

# Evidence of adaptive evolution in the cranial morphology of Tropicidurid lizards from coastal Peru

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**Abstract.** Head morphology is known for being a good predictor of diet characteristics in lizards. Head height and width are considered to be the most important head dimensions being related to a higher bite force, which allows certain lizards to consume different prey items. In this study head dimensions are compared between four species of the South American lava lizard genus *Microlophus*, which were paired considering their main diet (herbivorous and insectivorous). Head height, width and length were measured for each species and compared between them independently of body size. It was found that head height and width were strongly related with the diet of each species, as the ones with a semi-herbivore diet showed bigger values for these traits. Additionally, the head was longer in insectivorous species, which is a result not reported before. The different morphologies of the study species support a possible case of adaptive evolution, since both types of diet and head morphology arose from different clades in the *Microlophus* phylogeny. Consequently, the results of this study give evidence of a strong link between species and their constraints on specific diets. Further ecomorphological studies are needed to enrich the knowledge about this subject which is relatively understudied in South American desert lizards.

**Keywords:** Ecomorphology; diet; herbivory; lizards; desert; South America

## Introduction

Adaptive evolution allows organisms to take advantage of the environment that surrounds them through the development of traits which will increase their fitness. In this context, morphological traits are among the most useful characteristics to be studied because of their direct relation with ecology and performance (Pianka, 1973; Aerts et al., 2000). Several studies have linked the morphology of different species to distinct ecological characteristics, considering this relationship a product of natural selection (Collette, 1961; Carrascal et al., 1990; Winemiller, 1991; Aguirre et al., 2002). It is thus clear that ecomorphology is a relevant field in evolutionary biology. Distinct groups of species have been subject of this kind of studies but some of them are more useful as study systems because of their ease to show us different

evolutionary processes. Lizards are known to be a really good model system for several topics in evolutionary ecology because of their relatively fast evolution, specifically in terms of functional traits (Losos et al., Vitt et al., 1997; Losos et al., 1999; Herrel et al., 2008). Through their study, ecology and performance have been successfully related with morphological traits in past studies (Losos, 1990; Vitt et al, 1997; Vanhooydonck et al., 2000; Perry, 2007; among others), most of them showing repeated patterns that allow us to predict reciprocally the interactions between them.

Diet is one of the most relevant characteristics of the ecology of a lizard (as well as any other living being), and it is related with bite force as a measure of performance (Herrel, 2001a, b; McBrayer, 2004). Theoretically, bite force allows (or does not) a lizard to consume certain dietary items depending on their hardness, so that harder items need higher bite forces to be processed (Herrel et al., 2001a, b; Verwajen et al., 2002; Herrel et al., 2007; Huyghe et al., 2009). Hard dietary items include insect orders like Coleoptera and also plants (this last item needing a longer and more exhaustive digestion process, starting with a proper



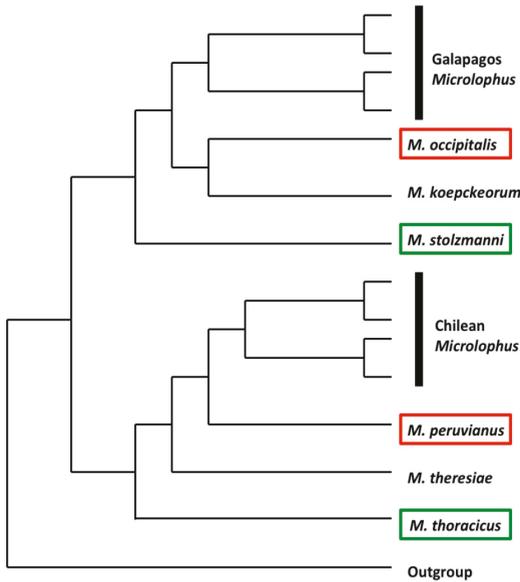
**Figure 1.** *Microlophus peruvianus* (A) and *Microlophus thoracicus* (B) are closely related species found in the same evolutionary clade, but their diets are very different, being *M. peruvianus* mostly an insectivore and *M. thoracicus* a semi-herbivore. Photographs by Ken Toyama.

management in the jaws) (Miles et al., 2007), while soft items would include dipterans, fleas, spiders, etc. In order to produce a certain bite force, lizards should present certain cranial characteristics, specifically related with simple head dimensions like head width and head height, among others (Herrel, 2001a, b; Lailvaux, 2004). It is generally accepted that higher and wider heads will produce stronger bite forces, so a lizard that consumes hard items should have a bigger and more robust head. As several studies support, the mentioned patterns repeat themselves all over the world (Miles et al., 2007; McBrayer and Corbin, 2007), but this particular topic has no representative studies developed in South America.

In this study a group of four species of lizards from the genus *Microlophus* is tested for their cranial morphology characteristics, being these traits expected to be related to the dietary ecology of the species. The *Microlophus* genus is spread in the South American western coast as well as on the Galapagos Islands, where they have radiated (Dixon and Wright, 1975; Wright, 1984; Benavides et al., 2008). Some data is published about the ecology of various species of this group (Dixon and Wright, 1975; Pérez and Jahnce, 1998; Catenazzi et al., 2005; Pérez and Balta, 2007; Guzmán, 2009; Quispitúpac and Pérez, 2009; Jordán and Pérez, 2012; Pérez et al., 2015), but morphological differences are poorly studied in the context of adaptive evolution (as taxonomical keys that use some morphological characteristics, with no apparent evolutionary relevance, exist in the literature). The species of this study (*M. occipitalis* (Peters, 1871), *M. peruvianus* (Lesson, 1830), *M. stolzmanni* (Steindachner, 1891) and *M. thoracicus* (Tschudi, 1845) can be paired considering their diet (i.e herbivorous or insectivorous). As *M. occipitalis* and *M. peruvianus* are insectivorous, predated mainly on soft insects, (Dixon and Wright, 1975; Pérez and Jahnce, 1998; Pérez and Balta, 2007) and *M. stolzmanni* and *M. thoracicus* are semi-herbivorous, showing a high consumption of vegetal items (Pérez et al, 2015; J. Pérez, pers. comm.) it is expected that these pairs show significant differences in their head dimensions. Interestingly, if we analyse the phylogeny of the *Microlophus* genus we should pair these four species in a different manner according to their evolutionary proximity. *M. peruvianus* and *M. thoracicus* (Figure 1A,B) are closely related species belonging to the “peruvianus” group while *M. occipitalis* and *M. stolzmanni* both lie in a different clade named the “occipitalis” group. (Dixon and Wright, 1975; Benavides et al., 2007; Figure 2). As phylogenetic relationships could mask the obtained results, this system allows us to examine if an expected pattern in morphology could probably be related to phylogeny or constitute evidence of adaptive evolution.

## Materials and Methods

Specimens of the four species were obtained from the collection of the Laboratorio de Estudios en Biodiversidad (LEB) – Universidad Peruana Cayetano Heredia. In total, 242 individuals were examined: Eighty-eight *M. thoracicus* (55 adults, 26 males and 29 females, and 33 juveniles), 49 *M. peruvianus* (34 adults, 21 males and 13 females, and 15 juveniles), 77 *M. occipitalis* (36 adults, 19 males and 17 females, and



**Figure 2.** Illustrative phylogeny of the *Microlophus* genus (not all species are shown). The four species of the study are indicated by colored squares, being insectivore species marked in red and semi-herbivore ones in green. Figure adapted from Benavides et al. 2007.

41 juveniles) and 28 *M. stolzmanni* (20 adults, 12 males and 8 females, and 8 juveniles). All the individuals were collected in different localities of coastal Peru.

### Morphometrics

Three principal dimensions were considered in this study aside snout-vent length (SVL): head length, head height and head width. The highest part of the head (considering a transversal central line) was considered as the head height and the widest part of the head posterior to the eyes, as the head width. The head length was measured from the anterior tip of the snout to the line formed by jaw junctions. Measurements were taken with a digital caliper (Mitutoyo, 0,01 mm precision).

### Analysis

Regressions were performed for every head dimension against snout-vent length for comparisons of the three dimensions among the species. A residual analysis was performed with the obtained results to avoid the influence of different SVL values within and between

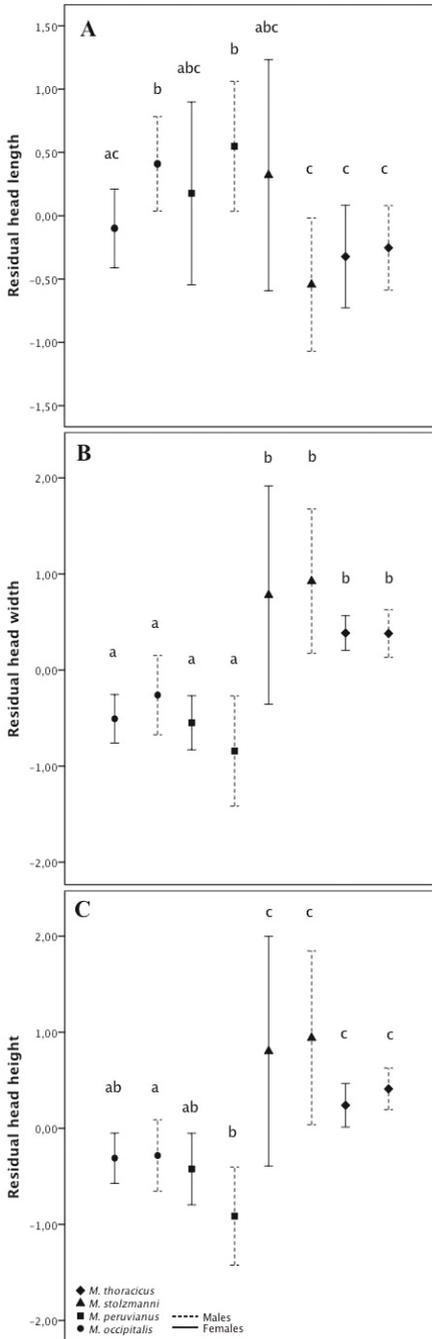
species. Before comparisons, the normality of residuals was tested (Shapiro-Wilk test) and depending on the result parametric or non-parametric tests were used (t-test or Mann-Whitney test respectively). SPSS Statistics 22 and Microsoft Excel 2010 were used for data analysis and management. A significance level of  $\alpha = 0.05$  was used for all the statistical tests performed.

### Results

Adults from the four species considered in this study showed different mean SVL values (ANOVA,  $p < 0.001$ ). *M. thoracicus* showed a mean SVL of  $69.05 \pm 8.61$  mm; *M. peruvianus*,  $82.93 \pm 12.46$  mm; *M. occipitalis*,  $52.00 \pm 6.19$  mm and *M. stolzmanni*,  $97.49 \pm 14.50$  mm. Now considering all the specimens, all the head dimensions were positively correlated with the SVL (Table 1). Regressions for every head dimension against SVL were performed with all the species and residuals were compared between them.

Within all the species there were no differences between juveniles and adults for all the head dimensions' residuals (all  $p > 0.05$ ). In respect to the head length, male *M. occipitalis* showed longer heads compared to females (t-test,  $t = -2.186$ ,  $p = 0.036$ ). Female *M. stolzmanni* showed longer heads when compared to males, with a difference near to significance (t-test,  $t = 2.018$ ,  $p = 0.06$ ) (Figure 3A). No other intraspecific differences between sexes were found for the other head dimensions (all  $p > 0.05$ ).

Only adults were considered for comparisons among species. The following differences were found for head length residuals: males of *M. stolzmanni* and both sexes in *M. thoracicus* showed significantly shorter heads when compared to males of *M. peruvianus* and *M. occipitalis* (all  $p < 0.02$ ). Females of *M. occipitalis* also showed significantly shorter heads when compared to males of *M. peruvianus* (Figure 3A). All other comparisons did not show significant differences. For head width residuals there were no interspecific differences between *M. occipitalis* and *M. peruvianus* comparing between and among sexes (all  $p > 0.05$ ). Also no significant differences were shown between *M. stolzmanni* and *M. thoracicus* comparing between and among sexes (all  $p > 0.05$ ). Both sexes of *M. occipitalis* and *M. peruvianus* showed significant differences when compared to any of the sex categories of both *M. stolzmanni* and *M. thoracicus* (all  $p < 0.03$  for comparisons with *M. stolzmanni* and all  $p < 0.02$  for the ones with *M. thoracicus*) (Figure 3B). For head height residuals a similar pattern was observed, there were no



**Figure 3.** Comparison between residuals of head length (A), width (B) and height (C) of each species-sex groups with error bars showed. Different lower case letters stand for significantly different means ( $p < 0.05$ ). Legend for species; circles: *Microlophus occipitalis*, squares: *M. peruvianus*, triangles: *M. stolzmanni* and rhombus: *M. thoracicus*. Continuous lines in the design of the error bars correspond to females and dotted lines for males.

interspecific differences between *M. occipitalis* and *M. peruvianus* comparing between and among sexes (all  $p > 0.05$ ), except the marginally significant head height residuals of *M. occipitalis* males when compared to *M. peruvianus* males ( $p = 0.05$ ) (Figure 3C). No significant differences were shown between *M. stolzmanni* and *M. thoracicus* comparing between and among sexes (all  $p > 0.05$ ). Both sexes of *M. occipitalis* and *M. peruvianus* showed significant differences when compared to any of the sex categories of both *M. stolzmanni* and *M. thoracicus* (all  $p < 0.02$  for comparisons with *M. stolzmanni* and all  $p < 0.05$  for the ones with *M. thoracicus*) (Figure 3C).

## Discussion

The patterns found in the comparisons were consistent with previous studies performed in other species (Herrel et al., 2001a, b; Verwajen et al., 2002; Herrel et al., 2007; Huyghe et al., 2009). The semi-herbivores *M. thoracicus* and *M. stolzmanni* showed significantly higher and wider heads in respect to the insectivore pair, *M. peruvianus* and *M. occipitalis*. There is some evidence that the diet and morphology of *M. thoracicus* is subjected to an ontogenic change, switching from an insectivore diet, in the case of juveniles, to a herbivore one in the adulthood (pers. observation). However, new evidence of *M. thoracicus* consuming vegetal items throughout its life has been found (Pérez et al., 2015), making this assumption questionable. The fact that no differences were found between juveniles and adults in any head dimension supports the latter evidence.

Head length behaved in an opposite way to head width and height in the sense that it presented higher values for insectivore species (Figure 3A). However, *M. stolzmanni* females showed high values for this trait and were not significantly different when compared to the insectivore species. Nonetheless a great uncertainty for this and the other traits in *M. stolzmanni* females does not allow us to appreciate a clear pattern in this particular case.

The only intraspecific difference was observed in one of the insectivore species. *M. occipitalis* males showed longer heads in comparison to females (Figure 3A), while no differences were apparent for the other traits. Head length is not considered to be an important trait when differentiating species of different diets (Herrel et al., 2001a, b; 2007) but might have a role in habitat use, prey search and competition. Focusing in the particular case of *M. occipitalis* it is tempting to explain the difference between sexes through the influence that

**Table 1.** Allometries of head dimensions (mm) versus SVL (mm).

Variable	Slope	Intercept	R <sup>2</sup>
<i>Microlophus occipitalis</i>			
Head length	0.18	3.60	0.78
Head width	0.13	1.97	0.84
Head height	0.09	1.35	0.72
<i>Microlophus peruvianus</i>			
Head length	0.22	-0.23	0.86
Head width	0.14	1.91	0.90
Head height	0.11	0.82	0.84
<i>Microlophus stolzmanni</i>			
Head length	0.15	4.53	0.97
Head width	0.16	1.38	0.95
Head height	0.14	-1.06	0.98
<i>Microlophus thoracicus</i>			
Head length	0.16	3.84	0.84
Head width	0.15	1.85	0.47
Head height	0.12	0.16	0.94

male-male competition might have in this species and the fact that head length has been found to be highly influential in these type of interactions (Herrel et al., 2001a, Lailvaux 2004). *M. occipitalis* males also showed higher heads when compared to *M. peruvianus* males (Figure 3C). It is interesting to notice that *M. peruvianus* males presented low values for head height and width, but the opposite for head length. Although in most of the cases these differences were not significant it is intriguing to observe that the means of these males were lower than the other insectivores. *M. peruvianus* is an opportunist and generalist lizard, conditions that allow this species to consume a large range of item types (Dixon & Wright, 1975; Pérez & Jahncke, 1998; Pérez & Balta, 2007; Guzmán, 2009). This wider range of utilizable resources might reduce the frequency of antagonistic interactions with conspecifics, reducing the need of traits beneficial for fighting, like higher heads (Herrel et al., 2001). Although a long head has been also identified to be an important trait for fighting (Herrel et al., 2001, Lailvaux 2004) it might also play an important role in other ecological functions like habitat use or foraging mode (Huey & Pianka, 1981; Kohlsdorf et al., 2008), which could be more relevant for this particular species.

The four species can be paired by their similar diets (and now by their morphologies) but if we consider their evolutionary history we could pair them differently. *M. peruvianus* and *M. thoracicus* are species that belong to the “peruvianus group” when looking into the *Microlophus* phylogeny. The same occurs with *M. occipitalis* and *M. stolzmanni*, both species belonging to the “occipitalis group” (Kizirian et al., 2004; Benavides et al., 2007) (Figure 2). We can see that a semi-herbivore diet arose in different branches in the *Microlophus* genus as reported previously for tropicidurids (Kohlsdorf et al., 2008) and we could also, with these results, conclude that an optimizing morphology also appeared in a convergent way (Figure 2). However, is important to consider that head shape has been found to be related not only to diet but also to other ecologically relevant interactions, like competition and habitat use (Lailvaux 2004, Kohlsdorf et al., 2008). To confer the morphological patterns observed in this study exclusively to diet it will be necessary to obtain complete information regarding the basic ecology of this group of lizards. Finally, these results also support the reported flexible evolutionary adaptability of tropicidurids (Kohlsdorf et al., 2001, 2004, 2008; Kohlsdorf and Navas, 2007) and represent the first ecomorphological study developed in this region. More studies, however, are needed since other patterns, similar and even new, might still be hidden in this poorly studied group of desert lizards.

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