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ORIGINAL ARTICLE

Note on the Mesopotamian spiny-tailed lizard, *Saara loricata* (Blanford, 1874): morphometrics and evidence for gender partitioning of hematological data

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Abstract

Although the knowledge of morphometrics and hematology of reptiles in health has grown substantially in recent years, there are still knowledge gaps in many species and from different geographical regions. The objectives of this study were to document morphometrical measurements and hematological data of clinically healthy free-ranging adult Mesopotamian spiny-tailed lizards (*Saara loricata*), the Iraqi Mastigure or Iraqi spiny-tailed lizard, from Khuzestan Province, Iran, and to investigate sex differences in evaluated parameters. Although we did not observe any gender differences in morphometrical measurements, the visually larger genital pores of males allowed for differentiation of study animals into females ($n = 6$) and males ($n = 5$). We obtained femoral vein blood samples for hematological analysis, including detailed red blood cell (RBC) measurements, RBC count, packed cell volume (PCV), white blood cell (WBC) counts, and blood film review. Gender differences were identified in some RBC measurements, notably length and width of erythrocytes and nucleus width, as well as RBC and WBC counts. Gametocytes of hemogregarines varied from absent to frequent. The information presented herein will be useful for interpretative considerations of health assessment data for this species from Iran in comparison to data from the same and closely related species from other geographical regions.

Keywords Erythrocyte count · Erythrocyte size · Reptile · White blood cell count

Introduction

The subfamily Uromasticinae are assigned to spiny-tailed lizards (Pyron et al. 2013), i.e., genus *Uromastyx* (sensu lato), which are small to medium-sized, ground-dwelling lizards distributed through desert habitats with high temperatures, low rainfall, and sparse vegetation from the African Sahara Desert to the Asian Thar Desert and across the Arabian Peninsula (Ramesh and Sankaran 2013; Wilms and Böhme 2007). The maximal body length of the species reportedly ranges between 25 and 50 cm, except for *Uromastyx aegyptia* group (sensu Wilms and Böhme 2007), which can extend up to or even more than 70 cm. Cunningham (2007) investigated morphological characteristics in *U. aegyptius* from the United Arab Emirates. However, two distinct clades are differentiated in this subfamily (Nasrabadi et al. 2017; Wilms et al. 2009): *Saara* Gray 1845 (tail whorls separated dorsally by 1–6 continuous rows of intercalary scales) and *Uromastyx* Merrem 1820 (tail whorls without dorsal intercalary scales). Maximum total length recorded for *S. loricata* (*Uromastyx loricata*) is 52 cm, and maximum snout–vent length (SVL) is 29 cm (Wilms et al. 2009). These size measurements are useful in differentiating adult from immature lizards of the species.

Reptile hematological studies date back almost 200 years (e.g., Gulliver 1840, 1842, 1875), and various extrinsic and

Morphometrics & hematology of the spiny-tailed lizard

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intrinsic factors influencing hematology data have been well documented (Duguy 1970; Wintrobe 1933). The most significant factors with effects on hematology data in reptiles include species, season, physiological state (e.g., hibernation, reproduction), nutrition, and age. Such data on the effects of extrinsic and intrinsic factors are essential in understanding physiological differences when interpreting hemogram data from reptiles as individual patients or when applying such data to a group or population (Stacy et al. 2011). For example, packed cell volume (PCV) provides information on the red blood cell (RBC) mass in its functional role for oxygen delivery, as an index for hydration, nutrition, and general health (Tavares-Dias et al. 2009; Zhang et al. 2011). To the authors' knowledge, to date, only Naldo et al. (2009) have described the hematology of free-ranging spiny-tailed lizard species (Egyptian spiny-tailed lizard [*Uromastyx aegyptia microlepis*] and Leptien's spiny-tailed lizard [*Uromastyx leptieni*]) in the United Arab Emirates.

The Iran Plateau Herpetological Research Group (IPHRG), an academic organization, is committed to the study of the Iranian herpetofauna. Several studies contributed to update the herpetofauna list (e.g., Anderson 1999; Nasrabadi et al. 2017; Rastegar-Pouyani et al. 2008; Safaei-Mahroo et al. 2015; Šmíd et al. 2014). The Mesopotamian spiny-tailed lizards, *S. loricata*, in its range in Iran, are distributed in the provinces of Kurdistan, Kermanshah, Ilam, Lorestan, Khuzestan, Bushehr, and Fars (Rastegar-Pouyani et al. 2006). Hitherto, the conservation biology of this species has been investigated by Frynta et al. (1997), but according to Nazari-Serenjeh and Torki (2017), no recent studies have evaluated its distribution and abundance in Iran. Papenfuss et al. (2009) indicated the conservation status as least concern (LC) because of its distribution in favorable habitats without any apparent immediate threats to the presumed large population. Nonetheless, population trends are unknown, and this species is presumed to be found at low densities in its habitat (www.iucn.org). However, Nazari-Serenjeh and Torki (2017) identified natural habitat modification, intensive agriculture, recent droughts, and overgrazing of livestock as potential threats to the population. Health data from representatives of its native habitat and effects of intrinsic factors have not been described to date. Therefore, the objectives of this study were to document morphometrical measurements and hematological data of apparently healthy free-ranging adult Mesopotamian spiny-tailed lizards (*S. loricata*) from Khuzestan Province, Iran, and to investigate sex differences in evaluated parameters.

Materials and methods

The study area is located in Khuzestan Province, Iran (30°34′01″N, 49°48′34″E). In adherence to conservation regulations, the wildlife habitat was entered through minimal attempts to capture a minimal number of study animals. The animals were included in the study if they were considered clinically normal, the definition of which was based on the presence of normal behavior, feeding, species-specific activities, and absence of external injuries. After visual and physical examination of each individual study animal, blood samples were collected within 24 h after transferring the lizards to the laboratory. For each lizard, 15 morphometrical measurements (Table 1) were determined, of which 11 were measured by a digital caliper (Pocket Caliper, Helios–Preisser, Gammertingen, Germany) and the remainder by a stereomicroscope (M8, Wild Heerbrugg, Heerbrugg, Switzerland) (Cunningham 2007; Rastegar-Pouyani and Nilson 2002). Sex determination was performed by visual observation of larger femoral pores in males compared to females (Wilms 2005).

From each individual lizard, non-anticoagulated whole blood (≈ 1–2 mL) was collected from the femoral vein using insulin syringes (27G) (Avapezeshk company, Tehran, Iran) and immediately processed for the following: preparation of blood films, determination of packed cell volume (PCV) by means of a 75-μL heparinized capillary tube (Haematokrit-Kapillaren, Hirschmann, Eberstadt, Germany) centrifuged at 13,000 rpm for 5 min (Haematokrit 200, Hettich, Tuttlingen, Germany), and red blood cell (RBC) and white blood cell (WBC) counts using the Natt–Herricks method per described methodology (Thrall et al. 2012). Blood films were stained with Giemsa stain (Sigma–Aldrich, Steinheim, Germany) for light microscopic evaluation (Fig. 1). Erythrocytes were photographed by means of a light microscope (CX22, Olympus, Tokyo, Japan) equipped with a camera (UCMOS10000KPA, Hangzhou ToupTek Photonics Co., Xiyuan, China). Erythrocyte length (EL) and width (EW) and nucleus length (NL) and width (NW) were measured. Erythrocyte size (ES) and nucleus size (NS) were calculated through the following formula (Arıkan and Çiçek 2010): $ES = EL \times EW \times \pi/4$ & $NS = NL \times NW \times \pi/4$. Cellular and nuclear shapes were compared to their EL/EW, NL/NW, and NS/ES ratios. Blood films were also evaluated for number and morphology of hemoparasites, using a subjective assessment at ×40 objective. The following subjective quantification was used for per 10 fields of view: less than 1–2 hemoparasites as rare, 1–2 as few, 2–5 as moderate, and more than 5 as frequent.

RBC and WBC data were statistically analyzed using one-way ANOVA, program SPSS 19.0, IBM for erythrocyte measurements, and Wilcoxon test in R project (R Core Team 2016) for testing gender differences in morphometrical measurements of study animals, RBC counts, WBC counts, and PCV. Data were considered statistically different at $p < 0.05$.

Table 1 Morphometrical measurements (mean ± SD) of metric (in millimeter) of 11 free–ranging Mesopotamian spiny–tailed lizards (*Saara loricata*) from Iran

Sex	SVL	TL	HL	HW	HH	RLFFL	RLHFL	EW	TyL	TyW	ENL	RFPN	LFPN	SN5CR	SN6CR
F + M	254 (±24)	180 (±15)	46 (±7)	43 (±5)	31 (±4)	87 (±11)	120 (±15)	11 (±1)	12 (±1)	6 (±1)	13 (±2)	17 (±3)	17 (±3)	29 (±2)	25 (±2)
F	243 (±11)	178 (±11)	43 (±5)	41 (±1)	31 (±4)	82 (±5)	112 (±2)	10 (±2)	11 (±1)	6 (±1)	12 (±2)	16 (±3)	17 (±3)	28 (±2)	25 (±2)
M	271 (±33)	186 (±23)	50 (±10)	46 (±9)	32 (±4)	96 (±15)	133 (±20)	12 (±2)	13 (±1)	7 (±2)	14 (±4)	18 (±2)	17 (±2)	30 (±3)	26 (±2)

There were no significant differences in morphometrical variables between females and males

SVL, snout–vent length; TL, tail length; HL, head length; HW, head width; HH, head height; RLFFL, right and left forefoot length; RLHFL, right and left hindfoot length; EW, eye width; TyL, tympanum length; TyW, tympanum width; ENL, eye–nose length; RFPN, right femoral pore number; LFPN, left femoral pore number; SN5CR, scale number for 5th caudal ring; SN6CR, scale number for 6th caudal ring

Results

Five male and six female specimens were captured during July 2016 to June 2017, except in October 2016 to February 2017 during hibernation. The time frame during which animals were captured and sampled was during the active period (i.e., outside hibernation) of the species. All eleven animals were considered clinically normal based on physical examination. This includes all selected individuals for the study. The mean weight of females and males was 504 g and 727 g, respectively, and the mean of both genders combined was around 588 g. The mean body lengths were 42.1 cm and 45.7 cm for females and males, respectively, and the mean of both genders combined was 43.4 cm. Table 1 shows a summary of the morphometrical measurements of studied animals. There were no significant differences in morphometrical variables between females and males.

Mature erythrocytes were elliptical and nucleated, with some minor shape variations. The nuclei were oval and centrally located. Table 2 represents a summary of all erythrocyte

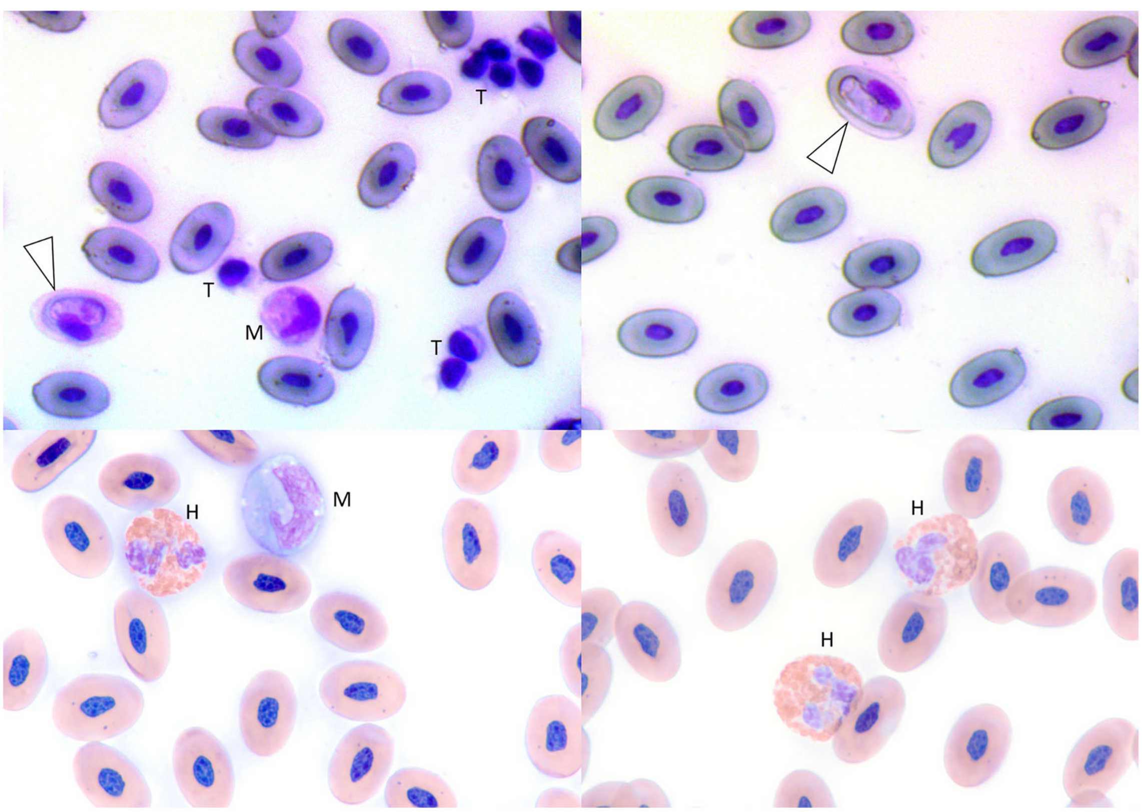

Fig. 1 Photomicrographs of blood films stained with Giemsa stain showing mature erythrocytes, two erythrocytes with hemogregarine gametocytes (arrowheads), heterophils (H), monocytes (M), and thrombocytes (T). ×100 objective

Table 2 Measurements (in μm) of erythrocytes and their nuclei from free-ranging adult Mesopotamian spiny-tailed lizards (*Saara loricata*) from Iran

Sex	Erythrocyte variables								
	EL ($X \pm$ SD) (min–max)	EW ($X \pm$ SD) (min–max)	EL/EW (X)	ES (X)	NL ($X \pm$ SD) (min–max)	NW ($X \pm$ SD) (min–max)	NL/NW (X)	NS (X)	NS/ES (X)
F + M $n = 3390$	17.72 ± 1.30 (10.25–25.04)	11.18 ± 0.88 (8.24–19.38)	1.58	155.59	7.00 ± 0.82 (4.16–10.21)	4.10 ± 0.55 (2.36–6.69)	1.71	22.54	0.14
F $n = 1862$	17.58 ± 1.26 (11.18–25.04)	11.11 ± 0.90 (8.24–14.88)	1.58	153.40	6.94 ± 0.80 (4.16–10.20)	4.07 ± 0.57 (2.48–6.64)	2.44	31.77	0.21
M $n = 1528$	17.90 ± 1.33 (10.25–24.07)	11.25 ± 0.84 (8.56–14.50)	1.59	158.16	6.98 ± 0.86 (4.31–10.21)	4.03 ± 0.52 (2.36–6.70)	1.73	22.09	0.14

EL, erythrocyte length; EW, erythrocyte width; EL/EW, erythrocyte length to width ratio; ES, erythrocyte size; NL, nucleus length; NW, nucleus width; NL/NW, nucleus length to width ratio; NS, nucleus size; ES/NS, erythrocyte nucleus size to cell size ratio; X, mean

variables and their measurements. Differences in erythrocyte parameters included significantly larger EL and EW and smaller NW in males compared to females. PCV, RBC, and WBC count data are summarized in Table 3. There was no sex difference in PCV, but RBC and WBC counts were significantly higher in males versus females.

Blood film review for hemoparasites resulted in observation of unpigmented gametocytes of hemogregarines (Fig. 1). The observed numbers of hemogregarines ranged from none ($n = 2$), rare ($n = 4$), to frequent ($n = 5$).

Discussion

This study provides new data on morphometrical measurements of the Mesopotamian spiny-tailed lizards of the Iranian Plateau and suggests gender partitioning of hematological data in this species. Therefore, these data contribute to increase the understanding of unique physiological differences in a reptile species from one habitat that will be applicable to comparing data from this species to other geographical regions.

Morphometrical measurements are commonly used to describe the phenotype of adult reptile species. *Uromastyx* spp. (Naldo et al. 2009) from a habitat in Abu Dhabi, United Arab Emirates, were comparatively heavier and larger compared to *S. loricata* from the present study. These differences may be attributed to interspecies and/or geographical variation. Cunningham (2007) succeeded in describing sexual dimorphism based on morphometrical characteristics in spiny-tailed lizards *U. aegyptius microlepis*. This is in contrast to the present study in which only the visually larger genital pores allowed for this differentiation, whereas all other numerical morphometrical measurements did not show any sex differences. This difference in study findings may be explained by the much larger sample size ($n = 93$) investigated by Cunningham (2007).

Detailed erythrocyte measurements have been documented in various reptile species. According to Ponsen et al. (2008), EL and EW of *Leiolepis belliana rubritaeniata* are slightly smaller than *S. loricata*, namely, 15.35–15.90 μm and 9.59–9.88 μm, respectively. The genus *Leiolepis* is a close relative of *Uromastyx* (sensu lato) in taxonomic classifications (Pyron et al. 2013; Wilms et al. 2009). Vaissi et al. (2013) reported

Table 3 Descriptive hematological data of free–ranging Mesopotamian spiny-tailed lizards (*Saara loricata*) from Iran

Parameter	Sex	n	$X \pm$ SD	Median	95%CI	Min	Max	Distribution
RBC	F	6	0.11 ± 0.01	0.11	0.10–0.12	0.09	0.12	normal
(×10^6/μl)	M	5	0.14 ± 0.00	0.14	0.13–0.15	0.14	0.14	normal
	F + M	11	0.12 ± 0.02	0.12	0.11–0.13	0.09	0.14	normal
WBC (×10^3/μl)	F	6	4.88 ± 0.09	4.89	4.81–4.94	4.75	4.97	normal
	M	5	5.96 ± 0.1	5.95	5.87–6.04	5.85	6.11	normal
	F + M	11	5.37 ± 0.57	4.97	5.03–5.7	4.75	6.11	normal
PCV (%)	F	6	34 ± 2	34.5	32–35	31	35	normal
	M	5	34 ± 1	35	33–35	33	35	normal
	F + M	11	34 ± 2	35	33–35	31	35	normal

CI, confidence interval; Max, maximum; Min, minimum; PCV, packed cell volume; RBC, red blood cells; SD, standard deviation; WBC, white blood cells much smaller measurements, 12.21 μm for EL and 6.82 μm for EW of *Laudakia nupta*, as well as 7.81 μm for EL and 4.50 μm for EW of *Trapelus lessonae*. According to Pyron et al. (2013), Uromasticinae, Leiolepidinae, Agaminae, and three other subfamilies classified within the family Agamidae. The EL and EW in *S. loricata*, hereupon, are closer to *Leiolepis belliana rubritaeniata* than *Laudakia nupta* and *Trapelus lessonae*. The Uromasticinae and Leiolepidinae are sister taxa and collectively located at a stem position in the family tree of Agamidae. The Agaminae and Draconinae, on the other hand, are sister taxa and altogether compose a crown group in the tree, thus revealing an interesting value of erythrocyte parameters as a part of taxonomic features. Sevinç et al. (2000) noted that lizard erythrocytes vary greatly in size depending on family and sometimes even within one family. Erythrocyte size can reflect the evolutionary position of a species (Wintrobe 1933). Nonmammalian vertebrates typically possess nucleated erythrocytes, whereas RBC of mammalian vertebrate species are small and nonnucleated (Gulliver 1875; Szarski and Czopek 1966). Nonetheless, other factors such as body weight might be a predictor of erythrocyte size (Frýdlová et al. 2013). We observed that the erythrocyte measures EL, EW, and NW and RBC count were significantly different in males compared to females. The larger RBC size and smaller nucleus in males may provide the ability for accommodation of more hemoglobin. Similarly, the higher RBC count is common in males of various reptile species (Campbell 2012). These differences provide an advantage for higher oxygen availability in males as needed for their behavioral differences; for example, males are territorial and defend access to several burrows; males expel other males if they approach burrows during the mating period; and males show increased physical activity during courtship and mating (Ramesh and Sankaran 2013). Similar differences have also been recorded in *Leiolepis belliana rubritaeniata* for RBC size, RBC count, and PCV. Such differences in PCV are usually attributed to the stimulating hormonal effects of androgenic steroids, such as testosterone, on hematopoietic stem cells (Burrows 2013; Saino et al. 1997). Some studies have also described higher RBC counts in males in other reptile species such as *Terrapene carolina* (Altland and Thompson 1958), *Cordylus vittifer* (Pienaar 1962), *Vipera aspis* (Duguy 1970), *Anguis fragilis* (Duguy 1963), and *Natrix maura* and *Emys orbicularis* (Duguy 1967). Hemogram data of our study was overall comparable to data from spiny-tailed lizards from Abu Dhabi reported by Naldo et al. (2009), with higher RBC counts, lower PCV, and similar WBC count averages. Naldo et al. (2009) report a wide range in their hemogram data, for example, PCV ranged from 4.9 to 44.5% and WBC counts from 1.00 to $8.10 \times 10^3/\mu l$, while we report more narrow ranges of PCV 31–35% and WBC count $4.75–6.11 \times 10^3/\mu l$, respectively. These variations are presumptively associated with differences in analytical methodology in addition to geographical/habitat differences of the species and highlight the importance of considering extrinsic and intrinsic factors in the comparison of hematological data from various studies of the same species.

Parasitological studies on the genus *Uromastyx* s.l. have formerly been conducted (e.g., Janakidevi 1961; *Naldo* et al. 2009; Telford et al. 2012). For example, Telford et al. (2012) recorded infection of *Uromastyx aegyptia microlepis* with *Haemocystidium apigmentada* and two parasite species of the genus *Hepatozoon*. Naldo et al. (2009) described presumptive new species of *Karyolysus* and *Hepatozoon* in *Uromastyx* spp. in the United Arab Emirates. In the present study, we describe the presence of unpigmented hemogregarines based on morphological evaluation. The observed gametocytes in our study were similar to those described in spiny-lizards from the United Arab Emirates (Naldo et al. 2009). Molecular studies will be necessary to further characterize the phylogeny of these hemoparasites in *S. loricata*.

Although this study is limited by the number of included animals, the data presented herein provide baseline data on the morphometrics and hematology of adult *S. loricata* inhabiting the Western and Southwestern regions of the Iranian Plateau, thus contributing to the knowledge on the physiology of this species. This data will be useful as a baseline for future studies and for comparison to data from the same and closely related species from other geographical regions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the ethics committee and was conducted in accordance with the ethical standards of the committee at Razi University (Permit code 396-2-026).

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