

Health status of *Polychrus gutturosus* based on physical examination, hematology and biochemistry parameters in Costa Rica (#53394)

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Health status of *Polychrus gutturossus* based on physical examination, hematology and biochemistry parameters in Costa Rica

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Studies evaluating the health status and characteristics of free-ranging **wildlife** populations are scarce or absent for most species. Saurian health assessments are usually performed in species that have conservation issues or that are kept in captivity. The Berthold's bush anole (*Polychrus gutturossus*) is one of eight species belonging to the genus *Polychrus*, the only representative of the family Polychrotidae. Only a handful **of** studies have been **reported concerning** ~~carried out about~~ **these lizards**, morphological variation, ecology, and natural history, probably because *P. gutturossus* is a canopy dweller and it **can be** difficult to locate individuals. It is believed that deforestation and habitat modification could pose a threat for this species, although **to date** no health assessment has **been done**. The aim of **this** study was to generate health baseline data on *P. gutturossus*. Forty Berthold's bush anoles (20 males and 20 females) **were sampled** at the Pacific versant in Costa Rica, where physical examination, skin and cloacal temperatures, and blood samples were obtained from individuals immediately after capture. Animals from the studied population were all healthy (body condition 2.5-3.0/**5.0**). No lesions or ectoparasites were detected, but **the** presence of hemoparasites **were found** in nine individuals. Hematological and biochemical values were obtained, **and** the morphology of leukocytes were **found to be** similar **to** other iguanians. A positive correlation was found between **the tissue enzymes** Aspartate amino transferase (AST) and Creatinine kinase (CK) and a negative correlation **was found** between skin and cloacal temperatures **and** AST and CK. There were positive correlations between female weight and total protein, Calcium, and **the** Calcium and Phosphorus ratio. No significant inter-sex differences were found **in biochemical values**, despite females being larger than males. **This** is the first health assessment performed on a free-ranging canopy dwelling

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All approved, however see comments and suggestions in edited paper here. For Figure 1, see question about yellow dots in legend but I see red dots on map, please correct. Also for Figure 5 and 6 (perhaps these should be called tables?), I think we need to include reference information in the table itself or possibly in the legend when writing out full latin name.

2. Experimental design
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3. Validity of findings
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4. General comments.
Overall a well put together study and presentation of important data. Please see and address my comments and suggestions.

5. No confidential notes for editor.

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changes in health status due to anthropogenic causes or natural disturbances.

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 2 **physical examination, hematology and biochemistry**
 3 **parameters in Costa Rica**

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23 **Abstract**

24 Studies evaluating the health status and characteristics of free-ranging populations are scarce or
25 absent for most species. Saurian health assessments are usually performed in species that have
26 conservation issues or that are kept in captivity. The Berthold's bush anole (*Polychrus*
27 *guturossus*) is one of eight species belonging to the genus *Polychrus*, the only representative of
28 the family Polychrotidae. Only a handful studies have been reported concerning these lizards,

29 morphological variation, ecology, and natural history, probably because *P. gutturossus* is a canopy dweller and
30 it can be difficult to locate individuals. It is believed that deforestation and habitat modification could

31 pose a threat for this species, although to date no health assessment has been done. The aim of this

32 study was to generate health baseline data on *P. gutturossus*. Forty Berthold's bush anoles (20
33 males and 20 females) were sampled at the Pacific versant in Costa Rica, where physical

34 examination, skin and cloacal temperatures, and blood samples were obtained from individuals
35 immediately after capture. Animals from the studied population were all healthy (body condition

36 2.5-3.0/5.0). No lesions or ectoparasites were detected, but the presence of hemoparasites were found in

37 nine individuals. Hematological and biochemical values were obtained, and the morphology of
38 leukocytes were found to be similar to other iguanians. A positive correlation was found between the tissue
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39 amino transferase (AST) and Creatinine kinase (CK) and a negative correlation was found between skin
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 41 and total protein, Calcium, and the Calcium and Phosphorus ratio. No significant inter-sex
 42 differences were found in biochemical values, despite females being larger than males. This is the first
 health
 43 assessment performed on a free-ranging canopy dwelling lizard. These findings provide baseline
 44 data that may be useful for future monitoring if the species faces changes in health status due to
 45 anthropogenic causes or natural disturbances.

47 Introduction

48 Population declines due to anthropogenic causes such as habitat fragmentation, pollution,
 49 invasive species, and global climate change, are widespread (Sinervo et al., 2010; Bruschi, Taylor
 50 & Whitfield, 2015). One way to understand how wild animals are impacted by and respond to
 51 these environmental stressors is through health assessments (Altizer et al., 2013). Hence, the
 52 quantification of hematological and biochemical parameters can be a valuable tool for assessing and
 53 monitoring the health and resilience of wild populations (Stacy, Alleman & Sayler, 2011;
 54 Campbell, 2014; Maceda-Veiga et al., 2015).

55 Health assessments are useful when baseline data on normal health parameter values
 56 from a clinically robust population are available (Valle et al., 2018). Therefore, it is important to
 57 assess the health of wild species, especially populations that have never been surveyed (Valle et
 58 al., 2018). This information helps to identify potential effects of disease, injury, pollutants, or
 59 other changing environmental conditions that would be difficult to understand without
 60 knowledge of normal species-specific variations in hematological and biochemical variables
 61 (Smyth et al., 2014; Lewbart et al., 2015). Performing health evaluations on wildlife populations is being
utilized more commonly by conservationists (Mathews et al., 2006) and has become a proactive
 62 management approach that allows further conservation actions to be taken (Madliger et al.,
 63 2017). For example, Henen, Hofmeyr & Baard (2013) found that confiscated adult tortoises
 64 showed poorer body condition and lower hematological values than wild ones, while Mathews
 65 et al. (2006) found that water voles (*Arvicola terrestris*) with better body condition and higher
 66 hematological values had greater survival probability when reintroduced into the wild.

67 Studies evaluating the health status and characteristics of free-ranging populations are,
 68 however, scarce or absent for most species, especially those that are rarely seen in the wild (Bell
 69 & Donnelly 2006; Whitfield et al., 2007; Dallwig et al., 2011). In lizards, health
 70 assessments reported in the literature have usually been done on species that are threatened (Alberts et
 71 al., 1998; Espinosa-Avilés, Salomón-Soto & Morales-Martínez, 2008; McEntire et al., 2018),
 72 endemic (Lewbart et al., 2015; Arguedas et al., 2018), or kept in captivity (Ellman, 1997; Mayer
 73 et al., 2005; Laube et al., 2016), providing information on the survival of species with
 74 conservation issues. However, free-ranging species with no apparent threats have generally not been
evaluated as well,

75 The Berthold's bush anole (*Polychrus gutturossus*) is one of eight species belonging to the
 76 genus *Polychrus* and the only representative of the family Polychrotidae in Middle America.
 77 This is a moderately large, diurnal lizard that is distinguished by its bright green body coloration

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78 and extremely long tail (over three times the length of the head and body) (Savage, 2002). The
 79 species is sexually dimorphic, with females being larger than males (Savage, 2002; Koch et al.,
 80 2011) and females having green eyelids, while males have yellow eyelids. The species ranges
 81 from Honduras to northwestern Ecuador, apparently restricted to moist and wet forests (Savage,
 82 2002; Leenders, 2019). Despite its large distribution, only a handful of studies have been carried
 83 out concerning its morphological variation, ecology, and natural history (Taylor, 1956; Roberts, 1997;
 84 Koch et al., 2011; Gómez-Hoyos et al., 2015; Bringsøe, Alfaro Sánchez & Hansen, 2016; Ruiz,
 85 Gutiérrez & Flóres Rocha, 2016). *P. gutturosus* is a canopy dweller and its body coloration
 86 makes it difficult to locate individuals during daylight hours. It is believed that deforestation and
 87 habitat modification could pose a threat for this species (Acosta Chaves et al., 2017), although no
 88 health assessment has ever been done and population status is unknown.

89 Health assessments of wildlife in Costa Rica are rare. To our knowledge, health
 90 evaluations of free-ranging species have been performed on 20 mammals (Schinnerl et al., 2011;
 91 Hagnauer Barrantes, 2012; Bernal-Valle, Jiménez-Soto & Meneses-Guevara, 2020) and only one
 92 reptile (green basilisk, *Basiliscus plumifrons*, Dallwig et al., 2011). Therefore, our aim is to
 93 generate data to improve our knowledge of the health status of more Costa Rican reptiles,
 94 by providing baseline data on a wild population of the unique lizard species (*P. gutturosus*). The
 95 following baseline data was included: (1) body temperature and weight, (2) presence of
 96 ectoparasites and external abnormalities through physical examination, and (3) hematological
 97 and biochemical values. Most of our knowledge on *P. gutturosus* comes from museum
 98 specimens (Savage, 2002; Koch et al., 2011) and sporadic observations of individuals in the field
 99 (Gómez-Hoyos et al., 2015; Bringsøe, Alfaro Sánchez & Hansen, 2016; Ruiz, Gutiérrez & Flóres
 100 Rocha, 2016). Therefore, this is the first long-term, empirical study on free-ranging *P. gutturosus*
 101 and one of the few studies overall that has been carried out on a species inhabiting the forest
 102 canopy. Our data was also compared to similar information previously published for close
 103 relatives of *P. gutturosus*.

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 105

106 Materials & Methods

107 Ethics statement

108 All research methods were authorized by Costa Rica's National System of Conservation Areas
 109 (SINAC) under permit numbers SINAC-ACC-PI-R-102-2018 and SINAC-ACC-257-2018.

110

111 Animal collection and handling

112 A total of 40 adult individuals (20 males and 20 females) were collected from October 2018 to
 113 May 2019, carrying out one field trip per month. Lizards were surveyed along a public, dirt road
 114 at El Rodeo (Cascante-Marín, 2012), Ciudad Colón, San José, Costa Rica (Fig. 1). The area has
 115 an irregular topography ranging from 400 to 1016 meters above sea level (masl) and with an
 116 annual average temperature of 23.4°C and an annual average rainfall of 2467 mm (Cascante-
 117 Marín, 2012). Two seasons are evident, a rainy season from May to October and a dry season

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118 from December to March, with two transitional months (November and April) (Cascante-Marín,
119 2012). The area of El Rodeo shows a landscape composed of pastureland and agricultural and
120 urban zones (Fig. 1), although the road sampled was surrounded by bushes, shrubs and trees on
121 both sides. The lizards were searched for only on such shrubs and trees at night, since resting
122 animals are easier to spot. Animals were located between 560 and 754 masl and air temperatures
123 ranged from 21.2 to 27.7°C.

124 Once an individual was observed, the skin temperature at the resting site was measured
125 using a digital laser infrared thermometer gun (Nubee®, NUB8550AT model). The lizards were
126 then hand-caught from shrubs or trees and taken to a workstation at the temporary mobile field
127 laboratory approximately 5 to 10 m from the collection site. A J/K/T/E thermocouple
128 thermometer (Professional Instruments®, 1312 model) was used to measure the lizard's cloacal
129 body temperature, which was taken by inserting the K probe into the cloaca, approximately 1-2
130 min after capture. A blood sample was taken after the temperature was measured. The process
131 from catching the animal to collecting its temperature and blood lasted ca. 12 min.

132

133 **Physical examination and tagging**

134 The individual was placed in a cloth bag and weighed on a digital scale (to the nearest 0.1 g).
135 Afterwards, the lizard was examined for obvious abnormalities or lesions. Physical examinations
136 were performed according to Divers (2019). Oral cavity inspection was easily performed since
137 they kept their mouths open as a defense mechanism. Any external parasites found on the skin
138 were noted and females were gently palpated to detect if they were gravid (~~feeling~~ for palpable
139 eggs). The body condition was assessed on a scale of 1-5; 1 being emaciated, 2 underweight, 3
140 normal, 4 overweight, 5 obese (Divers, 2019). After physical examination, a blood sample was
141 collected and each anole was measured to determine snout-vent length (SVL), and then tagged
142 subcutaneously in the left inguinal region ([https://www.wsava.org/Guidelines/Microchip-
143 Identification-Guidelines](https://www.wsava.org/Guidelines/Microchip-Identification-Guidelines)) with a Biomark® HPT12 radio frequency identification tag and
144 released back where it was collected.

145

146 **Hematology and biochemistry analyses**

147 Each lizard was manually restrained and 0.2-0.4 ml of blood was drawn from the ventral
148 coccygeal vein. If two attempts to collect blood from the tail were unsuccessful, then blood was
149 taken from the jugular vein. Blood sampling time varied between 3-5 min. For blood draws, a
150 heparinized 30-gauge needle attached to a 1.0 ml syringe was used. Two blood films were
151 immediately made on clean glass microscope slides and then the rest of the sample from the
152 syringe was placed in a 0.5 ml Eppendorf® tube. All samples were taken to the laboratory the
153 same night and stored at 4°C to be processed the following day. Red blood cell (RBC), white
154 blood cell (WBC) and thrombocyte count (TC) were performed using the standard method of a
155 Natt and Herrick solution (1/200) on a Boeco® Neubauer Improved chamber. Packed cell
156 volume (PCV) was determined using high-speed centrifugation (Digisystem® Laboratory
157 Instruments Inc.) of blood-filled microhematocrit tubes. Differential white blood cells were

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158 obtained by examining a peripheral smear stained with Diff-Quick® stain (Campbell, 2014).
159 Polychromatophil percentage was determined by counting the number of polychromatophils
160 among 1000 erythrocytes.

161 Total proteins were obtained by means of a clinical refractometer (REC-200ATC®,
162 RETK-70 model) using plasma from the microhematocrit tube. Biochemical parameters such as
163 aspartate aminotransferase (AST), albumin (Alb), calcium (Ca), cholesterol (Chol), creatinine
164 kinase (CK), glucose, phosphorus (P) and uric acid (UA) were measured with a Roche® analyzer
165 (Cobas c111 model) following the company's instructions.

166

167 Comparison with close relatives

168 Literature was reviewed for similar hematology and biochemistry information published on close
169 relatives of the Berthold's bush anole. The review focused on the infraorder Iguania, which
170 includes *Polychrus guttuosus*, according to the phylogeny proposed by Pyron, Burbrink &
171 Wiens (2013). Twenty-nine papers were found (see supplementary data) corresponding to eight
172 of the 14 families that make up Iguania, from which the mean and standard deviation (or range,
173 when SD was not reported) of hematological and biochemical parameters of free ranging
174 individuals was obtained. This information was used to place the physiological values generated
175 for *P. guttuosus* within a phylogenetic context.

176

177 Statistical analyses

178 The mean, standard deviation, range, and 95% confidence intervals for all blood parameters were
179 calculated. Differences in weight, biochemistry and hematological values between the sexes
180 were examined using t-tests. Differences between animals infected with hemoparasites and non-
181 infected animals in terms of PCV, RBC, heterophil to lymphocyte (H:L) ratio, WBC, weight and
182 SVL and SMI and sexes were determined using t-tests. A Pearson correlation was calculated to
183 look at the association between body temperature (skin and cloacal), weight and SVL with all the
184 hematologic and biochemistry values. To estimate body condition, the Scaled Mass Index (SMI)
185 was used. This index proved to be a better indicator of the relative size of energy reserves and
186 other body components, $SMI = Mi [Lo/Li]^{bSMA}$. The length (Li) variable has the strongest
187 correlation with mass (Mi) on a log-log scale, since this is likely to be the length that best
188 explains that fraction of mass associated with structural size. The scaling exponent (bSMA) is
189 calculated indirectly by dividing the slope from an ordinary least squares regression and Lo is the
190 mean of the total sample length (Peig & Green, 2009). All statistical analyses were performed
191 using IBM SPSS®v24 with a standard α level of 0.05. In addition, information from nine sample
192 points from 40 Berthold's bush anoles were geocoded and a map was generated using ArcGis
193 10.1 software (ESRI, Redlands, CA, USA).

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196 Results

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198 Physical examinations

199 All lizards appeared to be active and healthy. Female weight ranged from 27 g to 80 g (mean \pm
200 SD = 52.25 ± 13.56) and males weighed from 17 g to 52 g (mean \pm SD = 37.30 ± 8.86). No
201 evidence of lesions was detected during physical exams. No ectoparasites (acari, ticks, or other
202 macroscopic arthropods) were observed and none of the females had palpable oviductal eggs.

203 The general body condition of all individuals was between 2.5 to 3.0 and a body mass
204 index was also obtained. The SMI was $3.75 (\pm 0.15)$ CI [3.70-3.79]. No significant differences
205 were found between sexes ($t = 0.99$, $p = 0.33$).

206

207 Physiological parameters

208 No significant differences between sexes in any of the hematological or biochemical parameters
209 were found. Hematological values are presented in Table 1. The morphology of lymphocytes
210 (Fig. 2a), heterophils (Fig. 2b), eosinophils (Fig. 2c), basophils and monocytes (Fig. 2d) were
211 similar to other iguanian species.

212

213 Heterophil to lymphocyte (H:L) ratios were calculated (Table 1). Both shape and
214 appearance of erythrocytes and thrombocytes were similar to those reported for other reptiles.
215 Polychromatophilic erythrocyte percentage was $1.33 (\pm 0.69)$ (Table 1). Intraerythrocytic
216 parasites were found in nine (three females and six males) of the 40 individuals (22.5% of the
217 total sample) (Fig. 2e). No significant differences were found between individuals with and
218 without hemoparasites for the following variables: PCV ($t = -1.24$, $p = 0.22$), RBC, ($t = 1.11$, $p =$
221 0.27), WBC ($t = 0.64$, $p = 0.52$), H:L ratio ($t = 1.55$, $p = 0.28$), weight ($t = -0.16$, $p = 0.86$), SVL
222 ($t = -1.43$, $p = 0.16$), and polychromasia ($t = -0.64$, $p = 0.53$).

223

224 Clinical biochemistry values are reported in Table 2. A wide range was observed in AST
225 (15.1 U/L – 139.40 U/L) and CK (122.9 U/L – 6848.20 U/L), and both muscle enzymes were
226 highly correlated ($r = 0.795$, $p < 0.001$). Skin temperature varied between 18.8°C and 26.2°C
227 (mean \pm SD = 22.31 ± 1.74) and cloacal temperature varied between 21.2°C and 32.4°C (mean \pm
228 SD = 25.22 ± 2.11). A negative correlation was found between skin temperature ($r = -0.51$, $p =$
229 0.001) and cloacal temperature ($r = -0.42$, $p = 0.007$) with AST (Fig. 3a). The same occurred
230 between skin temperature ($r = -0.51$, $p = 0.001$) and cloacal temperature ($r = -0.42$, $p = 0.007$)
231 with CK (Fig. 3b). A positive correlation was found in females, but not in males, between
232 calcium ($r = 0.57$, $p = 0.009$), total protein ($r = 0.49$, $p = 0.03$) and the calcium/phosphorus
233 (Ca/P) ratio ($r = 0.71$, $p < 0.001$) with weight.

234

235 Phylogenetic comparison

236 Even though hematological and biochemical information is not available for a number of iguanian
237 families (e.g., Leiocephalidae, Crotaphytidae, Hoplocercidae, Opluridae and Leiosauridae), some
238 comparisons are still possible. For hematological parameters, WBC was found to be higher for
239 Polychrotidae, Liolaemidae and Corytophanidae (all three phylogenetically related) compared to

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240 other families, while the number of lymphocytes is high in Polychrotidae and comparable with
 241 Iguanidae and Tropicuridae (Fig 4). For biochemical parameters, Polychrotidae showed a higher
 242 value of total protein when compared to Tropicuridae, although no other value differed
 243 significantly (Fig. 5).

245 Discussion

246 Health assessments provide baseline information that can be used to understand future changes
 247 in the health status of wildlife populations. Both physical examination and internal physiological
 248 data (i.e. body temperature, hematology and biochemistry) can serve as valuable tools for
 249 evaluating and monitoring the health of wild populations (Stacy, Alleman & Saylor, 2011;
 250 Campbell, 2014), especially when such assessments provide the only available data for a given
 251 species (Innis, 2014). Although physical examinations are common in many taxa, including
 252 reptiles, there are no known reports assessing hematology and biochemistry parameters in free-
 253 ranging, canopy dwelling lizards. Therefore, this study is important to report such data for
 254 *Polychrus guttuerosus*.

255 Physical examination of Berthold's bush anoles showed no evident abnormalities,
 256 suggesting that all animals were apparently healthy. Body condition is assumed to influence
 257 an animal's health and fitness (Peig & Green, 2009) and although the body condition index cannot
 258 be compared with other studies, the fact that no differences were found between sexes indicates
 259 an evenness to our sample. These findings may indicate that environmental conditions such as
 260 availability of habitat, food and water are fulfilling the requirements of the individuals of the population
 261 studied, despite being located in an altered area (Fig. 1). Furthermore, healthy animals also
 262 suggest that physiological parameters, such as hematological and biochemical blood values, may
 263 be within a normal range. Blood cell counts and cell morphology, however, are highly variable
 264 between reptilian species, even among members of the same genus (Stacy, Alleman & Saylor,
 265 2011; Innis, 2014). Such variation is caused by both intrinsic and extrinsic factors like age, sex,
 266 season, presence of environmental stressors, parasite load, nutritional status, and capture and
 267 restraint (Campbell, 2014; Heatley & Russell, 2019). For that reason, the results in this study are compared
 268 with other related lizard species.

269 PCV and RBC counts were similar to closely related lizards (James et al., 2006; Dallwig
 270 et al., 2011; McEntire et al., 2018), and polychromatophilic cell mean was 1.33%. In normal
 271 reptiles, the percentage of polychromatophilic red cells is from >1 to 2.5% (Heatley & Russell,
 272 2019). Erythrocyte counts and the presence of a high percentage of polychromasia have been
 273 used as an important parameter for health assessments of wild lizards. For example, Smyth et al.
 274 (2014) found that sleepy lizards (*Tiliqua rugosa*) in agricultural environments had a regenerative
 275 anemia (low PCV and increased polychromatophils) compared to animals in non-agricultural
 276 areas.

277 In squamate species, lymphocytes are the predominating circulating cell, usually 80% of
 278 the leukogram (Sykes & Klaphake, 2015; Heatley & Russell, 2019), although in some species
 279 heterophils can be the main circulating leukocyte. Hematological data comparisons with other
 closely related families showed that

280 lymphocytes were the main white cell population in *P. guttuerosus*, followed by heterophils and
 281 monocytes (Fig. 5). For example, *Polychrus*, *Amblyrhynchus*, *Microlophus*, *Intellagama* and

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282 *Furcifer* include species that are predominantly lymphocytic, while heterophils are the predominant
 283 circulating leukocyte cell in *Basiliscus*, *Cyclura*, *Phrynosoma* and *Liolaemus* (Fig. 5). These blood
 284 circulating cells are important in calculating the heterophil to lymphocyte ratio (H:L ratio)
 285 which has been used as an indicator of stress in reptiles (Aguirre, et al., 1995; Cartledge, Gartrell
 286 & Jones, 2005; Davis, Maney & Maerz, 2008; French, Fokidis & Moore, 2008; Silvestre, 2014)
 287 and wild and domestic birds (Vleck, et al., 2000; Huff et al., 2005)

288 Normal H:L ratio in reptile species with more
 289 lymphocytes circulating than heterophils will have potentially delayed responses to heterophilia
 290 (Davis, Maney & Maerz, 2008; Campbell, 2014; Silvestre, 2014), which may be important when
 291 evaluating acute or chronic stress.

292 Most biochemistry analytes measured in *Polychrus guttuosus* were within similar ranges
 293 of other iguanian species. CK and AST values were similar to those found in *Cyclura* species
 294 (Alberts et al., 1998; James et al., 2006; Maria et al., 2007) and *Basiliscus plumifrons* (Dallwig et
 295 al., 2011), in which the length of the capture, holding period, restraint and venipuncture results in
 296 elevated CK and AST levels. In reptiles, CK is an enzyme considered to be specific to muscle cells
 297 and thus with muscle damage will elevate in the blood, while AST is a less specific enzyme and is found
 298 primarily in liver but also in muscle tissue,

299 (Anderson et al., 2013; Bogan & Mitchel, 2014; Petrosky, Knoll & Innis, 2015). A high positive
 299 correlation between AST and CK was found in *P. guttuosus*, suggesting that higher levels of the enzyme
 300 AST may be associated with muscle tissue along with CK in this lizard species.

301 A negative correlation was found between AST and CK and both skin and cloacal
 302 temperatures (Fig. 4). As ectotherms, reptiles experience temperature-induced changes in
 303 metabolic rate (Niewiarowski & Waldschmidt, 1992). When reptiles are resting and their body
 304 temperature is low, their metabolic rate and energy stay at basal levels (Vitt & Caldwell, 2014);
 305 however, movement or using anaerobic metabolism in specific situations requires more energy
 306 than the basal rate, so reptiles attain higher body temperatures (Randall et al., 2002). During
 307 high-intensity, short-duration activity (e.g., capture and sampling of the lizards [see Materials
 308 and Methods]), the concentration of ATP within muscles can be maintained constant by
 309 continuous re-phosphorylation of ADP by the CK reaction (Randall et al., 2002). As a result, an
 310 animal can use the large reserve of high-energy phosphate in CK to power muscle contraction
 311 until oxidative and anaerobic metabolism start to generate ATP, allowing it to move for much
 312 longer (Randall et al., 2002). Since our sampling (capture, restrain and venipuncture) was
 313 performed at night, individuals of *P. guttuosus* had lower body temperatures and thus likely lower
 314 oxygen consumption (Clark, Butler & Frappell, 2006), which with capture and struggle resulted in rapid
 315 muscle contraction initially utilizing

315 the anaerobic (glycolytic) pathway to keep its activity (Bennett, 1980). Anaerobic
 316 muscular metabolism also generates an electrolyte imbalance (mainly calcium) and releases
 317 oxygen and lactate, leading to muscle injury (Giannoglou, Chatzizisis & Misirli, 2007). Such muscle
 318 damage causes CK and AST enzymes to leak into the blood stream from muscle cells (Allison, 2005).
 319 Hence, increased plasma activities of both CK and AST suggest active or recent muscle injury

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Deleted: <#>In squamate species, lymphocytes are the
 predominating circulating cell, usually 80% of
 <#>the leukogram (Sykes & Klaphake, 2015; Heatley &
 Russell, 2019), although in some species
 heterophils can be the main circulating leukocyte.

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Commented [ss4]: We can't say that AST is less sensitive
 for liver damage in this species as we did not evaluate the
 liver specifically: in other words it may be an important
 enzyme of both liver and muscle but we can only comment
 on its association with the elevated CK in this study and no
 more.

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320 (Silvestre, 2014). Therefore, animals at lower temperatures, with lower oxygen consumption,
 321 utilized the anaerobic pathway at the moment of capture, leading to more muscle
 322 damage, resulting in the release of more CK and AST than lizards, captured at higher temperatures, which
 probably
 323 utilized anaerobic muscular activity later.

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324 No differences between sexes were found among hematological and biochemical
 325 variables. In other iguanian lizards where males are larger than females, significant differences
 326 in biochemical values have been found between sexes (Dallwig et al., 2011). For example, males
 327 of the San Cristóbal lava lizard (*Microlophus bivittatus*) had higher hemoglobin, PCV and
 328 glucose than females (Arguedas et al., 2018), and female green iguanas (*Iguana iguana*) had
 329 higher hemoglobin and PCV than males (Harr et al., 2001). Interestingly, in *Phrynosoma*
 330 *cornutum*, where females are larger than males, basophil counts were lower in females than in
 331 males (McEntire et al., 2018). Most explanations for differences between sexes in hematological
 332 and biochemical values are based on reproductive physiological status or hormonal biases
 333 (McEntire et al., 2018), although the reasons why *P. guttuosus* have no difference between
 334 sexes are unknown.

335 A positive correlation between calcium and proteins with body weight was found for
 336 females but not for males. It is known that during vitellogenesis, circulating estrogens raise
 337 calcium, phosphorus and proteins in plasma (Bonnet, Naulleau & Mauget, 1994; Jones, 2011),
 338 however, no correlation between P and weight was found. Calcium increases during
 339 vitellogenesis and folliculogenesis for most squamates, the investment of calcium in eggshells is
 340 considerably less than for yolk (Stuart & Ecay, 2010). We hypothesize that heavier females may
 341 be under active vitellogenesis, increasing their weight due to follicular development.

342 A correlation was found between calcium to phosphorus (Ca/P) ratio and weight in
 343 females but not in males. Ca and P homeostasis are directly interrelated because serum Ca
 344 interplays with serum P through the modulation of several hormones, such that serum
 345 concentration is approximately inversely related (a high Ca/P ratio means higher Ca than P)
 346 (Madeo et al., 2018). Calcium increases proportionally greater than P, resulting in a higher value
 347 of Ca/P. Although the reason for that is unknown, a possible explanation is that parathyroid
 348 activity may be higher in heavier females due to larger follicular development. Unfortunately, no
 349 literature is available regarding the breeding season on this species, so the reproductive
 350 stage of the animals sampled is unknown.

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351 Finally, intraerythrocytic parasites were found in nine individuals, but no differences
 352 were found between infected and non-infected animals with hematological values or physical
 353 measurements. The presence of hemoparasites in wild reptiles is common (Telford, 2009) and
 354 usually considered non-pathogenic (Stacy, Alleman & Saylor, 2011). Hemoparasite life cycles
 355 involve sexual reproduction in an invertebrate host (e.g. ticks, mites, mosquitoes and flies) and
 356 asexual reproduction in the reptilian host (Telford, 2009; Campbell, 2015). Since no mites or
 357 ticks were found in the lizards sampled (which may be due to their arboreal habits), it is possible
 358 that the hemoparasites were transmitted by mosquitoes or flies. Pathogenesis caused by
 359 hemoparasite infections in reptiles is unclear, with studies reporting from apparently non-

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360 detrimental infections in natural hosts to severe and life-threatening illness in unnatural hosts
 361 (Maia et al., 2014). Hence, more research is needed to identify the species of,
 362 hemoparasite identified here and continued, monitoring of these lizard populations, to establish actual
 prevalence of the disease.
 363 Health assessments allow for evaluation of body condition, disease, stress levels, hydration status
 and
 364 temperature changes of wild populations to be detected (Stacy, Alleman & Sayler, 2011; Innis,
 365 2014) and thus, determine whether a population faces any stress related to environmental
 366 changes or anthropogenic causes. In this study, the first baseline data of hematology and clinical
 367 biochemistry values for the Berthold's bush anole (*Polychrus guttuosus*) is reported. Poorly
 368 studied species with populations occurring in altered or non-protected environments can be at
 369 greater risk from human activity. Since *P. guttuosus* depends on its arboreal habits,
 370 deforestation due to urban or agricultural activities can affect their survival, reduce its habitat
 371 and increase the transmission of diseases. Preventing species from becoming threatened requires
 372 conservation actions based on scientific knowledge. This includes health assessments of wild
 373 populations that can be used for future management and protective actions.

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376 Conclusions

377 Hematological and biochemical values were obtained for the first time in this poorly studied,
 378 arboreal lizard species. The morphology of leukocytes were similar to other iguanians. A positive
 379 correlation was found between Aspartate amino transferase (AST) and Creatinine kinase (CK) and
 380 a negative correlation between skin and cloacal temperatures with AST and CK. There were
 381 positive correlations between female weight and total protein, Calcium, and the Calcium and
 382 Phosphorus ratio. No significant inter-sex differences were found, despite females being larger
 383 than males. These findings provide baseline data that may be useful if this,
 384 species faces changes in health status due to anthropogenic causes or natural disturbances in the future.

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 390 from the National University of Costa Rica School of Veterinary Medicine, for elaborating the
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Table 1 (on next page)

Table 1. Hematological values (n = 40) of the Berthold's bush anole (*Polychrus gutturosus*) in Costa Rica. PCV (Packed cell volume), RBC (Red blood cells), and WBC (White blood cells).

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1 **Table 1.** Hematological values (n = 40) of the Berthold's bush anole (*Polychrus gutturosus*) in
2 Costa Rica. PCV (Packed cell volume), RBC (Red blood cells), and WBC (White blood cells).

3

Analyte (Units)	Mean ± SD	Range	95% CI
PCV (%)	31.75 ± 4.53	23.00 - 44.00	30.35 - 33.15
RBC (10 ¹² /L ⁹)	0.94 ± 0.20	0.64 - 1.35	0.88 - 1.01
Polychromatophils (%)	1.33 ± 0.69	0.4-3.0	1.11-1.54
WBC (10 ⁹ /L)	19.44 ± 6.66	8.04 - 37.18	17.38 - 21.51
Thrombocyte Count (10 ⁹ /L)	2.13 ± 1.14	0.21 - 4.52	1.78 - 2.49
Heterophils (10 ⁹ /L)	2.66 ± 1.36	0.84 - 6.99	2.23 - 3.08
Heterophils (%)	13.78 ± 5.14	6.00 - 29.00	12.18 - 15.37
Lymphocytes (10 ⁹ /L)	14.37 ± 5.36	5.87 - 27.14	12.71 - 16.03
Lymphocytes (%)	74.13 ± 10.01	26.00 - 87.00	71.02 - 77.23
Monocytes (10 ⁹ /L)	1.76 ± 1.71	0.12 - 10.13	1.22 - 2.29
Monocytes (%)	8.60 ± 6.62	1.00 - 42.00	6.55 - 10.65
Eosinophils (10 ⁹ /L)	0.58 ± 0.41	0.00 - 1.57	0.45 - 0.70
Eosinophils (%)	3.03 ± 1.79	0.00 - 7.00	2.47 - 3.58
Basophils (10 ⁹ /L)	0.11 ± 0.15	0.00 - 0.45	0.06 - 0.16
Basophils (%)	0.58 ± 0.75	0.00 - 2.00	0.34 - 0.81
H:L Ratio	0.21 ± 0.17	0.08 - 1.12	0.15 - 0.26

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Table 2(on next page)

Table 2. Blood biochemical values ($n = 40$) of the Berthold's bush anole (*Polychrus gutturosus*) in Costa Rica. A/G Ratio (Albumin/Globulin ratio), AST (Aspartate amino transferase), CK (Creatinine kinase), and Ca:P Ratio (Calcium:Phosphorus rati

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Analyte (Units)	Mean \pm SD	Range	95% IC
Glucose (mmol/L)	11.96 \pm 2.04	8.38 - 16.10	11.32 - 12.59
Total Protein (g/L)	75.10 \pm 7.80	60.00 - 90.00	72.68-77.52
Albumin (g/L)	17.91 \pm 6.34	3.70 - 28.17	15.95 - 19.88
Globulins (g/L)	57.19 \pm 6.20	46.10 - 70.32	55.27 - 59.11
A/G Ratio	0.32 \pm 0.12	0.06 - 0.52	0.28 - 0.36
AST (U/L)	35.08 \pm 23.86	15.10 - 139.40	27.69 - 42.47
CK (U/L)	1283.56 \pm 1366.22	122.90 - 6848.20	860.17 - 1706.94
Calcium (mmol/L)	3.81 \pm 1.64	2.20 - 9.35	4.32 - 3.30
Phosphorus (mmol/L)	2.46 \pm 0.85	1.43 - 5.49	2.72 - 2.20
Ca:P Ratio	1.60 \pm 0.55	0.76 - 3.47	1.43 - 1.77
Uric acid (μ mol/L)	223.78 \pm 209.56	59.30 - 1164.90	158.84 - 288.72
Cholesterol (mmol/L)	8.97 \pm 3.16	4.25 - 17.50	7.99 - 9.95

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Figure 1

Map of Costa Rica showing the location of the Berthold's bush anole (*Polychrus guttuosus*) sampling site with exact coordinate points along an approximately 3 km trail showing where they were captured. The yellow marks refer to collection points, not to individuals.

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Commented [ss5]: Do you mean RED not Yellow? I don't see yellow marks

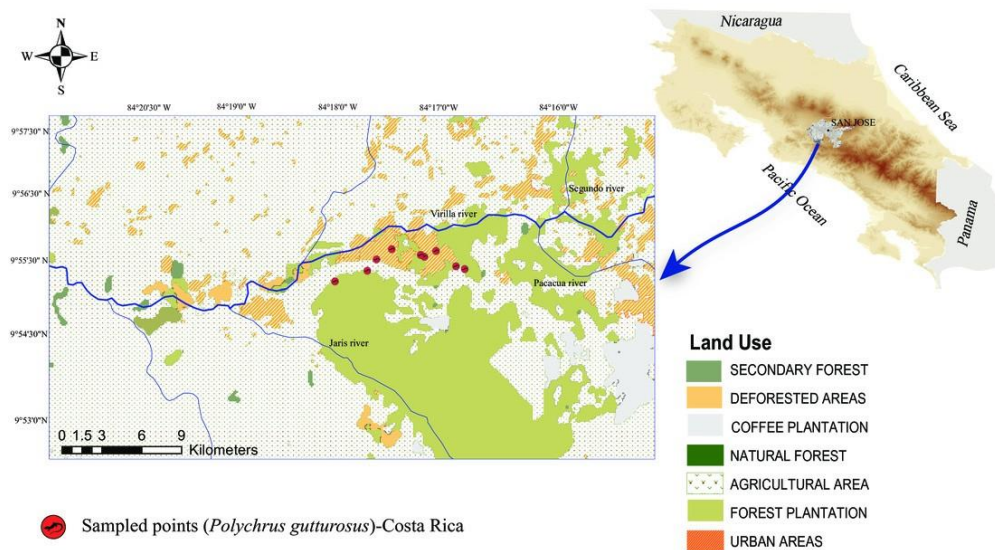


Figure 2

Photographs of selected Berthold's bush anole (*Polychrus gutturosus*) blood cells stained with Diff-Quick stain at 100x. (a) lymphocyte (b) heterophil (c) eosinophil with vacuolated cytoplasm (d) monocyte (e) intraerythrocytic hemoparasite.

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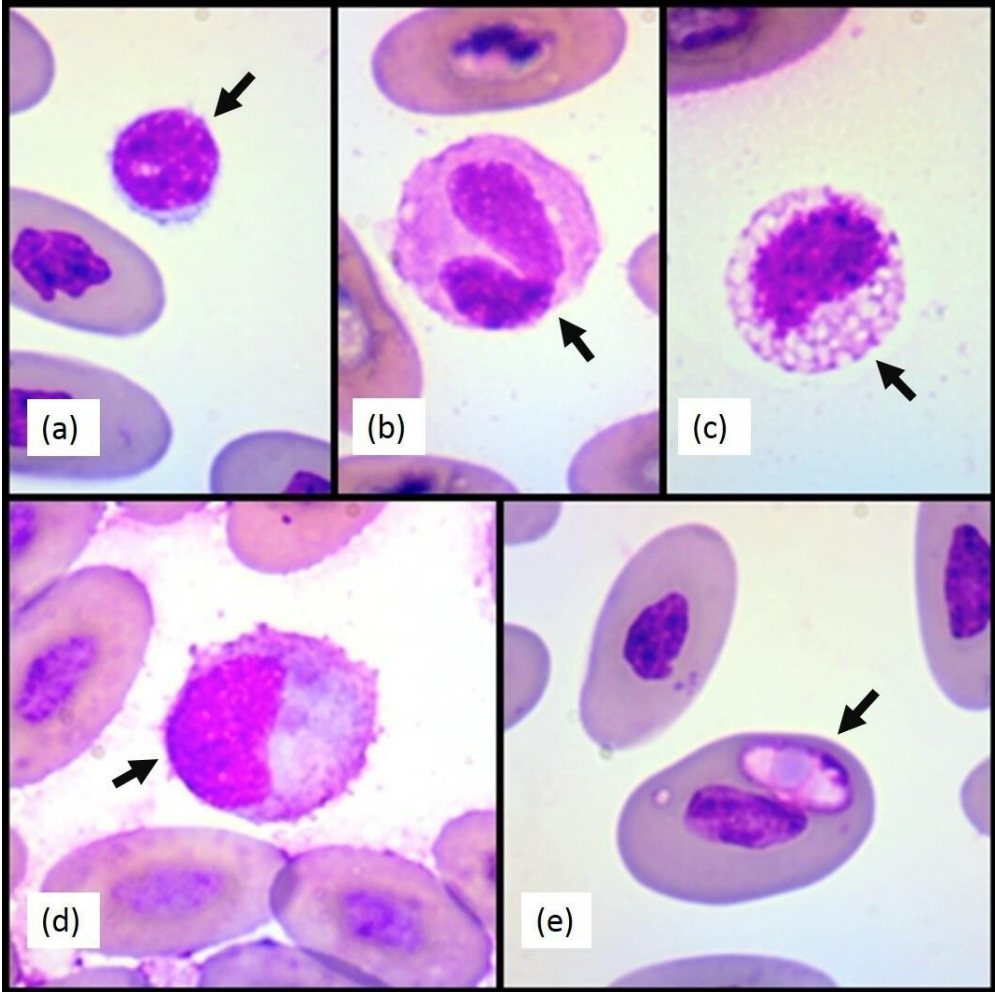


Figure 3

Linear correlations of skin and cloacal temperatures ($^{\circ}\text{C}$) of the Berthold's bush anole (*Polychrus gutturosus*) with blood values of (a) aspartate amino transferase (AST) and (b) creatinine kinase (CK).

This figure describes negative correlations between muscle tissue enzymes and body temperatures of the lizards

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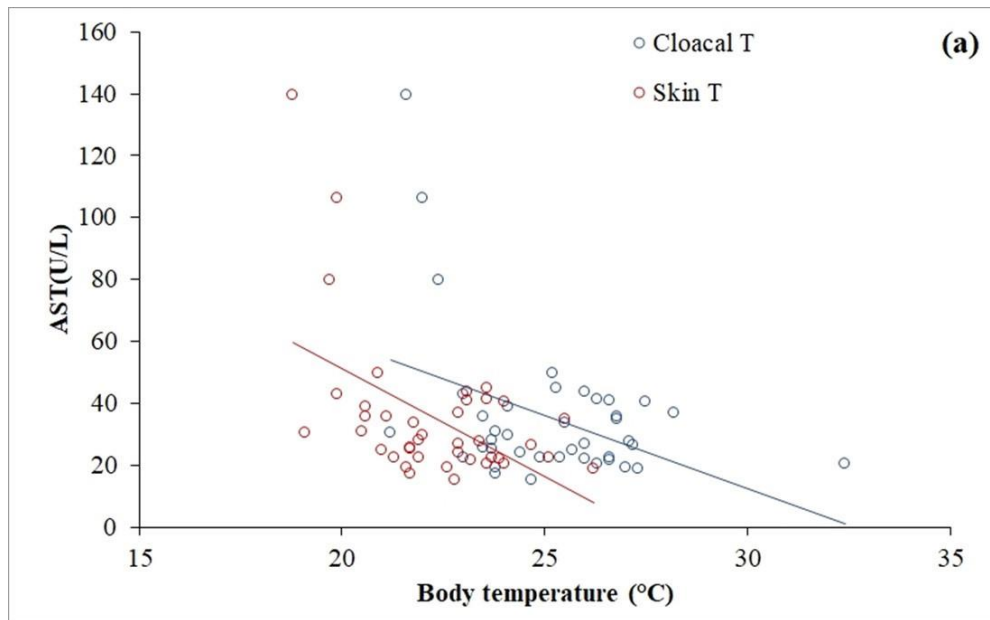


Figure 4

Linear correlations of skin and cloacal temperatures ($^{\circ}\text{C}$) of the Berthold's bush anole (*Polychrus gutturosus*) with blood values of (a) aspartate amino transferase (AST) and (b) creatinine kinase (CK).

This figure describes negative correlations between two muscle tissue enzymes and body temperatures

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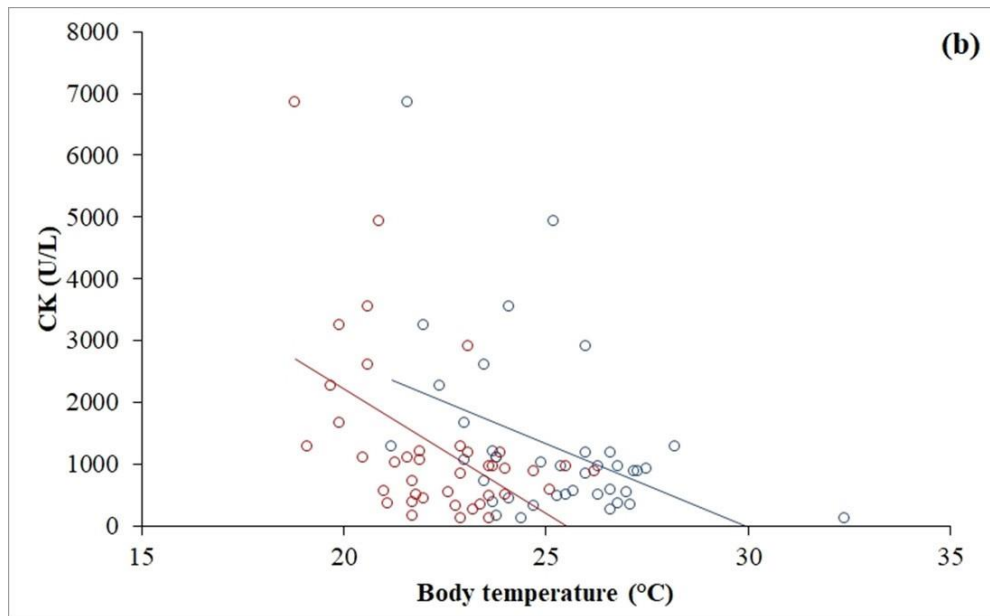
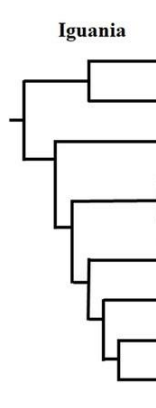


Figure 5

Mean \pm SD (or range) of hematological parameters extracted from the literature for species of the infraorder Iguania, as a comparison with the Berthold's bush anole (*Polychrus gutturosus*). Also depicted are the phylogenetic relationships (adapted from Pyron et al. [2013]) of iguanian families for which hematological information was available. The full name of each species is: *Furcifer pardalis* (panther chameleon), *Intelligama lesueurii* (Australian water dragon), *Microlophus bivittatus* (San Cristóbal lava lizard), *Amblyrhynchus cristatus* (marine iguana), *Cyclura cyclura* (Andros Island iguana), *Phrynosoma cornutum* (Texas horned lizard), *Liolaemus wiegmanni* (Wiegmann's lizard), and *Basiliscus plumifrons* (green basilisk.). ND = No Data.



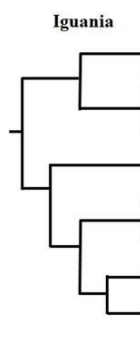
Iguania	Family	PCV (%)	WBC (10 ⁹ /L)	Heterophils (10 ⁹ /L)	Heterophils (%)	Lymphocytes (10 ⁹ /L)	Lymphocytes (%)	Monocytes (10 ⁹ /L)	Monocytes (%)
	Chamaeleonidae (<i>F. pardalis</i>)	26.30 \pm 6.10	7.30 \pm 3.10	ND	23.90 \pm 6.30	ND	67.30 \pm 7.80	ND	8.80 \pm 4.00
	Agamidae (<i>I. lesueurii</i>)	30.22 (21.00-43.00)	5.90 (0.88-9.90)	ND	35.09 (14.00-69.00)	ND	51.28 (18.00-76.00)	ND	8.06 (2.00-27.00)
	Tropiduridae (<i>M. bivittatus</i>)	33.39 \pm 5.90	ND	ND	5.40 \pm 4.98	ND	86.04 \pm 5.43	ND	7.16 \pm 7.45
	Iguanidae (<i>A. cristatus</i>)	27.05 \pm 5.67	ND	ND	10.09 \pm 8.29	ND	83.36 \pm 11.64	ND	6.05 \pm 3.92
	Iguanidae (<i>C. cyclura</i>)	29.06 \pm 3.70	6.91 \pm 2.57	4.25 \pm 1.84	ND	1.28 \pm 0.68	ND	0.24 \pm 0.19	ND
	Phrynosomatidae (<i>P. cornutum</i>)	26.80 \pm 5.44	3.04 \pm 0.94	1.41 \pm 0.58	45.40 \pm 9.00	1.12 \pm 0.37	37.30 \pm 8.60	0.17 \pm 0.11	5.48 \pm 3.07
	Polychrotidae (<i>P. gutturosus</i>)	31.75 \pm 4.53	19.44 \pm 6.66	2.66 \pm 1.36	13.78 \pm 5.14	14.37 \pm 5.36	74.13 \pm 10.01	1.76 \pm 1.71	8.60 \pm 6.62
	Liolaemidae (<i>L. wiegmanni</i>)	ND	19.13 (6.60-45.00)	ND	50.00 (23-90)	ND	36.00 (10-60)	ND	7.00 (4-32)
	Corytophanidae (<i>B. plumifrons</i>)	31.40 \pm 8.00	18.70 \pm 8.40	13.20 \pm 5.90	ND	3.60 \pm 2.20	ND	1.40 \pm 1.20	ND

Commented [ss6]: SHOULD THESE BE TABLES? For figures 5 and 6 do we need to reference where the data is from for these other species somewhere? I realize you have references for them but think unfortunately we need a call out either in the table or perhaps in the legend for the table after you provide the full latin name you could add the author reference. It will make for a long legend but must provide. Alternatively, you could provide as an asterisk and the information under the table.

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Figure 6

Mean \pm SD (or range) of biochemical parameters extracted from the literature for species of the infraorder Iguania, as a comparison with the Berthold's bush anole (*Polychrus gutturosus*). Also depicted are the phylogenetic relationships (adapted from Pyron et al. [2013]) of iguanian families for which biochemical information was available. The full name of each species is: *Furcifer pardalis* (panther chameleon), *Intellagama lesueurii* (Australian water dragon), *Pogona vitticeps* (bearded dragon), *Microlophus bivittatus* (San Cristóbal lava lizard), *Iguana iguana* (green iguana), *Cyclura cyclura* (Andros Island iguana), and *Basiliscus pluminifrons* (green basilisk.). ND = NoData.



Iguania	Family	Glucose (mmol/L)	Total Protein (g/L)	Albumin (g/L)	Calcium (mmol/L)	Phosphorus (mmol/L)	Uric acid (mmol/L)	Cholesterol (mmol/L)	AST (U/L)	CK (U/L)
	Chamaleonidae (<i>F. pardalis</i>)	3.90 \pm 1.71	48.0 \pm 7.00	19.00 \pm 4.00	2.40 \pm 0.30	1.90 \pm 0.30	0.32 \pm 0.22	ND	18.90 \pm 3.10	211.40 \pm 131.20
	Agamidae (<i>I. lesueurii</i>)	8.85 (5.27-12.17)	47.38 (28.00-72.00)	ND	6.07 (2.52-30.90)	1.99 (1.20-4.01)	0.21 (0.01-1.05)	ND	ND	1703.00 (42-7018)
	Agamidae (<i>P. vitticeps</i>)	11.70 \pm 2.20	66.00 \pm 12.00	ND	2.95 \pm 0.95	1.90 \pm 0.61	0.31 \pm 0.15	17.40 \pm 5.92	ND	ND
	Tropiduridae (<i>M. bivittatus</i>)	15.11 \pm 2.55	81.0 \pm 10.60	ND	ND	ND	ND	ND	ND	ND
	Iguanidae (<i>I. iguana</i>)	9.43 \pm 0.25	61.00 \pm 12.00	24.00 \pm 4.00	3.12 \pm 0.25	1.70 \pm 0.51	0.21 \pm 0.12	6.60 \pm 1.06	40.00 \pm 32.00	ND
	Iguanidae (<i>C. cyclura</i>)	10.50 \pm 2.18	48.0 \pm 8.80	20.30 \pm 3.80	3.25 \pm 2.30	1.72 \pm 0.74	0.10 \pm 0.12	2.51 \pm 0.87	29.47 \pm 16.38	2342.00 \pm 2572.79
	Polychrotidae (<i>P. gutturosus</i>)	11.96 \pm 2.04	75.10 \pm 7.80	17.91 \pm 6.34	3.81 \pm 1.64	2.46 \pm 0.85	0.22 \pm 0.21	8.97 \pm 3.16	35.08 \pm 23.86	1283.56 \pm 1366.22
	Corytophanidae (<i>B. pluminifrons</i>)	10.71 \pm 2.67	44.00 \pm 16.00	18.00 \pm 3.00	2.65 \pm 0.32	1.81 \pm 0.51	0.10 \pm 0.05	ND	48.30 \pm 26.20	6323.00 \pm 2074.00

Commented [ss7]: Should 5 and 6 be Tables. See comment on figure 5

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