

Seasonal and landscape patterns of *Fasciola* infection in cryptically diverse *Radix natalensis* snails in the Lake Albert region, Uganda

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Abstract

Fasciolosis is a zoonotic foodborne trematode infection involving freshwater lymnaeid snail intermediate hosts and is highly prevalent among livestock in Uganda's Lake Albert region. However, xenomonitoring data remain limited, particularly regarding cryptic *Radix* diversity and ecological predictors of infection. This study investigated *Radix* molecular diversity and the spatial and seasonal patterns of *Fasciola*-trematode infection. A longitudinal survey of 18 freshwater habitats in Kagadi District was conducted during the dry (June–July 2020) and wet seasons (October–November 2020). *Radix* snails were morphologically identified and screened for fasciolid and other trematode infections using multiplex rapid diagnostic PCR (RD-PCR) and genotyped using Cytochrome c Oxidase subunit I (COI) and Internal Transcribed Spacer (ITS) markers. Logistic regression was used to evaluate the association of spatio-temporal factors with infection patterns. Among 220 *Radix* snails, overall trematode and *Fasciola*Q3 prevalence were 63.18% (139/220) and 8.64% (19/220), respectively, with sequence analysis confirming *Fasciola* gigantica (99.41–100% identity-PZ297442). Phylogenetic and haplotype analyses revealed two sympatric *Radix natalensis* sensu lato subspecies, *R. n. debaizei* and *R. n. exsertus*, harbouring infections. *Fasciola* ($p=0.013$) and overall trematode ($p=0.005$) prevalence were significantly higher during the dry-season, while overall trematode prevalence was also higher below the escarpment ($p=0.003$). Seasonal and spatial variation are strongly associated with *Fasciola* spp. and other trematode infections in snail populations, with higher prevalence during the dry-season and at sites below the escarpment. Targeted dry-season snail control, particularly at infection hotspots, combined with water-trough use, may reduce livestock exposure.

Keywords Barcoding · Kagadi · Albertine Rift · Multiplex rapid diagnostic PCR · Trematode · Xenomonitoring

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Background

Fasciolosis is a foodborne trematode infection caused by liver flukes of the genus *Fasciola* (Trematoda: Fasciolidae), primarily *Fasciola hepatica* and *Fasciola gigantica*. *F. hepatica* occurs widely across Europe, Asia, Africa, the Americas, and Oceania, while *F. gigantica* is mainly restricted to Africa and Asia (World Health Organization (WHO) 2025). The disease is zoonotic, affecting both humans and herbivorous livestock, although its burden is most pronounced in ruminant production systems (Mas-Coma 2004). Fasciolosis persists through a complex life cycle involving mammalian definitive hosts, which acquire infection by ingesting metacercariae on vegetation or in contaminated water, and freshwater lymnaeid snails as intermediate hosts (Mas-Coma 2004, 2005). Its transmission is strongly modulated by climatic and ecological conditions, physiography, human behaviour, and population dynamics of the freshwater lymnaeid snail vectors.

Globally, fasciolosis affects an estimated 2.4 million people, with many millions more at risk (WHO 2025). Human infections have been reported in several African countries, including Egypt, Ethiopia, Nigeria, Ghana, Tanzania, South Africa, Angola, Senegal, Morocco, and Tunisia (Ashrafi et al. 2014; Black et al. 2013; Dermauw et al. 2021; El-Shazly et al. 2001; Fentie et al. 2013; Futagbi et al. 2015; Lukambagire et al. 2015; Monteiro Noel et al. 2013; Soliman 2008). Emerging evidence also indicates transmission in Uganda, with recent observations suggesting human cases in Mayuge District (Andriko, pers. comm., 2024). These reports underscore fasciolosis as a pertinent and emerging public health concern in the country.

Beyond human health, fasciolosis imposes substantial economic losses on livestock production systems, primarily through reduced productivity and liver condemnation. More than 300 million cattle are infected worldwide, with annual economic losses exceeding US\$3.2 billion (Malatji et al. 2020; Mehmood et al. 2017). However, in developing, highly endemic African countries, the disease's effect on the economy is occasionally underestimated (Mehmood et al. 2017). In Uganda, liver condemnation rates as high as 84% have been documented in abattoirs in Kampala, corresponding to an estimated US\$92.47 million in annual losses (Nambafu et al. 2015; Ssenkuba et al. 2022). In the Lake Albert basin, recent parasitological surveys detected *Fasciola* eggs in 56% of cattle faeces, with similarly high prevalence among sheep (50%) and goats (28.2%) (Namirembe et al. 2024). Furthermore, 57% of cattle livers inspected in abattoirs were infected with adult flukes. However, these estimates are based on faecal microscopy, a method limited by low sensitivity and difficulty in detecting prepatent infections (Calvani et al. 2017; WHO 2025). Furthermore, the study employed a cross-sectional survey, which does not capture the temporal variation in the prevalence of the disease. Importantly, the investigation did not explore the environmental source of infection or the role of snail intermediate hosts, an essential component for understanding transmission within a One Health framework. Ecological factors that shape snail distribution and infection risk therefore remain poorly characterised, limiting the development of targeted fasciolosis control strategies in the region.

Seasonality is a key driver of fasciolosis transmission. Seasonal temperature variations (also influenced by latitude and altitude), rainfall, and hydrological fluctuations shape snail population dynamics, producing mono-, bi-, or year-round transmission patterns in different environmental contexts (Mas-Coma et al. 2018). For example, in the Northern Bolivian Altiplano, a year-long transmission facilitated by a small monthly temperature variation, mono-seasonal transmission is experienced in extreme latitudes such as the monsoon period in South Central Asia and a bi-seasonal transmission (spring and autumn) is observed in areas of Europe, the United States of America and Australia (Mas-Coma et al. 2018). In East Africa, specifically in Kenya, *F. gigantica* transmission in *Radix natalensis* (Krauss, 1848) snails is predominantly wet-season associated, peaking from May to August (Biwott et al. 2025). Understanding these seasonal patterns is critical for optimising control strategies such as livestock treatment timing, pasture management, and water source protection (Mas-Coma et al. 2018).

Equally important, but less explored, is the genetic diversity of intermediate snail hosts. Species of the genus *Radix* play a central role in the transmission of *Fasciola* spp., yet their population structure and cryptic diversity remain poorly understood in many endemic regions (Mas-Coma 2004). Given that genetic variation influences species survival, adaptability, and susceptibility to parasites, investigating the population structure and cryptic

diversity of *Radix* spp. is highly relevant for elucidating the drivers that shape fasciolosis epidemiology (Gray 1995; Mirfendereski et al. 2021; Tantrawatpan et al. 2023). A recent study by Aksenova et al. (2024) has reported multiple subspecies of *R. natalensis* in East Africa, underscoring the need for integrative taxonomic and population genetic approaches to better understand host-parasite interactions and refine disease risk mapping (Mirfendereski et al. 2021; Vinarski et al. 2020).

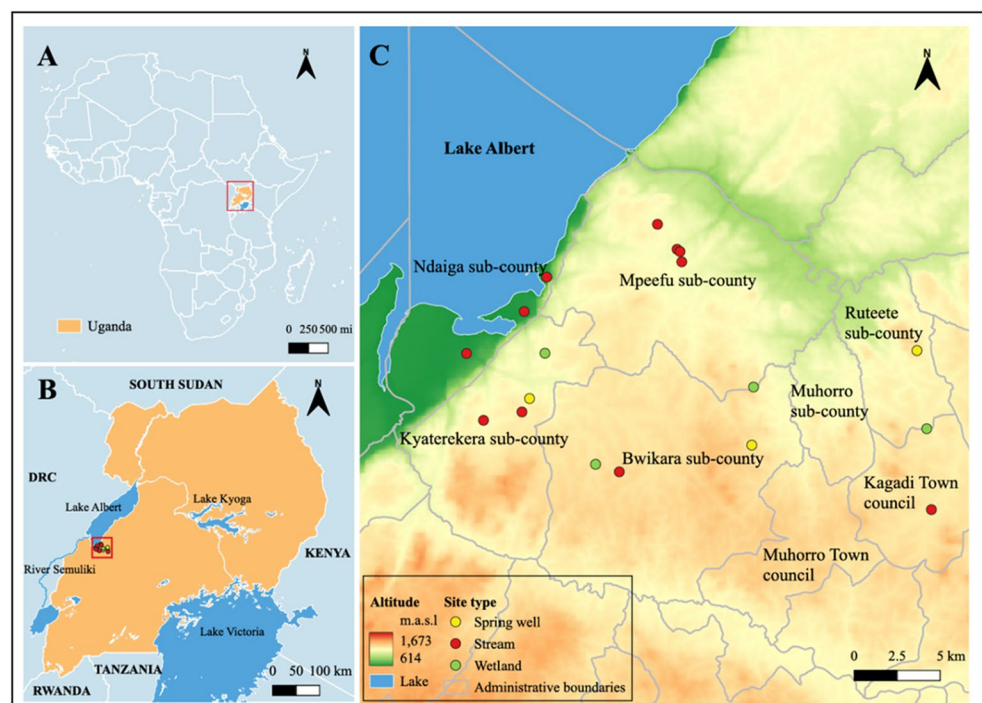
Despite the recognised importance of ecological, seasonal, and genetic drivers, fine-scale studies integrating malacological surveys, detection of prepatent infections in snails, microhabitat characterisation, and concurrent livestock infection remain scarce in the region. Most investigations in Uganda are cross-sectional, geographically narrow, and dependent on livestock diagnostics alone, constraints that hinder comprehensive risk assessment and intervention design. To address these knowledge gaps, we conducted a longitudinal survey along the Lake Albert shoreline region to: (i) identify the lymnaeid snail species present, (ii) assess the influence of seasonal variation on snail infection prevalence, and (iii) evaluate the role of microhabitat type and location relative to the Albertine Rift escarpment in shaping *Fasciola* infection patterns in snails using an Infection Multiplex Rapid Diagnostic (RD) PCR approach.

Methods

Sampling

Field sampling was conducted during both the dry season (June-July) and the wet season (October-November) in 2020 across Kagadi District, western Uganda (Fig. 1). Six administrative units were surveyed, comprising five sub-counties: Ndaiga, Kyaterekera, Mpeefu, Bwikara, and Ruteete, as well as Kagadi Town Council. Ndaiga sub-county lies on the floor of the western arm of the Great Rift Valley (Albertine Rift) in Western Uganda adjacent to Lake Albert, while the rest of the sub-counties plus Kyaterekera Town Council are situated upland, beyond the escarpment. The selection of these sampled areas reflects differences in cattle management systems, a key determinant of fasciolosis prevalence. The Ndaiga area is characterised predominantly by free-range livestock

Fig. 1 A Map of Africa showing the location of Uganda, B Map of Uganda showing the location of Lake Albert and the sampled Area, C Map of sampled sites in the Lake Albert region



rearing, where animals have unrestricted access to extensive water sources, including Lake Albert and the stream sites investigated in this study (Namirembe et al. 2024). In contrast, the upland sites are dominated by more controlled production systems, such as paddocking, zero-grazing, and tethering, which limit direct livestock–water contact and thereby reduce contamination pressure on aquatic habitats. Additionally, land tenure systems differ between upland and escarpment lowland areas. The lowlands are predominantly characterised by communal land tenure, resulting in open landscapes with minimal regulation of animal movements, whereas upland areas are largely privately owned, restricting livestock movement. A total of 18 freshwater habitats were purposively selected along the altitudinal gradient as representative sampling points, three sites from down the Albertine Rift escarpment and 15 sites above the Albertine Rift escarpment, including 11 streams, four wetlands, and three spring wells described in Tumusiime et al. (2024). All sites are located within the wider Albertine Rift region of south-western Uganda.

Snail sampling was conducted by one person using a hand scoop net (20 cm diameter, 1 mm mesh size) for a standardised 30-minute search period per site, actively agitating emergent and submerged macrophytes. Snails attached to rocky or hard substrates were manually collected using feather-weight forceps. All specimens were preserved in 80% ethanol, labelled with site and sampling-month metadata, and transported to the Royal Museum for Central Africa in Tervuren, Belgium. Voucher material is archived at the Royal Museum for Central Africa (Tervuren, Belgium). Snails were identified to genus level based on diagnostic shell morphology following Mandahl-Barth (1954) and Brown (1994).

DNA extraction

For molecular screening, ten *Radix* spp. individuals were randomly subsampled per site for DNA extraction and fasciolid infection detection. Soft tissue was carefully excised from each preserved gastropod specimen, removed from the shell, and air-dried on sterile absorbent paper. Whole tissue samples were then homogenised with a sterile scalpel on a glass surface. To prevent cross-contamination, forceps and scalpel blades were decontaminated between specimens with ethanol and held under a flame until they turned red hot, and the glass plate and gloves were treated with DNA AWAY™ (ThermoFisher™) after each sample preparation. Genomic DNA was extracted using the Chelex® (Bio-Rad™) protocol, selected to minimise cost and reduce processing time (Caron et al. 2011). Approximately 100 µL of a 5% Chelex suspension containing the homogenate was incubated at 56 °C for 1 h, followed by 95 °C for 30 min in a spinning thermocycler. Lysates were centrifuged at 13,000 ×g for 7 min, and the resulting supernatants were stored at −20 °C until use. DNA extracts were diluted 1:10 prior to PCR amplification to mitigate inhibition.

Xenomonitoring trematode infection in *Radix* spp. snails

Trematode infection in *Radix* spp. was detected using a *Fasciola* multiplex RD-PCR assay, with amplicon size as the diagnostic criterion, following Carolus et al. (2019). PCR reactions were prepared in 15 µL volumes and contained 1.5 µL of diluted gastropod DNA extract using the Multiplex Taq DNA Polymerase Kit (Qiagen, Venlo, The Netherlands). Final primer concentrations were 0.2 µM for 18S_Digenea_F and 18S_Digenea_R, 0.1 µM for FW_SNAIL2 and REV_SNAIL2, and 0.6 µM for Kaplan_F and Kaplan_R. The primer pair 18S_Digenea_F/18S_Digenea_R targets trematodes in general, whereas FW_SNAIL2/REV_SNAIL2 serves as an internal control targeting gastropods and confirms successful DNA amplification. The Kaplan_F/Kaplan_R primer set is specific for *Fasciola* spp (Carolus et al. 2019). Thermocycling conditions consisted of an initial denaturation at 95 °C for 15 min, followed by 39 cycles of 94 °C for 30 s, 60 °C for 90 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min on a Biometra™ T Professional thermocycler. Amplicons were visualised by gel electrophoresis on 3% agarose gels stained with Midori Green Direct® under UV illumination. Gels were run at 120 V for 1 h 45 min to resolve fragment sizes.

DNA sequencing and phylogenetic analysis

Gastropod specimens testing positive for *Fasciola* sp. by multiplex RD-PCR were subsequently sequenced to determine both host (gastropod) identity using COI and associated trematode species using ITS2_TREM and FAS ITS1. Of the 19 *Fasciola*-positive samples detected by RD-PCR, seven were purposively selected for sequencing, ensuring that at least two specimens were included from each infected site, two from down the escarpment sites, and five from the upland sites. These samples were successfully amplified using the *Fasciola*-specific ITS1 (Fas-ITS1) marker. Of the seven ITS1-confirmed *F. gigantica* infections, three specimens were additionally sequenced using the general trematode ITS2 marker (trem-ITS2). To characterise non-*Fasciola* trematode infections that were detected by RD-PCR, at least two trematode-positive specimens per site (from 10 infected snails) were selected for sequencing using the trem-ITS2 marker. Trematode identification targeted the ribosomal internal transcribed spacer 2 (ITS2) region using trematode-specific primers ITS2_TREM_F (5'- CAA HAA GTC GTG GMT TGG-3') and ITS2_TREM_R (5'- CCT AAA CAC CAC ATT GCC T -3'), yielding amplicons of approximately 364–879 bp as described by Hammoud et al. (2022). Gastropod genotyping was performed using the universal cytochrome c oxidase subunit I (COI) marker with primers LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G -3') and HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA -3'), generating a fragment of ~710 bp (Folmer et al. 1994). *Fasciola* spp. were further discriminated using a genus-specific ITS1 assay (FAS ITS1_F: 5'- TCT ACT CTT ACA CAA GCG ATA CAC-3'; FAS ITS1_R: 5'- GGC TTT CTG CCA AGA CAA G -3'), targeting a 716 bp region (Grabner et al. 2014).

PCR amplification was conducted in 15 µL reaction volumes containing 1.5 µL of 1:10 diluted genomic DNA template, 1.1 µM of each primer, and the Multiplex Taq DNA polymerase master mix (Qiagen, Venlo, The Netherlands). Cycling conditions consisted of an initial denaturation at 95 °C for 5 min; 39 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s; followed by a final extension at 72 °C for 10 min on a T Professional Thermocycler (Biometra™). Amplicons were resolved on 3% agarose gels stained with Midori Green Direct® and visualised under UV light. Products corresponding to the expected amplicon size were purified using ExoSAP (Fermentas™) PCR Product Cleanup procedure and submitted for Sanger sequencing in both directions (Big-Dye® chemistry) at Macrogen™.

Raw chromatograms were inspected and edited in BioEdit v7.2 (Hall 1999). Forward and reverse reads were trimmed for quality, manually corrected for ambiguous bases, and assembled into consensus sequences. Edited sequences were queried using the BLASTn tool against the NCBI GenBank database; top-hit matches were evaluated based on maximum alignment score. Species identity was inferred from BLAST similarity and further supported by phylogenetic placement in MEGA v11 (Tamura et al. 2021). All unique sequences generated in this study are deposited in GenBank (accession numbers: PZ295578-PZ295585, PZ297442-PZ297449). Three unique sequences generated in this study and ten reference sequences retrieved from NCBI GenBank were included in the analysis. Of these, seven i.e. OP084764.1, ON603552.1, OP084765.1, ON603553.1, OP084766.1, LC015513.1, and HG977206.1 originated from the taxonomic revision that resolved *Radix natalensis* to subspecies level (Aksenova et al. 2024), while three additional regional sequences pre-dated this revision i.e. MN601427.1, PP528793.1, and PP228864.1.

Also, for *Fasciola* spp., three unique sequences generated from this study together with four NCBI GenBank retrieved sequences from the region were included in the analysis (Table 1). All sequences were aligned using the Clustal algorithm and subsequently trimmed prior to phylogenetic reconstruction. The best-fit nucleotide substitution model was selected using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) implemented in MEGA v11. The Hasegawa-Kishino-Yano model was used to infer phylogenetic relationships using the Maximum Likelihood (ML) approach. Tree robustness was assessed through 1,000 bootstrap replicates. For species delimitation, pairwise genetic p-distances were calculated for the COI and ITS1 markers, with divergence values ≤5% considered indicative of intraspecific variation (Carolus et al. 2019).

The statistical parsimony network was constructed using the TCS algorithm (Clement et al. 2002) implemented in PopART software (Leigh et al. 2015) to analyse 13 haplotypes of *Radix natalensis*, comprising three generated

Table 1 Pairwise genetic distance (p-distance) between *Fasciola* sp. ITS1 sequences (676 bp). The *Fasciola* sp. genotypes collected from the Lake Albert region are indicated in bold. The GenBank Accession number is given after the ‘|’ separator

Species	1	2	3	4	5	6
1. <i>Fasciola</i> sp. (UG) E10_7						
2. <i>Fasciola</i> sp. (UG) KIJ_164	0.001					
3. <i>Fasciola</i> sp. (UG) Ru2_64	0.001	0.003				
4. <i>Fasciola gigantica</i> (Chad) MK321641.1	0.000	0.001	0.001			
5. <i>Fasciola gigantica</i> (Kenya) MZ396909.1	0.000	0.001	0.001	0.000		
6. <i>Fasciola gigantica</i> (Côte d’Ivoire) MK321638.1	0.000	0.001	0.001	0.000	0.000	
7. <i>Fasciola gigantica</i> (Algeria) ON661091.1	0.000	0.001	0.001	0.000	0.000	0.000

in the present study and ten sequences retrieved from GenBank. Haplotype diversity analysis was performed to characterise the genetic structure and variability of the sampled populations and to assess their relationships with reference specimens available in GenBank.

Data analysis

Prevalence of *Fasciola* spp. and trematode infection was calculated as the proportion of infected snails (%) relative to the total number examined at each site, location, season, and habitat type. Logistic regression analysis was performed with infection status (infected vs. non-infected) as the binary dependent variable to identify factors associated with *Fasciola* spp. and trematode infection. A binomial logistic model was considered appropriate given the dichotomous outcome and the presence of many zero observations, which limited the suitability of count-based models affected by overdispersion. Predictor variables included location (upland vs. down-escarpment), season (dry vs. wet), and site type (stream, spring well, and wetland). Reference categories were defined as upland for location, dry season for season, and stream for site type. Regression coefficients (β), standard errors (SE), odds ratios (OR), 95% confidence intervals (CI), and p-values were estimated. Odds ratios were obtained by exponentiating the regression coefficients. Model outputs were interpreted based on odds ratios and confidence intervals. In cases where zero infections were observed in a category (e.g., spring wells), regression estimates were unstable due to complete separation, and results were interpreted with caution. All statistical analyses were performed using R software (version 4.3.3) at a 5% level of significance.

Results

Molecular characterisation of *Radix* species

Lymnaeid snails collected in the study area were morphologically identified as *Radix natalensis* based on conchological features (Fig. 2). However, molecular analyses of nine specimens revealed underlying cryptic diversity within these specimens. BLAST searches of the partial COI gene sequences (496 bp) from nine individuals showed the highest similarity to GenBank records of *Radix natalensis exsertus* (one specimen) and *Radix natalensis debaizei* (eight specimens), despite the absence of appreciable morphological differentiation among these taxa (Fig. 2). Thus, based on close sequence matches (99.3–100% identity), two subspecies were recognised: *R. natalensis exsertus* (Fig. 2, right) and *R. natalensis debaizei* (Fig. 2, left). This assignment was further supported by p-distance analyses [See Additional File 2] and by well-resolved clades in the maximum likelihood phylogenetic tree (Fig. 3).

One unique haplotype clustered with *R. natalensis exsertus* specimens previously collected from Lake Albert (Accession no: ON603553.1, OP084766.1, Aksenova et al. (2024), and with a reference sequence from North Africa (Accession no: LC015513.1; Egypt), forming a strongly supported clade (bootstrap > 90%; Fig. 3B) and

Fig. 2 Photographs of the *Radix natalensis* snail sub-species collected in the Lake Albert region: *Radix natalensis debaizei* (left) and *Radix natalensis exsertus* (right)

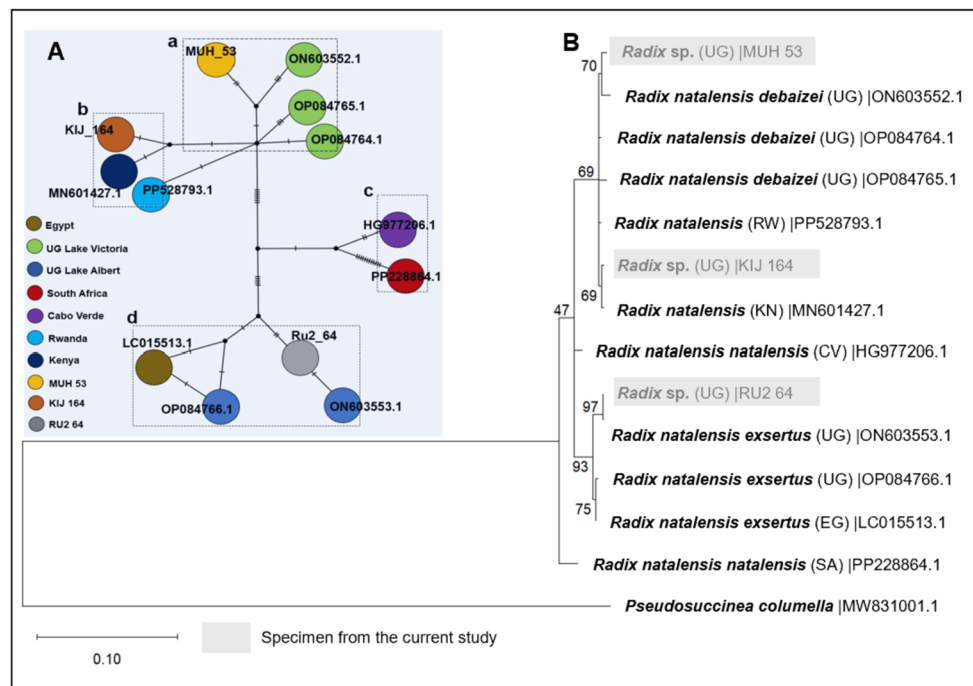


Fig. 3 A phylogenetic tree showing the genetic relationship of the *Radix* spp. collected from the sampled sites within the *Radix natalensis* complex represented by selected GenBank sequences, the accession numbers of which are given. **a** A haplotype network showing the distribution of the haplotypes among geographical locations. Haplotypes are coloured according to their geographical occurrence. **b** Maximum likelihood phylogenetic tree showing the genetic relationship between *Radix* spp. and the other *R. natalensis* subspecies in Africa. Bootstrap support values above 50% are shown. The scale bar represents the number of substitutions per site according to the Hasegawa-Kishino-Yano model of sequence evolution

with uncorrected pairwise genetic distances ranging from 0.2 to 0.8%. The haplotype network further indicated minimal mutational divergence, with only 1–4 mutations separating this haplotype from Lake Albert specimens and four mutations from the Egyptian specimen (Fig. 3A).

In contrast, two additional unique haplotypes clustered with *R. natalensis debaizei* from Lake Victoria (Accession no: OP084764.1 Aksenova et al. (2024), Uganda, with moderate to strong bootstrap support (69–70%; Fig. 3B). This relationship was corroborated by p-distances of 0.6–1.2%. The haplotype network revealed very low mutational differences between these haplotypes and *R. natalensis debaizei* reference sequences, ranging from three to six mutations (Fig. 3A), further supporting their conspecific status.

All haplotypes generated in this study were genetically distinct from *Radix natalensis natalensis sensu stricto* (Fig. 3A, B), highlighting previously unrecognised cryptic intraspecific diversity within *R. natalensis* in the Albertine Rift region. GenBank sequences from Rwanda and Kenya were excluded from subspecies-level comparisons because they pre-dated recent taxonomic revisions and lacked explicit subspecies delineation.

Of the nine sequenced specimens, eight were assigned to *R. natalensis debaizei* and one to *R. natalensis exsertus*. The single *R. natalensis exsertus* individual was collected together with *R. natalensis debaizei* in an upland stream, indicating sympatric occurrence. *Radix natalensis debaizei* was recorded throughout the study area, occurring in both streams below the escarpment and upland habitats.

Trematode identification

Multiplex RD-PCR screening revealed a high overall trematode prevalence of 63.18% (139/220) among the examined snails, whereas the prevalence of *Fasciola* spp. was comparatively low at 8.64% (19/220). Sequencing results confirmed the presence of a single *Fasciola* species, *Fasciola gigantica* [see Additional File 1]. The sequences showed 99.4–100% identity with reference specimens from Chad (MK321641.1). Species delineation based on pairwise genetic distance (p-distance) further supported this identification, with our specimens exhibiting $p\text{-distance} \leq 0.001$ relative to sequences from Chad (MK321641.1), Kenya (MZ396909.1), Côte d'Ivoire (MK321638.1), and Algeria (ON661091.1) (Table 1). Of the seven ITS1-confirmed *F. gigantica* infections, three specimens that were additionally sequenced using the general trematode ITS2 marker (trem-ITS2) further confirmed *F. gigantica*, exhibiting 99.7–100% similarity with GenBank isolates from Vietnam and Zimbabwe, providing independent validation of species identity.

Beyond *Fasciola*, multiple non-*Fasciola* trematode infections were detected by RD-PCR. Twelve specimens were successfully sequenced, of which eight were identified as *Plagiiorchis* spp. These sequences showed 98.9–100% similarity to GenBank references from Germany (KX160479.1) and Uganda (OQ549830.1) [see Additional File Table 1]. *Plagiiorchis* infections were detected in both upland and escarpment sites.

Three of the 12 ITS2 sequences corresponded to *F. gigantica*, while the last sequence matched *Isthmiophora hortensis*. This specimen showed 97.82% similarity to a reference isolate from Japan (AB189982.1) [see Additional file 1], representing the only record of this species in the current survey. However, due to the low genetic similarity, the delineation to species level is not reliable.

Factors associated with *Fasciola* spp. and trematode prevalence

Season was a significant predictor of *Fasciola* spp. infection. Prevalence was markedly higher during the dry season (13.33%) than in the wet season (3.00%), and snails collected in the wet season had significantly lower odds of infection compared to those from the dry season ($p=0.013$; Table 2), representing an approximate 80% reduction in the odds. A similar seasonal pattern was observed for overall trematode infections, with prevalence significantly greater in the dry season (71.67%) than in the wet season (53.00%; $p=0.005$; Table 2).

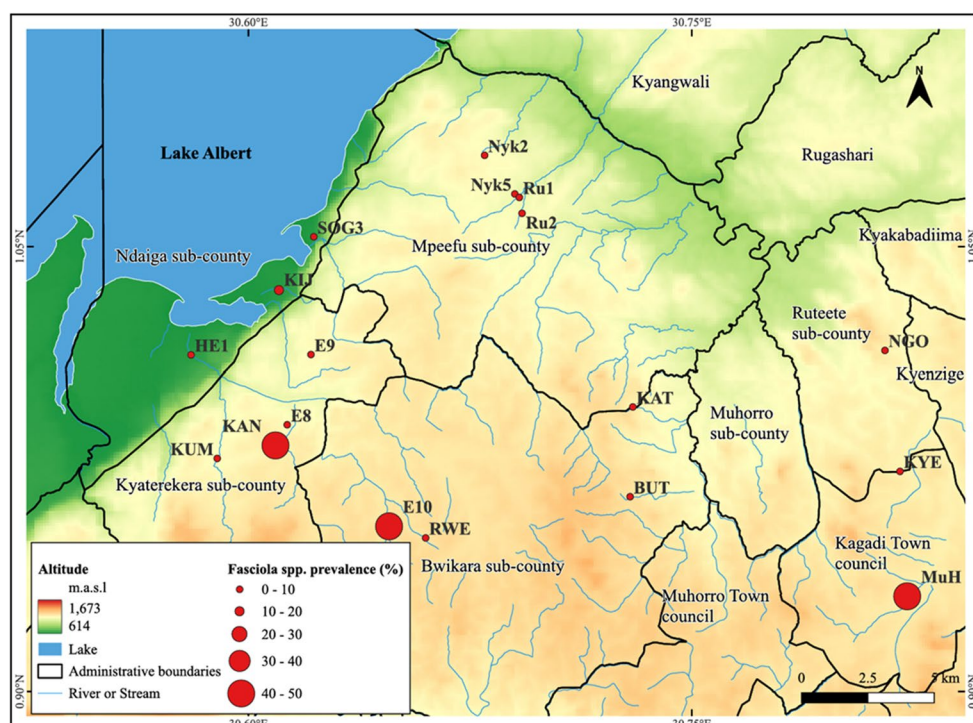
With respect to geographic location, *Fasciola* spp. prevalence was slightly higher in upland sites (8.95%) than in down-escarpment sites (6.67%), although this difference was not statistically significant ($p=0.681$; Table 2; Fig. 4). However, overall trematode prevalence was significantly greater in down-escarpment sites (90.00%) compared with upland sites (58.95%; $p=0.003$).

Site type was not significantly associated with *Fasciola* spp. prevalence, which was identical in streams and wetlands (10.00% each; $p=1.000$; Table 2), while no infections were detected in spring wells (0/30), resulting in unstable regression estimates and an undefined confidence interval. In contrast, trematode prevalence differed markedly among habitat types. Streams exhibited the highest prevalence (76.15%) and served as the reference category, whereas infection was significantly lower in spring wells (43.33%) and wetlands (45.00%; $p<0.001$).

Table 2 Prevalence of *Fasciola* spp. and other trematodes and their association with location, season, and site type

Predictor	Category	Prevalence (%)	β (SE)	95%CI	Odds ratio (OR)	P-value
Fasciola sp. prevalence						
Location	Upland (ref)	17/190 (8.95)				
	Down escarpment	2/30 (6.67)	-0.319 (0.775)	0.159–3.319	0.727	0.681
Season	Dry	16/120 (13.33)				
	Wet	03/100 (3.00)	-1.604 (0.645)	0.057–0.711	0.201	0.013*
Site type	Stream (ref)	13/130 (10.00)				
	Spring well	0/30 (0.00)	-16.92 (1569.624)	0.000– ∞	0.000	0.991
	Wetland	6/60 (10.00)	0.000 (0.520)	0.361–2.772	1.000	1.000
Trematode prevalence						
Location	Upland (ref)	112/190 (58.95)				
	Down escarpment	27/30 (90.00)	1.835 (0.626)	1.837–21.387	6.268	0.003**
Season	Dry	86/120 (71.67)				
	Wet	53/100 (53.00)	-0.808 (0.285)	0.255–0.779	0.446	0.005**
Site type	Stream (ref)	99/130 (76.15)				
	Spring well	13/30 (43.33)	-1.429 (0.422)	0.105–0.548	0.239	0.001***
	Wetland	27/60 (45.00)	-1.361 (0.331)	0.134–0.490	0.256	<0.001***

Fig. 4 Map of sampling sites showing the spatial distribution of *Fasciola* infection in *Radix* snails in the Lake Albert region



Discussion

Cryptic diversity among *Radix natalensis* snails

Despite the pivotal role of radicine pond snails in fasciolosis transmission, knowledge of their taxonomy in Africa remains poorly resolved because conchological and anatomical characteristics are highly plastic, environmentally influenced and may obscure cryptic diversity, leading to erroneous species delineation. Recent years have witnessed major reclassification within the *Radix natalensis* complex, largely driven by molecular approaches such as DNA barcoding (Aksenova et al. 2018, 2024; Lawton et al. 2015; Young et al. 2021).

Classical malacological studies in Africa treated *Radix natalensis exsertus*, *R. natalensis natalensis*, and *R. natalensis debaizei* as separate species based on shell morphology (Mandahl-Barth 1954). These taxa were later synonymised under *R. natalensis sensu lato* following broader revisions of African molluscs (Brown 1994). This broad species concept has dominated malacology research for decades, and most epidemiological surveys in East Africa have consequently identified snails only to species level (Biwott et al. 2025; Dube et al. 2025; Dusabe et al. 2024; Mahulu et al. 2019), overlooking intraspecific structure that might influence compatibility with *Fasciola* parasites, which might influence the epidemiological dynamics, though not yet tested. A recent continent-wide molecular reassessment has reinstated three subspecies within *R. natalensis*: *R. n. debaizei*, *R. n. exsertus*, and *R. n. natalensis*, highlighting substantial cryptic diversity within the group (Aksenova et al. 2024).

In the present study, we detected two sympatric subspecies, *R. n. debaizei* and *R. n. exsertus*, inhabiting lotic and wetland systems of the Lake Albert region (Fig. 3). Their distribution is biogeographically coherent with earlier reports that documented *R. n. exsertus* in Lake Albert and *R. n. debaizei* in the Lake Victoria basin within the Nile drainage (Aksenova et al. 2024). Phylogenetic clustering, COI p-distance estimates, and haplotype network structure supported their recognition as distinct evolutionary lineages while remaining clearly separated from *R. n. natalensis*. These findings challenge the long-standing practice of treating *R. natalensis* as a single homogeneous taxon in regional transmission studies.

Both subspecies were naturally infected with *F. gigantica*, suggesting that multiple cryptic lineages within *R. natalensis* may be involved in its transmission; however, their relative competence as intermediate hosts requires further investigation. Increasing evidence from lymnaeid systems indicates that subtle genetic differentiation among snail populations can structure parasite compatibility, thermal tolerance, and habitat associations, thereby modulating fasciolosis risk (Dube et al. 2023; Lawton et al. 2015). Similar patterns have been reported in other regions where host genetic background influences infection success and climate-driven transmission potential (Alba et al. 2021). However, the limited number of infected and sequenced individuals in our dataset precluded formal comparison of prevalence or susceptibility between subspecies, and the contribution of genetic variability to local epidemiology could not be tested.

Future investigations should integrate large-scale sampling with experimental infections to determine whether *R. n. debaizei* and *R. n. exsertus* differ in compatibility with *Fasciola* spp., parasite developmental rates, or ecological preferences. Incorporating subspecies-level resolution into ecological and climatic models may be useful for improving predictive risk mapping and guiding snail control strategies in the Nile basin.

***Fasciola* species prevalence is structured by spatio-temporal predictors**

In the current study, we observed a relatively low overall prevalence of *Fasciola* spp. of 8.64% (19/220) among the sampled *Radix* snails. This prevalence is markedly lower than estimates previously reported in bovine populations from the same region, which ranged from 18.1% to 47.1% based on coprological egg count methods (Namirembe et al. 2024). Such disparity is expected, as One Health investigations consistently demonstrate higher infection rates in definitive mammalian hosts than in intermediate snail hosts, further caused by overdispersion of the parasites in snail intermediate hosts. For example, a study in Vietnam reported a *Fasciola* prevalence of 3.2% in *Radix* snails using molecular techniques compared with 34.8% in cattle based on the coprological egg count method (Loan et al. 2025), reflecting differences perhaps due to parasite amplification within mammalian hosts and temporal dynamics of snail infection and parasite dispersion.

Conversely, we detected a high overall trematode prevalence of 63.18% (139/220) among the examined snails, with infections predominantly attributed to *Plagiorchis* spp., which have previously been reported to exhibit high infection rates through both primary and secondary infections (Soldánová and Kostadinova 2011). One hypothesis that warrants further investigation is whether the high prevalence of non-*Fasciola* trematodes influences the establishment of *Fasciola* spp. through antagonistic interspecific interactions within snail hosts. Martin and Cabrera (2018) reported extremely low co-infection rates (<1%) between xiphidiocercariae, to which *Plagiorchis* spp. belongs, and other cercarial types such as echinostomes in the Philippines. Similar competitive

exclusion mechanisms may operate in the Lake Albert region, where established trematode communities can suppress subsequent *Fasciola* spp. colonisation through resource competition, immune modulation, or alteration of host physiology that reduces suitability for additional parasites (Kuris and Lafferty 1994; Martin and Cabrera 2018). Field and experimental studies support such antagonistic interactions; for example, co-infection dynamics between *Calicophoron sukari* and *Schistosoma mansoni* in *Biomphalaria pfeifferi* suggest that *C. sukari* may release excretory–secretory products that inhibit the development of *S. mansoni* sporocysts (Laidemitt et al. 2019). These interactions highlight the importance of considering the broader trematode assemblage when interpreting patterns of *Fasciola* transmission in natural snail populations.

The prevalence of *Fasciola* spp. and other trematodes in the cryptically diverse *Radix* snails exhibited a pronounced seasonal pattern, with significantly higher infection levels recorded during the dry season. Specifically, *Fasciola* prevalence was higher in the dry season than in the wet season, with a similar seasonal pattern observed for overall trematode infections (Table 2). Snails collected during the wet season had significantly lower odds of *Fasciola* spp. infection than those from the dry season ($p=0.013$; Table 2), corresponding to an approximate 80% reduction in odds. These findings are consistent with observations from Indonesia, where *Fasciola gigantica* prevalence in lymnaeid snails and definitive hosts similarly peaked during the dry season (Purwaningsih et al. 2022).

The elevated prevalence during the dry season may be explained by the contraction of aquatic habitats, which promotes a concentration effect whereby livestock and wildlife congregate at a limited number of persistent water bodies. This aggregation increases faecal contamination of snail habitats and enhances exposure of *Radix* snails to *Fasciola* and other trematode miracidia (Purwaningsih et al. 2022). Furthermore, reduced water volume likely intensifies host–parasite contact rates, thereby increasing the probability of snail infection. Collectively, these findings underscore the importance of aligning fasciolosis control interventions with seasonal transmission dynamics, particularly by prioritising surveillance and integrated control measures during the dry season when transmission risk is highest. This approach may also enhance cost-effectiveness and reduce environmental impact, as fewer and more discrete snail habitats are available for targeted control during the dry season.

Spatial positioning (altitudinal gradient) of sampling sites in relation to the rift escarpment was not significantly associated with *Fasciola* spp. prevalence, although a marginally higher infection rate was observed in upland sites (8.95%) compared with down-escarpment sites (6.67%) ($p=0.681$; Table 2; Fig. 4). In contrast, overall trematode prevalence differed markedly between the two landscapes, with significantly higher infection in down-escarpment sites (90.00%) than in upland sites (58.95%; $p=0.003$). Snails collected below the escarpment were therefore more than six times more likely to harbour trematode infections, indicating substantial spatial heterogeneity in transmission risk. However, these spatial findings should be interpreted cautiously because the sampling design was unbalanced: only three down-escarpment sites were sampled, compared with 15 upland sites. The limited number of down-escarpment sites may have reduced the precision and generalisability of the estimated landscape effect, particularly for *Fasciola* spp., for which relatively few infections were detected.

Despite this limitation, the observed spatial pattern may be partly associated with differences in land use and livestock management practices. Communities along the lakeshore, particularly in Ndaiga Sub-county, predominantly practice free-range grazing, allowing livestock unrestricted access to shared water contact points (Namirembe et al. 2024). Such systems have repeatedly been associated with enhanced environmental contamination with trematode eggs and increased transmission to snail intermediate hosts across Africa and other endemic regions (Mereta et al. 2019, 2023; Nzalawahe et al. 2015). In contrast, upland communities largely rely on paddocking, zero-grazing, and tethering systems, which limit direct livestock–water contact and may reduce contamination pressure on aquatic habitats.

Additional contextual factors may further amplify transmission below the escarpment. The lakeshore zone is characterised by limited crop cultivation and predominantly communal land tenure, resulting in open landscapes with minimal regulation of animal movements. Proximity to Semliki Game Reserve facilitates frequent wildlife access to water bodies, potentially introducing additional reservoirs of trematode parasites and sustaining multi-host transmission cycles. The influence of land use on snail-borne trematode infections has been highlighted

in previous studies from Ugandan freshwater systems, where agricultural intensity and landscape modification were significant predictors of infection risk (Hammoud et al. 2025). Conversely, upland areas consist largely of privately managed farmlands located farther from wildlife corridors, conditions that may restrict both livestock and wildlife interactions at water contact sites and consequently lower the likelihood of trematode contamination.

Together, these findings underscore the importance of socio-ecological context in shaping trematode transmission dynamics and suggest that targeted interventions in high-risk down-escarpment communities, particularly those addressing livestock access to water and wildlife–livestock interface, such as providing non-contaminated water to cattle using water troughs, may be critical for reducing infection risk in *Radix* snail populations.

Habitat type was not significantly associated with *Fasciola* spp. prevalence ($p=1.000$; Table 2); however, no infections were detected in protected spring wells (0/30), possibly because restricted livestock access reduces faecal contamination and snail exposure to miracidia, consistent with observations from previous studies in East Africa (Mereta et al. 2019, 2023).

Overall trematode prevalence differed markedly among habitat types. Streams exhibited the highest prevalence (76.15%) and represented a substantially higher-risk environment compared with spring wells (43.33%) and wetlands (45.00%; $p<0.001$). This pattern may reflect more favourable physicochemical conditions in streams, such as lower salinity, cooler temperatures, and reduced turbidity, which attract livestock and wildlife to these water points, thus promoting trematode infection. The lower prevalence in wetlands and spring wells is likely due to poorer water quality and restricted animal access, respectively, both of which can limit transmission opportunities. Nevertheless, we could not conclusively assess the association of habitat type with *Fasciola* spp. prevalence, given the low number of infected snails screened (19/220), with similar prevalence reported at stream (13/130) and wetland (6/60) habitats only.

These findings underscore the importance of habitat characteristics and local water management in shaping trematode transmission, suggesting that protection of communal water sources and regulation of livestock access to streams could contribute to integrated control of fascioliasis and other snail-borne trematodiasis.

Conclusion

This study identified two sympatric cryptic subspecies of *Radix natalensis* explicitly, *R. n. debaizei* and *R. n. exsertus*, in the Lake Albert region, with *Fasciola* infection detected in both subspecies. However, their competence as intermediate hosts requires confirmation through experimental infection studies. These findings highlight the need to investigate how cryptic genetic diversity within radicine pond snails shapes transmission dynamics and to incorporate this variability into predictive models of fasciolosis risk. Seasonality emerged as a key predictor of infection, with significantly higher prevalence during the dry season, underscoring the importance of aligning snail surveillance and control interventions with periods of peak transmission. The exceptionally high prevalence of non-*Fasciola* trematodes, structured by season, micro-habitat, and landscape position relative to the Albertine Rift escarpment, further reveals a complex and largely unresolved trematode community. The application of high-resolution genomic tools, such as metabarcoding, may help to better characterise this hidden diversity, clarify its epidemiological significance, and refine assessments of actual rather than potential transmission risk. Targeted, seasonally timed snail control strategies will be critical for effective fasciolosis management in the region.

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Authors' contributions F.S.: Conceptualisation, investigation, data analysis, visualisation, writing—original draft, and writing—review and editing. T.H.: Conceptualisation, supervision, Investigation, writing—review and editing, and funding acquisition. A.B.: Investigation and writing—review and editing. C.U.T.: Supervision, Investigation, writing—review and editing, and funding acquisition. G.K.: Supervision, Investigation, writing—review and editing, and funding acquisition. J.T.: Supervision, investigation, writing—review and editing, and funding acquisition.

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Data availability The datasets supporting the conclusions of this article are included within the article (and its additional files). All unique sequences generated in this study are deposited in GenBank (accession numbers: PZ295578-PZ295585, PZ297442-PZ297449).

Declarations

Ethics approval and consent to participate This study received both institutional and national ethical approvals from the Mbarara University of Science and Technology Research Ethics Committee (MUST-2025-542) and the Uganda National Council for Science and Technology (NS1175ES), respectively.

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