

First Insight into the Genetic Diversity and Phylogenetic Analysis of Trichomonadida species Infesting *Gallus gallus domesticus* (Aves: Phasianidae) in Tunisia

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Introduction

In Tunisia, the domestic chicken *Gallus gallus domesticus* is one of the most valuable sources of protein. Despite its economic importance, research on protozoan infections affecting Tunisian poultry has been limited to parasitological surveys. This study addresses this gap by employing partial sequences of the 18S rRNA and α -actinin 1 genes as nuclear markers to molecularly characterize *Histomonas meleagridis* isolates from *G. gallus domesticus* across various geographical regions of Tunisia.

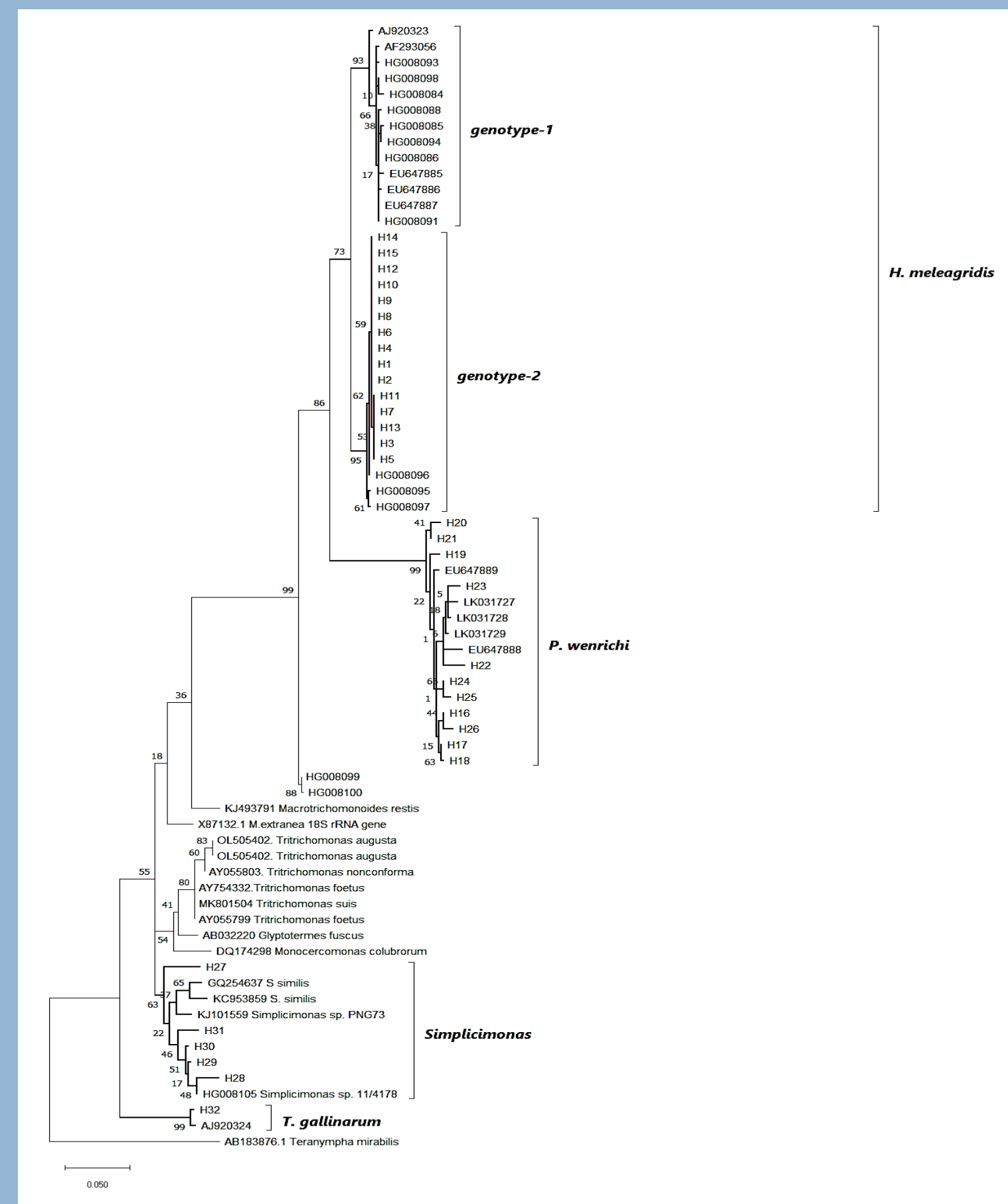
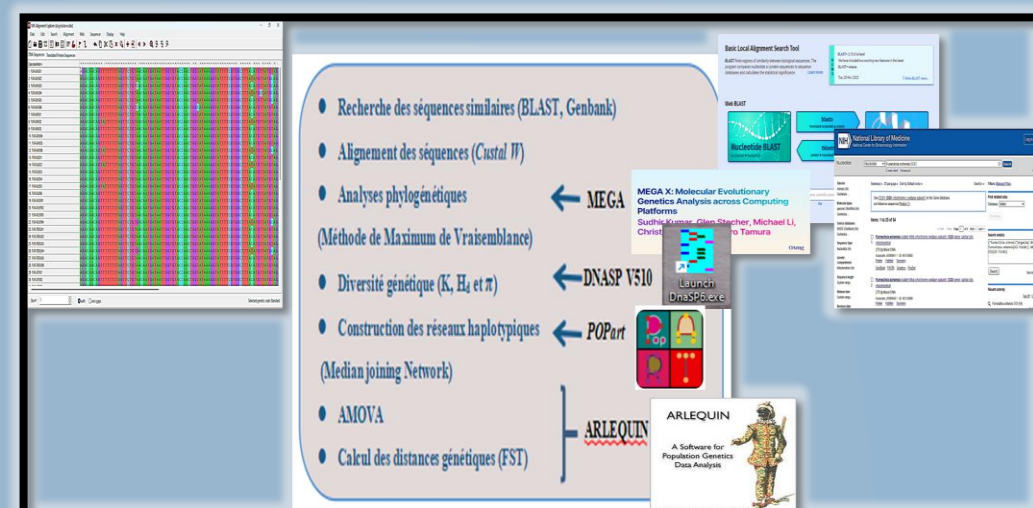
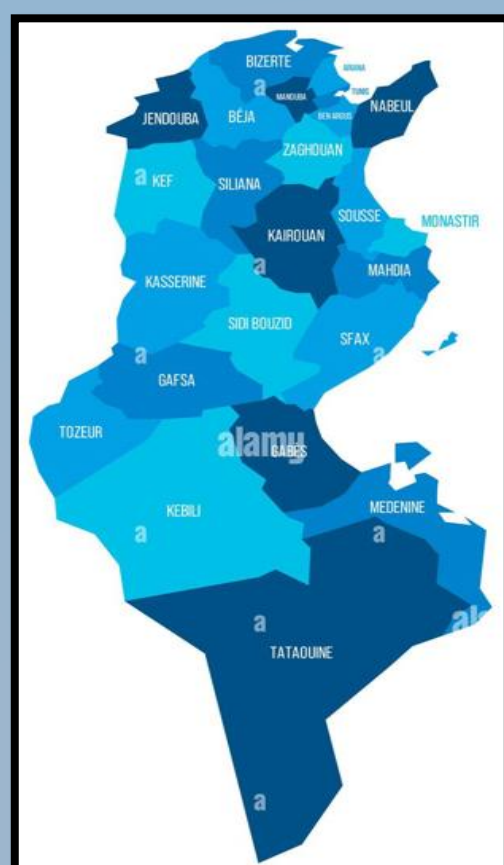
Aims

- Molecular characterization of *H. meleagridis* from Tunisia using the α -actinin 1 gene and partial 18S rRNA sequences as molecular markers.
- Genotyping of *H. meleagridis* could enhance genetic epidemiological studies and improve the assessment of treatment efficacy and virulence differences among eventual haplotypes.

Sample collection, DNA Extraction, Amplification, Sequencing and Population Genetic Analyses

Collection of parasites

DNA Extraction, Amplification, Sequencing and Population Genetic Analyses



- The genetic distance between clades ranged from 11.33% (*H. meleagridis* - *P. wenrichi*) to 21.76% (*T. gallinarum* - *P. wenrichi*).

	<i>H. meleagridis</i>	<i>P. wenrichi</i>	<i>Simplicimonas</i> sp.
<i>H. meleagridis</i>			
<i>P. wenrichi</i>	0.1133		
<i>Simplicimonas</i> sp.	0.1623	0.1890	
<i>T. gallinarum</i>	0.1913	0.2176	0.1180

Results and Discussion

18S rRNA dataset analysis

- 18S rRNA sequences were identified as: *H. meleagridis* (96-99% identity, HG008095); *Parahistomonas wenrichi* (94-97% identity, LK031729); *Simplicimonas* sp. (97-98% identity, HG008105) and *Tetratrichomonas gallinarum* (98% identity, AJ920324).
- Histomonas meleagridis* sequences generated 15 haplotypes, *P. wenrichi* 11, *Simplicimonas* 5 and *T. gallinarum* one haplotype.

Species	Geographical origin	Haplotypes	Accession numbers
<i>H. meleagridis</i>	All studied localities	H1-H15	PQ682583- PQ682597
<i>P. wenrichi</i>	All studied localities	H16-H26	PQ682598- PQ682608
<i>Simplicimonas</i>	Kasserine Tozeur	H27-H31	PQ682609- PQ682613
<i>T. gallinarum</i>	Ariana	H32	PQ682614

- Haplotype diversity (Hd) ranged from 0.875 in the case of *H. meleagridis* to 1 for *Simplicimonas* sp and *T. gallinarum*. Nucleotide diversity (Pi) values varied from 0.64% (*H. meleagridis*) to 4.9% (*Simplicimonas* sp.).

Clade	Number of sequences	Total number of mutations	PS	k	Hd	Pi
<i>H. meleagridis</i>	31	25	23	9.172	0.875	0.64%
<i>P. wenrichi</i>	16	26	20	6.075	0.92	1.138%
<i>Simplicimonas</i>	9	84	74	22.9%	1	4.965%
<i>T. gallinarum</i>	2	2	2	2	1	0.38%

Phylogenetic trees using α -actinin 1 sequences

- ML tree revealed two sub-clades, including the observed samples from 18SrRNA genotype-1 and genotype-2 of *H. meleagridis*. No genetic variability was found among the Tunisian sequences analyzed, all of which clustered within the sub-clade corresponding to genotype-2 (PQ682615-PQ682618).

Conclusion

- This study provides, for the first time, insights into the genetic diversity of Trichomonadida in Tunisia. The findings provide valuable insights into the genetic characteristics of these parasites in Tunisian poultry farms and contribute to the understanding of Trichomonadida diversity, enhancing disease control and prevention efforts.
- These findings suggest a complex protozoan community within the studied hosts. Phylogenetic analysis revealed a close relationship between *H. meleagridis* and *P. wenrichi*, as well as between *Simplicimonas* sp. and the *Monoceromonas-Tritrichomonas* group.

References

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- Bilic I., Jaskulska B., Souillard R., Liebhart D., Hess M. 2014: Multi-Locus Typing of *Histomonas meleagridis* Isolates Demonstrates the Existence of Two Different Genotypes. *Plos One* 9(3): e92438.