

CO-DISTRIBUTION PATTERN OF A HAEMOGREGARINE *HEMOLIVIA MAURITANICA* (APICOMPLEXA: HAEMOGREGARINIDAE) AND ITS VECTOR *HYALOMMA AEGYPTIUM* (METASTIGMATA: IXODIDAE)

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ABSTRACT: *Hyalomma aegyptium* ticks were collected from tortoises, *Testudo graeca*, at localities in northern Africa, the Balkans, and the Near and Middle East. The intensity of infestation ranged from 1–37 ticks per tortoise. The sex ratio of feeding ticks was male-biased in all tested populations. Larger tortoises carried more ticks than did the smaller tortoises. The juveniles were either not infested, or carried only a poor tick load. *Hyalomma aegyptium* was absent in the western Souss Valley and Ourika Valley in Morocco, the Cyrenaica Peninsula in Libya, Jordan, and the Antilebanon Mountains in Syria. *Hemolivia mauritanica*, a heteroxenous apicomplexan cycling between *T. graeca* and *H. aegyptium*, was confirmed in Algeria, Romania, Turkey, Syria, Lebanon, and Iran. Its prevalence ranged from 84% in Romania (n = 45), 82% in eastern Turkey (n = 28), and 82% in the area of northwestern Syria with adjacent Turkish borderland (n = 90), to 38% in Lebanon (n = 8) and in only 1 of 16 sampled tortoises in Algeria. The intensity of parasitemia in the studied areas ranged from 0.01% up to 28.17%. The percentage of *Hemolivia*-infected erythrocytes was significantly higher in adults. All tortoises from *Hyalomma*-free areas were *Hemolivia*-negative. Remarkably, all 29 *T. graeca* from Jabal Durūz (southwestern Syria) and 36 *T. graeca* from the area north of Middle Atlas (Morocco) were *Hemolivia*-negative, despite the fact that ticks parasitized all adult tortoises in these localities. Identical host preferences of *H. aegyptium* and *H. mauritanica* suggest the occurrence of co-evolution within the *Testudo-Hyalomma-Hemolivia* host–parasite complex.

Hemolivia mauritanica (Sergent and Sergent, 1904) is a heteroxenous haemogregarine apicomplexan that cycles between *Testudo graeca* Linnaeus, 1758 and the tick *Hyalomma aegyptium* (Linnaeus, 1758) (Sergent and Sergent, 1904; Laveran and Nègre, 1905). Originally, it was described from spur-thighed tortoise, *Testudo graeca*, collected in Algeria. The range of this haemogregarine was expected to overlap with the distribution area of its host species, *T. graeca*, despite the fact that only isolates collected from North Africa were known (Sergent and Sergent, 1904; Laveran and Nègre, 1905; Brumpt, 1938). Since Brumpt, however, little information has been published about the exact distribution area of *H. mauritanica*.

Hyalomma aegyptium almost exclusively infest tortoises; however, on occasion, other reptiles and mammals are also known to be infested (Hoogstraal, 1956). Specifically, species of *Testudo* are the principal hosts of the adult stages (Hoogstraal and Kaiser, 1960; Apanaskevich 2003, 2004; Široký et al., 2006). *Testudo* includes 5 species of predominantly herbivorous land tortoises that form a monophyletic group. They inhabit steppe and forest–steppe, as well as semi-desert and de-

sert, habitats of the western Palaearctic realm (Ernst and Barbour, 1989; Fritz and Bininda-Emonds, 2007).

Our recent studies confirmed occurrence of *Hemolivia* sp. on *T. graeca* in the Balkan countries and in western Turkish Anatolia and on *Testudo marginata* Schoepff, 1792 from Greece (Široký et al., 2005). Moreover, Paperna (2006) found *Hemolivia* sp.-infected tortoises on the Golan Heights of the Near East.

In the present paper, we report data on the occurrence of *H. mauritanica* and its vector tick *H. aegyptium* from previously poorly sampled areas over the western Palaearctic realm and discuss a possible co-evolution within the *Testudo-Hyalomma-Hemolivia* host–parasite complex.

MATERIALS AND METHODS

We examined more than 400 tortoises at 48 localities from Morocco (April 2008), Algeria (August 2006, July 2007), Libya (April 2006), Romania (April 2006), Turkey (June 2007, June 2008), Syria (April 2005, April 2007, June 2007), Lebanon (May and June 2006), Jordan (June 2003, April 2008), and Iran (July and August 2005). (For complete sampling list, see Table I.) For the purposes of our study, these 48 localities were grouped into the following natural geographical units: In northern Africa—Souss Valley (localities 1–2), Ourika Valley (loc. 3), the area north of Middle Atlas (loc. 4–5), northeastern Algeria (loc. 6), Cyrenaica Peninsula in Libya (loc. 7–9); in the Balkan Peninsula—eastern Romania (loc. 10); in the Near East region—borderland in southern Turkey (Antakya-Gaziantep region) and northwestern Syria (loc. 11–13, 21–24), Jabal an Nusayriyah (loc. 25–29), Antilebanon Mts. (loc. 30–31), Jabal Durūz (loc. 32–34), Lebanon (loc. 35–39), northern Jordan (loc. 40–41); and in the Middle East region—vicinity of the lake Van and Mt. Ararat in eastern Turkey (loc. 14–20), Iran (loc. 42–48).

Tortoises were found by walking through the habitat and were identified to species level according to their morphological characteristics (Ernst and Barbour, 1989; Iverson, 1992; Fritz and Cheylan, 2001). Each sampled tortoise was sexed using morphological criteria for *Testudo* spp. (Fritz and Cheylan, 2001) and marked by temporal paint to prevent repeated collection. Maximum straight carapace length (SCL) and carapace width (CW) were measured by vernier caliper to the nearest millimeter; body mass (BM) in g was obtained employing Pesola scales. Young specimens without recognizable sexual traits were con-

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TABLE I. List of sampling localities. Locality numbers correspond with those in the map (Fig. 3).

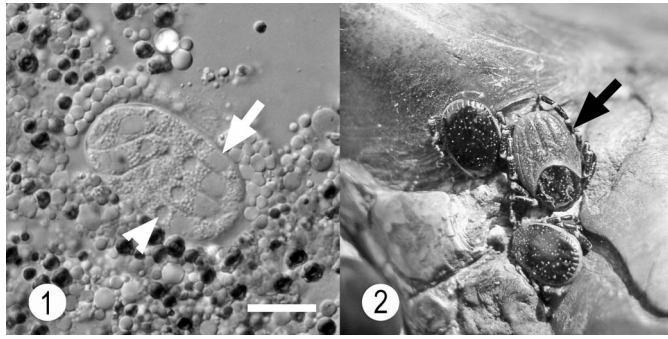
	Locality	Country	Latitude	Longitude	Altitude m (a.s.l.)	Number of sampled tortoises
1	Ademine	Morocco	30°22'N	9°22'W	68	16
2	Taroudannt	Morocco	30°29'N	8°52'W	242	13
3	Tnine de l'Ourika	Morocco	31°23'N	7°46'W	897	7
4	Oulmès	Morocco	33°26'N	6°01'W	1,153	22
5	Fès	Morocco	34°04'N	4°58'W	316	14
6	National Park El Kala	Algeria	36°50'N	8°10'E	12	16
7	Slonta	Libya	32°40'N	21°47'E	781	1
8	Täknis	Libya	32°29'N	21°07'E	437	1
9	Tukrah	Libya	32°31'N	20°33'E	12	1
10	Greci	Romania	45°12'N	28°13'E	17	45
11	Antakya	Turkey	36°12'N	36°10'E	102	20
12	Hassa	Turkey	36°48'N	36°29'E	862	20
13	Bogazkerim	Turkey	36°49'N	36°51'E	692	11
14	Van	Turkey	38°21'N	42°45'E	1,958	9
15	Mus	Turkey	38°42'N	41°28'E	1,574	2
16	Igdir	Turkey	39°51'N	44°04'E	860	10
17	Aralik	Turkey	39°50'N	44°30'E	902	1
18	Hakkari	Turkey	37°42'N	44°01'E	1,617	2
19	Yüksekova	Turkey	37°26'N	44°27'E	1,990	2
20	Semdinli	Turkey	37°21'N	44°30'E	1,717	2
21	Dar Taizzah	Syria	36°18'N	36°49'E	546	1
22	Qual'at Samaan	Syria	36°22'N	36°51'E	298	26
23	Cirrus	Syria	36°45'N	36°52'E	585	2
24	Kafr Takharim	Syria	36°07'N	36°28'E	689	14
25	Masyaf	Syria	35°05'N	36°18'E	1,058	2
26	Ayn al Bayda	Syria	35°02'N	36°17'E	984	13
27	Jourine	Syria	35°39'N	36°16'E	203	5
28	Krak des Chevaliers	Syria	34°45'N	36°17'E	578	20
29	Bmalkeh	Syria	34°56'N	35°58'E	302	6
30	Saydnaya	Syria	33°41'N	36°21'E	1,310	25
31	Maalüla	Syria	33°50'N	36°32'E	1,482	2
32	Suweida	Syria	32°42'N	36°34'E	1,126	9
33	Al Kafr	Syria	32°37'N	36°38'E	1,297	19
34	Rashiedeh	Syria	32°40'N	36°50'E	1,450	1
35	Saida	Lebanon	33°34'N	35°24'E	57	2
36	Jezzine	Lebanon	33°32'N	35°34'E	1,140	11
37	Aaitanit	Lebanon	33°34'N	35°40'E	1,150	6
38	Furzol	Lebanon	33°58'N	35°56'E	1,500	4
39	Batroun	Lebanon	34°16'N	35°40'E	23	1
40	Jarash	Jordan	32°16'N	35°54'E	559	1
41	Umm al Quttain	Jordan	32°19'N	36°38'E	989	4
42	Koheh Ghalajeh	Iran	34°16'N	47°02'E	1,561	2
43	Germi	Iran	39°00'N	48°05'E	1,152	1
44	Meshkinshahr	Iran	38°21'N	47°47'E	2,367	3
45	Manjil	Iran	36°44'N	49°25'E	411	5
46	Saghand	Iran	32°31'N	55°14'E	1,312	1
47	Anjir Avand	Iran	32°09'N	54°28'E	1,542	1
48	Shahr-e Babak	Iran	30°07'N	55°03'E	1,834	1

sidered as juveniles. All tortoises were released immediately afterwards at the location of capture.

Ticks were collected from tortoises using tweezers, were immediately placed into plastic tubes containing 70% ethanol, labeled with a field number of the tortoise, and then transported to the laboratory for species determination using available keys (Feldman-Muehsam, 1948; Pomerancev, 1950; Hoogstraal, 1956; Apanaskevich, 2003). Because haemogregarine species are not easy to determine based solely on their gamont morphology (Desser, 1993), when possible, a few live ticks were collected from a locality and later dissected in the laboratory to confirm

the presence of typical *H. mauritanica* sporocysts (Fig. 1) (Michel, 1973; Široký et al., 2004).

Blood was collected from the dorsal coccygeal vein; blood smears were air-dried, fixed in absolute methanol for 5 min, and stained with Giemsa (diluted 1:10 in water, pH 7) for 20 min. Stained smears were examined using an Olympus BX 41 microscope (Olympus Corporation, Tokyo, Japan) at $\times 1,000$ magnification; intensity of parasitemia was counted for each tortoise as the percentage of infected erythrocytes found in 10^4 cells. Mean, standard deviation (SD), and range were derived for intensity of parasitemia.



FIGURES 1–2. (1) Typical sporocyst of *Hemolivia mauritanica* recovered from dissected Turkish *Hyalomma aegyptium*. Note the sporozoite's refractile body, marked by an arrow, and the sporozoite's nucleus marked by an arrowhead. Scale bar = 10 μ m. (2) Three *H. aegyptium* feeding in situ in inguinal area of *Testudo graeca* collected in Romania. Only the female tick is marked by an arrow.

Statistical analyses were conducted using the STATISTICA version 8.0 (2007) program (StatSoft, Inc., Tulsa, Oklahoma). Main effects ANOVA and multiple regression were applied to analyze the effect of locality, sex, age, body size (SCL, CW, and BM), and maturity (adults vs. juveniles) on the number of ticks. In the second procedure, we analyzed the effect of the same variables (locality, sex, age, body size, and maturity), plus the number of ticks, on the intensity of *Hemolivia* sp. infection of tortoises.

Instead of a separate analysis of effect of SCL, CW, and BM, we performed analyses with the total body size index, calculated as the score of the PC1 component of every individual using principal components analysis (PCA). The eigenvalue of the PC1 axis was 2.964 and the first axis explained 98.8% of the total variance. Correlations between variables and PC1 axis were high (SCL = 0.995, CW = 0.993, and BM = 0.993). Relative contributions of each variable to the variance of the PC1 axis were equal (SCL 0.334, CW 0.333, and BM 0.333).

The observed sex ratio of ticks was compared with the expected sex ratio of 1:1 using a Chi-square (χ^2) test. Total sex ratio, as well as sex ratio in selected populations with large sample sizes (north of Middle Atlas, borderland in southern Turkey and northwestern Syria, Jabal an Nusayriyah, Jabal Durüz, and Lebanon), were tested.

Representative ticks and all blood smears were deposited in the col-

lection of the Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic.

RESULTS

Morphologically, the gamonts found in all examined tortoise populations, and the sporocysts from ticks feeding on tortoises in Romania, Turkey, and Syria, were nearly identical, fitting well to descriptions published for *H. mauritanica* (Michel, 1973; Široký et al., 2004, 2007). We detected a particularly high prevalence of *H. mauritanica*-infected tortoises in eastern Romania (84%, $n = 45$), eastern Turkey (82%, $n = 28$), and in borderland areas of northwestern Syria and southern Turkey (82%, $n = 90$). Further, *H. mauritanica* parasitized 59% ($n = 46$) of *T. graeca* in the Jabal and Nusayriah Mountains in western Syria. Among 8 examined blood smears from Lebanese tortoises, 3 were *H. mauritanica*-positive. We also found *H. mauritanica* gamonts in 3 *T. graeca* specimens ($n = 14$) from Iran. In northern Africa, we detected *H. mauritanica* only in Algeria (1 infected tortoise from 16 collected Algerian *T. graeca*), whereas all examined Moroccan ($n = 72$) and Libyan ($n = 3$) tortoises were haemogregarine-negative. In the Near East, all 5 Jordanian tortoises were haemogregarine-negative as well as those from the Syrian mountain ranges of Jabal Durüz ($n = 29$) and Antilebanon ($n = 27$). The intensity of parasitemia for all sampled areas is given in Table II.

In addition to 3 adult *Rhipicephalus sanguineus* (Latreille, 1806) collected from *T. graeca* at Bogazkerim in southern Turkey, we found 2,299 *H. aegyptium* (Fig. 2). All tortoises sampled in the arid area of the Antilebanon Mountains, as well as 5 tortoises from Jordan and 3 *T. graeca* from Libya, were devoid of ticks. Only 6 *H. aegyptium* associated with *T. graeca* were collected in the arid area of the Souss Valley in southern Morocco ($n = 29$). No ticks were found on 7 tortoises examined in the Ourika Valley in the High Atlas foothills. Data on ticks collected from *T. graeca* during our study are presented in Table II. For the list of localities where *H. mauritanica*, *H. aegyptium*, or both were detected, see Appendix 1.

TABLE II. Data on prevalence, infestation intensity by ticks *Hyalomma aegyptium* and parasitemia intensity of *Hemolivia mauritanica* collected from *Testudo graeca*. Sampling of ticks in Eastern Turkey and Romania was not complete and is not included. *All examined Libyan tortoises – were little juveniles; authors have reliable information that ticks are present on *T. graeca* on Cyrenaica Peninsula (C. Schneider, pers. obs.).

Sampling area	Number of tortoises	Infested by ticks	Male ticks (Σ ; mean \pm SD; range)	Female ticks (Σ ; mean \pm SD; range)	Prevalence of <i>H. mauritanica</i> (%)	Parasitemia (%): mean; SD; range
Ourika Valley	7	0	0	0	0	0; –; –
Souss Valley	29	3	3; 1.5 \pm 0.71; 1–2	3; 1 \pm 0; 1	0	0; –; –
North of Middle Atlas	36	33	236; 7.15 \pm 4.56; 1–21	78; 2.36 \pm 1.95; 1–6	0	0; –; –
Northeastern Algeria	16	11	16; 2 \pm 1.07; 1–4	11; 1.38 \pm 0.52; 1–2	6	0.65; –; –
Libya (Cyrenaica)	3	0*	0	0	0	0; –; –
Eastern Romania	46	30			84	1.89; 3.0; 0.04–13.47
Jabal Durüz	29	28	86; 3.07 \pm 1.78; 1–7	31; 2.07 \pm 1.39; 1–5	0	0; –; –
Antilebanon Mts.	27	0	0	0	0	0; –; –
Jabal an Nusayriyah	46	35	296; 8.5 \pm 8.37; 1–32	48; 2.18 \pm 1.33; 1–6	59	4.13; 6.7; 0.02–28.17
Northwest of Syria and adjacent area of Turkey	97	87	848; 9.86 \pm 5.54; 1–23	359; 4.79 \pm 3.67; 1–16	82	1.65; 2.46; 0.01–15.56
Eastern Turkey	28	28			82	1.92; 2.4; 0.02–10.86
Lebanon	24	22	100; 4.76 \pm 3.08; 2–13	39; 2.29 \pm 1.1; 1–5	38	1.64; 2.65; 0.07–4.7
Northern Jordan	5	0			0	0; –; –
Iran	14	4	10; 2.5 \pm 1.29; 1–4	9; 2.25 \pm 0.5; 2–3	21	0.61; 0.44; 0.11–0.92

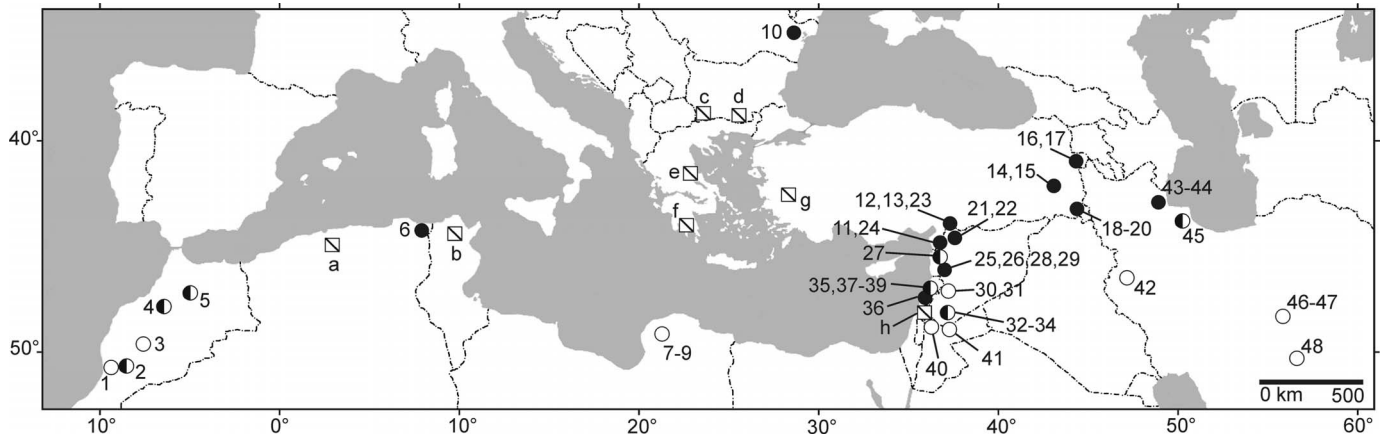


FIGURE 3. Distribution of sampling areas. Black dots represent localities with *Hyalomma aegyptium* and *Hemolivia mauritanica* co-occurrence, half-black dots represent localities where only *H. aegyptium* ticks were found, and empty circles mark localities where neither ticks nor hemogregarines were discovered. Numbers attributed to a particular mark correspond to locality numbers in Table I. Nearby localities are combined. *Hemolivia mauritanica* localities previously reported are marked by halved squares: **a** = Algeria, **b** = Tunisia, **c** = vicinity of Melnik, Bulgaria, **d** = Zhelezino, Bulgaria, **e** = Volos, Greece, **f** = Githio, Greece, **g** = western Anatolia, Turkey, **h** = Golan Heights (Brumpt, 1938; Široký et al., 2005, 2006; Paperna, 2006).

Of all measured variables, only locality (main effects ANOVA, $F = 18.853$, $df = 4$, $P < 0.001$), tortoise maturity (main effects ANOVA, $F = 29.605$, $df = 1$, $P < 0.001$), and body size (multiple regression, $F = 21.401$, $df = 1$, $P < 0.001$) affected the number of ticks per tortoise. Therefore, larger tortoises carried more ticks. Juveniles were either not parasitized at all or their tick load was poor. Sex (main effects ANOVA, $F = 2.670$, $df = 1$, $P = 0.104$) and age after maturity of tortoises (multiple regression, $F = 1.030$, $df = 1$, $P = 0.311$) did not influence tick load. Sex ratio of ticks was male-biased in the total ($\chi^2 = 475.973$, $P < 0.001$), as well as in particular tested populations: North of Middle Atlas in Morocco ($\chi^2 = 79.503$, $P < 0.001$), Syrian mountain ranges Jabal Durūz ($\chi^2 = 25.855$, $P < 0.001$) and Jabal an Nusayriyah ($\chi^2 = 178.791$, $P < 0.001$), areas in northwestern Syria and adjacent part of Turkey ($\chi^2 = 198.112$, $P < 0.001$), and in Lebanon ($\chi^2 = 26.770$, $P < 0.001$) (Table II). The percentage of *H. mauritanica*-infected erythrocytes differed according to locality (main effects ANOVA, $F = 5.078$, $df = 1$, $P < 0.05$) and was significantly higher in adults (main effects ANOVA, $F = 5.545$, $df = 1$, $P < 0.05$). However, it was not affected by sex (main effects ANOVA, $F = 0.257$, $df = 1$, $P = 0.613$), age (main effects ANOVA, $F = 0.927$, $df = 1$, $P = 0.338$), body size (multiple regression, $F = 0.061$, $df = 1$, $P = 0.805$), or tick load (multiple regression, $F = 0.019$, $df = 1$, $P = 0.889$).

DISCUSSION

Together with previous studies, our data provide evidence that the *H. mauritanica* range overlaps with a considerable portion of the distribution area of *T. graeca* in parts of northern Africa, southeastern Europe, and the Near and Middle East (Fig. 3) (Brumpt, 1938; Široký et al., 2005; Paperna, 2006). A high prevalence of *H. mauritanica* was detected in the majority of well-sampled areas in the Balkan countries, Turkey, and the adjacent part of Syria. Sampling in Algeria was limited, and the finding of a single *H. mauritanica*-infected *T. graeca* has confirmed Brumpt's (1938) results. Sampling in Iran considerably extends the known *H. mauritanica* range eastwards up to

the eastern limit of the *T. graeca* range. Thus, according to our current knowledge, its range extends from Algeria in the west to the Caspian region in northern Iran in the east and from eastern Romania in the north to Lebanon and the Golan Heights in the south (Brumpt, 1938; Široký et al., 2005; Paperna, 2006).

A notable distribution pattern was found in Syria, where none of 29 *T. graeca* specimens collected in the volcanic hills of Jabal Durūz was *H. mauritanica*-positive. This is surprising, as all 28 adult tortoises sampled there were infested by *H. aegyptium*. The same pattern was found in the area north of Middle Atlas in Morocco. The absence of *H. mauritanica* in these areas, with abundant numbers of both definitive and intermediate hosts, could be hypothesized to be a possible effect of dry periods in the past. Populations of hosts, vectors, or both might have decreased below the threshold level, which is necessary for life cycle maintenance (Swinton et al., 2002; Tompkins et al., 2002). Paperna (2006) suggests that *H. aegyptium* ticks are more dry-sensitive than are *T. graeca* tortoises. This trait could also explain the absence of *H. aegyptium* (and of *H. mauritanica* as well) in some arid areas of the *T. graeca* range, as in the Antilebanon Mountains of Syria, the Badia region of Jordan, and the Souss Valley in Morocco. Unfortunately, the ecological requirements of *H. aegyptium*, a tick species with little economic importance, have been poorly studied. Finally, one-way dependence of *H. mauritanica* on *H. aegyptium*, and of *H. aegyptium* on *Testudo* spp., may play a role in the present distribution pattern, where *H. mauritanica* has an apparently smaller range than *H. aegyptium* which, in turn, has a smaller distribution area than *Testudo* spp. (Kolonin, 1983; Iverson, 1992).

Despite the considerable proportion of *H. mauritanica*-infected *T. graeca*, all juveniles were *H. mauritanica*-negative. This can be explained by a higher probability of acquiring infection during a longer life span of tortoises in concert with the long persistence of *H. mauritanica* (parasitemia was observed in a single captive *T. marginata* for 14 years [Široký et al., 2004; P. Široký pers. obs.]). The independence of parasitemia intensity on the host's age, sex, size, and tick load may indicate either the absence of a cumulative re-infection effect, its rarity,

or the existence of a kind of regulation at the host–parasite level as, for example, a balance of the immune response and infection.

The significantly higher infestation by ticks of larger tortoises could be explained by the distance they move each day, which is greater than that of juveniles (Díaz-Paniagua et al., 1995; Keller et al., 1997). Thus, there is a higher probability for larger tortoises to acquire the ticks during movement and, finally, larger tortoises are larger targets for ticks.

Hemolivia mauritanica is transmitted by *H. aegyptium*, a tick with a strong preference for species of *Testudo*. Among them, *T. graeca* and *T. marginata* are the preferred hosts (Široký et al., 2006). In areas where *Testudo hermanni* Gmelin, 1789 is the only tortoise species, *H. aegyptium* is absent (Kolonin, 1983). In addition, *H. mauritanica* has been isolated from *T. graeca* and *T. marginata*, although not yet from *T. hermanni* (Široký et al., 2005). This matching host preference suggests the occurrence of co-evolution within the *Testudo-Hyalomma-Hemolivia* host–parasite complex. Thus, *H. mauritanica*, *H. aegyptium*, and *Testudo* spp. tortoises, all with large distribution areas, offer an excellent model for future studies focused on co-phylogeography and evolution at the infraspecific level.

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LITERATURE CITED

- APANASKEVICH, D. A. 2003. K diagnostike vida *Hyalomma* (*Hyalomma aegyptium* (Acari, Ixodidae) [To diagnostics of *Hyalomma* (*Hyalomma aegyptium* (Acari: Ixodidae)]. *Parazitologija* **37**: 47–59.
- . 2004. Parazito-khozjainye svjazi vidov roda *Hyalomma* Koch, 1844 (Acari, Ixodidae) i ikh svjaz s mikroevoljucionnym procesom [Host–parasite relationships of the genus *Hyalomma* Koch, 1844 (Acari, Ixodidae) and their connection with microevolutionary process]. *Parazitologija* **38**: 515–523.
- BRUMPT, E. 1938. Formes évolutives d'*Haemogregarina mauritanica* chez la tique *Hyalomma syriacum*. *Annales de Parasitologie* **16**: 350–361.
- DESSER, S. S. 1993. The Haemogregarinidae and Lankesterellidae. In *Parasitic Protozoa*. Vol. 4, 2nd ed., J. P. Kreier and J. R. Baker (eds.). Academic Press, New York, New York, p. 247–272.
- DÍAZ-PANIAGUA, C., C. KELLER, AND A. C. ANDREU. 1995. Annual variation of activity and daily distance moved in adult spur-thighed tortoises, *Testudo graeca*, in southwestern Spain. *Herpetologica* **51**: 225–233.
- ERNST, C. H., AND R. W. BARBOUR. 1989. *Turtles of the world*. Smithsonian Institution Press, Washington, D.C., 313 p.
- FELDMAN-MUEHSAM, B. 1948. On larvae and nymphs of some species of Palestinian *Hyalomma*. *Parasitology* **39**: 138–147.
- FRITZ, U., AND O. R. P. BININDA-EMONDS. 2007. When genes meet nomenclature: Tortoise phylogeny and the shifting generic concepts of *Testudo* and *Geochelone*. *Zoology* **110**: 298–307.
- , AND M. CHEYLAN. 2001. *Testudo* Linnaeus, 1758—Eigentliche Landschildkröten. In *Handbuch der reptilien und amphibien Europas*. Band 3/IIIA Schildkröten (Testudines) I (Bataguridae, Testudinidae, Emydidae), U. Fritz (ed.). AULA-Verlag GmbH, Wiesbaden, Germany, p. 113–124.
- HOOGSTRAAL, H. 1956. African Ixodoidea. I. Ticks of the Sudan. Department of the Navy, Bureau of Medicine and Surgery, Washington, D.C., 1,101 p.
- , AND M. N. KAISER. 1960. Some host relationships of the tortoise tick, *Hyalomma* (*Hyalommast*) *aegyptium* (L.) (Ixodoidea, Ixodidae) in Turkey. *Annals of the Entomological Society of America* **53**: 457–458.
- IVERSON, J. B. 1992. A revised checklist with distribution maps of the turtles of the world. Privately printed, Richmond, Indiana, 363 p.
- KELLER, C., C. DÍAZ-PANIAGUA, AND A. C. ANDREU. 1997. Post-emergent field activity and growth rates of hatchling spur-thighed tortoises, *Testudo graeca*. *Canadian Journal of Zoology* **75**: 1089–1098.
- KOLONIN, G. V. 1983. Mirovoe rasprostranenie iksodovykh kleshchey. Rody *Hyalomma*, *Aponomma*, *Amblyomma* [World distribution of ixodid ticks. Genera *Hyalomma*, *Aponomma*, *Amblyomma*]. Nauka, Moskva, SSSR, 121 p.
- LAVERAN, I., AND L. NÈGRE. 1905. Sur un protozoaire parasite de *Hyalomma aegyptium*. *Comptes Rendus des Séances et Mémoires de la Société de Biologie et de ses Filiales* **57**: 964–966.
- MICHEL, J. C. 1973. *Hepatozoon mauritanicum* (Et. et Ed. Sergent, 1904) n. comb., parasite de *Testudo graeca*: Redescription de la sporogonie chez *Hyalomma aegyptium* et de la schizogonie tissulaire d'après le matériel d' E. Brumpt. *Annales de Parasitologie Humaine et Comparée* **48**: 11–21.
- PAPERNA, I. 2006. *Hemolivia mauritanica* (Haemogregarinidae: Apicomplexa) infection in the tortoise *Testudo graeca* in the Near East with data on sporogonous development in the tick vector *Hyalomma aegyptium*. *Parasite* **13**: 267–273.
- POMERANCEV, B. I. 1950. Iksodovye kleshchi (Ixodidae) [Ixodid ticks (Ixodidae)]. *Fauna SSSR*, Tom. 4, Vyp. 2. Paukoobraznye. Izdatelstvo Akademii Nauk SSSR, Moskva, Leningrad, SSSR, 224 p.
- SERGENT, ED., AND ET. SERGENT. 1904. Sur une hémogrégarine, parasite de *Testudo mauritanica*. *Comptes Rendus des Séances et Mémoires de la Société de Biologie et de ses Filiales* **56**: 130–131.
- ŠIROKÝ, P., M. KAMLER, F. L. FRYE, P. FICTUM, AND D. MODRÝ. 2007. Endogenous development of *Hemolivia mauritanica* (Apicomplexa: Adeleina: Haemogregarinidae) in the marginated tortoise *Testudo marginata* (Reptilia: Testudinidae): Evidence from experimental infection. *Folia Parasitologica* **54**: 13–18.
- , AND D. MODRÝ. 2004. Long-term occurrence of *Hemolivia cf. mauritanica* (Apicomplexa: Adeleina: Haemogregarinidae) in captive *Testudo marginata* (Reptilia: Testudinidae): Evidence for cyclic merogony? *Journal of Parasitology* **90**: 1391–1393.
- , AND ———. 2005. Prevalence of *Hemolivia mauritanica* (Apicomplexa: Adeleina: Haemogregarinidae) in natural populations of tortoises of the genus *Testudo* in the east Mediterranean region. *Folia Parasitologica* **52**: 359–361.
- , K. J. PETRŽELKOVÁ, M. KAMLER, A. D. MIHALCA, AND D. MODRÝ. 2006. *Hyalomma aegyptium* as dominant tick in tortoises of the genus *Testudo* in Balkan countries, with notes on its host preferences. *Experimental and Applied Acarology* **40**: 279–290.
- SWINTON, J., M. E. J. WOOLHOUSE, M. E. BEGON, A. P. DOBSON, E. FERROGLIO, B. T. GRENFELL, V. GUBERTI, R. S. HAILS, J. A. P. HEESTERBEEK, A. LAVAZZA ET AL. 2002. Microparasite transmission and persistence. In *The ecology of wildlife diseases*, P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (eds.). Oxford University Press, New York, New York, p. 83–101.
- TOMPKINS, D. M., A. P. DOBSON, P. ARNEBERG, M. E. BEGON, I. M. CATTADORI, J. V. GREENMAN, J. A. P. HEESTERBEEK, P. J. HUDSON, D. NEWBORN, A. PUGLIESE ET AL. 2002. Parasites and host population dynamics. In *The ecology of wildlife diseases*, P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson (eds.). Oxford University Press, New York, New York, p. 45–62.

Appendix 1

Hemolivia mauritanica was confirmed from *Testudo graeca* at following localities (n = number of infected tortoises): Algeria—'Parc National d'El Kala' (August 2006, n = 1); Romania—Tulcea County: Greci (April 2006, n = 38); southern Turkey—Antakya-Gaziantep region: Antakya (June 2007, n = 16), Hassa (June 2007, n = 15), Bogazkerim (June 2007, n = 11), eastern Turkey-Lake Van region: Van (June 2008, n = 9), Mus (June 2008, n = 2), Hakkari (June 2008, n = 2), Semdinli

(June 2008, n = 2), Yüksekova (June 2008, n = 2); eastern Turkey—Mt. Ararat region: Iğdir (June 2008, n = 5), Aralık (June 2008, n = 1); Iran—Ardabil Province: Germe (July 2005, n = 2), Meshkinshahr (July 2005, n = 1); Syria—Jabal an Nusayriyah: Masyaf (April 2005, n = 1), Ayn al Bayda (April 2005, n = 10), Krak des Chevaliers (April 2007, n = 14), Bmalkeh (April 2007, n = 2); northwestern Syria—region north and west of Aleppo: Dar Taizzah (April 2005, n = 1), Qual'at Samaan (April 2005, n = 11; April 2007, n = 7), Cirrus (April 2007, n = 2), Kafr Takharim (April 2007, n = 11); Lebanon—Jezzine (June 2006, n = 3).

Hyalomma aegyptium was collected at following localities (n = number of collected ticks): Morocco—Souss Valley: Taroudannt (April 2008, n = 6); Morocco—north of Middle Atlas: Oulmès (April 2008, n = 217), Fès (April 2008, n = 90); Algeria—'Parc National d'El Kala' (August 2006, n = 27); Romania—Tulcea County: Greci (April 2006, n = 64); southern Turkey—Antakya-Gaziantep region: Antakya (June 2007, n = 305), Hassa (June 2007, n = 234), Bogazkerim (June 2007,

223); eastern Turkey—Lake Van region: Van (June 2008, n = 21), Mus (June 2008, n = 5), Hakkari (June 2008, n = 5), Semdinli (June 2008, n = 5), Yüksekova (June 2008, n = 5); eastern Turkey—Mt. Ararat region: Iğdir (June 2008, n = 20), Aralık (June 2008, n = 1); Iran—Ardabil Province: Germe (July 2005, n = 5), Meshkinshahr (July 2005, n = 4); Iran—Gilan Province: Manjil (August 2005, n = 10); Syria—Jabal Durūz: Suwayda (April 2005, n = 32), Al Kafr (April 2005, n = 82), Ar Rashiedeh (June 2007, n = 3); Syria—Jabal an Nusayriyah: Masyaf (April 2005, n = 10), Ayn al Bayda (April 2005, n = 81), Jourine (April 2005, n = 19), Krak des Chevaliers (April 2007, n = 224), Bmalkeh (April 2007, n = 10); Syria—region northwest of Aleppo: Qual'at Samaan (April 2005, n = 206; April 2007, n = 155), Cirrus (April 2007, n = 15), Kafr Takharim (April 2007, n = 69); Lebanon—Saida (Achmoun Temple, June 2006, n = 5), Jezzine (June 2006, n = 65), Aaitanit (at Karaoun Lake, June 2006, n = 47), Furzol (Wadi al Hamis, June 2006, n = 19), Batroune (Moussalayah Castle, June 2006, n = 3).