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Paper

First molecular detection of Leishmania major in the Greater Egyptian Jerboa (Jaculus orientalis) in Algeria (Djelfa)

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Abstract

Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is a major public health concern in Algeria, a recognized endemic hotspot. The parasite is maintained in a transmission cycle involving *Phlebotomus papatasi* sand flies as vectors and rodents as reservoirs, primarily *Psammomys obesus* and *Meriones shawi*. Investigating additional rodent species is essential to better understand transmission dynamics in emerging foci. This study assessed the potential role of wild rodent species as reservoirs of *Leishmania* parasites in the emerging focus of leishmaniasis in Djelfa, located in the Algerian central steppe. Rodents were trapped during the active season (February–November) using wire traps and identified morphologically. DNA was extracted from spleen and liver tissues. Samples were screened by PCR targeting the 18S rRNA gene for *Leishmania* detection, and positive samples were confirmed by ITS1 sequencing. Rodent species identification was molecularly validated by sequencing the cytochrome b (Cytb) gene. A total of 22 rodents were captured: 19 *Jaculus orientalis*, 2 *Psammomys obesus*, and 1 *Meriones shawi*. One asymptomatic *J. orientalis* tested positive for *L. major*. Phylogenetic analysis clustered the isolate within the *L. major* clade. Haplotype analysis revealed a unique haplotype differing by two mutations from the predominant North African haplotype. This study provides the first molecular evidence of natural infection of *J. orientalis* with *L. major* in Algeria. Although based on a single infected specimen, this species should be incorporated into eco-epidemiological surveillance, and its reservoir competence warrants further investigation through expanded sampling and xenodiagnostic studies.

Keywords

Leishmania major, Jaculus orientalis, Rodent reservoir, Algeria

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Introduction

Leishmaniasis is a neglected vector-borne disease caused by protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), transmitted through the bite of phlebotomine sand flies (Diptera: Psychodidae). The disease remains endemic in 98 countries and poses a significant health threat to more than 1 billion people worldwide (WHO, 2023). Among its clinical forms, cutaneous leishmaniasis (CL) is the most common form, is often caused by *Leishmania major,* and it is a zoonosis circulating among various rodents (Sadlova et al., 2023).

Rodents are among the most ecologically adaptable mammals, occupying a wide range of natural and human-modified habitats. Their ecological plasticity, high abundance, and close association with human settlements make them important reservoirs for numerous zoonotic pathogens, including *Leishmania* (Rodriguez-Morales et al., 2025).

In the Maghreb region, *L. major* is transmitted by *Phlebotomus papatasi* (Sergent et al., 1921) and primarily maintained in populations of two proven rodent reservoirs: the fat sand rat (*Psammomys obesus*) (Ashford et al., 1977), and *Meriones* spp., namely Shaw's jird (*M. shawi*) and Libyan jird (*M. libycus*) (Ashford et al., 1977; Rioux et al., 1982; Rioux et al., 1986).In Algeria, *Ph . papatasi* was confirmed as the main vector, sustaining a stable zoonotic cycle with *P. obesus* and *M. shawi* (Belazzoug, 1983; Belazzoug, 1986; Izri et al., 1992).

Although these species are recognised as the principal reservoirs of *L. major*, Algeria is home to at least 27 other rodent species (Ahmim, 2019; Meunier et al., 2020), some of which may play a significant role in pathogen maintenance and transmission. Indeed, *L. major* has already been detected in *Psammomys vexillaris* (Ben Othman et al., 2018), *Jaculus hirtipes ,* and *Jaculus jaculus* (Ghawar et al., 2022), and host competence of *Gerbillus amoenus* and *Acomys cahirinus seurati* has been demonstrated experimentally (Benallal et al., 2023; Karlin et al., 2026).

Beyond rodents, other mammalian hosts have been found to be naturally infected with *L. major* in Tunisia, including hedgehogs (*Atelerix algirus* and *Paraechinus aethiopicus*) and the least weasel (*Mustela nivalis*) (Derghal et al., 2022; Ghawar et al., 2011a; Tomás-Pérez et al., 2014).

In Algeria, CL is the leading parasitic disease, representing 35% of all notifiable infections (Messahel et al., 2021). The country ranks as the second-largest hotspot in the world after Afghanistan (Ruiz-Postigo et al., 2021; WHO, 2023), with more than 20,000 new cases annually and an incidence rate of 28.19 per 100,000 inhabitants. More than 252,000 cases have been recorded in the last four decades (Benikhlef et al., 2021). Despite this substantial disease burden, studies investigating animal reservoir hosts remain limited for this species compared with those for other species such as *L. infantum* (Djellouli et al., 2025; Ferdes et al., 2025; Medkour et al., 2019). This gap is particularly evident in the central steppe region, especially in Djelfa province, which represents a major endemic focus (Ouachek et al., 2026). In 2024, Djelfa recorded 3,252 cases, corresponding to an incidence rate of 168.19 per 100,000 inhabitants and a 12.9-fold increase (+1,193%) compared with 2023 (INSP, 2024).

In this context, this study aimed to investigate rodent species diversity in the newly emerging CL focus of Djelfa and to identify rodent species potentially involved in the transmission cycle of *Leishmania* parasites. This exploratory approach provides preliminary insights into host-parasite associations. However, the detection of *Leishmania* infection by molecular methods alone is insufficient to demonstrate reservoir competence. Nevertheless, these findings contribute to a better understanding of transmission cycles in the region and help identify potential host species for further investigation, ultimately supporting the design of more effective intervention strategies.

Materials and methods

Study area

The province of Djelfa is located about 300 km south of the capital, Algiers (2.01°-5.08° E and 32.85°-35.82° N). It lies at the heart of the steppe region, forming a transitional zone between the High Plateaus to the south of the Tell Atlas and the northern fringe of the Sahara. Djelfa covers 32,257 km^2, representing 1.35% of the national territory, the province is characterised by cold winters and hot dry summers. The climate is predominantly semi-arid in the central and northern sectors, average annual precipitation ranges between 200 and 500 mm, while the southern part is arid receiving less than 200 mm annually. Elevation ranges from 150 m to 1,613 m above sea level (Ouachek et al., 2026) (Figure 1).

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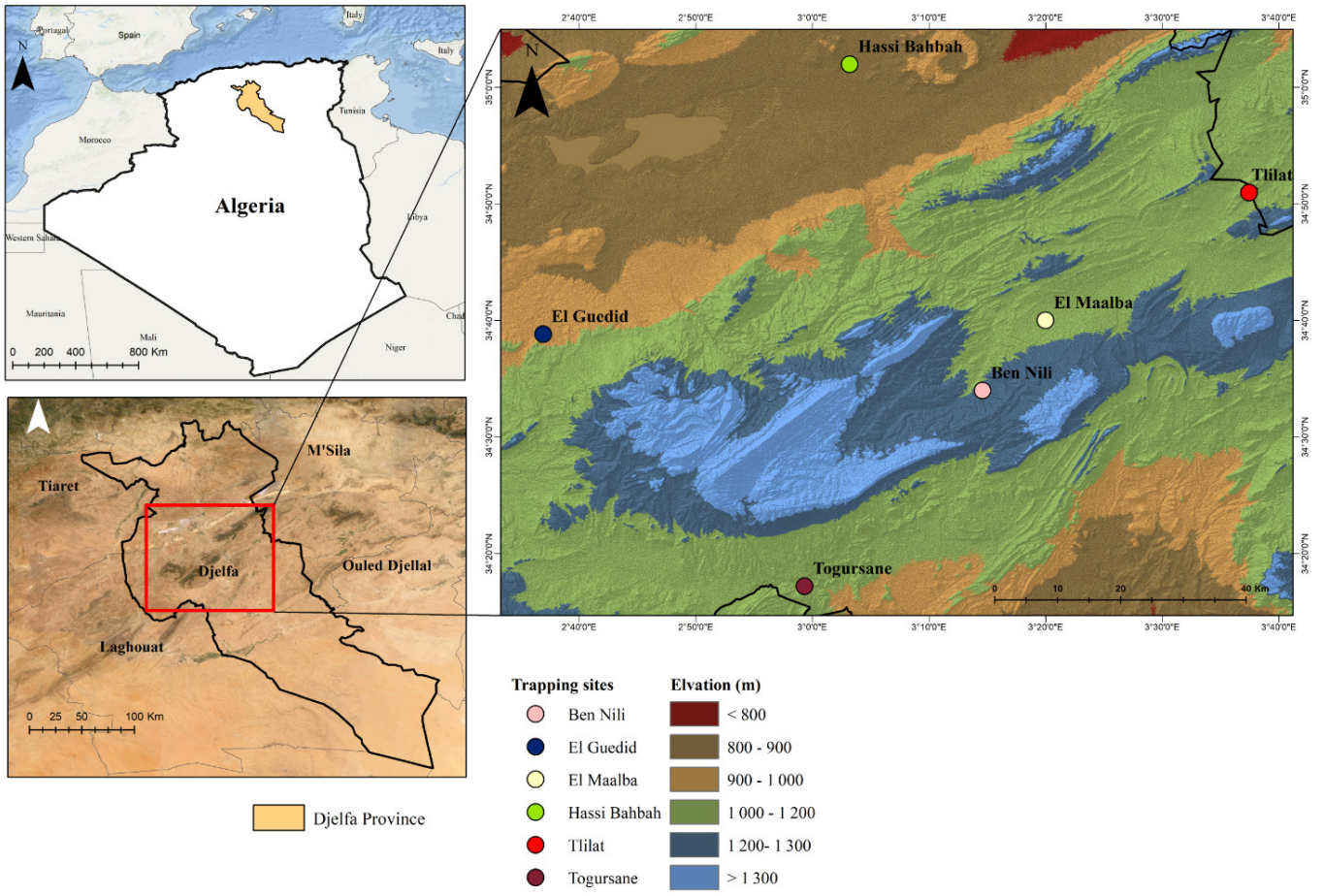

Figure 1. Study area. The map was generated using ArcMap 10.8. Administrative boundaries were obtained from the GADM database (https://gadm.org/), basemap layers were sourced from ArcGIS Online, and DEM data (30 m resolution) were retrieved from the USGS EarthExplorer platform (https://earthexplorer.usgs.gov).

Rodent trapping and manipulation

From February to November 2024, rodent trapping was conducted in areas reporting recent CL cases and showing clear signs of rodent activity, including active burrows, faeces at burrow entrances, and the presence of Chenopodiaceae vegetation. Sampling sites were located along dry riverbeds near animal shelters and human dwellings (Figure 1, Figure 2F). At each site, five baited wire traps containing bread, tomatoes, wheat, and barley were placed at sunset with approximately 10 m spacing between traps. Trap placement was guided by visible signs of rodent activity and habitat suitability to maximise the likelihood of capture. Trapping was conducted over 27 nights, representing a total effort of 135 trap-nights. Traps were checked the next morning, and captured animals were transferred to ventilated cages and transported to the Laboratory of Exploration and Valorization of Steppic Ecosystems (EVES), at the University of Djelfa.

Rodents were euthanised by cervical dislocation under anaesthesia with a mixture of ketamine/xylazine (66 mg/kg ketamine and 26 mg/kg xylazine per kilogram of body weight, respectively) injected intraperitoneally (Cold Spring Harbor Protocols, 2006). Death was confirmed in each animal by the absence of respiratory movement and the absence of a withdrawal reflex and then examined for the presence of *Leishmania* lesions on the muzzle, ears, paws, and tail (Figure 2D and E). Morphometric measurements, including tail length, body length, hind foot length, ear height and body weight (Table SM1) were taken for species identification according to Granjon and Duplantier (2009) and Ahmim (2019). Spleen and liver samples were collected aseptically from all rodents, placed individually in sterile Eppendorf microtubes, and stored at -20 °C until molecular analysis. Ear tissue samples were collected only from animals presenting cutaneous lesions. All tissue samples were processed and analysed individually.

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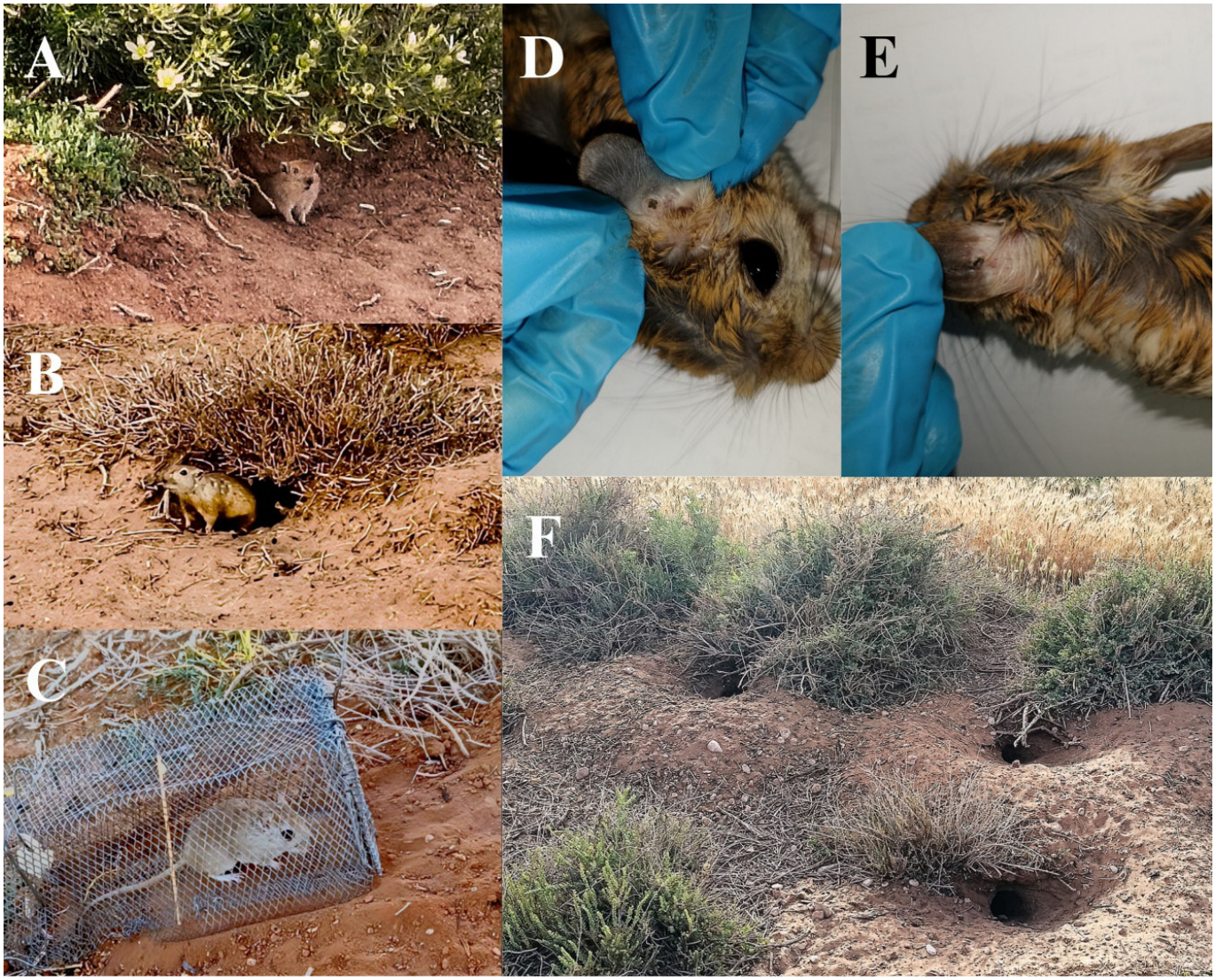

Figure 2. Rodent species and field evidence of rodent activity: (A, B) *Psammomys obesus*; (C) *Meriones shawi*; (D, E) examination of lesions on *Jaculus orientalis*; (F) active burrows.

DNA extraction, species identification and *Leishmania* screening

DNA extractions were performed from liver, spleen and ears using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. For rodent species confirmation, the mitochondrial cytochrome b (Cytb) gene was amplified using primers cyto 1 (5'-CCATCAAACATCTCAGCATGATGAAA-3') and cyto 2 (5'-CCCCTCAGAATGATATTTGTCCTC-3'). PCR reactions were carried out in a final volume of 25 µL containing 12.5 µL of Emerald Green Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan), 1 µL of each primer (10 µM), 1.5 µL of template DNA, and 9 µL of nuclease-free water. The thermocycling conditions consisted of 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min (Abbasi et al., 2009). PCR products were separated on a 2% agarose gel, and visualised under UV light.

Leishmania spp. were detected using a nested PCR assay targeting the 18S rRNA gene (detection limit: 10^{-2} parasites per sample). The first-round PCR amplified a 332-bp fragment using the outer primers 18SN1F (5′-GGATAACAAAGGAGCAGCCTCTA-3′) and 18SN1R (5′-CTCCACACTTTGGTTCTTGATTGA-3′). Reactions were performed in a final volume of 20 µL containing 3 µL of genomic DNA, 0.5 µL of each primer (10 µM), 10 µL of 2× Emerald Green Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan), and 6 µL of nuclease-free water. Thermal cycling consisted of an initial denaturation at 94 °C for 3 min and 30 s, followed by 35 cycles of at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 25 s, with a final extension at 72 °C for 7 min and a final hold at 12 °C.

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The second-round PCR amplified a 226-bp fragment using the inner primers 18SN2F (5′-AGATTATGGAGCTGTGCGACAA-3′) and 18SN2R (5′-TAGTTCGTCTTGGTGCGGTC-3′). Reactions were performed in a final volume of 20 µL containing 1 µL of the first-round PCR product, 0.5 µL of each primer (10 µM), 10 µL of 2× Emerald Green Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan), and 8 µL of nuclease-free water. Cycling conditions were identical to those used for the first-round PCR (Sadlova et al., 2022).

All samples that tested positive by nested PCR targeting the 18S rDNA gene were subsequently confirmed by amplification and sequencing of the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA, following the protocol described by Schönian et al. (2003). Amplification was performed using the previously published primers LITSR (5′-CTGGATCATTTTCCGATG-3′) as the forward primer and L5.8S (5′-TGATACCACTTATCGCACTT-3′) as the reverse primer. PCR reactions were carried out in a final volume of 25 µL containing 12.5 µL of Emerald Green Master Mix (Takara Bio Inc., Kusatsu, Shiga, Japan), 1 µL of each primer (10 µM), 1.5 µL of template DNA, and 9 µL of nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 34 cycles of 95 °C for 20 s, 53 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 6 min. Amplified products were resolved on a 2% agarose gel and visualised under UV illumination.

For all *Leishmania* PCR assays, DNA extracted from cultured *Leishmania major* was included as a positive control, while nuclease-free water served as the no-template control. To minimise the risk of contamination, PCR master mixes were prepared in a laminar-flow cabinet using dedicated pipettes and aerosol-resistant filter tips.

All positive Cytb and ITS1 amplicons were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany) and sequenced bidirectionally using the same amplification primers (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA, USA). Forward and reverse chromatograms were visually inspected, low-quality bases at both sequence ends were trimmed, and ambiguous base calls were manually checked. Forward and reverse reads were assembled into consensus sequences using BioEdit software v.7.2.5 (Hall, 1999). Consensus sequences were compared with those available in the GenBank database using BLAST. Species identification was considered reliable when sequences showed ≥98% identity, 100% query coverage, and an E-value of 0. Newly generated sequences were deposited in GenBank.

The evolutionary relationships among the sequences were inferred using MEGA 12 software (Kumar et al., 2024). For Cytb sequences, a maximum-likelihood (ML) tree was constructed under the TN93+I substitution model, with *Oryctolagus cuniculus* as the outgroup. For *Leishmania* ITS1 sequences, a neighbor-joining (NJ) tree was generated using *Leishmania tropica* as the outgroup. The robustness of all tree topologies was assessed with 1,000 bootstrap replicates.

Haplotype network analysis

Using the GenBank ITS1 dataset, a haplotype network for *Leishmania* sequences was constructed with DnaSP version 6 (Rozas et al., 2017) to calculate haplotype diversity indices based on a reduced alignment of 171 bp after the removal of gap-containing positions. A median-joining network was then generated and visualised with PopART (Leigh and Bryant, 2015).

Results

Captured rodents

A total of 22 rodents were captured and morphologically identified as *Jaculus orientalis* (Figure SM2), *Psammomys obesus* (Figure SM3), and *Meriones shawi* (Figure SM4) (Table I). Only one *J. orientalis* captured at El Maalba site exhibited a cutaneous lesion on its ear (Figure 2D and E).

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Province	Site	Genus	Species	Male	Female	Total	%
Djelfa	Ben Nili	*Jaculus*	*orientalis*	0	1	1	4.55 %
	El Guedid			0	1	1	4.55 %
	El Maalba			4	5	9	40.9 %
	Tlilat			3	4	7	31.81 %
	Tougursane			1	0	1	4.55 %
	Hassi Bahbah	*Psammomys*	*obesus*	1	1	2	9.09 %
		Meriones	*shawi*	0	1	1	4.55 %
			Total	9	13	22	100 %

Table I. Distribution of captured rodent species

Molecular identification of rodents

Mitochondrial Cytb gene characterisation confirmed the morphological identification of 21 of the 22 identified rodents. Only one specimen, morphologically identified as *P. obesus*, yielded a poor-quality sequence, impeding its identity confirmation by sequencing. Phylogenetic analysis further supported the identification of *J. orientalis*, *P. obesus*,and *M. shawi*, which clustered with their respective reference sequences from Algeria, Tunisia, Libya, and Morocco, highlighting the strong genetic similarity of these species across North Africa. The bootstrap values supported the robustness of these phylogenetic groupings (Figure 3).

The 21 sequences generated in this study were deposited in GenBank under the following accession numbers: 19 sequences of *J. orientalis* (PZ629108–PZ629126), one sequence of *P. obesus* (PZ629107), and one sequence of *M. shawi* (PZ629106).

Leishmania major analyses

Among the 22 analysed rodents, only one asymptomatic *J. orientalis*, captured in Tlilat, tested positive for *L. major* in its spleen by PCR targeting the 18S rRNA gene and ITS1 region. The phylogenetic tree showed that this sequence belonged to the *L. major* clade and clustered closely with strains from Tunisia (isolated from *Homo sapiens* and *J. jaculus*), while remaining distinct from the Iranian cluster (comprising a strain isolated from *M. libycus*) (Figure 4B).

The haplotype network (H = 4; *Hd*= 0.365) revealed low diversity among the analysed sequences and the positive sample corresponded to a unique haplotype (Hap 4) that differed from Hap 1 and Hap 2 by two and three mutations respectively (Figure 4A).

These results suggest that the *L. major* sequences detected in *J. orientalis* in Djelfa represents a genetically distinct haplotype. The sequence was deposited in GenBank under accession number PV973320.

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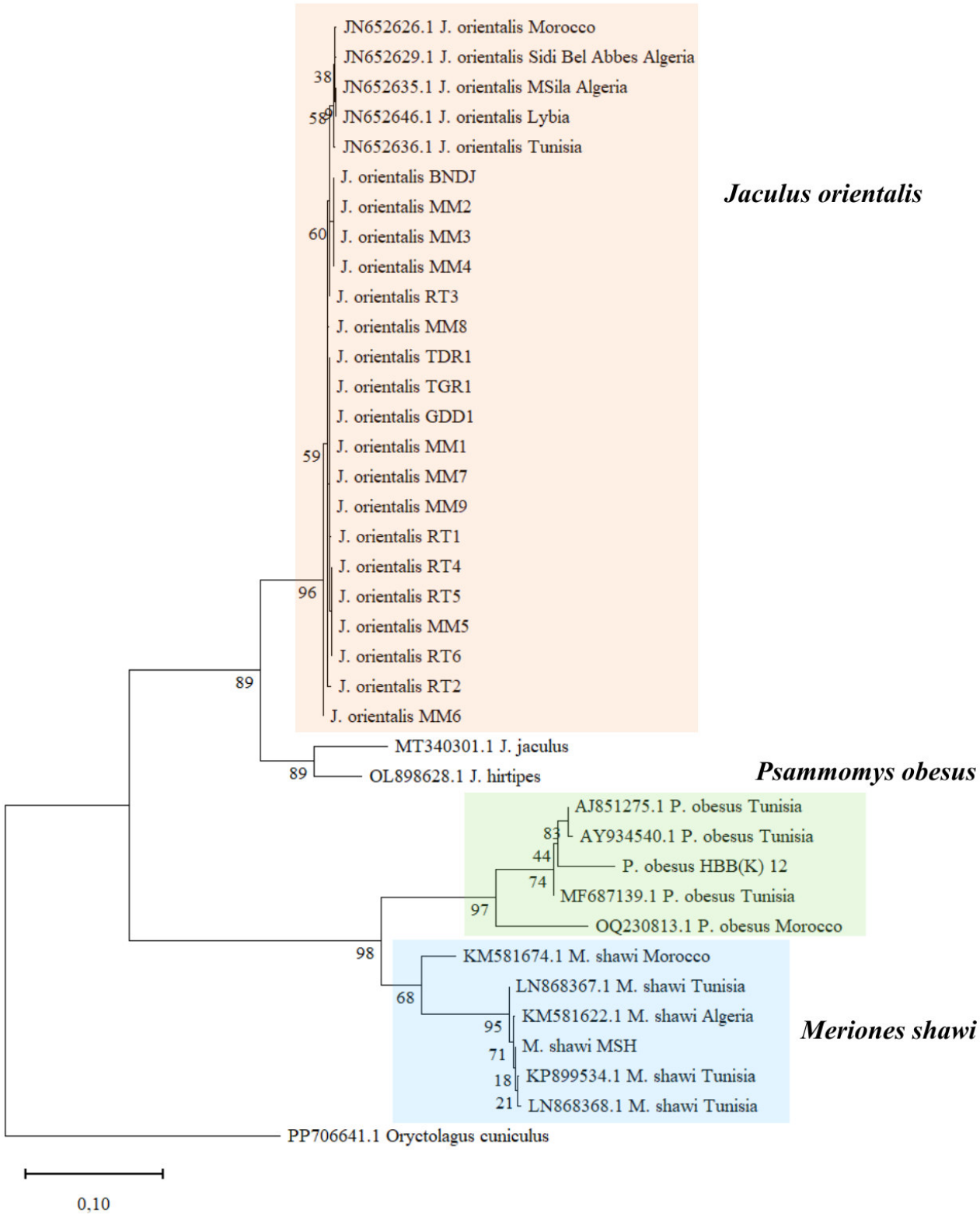

Figure 3. Maximum-likelihood (ML) phylogenetic tree based on the cytochrome b (Cytb) gene, showing the relationships among rodent sequences obtained in the present study together with homologous sequences retrieved from GenBank.

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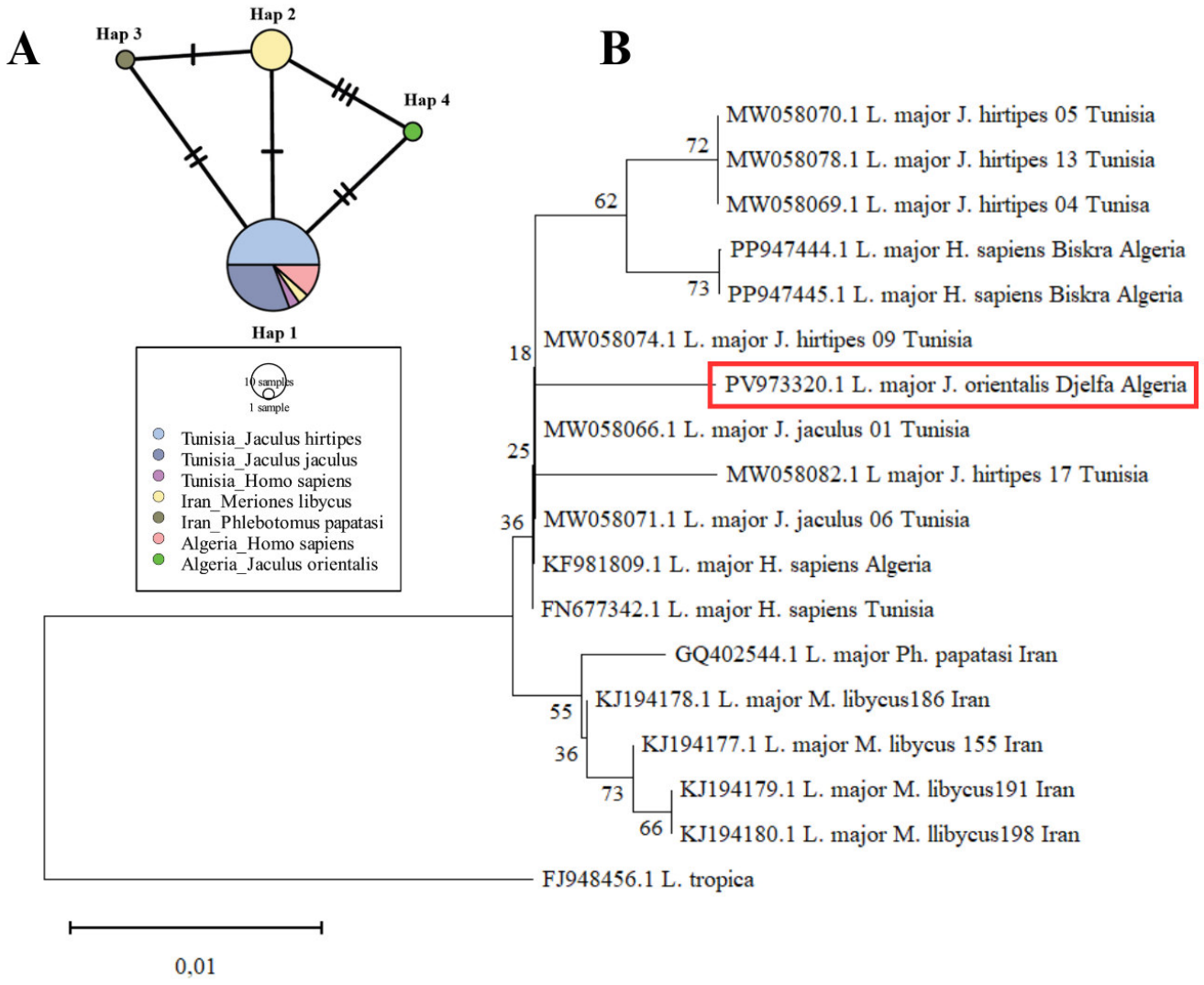

Figure 4. (A) Median-joining haplotype network based on the ITS1 region of *Leishmania* detected in *Jaculus orientalis* in the present study and homologous sequences retrieved from GenBank. (B) Neighbor-joining (NJ) phylogenetic tree reconstructed from the ITS1 dataset, including sequences from this study and reference sequences from GenBank, the sequence generated in the present study is outlined in red.

Discussion

The recent emergence of Djelfa as a major hotspot of zoonotic cutaneous leishmaniasis (ZCL) in central Algeria highlights the need to better understand the local transmission cycle (INSP, 2024). In this context, the present study provides the first molecular evidence of natural *L. major* infection in *J . orientalis* from this emerging focus. Although based on a single infected individual, this finding broadens the spectrum of wild rodent species naturally infected with *L. major* in North Africa and identifies *J. orientalis* as a species deserving further eco-epidemiological investigation.

To our knowledge, *J. orientalis* has not previously been reported to be naturally infected with *L eishmania*. This jerboa is widely distributed throughout North Africa and occupies a broad range of arid and semi-arid habitats in Algeria (Ahmim, 2019; Meunier et al., 2020).

To date, the endemicity of ZCL in the country has been associated with a classical transmission cycle maintained by *P. obesus* and *M. shawi* (Belazzoug, 1983; Belazzoug, 1986). Interestingly, none of the two *P. obesus* specimens nor the single *M. shawi* specimen examined in the present study tested positive for *Leishmania*, which may be explained by the very small sample size.

Rodents of the genus *Jaculus* have recently been identified as potential reservoirs of *L. major* in Tunisia, where natural infections were reported for the first time in two species, namely *J. hirtipes* and *J. jaculus* with infection rates of 66.7% and 54.4%, respectively (Ghawar et al., 2022). Our findings further expand on this evidence by documenting

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natural infection in *J. orientalis*. Together, these reports suggest that species of the genus *Jaculus* may be more frequently exposed to *L. major* than previously recognised. Nevertheless, the epidemiological significance of *J. orientalis* remains unknown and cannot be inferred from the present data alone.

In our study, the positive *J. orientalis* specimen did not show any external signs of infection, whereas the only individual presenting an ear lesion was PCR-negative. Similar asymptomatic infections have been reported (Ghawar et al., 2011b; Michel et al., 2011; Singh et al., 2014). A recent study focused on the reservoir-vector-pathogen complex *M. shawi-L. major-Ph. papatasi* demonstrated that even asymptomatic infected rodents can harbour and transmit parasites to sand flies, acting as silent reservoirs (Sadlova et al., 2023). However, given the small sample size and the detection of parasites in a single specimen, this finding remains insufficient to establish any epidemiological role or to consider *J. orientalis* a reservoir according to the previously defined criteria (Chaves et al., 2007).

Molecular-based approaches such as PCR and sequencing for the detection of *Leishmania* parasites in wild animals have proved important for identifying rodent species as potential reservoirs. However, the identification of parasite DNA in any rodent species is not sufficient to confirm its role as a reservoir host (Silva et al., 2005). Some hosts may simply serve as parasite sinks without contributing to transmission, meaning that even if they are infected, they do not contribute to the infection of naïve sand flies (Chaves et al., 2007). The most reliable approach for assessing a host's infectivity to sand flies and its involvement as a reservoir host is xenodiagnosis, which involves allowing laboratory-reared naïve sand flies to feed on the suspected or infected host and then examining their guts for the presence of parasites (Benallal et al., 2023; Sadlova et al., 2019).

Phylogenetic analysis based on the ITS1 region confirmed that the detected parasite belongs to the *L. major* clade circulating in North Africa. Haplotype network analysis revealed that the isolate belongs to a distinct haplotype (H4), indicating limited genetic differentiation. Although the biological significance of this haplotype cannot yet be determined, the overall low genetic diversity observed (*Hd* = 0.365) is consistent with previous studies reporting limited ITS1 variability among North African *L. major* isolates (Fotouhi-Ardakani et al., 2016; Ghawar et al., 2014). Whether this pattern reflects local geographic structuring, adaptation to particular ecological conditions, or limited sampling, remains to be investigated using larger datasets and higher-resolution genetic markers.

These preliminary results suggest that *J. orientalis* should be considered in future eco-epidemiological investigations of CL in arid and semi-arid areas of Algeria considering that infectivity is not synonymous with symptomatology. Further studies, including multilocus analyses, xenodiagnoses, and expanded sampling of rodents will be necessary to determine whether *J. orientalis* can act as a primary or accidental reservoir for *L. major*.

Conclusion

This study provides the first molecular evidence of natural *L . major* infection in *J . orientalis* (greater Egyptian jerboa) in Algeria. Although based on a single asymptomatic infected individual, this finding expands the spectrum of wild rodent species naturally infected with *L. major* in North Africa. However, molecular detection alone is insufficient to demonstrate reservoir competence, and the epidemiological role of *J. orientalis* therefore remains to be determined. Future studies integrating larger-scale rodent surveys, parasite isolation, and xenodiagnostic experiments will be essential to assess whether this species contributes to the maintenance of L. major transmission. Nevertheless, our findings identify *J. orientalis* as a potential host deserving further investigation and contribute to a better understanding of the eco-epidemiology of ZCL in Algeria and North Africa.

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Ethical approval

This study complied with Algerian legislation (Ordinance No. 88-08, January 26, 1988) and was approved by the Scientific Committee of the Faculty of Nature and Life Sciences, University of Djelfa.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Author Contributions

KO: Conceptualisation, Data curation, Investigation, Methodology, Project administration, Visualisation, Writing – original draft. KO and KEB: Formal analysis. JS, VD, IL, and KEB: Conceptualisation, Methodology, Project administration, Supervision, Writing – review & editing. TB, AH, KS, and AhH: Investigation, Writing – review & editing. All authors have read and approved to the final version of the manuscript.

Data availability

All data are available upon request.

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References

Abbasi, I., Cunio, R., & Warburg, A. (2009). Identification of Blood Meals Imbibed by Phlebotomine Sand Flies Using Cytochrome b PCR and Reverse Line Blotting. Vector-Borne and Zoonotic Diseases, 9(1), 79–86. https://doi.org/10.1089/vbz.2008.0064.

Ahmim, M. (2019). Les Mammiferes Sauvage D'Algerie Répartition et Biologie de la Conservation. Net.

Ashford, R. W., Schnur, L. F., Chance, M. L., Samaan, S. A., & Ahmed, H. N. (1977). Cutaneous leishmaniasis in the Libyan Arab Republic: Preliminary ecological findings. Annals of Tropical Medicine & Parasitology, 71(3), 265–271. https://doi.org/10.1080/00034983.1977.11687190.

Belazzoug, S. (1983). The new focus of cutaneous leishmaniasis of M'sila (Algeria). Natural infection of Psammomys obesus (Rodentia, Gerbillidae). Bull Soc Pathol Exot Filiales, 76(2), 146–149.

Belazzoug, S. (1986). Découverte d'un Meriones shawi (Rongeur, Gerbillidé) naturellement infesté par Leishmania dans le nouveau foyer de leishmaniose cutanée de Ksar Chellala (Algérie). Bulletin de La Société de Pathologie Exotique, 79(5), 630–633.

Ben Othman, S., Ghawar, W., Chaouch, M., Ayari, C., Chemkhi, J., Cancino-Faure, B., Tomás-Pérez, M., Alcover, M. M., Riera, C., Ben Salah, A., Fisa, R., Ben Ismail, R., & Ben Abderrazak, S. (2018). First detection of Leishmania DNA in Psammomys obesus and Psammomys vexillaris: Their potential involvement in the epidemiology of leishmaniasis in Tunisia. Infection, Genetics and Evolution, 59, 7–15. https://doi.org/10.1016/j.meegid.2018.01.013.

Benallal, K. E., Mezai, G., Mefissel, M., Klari, N., Lardjane, C., Khardine, A.-F., Kherachi, I., Dib, Y., Brahmi, K., Sadlova, J., Volf, P., & Harrat, Z. (2023). Host competence of Algerian Gerbillus amoenus for Leishmania major.

International Journal for Parasitology: Parasites and Wildlife, 21, 69–73. https://doi.org/10.1016/j.ijppaw.2023.04.007.

Benikhlef, R., Aoun, K., Boudrissa, A., Ben Abid, M., Cherif, K., Aissi, W., Benrekta, S., Boubidi, S. C., Späth, G. F., Bouratbine, A., Sereno, D., & Harrat, Z. (2021). Cutaneous Leishmaniasis in Algeria; Highlight on the Focus of M'Sila. Microorganisms, 9(5), 962. https://doi.org/10.3390/microorganisms9050962.

Chaves, L. F., Hernandez, M.-J., Dobson, A. P., & Pascual, M. (2007). Sources and sinks: Revisiting the criteria for identifying reservoirs for American cutaneous leishmaniasis. Trends in Parasitology, 23(7), 311–316. https://doi.org/10.1016/j.pt.2007.05.003.

Derghal, M., Tebai, A., Balti, G., Souguir-Omrani, H., Chemkhi, J., Rhim, A., Bouattour, A., Guizani, I., M'Ghirbi, Y., & Guerbouj, S. (2022). High-resolution melting analysis identifies reservoir hosts of zoonotic Leishmania parasites in Tunisia. Parasites & Vectors, 15(1), 12. https://doi.org/10.1186/s13071-021-05138-x.

Djellouli, M., Eddaikra, N., Beneldjouzi, A., Benikhlef, R., Ghimire, R., Wilkins, M., & Lafri, I. (2025). Serological, molecular, and epidemiological assessment of Leishmania spp. In equids within a hotspot endemic area for cutaneous leishmaniasis in southeastern Algeria. Veterinary Research Communications, 49(6), 328. https://doi.org/10.1007/s11259-025-10904-8.

Ferdes, I., Latrofa, M. S., Perles, L., Bezerra-Santos, M. A., Medrouh, B., Benmarce, M., Ouachek, K., Hakem, A., Lafri, I., & Otranto, D. (2025). Occurrence of Leishmania spp. In phlebotomine sand flies and dogs in Guelma region, North-eastern Algeria. Veterinary Parasitology: Regional Studies and Reports, 57, 101176. https://doi.org/10.1016/j.vprsr.2024.101176.

Fotouhi-Ardakani, R., Dabiri, S., Ajdari, S., Alimohammadian, M. H., AlaeeNovin, E., Taleshi, N., & Parvizi, P. (2016). Assessment of nuclear and mitochondrial genes in precise identification and analysis of genetic polymorphisms for the evaluation of Leishmania parasites. Infection, Genetics and Evolution, 46, 33–41. https://doi.org/10.1016/j.meegid.2016.10.011.

Ghawar, W., Snoussi, M. A., Hamida, N. B. H., Boukthir, A., Yazidi, R., Chaâbane, S., Chemkhi, J., Zâatour, A., & Salah, A. B. (2011a). First Report of Natural Infection of Least Weasel (Mustela nivalis Linnaeus, 1776) with Leishmania major in Tunisia. Vector-Borne and Zoonotic Diseases, 11(11), 1507–1509. https://doi.org/10.1089/vbz.2011.0673.

Ghawar, W., Toumi, A., Snoussi, M.-A., Chlif, S., Zâatour, A., Boukthir, A., Bel Haj Hamida, N., Chemkhi, J., Diouani, M. F., & Ben-Salah, A. (2011b). Leishmania Major Infection Among Psammomys Obesus and Meriones Shawi: Reservoirs of Zoonotic Cutaneous Leishmaniasis in Sidi Bouzid (Central Tunisia). Vector-Borne and Zoonotic Diseases, 11(12), 1561–1568. https://doi.org/10.1089/vbz.2011.0712.

Ghawar, W., Attia, H., Bettaieb, J., Yazidi, R., Laouini, D., & Salah, A. B. (2014). Genotype Profile of Leishmania major Strains Isolated from Tunisian Rodent Reservoir Hosts Revealed by Multilocus Microsatellite Typing. PLoS ONE, 9(9), e107043. https://doi.org/10.1371/journal.pone.0107043.

Ghawar, W., Chaouch, M., Ben Salah, A., Snoussi, M. A., Salem, S., Kharroubi, G., Chouchen, S., Bouaoun, A., Laouini, D., Bettaieb, J., & Ben Abderrazak, S. (2022). First Report of Two Jaculus Rodents as Potential Reservoir Hosts of Leishmania Parasites in Tunisia. Microorganisms, 10(8), 1502. https://doi.org/10.3390/microorganisms10081502.

Granjon, L., & Duplantier, J.M. (2009). Les rongeurs de l'Afrique sahélo-soudanienne. IRD Éditions; Publications scientifiques du Muséum national d'Histoire naturelle. https://www.documentation.ird.fr/hor/fdi:010048662.

Hall, T. A. (1999). Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/ NT. https://api.semanticscholar.org/CorpusID:82421255

INSP. (2024). Relevé Epidémiologique Mensuel «R.E.M » Algérie (p. 30). Institut National de Santé Publique.

Izri, M. A., Belazzoug, S., Pratlong, F., & Rioux, J.A. (1992). Isolement de Leishmania major chez Phlebotomus papatasi à Biskra (Algérie): Fin d'une épopée écoépidémiologique. Annales de Parasitologie Humaine et Comparée,

67(1), 31–32. https://doi.org/10.1051/parasite/199267131.

Karlin, S., Bečvářová, B., Benallal, K. E., Bečvář, T., Mezai, G., Zaabar, M., Mefissel, M., Volf, P., & Sádlová, J. (2026). Acomys cahirinus seurati as a Potential Reservoir Host of Leishmania major. Pathogens, 15(3), 268. https://doi.org/10.3390/pathogens15030268.

Cold Spring Harbor Protocols. (2006). Ketamine/Xylazine. pdb.rec702. https://doi.org/10.1101/pdb.rec702.

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing. Molecular Biology and Evolution, 41, 1–9. https://doi.org/10.1093/molbev/msae263.

Leigh, J. W., & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution, 6(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410.

Medkour, H., Laidoudi, Y., Lafri, I., Bitam, I., Mediannikov, O., & Davoust, B. (2019). Canine leishmaniosis and first report of Leishmania infantum in the blood of equids in Kabylia (Algeria). International Journal of Infectious Diseases, 79, 117–118. https://doi.org/10.1016/j.ijid.2018.11.290.

Messahel, N. E., Lafri, I., Moualek, I., Houali, K., & Hakem, A. (2021). Epidemiological situation analysis of cutaneous leishmaniasis in Batna (northeast): An important focus in Algeria. Veterinary Parasitology: Regional Studies and Reports, 26, 100621. https://doi.org/10.1016/j.vprsr.2021.100621.

Meunier, M., Stoetzel, E., Souttou, K., Sekour, M., Hadjoudj, M., Boukmenza, M., Doumandji, S., & Denys, C. (2020). Mise à jour de la liste des rongeurs d'Algérie, biogéographie et implications paléoécologiques. Bulletin de la Société zoologique de France, 145(4), 413–474.

Michel, G., Pomares, C., Ferrua, B., & Marty, P. (2011). Importance of worldwide asymptomatic carriers of Leishmania infantum (L. chagasi) in human. Acta Tropica, 119(2–3), 69–75. https://doi.org/10.1016/j.actatropica.2011.05.012.

Ouachek, K., Medrouh, B., Zebsa, R., Ferdes, I., Tahtah, A., Seddas, F., Souttou, K., Benallal, K. E., Hakem, A., & Lafri, I. (2026). Spatio-temporal modeling of zoonotic cutaneous leishmaniasis (ZCL) in the Algerian steppe: Epidemiological insights and climatic associations. Epidemics, 100930. https://doi.org/10.1016/j.epidem.2026.100930.

Rioux, J. A., Petter, F., Akalay, O., Lanotte, G., Ouazzani, A., Seguignes, M., & Mohcine, A. (1982). [Meriones shawi (Duvernoy, 1842) [Rodentia, Gerbillidae] a reservoir of Leishmania major, Yakimoff and Schokhor, 1914 [Kinetoplastida, Trypanosomatidae] in South Morocco (author's transl)]—PubMed. 294(11), 515–517.

Rioux, J. A., Petter, F., Zahaf, A., Lanotte, G., Houin, R., Jarry, D., Perieres, J., Martini, A., & Sarhani, S. (1986). Isolement de Leishmania major Yakimoff et Schokhor, 1914 (Kinetoplastida-Trypanosomatidae) chez Meriones shawi shawi (Duvernoy, 1842) (Rodentia-Gerbillidae) en Tunisie. Annales de Parasitologie Humaine et Comparée, 61(2), 139–145. https://doi.org/10.1051/parasite/1986612139.

Rodriguez-Morales, A. J., Shehata, A. A., Parvin, R., Tasnim, S., Duarte, P. M., & Basiouni, S. (2025). Rodent-Borne Parasites and Human Disease: A Growing Public Health Concern. Animals, 15(18), 2681. https://doi.org/10.3390/ani15182681.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution, 34(12), 3299–3302. https://doi.org/10.1093/molbev/msx248.

Ruiz-Postigo, J. A., Jain, S., Mikhailov, A., Maia-Elkhoury, A. N., Valadas, S., Warusavithana, S., Osman, M., Lin, Z., Beshah, A., Yajima, A., & Gasimov, E. (2021). Global leishmaniasis surveillance: 2019–2020, a baseline for the 2030 roadmap. Weekly Epidemiological Record, 96(35), 401–419. https://www.who.int/publications/i/item/who-wer9635-401-419.

Sadlova, J., Vojtkova, B., Hrncirova, K., Lestinova, T., Spitzova, T., Becvar, T., Votypka, J., Bates, P., & Volf, P.

(2019). Host competence of African rodents Arvicanthis neumanni, A. niloticus and Mastomys natalensis for Leishmania major. International Journal for Parasitology: Parasites and Wildlife, 8, 118–126. https://doi.org/10.1016/j.ijppaw.2019.01.004.

Sadlova, J., Bacikova, D., Becvar, T., Vojtkova, B., England, M., Shaw, J., & Volf, P. (2022). Porcisia transmission by prediuresis of sand flies. Frontiers in Cellular and Infection Microbiology, 12, 981071. https://doi.org/10.3389/fcimb.2022.981071.

Sadlova, J., Vojtkova, B., Lestinova, T., Becvar, T., Frynta, D., Benallal, K. E., Mekarnia, N., Harrat, Z., & Volf, P. (2023). Infectiousness of Asymptomatic Meriones shawi, Reservoir Host of Leishmania major. Pathogens, 12(4), 614. https://doi.org/10.3390/pathogens12040614.

Schönian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H. D. F. H., Presber, W., & Jaffe, C. L. (2003). PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diagnostic Microbiology and Infectious Disease, 47(1), 349–358. https://doi.org/10.1016/S0732-8893(03)00093-2.

Sergent, E., Sergent, E., Parrot, L., Donatien, A., & Beguet, M. (1921). Transmission du clou de Biskra par le phlébotome (Phlebotomus papatasi Scop.). CR Acad Sci, 173, 1030–1032.

Silva, E. S., Gontijo, C. M. F., & Melo, M. N. (2005). Contribution of molecular techniques to the epidemiology of neotropical Leishmania species. Trends in Parasitology, 21(12), 550–552. https://doi.org/10.1016/j.pt.2005.09.008.

Singh, O. P., Hasker, E., Sacks, D., Boelaert, M., & Sundar, S. (2014). Asymptomatic Leishmania Infection: A New Challenge for Leishmania Control. Clinical Infectious Diseases, 58(10), 1424–1429. https://doi.org/10.1093/cid/ciu102.

Tomás-Pérez, M., Khaldi, M., Riera, C., Mozo-León, D., Ribas, A., Hide, M., Barech, G., Benyettou, M., Seghiri, K., Doudou, S., & Fisa, R. (2014). First report of natural infection in hedgehogs with Leishmania major, a possible reservoir of zoonotic cutaneous leishmaniasis in Algeria. Acta Tropica, 135, 44–49. https://doi.org/10.1016/j.actatropica.2014.03.018.

WHO. (2023, January 12). Leishmaniasis. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis