



# Sexual dimorphism and interspecific head variation in the *Liolaemus melanops* complex (Squamata: Liolaemini) based on geometric morphometrics

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By analysing size and shape separately, geometric morphometric methods (GM) are a powerful tool to evaluate morphological differences within and between taxa. In this work, we used GM to investigate whether lizards of the *Liolaemus melanops* complex differ in shape and size. Specifically, we analysed head shape and size variation to quantify intraspecific sexual dimorphism and interspecific differences. We found sexual dimorphism in six of the seven investigated species (*L. canqueli*, *L. dumerili*, *L. goetschi*, *L. martorii*, *L. melanops* and *L. morenoi*). Five species (*L. canqueli*, *L. casamiquelai*, *L. martorii*, *L. melanops* and *L. morenoi*) were distinct in shape and size, whereas *L. goetschi* and *L. dumerili* were indiscernible from each other. This work illustrates the value of GM to study morphological variation in lizards, suggesting that similar studies would be valuable for testing species boundaries in other groups of *Liolaemus*.

**Key words:** Lizards, *Liolaemus*, morphological diversity, species' boundaries

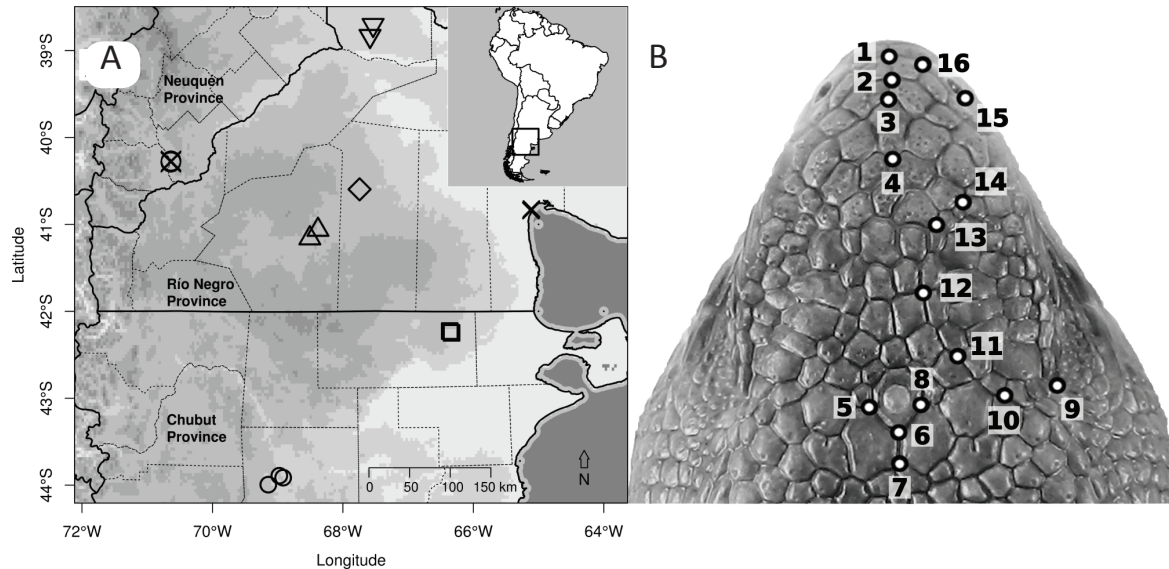
## INTRODUCTION

During the last two decades, several analytical approaches have changed the way we address morphological variation in ecological, systematic and taxonomic studies. One of these is the geometric morphometrics (GM) approach (Corti, 1993). GM establishes a mathematical basis for the study and quantification of organismal shape; it simultaneously upholds the geometric properties of the structures of interest (Zelditch et al., 2004; Kaliontzopoulou, 2011) while quantifying the ensemble of attributes or variables of objects used to describe shape and size (Claude, 2008). The methods implemented in GM represent a powerful tool for quantifying morphological differences in unified and pragmatic manner (see Bookstein, 1996; Rohlf, 2000; Zelditch et al., 2004). Many herpetological studies have used geometric morphometrics to assess intra and interspecific variation related to ecological variables (Daza et al., 2009; Kaliontzopoulou et al., 2010), ontogeny (Piras et al., 2010, 2011), sexual dimorphism (Vidal et al., 2005; Kaliontzopoulou et al., 2007, 2008; Alcorn et al., 2013), and other differences among species (Ivanović et al., 2008, 2009; Llorente et al., 2012; Tancioni et al., 2013; Showalter et al., 2014). Recent studies have implemented this approach to delimit taxa within species complexes (e.g., Florio et al., 2012) to achieve the conceptual framework of integrative taxonomy

(sensu Schlick-Steiner et al., 2010). Despite this, there are very few studies that have used this method for the highly diverse genus *Liolaemus*, and most of these were focused on single species (Vidal et al., 2005; Vidal Maldonado, 2011).

The South American lizard genus *Liolaemus* is of interest to evolutionary biologists because it shows a steady increase in lineage accumulation through time (Harmon et al., 2003). However, the total number of currently recognised species is at least 260 (Abdala & Quinteros, 2014 compared with 69 species used by Harmon et al., 2003), a number which is likely an underestimate given the pace of recent descriptions (reviewed in Abdala & Quinteros, 2014), the number of candidate species proposed in recent studies (e.g., Breitman et al., 2012; Olave et al., 2014), and ongoing systematic rearrangements (e.g., Pincheira-Donoso et al., 2008b; Lobo et al., 2010). The genus includes extensive morphological and ecological diversity (e.g., Schulte et al., 2004; Breitman et al., 2013), and several well-defined species complexes (e.g., Morando et al., 2003; Avila et al., 2006; Lobo et al., 2010; Breitman et al., 2012; Olave et al., 2014). Some well-sampled species complexes include cryptic species, candidate species (Lobo & Espinoza, 2004; Morando et al., 2007; Pincheira-Donoso et al., 2007a), and intraspecific clinal phenotypic variation (Pincheira-Donoso et al., 2007b; Pincheira-Donoso, 2008; Escudero et al., 2012). All of these factors

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**Fig. 1.** A) Geographic location of type localities and surroundings for each studied species. Circles: *Liolaemus canqueli*, triangles: *L. casamiquelai*, diamonds: *L. dumerili*, triangles downwards: *L. goetschi*, X: *L. martorii*, squares: *L. melanops* and circles with X: *L. morenoi*. B) Location of 16 landmarks used in this study. All landmarks were digitised from right dorsal view of the head. Description of the two dimensional homologue landmarks used in capturing head morphology shape: 1, Junction of the postrostrals and first pair of internasal scales; 2, Junction of first and second pair of internasal scales; 3, Junction of the second pair of internasal and median scale; 4, Posteriormost point of the median scale; 5, Anteriormost joint of left parietal and interparietal scales; 6, Anteriormost joint of both parietal and interparietal scales; 7, Posteriormost joint of both parietal scales; 8, Anteriormost joint of right parietal and interparietal scales; 9, Posteriormost point of the tenth circumorbital scale; 10, Central joint of ninth and eighth circumorbital scales; 11, Central joint of seventh and sixth circumorbital scales; 12, Central joint of fifth and fourth circumorbital scales; 13, Central joint of third and second circumorbital scales; 14, Anteriormost point of the first circumorbital scale; 15, Posteriormost point the nostril; 16, Anteriormost joint of the nasal and internasal scales.

make it difficult to assess morphological differences between phylogenetically closely related species. This issue is particularly relevant to the *Liolaemus fitzingerii* group *sensu* Avila et al. (2006), a morphologically diverse clade with many taxa diagnosed on the basis of traditional descriptive morphology, and without any statistical analysis (e.g., Etheridge & Christie, 2003).

The *fitzingerii* group is distributed in Patagonia Argentina from northern Neuquén and Río Negro provinces to the southern Santa Cruz province (Escudero et al. 2012) and includes two species complexes: *fitzingerii* and *melanops* (*sensu* Avila et al. 2006, 2010; Escudero et al. 2012). To evaluate sexual dimorphism (SD) and interspecific variation among closely related *Liolaemus* species, we studied seven species currently included within the *melanops* complex: *Liolaemus canqueli* (Ceí, 1975), *L. casamiquelai* (Avila et al., 2010), *L. dumerili* (Abdala et al., 2012b), *L. goetschi* (Müller & Hellmich, 1938), *L. martorii* (Abdala, 2003), *L. melanops* (Burmeister, 1888), and *L. morenoi* (Etheridge & Christie, 2003). Several incongruent taxonomic arrangements have been proposed for this species group since the 1970's (Ceí, 1973; Ceí & Scolaro, 1977; Scolaro & Ceí, 1977; Nori et al., 2010; Abdala et al., 2012a, 2012b). These works did not present detailed statistical analyses of shape and size with regard to intra vs interspecific variation. The objective of this work is to evaluate the ability of GM to detect statistically significant morphological differences between closely related species of the *L. melanops*

complex, and we focus explicitly on sexual size and shape dimorphism within and among all described species.

## MATERIALS AND METHODS

### Field work, examined material and operational criteria

We carried out surveys from March 2000 to March 2011 during spring-summer seasons throughout the complete geographic distribution of the *L. melanops* species complex (Fig. 1A). We collected lizards by hand or noose, and determined latitude, longitude and elevation with a Garmin GPS 12™. After capture, specimens were euthanized by a pericardiac injection of sodium thiopentotal Pentovet®, fixed in 10–20 % formalin, and then transferred to 70% ethanol. Based on the results of previous phylogeographic, phylogenetic and morphological studies (Avila et al., 2006, 2010; Abdala, 2007; Abdala et al., 2012b; Minoli, 2015), we performed morphological comparisons of the voucher specimens to establish individual identities. We included only adults in this study, and verified sex and sexual maturity by presence of precloacal pores and enlargement of the tail at the base of the cloaca. We analysed a total of 121 specimens (see Online Appendix) from the seven type localities and surrounding areas (Fig. 1A). We considered a species to be sexually dimorphic if it shows significant between-sex differences in shape (considering either a classification error >50% for both sexes and the graphic results), or in centroid size (alpha level ≤0.05).

**Table 1.** Values of the Discriminant Function Analysis using the leave-one-out cross-validation using a permutation test with 1000 iterations.

Species	Female - Male				
	Correct classification	Misclassified specimens	True n	Female % error	Male % error
<i>L. canqueli</i>	4 - 8	5 - 4	9 - 12	55.56	33.33
<i>L. casamiquelai</i>	3 - 2	5 - 5	8 - 7	62.50	71.43
<i>L. dumerili</i>	4 - 2	4 - 4	8 - 6	50.00	66.67
<i>L. goetschi</i>	6 - 4	6 - 3	12 - 7	50.00	42.86
<i>L. martorii</i>	6 - 7	3 - 2	9 - 9	33.33	22.22
<i>L. melanops</i>	4 - 7	4 - 6	8 - 13	50.00	46.15
<i>L. morenoi</i>	2 - 8	2 - 1	4 - 9	50.00	11.11

Our operational criterion for considering two species as different is if they present evidence of significant differences in shape and size of the centroid size for both sexes.

### Geometric morphometrics

We took high-resolution photos of all specimens inside a light box using a digital camera (Nikon P-500 Coolpix, resolution 12.10 MP) placed on a tripod-desk with the same height-distance and angle. We captured the dorsal view of each lizard's head with a millimetre ruler to record the scale. We downloaded all images to a PC and created a .tps file for each of the species using tpsUtil 1.58 (Rohlf, 1997b). We chose landmarks following Zelditch et al. (2004) for the purpose of quantifying any detectable differences between the specimens, to meet the following assumptions: (i) represent homologous anatomical landmarks, (ii) provide adequate coverage of the morphology, (iii) repeatedly and reliably located, and (iv) lay within the same plane. We also evaluated all literature on landmark selection used in earlier GM studies of *Liolaemus* (Vidal et al., 2005; Fontanella et al., 2012). We included a total of 16 Type 1 landmarks (see Bookstein, 1997; Zelditch et al., 2004) from adult specimens, and digitised all landmarks on the dorsal right

side of the head for each specimen (Fig. 1B) using tpsDig v. 2.17 (Rohlf, 1997a). We excluded all specimens that showed abnormalities or preservation artefacts (bumps, scars, bites, bent heads, etc.), that made one or more predefined landmarks impossible to establish accurately.

### Statistical analyses

Using MorphoJ v. 1.05f software (Klingenberg, 2011), we applied a full procrustes fit or superimposition (see Zelditch et al., 2004) to standardise the size and to translate and rotate the configurations of landmark coordinates (Rohlf & Slice, 1990). The full procrustes fit assigns less weight to observations that are far from the average shape, and is therefore more robust to outliers (Zelditch et al., 2004). We repeated digitisation of landmarks on 10 specimens of each species and performed the procrustes ANOVA (Klingenberg, 2011) to evaluate measurement error (which was negligible, results not shown). Then, we confirmed that variation in shape between individuals was sufficiently small, and accordingly the distribution of landmarks in the shape space could be represented satisfactorily in the tangent space. Additionally, we performed an outliers test (Viscosi & Cardini, 2011) to control and exclude any individual specimen that deviated strongly from the average, and an

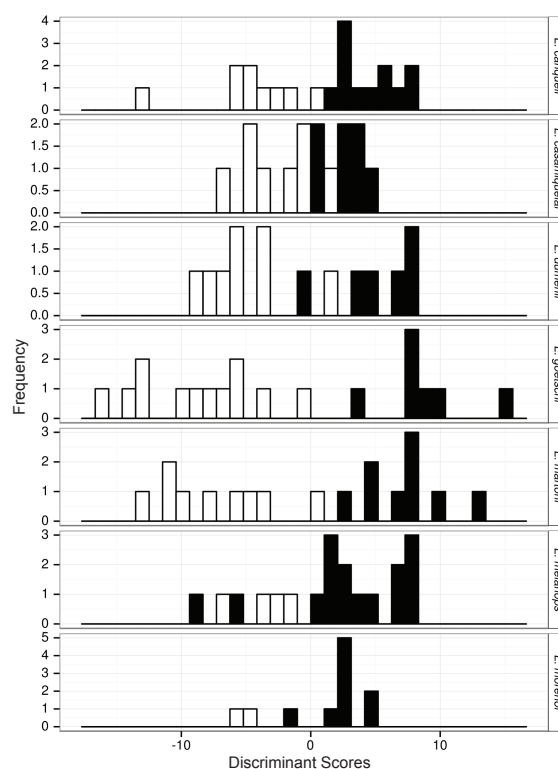
**Table 2.** Values of *t*-test of log centroid size from comparisons between sexes. Nor Test: Normality Test, StD: standard deviation, significant differences ( $p \leq 0.05$ ) in *italic*.

Species	Females			Males			Homogeneity of variance			Nor Test		<i>t</i> -test	
	mean	StD	<i>n</i>	mean	StD	<i>n</i>	F	df	<i>p</i>	<i>p</i>	df	<i>t</i>	<i>p</i>
<i>L. canqueli</i>	0.531	0.083	9	0.610	0.081	12	1.04	8	0.931	0.250	19	-2.20	0.041
<i>L. casamiquelai</i>	0.486	0.170	8	0.508	0.231	7	0.54	7	0.442	0.062	13	-0.22	0.833
<i>L. dumerili</i>	0.416	0.073	8	0.562	0.059	6	1.52	7	0.665	0.668	12	-4.00	0.002
<i>L. goetschi</i>	0.433	0.064	12	0.422	0.060	7	1.16	11	0.901	0.134	17	0.35	0.730
<i>L. martorii</i>	0.344	0.051	9	0.490	0.049	9	1.08	8	0.920	0.961	16	-6.25	<0.001
<i>L. melanops</i>	0.396	0.052	8	0.535	0.083	13	0.39	7	0.219	0.537	19	-4.25	<0.001
<i>L. morenoi</i>	0.471	0.047	4	0.610	0.066	9	0.50	3	0.617	0.957	11	-3.76	0.003

allometry test with a 4% threshold maximum to evaluate the potential for strong bias due correlation between size and shape (Zelditch et al., 2004; Claude, 2008).

Based on the refinement of the GM to detect subtle differences, we first assessed intraspecific variation between sexes before analysing interspecific differences. To evaluate sexual dimorphism (SD) within each species, we implemented a discriminant function analysis (DFA) and Student's *t*-tests with log-centroid size (LCS) values. DFA is useful in determining whether a set of variables is effective in predicting category membership (Harlow, 2005), particularly when only two groups are compared (Viscosi & Cardini, 2011), and we implemented a leave-one-out cross-validation using a permutation test with 1000 iterations. In order to validate *t*-tests assumptions, we implemented Fisher's F-test to evaluate the homogeneity of variances and the Shapiro-Wilks tests for the normality distribution. We averaged the shape by sex and species to represent the consensus configuration (see Kaliontzopoulou, 2011) to visualise differences between groups.

Once SD was validated for most of the species, we analysed differences among species considering males and females separately, and implemented a canonical variate analysis (CVA) and ANOVAs with LCS values. CVA is a method used to find shape features that maximise the separation between three or more groups (Viscosi & Cardini, 2011) by constructing variables to describe the relative positions of groups (or subsets of individuals) in the sample (Zelditch et al., 2004), and the grouping of specimens is assumed to be known *a priori*. If ANOVA *p* values were significant ( $p \leq 0.05$ ), we performed multiple *post hoc* comparisons using Tukey's honestly significant difference (HSD) tests for unequal sample sizes (Yandell, 1997; Miller & Haden, 2006). We checked for homoscedasticity and normality assumptions with Levene (Zar, 2010) and Shapiro-Wilks tests (Claude, 2008). We performed all statistical tests related to LCS



**Fig. 2.** Plot with the differences in shape from the Discriminant Function Analysis. References: black bars males, white bars females.

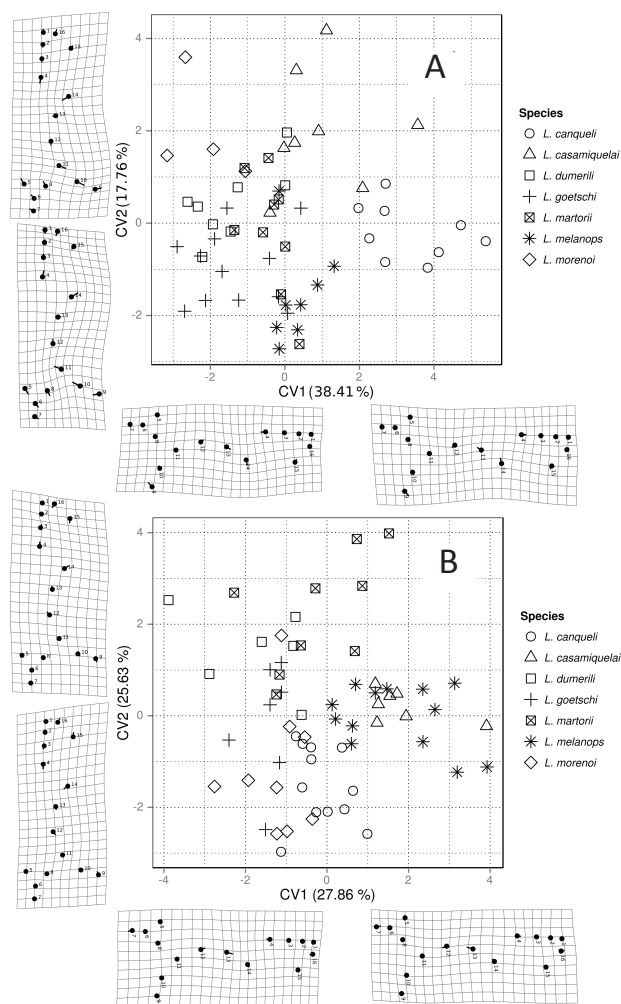
analyses with 'agricolae' (de Mendiburu, 2014) and 'multcompView' packages (Graves et al., 2012) in R v. 3.2.0 (R Core Team, 2015).

### Phylogenetic comparative methods

We analysed the correlation between head shape using an available molecular phylogeny based on 14 loci representing different parts of the genome (mtDNA, nuclear anonymous and protein-coding loci, Olave et

**Table 3.** Pairwise comparisons by the canonical variate analysis performed between the eight species. Results are reported as Mahalanobis distance and associated *p*-values (not significant values in *italic*), after 10000 permutation runs.

Females	<i>L. canqueli</i>	<i>L. casamiquelai</i>	<i>L. dumerili</i>	<i>L. goetschi</i>	<i>L. martorii</i>	<i>L. melanops</i>
<i>L. casamiquelai</i>	4.33 (<0.001)	--	--	--	--	--
<i>L. dumerili</i>	5.25 (<0.001)	4.11 (<0.001)	--	--	--	--
<i>L. goetschi</i>	5.10 (<0.001)	4.20 (<0.0001)	2.99 (0.063)	--	--	--
<i>L. martorii</i>	4.60 (<0.001)	4.10 (0.0001)	3.35 (0.003)	3.42 (<0.001)	--	--
<i>L. melanops</i>	4.43 (<0.001)	4.12 (<0.001)	4.00 (<0.001)	3.43 (<0.001)	3.58 (0.002)	--
<i>L. morenoi</i>	6.44 (<0.001)	5.17 (0.002)	4.24 (0.009)	4.51 (0.002)	4.56 (0.003)	5.30 (0.002)
Males	<i>L. canqueli</i>	<i>L. casamiquelai</i>	<i>L. dumerili</i>	<i>L. goetschi</i>	<i>L. martorii</i>	<i>L. melanops</i>
<i>L. casamiquelai</i>	3.66 (<0.001)	--	--	--	--	--
<i>L. dumerili</i>	4.15 (<0.001)	4.59 (<0.001)	--	--	--	--
<i>L. goetschi</i>	3.53 (<0.001)	4.32 (<0.001)	3.40 (0.057)	--	--	--
<i>L. martorii</i>	4.17 (<0.001)	3.57 (<0.001)	3.55 (0.011)	4.13 (0.0002)	--	--
<i>L. melanops</i>	3.37 (<0.001)	2.44 (0.440)	4.18 (<0.001)	3.79 (<0.001)	3.78 (<0.001)	--
<i>L. morenoi</i>	3.79 (<0.001)	4.04 (<0.001)	4.35 (0.002)	3.95 (<0.001)	4.33 (<0.001)	4.08 (<0.001)



**Fig. 3.** Plots of the two first Canonical Variate Analysis axis and deformation grids. A) females and B) males.

al., 2014). A strong phylogenetic signal often shows that closely related species tend to fall out closer in morphospace than more distantly related species (Klingenberg & Gidaszewski, 2010). The phylogenetic signal test implements a permutation approach to simulate the null hypothesis of the complete absence of phylogenetic structure, reassigning the phenotypic data randomly to the phylogeny's terminal nodes (Klingenberg & Gidaszewski, 2010). In order to do this, we performed a principal component analysis (PCA) in MorphoJ considering six of the seven investigated taxa: *L. canqueli*, *L. casamiquelai*, *L. goetschi*, *L. martorii*, *L. melanops*, *L. morenoi* (*L. dumerili* was not represented in the phylogeny published by Olave et al., 2014). We implemented three PCAs considering both sexes combined and analysing the sexes separately. We used the PC scores of each PCA with 10,000 permutations for the phylogenetic signal test. The null hypothesis of no phylogenetic signal is discarded if the permutation result is  $p \leq 0.05$  (calculated using unweighted squared-change parsimony).

## RESULTS

### Sexual dimorphism (SD)

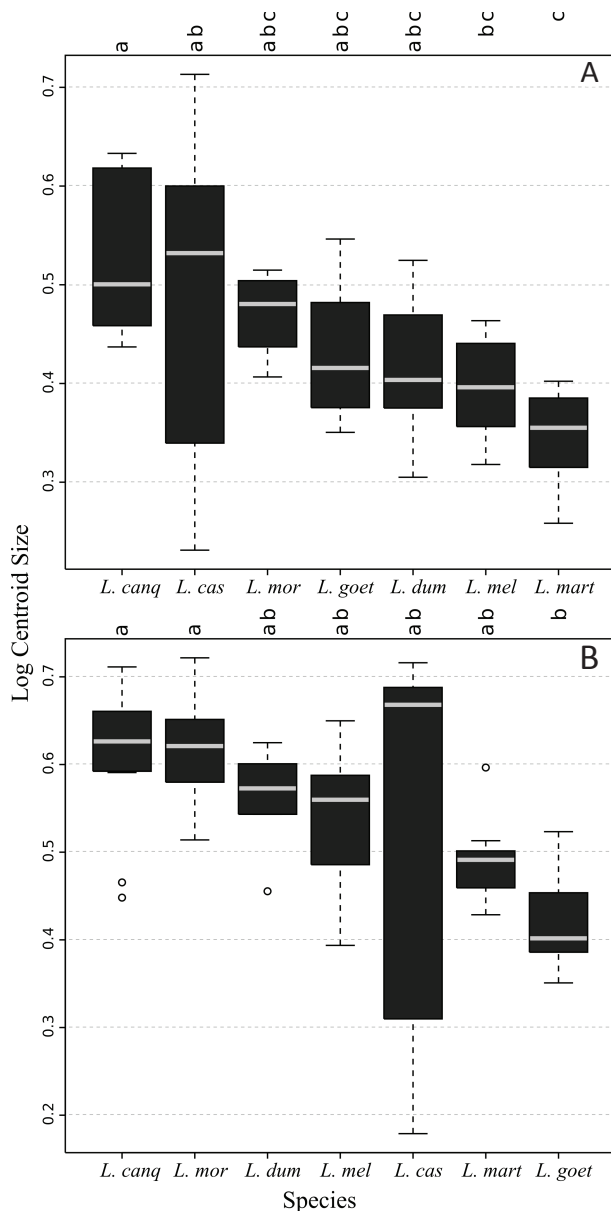
Intraspecific DFA on shape variables and *t*-tests of size based on the dorsal view of the head, showed that six (*L. canqueli*, *L. dumerili*, *L. goetschi*, *L. martorii*, *L. melanops* and *L. morenoi*) of the seven species significantly differed between sexes (Fig. 2, Tables 1 and 2). The DFA showed a moderate proportion of correctly assigned specimens for females (33.33–62.50%) and males (11.11–71.43%; Table 1). The best individual discrimination was achieved for *L. martorii* (33.33% of females and 22.22% of males misclassified), while the taxon with the highest error was *L. casamiquelai* (62.50%, for females and 71.43% for males, Table 1).

Intraspecific *t*-tests for LCS detected differences between sexes for five of the seven species (see Online Appendix). There was a significant difference in size between the sexes for *Liolaemus canqueli*, *L. dumerili*, *L. martorii*, *L. melanops* and *L. morenoi*. All species with sexual size dimorphism showed higher means in males than females (Table 2).

### Interspecific variation

The first two axes of the CVA on head shape described 56.17% and 53.49% of the total shape differences for females and males, respectively (Fig. 3). For females, the Mahalanobis distances from the CVA analysis showed significant morphological differences between all paired combinations for all species except *L. dumerili* versus *L. goetschi* (Table 3). The first two axes in females revealed a moderate discrimination for *L. canqueli*, *L. casamiquelai*, and a low discrimination for *L. dumerili*, *L. goetschi* and *L. martorii* (Fig. 3A). For *L. canqueli* and *L. goetschi*, the major differences in shape were described by the CV1 (Fig. 3A), which included the eye orbit (landmarks 11, 12, 13, 14) and nostril orientation (landmarks 15, 16). The scores for *L. melanops* and *L. casamiquelai* were clearly discriminated along the CV2 and included changes in snout shape and nostril position (landmarks 1, 2, 3, 15, 16). For males, the Mahalanobis distances from CVA analysis showed moderate morphological variability between the seven species, and no differences were found for *L. casamiquelai* versus *L. melanops*, and *L. dumerili* versus *L. goetschi* (Table 3). CVA results for males for the first two axes showed a clear discrimination for *L. canqueli* and a moderate discrimination for *L. morenoi* and *L. martorii* (Fig. 3B). The major differences in shape described by the CV1 (Fig. 3B) was the eye orbit (landmarks 9, 10) oriented close to the sagittal axis for *L. melanops*. The scores for *L. morenoi*, *L. canqueli* and *L. martorii* clustered separately along the CV2, which loads most heavily for changes in head shape, particularly in the post-rostral area (landmarks 1, 2, 3, 4; Fig. 3B). We calculated mean shape or consensus shape between species for both sexes separately to show a simple projection of interspecific differences (see Online Appendix).

The ANOVA for between-species size comparisons showed significant differences in females ( $F_{6,51}=4.35$ ;  $p=0.001$ ) and males ( $F_{6,56}=3.81$ ;  $p=0.003$ ). Multiple *post-hoc* comparisons among females (Fig. 4A) showed



**Fig. 4.** Differences of Log Centroid Size among species. Different letters shows significant differences from the post-hoc Tukey tests. A) females, B) males, *L. canq.*: *Liolaemus canqueli*, *L. cas.*: *L. casamiquelai*, *L. dum.*: *L. dumerili*, *L. goet.*: *L. goetschi*, *L. mart.*: *L. martorii*, *L. mel.*: *L. melanops*, *L. mor.*: *L. morenoi*.

differences for *L. martorii* vs. *L. canqueli* ( $p=0.001$ ), *L. martorii* vs. *L. casamiquelai* ( $p=0.024$ ) and *L. canqueli* vs. *L. melanops* ( $p=0.037$ ). For males (Fig. 4B), multiple comparisons showed differences for *L. goetschi* vs. *L. canqueli* ( $p=0.004$ ) and *L. goetschi* vs. *L. morenoi* ( $p=0.009$ ).

All three tests of phylogenetic signal with PCA scores indicated that head shape is not significantly correlated with phylogenetic relationships. The permutation tests in MorphoJ for all three data sets revealed no significant phylogenetic signal: A) 6-taxon data set, females: tree length=0.005,  $p=0.656$ ; B) 6-taxon data set, males: tree length=0.005,  $p=0.838$ ; and C) 6-taxon data set, both sexes: tree length=0.003,  $p=0.774$  (see Online Appendix).

## DISCUSSION

The study of shape and size is essential to analyse and quantify morphometric differences between closely related species. GM is an innovative approach that has not been previously applied for *Liolaemus* alpha taxonomy, and may prove useful to give further support to species designations based on traditional morphological diagnosis. Our multivariate study of head shape and size variation within and between species of the *L. melanops* complex showed some discrimination among taxa, with moderate differences in size and head shape detected by the independent contrasts. Because we did not detect a phylogenetic signal in the data, the observed character states are apparently not strongly influenced by shared descent. Moreover, considering size and head shape for both sexes, we found differences for five of the seven species. The interspecific differences were greater among females than males.

Based on our operational criteria for sexual dimorphism within species, *L. casamiquelai* showed no changes in shape or size, whereas three species had significant SD in both shape and size (*L. canqueli*, *L. martorii* and *L. morenoi*). Evidence of sexual dimorphism in lizard head shape or size, as quantified by GM, has been well documented for *Lacerta* (Bruner et al., 2005) and *Podarcis* (Kaliontzopoulou et al., 2007, 2008), but rarely for *Liolaemus* (Vidal et al., 2005; Fontanella et al., 2012). Consistent with these studies, we found that males always had larger heads than females. This trend implies that there may be a significant bias (by not discriminating males and females) in previous studies comparing related species (see, Kaliontzopoulou, 2011). The hypothesised explanations for SD in lizards are diverse, including ontogenetic aspects (Kaliontzopoulou et al., 2010), body size selection (Alcorn et al., 2013), sexual selection (Vidal Maldonado, 2011), and prey selection (e.g., *Liolaemus*, Vanhooydonck et al., 2010; Azócar & Acosta, 2011). The previous studies of GM in *Liolaemus* have shown that variation in head size and shape might result from adaptations related to sexual dimorphism (Vidal et al., 2005) or mating systems (Vidal Maldonado, 2011). Detection of SD (and any other intraspecific variation) can be challenging when making methodological decisions for studies of closely related species (see Viscosi & Cardini, 2011).

As with any other class of data, translating empirical GM data into taxonomic decisions should be based on explicit *a priori* threshold values as an operational criterion (Zelditch et al., 2004). The thresholds defined herein were statistically significant differences in the contrasts between species' head shapes through the ACV's Mahalanobis distances, and in head sizes as assessed by ANOVA and Tukey tests (after these comparisons had accommodated intraspecific between-sex differences). The major differences between species of the *L. melanops* complex reside in the shape of the head, which has been previously linked in other groups of lizards with differences in microhabitat use (e.g., Openshaw & Keogh, 2014). The overall results for these multiple comparisons resolved significant differences

among all taxa except for *L. dumerili* versus *L. goetschi*. These species were indiscernible in head shape and size, which in the absence of other evidence suggests that these taxa might be conspecifics. This is congruent with phylogenetic studies that included molecular data of *L. dumerili* and related species that do not show clear differences between individuals from type localities (see Avila et al., 2006; Abdala, 2007).

Given their common evolutionary and developmental history (Klingenberg & Gidaszewski, 2010; Urošević et al., 2012), we expected a phylogenetic signal in some phenotypic traits among species in a monophyletic group, although certain morphological differences can be attributed to adaptation to different ecological niches (e.g., *Anolis* sp.; Losos, 2010). Several studies have demonstrated the importance of selection as main factors influencing the phenotypic evolution of body size (Pincheira-Donoso, et al. 2008a) and head size as well as shape in lizards (Stayton, 2005). The lack of a phylogenetic signal on head shape suggests that evolutionary history does not limit or affect the variation in this character, or that the adaptations to current environmental factors override phylogenetic effects (Klingenberg, 2010; Klingenberg & Gidaszewski, 2010). Our findings are consistent with studies on other lizard groups (Vanhooydonck & Van Damme, 1999; Kohlsdorf et al., 2008).

GM has been widely used because of its mathematical robustness to test biological hypotheses related to shape-size variation, but it has been questioned on design and methodological issues. The design of the landmark configurations should be informative when evaluating differences; they must represent clearly reproducible homologous structures to avoid biased results and misinterpretations (Zelditch et al., 2004). Other studies have shown that the number of landmarks used in relation to the number of individuals may also bias results and interpretations (Cardini & Elton, 2007). Some authors favour the use of PCAs to test differences between individuals (Bruner et al., 2005; Alcorn et al., 2013), and software has been developed for which PCA is the only multivariate analysis (e.g., Adams & Otárola-Castillo, 2013). Alternatively, other authors use CVA to differentiate groups or species (Kaliontzopoulou et al., 2007; Ivanović et al., 2008; Moreno-Barajas et al., 2013). Here we implemented the CVA because it maximises the differences between groups defined *a priori* (Elewa, 2010), and it can be used if the specific identity of each individual is determined with certainty (Zelditch et al., 2004; Claude, 2008; Viscosi & Cardini, 2011). We define “certainty” as the current taxonomy of the *L. melanops* complex. Because we used samples collected from near each type locality for each taxon, all of which are allopatric, we considered the CVA as the best approach. Several studies show that the greatest biases in GM are due to intragroup sexual dimorphism, ontogeny, and geographic variation (Zelditch et al., 2004; Viscosi & Cardini, 2011), all of which were taken into account in our analyses.

The main theoretical criticisms of the use of GM in a phylogenetic context are related to the use of

shape variables combined with methods based on cladistic parsimony (Adams et al., 2013). This view argues that the main methodological difficulty relies on the transformation of continuous data into discrete multivariate states for analysis as parsimony characters (Monteiro, 2000). Alternatively, others validate their implementation to test the phylogenetic signal among species within a clade (Catalano et al., 2010; Klingenberg & Gidaszewski, 2010; González-José et al., 2011). Despite these different views, GM analyses in association with phylogenetic comparative methods are valid only if there is a detectable phylogenetic signal when comparing the shape of the morphological structure (via configuration of homologous landmarks) among taxa. The future challenge lies in integrating these two methods into a unified application for taxonomy and systematics.

The use of GM to detect differences among species is considered as one of the most promising approaches for morphological studies (Schlick-Steiner et al., 2010). It allows the analysis of size and shape independently while simultaneously enabling standardisation of variables, analyses and methods. In the present study we found the GM approach useful to investigate species delimitations. While caution is required, GM has been used successfully to assess shape differences in a cryptic species complex (Leaché et al., 2009), to show morphological differences between genetically defined lineages (Chiari & Claude, 2011), and to evaluate diagnostic taxonomic characters (e.g., Family Bataguridae, Jamniczky & Russell, 2004). The present study confirms that GM can be used to provide additional evidence to support or reject taxonomic hypotheses (e.g., Piras et al., 2010), along with other approaches such as molecular (Clemente-Carvalho et al. 2011; Florio et al. 2012) or linear morphometric (Kaliontzopoulou et al. 2007) methods. We suggest that the use of GM in integrative taxonomy (Schlick-Steiner et al., 2010; Kaliontzopoulou, 2011) is to incorporate these analyses into species hypotheses. In spite of being able to find differences between compared taxa, GM does not allow the incorporation of its results into a dichotomous key or diagnostic information that can be stored in museums, a problem which can be overcome by recording linear measurements between two landmarks that diagnose two taxa (Zelditch et al., 2012). Taking the necessary methodological precautions and when used together with other approaches and data sets, GM is a valuable tool for testing hypotheses related to *Liolaemus* taxonomy.

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