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Dirofilaria repens believed to be *D. immitis*: An erratic localization case in a golden jackal (*Canis aureus*)

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ABSTRACT

Dirofilariosis is a widespread mosquito-borne disease that primarily affects dogs, the main reservoir, but it can also infect other mammalian hosts. *Dirofilaria immitis* is the causative agent of a cardiopulmonary disease in dogs, whereas *Dirofilaria repens* typically inhabits subcutaneous tissues and is often associated with subclinical infections. Nevertheless, *D. repens* shows a greater tendency for aberrant localization in atypical anatomical sites. We report a rare case of cardiac localization of *D. repens* in a golden jackal from the Friuli Venezia Giulia region, Northeastern Italy. The golden jackal also presented a calcified nodular lesion on the calcaneal region of the right hind limb heel, in which a filiform worm was recovered. Species identification was achieved through morphometric identification, complemented by PCR amplification of the COI and 12S rRNA genes and phylogenetic analyses. This represents only the second documented case of aberrant cardiac localization of *D. repens* in a wild canid, following a report in a wolf from Kazakhstan. This finding highlights the importance of accurate identification, even when the anatomical location might itself suggest a diagnosis. Moreover, the ecological and anatomical plasticity of *D. repens* underscores the importance of including wild canids in epidemiological surveillance programs, particularly considering the expanding distribution of both the parasite and the golden jackal across Europe.

1. Introduction

Dirofilariosis is a mosquito-borne disease that affects primarily dogs (*Canis lupus familiaris*), but can also infect a wide range of other mammalian hosts. Two main species of *Dirofilaria* (syn. *Nochtiella*; Nematoda, Onchocercidae) currently circulate in Europe: *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* (Railliet and Henry, 1911) (Hattendorf and Lühken, 2025). Culicid mosquitoes serve as the main vector: females become infected during blood feeding on a microfilaremic host, allowing microfilariae to develop into infective third-stage larvae (L3). These larvae are then transmitted to a new host via the feeding wound and migrate into subcutaneous or subserosal tissues, where they undergo two moults (Ledesma and Harrington, 2011). Young adults ultimately reach their species-specific localization: in dogs, *D. immitis* resides in the pulmonary arteries and right ventricle, whereas *D. repens* is typically found within subcutaneous tissues (Hattendorf and Lühken, 2025).

The pathological impact differs markedly between the two nematode species. *Dirofilaria immitis* infections in dogs and, rarely, cats (*Felis catus*) may result in severe disease, including pulmonary endarteritis, pulmonary hypertension, and right-sided congestive heart failure (Vismarra et al., 2021). In contrast, most *D. repens* infections remain subclinical, with occasional cutaneous manifestations such as pruritus, dermal swelling, or subcutaneous nodules, and, more rarely, ocular involvement (Genchi and Kramer, 2017).

Reports describing pathological findings in wildlife are relatively scarce. Orioles et al. (2024) documented the pathogenic effects of co-infection with *Angiostrongylus vasorum* and *D. immitis* in golden jackals (*Canis aureus*), reporting right-sided cardiomegaly, multifocal granulomatous interstitial pneumonia, and proliferative villous endarteritis associated with the presence of *D. immitis*. Similarly, Gamboa-Suárez et al. (2026) described pathological findings, mostly in the lungs and kidneys, in a crab-eating fox (*Cerdocyon thous*) from Colombia with an adult *Dirofilaria* sp. located in the femoral vein. In the lungs, severe

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diffuse suppurative, fibrinous, and hemorrhagic bronchopneumonia, along with severe multifocal granulomatous vasculitis, was observed and was attributed to parasite migration through the pulmonary parenchyma. In the kidneys, glomerulonephritis was associated with the formation of nematode-specific antigen–antibody complexes and the deposition of *Dirofilaria* antigens along the glomerular basement membrane.

Humans may act as accidental hosts for both *D. repens* and *D. immitis*, and microfilaremia is generally rare (Ciuca et al., 2025). *Dirofilaria immitis* typically leads to the production of pulmonary nodules, which are often asymptomatic but may be misdiagnosed as neoplastic lesions (Hattendorf and Lühken, 2025). *Dirofilaria repens* is usually found in subcutaneous or ocular localizations in the human host (ocular dirofilariosis), potentially affecting the vision (Ciuca et al., 2025; Hattendorf and Lühken, 2025), but other aberrant locations have been reported (Ciuca et al., 2025).

Both *D. immitis* and *D. repens* are cosmopolitan nematodes considered endemic across Europe, Asia, and Africa (Genchi and Kramer, 2020). Both species have expanded their geographical range, with autochthonous cases now reported from Central and Northeastern Europe and even Siberia (Fuehrer et al., 2016; Genchi and Kramer, 2020; Genchi et al., 2025; Hattendorf and Lühken, 2025; Sałamatin et al., 2013), despite improved awareness and preventive chemoprophylaxis leading to a decline of *D. immitis* in formerly hyperendemic regions. Among the two species, *D. repens* has emerged as a significant public health concern: it is considered endemic throughout Italy, with prevalence in dogs ranging from 1.5% to 12%, and a recent study conducted on unowned, stray dogs in the Po River Valley reported a prevalence of 6.9% at Knott test (Genchi et al., 2025). Several factors may account for this expansion. First, prevention of canine subcutaneous dirofilariosis relies on macrocyclic lactones; however, not all compounds within this drug class are effective against *D. repens* (Genchi and Kramer, 2017; Genchi et al., 2025). Second, climate change, with rising temperatures and extended mosquito activity for a longer seasonal period, along with the establishment of invasive competent vectors such as *Aedes koreicus* and *Aedes japonicus*, plays a role in this expansion (Genchi and Kramer, 2017; Montarsi et al., 2014; Silaghi et al., 2017). Third, the facilitated movement of companion animals across the European Union (Pet Travel Scheme in 2000) (Genchi and Kramer, 2017) and the rise and spread of wild canid populations, such as red foxes (*Vulpes vulpes*), grey wolves (*Canis lupus*) and golden jackals, which can act as wild carriers, further contribute to the dissemination of these parasites (Ćirović et al., 2014; Dini et al., 2025; Ionică et al., 2017; Liulin et al., 2021; Magi et al., 2008; Moroni et al., 2020; Naletilić et al., 2024; Penezić et al., 2014).

Golden jackal is a highly adaptable wild canid with a generalist diet (Lange et al., 2021). Since the 1980s, its range has expanded markedly, likely driven by land-use changes, climate warming, taking advantage of the absence of larger competitors (e.g., wolves, in the past) (Frangini et al., 2025). This expansion from the Dalmatian and Balkan regions led to a westward dispersal, reaching the Istrian Peninsula and subsequently Northeastern Italy, where the species established a stable population in the Friuli Venezia Giulia region (Lapini et al., 2009). Their dietary flexibility, wide territorial range, and adaptability to urban environments make golden jackals potential vectors for transmitting canine and zoonotic parasites to domestic animals and humans (Veronesi et al., 2023). To date, *D. repens* in golden jackals has been reported in Romania with a relatively low prevalence when blood and spleen samples are tested (1.85%, 95% CI: 0.1–9.9, and 3.0%, 95% CI: 0.4–10.5, respectively). However, the prevalence of *D. repens* may be underestimated, as DNA detection in these sample types implies the presence of circulating microfilariae (Ionică et al., 2016, 2017).

In this epidemiological context, the present study reports an erratic localization of *D. repens* in a golden jackal from Friuli Venezia Giulia.

2. Materials & methods

As part of the regional wildlife health surveillance plan of the Friuli Venezia Giulia (FVG) region, Northeastern Italy, an adult male golden jackal, killed in a road accident, was collected and transported to the Department of Agricultural, Food, Environmental and Animal Sciences of the University of Udine, where it was stored at −20 °C until analysis. Since the study was conducted on a recovered carcass and did not involve the handling of live animals, no ethical approval was required. Necropsy was performed following standard procedures, inspecting the whole body. The heart was dissected and examined macroscopically to identify gross lesions and the presence of nematodes. Helminth specimens isolated during necropsy were collected, washed in saline, and fixed in 70% ethanol. Morphometric and molecular identification were carried out at the Department of Animal Medicine, Production and Health, University of Padova, while scanning electron microscopy (SEM) imaging was performed at the Department of Veterinary Medical Sciences, University of Bologna. Morphological identification of preserved specimens was reached upon clarification in Amman's lactophenol, according to the taxonomic criteria described by Yevstafieva et al. (2023). If the specimen was not sufficiently preserved for morphological identification, molecular analyses were directly performed. After light microscopy observation, the specimen was carefully rinsed and cleaned, then sectioned into smaller subsamples: selected portions were processed for SEM, while the remaining aliquots were molecularly analysed.

Sections for SEM imaging were fixed in 10% buffered formalin, then were dehydrated through a graded ethanol series, subjected to critical point drying, sputter-coated with gold–palladium, and examined using a Phenom XL G2 Desktop SEM (Thermo Fisher Scientific, Eindhoven, The Netherlands) operating at 5 kV, as described by Dini et al. (2025).

Aliquots were submitted to DNA extraction using the NucleoSpin® Tissue Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. PCR amplification was conducted using primer sets targeting the 12S rRNA gene (Diro12S-F/Diro12S-R), and the COI gene (Diro-cox1-F/Diro-cox1-R), obtaining nucleotide sequences of approximately 430 bp and 730 bp, respectively (Suzuki et al., 2015). The resulting nucleotide sequences were assembled using ContigExpress software and compared with homologous sequences deposited in the NCBI GenBank database through BLAST analysis. Phylogenetic inference was performed using MEGA 7 (Kumar et al., 2016). For 12S rRNA, the analysis was based on the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al., 1985), while for the COI gene, the Tamura-Nei (TN93) model (Tamura and Nei, 1993) was applied. The sequences obtained in this study have been deposited in GenBank.

3. Results

The necropsy was performed on an adult male golden jackal that was road-killed in San Canziano d'Isonzo (Gorizia province, FVG). The carcass weighed 12.1 kg and was in good condition of preservation, allowing a thorough examination. Subcutaneous and visceral fat deposits indicated an adequate nutritional status before death. Necropsy findings identified multiple traumatic injuries as the cause of death, including a cervical vertebral fracture and internal hemorrhaging resulting in hemoperitoneum. A calcified nodular lesion was observed on the calcaneal region of the right hind limb heel, from which a filiform worm was recovered (Fig. 1). Since the nematode located in the calcified nodule was poorly preserved, only the molecular investigation was performed for this sample. Moreover, a filaroid nematode was retrieved from the right ventricle of the heart. The comparison of the morphology of this nematode permitted the identification of the helminth as an adult male of *Dirofilaria repens* (Yevstafieva et al., 2023). The body was elongated and filiform. The cephalic extremity was obtusely rounded, with clearly expressed and thickened cuticle in the anterior region (Fig. 2 a). The mouth opening was small and weakly expressed, terminal,

Fig. 1. Macroscopic appearance of the tarsal region of a golden jackal after dissection. A well-defined whitish nodule, located subcutaneously at the calcaneal level, is observed to contain a coiled nematode, subsequently identified as *Dirofilaria repens*. Scale bar 2 cm.

leading to a shallow buccal cavity; four pairs of cephalic papillae and two amphids surrounded the oral opening (Fig. 2 b). The esophagus was short, slightly narrowed at the level of the nerve ring. The body cuticle was characterized by well-developed longitudinal ridges forming a rib-like pattern, associated with fine transverse striations along the entire body length. Each ridge was separated by an interspace wider than the ridge itself (Fig. 2 c and d). The posterior end of the male was rounded and short, bearing narrow lateral alae (Fig. 2 f). Six pairs of caudal papillae were present, interpreted as two pre-cloacal and four post-cloacal papillae (Fig. 2 f and g). The two spicula were unequal in length and morphology. The short spiculum was robust (Fig. 2 e), while the long spiculum had a wider anterior part and a thin posterior part; the distal portion was poorly defined, and its width could not be reliably measured. The typical triangular widening described for *D. repens* in the mid–distal region of the long spiculum was not clearly recognizable, possibly indicating an immature male or an anomalous individual. Morphometric parameters are presented in Table 1.

A central segment of approximately 10 mm of the nematode isolated from the heart ventricle and a portion of the filiform nematode in the calcified nodule were submitted to molecular analyses. Amplification of both 12S rRNA and COI genes yielded positive results (427 bp and 726 bp, respectively) and sequences of the two specimens were identical. Comparison with sequences available in GenBank database revealed a nucleotide identity of 100% for 12S rRNA and for COI with *D. repens* strains collected from a human in Greece and a dog in Italy (MK192091.1 and KX265048.1, respectively). Phylogenetic analysis of both mitochondrial genes clearly clustered our sequences with other *D. repens* isolates from dogs and humans across different countries, forming a well-supported and distinct clade (98-99/100 bootstrap value). This clade was clearly separated from *D. immitis* and *D. asiatica*, which formed their own distinct lineages. Interestingly, *D. asiatica* appeared as a more clearly separated clade in the COI phylogeny (Fig. 3). The sequences obtained from the cardiac individual were deposited in GenBank under accession numbers PZ016943 and PZ014620.

4. Discussion

The present study documents an aberrant cardiac localization of *D. repens* in a golden jackal, adding new evidence to the limited knowledge on unusual migratory patterns of this parasite in wild canids. Cardiac involvement is sporadic for *D. repens*, whose adult stages typically develop within subcutaneous tissues, and is scarcely reported in wild canids in the scientific literature. At necropsy, two filiform nematodes were recovered from the animal. One specimen was located within the right ventricle, a localization more commonly associated with *D. immitis*. The second parasite was retrieved from a calcified nodule in the heel region of the right hind limb. Unfortunately, this specimen was poorly preserved, allowing only molecular characterization.

The species identification of the isolated nematode in the cardiac chamber was performed by morphometric evaluation in conjunction with molecular analysis. Interestingly, the parasite exhibited morphological features consistent with *D. repens*, allowing differentiation from *D. immitis*. The specimen measured 5.5 cm in length, which falls within the reported range for *D. repens* male (4.8–6.6 cm) (Yevstafieva et al., 2023) and is considerably shorter than *D. immitis* male (13.9–17.0 cm) (Kryvoruchenko et al., 2021). The cuticle displayed longitudinal striations with interspaces wider than the ridges themselves along the entire body, a characteristic feature of *D. repens* that is absent in *D. immitis*. The short tail, bearing six pairs of caudal papillae (two pre-cloacal and four post-cloacal), further supported identification as *D. repens*, distinguishing it from *D. immitis*, which exhibits a more prominent tail and a different arrangement of caudal papillae (one pre-cloacal and four to five post-cloacal papillae) (Khedr et al., 2021; Kryvoruchenko et al., 2021; Perles et al., 2024; Wong and Brummer, 1978; Yevstafieva et al., 2023).

The sequences recovered from both nematodes were identical, providing molecular evidence that both parasites probably originated from a single infection event. This almost excludes the possibility of multiple independent exposures and points to a unique transmission episode in this golden jackal.

Amplification of 12S rRNA and the COI genes of both isolates confirmed *D. repens* as well as phylogenetic analyses consistently supported the monophyly of the genus *Dirofilaria*, with the three species (*D. repens, D. immitis,* and *D. asiatica*) forming well-supported, reciprocally monophyletic clades. The sequence obtained from the golden jackal in the present study clustered unambiguously within the *D. repens* clade, with high bootstrap support, alongside sequences from dogs, cats, and humans from across Europe, Russia, and Japan. *Dirofilaria asiatica,* represented by sequences from human and dog hosts in India, Sri Lanka, and Australia, formed a sister group to *D. repens* in both trees, consistent with its previously proposed status as a closely related but distinct taxon (Colella et al., 2025). *Dirofilaria immitis* sequences formed a separate, well-supported clade, confirming the clear molecular divergence between the two zoonotic species.

To date, only one comparable case has been reported: a female wolf (*Canis lupus*) from the Kostanay region of Kazakhstan, in which a single adult *D. repens* was recovered from the heart. The worm measured 14.7 cm in length and exhibited the characteristic longitudinal cuticular ridges typical of *D. repens*. Its identity was further confirmed as *D. repens* by molecular analysis (Uakhit et al., 2021). Similar to our findings, that case involved a solitary adult, suggesting that cardiac localization may occur sporadically and possibly as a consequence of aberrant migratory routes rather than a true tropism for the cardiopulmonary system.

Dirofilaria repens typically localizes in the subcutaneous or

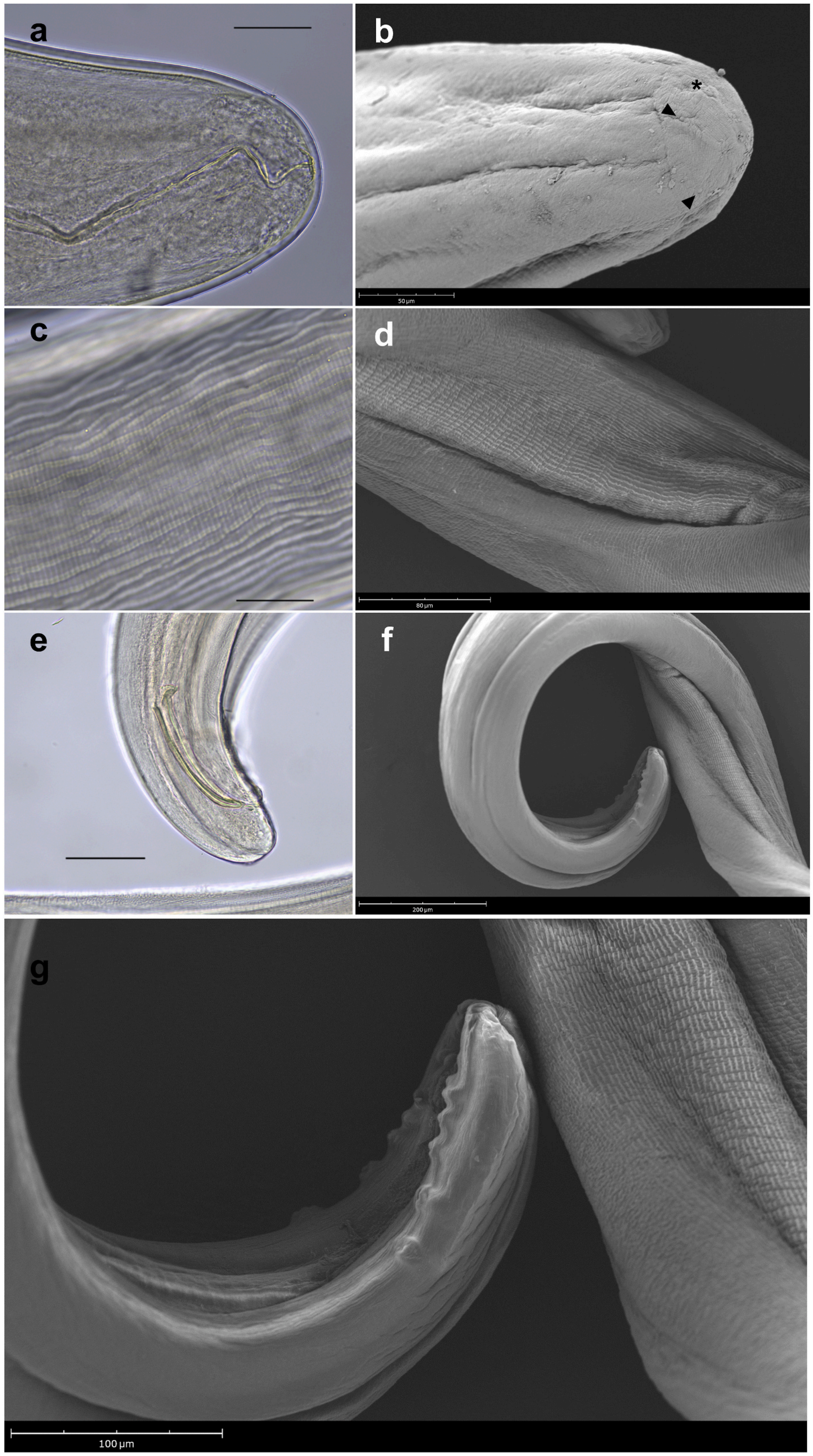

Fig. 2. Morphology of adult male of *Dirofilaria repens* in light microscopy (LM) and scanning electron microscopy (SEM). a) cephalic extremity showing characteristic thickened cuticle and anterior part of esophagus, LM (400x magnification, scale bar 50 μm); b) cephalic extremity showing characteristic cuticular striations and the appearance of cephalic papillae (arrowheads) and amphid (asterisk), SEM (scale bar 50 μm); c) detail of cuticle showing longitudinal ridges and fine transversal striations, LM (400x magnification, scale bar 50 μm); d) detail of cuticle surface with longitudinal ridges and fine transversal striations, SEM (scale bar 80 μm); e) caudal extremity showing the short spiculum, LM (200x magnification, scale bar 100 μm); f) coiled caudal extremity showing terminal lateral alae and six pairs of papillae, SEM (scale bar 200 μm); g) detail of caudal extremity showing a more clearly defined view of the terminal papillae; on the right, a portion of the body displaying the characteristic longitudinal striations, SEM (scale bar 100 μm).

Table 1
Metric parameters of males of *Dirofilaria repens* (following Yevstafieva et al., 2023). SD = standard deviation.

Metric parameters	Present study	Yevstafieva et al. (2023) Mean ± SD (min-max)
Overall body length (mm)	55	57.1 ± 6.5 (48.0–66.5)
Overall body width (μm)	450	406.7 ± 16.6 (390.1–432.4)
Body width at (μm):		
- Esophagus–intestine junction	437	382.7 ± 16.3 (359.4–402.1)
- Nerve ring	273	238.5 ± 11.9 (224.1–251.6)
- Cloacal region	110	118.8 ± 15.0 (98.6–137.2)
Thickness of the cuticle in the region of mouth opening (μm)	7.6	9.2 ± 0.8 (8.2–10.4)
Distance from anterior end to nerve ring (μm)	297	227.9 ± 10.5 (211.8–241.1)
Esophagus (μm):		
- Length	675	761.9 ± 39.2 (698.2–797.9)
- Width of the widest part	62	62.1 ± 2.1 (59.8–64.9)
- Width of the nerve ring	38	42.2 ± 2.1 (39.8–44.9)
- Width of the middle part	44	56.3 ± 4.6 (48.2–59.2)
Tail length (μm)	72	72.9 ± 7.1 (62.2–80.1)
Short spiculum (μm):		
- length	213	178.7 ± 9.4 (170.9–194.1)
- width of proximal end	24	21.9 ± 4.0 (14.9–25.1)
- width of distal end	10	14.4 ± 0.7 (13.2–15.1)
- width of middle part	24	31.4 ± 1.2 (30.1–33.1)
Long spiculum (μm):		
- length	479	432.9 ± 24.0 (392.1–450.9)
- width of proximal end	26	37.2 ± 1.8 (35.2 – 39.9)
- width of distal end	-	3.1 ± 0.2 (2.9–3.3)
- width of middle part	19.2	18.2 ± 0.7 (17.1–18.9)
Ratio between lengths of spicula	2.25:1	2.43:1 (2.23:1–2.58:1)

intramuscular connective tissues of canids. In particular, it is thought to induce an immunosuppressive response in canine host, allowing long-term survival, up to four years, and explaining why subcutaneous dirofilariasis is often asymptomatic; in some cases, live worms may even be visible beneath the skin (Albanese et al., 2013; Capelli et al., 2018; Genchi and Kramer, 2017).

Cutaneous signs arise from several mechanisms, including: (1) capillary embolization by microfilariae, (2) migration of adult worms through connective tissues, (3) immuno-allergic reactions to larval stages (L3-L5) and microfilariae, (4) parasite-derived toxins, and (5) the interaction of the immune system with the endosymbiont *Wolbachia*, leading to granuloma formation (Genchi and Kramer, 2017; Grandi et al., 2008; Tarello, 2011). Reported lesions range from pruritus and nodules to circular alopecic areas with lichenification, hyperpigmentation, and erythematous, scaly margins (Capelli et al., 2018; Tarello, 2011). No preferred anatomical site has been identified, and parasites may occur in multiple subcutaneous regions (Albanese et al., 2013).

Dirofilaria repens has also been associated with ocular lesions, including conjunctivitis and related signs (Hermosilla et al., 2006; Mircean et al., 2017). Moreover, scrotal and testicular involvement is increasingly recognized. In a recent study, 6% of dogs undergoing castration for medical or population-control reasons harbored adult *D. repens* between the layers of the *tunica vaginalis*, despite lacking any other subcutaneous abnormalities (Napoli et al., 2024). This and other reports suggest that testicular localization, traditionally considered uncommon, may be more frequent than previously believed, possibly due to a parasite tropism for regions with lower temperature and higher concentrations of sexual hormones (Capelli et al., 2018; Napoli et al., 2019, 2024; Omeragic et al., 2018).

Erratic localizations of *D. repens* have been reported in both dogs and humans. In the few massively infected animals with high microfilaremia, gross and histopathological lesions have been described in multiple organs, including spleen, liver, kidneys, lungs, heart, and brain, suggesting combined mechanical and immunopathological effects of both microfilariae and adult worms (Grandi et al., 2007). Experimental infections also indicate that aberrant sites are common: in one study, 5 out of 23 dogs harbored adult worms in the serosa of the abdominal or thoracic cavities (Petry et al., 2015), but the finding was not discussed further. Naturally occurring cases remain sparsely documented.

In Italy (Abruzzo, Central Italy), a dog presenting with dysorexia and exercise intolerance was found to carry *D. repens* adults in the peritoneal cavity, and microfilariae were detected in the peritoneal fluid (Pierantozzi et al., 2017). In Romania (Cluj-Napoca, Northwestern Romania), a dog co-infected with *D. immitis* and *D. repens* had over 300 *D. repens* adults subcutaneously across the body, including the testes; additionally, two adults of *D. repens* were found in the pelvic cavity and mesentery. The dog also showed chronic kidney disease with histological evidence of severe membranoproliferative glomerulonephritis and intralesional microfilariae, which were confirmed as *D. repens* by PCR (Mircean et al., 2017). A rare case of suspected *D. repens*-associated acute liver failure was also reported in a cat in Pretoria, South Africa. Numerous microfilariae were detected in blood and liver aspirates, and the animal showed a positive response to ivermectin, supporting *D. repens* as the probable causative agent (Schwan et al., 2000).

The present findings further highlight the importance of thorough and meticulous morphological identification of adult specimens in studies investigating parasites of wildlife. In unusual hosts such as wild canids, parasite localization and development may not strictly reflect the patterns commonly described in domestic species, such as dog. Therefore, assumptions based solely on the typical anatomical predilection sites observed in domestic hosts may lead to misidentification or oversimplification of host-parasite interactions. Careful morphological assessment, supported by molecular analyses, is essential to ensure accurate species determination, particularly when parasites are detected in atypical sites. This level of attention contributes to a more comprehensive understanding of parasite biology, host adaptability, and migratory behavior across different host species, ultimately refining our knowledge of their epidemiology and pathogenic potential in wildlife.

Although the pathophysiological mechanisms underlying such unusual localizations remain unclear, the detection of *D. repens* in the heart of a golden jackal underscores the parasite's capacity for ecologically and anatomically atypical behavior. When considered alongside previously documented cases of aberrant localizations in domestic animals, and its expanding range across Europe, this finding highlights the importance of including wild canids in epidemiological surveillance programs.

In conclusion, this report describes a unique case of erratic cardiac localization of *D. repens* in a golden jackal, expanding current knowledge on the spectrum of aberrant migratory behavior of this parasite in wild canids. Accurate identification of parasites detected during necroscopic examination of wildlife provides valuable information on the biological, ecological, and epidemiological characteristics of the parasites themselves and the diseases they cause. Wild animals subjected to monitoring programs represent a particularly valuable opportunity for such investigations, although thorough parasitological assessment is not always performed. This is especially relevant for parasites with zoonotic potential, such as *D. repens*, where comprehensive data collection contributes to a more accurate description of the epidemiological situation and, consequently, to a better estimation of the risk for human health. In this light, our findings further underscore the relevance of wildlife hosts in the epidemiology of *D. repens* and highlight the need for accurate morphological and/or molecular identification, even when the anatomical location might itself suggest a presumptive diagnosis.

CRediT authorship contribution statement

Elisabetta Ferraro: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Erica Marchiori:** Writing – review & editing, Supervision,

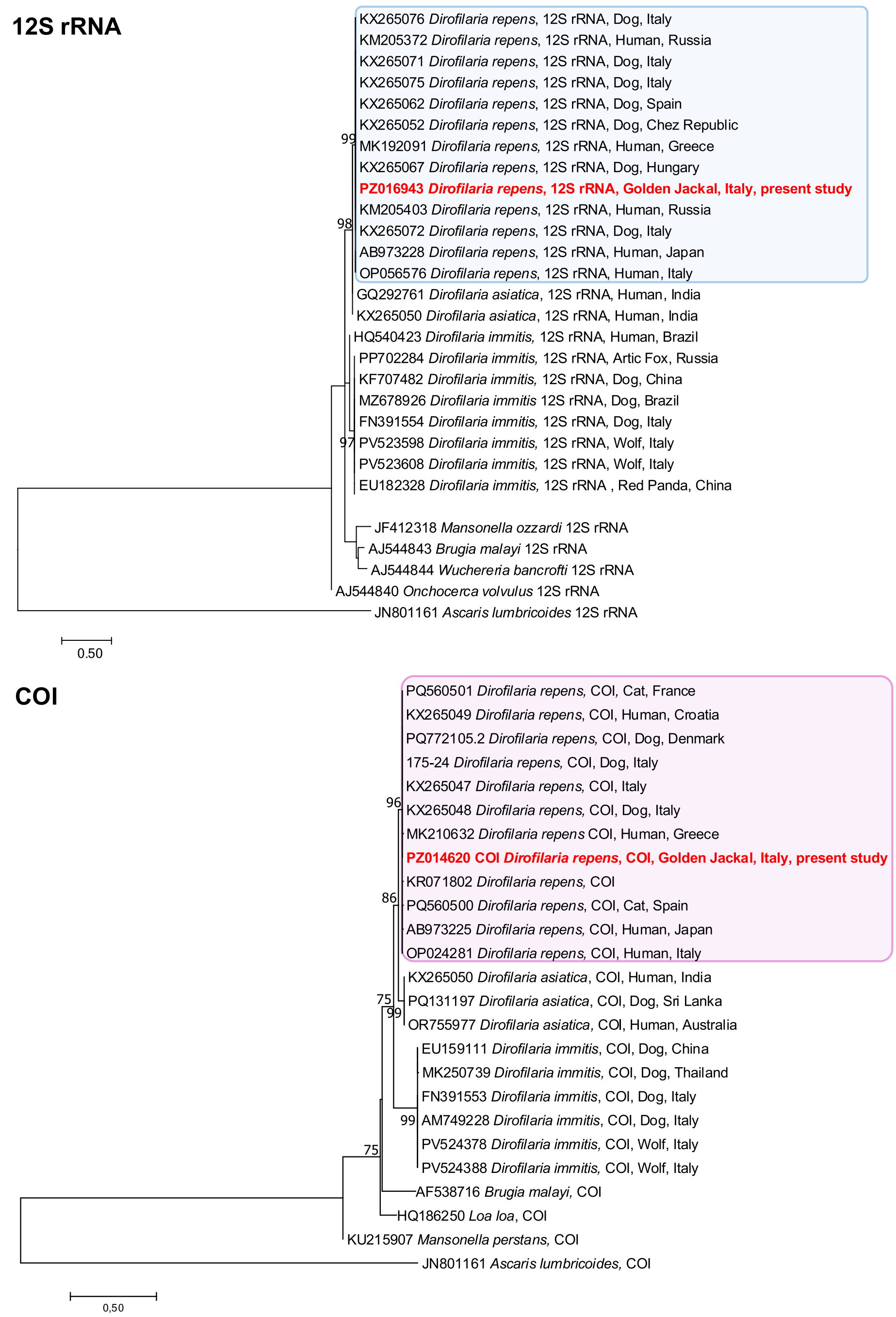

Fig. 3. Molecular phylogenetic analysis of the two mitochondrial markers (12s rRNA and COI). The evolutionary history was inferred using the Maximum Likelihood method with a gamma-distributed rate variation among sites. Only bootstrap values > 75 are shown on the trees. Original sequences obtained in this study are highlighted in red.

Methodology, Investigation, Conceptualization. **Filippo Maria Dini:** Writing – review & editing, Investigation, Data curation. **Marika Grillini:** Writing – review & editing, Formal analysis. **Perla Tedesco:** Writing – review & editing, Investigation. **Giulia Simonato:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Rudi Cassini:** Writing – review & editing, Supervision, Resources. **Paola Beraldo:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization.

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Declaration of competing interests

I have nothing to declare.

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