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Morphometry of *Anolis tropidonotus* (Peters 1863, Squamata: Dactyloidae) populations from contrasting habitats

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ABSTRACT

Objective: To evaluate variations in shape and size of two populations of *A. tropidonotus* from two habitats with contrasting vegetation and environmental characteristics.
Design/Methodology/Approach: Twenty-six *A. tropidonotus* specimens were collected and photographed using the TpsDig2 software. Subsequently, three type-I and eight type-II landmarks and nine semi-landmarks were placed. The landmark configuration patterns were evaluated using generalized procrustean and principal component analyses to detect microvariations in individuals of both populations.
Results: The results show intra- and inter-population geometric morphometric variations in *A. tropidonotus*.
Study Limitations/Implications: The geometric morphometric variations recorded in *A. tropidonotus* populations may be caused by biological barriers, clinal variations within the same habitat, competition for territory, different reproductive behaviors in part of the population, and reproductive and physical barriers that generate differentiation between and within *A. tropidonotus* populations.
Findings/Conclusions: The morphometric traits of *A. tropidonotus* showed a wide diversity of shapes. Geometric morphometry was used to differentiate various ecotypes in the evaluated populations.

Keywords: Lacertilidae, homologous landmarks, landmarks, TpsDig, principal component analysis.

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INTRODUCTION

Geometric morphometry is a biostatistical technique commonly used to distinguish, classify, and group individuals from a given population based on specific traits (Zelditch *et al.*, 2012).This technique has been used to study size and shape variations between individuals of one or more populations and even between species of the Squamata order (Meik *et al.*, 2020). The observed morphometric traits can be correlated with the environmental characteristics of the habitat to identify morphological adaptations to the ecosystem (Adams *et al.*, 2004).

Image by Jared Caraway at Pixabay

Within the Squamata order (lizards), the Dactyloidae family is a biologically diverse group in constant evolution. Its species serve as biological models for the development of geometric morphometry studies (Tinius and Russell, 2014; Manthey *et al.*, 2016). Specifically, the *Anolis* genus comprises more than 400 species, some of which inhabit islands and others the landmass of the American continent, from the United States to Brazil (Velasco *et al.*, 2016). The species of the *Anolis* genus are distributed from sea level to 2,000 m.a.s.l., and they can be found in most mainland and island ecosystems, which shows their capacity to adapt to the environmental conditions of their habitats (Stroud and Losos, 2020).

Fifty-five *Anolis* species have been recorded in Mexico. They have adaptive flexibility to tropical environments; their habitat is associated with shrub and tree vegetation close to bodies of water; they can be diurnal or nocturnal, feed on invertebrates, and their morphological trait changes as a result of selection and evolution pressures (Köhler, 2008; Flores and García, 2014; Köhler *et al.*, 2014).

No studies have been conducted about the expected changes in the shape and size of individuals of the *A. tropidonotus* species —commonly known as abaniquillo escamoso mayor or greater-scaled anole, which is native to Mexico, Belize, Guatemala, El Salvador, Honduras, and Nicaragua (Peters, 1863)—, according to the habitat where their populations develop. Likewise, no studies have taken geometric morphometry as the basis for detecting microvariations associated with the type of habitat of this species (Williams, 1983). This study describes variations in the geometric morphometric traits of individuals from two populations of *A. tropidonotus*: one from the tropical evergreen forest and the other from the tropical deciduous forest in central Veracruz, Mexico.

MATERIALS AND METHODS

Study area

The study was carried out in two locations in the central area of Veracruz, Mexico. The first location is the Fortín de las Flores ravine, Veracruz (18.892528, and 97.012), located at an altitude of 892 to 1,010 m.a.s.l., which has a warm temperate climate and evergreen tropical forest vegetation. The second location is Cerro de Lourdes, municipality of Amatlán de los Reyes, Veracruz (18.846811, −96.887268), located at an altitude of 640 to 840 m.a.s.l., with a mainly warm-humid climate and the presence of deciduous tropical forest (INEGI, 2009; Rivera, 2015).

Specimen collection

Between October and November 2019, five collection trips were conducted in each location, from 8 a.m. to noon, through transects, trails, and paths, searching for *A. tropidonotus* specimens. The lizards were caught with direct and manual techniques using elastic bands. The specimens were sacrificed by freezing or alcohol puncture and preserved in 90% ethanol in bottles labeled with information on the collection sites. Subsequently, the specimens were taken to the laboratory, where they were photographed and subjected to a geometric morphometry analysis. The specimen collection was authorized by SEMARNAT (Scientific Collection License no. SGPA/DGVS/001894/18).

Photo session of collected specimens

A millimeter paper was used to indicate where to position most of the animals' bodies. First, the organisms were placed on the millimeter paper and then the area was demarcated based on three parameters, to avoid positioning the samples in different places: 1) the edge of the muzzle; 2) the sides; and 3) the product of the division of the lateral perimeter of the animal (Figure 1a). The photographs were taken with a Canon Rebel T6 camera using an 18-mm lens. An angle limit was established to ensure that all the photographs covered the same space (Figure 1b). The specimens were placed in a standard posture to avoid bias during the extraction of the pure form (Figure 1c).

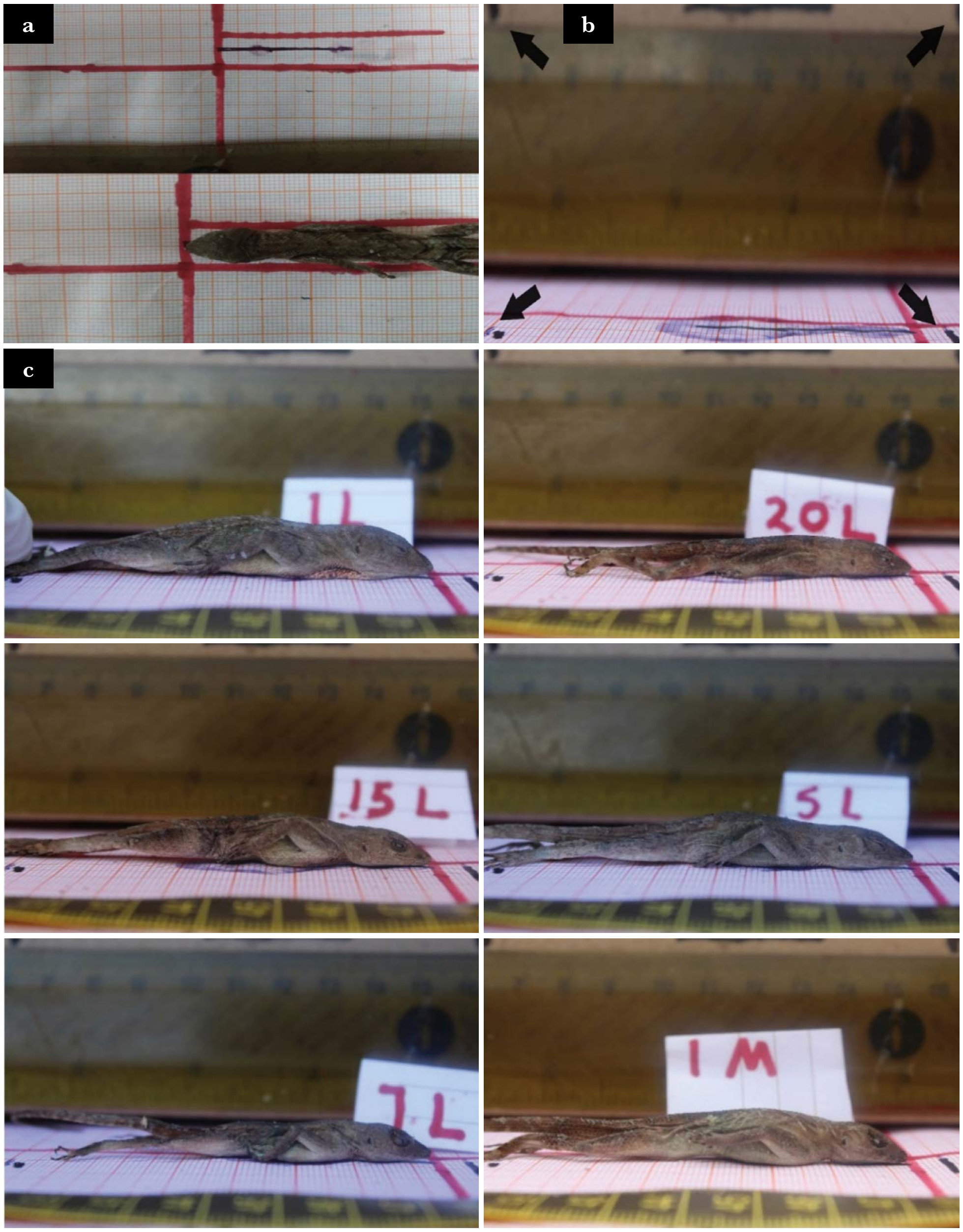

Figure 1. Stages of the photographic session of the *A. tropidonotus* specimens.

Landmark placement

All the photographs were digitized with the TpsDdig2 software (Rohlf, 2013). Landmarks were placed on the sides of the lizards' heads and semi-landmarks were set around the body outline (Vidal *et al.* 2006). Landmarks 7, 18 and 19 (red circles, Figure 2a) were placed at the intersection of the legs and the body; since they indicate the intersection of tissues, these are type-I landmarks. Eight type-II landmarks (white circles, Figure 2a) were positioned in local geometric structures: the maximum tip and the middle of the muzzle (landmarks 1, 2 and 13); the anterior and posterior ends of the eye and ear outline (landmarks 14, 15, 16 and 17); and the maximum point of the skull (landmark 10).

Lastly, semi-landmarks 3, 4, 5, 6, 7, 8, 9, 11 and 12 (yellow circles in Figure 2a) were placed around the outline of the animals' bodies, mainly in regions with no anatomical structures that could define type-I landmarks. Upon completion of landmark placement, the "Draw background curves" tool was used to connect the landmarks and estimate the consensus form of all samples, before starting the geometric morphometry analyses (Figure 2b).

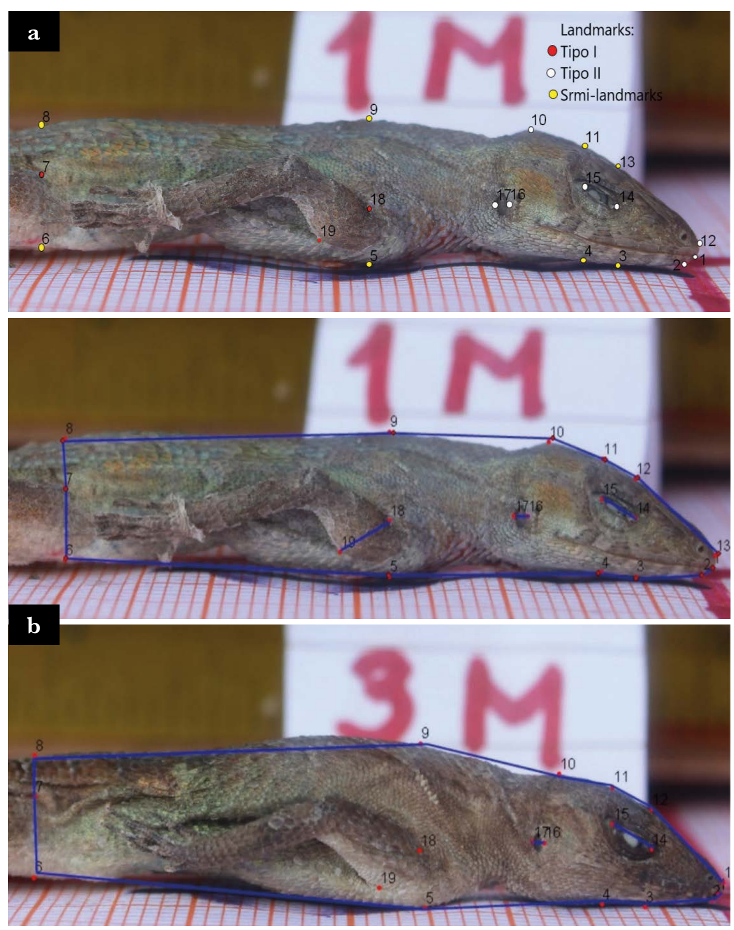

Figure 2. a) Placement of type-I and type-II landmarks and semi-landmarks in *A. tropidonotus* specimens; and b) estimation of the consensus form using the "Draw background curves" tool.

Geometric morphometry analyses

After the multivariate normal distribution of the data set was proven (comparing the expected theoretical distance distribution), the extreme or outlier values recorded were found to be below the expected value. Subsequently, the generalized procrustean analysis (GPA) was applied to create a "consensus" configuration representing the average of all the landmark configurations of the set of individuals. Morphometric analyses evaluate development pathways and structural design as a source of evolutionary diversification. Males and females were taken into consideration, since morphological variation, including sexual dimorphism (Benítez and Püschel 2014), is the focus of this study. Finally, the principal component analysis (PCA) was applied to explore form variation patterns between specimens. The MorphJ software was used to conduct these analyses (Klingenberg, 2011).

RESULTS AND DISCUSSION

Twenty-six specimens of *A. tropidonotus* were collected. Seven specimens (four ♂ and three ♀) inhabited tropical evergreen forests with a temperate climate (Fortín de las Flores ravine) and 19 (14 ♂ and five ♀) inhabited tropical deciduous forests with a tropical climate (Cerro de Lourdes).

The PCA produced 26 PC out of the 26 *A. tropidonotus* specimens, concentrating 34% of the variance. The lollipop graph showed that variation lies in landmarks 1 to 4 and 10 to 13, comprised of type-II landmarks and semi-landmarks (3-4 and 11-12), which are located on the head and muzzle. This set of landmarks and semi-landmarks showed variations in the shape of the head, the tympanic opening, and their relation to eye position (Figure 3). Out of the 26 deformation grids, the PC 2 landmarks expressed the full variance, particularly regarding the relationship between landmarks 1-4 and 10-13 (Figure 3).

The results obtained in this study showed intra- and inter-population variation resulting from sexual dimorphism. Two *A. tropidonotus* specimens of the same sex taken randomly from the same or different localities could have few morphological similarities. This variation could be due to environmental impact, low sample size, and sexual variation (Lafontaine *et al.*, 2018), as well as to the gradual change of phenotypic traits associated with geographic distance and microenvironmental or clinal variation (Futuyma, 1998).

Structural microvariations were identified in the head and body outline and at the intersection of the leg and body tissues of both *A. tropidonotus* populations. These microvariations may be the result of the wide morphological variability and the distinctive phenotypic characteristics at the individual level, which are part of the life history that each individual has experienced in its microhabitat (Losos, 1994; Losos and Thorpe 2004). For example, larger *A. tropidonotus* specimens were caught in areas with disturbed vegetation where a larger presence and diversity of insects was observed. These feeding conditions may provide an advantage regarding food resources and show their potential morphological development. In contrast, the morphological development of some relatively small specimens caught in areas with a higher state of plant conservation and with a lower presence of insects could have been limited by atypical and unfavorable weather conditions (Losos, 2009).

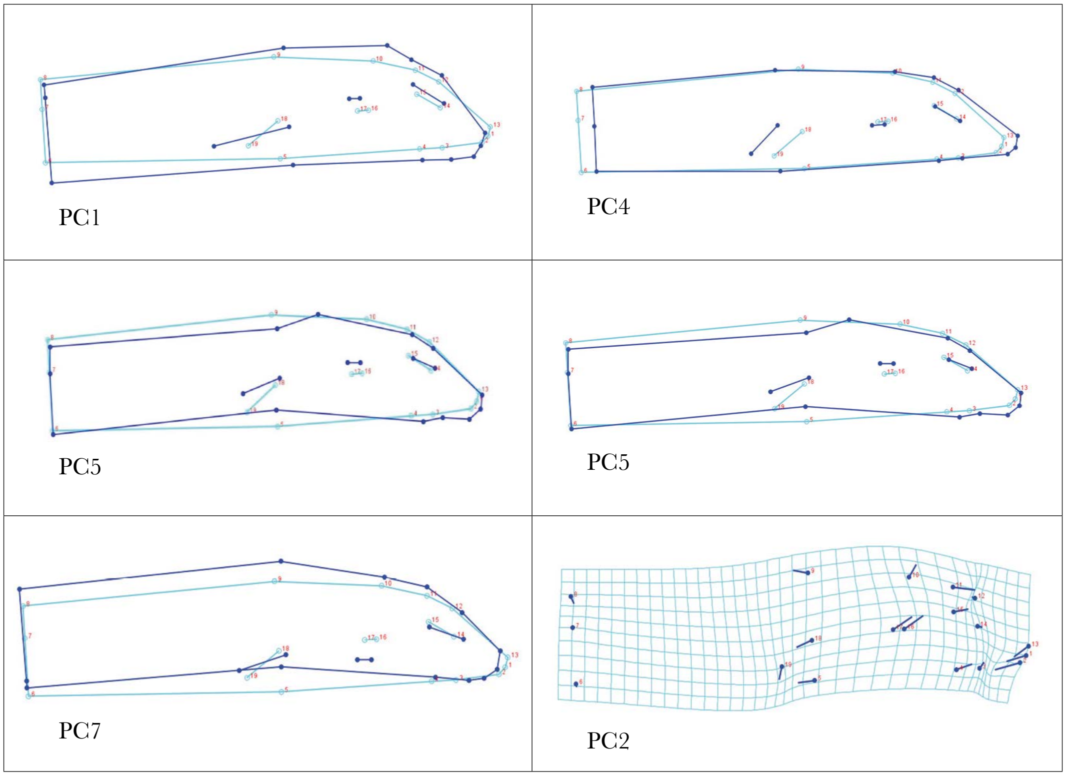

Figure 3. Lollipop graph comparing the principal components (PC) with shape variations in specimens of two populations of *A. tropidonotus* from contrasting environments, and deformation grid with PC2 showing the variation.

The study locations —in the central area of Veracruz, Mexico, where *A. tropidonotus* populations can be found— show variations in altitude, temperature, precipitation, humidity, soil types, vegetation, and food resources, which condition the differential development of *A. tropidonotus* individuals (Quatrini, 2001). This environmental variation in the geographic space inhabited by the *A. tropidonotus* populations impacts ecological processes and intra- and inter-population biogeographic patterns (Ghalambor *et al*., 2006; Prates *et al*., 2018). Therefore, the morphological variation observed may be associated with the selective and adaptive pressures resulting from the environmental, physical, and biological conditions experienced by each individual in its microhabitat (Hohenlohe *et al*., 2010).

The intra-population variation of *A. tropidonotus* identified in this study may be the result of a population subdivision marked by physical barriers at the microhabitat scale (Campbell-Staton *et al*., 2017). Climatic heterogeneity in their habitat, intra- and inter-specific relationships experienced by *A. tropidonotus* organisms, and geographic isolation of the populations shape the phenotypes of the species (Malhotra and Thorpe, 2000).

CONCLUSIONS

The intra- and inter-population morphological microvariations in five out of 19 landmarks evaluated in *A. tropidonotus* may be caused by the selective pressures that generate differences between individuals and sexes. Landmark 2, located at the edge and in the middle of the muzzle, was the trait that showed the greatest variation in both populations. Factors such as availability and quantity of prey may exert selective and adaptive pressure on this morphological trait of *A. tropidonotus* individuals —enough pressure to ensure that these sections of the muzzle are functional in each environment. Nevertheless, this hypothesis must be tested in future studies.

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