Population Structure and Genetic Diversity of Red Snapper (*Lutjanus campechanus*) in the U.S. South Atlantic and Connectivity with Red Snapper in the Gulf of Mexico

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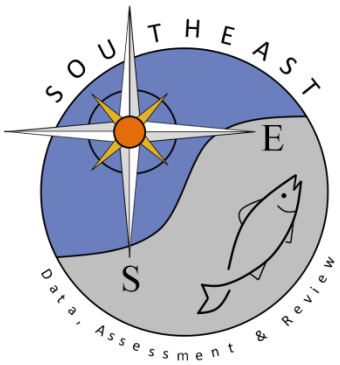

POPULATION STRUCTURE AND GENETIC DEMOGRAPHY OF RED SNAPPER (*LUTJANUS CAMPECHANUS*) IN THE U.S. SOUTH ATLANTIC AND CONNECTIVITY WITH RED SNAPPER IN THE GULF OF MEXICO

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I. EXECUTIVE SUMMARY

Genetic population structure of red snapper, *Lutjanus campechanus*, sampled from five localities along the southeastern coast of the United States (Atlantic) and from three localities in the northeastern Gulf of Mexico (eastern Gulf) was assessed using genotypes at16 nuclear-encoded microsatellites and 590 base pairs of the mitochondrialy encoded (mtDNA) ND4 gene. Genotypes at all 16 microsatellites in all localities sampled did not deviate significantly from the expectations of Hardy-Weinberg equilibrium, following Bonferroni correction. MtDNA haplotypes at the eight sample localities consisted primarily of two common haplotypes and numerous rare haplotypes. Conventional approaches (i.e., global exact tests, pairwise tests of F_{ST} or $\Phi_{ST} = 0$, hierarchical analysis of molecular variance) to test homogeneity of microsatellite alleles and genotypes and mtDNA haplotypes between and among localities and between pooled localities in the Atlantic versus pooled localities in the eastern Gulf, were non-significant. Mantel tests of a correlation between genetic and geographic distance (significant for mtDNA but non-significant for microsatellites) and a nearest-neighbor analysis of mtDNA suggested an isolation-by distance effect, possibly reflecting female philopatry or limited spatial geographic movement of females relative to males. A metric of selective neutrality of mtDNA haplotypes was significant at seven of the localities prior to Bonferroni correction and remained significant following correction at two localities, suggesting possible demographic differences among the localities. A Bayesian approach to estimation of F-statistics produced significant, non-zero estimates of the parameters θ^{I} and θ^{II} (reflecting historical and contemporaneous variance in microsatellite allele frequencies, respectively) among sample localities in the Atlantic, among sample localities in the eastern Gulf, and between sample localities in the Atlantic (pooled) versus sample localities in the eastern Gulf (pooled). Attempts to estimate contemporaneous effective size largely resulted in infinite point estimates or infinitely bounded confidence intervals. Estimates of average, long-term genetic effective size (N_{eLT}) among localities ranged from 826 to 2,111 but did not differ significantly from one another; estimates of N_{eLT} for localities from the Atlantic (pooled) and localities from the Gulf (pooled) were 3,930 and 4,114, respectively. The estimate of N_{eLT} for all eight localities (pooled) was 6,267. The sum of the estimates of N_{eLT} for the five localities in the Atlantic was 6,450, considerably larger that the global estimate (3,930) for the region. This is consistent with the 'propagule pool' model where migrants come primarily from a nearby subpopulation or stock and where a metapopulation is

subdivided into groups with different demographic rates (e.g., average survival and/or reproduction). The sum of the estimates of N_{eLT} for the three localities in the eastern Gulf was 4,414, close to the global estimate (4,114) for that region, suggesting few demographic differences among the three eastern-Gulf localities. The sum of the estimates of N_{eLT} for the two regions (8,005) is larger than the global estimate for all eight localities (6,267), suggesting that the two regions differ demographically. Estimates of average, long-term migration rates (m) between the two regions were 0.0033 (Gulf into the Atlantic) and 0.0021 (Atlantic into the Gulf) and did not differ significantly from one another. Because the estimates of N_{eLT} and of m for the two regions did not differ, we used an average estimate of N_{eLT} (4,022) and of m (0.0027) to generate a long-term mN_e estimate of 10.86, the effective number of migrants moving in either direction from one region to the other. Estimates of N_{eLT} represent a weighted harmonic mean of effective size (N_e) over a period of $4N_e$ generations, with greater weight on more recent generations and on smaller values of N_e. The estimate of m (0.33%) between the two regions is considerably less than the 10% rate, suggested for contemporaneous migration, beyond which populations are not considered to be demographically independent. Results of the study are consistent with slight genetic and demographic heterogeneity among localities within the two regions, particularly within the Atlantic, and between the two regions. The heterogeneity may reflect the metapopulation structure hypothesized previously for red snapper in the northern Gulf. In summary, there is evidence that genetic and demographic heterogeneity occurs among red snapper across the geographic region surveyed. The signal, however, is weak and precludes definition of geographic boundaries of subpopulations or stocks. More robust genetic approaches (e.g., RADseq) that utilize next-generation sequencing to screen thousands of genetic markers, and have the capability to identify genomic regions under selection are the next logical step in assessing population structure, genetic demography, and connectivity of red snapper across its range in U.S. waters.

II. PURPOSE

Red snapper *Lutjanus campechanus* (Poey 1860) have historically supported important commercial and recreational fisheries along the Atlantic Coast of the southeastern United States (hereafter Atlantic) and the northern (U.S.) coast of the Gulf of Mexico (hereafter Gulf) (Allman and Grimes 2002). Commercial landings of red snapper in the Gulf, for example, averaged 2.6

million pounds between 2007 and 2011, with an average dockside value of $9.6 million; while recreational fishing in the Gulf in 2011 involved > 375,000 target trips and $52.8 million in output impact (GMFMC 2013). Until recently, the major focus in terms of management has been red snapper in the Gulf, where the stock has been considered over-fished and to be undergoing overfishing since at least the late 1980s when the initial rebuilding plan was formulated (Strelcheck and Hood 2007). Although red snapper in the Gulf remain over-fished (NOAA 2012; SEDAR 2013), an assessment of red snapper in the U.S. South Atlantic indicated that red snapper are not only overfished since 1960 but that overfishing is occurring at several times the sustainable level (SEDAR 2008). Factors impacting the decline in red snapper are thought to include high mortality due to directed fisheries, habitat alteration and degradation and mortality of juveniles taken as unintentional harvest (bycatch) in the shrimp fishery, which appears to have been a factor in the Gulf (Schirripa and Legault 1997; Christman 1997; Ortiz et al. 2000).

Red snapper resources in the Atlantic and Gulf are currently managed as separate stocks (Cowan 2011). Genetics studies of adult red snapper, utilizing both nuclear and mitochondrial DNA (mtDNA) markers, have found little evidence of significant population structure across the northern Gulf (Gold et al. 1997: Pruett et al. 2005; Saillant and Gold 2006); Saillant et al. (2010), however, did detect a significant, positive spatial autocorrelation of microsatellite genotypes among age 0 fish sampled within a geographic range of 50–100 km. The lone genetic study of red snapper from both the Atlantic and the Gulf (Garber et al. 2004) involved sequences of the hypervariable, mitochondrial (mtDNA) control region among four localities in the Gulf (140 fish) and one locality in the Atlantic (35 fish). No differences in mtDNA haplotype frequencies were detected, consistent with the null hypothesis that red snapper in the five localities comprised a single, genetic population. MtDNA haplotype diversities (the probability of randomly sampling different haplotypes) in the localities sampled, however, ranged from 0.936 to 1.000 (average across localities of 0.973), indicating that most samples were comprised of unique, singleton mtDNA haplotypes. Effective testing of statistical homogeneity (the null hypothesis) was thus seriously constrained, leaving equivocal the question of whether red snapper from the two regions comprised a unit stock.

Alternatively, life-history data, results of tagging, and/or otolith microchemistry indicate there could be different stocks both within the Gulf and between the Gulf and Atlantic.

111 Significant differences in red snapper reproductive biology (Jackson et al. 2007), growth rate
112 (Fischer et al. 2004), and effective population size (Saillant and Gold 2006) have been found
113 among localities in the Gulf, and Brown-Peterson et al. (2009) found differences in reproductive
114 biology between red snapper sampled along the east coast of Florida and the Dry Tortugas.
115 Studies of red snapper in the Gulf, based on tagging and/or ultrasonic telemetry, have been more
116 equivocal as some (Fable 1980; Szedlmayer 1997; Schroepfer and Szedlmayer 2006; Strelcheck
117 et al. 2007) have shown relatively high site fidelity, while others (Watterson et al. 1998;
118 Patterson et al. 2001; Patterson and Cowan 2003) have reported lower site fidelity. However,
119 there is little to no evidence from tagging studies for movement of red snapper between the
120 Atlantic and the Gulf, and what limited data there are indicate high site fidelity, at least in the
121 Atlantic. Burns et al. (2004) tagged and released roughly 5,000 red snapper in the Atlantic and
122 Gulf (~40% were released in the Atlantic between Cape Canaveral, Florida, to Georgia).
123 Approximately 44% of the more than 400 recaptures were taken within less than 2 km of the
124 tagging site and only 2% or so of the recaptures had moved more than ≥160 km. Two smaller
125 studies carried out in the Atlantic also indicated relatively little movement away from the tagging
126 site (SEDAR 2008).
127 In this study, we used nuclear-encoded microsatellites and sequences of mtDNA to assess
128 genetic population structure of red snapper sampled in the Atlantic and the eastern Gulf.
129 Characterizing population structure is essential because failure to recognize population structure
130 within an exploited fishery may lead to over-exploitation and depletion of a localized, undetected
131 stock and result in the loss of unique genetic resources inherent in that stock (Carvalho and
132 Hauser 1994; Begg et al. 1999; Hilborn et al. 2003). Loss of genetic resources can compromise
133 long-term sustainability (Hilborn et al. 2003), and for fisheries undergoing rebuilding, failure to

recognize cryptic stocks can result in failure to anticipate recruitment in those non-identified units (Ruzzante et al. 1999). We also attempted to estimate the effective number of breeders (N_b) and the average, long-term effective (N_{eLT}) size at each sample locality. N_b is an estimate of the effective number of breeding individuals in a subpopulation (Waples 1990), while N_{eLT} reflects the long-term, relative effects of genetic drift and selection. As long-term sustainability requires maintenance of sufficient genetic resources (Allendorf and Waples 1996), stocks with small N_b and/or N_e potentially may suffer reduced capacity to respond to changing or novel environmental pressures (Frankham 1995; Higgins and Lynch 2001). Finally, we estimated the average, long-term migration rates between the Gulf and the Atlantic.

III. APPROACH

A total of 669 adult red snapper were sampled dockside between 2008 and 2011 from boats fishing offshore of North Carolina (NC), South Carolina (SC), Georgia (GA), Daytona, Florida (DA), and Melbourne, Florida (ML) in the U.S. South Atlantic (hereafter Atlantic), and offshore of Sarasota, Florida (SA), the Florida Middle Grounds (MG), and Panama City, Florida (PC) in the eastern Gulf of Mexico (hereafter Gulf). Approximate fishing localities are indicated in Figure 1. Tissue samples (fin clips) were obtained by personnel from several state or federal agencies (see Acknowledgements), fixed in 10% DMSO buffer (Seutin *et al*. 1991), and mailed to our laboratory in College Station. DNA was extracted following a modified chelex extraction protocol (Estoup *et al.* 1996); following final centrifugation, 1 µL of the supernatant was used as the template in subsequent polymerase-chain-reaction (PCR) amplification.

All fish were genotyped at 16 nuclear-encoded microsatellites, following multiplex PCR protocols described in Renshaw et al. (2006) and using PCR primers described in Bagley and Geller (1998) and Gold et al. (2001). Amplicons were electrophoresed and visualized on 6%

158 polyacrylamide gels, using an ABI Prism 377 automated sequencer (Applied Biosystems).
159 Allele-calling was conducted manually, using GENESCAN v.3.1.2 (Applied Biosystems Inc.,
160 Warrington, UK) and GENOTYPER v.2.5 (Perkin Elmer). A fragment of the mitochondrially-
161 encoded NADH dehydrogenase subunit 4 (ND4) gene was amplified for 20 individuals from
162 each locality, using primers ND4LB (Bielawski and Gold 2002) and NAP2 (Arevalo et al. 1994).
163 Thirty microliter PCR reactions consisted of 1x reaction buffer, 1.45 mM $MgCl_2$, 0.25 mM of
164 each dNTP, 30 pmol of each primer, 0.1 U/µL *Taq* polymerase, and 2 µL of DNA template.
165 Reaction conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles
166 of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 10 min.
167 Amplified products were purified with ExoSAP-IT^{TM} PCR cleanup kit (GE Healthcare,
168 Piscataway, NJ, USA) and sequenced bi-directionally, using BigDye Terminator v.3.1 Cycle
169 Sequencing Kit (Applied Biosystems). Five microliter sequencing reactions consisted of 10–50
170 ng of template, 0.5 µL of BigDye master mix, 0.875 µL of BigDye 5x reaction buffer, and 32
171 pmol of forward or reverse primer. Sequencing conditions consisted of denaturation at 96°C for
172 1 min followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Amplifications
173 were electrophoresed on an ABI 3100 Sequencer (Applied Biosystems) through 50 cm
174 capillaries. Sequence chromatograms were aligned and trimmed to a common 590 base pair
175 region, using SEQUENCHER 4.8 (Gene Codes Corporation).
176 Number of alleles, allelic richness, unbiased gene diversity (expected heterozygosity), and
177 the inbreeding coefficient F_{IS} (Weir and Cockerham1984)) were calculated for each
178 microsatellite in each locality, using FSTAT v.2.9.3.2 (Goudet 2001). Conformance to
179 expectations of Hardy-Weinberg equilibrium (HWE) was tested for each microsatellite in each
180 locality, using exact tests as implemented in GENEPOP v.4.0.7 (Raymond & Rousset 1995;

181 Rousset 2008). Parameters of the Markov Chain employed in estimation were 10,000
182 dememorizations, 1,000 batches, and 10,000 iterations per batch. Sequential Bonferroni
183 correction (Rice 1989) was applied for all multiple tests performed simultaneously. Possible
184 occurrence of scoring error due to stuttering, large allele dropout, and/or null alleles was
185 evaluated for each microsatellite in each locality, using MICROCHECKER (van Oosterhout *et al.*
186 2004). Likelihood-ratio tests of genotypic disequilibrium between pairs of microsatellite within
187 each locality were carried out using ARLEQUIN v.3.5 (Excoffier & Lischer 2010). Homogeneity
188 of allelic richness and unbiased gene diversity among localities was assessed using Friedman
189 rank tests, as implemented in R (R Core Team 2013). For mtDNA sequences, number of
190 haplotypes, haplotype diversity (h), and nucleotide diversity (π) were obtained for each sample,
191 using ARLEQUIN. Selective neutrality of mtDNA sequences was assessed using Fu and Li's
192 (1993) D^* and F^* statistics and Fu's (1997) F_S statistic, as implemented in DNASP v.5 (Librado
193 & Rozas 2009) and ARLEQUIN, respectively.
194 Tests of homogeneity of allele and genotype distributions (microsatellites) and haplotype
195 distribution (mtDNA) among localities employed exact tests as implemented in GENEPOP; exact
196 probabilities were estimated using the same Markov chain approach as above for tests of HWE.
197 The degree of divergence in microsatellites and mtDNA between pairs of localities was
198 estimated as F_{ST} and Φ_{ST}, respectively, using ARLEQUIN. For mtDNA, Φ_{ST} values were
199 estimated under a Tamura-Nei substitution model (Tamura and Nei 1993) with a gamma shape
200 parameter of 0.198, as selected by JMODELTEST v.2.1.1 (Guindon and Gascuel 2003, Darriba et
201 al. 2012). Significance of F_{ST} and Φ_{ST} values between pairs of localities was assessed by
202 permuting individuals between localities 10,000 times. Correction for multiple testing involved
203 sequential Bonferroni adjustment. Hierarchical analysis of molecular variance (AMOVA), as

implemented in ARLEQUIN, was conducted for both microsatellites and mtDNA by grouping Atlantic localities (NC, SC, GA, DA, and ML) and Gulf localities (SA, MG, and PC) separately; significance of the between-group component of variance was assessed by permuting sample localities between groups 10,000 times. Mantel tests, implemented in ARLEQUIN, were used to test for correlation between genetic distance and geographic distance for both microsatellites and mtDNA. Distance matrices contained pairwise measures of genetic distance, coded as F_{ST}/1-F_{ST} (microsatellites) or Φ_{ST}/1- Φ_{ST} (mtDNA), and linear coastline geographic distance, and were permuted 10,000 times to assess significance. Hudson's (2000) S_{nn} test was applied to the mtDNA data set to determine whether 'nearest neighbor' haplotypes (in terms of sequence identity) were sampled within the same locality more often than would be expected in a panmictic population. The test was performed considering each sample locality separately, the Atlantic sample localities pooled, and the Gulf sample localities pooled. To visualize relationships among haplotypes between the Atlantic and Gulf, a minimum-spanning network of mtDNA haplotypes was constructed using NETWORK v.4.6.11 (http://www.fluxus-engineering.com/).

Two alternative approaches to testing spatial genetic homogeneity, based on microsatellite data, were used to assess between/among population divergence. The first employed the Bayesian framework in HICKORY v 1.1 (Holsinger and Lewis 2002). This approach relaxes the assumption that allele frequencies are uncorrelated among populations, an assumption that does not necessarily hold when a small to moderately large number of populations are sampled (Song et al. 2006). Under this framework, HICKORY estimates a number of parameters, including: θ^I, which corresponds to Wright's (1951) F_{ST} and reflects variance in allele frequencies across evolutionary time, and θ^{II}, an analogue to Weir and Cockerham's (1984) θ and which reflects

contemporaneous variation between/among populations. HICKORY also provides estimates of rho (ρ), the among-population correlation of allele frequencies (Holsinger and Lewis 2002). Microsatellite data were separated into three partitions for separate HICKORY runs: (i) the five sample localities from the Atlantic; (ii) the three sample localities from the Gulf; and (iii) all sample localities from the Atlantic (pooled) and all sample localities from the Gulf (pooled). Each partition was were run in duplicate under the 'full' model in HICKORY, with a burn-in period of 500,000 steps, followed by 2 x 10^8 steps, with samples taken every 100 steps. The R package BOA (Smith 2005) was used to ensure convergence of posterior distributions, combine chains between replicates, and compute 95% HPD estimates for combined chains.

Estimates of the effective number of breeders (N_b) were generated for each sample locality, using microsatellite data and the linkage disequilibrium method implemented in LDNE (Waples 2006, Waples & Do 2008). Rare alleles below a frequency of 0.02 were excluded from calculations, following Portnoy *et al*. (2009); confidence intervals were obtained by jackknifing. Estimates of average, long-term effective population size (N_{eLT}) for each sample locality and for Atlantic localities (pooled) and Gulf localities (pooled) and estimates of average, long-term migration rate (m) between the Atlantic and Gulf were generated using microsatellite data and MIGRATE-N. A random subsample (n = 50; the smallest individual sample size) was drawn from each locality and replicate runs were combined to generate parameter estimates of theta (θ) and M (mutation-scaled migration rate), where $\theta = 4N_e\mu$ (N_e is the average, long-term effective population size [N_{eLT}] and μ is the modal mutation rate across all microsatellites per generation) and $M = m/\mu$. Estimates of μ were obtained using the Bayesian coalescent approach implemented in MSVAR v1.3 (Beaumont 1999, Storz and Beaumont 2002). BOA (Smith 2005) was used to estimate the 95% highest posterior density (HPD) interval for the modal value of μ.

Lower and upper bounds for N_{eLT} and m were estimated using 95% HPD intervals of θ and M generated by MIGRATE-N. Estimates of m (migration rate) were generated only for pooled sampled from the Atlantic versus pooled samples from the Gulf.

IV. FINDINGS

Summary statistics for microsatellite data are given in Appendix 1. The mean (± SE) number of alleles across microsatellites ranged from 7.44 ± 1.11(PA) to 9.69 ± 1.12 (ML); mean (± SE) allelic richness ranged from 7.40 ± 1.11 (PC) to 8.12 ± 0.97 (ML); and mean (± SE) gene diversity ranged from 0.583± 0.05 (PC) to 0.596 ± 0.05 (ML). Significant deviation from HWE equilibrium prior to Bonferroni correction was found in 13 of 128 tests; no significant deviations were found following correction. Possible null alleles, as inferred by MICROCHECKER, were detected at *Lca*107 (NC), *Ra*6 (SA), *Lca*43 (MG), and *Prs*221 (PC). Following Bonferroni correction, two pairwise tests of genotypic disequilibrium were significant: *Lca*20-*Lca*107 and *Lca*20-*Prs*328, both in SC. Friedman rank tests of homogeneity across sample localities in allelic richness (A_R) and gene diversity (H_E) were non-significant (A_R: $\chi^2_{[7,15]} = 4.01$, $P = 0.778$; H_E: $\chi^2_{[7,15]} = 3.12$, $P = 0.874$). Summary statistics for mtDNA data also are given in Appendix 1. A total of 39 haplotypes were found among the 160 individuals surveyed. Estimates of haplotype (h) and nucleotide (π) diversity ranged from 0.658 (PC) to 0.905 (ML) and 0.050 (MG) to 0.185 (ML), respectively. Fu & Li's (1993) D^* and F^* statistics were negative in all sample localities and significant prior to but not following Bonferroni correction in SC and MG; all other D^* and F^* statistics did not differ significantly from zero. Fu's (1997) F_S statistic was negative and significant prior to Bonferroni correction in all sample localities except GA; following correction, F_S statistics differed significantly from zero only in SC and SA.

Global exact tests of homogeneity in microsatellite allele and genotype distributions and in mtDNA haplotype distribution across all 16 microsatellites were non-significant (allelic: P = 0.072; genotypic: P = 0.150; haplotypic: P = 0.347). Pairwise estimates of F_{ST} (microsatellites) and Φ_{ST} (mtDNA) are given in Table 1; none of the pairwise comparisons F_{ST} or Φ_{ST} values differed significantly from zero. Hierarchical AMOVA revealed that the component of molecular variance allocated to between regions (Atlantic versus Gulf) was non-significant for both microsatellites (% variance = 0.02, P = 0.319) and mtDNA (% variance = 1.21, P = 0.106). Mantel tests of correlation between genetic and geographic distances were non-significant (P = 0.161) for microsatellites, but significant (P = 0.023) for mtDNA. Hudson's nearest-neighbor test (mtDNA only) was non-significant (P = 0.191) when considering all sample localities separately, but significant (P = 0.038) when samples were pooled within Gulf and Atlantic regions. The minimum-spanning network of mtDNA haplotypes by regional locality (Gulf and/or Atlantic) is presented in Figure 2. The distribution of haplotypes in all eight sample localities (Appendix 2) consisted primarily of two common haplotypes (#2 and #4) and numerous rare haplotypes. A total of 21 haplotypes were unique to the Atlantic, while 12 haplotypes were unique to the Gulf. At least four putative clades of two or three haplotypes were found in the Atlantic; none were found in the Gulf.

Bayesian analysis of population structure, using HICKORY, produced estimates of θ^I and θ^{II} (Table 2) that differed significantly from zero among sample localities in the Atlantic, among sample localities in the Gulf, and between sample localities in the Atlantic (pooled) versus sample localities in the Gulf (pooled). Estimate(s) θ^I were an order of magnitude greater than estimates of θ^{II}. For all three approaches, mean estimates of ρ from replicate runs were greater than 0.95 and differed significantly from zero.

296 Estimates of N_b (not shown but available upon request) for seven of the eight sample
297 localities had infinite upper bounds. The lone exception was SC where the upper bound was
298 greater than 10,000. Estimates (and 95% confidence limits) of theta (θ) and estimates of average
299 long-term effective size (N_{eLT}) for each sample locality, for localities from the Atlantic (pooled)
300 and localities from the Gulf (pooled) and for all eight localities (pooled) are given in Table 3;
301 N_{eLT} values were estimated according to $\theta = 4N_e\mu$ and a modal value of μ, obtained from MSVAR,
302 of 3.00×10^{-4}. Estimates of N_{eLT} across sample localities ranged from 826.15 (PC) to 2,111.28
303 (SA); none of the estimates of N_{eLT} differed significantly from one another. Estimates of N_{eLT} for
304 Atlantic localities (pooled) and Gulf localities (pooled) were 3,930.50 and 4,114.07, respectively,
305 and did not differ significantly from another. The estimate of N_{eLT} for all eight localities pooled
306 was 6,267.08. Finally, the estimate of average, long-term migration rate (m) from the Atlantic to
307 the Gulf was 0.21% (95% CI: 0.04% – 0.77%), while the rate from the Gulf to the Atlantic was
308 0.33% (95% CI: 0.07% – 1.11%). Because the estimates of N_{eLT} and of m for the two regions did
309 not differ, we used an average estimate of N_{eLT} (4,022) and of m (0.0027) to generate an average,
310 long-term estimate of 10.86 (mN_e), the effective number of migrants moving in either direction
311 from one region to the other.
312
313 **V. EVALUATION**
314 Conventional approaches (i.e., global exact tests, pairwise tests of F_{ST} or Φ_{ST}= 0,
315 hierarchical AMOVA) to test spatial homogeneity of microsatellite alleles and genotypes and
316 mtDNA haplotypes between and among localities and between pooled localities in the Atlantic
317 versus pooled localities in the Gulf, were non-significant. We also found no differences in levels
318 of (microsatellite) variability among localities. These findings are consistent with most prior
319 genetics studies (Gold et al. 1997, Heist and Gold 2000, Gold et al. 2001; Pruett et al. 2005;

320 Saillant and Gold 2006) of adult red snapper in the northern Gulf where significant spatial
321 heterogeneity of alleles, genotypes, or haplotypes was not detected. Spatial genetic differences
322 do exist in the Gulf, however, as Saillant et al. (2010) found significant heterogeneity in
323 microsatellite allele and genotype distributions among age 0 fish sampled at small spatial scales
324 in the western and central Gulf and a significant, positive spatial autocorrelation of microsatellite
325 genotypes within the geographic range 50–100 km. These findings were consistent with a
326 metapopulation model suggested by Pruett et al. (2005) and Saillant et al. (2006). In the latter,
327 significant differences in variance effective size (N_{eV}) also were reported across localities and
328 were correlated with findings of differences in growth rates (Fischer et al., 2004) and in size and
329 age of female maturation (Jackson e al. 2007). The lone genetic study of red snapper sampled
330 from both the Atlantic and Gulf (Garber et al. 2004) involved sequences of the hypervariable,
331 mitochondrial (mtDNA) control region sampled from four localities in the Gulf (140 fish) and
332 one locality in the Atlantic (35 fish). No differences in mtDNA haplotype frequencies were
333 detected, consistent with the null hypothesis that red snapper from the five localities comprised a
334 single, genetic population. Most of the mtDNA haplotypes in that study, however, were
335 singleton (unique) mtDNA haplotypes, seriously constraining the power to test the null
336 hypothesis of homogeneity.
337 Other approaches used to test spatial genetic homogeneity within and between the two
338 regions (Atlantic and Gulf) were not consistent with a single, well-mixed genetic unit. Mantel
339 tests revealed a weak but significant correlation between genetic and geographic distance in
340 mtDNA haplotypes, but not microsatellite genotypes, and analysis of mtDNA haplotypes
341 indicated that 'nearest-neighbor' haplotypes were sampled within the same geographic locality
342 more often than would be expected in a randomly sampled panmictic population. These results

343 indicate that mtDNA haplotypes are not distributed uniformly across the two regions and that
344 female-mediated gene flow, but not necessarily male-mediated, could be negatively correlated
345 with coastline geographic distance. This pattern of isolation by distance would then suggest
346 either female philopatry or limited spatial geographic movement of females relative to males.
347 Most examples of philopatry, including female philopatry, in non-anadromous, marine fishes not
348 restricted to coral reefs involve sharks (Heuter et al. 2004; Keeney et al 2005) although there is
349 evidence of philopatry in two estuarine-dependent sciaenids (Gold et al. 1999; Thorrold et al.
350 2001). Pruett et al. (2005) used nested-clade analysis of red snapper mtDNA haplotypes from
351 the Gulf and found historically restricted, female gene flow due to isolation by distance.
352 Alternatively, Gold et al. (1997) did not find evidence of a significant spatial autocorrelation
353 (also indicative of isolation by distance) of mtDNA haplotypes from adult red snapper sampled
354 over three years at nine localities in the Gulf stretching from northern through the Gulf to the
355 Florida panhandle. The isolation-by-distance of mtDNA haplotypes found in this study covered
356 the area from the Florida panhandle to the North Carolina coast, including samples from the west
357 and east coasts of Florida, possibly suggesting reduced (female) gene flow around peninsular
358 Florida. In addition, a network of mtDNA haplotypes revealed at least four clades of two or
359 three haplotypes occurring in the Atlantic but not the Gulf, and Fu's (1997) F_S metric was
360 negative and significant before Bonferroni correction at seven of the eight localities sampled and
361 at the samples from South Carolina (Atlantic) and Sarasota (Gulf) following correction. The
362 difference in number of small clades detected may stem in large part from the difference in
363 sample size (477 individuals from the Atlantic vs. 191 individuals from the Gulf). The F_S
364 approach, however, detects population growth and/or genetic hitch-hiking (Fu 1997), and
365 negative F_S values (based on mtDNA data) indicate either a 'selective sweep' or a recent

366 reduction in female effective size , both of which can result in an excess of rare mtDNA variants
367 over those expected under a neutral model (Kaplan et al. 1989). Together, these observations
368 suggest geographic limits to red snapper female gene flow and possible demographic differences
369 affecting female effective size.

370 Bayesian estimates of the population parameters θ^I (long-term heterogeneity) and θ^{II}
371 (contemporaneous heterogeneity) were significant and non-zero among localities in the Atlantic,
372 among localities in the Gulf, and between Atlantic localities (pooled) and Gulf localities
373 (pooled). Allele frequencies in all comparison groups were highly correlated ($\rho > 0.95$) and
374 significantly different from zero, violating the assumption of uncorrelated allele frequencies
375 implicit in approaches estimating parameters such as Φ_{ST} (Song et al. 2006). That both θ^I and θ^{II}
376 were significantly different than zero suggests that the pattern of genetic heterogeneity that exists
377 today within the Atlantic, within the Gulf, and between the two likely reflects patterns that have
378 existed historically. Estimates of θ^I, however, were more than an order of magnitude greater
379 than estimates of θ^{II}. Song et al. (2006) found that large estimates of θ^I may reflect a tendency
380 for estimators of θ^{II} to overestimate homogenizing effects of long-term gene flow, particularly if
381 allele frequencies between populations are highly correlated, suggesting that values of θ^{II}
382 estimated here could be underestimates. Regardless, the non-zero estimates of θ^I and θ^{II} are
383 consistent with genetic heterogeneity of red snapper within the Atlantic, within the Gulf, and
384 between the two.

385 Estimates of the effective number of breeders (N_b) for all but one of the localities sampled
386 had infinite upper bounds, suggesting a uniformly large N_b across the localities sampled (Waples
387 and Do 2010). However, estimates of N_b generated from adult samples are difficult to interpret
388 because they are influenced by the effective number of breeders (N_b) that generated each cohort

389 in a sample (Waples 2005). Red snapper can live for over 50 years (Wilson and Nieland 2001)
390 and mature sexually between age two and four (Schirripa and Legault 1997; Fitzhugh et al.
391 2004). Almost all fish sampled here were adults, meaning that fish in our samples from different
392 cohorts could have shared a parent or parents, severely compromising estimation of N_b.
393 Estimates of average, long-term genetic effective size (N_{eLT}) among localities ranged from
394 826 to 2,111 but did not differ significantly from one another; estimates of N_{eLT} for localities
395 from the Atlantic (pooled) and localities from the Gulf (pooled), respectively, were 3,930 and
396 4,114, respectively. The estimate of N_{eLT} for all eight localities (pooled) was 6,267. The sum of
397 the estimates of N_{eLT} for the five localities in the Atlantic was 6,450, considerably larger that the
398 global estimate (3,930) for the region. This is consistent with a 'propagule pool' model (Waples
399 2010) where migrants come primarily from a nearby subpopulation or stock and where a
400 metapopulation is subdivided into groups with different demographic rates (e.g., average survival
401 and/or reproduction) (Waples 2010). The sum of the estimates of N_{eLT} for the three localities in
402 the eastern Gulf was 4,414, close to the global estimate (4,114) for that region, perhaps
403 suggesting fewer demographic differences among the three eastern Gulf localities. The sum of
404 the estimates of N_{eLT} for the two regions (8,005) is larger than the global estimate for all eight
405 localities (6,267), suggesting that the two regions differ demographically. These comparisons
406 also are consistent with genetic and demographic heterogeneity among localities within the two
407 regions, particularly within the Atlantic, and between the two regions.
408 Estimates of average, long-term migration rates (m) between the two regions were 0.0033
409 (Gulf into the Atlantic) and 0.0021 (Atlantic into the Gulf) and did not differ significantly from
410 one another. The parameter m is defined as the proportion of individuals in a subpopulation that
411 are migrants from an outside subpopulation, and is most often expressed as the parameter mN_e,

412 the effective number of migrants entering a subpopulation each generation (Mills and Allendorf
413 1996). Because the estimates of N_{eLT} and of m for the two regions did not differ, we used an
414 average estimate of N_{eLT} (4,022) and of m (0.0027) to generate a long-term mN_e estimate of 10.86
415 which, theoretically, is the effective number of migrants moving in either direction from one
416 region to the other. Estimates of N_{eLT} represent a weighted harmonic mean of effective size (N_e)
417 over a period of $4N_e$ generations, with greater weight on more recent generations and on smaller
418 values of N_e (Beerli 2009; Hare et al. 2011). A final point to note is that the estimate of m
419 (0.33%) between the two regions is considerably less than the 10% rate, suggested for
420 contemporaneous migration (Waples 2010), beyond which populations are not considered to be
421 demographically independent.
422 To summarize, there is evidence that genetic and demographic heterogeneity occurs among
423 red snapper across the geographic region surveyed and that the species in U.S. waters is not
424 distributed spatially as a single, panmictic population (stock). The heterogeneity may reflect the
425 metapopulation structure previously hypothesized for red snapper in the northern Gulf (Pruett et
426 al. 2005; Saillant and Gold 2006) and reinforced by the study of Saillant et al. (2010) of age 0
427 fish. The genetic signal, however, is weak and precludes straightforward definition of
428 geographic boundaries of individual subpopulations or stocks. This is fairly typical for marine
429 fishes with large population densities and high dispersal capability assayed with selectively
430 neutral genetic markers such as microsatellites (Portnoy and Gold 2012). More robust genetic
431 approaches (e.g., RADseq) that utilize next-generation sequencing and allow genome-wide
432 surveys of variation in single-nucleotide polymorphisms (Davey et al. 2011) is the next logical
433 step in assessing population structure, genetic demography, and connectivity of red snapper (and
434 other species) across its (their) range in U.S. waters. These new approaches have the capability

435 to identify genetic markers (genome regions) that affect fitness on local scales and to quantify
436 allele frequencies at these markers across geographic space (Nielsen et al. 2009; Allendorf et al.
437 2010).
438

VI. TABLES

Table 1. Estimates of pairwise F_{ST} (microsatellites, upper diagonal) and Φ_{ST} (mtDNA, lower diagonal) between all eight sample localities.

	NC	SC	GA	DA	ML	SA	MG	PC
NC	0	0.001	0.001	0.001	0	0	0.002	-0.001
SC	0.046	0	-0.001	-0.001	0	-0.001	0	0.001
GA	-0.023	0.036	0	0.001	-0.001	-0.001	0	0.002
DA	0.005	-0.018	-0.004	0	0	0.002	0	0.002
ML	-0.024	0.02	-0.036	-0.076	0	0	0	-0.001
SA	0.015	0.027	0.011	-0.023	-0.025	0	0.001	-0.001
MG	0.012	-0.028	0.018	-0.012	-0.087	-0.04	0	0
PC	0.092	0.056	0.059	-0.04	-0.025	-0.019	-0.025	0

Table 2. Global estimates of θ^I, θ^{II}, and ρ estimated using the Bayesian framework in HICKORY. θ^I is an estimate of FST that corresponds to allele frequency variance across evolutionary time; θ^{II} is a measure of contemporaneous differentiation among sample locations, and ρ is the average correlation of allele frequencies among sample locations.

Samples	θ^I			θ^{II}				ρ	
	2.5%	Mode	97.5%	2.5%	Mode	97.5%	2.50%	Mean	97.50%
Within Atlantic	0.0375	0.0579	0.0641	0.0015	0.0017	0.0020	0.9552	0.9670	0.9759
Within Gulf	0.0491	0.0666	0.0861	0.0009	0.0011	0.0016	0.9750	0.9821	0.9877
Gulf/Atlantic (pooled)	0.0387	0.0548	0.0695	0.0007	0.0009	0.0010	0.9779	0.9843	0.9893

449 Table 3. Estimates and 95% confidence intervals for theta (θ), obtained from MIGRATE-N.

450 Estimates of N_{eLT} were generated from the relationship $\theta = 4N_{eLT}\mu$.

451

	θ			Ne_{LT}
	2.5%	Mode	97.5%	Mode
NC	0.86	1.61	2.36	1,343.54
SC	0.68	1.37	2.06	1,143.26
GA	0.52	1.21	1.90	1,009.74
DA	1.06	1.73	2.42	1,443.68
ML	1.08	1.81	2.58	1,510.44
SA	1.40	2.53	3.96	2,111.28
MG	1.08	1.77	2.44	1,477.06
PC	0.40	0.99	1.54	826.15
GULF	3.84	4.93	5.90	4,114.07
ATLANTIC	3.72	4.71	6.02	3,930.48
ALL	6.56	7.51	8.48	6,267.08

452

453

VII. FIGURES

Figure 1. Approximate sampling localities for red snapper in the U.S. Atlantic and eastern Gulf of Mexico.

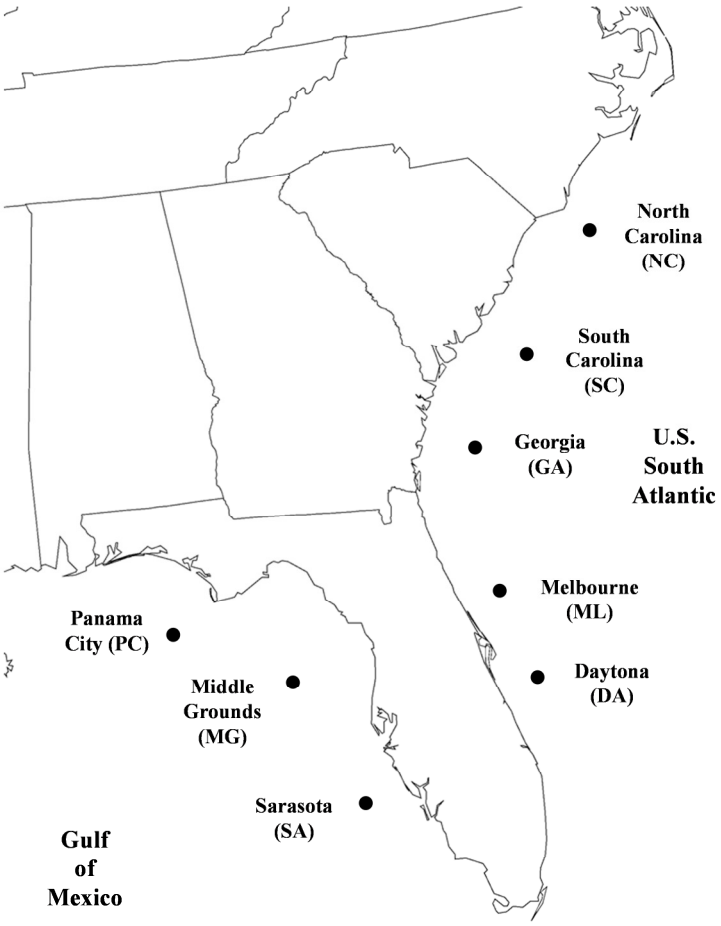
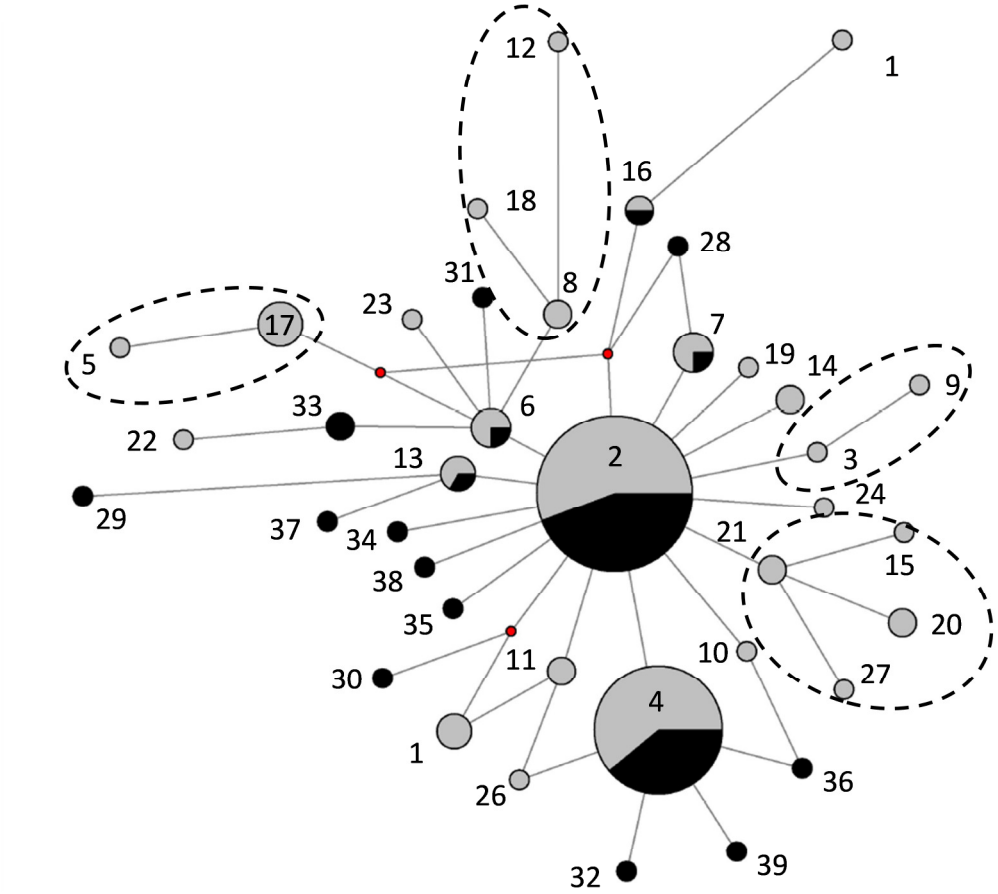


Figure 2. Minimum-spanning network of ND4 mtDNA haplotypes: gray, haplotypes found in the Atlantic; black, haplotypes found in the Gulf. Each node (small circle) represents a unique haplotype; sizes of nodes are scaled to reflect the relative frequency of each haplotype. Lengths of lines connecting haplotypes reflect number of single-base substitutions between haplotypes; the shortest line is one base-pair substitution. Small nodes indicated by very small circles are inferred mtDNA haplotypes. Dotted lines surround putative clades found in the Atlantic.



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704

Appendix 1: Summary statistics for microsatellite and mtDNA loci at each locality. For microsatellites: n = number of individuals sampled, #A = number of alleles, AR = allelic richness, H_E = expected heterozygosity, P_{HW} = probability of conformance to Hardy-Weinberg expectations, and F_{IS} = inbreeding coefficient. For mtDNA: n = number of individuals sampled, #H = number of unique haplotypes observed, h = haplotype diversity, π = nucleotide diversity, D^* and F^* from Fu and Li (1993), and F_S from Fu (1997). ‡ indicates a significant value ($P < 0.05$).

705

Sample		NC	SC	GA	DA	ML	SA	MG	PC
Microsatellite									
*Lca*20	*n*	93	84	101	98	101	48	97	46
	#A	4	5	5	4	5	5	5	3
	AR	3.80	4.05	3.72	3.30	4.25	4.93	4.03	2.98
	H_E	0.190	0.179	0.208	0.162	0.177	0.196	0.174	0.198
	P_{HW}	0.009	1.000	0.063	0.115	0.116	0.410	0.101	0.852
	F_{IS}	0.152	-0.067	0.094	0.182	0.049	0.150	-0.009	-0.099
*Lca*22	*n*	93	84	101	96	100	47	97	46
	#A	14	12	12	12	13	9	11	8
	AR	12.06	10.35	11.03	11.10	10.90	8.95	9.45	7.98
	H_E	0.751	0.726	0.727	0.783	0.725	0.693	0.752	0.761
	P_{HW}	0.265	0.629	0.210	0.077	0.304	0.032	0.230	0.661
	F_{IS}	0.055	-0.034	0.087	0.082	0.062	-0.043	0.040	-0.028
*Lca*43	*n*	93	85	101	98	101	48	97	46
	#A	7	8	9	9	9	7	8	7
	AR	6.40	7.00	7.61	7.58	7.80	6.99	7.34	6.94
	H_E	0.491	0.581	0.539	0.526	0.583	0.560	0.560	0.589
	P_{HW}	0.597	0.127	0.123	0.486	0.366	0.745	0.011	0.196
	F_{IS}	0.080	-0.013	0.026	-0.029	-0.018	0.033	0.190	-0.107
*Lca*64	n	93	84	101	98	101	48	97	46
	#A	11	12	11	12	9	8	9	6
	AR	9.17	10.23	8.67	9.48	7.80	7.81	7.62	5.98
	H_E	0.789	0.791	0.782	0.801	0.774	0.713	0.770	0.736
	P_{HW}	0.075	0.792	0.880	0.953	0.808	0.468	0.177	0.384
	F_{IS}	-0.077	0.007	0.013	-0.032	-0.036	0.065	0.023	-0.094
*Lca*91	n	93	85	101	98	101	47	97	46
	#A	6	7	6	7	6	6	7	4
	AR	5.19	5.89	5.65	5.91	5.17	5.96	5.60	3.98
	H_E	0.577	0.565	0.613	0.579	0.573	0.627	0.562	0.557
	P_{HW}	0.310	0.966	0.252	0.982	0.049	0.012	0.514	0.824
	F_{IS}	-0.025	0.042	0.046	-0.041	0.118	0.049	0.028	0.103

*Lca*107	*n*	93	84	98	98	101	48	97	46
	#A	9	8	9	10	10	9	10	10
	AR	8.46	7.98	8.41	9.15	9.22	8.93	9.36	9.96
	H_E	0.776	0.801	0.781	0.814	0.781	0.775	0.800	0.767
	P_{HW}	0.145	0.714	0.034	0.912	0.272	0.119	0.495	0.027
	F_{IS}	0.099	0.019	0.059	0.035	0.023	-0.021	0.021	0.065
*Prs*55	*n*	93	84	101	98	101	48	97	46
	#A	7	5	6	4	7	4	6	4
	AR	5.50	4.06	5.27	3.45	5.19	3.93	4.60	3.98
	H_E	0.271	0.265	0.253	0.256	0.297	0.193	0.251	0.255
	P_{HW}	0.100	0.272	0.753	0.426	0.942	1.000	0.867	0.611
	F_{IS}	0.166	-0.032	-0.057	0.082	-0.034	-0.077	-0.110	0.062
*Prs*137	*n*	93	85	101	98	99	47	97	46
	#A	13	12	11	11	12	12	11	8
	AR	10.24	9.78	9.09	9.34	9.63	11.74	8.83	7.96
	H_E	0.732	0.680	0.721	0.676	0.720	0.694	0.682	0.676
	P_{HW}	0.013	0.613	0.130	0.513	0.032	0.714	0.054	0.102
	F_{IS}	0.105	0.049	0.066	0.019	0.031	0.049	0.063	0.131
*Prs*221	*n*	93	84	101	98	101	48	97	46
	#A	12	12	12	13	15	13	16	11
	AR	10.67	10.23	10.78	10.93	11.93	12.68	12.54	10.91
	H_E	0.766	0.772	0.800	0.767	0.803	0.807	0.797	0.771
	P_{HW}	0.366	0.360	0.522	0.185	0.532	0.625	0.440	0.388
	F_{IS}	0.032	0.060	0.022	0.082	0.075	-0.007	0.056	0.069
*Prs*240	*n*	92	81	101	98	100	47	97	46
	#A	18	18	18	19	18	18	20	18
	AR	17.03	16.79	15.90	16.61	16.88	17.83	17.13	17.87
	H_E	0.902	0.901	0.889	0.899	0.892	0.902	0.907	0.875
	P_{HW}	0.269	0.298	0.983	0.681	0.850	0.926	0.022	0.042
	F_{IS}	-0.037	0.054	-0.002	-0.022	-0.009	-0.038	0.034	0.056
*Prs*248	*n*	93	84	101	98	101	48	97	46
	#A	18	17	20	23	18	14	19	16
	AR	15.30	14.29	15.50	16.60	14.64	13.80	16.05	15.89
	H_E	0.872	0.868	0.888	0.865	0.877	0.880	0.869	0.879
	P_{HW}	0.644	0.337	0.319	0.952	0.407	0.403	0.906	0.551
	F_{IS}	0.001	-0.056	0.052	-0.050	-0.005	0.029	0.015	0.035
*Prs*260	*n*	93	85	101	98	101	48	97	46
	#A	5	3	5	5	3	4	3	3

	AR	3.93	3.00	4.52	4.17	3.00	3.94	3.00	3.00
	H_E	0.305	0.397	0.398	0.439	0.394	0.379	0.357	0.393
	P_{HW}	0.064	0.218	0.309	0.017	0.814	1.000	1.000	0.400
	F_{IS}	0.118	-0.187	-0.019	-0.047	-0.029	-0.044	-0.011	-0.050
*Prs*275	*n*	93	85	101	98	101	48	97	45
	#A	6	8	6	7	10	5	7	5
	AR	5.34	6.64	5.05	5.75	7.33	4.94	5.36	5.00
	H_E	0.609	0.579	0.564	0.610	0.632	0.603	0.595	0.608
	P_{HW}	0.837	0.511	0.392	0.272	0.930	0.849	0.539	0.446
	F_{IS}	-0.095	-0.057	0.016	-0.121	-0.050	-0.002	0.099	0.050
*Prs*282	n	93	85	101	98	101	48	97	46
	#A	12	12	11	11	11	11	11	13
	AR	9.561	10.688	9.188	9.787	9.677	10.681	10.176	12.912
	H_E	0.599	0.66	0.636	0.64	0.684	0.565	0.655	0.693
	P_{HW}	0.270	0.443	0.399	0.895	0.261	0.630	0.594	0.006
	F_{IS}	0.049	0.091	0.082	-0.004	0.073	0.079	-0.022	0.027
*Prs*328	*n*	92	84	101	98	101	48	97	46
	#A	3	4	4	4	6	3	4	3
	AR	3.00	3.98	3.45	3.63	4.58	3.00	3.83	3.00
	H_E	0.547	0.567	0.568	0.531	0.559	0.516	0.546	0.563
	P_{HW}	0.243	0.487	0.106	0.856	0.930	0.592	0.702	0.842
	F_{IS}	-0.173	-0.008	0.128	0.039	-0.010	0.153	-0.075	-0.042
*Prs*333	n	93	85	101	98	101	46	97	46
	#A	5	5	7	5	7	5	6	6
	AR	4.85	4.09	5.08	3.76	5.53	4.98	5.01	5.96
	H_E	0.382	0.277	0.336	0.361	0.395	0.401	0.330	0.414
	P_{HW}	0.104	0.700	0.925	1.000	0.361	0.424	0.604	0.305
	F_{IS}	-0.040	-0.148	-0.032	-0.019	0.073	0.078	0.000	0.212
*Ra*6	*n*	92	85	101	98	101	48	97	46
	#A	7	7	7	6	7	7	7	7
	AR	6.44	5.95	5.72	5.41	6.02	6.88	6.03	6.96
	H_E	0.392	0.401	0.384	0.461	0.355	0.506	0.384	0.409
	P_{HW}	0.202	0.134	0.325	0.994	0.467	0.049	0.126	0.958
	F_{IS}	0.085	0.120	-0.032	-0.018	-0.003	0.218	0.140	-0.169
mtDNA ND4	*n*	20	20	20	20	20	20	20	20
	#H	8	11	7	8	11	10	7	6
	h	0.758	0.884	0.784	0.805	0.905	0.842	0.711	0.658

π	0.091	0.128	0.106	0.066	0.185	0.095	0.050	0.051
D^*	-2.392	-2.666‡	-1.441	-1.719	-1.211	-2.232	-2.616‡	-2.258
F^*	-2.534	-2.889‡	-1.582	-1.887	-1.472	-2.390	-2.713‡	-2.333
F_S	-3.376‡	-6.037‡	-1.413	-3.277‡	-4.584‡	-5.039‡	-3.054‡	-2.446‡

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Appendix 2: Spatial distribution of mitochondrial (ND4) haplotypes.

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Haplotype	NC	SC	GA	DA	ML	SA	MG	PC
#1	1				2			
#2	9	6	8	7	4	6	10	11
#3	1							
#4	5	4	5	6	5	6	5	5
#5	1							
#6	1	1		1		1		
#7	1			1	1			1
#8	1				1			
#9		1						
#10		1						
#11		2						
#12		1						
#13		1	1			1		
#14		1		1				
#15		1						
#16		1						1
#17			3		2			
#18			1					
#19			1					
#20			1		1			
#21				2				
#22				1				
#23				1				
#24					1			
#25					1			
#26					1			
#27					1			
#28						1		
#29						1		
#30						1		
#31						1		
#32						1		
#33						1	1	
#34							1	
#35							1	
#36							1	
#37							1	
#38								1
#39								1

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