GENETIC ASSESSMENT OF MACROBRACHIUM SPECIES IN TEXAS COASTAL STREAMS

A Thesis

by

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Freshwater shrimps in the genus *Macrobrachium* are ecologically and economically important as they provide essential services in habitats through a range in ecological roles such as detritus removal and predation as well as being prey items for large fish. Economically, *Macrobrachium* species have been exploited for aquaculture as well as commercial and recreation fisheries. Despite their importance, species distributions in *Macrobrachium* are poorly understood as the species exhibit similar morphologies making delineation difficult. The amphidromous lifestyle exhibited in many *Macrobrachium* species, including all that are native to the US, limits the dispersal capabilities potentially separating populations and allowing for localized adaptations. This lifestyle opens *Macrobrachium* species to multiple vulnerabilities such as habitat degradation and loss due to damming, land-use alterations, and pollution as well as past exploitation which have resulted in striking population declines of *Macrobrachium* species occurring in the United States. This study developed a robust phylogenetic hypothesis for *M. carcinus* throughout the species known distribution, including other North American species of *Macrobrachium* as outgroups using mitochondrial genes and made a preliminary assessment of *M. ohione* population structure between individuals occurring in different bay systems using mitochondrial cytochrome oxidase subunit I (COI). Phylogenetic trees created in BEAST and MEGA showed monophyly in the group as well as three genetically distinct clades of *M. carcinus* Puerto Rico, Central/South America, and Texas. Net p-distances of between these three clades were among species level divergences. Preliminary population assessment on *M. ohione* showed low levels of genetic diversity, which could be due to marker choice, but did show recent population expansion into Texas coastal streams.

DEDICATION

This thesis is dedicated to my husband, Mike Pineda, and parents, Kim and RJ Harbarger. Their unwavering support helped me see this through to the end.

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TABLE OF CONTENTS

PAGE

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I: INTRODUCTION

Macrobrachium is a diverse genus of freshwater shrimps, with greater than 240 species that exhibit a pan-tropical and pan-subtropical distribution (De Grave & Fransen 2011, Anger 2013). The origins of the genus *Macrobrachium* are not entirely resolved as both a marine and freshwater ancestor have been proposed (Murphy & Austin 2005, Liu et al. 2007, Wowor et al. 2009). Either way, this genus originated before the closure of the Tethys Sea and dispersed to its current location through marine currents and plate tectonics (Anger 2013). Establishment and subsequent radiation of species in freshwater habitats globally have contributed to their diversity (Anger 2013).

Additionally, the species within *Macrobrachium* exhibit different reproductive strategies which reflects their flexibility to live in various habitats. A small fraction approximately 31 of the species reproduce and develop solely within freshwater environments (Anger 2013), while most others exhibit varying degrees of amphidromy with extended larval development. First-stage zoea reside in freshwater but require a saline environment to metamorphose into a feeding second-stage zoea (Jilalah et al. 1993) and therefore must reach a saline environment within five days or die of starvation (Bauer & Delahoussaye 2008). Larval movement downstream to estuaries is typically achieved by drifting with the current but may be facilitated by downstream migration of females to ensure larvae reach the estuary within two days (Bauer & Delahoussaye 2008). The number of subsequent larval stages vary between species, ranging from three to 20 stages before becoming a freshwater juvenile that migrates upstream (Jilalah et al. 1993). Both the number of zoeal stages and the species-specific tolerance for salt water can determine how far species disperse through the estuary/marine environment to disjunct freshwater ecosystems (Jilalah et al. 1993, Rossi & Mantelatto 2013).

Macrobrachium species occupy diverse ecological roles in freshwater ecosystems. Many are opportunistic omnivores, filling detritivore roles in coastal rivers and streams (Lima et al. 2014, Pringle et al. 1999). Some species have an important carnivorous component to their diet (Lima et al. 2014). For example, stomach contents of the large-bodied *M. carcinus* contain other crustaceans, gastropods, and mollusks (Lima et al. 2014). Other species, e.g. *M. olfersii*, have been observed mutilating fish at night, clipping fins to feed on, suggesting a parasitic role (Sabino 1995). The omnivorous species fill an important ecological role by altering the carbon and nitrogen ratio within the systems they occupy, ensuring that the carbon from detritus is recycled back to their predators (Pringle 1999, Santos et al. 2001). Sediment transport is increased by *Macrobrachium* presence within streams due to foraging on fine particulate organic matter reducing sediment accrual by ingesting and resuspending sediments, increasing the overall water quality (Pringle 1999). Additionally, several large bodied predatory species of *Macrobrachium* have been shown to reduce the potential for schistosomiasis infections in humans, by feeding on gastropods that carry the trematode parasite (Sokolow et al. 2017). Even single species can occupy more than one ecological niche and subsequent roles. For example, *M. nipponense* fills a detritivore role in reservoir systems but has also been observed acting as a pelagic consumer in a canal system (Zhang 2020). Such diversity in reproductive and ecological roles can allow some species to fill other organisms' ecological roles when necessary. *Macrobrachium* also have been commercially developed for sale as a seafood product; specifically, Indo-Pacific species such as *M. rosenbergii* and *M. lar* (Kutty & Valenti 2010). In the US, large species such as *M. carcinus* supported fisheries in Texas and were typically put on display, while smaller species such as *M. ohione* supported commercial fisheries for food and recreational bait (Hedgepeth 1949, Gunter 1937).

Despite their important ecological functions, and the fact that many species of *Macrobrachium* have undergone drastic population declines (Bowles et al. 2000, Robison & McAllister 2011), species in the genus are afforded relatively few protections. For example, in the US, species have few state, and no federal protections due in part to their wide-ranging distributions and high local abundances (De Grave 2015). Dam construction is one of the primary proposed reasons for range reduction in *Macrobrachium* species. Different dam types have varying impacts on species, as in-channel withdrawal systems have no impact, while small low head dams may act as a partial barrier for migration, and large dams act as complete barriers (March 2003, Benstead et al. 1999). Impacts from dams likely vary between species and life stages, for example adult *M. carcinus* can leave the water and walk around dams, and other species can walk up dams with sufficient spillover (Horne & Beisser 1977, March 2003). However, dams heavily impact the downstream drift of first-stage larvae, increasing mortality because larvae must reach the estuaries soon after hatching (Benstead et al. 1999). Juveniles of *Macrobrachium* rely on flow cues for *en masse* up-stream migration (Hughes & Richard 1973). Therefore, juveniles can accumulate at below-dam reservoirs, increasing predation pressures (March 2003). *M. carcinus* distributional ranges within the US have been reduced with local extirpations due to large bottom release dams (Horne & Beisser 1977, Bowles et al. 2000). *M. ohione* had a historical distribution across Arkansas, Indiana, Illinois, Oklahoma, Ohio, Missouri, Kentucky, and Virginia (Bowles et al. 2000). Distributional ranges of *M. ohione* have depleted within Arkansas to a point that a "threatened" status has been recommended (Robinson & McAllister 2011); this species has been listed as critically imperiled in Missouri, Kentucky, and Virginia (NatureServe 2014).

Another factor attributed to the decline of *Macrobrachium* species is urban development. Development of land adjacent to freshwater habitats increases agricultural and urban runoff and reduces riparian zones (Allan 2004). Urban runoff such as sewage, oil, and pesticides can have a major impact on *Macrobrachium* species. *M. jelskii* in urban settings have been shown to exhibit oxidative stress, monitored through enzymatic response, while those in rural streams maintained normal activity (Mota et al. 2021). In addition, metals from runoff have been shown to significantly reduce immune function in *M. rosenbergii* (Kaoud & Ahmed 2013). Additionally, the reduction of riparian zones reduces the amount of woody refuge that species has access to, which is important for their diurnal behavior (Lammers et al. 2009). Besides the reduction in available refugia, the reduction in the shade associated with the reduction in riparian vegetation can lead to increased water temperature, causing the thermal stress and contributing to oxidative stress (Allan 2004, Manush et al. 2004).

Dispersal of these species depends on the habitat in which they occupy. Dispersal of adult *Macrobrachium* is limited to periods of connectivity associated with river flooding from climatic events or stream capture (Carini & Hughes 2004, Hurwood et al. 2014). Amphidromous species of *Macrobrachium* may also disperse within and between bay systems on oceanic currents during their salinity tolerant zoeal stages (Sharma & Hughes 2009, Rossi & Mantelatto 2013). Both the number of zoeal stages and the species-specific tolerance for salt water can determine how far species disperse through the estuary/marine environment to disjunct freshwater ecosystems and saline-tolerant species tend to be more widespread (Rossi & Manatello 2013, Dugger & Dobkin 1975).

Classification of North American species

Macrobrachium is understudied, with most studies focused on the aquaculture of only a few species of economic importance (Chong-Carrillo et al. 2015). Out of the phylogenetic studies, most focus is on South and East Asian species of *Macrobrachium* since it is the center of diversity for the genus, but *Macrobrachium* species are globally distributed, with several species also occurring in North America (Anger 2013, Chong-Carrillo et al. 2015, Bowles et al. 2000). Only six described species (four native and two potentially introduced to Florida) are present in the contiguous United States, most occurring in the southern coastal plains (Bowles et al. 2000). Out of the six described species, four occur in Texas (Horne & Beisser 1977).

All the species that occur in Texas, except *M. ohione*, have a geminate sister species in the eastern Pacific (Pileggi et al. 2014). The timing of separation between *M. tellenum* and *M. acanthurus* is estimated to have occurred around the closure of the Isthmus of Panama, while divergence between *M. carcinus* and *M. americanum* is estimated to have occurred after the formation of the Isthmus of Panama was complete (Acuna Gomez et al. 2013). Consistent with this hypothesized recent divergence, the morphology is so similar between *M. carcinus* and *M. americanum* that the primary diagnostic difference is specimen locale (Acuna Gomez et al. 2013, Anger 2013). *M. olfersii* forms a species complex with many other species but has putative geminate relationship with *M. hobbsi* (Anger 2013). The genetic variation between the *M. olfersii* and *M. hobbsi* in 16S mitochondrial DNA is only one percent (Acuna Gomez et al. 2013). Yet, the species exhibit distinct morphological characters limiting the synonymization of the species (Acuna Gomez et al. 2013). Furthermore, the species *M. olfersii* and *M. hobbsi* occur on both sides of the Isthmus of Panama and found within the Panama Canal, indicating that

human activities could increase the potential for other widespread species to disperse to previously unreported areas (Anger 2013, Abele & Kim 1989).

Phylogenetic Hypotheses

Cryptic diversity is continually being discovered in *Macrobrachium,* adding new species to the already diverse genus (De Grave & Fransen 2011). High morphological conservation seen in the genus makes species delineation difficult and attempts to describe diversity have become reliant on molecular approaches (Rossi et al. 2020). Various phylogenies show poor resolution of relationships, poor bootstrap support, and persistent polytomies, proposed to result from marker diversity and/or rapid radiation of the species (Pileggi & Mantelatto 2010, Chen et al. 2009, Lui et al. 2007, Murphy & Austin 2005). The monophyly of the genus has also been called into question, with a closely related genus, *Cryphiops*, being included within *Macrobrachium* in some studies (Mantelatto et al. 2021, Pileggi & Mantelatto 2010). Many taxonomic errors have been made because morphologically cryptic species may be sympatrically distributed (De Bruyn 2004) and single species have been described as multiple species across large geographic ranges on the basis of morphological variation (Castelin 2017). Therefore, genetic assessments of species across their geographic ranges are needed to thoroughly resolve taxonomic errors and to address conservation concerns.

Single copy mitochondrial DNA (mtDNA) is frequently used to generate phylogenies (Patwardhan et al. 2014). The ribosomal 16S DNA marker contains enough standing genetic variation to assist in resolving relationships of distantly related taxa while the cytochrome oxidase subunit I (COI) marker has been utilized as a 'barcoding' gene for distinguishing species from each other (Patwardhan et al. 2014). COI has been used in population assessment of geographically distant populations of *Macrobrachium* (Carini & Hughes 2004, Hurwood 2014).

Given that 16S is encoded in eukaryote mitochondrial DNA, 16S and COI are physically linked, and concatenation of the genes may provide better resolution needed to produce robust species trees (Patwardhan et al. 2014).

The objective of this study was to develop robust phylogenetic hypotheses for *M. carcinus* throughout the species known distribution and other North American species of *Macrobrachium* through independent and concatenated mtDNA genes. These phylogenetic hypotheses will address the known number of species occurring in Texas and the possibility of cryptic species, or spread of others throughout the Gulf of Mexico, as well as address the genetic distinction between *M. carcinus* in Texas and the Caribbean. Also, this study was to make a preliminary assessment of *M. ohione* population structure among individuals occurring in different bay systems utilizing COI and supply this information for the management of the species throughout the Texas coast.

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CHAPTER II: MOLECULAR ANALYSIS OF *MACROBRACHIUM CARCINUS* THROUGHOUT THE KNOWN DISTRIBUTION UNVEILS TWO CURRENTLY UNDESCRIBED SPECIES

Introduction

The genus *Macrobrachium* is diverse with >240 extant species, and cryptic species continually being discovered (De Grave & Fransen 2011). Morphological conservation seen in the genus makes species delineation difficult and attempts to describe diversity have become reliant on molecular approaches (Rossi et al. 2020). Various phylogenies show poor resolution of relationships, poor bootstrap support, and persistent polytomies, which could be attributed to the resolution of molecular markers applied, rapid radiation of species within the genus, potential misidentification and/or problems with alpha taxonomy (Pileggi & Mantelatto 2010, Murphy & Austin 2005, Chen et al. 2009). The monophyly of the genus has also been called into question, with a closely related genus, *Cryphiops*, likely included within *Macrobrachium* (Mantelatto et al. 2021, Pileggi & Mantelatto 2010).

Phylogenetic assessment of *Macrobrachium* is difficult due to over- and under-differentiation. Species within *Macrobrachium* exhibit morphological conservation with only subtle differences seen between species, making field identification difficult (Rossi et al. 2020). Species ranges are greatly influenced by dispersal potential, with more saline tolerant species found over wide geographic ranges, while other species that are more constrained to fresh or brackish water occupy a small number of bay or river systems (Rossi & Mantelatto 2013, Sharma & Hughes 2009). For some species with large geographic ranges there is genetic structure present that makes species delimitation difficult because differences can be interpreted as either intraspecific (i.e., strong population structure) or interspecific (i.e., recent speciation;

Vergamini et al. 2011, Weiss et al. 2015). In other cases, phenotypic variation can be observed between individuals of the same species, but little to no genetic variation is observed (García-Velazco et al. 2021). For example, some species occurring on both sides of the Isthmus of Panama exhibit morphological differences yet are genetically similar and have been placed into species complexes (Acuna Gomez et al. 2013, Anger 2013).

Despite the presence of six species in the United States (four native and two potentially introduced to Florida), most phylogenetic studies on *Macrobrachium* occurs in the Indo-Pacific due to being the center of highest diversity (Chong-Carrillo et al. 2015, Anger 2013, Bowles 2000). Most of the species occurring in the US are found in the southern coastal plains apart from *M. ohione* which has a historical range extending up the Mississippi River into other tributaries within Arkansas, Indiana, Illinois, Oklahoma, and Ohio (Bowles et al. 2000). All the native species in the US occur in Texas coastal streams, some of which supported commercial and recreational fisheries in the past (Horne & Beisser 1977, Hedgepeth 1949, Gunter 1937).

Some phylogenetic research has focused on the separation of the transisthmian geminate species pair *M. carcinus* and *M. americanum*. These species are thought to have diverged from a common ancestor around 1.9 - 2 million years ago (Pileggi & Mantelatto 2010, Pileggi et al. 2014, Acuna Gomez et al. 2013). *M. carcinus* has a wide geographic range from the Atlantic Coast of Florida, throughout the Gulf of Mexico, through Central America to the Caribbean islands, and as far south as the southern Brazil (Bowles et al. 2000). Despite this broad range, dispersal potential for *M. carcinus* is lower than some other *Macrobrachium* species such as *M. olfersii* and *M. acanthurus* (Dugger & Dobkin 1975, Choudhury 1971). Larval development of *M. carcinus* occurs in upper estuaries, optimally at 12 ppt which could limit the dispersal to within bay or estuarine systems, though individuals of M. carcinus in Brazil have optimal salinity

range of 16-28 (Lara & Wehrtmann 2009, Dugan et al. 1975, Sharma & Hughes 2009, Lima et al. 2021). Limited dispersal, along with distance and barriers such the Gulf Stream current could limit the dispersal potential between the Gulf of Mexico, the Caribbean, and South American populations of *M. carcinus* (Dugan et al. 1975, Oliveira et al. 2019). Other amphidromous shrimp species, such as *Atya scabra*; have shown discrete genetic groupings between the western Gulf of Mexico, Caribbean Sea, western South Atlantic, and eastern Atlantic (Oliveira et al. 2019), but no previous work has included *M. carcinus* from Texas or the Caribbean to assess the genetic diversity throughout the species wide range.

M. carcinus was originally listed as a species of concern and high priority for conservation measures in 2005 but was not included in the species of greatest conservation need or the updated conservation action plan (TPWD 2005, TPWD 2020). Distributional ranges and populations for *M. carcinus* have contracted due to instream barriers such as dams and the past fishery (Horne & Beisser 1977, Hedgepeth 1949, Bowles et al. 2000). Numbers have decreased enough that sightings of *M. carcinus* in Texas are rare, with populations in the upper reaches of San Marcos being virtually non-existent and only 5-12 individuals were observed through snorkeling on count nights within a three-to-four-month sampling time frame for a study in San Marcos River in the lower reaches (Scott et al. 2012). Besides the San Marcos River, only two other rivers (Rio Grande River and Sabine River) are known to have populations of M. carcinus in Texas, though turbidity in many Texas riverine systems may reduce potential detection of the species. Low abundances of the species could contribute to limited detection of individuals, and individuals within the species tend to be more cryptic, relying on boulder substrate for prey availability and predator refuge (Snyder et al. 2016).

With distributional ranges contracting and populations being extirpated due to human-induced pressures on the species, more data on the species throughout its distribution is needed to assess conservation concerns as genetic rescue may not be suitable along the vast distribution. Currently, the species is listed as Least Concern by the International Union for Conservation of Nature (IUCN) due in part to the large geographic distribution and high local abundances. The objective of this study was to develop a robust phylogenetic hypothesis for *M. carcinus* throughout the species known distribution, including other North American species of *Macrobrachium* as outgroups using mitochondrial genes. These phylogenetic hypotheses will address the known number of species occurring in Texas and the possibility of cryptic species, or potential spread of other introduced species (*M. faustinum* and *M. heterochirus*) from Florida, as well as address the genetic distinction between *M. carcinus* in Texas, the Caribbean, and Central and South America due to larval salinity tolerance, distance, and Gulf Stream Current factors potentially limiting dispersal of the species allowing for cryptic speciation along the distributional ranges. Genetic distinction throughout the known range could warrant greater conservation protections than what is currently awarded due to distributional ranges and not being as vast as what was previously thought.

Methods

DNA was extracted from tissues of *Macrobrachium* species previously collected by colleagues throughout their distribution in Texas and Puerto Rico and identified using morphological characteristics following several available keys (Table 2.1; Bowles 2001, Holthuis 1952, Thorpe & Rogers 2016). DNA was extracted using muscle tissue from the abdomen or legs with E.Z.N.A® Tissue DNA Kits (Omega Bio-Tek) following the manufacturer's protocols. A portion of the mitochondrial 16s RNA (16S) and cytochrome

oxidase I (COI) genes were amplified for each individual via PCR using primers 16sar-L (CGC CTG TTT ATC AAA AAC AT) and 16sbr-H (CCG GTC TGA ACT CAG ATC ACG T) as well as CO1a-H (AGT ATA AGC GTC TGG GTA GTC) and CO1-Lf (CCT GCA GGA GGA GGA GAC CC) respectively (Palumbi & Benzie 1991, Palumbi et al. 1991). Each PCR reaction was 25 µL with 1X buffer, 3 mM $MgCl_2$, 0.4 mM dNTPs each, 0.4 µM forward and reverse primer each, 0.05 units/µL Taq Polymerase, 1-3 µL DNA template, and ddH2O to reach a total 25 µl volume. Amplification of COI involved initial denaturation for 5 min at 95℃; followed by 40 cycles of 45 sec at 95℃ (denaturation), 45 sec at 48-55℃ (annealing), and 1 min at 72℃ (extension); with a final extension of 3 min at 72℃. Amplification of 16S involved an initial denaturation for 5 min at 95℃; followed by 45 cycles of 45 sec at 95℃ (denaturation), 45 sec at 49-50℃ (annealing), and 1 min at 72℃ (extension); with a final extension of 3 min at 72℃. PCR products were assessed visually after electrophoresis through 1% agarose gel. AMPure XP magnetic beads at a 1X ratio were used to purify PCR products prior to sequencing in an ABI 3730xl DNA sequencer in the Genomics Core Lab at Texas A&M University-Corpus Christi or at Retrogen, Inc, San Diego, CA. Sequences were manually edited and aligned in Sequencher v. 5.4.6 (GeneCodes Corporation). Additional 16S and COI sequences for *M. carcinus* and the Pacific geminate sister, *M. americanum*, were obtained from GenBank and added to the analysis.

Sequence alignments were loaded into jModelTest v.2.1.4 (Darriba et al. 2012) to analyze the best model of nucleotide evolution using of AIC, BIC, and DT (Table 2.2). Bayesian analyses were conducted in BEAST v.1.10.4 (Suchard et al. 2018) under a strict molecular clock for each gene separately and a concatenated data set. Independent runs consisted of a burn-in of 1 million steps followed by 10 million steps with a sampling interval set at 1,000 trees. Ten replicate runs were completed for each data set, then replicate runs were combined through

LogCombiner (BEAST suite). Combined log files were assessed in Tracer v.1.7.2 (Rambaut et al. 2018) to determine the effective sample size (ESS) with a cutoff value of 200 and to inspect run plots to see if the chain length, sampling interval, and burn-in was sufficient. TreeAnnotator (BEAST suite) was then used to create the maximum clade credibility tree and estimate posterior probabilities.

Maximum likelihood analysis was conducted in MEGA-X v 10.2.4 (Kumar et al. 2018) with 1,000 bootstrap replicates. Starting trees were determined through the default Neighbor-Joining/BioNJ with weak branch swap filter option and a fast subtree-pruning and regrafting Maximum Likelihood heuristic method.

Genetic pairwise distances were calculated using net group p-distance in MEGA-X v 10.2.4 (Kumar et al. 2018) using 1,000 bootstrap replicates. Groups were initially separated by species then separated by geographic locations (Texas, Puerto Rico and central/South America) for *M. carcinus*.

Results

Final alignment length for 16S was 324 bps with 198 variable sites, 142 parsimony informative sites, and an average nucleotide composition of T=37.3%, C=11.1%, A=29.3%, G=22.2%. The final alignment length for COI was 358 bps with 157 variable sites, 137 parsimony informative sites, and an average nucleotide composition of T=28.6%, C=21.7%, A=30.6%, G=19.1%. For the concatenated data set, the final alignment was 682 bps in length with 352 variable sites, 275 parsimony sites and an average nucleotide composition of T=32.8%, C=16.7%, A=30.0%, G=20.5%. Models of nucleotide evolution estimates selected using jModelTest v. 2.1.4 were TrN+I+G for 16S and HKY+G for COI (Table 2.2).

Five major clades were resolved in all Bayesian analyses. Three clades included individuals identified as *M. carcinus* (*M. carcinus*_Texas, *M. carcinus*_Central/South America and *M. carcinus*_Puerto Rico), one clade included individuals identified as *M. americanum,* and the final clade included individuals identified as *M. ohione*. In all Bayesian trees the three *M. carcinus* clades and the *M. americanum* clade formed a well-supported group, but *M. americanum* and *M. carcinus*_Puerto Rico formed a group that was sister to a group that contained *M. carcinus*_Texas and *M. carcinus*_Central/South America (Figure 2.1-3). For 16S, *M. acanthurus* (a single individual) was sister to *M. americanum*+*M. carcinus,* with *M. ohione* and *M. olfersii* occupying the same positions in all Bayesian trees. The 16S ML tree (Figure 2.4) had the same topology as the Bayesian trees but the ML trees based on COI and the concatenated data set split *M. americanum* into two groups, making *M. americanum* paraphyletic without the inclusion of *M. carcinus* and *M. carcinus*_Texas and *M. carcinus*_Central/South America were part of a grade that included *M. americanum*+*M. carcinus*_Puerto Rico (Figure 2.5 and 2.6). In addition, *M. ohione* was the outermost clade in the COI ML tree (Figure 2.5), while in all other trees *M. olfersii* was the outermost clade.

Support for clades varied between methods as well as between genes, with the highest support occurring in the Bayesian trees, except for the 16S ML tree that had good support for all nodes, but the *M. americanum* internal node. High posterior support was seen for the clade containing *M. carcinus*_Puerto Rico and *M. americanum*, while moderate support to low support occurred for the clade containing the *M. carcinus*_Texas and *M. carcinus*_Central/South America clade. The COI BEAST tree had lower support values than the 16S tree. The concatenated BEAST tree had high support values at all nodes, except a low value for the *M. carcinus*_Texas and *M. carcinus*_Central/South America clade. The ML trees had more support

in the COI topology with the *M. carcinus*_Texas clade being closer to the *M. carcinus*_Puerto Rico and *M. americanum*. In the concatenated ML tree, the *M. carcinus*_Central/South America support was <50%, but all other nodes were moderate to strongly supported.

Net pairwise distance for 16S ranged from 0.31% between *M. carcinus*_Puerto Rico and *M. americanum* to 33.49% between *M. olfersii* and *M. ohione;* with the distances between *M. carcinus* clades being *M. carcinus*_Central/South to *M. carcinus*_Texas 2.78%, *M. carcinus*_Central/South to *M. carcinus*_Puerto Rico 2.96%, and *M. carcinus*_Texas to *M. carcinus*_Puerto Rico 2.65% (Table 2.3). COI distances ranged from 8.69% between *M. carcinus*_Puerto Rico and *M. americanum* to 25.42% between *M. ohione* and *M. olfersii;* with the distances between *M. carcinus* clades being *M. carcinus*_Central/South to *M. carcinus*_Texas 10.13%, *M. carcinus*_Central/South to *M. carcinus*_Puerto Rico 14.46%, and *M. carcinus*_Texas to *M. carcinus*_Puerto Rico 16.54% (Table 2.4). Concatenated distances ranged from 4.9% between *M. carcinus* Puerto Rico and *M. americanum* to 29.26% between *M. olfersii* and *M. ohione*; with the distances between *M. carcinus* clades being *M. carcinus*_Central/South to *M. carcinus*_Texas 6.58%, *M. carcinus*_Central/South to *M. carcinus*_Puerto Rico 8.90%, and *M. carcinus*_Texas to *M. carcinus*_Puerto Rico 9.79% (Table 2.5).

Discussion

Morphological identifications of specimens included in analyses indicated four to five species, but phylogenetic hypotheses generated indicated that six to seven distinct genetic groups were present in the data. Individuals identified as *M. carcinus* collected in different localities (Texas, Puerto Rico, and Central/South America) formed clades, most with moderate to strong support, especially in the concatenated data set. Four out of the six phylogenetic hypotheses formed reciprocal monophyletic clades of *M. carcinus*_PR and *M. americanum*. For two trees,

maximum likelihood COI and MEGA concatenated, *M. americanum* was paraphyletic, with *M. carcinus*_PR embedded within *M. americanum*.

Net p-distances show genetic isolation between *M. carcinus* collected at different geographic locations. The net p-distance between *M. carcinus*_Puerto Rico and *M. americanum* was lower than the distances observed between other clades, especially for 16S where only 0.3% variation was observed between *M. carcinus*_Puerto Rico and *M. americanum*. Distances for 16S were larger between the *M. carcinus* clades, with 2.4-2.9% divergence observed, just below the 3% divergence in 16S that is potentially indicative of speciation (Lui et al. 2007). Net p-distances for COI were greater between all clades ranging from 6.8% between *M. carcinus*_Central/South America and *M. americanum* and the *M. carcinus* clades being 10.13% to 16.54% diverged, falling into the interspecific range recognized in other crustaceans, specifically brachyuran crabs (Servis et al. 2020). The greater support in the BEAST hypotheses and COI genetic divergence would seem to indicate that *M. carcinus*_PR and *M. americanum* are separate species. However, more studies may be needed to understand the implication of the 16S divergence estimate between *M. carcinus*_PR and *M. americanum*, which was much lower than what would be expected for species-level differences.

If *M. carcinus* and *M. americanum* are considered geminate species, the greater divergence between the other clades of *M. carcinus* and their relationship in the trees indicates the presence of two currently unrecognized species that diverged earlier than *M. carcinus* and *M. americanum*. When species groups have high intraspecific variation exceeding minimum genetic distances to sister taxa and near circumglobal distribution, there is potential for cryptic species presence (Goetze 2010). Taxonomic revision is beyond the scope of this study but recommended for what is currently recognized as *M. carcinus*.

The variation seen within both genes supports that these genetic clades of *M. carcinus* are separate species which warrants future work to assess the status of *M. carcinus* throughout the known ranges. The original descriptions of *M. carcinus* occurred using a sample believed to be collected in Jamaica in 1725, indicating that clade of *M. carcinus* collected in Puerto Rico would be the originally described species (Holthuis 1952). Therefore, the other clades of *M. carcinus* occurring in Texas and Central/South America need to be reassessed, holotypes should be collected and preserved, and previous synonyms should be resurrected, or new names created.

Current IUCN status for *M. carcinus* will also need to be reassessed as the distribution of the species is not as vast as was previously thought. Large geographic ranges with local abundances were used to determine that *M. carcinus* should not be listed as threatened or endangered (De Grave 2015). Given that *M. carcinus* in Texas is putatively a different species than the other *M. carcinus* clades, and the fact that the abundance and distribution of *M. carcinus* in Texas have decreased is concerning (Scott et al. 2012). Therefore, it is critical to revisit the taxonomic and conservation statuses of clades *M. carcinus* to ensure proper protections are put in place within their native ranges. In Texas, consideration should be given to ranking *M. carcinus* using the SGCN process. This will provide greater protections for this species and open up sources of funding for more conservation work.

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Appendix

Table 2.1. List of tissues used with species name, id used in phylogenetic hypotheses, sample identification used, location where the specimen was taken, and GenBank accession voucher.

Species	ID	Sample_ID	Location	GenBank: 16S	Genbank: COI
Macrobrachium acanthurus	*M. acan*	MaAc_AR_67	Aransas River, TX		
Macrobrachium carcinus	*M. car* 1 (PR)	MaCa_1_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 2 (PR)	MaCa_2_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 6 (PR)	MaCa_38_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 7 (PR)	MaCa_42_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 8 (PR)	MaCa_8_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 9 (PR)	MaCa_9_PR	Puerto Rico		
Macrobrachium carcinus	*M. car* 1 (TX)	MaCa_SM1_TX	San Marcos River, TX		
Macrobrachium carcinus	*M. car 5 (TX)*	MaCa_SM5_TX	San Marcos River, TX		
Macrobrachium carcinus	*M. car 6 (TX)*	MaCa_SM6_TX	San Marcos River, TX		
Macrobrachium carcinus	*M. car 7 (TX)*	MaCa_SM7_TX	San Marcos River, TX		
Macrobrachium carcinus	*M. car 8 (TX)*	MaCa_SM8_TX	San Marcos River, TX		
Macrobrachium carcinus	*M. car 9 (TX)*	MaCa_SM9_TX	San Marcos River, TX		
Macrobrachium ohione	*M. ohi 1(TX)*	MaOh_BR_138.2	Brazos River, TX		
Macrobrachium ohione	*M. ohi 2(TX)*	MaOh_BR_179a.6	Brazos River, TX		
Macrobrachium ohione	*M. ohi 3(TX)*	MaOh_BR_179b.1	Brazos River, TX		
Macrobrachium ohione	*M. ohi 4*	MaOh_WC 85.10	Wilson Creek, TX		
Macrobrachium ohione	*M. ohi 5*	MaOh_TR238.2	Trinity River, TX		
Macrobrachium ohione	*M. ohi 6*	MaOh_OC 180.2	Oyster Creek, TX		
Macrobrachium ohione	*M. ohi 7(TX)*	MaOh_OC_180.14	Oyster Creek, TX		

Macrobrachium ohione	*M. ohi 8(TX)*	MaOh_PL_91.4	Placedo River, TX		
Macrobrachium olfersii	*M. olf 1*	MaOl_MRO3	Mission River, TX		
Macrobrachium olfersii	*M. olf 2*	MaOl_Osc6	Mission River, TX		
Penaeus monodon	*P. monodon*	PM.10952.1	FL		
Macrobrachium carcinus	*M. car* 1 (BR)*		Brazil	HM352448	HM352490
Macrobrachium carcinus	*M. car 2* (BR)*		Brazil	HM352449	HM352491
Macrobrachium carcinus	*M. car (VEN)**		Venezuela	HM352450	HM352492
Macrobrachium carcinus	*M. car 2 (CR)**		Costa Rica	HM352449	KM101547
Macrobrachium americanum	*M. ame* 1 (CR)*		Costa Rica	HM352447	HM352489
Macrobrachium americanum	*M. ame 2* (CR)*		Costa Rica	KM101473	KM101547
Macrobrachium americanum	*M. ame 3* (CR)*		Costa Rica	KM101470	KM101544
Macrobrachium americanum	*M. ame 4* (CR)*		Costa Rica	KM101471	KM101545
Macrobrachium americanum	*M. ame 5* (CR)*		Costa Rica	KM101472	KM101546
Macrobrachium americanum	*M. ame* (PAN)*		Panama	KM101469	KM101543

Table 2.2. Final alignment information for 16S, COI, and concatenated dataset with jModeltest nucleotide evolution model outputs between different criteria: AIC, BIC, DT for 16S.

	Final Alignment			jModeltest		
	Length (bps)	Variable sites	Parismony informative	AIC	BIC	DT
16S	324	198	142	TrN+I+G	TrN+I+G	TrN+I+G
COI	358	157	137	HKY+I+G	HKY+G	HKY+G
Concatenated	682	352	275			

Table 2.3. 16S net p-distance calculated in MEGA-X. Abbreviations are TX- Texas, PR- Puerto Rico, C/S America- Central and South America. The number of base differences per site from estimation of net average between groups of sequences are shown. The rate variation among sites was modeled with a gamma distribution (shape parameter = 4). This analysis involved 30 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 324 positions in the final dataset.

	M. carcinus (C/S America)	*M. americanum*	*M. olfersii*	*M. ohione*	*M. carcinus* (TX)
M. carcinus (C/S America)					
M. americanum	0.0278				
M. olfersii	0.3233	0.3194			
M. ohione	0.2315	0.2150	0.3349		
M. carcinus (TX)	0.0278	0.0247	0.3225	0.2191	
M. carcinus (PR)	0.0296	0.0031	0.3145	0.2191	0.0265

Table 2.4. COI net p-distance calculated in MEGA-X. Abbreviations are TX- Texas, PR- Puerto Rico, C/S America- Central and South America. The number of base differences per site from estimation of net average between groups of sequences are shown. The rate variation among sites was modeled with a gamma distribution (shape parameter = 4). This analysis involved 30 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 358 positions in the final dataset.

	M. ohione	*M. carcinus* (PR)	*M. carcinus* (TX)	*M. olfersii*	*M. carcinus* (C/S America)
M. ohione					
M. carcinus (PR)	0.2351				
M. carcinus (TX)	0.1770	0.1654			
M. olfersii	0.2542	0.2443	0.2387		
M. carcinus (C/S America)	0.1695	0.1446	0.1013	0.2477	
M. americanum	0.1763	0.0869	0.0809	0.2359	0.0681

Table 2.5. Concatenate data set net p-distance calculated in MEGA-X. Abbreviations are TX-Texas, PR- Puerto Rico, C/S America- Central and South America. The number of base differences per site from estimation of net average between groups of sequences are shown. The rate variation among sites was modeled with a gamma distribution (shape parameter = 4). This analysis involved 31 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 682 positions in the final dataset.

	M. ohione	*M. carcinus* (PR)	*M. carcinus* (TX)	*M. olfersii*	*M. carcinus* (C/S America)
M. ohione					
M. carcinus (PR)	0.2276				
M. carcinus (TX)	0.1974	0.0979			
M. olfersii	0.2926	0.2787	0.2794		
M. carcinus (C/S America)	0.1981	0.0890	0.0658	0.2827	
M. americanum	0.1947	0.0464	0.0537	0.2756	0.0487

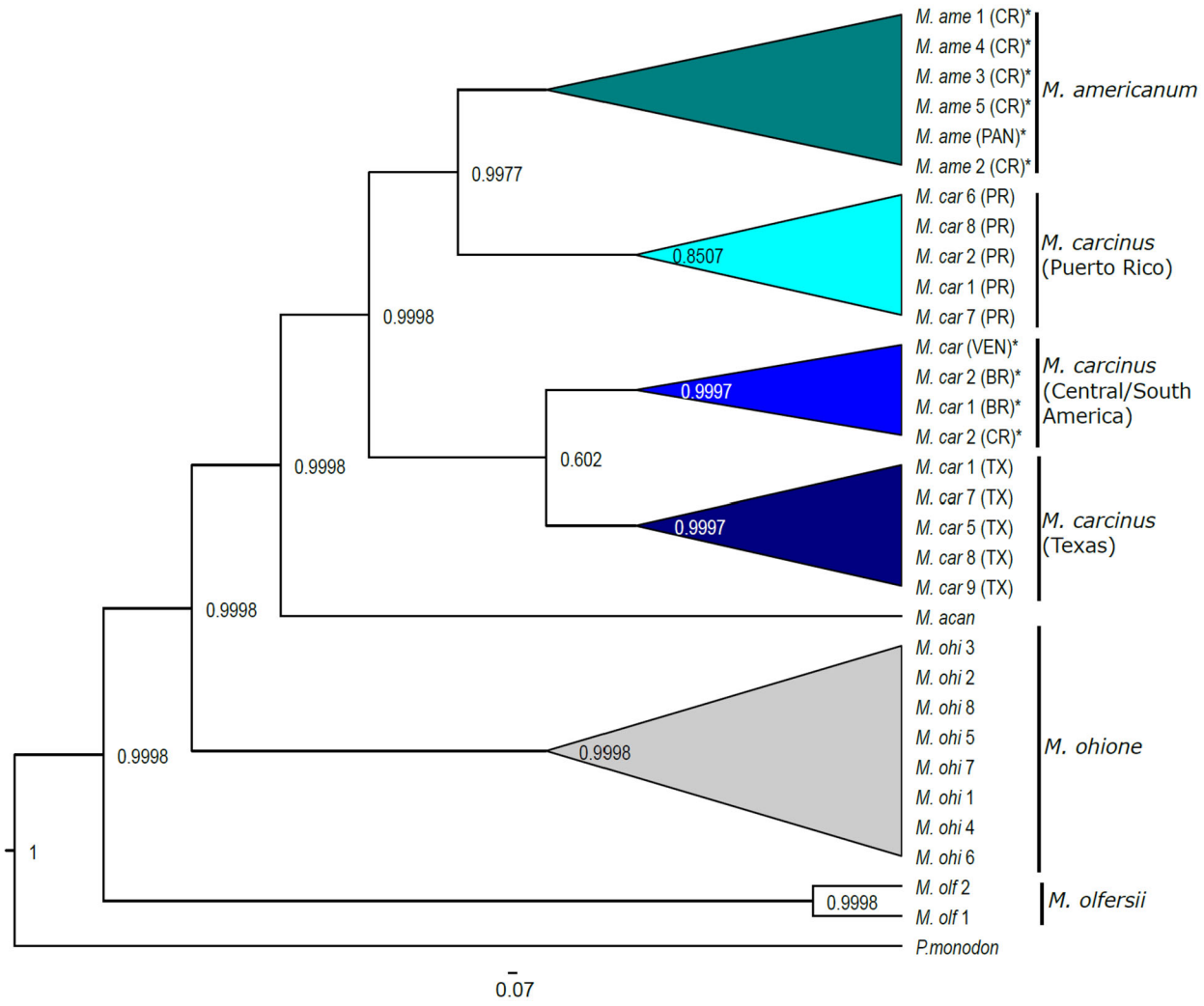

Figure 2.1. 16S BEAST phylogenetic hypothesis for *Macrobrachium* with posterior support values at the nodes. Nodes values <50% are removed. Scaled at 0.07 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum*, *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with *M. acanthurus* (*M. acan*) 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

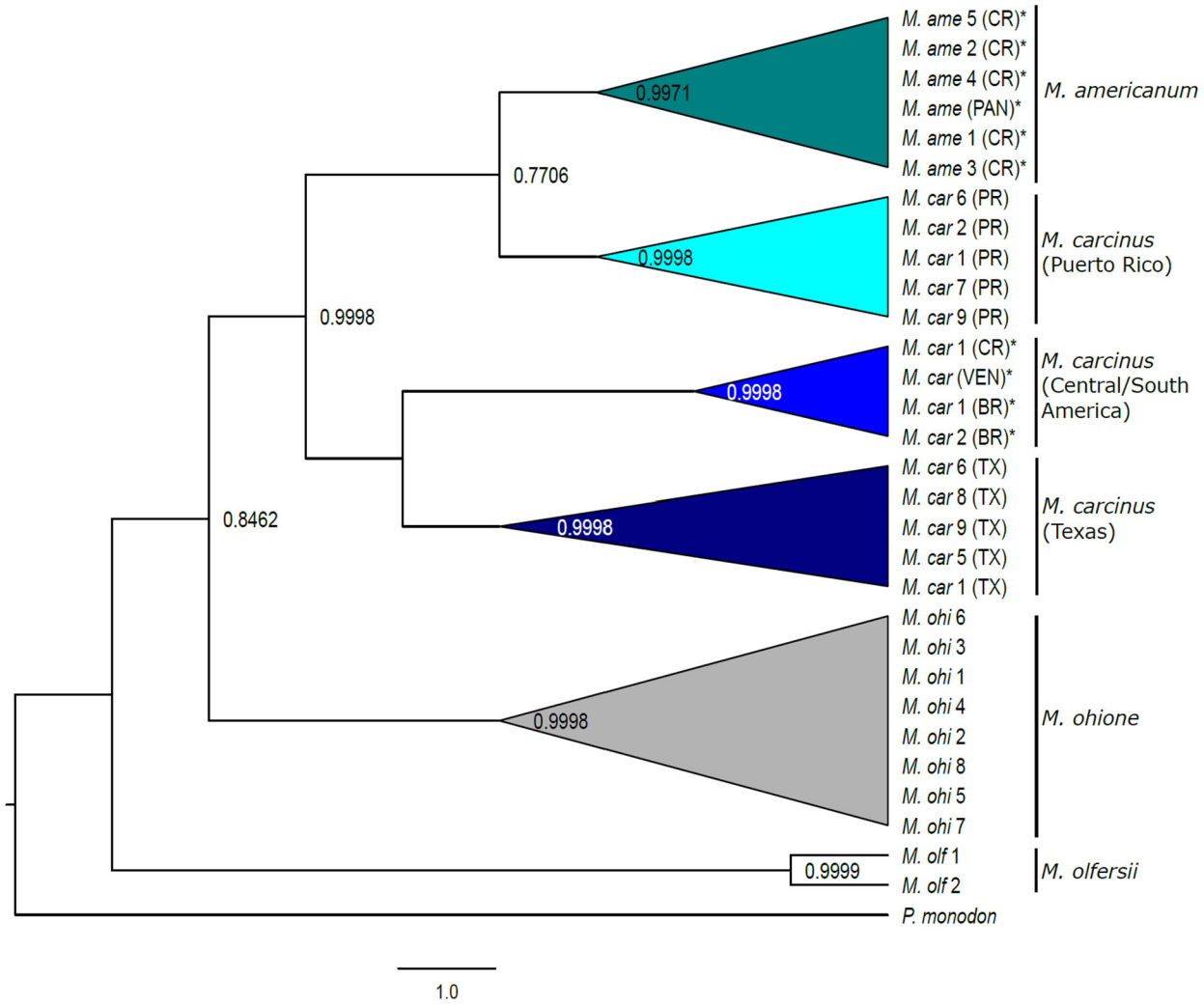

Figure 2.2. COI BEAST phylogenetic hypothesis for *Macrobrachium* with posterior support values at the nodes. Nodes values <50% are removed. Scaled at 1.0 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum*, *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

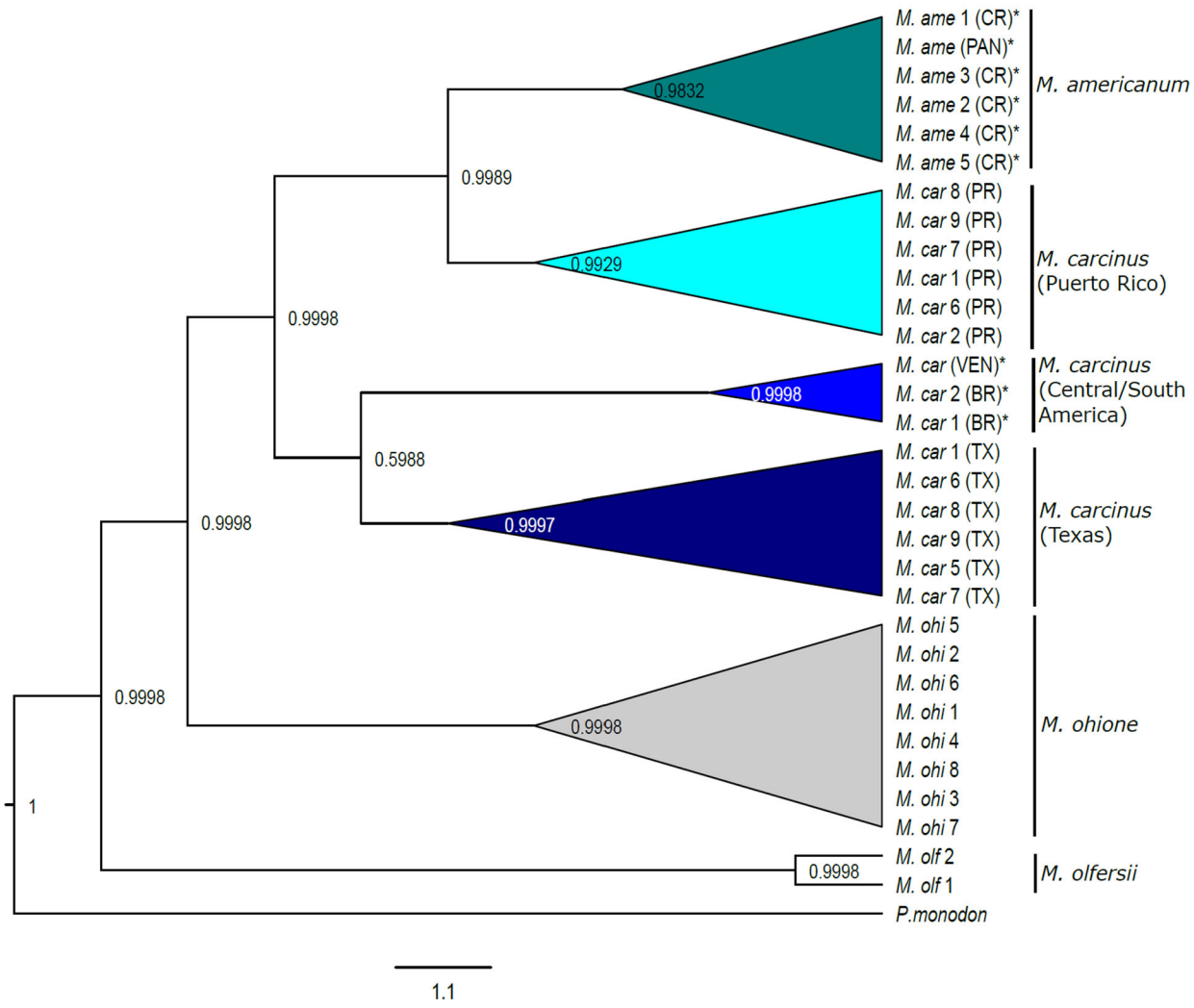

Figure 2.3. Concatenated data set BEAST phylogenetic hypothesis for *Macrobrachium* with posterior support values at the nodes. Scaled at 1.1 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum*, *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

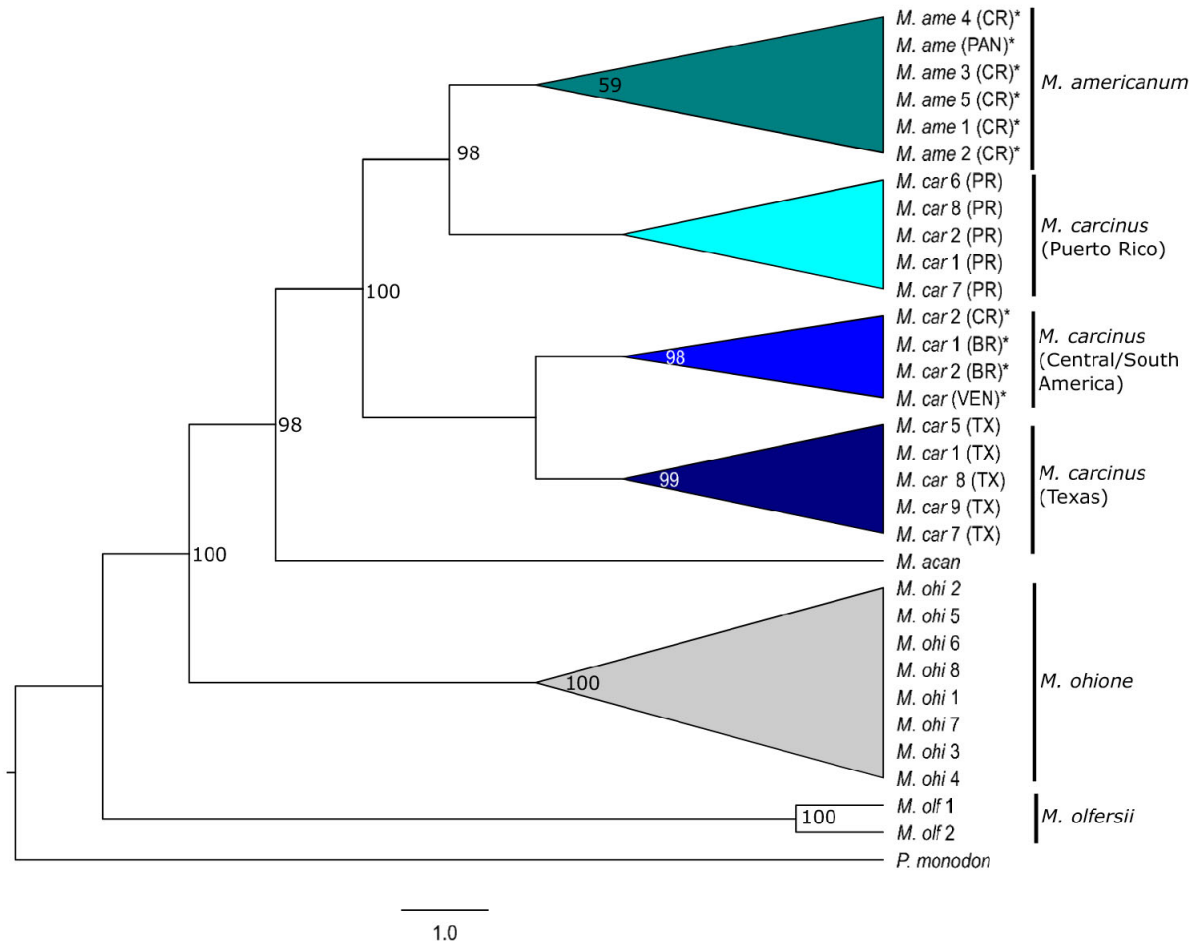

Figure 2.4. 16S MEGA phylogenetic hypothesis for *Macrobrachium* with bootstrap support values at the nodes. Nodes values <50 are removed. Scaled at 1.0 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum*, *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with *M. acanthurus* (*M. acan*) 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

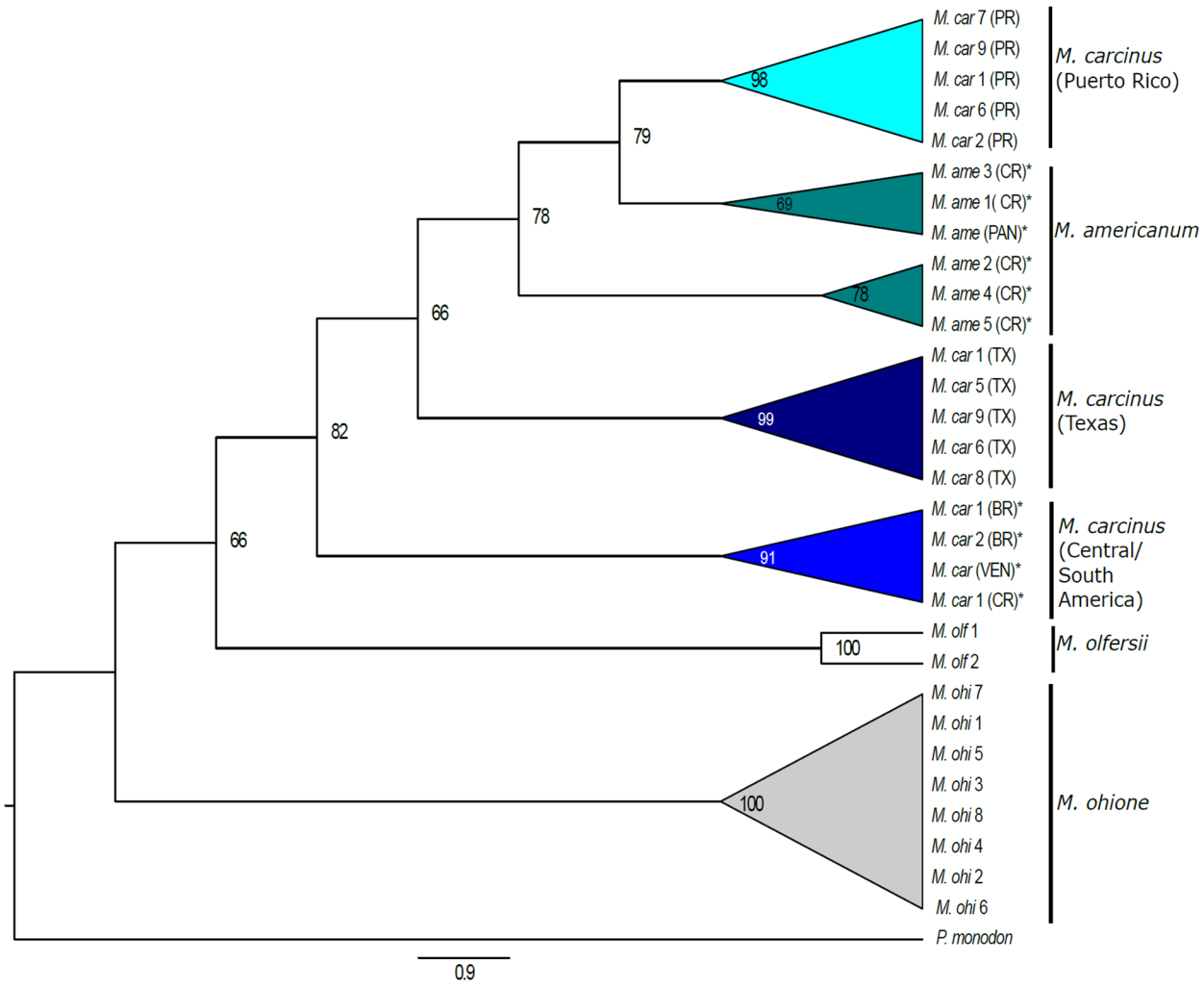

Figure 2.5. COI MEGA phylogenetic hypothesis for *Macrobrachium* with bootstrap support values at the nodes. Nodes values <50 are removed. Scaled at 0.9 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum* which is paraphyletic with one clade sister to *M. carcinus* (Puerto Rico) and the other sister to *M. americanum*+*M.carcinus* (Puerto Rico), *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

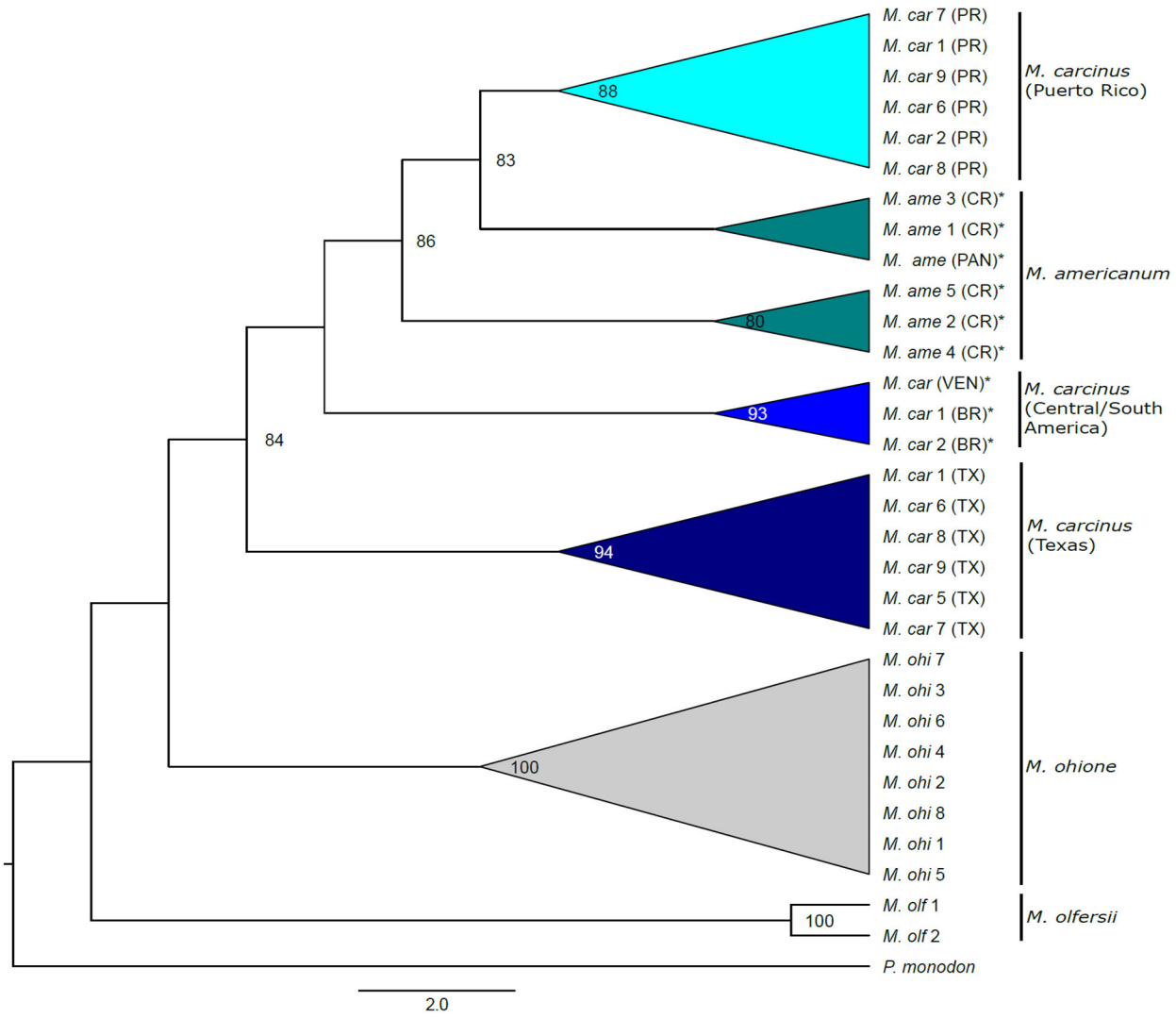

Figure 2.6. Concatenated data set MEGA phylogenetic hypothesis for *Macrobrachium* with bootstrap support values at the nodes. Nodes values <50 are removed. Scaled at 2.0 mutations per site with branches transformed into a cladogram layout for easier interpretation. Major clades represented are *M. americanum* which is paraphyletic with one clade sister to *M. carcinus* (Puerto Rico) and the other sister to *M. americanum*+*M.carcinus* (Puerto Rico), *M. carcinus* (Puerto Rico), *M. carcinus* (Central/South America), *M. carcinus* (Texas), and *M. ohione*, along with 2 individuals of *M. olfersii* and *Penaeus monodon* as an outgroup. Asterisks (*) represent sequences obtained from GenBank.

CHAPTER III: PRELIMINARY GENETIC ASSESSMENT OF *MACROBRACHIUM OHIONE* REVEALS LOW COI DIVERSITY AND EVIDENCE OF RECENT POPULATION EXPANSION IN TEXAS COASTAL STREAMS

Introduction

Understanding connectivity is important for the effective conservation and management of aquatic species, particularly those with complex life-histories (Jones et al. 2007). Greater connectivity can rescue populations from extirpation by maintaining or stabilizing population sizes, maintaining or increasing genetic diversity, and decreasing inbreeding in depleted, small populations (Brown & Kodric-Brown 1977, Ingvarsson 2001). The degree of connectivity among wild populations relies heavily on the habitat the species uses throughout its life cycle and the species life-history, as well as behavior, physiological limits and barriers to gene flow imposed by natural events or man-made structures.

Diadromous species exhibit a life-history in which connectivity can occur when individuals move between disjunct freshwater habitats through marine or estuarine environments. The degree of connectivity relies in part on the distance between freshwater habitats, with rivers within the same estuary system presumably more connected than rivers from different estuary systems (Bradbury et al. 2008). Dispersal among watersheds can also occur without movement through estuarine or marine systems, during high rainfall events, temporarily connecting freshwaters over short time scales, or via stream capture over geological timescales (Hurwood et al. 2014). However, it is during the marine/estuarine phase that dispersal is most likely to happen.

Many species within the genus *Macrobrachium* are amphidromous, relying on freshwater as first stage larvae, juveniles, and adults, while requiring a saline environment to metamorphose

through three to 20 different zoeal stages (Jalihal et al. 1993). The dispersal potential at the larval stages has been shown to be limited by salinity tolerance, as species with low salinity tolerance appear limited to dispersal within estuaries while more tolerant species exhibit greater dispersal potential (Sharma & Hughes 2009, Rossi & Mantelatto 2013). The degree of connectivity among estuaries determines the degree of genetic structuring and is of great importance to conservation and management approaches being used. However, there have not been population genetic assessments at a large scale for most taxa in this genus.

Migration is a vulnerable period in the life cycle of *Macrobrachium* species. Threats in adult and nursery habitat, and throughout the migratory corridor can have impacts on populations. One of the primary threats is the construction of dams which limits movements and can cut off access to suitable headwater refugia from predators (March 2003, Bauer 2011). Other factors such as fisheries, land-use alterations, pollution, invasive species, and climate factors such as droughts also threaten populations of these species (Hedgepeth 1949, Gunter 1937, Allan 2004, Bowles et al. 2000, Covich et al. 2006). In the U.S., *Macrobrachium* populations have been in decline, while in Texas, dams and other instream barriers have fragmented riverine habitats, and species are becoming more restricted to near-coastal habitats (Bowles et al. 2000). Population genetics can be used to investigate not only connectivity among streams, but also to determine within-population genetic diversity, an indicator of population health. The population status of *Macrobrachium* species and the degree of connectivity among catchments in Texas is currently unknown.

Macrobrachium ohione is widely distributed and in high abundance in the eastern U.S. and upper east coast of Mexico (Bowles et al. 2000). This species' historic range includes Texas, Louisiana, Mississippi, Florida, Arkansas, Indiana, Illinois, Oklahoma, and Ohio, with reports of

the species in Virginia (Bowles et al. 2000, Bauer 2011). Due to high local abundances and large distributional range, there are currently no protections for this species, and it is listed by the International Union for Conservation of Nature (IUCN) as Least Concern (De Grave 2015). Within Texas, *M. ohione* is the most abundant species of *Macrobrachium*, distributed throughout most Gulf of Mexico coastal streams. Currently no species of *Macrobrachium* are on the Species of Greatest Conservation Need list maintained by Texas Parks and Wildlife Department (TPWD 2020). However, the range for *M. ohione* has decreased since the 1940s, barely reaching into Arkansas, where the distribution has been constricted more so that a "threatened" conservation status was recommended for populations occurring in Arkansas (Bauer 2011, Robinson & McAllister 2011). There have been no population studies to assess the population structuring and connectivity of *M. ohione* within Texas.

M. ohione larvae have relatively low larval salinity tolerances, potentially limiting gene flow among populations through marine systems (Dugan et al. 1975, Brown & Kodric-Brown 1977, Ingvarsson 2001). Due to this, each bay system in Texas could have a unique genetic population connected only rarely with other bays during high flow events like storms. In addition, population connectivity may vary among catchments with areas of higher rainfall, like the upper eastern part of the Texas coast experiencing greater geneflow with neighboring populations than drier systems further south (Hurwood et al. 2014, Carini & Hughes 2004). The assessment of the population genetic structure of *M. ohione* among streams and bay systems in Texas can increase our understanding of dispersal and genetic diversity in this species and provide important information for conservation planning. The objective is to utilize a partial fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene to make a preliminary

assessment of population structure between individuals occurring in different bay systems and supply this information for the management of the species throughout the Texas coast.

Methods

A total of 130 individuals were collected in the mostly in the spring and summer months of 2019-2020 throughout the known distributions of *M. ohione* in Texas utilizing baited trap deployed an hour before sunset and retrieved an hour after sunset. Sample localities included river, stream, and bayou systems that that flow into the following main drainages; Sabine Lake, Galveston Bay, Brazos/San Bernard Gulf of Mexico drainage, Matagorda Bay, San Antonio Bay, Aransas Bay, and Corpus Christi Bay, with an additional sample from Lake Pontchartrain in Louisiana (Table 3.1, Figure 3.1). *Macrobrachium* specimens were collected by colleagues from TAMUCC and Louisiana Department of Wildlife and Fisheries. Species identity for each specimen was determined using morphological characteristics following several available keys (Bowles 2000, Holthuis 1952, Thorpe & Rogers 2016).

DNA was extracted from muscle tissue taken from the abdomen or legs using E.Z.N.A® Tissue DNA Kits (Omega Bio-Tek) following the manufacturer's protocols. A portion of the mitochondrial COI gene was amplified using CO1a-H (AGT ATA AGC GTC TGG GTA GTC) and CO1-Lf (CCT GCA GGA GGA GGA GAC CC;Palumbi & Benzie 1991). Each polymerase chain reaction (PCR) reaction was 25 µL with 1X buffer, 3 mM MgCl2, 0.4 mM dNTPs each, 0.4 µM forward and reverse primer each, 0.05 units/µL Taq Polymerase, 1-3 µL DNA template, and ddH2O to reach total 25 µl volume. PCR amplification involved initial denaturation for 2 min at 95℃; 40 cycles of 45s at 95℃ (denaturation), 45s at 49℃ (annealing), 1 in at 72℃ (extension), and final extension 3 min at 72℃. Amplicons were assessed visually after electrophoresis using 1% agarose gel, to confirm that there was enough amplification to proceed

to DNA sequencing. PCR products were sent to Genomics Core Lab at Texas A&M University-Corpus Christi for PCR clean-up and DNA sequencing. Sequences were manually edited and aligned in SEQUENCHER v. 5.4.6 (GeneCodes Corporation).

For all population genetic analysis only catchments that exceeded five individuals were included unless otherwise noted., To assess for homogeneity of haplotypes across catchments a single level analysis of molecular variance (AMOVA) and post hoc pairwise F_{ST} values estimated in ARLEQUIN v. 3.5 (Excoffier & Lischer 2010), with significance determined by permuting individuals between catchments 10,000 times. Haplotype (h) and nucleotide diversity (π) within catchments and the total sample set were also estimated in ARLEQUIN. Tajima's D and Fu's F_s were also estimated using in ARLEQUIN to assess for evidence of population expansion or selective sweeps within individual catchments as well as across the total sampled area. Parameter of demographic and spatial expansion were then estimated through mismatch distribution and Harpending's Raggedness (H_r) index using 100 bootstrap replicates. To visualize genetic structure a haplotype network and haplotype map were constructed in PopART v. 1.7 using the integer N.J. method (Leigh & Bryant 2015) and including catchments with less than five individuals.

Results

The final alignment length was 497 bps with 10 variable sites, 2 parsimony informative sites, and an average nucleotide composition of T= 28.0%, C= 24.1%, A= 30.2%, and G=17.7%. Homogeneity of haplotype variation among catchments could not be rejected with single level AMOVA ($\Phi_{ST} = 0.00$, $p = 0.554$, Table 3.2). Pairwise F_{ST} values also were all non-significant (Table 3.3); in agreement with the AMOVA results. Ten unique haplotypes were observed across out of 130 individuals and catchments, with the highest h occurring in Galveston Bay with (h =

0.2359, Table 3.4). Nucleotide diversity was low in all catchments and throughout the total data set (Table 3.4), with the highest diversity being in Galveston Bay ($\pi = 0.0007$). Tajima's *D* and Fu's F_s. were negative in all populations, and the total data set and several sites (Brazos/San Bernard River, Matagorda, and Galveston) deviated significantly from neutral expectations (Table 3.5). The SSD for Mismatch distribution and raggedness were non-significant for all sample sites and total sample area.

Out of 130 individuals sequenced, there were nine unique haplotypes in addition to the dominant haplotype, which occurred in 119 individuals. Most haplotypes differed by a single change from the dominant haplotype, except haplotype 9, which was separated from the dominant haplotype by three changes (Figure 3.2). More haplotype variation occurred in Galveston Bay and Matagorda Bay than other bay or estuarine systems. Only one individual from both Aransas and Brazos River (Gulf drainage) exhibits a different haplotype than the rest of their sample populations (Figure 3.3).

Discussion

Homogeneity in haplotype frequencies among populations could not be rejected in an AMOVA framework, and all pairwise comparisons were non-significant. These results could reflect widespread gene flow among the catchments, but could also be due to marker choice, as COI has a lower mutation rate relative to other protein-encoding mitochondrial markers (Patwardhan et al. 2014). COI has been used in previous population studies of *Macrobrachium* species and did show population genetic structure but over larger geographic scales or in non-migratory species where the COI divergence observed could reflect deep population isolation and/or cryptic species diversity (Chen et al. 2017, Cook et al. 2008, Yang et al. 2007, Carini & Hughes 2004). Further, species of *Macrobrachium* in those studies are older lineages and

showed no evidence of a recent population expansion, allowing for more mutations to accrue in those populations over time (Mantelatto et al. 2021). *M. olfersii* also exhibits low diversity and no population structure in COI throughout the distributional ranges in Central and South America and is a relatively recent lineage compared to the other species that have had success utilizing COI as a population marker (Rossi & Mantelatto 2013, Mantelatto et al. 2021).

Negative, significant values for Tajima's *D* and Fu's *F* indicate a departure from neutrality that can either be caused by a selective sweep or population expansion (Tajima 1989, Fu 1997). This study assessed variation in the mitochondrially-encoded COI gene, so the former seems less likely than the latter. Furthermore, non-significant values for the SSD in mismatch distribution and Harpending's Raggedness also suggest population expansion (Harpending 1994). The haplotype network is also star-like, and perhaps *M. ohione* might have recently colonized or recolonized the area of study post-glaciation (Cook et al. 2008, Flower et al. 2011). Previous glaciation periods restricted the distribution of populations of *M. nipponense*, and interglacial periods preceded demographic expansions (Chen et al. 2017). The Gulf of Mexico underwent an interglacial melting period where salinity dropped, and expansions of the *M. ohione* west of the Mississippi Delta were possible around 12,000-13,000 years ago (Flower et al. 2011). With a dominant haplotype observed in 91.5% of the population, a founder event is likely sometime in the recent evolutionary past.

There are limited studies that have utilized other markers for population studies of species in the genus *Macrobrachium*, with the exception of a microsatellite-based study that revealed only low to moderate levels of genetic diversity among populations of *M. rosenbergii* (Mohanty et al. 2016). The use of a population genomics approach to revisit the questions laid out in this study would provide more resolution because thousands of loci could be genotyped

simultaneously; furthermore, loci would be nuclear-encoded and could be used to directly assess for localized adaptive variation (Allendorf 2017). This local adaption could be critical in population assessments concerning the management of species because high gene flow may be suggested by neutral loci, while adaptive loci can be highly divergent among samples (Moody et al. 2015, O'Malley et al. 2019). Low to moderate population structuring is common in amphidromous species (Mohanty et al. 2016, Delgado et al. 2019), and population genomics can be used to assess genetic differentiation of adaptive loci across habitat types to identify ecotypes (Asaduzzaman et al. 2020). With *Macrobrachium*, isolation between estuarine systems is possible with potential localized ecotypes (Sharma & Hughes 2009) but this could not be assessed in the current study. The potential for localized ecotypes could shift conservation management decisions which need to balance increasing gene flow for population rescues with preserving locally adapted populations (McMahon et al. 2014).

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Appendix

Table 3.1. Sample site information with sampled rivers within the Texas and Louisiana coast, latitude, longitude, N representing the number of individuals obtained, and drainage.

River	Lat	Long	N	Drainage
Bonnet Carre Spillway, LA	30.15107	-90.0894	1	Lake Pontchartrain
North Fork Taylor Bayou	29.91172	-94.2933	7	Sabine Lake
Chocolate Bayou	29.212	-95.2086	1	Galveston Bay
Chocolate Bayou	28.5781	-96.7003	1	Galveston Bay
Dickson Bayou	29.4369	-95.0922	1	Galveston Bay
Eagle Gully	29.1966	-95.2992	1	Galveston Bay
Highland Bayou	29.3511	-95.0151	1	Galveston Bay
Juanita creek	28.9416	-96.1754	3	Galveston Bay
Moses Bayou	29.4214	-94.961	3	Galveston Bay
Mustang Bayou	29.4188	-95.2365	1	Galveston Bay
Oyster creek	29.1583	-95.4757	18	Galveston Bay
Oyster creek	29.6439	-95.7409	2	Galveston Bay
Oyster creek	29.05475	-95.4559	1	Galveston Bay
Oyster creek	29.6291	-95.6359	1	Galveston Bay
San Jacinto River	29.87623	-95.0941	3	Galveston Bay
Trinity River	29.8369	-94.7647	2	Galveston Bay
West Fork Chocolate Bayou	29.4809	-95.4318	1	Galveston Bay
Brazos river	29.1441	-95.6057	9	Gulf
Brazos river	29.6395	-95.9759	8	Gulf
San Bernard River	29.1118	-95.6769	8	Gulf
San Bernard River	29.0787	-95.6827	5	Gulf
San Bernard River	29.3136	-95.8936	5	Gulf
Linnville Bayou	28.98252	-95.7692	9	Matagorda Bay
Placedo creek	28.7269	-96.8434	14	Matagorda Bay
Tres Palacios	28.9959	-96.1368	4	Matagorda Bay
Wilson creek	28.8928	-96.0867	10	Matagorda Bay
San Antonio River	28.6131	-97.2139	1	San Antonio Bay
Aransas River	28.1275	-97.4278	8	Aransas Bay
Nueces	28.0384	-97.8607	1	Corpus Christi Bay

Table 3.2. Results of a single level analysis of molecular variance (AMOVA) calculations conducted in ARLEQUIN showing the source of variation, degrees of freedom (df), sum of squares (SS), variance components (cc), and percent variation (%V).

Variation	df	SS	vc	% V
Among populations	4	0.379	-0.001	-0.6
Within populations	122	13.432	0.110	100.6
TOTAL	126	13.811	0.109	

Table 3.3. Pairwise F_{ST} p-values estimated in ARLEQUIN with 20,022 permutations with significance level at= 0.05 Labels are ARA-Aransas, B/SBR-Brazos/San Bernard Gulf drainage, MAT-Matagorda, GAL-Galveston, SAB-Sabine.

	ARA	B/SBR	MAT	GAL	SAB
ARA	*				
B/SBR	0.342	*			
MAT	0.467	0.468	*		
GAL	0.539	0.484	0.553	*	
SAB	0.999	0.999	0.999	0.999	*

Table 3.4. Diversity calculations completed in ARLEQUIN within and among catchments as well as whole sample data set. The total number of individuals per site (N), number of haplotypes (H), haplotype diversity (*h*), and nucleotide diversity (π) are displayed.

	N	H	*h*	π
Sabine	7	1	0.000	0.0000
Galveston	40	5	0.236	0.0007
Brazos/SBR	35	2	0.057	0.0001
Matagorda	37	4	0.206	0.0005
Aransas	8	2	0.250	0.0005
Total	130	10	0.162	0.0004

Table 3.5. Neutrality tests: Tajima's *D*, Fu F_s, mismatch distribution sum of squared deviation (SSD) and Harpending's Raggedness (*Hr*) index conducted in Arlequin separated by population sample site. Sample sites with less than five individuals were not included for within catchment analysis but were included in total data set analysis (Corpus Christi, San Antonio, and Louisiana). Bold numbers indicate significant values.

	D	Fs	SSD	*Hr*
Sabine	0.000	0.000	0.000	0.000
Galveston	**-2.011**	**-3.270**	0.002	0.375
Brazos/SBR	**-1.136**	**-1.339**	0.000	0.788
Matagorda	**-1.754**	**-2.505**	0.001	0.428
Aransas	-1.055	-0.182	0.279	0.313
Total	**-2.177**	**-14.101**	0.0001	0.516

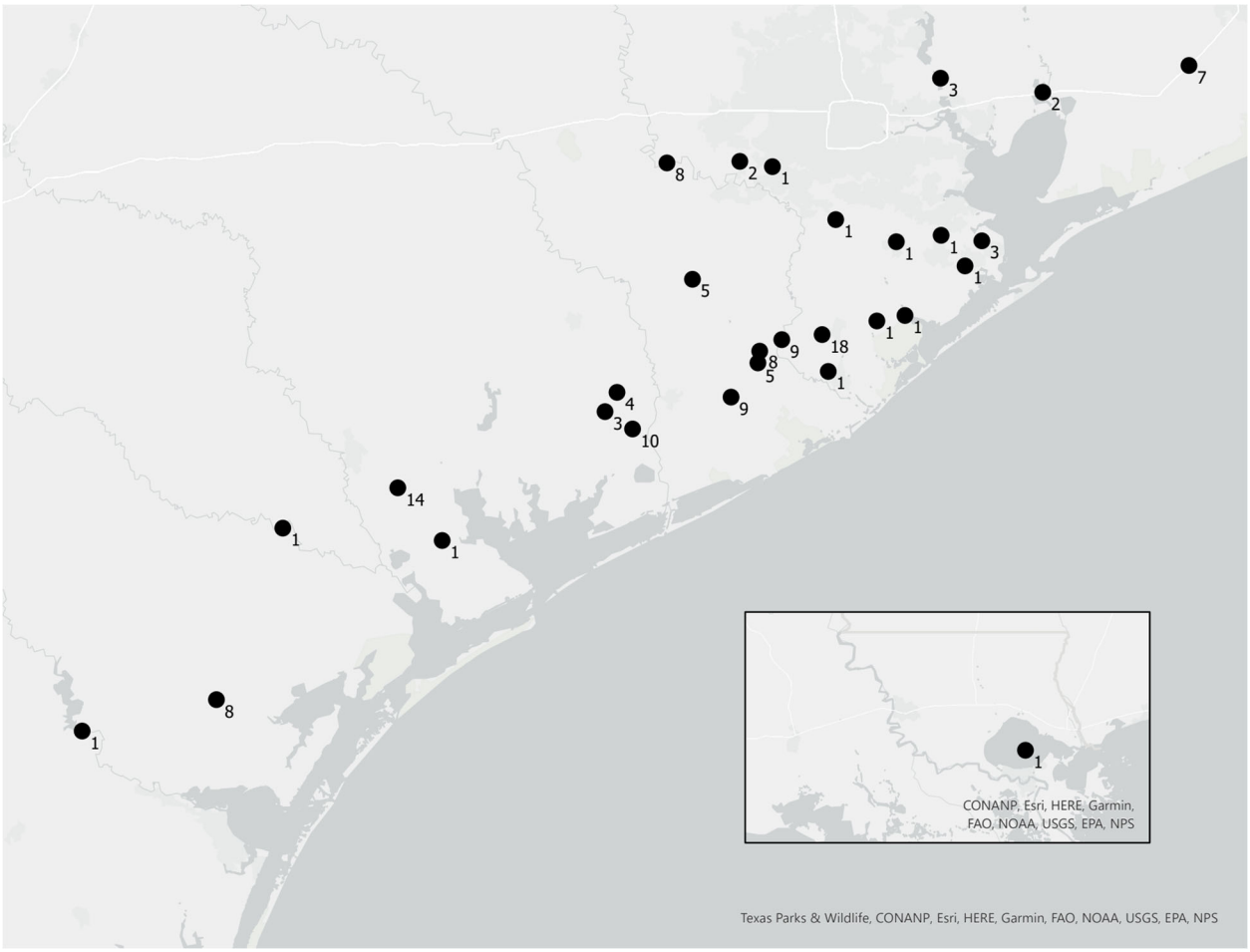

Figure 3.1. Map with all sample locations marked as circles throughout the Texas coast. Inset map in the bottom right corner shows the Bonnet Carre Spillway, Louisiana sample site. Circles depict the sample site with the numbers outside representing the number of individuals obtained from the sample site.

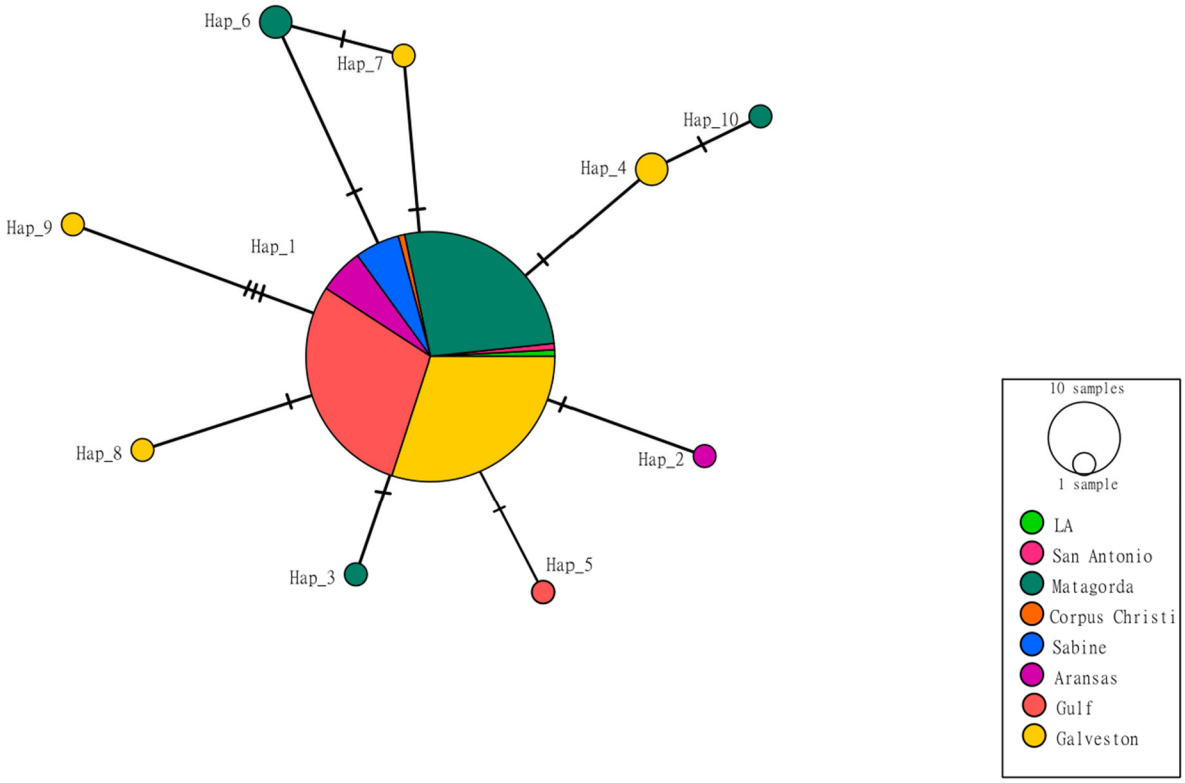

Figure 3.2. Haplotype network depicting the different haplotypes observed throughout *Macrobrachium ohione* with colors representing the population sample location. Sizes of the circles indicate the number of individuals with each haplotype. The dominant haplotype being the large center circle with a few branches to smaller circles. The hash marks on each branch show how many mutations occur between the different linked haplotypes. Sample sites are Lake Pontchartrain, Louisiana (LA), San Antonio, Matagorda, Corpus Christi, Sabine, Aransas, Gulf, and Galveston. Haplotype numbers are written next to the haplotype circle within the haplotype net and correspond to the haplotype map in Figure 2.

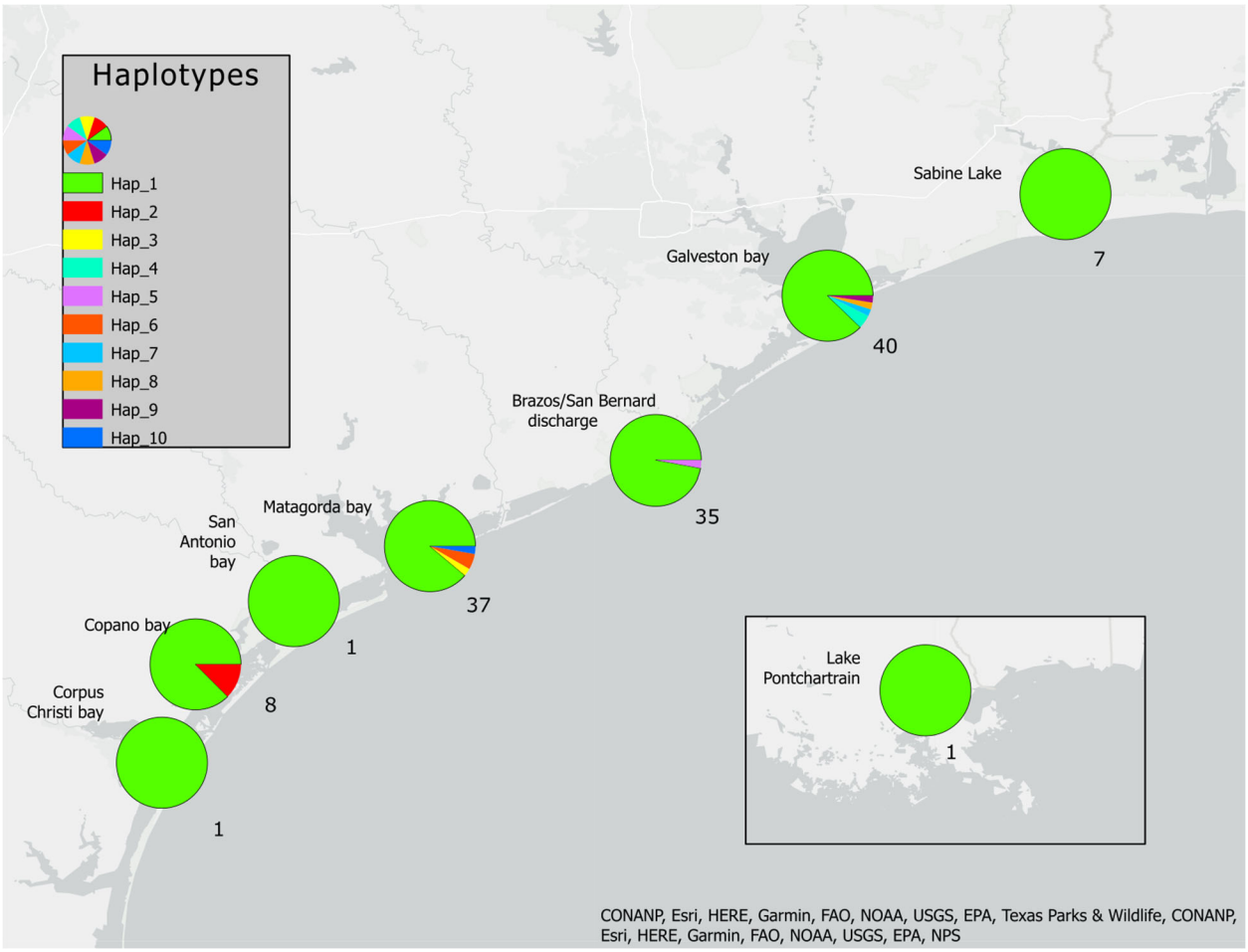

Figure 3.3. Map of Texas coast with an inset of Louisiana coast with colored circles depicting sample drainage sites. Color of the circles is indicative of the haplotypes found within the populations and sizes of the colored wedges indicates the number of individuals within the population that have that haplotype. The total number of individuals from each drainage is listed below the circles and the drainage name above.

CHAPTER IV: CONCLUSION

This study has concluded that there are multiple, genetically distinct clades of *M. carcinus* throughout its known distribution. The phylogenetic hypothesis presented here suggests that there are three distinct clades of *M. carcinus*. One clade found in Puerto Rico is sister to *M. americanum,* and this mixed-species clade is sister to a clade containing two distinct clades of *M. carcinus*. The hypothesis generated supports the idea that there are two currently unrecognized species, currently called *M. carcinus,* one occurring in Texas and one in Central/South America. Taxonomic revision is recommended for what is currently recognized as *M. carcinus* throughout the known distribution.

Macrobrachium ohione is the only species endemic to North America and patterns of population structure remain undetermined. The results of this study show that COI haplotype diversity is low among the Gulf coast populations of *M. ohione* and does not inform an assessment of population structure. The haplotype network formed a star-like topology consistent with recent population expansion in the Texas coastal plain. With this information, the use of less conserved genetic markers may show better population resolution for conservation and management decisions for the species.

Macrobrachium species are in decline due to increased human activities in areas adjacent to their habitats and declines could be a detriment to fragile ecosystems for which these species provide many services. One goal of this thesis went unmet, a full investigation of connectivity among populations of *M. ohione* along the Texas coastal plain was not possible due to the recent founding and expansion of the population and the standing genetic diversity of the marker used. Future studies should utilize more multiple unlinked genes with higher level of standing genetic variation to understand the population dynamics within and among Texas watersheds and a

population genomics is recommended. Overall, conservation decisions should be reassessed for what is currently known as *M. carcinus* due to different species occurring throughout the distributional ranges of what is currently called *M. carcinus*. The distinct nature of this species coupled with the census size and restricted range in Texas warrants consideration for conservation protection to ensure functional redundancy within the ecosystems to maintain the important ecosystem services provided by this species.