Genetic characterization of the endangered and endemic anchialine squat lobster *Munidopsis polymorpha* from Lanzarote (Canary Islands): management implications

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Anchialine species show restricted geographic ranges, high habitat specificity, and small population sizes. These factors make them particularly vulnerable to human activities, yet little is known about their ecology and evolutionary history. *Munidopsis polymorpha* is a decapod endemic to an anchialine cave system of the Corona lava tube in Lanzarote (Canary Islands). The present study, the first genetic survey conducted on this largely unknown species, was designed to characterize its genetic diversity, population structure and recent demographic history, using sequence data for the cytochrome oxidase I gene and eight microsatellites. A single haplotype was identified in the mitochondrial dataset. Nuclear genetic diversity was also low (average $\bar{N}_{m} = 4.375 \pm 1.685$). No significant genetic structure was detected between sampling sites and years, either by analysis of molecular variance ($F_{ST} = 0.006, p = 0.110$) or Bayesian clustering analysis ($K = 1$), indicating this species should be treated as a single management unit. Neither did we find evidence for a recent bottleneck event, and estimates of effective population size were extremely low ($\sim 50$). The lack of population structure, low genetic diversity and extremely low effective population size reinforce the high degree of isolation and endemicity of this species, and, consequently, the need to implement appropriate management actions.

**Keywords:** anchialine species, Canary Islands, conservation, effective population size, genetic diversity.

**Introduction**

Small or declining populations of threatened or endangered species are more prone to extinction than larger stable populations (Frankham *et al.*, 2002). When populations become geographically isolated, inbreeding is unavoidable and the subsequent loss of genetic variability is a risk for the long-term fitness of the population (Keller and Waller, 2002; Frankham and Ralls, 1998; Edmands, 2007). This problem is even more striking in closed populations with no migration, since mutation is the only mechanism for generating genetic diversity. All of these factors affect species inhabiting anchialine systems, and these typically show restricted geographic ranges, high habitat specificity and small population sizes. Anchialine habitats are defined as pools with no surface connection with the sea, containing salt or brackish water that fluctuates with tides (Holthuis, 1973). Such habitats are found worldwide, and harbour a fascinating and unique fauna, mainly dominated by crustaceans showing a high degree of endemism (Iliffe, 2000).

Despite a general interest in their conservation, these extraordinary and vulnerable habitats are becoming increasingly threatened, due to the negative impacts of anthropogenic activities, such as vandalism, rubbish dumping into cave openings, quarrying, deep-well injection waste or construction work (Glover and Earle, 2004). In the light of these threats, anchialine habitats are in urgent need of help. However, little is known about the ecology, demography, and evolutionary history of the species that inhabit them (Russ *et al.*, 2010). Interestingly, despite the great geographic isolation of these habitats, many anchialine taxa
show geographically widespread and anomalous disjunct distributions (Iliffe, 2000). It has been hypothesized that as a result of subterranean connections and marine dispersal, populations of anchialine organisms may experience higher levels of gene flow, but very low levels of genetic differentiation. However, so far, the few studies exploring the genetic connectivity of anchialine species have described sharply different patterns of genetic structure on the basis of mitochondrial DNA variation, from panmixia among populations hundreds of kilometres apart (Kano and Kase, 2004; Russ et al., 2010), restricted gene flow on small geographic scales (Craft et al., 2008; Santos, 2006). Accordingly, it is argued that the genetic structure observed in these systems is not determined exclusively by habitat or geographic features, but, to a large extent, by specific life-history traits (Kano and Kase, 2004; Craft et al., 2008; Russ et al., 2010).

Our study case focuses on the largest anchialine system in the East Atlantic region, the lava tube in the volcanic island of Lanzarote (Canary Islands, Spain). This anchialine system formed some 21 000 years ago under subaerial conditions, and experienced subsequent flooding during the post-glacial sea level rise (Carracedo et al., 2003). Three different formations constitute the Corona lava tube. Seawater penetrates 600 m inland, where it first encounters the Cueva de los Lagos. Hereafter, the lava tube section enters a tidal seawater pool, called the Jameos del Agua. At the seaward end of the Jameos del Agua, a second pool provides access to the Túnel de la Atlántida, a 1618-m-long subterranean tunnel that runs 64 m deep into the sea (Wilkens et al., 2009) (Figure 1). Several species of the marine crevicular and groundwater fauna of Lanzarote inhabit this anchialine cave (Martínez García et al., 2009; Wilkens et al., 1986), including several stygobiontic species characterized by their intense morphological, physiological and behavioural adaptations to cave environments. Among them, the decapod Munidopsis polymorpha (Koebel, 1892) is the most conspicuous and abundant taxon. This species is the only shallow-water representative of the genus Munidopsis (Macpherson, 2011), whose species usually inhabit the continental slope and abyssal domain (Baba et al., 2008). This endemic squat lobster from Lanzarote is listed as “Endangered” under the Spanish National Catalogue of Endangered Species (Templado et al., 2004) and the Regional Catalogue of the Canary Islands (see http://www.gobcan.es/boc/2001/097/004.html). Although the species is considered to be geographically restricted to the lava tube (Figure 1), isolated specimens have been sporadically reported in other flooded and subterranean environments in the north of the island (Gonzalez-Perez, 1995; Martínez Garcia et al., 2009). The species reaches its highest population density in the Jameos del Agua pool and the first few metres of the Túnel de la Atlántida, with isolated specimens inhabiting the Cueva de los Lagos (Figure 1). Early studies based on rough census surveys reported a high population density of up to 150 ind. m$^{-2}$ in the Jameos del Agua pool (Wilkens and Parzefall, 1974). However, more recent studies have witnessed a decline in the species, since the construction of a tourist resort in the area (Wilkens et al., 1990; Espino and Herrera, 2002). Unfortunately, the lack of systematic census data and the inconsistency of the methods applied in past studies preclude reliable comparisons and valid assessment of $M$. polymorpha population trends. Based on faunal affinities reported between the Jameos del Agua and Túnel de la Atlántida (Martínez Garcia et al., 2009), and the short geographic distance separating them (<65 m), a high degree of genetic connectivity would be expected on this scale. Nevertheless, this hypothesis has not been formally addressed.

Understanding patterns of genetic variation and connectivity among the populations of these particular habitats is crucial for management and conservation plans (Santos, 2006), since these serve as a benchmark against which changes can be monitored and measured. In effect, population genetics provides the appropriate tools to gain insight into the past history and current genetic variability of species, which in turn may determine the future success of their populations (Avise, 1994; Frankham et al.,

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**Figure 1.** Geographic location of the Lanzarote island and cross-section of the anchialine pools of the Corona lava tube: A) Cueva de los Lagos; B) Jameos del Agua; C) Túnel de la Atlántida; and D) subterranean portion of the Túnel de la Atlántida. Modified from Jantschke et al., 1994.
In this study, we used mitochondrial gene sequence data in combination with eight microsatellite nuclear markers to characterize the genetic diversity, population structure, and recent demographic history of *M. polymorpha*, to provide genetic data on which to base future management plans.

**Material and methods**

**Sample collection**

Over the period January 2005 to November 2009, specimens of *M. polymorpha* were collected from two sampling sites in the Corona lava tube system (Figure 1): Jameos del Agua in 2005, 2008 and 2009, and Túnel de la Atlántida in 2008 and 2009. Some isolated specimens have been reported in the Cueva de los Lagos (Figure 1), but caving techniques are needed to access these. Additionally, other groundwater systems (saline wells) in the northeastern part of the island, for which there are previous records of the species (Martínez García et al., 2009), were explored in the three sampling years, but, unfortunately, we could not confirm the presence of the species. Squat lobster specimens were captured just after dawn by snorkelling using hand nets, and preserved in absolute ethanol for subsequent molecular DNA analyses. Material from two specimens was deposited in the biological collections of the Centro de Estudios Avanzados de Blanes in Gerona, and the Museo Nacional de Ciencias Naturales in Madrid, Spain.

**DNA extraction, sequencing, and genotyping**

Total DNA was extracted from tissue samples using the magnetic Charge Switch gDNA Micro Tissue Kit (Invitrogen). A fragment of the cytochrome oxidase I (COI) gene was amplified by polymerase chain reaction (PCR) with the primers gala_COIF (5′-CAT CAC TWA GWT TRA TYA TTC GAG CAG AA-3′) and gala_COIR (5′-GAA YAG GRT CTC CTC CTC CTA C-3′) (Jones and Macpherson, 2007). Reactions were prepared in a final volume of 50 µL, and the PCR mix contained 2 µL of DNA template, 1 × standard reaction buffer containing final concentrations of 2 mM MgCl₂, 0.16 µM of each primer and 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), along with 0.5 µL of bovine serum albumin (10 mg ml⁻¹), 1.5 U of Taq DNA polymerase (Biotools) and double-distilled water (ddH₂O). Amplification was conducted as follows: 94°C (4 min), then 35 cycles at 94°C (1 min), 48.5°C (2 min) and 72°C (3.5 min), and a final extension at 72°C (10 min). Amplified fragments were purified by ethanol precipitation prior to sequencing both strands using the same PCR primers and Big Dye Terminator (Applied Biosystems). Sequences were run in an ABI 3730 Genetic Analyzer (Applied Biosystems).

All samples were genotyped for eight microsatellite loci: Mp-1, Mp-2, Mp-3, Mp-4, Mp-5, Mp-6, Mp-7 and Mp-8 (Cabezas et al., 2009). PCR amplifications were performed in 15 µL of reaction mix containing 2 µL of DNA template, 1 × standard reaction buffer containing final concentrations of 2 mM MgCl₂, 0.5 µM of each primer and 0.2 mM of each dNTP, along with 0.5 U of Taq polymerase (Biotools) and ddH₂O. The cycling profile was 94°C (4 min), followed by 35 cycles at 94°C (30 s), 56°C (30 s) and 72°C (45 s). Fluorescently labelled PCR products were analysed in an ABI 3730 Genetic Analyzer (Applied Biosystems) with the GeneScan500 internal size standard. Allele sizes were determined using GeneMapper 3.7 (Applied Biosystems).

**Data analysis**

Mitochondrial DNA sequences were edited and assembled using Sequencer 4.6 (Gene Codes) and aligned manually in Se-Al 2.0a11 (Ramant, 1996). The number of haplotypes (*h*) was explored using the program DnaSP 5 (Librado and Rozas, 2009).

Microsatellite data were checked for possible genotyping errors (i.e. null alleles, stuttering, and large allele dropout), using MicroChecker 2.2.3 (van Oosterhout et al., 2004). Genetic diversity based on microsatellites was assessed for each collection site and year, as well as for all the sampling sites together, by calculating the total number of alleles (*N_a*), observed (*H_o*) and expected (*H_e*) heterozogosities, and departures from Hardy–Weinberg equilibrium, using the package GenAlEx 6.4 (Peakall and Smouse, 2006). Allelic richness (*A_e*) and the inbreeding coefficient (*F_is*) were estimated, using FSTAT 2.9.3 (Goudet, 1995). Tests for differences in genetic diversity values (i.e. *H_o*, *H_e* and *A_e*) between localities and years were performed in FSTAT.

Genetic differentiation between collection sites (Jameos del Agua and Túnel de la Atlántida), and between sampling years (2005, 2008 and 2009), was tested by analysis of molecular variance performed in Genedive 2.0b20 (Meirmans and Van Tienderen, 2004). In addition, we assessed the genetic structure of *M. polymorpha* without prior assignment to the collection site origin using the Bayesian model-based clustering method implemented in Structure 2.3 (Pritchard et al., 2000), which allows the assignment of individuals to genetic clusters, the correlation of clusters with sampling sites, and the identification of admixed populations. To do this, we used the admixture model and correlated allele frequencies (F-model; Falush et al., 2003), assuming that frequencies in the different populations were likely to be similar. Ten independent runs were conducted for each *K*, from *K = 1* to *K = 6*. Then, for each value of *K*, we averaged the log-likelihood values and calculated their associated posterior probabilities according to Bayes’ rule (Pritchard et al., 2009).

Two methods were used to determine if the recent population decline in *M. polymorpha* in the Corona lava tube has generated a genetic signal of demographic contraction. The statistics tested were: Δ*H*, or the difference between the gene diversity *H_e* computed from the sample, and *H_A*, which is the heterozygosity expected from the observed number of alleles for a population in Wright–Fisher equilibrium (Cornuet and Luikart, 1996); and *M*, which is the ratio of the number of alleles over the range in allele size (Garza and Williamson, 2001). We used BOTTLENECK 1.2.02 (Piry et al., 1999) to calculate Δ*H*, employing the two-phase model with the 70% stepwise mutation model, and the Wilcoxon rank test to assess its significance with 10 000 iterations. *M_P_Val* (Garza and Williamson, 2001) was used to calculate *M*.

In small isolated populations, genetic drift and inbreeding erode genetic diversity through the loss of alleles and increasing homozygosity. These adverse consequences for genetic variability are largely dependent on the effective population size (*N_e*), which is a crucial parameter in wildlife management and conservation planning, because of its influence on population viability (Luikart et al., 2010). Estimates of contemporary *N_e* can be divided into: point estimates, based on one population sample (Hill, 1981); and temporal estimates, based on two or more samples of one population at different periods (Wang, 2005; Waples, 2005). There is currently no general consensus as to which method is best at estimating *N_e* (Luikart et al., 2010).
Thus, two one-sample estimator approaches and an additional two-sample \( N_e \) estimator, considering temporal samples, were used for comparative purposes. First, we estimated \( N_e \) using the approximate Bayesian computation method implemented in the program ONEsAMP 1.2 (Tallmon et al., 2008). Second, we used the program LDNe (Waples and Do, 2008), which estimates \( N_e \) based on linkage disequilibrium arising from genetic drift. A random mating system was assumed, and all alleles with frequencies <0.05 were excluded (Waples, 2006). Finally, we used the temporal method in Tempo Fs (Jorde and Ryman, 2007), which requires two or more samples separated in time to estimate \( N_e \) based on allele frequency changes between populations arising from drift. Each year was considered a temporal sample: three for Jameos del Agua and two for Tunel de la Atlantida (see the “Sample collection” section). For this approach, we used the F-statistic, which is unbiased for small sample sizes and skewed allele frequencies (as is characteristic of microsatellite data) (Jorde and Ryman, 2007), and sampling plan II, for which individuals are sampled before they reproduce, and not returned to the population. The generation time was assumed to be 1 year.

**Results**

In total, 121 specimens of *M. polymorpha* were captured: 76 individuals in Jameos del Agua (24 in 2005, 27 in 2008, and 25 in 2009), and 45 in Tunel de la Atlantida (23 in 2008, and 22 in 2009). A fragment of the mitochondrial COI gene was amplified and sequenced in 23 randomly selected individuals from all localities and for all 3 years, recovering a total of 547 bp. A single haplotype was detected in all samples (GenBank accession no. JQ622397).

All 121 samples were successfully genotyped for eight microsatellite loci (Table 1), and the genotypes have been deposited in the Dryad database under doi number http://dx.doi.org/10.5061/dryad.17s6621t. No evidence of null alleles, stuttering or large allele dropout was detected by MicroChecker. Overall, the observed heterozygosity ranged from 0.067 to 0.658 (mean \( H_o = 0.318 \pm 0.199 \)), the number of alleles per locus ranged from 2 to 6 (mean \( N_A = 4.375 \pm 1.685 \)), and the total number of alleles across all loci was 35 (Table 1). All allele frequencies were in agreement with the Hardy–Weinberg equilibrium, except for the Mp-1 microsatellite in specimens from Jameos del Agua. Genetic diversity, measured as \( H_o \), \( H_e \), and \( A_o \), did not differ significantly among collection sites or years (\( p > 0.8 \) in all cases). Four private alleles were observed in the Tunel de la Atlantida for the loci Mp-1, Mp-6 and Mp-7, and three were reported for the Jameos del Agua for the loci Mp-5 and Mp-8. In all cases, private alleles occurred at a frequency of <1.5% per site.

The fixation index was not significant among collection sites or years (\( F_{ST} = 0.006, p = 0.110 \)), or when samples were grouped according to their collection site and years (\( F_{CT} = 0.001, p = 0.066 \), and \( F_{CT} = -0.003, p = 0.312 \), respectively) (Table 2). Additionally, the analysis performed in the Structure program revealed that the most likely number of genetic clusters was \( K = 1 \) (posterior probability \( \approx 1 \)); thus, both collection sites and sampling years represent a single genetic pool.

The Wilcoxon rank test for heterozygosity excess performed in BOTTLENECK found no evidence of a recent bottleneck event for any of the sites sampled, when considered separately or together. Similarly, according to the \( M \) ratios estimated in M_P_Val, we detected no associations with population bottlenecks, given the observed values for the two localities, both separately and together (\( M_{FA} = 1.067, M_{TA} = 1.251, M_{FA+TA} = 1.458 \)), were larger than expected under a bottleneck event in 100% of the simulations performed.

The effective population size of *M. polymorpha* was low, overall. Estimates were slightly different among methods, but congruent and with largely overlapping confidence intervals (Table 3). Only the LDNe method failed to detect any evidence of genetic drift between generations for the Tunel de la Atlantida locality, thus rendering an infinite upper 95% confidence interval. When collection sites were considered separately, estimated mean \( N_e \) values were similar, ranging from 23.6 to 59 for Jameos del Agua, and from 27 to 46.6 for Tunel de la Atlantida. However, mean \( N_e \) estimates were higher when both collection sites were drawn together (\( N_e = 46.31–133 \); Table 3).

### Table 1. Genetic diversity of *M. polymorpha* based on microsatellite loci.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Mp-1</th>
<th>Mp-2</th>
<th>Mp-3</th>
<th>Mp-4</th>
<th>Mp-5</th>
<th>Mp-6</th>
<th>Mp-7</th>
<th>Mp-8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jameos del Agua (( N = 76 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_A )</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>3.875 ± 1.457</td>
</tr>
<tr>
<td>( A_o )</td>
<td>4.578</td>
<td>3.526</td>
<td>2</td>
<td>3.361</td>
<td>2.809</td>
<td>4.825</td>
<td>5.236</td>
<td>5.342 ± 1.248</td>
<td></td>
</tr>
<tr>
<td>( H_o )</td>
<td>0.211</td>
<td>0.461</td>
<td>0.184</td>
<td>0.421</td>
<td>0.079</td>
<td>0.079</td>
<td>0.658</td>
<td>0.382</td>
<td>0.309 ± 0.205</td>
</tr>
<tr>
<td>( H_e )</td>
<td>0.292</td>
<td>0.499</td>
<td>0.193</td>
<td>0.441</td>
<td>0.077</td>
<td>0.077</td>
<td>0.560</td>
<td>0.327</td>
<td>0.308 ± 0.185</td>
</tr>
<tr>
<td>( F_{IS} )</td>
<td>0.285</td>
<td>0.084</td>
<td>0.051</td>
<td>0.053</td>
<td>-0.022</td>
<td>-0.025</td>
<td>-0.169</td>
<td>-0.161</td>
<td>0.003 ± 0.145</td>
</tr>
<tr>
<td>Tunel de la Atlantida (( N = 45 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_A )</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4.375 ± 1.685</td>
<td></td>
</tr>
<tr>
<td>( A_o )</td>
<td>4.974</td>
<td>3.717</td>
<td>2</td>
<td>2.919</td>
<td>3.439</td>
<td>5.089</td>
<td>4.73</td>
<td>3.605 ± 1.249</td>
<td></td>
</tr>
<tr>
<td>( H_o )</td>
<td>0.311</td>
<td>0.556</td>
<td>0.333</td>
<td>0.422</td>
<td>0.067</td>
<td>0.111</td>
<td>0.644</td>
<td>0.178</td>
<td>0.328 ± 0.207</td>
</tr>
<tr>
<td>( H_e )</td>
<td>0.298</td>
<td>0.500</td>
<td>0.325</td>
<td>0.464</td>
<td>0.064</td>
<td>0.107</td>
<td>0.655</td>
<td>0.166</td>
<td>0.323 ± 0.207</td>
</tr>
<tr>
<td>( F_{IS} )</td>
<td>-0.034</td>
<td>-0.1</td>
<td>-0.015</td>
<td>0.102</td>
<td>-0.023</td>
<td>-0.026</td>
<td>0.028</td>
<td>0.059</td>
<td>-0.05 ± 0.06</td>
</tr>
<tr>
<td>All sampling sites (( N = 121 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( N_A )</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>4.375 ± 1.685</td>
<td></td>
</tr>
<tr>
<td>( A_o )</td>
<td>4.974</td>
<td>3.717</td>
<td>2</td>
<td>2.919</td>
<td>3.439</td>
<td>5.089</td>
<td>4.73</td>
<td>3.605 ± 1.249</td>
<td></td>
</tr>
<tr>
<td>( H_o )</td>
<td>0.248</td>
<td>0.496</td>
<td>0.240</td>
<td>0.421</td>
<td>0.074</td>
<td>0.091</td>
<td>0.653</td>
<td>0.306</td>
<td>0.318 ± 0.199</td>
</tr>
<tr>
<td>( H_e )</td>
<td>0.294</td>
<td>0.500</td>
<td>0.244</td>
<td>0.451</td>
<td>0.072</td>
<td>0.088</td>
<td>0.603</td>
<td>0.271</td>
<td>0.315 ± 0.190</td>
</tr>
<tr>
<td>( F_{IS} )</td>
<td>0.161</td>
<td>0.012</td>
<td>0.022</td>
<td>0.069</td>
<td>-0.025</td>
<td>-0.028</td>
<td>-0.079</td>
<td>-0.124</td>
<td>0.002 ± 0.088</td>
</tr>
</tbody>
</table>

\( N_A \) number of alleles; \( A_o \) allelic richness; \( H_o \) observed heterozygosity; \( H_e \) expected heterozygosity; \( F_{IS} \) inbreeding coefficient. Bold value indicates significant departures from Hardy–Weinberg equilibrium (\( P < 0.05 \)).
Table 2. Analysis of molecular variance among the M. polymorpha collection sites examined.

<table>
<thead>
<tr>
<th>Partition tested</th>
<th>Variation among groups (%)</th>
<th>$F_{IS}$</th>
<th>$F_{ST}$</th>
<th>$F_{CT}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among localities and years (all) ([J05, JA08, JA09, TA08, TA09])</td>
<td>0.6</td>
<td>-0.004 (0.557)</td>
<td>0.006 (0.110)</td>
<td></td>
</tr>
<tr>
<td>Between localities: (JA) vs. (TA) ([J05, JA08, JA09]) (TA08, TA09)</td>
<td>0.1</td>
<td>-0.004 (0.544)</td>
<td>0.005 (0.204)</td>
<td>0.001 (0.066)</td>
</tr>
<tr>
<td>Among years: (2005) vs. (2008) vs. (2009) ([J05]) (JA08, TA08) (JA09, TA09)</td>
<td>-0.3</td>
<td>-0.004 (0.514)</td>
<td>0.008 (0.137)</td>
<td>-0.003 (0.312)</td>
</tr>
</tbody>
</table>

$F_{IS}$, variation among individuals within localities; $F_{ST}$, variation among localities within the population; $F_{CT}$, variation among groups within the population; JA, Jameos del Agua; TA, Túnel de la Atlántida.

P-values are indicated between brackets.

Table 3. Effective population size ($N_e$) values estimated for M. polymorpha.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONeSAMP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jameos del Agua</td>
<td>35.65</td>
<td>26.51</td>
<td>55.45</td>
</tr>
<tr>
<td>Túnel de la Atlántida</td>
<td>33.88</td>
<td>25.57</td>
<td>51.22</td>
</tr>
<tr>
<td>All</td>
<td>46.31</td>
<td>34.13</td>
<td>75.30</td>
</tr>
<tr>
<td>LDNe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jameos del Agua</td>
<td>23.60</td>
<td>9.50</td>
<td>64.90</td>
</tr>
<tr>
<td>Túnel de la Atlántida</td>
<td>46.60</td>
<td>13.00</td>
<td>Infinity</td>
</tr>
<tr>
<td>All</td>
<td>53.40</td>
<td>21.50</td>
<td>202.70</td>
</tr>
<tr>
<td>Tempo Fs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jameos del Agua</td>
<td>59.00</td>
<td>21.00</td>
<td>69.00</td>
</tr>
<tr>
<td>Túnel de la Atlántida</td>
<td>27.00</td>
<td>11.00</td>
<td>73.00</td>
</tr>
<tr>
<td>All</td>
<td>133.00</td>
<td>61.00</td>
<td>204.00</td>
</tr>
</tbody>
</table>

Discussion

Prior studies examining patterns of genetic connectivity among anchialine species have yielded mosaics, spanning from panmixia to high population structure on different geographic scales (Kano and Kase, 2004; Santos, 2006; Russ et al., 2010; Craft et al., 2008). Accordingly, we used an intraspecific approach to address genetic variation and population structure.

Based on data from mitochondrial DNA sequences and microsatellite markers, we detected an overall lack of population structure, a low genetic diversity, and an extremely low effective population size for M. polymorpha. The homogeneity observed among individuals from the two collection sites indicates extensive bidirectional genetic interchange, between the Jameos del Agua and Túnel de la Atlántida. Private alleles were observed in both collection sites (see the “Results” section), which may indicate some restrictions to gene flow. The very low frequency (alleles found in only one or two specimens) might indicate that these alleles are randomly sampled in the two populations, and a larger sampling effort would be needed in order to detect the extent of gene-flow restriction.

Although isolated specimens have been observed in the Cueva de los Lagos lagoon, and other saline wells 5 km away from the cave system (Martínez García et al., 2009), the existence of additional stable populations is unlikely. Larval dispersal in M. polymorpha is lecithotrophic, with two zoal stages, both of which are practically incapable of locomotion (Wilkins et al., 1990; Baba et al., 2011). Sporadic dispersal events via oceanic routes have been reported in other anchialine species (Santos 2006), but given the brief larval development process in M. polymorpha, dispersion over long distances is improbable.

Moreover, adult stages show low vagility and usually feed in the same territory (Lovrich and Thiel, 2011; Wilkins et al., 1990). The lack of genetic structure suggests high connectivity on a very local scale within the Corona lava tube, along with rare, random, sporadic short-distance dispersal events through the groundwater systems of the island. Future studies will determine if specimens from other isolated localities are from the same genetic pool as those of the Corona lava tube system, or, on the contrary, belong to an as yet unknown divergent population.

Low levels of genetic diversity are often interpreted as declines in population size due to overexploitation, anthropogenic activities or past events, such as climate changes (Bouzat et al., 1998; Hewitt, 2000; Domínguez-Domínguez et al., 2007). However, this pattern can also be the outcome of historical genetic uniformity, and, thus, may not necessarily be related to recent demographic fluctuations (Matocq and Villablana, 2001; Rodríguez et al., 2011). Early studies in the 1970s reported a high population density of the species (150 ind. m$^{-2}$) in the Jameos del Agua pool (Wilkins and Parzefall, 1974), but a decade later, a considerable decline in the population density and fertility was detected (Wilkins et al., 1990). Our results, however, failed to unveil a genetic signal for a recent demographic contraction, such that the observed low level of genetic diversity could be the result of a long-standing low effective population size (Rodríguez et al., 2011), and/or of the colonization mode of the lava tube.

Considering that most representatives of the genus Munidopsis inhabit deep-sea waters (Macpherson and Segonzac, 2005), we could hypothesize that M. polymorpha, or its ancestor, colonized the groundwater system of the island and subsequently invaded the lava tube, or, alternatively, colonization may have been concurrent with the formation of the lava tube. The age of this putative colonization event is uncertain. The Corona lava tube formed during the Pliocene and Pleistocene, ≏ 21 ± 6.5 thousand years ago, coinciding with the Last Glacial Maximum (Carracedo et al., 2003). During this period, some extant deep water species exhibited shallower distributions (see Maggs et al., 2008 and references cited therein), which could have facilitated colonization of the lava tube from deeper environments. However, previous studies suggest that a colonization event as old as the emergence of the island of Lanzarote, ≏ 15 million years ago, offers a more realistic evolutionary scenario to explain taxonomic commonalities between the lava tube fauna and the groundwater system of the island (Wilkins et al., 2009; Martínez García et al., 2009). Thus, our low effective population size estimates could either point to a recent founder event involving very few individuals, or to an ancient colonization event. In the case of the latter, the effects of a reduction in the population size would pass unnoticed.
by the markers and methods used here. For both scenarios, the capacity of the species to achieve locally large and dense populations within the Corona lava tube might have prevented the effects of drift due to recent demographic changes, thus explaining our inability to detect a bottleneck genetic signal. We conclude that according to the genetic homogeneity within and between the Jameos del Agua and Túnel de la Atlántida, and the overall Hardy–Weinberg equilibrium, no significant gene flow exists with other populations outside the Corona lava tube system. This reinforces the high degree of isolation and endemicity of this species, and, consequently, the need to implement appropriate conservation actions.

Conservation and management implications
Historically, anchialine systems have suffered the negative impacts of anthropogenic activities (Glover and Earle, 2004). The Corona lava tube is no exception, and this unique habitat has been dramatically affected by the construction of a tourist complex in the area. Unfortunately, and despite the information provided at the entrance to the complex, visitors have wrongly considered cave pools as natural wishing wells in which to throw coins. Copper coins rapidly corrode in saltwater, with the subsequent accumulation of high levels of toxic copper ions, which could likely explain the marked decline in the abundance of M. polymorpha over the last years (Iliffe and Bishop, 2007). Significant efforts have been made to protect this anchialine habitat and its endemic biota, cataloguing M. polymorpha as “Endangered” in 1990 (Templado et al., 2004). However, the recognition of species that warrant prompt conservation efforts might be more effective if accompanied by the creation of protected areas, to preserve the ecological value of their habitats together with the evolutionary potential of the species. Their lack of genetic differentiation indicates that M. polymorpha specimens from the Corona lava tube should be managed as a single genetic pool. The low genetic diversity in combination with the extremely low effective population size, observed here, should warn of the compromised long-term viability of M. polymorpha, since a reduced genetic potential could affect the evolutionary flexibility of a species to face environmental changes (Frankham et al., 1999). Further, the effective population size of M. polymorpha is some orders of magnitude lower than that of numerous overexploited spiny lobsters (Palero et al., 2010), and also lower than that of endangered freshwater crayfish populations (Gouin et al., 2011), emphasizing the threatened status of this species. This study provides comprehensive baseline population genetic data, to aid the monitoring and development of management plans for the viability of this anchialine species. Based on our findings, we would strongly recommend regular monitoring of the species’ demographics, and its genetic consequences. We also recommend its conservation status of “Endangered” be maintained, and the necessary legal protection enforced, to preserve the ecological uniqueness of the Corona lava tube.

Acknowledgements
The authors thank the Gobierno de Canarias and Cabildo de Lanzarote for permission to collect samples of M. polymorpha. We are also very grateful to Alejandro Martínez for constructive comments and for providing samples of M. polymorpha. This study was funded by MEC project CTM 2008–00496. PC was supported by an I3P pre-doctoral grant from the Consejo Superior de Investigaciones Científicas and a grant from the Fundación Caja Madrid.

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*Handling editor: Rochelle Seitz*