

# Invasive *Pleurodema thaul* from Robinson Crusoe Island: molecular identification of its geographic origin and comments on the phylogeographic structure of this species in mainland Chile

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Robinson Crusoe Island, located 670 km west of the coast of central Chile, is characterized by a high number of endemic and introduced species of plants and animals. This island lacks native amphibians or reptiles, but somewhat more than 30 years ago the toad *Pleurodema thaul*, native to continental Chile, was introduced. The coast of central Chile was identified as the geographic source of the introduced population of Robinson Crusoe by means of phylogenetic analyses of mitochondrial sequences. These analyses also suggest a high degree of phylogeographic structure for this species in continental Chile.

*Key words:* anuran, Juan Fernández Islands, Leiuperidae, invasive species, phylogeography

## INTRODUCTION

Invasive species have been recognized as one of the main threats to global biodiversity (Alford & Richards, 1999; Mack et al., 2000). Amphibians have often been able to spread successfully to regions into which they have been introduced, and have generally had detrimental effects on the environment invaded (Kupferberg, 1997; Kraus et al., 1999; Crossland, 2000). For this reason, studies of the sources of origin and genetic diversity of organisms are indispensable for understanding the ecology of invading populations, and this type of data has proved valuable for designing and evaluating control and eradication strategies (Abdelkrim et al., 2007; Ficetola et al., 2008).

The Juan Fernández archipelago lies in the southeastern Pacific, 670 km west of the central Chilean coast. It is composed of three islands, Robinson Crusoe (14794 ha), Santa Clara (221 ha) and Alexander Selkirk (4952 ha), which extend between 33°37'00"S, 78°49'50"W and 33°46'00"S, 80°46'00"W (Pequeño & Sáez, 2000). As with most oceanic islands, the biota of the Juan Fernández archipelago presents a high degree of endemism (Matthei et al., 1993; Hulm & Thorsell, 1995; Ricci, 1996; Marticorena et al., 1998; Roy et al., 1999) as well as a great number of non-native species of plants and animals (Cuevas & Van Leersum, 2001; Iriarte et al., 2002; Cuevas et al., 2004). These islands lack native terrestrial reptiles or amphibians (Ceí, 1962; Donoso-Barros, 1966; Veloso & Navarro, 1988).

Somewhat more than 30 years ago, the toad *Pleurodema thaul* (family Leiuperidae; Frost, 2008), native to continental Chile, was introduced to Robinson Crusoe Island, and in recent years has reached a very

high population density (R. Solís, pers. comm.). The first mention of this species on the island was made by Zeiss & Hermosilla (1970), who reported:

“during our stay on the island we had the opportunity to hear the croak of an amphibian, probably the small four-eyed toad which was recently introduced, based on our inquiries”.

The density of *P. thaul* on the island must have been very low at that time, since the efforts of these authors to collect these amphibians were fruitless (W. Hermosilla, pers. comm.).

*P. thaul* is the most common and widely distributed amphibian in continental Chile, ranging latitudinally between 27° and 46°S (Correa et al., 2007). Throughout its wide distribution *P. thaul* presents a high degree of geographic variation in morphological traits, colour patterns, life history traits and reproductive characteristics (Ceí & Capurro, 1957; Ceí, 1958, 1962). From a taxonomic perspective, Duellman & Veloso (1977) postulated that *P. thaul* is a complex formed by three species, based on differences in sternal conditions, amplexic positions, egg deposition and karyotypes among populations. However, these authors did not formalize this subdivision. A morphological study by Rosset et al. (2001) demonstrated that it is not possible to distinguish morphotypes corresponding to the species recognized by Duellman & Veloso (1977). Furthermore, the higher degree of intra- compared to inter-population variability described by Rosset et al. (2001) with regard to all the variables considered, suggests that it is not possible to recognize the geographic origin of any individual of this species based on morphological attributes alone. Based on this, a phylogenetic approach was chosen to identify the geographic source

**Table 1.** Geographic coordinates of the localities of *Pleurodema thaul* and *P. bufonina* (Lago Cochrane) included in this study, ordered by latitude from north to south. The collection numbers of specimens and GenBank accession numbers for mitochondrial sequences are indicated. Localities are shown on the map (Fig. 1).

Locality	Latitude (S)	Longitude (W)	Collection number	Accession number
Carrera Pinto	27°06'40"	69°53'44"	DBGUCH0701067	EU445708
Cruce a Chungungo	29°35'13"	71°15'10"	DBGUCH0610059	EU445710
Socos	30°43'52"	71°29'28"	DBGUCH0511002	EU445709
Caimanes	31°55'38"	71°08'32"	DBGUCH0705007	EU445711
Quebrada de Córdova	33°26'28"	71°39'38"	DBGUCH0701295	EU445707
San Juan Bautista (Robinson Crusoe)	33°38'25"	78°50'02"	DBGUCH0512001	EU445705
Bahía El Padre (Robinson Crusoe)	33°40'10"	78°56'09"	DBGUCH0702008	EU445706
Radal Siete Tazas	35°27'33"	71°01'35"	DBGUCH0602001	EU445713
Nonguén	36°53'53"	72°58'58"	MZUC 28423	EU445712
Conguillío	38°35'35"	71°46'15"	DBGUCH0704085	EU445703
Mississippi	39°27'00"	73°12'00"	DBGUCH3144	EU445704
Llico Bajo	41°16'00"	73°40'00"	DBGUCH2918	DQ864560
Senda Darwin	41°53'03"	73°50'00"	DBGUCH0610062	EU445702
Futaleufú	43°15'11"	71°49'40"	DBGUCH3425	EU445701
Lago Cochrane	47°12'01"	72°27'42"	DBGUCH0702005	EU445700

of the *P. thaul* population present on Robinson Crusoe Island. For this, mitochondrial sequences were obtained from representatives from most of its geographic range in continental Chile. Another objective of this study was to contribute preliminary data regarding the phylogeographic structure of *P. thaul* in Chile.

## METHODS

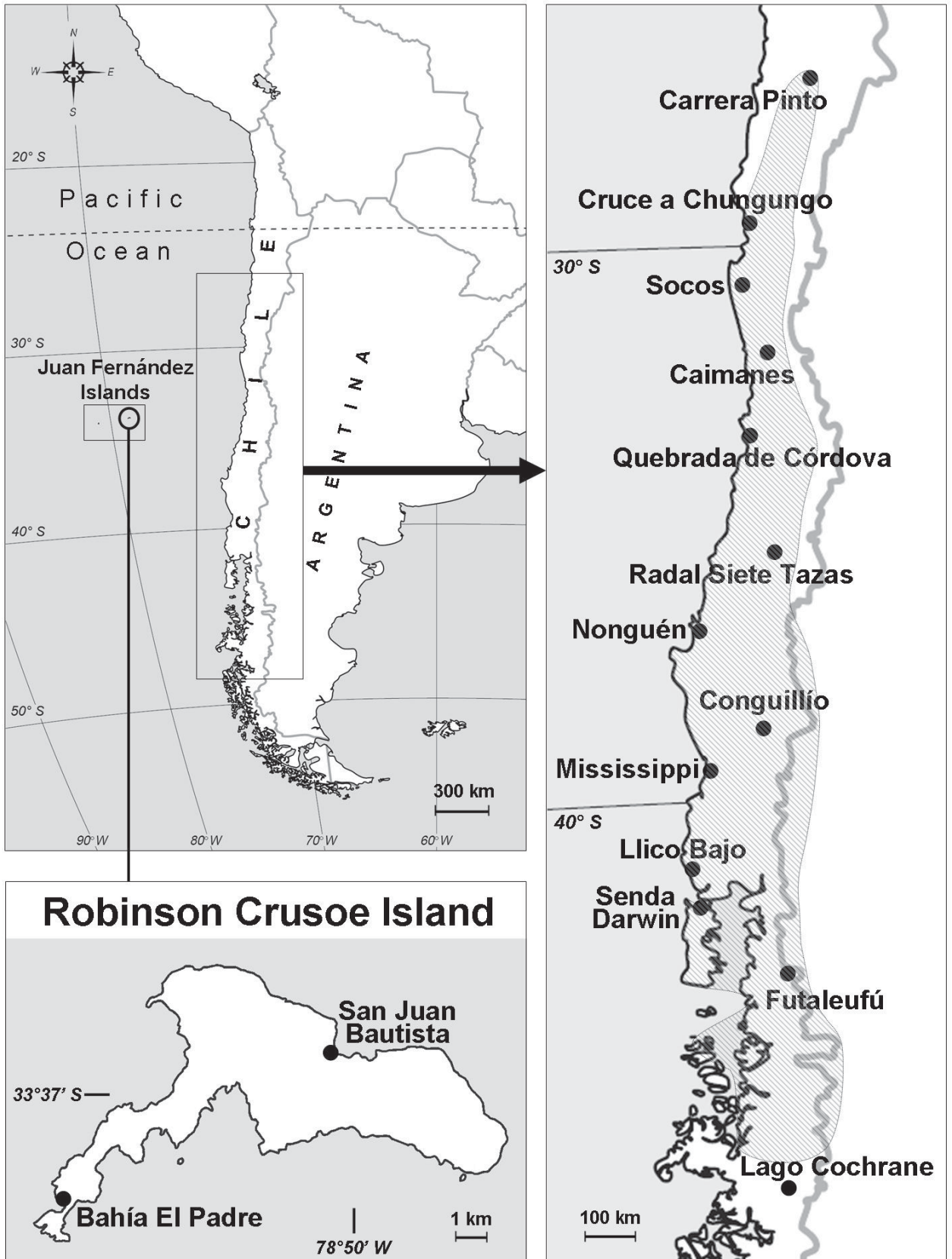
Twelve individuals from each of 12 locations throughout the distributional range (northern limit to 300 km from the southern limit; Correa et al., 2007) were included in the analyses. Also included were two individuals from Robinson Crusoe Island collected at different places on different dates (Fig. 1, Table 1). The individual from San Juan Bautista was collected in December 2005, while the one from Bahía El Padre was collected in February 2007. We further included an individual of *P. bufonina* from Lake Cochrane (Table 1) as the outgroup for phylogenetic analyses. This species has been previously identified as the sister species of *P. thaul* by Duellman & Veloso (1977) in the only phylogenetic analysis carried out for this genus to date.

All specimens studied have been deposited in the herpetological collection of the Departamento de Biología Celular y Genética de la Universidad de Chile (DBGUCH), except for the specimen from the Nonguén locality which belongs to the Museo de Zoología de la Universidad de Concepción (MZUC). The geographic coordinates of each collection locality plus the collection numbers for the specimens are listed in Table 1. A segment of mitochondrial DNA ranging from the 12S to the 16S ribosomal RNA gene was chosen for the phylogenetic analyses. Although these genes are the most conserved of the mitochondrial genome, they may present variable segments in comparisons at the intraspecific level, for which they were considered useful to describe the phylogeographic structure of *P. thaul* and therefore establish the geographic origin of the Robinson Crusoe population.

Total DNA was extracted from liver or muscle using a salt method. The primers used were: 1216LN (5'-CCAAAYACGTCAGGTCAAGGTG-3'), modified from the Hedges16H10, 1216H (5'-TGATTACGCTACCTTYGCA-CGGT-3'), modified from L2751, 16Sar-L and 16Sbr-H (Goebel et al., 1999). These primers produce two partially superposed fragments of approximately 1300pb (1216LN and 1216H) and 550pb (16Sar-L and 16Sbr-H). The phylogenetic reconstructions were carried out using the methods of maximum parsimony (MP), maximum likelihood (ML) (both with PAUP\* v4.0b10; Swofford, 2002) and Bayesian inference (BI) (with MrBayes v3.1.2; Huelsenbeck & Ronquist, 2001). For MP and ML analyses, heuristic searches were performed using the tree-bisection-reconnection branch-swapping algorithm. An additional MP analysis was done considering gaps as a fifth character state. For the analysis of ML and BI the nucleotide substitution model selected following the criterion of Akaike in the Modeltest v4b program (Posada & Crandall, 1998) was used. The BI analysis was run for 5,000,000 generations, sampling every 1000. The first 500 sampled trees were discarded as burn-in after verification of stationarity of the run. Statistical support for the nodes was calculated for MP and ML using 1000 replicates of non-parametric bootstrap (Felsenstein, 1985), while for the BI, posterior probability values were considered.

## RESULTS

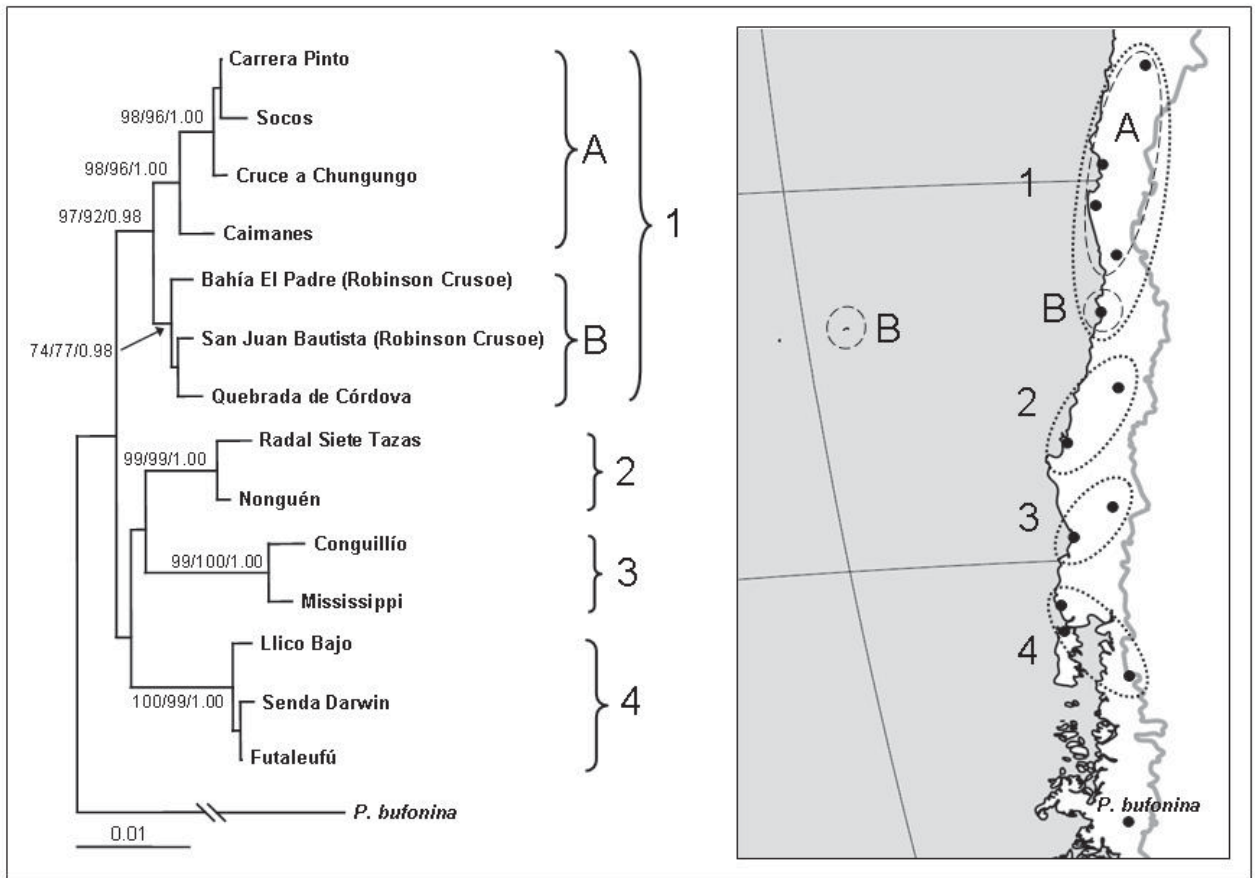
The sequences were deposited in GenBank with access numbers DQ864560 and EU445700–EU445713 (Table 1). The alignment of the two combined fragments had a length of 1720 nucleotide sites. Including the outgroup, there were 148 variable (89 for the 14 sequences of *P. thaul*), six with gaps (four for the 14 sequences of *P. thaul*) and 63 parsimony-informative sites (66 including those with gaps). The p-distances ranged from 0.0012 (Senda Darwin–Futaleufú) to 0.0239 (Socos–Conguillío) with an average of 0.0157. The distance between the two



**Fig. 1.** Localities of *Pleurodema thaul* and *P. bufonina* (Lago Cochrane) included in this study. The area with diagonal lines represents the distribution range of *P. thaul* in mainland Chile and Argentina.

individuals from Robinson Crusoe Island was 0.0035. All the phylogenetic analyses showed the same topology, with four clades that show a high degree of statistical support, formed of individuals from geographically con-

tiguous populations (Fig. 2). The individuals from Robinson Crusoe Island grouped with those from Quebrada de Córdoba in a clade that included representatives from the northern and central zones of Chile (from



**Fig. 2.** Phylogram of maximum likelihood showing the relationships among individuals from 12 populations of *Pleurodema thaul* from the continent and two individuals from Robinson Crusoe Island, rooted with an individual of the sister species *P. bufonina*. The analyses of maximum parsimony and Bayesian inference showed topologies identical to the tree shown. The values at the nodes represent the support values for the three phylogenetic analyses: maximum parsimony bootstrap, maximum likelihood bootstrap and posterior probability of Bayesian inference, respectively. Only values over 50 and 0.95 for bootstrap and posterior probability, respectively, are shown. The map on the right shows the geographic locations of the four main clades defined by the phylogram (numbers 1–4, dotted lines), and the main groupings into which the clade 1 is divided (A and B, dashed lines). The bar at the bottom left of the phylogram represents the nucleotide substitutions expected per site, based on the analysis of maximum likelihood.

Carrera Pinto to Quebrada de Córdoba), indicating that the population introduced to the island originated with individuals that came from the central Chilean coast (Fig. 2). Nevertheless, a more precise geographic identification requires an increase in sampling locations and the use of a marker having a higher degree of variation.

Another result of this study was the variation observed between the sequences of the two individuals that came from the island. The p-distance between these specimens was greater (0.0035) than that observed between those of more southerly populations (0.0012 between Senda Darwin and Futaleufú). This suggests that the genetic variation in the source population from which the island specimens came from is high, or that the two individuals collected are descendants of more than one event of introduction from different source populations (both from central Chile). These two possibilities are not mutually exclusive, and would require a more detailed study using other, more sensitive molecular

markers to evaluate the diversity and degree of genetic structuring of the island and mainland populations.

## DISCUSSION

The present report constitutes the first study of intraspecific genetic variation in *P. thaul* with DNA sequences and suggests that this species presents a high degree of phylogeographic structure in Chile (Fig. 2). Below, the subdivisions proposed by Duellman & Veloso (1977) are compared with those defined by the present phylogenetic analyses (Fig. 2). Those authors distinguished two lineages within the Chilean territory, one extending from the northern limit of the distribution (the city of Copiapó according to them, 27°20'S) to the Biobío river (37°S), and the other from the city of Valdivia to the Aisén region (from 40° to 46°S), suggesting that representatives of the two lineages occurred in the intermediate zone (from 37° to 40°S). The results of the

present study are not incompatible with the subdivision proposed by Duellman & Veloso (1977), but show more than two lineages within Chilean territory: the northern lineage (27°20'S–37°S) could be subdivided into two groups (clades 1 and 2 of Fig. 2), while the clade formed by the individuals from Conguillío and Mississippi (clade 3) may represent members from the intermediate zone (37°S–40°S) mentioned by those authors. Furthermore, the southern lineage of Duellman & Veloso (1977) corresponds to the clade located from Llico Bajo south (clade 4). In the only previous study of genetic variation in this species, Victoriano et al. (1995), using allozymes, were not able to differentiate the lineages proposed by Duellman & Veloso (1977). In relation to genetic divergence, the average p distance of 1.57% observed in *P. thaul* is within the range described between populations of other amphibian species for the 16S gene (Vences et al., 2005).

The presence of *P. thaul* on Robinson Crusoe Island may represent a threat to the integrity of its unique ecosystem, characterized by a high degree of endemism. The invasions of *Rhinella marina* in Australia and Hawaii (Slade & Moritz, 1998; Crossland, 2000; Urban et al., 2007) and of *Eleutherodactylus coqui* on the island of Hawaii (Kraus et al., 1999; Woolbright et al., 2006) are clear examples of this risk. In the absence of more concrete historical information about this invasion, the present study may be considered as a starting point for genetic, ecological and evolutionary research on this new component of the fauna of Robinson Crusoe Island.

## ACKNOWLEDGEMENTS

We thank Paola Jara, Alejandro Vera, Michel Sallaberry and Eduardo Soto for their help in sample collection, and also Rigoberto Solís, Jaime Bosch, Herman Núñez and Alberto Veloso for providing material and information on the population of Robinson Crusoe Island. We further thank Juan Carlos Ortiz and Marcela Vidal for lending us the sample from the Museo de Zoología de la Universidad de Concepción. We especially thank Wladimir Hermosilla for providing additional data concerning the first report of the presence of *P. thaul* on Robinson Crusoe Island. C. Correa and G. Lobos are grateful for a CONICYT Grant for Postgraduate Studies. This study was partially financed by projects FONDECYT 3000048, 1061256 and FC49 (“Biodiversity Assessment and Systematization of Existing Biological Knowledge on the Coastal Range of the Lakes, X Region, Chile”, World Wide Fund for Nature, WWF).

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Accepted: 14 November 2008