

Insights into *Mycoplasma* spp. genetic diversity through a long-term study in bank voles (*Clethrionomys glareolus*) in the Mazury Lake District in Poland

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Abstract

Hemotrophic mycoplasmas (hemoplasmas) are Gram-negative, uncultivable bacteria that parasitize red blood cells of diverse vertebrates. Rodent-associated hemoplasmas with zoonotic potential have been reported, highlighting the need to study these pathogens in wild rodent populations.

We investigated the prevalence, abundance, and genetic diversity of *Mycoplasma* spp. across three geographically separated populations of bank voles, *Clethrionomys glareolus* (n = 1514), sampled over 23 years. Additional positive samples were obtained from *Apodemus flavicollis*, *A. sylvaticus*, and *A. agrarius*. Prevalence was assessed using microscopic examination and molecular identification based on the 16S rRNA gene (595 bp). Infection abundance was quantified by counting infected erythrocytes in 200 microscopic fields. Genetic diversity and species delineation were evaluated using phylogenetic analyses and Minimum Spanning Network approaches.

Overall prevalence of *Mycoplasma* spp. was 69.4%, with a mean abundance of 12.6 ± 0.69 infected RBCs per 200 fields. Phylogenetic analyses revealed eight previously undescribed *Mycoplasma* species comprising 16 haplotypes.

These findings provide new insights into the prevalence, abundance, diversity, and host associations of *Mycoplasma* spp. in free-living rodents, demonstrating long-term infection stability and the influence of ecological and demographic factors. This study represents the first molecular investigation of rodent hemoplasmas in Poland.

Introduction

Hemotrophic mycoplasmas, commonly referred to as hemoplasmas, are Gram-negative, uncultivable, pleomorphic bacteria that parasitize red blood cells (RBCs) of a wide range of vertebrate hosts. They are classified within the genus *Mycoplasma*, class *Mollicutes*^{1–3}. Historically, these organisms were described as *Haemobartonella* and *Eperythrozoon* spp., with transmission attributed primarily to blood-sucking arthropods such as ticks, fleas, and lice^{1,2,4}. Despite this, the transmission routes of hemoplasmas remain incompletely understood. Current evidence highlights three main pathways: direct blood-to-blood contact during host fighting, flea-mediated transmission, and, in some cases, direct blood–saliva contact^{5–7}. In addition, vertical transmission has been documented across several species, including small mammals⁸, piglets⁹, humans¹⁰, and cats¹¹. Hemoplasma pathogenicity varies widely depending on both the *Mycoplasma* species and the mammalian host, ranging from chronic, subclinical infections with no obvious clinical signs to acute, life-threatening haemolytic anaemia^{12–15}.

Hemoplasma infections in domestic animals are well documented, however, much less is known about their prevalence, genetic diversity, transmission routes, bacteria–host–vector interactions, and the impact of infection on the health of wild animals^{16,17}. Rodentia is the most diversified and widespread order of mammals. Rodents act as hosts for a variety of zoonotic pathogens¹⁸ and several species of

ticks and fleas^{19–21}. Hemotrophic mycoplasmas have been documented in rodents in several regions of the world, including Egypt²², Brazil^{17,23}, Chile²¹, Japan³, and Poland^{24,25}. Most hemoplasmas exhibit a high degree of host specificity, although interspecies transmission has been also reported²⁶. Importantly, rodent-associated hemoplasmas with zoonotic potential have also been described^{27,28}, underscoring the relevance of studying these pathogens in rodent hosts.

In recent decades, growing attention has been directed toward the genetic diversity of *Mycoplasma* spp. isolated from a variety of hosts^{6,17,21,29}. In Poland, infections caused by *Mycoplasma* spp. have been documented in bank voles, rats, humans, turkeys, and wild geese^{24,25,30–32}. However, despite these reports, investigations specifically addressing the genetic diversity of *Mycoplasma* spp. in Poland remain limited. Therefore, in this study, we determined the abundance and prevalence of *Mycoplasma* spp. infections in three geographically separated bank vole populations and characterized the genetic diversity of the detected strains.

Results

In total, 1514 bank voles were screened for *Mycoplasma* spp. Infection of *Mycoplasma* spp. was detected in 69.4% of rodents, encompassing 1050 positive bank voles. Additionally, 19 positive samples from *A. flavicollis* (10), *A. sylvaticus* (2) and *A. agrarius* (7) were included in this study.

Prevalence of infection with *Mycoplasma* spp. in bank voles from three sites (n = 1514)

The overall prevalence of *Mycoplasma* spp. in bank voles was 69.4% (95% CI: 67.03–71.68), but there was a significant difference between the years (YEAR x *Mycoplasma* PA $\chi^2_6 = 129.6$, $P < 0.001$, Fig. 1A) and also a significant interaction with site (YEAR x SITE x *Mycoplasma* PA $\chi^2_{12} = 58.4$, $P < 0.001$, Fig. 1C). In some years prevalence was very similar in all three sites (e.g. 2018 and 2022), while in other years it differed markedly (e.g. 2014). The sites with the highest and lowest prevalence also differed between years. There was no independent variation in prevalence between sites (SITE x *Mycoplasma* PA $\chi^2_2 = 2.71$, $P = 0.26$, Tables 1, 2). Prevalence was significantly higher in female vs male voles (SEX x *Mycoplasma* PA $\chi^2_1 = 7.079$, $P = 0.008$, Table 2) and between age classes (AGE x *Mycoplasma* PA $\chi^2_1 = 26.103$, $P < 0.001$), declining significantly with increasing age of voles ($R_s = -0.125$, $n = 1510$, $P < 0.001$, Table 2). However, there was also a significant interaction of sex with age (AGE x SEX x *Mycoplasma* PA $\chi^2_2 = 10.345$, $P = 0.006$; Fig. 2A). In the youngest age class, the juvenile voles, prevalence was marginally higher in males, but among mature and old individuals, prevalence was higher in female voles (Fig. 2A).

Table 1
Prevalence of *Mycoplasma* spp. in bank voles by year and site of the study.

Site			Urwitalt	Talty	Pilchy	Total
Year	1999	i*	14	17	28	59
		n*	35	41	47	123
		%	40.0	41.5	59.6	48.0
		CL ₉₅	26.0155.12	26.3857.93	41.8075.54	40.3055.66
	2002	i	48	43	34	125
		n	67	73	73	213
		%	71.6	58.9	46.6	58.7
		CL ₉₅	60.5080.82	47.0570.13	35.1758.46	54.1863.09
	2006	i	77	73	68	218
		n	111	81	80	272
		%	69.4	90.1	85.0	80.1
		CL ₉₅	62.2975.70	80.0695.56	73.9592.17	75.8083.87
	2010	i	72	70	35	177
		n	88	95	56	239
		%	81.8	73.7	62.5	74.1
		CL ₉₅	69.8389.96	60.2184.01	52.0371.98	69.6778.03
	2014	i	41	25	60	126
		n	74	80	83	237
		%	55.4	31.3	72.3	53.2
		CL ₉₅	43.4566.70	20.8443.61	59.8582.42	48.4457.89
	2018	i	74	83	71	228
		n	89	94	77	260
		%	83.1	88.3	92.2	87.7
		CL ₉₅	71.1891.18	76.9594.67	83.0696.87	84.0890.61
	2022	i	46	39	32	117
* i = no. of infected voles, n = no. of voles assessed						

Site		Urwitałt	Tały	Pilchy	Total
	n	62	61	47	170
	%	74.2	63.9	68.1	68.8
	CL ₉₅	63.6782.68	53.0773.69	50.4182.43	59.8276.67
Total	i	372	350	328	1050
	n	526	525	463	1514
	%	70.7	66.7	70.8	69.4
	CL ₉₅	66.83–74.61	62.63–70.70	66.70–74.98	67.03–71.68
* i = no. of infected voles, n = no. of voles assessed					

Table 2
Prevalence of *Mycoplasma* spp. in bank voles by site, sex and age.

Factor	Level	n	Prevalence (%)	CL ₉₅
SITE	Urwitałt	526	70.7	67.75–73.54
	Tały	525	66.7	63.61–69.59
	Pilchy	463	70.8	64.44–76.61
SEX	Males	810	66.4	62.62–70.04
	Females	704	72.7	69.37–75.87
AGE	Juveniles	515	77.5	74.72–80.03
	Mature	540	66.9	63.76–69.80
	Old	455	63.3	56.70–69.47
* Four bank voles could not be assigned to an age class and were therefore excluded from the statistical analysis of age-related data.				

Abundance of infection with *Mycoplasma* spp. in bank voles

The mean abundance of *Mycoplasma* spp. was 12.6 ± 0.69 *Mycoplasma* spp. -infected RBC / 200 Fv. Overall abundance was very similar among voles from Urwitałt and Tały, but significantly higher among those from Pilchy (GLM with Negbin errors, main effect of SITE, $LR_{2,1498}=16.89$, $P = 0.0002$; Table 3). The greatest effect was the difference between years (main effect of YEAR, $LR_{6,1498}=97.33$, $P < 0.00001$; Fig. 1B), with particularly high abundance in 2006, and in the following years 2010, 2014 and 2018, before the fall in abundance that was evident in 2022. The high value in 2014 was mainly due to the voles from Pilchy, and the earlier peak in 2006 to the combination of voles from Pilchy and Tały

(Fig. 1D). In comparison to these two sites, abundance among voles from Urwitalt remained relatively stable (Fig. 1D), varying only from a low of 5.2/200 Fv in 2022 to a high of 14.2/200 Fv in 2018. These yearly differences between the sites were responsible for a highly significant 2-way interaction (YEAR x SITE, $LR_{12,1466}=75.61$, $P < 0.00001$).

Table 3
Abundance of *Mycoplasma* spp. in bank voles by site, sex and age.

Factor	Level	n	Abundance	SEM	Range/ 200 fv
SITE	Urwitalt	526	10.2	0.77	0-179
	Tały	525	11.2	0.95	0-260
	Pilchy	463	17.1	1.75	0-323
SEX	Males	810	11.5	0.96	0-323
	Females	704	14.0	0.98	0-260
AGE	Juveniles	515	17.3	1.48	0-323
	Mature	540	10.3	0.83	0-269
	Old	455	10.3	1.17	0-276

Abundance varied markedly between vole age classes. The highest abundance was in the youngest age class (main effect of AGE, $LR_{2,1498}=23.35$, $P < 0.0001$; Table 3), but there was no difference in abundance among the mature and old voles. The difference in abundance between the sexes was only just significant (main effect of SEX, $LR_{1,1498}=4.53$, $P = 0.033$; Table 3), but there was a significant interaction between these factors (2-way interaction SEX x AGE, $LR_{2,1466}=19.33$, $P < 0.0001$; Fig. 2B). While abundance was high and very similar in both sexes among juvenile voles (Fig. 2B), it then declined faster among male voles with increasing age ($R = 0.164$, $\beta = -5.803 \pm 1.231$, $t=-4.714$, $P < 0.001$), compared to female voles ($R = 0.048$, $\beta = -1.497 \pm 1.181$, $t=-1.268$, $P = 0.205$).

Two further interactions were significant. While abundance was higher in female voles overall (see above), the difference between the sexes varied between years (Fig. 2C). Abundance was higher in female voles in five surveys, markedly so in 2006 and marginally in 2002 and 2018, but lower than in males in 2014 and 2022 (2-way interaction SEX x YEAR, $LR_{6,1466}=19.17$, $P = 0.0039$; Fig. 2C). The age effect also showed some differences between years. Abundance was overall higher in juvenile voles compared to the older voles (see above), but not in 2010 or 2022 (AGE x YEAR, $LR_{2,1466}=25.21$, $P = 0.014$; Fig. 2D).

Molecular identification of *Mycoplasma* spp.

All *Mycoplasma* spp. DNA sequences obtained in this study were analyzed using the BLAST tool available through the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequence homology with

closely related GenBank sequences (MT345318, MT462251, U82963, OP271911, PP109119) ranged from 90% to 99%. Detailed homology results are provided in Supplementary File 1.

Phylogenetic analyses were performed on 66 newly obtained 16S rRNA gene sequences (595 bp) together with 37 reference *Mycoplasma* spp. sequences from GenBank (Fig. 3, Table 4). The MrBayes analysis revealed five major clades.

Table 4
Number of *Mycoplasma* spp. sequences obtained from rodents in 2018 and 2022 by site and host species

	2018			2022				Total
	Urwitałt	Tały	Pilchy	Urwitałt	Tały	Pilchy	Luknajno	
<i>C. glareolus</i>	10	21	5	9	0	2	0	47
<i>A. flavicollis</i>	0	0	0	8	1	1	0	10
<i>A. sylvaticus</i>	0	0	0	1	0	1	0	2
<i>A. agrarius</i>	0	3	0	0	0	0	4	7
Total	10	24	5	18	1	4	4	66

Clade I comprises 27 *Mycoplasma* spp. sequences generated in this study, together with 10 reference sequences originating from South American rodents. Sequence homology between our isolates and the reference sequences ranged from 99.3% to 99.5%, corresponding to 2–6 nucleotide differences (Supplementary file 1).

Within Clade I, several well-supported subgroups are evident. Our *Mycoplasma* spp. sequences segregate into two distinct branches (Fig. 3). The first branch contains 21 sequences obtained from *C. glareolus* across three study sites during the 2018 and 2022 sampling seasons. The second branch comprises six sequences originating from *A. flavicollis* (n = 2), *C. glareolus* (n = 1), and *A. agrarius* (n = 3).

The *Mycoplasma* spp. identified within this clade were assigned to two novel species: Candidatus *Mycoplasma glareolusae* sp. nov. (harbouring two haplotypes, G1 and G2) and Candidatus *Mycoplasma mazurae* sp. nov. (harbouring two haplotypes, M1 and M2). The former is named after the specific epithet of the bank vole, its primary host, whereas the latter derives its name from the region (Mazury Lake District) in which it was discovered. These relationships are illustrated in Fig. 3.

Clade II comprises 10 *Mycoplasma* spp. sequences generated in this study together with 13 reference sequences originating from rodents from Brazil and Japan. Sequence homology between our isolates and the reference sequences ranged from 99.0% and 99.5%, corresponding to 3–6 nucleotide differences (Supplementary file 1).

Within Clade II, two well-supported subgroups are apparent. The first subgroup contains reference sequences of *Mycoplasma* spp. derived from rodents originating in Brazil, Japan, and South Africa. The second subgroup comprises our *Mycoplasma* spp. sequences obtained from *A. flavicollis* (n = 4), *C. glareolus* (n = 3), *A. sylvaticus* (n = 2), and *A. agrarius* (n = 1). The *Mycoplasma* sp. identified within this clade represent novel species *Candidatus Mycoplasma rodentae* sp. nov., harboring 4 haplotypes: (R1, R2, R3 and R4). *Candidatus Mycoplasma rodentae* sp. nov. is named after the host's order.

Clade III included diverse *Mycoplasma* species from bats from Spain, Chile, and Brazil.

Clade IV comprises 22 *Mycoplasma* spp. sequences generated in this study and a single reference sequence of *Mycoplasma* sp. obtained from the black-footed colilargo (*Oligoryzomys nigripes*) in Brazil. Sequence homology within this clade ranged from 96.7% to 99.3%, corresponding to 4–14 nucleotide differences (Supplementary file 1).

Within Clade IV, three well-supported subgroups are evident. The first subgroup comprises 18 *Mycoplasma* spp. sequences obtained from *C. glareolus*, which cluster together with a single reference sequence from the black-footed colilargo from Brazil. The second subgroup contains two *Mycoplasma* spp. sequences identified in *A. agrarius* and *C. glareolus* from the Tałty study site. Their distinct clustering suggests the presence of a potentially novel *Mycoplasma* species occurring at this location (Fig. 3). The third subgroup consists of two *Mycoplasma* spp. sequences that are identical to each other and form a separate branch, indicating the possible presence of another novel *Mycoplasma* species associated with *C. glareolus* from Urwitałt. The *Mycoplasma* spp. identified within clade IV represent three novel species. The first, *Candidatus Mycoplasma clethrionimae* sp. nov., comprises four haplotypes (C1, C2, C3, and C4) and was named after the generic name for bank voles (Fig. 3). The second, *Candidatus Mycoplasma taltae* sp. nov., was named after the study site (Tałty) where it was discovered. Similarly, the third, *Candidatus Mycoplasma urwitaltae* sp. nov., was named after the study site of its discovery (Urwitałt).

Clade V. This clade comprises six *Mycoplasma* sp. sequences obtained in this study. All sequences are identical to one another and form a distinct clade, suggesting the presence of a potentially novel *Mycoplasma* species occurring in *A. agrarius*, *A. flavicollis*, and *C. glareolus* from two study sites (Łuknajno and Urwitałt). These relationships are shown in Fig. 3. The *Mycoplasma* sp. identified in this clade has been designated *Candidatus Mycoplasma kumakae* sp. nov. This species was named after our field station, the Masurian Center for Biodiversity and Nature Education in Urwitałt (KUMAK), where research on the epidemiology of parasitic infections in wild rodents has been conducted since 1999.

Finally, a single *Mycoplasma* sp. sequence obtained from *A. flavicollis* forms a separate branch clearly distant from all other clades, further indicating the possibility of a new *Mycoplasma* species. Thus has been named after its host genus, i.e. *Candidatus Mycoplasma apodemusae* sp. nov. (Fig. 3).

Haplotypes identified within *Mycoplasma* Species

Minimum Spanning Network (MSN) analysis of 16S *rRNA* gene sequences from *Mycoplasma* spp. (n = 66) revealed sixteen distinct haplotypes distributed across eight species (Fig. 4).

Candidatus *Mycoplasma glareolusae* sp. nov.

Two haplotypes were identified: G1 (n = 20), present across three study sites (Urwitałt, Tałty, Pilchy), and G2 (n = 1), restricted to Pilchy. Both haplotypes occurred in *C. glareolus*. The genetic divergence between G1 and G2 was 2 SNPs (Fig. 4A–B, Supplementary file 1).

Candidatus *Mycoplasma clethrionomae* sp. nov.

Four haplotypes were detected: C1 (n = 15), C2 (n = 1), C3 (n = 1), and C4 (n = 1). These haplotypes were distributed across Urwitałt, Tałty, and Pilchy, exclusively in *C. glareolus*. Pairwise differences ranged from 1–5 SNPs (Fig. 4A–B, Supplementary file 1).

Candidatus *Mycoplasma rodentae* sp. nov.

Four haplotypes were identified. R1 (n = 7) was found in *A. flavicollis*, *A. agrarius*, *A. sylvaticus*, and *C. glareolus* across Urwitałt, Tałty, and Pilchy. R2 and R3 were restricted to *C. glareolus* in Urwitałt, whereas R4 was detected in *A. flavicollis* at the same site. Genetic divergence among haplotypes ranged from 1–5 SNPs (Fig. 4A–B, Supplementary file 1).

Candidatus *Mycoplasma mazurae* sp. nov.

Two haplotypes were observed: M1 (n = 4), detected in three *A. agrarius* and one *C. glareolus* across Pilchy, Tałty, and Łuknajno; and M2 (n = 2), identified in *A. flavicollis* from Urwitałt and Tałty. M2 differed from M1 by 1 SNP and from G1 by 4 SNPs, while M1 diverged from R1 by 21 SNPs (Fig. 4A–B, Supplementary file 1).

Candidatus *Mycoplasma kumakae* sp. nov.

This species was identified in *A. flavicollis*, *A. agrarius*, and *C. glareolus* from Urwitałt and Łuknajno. This lineage was connected to C1, differing by 45 SNPs, and to Candidatus *Mycoplasma mazurae* by 41 SNPs (Fig. 4A–B, Supplementary file 1).

Candidatus *Mycoplasma urwitaltae* sp. nov

Identified in two *C. glareolus* from Urwitałt, this single haplotype was connected to C1, differing by 21 SNPs (Fig. 4A–B).

Candidatus *Mycoplasma apodemusae* sp. nov

Detected in one *A. flavicollis* from Urwitałt, this haplotype was related to Candidatus *Mycoplasma kumakae*, differing by 28 SNPs (Fig. 4A–B).

Candidatus *Mycoplasma taltae* sp. nov

Found in *C. glareolus* and *A. agrarius* from Talty, this haplotype differed from Candidatus *Mycoplasma clethrionomae* (C1) by 16 SNPs and from R1 by 37 SNPs (Fig. 4A–B).

Discussion

This study represents the first comprehensive investigation into the molecular and genetic diversity of *Mycoplasma* spp. in bank voles from Poland. Our analyses revealed an unexpectedly high prevalence of infection, with 69.4% of sampled individuals testing positive. Importantly, we identified eight novel *Mycoplasma* species and characterized 16 distinct haplotypes, underscoring the remarkable genetic heterogeneity of these organisms in wild rodent populations. These findings not only expand our current understanding of *Mycoplasma* diversity in Central Europe but also highlight Poland as a previously underexplored reservoir of hemotropic *Mycoplasma* lineages. The discovery of such extensive diversity has significant implications for epidemiology, host–pathogen interactions, and the potential zoonotic relevance of these bacteria.

Our findings confirm that *Mycoplasma* infections are widespread in bank voles, consistent with previous studies documenting high prevalence of *Mycoplasma* in small mammal communities^{6,22,24,25,29}. The overall prevalence detected in this study (69.4%) closely matches our earlier results from Poland (68.3%)²⁴, indicating remarkable stability of *Mycoplasma* infections in bank voles across the 23-year period covered by this long-term investigation. These findings align with earlier reports of high prevalence in rodents worldwide^{6,22,25,29} and exceed levels reported in mole rats from South Africa³³, rodents from Brazil³⁴, and rodents from Thailand³⁵. Variation in prevalence likely reflects biological and ecological factors, including host behavior (gregarious vs. solitary), age, habitat type, and whether animals are free-ranging or captive^{36–39}.

We hypothesized that extrinsic factors exert the strongest influence on *Mycoplasma* spp. community structure, particularly through long-term and unpredictable temporal variation. Previous short-term studies from Poland identified year and season as the most influential factors shaping haemoparasitic infections in rodents^{40–42}. Rodent populations often fluctuate temporally, sometimes following multi-year cycles⁴³, and similar fluctuations occur in their ectoparasites and vectors^{44–46}. Our findings support this hypothesis, showing significant between-year variation in haemoparasite infection.

In contrast, the prevalence of *Mycoplasma* spp. in bank voles from different study sites did not vary significantly, differing from earlier findings in Poland and Egypt^{22,24}. The long-term persistence of infections suggests that these forest habitats support stable communities of vectors capable of maintaining transmission. Sex differences in prevalence have been reported previously^{24,41}, with females often showing higher infection rates, as observed in *Microtus arvalis* infected with *Mycoplasma* and *Hepatozoon*⁴¹ and in *C. glareolus* infected with *Mastophorus muris*⁴⁷. Elevated prevalence in

females may reflect reproductive-associated immunosuppression^{48,49} and/or increased behavioural exposure, as females occupy smaller territories and spend extended periods in nests during breeding, increasing contact with vectors. Age also influenced infection, with juveniles showing the highest prevalence, consistent with Bajer et al. (2014)²⁴. Young rodents, with immature immune systems, are more susceptible to primary infections^{50,51}, and their increased movement within nests⁵² likely enhances exposure to ectoparasites.

Abundance patterns varied among sites: voles from Urwitałt and Tałty showed similar intensity levels, while those from Pilchy had significantly higher intensity. This contrasts with earlier findings where the abundance of infection with *Mycoplasma* spp. from Tałty and Pilchy were higher than in Urwitałt²⁴. The prevalence of flea infestations in 2018 was higher at Tałty and Pilchy compared with Urwitałt (Alsarraf unpublished), suggesting greater ectoparasite prevalence in these habitats. Overall, conditions for maintaining host-vector-parasite relationships appear more favourable at Tałty and Pilchy. However, we also observed a significant decline in infection abundance over time, consistent with previous findings in Poland²⁴ and Egypt²². These declines may be linked to climate warming and reduced rainfall in Poland, as well as increasing human activity altering local habitats.

Molecular identification of *Mycoplasma* spp.

Phylogenetic analyses of newly generated 16S *rRNA* sequences together with reference sequences resolved five major clades encompassing 18 *Mycoplasma* species including the novel species described in this study. The sequences detected in rodents clustered with *Mycoplasma* spp. previously reported from Brazil^{17,23}, Chile²¹, and Japan³, suggesting a shared common ancestor. Phylogenetic analysis and Minimum Spanning Network were consistent, supporting the presence of novel species identified in this study. Similar discoveries have recently been reported, such as Candidatus *Mycoplasma haematomyotis* and Candidatus *Mycoplasma haematophyllostomi* in bats from Spain⁵³.

Our findings provide valuable insights into the potential host specificity and spatial segregation of *Mycoplasma* spp. detected in rodents. Several of the newly identified species and haplotypes were restricted to a single host species, such as Candidatus *Mycoplasma glareolusae* sp. nov., Candidatus *Mycoplasma clethrionomae* sp. nov., Candidatus *Mycoplasma urwitaltae* sp. nov., and Candidatus *Mycoplasma apodemusae* sp. nov. Likewise, some taxa were found exclusively at a single sampling site, including Candidatus *Mycoplasma taltae* sp. nov., Candidatus *Mycoplasma urwitaltae* sp. nov., and Candidatus *Mycoplasma apodemusae* sp. nov., suggesting possible host adaptation or localized transmission dynamics. In contrast, other *Mycoplasma* lineages exhibited no evidence of host or site specificity, being detected across multiple rodent species and geographic locations. This pattern indicates that while certain taxa may display restricted distributions, others circulate broadly among sympatric rodent populations, reflecting a complex interplay of ecological and epidemiological factors.

The high genetic diversity observed here reflects the adaptive capacity of *Mycoplasma* spp. to colonize new hosts and environments, as reported in rodents from Brazil and Chile^{17,21}. Diversity may be

influenced by host community structure, environmental conditions, and ectoparasite abundance. Our Minimum Spanning Network revealed a tree-like topology with linear connections between haplotypes, with no reticulate (net-like) structures. This indicates that genetic relationships among the sequences are consistent with vertical inheritance and point mutations rather than recombination or horizontal gene transfer. Unlike examples described by Huson et al. (2006)⁵⁴ and Alabi et al. (2020)²¹, our results show simple branching patterns, supporting the interpretation that haplotype diversity is driven by accumulated mutations.

These genetic findings highlight the complexity of *Mycoplasma* species, the presence of uncharacterized genotypes, and unknown evolutionary pathways. They also reveal significant gaps in our understanding of cross-species spillover. Importantly, this study constitutes the first molecular and genetic investigation of *Mycoplasma* spp. in rodents from Poland. Previous reports in Poland have focused on rodents, rats, humans, and turkeys^{24,25,30–32}, but these relied on conventional or clinical approaches without molecular identification or genetic characterization.

Conclusion

Taken together, our findings provide novel insights into the prevalence, diversity, and host associations of *Mycoplasma* spp. in wild rodents, while also highlighting the stability of infections over decades and the influence of ecological factors. Importantly, this study constitutes the first molecular and genetic investigation of *Mycoplasma* spp. in rodents from Poland and describes eight novel Candidatus species of *Mycoplasma* identified through phylogenetic and haplotype network analyses.

Nevertheless, substantial gaps remain in our understanding of the evolutionary history, transmission dynamics, and potential for cross-species spillover. Further studies are required to address these gaps and to fully elucidate the role of *Mycoplasma* spp. in wildlife health and disease ecology.

Methods

Definition of terms

The taxonomic classification of bank voles has been revised multiple times in recent decades. Although they were recently placed within the genus *Myodes*, subsequent analyses indicated that the historically established name *Clethrionomys* is more appropriate. Therefore, this study follows the nomenclature recommended by the Mammal Diversity Database⁵⁵ and uses the designation *Clethrionomys glareolus*⁵⁶.

Study sites

Rodents were live-trapped in three sites, representing managed Scots pine forest in NE Poland. The study sites have been previously comprehensively described^{46,57,58,58}. They are localized in the Mazury

Lake District in the north-eastern corner of Poland, in the vicinity of Lake Śniardwy and the towns of Mikołajki, Ryn, and Pisz. Site 1 is referred to as Urwitałt (53.79838, 21.64549), site 2 as Tałty (53.89202, 21.53494), site 3 as Pilchy (53.70569, 21.80617). Additionally, we added a new trapping area in fallow lands near the Masurian Center for Biodiversity and Nature Education in Urwitałt (KUMAK) in 2022, site 4 'Łuknajno' (53.81395, 21.65199).

Collection of rodent samples

Rodent trapping was performed in late summer (August/September) in 1999, 2002, 2006, 2010, 2014, 2018 and 2022^{24,46,57–59}. At the field station in Urwitałt, live-trapped rodents were identified, culled, weighted (to the nearest 0.1 g), sexed and relevant morphometric data were recorded⁵⁸. The necropsies were carried out following terminal isoflurane (Merck, Darmstadt, Germany) anaesthesia. The rodents were placed in an anaesthetic chamber with an isoflurane concentration of 3–5% and then culled by cervical dislocation by an authorised researcher licensee (AB license no. 13/2015). The methodologies employed for rodent trapping, as well as the procedures for sampling and processing of captured specimens, have all been detailed in previous publications^{24,46,57,58}. The present study is based on a total of 1514 bank voles trapped in the years given above, and additionally we analysed 10 blood samples from *Apodemus flavicollis*, 2 from *Apodemus sylvaticus* and 7 samples from *Apodemus agrarius* (Tables 1 and 2).

Blood collection and DNA extraction

Thin blood smears were prepared from drops of blood taken from the heart. Blood smears were air-dried, fixed in absolute methanol and stained using Hemacolor kit (Merck, Germany). Smear was examined under oil immersion (×1,000 magnification). *Mycoplasma*-infected RBC were counted in 200 fields of vision (fv). Microscopical observation of stained blood smears was used as the only detection method for samples obtained in 1999–2014.

Since 2018, additional blood samples (200 µl) were collected into 0.001 M EDTA for subsequent molecular analysis and then stored at – 20°C. For individuals found dead in traps, the entire heart was removed and homogenized in 400 µl of 0.001 M EDTA. Genomic DNA was extracted from both whole-blood samples and heart homogenates using the DNeasy Blood and Tissue Kit (Qiagen, Germany), and all extracts were stored at – 20°C. The extracted DNA was used as a template for a polymerase chain reaction (PCR) assay targeting a 595 bp fragment of the 16S rRNA gene in samples collected in 2018 and 2022. Amplification was performed using universal primers specific for *Mycoplasma* spp.: forward primer HBT-F (ATACGGCCCATATTCCTACG) and reverse primer HBT-R (TGCTCCACCACTTGTTCA), as described by Criado-Fornelio et al. (2003)⁶⁰.

Sequencing and molecular typing

Selected PCR products (n = 66) were sequenced in both directions by private companies (Genomed S.A., Poland, and Eurofins Genomics, Germany). Both reads were checked for quality and then aligned and

edited to form a consensus sequence using BioEdit ⁶¹. Then sequences were checked in BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify *Mycoplasma* species/genotype. Obtained sequences were aligned with *Mycoplasma* reference sequences from GenBank using the ClustalW in MEGA11: Molecular Evolutionary Genetics Analysis version 11 ⁶², and AliView ⁶³. The number of haplotypes in the 16S *rRNA* gene sequences was calculated using DnaSP v6.12.03 ⁶⁴. A Minimum Spanning Network (MSN) for the 16S *rRNA* sequences was constructed in PopART v1.46 ⁶⁵, using only the sequences generated in this study.

Phylogenetic analysis

Phylogenetic relationships among the 16S *rRNA* gene sequences were assessed using Bayesian Inference, implemented in MrBayes v3.2.6 ⁶⁶. We fitted a GTR + G model with all six rate parameters free and with variation in the rate of evolution among sites in the alignment. A single run of 10,000,000 generations was sampled every 1,000 generations, with the first 25% discarded as burn-in. Convergence and effective sample sizes of all model parameters were assessed in Tracer v1.7. ⁶⁷.

Statistical analysis

For data subsets, prevalence values (% of voles infected with *Mycoplasma* spp.) are given with 95% exact confidence limits (CL₉₅) in the tables and text, and 95% confidence intervals (CI₉₅) illustrated on the Figs. These were calculated in bespoke software based on the tables of Rohlf and Sokal (1995)⁶⁸. For quantitative data (i.e. abundance as reflected in no. of infected RBC / 200 fv), mean values $\pm$ standard error of the mean (SEM) are provided and these are derived for all voles in a data subset, including the uninfected animals.

Prevalences were analyzed using maximum likelihood techniques based on log linear analysis of contingency tables in the software package IBM SPSS, version 28 (Armonk, New York, USA). Full factorial models included sex (two levels, male and female voles), age (three levels, juvenile, young adult and fully mature voles), geographical locality, i.e. SITE (three levels corresponding to the three study sites as specified above), year of survey (1999, 2002, 2006, 2010, 2014, 2018 and 2022), and these are referred to as the intrinsic (sex and age class) and extrinsic (site and year) factors. This approach is based on categorical values of factors of interest, which are used not only to fit hierarchical log-linear models to multidimensional cross-tabulations using an iterative proportional-fitting algorithm but also to detect associations between the fitted factors. Multi-factorial models were fitted as described previously ⁴⁷, beginning with the full factorial model, followed by simplification of the model until only significant terms remained. Thus, for each level of analysis in turn, beginning with the most complex model, involving all possible main effects and interactions, those combinations that did not contribute significantly to explaining variation in the data were eliminated in a stepwise fashion, beginning with the highest-level interaction (backward selection procedure). A minimum sufficient model (MSM) was then obtained, for which the likelihood ratio of Chi-squared was not significant, indicating that the model was sufficient in explaining the data. The importance of each term in interactions involving P/A of

Mycoplasma spp. infected RBCs in the final model was assessed by the probability that its exclusion would alter the model significantly and these values are given in the text, assessed by a likelihood ratio test between models with and without each term of interest.

For analysis of quantitative data, we fitted a full factorial generalized linear (GLM) statistical model in R (v. 4.3.2, R Core Development Team) based on the negative binomial error structure, with both extrinsic (year and site) and intrinsic (sex and age) fixed factors. The dependent variable was the number of infected RBC / 200 fv. We first used the “step” command in R to refine the model and then further simplified the model by a stepwise deletion of non-significant terms beginning with the highest order remaining interaction, until only significant main effects and interactions, and also main effects that were components of interactions but not significant in their own right, remained (the minimum sufficient model). The significance of each of the remaining terms was then assessed by comparison of models with and without that term. Values of likelihood ratios (LR) are provided. These procedures are fully described by Behnke et al. (2021)⁶⁹.

Declarations

Ethical declarations

All procedures were carried out under licenses issued by the First Warsaw Local Ethics Committee for Animal Experimentation in Poland (license numbers: 73/2010, 148/2011, 304/2012, and 706/2015). All rodents were culled by Prof. Anna Bajer, who is authorized by the Polish Laboratory Animal Science Association (POLASA) to perform experimental procedures and animal culling for scientific purposes (license no. 13/2015).

This study followed the Guidelines for the Care and Use of Laboratory Animals of the Polish National Ethics Committee on Animal Experimentation and complied with Polish national regulations governing field studies involving the trapping and culling of unprotected vertebrates for scientific purposes (Resolution No. 12/2022 of the Polish National Ethics Committee on Animal Experimentation, 11 March 2022). The study was conducted in accordance with the ARRIVE 2.0 guidelines.

Competing interests

The authors declare no competing interests.

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Author Contribution

MoA, Designed the study and performed the phylogenetic analysis. MoA, DW, MuA, WR, AB, JMB, JBB, MG, JG, MK, AG participated in a field work. JMB performed the statistical analysis. MoA, DDS, DW, FMF participated in laboratory work. MoA, JMB and AB drafted the manuscript. All authors read and approved the final manuscript.

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Data Availability

All relevant data are included in the article. All DNA sequences of *Mycoplasma* spp. have been deposited in GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>) under accession numbers PZ836163-PZ836228 and provided in main text.

References

1. Neimark, H., Johansson, K. E., Rikihisa, Y. & Tully, J. G. Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of 'Candidatus *Mycoplasma haemofelis*', 'Candidatus *Mycoplasma haemomuris*', 'Candidatus *Mycoplasma haemosuis*' and 'Candidatus *Mycoplasma wenyonii*'. *Int. J. Syst. Evol. Microbiol.* **51**, 891–899 (2001).
2. Messick, J. B. Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. *Vet. Clin. Pathol.* **33**, 2–13 (2004).
3. Sashida, H. et al. Two Clusters among *Mycoplasma haemomuris* Strains, Defined by the 16S-23S rRNA Intergenic Transcribed Spacer Sequences. *J. Vet. Med. Sci.* **75**, 643–648 (2013).
4. Neimark, H., Peters, W., Robinson, B. L. & Stewart, L. B. Phylogenetic analysis and description of *Eperythrozoon coccoides*, proposal to transfer to the genus *Mycoplasma* as *Mycoplasma coccoides* comb. nov. and Request for an Opinion. *Int. J. Syst. Evol. Microbiol.* **55**, 1385–1391 (2005).
5. Sykes, J. E. Feline hemotropic mycoplasmas. *Vet. Clin. North. Am. Small Anim. Pract.* **40**, 1157–1170 (2010).
6. Retief, L., Chimimba, C. T., Oosthuizen, M. C., Matshotshi, A. & Bastos, A. D. S. Haemoplasma Prevalence and Diversity in Three Invasive *Rattus* Species from Gauteng Province, South Africa.

- Microorganisms* **10**, 1632 (2022).
7. Cohen, C. et al. Haemoplasmas in wild rodents: Routes of transmission and infection dynamics. *Mol. Ecol.* **27**, 3714–3726 (2018).
 8. Millán, J., Martín-Maldonado, B., Rodríguez-Pastor, R., Martínez-Padilla, J. & Esperón, F. High diversity, novel genotypes, and vertical transmission of hemotropic Mycoplasma in micromammals. *Comp. Immunol. Microbiol. Infect. Dis.* **107**, 102151 (2024).
 9. Stadler, J. et al. Detection of Mycoplasma suis in pre-suckling piglets indicates a vertical transmission. *BMC Vet. Res.* **15**, 252 (2019).
 10. Huber, B. M. et al. Vertical Transmission of Mycoplasma pneumoniae Infection. *Neonatology* **114**, 332–336 (2018).
 11. Manvell, C., Ferris, K., Maggi, R., Breitschwerdt, E. B. & Lashnits, E. Prevalence of Vector-Borne Pathogens in Reproductive and Non-Reproductive Tissue Samples from Free-Roaming Domestic Cats in the South Atlantic USA. *Pathogens* **10**, 1221 (2021).
 12. Henry, S. C. Clinical observations on eperythrozoonosis. *J. Am. Vet. Med. Assoc.* **174**, 601–603 (1979).
 13. Hoelzle, L. Development of a diagnostic PCR assay based on novel DNA sequences for the detection of Mycoplasma suis (Eperythrozoon suis) in porcine blood. *Vet. Microbiol.* **93**, 185–196 (2003).
 14. Strait, E. L., Hawkins, P. A. & Wilson, W. D. Dysgalactia associated with Mycoplasma suis infection in a sow herd. *J. Am. Vet. Med. Assoc.* **241**, 1666–1667 (2012).
 15. Tasker, S. et al. Description of outcomes of experimental infection with feline haemoplasmas: Copy numbers, haematology, Coombs' testing and blood glucose concentrations. *Vet. Microbiol.* **139**, 323–332 (2009).
 16. Foley, J. E., Harrus, S., Poland, A., Chomel, B. & Pedersen, N. C. Molecular, clinical, and pathologic comparison of two distinct strains of Haemobartonella felis in domestic cats. *Am. J. Vet. Res.* **59**, 1581–1588 (1998).
 17. Gonçalves, L. R. et al. Diversity and molecular characterization of novel hemoplasmas infecting wild rodents from different Brazilian biomes. *Comp. Immunol. Microbiol. Infect. Dis.* **43**, 50–56 (2015).
 18. Hornok, S. et al. Synanthropic rodents and their ectoparasites as carriers of a novel haemoplasma and vector-borne, zoonotic pathogens indoors. *Parasit. Vectors.* **8**, 27 (2015).
 19. Berkenkamp, S. D. & Wescott, R. B. Arthropod transmission of Eperythrozoon coccoides in mice. *Lab. Anim. Sci.* **38**, 398–401 (1988).
 20. Fleer, K. A., Foley, P., Calder, L. & Foley, J. E. Arthropod vectors and vector-borne bacterial pathogens in Yosemite National Park. *J. Med. Entomol.* **48**, 101–110 (2011).
 21. Alabí, A. S. et al. Molecular Survey and Genetic Diversity of Hemoplasmas in Rodents from Chile. *Microorganisms* **8**, 1493 (2020).
 22. Alsarraf, M. et al. Long-term spatiotemporal stability and dynamic changes in the haemoparasite community of spiny mice (Acomys dimidiatus) in four montane wadis in the St. Katherine

- Protectorate, Sinai, Egypt. *Parasit. Vectors*. **9**, 195 (2016).
23. Gonçalves, L. R. et al. Genetic diversity and lack of molecular evidence for hemoplasma cross-species transmission between wild and synanthropic mammals from Central-Western Brazil. *Acta Trop*. **203**, 105303 (2020).
24. Bajer, A. et al. Long-Term Spatiotemporal Stability and Dynamic Changes in the Haemoparasite Community of Bank Voles (*Myodes glareolus*) in NE Poland. *Microb. Ecol*. **68**, 196–211 (2014).
25. Piasecki, T., Chrzastek, K. & Kasprzykowska, U. *Mycoplasma pulmonis* of Rodents as a Possible Human Pathogen. *Vector Borne Zoonotic Dis*. **17**, 475–477 (2017).
26. Obara, H., Fujihara, M., Watanabe, Y., Ono, H. K. & Harasawa, R. A feline hemoplasma, 'Candidatus *Mycoplasma haemominutum*', detected in dog in Japan. *J. Vet. Med. Sci*. **73**, 841–843 (2011).
27. Steer, J. A. et al. A novel hemotropic *Mycoplasma* (hemoplasma) in a patient with hemolytic anemia and pyrexia. *Clin. Infect. Dis. Off Publ Infect. Dis. Soc. Am*. **53**, e147–151 (2011).
28. Barker, E. N. et al. Development and use of real-time PCR to detect and quantify *Mycoplasma haemocanis* and 'Candidatus *Mycoplasma haematoparvum*' in dogs. *Vet. Microbiol*. **140**, 167–170 (2010).
29. Millán, J., López-Roig, M., Delicado, V., Serra-Cobo, J. & Esperón, F. Widespread infection with hemotropic mycoplasmas in bats in Spain, including a hemoplasma closely related to 'Candidatus *Mycoplasma hemohominis*'. *Comp. Immunol. Microbiol. Infect. Dis*. **39**, 9–12 (2015).
30. Wójcik-Fatla, A., Sawczyn-Domańska, A., Kloc, A., Krzowska-Firych, J. & Sroka, J. Seroprevalence of *Borrelia*, *Anaplasma*, *Bartonella*, *Toxoplasma*, *Mycoplasma*, *Yersinia*, and *Chlamydia* in Human Population from Eastern Poland. *Pathogens* **14**, 96 (2025).
31. Kursa, O., Tomczyk, G., Sieczkowska, A. & Kostka, S. Sawicka-Durkalec, A. *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Turkeys in Poland. *Pathogens* **13**, 78 (2024).
32. Sawicka-Durkalec, A., Tomczyk, G., Kursa, O., Stenzel, T. & Gyuranecz, M. Evidence of *Mycoplasma* spp. transmission by migratory wild geese. *Poult. Sci*. **101**, 101526 (2022).
33. Retief, L., Bennett, N. C. & Bastos, A. D. S. Molecular detection and characterization of novel haemotropic *Mycoplasma* in free-living mole rats from South Africa. *Infect. Genet. Evol*. **89**, 104739 (2021).
34. de Machado, E. Detection, genetic diversity, and factors associated with infection by hemotropic mycoplasmas in rodent and marsupial populations in regions of the states of Rio de Janeiro and Parana, Brazil. *Microb. Pathog*. **197**, 107017 (2024).
35. Bulanawichit, W. et al. Molecular detection and genetic diversity of hemotropic *Mycoplasma* species in synanthropic rodents across Thailand. *Curr. Res. Parasitol. Vector-Borne Dis*. **9**, 100370 (2026).
36. Vieira, R. F. C. et al. Detection of a novel hemoplasma based on 16S rRNA gene DNA in captive and free-ranging capybaras (*Hydrochaeris hydrochaeris*). *Vet. Microbiol*. **139**, 410–413 (2009).
37. de Sousa, K. C. M. et al. Occurrence and molecular characterization of hemoplasmas in domestic dogs and wild mammals in a Brazilian wetland. *Acta Trop*. **171**, 172–181 (2017).

38. Persichetti, M. F. et al. Clinical evaluation of outdoor cats exposed to ectoparasites and associated risk for vector-borne infections in southern Italy. *Parasit. Vectors*. **11**, 136 (2018).
39. Volokhov, D. V. et al. Genotype Richness, and Coinfection Patterns of Hemotropic Mycoplasmas in Raccoons (*Procyon lotor*) on Environmentally Protected and Urbanized Barrier Islands. *Appl. Environ. Microbiol.* **83**, e00211–e00217 (2017).
40. Bajer, A., Pawelczyk, A., Behnke, J. M., Gilbert, F. S. & Sinski, E. Factors affecting the component community structure of haemoparasites in bank voles (*Clethrionomys glareolus*) from the Mazury Lake District region of Poland. *Parasitology* **122 Pt 1**, 43–54 (2001).
41. Pawelczyk, A., Bajer, A., Behnke, J. M., Gilbert, F. S. & Sinski, E. Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland. *Parasitol. Res.* **92**, 270–284 (2004).
42. Welc-Faleciak, R., Paziewska, A., Bajer, A., Behnke, J. M. & Siński, E. *Bartonella* spp. Infection in Rodents from Different Habitats in the Mazury Lake District, Northeast Poland. *Vector-Borne Zoonotic Dis.* **8**, 467–474 (2008).
43. Cornulier, T. et al. Europe-wide dampening of population cycles in keystone herbivores. *Science* **340**, 63–66 (2013).
44. Paziewska, A., Zwolińska, L., Harris, P. D., Bajer, A. & Siński, E. Utilisation of rodent species by larvae and nymphs of hard ticks (Ixodidae) in two habitats in NE Poland. *Exp. Appl. Acarol.* **50**, 79–91 (2010).
45. Welc-Fałęciak, R., Bajer, A., Behnke, J. M. & Siński, E. Effects of host diversity and the community composition of hard ticks (Ixodidae) on *Babesia microti* infection. *Int. J. Med. Microbiol.* **298**, 235–242 (2008).
46. Alsarraf, M. et al. Genetic diversity of *Bartonella* spp. in rodents and fleas from Poland. *Sci. Rep.* **15**, 10710 (2025).
47. Grzybek, M., Bajer, A., Behnke-Borowczyk, J., Al-Sarraf, M. & Behnke, J. M. Female host sex-biased parasitism with the rodent stomach nematode *Mastophorus muris* in wild bank voles (*Myodes glareolus*). *Parasitol. Res.* **114**, 523–533 (2015).
48. Connan, R. M. The effect of host lactation on the self-cure of *Nippostrongylus brasiliensis* in rats. *Parasitology* **61**, 27–33 (1970).
49. Dineen, J. K. & Kelly, J. D. The suppression of rejection of *Nippostrongylus brasiliensis* in lactating rats: the nature of the immunological defect. *Immunology* **22**, 1–12 (1972).
50. Clerc, M., Babayan, S. A., Fenton, A. & Pedersen, A. B. Age affects antibody levels and anthelmintic treatment efficacy in a wild rodent. *Int. J. Parasitol. Parasites Wildl.* **8**, 240–247 (2019).
51. Jackson, J. A. et al. An immunological marker of tolerance to infection in wild rodents. *PLoS Biol.* **12**, e1001901 (2014).
52. Eccard, A., Firozpoor, J., Escobar, J. & Galan, M. Charbonnel, N. Individual activity of forest rodents correlates to pathogen communities. *Sci. Rep.* **16**, 14684 (2026).

53. Volokhov, D. V. et al. Expanded diversity of novel hemoplasmas in rare and undersampled Neotropical bats. *One Health*. **17**, 100633 (2023).
54. Huson, D. H. & Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254–267 (2006).
55. Upham, N. et al. Mammal diversity database. (2022).
56. Kryštufek, B. et al. Back to the future: the proper name for red-backed voles is *Clethrionomys Tilesius* and not *Myodes Pallas*. *Mammalia* **84**, 214–217 (2020).
57. Behnke, J. M. et al. Variation in the helminth community structure in bank voles (*Clethrionomys glareolus*) from three comparable localities in the Mazury Lake District region of Poland. *Parasitology* **123**, 401–414 (2001).
58. Behnke, J. M. et al. Temporal and between-site variation in helminth communities of bank voles (*Myodes glareolus*) from N.E. Poland. 1. Regional fauna and component community levels. *Parasitology* **135**, 985–997 (2008).
59. Grzybek, M. et al. Zoonotic Viruses in Three Species of Voles from Poland. *Animals* **10**, 1820 (2020).
60. Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A. & Barba-Carretero, J. C. Presence of *Mycoplasma haemofelis*, *Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: a molecular study. *Vet. Microbiol.* **93**, 307–317 (2003).
61. Hall, T. A. & BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. **41**, 95–98 (1999).
62. Tamura, K., Stecher, G. & Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **38**, 3022–3027 (2021).
63. Larsson, A. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* **30**, 3276–3278 (2014).
64. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452 (2009).
65. Bandelt, H. J., Forster, P. & Rohl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48 (1999).
66. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
67. Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **67**, 901–904 (2018).
68. Rohlf, F. J. & Sokal, R. R. *Statistical Tables* (Freeman, 2003).
69. Behnke, J. M., Rogan, M. T., Craig, P. S., Jackson, J. A. & Hide, G. Long-term trends in helminth infections of wood mice (*Apodemus sylvaticus*) from the vicinity of Malham Tarn in North Yorkshire, England. *Parasitology* **148**, 451–463 (2021).

Figures

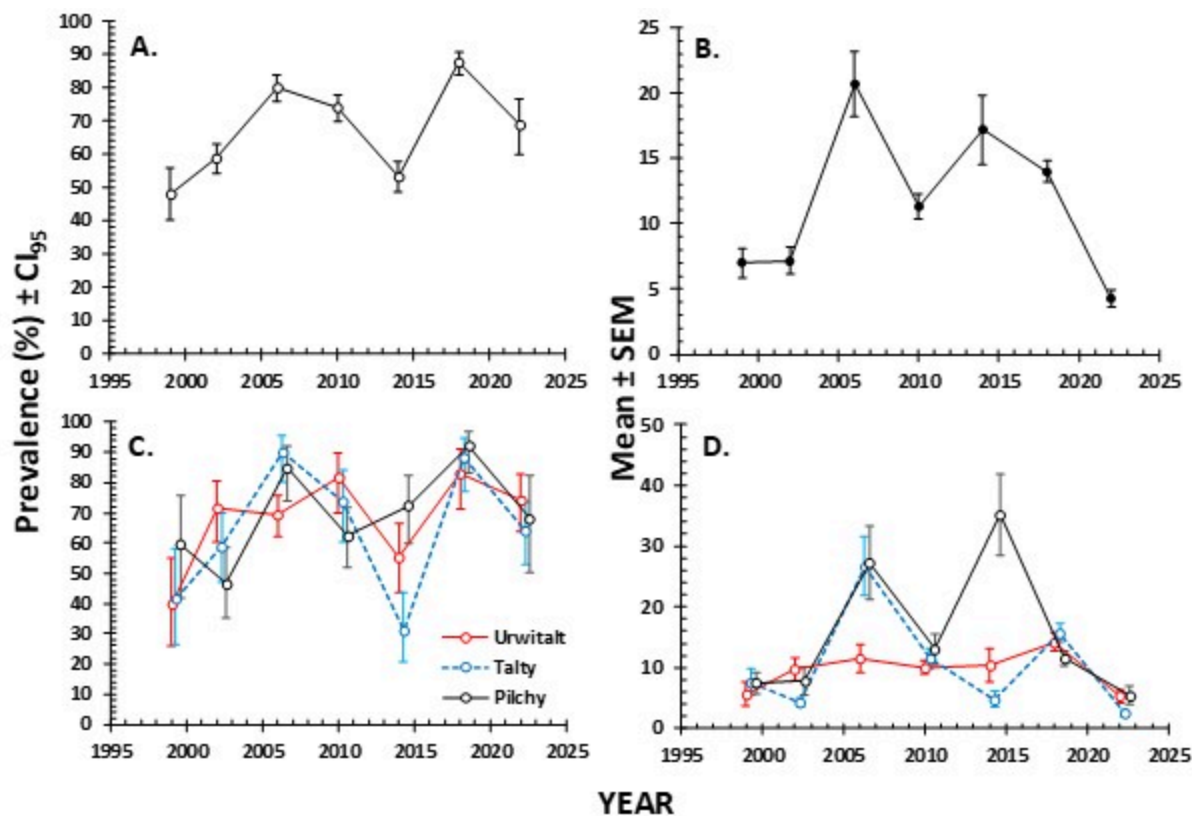


Figure 1

Prevalence (A and C) and abundance (B and D) of *Mycoplasmaspp.* by year of survey (A and B) and by year and site (C and D). For key to the symbols used in panel D, see panel C. For statistical analysis see text.

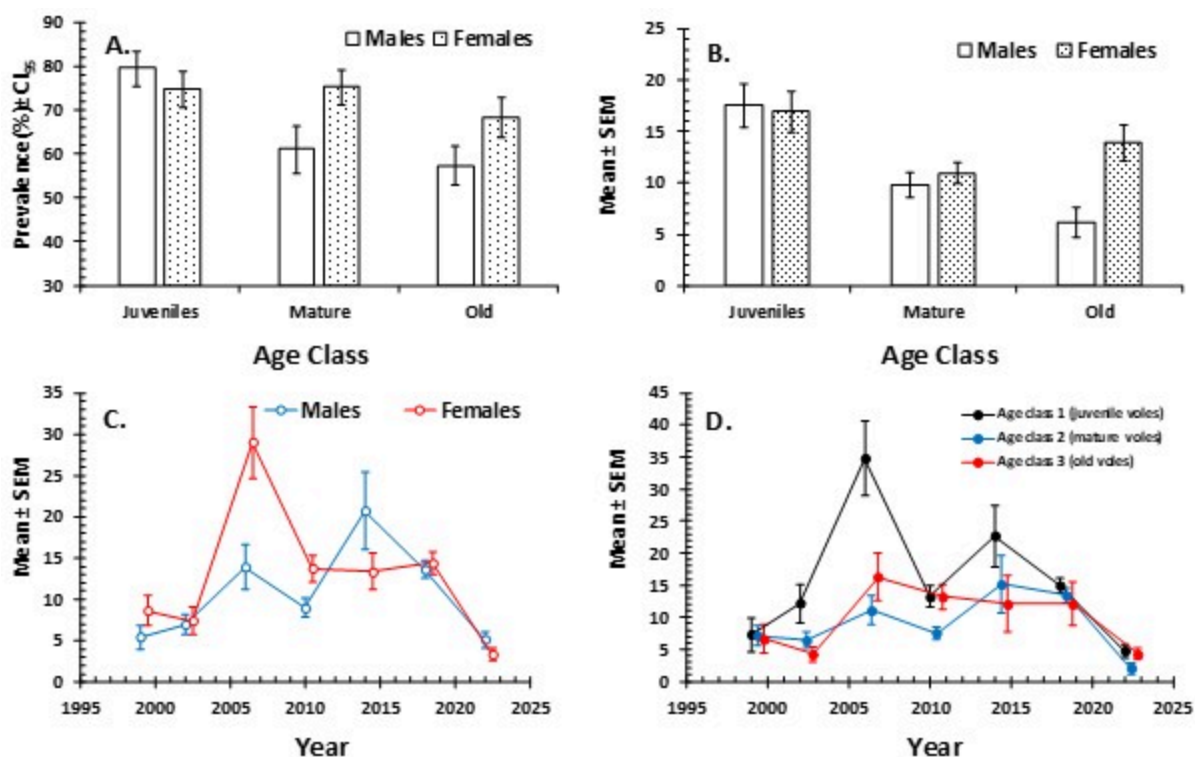


Figure 2

Significant interactions between factors affecting prevalence (A) and abundance (B, C and D) of *Mycoplasma* spp. For statistical analysis see text.

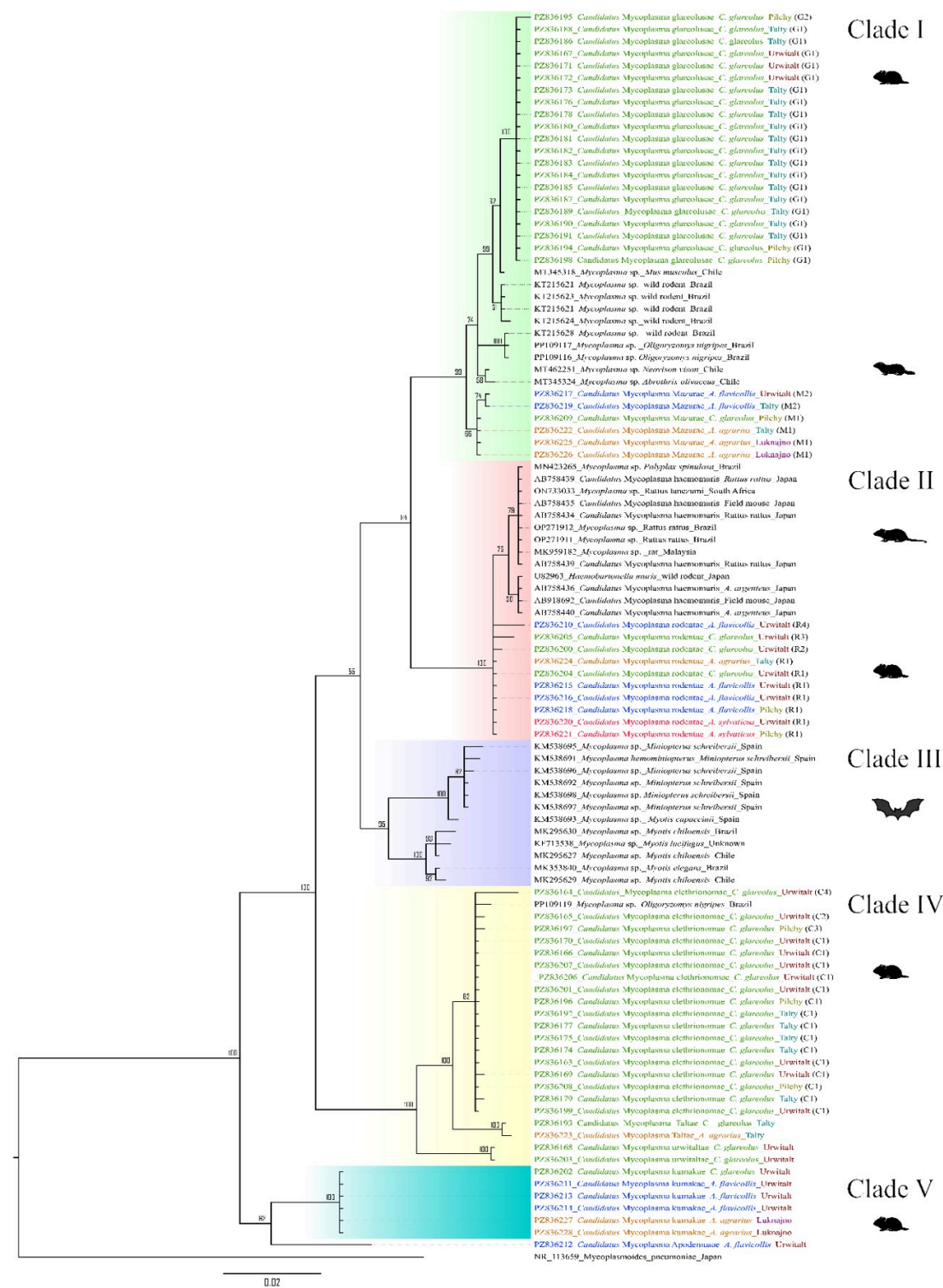


Figure 3

The phylogenetic tree of *Mycoplasma* spp. inferred from sequence variation of 595-bp *16S rDNA* gene fragment. The tree is 50%-majority rule consensus obtained using MrBayes (Bayesian Inference). Numbers along nodes represent *a posteriori* probability. The tree comprises 66 DNA sequences obtained in this study and 37 of references sequences from the GenBank.

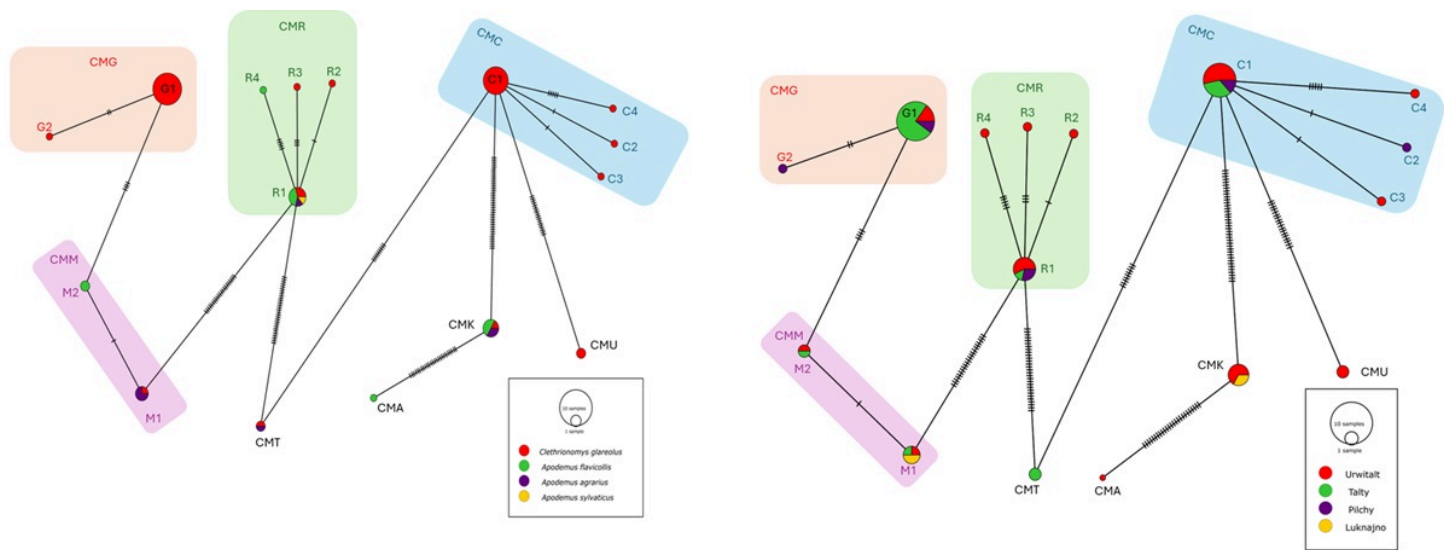


Figure 4

The Minimum Spanning Network of the *16S rDNA* gene showing the relationship between the haplotypes. (A) *Mycoplasma* spp. haplotypes based on the host and (B) *Mycoplasma* spp. haplotypes based on the study sites.

Supplementary Files

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