcribed spacer sequences from approximately 100 individuals distributed across the range. Phylogenetic analyses of the haplotypes revealed no genetic differentiation among the Gulf and Atlantic groups, which is consistent with previous studies. Both genes also showed little variation among individuals. A second objective was to determine how and when these two groups diverged. Based on BEAST and IM analyses, the divergence of the two groups appears to be recent, with massive expansion. These results support the potential for a glacial refugium and subsequent expansion into the current range during the last glacial maximum.

INTRODUCTION

Throughout the development of the discipline of phylogeography, the southeastern United States has been an important and intensely researched geographic area, primarily owing to the extensive work of John Avise and his collaborators (reviewed in Avise 1992). The area encompassed in this region includes Cape Cod, Massachusetts on the western Atlantic Ocean (with a northward extension in some species) continuing all the way to Texas in the Gulf of Mexico. It typifies a model system of neighboring sister areas that are influenced by the rise and fall of barriers to gene dispersal (Cunningham & Collins 1998). Coastal taxa in the southeastern U.S., labeled Carolina fauna by Briggs (1974), include both disjunct and continuous distribution patterns, with most marine taxa having good dispersal ability with planktonic larvae (Cunningham & Collins 1998). Due to the plethora of taxa sharing this disruption of gene flow among populations, a connection is suggested between microevolution processes and macroevolution differences among species in this region (Avise *et al.*1987; Avise 2000).

Much of the research done in this area has considered coastal marine taxa, with varying degrees of differentiation between Gulf and Atlantic groups, but many have the common feature of larval dispersal in the open ocean. In Avise (1992), three different scenarios were reviewed for southeastern U.S. taxa. First, some continuously distributed taxa did not have significant differentiation between groups, such as the coastal American eel (*Anguilla rostrata*) and hardhead catfish (*Arius felis*). However, other continuously distributed taxa, such as the estuarine horseshoe crab (*Limulus polyphemus*) and American oyster (*Crassostrea virginica*) did exhibit differentiation within their range. Other studies have found similar cryptic genetic species patterns within a continuous range, such as the mussel *Geukensia* (Sarver *et al.* 1992) and the

clam *Mercenaria* (Ó Foighil *et al.* 1996). Lee & Ó Foighil (2004) also found multiple points of differentiation in the continuous distribution of the scorched mussel (*Brachidontes exustus*), and the divergence between the Gulf/Atlantic disjunction was estimated to be fairly old. For another disjunctly distributed species, the brachyuran crab *Seasarma reticulatum,* Felder & Stanton (1994) found that genetic differentiation was significant enough to potentially cause speciation, a result that Schubart *et al.* (2000) also confirmed. Cunningham *et al.* (1992) studied the mtDNA of hermit crabs, which yielded a significantly degree of divergence between the disjunct Atlantic and Gulf groups for *P. pollicaris*, while there was little genetic variation across the disjunct distribution of *Pagarus longicarpus*. However, a later study of *P. longicarpus* confirmed divergence across the Atlantic and Gulf populations for allozymes, mtDNA, and morphology (Young *et al.* 2002). Another example using the stone crabs *Menippe mercenaria* and *M. adina*, though different from previous examples due to their hybrid zone, showed little genetic divergence between the Atlantic and Gulf groups, although they are considered separate species (Schneider-Broussard *et al.* 1998). And, two fish species, that inhabit both freshwater and estuarine/coastal waters during their life cycles, sturgeon (*Acipenser oxyrhynchus*) and menhaden (*Brevoortia* spp.), also had very similar genetic makeup, though in both species their distributions are disjunct.

In addition to these aforementioned taxa, fiddler crabs (genus *Uca*: family Ocypodidae) have been studied as part of this unique geographic area. *Uca* spp. burrow in mud or sand on the coast in tropical, subtropical, and temperate regions around the world, with thirteen fiddler crab species in the southeastern region of the United States. However, only one species is continuously distributed across most of this geographic area, *Uca pugilator* (Bosc 1802) while another species, *U. minax* (LeConte 1855), is the only fiddler crab disjunctly distributed around

Florida in the same region (Figure I). While *U. minax,* in particular, is not currently considered a threatened species (IUCN), fiddler crabs in general do play an essential role in coastal habitats. As a result of their importance as a food source at higher trophic levels, as well as their co-evolution with the mangrove ecosystem in which they live, fiddler crabs are considered an indicator species for coastal habitat quality and health (Crane 1975; Hogarth 2004; Beinlich & von Hagen 2006; Macintosh 1982; Bertness 1985; Bertness 2007). *Uca* spp. also have strong sexual dimorphism and are euryhaline. But a species can have a preferred habitat salinity level. For example, Brodie *et al.* (2005) showed that adult *U. minax* are usually found in areas with low salinity (< 10‰) and near-freshwater conditions, and can even be found many kilometers inland located in tidally influenced freshwater rivers and streams. However, such a distribution is atypical for fiddler crabs in general (Crane 1975).

The unique, disjunct distribution of *U. minax* among southeastern U.S. fiddler crab species is considered to be maintained by the difference in habitat type along peninsular Florida, as well as *U. minax*'s habitat preference. First, a substrate change at Apalachee Bay from terrigenous sediments to carbonate sediments on the Florida coast has the potential to break the distribution of fiddler crabs (Barnwell & Thurman 1984). Briggs (1974) also identified the transition from temperate to tropical species zones on the Gulf side at Naples, FL and on the Atlantic side at Cape Canaveral, FL. In *U. minax*, Cape Canaveral is the furthest extent of the Atlantic population, while the Crystal River on the west coast of Florida is the range limit for the Gulf populations (pers. comm. S.A. Borgianini).

Like many other marine invertebrates, most fiddler crab species, including *U. minax* and *U. pugilator*, have planktonic larvae (five zoeal stages in *U. minax*) that develop in the coastal ocean (salinity ~35 psu) for approximately 6-8 weeks and subsequently reinvade adult

communities within estuaries (salinity range from 0 to 32 psu) for juvenile development (Behum *et al.* 2005; Godley & Brodie 2007). Fiddler crabs have high fecundity, about 30,000-50,000 eggs, combined with high larval mortality (Thorson 1950; Young & Chiaa 1987). Little else is known about their pelagic period of development, but *Uca* larval forms have been collected as far as 25 km off the western Atlantic shore (Hyman 1920; Johnson & Allen 2005). They are not likely to return to their parental home but the effect of possible settlement cues have yet to be established for *U. minax* (Brodie *et al.* 2005), but oceanic currents do have the potential to be a primary dispersal mechanism.

On account of the paucity of available fossil records (Brito 1993), molecular evaluation is particularly important in order to study the distribution and genetic differentiation in *Uca* spp. Comparable to some of the already described coastal marine taxa, previous studies of *U. minax* have not found significant discrepancies in morphology, physiology, or allozymes between the Gulf and Atlantic distributions (Thurman 1982; Thurman 2002; Thurman 2003b; Felder & Staton 1994). However, genetic differentiation has not yet been adequately addressed. On account of incomplete genetic data for *U. minax*, the current study augments previous data by sequencing and analyzing both a nuclear (ITS) and a mitochondrial (COI) gene across the range of the species, in order to answer the first objective of our study: Is genetic variation of COI and ITS in *U. minax* consistent with patterns from previously published morphology, physiology, and allozyme data?

Due to the disjunct distribution of the species, it is expected that *U. minax* would have significant genetic differentiation. However, historical patterns that could influence gene flow, and therefore genetic structuring, must also be considered as a second objective of this study. Two potential, temporally distinct hypotheses for this region have been previously suggested in

other taxa to explain their evolutionary history of coalescence (Avise 1992; Schneider-Broussard *et al.* 1998; Lee & Ó Foighil 2004; Lee & Ó Foighil 2005). The two scenarios are: 1) an earlier Gulf and Atlantic connection through the Suwannee Straits, with possible expansion from the Gulf, and 2) a more recent colonization or re-colonization and expansion from a refugial population in southern Florida.

The first biogeographical scenario considers historical climatic changes that controlled the emergence of landmasses due to the rise and fall of sea levels around Florida. During the Miocene (13–25 million years before present: MYBP), the Okeefenokee Trough formed a shallow seaway called the Suwannee Straits, separating the states of Georgia, Alabama and Mississippi from the north end of what was insular Florida. This seaway could have allowed gene flow through larval dispersal between the Gulf and Atlantic bodies of water (Schneider-Broussard 1998). The last opening of the Suwannee Straits occurred during the late Pliocene. The lowered sea levels and subsequent closure of the seaway would have effectively isolated *U. minax*, a temperature zone species that does not survive in the tropical southern Florida coastline (Barnwell & Thurman 1984).

The second biogeographical scenario considers the impact from glacial advances and the creation of refugia during climatic cooling. The area currently under study appears to have been a refugial region for many species south of the Laurentide ice sheet, and postglacially these species could have colonized northwards (Hewitt 2004). Additionally, early work on the biogeography of Gulf fiddler crabs by Deevey (1950) proposed the southern tip of the Florida peninsula to be a refugium for temperature species displaced at the height of the Wisconsin glaciation (18,000 YBP) during the Pleistocene, due to the lower sea-surface temperatures. Average annual temperatures along the Gulf coast were about 5-10°C cooler than today, and

temperate species like *U. minax* would have been forced to seek warmer refuges, such as southern Florida or Mexico.

The combination of historical climatic change and its direct and indirect consequences including glacial advance/retreat, sea level rise/fall and oceanic current fluctuations, along with the climatic limitations on biotic habitat composition would have been influential on the expansion direction/rate in *U. minax*, and therefore their genetic structure and differentiation. In regards to time of coalescence, an earlier split, such as the last closing of the Suwannee Straits, would have allowed more time and therefore more genetic mutation and variation to arise between the two geographic areas. On the other hand, a more recent divergence between the two groups, such as the rise of a barrier (tropical south Florida) to gene flow after a glacial maximum, would imply less time for genetic mutations to develop and therefore less genetic differentiation.

MATERIALS AND METHODS

Sampling

In order to investigate the genetic structure of *U. minax*, tissue samples were obtained from 15 distinct localities along the Atlantic and Gulf coasts of North America between June 2005 and December 2007 (Table I). Immediately after collection the samples were stored in 95% ethanol. Based on recent phylogenetic analyses, we used *U. mordax* and *U. panacea* (COI only) as outgroups.

DNA sequencing

Total genomic DNA was isolated from the leg tissue of approximately ten individuals per locality using either standard guanidine thiocyanate extraction protocols for muscle tissue or Qiagen DNEasy kits (Qiagen). Tissues were digested overnight at 55°C in 300μL of cell lysis buffer (1.975 M NaCl, 125 mM Tris-Cl, 25 mM EDTA, 5% SDS) with 2μL Proteinase K. We purified the DNA with a protein precipitate solution (4M guanidine thiocyante, 100 mM Tris-Cl), 100% isoproponol precipitation, and 70% ethanol wash.

The complete nuclear internally transcribed spacer (ITS) and the mitochondrial cytochrome oxidase subunit I (COI) DNA regions were amplified using the polymerase chain reaction (PCR). PCR reactions were performed on a thermocycler (Bio-Rad, MyCycler™) in a total volume of 25 μl containing ~1.0 μg of DNA, 2 μM of each primer, 10X *Taq* salts (Promega GoTaq Green Master Mix), 10 mM dNTPs, and 1.25 units of *Taq* DNA polymerase (Promega). The following thermal profile was used: initial denaturation at 94°C (2 min); 40 cycles of 94°C (30 sec), 55.5°C (30 sec), 72°C (1 min); and a final extension at 72°C (7 min) before termination of the reaction at 4°C. The forward-strand primer for ITS (5' – CAC ACC GCC CGT CGC TAC TAC CGA TT – 3') and the reverse-strand primer for ITS (5' – ATC GAC CCA TGA GCC GAG TGA TC – 3') (Behum *et al.*, 2005) were used, as well as the forward-strand primer for COI (5' – CCT GCA GGA GGA GGA GAY CC – 3') and the reverse-strand primer for COI (5' – AGT ATA AGC GTC TGG GTA GTC – 3') (Schubart *et al.*, 1998). The resulting amplified product was run on 1% agarose gel, excised over UV light, to visually confirm DNA presence, and then purified with Exo Sap-it. Sequencing for both strands of the amplified fragments was completed at the DNA Sequencing Facility at Iowa State University in Ames, IA, USA. Sequences for outgroups were obtained using the same protocols.

Assemblage and manipulation of the sequences was done in SEQUENCHER 4.2 (Gene Codes Corp., Ann Arbor, MI, USA) in order to generate the data set. Due to the presence of indels (insertions and deletions) for the nuclear gene ITS, PHASE 2.1 (Stephens *et al.* 2001) was used to generate a haplotype reconstruction of the data set before beginning phylogenetic analyses. COI sequences were aligned easily due to an absence of indels. All novel DNA sequences will be deposited in GenBank upon publication of this study.

Data analysis

Unique haplotypes for each gene were determined in Collapse (Posada 2004), where missing data were not treated as a fifth state. The data set was then pruned to only include unique haplotypes for most of the phylogenetic analyses. The number of polymorphic sites and the spatial structure of genetic variation among localities were estimated using AMOVA (Analysis of molecular variance approach, Excoffier *et al.* 1992) in ARLEQUIN version 3.1 (Schneider *et al.* 2000).

We determined the models of sequence evolution using MrModeltest2.2 (Nylander 2004) software based on Akaike information criterion (AIC) (Pousada & Buckley 2004) for both the complete data sets, as well as the partitioned COI data set. Partitioning of COI was determined through a comparison with a complete COI gene (Genbank # FJ455507), with the sequence under study beginning at the 669th base pair and continuing to the 1328th base pair of the completely sequenced COI. Therefore the first base pair of the sequence was also a first codon position.

The models obtained from the partitioned COI data and ITS data were used as input parameters for the Bayesian analyses. The designated outgroup for ITS was *U. mordax* (Belize)

and *U. mordax* (Belize) and *U. panacea* (Florida) for COI. We conducted Bayesian analyses using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2001) on partitioned COI data and complete ITS data. Bayesian settings included random starting trees and all default priors. The Markov chain Monte-Carlo (MCMC) was run with four chains for 2 x 10^6 generations, with trees sampled every hundred generations. Branch lengths of sampled trees and burn-ins were saved. We used retained trees to generate a 50% majority rule consensus tree. The posterior probabilities of the nodes were determined by the percentage of times each node occurs among these trees (Huelsenbeck & Ronquist, 2001).

Likelihood analyses were performed using RAxML 2.2.3 (Stamatakis *et al.* 2008) using the RAxML "black box" at http://phyloench.vital-it.ch/raxml-bb/ to determine the relationships among haplotypes. Assumptions included independent branch length estimation of each gene segment and no sites were invariant in this analysis. One hundred replicates were analyzed for bootstrapping.

The age of extant *U. minax* groups were estimated in a Bayesian coalescence framework using BEAST v. 1.4.4 (Drummond *et al.* 2007). Because there are no external fossils for *U. minax* to estimate time to divergence, dates were calculated from substitution rate estimates under a molecular clock. The established mutation rates for mitochondrial data for arthropods are reviewed in Wares & Cunningham (2001), and for this study priors used in BEAST encompassed the range of mutation rates. A more general nuclear ITS mutation rate was used, with a prior that ranged for the generation time range for *U. minax* (3-5 years). For ITS, the MCMC chains were run for 120,000,000 generations, sampled every 1,000 generations. For COI, 60,000,000 generations of MCMC chains were run, sampled every 1,000 generations. Model parameters consisted of the GTR + I + G for ITS and HKY with codon partitioning for COI, with

a molecular clock enforced and the coalescent Bayesian skyline plot tree prior. The posterior probability graph from BEAST is the entity that an MCMC analysis attempts to obtain an estimate of.

In addition, the timing of genetic divergence between populations, migration rates, and population sizes were estimated using Isolation with Migration (IM) (Hey & Nielsen 2004) using the same established mutation rates (previously described) for each gene. Each run was continued until ESS reached at least 100 for every parameter, and three runs were completed in order to check marginal distributions. The program generates estimates of the marginal posterior probability densities for each of the model demographic parameters. Outgroup samples were excluded for both BEAST and IM estimates, and both programs were carried out using the resources of the Computational Biology Service Unit from Cornell University, which is partially funded by Microsoft Corporation.

Finally, Ramos-Onsins & Rozas' (2002) R_2 and Fu's F statistic (Fu 1997), were used to test for population expansion in each population. Simulation studies have shown that the R_2 test is more reliable for detecting population growth with small sample sizes (<20), whereas Fu's F performs well with larger (>20) (Ramos-Onsins & Rozas 2002). The significance of each test was determined using coalescent simulations with 10,000 replicates. The tests were performed using DNAsp 4.90.1 (Rozas *et al.* 2003). *P*-values < 0.05 are considered a significant departure from the null hypothesis of constant population size.

RESULTS

A total of 660 nucleotides were sequenced for the cytochrome oxidase I (COI) gene fragment for 97 fiddler crabs, and 495 nucleotides were sequenced for the internal transcribed spacer (ITS) gene fragment from 105 individuals. Seventy mtDNA COI haplotypes and 38 ITS nDNA haplotypes were observed among 97 and 196 sequences, respectively, sampled from 15 localities across the Gulf and Atlantic coasts of North America. Models of sequence evolution as determined by the AIC for COI were: 1^{st} codon, GTR + G; 2^{nd} codon, HKY; 3^{rd} codon, GTR + I ; and the entire COI region, HKY. For ITS, the model generated was GTR + I + G.

The maximum likelihood trees recovered a monophyletic *Uca minax* group. A phylogeny constructed from the nuclear ITS and a separate phylogeny constructed from the mitochondrial COI are shown in Figure II and Figure III, respectively. However, no structuring or differentiation is apparent in either gene tree. Relationships among the haplotypes were not resolved. AMOVA results show variation within populations (Gulf and Atlantic separately) best explains the genetic variation of ITS and COI (Table II). In addition, the F_{ST} value is low for both regions, as revealed by AMOVA, indicating low genetic differentiation.

Fu's Fs revealed values of -0.17379 and -0.13732 for Atlantic ITS and COI (respectively), and for the Gulf group, -0.21546 for ITS and -0.10088 for COI. All values of Fu's Fs were significant and all indicate expansion. The R_2 statistic (Ramos-Onsins and Rozas 2002) for the Atlantic group were calculated as 0.09354 (not significant) for ITS and 0.10726 for COI (significant). The R_2 value for Gulf ITS was not significant at 0.09403, but the Gulf COI R2 value of 0.10407 was significant.

Results from the BEAST skyline plot analysis indicate recent expansion due to posterior probability values that are much higher than zero for both ITS (Figure VIa) and COI (Figure VIb). The magnitude of expansion is higher in ITS than COI (Figure VI). IM graphs of posterior probability further supports a recent divergence from the ancestral population (Figures IV), as well as a less diverse ancestral population that split into the Atlantic and Gulf groups, but with more of the diversity ending up in the Gulf group (Figure V). IM graphs also indicate that migration from Gulf to Atlantic was larger in magnitude than Atlantic to Gulf migration.

DISCUSSION

Genetic structure and differentiation between the Gulf of Mexico and Atlantic Ocean population groups of *Uca minax* were not detected by our analyses. Both regions, COI and ITS, showed little variation among individuals, with some haplotypes representing as many as thirty individuals from both geographic areas. While this result is consistent with previous studies on this *U. minax*, the pattern of genetic similarity in a geographically disjunct taxon is unique. Most studies of the southeastern region of the United States have found taxa described as genetically similar only with a continuous distribution, or even genetically disjunct within a continuous distribution. However, of the previously studied taxa, such as the menhaden and sturgeon fishes (Avise 1992) that also exhibit *U. minax*'s unique pattern, a similar life history of freshwater habitat preference as adults with marine larval dispersal is apparent. This similarity suggests that genetic connectivity through larval dispersal could be a potential key for understanding the situation of *U.minax* because high mobility and large numbers of larvae may result in low genetic differentiation among groups.

Results of this study support a more recent divergence between these two population groups, which would best explain the low genetic diversity between them the two groups. In addition, the coalescent analyses employed here also support a more recent divergence. Based on BEAST and IM, the divergence of the two groups appears to be recent, with massive population expansion. The R_2 statistic and Fu's F_S values also support expansion. Therefore, these results support the hypothesis of a glacial refugium, followed by subsequent expansion into the current range during and then after, respectively, the last glacial maximum.

What is not detected by these analyses, though, is where *U. minax* was located before the glacial advance of the Pleistocene. It is unknown whether the species began on both the Atlantic and Gulf coasts, perhaps due to connection through the Suwannee Straits in the Miocene, or if the species was only distributed on one coast or the other until the glaciers receded, temperatures warmed, and *U. minax* initially colonized or re-colonized the two areas. Currently, the Gulf of Mexico is thought most likely to be the main evolutionary center for many marine taxa due to its richer fauna and significant amount of endemism (Briggs 1974). However, further analyses, such as a Mantel test, could help clarify this situation. Distinguishing between vicariance events, such as extinction in one area, with subsequent recolonization from the other, should also be at least considered as an alternative explanation for high genetic similarity, as opposed to panmixia due to good larval dispersal ability (Cunningham & Collins 1998).

However, good larval dispersal is a distinguishing characteristic of *Uca*, especially when considering their differing larval and adult habitat preferences. Therefore, the indirect approach to measure genetic connectivity utilized in this study would be complimented by direct methods of measuring connectivity at the opposite end of the temporal scale, i.e. the current range/ability of larval dispersal (Hedgecock et al. 2007). Direct methods would assist in understanding

whether or not the disjunct groups of *U. minax* will differentiate over a longer period of time, depending on gene flow (larval dispersal) currently taking place in this region. Though no *U. minax* are found between the Crystal River on the Gulf coast of Florida to Cape Canaveral on the Atlantic coast, oceanic currents have the potential to carry larvae past inadequate tropical habitat of southern Florida to appropriate temperate habitat. Direct methods of genetic evaluation, such as assignment or parentage tests, would be beneficial in a further understanding of *U. minax* evolutionary history.

ACKNOWLEDGEMENTS

This research was funded by the University of Northern Iowa (UNI) Undergraduate Research Program, the UNI College of Natural Sciences, an Alan Orr Research Grant, and two Dr. Swanson Research Grants. We thank R. Beer and J. Mraz from the Bell Baruch Lab in South Carolina, M. Swingl from the Virginia Aquarium, and L. Enzor from the University of West Florida for collection help. We also thank members of the UNI Fish Lab for technical assistance, including A. Avanesyan, K. Berge, A. Dewitt, and J. Hanna.

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Table I. Sampling localities (see Fig. 1), abbreviation, number of individuals sampled (*n*), number of haplotypes at each locality per gene, and collection date of the specimens

Collection site	Abbreviation	*n*	No of haplotypes COI	No of haplotypes ITS[a]	Collection date	Approximate Lat/Long
Atlantic localities						
MA, Berkley Bridge,	NEB	10	5	7	August 2007	41.50N/71.06W
VA, Virginia Beach	VVB	10	5	7	September 2007	36.48N/75.59W
SC, Bell Baruch Lab	SBB	10	6	9	June 2005, May/Aug06	33.20N/79.12W
SC, Great Pee Dee	SGP	10	10	8	July 2006	33.22N/79.16W
GA, Elliot's Bluff	GEB	10	9	8	December 2006	30.50N/81.34W
GA, St Mary's River	GSM	10	5	9	December 2006	30.44N/81.41W
Gulf localities						
FL, Ochlocknee	FOK	10	10	8	July 2006	29.59N/84.29W
FL, Eastpoint	FEP	2	1	2	July 2006	29.43N/84.53W
FL, Money Creek	FMC	4	2	5	July 2006	29.41N/85.15W
FL, White City	FOS	10	8	11	July 2006	29.52N/85.13W
FL, Overstreet	FWC	10	9	9	Aug 2005, July 06	29.59N/85.22W
FL, Escambia Co	FPE	10	7	2	December 2007	30.33N/87.13W
MS, Ocean Springs	MOS	10	8	7	August 2006	30.25N/88.48W
LA, Louisa	LLU	11	9	12	August 2006	29.46N/91.47W
LA, Cypremont Port	LCP	1	1	1	August 2006	29.43N/91.53W

[a]Note: Number of haplotypes for ITS can be greater than number of individuals sequenced due to adjustment for indels in PHASE 2.1 (Stephens *et al.* 2001).

Table II. AMOVA variance components, percentage variation explained at each spatial level and fixation indices for *U. minax* ITS and COI Gulf and Atlantic populations.

Source of variation	df[b]	Variance components	Percentage variation	Fixation index
ITS				
Among populations	1	0.12035 Va	5.92	
Within populations	194	1.91364 Vb	94.08	
Total	195	20.3399		F_{ST} = 0.05917
COI				
Among populations	1	0.22005 Va	8.90	
Within populations	104	2.25173 Vb	91.10	
Total	105	54.246		F_{ST} = 0.08903

[b] Note: Degrees of freedom are greater than actual number of individuals sequenced for ITS due to analysis in PHASE 2.1 (Stephens *et al.* 2001).

FIGURE LEGEND

FIGURE I. Current distribution of *Uca minax* (bold lines) and sampling localities (dots) with population codes. Light gray indicates Atlantic range and dark gray indicates Gulf range.

FIGURE II. Topologies produced in maximum likelihood analysis for ITS. Numbers above nodes indicate Bayesian posterior probabilities and numbers below indicate maximum likelihood bootstrap values. Numbers in parentheses indicate the number of individuals sharing a haplotype, if there is more than one. Three shades of gray indicate haplotype geographic distribution: dark gray indicates only Gulf, medium gray indicates individuals distributed in both the Atlantic and the Gulf, and light gray indicates haplotypes only distributed in the Atlantic. Outgroups are not shown.

FIGURE III. Topologies produced in maximum likelihood analysis for COI. Numbers above nodes indicate Bayesian posterior probabilities and numbers below indicate maximum likelihood bootstrap values. Numbers in parentheses indicate the number of individuals sharing a haplotype, if there is more than one. Three shades of gray indicate haplotype geographic distribution: dark gray indicates only Gulf, medium gray indicates individuals distributed in both the Atlantic and the Gulf, and light gray indicates haplotypes only distributed in the Atlantic. Outgroups are not shown.

FIGURE IV. IM estimates of the marginal posterior probability densities for each of the model demographic parameters: (a) Gulf population size, (b) ancestral population size, c) size of migration from Gulf to Atlantic, (d) Atlantic population size, (e) timing of divergence, and (f) size of migration from Atlantic to Gulf.

FIGURE V. A visual representation of the results of the IM analyses based upon the figure in Hey & Nielsen (2004). Relative width of the populations indicate effective population sizes, with change over time indicated by a vertical arrow. Horizontal arrows indicate migration between populations, with relative size indicating magnitude of migration.

FIGURE VI. BEAST skyline plots of posterior probabilities from the MCMC analysis, with (a) COI and (b) ITS. Expansion is indicated by positive values on the horizontal axis, as well as relative magnitude of expansion.

FIGURE I

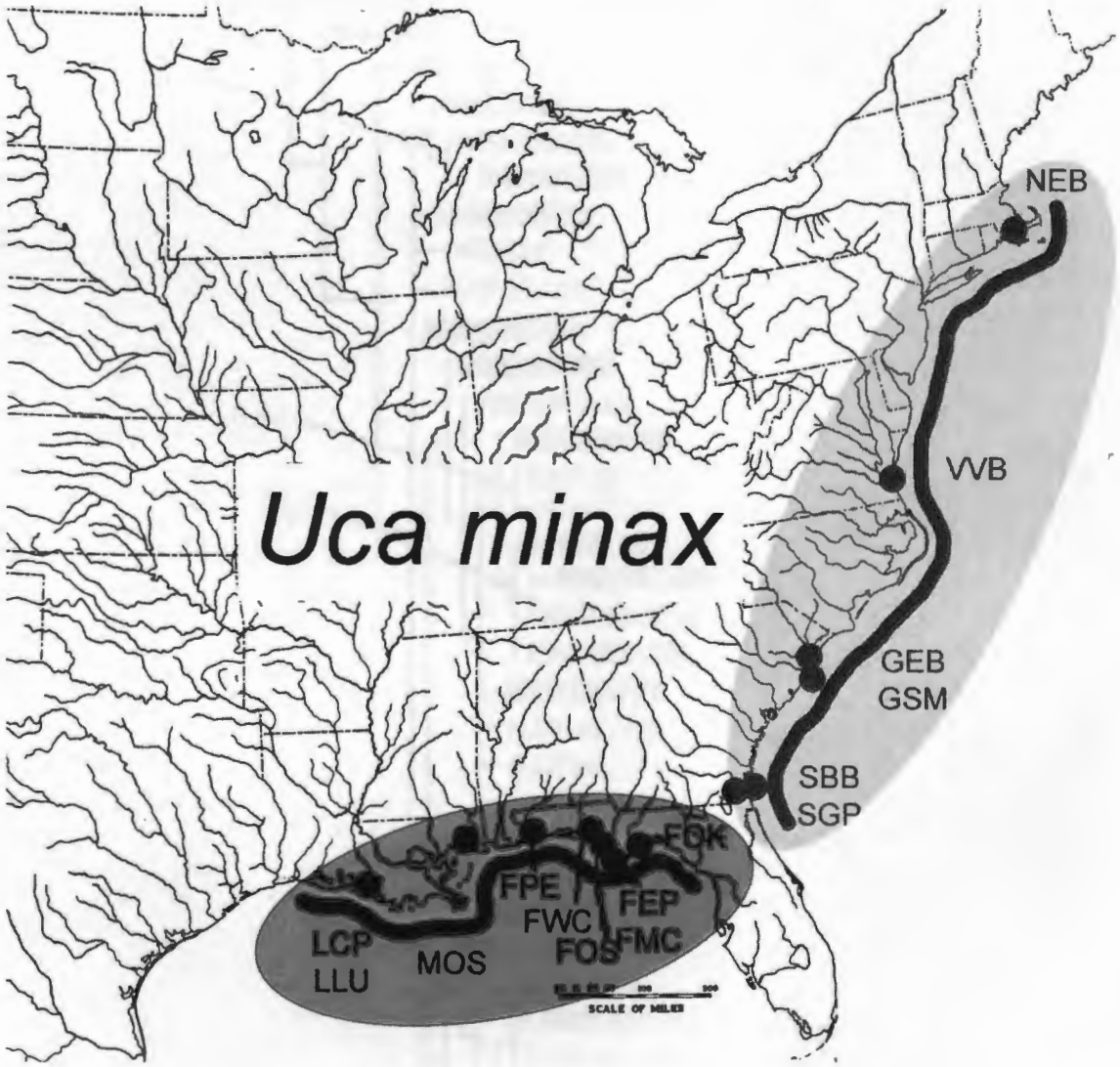

Uca minax
NEB
VVB
GEB
GSM
SBB
SGP
FOK
FPE
FEP
FWC
FMC
FOS
LCP
LLU
MOS
SCALE OF MILES

FIGURE II

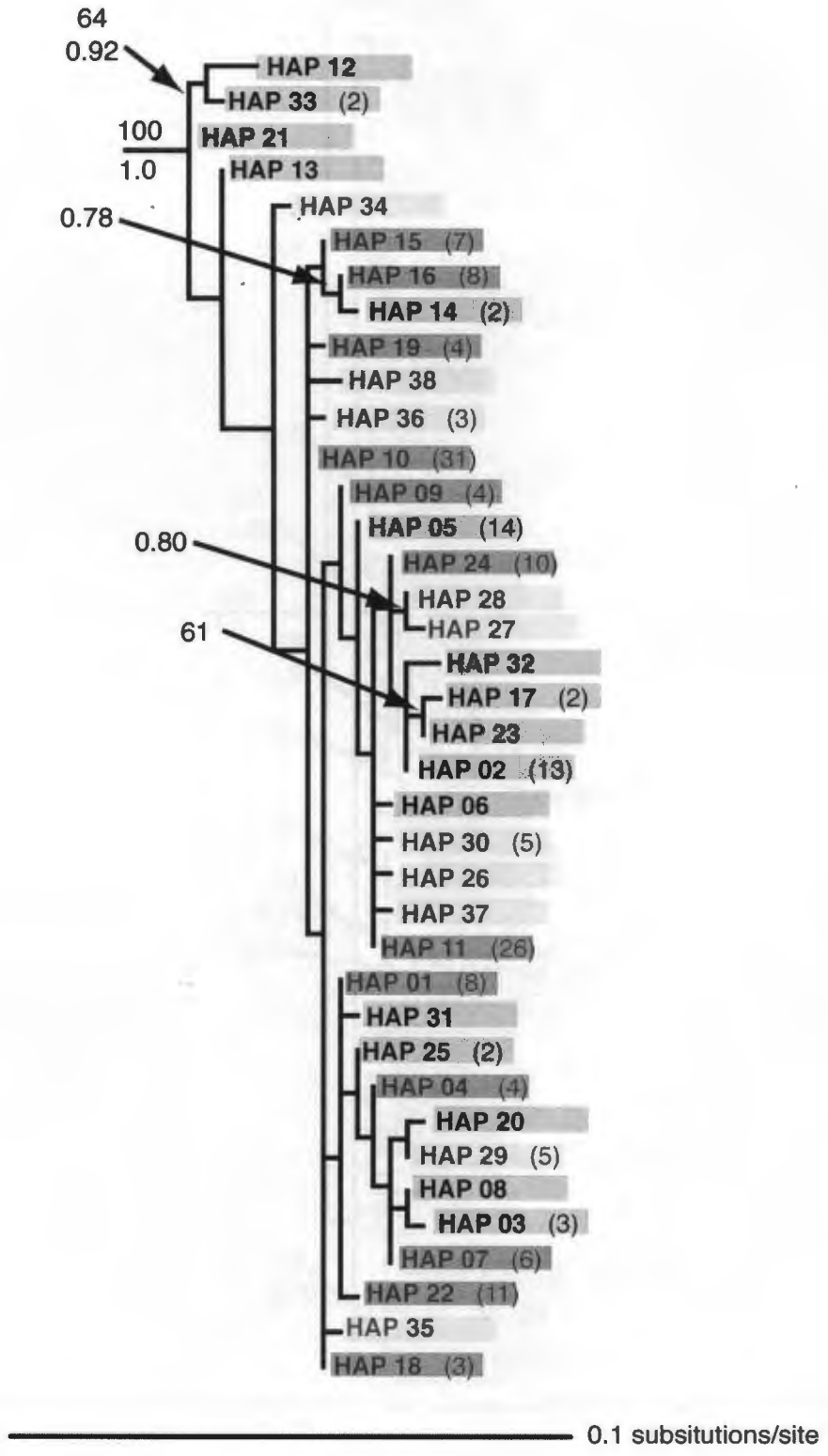
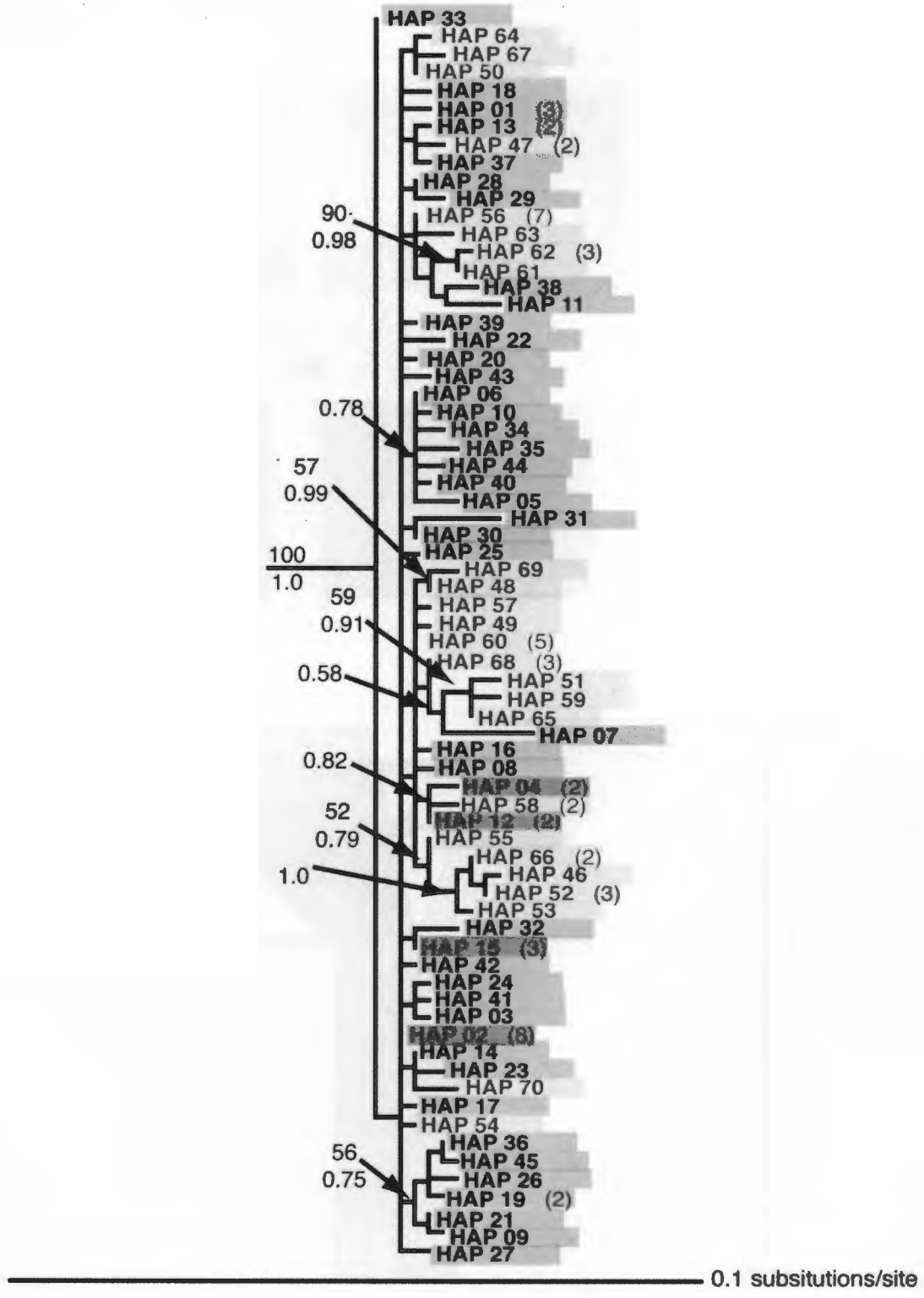

64
0.92
HAP 12
HAP 33 (2)
100
HAP 21
1.0
HAP 13
HAP 34
0.78
HAP 15 (7)
HAP 16 (8)
HAP 14 (2)
HAP 19 (4)
HAP 38
HAP 36 (3)
HAP 10 (31)
HAP 09 (4)
HAP 05 (14)
0.80
HAP 24 (10)
HAP 28
HAP 27
61
HAP 32
HAP 17 (2)
HAP 23
HAP 02 (13)
HAP 06
HAP 30 (5)
HAP 26
HAP 37
HAP 11 (26)
HAP 01 (8)
HAP 31
HAP 25 (2)
HAP 04 (4)
HAP 20
HAP 29 (5)
HAP 08
HAP 03 (3)
HAP 07 (6)
HAP 22 (11)
HAP 35
HAP 18 (3)
0.1 subsitutions/site

FIGURE III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HAP 64
HAP 67
HAP 50
HAP 18
HAP 01 (3)
HAP 13 (2)
HAP 47 (2)
HAP 37
HAP 28
HAP 29
90
HAP 56 (7)
0.98
HAP 63
HAP 62 (3)
HAP 61
HAP 38
HAP 11
HAP 39
HAP 22
HAP 20
HAP 43
HAP 06
0.78
HAP 10
HAP 34
HAP 35
57
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HAP 05
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100
HAP 25
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HAP 69
HAP 48
59
HAP 57
0.91
HAP 49
HAP 60 (5)
HAP 68 (3)
0.58
HAP 51
HAP 59
HAP 65
HAP 07
HAP 16
0.82
HAP 08
HAP 04 (2)
HAP 58 (2)
52
HAP 12 (2)
0.79
HAP 55
HAP 66 (2)
1.0
HAP 46
HAP 52 (3)
HAP 53
HAP 32
HAP 15 (3)
HAP 42
HAP 24
HAP 41
HAP 03
HAP 02 (8)
HAP 14
HAP 23
HAP 70
HAP 17
HAP 54
56
HAP 36
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HAP 45
HAP 26
HAP 19 (2)
HAP 21
HAP 09
HAP 27
0.1 subsitutions/site

FIGURE IV

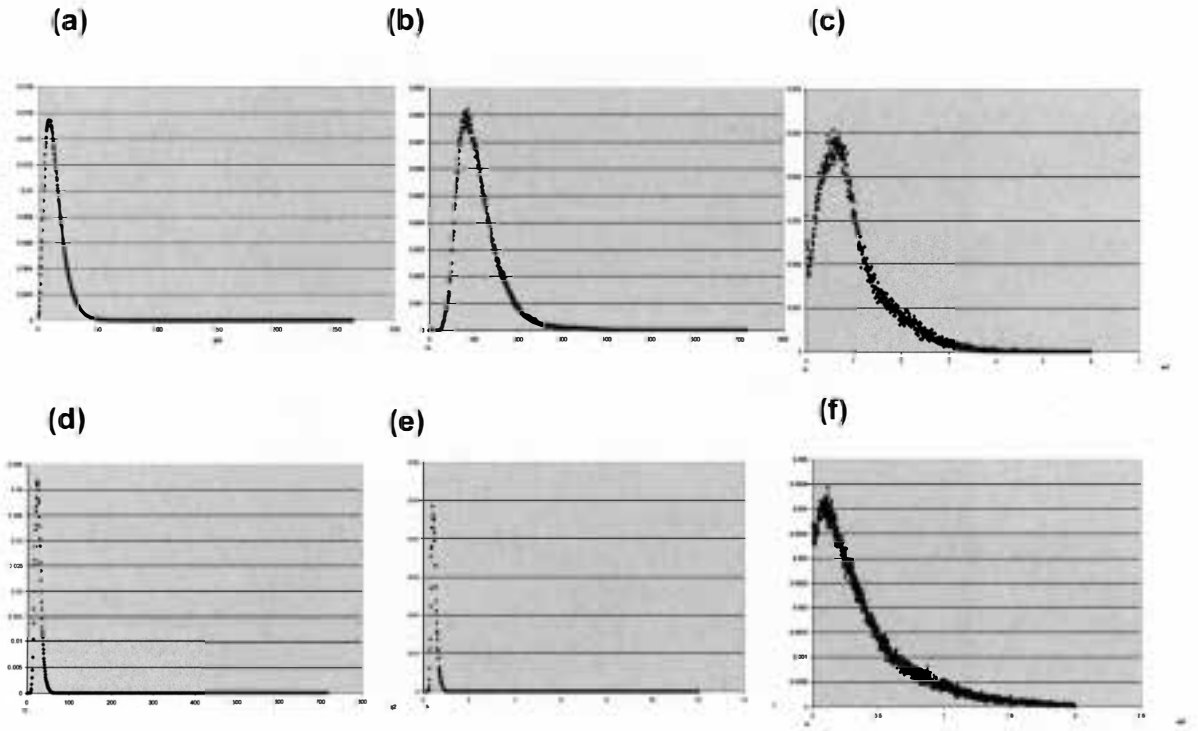


FIGURE V

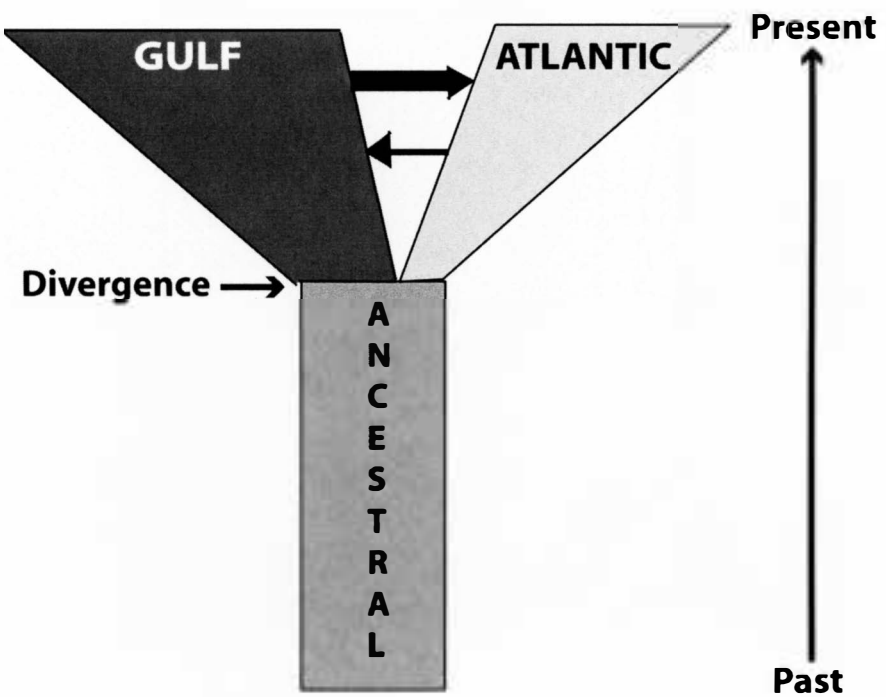
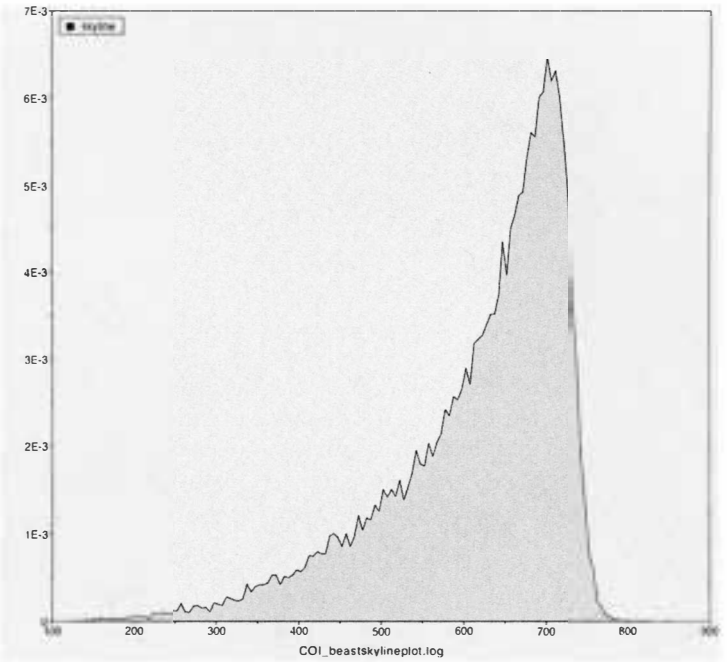


FIGURE VI

(a)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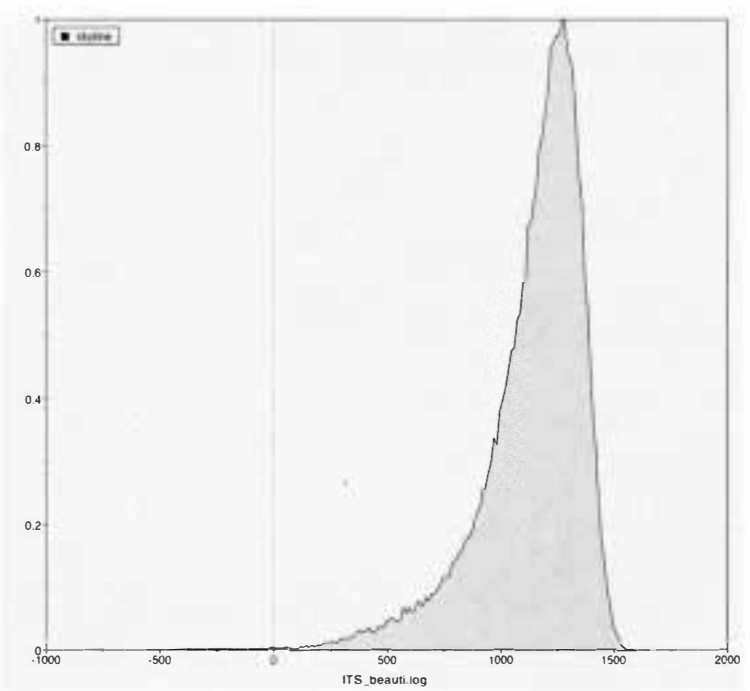
5E-3
4E-3
3E-3
2E-3
1E-3
200
300
400
500
600
700
800
COI_beastskylineplot.log

(b)


0.8
0.6
0.4
0.2
0
-1000
-500
500
1000
1500
2000
ITS_beauti.log