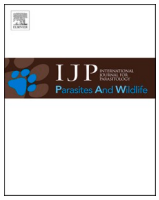
International Journal for Parasitology: Parasites and Wildlife 24 (2024) 100957

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw



Epidemiological investigation of *Trichomonas gallinae* in beijing, China

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ARTICLE INFO

Keywords:
Trichomonas gallinae
Bird species
Phylogeny
Genotype

ABSTRACT

Trichomonas gallinae is a globally distributed protozoan parasite that causes avian trichomoniasis, leading to significant morbidity and mortality in birds. The present study aims to investigate the prevalence, genetic diversity, and phylogenetic relationship of *T. gallinae* in various bird species in Beijing. A total of 413 oropharyngeal swab samples were collected from domestic pigeons, wild pigeons, and other bird species. The overall prevalence of *T. gallinae* infection was 32.0% (132/413). The infection was detected in domestic pigeons, wild pigeons, and red-necked turtledoves, but not in other wild birds. Molecular analysis identified two predominant genotypes, A and B, with genotype A found in wild pigeons and genotype B found in domestic pigeons. The present study provides valuable insights on the prevalence and genetic diversity of *T. gallinae* in Beijing. This can be useful for understanding its pathogen distribution and host range, and the development of strategies for the prevention and control of avian trichomoniasis.

1. Introduction

Avian Oropharyngeal Trichomonosis, which is caused by protozoan parasite *Trichomonas gallinae*, can lead to substantial morbidity and mortality among bird populations. This disease is characterized by the formation of a cheese-like substance in the affected area, which can obstruct the digestive and respiratory tracts, thereby hindering proper nutrition and respiration in affected individuals (Gómez-Muñoz et al., 2022). In addition, these parasites invade the pharyngeal glands, penetrate the underlying tissues, and ultimately reach the liver, causing abscess, and even death (Gómez-Muñoz et al., 2022). Through the transmission of trophozoites *via* shared water and food sources, Avian trichomonosis has become a globally distributed disease, affecting birds in Asia (Alrefaei, 2020; Chou et al., 2022), Europe (Tuska-Szalay et al., 2022), America (Amin et al., 2014; Martínez-Herrero et al., 2020) and Oceania (Brunthaler et al., 2022), and causing significant health issues in pigeons (*Columba livia*), as well as other domesticated and wild bird species (Chen et al., 2022). Given that China is the world's largest producer of pigeons, the meat and carrier breeding industries in the country are seriously threatened. A prevalence of approximately 30.0% has been reported in pigeon farms in Shandong Province (33.8%, 206/609) (Jiang et al., 2016), Beijing (28.3%, 161/569) (Feng et al., 2018), Guangdong Province (26.6%, 169/636) (Cai et al., 2022), and Anhui Province (35.1%, 565/1612) (Cai et al., 2022; Zhang et al., 2024). However, there is a paucity of research on the infection of *T. gallinae* in wild bird populations in China.

In order to address the challenge for *T. gallinae*, veterinarians have turned to a variety of treatment modalities. The most successful and non-toxic options include nitroimidazoles (metronidazole, ornidazole, carnidazole and ronidazole), as well as amides and aminoglycosides (Gómez-Muñoz et al., 2022). Nevertheless, the development of drug resistance has emerged as a substantial concern, which was largely due to the administration of excessive doses, or the inappropriate use of medications. In addition, the resistance development can be attributed

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https://doi.org/10.1016/j.ijppaw.2024.100957
Received 21 March 2024; Received in revised form 22 June 2024; Accepted 23 June 2024
Available online 24 June 2024

to the variable disease responses among individuals, and the delayed onset of symptoms in some infected birds (Luo et al., 2010; Zhu et al., 2007). These variations in disease presentation are compounded by the differential virulence exhibited by different lineages of the parasite. Some lineages are highly pathogenic, inflicting severe damage to the oropharynx and liver of infected birds, and often resulting in mortality (Zhu et al., 2007). Furthermore, the sequence analysis revealed that the heterogenic species of *T. gallinae* are present in different bird populations, and even within the same host species. This heterogeneity necessitates a nuanced understanding of the parasite's genetic makeup. Typically, the multilocus sequence typing analysis of *T. gallinae* target genes for internal transcribed spacer (ITS), the small subunit of ribosomal RNA (SSU rRNA), and iron hydrogenase (Fe-hyd) (Liu et al., 2024; Martínez-Herrero et al., 2020). *Trichomonas gallinae* genotypes A and B, and Trichomonas tenax-like strains have been identified in domestic pigeons in Shandong Province, China (Jiang et al., 2016), highlighting the need for further research and monitoring, and effective management and treatment of the parasite infection.

The present study aimed to investigate the prevalence, genetic diversity, and phylogenetic relationship of T. gallinae species across various bird species in Beijing. The investigators collected samples from domestic and wild pigeons, and other urbanized bird species, such as red-necked turtledoves (*Streptopelia tranquebarica*), common myna (*Acridotheres tristis*), magpie (*Pica pica*), crow (*Corvus*), red-billed Leiothrix (*Leiothrix lutea*), black swan (*Cygnus atratus*), mallard (*Anas platyrhynchos*), merlin (*Falco columbarius*), and peregrine falcon (*Falco peregrinus*). Focus was given on the prevalence of T. gallinae genotypes in birds from various bird species, geographical regions, age groups, months, and growth environments. In addition, the molecular epidemiology of trichomonads in different geographical regions and among different bird species was analyzed. The present study would provide valuable insights on the prevalence and genetic diversity of T. gallinae species, which can be useful for understanding the pathogen distribution and host range. Furthermore, these findings would contribute to the development of strategies for the prevention and control of avian trichomoniasis in susceptible bird species.

2. Materials and methods

2.1. Sample collection

The present study was conducted from January to March 2021. During this period, a total of 413 oropharyngeal swab smears were collected from domestic pigeons, *Columba livias*, and other avian species in pigeon farms and wildlife rehabilitation centers located in the administrative districts of Shunyi, Daxing, Miyun, Fangshan, Haidian and Chaoyang in Beijing (Table 1). The present study and experimental procedures were approved by the Institutional Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Sciences (approval no. IOZ-IACUC-2022-251). All sampling, animal housing, and experiments were conducted in strict accordance to the institutional Guidelines for Care and Use of Laboratory Animals. In order to collect the samples, a sterile cotton swab soaked in phosphate-buffered saline (PBS) was delicately manipulated to gently wipe the oral cavities of both domestic and wild birds for 2–3 times. At post-collection, the swabs were carefully transferred into 1.5 mL centrifuge tubes that contained the tryptone/yeast extract/maltose medium, as previously described in a literature (Diamond, 1957). Then, these tubes were labeled and placed in an insulated container, in order to maintain sample integrity. After the sampling was completed, all samples were promptly transported to the laboratory on the same day of excursion, and incubated at a temperature of 37 °C to facilitate the subsequent analysis and examination.

2.2. Parasite culture and sampling

After the 12-h incubation at 37 °C, the culture fluid was dripped onto a glass slide, covered with a coverslip, and transferred to an optical microscope for examination. At a magnification of 10× 10, the samples were inspected for irregular movements, and the presence of transparent parasites. The increase in magnification to 10× 40 allowed for the closer examination of the flagella and undulating membrane of any detected parasites.

Table 1
Sampling quantity of birds from different regions and species in 2021.

Group	January	February	March	Total
Geographical region				
Shunyi	0	14	66	80
Daxing	54	58	50	162
Miyun	50	9	20	79
Fangshan	0	60	26	86
Haidian	0	2	2	4
Chaoyang	0	1	1	2
Total	104	144	165	413
Avian species				
Wild pigeon (*Columba livias*)	4	53	39	96
Red-necked turtledoves (*Streptopelia tranquebaricas)*	0	6	12	18
Myna (*Acridotheres cristatellus)*	0	14	16	30
Magpie (*Pica pica*)	0	7	16	23
Crow (*Corvus*)	0	3	8	11
Red-billed Leiothrix (*Leiothrix lutea*)	0	0	2	2
Black swan (*Cygnus atratus*)	0	1	1	2
mallard (*Anas platyrhynchos*)	0	1	1	2
Merlin (*Falco columbarius*)	0	4	12	16
peregrine falcon (*Falco peregrinus*)	0	5	8	13
Nestling pigeon	50	30	20	100
Adolescent pigeon	50	20	30	100
Total	104	144	165	413

2.3. DNA extraction and polymerase chain reaction (PCR) methods

The DNA extraction was performed using the blood/tissue/cell base genomic DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's operation guide. Then, DNA templates from two randomly selected samples that contained active parasites were used. In order to ensure the absence of contamination, an equal volume of ddH_2O was used as the negative control. For the PCR detection of *T. gallinae* infection, the ITS regions of the samples were amplified using the following specific primers: ITS-1 (5′-TGCTTCAGCTCAGCGGGTCTTCC) and ITS-2 (5′-CGGTAGGTGAACCTGCCGTTGG) (Felleisen, 1997). Then, the PCR reaction was carried out at a volume of a 50 μL, which contained 2 μl of the extracted DNA template, 1 μL of each primer, 25 μL of 2 × Rapid Taq Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China), and 21 μL of ddH_2O. The PCR amplification was conducted using the Veriti™ Dx 96-Well Fast Thermal Cycler (Thermo Fisher Scientific) with the following thermal cycling conditions: initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C for denaturation, 30 s at 58.7 °C for annealing, and 30 s at 72 °C for extension. A final extension step of 10 min at 72 °C was included to ensure complete amplification. The PCR products were evaluated by gel electrophoresis using 1% agarose gel stained with GoldView II (Solarbio, Beijing, China), and visualized under ultraviolet light.

2.4. Sequencing, phylogenetic, and statistical analyses

The positive PCR products were sent to Beijing Liuhe Huada Genomics Technology Co., Ltd. for sequencing. The sequence was checked against the GenBank/EMBL/DDBJ database using the National Center for Biotechnology Information (NCBI) Blast tool (https://blast.ncbi.nlm.nih.gov). Those with the highest similarity to the PCR products were identified and downloaded as reference sequences for the phylogenetic analysis.

The phylogenetic analysis was conducted using the MEGA 7.0

software. The neighbor-joining and maximum likelihood method was employed to construct the phylogenetic tree. The Kimura 2-parameter model was chosen as the substitution model. This model is well-suited for estimating evolutionary distances between closely related sequences, and is particularly relevant for the present study, given the high degree of similarity among the *T. gallinae* sequences. In order to assess the reliability of the branches, these were analyzed using 1000 bootstrap pseudo-replicates. In addition to the neighbor-joining and maximum likelihood method, Bayesian inference (BI) analysis was conducted using MrBayes 3.2.2. Two separate analyses with 1,000,000 Metropolis-coupled MCMC generations each was executed in MrBayes 3.2.2, harvesting a tree from each generation. After the exclusion of the initial 250 trees as burn-in, the subsequent trees were retained for the computation of Bayesian posterior probabilities (BPP). Then, the phylogenetic diagrams were generated using FigTree version 1.44 (available at http://tree.bio.ed.ac.uk/software/figtree). The sequences obtained in the present study were deposited in GenBank, with accession numbers OQ807110-OQ807119, ensuring the transparency and reproducibility of the present findings.

For the statistical analysis, the SPSS software was used to assess the differences in the prevalence of *T. gallinae* infections. Chi-squared (X^2) test was applied to determine the significant differences in prevalence rates between the different groups, as appropriate. A p-value of <0.05 was considered statistically significant, indicating that the observed differences were unlikely to have occurred by chance.

3. Results

3.1. Prevalence of Trichomonas gallinae

The microscopic examination revealed an overall positive rate of 21.3% (88/413) for various bird species. The PCR assay, which was optimized for specificity, amplified a product of approximately 356 base pairs from the *T. gallinae* samples, but not from the heterologous control. Concurrent to the microscopy, all 88 positive samples by microscopy were confirmed by PCR, while an additional 44 negative microscopy samples yielded positive PCR results, indicating the heightened sensitivity of the PCR method. Thus, the subsequent analysis was based on the PCR outcomes.

Infestations were recorded in domestic pigeons, wild pigeons, and red-necked turtledoves, but not in other wild birds, such as mynas, magpies, crows, red-billed queleas, grey francolins, black swans, mallards, red kites, and peregrine falcons. The chi-squared test results revealed significant differences in prevalence among bird species ($X^2 = 132.88$, df $= 3$, $p < 0.001$), with the highest prevalence in domestic pigeons (55.0%, 110/200), followed by wild pigeons (16.7%, 16/96) and collared doves (33.3%, 6/18). Among the other wild birds, no infections were detected. On the region level, the highest prevalence was found in Miyun (45.6%, 36/79), while the other regions had a lower prevalence: 38.8% (31/80) in Shunyi, 33.7% (29/86) in Fangshan, and 22.2% (36/162) in Daxing. Domestic pigeons in Miyun (68.0%, 34/50) had the highest prevalence, while those in Daxing had the lowest prevalence (46.0%, 23/50). For wild pigeons, the highest prevalence was in Shunyi (23.1%, 3/13), and for red-necked turtledoves, the highest prevalence was in Daxing (50.0%, 4/8). No infections were detected in other wild birds across all districts.

The age-related prevalence trends indicated that domestic and wild pigeons, as well as red-necked turtledoves, were most commonly infected among the nestling birds, with domestic pigeons showing the highest prevalence (79.0%, 79/100). For adolescent birds, red-necked turtledoves had the highest prevalence (33.3%, 5/15), followed by domestic pigeons (31.0%, 31/100) and wild pigeons (13.8%, 12/87). *Trichomonas gallinae* infection was detected in domestic pigeons, wild pigeons, and red-necked turtledoves from January to March, with a particularly high incidence in January for domestic pigeons (54.8%, 57/104). A significant difference in *T. gallinae* incidence was observed across different growth environments ($X^2 = 94.65$, df $= 1$, $p < 0.001$), with domestic pigeons from large-scale breeding farms having the highest incidence (55.0%, 110/200), when compared to wild birds from wetlands, forest parks, and nature reserves (10.3%, 22/213) (Table 2).

3.2. PCR and sequence analysis

The PCR amplification of positive samples led to the sequencing of 132 *T. gallinae* ITS1/5.8S/ITS2 gene sequences. These sequences were processed and subjected to evolutionary analysis (Fig. 2). The results identified 10 distinct genotypes: OQ807110, OQ807111, OQ807112, OQ807113, OQ807114, OQ807115, OQ807116, OQ807117, OQ807118, and OQ807119. Among these, OQ807110, OQ807111, OQ807112, OQ807113, and OQ807114 exhibited evolutionary relationships similar to those of the reference sequences (KU954107, KT869155, KJ721785, MH733817 and EU215368) downloaded from GenBank. All of these belonged to the same branch, and were classified as genotype A. The remaining five sequences (OQ807115, OQ807116, OQ807117, OQ807118, and OQ807119) were identified on the same evolutionary branch as multiple sequences downloaded from GenBank, which included KJ721784, LC136936, KX459474, EU881912, and MH733820. These were classified as genotype B. Genotype A accounted for 41.7% (55/132), and was distributed in the Daxing and Fangshan districts, while genotype B constituted 58.3% (77/132), and was distributed in the Shunyi, Miyun, Daxing, and Fangshan districts. Wild pigeons predominantly harbored genotype A, while domestic pigeons were mainly associated to genotype B (Figs. 1 and 2).

4. Discussion

The common infection of *T. gallinae* in domestic pigeons and wild birds was observed in Beijing, China. Consistent with previous studies, the prevalence was highest for domestic pigeons, with over half of the tested samples yielding positive results. This incidence rate exceeds the findings of the survey conducted in Beijing in 2018 (28.3%, 161/569), and aligns with the median reported in previous studies from other regions of China (Jiang et al., 2016; Li, 2022; Zhu et al., 2007). Studies conducted in the UK (60.0%, 36/60) (Lennon et al., 2013) revealed a similar prevalence, while significantly lower rates were detected in Egypt (1.9%, 65/3315) (El-Khatam et al., 2016). It has been widely accepted that the infestation rate varies depending on the species, geographic location, and detection method (El-Khatam et al., 2016; Martínez-Herrero et al., 2014). The discrepancy in infestation rate is widely acknowledged to be influenced by the species, geographic location, and diagnostic method. Additional factors, including feeding habitat, climatic differences, management practices, and sample size, would likely contribute to the variability observed.

Diverging from prior studies conducted in China, the present study evaluated the prevalence in wild avian populations in Beijing. The infection rate for wild pigeons was identified to be 16.7% (16/96), while that for red-necked turtledoves reached 33.3% (6/18). Species, such as mynas, magpies, crows and peregrine falcons, exhibited an infection rate of 0.0%. The prevalence was lower for the wild birds in the present study, when compared to that in Western and Southern Europe, the UK, and Saudi Arabia (Alrefaei, 2020; Lennon et al., 2013; Marx et al., 2017). This may be attributed to the timing of the study, which was conducted from January to March, when wild pigeons and other wild bird species are known to reduce their foraging due to the harsh winter season, thereby affecting the representativeness of the sample collection. On the other hand, the prevalence was observed to be higher in artificial environments, when compared to natural settings. This suggests that *T. gallinae* has the propensity to disseminate within moist bird food, and is a significant factor in the transmission of trichomoniasis (McBurney et al., 2017). Wild birds have a stronger immune system in its natural, free-range environment. This makes these birds less susceptible to infection with *T. gallinae*, thereby resulting in lower infection rates for

Table 2
Prevalence of *Trichomonas gallinae* in the different characteristic groups of pigeons surveyed in Beijing, China in 2021.

Characteristic	Subgroup	Prevalence (positive/all)	Species			
			Pigeons (*Columba livia*)		Red-necked turtledoves (*Streptopelia tranquebarica*), *n* = 18	Other wild birds, *n* = 99
			Domestic, *n* = 200	Wild, *n* = 96		
Total	/	32.0% (132/413)	55.0% (110/200)	16.7% (16/96)	33.3% (6/18)	0
Region	Shunyi	38.8% (31/80)	54.0% (27/50)	23.1% (3/13)	20.0% (1/5)	0
	Daxing	22.2% (36/162)	46.0% (23/50)	20.9% (9/43)	50.0% (4/8)	0
	Miyun	45.6% (36/79)	68.0% (34/50)	7.7% (1/13)	33.3% (1/3)	0
	Fangshan	33.7% (29/86)	52.0% (26/50)	12.0% (3/25)	0	0
	Others	0	0	0	0	0
Age	Nestling	71.2% (84/118)	79.0% (79/100)	44.4% (4/9)	33.3% (1/3)	0
	Breeding	16.3% (48/295)	31.0% (31/100)	13.8% (12/87)	33.3% (5/15)	0
Month	January	54.8% (57/104)	57.0% (57/100)	0	0	0
	February	26.4% (38/144)	52.0% (26/50)	18.9% (10/53)	33.3% (2/6)	0
	March	22.4% (37/165)	54.0% (27/50)	15.4% (6/39)	33.3% (4/12)	0

wild pigeons and other wild birds. In contrast, domestic pigeons are more susceptible to influences from food, water sources, contaminated environments, and vectors of infection (Smith et al., 2023).

Notably, nestling birds were particularly susceptible to the disease. A study conducted in Beijing in 2018 revealed that the prevalence of pigeon *T. gallinae* infection was 28.3% (161/569), with the highest infection rate observed in nestling pigeons (33.2%, 65/196), followed by adolescent pigeons (30.1%, 61/203) and breeding pigeons (20.6%, 35/170) (Feng et al., 2018). In an epidemiological survey of *T. gallinae* infection in Guangzhou province, the prevalence was significantly greater for nestling pigeons (51.3%, 59/115), when compared to adult pigeons (18.1%, 19/105) (Martínez-Herrero et al., 2014). Furthermore, the parasite has been implicated in high nestling mortality rates in European Turtle Doves and Cooper's Hawks (Accipiter cooperi) (Stockdale et al., 2015; Urban and Mannan, 2014). The elevated infection rate in nestling birds may be due to the reliance on parents for direct oral feeding. This increases the susceptibility to infection, and leads to underdeveloped immune systems, causing these birds to lack sufficient resistance to diseases. During the rearing process, transmission can occur through contaminated equipment and drinking water (Xu and Li, 2014). A study conducted on Cooper's Hawks revealed that the oral pH of nestling hawks provide a conducive environment for *T. gallinae*, but this pH decreases as the hawks mature, rendering adults less susceptible to infection (Urban and Mannan, 2014). Being aware of these conditions can aid in the early detection of the parasite, and the implementation of medication and preventative measures to control the risk of disease outbreaks.

The comparison of samples across different months revealed a marked decline in infection rate for domestic pigeons from January to February and March, indicating that the prevalence of trichomoniasis in pigeons is influenced by climatic conditions (Robinson et al., 2010). Warm seasons, particularly spring, is associated to higher risk of transmission and infection due to the initiation of the breeding season. This surge in breeding activity leads to a concentrated bird population, which in turn facilitates the dissemination and contraction of *T. gallinae*. In addition, the high temperature and humidity during these seasons contribute to the reproduction and dissemination of the parasite, increasing the risk of infection. Conversely, during cold seasons, the infection risk diminishes due to the reduced number of birds, which constraints the parasite's ability to transmit and propagate, consequently lowering the infection risk (Lawson et al., 2012). Although the present study was constrained by its temporal scope, which covered only January to March, this provides valuable insights on the seasonal dynamics of trichomoniasis. However, the limited time frame reduced the statistical power of the analysis, limiting the scope of conclusions on the impact of seasonal patterns on infection rates. Future research with broader temporal coverage is necessary to fully elucidate the relationship between climatic conditions and the epidemiology of *T. gallinae*.

The evolutionary analysis of the ITS1/5.8S/ITS2 gene sequences of *T. gallinae* in the Beijing area identified two predominant genotypes (genotypes A and B), which aligns with the previous findings obtained from Beijing (Feng et al., 2018), and Shandong province (Jiang et al., 2016). This consistency suggests the stable distribution of these genotypes in these regions. The less common gene T type of *T. gallinae,* which is associated to oral trichomonad, was first detected in Spain, and this was also detected in Shandong (Jiang et al., 2016). The gene III type is less frequently reported, and is mostly distributed in Asia. In Western and Southern Europe, wood pigeons are mainly infected with the II (Lennon et al., 2013) and C/V/N lineage (Chi et al., 2013), while the infection in collared doves is mainly caused by the P and III (Chi et al., 2013) lineages. Most Italian collared dove samples belong to the P and III lineages(Chi et al., 2013). These research results suggest that there are genetic differences in *T. gallinae* from different geographical regions, which may be correlated to the living environment, host species, and climatic conditions (Martínez-Herrero et al., 2014). In addition, the genetic drift and mutations can lead to differences in genetic type, which may be influenced by factors, such as the use of antiparasitic drugs and environmental pollution.

The difference in predominant genotypes between domestic and wild pigeons suggest that there could be benefits to developing genotype-based therapies for managing trichomoniasis in pigeons. Genotype B is known to be less pathogenic, making this a preferable strain to target with lower doses of medication (Quillfeldt et al., 2018). However, the practice of farmers in administering lower doses of medication has led to the increased prevalence of drug-resistant strains of genotype B in domestic pigeon populations. On the other hand, genotype A is the predominant strain in wild pigeons, and is associated to supplementary food sources and water from artificial waterers. The research conducted by Purple K et al. reinforces the importance of maintaining clean bird waterers to reduce the risk of *T. gallinae* transmission (Purple et al., 2019). This genotype might be more effective in being transmitted through the environment, when compared to other strains. This could explain its prevalence in wild populations. Furthermore, genotype A is considered to have higher virulence. This means that this can cause more severe diseases, and potentially have a greater impact on pigeon populations. Given the potential population impact of the genotype A infection, it is crucial to manage supplementary food resources, in order to minimize the transmission risks (Stockdale et al., 2015). This could involve strategies to reduce the availability of supplementary food, which in turn, may help to reduce the overall prevalence of *T. gallinae* in pigeon populations. Furthermore, the effective management of supplementary food resources can help to mitigate the negative effects of

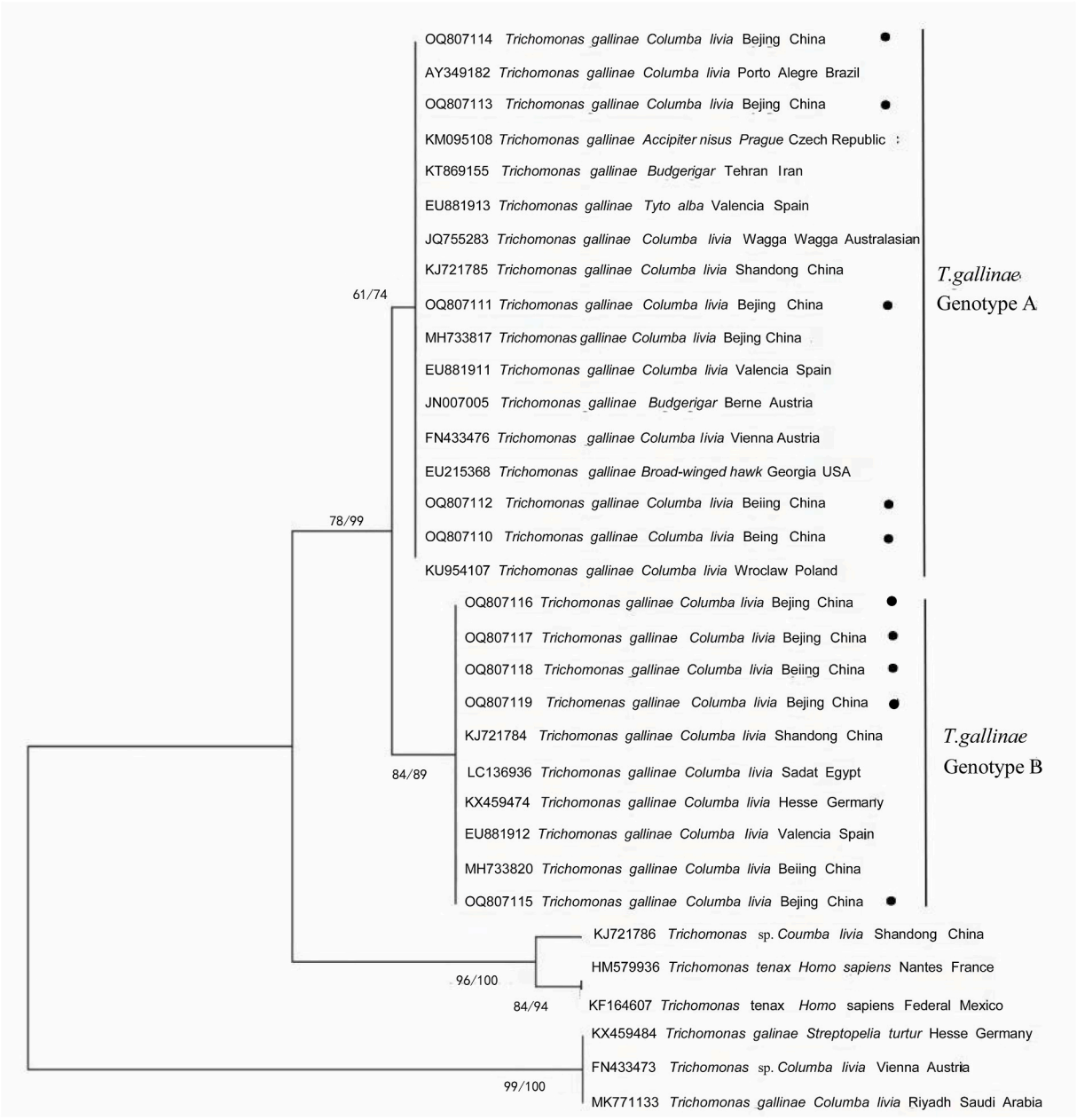

Fig. 1. Phylogenetic tree based on the ITS1/5.8S/ITS2 gene locus of *Trichomonas gallinae* using the neighbor-joining and maximum likelihood method. Note: ● represents the isolates in the study.

genotype A infection, and protect both domestic and wild pigeon populations from the harmful effects of *T. gallinae*.

The present study leads to the development of practical management strategies, and future prevention and control approaches. These results emphasize the importance of implementing a multi-faceted approach, including regular health screening, appropriate sanitation practices, and providing a healthy environment, in order to minimize the risk of *T. gallinae* infection. In addition, studies on the genetic diversity of *T. gallinae* would be crucial, since this can provide insights on the parasite's adaptive strategies, and potential resistance to intervention measures. Furthermore, understanding the ecological implications of *T. gallinae* is essential for effective conservation efforts within the avian community.

5. Conclusions

The survey revealed the significant infection rate of *T. gallinae* in the pigeon population in Beijing, China. The overall infection rate was 32.0% (132/413), with a particularly high prevalence in domestic pigeons (55.0%, 110/200). The parasite was also detected in wild pigeons (16.7%, 16/96) and red-necked turtledoves (33.3%, 6/18), but not in other wild bird species. The infection prevalence was primarily influenced by the bird species, age, and growth environment. Genotype A was predominantly found in wild pigeons, and genotype B was predominantly found in domestic pigeons. These findings provide valuable insights for the prevention and control of avian trichomoniasis.

CRediT authorship contribution statement

Shengfan Jing: Writing – original draft, Data curation, Conceptualization. **Yi Li:** Investigation. **Qiaoqiao Li:** Investigation. **Yanyi Huang:** Investigation. **Shuyi Han:** Writing – review & editing. **Qingxun Zhang:** Conceptualization. **Jinghui Fan:** Conceptualization. **Hongxuan He:** Project administration, Funding acquisition.

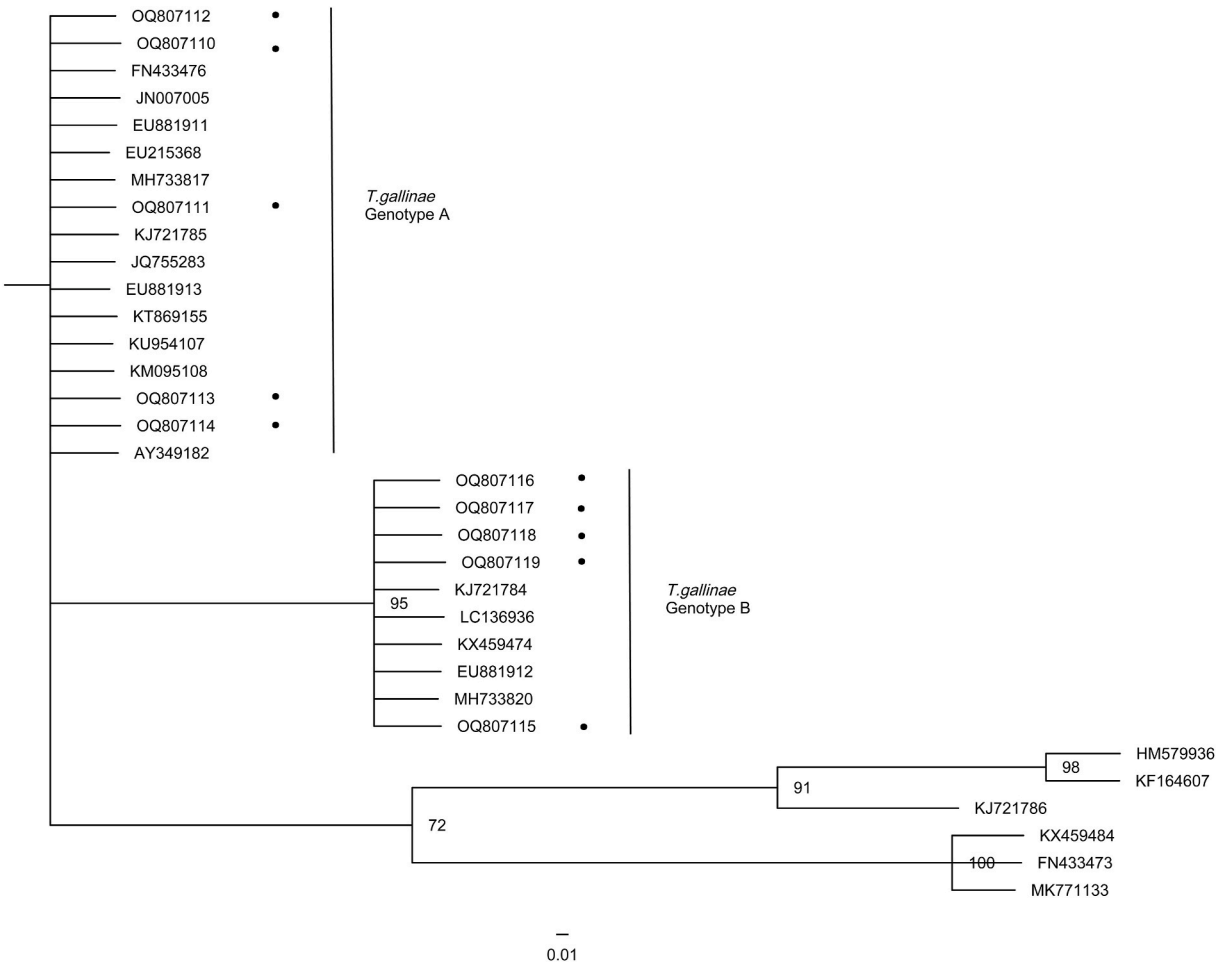

Fig. 2. Phylogenetic tree based on the ITS1/5.8S/ITS2 gene locus of *Trichomonas gallinae* using the Bayesian method. Note: ● represents the isolates in the study.

Declaration of competing 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.

Acknowledgments

This work was supported by the National Key R&D Program of China (2022YFC2601602), and the Science & Technology Fundamental Resources Investigation Program (2022FY100903).

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