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Leishmania tarentolae and Leishmania infantum in geckos from Mallorca Island, Spain

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ABSTRACT

Leishmania tarentolae and *Leishmania infantum* are two sympatric parasites of significant ecological and epidemiological interest in the Mediterranean basin. This study investigated the PCR prevalence of *L. tarentolae* and *L. infantum* in two gecko species (*Tarentola mauritanica* and *Hemidactylus turcicus*) present on Mallorca Island, Spain, using duplex quantitative PCR. A total of 59 geckos were sampled across the island, including 53 *T. mauritanica* and six *H. turcicus*. Tissue and blood samples were screened by PCR for both parasites. The results revealed the prevalence of *Leishmania* infection in adult *T. mauritanica*, with 10/49 (20.41 %) testing PCR positive for *L. tarentolae* only and with 1/49 (2.04 %) for *L. infantum* only. Coinfection with both parasites was detected in 3/49 geckos (6.12 %). No positives were identified in *H. turcicus*, probably due to small sample size. Regarding PCR positivity by tissues, coleomic organs were more likely to be positive for *L. tarentolae* in adult *T. mauritanica* than blood, with a slighter PCR positivity in the liver, spleen and lung. This study provides further insight into the interaction between *Leishmania* and geckos in leishmaniosis-endemic areas such as Mallorca.

1. Introduction

In Europe, zoonotic visceral leishmaniosis is caused by *Leishmania infantum*, dogs being their primary reservoir. This disease has a wide distribution throughout the Mediterranean basin (Martín-Sánchez et al., 2020; Otranto and Dantas-Torres, 2013). *L. infantum* is the dominant pathogenic species of *Leishmania* present in western and central Europe. In recent decades, different studies have shown that the epidemiology of leishmaniosis in Europe is much more complex than previously thought, broadening the range of reservoir hosts species (Taddei et al., 2022) or reporting an increase in drug resistance (Leprohon et al., 2015; Martí-Carreras et al., 2022; Ponte-Sucre et al., 2017). *Leishmania infantum* infections have been detected in other species of vertebrates, such as lagomorphs (i.e., rabbits and hares), which play an important role as sylvatic reservoirs in endemic areas of Europe (Barbero-Moyano et al., 2025; Martín-Sánchez et al., 2021; Molina et al., 2012; Tsokana et al., 2016). More recently, the infection of different Mediterranean reptile species with *L. infantum* has also been demonstrated, although its epidemiological role remains to be elucidated in this case (Mendoza-Roldan et al., 2022).

Among Mediterranean reptiles, the most frequently reported *Leishmania* species is *Leishmania tarentolae*. This species, subgenus *Sauroleishmania*, is limited to the Old World and it is found in association with geckos in southern Europe and North Africa (Latrofa et al., 2021; Wallbanks et al., 1985), and possibly also other areas with endemic Old World gecko populations, such as the Middle East (Rato et al., 2010). *Leishmania tarentolae* is transmitted by reptile-biting sandflies of the genus *Sergentomya* and is considered sympatric with *L. infantum*. It is regarded as non-pathogenic to mammals, including dogs (Iatta et al., 2023; Mendoza-Roldan et al., 2024), yet infection and parasite

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persistence can occur (Louzada-Flores et al., 2023). Studies in Italy have detected infection rates of *L. tarentolae* up to 7 % in geckos and 16.5 % in lacertids (Latrofa et al., 2021; Mendoza-Roldan et al., 2022). In Spain, where *Sergentomya minuta* is widely distributed (Bravo-Barriga et al., 2022) and where canine leishmaniosis shows high prevalence rates, especially in the Balearic Islands (Alcover et al., 2016, 2023; Cabezón et al., 2010), it is under research which *Leishmania* species infect reptiles and which role they may play on leishmaniosis epidemiology and *Leishmania* transmission cycles (Reimann et al., 2022).

Only two species of saurids inhabit the island of Mallorca, both of which are geckos: *Tarentola mauritanica* and *Hemidactylus turcicus* (Mayol Serra, 2003). There are no native representatives of the Lacertidae family, except for a few representatives of *Podarcis pituytensis,* recently introduced from the neighboring island of Eivissa (Mayol Serra, 2003). The two gecko species are very abundant throughout the island, with reported densities of over 1200 individuals/km^2, due to the presence of a very suitable biotope and the absence of competitors (Mayol Serra, 2003). Both species are anthropophilic, often living in houses and gardens. It is thought that these geckos have been introduced to Mallorca from North Africa (*T. mauritanica*) and the eastern Mediterranean (*H. turcicus*) more than 2000 years ago (Mayol Serra, 2003).

Considering the biology of these two gecko species and the high prevalence of canine leishmaniosis in Mallorca (Alcover et al., 2016; Cabezón et al., 2010), the study hypothesis was that the gecko populations in Mallorca could be parasitized by both *L. tarentolae* and *L. infantum*. Therefore, the aim of this study was to assess the prevalence of *L. tarentolae* and *L. infantum* in gecko species at different locations of Mallorca Island using nucleic acid detection by quantitative polymerase chain reaction (qPCR).

2. Material and methods

2.1. Field trapping and dissection

Tarentola mauritanica (common wall gecko, *dragó comú*) and *Hemidactylus turcicus* (Mediterranean house gecko, *dragonet* or *dragó rosat*), as illustrated in Fig. 1a, were captured over five days in May 2024 in the northern and central regions of Mallorca Island (Balearic Islands, Spain), as seen in Fig. 1b. Geckos were captured by hand and identified to species level using reference keys (Speybroek et al., 2016). Specimens were differentiated between adults and juvenile based on snout-to-vent size. For juveniles, snout-to-vent size is < 4 cm for *T. mauritanica* and < 3 cm for *H. turcicus*. They were then humanely euthanized in situ using intramuscular anesthesia consisting of ketamine (20 mg/kg) and medetomidine (0.2 mg/kg), followed by pitching, in accordance with the guidelines for the euthanasia of animals published by the American Veterinary Medical Association (Leary et al., 2020). Whole blood was immediately obtained via cardiac puncture and stored in a portable refrigerator before being frozen at −20 °C after each capture session. The geckos were dissected, and the spleen, heart, lungs, liver, and kidneys were individually collected and frozen at −20 °C for DNA extraction. For juvenile geckos with smaller body sizes, coelomic organs were not separated but frozen whole. Complete sample informa-

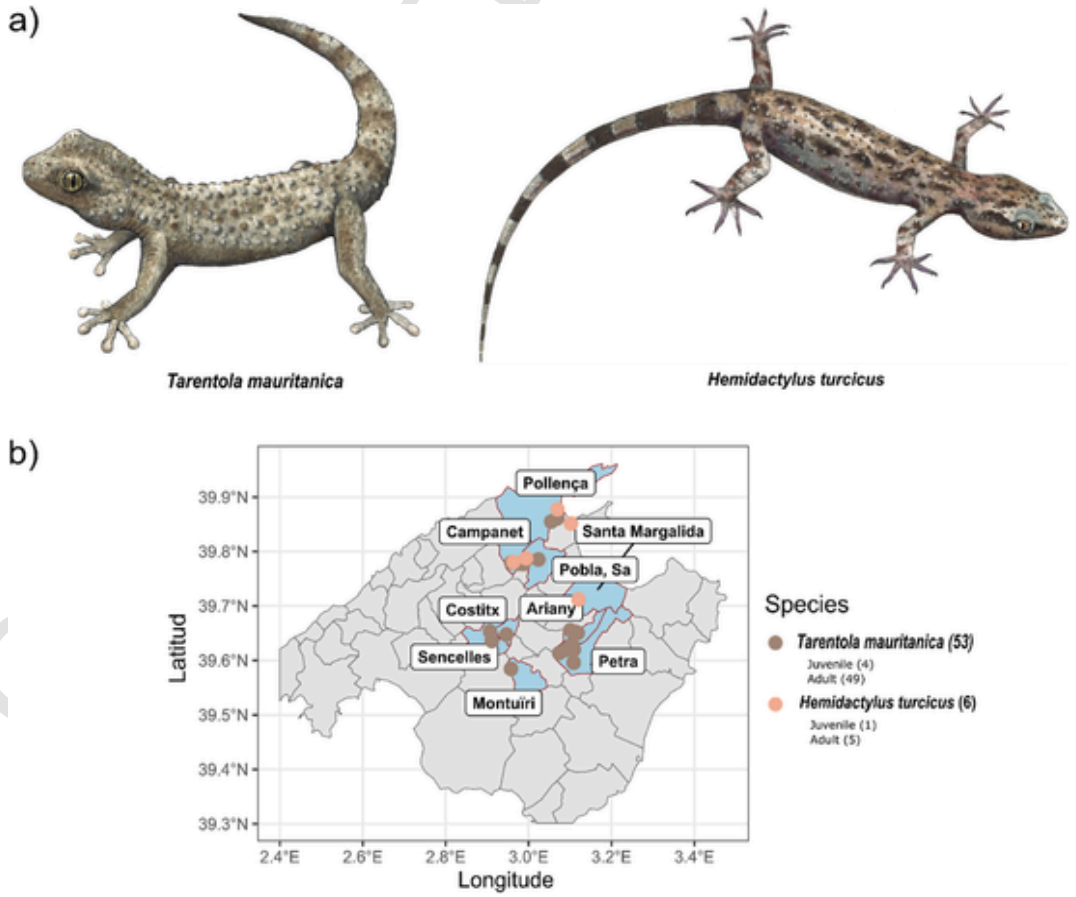

Fig. 1. Sampling distribution of *T. mauritanica* and *H. turcicus* in Mallorca Island in May 2024. Panel a) Colored illustration of adult *T. mauritanica* and *H. turcicus* by Bruna Roqué. Panel b) Sampling distribution of *T. mauritanica* (grey) and *H. turcicus* (pink) by place of sampling (municipalities of Mallorca) and life stage (juvenile or adult).

tion is provided in Supplementary Table 1. The capture, euthanasia and sampling in the present study were conducted following local law compliance on ethical and legal requirements for sampling wild animals (see **Ethical statements**). Isolation of *Leishmania* was attempted by using dissected organs stored in 9 % saline with penicillin. Organs were subsequently homogenized and inoculated in 5 ml Schneider medium supplemented with 20 % fetal bovine serum and 1–2 % sterile human urine at 25–26 °C. Cultures were inviable due to pervasive yeast contamination, likely coming during capture and dissection.

2.2. DNA extraction and qPCR screening

Tissue and blood samples were extracted on 96-well plates with an extraction robot Chemagic 360 (PerkinElmer, Waltham, Massachusetts) using two different extraction protocols: Chemagic DNA Tissue10 360 prefilling H96 VD150506 for solid tissue samples and Chemagic DNA Blood 250 prefilling 360 VD180628 for blood samples. Elution was performed in 80 µl. Samples were diluted 1/10 before proceeding to qPCR screening. The TaqMan Fast Advanced Master Mix (Themo Fisher Scientific, Waltham, Massachusetts, USA) was used for screening the presence of *L. tarentolae* and *L. infantum* with a discriminative duplex qPCR system previously described targeting the ITS1 region: Fw 5′-GCAGTAAAAAAAAGGCCG-3′, *L. tarentolae* probe 6-FAM-5′-CACGCCGCGTATACAAAAACAC-3′-f1-quencher-MGB and *L. infantum* probe VIC-5′-TAACGCACCGGCCTATACAAAAGCA-3′-f1-quencher-MGB (Latrofa et al., 2021) with a different reverse primer Rv L5.8S-5′-TGATACCACTTATCGCACTT-3’ (Schönian et al., 2003). Testing was conducted in duplicate with extraction controls for both blood and tissue samples. Positive controls for *L. tarentolae* and *L. infantum* were used. Non-template controls (NTC) were added to each qPCR plate. The analysis threshold for qPCR was set at 0.052 for *L. tarentolae* and 0.039 for *L. infantum*, and a sample was considered PCR positive if the Ct value was ≤38.5 for both replicates after revision of the amplification curves, similar thresholds to previous studies (Ct 38.0 for *L. infantum* and Ct 38.6 for *L. tarentolae*, Mendoza-Roldan et al., 2021). Kinetoplast qPCR: LEISH1 5′- AACTTTTCTGGTCCTCCGGGTAG -3′, probe FAM-5′-AAAAATGGGT GCAGAAAT- 3′ f1-quencher-MGB and LEISH2 5′-ACCCCCAGTTTCCCGCC-3′ was used for confirmation of *L. infantum* (Francino et al., 2006). Coinfection was considered when a gecko had the presence of both parasites in the same tissue sample or in different tissue samples from the same gecko. No significant amplification (Ct ≤ 38.5) was identified in extraction or NTC controls.

2.3. Data analysis

Primers and probes were evaluated for off-hit targets using blastn (Camacho et al., 2009) against the NCBI Nucleotide database (June 2024) using the recommended parameters for primer and probe design: expected threshold of 1000, word size of 7 and unmasking low complexity regions (Clore, 2011).

Eds qPCR files were processed with app.thermofisher (https://apps.thermofisher.com/apps/spa/), where amplification curves were manually revised. Data analysis was conducted with R v4.4.1 (R Core Team, 2019). Sample distribution was located with packages mapSpain v0.9.2, tidyverse v2.0.0 (Wickham et al., 2019). Generalized linear models (binomial logit) were employed to identify which organs were statistically more prone to be positive for *L. tarentolae* and *L. infantum* with packages for logistif v1.26.0 (Heinze et al., 2003) and emmeans v1.10.5 (Lenth, 2017). Analysis was only conducted for adult *T. mauritanica* (n = 49) as juvenile *T. mauritanica* or *H. turcicus* did not have enough sample size.

3. Results

A total of 59 geckos were collected, comprising five juveniles and 54 adults. Of these, 53 individuals were identified as *T. mauritanica* (four juveniles and 49 adults) and six as *H. turcicus* (one juvenile and five adults), as shown in Fig. 1b. Duplex qPCR was performed for the simultaneous detection of *L. tarentolae* and *L. infantum*. Counting both species, 14/59 (23.73 %) geckos were PCR positive for *Leishmania* spp. with 13/59 (22.03 %) for *L. tarentolae* and 4/59 (6.77 %) for *L. infantum*. Three of the four positives for *L. infantum* were also PCR positive for *L. tarentolae*, thus counting as coinfection cases (Fig. 2). All PCR-positive geckos were *T. mauritanica*, with no *Leishmania* spp. DNA detected in *H. turcicus*.

For adult *T. mauritanica* (n = 49), the prevalence of *L. tarentolae* was 13/49 (26.53 %) and 4/49 (8.16 %) for *L. infantum*, with a coinfection rate of 3/49 (6.12 %). No juvenile specimens (n = 4) were positive for *Leishmania*.

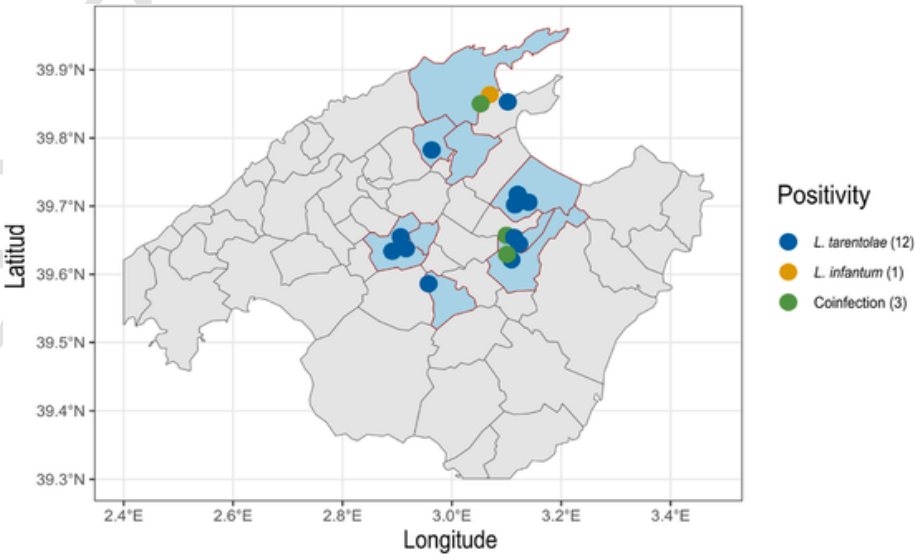

Fig. 2. Distribution of *L. tarentolae* and *L. infantum* PCR positive geckos in Mallorca Island in May 2024. Gecko positivity by RT-qPCR against *L. tarentolae* (blue), *L. infantum* (yellow) and coinfection with both parasites (green).

L. tarentolae tested PCR positive in 13 adults in liver (9), spleen (8), lung (9), kidney (7), heart (7) and blood (2) as summarized in Table 1. Discounting coinfections, *L. tarentolae* was detected either in all the tissues tested except blood, either as a single positive organ (specimens Dra24001, Dra24015, Dra24017 and Dra24038) or in multiple organs. Regarding *L. infantum*, four adults tested PCR positive in blood (2), spleen (2), liver (1), lung (1) and heart (1), three of them coinfected with *L. tarentolae*. The three coinfections were detected in liver and blood (specimen Dra24020), in spleen (specimen Dra24033), and in heart, lung and spleen (specimen 24029). In adult *T. mauritanica* (n = 49), *L. tarentolae* (including coinfections) is more likely to be identified in coelomic organs (heart, liver, spleen, lung and kidney), when compared to blood (glm, p-value = 0.0266, AIC 81.1), with a non-significant trend towards a slightly higher positivity in liver, spleen and lung. In fact, positive livers and spleens account for all *L. tarentolae* positive specimens (but Dra24015, were only the heart was positive).

For *L. tarentolae*, the average Ct of detection was 34.02 with a minimum Ct of 28.69 and maximum Ct of 38.42, meanwhile for *L. infantum*, the average Ct value of detection was 36.44 with a minimum Ct of 34.55 and a maximum Ct of 38.33. Additionally, positive samples for *L. infantum* Li TaqMan-MGB probe were also tested with a different qPCR assay targeting the kinetoplast for confirmation (Francino et al., 2006). Interestingly, the average Ct value of samples *L. tarentolae* no coinfections (35.49) and *L. tarentolae* coinfection-only (31.61) were statistically different (t-student, p-value = 2,08E-07).

Primer in silico analysis showed pan amplification of ITS1 of *Leishmania* and some *Leptomonas* and *Trypanosoma*, providing a high sensitivity to the assay. Contrarily, these analyses showed that TaqMan-MGB probes were highly specific against their targets. The TaqMan-MGB Probe Lt had 100 % identity and 100 % coverage against *L. tarentolae* (only taxon that matched). The TaqMan-MGB Probe Li had a 100 % identity and 100 % coverage against *L. donovani-infantum* complex. The complete specificity of TaqMan-MGB probes ensures the appropriate signal for each species.

4. Discussion

This study demonstrated the presence of *L. tarentolae* and *L. infantum* in a wild gecko population on Mallorca Island. The Balearic Islands, including Mallorca, are endemic for *L. infantum*, the primary causative agent of leishmaniosis in humans and companion animals (Alcover et al., 2016, 2023; Cabezón et al., 2010). The prevalence of *L. infantum* in geckos (6.77 %) was lower than in companion animals in the same area (Cabezón et al., 2010; Carbonara et al., 2024; Solano-Gallego et al., 2001) but was of similar magnitude to previous studies in southern Italy (Mendoza-Roldan et al., 2022; 2022, 2021), both endemic areas of canine leishmaniosis. It is important to note that serological tests for *L. infantum* may overestimate prevalence in mammals, as they may not discriminate between *L. infantum* and *L. tarentolae*, which is believed to be non-pathogenic but can infect and persist in mammals (Iatta et al., 2023; Louzada-Flores et al., 2023; Mendoza-Roldan et al., 2024).

Regarding *L. tarentolae*, this study found a notable prevalence (26.53 %) in *T. mauritanica*, which is higher than the prevalence reported in southern Italy (Mendoza-Roldan et al., 2022b; 2021). Such differences may be accounted for by (i) a high density of geckos on the island, which may facilitate parasite transmission; (ii) a high density and distribution of the sandfly vector, which contributes to the spread of *Leishmania*; and (iii) a high Ct ≤ 38.5 cutoff value used for considering a sample positive by PCR.

Furthermore, the approach in this study was able to detect coinfection with *L. tarentolae* and *L. infantum* in adult *T. mauritanica*, showing that such scenarios occur in wild populations. Interestingly, the average Ct value of positive samples for *L. tarentolae* with coinfections with *L. infantum* was lower than for only *L. tarentolae* was present, indicating a higher parasite load. This suggests new research endeavours on host-pathogen interaction or pathogen competition. Additionally, the Ct values were generally low, possibly due to the timing of the study (May), being early in *Sergentomyia minuta*'s season in Mallorca Island, making it is possible that not many geckos had had contact with the vector, thus having a low positivity signal (Ct) and overall abundance in the region.

Based on our findings, we can provide specific recommendations for future studies. All positive cases of *L. tarentolae*, including coinfections with *L. infantum*, could be recovered by PCR of the liver, spleen, and heart. Thus, for future studies, it is recommended to sample those organs for screening *L. tarentolae* and *L. infantum*. Conversely, it would not be advisable to collect blood for PCR testing, as it was not often positive.

To the authors' knowledge, this is the first study in Spain to examine the prevalence of *L. tarentolae* in reptile species. The presence of coinfections of *L. tarentolae* and *L. infantum* suggests that individual geckos can harbor multiple *Leishmania* species simultaneously. These findings highlight the potential role of reptiles in the local ecology of *Leishmania* species and underscore the need to investigate their ecological and epidemiological significance.

Table 1
Prevalence of *L. tarentolae* and *L. infantum* by qPCR in all coelomic organs and blood from *T. mauritanica* adult specimens. Detection is expressed as Ct value for positives, and Neg. for negative samples for coinfections. For each PCR positive organ and gecko, the average Ct value is provided for *L. tarentolae* and *L. infantum*. For coinfections the average Ct is first given for *L. infantum* and after double bar to *L. tarentolae*.

L. infantum						
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**
Dra24032						38.18
L. tarentolae						
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**
Dra24001			35.87			
Dra24011	33.06	34.05	34.02	34.39	37.51	
Dra24015	38.42					
Dra24017			37.84			
Dra24018		34.54		32.15	35.17	
Dra24022		34.13	36.49	32.53		
Dra24030	36.84	33.83		33.96	35.20	
Dra24036	37.49	37.74		36.02		
Dra24037		35.49	36.46	34.63	37.41	
Dra24038			37.68			
Coinfection						
GeckoID	**Heart**	**Liver**	**Spleen**	**Lung**	**Kidney**	**Blood**
Dra24020	Neg//30.92	36.67//30.09		Neg//30.92	Neg//34.55	38.09//33.33
Dra24029	35.38//29.65	Neg//29.97	33.93//28.69	34.55//28.17	Neg//30.40	Neg//34.00
Dra24033	Neg//32.64	Neg//31.42	38.30//35.94	Neg//31.52	Neg//33.61	

5. Conclusion

This study aimed to examine the prevalence of *L. tarentolae* and *L. infantum* in the wild gecko population, as possible reservoir host, on Mallorca Island. The findings contribute to identifying the presence of coinfecting *Leishmania* parasites in wildlife that cohabit with dogs and humans in isolated endemic regions. Such research opens the question whether *Leishmania* infection in geckos may contribute to *Leishmania* infection in humans and other mammals or remains confined to reptilian hosts.

CRediT authorship contribution statement

Joan Martí-Carreras: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Johan Espunyes:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Laura Carrera-Faja:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Carlotta Pasetto:** Writing – review & editing, Methodology, Investigation. **Maria Magdalena Alcover Amengual:** Writing – review & editing, Methodology, Investigation. **Sarah Chavez-Fisa:** Writing – review & editing, Methodology, Investigation. **Marina Carrasco:** Writing – review & editing, Investigation. **Xavier Roura:** Writing – review & editing, Supervision, Conceptualization. **Olga Francino:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Lluís Ferrer:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Ethical statement and consent to participate

The capture, euthanasia, and sampling of wild geckos in this study were authorized by the government of the Balearic Island (permit CEP 21/2023).

Consent for publication

All authors contributed and reviewed the final version of the manuscript, accept its submission as it is and its intent to be published.

Availability of data and material

Not applicable.

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Competing interests

Nano1Health SL is a for-profit organization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2025.101138.

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