

1 **RESEARCH ARTICLE**

2 **The Mystery of a Marine Monster: Morphological and Performance Modifications in the**
3 **World's only Marine Lizard, the Galápagos Marine Iguana**

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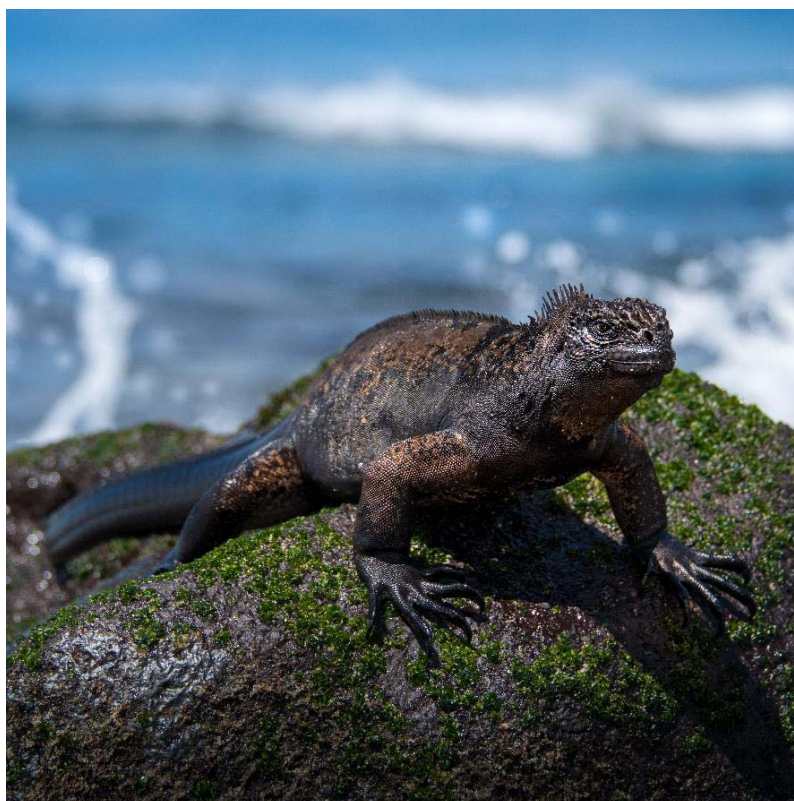
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11 **Keywords:**

12 Marine Iguana; Iguanidae; aquatic transition; performance; morphology; evolution

13
14 **Summary Statement:**

15 The Marine Iguana has undergone a unique evolutionary transition to aquatic behaviour,
16 we explore the extent of morphological and performance specialisation required and why there
17 are so few extant marine reptiles.



19 **Abstract:**

20 Reptiles have repeatedly invaded and thrived in aquatic environments throughout history,
21 however fewer than 8% of the 6000 extant species are primarily aquatic. The Galápagos Marine
22 Iguana (*Amblyrhynchus cristatus*), the world's only marine lizard, may have had one of the most
23 unique and challenging transitions to aquatic life. Curiously, previous studies have identified
24 relatively few physiological adaptations in Marine Iguanas, however, little is known about the
25 extent of morphological specialisation and performance trade-offs associated with the marine
26 environment. By examining the morphology and locomotory performance of the Marine Iguana in
27 comparison to their closely related mainland ancestors, the Black Spiny-tailed iguana (*Ctenosaura*
28 *similis*) and Green Iguana (*Iguana iguana*), we found variation reflected specialisation to ecological
29 niches. However, variation was more pronounced among subspecies of Marine Iguana, suggesting
30 that little morphological or performance modification is required for iguanids to successfully
31 invade aquatic environments, thus raising the question why there are so few extant aquatic
32 reptilian lineages. We found that specialisation for the marine environment resulted in a trade-off
33 in sprint speed in a terrestrial environment, similar to that seen in extant crocodylians. Reduced
34 performance in a terrestrial environment likely poses little risk to large-bodied apex predators,
35 whereas in iguanids, a performance trade-off would likely incur increased predation. As such, we
36 suggest that this may explain why iguanids and other ancestral lineages have not undergone
37 transitions to aquatic life. Additionally, we found that the magnitude of morphological and
38 performance variation was more pronounced between subspecies of Marine Iguana than between
39 iguanid species.

40 **Background:**

41 The extent to which an animal is adapted to the challenges of a specific habitat has long been a
42 subject of evolutionary studies. The link between morphology, performance and fitness was first
43 characterised in a framework described by Arnold (Arnold, 1983). This framework assumes that variation in
44 morphology and physiology determines an individual's fitness in a specific habitat only to the extent that it
45 influences intermediary traits, such as locomotory performance (Arnold, 1983, Garland Jr and Losos, 1994).
46 Under this view, natural selection acts most directly on locomotion, and therefore, locomotion is
47 recognised as the foundation of all behaviour undertaken in an ecological niche (Mcelroy et al., 2007). The
48 congruence between morphology, performance and habitat use is considered one of the most illustrative
49 outcomes of adaptive evolution (Vanhooydonck and Van Damme, 2003). However, adaptations which
50 promote performance in a single habitat can often reduce performance in other environments
51 (Vanhooydonck and Van Damme, 2003). As such, trade-offs in performance are expected in animals that
52 represent a transitional stage between terrestrial and aquatic life.

53 Reptiles are recognised as being one of the most evolutionary and ecologically remarkable
54 components of global biodiversity. Reptiles not only have successfully invaded most areas of the world,
55 they have also played a primary role in the origin and radiations of amniote vertebrates (Pincheira-Donoso
56 et al., 2013). Consequently, reptiles are extensively used as model organisms in ecomorphological studies
57 (Gomes et al., 2016). Although primarily terrestrial, reptiles have repeatedly invaded aquatic environments
58 throughout history (Seymour, 1982). The Mesozoic era gave rise to at least a dozen diverse groups of
59 marine reptiles, some of which were major oceanic predators for over 180 million years (Motani, 2009). Yet
60 today fewer than 8% of the 6000 reptilian species are primarily aquatic (Bauer and Jackman, 2007).
61 Previous studies have identified that the reptilian physiology allows for a simple transition into aquatic
62 environments (Seymour, 1982, Motani, 2009), thus raising the question as to why so few lineages of
63 aquatic reptiles exist today.

64 The Galápagos Marine Iguana (*Amblyrhynchus cristatus*) has arguably undergone one of the most
65 significant evolutionary transitions among extant reptiles, being the world's only marine lizard (Dawson et
66 al., 1977). Marine iguanas must overcome numerous challenges associated with feeding exclusively on
67 subtidal marine products, such as swimming through surf zones, overcoming buoyancy, tolerance of
68 anaerobic conditions, the thermal constraints of diving in cold water and the exertion associated with
69 underwater grazing (Seymour, 1982, Dawson et al., 1977). Surprisingly, Marine Iguana exhibit little
70 departure from the physiological patterns characteristic of terrestrial iguanids (Dawson et al., 1977). For
71 example, the species' aerobic scope, thermal dependences, circulatory responses to diving, nasal salt
72 secreting glands, reliance on anaerobiosis and restricted stamina all quantitatively resemble those of
73 terrestrial iguanids (Dawson et al., 1977, Bartholomew and Lasiewski, 1965). Furthermore, previous studies
74 have identified morphological modifications are often required for aquatic behaviour in reptiles, such as

75 laterally compressed tails, short necks and webbed feet (Seymour, 1982, Motani, 2009). In fact, the tail and
76 feet of Marine Iguana exhibited such little departure from the design of terrestrial iguanids that they did
77 not warrant comment in the initial description of the species (Dawson et al., 1977). To date no studies have
78 reappraised the morphological specialisations of Marine Iguana and quantified variation with terrestrial
79 ancestors. Likewise, despite the central role performance plays in determining an individual's fitness in a
80 specific environment, little information exists on the locomotory ability of Marine Iguana in comparison to
81 terrestrial ancestors.

82 In this study, we examine the morphology and locomotory performance of the Marine Iguana in
83 comparison to their closely related mainland ancestors, the Black Spiny-tailed iguana (*Ctenosaura similis*)
84 and the Green Iguana (*Iguana iguana*). This comparison explores the extent to which modification on the
85 reptilian system is required for successful invasion of the marine environment, thus addressing how an
86 iguanid evolved to become the world's only marine lizard. Further, despite evidence that inter-specific
87 studies require consideration of within-species variation, it is rarely considered in empirical works. As such,
88 we investigate the magnitude of morphological and performance variation between newly identified
89 subspecies of Marine Iguana as there is mounting evidence that the insular populations experience
90 contrasting selection pressures.

91 **Methods:**

92 **Sampling**

93 Morphometric and performance data was collected from 82 Marine Iguana across seven locations
94 within the Galápagos archipelago in 2018. Sampling locations were selected based on Miralles et.al.
95 (Miralles et al., 2017) taxonomic revision, resulting in data from seven populations representing six
96 subspecies of Marine Iguana. Due to both geographic and genetic isolation described in McLeod et al.
97 (MacLeod et al., 2015), we included two populations of *A.c.mertensi* in our subspecies analysis. A further 14
98 Black Spiny-tailed Iguana and 9 Green Iguana were collected from the Florida Keys in 2019. Data collection
99 for this project was authorized by the Galápagos National Park Service (Permit #PC-86-18 - Kate Berry) and
100 US Fish and Wildlife Service (FY 19-07). Ethics and animal handling protocols were approved by the
101 University of the Sunshine Coast (#ANA18130), UNC-Chapel Hill and Universidad San Francisco de Quito
102 (USFQ) Galápagos Science Center (GSC). Twenty morphological measurements, including limb, hip and head
103 dimensions, were collected for each specimen (Figure 1).

104

105 **Video Digitisation and Kinematics**

106 Three-dimensional kinematics of the iguana's stride, for each specimen was filmed running through
107 a portable racetrack. Two Basler Ace USB3 Cameras (aca1920-150um, Basler, Ahrensburg, Germany), with
108 either an 8mm or 16mm Tamron lens, were set on tripods perpendicular to the racetrack to capture both
109 dorsal and lateral views of the specimens. Cameras were synchronised through custom recording software
110 at 200 frames s^{-1} , with a 1920 x 650 pixel resolution. To resolve spatial information, the racetrack space was
111 calibrated using a two-point wand and DLT coefficients derived using easyWand software in the Matlab
112 environment (Hedrick, 2008) (MATLAB 2017b, The MathWorks, Inc., Massachusetts, USA). Seven landmarks
113 were painted on each iguana to mark the pelvis and hindlimb joints, to facilitate the video digitisation
114 (Figure 1). Digitised landmarks were converted to three-dimensional coordinates using 'DLTdv7' digitising
115 software within Matlab (Hedrick, 2008).

116 A total of 262 strides were digitised from videos of Marine Iguana (N=82, n=130), Black Spiny-tailed
117 Iguana (N=14, n=69) and Green Iguana (N=9, n=44). Stride frequency (Hz) stride length (m), mean speed
118 and maximal speed ($m \cdot s^{-1}$) was calculated for each stride. Further, twenty-five angular kinematic variables
119 were calculated to described the movement of the hindlimb segments in relations to one another (Supp.
120 Table S4). The maximum, minimum, midstance and the total angular change value was calculated for each
121 kinematic variable.

122

123 **Analysis**

124 Before analysis, mass, limb lengths and maximum speeds were log-transformed to normalise in R
125 version 3.1.1 (Team, 2015). Analyses were performed firstly to identify patterns of variation among species,
126 and secondly to identify patterns of variation among subspecies of Marine Iguana. Body size differences,
127 speed variation, and speed modulation strategies were explored using lm.R and aov.R models in R where
128 appropriate. The fastest two runs from each lizard were retained in speed analyses to reduce pseudo-
129 replication. We calculated residuals for each length estimate from mass to remove any effects of body size
130 and then explored shape variation using a linear discriminant analysis (LDA) with the lda.R function from
131 the MASS package. Kinematic variables were explored using the lda.R function from MASS. Scores of the
132 LDA were retained and used to estimate ancestral states using the fastANC.R function from the phytools
133 package (Revell, 2012). The topology for phylogenetic trees was modified from (Miralles et al., 2017, Pyron
134 et al., 2013).

135 **Results:**

136 **Body Size Variation in Iguanids**

137 Body mass varied significantly between iguanid species ($F_{2,101}=10.39$, $P<0.0001$; Supp. Figure S1). A
138 Tukey Post Hoc test reported significant variation between Spiny-tailed Iguana and Marine Iguana
139 ($P<0.001$). Marine Iguana had the greatest body mass (mean= 2.18 ± 0.193 , max=6.78kg), Green Iguana had
140 intermediate (mean= 0.582 ± 0.391 , max=3.267kg), and Black Spiny-tailed Iguana the lowest masses
141 (mean= 0.312 ± 0.068 , max=1.015kg). Patterns of SVL variation were consistent with those reported for
142 body mass ($F_{2,101}=10.04$, $P<0.001$).

143 Body mass of male Marine Iguana also varied significantly among subspecies ($F_{6,32}=6.866$, $P<0.001$;
144 Supp. Figure S2). A Tukey Post Hoc test revealed a significant difference in body mass between the two
145 populations of *A. c mertensi* ($P<0.001$), these being sampled from opposing coastlines of San Cristobal
146 (Figure 2).

147 **Evolution of Body Shape in Iguanids**

148 Variation in body shape, independent of size, was also visible among iguanids (Figure 2 & 3). An
149 LDA significantly separated species, with the first function explaining 62.57% of variation ($F_{2,99} = 105.8$,
150 $P<0.001$). Along LD1 Marine Iguana and Spiny-tailed Iguana had similar scores, whereas Green Iguana were
151 positive indicating long necks, tails, forelimbs, toes, and greatest hip depth (Supp. Table S1). The second
152 discriminant explained 37.43% of shape variation, and separated Marine Iguana from mainland ancestors,
153 by greater head depth, long forelimbs, shortest hindlimbs and widest hips.

154 Shape variation was also visible across subspecies of Marine Iguana (Figure 2). The first discriminant
155 function explained 34.80% of variation between species ($F_{6,73}=12.83$, $P<0.001$; Supp. Table S2) with a Tukey
156 Post Hoc test indicating significant variation between *A. c mertensi* (western coast population) and all other
157 subspecies (Figure 2).

158 **Evolution of Sprint Speed in Iguanids**

159 There was no significant effect of mass on maximal speed in iguanids ($F_{1,90}=1.913$, $P=0.17$), however
160 there was a significant effect of species ($F_{1,90}=22.477$, $P<0.0001$) and no significant interaction between the
161 two ($F_{1,90}=1.032$, $P=0.36$). Spiny-tailed Iguana had the greatest maximal speed recorded (Max $5.932 \text{ m}\cdot\text{s}^{-1}$,
162 Mass = 0.44 kg), Green Iguana had intermediate speeds of $4.82 \text{ m}\cdot\text{s}^{-1}$ max, (Mass = 0.705kg) and Marine
163 Iguana had the lowest maximal speed of $3.291 \text{ m}\cdot\text{s}^{-1}$ (Mass = 2.928kg; Supp. Figure S3).

164 Yet among Marine Iguana subspecies, there was a significant effect of mass on maximal speeds
165 ($F_{6,61}=6.373$, $P=0.042$), but no significant effect of subspecies ($F_{6,61}=2.090$, $P=0.067$) with a significant
166 interaction between the two ($F_{6,61}=2.325$, $P=0.043$; Supp. Figure S4). The only significant difference in
167 maximal speed reported in a Tukey Post Hoc test was between *A. c cristatus* and *A. c hassi* ($P<0.001$).

168 **Speed Modulation Strategies in Iguanids**

169 Stride length significantly influenced speed in iguanids ($F_{1,228}=45.32$, $P<0.0001$), but also varied
170 among species ($F_{2,228}=47.56$, $P<0.0001$) with a significant interaction between the two ($F_{2,228}=11.34$,
171 $P<0.0001$). A Tukey Post Hoc test revealed the Marine Iguana had the greatest mean stride length
172 (0.508m), with no difference between the Green Iguana (0.391m) and Spiny-tailed Iguana, (0.409m; Figure
173 4). Similarly, stride length significantly affected speed among Marine Iguana subspecies ($F_{1,114}=34.242$,
174 $P<0.001$), with a strong effect of subspecies ($F_{6,114}=45.32$, $P=0.005$) and no significant interaction of the two
175 ($F_{1,114}=1.911$, $P=0.08$). A Tukey Post Hoc test identified that the greatest variation in stride length was
176 between *A.c trillmichi* and the south east coast population of *A.c mertensi*.

177 Both Stride frequency and species significantly influenced stride speed in iguanids (Freq:
178 $F_{1,228}=76.55$, $P<0.001$; Species: $F_{2,228}=8.970$, $P<0.005$), with a significant interaction between frequency and
179 species ($F_{2,228}=8.970$, $P<0.0001$). Spiny-tailed Iguana had the highest stride frequency (mean=6.23 Hz),
180 followed by Green Iguana (mean=5.40 Hz) and Marine Iguana (mean=4.16 Hz). Further, across Marine
181 Iguana subspecies there was a significant effect of stride frequency on stride speed ($F_{6,114}=6.766$, $P<0.0001$),
182 a moderate effect on stride speed ($F_{1,114}=10.009$, $P<0.005$) and no significant interaction ($F_{1,114}=0.861$,
183 $P=0.526$). A Tukey Post Hoc test reported the *A.c trillmichi* (5.648 Hz) was significantly higher than all
184 subspecies except from *A.c cristatus* ($P<0.001$).

185 Combining the linear regression parameters from stride length and stride frequency indicated that
186 iguanids modulate speed primarily by increasing stride length, however, the extent of use varied (Supp.
187 Table S3). The intercept and slope reported that Marine Iguana take longer strides compared to their
188 mainland ancestors. Intraspecific investigation revealed that this trend isn't consistent across all Marine
189 Iguana subspecies, with *A.c mertensi*, *A.c godzilla* and *A.c hassi* all modulate speed through primarily with
190 their stride frequency.

191 **Iguanid Stride Kinematics**

192 Variation in iguanid stride kinematics was visible in an LDA, where the first discriminant explained
193 88.86% of variation ($F_{2,228}=195.8$, $P<0.0001$) and separated Marine Iguana from mainland ancestors. The
194 second discriminant explained 11.34% of variation ($F_{2,228}=25.05$, $P<0.0001$), with overlap in kinematics
195 being observed among iguanids (Figure 5a). Overall, Marine Iguana exhibited the greatest knee angles
196 (minimum and maximum), metatarsal angles (at midstance) and depression of the femur (maximum).

197 **Stride Kinematics in Marine Iguana Subspecies**

198 Differences in stride kinematics between Marine Iguana subspecies were also evident, with the first
199 discriminant explained 45.39% of the variation ($F_{6,121}=61.95$, $P<0.0001$; Figure 5c). A Tukey Post Hoc test
200 reported significant differences between *A.c godzilla* and *A.c hassi*, *A.c mertensi* (southeast and western
201 populations), *A.c trillmichi* and *A.c ventustissimus*. The second discriminant explained 26.29% variation

202 ($F_{6,121}=35.88$, $P<0.0001$), with a Tukey Post Hoc test reporting significant variation between *A.c hassi* and *A.c*
203 *cristatus*, as well as between *A.c godzilla* and *A.c trillmichi*, *A.c ventustissimus* and *A.c mertensi* (west coast
204 population). Overall, the two populations of *A.c mertensi* varied from one another significantly ($P<0.0001$).
205

206 **Discussion:**

207 **Ecomorphological Associations in Iguanids**

208 *Iguanidae* is one of the largest and most diverse families of lizards in the Western hemisphere, with
209 species having radiated extensively into habitats ranging from rainforests to deserts, urban environments
210 and rocky coastlines (Etheridge and de Queiroz, 1988). Studies suggest that specialisation for one habitat is
211 generally assumed to occur at the cost of reduced fitness in another (Clemente et al., 2013, Vanhooydonck
212 and Van Damme, 2003). As such, we hypothesised that the unique transition to a marine environment in
213 iguanids would induce variation in morphology and performance trade-offs in Marine Iguana.

214 Body size is arguably the most fundamental design characteristic defining differences in closely
215 related species and has been observed to change most readily on islands (Case and Schwaner, 1993, Petren
216 and Case, 1997). Previous studies have shown islands harbour either gigantic or dwarf forms compared to
217 mainland relatives, with size shifts often being a response to ecological pressures (Petren and Case, 1997).
218 Our findings of greater body size in Marine Iguanas align with those of Petren and Case (Petren and Case,
219 1997), who reported that the island endemic chuckwallas, were up to five-fold larger in body mass
220 compared to mainland species. Many theories exist that may explain the evolution of body size among
221 iguanids. Shaw (Shaw, 1945) hypothesised that larger size was simply retained in genera that were first to
222 diverge, whereas other studies suggest that recent evolution of small body size in mainland forms is
223 consistent with a pattern of Holocene dwarfism (Case and Schwaner, 1993, Pregill, 1986). Invasive Green
224 Iguana and Spiny-tailed Iguana were sampled from urban environments, therefore their smaller body sizes
225 may be associated with anthropogenic effects. Yet, the shift to larger sizes in *Amblyrhynchus* is likely a
226 response to island biogeography and depauperate environments (Case and Schwaner, 1993). Wikelski
227 (Wikelski, 2005) proposed that evolution of size in Marine Iguana was a result of larger sizes being favoured
228 because of the importance of thermal inertia on underwater foraging ability, as larger individuals are less
229 sensitive to heat loss (Pincheira-Donoso et al., 2013).

230 Variation in shape also reflected specialisation for different environments. Marine Iguanas
231 exhibited wide heads, blunt snouts, and short necks beneficial for underwater grazing and streamlined
232 aquatic locomotion (Seymour, 1982, Motani, 2009). Long forelimbs are often associated with arborealism,
233 as such we suggest that Marine Iguana have developed longer forelimbs to improve their manoeuvrability
234 and stability when grazing underwater (Damme et al., 1998). In contrast, mainland iguanids had greater
235 head neck lengths, longer tails, longer hindlimbs and toes than Marine Iguana, likely associated with
236 varying degrees of arborealism. Previous studies have identified correlations between relatively shorter
237 limbs and perch diameter in *Tropidurus*, *Draco* and Chameleons, hypothesised to be a trade-off between
238 sprint speed and clinging ability (Kohlsdorf and Navas, 2012, Ord and Klomp, 2014, Hagey et al., 2017). As
239 such, this trade-off likely explains differences in limb length between Spiny-tailed Iguana and Green Iguana.

240 As Green Iguana are arboreal specialists it is relatively intuitive that they would have longer limbs, toes and
241 tails as these traits benefit climbing ability and balance, whereas Spiny-tailed Iguana are only facultatively
242 arboreal and thus do not require as notable specialisations. These morphological specialisations for aquatic
243 behaviour may be associated with reduced performance in terrestrial environments (Clemente et al., 2013).

244 Sprint speed is used as a performance measure in ecomorphological studies as it can have a direct
245 impact on fitness and is associated to limb morphology, body size, muscle fibre composition and the natural
246 environment (Vanhooydonck et al., 2002). Our findings were consistent with existing literature, with the
247 world record sprint speed for lizards having been attained by a Black Spiny-tailed Iguana (Garland Jr, 1984).
248 It would be expected that a species from an open habitat, such as Spiny-tailed Iguana, would favour the
249 evolution of traits that increase predator escape, such as greater sprint speed (Clemente et al., 2009).
250 Whereas climbing species, such as Green Iguana, may be disadvantaged in terrestrial running as a result of
251 trade-offs associated with their performance in vertical environments, such as clinging ability and sure-
252 footedness (Clemente et al., 2013, Zaaf et al., 2001). Semi-aquatic reptiles may represent an intermediate
253 phenotype between terrestrial and aquatic lifestyles, which would require biomechanical construction that
254 enables locomotion on both land and in water (Seebacher et al., 2003). Therefore, we propose that
255 navigation of rocky and wet substratum imposes conflicting demands on the locomotory ability of Marine
256 Iguana, having likely occurred at the cost of reduced sprint speed in a terrestrial environment. This
257 performance trade-off may be a contributing factor as to why so few extant reptilian lineages have invaded
258 marine environments.

259 Sprint speed is a result of combining a particular stride length with a particular stride frequency,
260 where an organism is able attain an identical speed solely by increasing either stride frequency or length
261 (Clemente et al., 2013, Garland Jr and Losos, 1994). Iguanids were found to modulate speed primarily
262 through changes in stride length, however the percentage of change varied subtly between species (Figure
263 4 & Supp. Table S3). Previous studies have found that ground-dwelling lizards achieve high speeds by
264 increasing their stride length, whereas climbing species modulate speed through stride frequency
265 (Clemente et al., 2009, Clemente et al., 2013). Therefore, the lack of difference in speed modulation
266 strategies in iguanids, despite notable variation in sprint speed and habitat, suggests that predicted
267 performance differences may not be as general as previously thought. Further, variation in the percentage
268 of change in speed from stride length in iguanids may allow mainland ancestors to avoid lower speeds and
269 overcome challenges of arborealism by adjusting stride frequency. Marine Iguana took significantly longer
270 strides than mainland ancestors despite having relatively shorter hindlimbs. This association may be best
271 explained by their stride kinematics and axial bending, which can serve to effectively increase hindlimb
272 length.

273 Marine Iguana were found to increase knee, ankle and metatarsal angles, as well as femur rotation
274 and retraction, all of which significantly contribute to an increase in effective limb length and thus an

275 increase in stride length (Figure 5). An increase in effective limb length would increase the height of the
276 body, which we hypothesise is to improve navigation of wet substratum. Reily and Elias (Reilly and Elias,
277 1998) hypothesised that semi-erect locomotion derived in alligators reflects the varying degrees of
278 terrestrially. We observed striking similarities between the hip, femoral adduction and knee angles of
279 *Alligator mississippiensis* and Marine Iguana (Blob and Biewener, 2001, Gatesy, 1997, Reilly and Elias,
280 1998). Therefore, our findings support that postural changes in reptiles may be associated with
281 specialisation of aquatic environments (Reilly and Elias, 1998). Further, this effect may be disadvantageous
282 to mainland iguanids, as lengthening limbs would hinder climbing ability by moving the body centre of mass
283 away from the surface (Cartmill, 1985, Clemente et al., 2013, Zaaf et al., 2001). Unlike previous studies that
284 have found changes in kinematics to be more pronounced than changes in morphology, we found overlap
285 in iguanid joint angular changes (Clemente et al., 2013). This suggests that the increase in stride length in
286 Marine Iguana may be more complex than being achieved solely through hindlimb joint angular changes.
287 We hypothesise that lateral bending of the trunk, a common aspect of aquatic locomotion, may also
288 increase their stride length (Ritter, 1992). Previous studies have identified that a travelling wave of lateral
289 bending increases stride length and contributes to propulsive force of locomotion (Ritter, 1992). Therefore,
290 we recommend investigation into the extent of lateral bending in Marine Iguana and hypothesise that it,
291 along with a relative increase in hindlimb length through hindlimb joint changes, is likely the mechanism
292 behind their increased stride length. Furthermore, we suggest that these biomechanical differences are
293 associated with the demands of the marine environment, constraining terrestrial locomotory ability.

294 **Transitions to Aquatic Environments**

295 Dawson et al. (Dawson et al., 1977) suggested that the physiological grade represented by the
296 Iguanidae family was in a sense 'pre-adaptive' for aquatic habitats. Curiously, we found size and shape
297 variation was more pronounced among subspecies of Marine Iguana than between iguanids, with some
298 subspecies being up to four-fold larger in mass than others (Figure 2). Further, some subspecies were
299 smaller than mainland ancestors, suggesting that increased size is not associated to the aquatic transition,
300 and more likely associated with island biogeography and resource productivity (Wikelski, 2005, Wikelski
301 and Trillmich, 1997). Historic studies also identified divergence in morphology between different
302 populations of Marine Iguana (Chiari et al., 2016, Wikelski, 2005, Wikelski and Trillmich, 1997), although the
303 ecological considerations of such variation requires further investigation. Interestingly, *A.c mertensi*, *A.c*
304 *godzilla* and *A.c hassi* were found to modulate speed primarily with their stride frequency, thus differing to
305 the primary strategy used by iguanids (Supp. Table S3). Further evidence for significant subspecies
306 diversification was the extent of variation in their stride kinematics, differing on very localised scales, such
307 as between populations of *A.c mertensi* from opposing coastlines (Figure 5c). These intraspecific findings
308 demonstrate that the morphological and performance variation between iguanids would not inhibit the

309 initial transition to aquatic life, thus raising the question why so few reptiles have exploited aquatic
310 environments.

311 We found evidence that specialisation to the marine environment in Marine Iguana has led to a
312 trade-off in their performance (sprint speed) in terrestrial environments. Similarly, extant crocodylians,
313 despite having developed an array of movement patterns (high walk and gallop), have reduced locomotory
314 performance in terrestrial environments (Seebacher et al., 2003, Webb and Gans, 1982). Reduced
315 performance in a terrestrial environment likely poses little risk to large-bodied apex predators, whereas in
316 iguanids, a performance trade-off would likely incur increased predation, as nearly all continents are
317 occupied by mammalian predators (Dawson et al., 1977). Studies have suggested that the success of
318 Marine Iguana is largely due to the lack of predation in Galápagos, where there are few consequences of
319 being slow on land (Berger et al., 2007). Therefore, we suggest that other iguanids have not invaded
320 aquatic environments due to the trade-off in their terrestrial performance, which may be detrimental to
321 survival.

322 Further, the unique biogeography of the Galápagos may also explain why other lizards have not
323 invaded marine environments. The equatorial position of the Galápagos archipelago, and minimal variation
324 in seasons, is an important ecological consideration for heliothermic lizards (Seymour, 1982). Continuous
325 upwelling of nutrient-rich, cold waters, containing soft-bodied macrophytic algae, is quite localised, with
326 comparable upwelling regions found along the coasts of Namibia, Chile, California and Northwest Africa, all
327 being outside the geographic range of Iguanids (Berger et al., 2007). Furthermore, lizard diversity is low in
328 all of these regions and the presence of mammalian predators likely explains why other species have not
329 invaded marine environments in a similar manner to that of the Marine Iguana.

330 **The Mysteries of Marine Iguana – Subspecies Diversity**

331 Oceanic islands have long been recognised as natural laboratories for the study of evolution, as
332 significant genetic and morphological variation often occurs as a consequence of the exploitation of free
333 niches or multiple colonisation events (Van Valen, 1965, Losos et al., 1998). Studies have documented a
334 range of body size within Marine Iguana, however, majority of findings have been based off samples
335 collected over two decades ago (Wikelski, 2005, Wikelski and Thom, 2000, Wikelski and Trillmich, 1997,
336 Chiari et al., 2016, Miralles et al., 2017). Surprisingly, we found that subspecies of Marine Iguana exhibited
337 more variation in size, shape and performance than between iguanids, despite occupying similar niches
338 across the Galápagos.

339 We found significant variation in body size between subspecies of Marine Iguana, with some
340 subspecies being up to four-fold larger in mass than others. Further, some subspecies were smaller than
341 mainland ancestors, suggesting that increased size is not associated to marine life and supports greater
342 association with island biogeography and resource productivity (Wikelski, 2005).

343 Despite our interspecific findings suggesting that island biogeography cause shifts to larger body
344 sizes in iguanids, patterns of size variation in Marine Iguana varied significantly from historical records
345 documented by (Chiari et al., 2016) . We found there has been a six-fold reduction in body size in Marine
346 Iguana over the past two decades. In contrast, (Wikelski, 2005) found that size increased over the 19th
347 century. Over the past two decades, there have been two strong El Niño events (1997-1998 and 2015-
348 2016), which caused periodic disappearance of the iguana's preferred food source and thus population
349 crashes and depressed growth rates (Laurie, 1990, Romero and Wikelski, 2002). Larger body sizes are
350 strongly selected against during El Niños, whereas sexual selection drives an increase in size (Wikelski,
351 2005). During the 1997-1998 El Niño, individual Marine Iguana temporarily shrunk up to 20% of their body
352 length, hypothesised to be an adaptive response to low food availability (Wikelski and Trillmich, 1997). We
353 suggest that over time, climatic variation has had a more pronounced effect on body size than sexual
354 selection. As the frequency and severity of El Niño events increase, we suspect that this selection will
355 continue to favour a shift towards smaller sizes across the archipelago (Cai et al., 2014). Consequently, the
356 shift in body size is complex, historically being driven by island biogeography, whereas now, climate is likely
357 having a more pronounced effect on the patterns.

358 Historic studies also identified divergence in morphology between different populations of Marine
359 Iguana (Chiari et al., 2016, Miralles et al., 2017). Similar to our findings in body size, morphology and
360 locomotory ability was in some instances more varied among Marine Iguana subspecies than between
361 iguanid species, with *A.c mertensi*, *A.c godzilla* and *A.c hassi* modulating speed primarily with stride
362 frequency. However, modulating speed with stride frequency did not appear to be associated with the
363 highest attainable sprint speeds in Marine Iguana. Subspecies found on San Cristobal also had notable
364 differences in their stride kinematics, despite records of hybridisation and divergence times (MacLeod et
365 al., 2015). Therefore, we suggest that variation in design correlates with performance differences in Marine
366 Iguana, which is likely associated to localised, structural differences in habitat. This illustrates that the form-
367 function relationship is complex and highlights the ecomorphological mysteries of Marine Iguana. Further
368 investigation is recommended into microhabitat use by Marine Iguana to determine the mechanisms
369 behind their unique variation.

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375

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377

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380

381 **Data Accessibility:** Data will be submitted along the manuscript via Figshare.

382 <https://doi.org/10.6084/m9.figshare.12121449.v2>

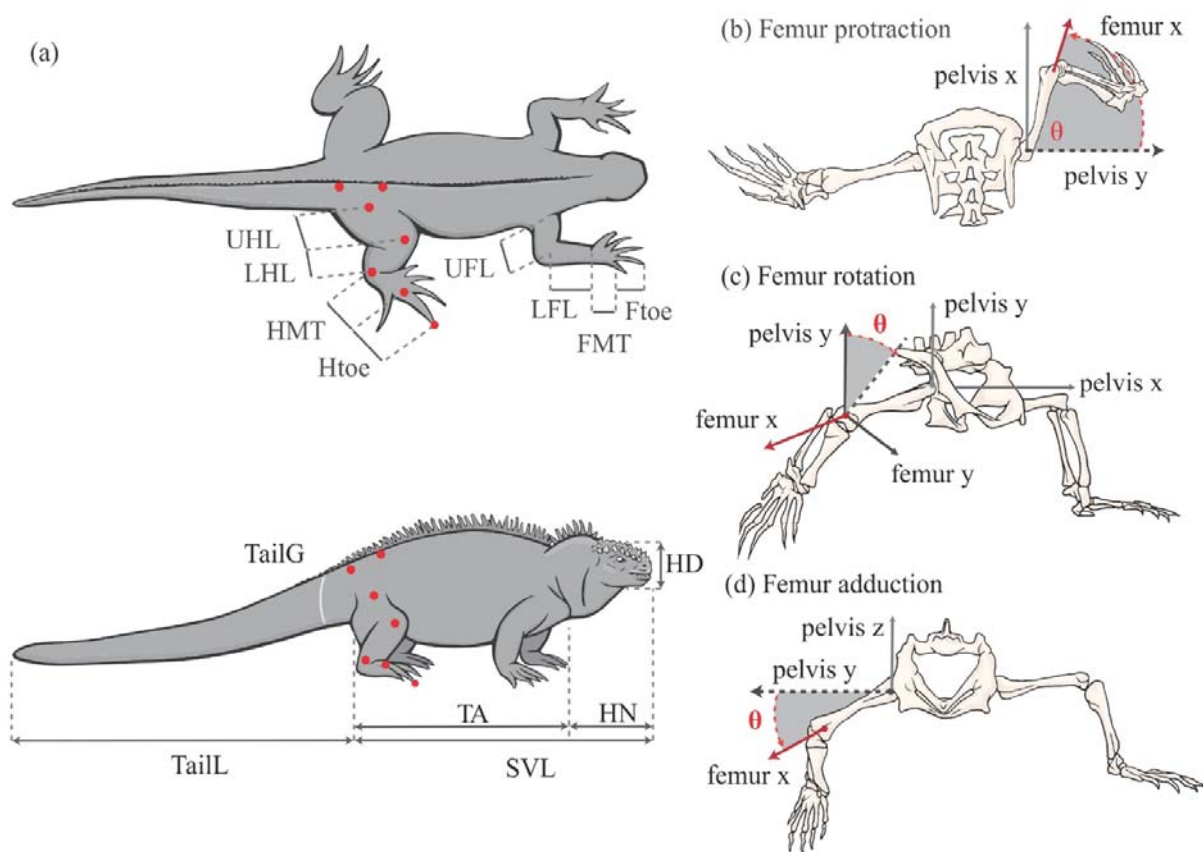
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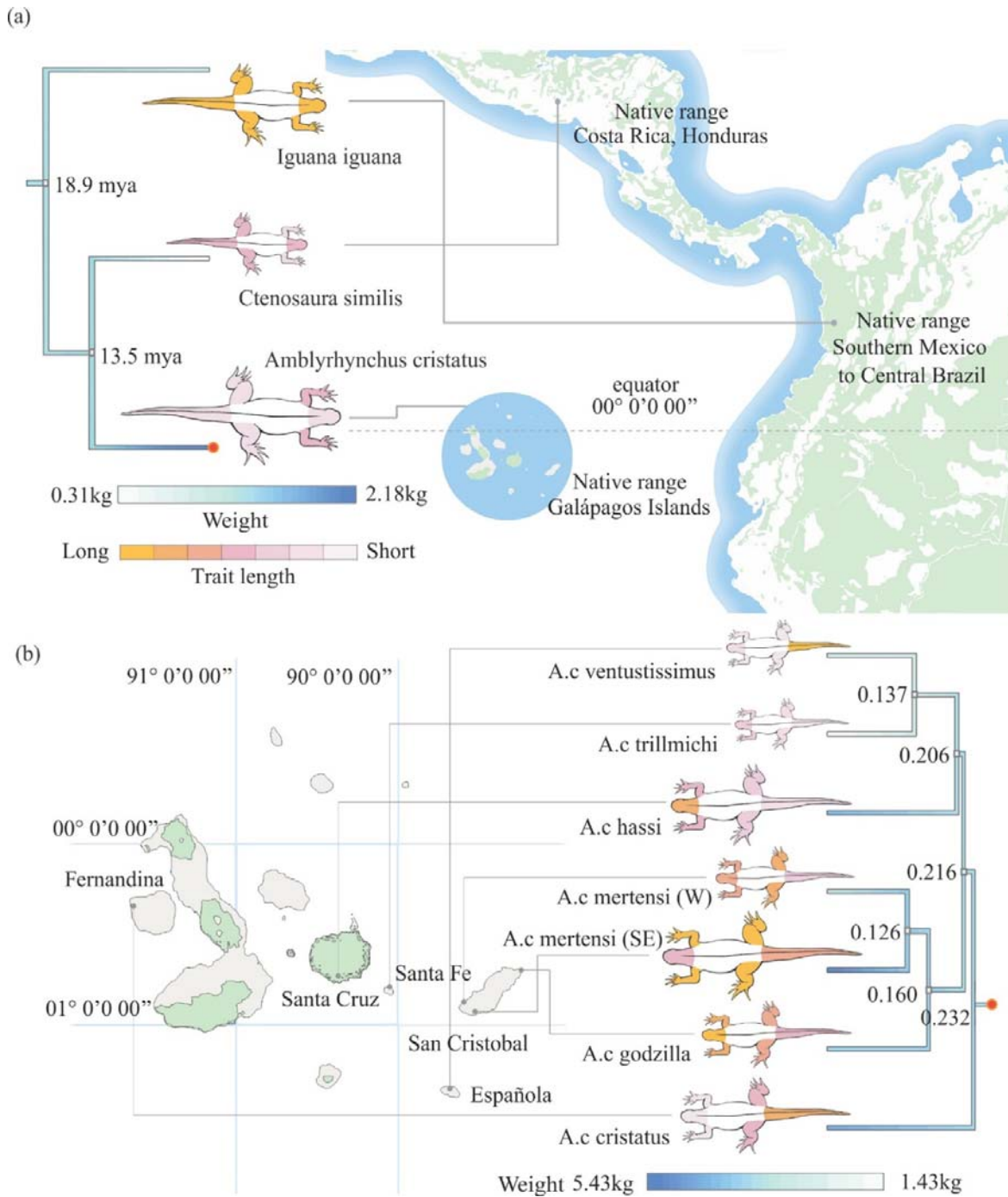
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486 **Figures:**



487

488 **Figure 1: a)** Morphological measurements and landmarks used in video digitisation. Also illustrated is the
489 three movements of the femur relative to the pelvis including **b) Femur protraction**, **(c) femur rotation** and
490 **(d) femur retraction**.

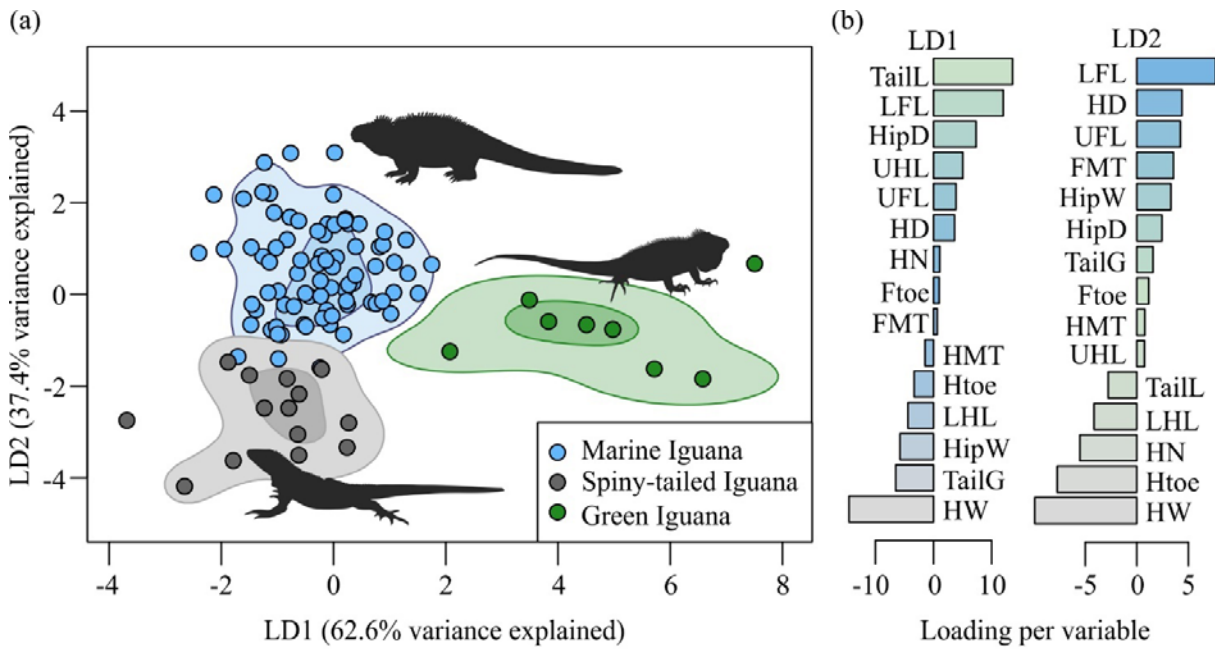


491

492 **Figure 2: (a)** Body shape and size variation between Marine Iguana, Green Iguana and Black Spiny-tailed
 493 Iguana. **(b)** Body size and shape variation in subspecies of Marine Iguana (juveniles excluded).

494 Residual means of morphometric traits are expressed on the iguanid bodies. The ancestral state estimates
 495 for body mass are shown along phylogenetic trees. Morphometrics represented include head-neck length
 496 (HN), forelimb length (FL), hindlimb length (HL) and tail length (tailL). Iguanid bodies are scaled according to
 497 mean weight.

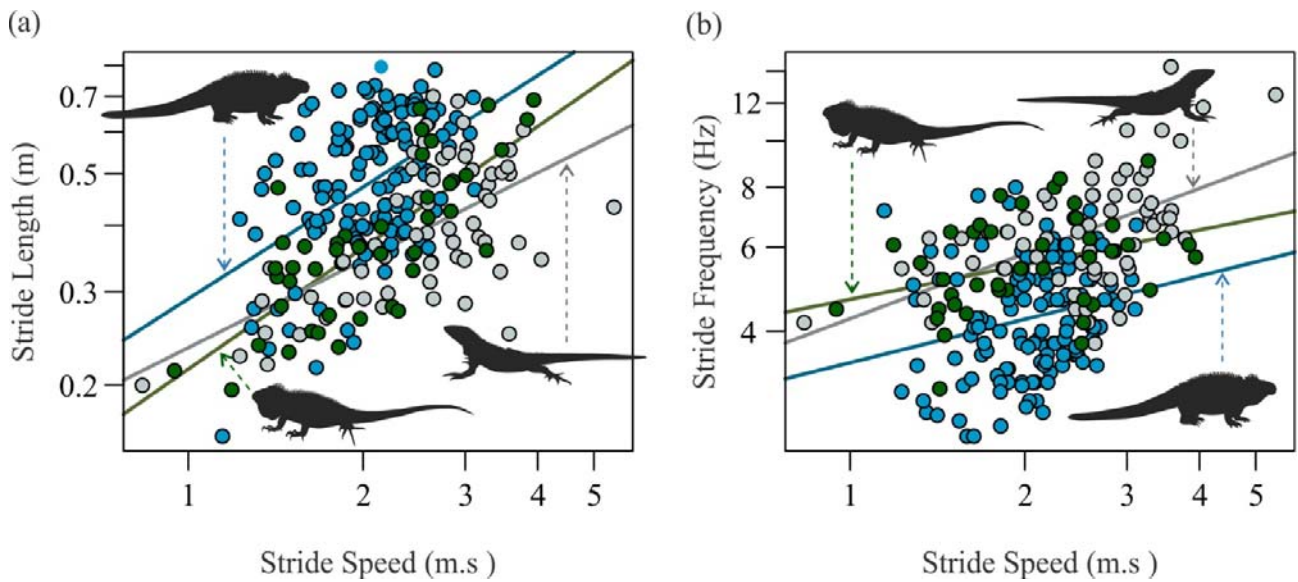
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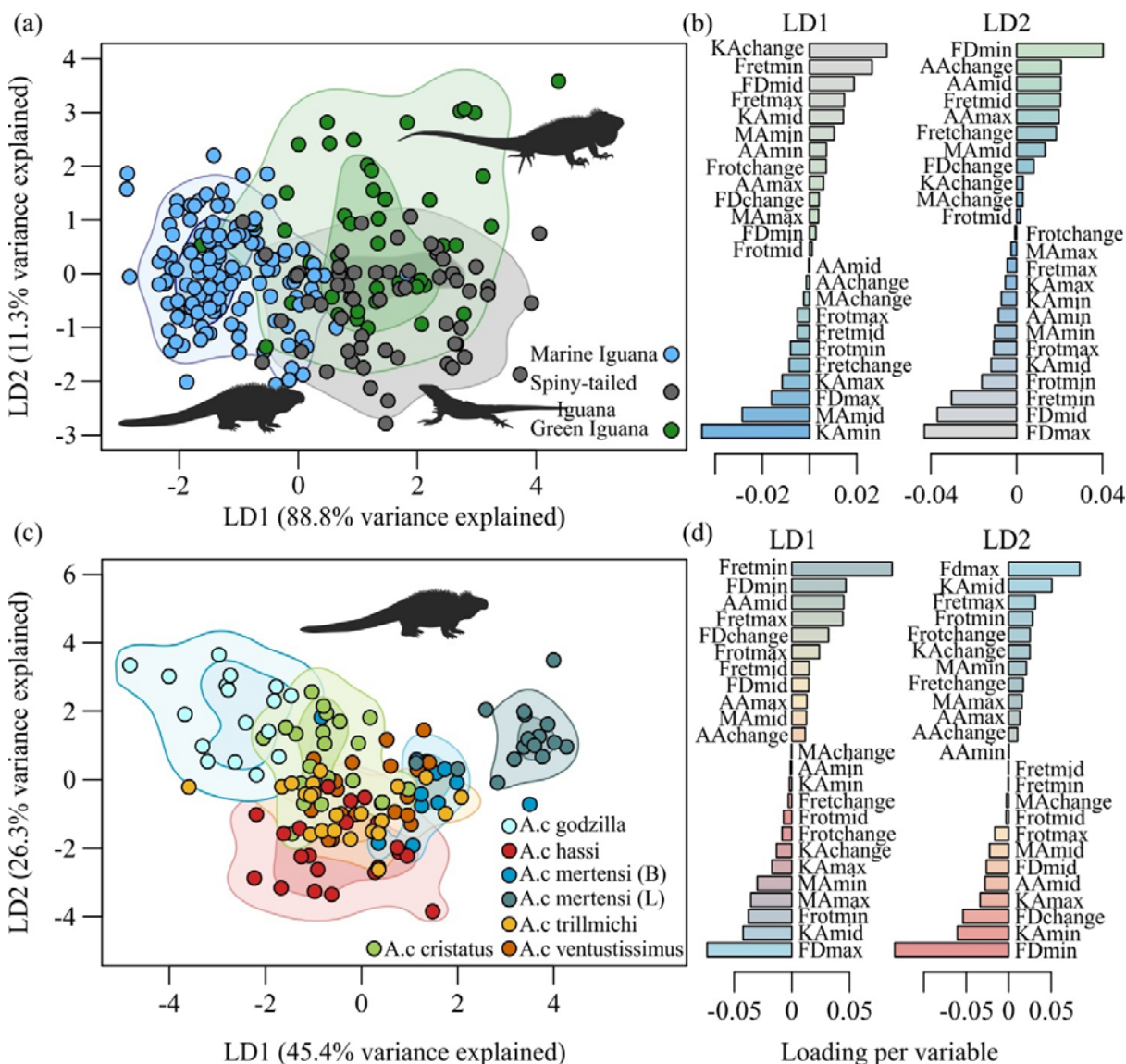
500

501 **Figure 3:** (a) A linear discriminant function illustrating shape variation between iguanids. Kernel density
 502 ellipses for each species illustrate 90% and 70% of the data distribution. (b) Graph of morphometric trait
 503 loadings from LD analysis.



504

505 **Figure 4:** (a) Linear regression illustrating the relationship between log10 stride frequency and log10 stride
 506 speed for the three species of iguanids. (b) Linear regression illustrating the relationship between and log10
 507 stride and log10 stride speed length for iguanids.



508

509 **Figure 5: (a)** LD function for variation in stride kinematics between iguanids, two of the fastest runs for
 510 each individual was retained (Marine Iguana N=65, Black Spiny-tailed Iguana N=35 & Green Iguana N=22).
 511 Kernel density ellipses for each species illustrate 90% and 70% of the data distribution. **(b)** Loadings for
 512 iguanid stride kinematics in (a). **(c)** LD function for variation in stride kinematics between subspecies of
 513 Marine Iguana. Kernel density ellipses as for (a). **(d)** Loadings for (c).