International Journal for Parasitology: Parasites and Wildlife 27 (2025) 101103

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Efficacy and safety of afoxolaner (NexGard®) in a collection of captive-bred lizards for the treatment and control of mite infestation

Jairo Alfonso Mendoza-Roldan [a], Livia Perles [a], Chiara Miuli [a], Pietro Larichiutta [b], Matteo Legrottaglie [b], Marcos Antônio Bezerra-Santos [a], Rossella Samarelli [a], Philippe Berny [c], Frederic Beugnet [d], Domenico Otranto [a,e,*]

[a] Department of Veterinary Medicine, University of Bari, Valenzano, Italy
[b] Zoo Safari di Fasano, Brindisi, Italy
[c] Toxicology Lab, Vetagro Sup, 1 Av Bourgelat, 69280, Marcy L'étoile, France
[d] Boehringer Ingelheim Animal Health, Lyon, France
[e] Department of Veterinary Clinical Sciences, City University of Hong Kong, China

ARTICLE INFO

Keywords:
Afoxolaner
Sauria
Ophionyssis natricis
Pterygosoma inermis
Pharmacokinetics

ABSTRACT

Lizards and other reptiles are increasingly gaining popularity as pets worldwide. These animals may be commonly parasitized by species of mites such as *Ophionyssus natricis*, which are deleterious, also because of their role as vectors of pathogens, including zoonotic ones. Treatment options are limited and may not be completely resolutive or safe. The aim of the present study was to assess the efficacy of afoxolaner (NexGard®; Boehringer Ingelheim, Germany) in a collection of mite-infested captive-bred lizards that were housed in mixed enclosures with other reptile classes, in a zoological park located in southern Italy. Lizards were clinically examined and weighed, and mite infestations were assessed on the animals and in their enclosures (environment). All animals were treated with a dose of 2.5 mg afoxolaner per kilogram body weight (2.5 mg/kg) administered orally. All animals were examined pre-treatment (T0) and at various time points post-treatment (T1, 24 h; T2, 14 days; T3, 28 days). The collected mites were morphologically identified at the species level and the species identity also confirmed molecularly and phylogenetically. Animals were morpho-molecularly screened for hemoparasites, endoparasites and vector-borne pathogens. Overall, 28 lizards were screened, and *Leishmania infantum* was detected in a Sudan plated lizard. Moreover, 6 (21.4 %) lizards had at least one mite. Mites were identified as *O. natricis* and *Pterygosoma inermis* infesting Indonesian blue-tongued skinks and Roughtail rock agamas, respectively. No mites were found on hosts after T1, and at T3 no mites were observed in the environment. No side effects were observed in the treated lizards. A single oral administration of afoxolaner at 2.5 mg/kg was a safe treatment in lizards and 100 % effective for the treatment and prevention of common mite species infestations.

1. Introduction

Herpetological medicine, the area dealing with the health of reptiles and amphibians, has been increasingly growing in knowledge and research, in order to offer the best medicine based specialized care and welfare to these non-traditional companion animals (NTCA; Wensley et al., 2016). Within the class Reptilia, lizards (Sauria: Squamata) have progressively gain popularity, and overall, reptiles are nowadays one of the most prevalent NTCAs in European and American markets, with more than 9 million animals being owned in both regions (Valdez, 2021). Despite the rising numbers of these reptiles in households, their susceptibility to ectoparasites and the subsequent effects on their health and behavior are still poorly understood. Mite and tick infestations in reptiles may cause significant discomfort, leading to dermatitis, dysecdysis, behavioral alterations, and potentially death (Mendoza-Roldan et al., 2020a, 2023). Lizards may be parasitized by several mite species, some of which have highly species-specific behavior, such as Pterygosomatids species like *Hirstiella diolli* and *Hirstiella stamii*, which commonly infest green iguanas (*Iguana iguana*) (Farmaki et al., 2013) and *Pterygosoma* spp. which are associated with Agamidae lizards

* Corresponding author. Department of Veterinary Medicine, University of Bari, Valenzano, Italy.
E-mail address: domenico.otranto@uniba.it (D. Otranto).

https://doi.org/10.1016/j.ijppaw.2025.101103
Received 22 April 2025; Received in revised form 14 June 2025; Accepted 14 June 2025
Available online 15 June 2025

(Fajfer, 2020). On the other hand, in captive conditions, lizards may also be infested by generalist species of mites, such as *Ophionyssus natricis*, which mainly infest snakes and may also infest humans (Mendoza-Roldan et al., 2023). Importantly, *O. natricis* is a vector of hemogregarines pathogens (i.e. *Hepatozoon* spp.) as well as of potential zoonotic pathogens such as *Aeromonas hydrophila*, *Rickettsia* spp. of the spotted fever group (Mendoza-Roldan et al., 2021a, 2021b). The great diversity of lizard species kept in captivity might further complicate the epidemiological picture linked to the wide range of mites that can parasitize them combined with the vector capacity. This scenario hampers the selection of appropriate treatments and effective management. Indeed, non-pharmacological strategies implemented to reduce the risk of mite infestations may be environmental measures, including increasing the temperature (>50 °C) as well as a decreasing humidity (<50 %), (Wozniak and DeNardo, 2000). In addition, isolation and quarantine of infested animals, disinfection and cleaning of the enclosure are also warranted (Divers and Mader, 2005). Importantly, the above control measures must be run frequently, considering that some mite species, such as *O. natricis*, can survive for up to three months in the environment without feeding on blood (Wozniak and DeNardo, 2000; Šlapeta et al., 2017). Conversely, safe pharmacological acaricidal treatment options in lizards remain scarce, being organophosphates, carbamates, pyrethrin or pyrethroid sprays, as well as ivermectin injections or sprays used (Mader et al., 1986; Harvey-Clark, 1995; Mader, 1995; d'Ovidio et al., 2023; Farmaki et al., 2013; Hellebuyck et al., 2012). For the drugs above, both safety and effectiveness of these treatment methods have not been thoroughly assessed. For instance, single whole-body application of 0.25 % Fipronil solution were considered safe and effective treatment for the reduction of parasitic burden in captive green iguanas infested by *Hirstiella* sp. mites, with no adverse reactions observed (Farmaki et al., 2013). Although these clinical observational studies are important, the lack of pharmacokinetic research hinders a comprehensive understanding of the toxicity of treatments and the appropriate frequency at which the treatment should be administered.

Isoxazolines, such as afoxolaner (NexGard® Boehringer Ingelheim, Germany) have demonstrated high safety and efficacy against various ectoparasite species, exhibiting no or minimal side effects when compared to other substances (Zhou et al., 2022). This compound was used to treat natural *O. natricis* infestation in captive snakes, and a single oral administration (i.e., 2.5 mg/kg) demonstrated to be a safe treatment with 100 % efficacy in eradicating the mite infestation (Mendoza-Roldan et al., 2023). Based on this previous study, and due to the necessity of new pharmacological mite control in lizards, the present study aimed to assess the efficacy of oral administration of afoxolaner against mite infestation in six different captive lizard species, as well as analyze drug concentration in plasma, and perform clinical follow-ups to determine the outcomes of the treatment.

2. Material and methods

2.1. Ethics statement

Protocols for reptile handling and sampling were approved by the Commission for Bioethics and Animal Welfare of the Department of Veterinary Medicine of the University of Bari, Italy (approval number 23/2023). The off-label use of afoxolaner was based on the European legal cascade authorizing the use of a veterinary formulation in another species when there is no existing registered product for the same indication in this species.

2.2. Animal screening and afoxolaner administration

The study took place in Fasano, Brindisi, Italy at the Zoosafari (40°50′00.6″N,17°20′18.96″E) a private zoo extending over 140 ha. The study focused on the Reptile House area, a room of around 700 square meters housing a variety of reptiles in mixed enclosures, invertebrates, and fish. During routine visits, the veterinarians observed the presence of mites in different lizard species and contacted the Parasitology unit of the Veterinary Medicine faculty, from the University of Bari, Italy. Prior to the pharmacological study 28 lizards of different species (Table 1) were clinically examined, blood samples were taken using the ventral coccygeal vein (Fig. 1A) and all animals and their environments (enclosures) were assessed for mite infestations (Fig. 1B). The blood was placed in 1.5 ml Eppendorf tubes, preserved at −20 °C and blood smears for hemoparasites (Telford, 2009) were performed using Diff-Quik staining and subsequently evaluated using an optical microscope (LEICA DM LB2, Germany). When possible, fecal samples were collected and stored in 1.5 ml Eppendorf at 4 °C. Due to the low volume obtained per individual lizard (~50 µl), all samples were only analyzed microscopically through direct smear (using saline solution) to observe motile protozoa, helminths, acanthocephalans, and pentastomids, as well as a flotation test with a low-density solution was performed (saturated $ZnCl_2$ solution, specific gravity 1.35) (Pasmans et al., 2008; Cervone et al., 2016).

All animals were treated with afoxolaner (NexGard®), administered orally, at a dose as close as possible to 2.5 mg/kg based on the animal's weigh, using 11.3 mg tablets [for small dogs (2–4 kg)] or 68 mg [for medium to large dogs (10–25 kg)] (Table 1). Animals were examined pre-treatment (T0) and at various post-treatment time points (T1, 24 h; T2, 14 days; T3, 28 days) to evaluate the presence of mites. At each time point, all animals were physically examined to assess mite presence, and the overall health of the animals, in order to determine any clinical sing compatible with adverse events. As isoxazolines have been associated to mainly neurological adverse effects in dogs (Bates et al., 2024), a throughout neurologic examination was performed to each animal (Mariani, 2007). In addition, the environment of the enclosure was daily checked for mites during routinely cleaning and maintenance by one of the authors (ML) as well as at each time point. No other environmental treatments were used to assess afoxolaner efficacy and avoid any interaction.

2.3. Pharmacokinetics of afoxolaner in sauria

Ten lizards were selected for pharmacokinetic follow-up (i.e., two *Broadleysaurus major*, two *Iguana iguana*, two *Tiliqua gigas*, two *Cyclura cornuta* and one *Salvator merianae*, *Tubinambis rufescens*) from which plasma was collected in the abovementioned time points (Table 1).

A specific analytical technique was developed using liquid chromatography and tandem mass spectrometry (LC-MSMS), based on (Kilp et al., 2016). Plasma samples (0.2 ml) were extracted with 100 mg QuEChERS salts (4 g MgSO4and 1 g NaCl) and 0.275 ml acetonitrile. Each sample was spiked with an internal standard (fluralaner) at a constant concentration for quantification. All samples were mixed for 1 min and centrifuged at 8000 g for 5 min. The supernatant layer was collected and used for analysis. The LC-MSMS equipment was the Agilent 1260 Affin-ity II Prime LC system, equipped with a 6470A triple-quadrupole mass spectrometer (Agilent Technologies France, Les Ulis, France). The column used was a VWR Lichrospher 100 C18e (100 mm × 4 mm ID, dp 5 µm). The mobile phase consisted of a mixture of acetonitrile and water (with solution A: ammonium carbonate 10 mM, pH 9) and was dispensed at 0.5 ml/min using a graded elution scheme (0–5 min: water 70 %, solution A 30 %; 5 min: water 90 %, solution A 10 %; 5.5–11 min: water 70 %, solution A 30 %). The total run time was 11 min. Afoxolaner was detected using the negative ion mode (precursor ion, 3 ions produced). The method was validated according to current standards. To cover matrix effects, samples were quantified by comparison with spiked samples on the same day. Linearity was checked between 2 and 100 µg/l and only accepted if $r^2 > 0.99$.

Descriptive statistics (mean, median, standard deviation, minimum, and maximum) were calculated for each time point (T1, T2, and T3) to evaluate plasma concentration trends and interindividual variability. To

Table 1
Species of lizards (scientific name) sampled, sex, weight (in Kg), clinical observations, presence/absence of ectoparasites and intensity, tablet size of afoxolaner used and animals selected for pharmacokinetics.

Family		Species	Sex	Weight	Tablet size of afoxolaner used	Clinical status	Ectoparasites (intensity)	Sample collection for pharmacokinetics
Gerrhosauridae	Sudan plated lizard	*Broadleysaurus major*	M	0.365	11.3	Healthy	No	Yes
		Broadleysaurus major	M	0.350	11.3	Healthy	No	–
		Broadleysaurus major	M	0.340	11.3	Healthy	No	–
		Broadleysaurus major	M	0.400	11.3	Healthy	No	–
		Broadleysaurus major	M	0.450	11.3	Respiratory signs	No	Yes
Agamidae	Roughtail rock agama	*Laudakia stellio*	M	0.130	11.3	Healthy	Yes (2) *Pterygosoma inermis*	–
		Laudakia stellio	F	0.100	11.3	Healthy	Yes (6) *P. inermis*	–
		Laudakia stellio	M	0.140	11.3	Healthy	Yes (3) *P. inermis*	–
		Laudakia stellio	F	0.100	11.3	Dorsal skin lesion	Yes (1) *P. inermis*	–
	Amboina sail-finned lizard	*Hydrosaurus amboinensis*	F	0.350		Healthy	No	–
Teiidae	Argentine black and white tegu	*Salvator merianae*	M	1.5	68	Healthy	No	Yes
		Salvator merianae	F	2.5	68	Healthy	No	–
	Red tegu	*Tupinambis rufescens*	F	2.280	68	Healthy	No	Yes
		Tupinambis rufescens	M	2.5	68	Healthy	No	–
Iguanidae		*Cyclura cornuta*	M	1.5	68	Healthy	No	Yes
	Rhinoceros iguana	*Cyclura cornuta*	F	1.5	68	Skin lesion	No	–
		Cyclura cornuta	F	1.5	68	Healthy	No	Yes
		Cyclura cornuta	F	1.5	68	Healthy	No	–
		Cyclura cornuta	M	1.5	68	Healthy	No	–
	Green iguana	*Iguana iguana*	M	0.990	68	Healthy	No	–
		Iguana iguana	F	0.800	68	Healthy	No	Yes
		Iguana iguana	M	0.900	68	Healthy	No	Yes
		Iguana iguana	M	0.900	68	Healthy	No	–
		Iguana iguana	M	0.901	68	Healthy	No	–
		Iguana iguana	F	0.902	68	Healthy	No	–
Varanidae	Cuming's water monitor	*Varanus cumingi*	F	5	68	Healthy	No	–
Scincidae	Indonesian blue-tongued skinks	*Tiligua gigas*	F	0.350	11.3	Healthy	Yes (8) *Ophionyssus natricis*	Yes
		Tiligua gigas	F	0.350	11.3	Healthy	Yes (3) *O. natricis*	Yes

assess whether pharmacokinetic profiles differed among species, non-parametric Kruskal–Wallis rank sum tests were performed separately for each time point, reporting the chi-squared statistic (χ^2), degrees of freedom (df), and p-value (p). All statistical analyses were conducted in R version 4.5.0 (R Core Team, 2025), using the tidyverse v2.0.0 for data processing. Line plots with standard deviation error bars were generated using ggplot2 v3.5.2 to illustrate mean plasma concentrations per species over time. Species were grouped by taxonomic families using facet-based visualization (facet_wrap).

2.4. Ectoparasite identification

Mites were collected and stored in Eppendorf tubes with absolute ethanol and genomic DNA (gDNA) was extracted from each mite individually, using an in-house protocol (Chomczynski, 1993), that avoid mite destruction and preserve the cuticule (Mendoza-Roldan et al., 2019). Mites were then clarified and mounted on slides in Hoyer's medium (Krantz and Walter, 2019) and morphologically identified using dichotomous keys (Fain and Bannert, 2000; Fajfer, 2020).

To assess the parasitic burden of mites, descriptive statistics were calculated using Quantitative Parasitology software, version 3.0 (Rózsa et al., 2000). The prevalence, average abundance (i.e., the number of mites per the total number of hosts) and intensity (i.e., the number of mites per the number of infested hosts) of the infestation were calculated.

2.4.1. Molecular screening

gDNA of mites was submitted to conventional PCRs (cPCR) assay targeting a 480 bp fragment of the endogenous 18S rRNA gene a fragment of ~700 bp of the *cox1* gene (Otto and Wilson, 2001). gDNA of blood and mites was submitted to cPCR for screening Anaplasmataceae agents using 16S rRNA gene (~345 bp, Martin et al., 2005), *Rickettsia* spp. using *gltA* gene (~400 bp, Wójcik-Fatla et al., 2009) and Apicomplexa using 18S rRNA (~460–520 bp, Gubbels et al., 1999). gDNA of blood was submitted to two real-time qPCRs (Francino et al., 2006; Latrofa et al., 2021) and cPCR (Sadlova et al., 2022) for *Leishmania* detection. cPCR results were observed in 2 % agarose gel stained with GelRed (VWR International PBI, Milan, Italy) and visualized on a Chemidoc imagining system (Bio-Rad Laboratories, Hercules, CA, USA). Amplicons were purified using an enzymatic method (Exol; Thermo

Fig. 1. Clinical examination and samplings performed in lizards. A) Blood sampling from ventral coccygeal of an *Iguana iguana*; A) Ectoparasite (red arrows) collection from the ventral abdomen of *Laudakia stellio*.

Fisher Scientific, Waltham, MA, USA) and were sequenced using Sanger methodology in an automated sequencer (ABI-PRISM 377; Applied Biosystems, Thermo Fisher Scientific). Consensus sequences were constructed with Geneious software version 11.14 and compared with those available in the GenBank database using the Nucleotide Basic Local Alignment Search Tool (nBLAST) (available at https://blast.ncbi.nlm.nih.gov/Blast.cgi). For phylogenetic inference, sequences from the present study were aligned with those retrieved from GenBank using MAFFT software version 7 (Katoh et al., 2018). The best evolutionary model was chosen under the Akaike Information Criterion (AIC) using CIPRES gateway (available at https://www.phylo.org/). Maximum likelihood phylogenetic analyses with 8000 bootstraps were performed using iqTree gateway (Trifinopoulos et al., 2016). The phylogenetic tree edition and rooting (outgroup) were performed using TreeGraph 2.0 beta software (Stöver and Müller, 2010).

3. Results

The 28 lizards enrolled in this study belonged to the Agamidae, Gerrhosauridae, Iguanidae, Teiidae, Scincidae, and Varanidae families (Table 1). Animals had different sizes and body weights, from 100 g (*Laudakia stellio*) to 5 kg (*Varanus cumingi*), with a mean body weight of 1.07 ± 1.05 kg. Out of the eight species screened, two had mite infestations, *L. stellio* and *T. gigas*, the latter individuals were housed with a carpet python (*Morelia spilota*) that also was infested with mites. Overall, screened lizards were apparently healthy, with two animals (i.e., *L. stellio* and *C. cornuta*) with skin lesions and one (i.e., *B. major*) with respiratory clinical signs (Table 1). None of the 28 blood smears (Fig. 2) and fecal samples examined had visible hemoparasites and endoparasites, respectively. All pathogens molecularly screened were negative, except for one sample (i.e., *B. major*) that resulted positive at qPCR targeting the kinetoplast DNA of *Leishmania infantum* (mean cycle threshold 35.99 ± 1). Despite additional attempts, no amplification was obtained at cPCR for further molecular characterization. Of the 28 animals screened, 6 were found to have at least one mite [21.4 %; 95 % confidence interval (CI): 40.9–82.9 %]. The mean infestation intensity was 3.83 (95 % CI: 2–5.67) and mean abundance was 0.82 (95 % CI: 0.29–1.75). Two *T. gigas* were parasitized by *Ophionyssus natricis* (Fig. 3A) and four *L. stellio* by *Pterygosoma inermis* (Fig. 1B; Fig. 3B; Table 1). Two gDNA from each mite species were randomly selected for sequencing, and results from 18S rRNA of samples obtained from *T. gigas* showed 100 % of query coverage and identity with several *O. natricis* (Acession number [AN] PP493245, MT163330), while results from sample obtained from two *L. stellio* showed 100 % of query coverage and 92 % of identity with a Pterygosomatidae sp. (AN ON555737).

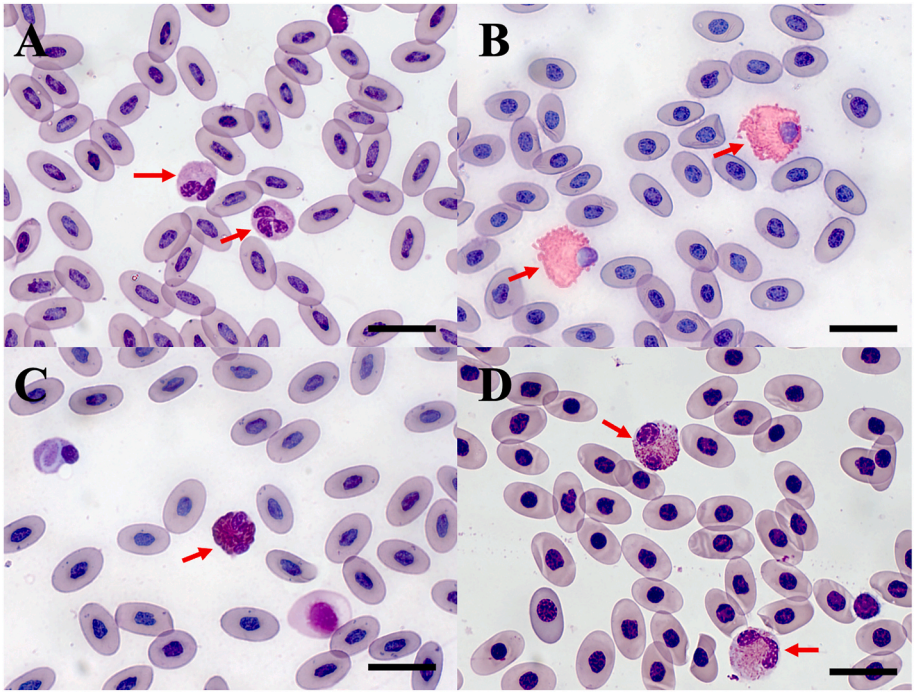

Fig. 2. Blood smears from different saurian species. A) *Laudakia stellio*; B) *Salvator merianae*; C) *Iguana iguana*; D) *Varanus cumingi*. Red arrows showing the heterophils. Scale bars 20 μm.

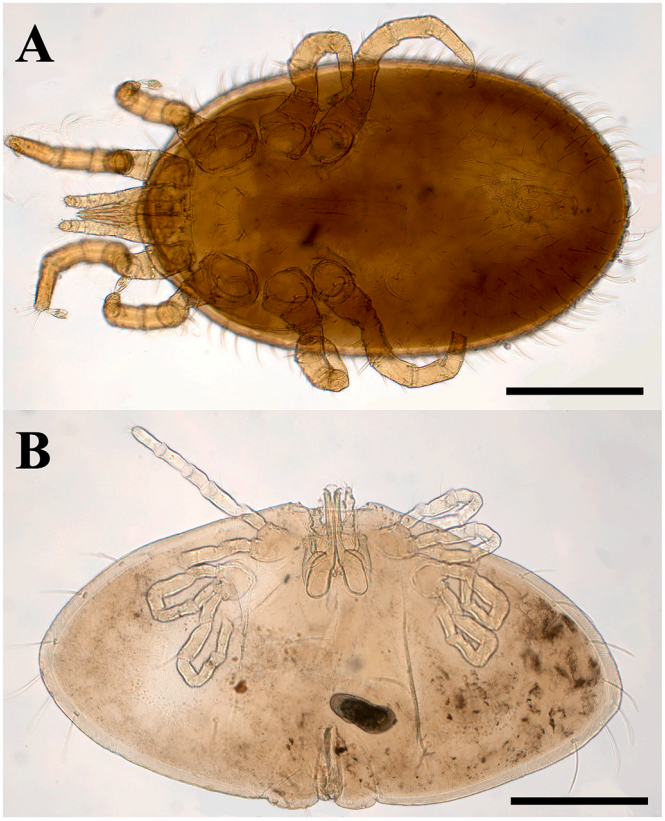

Fig. 3. Morphological features of collected mites. A) ventral view of female *Ophionyssus natricis*; B) ventral view of female *Pterygosoma inermis*. Scale bars A 20 μm; B 100 μm.

Phylogenetic analyses clustered sequences obtained from *T. gigas* with other *O. natricis* sequences, within the Macronyssidae clade, while sequences obtained from *L. stellio* clustered with the sequence of a Pterygosomatidae species, within the Anystoidea mites' clade (Fig. 4).

Following treatment, mites were observed in the enclosures after T1, but none were found on the lizards. From T2 up to T3 mites were no longer observed on animals nor in the environment. No related adverse events were recorded after oral administration of afoxolaner in any of the lizards. Afoxolaner availability in the plasma was found at different concentrations in samples collected from the 10 lizards sampled after 24 h up to day 28 (Table 2; Fig. 5). At 24h post oral administration (T1), all sampled lizards had plasma afoxolaner concentrations ranging from 56 to 722 ng/ml, with a mean value of 309.5 ng/ml. Plasma concentrations of afoxolaner decreased in all animals after 24h post oral administration. All lizards had detectable plasma concentration levels of afoxolaner at day 14 (i.e. T3) ranging from 5.02 ng/ml in *I. iguana*, to 330.57 ng/ml in a *Cyclura cornuta.* At day 28, most lizards, except for three iguanids, still had detectable plasma concentration afoxolaner (i.e. T4; mean of 64.7 ng/ml) ranging from 7 ng/ml in *T. gigas* to 144.7 ng/ml in a *B. major* (Table 2, Fig. 5).

Descriptive statistics revealed substantial interindividual variability in plasma afoxolaner concentrations, particularly at T1 (24 h post-treatment), with values ranging from 56.0 to 722.7 ng/ml (mean ± SD: 309.5 ± 222.3 ng/ml). This variation remained evident at later time points, though to a lesser extent (T2: 5.02–330.6 ng/ml; T3: 7.08–145.0 ng/ml) (Table 3).

No statistically significant differences were observed among species at T1 ($\chi^2 = 5.18$, df = 5, p = 0.394), T2 ($\chi^2 = 3.05$, df = 5, p = 0.692), or T3 ($\chi^2 = 2.36$, df = 4, p = 0.670), suggesting that systemic exposure to afoxolaner was broadly comparable across the different lizard species analyzed.

4. Discussion

Results from this study demonstrated the efficacy and safety of afoxolaner in different species of lizards. Indeed, a single oral administration of afoxolaner at a dose of 2.5 mg/kg had no adverse effects in any of the eight species of lizards treated and eliminated the natural infestation of two different, yet common, mite species. Importantly, as demonstrated by the concentration of afoxolaner in plasma up to day 28 post administration, the acaricidal efficacy and duration is comparable to that of mammals and snakes. Moreover, this study is the first to demonstrate the usefulness of isoxazolines in lizards, representing an alternative less stressful treatment that could avoid environmental strategies which could potentially target other invertebrates and leave residual toxicity.

The species of lizards screened represent families of pet reptiles that are commonly kept in Europe (Valdez, 2021). Moreover, in Italy most of these families of saurians (i.e., Agamidae, Iguanidae, Scincidae and Teiidae) have been screened for ectoparasites (Cervone et al., 2016; Mendoza-Roldan et al., 2019). Endoparasites have also been previously assessed in most of these families of saurians in Italy, identifying the presence of mostly non-pathogenic parasites, such as coccidia and oxyurids (Papini et al., 2011). The absence of endoparasites in the lizards investigated may be due to the established routine deworming program of the zoological collection or to the low amount of volume of feces collected (Rinaldi et al., 2012). Moreover, hemoparasites such as hemogregarines (i.e., *Hepatozoon*) are commonly found in wild caught animals, rather than captive bred, which is the case of most lizards analyzed in this study (Halla et al., 2014). Hence, even though hemoparasites were not detected, morpho-molecular screening is important and warranted to address the risk of introduction of parasites that may affect the autochthonous and allochthonous herpetofauna, especially when they are housed in mixed enclosures. Moreover, molecular screening of zoonotic pathogens is also recommended, especially in zoological settings where different species of animals are kept together possibly being blood source of shared arthropod vectors, such as mosquitoes, sand flies and ticks (Tuten et al., 2012; Hrnková et al., 2021; Pereira et al., 2023). The detection for the first time of *L. infantum* in the Sudan plated lizard may be explained by the fact that the zoological park herein assessed is in a hyper-endemic area in Apulia region, endemic for canine leishmaniosis (Mendoza-Roldan et al., 2020b). Accordingly, *L. infantum* was previously detected in tigers (*Panthera tigris*) and in sand flies in the same zoological park (Iatta et al., 2020), as well as in gekkonid and lacertid lizards from the same area (Mendoza-Roldan et al., 2022). All the above, along with the fact that *Sergentomyia minuta* sand flies, a typically herpetophilic species, were already found infected with *L. infantum* in the same hyperendemic context (Iatta et al., 2020), suggest that reptiles could be occasionally infected being non-natural hosts. In this case, although afoxolaner does not prevent against the transmission of *Leishmania* spp. if applied at a populational strategy level, it may reduce the incidence of infection, as seen in previous shelter dog studies (Panarese et al., 2021; Otranto et al., 2024). Future entomological studies are needed to address if the population of sand flies may diminish also in this herpetological context.

On the other hand, the present study further validated the use of afoxolaner in herpetological collections for the prevention, control and potential eradication of mite infestations when applied in the whole population at risk (Mendoza-Roldan et al., 2023). This strategy requires the selection of a safe product, such as afoxolaner, which has shown a high safety threshold with no adverse effects up to 5 times the maximum exposure dose in dogs (Drag et al., 2014). In addition, within the isoxazoline group, neurologic adverse events in dogs (e.g., ataxia, muscle tremor and convulsion) are lower than those reported for other isoxazolines (Bates et al., 2024). Given the limited number of animals, and that hematological and biochemical parameters were not performed, results from this study suggest that afoxolaner may be used in lizards with no apparent adverse effects, yet further studies are warranted to

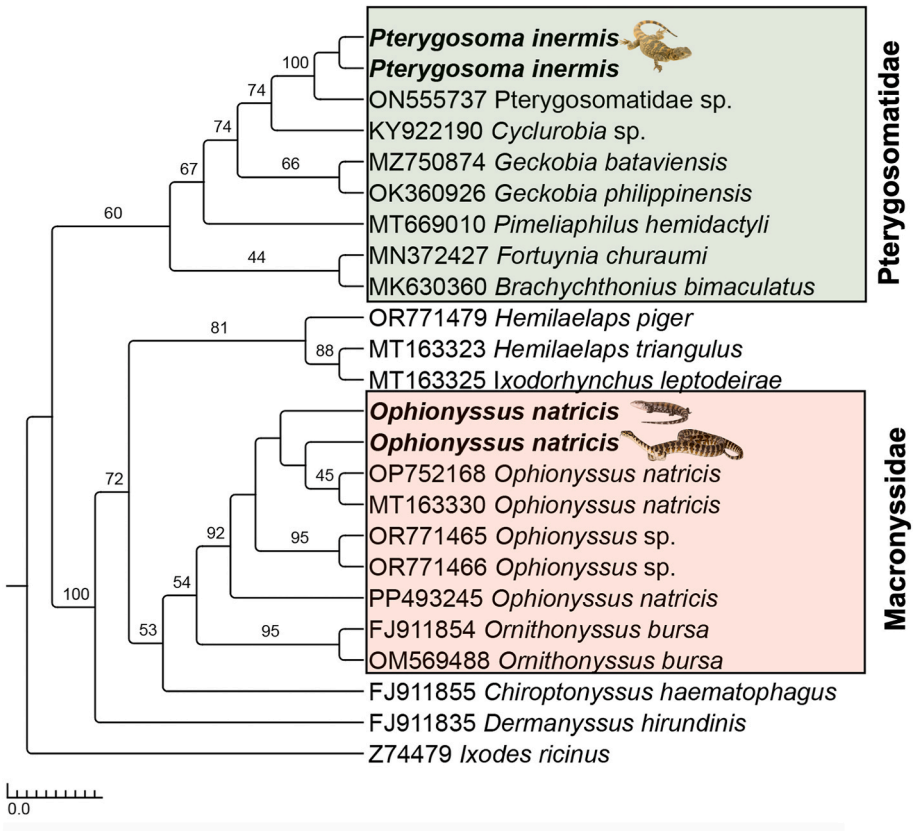

Fig. 4. Maximum-likelihood phylogenetic trees of 18S rRNA genes of mites *Ophionyssus natricis* and *Pterygosoma inermis*. Bootstrap values (>40 %) are shown near the nodes. *Ixodes ricinus* was used as outgroup. Scale bar indicates nucleotide substitution per site. Sequences of this study are in bold.

Table 2
Plasma concentration levels of afoxolaner from 10 sampled lizards are given for the four time points (i.e., T0, T1, T2, T3).

Family	Lizard species	T0	T1	T2	T3
Gerrhosauridae	*Broadleysaurus major*	0	64.46	27.92	12,87
	Broadleysaurus major	0	342.06	162.77	144,64
Teiidae	*Salvator merinae*	0	348.40	277.55	141,53
	Tupinabis rufescens	0	389.54	148.81	90,74
Iguanidae	*Cyclura cornuta*	0	722.67	330.57	<LOQ[a]
	Cyclura cornuta	0	308.70	7.50	<LOQ
	Iguana iguana	0	201.01	5.02	<LOQ
	Iguana iguana	0	581.65	75.16	25,91
Scincidae	*Tiligua gigas*	0	56.02	14.91	7,08
	Tiligua gigas	0	80.24	52.56	30,44

[a] Limit of Quantification.

fully assess the safety of isoxazolines in reptiles.

While *O. natricis* is commonly found in captive snakes worldwide (Orlova et al., 2024), it has been previously identified in wild *Tiliqua* skinks (Norval et al., 2021). Thus, skinks may be sentinels of mixed infestations, when the presence in snakes may be low, being competent hosts. Moreover, the catholic or generalist feeding behavior of *O. natricis*, which may also in certain conditions feed on humans, stresses and validates the need for a populational treatment with afoxolaner (Amanatfard et al., 2014). Conversely, although Pterygosomatidae mites have a low pathogenic effect on their saurian hosts, skin lesions have been recorded in humans following infestation with mites from iguanas (d'Ovidio et al., 2023). The mites *Pterygosoma inermis* identified herein, represent the first record of their presence in Italy, being originally described from Egypt (Fajfer et al., 2016).

Considering the confirmed acaricidal efficacy in lizards of a single dose of afoxolaner given orally, and the fact that it was also efficacious when administered to snakes *via* prey food (Mendoza-Roldan et al., 2023), afoxolaner may also be possibly given to lizards *via* their food (i.e., insects, meat or fruits and vegetables) reducing the need of stressing handling to apply topical acaricides (d'Ovidio et al., 2023). Importantly, afoxolaner had a rapid onset of absorption and persistency for several weeks, thus preventing future infestations of mites that feed on treated animals [i.e., persistent (residual) efficacy; Otranto et al., 2021]. This single strategy may reduce the number of environmental disinfections, that may be also toxic for the animals (Mendoza-Roldan et al., 2023). This is an important aspect to consider also when animals are kept in a zoological setting where visitors are constantly crossing these environments.

As previously observed in dogs and snakes, plasma levels were sufficient to be effective against ectoparasites at least for a month (Letendre et al., 2014; Mendoza-Roldan et al., 2023), except for iguanids that seemed to have a higher systemic clearance. Nonetheless, considering that Iguanids are generally more frequently parasitized by Pterygosomatid mites, that complete their whole cycle in a single host (Kalúz and Literák, 2019), as fleas and ixodid ticks, which afoxolaner has a 90 % lethal dose (LD_{90}) of 20 ng/ml and 110 ng/ml, respectively (Letendre et al., 2014), treatment with oral afoxolaner may also eliminate all type of ectoparasites. The above is also supported by the fact that the mean plasma concentration of afoxolaner in iguanids at T1 was 453.5 ng/ml. The reasons why iguanids may have a lower plasma half-life of afoxolaner remain unknown. Future studies with iguanids are advocated to better understand their particularities with isozaxolines pharmacokinetics. Despite results found in most iguanids, and the fact that pharmacokinetics was assessed in only 10 lizards, still the results suggest that the plasma concentration after 28 days post oral administration was enough to prevent new mite infestations in screened lizards.

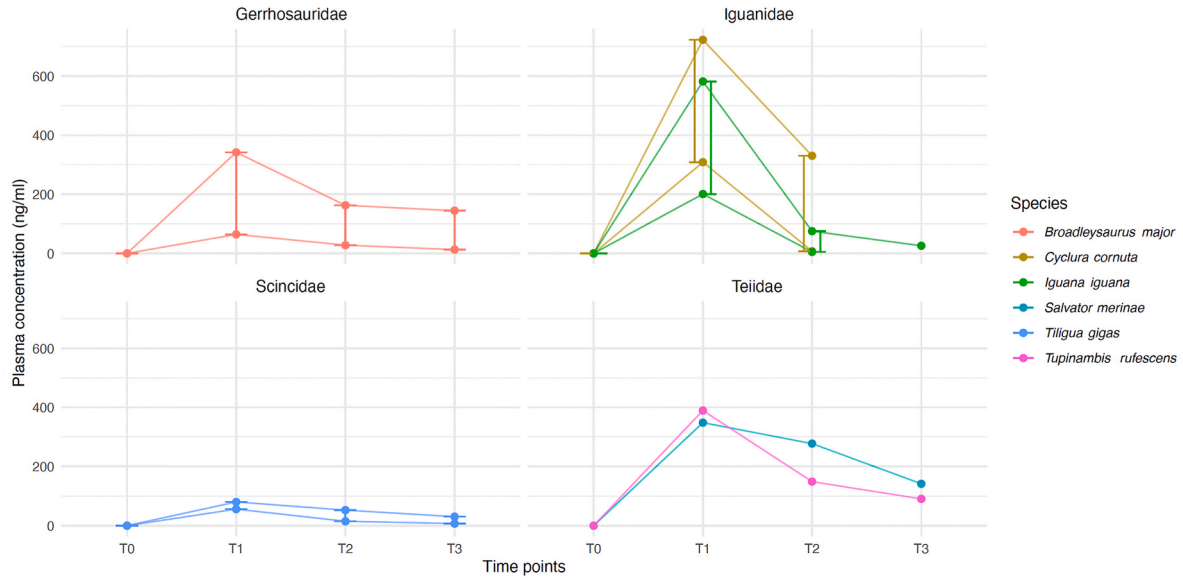

Fig. 5. Plasma concentration of afoxolaner over time in six lizard species. Each panel represents a different lizard family (Gerrhosauridae, Iguanidae, Scincidae, and Teiidae). Colored lines represent the mean concentration per species across four time points (T0, T1, T2, T3), with vertical bars indicating standard deviation. Peak plasma levels were observed at T1 (24 h post-treatment), followed by progressive decline.

Table 3
Descriptive statistics of plasma afoxolaner concentrations (ng/ml) in lizards at each sampling time point. Number of lizards, mean, standard deviation, median, minimum, and maximum values are reported for each time point (T1, T2, and T3).

Time Point	N (lizards)	Mean (ng/ml)	Standard deviation	Median	Minimum	Maximum
T1 (24 h)	10	309.5	222.3	325.0	56.0	722.7
T2 (14 days)	10	110.3	116.9	63.9	5.02	330.6
T3 (28 days)	7	64.7	60.1	30.4	7.08	145.0

5. Conclusion

Results from the present study confirmed that a single oral dose of afoxolaner is efficacious in eliminating and preventing infestations with two common mite families (Pterygosomatidae and Macronyssidae) in lizards kept under captive conditions. Additionally, using this single strategy in all housed animals may prevent future mite infestations, without the need of environmental control methods, also potentially reducing the hematophagous dipteran population (i.e., sand flies and mosquitoes), hence lessening the risk of transmission of *Leishmania*, which was detected for the first time in a Gerrhosauridae lizard. Moreover, the efficacy and potential safety of afoxolaner in lizards warrants its use during quarantine as an initial ectoparasitic treatment, in order to avoid mite and tick introduction to an animal collection. Results herein showcased suggest that monthly oral administration, also potentially *via* food, can control and prevent infestations with common mites of lizards, some of which have zoonotic potential.

CRediT authorship contribution statement

Jairo Alfonso Mendoza-Roldan: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Livia Perles:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis. **Chiara Miuli:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Pietro Larichiutta:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Matteo Legrottaglie:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Marcos Antônio Bezerra-Santos:** Writing – review & editing, Resources, Project administration, Methodology, Investigation. **Rossella Samarelli:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation. **Philippe Berny:** Writing – review & editing, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Frederic Beugnet:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Domenico Otranto:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Funding

The study was partially supported by Boehringer Ingelheim Animal Health (France, Europe). The study has been conducted under the frame of the EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

Declaration of interest

Frederic Beugnet is an employee of Boehringer Ingelheim Animal Health (France, Europe). The remaining authors have declared that no competing interests exist.

Acknowledgments

The authors thank the Zoosafari Zoological Park in Fasano for the herpetological assistance and allowing the follow-up of their reptile collection. The publication costs of this article were supported by the "*Fondo per la Qualità e l'Internazionalizzazione della Ricerca* – D.R. n. 2924 of 29/07/2024" (Bari, Italy).

References

Amanatfard, E., Youssefi, M.R., Barimani, A., 2014. Human dermatitis caused by *Ophionyssus natricis*, a snake mite. Iran. J. Parasitol. 9 (4), 594.

Bates, N., Dijkman, M.A., Edwards, J.N., 2024. Neurological adverse effects of isoxazoline exposure in cats and dogs. Vet. Rec. 194 (12), e4149.

Cervone, M., Fichi, G., Lami, A., Lanza, A., Damiani, G.M., Perrucci, S., 2016. Internal and external parasitic infections of pet reptiles in Italy. J. Herpetol. Med. Surg. 26, 122–130.

Chomczynski, P., 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15, 532–537.

Drag, M., Saik, J., Harriman, J., Larsen, D., 2014. Safety evaluation of orally administered afoxolaner in 8-week-old dogs. Vet. Parasitol. 201 (3–4), 198–203.

d'Ovidio, D., Pantchev, N., Santoro, D., 2023. *Hirstiella* spp. infestation in a client-owned iguana (*iguana Iguana*) associated with skin lesions on its owner. Top. Companion Anim. Med. 55, 100802.

Divers, S.J., Mader, D.R., 2005. Reptile Medicine and surgery-E-book. Elsevier Health Sciences, Amsterdam.

Fain, A., Bannert, B., 2000. New observations on species of the genus *ophionyssus mégnin* (acari: macronyssidae) parasitic on lizards of the genus *gallotia Boulenger* (reptilia: lacertidae) from the Canary Islands, Spain with description of a new species. Int. J. Acarol 28, 361–366.

Fajfer, M., 2020. A systematic revision of the scale mite genus *pterygosoma peters*, 1849 (acariformes: pterygosomatidae). Zootaxa 4805.

Fajfer, M., Melnikov, D., Dabert, M., 2016. Three new species of the genus *pterygosoma peters*, 1849 (acariformes: pterygosomatidae) from agamid lizards (sauria: Agaminae) with DNA barcode data. Syst. Parasitol. 93, 791–814.

Farmaki, R., Simou, C., Papadopoulos, E., Koutinas, A.F., Saridomichelakis, M.N., 2013. Effectiveness of a single application of 0·25% fipronil solution for the treatment of hirstiellosis in captive green iguanas (*iguana Iguana*): an open-label study. Parasitology 140 (9), 1144–1148.

Francino, O., Altet, L., Sánchez-Robert, E., Rodriguez, A., Solano-Gallego, L., Alberola, J., Ferrer, L., Sánchez, A., Roura, X., 2006. Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. Vet. Parasitol. 137, 214–221.

Gubbels, J.M., De Vos, A.P., Van der Weide, M., Viseras, J., Schouls, L.M., De Vries, E., Jongejan, F., 1999. Simultaneous detection of bovine *theileria* and *babesia* species by reverse line blot hybridization. J. Clin. Microbiol. 37, 1782–1789.

Halla, U., Korbel, R., Mutschmann, F., Rinder, M., 2014. Blood parasites in reptiles imported to Germany. Parasitol. Res. 113, 4587–4599.

Harvey-Clark, C.J., 1995. Common dermatologic problems in pet reptilia. Seminars Avian Exot. Pet Med. 4, 205–219.

Hellebuyck, T., Pasmans, F., Haesebrouck, F., Martel, A., 2012. Dermatological diseases in lizards. Vet. J. 193, 38–45.

Hrnková, J., Schneiderová, I., Golovchenko, M., Grubhoffer, L., Rudenko, N., Černý, J., 2021. Role of zoo-housed animals in the ecology of ticks and tick-borne Pathogens—A review. Pathogens 10 (2), 210.

Iatta, R., Zatelli, A., Laricchiuta, P., Legrottaglie, M., Modry, D., Dantas-Torres, F., Otranto, D., 2020. Leishmania infantum in tigers and sand flies from a leishmaniasis-endemic area, southern Italy. Emerg. Infect. Dis. 26 (6), 1311.

Kalúz, S., Literák, I., 2019. A description of the male of *Geckobiella donnae* Paredes-León, Klompen et Pérez, 2012 (Acari: pterygosomatidae) from captive iguanas in Honduras. Folia Parasitol. 66, 1–6.

Katoh, K., Rozewicki, J., Yamada, K.D., 2018. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings Bioinf. 20 (4), 1160–1166.

Kilp, S., Ramirez, D., Allan, M.J., Roepke, R.K., 2016. Comparative pharmacokinetics of furalaner in dogs and cats following single topical or intravenous administration. Parasites Vectors 31, 296.

Krantz, G.W., Walter, D.E., 2019. A Manual of Acarology, third ed. Texas Teech University Press, Lubbock.

Latrofa, M.S., Mendoza-Roldan, J.A., Dantas-Torres, F., Otranto, D., 2021. A duplex real-time PCR assay for the detection and differentiation of *Leishmania infantum* and *Leishmania tarentolae* in vectors and potential reservoir hosts. Entomol. Gen. 41, 543–551.

Letendre, L., Huang, R., Kvaternick, V., Harriman, J., Drag, M., Soll, M., 2014. The intravenous and oral pharmacokinetics of afoxolaner used as a monthly chewable antiparasitic for dogs. Vet. Parasitol. 201 (3–4), 190–197.

Mader, D.R., 1995. Acariasis. In: Mader, D.E. (Ed.), Reptile Medicine and Surgery. W.B. Saunders, Philadelphia, PA, USA, pp. 341–346.

Mader, D.R., Houston, R.S., Frye, F.L., 1986. *Hirstiella Trombidiiformis* infestation in a colony of chuckwallas. J. Am. Vet. Med. Assoc. 189, 1138–1139.

Mariani, C.L., 2007. The neurologic examination and neurodiagnostic techniques for reptiles. Vet. Clin. North. Am. Exot. Anim. Pract. 10 (3), 855–891 vii.

Martin, A.R., Brown, G.K., Dunstan, R.H., Roberts, T.K., 2005. *Anaplasma Platys*: an improved PCR for its detection in dogs. Exp. Parasitol. 109, 176–180.

Mendoza-Roldan, J.A., Colella, V., Lia, R.P., Nguyen, V.L., Barros-Battesti, D.M., Iatta, R., Dantas-Torres, F., Otranto, D., 2019. *Borrelia burgdorferi* (*Sensu lato*) in ectoparasites and reptiles in southern Italy. Parasites Vectors 12 (1), 35.

Mendoza-Roldan, J.A., Ribeiro, S.R., Castilho-Onofrio, V., Grazziotin, F.G., Rocha, B., Ferreto-Fiorillo, B., Pereira, J.S., Benelli, G., Otranto, D., Barros-Battesti, D.M., 2020a. Mites and ticks of reptiles and amphibians in Brazil. Acta Trop. 208, 105515.

Mendoza-Roldan, J.A., Benelli, G., Panarese, R., Iatta, R., Furlanello, T., Beugnet, F., Zatelli, A., Otranto, D., 2020b. *Leishmania infantum* and *Dirofilaria immitis* infections in Italy, 2009–2019: changing distribution patterns. Parasites Vectors 13, 1–8.

Mendoza-Roldan, J.A., Ribeiro, S.R., Castilho-Onofrio, V., Marcili, A., Simonato, B.B., Latrofa, M.S., Benelli, G., Otranto, D., Barros-Battesti, D.M., 2021a. Molecular detection of vector-borne agents in ectoparasites and reptiles from Brazil. Ticks Tick Borne Dis. 12, 101585.

Mendoza-Roldan, J.A., Mendoza-Roldan, M.A., Otranto, D., 2021b. Reptile vector-borne diseases of zoonotic concern. Int. J. Parasitol. Parasites. Wildl. 15, 132–142.

Mendoza-Roldan, J.A., Latrofa, M.S., Tarallo, V.D., Manoj, R.R., Bezerra-Santos, M.A., Annoscia, G., Iatta, R., Otranto, D., 2022. *Leishmania* spp. in squamata reptiles from the Mediterranean Basin. Transbound. Emerg. Dis. 69 (5), 2856–2866.

Mendoza-Roldan, J.A., Napoli, E., Perles, L., Marino, M., Spadola, F., Berny, P., España, B., Brianti, E., Beugnet, F., Otranto, D., 2023. Afoxolaner (NexGard®) in pet snakes for the treatment and control of *Ophionyssus natricis* (mesostigmata: macronyssidae). Parasites Vectors 16 (1), 6.

Norval, G., Halliday, B., Sharrad, R.D., Gardner, M.G., 2021. Additional instances of snake mite (*Ophionyssus natricis*) parasitism on sleepy lizards (*Tiliqua rugosa*) in South Australia. Trans. Roy. Soc. S. Aust. 145 (2), 183–193.

Orlova, M.V., Halliday, B., Reeves, W.K., Doronin, I.V., Mishchenko, V.A., Vyalykh, I.V., Kidov, A.A., 2024. Review of the distribution and biology of the snake mite *Ophionyssus natricis* (acari: macronyssidae). Acarologia 64 (2), 637–653.

Otranto, D., Dantas-Torres, F., Fourie, J.J., Lorusso, V., Varloud, M., Gradoni, L., Drake, J., Geurden, T., Kaminsky, R., Heckeroth, A.R., Schunack, B., Pollmeier, M., Beugnet, F., Holdsworth, P., 2021. World association for the advancement of veterinary parasitology (W.A.A.V.P.) guidelines for studies evaluating the efficacy of parasiticides in reducing the risk of vector-borne pathogen transmission in dogs and cats. Vet. Parasitol. 290, 109369, 2021.

Otranto, D., Mendoza-Roldan, J.A., Beugnet, F., Baneth, G., Dantas-Torres, F., 2024. New paradigms in the prevention of Canine vector-borne diseases. Trends Parasitol. 40 (6), 500–510.

Otto, J.C., Wilson, K., 2001. Assessment of the usefulness of ribosomal 18S and mitochondrial COI sequences in prostigmata phylogeny. In: Walter, E., Proctor, H., Norton, R.A., Collof, M., Halliday, R.B. (Eds.), Acarology: Proceedings of the 10th International Congress. CSIRO Publishing, Clayton, pp. 100–110.

Panarese, R., Iatta, R., Mendoza-Roldan, J.A., Zatelli, A., Beugnet, F., Otranto, D., 2021. Efficacy of afoxolaner (NexGard®) in preventing the transmission of *Leishmania infantum* and *Dirofilaria immitis* to sheltered dogs in a highly endemic area. Parasites Vectors 14, 1–8.

Papini, R., Manetti, C., Mancianti, F., 2011. Coprological survey in pet reptiles in Italy. Vet. Rec. 169 (8), 207.

Pasmans, F., Blahak, S., Martel, A., Pantchev, N., 2008. Introducing reptiles into a captive collection: the role of the veterinarian. Vet. J. 175 (1), 53–68.

Pereira, N.C.L., Michalsky, É.M., Alonso, C., Pinheiro, L.C., Lara-Silva, F.O., Lima, N.A., Nobi, R.C.A., de Mello, H.E.S., Fortes-Dias, C.L., 2023. Survey of phlebotomine sand fly fauna in a public zoo in Brazil: species diversity, seasonality, and host variety. Vet. Parasitol. Reg. Stud. Reports 44, 100917.

R Core Team, 2025. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rinaldi, L., Mihalca, A.D., Cirillo, R., Maurelli, M.P., Montesano, M., Capasso, M., Cringoli, G., 2012. FLOTAC can detect parasitic and pseudoparasitic elements in reptiles. Exp. Parasitol. 130 (3), 282–284.

Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. J. Parasitol. 86, 228–232.

Sadlova, J., Bacikova, D., Becvar, T., Vojtkova, B., England, M., Shaw, J., Volf, P., 2022. *Porcisia* transmission by prediuresis of sand flies. Front. Cell. Infect. Microbiol. 12, 981071.

Šlapeta, J., Modrý, D., Johnson, R., 2017. Reptile parasitology in health and disease. Reptile Medicine and Surgery in Clinical Practice, first ed. Wiley, Hoboken.

Stöver, B.C., Müller, K.F., 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinf. 11, 7.

Telford, S.R., 2009. Hemoparasites of the Reptilia: Color Atlas and Text (No. 21482), first ed. CRC Press, Taylor & Francis.

Trifinopoulos, J., Nguyen, L.T., von Haeseler, A., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44 (1), 232–235.

Tuten, H.C., Bridges, W.C., Paul, K.S., Adler, P.H., 2012. Blood-feeding ecology of mosquitoes in zoos. Med. Vet. Entomol. 26 (4), 407–416.

Valdez, J.W., 2021. Using google trends to determine current, past, and future trends in the reptile pet trade. Animals (Basel) 11 (3), 676.

Wensley, S., Dawson, S., Stidworthy, M., Soutar, R., 2016. Welfare of exotic pets. Vet. Rec. 178 (21), 540.

Wójcik-Fatla, A., Szymanska, J., Wdowiak, L., Buczek, A., Dutkiewicz, J., 2009. Coincidence of three pathogens [*borrelia burgdorferi sensu lato, Anaplasma phagocytophilum* and *babesia microti] in ixodes ricinus* ticks in the Lublin macroregion. Ann. Agric. Environ. Med. 16, 151–158.

Wozniak, E.J., DeNardo, D.F., 2000. The biology, clinical significance and control of the common snake mite, *Ophionyssus natricis*, in captive reptiles. J. Herpetol. Med. Surg. 10 (3), 4–10.

Zhou, X., Hohman, A.E., Hsu, W.H., 2022. Current review of isoxazolines ectoparasiticides used in veterinary medicine. J. Vet. Pharmacol. Therapeut. 45, 1–15.