# Genetic diversity and population structure of Caribbean marine invertebrates 

by

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#### Abstract

Natural populations of many marine invertebrates are under pressure by fisheries due to the increasing demand and unit prices leading to overexploitation. I examined the genetic variation of two marine invertebrates: the corallimorpharian Ricordea florida and the emerald crab Mithraculus sculptus both of which are heavily traded by the marine aquarium industry. A third species studied, the commensal barnacle Chelonibia caretta, was not under direct fishing pressure, but its host is an endangered species, the hawksbill sea turtle (Eretmochelys imbricata). Using direct sequencing of mitochondrial genes (COI and 16S) and the nuclear the ITS region (ITS1, 5.8S, and ITS2), the phylogeography and genetic population structure of these invertebrates were characterized. The genetic diversity in C. caretta was low compared to published sequences from populations of barnacles attached to fixed substrata. However, genetic diversity values from C. caretta were comparable to those reported from C. testudinaria, an epibiont barnacle on loggerhead turtles. Analysis of molecular variance and phylogenetic analysis of the sampled turtle epibionts indicated that they are genetically homogeneous, irrespective of the host or host gender. In contrast, the phylogeography of the ITS region of the coral $R$. florida uncovered two partial sympatric genetic lineages. These lineages showed two highly supported monophyletic groups when they were compared phylogenetically against published nuclear ribosomal sequences of other genera of Corallimorpharia (Actinodiscus, Amplexidiscus, Corynactis, and Rhodactis). Similarly to R. florida, the phylogeographic analysis of M. sculptus based on 16 S revealed two distinct lineages distributed throughout the sampled region. The analysis of molecular variance and the phylogeography of the sampled populations of M. sculptus reflected that most of the specimens within each lilneage were collected from demographically open populations within the western Caribbean.


In general, the three species studied are consistent with the prediction that higher rates of dispersal reduce population divergence and estimates of nucleotide diversity. As in previous studies, the absence of genetic differentiation between geographical distant populations of these species suggests high connectivity between all sampled areas. Additionally, the presence of cryptic lineages confirms concerns regarding that biodiversity can be vastly understudied for marine environment.

## RESUMEN

Las poblaciones naturales de muchos invertebrados están bajo presión por las pesquerías debido a su creciente demanda y valor comercial, que conducen a su sobreexplotación. Yo examiné la variación genética de dos especies de invertebrados marinos: el corallimorfario Ricorea florida y el cangrejo esmeralda Mithraculus sculptus, ambos cosechados extensamente para la industria de acuarios marinos. Una tercera especie fue estudiada, la bayoca comensalista Chelonibia caretta, la cual no está bajo presión directa de la pesquería, pero su hospedero, el carey de concha (Eretmochelys imbricata), es una especie de tortuga marina amenazada. Utilizando amplificaciones directas de genes mitocondriales (COI y 16S) y nucleares (ITS1, 5.8S y ITS2) se caracterizó la filogeografía y estructura genética poblacional de estos invertebrados. La diversidad genética de Chelonibia caretta fue baja al compararla con secuencias publicadas de otras especies de bayocas sobre substratos fijos. Sin embargo, los valores de la variabilidad genética para C. caretta son comparables con aquellos publicados para C. testudinaria, bayoca epibionte de la tortuga marina cabezona. Análisis de variación molecular y análisis filogenético de los epibiontes muestreados de la tortuga indicaron que éstos son genéticamente homogéneos, independientemente del hospedero o del género del hospedero. En contraste, los análisis de la región del ITS de R. florida revelaron dos linajes genéticos parcialmente solapados. Estos linajes fueron comparados filogenéticamente contra secuencias ribosomales nucleares publicadas de otros géneros de Corallimorpharia (Actinodiscus, Amplexidiscus, Corynactis y Rhodactis) y cada linaje formó un grupo monofilético altamente apoyado. Al igual que para R. florida, los análisis filogenéticos del 16S de Mithraculus sculptus, revelaron dos linajes distribuidos a través de la región muestreada. Los análisis de varianza molecular y la filogeografia de las poblaciones muestreadas de M. sculptus reflejaron que la mayor parte de los especímenes, dentro de cada linage, fueron colectados de poblaciones demográficamente
abiertas dentro del Caribe occidental.

En general, las tres especies estudiadas son consistentes con la predicción de que si los índices de dispersión son altos disminuyen la divergencia y los estimados de diversidad de nucleótidos. Como en estudios previos, la ausencia de la diferenciación genética entre poblaciones geográficamente distantes de estas especies sugiere una alta conectividad entre las áreas muestreadas. En adición, la presencia de linajes crípticos confirma la preocupación de que la biodiversidad está sub-estimada en los ambientes marinos.

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## DEDICATION

This work is dedicated to my family:

To my parents Hernán Torres Baez and Lauxelinda Pratts Luciano because without them I woulld not be here. And especially to my wife Sandra L. Maldonado and my kids Carmen Yuitza, Hernán, and María del Mar
"No hay palabras que puedan expresar mi agradecimiento a mi esposa Sandra por todo el apoyo que me ha dado."
"Soy una embarcación que partió para navegar en un mar de sueños y tú , Sandra, fuiste el ancla a la realidad que me permitió realizarlos. Tengo la dicha de que me dieras tres hijos Carmen, Hernán y María, son ellos tres faros de infinita luz e inagotable poder. Estos siempre iluminan mi camino y me guían a puerto seguro."

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## CHAPTER 1

## General Introduction

The origins of measurable attempts to describe the genetic structure of populations are attributed to Wahlund (1928) who reported heterozygote deficiencies in structured populations. Sewell Wright (1951) took the lead in the field of population genetics with the analysis of the expected distribution of neutral genetic variation in spatially subdivided populations (Wright's fixation index). Wright's approach to build on statistics that summarize the distribution of genetic variation within and among sampling units was based on the spatial (and sometimes temporal) distribution of gene and genotypic frequencies. The alloenzyme studies in the 1960's provided the data to test Wright's genetic models describing the spatial distribution of gene frequencies in natural populations. The discovery of the DNA double helix structure by Watson and Crick (1953) followed much later by the development of DNA sequencing methods in the 1970's by two groups, Maxam and Gilbert (1977) and Sanger and his colleagues (1977) setting up the basic tools for modern studies in population genetics and phylogenetics. In the beginning, sequencing 5,000 nucleotide bases took a week, but in 1987 the construction of the first automated DNA sequencer (ABI Model 370) brought the availability of faster, easier, affordable, and highly accurate DNA sequencing methodologies that generate numerous amounts of data at a low cost today. The continuing advances in molecular techniques have provided tools to contemporary molecular ecologists, conservationists, phylogeneticists, and other related fields to focus on the understandings of the source and retention of the genetic diversity within, between populations and species. These advances have modified our way of thinking on how evolutionary mechanisms work at different levels from the individual to the community level and have
promoted conservation genetics as an important tool for the assessment of biodiversity of the planet. According the United Nations Rio Convention: "Biological diversity is the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (Rio de Janeiro 1992).

The marine reserve areas in Puerto Rico have increased during the last 10 years. The designation of these areas was a response to pressures from environmental activists, decrease in fisheries production, and the humanitarian need to protect coral reefs for future generations. This initiative follows a global effort for designation of marine reserves for preserving biological diversity, increase fisheries yields, and protect particularly vulnerable life stages of marine species (Roberts 1997). However, the reserve's boundaries are usually created without having a clear understanding of the reserve's sustainability; rather, the boundaries are defined mainly by responding to fishing controls (Palumbi 2003) or other economic pressures. The knowledge of how larvae are transported in and out of reserves is crucial for the sustainability of the marine reserves (Palumbi 2003). Direct measurements of larval dispersal are needed to understand connectivity between the reserve and the surrounding environment, but research of this magnitude is extremely difficult and complex. Larvae are small and cannot be tagged as large fish or other marine vertebrates. Nevertheless, connectivity can be estimated indirectly though, through studying the patterns and the distribution of genetic variability through geographic space. The relationship between genetic differentiation and geographic distance may reveal some of the underlying properties of larval dispersal, and show the geographic scale over which populations are ecologically linked (Palumbi 2003). Studies of connectivity of marine populations should be integral parts of decisions regarding the conservation of marine
ecosystems. Measurements of genetic connectivity can help assess if populations are open or closed. Because many benthic marine species have larvae that can last days to months in the planktonic stage (Chapman 2002, Gaines and Bertness 1992, Scheltema 1986), it has lead to the prevailing belief that marine benthic populations are demographically open and highly "connected', resulting in genetically panmictic populations (Caley et al 1996). Recent works have shown more population structure and consequently more cryptic species complexes in the ocean than previously expected (Buroker 1983, Knowlton 2000, Dawson and Jacobs 2001). Recent research has also pointed out that planktonic larval stages of marine species are not good predictors of panmixia (Buroker 1983, Kyle and Boulding 2000, Bernatchez and Dodson 1991).

One way to interpret high genetic diversity is that a population contains many different alleles for each particular locus, providing a mechanism for adaptation to its changing environment. As the genetic diversity increases it enhances the chance that at least some of the individuals will overcome adversity. Identification of evolutionary lineages of a species is essential to retain maximum genetic diversity in the conservation of species and the areas that they occupy. I examined three Caribbean marine invertebrates to identify the connectivity patterns among populations across their geographic range using phylogeographic and population structure analyses. I estimated the phylogeography and genetic structure in three marine invertebrates with different dispersing potential in the Caribbean. Direct sequencing of mitochondrial and nuclear genes were used to characterize the genetic population structure of the turtle barnacle Chelonibia caretta Spengler, 1790, the emerald crab Mithraculus sculptus Lamarck, and the mushroom coral Ricordea florida Duchassaing and Michelotti, 1860 I used the relationship between genetic differentiation and geographic distance to show the geographic
scale over which the populations of C. caretta, M. sculptus, and R. florida are ecologically linked.

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## CHAPTER 2

# Genetic diversity of Chelonibia caretta, commensal barnacles of the endangered hawksbill sea turtle Eretmochelys imbricata from the Caribbean (Puerto Rico) 

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#### Abstract

The patterns of mitochondrial genetic diversity were studied in Chelonibia caretta, commensal barnacles of the endangered hawksbill sea turtle Eretmochelys imbricata from Mona Island of Puerto Rico. Two mitochondrial genes were sequenced: the large subunit of ribosomal RNA (16S rRNA) and the Cytochrome Oxidase Subunit I (COI). Haplotypic diversity (h) for $16 \mathrm{~S}(\mathrm{n}=34)$ and COI ( $\mathrm{n}=26$ ) varied from 0.763 to 0.468 , respectively. The nucleotide diversity $(\pi)$ of $16 \mathrm{~S} / \mathrm{COI}(0.00284 / 0.0013)$ of $C$. caretta was low compared to all other published sequences from population studies of barnacles attached to fixed substrate. However, the estimated levels of $\pi$ from C. caretta were comparable to those reported from C. testudinaria, an epibiont barnacle on loggerhead turtles. Analysis of molecular variance and parsimony analysis of the sampled turtle epibionts from Mona Island indicated that they are genetically homogeneous, irrespective of the host or its gender. Albeit the small sampling size, our results are consistent with the general prediction that higher rates of dispersal reduce population divergence and estimates of nucleotide diversity. Conservation concerns are raised about the status of Chelonibia caretta whose host (the hawksbill sea turtle) has been included in the Endangered Species List.


## INTRODUCTION

A conspicuous component of the marine turtle epibiotic community is the coronulid barnacle of the genus Chelonibia. Twenty-nine nominal species of "turtle barnacles" are currently recognized (Epibiont Research Cooperative 2007). These represent two balanomorph families: the Chelonibiidae (acorn barnacles) and the Platylepadidae (gooseneck barnacles). Studies of hawksbill sea turtle (Eretmochelys imbricata) epibiota revealed that two common obligate commensal barnacle species attached to the carapace are Chelonibia caretta and $C$. testudinaria with the former being the most common one (Frick et al. 2003; Schärer 2003).

Although the life history of C. caretta remains unknown in terms of dispersal potential, the complete life cycle of the congeneric C. testudinaria has been described by Zardus and Hadfield (2004), who documented that development from the first naupliar stage to the cyprid stage occurs in nine days. In laboratory essays, barnacle settlement occurred one or two days after completing development, however, it remains unknown how long C. testudinaria can remain in the cyprid stage in nature. Other barnacle species settle within two to six weeks (Lucus et al. 1979; Shanks 1986; Pineda et al. 2005).

One of largest nesting locations for hawksbills in the Caribbean Sea is Mona Island, Puerto Rico (Beggs et al. 2007; van Dam and Diez pers. comm.). Tagged adult hawksbill turtles from Mona Island, are capable of traveling to foraging habitats as geographically distant as Central America and the British Virgin Islands (van Dam et al. 2008). Unlike barnacles on fixed substrates, turtle barnacles are attached on a mobile substrate and are capable of dispersing both
as larvae and as adults. Higher rates of dispersal should prevent significant population subdivision as populations cease diverging due to drift or local adaptation. We have evaluated this hypothesis by analyzing the genetic variation at the mitochondrial genes COI/16S (914 bp) from 34/27 C. caretta collected from Mona Island and have compared our nucleotide diversity levels to previous population studies of barnacles inhabiting fixed substrates.

## MATERIALS AND METHODS

Barnacles were sampled from several adult and juvenile hawksbills at Mona Island, Puerto Rico a known breeding location of this species. The sampling sites were located on the southern coast of Mona Island ( $18^{\circ} 5^{\prime} 27^{\prime \prime} \mathrm{N}$ and $67^{\circ} 53^{\prime} 37^{\prime \prime} \mathrm{W}$ ). Barnacles were collected from nesting and foraging turtles during August and September of 2006 and 2007. Chelonibia sp. barnacles attached to the carapace were removed from three females (carrying 17 barnacles) and six males (carrying 18 barnacles). One of these barnacles was collected from a juvenile turtle from Culebra Island, eastern Puerto Rico. A specimen of Chelonibia testudinaria was collected from another juvenile turtle from Mona Island and was used as outgroup in the genealogical analysis. Barnacles were preserved in $100 \%$ ethanol for DNA analysis.

Total genomic DNA was extracted using a Gentra DNA purification kit (Qiagen, Inc.). A fragment of the 16 S rRNA gene was amplified using the primers from Crandall and Fitzpatrick (1996). A fragment of the COI was amplified with the universal primers from Folmer et al. (1994). Amplification was performed using the Master Taq ${ }^{\circledR}$ kit. To amplify the COI and the 16 S gene, the thermal cycler profile began with a denaturation step at $94^{\circ} \mathrm{C}$ for 2 min , followed by 40 cycles of $95^{\circ} \mathrm{C}$ for $15 \mathrm{sec}, 45^{\circ} \mathrm{C}$ for $30 \mathrm{sec}\left(\mathrm{COI} ; 42^{\circ} \mathrm{C}\right.$ for 50 sec$)$, and $72^{\circ} \mathrm{C}$ for 30 sec
(COI; 1 min ), ending with an extension step at $72^{\circ} \mathrm{C}$ for 5 min . Some DNA templates that were difficult to amplify were further cleaned by a Qiagen PCR Clean Up Kit and $\mathrm{Mg}^{+2}$ hot beads. PCR products were visualized by electrophoresis on a $1 \%$ agarose gel, and cleaned using ExoSap. Sequence reactions were prepared with the BigDye Terminator v.3.1 Cycle Sequencing Kit for both strands of the purified amplicon and loaded in an ABI 3130xl Genetic Analyzer. The resulting DNA sequences were verified by aligning reads from both 5 'and 3 ' directions for the majority of individuals, using the software CodonCode Aligner and further curated in MacClade (Maddison and Maddison 2000). The haplotypic ( $h$ ) and genetic diversity ( $\pi$ ) (Nei 1987) of barnacles was estimated in DNAsp (Rozas et al. 2003). The DNA neutrality tests Tajima's $D$ (Tajima 1989) and Fu's Fs (Fu 1997) were applied to test for significant departures from neutrality (Kimura 1968). Additionally, a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was applied to detect population differentiation between barnacles collected from male and female turtles. AMOVA analysis and pairwise $\Phi_{\text {ST }}$ comparisons were performed in Arlequin ver. 3.1 (Excoffier et al. 2005) by using the $\mathrm{K} 2 \mathrm{P}+\Gamma(0.222)$ (Kimura 1980) model of nucleotide substitution. We used the K2P model to increase resolution in our data set which was characterized by many closely related haplotypes. The significance of $\Phi_{\mathrm{ST}}$ was assessed by 10,000 permutations of groups and haplotypes. A parsimony haplotype was constructed for the concatenated sequences using the Templeton et al. (1992) algorithm as implemented in TCS v.1.21 (Clement et al. 2000). Genealogical trees of the mitochondrial sequences were built with the maximum parsimony method in MEGA 4 (Tamura et al. 2007). DNA sequences have been deposited in GenBank under Accession Numbers FJ385704FJ385766.

## RESULTS

After verifying and trimming of the DNA traces, a 389 bp segment of the mitochondrial gene 16S from 34 individual barnacles and a 525 bp segment of COI from 27 barnacles were included in the analysis. Two barnacles from turtles Female 1 and Male 1 did not yield high quality sequences for either gene. We identified 18 different haplotypes (Table 2.1). Four haplotypes of barnacles (Hap 3, 5, 6, 12) were shared by female and male turtles, while nine haplotypes were found only on females and six only on males (Table 2.1). Of the 18 haplotypes, 13 were singletons. There were 14 segregating sites (including two indels) in 16 S and six segregating sites in COI. There were seven transitions and five transversions in 16 S while all nucleotide substitutions in COI were transitions. Haplotypic diversity ( $h$ ) of barnacles ranged from 0.822 to 0.933 for male and female turtle hosts, respectively for the concatenated data (Table 2.2). Nucleotide diversity $(\pi)$ ranged from 0.00198 to 0.00213 for males and females, respectively for the concatenated data (Table 2.2). There were no significant differences in estimates of $h$ and $\pi$ when barnacles were grouped according to the gender of the host, for neither gene nor the combined data (Table 2.2).

When we included all barnacles with complete sequences (16S+COI, $n=25$ ), the increased number of singletons resulted in significant deviations from neutrality (Tajima's $D=-$ 1.92072, $P<0.05$; Fu's $F s=-10.675$ ). The significantly negative values of Tajimas's $D$ and Fu's Fs tests are consistent with an expanding population or a purifying selection scenario. When we applied the tests gene-by-gene, we detected significant deviations from neutrality with 16 S but borderline non-significant with COI, as most of the singletons were observed in the 16 S region.

When barnacle sequences were partitioned by individual hosts, AMOVA indicated that most of the genetic variation of barnacles $(98.24 \%)$ was observed within individual turtles, suggesting that the sampled $C$. caretta were not significantly differentiated between hosts $\left(\Phi_{\mathrm{ST}}=\right.$ $0.01761, \mathrm{P}=0.408 \pm 0.018)$. When partitioned by gender of the host turtles, AMOVA suggested that most of the genetic variance ( $89.52 \%$ ) was observed within rather than among gender for the concatenated genes. The overall $\Phi_{\mathrm{ST}}=0.1048$, which is not significant $(\mathrm{P}=0.077 \pm 0.003)$. The population specific $\Phi_{\text {ST }}$ 's were 0.1148 for barnacles attached to males and 0.0981 for barnacles attached to females. There was no genetic differentiation driven by the gender of the host, regardless if we concatenated or separated the genes. Pairwise $\Phi_{\text {ST }}$ comparisons did not indicate significant genetic differentiation of barnacles among hosts nor gender of hosts (data not shown). Genealogical analysis of the concatenated $16 \mathrm{~S}+\mathrm{COI}$ sequences ( 914 bp ) resulted in a mostly unresolved topology and the lack of genetic population structure in the barnacles (Figure 2.1). The haplotype relationships showed by tree topology are consistent with a demographic scenario of population expansion or purifying selection.

Table 2．1．Barnacle haplotypes and relative positions segregating sites from 914 bp of the concatenated sequences of the mitochondrial 16S and COI genes sampled across 35 specimens from 3 female（ $q$ ）， 6 male（ $O^{\top}$ ），and 1 juvenile（Juv）hawksbill turtles． Hap．$=$ Haplotypes，$-=$ Gaps，$?=$ missing data，$n=$ number of barnacles sharing this haplotype．The Specimen ID column refers to the GenBank codes of sequenced barnacles as well as the gender of their respective turtle host．

| Нар． | 16S（389 bp） |  |  |  |  |  |  |  |  |  |  |  |  |  | COI（525 bp） |  |  |  |  |  | Specimen ID |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 46 | 72 | 76 | 78 | 187 | 221 | 270 | 276 | 277 | 278 | 300 | 312 | 335 | 388 | 417 | 537 | 597 | 711 | 813 | 856 |  |
| 1 | C | C | A | A | － | A | A | T | A | A | A | A | T | G | G | G | T | C | G | A | ¢ 1 ＿1 |
| 2 | ． | T | ． | ． | － | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | A | ． | ． | ． | G | ¢1＿2 |
| 3 | ． | ． | ． | ． | － | ． | ． | ． | T | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | G | $\begin{aligned} & q 1 \_3, \not \subset 3 \_1, \partial_{1}^{\top} 2 \_1, \text { Juv } \\ & 1 \end{aligned}$ |
| 4 | ． | ． | G | ． | － | ． | ． | ． | ． | ． | ． | － | ． | ． | ． | － | ． | ． | ． | G | Q1＿4 |
| 5 | ． | ． | ． | ． | － | ． | ． | ． | T | ． | － | ． | － | ． | A | ． | ． | ． | ． | G | 운5，ơ6＿3 |
| 6 | $\cdot$ | ． | ． | ． | － | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | － | G |  |
| 7 | T | ． | ． | － | － | ． | ． | ． | － | － | ． | ． | ． | ． | － | － | － | － | － | G | 中1＿8 |
| 8 | ． | ． | ． | ． | － | ． | ． | ． | ． | － | ． | ． | ． | A | ． | ． | ． | ． | ． | G | 中1＿9 |
| 9 | ． | ． | ． | ． | － | G | ． | ． | T | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | G | ¢ 1 ＿14 |
| 10 |  | ． | ． | ． | － | ． | G | ． | T | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | G | ¢ 3 ＿2 |
| 11 | ． | ． | ． | － | － | ． | ． | ． | ． | － | C | ． | ． | ． | ． | ． | ． | ． | A | G | ¢3＿3 |
| 12 | ． | ． | ． | ． | － | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | C | T | ． | G | $\begin{aligned} & \text { す2_4, ó6_3, } q 1 \_10, \\ & q 1 \_12,{ }^{2} 1 \_2 \end{aligned}$ |
| 13 | ． | ． | ． | － | － | ． | ． | ． | T | － | － | ． | ． | ． | ． | ． | － | － | ． | ． |  |
| 14 | ． | ． | ． | G | － | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | ． | G | ò6＿2 |
| 15 | ． | ． | ． | ． | A | ． | ． | ． | ． | ． | ． | ． | ． | ． | ？ | ？ | ？ | ？ | ？ | ？ | ¢ 1 ＿13 |
| 16 | ． | ． | ． | ． | － | ． | ． | ． | － | ． | ． | ． | A | ． | ？ | ？ | ？ | ？ | ？ | ？ | ${ }^{\text {ond＿1 }}$ |
| 17 | ． | ． | ． | ． | － | ． | ． | ． | T | T | ． | ． | ． | ． | ？ | ？ | ？ | ？ | ？ | ？ | ${ }^{\text {on }} 2$ |
| 18 | ． | ． | ． | ． | － | ． | ． | A | ． | ． | ． | ． | ． | ． | ？ | ？ | ？ | ？ | ？ | ？ | ${ }^{\text {J2＿3}}$ |

Table 2.2. Host gender estimates of Chelonibia caretta haplotypic diversity $h( \pm \mathrm{SD})$ and nucleotide diversity $\pi$ ( $\pm \mathrm{SD})$ for 16 S and COI, separately and combined (16S+COI).

| Gene | Host sex | \# Host (turtles) | \# (barnacles) | $\boldsymbol{h}$ | $\boldsymbol{\Pi}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
| 16 S | Female | 3 | 18 | $0.797(0.090)$ | $0.00311(0.00059)$ |
|  | Male | 6 | 16 | $0.742(0.084)$ | $0.00258(0.00051)$ |
|  | Total | 9 | 34 | $0.763(0.063)$ | $0.00284(0.00041)$ |
|  | Female | 3 | 15 | $0.476(0.155)$ | $0.00102(0.00038)$ |
|  | Male | 5 | 11 | $0.473(0.473)$ | $0.0016(0.00062)$ |
| Total | 8 | 26 | $0.468(0.118)$ | $0.0013(0.0004)$ |  |
|  | Female | 3 | 15 | $0.933(0.054)$ | $0.00213(0.00034)$ |
|  | Male | 5 | 10 | $0.822(0.097)$ | $0.00174(0.00038)$ |
|  | Total | 8 | 25 | $0.883(0.052)$ | $0.00198(0.00029)$ |
|  |  |  |  |  |  |

Table 2.3. Estimates of genetic diversity ( $\pi$ ) of barnacle species from the literature. When population information was available, the range of genetic diversity is provided. $\left(\mathrm{CR}^{\#}\right)$. Studies containing less than 5 sequences per species were not included. Studies that did not include GenBank submission number were not included. Sequences also include flanking rRNA and tRNA sequences.

| Species | $\pi$ | Gene | Source | Accession Numbers |
| :--- | :--- | :--- | :--- | :--- |
| Balanus glandula | 0.0113 to 0.0188 | COI | Sotka et al. (2004) | AY62954-AY630026 |
|  | 0.0072 to 0.0106 | COI | Wares et al. (2001) | AF234351-AF234462 |
| Chelonibia testudinaria | 0.0020 to 0.0080 | COI | Rawson et al. (2003) | AY174289-AY174367 |
| Chthamalus proteus | 0.0110 to 0.0230 | COI | Zardus \& Hadfield (2005) | AY822764-AY823025 |
| Chthamalus fissus | 0.0112 to 0.0144 | COI | Wares et al. (2001) | AF234463-AF234527 |
| Chthamalus dalli | $0.00072^{*}$ | COI | Wares \& Castañeda (2005) | AY795282-AY795480 |
| Chthamalus stellatus | $0.01226^{*}$ | Ef1-a | Shemesh/Achituv ${ }^{*}$ | AM396207-AM396225 |
| Chthamalus montagui | $0.00265^{*}$ | Ef1-a | Shemesh/Achituv ${ }^{*}$ | AM396226-AM396249 |
| Pollicipes pollicipes | 0.0319 to 0.0669 | CR ${ }^{\#}$ | Quinteiro et al. (2007) | AY939545-AY939779, EF029129-E0F29178. |
| Semibalanus balanoides | 0.0520 to 0.0870 | CO1 | Quinteiro et al. (2007) | EF032142-EF032154 |
|  | $0.00993^{\dagger}$ to $0.01569^{\dagger \dagger}$ | COI | Wares \& Cunningham (2001) | AF242660-AF242728 |

Table 2.3 (continued).

| Tetraclita pacifica | 0.0000 | ITS-1 | Chan et al. (2007a) | DQ363725-DQ363740 |
| :--- | :--- | :--- | :--- | :--- |
|  | $0.0431^{*}$ | 12S | Chan et al. (2007a) | DQ363711-DQ363717 |
| Tetraclita squamosa | 0.00860 to 0.01190 | COI | Chan et al. (2007a) | DQ363680-DQ363695 |
|  | $0.0000 *$ | ITS-1 | Chan et al. (2007a) | DQ363741-DQ363746 |
|  | 0.00667 | 12S | Chan et al. (2007a) | DQ363718-DQ363721 |
| Tetraclita singaporensis | 0.00771 | COI | Chan et al. (2007a) | DQ363696-DQ363706 |
| Tetraclita japonica | 0.01002 to 0.01289 | COI | Tsang et al. (2007) | EF035162-EF035167 |
|  | 0.03749 to 0.06475 | CR | Tsang et al. (2007) | DQ647704-DQ647742 |
| Tetraclita formosana | 0.00943 | COI | Tsang et al. (2007) | DQ645847-DQ645887 |
|  | 0.04789 | CR | Tsang et al. (2007) | DQ647743-DQ647768 |

$(\dagger) \pi$ value was generated by pooling all European populations together. $(\dagger \dagger) \pi$ value was generated by pooling all North America populations together. $\left({ }^{*}\right) \pi$ value was generated by pooling all sequences of the study because sampled populations consisted of less than 5 individuals. ( ${ }^{*}$ ) Unpublished manuscript. $\mathrm{CR}=$ control region, Ef1-a $=$ elongation factor 1 alpha.


Figure 2.1. Majority rule consensus tree $(\mathrm{L}=148, \mathrm{CI}=0.636, \mathrm{RI}=0.636)$ of Chelonibea caretta haplotypes, based on 291 most parsimonious trees using the maximum parsimony algorithm in MEGA4. The barnacle haplotypes are based on the concatenated sequences of COI+16S. Branches reproduced in less than $50 \%$ of the trees are collapsed.

## DISCUSSION

Genetic diversity is the most basic unit of ecosystem diversity, playing a central role on how populations/species respond to changing environments. Comparisons of genetic diversity levels between Chelonibia caretta (Table 2.2) with those reported from barnacle species settled on fixed substrates (e.g. rocks, piers, mangrove roots) reveals that the commensal barnacles harbor low levels of genetic variability (Table 2.3). Chelonibia caretta exhibited similar to slightly lower values of genetic diversity than the congeneric C. testudinaria (0.002-0.008, Table 2.3), but the latter commensal species was collected from a much wider geographic region and from a different turtle host species (Rawson et al. 2003). The mitochondrial gene COI is the most frequently sequenced gene in population studies of barnacles and comparisons of nucleotide diversities reveal that previously reported values are about an order of magnitude higher than those of C. caretta (Table 2.3). Nucleotide diversities from other mitochondrial and nuclear genes yield similar or higher genetic diversities. However, comparisons may not be appropriate since different genes are usually under different selection constraints.

The population dynamics of hosts and their epibionts should be tightly linked. Barnacles attached to fixed substrates are capable of dispersing during the larval stage whereas barnacles attached to mobile substrates (e.g. turtles, boats, driftwood) can disperse during both larval and adult stages. Because C. caretta is an obligate commensal of marine turtles, its dispersal potential should be influenced by the migratory behavior of its hosts, as it has been shown for the congener C. testudinaria (Rawson et al. 2003). The parsimony analysis and the molecular variance of the sampled barnacles indicate that they are genetically homogeneous, irrespective of the host or its gender. Such pattern of molecular variance suggests that barnacles settle on turtles
in mating and/or foraging areas, where both sexes can be found concomitantly. Alternatively, rates of gene flow between barnacle populations are high enough to result in panmixia. The nesting population of Mona Island is genetically distinct from other Caribbean rookeries (DíazFernández et al. 1999; Velez-Zuazo et al. 2008), but the hawksbills feeding in the adjacent coral reef habitats are genetically composed of turtles from geographically distinct rookeries (Bowen et al. 1996). Because our samples were collected from Mona Island (and only one from Culebra Island), absence of population structure may not be indicative of the demography of the Caribbean populations of the barnacle. Additional sampling of C. caretta from genetically distinct Caribbean rookeries will be sufficient to test this hypothesis. On the other hand, the complex life history of the hawksbill turtle with the ontogenetically onset migration, the repeated movements between foraging, breeding and nesting habitats and the large variation of migratory behavior between and within sexes (van Dam et al. 2008; Velez-Zuazo et al. 2008) may explain the absence of population structure in the commensal barnacles. Significant recruitment to foraging habitats of Mona Island from other Caribbean rookeries (Velez-Zuazo et al. 2008) exposes resident hawksbills to migrant hawksbills with epibiont loads from other Caribbean regions. If the exposure to allochthonous turtles through habitat sharing is long enough, then a mechanism is provided to genetically homogenize the epibiont barnacle populations. In the foraging habitats, cyprids from different geographic origins can settle equally on resident and non-resident feeding turtles regardless the host or its gender. The success of cyprid settlement on the host carapace depends on the density of the hosts and barnacles, and the period of exposure of the host to the barnacles (Zardus and Hadfield 2004). Based on ours results, the most likely time and place for cyprid settlement should be nearshore habitats where turtles aggregate to mate or forage.

The hawksbill sea turtle Eretmochelys imbricata has declined as much as $80 \%$ during the last century (Meylan and Donelly 1999) and has been listed as critically endangered by the International Union for Conservation of Nature (Baillie and Groombridge 1996). Any reductions of the host population will be accompanied by a reduction of the epibiont populations, since hawksbill turtles constitute the primary habitat. The hawksbill turtle populations in the Atlantic are depleted (Meylan 1999), and with an estimated female census size of 5,000 turtles in the Caribbean (McClenachan et al. 2006), the number of suitable turtle hosts/habitats for barnacles is now limited. Because turtle barnacles have unique life histories tightly linked to their host's life history, they are susceptible to the same demographic processes, such as rapid population declines. Even though the observed excess of singletons in the mtDNA of barnacles may be explained by a population expansion, this pattern represents a genetic footprint of the past, when host populations were abundant. Similarly, other marine species [e.g. the sea urchin Diadema antillarum (Lessios et al. 2001) and whales (Roman and Palumbi 2003)] that have undergone recent drastic declines, harbor patterns of genetic variation indicative of much higher past population sizes than the current ones.

The U.S. Endangered Species list is comprised primarily of host species; however, these species are interconnected or affiliated with many other organisms (predators, preys, parasites, commensals, etc.). As many as 6,300 affiliated species have been estimated to be co-endangered along with the host species (Koh et al. 2004), indicating how closely linked the evolutionary destinies of affiliate-host groups are. The Chelonibia caretta-Eretmochelys imbricata is an affiliate-host species pair where the host has been included in the Endangered Species List; therefore, we suggest that the barnacle C. caretta should also be considered as a candidate for listing due to drastic declines of its primary habitat.

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## CHAPTER 3

## Two distinct, geographically overlapping lineages of the corallimorpharian Ricordea florida (Cnidaria: Hexacorallia: Ricordeidae)


#### Abstract

As part of a research initiative to estimate the connectivity patterns of Caribbean benthic communities, we examined the genetic variation of the corallimorpharian Ricordea florida. This corallimorpharian is distributed throughout the Caribbean region and is heavily harvested for the marine aquarium trade. Eighty-four distinct individuals of R. florida were sequenced from four geographically distant Caribbean locations (Curaçao, Florida, Guadeloupe, and Puerto Rico). Analysis of the ribosomal nuclear region (ITS1, 5.8S, ITS2) uncovered two geographically partially overlapping genetic lineages in R. florida. Lineage 1 was found in Florida and Puerto Rico and Lineage 2 was found in Florida, Puerto Rico, Guadeloupe, and Curaçao. Because of the multi-allelic nature of the ITS region, four individuals from Lineage 1 and six from Lineage 2 were cloned to evaluate the levels of hidden intra-individual variability. Pairwise genetic comparisons indicated that the levels of intra-individual and intra-lineage variability (< $1 \%$ ) were approximately an order of magnitude lower than the divergence (up to $9 \%$ ) observed between the two lineages. Ricordea florida, highly traded in the marine aquarium industry, is composed of two distinct genetic lineages that probably represent two cryptic species. The two lineages of R. florida were compared phylogenetically against published nuclear ribosomal sequences of other genera of Corallimorpharia (Actinodiscus, Amplexidiscus, Corynactis, and


Rhodactis) and the two lineages composed a highly supported monophyletic group. The monophyly of genera is supported and promotes the utility of the ITS region as one of the markers in resolving phylogenetic relationships in Corallimorpharia. The importance of precise taxonomy is emphasized because incorrectly defined taxa may lead to overharvesting of commercially important species stemming from the overestimation of geographical distribution and population abundance.

## INTRODUCTION

Corallimorpharians are distributed throughout the tropical reefs world-wide (Carlgren 1949; Chadwick-Furman and Spiegel 2000) and can be a dominant component of tropical shallow water ecosystems (Chen et al. 1996). This group of corals lacks a calcareous skeleton (Carlgren 1949; Schmidt 1974; den Hartog 1980; Pinto and da Costa Belem 2000; Daly et al. 2003, 2007; Fautin et al. 2007) and can be found either as solitary colonies (den Hartog 1980) or as aggregations of colonies that cover extensive sections of a reef (Gerald and Roger 1994; Muhando et al. 2002). Corallimorpharians may have a habitat preference of dead coral substrates and they have ranked second in percent living cover in disturbed corals reef areas in Tanzania (Muhando et al. 2002). Their affinity to disturbed habitats has been further documented in the mid-Pacific Palmyra Atoll where a corallimorph invasion was reported around a shipwreck (Work et al. 2008). The close phylogenetic affinity of Corallimorpharia to both Actiniaria and Scleractinia, has generated a multitude of hypotheses on how to best accommodate phylogenetically the corallimorpharians. According to Daly et al. (2007), the order Corallimorpharia includes four families (Corallimorphidae, Discosomatidae, Ricordeidae, and Sideractiidae), nine genera (12 genera according to Fautin et al. 2007), and at least 24 valid
species. Most likely, the number of extant species is a gross underestimation of the true number of existing species. A multitude of datasets including multi-character analysis (Daly et al. 2003), radioimmunology (Fautin and Löwenstein 1992), nuclear and mitochondrial DNA data (Berntson et al. 1999; Cappola and Fautin 2000; Romano and Cairns 2000) suggested that corallimorpharians are more related to scleractinians (Fautin and Löwenstein 1994; Medina et al. 2006) than to the actiniarians as originally hypothesized by Chen et al. (1995a). A more recent study of 26 complete mitochondrial genomes highly supported the corallimorpharians as a sistertaxon to the scleractinians (Brugler and France 2007).

Corallimorpharians in the Caribbean benthic communities are represented by at least six recognized species (Corynactis parvula, Pseudocorynactis caribberum, Discosoma sanctithomae, D. carlgreni, D. neglecta, and Ricordea florida) that comprise the families Corallimorphidae, Discosomatidae, and Ricordeidae (den Hartog 1980). Probably the most abundant Caribbean corallimorpharian is Ricordea florida Duchassaing and Michelotti, 1860 which is distributed throughout the region. The ecological importance of $R$. florida has not been well studied but is highly preyed upon by the hawksbill turtles in SW Dominican Republic (León and Bjorndal 2002) where R. florida consisted of up to $59 \%$ of stomach contents. In contrast, the commercial importance of $R$. florida is increasing because the species is among the most heavily harvested invertebrates in the marine aquarium trade.

The change in marine aquarium keeping, from fish tanks to reef mesocosms, has created a high demand for corallimorpharians and other "soft corals" (Rhyne et al. accepted). Ricordea is supplied to the aquarium trade from both a domestic fishery (Florida, USA) (Figure 3.1) and from imported specimens originating from Haiti. The recent spike in domestic collection is disconcerting when one takes into account changes in the management of the Florida marine life
fishery that took effect in July 2009 (FWC 2009). The ban of live rock collections in 1996 criminalized reef substrate collection in the Florida Keys National Marine Sanctuary (FKMNS). Because small quantities of substrate are removed with polyps, this ban on substrate effectively dampened fishing pressure on R. florida and other corallimorpharians. Fisherman risked fishery violations for possessing any reef substrata, including incidental amounts that were attached to anemones, zooanthids, or corallimorpharians. Recent regulatory changes and agreements between Florida Fish and Wildlife Commission and FKMNS allow fisherman to legally harvest small amounts of substrate (FWC 2009). These changes along with higher demand have created a perfect storm of demand and legality for a species of which we have very little knowledge of its reproductive biology, distribution, dispersal or genetic structure.

In order to assess the population structure of traded marine ornamental species we have recently begun analyzing the genetic structure of those that are heavily harvested. Here we report on the genetic divergence observed in the corallimorpharian R. florida. Using partial sequences of the internally transcribed spacer region (including ITS1, 5.8S, and ITS2), the present study investigated the genetic variability of $R$. florida in order to identify possible biogeographic genetic boundaries among populations across the sampled Caribbean area. We also evaluated the use of the ITS region in resolving phylogenetic relationships in Corallimorpharia.

## MATERIALS AND METHODS

Samples were collected by SCUBA from four Caribbean locations: Puerto Rico ( $n=46$ ), Florida ( $\mathrm{n}=28$ ), Curaçao $(\mathrm{n}=7)$, and Guadeloupe $(\mathrm{n}=3)$ during 2006 and 2007. Hereafter, samples
from each location will be considered as different populations. Specimens were either transported to the laboratory alive or preserved in $100 \%$ ethanol. Total genomic DNA was extracted using a DNA Easy kit (Qiagen, Inc. Valencia, CA) following the recommended protocol by the manufacturer. DNA extractions from ethanol preserved specimens yielded better quality template for PCR than those from living specimens. Presumably the large amounts of mucous excreted by the live Ricordea florida interfered with the PCR. The amplification of the ITS region was performed with the primers A18S (GATCGAACGGTTTAGTGAGG; Takabayashi et al. 1998) and ITS-4 (TCCTCCGCTTATTGATATGC; Meroz-Fine et al. 2003). The amplification profile began with a denaturation step at $95^{\circ} \mathrm{C}$ for 3 min , followed by 40 cycles of $95^{\circ} \mathrm{C}$ for $15 \mathrm{sec}, 45^{\circ} \mathrm{C}$ for 30 sec and $72^{\circ} \mathrm{C}$ for 1 min , ending with an extension step at $72^{\circ} \mathrm{C}$ for 5 min . Some DNA templates that were difficult to amplify were further cleaned by a PCR Clean Up Kit (Qiagen, Inc., Valencia CA). Amplicons were visualized by electrophoresis on a $1 \%$ agarose gel, and cleaned using the ExoSap-IT (USB Corporation, Cleveland, OH) procedure. PCR reactions produced from 10 randomly selected individuals were ligated into the pGEM-T Easy Vector (Promega). Plasmid DNA was isolated according to a QIAprep® Spin Miniprep protocol (Qiagen, Inc., Valencia CA). Multiple molecular clones from each individual were sequenced using the vector primers T7 and SP6. Sequence reactions were loaded in an ABI 3130xl Genetic Analyzer. The resulting DNA sequences were verified by aligning reads from both 5' and 3' directions, using the software CodonCode Aligner and further cleaning and end trimming was done with MacClade (Maddison and Maddison 2000). DNA sequences were aligned in ClustalX using the default parameters for multiple alignments (Larkin et al. 2007). The haplotypic (h) and genetic diversity ( $\pi$ ) (Nei 1987) of $R$. florida was estimated in DNAsp (Librado and Rozas 2009). Statistical parsimony networks were built in TCS (Clement et al.
2000) with the connection limit set at $95 \%$. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) in Arlequin 3.1 (Excoffier et al. 2005) was applied to test if population differentiation could be detected among populations. The significance of $\Phi$-statistics was assessed by 10,000 permutations of groups and haplotypes. Pairwise $F_{\text {ST }}$ values were estimated between populations of each lineage of $R$. florida as calculated in Arlequin.

Phylogenetic trees of selected Corallimorpharia (sensu Daly et al. 2007, Fautin et al. 2007) were reconstructed with the Neighbor-Joining (NJ, Saitou and Nei 1987), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods in PAUP (Swofford 2002). Bayesian analysis was performed in MrBayes v3.0b4 (Ronquist and Huelsenbeck 2003). The best-fit substitution model for the Bayesian analysis was selected by the Akaike Information Criterion in ModelTest v3.06 (Posada and Crandall 1998). The fast-heuristic method was used for MP. Nodal support for the NJ and MP trees was estimated by 1,000 bootstrap replicates (Felsenstein 1985). Bayesian analysis was run for $1,000,000$ generations, four independent chains, sampling every 1000 generations and discarding $15 \%$ of the sampled trees. Phylogenetic trees were built with the original alignment output from ClustalX using the default multiple alignment parameters and a more conservative alignment based on alignment criteria in the software Gblocks (Castresana 2000). Ribosomal regions are notorious for the degree of difficulty to obtain a homologous alignment and removing blocks of ambiguous alignment quality has been used successfully in the past (Porter et al. 2005). The Gblocks conditions were: minimum number of sequences for a conserved position 7, minimum number of sequences for a flanking position 7, maximum number of contiguous non-conserved positions 8 , minimum length of block 5 , and allowed gap positions with half. Corrected pairwise genetic distances between species and between genera were estimated in PAUP.

## RESULTS

Eighty-four direct sequences of the ITS region from four populations of Ricordea florida were obtained and were collapsed into seven haplotypes (Table 3.1). The Florida population had three haplotypes $\left(\mathrm{H}_{\mathrm{d}}=0.5741 \pm 0.0785\right.$ and $\left.\pi=0.3815 \pm 0.0192\right)$, Puerto Rico had five haplotypes $\left(\mathrm{H}_{\mathrm{d}}=0.2068 \pm 0.0791\right.$ and $\left.\pi=0.0068 \pm 0.0038\right)$, Guadeloupe had two haplotypes $\left(\mathrm{H}_{\mathrm{d}}=\right.$ $0.6667 \pm 0.3143$ and $\pi=0$ ), and Curaçao had 1 haplotype (Table 3.1). The amplified rDNA fragment contained a portion of the $3^{\prime}$ end of $18 \mathrm{~S}(20 \mathrm{bp})$, the ITS1 region (244-255 bp), all of $5.8 \mathrm{~S}(164-167 \mathrm{bp})$, the ITS2 region (196-241 bp) and a portion of the $5^{\prime}$ end of $28 \mathrm{~S}(15 \mathrm{bp})$ (Table 3.2). The sequenced region varied in length because of insertions/deletions. The G+C contents of the three regions varied between 49 to $63.3 \%$ (Table 3.2). The sequences were verified against the corallimorpharian sequences published by Chen et al. (1995a). The presence of multiple copies of ITS within individuals was confirmed with the presence of 15 ambiguities sites in the direct sequences. In order to evaluate the levels of intra-individual variability if the ITS region, up to fourteen clones from each of the 10 individuals of $R$, florida were sequenced from the PCR products (GenBank Accession Numbers: GQ465130-GQ465201).

Statistical parsimony analysis of seven haplotypes of the ITS region resulted in two disconnected networks (Figure 3.2), representing hereafter Lineage 1 and 2. The two highly divergent lineages (Lineage 1 and 2) of $R$. florida, attain partially overlapping distribution over the sampled region (Figure 3.2). Lineage 1 was detected in Florida and Puerto Rico and Lineage 2 was detected in Florida, Puerto Rico, Guadeloupe, and Curaçao (Figure 3.2). Cloning of the ITS region revealed some of the genetic diversity harbored within individuals and resulted in
higher levels of divergence within both lineages compared to estimates of divergence resulted from direct sequences alone (Table 3.3). However, the pairwise distance between lineages (8.8$9.1 \%$ ) was much higher than between individuals within each lineage ( 0.2 to $0.9 \%$ ), when using either the cloned sequences alone (Figure 3.3) or direct sequencing (Table 3.3). The reported divergence values are not likely an artifact of Taq cloning errors since the error estimates (about $0.01 \%$; Eckert and Kunkel, 1990) are much lower than our reported values. The withinindividual pairwise sequence divergence ranged from 0.5 to $2 \%$ in ITS1, $0.3-1.3 \%$ in 5.8 and $0.5-1.3 \%$ in ITS2. The levels of variation among lineages were 7.5-10\% in ITS1, 5.3-9.3 \% in 5.8 and $6.9-9.3 \%$ in ITS2. Lineage 2 showed higher values of nucleotide $(\pi)$ and haplotype diversity $\left(\mathrm{H}_{\mathrm{d}}\right)$ than Lineage 1 (Table 3.2). The Tajima's D test (Tajima 1989) indicated that the sequences of both lineages are evolving according to the neutral model of evolution (Table 3.2). Analysis of molecular variance indicated strong structure in the data set ( $\Phi_{\mathrm{ST}}=0.9965, \mathrm{P}<$ 0.0001 ). The vast majority of molecular variance ( $>99 \%$ ) was partitioned between the two lineages (Table 3.4). No population structure was detected within each lineage. Accordingly, none of the pairwise $F_{\mathrm{ST}}$ values between populations in each lineage were significant (Table 3.5).

The two lineages of $R$. florida were compared phylogenetically against published sequences of other species of Corallimorpharia. We obtained ITS sequences from GenBank for Actinodiscus nummiformis (AB441417), Amplexidiscus fenestrafer (AB441418), Corynactis californica (AB441415), Rhodactis bryoides (AB441416), and the scleractinian Montastraea annularis (AB065329). For the phylogenetic analysis of the alignment processed by Gblocks, the K81uf $+\mathrm{I}+\mathrm{G}$ model of substitution was applied with the following settings: Lset Base $=(0.2140$ $0.26760 .3073), \mathrm{Nst}=6$, Rmat $=(1.00001 .69131 .44631 .44631 .6913)$, Rates $=$ G, Shape $=$ 1.7690, and Pinvar $=0.3236$. For the phylogenetic analysis based on ClustalX alignment, the

TVM $+\mathrm{I}+\mathrm{G}$ model of substitution was applied with the following settings: Lset Base $=(0.2254$ $0.25630 .3070), \mathrm{Nst}=6$, Rmat $=(1.46791 .51161 .55991 .35561 .5116)$, Rates $=$ G, Shape $=$ 2.0177, and Pinvar $=0.2286)$.

Genealogies of tropical corallimorpharians inferred by maximum parsimony, maximum likelihood, neighbor-joining and Bayesian analyses showed well-supported nodes separating genera and species (Figs. 4a, b). The genera Actinodiscus, Amplexidiscus, Rhodactis and Ricordea appear monophyletic regardless the alignment procedure. The most represented family in the trees is Discosomatidae (sensu Daly et al. 2007) which is not recovered as a monophyletic group in either of the trees (Figs. 4a, b). The genus Actinodiscus is grouped with the corallimorphiid Corynactis californicus in the tree resulted from the Gblock alignment, however the clade is weakly supported (Figure 3.4a). In the phylogenetic analysis based on the default ClustalX alignment, the genus Actinodiscus was placed as a sister tax on Ricordea, however this placement was supported only by the Bayesian analysis (Figure 3.4b). The position of Actinodiscus is sensitive to the alignment conditions, therefore the monophyly of the family Discosomatidae should be examined with more genes. Consistently, the genera Rhodactis and Amplexidiscus are grouped together. Additionally, the two existing ITS sequences of Actinodiscus nummiformis are highly divergent (79\%). Since none of the previous studies (Chen et al. 1996; Fukami et al. 2008) cloned the ribosomal region of $A$. nummiformis, it is possible that one of these two known sequences is a highly divergent variant of the multiallelic region or that A. nummiformis is a species complex. The divergence observed between the two R. florida lineages ( $8.2 \%$-using only direct sequencing) is four to nine times less than the divergence (31.19-75.96\%) of the two lineages against other corallimorpharian genera (Table 3.5). The
smallest divergence is observed against Corynactis sp. and the largest divergence is observed against Actinodiscus sp.

Table 3.1. Locations, sampling size and accession numbers per haplotype of Ricordea florida.

|  | Locations | Sampling Size | GenBank Accession Numbers |
| :---: | :---: | :---: | :---: |
| Haplotype 1 | Florida Bay, Florida | 17 | GQ465046-GQ465062 |
|  | La Parguera, Puerto Rico | 41 | $\begin{aligned} & \text { GQ465063 - GQ465079 } \\ & \text { GQ465082 - GQ465103 } \\ & \text { GQ465105 - GQ465106 } \end{aligned}$ |
| Haplotype 2 | La Parguera, Puerto Rico | 1 | GQ465080 |
| Haplotype 3 | La Parguera, Puerto Rico | 1 | GQ465081 |
| Haplotype 4 | La Parguera, Puerto Rico | 1 | GQ465104 |
| Haplotype 5 | La Parguera, Puerto Rico | 2 | GQ465107-GQ465108 |
|  | Curaçao | 7 | GQ465109 - GQ465115 |
|  | Guadeloupe | 2 | GQ465116-GQ465117 |
|  | Hawk Channel, Florida | 6 | $\begin{aligned} & \text { GQ465120 - GQ465122 } \\ & \text { GQ465124 - GQ465126 } \end{aligned}$ |
| Haplotype 6 | Guadeloupe | 1 | GQ465118 |
| Haplotype 7 | Hawk Channel, Florida | 5 | $\begin{aligned} & \text { GQ465119, GQ465123 } \\ & \text { GQ465127-GQ465129 } \end{aligned}$ |

Table 3.2. Size of sequenced region by gene (bp), G+C content, and nucleotides diversity of the ITS region of Ricordea florida clones and other tropical corallimorpharians. Values from other corallimorpharian species are extracted from Chen et al. (1996).

|  | ITS-1 |  |  |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Species | Length <br> $(\mathrm{bp})$ | $\mathrm{G}+\mathrm{C}$ <br> $(\%)$ | $\pi \pm \mathrm{SD}$ | 5.8 S <br> $(\mathrm{bp})$ | Length <br> $(\%)$ | ITS2 | Length <br> $(\mathrm{bp})$ | $\mathrm{G}+\mathrm{C}$ <br> $(\%)$ | $\pi \pm \mathrm{SD}$ |
| Ricordea florida |  |  |  |  |  |  |  |  |  |
| Lineage 1 | $250-255$ | 55.4 | $0.0064 \pm 0.0012$ | $164-165$ | 49.6 | $0.0078 \pm 0.0022$ | $196-197$ | 63.3 | $0.0060 \pm 0.0013$ |
| N | 27 |  |  | 29 |  |  | 39 |  |  |
| Lineage 2 | $244-248$ | 54.8 | $0.0152 \pm 0.0023$ | $165-167$ | 49.2 | $0.0064 \pm 0.0021$ | $237-243$ | 062.6 | $0.0075 \pm 0.0021$ |
| N | 15 | ND | ND | 39 | ND | ND | 25 | 190 | 65.1 |

Table 3.3. Summary of DNA statistics in Ricordea florida using direct sequences. Multiallelic sites were treated as degenerative sites in Arlequin and gaps were regarded as a $5^{\text {th }}$ character. $\mathrm{N}=$ number of sequences, $\mathrm{S}=$ segregating sites, $\mathrm{h}=$ number of haplotypes, $\mathrm{SD}=$ Standard Deviation.

|  | N | S | h | $\mathrm{Hd} \pm \mathrm{SD}$ | $\pi \pm \mathrm{SD}$ | Tajima's D (P value) |
| :--- | :---: | :---: | :---: | :---: | :--- | :--- |
| Lineage 1 | 61 | 1 | 4 | $0.0967 \pm 0.0518$ | $0.0002 \pm 0.0003$ | $-1.3153(0.0510)$ |
| Lineage 2 | 23 | 1 | 3 | $0.4229 \pm 0.1041$ | $0.0256 \pm 0.0133$ | $0.9810(0.8100)$ |

Table 3.4. Summary of analysis of molecular variance (AMOVA) based on the ITS region of Ricordea florida (corrected by the Tamura-Nei distance).

| Source of variation | d.f. | Variance <br> components | $\%$ of <br> variance |
| :--- | :---: | :---: | :---: |
| Between lineages | 1 | 26.9356 | 99.57 |
| Among populations <br> within lineages | 4 | 0.0234 | 0.09 |
| Within populations <br> within lineages | 78 | 0.0921 | 0.34 |

Table 3.5. Corrected (Tamura-Nei distance) pairwise $F_{\text {ST }}$ values between populations of each lineage of Ricordea florida. L1 = Lineage 1, L2 = Lineage 2.

| Pairs of Sample locations | $F_{\text {ST }}$ |
| :--- | :---: |
| PR_L1 X FL_L1 | -0.0174 |
| PR_L2 X FL_L2 | 0.1081 |
| PR_L2 X Guadeloupe | 0.0000 |
| PR_L2 X Curaçao | 0.0000 |
| FL_L2 X Guadeloupe | 0.1955 |
| FL_L2 X Curaçao | 0.3285 |
| Guadeloupe X Curaçao | 0.0000 |

Table 3.6. Corrected sequence divergence $(\mathrm{F} 81+\mathrm{I}+\mathrm{G}, \mathrm{I}=0.2513, \mathrm{G}=2.3276)$ between Ricordea florida lineages and other corallimorpharians. The scleractinian Montastraea annularis was included for comparison. $\mathrm{L} 1=$ Lineage 1 .

| Average divergence between taxa |  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Lineage1 | $36.27 \%$ | $39.22 \%$ | $57.29 \%$ | $31.19 \%$ | $45.14 \%$ |  |
| Lineage2 | $41.01 \%$ | $45.14 \%$ | $75.96 \%$ | $37.58 \%$ | $47.66 \%$ | $8.20 \%$ |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  | Average divergence within taxa |  |  |  |  |  |
|  | Rhodactis | Amplexidiscus | Actinodiscus |  |  |  |
|  | $27.30 \%$ | $28.26 \%$ | $68.59 \%$ |  |  |  |



Figure 3.1. Reported landings and number of licenses for the Ricordea florida fishery from 2002-2008. Bars are number of licenses reporting landings and circles are number of landings reported. Solid line represents the linear regression line of landings over time. Data from the Florida Fish and Wildlife Commission Trip Ticket Program (modified from Rhyne et al. 2009).


Figure 3.2. Haplotype networks of the two lineages of Ricordea florida based on the ITS region. Unique haplotypes are shown as different ovals, and the size of the shape is proportional to the number of $R$. florida sharing this haplotype. The small solid circles between haplotypes represent mutational steps between alleles. Sampled locations are denoted by small ovals and geographic ranges of the two lineages are loosely depicted by the large clear ovals.


Figure 3.3. Average pairwise distances within and between lineages, with and without cloning. The genetic distances have been corrected by the Tamura-Nei model of substitution. Error bars denote 1 standard deviation.


Figure 3.4. A. Bayesian phylogeny of selected corallimorpharia based on the ITS region using the Gblocks alignment. Clade support values are shown along the corresponding branches (Maximum Parsimony/NJ/Maximum likelihood/Bayesian). NS = Non-supported clade, $\mathrm{GB}=\mathrm{DNA}$ sequences downloaded from GenBank.


Figure 3.4. B. Bayesian phylogeny of selected corallimorpharia based on the ITS region using the default alignment parameters in ClustalX. Clade support values are shown along the corresponding branches (Maximum Parsimony/Neighbor Joining/Maximum Likelihood/Bayesian). NS = Non-supported clade, GB = DNA sequences downloaded from GenBank.

## DISCUSSION

The genealogical analysis of the ITS region uncovered the presence of two distinct lineages in R. florida. The two lineages appeared to have a partially overlapping distribution (Figure 3.2). Lineage 1 was found in Florida and Puerto Rico while Lineage 2 was found in Florida, Puerto Rico, Guadeloupe, and Curaçao. The sampling regime emphasized the eastern Caribbean and since R. florida is distributed throughout the Caribbean region (den Hartog 1980) the suggested distribution of the lineages is preliminary. The AMOVA results indicated the absence of geographical sorting of the $R$. florida specimens within Lineage 1 and 2. In both lineages, identical or near-identical haplotypes were found on all locations where the lineages were sampled. Restricted gene flow has been discovered in three important scleractinian corals across large geographic scales (>500 km or east vs. west Caribbean; Fukami et al. 2004; Baums et al. 2005; Vollmer and Palumbi 2007), however, there are also coral species that exhibit no population structure (e.g. Montastraea carvenosa, Nunes et al. 2009). Significant population differentiation was observed between Acropora cervicornis from Bahamas and Curaçao (Vollmer and Palumbi 2007). In the contrary, no population subdivision was detected between $R$. florida samples of Lineage 2 between south Florida, which is relatively close to Bahamas, and Curaçao. The lack of evident geographic barriers in the region may allow high gene flow between populations, resulting in genetically homogeneous populations. Alternatively, the ITS region may not be an appropriate marker to evaluate the hypothesis of population structure within each lineage, at least in the sampled region. Even though the ITS sequences were useful to distinguish the two lineages, within each lineage, the levels of intraindividual variation were as high as those observed between individuals.

In the most heavily sampled location (La Parguera, southwestern Puerto Rico), Lineage 1 was predominantly more abundant. Five La Parguera reefs were sampled extensively and Lineage 1 of $R$. florida was recorded in all sampled reefs except Pinnacle Reef. Even though the geographic distance between the sampled reefs in La Parguera is small (about 5-8 km), Lineage 2 of $R$. florida was detected only in Pinnacle Reef ( $17^{\circ} 55.768^{\prime} \mathrm{N}-$ $67^{\circ} 00.704^{\prime} \mathrm{W}$ ). No obvious barriers or ecological differences were detected to explain the differential distribution of the two lineages within La Parguera. Alternatively, since we randomly sampled reefs in La Parguera, we may have missed locations where colonies of both lineages inhabit the same reef.

Ricordea florida is capable of sexual reproduction and asexual reproduction through basal expansion and longitudinal fission (den Hartog 1980). A shift in the reproductive strategies of R. florida was observed as depth increased (den Hartog 1980). Specimens collected between 10 to 15 m depth were either solitary or aggregated but well spaced from each other. Den Hartog (1980) interpreted the low densities of deeper R. florida as the result of slow rates or even absence of asexual reproduction. Ricordea florida is commonly found in dense numbers resembling carpets ( 10 s to 100 s of colonies) in shallow waters, indicating that asexual propagation is the predominant form of reproduction. Additionally, den Hartog (1980) detected larvae within the lumen of tentacles of isolated colonies and suggested that $R$. florida occasionally is viviparous (brooder?) in deeper waters. Although the process of sexual reproduction in R. florida remains understudied, studies in other corallimorpharians have revealed two variations of synchronous spawning: those that all polyps in each clonal group are the same sex (e.g. Corynactis californica, Holts and Beauchamp (1993)), and those that the small polyps at the edge of the colony are male, where the central larger polyps are female (e.g. Rhodactis, Chen et al. 1995b; Chadwick-Furman et al. 2000). The sampled colonies of Lineage 2 in Puerto Rico, Guadeloupe and Curacao were all distinct polyps (cm
to 1 m apart) where those of Lineage 1 in Puerto Rico and Florida were forming aggregation or mats. The colonies of Lineage 2 exhibited khaki-colors rather than the bright colors (e.g. orange, red and bright green) usually seen in $R$. florida. All sampled colonies of this study were collected from similar depths ( 3 to 8 m ), therefore further ecological work is necessary to determine if depth covaries with the mode of reproductive strategy and degree of aggregation. Genetic divergence is not necessarily coupled with obvious morphological divergence (as cryptic or sibling species complexes are continuously being discovered in the marine environment (Knowlton 1993, 2000). However, after very careful re-examination of morphological characters, it is possible to detect overlooked subtle morphological differences between genetic lineages (Carlon et al. 2002; Prada et al. 2008)

Ricordea florida is a complex of at least two distinct genetic lineages that do not exchange genetic material despite living in sympatry, at least in the sampled range. Even in the seemingly homogeneous marine environment, there are ample opportunities for genetic differentiation that may lead to speciation (Palumbi 1992, 1994). Disruption of gene flow in proximate populations and sympatric or parapatric speciation by ecological differentiation (Doebeli and Dieckmann 2003) is possible in the marine environment where very steep environmental gradients exist (e.g. depth, light, salinity). Ecological specialization to different habitats (Knowlton and Jackson 1994; Carlon et al. 2002; Prada et al. 2008) and asynchronous spawning (Knowlton et al. 1997; Levitan et al. 2004) may prevent individuals to reproduce randomly. With the absence of genetic exchange, spatially or temporally separated populations may diverge through random (i.e. genetic drift) or deterministic processes such as positive Darwinian selection in gamete recognition proteins (Vacquier et al. 1997; Palumbi 1998; Hellberg et al. 2000). In Caribbean corals, diversifying selection driven by depth has been proposed in at least two species, Favia fragum (Carlon et al. 2002) and Eunicea flexuosa (Prada et al. 2008). Similarly, depth preference may be a factor driving the
divergence of $R$. florida. Detailed depth distribution, molecular identification of each $R$. florida colony and transplant experiments are needed to evaluate the differential depth hypothesis. Apart from sympatric divergence, allopatric divergence is also plausible. The distinct Ricordea lineages in sympatry could be the result of secondary contact of once allopatrically diversified populations. A broader sampling effort could indicate if the two lineages coexist over a wider range or they attain partially overlapping distribution in the Caribbean.

The ITS region is one of the most widely use markers in molecular studies in corals (reviewed in Vollmer and Palumbi 2004, in addition: Pillay et al. 2006; Wei et al. 2006; Reimer et al. 2006, 2007a, b; Aguilar and Sánchez 2007; Forsman et al. 2009). This genetic marker has been used as an alternative marker for mtDNA as studies in anthozoans (France and Hoover 2001, 2002; Shearer et al. 2002; McFadden et al. 2004, 2006; Concepcion et al. 2008) have demonstrated the lack of resolution of mtDNA to discriminate species within many genera. Contrary to the mtDNA of cnidarians, nuclear DNA seems to accumulate mutations at a rate similar to other animal groups (Hellberg 2006). The ribosomal nuclear region undergoes homogenization through the process of concerted evolution (Hillis and Dixon 1991, Elder and Turner 1995), but if the homogenization is not rapid enough, high levels of intragenomic polymorphism could exist (Vogler and DeSalle 1994; Odorico and Miller 1997; Harris and Crandall 2000), rendering the use of ITS problematic (Coleman 2003). Molecular cloning should be used to understand the abundance of variant ITS regions within genomes of the same species, because direct sequences from PCR alone represent only a fraction of the ITS variants (Litaker et al. 2007).

The increasing number of investigations of the ITS regions has revealed a wide range of divergence levels within different anthozoans, from $2 \%$ variation in the Montastraea annularis complex (Medina et al. 1999) to $70 \%$ in Zoanthus spp. (Reimer et al. 2006). The

Ricordea florida complex showed an inter-lineage divergence an order of magnitude higher $(8.8-9.1 \%)$ than between individuals within each lineage ( 0.2 to $0.9 \%$ ). There are cases where the application of ITS regions is problematic (e.g. in resolving the genetic signature of reticulation in the Caribbean Acropora; Vollmer and Palumbi 2004), however, there are several more studies where ITS was used successfully to delineate species relationships (e.g. Chen and Miller 1996; Forsman et al. 2005, 2009; Pillay et al. 2006; Wei et al. 2006; Reimer et al. 2006; Reimer et al. 2007a, b) and document hybridization (Odorico and Miller 1997; McFadden and Hutchinson 2004). Undoubtedly though, the use of ITS will diminish as more nuclear markers become accessible to coral researchers.

The application of molecular techniques in the genetic boundaries of cnidarians, has resulted in the discovery of cryptic or sibling species complexes, previously undetected by traditional techniques (reviewed in Knowlton 1993, 2000) and more recent studies in Aurelia aurita (Dawson and Jacobs 2001), Favia fragum (Carlon et al. 2002), Palythoa (Reimer et al. 2007a), and Eunicea flexuosa (Prada et al. 2008). Determining the species boundaries provides the basis for formal taxonomic evaluation or for testable hypotheses concerning the diversity, distribution, population connectivity and phylogeny studies. By unknowingly including species complexes in biological studies, the results may be severely jeopardized or even invalid. While the discovery of cryptic species in the marine environment (reviewed by Knowlton 1993, 2000) is not unusual any longer, the presence of possible cryptic species in a commercially important species is significant. The polychaete Capitella capitata, which was considered to be one of the most sensitive pollution indicators, eventually proved to be a complex of several cryptic species (Grassle and Grassle 1976). Two other model organisms, the oligochaetes Lumbriculus variegatus (Gustafsson et al. 2009) and Tubifex tubifex (Sturmbauer et al. 1999) were found to consist of multiple species. Siddall et al. (2007) questioned the taxonomic integrity of the medicinal leeches and indicated that the
commercially available leeches are not Hirudo medicinalis, as has been assumed by the biomedical community for decades. The consequences of such basic taxonomic oversights may range from invalidation of results from a large number of studies to indiscriminate harvesting of unknown number of species to endangering public health.

## Phylogenetic issues in Corallimorpharia

Den Hartog (1980) speculated that the genus Ricordea exhibits intermediate morphologies between Corallimorphidae and Discosomatidae. Ricordea florida shares with the corallimorphiids the overall shape of the cnidom, simplicity of tentacles, and resembles discosomatiids in the number, size and motility of tentacles, and the increasing size of marginal tentacles. The topologies based on the ITS region (Figs. $4 \mathrm{a}, \mathrm{b}$ ) reflect to a degree the intermediateness of Ricordea since in neither of the trees a clear relationship was extracted among the three represented families (Ricordeidae, Discosomatidae, and Corallimorphidae). Systematic discrepancies (e.g. the grouping of Corynactis californica with the genus Actinodiscus -Figure 3.4a) is supported by a very low value of posterior probability with Bayesian inference, a weak bootstrap value in MP and not supported by NJ and ML. In Figure 3.4b, the genus Actinodiscus was the sister taxon of Ricordea however, the clade is supported only with Bayesian inference. A denser taxon sampling and additional genes will provide additional insights on the relationships of the three families. The ITS region proved to be appropriate to address questions at the genus level, as all genera were monophyletic.

## Ecological implications and the use of Ricordea florida in the marine ornamental trade

If the goals of conservation biology are to include the evolutionary potential of species then knowledge of basic biology and quantification of the genetic diversity and divergence within species, is integral to any informed management policy. A large increase in fishing pressure has occurred for R. florida (Figure 3.1), yet there is little information
available for managers to determine acceptable harvest limits. While Florida has a management plan, which include a license cap and bag limits most species are well below the total allowable catch (Rhyne et al. 2009). Ricordea florida provides an example of a species in which a bag limit was recently established but which the number of license reporting landings is far below the capped limit. This could result in an uncontrolled demand regulated fishery (Rhyne et al. 2009).

Are the two distinct lineages of Ricordea florida biologically meaningful for conservation? Moritz (2002) has argued that identification of distinct genetic lineages of a species is important to preserve genetic diversity in the conservation of species. If they are reproductively isolated species, each species should be managed separately by the marine aquarium trade. Even if they are not different species, genetically differentiated units should not be overlooked and deserve different management (Moritz 1995). The current findings highlight the assertion that genetic diversity and the number of marine species may be grossly underestimated. The discovery of cryptic species is especially troublesome when it pertains to commercially important groups.

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## CHAPTER 4

# Caribbean-wide population structure of the emerald crab Mithraculus sculptus 


#### Abstract

Natural populations are dynamic, they change over time in size, density, and location. Population distribution of marine species is determined by stochastic (e.g. rare migration events) and deterministic (e.g. larval stage duration) processes. Such knowledge is usually absent in commercially important species, especially those targeted by the marine aquarium industry. Using direct sequencing of the mitochondrial cytochrome c oxidase subunit I (COI) and 16 S ribosomal DNA ( 16 S rDNA) genes, we characterized the phylogeography and genetic population structure of the green emerald crab Mithraculus sculptus, the most heavily traded decapod species in the marine ornamental trade in the USA. Analysis of Molecular Variance (AMOVA) suggested that most of the genetic variation is observed within populations rather than among populations for both mitochondrial genes. As much as $91 \%$ (16S) and $99 \%$ (COI) of the variance was allocated within populations. The among population component of variance was $9 \%$ and $1 \%$ for COI and 16 S , respectively. The phylogeographic analysis showed two distinct lineages distributed throughout the sampled region for both genes. The $\Phi_{\mathrm{ST}}$ values and the phylogeographic analyses of the sampled populations of $M$. sculptus reflected that most of the sample localities are part of a demographically open population within the western Caribbean. The only exception appears to be Curaçao which harbors a genetically differentiated population of M. sculptus. Implications of increasing fishing pressure from the aquarium industry are discussed.


## INTRODUCTION

Fishing pressure has traditionally been defined as collecting organisms for human consumption. However, the growing marine ornamental trade now takes a strikingly large number of species from coral reefs. Over 1,200 species of fish and invertebrates are harvested from throughout the world fueling a multi-billion dollar industry (Rhyne et al. 2009). In an effort to assess the numbers and species traded, United Nations Environment Programme World Conservation Monitoring Centre (UNEP-WCMC) produced a report entitled "From Ocean to Aquarium" in which the trade was broken down by groups of animals (fish, corals, and non-coral invertebrates) and countries importing and exporting animals (Wabnitz et al. 2003). From the period of 1994 until 2005 a total of 54.7 million invertebrates were collected for the marine aquarium trade in the state of Florida alone. The increased catch is directly related to the demand from marine aquarium trade. It has been estimate that more than $4 \%$ of homes now have marine aquaria, compared to only about $1 \%$ in 1982 (Chapman et al. 1997).

Despite the dramatic changes of the fishery worldwide, for most traded invertebrates reproductive age, growth rate, population density, distribution, and population connectivity are unknown or poorly documented. The lack of such basic knowledge on harvested species is alarming given that it is rather common, with the application of molecular techniques, to discover distinct populations or cryptic or sibling species complexes, that have gone previously undetected by traditional methods [reviewed in Knowlton (1993, 2000)]. Indeed, the lack of ability to detect distinctive populations in the marine environment has led to the prevailing hypothesis that marine populations are mostly panmictic (Palumbi 1992, 1994; McQuinn 1997). This view has been strenghtened by the observation that most marine species have planktonic larvae with long-distance dispersal potential (Waples 1998). The application of high-resolution genetic markers have promoted the detection of genetic structure in organisms with high dispersal potential (Taylor and Hellberg 2003, Wirth and

Bernatchez 2001; Maes and Volckaert 2002). The redfish Sciaenops ocellatus of the northern Gulf of Mexico present a genetic differentiation explained by the isolation by distance model (Wright 1943, Gold and Richardson 1999) not easy to detect by traditional methods.

The emerald crab Mithraculus sculptus (Decapoda: Branchyura: Majidae), is a spider crab found in shallow waters throughout the Caribbean from Florida to coastal Atlantic Brazil (Williams 1984). They are usually found in association with the sea anemone Stichodactyla helianthus and seagrass-coral rubble substrata. Despite the ubiquity of the emerald crab, the only data on population density stems from La Parguera, southwestern Puerto Rico, where it ranged from 41 crabs per hectare in seagrass to 34,667 per hectare in shallow floats (LeGore et al. 2006). Emerald crabs are popular in the aquarium trade because they help in keeping the aquaria clean by controlling algae, especially the outbreak of bubble algae Ventricaria ventricosa (Rhyne et al. 2005, Figueiredo et al. 2008) which is regarded as an important "ecosystem service". Mithraculus sculptus is part of the so-called "clean-up crews" in marine fish tanks, and has attained a high value in the marine ornamental industry. During 2007 in the State of Florida only almost 100,000 specimens of M. sculptus were landed, making this species the $13^{\text {th }}$ most heavily collected invertebrate in the marine aquarium industry of the USA (Rhyne et al. 2009, Accepted). There are no reports on the effect of overfishing on this crab species. Moreover, there are few studies on the population biology of this species. The few available studies focus on taxonomy (Wagner 1990, Ng et al. 2008), development (Rhyne et al. 2005, Rhyne et al. 2006) and aquaculture (Rhyne 2006, Rhyne et al. 2004). O verfishing has an effect of increasing the isolation of populations by decreasing the connectedness between populations (Dawson et al. 2006).

In order to assess the population structure of traded marine ornamental species we have recently begun analyzing the genetic structure of those that are heavily harvested. Studies of genetic variability have been used to infer phylogeographic patterns and
demographic processes (Avise 2004). Identification of genetically distinct populations will help management decisions to preserve levels of genetic variation and safeguard the longterm fitness of the species. Here we report on the genetic variation and population structure of the emerald crab M. sculptus based on two mitochondrial genes and identify biogeographic boundaries among populations across the sampled Caribbean area.

## MATERIAL AND METHODS

Samples were collected by SCUBA from nine Caribbean locations: Florida ( $n=4$ ), Dominican Republic ( $n=10$ ), Vieques Island, $(\mathrm{n}=8)$, Mona Island $(\mathrm{n}=4)$, La Parguera, PR $(n=11)$, Buye, PR $(n=9)$, Curaçao $(n=10)$, and Honduras $(n=5)$. Each samples location will be considered as different populations. Specimens were either transported to the laboratory alive or preserved in $100 \%$ ethanol. All specimens have been deposited at the University of Puerto Rico Marine Invertebrates Collection (UPRM INV-COL).

Total genomic DNA was extracted using a Gentra DNA purification kit (Qiagen, Inc.). A fragment of the 16 S rRNA gene was amplified using the primers from Crandall and Fitzpatrick (1996). A fragment of the COI was amplified with the universal primers from Folmer et al. (1994). Amplification was performed using the Master Taq ${ }^{\circledR}$ kit. To amplify the COI and the 16 S gene, the thermal cycler profile began with a denaturation step at $94^{\circ} \mathrm{C}$ for 2 min , followed by 40 cycles of $95^{\circ} \mathrm{C}$ for $15 \mathrm{sec}, 45^{\circ} \mathrm{C}$ for $30 \sec \left(\mathrm{COI} ; 42^{\circ} \mathrm{C}\right.$ for 50 sec$)$, and $72^{\circ} \mathrm{C}$ for $30 \mathrm{sec}\left(\mathrm{COI} ; 1 \mathrm{~min}\right.$ ), ending with an extension step at $72^{\circ} \mathrm{C}$ for 5 min . Some DNA samples that were difficult to amplify were further cleaned by a Qiagen® PCR Clean Up Kit and amplified by substituting $\mathrm{MgCl}_{2}$ with $\mathrm{Mg}^{+2}$ hot beads. PCR products were visualized by electrophoresis on a $1 \%$ agarose gel, and cleaned using ExoSap. Cycle sequence reactions were prepared with the BigDye Terminator v.3.1 Cycle Sequencing Kit
for both strands of the purified amplicon and analyzed on an ABI 3130xl Genetic Analyzer at the University of Puerto Rico, Rio Piedras sequencing facility. The resulting DNA sequences were verified by aligning reads from both 5 'and 3 ' directions using the software CodonCode Aligner and checked by eye in MacClade (Maddison and Maddison, 2000).

Haplotype ( $h$ ) and Gene diversity ( $\pi$ ) (Nei 1987) were estimated in DNAsp (Librado and Rozas 2009). Departures from neutrality were tested using Tajima's $D$ (Tajima, 1989). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992)was applied to detect population differentiation between localities. Hereafter, localities and populations will be used interchangeably. AMOVA analysis and pairwise $\Phi_{\text {ST }}$ comparisons were performed in Arlequin ver. 3.1 (Excoffier et al., 2005) using the Tamura-Nei model of nucleotide substitution (Tamura and Nei 1993) as selected by AIC in Modeltest (Posada and Crandall 1998). The significance of $\Phi_{\text {ST }}$ was assessed by 10,000 permutations. We estimated rates of gene flow ( $N_{e} m$; where $N_{e}$ is the effective population size and $m$ is the fraction of migrants in a population) by using the Hudson et al. (1992) approximation, because $\Phi_{\text {ST }}$ values are directly proportional to the number of migrants exchanged among populations. Low $N_{e} m$ values signify almost total isolation $\left(\mathrm{N}_{\mathrm{e}} \mathrm{m} \ll 1\right)$ and four or more migrant per generation would result to a panmictic population $\left(\mathrm{N}_{\mathrm{e}} \mathrm{m}>4\right)$. A statistical parsimony network was constructed for each gene using the algorithm of Templeton et al. (1992) as implemented in TCS v.1. 21 (Clement et al. 2000). Uncorrected pairwise genetic distances between locations were estimated in MEGA 4 (Tamura et al., 2007). Genealogical analyses with Bayesian Inference (BI) and Maximum Parsimony (MP) were performed in MrBayes (Ronquist and Huelsenbeck 2003) and PAUP 4.0b10 (Swofford 2002), respectively. For outgroups, the related majid species Microphrys bicornutus, Micippa platipes, Micippa thalia, Mithraculus coryphe, Mithraculus cinctimanus, Mithraculus forceps, and Tiariniacor nigerarial were chosen.

## RESULTS

A total of 63 sequences of 16 S with an average length of 391 bp and 43 sequences with length of 437 bp of the COI gene were included in the analysis. Three 16S sequences (Accession Numbers EU682783- EU682785) and two COI sequences (EU682841EU682841) were extracted from GenBank. Genetic diversity indices across all populations are shown in Table 4.1. The 16S data set showed an average nucleotide diversity ( $\pi$ ) ranging from 0.0014 to 0.0061 for 16 S and from 0.0036 to 0.0146 for COI. Average haplotype diversity (h) ranged from 0.53 to 1.00 for 16 S and from 0.71 to 0.91 for COI gene (Table 4.1). Overall, there were 20 haplotypes for COI (Figure 4.1, Table 4.2) and 19 haplotypes for 16S (Figure 4.2, Table 4.3).

The parsimony network of COI was defined by two numerically dominant haplotypes observed at almost every location (Figure 4.1). The network for 16 S was dominated numerically by Haplotype 8 , found at all locations except Curaçao. Similar to COI, there was no relationship between haplotype and geographical location, however specimens from Curaçao formed a genetically distinct lineage (Figure 4.2). Haplotype 5 was found only in Puerto Rico (except Mona Island) and Dominican Republic. Haplotype 19 belongs to a Panamanian specimen, and the sequence was downloaded from GeneBank that can have noice that showed more genetic difference than should showed (Accession Numbers EU682785). The AMOVA analysis showed that most of the genetic variance was distributed within populations (Tables 4.4, 4.5). When the data were partitioned into two Caribbean regions (West: Honduras, Dominican Republic, Panama and Florida vs East: all Puerto Rico and Curaçao) AMOVA detected significant population structuring ( $\Phi_{\mathrm{ST}}=0.13076, \mathrm{P}=$ $0.0176 \pm 0.00392$ ). The divergence between regions $\Phi_{\mathrm{CT}}=-0.00965$ was not significant $(\mathrm{P}=$
$0.43402 \pm 0.01443$ ). Pairwise $\Phi_{\text {ST }}$ comparisons did not show any significant differences between pairs of populations except when one of the populations was Curaçao (Table 4.4.7). Pairwise $\Phi_{\mathrm{ST}}$ comparisons of 16 S gene between Curaçao and all other locations confirm that Curaçao population is genetically distinct from all other locations (Table 4.4.7). The low values of $N_{e}$ confirm the isolation of the Curaçao population. Regression analysis between genetic distance and geographic distance indicate the absence of significant correlation (Figure $4.5 \mathrm{~A}, \mathrm{~B}$ ). Phylogenetic trees based on 16 S reconstructed by Bayesian Inference and Maximum Parsimony showed two, well-supported nodes indicating the presence of two genetic lineages (Figure 4.3). The two lineages were not differentiated in the COI genealogy (Figure 4.4).


Figure 4.1. Haplotype network of Mithraculus sculptus based on COI sequences. Unique haplotypes are shown as different slim ovals, and the size of the oval is proportional to the number of individuals sharing a haplotype. Each line in the network represents a single mutation. The dots between haplotypes represent inferred intermediate haplotypes.


Figure 4.2. Haplotype network of Mithraculus sculptus based on 16S sequences. Unique haplotypes are shown as different slim ovals, and the size of the oval is proportional to the number of individuals sharing a haplotype. Each line in the network represents a single mutation. The dots between haplotypes represent inferred intermediate haplotypes.


Figure 4.3. Bayesian phylogeny of Mithraculus sculptus based on 16 S sequences. Clade support values (bootstrap support for MP and posterior probability for Bayesian) are shown along the corresponding branches. $\mathrm{GB}=\mathrm{DNA}$ sequences downloaded from GenBank.


Figure 4.4. Bayesian phylogeny of Mithraculus sculptus based on COI sequences. Clade support values (bootstrap support for MP and posterior probability for Bayesian) are shown along the corresponding branches. $\mathrm{GB}=\mathrm{DNA}$ sequences downloaded from GenBank.


B


Figure 4.5. A. Regression analysis ( $\mathrm{R}=0.17$, non-significant) between pairwise $\Phi_{\mathrm{ST}}$ and geographic distance between all population pairs of Mithraculus sculptus. B. Regression analysis ( $\mathrm{R}=0.38$, non-significant) between pairwise $\Phi_{\mathrm{ST}}$ and geographic distance between Curacao and all other populations of Mithraculus sculptus. Analysis is based on 16S sequences.

Table 4.1. Summary of statistics of Mithraculus sculptus for 16 S and COI. $\mathrm{N}=$ number of sequences, $\mathrm{S}=$ segregating sites, $\mathrm{h}=$ number of haplotypes, $\mathrm{SD}=$ Standard Deviation, $\pi=$ nucleotide diversity, $\mathrm{Hd}=$ haplotype diversity.

| Locality | N | S | h | $\mathrm{Hd}( \pm \mathrm{SD})$ | $\pi( \pm$ SD) | Tajima's D |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 16S |  |  |  |  |  |  |
| Curaçao | 10 | 3 | 4 | 0.5333 (0.1801) | $\begin{gathered} 0.0016 \\ (0.0016) \end{gathered}$ | -1.56222 (NS) |
| Dominican <br> Republic | 10 | 6 | 6 | 0.8667 (0.0850) | $\begin{gathered} 0.0061 \\ (0.0041) \end{gathered}$ | 0.28430 (NS) |
| Florida | 4 | 4 | 3 | 0.8333 (0.2224) | $\begin{gathered} 0.0068 \\ (0.0054) \end{gathered}$ | 1.36522 (NS) |
| Honduras | 5 | 6 | 4 | 0.9000 (0.1610) | $\begin{gathered} 0.0065 \\ (0.0049) \end{gathered}$ | -1.14554 (NS) |
| Panamá | 3 | 8 | 3 | 1.0000 (0.2722) | $\begin{gathered} 0.0146 \\ (0.0121) \end{gathered}$ | N/A |
| Mona (PR) | 4 | 4 | 3 | 0.8333 (0.2224) | $\begin{gathered} 0.0067 \\ (0.0054) \end{gathered}$ | 1.36522 (NS) |
| Vieques (PR) | 7 | 5 | 4 | 0.8095 (0.1298) | $\begin{gathered} 0.0062 \\ (0.0044) \end{gathered}$ | 0.59446 (NS) |
| La Parguera (PR) | 11 | 5 | 5 | 0.7818 (0.1073) | $\begin{gathered} 0.0054 \\ (0.0037) \end{gathered}$ | 0.65743 (NS) |
| Buyé (PR) | 9 | 3 | 2 | 0.5000 (0.1283) | $\begin{gathered} 0.0040 \\ (0.0030) \end{gathered}$ | 1.37122 (NS) |
| All locations | 63 | 20 | 19 | 0.7120 (0.0420) | $\begin{gathered} 0.0059 \\ (0.0005) \end{gathered}$ | -1.60189 (NS) |
| COI |  |  |  |  |  |  |
| Dominican <br> Republic | 10 | 7 | 4 | 0.7111 (0.1175) | $\begin{gathered} 0.0049 \\ (0.0033) \end{gathered}$ | -0.01324 (NS) |
| Honduras | 5 | 1 | 3 | 0.8000 (0.1640) | $\begin{gathered} 0.0036 \\ (0.0029) \end{gathered}$ | -0.81650 (NS) |
| Mona (PR) | 5 | 5 | 4 | 0.9000 (0.1610) | $\begin{gathered} 0.0072 \\ (0.0051) \end{gathered}$ | 0.0000 (NS) |
| Vieques (PR) | 10 | 13 | 8 | 0.9556 (0.0594) | $\begin{gathered} 0.0079 \\ (0.0050) \end{gathered}$ | -0.94159 (NS) |
| La Parguera (PR) | 11 | 8 | 7 | 0.9091 (0.0656) | $\begin{gathered} 0.0049 \\ (0.0032) \end{gathered}$ | -0.44682 (NS) |
| Florida |  |  |  |  |  |  |
| All locations | 41 | 22 | 18 | 0.8040 (0.0480) | $\begin{gathered} 0.0043 \\ (0.0007) \end{gathered}$ | $-2.29141(\mathrm{P}<0.01)$ |

Table 4.2. Frequency and geographic distribution of COI haplotypes of Mithraculus sculptus. DR = Dominican Republic.

| Haplotype | Honduras | DR | La Parguera | Vieques | Mona | Florida | Panamá |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hap 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 2 | 2 | 3 | 3 | 2 | 2 | 0 | 0 |
| Hap 3 | 1 | 5 | 2 | 2 | 1 | 0 | 0 |
| Hap 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Hap 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Hap 6 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hap 7 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hap 8 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hap 9 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hap 10 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Hap 11 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 12 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 13 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 14 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 15 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 16 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hap 17 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Hap 18 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Hap 19 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Hap 20 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

Table 4.3. Frequency and geographic distribution of 16 S haplotypes. $\mathrm{DR}=$ Dominican Republic.

| Haplotype | Curaçao | DR | Vieques | La Parguera | Buye | Mona | Florida | Honduras | Panamá |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hap 1 | 7 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |
| Hap 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 5 | 0 | 3 | 3 | 2 | 3 | 0 | 3 | 0 | 0 |
| Hap 6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 7 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 8 | 0 | 3 | 2 | 5 | 6 | 1 | 1 | 2 | 1 |
| Hap 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 10 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 11 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hap 12 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Hap 13 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Hap 14 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Hap 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Hap 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Hap 17 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Hap 18 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

Table 4.4. Analysis of molecular variance of Mithraculus sculptus based on COI sequences and the Tamura and Nei model of substitution.

| Source of <br> variation | df | Variance component | $\%$ of variation |  | P-value $\pm$ SD |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Among locations | 6 | 0.01357 | 1.19 | $\Phi_{\mathrm{ST}}=0.01190$ | $0.35376 \pm 0.00504$ |
| Within locations | 36 | 1.12677 | 98.81 |  |  |
| Total | 42 | 1.14034 |  |  |  |

Table 4.5. Analysis of molecular variance of Mithraculus sculptus based on 16 S sequences and the Tamura and Nei model of substitution.

|  | Variance <br> component |  |  |  | $\%$ of variation |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Source of variation | df |  |  |  |  |
| Among locations | 8 | 0.10315 | 9.23 | $\Phi_{\mathrm{ST}}=0.09232$ | $0.06119 \pm 0.00258$ |
| Within locations | 54 | 1.01414 | 90.77 |  |  |
| Total | 62 | 1.11729 |  |  |  |

Table 4.6. Analysis of molecular variance of Mithraculus sculptus based on 16S sequences and the Tamura and Nei model of substitution. Locations have been assigned to the east Caribbean (Curacao and all Puerto Rico) vs. west Caribbean regions (Dominican Republic, Florida, Honduras, and Panamá).

| Source of variation | df | Sum of <br> squares | Variance <br> component | \% of variation |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Between regions | 1 | 1.868 | -0.01079 | -0.97 | $\Phi_{\mathrm{CT}}=-0.00965$ | $0.43402 \pm 0.01443$ |
| Among locations within regions | 7 | 14.187 | 0.15697 | 14.04 | $\Phi_{\text {SC }}=0.13907$ | $0.02639 \pm 0.00365$ |
| Within locations | 54 | 52.473 | 0.97172 | 86.92 | $\Phi_{\text {ST }}=0.13076$ | $\mathbf{0 . 0 1 7 6 0} \pm \mathbf{0 . 0 0 3 9 2}$ |
| Total | 62 | 68.529 | 1.11790 |  |  |  |

Table 4.7. Pairwise $\Phi_{S T}$ comparisons between populations of Mithraculus sculptus based on 16 S . $\Phi_{\mathrm{ST}}$ values are shown below diagonal and significance values $\pm$ SD are shown above diagonal. Values in bold are those that are significant after sequential Bonferroni adjustment; $\mathrm{NS}=$ non-significant; * P < 0.05 before adjustment.

| Location | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 Curaçao |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 Dominican Republic | 0 | 0.02594 | 0.02594 | $\mathbf{0 . 0 0 6 1 4}$ | 0.09950 | $\mathbf{0 . 0 0 2 3 8}$ | $\mathbf{0 . 0 0 2 5 7}$ | 0.06683 | 0.01386 |  |  |  |
| 3 Vieques |  | $\pm 0.0015^{*}$ | $\pm 0.0015^{*}$ | $\pm \mathbf{0 . 0 0 0 8}$ | $\pm 0.0031$ | $\pm \mathbf{0 . 0 0 0 5}$ | $\pm \mathbf{0 . 0 0 0 6}$ | $\pm 0.0024$ | $\pm 0.0011^{*}$ |  |  |  |
| 4 La Parguera | 0 | NS | NS | NS | NS | NS | NS | NS |  |  |  |  |
| 5 Florida | 0.2689 | -0.0910 | 0 | NS | NS | NS | NS | NS | NS |  |  |  |
| 6 Buye | 0.378 | -0.0762 | -0.0755 | 0 | NS | NS | NS | NS | NS |  |  |  |
| 7 Honduras | 0.2636 | -0.1184 | -0.1556 | -0.0963 | 0 | NS | NS | NS | NS |  |  |  |
| 8 Mona | 0.5521 | -0.0488 | -0.0121 | -0.0717 | -0.0108 | 0 | NS | NS | NS |  |  |  |
| 9 Panamá | 0.6240 | 0.0152 | 0.0716 | 0.0121 | 0.0726 | -0.0628 | 0 | NS | NS |  |  |  |

Table 4.8. Pairwise $\Phi_{\text {ST }}$ comparisons and gene flow estimates of Mithraculus sculptus based on COI. The $\Phi_{\text {ST }}$ values are above diagonal and estimates of gene flow ( $N_{e} m$ ) are below diagonal.

| Location | 1 | 2 | 3 | 4 | 5 |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
| 1 Dominican Republic |  | -0.042 | -0.024 | 0.025 | -0.078 |
| 2 Vieques Island | -6.25 |  | 0.011 | 0.015 | -0.064 |
| 3 La Parguera | -10.52 | 22.49 |  | -0.040 | -0.034 |
| 4 Honduras | 9.7 | 16.62 | -6.50 |  | -0.058 |
| 5 Mona Island | -3.46 | -4.17 | -7.56 | -4.58 |  |

Table 4.9. Pairwise $\Phi_{\text {ST }}$ comparison and gene flow estimates of Mithraculus sculptus based on 16 S . The $\Phi_{\mathrm{ST}}$ values are above diagonal and estimates of gene flow $\left(N_{e} m\right)$ are below diagonal. $N_{e} m$ values in bold correspond to significant $\Phi_{\text {ST }}$ comparisons denoting population differentiation. $\inf =N_{e} m$ is very large.

| Location | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| 1 Curaçao |  |  |  |  |  |  |  |  |  |
| 2 Dominican Republic | $\mathbf{0 . 5 5}$ |  | -0.091 | $-\bar{l}^{\prime}$ | -0.117 | -0.046 | 0.011 | -0.163 | 0.155 |
| 3 Vieques | 0.229 | 0.386 | 0.261 | 0.543 | 0.545 | 0.244 | 0.51 |  |  |
| 4 La Parguera | $\mathbf{0 . 8 4}$ | -2.99 |  | 0.076 | -0.067 | -0.020 | 0.148 | -0.196 | 0.205 |
| 5 Florida | $\mathbf{0 . 4 0}$ | -3.52 | -5.20 |  | -0.067 | -0.076 | 0.022 | -0.064 | 0.125 |
| 6 Buye | 0.71 | -2.39 | -4.00 | -4.00 |  | -0.029 | 0.156 | -0.115 | 0.214 |
| 7 Honduras | $\mathbf{0 . 2 1}$ | -5.65 | -12.75 | -3.52 | -9.00 |  | -0.031 | -0.056 | 0.077 |
| 8 Mona | $\mathbf{0 . 7 8}$ | 21.88 | 1.44 | 11.08 | 1.35 | -3.25 |  | 0.065 | 0.000 |
| 9 Panamá | 0.74 | -1.79 | -1.52 | -4.17 | -2.43 | -4.75 | 3.61 |  | 0.139 |

## DISCUSSION

The Caribbean area is a relatively small biogeographic region and because of the direction and flow of oceanic currents should be characterized by genetically open populations, especially for species with long planktonic larval dispersion (PLD). The predicted relationship between PLD and homogeneity of populations has been challenged (REF) because even in marine environments, which are seemingly homogeneous habitats, distance can be a factor that segregates populations. It has been suggested that population structure may be observed in the Caribbean at large geographic scales (>500 km) (Vollmer and Palumbi 2007). Recent studies using molecular techniques have shown that in the absence of evident barriers, population genetic structure can occur on a scale much smaller than that expected based on larval dispersal potential (Taylor and Hellberg 2003, Baums et al. 2005, Fukami et al. 2004, Vollmer and Palumbi 2007, Gutierrez-Rodríguez et al. 2005)). The mitochondrial data here suggests that the emerald crab Mithraculus sculptus exhibits no population structure throughout its sampled distribution, which includes locations in the eastern and western Caribbean. High levels of gene flow were observed between all pairs of sampling localities, except those of Curaçao. The data are partially consistent with the historical subdivision observed in some benthic species between west and east Caribbean (Robins 1971; Briggs 1974). Other marine invertebrate species from Curaçao, an eastern Caribbean locality, were genetically distinct from populations of the western Caribbean (Baums et al. 2005, Vollmer and Palumbi 2007).

Although the emerald crab is a benthic species with lack of great vagility as adults, the larvae last on average 9.5 days in the water column under experimental conditions (Rhyne et al. 2005), which should provide enough time to disperse beyond
local reefs. They are probably reproductively active most of the year, because during most sampling times, ovigerous females were observed and collected (per. obs.). The continuous presence of crab zoea in the water column and the duration of the planktonic phase do not explain the patterns of genetic differentiation. There are no shared haplotypes between the populations of M. sculptus in Curaçao vs. Panamá and Curaçao vs. Florida (based on 16 S , because no COI are available from Curaçao). These populations are separated by 100 s of km however distance does not explain this pattern, since Curaçao shares haplotypes with Puerto Rico, Dominican Republic and the farthest location of our study, Honduras. Regression analysis of geographic vs. genetic distance confirmed the lack of isolation by distance model as proposed by Slatkin (1993). Curaçao is part of the Venezuelan continental shelf and crab populations do not have to cross a large body of water to reach continental reefs of the Caribbean coast of Panamá. Lack of genetic connectivity is possible if there is a cessation of suitable habitats but no obvious barriers, such as the Amazon River, between the Venezuelan continental shelf and Panamá. Adult and juvenile emerald crabs are usually found associated with sea anemones and/or substrata characterized by coral rubble and seagrass, a common habitat throughout the shallow waters of the Caribbean. Crab larvae are readily found in the water column and may be dispersed passively by coastal currents. The strongest Caribbean Current is found across the southern center of this water body (Gordon 1967; Kinder 1983) and passes through Curacao. This current has a dominant western flow $70 \mathrm{~cm} \mathrm{~s}-1$ along the coasts of Venezuela and the Netherland Antilles (Fratantoni 2001) that will promote the connectivity between Curaçao and western population of M. sculptus. This current has little flow over the Central American Rise, since most of the northwestward flow gets
channeled through a trough southwest of Jamaica, which could be preventing the dispersion of $M$. sculptus to Panamanian waters.

Marine barriers are not usually easy to detect unless there are abrupt chemical changes in the water (e.g. temperature, salinity) or impassable deep-water bodies that are separating (e.g. Eastern Pacific Barrier) populations. Other more subtle barriers can hinder passively moving larvae such as drag effect caused by water currents, by either retaining larvae in their source location or carrying them to substrates unsuitable for settlement (Gaylord and Gaines, 2000). Ocean currents not only act as impermeable barriers to organisms with planktonic dispersal kernels (Gaylord and Gaines, 2000) but they can also promote connectivity among geographically distant populations in the Caribbean. This may explain why M. sculptus from Curaçao are genetically connected to those of Puerto Rico and Dominican Republic. The relatively short distances between the Lesser Antilles may be suitable for dispersal of $M$. sculptus larvae northward to the Greater Antilles in a stepping-stone fashion but there is no data in the present study to address this question. Understanding the factors affecting connectedness between populations is important in the context of marine protected networks and the preservation of the biodiversity.

The worldwide marine aquarium trade involves millions of charismatic ornamental species and nearly $80 \%$ of the specimens collected are invertebrates (Rhyne et al. 2009). The high value of this trade, estimated globally at US \$200-330 million annually, is one of the factors fueling the growth of this industry during the last two decades, without full consideration of the impacts to reef inhabitants. Among the most traded species for marine aquaria are specialized invertebrates collectively known as "clean up crews" or "reef tank janitors" and are purchased for their maintenance ability. These once ignored species are now highly valued resources,
commending high market prices in the aquarium industry. In many of these species, such as in M. sculptus, there is a near complete void in the knowledge of life history, population size and genetic structure, or even numbers of specimens traded. Understanding the roles of organisms in their natural environments is critical to gauge the future demand for species, as well as the impact of their removal from the natural environment. While the discovery of cryptic species in the marine environment (reviewed by Knowlton 1993, 2000) is not unusual any longer, the presence of possible cryptic species in a commercially important species is significant.

The genealogical analysis of the 16 S uncovered the presence of two clades in M. sculptus (Figure 4.3). The two clades were not present in the genealogical analysis of COI (Figure 4.4). The discordant results could be related to the low variability present in COI compared to the 16 S region. The two clades appear to have an overlapping distribution, since they were recovered from all locations. The only exception was Curaçao where only one of the clades was found. It is unknown if the two clades are biologically meaningful. The overall morphology of the collected $M$. sculptus is similar, however no systematic analysis of the variation was undertaken. Recent studies in Favia fragum (Carlon et al. 2002) and Eunicea flexuosa (Prada et al. 2008) have shown the presence of cryptic species which after detailed morphological work the phenotypic divergence concur with the molecular divergence.

## Implications for the Marine Ornamental Trade in Puerto Rico

The marine ornamental trade has been active for several decades in Puerto Rico but is largely unregulated due to a weak fisheries law dating from the 1930s. New regulations were applied more recently (Fisheries Law 278 of 1998 and Coral Conservation Law of 1999) but their implementation was problematic because of
several reasons [reviewed in (Hardin and LeGore 2005)]. The fishery is comprised of less than 20 licensed fishers collecting primarily in west Puerto Rico. More than 100 fish species and more than 100 invertebrate species are collected from different habitats including coral reefs (LeGore et al. 2006). Even though there is no data on $M$. sculptus commercial landings in Puerto Rico, Mithraculus sculptus is listed prominently in the report by LeGore et al. (2006) as one of the surveyed species for establishing a baseline for meaningful regulations for this fishery. Data from La Parguera, southwestern Puerto Rico, showed that the density of emerald crabs was 41 crabs per hectare in seagrasses and 34,667 crabs per hectare in shallow floats LeGore et al. (2006). These densities are the only density measurements that exist for these species, and they are probably no impacted populations by the ornamental fishery. The "low" volume of marine ornamental harvesting in Puerto Rico is a reflection of the small contribution of the Caribbean region to the global ornamental trade (Bruckner 2005). A unique opportunity is presented in Puerto Rico, and more generally in the Caribbean, for development of sound regulations before the inevitable fishing pressure grows to satisfy global demand (Wood 2001). The absence of population structure in the Caribbean and the presence of most haplotypes in Puerto Rico suggest that there is high enough gene flow from geographically distant locations to replenish populations in case of increasing pressure from fisheries. The only exception is Curacao, where the population was genetically distinct compared to other Caribbean locations. In that case, stricter regulations should be taken into consideration as the majority of crabs are replenished by retention of local recruitment.

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## APPENDIX 1

Nexus file of the 16 S alignment of Mithraculus sculptus used for the parsimonious network and phylogenetic analysis

```
#NEXUS
[MacClade 4.08 registered to Hernan Torres, UPRM]
BEGIN DATA;
    DIMENSIONS NTAX=76 NCHAR=391;
    FORMAT DATATYPE=DNA MISSING=? GAP=- ;
MATRIX
\begin{tabular}{llllllllllllll}
{\([\)} & & & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 \\
110 & 120 & 130 & 140 & 150 & 160 & 170 & 180 & 190 & 200 & 210 & 220 & 230 \\
240 & 250 & 260 & 270 & 280 & 290 & 300 & 310 & 320 & 330 & 340 & 350 & 360 \\
370 & 380 & \(390]\) & & & & & & & & & &
\end{tabular}
[
_Mit_s_123_Panama GACCKCCTWWAGAGGAGCGWAWTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAAACTGTCTCCTTACTAAAAT-
TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA
AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Mit_s_122_Panama GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]
_Mit_s_17_Florida GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Curacaol GaCCGTGCAAaG-GTAGCATaGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTcTCCTtaCtAAAATTTGAAtTTaAcTTTTAAGTGAAAAGGCTTAAAtTTTTCAAGGgGACGATAAGAcCCTATAAAG CTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTaAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGAcTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTtaCTAAAAT-

TtGAATTTAAcTTTTaAGTGAAAAGGCTTAAaTTTTTCaaGGGGaCGATAAGACCCTATAAAG CtTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao3 GaCCGTGCAAaG-GTAGCATaGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTcTCCTTACTAAAATTTGAATTTaAcTTTTAAGTGAAAAGGCTTaAATTTTTCAAGGGGACGATAAGACCCTATAAA GCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAAcTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATcAAAAAAAGAGTTTGCGACC [376]

Curacao4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao7 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGTGA-AAAgTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao11 GaCCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGaAAGCTTGTATgAATGGTTGGACAAgAGAAAAACtGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTtTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGcGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao12 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao15 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACgATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAgATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao19 GACCGTGCaAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA

AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-
ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAgATAAAAA-TAC-AAGTAACTGTCAG-AGTAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Curacao20 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTAAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Dominican_Republic3 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
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Dominican_Republic4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
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Dominican_Republic5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Dominican_Republic6 GACCGTgCAaag-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATA-GACCCTATAAAGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAgATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic7 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAT-

AGGTAACTGTCAG-AATAAAA-TACA-ATTATTTTTG-ATTAA-TAATGATCCTT-
AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic8 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic9 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAGCTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic10 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Dominican_Republic1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Vieques1 gaccgtgcaaag-gtagcatagtaattagct-
tttaaattgaaagcttgtatgaatggttggacaagagaaaaactgtctccttactaaaat-
ttgaatttaactttaagtgaaaaggcttaaattttcaaggggacgataagaccetataaagctttataa-agtgcga-aaagtttaaaaatttatta-ataaaagaaaacttttaaaaattattatgttggggcgacacagataaaaa-tac-aagtaactgtcag-aataaaa-cacA-atcattttg-attaa-taatgatcctt-aataaagattaaaagactaagttactttagggataacagcgttattcttttgagagtccttatcaaaaaaagagttgcgacc [377]

Vieques2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Vieques 4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCaA-AAAGcTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-

AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTT-
AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Vieques6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Vieques5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Vieques 7 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Vieques8 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

La_pargueraPR15 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_pargueraPR1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGG-AAAGcTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR16 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-
AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTT-
AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT
TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR19 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA

AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-
ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

La_parguera_PR12 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

La_parguera_PR3 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAAcTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

La_parguera_PR8 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR14 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-
TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR17 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGcTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAt-AgGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

La_parguera_PR4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-

AAGTAACTGTCAG-AATAAAA-tACA-ATCATTTTTG-ATTAA-TAATGATCCTT-
AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT

TATCAAAAAAAGAGTTTGCGACC [376]

Florida2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Florida3 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACCAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Florida4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Florida4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

PRBUYE1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

PRBUYE5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

PRBUYE6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

PRBUYE7 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

PRBUYE2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

PRBUYE4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

PRBUYE8 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA

AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-
ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

PRBUYE9 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

PRBUYE10 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Honduras 1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Honduras2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAC-AAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Honduras 4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAGAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATTATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Honduras5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGGTCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAgTTTGCGACC [376]

Honduras6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAAA-TAT-

AGGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTT-
AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

Mona1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTgGGGcGaCACAGATAAAAAA-TACTAAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TAtcaAAAAAAGAGTTTGCGACC [378]

Mona 4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAATAACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [377]

Mona5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAA-AGTGCGA-AAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAAATTATTATGTTgGGGcGaCACAGATAAAAA-TACTAAGTAACTGTCAG-AATAAAA-TACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TAtcaAAAAAAGAGTTTGCGACC [378]

Mona6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAA-AGTGCGA-AAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACaCAGATAAAAA-TAT-AGGTAACTGTCAG-AATAAAA-TACA-ATCaTTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TAtcaAAAAAAGAGTTTGCGACc [376]
sMCM1 GACCGTGCAAAG-GTAGCATAGTAATTAGTT-TTTTAATTGGAAACTTGTATGAATGGTTGAACAAGAGAAAAACTGTCTCTTTTTTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATTAAACGGACGATAAGACCCTATAA AGCTTT-ATAA-AAGATTA-

AAAGTTTAAAAATCTAAATACATAAAAATAAACTTTTATAAATTTATTATGTTGGGGCGAC ACAGATATAAAATAA--GATAACTGTCAGTAAAATAA--ACA-ATTATTTTTGGATTTTTCATGATCCTTTAATGAAGATTAAAAGAAAAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC [379]

MCM4 GACCGTGCAAAG-GTAGCATAGTAATTAGTT-

TTTTAATTGGAAACTTGTATGAATGGTTGAACAAGAGAAAAACTGTCTCTTTTTTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATTAAACGGACGATAAGACCCTATAA AGCTTT-ATAA-AAGATTA-

AAAGTTTAAAAATCTAAATACATAAAAATAAACTTTTATAAATTTATTATGTTGGGGCGAC ACAGATATAAAATAA--GATAACTGTCAGTAAAATAA--ACA-ATTATTTTTGGATTTTTCATGATCCTTTAATGAAGATTAAAAGAAAAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC [379]

MCM2 GACCGTGCAAAG-GTAGCATAGTAATTAGTT-TTTTAATTGGAAACTTGTATGAATGGTTGAACAAGAGAAAAACTGTCTCTTTTTTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATTAAACGGACGATAAGACCCTATAA AGCTTT-ATAA-AAGATTA-

AAAGTTTAAAAATCTAAATACATAAAAATAAACTTTTATAAATTTATTATGTTGGGGCGAC ACAGATATAAAATAA--GATAACTGTCAGTAAAATAA--ACA-ATTATTTTTGGATTTT-

TCATGATCCTTTAATGAAGATTAAAAGAAAAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAGAGAGTTTGCGACC [379]

MCM3 GACCGTGCAAAG-GTAGCATAGTAATTAGTT-
TTTTAATTGGAAACTTGTATGAATGGTTGAACAAGAGAAAAACTGTCTCTTTTTTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATTAAACGGACGATAAGACCCTATAA AGCTTT-ATAA-AAGATTA-

AAAGTTTAAAAATCTAAATACATAAAAATAAACTTTTATAAATTTATTATGTTGGGGCGAC ACAGATATAAAATAA--GATAACTGTCAGTAAAATAA--ACA-ATTATTTTTGGAATTTTCATGATCCTTTAATGAAGATTAAAAGAAAAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAGAGAGTTTGCGACC [379]

C10Mcisankit 4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGAACAAGAGAAATACTGTCTcTTTATTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATCAAGTGGACGATAAGACCCTATAA AGCTTT-ATAA-ATTTGGA-AAAATTTTTAAAATTTAATA-ATAAAAAAAAATTTTTTAGAAGTTATTATGTTGGGGCGACACAGATAAAAAA-TTT-AAGTAACTGTCAGGAATAAAA--ACA-ATTATTTTTG--GTTTTTATGATCCTTTACTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC [376]

D03Mcisankit3 gaCCgTgcAAaG-GTAGcATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATgAATGgtTGAACAAgAGAAATACTGTCTCTTTATTAAAATTtGAATTTAACTTTTAAGTGAAAAGGCttAaATTTaTCAAGTGGACGATAAGACCCTATAAAG

CTTT-ATAA-ATTtGGA-AAAATTTTTaAAATTTAATA-
ATAAAAAAAAATTTTTTAgAAgTTaTTATGTTGGGGCGACACAgATaAAAA-TTT-
AAGTAACTGTCAGGAATAAAA--ACA-ATTATTTTTG--GTTT-
TtATgATCCTTTACTAAAGATTAAAAgATTAAgTTACTTTAGGGATAACAGCGTTATTCTTTT
TAaGAGTCCTTATCAAAAAAAGAGTTTGCGACC [376]

A10Mcisankit2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGAACAAGAGAAATACTGTCTCTTTATTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATCAAGTGGACGATAAGACCCTATAA

AGCTTT-ATAA-ATTTGGA-AAAATTTTTAAAATTTAATA-
ATAAAAAAAAATTTTTTAGAAGTTATTATGTTGGGGCGACACAGATAAAAA-TTT-AAGTAACTGTCAGGAATAAAA--ACA-ATTATTTTTG--GTTT-

TTATGATCCTTTACTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC [376]

H09Mcisankit1 GACCGTGCAAAG-GTAGCATAgTaATTAGcT-
TTTTAATTGaAAgCTtGTATGAATGGTTGAACAAGAGAAATACTGTCTctTTaTTaAAATTTGAAtTtAActTTTAAGTGAAAAGGcTTaAATTTATCAAGTGGACGATAAGACCCTATAAAG

CTTT-ATAA-ATTTGGA-AAAATTtTTaAAAtTTAATA-

ATAAAAAAAAATtTTTTaGAAGTTaTTaTgTtGGGGgcGaCACAgAtAaAAA-tTT-
aAgtaActgTCagGAAtAAAA--AcA-AtTaTTTTTg--GttT-
TtAtGaTCCtTTAcTaaAGatTaAAAgattAAgTTaCTTTaGGGaTaACaGcgTTatTcTTTTTAaGaGTCCtt ATCAAAAAAAGagtTTGCGACC [376]
__ TiariniacornigeraTiac16 GACTGTGCAAAG-GTAGCATAATCATTAGTT-
TTTTAATTGAAAACTTGTATGAAAGGTTAAACAAAAAAATTTCTGTCTC-

TTATTAAATGATTGAAATTGACTTTTAAGTGAAAAGGCTTAAATTTTTTAAGGGGACGATA AGACCCTATAAAGTTTA-ATAAGAATAAAG-AACTTTTTTGAAAAAGAT-A--

TAAAAGAAAGAGTTAGCTTT-

TTATTTTACTGGGGCGGTACAGATAAAAGTAATTTAATAACTGTCAGAATTGAAA--ACA-GCGATGCTTG-GGTAG-TAA-GATCCTA-

ACTAAAGATTACAAGACTAAGTTACTTTAGGGATAACAGCTTAATTTTTTTAAAGAGTTCA TATCGAAAGAAAAGTTTGAGACC [375]

MforcepsMitf120 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-

TTTTAATTGAAAGCTTGTATGAATGGTTGAACAAGAGAAACACTGTCTCTTTATTAAAGTTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTGTCAAGTGGACGATAAGACCCTATAA AGCTTT-ATAA-AACACAA-AAAATTTTAGAAACTTGTTT-

ATAAAAGAAAATTTTTTAGAAATTATTATGTTGGGGCGACACAGATAAAAA-TAT-

GAGTAACTGTCAGTAGAAAGA--ACA-ATTATTTTTG-ATTAA-CAATGATCCTT-

TGTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC [376]

MicrophrysbicornutusMicb121 ????????AAAG-
GTAGCATAATCATTAGCTCTTTTAATTGAAAGCTTGTATGAACGGTTAAACAAAAGAGGTT CTGTCTTCTTTTTTAGAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTGTTCAAAGG GACGATAAGACCCTATAAAGCTTT-ATAA--GTGATA-AAAATTTAATGAATTTTATA-ATAAAAAAAGA-TTTTTATTATTTATTGTGTTGGGGCGACACGGATATAAT-TCA-AGGTAACTGTTAG-AGAGAGC-AACATAAAATTTATG-AAAGT-TAATGATCCTTAATAAAGATTCAAAGACAAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTTCC TATCGAAATAAGAGTTTGCGACC [377]

MicippathaliaMict24 GACCGTGCAAAG-GTAGCATAATCATTAGTT-TTTTAATTGGAAACTTGTATGAATGGTTTGACAAGGGAAAAGCTGTCTCTGTTTGGTTGATTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAAGGGGACGATAAGACCCTATAA AGCTTATATAT--GTGTAA--AGTTTTTGTTGATATA--A-ATAATAATAAATTCTTTAGTGTTTATTTTGTTGGGGCGACATGAATAAAAT-TAT--AGTAACTGTTCATATAAAAA--TCA-TTTATAAATGAAAATT-TGTTGATCCTT-TTTAGAGATTTAA-

GATCAAGTTACTTTAGGGATAACAGCGTTATTTTTCCTGAGAGCTCTTATCGAAGGAAAAG ATTGCGACC [372]

MicippaplatipesMicp26 GACCGTGCAAAG-GTAGCATAATCATTAGTT-TTTTAATTGAAAACTTGTATGAATGGTTAGACAAGGGATCTACTGTCTCTGTTTAAAGAATTGAACTTAACTTTTAAGTGAAAAGGCTTAAATTTTTTAAGGGGACGATAAGACCCTATAA AGCTTT-ATAT--GTAAAA-AATTTTTGTTAAATAAAATA---AAAATAAAATTTTTTGTAT-TTATTTTGTTGGGGCGACATGAATAAAAT-TTT--AGTAACTGTTCAAAACTAAA-T-CA-TTTATAGATGATAAAATTAATGATCCTT-GGTAGAGATTAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTTTTTCTGAGAGCTCTTATCGAAGAAAAAG ATTGCGACC [372];

END;

\section*{APPENDIX 2}

Nexus file of the CO1 alignment of Mithraculus sculptus used for the parsimonious network and phylogenetic analysis
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\#NEXUS

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[MacClade 4.08 registered to Hernan Torres, UPRM]

\section*{BEGIN DATA;}

DIMENSIONS NTAX=43 NCHAR=437;

FORMAT DATATYPE=DNA MISSING=? GAP=- ;

MATRIX
\begin{tabular}{lllllllllllll} 
[ & & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 \\
110 & 120 & 130 & 140 & 150 & 160 & 170 & 180 & 190 & 200 & 210 \\
220 & 230 & 240 & 250 & 260 & 270 & 280 & 290 & 300 & 310 & 320 \\
330 & 340 & 350 & 360 & 370 & 380 & 390 & 400 & 410 & 420 & 430 \\
] & & & & & & & & & &
\end{tabular}
[

MSH1CO1R_1

TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSH4CO1R
???????????????????????????????????????????????????????????????CCAAAtGaGCGTATATTAATa ACTGTAGTTaTaAAATTAACTGCtcCAAGAATAGATGAAACCCCTGCAAGATGAAGAGAGA AAATACCCATATCAACCGAGGCTCCTGCATGAGCAATAGCAGCaGCCAAAGGGGGGTAAA CAGTTCACCCTGTCCCTACACCACTTTCAACCATGCCTCTtATTAAAAGTAAAGTTAAAGA AGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACGAGGGAAAGCTATATCAGGAGCACC AAGTATAAGCGGAACAAGTCAATTACCAAAACCACCAATTATAATAGGCATAACTATAAA GAAAATTATCACAAAAGCGTGGGCCGTCACAGCCACGTTGTAAATTTGATCA [437]

MSH5CO1F_1
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSH6CO1R

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC

TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSH2CO1R_1

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR3
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR4

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC

CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR5

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR6

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR7

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC тСттАТтАAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAACCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR8
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSdr9
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

MSDR10

GTAAAAGGATAGCGGTAATAAACACtGCCCAAACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAAAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGGAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCTGTCACAGCCA CGTTGTAAATTTGATCA [437]

DR11

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA

TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]
_MSdr12

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TСТтАТTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Green_E1

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL1

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AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATCTGATCA [437]

GrenL3
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCCC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL4

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTTACAGCCA CGTTGTAAATTTGATCA [437]

GrenL8
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG

AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL12

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL14
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATTCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL15
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAAAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC

CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL16
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GrenL17
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAAAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

\section*{GrenL19}

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GV1

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATGTCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GV2

GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCCCCTGCATGAGCAA TAGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGC CTCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATAC GAGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCA CCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCC ACGTTGTAAATTTGATCA [437]

GV4
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCTAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTATAAATTTGATCA [437]

GV3
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA

TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCtCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTtATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACGA GGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACC AATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCAC GTTGTAAATTTGATCA [437]

GV5
GTAAAAGGATAGCGGTAATAAACACTGCCCAaACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTtATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACGA GGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACC AATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCAC GTTGTAAATTTGATCA [437]

GV6
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAAtAATGGTATTTGGTCTATTGTTaT TCCAAATGaGCGTATATTAATAACTGtAGTTATAAAATTAACTGCTCCAaGAATAGATGAAA CCCCTGCAAGATGAAGAGAGAAAATAcCCATATCAACCGAGGCTCCTGCATGAGCAATAG CAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCCTC TTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACGAG GGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACCA ATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCACG TTGTAAATTTGATCA [437]

GV7
GTAAAAGGATAGCGgTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATaAAATTAACTGCTCCAAGAATAGATGAA ACCCCtGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAATA

GCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCCT CtTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACGAG GGAAAGCTATATCAGGAGCACCaaGTATAAGCGGAACAAGTCAATTACCAAAACCACCAA TTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCACGT TGTAAATTTGATCA [437]

GV8

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGACG AAACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCA ATAGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTTCCTACACCACTTTCAACCATG CCTCTtATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATAC GAGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCA CCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCC ACGTTGTAAATTTGATCA [437]

GV9

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCAGCGAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAA TAGCAGCAGCCAAAGGGGGGTAAACAGTTCATCCTGTCCCTACACCACTTTCAACCATGC CTCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATAC GAGGGAAAGCTATATCAGGAGCACCAAGTATAAGTGGAACAAGTCAATTACCAAAACCA CCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCC ACGTTGTAAATTTGATCA [437]

GV10
GTAAAAGGATAGCGGTAATaAACACTGCTCAaACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTtATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACGA

GGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACC AATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCAC GTTGTAAATTTGATCA [437]

GM1
GtAAAAGGATAGCGGTAATAAACACtGCcCAAACAAATAATGGTATTTGGTCTATTGTTATT CCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGAA ACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAATA GCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCCT CTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACGA GGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACC AATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCAC GTTGTAAATTTGaTCA [437]

GM2
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAaTTTgaTCA [437]

GM3
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC

CAATTATAATAGGCATAACTATAAaGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

GM5
GTAAAAGGATAGCGGTAATAAACACTGCtCAAACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACtGCTCCAAGAATAGATGAA ACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAATA GCAGCAGCCAGAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCCT CTAATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACGA GGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCACC AATTATAaTAGGCATAACTATAAaGAAAATTATCACAAAAGCGTGGgCCGTCACAGCCACG TTGTAaATTTGaTcA [437]

GM6
GTAAAAGGATAGCGGTAATAAACACTGCtCAAACAAATAATGGTATTTGGTCTATTGTTAT TCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCGACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATcACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGaTCA [437]

Mits 17
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGGGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGT????????????

Mits 122
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTTATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437] ;

END;

\section*{APPENDIX 3}

Nexus file of the 16S alignment of Mithraculus sculptus haplotypes used for phylogenetic analysis

\section*{\#NEXUS}
[MacClade 4.08 registered to Hernan Torres, UPRM]

\section*{BEGIN DATA;}

DIMENSIONS NTAX=26 NCHAR=391;

FORMAT DATATYPE=DNA MISSING=? GAP=- ;

MATRIX
\begin{tabular}{lllllllllllll} 
[ & & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 \\
110 & 120 & 130 & 140 & 150 & 160 & 170 & 180 & 190 & 200 & 210 \\
220 & 230 & 240 & 250 & 260 & 270 & 280 & 290 & 300 & 310 & 320 \\
330 & 340 & 350 & 360 & 370 & 380 & \(390]\) & & & &
\end{tabular}
[
__sMCM1 GACCGTGCAAAG-GTAGCATAGTAATTAGTT-
TTTTAATTGGAAACTTGTATGAATGGTTGAACAAGAGAAAAACTGTCTCTTTTTTAAAAAT-
TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATTAAACGGACGATAAGACCCTATAA
AGCTTT-

GTTGGGGCGACACAGATATAAAATAA--GATAACTGT-CAGTAAAATAAACA-ATTATTTTTGGATTTT-

TCATGATCCTTTAATGAAGATTAAAAGAAAAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC--- [379]

C10Mcisank GACCGTGCAAAG-GTAGCATAGTAATTAGCT-

TTTTAATTGAAAGCTTGTATGAATGGTTGAACAAGAGAAATACTGTCTCTTTATTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTATCAAGTGGACGATAAGACCCTATAA AGCTTT-ATAAATTTGGAAAAATTTTTAAAATTTAATA-ATAAAAAAAAATTTTTTAGAAGTTATTATGTTGGGGCGACACAGATAAAAATTTA--AGTAACTGT-CAGGAATAAAAACA-ATTATTTTTG--GTTTTTATGATCCTTTACTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTCTT TTTAAGAGTCCTTATCAAAAAAAGAGTTTGCGACC--- [376]

Tiariniaco GACTGTGCAAAG-GTAGCATAATCATTAGTT-

TTTTAATTGAAAACTTGTATGAAAGGTTAAACAAAAAAATTTCTGTCTC-
TTATTAAATGATTGAAATTGACTTTTAAGTGAAAAGGCTTAAATTTTTTAAGGGGACGATA AGACCCTATAAAGTTTA-ATAAGAATAAAGAACTTTTTTGAAAAAGATA--

TAAAAGAAAGAGTTAGCTTT-

TTATTTTACTGGGGCGGTACAGATAAAAGTAATTTAATAACTGT-CAGAATTGAAAACA-GCGATGCTTG-GGTAG-TAA-GATCCTAACTAAAGATTACAAGACTAAGTTACTTTAGGGATAACAGCTTAATTTTTTTAAAGAGTTCA TATCGAAAGAAAAGTTTGAGACC--- [375]

MforcepsMi GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGAACAAGAGAAACACTGTCTCTTTATTAAAGTTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTGTCAAGTGGACGATAAGACCCTATAA AGCTTT-ATAAAACACAAAAAATTTTAGAAACTTGTTT-ATAAAAGAAAATTTTTTAGAAATTATTATGTTGGGGCGACACAGATAAAAATATG--AGTAACTGT-CAGTAGAAAGAACA-ATTATTTTTG-ATTAA-CAATGATCCTTTGTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Micippatha GACCGTGCAAAG-GTAGCATAATCATTAGTT-
TTTTAATTGGAAACTTGTATGAATGGTTTGACAAGGGAAAAGCTGTCTCTGTTTGGTTGA-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTATTTAAGGGGACGATAAGACCCTATAA AGCTT--ATATA-TGTGTAAAGTTTTTGTTGAT-ATAA-

ATAATAATAAATTCTTTAGTGTTTATTTTGTTGGGGCGACATGAATAAAATTATA---GTAACTGT-TCATATAAAAATCA-TTTATAAATGAAAATT-TGTTGATCCTT-TTTAGAGATTTAA-

GATCAAGTTACTTTAGGGATAACAGCGTTATTTTTCCTGAGAGCTCTTATCGAAGGAAAAG ATTGCGACC--- [372]

Microphrys --------AAAG-
GTAGCATAATCATTAGCTCTTTTAATTGAAAGCTTGTATGAACGGTTAAACAAAAGAGGTT CTGTCTTCTTTTTTAGAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTGTTCAAAGG GACGATAAGACCCTATAAAGCTTT-ATAA-GTGATAAAAATTTAATGAATTTTATA-ATAAAAAAAGA-TTTTTATTATTTATTGTGTTGGGGCGACACGGATATAATTCAAG--GTAACTGT-TAGAGAGAGCAACATAAAATTTATG-AAAGT-TAATGATCCTTAATAAAGATTCAAAGACAAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTTCC TATCGAAATAAGAGTTTGCGACC--- [369]

Micippapla GACCGTGCAAAG-GTAGCATAATCATTAGTT-
TTTTAATTGAAAACTTGTATGAATGGTTAGACAAGGGATCTACTGTCTCTGTTTAAAGAATTGAACTTAACTTTTAAGTGAAAAGGCTTAAATTTTTTAAGGGGACGATAAGACCCTATAA AGCTTT-ATA---TGTAAAAAATTTTTGTTAAATAAAA--TAAAAATAAAATTTTTTGTATTTATTTTGTTGGGGCGACATGAATAAAATTTTA---GTAACTGTTCAAAACTAAAT-CA-TTTATAGATGATAAAATTAATGATCCTT-GGTAGAGATTAAAGATTAAGTTACTTTAGGGATAACAGCGTTATTTTTTCTGAGAGCTCTTATCGAAGAAAAAG ATTGCGACC--- [372]

Hap_1 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA

AGCTTT-ATAAAGTGCGAAAAGTTTTAAAAATTTATTA-
ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT

TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_2 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGTGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_3 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAGTAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_4 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGTTTAAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--
AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_5 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAAAGTGCGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [377]

Hap_6 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATA-GACCCTATAAAGCTTTTATAAAGTGCGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_7 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATTATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_8 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-тTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--

GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT

TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_9 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAGCTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_10 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-

TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAAAGTGCGAAAAGTTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAACACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [377]

Hap_11 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCAAAAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_12 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAAT-

TTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGGAAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_13 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAACTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAAAGTGCGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACA--AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [377]

Hap_14 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-

TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACCAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_15 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAGAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATTATTTTTG-ATTAA-TAATGATCCTT-

AATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_16 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-

ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGGTCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [376]

Hap_17 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTTTATAAAGTGCGAAAAGTTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATACTA-AGTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [378]

Hap_18 GACCGTGCAAAG-GTAGCATAGTAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATAACAATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [377]

Hap_19 GACC?CCT??AGAGGAGCG?A?TAATTAGCT-
TTTTAATTGAAAGCTTGTATGAATGGTTGGACAAGAGAAAAACTGTCTCCTTACTAAAATTTGAATTTAACTTTTAAGTGAAAAGGCTTAAATTTTTCAAGGGGACGATAAGACCCTATAA

AGCTTT-ATAAAGTGCGAAAAGCTTTAAAAATTTATTA-
ATAAAAGAAAACTTTTTAAAAATTATTATGTTGGGGCGACACAGATAAAAATATAG--GTAACTGT-CAGAATAAAATACA-ATCATTTTTG-ATTAA-TAATGATCCTTAATAAAGATTAAAAGACTAAGTTACTTTAGGGATAACAGCGTTATTCTTTTTGAGAGTCCT TATCAAAAAAAGAGTTTGCGACC--- [377]
;

END;

\section*{APPENDIX 4}

Nexus file of the CO1 alignment of Mithraculus sculptus haplotypes used for phylogenetic analysis

\section*{\#NEXUS}
[MacClade 4.08 registered to Hernan Torres, UPRM]

\section*{BEGIN DATA;}

DIMENSIONS NTAX=25 NCHAR=437;

FORMAT DATATYPE=DNA MISSING=? GAP=- ;

MATRIX
\begin{tabular}{lllllllllllllll} 
[ & & & 10 & 20 & 30 & 40 & 50 & 60 & 70 & 80 & 90 & 100 & 110 \\
120 & 130 & 140 & 150 & 160 & 170 & 180 & 190 & 200 & 210 & 220 & 230 & 240 \\
250 & 260 & 270 & 280 & 290 & 300 & 310 & 320 & 330 & 340 & 350 & 360 & 370 \\
380 & 390 & 400 & 410 & 420 & 430 & \(]\) & & & & & & &
\end{tabular}
[

MCM1
CGAGCTGAACTTGGACAGCCTGGTACCTTTattGGTAAtGaTCAAAttTATAATGTCGCGGTTA
CAGCACATGCTTTTGTTATAATTTTTTTTATAGTTATACCTATTATAATTGGGGGATTTGGA

AATTGACTAATTCCACTTATGTTAGGAGCTCCTGATATAGCTTTTCCTCGTATAAATAATAT AAGATTTTGACTTCTTCCTCCTTCATTAACTTTACTTTTAATAAGAGGTATAGTTGAAAGTG GAGTTGGTACTGGATGAACTGTATATCCTCCTCTCTCTGCCGCTATTGCTCATGCAGGTGC TTCAGTTGATATAGGAATTTTTTCTCTTCATCTTGCAGGTGTATCTTCTATTTTAGGTGCAG TTAACTTTATAACGACAGTAATTAATATACGCTCTTATGGTATATCTATAGATCAAATACC CTT [437]

MCiP5CO1
CGAGCCGAACTTGGTCAGCCTGGTACTTTTATTGGCAATGACCAAATTTATAATGTAGCCG TAACAGCTCACGCTTTTGTTATAATTTTTTTTATAGTTATACCAATTATAATTGGTGGATTT GGTAATTGGCTTGTACCACTTATACTTGGTGCTCCTGATATGGCGTTTCCCCGTATAAACA ATATAAGATTTTGACTTCTTCCTCCTTCTTTAACTTTACTTTTAATAAGAGGTATGGTTGAA AGTGGTGTTGGTACAGGATGAACCGTCTACCCTCCTCTTGCTGCTGCTATTGCCCACGCAG GAGCTTCTGTTGATATGGGTATTTTTTCTCTTCACCTAGCAGGGGTTTCATCTATTCTTGGG GCAGTTAATTTTATAACTACAGTTATTAATATACGTTCTTATGGAATAACAATAGATCAAA TACCATT [437]

MicippathaliaGB
CGAGCTGAACTAGGTCAACCAGGAACTTTGATTGGTAATGACCAGATTTATAACGTTATTG TGACTGCTCATGCTTTTGTTATAATTTTTTTTATAGTTATACCTATTATAATTGGTGGATTTG GAAACTGATTAGTTCCTTTAATGTTAGGTGCCCCTGATATAGCTTTCCCTCGTATAAACAA TATAAGATTCTGACTCCTTCCTCCTTCTCTTAСTCTTCTTCTCATAAGAGGAATAGTAGAGA GAGGTGTCGGAACAGGATGAACTGTTTATCCTCCCCTTGCTTCTGCCATTGCCCATGCTGG AGCTTCTGTTGATATAGGCATTTTCTCTCTTCACTTAGCTGGTGTGTCCTCTATTTTAGGTG CAATTAATTTTATAACTACAGTTCTGAACATACGGTCTTATGGAATAACTTTAGATCAGAT ACCTTT [437]

Microphrysbicornutus_GB
CGAGCTGAACTTGGCCAACCTGGAACATTTATTGGAAATGATCAGATTTATAATGTAGCTG TTACAGCCCATGCATTTGTTATAATTTTCTTTATAGTTATACCTATTATAATTGGTGGATTT GGAAATTGACTTATTCCCCTTATACTTGGTGCTCCTGATATAGCCTTCCCTCGAATAAACA ATATAAGATTTTGACTTCTTCСТССТTСТСTTACACTCСТССTAATAAGAGGAATGGTTGAA

AGAGGTGTTGGAACAGGATGAACTGTATATCCTCCATTAGCTGCTGCTATTGCTCATGCAG GTGCTTCAGTAGATATGGGGATTTTTTCGCTTCATCTGGYTGGTGTTTCATCTATTTTAGGA GCAGTAAACTTTATAACTACAGTTATTAATATACGATCTTATGGAATAACTATAGACCAAA TACCACT [437]

MithraculusforcepsGB
CGAGCTGAACTTGGTCAGCCCGGTACTTTTTATTGGTAATGACCAGATTTATAATGTAGCTG TTACAGCCCACGCTTTTGTTATGATTTTCTTTATAGTTATACCAATTATAATTGGTGGCTTT GGTAACTGACTTGTCCCTCTTATACTTGGTGCCCCTGATATAGCCTTCCCTCNNNTGAACA ACATAAGGTTTTGACTTCTTCCACCTTYTTTAACTTTACTTCTGATAAGAGGTATAGTTGAA AGTGGTGTTGGAACAGGTTGAACCGTTTACCCTCCGCTTGCGGCTGCTATTGCTCACGCGG GGGCCTCAGTTGATATGGGTATTTTTTCTCTCCATCTTGCTGGGGTTTCATCTATTCTTGGA GCTGTAAACTTTATAACTACAGTTATTAATATACGCTCATACGGAATAACGATGGATCAAA TACCACT [437]

Hap_1
GTAAAAGGATAGCGGTAATAAAGACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_2
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC

CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_3
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_4
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAACCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_5
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAAAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC тСТтАТТААААGTAAAGTTAAAGAAGGCGGGAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCTGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_6
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATCTGATCA [437]

Hap_7
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCCC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_8
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTTACAGCCA CGTTGTAAATTTGATCA [437]

Hap_9
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA

TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATTCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_10
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAAAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_11

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATGTCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_12
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCCCCTGCATGAGCAA

TAGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGC CTCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATAC GAGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCA CCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCC ACGTTGTAAATTTGATCA [437]

Hap_13
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCTAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC СААТтАТААТАGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTATAAATTTGATCA [437]

Hap_14

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_15
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGACG AAACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCA ATAGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTTCCTACACCACTTTCAACCATG CCTCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATA

CGAGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACC ACCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGC CACGTTGTAAATTTGATCA [437]

Hap_16
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCAGCGAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAA TAGCAGCAGCCAAAGGGGGGTAAACAGTTCATCCTGTCCCTACACCACTTTCAACCATGC CTCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATAC GAGGGAAAGCTATATCAGGAGCACCAAGTATAAGTGGAACAAGTCAATTACCAAAACCA CCAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCC ACGTTGTAAATTTGATCA [437]
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Hap_17

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GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAGAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTAATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_18
GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCGACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC

CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]

Hap_19

GTAAAAGGATAGCGGTAATAAACACTGCTCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGTGGAAGAAGTCAAAATCTTATGTTGTTCATACG AGGGAAAGCTATATCAGGGGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGT???????????? ????????????? [437]

Hap_20
GTAAAAGGATAGCGGTAATAAACACTGCCCAAACAAATAATGGTATTTGGTCTATTGTTA TTCCAAATGAGCGTATATTAATAACTGTAGTTATAAAATTAACTGCTCCAAGAATAGATGA AACCCCTGCAAGATGAAGAGAGAAAATACCCATATCAACCGAGGCTCCTGCATGAGCAAT AGCAGCAGCCAAAGGGGGGTAAACAGTTCACCCTGTCCCTACACCACTTTCAACCATGCC TCTTATTAAAAGTAAAGTTAAAGAAGGCGGAAGAAGTCAAAATCTTATGTTGTTTATACG AGGGAAAGCTATATCAGGAGCACCAAGTATAAGCGGAACAAGTCAATTACCAAAACCAC CAATTATAATAGGCATAACTATAAAGAAAATTATCACAAAAGCGTGGGCCGTCACAGCCA CGTTGTAAATTTGATCA [437]
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