

1 **Bipedalism in Mexican Albian lizard (Squamata) and the locomotion type in other**

2 **Cretaceous lizards**

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20 **ABSTRACT**

21 Representative locomotion types in lizards include terrestrial, arboreal, grass swimmer, sand
22 swimmer and bipedal. Few studies explain the locomotion habit of extinct lizards, and even less
23 asses those of bipedal ones. Here, we use quantitative methods to infer the type of locomotion of
24 two Albian Mexican lizards (Lower Cretaceous) and three Cretaceous lizards from Brazil, North
25 America and Spain, assessing the similarities of the hindlimb-forelimb length ratio amongst
26 extinct and extant species. Additionally, an ancestral character state reconstruction analysis was
27 performed, to evaluate the evolution of lizard locomotion habits. The species *Huehuecuetzpalli*
28 *mixtecus* was bipedal while *Tijubina ponteii* was facultative bipedal, *Hoyalacerta sanzi*,
29 *Tepexisaurus tepexii* and *Polyglyphanodon sternbergi* cannot be differentiated amongst terrestrial
30 or arboreal with the approach used in this work. The ancestral character state reconstruction
31 analysis showed a terrestrial ancestral locomotion type, with a basal character state of hindlimbs
32 longer than forelimbs. Equal length between hind and forelimbs appear to be a derivate state that
33 evolved multiple times in lizard evolutionary history.

34 **Keywords:** Ecomorphology, Tlayúa Quarry, *Huehuecuetzpalli mixtecus*, México, *Tepexisaurus*
35 *tepexii*.

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37 **1. INTRODUCTION**

38 Lizard bipedalism is found in nine of the 46 extant known families, such as Iguania (Agamidae,
39 Crotaphytidae, Iguanidae, Liolaemidae, Phrynosomatidae, Tropiduridae), Lacertoidea
40 (Lacertidae, Teiidae) and Anguimorpha (Varanidae) (Clemente, 2014; Pyron, 2017; Vitt and
41 Caldwell, 2014). The emergence of bipedalism is believed to have been an exaptation, as a trait to
42 acquire greater maneuverability as speed increased (Clemente, 2014). However, succeeding
43 lineages exploited this trait because of its evolutionary advantages, suggesting a possible

44 adaptative radiation in some groups (Clemente, 2014). Recent works point to the emergence of
45 bipedalism in Squamata in the Lower Cretaceous somewhere during the Aptian-Albian (Simoes
46 et al., 2015; Lee et al., 2018). The most ancient inferred bipedal lizard, due to its hindlimb-
47 forelimb ratio, is *Tijubina ponteii* from the Cretaceous of Brazil (Aptian-Albian ~113 Ma), a
48 species closely related to Polyglyphanodontidae (Simões et al., 2015), an extinct family basal to
49 Lacertoidea (Pyron et al., 2017). Direct fossil evidence of bipedalism in lizards was reported by
50 Lee et al. (2018), who found ichnofossils of lizard footprints belonging to *Sauripes hadongensis*,
51 from 110 Ma ago in the Cretaceous of South Korea.

52 *Huehuecuetzpalli mixtecus* is a fossil lizard from 105 Ma ago of the Cretaceous Albian of Mexico
53 (Reynoso, 1998). The mention of it being a bipedal lizard exists (Reynoso, 1998; Reynoso and
54 Cruz, 2014), but there is not any study supporting this statement. *H. mixtecus* has been placed at
55 the base of the Iguania clade (Pyron, 2017), alongside *Hoyalacerta* from the Lower Cretaceous of
56 Spain (Evans and Barbadillo, 1999), becoming the sister group to all iguanids (Pyron et al. 2017).
57 *Huehuecuetzpalli mixtecus* was found in the Tlayúa quarry, Tepexi de Rodríguez, Puebla
58 (Reynoso, 1998), a fossiliferous site regarded as Lagerstätte for its excellent fossil preservation
59 (Espinosa-Arrubarrena and Applegate, 1996). The Tlayúa quarry is a small outcrop located in the
60 west side of Tepexi de Rodríguez municipality, Puebla, México, at 18°35'7.24" N and
61 97°54'38.09" W, 1740 masl, with an age about 105 Ma (Benammi et al., 2006). The fossil
62 lepidosaurians of Tlayúa are represented by two rhynchocephalian sphenodontids
63 (*Ankylosphenodon pachyostosus* and *Pamizinsaurus tlayuensis*) and the lizards *Huehuecuetzpalli*
64 *mixtecus* and *Tepexisaurus tepexii* (Reynoso and Cruz, 2014) (Figure 1 A, B). *Tepexisaurus*
65 *tepexii* had short limbs and tail, indicative of crawling movements (Reynoso and Callison, 2000),
66 and *H. mixtecus* had long hindlimbs and tail, which has led to believe it had a bipedal
67 locomotion, with tail autotomy (Reynoso, 1998; Reynoso and Cruz, 2014). Nevertheless, the

68 locomotion inferences of these lizards have only been made using uniformitarianism (Reynoso
69 and Cruz, 2014), without carrying out a quantitative analysis. Using uniformitarianism and
70 quantitative data we can explain Earth history in terms of gradual change by processes observed
71 today (Polly and Spang, 2002), which includes evolutive inferences derived from paleontological
72 records. In this study we quantitatively infer the locomotion type of *H. mixtecus* and *T. tepexii*,
73 and the significance of these within the evolution of bipedalism in Squamata by comparing them
74 to other extinct and extant lizards.

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77 **Figure 1.** Cretaceous lizards from the Tlayúa Quarry, Mexico (A, B), and locomotion types of
78 extant lizards. Bipedal (C, D), sand swimmer (E), grass swimmer (F), arboreal (G) and terrestrial
79 (H). A) *Huehuecuetzpalli mixtecus* (IGM 7389), B) *Tepexisaurus tepexii* (IGM 7466), C)
80 *Basiliscus vittatus*, D) *Corythopanes hernandezi*, E) *Aspiloscelis inornata*, F) *Plestiodon lotus*,
81 G) *Abronia graminea* and H) *Phrynosoma sherbrookei*. Photographs by Eric Centenero Alcalá.

82 Scale bar = 3 cm.

83

84 **2. MATERIAL AND METHODS**

85 Photographs were taken of the forelimbs (humerus, radius, ulna) and hindlimbs (femur, tibia,
86 fibula) of 36 existing species of lizards, all with their respective measurement scale. The
87 specimens belong to the osteological reference collection of the paleontology laboratory of the
88 FCB, BUAP (BUAPALO, Table S1). For the digital capture, we used a VE-LX1800 camera
89 mounted in a Miotic SMZ-168 stereoscopic microscope. Bone length was measured with the
90 program Image J v1.8.0 (Ferreira and Rasband, 2012). The work by Gans et al. (2008) was used
91 for identification of disarticulated lizard limb bones. Our current sample of organisms is not very
92 extensive; however recent osteological studies with small sample sizes due to the availability of
93 collection specimens have been known to yield good results with fewer than 30 specimens
94 (Yildirim et al., 2017; Ledesma and Scarpetta, 2018; Paparella et al., 2020).

95 The locomotion habit by which extant species were catalogued included: bipedal, arboreal,
96 terrestrial, sand swimmer and grass swimmer (Figure 1). Bipedalism involves moving on the
97 hindlimbs alone (Russell and Bels, 2001); many lizards are known to run bipedally, but unlike
98 theropod dinosaurs or birds they do not have bipedal striding gaits (Aerts et al., 2003). The
99 arboreal term is used to describe tree-dweller lizards (Fischer et al., 2010), while ground-dwellers
100 are defined as terrestrial (Russell and Bels 2001). If a ground-dweller lizard shows serpentine
101 movements it will be called sand swimmer if it dwells on sandy environments, or grass swimmer
102 if it is found on grasslands (Maladen, 2009).

103 Regarding extinct species, holotype high resolution images were consulted for *H. mixtecus* (IGM
104 7389, 4185) and *T. tepexii* (IGM 7466), both belonging to the paleontological collection of the
105 Institute of Geology, UNAM (Figure 1A, B). The limb bone length was obtained following the
106 same procedure than before. *Polyglyphanodon sternbergi*, *Hoyalacerta sanzi* and *Tijubina ponteii*

107 limb lengths were taken from Gilmore (1942), Evans and Barbadillo (1999) and Bonfim-Júnior
108 and Rocha-Barbosa (2006), respectively.

109 The hindlimb-forelimb length ratio was calculated as the sum of the femur and tibia longitudes
110 (hindlimb length) divided by the sum of the humerus and ulna longitudes (forelimb length), in
111 mm. Values equal to one, indicated limbs of equal length. Values higher than one showed longer
112 hindlimbs, while values below one showed longer forelimbs. A simple linear regression analysis
113 was performed to evaluate hindlimb-forelimb length correlation. Other measures used were the
114 femur/humerus, tibia/ulna, and fibula/radius ratios which together with the hindlimb-forelimb
115 length ratio were used to construct a Principal Component Analysis (PCA). All analyses were
116 carried out in Rstudio (Rstudio Team 2020; <http://www.rstudio.com/>).

117 To analyze the evolution of bipedalism, we used the squamate phylogeny of Pyron et al. (2017).
118 Serpentes and Amphisbaenia groups were excluded, in order to work only with lizards. This
119 phylogeny was chosen because it includes *Huehucuetzpalli*, *Hoyalacerta* and *Polyglyphanodon*.
120 *Tijubina ponteii* was placed alongside *Polyglyphanodon*, according to the classification by Simões
121 et al. (2015). A pruned 40 taxa ultrametric tree was obtained. For each taxon, the corresponding
122 species name, hindlimb-forelimb length ratio and locomotion type were provided (Table S1). It is
123 known that analyses based on osteological data of extinct and extant organisms tend to have
124 relatively small sample sizes (Cardini and Elton, 2007; Duan et al., 2020) because of rarity and
125 collection difficulty (Brown and Vavrek, 2015). In our case we have a limited number of
126 specimens due to availability constrains (see above). However scarce fossil taxa may be, it can
127 improve the accuracy of phylogenetic analysis of morphological datasets (Koch et al., 2020).
128 Two ancestral character state reconstructions were carried out. The first one was issued discrete
129 values: the types of locomotion for each of the extant and fossil lizards. The extinct species
130 locomotion type was implied from the statistical analysis of this work (Table 1). To identify the

131 likely plesiomorphic condition for the type of locomotion, of the studied lizard taxa, a stochastic
132 mapping for discrete phylogeny traits was performed (Bollback, 2006; Revell, 2012), with a
133 bootstrapping of 1000 iterations. The analysis was carried out under the maximum likelihood
134 approach (Bollback, 2006) and an equal-rates model (Pagel, 1994; Pagel, 1999) was selected as
135 the model of character state evolution, with equal probability for any change. The equal-rates
136 model recognizes *a priori* information, such as the fossil records used in this work (Pagel, 1994;
137 Pagel, 1999; Skinner, 2010).

138 For the second ancestral character state reconstructions analysis, a continuous trait was given: the
139 hindlimb-forelimb length ratio (Table 1). Classification of species as bipedal, or other locomotion
140 habit, was mapped onto a phylogenetic tree. In order to characterize hindlimb-forelimb length
141 ratio evolution, seven models of evolution were tested, and the one providing the best fit was
142 chosen. The evolution models are listed as follows: *i*) a single-rate Brownian motion model
143 (BM1); *ii*) an Ornstein–Uhlenbeck model with one optimum for all lizard species (OU1); *iii*) a
144 Brownian motion model that allowed for separate rates for each locomotion habit regime (BMS);
145 *iv*) an OU_M model that allows for different optima for each regime. And three OU models
146 allowing for *v*) different Brownian motion rates (OU_{MV}), *vi*) different strength of selection
147 parameters (OU_{MA}) or *vii*) different Brownian motion rates and different strength of selection
148 parameters (OU_{MVA}). The evolution model that best described continuous traits evolving under
149 discrete selective pressures (O’Meara et al., 2006), was chosen as the one providing the best
150 Akaike Information Criterion corrected for small sample sizes (AICc) fit (O’Meara et al., 2006).

151 In order to infer the historic change of lizard hindlimb-forelimb proportion, a phylogenetic
152 mapping estimating the transitional states, using maximum likelihood approach (Revell, 2012)
153 and Felsenstein methodology (Felsenstein, 1985), was carried out.

154 All analyses were performed in R v4.0.3 (R Core Team, 2020). For stochastic and transitional
155 states phylogenetic mapping, we used the *phytools* R package v0.6.99 (Revell, 2012) and for the
156 evolution model testing, the *OUwie.R* function from the *OUwie* R package v2.6 (Beaulieu et al.,
157 2012; Beaulieu and O’Meara, 2019; Beaulieu and O’Meara, 2021). and for the delta AICc scores
158 the *akaike.weights* function from the *qpcR* R package v1.4-1 (Spiess, 2018).

159

160 3. RESULTS

161 3.1 Extant and extinct lizard locomotion habits

162 Hindlimb-forelimb length ratio shows that grass swimmer species are the lizards with the most
163 similar limb length (Q1 – Q3 = 1.05 – 1.12). Of all the groups, terrestrial (Q1 – Q3 = 1.14 – 1.28)
164 and arboreal (Q1 – Q3 = 1.05 – 1.23) lizards display the largest length variation.

165 Sand swimmer species appear to have slightly longer hindlimbs (Q1 – Q3 = 1.33 – 1.46), while
166 bipedal ones show the highest ratio values of them all (Q1 – Q3 = 1.63 – 1.87) (Table 1, Figure
167 2A). For the Cretaceous lizards, it is not possible to determine the locomotion habit of
168 *Tepexisaurus tepexii* and *Polyglyphanodon sternbergi*, due to its hindlimb-forelimb length ratio
169 (1.19 and 1.14, respectively) being inside the terrestrial-arboreal interval (Figure 2A).

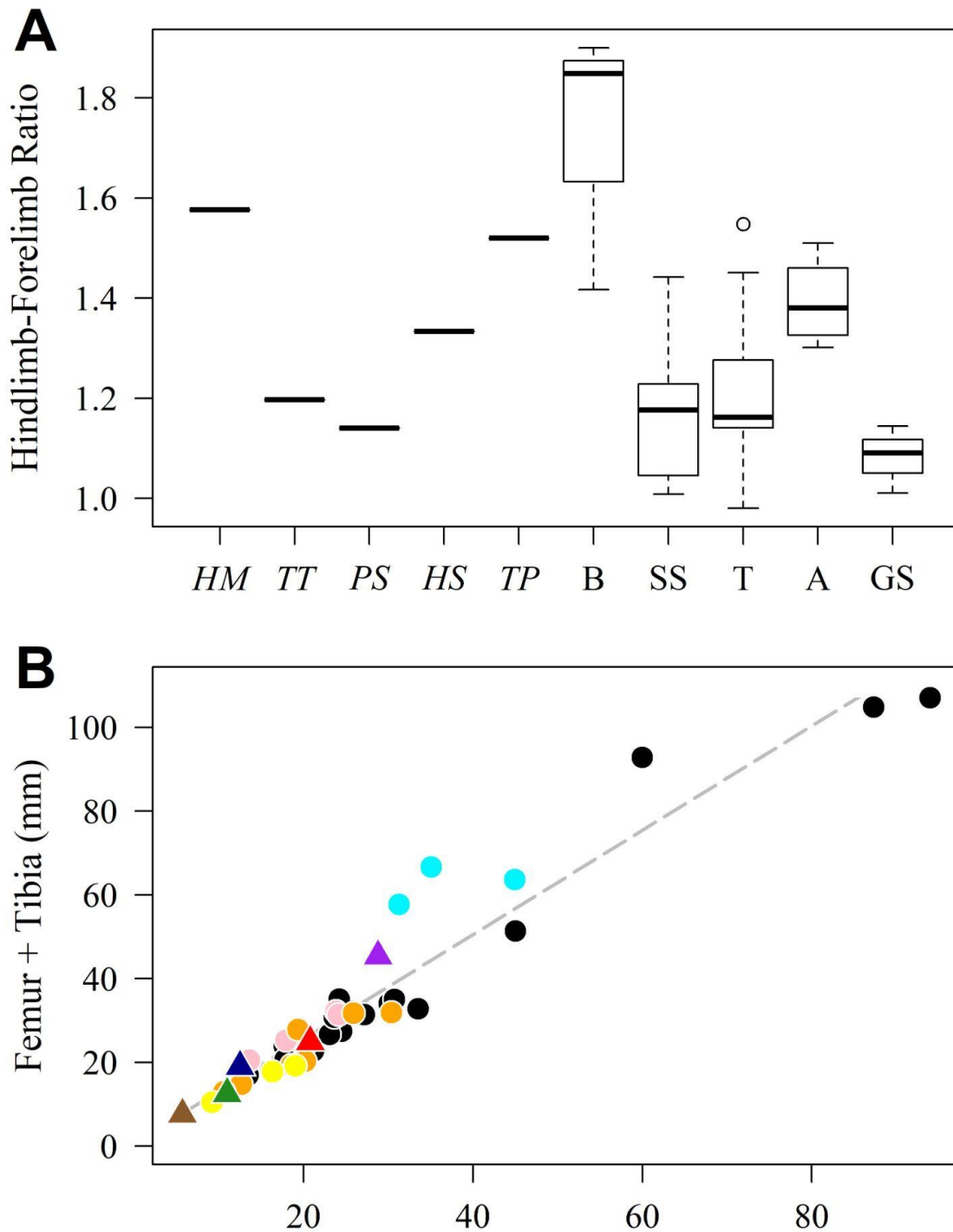
170 Nevertheless, a terrestrial locomotion type is assumed for both species, according to what has
171 been published (Gilmore, 1942; Reynoso and Callison, 2000). *Hoyalacerta sanzi* has marginally
172 longer hindlimbs (1.33), which fall in between the quartiles of the sand swimmer, terrestrial and
173 arboreal locomotion habit (Figure 2A). Both *Tijubina ponteii* and *Huehucuetzpalli mixtecus*
174 hindlimb-forelimb length ratios (Table 1) are above sand swimmer variation values (Min – Max
175 = 1.3 – 1.51) but are encompassed by bipedal ones (Min -Max = 1.42 – 1.9).

176

177 **Table 1.** Minimum, maximum, median and quartiles (Q), in millimeters, of the relationship
178 between hind (femur + tibia) and forelimb (humerus + ulna) bone length.

Locomotion type	Minimum	Q1	Median	Q3	Maximum
Bipedal	1.42	1.63	1.85	1.87	1.9
Arboreal	1.01	1.05	1.18	1.23	1.44
Terrestrial	0.98	1.14	1.16	1.28	1.45
Sand swimmer	1.3	1.33	1.38	1.46	1.51
Grass swimmer	1.01	1.05	1.09	1.12	1.15
<i>Huehuecuetzpalli mixtecus</i>	1.58	1.58	1.58	1.58	1.58
<i>Tijubina pontei</i>	1.52	1.52	1.52	1.52	1.52
<i>Hoyalacerta sanzi</i>	1.33	1.33	1.33	1.33	1.33
<i>Tepexisaurus tepexii</i>	1.20	1.20	1.20	1.20	1.20
<i>Polyglyphanodon sternbergi</i>	1.14	1.14	1.14	1.14	1.14

179



180
181 **Figure 2.** A) Hindlimb-forelimb length ratio boxplot, for the Cretaceous lizards *Huehuecuetzpalli*
182 *mixtecus* (HM), *Tepexisaurus tepexii* (TT), *Polyglyphanodon sternbergi* (PS), *Hoyalacerta sanzi*
183 (HS), *Tijubina ponteii* (TP) and the locomotion types of 36 extant species. Abbreviations: B

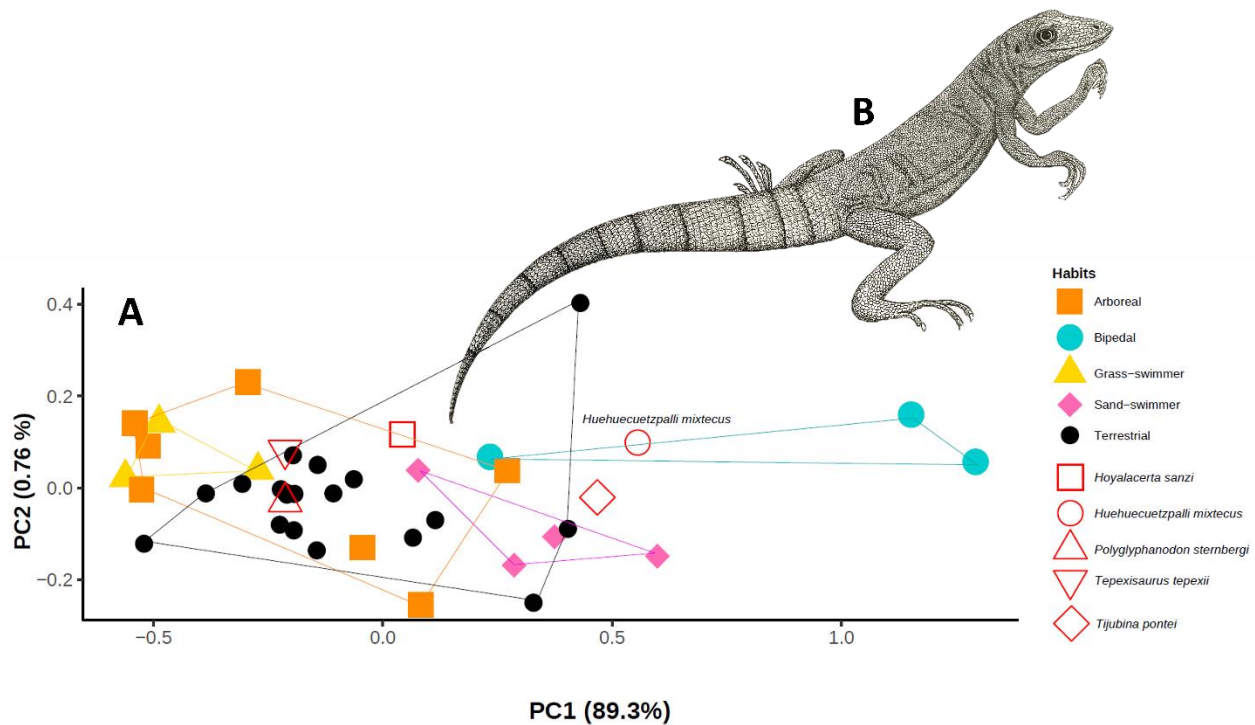
184 stands for bipedal; SS = sand swimmer; T = terrestrial; A = arboreal; GS = grass swimmer. B)
185 Linear regression for the longitude of hindlimb (femur + tibia) by forelimb (humerus + ulna) of
186 extant (circles) and fossil (triangles) lizards. Black stands for terrestrial locomotion habit; sky
187 blue = bipedal; pink = sand swimmer; orange = arboreal; yellow = grass swimmer; purple =
188 *Huehuecuetzpalli mixtecus*; red = *Tepexisaurus tepexii*; dark blue = *Tijubina pontei*; green =
189 *Polyglyphanodon sternbergi*; gray = *Hoyalacerta sanzi*.

190

191 Simple linear regression analysis shows a positive correlation between hind and forelimbs
192 ($R^2=0.55$, $P<0.0001$). All extant lizards are near or below the regression line, except the bipedal
193 species and one terrestrial species (Figure 2B). These last four have longer hindlimbs than
194 forelimbs. Of the Cretaceous fossils, *Huehuecuetzpalli mixtecus* raises above the regression line,
195 grouped with the extant bipedal lizards. Meanwhile, *Tijubina pontei* barely goes above the line,
196 near the sand swimmer locomotion type lizards. *Hoyalacerta sanzi*, *Polyglyphanodon sternbergi*
197 and *Tepexisaurus tepexii* are inside the regression line, being unable with this analysis to
198 determine their locomotion habit.

199 PCA shows to *Polyglyphanodon sternbergi*, *Tepexisaurus tepexii*, and *Hoyalacerta sanzi* inside
200 the terrestrial and arboreal variation, *Huehuecuetzpalli mixtecus* is clear inside the bidpedal
201 locomotion type and *Tijubina pontei* is outside of all locomotion type (Figure 3A). This led us to
202 infer that *Huehuecuetzpalli mixtecus* was a bipedal lizard supported by all analyses, like extant
203 lizards *Basiliscus*, *Laemanctus*, and *Corytophanes* (Figure 3B). *Tijubina pontei* was possibly a
204 facultative bipedal lizard because the hindlimb-forelimb length ratios (Table 1) are above sand
205 swimmer values close to bipedal variation values (Figure 2A); however, it is possible that
206 bidepalism is not supported by the regression or PCA analyzes (Figure 2, 3). We could not infer

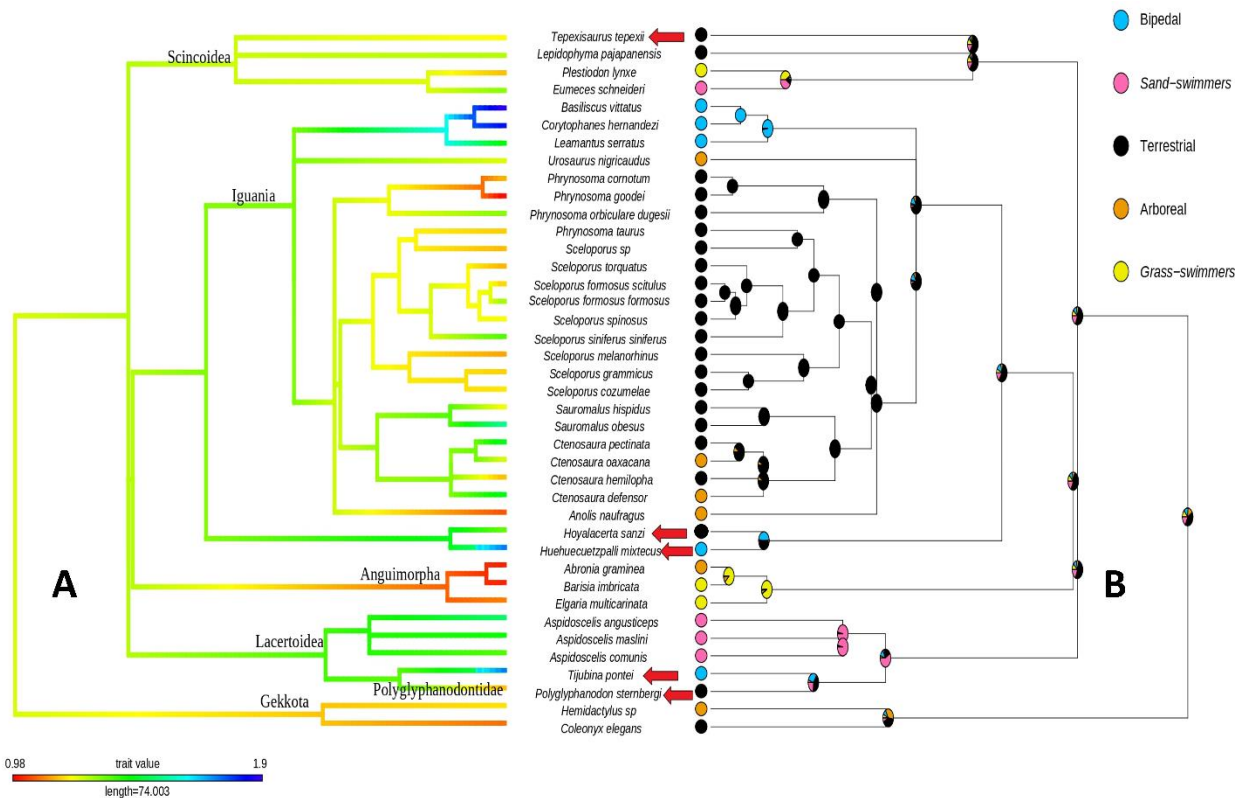
207 the locomotion type for *Polyglyphanodon sternbergi*, *Tepexisaurus tepexii*, and *Hoyalacerta*
208 *sanzi*, since the fossils fell within terrestrial and arboreal locomotion (Figure 2, 3).



209
210 **Figure 3.** A) PCA of the locomotion types in extant (filled figures) and extinct (empty figures)
211 lizards. B) Reconstruction of a lizard *Huehuecuetzpalli mixtecus* in bipedal position, iguanid
212 body and varanid head, based on the description of Reynoso and Cruz (2014). Drawn by Gerardo
213 García Demeneghi.

214
215 *3.2 Evolution of lizard bipedalism*
216 Ancestral state reconstruction of discrete character infers terrestrial locomotion habit as the most
217 likely condition at the base of the phylogeny (51% inference value, Figure 3B). Other habits
218 show similar inference values (bipedal = 12%, arboreal = 10%, sand swimmer = 17% and grass
219 swimmer = 10%). No group displays a unique locomotion type, for all the habits emerge rapidly
220 in different groups (Figure 4B), and terrestrial locomotion habit seems to be the ancestral

221 condition at internal nodes of the phylogeny. Nonetheless, bipedalism only develops in
 222 Lacertoidea with *Tijubina ponteii*, and Iguania with *Huehuecuetzpalli mixtecus*, *Basiliscus*
 223 *vittatus*, *Corytophanes hernandezii* and *Laemanctus serratus*.
 224



225
 226 **Figure 4.** Phylogenetic tree and evolution model of lizard hindlimb-forelimb length ratio. A)
 227 Values close to one (orange - red) show a similar length ratio for arboreal (e.g. *Abronia*
 228 *gramminea*), grass swimmer (e.g. *Barisia imbricata*) and terrestrial (e.g. *Phrynosoma goodei*)
 229 species. Values different from one (light-dark blue) indicate longer hindlimbs for bipedal (e.g.
 230 *Basiliscus vittatus*, *Huehuecuetzpalli mixtecus*, *Tijubina ponteii*), sand swimmer (*Aspidoscelis*
 231 *angusticeps*) and terrestrial (e.g. *Sauromalus obesus*) lizards. Intermediate values (green - yellow)
 232 denote marginally longer forelimbs for terrestrial and sand swimmer locomotion habits. B)
 233 Mapping of the locomotion types of extant and fossil (arrows) lizard species. The ancestral state

234 is inferred to be terrestrial at the base of the phylogeny (51%) in relation to other habits: bipedal
235 (12%), arboreal (10%), sand swimmer (17%) and grass swimmer (10%).

236

237 The evolution model that provided the best fit for characterizing the hind-forelimb length
238 relationship, was the OU_{MA} (Table 2). Which indicates that strength of selection (α) and the
239 optima (θ) differ between the selective regimes (locomotion habits), but not so the rate of
240 stochastic motion (σ^2). The terrestrial state had the highest strength of selection (0.5408),
241 followed by bipedal (0.0135), grass-swimmer (0.0047), arboreal (0.0005) and sand-swimmer
242 (0.0002). The optima for terrestrial lizards was 1.14, for all others it was 0. Longer hindlimbs are
243 only present in modern day family Corytophanidae (*Basiliscus*, *Corytophanes* and *Laemanctus*)
244 and the fossil lizards *Huehuecuetzpalli mixtecus* (Iguania) and *Tijubina ponteii* (Lacertoidea).
245 Species with slightly longer hindlimbs were the extant terrestrial iguanas *Sauromalus obesus* and
246 *Ctenosaura pectinata*, as well as the sand swimmer teiid *Aspidoscelis angusticeps* (Figure 3A).
247 Limb length relationship points to a basal character state of marginally longer hindlimbs. Equal
248 length between hind and forelimbs appear to be a derivate state that evolved multiple times in
249 lizard evolutionary history, among terrestrial (e.g. *Phrynosoma goodei*), arboreal (e.g. *Abronia*
250 *gramminea*) and sand swimmer (e.g. *Barisia imbricata*) species (Figure 3A). On the other hand,
251 the development of larger hindlimbs can be discerned in bipedal extant (*Basiliscus*, *Corytophanes*
252 and *Laemanctus*) and extinct (*H. mixtecus* and *T. ponteii*) taxa, as well as some sand swimmer
253 (*Aspidoscelis angusticeps*) and terrestrial (*Sauromalus obesus*) species (Figure 3A).

254

255 **Table 2.** Akaike Information Criterion corrected for small sample sizes (AICc) and Bayesian
256 Information Criteria (BIC) for the seven evolutionary models tested in this work. OU_{MA} model
257 best describes the evolution of hindlimb-forelimb length relationship for extant and extinct

258 lizards. Likelihood, AICc and BIC scores were determined using the function *OUwie* in the
259 *OUwie* R package (Beaulieu & O'Meara, 2021). Delta AICc scores were then calculated using the
260 function *akaike.weights* in the *qpcR* R package (Spiess, 2018).

Model	-lnL	AICc	Δ AICc	BIC
BM1	8.65	-13.29	-12.97	-9.91
BMS	13.43	-12.32	703.95	-4.73
OU1	13.06	-19.45	696.82	-15.05
OU _M	25.21	-32.93	683.34	-24.60
OU _{MV}	28.56	-25.69	690.58	-16.54
OU _{MA}	373.85	-716.27	0	-707.12
OU _{MVA}	27.99	-5.97	710.30	-0.64

261

262 4. DISCUSSION

263 The hindlimb-forelimb length ratio indicates a clear difference between lizard bipedalism and
264 other locomotion types (Figures 2, 3). Differences were also shown among sand swimmer and
265 grass swimmer species, but were not between terrestrial and arboreal ones, due to their wide
266 variation range (Table 1, Figure 2, 3). It was inferred that *Huehuecuetzpalli mixtecus* exhibited a
267 bipedal locomotion, while *Tijubina ponteii* exhibited a facultative bipedal locomotion type, due to
268 the hindlimb-forelimb length ratio values not being as high as those of extant lizards (Table 1,
269 Figures 2, 3). Because length values of *Hoyalacerta sanzi*, *Tepexisaurus tepexii* and
270 *Polyglyphanodon sternbergi* are similar between hind and forelimbs, it was not possible to
271 discern if these species were either terrestrial or arboreal (Table 1, Figure 2, 3). In order to
272 distinguish between terrestrial and arboreal locomotion it will be necessary to carry out further

273 studies, either with claw (Herrel et al., 2002; Zani, 2008) or hip (Fischer et al., 2010; Russell and
274 Bels, 2001) measurements.

275 Linear bone limb measurement, as well as hindlimb-forelimb length ratio, have already been used
276 to infer bipedal locomotion habits, such as those in the extinct reptiles *Eudibamus cursoris* from
277 the Permian of Germany (Berman et al., 2000) and *Langobardisaurus* from the Triassic of Italy
278 (Renesto et al., 2002). Multiple times, these parameters have also been used to infer extant lizard
279 locomotion types (Abou Egla et al., 2008; Druelle et al., 2019; Foster et al., 2018; Herrel et al.,
280 2002; Snyder, 1962), demonstrating their effectivity at studying the locomotion of extinct
281 organisms.

282 Lizard bipedalism is the easiest locomotion type to differentiate, due to the fact that hindlimbs
283 will always be longer than forelimbs (Grinham and Norman, 2020; Schuett et al., 2009; Snyder,
284 1962). This allowed us to infer bipedalism and the facultative bipedalism of the Cretaceous
285 lizards *Huehuecuetzpalli mixtecus* and *Tijubina ponteii*, respectively. It is facultative because their
286 hindlimb-forelimb length ratio is less than extant bipedal lizards, such as *Basiliscus*, *Corytophanes*
287 and *Leamantus* (Figure 2, 3). Bipedal locomotion of *T. ponteii* was possibly a consequence of
288 morphology (hindlimb longer than forelimbs) and acceleration, allowing more maneuverability as
289 speed increased (Clemente, 2014).

290 With the inclusion in the analysis of other Cretaceous lizard fossils (*Hoyalacerta sanzi*,
291 *Huehuecuetzpalli mixtecus*, *Polyglyphanodon sternbergi*, *Tepexisaurus tepexii* and *Tijubina*
292 *ponteii*), terrestrial locomotion habit was inferred to be the ancestral trait. This is in accordance
293 with the hypothesis that several locomotion habits are derivatives from the quadrupedal terrestrial
294 type (Russell and Bels, 2001). Strength of selection values (α) of the OU_{MA} model further support
295 this: the highest α value was for the terrestrial locomotion habit (0.5408). Bipedal locomotion had
296 the second highest a value (0.0135). The hindlimb-forelimb ratio optimum (θ) was the terrestrial

297 habit (1.14) while all the others locomotion types tended to 0. It is known that OU_{MA} , OU_{MV} and
298 OU_{MVA} models tend to overestimate α and underestimate θ , but this happens even with big
299 sample sizes (i.e. 512 taxa) (Beaulieu et al., 2012). Nevertheless, our interpretations should be
300 considered with caution.

301 *Huehucuetzpalli mixtecus* and *T. pontei* are basal to Iguania and Lacertoidea which are two out
302 of the three clades that possess modern day bipedal lizards (the third being Anguimorpha)
303 (Clemente, 2014). This strengthens the hypothesis of lizard bipedalism emergence as an
304 exaptation, which later favored certain organisms as an evolutive advantage (Clemente, 2014).
305 Among these are some corytophanids, agamids and varanids (Clemente, 2014; Schuett et al.,
306 2009). We acknowledge that most of the specimens used in our study correspond to specific
307 lineages in Iguania and this overrepresentation could lead to biased conclusions. Nonetheless the
308 objective of our study was to evaluate the significance of *H. mixtecus* and *T. tepexii* in the
309 evolution of bipedalism in lizards, because *H. mixtecus* is basal to Iguania (Pyron et al., 2017).
310 Ancestral character state reconstructions of the hindlimb-forelimb length ratio showed that the
311 ancestral locomotion type belonged to a terrestrial lizard with marginally longer hindlimbs
312 (Figure 3A). This is consistent with the oldest fossil record of lizard-like diapsid limbs, such as
313 those of the species: *Paleagama vielhaueri* and *Saurosternon bainii* from the Permian-Jurassic
314 (Carroll, 1975) and *Hongshanxi xiei* from the Jurassic (Dong et al., 2019); all three species had
315 longer hindlimbs (Table 3). From the ancestral state of hindlimbs slightly longer than forelimbs,
316 the extant lizard limb proportions arose. For example, those of the species with same limb lengths
317 (arboreal, terrestrial and grass swimmer), and those of the taxa with longer hindlimbs (bipedal,
318 sand swimmer and, occasionally, terrestrial). However, further research is needed to explain the
319 origin and diversification of lizard locomotion habits.

320

321 **Table 3.** Hind and forelimb length of the Permian-Jurassic diapsids *Paleagama vielhaueri*,
322 *Saurosternon bainii* and *Hongshanxi xiei*. Note the higher hindlimb values.

Fossil	Forelimb (humerus + ulna length)	Hindlimb (femur + tibia length)
<i>Paleagama vielhaueri</i>	38.2 mm	55.4 mm
<i>Saurosternon bainii</i>	28.8 mm	38.8 mm
<i>Hongshanxi xiei</i>	13.8 mm	22.5 mm

323

324 **5. CONCLUSIONS**

325 Bipedal locomotion habit is inferred for the Cretaceous lizard *Huehuecuetzpalli mixtecus* and
326 facultative bipedal locomotion habit is inferred for *Tijubina pontei*.

327 The locomotion type of *Hoyalacerta sanzi*, *Tepexisaurus tepexii* and *Polyglyphanodon sternbergi*
328 cannot be differentiated between terrestrial or arboreal, with the approach used in this work.

329 We infer that the last common lizard ancestor had a terrestrial locomotion habit, with hindlimbs
330 slightly longer than forelimbs, whereas hindlimbs equally elongated as forelimbs or strongly
331 longer than forelimbs is a derived condition.

332 Lizards with longer hindlimbs can be interpreted as being bipedal or facultative bipedal. By
333 contrast, grass swimmers have the same length between their limbs. Sand swimmers have long
334 hindlimbs, but without being particularly long as in the case of bipedal lizards. Finally, the most
335 variation in hindlimb-forelimb length ratio was observed for the terrestrial and arboreal habits.

336 However, because of the overrepresentation of Iguanid lineages in our study and specimen
337 availability limitations, further research with more diverse samples is needed.

338

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347

348 **SUPPLEMENTARY MATERIAL**

349 Table S1. Excel spreadsheet with bones measurements, type of locomotion and catalog number of
350 extinct and extant lizards used in this study.

351

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