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**Supplementary materials** (and Elsewhere): Three-Legged Lizards and the Study of Evolutionary Adaptation."  
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**Title:** Pirates of the Caribbean (and elsewhere): Three-Legged Lizards and the Study of Evolutionary Adaptation

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## **Cause of Limb Loss: Direct observation**

One set of longitudinal observations reveals how a limb may be lost via interactions with the abiotic environment. In early July 2016, a male *A. sagrei* was found with its hindlimb pinned by a trash can lid in Jacksonville, north Florida (USA). The lid was removed, and the lizard fell to the ground and ran away. A few hours later, it was seen perched on a fencepost with its upper hindlimb swollen and the limb below the knee mangled. The animal was seen repeatedly over the course of the next eight days and appeared to behave normally, but its lower hindlimb withered away to the point that it was attached to the upper hindlimb by a narrow band of tissue, while the stump at the bottom of the upper hindlimb appeared to be healing (Losos, pers. obs.). Three days later, the lower limb had fallen off and the lizard was seen mating with a large female, as well as directing dewlap extensions and lateral pushup displays toward a smaller male. Five weeks after the injury, the leg appeared to have completely healed and the lizard continued to be active and in good condition. Following the observation that the wound had successfully healed in the wild and the lizard appeared to be healthy and behaving normally, JBL travelled to Jacksonville to observe this lizard in person. On capture, it was confirmed that the lizard (hereafter, "Lizard 38") had lost its right hindlimb up to the knee (i.e., 60% loss; Table 1, ID 38). Lizard 38 was collected for laboratory measurements, and we include assessments of Lizard 38's locomotor performance relative to other brown anoles in an analysis below.

## **Comparative performance: sprint speed, endurance, and jumping**

We start with the caveat that statistical power is weak in our comparative tests as most datasets only included a single limb-reduced lizard. Therefore, caution should be applied when interpreting model outputs from all statistical tests. Nevertheless, we present a detail analysis of the datasets available to us.

1. Eastern fence lizard (*Sceloporus undulatus*) missing 60% of its right hind limb (Fig S52). This lizard demonstrated comparable maximal sprint speed to other healthy male lizards in the population ( $\chi^2 = 0.15$ ,  $p = 0.703$ ; methods described in (Boronow and Langkilde 2010). The relationship between maximum sprint speed, injury status, and body size (snout-vent length, SVL) in male *S. undulatus* was investigated using a generalized linear model (GLM) fitted using the *glm* function in R (R Core Team 2021). Maximum sprint speed (m/s) was the response variable, injury was included as a categorical predictor, and log-transformed svl as a continuous covariate. The model's assumptions were assessed using the *check\_model* function from the *performance* package (Lüdecke et al. 2021), which included checks for linearity, homoscedasticity,

normality of residuals. *P*-values were obtained from a Type III ANOVA using the *Anova* function from the *car* package (Fox and Weisberg 2019) to determine the significance of the fixed effects (limb loss and log(sv)) on maximal sprint speed.

2. European common lizard (*Zootoca vivipara*; Fig S65; Miles, *unpub. data*). Although the extent of limb loss was not recorded in this study, injured female lizards had comparable maximal sprint speed compared to other adult females from the same population. Two females with missing forelimbs could sprint as fast as uninjured adult females (n=57 uninjured lizards;  $\chi^2 = 0.001$ ,  $p = 0.973$ ), while one female missing a hindlimb also showed no significant difference in maximal sprint speed (n=1 injured vs. n=57 uninjured lizards;  $\chi^2 = 2.33$ ,  $p = 0.127$ ). Standard methods were followed to assess sprinting and endurance in these lizards. To test for differences in endurance based on forelimb injury, a linear mixed-effects model was employed using the *lmer* function from the *lme4* package (Bates et al. 2015). The model included log-transformed max\_vel as the response variable, forelimb injury as a fixed effect, and SVL as a random effect to account for potential size-related variations. The significance of the fixed effect was determined using a Type III ANOVA with the *Anova* function from the *car* package. The resulting *p*-value for the effect of forelimb injury on max\_vel was obtained and reported.

3. Cuban brown anole (*Anolis sagrei*; Table 1, ID 38, “Lizard 38”; see “Cause of Limb Loss” section in the main manuscript). Sprint speed was measured on a 3m long, flat racetrack. A white dot was drawn on the dorsum and lizards were placed at the start of the track; if lizards did not immediately sprint then locomotion was induced via clapping behind it. All sprint trials were conducted by C.V. Anderson. Two synchronized high-speed cameras recorded both lateral and dorsal views at 500 frames per second (see (Bergeson 2022) for full methods). Camera calibration and video digitization was conducted using DLTdv8 (Hedrick 2008) by B. Doshna, and statistical analyses were conducted in R. All data and code are available as supplementary materials. We compared the sprinting ability of Lizard 38 to eight similar sized adult male conspecifics wild-collected from the same populations. Only maximal sprint data were assessed for each individual lizard and body size was included as a covariate in all models. Surprisingly, we observed no differences in maximal velocity (Fig 3A;  $\beta = 0.11$ ,  $t(6) = 0.27$ ,  $p = 0.785$ ) or maximal acceleration ( $\beta = 0.36$ ,  $t(6) = 0.71$ ,  $p = 0.476$ ) between Lizard 38 and fully-limbed conspecifics (n=8). The effect of body size was not statically significant for either analysis (velocity,  $\beta = -1.30$ ,  $t(6) = -0.92$ ,  $p = 0.358$ ; acceleration,  $\beta = -2.39$ ,  $t(6) = -1.33$ ,  $p = 0.184$ ). The relationship between maximum velocity or acceleration, injury status, and body size (snout-vent length, svl) in *A. sagrei* was investigated using a generalized linear model (GLM) fitted using the *glm* function in R. Log-transformed max velocity or acceleration were the response variables, injury was included as a categorical predictor, and log-transformed SVL as a continuous covariate. The model's assumptions were assessed using the *check\_model* function from the *performance* package (Lüdecke et al. 2021), which included checks for linearity, homoscedasticity, and normality of residuals. *P*-values were obtained from a Type III

ANOVA using the Anova function from the car package (Fox and Weisberg 2019) and from the model summary to determine the significance of the fixed effects (injury and log(sv)) on log-transformed maximal velocity.

Instead, there were substantial differences in the degree of trunk undulation as measured across eight discrete points on the caudal spine (Fig S3C). Using a MANOVA of all spinal points combined as the response variable with limb loss and body size (SVL) as fixed effects, we found no effect of body size on the combined patterns of lateral flexion along these spinal points ( $F_{(1,23)} = 0.22$ ,  $p = 0.982$ ), but there was a strong effect of limb loss ( $F_{(1,23)} = 7.73$ ,  $p < 0.001$ ). We then conducted individual ANOVAs on each spinal point with the same fixed effects; p-values are reported in Fig 3B. In short, Lizard 38 exhibited substantially higher lateral flexion of the mid-spine (spinal points 4-6).

4. Puerto Rican crested anole (*Anolis cristatellus*; Table 1, ID 10, “Lizard 10”). Sprint speed was measured as the vertical sprinting ability on a 1.5m long, 7.5cm wide racetrack positioned at 104.5° incline. The racetrack consisted of a large oak log (*Quercus* sp.) that was cut to 7.5cm wide: curvature of the log was negligible, so the racetrack was effectively flat but with natural bark substrate. Thin white tape was placed horizontally every 10cm on the log. A dark hide filled with paper towels was fixed to the top of the racetrack as a refuge toward which lizards could run. Sprinting trials were filmed using Canon EOS R50 mirrorless cameras at 120 FPS. Maximal sprint data were assessed for each individual lizard and body size was included as a covariate in all models. We compared the sprinting ability of Lizard 10 to nine similar-sized adult male conspecifics wild-collected from the same populations. As with Lizard 38, we observed no difference in maximum velocity (Fig 3D;  $\beta = -0.15$ ,  $t(7) = -0.57$ ,  $p = 0.571$ ) or maximum acceleration ( $\beta = -0.18$ ,  $t(7) = -0.38$ ,  $p = 0.702$ ) between Lizard 10 and other male *A. cristatellus*. There was no significant effect of body size in either test (velocity,  $\beta = 3.70$ ,  $t(7) = 0.131$ ,  $p = 0.191$ ; acceleration,  $\beta = 2.01$ ,  $t(7) = 0.39$ ,  $p = 0.697$ ). The same statistical approaches were used here as with *A. sagrei* previously.

To assess jumping, Lizard 10 and ten other male *A. cristatellus* were encouraged to jump from a horizontal palm branch that was approximately 10cm wide and positioned approximately 50cm from the ground; this represents an ecologically relevant performance test for this species, which usually perches on branches of this size at this height and will jump to the ground to forage. Each lizard conducted three jumping trials. The landing location of each jump was recorded, and the jump distance was measured as the horizontal distance across the ground from the launch location to the landing location. Only the furthest (maximal) jump was analyzed for each lizard. We investigated the relationship between a lizard's maximum jump distance, snout-vent length (SVL), and injury status using a linear model with maximum jump distance as the response variable and SVL and injury status as predictor variables. To test the significance of each predictor variable while controlling for the other, we conducted a Type III ANOVA (as in previous methods). The main effect of body size (SVL) was not statistically

significant ( $F_{(1,8)} = 1.19, p = 0.307$ ), but the main effect of injury status (Lizard 10 versus all other lizards which did not have any limb loss) was statistically significant ( $F_{(1,8)} = 5.86, p = 0.042$ ). Taken together, when accounting for differences in body size among lizards, Lizard 10 jumped shorter distances than fully limbed lizards.

### **Sprinting: Compensating for limb loss**

Lizards 10 and 38 appeared to employ different compensatory strategies to maintain comparable sprinting speed with uninjured lizards. In Lizard 38 (*A. sagrei*; Fig 3A-C), an increase in mid-body lateral body flexion may have increased stride length, allowing him to move faster than if he had exhibited the same level of flexion as uninjured lizards. This strategy appears effective, as Lizard 38 had a faster maximal sprinting speed than expected for his body size despite substantial hindlimb loss. Lizard 10 (*A. cristatellus*), however, had slower maximal sprint speed than expected for his body size. Lizard 10 did exhibit substantially faster stride frequency than the fully limbed lizards, but the effect on sprint speed appeared weaker. Further increases in stride frequency to compensate may not be possible, as the mechanistic drivers of this strategy (muscle twitch times and associated contractile properties; Marsh and Bennett 1985) appear conserved within species (C.V. Anderson & T.J. Roberts, unpub. data).

### **Habitat use**

If a lizard were to incur performance costs associated with limb loss or damage, then one might expect the lizard to exhibit different patterns of resource use to fully limbed members of the population. Only one dataset provides an insight to this prediction. In Miami, an adult male brown anole that had lost half of its right hindlimb (*A. sagrei*; Fig S32), an adult female brown anole that had lost nearly a whole forelimb (*A. sagrei*; Fig S33), and an adult male crested anole that had lost 40% of its hindlimb ("Lizard 10"; *A. cristatellus*; Fig 3F and Fig S8) all exhibited similar patterns of habitat use relative to other lizards in the population (i.e., within one standard deviation of the population mean). Specifically, the male *A. sagrei* was observed perching 132 cm above the ground (versus 80 cm adult male population mean [1 S.D. = 68 cm]) on a 2 cm wide branch (versus 8.5 cm population mean [1 S.D. = 11cm]; Stroud, pers. obs.). The female *A. sagrei* was observed perching 26 cm above the ground (versus 48 cm adult female population mean [1 S.D. = 55cm]) and on a 2 cm wide branch (versus 6.3 cm population mean [1 S.D. = 10 cm]). And, finally, the male *A. cristatellus* was observed perching on a 4 cm wide branch 92 cm above the ground, compared to a population average for other male *A. cristatellus* of 14 cm and 105 cm, respectively. Of course, caution must be exercised in gleaning too much information from these data as habitat use was only recorded once, at the time of capture, for each individual lizard in this population. The extent to which a single snapshot of habitat use reflects the general behavior of an individual lizard is unclear (Kamath and Losos 2017).

## Survival

The probability that by chance all seven limb-damaged side-blotched lizards (*Uta stansburiana*) would not survive (i.e., survival unrelated to limb condition) is approximately 7.5-13.6%, given background survival rates of 24.8-30.9% (i.e.,  $P_{\text{(not capture)}} = (1 - \text{survival rate})^7$ ). In May 2018, the lead author (JTS) encountered two three-legged adults (Figs S32 and S33) in a brown anole (*Anolis sagrei*) population that was the focus of a long-term survival study in Miami, Florida USA (Stroud et al. 2023). While the individuals appeared to have healthy body condition (Table 2, ID 43 and 44), neither survived to the next sampling period 6 months later. Whether this was due to limb loss is unclear because most other conspecifics also did not survive that time period. For example, adult male brown anoles that were similar in either body size (7.1% predicted survival rate), body mass (8.7%), or body condition (15.4%) had relatively low survival rates even without limb loss.

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**Table S1.** Observations of limb damage in wild lizards. Percentages were used to demonstrate the extent of loss on forelimb or hindlimb and do not represent accurate proportions. Forelimb loss: entire hand, 20%; entire hand and part of radius/ulna, 40%; entire hand and entire radius/ulna, 60%; entire hand, radius/ulna, and part of humerus, 80%; entire forelimb, 100%. Hindlimb: entire foot, 20%; entire foot and part of tibia, 40%; entire foot and entire tibia, 60%; entire foot, tibia, and part of femur, 80%; entire hindlimb, 100%. <sup>1</sup>Limb present but was in the process of detaching. <sup>2</sup>Injuries to both forelimbs. <sup>3</sup>Limb loss was noted, but detailed information not collected. References (indicated by superscript numbers next to species names): 1. (Gandia et al. 2018), 2. (Mora 2020), 3. (Cavalcante and Passos 2024), 4. (Pasachnik 2011), 5. (Kolenda et al. 2017), 6. (Cortada et al. 2017), 7. (Gleed-Owen 2012), 8. (Langner 2017), 9. (Carbajal-Márquez and Valdez-Villavicencio 2012), 10. (Marín et al. 2023), 11. (Christopoulos and Pafilis 2020).

Record ID	Species	Family	Location	Country	Forelimb lost	Hindlimb lost	Record
1	<i>Sitana spinaecephalus</i>	Agamidae	Gujarat	India	na	20%	Fig S1
2	<i>Elgaria sp.</i>	Anguidae	California	USA	na	60%	Fig S2
3	<i>Anolis carolinensis</i>	Anolidae	Hawaii	USA	20%	na	Fig S3
4	<i>Anolis carolinensis</i>	Anolidae	Arkansas	USA	na	60%	Fig S4
5	<i>Anolis carolinensis</i>	Anolidae	Tennessee	USA	60%	na	Echternacht pers. obs.
6	<i>Anolis carolinensis</i>	Anolidae	Alabama	USA	100%	na	Fig S5
7	<i>Anolis cristatellus</i>	Anolidae	-	Puerto Rico	100%	na	Fig 1A
8	<i>Anolis cristatellus</i>	Anolidae	-	Puerto Rico	60%	na	Fig S6
9	<i>Anolis cristatellus</i>	Anolidae	Miami, FL	USA	80%	na	Fig S7
10	<i>Anolis cristatellus</i>	Anolidae	Miami, FL	USA	na	40%	Fig S8
11	<i>Anolis cristatellus</i>	Anolidae	Miami, FL	USA	na	20%	Fig S9
12	<i>Anolis cybotes</i>	Anolidae	-	Dominican Rep.	na	80%	Fig S10
13	<i>Anolis distichus</i>	Anolidae	Abaco	Bahamas	na	40%	Fig S11
14	<i>Anolis distichus</i>	Anolidae	Miami, FL	USA	60%	na	Fig S12
15	<i>Anolis equestris</i>	Anolidae	Havana	Cuba	na	100%	Ref 1.
16	<i>Anolis lineatopus</i> <sup>2</sup>	Anolidae	Kingston	Jamaica	100-100%	na	Fig 2C
17	<i>Anolis lineatopus</i>	Anolidae	-	Jamaica	40%	na	Fig S13
18	<i>Anolis longitibialis</i> <sup>1</sup>	Anolidae	-	Dominican Rep.	40%	na	Fig S14
19	<i>Anolis oculatus</i>	Anolidae	-	Dominica	40%	na	Fig S15

20	<i>Anolis opalinus</i>	Anolidae	-	Jamaica	80%	60%	Fig. 2D
21	<i>Anolis ophiolepis</i>	Anolidae	Guantanamo Bay	Cuba	na	60%	Losos pers. obs.
22	<i>Anolis sabanus</i>	Anolidae	-	Saba Island	60%	na	Fig S16
23	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	na	40%	Fig S17
24	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	40%	na	Fig S18
25	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	40%	na	Fig S19
26	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	na	40%	Fig S20
27	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	na	80%	Fig S21
28	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	na	100%	Fig S22
29	<i>Anolis sagrei</i>	Anolidae	Staniel Cay	Bahamas	40%	na	Fig. 1B
30	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	40%	na	Fig S23
31	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	40%	na	Fig S24
32	<i>Anolis sagrei</i>	Anolidae	Staniel Cay	Bahamas	60%	na	Losos pers. obs.
33	<i>Anolis sagrei</i>	Anolidae	Abaco	Bahamas	80%	na	Fig S25
34	<i>Anolis sagrei</i>	Anolidae	Staniel Cay	Bahamas	na	80%	Fig S26
35	<i>Anolis sagrei</i>	Anolidae	Staniel Cay	Bahamas	20%	na	Losos pers. obs.
36	<i>Anolis sagrei</i>	Anolidae	Staniel Cay	Bahamas	na	80%	Losos pers. obs.
37	<i>Anolis sagrei</i>	Anolidae	Grand Cayman	Cayman Islands	na	80%	Fig S27
38	<i>Anolis sagrei</i>	Anolidae	Gainesville, FL	USA	na	60%	Fig 3C
39	<i>Anolis sagrei</i>	Anolidae	Jacksonville, FL	USA	na	60%	Fig S28
40	<i>Anolis sagrei</i>	Anolidae	Jacksonville, FL	USA	na	80%	Fig S29
41	<i>Anolis sagrei</i>	Anolidae	Miami, FL	USA	60%	na	Fig S30
42	<i>Anolis sagrei</i>	Anolidae	Miami, FL	USA	na	60%	Fig S31
43	<i>Anolis sagrei</i>	Anolidae	Miami, FL	USA	na	40%	Fig S32
44	<i>Anolis sagrei</i>	Anolidae	Miami, FL	USA	80%	na	Fig S33
45	<i>Anolis sagrei</i>	Anolidae	New Orleans, LA	USA	100%	na	SM Video 1
46	<i>Anolis sagrei</i>	Anolidae	-	Taiwan	na	80%	Fig S34
47	<i>Anolis sagrei</i>	Anolidae	-	Taiwan	na	80%	Fig S35
48	<i>Anolis sagrei</i>	Anolidae	-	Taiwan	na	20%	Fig S36
49	<i>Anolis sagrei</i> <sup>2</sup>	Anolidae	Winter Park, FL	USA	20-40%	na	Brown pers. obs.
50	<i>Anolis sericeus</i>	Anolidae	-	Mexico	80%	na	Fig S37
51	<i>Anolis smaragdinus</i>	Anolidae	Abaco	Bahamas	20%	na	Fig. 1G
52	<i>Anolis ventrimaculatus</i>	Anolidae	Valle de Cauca	Colombia	na	40%	Fig S38
53	<i>Furcifer pardalis</i> <sup>3</sup>	Chamaeleonidae	-	Madagascar	na	na	Anderson pers. obs.

54	<i>Trioceros quadricornis</i>	Chamaeleonidae	Mount Kupe	Cameroon	100%	na	Fig. 1E
55	<i>Platysaurus broadlevi</i>	Cordylidae	-	South Africa	20%	na	Whiting pers. obs.
56	<i>Platysaurus broadlevi</i>	Cordylidae	-	South Africa	60%	na	Whiting pers. obs.
57	<i>Platysaurus broadlevi</i>	Cordylidae	-	South Africa	100%	na	Whiting pers. obs.
58	<i>Platysaurus broadlevi</i>	Cordylidae	-	South Africa	na	40%	Fig S39
59	<i>Platysaurus broadlevi</i>	Cordylidae	-	South Africa	na	100%	Whiting pers. obs.
60	<i>Basiliscus plumifrons</i>	Corytophanidae	Limon	Costa Rica	100%	na	Fig 1 G; Ref. 2
61	<i>Basiliscus vittatus</i>	Corytophanidae	Miami, FL	USA	100%	20%	Fig 2A
62	<i>Crotaphytus collaris</i>	Crotaphytidae	Missouri	USA	80%	na	Fig S40
63	<i>Crotaphytus collaris</i>	Crotaphytidae	Taum Sauk, MO	USA	20%	na	Templeton pers. obs.
64	<i>Crotaphytus collaris</i> <sup>2</sup>	Crotaphytidae	Peck Ranch, MO	USA	20-100%	na	Templeton pers. obs.
65	<i>Crotaphytus collaris</i>	Crotaphytidae	Peck Ranch, MO	USA	20%	na	Templeton pers. obs.
66	<i>Crotaphytus collaris</i>	Crotaphytidae	Peck Ranch, MO	USA	na	20%	Templeton pers. obs.
67	<i>Crotaphytus collaris</i>	Crotaphytidae	Peck Ranch, MO	USA	20%	na	Templeton pers. obs.
68	<i>Crotaphytus collaris</i>	Crotaphytidae	Peck Ranch, MO	USA	na	20%	Templeton pers. obs.
69	<i>Oedura lesueurii</i>	Diplodactylidae	-	Australia	na	20%	Pike pers. obs.
70	<i>Diploglossus lessonae</i>	Diploglossidae	Ceará	Brazil	100%	na	Fig S41
71	<i>Gehyra dubia</i>	Gekkonidae	-	Australia	na	20%	Pike pers. obs.
72	<i>Hemidactylus agrius</i>	Gekkonidae	Rio Grande Norte	Brazil	na	20%	Ref. 3
73	<i>Hemidactylus agrius</i>	Gekkonidae	Rio Grande Norte	Brazil	na	100%	Ref. 3
74	<i>Micrablepharus maximiliani</i>	Gymnophthalmidae	Ceará	Brazil	na	60%	Fig S42
75	<i>Vanzosaura rubricauda</i> <sup>2</sup>	Gymnophthalmidae	Ceará	Brazil	40-60%	na	Fig S43
76	<i>Amblyrhynchus cristatus</i>	Iguanidae	Galápagos	Ecuador	na	60%	Fig. 1D
77	<i>Ctenosaura oedirhina</i>	Iguanidae	Roatan	Honduras	na	60%	Ref. 4
78	<i>Iguana iguana</i>	Iguanidae	Rio Grande Norte	Brazil	na	40%	Ref. 3
79	<i>Sauromalus obesus</i>	Iguanidae	Utah	USA	na	20%	Fig S44
80	<i>Lacerta agilis</i>	Lacertidae	Ruda Milicka	Poland	na	60%	Ref. 5
81	<i>Podarcis lilfordi</i>	Lacertidae	Mallorca	Spain	na	60%	Ref. 6
82	<i>Zootoca vivipara</i> <sup>2</sup>	Lacertidae	Dorset	UK	60-60%	na	Ref. 7
83	<i>Zootoca vivipara</i>	Lacertidae	Milsko	Poland	na	20%	Ref. 5
84	<i>Lanthanotus borneensis</i>	Lanthanotidae	Borneo	Indonesia	na	80%	Ref. 8
85	<i>Lanthanotus borneensis</i>	Lanthanotidae	Borneo	Indonesia	na	80%	Ref. 8
86	<i>Leiocephalus carinatus</i>	Leiocephalidae	Abaco	Bahamas	na	40%	Fig S45
87	<i>Leiocephalus carinatus</i>	Leiocephalidae	Abaco	Bahamas	100%	na	Fig S46

88	<i>Leiocephalus carinatus</i>	Leiocephalidae	Fort Lauderdale, FL	USA	na	40%	Kolbe pers. obs.
89	<i>Leiocephalus carinatus</i>	Leiocephalidae	Thomas Cay	Bahamas	20%	na	Fig S47
90	<i>Leiocephalus loxogrammus</i>	Leiocephalidae	Rum Cay	Bahamas	20%	na	Fig. 1C
91	<i>Petrosaurus thalassinus</i>	Phrynosomatidae	-	Mexico	na	80%	Ref. 9
92	<i>Phrynosoma orbiculare</i>	Phrynosomatidae	Aguascalientes	Mexico	60%	na	Ref. 10
93	<i>Sceloporus grammicus</i>	Phrynosomatidae	Zoquiapán	Mexico	80%	na	Fig S48
94	<i>Sceloporus grammicus</i>	Phrynosomatidae	San Antonio	Mexico	na	40%	Fig S49
95	<i>Sceloporus grammicus</i>	Phrynosomatidae	Ciclopista	Mexico	na	60%	Fig S50
96	<i>Sceloporus undulatus</i>	Phrynosomatidae	Alabama	USA	na	60%	Fig S51
97	<i>Sceloporus undulatus</i>	Phrynosomatidae	Missouri	USA	100%	na	Fig S52
98	<i>Urosaurus ornatus</i>	Phrynosomatidae	Arizona	USA	na	20%	French pers. obs.
99	<i>Uta stansburiana</i>	Phrynosomatidae	Nevada	USA	na	80%	Fig S53
100	<i>Uta stansburiana</i>	Phrynosomatidae	Nevada	USA	na	40%	Zani pers. obs.
101	<i>Uta stansburiana</i>	Phrynosomatidae	Oregon	USA	na	60%	Fig S54
102	<i>Uta stansburiana</i>	Phrynosomatidae	Oregon	USA	20%	na	Zani pers. obs.
103	<i>Uta stansburiana</i>	Phrynosomatidae	Oregon	USA	20%	na	Zani pers. obs.
104	<i>Uta stansburiana</i>	Phrynosomatidae	Oregon	USA	na	20%	Zani pers. obs.
105	<i>Uta stansburiana</i>	Phrynosomatidae	Oregon	USA	na	20%	Zani pers. obs.
106	<i>Uta stansburiana</i>	Phrynosomatidae	Utah	USA	20%	na	French pers obs.
107	<i>Uta stansburiana</i>	Phrynosomatidae	Utah	USA	20%	na	French pers obs.
108	<i>Uta stansburiana</i>	Phrynosomatidae	Utah	USA	na	100%	French pers obs.
109	<i>Phyllopezus pollicaris</i>	Phylodactylidae	Rio Grande Norte	Brazil	na	20%	Ref. 3
110	<i>Chalcides ocellatus</i>	Scincidae	Boeotia	Greece	na	60%	Ref. 11
111	<i>Cryptoblepharus pulcher</i>	Scincidae	-	Australia	na	100%	Pike pers. obs.
112	<i>Eutropis rudis</i>	Scincidae	Sarawak, Borneo	Malaysia	40%	na	Whiting pers. obs.
113	<i>Plestiodon fasciatus</i>	Scincidae	Minnesota	USA	60%	na	Fig S55
114	<i>Psychosaura agmosticha</i>	Scincidae	Bahia	Brazil	na	60%	Ref. 3
115	<i>Coleodactylus meridionalis</i>	Sphaerodactylidae	Ceará	Brazil	20%	na	Fig S56
116	<i>Sphaerodactylus nicholsi</i>	Sphaerodactylidae	Boqueron	Puerto Rico	na	20%	Fig S57
117	<i>Sphaerodactylus nicholsi</i>	Sphaerodactylidae	Boqueron	Puerto Rico	na	20%	Fig S58
118	<i>Ameivula ocellifera</i>	Teiidae	Piauí	Brazil	80%	na	Fig S59
119	<i>Dracaena paraguayensis</i>	Teiidae	Mato Grosso do Sul	Brazil	80%	na	Fig. 1F
120	<i>Glaucomastix littoralis</i>	Teiidae	Rio de Janeiro	Brazil	80%	na	Fig S60
121	<i>Tropidurus hispidus</i>	Tropiduridae	Rio Grande Norte	Brazil	80%	na	Ref. 3

122      *Tropidurus semitaeniatus*      Tropiduridae      Ceará      Brazil      na      40%      Fig S61

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**Table S2.** Limb loss in lizard populations across various species, families, locations, and continents. Information on the total population size (Pop. size), the number of lizards in the population with limb loss (N inds. limb loss), and the proportion of lizards in the population with limb loss (% limb loss) are included. The proportion was calculated by dividing the number of lizards with limb loss by the total population size for each species and location.

Species	Family	Location	Continent	Pop. size	N inds. limb loss	% limb loss	Reference
<i>Anolis sagrei</i>	Anolidae	Miami, FL USA	North America	587	2	0.34%	Stroud unpub. data
<i>Anolis cristatellus</i>	Anolidae	Miami, FL USA	North America	666	1	0.15%	Stroud unpub. data
<i>Crotaphytus collaris</i>	Crotaphytidae	Taum Sauk, MO USA	North America	235	2	0.85%	Templeton unpub. data; Brisson et al. 2003
<i>Crotaphytus collaris</i>	Crotaphytidae	Peck Ranch, MO USA	North America	1212	5	0.41%	Templeton unpub. data; Templeton et al. 2024
<i>Cryptoblepharus pulcher</i>	Scincidae	Australia	Australia	195	1	0.51%	Pike unpub. data
<i>Oedura lesueurii</i>	Diplodactylidae	Australia	Australia	262	1	0.38%	Pike unpub. data
<i>Platysaurus broadleyi</i>	Cordylidae	South Africa	Africa	1046	5	0.48%	Whiting unpub. data
<i>Sceloporus grammicus</i>	Phrynosomatidae	Zoquiapán, Mexico	North America	31	1	3.23%	Bastiaans unpub. data
<i>Sceloporus grammicus</i>	Phrynosomatidae	San Antonio de las Alazanas, Coahuila, Mexico	North America	191	1	0.52%	Bastiaans unpub. data
<i>Uta stansburiana</i>	Phrynosomatidae	Wright's Point, Oregon, USA	North America	617	3	0.49%	Zani unpub. data
<i>Uta stansburiana</i>	Phrynosomatidae	Wright's Point, Oregon, USA	North America	530	3	0.57%	Zani unpub. data
<i>Uta stansburiana</i>	Phrynosomatidae	Wright's Point, Oregon, USA	North America	153	1	0.65%	Zani unpub. data
<i>Zootoca vivipara</i>	Lacertidae	PIM, Massif Central, France	Europe	51	2	3.92%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	ROB , Massif Central, France	Europe	72	6	8.33%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	AUBISQUE, Pyrenees, France	Europe	21	2	9.52%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BAR, Pyrenees, France	Europe	10	1	10.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BEL, Pyrenees, France	Europe	25	1	4.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BES, Pyrenees, France	Europe	66	4	6.06%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	Bigadou, Pyrenees, France	Europe	27	2	7.41%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BOB, Pyrenees, France	Europe	27	2	7.41%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BON, Pyrenees, France	Europe	10	1	10.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	BOU, Pyrenees, France	Europe	26	2	7.69%	Miles unpub. data

<i>Zootoca vivipara</i>	Lacertidae	CHALET, Pyrenees, France	Europe	20	1	5.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	COM, Pyrenees, France	Europe	44	3	6.82%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	COP, Pyrenees, France	Europe	21	1	4.76%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	Gabas, Pyrenees, France	Europe	40	4	10.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	Jon, Pyrenees, France	Europe	10	1	10.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	LAJO, Pyrenees, France	Europe	23	1	4.35%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	Louvie, Pyrenees, France	Europe	47	3	6.38%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	MON, Pyrenees, France	Europe	20	1	5.00%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	PUY, Pyrenees, France	Europe	43	2	4.65%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	ROB , Pyrenees, France	Europe	21	1	4.76%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	TIO, Pyrenees, France	Europe	14	1	7.14%	Miles unpub. data
<i>Zootoca vivipara</i>	Lacertidae	Viala, Pyrenees, France	Europe	25	1	4.00%	Miles unpub. data

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Figure S1. *Sitana spinaecephalus* (Moti Virani, Gujarat, India). Photo: Ambika Kamath.



Figure S2. *Elgaria* sp. (Martinez, California, USA). Photo: Ambika Kamath.

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Figure S3. *Anolis carolinensis* (Hawaii, USA). Photo: Tony Gamble.



Figure S4. *Anolis carolinensis* (Arkansas, USA). Photo: Matthew Connior.



Figure S5. *Anolis carolinensis* (Auburn, AL, USA). Photo: Joshua Hall.

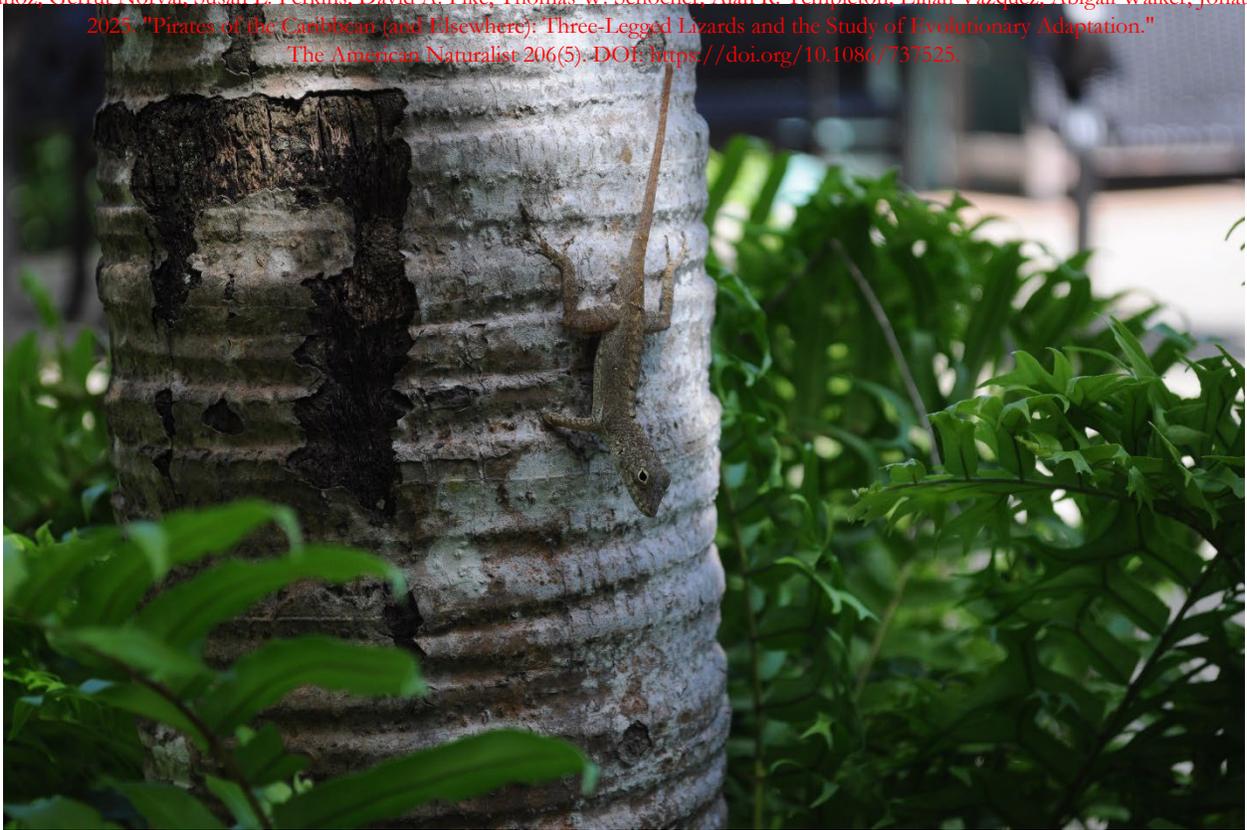


Figure S6. *Anolis cristatellus* (Puerto Rico). Photo: Melissa Aja.

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Figure S7. *Anolis cristatellus* (Miami, FL, USA). Photo: James Stroud.

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Figure S8. *Anolis cristatellus* (Miami, FL, USA). Photo: Jonathan Suh/Abigail Arnashus.

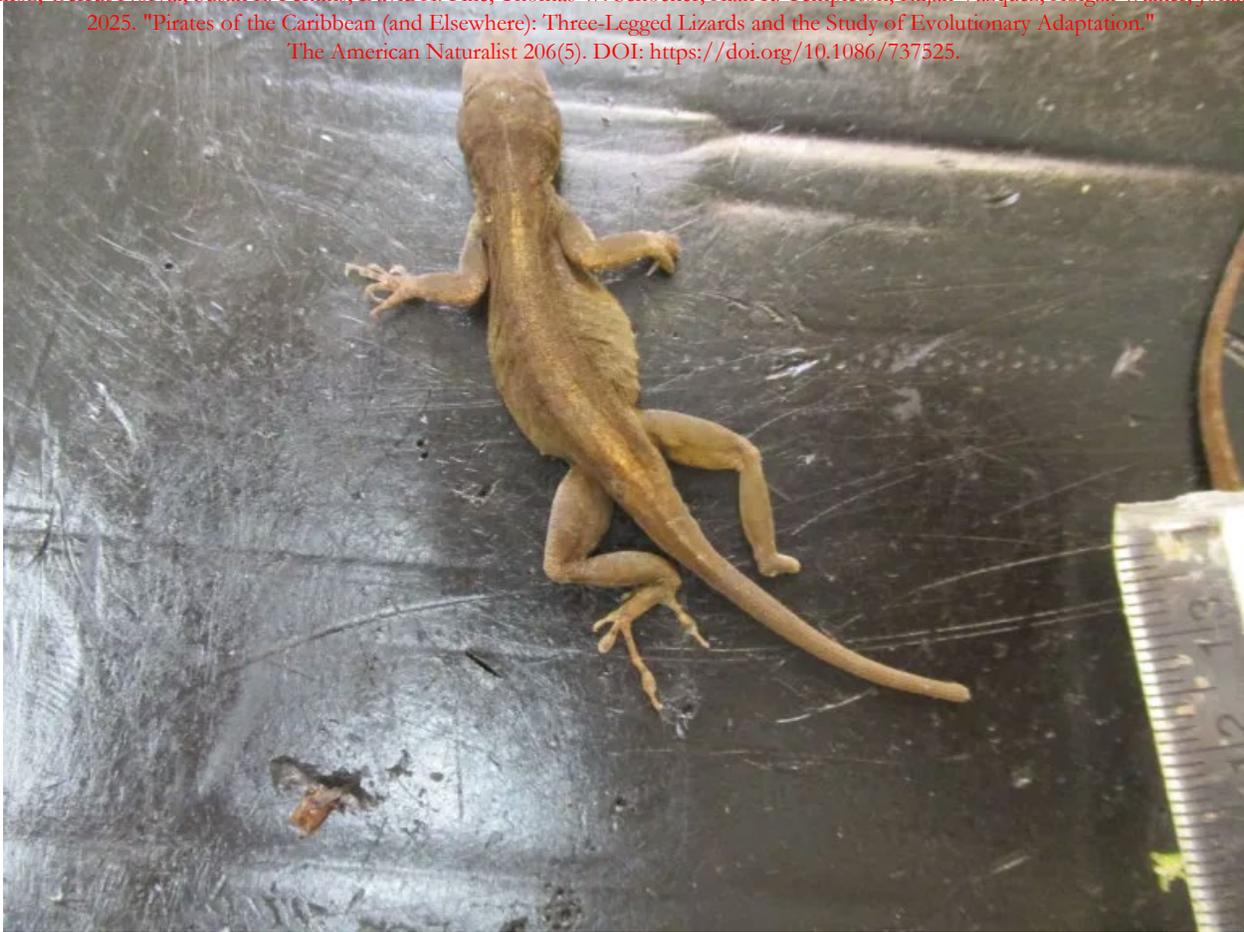


Figure S9. *Anolis cristatellus* (Miami, FL, USA). Photo: Joshua Hall/James Stroud.

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Figure S10. *Anolis cybotes* (Dominican Republic). Photo: Susan Perkins.



Figure S11. *Anolis distichus* (Abaco, Bahamas). Photo: Jonathan Losos.

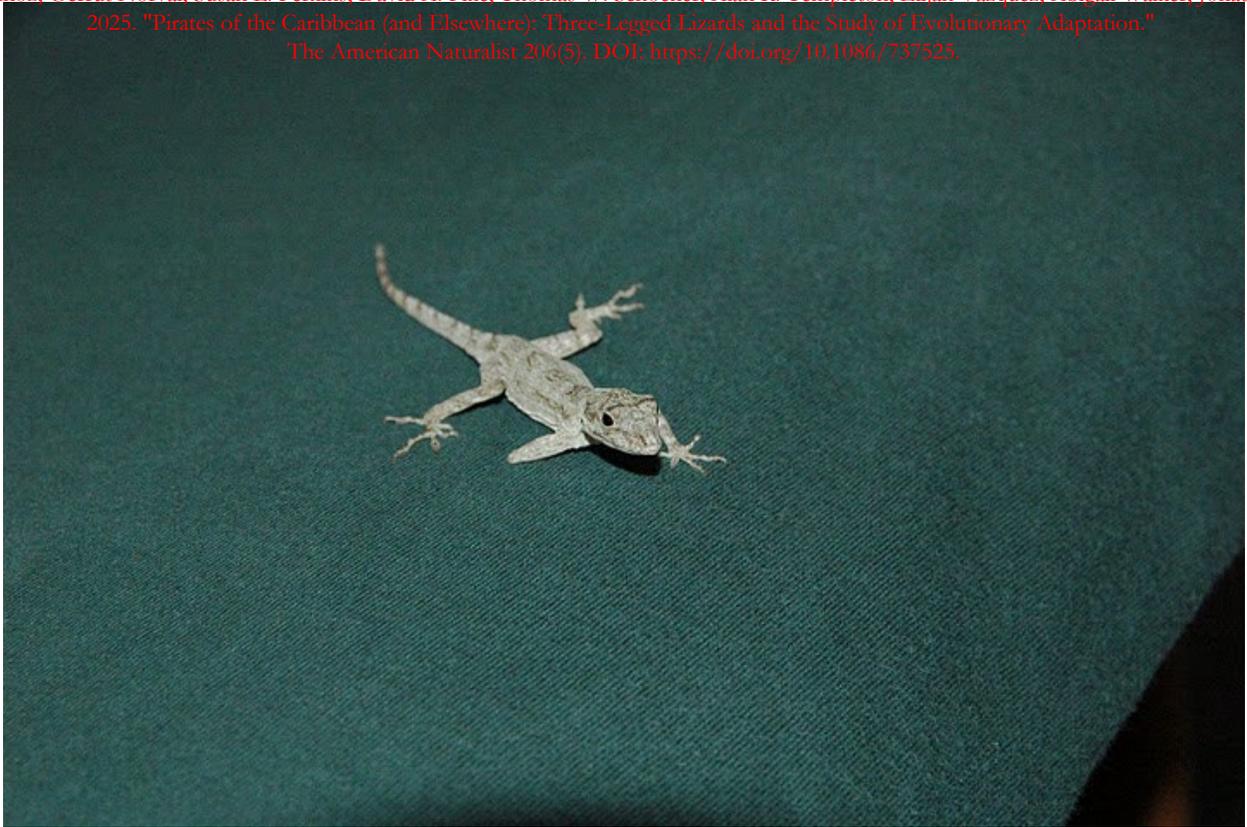


Figure S12. *Anolis distichus* (Miami, FL, USA). Photo: Melissa Losos.

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Figure S13 *Anolis lineatopus* (Jamaica). Photo: Kareen Barnett.



Figure S14. *Anolis longitibialis* (Dominican Republic). Photo: Martha Muñoz.

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Figure S15. *Anolis oculatus* (Dominica). Photo: Claire Dufour.

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Figure S16. *Anolis sabanus* (Saba Island). Photo: Susan Perkins.



Figure S17. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard k74)

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Figure S18. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard s46).



Figure S19. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard x14).

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Figure S20. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard k158).

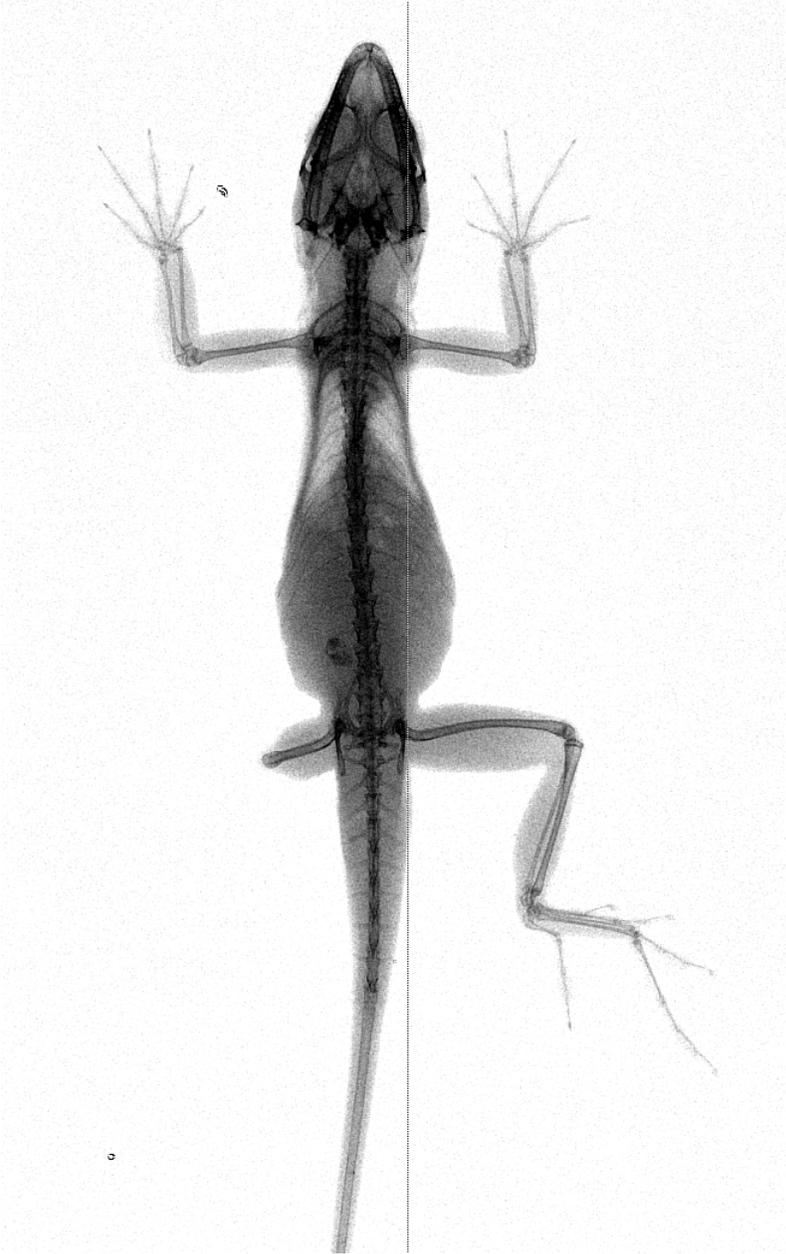


Figure S21. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard k25).



Figure S22. *Anolis sagrei* (Abaco, Bahamas). Photo: Jonathan Losos (2006 Abaco male).

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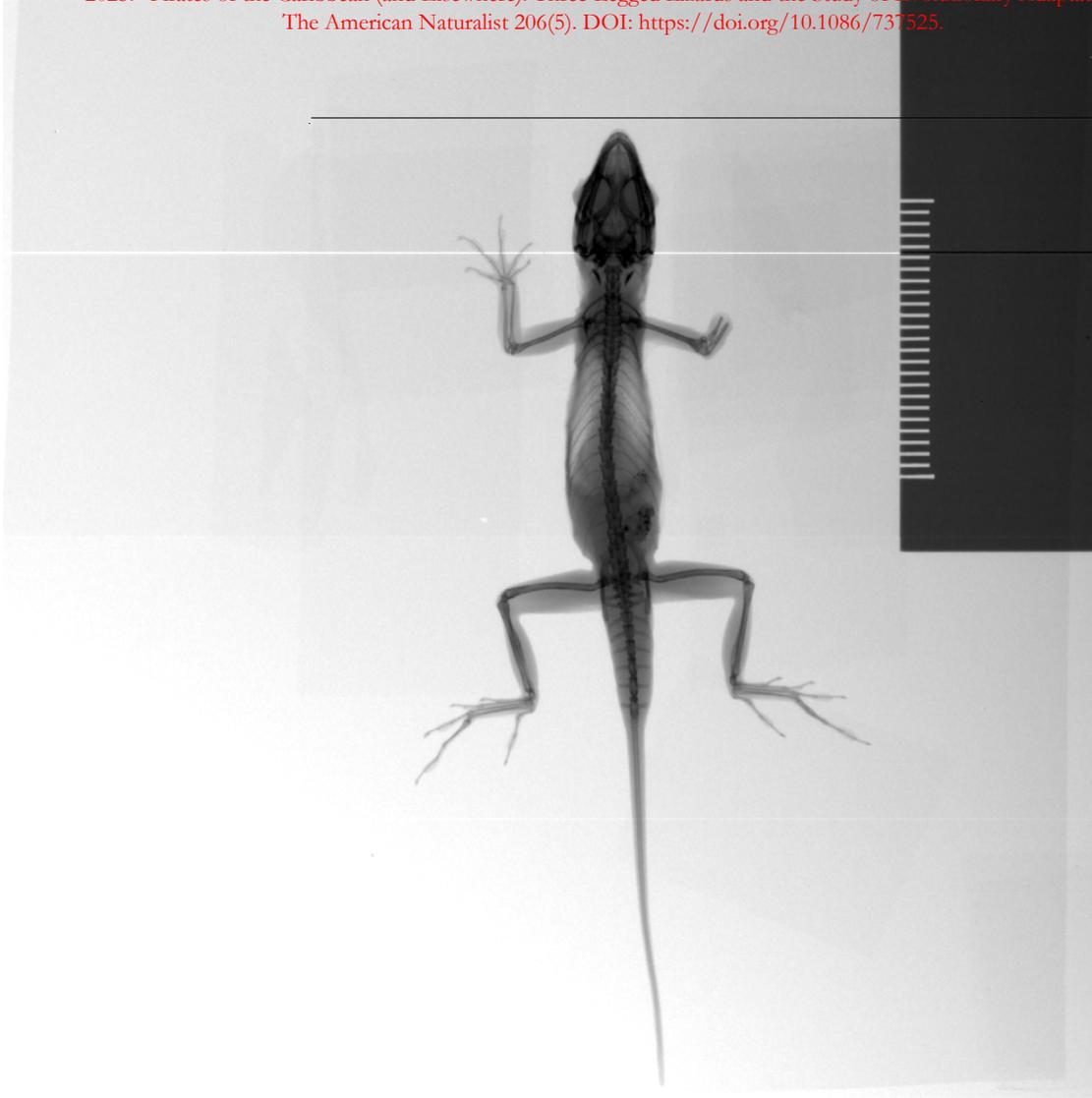


Figure S23. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard s103).

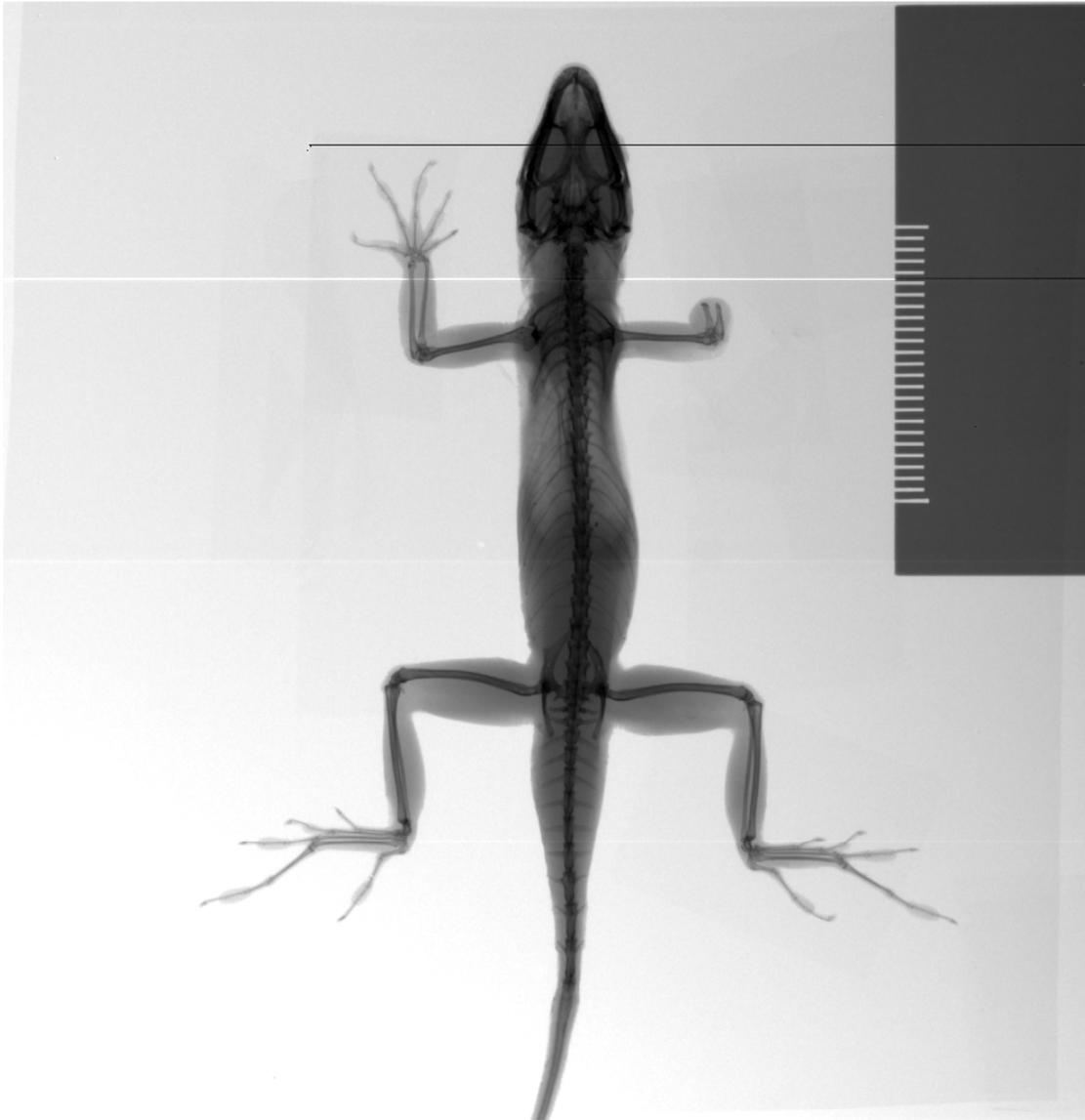


Figure S24. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener (lizard s133).

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Figure S25. *Anolis sagrei* (Abaco, Bahamas). Photo: Jason Kolbe/Jonathan Losos/Tom Schoener.



Figure S26. *Anolis sagrei* (Staniel Cay, Bahamas). Photo: Oriol Lapiedra.

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Figure S27. *Anolis sagrei* (Grand Cayman, Cayman Islands). Photo: Jonathan Losos.



Figure S28. *Anolis sagrei* (Jacksonville, FL, USA). Photo: Karen Cusick.



Figure S29. *Anolis sagrei* (female, bottom; Jacksonville, FL, USA). Photo: Karen Cusick.

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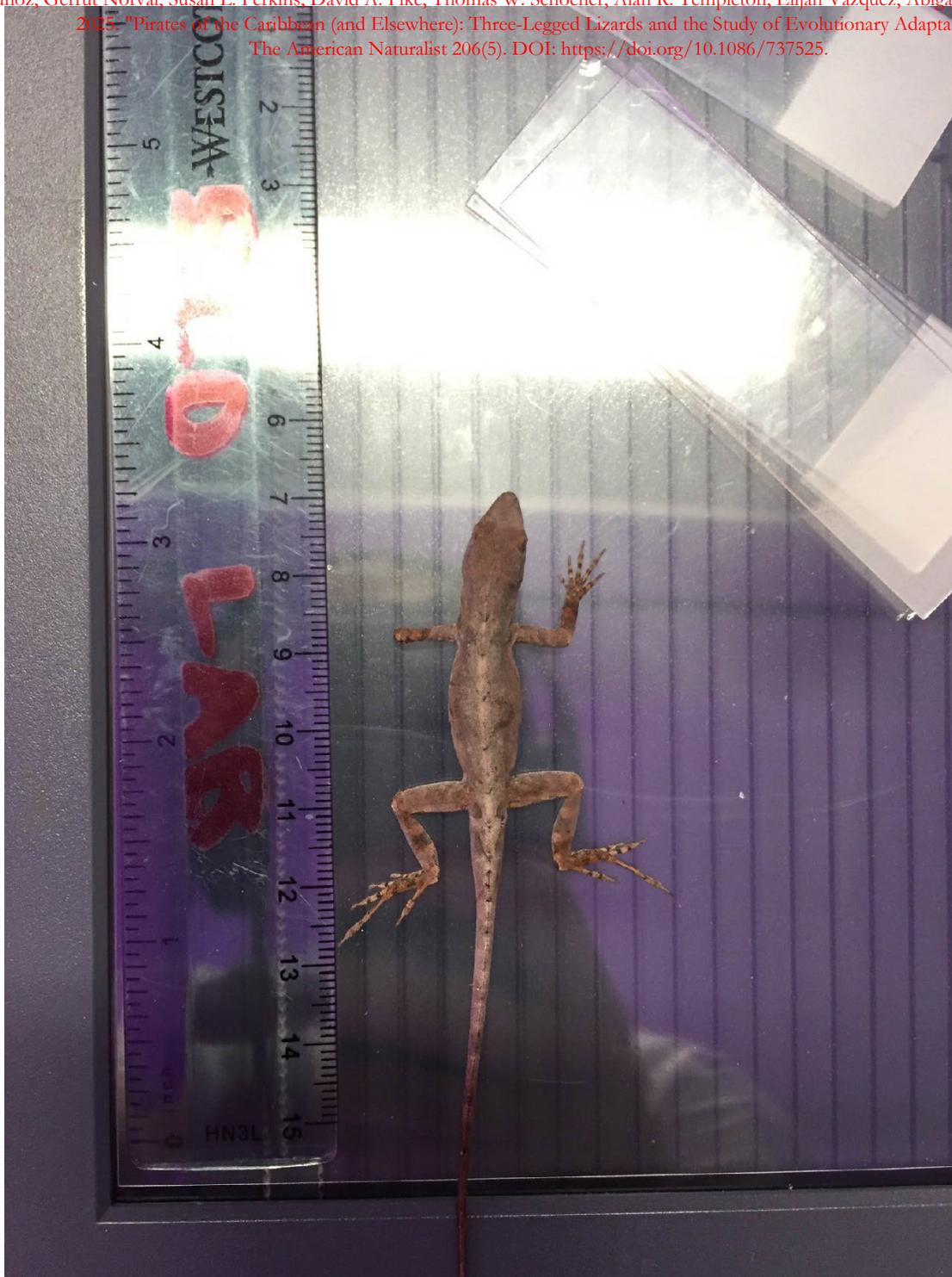


Figure S30. *Anolis sagrei* (Miami, FL, USA). Photo: James Stroud.

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Figure S31. *Anolis sagrei* (Miami, FL, USA). Photo: Jason Kolbe/James Stroud.



Figure S32. *Anolis sagrei* (Miami, FL, USA). Photo: James Stroud.

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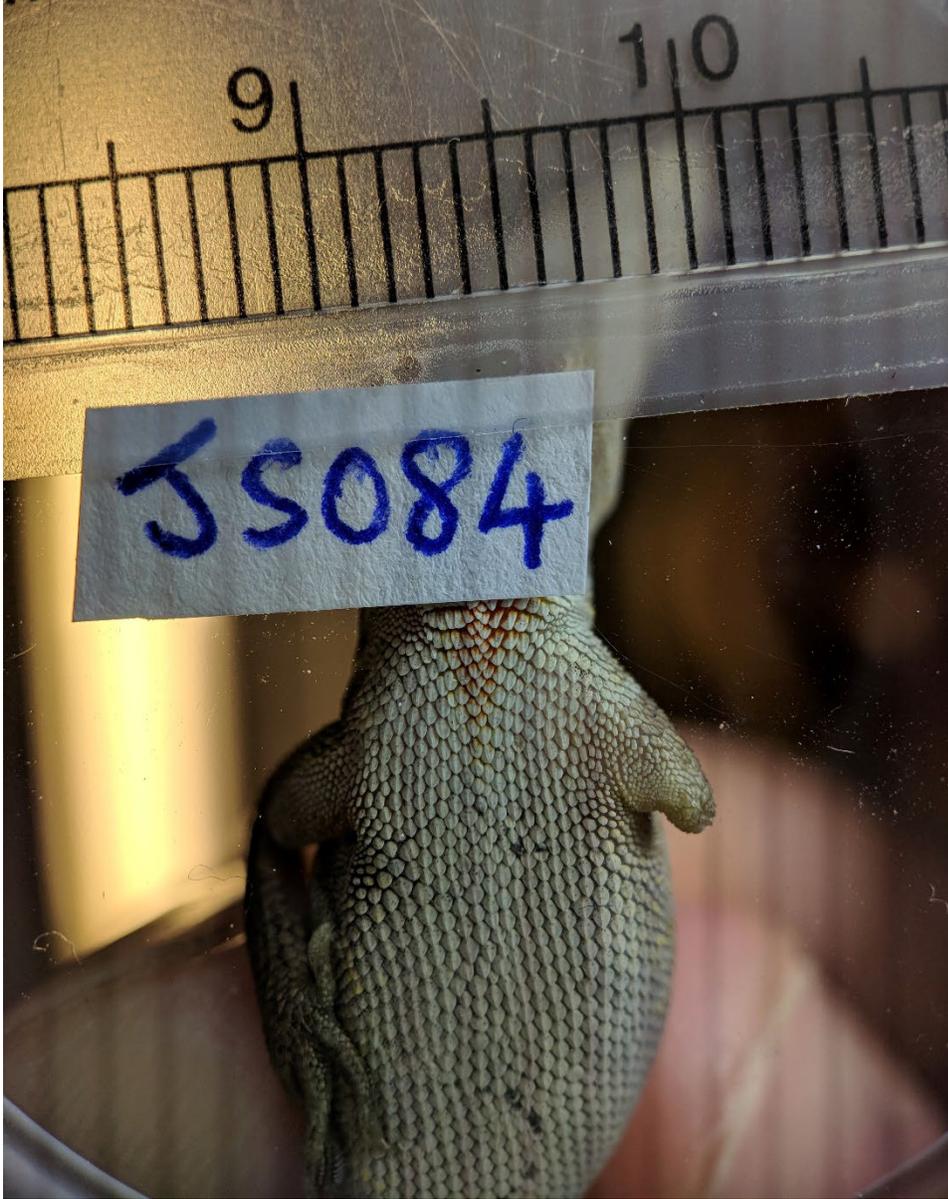


Figure S33. *Anolis sagrei* (Miami, FL, USA). Photo: James Stroud.



Figure S34. *Anolis sagrei* (Taiwan). Photo: Gerrut Norval.

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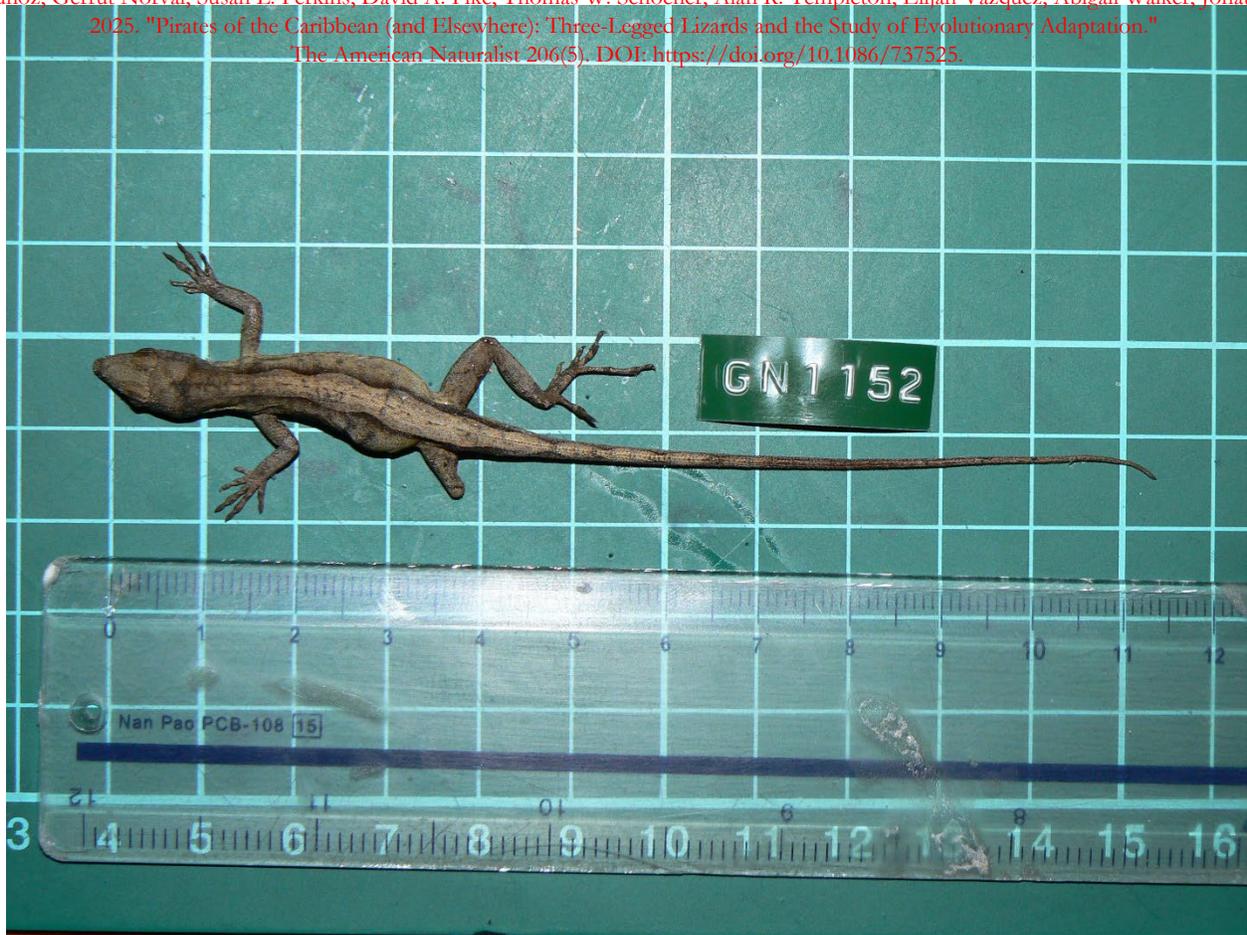


Figure S35. *Anolis sagrei* (Taiwan). Photo: Gerrut Norval.



Figure S36. *Anolis sagrei* (Taiwan). Photo: Gerrut Norval.

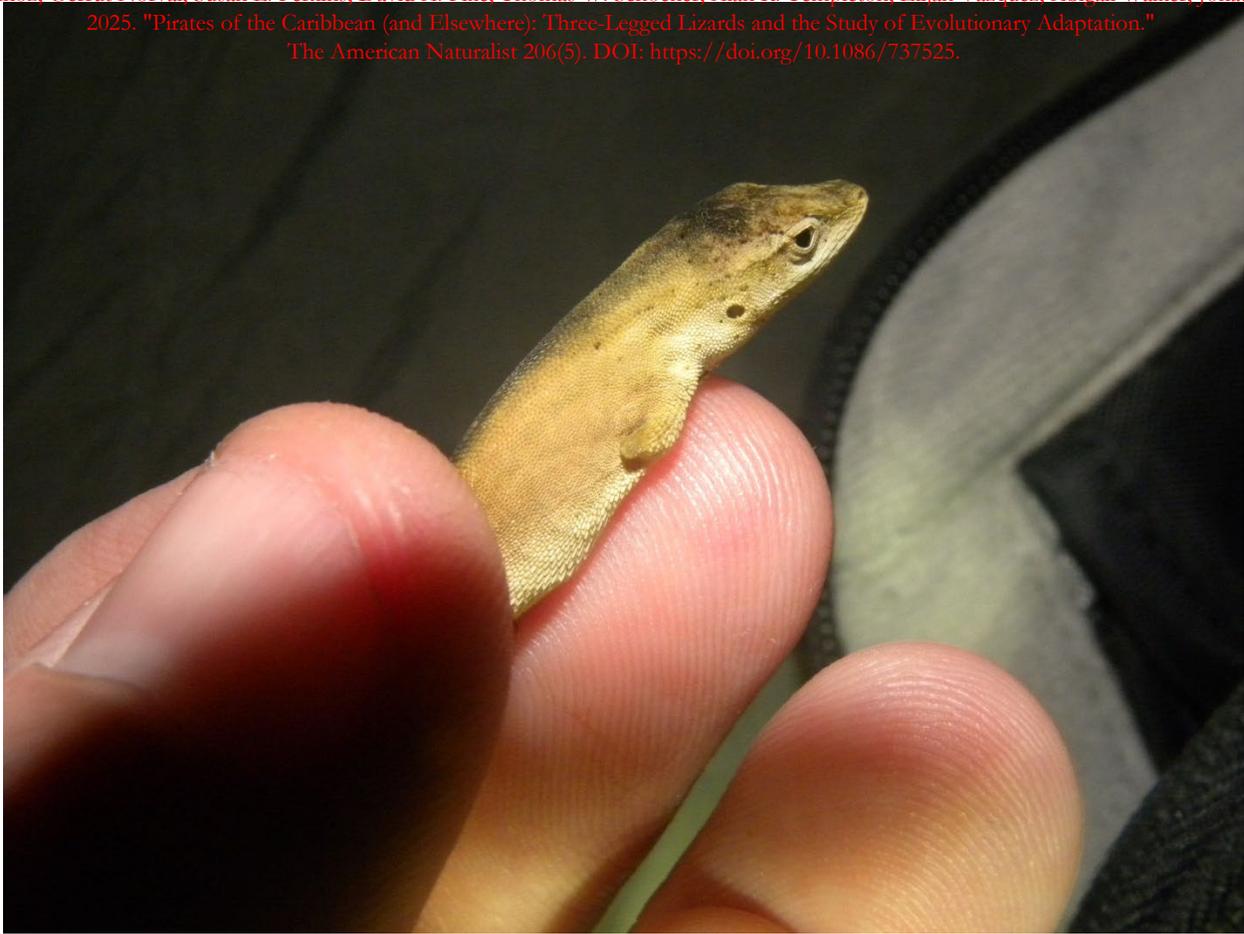


Figure S37. *Anolis sericeus* (Mexico). Photo: Levi Gray.



Figure S38. *Anolis ventrimaculatus* (Colombia). Photo: María del Rosario Castañeda.

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Figure S39. *Platysaurus broadleyi* (South Africa). Photo: Martin Whiting.

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Figure S40. *Crotaphytus collaris* (Missouri, USA). Both photographs are of the same individual. (A) Photo: Alan Templeton, (B) Photo: Jennifer Brisson.

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Figure S41. *Diploglossus lessonae* (Ceará, Brazil). Photo: Daniel Passos.

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The American Naturalist 206(5). DOI: <https://doi.org/10.1086/737525>.



Figure S42. *Micrablepharus maximiliani* (Ceará, Brazil). Photo: Daniel Passos.



Figure S43. *Vanzosaura rubricauda* (Ceará, Brazil). Photo: Daniel Passos.

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Figure S44. *Sauromalus obesus* (Utah, USA). Photo: Daniel Rabosky.

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Figure S45. *Leiocephalus carinatus* (Abaco, Bahamas). Photo: Jonathan Losos.



Figure S46. *Leiocephalus carinatus* (Abaco, Bahamas). Photo: Oriol Lapiedra.

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Figure S47. *Leiocephalus carinatus* (Abaco, Bahamas). Photo: Jonathan Losos.

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Figure S48. *Sceloporus grammicus* (Zoquiapán, Mexico). Photo: Elizabeth Bastiaans.



Figure S49. *Sceloporus grammicus* (San Antonio de las Alazanas, Mexico). (A) Lateral view. (B) Ventral view. Photo: Elizabeth Bastiaans.

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Figure S50. *Sceloporus grammicus* (Ciclopista, Mexico). Photo: Elizabeth Bastiaans.

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Figure S51. *Sceloporus undulatus* (Alabama, USA). Photo: Katie Boronow.

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Figure S52. *Sceloporus undulatus* (Missouri, USA). Photo: Jonathan Losos.



Figure S53. *Uta stansburiana* (Nevada, USA). Photo: Peter Zani.

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Figure S54. *Uta stansburiana* (Oregon, USA). Photo: Peter Zani.

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Figure S55. *Plestiodon fasciatus* (Minnesota, USA). Photo: Tony Gamble.



Figure S56. *Coleodactylus meridionalis* (Ceará, Brazil). Photo: Daniel Passos.

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Figure S57. *Sphaerodactylus nicholsi* (Puerto Rico). Photo: Tony Gamble.

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Figure S58. *Sphaerodactylus nicholsi* (Puerto Rico). Photo: Tony Gamble.

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Figure S59. *Ameivula ocellifera* (Brazil). Photo: Daniel Passos.

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Figure S60. *Glaucomastix littoralis* (Rio de Janeiro, Brazil). Photo: Daniel Passos.

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Figure S61. *Tropidurus semitaeniatus* (Ceará, Brazil). Photo: Daniel Passos.

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Figure S62. *Uta stansburiana* (Nevada, USA). Photo: Peter Zani.

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**Males: Max velocity**  
**p-value: 0.706**

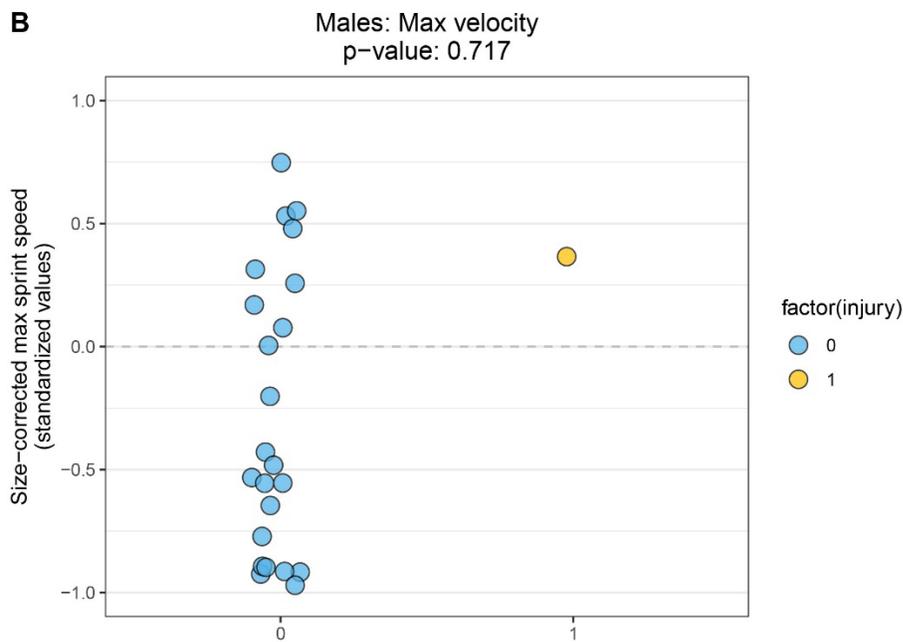
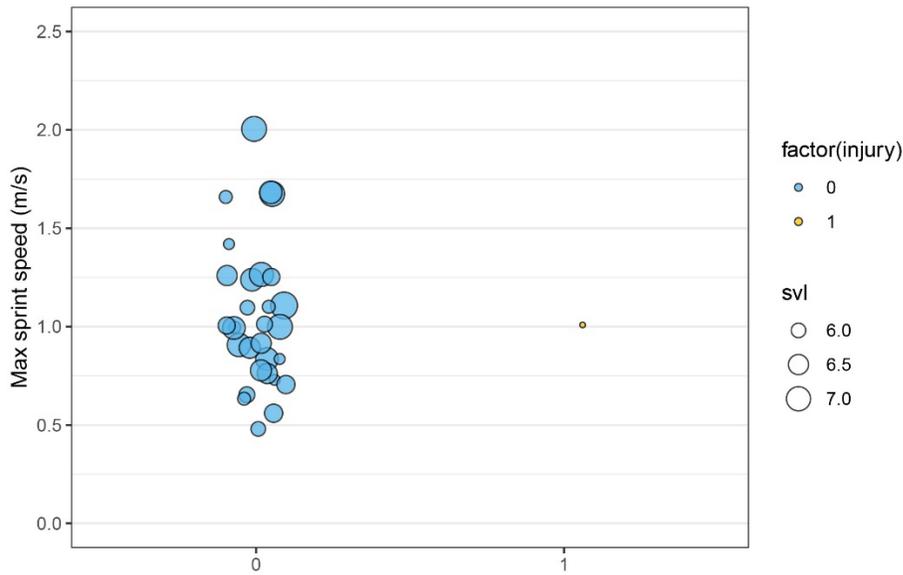


Figure S63. Max sprint speed assessments of *Sceloporus undulatus* (Boronow, unpub. data) relative to limb loss. (A) Absolute sprint speed values are shown; point size is scaled to represent body size. (B) Sprint speed values corrected for body size are shown with a standardized distribution (mean=0, standard deviation=1). Snout-vent lengths (SVL) are shown in centimeters. Points are jittered horizontally (along the x-axis) for visualization purposes.

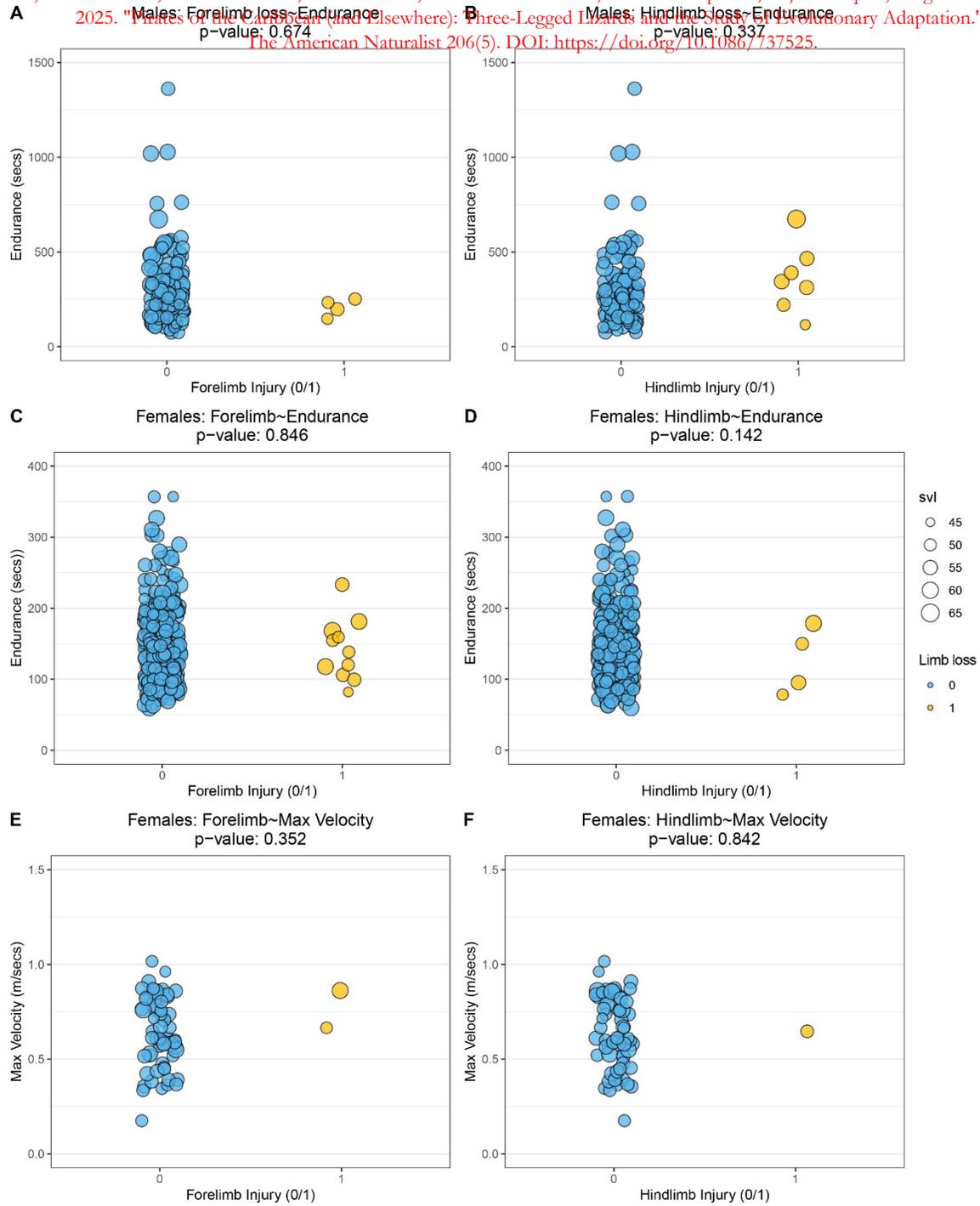


Figure S64. Performance assessments of *Zootoca vivipara* (Miles, unpub. data) relative to limb loss. (A-D) Endurance values in seconds. (E-F) Absolute sprint speed values are shown. In all plots, point size is scaled to represent body size. Snout-vent lengths (SVL) are shown in millimeters. Points are jittered horizontally (along the x-axis) for visualization purposes.