


# Molecular investigation of hindgut flagellates from turkeys and pheasants in Hungary confirms the endemicity of a new species closely related to *Histomonas meleagridis*

Acta Veterinaria  
Hungarica

73 (2025) 3, 199-206

DOI:  
10.1556/004.2025.01119  
© 2025 The Author(s)

SÁNDOR SZEKERES<sup>1,2\*</sup> , NÓRA TAKÁCS<sup>1,2</sup>,  
LÁSZLÓ ÓZSVÁRI<sup>3,8</sup>, BARBARA TUSKA-SZALAY<sup>1</sup>,  
KRISZTINA BÁRDOS<sup>3,8</sup>, ÁDÁM KEREK<sup>4,8</sup>,  
PÉTER FERENC DOBRA<sup>5,8</sup>, LÁSZLÓ KOVÁCS<sup>6,8</sup>,  
GERGŐ KEVE<sup>1,2</sup>, VIVIÁNA MOLNÁR-NAGY<sup>7</sup>,  
ZSÓFIA BATA<sup>7</sup> and SÁNDOR HORNOK<sup>1,2</sup>

<sup>1</sup> Department of Parasitology and Zoology, University of Veterinary Medicine, Budapest, Hungary

<sup>2</sup> HUN-REN-UVMB Climate Change: New Blood-sucking Parasites and Vector-borne Pathogens Research Group, Hungary

<sup>3</sup> Department of Veterinary Forensics and Economics, University of Veterinary Medicine, Budapest, Hungary

<sup>4</sup> Department of Pharmacology and Toxicology, University of Veterinary Medicine, Budapest, Hungary

<sup>5</sup> Department of Pathology, University of Veterinary Medicine, Budapest, Hungary

<sup>6</sup> Department of Animal Hygiene, Herd Health and Mobile Clinic, University of Veterinary Medicine, Budapest, Hungary

<sup>7</sup> Dr. Bata Ltd., 2364 Ócsa, Hungary

<sup>8</sup> National Laboratory of Infectious Animal Diseases, Antimicrobial Resistance, Veterinary Public Health and Food Chain Safety, University of Veterinary Medicine, Budapest, Hungary

Received: 21 August 2024 • Revised manuscript received: 7 February 2025 • Accepted: 22 February 2025  
Published online: 16 July 2025

## RESEARCH ARTICLE



## ABSTRACT

To compensate the lack of molecular-phylogenetic data on hindgut flagellates in Hungary, galliform birds were monitored in five regions for two years. Samples were collected from 11 turkeys (from 4 flocks) and 9 pheasants (from 3 farm-raised flocks) suspected to have histomonosis. These samples were molecularly and phylogenetically analysed. In nine turkeys, five 18S rRNA genes and two ITS sequence variants of *Histomonas meleagridis* were identified. These variants were identical between the caecum and liver of the same bird in most cases, but different 18S variants were identified between sampling sites. In one turkey, an unnamed species, here designated as “Dientamoebidae sp. HUN35”, was identified. Its 18S rRNA gene sequence was near-identical (99.6–99.3%) to the sequence reported previously; and the ITS-1/5.8S/ITS-2 region confirmed a close relationship with *H. meleagridis* and *Dientamoeba fragilis*. In one pheasant, *Tetratrichomonas gallinarum* was detected. Different 18S rRNA variants had either identical or different ITS sequences, thus optimally, both should be used for molecular epidemiological studies. Our results suggest the unnamed Dientamoebidae sp. has been present in Hungary since its first detection in 2010 and the host range of this species as well as that of *T. gallinarum* is broader than previously thought.

\*Corresponding author.  
Tel.: +36306363725.  
E-mail: sanyi.szekeres@gmail.com

## KEYWORDS

*Histomonas*, *Tetratrichomonas*, *Dientamoeba*, turkey, pheasant

## INTRODUCTION

The unranked group, Parabasalia (Ph: Metamonada) includes at least 500 known species of flagellate protozoa, the majority of which are intestinal symbionts, commensals or parasites of insects and vertebrates (Céza et al., 2022). In vertebrate hosts, two orders, Trichomonadida and Tritrichomonadida, have the highest importance for veterinary medicine. Although most species are harmless oral or intestinal commensals that can be found in fish, amphibians, reptiles, birds and mammals, they live and multiply epically while associated with mucosal cells and this can result in mild to severe pathogenic consequences (Cepicka et al., 2016).

In avian hosts, some trichomonadids and tritrichomonadids are exclusively present in the gastrointestinal tract as parasites or commensals in the oral cavity or the hindgut, such as *Trichomonas gallinae* and *Tritrichomonas eberthi*, respectively (Amin et al., 2014). However, some other species, most notably *Histomonas meleagridis* and *Tetratrichomonas gallinarum* in galliform and other birds are able to penetrate the colon wall and thus to reach viscera (usually the liver) via the blood stream (Hess et al., 2015). Infection with *H. meleagridis* can result in high mortality among turkeys, but it tends to affect other galliform birds less severely (Beer et al., 2022). At the same time, the economic losses due to histomonosis appear to be increasing since the withdrawal of “histomonocidal” drugs from use in poultry reared for human consumption (Liebhart et al., 2017).

Since trichomonadids can be relatively easily maintained in culture, and their pathogenic effects (organ location and type of the elicited lesions) are characteristic, methods have been available to detect their infections for decades (Amin et al., 2014; Hess et al., 2015). However, the availability of molecular tools allowed a more sensitive and specific identification on the species level, as well as a more accurate assessment of their taxonomy. Consequently, in the past few years, several new trichomonadid species were discovered (e.g., Girard et al., 2014; Martínez-Díaz et al., 2015).

From birds, the genetic diversity of various hindgut trichomonadids was assessed and reported from a limited number of countries in Eurasia, including *H. meleagridis* from various western and central European countries (Bilic et al., 2014), *Dientamoeba fragilis* in Anatolia (Yetismis et al., 2022) and *T. gallinarum* in China (Feng et al., 2021). Nevertheless, several papers on clinical cases involved a single trichomonadid species from the same host species and large-scale studies on histomonosis and/or trichomonadid infection of domestic poultry, or other galliform/anseriform birds were reported from western Europe and the northern part of central Europe (Germany: Hauck et al., 2010; Popp et al., 2011; Poland: Dolka et al., 2015; Falkowski et al., 2020; France: Callait-Cardinal et al., 2010; The Netherlands: van der Heijden and Landman 2011). A few studies also targeted wild, wetland-associated bird species (The Netherlands: Landman et al., 2021) or ducks kept in zoo (Germany: Richter et al., 2010).

Although samples of chickens from Hungary have been used in international molecular studies on histomonosis previously (Zaragatzki et al., 2010; Bilic et al., 2014), the molecular diversity of *H. meleagridis* and *Tetratrichomonas* species has not been examined in this country until now. The aim of this study was to compensate for this lack of data, i.e., to obtain molecular-phylogenetic update on hindgut flagellates of turkeys and pheasants in Hungary. This can be regarded as an attempt and an initiative highly relevant to the southern and eastern part of Europe or even in a broader geographical context, because studies on histomonosis and *Tetratrichomonas*-infection in poultry are almost exclusively reported from countries north or west of Hungary.

## MATERIALS AND METHODS

In this study, *postmortem* samples (caecal wall, liver parenchyma), or faeces were collected from 11 turkeys and 9 pheasants from five sampling sites (Table 1), that were suspected to have a Trichomonadida infection during veterinarian care or supervision in a two-year-long period (between June 2021 and May 2023). For turkeys, the birds/carcasses were collected from the flock on a given collection day in each of the four flocks. Pheasant faecal samples were collected from the ground of the bird housing areas at three different locations. During the pathological inspection, lesions characteristic of histomonosis were seen in the caecum and/or the liver of 5 turkeys. Tissue samples were collected from all clinically suspected carcasses and were kept frozen at  $-20^{\circ}\text{C}$  until processing.

DNA was extracted from tissues and faecal samples with the QIAamp DNA or Fast Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer’s instruction and including extraction control (180  $\mu\text{L}$  tissue lysis buffer) in each set of samples to monitor cross-contamination. In total, 28 DNA extracts and 4 extraction controls were screened for trichomonadid parasites with conventional PCRs (PCR-A: *Histomonas-Dientamoeba-Tetratrichomonas* 18S rRNA gene; PCR-B: *Histomonas-Dientamoeba* ITS nested; PCR-C: *Dientamoeba-Tetratrichomonas* ITS) (van der Heijden et al., 2006; Bart et al., 2008; Bilic et al., 2014; Landman et al., 2019) (Table 2). In each PCR, 5  $\mu\text{L}$  of extracted DNA was added to 20  $\mu\text{L}$  of reaction mixture containing 1.0 U HotStar Taq Plus DNA Polymerase (5 U/ $\mu\text{L}$ ) (Qiagen, Hilden, Germany), 0.5  $\mu\text{L}$  dNTP Mix (10 mM), 0.5  $\mu\text{L}$  of each primer (50  $\mu\text{M}$ ), 2.5  $\mu\text{L}$  of 10  $\times$  CoralLoad PCR buffer (15 mM  $\text{MgCl}_2$  included) and 15.8  $\mu\text{L}$  PCR grade water. In all PCRs, sequence-verified positive controls were used. In order to complement the molecular results from turkeys and pheasants with *T. gallinarum* from chickens, a positive control sample was included in the molecular analysis of the 18S rRNA gene: this sample was originally collected in Poland (Zielona Gora) and maintained in culture in modified Dwyer’s medium (van der Heijden and Landman 2007) at  $41^{\circ}\text{C}$ .

Table 1. Data of sample origin by DNA (isolate) code the and results of molecular analyses. PCR codes are shown according to Table 2

Host (number of individuals)	Origin and DNA/isolate code			Date (day-month-year)	Origin	Results of PCR and sequencing	GenBank accession numbers		
	Liver	Caecum	Faeces				18S rRNA gene (PCR-A)	ITS-1 -5.8S - ITS-2 nested (PCR-B)	ITS-1 - 5.8S -ITS-2 (PCR-C)
Turkey ( <i>Meleagris gallopavo</i> ) (n = 11)	HUN2*	HUN1*	-	07-2021	Jászalsószentgyörgy	<i>H. meleagridis</i>	PP853592	PP851383	ns
	-	KP1V*	-	07-08-2022	Ócsa	<i>H. meleagridis</i>	PP853593		ns
	-	KP2V*				<i>H. meleagridis</i>			ns
	-	NPV*				<i>H. meleagridis</i>		PP851384	ns
	HUN7*	HUN12*	-	13-10-2022	Ócsa	<i>H. meleagridis</i>		PP851383	ns
	HUN8	HUN13				<i>H. meleagridis</i>			ns
	HUN9	HUN14				<i>H. meleagridis</i>			ns
	HUN10	HUN15				<i>H. meleagridis</i>	PP853594		ns
	HUN11	HUN16				<i>H. meleagridis</i>	PP853595-6		ns
	HUN35	HUN36	-	04-2023	Ócsa	Dientamoebidae sp.	PP853597	ns	PP853600
Pheasant ( <i>Phasianus colchicus</i> ) (n = 9)	HUN37	HUN38				negative	-	-	-
	-	-	HUN4/A-G	06-06-2022	Tarnaszentmiklós	negative	-	-	-
	-	-	HUN5	31-08-2022	Ásotthalom	negative	-	-	-
	-	-	HUN6	29-08-2022	Nagykőrös	<i>T. gallinarum</i>	PP853598	ns	ns

\*these samples were collected from birds with lesions characteristic of histomonosis.

Abbreviations: H - *Histomonas*, T - *Tetratrichomonas*, ns - not successful.

Table 2. Data of PCRs used in this study: target gene, oligonucleotide (primer) sequences and parameters of 40 cycles

PCR code	Target group	Target gene (amplicon size)	Oligonucleotides (5'-3')	Initial denaturation	Cycle denaturation	Cycle annealing	Cycle extension	Final extension	Reference
A	<i>Histomonas-Dientamoeba-Tetratrichomonas</i>	18S rRNA gene (603 bp)	18S-F (GCA GTT AAA ACG CTC GTA GTC) 18S-R (AAC GCT AGA CAG GTC AAC CC)	95 °C, 5 m	95 °C, 30 s	53 °C, 40 s	72 °C, 1 m	72 °C, 10 m	Bilic et al. (2014)
B	<i>Histomonas-Dientamoeba</i>	ITS outer (320-380 bp)	TRICHO-F (CGG TAG GTG AAC CTG CCG TT) TRICHO-R (TGC TTC AGT TCA GCG GGT CT)	95 °C, 5 m	95 °C, 30 s	58 °C, 40 s	72 °C, 40 s	72 °C, 7 m	Duboucher et al. (2006), Landman et al. (2019).
		ITS nested (340 bp)	TRICHO-FBIS (GGT GAA CCT GCC GTT GGA TC) TRICHO-RBIS (TCA GTT CAG CGG GTC TTC CT)	95 °C, 5 m	95 °C, 30 s	58 °C, 40 s	72 °C, 40 s	72 °C, 7 m	
C	<i>Dientamoeba-Tetratrichomonas</i>	ITS (350 bp)	Histo-ssu2 (GGA ATC CCT TGT AAA TGC GT) Histo-5.8s1 (TGT GAG GAG CCA AGA CAT CC)	95 °C, 5 m	95 °C, 30 s	55 °C, 40 s	72 °C, 40 s	72 °C, 7 m	Bart et al. (2008)

Purification and sequencing of the selected PCR products were done by Eurofins Biomi Ltd. (Gödöllő, Hungary). Obtained DNA sequences were manually edited using the BioEdit program, then compared to each other and with GenBank sequences using the nucleotide BLASTn program (<https://blast.ncbi.nlm.nih.gov>). New sequences were submitted to GenBank (accession numbers: PP853592–PP853599 for the 18S rRNA gene, PP853600, PP851383–PP851384 for the internal transcribed spacer (ITS-1 – 5.8S rRNA gene – ITS-2) region). All sequences retrieved from GenBank had (nearly) 100% coverage with sequences generated in this study and were trimmed to the same length prior to phylogenetic analysis. This dataset was resampled 1,000 times to generate bootstrap values. Phylogenetic analyses were conducted with the neighbour-joining method, p-distance model using MEGA 11 (Tamura et al., 2021).

## RESULTS

In nine of the 11 turkeys, sampled during summer or autumn, *H. meleagridis* was identified with PCR-A (Table 1). Based on a 545-bp-long part of the 18S rRNA gene, five sequence variants (PP853592–6) were detected, which differed in up to 3 nucleotides (meaning 99.4–100% identity). These variants were 100% identical in the caecum and liver samples of the same bird in four turkeys but differed in a single nucleotide in one case. Different 18S sequence variants were identified in both sampling sites (i.e. Jászalsószentgyörgy and Ócsa), but either identical or different 18S sequence variants were also detected on separate dates at the same location (e.g. same sequence variant, PP853593, collected on 07-08-2022 and 13-10-2022 at Ócsa; different sequence variants, PP851383 and PP851384, collected on the same date, 07-08-2022 at Ócsa) (Table 1). However, *H. meleagridis* collected in Hungary showed a more pronounced, 2–5 bp difference in its 18S rRNA gene sequence when compared to isolates reported from Austria (AJ920323) and France (AF293056, EU647884, EU647887), with only 99.1–99.6% similarity. This was also confirmed phylogenetically, because the 18S rRNA gene sequences from this study clustered separately from the above isolates, i.e., those reported in Western European countries. These differences between the 18S rRNA gene sequences from this study and a laboratory strain from Austria (AJ920323) always showed single nucleotide polymorphism (SNP) located on the same sites (positions 807 and/or 864, and 879 as well as 1039 of the latter) and a switch of G to A.

The ITS region of *H. meleagridis* was successfully amplified with PCR-B (PP851383–PP851384) and showed a single nucleotide difference (i.e., 99.6–100% identity) between the samples of this study (Table 1). Interestingly, different 18S rRNA gene variants had either identical (e.g., HUN2 and HUN7) or different ITS sequences (HUN2 vs NPV). The predominant Hungarian variant of *H. meleagridis* was 97.3%–100% identical to conspecific sequences reported from Germany (e.g., HM229780 to HM229784) and the USA (e.g., HQ334183 to HQ334189). In the lack of sequences of the corresponding region with high coverage

from further trichomonadids in GenBank, phylogenetic analysis could not be performed.

In one turkey sampled in the springtime, an unnamed species (designated here as “Dientamoebidae sp. HUN35”) was identified with PCR-A and C assays (Table 1). The 18S rRNA gene sequence of this species (PP853597) had the highest, 99.6% (542/544 bp) identity to a Dientamoebidae sp. 10/1711 DNA sequence (HG008099) reported from chicken sampled in Hungary in 2010, and a lower, 99.3% (540/544 bp) identity to another variant from turkey sampled in 2009 in France (HG008100, Dientamoebidae sp. 9-5-32). At the same time, Dientamoebidae sp. HUN35 showed only 93.9% (522/556 bp) identity to *H. meleagridis* (AJ920323) and their phylogenetic relationships reflected that they are sister species (Fig. 1). In addition, the ITS region of this unnamed species (PP853600) was also successfully identified with PCR-C (Table 1). Based on this, its clustering with *D. fragilis* (human isolate from the UK; JQ677164) received moderately strong (81%) bootstrap support on the phylogenetic tree (Fig. 2).

In one of the nine pheasants, *T. gallinarum* was identified (Table 1). The corresponding 18S rRNA gene sequence (PP853598) had the highest, 99.8% (556/557 bp) identity with *T. gallinarum* isolate “rooster2” reported from chicken (JX565085), duck (AF124608), swan (AY245112) and a human (AY247749). Their clustering in one phylogenetic group was strongly supported (Fig. 1). However, amplification of the ITS region of this species was not successful.

## DISCUSSION

This study compensates for the lack of the molecular-phylogenetic analysis of hindgut flagellates of turkeys and pheasants in Hungary. Nevertheless, a few samples obtained from chickens in Hungary (dating back to 2010) were used for similar purposes in an international study (Bilic et al., 2014) in which the 18S rRNA gene of an undescribed Dientamoebidae species was detected in a chicken sample from this country. These previous findings highlight the importance of this region in the above context of hindgut flagellates, and we confirmed the prolonged presence of an unnamed Dientamoebidae species, this time in turkey.

First, a fragment of the 18S rRNA gene, which is the most frequently used genetic marker in genotyping protozoan parasites, was simultaneously amplified and analysed here for three hindgut flagellates from two galliform bird species as hosts. It is noteworthy that the three trichomonadid parasites compared in this study have different host spectra and this might have had an impact on their genetic polymorphism. In particular, *H. meleagridis* and *T. gallinarum* can both cause fatal typhlohepatitis in galliform birds (Liebhart et al., 2014; Beer et al., 2022), but the latter more frequently affects anseriforms (Richter et al., 2010; Falkowski et al., 2020). While *H. meleagridis* is not known to cause infection in vertebrates other than birds, mammals (even primates) are among the potential hosts of *T. gallinarum* and closely related species (Kutisova et al., 2005; Smejkalová

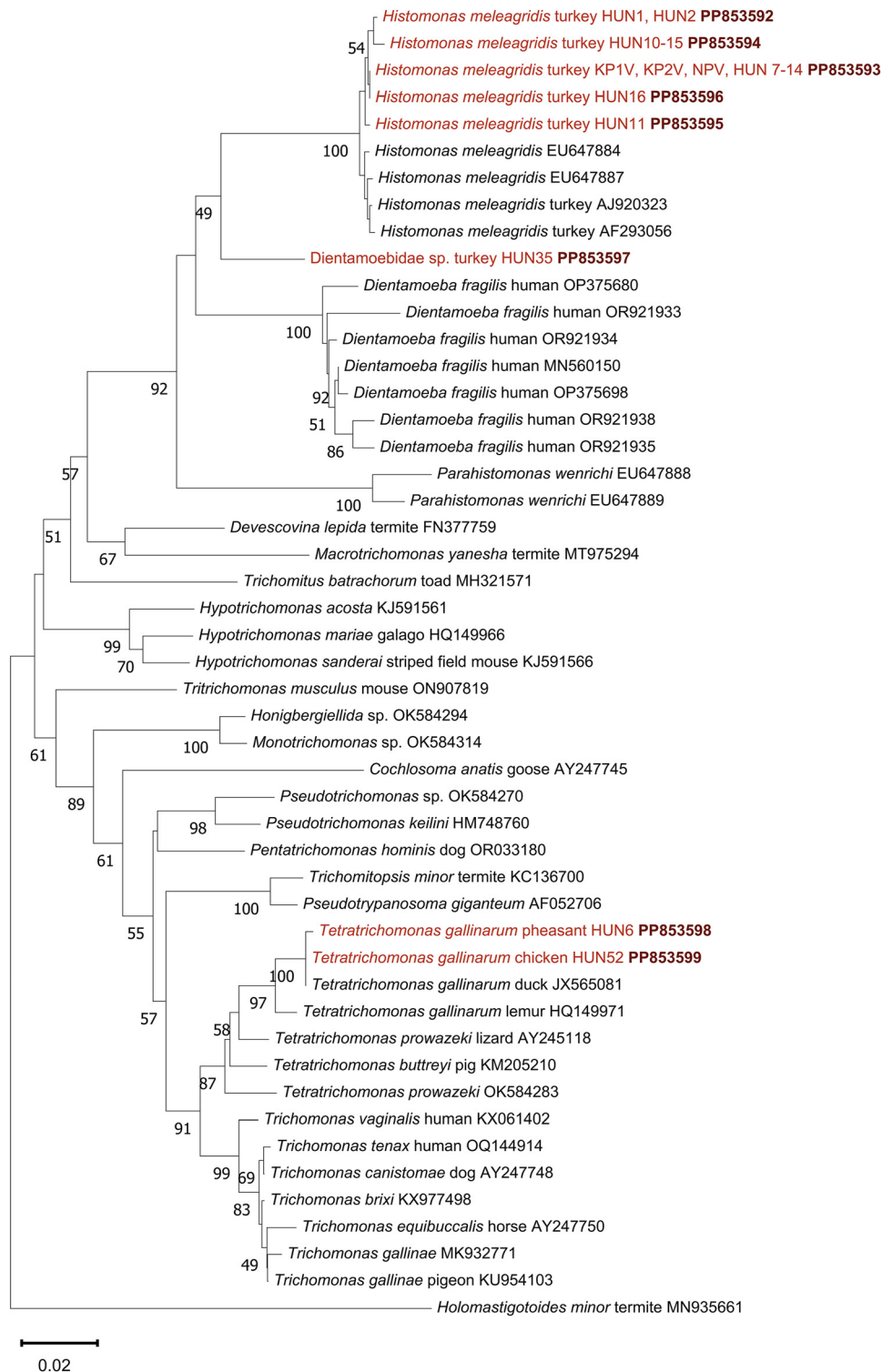


Fig. 1. Phylogenetic tree of trichomonads based on 18S rRNA gene sequences. In each row, after the species or genus name, the isolation source and the GenBank accession number are shown. Sequences obtained in this study are in red and bold accession numbers. The evolutionary history was inferred using the neighbour-joining method. The scale-bar indicates the number of substitutions per site. The evolutionary distances were computed using the p-distance method. This analysis involved 49 nucleotide sequences. There was a total of 553 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

et al., 2012). At the same time, although *D. fragilis* is essentially a human parasite, it has also been demonstrated to pass from birds (Yetismis et al., 2022). Nowadays, *D. fragilis* is considered as an emerging pathogen of humans

(Guadano-Procesi et al., 2024). However, it has not been studied since the discovery of its close relative, *Dientamoebidae* sp. in samples from Hungary and France for the first time more than a decade ago.

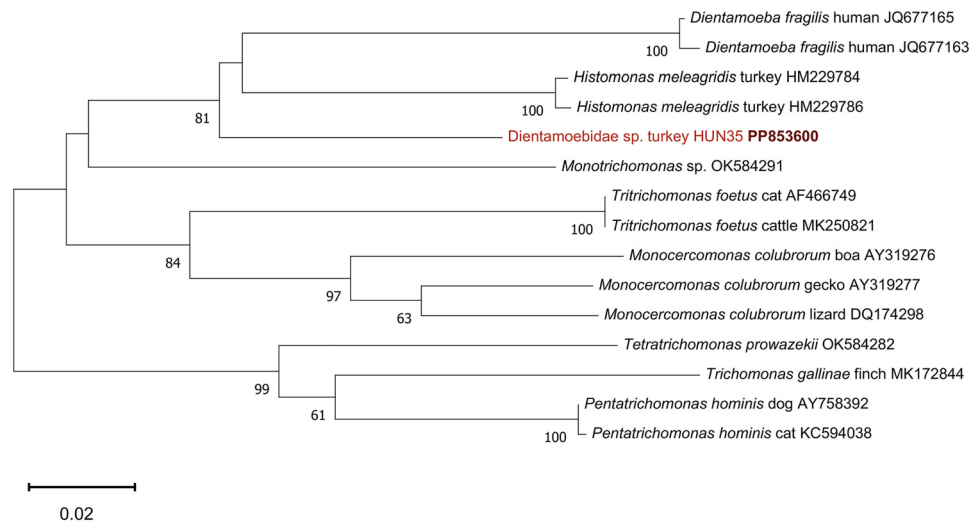


Fig. 2. Phylogenetic tree of trichomonads based on ITS-1 – 5.8S – ITS-2 sequences. In each row, after the species or genus name, the isolation source and the GenBank accession number are shown. Sequences obtained in this study are in red and bold accession numbers. The evolutionary history was inferred using the neighbour-joining method. The scale-bar indicates the number of substitutions per site. The evolutionary distances were computed using the p-distance method. This analysis involved 15 nucleotide sequences. There was a total of 234 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

In this study, the 18S rRNA gene sequences of *H. meleagridis* were mostly identical when obtained from the caecum or the liver of the same bird, but it also presented as a mixed infection with different variants. There was no clear spatiotemporal difference in the occurrence of 18S variants, although the limited sample size did not allow a final conclusion in this respect. In particular, the 18S rRNA gene sequence variant was predominantly (but not exclusively) identical among the samples obtained at the repeatedly sampled site (Ócsa), but differed from the other sampled site (Jászalsószentgyörgy) (Table 1). The 18S rRNA gene sequence identity of *H. meleagridis* within the same sampling site can be explained by local, short-distance routes of transmission, e.g. with dust, as also reported in Austria and in France (Sulejmanovic et al., 2017, 2019). However, different 18S rRNA gene sequence variants were also shown to be present at the same location, probably owing to the long-distance spread of this parasite via animal trading. This finding is slightly different from the previous observation that a single sequence variant was detected among all samples belonging to the same flock (Bilic et al., 2014). At the same time, 18S rRNA gene sequences obtained from samples collected in Hungary clustered phylogenetically separately from others reported from other countries, i.e. from Austria and France (AF293056, AJ920323, EU647887, EU647884) suggesting that certain variants might be endemic to a peculiar geographical region where international turkey trading is restricted. The observation of SNP in certain positions among variants and the same G-to-A switch might also indicate paralogs, i.e., that multiple copies of the 18S rRNA gene in the same genome are different (e.g., as a result of point mutations affecting the same loci). A similar phenomenon was reported in other genes of the *H. meleagridis* genome (Shinde et al., 2024).

On the other hand, the findings were different for the second genetic marker ITS-1– 5.8S – ITS-2 analysed here.

While the relative proportions of the ITS-1 region tends to be conserved in *H. meleagridis* (van der Heijden et al., 2006), SNP have been detected even among sequences originating from the same *H. meleagridis* clonal culture (Bilic et al., 2014). In this study, ITS sequences were consistent between different 18S rRNA genotypes, except for one case where 18S rRNA gene variants differed in this region. At the same time, when comparing sequences from this study to those from previous reports, a higher ratio of heterogeneity was observed in the ITS region (up to 2.7%) than in the 18S rRNA gene (below 0.9%); and similar rates of genetic variation were demonstrated on smaller and larger geographical scales (within Central Europe or between Europe and North America). This may indicate that there is no geographical pattern in heterogeneity in relation to distance, probably due to the commercial trade of turkeys.

In the examined turkeys, an undescribed *Dientamoebidae sp.* (isolate HUN35) that is closely related to *H. meleagridis*, was also detected. This species was previously reported in an Austrian study from chicken sampled in Hungary, but only based on one genetic marker, the 18S rRNA gene (Bilic et al., 2014; Liebhart et al., 2014). It may be relevant to note that the search for genetic variation in the closely related species, *D. fragilis* was based on the 18S rRNA gene, which was not considered useful for molecular epidemiology (Bart et al., 2008). However, the ITS region was found to be highly variable. For instance, it was shown that human individuals within a family can be infected with the same or different strains of *D. fragilis*. It was concluded that the ITS region can serve as a molecular epidemiological tool for the subtyping of *D. fragilis* (Bart et al., 2008). This is the first study where the ITS region of this unnamed *Dientamoebidae sp.* (isolate HUN35) was successfully amplified and phylogenetically analysed. This genetic marker confirmed its taxonomy on the family level, i.e., that it is closely related to both *H. meleagridis* and *D. fragilis*.

No pathological lesions were observed in the turkey infected with *Dientamoebidae* sp. HUN35, this was also true in the case of several other birds infected with *H. meleagridis* in this study. Therefore, based on the present results, the pathogenic role (if any) of this tentatively named new species cannot be completely excluded.

In this study, only *T. gallinarum* was shown to be present in pheasant. Although *T. gallinarum* was already reported in this host (Cepicka et al., 2005), corresponding sequences were not available in GenBank prior to this study. The pathological lesions caused by *T. gallinarum* in the caecum and the liver are very similar to those elicited by *H. meleagridis*, especially in turkeys (Ayan, 2020). Therefore, the molecular identification is very useful in distinguishing these two species. For this, the DNA sequence provided here may serve as an initiative for genotyping *T. gallinarum* in other galliform hosts in which it occurs less frequently than in anseriform birds (Richter et al., 2010). This is especially important to consider for future large-scale studies, because previous results suggest that some species of the birds might be susceptible to a particular strain of *T. gallinarum* (Liebhart et al., 2014).

In summary, based on the isolates we analysed, there was no consistency between the two evaluated genetic markers (i.e., individuals sampled on different dates). Therefore, optimally, the 18S rRNA gene and the ITS region should be used simultaneously for molecular epidemiological or subtyping studies. The unnamed *Dientamoebidae* sp. is still present (endemic) in Hungary, and the host range of this species as well as that of *T. gallinarum* is broader than previously thought, now including the turkey and the pheasant, too.

**Ethical approval:** Samples in this study were collected during regular veterinary care. Investigation of the cadavers is a daily routine in the poultry farms, therefore no ethical permission was needed.

**Competing interest:** There are no competing interests.

**Authors' contributions:** SS: study design, pathology, sample collection, data analysis, DNA extraction. NT: molecular work. LÓ: supervision, study design, data curation, financing. BTS: sample collection, DNA extraction. KB: study design, data curation, financing. ÁK: culturing. PFD: pathological examination. LK: sample collection. V M-N: sample collection, data curation. ZB: sample collection, data curation. GK: phylogenetic analysis. SH: conceptualization, data analysis, GenBank processing, manuscript writing. All authors reviewed the manuscript.

**Funding:** The research was supported by the project 2020-1.1.2-PIACI-KFI-2021-00202 "Development of natural-based complex feed additives for the optimization of the poultry microbiome", and Project No. RRF-2.3.1-21-2022-00001, which was implemented with the support provided by the Recovery and Resilience Facility (RRF), financed under the National Recovery Fund budget estimate, RRF-2.3.1-21 funding scheme.

**Availability of data and materials:** Data (i.e. accession numbers, PCR protocols) are provided within the manuscript.

## ACKNOWLEDGEMENTS

The authors are grateful to Ms. Lili Veronika Németh for administrative support. The authors are grateful to Dr. Gábor Majoros for his indispensable contribution to methodology during sample collection and processing.

## REFERENCES

- Amin, A., Bilic, I., Liebhart, D. and Hess, M. (2014): Trichomonads in birds – a review. *Parasitology* **141**, 733–747. <https://doi.org/10.1017/S0031182013002096>
- Ayan, A. (2020): Molecular characterization of *Tetratrichomonas gallinarum* in domestic turkeys (*Meleagris gallopavo*) in van province, Turkey. *Int. J. Ecosyst. Ecol. Sci (IJEES)* **10**, 285–292. <https://doi.org/10.31407/ijeec10.207>
- Bart, A., van der Heijden, H. M., Greve, S., Speijer, D., Landman, W. J. and van Gool, T. (2008): Intragenomic variation in the internal transcribed spacer 1 region of *Dientamoeba fragilis* as a molecular epidemiological marker. *J. Clin. Microbiol.* **46**, 3270–3275. <https://doi.org/10.1128/JCM.00680-08>
- Beer, L. C., Petrone-Garcia, V. M., Graham, B. D., Hargis, B. M., Tellez-Isaias, G. and Vuong, C. N. (2022): Histomonosis in poultry: a comprehensive review. *Front. Vet. Sci.* **6**(9), 880738. <https://doi.org/10.3389/fvets.2022.880738>
- Bilic, I., Jaskulska, B., Souillard, R., Liebhart, D. and Hess, M. (2014): Multi-locus typing of *Histomonas meleagridis* isolates demonstrates the existence of two different genotypes. *PLoS One* **9**. <https://doi.org/10.1371/journal.pone.0092438>
- Callait-Cardinal, M. P., Gilot-Fromont, E., Chossat, L., Gonthier, A., Chauve, C. and Zenner, L. (2010): Flock management and histomoniasis in free-range turkeys in France: description and search for potential risk factors. *Epidemiol. Infect.* **138**, 353–363. <https://doi.org/10.1017/S0950268809990562>
- Cepicka, I., Dolan, M. F. and Gile, G. H. (2016): Parabasalia. In: *Handbook of the Protists*. Springer International Publishing, Cham, pp. 1–44
- Cepicka, I., Kutisová, K., Tachezy, J., Kulda, J. and Flegr, J. (2005): Cryptic species within the *Tetratrichomonas gallinarum* species complex revealed by molecular polymorphism. *Vet. Parasitol.* **128**, 11–21. <https://doi.org/10.1016/j.vetpar.2004.11.003>
- Céza, V., Kotyk, M., Kubánková, A., Yubuki, N., Stáhlavský, F., Silberman, J. D. and Cepicka, I. (2022): Free-living trichomonads are unexpectedly diverse. *Protist* **173**. <https://doi.org/10.1016/j.protis.2022.125883>
- Dolka, B., Zbikowski, A., Dolka, I. and Szeleszczuk, P. (2015): Histomonosis - an existing problem in chicken flocks in Poland. *Vet. Res. Commun.* **39**, 189–195. <https://doi.org/10.1007/s11259-015-9637-2>
- Duboucher, C., Caby, S., Dufernez, F., Chabé, M., Gantois, N., Delgado-Viscogliosi, P., Billy, C., Barré, E., Torabi, E., Capron, M., Pierce, R. J., Dei-Cas, E. and Viscogliosi, E. (2006):

- Molecular identification of *Trichomonas foetus*-like organisms as coinfecting agents of human *Pneumocystis pneumonia*. *J. Clin. Microbiol.* 0095-1137, **44**(3), 1165–1168. <https://doi.org/10.1128/JCM.44.3.1165-1168.2006>. 16517921.
- Falkowski, P., Liebhart, D., Bobrek, K. and Gawel, A. (2020): The prevalence of *Tetratrichomonas* spp. in reproductive geese flocks. *Avian Dis.* **64**. <https://doi.org/10.1637/aviandiseases-D20-00042>
- Feng, S., Chang, H., Wang, Y., Luo, F., Wu, Q., Han, S. and He, H. (2021): Lethal infection caused by *Tetratrichomonas gallinarum* in black swans (*Cygnus atratus*). *BMC Vet. Res.* **17**, 191. <https://doi.org/10.1186/s12917-021-02894-x>
- Girard, Y. A., Rogers, K. H., Gerhold, R., Land, K. M., Lenaghan, S. C., Woods, L. W., Haberkern, N., Hopper, M., Cann, J. D. and Johnson, C. K. (2014): *Trichomonas stableri* n. sp., an agent of trichomonosis in Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*). *Int. J. Parasitol. Parasites. Wildl.* **3**, 32–40. <https://doi.org/10.1016/j.ijppaw.2013.12.002>
- Guadano-Procesi, I., Berrilli, F., Montalbano Di Filippo, M. and Di Cave, D. (2024): Detection and genotyping of *Dientamoeba fragilis* from symptomatic patients: new insights from Italy into a little-known gastrointestinal protozoan. *Parasitol. Int.* **98**. <https://doi.org/10.1016/j.parint.2023.102816>
- Hauck, R., Balczulat, S. and Hafez, H. M. (2010): Detection of DNA of *Histomonas meleagridis* and *Tetratrichomonas gallinarum* in German poultry flocks between 2004 and 2008. *Avian Dis.* **54**, 1021–1025. <https://doi.org/10.1637/9261-012910-Reg.1>
- Hess, M., Liebhart, D., Bilic, I. and Ganas, P. (2015): *Histomonas meleagridis*—new insights into an old pathogen. *Vet. Parasitol.* **208**, 67–76. <https://doi.org/10.1016/j.vetpar.2014.12.018>
- Kutisova K, Kulda J, Cepicka I, Flegr, J., Koudela, B., Teras, J. and Tachezy, J. (2005): *Tetratrichomonads* from the oral cavity and respiratory tract of humans. *Parasitology* **131**, 309–319. <https://doi.org/10.1017/S0031182005008000>
- Landman, W. J. M., Gantois, N., Sawant, M., Majoor, F. A., van Eck, J. H. H. and Viscogliosi, E. (2021): Prevalence of trichomonads in the cloaca of wild wetland birds in The Netherlands. *Avian Pathol.* **50**, 465–476. <https://doi.org/10.1080/03079457.2021.1967876>
- Landman, W. J. M., Gantois, N., van Eck, J. H. H., van der Heijden, H. M. J. F. and Viscogliosi, E. (2019): *Tetratrichomonas gallinarum* granuloma disease in a flock of free range layers. *Vet. Q.* **39**, 153–160. <https://doi.org/10.1080/01652176.2019.1682714>
- Liebhart, D., Ganas, P., Sulejmanovic, T. and Hess, M. (2017): Histomonosis in poultry: previous and current strategies for prevention and therapy. *Avian Pathol.* **46**, 1–18.
- Liebhart, D., Neale, S., Garcia-Rueda, C., Wood, A. M., Bilic, I., Wernsdorf, P., Jaskulska, B. and Hess, M. (2014): A single strain of *Tetratrichomonas gallinarum* causes fatal typhlohepatitis in red-legged partridges (*Alectoris rufa*) to be distinguished from histomonosis. *Avian Path.* **43**, 473–480. <https://doi.org/10.1080/03079457.2014.959435>
- Martínez-Díaz, R. A., Ponce-Gordo, F., Rodríguez-Arce, I., del Martínez-Herrero, M. C., González, F. G., Molina-López, R. Á. and Gómez-Muñoz, M. T. (2015) *Trichomonas gypaetini* n. sp., a new trichomonad from the upper gastrointestinal tract of scavenging birds of prey. *Parasitol. Res.* **114**, 101–112. <https://doi.org/10.1007/s00436-014-4165-5>
- Popp, C., Hauck, R., Balczulat, S. and Hafez, H. M. (2011): Recurring histomonosis on an organic farm. *Avian Dis.* **55**, 328–330. <https://doi.org/10.1637/9596-110810-Case.1>
- Richter, B., Schulze, C., Kämmerling, J., Mostegl, M. and Weissenböck, H. (2010): First report of typhlitis/typhlohepatitis caused by *Tetratrichomonas gallinarum* in three duck species. *Avian Path.* **39**, 499–503. <https://doi.org/10.1080/03079457.2010.518137>
- Shinde, A. P., Kucerová, J., Dacks, J. B. and Tachezy, J. (2024): Tracing back the expansion of the endomembrane rescue systems in parasitic Parabasalids to the ancestral origin in its free-living sister lineage. *BioRxiv*. <https://doi.org/10.1101/2024.01.09.574884>
- Smejkalová, P., Petrzelková, K. J., Pomajbíková, K., Modrý, D. and Cepicka, I. (2012): Extensive diversity of intestinal trichomonads of non-human primates. *Parasitology* **139**, 92–102. <https://doi.org/10.1017/S0031182011001624>
- Sulejmanovic, T., Liebhart, D., Mägdefrau-Pollan, B., Sanglhuber, E. M., Wiesinger, E., Bilic, I. and Hess, M. (2017): Emergence of fatal histomonosis in meat Turkey flocks in Austria from 2014 to 2016. *Wien. Tierarztl. Monatsschrift.* **104**, 277–287.
- Sulejmanovic, T., Turblin, V., Bilic, I., Jaskulska, B. and Hess, M. (2019): Detection of *Histomonas meleagridis* DNA in dust samples obtained from apparently healthy meat Turkey flocks without effect on performance. *Avian Path.* **48**, 329–333. <https://doi.org/10.1080/03079457.2019.1599819>
- Tamura, K., Stecher, G. and Kumar, S. (2021): MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **38**, 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- van der Heijden, H. M. J. F. and Landman, W. J. M. (2007): Improved culture of *Histomonas meleagridis* in a modification of Dwyer medium. *Avian Dis.* **51**, 986–988. <https://doi.org/10.1637/8018-051007-REVIEWWR.1>
- van der Heijden, H. M. J. F. and Landman, W. J. M. (2011): High seroprevalence of *Histomonas meleagridis* in Dutch layer chickens. *Avian Dis.* **55**, 324–327. <https://doi.org/10.1637/9609-120610-ResNote.1>
- van der Heijden, H. M. J. F., Landman, W. J. M., Greve, S. and Peek, R. (2006): Genotyping of *Histomonas meleagridis* isolates based on internal transcribed spacer-1 sequences. *Avian Path.* **35**, 330–334. <https://doi.org/10.1080/03079450600815499>
- Yetismis, G., Yildirim, A., Pekmezci, D., Duzlu, O., Ciloglu, A., Onder, Z., Simsek, E., Ercan, N., Pekmezci, G. Z. and Inci, A. (2022): First report and genotyping of *Dientamoeba fragilis* in pet budgerigars (*Melopsittacus undulatus*), with zoonotic importance. *Zoonoses Public Health* **69**, 572–578. <https://doi.org/10.1111/zph.12949>
- Zaragatzki, E., Mehlhorn, H., Abdel-Ghaffar, F., Rasheid, K. A., Grabensteiner, E. and Hess, M. (2010): Experiments to produce cysts in cultures of *Histomonas meleagridis* - the agent of histomonosis in poultry. *Parasitol. Res.* **106**, 1005–1007. <https://doi.org/10.1007/s00436-010-1776-3>