**Substitution rate estimated for demographic analysis.**

The substitution rate for the demographic analysis of *Orestias* was estimated using the phylogenetic relationships and divergence times found for D-loop sequences. We used a total of 254 sequences where 61 was from of chilean *Orestias* phylogeny on Vila et al. (2013) with GenBank accession numbers JX134506.1- JX134566.1 and 117 from Guerrero et al. (2017) with the GenBank accession numbers KX498242.1- KX498358.1. We added 76 new sequences from this work. As was performed on Vila et al. 2013, *Orestias* *puni* sequences available on their dataset were used as outgroup. Phylogenetic relationships and divergence times were determined by BEAST software v. 1.8.4 (Drummond, Suchard, Xie & Rambaut, 2012). A Bayesian framework which allowed for variable divergence time estimations among lineages was used and an uncorrelated relaxed clock with a lognormal distribution model was used as prior based on the relevance of evolutionary rates. The General Time Reversible model (GTR) was selected on jModelTest (Posada, 2008).

Markov Chain Monte Carlo (MCMC) analyses were run for 20 million of generations and trees were sampled every 1000 generations. Two million generations were considered as a burn-in to approximate the stabilization levels of the Markov chains. The convergence of the MCMC algorithm was checked by repeating each analysis at least four times, to find suitable effective sample sizes (ESS) and to improve the credibility of the parameters. The analyses were performed on Tracer 1.5 (Rambaut & Drummond, 2009). To estimate the divergence times, the molecular clock was adjusted using three calibration points as priors.

Despite not having a fossil record, it is possible to use geologic events as a starting point for population growth and consequent genetic diversification. According to these and that Lake Titicaca that rises at 3805 masl has been proposed as a center of radiation for the genus towards the Altiplano, as first calibration point, we used the high rise of Andes Mountains occurred 5-2 Mybp where our interest places of Chilean Altiplano reached heights greater than 4000 masl together with the elevation of the western vergent of Andes Mountains (Villwock 1983; Kött, Gaup & Wörner 1995; Muñoz & Charrier 1996; Lamb & Davis 2003) and also, considering that the paleolake that reached the highest altitude in the Altiplano basin was Lake Mataro with 3950 masl (Fornari, Risacher & Féraud 2001). The prior values followed a gamma distribution with the following parameters: offset= 5; shape= 2; scale= 0.5. As second calibration point, we used the regression of Tauca paleo Lake 14,500-13,500 ybp which meant the isolation of one of the southern basins on Chilean altiplano, The Carcote saltpan basin (Placzek, Quade & Patchett 2006; Blard et al 2011) where 13,500 ybp was considered as nearest to the isolation event. The prior values followed a gamma distribution with the following parameters: offset= 0.013; shape= 1.2; scale= 0.0005. The third point of calibration was the collapse of Parinacota volcano that according to latest analysis occurred 8,800 ybp and restructured aquatic systems of Lauca Basin (Clavero et al 2002, 2004; Sáez et al., 2007; Giralt et al., 2008; Jicha et al 2015). The prior values followed a gamma distribution with the following parameters: offset= 0.008; shape= 2; scale= 0.001.

The estimated mutational rate of D-loop for *Orestias* populations was approximately 3.99% per million years.

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