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First molecular characterisation of *Hydatigera taeniaeformis* (Cestoda: Taeniidae) from *Rattus rattus* in urban market centres from Tamale, Ghana

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Abstract

Background The common cat tapeworm, *Hydatigera taeniaeformis*, is widely distributed across many regions of the world but its global genetic diversity, ecology, and other biological features are not fully understood. The predator-prey relationship of felids and rodents sustains the transmission in both sylvatic and synanthropic or urban settings. This study was carried out in three urban market centres in Tamale, Ghana to determine the prevalence of *H. taeniaeformis* infection in urban rats and characterise the local population genetics using the mitochondrial *nad1* gene sequences.

Methods From January to May 2023, rats were captured and euthanized to identity and isolate the *H. taeniaeformis* metacestodes. The full mitochondrial nad1 gene was amplified, sequenced and used to characterise the cestode population.

Results In total 64/80 rats from the three market centres were found naturally infected with metacestodes of *H. taeniaeformis*. Averagely, infected rats harboured two cysts with one exceptional case of 25 cysts in one liver. Each cyst harboured one metacestode. The DNA sequences revealed four haplotypes in a star-like distribution network across the three market centres. All four haplotypes were found to be unique, clustered with *H. taeniaeformis* sensu stricto (s.s.) global isolates and had only marginal variation among themselves.

Conclusion This is the first account of the genetic variability within *H. taeniaeformis* in Ghana and would contribute to ongoing and future studies on African regional biogeographic distribution of the common cat tapeworm.

Keywords *Hydatigera taeniaeformis*, Rats, Urban market, Tamale, Ghana

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Introduction

Hydatigera Lamarck 1816 is one of the four important genera of the family Taeniidae with the others being, *Echinococcus*, *Taenia* and *Versteria* [1]. *Hydatigera* contains cryptic genetic variants that are collectively referred to as *Hydatigera taeniaeformis* sensu lato (s.l.), until a recent taxonomic revision that differentiated them into four cryptic species based on host specificity, molecular genetic variability and geographical spread [1, 2] namely, *H. taeniaeformis* sensu stricto (s.s), *H. kamiyai*, *H. parva* and *H. krepkogorski*. The common cat tapeworm (*H. taeniaeformis* s.s.) is the most cosmopolitan of the species complex [2–4] It uses domestic cat and other felids as definitive hosts and rats and mice as intermediate hosts. The adult tapeworm grows and lodges in the small intestine of cats and the parasite's eggs and/or gravid proglottids are released into the environment via the faeces of its felid hosts. Rodent hosts become infected by ingesting eggs in contaminated food or water. The parasitic eggs will hatch and eventually forms metacestodes, usually in the liver. The transmission cycle is completed when cats feed on infected rodents. *Hydatigera taeniaeformis* is generally assumed to posse low zoonotic risk, but a few old accounts of human infections with the metacestodes in the liver and intestinal adult *H. taeniaeformis* infection are also known [5].

The taxonomic revision of the *H. taeniaeformis* s.l. complex has been helpful in appraising the global genetic diversity of the cestode and brought some clarity in understanding of its biology. Meanwhile, the revision exercise was only possible after a sizeable molecular data became available from a few endemic localities [1, 2]. For a comprehensive appreciation of the genetic diversity, host range, biogeographic distribution and transmission dynamics of the cestodes, more population studies of natural infections across different hosts, localities and habitats are needed [2, 3]. *Hydatigena taeniaeformis* occurrence has been reported from much part of the world including Africa, Asia, Middle East, Europe, North and South America [1–4]. Meanwhile, there is a huge gap in data from Africa. Current review of published literature shows endemicity across the sub-regions of sub-Saharan Africa [3] and the north but data on molecular identification of *Hydatigera*, isolates are only available from Ethiopia and South Africa [2], Kenya [4] and Senegal [3]. The wide distribution of *H. taeniaeformis* in all parts of Africa shows the apparent importance of the cestode in the region although many aspects of it including the population genetics remain under researched.

So far, molecular data on *H. taeniaeformis* s.l. from Africa identified *H. parva* and *H. taeniaeformis* s.s. as the species in circulation [2–4]. In the present study, we determined the infection rate and genetic variability of *H. taeniaeformis* in rats from urban market centres in the Tamale metropolis of northern Ghana. Our data revealed abundance of the taeniid parasite in the urban space and positioned Ghanaian isolates within the *H. taeniaeformis* s. s. clade in the cryptic species complex.

Materials and methods

Study area

The study was conducted in market centres in the Tamale metropolis. Tamale is the capital town of the Northern Region, and the capital of the Tamale metropolis, located in the guinea savannah ecological zone (latitudes 9.16° & 9.34° North and longitudes 00.36° & 00.57° West). The city has a human population of 374,744 [6]. Tamale city is cosmopolitan with five large urban markets and many smaller markets in the suburbs [7]. The present study was conducted in three out of the five major markets in Tamale, namely, Aboabo, Central and Lamashegu markets. All the three markets are heterogenous in produce/wares sold but the major category is food.

Trapping and dissection of rats

The market was zoned into fairly homogeneous areas to aid in trapping. Locally designed wooden live traps were set in dark areas in the night using groundnut or dry fish as bait and traps were inspected for catch the following morning before sun rise. Catches were transferred live into individual compartment of a transporting cage and taken to the biology laboratory at the University for Development Studies, Nyankpala campus, for dissection.

Rats were euthanized by cervical dislocation following the guidelines of the American Veterinary Medical Association (AVMA) [8] and dissected to isolate the liver for inspection for cysts. In infected livers, the translucent cysts were carefully opened into a petridish and the number of *H. taeniaeformis* metacestodes were noted. Individual larvae were identified using the scolex, fixed and stored in 75% ethanol until DNA extraction.

The intestines of the dissected rats were fixed in ethanol for later screening for intestinal helminths (the report is not contained here). A tissue of the spleen of each rat was also fixed in 75% ethanol for molecular identification of the rat species.

DNA extraction and polymerase chain reaction for amplification of the cestode mitochondrial *nad1* gene

DNA was obtained from the ethanol fixed metacestode isolates using the NaOH-tissue lysing method [9]. In

brief, a small tissue piece from the strobila was cut, chopped into tiny pieces and transferred into 30 µl 0.02 M NaOH and the tissue lysed at 99 ºC for 30 min. The resultant lysate was centrifuges at 300 g for 60 s and the crude supernatant used as source of DNA.

The mitochondrial NADH dehydrogenase subunit 1 (*nad1*) gene (894 bp) was amplified using the newly designed taeniid primers, Forward – 5'-AAAACGTTG GGTTTGCGTCTC-3' and Reverse – 5'- AGACCAAA GGTCCCCAAAACC-3'. The PCR was carried out in a 25 µl reaction volume including 12.5 µl Red Taq 2x Master Mix (VWR International Ltd. UK), 0.4 µM of each primer and 2 µl of the lysate. The PCR was cycled under the following thermal conditions: initial denaturation at 95 ºC for 5 min, followed by 40 cycles of denaturation at 95 ºC for 30 s, annealing at 50 ºC for 30 s and elongation at 72 ºC for 60 s, and an extension at 72 ºC for 5 min. The PCR amplicons were resolved on 1.5% agarose gel stained with ethidium bromide. The amplicons were sequenced in forward and reverse directions using the PCR primers at Inqaba Biotec West Africa ltd, Pretoria South Africa.

Rat species identification by PCR-RFLP and DNA sequencing of the *cytb* and 16 S rRNA genes

For identification of the rat species, DNA was obtained from the spleen tissues of all samples using the same NaOH-tissue lysing approach as described above and subjected it to the polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) protocol developed by Akbary Rad et al. [10]. The mitochondrial cytochrome b (*cytb*) gene (1200 bp) was amplified using the primer pairs 5'-ACTAATGACAT GAAAAATCATCGTT-3' and 5'-TCTTCATTTTTG GTTTACAAGAC-3' [11]. The PCRs were performed in 25 µl reaction volume including OneTaq® 2x Master Mix (New England Biolabs® Inc), 0.4 µM of each primer and 2 µl of DNA template. The reaction was cycled under the following thermal conditions: initial denaturation at 94 °C for 5 min and followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 48 ºC for 30 s and elongation at 72 ºC for 60 s and a post cycling extension at 72 °C for 7 min. The PCR amplicons were subjected to restriction fragment length polymorphism (RFLP) using Hinfl endonuclease. The restriction was done in 25 µl reaction comprising 10 µl of PCR product, 0.5 µl of Hinfl, 2.5 µl of 10x buffer (provided in the kit) and 12 µl of nuclease free water to make up the volume. The digestion reaction was incubated at 37 °C for 3 h and the enzyme inactivated at 80 °C for 20 min. The restricted fragmented were resolved on 2% agarose gel. To rule out ambiguity in restriction banding patterns, the *cytb* amplicons and additionally the 16 S rRNA gene (600 bp) of 10 randomly selected isolates were sequenced. The 16 S rRNA gene was amplified using the primers 5'-CGCC TGTTTACCAAAAACAT-3' and 5'-CCGGTCTGAA CTCAGATCACGT-3' [12] with similar reaction mixture and conditions as the *cytb* PCR except a primer annealing at 50 °C for 30 s, elongation at 72 °C for 30 s. Rat species were conclusively determined using the DNA sequences in a nucleotide BLASTn query against previous deposits in NCBI-GenBank.

DNA sequence analysis

DNA sequences were viewed and cleaned using GENtle (Manske M., 2003, University of Cologne, Germany). Sequences (isolates) were identified by comparison with previous deposits in GenBank and subsequently a multiple sequence alignment was performed using CLUSTAL Omega on the Ebi online platform (https://www.ebi.ac.uk/jdispatcher/msa). The alignment data was used to infer haplotype networks in PopART (http://popart.otago.ac.nz) based on the TCS network [13]. Genetic diversity and neutrality indices were calculated in DnaSP v6 [14]. Similarly, the alignment data was imported into MEGA 11 [15] and used to reconstruct a neighbour-joining phylogram [16] using the maximum composite likelihood method [17]. All DNA sequences generated in the present study were deposited in the GenBank database under the accessions: PP933736 – PP933739, PQ038945 – PQ038954 and PQ044170 – PQ044179.

Results

Rat species identification

Black rats were successfully trapped in all the three market centres, namely, Aboabo market (n = 27), Central market (n = 28) and Lamashegu market (n = 25). The PCR-RFLP of the rat mitochondrial *cytb* gave a uniform banding pattern (620 bp, 530 bp) that was consistent with *Rattus rattus* across all the 80 isolates used. Subsequently, DNA sequences of 10 randomly selected isolates were seen to be nonpolymorphic and a comparison with previous deposits in GenBank confirmed all isolates to be *Rattus rattus* (black rat). The nuclear 16 S rRNA and mitochondrial *cytb* sequences were 100% identical to MT256377 (Gabon) and OM574963 (China), respectively. The present 16 S rRNA and *cytb* sequences were deposited in GenBank under the accessions PQ038945 – PQ038954 and PQ044170 – PQ044179, respectively.

Hydatigera taeniaeformis larval cestode isolation

Generally, liver cysts were recorded in four out of every five rats (64/80 = 80% prevalence) (Fig. 1; Table 1). Most of these infected rats had just one cyst in the liver but there were a few instances where

Fig. 1 Dissected liver of a black rat (*Rattus rattus*) showing translucent cysts of *H. taeniaeformis*

Table 1 *Hydatigera taeniaeformis* prevalence and intensity of infection load in rat population from three market centres in Tamale, Ghana

Market centre	*n* rat	*n* rats with liver cysts	Liver cyst prevalence (%)	Mean intensity of infection (range)	Total number of cestode larvae recovered
Aboabo	27	20	74.07	2 (1–4)	41
Central	28	24	85.71	1	30
Lamashegu	25	20	80.00	3 (1–25)	64
Total	80	64	80.00	2 (1–25)	135

affected animals had multiple cysts with the highest being 25 cysts (Fig. 1). Likewise, each liver cyst harboured one metacestode (the larvae of *H. taeniaeformis*). A total of 135 *H. taeniaeformis* larvae were isolated from 124 cysts of the 64 infected rats.

Variation of the mitochondrial *nad1* gene (894 bp) sequences of *H. taeniaeformis*

To assess the genetic variability of the *H. taeniaeformis* larvae, sequences of the *nad1* gene of 28 randomly selected isolates were obtained and analysed.

Four unique sequences (haplotypes, Ht01-Ht04) were recorded of which two, thus, Ht01 and Ht02, had occurrence in all the three market centres where rats were trapped (Table 2; Fig. 2). Genetic diversity and neutrality tests showed 0.0013 nucleotide diversity with a pairwise nucleotide difference, k = 1.167, and nonsignificant Tajima's D = -0.25554).

Polymorphism within the nad1 sequences revealed five segregation sites (5 mutations) of which singleton variable sites = 3 and parsimony informative sites = 2. Of these mutational sites, changes at the polymorphic sites, 18 bp, 277 bp and 576 bp resulted in no amino acid change, but the change at position 98 bp and 245 bp caused replacement in the amino acids. The phenomenon of multiple haplotypes occurrence in single host was observed in two rats where Ht01 & Ht02 were found in multiple cysts of different hosts, and haplotypes Ht02 & Ht03 in two different cysts in one rat host. All the four haplotypes had identity to previous *H. taeniaeformis* s.s. *nad1* sequence deposits in GenBank: Ht01 & Ht02 were 99.66% identical to NC056571 and Ht03 & Ht04 were 99.44% and 99.55% identical, respectively to NC056571. Sequences of the haplotypes Ht01-Ht04 were deposited in GenBank under the accessions PP933736 – PP933739.

A local haplotype network of the Ghanaian sequences depicts a star-like distribution with a rather minimal variations between sequences (Fig. 2A). Comparison of the present data with previous *H. taeniaeformis* deposits from other populations was possible only with the short consensus sequences (220 bp) but it revealed a similar star-like distribution of isolates, with biogeographic clustering. The Ghanaian isolates loosely clustered with isolates from Australia, China and India, and separated from isolates from Pakistan, Europe and American (Fig. 2B).

The *H. taeniaeformis* s.s. isolates from the present and previous studies clearly clustered away from *H. kamiyai*, *H. krepkogorski* and *Hydatigera* sp. (Fig. 3). The longest mutational distance (mutational steps) was recorded between *H. taeniaeformis* s.s. vs. *H. krepkogorski*, followed by *H. taeniaeformis* s.s. vs. *Hydatigera* sp. (NC061206), and *H. taeniaeformis* s.s. vs. *H. kamiyai*, respectively. This genetic distance was also apparent in the neighbour-joining phylogram (Fig. 4), whereby the four species formed clearly distinguishable clades with *H. krepkogorski* occupying the basal clade and *H. taeniaeformis* s.s. the apex clade.

Table 2 *Hydatigera taeniaeformis* population genetics indices based on the mitochondrial *nad1* gene sequences (894 bp)

Haplotype (GenBank accession)	Nucleotide Substitution sites					Haplotype Frequencies				Diversity indices		Neutrality indices
	18	98	245	477	576	AM	CM	LM	Total	ND	HD	Tajima's D
	T	**A**	T	T	C	3	1	14	18	0.0013	0.521	-0.25554
Ht01 (PP933736)												
Ht02 (PP933737)	*	*	*	C	T	3	2	3	8			
Ht03 (PP933738)	C	G	*	C	T	-	-	1	1			
Ht04 (PP933739)	*	*	C	C	T	-	-	1	1			
Total						6	3	19	28			

AM: Aboabo market, CM: Central market, LM: Lamashegu market, ND: nucleotide diversity, HD: haplotype diversity

Discussion

Wild rodents in urban areas are widespread with accompanying records of rising incidence of emerging and re-emerging rodent-borne infections in humans and domestic animals that may have severe impact in

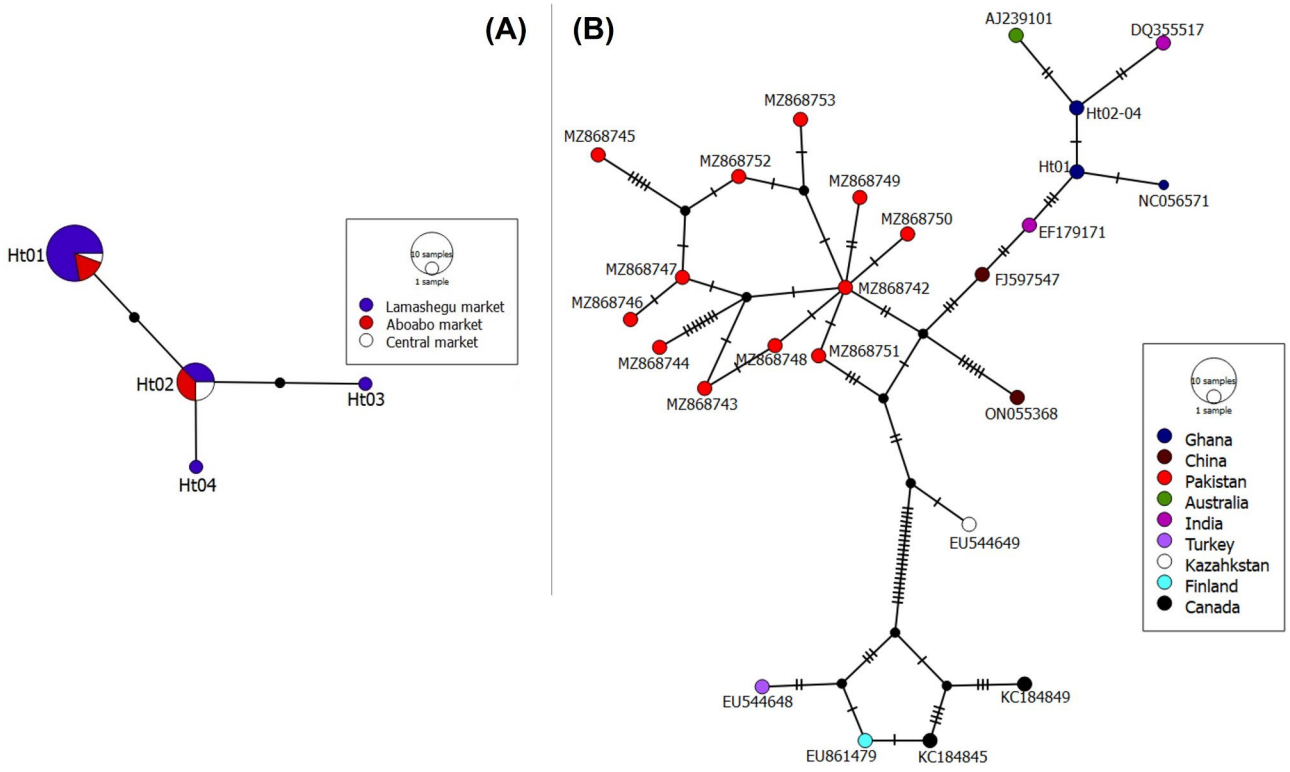

Fig. 2 Local (Ghana) statistical parsimony network of *Hydatigera taeniaeformis* mtDNA haplotypes derived from sequences of *nad1* (894 bp) (**A**) vs. global comparative network using 220 bp *nad1* sequences (**B**). Vertex size indicates frequency of the haplotype and mutational steps between haplotypes is denoted using wedges (A). Ht01-Ht04: PP933736-9. The country of origin of the sequence NC056571 could not readily be determined from the GenBank accession but it is probably of Asian origin

rural and other settings of developing countries where domestic – wildlife interactions could be frequent [18, 19]. We have presented data in the present study that draws attention to the potential risk of urban rats to the health of cats and probably humans in Tamale, Ghana.

The *H. taeniaeformis* prevalence value of 80% in the 'urban' rats could only be possible in settings where the predator – prey (cat-rat) relationship is active. We attribute the high infection rate in rats to the abundance of cats seen at the market premises and vicinity during the rat trapping exercise. Both rats and cats are in the market area for easy access to food, but this may create trophic interaction that would benefit diseases transmission, like noted in the present study and other endemic rodent-borne infections of public health importance in the area.

To the best of our knowledge, the present study is the first to use molecular tools to identify or characterize *Hydatigera* isolates from Ghana. Most accounts of *Hydatigera* infection in animals in Africa used morphological identification of adult worm, the eggs or using morphological features of the metacestode in rodents (as reviewed in Catalano et al. [3] and Ivoke [20]. Meanwhile, the cryptic nature of the *H. taeniaeformis* s. l., coupled with the multi-trophic interactions as noticed by Catalano et al. [3] demands the need to complement epidemiological studies with molecular analysis or diagnosis of the *Hydatigera* spp. in sub-Saharan Africa. The present *Hydatigera* data is based on specimens taken from a few black rats (*R. rattus*) which may not reflect the genetic diversity of the cestode in Tamale since only 28/135 were genetically characterised and no morphological description was done. In Ghana where varied rodent species are present [21], it will be beneficial to evaluate their importance in the transmission of *Hydatigera* infections or maintenance of the cestode population, as well as investigate the diversity of *Hydatigera* across trophic levels.

All the Ghanaian *Hydatigera* isolates reported here were identified as *H. taeniaeformis* s.s., and were unique of any reported *H. taeniaeformis* isolates available yet in the global databases. Regional comparison to appraise the distribution of the variants obtained in the present study was not possible because of the apparent lack of molecular data from West Africa. So far, molecular data of *Hydatigera* spp. in West Africa is available only from Senegal where Catalano et al. [3] used partial cox1 sequences to diagnose *H. parva* in

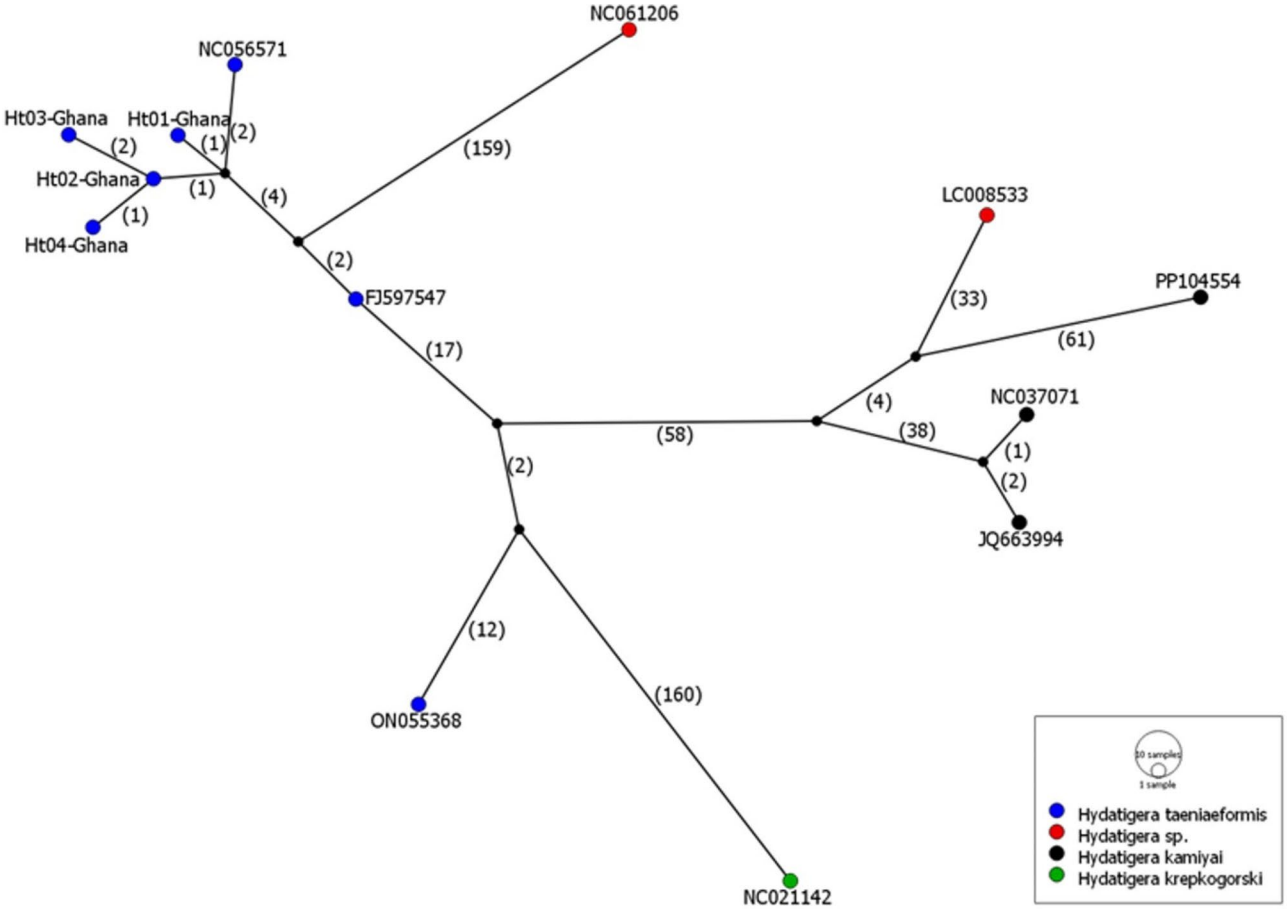

Fig. 3 Global statistical parsimony network of *Hydatigera* spp. mtDNA (*nad1*) haplotypes depicting the position of the *Hydatgera taeniaeformis* isolates from Ghana. Number in parenthesis indicate the mutational steps between haplotypes

Mastomys huberti and *Taterillus* sp., and *H. taeniaeformis* s.s. in *Arvicanthis niloticus*. The *nad1* genetic marker employed in our study revealed very minimal microvariations indicating only four haplotypes but gave an outlook of a shrinking population from a recent bottleneck event. The present molecular data (*nad1* gene sequences) is therefore the second from West Africa and together with Catalano et al. [3] have shown the widespread of the common cat tapeworm *H. taeniaeformis* s.s. in different rodent species in the region though the different genetic markers used (*cox1* vs. *nad1*) precluded data comparison. The *H. taeniaeformis* s.s. lineage is thought to have originated from Asia and subsequently distributed to Australia, Africa, America and Europe through anthropogenic introductions and host migration events [1–3, 22]. In sub-Saharan Africa, molecular diagnosis has identified *H. taeniaeformis* s.s. from the black rat (*R. rattus*), brown rat (*R. norvegicus*) and the Nile rat (*Arvicanthis niloticus*). Our data of the cestode population in 'urban' black rats that would have had a unique ecological niche compared to the populations in rural and anthropogenically undisturbed wildlife settings could explain the shrinking population genetics outlook seen. It will be interesting to show how the genetics of *H. taeniaeformis* s. l. from rodent hosts in urban, peri-urban/rural and wildlife settings compare.

Conclusion

The present study has shown high prevalence of *H. taeniaeformis* infection in urban *R. rattus* in Tamale, Ghana. The *H. taeniaeformis* isolates grouped into four globally unique haplotypes that clearly identify as *H. taeniaeformis* s.s. This data is a proxy of the abundance of rodents in urban spaces in Tamale, and the important role these rodents could play in transmitting other rodent-borne infections that may be endemic in the area. It is essential to characterize the pathogens profile of such rodent species in human habitations in Ghana to appraise their local public health risks to humans and domestic animals.

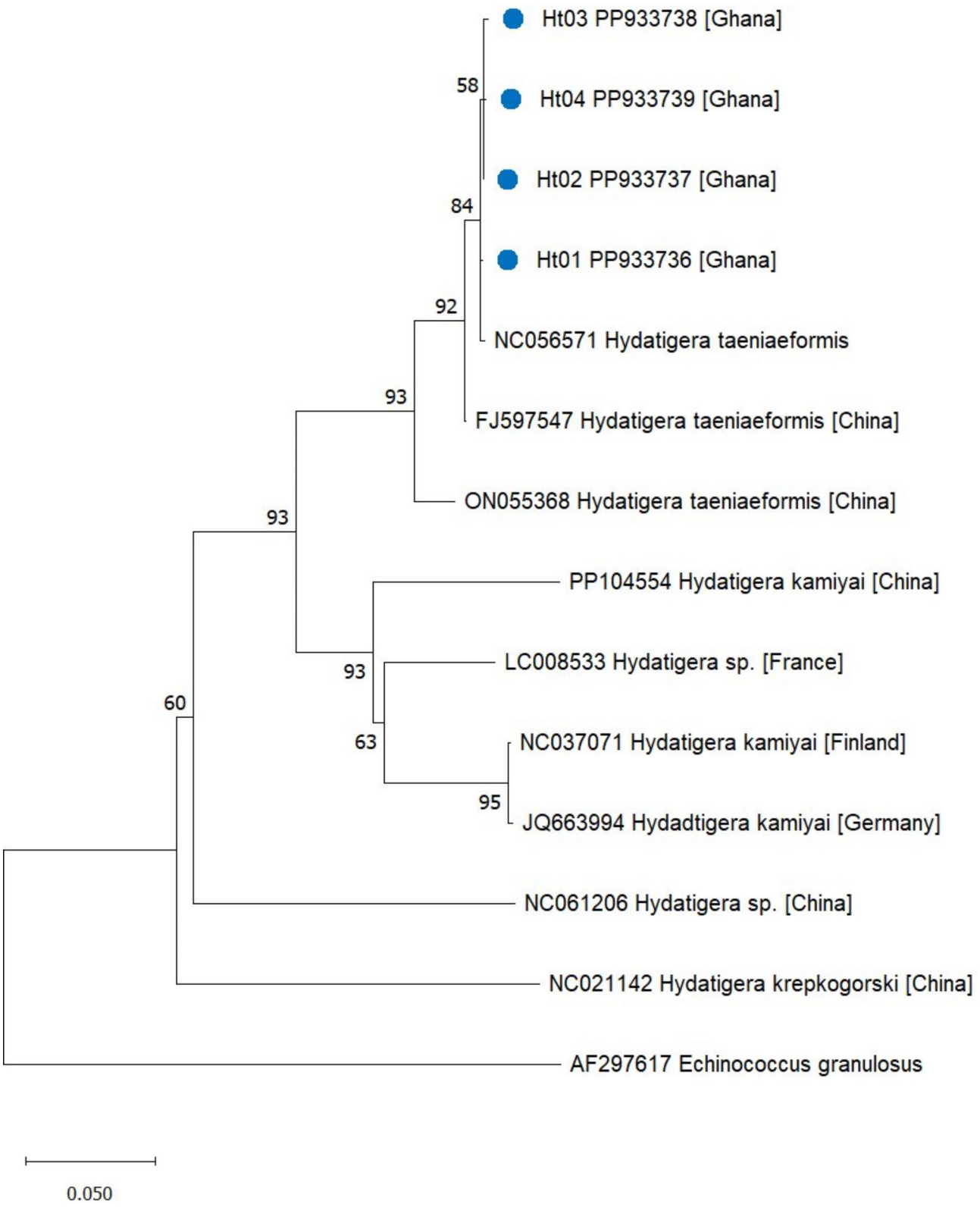

Fig. 4 Reconstructed neighbour-joining phylogeny of *Hydatigera taeniaeformis* sensu lato using the full sequences of the *nad1* gene to depict the cladal position of the Ghanaian *H.taeniaeformis* isolates. *Echinococcus granulosus* was used as an outgroup. The NJ phylogram was inferred using the Maximum Composite Likelihood model with 1000 bootstrap test (Felsenstein, 1985). The accession JQ663994 was reclassified as *H. kamiyai* in Lavikainen et al. (2016)

Abbreviations

16 S rRNA	16 S ribosomal ribonucleic acid
Cytb	Cytochrome b gene
DNA	Deoxyribose nucleic acid
Ht	*Hydatigera taeniaeformis* haplotype
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
s.l.	Sensu lato
s.s.	Sensu stricto
vs.	Versus

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Author contributions
F.A. conceived, designed, supervised the study, analysed the parasite genetics and wrote the main manuscript text. A.R.A. conducted the rat trapping and dissection. A.F.M. carried out the rat species identification.All authors reviewed the manuscript.

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Data availability
DNA sequences generated from the present study were deposited in the global public database of GenBank under the following accession numbers: PQ038945 – PQ038954, PQ044170 – PQ044179, PP933736 – PP933739. These accessions will be available to the public upon publication of this manuscript.

Declarations

Ethical approval and consent to participate
The study received ethical approval from the institutional review board of the University for Development Studies, reference number: UDS/RB/139/23. We also obtained permission from management of the respective markets to trap the rodents at the market centres.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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