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# Intraspecific Relationships in Paracalanus quasimodo [Calinoideae] and Temora turbinata [Calinoideae] along the Southeastern Coast of the United States

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# INTRASPECIFIC RELATIONSHIPS IN *PARACALANUS QUASIMODO* [CALINOIDEAE] AND *TEMORA TURBINATA* [CALINOIDEAE] ALONG THE SOUTHEASTERN COAST OF THE UNITED STATES

A dissertation submitted in partial fulfillment of the

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by

Richard Yen-Ching Chang

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To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Richard Yen-Ching Chang, and entitled Intraspecific Relationships in *Paracalanus quasimodo* [Calinoideae] and *Temora turbinata* [Calinoideae] along the Southeastern Coast of the United States, having been approved in respect to style and intellectual content, is referred to you for judgement.

We have read this dissertation and recommend that it be approved.

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Timothy Collins, Major Professor

Date of Defense: July 3, 2013

The dissertation of Richard Yen-Ching Chang is approved

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DEDICATION

To my family, who have supported and encouraged me through the years.

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ABSTRACT OF THE DISSERTATION

INTRASPECIFIC RELATIONSHIPS IN *PARACALANUS QUASIMODO* [CALINOIDEAE] AND *TEMORA TURBINATA* [CALINOIDEAE] ALONG THE SOUTHEASTERN COAST OF THE UNITED STATES

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*Paracalanus quasimodo* and *Temora turbinata* are two calanoid copepods prominent in the planktonic communities of the southeastern United States. Despite their prominence, the species and population level structure of these copepods is yet unexplored. The phylogeographic, temporal and phylogenetic structure of *P. quasimodo* and *T. turbinata* are examined in my study. Samples were collected from ten sites along the Gulf of Mexico and Florida peninsular coasts. Three sites were sampled quarterly for two years. Individuals were screened for unique ITS-1 sequences with denaturing gradient gel electrophoresis. Unique variants were sequenced at the nuclear ITS-1 and mitochondrial COI loci. Sampling sites were analyzed for pairwise community differences and for variances between geographic and temporal groupings. Genetic variants were analyzed for phylogenetic and coalescent topology. *Paracalanus quasimodo* is highly structured geographically with populations divided between the Gulf of Mexico, temperate Atlantic and subtropical Atlantic, in addition to isolation by distance. No significant differences were detected between the *T. turbinata* samples. Both *P. quasimodo* and *T. turbinata* are

stable within sites over time and between sites within a sampling period, with two exceptions. The first was a pilot sample from Miami taken two years prior to the general sampling whose community showed significant differences from most of the other Miami samples. *Paracalanus quasimodo* had a positive correlation of $F_{st}$ with time. The second was high temporal variability detected in the samples from Fort Pierce. Phylogenetically, both *P. quasimodo* and *T. turbinata* were in well supported, congeneric clades. *Paracalanus quasimodo* was not monophyletic, divided into two well-supported clades. *Temora turbinata* variants were in one clade with insignificant support for topology within the clade and very little intraspecific variation. *Paracalanus quasimodo* and *T. turbinata* populations show opposite trends. *Paracalanus quasimodo* occurs near shore and shows population structure mediated by hydrological features and distance, both geographic and temporal. The phylogeny shows two deeply divergent clades suggestive of cryptic speciation. In contrast, *T. turbinata* populations range further offshore and show little geographic or temporal structure. However, the low genetic variation detected in this region suggests a recent bottleneck event.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AMOVA | Analysis of Molecular Variance |
| COI | Cytochrome Oxidase subunit 1 |
| DGGE | Denaturing gradient gel electrophoresis |
| FP | Fort Pierce |
| GoM | Gulf of Mexico |
| IM | Isla Mujeres |
| ITS-1 | Internal transcribed spacer region 1 |
| Ja | Jacksonville |
| Lu | Louisiana |
| LW | Louisiana West |
| Mi | Miami |
| MSN | Minimum spanning network |
| PA | Port Aransas |
| PC | Panama City |
| PCR | Polymerase chain reaction |
| SK | Summerland Key |
| SNP | Single nucleotide polymorphism |
| Ta | Tampa |

# CHAPTER I

# INTRODUCTION

The genetic structure of populations can provide insight into the history and population dynamics of a species as well as practical information for management. Spatial variation in allele frequencies has been used to detect vicariance events (Schneider-Broussard et al. 1998; Craig et al. 2004; Satoh et al. 2004), patterns of expansion (Caudill and Bucklin 2004; Gil et al. 2004; Mobley et al. 2010; Chen and Hare 2011), and evolutionary bottlenecks (Ball and Chapman 2003). Understanding the history of populations and patterns of population subdivision is critical for defining evolutionary significant units and drafting adequate conservation strategies (Lankford et al. 1999; Gold et al. 2002; Chiswell et al. 2003). Several studies have examined population structure in marine organisms inhabiting the southeastern United States coast, relating life history, mobility, habitat continuity and hydrography to genetic diversity and distribution (Hare and Avise 1996; Garcia-Rodriguez et al. 1998; Schneider-Broussard et al. 1998; Bagley et al. 1999; Lankford et al. 1999; Schizas et al. 1999; Broughton et al. 2002; Gold et al. 2002; Herke and Foltz 2002; Ball and Chapman 2003; McMillen-Jackson and Bert 2004; Mobley et al. 2010). However, no study has investigated the influence of life history and the currents on population structure across the Gulf of Mexico (GoM) and the northwest Atlantic Ocean. A comprehensive study is required to understand the patterns of gene flow in marine organisms with a planktonic life stage in the southeastern United States.

Dispersal influences population subdivision, with greater dispersal leading to less structured populations because of exchange of alleles. High mobility in at least one life

stage facilitates gene flow, as is seen in vermilion snapper, *Rhomboplites aurorubens*, in the GoM and the South Atlantic Bight (Bagley et al. 1999) and king mackerel, *Scomberomorus cavalla*, in the GoM and the Atlantic Ocean (Broughton et al. 2002; Gold et al. 2002). *Rhomboplites aurorubens* is a reef fish with planktonic eggs and larvae, and *S. cavalla* is highly mobile as an adult. Species with high dispersal potential are likely to exhibit low levels of population differentiation even across purported biogeographic barriers (McMillen-Jackson and Bert 2004; but see Burton and Lee 1994; Edmands 2001). In contrast, limited dispersal reduces the frequency that individuals from geographically distant populations interbreed, leading to population subdivision through limited gene flow.

The copepod *Acartia tonsa* may be an example of the effects of limited dispersal. The primarily estuarine habitat used by *A. tonsa* reduces the migration rate as a consequence of the difficulty of surviving the journey between habitats. Gulf and Atlantic populations of *A. tonsa* are genetically distinct (Caudill and Bucklin 2004), but the sampling regime was insufficient to determine whether this was a result of isolation by distance or biogeographic barriers. The latter is likely as a large proportion of genetic types found in the Gulf were rare in the Atlantic samples (Caudill and Bucklin 2004).

Many marine organisms undergo a planktonic stage that increases their dispersal range (Hoskin 1997). Species can be planktonic throughout their life, during particular life stages or only for certain periods of the day. Dispersal potential increases with plankton residence times (McMillan et al. 1992; Kirkendale and Meyer 2004).

Planktonic residence times alone are, however, insufficient to predict rates of gene flow. Habitat continuity can also influence population structure. Manatees are

restricted to coastal habitats with abundant vegetation and freshwater; they show a population structure attributed to discontinuous habitat (Garcia-Rodriguez et al. 1998). Pink shrimp populations are homogenous between the GoM and the Atlantic, attributed to high gene flow facilitated by a continuous habitat distribution along south Florida (McMillen-Jackson and Bert 2004). In contrast, white shrimp have more stringent habitat requirements (McMillen-Jackson and Bert 2004), resulting in significant genetic differences between Gulf and Atlantic populations (Ball and Chapman 2003). However, whether oceanic or estuarine, habitat does not appear to affect penaeid population structure in the absence of biogeographic barriers (Benzie 2000).

In contrast to penaeid shrimp, other species exhibit population structure despite dispersal potential and habitat continuity. Geographically proximate populations of the tide pool harpacticoid copepod *Tigriopus californicus* show high divergence in mitochondrial alleles (Burton and Feldman 1981; Edmands 2001; Burton et al. 2007). Allele frequencies in American Oyster (*Crassostrea virginica*) populations shift from Gulf to Atlantic haplotype dominance at Cape Canaveral (Hare and Avise 1996). The shift in alleles is attributed to the subtropical to temperate ecotone along the central and northeast coasts of the Florida peninsula and to local hydrography (Hare and Avise, 1996). The ghost shrimp, *Callichirus islagrande*, exhibits heterogeneity between populations on both regional to smaller scales of 10s of kilometers (Bilodeau et al. 2005).

The forces driving larval movement can result in retention of gametes rather than wide dispersal. Bluehead wrasse populations, *Thalassoma bifasciatum*, at St. Croix, US Virgin Islands, were found to recruit primarily from retained young rather than from immigration (Swearer et al. 1999). A model of larval dispersal based on Lagrangian

drifters off the Chilean coast and on simulations found sources and sinks on scales of less than 10 km (Aiken et al. 2007), which indicates that populations can be isolated from their near neighbors. The population structure of the ghost shrimp *C. islagrande* in the northeastern GoM supports this model as it exhibits significant genetic differentiation (Bilodeau et al. 2005). An extreme case of isolation occurs in the tide pool inhabiting copepod, *Tigriopus californicus*, where populations separated by only hundreds of meters are genetically distinct (Edmands 2001).

Comparing the phylogeography of copepods in the GoM and the northwest Atlantic will provide insight into the mechanisms influencing gene flow within this region. Currents (Hare and Avise 1996), life history (Kirkendale and Meyer 2004; Lee and Boulding 2009) and habitat continuity (Garcia-Rodriguez et al. 1998; McMillen-Jackson and Bert 2004) have been named as factors influencing population structure within geographic regions and across biogeographic barriers. Assessing patterns of genetic type distribution in species that differ in life history will help deduce how currents and life history interact to affects gene flow.

In addition to geographic patterns, populations may exhibit temporal patterns over the course of mere months. The haplotype frequencies of *A. tonsa* appear to change between sampling years (Caudill and Bucklin 2004). The difference may have been an artifact of the sampling regime, yet is suggestive that migration can have a significant effect even in resident populations. Should this be, wide dispersing plankton may exhibit phylogeographic changes over short temporal periods as populations rove between locations.

Dispersal range should be affected by life history. Zooplankton limited to coastal habitats have theoretically limited dispersal. *Paracalanus quasimodo* are calanoid copepods that fall under this category, found primarily in the neritic zone. Ubiquitous plankton have a greater probability of high gene flow. Their ability to exist from coastal to open ocean habitats allows them to be entrained in long-distance currents such as the Florida Current and the Gulf Stream. *Temora turbinata*, a calanoid copepod, is one such ubiquitous species.

The predominant currents along the tropical to subtropical coasts of the American continent provide a planktonic vector for gene flow. The Caribbean Current either merges into the Florida Current (FC) or the Gulf Loop Current (LC) when it passes through the Strait of Yucatan, between the Yucatan Peninsula and Cuba. The FC sweeps along the southern edge of the Florida Keys and feeds into the Gulf Stream flowing north along the northeast American coast. The LC, instead, travels north through the GoM and veers east between 26°N and 28°N, then south as it nears the continental shelf. At this point, the LC either merges into the FC or it completes the circle and flows back into its northward component. There are occasional vectors towards the western Gulf, either along the Yucatan Peninsula or further north along the LC. Particles entrained in the western Gulf have little opportunity to be carried to the eastern Gulf; hydrographic eddies in the western Gulf limit the frequency of eastern vectors moving particles into the LC (Tomczak and Godfrey 1994).

The present study will examine gene flow in copepods with different habitat ranges in the Gulf of Mexico and along the coasts of the Florida peninsula. Both *Paracalanus quasimodo* and *Temora turbinata* occur in the neritic zone, but *T. turbinata*

can survive further offshore. The nuclear internal transcribed spacer region 1 (ITS-1) and the mitochondrial Cytochrome Oxidase subunit 1 (COI) loci will be examined for nucleotide polymorphisms. The sampled communities will be examined for geographic and temporal structure, and the phylogeny of the genetic variants will provide insight on the intraspecific relationships.

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# CHAPTER II

# GEOGRAPHIC POPULATION STRUCTURE IN *PARACALANUS QUASIMODO* [CALINOIDEAE] AND *TEMORA TURBINATA* [CALINOIDEAE] ALONG THE SOUTHEASTER COAST OF THE UNITED STATES

## Introduction

Phylogeographic studies can provide insights about the factors shaping variation within species, as well as the process of species origination. Patterns of genetic variation over a species range can be used to detect, among others, ecologically significant population boundaries and connectivity between sites. In addition, proper management of commercial and endangered species requires knowledge of reproductive population boundaries (Garcia-Rodriguez et al. 1998; Bagley et al. 1999; Lankford et al. 1999; Gold et al. 2002; Kovach et al. 2010; Saillant et al. 2010; Vinas et al. 2010) and the degree of gene flow between discrete populations (Broughton et al. 2002; Ball and Chapman 2003). Genetic distribution and diversity also provide hints to a species evolutionary history, such as population expansions (Mobley et al. 2010) and evolution of populations divided by geographic shifts (Schneider-Broussard et al. 1998). Comparisons of population structure in conspecific taxa gauge the strength of barriers to gene flow and connectivity. (Lambert et al. 2003; McMillen-Jackson and Bert 2003; Larsson et al. 2010). My study aims to investigate phylogeographic patterns in the southeastern US by examining the population structure of two copepod species, *Paracalanus quasimodo* and *Temora turbinata*.

Both deep and recent histories of gene flow create the observed geographic patterns of the present. Whether as gametes, spores, eggs, larvae or adults, mobility in reproductive units is key to gene flow and connectivity, and plankton have limited autonomous mobility. A planktonic life stage, whether obligate or meroplankton, is important in dispersal of, and maintaining connectivity between, marine populations (Yeung et al. 2001; Barber et al. 2002; Hare and Walsh 2007; Watson et al. 2010; Paz-Garcia et al. 2012). Though some marine taxa can travel long distances as adults to breed, particularly pelagic fish (Broughton et al. 2002), many mobile adults remain close to where they settled as juveniles, and sessile organisms have no other mode of long distance dispersal (Hare and Avise 1996; Domingues et al. 2010).

Phylogeographic studies in the southeastern United States (US) have examined population structure in diverse marine taxa. Some species are panmictic, with one breeding population spanning the entire region and little evidence of genetic partitioning (vermilion snapper, *Rhomboplites aurorubens*, Bagley et al. 1999; king mackerel, *Scomberomorus cavalla*, Broughton et al. 2002, Gold et al. 2002; brown shrimp, *Farfantepenaeus aztecus*, McMillen-Jackson and Bert 2003; pink shrimp, *F. duorarum*, McMillen-Jackson and Bert 2004). Many more species have multiple breeding populations in the southeast US.

For genetically structured populations, five general patterns have been observed in the southeast US. One common pattern is a split between the Gulf of Mexico (GoM) and the Atlantic (American oyster, *Crassostrea viginica*, Hare and Avise 1996; West Indian manatee, *Trichechus manatus*, Garcia Rodriguez et al. 1998; Atlantic croaker, *Micropogonias undulates*, Lankford et al. 1999; *EuryTemora affinis* Lee 2000; longfin

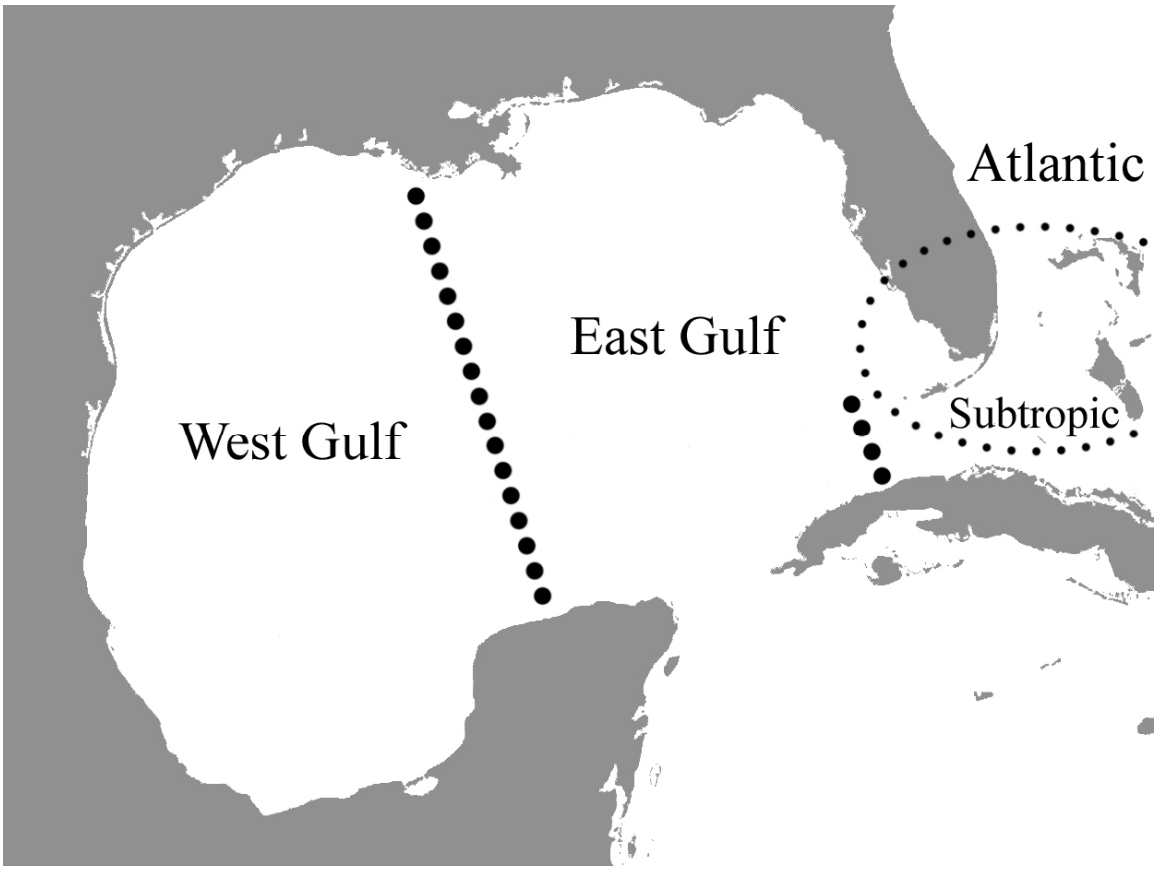

Figure 1: General marine phylogeographic boundaries in the southeastern United States.

squid, *Loligo pealei*, Herke and Foltz 2002; *Acartia tonsa* Caudill and Bucklin 2004; Figure 1). A second has a division between the western GoM and a combined eastern GoM/Atlantic (arrow squid, *Loligo plei*, Herke and Foltz 2002; white shrimp *Litopenaeus setiferus*, McMillen-Jackson and Bert 2003, but see Ball and Chapman 2003). The third returns to the division between the GoM and the Atlantic with an additional sub-tropic population (Lee and O'Foighil, 2004). A fourth is similar to the previous with GoM, sub-tropic and Atlantic populations, but introduces an additional division between the eastern and western GoM (*Gracilaria tikvahiae*, Gurgel et al. 2004). Finally, there is regional and local structure independent of ocean basins (*Microarthridion littorale*, Schizas et al. 1999; dusky pipefish, *Syngnathus floridae*, Mobley et al. 2010). Although some of the studies have only one sampling site in a purportedly isolated region, the differences found are sufficient to indicate the presence of structure over the range of the respective taxa.

At a basic level, currents may influence patterns in population structure (Roberts, 1997) with additional factors and interactions contributing to diverse phylogeographic patterns. Hydrographic (Lee et al., 1994; Limouzy-Paris et al. 1997; Olascoaga 2010), atmospheric (Feichter et al., 2008; Smith, 2009) and geographic factors influence oceanic and local current directions and velocities. The strongest currents off the southeastern US coasts are the Loop Current in the GoM, which becomes the Florida Current and the Gulf Stream (Figure 2). However, tidal currents perpendicular to the shore, along shore counter currents and eddies are also present, which complicates prediction of the path of a plankter. Behavior, such as vertical migration and the timing thereof, influence which currents carry plankton, thus whether and how far they disperse or how well they maintain their geographic position (Criales et al., 2006; Aiken et al., 2007).

*Paracalanus quasimodo* and *Temora turbinata* are epipelagic species, but *P. quasimodo* is strictly coastal with a limited latitudinal distribution while *T. turbinata* occurs both along coasts and in oceanic waters of the tropics and subtropics over most of the globe, except the east Pacific (Owre and Foyo, 1967; Boltovskoy 1999). Despite the differences in global distribution, *P. quasimodo* and *T. turbinata* inhabit the same waters in the GoM and the Atlantic coast of the Florida peninsula. However, as *T. turbinata* ranges further offshore than *P. quasimodo*, *T. turbinata* will encounter the stronger currents typically found beyond the continental shelf more frequently than will *P. quasimodo*. Comparing *P. quasimodo* and *T. turbinata* population structures will demonstrate how connectivity changes in the southeast US with different degrees of dispersal potential.

Although there is no definite correlation between mobility and patterns of population structure in previous studies, there is a general association between greater mobility and more homogenous population structure in the southeast US (Herke and Foltz 2002). As a consequence of the differences in potential mobility, we hypothesized that *P. quasimodo* would have more structured populations than *T. turbinata*. To test the hypothesis, the two copepod species were sampled across the GoM and the Atlantic coast of the Florida peninsula. Sampled populations were tested for connectivity and gene flow restrictions to detect which, if any, were isolated. They were further analyzed for broader geographic patterns of isolation-by-distance (IBD), genetic variant clustering and hierarchal, regional variation.

## Materials and Methods

### *Collection*

For the purposes of the present study, we assumed that a sampling site represented a population. Samples were collected from ten sites around the Gulf of Mexico and the Florida peninsula (Table 1, Figure 2). The majority of the samples were collected between November 2007 and January 2008, the exceptions being the Lu samples in August of 2006, an earlier FP sample in September of 2007 and the IM samples in September of 2008. With the exception of Lu and LW, samples were collected by ten-minute surface tows with both a 150μm and 366μm mesh nets; Lu and LW samples were collected with five vertical hauls with a 150μm mesh net beginning 5m below the surface. Surface tows were conducted along transects beginning ten miles offshore to inshore with one tow at the beginning, middle and end of the transect. Samples were named by location followed by the three-digit number of the storage bottle.

Excepting PA and IM, samples were immediately preserved in 95% ethanol. The PA samples were preserved in 60% isopropanol, chilled with dry ice, transported in a cooler with blue ice and transferred to 95% ethanol upon return to the laboratory. The IM samples were preserved in 60% ethanol for transport and transferred to 95% ethanol upon return. Selected individuals were identified to species by dichotomous keys (Owre and Foyo 1967; Boltovskoy 1999).

Table 1: Sampling sites, sample size, number of variants and coordinates for *Paracalanus quasimodo* and *Temora turbinata*. FP072 = Fort Pierce 072; FP 091 = Fort Pierce 091; IM = Isla Mujeres; Ja = Jacksonville; LW = Louisiana West; Lu = Louisiana; Mi = Miami; PC = Panama City; PA = Port Aransas; SK = Summerland Key; Ta = Tampa

| | *P. quasimodo* | | | *T. turbinata* | | |
|---|---|---|---|---|---|---|
| Site | n | #v | Latitude/Longitude | n | #v | Latitude/Longitude |
| FP072 | 40 | 6 | N27°49.396 W97°02.602 | n/a | n/a | n/a |
| FP091 | 65 | 14 | N27°26.960 W80°05.025 | 39 | 9 | N27°26.960 W80°05.025 |
| IM | 39 | 10 | N21°17 W86°46 | 38 | 9 | N21°19 W86°47 |
| Ja | 40 | 3 | N30°23.477 W81°12.867 | 38 | 11 | N30°23.477 W81°12.867 |
| LW | 40 | 5 | N29°19.524 W93°25.044 | 40 | 5 | N29°19.524 W93°25.044 |
| Lu | 59 | 7 | N29°02.508 W90°31.314 | 38 | 6 | N28°51.444 W90°27.816 |
| Mi | 40 | 4 | N25°54.001 W80°07.521 | 40 | 5 | N25°54.001 W80°07.521 |
| PC | 39 | 10 | N29°59.172 W85°47.214 | 16 | 6 | N30°07.305 W85°44.734 |
| PA | 40 | 8 | N27°49.396 W97°02.602 | 40 | 5 | N27°41.555 W96°57.026 |
| SK | 39 | 4 | N24°33.746 W81°27.597 | 39 | 7 | N24°33.746 W81°27.597 |
| Ta | 40 | 2 | N27°44.087 W82°51.913 | 40 | 9 | N27°44.087 W82°51.913 |

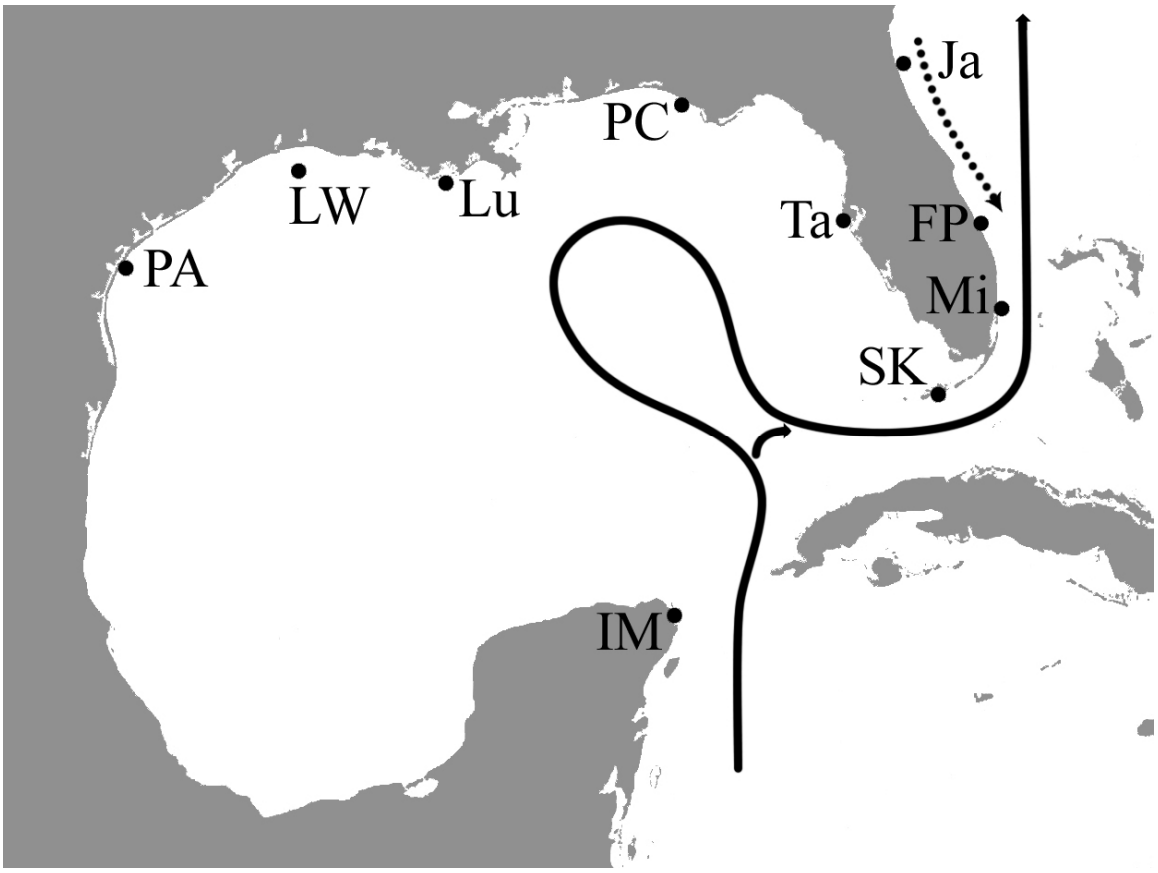


Figure 2: Sampling sites and major currents in the southeast United States. The dotted line represents a coastal countercurrent. FP=Fort Pierce, IM=Isla Mujeres, Ja=Jacksonville, Lu=Louisiana, LW=Louisiana West, Mi=Miami, PC=Panama City, PA=Port Aransas, SK=Summerland Key, Ta=Tampa. Map base modified from Google Earth.

*DNA Extraction*

Forty individuals each of *Paracalanus quasimodo* and *Temora turbinata* were selected from site samples with two exceptions. Sixty *P. quasimodo* were selected from the Lu sample as part of a prior feasibility study (data not shown). The first 40 FP091 *P. quasimodo* formed an anomalous community, thus forty additional *P. quasimodo* were selected from this sample to confirm the variant distribution. Only 38 and 16 *T. turbinata* were found in the Lu and PC samples, respectively. Individual copepods were transferred directly from ethanol to a 1.5µl microcentrifuge tube. Extractions were conducted with the MasterPure DNA extraction kit (Epicentre, Madison, WI) following manufacturer's protocols and stored in 50µl of UV sterilized, micropore filtered, deionized water at –80°C. DNA concentration was quantified on a DyNA Quant 200 spectrophotometer (Hoefer) and varied between 3nM and 30nM.

*PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)*

Denaturing gradient gel electrophoresis is a useful tool for screening multiple populations with a large sampling size for genetic variation (Abrams and Stanton, 1992; LaJeunesse, 2001). Because of its sensitivity to nucleotide changes, DGGE has been used to describe microbial assemblages (Schauer et al., 2000; Diez et al., 2001; Martin et al., 2006; Rigonato et al., 2012) and screen for *Symbiodinium* lineages within cnidarians (LaJeunesse and Trench, 2000; LaJeunesse 2001). The potential to detect one base pair (bp) changes between individuals facilitates the process of sorting intraspecies polymorphisms. The nuclear internal transcribed spacer I (ITS-1), located between the 18s and 5.8s genes, was chosen because, as it is a non-encoding region, this locus has a

potentially high mutation rate (LaJeunesse and Trench, 2000; LaJeunesse, 2001; LaJeunesse and Pinzon, 2007).

The ITS-1 region was amplified with the primers ITS-1f (Coleman et al. 1994, Table 2) with the GC clamp added to the 5' end of the sequence and ITS-1r (Schizas et al. 1999). The PCR reactions were run as quantitative reactions in lieu of sacrificing PCR product to check for successful amplification through electrophoresis. Amplifications were run in 10µl PCR reactions composed of 5µl iQ SybrGreen 2x (BioRad, Hercules, CA), 0.5µl ITS-1r (0.5 µM final concentration), 0.5µl ITS-1fCol with GC clamp (1 µM final concentration), 1µl DNA extract (0.3 nM to 3 nM final concentration), and 3µl nuclease free water.

The ITS-1 primer pair produced amplicons of approximately 320bp in length. The thermal profile for the reactions was: 95°C (5 min), 10 touchdown cycles beginning with 95-60-72°C for 20-20-60s with a 0.5°C drop in the annealing temperature per cycle, 25 cycles of 95-55-72°C for 20-20-60s and a final extension at 72°C for 10 minutes in a DNA Engine Opticon 2 (MJ Research).

The optimal urea gradient for parallel DGGE was determined to be 30-50%. The gels were run in aquaria (C.B.S. Scientific, Del Mar, CA) of TAE buffer at 65°C for 18 hours at 90 volts. To confirm that these were ITS-1 sequences, the brightest lower bands from the three dominant variants were cut from the DGGE gel, eluted in 500 µl of water for 24 hours, cleaned with the Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI), re-amplified and prepared for sequencing with BigDye terminator, ver 3.1 (Applied Biosystems, Grand Island, NY).

Table 2: Primer sequences.

| Primer Name | Sequence |
|---|---|
| GC Clamp | CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC |
| ITS-1f | GGG ATC CGT TTC CGT AGG TGA ACC TGC |
| ITS-1r | ATC GAC CCA TGA GCC GAG TGA TC |
| LCOI-1490 | GGT CAA CAA ATC ATA AAG ATA TTG G |
| LCOI-1490c | GGT CAT GTA ATC ATA AAG ATA TTG G |
| LCOI-1528P | GTT AGC AGG AGC TTG ATC AG |
| HCOI-2198 | TAA ACT TCA GGG TGA CCA AAA AAT CA |
| HCOI-2198Par | TAG ACT TCA GGA TGT CCA AAG AAT CA |

*Sequencing*

Polymorphic sequences can change the DGGE banding pattern between individuals with the same dominant genetic variant. However, the dominant variant provides strong evidence of the evolutionary history in an organism (LaJeunesse and Pinzon, 2007). Thus, ten individuals from each sample site were selected for DNA sequencing on the basis of DGGE variants. Where possible, at least one of each variant present at a sample site was selected. The nuclear ITS-1 and mitochondrial cytochrome oxidase I (COI) regions were amplified. The ITS-1 primers were the same as that for DGGE analysis, minus the GC clamp. Standard Folmer COI primers (LCOI-1490 and HCOI-2198) were unable to amplify a majority of the *Paracalnus quasimodo* samples and a large number of the *Temora turbinata*. Modified Folmer primers (LCOI-1490c and HCOI-2198Par) were designed based on whole copepod mitochondrial sequences found in GenBank worked well with *T. turbinata*, but were only moderately successful with *P. quasimodo*. A COI primer was designed specifically for *P. quasimodo* (LCOI-1528p), which began 38bp downstream of LCOI-1490, and was paired with HCOI-2198Par to sequence the remaining *P. quasimodo*.

The PCR amplifications were conducted in a PTC-200 DNA Engine (MJ Research) with Promega GoTaq® Flexi reagents. Reactions were composed of 2µl of 5x Buffer, 0.6µl of 25mM $MgCl_2$ (1.5mM final concentration), 0.5µl each of forward and reverse primers (0.5µM, ITS-1 primers, and 1.0µM, COI primers, final concentration), 0.2µl of 10 µM dNTPs (0.2µM final concentration), 0.2µl of 2.5U/µl DNA Polymerase (0.05U/µl final concentration) and 2 to 5µl of DNA (0.6-6nM to 1-10nM final concentration) with nuclease free water added to make a final volume of 10µl. The higher

concentration of DNA was used when the lower concentration resulted in low amplification.

Three µl of PCR product were ran on an 0.8% agarose gel to determine whether the amplification was successful. The PCR products were then cleaned with ExoSap (Affymetrix, Santa Clara, CA) following manufacturer's protocols. Cleaned products were prepared for sequencing with BigDye terminator, ver 3.1 (Applied Biosystems, Grand Island, NY) for both forward and reverse strands in a PTC-200 DNA Engine (MJ Research). The 10µl cycle sequencing reaction mix was composed of 0.5µl of 1.87µM primer (0.935 µM final concentration), 2-4µl of amplified product, depending on gel band intensity (2µl if the band was bright, otherwise 4µl), 2µl of 5x sequencing buffer, 1µl of BigDye v3.1 and the rest with double distilled deionized water. The reaction cycle was 15 seconds at 95ºC, 10 seconds at 50ºC and 4 minutes at 60ºC, repeated 35 times. Products were sequenced in an AB 3100 Genetic Analyzer (Applied Biosystems, Grand Island, NY) at the Florida International University DNA Core. Several sequences were unreadable due to contamination or, in the case of ITS-1, the presence of multiple sequence varieties. Samples where standard sequencing failed were cloned with a TOPO TA cloning kit for sequencing (Invitrogen, Grand Island, NY) following the manufacturer's protocols.

Sequences were edited and reconciled in FinchTV (Geospiza, Seattle, WA), trimmed in BioEdit (Hall, 1999) and aligned with ClustalW (Larkin et al., 2007) through BioEdit. A consensus sequence was constructed in BioEdit for each variant with multiple sequences. All polymorphic sites were marked as such, even if the alternate nucleotide was represented in only one sequence. Individuals were designated as separate variants

when the concatenated sequences were different. Thus, variants could be identical in either ITS-1 or COI, but not both.

*Analysis*

Population comparisons and Mantel tests were conducted in Arlequin 3.5 (Excoffier and Lischer 2010) with sequence data. Pairwise $F_{st}$ comparisons were conducted to determine whether there was significant restriction in gene flow between sites. Pairwise comparisons were run with 99,999 permutations. A Bonferroni correction for multiple tests was applied to the results of the pairwise comparisons. Mantel tests examined the data for evidence of isolation-by-distance (IBD; Barber et al. 2002). The $F_{st}$ matrices produced by the $F_{st}$ pairwise comparisons were tested against three distance matrices: straight-line distances between sites, chord distances from Ja to SK, then straight line to all other sites and chord distance along the southeast US coast. Straight-line distances were the shortest lines between sites. Chord distances were calculated by adding straight-line distances of adjacent sites (e.g., the chord distance from FP to SK would be the sum of the straight line distances from FP to Mi and from Mi to SK). IM distances were the same for the second and third distance matrices.

Genetic data was converted into numerical sequences with GenAlEx 6.41 (Peakall and Smouse, 2006) in preparation for cluster analysis by Structure 2.3.3 (Hubisz et al., 2009). Cluster analysis groups the variants into populations by similarity and the distribution of the populations within and between sites can be examined for geographic patterns. Analysis was set to a 60,000 MCMC chain with a 10,000 step burn-in period. Both alpha and lambda values were inferred by Structure. The number of populations (K)

was set for 1 through 10, with 20 replicate runs for each K. The results were analyzed with Structure Harvester (Dean and vonHoldt, 2012) to determine the most likely value for K.

Analyses of molecular variance were used to test whether sites were part of distinct geographic regions. AMOVAs were run in Arlequin 3.5 (Excoffier and Lischer, 2010) with the maximum number of permutations (99,999) with three sets of a priori groups found in other taxa. The first set was a single population. The second set separated sites between the GoM and the Atlantic. The third set had GoM, Atlantic and subtropical groups. Because the *P. quasimodo* population composition of FP091 was inconsistent with all other samples collected from FP over a two year period (data not shown), this sample was excluded from the *P. quasimodo* AMOVA analyses.

## RESULTS

Of the 500 *Paracalanus quasimodo* and 374 *Temora turbinata* DNA extractions, 498 and 368 individuals were successfully amplified for DGGE, respectively. The eluted DGGE bands were confirmed to be ITS-1 sequences. *Euchatea ramina* was morphologically similar to *P. quasimodo*, but had a distinct ITS-1 sequence. *P. quasimodo* genetic variants 26 and 38 were removed from analysis as a BLAST search returned a 99% similarity to *Euchatea ramina* (Accession HM045386.1). There were a total of 37 *P. quasimodo* and 27 *T. turbinata* variants. The greatest number of *P. quasimodo* variants was found at FP, with 14 variants, and the least number of variants were found at Ta, with two. The greatest and least numbers of *T. turbinata* variants were found at Ja, with 11 variants, and PA, LW, and Mi, with 5 variants, respectively. The variant proportions from the December *P. quasimodo* sample for FP (FP 091) differed widely from the other FP samples (data not shown), thus an earlier sample from September (FP 072) was added to the analyses.

### *Paracalanus quasimodo*

The most frequent variants were one, two and three, followed by variants 21, 22 and 23 (Figure 3). Pairwise comparisons indicate restricted gene flow between most of the sampling sites (Table 3). ITS-1, COI and concatenated sequences shared the pattern of no significant restrictions in gene flow detected between LW, Lu, Ta and Ja and between SK, Mi and FP (Table 3). Additional connections were found depending on the sequence. For ITS-1, there was no significant restriction between Ja and FP 072. For

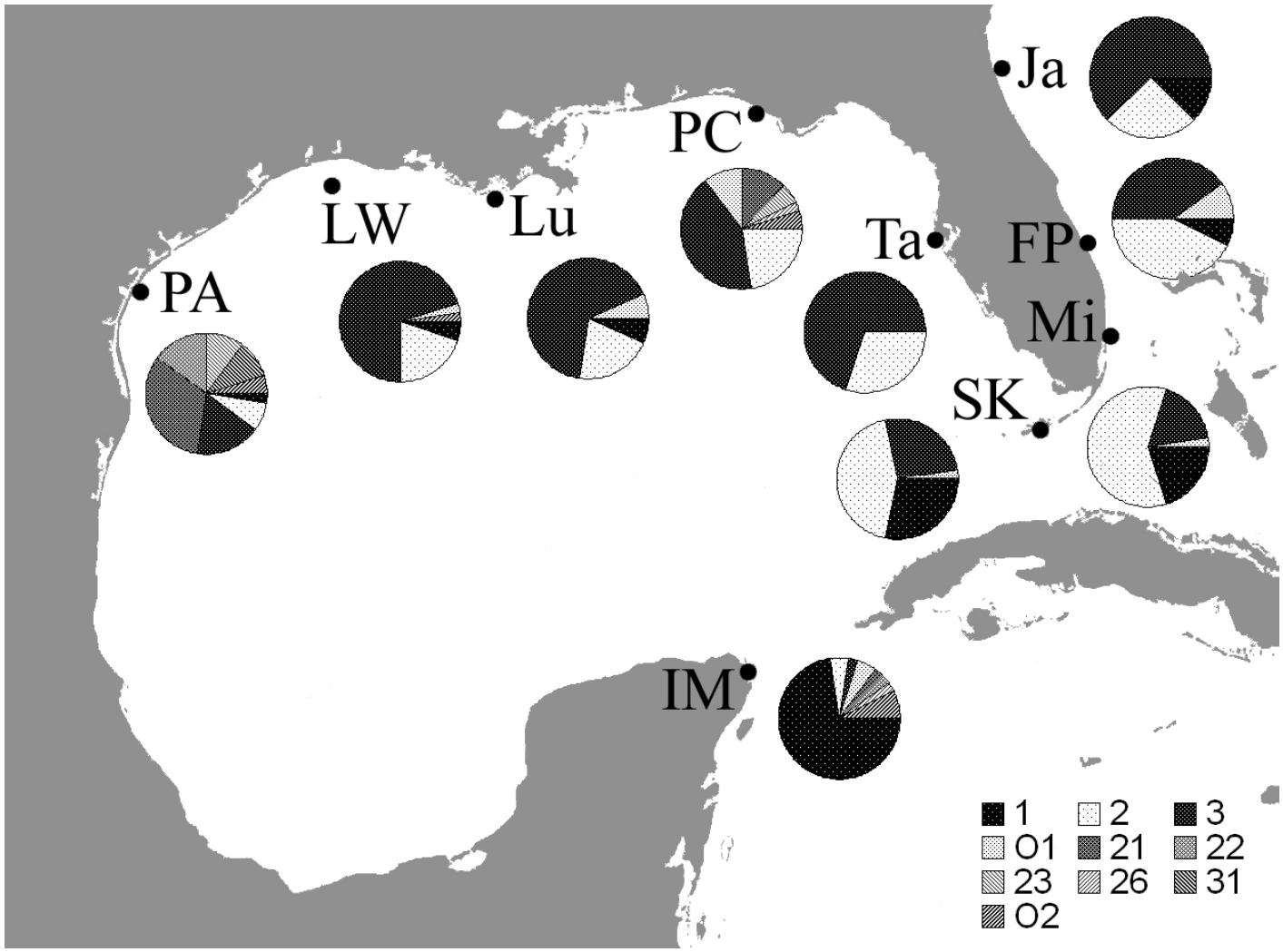


Figure 3: *Paracalanus* quasimodo population composition. The most abundant genetic variants are labeled by number and the rare variants were grouped into O1 and O2 for populations one and two, respectively.

Table 3: $F_{st}$ pairwise comparisons for *Paracalanus quasimodo*. Fst values are below the diagonal. Probabilities are above the diagonal. Probabilities in bold remained significant with a Bonferroni correction. - = not significant; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

ITS-1

| | PA | LW | Lu | PC | Ta | SK | Mi | FP091 | FP072 | Ja | IM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PA | 0 | ******* | ******* | ******* | ******* | ******* | ******* | * | ******* | ******* | ******* |
| LW | 0.606 | 0 | - | ******* | - | ******* | ******* | ******* | * | - | ******* |
| Lu | 0.644 | -0.019 | 0 | ******* | - | ******* | ******* | ******* | * | - | ******* |
| PC | 0.274 | 0.179 | 0.206 | 0 | ******* | ******* | ******* | ** | ******* | ******* | ******* |
| Ta | 0.609 | -0.005 | 0.002 | 0.182 | 0 | ******* | ******* | ******* | * | - | ******* |
| SK | 0.559 | 0.164 | 0.158 | 0.156 | 0.167 | 0 | - | ******* | - | ** | ******* |
| Mi | 0.571 | 0.232 | 0.226 | 0.177 | 0.217 | -0.005 | 0 | ******* | * | ******* | ******* |
| FP091 | 0.064 | 0.344 | 0.389 | 0.084 | 0.348 | 0.293 | 0.305 | 0 | ******* | ******* | ******* |
| FP072 | 0.595 | 0.081 | 0.069 | 0.174 | 0.065 | 0.031 | 0.054 | 0.330 | 0 | - | ******* |
| Ja | 0.600 | -0.003 | -0.007 | 0.175 | 0.009 | 0.088 | 0.147 | 0.336 | 0.023 | 0 | ******* |
| IM | 0.492 | 0.353 | 0.366 | 0.186 | 0.381 | 0.141 | 0.197 | 0.234 | 0.281 | 0.297 | 0 |

COI

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PA082 | 0 | ******* | ******* | ******* | ******* | ******* | ******* | - | ******* | ******* | ******* |
| LW032 | 0.664 | 0 | - | ******* | - | ** | ******* | ******* | * | - | ******* |
| Lu022 | 0.697 | -0.020 | 0 | ******* | - | ** | ******* | ******* | * | - | ******* |
| PC099 | 0.337 | 0.173 | 0.200 | 0 | ******* | ** | *** | ** | ******* | ******* | ** |
| Ta105 | 0.674 | -0.001 | 0.004 | 0.183 | 0 | ** | ******* | ******* | - | - | ******* |
| SK085 | 0.615 | 0.087 | 0.084 | 0.133 | 0.088 | 0 | - | ******* | - | * | ******* |
| Mi089 | 0.624 | 0.167 | 0.168 | 0.150 | 0.142 | 0.003 | 0 | ******* | - | ** | ******* |
| FP091 | 0.049 | 0.473 | 0.515 | 0.133 | 0.486 | 0.411 | 0.425 | 0 | ******* | ******* | ******* |
| FP072 | 0.649 | 0.068 | 0.065 | 0.157 | 0.050 | 0.009 | 0.021 | 0.454 | 0 | * | ******* |
| Ja097 | 0.666 | -0.012 | -0.013 | 0.179 | 0.002 | 0.053 | 0.133 | 0.475 | 0.053 | 0 | ******* |
| IM134 | 0.533 | 0.275 | 0.291 | 0.150 | 0.325 | 0.160 | 0.244 | 0.318 | 0.269 | 0.254 | 0 |

Concatenated

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PA082 | 0 | ******* | ******* | ** | ******* | ******* | ******* | * | ******* | ******* | ******* |
| LW032 | 0.245 | 0 | - | * | - | ******* | ******* | ******* | ** | - | ******* |
| Lu022 | 0.240 | -0.019 | 0 | * | - | ******* | ******* | ******* | ** | - | ******* |
| PC099 | 0.080 | 0.0529 | 0.048 | 0 | * | ** | ******* | ******* | - | - | ******* |
| Ta105 | 0.267 | -0.010 | -0.005 | 0.056 | 0 | ******* | ******* | ******* | * | - | ******* |
| SK085 | 0.171 | 0.191 | 0.173 | 0.086 | 0.191 | 0 | - | ** | - | ** | ******* |
| Mi089 | 0.228 | 0.290 | 0.270 | 0.147 | 0.273 | 0.009 | 0 | ******* | * | ******* | ******* |
| FP091 | 0.028 | 0.227 | 0.218 | 0.078 | 0.250 | 0.078 | 0.142 | 0 | ******* | ******* | ******* |
| FP072 | 0.169 | 0.093 | 0.080 | 0.026 | 0.074 | 0.022 | 0.053 | 0.115 | 0 | - | ******* |
| Ja097 | 0.213 | -0.010 | -0.014 | 0.033 | -0.001 | 0.117 | 0.212 | 0.178 | 0.045 | 0 | ******* |
| IM134 | 0.350 | 0.518 | 0.490 | 0.397 | 0.560 | 0.265 | 0.381 | 0.230 | 0.402 | 0.448 | |

COI, comparisons between Mi and FP 072, Ta and FP 072 and PA and FP 091 were not significant. For the concatenated sequences, no significant restrictions in gene flow were detected between PC and Ja and between PC and FP 072. After applying a Bonferroni correction for multiple tests, a majority of the gene flow restrictions between sites remained significant (Table 3). In addition to local structure, both ITS-1 and COI follow an IBD gradient, but there was no significant correlation with the concatenated sequences (Table 4; Figure 4).

Structure Harvester indicated that there were two populations of *P. quasimodo* in the southeast US. Under K=2, Structure clustered the major variants, variants 1, 2 and 3, and the rare variants 4 through 18, 36, and 40 in one population (Table 5). With the exception of variant 52, the remaining variants clustered in the second population. Variant 52 identified with different populations depending on which locus was examined: population one with ITS-1 and the concatenated sequences and population two with COI. The majority of population two was found in the Gulf (Figure 5), although 53% (19/36) of FP091 came from population two.

The AMOVA detected significant variation between populations (Table 6). Separating populations into GoM and Atlantic groups results with no significant variation among the groups, but highly significant variation among the populations within the groups, which does not support a division between the GoM and Atlantic. As Structure indicated that there were two populations, and there was a large genetic divergence between populations one and two, sites with a larger proportion from population two were excluded from the following AMOVAs. Under these conditions, AMOVA detected significant variation between GoM and the Atlantic. However, there was also significant

Table 4: Mantel tests for isolation-by-distance.

| | | Straight Line | | Partial Chord | | Chord | |
|---|---|---|---|---|---|---|---|
| Species | Loci | p | $R^2$ | p | $R^2$ | p | $R^2$ |
| *P. quasimodo* | ITS-1 | 0.011* | 0.290 | 0.038* | 0.125 | 0.038* | 0.125 |
| | COI | 0.025* | 0.249 | 0.035* | 0.133 | 0.034* | 0.139 |
| | Concatenated | 0.077 | 0.117 | 0.270 | 0.011 | 0.304 | 0.005 |
| *T. turbinata* | ITS-1 | 0.495 | 0.001 | 0.540 | 0.001 | 0.572 | 0.003 |
| | COI | 0.041* | 0.107 | 0.026* | 0.131 | 0.022* | 0.150 |
| | Concatenated | 0.357 | 0.002 | 0.352 | 0.002 | 0.359 | 0.001 |

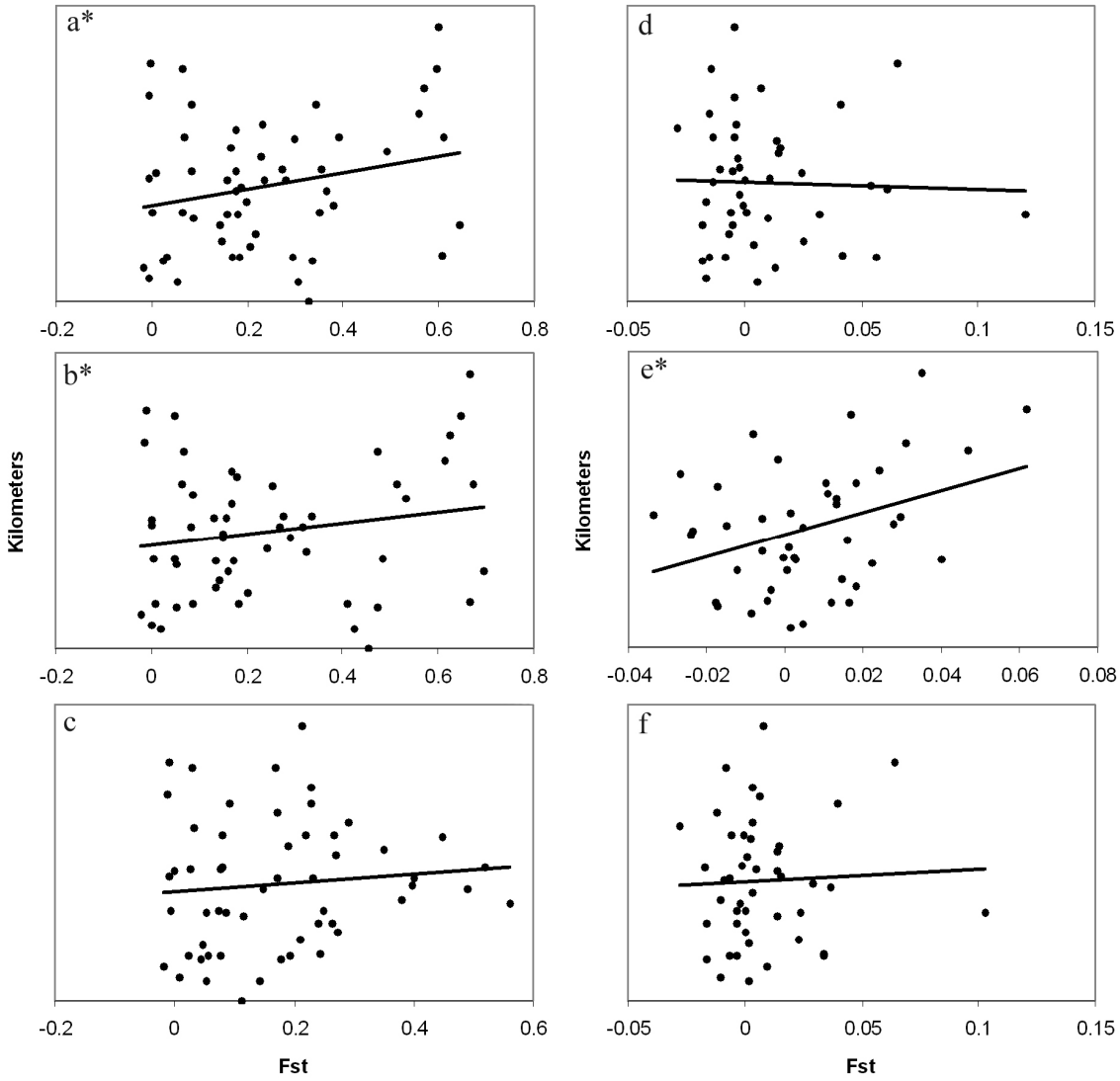


Figure 4: Scatter plots of $F_{st}$ versus distance with a least squares regression line. As straight-line distance, partial chord and chord distances resulted in the same probabilities in the Mantel test, only the partial chord plots are shown here. a) *P. quasimodo* ITS-1; b) *P. quasimodo* COI; c) *P. quasimodo* concatenated; d) *T. turbinata* ITS-1; e) *T. turbinata* COI; f) *T. turbinata* concatenated. Significant correlations at $\alpha = 0.5$ are denoted by an asterisk (a, b and e).

Table 5: *Paracalanus quasimodo* variant clusters as designated by Structure.

| | Variant | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| ITS-1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| COI | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| Concatenated | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |

| | Variant | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 21 | 22 | 23 | 25 | 28 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 40 | 44 | 50 | 51 | 52 |
| ITS-1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 |
| COI | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 2 |
| Concatenated | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 1 |

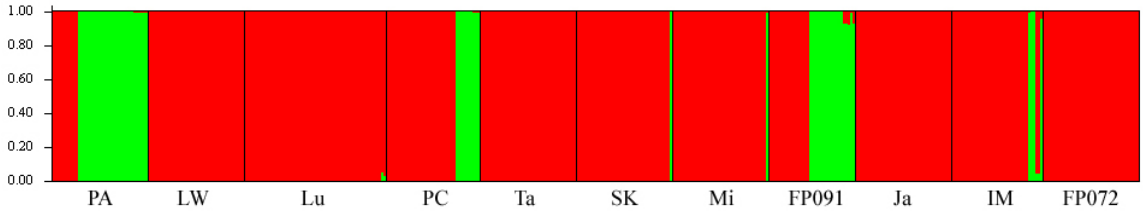


Figure 5: Structure bar plot of inferred ancestry of *P. quasimodo* from ITS-1 sequences.

Table 6: AMOVA results. PA, LW, Lu, PC, Ta and IM comprise the Gulf sites. SK Mi, FP and Ja comprise the Atlantic sites. SK and Mi were removed from the Atlantic group to form the subtropics group. PA, PC and IM were not included in the population one analysis, as there was a large proportion of population two at these sites. *T. turbinata* results were similar for both loci and when the loci were combined. ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

| Source of variation | df | Sum of squares | Variance components | Percentage of variation | F index | p |
|---|---|---|---|---|---|---|
| | | | *Paracalanus quasimodo* ITS-1 | | | |
| One population | | | | | | |
| Among populations | 9 | 1637.192 | 4.21703 | 38.05 | Fst=0.38052 | *** |
| Within populations | 406 | 2787.336 | 6.86536 | 61.95 | | |
| Gulf and Atlantic | | | | | | |
| Among groups | 1 | 203.269 | 0.12197 | 1.09 | Fct=0.01095 | ns |
| Within groups | 8 | 1433.923 | 4.15274 | 37.28 | Fsc=0.37690 | *** |
| Within populations | 406 | 2787.336 | 6.86536 | 61.63 | Fst=0.38372 | *** |
| Population 1 Gulf and Atlantic | | | | | | |
| Among groups | 1 | 58.053 | 0.33188 | 9.54 | Fct=0.09543 | ns |
| Within groups | 5 | 42.045 | 0.12779 | 3.67 | Fsc=0.04062 | ** |
| Within populations | 292 | 881.254 | 3.01799 | 86.78 | Fst=0.13218 | *** |
| Population 1 Gulf, Subtropics and Atlantic | | | | | | |
| Among Groups | 2 | 88.296 | 0.13009 | 12.48 | Fct=0.12479 | * |
| Within Groups | 4 | 11.801 | -0.00158 | -0.05 | Fsc=-0.00053 | ns |
| Within populations | 292 | 881.254 | 3.01799 | 87.57 | Fst=0.12433 | *** |
| | | | *Paracalanus quasimodo* COI | | | |
| One population | | | | | | |
| Among populations | 9 | 2049.664 | 5.30026 | 39.22 | Fst=0.39218 | *** |
| Within groups | 405 | 3326.916 | 8.21461 | 60.78 | | |
| Gulf and Atlantic | | | | | | |
| Among groups | 1 | 260.256 | 0.18644 | 1.37 | Fct=0.01371 | ns |
| Within groups | 8 | 1789.408 | 5.20192 | 38.24 | Fsc=0.38772 | *** |
| Within populations | 405 | 3326.916 | 8.21461 | 60.39 | Fst=0.39612 | *** |
| Population 1 Gulf and Atlantic | | | | | | |
| Among groups | 1 | 49.684 | 0.26673 | 5.66 | Fct=0.05660 | ns |
| Within groups | 5 | 49.477 | 0.13264 | 2.81 | Fsc=0.02984 | * |
| Within populations | 291 | 1255.091 | 4.31303 | 91.53 | Fst=0.08475 | *** |
| Population 1 Gulf, Subtropics and Atlantic | | | | | | |
| Among groups | 2 | 74.434 | 0.32500 | 6.94 | Fct=0.06942 | * |
| Within groups | 4 | 24.727 | 0.04386 | 0.94 | Fsc=0.01007 | ns |
| Within populations | 291 | 1255.091 | 4.31303 | 92.12 | Fst=0.07878 | *** |
| | | | *Paracalanus quasimodo* concatenated | | | |
| One population | | | | | | |
| Among populations | 9 | 29.131 | 0.07105 | 19.46 | Fst=0.19458 | *** |
| Within groups | 405 | 119.103 | 0.29408 | 80.54 | | |
| Gulf and Atlantic | | | | | | |
| Among groups | 1 | 3.891 | 0.00375 | 1.02 | Fct=0.01022 | ns |
| Within groups | 8 | 25.239 | 0.06907 | 18.83 | Fsc=0.19020 | *** |
| Within populations | 405 | 119.103 | 0.29408 | 80.15 | Fst=0.19847 | *** |
| Population 1 Gulf and Atlantic | | | | | | |
| Among groups | 1 | 5.916 | 0.03386 | 10.40 | Fct=0.10399 | ns |
| Within groups | 5 | 4.353 | 0.01409 | 4.33 | Fsc=0.04828 | ** |
| Within populations | 291 | 80.808 | 0.27769 | 85.28 | Fst=0.14725 | *** |
| Population 1 Gulf, Subtropics and Atlantic | | | | | | |
| Among groups | 2 | 8.723 | 0.04160 | 12.93 | Fct=0.12926 | * |
| Within groups | 4 | 1.547 | 0.00256 | 0.79 | Fsc=0.00913 | ns |
| Within populations | 291 | 80.808 | 0.27769 | 86.28 | Fst=0.12926 | *** |
| | | | *Temora turbinata* ITS-1 | | | |
| Among populations | 9 | 3264 | 0.00175 | 0.58 | Fst=0.00581 | ns |
| Within groups | 357 | 106.684 | 0.29883 | 99.42 | | |

variation among populations within the groups, indicating unresolved structure. When the two southern populations of the Atlantic, SK and Mi, were set in their own group, there was significant variation between the three groups, but no significant variation within the groups.

*Temora turbinata*

*T. turbinata* showed a pattern that suggested panmixia. All significant restrictions in gene flow occurred between LW and PA, PC and Ja (Table 7). Significant restrictions between LW and Ja were consistent across the three sequence analyses. Additional significant restrictions were detected between LW and PC with ITS-1, between LW and FP091 with COI and between LW and PA and LW and FP091 with the concatenated sequences. However, none of the restrictions in gene flow between pairs remained significant after applying the Bonferroni correction. A Mantel test with COI pairwise $F_{st}$ values showed a significant IBD correlation (Table 4), but no such correlation was detected with either ITS-1 or the concatenated sequences. There were no significant hierarchal differences detected by AMOVA (Table 6).

Structure Harvester suggests two, three and three populations for ITS-1, COI and concatenated sequences, respectively (Table 8; Figure 6). However, populations one, two and four from the COI analysis coincided with population one from the ITS-1 and concatenated data, thus they are henceforth referred to as population A. The patterns from the three analyses are similar, with minor variations; populations one and A dominate.

Table 7: $F_{st}$ Pairwise comparisons for *Temora turbinata*. Fst values are below the diagonal. Probabilities are above the diagonal. - = not significant; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

ITS-1

| | PA | LW | Lu | PC | Ta | SK | Mi | FP | Ja | IM |
|---|---|---|---|---|---|---|---|---|---|---|
| PA | 0 | - | - | - | - | - | - | - | - | - |
| LW | 0.042 | 0 | - | * | - | - | - | 0.052 | * | - |
| Lu | -0.018 | 0.013 | 0 | - | - | - | - | - | - | - |
| PC | -0.002 | 0.121 | 0.004 | 0 | - | - | - | - | - | - |
| Ta | -0.004 | 0.011 | -0.006 | 0.056 | 0 | - | - | - | - | - |
| SK | -0.015 | 0.016 | -0.013 | 0.032 | -0.015 | 0 | - | - | - | - |
| Mi | 0.007 | -0.003 | -0.003 | 0.061 | -0.007 | -0.016 | 0 | - | - | - |
| FP | -0.014 | 0.041 | -0.014 | -0.010 | 0.001 | -0.009 | 0.006 | 0 | - | - |
| Ja | -0.004 | 0.066 | -0.004 | -0.029 | 0.024 | 0.010 | 0.025 | -0.018 | 0 | - |
| IM | 0.015 | -0.005 | -0.002 | 0.054 | -0.000 | -0.005 | -0.017 | -0.000 | 0.014 | 0 |

COI

| | PA | LW | Lu | PC | Ta | SK | Mi | FP | Ja | IM |
|---|---|---|---|---|---|---|---|---|---|---|
| PA | 0 | - | - | - | - | - | - | - | - | - |
| LW | -0.004 | 0 | - | - | - | - | - | * | ** | - |
| Lu | -0.012 | -0.009 | 0 | - | - | - | - | - | - | - |
| PC | 0.001 | 0.040 | -0.004 | 0 | - | - | - | - | - | - |
| Ta | 0.011 | 0.028 | 0.002 | -0.018 | 0 | - | - | - | - | - |
| SK | -0.002 | 0.011 | 0.005 | 0.003 | 0.012 | 0 | - | - | - | - |
| Mi | -0.008 | 0.024 | 0.013 | -0.024 | 0.014 | 0.004 | 0 | - | - | - |
| FP | 0.017 | 0.047 | 0.018 | -0.034 | -0.001 | 0.016 | 0.002 | 0 | - | - |
| Ja | 0.035 | 0.062 | 0.031 | -0.027 | -0.006 | 0.022 | 0.018 | -0.017 | 0 | - |
| IM | 0.013 | 0.030 | 0.016 | -0.023 | -0.006 | 0.001 | 0.001 | -0.015 | -0.017 | 0 |

Concatenated

| | PA | LW | Lu | PC | Ta | SK | Mi | FP | Ja | IM |
|---|---|---|---|---|---|---|---|---|---|---|
| PA | 0 | - | - | - | - | - | - | - | - | - |
| LW | 0.033 | 0 | - | ** | - | - | - | * | ** | - |
| Lu | -0.017 | 0.009 | 0 | - | - | - | - | - | - | - |
| PC | -0.001 | 0.103 | 0.002 | 0 | - | - | - | - | - | - |
| Ta | -0.000 | 0.015 | -0.004 | 0.034 | 0 | - | - | - | - | - |
| SK | -0.012 | 0.015 | -0.009 | 0.024 | -0.007 | 0 | - | - | - | - |
| Mi | 0.003 | 0.004 | 0.001 | 0.037 | 0.000 | -0.010 | 0 | - | - | - |
| FP | -0.008 | 0.040 | -0.006 | -0.017 | 0.000 | -0.003 | 0.002 | 0 | - | - |
| Ja | 0.007 | 0.064 | 0.007 | -0.028 | 0.014 | 0.014 | 0.023 | -0.016 | 0 | - |
| IM | 0.014 | 0.005 | 0.003 | 0.0288 | -0.002 | -0.003 | -0.011 | -0.006 | 0.003 | 0 |

Table 8: *Temora turbinata* variant clusters as designated by Structure. 'A' is a combination of clusters 1 and 2. 'B' is a combination of 1, 2 and 3. 'C' is a combination of 2 and 3.

| | Variant | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | 1 | 2 | 3 | 4 | 5 | 6 | 9 | 12 | 13 | 15 | 16 | 17 | 19 | 21 | 22 | 23 | 24 | 26 |
| ITS-1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| COI | A | A | A | B | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | B | 3 | 3 | 3 |
| Concatenated | 1 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

| | Variant | | | | | |
|---|---|---|---|---|---|---|
| | 27 | 28 | 29 | 30 | 31 | 33 |
| ITS-1 | 3 | 3 | 3 | 3 | 3 | 3 |
| COI | 3 | 3 | C | 3 | 3 | 3 |
| Concatenated | 3 | 3 | 3 | 3 | 3 | 3 |

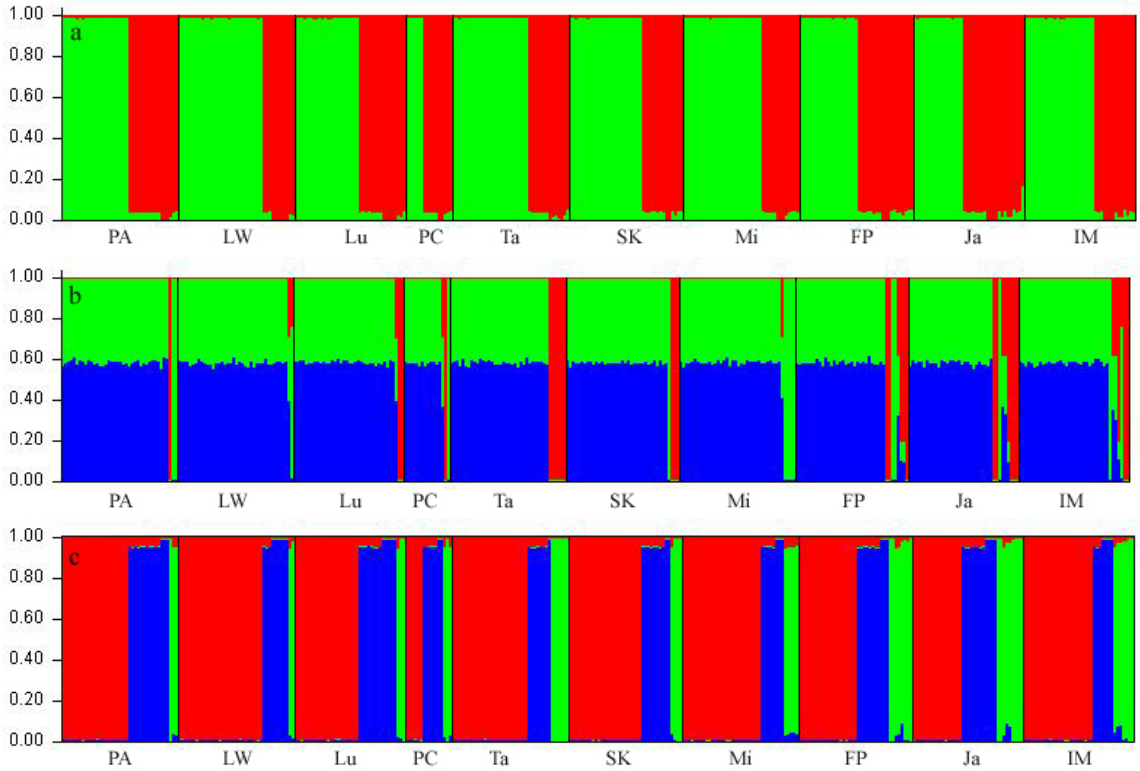


Figure 6: Structure bar plot of inferred ancestry of *T. turbinata* from ITS-1 (a), COI (b) and concatenated (c) sequences.

## DISCUSSION

*Paracalanus quasimodo* and *Temora turbinata* population structures differ greatly around the Gulf of Mexico. *Paracalanus quasimodo* shows a high degree of gene flow restriction while *T. turbinata* appears to be near panmixia. The contrast between the homogeneous distribution of *T. turbinata* and the structured distribution of *P. quasimodo* supports observations of diminished gene flow in taxa limited to near shore habitats (Herke and Foltz, 2002). Taxa with greater habitat restrictions, proximity to shore in this case, are less likely to encounter suitable habitat (McMillan-Jackson and Bert, 2004). *P. quasimodo* enters an inhospitable environment should it drift too far from shore, restricting dispersal to near shore currents. Alongshore advection is slower than can occur in the primary currents, thus limiting dispersal range.

Despite limited dispersal potential in *P. quasimodo*, the connections between LW, Lu, Ta, FP072 and Ja demonstrate gene flow between sites separated by over 1000km. With significant restrictions between sites separated by less than 200km, some factor must enable connectivity between these sites. Similar environments at LW, Lu, Ta, FP and Ja may support concerted adaptation, but significantly restricted gene flow to similar sites suggests that the environment is not the driving force behind the similar genetic signatures. The lack of significant gene flow restrictions between FP, on the border between subtropical and temperate zones, and Ja, a temperate site, and SK and Mi, subtropical sites, suggests a latitudinal gradient. However, PA and PC show significantly restricted gene flow to LW, Lu, Ta, FP and Ja, but are at roughly equivalent latitudes. Another shared factor is proximity to an estuary with PC and Mi as the discrepant sites.

A degree of gene flow between sites is necessary, as environment cannot fully explain the similarity between sites. Gene flow likely occurred between the Gulf and Atlantic sites across the Suwannnee Strait, prior to the Miocene. Though the strong currents through the strait (Popenoe et al. 1987) could sweep plankton from the Gulf to Atlantic coast, it is unlikely for the populations to maintain the similar genetic profile without continuous gene flow for over five million years. Without continuous gene flow, speciation would likely occur, as has occurred in marine taxa with longer generation times whose populations were divided by the closing of the Suwannee strait (Bert, 1986; Bert and Harrison, 1988; Schneider-Broussard et al., 1998; Drumm and Kreiser 2012).

Considering that some degree of migration occurs between the western and eastern GoM, as supported by the presence of both genetic clusters at PA and PC, yet *P. quasimodo* populations have developed distinct, genetic signatures across permeable hydrographic barriers, LW, Lu, Ta, FP and Ja likely experience continued gene flow across the geographic barrier of the Florida peninsula. A possible scenario is the close proximity the connected sites have to the primary GoM and Atlantic currents. Lu and Ta are relatively close to the Gulf Loop Current, which eventually becomes the Gulf Stream, passing FP and Ja. Louisiana West is directly downstream of Lu via the Mississippi River plume (Schiller et al., 2011) and surface currents (Chu et al. 2005), thus, individuals from Lu could easily drift to LW (Johnson et al. 2009).

The population structure patterns of *P. quasimodo* and the regional currents indicate that the population at Lu has a large influence on Florida populations. Isla Mujeres is the site furthest upstream of the primary currents in this study, yet there are significant gene flow restrictions to all other sites. Depending on whether the Loop

Current intrudes into the northern GoM or veers directly into the Florida current, either Lu or SK would be the next population downstream of IM. The Loop Current entrains water from the Mississippi River plume (Schiller et al. 2011), thus Lu will influence the composition of downstream populations. Olascoaga (2010) found that passive drifters released in the Mississippi River plume have the potential to reach all of the downstream sites of this study.

The Mississippi river plume flows west from the river mouth and the currents can carry particles to the Texas coast (Olascoaga 2010), but there is a genetic break that isolates PA *P. quasimodo* from the other sampled sites. Bilodeau et al. (2005) found the same partition in populations of the beach ghost shrimp *Callichirus islagrande* and suggested that the Louisiana Chenier Plain as the dividing point. The near shore currents in the region are not amenable to longitudinal transport of particles across the plains.

A hydrographic barrier associated with current vectors like the one at the Chenier Plain is unlikely to have caused restricted gene flow detected between Lu and SK and Mi. No significant reductions in gene flow were detected between SK and Mi and FP. As Mi and FP do not share a connection with ITS-1 or the concatenated sequences prior to Bonferroni corrections, SK likely influences the downstream sites. With a potential rate of 160cm/s (Johns et al., 1999), the Florida Current can carry particles from SK to Mi in 1.5 days, thus, any changes in the population at SK can quickly affect the Mi population.

Two bodies of water influence SK, a unique situation in this study. To the south, there is the Florida Channel and the Florida Current. To the north is Florida Bay. Although the Keys provide a physical barrier, the many channels between the Keys provides a net flow from Florida Bay to the Florida Channel (Pitts, 2002; Smith, 2009),

resulting in a mix of water with origins from Florida Bay, the GoM and the Florida Keys (Willemsen, 2005). Under normal conditions, hydrographic features and weak cross shelf transport restrict transport into Florida Bay from passive drifters originating in the GoM (Criales et al., 2006; Hare and Walsh, 2007; Olascoaga, 2010). Isolation from the GoM and a low degree of mixing from the north are ideal conditions for developing a genetically distinct population. Mixing with such a population may be responsible for the distinctive population found at SK, which spreads to Mi. Such a shift in population composition is seen in the scorched mussel (Lee and O'Foighil, 2004). Sampling between the Florida Keys and Tampa is required to determine whether Florida Bay populations also influence *P. quasimodo* community composition south of the Florida Keys. A third scenario is that SK and Mi belong to a subtropical population similar to the scorched mussel, *Brachidontes exustus*, (Lee and O'Foighil, 2004). The influence of populations from the Bahamas and Cuba are unknown and should be examined in future studies.

In both composition and $F_{st}$ pairwise comparisons, FP is intermediate to SK and Ja. FP is in a mixing zone between north Atlantic Florida and south Florida populations. The large swath of coast running from Mi to Georgia was identified as a region where Atlantic and Gulf of Mexico variants of *Crassostrea virginica*, the American oyster, intermix (Hare and Avise, 1996). The Florida Current and Gulf Stream transports particles from SK towards FP (Figure 2; Roberts, 1997). Countercurrents along the Atlantic coast will transport passive drifters from Ja towards FP inshore of the Gulf Stream (Hare and Walsh, 2007). The population composition of FP, intermediate to SK and Ja, supports FP as a mixing zone (Figure 3).

Although IM borders the Yucatan Strait, the Caribbean gateway into the Gulf of Mexico, there are significant restrictions in gene flow to all the downstream populations. Variant 1, the dominant variant at IM, does not appear in either PC or in Ta, the two sites in one of the most isolated regions of this study (Olascoaga 2010). The reduction in variant 1 proportions at other sites suggests several scenarios, one of which is that variant 1, whether through low original frequency or environmental factors, is unsuccessful at maintaining a presence over the west Florida shelf. An alternate scenario is that the presence of variant 1 is primarily maintained by migrants originating from IM. Lu is the closest site to IM should the Loop Current intrude into the northern Gulf, and when the Loop Current veers directly east into the Florida current, the Florida Keys provide the closest coasts. The additional time required to reach Ta and PC may be too great for migrants from IM.

With a few exceptions, *Temora turbinata* were collected from the same sample of a site transect as *P. quasimodo*. Unlike *P. quasimodo*, *T. turbinata* appears to be near panmixia. Although Structure Harvester suggests multiple populations, all purported populations were represented in the majority of the sites. If there are multiple populations, they range beyond, and overlap in the southeast United States.

Louisiana West was the only site that showed significant restrictions in gene flow to multiple sites, although these restrictions were no longer significant after Bonferroni corrections. All other sites that showed significant restrictions were reciprocal to LW. Situated in an area with low mixing (Olascoaga 2010) with a phylogeographic break between the Louisiana and Texas shelf (Bilodeau et al., 2005), LW is likely a planktonic sink. Population composition and drifter patterns (Olascoaga, 2010) indicate that PC is

also a sink. Although LW and PC still receive migrants from other populations, as sinks, they would have minimal influence on how the species evolves in the region and begin to develop a distinct community. Ja does not have obvious hydrographic barriers that would retard gene flow, but this is the geographically furthest site from LW, so the restricted gene flow can be attributed to IBD.

## CONCLUSIONS

*Paracalanus quasimodo* and *Temora turbinata* populations overlap throughout the SE United States. However, *T. turbinata* range further offshore than *P. quasimodo*. Different degrees of access to strong currents and habitat restrictions produce different patterns of population structure. The near shore *P. quasimodo* exhibited greater restriction in gene flow between sites than the oceanic *T. turbinata*. While both showed evidence of IBD, *T. turbinata* was nearly panmictic, but *P. quasimodo* population structure cannot be explained by distance alone. Though plankton with a wide habitat range, such as *T. turbinata*, can overcome hydrographic barriers in the southeastern US, the current patterns shape the population structure of taxa restricted to coastal habitats.

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# CHAPTER III

# TEMPORAL STABILITY IN POPULATIONS OF TWO COPEPOD SPECIES (*PARACALANUS QUASIMODO* [CALINOIDEAE] AND *TEMORA TURBINATA* [CALINOIDEAE]) ALONG THE SOUTH EASTERN FLORIDA COAST

## Introduction

Phylogeographic studies typically sample a population one time, an implicit assumption of temporal stability, with changes occurring over evolutionary time. Relatively few phylogenetic or phylogeographic studies have sampled populations of a species repeatedly over time. In those studies that resampled, some populations are temporally stable (Ball and Chapman 2003; Lambert et al.2003; Kovach et al. 2010), while the genetic signature of other populations change (Smolenski et al. 1993; Purcell et al. 1996; Cook et al. 2007; Larsson et al. 2010; Horne et al. 2012). Populations that undergo detectable change are generally a minority among temporally stable populations across a species range (Cook et al. 2007; Calderon et al. 2012). The present study aims to examine the phylogeographic structure of *Paracalanus quasimodo* and *Temora turbinata* combined with temporal sampling to determine the stability of populations within a site and of the relationships between sites.

The majority of temporal studies to date have focused on genetic monitoring, examining populations for evidence of genetic drift, indicating critical reductions in the effective breeding population, or for changes in the genetic signature of a population due to anthropogenic and environmental pressures. Most studies find that the populations are

stable and resilient to stressors such as population decline exacerbated by harvesting pressure (Cuveliers et al. 2011; Van Doornik et al. 2011). However, some species show a significant change in genetic signature over the course of the study, whether over generations (Larsson et al. 2010) or over seasons (Dunton et al. 2012). These genetic monitoring studies generally focus on one population and do not address phylogeographic relationships between populations.

Phylogeographic studies delve into the biological and geographic history of a species. Examining species across their ranges can detect isolation and connectivity between populations (Broughton et al. 2002; Ball and Chapman 2003). Isolated populations may develop novel variants that have the potential for speciation, if this had not already occurred (Bert 1986; Bert and Harrison 1988; Craig et al. 2004). In the practical view of natural resource management, phylogeographic studies are used to determine the range and the aforementioned connectivity between populations, which can aid in MPA delineation and determining management regions (Bagley et la. 1999; Broughton et al. 2002; Gold et al. 2002; Kovach et al. 2010; Lankford et al. 1999; Vinas et al. 2010). Population patterns can also reveal past vicariant history.

More temporal sampling in phylogeographic studies is needed in order to evaluate the degree of short-term variability within populations (Horne et al. 2012), as most studies to date are limited in the numbers of temporal samples (Purcell et al. 1996; Lambert et al. 2003; Cook et al. 2007; Horne et al. 2012), or the numbers of sites sampled (Smolenski et al. 1993; Ball and Chapman 2003; Calderon et al. 2012). Purcell (1996) demonstrates how populations of the same species undergo different pressures resulting

in varied changes in genetic identity. Thus, it cannot be assumed that all populations are temporally stable or unstable based on a limited number of sampling sites.

Species with planktonic larval stages may be especially subject to short-term temporal variation. A shift in the local current pattern can facilitate the exchange of novel genetic variants between formerly separated populations (Calderon et al. 2012). Despite the large number produced, low survivorship of planktonic larvae reduces the effective population size, amplifying the impact of successful breeders on the genetic signature of the following generation (Hedgecock 1994; Lee and Boulding 2009). The genetic composition will change significantly should a minor genetic variant succeed in this sweepstake recruitment. Though major vectors remain, on average, constant, local hydrographic features can change or acute disturbances, like cyclonic activity, can alter dispersal patterns between generations (Calderon et al. 2012). Major recruitment events may change populations, especially following severe disturbances (Paz-Garcia et al. 2012) or throughout cyclical extinction and colonization events (Lambert et al 2003).

By examining populations at geographically adjacent sites, it should be possible to determine if populations are stable over time or, should they change, the degree upstream sites influence downstream populations. Communities in train of the currents should be directly sampled, whether by periodic sampling while adrift or frequent sampling at a fixed point within the current. Such a study can address whether the communities along the path of major currents are genetically homogeneous or if they are geographically structured. An alternate scenario, particularly for species occurring further from shore, is nomadic populations that inhabit a specific parcel of water in the currents rather than a geographic location. Like two objects on a conveyor belt, two populations

are nomadic relative to a fixed, geographic point, but separated by a constant distance along the current path; both populations will pass through a hypothetical point 'A,' but at different times. Here, time at location, rather than geography, separates the populations. Isolation occurs because it is difficult for individuals to travel upstream or downstream relative to their natal population.

The present study examines populations of two copepod species, *Paracalanus quasimodo* and *Temora turbinata*, at three locations along the southeast Florida coast. The coastal *P. quasimodo* is expected to show less of a temporal gradient than *T. turbinata*, which has a higher dispersal potential (Lambert et al. 2003). To test for temporal stability, sites were sampled quarterly over a period of two years and examined for temporal patterns within and among sites. Within site comparisons were performed to determine whether there were temporal variations and patterns supporting resident or nomadic populations. Among-site patterns were examined for connectivity between sites and whether changes in a population will affect downstream sites.

## MATERIALS AND METHODS

### *Collection*

For the purposes of this study, we assumed that a sampling site represented a population. Samples were collected from three sites off the Atlantic coast of the Florida peninsula (Table 1, Figure 1), repeated quarterly from 2007 to 2008. Weather permitting, quarterly sampling was conducted over three consecutive days, beginning with SK and ending with FP. Two samples from a 2005 pilot study were included, one from SK and one from Mi. Samples were collected by ten-minute surface tows with both a 150μm and 366μm mesh nets. Surface tows were conducted along transects beginning ten miles offshore to inshore with one tow at the beginning, middle and end of the transect. Samples were named by a two-digit abbreviation for the quarter, the year and then the site. Samples were immediately preserved in 95% ethanol. Species were identified by dichotomous keys (Owre and Foyo 1967; Boltovskoy 1999).

### *DNA Extraction*

Forty individuals each of *Paracalanus quasimodo* and *Temora turbinata* were selected from each site sample with two exceptions. Sixty *P. quasimodo* were selected from the 2005 SK and Mi samples as part of a feasibility study (data not shown). The first 40 Fa07-FP *P. quasimodo* formed an anomalous community, thus forty additional *P. quasimodo* were selected to confirm the variant distribution. The Su07-Mi sample captured few of the two target species with only 10 *P. quasimodo* and 6 *T. turbinata*. Individual copepods were transferred directly from ethanol to a 1.5μl microcentrifuge

Table 1: Sampling sites, year and quarter collected, sample size and coordinates for *Paracalanus quasimodo* and *Temora turbinata*. *T. turbinata* samples from fall 2008 were combined from two tows. SK = Summerland Key; Mi = Miami; FP = Fort Pierce

| | | | *Paracalanus quasimodo* | | | *Temora turbinata* | | |
|---|---|---|---|---|---|---|---|---|
| Site | Year | Quarter | n | Latitude/Longitude | | n | Latitude/Longitude | |
| SK | 2005 | Summer | 60 | N24°32.95 | W81°21.792 | | | |
| | 2007 | Winter | | | | 39 | N24°35.469 | W81°28.559 |
| | 2007 | Spring | | | | 34 | N24°33.643 | W81°27.629 |
| | 2007 | Fall | 40 | N24°33.746 | W81°27.597 | 39 | N24°33.746 | W81°27.597 |
| | 2008 | Winter | 40 | N24°35.487 | W81°28.540 | 39 | N24°32.117 | W81°26.180 |
| | 2008 | Spring | 40 | N24°35.437 | W81°28.692 | | | |
| | 2008 | Summer | 40 | N24°35.5 | W81°28.5 | | | |
| | 2008 | Fall | 40 | N24°36.509 | W81°29.071 | 38 | N24°36.509 | W81°29.071 |
| | | | | | | | N24°35.516 | W81°28.621 |
| Mi | 2005 | Spring | 54 | N25°51 | W79°56.25 | | | |
| | 2007 | Winter | | | | 34 | N25°54.591 | W80°07.517 |
| | 2007 | Spring | 39 | N25°54.053 | W80°06.026 | 40 | N25°54.053 | W80°06.026 |
| | 2007 | Summer | 10 | N25°53.775 | W80°06.438 | 06 | N25°53.775 | W80°06.438 |
| | 2007 | Fall | 40 | N25°53.887 | W80°06.636 | 40 | N25°53.887 | W80°06.636 |
| | 2008 | Winter | 40 | N25°53.786 | W80°06.541 | 40 | N25°53.786 | W80°06.541 |
| | 2008 | Spring | 40 | N25°53.803 | W80°06.600 | | | |
| | 2008 | Summer | 40 | N25°53.964 | W80°01.979 | 39 | N25°53.870 | W80°06.414 |
| | 2008 | Fall | 40 | N25°54.130 | W79°56.104 | 36 | N25°54.130 | W79°56.104 |
| FP | 2007 | Winter | | | | 37 | N27°28.013 | W80°18.427 |
| | 2007 | Spring | 40 | N27°27.071 | W80°04.978 | 39 | N27°27.047 | W80°15.019 |
| | 2007 | Summer | 40 | N27°27.009 | W80°04.997 | 38 | N27°27.007 | W80°10.998 |
| | 2007 | Fall | 66 | N27°26.960 | W80°05.025 | 39 | N27°26.960 | W80°05.025 |
| | 2008 | Winter | 40 | N27°27.094 | W80°15.042 | 38 | N27°27.210 | W80°11.156 |
| | 2008 | Summer | 37 | N27°26.838 | W80°05.067 | 37 | N27°26.997 | W80°10.924 |
| | 2008 | Fall | 40 | N27°26.965 | W80°15.016 | | | |

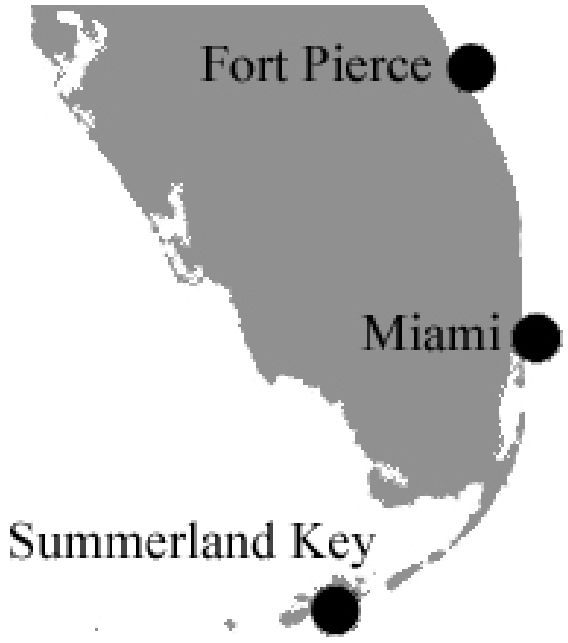


Figure 1: Sampling sites along the southeast Florida coast.

tube. Extractions were conducted with the MasterPure DNA extraction kit (Epicentre, Madison, WI) following manufacturer protocols and stored in 50µl of UV sterilized, micropore filtered, deionized water at –80°C. DNA concentration was quantified on a DyNA Quant 200 spectrophotometer (Hoefer) and varied between 3nM and 30nM.

*PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)*

The ITS-1 region was amplified with the primers ITS-1f (Coleman et al. 1994; Table 2) with the GC clamp added to the 5' end of the sequence and ITS-1r (Schizas et al. 1999). The PCR reactions were run as quantitative reactions in lieu of sacrificing PCR product to check for successful amplification through electrophoresis. Amplifications were run in 10µl PCR reactions composed of 5µl iQ SybrGreen 2x (BioRad, Hercules, CA), 0.5µl ITS-1r (0.5 µM final concentration), 0.5µl ITS-1fCol with GC clamp (1 µM final concentration), 1µl DNA extract (0.3 nM to 3 nM final concentration), and 3µl nuclease free water.

The ITS-1 primer pair produced amplicons of approximately 320bp in length. The thermal profile for the reactions was: 95°C (5 min), 10 touchdown cycles beginning with 95-60-72°C for 20-20-60s with a 0.5°C drop in the annealing temperature per cycle, 25 cycles of 95-55-72°C for 20-20-60s and a final extension at 72°C for 10 minutes in a DNA Engine Opticon 2 (MJ Research).

The optimal urea gradient for parallel was determined to be 30-50%. The gels were allowed to run in aquaria (C.B.S. Scientific, Del Mar, CA) filled with 20L of TAE buffer at 65°C for 18 hours at 90 volts. To confirm that these were ITS-1 sequences, the brightest lower bands from the three dominant variants were cut from the DGGE gel,

Table 2: Primer sequences.

| Primer Name | Sequence |
|---|---|
| GC Clamp | CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC |
| ITS-1f | GGG ATC CGT TTC CGT AGG TGA ACC TGC |
| ITS-1r | ATC GAC CCA TGA GCC GAG TGA TC |
| LCOI-1490 | GGT CAA CAA ATC ATA AAG ATA TTG G |
| LCOI-1490c | GGT CAT GTA ATC ATA AAG ATA TTG G |
| LCOI-1528P | GTT AGC AGG AGC TTG ATC AG |
| HCOI-2198 | TAA ACT TCA GGG TGA CCA AAA AAT CA |
| HCOI-2198Par | TAG ACT TCA GGA TGT CCA AAG AAT CA |

eluted in 500 µl of water for 24 hours, cleaned with the Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI), re-amplified and prepared for sequencing with BigDye terminator, ver 3.1 (Applied Biosystems, Grand Island, NY).

*Sequencing*

Polymorphic sequences can change the DGGE banding pattern between individuals with the same dominant genetic variant. However, the dominant variant provides strong evidence of the evolutionary history in an organism (LaJeunesse and Pinzon, 2007). Thus, ten individuals from each sample site were selected for DNA sequencing based on DGGE variants. When possible, at least one of each variant present at a sample site was selected. The nuclear ITS-1 and mitochondrial cytochrome oxidase I (COI) regions were amplified. ITS-1 primers were the same as that for DGGE analysis, minus the GC clamp. Standard Folmer COI primers (LCOI-1490 and HCOI-2198) were unable to amplify a majority of the *Paracalnus quasimodo* samples and a large number of the *Temora turbinata*. Modified Folmer primers (LCOI-1490c and HCOI-2198Par) were designed using whole copepod mitochondrial sequences found in GenBank worked well with *T. turbinata*, but only had moderate success with *P. quasimodo*. A COI primer tailored for *P. quasimodo* (LCOI-1528p), which began 38bp downstream of LCOI-1490, was paired with HCOI-2198Par to sequence the remaining *P. quasimodo*.

The PCR amplifications were conducted in a PTC-200 DNA Engine (MJ Research) with Promega GoTaq® Flexi reagents. Reactions were composed of 2µl of 5x Buffer, 0.6µl of 25mM $MgCl_2$ (1.5mM final concentration), 0.5µl each of forward and reverse primers (0.5µM, ITS-1 primers, and 1.0µM, COI primers, final concentration),

0.2µl of 10 µM dNTPs (0.2µM final concentration), 0.2µl of 2.5u/µl DNA Polymerase (0.05u/µl final concentration) and 2 to 5µl of DNA (0.6-6nM to 1-10nM final concentration) with nuclease free water added to make a final volume of 10µl. The higher concentration of DNA was used when the lower concentration resulted in low amplification.

Three µl of PCR product were ran on an 0.8% agarose gel to determine whether the amplification was successful. PCR products were cleaned with ExoSap (Affymetrix, Santa Clara, CA) following manufacturer's protocols. Cleaned products were sequenced with BigDye terminator, ver 3.1 Applied Biosystems, Grand Island, NY) for both forward and reverse strands in a PTC-200 DNA Engine (MJ Research). The 10µl cycle sequencing reaction mix was composed of 0.5µl of 1.87µM primer (0.935 µM final concentration), 2-4µl of amplified product, depending on gel band intensity (2µl if the band was bright, otherwise 4µl), 2µl of 5x sequencing buffer, 1µl of BigDye v3.1 and double distilled deionized water to a total volume of 10µl. The reaction cycle was 15 seconds at 95ºC, 10 seconds at 50ºC and 4 minutes at 60ºC, repeated 35 times. The products were sequenced in an AB 3100 genetic analyzer (Applied Biosystems, Grand Island, NY) in the Florida International University DNA Core. Several sequences were unreadable due to contamination or, in the case of ITS-1, the presence of multiple sequence varieties. Samples where standard sequencing failed were cloned with a TOPO TA cloning kit for sequencing (Invitrogen, Grand Island, NY) following the manufacturer's protocols.

Sequences were proofread in FinchTV (Geospiza, Seattle, WA), trimmed in BioEdit (Hall, 1999) and aligned with ClustalW (Larkin et al., 2007) through BioEdit. As ITS-1 is a non-coding region of the nuclear DNA, neither genotype nor haplotype are appropriate descriptors for unique sequences. The term 'variant' will be used as per LaJeunesse and Pinzon (2007) expanded to unique concatenated sequences. For example, should two sequences have identical ITS-1 sequences, yet have different COI sequences, or vice versa, they would be described as different variants. A consensus sequence was constructed in BioEdit for each variant with multiple sequences. All polymorphic sites were marked as such, even if the alternate nucleotide was represented in only one sequence. DGGE variants that were identical in both ITS-1 and COI sequences were grouped under the numerically lowest variant number.

*Analysis*

Population comparisons and Mantel tests were conducted in Arelequin 3.5 (Excoffier and Lischer 2010) with sequence data. Pairwise $F_{st}$ comparisons were conducted to determine whether there was significant restriction in gene flow over space and time. Pairwise comparisons were run with 99,999 permutations. A Bonferroni correction for multiple tests was applied to the results of the pairwise comparisons. Mantel tests examined the data for evidence of a temporal gradient similar to isolation-by-distance (Barber et al. 2002), but with a matrix based on time rather than by distance. The $F_{st}$ matrices produced by the $F_{st}$ pairwise comparisons were tested against a temporal matrix of days between samples, with the spring 2005 Miami sample (02 May 2005) as day zero.

Genetic data were converted into numerical sequences with GenAlEx 6.41 (Peakall and Smouse, 2006) in preparation for cluster analysis by Structure 2.3.3 (Hubisz et al., 2009). Cluster analysis groups the variants by similarity and the distribution of the clusters within and between sites can be examined for geographic patterns and temporal patterns. Analysis was set to a 60,000 MCMC chain with a 10,000 step burn-in period. Both alpha and lambda values were inferred by Structure. The number of clusters (K) was set for 1 through 10, with 20 replicate runs of each K. The results were analyzed with Structure Harvester (Dean and vonHoldt, 2012) to determine the most likely value for K.

I used AMOVAs to test whether sites were independent and whether there was seasonal variation. The AMOVAs were run in Arlequin 3.5 (Excoffier and Lischer, 2010) with the maximum number of permutations (99,999) with four sets of a priori groups. The first set was a stable, single population. The second set separated samples by site. The third set grouped samples by sampling quarter (e.g. Spring 2007) and the fourth by season.

## RESULTS

Eight-hundred twenty-five of 850 *Paracalanus quasimodo* and 652 of 686 *Temora turbinata* successfully amplified for DGGE with an additional 14 *Euchatea ramina* from Fa07-FP misidentified as *P. quasimodo*. Thirty-five variants were found in both *P. quasimodo* and *T. turbinata*. *Paracalanus quasimodo* was not found in any of the SK tows from winter 2007, spring 2007 and summer 2007, in Mi tows from winter 2007 and in FP tows from winter 2007 and spring 2008. *Temora turbinata* was not found in the SK tows from summer 2007, spring 2008 and summer 2008, in Mi tows from spring 2008 and in FP tows from spring 2008 and fall 2008.

*Paracalanus quasimodo*

After Bonferroni corrections, Sp05-Mi, Sp07-FP and Fa07-FP show a pattern of significant differences from the majority of other samples (Table 3; Figure 2). The Wi08-FP and Sp08-Mi also have multiple differences, but were not significant after applying the Bonferroni corrections. Within the sampling quarters, the only site that showed significant restrictions from the others was FP from Spring and Fall 2007 and Winter 2008 (Table 4). Fall 2007 was the only sample that remained significant after Bonferroni corrections.

Within sites, no significant changes were detected in SK over the course of this study (Tables 5). At Mi, a high proportion of variant 3 differentiated the 2005 pilot sample all other Mi samples. The spring 2008 sample from Mi also shows significant differences, but only before the Bonferroni corrections. The genetic signature at FP

Table 3: Pairwise $F_{st}$ comparisons between samples for *Paracalanus quasimodo* and *Temora turbinata*. Samples are named by a two-letter abbreviation for the quarter (Wi = Winter, Sp = spring, Su = summer, Fa = fall), the year and the site at which they were collected. $F_{st}$ values for ITS-1 are below the diagonal; probabilities are above the diagonal. Pairs with three probability values are for ITS-1, COI and concatenated sequences, respectively, otherwise, the probabilities share the same degree of significance. P-values that remained significant after Bonferroni corrections are in bold. - = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

*Paracalanus quasimodo*

| | Sp05-Mi | Su05-SK | Sp07-Mi | Sp07-FP | Su07-Mi | Su07-FP | Fa07-SK | Fa07-Mi | Fa07-FP | Wi08-SK | Wi08-Mi |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sp05-Mi | 0 | ******* | ******* | - | * | *** | ******* | ******* | ******* | ******* | ******* |
| Su05-SK | 0.19194 | 0 | - | *** | - | - | - | - | ******* | - | - |
| Sp07-Mi | 0.17633 | -0.01107 | 0 | *** | - | - | - | - | ******* | - | - |
| Sp07-FP | 0.03045 | 0.1279 | 0.13693 | 0 | - | - | *** | ******* | ******* | ******* | ** |
| Su07-Mi | 0.13478 | -0.0299 | -0.05336 | 0.1115 | 0 | - | - | - | * | - | - |
| Su07-FP | 0.12885 | 0.0097 | 0.02494 | 0.04477 | 0.00255 | 0 | - | * | ******* | * | - |
| Fa07-SK | 0.20398 | -0.015 | -0.02085 | 0.1494 | -0.04337 | 0.02215 | 0 | - | ******* | - | - |
| Fa07-Mi | 0.31683 | 0.00596 | 0.03019 | 0.22848 | 0.03723 | 0.05303 | 0.00908 | 0 | ******* | - | - |
| Fa07-FP | 0.22738 | 0.10585 | 0.08898 | 0.21911 | 0.05551 | 0.13264 | 0.10618 | 0.1621 | 0 | ******* | ******* |
| Wi08-SK | 0.30609 | 0.00319 | 0.00924 | 0.2397 | 0.00679 | 0.06719 | -0.00457 | -0.01194 | 0.13205 | 0 | - |
| Wi08-Mi | 0.23679 | -0.0069 | 0.0163 | 0.14483 | 0.00855 | 0.00701 | 0.00264 | -0.00796 | 0.12202 | 0.00842 | 0 |
| Wi08-FP | 0.12207 | 0.01358 | 0.02103 | 0.06007 | -0.00923 | -0.00566 | 0.02439 | 0.06417 | 0.06976 | 0.06899 | 0.01031 |
| Sp08-SK | 0.35145 | 0.02101 | 0.04165 | 0.27229 | 0.0526 | 0.08531 | 0.02111 | -0.01676 | 0.16177 | -0.01513 | 0.01088 |
| Sp08-Mi | 0.40331 | 0.0492 | 0.08591 | 0.3171 | 0.1152 | 0.11596 | 0.05966 | -0.00689 | 0.19899 | 0.00924 | 0.02532 |
| Su08-SK | 0.25054 | -0.00188 | -0.01256 | 0.20323 | -0.0321 | 0.05924 | -0.0163 | 0.01384 | 0.10777 | -0.01023 | 0.02064 |
| Su08-Mi | 0.26984 | -0.00349 | 0.01994 | 0.17522 | 0.01943 | 0.02125 | 0.00119 | -0.01897 | 0.15731 | -0.00078 | -0.01833 |
| Su08-FP | 0.32549 | 0.00728 | 0.01908 | 0.2521 | 0.02201 | 0.07293 | 0.00124 | -0.01882 | 0.15013 | -0.0229 | 0.00608 |
| Fa08-SK | 0.19983 | -0.01052 | -0.02168 | 0.15312 | -0.04827 | 0.03144 | -0.02283 | 0.01929 | 0.10354 | 0.0009 | 0.01245 |
| Fa08-Mi | 0.32079 | 0.0075 | 0.01678 | 0.25072 | 0.01745 | 0.0748 | 0.00061 | -0.01483 | 0.13943 | -0.02254 | 0.00815 |
| Fa08-FP | 0.20555 | -0.00586 | 0.00531 | 0.13215 | -0.01215 | 0.0102 | -0.00109 | 0.01148 | 0.10879 | 0.01427 | -0.00592 |

| | Wi08-FP | Sp08-SK | Sp08-Mi | Su08-SK | Su08-Mi | Su08-FP | Fa08-SK | Fa08-Mi | Fa08-FP |
|---|---|---|---|---|---|---|---|---|---|
| Sp05-Mi | ******* | ******* | ******* | ******* | ******* | ******* | ******* | ******* | ******* |
| Su05-SK | - | - | * | - | - | - | - | - | - |
| Sp07-Mi | - | -/-/* | ** | - | - | - | - | - | - |
| Sp07-FP | * | ******* | ******* | ******* | *** | ******* | *** | ******* | ** |
| Su07-Mi | - | - | * | - | - | - | - | - | - |
| Su07-FP | - | ** | ** | * | - | * | - | * | - |
| Fa07-SK | - | - | * | - | - | - | - | - | - |
| Fa07-Mi | * | - | - | - | - | - | - | - | - |
| Fa07-FP | ******* | ******* | ******* | ******* | ******* | ******* | ******* | ******* | ******* |
| Wi08-SK | **/*/** | - | - | - | - | - | - | - | - |
| Wi08-Mi | - | - | - | - | - | - | - | - | - |
| Wi08-FP | 0 | ** | *** | */**/* | - | ** | - | ** | - |
| Sp08-SK | 0.08986 | 0 | - | - | - | - | - | - | - |
| Sp08-Mi | 0.12303 | -0.01161 | 0 | * | - | - | * | - | * |
| Su08-SK | 0.05306 | 0.0155 | 0.05509 | 0 | - | - | - | - | - |
| Su08-Mi | 0.03772 | -0.00068 | 0.01425 | 0.01644 | 0 | - | - | - | - |
| Su08-FP | 0.07642 | -0.02188 | -0.00086 | -0.00396 | -0.00673 | 0 | - | - | - |
| Fa08-SK | 0.02906 | 0.03006 | 0.07264 | -0.01911 | 0.01155 | 0.00762 | 0 | - | - |
| Fa08-Mi | 0.07459 | -0.01703 | 0.00353 | -0.00607 | -0.00231 | -0.02463 | 0.00611 | 0 | - |
| Fa08-FP | 0.01401 | 0.02784 | 0.05409 | 0.01463 | -0.0001 | 0.01793 | 0.00495 | 0.01867 | 0 |

Table 3 (continued): Pairwise $F_{st}$ comparisons between samples for *Paracalanus quasimodo* and *Temora turbinata*.

*Temora turbinata*

| | Wi07-SK | Wi07-Mi | Wi07-FP | Sp07-SK | Sp07-Mi | Sp07-FP | Su07-Mi | Su07-FP | Fa07-SK | Fa07-Mi | Fa07-FP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wi07-SK | 0 | - | - | - | - | - | - | - | - | - | - |
| Wi07-Mi | 0.02197 | 0 | - | - | - | - | - | - | - | - | - |
| Wi07-FP | 0.02662 | -0.00011 | 0 | - | - | - | - | - | - | - | - |
| Sp07-SK | 0.00784 | 0.00494 | 0.02553 | 0 | - | - | - | -/*/- | -/*/- | - | - |
| Sp07-Mi | 0.02189 | -0.01505 | 0.0188 | -0.00922 | 0 | - | - | - | - | - | - |
| Sp07-FP | 0.02637 | -0.0185 | 0.00025 | 0.00964 | -0.01067 | 0 | - | - | - | - | - |
| Su07-Mi | -0.03469 | -0.04598 | -0.091 | -0.05298 | -0.03597 | -0.04635 | 0 | - | - | - | - |
| Su07-FP | -0.01729 | 0.00066 | 0.00893 | 0.00305 | 0.00472 | 0.00505 | -0.04691 | 0 | - | - | - |
| Fa07-SK | -0.0078 | -0.00402 | 0.0194 | -0.01248 | -0.00898 | 0.00338 | -0.03958 | -0.01738 | 0 | - | - |
| Fa07-Mi | -0.01243 | 0.00465 | 0.00094 | -0.01117 | 0.0039 | 0.00525 | -0.07357 | -0.01766 | -0.01634 | 0 | - |
| Fa07-FP | 0.02432 | -0.01734 | 0.02206 | -0.00445 | -0.01708 | -0.00543 | -0.0319 | 0.00935 | -0.00562 | 0.00839 | 0 |
| Wi08-SK | 0.00046 | 0.09526 | 0.07731 | 0.06688 | 0.09233 | 0.09344 | 0.03301 | 0.01627 | 0.04065 | 0.0217 | 0.10088 |
| Wi08-Mi | -0.0138 | 0.00666 | 0.01176 | -0.01303 | 0.00194 | 0.01162 | -0.06041 | -0.01782 | -0.02012 | -0.02314 | 0.00634 |
| Wi08-FP | -0.01557 | 0.00146 | 0.02473 | -0.00957 | -0.00467 | 0.007 | -0.03501 | -0.01878 | -0.02219 | -0.01548 | -0.00183 |
| Su08-Mi | 0.00822 | -0.00114 | -0.01297 | 0.00813 | 0.00808 | -0.00304 | -0.0802 | -0.00303 | 0.00716 | -0.00763 | 0.01158 |
| Su08-FP | 0.07234 | -0.00729 | 0.03034 | 0.01344 | -0.00768 | 0.00177 | -0.02518 | 0.04755 | 0.02611 | 0.03872 | -0.01019 |
| Fa08-SK | -0.0112 | -0.00546 | 0.00747 | -0.01017 | -0.00644 | 0.00068 | -0.05371 | -0.02148 | -0.02459 | -0.02151 | -0.00264 |
| Fa08-Mi | 0.03909 | -0.02395 | 0.00685 | 0.02224 | -0.00897 | -0.01501 | -0.02857 | 0.01089 | 0.00452 | 0.01775 | -0.01164 |

| | Wi08-SK | Wi08-Mi | Wi08-FP | Su08-Mi | Su08-FP | Fa08-SK | Fa08-Mi |
|---|---|---|---|---|---|---|---|
| Wi07-SK | - | - | - | - | **/-/** | - | - |
| Wi07-Mi | **/-/* | - | - | - | - | - | - |
| Wi07-FP | */-/* | - | - | - | - | - | - |
| Sp07-SK | */**/* | - | - | - | - | - | - |
| Sp07-Mi | **/*/** | - | - | - | - | - | - |
| Sp07-FP | **/*/** | - | - | - | - | - | - |
| Su07-Mi | - | - | - | - | - | - | - |
| Su07-FP | - | - | - | - | * | - | - |
| Fa07-SK | - | - | - | - | -/*/- | - | - |
| Fa07-Mi | - | - | - | - | */-/* | - | - |
| Fa07-FP | **/*/** | - | - | - | - | - | - |
| Wi08-SK | 0 | - | - | * | ***/**/*** | - | ** |
| Wi08-Mi | 0.0218 | 0 | - | - | -/-/* | - | - |
| Wi08-FP | 0.02813 | -0.02076 | 0 | - | -/-/* | - | - |
| Su08-Mi | 0.05418 | 0.00035 | 0.00741 | 0 | - | - | - |
| Su08-FP | 0.16167 | 0.03869 | 0.03559 | 0.02888 | 0 | - | - |
| Fa08-SK | 0.03191 | -0.02298 | -0.02163 | -0.00043 | 0.02868 | 0 | - |
| Fa08-Mi | 0.1164 | 0.01985 | 0.01365 | 0.01224 | -0.00367 | 0.00264 | 0 |

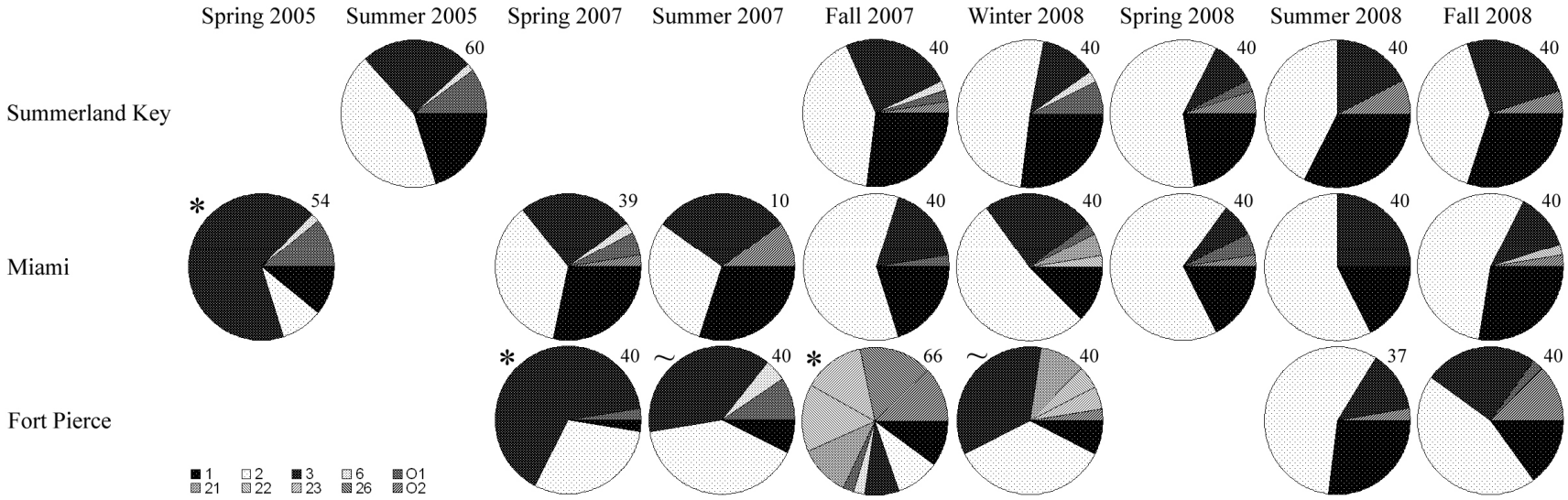


Figure 2: Proportions of *Paracalanus quasimodo* variants. Dominant variants are shown individually (cluster one: 1, 2, 3, 6; cluster two: 21, 22, 23, 26), while rare variants are grouped into O1 and O2 for populations one and two, respectively. Samples with patterns of pairwise significant restrictions after Bonferroni corrections are marked with an *. Samples with patterns of pairwise significant restrictions only before Bonferroni corrections are marked with a ~. Sample sizes are noted to the top right of their respective pie chart.

Table 4: Pairwise Fst comparisons for *Paracalanus quasimodo* and *Temora turbinata* by quarter sampled. Fst values for ITS-1 are below the diagonal; probabilities are above the diagonal. Pairs with three probability values are for ITS-1, COI and concatenated sequences, respectively, otherwise, the probabilities share the same degree of significance. P-values that remained significant after Bonferroni corrections are in bold. - = ns; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

*Paracalanus quasimodo*

| | Sp05-Mi |
|---|---|
| Sp05-Mi | 0 |

| | Fa05-SK |
|---|---|
| Fa05-SK | 0 |

| | Sp07-Mi | Sp07-FP |
|---|---|---|
| Sp07-Mi | 0 | *** |
| Sp07-FP | 0.13693 | 0 |

| | Su07-Mi | Su07-FP |
|---|---|---|
| Su07-Mi | 0 | - |
| Su07-FP | 0.00255 | 0 |

| | Fa07-SK | Fa07-Mi | Fa07-FP |
|---|---|---|---|
| Fa07-SK | 0 | - | ******* |
| Fa07-Mi | 0.00908 | 0 | ******* |
| Fa07-FP | 0.10618 | 0.1621 | 0 |

| | Wi08-SK | Wi08-Mi | Wi08-FP |
|---|---|---|---|
| Wi08-SK | 0 | - | ****/*/**** |
| Wi08-Mi | 0.00842 | 0 | - |
| Wi08-FP | 0.06899 | 0.01031 | 0 |

| | Sp08-SK | Sp08-Mi |
|---|---|---|
| Sp08-SK | 0 | - |
| Sp08-Mi | -0.01161 | 0 |

| | Su08-SK | Su08-Mi | Su08-FP |
|---|---|---|---|
| Su08-SK | 0 | - | - |
| Su08-Mi | 0.01644 | 0 | - |
| Su08-FP | -0.00396 | -0.00673 | 0 |

| | Fa08-SK | Fa08-Mi | Fa08-FP |
|---|---|---|---|
| Fa08-SK | 0 | - | - |
| Fa08-Mi | 0.00611 | 0 | - |
| Fa08-FP | 0.00495 | 0.01867 | 0 |

*Temora turbinata*

| | Wi07-SK | Wi07-Mi | Wi07-FP |
|---|---|---|---|
| Wi07-SK | 0 | - | - |
| Wi07-Mi | 0.02197 | 0 | - |
| Wi07-FP | 0.02662 | -0.00011 | 0 |

| | Sp07-SK | Sp07-Mi | Sp07-FP |
|---|---|---|---|
| Sp07-SK | 0 | - | - |
| Sp07-Mi | -0.00922 | 0 | - |
| Sp07-FP | 0.00964 | -0.01067 | 0 |

| | Su07-Mi | Su07-FP |
|---|---|---|
| Su07-Mi | 0 | - |
| Su07-FP | -0.04691 | 0 |

| | Fa07-SK | Fa07-Mi | Fa07-FP |
|---|---|---|---|
| Fa07-SK | 0 | - | - |
| Fa07-Mi | -0.01634 | 0 | - |
| Fa07-FP | -0.00562 | 0.00839 | 0 |

| | Wi08-SK | Wi08-Mi | Wi08-FP |
|---|---|---|---|
| Wi08-SK | 0 | - | - |
| Wi08-Mi | 0.0218 | 0 | - |
| Wi08-FP | 0.02813 | -0.02076 | 0 |

| | Su08-Mi | Su08-FP |
|---|---|---|
| Su08-Mi | 0 | - |
| Su08-FP | 0.02888 | 0 |

| | Fa08-SK | Fa08-Mi |
|---|---|---|
| Fa08-SK | 0 | - |
| Fa08-Mi | 0.00264 | 0 |

Table 5: Pairwise Fst comparisons for Paracalanus quasimodo and Temora turbinata by sampling site. Fst values for ITS-1 are below the diagonal; probabilities are above the diagonal. Pairs with three probability values are for ITS-1, COI and concatenated sequences, respectively, otherwise, the probabilities share the same degree of significance. P-values that remained significant after Bonferroni corrections are in bold. - = ns; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

*Paracalanus quasimodo*

Summerand Key

| | Fa05 | Fa07 | Wi08 | Sp08 | Su08 | Fa08 |
|---|---|---|---|---|---|---|
| Fa05 | 0 | - | - | - | - | - |
| Fa07 | -0.015 | 0 | - | - | - | - |
| Wi08 | 0.003 | -0.005 | 0 | - | - | - |
| Sp08 | 0.021 | 0.021 | -0.015 | 0 | - | - |
| Su08 | -0.002 | -0.016 | -0.010 | 0.016 | 0 | - |
| Fa08 | -0.011 | -0.023 | 0.001 | 0.031 | -0.019 | 0 |

Miami

| | Sp05 | Sp07 | Su07 | Fa07 | Wi08 | Sp08 | Su08 | Fa08 |
|---|---|---|---|---|---|---|---|---|
| Sp05 | 0 | ******* | * | ******* | ******* | ******* | ******* | ******* |
| Sp07 | 0.176 | 0 | - | - | - | ** | - | - |
| Su07 | 0.135 | -0.053 | 0 | - | - | * | - | - |
| Fa07 | 0.317 | 0.030 | 0.037 | 0 | - | - | - | - |
| Wi08 | 0.237 | 0.016 | 0.009 | -0.008 | 0 | - | - | - |
| Sp08 | 0.403 | 0.086 | 0.115 | -0.007 | 0.025 | 0 | - | - |
| Su08 | 0.270 | 0.020 | 0.019 | -0.019 | -0.018 | 0.014 | 0 | - |
| Fa08 | 0.321 | 0.017 | 0.017 | -0.015 | 0.008 | 0.004 | -0.002 | 0 |

Fort Pierce

| | Sp07 | Su07 | Fa07 | Wi08 | Su08 | Fa08 |
|---|---|---|---|---|---|---|
| Sp07 | 0 | - | ******* | * | ******* | ** |
| Su07 | 0.045 | 0 | ******* | - | * | - |
| Fa07 | 0.219 | 0.133 | 0 | ******* | ******* | ******* |
| Wi08 | 0.060 | -0.006 | 0.070 | 0 | ** | - |
| Su08 | 0.252 | 0.073 | 0.150 | 0.076 | 0 | - |
| Fa08 | 0.132 | 0.010 | 0.109 | 0.014 | 0.018 | 0 |

Temora turbinata

Summerland Key

| | Wi07 | Sp07 | Fa07 | Wi08 | Fa08 |
|---|---|---|---|---|---|
| Wi07 | 0 | - | - | - | - |
| Sp07 | 0.008 | 0 | -/*/- | */**/* | - |
| Fa07 | -0.008 | -0.012 | 0 | - | - |
| Wi08 | 0.000 | 0.067 | 0.041 | 0 | - |
| Fa08 | -0.011 | -0.010 | -0.025 | 0.032 | 0 |

Miami

| | Wi07 | Sp07 | Su07 | Fa07 | Wi08 | Su08 | Fa08 |
|---|---|---|---|---|---|---|---|
| Wi07 | 0 | - | - | - | - | - | - |
| Sp07 | -0.015 | 0 | - | - | - | - | - |
| Su07 | -0.046 | -0.036 | 0 | - | - | - | - |
| Fa07 | 0.005 | 0.004 | -0.074 | 0 | - | - | - |
| Wi08 | 0.007 | 0.002 | -0.060 | -0.023 | 0 | - | - |
| Su08 | -0.001 | 0.008 | -0.080 | -0.008 | 0.000 | 0 | - |
| Fa08 | -0.024 | -0.009 | -0.029 | 0.018 | 0.020 | 0.012 | 0 |

Fort Pierce

| | Wi07 | Sp07 | Su07 | Fa07 | Wi08 | Su08 |
|---|---|---|---|---|---|---|
| Wi07 | 0 | - | - | - | - | - |
| Sp07 | 0.000 | 0 | - | - | - | - |
| Su07 | 0.009 | 0.005 | 0 | - | - | * |
| Fa07 | 0.022 | -0.005 | 0.009 | 0 | - | - |
| Wi08 | 0.025 | 0.007 | -0.019 | -0.002 | 0 | -/-/* |
| Su08P | 0.030 | 0.002 | 0.048 | -0.010 | 0.036 | 0 |

shows multiple, significant changes over the course of this study. Fort Pierce shifted between variant 03 dominance to variant 01 dominance and an anomalous incursion of cluster two during Fall 2007.

Structure 2.3.3 and Structure Harvester detected two P. quasimodo clusters (Table 6; Figure 3). The first cluster dominated most of the samples and the second, centered in the Gulf of Mexico (data not shown), were found intermittently at low frequency. The exception was Fa07FP, where cluster two dominated. No members of cluster two were detected in the previous FP sample and the proportion of population two at FP decreased over time. However, variants from cluster two were detected in the last FP sample as well. AMOVA results were similar for both loci and the concatenated sequence. There is significant variation among and within populations, but no significant variation among groups (Table 7). Mantel tests on both loci and the concatenated sequence show a significant correlation between Fst and time (Table 8; Figure 4). Because the Mi sample from spring 2005 showed significant restriction from most other samples, the tests were ran again without the 2005 samples, which resulted in a weaker, but still significant, correlation.

Temora turbinata

Variant 01 was generally dominant in the populations, followed by 02 and 03 (Table 6; Figure 5). Variant 03 proportions varied more than variant 02. Following Bonferroni corrections, there were no significant restrictions within quarters and sites (Tables 4 and 5). The only significant restriction was between Wi08SK and Su08FP for

Table 6: Genetic variant distribution between temporal samples.

*Paracalanus quasimodo*

| | Cluster one | | | | | Cluster two | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 6 | O1 | 21 | 22 | 23 | 26 | O2 |
| SK010Fa05 | 12 | 26 | 15 | 1 | 6 | 0 | 0 | 0 | 0 | 0 |
| Mi006Sp05 | 6 | 5 | 37 | 1 | 6 | 0 | 0 | 0 | 0 | 0 |
| Mi055Sp07 | 11 | 14 | 10 | 1 | 2 | 0 | 0 | 0 | 0 | 1 |
| FP057Sp07 | 1 | 12 | 26 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Mi070Su07 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| FP072Su07 | 3 | 17 | 16 | 2 | 4 | 0 | 0 | 0 | 0 | 0 |
| SK085Fa07 | 11 | 17 | 10 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| Mi089Fa07 | 8 | 24 | 7 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| FP091Fa07 | 8 | 8 | 6 | 2 | 2 | 9 | 12 | 11 | 13 | 10 |
| SK108Wi08 | 11 | 21 | 5 | 1 | 3 | 0 | 0 | 0 | 0 | 0 |
| Mi113Wi08 | 5 | 21 | 10 | 0 | 1 | 2 | 1 | 0 | 0 | 0 |
| FP115Wi08 | 3 | 14 | 14 | 0 | 0 | 4 | 2 | 2 | 0 | 1 |
| SK118Sp08 | 9 | 24 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| Mi123Sp08 | 7 | 27 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 1 |
| SK135Su08 | 13 | 17 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Mi139Su08 | 7 | 23 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FP143Su08 | 10 | 21 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| SK147Fa08 | 12 | 16 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Mi152Fa08 | 11 | 22 | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| FP156Fa08 | 6 | 18 | 10 | 0 | 1 | 0 | 0 | 0 | 0 | 5 |

*Temora turbinata*

| | 1 | 2 | 3 | 4 | 15 | 22 | 23 | 35 | O |
|---|---|---|---|---|---|---|---|---|---|
| SK037Wi07 | 28 | 5 | 1 | 0 | 1 | 0 | 1 | 0 | 4 |
| Mi043Wi07 | 18 | 6 | 5 | 0 | 0 | 0 | 1 | 0 | 5 |
| FP048Wi07 | 23 | 2 | 8 | 0 | 2 | 0 | 0 | 1 | 1 |
| SK051Sp07 | 20 | 5 | 0 | 1 | 5 | 0 | 0 | 0 | 3 |
| Mi055Sp07 | 21 | 8 | 3 | 0 | 2 | 1 | 0 | 0 | 5 |
| FP058Sp07 | 21 | 6 | 6 | 3 | 1 | 0 | 0 | 0 | 2 |
| Mi070Su07 | 4 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| FP073Su07 | 26 | 6 | 3 | 0 | 0 | 0 | 0 | 1 | 2 |
| SK085Fa07 | 25 | 8 | 2 | 0 | 1 | 0 | 0 | 0 | 3 |
| Mi089Fa07 | 27 | 5 | 3 | 1 | 4 | 0 | 0 | 0 | 0 |
| FP091Fa07 | 20 | 8 | 3 | 0 | 2 | 1 | 2 | 0 | 5 |
| SK110Wi08 | 33 | 4 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Mi113Wi08 | 27 | 6 | 2 | 0 | 0 | 0 | 0 | 2 | 3 |
| FP114Wi08 | 25 | 7 | 1 | 0 | 0 | 0 | 0 | 1 | 4 |
| Mi141Su08 | 24 | 2 | 5 | 1 | 1 | 2 | 0 | 0 | 4 |
| FP146Su08 | 15 | 7 | 5 | 0 | 4 | 1 | 0 | 0 | 5 |
| SK147Fa08 | 25 | 7 | 3 | 0 | 1 | 0 | 0 | 0 | 2 |
| Mi152Fa08 | 19 | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 2 |

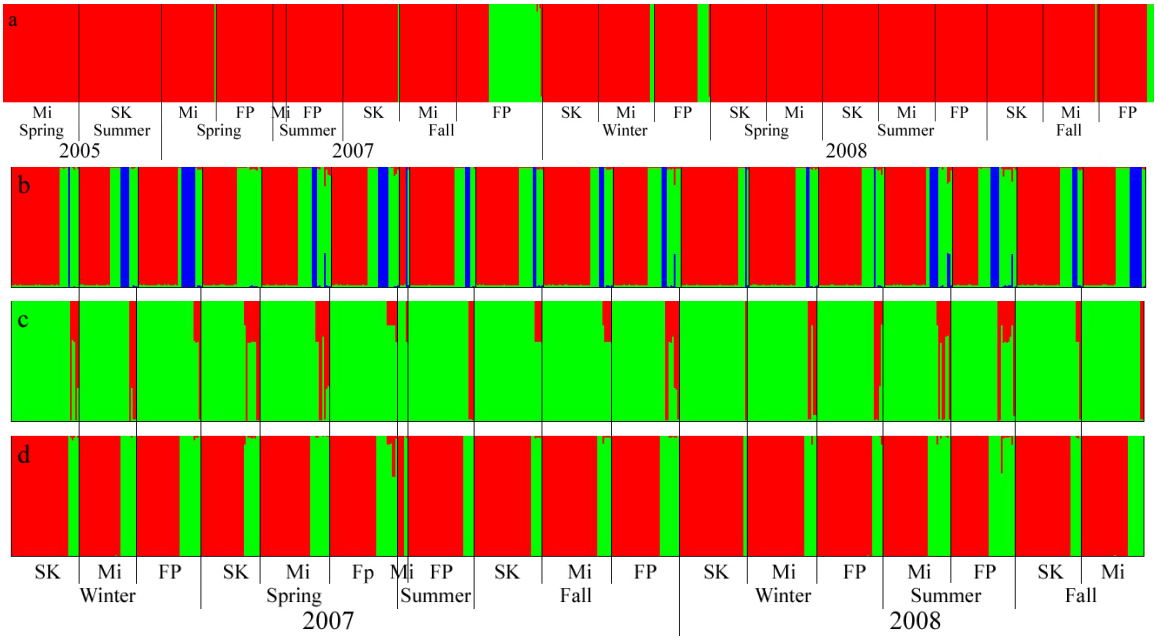


Figure 3: Structure bar plots of inferred ancestry. (a) *Paracalanus quasimodo* from ITS-1 sequences; COI and concatenated sequences have similar distributions. *T. turbinata* from ITS-1 (b), COI (c) and concatenated (d) sequences.

Table 7: AMOVA results for temporal variation. Four group structures were analyzed. The first was grouped as a single population, the second group by sampling site, the third by the quarter the samples were taken, and the third by season. For *Paracalanus quasimodo*, as ITS-1, COI and concatenated analyses result in the same significance pattern, only the ITS-1 results are shown. ns = p > 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001

| Source of variation | df | Sum of squares | Variance components | % of variation | F index | p |
|---|---|---|---|---|---|---|
| *Paracalanus quasimodo* ITS-1 | | | | | | |
| *One population* | | | | | | |
| | Among populations | 19 | 28.782 | 0.02892 | 8.06 | Fst= 0.08060 | *** |
| | Within populations | 802 | 264.583 | 0.3290 | 91.94 | | |
| *By Site* | | | | | | |
| | Among groups | 2 | 4.001 | 0.00168 | 0.47 | Fct= 0.00468 | ns |
| | Within groups | 17 | 24.782 | 0.02774 | 7.72 | Fsc= 0.07756 | *** |
| | Within populations | 802 | 264.583 | 0.32990 | 91.81 | Fst= 0.08188 | *** |
| *By quarter* | | | | | | |
| | Among groups | 8 | 17.763 | 0.01251 | 3.48 | Fct= 0.03478 | ns |
| | Within groups | 11 | 11.019 | 0.01735 | 4.82 | Fsc= 0.04998 | *** |
| | Within populations | 802 | 264.583 | 0.32990 | 91.70 | Fst= 0.08302 | *** |
| *By season* | | | | | | |
| | Among groups | 3 | 3.538 | -0.00218 | -0.61 | Fct=-0.00608 | ns |
| | Within groups | 16 | 25.244 | 0.03057 | 8.53 | Fsc= 0.08480 | *** |
| | Within populations | 802 | 264.583 | 0.32990 | 92.08 | Fst= 0.07924 | *** |
| *Temora turbinata* ITS-1 | | | | | | |
| *One population* | | | | | | |
| | Among populations | 17 | 6.372 | 0.00233 | 0.79 | Fst= 0.00794 | 0.0834 |
| | Within populations | 634 | 184.332 | 0.29074 | 99.21 | | |
| *By Site* | | | | | | |
| | Among groups | 2 | 1.310 | 0.00146 | 0.50 | Fct= 0.00496 | ns |
| | Within groups | 15 | 5.062 | 0.00130 | 0.44 | Fsc= 0.00446 | ns |
| | Within populations | 634 | 184.332 | 0.29074 | 99.06 | Fst= 0.00940 | 0.0853 |
| *By quarter* | | | | | | |
| | Among groups | 6 | 2.617 | 0.00100 | 0.34 | Fct= 0.00489 | ns |
| | Within groups | 11 | 3.756 | 0.00143 | 0.49 | Fsc= 0.00489 | ns |
| | Within populations | 634 | 184.332 | 0.29074 | 99.17 | Fst= 0.00829 | 0.0833 |
| *By season* | | | | | | |
| | Among groups | 3 | 1.371 | 0.00061 | 0.21 | Fct= 0.00208 | ns |
| | Within groups | 14 | 5.001 | 0.00186 | 0.63 | Fsc= 0.00634 | ns |
| | Within populations | 634 | 184.332 | 0.29074 | 99.16 | Fst= 0.00841 | 0.0838 |
| *Temora turbinata* COI | | | | | | |
| *One population* | | | | | | |
| | Among populations | 17 | 3.143 | 0.00160 | 1.24 | Fst= 0.01239 | * |
| | Within populations | 633 | 80.572 | 0.12729 | 98.76 | | |
| *By Site* | | | | | | |
| | Among groups | 2 | 0.224 | -0.00040 | -0.31 | Fct=-0.00310 | ns |
| | Within groups | 15 | 2.919 | 0.00188 | 1.46 | Fsc= 0.01454 | * |
| | Within populations | 633 | 80.572 | 0.12729 | 98.85 | Fst= 0.01149 | * |
| *By quarter* | | | | | | |
| | Among groups | 6 | 1.653 | 0.00152 | 1.18 | Fct= 0.01180 | 0.0559 |
| | Within groups | 11 | 1.490 | 0.00023 | 0.18 | Fsc= 0.00181 | ns |
| | Within populations | 633 | 80.572 | 0.12729 | 98.64 | Fst= 0.01359 | * |
| *By season* | | | | | | |
| | Among groups | 3 | 0.934 | 0.00096 | 0.74 | Fct= 0.00744 | 0.0803 |
| | Within groups | 14 | 2.210 | 0.00085 | 0.66 | Fsc= 0.00667 | ns |
| | Within populations | 633 | 80.572 | 0.12729 | 98.59 | Fst= 0.01406 | * |
| *Temora turbinata* concatenated | | | | | | |
| *One population* | | | | | | |
| | Among populations | 17 | 6.675 | 0.00281 | 0.96 | Fst= 0.00958 | 0.0516 |
| | Within populations | 633 | 184.270 | 0.29111 | 99.04 | | |
| *By Site* | | | | | | |
| | Among groups | 2 | 1.316 | 0.00137 | 0.47 | Fct= 0.00467 | ns |
| | Within groups | 15 | 5.359 | 0.00185 | 0.63 | Fsc= 0.00630 | ns |
| | Within populations | 633 | 184.270 | 0.29111 | 98.91 | Fst= 0.01094 | 0.0509 |
| *By quarter* | | | | | | |
| | Among groups | 6 | 2.840 | 0.00133 | 0.45 | Fct= 0.00451 | ns |
| | Within groups | 11 | 3.835 | 0.00162 | 0.55 | Fsc= 0.00554 | ns |
| | Within populations | 633 | 184.270 | 0.29111 | 99.00 | Fst= 0.01003 | 0.049* |
| *By season* | | | | | | |
| | Among groups | 3 | 1.534 | 0.00089 | 0.30 | Fct= 0.00301 | ns |
| | Within groups | 14 | 5.141 | 0.00213 | 0.72 | Fsc= 0.00726 | ns |
| | Within populations | 633 | 184.270 | 0.29111 | 98.97 | Fst= 0.01025 | 0.0505 |

Table 8: Mantel test results as p-values.

| | ITS-1 | COI | concatenated |
|---|---|---|---|
| *Paracalanus quasimodo* | 0.017915* | 0.019099* | 0.017874* |
| *Temora turbinata* | 0.315851 | 0.468501 | 0.342074 |

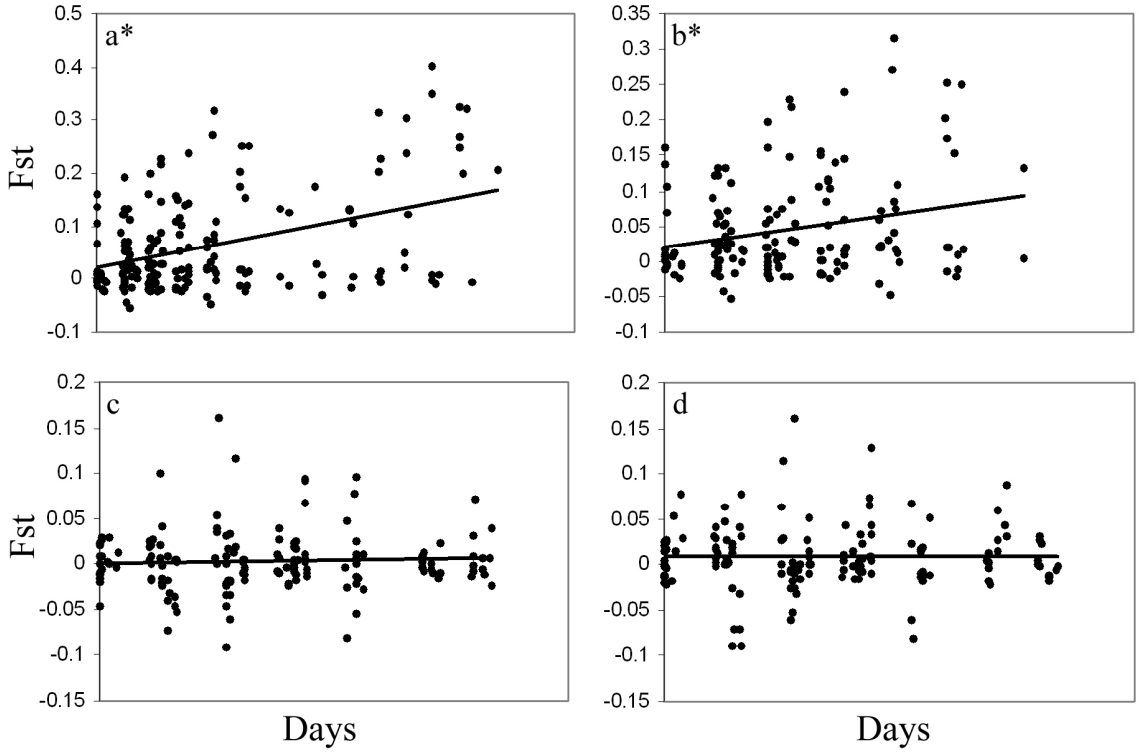


Figure 4: Scatter plot of $F_{st}$ versus time with a least squares regression line. As ITS-1, COI and concatenated plots for *Paracalanus quasimodo* and ITS- 1 and concatenated for *Temora turbinata* are nearly identical, only the ITS-1 plots for *P. quasimodo* and the ITS-1 and COI plots for *T. turbinata* are shown. a) *P. quasimodo* ITS-1; b) *P. quasimodo* ITS-1 without 2005 samples; c) *T. turbinata* ITS-1; d) *T. turbinata* COI. Significant correlations at $\alpha = 0.5$ are denoted by an asterisk (a and b).

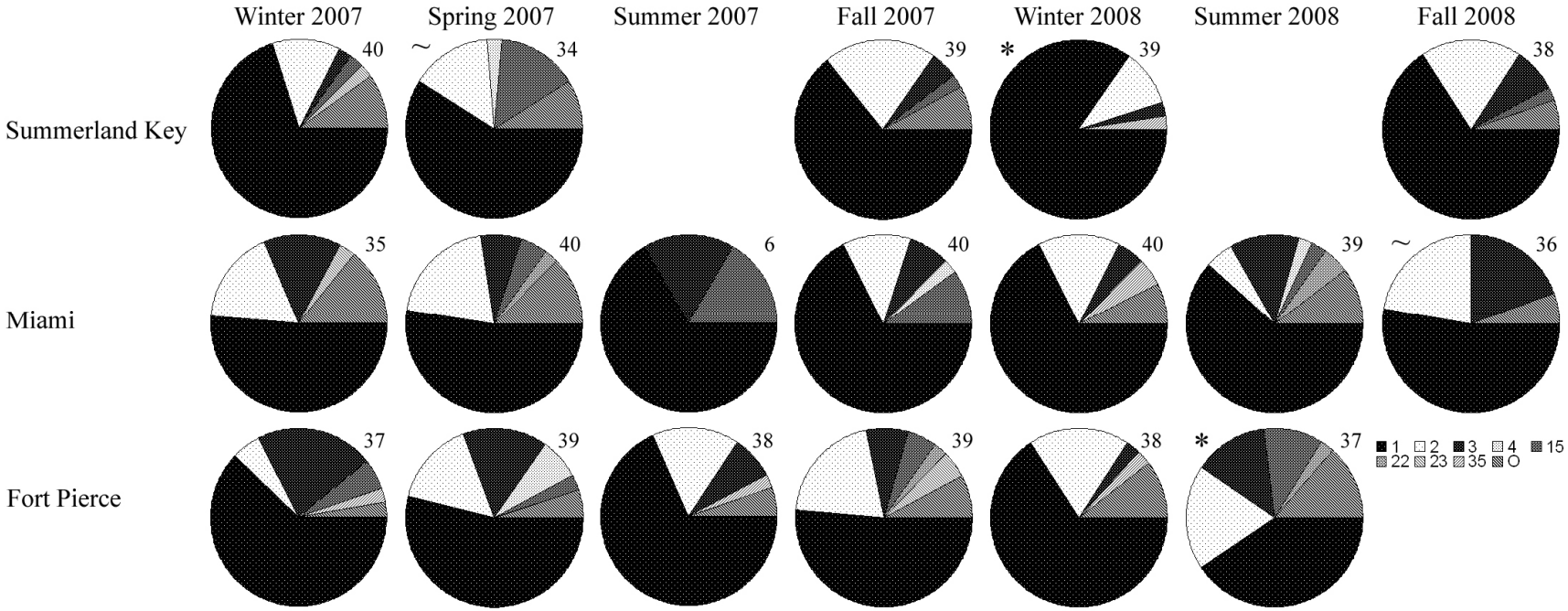


Figure 5: Proportions of *Temora turbinata* variants. Dominant variants are shown individually, while rare variants are grouped into 'O.' Samples with patterns of pairwise significant restrictions after Bonferroni corrections are marked with an *. Samples with patterns of pairwise significant restrictions only before Bonferroni corrections are marked with a ~. Sample sizes are noted to the top right of their respective pie chart.

the ITS-1 and concatenated sequences. There were no significant changes at Mi over the course of this study.

There were no significant correlations between $F_{st}$ values and time (Table 8; Figure 4). Structure 2.3.3 and Structure Harvester detected three clusters with ITS-1, but only two with COI and concatenated sequences. All genetic clusters were present in all samples (Table 6; Figure 3). AMOVAs found no significant variation in ITS-1 sequences among groups, among populations within the groups and within populations (Table 7). AMOVAs of COI sequences grouped by sampling site found no significant variation among the sites, but populations within groups and individuals within populations varied significantly. AMOVAs of COI sequences grouped by quarter and season found low, but non-significant p-values among the groups, no significant variation among populations within the groups and significant variation within the populations.

## DISCUSSION

Both *Paracalanus quasimodo* and *Temora turbinata* populations show temporal fluctuations. The changes in the genetic signature of *P. quasimodo* populations occurs over both the long term of multiple generations over the course of this study and, in the case of FP, between sampling quarters. In general, *T. turbinata* populations were stable over the course of this study, but showed a seasonal component.

Summerland Key and Mi appear to have stable, resident populations of *P. quasimodo*. Although upstream populations influence both sites, the populations do not undergo rapid changes over a quarterly timescale. The pairwise $F_{st}$ values between the first sample, Sp05Mi, and most later samples, coupled with a correlation between Fst values and time, suggest a slow shift in composition. Assuming two generations per month (Paffenhofer and Gibson 1999) and a small effective population size, a significant shift in the genetic signature could occur within 48 generations (Larsson et al 2010). No significant variations in SK and Mi populations were detected when sampled two years apart, which supports the typical assumption in single site sample phylogeographic studies that the sites are stable and the relationships between sites are stable.

However, the population at FP experienced large shifts in population composition over the period of this study. FP lies in the region identified as a mixing zone between Gulf and Atlantic marine populations (Hare and Avise 1996). The temporal instability may be a cycle of colonization from the Gulf and Atlantic populations or a continuous shift in population boundaries. However, this is only supposition as no known Atlantic populations were sampled to define the population composition.

The proportionate decline of cluster two at FP since the Fall 2007 sample suggests that this was a single, mass introduction of a foreign population. Although individuals from cluster two are occasionally found at SK and Mi, cluster two variants are more common in the Gulf of Mexico, and there was no significant difference between Fa07FP and a sample taken from Port Aransas (data not shown). The lower, but still relatively high, proportion of population two found at FP during the next quarter suggests that these variants were either unable to compete against population one variants or diluted by population one variants from the normal FP upstream sources. As the population shifts rapidly at FP, the latter is the more likely scenario.

As cluster two variants do not persist at FP, they were likely introduced. Two possible sources are as ballast and as hurricane castaways. Cyclones in the northern hemisphere create net, eastward flow (Price et al. 1994). Hurricanes Erin and Humberto moved through the northwestern Gulf of Mexico in 2007 during the interim between sampling quarters, thus would disturb local current patterns and send particles into the eastern Gulf of Mexico, where they can then be transported to the Atlantic coast. However, entrainment of western Gulf populations into the Loop Current is tenuous as no other sites had signs of western Gulf influence. An alternative is transfer from PA to FP through ballast water. Many plankton can survive for short trips (Lavoie et al. 1999) and, even should a ship following ballast clearing protocol at sea to prevent invasion of alien species, the effectiveness of removing plankton from ballast tanks are dependent on the method used (Simard et al. 2010). However, sampling a community from a ballast transfer event so close to shore and by accident is unlikely. With the limited data available, explanations for the influx of cluster two variants are speculative.

*Temora turbinata* populations fluctuate less than those of *P. quasimodo*. Only two samples show significant restrictions in gene flow to other sites and sampling periods over the course of this study. However, as gene flow does occur to all other sampling sites and times, this significant pairwise comparison may be a result of sampling extremes within the natural population variation. Fort Pierce is in a mixing zone (Hare and Avise 1996), which may facilitate production of outlier populations. Summerland Key is along the path of anticyclonic gyres formed along the loop current (Lee et al. 1994; Willemsen 2005), which can alter population composition by introducing novel variants or altering the proportion of variants.

The lack of restrictions between most sites and sampling periods indicates either recently segregated populations or one with a large range. The data support panmixia over recent separation. There is low genetic variation across the southeastern United States (data not shown). Larger populations where individuals are well mixed take longer to change through genetic drift, and the lack of significant correlation between $F_{st}$ shows that there is no concerted change over the course of this study. There is no significant variation of ITS-1 within samples. Contrary to expectations, COI showed greater variation. However, the three sites did not vary significantly from each other, further supporting panmixia.

*Temora turbinata* does not show a temporal correlation, but the COI sequences suggest seasonal variation. Although the p-values when grouped by quarter and by season are not significant, they are low (0.0559 and 0.0803, respectively). Further, contrary to grouping by site, there was no significant variation between the samples within the quarters and seasons. The lower p-value for grouping by quarter suggests that there is

also some annual fluctuation. There is too much variation in the *P. quasimodo* data to determine whether this species also has a seasonal component.

## CONCLUSION

Despite the temporal correlation in *P. quasimodo* and the seasonal variation in *T. turbinata*, these copepods are more likely to have resident rather than nomadic populations. As such, snapshot phylogeographic studies are unlikely to be affected by temporal variation. Even with the short generation times of *P. quasimodo* and *T. turbinata* (Paffenhofer and Gibson 1999; Chisholm and Roff 1990), and the assumed lower effective population size of *P. quasimodo* because of distinct population signatures in the southeast US (data not shown), no significant restrictions were detected between the majority of the temporal samples. As most samples for phylogeographic studies are taken within the span of a few years and a majority of that research in the southeast United States is on commercial species that have generation times of at least one year, significant genetic shifts are unlikely to occur during the short span of a phylogeographic study. However, caution should be taken in known or suspected mixing zones as evidenced by the multiple changes in the FP population over two years. Seasonal variation may also occur at a sampling site in migratory species. Low quality habitats may also experience cycles of local extinction and re-colonization, resulting in large shifts in genetic composition between colonies. These factors must be taken in consideration before applying the assumption of temporal stability.

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# CHAPTER IV

# INTRASPECIFIC RELATIONSHIPS IN TWO CALANOID COPEPODS

## Introduction

*Paracalanus* and *Temora* are prominent copepod genera in the coasts of the western Atlantic. Species from these genera dominate many planktonic communities (Bradford 1977; Lopes et al. 1999; Suarez-Morales and Gasca 2000a; Suarez-Morales and Gasca 2000b; Ara 2002; Dunbar and Webber 2003; Lester et al. 2008; Miyashita et al. 2009; Zhang et al. 2010; Hsiao et al. 2011; Lin et al. 2011). Despite their abundance, little is known regarding the evolutionary relationships within and between species. At the level of genera, only six of 18 *Paracalanus* species and three of four *Temora* species have been reported in the western Atlantic. The present study examines the intraspecific relationships in one *Paracalanus* and one *Temora* species through the nuclear internal transcribed spacer one (ITS-1) and mitochondrial cytochrome oxidase one (COI) loci.

The six *Paracalanus* species detected in the western Atlantic are *P. aculeatus*, *P. denudatus*, *P. indicus*, *P. nanus*, *P. parvus*, and *P. quasimodo*. *P. aculeatus* was reported in the Gulf of Mexico (Owre and Foyo 1967; Lopez-Salgado and Suarez-Morales 1998), Caribbean (Owre and Foyo 1967; Webber and Roff 1995; Suarez-Morales and Gasca 1997), Jamaica (Dunbar and Webber 2003; Webber et al. 2005), western Atlantic (Owre and Foyo 1967) and along the coast of Brazil (Eskinazi-Sant'Anna and Bjornberg 2006; Neumann-Leitao et al. 2008). *P. denudatus* was reported from the Bermudas (Paffenhofer and Mazzocchi 2003), *P. indicus* from southeast Brazil (Neumann-Leitao et

al. 2008) and *P. nanus* from the Bermudas (Paffenhofer and Mazzocchi 2003) and the Sargasso Sea (Andersen et al. 2011). *P. parvus* has been reported in Massachusetts Bay (Turner et al. 2000), Georges Bank (Cohen and Lough 1981) Brazil (Guenther et al. 2008) and on the coasts between the Argentina and Uruguay border (Berasategui et al 2006; Di Mauro et al 2009; Cepeda et al 2012). *P. quasimodo* has been reported in the Gulf of Mexico (Turner 1984a; Lester et al. 2008), in the Caribbean (Suarez-Morales and Gasca 1997) and on the coasts of Brazil (Lopes et al. 1999; Eskinazi-Sant'Anna and Bjornberg 2006; Neumann-Leitao et al. 2008; Dias and Bonecker 2009).

Of the four *Temora* species, only *T. stylifera* and *T. turbinata* have been reported in the tropical and subtropical western Atlantic. *T. stylifera* has been reported in the Gulf of Mexico (Owre and Foyo 1967; Turner 1984b; Lopez-Salgado and Suarez-Morales 1998; Lopez-Salgado et al. 2000), along the coast from the Gulf of St. Lawrence to the north shore of Venezuela (Owre and Foyo 1967), in the Sargasso Sea (Anderson et al. 2011), in the Caribbean (Suarez-Morales and Gasca 1997), along the Brazilian coast (Lopes et al. 1999; Eskinazi-Sant'Anna and Bjornberg 2006; Neumann-Leitao et al. 2008; Miyashita et al. 2009; Dias et al 2010) and in Jamaica (Webber and Roff 1995; Webber et al 2005). *T. turbinata* has been reported in the Gulf of Mexico (Owre and Foyo 1967; Thayer et al. 1983; Turner 1984b; Lopez-Salgado and Suarez-Morales 1998; Green and Dagg 1997; Lopez-Salgado et al. 2000; Lester et al. 2008), along the Atlantic coast from south of the Gulf of Maine to the north shore of Venezuela (Owre and Foyo 1967), in the Sargasso Sea (Anderson et al. 2011), in the Caribbean (Suarez-Morales and Gasca 1997, 2000a, 2000b), Jamaica (Webber and Roff 1995; Dunbar and Webber 2003;

Webber et al. 2005) and along the coasts of Brazil (Lopes et al 1999; Eskinazi-Sant'Anna and Bjornberg 2006; Dias and Bonecker 2009; Miyashita et al. 2009).

The remaining species within *Temora* are T. *longicornis* and *T. discaudata*. Contrary to the tropical and subtropical range indicated by Boltovskoy (1999), *T. longicornis* has only been reported in temperate north Atlantic (Licandro et al. 2001; Manning and Bucklin 2005; Durbin and Casas 2006; Wishner et al. 2006; Kane 2007; Kane and Prezioso 2008; Turner et al. 2011). The *T. longicornis* range appears to begin where the *T. turbinata* range ends. Along the coasts of the American continent, *T. discaudata* inhabits the eastern Pacific (Palomares-Garcia and Gomez-Gutierrez 1996; Fernandez-Alamo et al. 2000; Lavaniegos et al. 2012), a region where *T. turbinata* is reported as absent (Boltovskoy 1999).

Little is known of the molecular phylogeny of the *Paracalanus* and *Temora* genera. The position of *Paracalanus* and *Temora* within the calanoid copepod phylogeny is known only through *Paracalanus parvus* and *Temora discaudata* (Blanco-Bercial et al. 2011b). Though marine copepod species have been analyzed for intraspecific relationships, none were either *Paracalanus* or *Temora* . Intraspecific divergences can reveal biogeographic barriers (Burton and Lee 1993; Hare and Avise 1996), genetic bottlenecks (Edmands 2011) and cryptic speciation (Knowlton 1993; da Costa 2011). We propose to examine the intra and interspecific phylogenetic relationships within *P. quasimodo* and *T. turbinata* by Bayesian and coalescent analyses. Intraspecific relationships will further be explored with minimum spanning networks (MSN).

## MATERIALS AND METHODS

### *Collection*

Samples were collected from ten sites around the Gulf of Mexico and the Florida peninsula between March 2005 and January 2009 (Table 1, Figure 1). Except for Lu and LW, samples were collected by ten-minute surface tows with both 150µm and 366µm mesh nets; Lu and LW samples were collected with five vertical hauls with a 150µm mesh net beginning 5m below the surface. Surface tows were conducted along transects beginning ten miles offshore to inshore with one tow at the beginning, middle and end. Samples were named by site followed by the three-digit number of the storage bottle. Excepting PA and IM, samples were immediately preserved in 95% ethanol. The PA samples were preserved in 60% isopropanol, chilled, transported in a cooler and transferred to 95% ethanol upon return to the laboratory. The IM samples were preserved in 60% ethanol for transport and transferred to 95% ethanol upon return.

Selected individuals were first identified to species by dichotomous keys (Owre and Foyo 1967; Boltovskoy 1999). Once key features were identified, individuals were selected by morphological characteristics. For *Paracalanus quasimodo*, the characteristics were the lateral profile, pleiopod four morphology, particularly the serration along the distal edge, and pleiopod five morphology. For *Temora turbinata*, the characteristics were the dorsal and lateral profile, the caudal rami and pleiopods four and five. Samples were screened with DGGE and unique variants were chosen for sequencing. The term ‘variant’ will be used as per LaJeunesse and Pinzon (2007) expanded to unique concatenated sequences. Should two sequences have identical ITS-1

Table 1: Sampling sites and coordinates for *Paracalanus quasimodo* and *Temora turbinata*. Coordinates for FP, Mi and SK are approximate as multiple samples were taken from these sites.

| | *P. quasimodo* | | *T. turbinata* | |
|---|---|---|---|---|
| Site | Latitude/Longitude | | Latitude/Longitude | |
| Fort Pierce | N27°27 | W80°05 | N27°27 | W80°05 |
| Isla Mujeres | N21°17 | W86°46 | N21°19 | W86°47 |
| Jacksonville | N30°23.477 | W81°12.867 | N30°23.477 | W81°12.867 |
| Louisiana West | N29°19.524 | W93°25.044 | N29°19.524 | W93°25.044 |
| Louisiana | N29°02.508 | W90°31.314 | N28°51.444 | W90°27.816 |
| Miami | N25°54 | W80°07.5 | N25°54 | W80°07.5 |
| Panama City | N29°59.172 | W85°47.214 | N30°07.305 | W85°44.734 |
| Port Aransas | N27°49.396 | W97°02.602 | N27°41.555 | W96°57.026 |
| Summerland Key | N24°34 | W81°27.5 | N24°34 | W81°27.5 |
| Tampa | N27°44.087 | W82°51.913 | N27°44.087 | W82°51.913 |

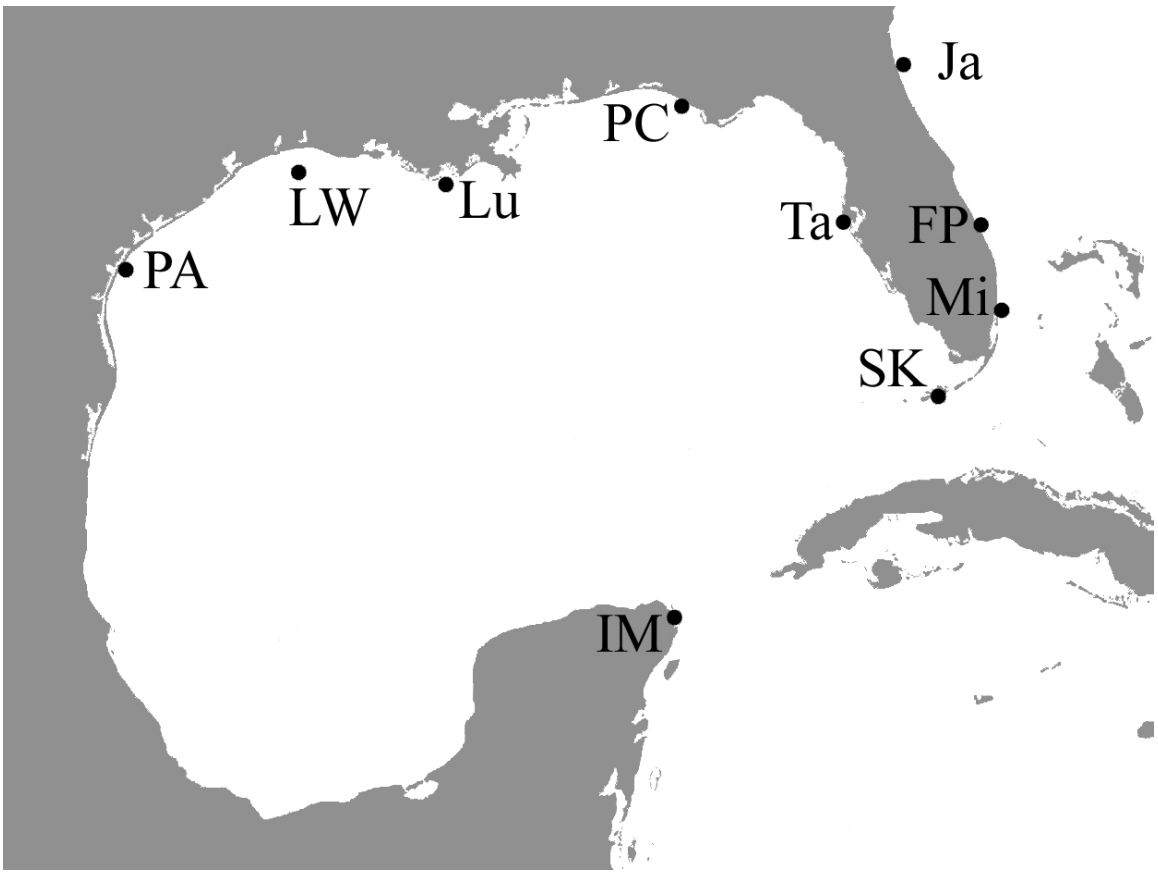


Figure 1: Sampling sites and major currents in the southeast United States. The dotted line represents a coastal countercurrent. FP=Fort Pierce, IM=Isla Mujeres, Ja=Jacksonville, Lu=Louisiana, LW=Louisiana West, Mi=Miami, PC=Panama City, PA=Port Aransas, SK=Summerland Key, Ta=Tampa. Map base modified from Google Earth.

sequences, yet have different COI sequences, or vice versa, they would be considered different variants. A consensus sequence was constructed in BioEdit (Hall, 1999) for each variant with multiple sequences. All polymorphic sites were marked as such, even if the alternate nucleotide was represented in only one sequence. DGGE variants that were identical in both ITS-1 and COI sequences were grouped under the numerically lowest variant number.

*DNA Extraction*

Individual copepods were transferred directly from ethanol to a 1.5µl microcentrifuge tube. Extractions were conducted with the MasterPure DNA extraction kit (Epicentre, Madison, WI) following manufacturer protocols and stored in 50µl of deionized water at –80°C. DNA concentration was quantified on a DyNA Quant 200 spectrophotometer (Hoefer) and varied between 3nM and 30nM.

*PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)*

The ITS-1 region was amplified with the primers ITS-1f (Coleman et al. 1994, Table 2) with the GC clamp added to the 5' end of the sequence and ITS-1r (Schizas et al. 1999). PCR reactions were run as quantitative reactions in lieu of sacrificing PCR product to check for successful amplification through electrophoresis. Amplifications were run in 10µl PCR reactions composed of 5µl iQ SybrGreen 2x (BioRad, Hercules, CA), 0.5µl ITS-1r (0.5 µM final concentration), 0.5µl ITS-1fCol with GC clamp (1 µM final concentration), 1µl DNA extract (0.3 nM to 3 nM final concentration), and 3µl nuclease free water.

Table 2: Primer sequences.

| Primer Name | Sequence |
|---|---|
| GC Clamp | CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC |
| ITS-1f | GGG ATC CGT TTC CGT AGG TGA ACC TGC |
| ITS-1r | ATC GAC CCA TGA GCC GAG TGA TC |
| LCOI-1490 | GGT CAA CAA ATC ATA AAG ATA TTG G |
| LCOI-1490c | GGT CAT GTA ATC ATA AAG ATA TTG G |
| LCOI-1528P | GTT AGC AGG AGC TTG ATC AG |
| HCOI-2198 | TAA ACT TCA GGG TGA CCA AAA AAT CA |
| HCOI-2198Par | TAG ACT TCA GGA TGT CCA AAG AAT CA |

The ITS-1 primer pair produced amplicons of approximately 320bp in length. The thermal profile for the reactions was: 95°C (5 min), 10 touchdown cycles beginning with 95-60-72°C for 20-20-60s with a 0.5°C drop in the annealing temperature per cycle, 25 cycles of 95-55-72°C for 20-20-60s and a final extension at 72°C for 10 minutes in a DNA Engine Opticon 2 (MJ Research).

The optimal urea gradient for parallel DGGE was determined to be 30-50%. The gels were run in aquaria (C.B.S. Scientific, Del Mar, CA) of TAE buffer at 65°C for 18 hours at 90 volts. To confirm that these were ITS-1 sequences, the brightest lower bands from the three dominant variants were cut from the DGGE gel, eluted in 500 μl of water for 24 hours, cleaned with the Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI), re-amplified and prepared for sequencing with BigDye terminator, ver 3.1 (Applied Biosystems, Grand Island, NY).

*Sequencing*

Polymorphic sequences can change the DGGE banding pattern between individuals with the same dominant genetic variant. However, the dominant variant provides strong evidence of the evolutionary history in an organism (LaJeunesse and Pinzon, 2007). Thus, ten individuals from each sample site were selected for DNA sequencing based on DGGE variants. Where possible, at least one of each variant present at a sample site was selected. The nuclear ITS-1 and mitochondrial cytochrome oxidase I (COI) regions were amplified. ITS-1 primers were the same as that for DGGE analysis, minus the GC clamp. Standard Folmer COI primers (LCOI-1490 and HCOI-2198) were unable to amplify a majority of the *Paracalnus quasimodo* samples and a large number of

the *Temora turbinata*. Modified Folmer primers (LCOI-1490c and HCOI-2198Par) were designed based on whole copepod mitochondrial sequences found in GenBank worked well with *T. turbinata*, but were only moderately successful with *P. quasimodo*. A COI primer was designed specifically for *P. quasimodo* (LCOI-1528p), which began 38bp downstream of LCOI-1490, and was paired with HCOI-2198Par to sequence the remaining *P. quasimodo*.

The PCR amplifications were conducted in a PTC-200 DNA Engine (MJ Research) with Promega GoTaq® Flexi reagents. Reactions were composed of 2µl of 5x Buffer, 0.6µl of 25mM $MgCl_2$ (1.5mM final concentration), 0.5µl each of forward and reverse primers (0.5µM, ITS-1 primers, and 1.0µM, COI primers, final concentration), 0.2µl of 10 µM dNTPs (0.2µM final concentration), 0.2µl of 2.5U/µl DNA Polymerase (0.05U/µl final concentration) and 2 to 5µl of DNA (0.6-6nM to 1-10nM final concentration) with nuclease free water added to make a final volume of 10µl. The higher concentration of DNA was used when the lower concentration resulted in low amplification.

Three µl of PCR product were ran on an 0.8% agarose gel to determine whether the amplification was successful. PCR products were then cleaned with ExoSap (Affymetrix, Santa Clara, CA) following manufacturer's protocols. Cleaned products were prepared for sequencing with BigDye terminator, ver 3.1 (Applied Biosystems, Grand Island, NY) for both forward and reverse strands in a PTC-200 DNA Engine (MJ Research). The 10µl cycle sequencing reaction mix was composed of 0.5µl of 1.87µM primer (0.935 µM final concentration), 2-4µl of amplified product, depending on gel

band intensity (2µl if the band was bright, otherwise 4µl), 2µl of 5x sequencing buffer, 1µl of BigDye v3.1 and the rest with double distilled deionized water. The reaction cycle was 15 seconds at 95ºC, 10 seconds at 50ºC and 4 minutes at 60ºC, repeated 35 times. The products were sequenced in an AB 3100 Genetic Analyzer (Applied Biosystems, Grand Island, NY) at the Florida International University DNA Core. Several sequences were unreadable due to contamination or, in the case of ITS-1, the presence of multiple sequence varieties. Samples where standard sequencing failed were cloned with a TOPO TA cloning kit for sequencing (Invitrogen, Grand Island, NY) following the manufacturer's protocols. Sequences were proofread in FinchTV (Geospiza, Seattle, WA), trimmed in BioEdit (Hall, 1999) and aligned with ClustalX 2.1 (Larkin et al., 2007).

*Analysis*

Separate analyses were conducted for ITS-1, COI and concatenated sequences. An additional analysis was conducted with the COI sequences combined with copepod COI sequences from published studies and found in GenBank (Table 3). *Caligus elongatus* and *Rhincalanus cornutus* were excluded from this analysis as the first had high divergence due to its parasitic lifestyle and the identity of the latter was in question as the two sequences were widely separated in a preliminary examination. Sequences were analyzed for the best model in jModelTest 2.13 (Darriba et al. 2012) with default settings, testing 88 models. Phylogenetic analysis was conducted with MrBayes 3.2 (Ronquist and Huelsenbeck 2003) with the model suggested by jModelTest 2.13. Models were run until the split frequencies fell below 0.01. Burnin was left at 25%. No ITS-1

Table 3: GenBank copepod COI sequences used for general copepod phylogeny analysis. Names have been modified to differentiate between multiple sequences for the same species.

| Name | Accession # | Publication |
|---|---|---|
| Artemia franciscana | DQ119645 | Hou et al. 2006 |
| Acartia hudsonica | EU016218 | Durbin et al. 2008 |
| Acartia pacifica | DQ071177 | Ueda and Bucklin 2006 |
| Acartia tonsa EU016219 | Durbin et al. 2008 | |
| Calanus euxinus | AY604518 | Unal et al. 2006 |
| Calanus helgolandicus | AY604520 | Unal et al. 2006 |
| Calanus pacificus | AF315013 | Rocha-Olivares et al. 2001 |
| Calanus sinicus | JF430043 | Kozol et al. 2012 |
| Centropages abdominalis | FJ602518 | Bucklin et al. 2010a |
| Centropages typicus | EU016221 | Durbin et al. 2008 |
| Chiridius armatus | AY660604 | Vestheim et al. 2005 |
| Clausocalanus arcuicornis B1 | GU171291 | Bucklin et al. 2010b |
| Clausocalanus arcuicornis B2 | GU171292 | Bucklin et al. 2010b |
| Clausocalanus arcuicornis B3 | HQ150077 | Blanco-Bercial et al. 2011b |
| Clausocalanus arcuicornis h1to h43 | JF279610 to JF279652 | Blanco-Bercial et al. 2011a |
| Clausocalanus furcatis | EF554837 | Bucklin and Frost 2009 |
| Clausocalanus jobei | EF554836 | Bucklin and Frost 2009 |
| Clausocalanus lividus B | GU171293 | Bucklin et al. 2010b |
| Clausocalanus lividus h1 to h33 | JF279653 to JF279685 | Blanco-Bercial et al. 2011a |
| Clausocalanus mastigophorus | GU171296 | Bucklin et al. 2010b |
| Clausocalanus parapergens | EF554835 | Bucklin and Frost 2009 |
| Cletocamptus deitersi | AF315009 | Rocha-Olivares et al. 2001 |
| Cletocamptus helobius | AF315014 | Rocha-Olivares et al. 2001 |
| Centropages hamatus | EU016220 | Durbin et al. 2008 |
| Ctenocalanus citer | FJ960446 | Tobe et al. 2010 |
| Lucicutia flavicornis | HQ150055 | Blanco-Bercial et al. 2011a |
| Nannocalanus minor B1 | GU171285 | Bucklin et al. 2010b |
| Nannocalanus minor B2 | GU171286 | Bucklin et al. 2010b |
| Nannocalanus minor B3 | GU171287 | Bucklin et al. 2010b |
| Neocalanus cristatus B1 | FJ602506 | Bucklin et al. 2010a |
| Neocalanus cristatus B2 | FJ602507 | Bucklin et al. 2010a |
| *Paracalanus* parvus a | HQ150069 | Blanco-Bercial et al. 2011b |
| *Paracalanus* parvus b | KC594152 | Jungbluth et al. (In Press) |
| *Paracalanus* parvus c | JF905687 | unpublished |
| Pareuchaeta norvegica | AY660600 | Vestheim et al. 2005 |
| Pontellina plumata B1 | GU171322 | Bucklin et al. 2010b |
| Pontellina plumata B2 | GU171323 | Bucklin et al. 2010b |
| Pontellina plumata B3 | GU171324 | Bucklin et al. 2010b |
| Pontellina plumata B4 | HQ150060 | Blanco-Bercial et al. 2011b |
| Pseudocalanus elongatus a | AY604522 | Unal et al. 2006 |
| Pseudocalanus elongatus b | AY604523 | Unal et al. 2006 |
| Pseudocalanus elongatus c | HM770077 | Holmborn et al. 2010 |
| Pseudocalanus elongatus d | HM770078 | Holmborn et al. 2010 |
| Pseudocalanus minutus a | HM770075 | Holmborn et al. 2010 |
| Pseudocalanus minutus b | HM770076 | Holmborn et al. 2010 |
| Pseudocalanus moultoni | AF242842 | Bucklin et al. 1999 |
| Pseudocalanus newmani | AF242841 | Bucklin et al. 1999 |
| *Temora* discaudata | HQ150061 | Blanco-Bercial et al. 2011b |

Table 3 (continued): GenBank copepod COI sequences used for general copepod phylogeny analysis.

| Name | Accession # | Publication |
|---|---|---|
| Undinula vulgaris B1 | GU171332 | Bucklin et al. 2010b |
| Undinula vulgaris B2 | GU171333 | Bucklin et al. 2010b |
| Undinula vulgaris B3 | GU171334 | Bucklin et al. 2010b |
| Undinula vulgaris B4 | GU171335 | Bucklin et al. 2010b |
| Undinula vulgaris J1 | KC594158 | Jungbluth et al. (In Press) |
| Undinula vulgaris J2 | KC594159 | Jungbluth et al. (In Press) |
| Undinula vulgaris J3 | KC594160 | Jungbluth et al. (In Press) |
| Undinula vulgaris J4 | KC594161 | Jungbluth et al. (In Press) |
| Undinula vulgaris J5 | KC594162 | Jungbluth et al. (In Press) |
| Undinula vulgaris J6 | KC594163 | Jungbluth et al. (In Press) |
| Undinula vulgaris J7 | KC594164 | Jungbluth et al. (In Press) |
| Undinula vulgaris J8 | KC594165 | Jungbluth et al. (In Press) |
| Undinula vulgaris J9 | KC594166 | Jungbluth et al. (In Press) |

sequences within *Paracalanus* or *Temora* were available in GenBank, so the *P. quasimodo* ITS-1 phylogeny was left unrooted and the *Paracalanus* variant 20 ITS-1 sequence was used as the outgroup for *T. turbinata*. As the *T. turbinata* variants were all closely related, there is minimal risk of long branch attraction confounding the results. *Paracalanus parvus* (Accession HQ150069) was the outgroup for the *P. quasimodo* COI analysis. For the *T. turbinata* COI analysis, the outgroup was *T. discaudata* (Accession HQ150061). The concatenated outgroups were the COI sequence with missing data for the ITS-1 segment.

Coalescent analyses were run in BEAST 1.7.5 (Drummond et al. In Press) following the same model as for the MrBayes analyses where possible. Other settings were left at default. All analyses were run for ten million generations, with the exception of the *T. turbinata* ITS-1 and concatenated sequences, which ran for 100 million and 20 million generations. Runs were examined for a sufficiently large effective sample size with Tracer 1.5 (Rambaut and Drummond 2007). Bayesian and coalescent trees were edited in Mesquite 2.75 (Maddison and Maddison 2011). MSNs were calculated in Arlequin 3.5.1.2 (Excoffier and Lischer 2010). The resultant network was visualized and edited with HapStar 0.7 (Teacher and Griffiths 2011).

## RESULTS

Of 1,293 *Paracalanus quasimodo* and 902 *Temora turbinata* successfully screened by DGGE, 42 and 29 variants were detected, respectively. Variant 20 was not included in the previous total as the COI sequence had 99% similarity to a *P. parvus* sequence on GenBank (Accession AF474111, HQ150069 and KC594152). Average standard deviation of the split frequencies fell below 0.01 within one million generations for all *P. quasimodo* analyses. *T. turbinata* analyses ran for one, two and one million generations for ITS-1, COI and concatenated sequences, respectively. Analysis of the GenBank copepod COI sequences ran for 19 million generations with a final split frequency of 0.009558.

*Paracalanus quasimodo* variants were divided between two distinct clades (Figure 2) with little significant support for topology within the clades (Figures 3-5). With the exception of ITS-1 the Bayesian and coalescent analyses concluded with the same tree shape. The coalescent ITS-1 analysis places clade one, named such as it contains the most abundant variants, as a subset of clade two, whereas all other analyses of *P. quasimodo* support a distinct separation of the two clades. The divergence between clades one and two ranged from 17 to 21% in the concatenated sequences. All variants except for variant 52 fell in the same clades for the ITS-1, COI and concatenated analyses. Variant 52 was found in clade one with ITS-1 and in clade two with the COI and concatenated sequences.

The MSNs reflect the phylogenetic trees, with two distinct clusters (Figures 6-8). The variants in the MSN clusters correspond with the clades. As with the phylograms,


Figure 2: Copepod phylogeny based on COI sequences. Excepting *Paracalanus parvus*, single species clades have been collapsed for brevity. Nodes with probabilities lower than 0.9 have been collapsed.

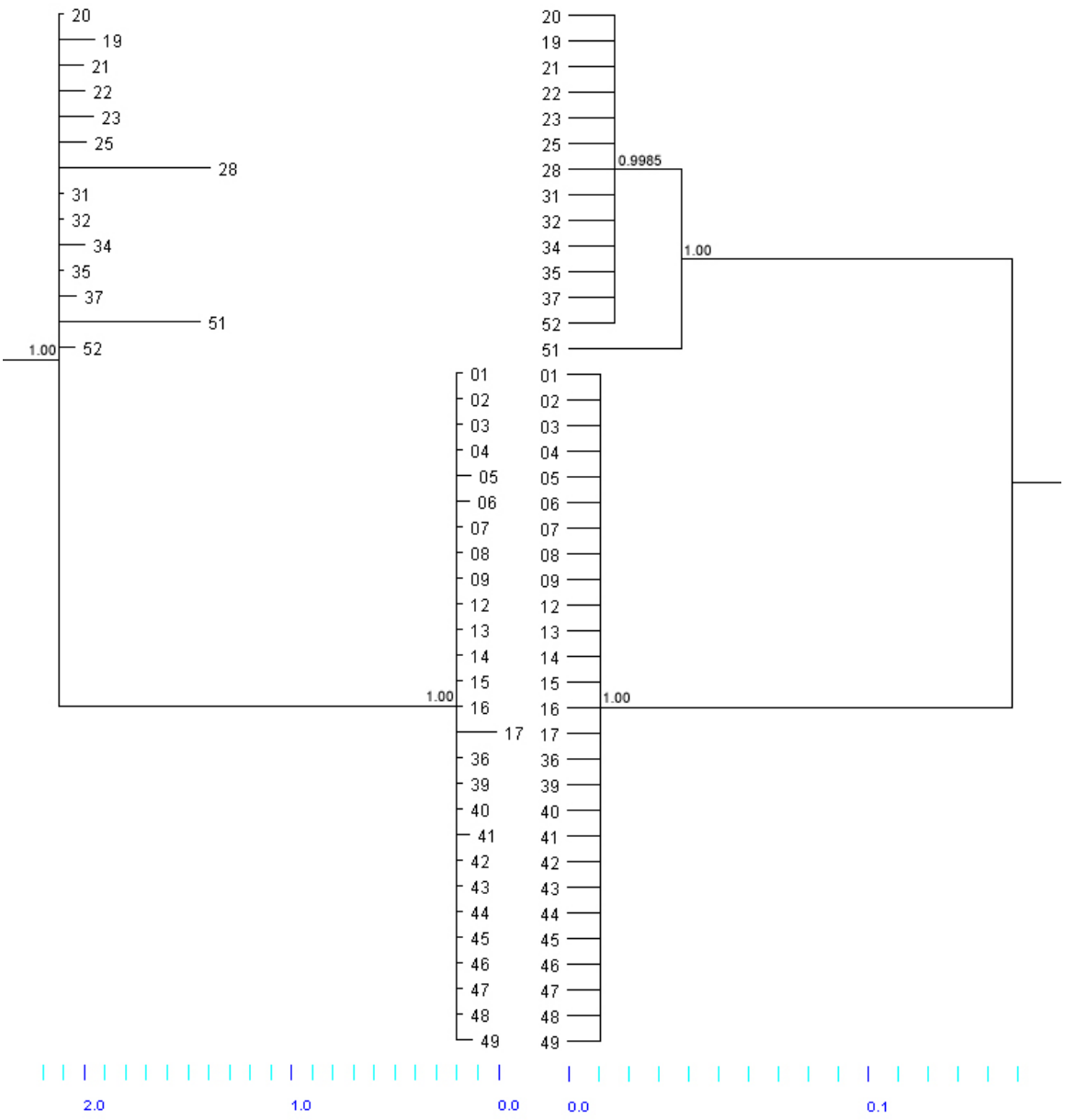


Figure 3: *Paracalanus quasimodo* variant phylogeny based on ITS-1 sequences. The Bayesian phylogram is on the left and the coalescent phylogram is on the right. These trees are unrooted. Nodes with probabilities below 0.9 have been collapsed.

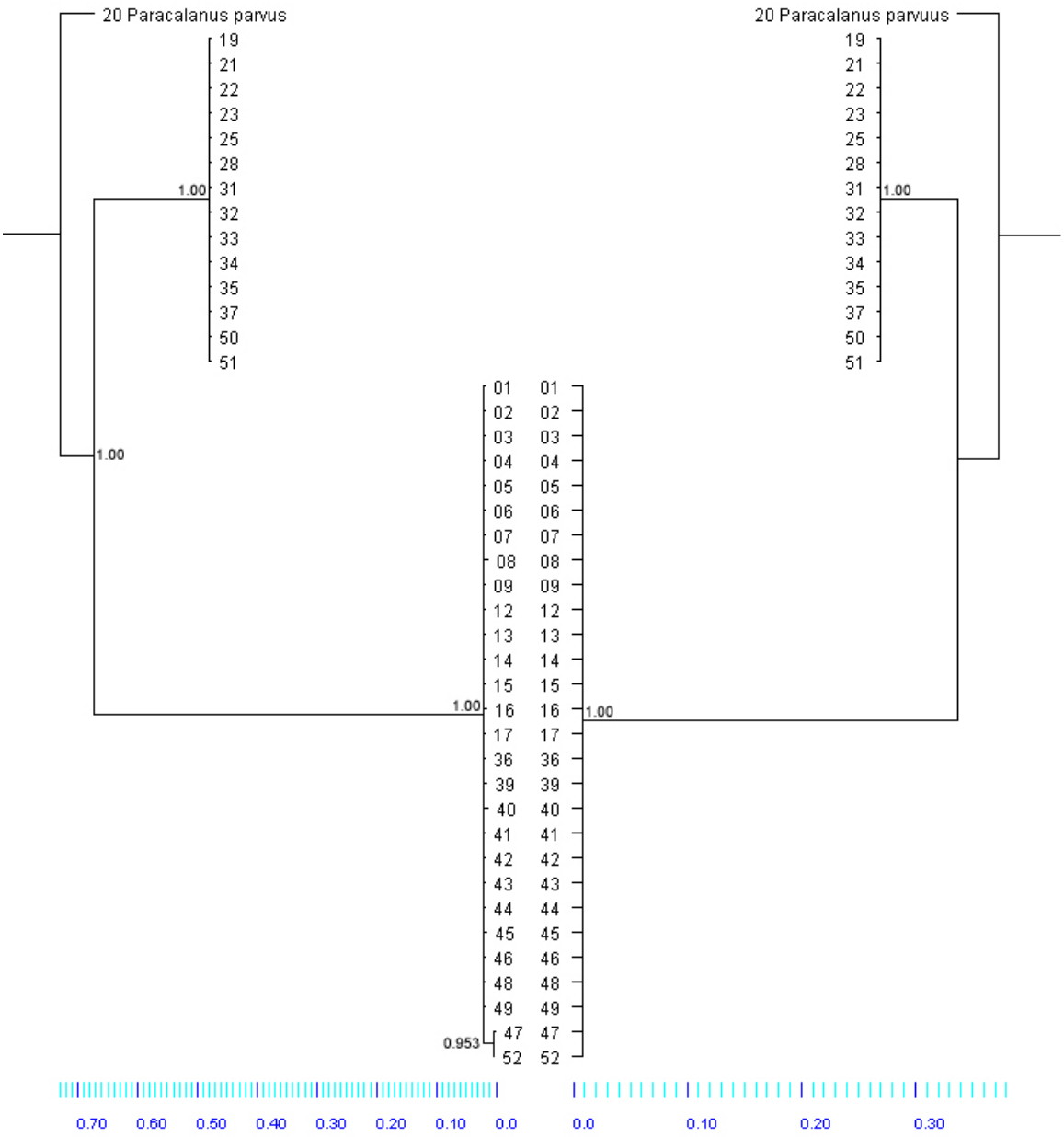


Figure 4: *Paracalanus quasimodo* variant phylogeny based on COI sequences. The Bayesian phylogram is on the left and the coalescent phylogram is on the right. Nodes with probabilities below 0.9 have been collapsed.

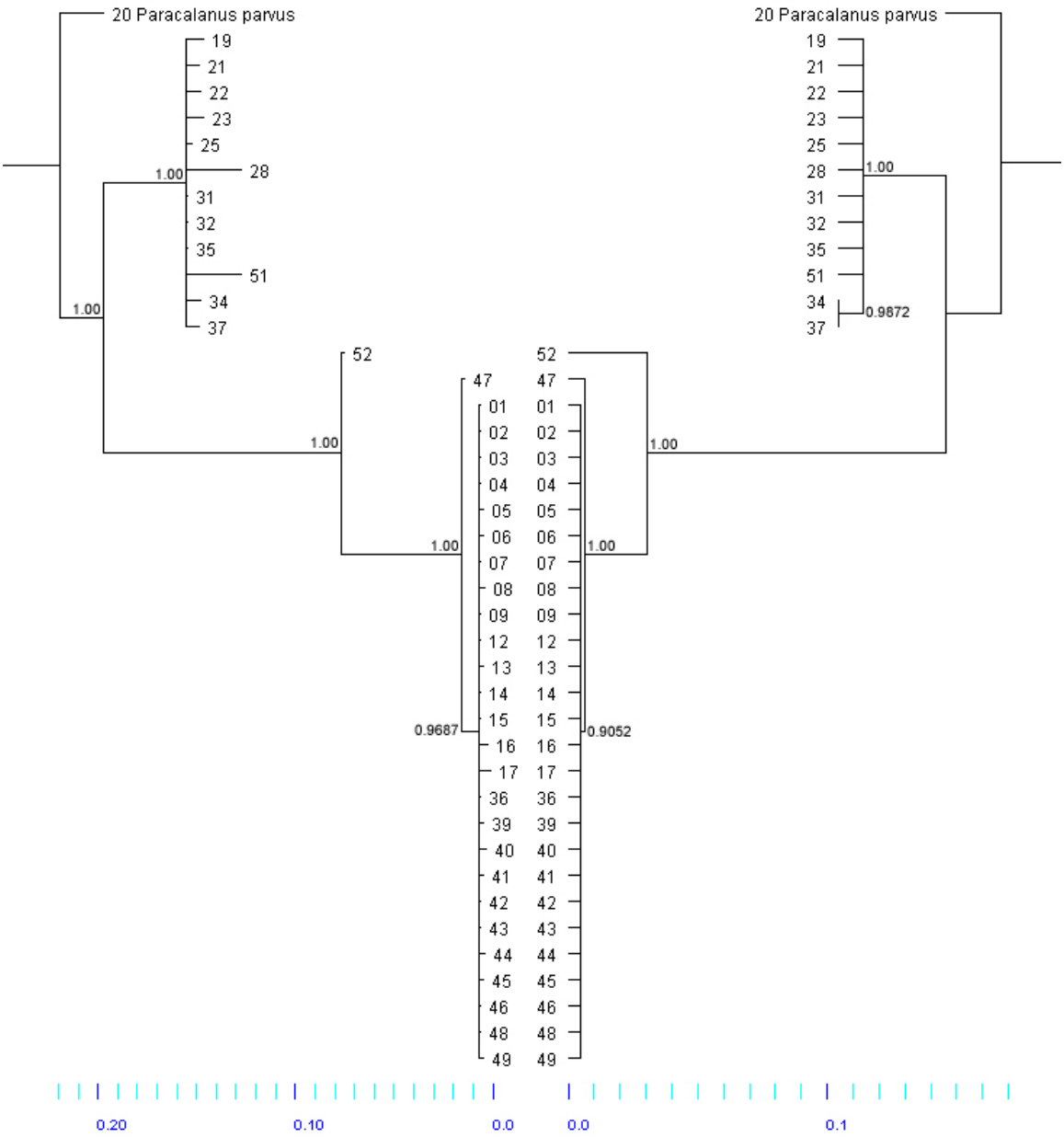


Figure 5: *Paracalanus quasimodo* variant phylogeny based on COI sequences. The Bayesian phylogram is on the left and the coalescent phylogram is on the right. Nodes with probabilities below 0.9 have been collapsed.

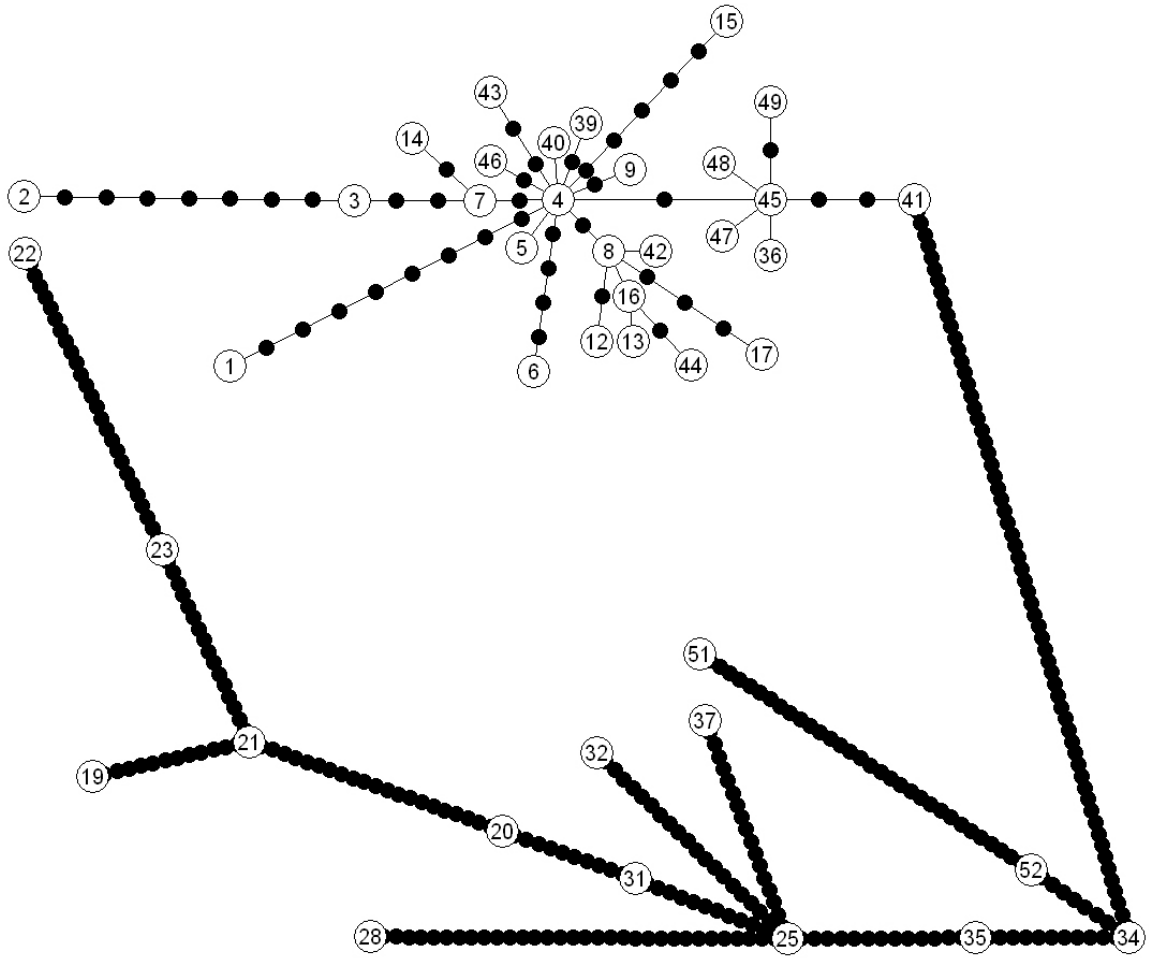

Figure 6: MSN of *Paracalanus quasimodo* ITS-1 variants and *Paracalanus parvus* (20).

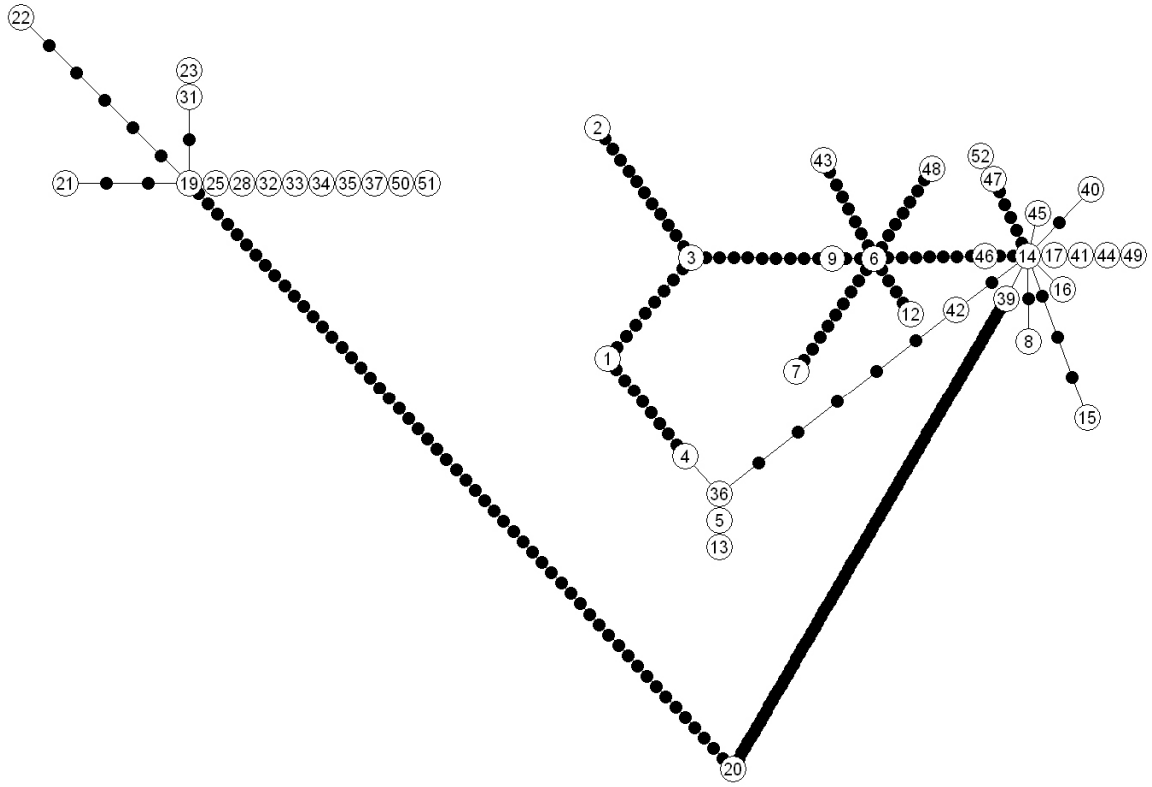


Figure 7: MSN of *Paracalanus quasimodo* COI variants and *Paracalanus parvus* (20). Variants that touch have zero steps between them on the MSN.

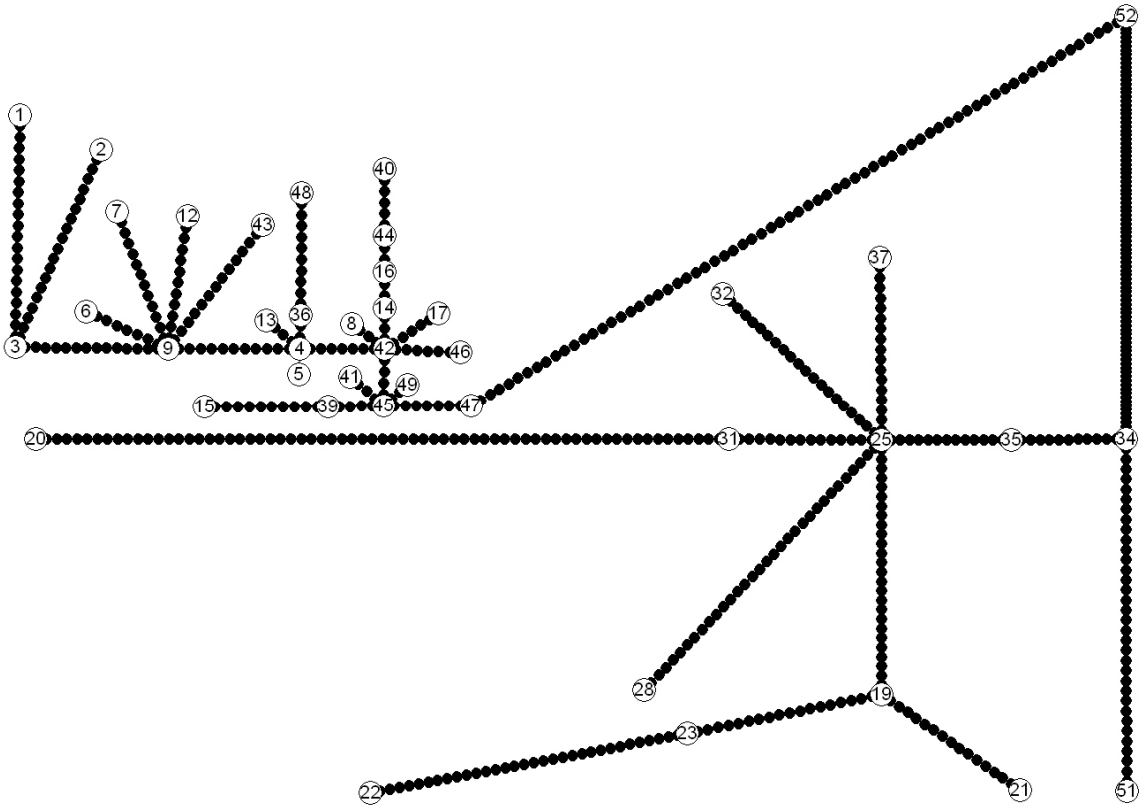


Figure 8: MSN of *Paracalanus quasimodo* concatenated variants and *Paracalanus parvus* (20).

variant 52 is found in different clusters depending on the loci. The MSN for the concatenated sequences shows two clusters corresponding to the clades, with 100 steps between the closest two variants, variant 52 of clade one and variant 34 of clade two (Figure 8), with 10.9% difference between the two variants. The most steps between variants within clade one were 70 between variants 52 and 47 (7.7% divergence), two steps more than between variant 31 in clade two and 20, *P. parvus* (7.4% divergence). There were fewer steps between variants in clade one with ITS-1 and concatenated loci and in clade two with COI. *P. parvus* was located in the interior of the ITS-1 and COI MSNs. The most abundant variants, 1, 2 and 3 from clade one and 21, 22 and 23 from clade two (data not shown), were consistently at opposite ends of the networks.

The nearest neighbor to *T. turbinata* was *T. discaudata* (Figure 2). Bayesian and coalescent analyses places all *T. turbinata* variants in one clade (Figure 9). The maximum separation between variants in the MSNs was one step (Figure 10). Sequence variations between the variants were minimal. The largest difference between concatenated variants was 2% between variants 3 and 17. Eighteen of the nineteen deviations between the two variants were due to single-nucleotide polymorphisms (SNPs) where the SNP on one variant was ambiguous two nucleotides, one of which was the same as on the other variant. For example, variant 3 had cytosine at position 26 while variant 17 was ambiguous with cytosine and guanine. The remaining difference was an indel at position 285. Overall, there were 33 divergent sites in 995 bases. Two of the divergences were indels at position 285 and 286 and 31 were SNPs. Eleven of the SNPs were due to unambiguous nucleotides between at least two variants.

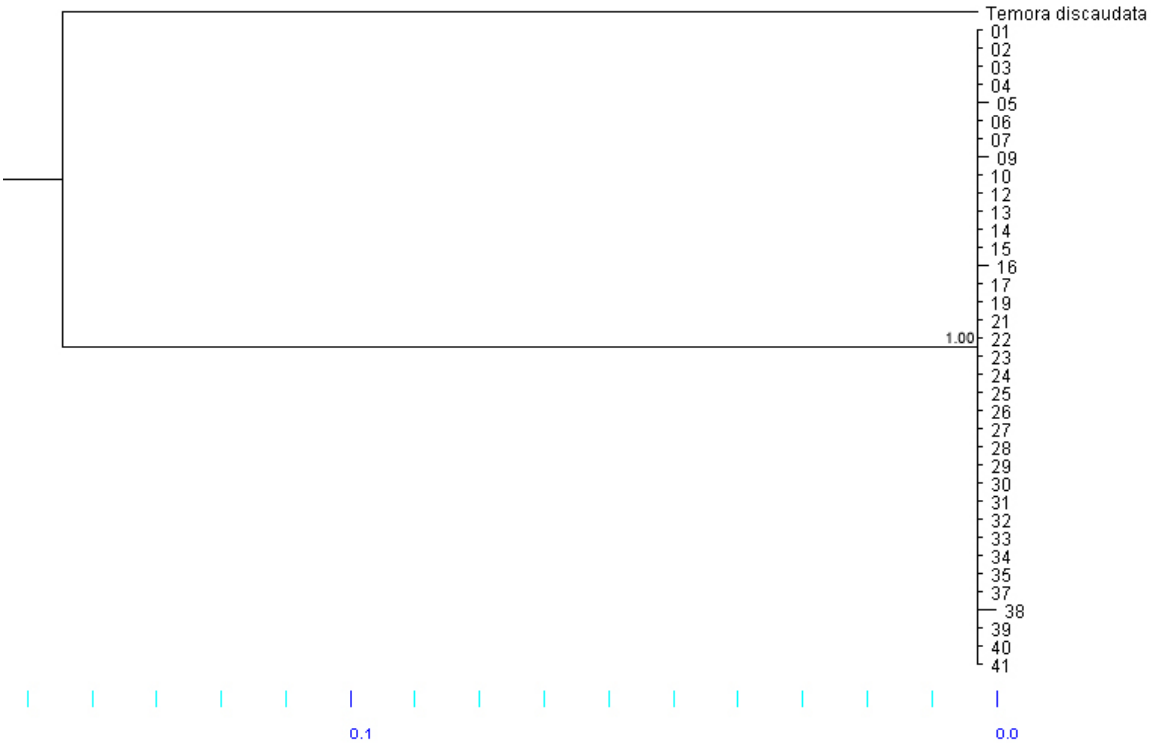


Figure 9: *Temora turbinata* variant phylogeny based on concatenated sequences. Excepting scale, the supported topology is identical for ITS-1, COI and concatenated sequences for both Bayesian and coalescent analyses.

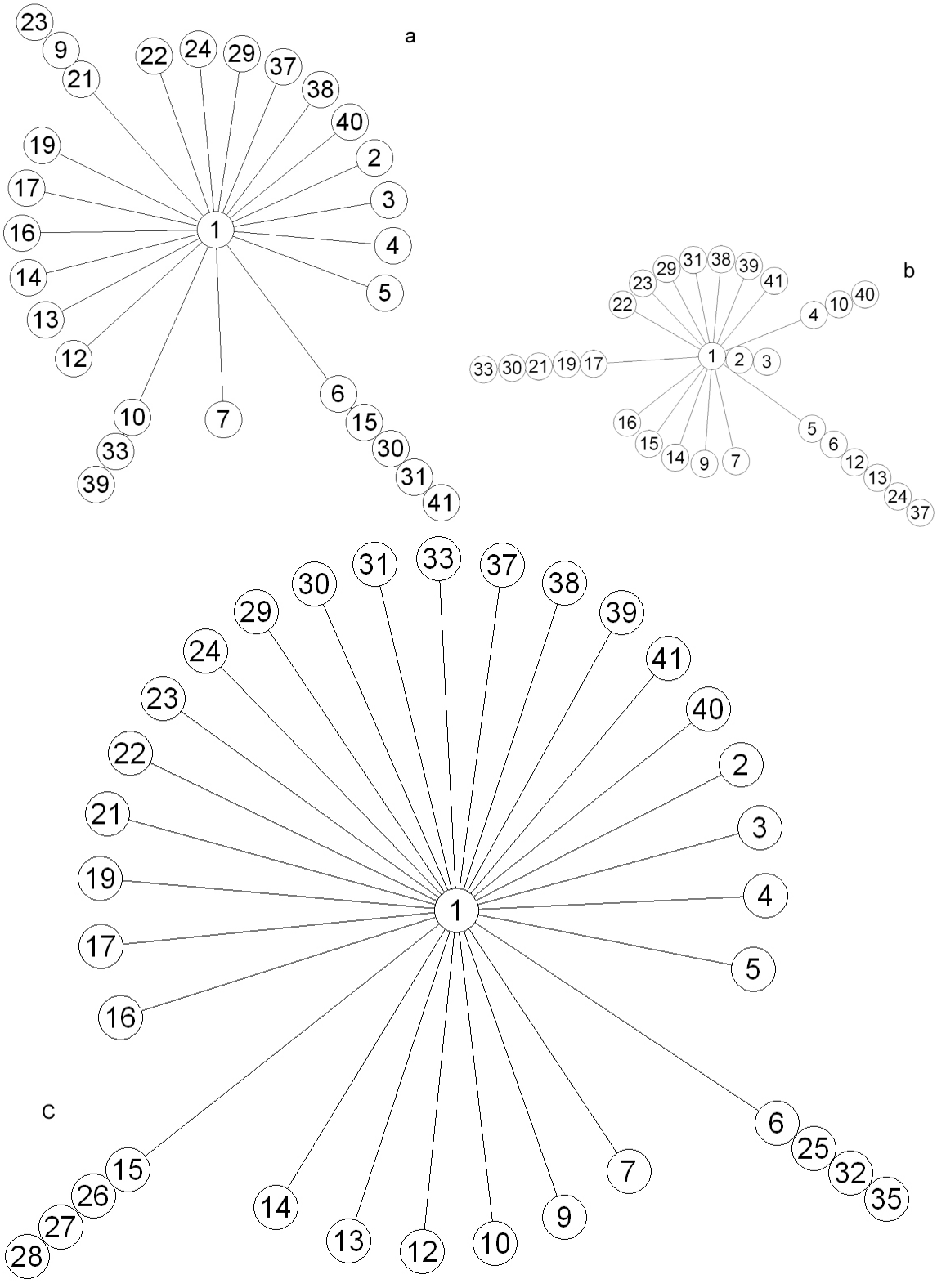


Figure 10: MSNs of *Temora turbinata* (a) ITS-1, (b) COI and (c) concatenated variants. Variants that touch have zero steps between them on the MSN.

## DISCUSSION

*Paracalanus quasimodo* and *Temora turbinata* formed clades with congeneric species. *P. quasimodo* variants were divided into two highly divergent clades. *T. turbinata* variants formed a single clade with minimal differentiation between the variants. There was no significant support for intraclade topology in either species.

The sequence divergence between the two *P. quasimodo* clades is greater than the intraspecific variation in many copepod species (Bucklin and Wiebe 1998; Bucklin et al. 1999; Bucklin et al. 2003; Caudill and Bucklin 2004; Bucklin et al. 2010b; Blanco-Bercial et al. 2011a; da Costa et al. 2011; Winkler et al. 2011). Although morphological misidentification is always a possibility, clade two is unlikely to be another *Paracalanus* species extant in the southeast United States. Despite variant 20, clade two is unlikely to be misidentified *P. parvus*, as both clade 2 and the *P. parvus* clade are well supported, and the divergence between clade two and *P. parvus* was greater than 8%. Similarly, clade two is unlikely to be the morphologically similar *P. aculeatus* as *P. aculeatus* was not detected in the samples with high proportions of clade two. There is also an approximate 12% divergence between clade two and *P. aculeatus*, as with *P. aculeatus* and *P. parvus*.

The large divergence between clades one and two suggests cryptic speciation. Morphological stasis with genetic divergence has been discovered in numerous crustacean taxa (Knowlton 1986, 1993; Williams et al. 2001; Lee and Frost 2002; Chen and Hares 2011; da Costa 2011), and individuals from the two clades are indistinguishable by the dichotomous key produced by Boltovskoy (1999). However, the

position of clade two on the phylogenetic tree and the degree of sequence divergence, suggesting that clade two is closer related to *P. parvus*, argues against cryptic speciation. An alternate scenario is that clade one is *P. quasimodo* and clade two is an unknown species in the genera of *Paracalanus*. Finally, the divergence between clades one and two is within the unusual intraspecific variation of *Tigriopus californicus* (Burton and Lee 1994; Burton et al. 2007), which demonstrates that it is possible for widely divergent clades to belong to the same species. A thorough morphometric analysis is required to determine whether clade two is morphologically identical to clade one, a different known species or a new species entirely.

*Paracalanus quasimodo* variant 52 changes clades depending on the locus. In the MSNs, variant 52 is intermediate of clades one and two with concatenated sequences, identifies with clade one with ITS-1 and clade two with COI. The clade hopping with different loci indicates a hybrid individual rather than a genetic intermediate to the two clades. Variant 52 was collected from Panama City, where both clades were prominent (data not shown). If this is truly a hybrid, then clades one and two have not diverged to the point of reproductive exclusion. This suggests that the two clades are closer related to each other than to *P. parvus* and a possible case of cryptic speciation. However, whether *P. quasimodo* is able to hybridize with congeneric species has not been tested.

The placement of variant 20 within the clade two cluster of the ITS-1 and COI MSNs suggests a close relationship between *P. parvus* and the taxa of clade two. Although the MSN from the concatenated loci placed variant 20 outside of the clade two cluster and *P. parvus* is well supported as a distinct clade, the gene history suggests that

the two groups are closely related. Although there is a marked separation from clade one, it is unclear whether clade two or *P. parvus* is the closer relative.

The low level of genetic differentiation in *T. turbinata* and the star pattern in the MSNs are indicative of a population bottleneck. As *T. turbinata* occurs throughout the tropics and subtropics (Boltovskoy 1999), it is unlikely that the species had to retreat to refugia due to glaciation, as was required of more temperate species. However, glaciation would have reduced the *T. turbinata* range and provided an opportunity for range expansion at the end of the last ice age and the apparently panmictic distribution in the western Atlantic would be due to insufficient time for populations to differentiate.

An alternative is that the presence of *T. turbinata* in the western Pacific is relatively new and the bottleneck is due to a founder event. Though reported to inhabit opposite ends of the earth, *T. turbinata* is not found in the eastern Pacific (Boltovskoy 1999) and the only study reporting this species between the Chinese coast and the western Atlantic was off the west coast of India (Goswami and Padmavati 1996). *T. turbinata* may be absent from European and African coasts, or they may not have been reported. Should *T. turbinata* be absent from European and African coasts, then there is a large geographic break between populations, then the homogeneity within the southeast United States supports a recently introduced population. As the genetic signature of *T. turbinata* outside of the southeastern United States is unknown, leaving any conclusion regarding the origin of this species as mere conjecture.

## CONCLUSION

*Paracalanus quasimodo* and *Temora turbinata* show opposite trends of divergence in the southeast United States. While *P. quasimodo* has two deeply divergent clades, *T. turbinata* has minimal diversity suggestive of a bottleneck. Both species require further sampling of distant populations to test whether these trends in molecular variation are local or a general characteristic of the respective species.

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# CHAPTER V

# CONCLUSIONS

The population structure of *Paracalanus quasimodo* and *Temora turbinata* supports the paradigm that, generally, population structure increases with a decrease in dispersal potential. There were geographic and temporal differentiations in *P. quasimodo* samples that were not evident in the farther ranging *T. turbinata*. Further, *P. quasimodo* variants were divided between two, deeply divergent clades. Clade one was present throughout the sampling region, while, with the exception of one aberrant sample from Fort Pierce, clade two was prominent only in the samples from the Gulf of Mexico.

As the *P. quasimodo* clades are sympatric, dispersal potential alone cannot explain the development of said clades. Clade two may have originated from the southwest Gulf of Mexico and were carried into the northern gulf to mix with clade one. Further sampling along the Mexican coast of the GoM would be required to test this hypothesis. An alternate hypothesis is that clade two is a cryptic species that was able to exploit a niche present in the GoM, but minimal or missing on the Atlantic coast. This would be an explanation for the inability of clade two to establish a successful colony at Fort Pierce despite the large influx detected in fall of 2007.

Minimal variation in *T. turbinata* suggests a founder affect in a recently established colony or similar bottleneck. The low genetic variation would make it difficult to detect population structure, except in extreme cases. Thus, *T. turbinata* may be panmictic or it may be divided into populations similar to *P. quasimodo*, but with insufficient time for the populations to develop a distinct signature. An examination of *T.*

*turbinata* populations from other regions of the earth would provide further insight as to the intraspecific variation found in this species and whether this variation was anomalous.

# VITA

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