

2013-01-01

Genetic Diversity In Populations Of Epiphanes Chihuahuensis (rotifera: Monogononta) In The Northern Chihuahuan Desert

Diego E. Reyes

University of Texas at El Paso, dereyes@miners.utep.edu

Follow this and additional works at: https://digitalcommons.utep.edu/open_etd



Part of the [Biology Commons](#), [Developmental Biology Commons](#), [Evolution Commons](#), and the [Genetics Commons](#)

Recommended Citation

Reyes, Diego E., "Genetic Diversity In Populations Of Epiphanes Chihuahuensis (rotifera: Monogononta) In The Northern Chihuahuan Desert" (2013). *Open Access Theses & Dissertations*. 1716.
https://digitalcommons.utep.edu/open_etd/1716

This is brought to you for free and open access by DigitalCommons@UTEP. It has been accepted for inclusion in Open Access Theses & Dissertations by an authorized administrator of DigitalCommons@UTEP. For more information, please contact lweber@utep.edu.

GENETIC DIVERSITY IN POPULATIONS OF *EIPHANES*
CHIHUAHUAENSIS (ROTIFERA: MONOGONONTA) IN THE NORTHERN
CHIHUAHUAN DESERT

DIEGO E. REYES

Department of Biological Sciences

APPROVED:

Elizabeth J. Walsh, Ph.D., Chair

Max Shpak, Ph.D.

Ming-Ying Leung, Ph.D.

Benjamin C. Flores, Ph.D.
Dean of the Graduate School

Copyright ©

by

Diego E. Reyes

2013

GENETIC DIVERSITY IN POPULATIONS OF *EPIPHANES*
CHIHUAHUAENSIS (ROTIFERA: MONOGONONTA) IN THE NORTHERN
CHIHUAHUAN DESERT

by

DIEGO E. REYES, B.S.

THESIS

Presented to the Faculty of the Graduate School of
The University of Texas at El Paso
in Partial Fulfillment
of the Requirements
for the Degree of
MASTER OF SCIENCE

Department of Biological Sciences
THE UNIVERSITY OF TEXAS AT EL PASO

August 2013

ACKNOWLEDGEMENTS

I would like to express my most sincere appreciation to Dr. Elizabeth J. Walsh for her guidance during the course of the project. This project would not have been possible without her expert advice, support and knowledge. Many thanks to the members of my committee Dr. Max Shpak and Dr. Ming-Ying Leung for their all their help, guidance, and knowledge shared.

Special thanks to Ivan Alvarez for his help in sample collecting in the field and with experiments in the laboratory. Thanks to Dr. Walsh's laboratory students and staff for helping me in the field and laboratory during the course of my research. Thanks to Dr. Musa Hussein for preparing the map of the Chihuahuan Desert. I would also like to thank faculty and staff from the Department of Biological Sciences for all of their help. Thanks to Ana Betancourt from UTEP'S BBRC DNA Analysis Core Facility for conducting DNA sequencing. Thanks to Dr. Julia Bader from UTEP's BBRC Statistical Consulting Laboratory for her help with statistical analysis of the cross-mating experiments. Much appreciation to the staff at Hueco Tanks State Park and Historic Site (TPWD permit: #02-09) and to John Sproul for facilitating sample collection at Rio Bosque Wetlands Park.

Funds for this project were provided by NIH grant NCRR S612RR008124-17 which includes UTEP's BBRC DNA Analysis Core Facility. NSF DEB 0516032 and NSF DEB 1257068 also provided support for research supplies and equipment. Additional support was provided by Sigma Xi GIAR # G20111015158706, and UTEP's Provost office.

I would like to thank my mother for supporting me throughout the course of my life, my brother and sisters and all other family members. Finally, I would like to thank my girlfriend, Stephany Herrera, for all her support and encouragement.

ABSTRACT

1. Cryptic speciation in zooplankton is a phenomenon that has been recently gaining much attention. This is due in part to advances in molecular techniques which help in the identification of morphologically indistinct species. Organisms that have been traditionally believed to have cosmopolitan distributions are being found to be composed of cryptic species complexes with high levels of genetic divergence among lineages.
2. *Epiphanes chihuahuensis* is a member in the *Epiphanes senta* species complex. In a previous study by Schröder & Walsh (2007), genetic data, along with morphological and reproductive isolation data were employed to help delineate four species within the complex. A more recent study based on DNA fingerprinting demonstrated the presence of two distinct lineages of *Epiphanes chihuahuensis* coexisting within a site in the northern Chihuahuan Desert. Using genetic sequence data in combination with DNA fingerprinting and reproductive isolation, I investigated genetic population structure and differentiation in populations of *Epiphanes chihuahuensis* to better understand the diversity of the *E. senta* cryptic species complex.
3. Individuals were collected from two sites in the northern Chihuahuan Desert separated by approximately 40 km. From these individuals, 47 COI gene (649 bp) and 43 ITS region (673 bp) sequences were obtained for phylogenetic analyses using Bayesian and Maximum Likelihood approaches. I also conducted a Bayesian phylogenetic analysis including other isolates from *E. senta* species complex obtained through GenBank. In addition, DNA fingerprints for some of these individuals were generated using 4 Randomly Amplified Polymorphic DNA (RAPD) primers for Bayesian population structure analysis. Cross-mating experiments were conducted to assess the level of reproductive isolation in the *E. senta* species complex.
4. Analyses of COI gene sequences demonstrated a high level of differentiation between populations of *E. senta* species complex occurring at the two sites in the northern Chihuahuan Desert. Mean COI

sequence divergence between lineages from each site was 19.5%. Less divergence was seen in ITS region gene sequences with a mean of 4.3% between the two lineages. Phylogenetic analyses of COI gene and ITS region sequences produced two distinct monophyletic clusters with high clade support when using isolates from the northern Chihuahuan Desert only. When other isolates from the *E. senta* complex were included in a Bayesian phylogenetic analysis, two main clades were formed. One clade was formed by individuals from Hueco Tanks State Park and Historic Site, which corresponded to *E. chihuahuensis*. The other clade was composed by the individuals from Rio Bosque Wetlands Park, a representative of *E. hawaiiensis* and isolates from the *E. senta* species complex obtained from GenBank. Within *E. chihuahuensis* low levels of genetic divergence were found with 0-1.6% for the COI. For the ITS region, percent sequence differences were lower (0-0.4%). COI gene sequences of *E. chihuahuensis* yielded 11 haplotypes and a haplotype diversity of 0.69 while 9 haplotypes and a haplotype diversity of 0.75 were detected based on ITS region sequences. Two distinct clusters with high levels of support were identified by Bayesian analysis of RAPD band patterns for individuals from Rio Bosque Wetlands Park and Hueco Tanks State Park and Historic Site. Cross-mating experiments between individuals from the two populations resulted in the occasional production of fertilized eggs. Only in 9% of cross-matings were diapausing eggs successfully produced. Viability of these embryos was not assessed.

5. The high levels of COI gene sequence divergence between *E. senta* lineages from this region are comparable to those of other rotifer species and aquatic zooplankton species. The low levels of genetic divergence within individuals from HTSPHS do not support the idea of further cryptic speciation in *E. chihuahuensis*. Moreover, no coherent structure was found between the two populations sampled in this region by Analysis of Molecular Variation, again supporting the distinctness of the lineages studied here. Finally population structure analysis did not support cryptic speciation in *E. chihuahuensis* but it detected the presence of two distinct lineages. Although reproductive isolation is incomplete among

these populations of the *E. senta* species complex, it is presently unknown if the fertilized eggs are viable. As demonstrated in this study, the level of complexity underlying the diversity of these animals is just beginning to be understood. While there are some lineages having large scale distributions, other lineages have only been found in few localities in the region. Understanding the diversity of aquatic ecosystems is important as different species, even if morphologically undistinguishable as in the case of cryptic species, likely contribute differently to their habitats.

Keywords: zooplankton, cryptic speciation, species complex.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iv
Abstract.....	v
TABLE OF CONTENTS	viii
LIST OF TABLES.....	x
LIST OF FIGURES	xiii
Introduction.....	1
MATERIALS AND METHODS	5
Study Sites	5
A. Rio Bosque Wetlands Park	5
B. Hueco Tanks State Park and Historic Site	5
Sample Collection, Culture and Preservation	5
DNA Extraction, Amplification, and Sequencing of COI gene and ITS region	6
Sequence Analyses of COI gene and ITS region.....	7
DNA Extraction, Amplification, and scoring for RAPD Analyses	9
Crossbreeding Experiments	10
RESULTS	12
Genetic divergence based on COI and ITS sequences	12
RAPD Analysis.....	14
DISCUSSION.....	15
REFERENCES	24
TABLES	33
FIGURES.....	41
APPENDICES	48
Appendix 1: COI <i>E. senta</i> sequences from northern Chihuahuan Desert populations.....	48
Appendix 2: ITS <i>E. senta</i> sequences from northern Chihuahuan Desert populations.....	54

Appendix 3: COI uncorrected (“p”) distance matrix for <i>E. senta</i> populations from the northern Chihuahuan Desert and outgroups	61
Appendix 4: ITS uncorrected (“p”) distance matrix of <i>E. senta</i> populations from the northern Chihuahuan Desert and outgroup	64
CURRICULUM VITAE.....	67

LIST OF TABLES

Table 1. Populations of <i>E. senta</i> species complex and <i>E. brachionus</i> included in this study.	33
Table 2. Geographic (below diagonal; m) and pairwise genetic distances (above diagonal; %) of 47 clonal lineages of <i>E. senta</i> species complex from the northern Chihuahuan Desert. Geographic distances were generated by Geographic Distance Generator v1.2.3. Uncorrected “p” pairwise distances for COI sequences were generated by PAUP*4.0 β.	35
Table 3. Geographic (below diagonal; m) and pairwise genetic distances (above diagonal; %) of 43 clonal lineages of <i>E. senta</i> species complex from the northern Chihuahuan Desert. Geographic distances were generated by Geographic Distance Generator v1.2.3. Uncorrected “p” pairwise distances for ITS region sequences were generated by PAUP*4.0 β.	36
Table 4. Haplotype analysis of COI gene sequences derived from <i>E. chihuahuensis</i> isolates produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.	36
Table 5. Haplotype analysis of ITS region sequences derived from <i>E. chihuahuensis</i> isolates produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.	36
Table 6. Haplotype analysis of COI gene sequences derived from Chihuahuan Desert populations of <i>E. senta</i> species complex produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.	37

Table 7. Haplotype analysis of ITS region sequences derived from Chihuahuan Desert populations of <i>E. senta</i> species complex produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.	37
Table 8. Xia's test for saturation of nucleotide substitution of 47 <i>E. senta</i> species complex sequences and 3 outgroup sequences (<i>E. brachionus</i>) for COI genetic sequences implemented in DAMBE. Iss, index of substitution saturation. Iss.cSym, critical value of Iss under assuming symmetric tree topology. Iss.cAsym, critical value of Iss assuming an asymmetric tree topology. DF, degrees of freedom. NumOTU, number of taxa included in analysis.	38
Table 9. Most likely number of populations (Delta K) in <i>E. senta</i> species complex from the northern Chihuahuan Desert as determined by the Evanno method. K was run from K=1 to K=10. The highest Delta K is highlighted in yellow. Delta K is computed by taking the absolute value of the 2 nd order rate of change of the likelihood distribution (mean) and dividing it by the standard deviation of the likelihood distribution. K is the number of assumed populations for structure analysis.	38
Table 10. Cross-matings for <i>E. senta</i> species complex populations from 5 different sites in the northern Chihuahuan Desert. Mictic female eggs are placed together with 1-2 males from a different lineage for possible mating and resting egg production. Table contains the number of eggs produced with the number of trials in parenthesis.	39
Table 11. Fisher's exact test to determine whether there is a significant difference in egg production between crosses of <i>E. senta</i> species complex lineages from the northern Chihuahuan Desert and crosses within <i>E. senta</i> species complex lineages from the northern Chihuahuan Desert.	39

Table 12. Groups of *E. senta* species complex lineages from the northern Chihuahuan Desert arranged by sampling location and clusters according to Bayesian and Maximum Likelihood analyses used for Analysis of Molecular Variation (AMOVA).....40

Table 13. Analysis of Molecular Variance (AMOVA) of *E. senta* species complex populations from two sites in the northern Chihuahuan Desert. Grouping was based on geographic location and COI gene sequence clustering. d.f., degrees of freedom.....40

LIST OF FIGURES

- Figure 1. A. Map of the Chihuahuan Desert (Courtesy of Mr. Musa Hussein). Inset shows study sites in the El Paso, TX region (<http://maps.google.com/>). Red dot corresponds to Hueco Tanks State Park and Historic Site (HTSPHS) and green dot represents Rio Bosque Wetlands Park (RBWP). Distance between sites is 40 km. B. Sampling sites at HTSPHS (A-C) and RBWP (E-F).41
- Figure 2. Mantel test for correlation of geographic distance with genetic distance (COI) of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert produced by Isolation by Distance Web Service v3.23.42
- Figure 3. Mantel test for correlation of geographic distance with ITS genetic distance of 42 *E. senta* species complex lineages from the northern Chihuahuan Desert produced by Isolation by Distance Web Service v3.23.42
- Figure 4. Phylogenetic tree of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert and three members of the outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of COI gene sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.43
- Figure 5. Phylogenetic tree of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert and three members of the outgroup (*E. brachionus*) based on Maximum Likelihood analysis (RAxML v7.6.3) of COI gene region sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.44
- Figure 6. Phylogenetic tree of 43 *E. senta* species complex lineages from the northern Chihuahuan Desert and one outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of ITS region

sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.....45

Figure 7. Phylogenetic tree of 51 *E. senta* species complex lineages and one outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of COI gene region sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location and/or GenBank accession number, red = HTSPHS (USA), purple = JF714413 (Norway), green = RBWP (USA), yellow = RBWP (USA), JF714414 (Germany), and DQ089728, and blue = Hawaii and RBWP (USA).46

Figure 8. Estimated Delta K (ΔK) for K=2 through K=9 *E. senta* species complex populations from the northern Chihuahuan Desert using the Evanno method (Evanno *et al.*, 2005) and produced by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Delta K reaches the highest peak at K = 2. K is the number of populations assumed for genetic structure analysis.....47

Figure 9. Bar plot of *E. senta* species complex populations (number of individuals=125) from the northern Chihuahuan Desert genotype assignments produced by STRUCTURE v2.3.3 and modified in Distruct v1.1 based on four RAPD primers. Bayesian analysis shows two clusters (green and red) and estimated probability of each individual of belonging to one of the two clusters (K = 2, probability of belonging to green cluster for RBC1 and RBC2 0.98; probability of belonging to the red cluster for HT Heart= 0.90, HT North=0.98 and HT South=0.93).47

INTRODUCTION

Recent advances in molecular systematics have allowed researchers uncover cryptic species in a wide variety of taxa. In recent years this phenomenon has been of particular importance in aquatic microinvertebrates. Previously undetected differences in these organisms have been discovered thanks in part to these advances. Cryptic speciation has been detected in a wide variety of aquatic taxa including copepods (Chen & Hare, 2011; Thum & Derry 2008; Thum & Harrison, 2009; Goetze, 2010, 2003), ostracods (Schon *et al.*, 2012; Bode *et al.*, 2010), amphipods (Bradford *et al.*, 2010; Witt, Threlloff & Hebert, 2006; Murphy, Adams, & Austin, 2009; Seidel, Lang, & Berg, 2009), cladocerans (Belyaeva & Taylor, 2009; Forro *et al.*, 2008) and rotifers (Monogonont: Schröder & Walsh, 2010; Walsh *et al.*, 2009; Gilbert & Walsh, 2005; Suatoni *et al.*, 2006; Mills, Lunt, & Gomez, 2007 Garcia-Morales & Elias-Gutierrez, 2013; Leasi *et al.*, 2013; Bdelloid: Fontaneto *et al.*, 2011; Fontaneto, Boschetti, & Ricci, 2008b; Fontaneto *et al.*, 2009;). Historically, many zooplankton species had been considered cosmopolitan species. In the traditional view of The Baas-Becking or “everything is everywhere” hypothesis, small organisms, usually smaller than 2mm, are assumed to be globally distributed (De Wit & Bouvier, 2006). The reasoning behind this idea is that small organisms usually have high dispersal capabilities and dormant stages or propagules. Another reason that microscopic organisms are often considered to be cosmopolitan is the lack of easily identifiable morphological characteristics. This more traditional morphological approach for delimiting a species has been unable to uncover the presence of cryptic species. In more recent years, the concept of cosmopolitanism in microscopic organisms has been challenged thanks to phylogenetic studies which have shown that many of these so called cosmopolitan species are in fact cryptic species complexes.

Cryptic species complexes can show a great extent of genetic differentiation among populations. A variety of methods including DNA sequencing of mitochondrial and nuclear genes and DNA fingerprinting such as Amplified Fragment Length Polymorphisms (AFLPs) and Randomly Amplified

Polymorphic DNA (RAPD) have been used to aid in the identification of unique lineages and resolution of genetic relationships in cryptic species. For example cryptic speciation was detected in a genetic study of pelagic copepods with 12 new genetic lineages delineated within the Eucalanidae family. Genetic distances of mitochondrially encoded cytochrome c oxidase I (COI) sequences in these lineages ranged from about 5-24% while nuclear DNA (16S rRNA) sequence divergence ranged from ~2%-23%. At least four of the 12 lineages in that study were identified as cryptic species (Goetze, 2003). This phenomenon has also been detected in bdelloid rotifers, which have traditionally been believed to have cosmopolitan distributions, but recent studies have shown that they have evolved into distinctive clades, indicating that they are independently evolving entities (Hamdan, 2010; Birky *et al.*, 2005; Fontaneto *et al.*, 2008a; Fontaneto *et al.*, 2008b, Fontaneto *et al.*, 2011, Birky *et al.*, 2011). The occurrence of cryptic speciation and high genetic differentiation also takes place in monogonont rotifers. For example in a phylogenetic study of *Brachionus plicatilis*, a monogonont rotifer thought to be cosmopolitan, contained up to 23% sequence divergence and at least twenty-two independently evolving lineages (Suatoni *et al.*, 2006; Fontaneto *et al.*, 2009). Similar levels of high sequence divergence were also found in a broader study of 63 monogonont rotifer taxa with a COI divergence level mean of 21% among cryptic species sequenced (Garcia-Morales & Elias-Gutierrez, 2013).

Epiphanes senta is a rotifer that inhabits mainly freshwater habitats, most of which are ephemeral. A recent study showed *Epiphanes senta* as a cryptic species complex with three newly described species: *Epiphanes hawaiiensis*, *E. ukera*, and *E. chihuahuaensis* (Schröder & Walsh, 2007). To designate the three species, Schröder & Walsh (2007) used partial COI genetic sequence data. Diapausing egg and trophi morphology as well as cross-mating experiments were used in conjunction with DNA sequence data to confirm the existence of the three sibling species. In a more recent study differences in mating behavior such as mixis cues, egg guarding, and mate choice behavior, confirmed that the three species are on different evolutionary trajectories (Schröder & Walsh, 2010). DNA

fingerprinting results in the same study identified *E. ukera*, *E. hawaiiensis*, and *E. chihuahuaensis* as separate entities. DNA fingerprinting also showed two distinct co-occurring lineages of *E. chihuahuaensis*.

As noted above, COI gene has been widely used for constructing species level phylogenies and used in conjunction with other genetic regions such as the nuclear ribosomal internal transcribed spacer region (ITS) to uncover cryptic species and to aid in species delimitation. COI has been identified as a better indicator of true diversity in small invertebrates when compared to traditional morphology alone (Tang et al., 2012). In a broad survey of several microscopic species taxa, Tang et al. (2012) demonstrated that analysis of COI gene sequences with a variety of species delimitation metrics (e.g., K/Θ method (Birky et al., 2011, 2005), Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), Generalized Mixed Yule Coalescent (GMYC) (Pons et al., 2006), Nucleotide Divergence Threshold (NDT) (Hebert et al., 2003)), show an increase in diversity when compared to traditional morphological analyses. This increase in uncovered diversity by using the methods mentioned above provides a better understanding of biodiversity in aquatic microinvertebrates. In the same study, a high degree of congruence was found among the K/Θ, GMYC, and NDT species delineation methods when using COI gene sequences. In this study we make use of the COI gene and ITS region DNA sequences and apply the K/Θ method to investigate the phenomenon of cryptic speciation in the monogonont rotifer *Epiphanes chihuahuaensis*. In combination with this approach, DNA fingerprinting and reproductive isolation experiments were conducted to provide a better estimate of the extent of diversification in the *Epiphanes senta* complex.

This research focuses on populations occurring at two sites in the northern Chihuahuan Desert. These sites both contain temporary water bodies – rock pools and periodically flooded wetland ponds. These aquatic habitats can hold water from rainfall and runoff, and can support a high biodiversity of Chihuahuan Desert aquatic biota (Wallace *et al.*, 2005). Some of these water bodies are more sheltered

from the sun and wind, and therefore hold water for a longer period of time (up to months). On the other hand the more exposed and shallow rock pools may hold water for just a few days. These differences in water retention mean that organisms living in these habitats are likely adapted to highly variable environments (i.e., wet and dry conditions) by increased rates of sexual reproduction. As a result of sexual reproduction, a diapausing embryo or resting egg is produced. Because of this there is a selective pressure for those organisms to produce diapausing stages to survive dry conditions; therefore high rates of sexual reproduction have been found in ephemeral habitats (Schröder, 2003; Schröder et al., 2007).

E. chihuahuensis inhabits some of the temporary ponds at HTSPHS and individuals from different rock pools have been used in previous studies to test for the possibility of cryptic speciation and genetic differentiation in the *Epiphanes senta* species complex (Schröder & Walsh, 2007; Schröder & Walsh, 2010). Here, I specifically address the following questions: 1) What is the extent of cryptic speciation in *E. chihuahuensis*? 2) Are populations of *E. chihuahuensis* genetically differentiated in the northern Chihuahuan Desert? and 3) If so, are they reproductively isolated?

MATERIALS AND METHODS

Study Sites

A. Rio Bosque Wetlands Park

Rio Bosque Wetlands Park (RBWP) is a 1.5 km² park owned by the City of El Paso. Since 1966 the park has been managed by the Center of Environmental Research & Management at the University of Texas at El Paso (UTEP). The park is located adjacent to the Rio Grande which forms the U.S. and Mexico border (GPS N31°38'34.8612", W106°18'42.537"). The park has irrigation canals that channel water on three sides by redirecting its flow into two large wetland cells (Watts *et al.*, 2002). However, the water does not come from the Rio Grande but rather from Roberto Bustamante Wastewater Treatment Plant which is located near the park. The purpose of the park is to re-establish the aquatic habitat types that once characterized the Rio Grande and its floodplain (Watts *et al.*, 2002).

B. Hueco Tanks State Park and Historic Site

Hueco Tanks State Park and Historic Site (HTSPHS) is a 3.48 km² park located 52 km northeast of El Paso, El Paso Co, TX (GPS N31°55'29.0994", W106°2'32.2794"). The Park is named after the “huecos” (holes or hollows in Spanish) formed in the rocks and boulders of the mountains in the park. Huecos and other temporary ponds trap and retain some water during the rainy season, which lasts typically from late June to October.

Sample Collection, Culture and Preservation

Individuals were collected from two sites in the northern Chihuahuan Desert including two large wetlands cells at RBWP (Cell 1 and Cell 2), and three small desert rock pools at HTSPHS (North,

South, and Heart) (Figure 1). Most sample collections took place immediately (typically one to three days) after a rain event. Samples were taken using a 67- μ m plankton net. Immediately following collection, individual rotifers were isolated in 24-well tissue culture plate containing modified MBL media (Stemberger, 1981) and a mixture of the algae *Chlamydomonas reinhardtii* (UTEX strain 90) and *Rhodomonas minuta*. Individuals were isolated in order to establish clonal lineages. Clonal lineages are generated in monogonont rotifers by taking advantage of the asexual portion of their partially parthenogenetic life cycle. Clonal isolates were cultured until sufficient numbers of individuals were obtained for genetic analyses. Mictic females and males produced in the sexual phase of their life cycle were used for cross-mating experiments. When insufficient numbers were available from active ponds, rotifer diapausing eggs were obtained from sediment samples from localities. Sediment (~10 grams) was placed into a container with MBL media and kept at 13°C in alternating 12 hour light and dark cycle. Containers were examined for rotifers every day for the first week and every two days thereafter. Diapausing eggs produced by clonal lineages were also dehydrated and stored in a dark container at 4°C for a minimum of three weeks for later use when active clonal lineages were lost. These eggs were then rehydrated in a mixture of MBL and media to restore lost lineages. Rotifers were preserved in 70% EtOH prior to DNA extraction. The samples were changed from EtOH to TE buffer for 24 to 48 hours to remove excess EtOH before extraction.

DNA Extraction, Amplification, and Sequencing of COI gene and ITS region

DNA was extracted from rotifers with lysis buffer (as described in Schröder & Walsh, 2010). Product from lysis was homogenized using a sterile glass pestle and frozen at -80°C. This DNA template was then used for PCR reactions. A Techne TC-412 thermocycler was used to perform DNA amplification by PCR. Parameters for PCR were an initial denaturation at 94°C for 15 minutes, 35 cycles at 94°C for 1 minute, 47°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 7

minutes. Primers used for (PCR) mitochondrial DNA amplifications were the primer pair LCO (5'-GGTCAAAAATCATAAAGATAT-3') and HCO (5'-TAAACTTCAGGGTGACCAAAA-3') for mitochondrial cytochrome c oxidase subunit 1 (COI) (Folmer *et al.*, 1994). In addition to the mtDNA amplifications, ribosomal spacer regions amplifications were conducted with the primer pair ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al.*, 1990). Following DNA amplification, electrophoresis in a 1.5% agarose 3:1 gel was carried out to separate and visualize DNA fragments. DNA was excised from the gel and purified using Gene Clean III® kits. A NanoDrop 1000 spectrophotometer was utilized for DNA quantification prior to sequencing (COI, ITS) at UTEP's BBRC DNA Analysis Core Facility. Additionally, three sequences of COI were obtained from GenBank [GenBank accession numbers: JF714414, JF714413, DQ089728].

Sequence Analyses of COI gene and ITS region

Sequences were manually inspected and trimmed to check for DNA code ambiguity and to improve sequence quality using Sequence Scanner v1.0 (www.appliedbiosystems.com). Contiguous DNA assembly was done using CAP3 (Huang & Madan, 1999). Multiple sequence alignment was performed in Clustal Omega (Goujon *et al.*, 2010; Sievers *et al.*, 2011) and adjusted manually. Sequence alignments were evaluated for saturation of nucleotide substitution using Xia's test implemented in DAMBE v5.3.4 (Xia *et al.*, 2003; Xia & Lemey 2009). Pairwise genetic distances using uncorrected "p" values were calculated in PAUP*4.0β by implementing the Neighbor Joining algorithm (Swofford, 2002). The genetic distances were collapsed by site and put into genetic distance matrices. These data were combined with geographic distances calculated by Geographic Distance Generator v1.2.3 and put into geographic and genetic distance matrices. The geographic and genetic distance matrices were used to perform a Mantel test for correlation of geographic and genetic distances using Isolation by Distance Web Service v3.23 (Jensen, Bohonak, & Kelley, 2005).

Bayesian analysis was performed in MrBayes v3.2.1 (Ronquist & Huelsenbeck, 2003). jModelTest v2.1.3 identified TPM2uf+G as the best model using the Akaike information criterion corrected for small sample sizes (AICc) and NumModels=88 and the GTR+I model using the AICc with NumModels=24 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). Since MrBayes v3.2.1 does not implement the TPM2uf+G, the GTR+I model was selected as the best model for Bayesian inference. Bayesian analysis was performed with 2 million generations with sample topologies recorded every 1000 generations in two independent runs. 25% of tree topologies were removed from the final analysis as burn-in and the remaining were used to produce a consensus tree. Maximum likelihood was conducted in RAxML v7.6.3 (Stamatakis, 2006) through CIPRES Science Gateway web portal (www.phylo.org). Maximum likelihood analyses were run with 1000 bootstrap replicates using the GTR+G model since RAxML v7.6.3 does not support the TPM2uf+G model. DnaSP v5.0 was used to determine the number of haplotypes as well as haplotype diversity from DNA sequence data (Librado & Rozas, 2009). Analysis of Molecular Variance (AMOVA) to determine variation within and between populations was completed in Arlequin v3.5.1.3 (Excoffier & Lischer, 2010). The analysis was based on partitioning populations by sampling location and clusters as determined by Bayesian and maximum likelihood analyses.

To determine species boundaries the K/Θ method was implemented (Birky *et al.*, 2005, 2011). This method uses genetic pairwise sequence data to identify distinct lineages. The pairwise genetic differences in this method are expected to be greater by a magnitude of 4 among clades as compared to within clades. The implementation of this method calls for well supported clades previously determined by phylogenetic analyses as the starting point. Then for each clade $\Theta = \pi / (1 - 4\pi/3)$ was calculated, where π = nucleotide diversity (estimated from mean pairwise differences within each clade with correction for sample size). K is calculated by the mean pairwise corrected sequence difference between the clades. If

the ratio $K/\Theta \geq 4$, then there is a 95% probability that the two clades being compared are independently evolving lineages (Birky *et al.*, 2005, 2011).

DNA Extraction, Amplification, and scoring for RAPD Analyses

DNA for RAPD analyses was obtained using the procedure described in the section above. Four primers from Operon Technologies were used for RAPD PCR analyses: OP-G5 (5'-CTGAGACGGA-3'), OP-H6 (5'-ACGCATCGCA-3'), OP-H18 (5'-GAATCGGCCA-3'), and OP-W3 (5'-GTCCGGAGTG-3'). A master mix was prepared for RAPD amplification reactions. The master mix contained 0.75 μ L Taq polymerase (5 U/ μ L), 5 μ L of 5X PCR buffer, 1.5 μ L of 50 mM MgCl₂, 2.5 μ L of dNTP mix each of dATP, dCTP, dGTP, and dTTP at 1 mM, and 5 μ L of primer at 5 μ M. 22 μ L of master mix were added per reaction along with 3 μ L of template to a final volume of 25 μ L. A Techne TC-412 thermocycler was used for RAPD PCR with initial denaturation of 5 minutes at 95°C, 45 cycles of 1 minute at 95°C, 1 minute at 36°C, 2 minutes at 72°C and a final extension of 1 minute at 72°C. RAPD reactions were performed in duplicate for 75% of total number of reactions to verify the reproducibility of RAPD bands. Separation of DNA was done by electrophoresis at 100 V for 20 minutes and 70 V for 4 hours in a 2.0% low melting point agarose gel on 1 X TAE buffer. RAPD band fragment size was determined using a 1Kb plus DNA ladder (Invitrogen) and GeneImagIR 3.56 (Scanalytics, Inc) software. Bands of different sizes were considered different loci and presence and absence of a band were denoted by a 1 and a 0 respectively and arranged in a data matrix that was used for genetic analyses.

Bayesian inference of population structure was performed in STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). Briefly, STRUCTURE assigns sampled individuals to K populations based on genotype data. Each individual is given a probability of belonging to one or more of K clusters. Run length for population structure analyses was set to initial 25,000 iterations for the burn-in period and

100,000 MCMC repetitions after that, as suggested by Pritchard *et al.* (2000). Initially, the ancestry model was set to admixture using sampling locations as prior conditions because of the small data set as in Schröder and Walsh 2010. The allele frequencies model was set to frequencies correlated (Falush, Stephens & Pritchard, 2003). K was tested from K=1 to K=10, corresponding to five more than the actual number of populations because there is a possibility of having more than one lineage per site. Each K was run with 20 iterations for stability and for quantification of likelihood variation following the Evanno method (Evanno, Regnaut, & Goudet, 2005). Results from all the runs were uploaded to STRUCTURE HARVESTER v0.6.93 (Earl & vonHoldt, 2012) to compute the most probable K. STRUCTURE HARVESTER helps select the most likely value of K by calculating a ΔK value. ΔK is then determined by taking the absolute value of the 2nd order rate of change of the likelihood distribution (mean) and dividing it by the standard deviation of the likelihood distribution (Evanno *et al.*, 2005). Graphical representation and improved appearance of the results generated by STRUCTURE v2.3.4 was produced by Distruct v1.1 (Rosenberg, 2004).

Crossbreeding Experiments

Single females were isolated from available clonal lineages and placed in a 24-well tissue culture plate with 2 ml of medium composed of MBL and a mixture of *Chlamydomonas reinhardtii* plus *Rhodomonas minuta*. Culture medium was exchanged every 2 to 3 days to avoid overgrowth of bacteria and fungi. Individual eggs with fully developed embryos were collected from mictic females from each clonal lineage. These eggs were then placed in a 96-well tissue culture plate along with males from another clonal lineage (cross-mating) or from the same lineage (positive control). Two to four males were used per trial to increase the chances of mating success as occasionally some males would die before the female hatched from the egg [nb: males typically live only 1-3 days after hatching because they do not feed (Schröder, 2003)]. 50 μ l of culture medium were added to each well after adding the

mictic female egg and the male rotifers. 200 µl of culture medium were added the following day after females had hatched and the surviving males were removed. Females were cultured until they deposited eggs. Diapausing eggs were then dehydrated and put in a dark container at 4°C for a minimum of three weeks to check for viability at a later time. A Fisher's exact test implemented in Minitab 16 statistical software was used to determine significance of cross-mating experiments of two sets. One set was comprised of between population (HTSPHS and RBWP) crosses and another set of within population crosses.

RESULTS

Genetic divergence based on COI and ITS sequences

A total of 47 isolates from 5 populations from 2 sites were used for phylogenetic reconstruction based on partial COI gene sequences in *E. senta* species complex populations from the northern Chihuahuan Desert. For the nuclear ribosomal ITS region, a total of 42 isolates were used. An additional Bayesian phylogenetic analysis was included with four *E. senta* species complex sequences obtained from GenBank (Table 1). *E. brachionus* was used as outgroup for Bayesian and maximum likelihood analyses based on sequences for both genetic markers. A total of 649 base pairs for the COI gene and 673 base pairs for the ITS region were sequenced. According to Xia's test for saturation of nucleotide substitution, the COI sequences are not saturated since index of substitution saturation (Iss) was significantly smaller than the critical Iss value (Iss.c) ($p < 0.001$; Table 8) and thus are adequate for phylogenetic analyses. Substantial genetic differences were found for the COI gene, ranging from 18.8-21.4% between individuals of RBWP and HTSPHS (Table 2). Genetic differences within populations ranged from 0-3.2% in the COI gene. On the other hand little genetic differentiation ($< 5\%$) was found in the ITS region sequences among RBWP and HTSPHS individuals (Table 3). Genetic differences for the ITS region ranged from 0-0.4% and 0-0.8% when comparing individuals from the same sampling location (RBWP and HTSPHS, respectively). When comparing genetic differences among individuals from different locations, uncorrected p distances for these sequences ranged from 3.8% to 5.0%.

Correlation of genetic to geographic distance was tested by a Mantel test with four different analyses for each genetic marker (COI and ITS). The first analysis compared genetic distance to geographic distance and had a very high positive correlation but this relationship was not statistically significant (Figure 2, $r = 0.999$, $p = 0.08$ and Figure 3, $r = 0.999$, $p = 0.075$). Similarly, the other three analyses showed positive, but not statistically significant, correlations of geographic and genetic

distances. Only one of the four analyses for each genetic region (COI and ITS) is included here as all of them had similar results.

An initial Bayesian analysis including only individuals from the northern Chihuahuan Desert for the COI gene supported monophyly of populations from each locality (RBWP and HTSPHS) with a high posterior probability (Figure 4). Maximum likelihood results were congruent with Bayesian analysis and showed similar results also forming two main clades with high support (bootstrap value = 100) (Figure 5). An additional Bayesian phylogenetic analysis including other members of the *E. senta* species complex formed two main groups (posterior probability = 1). One group was a monophyletic cluster composed of individuals of HTSPHS only; representing *E. chihuahuensis* (Figure 7). The other group was composed of the other members of the *E. senta* species complex with the isolates from RBWP. This cluster was further divided and had the isolate from Norway (JF714413) splitting from the rest of the RBWP and *E. senta* species complex isolates (posterior probability = 1). The other cluster had a polytomy with three groups formed: a group formed by most of the isolates from RBWP, a group that included an isolate from RBWP, an isolate from Germany (JF714414) and the other isolate obtained from GenBank (Garcia-Varela & Nadler 2006) (DQ089728), and a group that included an isolate from Hawaii with the rest of the RBWP isolates. Using the K/Θ method for isolates from the northern Chihuahuan Desert, two independently evolving lineages were confirmed, with a probability higher than 95%.

For 29 *E. chihuahuensis* COI sequences, 11 haplotypes were identified by DnaSP with haplotype diversity of 0.69. *E. chihuahuensis* individuals shared haplotypes among individuals from all three ponds at HTSPHS. Haplotype analysis of the ITS region yielded 9 haplotypes with a haplotype diversity of 0.75. Haplotypes in the ITS region were also shared among individuals from all three ponds at HTSPHS. For 47 COI sequences from isolates from the northern Chihuahuan Desert, 21 haplotypes were identified by DnaSP with haplotype diversity of 0.87 (Table 6). RBWP and HTSPHS individuals

shared haplotypes with individuals from the same location but did not share any haplotypes with individuals from other locations. Similarly, individuals from RBWP and HTSPHS did not share any haplotypes based on ITS sequences. For this region, 15 haplotypes were detected among 42 DNA sequences from northern Chihuahuan Desert individuals, with a haplotype diversity of 0.77 (Table 7).

Two groups, one composed of isolates from RBWP and the other composed of isolates from HTSPHS were used for AMOVA. The groups were formed based on geographic location as well as Bayesian and maximum likelihood analyses from the five samples (three huecos at HTSPHS and two wetland cells at RBWP) (Table 12). AMOVA results show that most of the variation in these isolates is found among groups ($\phi_{st} = -0.007$; 95.7%; $p < 0.0001$). This indicates that most of the variation is found between the RBWP clade and the HTSPHS clade. Variation within populations accounted for 4.3% of the variation but this result was not statistically significant ($p = 0.099$).

RAPD Analysis

Analysis of Randomly Amplified Polymorphic DNA fingerprinting yielded 65 polymorphic loci. Band reproducibility was tested in 75% of the reactions and 92% were reproducible. Bands that were not reproducible were not included in the analysis. A total of 125 individuals were included in the final analysis: RBWP Cell 1 ($n=27$), RBWP Cell 2 ($n=34$), HTSPHS Heart ($n=20$), North ($n=22$), and South ($n=22$). Individuals from RBWP had four loci that were exclusive to their group. HTSPHS individuals did not have any exclusive loci. Population structure analysis showed the most appropriate number for K is 2 ($\Delta K = 39.68$) (Figure 8). Two discrete clusters were formed, each corresponding to one of the two main sampling locations (RBWP and HTSPHS) (Figure 9). When $K=2$, individuals from both RBWP Cell 1 and Cell 2 had an average 0.98 probability of belonging to RBWP cluster (Figure 9). Individuals from HTSPHS Heart, North and South had average probabilities of 0.90, 0.98 and 0.93, respectively, of belonging to HTSPHS cluster (Figure 9).

DISCUSSION

Although results do not demonstrate further cryptic speciation in *E. chihuahuensis*, results demonstrate the presence of different lineages occurring in a relatively small region of the northern Chihuahuan Desert. DNA genetic sequence data and DNA fingerprinting analysis showed that isolates from HTSPHS and RBWP are distinct and are not members of the same species. It is presently unknown if the diapausing eggs produced by cross-mating experiments are viable or not, as a result reproductive isolation between the lineages cannot be completely ruled out. The high amount of genetic sequence variation between the HTSPHS and the RBWP lineages in congruence with the patterns shown by phylogeny reconstruction and population structure suggest that the distinct lineages found in the northern Chihuahuan Desert are different species, however this does not correspond to cryptic speciation in *E. chihuahuensis*. Levels of genetic divergence between the two populations found in this region compares to what has been found recently in other rotifer taxa (Monogonont: Garcia-Morales & Elias-Gutierrez, 2013; Leasi *et al.*, 2013, Schröder & Walsh, 2010; Walsh *et al.*, 2009; Mills, Lunt, & Gomez, 2007; Schröder & Walsh, 2007; Gilbert & Walsh, 2005; Suatoni *et al.*, 2006; Bdelloid: Fontaneto *et al.*, 2011; Fontaneto *et al.*, 2008b; Fontaneto *et al.*, 2009) as well as in other aquatic invertebrates (Chen & Hare, 2011; Goetze, 2010, Schon *et al.*, 2012, Bradford *et al.*, 2010; Belyaeva & Taylor, 2009; Forro *et al.*, 2008).

Genetic distances within isolates from HTSPHS indicate that they are member of the same species corresponding to *E. chihuahuensis* and no signs of cryptic speciation were detected within this species. On the other hand a high degree of genetic divergence was detected between isolates from HTSPHS and RBWP. Genetic distances among lineages from the two sites are similar to those of other species based on traditional morphology. High levels of divergence of COI sequences (18.8-21.4%) between isolate of HTSPHS and RBWP compares to what has been found in cryptic species of

monogonont rotifers (Garcia-Morales & Elias-Gutierrez, 2013; Leasi *et al.*, 2013; Fontaneto *et al.*, 2009; Schröder & Walsh, 2007) and bdelloid rotifers (Fontaneto *et al.*, 2008b, 2011, Hamdan, 2010). COI sequence divergence from this study, ca. 19-21% between members of the two major clades (HTSPHS and RBWP), compares to and exceeds that of Schröder & Walsh (2007), where sequence divergence ranged from ca. 11-14% between Hawaiian, German, and Texan populations. In this study RBWP isolates were nearly identical to an isolate from Hawaii indicating that those RBWP isolates could be members of *E. hawaiiensis* and not *E. chihuahuaensis* as previously believed. Two isolates obtained from GenBank (JF714414 and DQ089728) formed a cluster with an isolate from RBWP; this could indicate that those isolates are also from *E. hawaiiensis*. It is important to note that the German isolate obtained from GenBank (JF714414) is different from *E. ukera* isolates characterized by Schröder and Walsh (2007). This suggests that the German isolate may not be a member of *E. ukera* as it forms a highly supported (0.96 posterior probability) cluster with *E. hawaiiensis*. The Norwegian isolate obtained from GenBank (JF714413) formed a group with RBWP and the other isolates obtained from GenBank, which was very distinct from HTSPHS clade. Within this group, the Norwegian isolate branches off to its own group, suggesting possible further cryptic speciation in the *E. senta* species complex.

Isolates from the Chihuahuan Desert had a genetic divergence range that compares to that of *Testudinella clypeata*, which ranges from ca.17-28% (Leasi *et al.*, 2013) and *Brachionus plicatilis*, which ranges from ca. 6-24% (Fontaneto *et al.*, 2009). Mean sequence divergence between the two main genetic clusters of populations from the northern Chihuahuan Desert (19.5%) also compares with the mean sequence divergence of other monogonont rotifer taxa such as *Platyia quadricornis* (~21%), *Lecane lunaris* (21%), *Brachionus quadridentatus f. brevespinus* (18%), and *Mytilina ventralis var. macracantha*, (20%) (Garcia-Morales & Elias-Gutierrez, 2013). These results from partial COI gene sequences divergence also compare to amount of sequence divergence that has been found in some

bdelloid rotifers (Birky *et al.*, 2011; Hamdan, 2010; Fontaneto *et al.*, 2009). This degree of COI sequence differentiation is also similar to levels found in other aquatic invertebrate taxa such as the copepods *Skiptodiaptomus* (>15%; Thum & Harrison, 2009) and *Rhincalanus nasutus* (20-24%; Goetze, 2003).

Phylogenetic analyses demonstrate the existence of distinct lineages in the *E. senta* complex occurring in close proximity in the northern Chihuahuan Desert. HTSPHS and RBWP lineages formed two separate monophyletic clades in analyses including only populations from the Chihuahuan Desert. This was supported by Bayesian and maximum likelihood methods of phylogeny inference. Although ITS sequence divergence was relatively low, ranging from 3.8-5.0% between populations, it was consistent with phylogenetic analyses of COI and also formed two separate clades (HTSPHS and RBWP). Also, mean ITS sequence divergence in this study was similar to that found in *Brachionus calyciflorus* populations by Gilbert & Walsh (2005). In their study, they found a mean ITS sequence divergence of 5.2% between populations from Georgia, Florida, Texas, and Australia whereas in *E. senta* isolates from the Chihuahuan Desert, it was slightly smaller with a mean sequence divergence of 4.2% between HTSPHS and RBWP populations. Results from this study are also similar to those found in *Brachionus plicatilis* where a maximum sequence divergence within lineages was 0.6% and maximum between lineages sequence divergence was 3% (Gomez *et al.*, 2002). Results from Gomez *et al.* (2002) compare to the present study since ITS sequence divergence had a maximum of 0.8% within lineages and a maximum of 5% between lineages. ITS sequence divergences in this study are probably higher by the addition of the ITS2 region, which is more variable. Analysis of ITS also support the existence of distinct genetic lineages of the *E. senta* species complex in this region.

Analyses of geographic and genetic distance using only isolates from the northern Chihuahuan Desert showed a strong positive correlation but this correlation was not statistically significant ($r=0.999$, $r^2=1.00$, $p=0.08$). This is unlike the findings of Mills *et al.* (2007) in which they found that geographic

distance explained the variance in genetic distance between *Brachionus plicatilis* populations ($r^2=0.73$, $p<0.01$). Hamdan (2010) also found positive correlation between genetic and geographic distance, in the bdelloid rotifer *Philodina megalotrocha*, when using log transformed data ($r=0.178$ $p<0.007$) contrasting with our results in which strong correlation was found when using log transformed data, although it was not statistically significant. The results in this study may not be significant due to the small sample size. Although these results did not yield a statistically significant correlation between geographic and genetic distances, they show a pattern in which two discrete clusters are formed. One is formed by comparing distances within populations and the other by comparing distances between populations. Again, this provides evidence for the existence of two discrete clusters in *E. senta* populations from the northern Chihuahuan Desert.

COI haplotype diversity in *E. chihuahuensis* ($H_d=0.69$) is similar to that found in populations of the monogonont rotifer *Brachionus calyciflorus* ($H_d=0.614-0.879$) (Li *et al.*, 2010). Higher levels of haplotype diversity have been found in the bdelloid rotifer *Philodina megalotrocha* ($H_d=0.98$) (Hamdan, 2010). Although haplotype diversity levels are similar to Li *et al.* (2010) and Hamdan (2010), no distinct lineages were detected within *E. chihuahuensis*. Haplotypes were also shared among all three locations at HTSPHS and sequence divergence among the COI examined haplotypes of *E. chihuahuensis* was very low (0-1.6%). Genetic divergence among haplotypes in Li *et al.* (2010) was higher (12.1%) with two distinct lineages detected in *B. calyciflorus* occurring in a pond. Genetic distance between haplotypes was also higher (up to 19.7%) in Hamdan (2010). Even though no distinct lineages within *E. chihuahuensis* were found in this study, genetic distance between haplotypes from two populations (HTSPHS and RBWP) was high (19.5%) and no haplotypes were shared between individuals from the two locations. This shows the presence of distinct lineages of *E. senta* complex occurring in the northern Chihuahuan Desert.

Analysis of molecular variance also supports the idea of different lineages of *E. senta* occurring in the Chihuahuan Desert as results do not show apparent structure between the two groups (HTSPHS and RBWP). These two groups of *E. senta* complex from the Chihuahuan Desert are also genetically differentiated. This is similar to what was found in Schröder & Walsh (2010) in which AMOVA of RAPD data did not show genetic structuring between the three species of the *E. senta* complex indicating that they were separate genetic lineages. Therefore, AMOVA indicates that the two populations in northern Chihuahuan Desert are not members of the same species.

The existence of two distinct lineages of the *E. senta* species complex occurring in the Chihuahuan Desert region of El Paso was also verified by RAPD analysis as it placed individuals from HTSPHS and RBWP into two distinct clades. No individuals from each of the two populations (HTSPHS and RBWP) were designated to the other lineage with a high probability (highest probability of belonging to the other cluster was 0.10 for individuals from HTSPHS Heart). Within *E. chihuahuensis* only one genetic cluster was found showing no evidence for cryptic speciation within the species. These results do not support Schröder & Walsh (2010) since only one lineage was detected at HTSPHS while two were detected in their study. This may be due to the fact that different primers were used in each study. Primers for their study were OP-H7, OP-H16, and OP-H18 while primers for this study were OP-G5, OP-H6, OPH18, AND OP-W3. Initially we evaluated OP-H7 and OP-H16 for band polymorphism and banding pattern reproducibility in this study, but we did not yield positive results. The selected primers gave the best results in terms of polymorphisms and reproducibility. Another reason that the results here differ from those of Schröder and Walsh (2010) may be due to the ability to detect distinct lineages at the time of sampling. Even though sampling took place on numerous occasions in the course of three years, *E. chihuahuensis* was only detected a few times thus there may be a possibility that the other lineage was not present during sampling. Additional populations from *E.*

chihuahuensis from other sites are necessary to fully determine if further cryptic speciation exists in the *E. senta* species complex.

Reproductive isolation experiments show that gene flow is still possible between the HTSPHS and RBWP groups as diapausing embryos were produced in a few of the cross-matings (9%): males from Rio Bosque Cell 2 crossed with females of Hueco Tanks North and Heart, and males from Hueco Tanks Heart with females from Rio Bosque Cell 1. Presently, it is unknown whether the embryos produced from these cross-matings are viable. Even though mating can still occur under laboratory conditions it is currently unknown if they are reproductively isolated in nature.

Even though no evidence for cryptic speciation was found within *E. chihuahuensis*, occurrence of very distinct lineages existing in very close proximity in the northern Chihuahuan Desert was found. As demonstrated by this and previous studies, very distinct lineages can occur not only at large spatial scales (Belyaeva & Taylor, 2009; Bode et al., 2010; Goetze, 2010, Schröder & Walsh, 2007) but also at very small spatial scales (Montero-Pau et al., 2011; Gomez et al., 2002; Li et al., 2010). For example, Hamdan (2010) found distinct lineages of a bdelloid rotifer (*Philodina megalotrocha*) at both large and small spatial scales. In the present study we found very distinct lineages occurring at a small spatial scale, within 40 km. While there have been studies that have shown that cryptic species can even exist in the same body of water (e.g., the presence of distinct lineages in a lake (Montero-Pau et al., 2011; Dionne et al., 2011) or in the same pond (Li et al., 2010)), as was the case of Schröder & Walsh (2010), in this study we did not detect cryptic speciation within *E. chihuahuensis* or distinct lineages within each site. This might be explained by a possible seasonal fluctuation where individuals from other lineages were not present at the time of sampling. Other studies have shown that sampling effort has an impact on the ability to detect certain lineages (Hamdan, 2010) which may also explain why only one lineage was found at HTSPHS and not two as in Schröder & Walsh (2010). In this study however, very different lineages were found living within a small geographic region. The fact that such high

genetically distinct lineages can be found living in such close proximity can most likely be explained by drastic environmental changes. For example desert rock pools at HTSPHS fill up quickly with rain water but also dry very fast because of their small size. This can put selective pressure in individuals to go into the sexual phase of their lifecycle faster than would be the case if they inhabited a more permanent habitat. Individuals that can produce diapausing eggs faster have a higher chance of thriving in these extreme environments as they would be able to produce the resistant cysts that can survive the extreme conditions and hatch once conditions are favorable again. Individuals that do not produce diapausing eggs before the pond dries will die before having produced the resistant stage and will not be able to successfully colonize the site. These rapid changes in the environment may favor the occurrence of sibling species in such close proximity. These changes can also differ depending not only on the size of the ponds as mentioned above (small vs large), but also on the location (shaded vs fully exposed) of the water body. For example, the ponds and wetlands in this region can fill after a rain event at different rates then dry also at different rates depending on the factors discussed above thus promoting diversification (Schröder et al., 2007). Even though the exact mechanism that allows sibling species to live in close proximity is unknown, their maintained coexistence could be explained by niche partitioning or seasonal fluctuation (Montero-Pau *et al.*, 2011; Li et al., 2010). As for *E. senta* species complex populations in the northern Chihuahuan Desert, it is presently unknown what may be keeping the two distinct populations isolated, given the high dispersal capability of these microscopic organisms. But given that no sampling event coincided, as one location was dry when the other was wet, gives the idea that there may be a change in mixis pattern or a change in the time of egg hatching.

Our results are beginning to give us an idea of the level of complexity underlying the biodiversity of zooplankton. While we see that some rotifers from desert regions seem to have cosmopolitan distribution (Wallace et al., 2008), some others have not yet been discovered elsewhere. The isolates found at RBWP seem to have a global distribution but *E. chihuahuensis* has yet to be

discovered at other places besides HTSPHS. Local adaptations, dispersal and colonization ability likely determine what species would be able to inhabit particular temporary pools. For example, diapausing eggs could be transported into one of these desert ponds by wind, water or other animals (De Meester et al., 2002). Once in the pond a subset of individuals may emerge from diapausing eggs, if conditions are favorable, and become adults if they survive predation and disease. The adults would then, through an environmental queue, produce other diapausing eggs that can be deposited into a diapausing egg bank in the pond. Some of these eggs could either hatch in the site once conditions are favorable again or will be transported out of the pond by the previously mentioned mechanisms. Some eggs deposited into the resting egg bank may be buried and may not hatch until they emerge from the sediment, which can happen very quickly or may take a very long time. Another possibility is that the egg being deposited in the pond may be directly taken out before having the time to hatch and reproduce. The situation of the complexity of biodiversity in the northern Chihuahuan Desert may be explained by these mechanisms of adaptation, colonization and dispersal (Hamdan, 2010). Different lineages have been found in this area, for example the individuals found at RBWP could be members of *E. hawaiiensis*, which would explain why they were able to successfully colonize RBWP since it is a more permanent habitat, which is similar to where *E. hawaiiensis* was found (Schröder and Walsh, 2007) and not the rock pools at HTSPHS, which are ephemeral pools with very drastic changes. On the other hand the individuals from *E. chihuahuensis* have not yet been detected outside HTSPHS. This could mean that *E. chihuahuensis* are highly adapted to temporary small desert rock pools and can successfully colonize them. As mentioned previously sampling additional pools, including those with similar characteristics, would give a better understanding on the diversity of these animals and how they adapt to different types of environments. As seen in this study, the biodiversity of invertebrates in these types of habitats is highly dependent upon our ability to detect it and also that we are just beginning to understand hidden diversity and its importance in these ecosystems. Therefore it is necessary to look more carefully at the

phenomenon of cryptic speciation. This will allow us to better understand the dynamics of aquatic ecosystems as each species likely contributes differently to their habitat.

REFERENCES

- Arndt, H. (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. *Hydrobiologia* 255/256:231-246.
- Belyaeva, M., and Taylor, D.J. (2009) Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology. *Mol Phylogenet Evol.* 50(3):534-46.
- Bickford, D., Lohman, D.J., Sodhi N.S., Ng, P.K.L., Meier, R., Winker, K., *et al.* (2006) Cryptic species as a window on diversity and conservation. *TREE* 22: 148-155.
- Birky, C.W.Jr., Wolf, C., Maughan, H., Herbertson, L., and Henry, E. (2005) Speciation and selection without sex. *Hydrobiologia* 546:29-45.
- Birky, C.W.Jr., Ricci, C., Melone, G., and Fontaneto, D. (2011) Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). *Zoological Journal of the Linnean Society* 161:723-734.
- Bode, S.N.S., Adolfsson, S., Lamatsch, D.K., Martins, M.J.F., Schmit, O., Vandekerkhove, J., *et al.* (2010) Exceptional cryptic diversity and multiple origins of parthenogenesis in a freshwater ostracod. *Molecular Phylogenetics and Evolution* 54:542-552.
- Bradford, T., Adams, M., Humphreys, W.F., Austin, A.D., Cooper, S.J.B. (2010) DNA barcoding of stygofauna uncovers cryptic amphipod diversity in a calcrete aquifer in Western Australia's arid zone. *Molecular Ecology Resources* 10:41-50.
- Chen, G., and Hare, M.P. (2011) Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. *Molecular Ecology*, 20, 2425–244.

Climatography of the United States No. 20: El Paso Intl AP, TX 1971–2000" National Oceanic and Atmospheric Administration. Web accessed May 16, 2013.

<<http://cdo.ncdc.noaa.gov/climatenormals/clim20/tx/412797.pdf>>

Darriba D, Taboada GL, Doallo R, Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8), 772.

De Meester, L., Gomez, A., Okamura, B., and Schwenk, K. (2002) The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica* 23:121-135.

De Wit, R., and Bouvier, T. (2006) ‘Everything is everywhere, but, the environment selects’; what did Baas Becking and Beijerinck really say? *Environmental Microbiology* 8(4):755-758.

Dionne, K., Vergilino, R., Dufresne, F., Charles, F., and Nozais, C. (2011) No evidence for temporal variation in a cryptic species community of freshwater amphipods of the *Hyalella azteca* species complex. *Diversity* 3:390-404.

Drummond, A., and Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7:214-221.

Earl, D.A. and vonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4 (2):359-361 doi: 10.1007/s12686-011-9548-7 Core version: vA.1 March 2012 Plot version: vA.1 November 2012 Web version: v0.6.93 November 2012. Web accessed June 26, 2013.

Ersts, P.J. [Internet] Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available from http://biodiversityinformatics.amnh.org/open_source/gdmg. Web accessed February 12, 2013.

Evanno, G., Regnaut, S., and Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611-2620.

- Excoffier, L., and Lischer, H.E.L. (2010) Arlequin suite ver 3.11: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.
- Falush, D., Stephens, M., and Pritchard, J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567–1587.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I form diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3:294-299.
- Fontaneto, D., Barraclough, T.G., Chen, K., Ricci, C., and Herniou, E.A. (2008a) Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. *Mol. Ecol.* 17:3136–3146.
- Fontaneto, D., Boschetti, C., and Ricci, C. (2008b) Cryptic speciation in ancient asexuals: evidences from the bdelloid rotifer *Philodina flaviceps*. *J. Evol. Biol.* 21:580–587.
- Fontaneto, D., Kaya, M., Herniou, E., and Barraclough, T. (2009) Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution* 53:182–189.
- Fontaneto, D., Iakovenko, N., Eyres, I., Kaya, M., Wyman, M., and Barraclough, T.G. (2011) Cryptic diversity in the genus *Adineta* Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. *Hydrobiologia* 662:27-33.
- Forro, L., Korovchinsky N.M., Kotov, A.A., and Petrusek, A (2008) Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia* 595:177-184.
- Garcia-Morales, A.E., and Elias-Gutierrez, M. (2013) DNA barcoding of freshwater Rotifera in Mexico: Evidence of cryptic speciation in common rotifers. *Molecular Ecology Resources*. doi: 10.1111/1755-0998.12080

- Garcia-Varela, M., Nadler S.A. (2006) Phylogenetic relationships among Syndermata inferred from nuclear and mitochondrial gene sequences. *Mol. Phylogenet. Evol.* 40(1):61-72
- Gilbert, J.J., and Walsh, E.J. (2005) *Brachionus calyciflorus* is a species complex: Mating behavior and genetic differentiation among four geographically isolated strains. *Hydrobiologia* 546:257-265.
- Gomez, A., and Carvalho, G.R. (2000) Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology* 9:203–214.
- Goetze, E. (2003) Cryptic speciation in the high seas; global phylogenetics of the copepod family Eucalinidae. *Proc. R. Soc. Lond. B* 270: 2321-2331.
- Goetze, E. (2010) Species discovery in marine planktonic invertebrates through global molecular screening. *Molecular Ecology* 19: 952-967
- Gomez, A., Clabby, C., and Carvalho, G.R. (1998) Isolation and characterization of microsatellite loci in a cyclically parthenogenetic rotifer, *Brachionus plicatilis*. *Molecular Ecology* 7:1613-1621.
- Gomez, A., Serra, M., Carvalho, G.R., and Lunt, D.H. (2002) Speciation in ancient cryptic species complexes: Evidence from molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution* 56:1431–1444.
- Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, *et al.* (2010) A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res.* 38:695-699
[doi:10.1093/nar/gkq313](https://doi.org/10.1093/nar/gkq313)
- Gribble, K.E., and Mark Welch, D.B. (2012) The mate recognition gene mediates reproductive isolation and speciation in the *Brachionus plicatilis* cryptic species complex. *BMC Evolutionary Biology* 12:134
- Guindon, S., and Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol.* 52:696-704.

- Hamdan, L. K. (2010) *Ecology and genetics of Philodina megalotrocha (Rotifera, Bdelloidea) from Chihuahuan Desert populations*. (Order No. 1477791, The University of Texas at El Paso). *ProQuest Dissertations and Theses*, , 85. Retrieved from <http://search.proquest.com/docview/604829417?accountid=7121>. (prod.academic_MSTAR_604829417).
- Hebert, P.D.N., Cywinska, A., Ball S.L., and deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proc Biol Sci* 270:313–321.
- Huang, X., and Madan, A. (1999) CAP3: A DNA sequence assembly program. *Genome Res.*, 9:868-877.
- “Hueco Tanks: Crossroads through Time.” *Texas Beyond History*. The University of Texas at Austin. January 2008. Web accessed March 6 2013.
- Huelsenbeck, J.P., and Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754-755.
- Jensen, J.L., Bohonak, A.J., and Kelley, S.T. (2005) Isolation by distance, web service. *BMC Genetics* 6: 13. v.3.23. <<http://ibdws.sdsu.edu/>>
- Koufopanou, V., Burt, A., Szaro, T., and Taylor, J. (2001) Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). *Mol Biol Evol* 18:1246-1258.
- Leasi, F., Tang, C.Q., De Smet, W.H., and Fontaneto, D. (2013) Cryptic diversity with a wide salinity tolerance in the putative euryhaline *Testudinella chlypeata* (Rotifera, Monogononta). *Zoological Journal of the Linnean Society*, doi: 10.1111/zoj.12020
- Li, Li, Cuijuan Niu, and Rui Ma (2010) Rapid temporal succession identified by COI of the rotifer *Brachionus calyciflorus* Pallas in Xihai Pond, Beijing, China, in relation to ecological traits. *Journal of Plankton Research* 32(6):915-959.

- Librado, P., and Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451-1452
- Mark Welch, D., and Messelson, M. (2000) Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* 288:1211-1214.
- Mark Welch, J., Mark Welch, D., and Messelson, M. (2004) Cytogenetic evidence for asexual evolution of bdelloid rotifers. *PNAS* 101:1618-1621.
- Mills, S., Lunt, D.H., and Gomez, A. (2007) Global isolation by distance despite strong regional phylogeography in a small metazoan. *BMC evolutionary biology* 7:225-235
- Montero-Pau, J., Ramos-Rodriguez, E., Serra, M., and Gomez, A. (2011) Long-term coexistence of rotifer cryptic species. *PLoS ONE*, 6, e21530.
- Murphy, N.P., Adams, M., and Austin, A.D. (2009) Independent colonization and extensive cryptic speciation of freshwater amphipods in the isolated groundwater springs of Australia's Great Artesian Basin. *Molecular Ecology* 18:109-122
- Okin, G. S., D'Odorico, P., and Archer, S.R. (2009) Impact of feedbacks on Chihuahuan Desert grasslands: Transience and metastability, *J. Geophys. Res.*, 114, G01004, doi:10.1029/2008JG000833.
- Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., et al. (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst Biol* 55:595–609.
- Pritchard, J. K., Stephens, M., and Donnelly P. J. (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- Puillandre, N., Lambert, A., Brouillet, S., Achaz, G. (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol* 21:1864–1877.

- Ricci, C., and Balsamo, M. (2000) The biology and ecology of lotic rotifers and gastrotrichs. *Freshwater Biology* 44:15-28.
- Rico-Martinez, R. (1998) Cross-mating tests re-discovered: a tool to asses species boundaries in rotifers. *Hydrobiologia* 387/388:109-115.
- Ronquist, F. and Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.
- Rosenberg, N. A., (2004) Distruct: a program for the graphical display of population structure. *Mol.Ecol. Notes* 4:137–138.
- Rozen, S., and Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. Pp. 365-386 In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz, S., Misener, S.). Humana Press, Totowa, NJ.
- Schröder, T. (2003) Precopulatory mate guarding and mating behaviour in the rotifer *Epiphanes senta* (Monogononta:Rotifera). *Proc. R. Soc. Lond. B* 270:1965-1970.
- Schröder, T., Howard, S., Arroyo, L., and Walsh, E.J. (2007) Sexual reproduction and diapause of *Hexarthra* sp. (Rotifera) in short-lived Chihuahuan Desert ponds. *Freshwater Biology* 52:1033-1032.
- Schröder, T., and Walsh, E.J. (2007) Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of three new species. *Hydrobiologia* 593:129-140.
- Schröder, T., and Walsh, E.J. (2010) Genetic differentiation, behavioural reproductive isolation and mixis cues in three sibling species of monogonont rotifers. *Freshwater Biology* 55: 2570–2584.
- Schön I, Pinto RL, Halse S, Smith AJ, Martens K, et al. (2012) Cryptic Species in Putative Ancient Asexual Darwinulids (Crustacea, Ostracoda). *PLoS ONE* 7(7): e39844. doi:10.1371/journal.pone.0039844

- Seidel, R.A., Lang, B.K., and Berg, D.J. (2009) Phylogeographic analysis reveals multiple cryptic species of amphipods (Crustacea: Amphipoda) in Chihuahuan Desert springs. *Biological Conservation* 142:2303-2313
- Sievers, F., Wilm, A., Dineen, D.G., Gibson T.J., Karplus, K., Li, W. *et al.* (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology* 7:539 [doi:10.1038/msb.2011.75](https://doi.org/10.1038/msb.2011.75)
- Sorensen, M., and Giribet, G. (2006) A modern approach to rotiferan phylogeny: Combining morphological and molecular data. *Molecular Phylogenetics and Evolution* 40:585–608.
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models, *Bioinformatics* 22(21):2688–2690.
- Suatoni, E., Vicario, S., Rice, S., Snell, T., and Caccone, A. (2006) An analysis of species boundaries and biogeographic patterns in a cryptic species complex: The rotifer—*Brachionus plicatilis*. *Molecular Phylogenetics and Evolution* 41:86–98.
- Swofford, D.L. (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods), vers. 4.0. Sinauer Assoc., Sunderland, MA.
- Stemberger, R.S. (1981) A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences* 38:721–724.
- Thum, R.A., and Derry, A.M. (2008) Taxonomic implications for diaptomid copepods based on contrasting patterns of mitochondrial DNA sequence divergences in four morphospecies. *Hydrobiologia* 614:197-207
- Thum, R.A., and Harrison, R.G. (2009) Deep genetic divergences among morphologically similar and parapatric *Skistodaptomus* (Copepoda: Calanoida: Diaptomidae) challenge the hypothesis of Pleistocene speciation. *Biological Journal of the Linnean Society* 96:150-165

- Wallace, R.L., Walsh, E.J., Arroyo, M.L., and Starkweather, P.L. (2005) Life on the Edge: Rotifers From Springs and Ephemeral Waters in the Chihuahuan Desert, Big Bend National Park (Texas, USA). *Hydrobiologia* 546:147-157.
- Wallace, R.L., Walsh, E.J., Schroder, T., Rico-Martinez, R., and Rios-Arana, J.V. (2008) Species composition and distribution of rotifers in Chihuahuan Desert waters of Mexico: is everything everywhere? *Verh Internat. Verein. Limnol.* 30:73-76.
- Walsh, E.J., Schröder, T., Wallace, R.L., and Rico-Martinez, R. (2009) Cryptic speciation in *Lecane bulla* (Monogononta: Rotifera) in Chihuahuan Desert waters. *Verh Internat. Verein. Limnol.* 30:1046-1050.
- Watts, S.H., Sproul, J., and Hamlyn, E. (2002) A Biological Management Plan for the Rio Bosque Wetlands Park. Center for Environmental Resource Management. University of Texas at El Paso, 500 West University Avenue, El Paso, Texas 79968.
- White, T.J., Bruns, T., Lee, S., and Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: *PCR Protocols: A Guide to Methods and Applications*, Eds. Innis, M.A., D. H., Gelfand, J.J., Sninsky, and T.J. White. Academic Press, Inc., New York.
- Witt, J.D.S., Threlhoff, D.L., and Hebert, P.D.N. (2006) DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. *Molecular Ecology* 15:3073-3082
- Xia, X., Xie, Z., Salemi, M., Chen, L., and Wang, Y. (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* 26:1-7.
- Xia, X., and Lemey, P. (2009) Assessing substitution saturation with DAMBE. Pp. 615-630 In: Philippe Lemey, Marco Salemi and Anne-Mieke Vandamme, eds. *The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny*. 2nd edition Cambridge University Press.

TABLES

Table 1. Populations of *E. senta* species complex and *E. brachionus* included in this study.

Population	Abbreviation	Locality	Date of Collection	GPS Coordinates
Hueco Tanks Heart	HT_HD17	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD4	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD36	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD8	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD32	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_H3	HTSPHS, El Paso Co., USA	12/3/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_H2	HTSPHS, El Paso Co., USA	<8/23/2010	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_H1	HTSPHS, El Paso Co., USA	<8/23/2010	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD11	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks Heart	HT_HD9	HTSPHS, El Paso Co., USA	12/27/2011	31.9248667 N, -106.0424 W
Hueco Tanks North	HT_NTD19	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTD5	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTD1	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTD7	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTD9	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTB4	HTSPHS, El Paso Co., USA	12/29/2010	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTMA1	HTSPHS, El Paso Co., USA	5/10/2013	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NT23	HTSPHS, El Paso Co., USA	12/29/2010	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NT11	HTSPHS, El Paso Co., USA	12/29/2010	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTB2	HTSPHS, El Paso Co., USA	12/29/2010	31.92475 N, -106.0423 W
Hueco Tanks North	HT_NTD11	HTSPHS, El Paso Co., USA	12/27/2011	31.92475 N, -106.0423 W
Hueco Tanks South	HT_STD12	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD14	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD9	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_ST1	HTSPHS, El Paso Co., USA	12/29/2010	31.9247333 N, -106.04225 W

Hueco Tanks South	HT_STM16	HTSPHS, El Paso Co., USA	3/10/2012	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STM3	HTSPHS, El Paso Co., USA	3/10/2012	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STM13	HTSPHS, El Paso Co., USA	3/10/2012	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD24	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_ST10	HTSPHS, El Paso Co., USA	12/29/2010	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STM15	HTSPHS, El Paso Co., USA	3/10/2012	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD11	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD5	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Hueco Tanks South	HT_STD22	HTSPHS, El Paso Co., USA	12/27/2011	31.9247333 N, -106.04225 W
Rio Bosque Cell 1	RBC1_107	RBWP, El Paso Co., USA	3/4/2011	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_4	RBWP, El Paso Co., USA	2010	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_1	RBWP, El Paso Co., USA	11/11/2010	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_D40	RBWP, El Paso Co., USA	11/27/2012	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_65	RBWP, El Paso Co., USA	3/4/2011	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_F11	RBWP, El Paso Co., USA	1/31/2013	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_138	RBWP, El Paso Co., USA	3/4/2011	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_71	RBWP, El Paso Co., USA	3/4/2011	31.6430167 N, -106.31181 W
Rio Bosque Cell 1	RBC1_60	RBWP, El Paso Co., USA	3/4/2011	31.6430167 N, -106.31181 W
Rio Bosque Cell 2	RBC2_35	RBWP, El Paso Co., USA	2010	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_26	RBWP, El Paso Co., USA	2010	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_142	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_92	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_121	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_89	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_124	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_95	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_29	RBWP, El Paso Co., USA	2010	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_922	RBWP, El Paso Co., USA	1/6/2011	31.6364667 N, -106.31083 W

Rio Bosque Cell 2	RBC2_115	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_4	RBWP, El Paso Co., USA	2010	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_96	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Rio Bosque Cell 2	RBC2_71	RBWP, El Paso Co., USA	3/4/2011	31.6364667 N, -106.31083 W
Norway (JF714413)	EsentaF	Svalbard, Norway	-	-
Germany (JF714414)	EsentaGer1	Germany	-	-
Garcia-Varela & Nadler (2006) (DQ089728)	EsentaG	-	-	-
Hawaii	Ehawaii	Hawaii, USA	-	-
<i>Epiphanes brachionus</i>				
Hueco Tanks Heart	Ebrach	HTSPHS, El Paso Co., USA	10/09/2012	31.9248667 N, -106.0424 W
Hueco Tanks Heart	Ebrach2	HTSPHS, El Paso Co., USA	10/09/2012	31.9248667 N, -106.0424 W
Hueco Tanks Heart	Ebrach3	HTSPHS, El Paso Co., USA	10/09/2012	31.9248667 N, -106.0424 W

Table 2. Geographic (below diagonal; m) and pairwise genetic distances (above diagonal; %) of 47 clonal lineages of *E. senta* species complex from the northern Chihuahuan Desert. Geographic distances were generated by Geographic Distance Generator v1.2.3. Uncorrected “p” pairwise distances for COI sequences were generated by PAUP*4.0 β.

	Hueco Tanks Heart	Hueco Tanks South	Hueco Tanks North	Rio Bosque Cell 1	Rio Bosque Cell 2
Hueco Tanks Heart	-	0-1.6	0-1.6	19-21.2	19-21.2
Hueco Tanks South	21	-	0-1.6	18.8-21.4	18.8-21.4
Hueco Tanks North	16	5	-	18.8-21.2	18.8-21.2
Rio Bosque Cell 1	40427	40424	40423	-	0-3.2
Rio Bosque Cell 2	40938	40935	40934	735	-

Table 3. Geographic (below diagonal; m) and pairwise genetic distances (above diagonal; %) of 43 clonal lineages of *E. senta* species complex from the northern Chihuahuan Desert. Geographic distances were generated by Geographic Distance Generator v1.2.3. Uncorrected “p” pairwise distances for ITS region sequences were generated by PAUP*4.0 β.

	Hueco Tanks Heart	Hueco Tanks South	Hueco Tanks North	Rio Bosque Cell 1	Rio Bosque Cell 2
Hueco Tanks Heart	-	0-0.4	0-0.4	3.8-4.4	3.8-5.0
Hueco Tanks South	21	-	0-0.4	3.8-4.3	3.8-4.8
Hueco Tanks North	16	5	-	4.0-4.3	4.0-4.8
Rio Bosque Cell 1	40427	40424	40423	-	0-0.8
Rio Bosque Cell 2	40938	40935	40934	735	-

Table 4. Haplotype analysis of COI gene sequences derived from *E. chihuahuensis* isolates produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.

COI			
Number of sequences 29			
Number of haplotypes, h: 11			
Haplotype diversity, Hd: 0.69			
Hueco Tanks			
	Heart	South	North
Number of sequences	9	11	9
Number of haplotypes	4	4	6
Shared haplotypes			
	with Hueco Tanks South and North	with Hueco Tanks Heart and North	with Hueco Tanks Heart and South

Table 5. Haplotype analysis of ITS region sequences derived from *E. chihuahuensis* isolates produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.

ITS			
Number of sequences 28			
Number of haplotypes, h: 9			
Haplotype diversity, Hd: 0.75			
Hueco Tanks			
	Heart	South	North
Number of	10	11	7

sequences			
Number of haplotypes	4	3	3
Shared haplotypes	with Hueco Tanks South and North	with Hueco Tanks Heart and North	with Hueco Tanks Heart and South

Table 6. Haplotype analysis of COI gene sequences derived from Chihuahuan Desert populations of *E. senta* species complex produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.

COI						
Number of sequences 47						
Number of haplotypes, h: 21						
Haplotype diversity, Hd: 0.87						
	Rio Bosque		Hueco Tanks			
	Cell 1	Cell 2	Heart	South	North	
Number of sequences	7	11	9	11	9	
Number of haplotypes	6	7	4	4	6	
Shared haplotypes	with Rio Bosque Cell 2	with Rio Bosque Cell 1	with Hueco Tanks South and North	with Hueco Tanks Heart and North	with Hueco Tanks Heart and South	

Table 7. Haplotype analysis of ITS region sequences derived from Chihuahuan Desert populations of *E. senta* species complex produced by DnaSP v5.10.1 where Hd=1: all sequences have unique haplotypes, Hd=0: all sequences share a common haplotype.

ITS						
Number of sequences 43						
Number of haplotypes, h: 15						
Haplotype diversity, Hd: 0.77						
	Rio Bosque		Hueco Tanks			
	Cell 1	Cell 2	Heart	South	North	
Number of sequences	4	11	10	11	7	
Number of haplotypes	2	7	4	3	3	
Shared haplotypes	with Rio Bosque Cell 2	with Rio Bosque Cell 1	with Hueco Tanks South and North	with Hueco Tanks Heart and North	with Hueco Tanks Heart and South	

Table 8. Xia's test for saturation of nucleotide substitution of 47 *E. senta* species complex sequences and 3 outgroup sequences (*E. brachionus*) for COI genetic sequences implemented in DAMBE. Iss, index of substitution saturation. Iss.cSym, critical value of Iss under assuming symmetric tree topology. Iss.cAsym, critical value of Iss assuming an asymmetric tree topology. DF, degrees of freedom. NumOTU, number of taxa included in analysis.

NumOTU	Iss	Iss.cSym	DF	P-value	Iss.cAsym	DF	P-value
4	0.29	0.805	658	<0.001	0.77	658	<0.001
8	0.26	0.766	658	<0.001	0.66	658	<0.001
16	0.25	0.744	658	<0.001	0.54	658	<0.001
32	0.25	0.718	658	<0.001	0.39	658	<0.001

Table 9. Most likely number of populations (Delta K) in *E. senta* species complex from the northern Chihuahuan Desert as determined by the Evanno method. K was run from K=1 to K=10. The highest Delta K is highlighted in yellow. Delta K is computed by taking the absolute value of the 2nd order rate of change of the likelihood distribution (mean) and dividing it by the standard deviation of the likelihood distribution. K is the number of assumed populations for structure analysis.

K	Repetitions	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	Ln''(K)	Delta K
1	20	-3358.1	0.43	—	—	—
2	20	-3195.94	2.93	162.16	116.28	39.68
3	20	-3150.06	9.75	45.88	10.61	1.09
4	20	-3114.79	45.06	35.28	4.62	0.10
5	20	-3074.89	23.66	39.90	106.30	4.49
6	20	-3141.29	133.37	-66.4	37.21	0.28
7	20	-3244.9	144.62	-103.61	184.32	1.27
8	20	-3164.19	52.88	80.71	152.33	2.88
9	20	-3235.82	66.26	-71.62	23.42	0.35
10	20	-3284.02	115.36	-48.21	—	—

Table 10. Cross-matings for *E. senta* species complex populations from 5 different sites in the northern Chihuahuan Desert. Mictic female eggs are placed together with 1-2 males from a different lineage for possible mating and resting egg production. Table contains the number of eggs produced with the number of trials in parenthesis.

MALES						
FEMALES		Rio Bosque Cell 1	Rio Bosque Cell 2	Hueco Tanks Heart	Hueco Tanks North	Hueco Tanks South
	Rio Bosque Cell 1	5(40)	6(12)	1(16)	0(2)	NA
	Rio Bosque Cell 2	1(23)	3(8)	0(4)	0(2)	NA
	Hueco Tanks Heart	0(2)	2(5)	0(5)	2(3)	1(2)
	Hueco Tanks North	0(5)	1(4)	0(6)	NA	1(1)
	Hueco Tanks South	NA	NA	0(6)	NA	NA

Table 11. Fisher's exact test to determine whether there is a significant difference in egg production between crosses of *E. senta* species complex lineages from the northern Chihuahuan Desert and crosses within *E. senta* species complex lineages from the northern Chihuahuan Desert.

Rows: group	Columns: successfailure		
	failure	success	All
cross	40	4	44
	90.91	9.09	100.00
	27.40	17.39	26.04
	23.67	2.37	26.04
within	106	19	125
	84.80	15.20	100.00
	72.60	82.61	73.96
	62.72	11.24	73.96
All	146	23	169
	86.39	13.61	100.00
	100.00	100.00	100.00
	86.39	13.61	100.00
Cell Contents:	Count		
	% of Row		
	% of Column		
	% of Total		
Pearson Chi-Square = 1.033, DF = 1, P-Value = 0.309			
Likelihood Ratio Chi-Square = 1.111, DF = 1, P-Value = 0.292			
Fisher's exact test: P-Value = 0.444106			

Table 12. Groups of *E. senta* species complex lineages from the northern Chihuahuan Desert arranged by sampling location and clusters according to Bayesian and Maximum Likelihood analyses used for Analysis of Molecular Variation (AMOVA).

Group	Number of Sequences
Rio Bosque	18
Cell 1	7
Cell 2	11
Hueco Tanks	29
Heart	9
North	9
South	11

Table 13. Analysis of Molecular Variance (AMOVA) of *E. senta* species complex populations from two sites in the northern Chihuahuan Desert. Grouping was based on geographic location and COI gene sequence clustering. d.f., degrees of freedom.

Source of variation	d.f.	Percentage of variation	P-value
Among groups	1	95.74	<0.001
Among populations within groups	3	-0.03	0.462
Within populations	42	4.29	0.099
Fixation indices	$\phi_{ct} = -0.007$ $\phi_{sc} = 0.957$ $\phi_{st} = 0.957$		

FIGURES

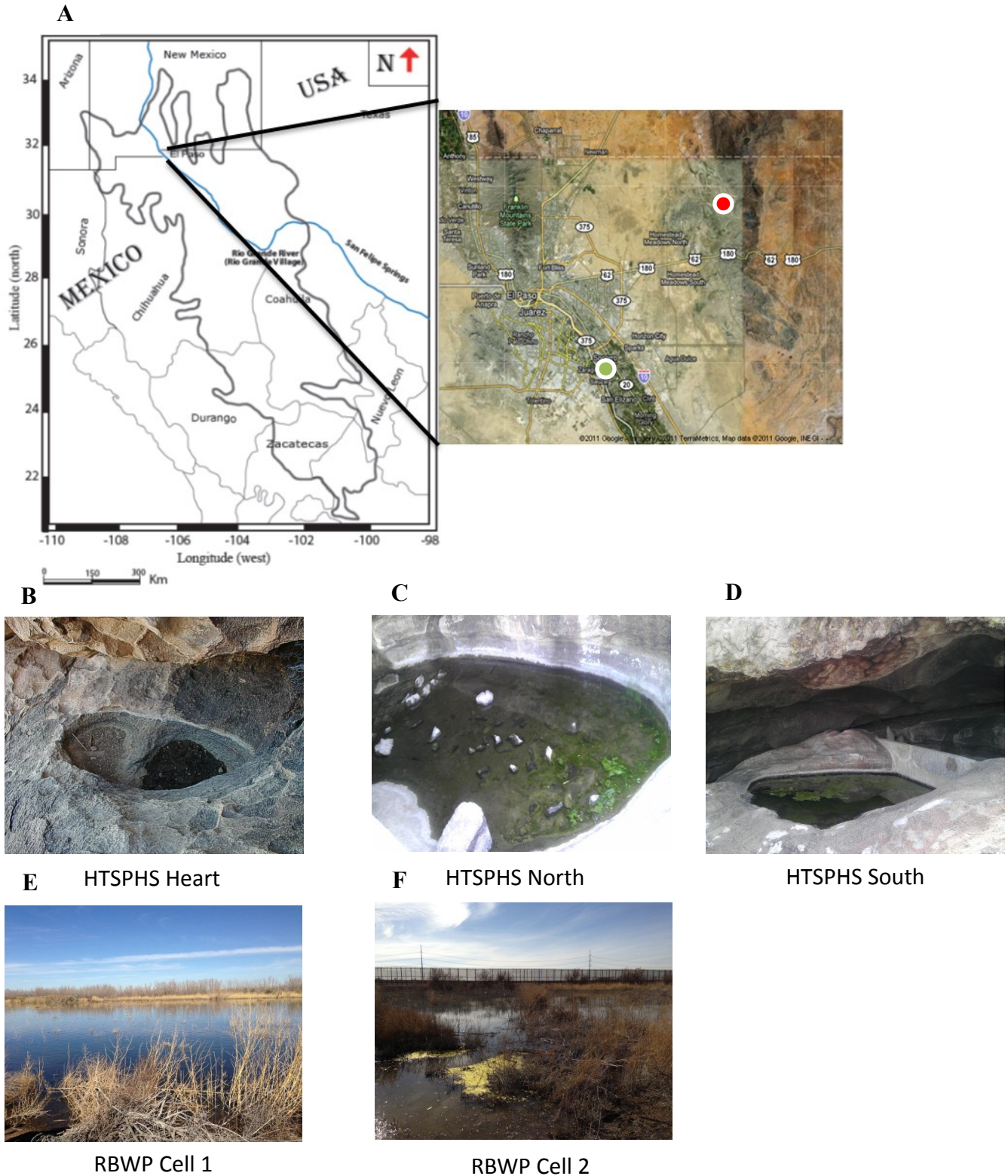


Figure 1. A. Map of the Chihuahuan Desert (Courtesy of Mr. Musa Hussein). Inset shows study sites in the El Paso, TX region (<http://maps.google.com/>). Red dot corresponds to Hueco Tanks State Park and Historic Site (HTSPHS) and green dot represents Rio Bosque Wetlands Park (RBWP). Distance between sites is 40 km. B. Sampling sites at HTSPHS (A-C) and RBWP (E-F).

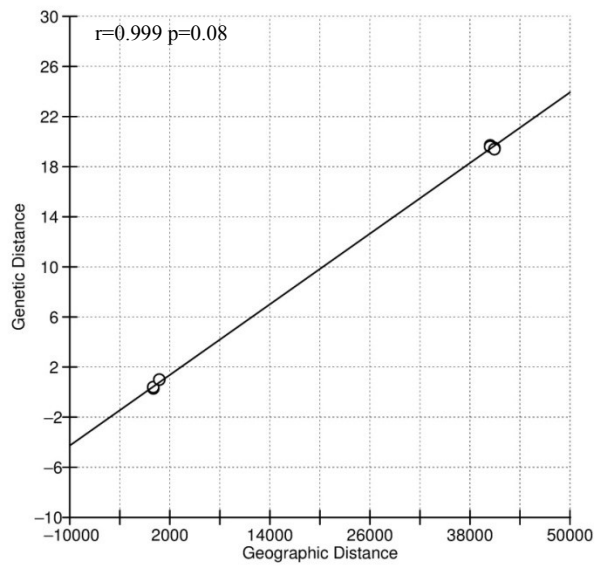


Figure 2. Mantel test for correlation of geographic distance with genetic distance (COI) of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert produced by Isolation by Distance Web Service v3.23.

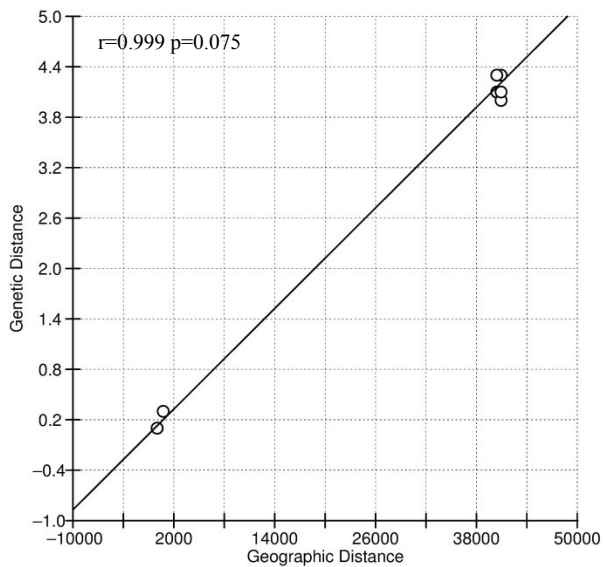


Figure 3. Mantel test for correlation of geographic distance with ITS genetic distance of 42 *E. senta* species complex lineages from the northern Chihuahuan Desert produced by Isolation by Distance Web Service v3.23.

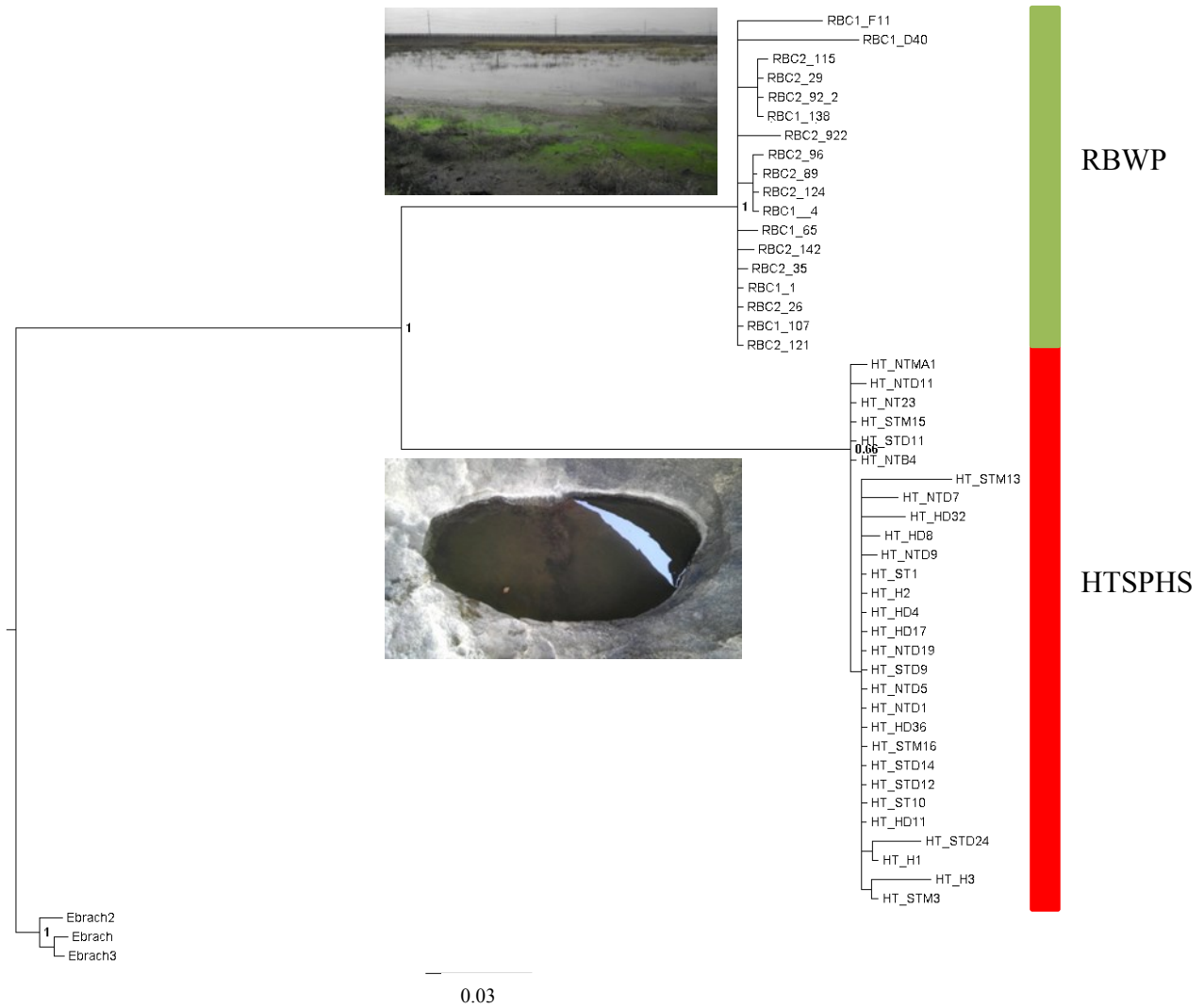


Figure 4. Phylogenetic tree of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert and three members of the outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of COI gene sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.

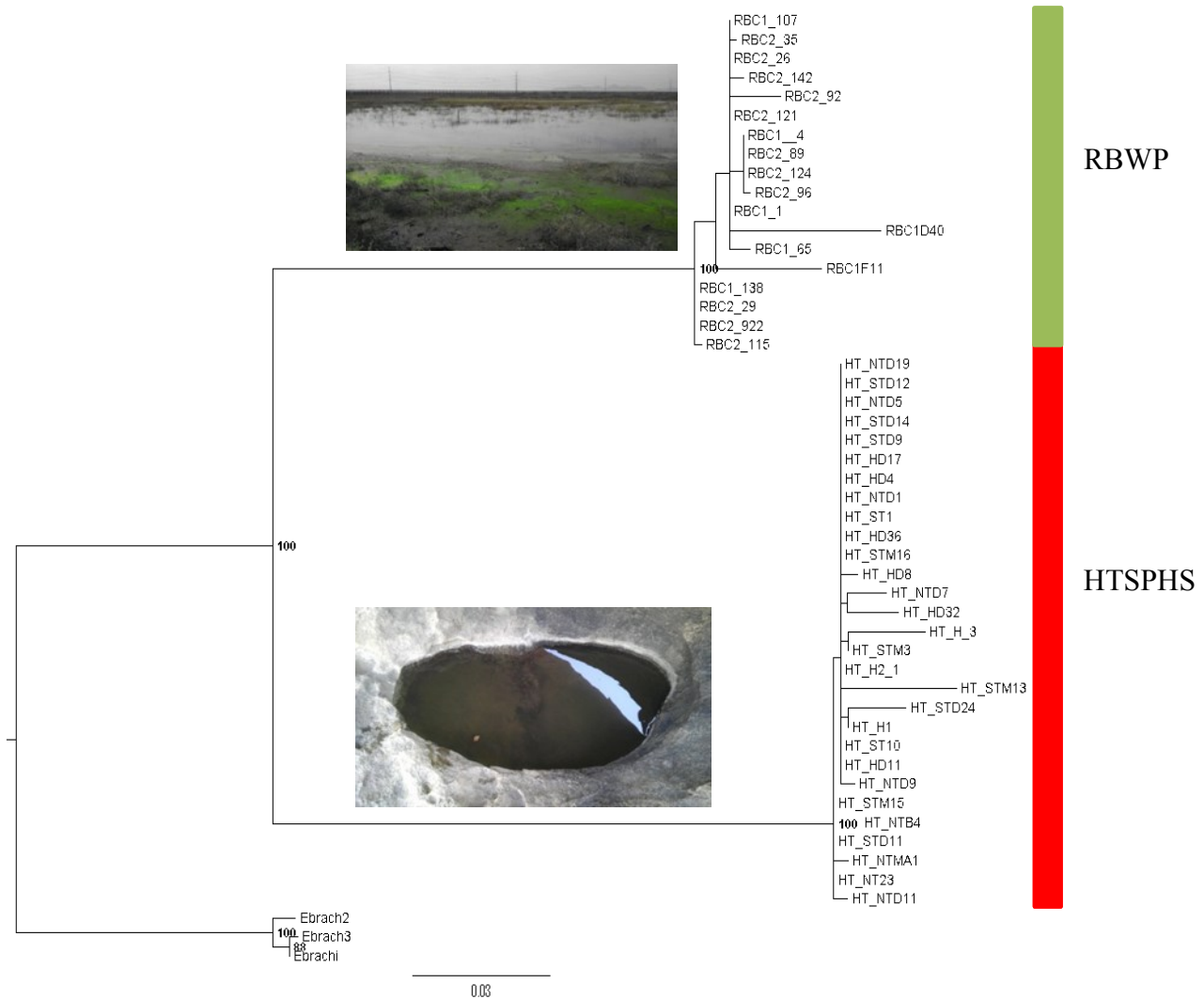


Figure 5. Phylogenetic tree of 47 *E. senta* species complex lineages from the northern Chihuahuan Desert and three members of the outgroup (*E. brachionus*) based on Maximum Likelihood analysis (RAxML v7.6.3) of COI gene region sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.

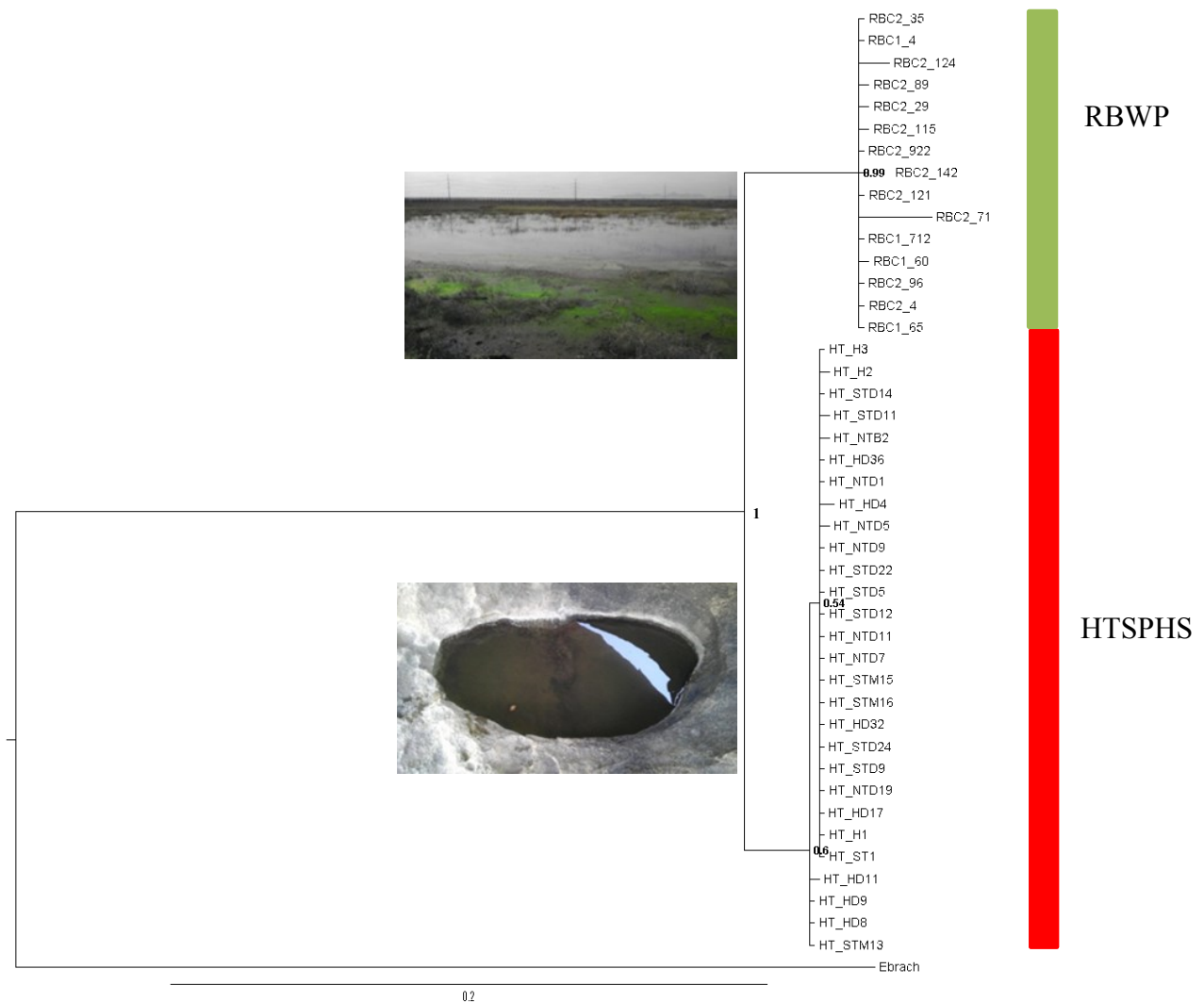


Figure 6. Phylogenetic tree of 43 *E. senta* species complex lineages from the northern Chihuahuan Desert and one outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of ITS region sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location, red = HTSPHS and green = RBWP.

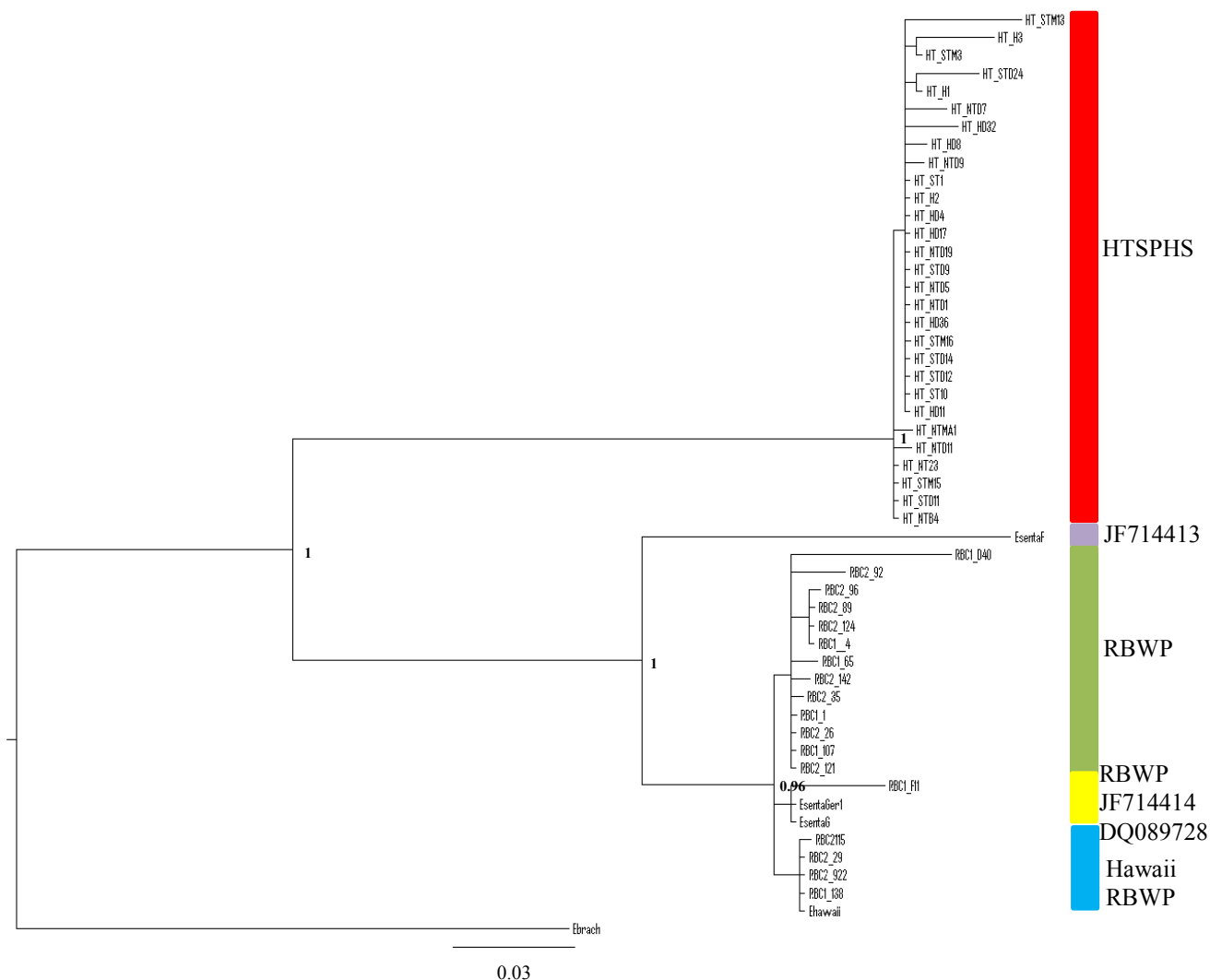


Figure 7. Phylogenetic tree of 51 *E. senta* species complex lineages and one outgroup (*E. brachionus*) based on Bayesian analysis (MrBayes v3.2.1) of COI gene region sequences. Abbreviations refer to species and population origin given in Table 1. Colors refer to sampling location and/or GenBank accession number, red = HTSPHS (USA), purple = JF714413 (Norway), green = RBWP (USA), yellow = RBWP (USA), JF714414 (Germany), and DQ089728, and blue = Hawaii and RBWP (USA).

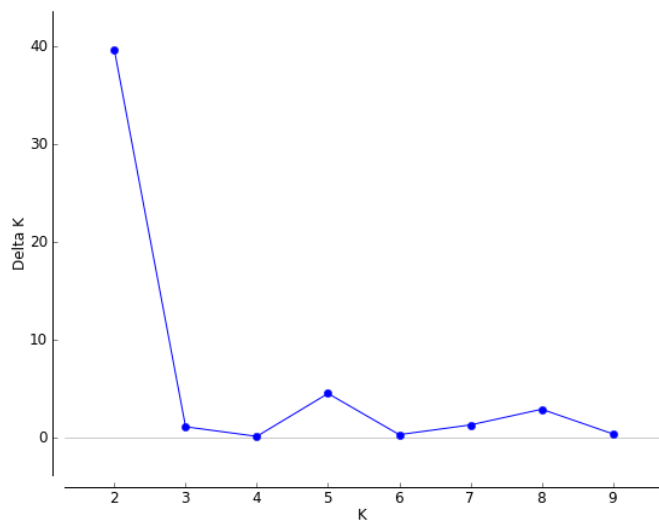


Figure 8. Estimated Delta K (ΔK) for K=2 through K=9 *E. senta* species complex populations from the northern Chihuahuan Desert using the Evanno method (Evanno *et al.*, 2005) and produced by STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Delta K reaches the highest peak at K = 2. K is the number of populations assumed for genetic structure analysis.

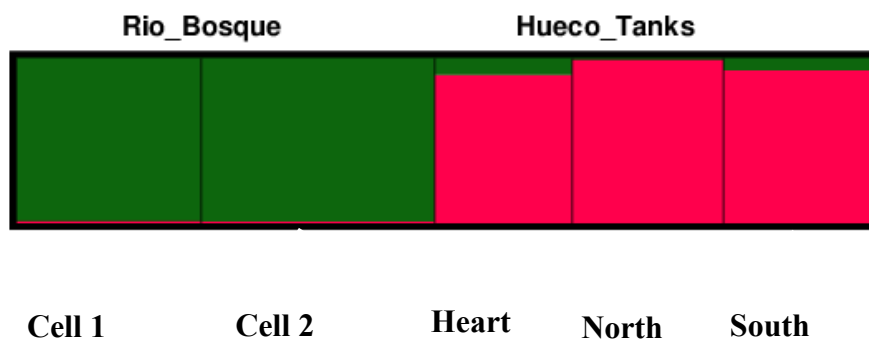


Figure 9. Bar plot of *E. senta* species complex populations (number of individuals=125) from the northern Chihuahuan Desert genotype assignments produced by STRUCTURE v2.3.3 and modified in Distruct v1.1 based on four RAPD primers. Bayesian analysis shows two clusters (green and red) and estimated probability of each individual of belonging to one of the two clusters (K = 2, probability of belonging to green cluster for RBC1 and RBC2 0.98; probability of belonging to the red cluster for HT Heart= 0.90, HT North=0.98 and HT South=0.93).

APPENDICES

Appendix 1: COI *E. senta* sequences from northern Chihuahuan Desert populations

RBWP

>RBC1F11

TTATTCGTTTATAGAGCTTGCCCTTGATAGGCCCTACCTTGGTGATGAGCATCTTTATAACGTTTATAGTTACGGCTCATGCTTTTG
TTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATA
TGGCTTTTCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTTCTTTTCTTTCTTACTTCTGTCTTCTATTTTAGATGC
GGGAGCAGGTACTGGATGAAGTGTATCTCTCTCTTTCTGATTCTAAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTT
TAGGTTACATCTAGCTGGTGTCTTCTATTCTAGGCAGAAATTAATTCCTTACAACATATTATTTGTTCTCGTACTACTAAAGC
AGTTTCTCTTGATCGTCTCCCTCTTATGCTTTGGGCTATTGCAGTTACTGCAGTTTATTAGTCACTAGACTTCCTGTTCTTGC
AGGAGCTATCACTATACTTTAACTGATCGTAATTTAACACTTCTTTCTTTGATCCTGCTTGTGGCGGTAAATCCTGTT

>RBC1D40

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTATAGAGCTTGGGGTTGTAGGCCCTAC
CTTGGTGATGAGCATCTTTATAACGTTTATAGTTACGGCTCATGCTTTTGTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCTCGTATAAAATAACTTATCTTTCTGA
CTTTAGTCCCTTCTTTTCTTTTCTTACTTCTATCTCTATTTTAGATGCGGGAGCAGGTACTGGATGAAGTGTATCTCTCTC
TTTCTGATTCAAAGTATCATAGTGGTATTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTATTCTAG
GCAGAATTAATTCCTTACAACATATTATTTGCTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTATTAGTTACTAGACTTCCTGTTCTTTCCCGAGCTATCATTACGCTTTAACTGATCGTATTT
TAACTCTTCTTTTCTTGTTCCTGCAGGTGGTGGTAATCCTGTTTTTATTTCATCTCTTTT

>RBC1_138

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTATAGAGCTTGGGGTTGTAGGTCCCTAC
CTTGGTGATGAGCATCTTTATAACGTTTATAGTTACGGCTCATGCTTTTGTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATAGCTTTTCTCGTATAAAATAACTTATCTTTCTGA
CTTTAGTCCCTTCTTTTCTTTTCTTACTTCTATCTCTATTTTAGATGCGGGAGCAGGTACTGGATGAAGTGTATCTCTCTC
TTTCTGATTCTAAGTATCATAGTGGTATTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTATTCTAG
GCAGAATTAATTCCTTACAACATATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTACTAGTTACTAGACTTCCTGTTCTTGCAGGAGCTATCACTATACTTTAACTGATCGTAATT
TTAACACTTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC1_4

CGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTATAGAGCTTGGGGTTGTAGGCCCTACCTTGGTG
ATGAACATCTTTATAACGTTTATAGTTACGGCTCATGCTTTTGTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTT
TTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCTCGTATAAAATAACTTATCTTTCTGACTTTT
TCCCTTCTTTTCTTTTCTTACTTCTATCTCTATTTAGATGCGGGAGCAGGTACTGGATGAAGTGTATCTCTCTCTTCTG
ATTCTAAGTATCATAGTGGTATTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTATTCTAGGCAGAA
TTAATTTCTTACAACATATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCCTCTTATGCTTTGGGCTATTGC
AGTTACTGCAGTTTATTAGTTACTAGACTTCCTGTTCTTGCAGGAGCTATCACTATGCTTTAACTGATCGTAATTTAACAC
TTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC1_65

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTATAGAGCTTGGGGATGAAGGCCCTAC
CTTGGTGATGAGCATCTTTATAACGCTTTAGTTACGGCTCATGCTTTTGTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCTCGTATAAAATAACTTATCTTTCTGA
CTTTAGTCCCTTCTTTTCTTTTCTTACTTCTATCTCTATTTTAGATGCGGGAGCAGGTACTGGATGAAGTGTATCTCTCTC
TTTCTGATTCTAAGTATCATAGTGGTATTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTATTCTAG
GCAGAATTAATTTCTTACAACATATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTATTAGTTACTAGACTTCCTGTTCTTGCAGGAGCTATCACTATGCTTTAACTGATCGTAATT
TTAACACTTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC1_1

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTATAGAGCTTGGGGTTGTAGGCCCTAC
CTTGGTGATGAGCATCTTTATAACGTTTATAGTTACGGCTCATGCTTTTGTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCTCGTATAAAATAACTTATCTTTCTGA
CTTTAGTCCCTTCTTTTCTTTTCTTACTTCTATCTCTATTTTAGATGCGGGAGCAGGTACTGGATGAAGTGTATCTCTCTC
TTTCTGATTCTAAGTATCATAGTGGTATTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTATTCTAG
GCAGAATTAATTTCTTACAACATATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTATTAGTTACTAGACTTCCTGTTCTTGCAGGAGCTATCACTATGCTTTAACTGATCGTAATT
TTAACACTTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC1_107

GTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAGC
ATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTA
ATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTT
CTTTCTTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTCTTTCTGATTCTA
AGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTATTCTAGGCAGAATTAATT
TCCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTCCCTCTTATGCTTTGGGCTATTGCAGTTA
CTGCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTTCTT
TCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_115

GTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGTCCCTACCTTGGTGATGAGC
ATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTA
ATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATAGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTT
CTTTCTTTTCTTACTTCTATCCTCTATTTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTCTTTCTGATTCTA
AGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTATTCTAGGCAGAATTAATT
TCCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTCCCTCTTATGCTTTGGGCTATTGCAGTTA
CTGCAGTTTTACTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATACTTTTAACTGATCGTAATTTTAACTTCTT
TCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_29

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGTCCCTAC
CTTGGTGATGAGCATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATAGCTTTTCCTCGTATAAAATAACTTATCTTTCTGA
CTTTTAGTCCCTTCTTTTCTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTC
TTTCTGATTCTAAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTATTCTAG
GCAGAATTAATTTCCCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTCCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTTACTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATACTTTTAACTGATCGTAATT
TTAACTTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_922

GTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGTCCCTACCTTGGTGATGAGC
ATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTA
ATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATAGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTT
CTTTCTTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTCTTTCTGATTCTA
AGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTATTCTAGGCAGAATTAATT
TCCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTCCCTCTTATGCTTTGGGCTATTGCAGTTA
CTGCAGTTTTACTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATACTTTTAACTGATCGTAATTTTAACTTCTT
TCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_92

GTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAGC
ATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATTATTTTCTTTATAGTTATACCTGTGTCTTATTGGTGGTTTTGGTAA
TTGATTAAATCTTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTGGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTCT
TTTCTTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTCTTTCTGATTCTAAG
TATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTCTTATTCTAGGCAGAATTAATTT
CTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCTCTTATGCTTTGGGCTATTGCAGTTACT
GCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTTCTTTCT
TTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_96

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTAC
CTTGGTGATGAACATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGA
CTTTTAGTCCCTTCTTTTCTTTTCTTACTTCTATCCTCTATTTCTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTC
CTTCTGATTCTAAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTCTTATTCTAG
GCAGAATTAATTTCCCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTATATTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATGCTTTTAACTGATCGTAATT
TTAACTTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_89

ATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGA
ACATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGG
TAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCC
TTCTTTTCTTTTCTTACTTCTATCCTCTATTTCTAGATGCGGGAGCAGGACTGGATGAAGTGTATCCTCCTCTTTCTGATTCT
AAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTTCCTTCTTATTCTAGGCAGAATTAAT
TTCTTACAACATAATTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCTCTTATGCTTTGGGCTATTGCAGTT
ACTGCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGACAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTTCT
TTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_124

TGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAA
CATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGT
AATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCT
TCTTTTCTTTCTTACTTCTATCCTCTATTCTAGATGCGGGAGCAGGTAAGTGAAGTGTATCCTCCTCTTTCTGATTCT
AAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTTAGGTTACATCTAGCTGGTGTCTTCTTATTCTAGGCAGAATTAAT
TTCCTTACAACTATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCTCTTATGCTTTGGGCTATTGCAGTT
ACTGCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGCGAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTCTCT
TTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_142

GAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAGCAT
CTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTAAT
TGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTTCTT
TTCTTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGTAAGTGAAGTGTATCCTCCTCTTTCTGATTCTAAGT
ATCATAGTGGTATTTCTGTTGATCTTGCTATTTTAGGTTACATCTAGCTGGTGTCTTCTTATTCTAGGCAGAATTAATTTCC
TTACAACTATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCTCTTATGCTTTGGGCTATTGCAGTTACTG
CAGTTTTATTAGTTACTAGACTTCCTGTTCTTGCGAGGAGCTATCCCTTTGCTTTTAACTGATCGTAATTTTAACTCTCTTTCT
TTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_35

GTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAGC
ATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTA
ATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTTCTT
CTTTTCTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGTAAGTGAAGTGTATCCTCCTCTTTCTGATTCTA
AGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTAGGTTACATCTAGCTGGTGTCTTCTTATTCTAGGCAGAATTAATTT
TCCTTACAACTATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCTCTTATGCTTTGGGCTATTGCAGTTA
CTGCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGCGAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTCTCTTTCT
TCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_26

TGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTACCTTGGTGATGAGCA
TCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATGGGTGGTTTTGGTAA
TTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGACTTTTAGTCCCTTCTT
TTTCTTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGTAAGTGAAGTGTATCCTCCTCTTTCTGATTCTAAG
TATCATAGTGGTATTTCTGTTGATCTTGCTATTTTAGGTTACATCTAGCTGGTGTCTTCTTATTCTAGGCAGAATTAATTTCT
CTTACAACTATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCTCTTATGCTTTGGGCTATTGCAGTTACT
GCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGCGAGGAGCTATCACTATGCTTTTAACTGATCGTAATTTTAACTCTCTTTCT
TTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

>RBC2_121

TTATCTTCGGTATGTGAGCTGGGTTTATTGGTCTTAGAATGAGTTTACTTATTCGTTTAGAGCTTGGGGTTGTAGGCCCTAC
CTTGGTGATGAGCATCTTTATAACGTTTTAGTTACGGCTCATGCTTTTGTTATGATTTTCTTTATAGTTATACCTGTGTCTATG
GGTGGTTTTGGTAATTGACTAATTCCTCTTATGTTAGGTGTTGCTGATATGGCTTTTCCTCGTATAAAATAACTTATCTTTCTGA
CTTTTAGTCCCTTCTTTTCTTTCTTACTTCTATCCTCTATTTTAGATGCGGGAGCAGGTAAGTGAAGTGTATCCTCCTCT
TTTCTGATTCTAAGTATCATAGTGGTATTTCTGTTGATCTTGCTATTTTAGGTTACATCTAGCTGGTGTCTTCTTATTCTAG
GCAGAATTAATTTCTTACAACTATTATTTGTTCTCGTACTACTAAAGCAGTTTCTCTTGATCGTCTTCCTCTTATGCTTTGGG
CTATTGCAGTTACTGCAGTTTTATTAGTTACTAGACTTCCTGTTCTTGCGAGGAGCTATCACTATGCTTTTAACTGATCGTAATT
TTAACTCTCTTTCTTTGATCCTGCTGGTGGTGGTAATCCTGTTTTATATCAACATCTTTT

HTSPHS

>HT_H3

TGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCTTTA
CAATGTTTTGGTACTGCTCATGCTTTTGTTATAATCTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGACT
TATTCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAAACAATCTTTCTTCTGACTTTTAATTCCATCTTTCTT
ATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAAGTACCA
TAGGGGTATCTCAGTTGATTTAGCTATTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCTTAC
AACTATCATTTGCTCTCGTACCACTAAATCGGTTTCTTAGGCCGCCTTCCATTAAATGTTATGAGCTATTGCTGTTACTGCTGT
GCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTACCCTTCTTTCTTTGA
TCCTGCTTGGTGGGAGGGG

>HT_HD32

GCTGGTTTTTTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCTTT
ACAATGTTTTGGTACTGCTCATGCTTTTGTTATAATCTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGA
CTTATTCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAAACAATCTTTCTTCTGACTTTTAATTTCCATCTTTCT
TTATCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAAGTAC
CATAGGGGTATCTCAGTTGATTTAGCTATTTTATTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCTT
ACAATATCATTTGCTCTCTTACCCTAAATCGGTTTCTTAGGCCGCCTTCCATTAAATGTTATGAGCTATTGCTGTTACTGCT
GTGCTGCTTGTACTATTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACTCTCTTTCTTT
GATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_HD8

ATGAACATCTTTACAATGTTTTGGTTACTACTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCT
TTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAA
TTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTG
ATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTA
TTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTG
CTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTAACA
CTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTT

>HT_H2

GTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAAC
ATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGA
ATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCAT
CTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTA
AGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTTCACCTGGTGTCTCTTCTATTTTAGGTAGTATTAATT
TCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTGA
CTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTAACACTTCTT
TCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_H1

GTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAAC
ATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGA
ATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCAT
CTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTA
AGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTTCACCTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATT
TCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTGA
CTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGTCTGGTGCAATCCCATGCTTCTTACGGACCGTAATTTAACACTTCTT
TCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_HD4

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTTCACCTTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_HD17

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTTCACCTTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_HD36

TCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCTTTACAATGTTTTGGT
TACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGACTTATTCCCCTCAT
GTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTTTCTTATTCTTATTACT
GTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTAAGTACCATAGGGGTATCT
CAGTTGATTAGCTATTTTATGCTTTCACCTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCCCTTACAACATCATTT
GCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTTACTGCTGTGCTGCTTGTGA
CTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACACTTCTTTCTTTGATCCTGCTGGTG
GAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_HD11

AGCTGGTTTTATTGGTCTTAGTATGAGTCTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCT
TTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTG
ACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTTT
CTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTAAGTA
CCATAGGGGTATCTCAGTTGATTTAGCTATTTTATGCTTTCACCTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCT
TACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTTACTGCT
TGTGCTGCTTGTACTAGTTTACCTGTTCTTGTCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACACTTCTTTCTT
TGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HTNTD7

TTATCTTTGGTATGTGAGGTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATATTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCGTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTMA1_1
 TGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCTTTA
 CAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGACT
 TATCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTCTGACTTTTAAATCCATCTTTCTT
 ATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCCTTTCTGATTCTAAGTACCA
 TAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTATAGGTAGTATTAATTTCTTAC
 AACTATTCATTGCTCTCGTACCCTAAATCGGTTTCTTAGACCGCCTCCATTAATGTTATGAGCTATTGCTGTTACTGCTGT
 GCTGCTTGTACTAGTTTACCTGTTCTTGTGCTGGGGCAATCACCATGCTTCTTACGGACCGTAATTTTAACTTCTTCTTTGA
 TCCTGCTGGTGGGAAGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTD9
 TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGGTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCGTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTD11
 TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTD19
 TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTD5
 TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNTD1
 TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
 TTGGTGATGAACATCTTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
 GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
 CTTTAAATCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
 CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTATAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
 GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCCTAAATCGGTTTCTTTAGGCCGCCCTCCATTAATGTTATGA
 GCTATTGCTGTTACTGCTGTGCTGCTGTTACTAGTTTACCTGTTCTTGTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
 TTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT
 >HTNT23
 GAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATC
 TTTACAATGTTTTGGTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATT

GACTTATTCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTT
TCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAAGT
ACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTTCTTATTTTAGGTAGTATTAATTTCC
TTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGACCGCCTTCCATTAATGTTATGAGCTATTGCTGTTACTG
CTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACACTTCTTTCT
TTGATCCTGCTGGTGGAGGTA

>HTNTB4

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGACCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGTGTTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STM13

TGAGCTAGTTATAATAGTATTAATATGAGTTTCTTAATTCGCTTAGAGTTGGGTGTAGTTGTTTCCTTATATTGGTGATGAACA
TCTTTACAATGTTTTGGTTAATGCTCATGCTTTTATTATAATCTTTTTCATGGTTATCCCGGTCTCAATGGGAGGCTTTGGGA
TTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATC
TTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAA
GTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTT
CCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTTAC
TGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACACTTCTTT
CTTTGATCCTGCTGGTGGAGGTAATC

>HT_STD24

AGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGAGGAGGGTCCTTATCTTGGTGATGAACATC
TTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATT
GACTTATTCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTT
TCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAAGT
ACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCC
TTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTTACTG
CTGTAGTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCCCTTTCTTCTTACGGACCGTAATTTTAACACTTCTTTCTT
TGATCCTGCTGGTGGAGGTAATCCTGTTTGTACCAACATCTGTT

>HT_STM16

GGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGA
GGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTT
TTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCTT
TCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGT
AGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCT
ATTGCTGTTACTGCTGTGCTGCTGTGTTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTT
AACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STM3

TGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATCTTGGTGATGAACATCTTTACAATGTTTT
GGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGACTTATTCCCCT
CATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTTTCTTATTCTTATT
ACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCCTTTCTGATTCTAAGTACCATAGGGGT
TCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCCCTTACAACATCAT
TTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTTACTGCTGTGCTGCTTGT
TACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTTAACACTTCTTTCTTTGATCCTGCTGG
GGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_ST1

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACTTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGTGTTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTTAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STD9

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCTTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGCTGGTACAGGGTGGACCGTTTATCCTCCC

CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTAAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STD14

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCCTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTAAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STD12

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCCTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTAAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_ST10

GAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCCTATCTTGGTGATGAACATC
TTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATT
GACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTT
TCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTAAGT
ACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCC
TTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTACTG
CTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTAAACACTTCTTTCT
TTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STM15

TTATCTTTGGTATGTGAGCTGGTTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCCTATC
TTGGTGATGAACATCTTTACAATGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGG
GAGGCTTTGGGAATTGACTTATTCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGA
CTTTTAATTCCATCTTTCTTATTCTTATTACTGTCTTCTATTCTAGATGCTGGTGTGCTGGTACAGGGTGGACCGTTTATCCTCCC
CTTCTGATTCTAAGTACCATAGGGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTA
GGTAGTATTAATTTCCCTTACAACATCATTTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGA
GCTATTGCTGTTACTGCTGTGCTGCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAAT
TTAAACACTTCTTTCTTTGATCCTGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

>HT_STD11

TTTTATTGGTCTTAGTATGAGTCTTTTAATTCGCTTAGAGTTGGGTGTTGTTGGTCCCTATCTTGGTGATGAACATCTTTACAA
TGTTTTGGTTACTGCTCATGCTTTTGTATAATCTTTTTCATGGTTATACCGGTCTCAATGGGAGGCTTTGGGAATTGACTTAT
TCCCCTCATGTTAGGAGTTGCTGATATAGCTTTCCACGTATAAACAATCTTTCTTTCTGACTTTTAATTCCATCTTTCTTATT
CTTATTACTGTCTTCTATTCTAGATGCTGGTGTGCTGGTACAGGGTGGACCGTTTATCCTCCCCTTTCTGATTCTAAGTACCATAG
GGGTATCTCAGTTGATTTAGCTATTTTTAGTCTTCACCTGGCTGGTGTCTCTTCTATTTTAGGTAGTATTAATTTCCCTTACAAC
TATCATTGCTCTCGTACCACTAAATCGGTTTCTTTAGGCCGCCTTCCATTAATGTTATGAGCTATTGCTGTACTGCTGTGCT
GCTTGTACTAGTTTACCTGTTCTTGCTGGTGCAATCACCATGCTTCTTACGGACCGTAATTTAAACACTTCTTTCTTTGATCC
TGCTGGTGGAGGTAATCCTGTTTTGTACCAACATCTGTT

Appendix 2: ITS *E. senta* sequences from northern Chihuahuan Desert populations

RBWP

>RBC2_35

TTTAATCATAAATATCACTCAGTAAAAATGCAAAATAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACTTAACTAATATAGGATTAGTTTTACAAATCTAAAA
TCGATAAATATATTATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTAAATGTAAATGTGTTTGTAAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
TAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC2_121

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC2_71

AAATATCAATCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGCATGAGCAA
GCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAAATCGATAAA
TATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAGGCGTGGTC
ATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTCACGCAGTT
TGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAATATAAAAAAT
ATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGCGCAATTCA
TTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATTTCATAGGTA
CCCGACCATACTAAGCCAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAAACGCAAAGT
AGCGCTTG

>RBC1_712

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC1_60

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC2_96

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACATAAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTTTAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC2_4

TTTAATCATAAATATCACTCAGTAAAAATGCAAATTAAACTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAAATATATTATTTAACGACCGATCAAAAGTGGAACCACCTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC

ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACATAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTAAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

>RBC1_65

TTTAATCATAAATATCACTCAGTAAAAATGCAAAATTAAGTCAAGCAAACAAATTTGATTATTCAAAGCAATTTTTGTTTGC
ATGAGCAAGCGTAAATTAGCATCTTCAGTGATATAAATAGTGCACCTTAACTAATATAGGATTAGTTTTTACAAATCTAAAA
TCGATAAATATATTATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACATAAAAACAACATAATAAATTTTTTAAATGTAAATGTGTTTGTAAAAAGCTCGACGGGC
GCAATTCATTTAGAATGAAATGTCTTTAAACCATACGGTAAAAACTATACCGCTTATGATGCAATTGCATGACAACAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGTAGCGCTTG

HTSPHS

>HT_H3

TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACCTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_HD11

TTAAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACCTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_H2

TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAAAAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACCTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_STD14

TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACCTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAATTTTGATTATAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_STD11

TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACCTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACTTTTCACGATTTTACATATAAAACCGACCCCTCAGACAG
GCGTGGTCATGGAAGCGAACCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC

CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAAC TTTGATTATAAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_NTD19
TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACCTTTTACGATTTTACATATAAAACCGACCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAAC TTTGATTATAAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_HD17
TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACCTTTTACGATTTTACATATAAAACCGACCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAAC TTTGATTATAAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_HI
TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACCTTTTACGATTTTACATATAAAACCGACCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAAC TTTGATTATAAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

>HT_ST1
TTTAATTAAATATATCACTCAGTAAAAATGCAAGTTCAACTCAGGCAAACAAATTTGATGATTCAACGCAATTTTTGTTTGC
AAGAGCAAGCTCAAATTTGCATTTTTAGTGATATAAATAGTGCACTTAACTAATATATGATTAGTTTTTACAAATCTAAAG
TCGATAAAATAAAATATTTAACGACCGATCAAAAGTGGAACCACCTTTTACGATTTTACATATAAAACCGACCTCAGACAG
GCGTGGTCATGGAAGCGATCCATAACCGCAATATGCGTTCAAGATATCGATGATCAATGTGTCTCTGCAAATCACATTAATTC
ACGCAGTTTGCTGCGCTCTTCATCGACTCGCGAGCCAAGTGATCCACCGCATAGGGTTGTTTAAATGTTTAAATGCTCAAAT
ATAAAAAATATTTTCGCTTTTTACACAAAAACAACATGATAAATTTTTTAAATGTAAATGTGTTTGTATAAGCTCTACGGGC
GCAATTCATTTTGAATGAAGTGTCTTTAAACCATACGGTAAAAGCTATACCGCTTATGATGCAATTGCATGACAAAAGCATT
CATAGGTACCCGACCATACTAAGACAAGCATGGATATTAAC TTTGATTATAAAAAAGGCATTTATAATCAGCGTGTTTTTAA
CGCAAAGAAGCGCTTG

Appendix 3: COI uncorrected (“p”) distance matrix for *E. senta* populations from the northern Chihuahuan Desert and outgroups

	1	2	3	4	5	6	7	8
1 HTSTM13	-							
2 HTH3	0.04282	-						
3 HTSTD24	0.03753	0.03287	-					
4 HTNTD7	0.03297	0.00879	0.02275	-				
5 HTNTMA1	0.02958	0.02296	0.01908	0.01238	-			
6 HTNTD11	0.02928	0.02299	0.01892	0.01340	0.00634	-		
7 HTNT23	0.02629	0.01666	0.01638	0.01219	0.00329	0.00328	-	
8 HTSTM15	0.02603	0.01967	0.01579	0.01170	0.00319	0.00303	0.00000	-
9 HTSTD11	0.02484	0.01990	0.01602	0.01071	0.00320	0.00315	0.00000	0.00000
10 HTNTB4	0.02603	0.01967	0.01579	0.01170	0.00319	0.00303	0.00000	0.00000
11 HTHD32	0.03616	0.02960	0.02700	0.02119	0.01589	0.01740	0.01487	0.01428
12 HTHD8	0.01109	0.02472	0.01295	0.01438	0.00921	0.00891	0.00554	0.00546
13 HTNTD9	0.02761	0.02130	0.01579	0.01336	0.00796	0.00758	0.00481	0.00455
14 HTSTM3	0.02182	0.01667	0.01612	0.00901	0.00644	0.00637	0.00330	0.00322
15 HTH1	0.02611	0.01975	0.01265	0.01217	0.00639	0.00626	0.00328	0.00313
16 HTST1	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152

17	HTH 2	0.02447	0.01809	0.01423	0.01042	0.00480	0.00469	0.00163	0.00157
18	HTHD4	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
19	HTHD17	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
20	HTNTD19	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
21	HTSTD9	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
22	HTNTD5	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
23	HTNTD1	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
24	HTHD36	0.01860	0.01854	0.01460	0.00909	0.00484	0.00474	0.00165	0.00159
25	HTSTM16	0.00741	0.02067	0.00890	0.01022	0.00529	0.00505	0.00178	0.00171
26	HTSTD14	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
27	HTSTD12	0.02441	0.01804	0.01423	0.01004	0.00478	0.00455	0.00161	0.00152
28	HTST10	0.02446	0.01809	0.01422	0.01048	0.00478	0.00469	0.00162	0.00157
29	HTHD11	0.02447	0.01807	0.01422	0.01052	0.00477	0.00469	0.00162	0.00157
30	Ebrach2	0.21213	0.19622	0.20757	0.20041	0.19631	0.19497	0.19318	0.19294
31	Ebrach3	0.18809	0.19951	0.18753	0.19036	0.18367	0.17965	0.18179	0.17981
32	Ebrach	0.20461	0.20638	0.19916	0.19910	0.19045	0.18602	0.18816	0.18605
33	RBC1F11	0.20522	0.20347	0.20476	0.20533	0.19607	0.19753	0.18865	0.19421
34	RBC1D40	0.21906	0.21617	0.21657	0.20625	0.20799	0.20455	0.19957	0.20152
35	RBC2 29	0.19947	0.19811	0.18653	0.19460	0.17940	0.17727	0.17981	0.17424
36	RBC2 922	0.19927	0.19786	0.18616	0.19744	0.17910	0.17914	0.17947	0.17601
37	RBC1 138	0.19947	0.19811	0.18653	0.19460	0.17940	0.17727	0.17981	0.17424
38	RBC2 115	0.20089	0.19951	0.18774	0.19916	0.18069	0.18069	0.18110	0.17756
39	RBC2 92	0.21071	0.20939	0.19882	0.20970	0.19018	0.19008	0.19098	0.18696
40	RBC2 96	0.19783	0.19646	0.18651	0.19293	0.17781	0.17576	0.17816	0.17273
41	RBC2 89	0.19769	0.19632	0.18629	0.19510	0.17758	0.17705	0.17790	0.17393
42	RBC2 124	0.19765	0.19624	0.18622	0.19544	0.17754	0.17734	0.17786	0.17421
43	RBC1 4	0.19754	0.19610	0.18604	0.19571	0.17739	0.17773	0.17769	0.17461
44	RBC1 65	0.20600	0.20471	0.18809	0.20132	0.18575	0.18333	0.18639	0.18030
45	RBC2 142	0.20453	0.20292	0.18630	0.19981	0.18395	0.18430	0.18450	0.18117
46	RBC2 35	0.20250	0.20115	0.19096	0.20095	0.18231	0.18228	0.18271	0.17915
47	RBC1 1	0.20265	0.18861	0.19342	0.19615	0.18267	0.18172	0.18124	0.17856
48	RBC2 26	0.20097	0.19962	0.18948	0.19950	0.18081	0.18092	0.18122	0.17780
49	RBC1 107	0.20089	0.19951	0.18936	0.19923	0.18070	0.18071	0.18110	0.17759
50	RBC2 121	0.20110	0.19977	0.18970	0.19631	0.18100	0.17879	0.18144	0.17576

Uncorrected ("p") distance matrix (continued)

	9	10	11	12	13	14	15	16
9 HTSTD11	-							
10 HTNTB4	0.00000	-						
11 HTHD32	0.00959	0.01428	-					
12 HTHD8	0.00547	0.00546	0.00930	-				
13 HTNTD9	0.00485	0.00455	0.01587	0.00566	-			
14 HTSTM3	0.00323	0.00322	0.00648	0.00564	0.00486	-		
15 HTH1	0.00322	0.00313	0.01433	0.00545	0.00467	0.00320	-	
16 HTST1	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	-
17 HTH 2	0.00164	0.00157	0.01276	0.00372	0.00310	0.00161	0.00157	0.00000
18 HTHD4	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
19 HTHD17	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
20 HTNTD19	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
21 HTSTD9	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
22 HTNTD5	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
23 HTNTD1	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
24 HTHD36	0.00161	0.00159	0.00488	0.00369	0.00329	0.00164	0.00158	0.00000
25 HTSTM16	0.00173	0.00171	0.00543	0.00371	0.00187	0.00184	0.00168	0.00000
26 HTSTD14	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
27 HTSTD12	0.00162	0.00152	0.01270	0.00368	0.00303	0.00163	0.00157	0.00000
28 HTST10	0.00163	0.00157	0.01274	0.00371	0.00313	0.00161	0.00157	0.00000
29 HTHD11	0.00162	0.00157	0.01270	0.00370	0.00315	0.00162	0.00157	0.00000
30 Ebrach2	0.19334	0.19294	0.20847	0.19800	0.19797	0.19745	0.19797	0.19462
31 Ebrach3	0.18021	0.17981	0.18761	0.18635	0.18357	0.18436	0.18370	0.18164
32 Ebrach	0.18609	0.18605	0.19982	0.18487	0.19070	0.19141	0.19046	0.18760
33 RBC1F11	0.19452	0.19421	0.20132	0.19458	0.19938	0.19812	0.19775	0.19594
34 RBC1D40	0.20711	0.20152	0.21712	0.20128	0.20606	0.21073	0.20796	0.20303
35 RBC2 29	0.17844	0.17424	0.18860	0.18126	0.17879	0.18180	0.17957	0.17576
36 RBC2 922	0.17811	0.17601	0.18837	0.18095	0.18068	0.18142	0.17925	0.17757
37 RBC1 138	0.17844	0.17424	0.18860	0.18126	0.17879	0.18180	0.17957	0.17576
38 RBC2 115	0.17970	0.17756	0.18995	0.18104	0.18224	0.18301	0.18082	0.17912
39 RBC2 92	0.18925	0.18696	0.19943	0.19183	0.19163	0.19269	0.19025	0.18852
40 RBC2 96	0.17685	0.17273	0.18701	0.17760	0.17727	0.18019	0.17799	0.17424
41 RBC2 89	0.17660	0.17393	0.18686	0.17736	0.17860	0.17991	0.17777	0.17548
42 RBC2 124	0.17655	0.17421	0.18680	0.17731	0.17889	0.17985	0.17770	0.17577
43 RBC1 4	0.17639	0.17461	0.18667	0.17716	0.17921	0.17966	0.17753	0.17614

44	RBC1 65	0.18483	0.18030	0.19494	0.18321	0.18333	0.18824	0.18586	0.18182
45	RBC2 142	0.18301	0.18117	0.19320	0.18476	0.18588	0.18637	0.18128	0.18274
46	RBC2 35	0.18138	0.17915	0.19158	0.18303	0.18383	0.18473	0.18239	0.18071
47	RBC1 1	0.18326	0.17856	0.19391	0.18502	0.18338	0.18511	0.18433	0.18017
48	RBC2 26	0.17986	0.17780	0.19006	0.18116	0.18250	0.18321	0.18104	0.17936
49	RBC1 107	0.17975	0.17759	0.18997	0.18106	0.18226	0.18308	0.18082	0.17915
50	RBC2 121	0.18008	0.17576	0.19021	0.18138	0.18030	0.18346	0.18115	0.17727

Uncorrected ("p") distance matrix (continued)

		17	18	19	20	21	22	23	24
17	HTH 2	-							
18	HTHD4	0.00000	-						
19	HTHD17	0.00000	0.00000	-					
20	HTNTD19	0.00000	0.00000	0.00000	-				
21	HTSTD9	0.00000	0.00000	0.00000	0.00000	-			
22	HTNTD5	0.00000	0.00000	0.00000	0.00000	0.00000	-		
23	HTNTD1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-	
24	HTHD36	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-
25	HTSTM16	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
26	HTSTD14	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
27	HTSTD12	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
28	HTST10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
29	HTHD11	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
30	Ebrach2	0.19626	0.19462	0.19462	0.19462	0.19462	0.19462	0.19462	0.19814
31	Ebrach3	0.18206	0.18164	0.18164	0.18164	0.18164	0.18164	0.18164	0.18263
32	Ebrach	0.18888	0.18760	0.18760	0.18760	0.18760	0.18760	0.18760	0.19042
33	RBC1F11	0.19608	0.19594	0.19594	0.19594	0.19594	0.19594	0.19594	0.19653
34	RBC1D40	0.20640	0.20303	0.20303	0.20303	0.20303	0.20303	0.20303	0.20981
35	RBC2 29	0.17800	0.17576	0.17576	0.17576	0.17576	0.17576	0.17576	0.18080
36	RBC2 922	0.17767	0.17757	0.17757	0.17757	0.17757	0.17757	0.17757	0.18044
37	RBC1 138	0.17800	0.17576	0.17576	0.17576	0.17576	0.17576	0.17576	0.18080
38	RBC2 115	0.17925	0.17912	0.17912	0.17912	0.17912	0.17912	0.17912	0.18204
39	RBC2 92	0.18868	0.18852	0.18852	0.18852	0.18852	0.18852	0.18852	0.19177
40	RBC2 96	0.17643	0.17424	0.17424	0.17424	0.17424	0.17424	0.17424	0.17918
41	RBC2 89	0.17619	0.17548	0.17548	0.17548	0.17548	0.17548	0.17548	0.17893
42	RBC2 124	0.17613	0.17577	0.17577	0.17577	0.17577	0.17577	0.17577	0.17887
43	RBC1 4	0.17596	0.17614	0.17614	0.17614	0.17614	0.17614	0.17614	0.17869
44	RBC1 65	0.18429	0.18182	0.18182	0.18182	0.18182	0.18182	0.18182	0.18728
45	RBC2 142	0.18286	0.18274	0.18274	0.18274	0.18274	0.18274	0.18274	0.18543
46	RBC2 35	0.18082	0.18071	0.18071	0.18071	0.18071	0.18071	0.18071	0.18380
47	RBC1 1	0.18268	0.18017	0.18017	0.18017	0.18017	0.18017	0.18017	0.18587
48	RBC2 26	0.17947	0.17936	0.17936	0.17936	0.17936	0.17936	0.17936	0.18226
49	RBC1 107	0.17925	0.17915	0.17915	0.17915	0.17915	0.17915	0.17915	0.18213
50	RBC2 121	0.17958	0.17727	0.17727	0.17727	0.17727	0.17727	0.17727	0.18249

Uncorrected ("p") distance matrix (continued)

		25	26	27	28	29	30	31	32
25	HTSTM16	-							
26	HTSTD14	0.00000	-						
27	HTSTD12	0.00000	0.00000	-					
28	HTST10	0.00000	0.00000	0.00000	-				
29	HTHD11	0.00000	0.00000	0.00000	0.00000	-			
30	Ebrach2	0.19308	0.19462	0.19462	0.19523	0.19542	-		
31	Ebrach3	0.18327	0.18164	0.18164	0.18215	0.18214	0.00584	-	
32	Ebrach	0.18190	0.18760	0.18760	0.18796	0.18810	0.00873	0.00176	-
33	RBC1F11	0.19002	0.19594	0.19594	0.19616	0.19619	0.17394	0.17395	0.17361
34	RBC1D40	0.20664	0.20303	0.20303	0.20692	0.20708	0.18812	0.18832	0.19419
35	RBC2 29	0.17674	0.17576	0.17576	0.17846	0.17861	0.17619	0.16035	0.16754
36	RBC2 922	0.17636	0.17757	0.17757	0.17813	0.17828	0.17713	0.16021	0.16841
37	RBC1 138	0.17674	0.17576	0.17576	0.17846	0.17861	0.17619	0.16035	0.16754
38	RBC2 115	0.17646	0.17912	0.17912	0.17970	0.17986	0.17886	0.16056	0.16685
39	RBC2 92	0.18689	0.18852	0.18852	0.18918	0.18935	0.18936	0.17136	0.17938
40	RBC2 96	0.17316	0.17424	0.17424	0.17688	0.17703	0.17956	0.16253	0.17064
41	RBC2 89	0.17290	0.17548	0.17548	0.17664	0.17679	0.17977	0.16241	0.17087
42	RBC2 124	0.17284	0.17577	0.17577	0.17658	0.17674	0.18026	0.16241	0.17127
43	RBC1 4	0.17266	0.17614	0.17614	0.17642	0.17657	0.17944	0.16233	0.17064
44	RBC1 65	0.17861	0.18182	0.18182	0.18477	0.18493	0.18132	0.16088	0.17222
45	RBC2 142	0.18004	0.18274	0.18274	0.18297	0.18313	0.17950	0.16259	0.17056
46	RBC2 35	0.17836	0.18071	0.18071	0.18130	0.18148	0.17711	0.15927	0.16842
47	RBC1 1	0.18030	0.18017	0.18017	0.18319	0.18337	0.17631	0.16382	0.17364
48	RBC2 26	0.17657	0.17936	0.17936	0.17984	0.18000	0.17572	0.15915	0.16713

49 RBC1 107	0.17646	0.17915	0.17915	0.17972	0.17988	0.17712	0.15903	0.16842
50 RBC2 121	0.17684	0.17727	0.17727	0.18005	0.18022	0.17620	0.15919	0.16755

Uncorrected ("p") distance matrix (continued)

	33	34	35	36	37	38	39	40
33 RBC1F11	-							
34 RBC1D40	0.04947	-						
35 RBC2 29	0.02730	0.03939	-					
36 RBC2 922	0.02738	0.04096	0.00000	-				
37 RBC1 138	0.02730	0.03939	0.00000	0.00000	-			
38 RBC2 115	0.02565	0.04250	0.00154	0.00157	0.00154	-		
39 RBC2 92	0.03765	0.04405	0.01878	0.01887	0.01878	0.02044	-	
40 RBC2 96	0.03073	0.03636	0.01212	0.01253	0.01212	0.01407	0.01562	-
41 RBC2 89	0.02909	0.03614	0.01095	0.01100	0.01095	0.01256	0.01412	0.00155
42 RBC2 124	0.02911	0.03620	0.01098	0.01100	0.01098	0.01257	0.01413	0.00156
43 RBC1 4	0.02916	0.03596	0.01089	0.01102	0.01089	0.01260	0.01417	0.00155
44 RBC1 65	0.03072	0.03636	0.01212	0.01253	0.01212	0.01407	0.01562	0.00909
45 RBC2 142	0.02902	0.03632	0.01099	0.01101	0.01099	0.01258	0.01420	0.00783
46 RBC2 35	0.02743	0.03470	0.00942	0.00943	0.00942	0.01101	0.01258	0.00627
47 RBC1 1	0.01599	0.02231	0.00797	0.00826	0.00797	0.00987	0.01154	0.00479
48 RBC2 26	0.02565	0.03313	0.00786	0.00786	0.00786	0.00943	0.01102	0.00470
49 RBC1 107	0.02570	0.03313	0.00786	0.00786	0.00786	0.00943	0.01101	0.00470
50 RBC2 121	0.02559	0.03182	0.00758	0.00783	0.00758	0.00937	0.01093	0.00455

Uncorrected ("p") distance matrix (continued)

	41	42	43	44	45	46	47	48
41 RBC2 89	-							
42 RBC2 124	0.00000	-						
43 RBC1 4	0.00000	0.00000	-					
44 RBC1 65	0.00780	0.00782	0.00775	-				
45 RBC2 142	0.00631	0.00628	0.00628	0.00783	-			
46 RBC2 35	0.00472	0.00471	0.00471	0.00627	0.00473	-		
47 RBC1 1	0.00329	0.00329	0.00326	0.00479	0.00330	0.00164	-	
48 RBC2 26	0.00315	0.00314	0.00314	0.00470	0.00315	0.00159	0.00000	-
49 RBC1 107	0.00315	0.00314	0.00314	0.00470	0.00314	0.00157	0.00000	0.00000
50 RBC2 121	0.00313	0.00313	0.00309	0.00455	0.00313	0.00157	0.00000	0.00000

Uncorrected ("p") distance matrix (continued)

	49	50
49 RBC1 107	-	
50 RBC2 121	0.00000	-

Appendix 4: ITS uncorrected ("p") distance matrix of *E. senta* populations from the northern Chihuahuan Desert and outgroup

	1	2	3	4	5	6	7	8
1 Ebrach	-							
2 RBC2 35	0.25909	-						
3 RBC1 4	0.25909	0.00000	-					
4 RBC2 124	0.27412	0.02651	0.02651	-				
5 RBC2 89	0.26506	0.01473	0.01473	0.01620	-			
6 RBC2 29	0.26361	0.01620	0.01620	0.01325	0.00736	-		
7 RBC2 115	0.26964	0.01915	0.01915	0.01031	0.01031	0.00884	-	
8 RBC2 922	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	-
9 RBC2 142	0.26662	0.02062	0.02062	0.00884	0.01031	0.00736	0.00442	0.00147
10 RBC2 121	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	0.00000
11 RBC2 71	0.26813	0.02062	0.02062	0.00884	0.01031	0.00736	0.00442	0.00147
12 RBC1 712	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	0.00000
13 RBC1 60	0.26964	0.02062	0.02062	0.00884	0.01031	0.00736	0.00442	0.00147
14 RBC2 96	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	0.00000
15 RBC2 4	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	0.00000
16 RBC1 65	0.26813	0.01915	0.01915	0.00736	0.00884	0.00589	0.00295	0.00000
17 HT H3	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
18 HT HD11	0.26807	0.05449	0.05449	0.04271	0.04418	0.04124	0.03829	0.03535

19	HT	H2	0.27110	0.05744	0.05744	0.04566	0.04713	0.04418	0.04124	0.03829
20	HT	STD14	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
21	HT	STD11	0.27110	0.05744	0.05744	0.04566	0.04713	0.04418	0.04124	0.03829
22	HT	NTB2	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
23	HT	HD36	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
24	HT	NTD1	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
25	HT	HD4	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
26	HT	HD9	0.26807	0.05449	0.05449	0.04271	0.04418	0.04124	0.03829	0.03535
27	HT	NTD5	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
28	HT	HD8	0.26807	0.05449	0.05449	0.04271	0.04418	0.04124	0.03829	0.03535
29	HT	STM13	0.26807	0.05449	0.05449	0.04271	0.04418	0.04124	0.03829	0.03535
30	HT	NTD9	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
31	HT	STD22	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
32	HT	STD5	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
33	HT	STD12	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
34	HT	NTD11	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
35	HT	NTD7	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
36	HT	STM15	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
37	HT	STM16	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
38	HT	HD32	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
39	HT	STD24	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
40	HT	STD9	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
41	HT	NTD19	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
42	HT	HD17	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
43	HT	H1	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682
44	HT	ST1	0.26959	0.05596	0.05596	0.04418	0.04566	0.04271	0.03976	0.03682

Uncorrected ("p") distance matrix (continued)

		9	10	11	12	13	14	15	16
9	RBC2 142	-							
10	RBC2 121	0.00147	-						
11	RBC2 71	0.00295	0.00147	-					
12	RBC1 712	0.00147	0.00000	0.00147	-				
13	RBC1 60	0.00295	0.00147	0.00295	0.00147	-			
14	RBC2 96	0.00147	0.00000	0.00147	0.00000	0.00147	-		
15	RBC2 4	0.00147	0.00000	0.00147	0.00000	0.00147	0.00000	-	
16	RBC1 65	0.00147	0.00000	0.00147	0.00000	0.00147	0.00000	0.00000	-
17	HT H3	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
18	HT HD11	0.03682	0.03535	0.03682	0.03535	0.03682	0.03535	0.03535	0.03535
19	HT H2	0.03976	0.03829	0.03976	0.03829	0.03976	0.03829	0.03829	0.03829
20	HT STD14	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
21	HT STD11	0.03976	0.03829	0.03976	0.03829	0.03976	0.03829	0.03829	0.03829
22	HT NTB2	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
23	HT HD36	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
24	HT NTD1	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
25	HT HD4	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
26	HT HD9	0.03682	0.03535	0.03682	0.03535	0.03682	0.03535	0.03535	0.03535
27	HT NTD5	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
28	HT HD8	0.03682	0.03535	0.03682	0.03535	0.03682	0.03535	0.03535	0.03535
29	HT STM13	0.03682	0.03535	0.03682	0.03535	0.03682	0.03535	0.03535	0.03535
30	HT NTD9	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
31	HT STD22	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
32	HT STD5	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
33	HT STD12	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
34	HT NTD11	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
35	HT NTD7	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
36	HT STM15	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
37	HT STM16	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
38	HT HD32	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
39	HT STD24	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
40	HT STD9	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
41	HT NTD19	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
42	HT HD17	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
43	HT H1	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682
44	HT ST1	0.03829	0.03682	0.03829	0.03682	0.03829	0.03682	0.03682	0.03682

Uncorrected ("p") distance matrix (continued)

		17	18	19	20	21	22	23	24
17	HT H3	-							
18	HT HD11	0.00147	-						
19	HT H2	0.00147	0.00295	-					

20	HT	STD14	0.00000	0.00147	0.00147	-			
21	HT	STD11	0.00147	0.00295	0.00295	0.00147	-		
22	HT	NTB2	0.00000	0.00147	0.00147	0.00000	0.00147	-	
23	HT	HD36	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	-
24	HT	NTD1	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
25	HT	HD4	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
26	HT	HD9	0.00147	0.00000	0.00295	0.00147	0.00295	0.00147	0.00147
27	HT	NTD5	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
28	HT	HD8	0.00147	0.00000	0.00295	0.00147	0.00295	0.00147	0.00147
29	HT	STM13	0.00147	0.00000	0.00295	0.00147	0.00295	0.00147	0.00147
30	HT	NTD9	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
31	HT	STD22	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
32	HT	STD5	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
33	HT	STD12	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
34	HT	NTD11	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
35	HT	NTD7	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
36	HT	STM15	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
37	HT	STM16	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
38	HT	HD32	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
39	HT	STD24	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
40	HT	STD9	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
41	HT	NTD19	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
42	HT	HD17	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
43	HT	H1	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000
44	HT	ST1	0.00000	0.00147	0.00147	0.00000	0.00147	0.00000	0.00000

Uncorrected ("p") distance matrix (continued)

		25	26	27	28	29	30	31	32
25	HT	HD4	-						
26	HT	HD9	0.00147	-					
27	HT	NTD5	0.00000	0.00147	-				
28	HT	HD8	0.00147	0.00000	0.00147	-			
29	HT	STM13	0.00147	0.00000	0.00147	0.00000	-		
30	HT	NTD9	0.00000	0.00147	0.00000	0.00147	0.00147	-	
31	HT	STD22	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	-
32	HT	STD5	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
33	HT	STD12	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
34	HT	NTD11	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
35	HT	NTD7	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
36	HT	STM15	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
37	HT	STM16	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
38	HT	HD32	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
39	HT	STD24	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
40	HT	STD9	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
41	HT	NTD19	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
42	HT	HD17	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
43	HT	H1	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000
44	HT	ST1	0.00000	0.00147	0.00000	0.00147	0.00147	0.00000	0.00000

Uncorrected ("p") distance matrix (continued)

		33	34	35	36	37	38	39	40
33	HT	STD12	-						
34	HT	NTD11	0.00000	-					
35	HT	NTD7	0.00000	0.00000	-				
36	HT	STM15	0.00000	0.00000	0.00000	-			
37	HT	STM16	0.00000	0.00000	0.00000	0.00000	-		
38	HT	HD32	0.00000	0.00000	0.00000	0.00000	0.00000	-	
39	HT	STD24	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-
40	HT	STD9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
41	HT	NTD19	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
42	HT	HD17	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
43	HT	H1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
44	HT	ST1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Uncorrected ("p") distance matrix (continued)

		41	42	43	44
41	HT	NTD19	-		
42	HT	HD17	0.00000	-	
43	HT	H1	0.00000	0.00000	-
44	HT	ST1	0.00000	0.00000	0.00000

CURRICULUM VITAE

Diego E. Reyes was born in Chihuahua, Mexico in 1982. He moved to the United States in the late 1990's where he attended Jefferson High School. He obtained a Bachelor of Science degree in Biology from the University of Texas at El Paso in 2008. After graduating, he entered an alternative teacher certification program where he completed his internship at Del Valle High School and became a biology and physics instructor at Canutillo High School before seeking a graduate degree. In the fall of 2010 he began pursuing a Masters degree in Biological Sciences at the University of Texas at El Paso. During this time he functioned as a teaching assistant of two biology introductory courses until the spring of 2013. He also worked as a Research Assistant in the summers of 2012 and 2013 and participated in curriculum development of biology courses in spring and summer 2013. He became a member of the Ecological Society of America (ESA) in 2011 and presented a poster in the annual meeting held in Austin, TX. He is a member of the Association for the Sciences of Limnology and Oceanography (ASLO). He presented his research in the 2013 ASLO Aquatic Sciences Meeting in New Orleans, LA.

Contact information:

dereyes@miners.utep.edu or zeta1982@hotmail.com

Permanent Address:

548 Encino

El Paso, TX, 79905