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## Hematology and biochemistry parameters of the Central American bushmaster (*Lachesis stenophrys*) under human care in Costa Rica

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### Abstract

**Background:** The Central American bushmaster (*Lachesis stenophrys*) is one of the largest pitvipers in the Americas, with relatively low abundance, suspected population declines, and continuing loss, fragmentation, and habitat degradation.

**Aim:** Conservation actions, both in the wild and in captivity, bear the need for health parameters that allow managers and veterinarians to have a better understanding of health, especially when there are relatively few individuals in captivity to obtain robust information since there is no published information on the genus. To have hematological and biochemical reference ranges on the genus *Lachesis*.

**Methods:** Blood samples were collected from 32 individuals (18 females and 14 males) under human care from 7 zoological institutions from August 2022 to January 2023 and performed hematological and biochemical analyses.

**Results:** Reference intervals of hematological analytes included packed cell volume (17.51%–37.27%), total red blood cell count ( $0.36\text{--}0.92 \times 10^{12}/\text{l}$ ), hemoglobin (61.73–145.23 g/l), white blood cell count ( $3.18\text{--}13.79 \times 10^9/\text{l}$ ), lymphocytes (2.16%–11.23%), azurophils (0.50%–4.20%), monocytes (0.00%–0.21%), heterophils (0.05%–1.12%), eosinophils (0.00%–0.19%), basophils (0.00%–2.00%), and total thrombocyte count ( $0.68\text{--}6.68 \times 10^9/\text{l}$ ), and biochemistry reference intervals included total protein (41.76–111.31 g/l), albumin (11.46–28.69 g/l), globulins (29.25–85.14 g/l), aspartate aminotransferase (1.44–68.75 U/l), creatinine kinase (52.72–625.00 U/l), uric acid (20.02–438.53  $\mu\text{mol/l}$ ), glucose (0.68–3.29 mmol/l), cholesterol (41.74–13.25 mmol/l), calcium (1.78–6.06 mmol/l), and phosphorus (0.72–2.26 mmol/l).

**Conclusion:** This is the first report on the genus *Lachesis* reporting hematological and biochemical reference ranges.

**Keywords:** Biochemistry, Bushmaster, Costa Rica, Hematology, *Lachesis stenophrys*.

### Introduction

The genus *Lachesis* has four species, which are the largest pitvipers in the Americas and the only pitvipers that lay eggs (Savage, 2002; Leenders, 2019). This is likely one of the factors that limit their distribution to hot and humid tropical forests since incubation requires a high ambient temperature and a high relative humidity (Leenders, 2019).

The Central American bushmaster (*Lachesis stenophrys*) is one of the longest vipers, with males being slightly longer than females, averaging 2–2.10 and 1.9–2.05 m, respectively (Solórzano, 2004), and frequently over 4 kg in weight (Corrales *et al.*, 2014). It is distributed along the Caribbean versant of Nicaragua to western and central Panama (Campbell and Lamar, 2004), and in Costa Rica, it is found in tropical and subtropical rainforests on the Caribbean versant (Leenders, 2019). The breeding season occurs from February to March. The species is cataloged as Near Threatened (A2c

criterion), given the species' relatively low abundance, suspected population declines, and continuing loss, fragmentation, and habitat degradation, since this species lives in close association with unaltered humid forests (Solórzano, 2004). The rate of decline is likely less than 30% over 10 years or three generations (Acosta-Chaves *et al.*, 2021).

On the other hand, this species has public health importance since it may be implicated in ophidic accidents (e.g., snake bites) throughout its distribution, even though other viper species, such as *Bothrops asper*, are the most frequently involved in accidents (Sasa and Cano, 2020). The size and venom composition make this species very dangerous (Lomonte *et al.*, 2014). This is one of the reasons why it is kept and bred in captivity in institutions in Costa Rica, both for venom extraction and antivenom production. At the same time, it is maintained in some other captive institutions for

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breeding and conservation programs and exhibitions in Costa Rica and worldwide.

Because this is an uncommon species, the captive population is limited to a small number of individuals in the country. Many of these individuals came from the wild since many people in rural areas see the snake as a menace to their family or community, but they prefer to call the local authorities or emergency services before killing a snake (Quesada-Acuña, 2019); therefore, because some individuals cannot be relocated near or have serious injuries, they have to be taken to captive institutions. Other individuals were rescued from illegal traffic since this is a very expensive specimen on the wild animal black market.

For reptile collections, blood analyses are necessary for health assessment, entry or exit of collection, baseline determination, presurgical or therapeutic assessment, and diagnosing clinical suspicion of inflammation or organ dysfunction (Heatley and Russel, 2019). Hematology is commonly used to evaluate conditions such as anemia, inflammatory diseases, or parasitemia, while blood chemistry profiles are used to reflect the physiological function and activity of certain organs and physiological status (Nardini *et al.*, 2013; Campbell, 2015).

However, reference intervals for total erythrocyte count, hemoglobin, hematocrit, total leukocyte count, and leukocyte differential are difficult to establish for reptiles, mainly due to physiological adaptations that can occur in response to a large number of intrinsic and extrinsic factors. Likewise, studies designed to clarify the relationship between the function of certain organs and changes in chemical values or hematology in reptiles, compared to domestic mammals, are lacking (Campbell, 2015), and most hematology information available for reptiles does not conform to the current American Society for Veterinary Clinical Pathology (ASVCP) guidelines for reference range creation (Heatley and Russel, 2019).

For neotropical viperids, there is a lack of information regarding hematology and biochemistry values, both for free-living species and those in captivity (Grego *et al.*, 2006). Conservation actions of the Central American bushmaster both in the wild and in captivity bear the need for health parameters that allow managers and veterinarians to have a better understanding of health, especially when there are relatively few individuals in captivity to obtain robust information.

## Materials and Methods

### *Study population and inclusion criteria*

We looked for all the zoological institutions that kept bushmasters in their facilities and then offered to do complete blood work as part of their annual veterinary checkup in our laboratory, since all zoological facilities in Costa Rican legislation are required to provide health assessments to all of their collection. We found eight authorized centers with *L. stenophrys* in their collection,

but only seven wanted to be part of the study. The total population of the species in captivity according to the information given to us was 47 individuals. The institution that did not want to participate in the study held 14 adult individuals.

We found 33 individuals distributed in the following ways in the captive centers who agreed to participate in the study: 16, 8, 4, 2, 1, 1, and 1.

### *Inclusion criteria*

We included clinically healthy animals (no visible lesions, eating regularly, no ectoparasites, signs of dysecdysis), more than 2 years of being in captivity, and more than 1 m of total length. We did not include sex or origin (if it was wild-caught or born in captivity). Only one individual from a facility was excluded because it was less than a meter long and had 1 year of being in captivity. Thus, we ultimately selected 32 individuals (18 females and 14 males).

Captive conditions were different between facilities in terms of enclosure size and enrichment, although all facilities maintained controlled temperature and humidity and fed on mice or rats. Most of the enclosures have adequate size but differ in vegetation, furniture, and substrate. Each individual was kept alone in all the institutions.

### *Sampling methods*

We sampled from August 2022 to January 2023, but in Costa Rica, there was no important change in temperature throughout the year, and they were not in the breeding season.

All the animals were sampled in the zoological institution, so we did not transport them to avoid stress. Before venipuncture, each snake was scrutinized for a routine physical examination to rule out any obvious lesion, external parasites, dysecdysis, internal palpable abnormalities, or abnormal secretions, as well as body condition.

Each snake was manually restrained within an acrylic tube, while 2.5–3 ml of blood was obtained from the ventral coccygeal vein with a 23-gauge needle attached to a 3.0 ml syringe. Blood films were made immediately on clean glass microscope slides, and after that, the samples were placed on 3 ml VACUETTE® LH lithium heparin tubes, taken to the laboratory, and processed the same day. After blood collection, each individual was measured for total body length and snout-vent length (SVL), weighed, and sexed using stainless steel probes.

Total red blood cell count (RBC), total white blood cell count (WBC), and total thrombocyte count (TBC) were performed using standard methods with a Natt and Herrick solution (1/200) Neubauer improved chamber. To calculate RBCs, we used the following formula:  $RBC = [(# \text{ erythrocytes counted in 5 squares}) \times 10]$ . To calculate WBC and TBC, we used the following formula:  $WBC/TBC = [(# \text{ cells in 9 large squares}) \times 1.1 \times 0.2]$ . Hematocrit was determined using high-speed centrifugation (15,000 rpm) of blood-filled Vitrex®

nonheparinized microhematocrit tubes for 5 minutes. Hemoglobin was measured with a HemoCue® 201+ System, which uses an azide methemoglobin reaction with dual wavelengths (570 and 880 nm). Differential WBCs were made by examining a peripheral smear stained with Wright stain at 100× with a Delphi-X Observer™, Euromex®, with ocular eyepieces 10 × 25 mm. Total proteins were determined utilizing a clinical refractometer using plasma from the microhematocrit tube.

Biochemistry parameters were determined by spectrophotometry in a Beckman Coulter AU480® analyzer following the manufacturer's specifications.

All diagnostic laboratory analyses were performed by the same person to minimize result bias.

#### Statistical analysis

The mean, standard deviation, median, minimum and maximum values, reference intervals, 90% confidence intervals around each reference limit, distribution, and *p*-values for all blood parameters were calculated. Differences in weight, biochemistry, and hematological values between the sexes were examined using the Mann–Whitney *U* test with a standard  $\alpha$  level of 0.05. Tests of normality were performed using Shapiro–Wilks with an  $\alpha$  level of 0.05. Logarithmic transformation was used, and reference intervals were calculated with the Robust method with 1,000 iterations, according to the ASVCP guidelines (Gunn-Christie *et al.*, 2012) using Med-Calc®v22.009 software. The other statistical analyses were performed using IBM SPSS®v24 software.

#### Ethical approval

In Costa Rica, it is only legal to have native species in captive institutions authorized by the government, specifically by the Sistema Nacional de Áreas de Conservación (SINAC) (which means that people cannot have them as pets or in private collections). Here, we provide the permit number given by SINAC for each institution: SINAC-ACC-OH-RES-575-2021, SINAC-ACC-OA-VS-039-2020, ACAT-D-VS-006-2019, ACLA-P-D-219-2019, ACLAC-DRFVS-002-2021, SINAC-ACC-OSJ-re428-2021, and SINAC-ACC-OH-R-1515-2020.

### Results

We sampled a total of 32 individuals ranging from 2 to 8 years of being in captivity. All snakes appeared to be active and healthy. Weight ranged from 470 to 5,130 g (median 2,560 g, range 4,660 g). There were 18 females, with weights ranging from 470 to 5,130 g (median 3,110, range 4,660), and 14 males, with weights ranging from 1,210 to 4,330 g (median 1,370, range 3,120). The SVL ranged from 980 to 1,910 cm (median 1.56 cm, range 0.93 cm), and the total length ranged from 1,070 to 2,003 cm (median 1.73 cm, range 0.96 cm). No evidence of lesions was detected during physical examinations. No ectoparasites or dysecdysis were observed, and none of the females had palpable

eggs. The general body condition of all individuals was between 3 and 4/5 (3/5 (*n* = 30) and 4/5 (*n* = 2).

No significant differences between sexes in any of the hematological or biochemical parameters were found. Hematological values are presented in Table 1. The morphologies of lymphocytes (Fig. 1a), azurophils (Fig. 1b–d), heterophils (Fig. 1e), eosinophils (Fig. 1f), and basophils (Fig. 1g) are shown. Azurophils were the leukocytes with more morphologic variation ranging from a blue to reddish cytoplasm with no vacuoles to different degrees of vacuolization (Fig. 1b–d).

Both the shape and appearance of erythrocytes and thrombocytes (Fig. 1j) were similar to those reported for other reptiles. Erythrocytes were ellipsoid with a central positioned oval nucleus that contained dense purple chromatin with irregular margins. The cytoplasm is stained orange or pale pink. Thrombocytes are rounded with the nucleus located in a central position, containing dense chromatin that is stained purple and with colorless cytoplasm. Intraerythrocytic parasites were found in 3 of the 32 individuals (9.37% of the total sample) (Fig. 1h and i). Biochemistry values are included in Table 2.

### Discussion

Our results provide a first view of the hematological and biochemical parameters of *L. stenophrys* based on the values of almost all captive individuals in the country.

Although samples were taken from several captive populations, all the animals that were used in this research were clinically healthy, even though there were two individuals who we considered to be in a high corporal condition. One important aspect to be said is that all of them were at similar temperature and humidity ranges, and all of them ate adult rats (from a frozen stock kept by each institution). This is a very important aspect to consider in ectotherms, as extrinsic factors such as nutrition, temperature, and relative humidity may cause variations in biochemical and hematological values (Campbell, 2015).

Most of the information on hematology and blood biochemistry in neotropical vipers comes from studies carried out in Brazil, Argentina, Peru, and Costa Rica (Troiano *et al.*, 1997, 1999, 2000, 2001; Grego *et al.*, 2006; Rameh-de-Albuquerque *et al.*, 2007; Silva *et al.*, 2010; Ghilardi and Zacariotti, 2013; Glaser *et al.*, 2013; Gómez *et al.*, 2016; Trujillo *et al.*, 2016; Pereira *et al.*, 2022). These investigations were made for the genera *Crotalus*, *Bothrops*, *Bothropoides*, and *Caudisoma*, obtaining ranges of values. Studies with free-living animals have been carried out by Grego *et al.* (2006) and Gómez *et al.* (2016) in *Bothrops leucurus* and *Crotalus simus*, respectively. Grego *et al.* (2006) worked only with hematology and reported several hemoparasites. There are no data for the genus *Lachesis*.

In general, most of the hematological parameters do not differ significantly from those of other tropical

**Table 1.** Hematological values ( $n = 32$ ) of the Central America bushmaster (*L. stenophrys*) under human care in Costa Rica.

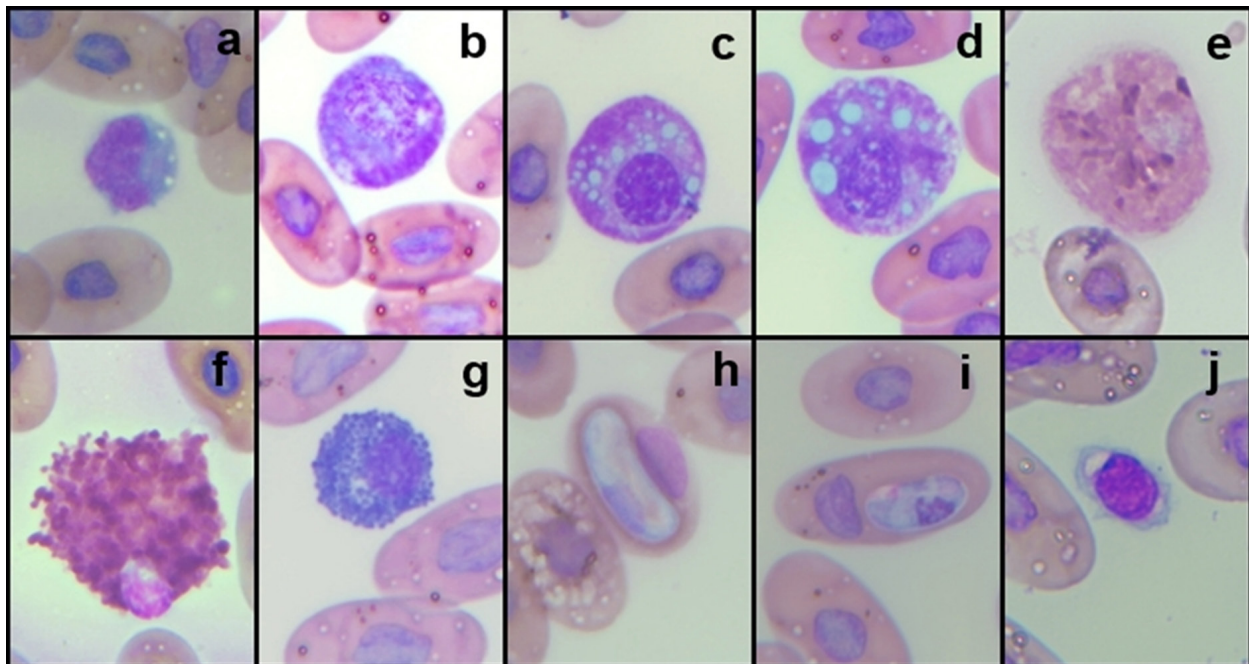
Analyte (units)	Mean $\pm$ SD	Median	Min–max	RI	LRL 90% CI	URL 90% CI	D	p-value
PCV (%)	24.21 $\pm$ 4.81	25.00	12.00–32.00	17.51–37.27	15.23–20.21	32.78–41.95	NG	0.000
RBC ( $10^{12}/l$ )	0.61 $\pm$ 0.18	0.58	0.38–1.09	0.36–0.92	0.34–0.41	0.82–1.03	G	0.325
Hb (g/l)	88.75 $\pm$ 19.50	93.50	42.03–118.00	61.73–145.23	53.60–72.88	125.75–167.93	NG	0.000
MCV (fl)	401.80 $\pm$ 16.72	400.00	229.36–552.63	269.20–617.06	239.67–305.08	561.15–672.90	G	0.12
MCHC (g/l)	366.65 $\pm$ 43.21	373.50	233.30–428.64	302.28–467.39	279.30–327.90	429.79–502.04	NG	0.001
MCH (pg)	147.27 $\pm$ 40.52	141.78	62.69–223.70	92.06–253.21	79.12–108.60	221.34–291.60	G	0.06
WBC ( $10^9/l$ )	6.85 $\pm$ 2.55	6.78	3.52–19.10	3.18–13.79	2.75–3.85	11.14–16.89	G	0.45
Lymphocytes ( $10^9/l$ )	4.91 $\pm$ 2.5	5.00	2.01–14.89	2.16–11.23	1.74–2.74	9.05–13.87	G	0.53
Lymphocytes (%)	72.43 $\pm$ 10.79	74.50	50.00–87.00	54.33–89.35	49.47–60.85	83.02–95.68	NG	0.22
Azuropils ( $10^9/l$ )	1.42 $\pm$ 0.76	1.57	0.46–4.31	0.50–4.20	0.38–0.70	3.26–5.41	G	0.37
Azuropils (%)	20.74 $\pm$ 9.34	21.50	11.00–41.00	9.57–44.81	8.08–11.64	37.05–53.27	NG	0.04
Monocytes ( $10^9/l$ )	0.02 $\pm$ 0.06	0.00	0.00–0.24	0.00–0.21	0.00–0.42	0.00–0.63	NG	0.00
Monocytes (%)	0.20 $\pm$ 0.55	0.00	0.00–2.00	0.00–1.40	0.00–0.44	0.96–1.84	NG	0.00
Heterophils ( $10^9/l$ )	0.19 $\pm$ 0.16	0.26	0.04–0.78	0.05–1.12	0.03–0.08	0.56–1.37	NG	0.03
Heterophils (%)	2.72 $\pm$ 1.98	3.00	1.00–8.00	0.90–8.90	0.65–1.26	6.98–11.04	NG	0.01
Eosinophils ( $10^9/l$ )	0.04 $\pm$ 0.09	0.00	0.00–0.39	0.00–0.19	0.00–0.42	0.00–0.61	NG	0.00
Eosinophils (%)	0.57 $\pm$ 1.36	0.00	0.00–6.00	0.00–2.00	0.00–0.57	1.43–2.57	NG	0.00
Basophils ( $10^9/l$ )	0.04 $\pm$ 0.05	0.00	0.00–0.13	0.00–0.14	0.00–0.42	0.00–0.56	NG	0.00
Basophils (%)	0.53 $\pm$ 0.73	0.00	0.00–2.00	0.00–2.00	0.00–0.47	1.53–2.47	NG	0.00
Thrombocytes ( $10^9/l$ )	2.10 $\pm$ 1.29	2.23	0.50–5.53	0.68–6.68	0.50–0.96	5.19–8.55	G	0.06

PCV: packed cell volume; RBC: red blood cells; WBC: white blood cells; Hb: haemoglobin; RI: reference interval; LRL: lower reference limit; URL: upper reference limit; CI: confidence interval; D: distribution; G: Gaussian; NG: non-Gaussian.

vipers, and the leukocyte distribution is similar to that of *Crotalus durissus*, *B. leucurus*, *Bothrops jararaca*, and *Bothriopsis jararacussu*, where lymphocytes are the most prevalent cell population, followed by azurophils. However, for example, *Bothrops ammodytes* have lymphocytes as the first cell but eosinophils and then heterophils as the second and third cells, leaving the azurophil as the fourth cell population, which is different from what Pereira *et al.* (2022) found for *Bothrops atrox*, where heterophils were the first line, followed by lymphocytes. Although *Lachesis* is a genus confined to tropical areas, it is more closely related phylogenetically to the genera *Ophryacus* (a genus endemic to Mexico) and *Agkistrodon* (Castoe and Parkinson, 2006; Alencar *et al.*, 2016; Pyron, 2017); therefore, it is probably more accurate to compare its parameters with those of copperheads. An article published by Cerreta *et al.* (2020), with *Agkistrodon*

*contortix*, has a very similar leukocyte population percentage to our study, where lymphocytes are the first line, and azurophils and heterophils are in third place. Biochemistry parameters are also similar when compared with some of the investigations mentioned above (Troiano *et al.*, 1999; Grego *et al.*, 2006; Rameh-de-Albuquerque *et al.*, 2007; Silva *et al.*, 2010; Ghilardi and Zacariotti, 2013; Glaser *et al.*, 2013; Gómez *et al.*, 2016; Trujillo *et al.*, 2016; Pereira *et al.*, 2022); however, comparing different biochemistry results done with different equipment may be handled with care, since there can be an important degree of heterogeneity, especially from in-house analyzers (Campbell, 2015). Thus, every clinician must take into consideration the specific methodology because these differences would influence the interpretation of test results and may affect patient management (Campbell, 2015).





**Fig. 1.** Photographs taken from blood smears of selected Central America bushmaster (*L. stenophrys*) under human care in Costa Rica. Wright stains at 100×. (a) Lymphocytes; (b–d) azurophilic; (e) heterophil; (f) eosinophil; (g) basophil; (h and i) hemoparasites; and (j) thrombocyte.

**Table 2.** Biochemical values ( $n = 32$ ) of the Central America bushmaster (*L. stenophrys*) under human care in Costa Rica.

Analyte (units)	Mean $\pm$ SD	Median	Min–max	RI	LRL 90% CI	URL 90% CI	D	p-value
Total protein (g/l)	65.18 $\pm$ 15.36	68.50	21.00–92.00	41.76–111.31	34.69–52.49	89.73–134.04	NG	0.00
Albumin (g/l)	17.41 $\pm$ 4.21	17.68	6.01–29.66	11.46–28.69	9.57–14.03	23.57–34.50	NG	0.00
Globulins (g/l)	49.44 $\pm$ 12.89	49.05	14.99–71.16	29.25–85.14	24.26–36.29	68.72–103.17	NG	0.00
A/G ratio	0.36 $\pm$ 0.10	0.37	0.17–0.73	0.24–0.56	0.21–0.28	0.47–0.64	NG	0.00
AST (U/l)	19.46 $\pm$ 22.71	8.62	2.13–87.22	1.44–68.75	0.93–2.22	35.16–109.00	G	0.06
CK (U/l)	262.53 $\pm$ 275.90	173.00	48.00–1,530.00	52.72–625.00	36.91–75.31	409.32–884.37	G	0.36
Calcium (mmol/l)	3.55 $\pm$ 1.50	3.27	1.46–9.75	1.78–6.06	1.47–2.31	4.69–7.36	NG	0.00
Phosphorus (mmol/l)	1.30 $\pm$ 0.44	1.29	0.73–2.45	0.72–2.26	0.61–0.82	1.91–2.62	G	0.26
Uric acid ( $\mu$ mol/l)	184.06 $\pm$ 255.09	94.00	17.10–1,062.80	20.02–438.53	13.22–33.65	272.17–665.05	NG	0.02
Glucose (mmol/l)	1.47 $\pm$ 0.76	1.48	0.53–3.62	0.68–3.29	0.55–0.85	2.69–4.01	G	0.68
Cholesterol (mmol/l)	4.52 $\pm$ 1.65	4.76	0.38–7.15	1.74–13.25	1.13–2.94	7.98–20.12	NG	0.00

A/G ratio: albumin/globulin ratio; AST: aspartate aminotransferase; CK: creatinine kinase; RI: reference interval; LRL: lower reference limit; URL: upper reference limit; CI: confidence interval; D: distribution; G: Gaussian; NG: non-Gaussian

We did not find differences between sexes in any of the values. Some studies in snakes and lizards have found differences between sexes in some parameters; for example, in *Natrix natrix*, there were differences in the red blood count (hematocrit, RBC, and Hb), and in *Sistrurus catenatus*, there were lower blood glucose, packed cell volumes, absolute azurophil counts, and higher plasma calcium and phosphorus concentrations

than in males (Allender *et al.*, 2006). Even so, our data should be taken cautiously, since we have a small sample and had to use nonparametric analyses. Hemoparasites were also found in three individuals. The presence of hemoparasites in wild reptiles is common (Telford, 2009), and hemoparasites are usually considered nonpathogenic (Stacy *et al.*, 2011) and are usually transmitted by an intermediate host, such as

ticks or mites (Telford, 2009; Campbell, 2015). We did not find any individuals with ectoparasites, and since asexual reproduction can occur in the reptilian host, they were already present in animals that came from the wild, which we could verify on their records.

We are aware of limitations in our study that include a small sample of individuals, undetected underlying causes of disease, and, as with other reptiles, any environmental factor that could possibly affect an individual at the sampling time.

This is the first study on the clinical pathology values in *L. stenophrys*, an uncommon threatened viper species. We consider that the generated data can be very valuable and can be used as baseline data for clinicians, researchers, and wildlife managers for both captive and wild individuals or population health assessments of bushmasters. However, more studies must be performed on these viper species to build robust information.

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### Conflict of interest

The authors declare that there is no conflict of interest.

### Author contribution

Randall Arguedas: Main idea, field work, and sampling, laboratory analyses, statistical analyses, and manuscript writing. Esteban Castro: Field work and sampling, laboratory analyses, and manuscript writing. Lizbeth Ovaes: Field work and sampling, laboratory analyses, and manuscript writing.

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### Data availability

Data are available from the authors upon reasonable request.

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