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Short Communication

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High genetic diversity in the harvestman *Geraeocormobius sylvarum* (Arachnida, Opiliones, Gonyleptidae) from subtropical forests in north-eastern Argentina revealed by mitochondrial DNA sequences

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Abstract

This research was aimed to analyse the genetic diversity of *Geraeocormobius sylvarum*, a forest-dwelling Neotropical harvestman with a disjunct distribution, separated by approximately 630 km of semi-arid environments. The usefulness of a fragment of the cytochrome c oxidase subunit I (COI) mitochondrial gene as molecular marker was tested in 109 individuals. Results showed high levels of both haplotype and nucleotide diversity in populations corresponding to north-eastern Argentina, the core area of the species range. A strong genetic structuring was detected, supported by both the phylogenetic trees and the haplotype network, with six identifiable haplogroups. Populations of the Yungas ecoregion did not show significant diversity levels, suggesting a putative recent introduction of the species into that region. The overall results suggest that the present genetic diversity of the species is consistent with past fragmentation events of the species range (in refuges?), probably during the Last Glacial Maximum. The COI gene was concluded to be a well-suited marker to associate past environmental events with the high genetic diversity observed in this species.

Key words: Neotropics – harvestmen – disjunct distribution – cytochrome oxidase I – genetic diversity

Introduction

Geraeocormobius sylvarum Holmberg 1887 (Opiliones, Gonyleptidae, Gonyleptinae) is a conspicuous harvestman species, widely spread over north-eastern Argentina, as well as in adjacent areas of Brazil and Paraguay (Acosta et al. 2007; Acosta 2008). The distribution of *G. sylvarum* encompasses predominantly the Paranense biogeographic region (Cabrera and Willink 1973), thereby matching the 'Misiones opiliogeographical area' recognized by Acosta (2002). The species also extends marginally through gallery forests along the Paraná and Uruguay Rivers, into the adjacent 'Mesopotamian *sensu stricto* (*ss*)' opiliogeographic area. It was also found in the Yungas ecoregion (montane rainforests) of the Province of Tucumán, north-western Argentina, being these disjunct populations separated from the core area by a gap of approximately 650 km (Fig. 1). Between both regions lies the thorny semi-arid Chaco, whose climatic conditions – principally the marked decrease of humidity – likely act as an effective barrier for the distribution of this species (Acosta 2008). Although the causes of this disjunction are so far unknown, Nores (1992) and Acosta (2002) postulated for birds and other harvestmen, respectively, a hypothetical paleoconnection between the Paranense or Mesopotamian areas with the Yungas during warmer/more humid phases of the Pleistocene, in which forests are deemed to have formed an ephemeral corridor across the sub-xeric Chaco (thus enabling Mesopotamian or Paranense species to reach the Yungas). However, the evidence for *G. sylvarum* is not conclusive to support that paleobridge; in fact, data at hand cannot exclude the possibility that *G. sylvarum* has actually been introduced in the Yungas by human action (Acosta 2008).

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One powerful strategy to test these alternative hypotheses for the disjunction (historical versus introduction) is the population analysis using suitable molecular markers. Understanding the organismal biodiversity based on the analysis of genetic variation has important implications for the study of the evolutionary history and the population genetic structure (Zhou et al. 2013). Population genetics analyses in harvestmen are relatively scarce (e.g. Boyer et al. 2007; Thomas and Hedin 2008; Derkarabetian et al. 2010; Arthofer et al. 2013; Fernández and Giribet 2014; Kumekawa et al. 2014). Moreover, the species analysed in those studies belong to taxonomic groups phylogenetically distant to *G. sylvarum*, emphasizing the importance of performing a population genetic study in the latter species, the first research of this kind in a member of the family Gonyleptidae.

The genetic diversity detected with mitochondrial markers can help explain the demographic history of a given population and to propose hypotheses about the boundaries among genetically divergent groups (Avise 2000). Additionally, these markers, which follow a matrilineal inheritance, do not undergo rearrangements or recombination and have a reduced coalescent time and high mutation rates. Therefore, they have been extensively used for analysing relationships between species and populations of recent divergence, patterns of colonization, occurrence of bottlenecks and other related processes in natural populations (Templeton 2006). Accordingly, the purpose of the present study was to test the usefulness of the mitochondrial cytochrome c oxidase subunit I (COI) gene diversity as a valid marker, to get a better knowledge of the genetic structure of natural populations of *G. sylvarum* occurring in both portions of its disjunct range. In addition, the historical and environmental processes suffered by populations of *G. sylvarum* in their different areas of distribution were also examined.

Materials and Methods

Sampling

Molecular data were obtained from 109 individuals collected in 30 localities in NE and NW Argentina, in Provinces of Misiones (15 localities),

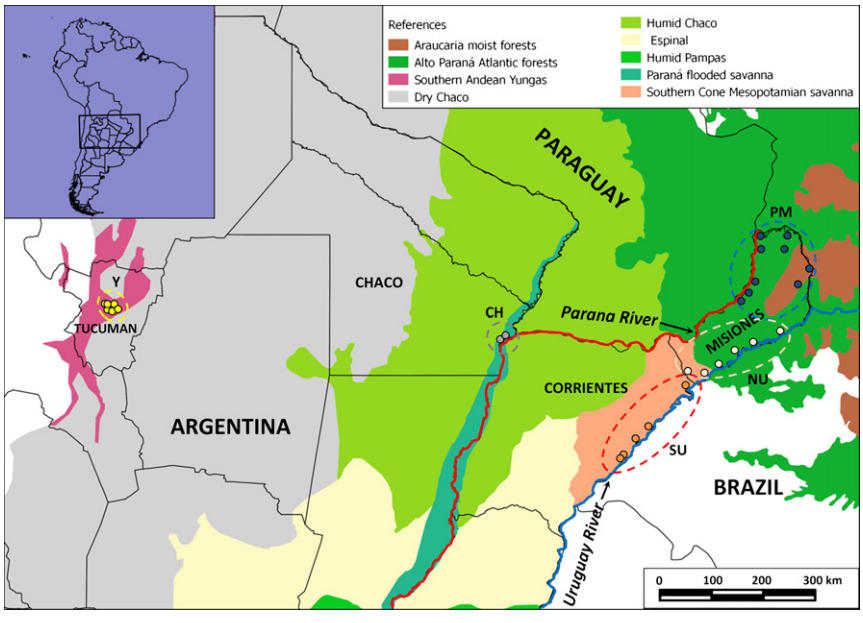

Fig. 1. Sampled localities of *Geraeocormobius sylvarum*. Records are grouped according to the different geographic sectors of the species, as defined in the text and Table 1: Tucumán Yungas (Y); Paraná River + northern Misiones (PM); northern Uruguay River (NU); southern Uruguay River (SU); Chaco (CH). Ecoregional names are according to Olson et al. (2001).

Table 1. Diversity measures for populations of *Geraeocormobius sylvarum* included in this study

Locality	Province	*N*	*Nh*	*Np*	*h*	π_n
Bañado Alto Paraná	Misiones – PM	2	2	6	1.0000 ± 0.5000	0.056075 ± 0.060568
Bernardo de Irigoyen	Misiones – PM	3	3	26	1.0000 ± 0.2722	0.161994 ± 0.124853
Comandante Andresito	Misiones – PM	4	4	4	1.0000 ± 0.1768	0.020249 ± 0.016727
Cruce Caballero	Misiones – PM	5	5	18	1.0000 ± 0.1265	0.077570 ± 0.050683
Eldorado	Misiones – PM	3	3	48	1.0000 ± 0.2722	0.299065 ± 0.227051
Montecarlo	Misiones – PM	1	1	NA	NA	NA
Garuhapé	Misiones – PM	3	1	NA	NA	NA
Urugua-í	Misiones – PM	4	3	2	0.8333 ± 0.2224	0.009346 ± 0.009259
Puerto Iguazú	Misiones – PM	6	5	32	0.9333 ± 0.1217	0.142679 ± 0.086070
Alba Posse	Misiones – NU	5	4	21	0.9000 ± 0.1610	0.110280 ± 0.070531
Apóstoles	Misiones – NU	5	1	NA	NA	NA
Concepción de la Sierra	Misiones – NU	3	1	NA	NA	NA
El Soberbio	Misiones – NU	5	4	33	0.9000 ± 0.1610	0.138318 ± 0.087529
Itacaruaré	Misiones – NU	5	2	16	0.4000 ± 0.2373	0.059813 ± 0.039891
Panambí	Misiones – NU	5	3	3	0.7000 ± 0.2184	0.014953 ± 0.012323
Agua Santa	Corrientes – SU	3	1	NA	NA	NA
Garruchos	Corrientes – SU	4	3	22	0.8333 ± 0.2224	0.115265 ± 0.079103
Cuays 1	Corrientes – SU	4	2	8	0.5000 ± 0.2652	0.037383 ± 0.028095
Yapeyú 0	Corrientes – SU	3	1	NA	NA	NA
Yapeyú 1	Corrientes – SU	2	1	NA	NA	NA
Yapeyú 2	Corrientes – SU	2	2	18	1.0000 ± 0.5000	0.168224 ± 0.172834
10 km Pt. Antequera	Chaco – CH	1	1	NA	NA	NA
7 km Pt. Antequera	Chaco – CH	5	2	5	0.6000 ± 0.1753	0.028037 ± 0.020476
2 km Villa Nougués	Tucumán – Y	5	2	1	0.6000 ± 0.1753	0.005607 ± 0.006143
2 km El Cristo	Tucumán– Y	1	1	NA	NA	NA
M. García Fernández	Tucumán– Y	3	2	1	0.6667 ± 0.3143	0.006231 ± 0.007771
Road to Villa Nougués	Tucumán– Y	4	2	1	0.5000 ± 0.2652	0.004673 ± 0.005793
San Javier-1a	Tucumán– Y	4	2	3	0.5000 ± 0.2652	0.014019 ± 0.012510
San Javier-1b	Tucumán– Y	4	2	1	0.6667 ± 0.2041	0.006231 ± 0.006992
San Javier-2	Tucumán– Y	5	1	NA	NA	NA

Acronym after the Province name indicates the sector of the species' range to which these localities belong (see also Fig. 1): Y – Tucuman Yungas; PM – Paraná River + northern Misiones; SU – southern Uruguay River; NU – northern Uruguay River and CH – Chaco. References: sample size (*N*), number of haplotypes (*Nh*), number of polymorphic sites (*Np*), haplotype diversity (*h*) and nucleotide diversity (π_n). Collection details for samples are given in Appendix I. Very close localities with the same name which are only differentiated by a number or a letter were considered as one group in AMOVA analysis (Table 2).

Tucumán (7), Corrientes (6) and Chaco (2) (Table 1 and Fig. 1). All the specimens are stored in the freezer collection (CDA-F) of the Cátedra de Diversidad Animal I, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (see Appendix for a detail of analysed samples). To enable comparisons, sampled localities from the 'core area' of *G. sylvarum* ('Paranense region' from now on) were grouped in differ-

ent geographic sectors: Paraná River + northern Misiones (PM), northern Uruguay River (NU), southern Uruguay River (SU) and Chaco (CH); localities from the Yungas are referred to as 'Y' (Table 1 and Fig. 1).

DNA extraction, amplification, sequencing and alignment

DNA was extracted using a standard phenol/chloroform technique, precipitated with ethanol and resuspended in Tris-EDTA buffer (Sambrook et al. 1989), then stored at −20°C. The COI gene was amplified using primers LCO1490 and HCO2198 (Edgecombe et al. 2002). PCR amplifications were performed in a volume of 25 μl containing 2.5 μl of buffer 10X with $(NH_4)SO_4$, 2.5 μl $MgCl_2$ (25 mM), 0.25 mM of each dNTPs, 0.7 μM of each primer, 0.15 μl (5 $U/\mu l^{-1}$) of Taq polymerase (Fermentas) and 1 μl of DNA (≈15 $ng/\mu l^{-1}$) template. The reaction conditions were as follows: initial denaturation at 94°C for 3 min, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 1 min and 30 s and finally elongation at 72°C for 1 min and 30 s; after the last cycle, a final extension step of 7 min at 72°C was implemented. The reactions were performed in an automated thermal cycler Biometra (Göttingen, Germany); PCR products were controlled in 1% agarose gels, stained with ethidium bromide and visualized under UV light.

PCR products were purified with the Silica Bead DNA Gel Extraction Kit (Fermentas, USA) and sequenced directly at the Instituto de Biotecnología, Unidad de Genómica, INTA Castelar Sequencing Center (Hurlingham, Argentina). Both forward and reverse sequences were obtained using the same primers set used for PCR amplifications. The edition of the sequences was controlled using the CHROMAS v2.4.1 (http://technelysium.com.au/) software. Nucleotide alignments were produced using MAFFT v7 (Katoh and Standley 2013). We used the G-INS-I strategy, with gap opening penalty: 1.53, gap extension penalty: 0.1. The aligned matrix was checked manually. Details about GenBank accession numbers of these sequences (KM387731–KM387839) are available in Table S1.

Statistical analyses

The average base frequencies were obtained using ARLEQUIN v.3.5.1.3 (Excoffier and Lischer 2010). To estimate the genetic diversity, we used the following indexes: number of haplotypes, haplotype diversity (*h*) and nucleotide diversity (π), using DNASP v5 (Rozas et al. 2003) and ARLEQUIN softwares. Saturation of nucleotide substitution was checked using the Xia test (Xia and Lemey 2009), implemented in DAMBE version 5.3.108 (Xia 2013); this test calculates whether the observed index of substitution saturation (Iss) of nucleotide sequences is significantly lower than the critical value (Iss.c) calculated for the same sequences, being Iss.c equivalent to the value at which saturation occurs (Xia et al. 2003).

The population structure was determined with ARLEQUIN. Divergence among populations was calculated through a global F_{ST} estimation. We also performed an analysis of molecular variance (AMOVA) (Weir and Cockerham 1984; Excoffier et al. 1992), with a significance level (p) of 0.01 and 1.000 non-parametric permutations. Two different AMOVA tests were run: (1) comparing populations according to the disjunct parts of the range, that is Paranense region versus Yungas, (2) comparing populations of the Paranense region considering the four groups showed in Figure 1: PM, NU, SU and CH.

Phylogenetic structure

A phylogenetic consensus tree of the mtDNA haplotypes was estimated by three different methods: maximun parsimony (MP), maximum likelihood (ML) and Bayesian analyses. For the ML and Bayesian analyses, JMODELTEST 2 (Darriba et al. 2012) was used to find the best-fit substitution model: the TrNG model was selected. Bayesian inference analyses (BI) were executed in MRBAYES 3.2 (Ronquist et al. 2012), MP analysis was completed in PAUP*4.0b10 (Swofford 2003), and ML trees were constructed using the on line program PHYMLVER 3.0 (http://www.atgc-montpellier.fr/phyml/) (Guindon et al. 2010). Node supports for MP and ML trees were evaluated by 1000 bootstrap replicates. To obtain the posterior probability values among clades in MrBayes, 10 million generations and four independent Markov chains were simultaneously run. We used as outgroups the sequences of the congener *Geraeocormobius clavifemur* Mello-Leitão, 1927 (KF726747; Pinto-da-Rocha et al. 2013) and an additional member of the same subfamily, *Megapachylus grandis* Roewer, 1913 (JF786442; Sharma and Giribet 2011). We also used NETWORK v4.6.1.2 (Bandelt et al. 1999) software to draw a median-joining network and to analyse the relationships among the haplotypes detected. To solve alternative node connection, the maximum parsimony post-processing analysis was applied.

Demographic history analysis

This analysis was performed with ARLEQUIN, to assess possible population expansions in the Yungas and Paranense regions of the species' range. Under the assumption of neutrality, both the Tajima and Fu tests (Tajima 1996; Fu 1997) are sensitive to bottleneck effects or to population expansion, causing these values to be significantly negative. The Fu's F_S is particularly sensitive to recent population growth. In addition, population expansion events were also determined with ARLEQUIN by mismatch analyses, with the number of bootstrap replicates set to 1000. Parameters of demographic expansion were estimated, and the validity of the model was tested using the sum of squared deviations (SSD) and the Harpending's raggedness index (*R*) between observed and expected mismatch distributions.

Results

Genetic diversity, structure and differentiation

The DNA matrix presented 681 nucleotides, showing 107 polymorphic sites with 20 substitutions (singleton) and 87 parsimony informative sites. The average base frequencies were A = 21.72%, C = 31.52%, G = 7.08% and T = 39.68%.

The number of haplotypes (*Nh*), nucleotide diversity (π_n) and haplotype diversity (*h*) in each population are summarized in Table 1. The Paranense group showed on average a very high haplotype diversity index (0.9821 ± 0.0050) while that of the Yungas group was considerably lower (0.4277 ± 0.0949). The Paranense area exhibited some representative cases of high genetic variability, for example, the nucleotide diversity in Eldorado reached a considerable high value (0.299; see Table 1). On the other hand, COI sequences were not saturated, showing a site saturation (Iss) of 0.075, which was significantly lower ($p < 0.0001$) than the critical saturation value (Iss.c) of 0.721.

The global F_{ST} considering all populations together was high ($F_{ST} = 0.49$); the global F_{ST} for the Paranense region was 0.25, and the global F_{ST} for the Yungas region was almost 0. The AMOVA comparing Paranense versus Yungas regions showed that variation was of 35.60% among groups and of 40.49% among populations within groups (Table 2). When comparing the four groups within the Paranense region, AMOVA values were of 17.33% ($p < 0.01$) among groups and of 47.07% ($p < 0.01$) for differences among population within groups (Table 2).

Concerning the phylogenetic relationships among haplotypes, Bayesian, ML and parsimony consensus trees yielded different results: three clades (I, II and V) were recovered by all three methods, but Clades III, IV and VI were recovered only in the Bayesian inference (Figure S1). Interestingly, Clade I (which comprises haplotypes H44, H1, H18 and H22) is mainly composed by all specimens from the Yungas (corresponding to H1, H18 and H22), along with a few individuals from de core area. In addition, Clade

Table 2. Results of analysis of molecular variance (AMOVA). Very close localities with the same name were considered as part of one population (see Table 1 for a detail).

Source of variation	Percentage of total variation	Fixation indices
AMOVA by disjunct biogeographic regions (Paranense versus Yungas)		
Among groups	35.60	0.35604*
Within groups	40.49	0.62870*
Within population	23.91	0.76090*
AMOVA by groups within the Paranense region		
Among groups	17.33	0.17327*
Within groups	47.07	0.56941*
Within population	35.60	0.64402*

Asterisks indicate significance at the $p < 0.05$ level.

II consisted entirely of localities belonging to the Uruguay River area. Finally, haplotypes H10 and H28, corresponding to Puerto Iguazú (Paraná River + northern Misiones sector) formed the most divergent clade (Clade V), as supported by both bootstrap values (MP and ML) and Bayesian posterior probabilities.

The median-joining network is displayed in Fig. 2. On the one hand, a considerable number of haplotypes are separated by large genetic gaps, being remarkable the case of haplotypes H43, H14, H15, H10 and H28, which are separated by more than 10 mutational steps from the rest of the network. On the other hand, it is also worth noting that two star-like patterns were observed: one around haplotype H32 (n = 5), comprising representatives of the Paraná River + northern Misiones (Comandante Andresito and Urugua-í) and Chaco (7 km Pt. Antequera) sectors; the second one around H18 (n = 20), which together with its derived haplotypes H1 and H22, included all representatives from the Yungas.

Demographic history analysis

The results of Tajima's D and Fu's F_S neutrality tests indicated a highly significant F_S value for the Paranense group (−11.80765, $p < 0.05$), while that for the Yungas was not statistically significant (0.33066, $p > 0.05$). Mismatch distribution analyses (Figure S2) showed a bimodal frequency distribution of pairwise differences in the Paranense region (Figure S2a), a pattern expected when populations are geographically subdivided (Marjoram and Donnelly 1994). Moreover, the SSD value of 0.004 (ns) and a Harpending′s raggedness index determining a good fit of data for the Paranense area (0.003; $p > 0.05$) do not reject the model for sudden expansion of the species in this region. In the case of the Yungas, both the SSD (0.011; ns) and the Harpending raggedness (0.177; ns) do not reject this sudden expansion model either.

Discussion

The present study, although preliminary, demonstrated different levels of haplotype and nucleotide diversity for *G. sylvarum,* depending on the range sector considered. Variability was very high for the Paranese region – the species′ core area – and considerably low in the Yungas. COI sequences were not saturated, indicating that the fragment employed in this study is an informative marker and that it would be useful to make inferences on

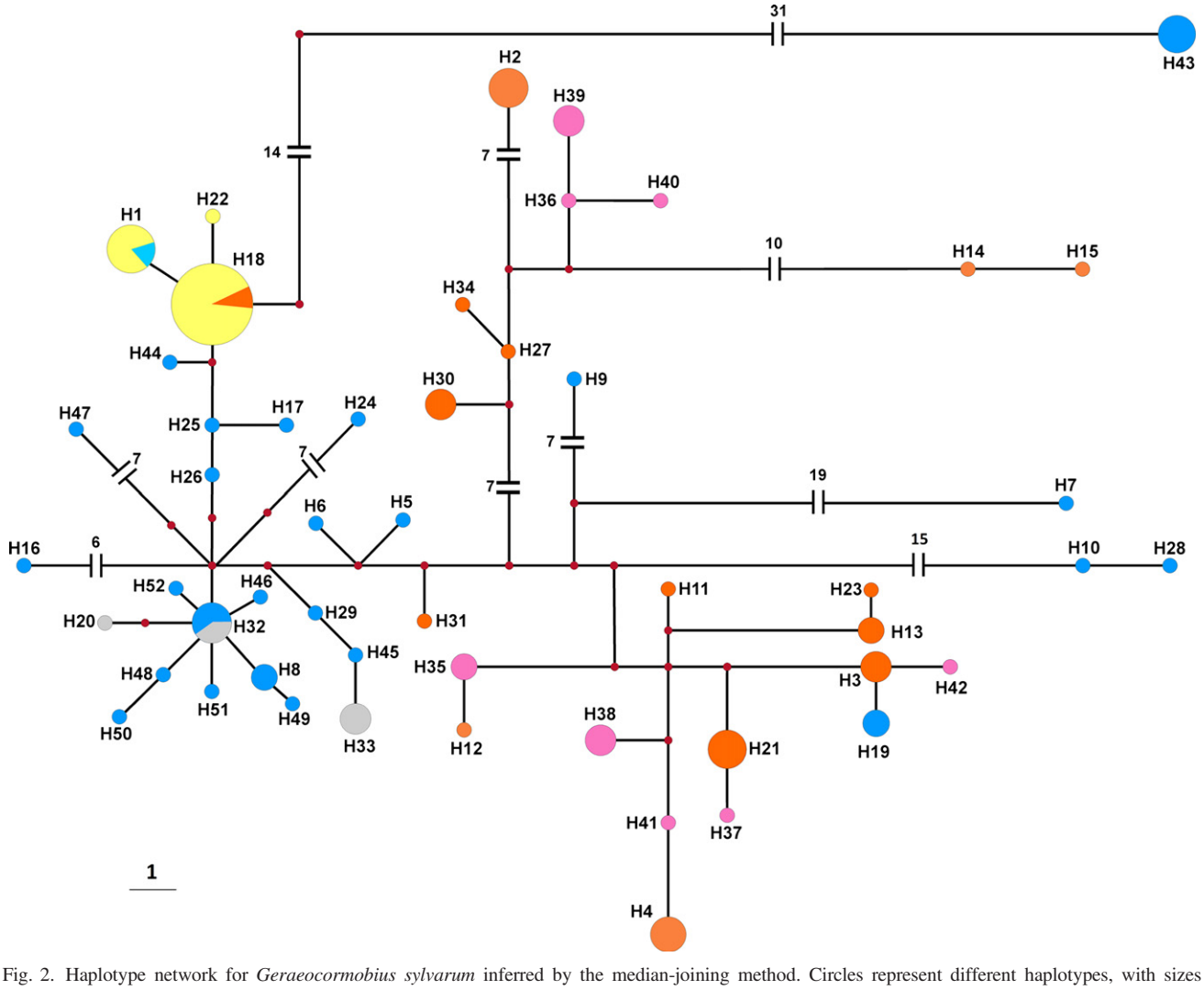

Fig. 2. Haplotype network for *Geraeocormobius sylvarum* inferred by the median-joining method. Circles represent different haplotypes, with sizes proportional to haplotype frequencies. Branches with more than 5 step mutations have that number indicated; otherwise the length represents the number of mutations between contiguous haplotypes. Colours indicate the main geographical sector of the species' range: Tucumán Yungas (Y – yellow); Paraná River + northern Misiones (PM – blue); southern Uruguay River (SU – pink); northern Uruguay River (NU – orange); Chaco (CH – grey).

the possible historical origin of the disjunct pattern observed in the species.

As stated, three clades were recovered with all phylogenetic methods, whereas Clades III, IV and VI were recovered only in the Bayesian inference (Figure S1). On the one hand, Clades I and II suggest two separate lineages, which are in agreement to their geographic localization: in fact, Clade I comprises all representatives of the Yungas (H1, H18 and H22), while Clade II encompasses exclusively populations of the Uruguay River. On the other hand, haplotypes H10 and H28 (Puerto Iguazú) formed the divergent Clade V, which could be explained by a possible ancestral split in populations of this locality.

The median-joining network (Fig. 2) allowed the identification of a two star-like pattern. One is arranged around haplotype H32, which encompassed representatives located in separated sectors of the Paranense distribution: (1) Comandante Andresito and Urugua-í populations corresponding to northern Misiones and (2) 7 km Pt. Antequera from Chaco; these two localities are separated by about 520 km. The second star-like shape was around H18 ($n = 20$) and included H1 and H22. This group of haplotypes comprises all the representatives of the Yungas and sparse individuals of other regions (NU, $n = 1$ in H18 and PM, $n = 1$ in H1). Although the mismatch distribution analyses (Figure S2) do not reject the sudden expansion model for the Yungas, this evidence suggests a very short evolutionary history or a recent introduction of the species in this region (as further discussed below).

In general, in the Paranense region, most haplotypes of *G. sylvarum* were not shared between populations (resulting in a high $F_{ST} = 0.25$ and a very high value of among groups comparison), which to some extent would be expected whether taking into account the alleged low dispersal capabilities and high endemicity of harvestmen (Giribet and Kury 2007). Additionally, very high haplotype and nucleotide diversity levels within populations were found for this region. The Yungas represent a contrasting situation, with very low diversity (and a very low value of $F_{ST} = -0.03360$), a pattern more consistent with (again) a process of recent introduction in this region, likely anthropic, as suggested by Acosta (2008). While the Yungas area has been quite exhaustively surveyed since the early 90s (Acosta et al. 2007; Acosta 2008), *G. sylvarum* was collected only recently in and few, very nearby localities (the most distant separated by <15 km from the rest; Fig. 1). This might support the idea of a recent introduction into this humid area. The high level of haplotype variability found in the present study demonstrated the usefulness of the COI gene as suitable molecular marker to investigate the geographic structure of *G. sylvarum*.

Aside the paleoconnection hypothesis, little supported for *G. sylvarum*, it is possible that the Paranense region became split into forest refuges during the Last Glacial Maximum (LGM), as consequence of the arid conditions that would have dominated South America during this period, causing the generalized retraction of tropical and subtropical forests (Ab'Saber 2000). This is the basis for the so-called refuges theory (Haffer 1982; Lessa et al. 2003), a historical event that could have left genetic discontinuities detectable from population analyses. In this respect, Boyer et al. (2007) suggested that the high genetic differentiation in the harvestman *Aoraki denticulata denticulata* (Forster, 1948) (Cyphophthalmi, Pettalidae) from New Zealand would have been produced by genetic admixture of haplotypes following incipient differentiation, as a consequence of habitat fragmentation during the Pliocene orogeny or the Pleistocene glaciation. Results obtained in our analysis are in agreement with a presumable fragmentation scenario, which might strengthen the hypothesis of the so-called Iguazú refuge, as proposed by Nores (1989) for birds. This hypothesis sought to explain the high rate of bird diversity observed in the northern region of Province of Misiones and neighbouring areas in Brazil and Paraguay. The 'Iguazú refuge' hypothesis proposed the persistence of forest patches in a restricted location during arid phases of the Pleistocene, during which most forests retreated. According to Nores (1989), this area would have remained as a Pleistocene refuge where birds and other animals were isolated and could have differentiated in the course of time. For *G. sylvarum*, a widespread sampling of additional populations, covering the distribution range of the species, is currently being conducted by our team, in order to properly analyse the phylogeographic pattern of populations of the species and to shed light on the 'Iguazú refuge' hypothesis, which might have an important meaning for the conservation of Paranense forests and adjacent areas.

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Resumen

Elevada diversidad genética en el opilión Geraeocormobius sylvarum (Arachnida, Opiliones, Gonyleptidae) en la selva subtropical del noreste argentino, revelada a partir de secuencias de ADN mitocondrial

El objetivo de este trabajo fue analizar la diversidad genética de *Geraeocormobius sylvarum*, un opilión neotropical habitante de selva con distribución disyunta, con poblaciones separadas del área principal por aproximadamente 630 km de ambientes semi-áridos. Se evaluó la utilidad de un fragmento de la subunidad I del gen mitocondrial Citocromo Oxidasa (COI) como marcador molecular en 109 individuos. Los resultados revelaron altos niveles de diversidad haplotípica y nucleotídica en poblaciones correspondientes al noreste de Argentina, el área principal de distribución de la especie. Se detectó una fuerte estructuración genética para la especie, un resultado apoyado tanto por los árboles filogenéticos como por la red haplotípica, con 6 haplogrupos identificables. Las poblaciones de las Yungas no mostraron niveles significativos de diversidad, sugiriendo la reciente introducción de la especie en la región. En general, los resultados sugieren que la diversidad genética actual es consistente con eventos pasados de fragmentación del rango de la especie (¿refugios?), probablemente durante el Último Máximo Glacial. Se concluye que el gen COI es un marcador apropiado para asociar eventos ambientales pasados con la alta diversidad genética observada en esta especie.

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Appendix 1. List of analysed materials of *Geraeocormobius sylvarum*, with geographical coordinates (Lat/Lon, decimal degrees) of localities and collecting information. All specimens were collected in Argentina and are stored in the freezer collection, at Cátedra de Diversidad Animal I, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba (CDA-F). Localities representing new records for the species are indicated. Where applicable, the abbreviated locality name as used in Table 1 is stated in this list in square brackets.

Province	Locality	Latitude (S)	Longitude (W)	Collection data
Misiones	Puerto Iguazú	−25.5957	−54.5825	1 ♂, 5 ♀ (CDA-F), 18-xi-2003 (J. Farina) – NEW RECORD
Misiones	Bañado Alto Paraná	−25.7604	−54.5378	2 ♂ (CDA-F), 16-xi-2012 (L.M. Vaschetto, M. Ferro, N. Acuña) – NEW RECORD
Misiones	Comandante Andresito	−25.7029	−54.0526	1 ♂, 3 ♀ (CDA-F), 13-xii-2012 (L.M. Vaschetto, R. González-Ittig, S. Poljak) – NEW RECORD
Misiones	Urugua-í (Provincial Park)	−25.8578	−54.1705	4 ♂ (CDA-F), 13-xii-2012 (L.M. Vaschetto, R. González-Ittig, S. Poljak) – NEW RECORD
Misiones	Bernardo de Irigoyen	−26.2500	−53.6500	1 ♂, 2 ♀ (CDA-F), 27-x-2006 (D. Martí). Record cited by Acosta et al. 2007
Misiones	Cruce Caballero	−26.5201	−53.9860	1 ♂, 4 ♀ (CDA-F), 26-ix-2010 (M. Pereyra) – NEW RECORD
Misiones	Eldorado (nearby)	−26.4561	−54.6441	3 ♂ (CDA-F), 16-xi-2012 (L.M. Vaschetto, M. Ferro, N. Acuña) – NEW RECORD
Misiones	Montecarlo	−26.6014	−54.7278	1 ♂ (CDA-F), 15-xi-2012 (L.M. Vaschetto, M. Ferro, N. Acuña) – NEW RECORD
Misiones	Garuhapé (near Puerto Rico)	−26.8195	−54.9903	3♂ (CDA-F), 15-xi-2012 (L.M. Vaschetto, M. Ferro, N. Acuña) – NEW RECORD
Misiones	El Soberbio	−27.2830	−54.1955	3♂, 2♀ (CDA-F), 29-x-2010 (P. Iglesias) – NEW RECORD
Misiones	Alba Posse	−27.5697	−54.6793	3♂, 2♀ (CDA-F), 28-x-2010 (P. Iglesias) – NEW RECORD
Misiones	Panambí	−27.7233	−54.9148	2 ♂, 3♀ (CDA-F), 27-x-2010 (P. Iglesias) – NEW RECORD
Misiones	Itacaruaré	−27.8698	−55.2633	2 ♂, 3♀ (CDA-F), 26-x-2010 (P. Iglesias) – NEW RECORD
Misiones	Concepción de la Sierra	−27.9812	−55.5220	1 ♂, 1 ♀, 1 juv. (CDA-F), 26-x-2010 (P. Iglesias) – NEW RECORD
Misiones	Apóstoles	−27.9167	−55.7667	2 ♂, 3 ♀ (CDA-F), 25-x-2010 (P. Iglesias) – NEW RECORD
Corrientes	Garruchos, near Naval Prefecture post [Garruchos]	−28.1767	−55.6438	1 ♂, 3 ♀ (CDA-F), 25-ii-2012 (L. Acosta, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Corrientes	Los Cuays, near Naval Prefecture post [Cuays 1]	−28.8530	−56.3036	4♂(CDA-F), 26-ii-2012 (L. Acosta, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Corrientes	La Cruz, Gruta del Agua Santa ('Holy Water grotto')	−29.1648	−56.6255	3♂ (CDA-F), 26-ii-2012 (L. Acosta, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Corrientes	400 m S of Yapeyú [Yapeyú 0]	−29.4790	−56.8202	2 ♂, 1 ♀ (CDA-F), 21-v-2010 (J. Vergara, L. Paoloni) – NEW RECORD
Corrientes	1 km S of Yapeyú [Yapeyú 1]	−29.4837	−56.8259	1♂, 1 ♀ (CDA-F), 26-ii-2012 (L. Acosta, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Corrientes	Arroyo Guaviraríi, nearYapeyú [Yapeyú 2]	−29.4936	−56.8425	2♀ (CDA-F), 26-ii-2012 (L. Acosta, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Chaco	10 km from Puerto Antequera, to Isla del Cerrito [10 km Pt. Antequera]	−27.3873	−58.8004	1 ♂ (CDA-F),24-ii-2005 (L. Acosta, G. Rubio, P. Olivero, M. Garcia). Record cited by Acosta et al. 2007
Chaco	7 km from Puerto Antequera [7 km Pt. Antequera]	−27.3996	−58.8176	4♂, 1♀ (CDA-F), 2-xii-2011 (J. Vergara, R. González-Ittig, L.M. Vaschetto) – NEW RECORD
Tucumán	Road to Cerro San Javier, between El Corte and 'primera confitería' (ca. 500-700 m) [San Javier 1a]	−26.8082	−65.3378	2 ♂, 2 ♀ (CDA-F),24-viii-2003 (L. Acosta). Record cited by Acosta et al. 2007
Tucumán	El Corte, same coordinates as the former [San Javier-1b]	–	–	1 ♂, 3 ♀ (CDA-F), 16-viii-2005 (L. Acosta, M.L. Juaréz)
Tucumán	Road to Cerro San Javier, same coordinates as the former [San Javier 2]	–	–	3 ♂, 2 ♀ (CDA-F), 24-xi-2008 (G. Rubio).
Tucumán	2 km from El Cristo to Villa Nougués [2 km El Cristo]	−26.8096	−65.3635	1 ♂ (CDA-F),16-viii-2005 (L. Acosta, M.L. Juárez) Record cited by Acosta et al. 2007

Appendix 1. (continued)

Province	Locality	Latitude (S)	Longitude (W)	Collection data
Tucumán	2 km down Villa Nougués [2 km Villa Nougués]	−26.8559	−65.3680	1 ♂, 3 ♀, 1 juv. (CDA-F),10-i-2010 (L. Acosta, M. García) – NEW RECORD
Tucumán	Road up to Villa Nougués, ca. 750 m [Road to Villa Nougués]	−26.8603	−65.3507	4 ♂ (CDA-F), 25-xi-2008 (G. Rubio) – NEW RECORD
Tucumán	1.8 km E of Manuel García Fernández [M. García Fernández]	−26.9615	−65.2570	3 ♂ (CDA-F), 21-ii-2011 (J. Vergara) – NEW RECORD

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Bayesian 50% majority rule consensus tree based on COI sequences showing relationships among haplotypes of *Geraeocormobius sylvarum*.

Figure S2 Mismatch distribution analyses for 'Paranense Forest + Mesopotamia *s.s.*' and 'Yungas' regions of the range of *Geraeocormobius sylvarum*.

Table S1 GenBank Accession numbers and nucleotide sequences of *Geraeocormobius sylvarum*