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High phylogeographic structure in sylvatic vectors of Chagas disease of the genus *Mepraia* (Hemiptera: Reduviidae)

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ABSTRACT

The hematophagous Hemiptera of the subfamily Triatominae are a very diverse group with a variety of morphs, behaviors and distributions. They have great epidemiological importance because many of its members are vectors of the protozoan *Trypanosoma cruzi*, the agent of Chagas disease. *Mepraia* is a genus of Triatominae endemic to Chile responsible for transmitting *T. cruzi* in the sylvatic cycle. *Mepraia* includes three species, *M. gajardoi* (18° 30′–26° 30′ S) *M. spinolai* (26° 30′–34° 20′ S) and the recently described *M. parapatrica* in intermediate zones (24° 36′–26° 51′ S). Using mitochondrial DNA sequences, we inferred historical processes that led to the current structure of populations. Phylogeographic analyses identified three lineages, congruent with current taxonomy, and populations were highly structured. The times to the most recent common ancestor suggest that *M. spinolai* is the oldest lineage. We discuss the taxonomic and biogeographic implications of our results.

1. Introduction

Chagas disease is a serious human parasitic disease in the Americas caused by the flagellate protozoan *Trypanosoma cruzi*, and transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera: Reduviidae) (Lent and Wygodzinsky, 1979; Schofield et al., 2006). Despite the research effort dedicated to the subfamily in recent years, the taxonomic status, evolutionary relationships and phylogeographic structure of several species remain controversial, due in part to their high degree of morphological plasticity (Dujardin et al., 1999). Two genera of Triatominae occur in Chile: *Triatoma* and *Mepraia*. *Triatoma infestans* is the main domestic vector of *T. cruzi*, now controlled in several countries including Chile through widespread interventions under the Southern Cone Initiative (Dias and Schofield, 1999). *Mepraia* is endemic to the semiarid and arid regions distributed in coastal and interior valleys of northern and central Chile. While *Triatoma* is mainly associated with domestic settings in Chile, *Mepraia* is found in sylvatic areas mainly among rock piles, but it occasionally colonizes domestic and peridomestic habitats (Frías and Atria, 1998; Schofield et al., 1998; Cattan et al., 2002). *Mepraia* was first described with the inclusion of *Triatoma spinolai* Porter 1934 as *Mepraia spinolai* by Mazza et al. (1940), and revalidated by Lent et al. (1994). Lent and Wygodzinsky (1979) described the *spinolai* complex as a taxonomic group composed of *Triatoma eratyrusiformis* Del Ponte 1929, *Triatoma breyeri* Del Ponte 1929 and *Triatoma* (*Mepraia*) *spinolai* Porter 1934. *T. eratyrusiformis* and *T. breyeri* are geographically separated from *T. spinolai* (*M. spinolai*) by the Andes Range (Lent and Wygodzinsky, 1979). The same authors proposed that the species of the *spinolai* complex share a common ancestor that was separated by the uplifting of the Andes during the Miocene about 20 million years ago (Moreno et al., 2006; Campos et al., 2013).

Three species currently are included in the genus: *M. spinolai*, *M. gajardoi* and *Mepraia parapatrica* (Frías and Atria, 1998; Frías et al., 1998; Frías, 2010). Until 1998, *Mepraia spinolai* was the only species of the genus, distributed in coastal and interior valleys in Chile between 18° and 34° S (Lent and Wygodzinsky, 1979). However, on the basis of karyotypes, morphological characters and experimental crosses, coastal desert populations between 18° and 26° S were described as *M. gajardoi* (Frías et al., 1998; Jurberg et al., 2002). The remaining populations from 26° to 34° S maintained the status of *M. spinolai* inhabiting in the interior mountains from the Atacama Region to the Metropolitan Region (Frías et al., 1998). Studies using molecular markers suggested that the geographical criterion initially proposed by Frías et al. (1998) to separate the two *Mepraia* species should be reviewed (Calleros et al., 2010). A recent study using geometric morphometrics on wings of *Mepraia* species identified two distinct groups (congruent with *M. gajardoi* and *M. spinolai*), but also reported a new wing phenotype; insects with

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vestigial wings in the areas that limit the range of the two species (Campos et al., 2011). In that area (border of the Antofagasta and Atacama Regions; 24°–26° S) a new species (*M. parapatrica*) was recently described (Frías, 2010). The complex taxonomy of *Mepraia* may be resolved using an integrative approach that includes morphological and molecular characters. To our knowledge, no study has evaluated the historical processes that may have occurred within *Mepraia* leading to its current phylogeographic pattern.

In this study, we used molecular data from natural populations of *Mepraia* to explore genetic structure across northern Chile and the phylogeographic relationships of *M. parapatrica* with *M. gajardoi* and *M. spinolai*. Our study also aims to estimate the date of the most recent common ancestor (MRCA) of *Mepraia*, and discuss the biogeographic scenario that yielded the current phylogeographic structure of the three species.

2. Materials and methods

2.1. Insect collection

A total of 164 nymphs and adults of *Mepraia* from 13 localities in Chile ranging from 18° to 33° S were collected between 2007 and 2010 in the coast and interior valleys (Table 1). Two *Triatoma eratirusiformis* from Salinas de Bustos, and two *Triatoma breyeri* from Patquia Viejo were also collected; both locations belong to the Department of Independencia, Province of La Rioja, Argentina (courtesy of S Catalá). Insects were manually collected by trained people. Bugs were dissected in the laboratory and the limbs were kept in 70% ethanol at −20 °C.

2.2. Mitochondrial DNA extraction, amplification, and sequencing

Genomic DNA from legs was extracted using the DNA extraction kit EZNA Tissue DNA® according to manufacturer's instructions. A 636-bp fragment of the mitochondrial cytochrome oxidase subunit-I (COI) gene and a 682-bp fragment of the cytochrome b (Cyt b) gene were amplified via polymerase chain reaction (PCR) using the primers LCO1490 (forward) (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (reverse) (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) for the Cyt b gene (Folmer et al., 1994), and the primers 7432 (forward) (5′-GGACGWGGWATTTATTATGGATC-3′) and 7433 (reverse) (5′-GCWCCAATTCARGTTARTAA-3′) for the COI gene (Monteiro et al., 2003). The following conditions were used to amplify both Cyt b and COI genes: an initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 1 min of denaturation, 45 °C for 1 min of annealing, and 72 °C for 1 min of extension, followed by a final extension of 10 min. Verification of successful amplification was assessed by 2% agarose gel electrophoresis. Sequencing reactions were conducted by Macrogen Inc. (Korea) using the same PCR primers. Sequences were edited using Bioedit 7.0.8.0 (Hall, 1999) and aligned using Clustal W (Thompson et al., 1994) as implemented in Bioedit (Hall, 1999). After the alignments, sites that showed nucleotide substitutions were re-examined by visual inspection of each individual's raw chromatogram. COI and Cyt b gene sequences were deposited in Genbank with accession numbers KC236913-KC236980 and manually concatenated.

2.3. Phylogenetic analyses and population structure

The number of haplotypes was calculated with DnaSp 5.1 (Librado and Rozas, 2009). Phylogenetic trees were inferred by maximum likelihood (ML) and Bayesian inference. ML analysis was inferred using the online platform PhyML 3.0 (Guindon and Gascuel, 2003). The best-fitting model of nucleotide substitution (TrN + G = 0.129) was selected using the Akaike information criterion (Akaike, 1974) on the concatenated matrix implemented in the program jmodelTest 0.1.1 (Posada, 2008). Nodal supports were estimated by the bootstrap method (Felsenstein, 1985) with 1000 replicates using PhyML 3.0 (Guindon and Gascuel, 2003). We considered branches receiving >70% bootstrap support to be well-supported (Hillis and Bull, 1993; Wilcox et al., 2002).

Bayesian analyses were performed using MrBAYES v.3.1.2b (Ronquist and Huelsenbeck, 2003), using as prior parameters values obtained from jModeltest. We ran Metropolis-coupled Markov Chain Monte Carlo simulations (MCMCMC) with four incrementally heated chains. From random starting trees, four independent runs (two replicas of two simultaneous, independent runs each) of 1×10^7 generations each were performed, with the resulting trees sampled every 1000 generations. We determined when a stationary state was reached (to discard the burn-in samples; 1000 trees) by plotting the logarithmic likelihood scores of sample points against generations. The last 9000 trees were used to compute a 50% majority rule consensus tree. The percentage of samples that recover any particular clade in this tree represents that clade's posterior probability; we considered $P \geqslant 95\%$ as evidence for significant support (Alfaro et al., 2003). Trees were visualized using the FigTree v1.1.2 program, available at http://tree.bio.ed.ac.uk/software/figtree/. *T. infestans*, *T. breyeri* and *T. eraturiformis* were used as outgroups based on their phylogenetic proximity to the *spinolai* complex (Hypsa et al., 2002; de Paula et al., 2005). The Median Joining method (Bandelt et al., 1999) implemented in Network 4.2.0.1 software was used alternatively to assess intraspecific relationships of all *Mepraia* samples. The pairwise differences between populations were calculated using the fixation index (Fst) and the significance of the values was obtained by 1000 permutations using Arlequin 3.5.1 (Excoffier and Lischer, 2010). Genetic structure was estimated using an analysis of molecular variance (AMOVA; Excoffier et al., 1992) using Arlequin 3.5.1 (Excoffier and Lischer, 2010); the analysis assesses the proportion of genetic variation explained by different population hierarchies. The level hierarchies were defined on the basis of the phylogenetic tree and haplotype network.

2.4. Molecular diversity and neutrality tests

We evaluated genetic variability of concatenated COI and Cyt b sequences (1022-nt) for each population using DnaSp 5.1 (Librado and Rozas, 2009) by estimating the number of segregating sites (S), number of haplotypes (h), the average number of nucleotide differences (k), haplotype (Hd) and nucleotide diversity (π). Neutrality tests assume that populations are in mutation-drift and migration-drift equilibrium, so we assessed population equilibrium in *Mepraia* sp. by performing Tajima's D-test (Tajima, 1989), Fu's Fs-test (Fu, 1997), and the R_2-test (Ramos-Onsins and Rozas, 2002), testing the significance of the statistics from 5000 simulated samples. Significantly negative values in neutrality tests reflect an excess of low frequency (Tajima's D) mutations in a population relative to what is expected under a standard neutral model, which is consistent with either directional or purifying selection (i.e., they are not in mutation-drift equilibrium) or an increase in population size (i.e., they are not in migration-drift equilibrium). Fu's Fs statistics measures departure from neutrality by detecting excess or deficiency of alleles, consistent with recent population expansion or genetic hitchhiking (negative values), and recent population bottlenecks or selection (positive values). The Ramos-Onsis R_2-test is based on the difference between the number of singleton mutations and the average number of nucleotide differences, with lower values of the statistic expected under a population growth event. Population equilibrium tests were performed using DnaSp 5.1 (Librado and Rozas, 2009).

Table 1
Descriptive statistics of genetic variation and neutrality tests of *Mepraia* sequences.

Lineage/sampling site	Coordinates	*N*	*S*	*h*	*k*	Hd ± SD	Π ± SD	Fu's-Fs	R_2	Tajima's D
M. gajardoi		65	46	15	15.62	0.920 ± 0.011	0.01503 ± 0.00046	7.6189 (*P* = 0.977)	0.1664 (*P* = 0.981)	1.9393 (*P* = 0.979)
M. parapatrica		27	22	3	10.84	0.658 ± 0.042	0.01061 ± 0.00087	16.6243 (*P* = 1.000)	0.1574 (*P* = 0.994)	3.2310 (*P* = 0.999)
M. spinolai		72	104	15	35.21	0.896 ± 0.016	0.03445 ± 0.00198	24.7310 (*P* = 1.000)	0.1690 (*P* = 0.990)	2.1849 (*P* = 0.988)
Corazones, Arica, APR	18°28′47″ S; 70°19′27″ W	13	5	5	1.026	0.628 ± 0.143	0.00100 ± 0.00036	−1.6933 (*P* = 0.151)	0.1282 (*P* = 0.169)	−1.2958 (*P* = 0.113)
Caleta Vitor, APR	18°45′45″ S; 70°20′34″ W	13	5	4	1.359	0.679 ± 0.112	0.00133 ± 0.00032	0.1571 (*P* = 0.577)	0.1497 (*P* = 0.404)	−0.5582 (*P* = 0.301)
Caleta Camarones, APR	19°12′16″ S; 70°16′08″ W	13	5	2	2.692	0.538 ± 0.060	0.00263 ± 0.00029	5.6491 (*P* = 1.000)	0.2692 (*P* = 1.000)	2.3921 (*P* = 0.997)
Rio Seco, TR	21°00′06″ S; 70°09′52″ W	15	6	4	1.676	0.619 ± 0.120	0.00164 ± 0.00060	0.8573 (*P* = 0.745)	0.1397 (*P* = 0.414)	−0.3208 (*P* = 0.421)
San Marcos, TR	21°06′56″ S; 70°07′30″ W	11	1	2	0.545	0.545 ± 0.072	0.00053 ± 0.00007	1.1365 (*P* = 0.899)	0.2727 (*P* = 0.491)	1.4427 (*P* = 0.865)
El Medano, ANR	24°36′51″ S; 70°33′31″ W	11	0	1	0.000	0.000 ± 0.000	0.00000 ± 0.00000			
Caleta Zenteno, ATR	26°51′08″ S; 70°48′36″ W	16	1	2	0.458	0.458 ± 0.095	0.00045 ± 0.00009	1.0962 (*P* = 0.816)	0.2292 (*P* = 0.475)	1.0344 (*P* = 0.745)
Llanos de Challe, ATR	28°08′52″ S; 71°04′32″ W	8	6	4	2.607	0.821 ± 0.101	0.00255 ± 0.00040	0.7016 (*P* = 0.650)	0.2040 (*P* = 0.637)	0.5904 (*P* = 0.751)
Peral Norte, ATR	28°43′21″ S; 70°31′02″ W	14	1	2	0.143	0.143 ± 0.119	0.00014 ± 0.00012	−0.5948 (*P* = 0.347)	0.2575 (*P* = 0.494)	−1.1552 (*P* = 0.369)
Caleta Toro, CR	30°44′30″ S; 71°42′05″ W	14	0	1	0.000	0.000 ± 0.000	0.00000 ± 0.00000			
Monte Patria, CR	30°51′16″ S; 70°41′51″ W	14	8	3	3.604	0.582 ± 0.092	0.00353 ± 0.00059	4.6932 (*P* = 0.982)	0.2187 (*P* = 0.958)	1.6321 (*P* = 0.959)
Reserva Las Chinchillas, CR	31°30′28″ S; 71°06′19″ W	11	4	3	1.891	0,691 ± 0.086	0.00185 ± 0.00023	2,0093 (*P* = 0,915)	0.2364 (*P* = 0.945)	1.4083 (*P* = 0.950)
Til Til, MR	33°06′19″ S; 70°55′53″ W	11	1	2	0.327	0.327 ± 0.153	0.00032 ± 0.00015	0.3563 (*P* = 0.611)	0.1636 (*P* = 0.000)	−0.1000 (*P* = 0.606)

APR, Arica and Parinacota Region; TR, Tarapacá Region; ANR, Antofagasta Region; ATR, Atacama Region; CR, Coquimbo Region; MR, Metropolitan Region; N, Number of individuals; S, number of segregating sites; h, number of haplotype; k, pairwise difference; Hd, haplotype diversity; Π, nucleotide diversity; SD, standard deviation. Significance (*P*-values) of the statistics is shown in parenthesis.

2.5. Dates of the MRCA

We used the Bayesian Markov Chain Monte Carlo (BMCMC) method available in BEAST v1.6.2 (Drummond and Rambaut, 2007) to estimate dates of MRCA for *Mepraia* lineages (*gajardoi*, *parapatrica* and *spinolai*). This analysis employed the conservative Bayesian skyline coalescent prior, using both strict, relaxed (uncorrelated lognormal), and random local molecular clocks, which produced similar results. Convergence of the MCMC chains was reached after running the BEAST program for 2×10^8 steps; we discarded 10% burn-in and the statistical uncertainty was depicted in values of the 95% Highest Probability Density (HPD). Convergence of the chain, sampling and mixing were confirmed by inspection of the MCMC samples using the program Tracer v.1.5 (Rambaut and Drummond, 2009). We assumed a prior fixed mean substitution rate of 0.016 sub/site/my calculated for COI and COII (Arensburger et al., 2004).

3. Results

3.1. Phylogenetic analyses and population structure

After editing the COI (508 bp) and Cyt b (514 bp) gene sequences, we obtained a complete matrix of 164 concatenated sequences of *Mepraia* with 1022 bp that produced 33 haplotypes. *T. eratyrusiformis* and *T. breyeri* (outgroups) only have the Cyt b gene available. Fig. 1 shows the ML haplotype tree. Bayesian topology (see supplementary data) differed from that of ML by not resolving haplotype from Medano locality (haplotype 16) within lineage C (Fig. 1). In the ML topology three well-defined lineages are observed. The first lineage (lineage A) included the haplotypes from Corazones, Caleta Vitor, Caleta Camarones, Río Seco and San Marcos, grouping all samples currently assigned to *M. gajardoi*. Within the second lineage (lineage B) the haplotypes from Llanos de Challe National Park, Peral Norte, Caleta Toro, Monte Patria, Las Chinchillas National Reserve and Til Til were included (*M. spinolai* lineage). The samples from Medano and Caleta Zenteno were grouped (except in the Bayesian topology) in the third lineage (*M. parapatrica*; lineage C). The *M. gajardoi* and *M. spinolai* lineages were supported by likelihood bootstrap and posterior probabilities while the *M. parapatrica* lineage was supported only by likelihood bootstrap. The *M. gajardoi* lineage appears close to the *M. spinolai* lineage, but unsupported.

The unrooted phylogeographic network shows high population structure (Fig. 2), with haplotypes primarily associated with geographic locations. Only two haplotypes were shared across different localities of the same species (*M. gajardoi*), between Caleta Vitor and Caleta Camarones (haplotype 9) and between Río Seco and San Marcos (haplotype 12).

AMOVA was performed to assess substructure within *Mepraia* populations. Three groups were defined based on the three lineages of the phylogenetic hypothesis. The percentage of variance contributed by different levels was 56.4% between groups, 41.8% among populations within their group, and 1.8% of the variation contributed by the variation within populations. The values of the fixation index were high and significant ($P < 0.05$): F_{CT}: 0.56376 (associated with the variation between groups), F_{SC}: 0.95880 (associated with the variation among populations within its group), F_{ST}: 0.98203 (total coefficient of differentiation). $F_{CT} = 0.564$, ($P < 0.05$); F_{SC}: 0.959, ($P < 0.05$); $F_{ST} = 0.982$, ($P < 0.05$). Paired differentiation among all *Mepraia* populations using the F_{ST} index showed high and significant ($P < 0.05$) values in all comparisons.

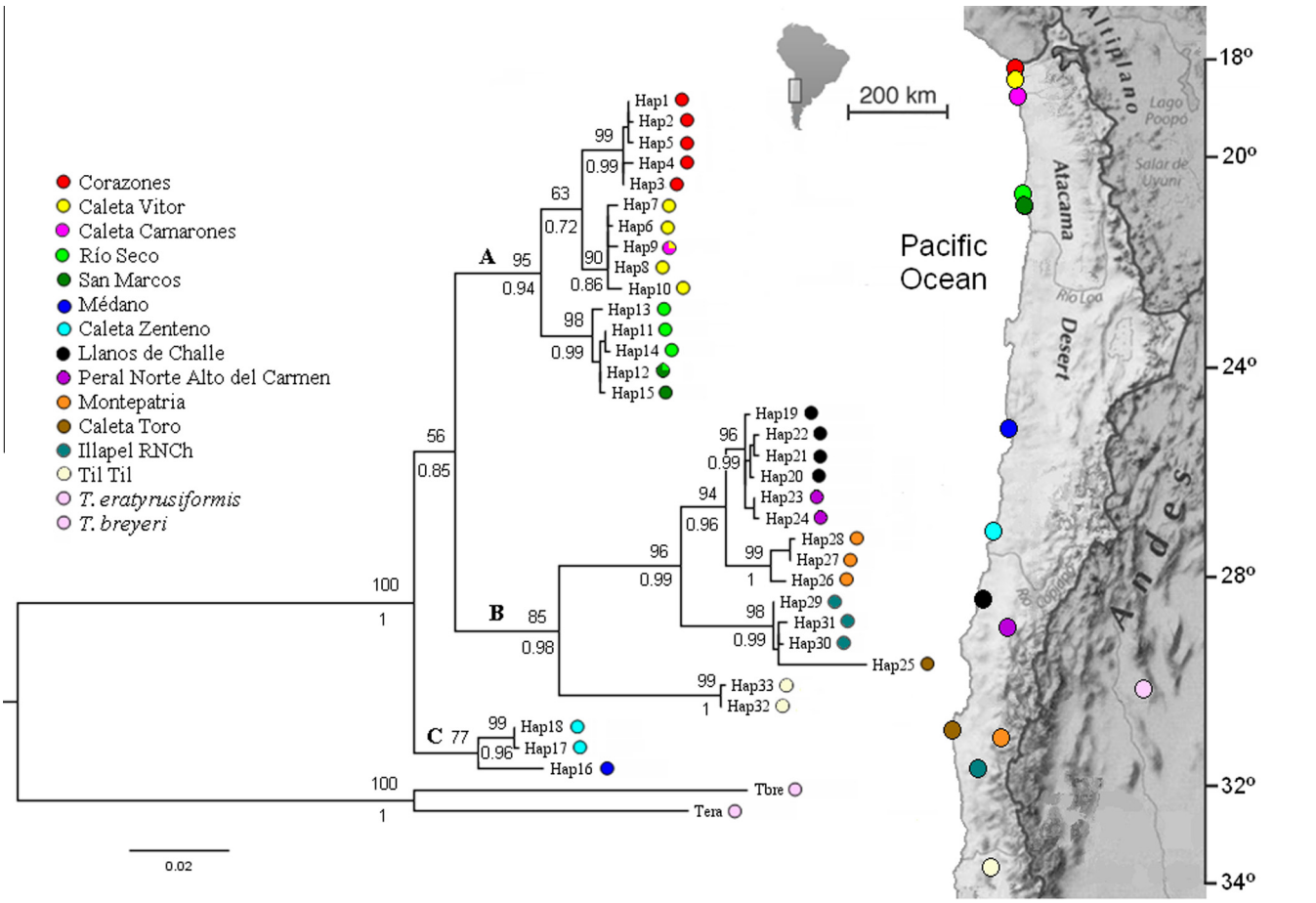

Fig. 1. Maximum likelihood phylogenetic haplotype tree of *Mepraia* species based on Cyt b and COI concatenated gene sequences (1022 bp) inferred with the model TrN + G ($G = 0.129$). A: *M. gagardoi* lineage, B: *M. spinolai* lineage, C: *M. parapatrica* lineage. Numbers above the branches are the support values derived from bootstrap resampling with 1000 pseudoreplicates, and numbers below the branches are the Bayesian posterior probabilities. Colors represent geographic distribution of haplotypes. The bar represents the number of substitutions per site.

3.2. Molecular diversity and neutrality tests

Genetic variability index and neutrality tests are shown in Table 1. Estimates of genetic variation varied across lineages. For *M. gajardoi*, haplotype diversity (Hd) was 0.920 ± 0.011 and nucleotide diversity (Π) was 0.01503 ± 0.00046. *M. spinolai* Hd was 0.896 ± 0.016 and Π was 0.03445 ± 0.00198. However, the *M. parapatrica* lineage showed lower Hd (0.658 ± 0.042) and Π (0.01061 ± 0.00087) values. Historical processes such as demographic contraction and expansion were evaluated under the assumption of neutrality using Cyt b and COI sequences of *Mepraia*. Neutrality tests were non-significant in all lineages analyzed, rejecting the hypothesis of sudden expansion (Table 1).

3.3. Dates of the MRCA

The time of the MRCA for the *Mepraia* genus was estimated at 3.6 million years ago (Mya; 95% HPD = 2.8–4.5). The estimated MRCA for the *M. spinolai* lineage was 2.9 Mya (95% HPD = 2.1–3.7). The MRCA for the *M. gajardoi* lineage was estimated to be between 0.66–1.36 Mya (95% HPD; mean of 0.99 Mya), and for *M. parapatrica* between 0.40–1.01 Mya (95% HPD; mean of 0.69 Mya).

4. Discussion

The haplotype phylogeny of *Mepraia* showed three lineages, each congruent with the three current described species: *M. gajardoi*, *M. spinolai*, and *M. parapatrica*. Two lineages were supported by likelihood bootstrap and posterior probabilities (*M. gajardoi* and *M. spinolai*), while the *M. parapatrica* lineage was supported only by likelihood bootstrap. This finding agrees with the highly structured haplotype network in which each group appears separated by more than 40 mutational steps (Fig. 2). Our results are concordant with a previous reported topology using COI (Calleros et al., 2010), but disagree in that localities from Antofagasta Region (assigned to *M. parapatrica*) were grouped in a supported clade, while the locality of Caleta Bandurrias (from Antofagasta Region) appeared close (but unsupported) to the *gajardoi* lineage in Calleros et al. (2010). Previous analyses with nuclear markers also showed two clades, but differed from the mitochondrial topology in including Caleta Bandurrias within the *spinolai* lineage (Calleros et al., 2010), a finding interpreted as result of ancient mitochondrial introgression or retention of ancestral polymorphisms. Geometric morphometric analyses on wings of *Mepraia* have allowed identification of two distinct groups consistent with *M. gajardoi* and *M. spinolai* (Campos et al., 2011), although the same study reported that male kissing bugs collected in the Antofagasta Region have vestigial wings, an alternative morphological feature to brachypterous *M. gajardoi* and micropterous, brachypterous and macropterous *M. spinolai*. Also, cytogenetic evidence (heterochromatizination of telomeres of autosomes) of the *M. parapatrica* lineage shows agreement with *M. spinolai* lineages as suggested by ITSs (Calleros et al., 2010; Frías, 2010). Ecological features show that *Mepraia* species inhabit areas with different characteristics. *M. gajardoi* inhabits northern coastal

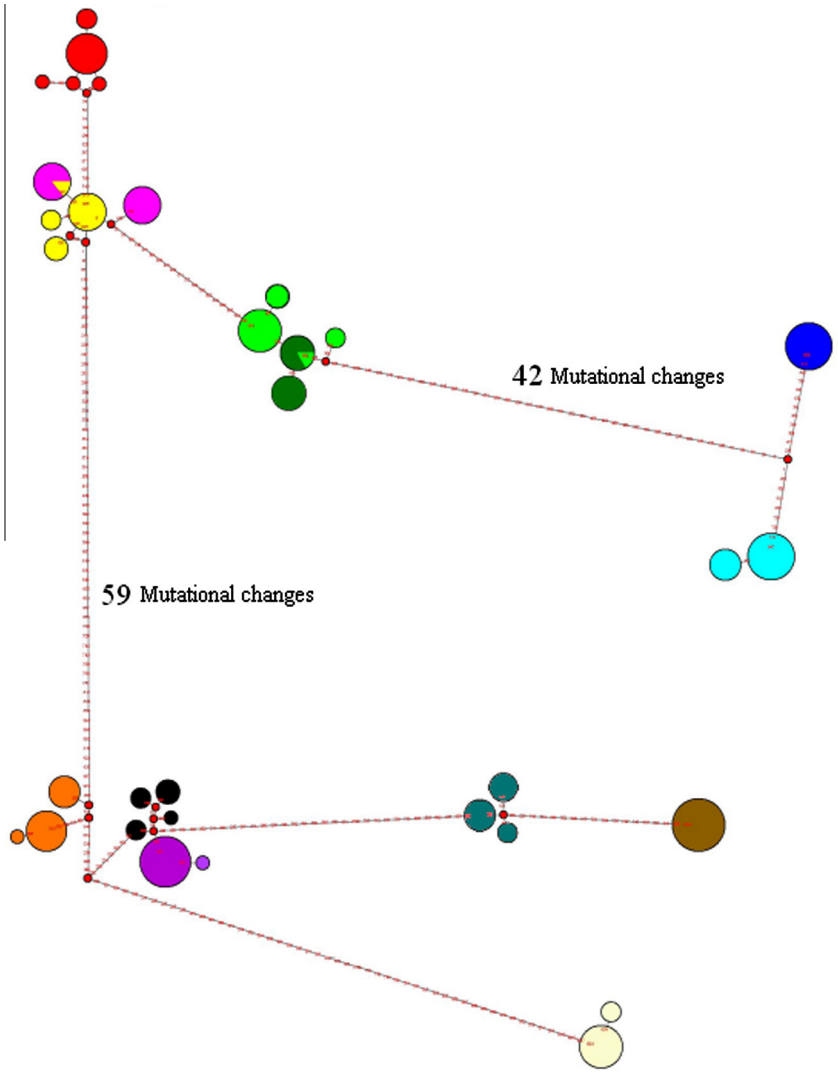

Fig. 2. Unrooted phylogeographic network of *Mepraia* using Cyt b and COI sequences with haplotypes depicted according to sampled localities. Size of circles represents the number of individuals per haplotypes. Colors depicted according to Fig. 1.

areas, while *M. spinolai* dwells within the semiarid valleys and some coastal areas (Frías et al., 1998). *M. parapatrica* inhabits the coastal area of the Antofagasta Region with climatic and ecological intermediate characteristics with higher ecological diversity than those inhabited by *M. gajardoi*. Therefore, ecological together with molecular (Frías, 2010; Calleros et al., 2010, and morphological (Campos et al., 2011) characteristics indicate that populations of *Mepraia* in the Antofagasta Region of Chile harbor particular features that differ from those of the *gajardoi* and *spinolai* lineages. However, the specific status of *M. parapatrica* remains controversial given the widely recognized morphological plasticity within the subfamily Triatominae (Dujardin et al., 1999), and the presumptive introgression/hybridization processes acting within *Mepraia* (Calleros et al., 2010). An integrative taxonomic approach (Fujita et al., 2012; Mas-Coma and Bargues, 2009) may be implemented to elucidate the specific status of these populations.

The phylogeographic network showed that haplotypes appeared mostly segregated according to their geographic origin. Only two haplotypes were shared by individuals from different but geographically close localities within *M. gajardoi* (haplotype 9 shared between Caleta Vitor and Caleta Camarones; haplotype 12 shared between Río Seco and San Marcos). These findings are congruent with our AMOVA results, which show that the highest molecular variance was explained among groups (lineages), and among populations within groups. The highly structured haplotype distribution (high and significant Fst values) was clear also at a small spatial scale (close populations such as Corazones vs. Caleta Vitor) suggesting low interchange of alleles probably derived from reduced dispersal capacity and the generation of colonies with low intrapopulation variability and high inbreeding. Also, a very low home range and patch distribution reported in *M. spinolai* (Botto-Mahan et al., 2005) may contribute to the observed high population structure. The high genetic structure in *Mepraia* is a pattern found at different spatial scales within Triatominae (e.g., Piccinali et al., 2011; Waleckx et al., 2011). Although the two mtDNA markers produced a reliable dataset with a significant nucleotide length analyzed (e.g., 40–50 mutational steps are a signal of strong genetic differentiation), they are not independent markers, since the genes evolved together. Studies should be corroborated with independent nuclear/ribosomal markers for further reliability.

The major geographical barrier that explains the distribution of this genealogy of genes is the emergence of the Andes highlands that began to have significant heights in the Miocene. After the formation of the Atacama Desert in the late Pliocene (Hartley and Chong, 2002) later population structure was shaped by orogenic changes and volcanic climate during the Late Pleistocene (Vuilleumier, 1971; Solari, 2011). Thus, the bifurcation of the ancestral population with the rise of the Andes originated *Mepraia* and

Argentine lineages, and the present northern populations remained in the coastal zone after the Atacama Desert formation in the late Pliocene.

Mitochondrial estimations of the MRCA suggest that the origin of the genus *Mepraia* is pre-Pleistocene (approximately 3.6 Mya). The *M. spinolai* lineage was inferred as the oldest, followed by *M. gajardoi* (0.99 Mya), with *M. parapatrica* as the most recent (0.66 Mya). Ancient (although more recent) estimations of MRCA were also found for the kissing bug *T. infestans* (Torres-Pérez et al., 2011), showing that the evolutionary history of the kissing bugs in South America is not recent, and that current lineages diverged a long time ago. Neutrality tests suggest that populations are in demographic equilibrium, which may be explained by the persistence of old alleles in the genetic pool of populations that have not been affected by strong bottlenecks and/or have not experienced sudden demographic changes due to repeated climatic fluctuations. In fact, Quaternary glaciations affected mainly areas in both hemispheres at high latitudes (Hewitt, 2004; Rabassa et al., 2011). Although climate changes formed glaciers in different places in the Andes (Rodbell et al., 2009), we speculate that the areas where *Mepraia* species inhabit (low lands in coastal and interior areas of north-central Chile) were not deeply affected by those climatic fluctuations. Previous morphological and chromosome analyses produced the suggestion that speciation within *Mepraia* would have followed a north–south dispersal, starting with the *gajardoi* lineage as the most ancestral, followed by the *parapatrica* lineage, with *spinolai* as the most derived lineage (Frías, 2010). Our estimation of *spinolai* as the lineage harboring the ancestral haplotypes suggests a different timing and mode of speciation. However, we acknowledge the limitations of our study based on two mitochondrial markers and the very large variance of our estimations; therefore additional molecular markers are necessary to investigate further this dynamics.

Our study assesses the phylogeographic structure observed for *Mepraia* in Chile, and raises new questions regarding the impact of the highly structured populations in the maintenance and transmission of *T. cruzi* among wild animals, and ultimately in humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.04.036.

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