Research Article

Molecular Characterization of *Paramphistomum cervi* (Trematoda: Digenea: Paramphistomatidae) from Uzbekistan

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Abstract | In this study, morphological and morphometric analyses were conducted on *Paramphistomum cervi* (*P. cervi*) species isolated from the digestive systems of cattle and sheep. The total body length of the parasite was found to range from 2.8 to 8.3 mm (average 6.4 mm), and the width ranged from 1.2 to 3.6 mm (average 2.7 mm). Molecular-genetic identification of *P. cervi* was carried out, and a 286 base-pair fragment of the rDNA ITS2 region was amplified and sequenced. Nucleotide variations were detected among the *P. cervi* samples collected from different regions of Uzbekistan, indicating interspecies and intraspecies (population-level) differences. Phylogenetic analysis revealed that the *Paramphistomum* genus samples formed a strong cluster (bootstrap = 94), showing a high level of genetic similarity. In contrast, samples of the *Calicophoron* genus this sentence is cut off, indicating relatively weak genetic differentiation within the group. Molecular analyses clearly demonstrated the genetic divergence between the *Paramphistomum* and *Calicophoron* genera and confirmed the genetic integrity of the *P. cervi* samples.

Keywords | Genus, species, *Paramphistomum cervi,* rDNA, ITS2, Phylogeny

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INTRODUCTION

The genus *Paramphistomum* (Fischoeder, 1901) currently includes several species, among them *P. cervi* (Zeder, 1790), *P. liorchis* (Fischoeder, 1901), *P. gracile* (Fischoeder, 1901), *P. epiclitum* (Fischoeder, 1904), *P. gotoi* (Fukui, 1922), *P. ichikawai* (Fukui, 1922), *P. leydeni* (Näsmark, 1937), and *P. hiberniae* (Willmott, 1950). One of the most widely studied representatives, *Paramphistomum cervi* (Zeder, 1790) Fischoeder, 1901, was initially recorded by Daubenton in 1754 in the digestive tract of cattle and later confirmed by Zeder in 1790 in deer (Kennedy *et al.*, 1985). In subsequent decades, some researchers, relying on limited morphological descriptions, proposed different names for the same species, including *Fasciola cervi* (Schrank, 1790), *Fasciola elaphi* (Sinha, 1950), and *Monostoma conicum* (Zeder, 1790).

P. cervi (Trematoda: Digenea: Paramphistomatidae) is a parasitic fluke whose adult form colonizes the rumen of ruminants, whereas the immature stages are usually found in the duodenum. This parasite has been documented in domestic livestock such as cattle, sheep, and goats, as well as in several wild ungulates (Adane and Demelash, 2020). Taxonomic classification within Digenea is still under revision, with modern molecular data suggesting

reductions of certain orders and regrouping at the superfamily level. Nevertheless, families remain the most stable taxonomic units. Currently, the order Echinostomida (subclass Digenea) encompasses the superfamily Paramphistomoidea (Olson *et al.*, 2003), which comprises the families Paramphistomidae and Gastrothylacidae, both of which harbor the majority of paramphistome species found in ruminants.

This parasite has a wide geographical range and has been reported from many parts of the world (Rangel-Ruiz *et al.*, 2003; Ayaz *et al.*, 2013). While infections with adult flukes are generally mild, infestations in young animals may result in severe gastroenteritis (Ayaz *et al.*, 2013; Horak, 1971; Olsen, 1974). Immature paramphistomosis is still considered a neglected parasitic disease (Hajipour *et al.*, 2021), frequently overlooked in diagnosis (Phiri *et al.*, 2007). Nonetheless, it can lead to substantial losses due to mortality, reduced growth, and diminished milk production (Chaudhry *et al.*, 2017). Even in areas where prevalence is reported to be low, such as the Pakong and Pasean regions, outbreaks in calves can result in high mortality and significant tissue damage, thereby reducing the economic value of livestock (Aryani *et al.*, 2022; Hassan *et al.*, 2011; Zhang and Hewitt, 1997).

Research on *P. cervi* to date has mostly focused on its morphology, life cycle, and epidemiology (Rangel-Ruiz *et al.*, 2003; Ayaz *et al.*, 2013; Wang *et al.*, 2006), while molecular-level studies are comparatively scarce. More recently, however, both the complete mitochondrial genome and ITS2 rDNA region of *P. cervi* have been reported (Yan *et al.*, 2013; Bazsalovicsova *et al.*, 2010).

In eukaryotes, nuclear rDNA occurs as tandem repeats, each repeat containing three coding regions (18S, 5.8S and 28S rRNA genes), two internal transcribed spacers (ITS1 and ITS2), and an intergenic spacer (IGS) between transcriptional units (Long and Dawid, 1980). Because these rDNA regions evolve at different rates, they represent valuable genetic markers for phylogenetic and taxonomic analyses. Among them, the ITS sequences are particularly informative for parasite identification (Dai *et al.*, 2012; Orosova *et al.*, 2010; Yamada *et al.*, 2011; Wang *et al.*, 2012). The IGS region, which often includes repetitive elements, shows significant variability within and between parasite species (Zhao *et al.*, 2011).

The present study aims to characterize *Paramphistomum cervi* (Zeder, 1790) from small and large ruminants using molecular analysis of the rDNA ITS2 region.

MATERIALS AND METHODS

Collection of helminthological samples

In the present study, parasitic specimens were recovered from the digestive tracts of both large ruminants (cattle) is correct and small ruminants (sheep). Helminthological samples were collected from privately owned farms across four districts of the Samarkand region: Ishtikhon (sheep, n = 2), Payariq (sheep, n = 3), Pastdargom (cattle, n = 1), and Akdarya (sheep, n = 2). All specimens were preserved in a 70% ethanol solution for subsequent morphological and molecular analyses (Figure 1).

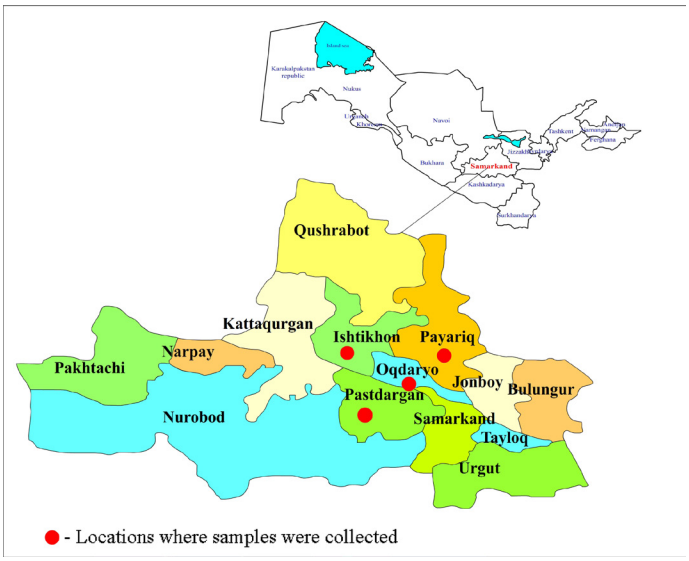

Figure 1: Regions where helminthological studies were conducted.

Morphological and morphometric analysis

Species identification of *Paramphistomum cervi* (genus *Paramphistomum*) was carried out using Meiji ML5000 and Nexcope NSZ818 light microscopes. Diagnostic features such as the overall body shape, structure of anterior and posterior suckers, reproductive organs and distribution of tegumental papillae were examined to confirm species identity (Choudhary *et al.*, 2015).

DNA extraction

Genomic DNA was isolated from specimens preserved in 70% ethanol using the DNeasy Blood and Tissue Kit (Qiagen, https://www.qiagen.com). DNA concentration and purity were assessed with a Thermo Fisher Scientific spectrophotometer (China). All DNA extracts were kept at –20 ˚C until further PCR amplification.

PCR amplification

Molecular identification was performed by targeting the rDNA ITS1–5.8S–ITS2 region, a widely applied marker for helminth taxonomy (Subbotin *et al.*, 2001). PCR reactions (20 µl total volume) consisted of 16.1 µl nuclease-free water, 2 µl 10× PCR buffer, 0.4 µl dNTPs, 2 µl of each primer [forward primer TW81 (5`-GTTTCCGTAGGTGAACCTGC-3`) and reverse primer AB28 (5`-ATATGCTTAAGTTCAGCGGGT-3`)], and 0.4 µl Taq DNA polymerase. The amplification protocol consisted of an

Table 1: Information on genetic samples obtained from the GenBank database.

No.	Species name	Accession number	Geographic origin	Host species
1	*Paramphistomum cervi*	KX274233	Croatia	*Cervus elaphus*
2	*Paramphistomum cervi*	KJ459936	China	Sheep
3	*Paramphistomum cervi*	KJ459935	China	Sheep
4	*Paramphistomum cervi*	OK216191	Egypt	Rumen
5	*Paramphistomum cervi*	OK216190	Egypt	Rumen
6	*Paramphistomum cervi*	OK216189	Egypt	Rumen
7	*Paramphistomum cervi*	HM026462	Slovakia	*Cervus elaphus*
8	*Calicophoron clavula*	MK416145	Egypt	–
9	*Calicophoron phillerouxi*	PP854140	South Africa	African buffalo
10	*Calicophoron clavula*	KX668944	USA	Kenyan ruminants
11	*Calicophoron clavula*	OQ842708	Germany	Cattle
12	*Calicophoron microbothrium*	MN912249	Egypt	–
13	*Calicophoron phillerouxi*	KX668977	USA	Kenyan ruminants
14	*Calicophoron phillerouxi*	KX668965	USA	Kenyan ruminants
15	*Calicophoron phillerouxi*	KX668964	USA	Kenyan ruminants
16	*Calicophoron microbothrium*	KX668921	USA	Kenyan ruminants
17	*Calicophoron microbothrium*	KX668919	USA	Kenyan ruminants
18	*Calicophoron microbothrium*	KX668918	USA	Kenyan ruminants
19	*Calicophoron microbothrium*	KP639631	Zimbabwe	Cattle
20	*Carmyerius gregarius*	OQ842678	Germany	Sheep

initial denaturation step at 98 °C for 30 s, followed by 40 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min (Kuchboev *et al*., 2020).

Gel electrophoresis, purification, and sequencing

PCR products were visualized on a 1% agarose gel under 100 V. Target DNA bands were excised and purified using reagents supplied by "Sileks M" (Moscow, Russia), following the manufacturer's instructions. Sequencing was performed using the ABI PRISM® BigDye™ Terminator v3.1 Cycle Sequencing Kit, with services provided by GATC Biotech AG. Sequence data were subsequently processed and aligned using *BioEdit**, *ClustalX2**, *DNASTAR™** and *PAUP** software packages (Larkin *et al*., 2007).

Phylogenetic tree construction

To investigate the molecular phylogenetic relationships of Paramphistomum cervi, nucleotide sequences of the ITS2 region were analyzed. These sequences were compared with homologous genetic data of other species retrieved from the GenBank database (Table 1).

Nucleotide sequences of the ITS2 region were used for phylogenetic inference. A sequence from a different genus was incorporated as an outgroup. Multiple sequence alignment was first conducted with the MAFFT algorithm (Katoh *et al*., 2002), and subsequently, ambiguous regions and evident misalignments were manually refined using BioEdit* software (version 7.0.5.2; Hall, 1999). Gaps and missing data were retained and treated as missing characters rather than being removed, as this approach helps preserve phylogenetic signal and provides a more reliable representation of evolutionary relationships. Phylogenetic tree reconstruction was performed in IQ-TREE 2 (Minh *et al*., 2020) under the Maximum Likelihood (ML) framework. The most appropriate nucleotide substitution model was identified automatically with the Model Finder module. Node support was assessed with 1000 bootstrap replicates (Felsenstein, 1985). The resulting tree was visualized and graphically customized using the Interactive Tree of Life (iTOL) web tool, which provides a clear and flexible representation of phylogenetic relationships.

RESULTS AND DISCUSSION

Morphological and morphometric analysis

The sentence is very long. Consider breaking it up for clarity: Helminthological studies revealed that *P. cervi*, isolated from the digestive systems of cattle and sheep, had a pale reddish coloration and a pear-shaped body. An oral sucker was located at the anterior end, and a ventral sucker (acetabulum) was positioned at the posterior end. The total body length (2.8 mm to 8.3 mm) indicates notable intraspecific variability among the parasites. The mean body

length was 6.4 mm, suggesting these specimens belong to a relatively large group of organisms. The standard deviation of 1.07 mm reflects a moderate degree of variation (Table 2, Figure 2).

Table 2: Morphometric characteristics of *Paramphistomum cervi* (n = 10).

Morphological features	Measurements (mm), n=10
Body length	2.8 – 8.3, (6.4±1.07)
Body width	1.2 – 3.6, (2.7±0.9)
Diameter of oral sucker	0.18 – 0.32, (0.23±0.3)
Diameter of ventral sucker	0.45 – 0.80, (0.61±0.4)
Egg size (length × width)	0.11–0.16 × 0.7–0.9

Body width also demonstrated a certain level of variability. The average width was 2.7 mm, which appears proportionate to body length. A relatively high standard deviation (±0.9 mm) indicates noticeable morphological differences in this parameter as well. The diameter of the oral sucker was relatively small, with an average of 0.23 mm and a very low standard deviation (±0.03 mm), suggesting that this structure is morphologically stable and shows minimal variation among individuals.

The ventral sucker (acetabulum) was significantly larger than the oral sucker, likely to facilitate firm attachment within the host's tissue. Its average diameter was 0.61 mm, with a low standard deviation (±0.04 mm), indicating structural consistency. Egg dimensions fall within the micrometric range, which requires specialized measurement techniques. The differences between length and width were minimal, and overall, the egg size is within the typical range reported for trematodes. Therefore, egg morphology may serve as a useful criterion for species identification of *P. cervi*.

Figure 2: Morphological features of *Paramphistomum cervi*. a: Specimens collected from sheep; b: General body shape; c: Anterior end showing the oral sucker; d: Posterior end with the acetabulum.

The oral sucker is funnel-shaped, widening towards the posterior. The intestines are broad, interconnected in a serpentine manner, and their terminal portions are positioned more dorsally than laterally. The genital pore is located anterior to the intestinal bifurcation. The acetabulum (posterior sucker) is situated near the posterior end of the body (subterminal position), measuring approximately one-fourth to one-fifth of the body length.

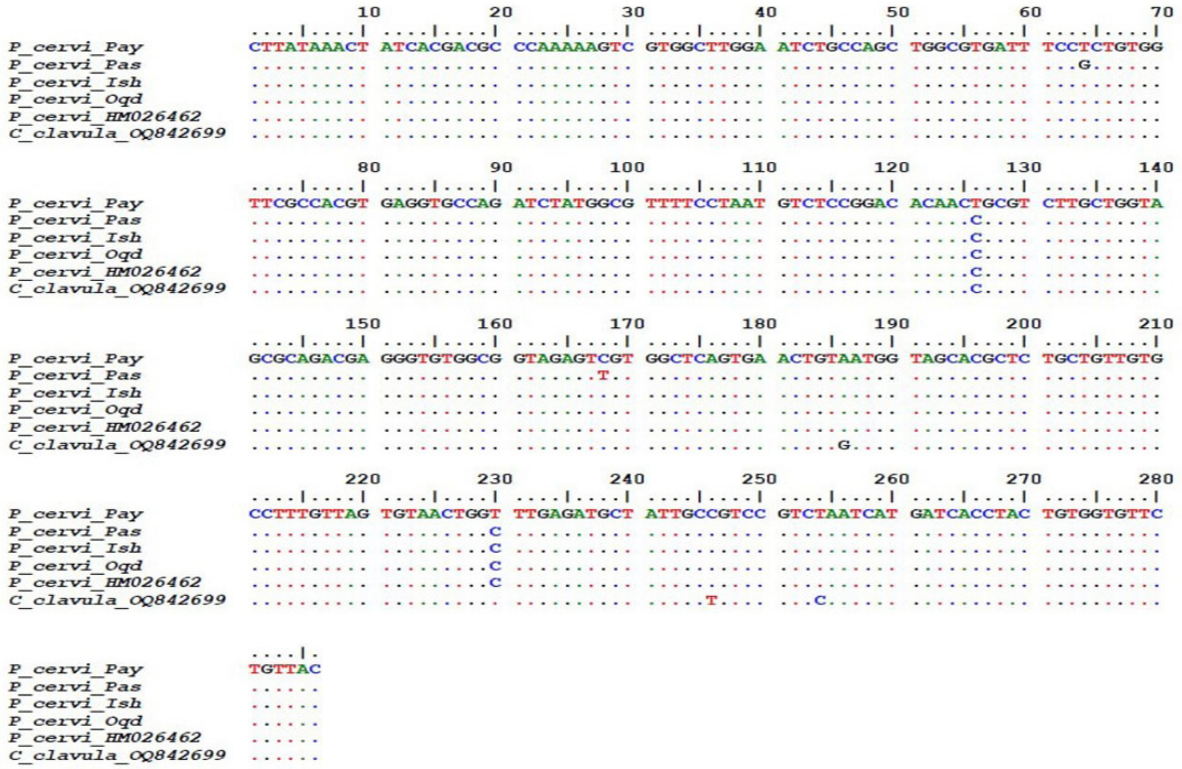

Figure 3: Alignment of the ITS2 region nucleotide sequences from *P. cervi* samples and reference sequences.

Molecular-genetic analysis

Following sequencing and chromatogram analysis, a 286-base-pair fragment of the rDNA ITS2 region was successfully amplified and identified from *P. cervi* (genus *Paramphistomum*, Fischoeder, 1901). For comparative analysis, reference sequences were obtained from the international GenBank database (https://blast.ncbi.nlm.nih.gov), including *Paramphistomum cervi* (accession number: HM026462) and *Calicophoron clavula* (genus *Calicophoron*, Näsmark, 1937; accession number: OQ842699) (Figure 3).

This section is very repetitive and hard to follow. It should be significantly condensed and presented in a more synthetic way. For example: Nucleotide variations were observed among the *P. cervi* samples from different regions (Payariq, Pastdargom, Ishtikhon, Akdarya) and the reference sequence (HM026462). Key variable positions included sites 64, 126, 168, 186, 230, 246, and 254. For instance, at position 126, samples from Payariq (*P. cervi*_Pay) had a thymine (T), whereas samples from other regions and the reference sequence had a cytosine (C). Greater sequence divergence was observed between all *P. cervi* samples and the congeneric species *Calicophoron clavula* (OQ842699), with consistent differences at positions 186 (A in *P. cervi* vs. G in *C. clavula*), 246 (C vs. T), and 254 (T vs. C).

In *P. cervi_Pas*, at position 64, G-guanine was found, while in *P. cervi_Ish*, T-thymine was present. At position 168, *P. cervi_Pas* contained T-thymine, while *P. cervi_Ish* contained C-cytosine. At position 64, *P. cervi_Pas* contained G-guanine, while *P. cervi_Oqd* contained T-thymine. At position 64, *P. cervi_Pas* contained G-guanine, while *C. clavula* OQ842699 contained T-thymine. At positions 168 and 254, *P. cervi_Pas* contained T-thymine, whereas *C. clavula* OQ842699 contained C-cytosine. At position 186, *P. cervi_Pas* contained A-adenine, while *C. clavula* OQ842699 contained G-guanine. At position 246, *P. cervi_Pas* contained C-cytosine, while *C. clavula* OQ842699 contained T-thymine.

In *P. cervi_Ish*, at position 186 A-adenine was present, while in *C. clavula*, OQ842699 contained a G-guanine; at position 246, *P. cervi_Ish* contained a C-cytosine, while *C. clavula* OQ842699 contained T-thymine; at position 254, *P. cervi_Ish* contained T-thymine, while *C. clavula* OQ842699 contained C-cytosine. Similarly, in *P. cervi_Oqd*, at position 186 A-adenine was present, while in *C. clavula*, OQ842699 contained a G-guanine; at position 246, *P. cervi_Oqd* contained a C-cytosine, while *C. clavula* OQ842699 contained T-thymine; and at position 254, *P. cervi_Oqd* contained T-thymine, while *C. clavula* OQ842699 contained C-cytosine.

The observed substitutions within the ITS2 region are located in a non-coding segment of rDNA. Although these mutations are not expected to alter protein structure, they may still influence secondary DNA structure or ribosomal RNA processing. In this study, recurrent substitutions at positions 126 and 230 consistently differentiated *P. cervi* from *Calicophoron* species and also showed variability among local *P. cervi* populations. Such patterns suggest that these mutations, while synonymous in nature, may hold phylogenetic and taxonomic value for distinguishing closely related species and geographic isolates.

Phylogenetic analysis

The *Paramphistomum* cluster (marked with a blue line) includes only *P. cervi* samples. The genetic similarity among these species is very high, and the main node is strongly supported with a bootstrap value of 94. Within the group, samples obtained from different geographical regions (Ishtikhon (Sheep), Payariq (Sheep), Pastdargom (Cattle), Akdarya (Sheep)), as well as sequences retrieved from GenBank, are present, and they are positioned almost identically in the phylogenetic tree (Figure 4).

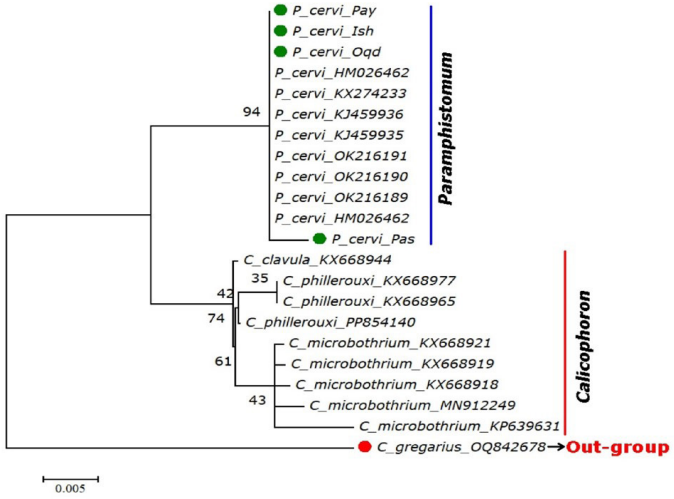

Figure 4: Maximum Likelihood phylogenetic tree based on ITS2 sequences, showing the relationship between *Paramphistomum* and *Calicophoron* species. Bootstrap values (>35%) are shown at nodes.

C. gregarious (OQ842678) was used as an outgroup to root the tree and provide phylogenetic orientation. The molecular distance scale (0.005) represents the number of substitutions per site. Bootstrap support values (shown at the nodes) indicate the statistical reliability of branching, where values above 70% are generally considered strong support.

The *Calicophoron cluster* (separated by a red line) is divided into two sub-branches. The first branch consists of *C. clavula* and *C. phillerouxi* species, with the main node showing a bootstrap value of 35. The second branch unites

Links Researchers

C. microbothrium samples, with a bootstrap value of 43 at its main node. The overall node for the entire *Calicophoron* group has a bootstrap value of 42, indicating a moderate level of confidence in interspecific differentiation. As an outgroup, the sample *Carmyerius gregarius* (OQ842678) was used, and it was clearly separated from all other species. The molecular distance scale represents 0.005 units. The results demonstrate that species belonging to the genera *Paramphistomum* and *Calicophoron* are well distinguished phylogenetically. The high bootstrap values for *P. cervi* samples confirm their genetic integrity, whereas the internal sub-branches within the *Calicophoron* group are separated with relatively low confidence.

DISCUSSION

Paramphistomiasis is highly prevalent in ruminants, and morphological studies along with genetic approaches are used in species discrimination. The morphological analysis of the current specimens revealed that they belong to the superfamily Paramphistomidea by comparison with other trematodes (Jones *et al*., 2002).

During this study, the morphological and morphometric characteristics of *Paramphistomum cervi* were analyzed and compared with data from existing literature to clarify the diagnostic features of the species. The parasite's light reddish coloration, pear-shaped body structure, and the presence of two suckers one funnel-shaped oral sucker located anteriorly and a posterior acetabulum represent classical morphological traits of this species.

The observed body length (ranging from 2.8 to 8.3 mm, with an average of 6.4 mm) and width (1.2 to 3.6 mm, average 2.7 mm) fall within the range reported by many authors and indicate the species' morphometric variability. Notably, the subterminal position of the acetabulum, located near the posterior end and occupying approximately one-fourth to one-fifth of the body, serves as a key diagnostic feature that distinguishes this species from other representatives of the *Paramphistomum* genus.

The wide, serpentine-shaped intestinal branches and the posteriorly expanding funnel-like oral opening reflect adaptations in the digestive system of the species. Additionally, the genital pore located just posterior to the intestinal bifurcation is also an important morphological trait for species identification.

Furthermore, the identification of *P. cervi* in the digestive systems of sheep and cattle indicates its widespread distribution among ruminants and highlights its ecological adaptability. These morphological analyses play a crucial role in the accurate identification, differential diagnosis, and development of effective treatment and prevention strategies for this parasitic species.

The results of the phylogenetic analysis based on ITS rRNA gene sequences demonstrated clear phylogenetic differentiation between representatives of the genera Paramphistomum and Calicophoron. In the constructed phylogenetic tree, all P. cervi samples formed a monophyletic cluster, and the high bootstrap value (94) at the main node confirmed their genetic coherence and phylogenetic stability. This cluster included four autochthonous samples obtained from the study areas (*P. cervi*_Pay, *P. cervi*_Ish, *P. cervi*_Oqd – isolated from sheep; *P. cervi*_Pas – isolated from cattle) as well as sequences available in the GenBank database, originating from Croatia (*Cervus elaphus*), China (sheep), Egypt (rumen sample), and Slovakia (*Cervus* elaphus).

In Uzbekistan, molecular investigations of the local fauna have been expanding in recent years, encompassing studies on insects (Kimyonazarov *et al*., 2024; Kadirov *et al*., 2024), nematodes (Aliyev *et al*., 2024; Mirzaev *et al*., 2024; Turgunov *et al*., 2024), and fish (Quvatov *et al*., 2023; Ubaydullayev *et al*., 2025).

Earlier research has shown that the ITS2 region of rDNA tends to be more conserved than ITS1 (Luton *et al*., 1992), potentially due to the presence of varying types and quantities of repeat sequences. Both long and short repeats, which can cause size differences, have been documented in various helminth groups such as trematodes (Warberg *et al*., 2005), cestodes (Bowles *et al*., 1995), and nematodes (Subbotin *et al*., 2011). However, in the current study, no length variation was observed among the ITS2 sequences of any *P. cervi* specimens.

The minimal genetic distance observed between local and international samples suggests that *P. cervi*, despite its wide host range (including sheep, cattle, and wild ungulates), exhibits a high level of genetic conservation. This suggests that its evolutionary history may reflect its ability to parasitize a broad spectrum of hosts with limited host-specific adaptations. Moreover, the monophyletic structure of the cluster provides strong evidence of the intraspecific phylogenetic homogeneity of *P. cervi*.

In contrast, the Calicophoron group was divided into two subclusters: The first comprising *C. clavula* and *C. phillerouxi*, and the second consisting of *C. microbothrium* samples. The bootstrap values for these subclusters were relatively low (35 and 43), indicating weaker genetic differentiation among these species compared to *P. cervi*. According to the data table, these species are geographically widespread, found in Egypt, the USA (including Kenyan domestic animals), Germany (cattle), South Africa (African buffalo), and Zimbabwe (cattle). The wide distribution range,

particularly in species such as *C. microbothrium*-suggests a broad ecological amplitude and a high degree of host plasticity or low host specificity.

The outgroup Carmyerius gregarius (Germany, sheep) was clearly separated from the main clusters and placed at the root of the tree, demonstrating the phylogenetic adequacy of the chosen outgroup. The low value of the molecular distance scale (0.005) indicates relatively low nucleotide divergence among samples, while still confirming clear phylogenetic separation at the genus level.

While recent studies have highlighted the scarcity of molecular investigations on paramphistomid flukes (Mitchell *et al*., 2020), they have generally focused on broad species-level identification and global distribution without providing a critical assessment of existing phylogenies. In this context, the present work introduces a novel contribution by presenting the first molecular-genetic characterization of *Paramphistomum cervi* from Uzbekistan, including isolates from both sheep and cattle. Unlike previous studies that primarily emphasized the universal applicability of ITS2 sequencing, our research provides a detailed comparative analysis of nucleotide polymorphisms across local isolates and reference sequences. The identification of consistent diagnostic substitutions at specific positions (64, 126, 168, 230, 246, and 254) not only confirms the high conservatism of *P. cervi* but also establishes reliable markers for distinguishing between closely related taxa (*Paramphistomum* vs. *Calicophoron*). Furthermore, by integrating newly generated sequences with international GenBank data, we critically evaluated the robustness of genus-level clades, demonstrating strong phylogenetic stability in *P. cervi* and revealing relatively weaker resolution within *Calicophoron*. This dual approach local molecular characterization combined with a critical evaluation of existing phylogenies-extends beyond earlier reports and provides a regional reference dataset that can be applied to future phylogeographic and epidemiological studies in Central Asia.

Overall, the findings indicate that *P. cervi*, despite its wide geographical distribution and broad host range, remains genetically stable and conservative, whereas the genus Calicophoron exhibits comparatively higher levels of internal genetic diversity. These results provide important insights into host adaptation strategies, dispersal dynamics, and phylogenetic evolution of these parasites.

CONCLUSION

The present study provides a comprehensive morphological, morphometric, and molecular characterization of *Paramphistomum cervi*, a trematode parasite isolated from the digestive systems of cattle and sheep in the Samarkand region. Morphologically, *P. cervi* exhibits a light reddish, pear-shaped body with distinct anatomical features including anterior and posterior suckers, a funnel-shaped oral opening, serpentine intestinal branches, and clearly positioned genital pore and acetabulum. These features are consistent with previously described characteristics of the species.

Morphometric analysis revealed that body length ranged from 2.8 to 8.3 mm (mean ± SD: 6.4 ± 1.07 mm) and body width from 1.2 to 3.6 mm (2.7 ± 0.9 mm), indicating moderate intraspecific variation. The oral sucker was relatively small (0.23 ± 0.03 mm), while the ventral sucker (acetabulum) was significantly larger (0.61 ± 0.04 mm), suggesting functional adaptation for host attachment. Egg dimensions were within typical micrometric ranges reported for trematodes, showing minimal variation and offering diagnostic value for species identification.

Molecular and bioinformatic analysis targeting the ITS2 region of rDNA confirmed the genetic identity of the species. Despite minor nucleotide variations indicating potential inter-population differences, phylogenetic reconstruction revealed that all *P. cervi* samples formed a strongly supported clade (bootstrap = 94), highlighting a high degree of genetic homogeneity. Furthermore, the analysis distinctly separated the genera *Paramphistomum* and *Calicophoron*, underscoring their evolutionary divergence.

Collectively, these findings confirm the taxonomic status of *P. cervi* and emphasize the importance of integrating morphological and molecular approaches for accurate parasite identification. The results hold practical relevance for parasitological diagnostics, epidemiological surveillance, and the development of targeted control and prevention strategies against paramphistomosis in ruminants.

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NOVELTY STATEMENT

This study provides the first molecular characterization of *P. cervi* from Uzbekistan, thereby filling a significant gap in the parasitological database of Central Asia. These results

not only expand the regional knowledge of trematode biodiversity but also contribute novel reference data for future epidemiological and phylogenetic research.

AUTHOR'S CONTRIBUTION

Field sampling, morphological identification, and statistical analyses were carried out by MT, AD, KU, and LX. Molecular experiments and sequencing were performed by RS. Data interpretation and manuscript preparation were undertaken by OA. All authors reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI was used to conceive, analyze, or interpret the research data.

Conflict of interest

The authors have declared no conflict interests related to this work.

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