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First Molecular Characterization of Adult *Physaloptera praeputialis* von Linstow, 1889, Infecting African Lion (*Panthera leo*) in Tanzania

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ABSTRACT

Physalopterid nematodes are common gastric parasites of carnivorous mammals, yet their diversity, host associations, and evolutionary relationships in African wildlife remain poorly understood. The present study aimed to investigate the adult *Physaloptera* specimens recovered from African lions (*Panthera leo*) in the Serengeti National Park in the Mara region, Tanzania, using morphological and molecular techniques. Seven non-captive lions comprising three males and four females were opportunistically necropsied between 2023 and 2025. The sampled lions included two subadults (4-5 years), three prime adults (6-9 years), and two older individuals (10-14 years). A total of 157 adult spirurid nematodes were recovered from three infected hosts with infection intensities ranging from 48 to 57 worms per host. Morphometric characterizations, such as cephalic collarette structures, marked unequal spicules, well-developed caudal alae in males, and an anterior positioned vulva in females, together with typical eggs, were consistent with *Physaloptera praeputialis*. Nuclear *18S rRNA* sequences showed high similarity of 98-99.5% to *P. praeputialis* reference sequences. In contrast, *mitochondrial cytochrome c oxidase subunit I* (*Cox1*) sequences formed a distinct lineage, exhibiting approximately 12% divergence from available *P. praeputialis* sequences retrieved from the GenBank database at the time of analysis. A molecularly characterized record of adult *Physaloptera* from an African lion in Tanzania is presented, revealing previously unrecognized genetic diversity among African physalopterids. Findings highlight the role of apex predators as indicator hosts in trophic parasite transmission and emphasize the importance of multilocus approaches for resolving spirurid taxonomy, parasite biogeography, and host–parasite associations in wildlife ecosystems.

Keywords: African lion, Molecular characterization, Parasitic nematode, Phylogenetic, Serengeti ecosystem

INTRODUCTION

The genus *Physaloptera* Rudolphi, 1819 (Nematoda: *Spirurida*: Superfamily *Physalopteridae*) comprises gastric parasitic nematodes infecting a broad range of vertebrate hosts, including mammals, birds, reptiles, and amphibians (Mohamadain and Ammar, 2012). Members of the genus *Physaloptera* are prevalent in carnivorous mammals, where adult worms inhabit the stomach and attach firmly to the gastric mucosa using a well-developed cephalic collarette and paired pseudolabia (Maharana et al., 2021). Several species, including *Physaloptera rara*, *P. felidis*, *P. maxillaris*, *P. canis*, *P. dilatata*, *P. tumefaciens*, and *P. praeputialis*, have been reported from domestic to wild carnivores across Asia, North America, and Europe (Faria et al., 2019; et al., 2023; Macedo et al., 2023).

Physaloptera species exhibit an indirect life cycle involving orthopteran and coleopteran insects, particularly cockroaches, beetles, crickets, and grasshoppers, as intermediate hosts in which infective third-stage larvae develop (Naem S and Asadi, 2013). Rodents, reptiles, amphibians, and birds may serve as paratenic hosts (Kalyanasundaram et al., 2018). Carnivores acquire infection primarily through predation or accidental ingestion of infected intermediate or paratenic hosts (Maharana et al., 2021). Adult *Physaloptera* worms embed in the gastric mucosa of carnivorous definitive hosts and feed on host tissues and blood, often causing focal erosions, hemorrhages, and chronic gastritis (Alves et al., 2022).

Physaloptera infections in carnivorous definitive hosts, particularly domestic dogs and cats and occasionally wild carnivores, have been associated with vomiting, anorexia, weight loss, anemia, and, in severe cases, gastric ulceration and chronic gastritis (Alves et al., 2022; Chen et al., 2023). Despite the rarity of human infections, sporadic cases of physalopteriasis have been reported, suggesting a limited but recognized zoonotic potential (Mohamadain and Ammar,

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2012). Species-level identification within *Physaloptera* remains challenging and has traditionally relied on morphological features such as pseudolabia structure, the number and arrangement of male caudal papillae, spicules morphology, vulvar position, and cuticular ornamentation (Soulsby, 1965). However, morphological characteristics of *Physaloptera* species often overlap among congeners and may vary according to host species, developmental stage, or preservation quality (Pereira et al., 2012). As a result, historical records lack sufficient diagnostic resolution, leading to frequent misidentifications among closely related species (Chen et al., 2023).

In recent years, molecular approaches have been used in spirurid nematodes to clarify classical morphology and improve taxonomic resolution (Chen et al., 2023). Nuclear ribosomal genes such as *18S rRNA*, along with mitochondrial markers, particularly *cytochrome c oxidase subunit I* (*Cox1*), have proven valuable for reconstructing phylogenetic relationships, resolving cryptic diversity, and verifying species boundaries (Maharana et al., 2021; Chen et al., 2023). However, molecular information for physalopterid nematodes remains sparse, and available reference sequences are largely skewed towards isolates from domestic animals from Asia and Europe, with minimal representation from African wildlife (Ferroglio et al., 2009; Maharana et al., 2021).

In Tanzania, *Physaloptera praeputialis* (*P. praeputialis*) has been reported only once based on a coprological study, without genetic confirmation or morphological observation (Müller-Graf, 1995). To date, there are no published integrative morphological and molecular characterizations of adult *Physaloptera* species from wildlife hosts in Tanzania, despite the high diversity of carnivores and the ecological conditions favorable for trophic-transmitted parasites in the Serengeti ecosystem. Large felids, including African lions (*Panthera leo*), are apex predators that frequently consume insectivorous and small vertebrate prey that may act as paratenic hosts for *Physaloptera* spp. Apex predators represent valuable sentinel hosts for investigating the diversity, transmission ecology, and population structure of trophically transmitted helminths (Palomba et al., 2023). Identification of gastric nematodes in apex predators is essential for understanding host–parasite associations, parasite diversity, and the epidemiology of infections in wildlife populations (Moudgil et al., 2015). Characterizing these parasites contributes to improved knowledge of disease ecology and provides baseline information for wildlife health monitoring and conservation. Therefore, the present study aimed to provide the first integrative morphological and molecular characterization of adult *Physaloptera* specimens recovered from the African lions in the Serengeti ecosystem of Tanzania.

MATERIALS AND METHODS

Ethical approval

The objectives and procedures of the present study, which is part of an ongoing project, were reviewed and approved by the Joint Management Research Committee of the Tanzania Wildlife Research Institute. Additionally, research permits were granted by the Commission for Science and Technology Tanzania (CST00001738-2025-2025-01459). Field collections of Physalopterid nematodes were conducted in the Serengeti National Park, following the necessary permissions to enter these protected areas. All nematodes were isolated exclusively from naturally dead African lions encountered during the study.

Study area and specimen collection

The present study was conducted in the Central and Eastern Serengeti National Park within the Serengeti ecosystem in northern Tanzania, between 2023 and 2025 (Figure 1). The area is characterized by semi-arid savannah with woodland mosaics, dominated by open grasslands, acacia woodlands, and riverine vegetation, and supports a large, well-studied population of African lions. The ecosystem experiences a bimodal rainfall pattern with annual precipitation ranging approximately from 500 to 1,200 mm. Mean annual temperatures range from 20°C to 27°C, with daytime maxima often exceeding 30°C during the dry season. Relative humidity varies seasonally, averaging about 40-60% during the dry season and increasing to 65-80% during the wet season, particularly in riverine and grassland habitats (Sinclair et al., 2015). These environmental conditions support diverse intermediate arthropod hosts and contribute to parasite transmission dynamics within the ecosystem. Lion carcasses were found through routine wildlife health surveillance and carcass monitoring programs jointly implemented by the Tanzania Wildlife Research Institute (TAWIRI) and Serengeti National Park veterinary and research units.

Sampling was opportunistic and non-invasive; no lions were euthanized or intentionally sacrificed during sample collection. A total of seven lion carcasses were recovered at different locations and time points within the study area. The carcasses comprised 3 males and 4 females, collected between 2023 and 2025 (2 in 2023, 3 in 2024, and 2 in 2025). All carcasses were classified as fresh to moderately decomposed within a post-mortem interval of ≤ 48-72 hours based on field assessment and were considered suitable for gastrointestinal parasitological examination. Age estimation was conducted by trained wildlife veterinarians and carnivore specialists during post-mortem examination using established

criteria based on dental eruption and wear patterns, combined with body condition assessment and cranial morphology (Whitman et al., 2007). Two lions were classified as subadults, approximately 4-5 years, three as prime adults of 6-9 years, and two older adult lions of about 10-14 years. Three lions had been observed intermittently for 2-3 weeks prior to death and showed signs of poor body condition, including marked emaciation and reduced mobility. However, no definitive cause of morbidity could be established prior to death, and the observed clinical deterioration was considered multifactorial and potentially associated with age-related decline, injury, social displacement, or underlying disease processes.

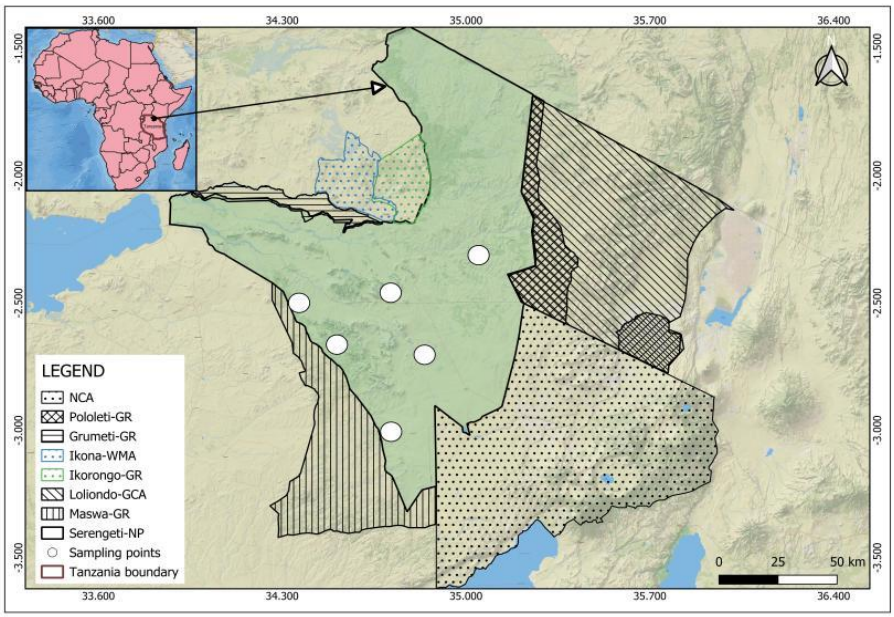

Figure 1. Location of the study sites in the Serengeti National Park within the Serengeti ecosystem, Tanzania

Necropsy and ecological observations

The isolation of nematodes was performed following standard gastrointestinal parasitological necropsy procedures with minor modifications (Anderson, 2000; Wobeser, 2007). The abdominal cavity was opened using sterile dissection tools, and the stomach and small intestine were removed sequentially to minimize cross-contamination between organs. Careful separation of gastrointestinal compartments was maintained throughout the procedure to ensure accurate parasite recovery and identification (Wobeser, 2007). The nematode worms were recovered using fine forceps, rinsed in 0.85% physiological saline solution (Oxoid Ltd., United Kingdom), counted, and preserved in 70% ethanol (Sigma-Aldrich, USA) for molecular studies and in 10% neutral buffered formalin (Sigma-Aldrich, USA) for subsequent morphological examination, following standard helminth preservation protocols (Wobeser, 2007). During necropsy, stomach content composition, parasite burden, gross pathological changes, and overall organ condition were recorded.

Coprological investigation

Fecal samples collected from the rectum were examined using the formalin ether concentration technique (Sato et al., 2014; Khanna et al., 2018) with minor modifications. Briefly, approximately 10g of fecal sample was thoroughly homogenized to ensure uniform distribution of parasite stages, after which 1g aliquots were transferred into 15 mL conical-bottom polypropylene tubes (Falcon®, USA) containing 7 mL of 10% formalin. The reduced aliquot volume was used to optimize sedimentation efficiency and microscopic clarity during concentration procedures. Subsequently, 3 mL of diethyl ether (Sigma-Aldrich, USA) was added, and the suspension was vigorously mixed for approximately one minute with intermittent venting to release internal pressure. Samples were centrifuged at 500 × g for 10 minutes, after which the supernatant layers were carefully decanted. The sediment was washed twice to improve preparation clarity and then resuspended in 200 µL of formalin-saline. The remaining pellets were resuspended in 200 µL of formalin-saline. Two slides per sample were prepared using 30 µL aliquots and examined systematically under a light microscope (Olympus, Japan) at 100× and 400× magnification. Helminth eggs were identified based on morphological characteristics using standard diagnostic keys (Sohn and Chai, 2024).

Morphological identification of *Physaloptera* species

Prior to morphological identification, recovered nematodes were gently rinsed in 0.85% physiological saline solution (Oxoid Ltd., United Kingdom) to remove adherent debris and subsequently preserved in pre-heated 10% buffered formalin (Sigma-Aldrich, USA). Specimens were cleared in lactophenol prepared from phenol crystals

(C_6H_5OH; Sigma-Aldrich, USA), glycerol (Merck KGaA, Germany), lactic acid ($CH_3CHOHCOOH$; Thermo Fisher Scientific, USA), and distilled water in proportions of 20 g, 40 mL, 20 mL, and 20 mL, respectively, to enhance visualization of diagnostic morphological structures (Rana et al., 2020). Morphological identification was performed using a light microscope (Olympus, Japan) following established taxonomic keys (Anderson et al., 2009). Diagnostic characters examined included cephalic collarette and pseudolabia, spicule length and morphology, arrangement of pedunculate and sessile caudal papillae, and vulvar position and morphology in females. Representative specimens were measured using calibrated ocular micrometry, and images were captured for documentation (Maggenti, 2020).

Molecular identification of *Physaloptera* species

PCR and DNA sequencing

Nine adult nematodes (three specimens per host) obtained from three representative lions were selected for molecular analyses to assess species identity and intra-population genetic variation. Specimens were rinsed three times in sterile phosphate-buffered saline (PBS) solution (Thermo Fisher Scientific, USA) to remove residual host material. Worms were then mechanically homogenized in 180 µL ATL tissue lysis buffer (Qiagen, Germany) supplemented with 20 µL proteinase K (20 mg/mL) and incubated overnight at 56°C until complete tissue digestion. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, USA) following the manufacturer's protocol and eluted in 50 µL of TE buffer composed of 10 mM Tris-HCl and 1 mM EDTA at pH 8.0. DNA concentration and purity were quantified using a UV–Vis spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific, USA), and DNA integrity was confirmed by agarose gel electrophoresis. Partial fragments of the *mitochondrial cytochrome c oxidase subunit I* (*cox1*) gene and the nuclear *small subunit ribosomal RNA* (*18S rRNA*) gene were amplified using species-specific conserved primers listed in Table 1. Polymerase chain reaction assays were performed in 25 µL reaction volumes containing 2.5 µL of 10× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.4 µM of each primer, 1 U Taq DNA polymerase (Takara Bio Inc., Japan), and approximately 50 ng of genomic DNA template with nuclease-free water added to the final volume. Thermal cycling conditions for both *cox1* and *18S rRNA* consisted of initial denaturation at 95°C for three minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at primer-specific temperatures for 30 seconds, and extension at 72°C for 60 seconds, with a final extension step at 72°C for seven minutes. Negative controls included a no-template control containing nuclease-free water instead of DNA and an extraction blank processed alongside samples to monitor contamination. Polymerase chain reaction products were separated by electrophoresis on 1.5% agarose gels in 1× TBE buffer at 100 V for 45 minutes, stained with ethidium bromide, and visualized under UV illumination. Amplified products were purified prior to bidirectional Sanger sequencing.

Table 1. Primers of *Cytochrome oxidase 1* and *18s ribosomal RNA* used in the study

Target gene	Primer name	Primer sequence (5′→3′)	Product length (bp)	Reference
Cox 1	Cophy F Cophy R	GGGCAGGATTAGGAGGTTCTG AAGCCCCAGCCAAAACTGGAA	525	(Kalyanasundaram et al., 2018)
18srRNA	Physa F Physa R	GCGAACGGCTCATTATAA AATTTCACCTCTCAGCA	745	(Maldonado et al., 2019)

DNA sequence analyses

DNA sequences of the mitochondrial *cox1* and *18S rRNA* genes were assembled and edited using Geneious R9.1 software (Biomatters Ltd., New Zealand) following standard sequence processing workflows (Kearse et al., 2012). Consensus sequences were aligned using MUSCLE and trimmed to uniform lengths. Comparative *Cox1* sequences were retrieved from Genbank, including *Physaloptera* spp. WTL (LC596961), *Physaloptera* spp. SNM (MH752202), *Physaloptera* spp. BRM (MW517846) and *Physaloptera* spp. haplotype D KG (OP589140), *Onchocerca lupi* (JX080028), *Dirofilaria immitis* (KT282097), *Brugia malayi* (OQ727412), and the outgroup was *Gnathostoma turgidum* (KT894798) was selected as an outgroup based on its phylogenetic distance within Spirurida. For *18S rRNA* analyses, reference sequences included *Physaloptera praeputialis* (MW410927), *Physaloptera retusa* (KT894814), *Physaloptera turgida* (MH748145), *Physaloptera rara* (MH938367), *Physaloptera sibirica* (OQ846902), *Heliconema longissimum* voucher IPCAS N-862/2 (JF803949), *Abbreviata antarctica* (KX255660), *Abbreviata hastaspicula* (KX255661), *Dirofilaria immitis* (OP811190), *Brugia malayi* (OQ727413), with *Gnathostoma turgidum* (Z96948) as the outgroup.

Phylogenetic analysis using the partial sequences of *Cox1* and *18S RNA* was evaluated by the most appropriate nucleotide substitution model using the Bayesian Information Criterion in Molecular Evolution Genetics Analysis, MEGA v11.0 (Tamura et al., 2021). Maximum-likelihood (ML) phylogenies were reconstructed in MEGA with 1,000 bootstrap replicates. Bayesian phylogenetic analyses were performed using BEAST v1.10.4 (Suchard et al., 2018), which implements a Bayesian inference framework for evolutionary reconstruction. Population genetic indices, including

number of haplotypes, haplotype diversity (Hd), nucleotide diversity (π), number of polymorphic sites, total mutations, and mean pairwise nucleotide differences, were calculated using DnaSP v6.12.03 (Librado and Rozas, 2009).

RESULTS

Parasite burden and host infection intensity

A total of 157 adult spirurid nematodes were recovered from the stomachs of three infected African lions (Figure 2). The remaining four lions examined during the study were negative for *Physaloptera* infection based on necropsy and gastrointestinal inspection. Of the total parasite collection, 105 specimens were recovered from two infected hosts, a 9-year-old female (n = 57) and a 12-year-old male (n = 48), while 52 worms were recovered from a third female lion estimated to be approximately 10 years old. Both sexes exhibited a thick cuticle forming a posteriorly extended caudal sheath (Figure 2). The anterior extremity bore a well-developed cephalic collarette with partially submerged pseudolabia armed with flattened internal tooth-like projections and a single external conical tooth, a configuration characteristic of the genus *Physaloptera* (Figure 3).

Morphometrics and key diagnostic features of male specimens

Male *Physaloptera* specimens measured 48.5-50.2 mm in length and 1.8-2.1 mm in maximum width (Table 2). The muscular-glandular esophagus of male *Physaloptera* spp. was long and robust, measuring 6.8-7.1 mm, representing approximately 14% of total body length. In male *Physaloptera* spp., the nerve ring, deirids, and excretory pore were located at 1.1-1.4 mm, 1.2-1.4 mm, and 7.9-8.3 mm from the anterior extremity, respectively (Table 2). The posterior end of male *Physaloptera* spp. bore prominent lateral alae forming a wing-like bursa structure (Figure 3). Spicules of male *Physaloptera* spp. were markedly unequal and dissimilar; the left spicule was longer and lanceolate, measuring 1.5-1.7 mm in length, while the right spicule was shorter and curved, measuring 1.1-1.3 mm, without terminal dilation. The cloaca of male *Physaloptera* spp. was situated 0.9-1.2 mm from the posterior extremity (Figure 3).

Morphometrics and reproductive features of female *Physaloptera* species

Female *Physaloptera* spp. were larger than male worms, measuring 59.5-77.5 mm in length and 2.1-2.4 mm in width (Table 3). The total esophageal length in female *Physaloptera* spp. was 8.5-9.3 mm. The vulva of female *Physaloptera* spp. opened at 25.2-27.5 mm from the anterior extremity, corresponding to 34-42% of total body length. The anus of female *Physaloptera* spp. was located 1.5-1.6 mm from the posterior extremity. Uteri of female *Physaloptera* spp. were filled with numerous embryonated eggs morphologically identical to those recovered from fecal samples (Figure 4). Coprological examination of *Physaloptera* infection in African lions revealed oval, thick-shelled larvated eggs measuring 33-36 × 40-45 µm in all infected hosts.

Molecular identification of *Physaloptera* species and phylogenetic analysis

Partial fragments of the mitochondrial *cytochrome c oxidase subunit I* (*cox1*, approximately 525 bp) and nuclear *18S rRNA* (approximately 748 bp) genes were successfully amplified from all nematode specimens selected for molecular analysis and yielded clear single bands on 1.5% agarose gels (Figure 5). Sequencing was performed on representative PCR-positive amplicons, and all obtained sequences were identical across specimens with no observed intraspecific variation. Representative sequences were deposited in GenBank under accession numbers OR821828 (*cox1*) and OR821831 (*18S rRNA*).

BLAST analysis confirmed that the *Cox1* sequences belong to the genus *Physaloptera*. Pairwise sequence similarities with available *Physaloptera* references ranged from 88% to 88.6% identity, with the highest similarity to *P. retusa (*KT894803), *P. mirandai* (KP981418), and other *Physaloptera* isolates with accession numbers MW517846, LC381943, and LC596961 (Figure 6). The alignment contained 141 polymorphic sites and 193 mutational events, yielding 10 haplotypes with high haplotype diversity (Hd: 0.982 ± 0.046). Phylogenetic reconstruction based on *Cox1* sequences grouped the Tanzanian isolate within the Physalopteridae clade; however, as a distinct mitochondrial lineage, separated from clusters corresponding to *P. retusa* and *P. mirandai*, and formed a sister lineage to reference sequences identified as *Physaloptera* spp. (Figure 6).

The partial *18S rRNA* sequence exhibited high similarity of 98.1-99.5% to multiple *Physaloptera* species, including *P. praeputialis* (MW410927), *P. rara* (MH938367), *P. sibirica* (OQ846902), *P. retusa* (KT894814), and *P. turgida* (MH748145). Pairwise divergence between the Tanzanian isolate and *P. praeputialis* from domestic cats in India was 0.5%. Across 15 aligned sequences, 51 polymorphic sites and 65 mutational events were detected, generating 14 haplotypes (Hd: 0.9905 ± 0.028; π: 0.0607). Phylogenetic inference based on *18S rRNA* placed the Tanzanian isolate within a well-supported *Physaloptera clade*, clustering closely with reference sequences of *P. praeputialis* (Figure 7).

Table 2. Comparative morphometric features of male *Physaloptera* species recovered from different carnivore hosts, including specimens obtained from African lions in the Serengeti ecosystem, Tanzania, between 2023 and 2025

Species	***P. torquata***	***P. maxillaris***	***P. rara***	***P. praeputialis***	***P. praeputialis***	***P. praeputialis***
Reference	**Morgan (1942)**	**Morgan (1943)**	**Ackert (1941)**	**Linstow (1889)**	**Linstow (1889)**	**Current study**
Host	***Taxidea taxus***	***Mephitis mephitis***	***felis domestica***	***Felis catus***	***Felids***	***Panthera leo***
Body length (mm)	20 - 23	20 - 26	25 - 29	21	13 -40	48.5 - 50.2
Maximum width (mm)	0.56 - 0.79	0.55 - 0.88	0.71 - 0.80	1.5	--	1.8 - 2.1
Esophagus length (mm)	3.4 - 4.1	3.1 - 5.3	4.7 - 5.3	--	--	6.8 - 7.1
Nerve ring (mm)	--	--	--	--	--	1.1 - 1.4
Deirids (mm)	--	--	--	--	--	1.2 - 1.4
Excretory pore (mm)	--	--	--	--	--	7.9 - 8.3
Right spicule length (mm)	0.53 - 0.80	0.55 - 0.62	0.51 - 0.60	--	0.84 - 0.9	1.1-1.3
Left spicule length (mm)	0.604 - 0.854	0.84 - 0.99	0.67 - 0.83	--	1 - 1.2	1.5 - 1.7
Tail length/ cloaca (mm)	--	--	--	--	--	0.9 - 1.2

Table 3. Comparative morphometric characteristics of female *Physaloptera* species recovered from different carnivore hosts, including specimens obtained from African lions in the Serengeti ecosystem, Tanzania, between 2023 and 2025

Species	***P. torquata***	***P. maxillaris***	***P. rara***	***P. praeputialis***	***P. praeputialis***
Reference	**Morgan (1942)**	**Morgan (1943)**	**Ackert (1941)**	**Linstow (1889)**	**Current study**
Host	***Taxidea taxus***	***Mephitis mephitis***	***Felis domestica***	***Felis catus***	***Panthera leo***
Body length (mm)	30 - 38	21 - 29	27 - 44	30	59.5 - 77.5
Maximum width (mm)	1.2 - 1.6	0.84 - 1.2	0.56 - 1.1	2	2.1 - 2.4
Esophagus length (mm)	3.4 - 4.1	4.3 - 6.1	6.6 - 7.8	--	8.5 - 9.3
Muscular portion length (mm)	--	--	--	--	6.1 - 6.5
Glandular portion length (mm)	--	--	--	--	8.8 - 9.3
Nerve ring from the anterior extremity (mm)	--	--	--	--	0.8 - 1.0
Deirids (mm)	--	--	--	--	1.8 - 2.2
Excretory pore (mm)	--	--	--	--	8.9 - 9.7
Vulva opening (mm)	5.5 - 10.1	4.6 - 8.3	3.2 - 5.8	8	25.2 - 27.5
Tail length/ cloaca (mm)	--	--	--	--	1.5 - 1.6
Egg (µm)	51 - 33	47 × 32	29-35 × 42-46	33 - 55	32.5 -35.5 × 40-45

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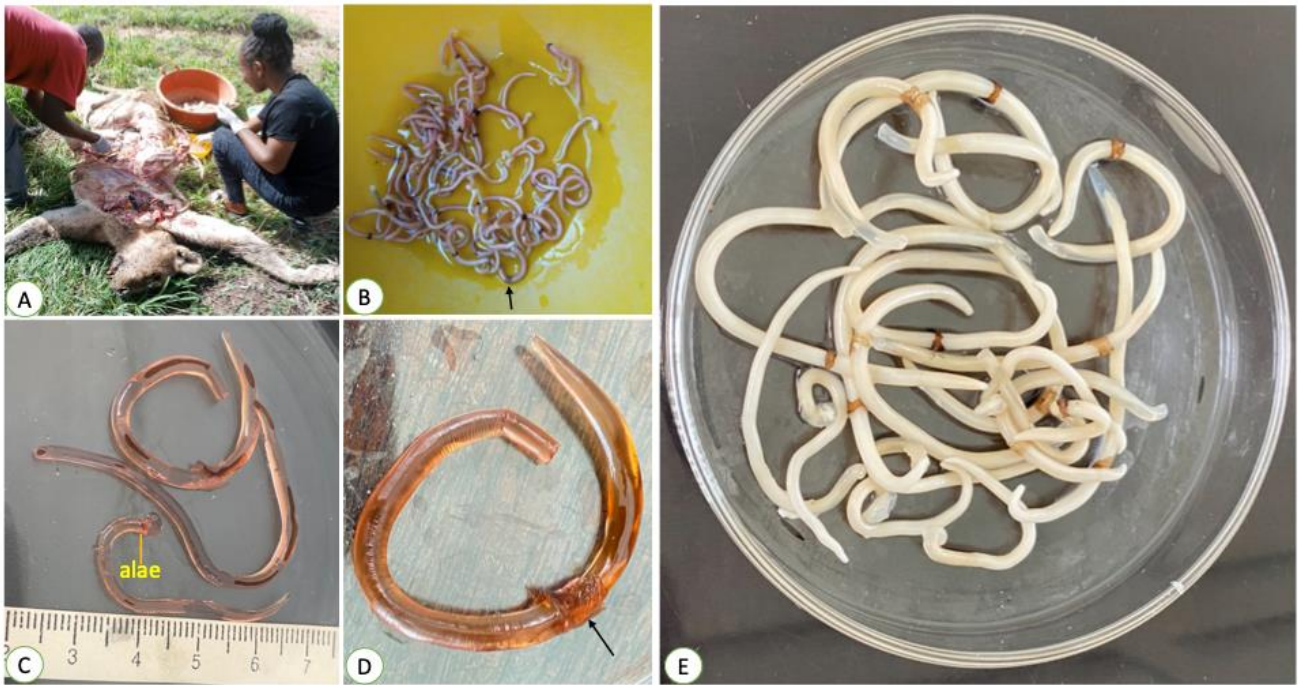

Figure 2. Adult *Physaloptera praeputialis* recovered from African lions (*Panthera leo*) in the Serengeti ecosystem, Tanzania, between 2023 and 2025. **A:** Freshly recovered specimens of *P. praeputialis* immediately after collection from the host are maintained in a collection container prior to processing. **B:** Additional adult specimens separated during initial handling. **C:** Posterior end of adult male showing the caudal bursa with lateral alae (wing-like structures). **D:** Female specimen showing brown cement-like material adherent to the cuticle (black arrow). **E:** Specimens were transferred to a Petri dish for cleaning and detailed morphological examination, showing improved visibility of diagnostic structures compared with the initial collection state.

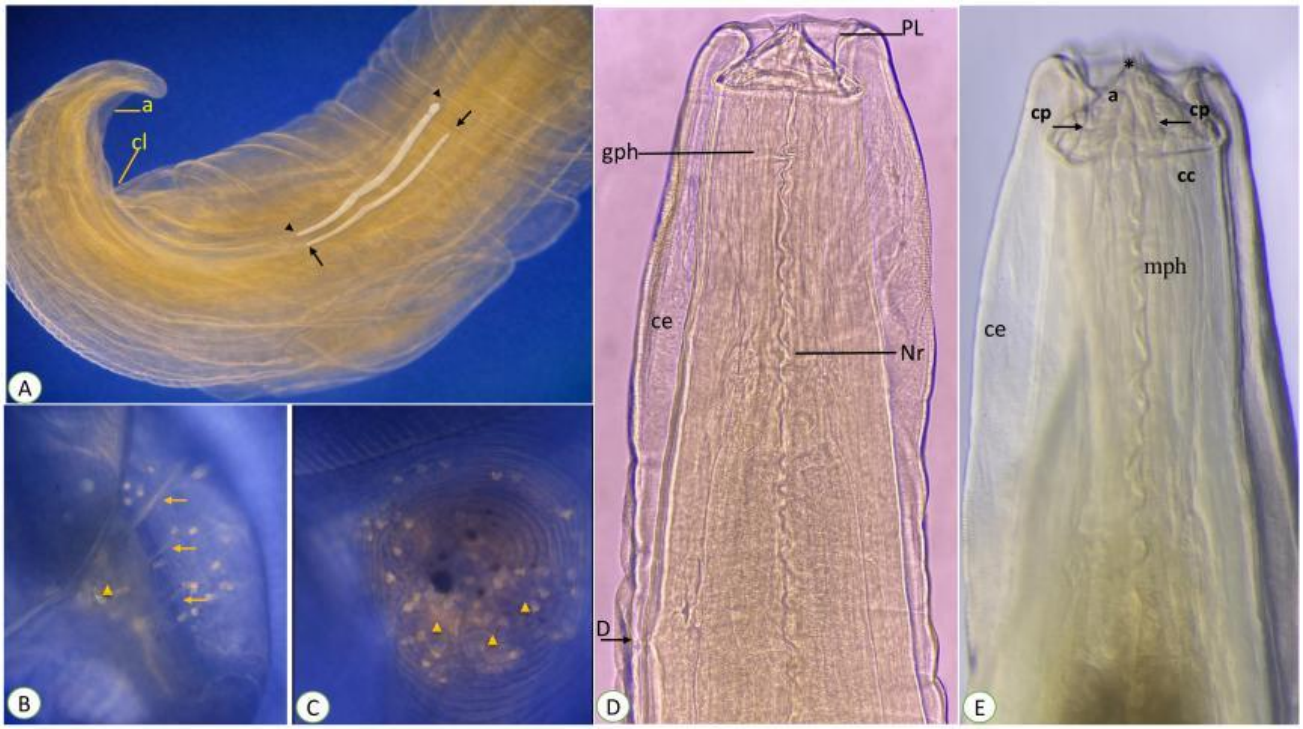

Figure 3. Morphological features of male *Physaloptera praeputialis* recovered from African lions (*Panthera leo*) in the Serengeti ecosystem, Tanzania, between 2023 and 2025. **A:** Ventral view of posterior region showing alae (a), cloaca (cl), right spicule (arrow), and left spicule (arrowheads). **B and C:** Ventral view of posterior extremity showing pedunculate papillae (arrows) and sessile papillae (arrowheads). **D:** Anterior extremity showing pseudolabia (PL), glandular pharynx (gph), cuticular expansion (ce), deirid (D), and nerve ring (Nr). **E:** Cephalic region showing cephalic papillae (cp), amphids (a), cephalic collar (cc), muscular pharynx (mph), and internal tooth (asterisk). Scale bars: 200 µm **(A-D**, **F-G)**; 100 µm **(E)**.

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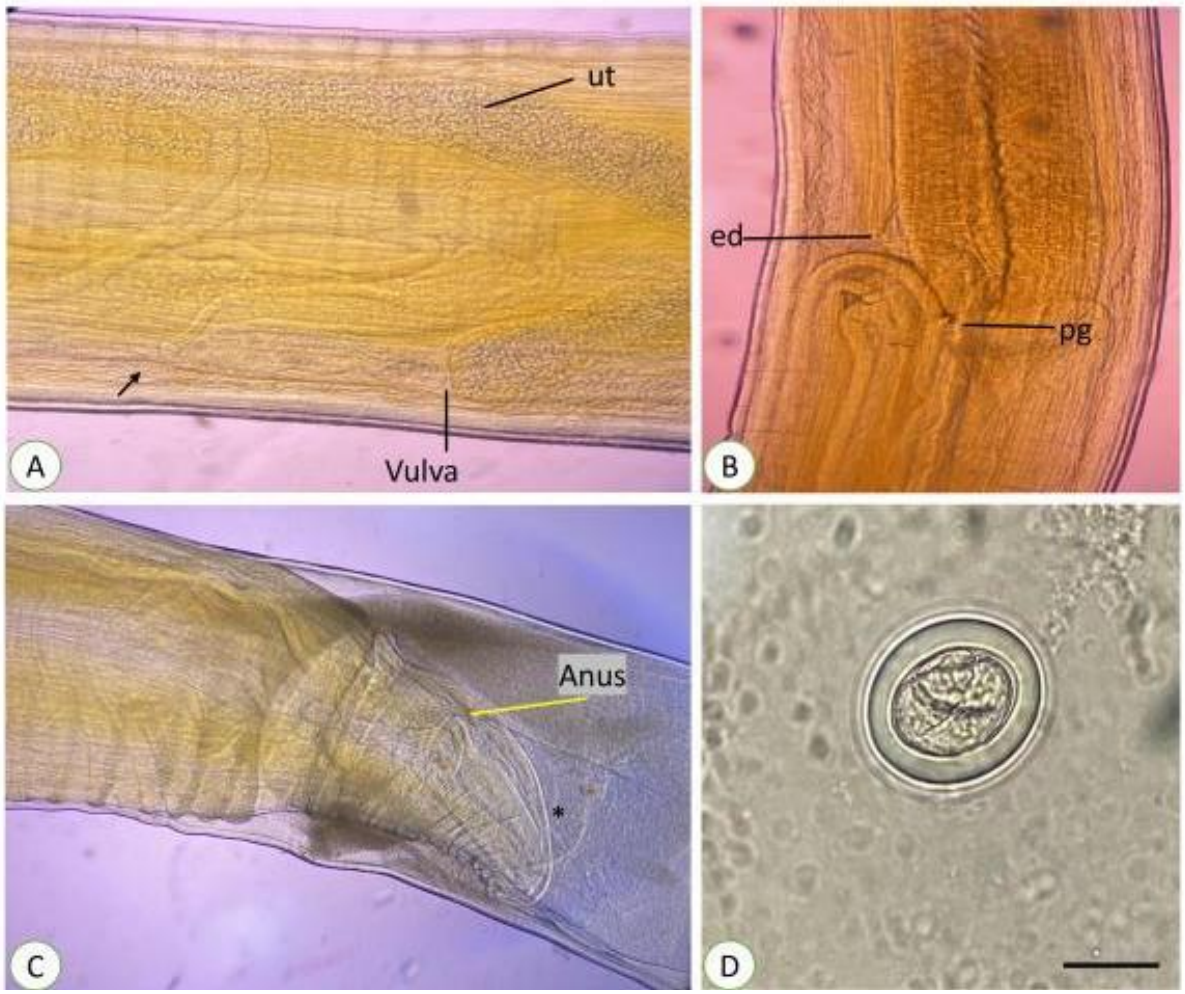

Figure 4. Female reproductive system of *Physaloptera praeputialis*. Recovered from African lions (*Panthera leo*) in the Serengeti ecosystem, Tanzania, between 2023 and 2025. **A:** Uterus (ut) and vaginal duct (arrows). **B:** Anterior region of female showing the excretory duct (ed) and pharyngeal-gastric valve (pg). **C:** Posterior end of adult female showing the anus and anal pouch (asterisk). **D:** Larvated egg recovered from fecal sample (×40 magnification).

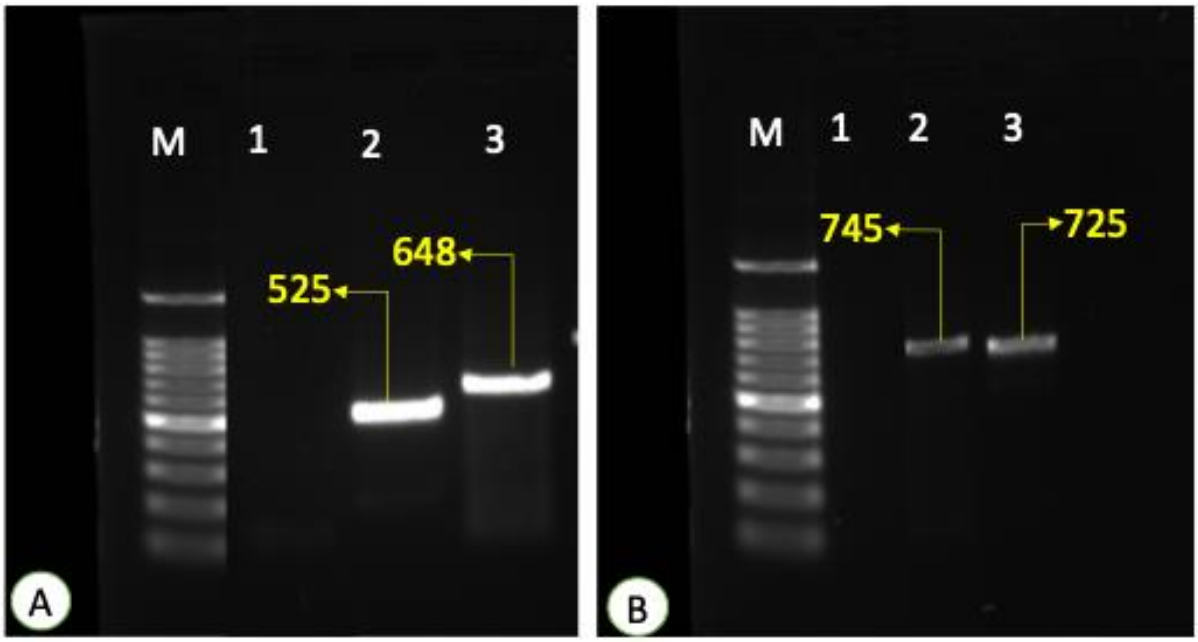

Figure 5. Agarose gel electrophoresis (1.5%) showing PCR amplification of mitochondrial *cox1* and nuclear *18S rRNA* genes of *Physaloptera praeputialis* isolated from African lions (*Panthera leo*) in the Serengeti ecosystem, Tanzania, collected between 2023 and 2025. **A:** Amplification of the *cox1* gene showing positive bands at approximately 525 bp (lane 2) and 648 bp (lane 3), negative control (lane 1; no-template control), and molecular weight marker (100 bp DNA ladder, lane M). **B:** Amplification of the *18S rRNA* gene showing positive bands at approximately 745 bp (lane 2) and 725 bp (lane 3), negative control (lane 1; no-template control), and molecular weight marker (100 bp DNA ladder, lane M).

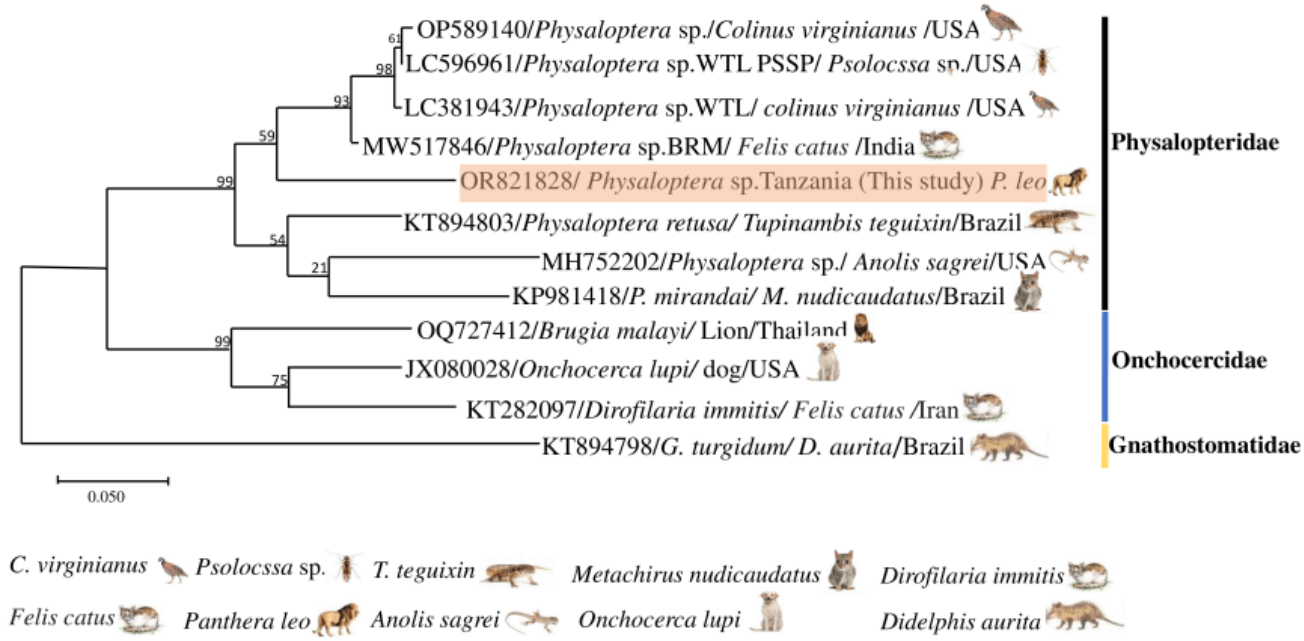

Figure 6. Phylogenetic tree based on mitochondrial *cox1* sequences showing the relationships of *Physaloptera* spp. (OR821828) isolates obtained from *Panthera leo* in the Serengeti ecosystem, Tanzania, collected between 2023 and 2025, together with reference sequences retrieved from GenBank.

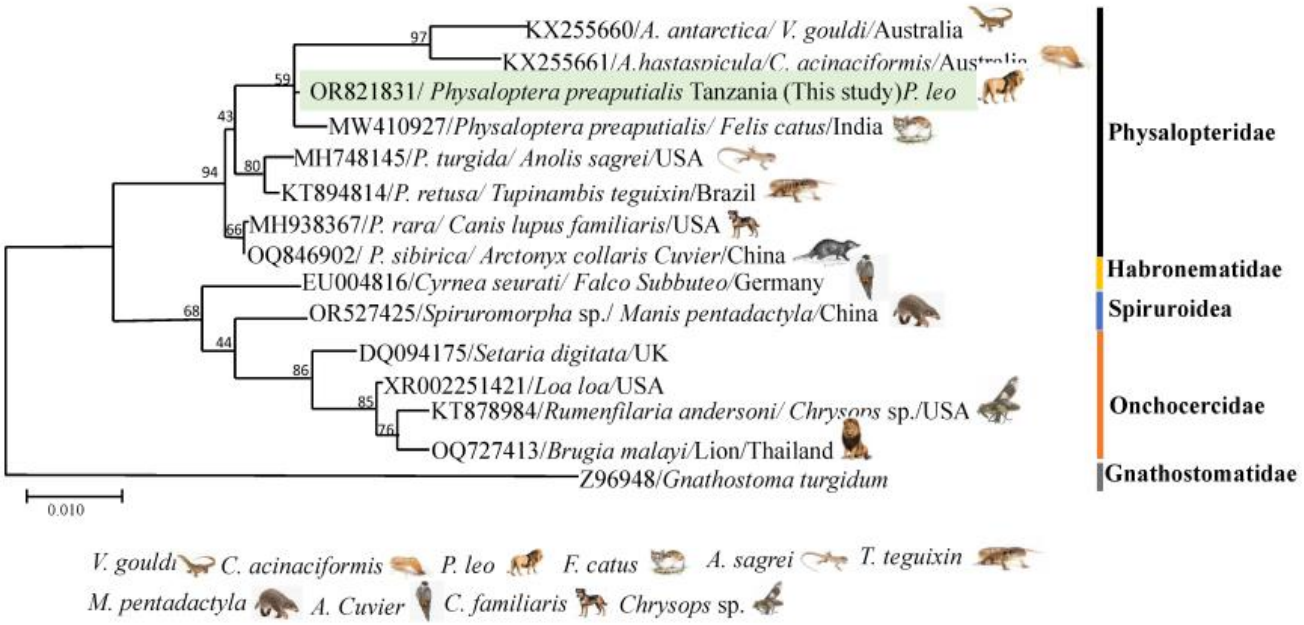

Figure 7. Phylogenetic tree based on nuclear *18S rRNA* gene sequences showing the relationships of *Physaloptera praeputialis* isolates obtained from *Panthera leo* in the Serengeti ecosystem, Tanzania, collected between 2023 and 2025, together with reference sequences retrieved from GenBank.

DISCUSSION

The combined morphological and molecular evidence supports the identification of the Tanzanian *Physaloptera* specimens as *P. praeputialis*, extending current knowledge of its host range to African lions in Tanzania. Previous

reports of physalopterids infections in Tanzania were based primarily on coprological examinations and identified parasites only to the genus level without attempting species-level identification (Müller-Graf, 1995). The present findings establish an essential taxonomic and molecular baseline for future ecological, epidemiological, and evolutionary investigations of spirurid nematodes in African wildlife.

In the current study, adult *P. praeputialis* specimens were associated with erosive and hemorrhagic gastritis in the stomach of the African lions. Similar lesions have been reported in domestic and wildlife infected with *Physaloptera* spp., where persistent mucosal attachment, mechanical abrasion, hematophagy, and secondary bacterial invasion contribute to chronic inflammation (Soulsby,1965; Naem and Asadi, 2013). Although parasite burden was not quantified, the observed mucosal damage suggests that *P. praeputialis* may contribute to subclinical morbidity in lions, particularly in aged or nutritionally compromised individuals (Anderson et al., 2009). In apex predators such as lions, chronic gastric inflammation has the potential to reduce digestive efficiency and exacerbate energy stress during periods of prey scarcity (Anderson, 2000; Wobeser, 2007). However, direct impacts on fitness, hunting success, or survival remain speculative and should be evaluated through quantitative parasitological and longitudinal health studies (Packer et al., 2005).

Morphological characterization observed in adult worms, such as cephalic structures, spicule morphology, and caudal papillae arrangement, is consistent with classical descriptions of genus *P. praeputialis*, yet precise species validation remains challenging due to morphological similarity and phenotypic plasticity (Anderson et al., 2009). In the present study, the concordance between morphology and nuclear genetic data supports placement within the *P. praeputialis* species complex, but does not clearly resolve species boundaries. Integrative taxonomy combining morphology, multiple genetic loci, host associations, and biogeography is necessary for resolving spirurid systematics (Dayrat, 2005; Ailán-Choke and Pereira, 2021). Phylogenetic analyses based on both nuclear and mitochondrial loci place the Tanzanian isolates within the *P. praeputialis* clade. The high genetic similarity observed in the *18S rRNA* gene relative to reference sequences reflects the slow evolutionary rate and confirmation of generic placement and deep phylogenetic relationships (Blouin, 2002). Mitochondrial *cox1* sequences from the present *Physaloptera* specimens exhibited substantial nucleotide divergence and high haplotype diversity compared with nuclear *18S rRNA* sequences, a pattern commonly reported in spirurid nematodes and other parasitic helminth groups, including cestodes and trematodes (Maharana et al., 2021; Chen et al., 2023). The mean sequence divergence relative to other physalopterid sequences exceeds typical intraspecific variation reported for nematodes and overlaps with divergence levels among closely related species, consistent with species-level generic differentiation (Blouin et al., 1995; Powers, 2004). Phylogenetic reconstruction places the Tanzanian isolates within the *P. praeputialis* clade, consistent with long-term population subdivision and restricted gene flow among geographically separated parasite populations (Blouin et al., 1995; Nadler and De León, 2011). The magnitude of *Cox1* divergence observed in the present *P. praeputialis* isolates falls within previously reported interspecific divergence levels for spirurid nematodes (Maharana et al., 2021). The observed pattern may reflect deep phylogeographic structuring within a broadly distributed *P. praeputialis* species complex or cryptic diversification in African populations that is not detectable using conserved nuclear markers alone (Maharana et al., 2021).

Lions occupy the apex of a highly connected trophic web linking herbivorous prey, insect communities, scavengers, and domestic carnivores at the wildlife-livestock interface (Sinclair et al., 2015). Such ecological connectivity enables *Physaloptera* spp. to utilize insects as intermediate hosts and vertebrates as paratenic hosts, expecting transmission to be strongly structured by both food-web architecture and host association (Dobson et al., 2009). The Serengeti ecosystem is characterized by high prey biomass and abundant coprophagous insects, which likely sustain stable enzootic transmission cycles (Sinclair et al., 2015). African lions may function as terminal accumulation hosts rather than key maintenance hosts, acquiring infections through repeated ingestion of infected prey (Bjork et al., 2000; Green et al., 2020; Tull et al., 2022). Such ecological connectivity can promote higher mitochondrial genetic variation while nuclear genes remain more conserved due to occasional gene flow among host species and across different habitats (Nadler et al., 2011). Although numerous parasitological investigations in East Africa have focused on domestic animals, molecular characterization of helminths infecting wildlife remains comparatively limited, highlighting the need for integrative studies to improve understanding of parasite diversity and transmission dynamics in the region (Brooks and Hoberg, 2007).

As a result, the diversity and distribution of spirurid nematodes in Tanzania remain poorly resolved. The presence of *P. praeputialis* in the Serengeti ecosystem highlights the hidden parasite diversity in protected areas and the role of apex predators as indicators of ecosystem health (Bjork et al., 2000; Han et al., 2021; Moraes et al., 2024).

Although *Physaloptera* spp. are primarily parasites of carnivorous vertebrates, human exposure may occasionally occur through accidental ingestion of infected intermediate hosts such as insects; however, confirmed human infections are extremely rare and not well documented in the literature (Anderson, 2000). While the public health risk is likely low,

the detection of *P. praeputialis* populations in human-wildlife proximity highlights the importance of incorporating spirurid nematodes into broader helminth surveillance frameworks (Ma et al., 2020). Establishing molecular reference sequences from Tanzania also strengthens regional parasite biobanking initiatives and enables future monitoring of parasite dispersal associated with land-use change, climate variability, and carnivore population dynamics.

CONCLUSION

The current study presents the first characterization of adult *P. praeputialis* recovered from the African lion in Tanzania using integrative morphological and molecular approaches. The parasite exhibits substantial mitochondrial diversity while maintaining nuclear genetic cohesion, consistent with geographically structured populations circulating within complex Serengeti food webs. The findings expand the known range of *P. praeputialis*, emphasize the ecological role of apex predators in helminth transmission networks, and provide a molecular baseline for future investigations on spirurid diversity, wildlife health, and ecosystem stability in Tanzania. Interpretation of the findings should consider the limited sample size, opportunistic recovery from naturally deceased hosts, absence of intermediate host investigations, and analysis based on only two genetic loci.

DECLARATIONS

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Authors' contributions

Barakaeli Abdieli Ndossi, Maulid Mzinga Mdaki, and Eblate Ernest Mjingo were responsible for the conceptualization. Mohammed Mebarek Bia and Barakaeli Abdieli Ndossi contributed to the formal analysis. Barakaeli Abdieli, Ndossi, and Mohammed Mebarek Biawere conducted the methodology. Barakaeli Abdieli Ndossi, Maulid Mzinga Mdaki, Heejae Yang, and Marry Wokusima Zebedayo participated in the resources. Keeseon Eom, Mohammed Mebarek Bia, and Eblate Ernest Mjingo were responsible for the validation. Barakaeli Abdieli Ndossi prepared the original draft. Mohammed Mebarek Biaand Barakaeli Abdieli Ndossi contributed to writing, reviewing, and editing. All authors have read and approved the final version of the manuscript before publication in the present journal.

Availability of data and materials

The data to support the findings of the present study are available upon reasonable request from the corresponding author.

Competing interests

We have no conflict of interest related to the present study.

Ethical considerations

The authors declared that the manuscript is original, has not been published elsewhere, and confirmed the latest version before publication. The authors declared that no AI tools were used for writing and preparing the article.

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REFERENCES

Ailán-Choke LG and Pereira FB (2021). Deep in the systematics of *Camallanidae* (Nematoda): Using integrative taxonomy to better understand the phylogeny and consistency of diagnostic traits. Parasitology, 148(8): 962-974. DOI: https://www.doi.org/10.1017/S0031182021000706

Alves PV, Couto JV, and Pereira FB (2022). Redescription of the two most recorded *Physaloptera* (Nematoda: *Physalopteridae*) parasitizing lizards in the Americas: First step towards a robust species identification framework. Systematic Parasitology, 99(1): 63-81. DOI: https://www.doi.org/10.1007/s11230-021-10016-2

Anderson RC (2000). Nematode parasites of vertebrates: Their development and transmission. CABI., New York, pp. 1-16. DOI: https://www.doi.org/10.1079/9780851994215.0001

Anderson RC, Chabaud AG, and Willmott S (2009). Keys to the nematode parasites of vertebrates. Archival volume 2. Parasites & Vectors, 2: 42. DOI: https://www.doi.org/10.1186/1756-3305-2-42

Blouin MS (2002). Molecular prospecting for cryptic species of nematodes: Mitochondrial DNA versus internal transcribed spacer. International Journal for Parasitology, 32(5): 527-531. DOI: https://www.doi.org/10.1016/S0020-7519(01)00357-5

Blouin MS, Yowell CA, Courtney CH, and Dame JB (1995). Host movement and the genetic structure of populations of parasitic nematodes. Genetics, 141(3): 1007-1014. DOI: https://www.doi.org/10.1093/genetics/141.3.1007

Bjork KE, Averbeck GA, and Stromberg BE (2000). Parasites and parasite stages of free-ranging wild lions (*Panthera leo*) of northern Tanzania. Journal of Zoo and Wildlife Medicine, 31(1): 56-61. DOI: https://www.doi.org/10.1638/1042-7260(2000)031[0056:PAPSOF]2.0.CO;2

Brooks DR and Hoberg EP (2007). How will global climate change affect parasite–Host assemblages?. Trends in parasitology, 23(12): 571-574. DOI: https://www.doi:10.1016/j.pt.2007.08.016

Chen HX, Zeng JL, Gao YY, Zhang D, Li Y, and Li L (2023). Morphology and genetic characterization of *Physaloptera sibirica* Petrow & Gorbunov, 1931 (Spirurida: *Physalopteridae*), from the hog-badger Arctonyx collaris Cuvier (Carnivora: *Mustelidae*), with molecular phylogeny of *Physalopteridae*. Parasites & Vectors, 16(1): 227. DOI: https://www.doi.org/10.1186/s13071-023-05838-6

Dayrat B (2005). Towards integrative taxonomy. Biological Journal of the Linnean Society, 85(3): 407-417. DOI: https://www.doi.org/10.1111/j.1095-8312.2005.00503.x

Dobson A, Lafferty KD, Kuris AM, Hechinger RF, and Jetz W (2008). Homage to Linnaeus: How many parasites? How many hosts?. Proceedings of the National Academy of Sciences, 105(1): 11482-11489. DOI: https://www.doi.org/10.1073/pnas.0803232105

Faria RV, Giese EG, and Lanfredi RM (2019). Taxonomy of *Physaloptera mirandai* (Nematoda: *Physalopteridae*) based on three-dimensional microscopy and phylogenetic positioning. Acta Tropica, 195: 115-126. DOI: https://www.doi.org/10.1016/j.actatropica.2019.04.002

Ferroglio E, Ragagli C, and Trisciuoglio A (2009). *Physaloptera sibirica* in foxes and badgers from the Western Alps (Italy). Veterinary Parasitology, 163(1-2): 164-166. DOI: https://www.doi.org/10.1016/j.vetpar.2009.04.005

Green J, Jakins C, Asfaw E, Bruschi N, Parker A, de Waal L, and D'Cruze N (2020). African lions and zoonotic diseases: Implications for commercial lion farms in South Africa. Animals, 10(9): 1692. DOI: https://www.doi.org/10.3390/ani10091692

Han BA, Castellanos AA, Schmidt JP, Fischhoff IR, and Drake JM (2021). The ecology of zoonotic parasites in the Carnivora. Trends in Parasitology, 37(12): 1096-1110. DOI: https://www.doi.org/10.1016/j.pt.2021.08.006

Kalyanasundaram A, Henry C, Brym MZ, and Kendall RJ (2018). Molecular identification of *Physaloptera* spp. from wild northern bobwhite (*Colinus virginianus*) in the Rolling Plains ecoregion of Texas. Parasitology Research, 117(9): 2963-2969. DOI: https://www.doi.org/10.1007/s00436-018-5993-5

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C et al. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12): 1647-1649. DOI: https://www.doi.org/10.1093/bioinformatics/bts199

Khanna V, Sagar S, Khanna R, and Chawla K (2018). A comparative study of formalin-ethyl acetate sedimentation technique and Mini Parasep® solvent-free method in the rapid diagnosis of intestinal parasites. Tropical Parasitology, 8(1): 29-32. DOI: https://www.doi.org/10.4103/tp.TP_44_17

Librado P and Rozas J (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11): 1451-1452. DOI: https://www.doi.org/10.1093/bioinformatics/btp187

Ma G, Wang T, Korhonen PK, Hofmann A, Sternberg PW, Young ND, and Gasser RB (2020). Elucidating the molecular and developmental biology of parasitic nematodes: Moving to a multiomics paradigm. Advances in Parasitology, 108: 175-229. DOI: https://www.doi.org/10.1016/bs.apar.2019.12.005

Macedo LC, Willkens Y, Silva LM, Gardner SL, Melo FT, and Santos JN (2023). Revisitando o passado: Uma redescrição de *Physaloptera retusa* (Nemata, Physalopteridae) a partir de material depositado em museus e novo material de lagartos amazônicos [Revisiting the past: A redescription of *Physaloptera retusa* (Nemata: Physalopteridae) based on museum specimens and new material from Amazonian lizards]. Revista Brasileira de Parasitologia Veterinária, 32: 017422. DOI: https://www.doi.org/10.1590/S1984-29612023016

Maggenti AR (2020). General nematode morphology. Manual of agricultural nematology, 1st Edition. CRC Press., 5 Howick Place, London, pp. 3-46. DOI: https://www.doi.org/10.1201/9781003066576

Maharana BR, Gupta S, Gupta S, Ganguly A, Kumar B, Chandratre GA, and Bisla RS (2021). First report of molecular and phylogenetic analysis of *Physaloptera praeputialis* in naturally infected stray cats from India. Parasitology Research, 120(6): 2047-2056. DOI: https://www.doi.org/10.1007/s00436-021-07163-2

Maldonado Jr A, Simões RO, São Luiz J, Costa-Neto SF, and Vilela RV (2020). A new species of *Physaloptera* (Nematoda: Spirurida) from *Proechimys gardneri* (Rodentia: Echimyidae) from the Amazon rainforest and molecular phylogenetic analyses of the genus. Journal of Helminthology, 94: 68. DOI: https://www.doi.org/10.1017/S0022149X19000610

Mohamadain HS and Ammar K (2012). Redescription of *Physaloptera praeputialis* von Linstow, 1889 (Nematoda: *Spirurida*) infecting stray cats (*Felis catus* Linnaeus, 1758) in Qena, Egypt and overview of the genus taxonomy. Journal of the Egyptian Society of Parasitology, 42(3): 675-690. DOI: https://www.doi.org/10.12816/0006351

Moraes ID, Silva S, Andrade-Silva BED, Gomes APN, Urzedo NFD, Abolis VB, and Ramos DG (2024). Gastrointestinal helminths in wild felids in the cerrado and pantanal: Zoonotic bioindicators in important Brazilian biomes. Animals, 14(11): 1622. DOI: https://www.doi.org/10.3390/ani14111622

Moudgil AD, Singla LD, and Pallavi (2015). Parasitosis in wild felids of India: an overview. Journal of Threatened Taxa, 7(10):7 641-7648. DOI: https://www.doi.org/10.11609/JoTT.o4236.7641-8

Müller-Graf CD (1995). A coprological survey of intestinal parasites of wild lions (*Panthera leo*) in the Serengeti and the Ngorongoro Crater, Tanzania, East Africa. The Journal of Parasitology, 81(5): 12-814. DOI: https://www.doi.org/10.2307/3283987

Nadler SA and De León GP (2011). Integrating molecular and morphological approaches for characterizing parasite cryptic species: Implications for parasitology. Parasitology, 138(13): 1688-1709. DOI: https://www.doi.org/10.1017/S003118201000168X

Naem S and Asadi R (2013). Ultrastructural characterization of male and female *Physaloptera rara* (Spirurida: *Physalopteridae*): Feline stomach worms. Parasitology Research, 112(5): 1983-1990. DOI: https://www.doi.org/10.1007/s00436-013-3356-9

Packer C, Hilborn R, Mosser A, Kissui B, Borner M, Hopcraft G, and Sinclair AR (2005). Ecological change, group territoriality, and population dynamics in Serengeti lions. Science, 307(5708): 390-393. DOI: https://www.doi.org/10.1126/science.1105122

Palomba M, Marchiori E, Tedesco P, Fioravanti M, Marcer F, Gustinelli A, and Mattiucci S (2023). An update and ecological perspective on certain sentinel helminth endoparasites within the Mediterranean Sea. Parasitology, 150(12): 1139-1157. DOI: https://www.doi.org/10.1017/S0031182023000951

Pereira FB, Alves PV, Rocha BM, de Souza Lima S, and Luque JL (2012). A new *Physaloptera* (Nematoda: *Physalopteridae*) parasite of *Tupinambis merianae* (Squamata: Teiidae) from southeastern Brazil. The Journal of Parasitology, 98(6): 1227-1235. DOI: https://www.doi.org/10.1645/GE-3159.1

Powers T (2004). Nematode molecular diagnostics: From bands to barcodes. Annual Review of Phytopathology, 42(1): 367-383. DOI: https://www.doi.org/10.1146/annurev.phyto.42.040803.140348

Rana A, Yadav A, Bhat AH, Chaubey AK, and Malhotra SK (2020). Techniques to conduct morphological and molecular investigations on nematodes. Experimental protocols in biotechnology. Springer., New York, pp. 75-94. DOI: https://www.doi.org/10.1007/978-1-4939-9830-8

Sato C, Rai SK, and Uga S (2014). Re-evaluation of the formalin-ether sedimentation method for the improvement of parasite egg recovery efficiency. Nepal Medical College Journal, 16(1): 20-25. Available at: https://europepmc.org/article/med/25799805

Sinclair AR, Metzger KL, Mduma SA, and Fryxell JM (2015). Serengeti IV: Sustaining biodiversity in a coupled human-natural system. University of Chicago Press., Chicago, pp. 419-447. DOI: https://www.doi.org/10.7208/9780226196336

Sohn WM and Chai JY (2024). Practical guide for the diagnosis of helminth ova in stools. Annals of Clinical Microbiology, 27(2): 49-67. DOI: https://www.doi.org/10.5145/ACM.2024.27.2.3

Soulsby E (1965). Textbook of veterinary clinical parasitology. Volume I. Helminths. CABI., Oxford, UK. pp. xxxii+-1120. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/19662901092

Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, and Rambaut A (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus, 4: 16. DOI: https://www.doi.org/10.1093/ve/vey01

Tamura K, Stecher G, and Kumar S (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. DOI: https://www.doi.org/10.1093/molbev/msab120

Tull A, Valdmann H, Tammeleht E, Kaasiku T, Rannap R, and Saarma U (2022). High overlap of zoonotic helminths between wild mammalian predators and rural dogs–an emerging One Health concern?. Parasitology, 149(12): 1565-1574. DOI: https://www.doi.org/10.1017/S0031182022001032

Wobeser GA (2007). Disease in wild animals: Investigation and management. Springer., Berlin, Heidelberg, pp. 217-245. DOI: https://www.doi.org/10.1007/978-3-540-48978-8_12

Whitman KL, Starfield AM, Quadling H, and Packer C (2007). Modeling the effects of trophy selection and environmental disturbance on a simulated population of African lions. Conservation Biology, 21(3): 591-601. DOI: https://www.doi.org/10.1111/j.1523-1739.2007.00700.x