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Hybridization could be a common phenomenon within the highly diverse lizard genus *Liolaemus*

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Liolaemus;
model-based approach;
Patagonia.

Abstract

Hybridization is likely to occur more often between closely related taxa that have had insufficient time to diverge to the point of reproductive incompatibility; hybridization between deeply divergent lineages is rare. In squamate reptiles, hybridization has been proposed as a possible explanation for the extensive paraphyly observed in mitochondrial gene trees in several species complexes of the South American lizard genus *Liolaemus*. One of the best-documented cases is within the *L. boulengeri* and *L. rothi* complexes, which diverged ~5.5 million years ago. Here, we describe a comprehensive study for approaching the hybridization hypothesis between these lizard species complexes. We explored the level of gene tree discordance using the novel 'extra lineage contribution' statistics (XLC, presented in this study) that quantifies the level of gene tree discordance contribution per individual within a species. We included molecular data (12 nuclear and two mitochondrial genes) from 127 individuals, and results of a coalescent model-based analysis show that the most likely explanation for the gene tree-species tree discordance is interspecific hybridization. Our best-supported hypothesis suggests current and past hybridization between *L. rothi* (*rothi* complex) and *L. tehuelche* (*boulengeri* complex), and independently between *L. rothi* and *L. boulengeri* and *L. telsen* (*boulengeri* complex). The hybrid descendants are characterized by intermediate phenotypes between the parental species, but are more similar to *L. rothi* in body size. We discuss the possible role of hybridization in *Liolaemus* evolution.

Introduction

Why two different species are in some cases able to hybridize is a question that has been debated for the last half century (Ellstrand & Rieseberg, 2016). It is becoming clear that hybridization is not as uncommon as previously thought, and many new studies are reporting evidence of gene flow between different species (Mallet, 2005, 2007; Ellstrand & Rieseberg, 2016). Hybridization might be more likely to happen between closely related taxa, because with time genetic differences accumulate and lead to complete reproductive isolation (Nosil, 2008; Abbott *et al.*, 2013). For instance, hybrid offspring from distant parental species are more likely to be under negative selection pressures due to the effects of the Dobzhansky–Muller hybrid or other chromosomal incompatibilities (see Abbott *et al.*, 2013). However, recent molecular studies have demonstrated hybridization between deeply divergent plant (e.g. Worth *et al.*, 2016) and animal species (e.g. Olave *et al.*, 2011, 2017a; Canestrelli *et al.*, 2017). In these cases, the underling genetic processes of how species that diverged millions of years ago, yet are still able to produce viable offspring, remain unknown.

Detecting hybridization is often challenging because similar patterns may result from random segregation of

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alleles during speciation. Both processes will increase the levels of gene tree – species tree discordance (Maddison, 1997; Olave *et al.*, 2017a), and whereas these patterns are commonly attributed to the retention of ancestral polymorphism during the speciation continuum (i.e. incomplete lineage sorting [ILS] or 'deep coalescence'; Maddison, [1997]), new methods can now accommodate both ILS and hybridization and partition the contributions of each to the evolution of the species (e.g. Gerald *et al.*, 2011; Yu & Nakhleh, 2015; Solis-Lemus & Ané, 2016; Olave *et al.*, 2017a). The population size and the number of generations since the divergence are the parameters conditioning the impact of ILS, where a larger number of generations since divergence decreases the amount of expected ILS (Degnan & Salter, 2005; Rosenberg, 2013; Than & Rosenberg, 2013; Gao *et al.*, 2015). Although deeply divergent species may still share ancestral polymorphisms, there is also a trade-off between ancestral population size and the number of generations that might result in the persistence of ILS effects over longer time frames. Thus, looking at gene trees within species trees in model-based analyses is required to distinguish between ILS and hybridization.

The lizard genus *Liolaemus* is distributed across most of the temperate part of continental South America and is associated with climatic regimes ranging from the extremely arid Atacama desert (southern Peru) to the temperate *Nothofagus* rainforests (southern Chile) (Cei, 1986; Lobo, 2001). *Liolaemus* currently includes 267 described species and represents globally the most species-rich temperate zone amniote genus. Among many interesting aspects of the evolutionary history of *Liolaemus*, interspecific hybridization has been documented between nonsister species (*L. gracilis* and *L. bibronii*; Olave *et al.*, 2011, 2017a), and many other cases of hybridization have been hypothesized for several other groups within the genus (Morando *et al.*, 2004; Avila *et al.*, 2006; Breitman *et al.*, 2011; Feltrin, 2014; Medina *et al.*, 2014; Olave *et al.*, 2014). Particularly, hybridization has been suggested between two deeply divergent species complexes, the *boulengeri* (five species) and *rothi* complexes (six species) (Avila *et al.*, 2006), based on extensive mitochondrial paraphyly.

Specifically, because the mitochondrial genome is haploid and segregates matrilineally, its effective population size is four times smaller than that for a nuclear locus and, thus, the probability of observing deep coalescences in a mitochondrial gene tree over time decreases at a higher rate than nuclear loci (Funk & Omland, 2003). We recently estimated the coalescence between the *boulengeri* and *rothi* clades to be ~5.5 million years ago (Olave *et al.*, 2015), which represents a minimum of 2.25 million post-divergence generations (assuming a 2-yr generation time; e.g. Rocha, 1992; Martori & Aun, 1997; Ibargüengoytía & Cussac, 1998). Moreover, the adult phenotypes have already evolved clear differences; on average, the *rothi* complex species are larger and more robust compared with species of the *boulengeri* complex, and the two groups clearly differ in colour patterns. Thus, the deeply divergent *boulengeri* and *rothi* complexes represent interesting groups in which to evaluate the role of hybridization as a plausible explanation for the mitochondrial paraphyly.

In this study, we included sequences for two mitochondrial and 12 nuclear loci for 127 individuals representing all described species for both complexes ($n = 11$). We explored the level of gene tree discordance by presenting the novel 'extra lineage contribution' statistics (XLC), which identifies specific samples (or individuals or alleles) likely to contribute to observed gene tree – species tree discordance. We then propose a set of explicit hypotheses and tested for hybridization using a coalescent model-based approach. Results show that individuals carrying an introgressed mitochondrial haplotype still display intermediate genetic background similar to their parental genotypes and make the largest contributions to the gene tree discordance. We detected current and past gene flow between three species of the *boulengeri* complex (*L. tehuelche*, *L. boulengeri* and *L. telsen*), each separately with the same species of the *rothi* complex (*L. rothi*), and show that is the most likely hypothesis to explain the extensive gene tree discordance observed for these groups. Lastly, we integrated morphological data from morphometric and colour pattern variables into these analyses and show that these hybrid individuals are more similar to *L. rothi* in body size, but display intermediate colour patterns to both parental species. We discuss the role of hybridization and its impact on *Liolaemus* evolution.

Materials and methods

Field sampling

We included a total of 127 terminals representing all described species of both clades; including five in the *boulengeri* complex (*L. boulengeri*, *L. senguer*, *L. inacayali*, *L. telsen* and *L. tehuelche*), and six in the *rothi* complex (*L. sagei*, *L. hermannunezi*, *L. tromen*, *L. sitesi*, *L. loboi* and *L. rothi*), sampled mostly from Argentina, with a small number from Chile (Appendix S3). We sequenced two mitochondrial genes and 12 nuclear loci (10 049 bp total). For further details of field sampling and laboratory procedures, see Appendix S1.

Genetic structure, gene trees and species tree

We calculated a principal component analysis (PCA) using all variable sites from a concatenated matrix with all 14 loci, using the R function pca(). We reconstructed independent gene trees to explore levels of

gene tree – species tree discordance for all species in both species complexes. Gene trees were reconstructed using BEAST v1.6.1 (Heled & Drummond, 2010), and for the mitochondrial data set, we also estimated coalescence times by calibrating the clock hyperpriors using substitution rates for both mitochondrial genes estimated for the genus *Liolaemus* (Olave *et al.*, 2015). Finally, we generated a species tree topology for use as a prior input to test the hybridization hypothesis (see below) using the full matrix in *BEAST and including the focal species *L. rothi, L. boulengeri, L. telsen* and *L. tehuelche*. For details of these analyses, see Appendix S1.

Exploration of gene tree – species tree discordance and the extra lineage contribution statistics

We are interested in exploring the contribution of each individual to the overall gene tree discordance. We aim to see whether the individuals with mitochondrial introgression (candidate hybrids) also contribute to a high level of discordance in nuclear gene trees, as opposed to a general contribution to gene tree discordance. Thus, we introduce in this study the novel 'extra lineage contribution' statistic (XLC). The XLC is informed by the contribution of each sample (i.e. individual or allele) to the total number of extra lineages (Maddison, 1997), as a measure of gene tree discordance (see also Olave *et al.*, 2017a).

Consider a n-taxon species tree, and a sample of gene trees $g = (g_1, g_2, \ldots, g_i)$. Then, the XLC matrix is determined by the gene trees and the alleles (or individuals, or tips) $a = (a_1, a_2, \ldots, a_j)$. The XLC_{ij} element is calculated considering the extra lineages XL_i require for the reconciliation of the g_i with the species tree (as described by Than & Rosenberg, 2013) and the extra lineages XL_n required under the absence of the nth species. Finally, the XL_{ij} denotes the extra lineages counted with the only presence of the a_j of the nth species. Thus, the extra lineage contribution of a_j for the g_i is calculated as:

$$XLC_{ij} = \frac{XL_{ij} - XL_n}{XL_i}$$

where $XL_i \geq XL_{ij} \geq XL_n$. Then, the total contribution of the allele a_j can be obtained as:

$$XLC_j = \frac{\sum XL_{ij} - XL_n}{\sum XL_i}$$

The XLC statistics ranges between 0 and 1, where 0 means no contribution and 1 maximum contribution to the observed gene tree – species tree discordance. Our R function getXLC() available online (www.github.com/melisaolave/Olave_etal2018-JEB) returns the XLC matrix, summary statistics (mean, standard deviation, percentiles) per allele and gene trees, and allows running calculations for each gene tree in multiple cores in parallel, thus it is feasible to explore large genomic data sets. We have also made available the function plotXLCmatrix() to obtain a similar plot to our Fig. 4a.

Testing the hybridization hypotheses

We recently introduced a new method to explicitly test gene flow in the presence of ILS (Olave *et al.*, 2017a). This approach tests if the observed number of extra lineages can be explained by ILS alone (null model), or whether including one or more gene flow parameters provides a better fit. Simply, the method simulates a set of gene trees to infer a distribution of expected extra lineages and calculates a likelihood function by comparing the fit of the real gene trees to the expected distributions.

Here, we propose a null model (strict ILS) to explain the observed gene tree discordance, relative to alternative models of gene flow parameters. The rational for these models is based on exploratory analyses (see Results: Gene tree discordance) suggesting that at least three patterns of introgression could have occurred: *L. rothi* × *L. boulengeri*, *L. rothi* × *L. telsen* and *L. rothi* × *L. tehuelche*. We included different M parameters in the model as described in the Fig. 1. Specifically, the null hypothesis restricts $M_{1,2,3,4,5} = 0$, and thus all gene tree discordance is strictly explained due to ILS. Alternative hypotheses include current gene flow (M_1 and M_2), as well as past gene flow with the combination of (M_3, M_4 and M_5). For simulation details, see Appendix S1.

Morphological comparisons

We compared the morphology of the mtDNA-introgressed individuals to the remaining samples (see

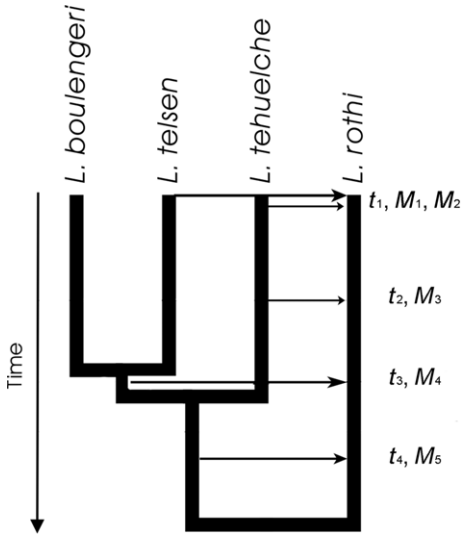

Fig. 1 Illustration of a general model describing the topology and gene flow parameters ($M_{1,2,3,4,5}$).

Results: Gene tree discordance). We collected morphological data for a total of 118 samples (18 *L. boulengeri*, nine *L. telsen*, 23 *L. tehuelche* and 68 *L. rothi*). Within *L. rothi*, based on the mtDNA results, we separated the individuals with introgressed mitochondria into two groups (introgressed mitochondria 1 [i.mt (i)] and 2 [i.mt (ii)]), to compare their morphologies with respect to the other species. We included 10 morphometric and 16 colour pattern variables (see Appendix S1 for details). Using R, we calculated summary statistics for all variables for each group, and to evaluate differences in morphology, we performed multivariate tests for the quantitative characters. We first performed a MANOVA test and a Hotelling contrast analysis, and then a discriminate function analysis. Following Escudero *et al.* (2012) and Olave *et al.* (2017b), we also used R package to perform Pearson's chi-squared test with 5000 Markov permutations, using a contingency table for comparisons of qualitative variables among different groups.

Results

Gene tree discordance

The mitochondrial tree (Fig. 2) resolved two major clades with strong support: the *boulengeri* complex and the *rothi* complex. We recovered most of the 11 species as monophyletic, but *L. rothi* was resolved as polyphyletic within the *boulengeri* complex. One mitochondrial lineage of 15 individuals was recovered within an unresolved clade with *L. telsen* and *L. boulengeri*, and nested within this clade a single *L. rothi* was also nested within a clade of eight *L. telsen* individuals (i.mt [i] group). Six samples of *L. rothi* were inferred as the sister clade of 18 individuals of *L. tehuelche*, and two individuals of *L. rothi* are nested within *L. tehuelche* (i.mt [ii] group).

Figure 3a maps the overlapping geographic distributions of *L. rothi*, *L. telsen*, *L. tehuelche* and *L. boulengeri*; all species are restricted to the Chubut and Río Negro provinces in southern Argentina. In particular, *L. rothi* has the largest distribution and overlaps *L. tehuelche* in the north-western part of its range, with *L. boulengeri* in the central-western range, and in its eastern range, *L. rothi* overlaps with *L. telsen*. *Liolaemus rothi* individuals with mitochondrial haplotypes related to the *L. boulengeri* complex were collected from throughout most of the *L. rothi* distribution. The multilocus PCA (Fig. 3b) displays *L. rothi* individuals with introgressed mitochondria (i.mt [i] [green squares] and i.mt [ii] [open diamonds]) clearly as separate clusters, close to the middle of others representing the remaining species. Further, two individuals of the group i.mt [ii] appear closer to the *L. tehuelche* than *L. rothi*. These two individuals are the same ones nested within *L. tehuelche* clade in the mitochondrial gene tree (note that we have discarded laboratory errors by repeating DNA extractions and sequencing, and discarded species association errors).

The XLC matrix shows extended contributions of extra lineages affecting all gene trees and individuals

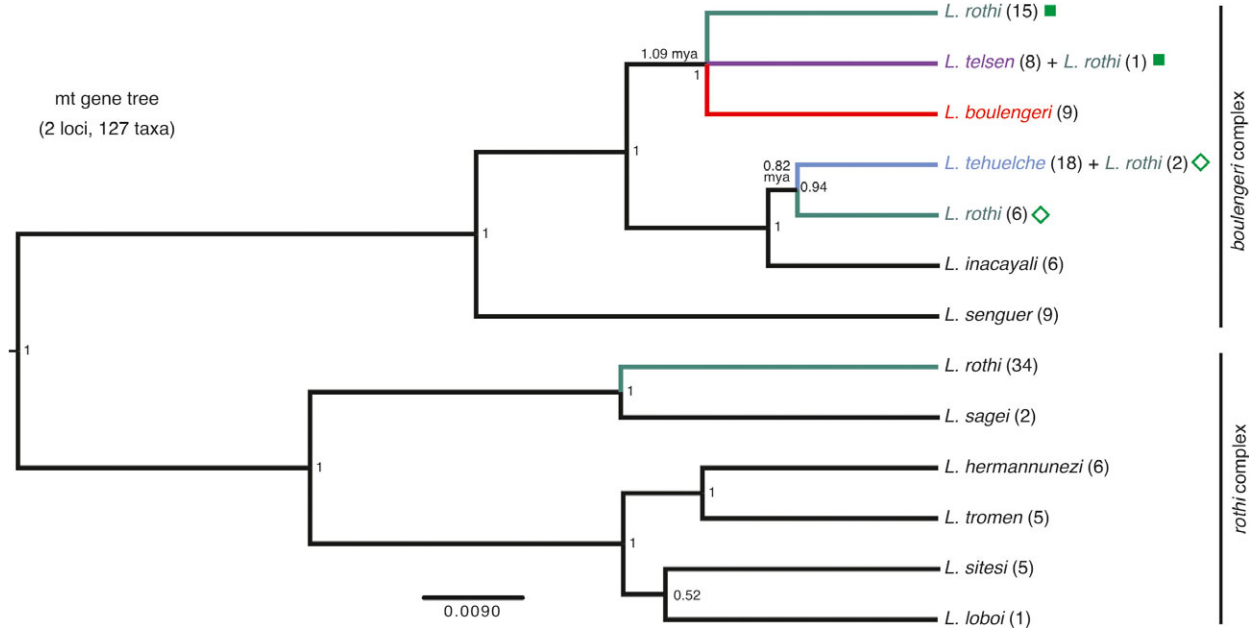

Fig. 2 Mitochondrial gene tree of the *boulengeri-rothi* complex (127 individuals, two mitochondrial loci). Morphological individuals of *L. rothi* recovered within *L. telsen* and *L. boulengeri* clades are identified as green squares (i.mt [i]), whereas individuals of *L. rothi* recovered as the sister lineage of *L. tehuelche* are identified as open diamonds (i.mt [ii]). Numbers in parentheses indicate samples sizes for each terminal.

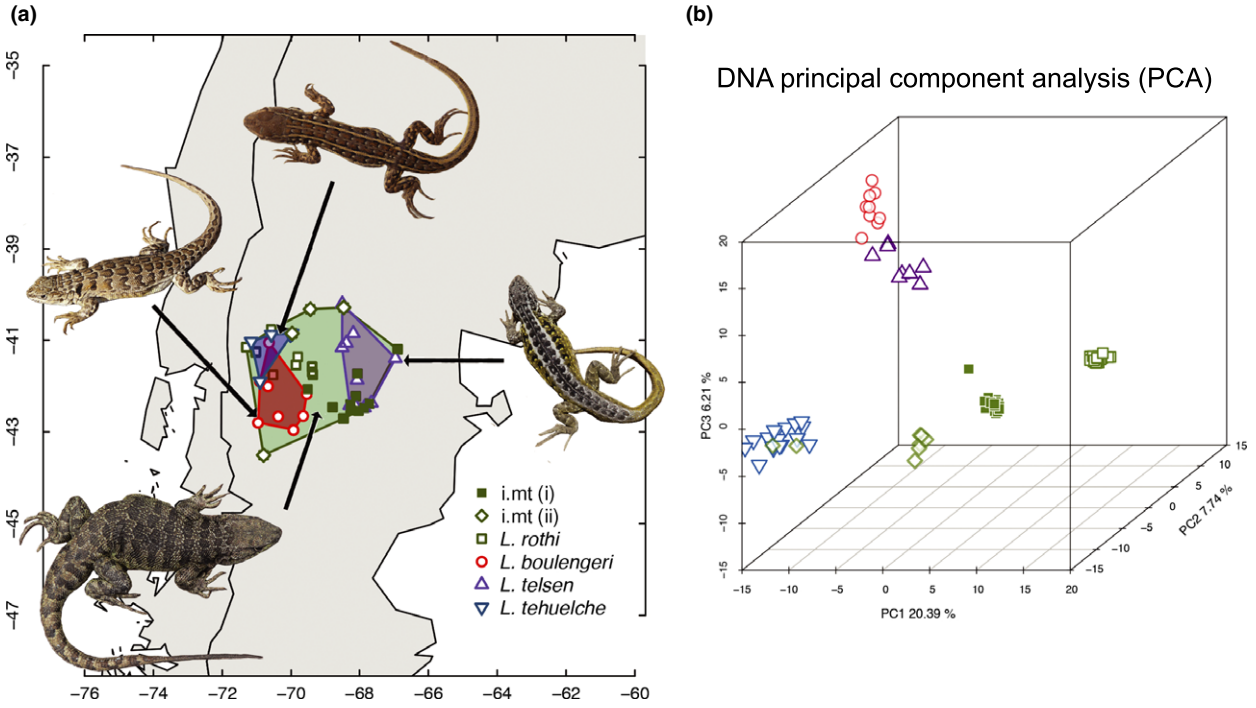

Fig. 3 (a) Distribution map of focal species of the hybridization test, showing overlapping distributions between *L. rothi* and *L. tehuelche*, *L. telsen*, and *L. boulengeri*. (b) PCA using all variable sites from a concatenated DNA matrix (14 loci). Samples showing possible mitochondria introgression (Fig. 2) are shown with green squares (i.mt [i]) and diamonds (i.mt [ii]), clearly as separate clusters, close to the middle of others representing the remaining species.

(Fig. 4a). Because all XLC_{ij} ranges from 0 to 0.4 and the overall XLC maximum is equal to 0.2, there are multiple individuals contributing to the observed gene tree discordance in each locus (no sample XLC_{ij} or XLC = 1). However, individuals of groups i.mt[i] and especially i.mt[ii] represent most extremes in the XLC histogram (Fig. 4b), and both also resulted in the largest means (Fig. 4c). Further, the gene tree discordance was present in all the estimated gene trees (Fig. 4d), with the highest mean values in the nuclear loci A4B, A9C and MXRA5. Gene tree discordance is present in all 13 loci, but most of the gene tree/species tree incongruence is contributed by the i.mt[i] and [ii] samples (Fig. 4b,c).

The divergence time estimates (Fig. 2) give very similar mean values for mitochondrial coalescence in the first two cases of observed paraphyly. Interestingly, the estimated coalescence of the *L. rothi* mitochondria with *L. telsen* and *L. boulengeri* was dated 1.09 mya (HPD: 0.23–3.16), and with *L. tehuelche*, 0.82 mya (HPD: 0.15–2.27), which are earlier dates than the estimated divergence time for the *boulengeri* and *rothi* complexes (=5.5 mya; Olave *et al.*, 2015). The *posterior* estimation of substitution rates for cyt-b and 12S is presented in Table S2.

Testing hybridization hypotheses

Results show that the level of gene tree discordance is insufficiently explained by ILS alone (Table 1). All models including M_1–M_4 showed a greater likelihood than a strict ILS model. The likelihood ratio test selected the model $M_{1,2,3,4}$ = 1 (ln L = −50.3897; likelihood ratio test: $G_{df=2}$ = 119.9792, *P*-value = 8.8477 × 10^{-27}), suggesting that current and past hybridization between *L. rothi* × *L. tehuelche*, *L. rothi* × *L. telsen* and *L. rothi* × *L. boulengeri* is the most likely explanation for the gene tree discordance.

Morphological comparisons

The result of the MANOVA test is significant ($F_{df=98}$ = 3.722; *P*-value < 0.001), and Hotelling contrast analyses are shown in Table 2. The three species of the *boulengeri* complex (*L. telsen*, *L. tehuelche* and *L. boulengeri*) are inferred as a separate group (labelled class 'C'), whereas *L. rothi* overlaps with i.mt (ii) (class 'A'), and i.mt (ii) overlaps with i.mt (i) (class 'B').

Discriminate plots showed overlapping point clouds among most groups, with some level of separation

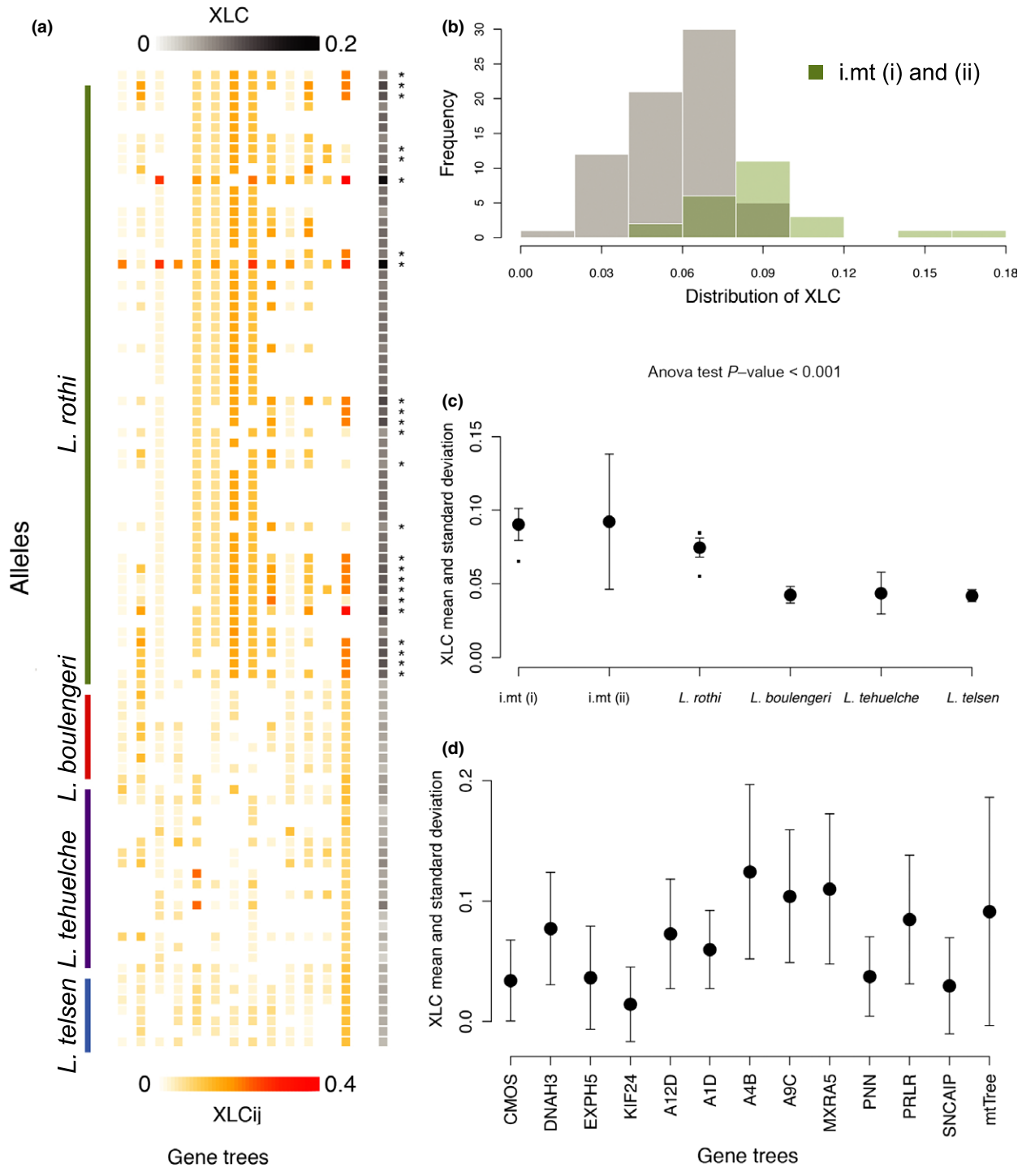

Fig. 4 (a) Graphical representation of the XLC matrix (yellow-red gradient) and the overall XLC per allele (grey-black gradient). Rows represent different alleles within species (or individuals), and columns are the 13 gene trees (first 12 columns are nuclear genes; last column yellow column is the mt tree). The colour gradient represents the magnitude of the XLC, ranging from 0 (or no contribution, white cells), to 0.4 (maximum contribution in this data set, red or black). Alleles are sorted by species. Asterisk (*) highlights the individuals part of i.mt (i) and (ii) groups from Fig. 2. (b) Histogram of the overall XLC distribution. Green bars correspond to the i.mt (i) and (ii) groups and the grey bars to all remaining groups. (c) Mean and standard deviation obtained for the overall XLC per allele among different species/groups. (d) XLC index mean and standard deviation per gene tree.

Table 1 The parameter estimation for each model. Results of likelihood calculations and model selection following the likelihood ratio test; the selected model is shown in grey (ln L = −50.3897; likelihood ratio test: $G_{df=2}$ = 119.9792, *P*-value = 8.8477×10^{-27}).

Models						
M1	*M2*	*M3*	*M4*	*M5*	*M* parameters	Ln likelihood
–	–	–	–	–	0	−210.7909
–	–	–	1	–	1	−208.6641
–	–	1	–	–	1	−191.4251
–	–	–	–	5	1	−209.5037
–	–	3	4	–	2	−110.3794
1	1	1	1	–	4	−50.3897

between *L. rothi* and the introgressed samples (i.mt [i] and [ii]) with respect to the *boulengeri* complex species, Fig. S1). The centroids of the groups are summarized in Table S3, and the cross-classification tests are summarized in Table S4. Results in body size associate the i.mt groups closer to *L. rothi* group (Fig. S1; Table S4).

Chi-squared tests for qualitative variables of colour patterns showed no significant differences between groups i.mt (i) and (ii), as well as their comparison with *L. rothi*, *L. boulengeri*, *L. telsen* and *L. tehuelche* (Table 3). On the other hand, we found significant differences between *L. rothi* with respect to the species of the *boulengeri* complex. Thus, groups i.mt (i) and (ii) showed a tendency to have intermediate phenotypes based on the qualitative assessments of colour patterns.

Table 3 Pearson's $\chi^2_{df=15}$ results for qualitative variables. Comparisons between pairs of pure parental (*L. rothi* vs. *L. boulengeri, L. telsen* and *L. tehuelche*) resulted in significant differences in colour patterns variables (all *P*-values < 0.05; highlighted by asterisks), and comparison between them and the hybrid candidates i.mt (i) and (ii) returned not significant differences (all *P*-values > 0.05).

Group 1	Group 2	Group 3	χ^2	*P*-value
L. rothi	*L. boulengeri*		60.5519	0.0002***
L. rothi	*L. telsen*		42.6945	0.0006***
L. rothi	*L. tehuelche*		43.3627	0.0018**
i.mt (i)	i.mt (ii)	*L. rothi*	7.8111	0.9998
i.mt (i)	i.mt (ii)	*L. boulengeri*	25.17	0.2877
i.mt (i)	i.mt (ii)	*L. telsen*	8.8445	0.9418
i.mt (i)	i.mt (ii)	*L. tehuelche*	21.7962	0.6123
i.mt (i)	i.mt (ii)		5.8218	0.9016

Discussion

Hybridization could be a common phenomenon among lizards of the genus *Liolaemus*

In this study, we tested the hybridization hypothesis using a recently developed coalescent-based method and found strong evidence for interspecific gene flow between the *boulengeri* and *rothi* complexes based on molecular as well as morphological evidence. Although the divergence between these two species complexes is dated ~5.5 million years ago (Olave *et al.*, 2015), we document signal for current and past hybridization between *L. rothi* and *L. boulengeri* – *L. telsen* as well as between *L. rothi* and *L. tehuelche*. We show evidence of extensive gene tree discordance in both complexes by quantifying the level of extra lineage contributions (Fig. 4a–c), as well as discordance in all included gene trees (Fig. 4d). Although all samples and species contribute to the observed gene tree discordance (Fig. 4a–c), interestingly, the largest incongruence concentrated in samples of the i.mt group (Fig. 4b,c). We have also shown the intermediate genetic configuration of these samples (Fig. 3b), as well as the retention of intermediate colour patterns (Table 3).

The past mitochondrial introgression was dated 1.09 mya and 0.82 mya for two nodes in the mtDNA gene tree (Fig. 2). If the observed mitochondrial paraphyly resulted from hybridization, dating the coalescence times in the mitochondrial genealogy could lead to an approximation of the occurrence of that event on a time-calibrated tree (see Enciso-Romero *et al.*, 2017 for a similar approach). Given these estimates, mitochondrial introgression occurred over four million years after divergence of the species complex, but we note

Table 2 Results of Hotelling contrast tests for all quantitative variables (MANOVA test: $F_{df=98}$ = 3.722; *P*-value < 0.001). Class column corresponds to associations of groups. The three species parts of the *boulengeri* complex (*L. telsen*, *L. tehuelche* and *L. boulengeri*) were recovered as a group (class 'C'), whereas *L. rothi* was recovered with an overlap with i.mt (ii) ('A'), and i.mt (ii) is overlapped with i.mt (i) ('B').

	Variables															
Species/group	SVL	AGD	HaL	RUL	HL	FoL	TFL	FL	KKD	NSLVL	NSRVL	NLPS	NRPS	*n*	Class	
i.mt (i)	77.57	36.81	12.06	13.37	11.55	21.68	15	14.94	34.2	7.71	7.79	6.86	7.21	14		B
i.mt (ii)	73.54	35.74	11.26	12.25	10.93	20.54	12.72	13.3	29.87	8.5	8.5	7.67	8	6	A	B
L. rothi	75.16	36.67	12.02	13.43	11.74	20.91	14.86	15.66	32.75	8.51	8.44	7.47	7.4	43	A	
L. telsen	59.87	29.36	8.96	9.89	9	16.17	11.11	10.81	25.99	10.17	10.17	9	9.33	6		C
L. tehuelche	63.94	31.28	9.33	10.29	9	16.9	11.84	11.89	26.74	10.59	10.59	10.06	10.18	17		C
L. boulengeri	56.76	26.57	8.64	9.4	8.14	15.91	10.8	10.45	24.38	10.24	10.24	9.41	9.71	17		C

that the coalescence of a single gene is predicted to be deeper in time than population divergence in the species tree (Edwards & Beerli, 2000; McCormack *et al.*, 2011).

In earlier studies, we found evidence for hybridization in another *Liolaemus* species group; this was well documented in the subgenus *Liolaemus sensu stricto*, in which *L. gracilis* × *L. bibronii* hybrids are common where their ranges overlap (Olave *et al.*, 2011, 2017a). Further, hybridization has also been suggested for several other groups within the genus, with similar patterns of high mitochondrial paraphyly, including the *darwinii* complex (Morando *et al.*, 2004); the *lineomaculatus* series (Breitman *et al.*, 2011; Olave *et al.*, 2014), the *petrophilus* complex (Feltrin, 2014), the *fitzingerii* group (Avila *et al.*, 2006), and the *kriegi* complex (Medina *et al.*, 2014). However, further analyses are needed to actually test alternative hypotheses in these groups.

We compared the morphology of the introgressed samples to the other ingroup species, and multivariate analyses of the quantitative variables showed that morphometrically hybrid individuals are generally more similar to *L. rothi* than to any of the species in the *boulengeri* complex (Fig. S1; Table S4). However, these hybrid lizards did not differ significantly in colour patterns from any of the other species (Table 3), suggesting that the intermediate phenotypes of hybrids between their hypothesized parental species are stabilized through subsequent generations. Similar results were reported for intermediate hybrid descendants in *L. gracilis* × *L. bibronii* study (Olave *et al.*, 2011). The common pattern of intermediate-like hybrids raises the question of how this could influence the evolution of *Liolaemus*.

The role of hybridization in *Liolaemus*

In a broader evolutionary context, hybridization can be (i) neutral, (ii) disadvantageous (e.g. Martin *et al.*, 2013; Canestrelli *et al.*, 2017) or (iii) adaptive (e.g. Becker *et al.*, 2013; Clarkson *et al.*, 2014; Enciso-Romero *et al.*, 2017; Meier *et al.*, 2017). How gene flow affects the different species' persistence and adaptation will depend on the role of natural selection in 'sweeping' the new introgressed alleles to fixation in the introgressed species (see Feder *et al.*, 2012). The four independent events of interspecific gene flow between *L. rothi* and the species of the *boulengeri* complex we document here, in combination with earlier studies describing similar patterns in other complexes within the genus *Liolaemus*, suggest that hybridization could be a common phenomenon within these lizards.

Liolaemus is a species-rich clade characterized by an extraordinary diversification rate, comparable to the *Anolis* lizards in the tropics (Harmon *et al.*, 2003). In contrast to these highly specialized and phenotypically diverse tropical lizards (Yoder *et al.*, 2010), *Liolaemus* has been characterized by morphological stasis (Olave *et al.*, 2017b), and as a rule, *Liolaemus* species tend to be more generalists than specialists, and to share what appears to be a conserved morphology (Abdala *et al.*, 2014). Morphological generalists are considered to have advantages over the specialists in fluctuating and heterogeneous environments, because the former are more tolerant to climatic changes and more likely to find suitable habitats (Jansson & Dynesius, 2002; Lanchier & Neuhauser, 2006). Southern South America is an example of a large heterogeneous landscape with a complex geo-climatic history, including extensive tectonic uplift, volcanism, and many cycles of glacial advance and retreat (Ponce *et al.*, 2011). Some *Liolaemus* lineages show a genetic signature of persistence in climate refugia (e.g. Breitman *et al.*, 2012; see Sérsic *et al.*, 2011 for a general review of the regional biota) during glacial advances, followed by expansions into new geographic areas when climates became optimal (e.g. Olave *et al.*, 2011; Breitman *et al.*, 2012). The genus is characterized by at least two rapid radiations that were dated to the Late Miocene (~10 mya), and the early Pliocene (~5 mya; Pincheira-Donoso *et al.*, 2015; Olave *et al.*, 2015); in both cases, the genus radiated to colonize a wide array of different environments (Abdala & Quinteros, 2014; Pincheira-Donoso *et al.*, 2015). Thus, as a future direction promoted by the research presented here, it would be interesting to test whether the hybridization is one of the evolutionary processes promoting the success of *Liolaemus* diversification (see Seehausen, 2013). Hybridization could have benefitted species that occupy fluctuating environments in at least two ways, either by (i) rapidly increasing genetic variability after population bottlenecks, and/or (ii) contributing to the maintenance of an adaptive 'generalist biology' (i.e. preventing specialization) by rapidly generating a wide distribution of intermediate phenotypes in cohorts of hybrid descendants. It would be interesting as well to explore whether a generalist morphology and apparent phenotypic stasis in *Liolaemus* (Olave *et al.*, 2017b) might facilitate the phenomenon of still ongoing gene flow among deep divergent species. New tools provided by emerging genomic technologies, coupled with eco-physiological field studies, as well as clade-wide statistical assessments of phenotypic diversity, will be needed to critically test the influence of hybridization on the diversification of this genus.

Gene tree discordance and the extra lineage contribution statistics

Hybridization was traditionally interpreted as a rare process that limits speciation and is also considered as an evolutionary dead-end (Seehausen, 2013). However, in the last decade, hybridization has been highlighted as more common than previously thought (Mallet, 2005, 2007), and novel views have suggested a new

role for hybridization as a promotor of diversification under some conditions. These include transferring adaptive traits via introgression, through establishment of recombinant forms (homoploid hybrid speciation), or via allopolyploidization, which may lead to instantaneous speciation (Abbott *et al.*, 2013; Soltis 2013). These new perspectives have increased the focus on hybridization studies and the development of new theoretical methods to approach these questions.

Although many programs that have been developed to explore the ancestry of sets of genetic samples (e.g. ABBA/BABA [Kulathinal *et al.*, 2009; Green *et al.*, 2010; Durand *et al.*, 2011], ADMIXTURE [Alexander *et al.*, 2009], and Treemix [Pickrell & Pritchard, 2012]), there has also been increased focus on individual gene trees (Than & Nakhleh, 2009; Yu & Nakhleh, 2015; Solis-Lemus & Ané, 2016; Olave *et al.*, 2017a). Such interest is probably due to major improvements in the efficiency with which large data sets can be analysed in a fraction of the computational time compared with earlier methodologies (Than & Nakhleh, 2009; Olave *et al.*, 2017a).

Here, we have presented the first statistic that captures the individual contributions of each sample to the gene tree discordance. We showed that this statistic represents a useful way of exploring data sets *a priori*, and it provides the advantages of investigating whether the discordance is associated to specific hybrid candidates vs. more general contributions. Further, as shown in Fig. 4d, it is also possible to explore the level of discordance in each locus. Thus, the XLC matrix highlights the extra lineage contribution of each gene tree, which provides useful information in detecting asymmetry in the introgression of specific genes. In other words, comparisons of the XLC calculations among specific genes vs. neutral genes could be used to explore whether some genes introgress more often than others (or the opposite, i.e. do not introgress). For example, under the scenario of adaptive introgression of specific genes, these loci should display a greater XLC value than neutral nonlinked genes. This makes the implementation of XLC a promising approach for future hybridization studies, allowing exploration of the advantages or disadvantages of gene flow in a given hybridizing set of taxa.

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Authors' contributions

M.O. and M.M. conceived the ideas and designed methodology. M.O. analysed the data and led the writing of the manuscript. M.M., L.J.A. and J.W.S., Jr. participated in some field work and provided the funding and facilities for collection of the molecular data. All authors contributed critically to revision of the drafts and gave final approval for publication.

Data accessibility

DNA sequences: GenBank accessions MH118747-MH118923.
Final DNA sequence assembly: Dryad https://doi.org/10.5061/dryad.fm0g3m5.
Sampling locations: Appendix S3.
Extended Methods and Results: Appendix S1.
Topology used for simulations and model test: Appendix S2.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1 Extended Methods and Results.
Table S1 Substitution models selected by jModeltest.
Table S2 Results of *posterior* estimation of substitution rates for the *boulengeri-rothi* complexes.
Table S3 Centroid coordinates estimated by the multivariate discriminant analysis (Fig. S1).
Table S4 Cross-classification table from the discriminant analysis (Fig. S1).
Figure S1 Discriminate analysis plot estimated for the quantitative variables.
Appendix S2 Species tree in newick format used to test the hybridization hypotheses.
Appendix S3 Details of samples employed.

Data deposited at Dryad: https://doi.org/10.5061/dryad.fm0g3m5

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