*Zootaxa* 4438 (2): 283–298
http://www.mapress.com/j/zt/

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4438.2.4
http://zoobank.org/urn:lsid:zoobank.org:pub:FD91E364-3B03-4ADC-B70F-D98DA9A7FB51

# Molecular evidence for conspecificity of two desert *Liolaemus* lizards (Iguania: Liolaemidae)

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## Abstract

*Liolaemus audituvelatus* (Núñez & Yáñez 1983) and *L. manueli* (Núñez, Navarro, Garín, Pincheira-Donoso & Meriggio 2003) are endemic species of the Atacama Desert of northern Chile that belong to the *montanus* group. Both species are considered cryptic from each other and can only be distinguished by their distribution ranges and karyotypes. Originally, there was a wide separation zone between their known distribution ranges, but later collections reduced the gap from 430 km to only 150 km. In this study, we review the geographic information about both species and report new localities within the distribution gap, where species identification becomes difficult. We performed a molecular phylogenetic analysis and applied several species delimitation methods to reassess the taxonomic status of both nominal species and new intermediate populations. Our analyses support the placement of *L. manueli* in the synonymy of *L. audituvelatus*. We discuss the biogeographic and conservation implications of this new synonymy.

**Key words:** Atacama Desert, synonymy, species delimitation, *Liolaemus audituvelatus*, *L. manueli*, *Phrynosaura*

## Introduction

*Liolaemus* is a lizard genus widely distributed throughout South America, ranging from central Peru and southeastern Brazil to Tierra del Fuego in Argentina and Chile (Pincheira-Donoso *et al.* 2008). The more than 250 species of the genus (Abdala & Quinteros 2014; Uetz & Hošek 2016) occupy the most diverse environments with altitudes ranging from sea level to approximately 5000 m in the Andean highlands (Donoso-Barros 1966; Cei 1993; Pincheira-Donoso *et al.* 2008; Aparicio & Ocampo 2010).

Two major clades have been recognized within *Liolaemus*: The Chilean group proposed as the subgenus *Liolaemus*, and the Argentinian group, proposed as the subgenus *Eulaemus* (Laurent 1983; 1985; Etheridge 1995; Schulte *et al.* 2000). A subset of species belonging to the Argentinian group have been identified as the *montanus* group (Etheridge 1995), where the knowledge about many species is still poor and where there have been some taxonomic problems (Núñez & Jaksic 1992; Pincheira-Donoso & Núñez 2002; Valladares *et al.* 2002; Pincheira-Donoso & Núñez 2005; Troncoso-Palacios 2014; Ruiz de Gamboa & Ortiz 2016; Aguilar *et al.* 2016). This is probably because these species inhabit the desert or highlands where is difficult to study them.

Within this group, two species of similar morphology were described in northern Chile: *Liolaemus audituvelatus* (Núñez & Yáñez 1983) and *L. manueli* (Núñez, Navarro, Garín, Pincheira-Donoso & Meriggio 2003). Originally, *L. audituvelatus* was known only in the type locality Llano de Vilama, 10 km east of San Pedro

*Accepted by L. Avila: 18 Apr. 2018; published: 21 Jun. 2018*

de Atacama (Núñez & Yáñez 1983). Years later, Núñez *et al.* (2003) mentioned that the distribution of *L. audituvelatus* was restricted to the Salar de Atacama (50 km south of San Pedro de Atacama) in Antofagasta Region. *Liolaemus manueli* was known only from its type locality: Diego de Almagro in the Antofagasta Region (Núñez *et al.* 2003). Between their type localities, there existed a wide separation zone (430 km in straight line), where it was assumed that there were no populations connecting both species. Differentiated morphologically from each other only by imprecise subtleties, they were considered cryptic species, distinguishable only by their distribution and karyotypes (Núñez *et al.* 2003).

Later, Núñez *et al.* (2012) reported new localities for both species: *L. manueli* was recorded 124 km southwest of its type locality and *L. audituvelatus* was recorded 280 km southwest of its type locality. This last record reduced the gap separating the known localities of the two species, from 430 km to only 150 km. Due to the lack of diagnostic morphological characters between these species, the correct assignment of individuals to species is complicated in intermediate records. In this study, we update the geographic data of both species and evaluate whether *L. audituvelatus* and *L. manueli* correspond to different phylogenetic taxa by applying a series of species delimitation methods.

## Materials and methods

For this study, we compiled all the geographic records of the literature and the database of the Museo Nacional de Historia Natural de Chile (MNHN) herpetological collection (GBIF, 2016), to which we added new undescribed localities (Figure 1, Appendix S1) assigned to *Liolaemus audituvelatus* (Figure 2a) or *L. manueli* by geographic criteria. We also collected specimens near the type locality of *L. audituvelatus* (near Caspana), and other intermediate localities between the known distributions of *L. manueli* (Figures 2d, e and f) and *L. audituvelatus* (Figure 1) (Figure 2b and c). Additionally, a total of 16 individuals belonging to seven species of the *montanus* group were included in the phylogenetic and species delimitation analyses (Appendix S2).

The collected individuals were sacrificed by injection of sodium thiopental, applied in the pericardial cavity. For the molecular study, we cut a small piece of the tail or liver, which were immediately stored in absolute ethanol (>99.5%).

**Genetic data and analyses.** Genomic DNA was extracted from the tail or liver using a Promega Wizard SV Genomic DNA Purification System Kit, following the manufacturer's protocol. A fragment of the mitochondrial gene cytochrome *b* (Kocher *et al.* 1989) was amplified using the PCR protocol of Vidal *et al.* (2012) and sequenced with an Applied Biosystems 3730XL sequencer (Macrogen, Korea). Sequences were edited with CodonCode Aligner software (CodonCode Corporation, Dedham, MA.) and translated to amino acids to identify open reading frames.

As an ingroup, we used specimens that could be assigned to *L. audituvelatus* by distribution range, including one found near the type locality (Figure 3), one specimen from the type locality of *L. manueli* and another one from a known locality, specimens of intermediate populations between the type localities of these two species, and representatives of other species of the *Liolaemus* (*Eulaemus*) *montanus* group (Schulte *et al*. 2000) from Chile and another for which cytb sequences are available in GenBank (Appendix S2). As an outgroup, we used *L. ornatus* Koslowsky, belonging to the *Liolaemus(Eulaemus) darwinii* group (Aguilar *et al.* 2016). All new sequences were deposited in GenBank (Appendix S2).

Sequences were aligned with MUSCLE (Edgar 2004). We evaluated whether the sequences were saturated and thus useful for the phylogenetic analysis, using the Xia test (Xia *et al.* 2003; Xia & Lemey 2009) implemented in DAMBE v6.4.29 (Xia 2013). This is an entropy-based index that estimates a substitution saturation index (Iss) and compares it to a critical substitution saturation index (Iss.c) via a randomization process with 95% confidence intervals (Xia 2000; Xia & Lemey 2009). Phylogenetic relationships were inferred using a Markov Chain Monte Carlo (MCMC) method within a Bayesian framework (BMCMC) to estimate the posterior probability of phylogenetic trees in BayesPhylogenies v1.1 software (http://www.evolution.rdg.ac.uk/ BayesPhy.html). We ran BMCMC analyses consisting of 10,000,000 iterations of phylogenetic trees, sampled every 1000th to assure that successive samples were independent. From this sample of trees, we removed the first thousand to avoid including samples before the convergence of the Markov Chains.

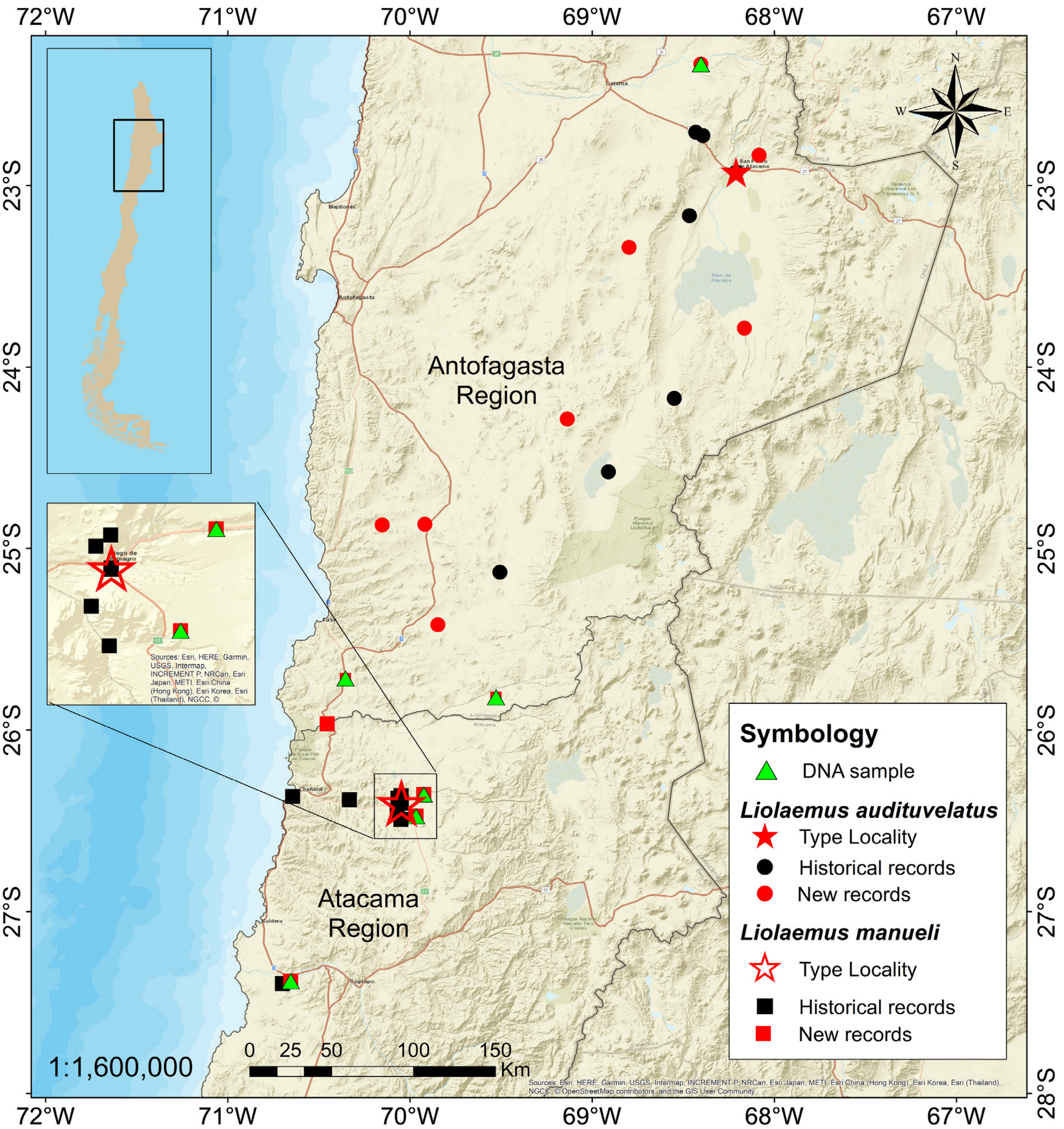

**FIGURE 1.** Distribution maps of *Liolaemus audituvelatus* and *L. manueli* based on historical and new records. Type localities of both species are indicated by stars. Historical records of both species gathered from Núñez *et al.* (1998, 2003, 2012) and GBIF (2016) also are indicated by black circles and squares. New localities of *L. audituvelatus* are indicated by red circles and red squares for *L. manueli*. Localities from which DNA samples were obtained for the analyses of this study are indicated with green triangles. The geographic references are in Appendix S2.

**Species delimitation.** We applied three species delimitation methods to evaluate whether *L. audituvelatus* and *L. manueli* are phylogenetically distinct. We used Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.* 2012), Poisson Tree Process (PTP) (Zhang *et al.* 2013) and multi-rate Poisson Tree Process (mPTP) (Kapli *et al.* 2017), which are based on different aspects of molecular evolution. The Automatic Barcode Gap Discovery (ABGD) method was used to divide data into candidate species based on a statistically inferred barcode gap (Puillandre *et al.* 2012), employing three distance options (Simple, Jukes-Cantor and Kimura 2-parameter). We used a relative gap width (X) of 1.0 and tested a range between 0.001 and 0.25 of prior intraspecific divergence values (Puillandre *et al.* 2012). The corresponding analysis was conducted on the web server for ABGD (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). The PTP method models speciation rate by directly using the number of substitutions and applying two independent Poisson process classes, one which describes speciation and another which describes the coalescent intraspecific processes. Thus, the adjustment of both processes delimits the species in a given topology (Zhang *et al.* 2013). A new method based on the PTP algorithm, multi-rate PTP (mPTP), improves the estimate considering different rates of speciation-coalescence (Kapli *et al.* 2016). Although the speciation rate can be assumed as constant between closely related species, intraspecific coalescence rate and genetic diversity can differ significantly even among sister species, then mPTP allows to consider different rates of branching events within each delimited species (Kapli *et al.* 2016). To perform PTP and mPTP we used the web server (http://mptp.h-its.org/) and the Bayesian consensus tree. For all these analyses, the sequence of the outgroup was excluded.

Moreover, we complemented the species delimitation analyses by computing pairwise distances among all specimens included in the phylogenetic analysis with BioEdit v.7.0.9 (Hall 1999).

**FIGURE 2.** Individuals from a) near Caspana (MUAP-114), near type locality for *Liolaemus audituvelatus*; b) Diego de Almagro, type locality of *L. manueli*; c) and d) Altos Quebrada Agua Colorada; e) and f) Barranquilla (near Caseron). f) is a gravid female. Note that b) male of *L. audituvelatus* from known distribution and d) male of *L. manueli* from type locality, are identical and were found 300 km away (in straight line); and, a) and e) are also similar and were found 600 km away (in straight line).

## Results

**Phylogenetic Analysis.** The Bayesian analysis included cytb sequences from a total of 29 specimens assigned to 15 nominal species of the *montanus* group, seven from Argentina and eight from Chile. The consensus tree with Bayesian posterior probabilities, based on 705 nucleotide sites, is shown in Figure 4. The analysis recovered *L. audituvelatus* and *L. manueli* as a monophyletic group with high support (BPP = 1), but nevertheless, the two specimens of *L. audituvelatus* from near Caspana are paraphyletic with respect to all samples assigned to *L. manueli*.

**FIGURE 3.** Specimens of *Liolaemus audituvelatus* collected near Caspana, 65 km north of San Pedro de Atacama, Antofagasta Region (MUAP-113 (LNC135) and MUAP-114 (LNC136)).

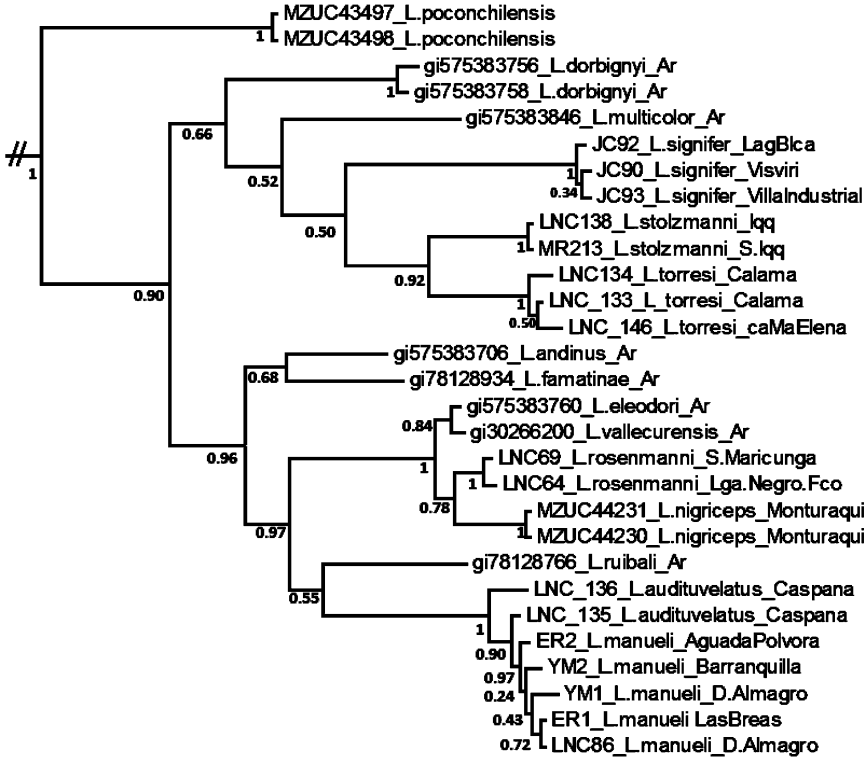

**FIGURE 4.** Bayesian consensus tree showing the phylogenetic relationships among species of the *montanus* group and new populations related to *L. audituvelatus* and *L. manueli*. Posterior probability is indicated at each node.

**Species delimitation analyses.** Each species delimitation method recognized a different number of candidate species (Figure 5). ABGD recognized 11 candidate species within each of the three distances used. This method suggests that *L. audituvelatus* and *L. manueli* correspond to a single species, as was also the case for the clade including *L. eleodori* Cei, Etheridge & Videla, *L. vallecurensis* Pereyra, *L. rosenmanni* Núñez & Navarro and *L. nigriceps* (Philippi).

The mPTP model recognized 10 candidate species and also suggested that *L. audituvelatus* and *L. manueli* correspond to a single species. Likewise, the clade including *L. eleodori, L. vallecurensis, L. rosenmanni* and *L. nigriceps* was also found to correspond to a single species as was the clade including *L. andinus* Koslowsky and *L. famatinae* Cei.

The PTP model recognized 14 candidate species. This method also suggests that *L. audituvelatus* and *L. manueli* are conspecifics, but one individual of *L. audituvelatus* (MUAP-114 (LNC136) was recognized as a different species. Also, *L. eleodori* with *L. vallecurensis* likely correspond to the same species.

While each of the three species delimitation methods found *Liolaemus audituvelatus* and *L. manueli* to belong to a single species, one of the two specimens from near Caspana (MUAP-114 (LNC136); Figure 3) was assigned to *L. audituvelatus* was recognized as a separate candidate species by the mPTP analysis. All methods placed the other Caspana specimen (MUAP-113 (LNC135)) in the same species as all samples assigned to *L. manueli* (Figure 5). It is necessary to emphasize that both specimens of *L. audituvelatus* collected near Caspana are indistinguishable morphologically (Figure 3) and that the genetic distance between specimen MUAP-113 (LNC135) and the specimen from the type locality of *L. manueli* (Diego of Almagro) is extremely low (0.0043, Appendix S3), even though the localities are 480 km apart.

**Genetic distances.** Considering the current nominal taxonomy, intraspecies genetic distances fluctuated between 0 and 0.0172 (Appendix S3). The highest value corresponded to the two individuals of *Liolaemus audituvelatus* that were collected near Caspana. The second highest value (0.0143) corresponds to the two individuals of *L. torresi* (Núñez, Navarro, Garín, Pincheira-Donoso & Meriggio), which were collected 60 km apart. Lower intraspecies genetic distances were obtained among the samples of *L. manueli*, with a maximum value of 0.0082 between two individuals collected 120 km apart.

Interspecies genetic distances with the sampled species of the *montanus* group fluctuated between 0 and 0.1407 (Appendix S3). The highest distance was observed between *L. signifer* (Duméril & Bibron) and *L. poconchilensis* Valladares, whereas *L. eleodori* and *L. vallecurensis* exhibited the lowest distance according to sequences generated in other studies (Morando *et al.* 2003; Olave *et al.* 2014). *Liolaemus audituvelatus* and *L. manueli* have the second lowest distance (0.0043) between individuals which were collected at 480 km away.

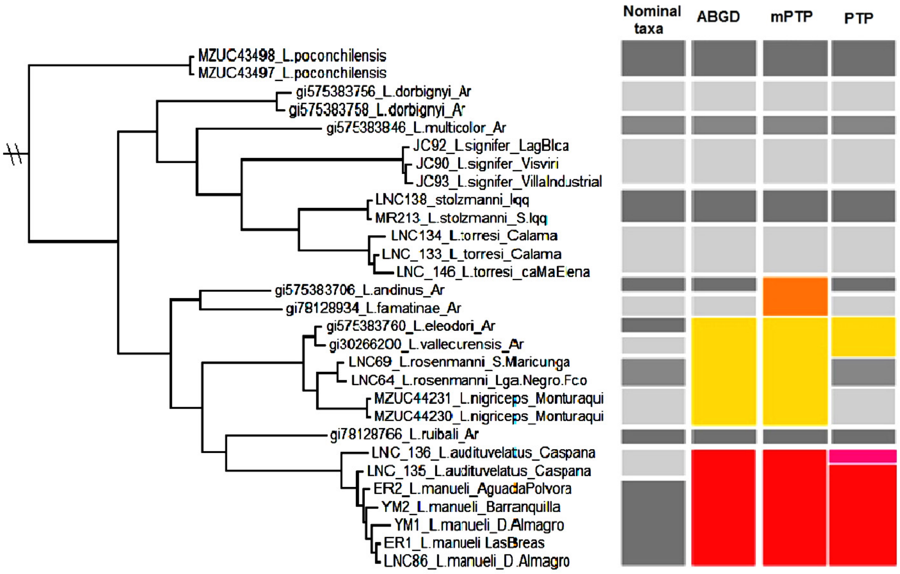

**FIGURE 5.** Results of the species delimitation analyses. On the left, the Bayesian consensus topology used for PTP and mPTP analyses is shown. On the right, boxes represent the species delimitations obtained with the three methods, in comparison with the current taxonomy (column nominal taxa).

## Discussion

Although the delimitation methods we employed differ in the number of candidate species recognized, they are congruent enough to suggest that *L. audituvelatus* and *L. manueli* belong to a single species. The discrepancies among analyses are few and are found in non-focal species. For example, the clade (*L. eleodori* + *L. vallecurensis*) + (*L. rosenmanni* + *L. nigriceps*) was recognized as one species in the ABGD and mPTP analyses. Species of this clade inhabit Argentina and Chile and exhibit enough morphological differences (Philippi 1860, Cei *et al.* 1985, Núñez & Navarro 1992, Pereyra 1992) to have been considered valid taxa (Pincheira-Donoso & Núñez 2005; Pincheira-Donoso *et al.* 2008; Lobo *et al.* 2010; Abdala & Quinteros 2014), but they are separated by low genetic distances (< 2%) according to all available data (Morando *et al.* 2003; Olave *et al.* 2014; this study). Nevertheless, there exists a possibility that some of these species are not valid because *L. rosenmanni* was initially reported as *L. eleodori* in Chile (Núñez & Torres-Mura 1992; Núñez & Jaksic 1992; Núñez & Navarro 1992). Moreover, it would be useful to review the identification of the individuals recognized as *L. vallecurensis* by Morando *et al.* (2003) and *L. eleodori* by Olave *et al.* (2014) because they display low genetic differences in cytochrome b (Appendix S3). Consequently, our delimitation analyses recovered them as conspecifics. These results suggest that more in-depth studies are needed, specifically studies including more specimens that also expand the geographical distribution of the samples in order to morphologically and phylogenetically delimit the valid taxa among these species.

Although mPTP is the most sophisticated methodology used here, in our case it underestimates the number of species when current taxonomy is taken into account. PTP was the method that most coincided with the currently accepted taxonomy.

The mPTP analysis recognized both specimens collected near Caspana to be belong to different species, this suggests that there are two cryptic species at Caspana, with one restricted to that locality and the other with a wide distribution towards the south. This would imply the sympatry of two sister species in one locality, without evident morphological differences, which poses a more complex and improbable evolutionary scenario. Moreover, it is noteworthy that the other two analyses (ABGD and PTP) support that they are a single species together with all samples assigned to *L. manueli*. We infer that there would a higher intraspecific genetic divergence in the population of Caspana which now defines the northern end of the distribution of *L. audituvelatus*.

So far, only the karyotype allows for the differentiation of *L. audituvelatus* from *L. manueli.* Nevertheless, chromosomes may vary in form, size and even in number, within and between populations of *Liolaemus* (Lamborot *et al.* 1979; 1981; 1993; 2006; Lamborot & Álvarez-Sarret 1989; 1993; Lamborot 1991; 1998; 2001; Iturra *et al.* 1994; Lamborot & Vásquez 1998); even the descriptions of karyotypes of the same species may differ when comparing different sources (e.g. Navarro Barón 1991; Aiassa *et al.* 1999; Aiassa *et al.* 2005, Aiassa & Gorla 2010). Due to this variability in the karyotype as a character, this would not be reliable enough to define species in *Liolaemus* and therefore is not enough to differentiate to *L. manueli* of *L. audituvelatus*.

*Liolaemus audituvelatus* was described in the genus *Ctenoblepharys* (Núñez & Yañez 1983) and *L. manueli* in *Phrynosaura* (Núñez *et al.* 2003). Later, *C. audituvelatus* was incorporated into *Phrynosaura* (Laurent 1984; Núñez & Yañez 1984). Although *Phrynosaura* was synonymized with *Liolaemus* more than twenty years ago (Frost & Etheridge 1989; Etheridge 1995), several authors have continued considering it a valid genus (Núñez & Jaksic 1992; Núñez *et al.* 1998; 2003; Pincheira-Donoso & Núñez 2005; Vidal *et al.* 2009) or a group of species within *Liolaemus* (*L. reichei* group in Pincheira-Donoso *et al.* 2008). Our phylogenetic hypothesis includes all species described or included in *Phrynosaura* (*L. audituvelatus, L. manueli, L. poconchilensis, L. stolzmanni* and *L. torresi*) except *L. erroneus*, which was described by a single specimen, now lost, and whose type locality is not known with exactitude. For this reason, no later records exist. Our results support and confirms that *Phrynosaura* or the *L. reichei* group are not monophyletic (Valladares *et al.* 2002; Lobo *et al.* 2010), because that the species formerly recognized by some authors as belonging to *Phrynosaura* are placed in separate clades by the present phylogenetic hypothesis.

Based on their great morphological similarity and their close phylogenetic affinity, we consider *L. audituvelatus* and *L. manueli* to be conspecific and here recognize *L. manueli* to be a junior synonym of *L. audituvelatus*. Our placement of *Liolaemus manueli* in the synonymy of *L. audituvelatus* implies a considerable range extension of the senior species, which affects its conservation status. *Liolaemus audituvelatus* is categorized as Vulnerable B1ab(iii); D2 by the IUCN (Núñez *et al.* 2017) and is considered Vulnerable B1ab(iii)+2ab(iii) by Chilean Law (D.S. Nº 16/2016 MMA), while *L. manueli* is considered Endangered B1ab(iii)+2ab(iii) in Chile

(D.S. Nº 16/2016 MMA) and Endangered B1ab(iii) by the IUCN (Valladares *et al.* 2016). It is worth noting that *L. audituvelatus* is a desert species, living in places of very scant to no vegetation and populations of low densities. Thus, it is hard to observe in the field. Moreover, the places where it lives are under pressure from intensive mining activities and vehicular traffic due to recreational activities like raids. Based on the considered range expansion of this taxon, we recommend that *L. audituvelatus*, in its new definition, remains Vulnerable.

## Acknowledgments

We thank Servicio Agrícola y Ganadero (Permit # 9487/2014) for collecting permit, Marcos Ferrú and Gilmar Ramirez for field work assistance, Carlos Garín for giving us valuable field information and Tania Coronado for laboratory support. MR thanks the project EDPG LPR-161 of Dirección de Postgrado of Universidad de Concepción. YM thanks Jorge Valdes for field work assistance. E. Riveros-Riffo thanks Marina Lemus, Andrea Zapata, Juan Carlos Torres-Mura, Víctor Escobar and Maximiliano Daigre for their valuable information and field work assistance. Financial support was provided by CONICYT Chile Doctoral Fellowship # 21120577 to M. Ruiz De Gamboa. C. Correa acknowledges financial support from Project PAI 79130032 and J.C. Ortiz acknowledges financial support from Grant Fondecyt 1131009. Finally, we thank the editor and the reviewers who helped us to improve the manuscript.

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**APPENDIX S1.** Geographic information of localities included on map of Figure 1.

| SPECIES | LOCALITY | ADMINISTRATIVE REGION | LATITUDE | LONGITUDE | REFERENCE | OBSERVATION |
|---|---|---|---|---|---|---|
| *L. audituvelatus* | Caspana | Antofagasta Region | -22.3311 | -68.4030 | Personal record (MRdeG) | DNA sample |
| *L. audituvelatus* | Cuesta Barros Arana, cima. | Antofagasta Region | -22.7067 | -68.4303 | occ_search_1385755319688.xls | |
| *L. audituvelatus* | Cuesta Barros Arana km 60. Cruce a Río Grande | Antofagasta Region | -22.7257 | -68.3915 | Núñez *et al.* (2000) in Núñez *et al.* (2012) | |
| *L. audituvelatus* | San Pedro de Atacama | Antofagasta Region | -22.9153 | -68.1642 | occ_search_1385755319688.xls | |
| *L. audituvelatus* | 10 km E de San Perdro de Atacama | Antofagasta Region | -22.9160 | -68.1200 | Núñez & Yañez 1983; Núñez *et al.* 1998 | Type locality |
| *L. audituvelatus* | Alto Qda Agua Colorada | Antofagasta Region | -23.1769 | -68.4752 | Núñez & Yañez 1983; Núñez *et al.* 1997; Núñez *et al.* 1998; Veloso & Núñez 1998; Núñez & Veloso 2001 en Núñez *et al.* 2012 | |
| *L. audituvelatus* | Mina Gaby | Antofagasta Region | -23.3409 | -68.7969 | Personal record (ER) | |
| *L. audituvelatus* | Borde S Salar de Atacama | Antofagasta Region | -23.7855 | -68.1639 | Personal record (ER) | |
| *L. audituvelatus* | Agua Colorada | Antofagasta Region | -24.1731 | -68.5486 | occ_search_1385755319688.xls | |
| *L. audituvelatus* | Mina Escondida | Antofagasta Region | -24.2856 | -69.1361 | Personal record (ER) | |
| *L. audituvelatus* | Salar de Punta Negra | Antofagasta Region | -24.5778 | -68.9108 | occ_search_1385755319688.xls | |
| *L. audituvelatus* | Pampa Los Changos 1 | Antofagasta Region | -24.8655 | -69.9181 | Personal record (ER) | |
| *L. audituvelatus* | Pampa Los Changos 2 | Antofagasta Region | -24.8699 | -70.1522 | Personal record (ER) | |
| *L. audituvelatus* | desierto interior de Taltal, mina Guanaco | Antofagasta Region | -25.1294 | -69.5061 | Núñez *et al.* 2012 | |
| *L. audituvelatus* | Agua Verde | Antofagasta Region | -25.4191 | -69.8459 | Personal record (ER) | |
| *L. audituvelatus* | Ruta B-950 | Antofagasta Region | -25.6614 | -70.5938 | Personal record (MRdeG) | |
| *L. manueli* | 17 km S of Las Breas. Ruta A-5 near cruce B-955. | Antofagasta Region | -25.7135 | -70.3549 | Personal record (ER) | DNA sample |
| *L. manueli* | Llano de La Polvora | Antofagasta Region | -25.8171 | -69.5280 | Personal record (ER) | DNA sample |
| *L. manueli* | Ruta Panamericana 5-N | Atacama Region | -25.9647 | -70.4544 | Personal record (ER) | |
| *L. manueli* | km 65 ruta C-13 D. Almagro | Atacama Region | -26.3536 | -69.9245 | Personal record (MRdeG) | DNA sample |
| *L. manueli* | Cerro La Estrella | Atacama Region | -26.3608 | -70.0483 | occ_search_1385755319688.xls | |
| *L. manueli* | Cerro Azul | Atacama Region | -26.3653 | -70.6439 | occ_search_1385755319688.xls | |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.3739 | -70.0658 | occ_search_1385755319688.xls | Type Locality |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.3830 | -70.0330 | Schulte II *et al.* 2003; Núñez *et al.* 2003 | |

......*continued on the next page*

**APPENDIX S1. (Continued)**

| SPECIES | LOCALITY | ADMINISTRATIVE REGION | LATITUDE | LONGITUDE | REFERENCE | OBSERVATION |
|---|---|---|---|---|---|---|
| *L. manueli* | Diego de Almagro | Atacama Region | -26.3830 | -70.0330 | Núñez *et al.* 1998; Schulte II *et al.* 2003; Núñez *et al.* 2003 | |
| *L. manueli* | Finca de Chañaral | Atacama Region | -26.3830 | -70.3330 | Núñez *et al.* 1998 | |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.3994 | -70.0475 | Núñez *et al.* 2003 | |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.4008 | -70.0481 | occ_search_1385755319688.xls | |
| *L. manueli* | Finca de Chañaral | Atacama Region | -26.4026 | -70.0591 | Valladares 2011 | |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.4444 | -70.0708 | occ_search_1385755319688.xls | |
| *L. manueli* | Diego de Almagro | Atacama Region | -26.4733 | -69.9664 | Personal record (YM) | DNA sample |
| *L. manueli* | Diego de Almagro, 16 km al Sur, Camino a la Finca | Atacama Region | -26.4908 | -70.0497 | occ_search_1385755319688.xls | |
| *L. manueli* | Llanos de Barranquilla | Atacama Region | -27.3803 | -70.6554 | Personal record (YM) | DNA sample |
| *L. manueli* | Caseron, Ruta C370, Copiapo | Atacama Region | -27.3958 | -70.7001 | Núñez *et al.* 2012 | |

**APPENDIX S2.** Voucher codes, GenBank accession numbers and localities of the specimens used in the phylogenetic/delimitation analyses. MUAP= Museo de la Universidad Arturo Prat de Iquique; MZUC= Museo de Zoología Universidad de Concepción and SSUC-Re = Colección Patricio Sánchez Reyes – Reptiles, Pontificia Universidad Católica de Chile.

| VOUCHER | GENBANK ACCESSION | SPECIES | LOCALITY | LATITUDE | LONGITUDE | OBSERVATION |
|---|---|---|---|---|---|---|
| | KF968823 | *L. andinus* | Olave *et al.* (2014) | | | |
| MUAP-113 | MH184784 | *L. audituvelatus* | Caspana, San Pedro de Atacama. Antofagasta Region | -22.33112 | -68.40303 | LNC135 |
| MUAP-114 | MH184785 | *L. audituvelatus* | Caspana, San Pedro de Atacama. Antofagasta Region | -22.33112 | -68.40303 | LNC136 |
| | KF968848 | *L. dorbignyi* | Olave *et al.* (2014) | | | |
| | KF968849 | *L. dorbignyi* | Olave *et al.* 2014 | | | |
| | KF968850 | *L. eleodori* | Olave *et al.* 2014 | | | |
| | DQ237485 | *L. famatinae* | Morando *et al.* 2003 | | | |
| MUAP-104 | MH184782 | *L. manueli* | Llano de Barranquilla. Atacama Region | -27.3803 | -70.6554 | YM1 |
| MUAP-118 | MH184781 | *L. manueli* | Diego de Almagro. Atacama Region | -26.4733 | -69.9664 | YM2 |
| MUAP-110 | MH184779 | *L. manueli* | Diego de Alamgro. km 65 ruta C-13. Atacama Region | -26.35357 | -69.92448 | LNC86 |

......*continued on the next page*

**APPENDIX S2. (Continued)**

| VOUCHER | GENBANK ACCESSION | SPECIES | LOCALITY | LATITUDE | LONGITUDE | OBSERVATION |
|---|---|---|---|---|---|---|
| SSUC-Re 760 | MH184783 | *L. manueli* | Llano La Polvora. Antofagasta Region | -25.81741 | -69.527632 | ER2 |
| ER1 | MH184780 | *L. manueli* | 17 km S of Las Breas. Ruta A-5 near cruce B-955. Antofagasta Region | -25.71352 | -70.354936 | Released on the same place |
|  | KF968893 | *L. multicolor* | Olave *et al.* 2014 |  |  |  |
| MZUC-44230 | MH184788 | *L. nigriceps* | Ruta B-55. camino a Monturaqui. Antofagasta Region | -24.24781 | -68.501778 |  |
| MZUC-44231 | MH184789 | *L. nigriceps* | Ruta B-55. camino a Monturaqui. Antofagasta Region | -24.24781 | -68.501778 |  |
| MZUC-33901 | MH184800 | *L. ornatus* | Cariquima. Tarapacá Region | -19.51 | -68.62 | JC60 |
| MZUC-43497 | MH184799 | *L. poconchilensis* | Portezuelo de Poconchile. Arica y Parinacota Region | -18.47433 | -70.08366 |  |
| MZUC-43498 | MH184798 | *L. poconchilensis* | Portezuelo de Poconchile. Arica y Parinacota Region | -18.47433 | -70.08366 |  |
| MUAP-108 | MH184786 | *L. rosenmanni* | Laguna del Negro Francisco. Atacama Region | -27.48609 | -69.236554 | LNC64 |
| MUAP-109 | MH184787 | *L. rosenmanni* | Between aduana y Ola. Camino a Salar Pedernales. Atacama Region | -26.74274 | -69.07014 | LNC69 |
|  | DQ237646 | *L. ruibali* | Morando *et al.* 2003 |  |  |  |
| MZUC-34183 | MH184792 | *L. signifer* | Villa Industrial norte, Tacora. Arica y Parinacota Region | -17.84004 | -69.701143 | JC93 |
| MZUC-34182 | MH184790 | *L. signifer* | Laguna Blanca. Arica y Parinacota Region | -17.66664 | -69.669252 | JC92 |
| MZUC-34180 | MH184791 | *L. signifer* | Visviri. Arica y Parinacota Region | -17.59052 | -69.480127 | JC90 |
| MUAP-117 | MH184794 | *L. stolzmanni* | Altos de Chipana, Iquique. Tarapacá Region | -21.25142 | -70.014006 | MR213 |
| MUAP-115 | MH184793 | *L. stolzmanni* | Cerro Huantajaya, Alto Hospicio. Tarapacá Region | -20.23364 | -70.007514 | LNC138 |
| MUAP-112 | MH184795 | *L. torresi* | Salar El Indio, Calama. Antofagasta Region | -22.39052 | -68.87062 | LNC134 |
| MUAP-111 | MH184796 | *L. torresi* | Salar El Indio, Calama. Antofagasta Region | -22.39048 | -68.86941 | LNC133 |
| MUAP-116 | MH184797 | *L. torresi* | near Maria Elena. Antofagasta Region |  |  | LNC146 |
|  | AY173546 | *L. vallecurensis* | Morando *et al.* 2003 |  |  |  |

**APPENDIX S3.** Pairwise genetic distances among the specimens used in the phylogenetic/delimitation analyses.

| N° | SEQUENCE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LNC86 *L.manueli* | | | | | | | | | | | | | | |
| 2 | ER1 *L.manueli* | 0.0000 | | | | | | | | | | | | | |
| 3 | YM *L.manueli* | 0.0055 | 0.0055 | | | | | | | | | | | | |
| 4 | MUAP-104 *L.manueli* | 0.0057 | 0.0057 | 0.0110 | | | | | | | | | | | |
| 5 | ER2 *L.manueli* | 0.0028 | 0.0028 | 0.0082 | 0.0057 | | | | | | | | | | |
| 6 | LNC135 *L.audituvelatus* | 0.0043 | 0.0043 | 0.0082 | 0.0071 | 0.0043 | | | | | | | | | |
| 7 | LNC136 *L.audituvelatus* | 0.0187 | 0.0187 | 0.0277 | 0.0216 | 0.0187 | 0.0172 | | | | | | | | |
| 8 | gi78128766 *L.ruibali* | 0.0829 | 0.0829 | 0.0801 | 0.0865 | 0.0865 | 0.0828 | 0.0865 | | | | | | | |
| 9 | gi30266200 *L.vallecurensis* | 0.0793 | 0.0793 | 0.0946 | 0.0814 | 0.0793 | 0.0792 | 0.0813 | 0.0731 | | | | | | |
| 10 | gi575383760 *L.eleodori* | 0.0987 | 0.0987 | 0.0986 | 0.1018 | 0.0987 | 0.0987 | 0.1003 | 0.0809 | 0.0039 | | | | | |
| 11 | LNC64 *L.rosenmanni* | 0.1005 | 0.1005 | 0.0985 | 0.1004 | 0.0973 | 0.0972 | 0.1021 | 0.0901 | 0.0214 | 0.0187 | | | | |
| 12 | LNC69 *L.rosenmanni* | 0.1005 | 0.1005 | 0.0955 | 0.1004 | 0.0973 | 0.0972 | 0.1021 | 0.0883 | 0.0194 | 0.0173 | 0.0043 | | | |
| 13 | MZUC44230 *L.nigriceps* | 0.1034 | 0.1034 | 0.0986 | 0.1065 | 0.1034 | 0.1034 | 0.1018 | 0.1010 | 0.0374 | 0.0319 | 0.0334 | 0.0319 | | |
| 14 | MZUC44231 *L.nigriceps* | 0.1034 | 0.1034 | 0.0986 | 0.1065 | 0.1034 | 0.1034 | 0.1018 | 0.1010 | 0.0374 | 0.0319 | 0.0334 | 0.0319 | 0.0000 | |
| 15 | gi575383706 *L.andinus* | 0.0875 | 0.0875 | 0.0808 | 0.0906 | 0.0907 | 0.0874 | 0.0922 | 0.0773 | 0.0727 | 0.0809 | 0.0873 | 0.0857 | 0.0905 | 0.0905 |
| 16 | gi78128934 *L.famatinae* | 0.0974 | 0.0974 | 0.0871 | 0.1005 | 0.1006 | 0.0973 | 0.1022 | 0.0741 | 0.0665 | 0.0765 | 0.0908 | 0.0892 | 0.0940 | 0.0940 |
| 17 | gi575383756 *L.dorbignyi* | 0.1060 | 0.1060 | 0.1122 | 0.1092 | 0.1093 | 0.1060 | 0.1076 | 0.0984 | 0.0928 | 0.1044 | 0.1075 | 0.1092 | 0.1045 | 0.1045 |
| 18 | gi575383758 *L.dorbignyi* | 0.0990 | 0.0990 | 0.1054 | 0.1021 | 0.1022 | 0.0990 | 0.1006 | 0.0978 | 0.0944 | 0.1054 | 0.1086 | 0.1102 | 0.1023 | 0.1023 |
| 19 | gi575383846 *L.multicolor* | 0.1268 | 0.1268 | 0.1176 | 0.1316 | 0.1268 | 0.1267 | 0.1234 | 0.1258 | 0.0922 | 0.1069 | 0.1053 | 0.1069 | 0.1071 | 0.1071 |
| 20 | JC92 *L.signifer* | 0.1214 | 0.1214 | 0.1274 | 0.1246 | 0.1247 | 0.1213 | 0.1263 | 0.1216 | 0.1138 | 0.1196 | 0.1248 | 0.1231 | 0.1294 | 0.1294 |
| 21 | JC90 *L.signifer* | 0.1248 | 0.1248 | 0.1274 | 0.1279 | 0.1281 | 0.1247 | 0.1264 | 0.1217 | 0.1138 | 0.1197 | 0.1248 | 0.1232 | 0.1295 | 0.1295 |
| 22 | JC93 *L.signifer* | 0.1232 | 0.1232 | 0.1276 | 0.1264 | 0.1265 | 0.1232 | 0.1248 | 0.1199 | 0.1118 | 0.1182 | 0.1233 | 0.1216 | 0.1279 | 0.1279 |
| 23 | LNC138 *L.stolzmanni* | 0.1265 | 0.1265 | 0.1338 | 0.1313 | 0.1299 | 0.1281 | 0.1248 | 0.1275 | 0.1091 | 0.1200 | 0.1234 | 0.1218 | 0.1234 | 0.1234 |
| 24 | MR213 *L.stolzmanni* | 0.1265 | 0.1265 | 0.1338 | 0.1313 | 0.1299 | 0.1281 | 0.1248 | 0.1275 | 0.1091 | 0.1200 | 0.1234 | 0.1218 | 0.1234 | 0.1234 |
| 25 | LNC134 *L.torresi* | 0.1182 | 0.1182 | 0.0957 | 0.1196 | 0.1182 | 0.1148 | 0.1198 | 0.1273 | 0.1176 | 0.1292 | 0.1294 | 0.1311 | 0.1327 | 0.1327 |
| 26 | LNC133 *L.torresi* | 0.1199 | 0.1199 | 0.1049 | 0.1213 | 0.1199 | 0.1165 | 0.1215 | 0.1255 | 0.1177 | 0.1310 | 0.1278 | 0.1295 | 0.1311 | 0.1311 |
| 27 | LNC146 *L.torresi* | 0.1215 | 0.1215 | 0.1081 | 0.1230 | 0.1215 | 0.1182 | 0.1231 | 0.1256 | 0.1157 | 0.1327 | 0.1328 | 0.1345 | 0.1328 | 0.1328 |
| 28 | MZUC43498 *L.poconchilensis* | 0.1183 | 0.1183 | 0.1306 | 0.1214 | 0.1183 | 0.1182 | 0.1281 | 0.1239 | 0.1075 | 0.1183 | 0.1218 | 0.1235 | 0.1185 | 0.1185 |
| 29 | MZUC43497 *L.poconchilensis* | 0.1183 | 0.1183 | 0.1306 | 0.1214 | 0.1183 | 0.1182 | 0.1281 | 0.1239 | 0.1075 | 0.1183 | 0.1218 | 0.1235 | 0.1185 | 0.1185 |
| 30 | JC60 *L.ornatus* | 0.1883 | 0.1883 | 0.1813 | 0.1920 | 0.1883 | 0.1846 | 0.1793 | 0.1910 | 0.1702 | 0.1711 | 0.1711 | 0.1728 | 0.1780 | 0.1780 |

……*continued on the next page*

**APPENDIX S3. (Continued)**

| N° | SEQUENCE | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LNC86 *L.manueli* | | | | | | | | | | | | | | | |
| 2 | ER1 *L.manueli* | | | | | | | | | | | | | | | |
| 3 | YM *L.manueli* | | | | | | | | | | | | | | | |
| 4 | MUAP-104 *L.manueli* | | | | | | | | | | | | | | | |
| 5 | ER2 *L.manueli* | | | | | | | | | | | | | | | |
| 6 | LNC135 *L.audituvelatus* | | | | | | | | | | | | | | | |
| 7 | LNC136 *L.audituvelatus* | | | | | | | | | | | | | | | |
| 8 | gi78128766 *L.ruibali* | | | | | | | | | | | | | | | |
| 9 | gi30266200 *L.vallecurensis* | | | | | | | | | | | | | | | |
| 10 | gi575383760 *L.eleodori* | | | | | | | | | | | | | | | |
| 11 | LNC64 *L.rosenmanni* | | | | | | | | | | | | | | | |
| 12 | LNC69 *L.rosenmanni* | | | | | | | | | | | | | | | |
| 13 | MZUC44230 *L.nigriceps* | | | | | | | | | | | | | | | |
| 14 | MZUC44231 *L.nigriceps* | | | | | | | | | | | | | | | |
| 15 | gi575383706 *L.andinus* | | | | | | | | | | | | | | | |
| 16 | gi78128934 *L.famatinae* | 0.0606 | | | | | | | | | | | | | | |
| 17 | gi575383756 *L.dorbignyi* | 0.0910 | 0.1044 | | | | | | | | | | | | | |
| 18 | gi575383758 *L.dorbignyi* | 0.0858 | 0.0990 | 0.0072 | | | | | | | | | | | | |
| 19 | gi575383846 *L.multicolor* | 0.1129 | 0.1266 | 0.0941 | 0.0952 | | | | | | | | | | | |
| 20 | JC92 *L.signifer* | 0.1013 | 0.1098 | 0.1200 | 0.1210 | 0.1113 | | | | | | | | | | |
| 21 | JC90 *L.signifer* | 0.1013 | 0.1099 | 0.1201 | 0.1211 | 0.1113 | 0.0028 | | | | | | | | | |
| 22 | JC93 *L.signifer* | 0.0998 | 0.1084 | 0.1202 | 0.1212 | 0.1098 | 0.0028 | 0.0028 | | | | | | | | |
| 23 | LNC138 *L.stolzmanni* | 0.1113 | 0.1233 | 0.1021 | 0.1000 | 0.0935 | 0.0946 | 0.0947 | 0.0948 | | | | | | | |
| 24 | MR213 *L.stolzmanni* | 0.1113 | 0.1233 | 0.1021 | 0.1000 | 0.0935 | 0.0946 | 0.0947 | 0.0948 | 0.0000 | | | | | | |
| 25 | LNC134 *L.torresi* | 0.1142 | 0.1113 | 0.1147 | 0.1060 | 0.1012 | 0.1090 | 0.1091 | 0.1092 | 0.0619 | 0.0619 | | | | | |
| 26 | LNC133 *L.torresi* | 0.1126 | 0.1097 | 0.1099 | 0.1045 | 0.0997 | 0.1043 | 0.1043 | 0.1044 | 0.0573 | 0.0573 | 0.0071 | | | | |
| 27 | LNC146 *L.torresi* | 0.1175 | 0.1114 | 0.1116 | 0.1061 | 0.1013 | 0.1091 | 0.1092 | 0.1093 | 0.0619 | 0.0619 | 0.0143 | 0.0071 | | | |
| 28 | MZUC43498 *L.poconchilensis* | 0.1080 | 0.1282 | 0.1251 | 0.1228 | 0.1261 | 0.1371 | 0.1405 | 0.1407 | 0.1291 | 0.1291 | 0.1303 | 0.1222 | 0.1239 | | |
| 29 | MZUC43497 *L.poconchilensis* | 0.1080 | 0.1282 | 0.1251 | 0.1228 | 0.1261 | 0.1371 | 0.1405 | 0.1407 | 0.1291 | 0.1291 | 0.1303 | 0.1222 | 0.1239 | 0.0000 | |
| 30 | JC60 *L.ornatus* | 0.1599 | 0.1725 | 0.1809 | 0.1817 | 0.1726 | 0.1825 | 0.1790 | 0.1810 | 0.1711 | 0.1711 | 0.1775 | 0.1723 | 0.1742 | 0.1631 | 0.1631 |