

2010-01-01

Ecology and Genetics of *Philodina megalotrocha* (Rotifera, Bdelloidea) From Chihuahuan Desert Populations

Lina Kamel Hamdan

University of Texas at El Paso, lkhamdan2@miners.utep.edu

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



Part of the [Ecology and Evolutionary Biology Commons](#), and the [Genetics Commons](#)

Recommended Citation

Hamdan, Lina Kamel, "Ecology and Genetics of *Philodina megalotrocha* (Rotifera, Bdelloidea) From Chihuahuan Desert Populations" (2010). *Open Access Theses & Dissertations*. 2703.
https://digitalcommons.utep.edu/open_etd/2703

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.

ECOLOGY AND GENETICS OF *PHILODINA MEGALOTROCHA*
(ROTIFERA, BDELLOIDEA) FROM CHIHUAHUAN DESERT
POPULATIONS

LINA KAMEL HAMDAN

Department of Biological Sciences

APPROVED

Elizabeth J. Walsh, Ph.D., Chair

Carl S. Lieb, Ph.D.

Diane I. Doser, Ph.D.

Robert L. Wallace, Ph.D.

Patricia D. Witherspoon, Ph.D.
Dean of the Graduate School

Copyright ©

by

Lina Kamel Hamdan

2010

ECOLOGY AND GENETICS OF *PHILODINA MEGALOTROCHA*
(ROTIFERA, BDELLOIDEA) FROM CHIHUAHUAN DESERT
POPULATIONS

by

LINA KAMEL HAMDAN, 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

May 2010

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Elizabeth Walsh for her efforts, supervision, constructive criticism, advice, help and general support during the progress of this work. I also would like to thank her for the great knowledge she shared with me.

Many thanks go to my graduate committee, Dr. Robert Wallace, Dr. Carl Lieb, and Dr. Diane Doser, for their guidance.

I wish to thank Dr. Thomas Schröder for his help in the field and laboratory. Dr. Musa Hussein kindly prepared the map of the Chihuahuan Desert. Dr. Carl Lieb provided samples from Kendall Co., TX; Comal Co., TX, Valverde Co., TX; and La Paz County Park, AZ. Nic Lannutti provided sample from Yavapai Co., AZ. Dr. Judith Riós-Arana provided samples from Chihuahua, MX and the Rio Grande, El Paso Co., TX. Dr. Roberto Rico-Martinez kindly provided the sample from San Luis Potosí, MX. Finally I thank Dr. Diego Fontaneto for providing *Philodina megalotrocha* sequences for two individuals from the Cam River, United Kingdom.

I would like to thank the faculty and staff of the Department of Biological Sciences for their help. I thank Omar Hernandez from the BBRC Sequencing Facility for his expert sequencing. Thanks are expressed to the staff and students in our laboratory for their help with field collections and support.

Collections from U.S. were taken under permits to Dr. E. Walsh (Big Bend National Park, permit # BIBE-2006-SCI-0003; Guadalupe Mountains National Park, permit # GUMO-2009-SCI-0009; Carlsbad Caverns National Park, permit # CAVE-2008-SCI-0005; Balmorhea Canal, Texas Parks and Wildlife Department, permit # 02-04). Collections from México (Ojo de la

Punta, Ojo de Santa Maria B, Ojo de en Medio, Poza Tortugas, and Manantial San Sebastian) were taken under a permit to Dr. Marcelo Silva Briano (permit # DGOPA/16216/281105-07984).

This research was supported by National Science Foundation under Grant No. DEB 0516032 (to E.J. Walsh). NSF ADVANCE No. 0245071 provided GRA support for 1.5 years. UTEP's BBRC DNA Sequencing Core Facility (NIH NCRR No. 5G12RR008124) provided facilities and technical support that for DNA quantification and generating some sequences.

Funds that allowed me to present my results at conferences were provided by the Department of Biological Sciences, the College of Science, the Graduate School, and the Student Government Association (SGA) at UTEP. Additional funding was provided by a Department of Labor-Employment and Training Administration "Science, Technology, Engineering and Mathematics (STEM) Opportunities in the Workforce System Initiative" grant no. SGA-DFA-PY-07-03.

Finally, all my appreciation go to my husband, Musa, and my daughter, Lana, for their patience, help, continuous encouragement and support, and to all my family members, parents, brothers and sisters.

ABSTRACT

The Chihuahuan Desert is a unique region with high biodiversity. There is a high degree of endemism of freshwater biota, but relatively few studies have been focused on zooplankton. Bdelloid rotifers are one component of these ecosystems and often dominate very temporary habitats. Bdelloids are usually assumed to be cosmopolitan species due to their potentially high dispersal rates. Distribution records of the bdelloid *Philodina megalotrocha* extend from North America to New Zealand. However, little is known about its ecology or genetics. The aims of this study are to: 1) determine whether the distribution of *P. megalotrocha* in the Chihuahuan Desert is associated with ecological characteristics of habitats, 2) investigate whether *P. megalotrocha* is truly a cosmopolitan species and not a complex of cryptic species and 3) determine patterns of gene flow among populations of *P. megalotrocha* with respect to their geographical distribution. Redundancy Analysis was applied to investigate relationships between rotifer species and 30 environmental factors. To determine the extent of genetic differentiation among geographically isolated populations, *cox1* sequences were used to construct phylogenetic hypotheses using Neighbor Joining, Maximum Parsimony, and Bayesian Analysis. The first 4 canonical axes in the Redundancy Analysis explained only 7.6% of the variance in the species data. Although environmental factors did not explain much variation in the distribution of rotifers among ecological habitats, this analysis indicated that *P. megalotrocha* is associated with the Cattail Falls Spring Pools (Big Bend National Park), presence of macrophytes, elevation, and to some degree with flowing waters and the summer season. High levels of genetic variation among populations of *P. megalotrocha* were found in *cox1* sequences (ranging from 0% to 19.7%). Levels of sequence divergence among some *P. megalotrocha* Chihuahuan Desert populations were equivalent to or greater than those of other well-defined bdelloid species.

P. megalotrocha was monophyletic in two of three analyses and contained at least five independent clades. Haplotype diversity was 0.98, with 22 haplotypes identified from 28 sequences. This may indicate that *P. megalotrocha* populations in the Chihuahuan Desert are, in fact, members of a complex of cryptic species. This study is the first to provide a detailed ecological survey of *P. megalotrocha* in a large geographic area. Further, this is the first analysis of phylogenetic relationships within *P. megalotrocha* populations using a molecular approach. Phylogenetic analyses demonstrate that morphological taxonomy in this group underestimates genetic diversity as has been recently found other bdelloid species.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	vi
TABLE OF CONTENTS.....	viii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
CHAPTER	
1. GENERAL INTRODUCTION.....	1
2. ECOLOGY STUDY	6
2.1 Materials and Methods	6
2.1.1 Sample Collection	6
2.1.2 Water Chemistry and Site Characterization.....	6
2.1.3 Redundancy Analysis (RDA).....	7
2.2 Results.....	7
3. GENETICS STUDY.....	9
3.1 Materials and Methods.....	9
3.1.1 Genetic Analyses.....	9
3.1.1.1 Sample Collection.....	9
3.1.1.2 Rotifer Culture and Preservation for DNA extraction.....	9
3.1.1.3 DNA extraction	9
3.1.1.4 DNA Amplification and Quantification.....	10
3.1.1.5 DNA Sequencing and Alignment	11

3.1.2 Distance Analysis.....	11
3.1.3 Phylogenetic Analyses.....	11
3.1.4 Population Diversity	12
3.2 Results	12
3.2.1 Distance Analysis.....	12
3.2.2 Phylogenetic Analyses.....	14
3.2.3 Population Diversity.....	15
4. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS.....	16
4.1 General Discussion.....	16
4.1.1 Ecology.....	16
4.1.2 Genetics.....	20
4.1.3 Synthesis of Ecology and Genetics.....	23
4.2 Conclusions and Future Directions	23
REFERENCES.....	25
APPENDICES.....	58
Appendix 1: Multiple sequence alignment for <i>P. megalotrocha</i> populations and outgroups using ClustalW	58
Appendix 2: Uncorrected ("p") distance matrix for <i>P. megalotrocha</i> populations and outgroups	67
Appendix 3: Mantel test for the correlation between genetic distance and geographic distance between <i>P. megalotrocha</i> populations.....	70
CURRICULUM VITAE.....	74

LIST OF TABLES

		page
Table 2.1	Localities of <i>P. megalotrocha</i> populations from the Chihuahuan Desert and outgroups that were included in the ecological analyses.....	35
Table 2.2	Summary of RDA statistics for rotifers species and environmental factors in the Chihuahuan Desert.....	40
Table 3.1	Populations of <i>P. megalotrocha</i> from the Chihuahuan Desert and outgroups that were included in the genetic study.....	41
Table 3.2	Correlation between genetic and geographic distances (Isolation by Distance) in <i>P. megalotrocha</i> populations	43
Table 3.3a	Genetic structure of <i>P. megalotrocha</i> populations used in AMOVA analysis, based on geographic locality.....	44
Table 3.3b	Genetic structure of <i>P. megalotrocha</i> populations used in AMOVA analysis, based on phylogenetic clades.....	45
Table 3.4a	Molecular variance of <i>P. megalotrocha</i> populations for sites included in the genetic study, based on geographic locality.....	46
Table 3.4b	Molecular variance of <i>P. megalotrocha</i> populations for sites included in the genetic study, based on phylogenetic clades	46
Table 4.1	Occurrence of <i>P. megalotrocha</i> in relation to some environmental variables.	47
Table 4.2	Summary of genetic diversification in some monogonont and bdelloid rotifer species based on partial <i>cox1</i> gene sequences.....	48

LIST OF FIGURES

		Page
Figure 2.1	Redundancy Analyses (RDA) of rotifer species and 30 selected environmental factors from the Chihuahuan Desert.....	50
Figure 3.1	Site names and locations of <i>P. megalotrocha</i> populations sampled in the Chihuahuan Desert.....	51
Figure 3.2	Correlation between genetic distance and Log-transformed geographic distance between <i>P. megalotrocha</i> populations.....	53
Figure 3.3	Phylogenetic relationships of <i>P. megalotrocha</i> populations inferred by Neighbor Joining analysis of <i>cox1</i> sequences.....	54
Figure 3.4	Phylogenetic relationships within <i>P. megalotrocha</i> populations, consensus of Maximum Parsimony analysis of <i>cox1</i> sequences.....	55
Figure 3.5	Phylogenetic relationships within <i>P. megalotrocha</i> populations based on Bayesian analysis of <i>cox1</i> sequences under a (GTR+I+G) substitution model.....	56
Figure 4.1	Occurrence of <i>P. megalotrocha</i> as a function of sampling effort for all sites.....	57

Chapter 1

GENERAL INTRODUCTION

Historically, many freshwater and marine invertebrates have been believed to be cosmopolitan. The Baas Becking concept (Quispel, 1998; de Wet and Bovier, 2006), ‘Everything is everywhere, but, the environment selects’, argues that microscopic organisms (organisms ≤ 1 mm) such as prokaryotes and protists are distributed worldwide due to 1) their highly dispersal potential (Kellogg and Griffin, 2006) and 2) their small size and ability to enter dormancy and produce dormant propagules (Cáceres, 1997; Bohonak and Jenkins, 2003; Fenchel and Finlay, 2004). Because of their small size, the Baas Becking concept should pertain to rotifers (0.05–2 mm). In fact, rotifers have long been considered as cosmopolitan organisms due to their dormant propagules, which allows for long distance transport through wind, water and animal vectors (Ruttner-Kolisko, 1974; Wallace and Snell, 2010). However, the ‘Everything is everywhere’ concept has been challenged recently as application of molecular tools has revealed high levels of genetic differentiation and restricted dispersal in many zooplankton species (e.g., De Meester *et al.*, 2002; Gómez *et al.*, 2002; Suatoni *et al.*, 2006; Schröder and Walsh, 2007; Walsh *et al.* 2009). Molecular studies also have shown cryptic speciation in copepods (Lee, 2000; Lee and Frost, 2002; Goetze, 2003) and amphipods (Finston *et al.*, 2007). For instance, the copepod *Eurytemora affinis*, which has a broad geographic range within the Northern Hemisphere, was found to be a complex of sibling species, as revealed by genetic divergences and reproductive isolation (Lee, 2000).

Like other zooplankton, some rotifer species have undergone cryptic speciation. Phylum Rotifera consists of four classes; Bdelloidea (only asexual reproduction), Monogononta (sexual and asexual reproduction), Seisonida (sexual reproduction only), and Acanthocephala (sexual

reproduction only) (Melone, 1998; Min and Park, 2009). Monogononts are a striking example where cryptic speciation has been detected using molecular, ecological, morphological approaches (Gómez *et al.*, 2002; Derry *et al.*, 2003; Suatoni *et al.*, 2006; Gilbert and Walsh, 2005; Schröder and Walsh, 2007; Walsh *et al.*, 2009). High levels of sequence divergence and reproductive isolation supported cryptic speciation in *Brachionus calyciflorus* (Gilbert and Walsh, 2005) and *Epiphanes senta* (Schröder and Walsh, 2007). Molecular data and absence of hybridization in sympatric populations of *Brachionus plicatilis* supports up to 14 distinct lineages in this species complex (Gómez *et al.*, 2002; Suatoni *et al.*, 2006). Walsh *et al.*, (2009) reported high genetic variation in sympatric *Lecane bulla* populations in the Chihuahuan Desert. Monogononts have been considered to be the most closely related group to bdelloids (Melone *et al.*, 1998; Poinar and Ricci, 1992; Mark Welch *et al.*, 2004). However, Min and Park, (2009) recently investigated the phylogenetic relationships among Monogononta, Bdelloidea, and Acanthocephala using a comparative analysis of the complete mitochondrial genome of a bdelloid (*Rotaria rotatoria*), a representative monogonont, and an Acanthocephalan. Their results indicate that Bdelloidea and Acanthocephala are a sister group and that together they are sister group to the Monogononta. Their analysis supports the hypothesis that Bdelloidea shares most common recent ancestry with Acanthocephala rather than with Monogononta, as was earlier proposed by Lorenzen (1985) using morphological evidence (based on putative synapomorphic characters of lemnisci and proboscis (*cf.* Ricci 1998a).

Bdelloids are distinguished by two characteristics that are autapomorphic, and potentially affect their distribution: 1) obligatory parthenogenetic reproduction and 2) ability to withstand unfavorable conditions through anhydrobiosis (Ricci, 1987; Ricci, 1998b). Bdelloids are found in freshwater and wet terrestrial habitats worldwide (Wallace and Snell, 2010). They have survived

for more than 100 million years (Mark Welch and Meselson, 2000) and comprise more than 400 morphologically recognizable species (Segers, 2007). No males have been observed in bdelloids and molecular studies support the view that they reproduce only through parthenogenesis (e.g., Mark Welch and Meselson, 2000; Mark Welch *et al.*, 2004; Barraclough *et al.*, 2007). Bdelloid species are usually assumed to be cosmopolitan (Fontaneto *et al.*, 2007b). The potential for dispersal by their dormant propagules, which can be transported passively and become the founders of new populations, makes the assumption of cosmopolitan distribution more reasonable. However, bdelloids suffer from a lack of extensive faunistic studies. The main reason for the poor knowledge of the diversity and distribution of bdelloid species is the difficulty of identification because of their soft bodies and lack of easily recognized morphological features. Notably, most of the ~ 400 species described worldwide are known from Europe. But, our knowledge of the biogeography of bdelloids in Europe and other geographic regions is still not complete and the number of undescribed species is increasing (Örstan, 1995; Yakovenko, 2000a; 2000b; Fontaneto and Melone, 2003). Moreover, a complicating factor is that bdelloids occupy unusual habitats (e.g., pitcher plants, gill chambers of crustaceans) that have not been studied in detail (Petersen *et al.*, 1997; Fontaneto *et al.*, 2004), thus many more species may be discovered. In a recent study of bdelloids (Fontaneto *et al.*, 2007a), 61 species were reported from 185 collections around the world. This study found that some species have cosmopolitan distributions, while other seems to be geographically limited to certain areas. In addition, some bdelloid species traditionally known as cosmopolitan have recently been recognized as cryptic species (e.g. Birky *et al.*, 2005; Fontaneto *et al.*, 2007b; 2008a, b; 2009; Kaya *et al.*, 2009), which suggests that actual bdelloid diversity can be expected to be much higher than that recognized by a morphological approach.

In this study I focused my efforts on *Philodina megalotrocha*, a relatively common bdelloid present in ephemeral pools of the Chihuahuan Desert. This desert region covers parts of the states of Arizona, New Mexico, and Texas in the United States, as well as parts of the states of Chihuahua, Coahuila, Nuevo Leon, Durango, Zacatecas, and San Luis Potosí in México. It is one of the largest deserts in North America. It has an area of about 362,600 km². The Chihuahuan Desert is rather dry and the rainy seasons occur more often in summer (usually in June and July) rather than in winter. The mean annual precipitation for the Chihuahuan Desert is 235 mm, ranging from 150 to 400 mm (Davis *et al.*, 1997). The Chihuahuan Desert is a unique region recognized for its biodiversity and high levels of endemism. About 3,500 plant species live in this desert, and up to 1000 (29%) are considered to be endemic species (Toledo 1988). It also contains a high diversity of mammals, birds, and insects (Dinerstein, *et al.*, 2000). Despite the arid conditions, there are ample water sources in the Chihuahuan Desert to support aquatic life and a high degree of local endemism has been found in its freshwater biota (Dinerstein, *et al.*, 2000). However, relatively few studies have been focused on its aquatic systems.

The freshwater habitats of the Chihuahuan Desert include: permanent rivers (e.g., Rio Grande, Rio Mesquites) and reservoirs (e.g., Presa Chihuahua, Presa Alvaro Obregon), free flowing springs (e.g., Cattail Falls Spring Pools, Burro Spring Pools), spring seeps (e.g., Glenn Spring, Burro Springs), ephemeral playas (e.g., Laguna La Leche, Stinky Playa), and artificial cattle tanks (e.g., Paint Gap Cattle Tank, Tule Cattle Tank) and rock pools (e.g., Window Trail Tinajas, Ernst Tinaja). Furthermore, Chihuahuan Desert supports a large number of high levels of local endemism including fishes, amphipods, and springsnails. This arid region is at significant risk from a variety of anthropogenic activities including human habitations, industrial

and recreation development, introduction of exotic species, overgrazing by non-native stock animals, and aquifer depletion (Shepard, 1993; Olson and Dinerstein, 1998; Abel *et al.*, 2000).

This study is the first molecular and ecological study of *P. megalotrocha*. *Philodina megalotrocha* (Bdelloidea, Eurotatoria) has been considered to be a cosmopolitan species (Donner, 1965; Koste and Shiel, 1986), with distribution records extending from North America (Wallace *et al.*, 2005; Walsh *et al.*, 2007; Wallace *et al.*, 2008; Walsh *et al.*, 2008) to New Zealand (Shiel and Green, 1996). Although *P. megalotrocha* is recognized as possessing this wide distribution, relatively little is known concerning its ecology or genetics. The general ecological features of *P. megalotrocha* were summarized by Donner (1965). According to his review, *P. megalotrocha* is epibenthic, typically found among aquatic plants in running waters, pools, ponds, and lakes. *Philodina megalotrocha* can attach to the loricae of other rotifers and to other aquatic organisms, while eggs are usually attached to roots and algae. It is generally believed that this species is eurytopic as a consequence of its distribution; however detailed studies have not tested this assumption. Likewise genetic study of *P. megalotrocha* has not been previously done at any level. Thus, the present study investigated ecological and genetic features of *P. megalotrocha* in the Chihuahuan Desert.

This research addresses the following questions:

- 1) Does distribution of *P. megalotrocha* in the Chihuahuan Desert correlate with specific ecological factors and habitats? 2) Is *P. megalotrocha* truly a cosmopolitan species or is it a species complex? 3) Is gene flow among Chihuahuan Desert populations of *P. megalotrocha* restricted with respect to their geographical distribution?

Chapter 2

ECOLOGY STUDY

2.1 Materials and Methods

2.1.1 Sample Collection

Individuals of *P. megalotrocha* were collected from 17 populations in the Chihuahuan Desert of the U.S. and México; these collections were integrated with samples from 57 populations collected previously as part of a large study on Chihuahuan Desert waters (Wallace *et al.*, 2005; Wallace *et al.*, 2008; Walsh *et al.*, 2007; Walsh *et al.*, 2009). Samples from several populations outside the Chihuahuan Desert (n=8) but within the southwest U.S. were also included (Table 2.1). The total number of sampled sites was 259. Sites included a variety of systems: springs, rivers, reservoirs, tinajas, tanks and artificial ponds. Samples were collected using different techniques such as plankton nets (64 µm), grab samples, and aspirating samplers. Further details of methods are described in Wallace *et al.* (2005) and references listed above. The taxonomic keys available for bdelloids (Donner, 1965; Koste and Shiel, 1986; Ricci and Melone, 2000) were followed to recognize individuals of *P. megalotrocha* in the samples.

2.1.2 Water Chemistry and Site Characterization

Water chemistry data were recorded following protocols in Wallace *et al.* (2005) and Walsh *et al.* (2007). A YSI 556 multiprobe was used for determining water temperature, pH, dissolved oxygen (DO), oxidation reduction potential (ORP), conductivity, and total dissolved solids (TDS). Turbidity, ammonia, nitrite, nitrate, phosphate, alkalinity, hardness, silica, chloride, sulfate, and watercolor were determined using a YSI 9000 Photometer and YSI test kits. Additional information recorded at each collection site included: elevation, habitat size (e.g., length, width, depth), aquatic system description (e.g., flow rate, interconnectivity,

watercolor), characterization of vegetation (e.g., algae, macrophytes, decaying material), and site exposure. GPS coordinates and elevation were recorded using Brunton Multi-Navigator[®] and verified using GoogleEarth[®].

2.1.3 Redundancy Analysis (RDA)

Statistical analyses of habitat characteristics and *P. megalotrocha* distribution were conducted using Redundancy Analysis (RDA) in CANOCO for windows 4.54 (ter Braak and Smilauer, 2002). RDA was conducted using the complete dataset (provided by E.J. Walsh and R.L. Wallace (pers. comm.)). Environmental variables were sequentially added to the model when they provided additional fit at a significance level $p < 0.05$. The significance of variables was determined with Monte Carlo tests running 9999 permutations.

2.2 Results

Philodina megalotrocha was found in 73 of the 259 Chihuahuan desert sites (28%). *Philodina megalotrocha* is found in habitats with wide ranges of temperature, pH, conductivity, ORP, and dissolved oxygen (Table 4.1).

Redundancy analysis is a statistical technique that can be applied to make predictions of how species respond to multiple environmental factors (ter Braak and Smilauer, 2002). I applied this technique to investigate relationships between *P. megalotrocha* distribution and habitat features. Thirty environmental variables were included in the RDA analysis. RDA showed a significant relationship between environmental parameters and species composition. The first four canonical axes in the RDA explained 7.6% of the variance in the total species data (Table 2.2). The most important environmental variables in the model were: tinajas rarely filled with waters, Cattail Falls Spring Pools at Big Bend National Park (BBNP), alkalinity, springs that pond, and presence of macrophytes. The first axis explained more than half of the variance of the

species distribution that is attributable to environmental factors; the remaining axes had minor contributions to the total variance (Table 2.2).

RDA analysis indicates that *P. megalotrocha* is associated mostly with the Cattail Falls Spring Pools at BBNP. This may reflect a combination of factors associated with the complexity of the physical structure of these pools as *P. megalotrocha* was also correlated with presence of macrophytes, water flow, and elevation. Furthermore, *P. megalotrocha* was somewhat associated with seasonality; positively correlated with summer, but negatively correlated with spring. In contrast, there is no strong correlation between *P. megalotrocha*'s distribution and water chemistry parameters such as ORP, silica, pH, and nitrate (Fig. 2.1).

Chapter 3

GENETICS STUDY

3.1 Materials and Methods

3.1.1 Genetic Analyses

3.1.1.1 Sample Collection

Individuals of *P. megalotrocha* were collected as described above from different populations (n=17) in the Chihuahuan Desert of the U.S. and México. Samples from several populations outside the Chihuahuan Desert but within the southwest U.S. (n=7); San Felipe Springs, Comal Springs, Cibolo Creek, and Bear Creek from Texas; Lake Jennings from California, Willow Lake and La Paz County Park from Arizona and a population from Cam River in England were also included. Eleven additional *cox1* sequences from other *Philodina* species obtained from GENBANK or Dr. Roberto Rico-Martinez were used as outgroups (Fig.3.1 and Table 3.1).

3.1.1.2 Rotifer Culture and Preservation for DNA extraction

Individuals of *P. megalotrocha* were isolated and cultured in modified MBL (Marine Biological Laboratory) media (Stemberger, 1981) and fed the green algae *Chlamydomonas reinhardtii* (UTEX strain 90) for a minimum of one week. Animals were serially transferred in MBL media several times to remove algae and other contaminants before preservation in 70% ethanol for storage until DNA extraction.

3.1.1.3 DNA extraction

DNA was extracted using a Chelex preparation (InstaGene Matrix; Bio-Rad.) after removing EtOH from samples by drying at 65°C or by using live animals after rinsing them with distilled water. Then InstaGene Matrix (35 µl/100 individuals) was added and samples were

incubated at 56°C for 15-30 minutes, followed by vortexing at high speed for 10 seconds. Next samples were heated at 100°C for 8 minutes and then vortexed at high speed for 10 seconds and centrifuged for 2-3 minutes. This product was used as template for PCR reactions. Alternatively, a lysis buffer was used. In this case 3 µl per 10 individuals were added before heating samples at 55°C for 90 minutes, homogenizing, and freezing at -70°C for 24 hours. Fresh lysis buffer was prepared for each extraction series by adding 5 µl of Proteinase K solution (20 mg/ml) to 95 µl of 1XPCR buffer. 1XPCR buffer was prepared by adding 100 µl 10xPCR buffer to 30 µl 50 mM MgCl₂ and 870 µl sterile H₂O (HPLC grade), then 1XPCR buffer was stored at -20°C. To prepare Proteinase K Solution, buffer was prepared by adding 63 mg Tris HCL and 2.9 mg CaCl₂*2H₂O then brought-up to 10 ml with sterile H₂O, then 10 ml glycerol was added. Next, 10 mg lyophilized Proteinase K (Sigma P-8044) was dissolved in 500 µl of storage buffer and finally the Proteinase K buffer was stored at -20°C.

3.1.1.4 DNA Amplification and Quantification

A 583 base-pair (bp) fragment of *cox1* gene was amplified using the primers LCO and HCO (Folmer *et al.*, 1994). All amplifications included a negative control to detect amplification of contaminating DNA. Contaminated DNA samples were ignored and not included in the analysis. The *cox1* gene was amplified with denaturation, annealing, and extension temperatures of 94°C, 47°C, and 72°C, respectively. Amplification products were examined by electrophoresis; bands were removed and cleaned with Gene Clean III[®] Kits. Then DNA was quantified using Pico Green[®] fluorescent staining and a Fluoroscan or by a NanoDrop 1000 spectrophotometer before sequencing.

3.1.1.5 DNA Sequencing and Alignment

Samples were sequenced on a capillary sequencer at The University of Arizona (ABI 3700) or The University of Texas at El Paso (ABI 3130xl) following standard protocols. All sequences were run at least twice in both directions. Sequences were checked against GENBANK to ensure that amplification products were from phylum Rotifera. Then, verified sequences were aligned using ClustalW (Thompson *et al.*, 1994) with some manual editing. Sequences are given in Appendix 1.

3.1.2 Distance Analysis

Pairwise genetic distances were calculated using the Neighbor Joining algorithm in PAUP*4.0 β (Swofford, 2003) using uncorrected ("p") values. These genetic distances were used to construct dendrograms to compare genetic similarity among populations. Geographical distances were calculated as distances between paired coordinates. Genetic and geographic values were used to conduct Mantel tests to determine whether genetic distances and geographic distances between populations were correlated using Isolation by Distance (IBD, Bohonak, 2002).

3.1.3 Phylogenetic Analyses

Phylogenetic hypotheses for relationships among *P. megalotrocha* populations were constructed based on *cox1* gene sequences using Maximum Parsimony (MP) and Neighbor Joining (NJ) algorithms implemented in PAUP*4.0 β (Swofford, 2003) and Bayesian Analysis conducted with MrBayes v3 (Ronquist and Huelsenbeck, 2003). Clade support for MP was assessed using 1000 bootstrap replicates heuristically searched with 1000 random addition replicates and TBR branch swapping. Only informative characters were included in MP analyses (206 of 377). Bayesian Analysis was run for 5 million generations, after an initial burn-in period

of 250 generations, using a general time reversible model with a proportion of variable sites and a gamma-shaped distribution of rates across sites (GTR+I+G), which was identified by MrModeltest v2.3 (Nylander, 2004) and Modeltest v3.7 (Posada and Crandall, 1998) as the best-fit model for *cox1* evolution for the dataset. Trees were examined by eye to determine congruency.

3.1.4 Population Diversity

Populations of *P. megalotrocha* were divided into 7 groups by geographic locality and by phylogenetic clades. The number of haplotypes per group and haplotype diversity (Hd) were computed using DnaSP 5.0 (Librado and Rozas, 2009). To analyze the spatial pattern of *P. megalotrocha* genotypes, Analysis of Molecular Variance (AMOVA; Excoffier *et al.*, 1992) based on geographic locality or clades identified by MrBayes analysis was performed using Arlequin v3.01 (Excoffier *et al.*, 2005) at three levels: variation among individuals in the same population, variation among individuals in different populations in the same region, and variation among individuals in different regions. If the taxa are widely distributed, more haplotypic variation is expected to occur among individuals from the same population. If the species shows high genetic connectivity throughout its geographical range, most of the variation would occur among regions. If natural gene flow occurs, a clinal change in genotype would be predicted. If dispersal occurs frequently, we would expect an array of different haplotypes to be present within a given region.

3.2 Results

3.2.1 Distance Analysis

Genetic variation was investigated using *cox1* sequences from 25 populations of

P. megalotrocha: 17 populations located in the Chihuahuan Desert and 8 populations outside the Chihuahuan Desert (total number of sequences included was 28, some populations have duplicate samples and two sequences from a United Kingdom population were provided by Dr. Diego Fontaneto). In addition, 11 *cox1* sequences of other *Philodina* species were used as out-groups: two sequences of *Philodina sp.*, two sequences of *P. acuticornis*, three sequences of *P. citrina*, and four sequences of *P. flaviceps* (Table 3.1). Pairwise distances showed high genetic variation among populations of *P. megalotrocha*, ranging from 0% to 19.7% (Appendix 2). The greatest genetic distance was found between the San Felipe Springs (TX, USA) and Media Luna Canal (San Luis Potosí, México) populations (19.7% sequence divergence, these populations are 833 km apart). Other Chihuahuan desert populations showing high genetic variation include Croton Spring isolate-2 (BBNP) and Ojo de la Punta isolate-1 (Chihuahua) (18.7% sequence divergence). High genetic variation was also found between Chihuahuan Desert and Non-Chihuahuan Desert populations, for instance Dripping Springs (NM) and Bear Creek (TX) populations have 18% sequence divergence and a geographic distance of 769 km. Moreover, high genetic variation was found between isolates from the same location. The two isolates from Croton Spring have 12.5% sequence divergence and Ojo de la Punta isolates showed 15.8% sequence divergence. Both of these were taken in different times, one in different years (Croton) and the other in different seasons (Ojo de la Punta) (Table 3.1). However, other populations showed little genetic differentiation independent of geographic distance. For instance, individuals from two pools in a series (Cattail Falls Spring Pools A and H, BBNP) showed only 0.52% sequence divergence and those from the Rio Grande (near BBNP, Brewster Co., TX) and Ascarate Lake (El Paso, Co. TX) have only 0.35% sequence divergence although they are 435 km apart.

As described above, patterns of genetic variation in *P. megalotrocha* populations do not correspond directly with their geographic distributions. High and low genetic divergences among *P. megalotrocha* populations were found at very short or very long geographical distances. I applied the Mantel test using the IBD program to calculate correlations between the genetic distance matrix and the geographical distance matrix. The results indicated a significant but weak correlation between genetic distance and geographic distance using Log transformation of both or any of the combination of the data ($r=0.178$, $p<0.007$). Using untransformed data there was no significant relationship between genetic and geographical distances ($r = 0.1214$, $p = 0.1450$) (Table 3.2). A scatter plot of genetic distance versus Log-transformed geographic distance shows that genetic and geographic distances are not strongly related (Fig. 3.2).

3.2.2 Phylogenetic Analyses

Slight differences in topology were observed among MP, NJ, and Bayesian *cox1* trees. For MP analysis, 6 equally parsimonious trees were found. In MP and NJ trees, *P. megalotrocha* populations formed a monophyletic with at least 5 distinct clades; (A-E, Fig. 3.3 and Fig. 3.4). Bayesian analysis produced a consensus tree with a high number of well-supported clades within *P. megalotrocha* (Fig. 3.5). Even though, *P. megalotrocha* populations formed a polyphyletic clade, most of the populations were found in one monophyletic clade, which has 4 clades (A-D) and was highly supported (95%), while the other clade (clade E) was weakly supported. Generally, the same relationships among *P. megalotrocha* populations were found in the three phylogenetic hypotheses. For example, Cibolo Creek and Balmorhea Canal populations were found as a sister group in all phylogeny hypotheses. Ojo de la Punta isolate-1 and Media Luna Canal populations were found as a sister group with Rattlesnake Springs and Bear Creek populations. Also, Rio Grande and Ojo de Santa Maria B populations formed a sister group in all

analyses. On the other hand, some of the duplicate samples of the same geographic population were found in different clades (Croton Spring isolate-1 and isolate-2, and Ojo de la Punta isolate-1 (clade E) and isolate-2 (clade D)), which was unexpected. Similarly, some populations from interconnected pools were found in different clades (i.e., Cattail Falls Spring Pools E, A, and H). Furthermore, close examination of the genetic relationships between *P. megalotrocha* populations and their geographic localities, indicates that most clades do not correspond to geographic origin in any of the tree analysis.

Thus the null hypothesis, predicting that *P. megalotrocha* is a cosmopolitan species and can be readily dispersed, is rejected. The alternative assumption, of cryptic speciation, is supported by the existence of at least 5 divergent independent evolving entities. This indicates that populations of *P. megalotrocha* are geographically isolated and gene flow is restricted among them.

3.2.3 Population Diversity

Twenty-two haplotypes were found from 28 sequences divided into 7 groups either by geographic locality (Table 3.3a) or by clades identified by Bayesian analysis (Table 3.3b). Haplotype diversity (H_d) is 0.98. AMOVA based on the geographic locality of these 7 groupings shows that most the variation in *P. megalotrocha* populations occurs within populations (69.97%) followed by variation among populations within groups (21.9%). Only a small amount of variation among groups was observed (8.0%) (Table 3.4a). Conversely, in the analysis based on the phylogenetic clades, variation among clades (54.3%) was higher than the variation between clades (45.6%) and no detectable variation was observed within populations. The lack of variation within populations in this analysis is an artifact of small sample size ($n=2$).

Chapter 4

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS

4.1 General Discussion

4.1.1 Ecology

One of the main objectives of this study was to determine whether the distribution of *P. megalotrocha* correlates with specific ecological factors and habitats. The null hypothesis was that *P. megalotrocha* populations are found in habitats possessing non-similar ecological characteristics. My results showed that *P. megalotrocha* populations were found in different habitat types (i.e., springs, streams, rivers, huecos, tinajas, artificial and natural ponds, lakes; Table 2.1). It is important to mention that sampling effort was not equal among all sites; some sites were sampled only once while others were sampled up to twelve times, which could bias the ability to detect the presence of *P. megalotrocha*. With increased sampling effort, the probability of detecting *P. megalotrocha* increased (Fig. 4.1). For instance, out of 135 sites that were sampled only once, *P. megalotrocha* was found only in 16 sites (12%). On the other hand, *P. megalotrocha* was found frequently in sites that were sampled more than 10 times.

Philodina megalotrocha has a reported cosmopolitan distribution (Donner, 1965). Many microscopic organisms are known to have a cosmopolitan distributions and general biogeographical patterns, due to their high dispersal potential (Fenchel and Finlay, 2004) and rapid adaptation of resident population to local conditions (De Meester, 1993, 1996; Okamura and Freeland, 2002). The concept of “everything is everywhere” formulated by the studies of Beijerinck and Baas Becking (Quispel, 1998; de Wet and Bovier, 2006) has been used to characterize distribution of microorganisms on the scale of bacteria and protists. Although, some previous studies have disagreed with the assumption of cosmopolitanism (Fenchel *et al.*, 1997;

Finlay, 2002; Presson, 2002), it remains that small organisms (≤ 1 mm) have a wider distribution than larger organisms (Fenchel and Finlay, 2004). If this is true in general and given that many rotifers are smaller than 1 mm, they are likely to have worldwide distributions. Many monogonont species are recorded to be cosmopolitan (Dumont, 1983). In a recent study conducted by Walsh *et al.* (2007), 84 species of monogonont rotifers were identified from 4 different habitats in BBNP (TX, USA). The majority of species found had reportedly cosmopolitan distributions. On the other hand, some monogonont rotifers have restricted biogeography due to authentic endemism (Dumont and Segers, 1996) or as a result of cryptic speciation (Ortells *et al.* 2003).

Apparently both bdelloid and monogonont rotifers can potentially achieve global distribution (Fontaneto *et al.*, 2007a). Due to the potential of dispersal provided by their dormant xerosomes and xerova (anhydrobiotic adults and eggs, respectively; Wallace and Smith, 2009; Wallace and Snell, 2010), the assumption of cosmopolitan distribution is even more likely for bdelloid rotifers. However, Fontaneto *et al.* (2005, 2006) analyzed 171 assemblages of bdelloid rotifers living in different habitats in Italy and found that bdelloids have low species richness with strong habitat selection. These authors suggested that dispersal is very rare so that not all propagules arrive everywhere. They also argued that habitat availability prevents many species from colonizing. In addition, Fontaneto *et al.* (2007a) found that some bdelloid species have cosmopolitan distribution. For example, *Adineta vaga* was found in almost every habitat, while other species seem to be geographically limited to certain areas or habitats (i.e., *Philodina nitida* seems to be localized to areas of Gondwanaland origin, *Philodina citrina* and *Rotaria macrura* are found only among submerged plants in lentic waters). Thus, studies have not fully uncovered the relationships between habitat types and bdelloid species distribution (Fontaneto *et al.*, 2009).

Philodina megalotrocha not only has a reportedly cosmopolitan distribution, it has been found in variety habitats: streams (Zullini and Ricci, 1980); rivers (Fontaneto et. al., 2007a); lakes (Jennings, 1994; Tasevska *et al.*, 2004; Aydin and Ahiska, 2009). In a large research effort that was conducted in Chihuahuan Desert waters, Walsh and her colleagues have found significant associations between environmental parameters and rotifers distribution among water systems. *Philodina megalotrocha* was associated with elevation in the analysis restricted to tinajas at BBNP (Wallace *et al.*, 2005), which is similar to the findings of this study. Also, they reported that *P. megalotrocha* was widespread among 10 aquatic systems comprising different habitat types (Walsh *et al.*, 2007). Additionally, in other analyses restricted to saline systems in the Mexican portion of the Chihuahuan Desert (Walsh *et al.*, 2008), that include some of the sites reported here, *P. megalotrocha* was ordinated with presence of macrophytes and chloride concentration. The analysis reported here (Fig 2.1, Table 2.2) includes the data used in these previous studies along with several new collections. Using this updated dataset, *P. megalotrocha* was correlated with the Cattail Falls Spring Pools at BBNP and presence or absence of macrophytes, but it was not associated with chloride concentration. My results showed that connectivity among systems is an important factor that affects presence of *P. megalotrocha* in these systems. This would account for the presence of *P. megalotrocha* in many connected systems in the Chihuahuan Desert, such as: Cattail Falls Spring Pools, Ernst tinajas, Glenn Springs, Window Trail tinajas, Ojo de Santa Maria Springs, and the Balmorhea canal and wetland. Thus, while habitat types do not limit the existence of *P. megalotrocha* in different locations, some environmental variables are significant factors in determining its occurrence.

Donner (1965) reported that *P. megalotrocha* was found among plants in all types of waters or attached to other rotifers or other aquatic organisms. In this study (in both field

collections and laboratory cultures) it was noted that *P. megalotrocha* was attached mostly to algae and not to plants, which is logical since there are few macrophytes in desert habitats as compared with other more mesic habitats. Donner also found that *P. megalotrocha* is associated with pH above 6.8 and this is consistent with this study (Table 4.1). In a previous study of the occurrence of rotifers in relation to watercolor in a large number of Swedish waters (Bērzinš and Pejler, 1989), *P. megalotrocha* had a wide tolerance range for watercolor (3-260 mg/L Pt.), and high abundances of *P. megalotrocha* were recorded when watercolor was < 50 mg/L Pt. The range that I found (0-130 mg/L Pt) was lower than that of Bērzinš and Pejler. Occurrence of *P. megalotrocha* in the Chihuahuan Desert is similar to these values, with 82% of occurrences \leq 40 mg/L Pt (Table 4.1). Finally, Ricci (1984) found that bacteria and yeast are the best diet for bdelloids and that algae do not appear to be sufficient food for some bdelloid species. These observations support my findings: *P. megalotrocha* survived to the third generation when it was cultured in yeast or bacteria and no reproduction was observed when it was cultured in algae. However, I found that when *P. megalotrocha* was cultured on the green alga *Chlamydomonas reinhardtii* clonal lineages were able to survive for more than 3 generations.

Overall, environmental factors did not explain much variation in the distribution of *P. megalotrocha* among ecological habitats, which suggests that environmental factors and habitats do not strongly affect the distribution of *P. megalotrocha* populations. My results suggest that geographical distribution of *P. megalotrocha* may be influenced by other factors such as cryptic speciation. I discuss this in the following section.

4.1.2 Genetics

This is the first study to address phylogenetic relationships within *P. megalotrocha* populations, and thus is the first using a molecular approach (mitochondrial DNA *cox1* sequence data). The high levels of genetic divergence among populations of *P. megalotrocha* (ranging from 0% to 19.7%) suggest that some *P. megalotrocha* populations are genetically isolated. This may indicate that *P. megalotrocha* populations in the Chihuahuan Desert are, in fact, members of a complex of cryptic species. Levels of sequence divergence among some *P. megalotrocha* Chihuahuan Desert populations were equivalent to or greater than those of other well-defined bdelloid species, such as those incorporated in the analysis as outgroups (e.g., *P. citrina* isolate IT.1.3 and *P. flaviceps* isolate dv2e at 13%; *P. acuticornis* isolate UT.a and *P. flaviceps* isolate Ro3a at 11%; Appendix 2).

To explore genetic diversification among rotifer lineages, I compared my results with previous studies conducted on monogonont and bdelloid rotifers using the same mitochondrial gene (*cox1*) (Table 4.2). There was similarity in levels of nucleotide sequence divergence between *P. megalotrocha* populations and that found in monogonont rotifers. The level of divergence found in *P. megalotrocha* populations was similar to that found by Gómez *et al.* (2002) in the *Brachionus plicatilis* species complex where differences ranged from 12-23%. Levels of divergence in *cox1* sequences are higher than those reported in some studies. For example, genetic variation ranging from 10.9 to 12.5% was found in the *Epiphanes senta* species complex (Schröder and Walsh, 2007). As well, DNA sequence divergences ranging from 0-13% were found among members of the *Brachionus calyciflorus* species complex (Gilbert and Walsh, 2005) and from 0.9 to 16% in *Lecane bulla* (Walsh *et al.*, 2009).

Moreover, genetic divergences within family Brachionidae were slightly higher than those reported in this study (ranged from 20-25% among species of *Brachionus* and from 23-27% among species of *Keratella*) (Derry *et al.*, 2003).

Cryptic diversification has also occurred in bdelloid morphospecies. Birky *et al.* (2005) demonstrated that bdelloids have undergone speciation similar to sexual organisms: more than 8% genetic diversification was found among 21 clades belonging to 3 bdelloid families (Adinetidae, Philodinidae, and Habrotrochidae). Fontaneto *et al.* (2007b) showed that genetic clades of bdelloids (represented by genus *Rotaria*) matched well with traditional species, as identified by morphological basis. Such diversification occurs also at lower taxonomic levels. For example, within a single traditional species, *Philodina flaviceps*, 25 haplotypes corresponding to at least 9 clusters have been recognized by analyzing sequences from 86 individuals (Fontaneto *et al.*, 2008b). These molecular studies confirmed that DNA taxonomy provides much higher species richness in bdelloids than traditional taxonomy: for example, 117 haplotypes belonging to 22 traditional bdelloid species with 57 independently evolving entities were found out of 264 examined sequences (Kaya *et al.*, 2009). Recently, Fontaneto *et al.* (2009) measured cryptic diversity for eight traditional species of bdelloids and up to 34 potential cryptic taxa were found, suggesting that different lineages within each traditional species may be spatially isolated, but do not have narrower ecological niches.

As shown here, *P. megalotrocha* has been able to diversify into at least 5 distinct clades that are comparable to the other traditional species of the same genus (Fig. 3.3, Fig. 3.4, and Fig. 3.5). Twenty-two haplotypes were identified from 28 sequences. The extent of haplotype divergence indicates the existence of distinct lineages, which support cryptic speciation in some *P. megalotrocha* populations. At the same time, similar haplotypes were found in some

populations, for instance in Cattail Falls Spring Pools A and H populations. Since these pools are interconnected there is likely localized gene flow between populations. Although competition between individuals may take place, in this case only strong lineages might co-exist. On the other hand, different haplotypes and independent clades of *P. megalotrocha* coexist both locally and regionally. These findings provide evidence of restricted gene flow among *P. megalotrocha* populations, which may suggest isolation due to either distance or ecological differences. Thus, the occurrence of the three separate assemblages in the scatter plot of genetic distance versus log transformed geographic distance (Fig. 3.2) may be explained as follows. The first cluster represents populations with low genetic divergences, which may indicate that gene flow occurs among those populations. The second cluster corresponds to populations with intermediate genetic divergences (range from 5-10%) found at different geographical scales (approximately from 100-1000 km). In this case, two possibilities are: 1) dispersal occurs sporadically between some populations separated by larger geographic distances which may explain the scenario of intermediate genetic divergence; or 2) cryptic speciation is occurring among some populations. While the third cluster represents high genetic variation among *P. megalotrocha* populations that were found at various geographic distances, which may be interpreted as cryptic speciation among populations. Estimating divergence time for *P. megalotrocha* was not undertaken in this study for two reasons. (1) No fossils of *P. megalotrocha* are available to calibrate a molecular clock. (2) When different species have different mutation rates, body size and mutation rate will not be correlated, thus, making inferences about divergence rates in invertebrate taxa questionable (Thomas *et al.*, 2006). However, the large degree of genetic divergence (up to 20%) among *P. megalotrocha* populations reflects separate evolutionary paths for lineages.

4.1.3 Synthesis of Ecology and Genetics

Philodina megalotrocha was found in a wide variety of geographic localities and habitat types, supporting the idea of cosmopolitan distribution. Nevertheless, my phylogenetic analysis indicates considerable cryptic speciation. Employing different methods for phylogenetic trees construction resulted mostly in consistent tree topologies for *P. megalotrocha* based on partial *cox1* sequences. *Philodina megalotrocha* was monophyletic in two of three analyses, but contained significantly distinct genetic clades. Overall, it is obvious that independent lineages are present at both wide and narrow geographic scales. These lineages may have originated either allopatrically or sympatrically, which suggests geographic isolation or adaptation to a new environment leading to divergence evolution of distinct lineages. The geographical distribution provides a poor match to patterns of genetic diversity. As for habitat types, genetic clades grouped together individuals collected in different habitats, like clade D: comprising populations from springs (e.g., Ojo De Santa Maria), from lakes (e.g., Ascarate Lake), and from rivers (e.g., Rio Grande, Borderland, El Paso, Co., TX). No indication was found to connect cryptic speciation with habitat, as was concluded by Fontaneto *et al.*, (2009). These results are consistent with the view that asexual organisms are expected to diversify into discrete genetic clades similar to those found in sexual organisms.

4.2 Conclusions and Future Directions

This research provides the first evidence of species diversity in *P. megalotrocha*. The findings of this study demonstrate that taxonomy based on morphology underestimates the genetic diversity of this bdelloid species. Thus, mitochondrial DNA sequence analyses show that this traditional morphospecies is not a cosmopolitan species, but rather a species complex containing a minimum of five distinct clades with genetic variation between lineages ranged

from 0.0 to 19.7%, indicating that some populations are genetically isolated. Levels of divergence among some populations were equivalent to or greater than those of other well-defined bdelloid species. This result supports the conclusion that gene flow is restricted among Chihuahuan Desert populations of *P. megalotrocha*. These findings are similar to those for other well-studied bdelloid species (Kaya *et al.*, 2009; Fontaneto *et al.*, 2008b; 2009). There is a significant, but weak, correlation between geographical distribution and genetic diversity patterns in *P. megalotrocha* populations. According to my study, existence of *P. megalotrocha* is not restricted by habitat types, but some environmental variables are significant factors in determining occurrence of *P. megalotrocha*. For the most part, populations are geographically isolated and may be in process of speciation. Also, sympatric divergence may be taking place among *P. megalotrocha* populations through rapid local adaptation to environmental conditions.

To understand the evolutionary history of *P. megalotrocha* and to resolve relationships among its populations will require additional samples. Moreover, additional gene regions should be sequenced to validate my findings. Furthermore, combined analyses of morphological and molecular data are required to reveal the full extent of genetic differentiation within the *P. megalotrocha* species complex, which will enhance our understanding of diversity within this species. In addition, detailed studies of global populations of *P. megalotrocha* are likely to yield similar levels of genetic divergence, which will add further support to the hypothesis the *P. megalotrocha* is a complex of cryptic species.

LIST OF REFERENCES

- Abel, R. A., Olson, D. M., Dinerstein, E., Hurley, P. T., Diggs, J. T., Eichbaum, W., Walter, S., Wettengel, W., Allnutt, T., Loucks, C. J., and Heado, P. 2000. Freshwater Ecoregions of North America: A conservation assessment. Island Press, Washington D.C., 319 pp.
- Aydin, D. and Ahiska, S. 2009. Determination of trophic situation of Sarimasakli Dam Lake (Kayseri-Turkey). *African Journal of Biotechnology*, 8(22): 6295-6300
- Barracclough, T. G., Fontaneto, D., Ricci, C. and Herniou, E. A. 2007. Evidence of inefficient selection against deleterious mutations in cytochrome oxidase I of asexual bdelloid rotifers. *Molecular Biology and Evolution*, 24: 1952-1962.
- Bērziņš, B. and Pejler, B. 1989. Rotifer occurrence in relation to water color. *Hydrobiologia*, 184: 23-28.
- Birky, C. W., Wolf, C., Maughan, H., Herbertson, L., and Henry, E. 2005. Speciation and selection without sex. *Hydrobiologia*, 546: 29-45.
- Bohonak, A. J. 2002. IBD (Isolation By Distance): A program for analyses of isolation by distance. *Journal of Heredity*, 93: 153-154.
- Bohonak, A. J. and Jenkins, D. G. 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters*, 6: 783-796.
- Cáceres, C. E. 1997. Dormancy in invertebrates. *Invertebrates Biology*, 116: 371-383.
- Davis, S. D., Heywood, V. H., Herrera-MacBryde, O., Villa-Lobos, J., and Hamilton, A. C. 1997. Centres of Plant Diversity. The Americas. WWF, IUCN, Oxford, U.K. v. 3.
- De Meester, L. 1993. Inbreeding and outbreeding depression in *Daphnia*. *Oecologia*, 96: 80-84.
- De Meester, L. 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. *Ecoscience*, 3: 385-399.

- De Meester, L., Gómez, 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.
- Derry, A. M., Hebert, P. D. N., and Prepas, E. E. 2003. Evolution of rotifers in saline and subsaline lakes: A molecular phylogenetic approach. *Limnology and Oceanography*, 48(2): 675-685.
- Dinerstein, E., Olson, D., Atchley, J., Loucks, C., Cotreras-Balderas, S., Abel, R., Inigo, E., Enkerlin, E., Williams, C., and Gastilleja, G. 2000. Ecoregion-based conservation in the Chihuahuan Desert: A biological Assessment. World Wildlife Fund, 1-128.
- Donner, J. 1965. Ordnung Bdelloidae (Rotatoria, Rädertiere). *Bestimmungsbücher Bodenfauna Europas* Berlin: Akademie-Verlag, 6: 1-297.
- Dumont, H. J. 1983. Biogeography of rotifers. *Hydrobiologia*, 104: 19-30.
- Dumont, H. J. and Segers, H. 1996. Estimating lacustrine zooplankton species richness and complementarity. *Hydrobiologia*, 341: 125-132.
- Excoffier, L., Laval, G., and Schneider, S., 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1:47-50.
- Excoffier, L., Smouse, P. E. and Quatro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Applications to human mitochondrial DNA restriction data. *Genetics*, 131: 479-491.

- Fenchel, T. and Finlay, B. J. 2004. The ubiquity of small species. Patterns of local and global diversity. *Bioscience*, 54: 777-784.
- Fenchel, T., Esteban, G. F. and Finlay, B. J. 1997. Local versus global diversity of microorganisms: Cryptic diversity of ciliated protozoa. *Oikos*, 80: 220-225.
- Finlay, B. J., 2002. Global dispersal of free-living microbial eukaryotic species. *Science*, 296: 1061-1063.
- Finston, T. L., Johnson, M. S., Humphreys, W. F., Eberhard, S. M., and Halse, S. A. 2007. Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Molecular Ecology*, 16: 355-365.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers from application of mitochondrial cytochrome c oxidase subunit1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294-299.
- Fontaneto D., Melone, G., and Ricci, C. 2005. Connectivity and nestedness of the meta-community structure of moss dwelling rotifers along a stream. *Hydrobiologia*, 542: 131-136.
- Fontaneto, D. and Melone, G. 2003. On some rotifers new for the Italian fauna. *Italian Journal of Zoology*, 70: 253-259.
- 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. *Molecular Ecology*, 17: 3136-3146.
- Fontaneto, D., Boschetti, C., and Ricci, C. 2008b. Cryptic diversification in ancient asexuals: Evidence from the bdelloid rotifer *Philodina flaviceps*. *Evolutionary Biology*, 21(2): 580-587.

- Fontaneto, D., Ficetola, G. F., Ambrosinic, R., and Ricci, C. 2006. Patterns of diversity in microscopic animal: Are they comparable to those in protists or in large animals? *Global Ecology and Biogeography*, 15: 153-162.
- Fontaneto, D., Herniou, E. A., Barracough, T. G., and Ricci, C. 2007a. On the global distribution of microscopic animals: New worldwide data on bdelloid rotifers. *Zoological Studies*, 46(3): 336-346.
- Fontaneto, D., Herniou, E. A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C., and Barracough, T. G. 2007b. Independently evolving species in asexual bdelloid rotifers. *PLoS Biology*, 5: 914-921.
- Fontaneto, D., Kaya, M., Barracough, T. G. and Herniou, E. A. 2009. Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, 35: 182-189.
- Fontaneto, D., Segers, H., and Melone, G. 2004. Epizoic rotifers (Rotifera: Monogononta, Bdelloidea) from the gill chambers of *Potamon fluviatile* (Herbst, 1785). *Journal of Natural History*, 38: 1225-1232.
- Gilbert, J. J. and Walsh, E. J. 2005. *Brachionus calyciflorus* is a species complex: Mating behaviour and genetic differentiation among four geographically isolated strains. *Hydrobiologia*, 546: 257-265.
- Goetze, E. 2003. Cryptic speciation on the high seas; Global phylogenetics of the copepod family Eucalanidae. *Proceeding of the Royal Society B.*, 270: 2321-2331.
- Gómez, A., Serra, M., Carvalho, G. R. and Lunt, D. H. 2002. Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, 56: 1431-1444.

- Jennings, H. S. 1994. The Rotatoria of the Great Lakes and some of the inland lakes of Michigan. Bulletin of the Michigan Fish Commission, No.3.
- Julio Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19: 2469-3467.
- Kaya, M., Herniou, E. A., Barraclough, T. G., and Fontaneto, D. 2009. Inconsistent estimates of diversity between traditional and DNA taxonomy in bdelloid rotifers. *Organisms Diversity and Evolution*, 9: 3-12.
- Kellogg, C. A. and Griffin, D. W. 2006. Aerobiology and the global transport of desert dust. *Trends in Ecology and Evolution*, 21: 638-644.
- Koste, W. and Shiel, R. J. 1986. Rotifera from Australian inland waters. I. Bdelloidea (Rotifera: Digononta). *Australian Journal of Marine Freshwater Research*, 37: 765-792.
- Lee, C. E. 2000. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate 'populations'. *Evolution*, 54: 2014-2027.
- Lee, C. E. and Frost, B. W. 2002. Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). *Hydrobiologia*, 480: 111-128.
- Librado, P. and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452 | doi: 10.1093/bioinformatics/btp187.
- Lorenzen, S. 1985. Phylogenetic aspects of pseudocoelomate evolution. In: Gonway, M. S., George, J. D., Gibson, R., Platt, H. M. (eds.), *The origins and relationships of lower invertebrates*. Oxford University Press, Oxford, pp. 210-223.
- Mark Welch, D. and Meselson, M. 2000. Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science*, 288: 1211-1215.

- Mark Welch, D. B., Cummings, M. P., Hillis, D. M., and Meselson, M. 2004. Divergent gene copies in the asexual class Bdelloidea (Rotifera) separated before the bdelloid radiation or within bdelloid families. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 1622-1625.
- Mark Welch, J. L., Mark Welch D. B., and Meselson, M. 2004. Cytogenetic evidence for sexual evolution of bdelloid rotifers. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 1618-1621.
- Melone, G., Ricci, C., Segers, H., and Wallace, R. L. 1998. Phylogenetic relationships of Phylum Rotifera with emphasis on the families of Bdelloidea. *Hydrobiologia*, 387/388: 101-107.
- Min, G. S. and Park J. K. 2009. Eurotatorian paraphyly: Revising phylogenetic relationships based on the complete mitochondrial genome sequence of *Rotaria rotatoria* (Bdelloidea: Rotifera: Syndermata). *BioMed Central Genomics*. doi:10.1186/1471-2164-10-533.
- Nylander, J. A. A. 2004. MrModeltest 2.3. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Okamura, B. and Freeland, J. R. 2002. Gene flow and the evolutionary ecology of passively dispersing aquatic invertebrates. In: Bullock, J. M., Kenward, R. E. and Halis, R. S. (eds.), *Dispersal Ecology*. Blackwell Science, Oxford, pp. 194-216.
- Olson, D. M. and Dinerstein, E. 1998. The Global 200: A representation approach to conserving the Earth's most biologically valuable ecoregions. *Conservation Biology*, 12(3): 502-515.
- Örstan, A. 1995. A new species of bdelloid rotifer from Sonora, México. *The Southwestern Naturalist*, 40(3): 255-258.
- Ortells, R., Gómez, A., and Serra, M. 2003. Coexistence of cryptic rotifer species: Ecological and genetic characterization of *Brachionus plicatilis*. *Freshwater Biology*, 48: 2194-2202.

- Petersen R. L., Hanley, L., Walsh, E. J., Hunt, H., and Duffield, R. M. 1997. Occurrence of the rotifer, *Habrotrocha* cf. *rosa* donner, in the purple pitcher plant, *Sarracenia purpurea* L., (Sarraceniaceae) along the eastern seaboard of North America. *Hydrobiologia*, 354: 63-66.
- Poinar, G. O. and Ricci, C. 1992. Bdelloid rotifers in Dominican amber: Evidence for parthenogenetic continuity. *Experientia*, 48: 408-410.
- Posada, D., and Crandall, K. A. 1998 Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14 (9): 817-818.
- Presson, A. 2002. Proliferation of cryptic protists and germination of resting stages from untreated sediment samples with emphasis on dinoflagellates. *Ophelia*, 55: 152-166.
- Quispel, A. 1998. Lourens G. Becking (1895-1963), inspirator for many (micro) biologists. *International Microbiology*, 1: 69-72.
- Ricci, C. 1987. Ecology of bdelloids: How to be successful. *Hydrobiologia*, 147: 117-127.
- Ricci, C. 1998a. Are lemnisci and proboscis present in the Bdelloidea? *Hydrobiologia*, 387/388: 93-96.
- Ricci, C. 1998b. Anhydrobiotic capabilities of bdelloid rotifers. *Hydrobiologia*, 387/388: 321-326.
- Ricci, C. and Melone, G., 2000. Key to the identification of the genera of bdelloid rotifers. *Hydrobiologia*, 418: 73-80.
- Ricci, C., 1984. Culturing of some bdelloid rotifers. *Hydrobiologia*, 112: 45-51.
- Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572-1574.

- Ruttner-Kolisko, A. 1974. Plankton rotifers. In: Elster, H. J. and Ohle, W. (eds.), Die Binnengewasser. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 26(1): 1-146.
- Schmidt, R. H., Jr. 1979. A climatic delineation of the "real" Chihuahuan Desert. *Journal of Arid Environments* 2:243-250
- Schröder, T. and Walsh, E. 2007. Cryptic speciation in the cosmopolitan *Epiphanes senta* complex (Monogononta, Rotifera) with the description of new species. *Hydrobiologia*, 593: 129-140.
- Segers, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera) with notes on nomenclature, taxonomy and distribution. *Zootaxa*, 1546: 1-104.
- Shepard, W. D. 1993. Desert springs – both rare and endangered. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 3: 351-359.
- Shiel, R. T. and Green, J. D. 1996. Rotifera recorded from New Zealand, 1859-1995, with comments on zoogeography. *New Zealand Journal of Zoology*, 23: 193-209.
- Stemberger, R. S. 1981. A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences*, 38(6): 721-724.
- 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. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4 beta 10. Sinauer Associates, Sunderland, Massachusetts.
- Tasevska, O., Kostoski, G., and Guseska, D. 2004. Composition and dynamic of Rotifera fauna from eastern littoral zone of Lake Ohrid as parameter of water quality. *BALWOIS*, 25-29.

- ter Braak, C. J. F. And Smilauer, P. 2002. CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5) Ithaca: Microcomputer Power.
- Thomas, J. A., Welch, J. J., Wolfit, M., and Bromham, L. 2006. There is no universal molecular clock for invertebrates but rate variation does not scale with body size. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 7366-7371.
- Thompson, J. D., Higgings, D.G., and Gibson, T. J. 1994. ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22: 4676-4680.
- Toledo, V. M. 1988. La diversidad biológica de México. *Ciencia y Desarrollo XIV.*, 81: 17-30.
- Wallace, R. L. and Smith, H. A. 2009. Rotifera. In: Likens, G. E. (ed.), *Encyclopedia of Inland Waters*. Elsevier, Oxford, 3: 689-703.
- Wallace, R. L. and Snell, T. W. 2010. Rotifera. In Thorp J. H. and Covich, A. P. (eds.), *Ecology and classification of North American freshwater invertebrates*, 3rd ed., Elsevier, Oxford, pp. 173-235.
- 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, U.S.A.). *Hydrobiologia*, 546: 147-157.
- Wallace, R. L., Walsh, E. J., Schröder, T., Ricco-Martinez, and R., Ríos-Arana, J. V. 2008. Species composition and distribution of rotifers in Chihuahuan Desert waters of México: is everything everywhere? *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 30: 73-76.

- Walsh, E. J., Schröder, T., and Wallace, R. L. 2009. Cryptic speciation in *Lecane bulla* (Monogononta: Rotifera) in Chihuahuan Desert waters. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 30: 1046-1050.
- Walsh, E. J., Schröder, T., Wallace, R. L., Ríos-Arana J. V., and Rico-Martinez, R. 2008. Rotifers from selected inland saline waters in the Chihuahuan Desert of México. *Saline Systems*. doi 10.1186/1746-1448-4-7.
- Walsh, E., Schröder, T., Arroyo, M. L. and Wallace, R. L. 2007. How do single samples reflect rotifer species diversity? A test based on interannual variation of rotifers communities in Big Bend National Park (Texas, USA). *Hydrobiologia*, 593: 39-47.
- Yakovenko, N. S. 2000a. New for the fauna of Ukraine rotifers (Rotifera, Bdelloidea) of Adinetidae and Habrotrochidae families. *Vestnik Zoologii*, 34: 11-19.
- Yakovenko, N. S. 2000b. New for the fauna of Ukraine rotifers (Rotifera, Bdelloidea) of Philodinidae family. *Vestnik Zoologii*, 14: 26-32.
- Zullini, A. and Ricci, C. 1980. Bdelloid rotifers and nematodes in a small Italian stream. *Freshwater Biology*, 10: 67-72.

Website:

CDRI “Chihuahuan Desert Research Institution”. July 1. 2009 <<http://www.cdri.org>>.

Table 2.1: Localities of *P. megalotrocha* from the Chihuahuan Desert and outgroups that were included in the ecological analyses. BBNP refers to Big Bend National Park.

Site name	Locality and county	Frequency of <i>P. megalotrocha</i>	%	Habitat	GPS coordinates
<i>P. megalotrocha</i> - Chihuahuan Sites					
Burro Spring pool A	BBNP, Brewster Co., USA	1/1	100	Spring	29.2373000 N, -103.4259000 W
Cattail Falls Spring Pool A	BBNP, Brewster Co., USA	11/11	100	Spring	29.2731805 N, -103.3355138 W
Cattail Falls Spring Pool B	BBNP, Brewster Co., USA	10/11	83	Spring	29.2731833 N, -103.3355500 W
Cattail Falls Spring Pool C	BBNP, Brewster Co., USA	4/11	36	Spring	29.2731833 N, -103.3355861 W
Cattail Falls Spring Pool C'	BBNP, Brewster Co., USA	3/8	38	Spring	29.2731833 N, -103.3355861 W
Cattail Falls Spring Pool C''	BBNP, Brewster Co., USA	5/7	71	Spring	29.2731833 N, -103.3355861 W
Cattail Falls Spring Pool C-D	BBNP, Brewster Co., USA	4/9	44	Spring	29.2731555 N, -103.3357336 W
Cattail Falls Spring Pool D	BBNP, Brewster Co., USA	5/11	46	Spring	29.2731527 N, -103.3358277 W
Cattail Falls Spring Pool E	BBNP, Brewster Co., USA	4/10	46	Spring	29.2731444 N, -103.3359666 W
Cattail Falls Spring Pool F	BBNP, Brewster Co., USA	4/11	36	Spring	29.2731333 N, -103.3360833 W
Cattail Falls Spring Pool G	BBNP, Brewster Co., USA	3/11	27	Spring	29.2731666 N, -103.3361638 W
Cattail Falls Spring Pool H	BBNP, Brewster Co., USA	8/11	73	Spring	29.2731694 N, -103.3362388 W
Croton Stream (stream ephemeral)	BBNP, Brewster Co., USA	1/1	100	Stream	29.3437833 N, -103.3465000 W

Dripping Spring Upper	BBNP, Brewster Co., USA	1/1	100	Spring	29.4049666 N, -103.3078583 W
Dripping Spring	BBNP, Brewster Co., USA	1/3	33	Spring	29.4049666 N, -103.3078583 W
Ernst Tinaja 01	BBNP, Brewster Co., USA	1/7	14	Hueco	29.2568666 N, -103.0100833 W
Ernst Tinaja 02	BBNP, Brewster Co., USA	1/6	17	Hueco	29.2567416 N, -103.0103583 W
Ernst Tinaja Hueco	BBNP, Brewster Co., USA	1/1	100	Hueco	29.2551000 N, -103.0148833 W
Glenn Spring 02	BBNP, Brewster Co., USA	1/6	17	Spring	29.1744166 N, -103.1575000 W
Glenn Spring 03	BBNP, Brewster Co., USA	2/6	40	Spring	29.1744166 N, -103.1575000 W
Glenn Spring 04	BBNP, Brewster Co., USA	3/6	33	Spring	29.1744166 N, -103.1575000 W
Glenn Spring 06	BBNP, Brewster Co., USA	2/4	50	Spring	29.1744166 N, -103.1575000 W
Glenn Spring 07	BBNP, Brewster Co., USA	1/2	50	Spring	29.1744166 N, -103.1575000 W
Glenn Spring Stream South	BBNP, Brewster Co., USA	1/1	100	Spring	29.1744166 N, -103.1575000 W
Government Spring	BBNP, Brewster Co., USA	1/9	11	Spring	29.3405666 N, -103.2560833 W
Mule Ears Spring (Lower)	BBNP, Brewster Co., USA	1/2	50	Spring	29.1623500 N, -103.4082833 W
Rio Grande River	BBNP, Brewster Co., USA	2/5	40	River	29.1855500 N, -102.9729666 W
Rio Grande River at Rio Grande Village	BBNP, Brewster Co., USA	1/1	100	Stream	29.1855500 N, -102.9729666 W
Rio Grande Village Canal	BBNP, Brewster Co., USA	1/3	33	River	29.1861500 N, -102.9722500 W
Rio Grande Village Cattail	BBNP, Brewster	4/7	57	Pond	29.1890000 N, -102.9716166 W

Pond	Co., USA				
Rio Grande Village Lower pond	BBNP, Brewster Co., USA	8/12	67	Pond	29.1785166 N, -102.9537500 W
Rio Grande Village Upper pond	BBNP, Brewster Co., USA	4/12	33	Pond	29.1855500 N, -102.9729666 W
Window Trail Pool A	BBNP, Brewster Co., USA	1/5	20	Tinaja	29.2800300 N, -103.3299472 W
Window Trail Pool B	BBNP, Brewster Co., USA	3/4	75	Tinaja	29.2800300 N, -103.3299472 W
Window Trail Pool C	BBNP, Brewster Co., USA	1/3	33	Tinaja	29.2800900 N, -103.3301800 W
Window Trail Pool D	BBNP, Brewster Co., USA	1/2	50	Tinaja	29.2802000 N, -103.3303800 W
Window Trail Pool E	BBNP, Brewster Co., USA	3/6	50	Tinaja	29.2802500 N, -103.3304300 W
Window Trail Pool F	BBNP, Brewster Co., USA	3/6	50	Tinaja	29.2803100 N, -103.3305000 W
Window Trail Pool G	BBNP, Brewster Co., USA	1/6	17	Tinaja	29.2803500 N, -103.3305388 W
Window Trail Pool H	BBNP, Brewster Co., USA	3/5	60	Tinaja	29.2804138 N, -103.3305388 W
Window Trail Pool I	BBNP, Brewster Co., USA	1/3	33	Tinaja	29.2804611 N, -103.3305388 W
Croton Spring	BBNP, Brewster Co., USA	2/7	29	Spring	29.3446166 N, -103.3471166 W
Ascarate Lake	El Paso Co., TX, USA	3/3	100	Lake	31.7501777 N, -106.4047527 W
Rio Grande Borderland	El Paso Co., TX, USA	1/1	100	River	31.8859527 N, -106.5988777 W
Fuentes Shafter	Presidio Co., TX, USA	1/1	100	Pond	29.8143166 N, -104.3071333 W
Stream Shafter	Presidio Co., TX, USA	1/1	100	Stream	29.8143166 N, -104.3071333 W

Balmorhea Wetland	Reeves Co., TX, USA	3/7	43	Spring	30.9449166 N, -103.7843500 W
Balmorhea Canal	Reeves Co., TX, USA	2/6	50	Canal	30.9444472 N, -103.7851583 W
Dripping Spring	Doña Ana Co., NM, USA	1/1	100	Spring	32.3231888 N, -106.5725138 W
Manzanita Spring	Guadalupe National Monument, NM, USA	2/3	67	Spring	31.9103194 N, -104.7985500 W
Rattlesnake Springs	Carlsbad Caverns National Monument, NM, USA	2/2	100	Spring	32.1097000 N, -104.4716250 W
Sitting Bull Falls	Eddy Co., NM, USA	1/1	100	Spring	32.2434916 N, -104.6962916 W
Sitting Bull Falls Pool 1	Eddy Co., NM, USA	1/1	100	Spring	32.2390333 N, -104.7025333 W
Tamosposa Upper Pool 1	San Luis Potosí, MX	1/1	100	Pond	21.9395400 N, -99.3967900 W
Presa Alvaro Obregon outflow pond	San Luis Potosí, MX	1/1	100	Stream	22.1853055 N, -99.6333611 W
Presa Alvaro Obregon	San Luis Potosí, MX	2/2	100	Lake	22.1844444 N, -99.6302777 W
Media Luna Laguna	San Luis Potosí, MX	1/3	33	Spring	21.8608611 N, -100.0282500 W
Media Luna Canal de Riego	San Luis Potosí, MX	1/1	100	Canal	21.8891388 N, -100.0373888 W
Manantial San Sebastian	San Luis Potosí, MX	1/3	33	Spring	21.7747777 N, -99.8327222 W
Presa Alvaro Obregon outflow channel	San Luis Potosí, MX	1/1	100	Canal	22.1886111 N, -99.6401111 W
Rio Mesquites	Coahuila, MX	2/3	67	River	26.9222222 N, -102.1083333 W
Poza Tortugas	Coahuila, MX	2/3	67	Spring	26.9314500 N, -102.1247000 W

Poza Churince	Coahuila, MX	3/3	100	Spring	26.8404166 N, -102.1342333 W
Poza Marcelo	Coahuila, MX	1/2	50	Spring	26.9104000 N, -102.0363166 W
San Jose del Anteojo	Coahuila, MX	2/2	100	Spring	26.9693166 N, -102.1208166 W
Ojo de en Medio	Chihuahua, MX	4/8	50	Spring	31.3788500 N, -106.5877833 W
Ojo de la Punta	Chihuahua, MX	9/11	82	Spring	31.3859166 N, -106.6022666 W
Presa Chihuahua	Chihuahua, MX	1/3	33	Lake	28.5762166 N, -106.1711833 W
Lago Colina	Chihuahua, MX	1/4	25	Lake	27.5724000 N, -105.4004666 W
Presa de la Boquilla	Chihuahua, MX	1/4	25	Lake	27.5361333 N, -105.4011333 W
Presa Francisco Ignacio Madero	Chihuahua, MX	1/1	100	Lake	28.1626166 N, -105.6321833 W
Ojo de Santa Maria A	Chihuahua, MX	2/2	100	Spring	31.1552777 N, -107.3172222 W
Ojo de Santa Maria B	Chihuahua, MX	2/2	100	Spring	31.1550000 N, -107.3172222 W
Ojo de Santa Maria C	Chihuahua, MX	1/2	50	Spring	31.1550000 N, -107.3172222 W
<i>P. megalotrocha</i> - Outgroup Sites					
San Felipe Springs	Valverde Co., TX, USA	1/1	100	Spring	29.3698500 N, -100.8838166 W
Comal Springs	Comal Co., TX, USA	1/1	100	Spring	29.7132333 N, -98.1373333 W
Cibolo Creek	Kendall Co., TX, USA	1/1	100	Stream	29.7893666 N, -98.7240500 W
Bear Creek	Kendall Co., TX, USA	1/1	100	Spring	30.1235388 N, -98.9112222 W
Lake Jennings	San Diego Co., CA, USA	1/1	100	Lake	32.8575550 N, -116.8874638 W
Willow Lake	Yavapai Co., AZ, USA	1/1	100	Lake	34.6086000 N, -112.4393000 W
La Paz County Park	La Paz Co., AZ, USA	1/1	100	Lagoon	34.2227166 N, -114.1971333 W
Cam River	Cambridge, UK	na	na	River	52.1977777 N, 0.1161111 E

Table 2.2: Summary of RDA statistics for rotifer species and environmental factors in the Chihuahuan Desert.

Axes	1	2	3	4	Total Variance
Eigenvalues	0.041	0.014	0.011	0.009	1.000
Species-environment correlations:	0.564	0.655	0.556	0.591	
Cumulative percentage variance					
of species data:	4.1	5.5	6.6	7.6	
of species-environment relation:	30.8	41.1	49.7	56.5	
Sum of all eigenvalues:					1.000
Sum of all canonical eigenvalues:					0.134
All four eigenvalues reported above are canonical and correspond to axes that are constrained by the environmental variables					

Table 3.1: Populations of *P. megalotrocha* from the Chihuahuan Desert and outgroups that were included in the genetic study. BBNP refers to Big Bend National Park.

Population/Species name	Abbreviation	Locality	Date of collection	GPS coordinates
<i>P. megalotrocha</i> - Chihuahuan Populations:				
Cattail Falls Spring Pool A	P.meg.CattailA	BBNP, Brewster Co., USA	01.20.2008	29.2731805 N, -103.3355138 W
Cattail Falls Spring Pool E	P.meg.CattailE	BBNP, Brewster Co., USA	05.18.2006	29.2731444 N, -103.3359666 W
Cattail Falls Spring Pool H	P.meg.CattailH	BBNP, Brewster Co., USA	01.20.2008	29.2731694 N, -103.3362388 W
Croton Spring isolate-1	P.meg.CrotonSpr1	BBNP, Brewster Co., USA	12.17.2006	29.3446166 N, -103.3471166 W
Croton Spring isolate-2	P.meg.CrotonSpr2	BBNP, Brewster Co., USA	01.19.2008	29.3446166 N, -103.3471166 W
Rio Grande River (Rio Grande Village)	P.meg.RioGrandeRGV	BBNP, Brewster Co., USA	01.19.2008	29.1855500 N, -102.9729666 W
Rio Grande Borderland	P.meg.RioGrandeBorderland	El Paso Co., TX, USA	09.25.2008	31.8859527 N, -106.5988777 W
Ascarate Lake	P.meg.AscarateLake	El Paso Co., TX, USA	05.20.2008	31.7501777 N, -106.4047527 W
Balmorhea Canal	P.meg.BalmorheaCanal	Reeves Co., TX, USA	01.21.2008	30.9444472 N, -103.7851583 W
Manzanita Spring	P.meg.ManzanitaSpr	Guadalupe Mountains, NM, USA	06.05.2008	31.9103194 N, -104.7985500 W
Rattlesnake Springs	P.meg.RattlesnakeSpr	Guadalupe Mountains, NM, USA	06.05.2008	32.1097000 N, -104.4716250 W
Dripping Spring	P.meg.DrippingSpr	Doña Ana Co., NM, USA	08.12.2006	32.3231888 N, -106.5725138 W
Ojo de la Punta isolate-1	P.meg.OjoDeLaPunta1	Chihuahua, MX	09.01.2007	31.3859166 N, -106.6022666 W
Ojo de la Punta isolate-2	P.meg.OjoDeLaPunta2	Chihuahua, MX	11.29.2007	31.3859166 N, -106.6022666 W
Ojo de Santa Maria B	P.meg.SantaMariaB	Chihuahua, MX	06.11.2009	31.1550000 N, -107.3172222 W
Ojo de en Medio	P.meg.OjoDeEnMedio	Chihuahua, MX	09.01.2007	31.3788500 N, -106.5877833 W
Poza Tortugas	P.meg.PozaTortugas	Coahuila, MX	01.08.2006	26.9314500 N, -102.1247000 W
Media Luna Canal	P.meg.MediaLunaCanal	San Luis Potosí, MX	02.17.2008	21.8891388 N, -100.0373888 W
Manantial San Sebastian	P.meg.SanSebastian	San Luis Potosí, MX	02.17.2008	21.7747777 N, -99.8327222 W
<i>Outgroups:</i>				
<i>P. megalotrocha</i> , San Felipe Springs	P.meg.SanFelipeSpr	Valverde Co., TX, USA	07.08.2008	29.3698500 N, -100.8838166 W
<i>P. megalotrocha</i> , Comal Springs	P.meg.ComalSpr	Comal Co., TX, USA	03.29.2008	29.7132333 N, -98.1373333 W
<i>P. megalotrocha</i> , Cibolo Creek	P.meg.CiboloCreek	Kendall Co., TX, USA	03.29.2008	29.7893666 N, -98.7240500 W

<i>P. megalotrocha</i> , Bear Creek	P.meg.BearCreek	Kendall Co., TX, USA	07.04.2008	30.1235388 N, -98.9112222 W
<i>P. megalotrocha</i> , Lake Jennings	P.meg.LakeJennings	San Diego Co., CA, USA	06.07.2008	32.8575550 N, -116.8874638 W
<i>P. megalotrocha</i> , Willow Lake	P.meg.WillowLake	Yavapai Co., AZ, USA	08.07.2008	34.6086000 N, -112.4393000 W
<i>P. megalotrocha</i> , La Paz County Park	P.meg.LaPazCoPark	La Paz Co., AZ, USA	05.30.2008	34.2227166 N, -114.1971333 W
<i>P. megalotrocha</i> -1, Cam River	P.meg.1.UK	Cambridge, UK	05.06.2007	52.1977777 N, 0.1161111 E
<i>P. megalotrocha</i> -2, Cam River	P.meg.2.UK	Cambridge, UK	05.06.2007	52.1977777 N, 0.1161111 E
<i>Philodina</i> sp.1	Philodina.spa.2.MX	San Luis Potosí, MX	-	-
<i>Philodina</i> sp. CWB-2005 (GENBANK accession number: DQ078621)	Philodina.sp.1.US	Pima Co., AZ, USA	-	-
<i>P. acuticornis</i> (EU751195)	P. acut.TU.a	Turkey	-	-
<i>P. acuticornis</i> (EU751198)	P. acut.TU.b	Turkey	-	-
<i>P. citrine</i> (EF650599)	P. citr.IT.2.1	Italy	-	-
<i>P. citrine</i> (EF650601)	P. citr.IT.2.2	Italy	-	-
<i>P. citrine</i> (EF650602)	P. citr.IT.1.3	Italy	-	-
<i>P. flaviceps</i> (DQ890120)	P. flav.dv2e	Italy	-	-
<i>P. flaviceps</i> (DQ890071)	P. flav.Ro2h	Italy	-	-
<i>P. flaviceps</i> (DQ890090)	P. flav.Ro3a	Italy	-	-
<i>P. flaviceps</i> (DQ890156)	P. flav.Va3e	Italy	-	-

Table 3.2 Correlation between genetic and geographic distances (Isolation by Distance) in *P. megalotrocha* populations.

Correlation	z	r	p
Genetic distance and geographic distance	90031	0.12	0.145
Genetic distance and Log (geographic distance)	137	0.18	0.007
Log (genetic distance) and geographic distance	-562658	0.12	0.019
Log (genetic distance) and Log (geographic distance)	-929	0.23	0.002

Table 3.3a: Grouping of *P. megalotrocha* populations used in AMOVA analysis, based on geographic locality.

Groups	Number of sequences
Group 1, TX- Chihuahuan Desert:	
Cattail Falls Spring Pool A	1
Cattail Falls Spring Pool E	1
Cattail Falls Spring Pool H	1
Croton Spring	2
Rio Grande River (Rio Grande Village, BBNP)	1
Rio Grande Borderland	1
Ascarate Lake	1
Balmorhea Canal	1
Group 2, NM	
Manzanita Spring	1
Rattlesnake Springs	1
Dripping Spring	1
Group 3, MX	
Ojo de la Punta	2
Ojo de Santa Maria B	1
Ojo de en Medio	1
Poza Tortugas	1
Media Luna Canal	1
Manantial San Sebastian	1
Group 4: TX-Non-Chihuahuan Desert	
San Felipe Springs	1
Comal Springs	1
Cibolo Creek	1
Bear Creek	1
Group 5, AZ:	
Willow Lake	1
La Paz County Park	1
Group 6, CA:	
Lake Jennings	1
Group 7, UK:	
Cam River	2

Table 3.3b: Grouping of *P. megalotrocha* populations used in AMOVA analysis, based on phylogenetic clades derived by Bayesian analysis, (see Fig. 3.5). Abbreviations are given in Table 3.1.

Groups	Number of sequences
Group 1:	
P.meg.CattailA	1
P.meg.CattailH	1
P.meg.CrotonSpr1	1
P.meg.OjoDeEnMedio	1
P.meg.DrippingSpr	1
Group 2:	
P.meg.RioGrandeRGV	1
P.meg.RioGrandeBorderland	1
P.meg.AscarateLake	1
P.meg.SantaMariaB	1
P.meg.OjoDeLaPunta2	1
P.meg.LakeJennings	1
P.meg.WillowLake	1
P.meg.LaPazCoPark	1
P.meg.ManzanitaSpr	1
Group 3:	
P.meg.OjoDeLaPunta1	1
P.meg.BearCreek	1
P.meg.MediaLunaCanal	1
P.meg.RattlesnakeSpr	1
Group 4:	
P.meg.SanSebastian	1
P.meg.CiboloCreek	1
P.meg.BalmorheaCanal	1
Group 5:	
P.meg.ComalSpr	1
Group 6:	
P.meg.PozaTortugas	1
Group 7:	
UK (P.meg.1.UK and P.meg.2.UK)	2
P.meg.SanFelipeSpr	1
P.meg.CrotonSpr2	1
P.meg.CattailE	1

Table 3.4a: Analysis of Molecular Variance of *P. megalotrocha* populations from sites included in the genetic study, based on geographic locality. Significance tests were based on 10100 permutations. df, degrees of freedom.

Source of variation	df	Variance components	Percentage of variation	P-value
Among groups	6	3.1	8.05	0.05
Among populations within groups	18	8.6	21.98	0.53
Within populations	3	27.3	69.97	0.08
Total	27	39.1		

Table 3.4b: Analysis of Molecular Variance of *P. megalotrocha* populations from sites included in the genetic study, based on phylogenetic clades derived from Bayesian analyses. Significance tests were based on 10100 permutations. df, degrees of freedom.

Source of variation	df	Variance components	Percentage of variation	P-value
Among clades	6	23.1	54.37	0.00
Between clades within groups	20	19.4	45.63	0.20
Within populations	1	0.0	0.00	0.01
Total	27	42.5		

Table 4.1: Occurrence of *P. megalotrocha* in relation to some environmental variables in this study.

Environmental variable	Range	% of records
Temperature	1.6 - 34.7 °C	74% between 15-30 °C
pH	5.05 - 9.79	83% > 6.8
Conductivity	0.55 – 3478 $\mu\text{S cm}^{-3}$	-
DO	15.3 - 279.9%	-
TDS	0.066 - 2.261 g/L	63% \leq 0.5
ORP	-239.7 - 309.8	-
Elevation	288 - 1899 m	73% > 1000 m
Ammonia	0.0 - 1.0 mg/L $\text{NH}_4\text{-N}$	73% \leq 0.09 mg/L
Nitrite	0.0 - 0.044 mg/L $\text{NO}_2\text{-N}$	71% \leq 0.004 mg/L
Nitrate	0.0 - 2.53 mg/L $\text{NO}_3\text{-N}$	81% \leq 0.2 mg/L
Phosphate	0.01 - 0.99 mg/L $\text{PO}_4\text{-P}$	77% \leq 0.1 mg/L
Turbidity	0.0 – 270 FTU	94% \leq 50 FTU
Alkalinity	56 – 405 mg/L CaCO_3	73% (100 – 300) mg/L
Hardness	12.5 – 2650 mg/L CaCO_3	84% \leq 1000 mg/L
Silica	0.5 - 74.7 mg/L SiO_2	82% \leq 40 mg/L
Chloride	0.0 – 300 mg/L Cl^-	75% \leq 50 mg/L
Sulfate	0.0 – 1780 mg/L SO_4	89% \leq 1000 mg/L
Watercolor	0 – 130 mg/L Pt	82% \leq 40 mg/L Pt

Table 4.2: Summary of genetic diversification in some monogonont and bdelloid rotifer species based on partial *cox1* gene sequences.

Species	Sample Locations	Number of genetic clusters/ lineages	Genetic Divergence	Number of Haplotypes	Reference
A. Monogononts					
<i>Brachionus plicatilis</i>	48 salt and brackish ponds, lakes, and lagoons in the Iberian Peninsula and 6 non-Iberian locations	9 lineages within <i>B.plicatilis</i>	0.0-23% among all sequences 0-12% within clades 12-23% between clades	-	Gómez <i>et al.</i> , 2002
<i>Brachionus plicatilis</i>	25 localities from around the world: 13 countries in North America, Europe, Asia, Africa, Australia, and the Caribbean	14 lineages	>16% among populations	-	Suatoni <i>et al.</i> , 2006
Species from Brachionidae and Synchaetidae families: <i>Brachionus calyciflorus</i> <i>B. plicatilis</i> <i>B. urceolaris</i> <i>Keratella cochlearis</i> <i>K. hiemalis</i> <i>K. quadrata</i> <i>Synchaeta</i> sp. <i>S. c.f. pectinata</i>	from 9 lakes and 3 ponds in Alberta, Canada plus samples from Florida	-	- between species of <i>Brachionus</i> : 20-25% - between species of <i>Keratella</i> : 23-27% - between species of <i>Synchaeta</i> : 1-20%	<i>B. calyciflorus</i> : 1 out of 2 sequences <i>B. plicatilis</i> : 16 out of 22 <i>B. urceolaris</i> : 4 out of 7 <i>K. cochlearis</i> : 5 out of 9 <i>K. hiemalis</i> : 3 out of 7 <i>K. quadrata</i> : 7 out of 26 <i>Synchaeta</i> sp.: 3 out of 5 <i>S. c.f. pectinata</i> : 3 out of 7	Derry <i>et al.</i> , 2003
<i>Brachionus calyciflorus</i>	4 strains: 3 from USA (FL, GA, TX) and one from Australia	-	0.0-13%	-	Gilbert and Walsh, 2005
<i>Epiphanes senta</i>	Germany, USA (TX, HA)	-	10.9-12.5%	-	Schröder and Walsh, 2007
<i>Lecane bulla</i>	Chihuahuan Desert Waters in USA (TX, NM) and MX	3 lineages	0.9-16%	-	Walsh <i>et al.</i> , 2009
B. Bdelloids					
<i>Adineta vaga</i> <i>A. oculata</i> <i>Adineta</i> sp. <i>Habrotrocha</i> sp. <i>H. constricta</i> <i>Abrochtha</i> sp. <i>Macrotrachela quadricornifera</i> <i>Macrotrachela</i> sp.	Italy, USA (AZ, MA, TN, NM, TX, CO, WY, IL, and OH)	21 clades	≤ 2% within clade > 8% between clades	-	Birky <i>et al.</i> , 2005

<i>Rotaria</i> sp. <i>Philodina roseola</i> <i>Philodina</i> sp.					
9 species of <i>Rotaria</i> genus: <i>R. magnacalcarata</i> <i>R. socialis</i> , <i>R. rotatoria</i> <i>R. citrina</i> , <i>R. tardigrada</i> <i>R. sordida</i> , <i>R. neptunia</i> <i>R. macrura</i> , <i>R. neptunoida</i>	UK, Italy, Finland, Belgium, France, Denmark, Switzerland, Sweden, Australia, Tanzania, México, and USA	13 clusters within <i>Rotaria</i>	0.0-3.3% within clusters 4.1-23.1% between clusters	-	Fontanet <i>et al.</i> , 2007b
<i>Philodina flaviceps</i>	7 streams in Piedmont region north-wertern Italy	9 clusters	0.0-3.2% within cluster 4.5-18.8% between clusters 9.5-16.9% between <i>P. flaviceps</i> and other <i>Philodina</i> species (used as OG)	25 out of 86 sequences Haplotype diversity (Hd) for streams ranged from 0.429 to 1	Fontanet <i>et al.</i> , 2008a
6 species of <i>Adineta</i> genus: <i>A. gracilis</i> , <i>A. grandis</i> <i>A. oculata</i> , <i>A. ricciae</i> <i>A. steineri</i> , <i>A. vaga</i> 8 species of <i>Rotaria</i> genus: <i>R. magnacalcarata</i> <i>R. socialis</i> , <i>R. citrina</i> , <i>R. tardigrada</i> , <i>R. sordida</i> , <i>R. neptunia</i> , <i>R. macrura</i> <i>R. rotatoria</i>	Most samples from Italy and UK, some from other 7 countries in Europe, and 25 samples from Africa, Australia, Antarctica, New Zealand, North America and Southeast Asia	<i>Adineta</i> : 19 clusters <i>Rotaria</i> : 25 clusters	-	-	Fontaneto <i>et al.</i> , 2008a
22 species related to: <i>Adineta</i> <i>Macrotrachela</i> <i>Philodina</i> <i>Pleureta</i> <i>Rotaria</i> <i>Habrotrochidae</i>	Turkey and UK	57 independently evolving entities	-	117 haplotypes out of 264 sequences	Kaya <i>et al.</i> , 2009
8 species: <i>P. citrina</i> , <i>P. flaviceps</i> , <i>M. quadricornifera</i> , <i>R. magnacalcarata</i> , <i>R. rotatoria</i> , <i>R. socialis</i> , <i>R. sordida</i> , <i>P. Lineate</i>	All <i>cox1</i> sequences already published and available in GENBANK plus 210 new sequences	<i>P. citrina</i> : 8 clusters <i>P. flaviceps</i> : 9 <i>M. quadricornifera</i> : 21 <i>R. magnacalcarata</i> : 2 <i>R. rotatoria</i> : 34 <i>R. socialis</i> : 1 <i>R. sordida</i> : 17 <i>P. lineate</i> : 5	-	<i>P. citrina</i> : 18 haplotypes out of 39 sequences <i>P. flaviceps</i> : 41 out of 86 <i>M. quadricornifera</i> : 41 out of 80 <i>R. magnacalcarata</i> : 24 out of 54 <i>R. rotatoria</i> : 87 out of 175 <i>R. socialis</i> : 30 out of 56 <i>R. sordida</i> : 33 out of 50 <i>P. lineate</i> : 20 out of 48	Fontaneto <i>et al.</i> , 2009

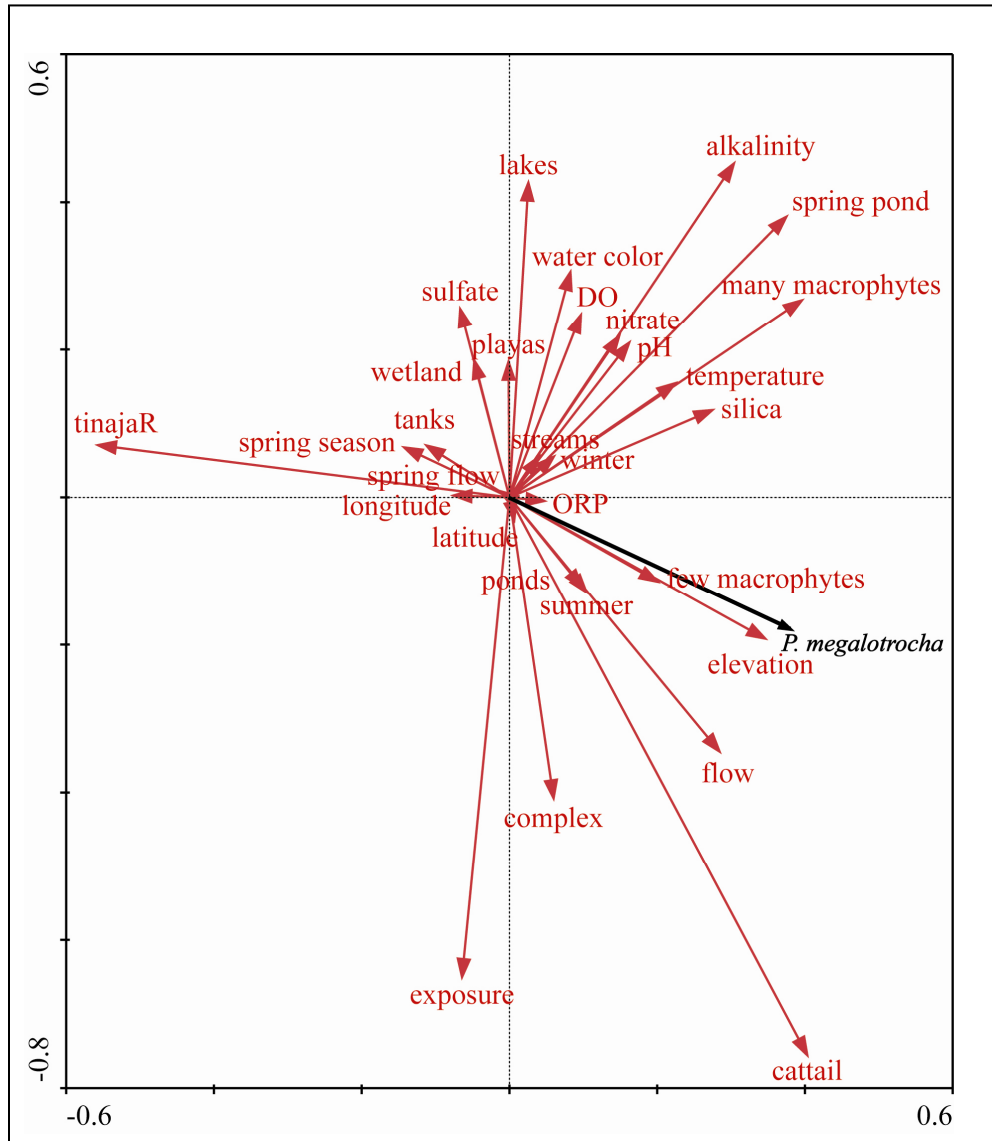


Fig. 2.1: RDA of rotifer species and 30 selected environmental factors. tinajaR refers to tinajas rarely filled with water, all factors are described in the Methods. All species vectors were removed from the figure except *P. megalotrocha*.

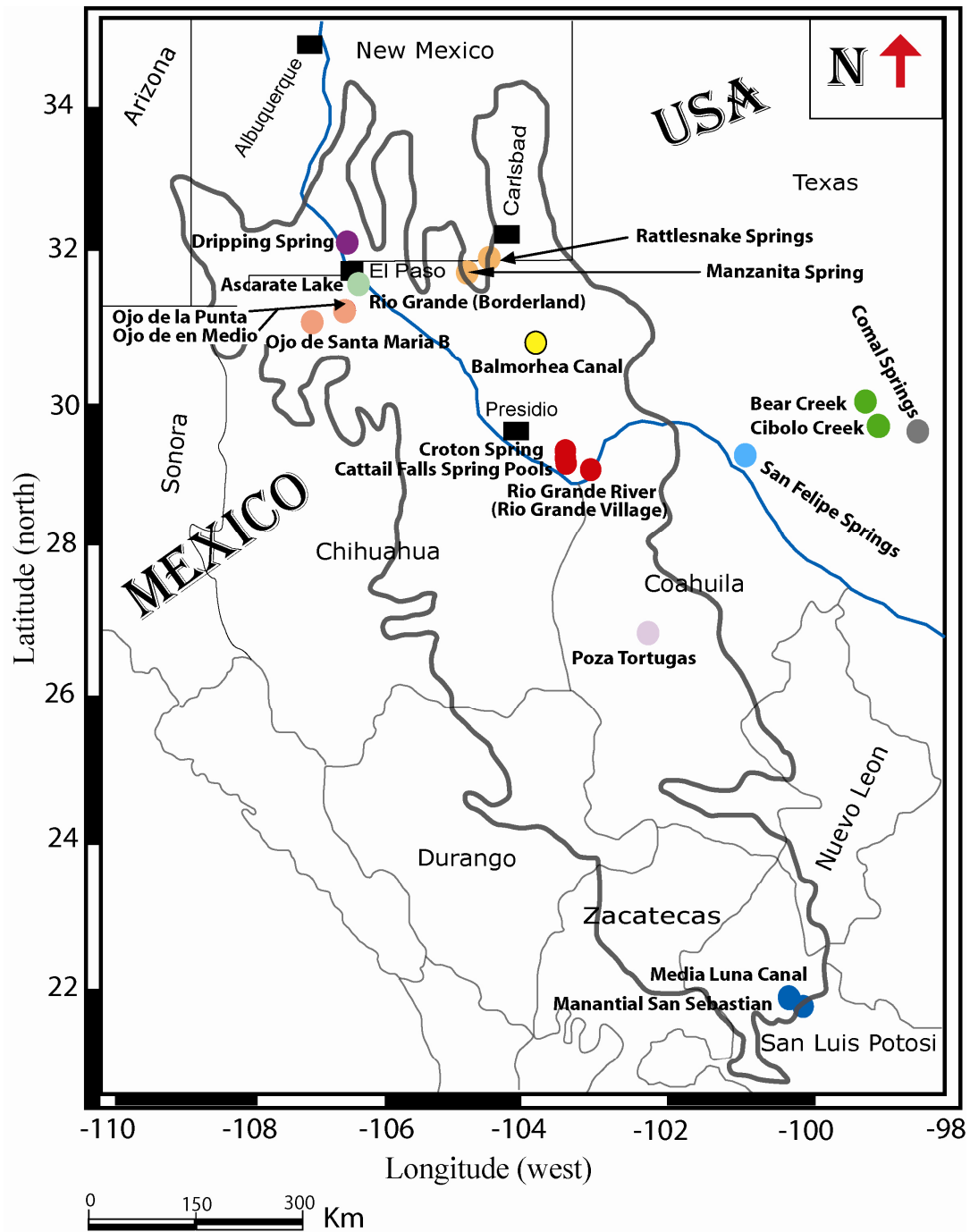














Fig.3.1: Site names and locations of *P. megalotrocha* populations sampled in the Chihuahuan Desert. The Map was modified after that displayed by the Chihuahuan Desert Research Institution (www.cdri.org) and Schmidt (1979). (Legend on next page)

Legend:

- | | | | |
|---|---|---|--|
|  | BBNP, Brewster Co., USA |  | Doña Ana Co., NM, USA |
|  | El Paso Co., TX, USA |  | San Luis Potosí, MX |
|  | Reeves Co., TX, USA |  | Chihuahua, MX |
|  | Valverde Co., TX, USA |  | Coahuila, MX |
|  | Comal Co., TX, USA |  | Cities |
|  | Kendall Co., TX, USA | | Blue line: Rio Grande |
|  | Guadalupe National Monument/Carlsbad National Monument, NM, USA | | Solid black line: Chihuahuan Desert boundaries |

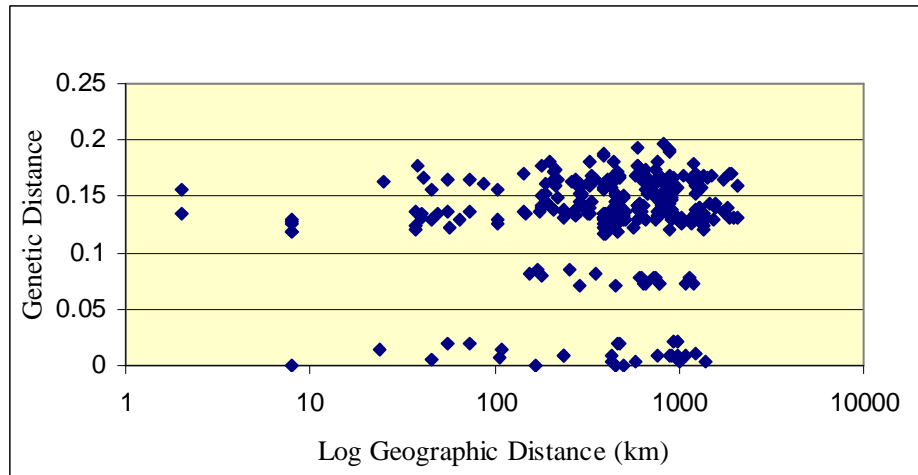


Fig. 3.2: A scatter plot showing the correlation between genetic distance and Log-transformed geographic distance between *P. megalotrocha* populations ($r = 0.178$; $p \leq 0.007$, from 1000 randomizations).

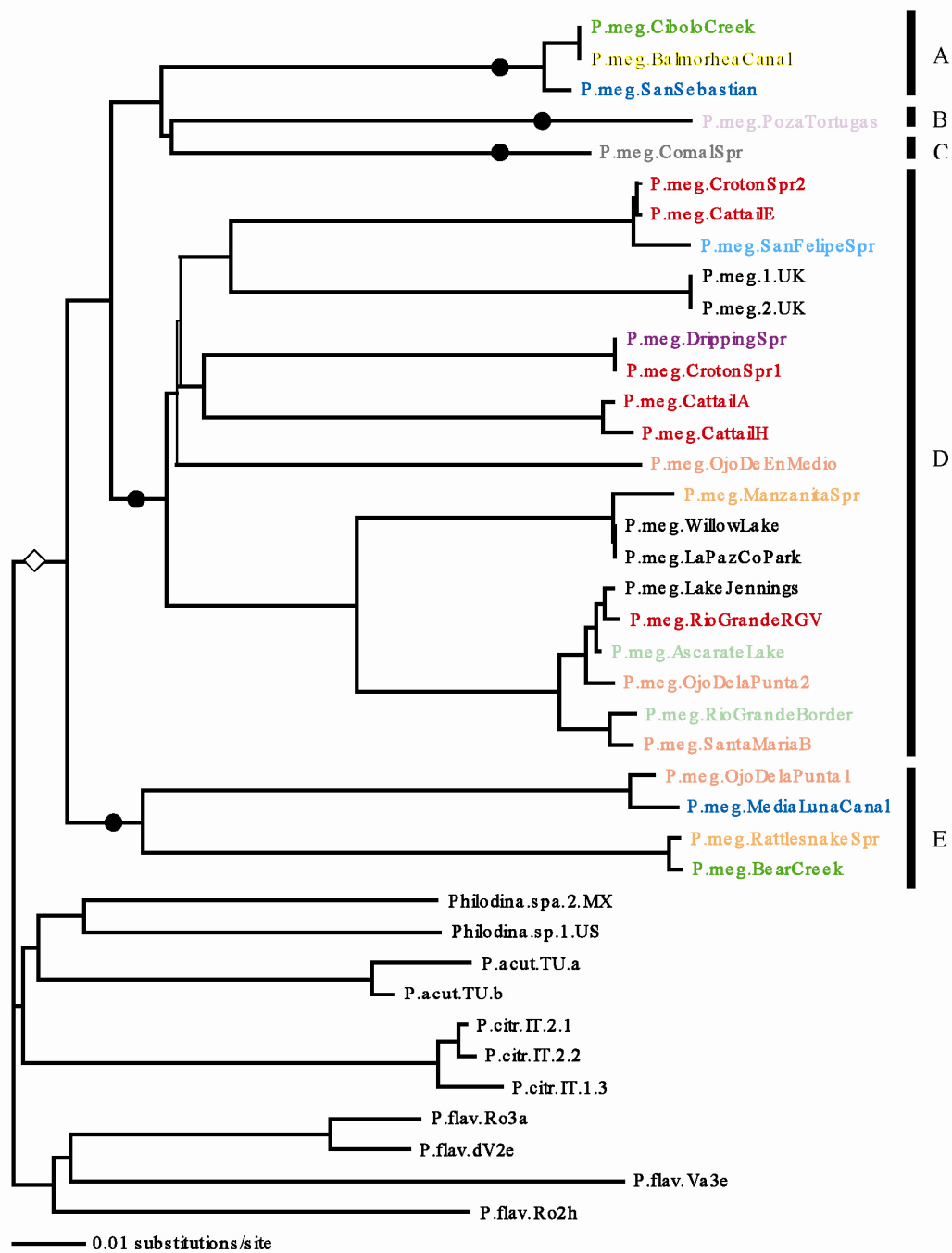


Fig. 3.3: Phylogenetic relationships within *Philodina megalotrocha* inferred by Neighbor Joining analysis of *cox1* sequences using uncorrected ("p") genetic distances. Closed circles indicate independent clades, which are designated as A to E. Diamond indicates *P. megalotrocha* monophyly. Names refer to species and population origin (see Table 3.1). Scale bar indicates substitutions/site. Colors correspond to localities given in the legend to Fig. 3.1.

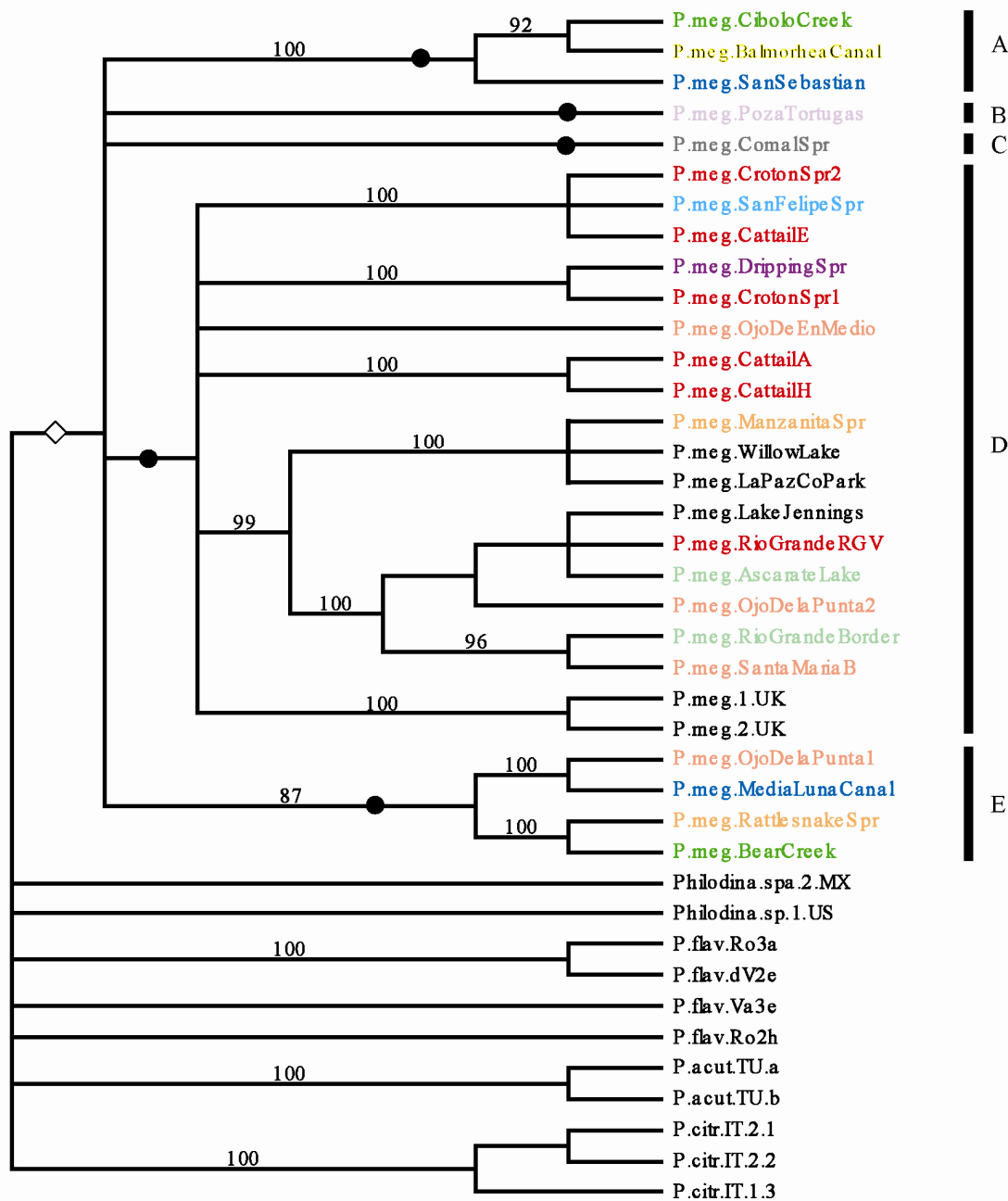


Fig. 3.4: Phylogenetic relationships within *Philodina megalotrocha* populations. Consensus of 6 retained trees from Maximum Parsimony analysis of *cox1* sequences using 1000 bootstrap replicates heuristically searched with 1000 random addition replicates and TBR branch swapping. Bootstrap support > 80% is shown above each branch. Closed circles indicate independent clades, which are designated as A to E. Diamond indicates *P. megalotrocha* monophyly. Names refer to the species and population origin (see Table 3.1). Scale bar indicates substitutions/site. Colors correspond to localities given in the legend to Fig. 3.1.

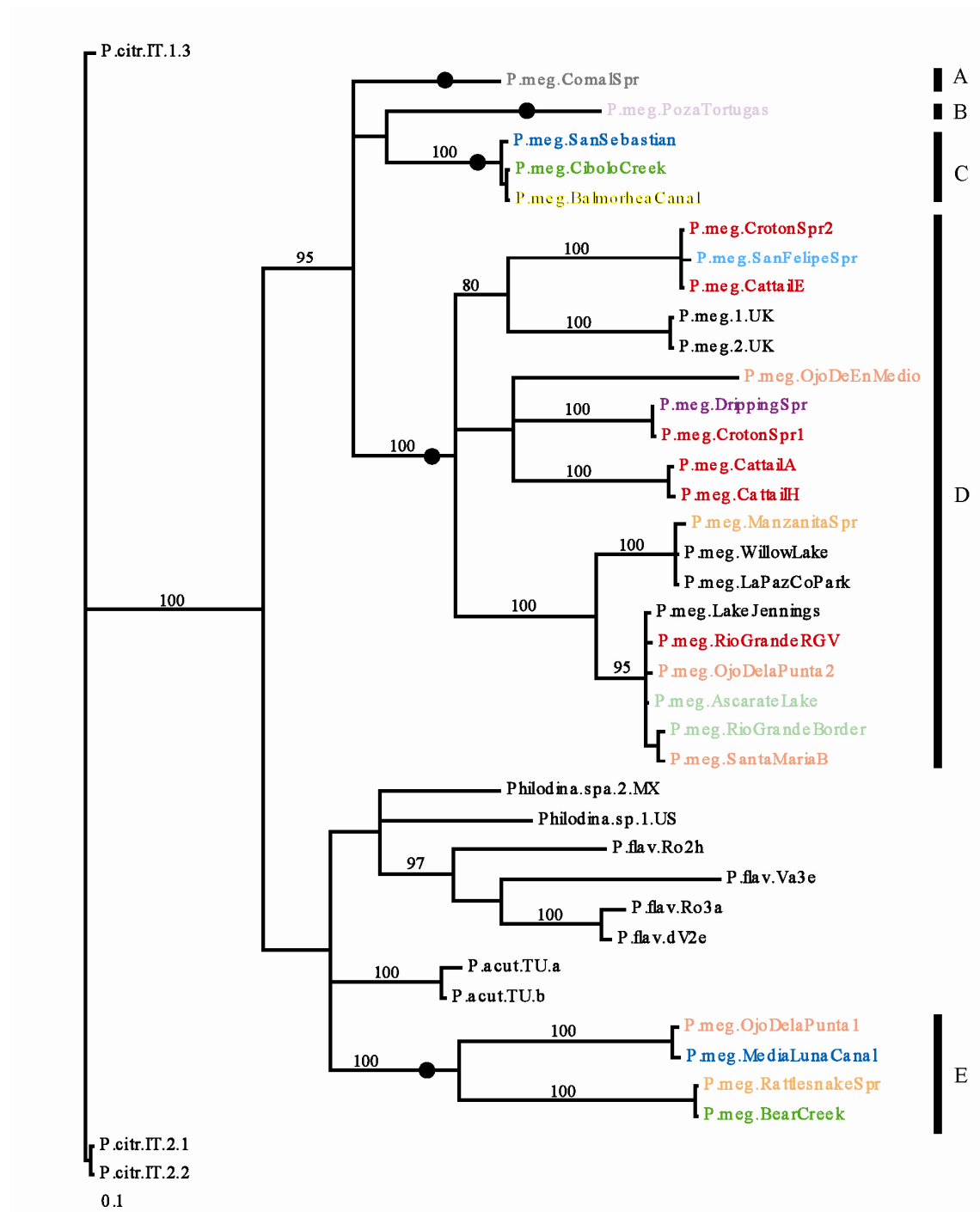


Fig. 3.5: Phylogenetic relationships within *Philodina megalotrocha* populations. The consensus of 999502 sampled trees from Bayesian analysis of *cox1* sequences under a (GTR+I+G) substitution model. Posterior probabilities $\geq 80\%$ are shown above each branch, except for very short branches which are all over 80%. Closed circles indicate independent clades, which are designated as A to E. Names refer to the species and population origin (see Table 3.1). Scale bar indicates substitutions/site. Colors correspond to localities given in the legend to Fig. 3.1.

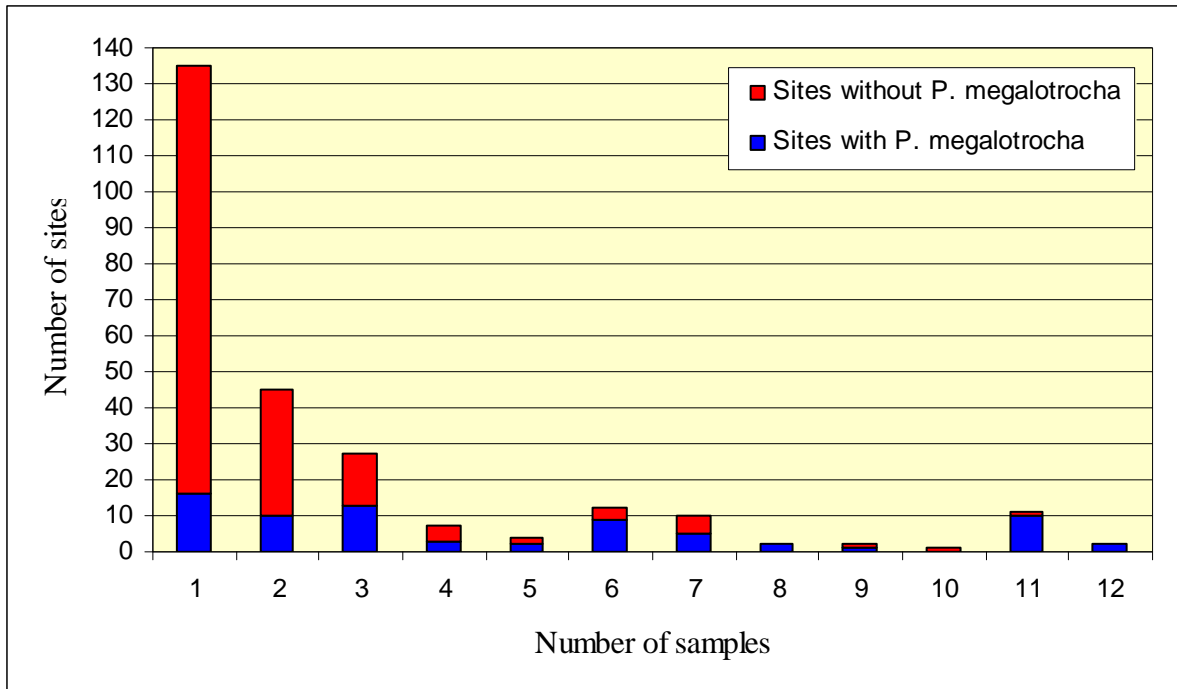


Fig. 4.1: Occurrence of *Philodina megalotrocha* as a function of sampling effort for all sites (n=259) in Chihuahuan Desert.

APPENDICES

Appendix 1: Multiple sequence alignment for *Philodina megalotrocha* populations and outgroups using ClustalW - names refer to species and population origin (see Table 3.1).

P.meg.CiboloCreek	GTTTTTAGGAGCTAGAATAAGATTAATTATTCGTAC
P.meg.BalморheaCanal	GTTTTTAGGAGCTAGAATAAGATTAATTATTCGTAC
P.meg.SanSebastian	GTTTTTAGGAGCTAGAATAAGATTAATTATTCGTAC
P.meg.PozaTortugas	TTTTTTAGGTGCAAGAATAAGGTTAATTATTCGTAC
P.meg.ComalSpr	TTTTTTAGGTGCAAGAATAAGGTTGATTATTCGTAC
P.meg.CrotonSpr2	TTTTTTGGGAGCAAGAATAAGACTTATTATTCGTAC
P.meg.SanFelipeSpr	TTTTTTGGGAGCAAGAATAAGACTTATTATTCGTAC
P.meg.CattailE	TTTTTTGGGAGCAAGAATAAGACTTATTATTCGTAC
P.meg.DrippingSpr	GTTTTTGGGGGCTAGGATAAGATTGATTATTCGAAC
P.meg.CrotonSpr1	GTTTTTGGGGGCTAGGATAAGATTGATTATTCGAAC
P.meg.OjoDeEnMedio	GTTTTTAGGAGCAAGTATAAGATTAATTATTCGAAC
P.meg.CattailA	GTTTTTGGGAGCTAGGATAAGGTTAATTATTCGTAC
P.meg.CattailH	GTTTTTGGGAGCTAGGATAAGGTTAATTATCCGTAC
P.meg.ManzanitaSpr	TTTTTTAGGGGCTAGAATAAGTTTAATTATTCGTAC
P.meg.WillowLake	TTTTTTAGGGGCTAGAATAAGTTTAATTATTCGTAC
P.meg.LaPazCoPark	TTTTTTAGGGGCTAGAATAAGTTTAATTATTCGTAC
P.meg.LakeJennings	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.RioGrandeRGV	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.OjoDeLaPunta2	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.AscarateLake	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.RioGrandeBorder	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.SantaMariaB	GTTCTTAGGAGCTAGAATAAGTTTAATTATTCGTAC
P.meg.1.UK	GTTTTTAGGGGCTAGTATGAGTTTAATTATTCGCAC
P.meg.2.UK	GTTTTTAGGGGCTAGTATGAGTTTAATTATTCGCAC
Philodina.spa.2.MX	ATTTCTTGGTGCGAGAATTAGTTTAATTATTCGTAC
Philodina.sp.1.US	ATTTTLAGGTGCAAGTATAAGTTTAATTATTCGAAC
P.flav.Ro3a	GTTTTTGGGTGCAAGAATTAGATTAATTATCCGCAC
P.flav.dV2e	GTTTTTGGGTGCAAGAATTAGATTAATTATTCGTAC
P.flav.Va3e	GTTTTTAGGAGCTAGGATTAGGTTAATTATTCGTAC
P.flav.Ro2h	ATTTTLAGGTGCAAGAATTAGATTAATTATTCGTAC
P.acut.TU.a	GTTTTTGGGGNCAAGAATAAGAATAATTATTCGTTT
P.acut.TU.b	GTTTTTAGGTGCAAGAATAAGAATAATTATTCGTAC
P.citr.IT.2.1	GTTTTTAGGTGCAAGAATAAGATTAATTATTCGTAC
P.citr.IT.2.2	GTTTTTAGGTGCAAGAATAAGATTAATTATTCGTAC
P.citr.IT.1.3	GTTTTTAGGTGCAAGAATAAGATTAATTATTCGTAC
P.meg.OjoDeLaPunta1	ATTTTLAGGTGCGAGTATAAGTTTAATTATTCGTAC
P.meg.MediaLunaCanal	ATTTTLAGGTGCGAGTATAAGTTTAATTATTCGTAC
P.meg.RattlesnakeSpr	TTTCTTAGGTGCTAGAATAAGATTGATTATTCGTAC
P.meg.BearCreek	TTTCTTAGGTGCTAGAATAAGATTGATTATTCGTAC
P.meg.CiboloCreek	AGAACTTGGAATAGTAGGGAGTGTGATTATAGATGAGCAAATTTATAATA
P.meg.BalморheaCanal	AGAACTTGGAATAGTAGGGAGTGTGATTATAGATGAGCAAATTTATAATA
P.meg.SanSebastian	AGAACTTGGAATAGTAGGGAGTGTGATTATAGATGAGCAAATTTATAATA
P.meg.PozaTortugas	AGAGTTAGGTATAGTAGGAAGAGTAATTATAGATGAGCAAATTTATAATA
P.meg.ComalSpr	TGAATTAGGAATAGTCGGAAGAGTTATTATAGATGAGCATATTTATAATA
P.meg.CrotonSpr2	AGAGCTAGGAATGGTAGGCAGAGTTATTATAGATGATCAAATTTATAATA
P.meg.SanFelipeSpr	AGAGAAGAGAATGGTAGGCAGAGTTATTATAGATGATCAAATTTATAATA
P.meg.CattailE	AGAGCTAGGAATGGTAGGCAGAGTTATTATAGATGATCAAATTTATAATA
P.meg.DrippingSpr	AGAATTAGGAATAGTAGGGAGTGTATTATAGATGAGCAGATTTATAATA
P.meg.CrotonSpr1	AGAATTAGGAATAGTAGGGAGTGTATTATAGATGAGCAGATTTATAATA

P.meg.OjoDeEnMedio
P.meg.CattailA
P.meg.CattailH
P.meg.ManzanitaSpr
P.meg.WillowLake
P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDeLaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDeLaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

AGAGTTAGGAATAGTTGGGAGTGTGATTATAGATGAGCAGATTTATAATA
AGAACTAGGGATAGTAGGTAGTGTAAATTATAGATGAACAAATCTATAATA
AGAACTAGGCATAGTAGGGAGTGTAAATTATAGATGAACAAATCTATAATA
GGAGCTAGGGATAGTAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
GGAGCTAGGGATAGTAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
GGAGCTAGGGATAGTAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAGGCAGGGTTATTATAGATGAGCAAATTTATAATA
ACAGTAAGGGATAGTAGGCAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAGGCAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
AGAGTTAGGGATAGTAAGGTAGGGTTATTATAGATGAGCAAATTTATAATA
TGAACTAGGAATAGTAGGAAGAGTGATCATAGATGATCAAATCTATAATA
TGAACTAGGAATAGTAGGAAGAGTGATCATAGATGATCAAATCTATAATA
AGAATTAGGAATAGTTAGAAGAATTATTATAGATGAGCAAATTTATAATG
AGAATTAGGAATAGTAGGTAGAATTATTATAGATGAACAAATTTATAATG
AGAGTTAGGGATAGTAGGTAGAATCCTTATAGATGAGCAAATTTATAATG
TGAGTTAGGGATAGTAGGTAGAATTATTATAGATGAGCAAATTTATAATG
TGAGTTAGGTATAGTAGGGAGAATTATCATAGATGAACAAATTCATAATG
TGAATTAGGAATGGTGGGAAGTATTATCATAGATGAGCAAATTTATAACT
TGAATTAGGAATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATG
TGAATTAGGAATAGTTGGTAGAATTATTATAGATGAGCAAATTTATAATG
AGAATTAGGAATAATTGGCAGAATTATTATAGACGAACAAATTTATAATA
AGAATTAGGAATAATTGGCAGAATTATTATAGACGAACAAATTTATAATA
AGAATTAGGAATAGTTGGCAGAATTATTATAGACGAACAAATTTATAATA
TGAGTTAGGGATAGTAGGAAGGGTGATTATAGATGAGCAGATTTATAATG
TGAGTTAGGGATAGTAGGAAGGGTAATTATAGATGAGCAGATTTATAATG
TGAACTAGGGATAGTTGGTAGAGTTATTATAGATGAGCAAATTTATAATG
TGAACTTGGGATAGTTGGTAGAGTTATTATAGATGAGCAAATTTATAATG

P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDelaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

CTATAGTAACGGCTCATGCTTTTTTAATAATTTTTTTTTTTGTTATGCCT
CCATAGTTACGGCTCATGCTTTTCCTAATAATTTTTTTTTTTGTAATGCCT
CTATAGTTACTGCTCATGCTTTTTTAATAATTTTTTTTTTTGTTATACCA
CTATAGGAACCGCTCACGCTTTTTTAATAATTTTTTTTTTTGTTATCCCT
CTATAGTAAGTCTCATGCTTTTTTAATAATTTTTTTTTTTGTTATACCT
GAATAGTAACAGCTCATGCTTTTTCTAATAATTTTTTTTTCTTTGTTATGCCT
GAATAGTAACAGCTCATGCTTTTTCTAATAATTTTTTTTTCTTTGTTATGCCT
GAATAGTAACAGCTCATGCTTTTTCTAATAATTTTTTTTTCTTTGTTATGCCT
CTATAGTGACAGCTCATGCTTTTTTAATGATTTTTTTTTCTTTGTTATACCT
CTATAGTGACAGCTCACGCTTTTTTAATGATTTTTTTTTTTGTTATACCT
CTATAGTAAGTCTCATGCTTTTTTAATAATTTTTTTTTTTGTTATACCT
CTATAGTAAGTCTCATGCTTTTTTAATAATTTTTTTTTTTGTTATACCT

P.meg.CiboloCreek
P.meg.BalморheaCanal
P.meg.SanSebastian
P.meg.PozaTortugas
P.meg.ComalSpr
P.meg.CrotonSpr2
P.meg.SanFelipeSpr
P.meg.CattailE
P.meg.DrippingSpr
P.meg.CrotonSpr1
P.meg.OjoDeEnMedio
P.meg.CattailA
P.meg.CattailH
P.meg.ManzanitaSpr
P.meg.WillowLake
P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDelaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDelaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

GTAGCTGTAGGGGGGTTTGGAAATTGATTACTTCCTTTAATAATAAATGC
GTAGCTGTAGGGGGGTTTGGAAATTGATTACTTCCTTTAATAATAAATGC
GTAGCTGTAGGGGGGTTTGGGAATTGATTACTTCCTTTAATAATAAATGC
GTTGCAGTAGGGGGATTGGTAATTGACTTCTTCCTTTAATAATAAATGC
GTTGCTGTAGGGGGATTGGTAATTGACTTCTACCATTGATAATGAATGC
GTTGCTGTAGGTGGGTTTGGGAATTGACTTCTCCCTTTAATAATAAATGC
GTTGCTGTAGGTGGGTTTGGGAATTGACTTCTCCCTTTAATAATAAATGC
GTTGCTGTAGGTGGGTTTGGGAATTGACTTCTCCCTTTAATAATAAATGC
GTAGCTGTAGGGGGATTGCGGAATTGATTATTACCCTTAATAATAAATGC
GTAGCTGTAGGGGGATTGCGGAATTGATTATTACCCTTAATAATAAATGC
GTTGCTGTAGGGGGTTTGGAAATTGATTGCTTCCTTTGATAATAAATGC
GTGGCTGTAGGGGGGTTTGGTAATTGATTACTTCCTTTAATAATAAATGC
GTGGCTGTAGGGGGGTTTGGTAATTGATTATTGCCCTTGATAATAAATGC
GTTGCTGTGGGGGGGTTTGGTAATTGATTATTGCCCTTGATAATAAATGC
GTTGCTGTGGGGGGGTTTGGTAATTGATTATTGCCCTTGATAATAAATGC
GTTGCTGTAGGGGGTTTGGTAATTGATTATTGCCCTTTAATAATAAATGC
GTTGCTGTAGGGGGTTTGGTAATTGATTATTGCCCTTTAATAATAAATGC
GTTGCTGTAGGGGGTTTGGTAATTGACTATTGCCCTTTAATAATAAATGC
GTTGCTGTAGGGGGTTTGGTAATTGACTATTGCCCTTTAATAATAAATGC
GTAGCGGTAGGGGGGTTTGGAAATTGGCTTCTTCCTTTAATAATAAATGC
GTAGCGGTAGGGGGGTTTGGAAATTGGCTTCTTCCTTTAATAATAAATGC
GTAGCTGTAGGTGGTTTTGGTAATTGATTATTACCTTTGATAATAAATGT
GTTGCTGTTGGTGGTTTTGGAAATTGATTATTACCTTTAATAATGAATGT
GTTGCTGTAGGGGGTTTGGAAATTGATTATTACCTTTAATAATAAATGT
GTTGCTGTAGGGGGTTTGGAAATTGATTATTACCTTTAATAATAAATGT
GTAGCTGTGGGGGGATTGGTAATTGGTTGTTGCCTTTAATAATAAATGT
GTAGCTGTTGGAGGCTTTGGAAATTGATTATTCCCTTTAATAATGAATGT
GTTGCTGTTGGAGGCTTTGGAAATTGATTATTACCTTTAATAATGAATGT
GTAGCTGTGCGGAGGATTGGTAAGTATTGCTTTAATAATAAATGC
GTAGCTGTGCGGAGGATTGGTAAGTATTGCTTTAATAATAAATGC
GTAGCTGTGCGGAGGATTGGTAAGTATTGCTTTAATAATAAATGC
GTAGCTGTGCGGAGGTTTGGTAATTGGTTATTACCTTTAATAATAAATGT
GTTGCTGTTGGAGGATTGGTAATTGACTTCTTCCTTTAATAATAAATGT
GTTGCTGTTGGAGGATTGGTAATTGACTTCTTCCTTTAATAATAAATGT

P.meg.CiboloCreek
P.meg.BalморheaCanal
P.meg.SanSebastian
P.meg.PozaTortugas

TATAGATATAGCATTCCCTCGTTTGAATAATTTAAGATTTTGACTTGTAC
TATAGATATAGCATTCCCTCGTTTGAATAATTTAAGATTTTGACTTGTAC
TATAGATATAGCATTCCCTCGTTTGAATAATTTAAGATTTTGACTTGTAC
AATGGATATAGCATTTCCCTCGTTTGAATAATTTAAGATTTTGTTAGTTC

P.meg.ComalSpr
P.meg.CrotonSpr2
P.meg.SanFelipeSpr
P.meg.CattailE
P.meg.DrippingSpr
P.meg.CrotonSpr1
P.meg.OjoDeEnMedio
P.meg.CattailA
P.meg.CattailH
P.meg.ManzanitaSpr
P.meg.WillowLake
P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDeLaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDeLaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

TATAGATATAGCATTTCCTCGTTTAAATAACTTAAGGTTTTGATTAGTAC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGGTTTTGATTAGTTC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGGTTTTGATTAGTTC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGGTTTTGATTAGTTC
AATAGATATAGCTTTTTCTCGTTTAAATAAATTTGAGGTTTTGATTAGTAC
AATAGATATAGCTTTTTCTCGTTTAAATAAATTTGAGGTTTTGATTAGTAC
AATGGATATGGCTTTTTCTCGTTTAAATAAATTTAAGTTTTGATTAGTTC
AATAGATATGGCTTTTTCTCGTTTGAATAAATTTAAGTTTTGATTAGTAC
AATAGATATGGCTTTTTCTCGTTTGAATAAATTTAAGTTTTGATTAGTAC
TATAGATATAGCTTTTTCCCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATGGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATGGATATAGCATTTCCTCGTTTAAATAAATTTAAGGTTTTGATTGGTTC
TATGGATATAGCATTTCCTCGTTTAAATAAATTTAAGGTTTTGATTGGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTGC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTGC
TATGGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGATTAAATAAATTTAAGATCTTGATTGGTTC
TATAGATATAGCTTTTTCTCGTTTAAATAAATTTGAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTGAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCTTTTTCTCGTTTGAATAAATTTAAGATTTTGATTAGTTC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTGGTGC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTGGTGC
TATAGATATAGCATTTCCTCGTTTAAATAAATTTAAGATTTTGATTGGTGC
AATAGATATAGCTTTTTCTCGTTTAAATAAATTTGAGTTTTGACTAGTGC
AATAGATATAGCTTTTTCTCGTTTAAATAAATTTGAGTTTTGACTAGTGC
TATGGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTGGTTC
TATGGATATAGCTTTTTCTCGTTTAAATAAATTTAAGATTTTGATTGGTTC

P.meg.CiboloCreek
P.meg.BalморheaCanal
P.meg.SanSebastian
P.meg.PozaTortugas
P.meg.ComalSpr
P.meg.CrotonSpr2
P.meg.SanFelipeSpr
P.meg.CattailE
P.meg.DrippingSpr
P.meg.CrotonSpr1
P.meg.OjoDeEnMedio
P.meg.CattailA
P.meg.CattailH
P.meg.ManzanitaSpr
P.meg.WillowLake
P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDeLaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDeLaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr

[illegible]

P.meg.BearCreek	GGCACTGGTTGAACAGTGTATCCTCCTTTATCAAATTCAGTCTATCATTT
P.meg.CiboloCreek	TGGGGGGTTCGGTAGATTTTGCTATTTTTAGTCTTCATGTAGCAGGGGTTT
P.meg.BalморheaCanal	TGGGGGGTTCGGTAGATTTTGCTATTTTTAGTCTTCATGTAGCAGGGGTTT
P.meg.SanSebastian	TGGGGGGTTCGGTAGATTTTGCTATTTTTAGTCTTCATGTAGCAGGGGTTT
P.meg.PozaTortugas	TGGGGGGTTCGGTAGATTTTGCTATTTTTAGATTACATGTTGCAGGTGTTT
P.meg.ComalSpr	TGGGGGGTTCGGTAGATTTTGCTATTTTTAGTCTTCATGTGGCAGGTGTCT
P.meg.CrotonSpr2	TGGTGGATCAGTAGATTTTGCTATTTTTAGGTTGCATGTTGCTGGTGTAT
P.meg.SanFelipeSpr	TGGTGGATCAGTAGATTTTGCTATTTTTAGGTTGCTTGTTGCTGGTGTAT
P.meg.CattailE	TGGTGGATCAGTAGATTTTGCTATTTTTAGGTTGCATGTTGCTGGTGTAT
P.meg.DrippingSpr	TGGGGGTTCTGTTGATTTTGCTATTTTTAGATTACATGTTGCAGGGGTTT
P.meg.CrotonSpr1	TGGGGGTTCTGTTGATTTTGCTATTTTTAGATTACATGTTGCAGGGGTTT
P.meg.OjoDeEnMedio	TGGGGGGTTCAGTAGATTTTGCTATTTTTAGTCTTCATGTTGCTGGGGTGT
P.meg.CattailA	TGGTGGTTCTGTTGATTTTGCTATTTTTAGCTTACATGTGGCTGGTGT
P.meg.CattailH	TGGTGGTTCTGTTGATTTTGCTATTTTTAGCTTACATGTGGCTGGTGT
P.meg.ManzanitaSpr	TGGAGGGCTGTTGATTTTGCTATTTTTAGATTGCATGTAGCTGGGGTGT
P.meg.WillowLake	TGGAGGGCTGTTGATTTTGCTATTTTTAGATTGCATGTAGCTGGGGTGT
P.meg.LaPazCoPark	TGGAGGGCTGTTGATTTTGCTATTTTTAGATTGCATGTAGCTGGGGTGT
P.meg.LakeJennings	TGGAGGGCTGTTGATTTTGCTATTTTTAGATTGCATGTGGCCGGGGTAT
P.meg.RioGrandeRGV	TGGAGGGCTGTTGATTTTGCTATTTTTAGATTNCATGTGGCCGGGGTAT
P.meg.OjoDeLaPunta2	TGGAGGATCTGTTGATTTTGCTATTTTTAGATTACATGTGGCCGGGGTAT
P.meg.AscarateLake	TGGAGGGCTGTTGATTTTGCTATTTTTAGNTTACATGTGGCCGGGGTAT
P.meg.RioGrandeBorder	TGGAGGGCTGTTGATTTTGCTATTTTTAGGTTACATGTGGCCGGGGTAT
P.meg.SantaMariaB	TGGAGGGCTGTTGATTTTGCTATTTTTAGGTTACATGTGGCCGGGGTAT
P.meg.1.UK	TGGGGGCTCTGTTGATTTTGCTATTTTTAGATTACATGTGGCTGGTGT
P.meg.2.UK	TGGGGGCTCTGTTGATTTTGCTATTTTTAGATTACATGTGGCTGGTGT
Philodina.spa.2.MX	TGGAGGTTCTGTAGATTTAGCAATTTTTAGTTTACATGTAGCAGGGGTCT
Philodina.sp.1.US	TGGAGGTTCTGTGATTTAGCAATTTTTAGTTTACATGTGGCTGGTGTGT
P.flav.Ro3a	TGGCGGATCTGTTGATTTAGCAATTTTTAGTTTACATGTGCCGGGTGTGT
P.flav.dV2e	CGGTGGATCTGTTGATTTAGCAATTTTTAGTTTGCATGTTGCTGGTGTGT
P.flav.Va3e	TGGGGGATCAGTGGATTTAGCAATTTTTAGATTGCATGTGGCAGGCGTTT
P.flav.Ro2h	TGGGGGGTCTGTTGATTTAGCAATTTTTAGGTTACATGTAGCTGGAGTGT
P.acut.TU.a	TGGAGGTTCTGTTGATTTAGCTATTTTTAGTTTACATGTTGCGGGAGTTT
P.acut.TU.b	TGGAGGTTCTGTTGATTTAGCTATTTTTAGTTTACATGTTGCTGGAGT
P.citr.IT.2.1	TGGAGGTTCTGTAGATTTAGCTATTTTTAGTTTACATGTCGCTGGGGT
P.citr.IT.2.2	TGGAGGTTCTGTAGATTTAGCTATTTTTAGTTTACATGTCGCTGGGGT
P.citr.IT.1.3	TGGAGGTTCTGTAGATTTAGCTATTTTTAGTTTACATGTCGCTGGGGT
P.meg.OjoDeLaPunta1	TGGAGGTTCTGTTGACTTTGCTATTTTTAGTCTTCATGTTGCGGGGGT
P.meg.MediaLunaCanal	TGGAGGTTCTGTTGACTTTGCTATTTTTAGTCTTCATGTTGCGGGGGT
P.meg.RattlesnakeSpr	TGGGGGTTCTGTTGATTTTGCTATTTTTAGTTTACATGTAGCGGGTGTGT
P.meg.BearCreek	TGGGGGTTCTGTTGATTTTGCTATTTTTAGTTTACATGTAGCGGGTGTGT
P.meg.CiboloCreek	CCTCTATTTTAGGAGCCATTAATTTTATTACTACATGTATAAAAGGTAAG
P.meg.BalморheaCanal	CCTCTATTTTAGGAGCCATTAATTTTATTACTACATGTATAAAAGGTAAG
P.meg.SanSebastian	CTTCTATTTTAGGAGCCATTAATTTTATTACTACATGTATAAAAGGTAAG
P.meg.PozaTortugas	CTTCAATTTTAGGGGCTATTAATTTTATTACTACTGTATAAAAGGAAAA
P.meg.ComalSpr	CTTCTATTTTAGGGGCTATTAATTTTATTACTACTGTATAAAAGGAAAA
P.meg.CrotonSpr2	CTTCAATTTTAGGTGCTATTAATTTTATTACTACTTGTTTAAAGGGTAAA
P.meg.SanFelipeSpr	CTTCAATTTTAGGTGCTATTAATTTTATTACTACTTGTTTAAAGGGTAAA
P.meg.CattailE	CTTCAATTTTAGGTGCTATTAATTTTATTACTACTTGTTTAAAGGGTAAA
P.meg.DrippingSpr	CTTCAATTTTAGGAGCTATTAATTTTATTACTACTTGTTTAAAGGGTAAA
P.meg.CrotonSpr1	CTTCAATTTTAGGAGCTATTAATTTTATTACTACTTGTTTAAAGGGTAAA
P.meg.OjoDeEnMedio	CTTCAATTTTAGGAGCTATTAATTTTATTACAACCTGTTTGAAGGGTAAAG
P.meg.CattailA	CTTCTATTTTAGGAGCTATTAATTTTATTACAACCTGTTTAAAGGGTAAA
P.meg.CattailH	CTTCTATTTTAGGAGCTATTAATTTTATTACAACCTGTTTAAAGGGTAAA
P.meg.ManzanitaSpr	CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAGGTAAG
P.meg.WillowLake	CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAGGTAAG

P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDelaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDelaPuntal
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGTAAG
CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGTAAG
CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGTAAG
CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGTAAG
CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGCAAG
CTTCTATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGCAAG
CTTCGATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGAAAG
CTTCGATTTTAGGGGCTATTAATTTTATTACTACATGTTTAAAAGGAAAG
CTTCAATTTTAGGGGGGATTAATTTTATTACTACATGTTTAAAAGGAAAA
CTTCAATTTTAGGAGGAATTAATTTTATTACTACTTGTTTAAAGGGTAAA
CTTCTATTTTAGGAGGTATTAATTTTATTACTACTTGTTTAAAGGAAAA
CTTCTATTTTAGGAGGTATTAATTTTATTACCACTTGTTTAAAGGAAAA
CTTCTATTTTAGGAGGAATTAATTTTATTACGACATGTTTGAAAGGAAAA
CTTCTATTTTAGGAGGAATTAATTTTATTACTACTTGTCTTAAAGGGAAA
CTTCTATTTTAGGAGCTATTAATTTTATTACTACATGTTTAAAAGGAAAA
CTTCTATTTTAGGAGCTATTAATTTTATTACTACATGTTTAAAAGGAAAA
CTTCAATTTTAGGAGGAATTAATTTTATTACAACATGCATAAAAGGTAAG
CTTCAATTTTAGGAGGAATTAATTTTATTACAACATGCATAAAAGGTAAG
CTTCGATTTTAGGGGGGATTAATTTTATTACTACTTGTATAAAAGGAAAG
CTTCGATTTTAGGGGGGATTAATTTTATTACTACTTGTATAAAAGGGAAG
CGTCTATTTTGGGTGCGATTAATTTTATTACAACCTTGTATGAAAGGTAAG
CGTCTATTTTGGGTGCGATTAATTTTATTACAACCTTGTATGAAAGGTAAG

P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDelaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

P.meg.CiboloCreek
P.meg.BalmoreheaCanal
P.meg.SanSebastian
P.meg.PozaTortugas
P.meg.ComalSpr
P.meg.CrotonSpr2
P.meg.SanFelipeSpr
P.meg.Cattaile
P.meg.DrippingSpr
P.meg.CrotonSpr1
P.meg.OjoDeEnMedio
P.meg.CattailA
P.meg.CattailH
P.meg.ManzanitaSpr
P.meg.WillowLake
P.meg.LaPazCoPark
P.meg.LakeJennings
P.meg.RioGrandeRGV
P.meg.OjoDelaPunta2
P.meg.AscarateLake
P.meg.RioGrandeBorder
P.meg.SantaMariaB
P.meg.1.UK
P.meg.2.UK
Philodina.spa.2.MX
Philodina.sp.1.US
P.flav.Ro3a
P.flav.dV2e
P.flav.Va3e
P.flav.Ro2h
P.acut.TU.a
P.acut.TU.b
P.citr.IT.2.1
P.citr.IT.2.2
P.citr.IT.1.3
P.meg.OjoDelaPunta1
P.meg.MediaLunaCanal
P.meg.RattlesnakeSpr
P.meg.BearCreek

P.meg.CiboloCreek
P.meg.BalmoreheaCanal
P.meg.SanSebastian
P.meg.PozaTortugas
P.meg.ComalSpr
P.meg.CrotonSpr2
P.meg.SanFelipeSpr
P.meg.Cattaile
P.meg.DrippingSpr

ATCAGATATGTTTTGTCTTTTGAATTTTAAATTTATTTGTTTGAGCTAT
ATCAGATATGTTTTGTCTTTTGAATTTTAAATTTATTTGTTTGAGCTAT
ATCAGATATGTTTTGTCTTTTGAATTTTAAATTTATTTGTTTGAGCTAT
ATTAATTTTGTAAATTCGTTTCGAGTTTTTAACCTTTATTTGTTTGAGCAAT
ATTAATTTTGTAAATTCGTTTCGAGTTTTTAACCTTTATTTGTTTGAGCAAT
ATTAATTTTGTATTTCTTTTGAGTTTTTAACCTTTATTTGTTTGGGCTAT
ATTAATTTTGTATTTCTTTTGAGTTTTTAACCTTTATTTGTTTGGGCTAT

AATTGTAACAAGATTTTTATTAGTTTTAAGACTACCTGTATTAGCAGGTG
AATTGTAACAAGATTTTTATTAGTTTTAAGACTACCTGTATTAGCAGGTG
GATTGTAACAAGATTTTTATTAGTTTTAAGACTACCTGTATTAGCAGGTG
GATTGTAACAAGATTTTTATTAGTTCTGAGTTTACCTGTTTTAGCTGGTG
GATTGTTACAAGTTTTTTATTAGTTTTAAGTTTACCGGTATTGGCNGGGG
AATTGTTACAAGTTTTTTATTAGTTTTAAGTTTACCGGTATTGGCTGGGG
AATTGTTACAAGTTTTTTATTAGTTTTAAGTTTACCGGTATTGGCTGGGG
GATTGTAACAAGATTTTTATTAGTCTTAAGTTTACCAGTATTGGCTGGTG
GATTGTAACAAGATTTTTATTAGTCTTAAGTTTACCAGTATTGGCTGGTG
AATTGTTACGAGTTTTTTATTAGTCTTAAGTTTACCTGTCTTAGCAGGTG
GATTGTTACTAGGTTTTTACTAGTATTAAGATTACCTGTTTTAGCGGGAG
GATTGTTACTAGGTTTTTACTAGTATTAAGATTACCTGTTTTAGCGGGAG
AATTGTTACTAGATTTTTATTAGTTTTAAGATTGCCTGTATTAGCAGGTG
AATTGTTACTAGATTTTTATTAGTTTTAAGATTGCCTGTATTAGCAGGTG
AATTGTTACTAGATTTTTATTAGTTTTAAGATTGCCTGTATTAGCAGGTG
GATTGTAAGTAGGTTTTTATTAGTGTTAAGTCTTCCTGTTTTAGCAGGGG
GATTGTAAGTAGGTTTTTATTAGTGTTAAGTCTTCCTGTTTTAGCAGGGG
GATTGTAAGTAGGTTTTTATTAGTGTTAAGTCTTCCTGTTTTAGCAGGGG
GATTGTAAGTAGGTTTTTATTAGTGTTAAGTCTTCCTGTTTTAGCAGGGG
GATTGTAAGTAGGTTTTTATTAGTGTTAAGTCTTCCTGTTTTAGCAGGGG
AATTGTGACTAGTTTTTTATTAGTATTAAGTTTACCTGTGTTAGCGGGTG
AATTGTGACTAGTTTTTTATTAGTATTAAGTTTACCTGTGTTAGCGGGTG
AATTGTGACTAGTTTTTTATTAGTATTAAGTTTACCTGTGTTAGCGGGTG
AATTGTGACTAGTTTTTTATTAGTTTTAAGTTTACCTGTATTAGCTGGAG
AATTGTTACTAGATTTTTATTGGTTTTGAGGTTACCTGTTTTAGCTGGTG
AATTGTTACAAGATTTTTATTAGTTTTAAGTTTACCTGTTTTAGCAGGTG
AATTGTTACAAGATTTTTATTAGTTTTAAGTTTACCTGTTTTAGCAGGTG
AATTGTAAGTAGGTTTTTGTAGTATTAAGGTTACCTGTTTTAGCGGGTG
GATTGTCACTAGTTTTTTATTAGTTTTAAGTCTTCCTGTTTTAGCAGGTG
AATTGTTACTAGATTTTTATTAGTTCTAAGATTACCTGTTTTAGCTGGAG
AATTGTTACTAGATTTTTATTAGTTCTAAGATTACCTGTTTTAGCTGGAG
AATTGTAACAAGATTTCTTTTAGTTTTAAGTCTTCCTGTATTAGCTGGAG
AATTGTAACAAGATTTCTTTTAGTTTTAAGTCTTCCTGTATTAGCTGGAG
AATTGTAACAAGATTCCTTTTAGTTTTAAGTCTTCCTGTGTTAGCGGGAG
GATTATTACTAGGTTTCTTTTAGTATTAAGGCTACCTGTGTTAGCTGGTG
GATTATTACTAGGTTTCTTTTAGTATTAAGGCTACCTGTGTTAGCTGGTG
AATTATTACTAGGTTTTTGTAGTTTTAAGTCTTCCTGTGTTAGCTGGGG
AATTATTACTAGGTTTTTGTAGTTTTAAGTCTTCCTGTGTTAGCTGGGG

GGATTACTATATTATTATTAGATCGAAATTTTGGGTCTTCTTTTTTTT
GGATTACTATATTATTATTAGATCGAAATTTTGGGTCTTCTTTTTTTT
GGATTACTATATTATTATTAGATCGAAATTTTGGGTCTTCTTTTTTTT
GTATTACTATGCTTCTTTTAGATCGTAATTTTGGGTCTTCTTTTTTTT
GAATTACTATGTTATTATTAGATCGTAATTTTGGGTCTTCTTTTTTTT
GTATTACTATGTTGTTATTAGATCGTAATTTTGGGTCTTCTTTTTTTT
GTATTACTATGTTGTTATTAGATCGTAATTTTGGGTCTTCTTTTTTTT
GTATTACTATGTTGTTATTAGATCGTAATTTTGGGTCTTCTTTTTTTT
GGATCACTATATTATTATTAGACCGTAATTTTGGATCCTCTTTTTTTT

P.meg.CrotonSpr1
 P.meg.OjoDeEnMedio
 P.meg.CattailA
 P.meg.CattailH
 P.meg.ManzanitaSpr
 P.meg.WillowLake
 P.meg.LaPazCoPark
 P.meg.LakeJennings
 P.meg.RioGrandeRGV
 P.meg.OjoDelaPunta2
 P.meg.AscarateLake
 P.meg.RioGrandeBorder
 P.meg.SantaMariaB
 P.meg.1.UK
 P.meg.2.UK
 Philodina.spa.2.MX
 Philodina.sp.1.US
 P.flav.Ro3a
 P.flav.dV2e
 P.flav.Va3e
 P.flav.Ro2h
 P.acut.TU.a
 P.acut.TU.b
 P.citr.IT.2.1
 P.citr.IT.2.2
 P.citr.IT.1.3
 P.meg.OjoDelaPunta1
 P.meg.MediaLunaCanal
 P.meg.RattlesnakeSpr
 P.meg.BearCreek

GGATCACTATATTATTATTAGACCGTAATTTTGGATCCTCTTTTTTTT
 GTATTACAATACTATTACTAGATCGTAATTTTGGTTCTTCTTTTTTTT
 GTATTACTATGTTGTTATTAGATCGTAATTTTGGATCTTCCTTTTTTTT
 GTATTACTATGTTGTTATTAGATCGTAATTTTGGATCTTCCTTTTTTTT
 GGATTACCATATTATTATTAGATCGAAATTTTGGGTCGCCTTTTTTTT
 GGATTACTATATTATTATTAGATCGAAATTTTGGGTCGTCTTTTTTTT
 GGATTACTATATTATTATTAGATCGAAATTTTGGGTCGTCTTTTTTTT
 GGATTACTATGTTATTATTAGATCGAAATTTTGGTTTCGTCTTTTTTTT
 GGATTACTATGTTATTATTAGATCGAAATTTTGGTTTCGTCTTTTTTTT
 GGATTACTATGTTATTATTAGATCGAAATTTTGGTTTCGTCTTTTTTTT
 GGATTACTATGTTATTATTAGATCGAAATTTTGGTTTCGTCTTTTTTTT
 GTATTACTATGTTATTATTAGATCGAAATTTTCGGTTCTTCTTTTTTTT
 GTATTACTATGTTATTATTAGATCGAAATTTTCGGTTCTTCTTTTTTTT
 GAATTACTATGTTATTATTGGATCGTAATTTTGGTTCTTCTTTTTTTT
 GAATTACTATATTATTATTAGATCGAAATTTTGGTTCTTCTTTTTTTT
 GTATTACTATATTATTGTTGGATCGTAATTTTGGATCTTCATTTTTTTT
 GTATTACTATGTTATTGTTGGATCGTAATTTTGGATCTTCATTTTTTTT
 GTATTACTATGCTATTGTTAGATAATAATTTTGGCTCCTCTTTTTTTT
 GAATTACTATGTTATTATTAGATCGTAATTTTGGTTCTTCATTTTTNT
 GTATTACAATGTTATTATTAGATCGAAATTTTGGTTCTTCCTTTTTTTT
 GTATTACAATGTTATTATTAGATCGAAATTTTGGTTCTTCCTTTTTTTT
 GGATTACAATATTATTATTAGATCGTAATTTTNGGATCTTCGTTTTTTT
 GGATTACAATATTATTATTAGATCGTAATTTTGGATCTTCATTCTTTT
 GGATTACAATATTATTATTAGATCGTAATTTTGGATCTTCATTTTAT
 GTATTACTATGTTATTATTAGATCGTAATTTTGGTTCTTCTTTTTTTC
 GCATTACTATGTTATTATTAGATCGTAATTTTGGTTCTTCTTTTTTTC
 GTATTACTATGTTACTTTTTGGATCGTAATTTTGGTTTCGTCTTCCTTC
 GTATTACTATGTTACTTTTTGGATCGTAATTTTGGTTTCGTCTTCCTTC

Appendix 2: Uncorrected ("p") distance matrix for *Philodina megalotrocha* populations and outgroups - names refer to the species and to each population (see Table 3.1).

	1	2	3	4	5	6	7
1 P.meg.CiboloCree	-						
2 P.meg.BalmorheaC	0.00000	-					
3 P.meg.SanSebasti	0.00858	0.00858	-				
4 P.meg.PozaTortug	0.13551	0.13551	0.13208	-			
5 P.meg.ComalSpr	0.12178	0.12178	0.12007	0.13379	-		
6 P.meg.CrotonSpr2	0.15137	0.15137	0.14793	0.15133	0.12877	-	
7 P.meg.SanFelipeS	0.15952	0.15952	0.15609	0.15952	0.13551	0.00858	-
8 P.meg.CattailE	0.15266	0.15266	0.14923	0.15266	0.12864	0.00000	0.00858
9 P.meg.DrippingSp	0.14237	0.14237	0.13551	0.15266	0.13722	0.12534	0.13208
10 P.meg.CrotonSpr1	0.14237	0.14237	0.13551	0.15266	0.13722	0.12534	0.13208
11 P.meg.OjoDeEnMed	0.13379	0.13379	0.13722	0.15609	0.14580	0.13063	0.13894
12 P.meg.CattailA	0.14580	0.14580	0.13894	0.15952	0.14923	0.12722	0.13722
13 P.meg.CattailH	0.14580	0.14580	0.13894	0.16123	0.15094	0.12894	0.13894
14 P.meg.ManzanitaS	0.13722	0.13722	0.13894	0.17496	0.15266	0.13406	0.14408
15 P.meg.WillowLake	0.12864	0.12864	0.13036	0.16638	0.14408	0.12549	0.13551
16 P.meg.LaPazCoPar	0.12864	0.12864	0.13036	0.16638	0.14408	0.12549	0.13551
17 P.meg.LakeJennin	0.13722	0.13722	0.13208	0.16295	0.14065	0.13234	0.14065
18 P.meg.RioGrandeR	0.13762	0.13762	0.13247	0.16375	0.14135	0.13472	0.13962
19 P.meg.OjoDeLaPun	0.13894	0.13894	0.13379	0.16467	0.14237	0.13406	0.14237
20 P.meg.AscarateLa	0.13577	0.13577	0.13063	0.16183	0.13947	0.13280	0.14114
21 P.meg.RioGrandeB	0.14580	0.14580	0.14065	0.16295	0.14408	0.13579	0.14408
22 P.meg.SantaMaria	0.14580	0.14580	0.14065	0.15952	0.14408	0.13407	0.14237
23 P.meg.1.UK	0.15266	0.15266	0.15266	0.15952	0.15780	0.12210	0.13208
24 P.meg.2.UK	0.15266	0.15266	0.15266	0.15952	0.15780	0.12210	0.13208
25 Philodina.spa.2.	0.14065	0.14065	0.13894	0.14408	0.13208	0.15111	0.15780
26 Philodina.sp.1.U	0.14408	0.14408	0.14923	0.16638	0.13208	0.14599	0.15266
27 P.flav.Ro3a	0.14580	0.14580	0.14751	0.15609	0.14580	0.14276	0.15094
28 P.flav.dV2e	0.14408	0.14408	0.14923	0.16123	0.13722	0.13416	0.14237
29 P.flav.Va3e	0.16981	0.16981	0.17324	0.17496	0.16810	0.17882	0.18696
30 P.flav.Ro2h	0.14764	0.14764	0.14249	0.16826	0.14252	0.15497	0.16314
31 P.acut.TU.a	0.14640	0.14640	0.14644	0.16686	0.13597	0.15685	0.16355
32 P.acut.TU.b	0.13722	0.13722	0.13722	0.15609	0.12007	0.14940	0.15609
33 P.citr.IT.2.1	0.12536	0.12536	0.12536	0.16489	0.14772	0.15305	0.15976
34 P.citr.IT.2.2	0.12693	0.12693	0.12693	0.16638	0.14923	0.15452	0.16123
35 P.citr.IT.1.3	0.13208	0.13208	0.13208	0.17153	0.15437	0.15976	0.16810
36 P.meg.OjoDeLaPun	0.16467	0.16467	0.15952	0.17324	0.14923	0.18720	0.19383
37 P.meg.MediaLunaC	0.16810	0.16810	0.16295	0.17667	0.15094	0.19063	0.19726
38 P.meg.Rattlesnak	0.16981	0.16981	0.16981	0.16638	0.16123	0.16314	0.17153
39 P.meg.BearCreek	0.16638	0.16638	0.16638	0.16810	0.16123	0.16486	0.17153

Uncorrected ("p") distance matrix(continued)

	8	9	10	11	12	13	14
8 P.meg.CattailE	-						
9 P.meg.DrippingSp	0.12521	-					
10 P.meg.CrotonSpr1	0.12521	0.00000	-				
11 P.meg.OjoDeEnMed	0.13208	0.12521	0.12521	-			
12 P.meg.CattailA	0.12864	0.11835	0.11835	0.13208	-		
13 P.meg.CattailH	0.13036	0.11835	0.11835	0.13208	0.00515	-	
14 P.meg.ManzanitaS	0.13551	0.13722	0.13722	0.14065	0.13551	0.14065	-
15 P.meg.WillowLake	0.12693	0.12864	0.12864	0.13379	0.12693	0.13208	0.00858
16 P.meg.LaPazCoPar	0.12693	0.12864	0.12864	0.13379	0.12693	0.13208	0.00858
17 P.meg.LakeJennin	0.13379	0.13036	0.13036	0.13036	0.12007	0.12350	0.07890
18 P.meg.RioGrandeR	0.13617	0.13099	0.13099	0.13089	0.12065	0.12407	0.08097
19 P.meg.OjoDeLaPun	0.13551	0.12864	0.12864	0.13551	0.11664	0.12178	0.08062
20 P.meg.AscarateLa	0.13426	0.12913	0.12913	0.12900	0.11697	0.12044	0.08085
21 P.meg.RioGrandeB	0.13722	0.13551	0.13551	0.13722	0.12350	0.12864	0.08576
22 P.meg.SantaMaria	0.13551	0.13551	0.13551	0.13722	0.12350	0.12864	0.08576
23 P.meg.1.UK	0.12350	0.14065	0.14065	0.14065	0.13036	0.13208	0.14923
24 P.meg.2.UK	0.12350	0.14065	0.14065	0.14065	0.13036	0.13208	0.14923
25 Philodina.spa.2.	0.15094	0.15266	0.15266	0.15780	0.16467	0.16638	0.15780
26 Philodina.sp.1.U	0.14580	0.15094	0.15094	0.15094	0.14751	0.15094	0.14580
27 P.flav.Ro3a	0.14408	0.14408	0.14408	0.14237	0.13894	0.14065	0.14751
28 P.flav.dV2e	0.13551	0.14923	0.14923	0.14408	0.13551	0.14065	0.13894
29 P.flav.Va3e	0.18010	0.18010	0.18010	0.16981	0.16810	0.16810	0.16981
30 P.flav.Ro2h	0.15627	0.14594	0.14594	0.15284	0.15627	0.15798	0.16141
31 P.acut.TU.a	0.15667	0.14971	0.14971	0.16529	0.14634	0.14978	0.15499
32 P.acut.TU.b	0.14923	0.14408	0.14408	0.15266	0.13551	0.13894	0.14408
33 P.citr.IT.2.1	0.15290	0.14771	0.14771	0.15632	0.15288	0.15459	0.15632
34 P.citr.IT.2.2	0.15437	0.14923	0.14923	0.15780	0.15437	0.15609	0.15780
35 P.citr.IT.1.3	0.16123	0.15609	0.15609	0.16123	0.15609	0.15780	0.16123
36 P.meg.OjoDeLaPun	0.18696	0.15609	0.15609	0.15609	0.15780	0.16123	0.17667
37 P.meg.MediaLunaC	0.19039	0.15780	0.15780	0.16123	0.15952	0.16295	0.17839

38	P.meg.Rattlesnak	0.16295	0.18010	0.18010	0.17324	0.16295	0.16810	0.17667
39	P.meg.BearCreek	0.16467	0.18010	0.18010	0.17324	0.16295	0.16810	0.17667

Uncorrected ("p") distance matrix(continued)

	15	16	17	18	19	20	21
15 P.meg.WillowLake	-						
16 P.meg.LaPazCoPar	0.00000	-					
17 P.meg.LakeJennin	0.07033	0.07033	-				
18 P.meg.RioGrandeR	0.07239	0.07239	0.00345	-			
19 P.meg.OjoDelaPun	0.07204	0.07204	0.00858	0.00860	-		
20 P.meg.AscarateLa	0.07227	0.07227	0.00343	0.00345	0.00517	-	
21 P.meg.RioGrandeB	0.07719	0.07719	0.02058	0.01901	0.01887	0.01374	-
22 P.meg.SantaMaria	0.07719	0.07719	0.02058	0.01902	0.01887	0.01374	0.00686
23 P.meg.1.UK	0.14065	0.14065	0.13379	0.13435	0.13551	0.13247	0.13379
24 P.meg.2.UK	0.14065	0.14065	0.13379	0.13435	0.13551	0.13247	0.13379
25 Philodina.spa.2.	0.14923	0.14923	0.14923	0.15002	0.14751	0.14614	0.15094
26 Philodina.sp.1.U	0.13722	0.13722	0.15094	0.15174	0.14751	0.14790	0.15266
27 P.flav.Ro3a	0.13894	0.13894	0.14237	0.14312	0.14065	0.13929	0.14237
28 P.flav.dV2e	0.13036	0.13036	0.14408	0.14643	0.14237	0.14447	0.14751
29 P.flav.Va3e	0.16123	0.16123	0.16467	0.16706	0.16467	0.16692	0.16810
30 P.flav.Ro2h	0.15283	0.15283	0.15454	0.15537	0.15454	0.15153	0.15454
31 P.acut.TU.a	0.14813	0.14813	0.15153	0.15230	0.14807	0.14844	0.15324
32 P.acut.TU.b	0.13722	0.13722	0.13894	0.13971	0.13551	0.13587	0.14065
33 P.citr.IT.2.1	0.14946	0.14946	0.14258	0.14332	0.14258	0.13944	0.14773
34 P.citr.IT.2.2	0.15094	0.15094	0.14408	0.14483	0.14408	0.14094	0.14923
35 P.citr.IT.1.3	0.15609	0.15609	0.14751	0.14824	0.14751	0.14436	0.15266
36 P.meg.OjoDelaPun	0.16810	0.16810	0.15780	0.15854	0.15780	0.15661	0.16467
37 P.meg.MediaLunaC	0.16981	0.16981	0.15952	0.16025	0.15952	0.15832	0.16638
38 P.meg.Rattlesnak	0.16810	0.16810	0.16467	0.16544	0.16467	0.16168	0.16295
39 P.meg.BearCreek	0.16810	0.16810	0.16467	0.16545	0.16467	0.16169	0.16295

Uncorrected ("p") distance matrix(continued)

	22	23	24	25	26	27	28
22 P.meg.SantaMaria	-						
23 P.meg.1.UK	0.13208	-					
24 P.meg.2.UK	0.13208	0.00000	-				
25 Philodina.spa.2.	0.14751	0.16467	0.16467	-			
26 Philodina.sp.1.U	0.15266	0.15952	0.15952	0.10120	-		
27 P.flav.Ro3a	0.14065	0.16123	0.16123	0.11492	0.10635	-	
28 P.flav.dV2e	0.14580	0.16295	0.16295	0.11321	0.10463	0.02401	-
29 P.flav.Va3e	0.17153	0.17324	0.17324	0.15266	0.15094	0.13379	0.12178
30 P.flav.Ro2h	0.15454	0.15967	0.15967	0.11676	0.12193	0.11506	0.10992
31 P.acut.TU.a	0.15325	0.16702	0.16702	0.12226	0.11710	0.11194	0.10850
32 P.acut.TU.b	0.14065	0.15437	0.15437	0.11149	0.10120	0.10463	0.09949
33 P.citr.IT.2.1	0.14773	0.17362	0.17362	0.11681	0.12543	0.12886	0.12886
34 P.citr.IT.2.2	0.14923	0.17667	0.17667	0.11835	0.12693	0.12864	0.12864
35 P.citr.IT.1.3	0.15266	0.17324	0.17324	0.12521	0.13036	0.13208	0.13208
36 P.meg.OjoDelaPun	0.16467	0.16123	0.16123	0.14065	0.15266	0.14923	0.14580
37 P.meg.MediaLunaC	0.16638	0.16810	0.16810	0.14408	0.15437	0.15437	0.15094
38 P.meg.Rattlesnak	0.16123	0.16467	0.16467	0.15609	0.16295	0.14923	0.14580
39 P.meg.BearCreek	0.16295	0.16638	0.16638	0.15780	0.16295	0.15266	0.14923

Uncorrected ("p") distance matrix(continued)

	29	30	31	32	33	34	35
29 P.flav.Va3e	-						
30 P.flav.Ro2h	0.13903	-					
31 P.acut.TU.a	0.16370	0.12757	-				
32 P.acut.TU.b	0.15609	0.11679	0.01719	-			
33 P.citr.IT.2.1	0.15456	0.13414	0.12593	0.11512	-		
34 P.citr.IT.2.2	0.15609	0.13391	0.12745	0.11664	0.00343	-	
35 P.citr.IT.1.3	0.15780	0.13244	0.13262	0.12178	0.01372	0.01372	-
36 P.meg.OjoDelaPun	0.18010	0.16314	0.15517	0.14408	0.16146	0.16295	0.16467
37 P.meg.MediaLunaC	0.18525	0.16314	0.15689	0.14923	0.16661	0.16810	0.16981
38 P.meg.Rattlesnak	0.18010	0.15629	0.15150	0.13894	0.16150	0.15952	0.16638
39 P.meg.BearCreek	0.18010	0.15629	0.15150	0.13894	0.16150	0.15952	0.16638

Uncorrected ("p") distance matrix(continued)

	36	37	38	39
36 P.meg.OjoDelaPun	-			
37 P.meg.MediaLunaC	0.01029	-		
38 P.meg.Rattlesnak	0.14923	0.15266	-	
39 P.meg.BearCreek	0.14923	0.15266	0.00343	-

Appendix 3: Mantel test for the correlation between genetic distance and geographic distance between *Philodina megalotrocha* populations.

ANALYSIS 1

```

      MANTEL TEST for matrix correlation
      between genetic distance and geographic distance
      [user-entered genetic distances]

Correlation of genetics and geographic distance:
Z = 90031.8765, r = 0.1214, one-sided p <= 0.1450 from 1000 randomizations
(for test of negative correlations, one-sided p <= 0.8550)

= = = = =

      REDUCED MAJOR AXIS REGRESSION to calculate intercept and slope
      of genetic distance vs geographic distance
      [user-entered genetic distances]

      INTERCEPT          SLOPE          R^2          n
      -----          -----          ---          ----
Linear model:
estimate          0.1062          1.548e-05          0.0147          378
st.error          0.0025          7.926e-07
Jackknife over...
all points
  estimate          0.1061          1.544e-05          378
  st.error          0.0035          1.320e-06
populations
  estimate          0.1070          1.215e-05          28
  st.error          0.0090          7.123e-06

95% CI:
lin.model          0.1012, 0.1111          1.393e-05, 1.704e-05

jackknife over...
all points          0.0992, 0.1130          1.285e-05, 1.803e-05          378
populations          0.0886, 0.1254          -2.469e-06, 2.676e-05          28

1000 bootstraps over...
all points          0.0995, 0.1127          1.310e-05, 1.841e-05          0.0055, 0.0278          378
ind.pairs          0.0624, 0.1621          -1.473e-05, 9.477e-05          0.000, 0.152          14

99% CI:
lin.model          0.0996, 0.1127          1.344e-05, 1.753e-05

jackknife over...
all points          0.0970, 0.1152          1.204e-05, 1.884e-05          378
populations          0.0821, 0.1318          -7.590e-06, 3.188e-05          28

1000 bootstraps over...
all points          0.0973, 0.1154          1.186e-05, 1.936e-05          0.0028, 0.0322          378
ind.pairs          0.0356, 0.1669          -1.638e-05, 1.571e-04          0.000, 0.265          14

= = = = =
ANALYSIS 2

```

```

      MANTEL TEST for matrix correlation
      between genetic distance and Log(geographic distance)
      [user-entered genetic distances]

Correlation of genetics and geographic distance:
Z = 137.4232, r = 0.1780, one-sided p <= 0.0070 from 1000 randomizations
(for test of negative correlations, one-sided p <= 0.9930)

= = = = =

```

```

REDUCED MAJOR AXIS REGRESSION to calculate intercept and slope
of genetic distance vs Log(geographic distance)
[user-entered genetic distances]

          INTERCEPT          SLOPE          R^2          n
          -----          -
Linear model:
  estimate      0.04458      0.03284      0.0317      378
  st.error      0.00494      0.00167
Jackknife over...
  all points
    estimate      0.04788      0.03171      378
    st.error      0.01700      0.00551
  populations
    estimate      0.05479      0.02929      28
    st.error      0.03396      0.01095

95% CI:
  lin.model      0.03491, 0.05425      0.02957, 0.03611

  jackknife over...
  all points      0.01456, 0.08120      0.02091, 0.04251      378
  populations      -0.01488, 0.12447      0.00683, 0.05175      28

  1000 bootstraps over...
  all points      0.00107, 0.06894      0.02545, 0.04775      378
  ind.pairs      -0.18214, 0.38660      -0.08800, 0.10626      14
          0.000, 0.467

99% CI:
  lin.model      0.03187, 0.05729      0.02855, 0.03713

  jackknife over...
  all points      0.00409, 0.09167      0.01752, 0.04590      378
  populations      -0.03929, 0.14888      -0.00104, 0.05961      28

  1000 bootstraps over...
  all points      -0.02291, 0.07535      0.02311, 0.05525      378
  ind.pairs      -0.25716, 0.43750      -0.10866, 0.12737      14
          0.000, 0.808

```

=====

ANALYSIS 3

MANTEL TEST for matrix correlation
between Log(genetic distance) and geographic distance
[user-entered genetic distances]

In 5 cases, a genetic distance of zero was set to 0.000100 before logging

Correlation of genetics and geographic distance:

Z = -562658.9566, r = 0.1235, one-sided p <= 0.0190 from 1000 randomizations
(for test of negative correlations, one-sided p <= 0.9810)

=====

REDUCED MAJOR AXIS REGRESSION to calculate intercept and slope
of Log(genetic distance) vs geographic distance
[user-entered genetic distances]

In 5 cases, a genetic distance of zero was set to 0.000100 before logging

```

          INTERCEPT          SLOPE          R^2          n
          -----          -
Linear model:
  estimate      -1.263      1.730e-04      0.0152      378
  st.error       0.028      8.852e-06

```

```

Jackknife over...
all points
  estimate      -1.266          1.737e-04          378
  st.error       0.061          2.522e-05
populations
  estimate      -1.250          1.390e-04          28
  st.error       0.083          6.747e-05

95% CI:
lin.model      -1.318,-1.208      1.556e-04, 1.903e-04

jackknife over...
all points     -1.385,-1.146      1.242e-04, 2.231e-04          378
populations    -1.421,-1.079      5.253e-07, 2.774e-04          28

1000 bootstraps over...
all points     -1.377,-1.140      1.242e-04, 2.215e-04      0.0090, 0.0233          378
ind.pairs      -2.072,-0.799      -3.233e-05, 1.396e-03      0.000, 0.127          14

99% CI:
lin.model      -1.335,-1.190      1.502e-04, 1.958e-04

jackknife over...
all points     -1.422,-1.109      1.087e-04, 2.387e-04          378
populations    -1.481,-1.019      -4.798e-05, 3.259e-04          28

1000 bootstraps over...
all points     -1.412,-1.080      9.869e-05, 2.420e-04      0.0063, 0.0270          378
ind.pairs      -2.584,-0.790      -3.667e-05, 2.214e-03      0.000, 0.230          14

= = = = =
ANALYSIS 4

      MANTEL TEST for matrix correlation
      between Log(genetic distance) and Log(geographic distance)
      [user-entered genetic distances]

In 5 cases, a genetic distance of zero was set to 0.000100 before logging

Correlation of genetics and geographic distance:
Z = -929.3083, r = 0.2263, one-sided p <= 0.0020 from 1000 randomizations
(for test of negative correlations, one-sided p <= 0.9980)

= = = = =

      REDUCED MAJOR AXIS REGRESSION to calculate intercept and slope
      of Log(genetic distance) vs Log(geographic distance)
      [user-entered genetic distances]

In 5 cases, a genetic distance of zero was set to 0.000100 before logging

      INTERCEPT      SLOPE      R^2      n
      -----      -----      ---      ----
Linear model:
  estimate      -1.951      0.3668      0.0512      378
  st.error       0.055      0.0184
Jackknife over...
all points
  estimate      -1.924      0.3579      378
  st.error       0.214      0.0699
populations
  estimate      -1.843      0.3299      28
  st.error       0.342      0.1086

95% CI:
lin.model      -2.057,-1.844      0.3307, 0.4029

```

jackknife over...				
all points	-2.343,-1.505	0.2209, 0.4948		378
populations	-2.544,-1.141	0.1071, 0.5526		28
1000 bootstraps over...				
all points	-2.519,-1.616	0.2619, 0.5561	0.002, 0.198	378
ind.pairs	-5.561, 1.173	-0.7595, 1.5560	0.000, 0.779	14
99% CI:				
lin.model	-2.091,-1.810	0.3194, 0.4143		
jackknife over...				
all points	-2.475,-1.373	0.1779, 0.5378		378
populations	-2.790,-0.895	0.0291, 0.6307		28
1000 bootstraps over...				
all points	-2.838,-1.535	0.2319, 0.6579	0.000, 0.285	378
ind.pairs	-6.670, 2.138	-1.0887, 1.8813	0.000, 0.961	14

CURRICULUM VITAE

Lina Hamdan was born in Kuwait in 1973, graduated from Al-Jubeiha High School, Amman, Jordan in the spring of 1991 and entered University of Jordan in the fall of 1991. She graduated with a Bachelors degree in Biological Sciences from University of Jordan in 1995. She entered the University of Texas at El Paso, Texas, in the spring of 2007. While pursuing her Masters degree, Lina received a Research Assistant funded by the NSF ADVANCE Program and was a Teaching Assistant for the Department of Biological Sciences. She is a member of the American Microscopical Society (AMS) and the Ecological Society of America (ESA). She presented posters at the Dynamic Deserts Conference at Arizona State University, Tempe, AZ (February 2009), at Ecological Society of America meeting in Albuquerque, NM (August 2009), at the XII International Rotifer Symposium in Berlin, Germany (August 2009), and at the Southwestern Association of Naturalist meeting in Junction, TX (April 2010).

Permanent Address

Lina Kamel Hamdan

368 Shadow Mountains, Apt # 230U

El Paso, TX, 79912