



SHORT COMMUNICATIONS

Genetic characterization of *Giardia duodenalis* in wild rats in China: Identification of a divergent lineage within Assemblage B

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ABSTRACT

Giardia duodenalis is a zoonotic intestinal protozoan parasite that causes gastrointestinal disease in both humans and a wide range of animals. This study investigated the prevalence and genetic diversity of *G. duodenalis* in wild rats in China to assess the potential role of these wild rats in zoonotic transmission. Fecal samples were collected from 228 wild rats in Chongqing Municipality and Guangdong Province. The parasite was detected and characterized by nested PCR amplification, sequencing, and phylogenetic analysis of the β -giardin (*bg*), glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) genes. A distinct genetic lineage of *G. duodenalis* Assemblage B was identified in wild rats, with an overall prevalence of 6.6%. Phylogenetic analysis revealed that these isolates formed a unique, strongly supported clade within Assemblage B, showing significant genetic divergence from previously documented subtypes. No other assemblages were detected in this study. These findings highlight the presence of a divergent lineage *G. duodenalis* lineage in wild rats and underscore the need for further research to determine the prevalence and molecular detection status and genetic diversity of this parasite in rodent populations across China.

Keywords: *Giardia duodenalis*; prevalence; assemblage B; rat; China.

INTRODUCTION

Giardiasis, caused by the protozoan parasite *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*), is a neglected global parasitic disease affecting over 200 million people annually, with a significant burden among children (Coelho *et al.*, 2017). Transmission occurs via the fecal-oral route, either directly or through contaminated food and water. While human prevalence is relatively low in developed countries (0.4–7.5%), it can reach up to 30% in developing regions (Feng & Xiao, 2011). Infections range from asymptomatic to symptomatic, presenting with diarrhea, dehydration, abdominal pain, nausea, vomiting, and weight loss. *G. duodenalis* also infects a wide range of animals, leading to morbidity and economic losses (Li *et al.*, 2017).

Giardia duodenalis is considered a species complex, currently classified into eight (A–H) genetically distinct assemblages (Ryan & Cacciò, 2013). Assemblages A and B are zoonotic, infecting a broad spectrum of vertebrates, including humans. The remaining assemblages exhibit greater host specificity: C and D in canids, E in ruminants, F in felids, G in rodents, and H in marine mammals (Cacciò *et al.*, 2018). Although high prevalence has been reported in many animal species, data on *G. duodenalis* infection in wild rats remain limited, including in China (Zhao *et al.*, 2015; Feng *et al.*, 2024; Davoodi *et al.*, 2025). Rodents are primarily infected with the host-adapted Assemblage G, but assemblages with known zoonotic potential (A, B, C, and E) have also been detected in species such

as bamboo rats (Ma *et al.*, 2018), commensal rodents (Zhao *et al.*, 2015), chinchillas, and muskrats (Ryan & Cacciò, 2013). These findings suggest that rodents can harbor diverse *G. duodenalis* genotypes; however, the assemblage diversity, prevalence, and distribution in wild rat populations are poorly understood and characterized across China (Zhao *et al.*, 2015; Feng *et al.*, 2024; Davoodi *et al.*, 2025).

Leopoldamys edwardsi and *Berylmys bowersi* are two common rat species widely distributed across Asia. Their ecological habits bring them into frequent contact with human environments, making them potential reservoirs for zoonotic pathogens (Meerburg *et al.*, 2009). However, there is a notable lack of information regarding the prevalence and genetic characteristics of *G. duodenalis* in these specific wild rat species. To elucidate the population biology of *G. duodenalis* in wild rats, this study investigated its prevalence and assessed assemblage distribution in these two species from two regions of China.

METHODOLOGY

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of HuaiHua Vocational & Technical College (No. 2017003). The wild rats used for the study were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Specimen collection

Two hundred and twenty-eight wild rats of two species were purchased on the markets by our collaborators. Fecal samples were collected directly from the intestinal contents of each rat, immediately transferred into aseptically treated centrifuge tubes containing 2.5% potassium dichromate solution, placed in an ice box, transported to the laboratory, and stored at -20°C. The detailed information of these samples was recorded. Information regarding geographic origin, species and sex was recorded (Table 1). Of these, 111 samples from Edwards's giant rats (*L. edwardsi*) were collected from Chongqing city (28°102'~32°132' N, 105°112'~110°112' E) and 117 samples from Bower's white-toothed rats (*B. bowersi*) were collected from Guangdong province (20°132'~25°312' N, 109°392'~117°192' E), China in 2017.

PCR amplification and sequence analyses

The specific identity of the examined wild rats was determined by PCR-based sequencing of the mitochondrial *cox1* gene using an established method (Robins et al., 2007). Genomic DNA was extracted from fresh fecal samples (or from fresh feces) using an E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) following the manufacturer's instructions. The prevalence and genotypes of *G. duodenalis* were determined by the nested PCR amplification of the triosephosphate isomerase (*tpi*), beta giardin (*bg*) and glutamate dehydrogenase (*gdh*) genes as previously reported (Zhang et al., 2017). Positive controls consisted of genomic DNA extracted from a known *G. duodenalis* Assemblage B isolate (human clinical origin, previously confirmed by sequencing). Negative controls consisted of nuclease-free water substituted for template DNA in both primary and secondary nested PCR reactions. No-template controls were included in each PCR run. The secondary PCR amplicons were examined on agarose (1.0%) gels containing GoldView™. The positive amplicons were purified and then sent to Sangon Biotech (Shanghai, China) for sequencing in both directions.

The sequencing results were aligned with known reference sequences available in GenBank using the Basic Local Alignment Search Tool (BLAST), and the genotypes of *G. duodenalis* were identified by MAFFT 7.122 with G-INS-i strategy (Katoh & Standley, 2013). The sequences with mutations (nucleotide substitutions, deletions and insertions) or mixed *G. duodenalis* genotypes were detected by chromatograms. The obtained sequences and the reference sequence were aligned individually using MAFFT 7.245. Phylogenetic analyses were performed using Bayesian inference (BI) and neighbor-joining (NJ). BI was conducted with four independent Markov chains for 100,000 Metropolis-coupled MCMC generations, sampling trees every 100 generations in MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003); the first 2,500 trees represented burn-in and the remaining trees were used to calculate Bayesian posterior probabilities (Bpp). The analysis was run until the potential scale reduction factor approached 1 and the average standard deviation of split frequencies was < 0.01. NJ using MEGA 7 (Kumar et al., 2016), with the Kimura 2-parameter substitution model. Bootstrap frequency was calculated using 1000 bootstrap

replicates. Phylograms were drawn using the program FigTree v.1.4 (<http://tree.bio.ed.ac.uk/software/figtree>).

Statistical analyses

The prevalence of *G. duodenalis* was calculated for each host species, sex, and sampling region, and exact binomial 95% confidence intervals were estimated. Because the number of positive samples was small and some categories contained zero positive samples, Fisher's exact test rather than the chi-square test was used to compare prevalence among groups. Two-sided exact *P* values were reported, and *P*<0.05 was considered statistically significant. Conventional binary logistic regression was not performed because sparse data and complete or quasi-complete separation prevented reliable estimation of odds ratios and independent effects. When zero cells were present, odds ratios were considered not estimable and the results were interpreted descriptively

RESULTS AND DISCUSSION

In this study, 15 (6.6%) out of 228 rat samples were PCR-positive for *G. duodenalis*. A total of 2 *tpi*, 15 *bg* and 5 *gdh* gene sequences were obtained in *L. edwardsi* samples, but no positivity was observed in *B. bowersi* samples. These sequences were deposited in GenBank (Accession nos. MQ751592-MQ751597). The overall prevalence of 6.6% in wild rats from Chongqing and Guangdong, China, is slightly higher than the 6% (14/232) reported in commensal rats from Fujian province, China (Zhao et al., 2015). This rate falls within the wide range reported globally for rodents. A recent global meta-analysis estimated a pooled prevalence of 7.4% in rodents, with significant geographical heterogeneity (Davoodi et al., 2025). For instance, studies in Central Europe, such as in Hungary, reported a lower prevalence of 3.2% in pet and wild rodents (Tuska-Szalay et al., 2025), while investigations in specific regions of China have revealed varying rates. A comprehensive survey across multiple provinces in China detected *G. duodenalis* in 19.8% (75/378) of wild rodents, with prevalence differing markedly among species and sites (Feng et al., 2024). The exclusive detection of *G. duodenalis* DNA in *L. edwardsi* and absence in *B. bowersi* within our study aligns with findings of significant species-specific differences in *Giardia* carriage. This underscores that prevalence is influenced by a complex interplay of host ecology, species susceptibility, and local environmental factors.

Sequence analysis revealed that these *tpi*, *bg*, and *gdh* sequences derived from *L. edwardsi* samples shared 99.3–100%, 99.6–100%, and 99.8–100% sequence homology, respectively. BLAST analysis and phylogenetic reconstruction indicated that these isolates belong to Assemblage B. However, they exhibited high genetic divergence compared to reference sequences. The nucleotide sequence difference was ~8%, ~4%, and ~6% between these *tpi*, *bg*, and *gdh* sequences and those of reference *G. duodenalis* Assemblage B isolates, respectively. Although these divergence levels are comparable to those recorded between some distinct assemblages (e.g., sequence difference in *tpi*, *bg*, *gdh* genes

Table 1. Factors associated with prevalence of *Giardia duodenalis* in wild rats

Factor	Category	No. tested	No. positive	Genetic locus		
				TPI	BG	GDH
Region/Species	Chongqing/ <i>Leopoldamys edwardsi</i>	111	15	2	15	5
	Guangdong/ <i>Berylmys bowersi</i>	117	0	0	0	0
Sex	Female	113	6	0	6	2
	Male	115	9	2	9	3
Total		228	15	2	15	5

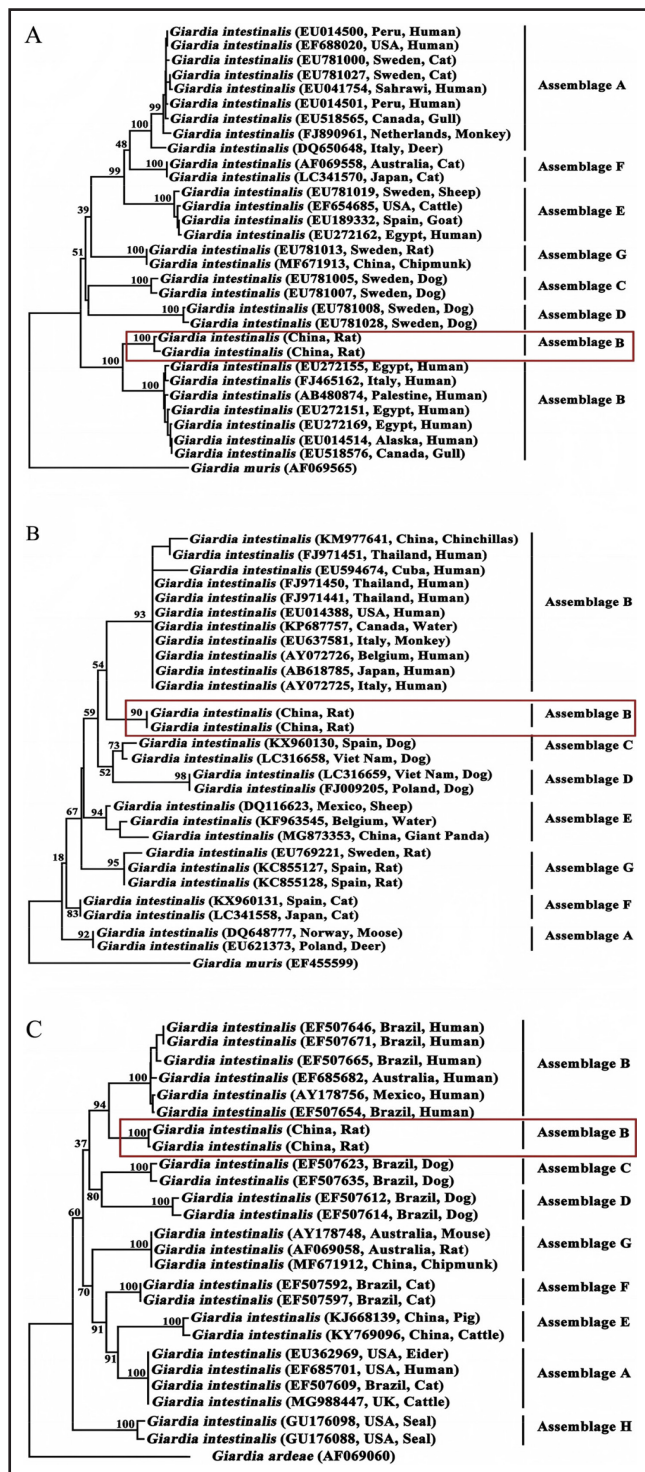


Figure 1. Phylogenetic analysis based on the *tpi* (A), *bg* (B) and *gdh* (C) gene sequences of *Giardia duodenalis* from different geographical origin and hosts. The isolate name, GenBank accession number, host, and area of origin are listed. *Giardia muris* was used as an outgroup.

is ~ 19%, ~ 7% and ~ 9% between assemblages C and D, respectively), our phylogenetic analysis placed this divergent lineage within the Assemblage B clade (Figure 1).

The divergent lineage identified in the present study clustered into a monophyletic subclade within Assemblage B with strong support in all phylogenetic analyses (Figure 1). This lineage was sister to other Assemblage B sequences with strong support in *tpi* (bootstrap = 100) and *gdh* (bootstrap = 94) sequence analyses,

and moderate support in *bg* (bootstrap = 54) sequence analyses (Figure 1). Assemblage B is known to be highly polymorphic (Ryan & Cacciò, 2013). The significant genetic distance observed here suggests that wild rats in this region may harbor a specific, potentially host-adapted variant of Assemblage B. No other assemblages were detected in the present study, although assemblages A, B, C, E and G have been reported previously in rodents (Ryan & Cacciò, 2013; Zhao et al., 2015; Ma et al., 2018; Ryan et al., 2019). Our finding of only Assemblage B differs from the assemblage diversity often reported in rodents globally. While Assemblage G is frequently identified as the predominant host-adapted genotype in rodents worldwide (Davoodi et al., 2025; Asghari et al., 2022; Zhao et al., 2015), zoonotic assemblages are also commonly detected. For example, in Europe, wild rodents have been found to carry both Assemblages A and B (Klotz et al., 2021). In China, a large-scale study identified multiple assemblages (A, B, and G) circulating in wild rodent populations, with assemblage distribution showing geographical and host-related variations (Feng et al., 2024). The sole detection of a divergent Assemblage B lineage in our *L. edwardsi* samples may indicate a unique transmission cycle or host adaptation within this particular species and region. This contrasts with findings in some European rodents where Assemblage A was more prevalent than B (Tuska-Szalay et al., 2025), and with studies in bamboo rats from China, which reported Assemblage A (Ma et al., 2018). The high degree of genetic divergence observed within our Assemblage B sequences echoes findings from other wildlife studies, highlighting the extensive cryptic diversity within established assemblages that may be associated with specific host groups or ecosystems (Hillman et al., 2019).

Several limitations should be considered. First, samples were obtained from markets rather than through systematic field trapping, which may not fully capture the natural dynamics of *G. duodenalis* carriage. Second, detection relied solely on molecular methods; coupling this with morphological examination could provide a more complete diagnostic picture. Finally, the uneven sample distribution between species and locations may affect the generalizability of the prevalence estimates. Despite these limitations, this study provides the first report and genetic characterization of *G. duodenalis* Assemblage B in *Leopoldamys edwardsi*, revealing a highly divergent genetic variant. These findings contribute to the growing understanding of the complex genetic landscape of *G. duodenalis* in wildlife reservoirs. The presence of a potentially host-adapted or geographically distinct variant of the zoonotic Assemblage B (a genotype with known zoonotic potential) in wild rats underscores their possible role in the parasite's ecology. However, as no human isolates were analyzed, no environmental sampling was performed, and no direct transmission evidence was obtained in this study, the actual zoonotic risk remains speculative. It emphasizes the need for sustained surveillance of synanthropic wildlife combined with human clinical and environmental investigations to better assess whether transmission occurs and to understand the ecology of *Giardia* at the human-animal-environment interface (Ryan et al., 2019; Sanhueza Teneo et al., 2025).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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