

New locality and host records of mites and ticks (Chelicerata: Acari) parasitizing lizards of Morocco

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Open Science in Acarology

New locality and host records of mites and ticks (Chelicerata: Acari) parasitizing lizards of Morocco

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Original research

ABSTRACT

The aim of this study was to investigate the parasitic relationships of mites and ticks infesting lizards in Morocco and their determinant factors. A total of 1498 lizards representing 27 species, 8 families and 18 genera were randomly captured and examined in their natural habitats. The overall prevalence of ectoparasite infestation was 22.4%, with a mean intensity of 14.5 (95% CI: 12.2 - 16.8). Four families of Acari (Chelicerata) were identified: Ixodidae, Macronyssidae, Pterygosomatidae, and Trombiculidae, with eleven new host records and new data on geographical distributions. Trombiculid mites were the most prevalent (17.2%), followed by the Pterygosomatidae (4.1%), the Ixodidae (1.5%), and the Macronyssidae (0.4%). Nevertheless, Macronyssids showed the highest mean intensity 31.7 (14.3 - 54.2), followed by the Pterygosomatidae 22.0 (16.2 -30.5), the Trombiculidae 12.7 (10.9 - 14.8), and the Ixodidae 1.7 (1.3 - 2.2). In total, nine species of parasites were found, and co-infestations were recorded on three lizard species. Ixodid prevalence and intensity were significantly related to altitude and host species, whereas Macronyssid prevalence and intensity were associated with host age and altitude. Ectoparasite intensity was associated with lizard sex for all mite families, with males carrying heavier loads than females, while prevalence was related to sex only for Pterygosomatid and Trombiculid mites. Season was important for Macronyssid and Trombiculid infestation intensity. Finally, host characteristics (species, size, age class), along with altitude, were determinant factors for infestation prevalence and intensities in Pterygosomatid and Trombiculid mites. The Ixodidae and Macronyssidae were mainly attached to the ventral side of hosts, whereas the Trombiculidae and Pterygosomatidae were attached to different parts of the host body with aggregations in skin folds. This updated information should prove useful for modeling biodiversity and predicting zoonotic disease outbreaks.

Keywords Ixodidae; Macronyssidae; Pterygosomatidae; Trombiculidae; parasitic niches; reptiles

Introduction

Information on host-arthropod associations and data on arthropods acting as vectors of pathogens is important for modeling and predicting zoonotic disease outbreaks (Benelli &

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Er-Rguibi O. et al.

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ISSN 0044-586X (print) ISSN 2107-7207 (electronic) Duggan 2018). In addition, studying parasite communities can help us better understand the structure and functioning of natural ecological systems. Such knowledge is of particular importance when host species are endemic, but of threatened conservation status (Er-Rguibi et al. 2021). Remarkably, more than 400 species of ectoparasites (mites and ticks of the subclass Acari) have been recorded parasitizing reptiles worldwide (Fajfer 2012). Reptilian ectoparasites belong to three Acari orders: Mesostigmata, Metastigmata and Prostigmata, and are represented by 15 families of temporary or permanent parasites (Fajfer 2012). Mites and ticks attached to the host skin extract blood and fluids from the host tissues, inducing local lesions, such as excoriation, papules, scaling, and inflammation (Lehmann 1993). Infestation with a high number of ectoparasites can reduce the density of blood cells, and consequently affect host physiology and behavior (Main & Bull 2000; Huyghe et al. 2010). These impacts depend in part on the intensity of infestation and where the ectoparasites are attached (Chilton et al. 1992; Bertrand et al. 1995). In addition, ectoparasites can be vectors of many other parasites and pathogens, such as haemogregarines, viruses, bacteria, and helminths, all of which can have strong negative effects on host body condition, survival and reproduction (Lehmann 1993; Smallridge & Bull 2000; Holmstad et al. 2008).

Morocco is a hot spot of reptilian biodiversity and endemism in North Africa and in the western Mediterranean region (Bons & Geniez 1996; Bouazza *et al.* 2021). The new updated checklist for Morocco showed the presence of 80 species of lizards, from 10 families including Agamidae (7 species), Anguidae (1 species), Gekkonidae (7 species), Lacertidae (25 species), Phyllodactylidae (8 species), Scincidae (19 species), and Sphaerodactylidae (9 species) (Bouazza *et al.* 2021). However, host-parasite interactions between Acari and lizards in Morocco have received limited attention, even if several families of Acari have been described as reptile ectoparasites worldwide (Fajfer 2012; Heath & Whitaker 2015; Mendoza-Roldan *et al.* 2020a). To date, two families of Trombidiformes were recorded on lizards in Morocco: Pterygosomatidae and Trombiculidae, with 17 species from 12 genera (Jack 1961, 1962; Jack & Girot 1965; Girot 1968; Stekolnikov 2018a, 2018b; Fajfer 2020). Moreover, one tick family has been reported, Ixodidae, with 3 species belonging to 2 genera (Bailly-Choumara *et al.* 1976). However, little information is currently available on their patterns of infestation (prevalence and intensity) and geographical distribution in Morocco.

The purpose of this article is therefore to: a) evaluate the overall prevalence and intensity of infestation by Acari in 27 lizard species in order to better understand host-ectoparasite relationships in these species; b) establish which ectoparasites most frequently infest lizards in Morocco, and evaluate their levels of co-infestation, c) examine parasitic niches and preferred attachment sites to better understand host exploitation, and d) evaluate the relationship between patterns of infestation (prevalence and intensity) and some specific host and environmental factors that may determine infestation (host species, sex, age class, host size, altitude and season).

Material and methods

Sample collection

Lizard specimens were collected between 2019 and 2021 in Morocco. Individuals were randomly captured by hand or using a noose in 51 prospected localities (Figure 1). The study sites were visited twice per year (in spring and summer) for the three years of the study. A total of 1498 lizards were collected, comprising 27 species, 18 genera, and 8 families (Table 1). After capture, lizards were identified, weighted with a digital scale (\pm 0.01 g), measured (Snout-vent Length "SVL") using digital calipers (\pm 0.01mm), sexed according to morphological characteristics (Bons & Geniez 1996), and digitally photographed. Coordinates of the collection localities were registered using a GPS device. After measurements, lizards were immediately examined for mite and tick infestation. For the infested individuals, the number, the type of parasite (tick/mite), and the occupied niche were recorded (head, front

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Figure 1 Map showing the distribution of sampling localities in Morocco. Locality numbers and altitude: 1 – Moulay Bouzerktoune, Essaouira 20 m; 2 – El Ghazoua, Essaouira 132 m; 3 – Sidi Kaouki, Essaouira 130 m; 4 – Had Draa, Essaouira 135 m; 5 – Ounagha, Essaouira 304 m; 6 – Ain Nakhla, Agadir 104 m; 7 – Bigoudine, Argana 698 m; 8 – Fom Jrana, Chichaoua 1234 m; 9 – Ouled Abbas, Echemmaia 465 m; 10 – Zaouiat Echerradi, Marrakech 354 m; 11 – Guemassa, Marrakech 518 m; 12 – Kettara, Tamansourt 483 m; 13 – Tensift, Marrakech 395 m; 14 – Fssm, Marrakech 490 m; 15 – Ouirgane, Asni 917 m; 16 – Central Jbilet, Sidi Bou Othmane 528 m; 17 – Imlil, Marrakech 1900 m; 18 – Oukaïmeden, Marrakech 2640 m; 19 – Ait El Qaq, Marrakech 2000 m; 20 – Dar Bouazza, Casablanca 9 m; 21 – Taferiate, Aït Ourir 820 m; 22 – Taddart Izdar 1600 m; 23 – Tizi n'Tichka, Taddart Oufella 2120 m; 24 – Assif Iriri, Ouarzazate 1271 m; 25 – Aghbalou, Bzou 576 m; 26 – Ouzoud, Azilal 837 m; 27 – Ouarzazate 1134 m; 28 – Ouled Arbia, Ouarzazate 1188 m; 29 – Toundoute, Ouarzazate 1455 m; 30 – Oued El Hajjaj, Ouarzazate 1335 m; 31 – Skoura, Ouarzazate 1209 m; 32 – Sidi Flah, Ouarzazate 1197 m; 33 – Jbel Saghro, Skoura 1300 m; 34 – Azilal 1907 m; 35 – Imassine, Ouarzazate 1360 m; 36 – Oum laachar, Zagora 800 m; 37 – Zaouiat Ahansal, Azilal 2666 m; 38 – Anounizeme, Boumalne Dadès 1570 m; 39 – Ouled Ayad, Béni Mellal 641 m; 40 – Tassent, Imilchil 1919 m; 41 – Lac Tislit, Imilchil 2333 m; 42 – Jbel Chendir, Belyounech 440 m; 43 – Aït Aammour ou Ali, Azrou 1077 m; 44 – Lac Zerouka, Ifrane 1482 m; 45 – Daït Aoua, Ifrane 1462 m; 46 – ksar Tizougaghine, Tinejdad 999 m; 47 – Tighejdet, Er-Rich 1444 m; 48 – El Khank, Errachidia 1124 m; 49 – Touz Azi El Bide, Merzouga 720 m; 50 – Ksar Tanamouste, Merzouga 766 m; 51 – Refria, Missour 1120 m.

and hind legs, back, vent, cloaca, and tail). The ectoparasites were then removed delicately using entomological tweezers and conserved in absolute ethanol (99.8%) for later identification. Lizards were released at the exact location of capture.

Ectoparasite identification

In the laboratory, mites and ticks were identified according to morphological characteristics based on original descriptions and systematic reviews (Jack 1961, 1962; Girot 1968; Bertrand *et al.* 1995, 2000, 2013; Moraza *et al.* 2009; Walker *et al.* 2014; Estrada-Peña *et al.*

 Table 1
 Number of individuals, mean size (SVL) in mm and mean weight (MW) in g with the range of values (R), and sampled localities where the host species was found. See Figure 1 for the correspondence between locality numbers and map locations.

YY	Number of compled individuals							1.000	Localitics (by symbol)	
Host species	Malac	Eemalee	Immature	Total	MS + SD	P	MW + SD	p	Localities (by number)	
Agamidae Sniv 1825	wates	Temates	minature	Totai	MS ± SD	K	MW ± 5D	K		
Agama impalearis Boettger, 1874	5	6	19	30	61.8 ± 25.5	32.5 - 124.0	22.9 ± 48.6	1.2 - 198.9	2; 3; 5; 6; 10; 11; 12; 15; 24: 35: 42: 43	
Uromastyx nigriventris Rothschild & Hartert, 1912 Anguidae Gray, 1825		1	3	4	56.1 ± 10.4	48.15 - 73.9	7.3 ± 1.0	6.3 - 8.8	30; 32; 33	
Hyalosaurus koellikeri (Günther, 1873)	1			1	144.0 ± 0.0	144	44.0 ± 0.0	44	22	
Gekkonidae Gray, 1825										
Stenodactylus mauritanicus Guichenot, 1850	2			2	39.5 ± 3.3	37.1 - 41.8	2.0 ± 0.8	1.4 - 2.5	47	
Tropiocolotes algericus Loveridge, 1947 Lacertidae Batsch, 1788	1	1		2	27.2 ± 2.0	25.8 - 28.6	1.0 ± 0.1	0.9 - 1.0	48	
Acanthodactylus boskianus (Daudin 1802)	1			1	58.5 ± 0.0	58.5	$7.8\ \pm 0.0$	7.8	51	
Acanthodactylus dumerilii (Milne- Edwards, 1829)	6	1		7	55.0 ± 3.9	54.9 - 59.3	6.5 ± 1.0	4.7 - 8.0	36; 50	
Acanthodactylus erythrurus (Schinz, 1833)	19	12		31	54.7 ± 8.5	38.8 - 81.9	4.6 ± 2.2	1.2 - 9.0	2; 6; 10; 20; 35; 38; 45	
Acanthodactylus maculatus (Gray, 1838)	3	1	4	8	24.9 ± 12.2	30.1 - 57.3	3.0 ± 2.3	6.6 - 0.6	11	
Acanthodactylus margaritae Tamar et al., 2017	2			2	46.6 ± 5.4	42.8 - 50.4	2.1 ± 0.6	1.7 – 2.5	6	
Atlantolacerta andreanskyi	59	38	9	106	40.2 ± 7.7	10,3 - 66.5	1.9 ± 0.9	0.3 - 4.8	18; 37	
Werner, 1929 Mesalina guttulata (Lichtenstein,	5	3		8	42.9 ± 3.7	39.2 - 50.5	1.5 ± 0.3	1.2 - 1.9	29; 32; 38; 47; 49	
1823) Mesalina simoni (Boettger, 1881)	33	36	5	74	39.8 ± 8.4	8.8 - 72.1	1.5 ± 0.6	0.4 - 2.6	3; 4; 11; 12; 16	
Podarcis vaucheri (Boulenger,	120	81	7	208	46.0 ± 7.1	22.1 - 62.4	2.5 ± 1.1	0.3 - 5.6	18; 19; 23; 34; 37; 40;	
Psammodromus algirus	9	10		19	52.6 ± 12.8	31.7 - 72.4	5.0 ± 2.9	0.9 - 9.6	13; 18; 22; 34; 42; 45	
Scelarcis perspicillata (Duméril and Bibron 1839)	15	18	3	36	48.2 ± 10.1	26.6 - 68.9	2.8 ± 1.6	0.4 - 5.8	18; 19; 26	
Timon tangitanus (Boulenger, 1891)	2	8		10	54.0 ± 29.0	33.9 - 129.1	19.0 ± 35.1	1.2 - 90.6	18; 19; 40; 45	
Phyllodactylidae Gamble et al., 2008										
Tarentola mauritanica (Linnaeus, 1758)	77	29	10	116	42.8 ± 11.1	23.6 - 74.2	2.8 ± 2.2	0.4 - 10.4	1; 2; 3; 5; 6; 8; 10; 9; 11; 12; 13; 14; 16; 19; 20; 27; 31; 43; 44	
Scincidae Oppel, 1811										
Chalcides mionecton (Boettger, 1874)	31	26	7	64	74.0 ± 14.5	53.0 - 133.4	3.4 ± 1.5	1.2 - 8.0	1; 2; 3; 6; 13; 20	
Chalcides montanus Werner, 1931	1	2		3	91.9 ± 6.6	86.9 - 99.4	12.3 ± 1.9	10.1 - 13.4	18	
Chalcides polylepis Boulenger, 1890	19	25	3	47	79.2 ± 21.8	38.7 - 140.2	11.2 ± 9.2	0.8 - 40.0	3; 4; 10; 13; 22; 25; 44	
Eumeces algeriensis Peters, 1864	2	2		4	130.5 ± 42.5	82.2 _ 171.0	17.8 ± 11.1	4.8 _ 28.5	3; 13; 20	
Sphaerodactylidae Underwood, 1954										
Quedenfeldtia moerens (Chabanaud, 1916)	30	17	1	48	43.3 ± 4.3	31.1 - 51.9	2.5 ± 0.9	1.2 - 6.9	7; 8	
Quedenfeldtia trachyblepharus (Boettger, 1874)	188	162	36	386	40.5 ± 9.6	28.3 - 61.7	2.3 ± 0.9	0.3 - 8.1	17; 18; 19	
Saurodactylus brosseti Bons & Pasteur, 1957	114	81	63	258	26.2 ± 5.3	8.3 - 41.6	0.7 ± 0.3	0.2 - 1.9	1; 2; 3; 10; 11; 12; 13; 16; 21	
Saurodactylus fasciatus Werner, 1931 Tragononbiidae Croy, 1865		1		1	16.0 ± 0.0	16	0.5 ± 0.0	0.5	39	
Trogonophis wiegmanni Kaup, 1830	11	6	5	22	117.5 ± 30.5	69.8 - 169.4	5.8 ± 3.9	0.8 - 13.9	1; 2; 20	

2017; Stekolnikov 2018a, 2018b; Fajfer 2020; Stekolnikov *et al.* 2021). Macronyssidae, Pterygosomatidae, and ticks (Ixodidae) were mounted on microscope slides using Berlese's medium (Walter and Krantz 2009). The slides were examined under a stereomicroscope (DM-143-FBLED-A5) and microscope (Euromex BScope series) using different lenses. Slides of chigger mites (Acariformes: Trombiculidae) were examined under a Leica DM 2500 compound microscope (Leica Microsystems GmbH, Wetzlar, Germany) using differential interference contrast (DIC). Microphotographs were taken by means of a Leica DMC 4500 digital camera. Identified mites and ticks were deposited in the collection of the Laboratory of Water, Biodiversity and Climate Changes (WaterBiodiCC), Faculty of Sciences, Semlalia, Cadi Ayyad University, Marrakech. Specimens of chigger mites were also deposited in the Zoological Institute of the Russian Academy of Sciences (ZIN, Saint Petersburg, Russia).

Data analysis

To evaluate the infestation data, two parasitological parameters were calculated using the Quantitative Parasitology software, version 3.0 (Rózsa *et al.* 2000): 1) infestation prevalence (%) (P) = $100 \times$ (number of infested lizards/total number of lizards); 2) mean intensity (MI) = number of parasites/number of infested lizards. The values of P and MI are given with 95% confidence interval (CI) and the range of infestation values.

To examine the effect of different intrinsic [host species (infested species), sex (two levels), age class (two levels), body size "SVL" (continuous)], and extrinsic [season (two levels), altitude (continuous)] variables on ectoparasite prevalence a generalized linear model (GLZM) was used, with parasite prevalence as the dependent variable, following a binomial distribution, with size as a covariate, and including the interactions between different factors in the model. Likewise, the infestation intensity was studied using a general linear model (GLM), with parasite intensity as the dependent variable, size as a covariate, and with the aforementioned factors and their interactions. Some of the data concerning *Quedenfeldtia trachyblepharus* (n=227) were already published in Er-Rguibi *et al.* (2021). Separate tests were performed for each family of ectoparasites to investigate the impact of the six studied factors and their interactions on ectoparasitism patterns. We used a two-way ANOVA test to examine differences in parasitic niche use on the host among ectoparasite families. All statistical tests were performed using SPSS Statistics 19.0 software (SPSS, Chicago, IL, USA), and a *p* value <0.05 was considered statistically significant.

Results

Mite and tick diversity

From the 1498 lizards examined, 335 were found infested with ectoparasites, with an overall prevalence of 22.4% and mean intensity of 14.5 (95% confidence interval: 12.2 - 16.8). Three mite families (Macronyssidae, Pterygosomatidae, and Trombiculidae) and one tick family (Ixodidae) were found in the sampled lizards. Trombiculid mites presented the highest prevalence of infestation (17.2%), followed by Pterygosomatidae (4.1%), Ixodidae (1.5%), and Macronyssidae (0.4%). However, the latter showed the highest mean intensity 31.7 (14.3 – 54.2), followed by Pterygosomatidae 22.0 (16.2 – 30.5), Trombiculidae 12.7 (10.9 – 14.8), and Ixodidae 1.7 (1.3 – 2.2) (Table 2). The spatial distribution of ectoparasites infesting Moroccan lizards is shown in Figure 1.

The family Ixodidae, represented by larvae and nymphs of *Ixodes ricinus*, were found infesting five lizard species: *Podarcis vaucheri*, *Psammodromus algirus* (Figure 2A), *Scelarcis perspicillata*, *Quedenfeldtia moerens* (Figure 2B), and *Q. trachyblepharus* (Figures 2C), with different patterns of infestation (Table 2). The Macronyssidae, represented by *Ophionyssus natricis*, was recorded for the first time in Morocco, and was found infesting *Mesalina simoni*. The Pterygosomatidae were represented by two species: an unidentified species of the genus



Figure 2 Lizards infested with the tick *Ixodes ricinus*: A – *Psammodromus algirus*; B –*Quedenfeldtia moerens*; C – *Quedenfeldtia trachyblepharus*.

Geckobia infesting Tarentola mauritanica (Figure 3A), and Pterygosoma bibronii that was found on Agama impalearis (Figure 3B) and Chalcides polylepis. The infestation of C. polylepis is the first record of scale mites on the genus Chalcides and for the family Scincidae. The Trombiculidae infesting lizards in Morocco (Figure 3C - D) were represented by five species – Ericotrombidium tarentolae, Neotrombicula orycti, Pentidionis maura, Pentidionis meridialis, and Xinjiangsha imlilica (Figure 4). Seven new host records for these species were reported (Table 2). In addition, co-infestation by two ectoparasites was recorded in three host species. Twelve of 386 examined individuals of the Atlas day gecko Q. trachyblepharus were simultaneously infested with the tick I. ricinus and one of the two chigger mites: E. tarentolae or N. orycti. One of 74 examined individuals of M. simoni was infested with O. natricis and P.

maura. Finally, the one examined individual *A. boskianus* was parasitized by *P. maura* and *P. meridialis*.

The number of individuals and lizard species examined and infested by different Acari families, as well as their patterns of infestation, are summarized in Table 2. The highest prevalence of infestation was recorded in *A. maculatus*, where 50.0% of individuals were infested with the chigger species *P. maura*. In addition, notable cases of infestation were those of *Q. moerens* by *E. tarentolae* (45.8%) and *T. mauritanica* by *Geckobia* sp. (44.8%) (Table 2). The lowest infestation was recorded in *P. vaucheri* infested with the tick *I. ricinus* (0.5%). The maximum mean intensities were observed in *A. maculatus* infested with *P. maura* 45.3 (10.3 – 72.0), *M. simoni* infested with *O. natricis* 31.7 (13.5 – 54.0) and *T. mauritanica* infested with *Geckobia* sp. 23.7 (16.2 – 32.8). The lowest mean intensities were recorded for *P. vaucheri*, *Q. moerens*, and *S. perspicillata* infested with *I. ricinus* 1.0 (CI uncertain).

 Table 2
 Prevalence (P), mean intensity (MI), 95% confidence interval (CI), and range (R) of infestation by mites and ticks in Moroccan lizards (* – new host record).

Mite and tick parasites	Host species	Number of sampled individuals	Number of infested individuals	Prevalence (%) (95% CI)	Mean Intensity	· (MI)	Localities for infested individuals (by numbers)	
					MI (95% CI)	R	-	
Ixodidae Koch, 1844		1498	23	1.5 (1.0 - 2.3)	1.7 (1.3 – 2.2)	1-5		
Ixodes ricinus (Linnaeus, 1758)	Po. vaucheri	208	1	0.5 (0.0 - 2.7)	1.0 (0.0 - 0.0)	1	18	
	Ps. algirus	19	3	15.8 (3.4 - 39.6)	2.3 (2.0 – 2.7)	2-3	42	
	Q. moerens *	48	1	2.1 (0.1 – 11.1)	1.0(0.0-0.0)	1	7	
	Q. trachyblepharus	386	17	4.4 (2.6 - 6.9)	1.7 (1.2 – 2.3)	1 – 5	17; 18; 19	
	S. perspicillata *	36	1	2.8 (0.1-14.5)	1.0 (0.0 - 0.0)	1	18	
Macronyssidae Oudemans, 1936		1498	6	0.4 (0.1 - 0.9)	31.7 (14.3 - 54.2)	2-74		
Ophionyssus natricis (Gervais, 1844)	M. simoni *	74	6	8.1 (3.0 - 16.8)	31.7 (13.5 - 54.0)	2-74	11; 16	
Pterygosomatidae Oudemans, 1910		1498	62	4.1 (3.1 – 5.2)	22.0 (16.2 - 30.5)	1 - 113		
<i>Geckobia</i> sp. Mégnin, 1878	T. mauritanica	116	52	44.8 (35.6 - 54.4)	23.7 (16.9 - 32.8) 1 - 113		1; 2; 3; 5; 6; 8; 9; 10; 11; 14; 16; 19; 20; 27; 31; 43; 44	
Pterygosoma bibronii Jack, 1962	Ag. impalearis	30	9	30.0 (14.7 - 49.4)	14.7 (7.6 – 28.2)	1-51	10; 11; 30; 35; 43	
	C. polylepis *	47	1	2.1 (0.1 – 11.3)	3.0(0.0-0.0)	3	10	
Trombiculidae Ewing, 1944		1498	257	17.2 (15.3 - 19.2)	12.7 (10.92 - 14.8)	1 - 100		
Ericotrombidium tarentolae (Vercammen- Grandjean & Langston, 1976)	Q. moerens	48	22	45.8 (31.4 - 60.8)	18.6 (11.2 - 30.4)	2 - 78	7; 8	
	Q. trachyblepharus*	386	73	18.9 (15.1 – 23.2)	13.1 (10.3 - 16.88)	1 - 72	17; 18; 19	
Neotrombicula orycti Taufflieb, 1960	Q. trachyblepharus	386	148	38.3 (33.5 - 43.4)	11.2 (9.2 – 14.1)	1 - 100	18	
Pentidionis maura (Taufflieb, 1960)	Ac. boskianus*	1	1	100 (2.5 - 100)	2.0 (0.0 - 0.0)	2	51	
	Ac. dumerilii*	7	1	14.3 (0.36 - 57.9)	5.0(0.0-0.0)	5	36	
	Ac. maculatus *	8	4	50.0 (15.7 - 84.3)	45.3 (10.3 - 72.0)	2 - 90	11	
	M. simoni *	74	5	6.8 (2.2 - 15.1)	2.2 (1.2 - 3.8)	1 - 5	16	
Pentidionis meridialis (Taufflieb, 1960)	Ac. boskianus*	1	1	100 (2.5 - 100)	3.0 (0.0 - 0.0)	3	51	
Xinjiangsha imlilica (Brown, 2008)	Po. vaucheri *	208	3	1.4 (0.3 - 4.2)	15.0 (10.3 - 16.9)	10 - 20	41	



Figure 3 Lizards infested with mites: A – Tarentola mauritanica infested with Geckobia sp. B – Agama impalearis infested with Pterygosoma bibronii. C –Quedenfeldtia trachyblepharus infested with Neotrombicula orycti. D – Acanthodactylus maculatus infested with Pentidionis maura.

Effects of host species, host size, sex, age class, altitude, and season on ectoparasitism

The prevalence of infestation by Ixodidae was related to host species (GLZM, Wald's $\chi^2 = 10.639$, df = 4, p = 0.014) and altitude (Wald's $\chi^2 = 34.166$, df = 20, p = 0.018). No other factor nor their interactions were found to significantly affect the prevalence of Ixodidae infestations (Table 3). Likewise, the intensity of infestation by this tick family was linked only to host species (GLM, $r^2 = 0.101$, $F \sim (4, 692) \sim = 4.574$, p = 0.004) and altitude ($F \sim (20, 676) \sim = 1.904$, p = 0.012) (Table 4).

The prevalence of Macronyssidae infestation was related to age class (GLZM, Wald's χ^2 = 44.079, df = 1, p < 0.001) and altitude (Wald's $\chi^2 = 49.243, df = 5, p < 0.001$), but was not related to the other studied factors (Table 3). The intensity of infestation by Macronyssid mites significantly differed between males and females (GLM, r² = 0.930, F~(1, 72)~ = 4.718, p = 0.035), adults and juveniles (F~(1, 72)~ = 4.848, p = 0.033), altitude (F~(5, 68)~ = 10.539, p < 0.001), and season (F~(1, 72)~ = 6.478, p = 0.014). Likewise, the interactions between altitude × SVL, altitude × season, sex × SVL, and season × sex were statistically significant (Table 4). All the other non-statistically significant interactions were removed from the model.

The prevalence of Pterygosomatidae infestation was related to host species (GLZM, Wald's $\chi^2 = 6.170$, df = 2, p = 0.046), sex (Wald's $\chi^2 = 6.437$, df = 1, p = 0.040), age class (Wald's $\chi^2 = 16.629$, df = 1, p = 0.002), body size (Wald's $\chi^2 = 6.749$, df = 1, p = 0.009), and altitude (Wald's $\chi^2 = 44.247$, df = 29, p = 0.001). Furthermore, the interactions between season ×



Figure 4 Microphotographs of chigger mites (dorsal aspect of body): A – *Ericotrombidium tarentolae*; B – *Pentidionis maura*; C – *Pentidionis meridialis*; D – *Xinjiangsha imlilica* Scale bars: 50 µm.

altitude, host species × altitude, sex × altitude, sex × age classes, altitude × age classes, altitude × SVL, and age classes × SVL were statistically significant (Table 3). All the other non-statistically significant interactions were removed from the model. The intensity of infestation by Pterygosomatidae was linked to host species (GLM, $r^2 = 0.625$, $F\sim(2, 190)\sim = 4.350$, p = 0.016), sex ($F\sim(1, 192)\sim = 7.025$, p = 0.002), age class ($F\sim(1, 192)\sim = 4.065$, p = 0.005), and

Table 3 Summary table of the effect of studied factors on ectoparasite prevalence using generalized linear models (GLZM).

	Ixodidae			Macronyssidae			Pterygosomatidae			Trombiculidae		
Effect	df	Wald's χ2	p -value	df	Wald's χ2	p -value	df	Wald's χ2	p -value	df	Wald's χ2	p -value
Host species	4	10.639	0.014				2	6.17	0.046	6	181.44	< 0.001
Sex	1	0.881	0.644	1	0.001	0.975	1	6.437	0.04	1	6.602	0.037
Age class	2	1.684	0.64	1	44.079	< 0.001	1	16.629	0.002	2	4.723	0.094
Body size	1	1.333	0.248	1	0.001	0.977	1	6.749	0.009	1	0.03	0.862
Season	1	0.515	0.473	1	0.565	0.452	1	0.307	0.046	1	0.824	0.364
Altitude	20	34.166	0.018	5	49.243	< 0.001	29	44.247	0.001	25	108.22	< 0.001
Season × Altitude							2	6.678	0.035			
Host species × Altitude							2	8.42	0.015			
$\text{Sex} \times \text{Altitude}$							8	39.453	< 0.001			
$\mathbf{Sex} \times \mathbf{Age\ class}$							4	18.602	0.001			
Altitude × Age class							3	10.554	0.014	9	22.572	0.007
Altitude × Body size							13	41.374	< 0.001			
Age class × Body size							4	12.898	0.012			

body size (F~(1, 192)~ = 11.983, p = 0.001). Only the interactions between host species × altitude and altitude × SVL were statistically significant (Table 4), all the other interactions were not significant and were removed from the model.

The prevalence of Trombiculidae infestation was significantly different between host species (GLZM, Wald's $\chi^2 = 181.440$, df = 6, p < 0.001), males and females (Wald's $\chi^2 = 6.602$, df = 1, p = 0.037), and altitude (Wald's $\chi^2 = 108.220$, df = 25, p < 0.001). Only the interaction between altitude × age classes was statistically significant; all other interactions were removed from the model (Table 3). The intensity of infestation by chigger mites was related to host species (GLM, $r^2 = 0.228$, $F\sim(6, 725)\sim = 18.202$, p < 0.001), sex ($F\sim(1, 730)\sim = 11.839$, p < 0.001), body size ($F\sim(1, 730)\sim = 4.745$, p = 0.030), altitude ($F\sim(24, 706)\sim = 3.069$, p < 0.001), and season ($F\sim(1, 730)\sim = 5.410$, p = 0.020) (Table 4). The interactions between analyzed factors were not statistically significant and were removed from the model.

Parasitic niches and preferred locations

Concerning the parasitic niche on the animal (Figure 5), an important difference was observed in the attachment sites of the different ectoparasite families (two-way ANOVA test: Ectoparasite family $F\sim(3, 720)\sim = 10.51$, p < 0.001; Parasitic niche $F\sim(6, 717)\sim = 3.336$, p = 0.003; Interaction $F\sim(18, 705)\sim = 1.85$, p = 0.017). The tick *I. ricinus* was found mainly attached on the ventral side of lizard hosts (vent) (Figure 2B). The Macronyssid mites *O. natricis* were found attached under the scales on the ventral side of front and hind legs, and on vent and tail of *M. simoni*. The Pterygosomatidae were found attached in different parts of the host body (head, front and hind legs, back, vent, cloaca, and tail). Mites of the genus *Geckobia* were found in different parts of *T. mauritanica*, especially on the dorsal side, between toes (Figure 3A), at the base of the claws, and in clusters near the cloaca. *P. bibronii* attached mainly under the scales of the vent, throat, and tail of *A. impalearis* (Figure 3B), and under the scales of the vent in *C. polylepis*. Attached chigger mites formed clusters on different body parts, but were especially frequent in skin fold regions at the base of the legs, eyes, ears, cloaca and between the fingers (Figure 3C – D).

Discussion

To the best of our knowledge, this work represents the most comprehensive investigation of mites and ticks parasitizing natural populations of Moroccan lizards, in terms of the number of lizard species (27), the number of individuals examined (1498), and the geographical coverage. Trombiculidae and Pterygosomatidae were the most prevalent families exploiting Moroccan lizards, probably due to the high diversity of hosts in the region. Indeed, the last

Table 4 Summary table of the effect of studied factors on ectoparasite intensity using general linear models (GLM).

	Ixodidae (GLM, $r^2 = 0.101$)			Macronyssidae (GLM, $r^2 = 0.930$)			Ptervgosomatidae (GLM, $r^2 = 0.625$)			Trombiculidae (GLM, $r^2 = 0.228$)		
Effect	df_1, df_2	Statistic (F)	p -value	df_1, df_2	Statistic (F)	p -value	df_1, df_2	Statistic (F)	p -value	df_1, df_2	Statistic (F)	p -value
Host species	4,692	4.574	0.004			-	2, 190	4.35	0.016	6,725	18.202	< 0.001
Sex	1,695	1.326	0.266	1,72	4.718	0.035	1, 192	7.025	0.002	1,730	11.839	< 0.001
Age class	1,695	0.379	0.768	1,72	4.848	0.033	1, 192	4.065	0.005	1,730	0.438	0.646
Body size	1,696	2.862	0.091	1,72	< 0.001	0.996	1, 192	11.983	0.001	1,730	4.745	0.03
Season	1,695	0.088	0.767	1,72	6.478	0.014	1, 192	0.184	0.669	1,730	5.41	0.02
Altitude	20, 676	1.904	0.012	5,68	10.539	< 0.001	29, 163	1.169	0.301	1,706	3.069	< 0.001
Altitude \times Body size				2, 71	7.754	0.001	13, 179	2.037	0.027			
$Altitude \times Season$				1, 72	72.664	< 0.001						
$\mathbf{Sex} \times \mathbf{Body}$ size				1, 72	5.107	0.028						
$Season \times Sex$				1,72	4.402	0.041						
Host species × Altitude							2, 190	7.223	0.001			



Figure 5 Parasitic niches of Ixodidae, Macronyssidae, Pterygosomidae and Trombiculidae in Moroccan lizards.

taxonomic update showed that Morocco has one of the highest levels of lizard biodiversity and endemism in the Western Palaearctic, with Agamidae (7 species), Phyllodactylidae (8 species), Gekkonidae (7 species), Sphaerodactylidae (9 species), and Lacertidae (25 species) (Bouazza *et al.* 2021). The high prevalence of Trombiculd (chigger) mites is likely due to their low host specificity (Azad 1986), whereas Pterygosomatid mites spend their entire life cycle on the host, from larva to adult (Bertrand *et al.* 1995; Bertrand *et al.* 2000). In addition, the origin of examined lizards (captive or wild) can affect the diversity of ectoparasites (Castro *et al.* 2019; Mendoza-Roldan *et al.* 2020a), with the Trombiculidae and Pterygosomatidae families being most prevalent in natural populations. The same finding was also shown in the examination of Brazilian lizards (Mendoza-Roldan *et al.* 2020a).

Only one species of Ixodidae, *I. ricinus*, was found in our study, infesting five lizard species. Two additional tick species were found previously in Moroccan lizards, *I. ventalloi* and *Haemaphysalis sulcata* (Bailly-Choumara *et al.* 1976) (Supplementary Table 1). The absence of these species in the present study may be due to changes in the presence of other host species involved in the life cycle. It is well known that *I. ricinus* is a broad host generalist, with immature stages readily exploiting lizards, small mammals, and birds (Bauwens *et al.* 1983; Medlock *et al.* 2013; Nicholson *et al.* 2019).

Our results indicated the presence of mites from the genus *Ophionyssus* in Moroccan lizards for the first time; these mites were found in two localities in the Marrakech-Safi region (Guemassa and Central Jbilet) on *M. simoni* with a prevalence of infestation of 8.1% (6/74) and a high mean intensity of 31.7 mites/infested lizard (2–74 mites/infested lizard). This finding suggests that this mite uses *M. simoni* as a host species and our observations do not represent an accidental infestation event. This genus has been previously found in different lizard families (Agamidae, Scincidae, and Lacertidae) (Fain & Bannert 2000; Fajfer 2012), and snakes are actually considered the preferred host of these mites. It has been suggested that these mites exploit lizards when the preferred host is not available or when a wide variety of reptiles share the same habitat (Castro *et al.* 2019). This mite parasite is a mechanical vector for several blood-borne infectious diseases in reptiles (e.g. *Aeromonas hydrophila, Anaplasma* sp. and

Rickettsia sp.) (Mendoza-Roldan *et al.* 2020b; Mendoza-Roldan *et al.* 2021). Unfortunately, it may also accidentally infest humans and can cause severe mite-associated dermatitis (Schultz 1975; Amanatfard *et al.* 2014).

In our study, the Pterygosomatidae were represented by two genera: *Geckobia* and *Pterygosoma*. These two genera have been reported in the literature parasitizing *T. mauritanica* (Girot 1968), and *A. impalearis* (Jack 1961; Bertrand *et al.* 2000; Fajfer 2020), respectively. (Supplementary Table 1). Previously, *Pterygosoma* were found infesting only Agamidae; here we reported this genus from Scincidae for the first time. However, this finding may represent an accidental infestation. Further examinations for infestation of Scincidae by *Pterygosoma* are needed to evaluate this relationship.

The Trombiculidae are the most diverse lizard ectoparasites in Morocco. Previously, 10 chigger genera were reported from Moroccan lizards: *Acomatacarus, Ericotrombidium, Eutrombicula, Hypotrombidium, Matacarus, Neoschoengastia, Neotrombicula, Ornithogastia, Pentidionis,* and *Schoutedenichia* (Supplementary Table 1) (Taufflieb 1958; Stekolnikov 2018b, 2019; Stekolnikov *et al.* 2021). Here, we found five chigger mite species of four genera infesting nine lizard hosts, a difference likely explained by the number of examined hosts compared to existent lizard diversity in Morocco (27/86) (Bouazza *et al.* 2021).

Previously, a series of studies reported the co-infestation of lizards by different mite and tick species (Girot 1968; Tomassone *et al.* 2017; Mendoza-Roldan *et al.* 2019). Indeed, the high diversity of parasitic species may lead to regular co-infestation events. In the present work, we recorded four cases of co-infestation. For instance, the presence of two closely related chigger species, *P. maura* and *P. meridialis*, on the same host individual.

In addition to host specificity, patterns of infestation may be related to other biotic and abiotic factors, including differences between the environmental conditions in the habitats (Álvarez-Ruiz et al. 2018; Mendoza-Roldan et al. 2020a), and host behavior and immunity (Eisen & Wright 2001; Hawley & Altizer 2011). The behavior of the host can, for example, influence the degree of exposure to parasites, which could explain the observed differences in the patterns of infestation (Sol et al. 2000). Indeed, in agreement with the findings of Gwiazdowicz et al. (2020); Coêlho et al. (2021); and Er-Rguibi et al. (2021), infestations by Macronyssidae, Pterygosomatidae, and Trombiculidae were significantly different between males and females. We attribute these differences to differences in behavior (Er-Rguibi et al. 2021; Er-Rguibi et al. 2022); adult males are likely more active and can occupy more favorable areas where they are exposed to the ectoparasites. In addition, the maintenance of high concentrations of testosterone during the mating season, may act as an immune suppressor effect (Olsson et al. 2000; Foo et al. 2017) which indirectly affects the ectoparasite loads of male lizards (Halliday et al. 2014). This steroid hormone may also increase activity, competition, and aggression with other males to maintain a territory and a female mate, which can again lead to higher exposure risks to mite and tick infestations (Olsson et al. 2000).

The prevalence and intensity of Macronyssidae, Pterygosomatidae, and Trombiculidae infestations were related to age class, host body size or both. The same result was found in previous studies (e.g. Scali *et al.* 2001; Obi *et al.* 2013; Gwiazdowicz *et al.* 2020; Coêlho *et al.* 2021; Er-Rguibi *et al.* 2021). Higher infestations in adult hosts may be due to their long exposure time to the parasite's infective stages (Obi *et al.* 2013; Er-Rguibi *et al.* 2021). Furthermore, the adult lizards occupy favorable habitats, which may increase the probability of contact with the parasites; juveniles are often limited to marginal areas and are not physically associated with their parents or siblings (Amo *et al.* 2004; Er-Rguibi *et al.* 2021). Finally, older lizards have large body sizes that facilitate more place for mite and tick fixation (Amo *et al.* 2004; Gwiazdowicz *et al.* 2020; Coêlho *et al.* 2021).

The prevalence and intensity of Ixodidae, Macronyssidae, Pterygosomatidae, and Trombiculidae infestations were linked to altitude. This is in line with the results of Álvarez-Ruiz *et al.* (2018) and Er-Rguibi *et al.* (2021), which indicated that ectoparasite infestations varied with elevation. This finding could be explained by changes in environmental conditions, such as humidity and temperature that affect ectoparasite survival, and consequently lead to geographic variation of host-ectoparasite relationships (Medlock *et al.* 2013; Poisot *et al.* 2017). Variation in abiotic and biotic conditions in high elevations could also cause longer hibernation durations, resulting in shorter periods of activity for both host and parasite to reproduce and grow (Comas & Ribas 2015). Long periods of lizard hibernation likely contribute to increasing winter ectoparasite mortality (Postawa & Nagy 2016).

A significant difference in infestation by Macronyssid and Trombiculid mites between seasons was observed. The same results were recorded in other studies (e.g. Huyghe *et al.* 2010; Er-Rguibi *et al.* 2021). This difference may be due to changes in lizard activity and behavior between seasons. For example, changes in testosterone concentrations during reproduction leads to strong variation in activity patterns which can modify exposure rates (Olsson *et al.* 2000; Foo *et al.* 2017). Moreover, as above, the modification of environmental conditions between seasons can alter lizard and ectoparasite interactions (Medlock *et al.* 2013; Poisot *et al.* 2017).

Regarding the on-host parasitic niches, the highest number of *I. ricinus* was found on the ventral side of lizards, where this tick could find an area protected from unfavorable conditions (temperature and wind) (Coêlho et al. 2021). The same result was found between the tick Amblyomma dissimile Koch, 1844 and its host Tropidurus oreadicus Rodrigues, 1987 in Brazil (Coêlho et al. 2021). Concerning the Macronyssidae, these mites were attached under the scales of the vent, legs, and tail. This result was also reported in previous studies, where Ophionyssus saurarum (Oudemans, 1901), infesting Lacerta viridis viridis (Laurenti, 1768), was found around the tail base and hind limb axillae (Masan et al. 2009). O. galloticolus Fain & Bannert, 2000, a parasite of Gallotia galloti eisentrauti Bischoff, 1982 from the Canary Island of Tenerife, was attached around the tail right behind the cloaca, and under the large scales of the belly (Bannert et al. 2000). The Pterygosomatidae were found in different parts of the host body. Thus, Geckobia sp. were found especially on back (dorsal side), between toes, at the base of the claws, and in clusters near the cloaca. The same attachment site was observed for G. loricata and G. latastei parasitizing T. mauritanica in Morocco (Girot 1968). Geckobia spp. infesting Hemidactylus frenatus Duméril & Bibron, 1836 in Colombia were observed mostly on the dorsum of the posterior legs and between the claws (Díaz et al. 2020). Concerning the scale-mite P. bibronii, they tended to be attached under the scales of the ventral side of hosts. However, other studies reported that species of this genus attached to multiple sites on the host body— under the scales of back, vent, tail, and legs (Mostafa 1974; Bertrand et al. 1995). This finding suggest that the body location of the mites of this group was not specified. Regarding the chigger mites, previous findings showed that they prefer small folds of skin in the cervical, axillary, and inguinal regions or the tail base of lizard hosts (Bertrand & Modrý 2004). In our study, chiggers were attached to different parts of the host body, mainly in clusters at the base of limbs, eyes, ears, cloaca and between fingers. For instance, P. maura was found attached in clusters at the tail base and at the leg base of A. maculatus. Thus, infesting these protected areas may help them either avoid being physically removed (either by host grooming or by physical contact with environmental substrates) or penetrate tissues more easily (because the host tissue is maybe more tender in these areas of the body).

In conclusion, Moroccan lizards were infested by nine species of ectoparasite of four mite families (Ixodidae, Macronyssidae, Pterygosomatidae, and Trombiculidae), with the dominance of Acari within the Trombidiformes order (Trombiculidae and Pterygosomatidae) in term of both prevalence and intensity of infestation. The diversity of ectoparasite species associated with lizards in Morocco remains low compared to Brazilian and New Zealand lizards (Heath & Whitaker 2015; Mendoza-Roldan *et al.* 2020a). This is likely linked to the higher diversity of hosts in these countries. Currently, much remains unknown concerning the impacts of the mite and tick parasites on lizard hosts, their determining factors, and the infectious agents they transmit. Thus, larger examination will be necessary to better understand the relationship between lizards and their ectoparasites.

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