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Spatial and temporal analysis of the genetic diversity and population structure of a freshwater ostracod from the high Andean plateau

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ABSTRACT

Background: The endorheic basins (basins with no outflow to another body of water) of the Andes Mountains contain several populations of small invertebrates with varying levels of isolation. Little is known about their genetic composition.

Hypothesis: Population isolation and changes in population size over time will increase genetic diversity.

Organisms: Three populations of the ostracod *Limnocythere* sp. with varying levels of isolation and population stability.

Field site: El Monumento Natural Salar de Surire, Chile, in the high Andean Plateau at 4200–4300 m above sea level.

Methods: We surveyed two molecular markers: the cytochrome oxidase I (COI) region and the internal transcribed spacer (ITS) region. With these data, we estimated the genetic diversity and spatial genetic structure during four consecutive years.

Conclusions: Genetic structure within populations did not differ among years, although there were differences among populations.

Keywords: population, molecular markers, gene flow, environmental stochasticity, ostracod, high Andean plateau.

INTRODUCTION

Gene flow, population subdivision, selection, and drift are the most important forces determining the distribution of gene frequencies in populations (Cunningham and Moritz, 1998; Bulgin *et al.*, 2003; Miller and Waits, 2003; Williams *et al.*, 2003; Reding *et al.*, 2010). Hence, the spatio-temporal patterns of genetic structuring provide an important link between evolutionary and ecological processes (Hedgecock, 1994; Jehle *et al.*, 2005; Reding *et al.*, 2010). The empirical study of such

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micro-evolutionary processes is largely constrained by the availability of suitable systems of populations that can be studied empirically and also by the availability of neutral genetic markers. In South America, the high Andean Plateau is considered a 'natural laboratory' on which small ponds offer a unique system of populations with varying levels of isolation among them. In fact, a large number of aquatic invertebrates inhabiting the endorheic basins of the salars are endemic (Zuñiga *et al.*, 1999; Menu-Marque *et al.*, 2000; Gonzalez and Wattling, 2001; Scheihing *et al.*, 2010) and are able to tolerate large fluctuations in environmental parameters (Bayly, 1993; Williams *et al.*, 1995; Menu-Marque and Locascio de Mitrovich, 1998; Williams, 1998; Oyanedel *et al.*, 2008). However, despite the ecological importance of these habitats, a large number of these species remain unstudied, which means that our knowledge of these habitats is rather basic (Scheihing *et al.*, 2010). Among them, Ostracoda is the most conspicuous group. Ostracods are considered ideal models for testing hypotheses in evolutionary ecology, because of their striking life histories, including sexual and asexual reproduction (Yo, 2000), and also because of their excellent fossil record, which is commonly used for past reconstruction (Holmes and Chivas, 2002). In the Salar de Surire, there are three permanent ponds containing populations of *Limnocythere* sp., two of them characterized by relatively cold water (~18°C) and one by hot water (~40°C).

Generally, genetic variation of these ostracods has been characterized by allozyme markers (Sywula *et al.*, 1985; Havel and Hebert, 1993; Butlin *et al.*, 1998; Rossi *et al.*, 1998; Little, 2005), with a few researchers using the cytochrome oxidase subunit I (COI) and ribosomal internal transcribed spacer (ITS) markers (Schön *et al.*, 2000; Gandolfi *et al.*, 2001; Martens *et al.*, 2005). The information available to date is mostly from ponds from Europe and North America, but for the Southern hemisphere such studies are scarce (Smith and Martens, 2000). These authors have suggested low levels of genetic variability within species as well as among species, mainly due to their asexual mode of reproduction (Smith and Martens, 2000; Gandolfi *et al.*, 2001; Schön *et al.*, 2003). Within populations, abiotic environmental stress may affect the dynamics of population genetic structure in a number of ways (Martins *et al.*, 2010). For instance, population cycles could determine expansions and contractions in genetic diversity (Rossi *et al.*, 2010). Gene flow could homogenize the genetic composition of populations provoking a homogenizing effect and environmental instability could reduce genetic variation while spatial heterogeneity within populations could increase genetic variation (Little, 2005; Martins *et al.*, 2010; Rossi *et al.*, 2010; Junge *et al.*, 2011).

The *Limnocythere atacamae* population present in the Salar de Surire offers an excellent opportunity to test for an interaction among environmental stability and dispersal. Thus, in this study we explored the temporal and spatial variation in genetic diversity among three populations of *L. atacamae*. The populations of *L. atacamae* differ mostly in their stability, with the hot spring being more stable than the two cold ponds, which exhibit periodic fluctuations in temperature and water levels, which in turn could be associated with periodic contractions and expansions in genetic diversity. Also, the two cold ponds are visited by flamingos, which could transport individuals between the two ponds, generating gene flow between these ponds. Three main predictions arise from this system. First, because of periodic bottlenecks, in a given year genetic diversity should be lower in both cold ponds compared with the hot spring. Second, because of gene flow, more genetic homogeneity should be found between the two cold ponds compared with the hot spring. Third, less inter-annual variation in genetic composition should be expected in the hot spring than the cold ponds.

MATERIALS AND METHODS

Sampling locations, collection and preservation of specimens

The Salar de Surire is located in the Chilean high Andean Plateau (18°47′24.7″S; 69°05′17.6″W) at an altitude of 4200–4300 m (Fig. 1). It comprises an area of about 129 km^2 and has a variety of permanent shallow ponds of different shapes and sizes, some of which are fed by freshwater streams. We selected three permanent sites, based on the existence of dense populations of *L. atacamae*. The sites can be described as follows: (1) a pond with a sandy bottom ('pond 1'), 5–10 cm deep (18°47′41.6″S; 69°05′20.0″W; 14.6×10^4 m^2); (2) a pond with a sandy bottom ('pond 2'), 35–40 cm deep (18°51′43.3″S; 69°07′57.8″W; 7.4×10^4 m^2); and (3) a hot spring with a sandy bottom (the 'hot spring' site), 45–50 cm deep (18°54′45.9″S; 68°59′57.2″W; 5×10^3 m^2). The samplings were carried out during the months of austral spring (October–November) in four consecutive years: 2004, 2005, 2006, and 2007. Sampling was performed by inserting a hand-held plastic core 2 cm into the sediment (Higgins and Thiel, 1988). Specimens from each site were used for morphological and DNA analysis. In each case, specimens were preserved in absolute ethanol until analysis in the laboratory at the Centro de Estudios Científicos (CECS). The taxonomic classification of samples was performed using a Zeiss Axioskop stereomicroscope. Ostracods were individually dissected and identified (Brehm, 1935; Smith and Martens, 2000; Smith and Kamiya, 2003).

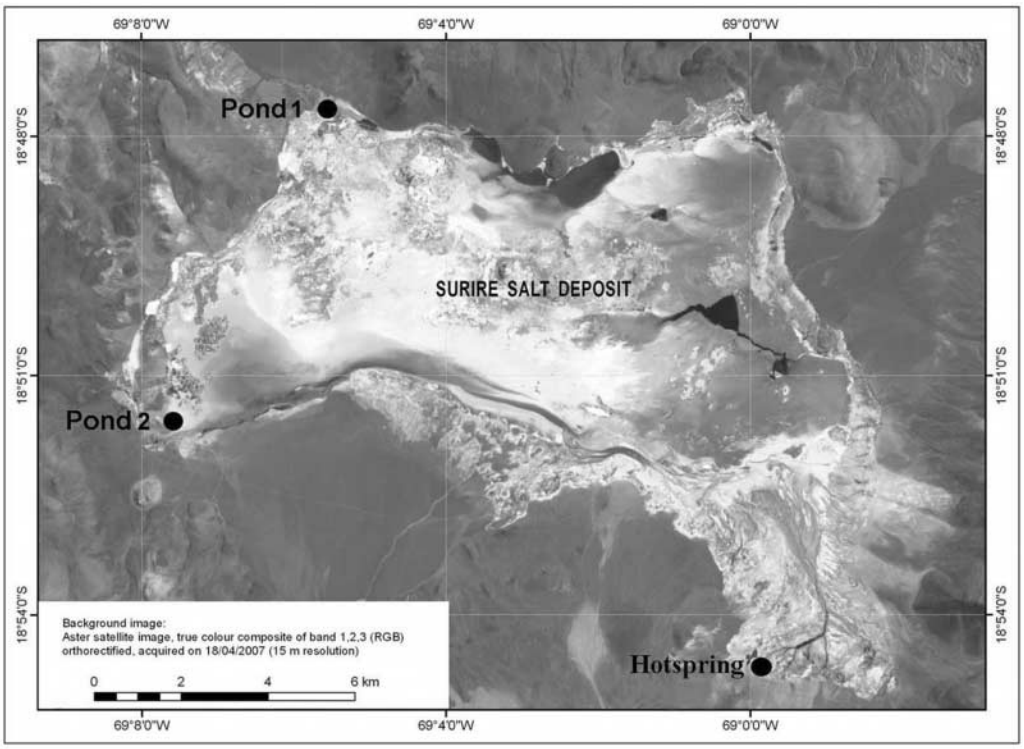

Fig. 1. Geographic locations of the three studied populations of *Limnocythere atacamae* at Salar de Surire, in the Chilean high Andean plateau.

We also determined *in situ* environmental parameters (water temperature, dissolved oxygen, and pH) using an ORION 3 Strar sensor (Thermo Electro Corporation) at each site studied. Environmental parameters at each site were estimated yearly in 36 sampling spots. These 36 spots were distributed in six different transects that crossed the diameter of each pond in order to maximize the sampling area at each site.

DNA extraction

Before DNA extraction, individuals were soaked in double-distilled water for a minimum of one hour. DNA extractions from a single individual (50 μL final volume) were achieved using an Easy-DNA Kit (Invitrogen). Seventeen individuals for each population and year were amplified. A 510 base pair (bp) fragment of cytochrome oxidase I (COI) was amplified using the primers LCOI490 and HCO2918 (Folmer *et al.*, 1994), while a 315 bp fragment of the 5.8s internal transcribed spacer (ITS) region was amplified using the primer ITS (White *et al.*, 1990). Both genes have been widely used in invertebrate species and also in ostracod species, allowing comparison of the present data with those in the literature. The polymerase chain reaction (PCR; 50-μL total volume) consisted of 3–5 μL of DNA template, 5 μL of 10 × PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 0.2 μM of each primer, 2.5 mM $MgCl_2$, 0.4 mM of each dNTP, and 1.25 units of Taq DNA polymerase. The PCR regime was as follows: an initial cycle of denaturation at 94°C for 1 min followed by 35 cycles of 95° C for 1 min, 45°C for 1.5 min, and 72°C for 1.5 min. Finally, the PCR ended with a final extension of 72°C for 10 min. The PCR products were purified using QIAquick purification kit (QIAGEN). Double-stranded PCR products were sequenced for each individual using an ABI PRISM® 3100 automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). Sequences were performed at Macrogen, Seoul, Korea (http://www.macrogen.com).

Data analysis

Sequences were edited and aligned using the ClustalW 2.0 multiple sequence alignment program (Chenna *et al.*, 2003). Variable positions were confirmed by visual inspection and verified on both strands. Genetic diversity was screened for each pond and year. The following standard genetic diversity indexes were calculated: polymorphic sites (S); number of haplotypes (Nhap); haplotype diversity (He); average number of differences (Π); and nucleotide diversity (π). The program Arlequin v.3.5.1.2 (Excoffier and Lischer, 2010) was used to undertake the analysis. To test the interaction between genetic diversity and ponds, we performed a two-factor analysis of variance (ANOVA) using molecular marker (two levels) and pond (three levels) as factors. Data satisfied the assumptions of ANOVA (normality and homogeneity of variance) according to Levene's test ($P > 0.05$).

Spatio-temporal genetic structure

Inferences of genetic structure were performed using an analysis of genetic variance as defined by Excoffier *et al.* (1992), Weir and Cockerham (1984), and others and implemented in the Arlequin program. First, to determinate the influence of years versus ponds on the genetic differentiation, we performed a hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). In this analysis, genetic variance was partitioned into three components, variations among ponds (ΦCT), among years within ponds (ΦSC), and

among years over all ponds (ΦST). Second, we calculated a pair-wise Φst to compare the genetic differentiation among the three ponds. Finally, a neighbour-joining tree (with random input order and the options heuristic search and step-wise addition) based on ITS sequences among sites and bootstrapping with 1000 replicates was constructed using the program Mega, version 4 (Tamura *et al.*, 2007).

RESULTS

We found differences in the average values of the environmental parameters between sites (Table 1). The main differences among ponds were registered in temperature and conductivity (i.e. salinity). Altogether, the MtDNA COI gene and ITS gene of 204 individuals were analysed. We identified 47 haplotypes for the COI gene and 68 for the ITS gene (see Tables 2 and 3 respectively). We did not find shared haplotypes of the COI gene among populations, and the most abundant haplotype was H13, with a frequency of 17% and present only at the hot spring. Only one haplotype (H12) was shared between two populations (i.e. between pond 2 and the hot spring), and only in 2005. Moreover, we found significant differences in the genetic diversity among sites, yet the genetic diversity was similar within each population across time. In pond 1, the genetic diversity of the COI gene was the highest with a diversity (He) of between 0.85 and 0.98, whereas the hotspring had a

Table 1. Environmental parameters recorded at the study sites (see Methods for details) (mean ± S.D.)

Site	Latitude	Longitude	Temperature (°C)	Oxygen ($mg \cdot L^{-1}$)	pH	Conductivity ($mS \cdot cm^{-1}$)	Depth (cm)
Pond 1	18°47′41.6″	69°05′20.0″	18.9 ± 1.7	6.4 ± 0.7	9.0 ± 0.1	24.8 ± 3.3	10 ± 5
Pond 2	18°54′45.9″	68°59′57.2″	16.7 ± 1.4	5.8 ± 0.3	8.9 ± 0.5	7.2 ± 0.6	30 ± 10
Hot spring	18°51′43.3″	69°07′57.8″	43.4 ± 6.0	4.4 ± 0.3	7.1 ± 0.4	18.4 ± 1.8	40 ± 10

Table 2. COI genetic diversity of *Limnocythere atacamae*

Year	Site	S	Nhap	He	π	Π
2004	Pond 1	37	5	0.66 ± 0.09	0.009 ± 0.005	4.83 ± 2.48
	Pond 2	28	7	0.85 ± 0.05	0.008 ± 0.004	4.22 ± 2.21
	Hot spring	1	3	0.66 ± 0.07	0.001 ± 0.001	0.66 ± 0.53
2005	Pond 1	29	4	0.65 ± 0.09	0.011 ± 0.006	5.66 ± 2.86
	Pond 2	21	7	0.84 ± 0.06	0.065 ± 0.003	3.39 ± 1.83
	Hot spring	12	3	0.56 ± 0.08	0.003 ± 0.002	1.78 ± 1.08
2006	Pond 1	38	9	0.911 ± 0.04	0.012 ± 0.007	6.676 ± 3.31
	Pond 2	27	14	0.978 ± 0.02	0.008 ± 0.005	4.639 ± 2.39
	Hot spring	3	6	0.743 ± 0.08	0.002 ± 0.010	1.279 ± 0.84
2007	Pond 1	36	7	0.838 ± 0.05	0.011 ± 0.005	5.066 ± 2.58
	Pond 2	26	14	0.955 ± 0.04	0.008 ± 0.005	4.566 ± 2.36
	Hot spring	1	3	0.661 ± 0.06	0.001 ± 0.010	0.661 ± 0.53

Table 3. ITS DNA standard diversity index of *Limnocythere atacamae* populations studied

Year	Site	S	Nhap	He	π	Π
2004	Pond 1	3	4	0.698 ± 0.075	0.003 ± 0.002	0.955 ± 0.68
	Pond 2	3	6	0.721 ± 0.08	0.003 ± 0.002	0.976 ± 0.63
	Hot spring	2	3	0.404 ± 0.13	0.001 ± 0.001	0.500 ± 0.44
2005	Pond 1	4	6	0.816 ± 0.06	0.004 ± 0.002	1.264 ± 0.83
	Pond 2	4	7	0.794 ± 0.07	0.005 ± 0.003	1.470 ± 0.93
	Hot spring	3	5	0.581 ± 0.13	0.003 ± 0.002	0.808 ± 0.61
2006	Pond 1	5	8	0.838 ± 0.06	0.004 ± 0.003	1.367 ± 0.88
	Pond 2	5	9	0.882 ± 0.05	0.003 ± 0.002	1.463 ± 0.93
	Hot spring	3	6	0.706 ± 0.10	0.004 ± 0.003	1.191 ± 0.80
2007	Pond 1	4	5	0.794 ± 0.05	0.005 ± 0.003	1.426 ± 0.91
	Pond 2	3	6	0.691 ± 0.10	0.003 ± 0.002	0.860 ± 0.63
	Hot spring	4	3	0.324 ± 0.13	0.003 ± 0.002	0.779 ± 0.59

lower diversity index (He = 0.56–0.66) (Table 2). The genetic diversity was lower with the ITS gene than the COI gene (Table 3). The genetic diversity in pond 1 from years 2004 and 2005 was higher than in other years. Pond 1was the most diverse location during 2005 and 2007, and for pond 2 the highest diversity was observed in 2004 and 2006. The hot spring showed the least genetic diversity (He = 0.32–0.71) (see Table 3). Finally, the ANOVA of differences in genetic diversity among markers and ponds showed significant differences in both markers ($F_{1,30} = 8.63$, $P \ll 0.001$ and $F_{2,30} = 26.9$, $P \ll 0.001$ respectively) (Fig. 2). Also, the Tukey *post-hoc* test indicated that in the hot spring there were significant differences between genetic markers among years. In addition, pond 1 and pond 2 did not exhibit significant differences in genetic diversity. There was a non-significant interaction in molecular markers among ponds and years ($F_{2,30} = 2.3$, $P = 0.114$).

We analysed the genetic structure using three different approaches. First, the AMOVA indicated that there were significant differences among ponds (COI: ΦST = 0.69, $P \ll 0.0001$; ITS = 0.72, $P \ll 0.0001$) (Table 4). However, these differences were due to genetic differentiation among the three ponds (COI: ΦCT = 0.68, $P \ll 0.0001$; ITS = 0.63, $P \ll 0.001$), and differences among years, within ponds (COI: ΦCS = 0.03, $P = 0.52$; ITS = 0.22, $P = 0.56$). Second, pairwise F_{ST} analysis confirmed the high levels of genetic structuring among ponds, with almost no variation between years within ponds, except 2005 when samples from pond 1 and the hot spring were significantly different (Table 5). The inferred relationships among lineages, developed as a haplotype network, revealed three main clusters (Fig. 3). There were significant differences, with several mutational steps among them. There were 12 mutational steps between pond 1 and pond 2, another 12 mutational steps between pond 1 and the hot spring, and 5 mutational steps between pond 2 and hot spring. Finally, the neighbour-joining tree built using Kimura two-parameter distances, and based on a 315 bp alignment of ITS1 sequence data, revealed three major branches (Fig. 4), which coincide with those established by the COI network. In addition, the bootstrap values indicated lower levels of temporal structuring but stronger levels of spatial structuring.

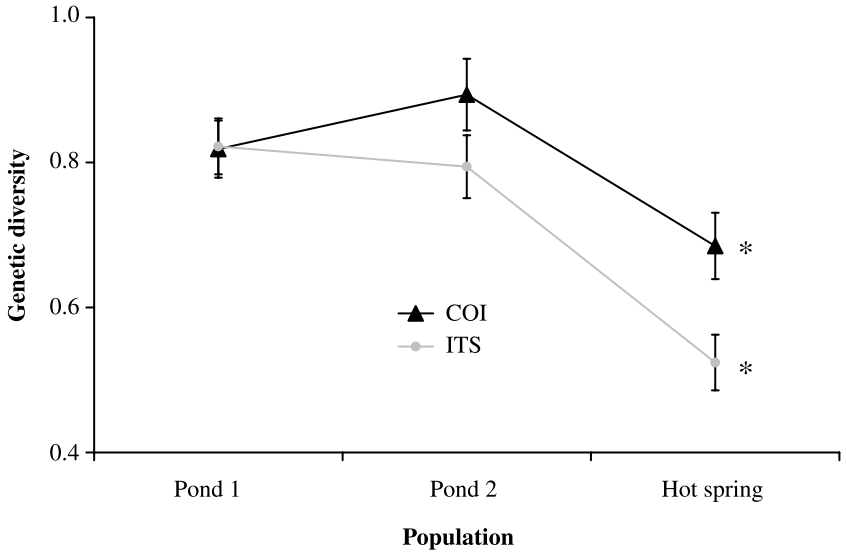

Fig. 2. Interaction plot from the two-way ANOVA, including marker (two levels) and population (three levels) as factors. See Results for details. *Significant differences in both markers.

Table 4. Analysis of molecular variance (AMOVA) of population structure in *Limnocythere atacamae*

Locus	Source of variation	Fixation indices	*P*-value
COI	Among ponds	$F_{ct} = 0.68$	⩽0.001
	Among years, within ponds	$F_{sc} = 0.03$	0.051
	Within years	$F_{st} = 0.69$	⩽0.001
ITS	Among ponds	$F_{ct} = 0.63$	⩽0.001
	Among years, within ponds	$F_{sc} = 0.22$	0.056
	Within years	$F_{st} = 0.72$	⩽0.001

DISCUSSION

We studied a system of three populations of a non-marine ostracod for four consecutive years. Three main conclusions can be drawn from the present work. First, cold ponds exhibited higher genetic diversity than the hot spring, independently of year. Second, there was a consistent genetic structure among ponds, suggesting no gene flow in this system. Third, there was no inter-annual variation inside any pond.

Several species of ostracods from the genus *Limnocythere* exhibit high genetic diversity as well as sexual and asexual reproduction (Schön and Martens, 1998; Smith and Martens, 2000; Schön *et al.*, 2003; Martens *et al.*, 2008; Martins *et al.*, 2010; Rossi *et al.*, 2010). In fact, sexual populations of these species can show divergences of up to 19.6% for COI and 4.3% for ITS1, while differences within asexual lineages can reach 24.3% for COI and 10.3% for ITS1. Our results suggest that the observed genetic diversity is typical of a sexually reproducing species (Schön *et al.*, 2003; Martens *et al.*, 2008).

Table 5. Pairwise F_{st} values among populations collected

	P1 2004	P2 2004	HT 2004	P1 2005	P2 2005	HT 2005	P1 2006	P2 2006	HT 2006	P1 2007	P2 2007	HT 2007
P1 2004	—	**0.72**	**0.81**	−0.03	**0.68**	**0.78**	−0.04	**0.66**	**0.74**	−0.02	**0.78**	**0.82**
P2 2004	**0.70**	—	**0.74**	**0.69**	−0.01	**0.70**	**0.68**	0.03	**0.65**	**0.64**	**0.54**	**0.75**
HT 2004	**0.54**	**0.80**	—	**0.78**	**0.69**	−0.01	**0.77**	**0.67**	0.03	**0.76**	**0.81**	**0.54**
P1 2005	**0.13**	**0.66**	**0.59**	—	**0.65**	**0.76**	−0.02	**0.63**	**0.71**	0.01	**0.76**	**0.79**
P2 2005	**0.72**	−0.05	**0.83**	**0.68**	—	**0.66**	**0.64**	**0.07**	**0.61**	**0.60**	**0.49**	**0.71**
HT 2005	**0.52**	**0.76**	**0.19**	**0.56**	**0.78**	—	**0.75**	**0.64**	0.05	**0.73**	**0.78**	**0.50**
P1 2006	−0.04	**0.66**	**0.48**	**0.12**	**0.68**	**0.47**	—	**0.62**	**0.70**	−0.02	**0.75**	**0.78**
P2 2006	**0.68**	**−0.04**	**0.79**	**0.65**	−0.03	**0.74**	**0.65**	—	**0.57**	**0.58**	**0.48**	**0.70**
HT 2006	**0.52**	0.78	0.01	**0.56**	**0.81**	**0.17**	**0.46**	**0.77**	—	**0.69**	**0.73**	**0.45**
P1 2007	−0.04	**0.69**	**0.54**	**0.14**	**0.71**	**0.51**	−0.02	**0.68**	**0.51**	—	**0.73**	**0.77**
P2 2007	**0.69**	**−0.03**	**0.79**	**0.65**	−0.02	**0.74**	**0.65**	−0.04	**0.77**	**0.68**	—	**0.81**
HT 2007	**0.54**	0.80	−0.06	**0.59**	**0.83**	**0.19**	**0.48**	**0.79**	0.01	**0.54**	**0.79**	—

Note: ITS F_{st} values are given above the diagonal and COI values below the diagonal. P1 = pond 1, P2 = pond 2, and HT = hot spring. Significant *P* values are in **bold** ($P \leqslant$ **0.001).**

We hypothesized that given that cold ponds are temporally unstable, their genetic diversity would be lower than that in the hot spring. This prediction is associated with the expectation that populations that experience a constant process of extinction/colonization are characterized by reduced genetic diversity, principally due to founder effects and the strength of genetic drift (only a few individuals are needed to found the new population). However, our results show that genetic diversity in the cold ponds is higher than in the hot spring, suggesting that the strength of genetic drift during the founder process is overridden by other factors.

Dormant eggs can build up large egg banks in the sediments of ponds and lakes and may remain viable for decades (Hairston *et al.*, 1995; Brendonck and De Meester, 2003). At the start of a new growing season, a fraction of the dormant eggs residing in the egg bank will hatch and start a new phase of clonal reproduction. The presence of a large resting propagule bank would produce enough material to maintain the level of genetic diversity even on a long temporal scale (De Meester *et al.*, 2002). Then, because a sexual population could be present in the cold ponds, we suggest that sexual reproduction is the main process that maintains the

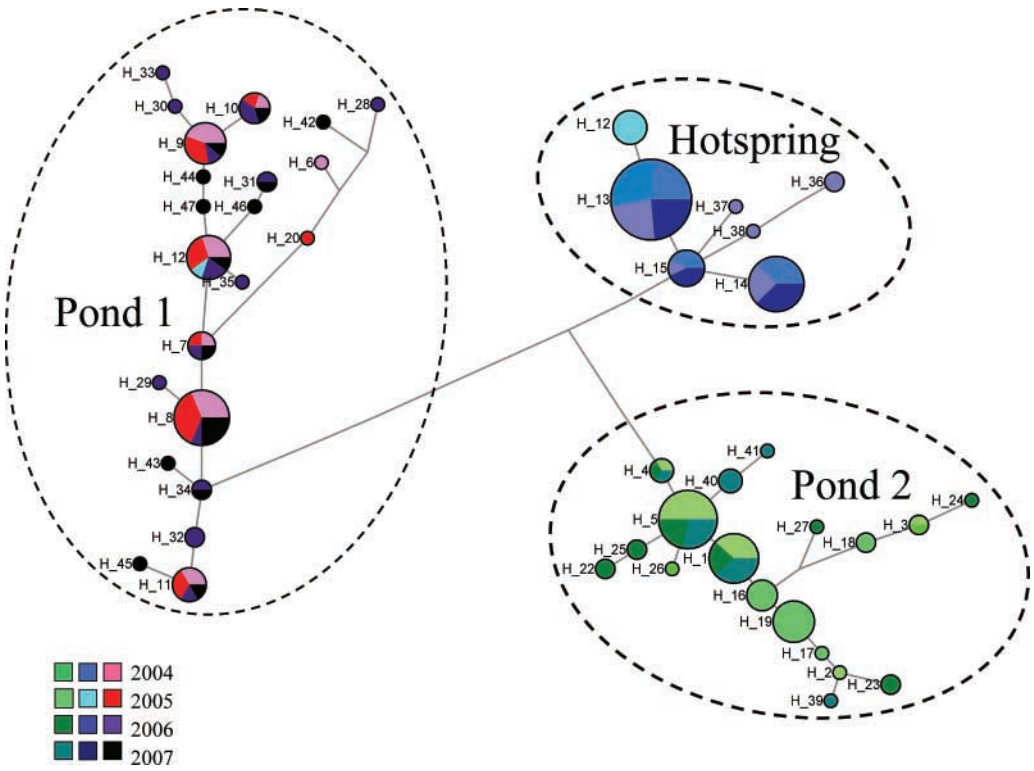

Fig. 3. Haplotype network of the 204 *Limnocythere atacamae* mtDNA haplotypes from the COI gene. Each haplotype is represented by a circle with its size proportional to the number of individuals bearing the haplotype over the whole data set. Colours indicate the year to which the haplotypes correspond.

genetic diversity in these ponds associated with the ability to store propagules. In this context, we argue that founder events combined with rapid local adaptation may underlie the striking patterns of genetic differentiation for neutral markers in this species, a process described in the monopolization hypothesis (De Meester *et al.*, 2002). Following this hypothesis, rapid population growth and local adaptation upon colonization of a new habitat result in the effective monopolization of resources, yielding a strong priority effect. Once a population is locally adapted, the presence of a large resting propagule bank provides a powerful buffer against newly invading genotypes, so enhancing priority effects. Thus, under this monopolization hypothesis, high genetic differentiation among nearby populations is also expected (De Meester *et al.*, 2002). Our data show in both markers a strong genetic differentiation among the three populations isolated by only a few kilometres.

Dispersal promotes gene flow and homogeneity of genes among populations, whereas limited dispersal leads to divergence of populations by genetic drift and natural selection (Slatkin, 1985; Broquet and Petit, 2009). The *L. atacamae* population system would be considered an island-like system where the three ponds are isolated and the only possibility to exchange genotypes is by effective dispersal. Two observations would suggest an important level of gene flow among populations. First, flamingos feeding over the two cold ponds could act as

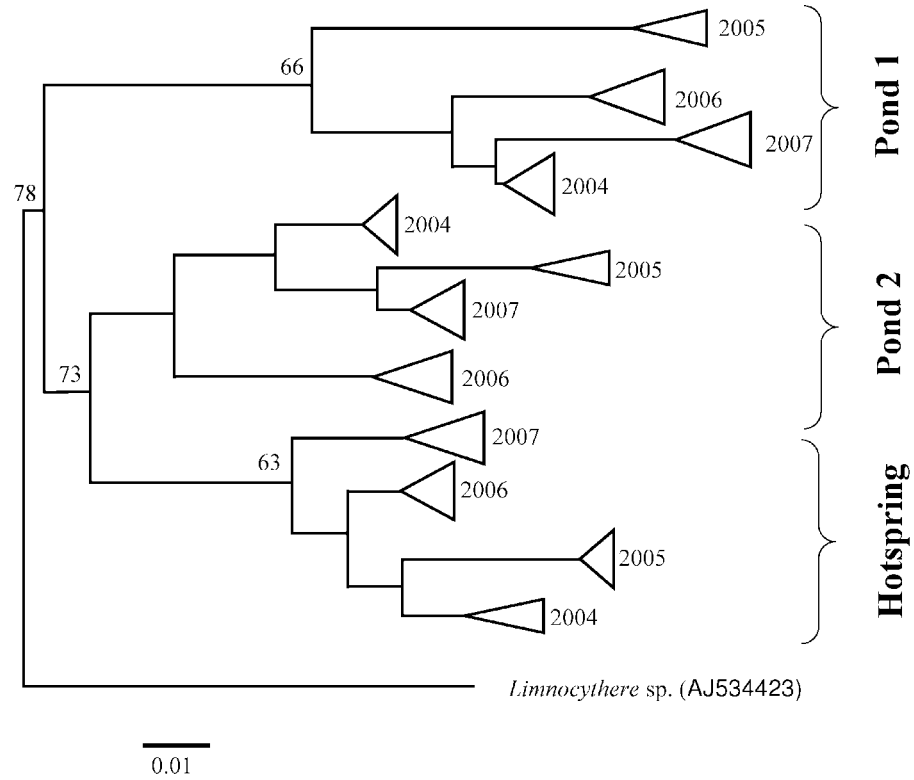

Fig. 4. Neighbour-joining tree constructed from ITS data for *Limnocythere atacamae* using K2P distances (substitutions per site) and including the whole data set ($n = 204$). Bootstraps were calculated based on 1000 replicates, the values are given above the branches; values less than 50% are not included.

a dispersal vector. Second, during the Bolivian winter periodic floods could invade all three populations, thus the three populations could be connected during this period. However, our data show a pattern of temporally stable genetic differentiation among the cold ponds and hot spring, suggesting the absence of effective dispersal and consequently gene flow. Only one COI haplotype was shared among ponds and this occurred in one year only (2005). We believe that this could be a stochastic event related to the propagule bank and an upsurge of ancestral genotypes, as this haplotype was absent in others years in the present analysis. In summary, spatial differentiation is strongly associated with genetic structure, as dispersal capacity is limited by geographic barriers (Hebert *et al.*, 2002; Adamowicz *et al.*, 2004; Mura *et al.*, 2006; Rossi *et al.*, 2010).

Information available on genetic structure, dispersion modes, and gene flow in non-marine ostracods is scarce. There are some habitats that contain higher numbers of endemic species than others, such as ancient lakes (Wouters and Martens, 2001) and younger ancient lakes such as Lakes of the Andean region (Martens and Behen, 1994; Mourguiart, 2000; Laprida, 2006; Laprida *et al.*, 2006). The information obtained to date (including this work) suggests that the high endemism (≈60%) of the aquatic communities of endorheic basins in the freshwater bodies of the high Andean plateau can be explained by their isolation (Gonzalez and Wattling, 2001;

Menu-Marque *et al.*, 2000; Zuñiga *et al.*, 1999), and also by the capacity of these communities to tolerate strong fluctuations in environmental parameters (Bayly, 1993; Williams *et al.*, 1995; Menu-Marque and Locascio de Mitrovich, 1998; Williams, 1998; Oyanedel *et al.*, 2008; Scheihing *et al.*, 2010).

In general, our results suggest that geographical differentiation is strongly associated with genetic differentiation, probably because the capacity for dispersion is severely limited by geographic barriers or the absence of dispersers in the area. Thus, this system is unique for studying allopatric speciation, and invites further research into the adaptive micro-evolutionary processes that explain the local differentiation they exhibit.

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