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Mycoplasma aquilae sp. nov., *Mycoplasma paraquilae* sp. nov., *Mycoplasma haliaeeti* sp. nov., *Mycoplasma milvi* sp. nov., and *Mycoplasma razini* sp. nov., isolated from predatory birds of the *Accipitridae* family

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ABSTRACT

Mycoplasmas are regularly isolated from the upper respiratory tract of predatory birds; however, most of these *Mycoplasma* isolates remain unidentified. A cohort of such unidentified *Mycoplasma* strains (n = 42) recovered from birds of the *Accipitridae* family was subjected to a comprehensive taxonomic study. All strains grew well in modified Hayflick's medium, and colonies exhibited typical fried egg morphology. The strains neither produced acid from sugar carbon sources nor hydrolysed arginine or urea. Analyses of 16S rRNA gene, 16S—23S intergenic spacer, and partial *rpoB* gene sequences placed the strains within the *Mycoplasma* (*M.*) *synoviae* cluster (Hominis group) with *M. verecundum* and *M. seminis* being their closest relatives. Phylogenetic trees inferred from 16S rRNA and *rpoB* gene sequences subdivided the 42 strains into five strain clusters. MALDI-ToF mass spectrometry allowed the differentiation of one strain group from the others but failed to distinguish the remaining four strain groups. Genome and proteome similarity metrics (ANIb, ANIm, TETRA, dDDH, AAI) and phylogenomic analysis provided solid evidence that the strains examined are indeed representatives of five hitherto unclassified species of genus *Mycoplasma* for which the names *Mycoplasma aquilae* sp. nov., *Mycoplasma paraquilae* sp. nov., *Mycoplasma haliaeeti* sp. nov., *Mycoplasma milvi* sp. nov., constituting the newly defined *Mycoplasma aquilae* complex, and *Mycoplasma razini* sp. nov. are proposed, with their designated type strains 1449^T (ATCC BAA-1896^T = DSM 22458^T), 654^T (DSM 113738^T = NCTC 14855^T), VS42A^T (DSM 113741^T = NCTC 14856^T), Z331B^T (DSM 113740^T = NCTC 14858^T), and 005V^T (DSM 113739^T = NCTC 14838^T), respectively.

Introduction

Members of the class *Mollicutes* (phylum *Mycoplasmatota*) are distinguished from other bacteria by a complete lack of a cell wall, a small cell and genome size, and a low genomic G + C content (Razin et al., 1998). Within the class *Mollicutes,* the genus *Mycoplasma* (trivial name mycoplasmas) is highly diverse and currently contains 136 recognized species that are distributed in a broad range of hosts like humans and other mammals, birds, reptiles, fish, and molluscs, where they live as commensals and/or pathogens (Balish et al., 2024). Among avian hosts, mycoplasmas are mainly found in domestic poultry and waterfowl (Bradbury, 2005; Grózner et al., 2019) but are also considered frequent inhabitants of diurnal birds of prey, especially of the order *Falconiformes* (Lierz et al., 2008a, 2008b, 2008c). However, relatively little is known about the occurrence of mycoplasmas in predatory birds of the *Accipitridae* family (order *Accipitriformes*), which comprises well-known birds of prey such as eagles, hawks, buzzards, kites, harriers, and Old-World vultures. The only recognized *Mycoplasma* species known to predominantly colonise *Accipitridae* are *Mycoplasma* (*M.*) *buteonis*, *M. gypis*, and *M. neophronis*, which have primarily been detected in common buzzards (*Buteo buteo*), Eurasian griffon vultures (*Gyps fulvus*), and Canarian Egyptian vultures (*Neophron percnopterus* subsp.

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Table 1
Attributes of strains included in the study and accession numbers for 16S rRNA genes, the 16S—23S intergenic spacer region (ISR), partial *rpoB* gene, and genome sequences.

Proposed names	Strain designation	Host species	Isolation site	Year of isolation	Country	Acc. no. 16S rDNA	Acc. no. ISR	Acc. no. *rpoB*	Acc. no. genome sequence
Mycoplasma aquilae sp. nov.	1449^T	*Aquila adalberti*	lung	2002	Spain	FM196532	FM196533	PP706177	CP182208
	Z244B	*Aquila heliaca*	trachea	2021	Austria	PP693538	PP694101	PP706178	JBLVZI000000000
	VS424B	*Aquila heliaca*	trachea	2023	Austria	PP693539	PP694102	PP706179	JBLVZJ000000000
	654^T	*Aquila heliaca*	lung	2008	Austria	PP693540	PP694103	PP706180	CP182209
	1458C	*Aquila adalberti*	lung	2002	Spain	PP693541	PP694104	PP706181	JBLVZK000000000
	3118	*Aquila adalberti*	trachea	2003	Spain	PP693542	PP694105	PP706182	JBLVZL000000000
	31_09	*Aquila adalberti*	choana	2006	Spain	PP693543	PP694106	PP706183	JBLVZM000000000
	4F	*Aquila adalberti*	choana	2006	Spain	PP693544	PP694107	PP706184	JBLVZN000000000
Mycoplasma paraquilae sp. nov.	AA7A	*Aquila adalberti*	oesophagus	2007	Spain	PP693545	PP694108	PP706185	JBLVZO000000000
	T193	*Necrosyrtes monachus*	trachea	2007	Spain	PP693546	PP694109	PP706186	JBLVZP000000000
	Z473B	*Aquila chrysaetos*	trachea	2019	Austria	PP693547	PP694110	PP706187	JBLVZQ000000000
	VS403A	*Aquila heliaca*	trachea	2020	Austria	PP693548	PP694111	PP706188	JBLVZR000000000
	VS509_3	*Aquila heliaca*	trachea	2021	Austria	PP693549	PP694112	PP706189	JBLVZS000000000
	1932B	*Aquila heliaca*	mouth	2021	Austria	PP693550	PP694113	PP706190	JBLVZT000000000
	SK928A	*Aquila heliaca*	trachea	2022	Austria	PP693551	PP694114	PP706191	JBLVZU000000000
	VS42A^T	*Haliaeetus albicilla*	trachea	2020	Austria	PP693552	PP694115	PP706192	CP182210
	SH20	*Haliaeetus albicilla*	choana	2009	Germany	MK615071	PP694116	MT721009	JBLVZV000000000
	B6188	*Haliaeetus albicilla*	choana	2009	Germany	PP693553	PP694117	PP706193	JBLVZW000000000
	B6400	*Haliaeetus albicilla*	choana	2009	Germany	PP693554	PP694118	PP706194	JBLVZX000000000
Mycoplasma haliaeeti sp. nov.	BRA290	*Haliaeetus albicilla*	choana	2009	Germany	PP693555	PP694119	PP706195	JBLVZY000000000
	MV126	*Haliaeetus albicilla*	choana	2009	Germany	PP693556	PP694120	PP706196	JBLVZZ000000000
	653B	*Haliaeetus albicilla*	lung	2019	Austria	PP693557	PP694121	PP706197	JBLWAA000000000
	Z407A	*Haliaeetus albicilla*	trachea	2020	Austria	PP693558	PP694122	PP706198	JBLWAB000000000
	SK341A	*Haliaeetus albicilla*	trachea	2023	Austria	PP693559	PP694123	PP706199	JBLWAC000000000
	VS292A	*Haliaeetus albicilla*	trachea	2023	Austria	PP693560	PP694124	PP706200	JBLWAD000000000
	Z331B^T	*Milvus milvus*	trachea	2020	Austria	PP693561	PP694125	PP706201	CP182211
	HF11B	*Aquila fasciata*	trachea	2005	Spain	PP693562	PP694126	PP706202	JBLWAE000000000
	Sp48II	*Aquila fasciata*	trachea	2006	Spain	PP693563	PP694127	PP706203	JBLWAF000000000
	46,852	*Buteo buteo*	choana	2009	Germany	PP693564	PP694128	PP706204	JBLWAG000000000
	VS428	*Milvus milvus*	trachea	2020	Austria	PP693565	PP694129	PP706205	JBLWAH000000000
Mycoplasma milvi sp. nov.	Z631	*Milvus milvus*	trachea	2020	Austria	PP693566	PP694130	PP706206	JBLWAI000000000
	Z663	*Milvus milvus*	trachea	2020	Austria	PP693567	PP694131	PP706207	JBLWAJ000000000
	3137	*Milvus milvus*	lung	2021	Austria	PP693568	PP694132	PP706208	JBLWAK000000000
	VS410B	*Accipiter gentilis*	trachea	2022	Austria	PP693569	PP694133	PP706209	JBLWAL000000000
	Z355B	*Milvus milvus*	trachea	2022	Austria	PP693570	PP694134	PP706210	JBLWAM000000000
	VS299A	*Milvus milvus*	trachea	2023	Austria	PP693571	PP694135	PP706211	JBLWAN000000000
	Z707	*Milvus milvus*	trachea	2023	Austria	PP693572	PP694136	PP706212	JBLWAO000000000
	005V^T	*Hieraaetus pennatus*	trachea	2005	Spain	MK615070	PP694137	MT721031	CP182212
Mycoplasma razini sp. nov.	2634B	*Aegypius monachus*	conjunctiva	2006	Spain	PP693573	PP694138	PP706213	JBLWAP000000000
	CR	*Circaetus gallicus*	trachea	2006	Spain	PP693574	PP694139	PP706214	JBLWAQ000000000
	HF3V	*Aquila fasciata*	choana	2005	Spain	PP693575	PP694140	PP706215	JBLWAR000000000
	Sp33II	*Aquila fasciata*	choana	2006	Spain	PP693576	PP694141	PP706216	JBLWAS000000000

majorensis), respectively (Poveda et al., 1994; Suárez-Pérez et al., 2012). In addition, Oaks et al. (Oaks et al., 2004) reported a novel, non-cultivable (on/in standard mycoplasma culture media) *Mycoplasma* species, tentatively named '*Mycoplasma vulturii*', in Oriental white-backed vultures (*Gyps bengalensis*) that died from diclofenac poisoning. Other known *Mycoplasma* species inhabiting birds of prey include *M. falconis*, *M. hafezii*, and *M. seminis*, which are regularly isolated from falcons and occasionally from *Accipitridae* (Lierz et al., 2007a; Lierz et al., 2008a, 2008b, 2008c; Lierz and Hafez, 2008; Ziegler et al., 2019; Fischer et al., 2020), and *M. corogypsi*, which has so far only been isolated from black vultures (*Coragyps atratus,* a New-World vulture of family *Cathartidae*) displaying pododermatitis, polyarthritis and/or tenosynovitis (Panangala et al., 1993; Ruder et al., 2009; Van Wettere et al., 2013). Furthermore, mycoplasmas that usually colonise chickens such as *M. gallinarum, M. gallinaceum, M. iners* and *M. lipofaciens* have occasionally been detected in birds of prey (Poveda et al., 1990; Lierz et al., 2007b).

Previous and more recent studies have also reported the presence of several unknown *Mycoplasma* species in predatory birds (Loria et al., 2008; Lecis et al., 2010, 2016; Sawicka-Durkalec et al., 2021). Likewise, a considerable number of unidentified *Mycoplasma* isolates have been recovered at the mycoplasma diagnostic laboratory, University of Veterinary Medicine, Vienna, Austria, from samples of various predatory birds originating from native bird populations in Austria, Germany, and Spain. One cohort of unidentified *Mycoplasma* strains, consisting of more than 80 isolates recovered from *Accipitridae* over the last 23 years, exhibited the highest 16S rRNA gene sequence similarity to *M. verecundum* 107^T (98.2 %) and > 99 % similarity to each other. Furthermore, the strains showed no match with matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) mass spectrometry (MS) reference spectra contained in a large in-house mycoplasma research library that included all type and several field strains of cultivable *Mycoplasma* species isolated from animals (Spergser et al., 2019). Based on these preliminary findings, the current study aimed to comprehensively characterise this group of related and yet unidentified *Mycoplasma* strains using a polyphasic approach that followed the guidelines for the description of new species in the class *Mollicutes* (Brown et al., 2007) and other recommendations of the Subcommittee on the Taxonomy of *Mollicutes* (Firrao and Brown, 2013; May and Brown, 2019), and also included in-depth genomic analyses. A total of 42 selected *Mycoplasma* strains, all from *Accipitridae* and almost exclusively isolated from the respiratory tract, were included in this study. They were identified to represent five novel *Mycoplasma* species for which the names *Mycoplasma aquilae* sp. nov., *Mycoplasma paraquilae* sp.

nov., *Mycoplasma haliaeeti* sp. nov., *Mycoplasma milvi* sp. nov., which together constitute the newly defined *Mycoplasma aquilae* complex, and *Mycoplasma razini* sp. nov. are proposed.

Material and methods

Culture, morphology, and gliding motility

Samples were taken from *Accipitridae* during field studies and veterinary revisions of captive breeding individuals by researchers of the SaBio research group at the Institute for Game and Wildlife Research, University of Castilla-La Mancha, Ciudad Real, Spain, as well as during necropsy at the Research Institute of Wildlife Ecology, University of Veterinary Medicine, Vienna, Austria, and during field studies on birds of prey conducted in Germany. Samples were maintained at approximately 4 °C during transport to the diagnostic unit of the Institute of Microbiology, University of Veterinary Medicine Vienna, Austria, and submitted for primary isolation of mycoplasmas. After arrival, samples were cultivated on modified Hayflick's agar (Tully, 1995) incubated at 37 °C under 5 % CO_2 atmosphere for up to 7 days. Once typical mycoplasma colonies were observed, single colonies were picked and transferred into modified Hayflick's medium (Tully, 1995) and incubated at 37 °C in ambient air for 3–4 days. Cultures were then identified to the species level using 16S rRNA gene sequencing (isolates before 2017) and/or MALDI-ToF MS (isolates from 2017 on) (Spergser et al., 2019), and unidentified *Mycoplasma* isolates were stored at −80 °C until further investigation. From a large group of unknown but related *Mycoplasma* isolates exhibiting closest relationship to *M. verecundum* (>80 isolates), 42 representative strains were selected based on host species and epidemiologic unrelatedness (e.g., isolates from different countries and years of isolation, one isolate per host) and subjected to comprehensive taxonomic analyses (Table 1). All strains were triple filter cloned (Tully, 1983) and cultivated in/on modified Hayflick's medium (Tully, 1995) to determine their culture characteristics (Spergser et al., 2020). The cell morphology of the proposed type strains (see below) was assessed by transmission electron microscopy as described previously (Suárez-Pérez et al., 2012). In addition, cells of proposed type strains were also tested for motility by applying a microcinematography motility assay (Indikova et al., 2014).

Phylogenetic analyses

DNA was extracted from cell pellets harvested by centrifugation of 1 ml broth cultures at 20,000 x g for 10 min using the DNeasy Blood & Tissue Kit (Qiagen). Then, 16S rRNA genes and 16S—23S intergenic spacer region (ISR) sequences of all 42 strains were amplified by PCR as previously described (Lane, 1991; Harasawa et al., 2004). Amplicons were purified using GenElute Purification Kit (Sigma) and Sanger sequenced in both directions at LGC Genomics, Berlin, Germany. Similarities of assembled 16S rRNA gene and ISR sequences were determined using EzBioCloud (Yoon et al., 2017) and blastn search to GenBank entries (Altschul et al., 1990), respectively. In addition, 16S rRNA gene sequences were aligned using ClustalW (Thompson et al., 1997) and a phylogenetic tree comprising closest relatives constructed in MEGA11 applying the maximum likelihood algorithm with Tamura-Nei model adjustment and 1000 bootstrap replicate analysis (Tamura et al., 2021). The relatedness of the 42 studied strains to recognized *Mycoplasma* species was further determined by PCR amplification of the partial *rpoB* gene (Volokhov et al., 2012). Since no amplicons could be generated employing the published primer sequences, new primers flanking the described *rpoB* fragment of *M. verecundum* 107^T were designed (rpoBv-f: 5'-TTGAAGGCCAATTATCAGTTGCTTT-3′, rpoBv-r: 5'-TGTAGCTCCATAAGATTCAATAGCTCA-3′) using Primer 3 plugin in Geneious Prime® 2024.02 (Biomatters Ltd.) and a PCR performed with the following cycling conditions: 95 °C for 5 min followed by 45 cycles of 95 °C for 1 min, 52 °C for 1 min and 68 °C for 2 min, and a final elongation step at 68 °C for 5 min. PCR products were purified and sequenced in both directions (LGC Genomics). After blastn search, partial *rpoB* sequences of investigated strains and closely related *Mycoplasma* species were aligned and similarity values calculated using BioEdit 7.2.5 (Hall, 1999), and a phylogenetic tree constructed in MEGA11 as described above for the 16S rRNA gene. Visualisation and annotation of both trees were performed using iTOL v6 (Letunic and Bork, 2024). In a follow-up study the partial *rpoB* genes of a further 31 strains (remaining strains recovered from the respiratory tract of 31 birds of the three genera *Aquila*, *Haliaeetus*, and *Milvus*) were sequenced and a phylogenetic tree including the 42 investigated strains was constructed as described above.

Phenotypic tests and MALDI-ToF mass spectrometry

Strains were examined for metabolism of glucose, for hydrolysis of arginine and urea (Aluotto et al., 1970), for alkaline phosphatase activity (Bradbury, 1983), for haemolysis (Gardella and Del Giudice, 1983), and for 'film and spots' production (Freundt, 1983).

MALDI-ToF MS of broth cultures of the 42 strains investigated and strains of related *Mycoplasma* species was performed as described before (Spergser et al., 2019). Main spectrum profiles (MSPs) were generated, compared and spectral concordance expressed by log score values determined. In addition, a score-oriented dendrogram based on arbitrary distance matrix was constructed, applying the correlation distance measure with the average linkage algorithm (Bruker Daltonics).

Genomic and phylogenomic analyses

For genome sequencing, the 42 strains as well as strains of their closest relatives, *M. verecundum* 107^T and *M. seminis* 2200 (isolated from the lung of a gyrfalcon in 2008) were grown in 25 ml modified Hayflick's medium for 5 days at 37 °C and ambient air. After centrifugation at 20,000 ×*g* for 10 min, DNA was extracted from the pellet using the DNeasy Blood and Tissue Kit (Qiagen), and the quantity and quality of extracted genomic DNA were assessed by fluorometry (Qubit™ 4 fluorometer, Thermo Scientific) and by employing an automated electrophoresis system (Tape Station 4150, Agilent). All strains were sequenced on an Illumina MiniSeq platform (2 × 150 bp, Illumina). Raw reads with low quality scores and adaptor contamination were trimmed using BBDuk plugin in Geneious Prime® 2024.02 (Biomatters Ltd.) and qualified reads were finally assembled into contigs using SPAdes 4.0.0. (Bankevich et al., 2012) with default parameters. Resulting draft genomes were annotated utilising the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP). In addition, the proposed type strains (1449^T, 654^T, $VS42A^T$, $Z331B^T$, $005V^T$), *M. verecundum* 107^T, and *M. seminis* 2200 were sequenced on a MinION device (Oxford Nanopore Technologies) as previously described (Spergser et al., 2022), and Nanopore long reads along with Illumina short reads hybrid assembled applying the Unicycler pipeline version 0.5.1 with default settings (Wick et al., 2017). Finally, completed genomes were annotated using PGAP.

For genome coherence analyses, average nucleotide identity based on the blast algorithm (ANIb) or MUMmer (ANIm) and tetranucleotide signature correlation index (TETRA) scores were calculated using the JSpeciesWS online software package (Richter et al., 2015). In addition, digital DNA-DNA hybridization (dDDH) values were determined by Genome-to-Genome Distance Calculator (GGDC, version 3.0) with distances inferred by applying the recommended formula 2 (identities/HSP [High Scoring segment Pairs] length) (Meier-Kolthoff et al., 2022). Proteome-wide comparison was performed by determining average amino acid identity (AAI) scores employing AAI calculator (Konstantinidis and Tiedje, 2005). Furthermore, a phylogenetic tree was constructed from the genomes of the 42 strains, *M. verecundum* 107^T, and *M. seminis* 2200 utilising the Codon Tree method at the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) which selects single-

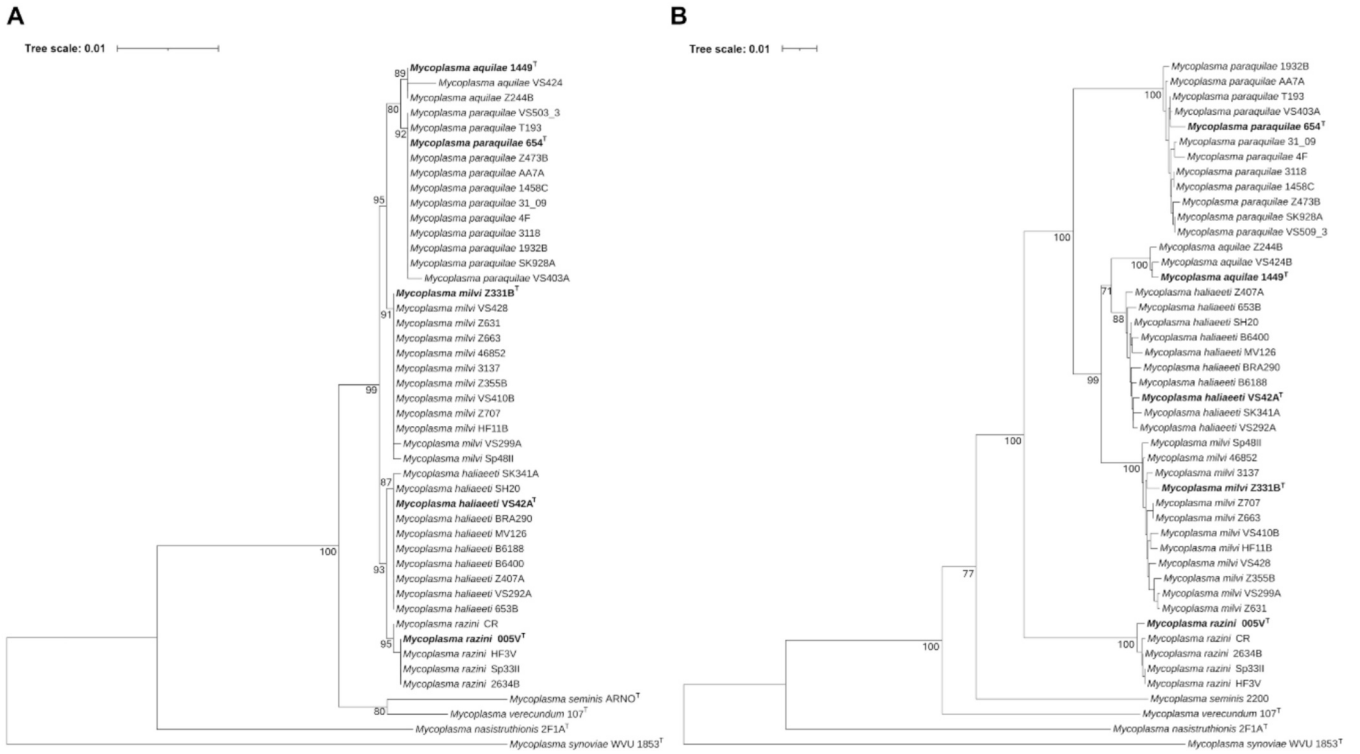

Fig. 1. Maximum likelihood tree illustrating the phylogenetic position of the 42 investigated strains and closely related strains of the *M. synoviae* cluster based on (A) 16S rRNA genes and (B) partial *rpoB* gene sequences. The sequence of *M. synoviae* WVU 1853^T was used as out-group organism. Numbers at nodes represent bootstrap confidence values (1000 replications). Only values ≥ 70 % are shown. Tree scale, number of nucleotide substitutions per site.

copy BV-BRC global protein families (PGFams) and analyses protein and coding DNA aligned by MUSCLE (Edgar, 2004) by Randomized Axelerated Maximum Likelihood (RAxML, version 8.2.11) and Fast Bootstrapping (Stamatakis, 2014). Visualisation and annotation of the tree were performed using iTOL v6 (Letunic and Bork, 2024).

Comparative genomics of closed genomes generated from proposed type strains (1449^T, 654^T, VS42A^T, Z331B^T, 005V^T) was performed by analysing whole-genome alignments using progressive Mauve (Darling et al., 2004) implemented in Geneious Prime® (Biomatters Ltd.). Functional genomic analysis was achieved by comparing genomic loci and proteins of interest to the non-redundant protein sequences database at NCBI using the blastp algorithm with default settings. Only hits with ≥ 60 % coverage and ≥ 40 % amino acid (aa) identity were considered significant.

Genome and 16S rRNA gene screening in metagenomic datasets

Metagenomes from bird-associated microbiomes, such as gut, cloacal, faecal, digestive tract, and intestinal microbiomes, were retrieved from the European Nucleotide Archive (ENA) public repository, resulting in a total of 110 whole-cell shotgun metagenomes and 1224 16S rRNA amplicon sequencing datasets. In addition, metagenomes were selected from the Sandpiper database (Woodcroft et al., 2024) by performing a taxonomy-based search targeting members of the family *Mycoplasmataceae* across all environmental sample types, selecting a total of 835 metagenomes.

The selected metagenomes were trimmed using the bbduk v38.82 tool (https://jgi.doe.gov) with the parameters ktrim = r, k = 28, mink = 12, hdist = 1, tbo = t, tpe = t, qtrim = rl, trimq = 20, and minlength = 100. Trimmed reads were mapped against the type strains genomes of the newly characterised *Mycoplasma* species using the blastn option of Magic-BLAST +version 2.12.0 tool with default settings (Boratyn et al., 2019). Reads that found a match higher than the 97 % sequence identity and an (alignment length) / (query read length) greater than 0.8 were retained for further analysis. The genome sequencing depth and breadth for each metagenome was calculated using a Python script from https://github.com/rotheconrad/00_in-situ_GeneCoverage (Conrad et al., 2022).

Moreover, the 16S rRNA sequences were compared against the SILVA database (Quast et al., 2013). Briefly, the sequences were imported into the non-redundant SILVA REF 138.2 database and aligned using the SINA v1.3.1 aligner (Pruesse et al., 2007), as implemented in the ARB program package v6.0.6 (Ludwig et al., 2004). The aligned sequences were inserted in the reference phylogenetic tree using the parsimony algorithm.

Results and discussion

Cultural and morphological characteristics, gliding motility

All strains were cultivated on modified Hayflick's agar at 37 °C under 5 % CO_2 atmosphere for up to 10 days. After 4 days of incubation, colonies with diameters from 400 to 800 µm were produced that exhibited the typical fried egg morphology and showed irregular margins (Fig. S1). Strains grew rapidly at 37 °C and slowly at 25, 30, 42, and 45 °C. No growth was observed at 15 °C. They grew also anaerobically at 30 and 37 °C using the GasPak™ anaerobic system (BD Diagnostics). Strains were filterable through membrane filters with pore sizes of 450 and 220 nm. The cell morphology of the proposed type strains (see below) was determined by transmission electron microscopy presenting cells devoid of a cell wall and bounded by a single cell membrane with a fuzz-like coating. Cells were mostly spherical or oval in shape (Fig. S1). No gliding mycoplasma cells were observed using a micro-cinematographic motility assay at various temperatures, viscosities, and serum sources.

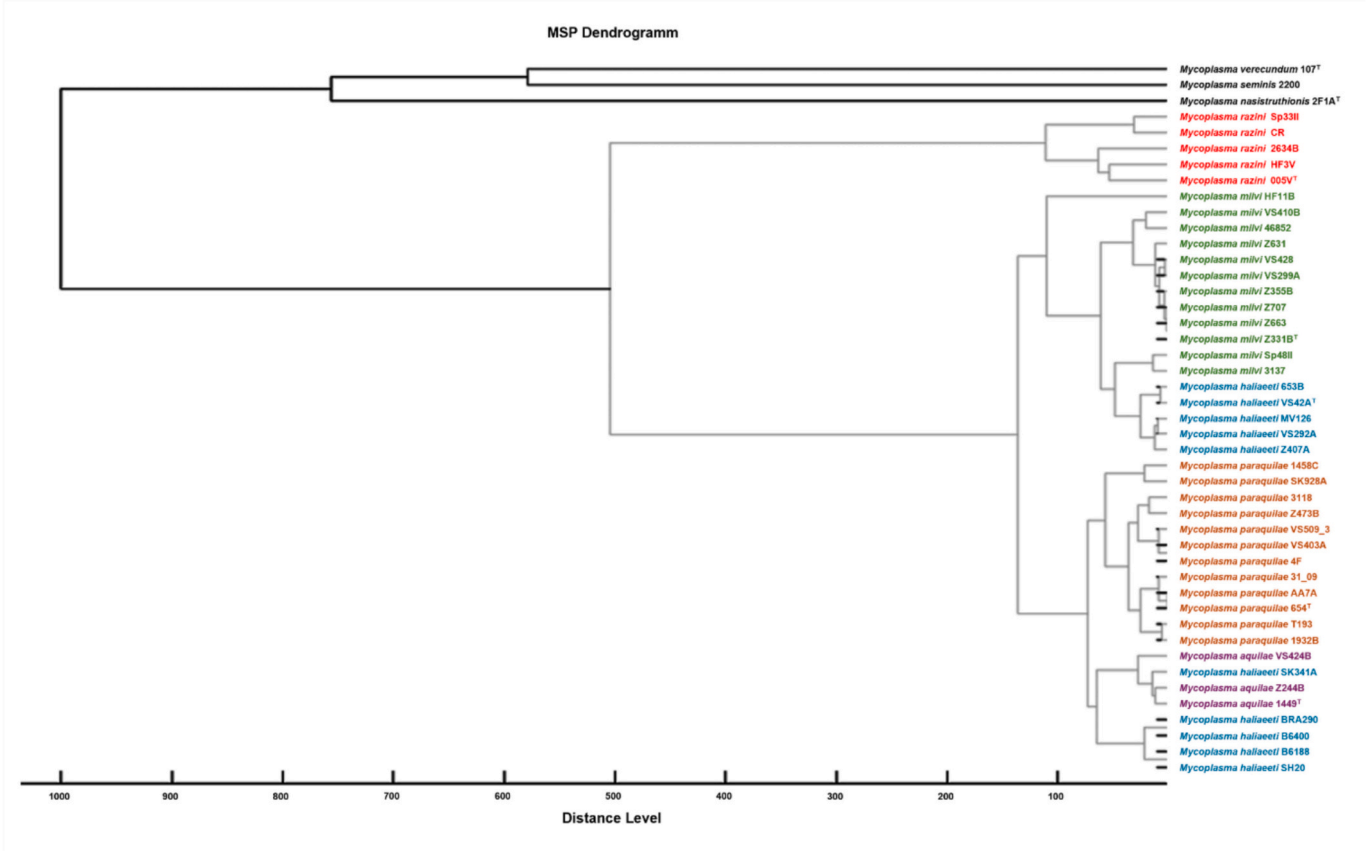

Fig. 2. MALDI-ToF MS score-oriented dendrogram based on distances between MSPs from the 42 strains investigated in this study (strain labels are coloured differently according to their phylogenetic relatedness) and their closest relatives *M. seminis* (strain 2200), *M. verecundum* (107^T), and *M. nasistruthionis* (2F1A^T).

Phylogenetic positioning

Analysis of 16S rRNA gene sequences of the 42 investigated strains (1436–1437 nt lengths) revealed that they belong to genus *Mycoplasma*, expressing highest sequence similarity values with *M. verecundum* 107^T (98.38–98.52 %), *M. seminis* ARNO^T (97.88–98.17 %), and *M. nasistruthionis* 2F1 A^T (95.21–95.57 %). Furthermore, the 16S rRNA gene sequences of the 42 strains differed only slightly (two to seven bases difference), exhibiting similarity values of 99.49–99.86 % among each other. However, in a phylogenetic tree constructed, the 42 strains were clearly separated into five clusters supported by high bootstrap values (Fig. 1A), indicating that the strain cohort may possibly be subdivided into five different species. Based on these preliminary results, five strains were selected and proposed as potential type strains, representing the five clusters formed by 16S rRNA gene sequence analysis, namely 1449^T, 654^T, VS42A^T, Z331B^T, and 005V^T.

The ISR sequences of the 42 strains showed highest similarity to *M. seminis* ARNO^T (90.77–95.38 %) and *M. verecundum* 107^T (86.81–90.87 %) considering full coverage of compared ISR sequences. The lengths of the ISR sequences ranged from 264 to 276 bp, and same as in 16S rRNA gene sequence analysis, the 42 strains were grouped into five clusters presenting identical ISR sequences and lengths for strains within a cluster, and similarity values between clusters ranging from 94.81 (1449^T vs. Z331B^T) to 99.26 (VS42A^T vs. Z331B^T) and 99.61 % (1449^T vs. 654^T), the latter values corresponding to two and one nucleotide differences between ISR sequences, respectively (Table S1).

Partial *rpoB* gene sequences of the 42 strains were of 1698 nt length and showed again highest similarity to *M. seminis* 2200 (89.95–90.83 %, MT731030) (the *rpoB* sequence of ARNO^T is not publicly available), *M. verecundum* 107^T (88.82–89.23 %, DQ219485), and *M. nasistruthionis* 2F1A^T (82.75–83.71 %, MF197783). As with 16S rRNA gene sequences, the phylogenetic tree constructed illustrates a clear separation of the 42 strains into five clusters (Fig. 1B). The partial *rpoB* sequences of strains within a cluster were highly similar, with similarity values ranging from 99.12 to 99.94 %, while the similarity of strains belonging to different clusters ranged from 92.17 to 98.06 % (Table S2). Although these values were above the generally proposed threshold of 90–91 % for species delineation in *Mycoplasmataceae* using *rpoB*, they were still below or similar to the threshold of 97–98 % suggested to differentiate members of the *M. mycoides* cluster, a phylogenetic group within genus *Mycoplasma* composed of closely related species that infect ruminants (Volokhov et al., 2012). To place the proposed clusters into a broader context, the partial *rpoB* genes of 31 additional strains (isolated from the respiratory tract of the three bird genera *Aquila, Haliaeetus,* and *Milvus*) were sequenced. Phylogenetic analysis revealed that these strains grouped consistently within four of the five defined clusters (Fig. S2, Table S3), and no evidence of an intermediate continuum between clusters was observed. Instead, the additional strains reinforced that the clusters represent stable ecological and host-associated lineages rather than transient subdivisions.

Altogether, sequence similarities of all three phylogenetic markers examined indicate that the investigated strains may represent five novel but closely related species, phylogenetically positioned within the *M. synoviae* cluster of the Hominis group of genus *Mycoplasma*.

Phenotypic characteristics and MALDI-ToF mass spectra

All 42 strains tested did not produce acid from glucose indicating that they may preferentially utilise other carbon sources for energy generation, which was confirmed by functional genomic analysis (see below). Further phenotypic tests revealed that the 42 strains were unable to hydrolyse arginine confirmed by the absence of genes

Table 2
Genomic features of proposed type strains 1449^T, 654^T, VS42A^T, Z331B^T, and 005V^T.

	1449^T	654^T	VS42A^T	Z331B^T	005V^T
Genome acc. no.	CP182208	CP182209	CP182210	CP182211	CP182212
Genome size (bp)	941,164	885,996	887,399	844,529	823,238
G + C (%)	29.7	29.2	30.2	29.9	29.2
Genes (total)	796	724	729	696	643
CDSs (total)	753	681	686	653	600
CDSs (with protein)	731	677	674	648	597
Genes (RNA)	43	43	43	43	43
rRNAs (complete) (5S, 16S, 23S)	3, 3, 3	3, 3, 3	3, 3, 3	3, 3, 3	3, 3, 3
tRNAs	31	31	31	31	31
ncRNAs	3	3	3	3	3
Pseudogenes	22	4	12	5	3
Transposases	33 (+17 pseudo)[a]	5[b]	11 (+7 pseudo)[c]	11[d]	–
MICE	ICEAq$_{1449}$	ICEPaq$_{654}$	ICEHA$_{VS42A}$	–	–
Prophages	–	MAV1-like	MAV1-like	MAgV1-like	–
CRISPR/Cas9	–	type II-A	–	type II-A	type II-A

[a] 12 IS1634- (10 of them pseudogenes), 13 IS256- (7 of them pseudogenes), nine IS30-, and two IS3-family transposases, 14 unclassified transposases.
[b] five IS30-family transposases.
[c] eight IS1634- (seven of them pseudogenes), two IS256-, two IS30-, and three IS3-family transposases, three unclassified transposases.
[d] six IS30- and three IS3-family transposases, two unclassified transposases.

constituting the arginine dihydrolase pathway in their genomes. In addition, strains were unable to hydrolyse urea but exhibited alkaline phosphatase activity. 'Film and spots' were not produced by colonies grown on modified Hayflick's agar for 7 days, but α-haemolysis was observed when strains were cultured on modified Hayflick's agar containing 5 % (*v*/v) sheep blood. Overall, no differentiating phenotypic characteristics could be identified that distinguish the tested strains from their relatives (*M. seminis, M. verecundum, M. nasistruthionis*) and according to their assignment to phylogenetic clusters.

MALDI-ToF MS analysis, on the other hand, not only allowed the 42 strains to be distinguished from *M. seminis*, *M. verecundum*, and *M. nasistruthionis* but also clearly separated the 005V^T strain group from the other strains (log score < 1.7). Although most of the remaining strains (1449^T, 654^T, and Z331B^T strain groups) were also grouped according to their phylogenetic relatedness (except for VS42A^T and its related strains that split into three groups), they could not be differentiated by MALDI-ToF MS but only identified as belonging to the same species due to their high spectral concordances, expressed by log score values >2.0 (Fig. 2).

Genomic features, genomic coherence and phylogenomics

Illumina short reads and MinION long reads generated from the proposed type strains 1449^T, 654^T, VS42A^T, Z331B^T, and 005V^T were hybrid assembled resulting in circular chromosomes exhibiting features typical for *Mycoplasma* genomes such as a small genome size and a low genomic G + C content (Table 2). In all proposed type strains, three operons of complete 5S, 16S, and 23S rRNA genes were present. Comparison of the three 16S rRNA gene copies in each genome revealed minor inter-operon polymorphisms reflecting variations in the Sanger consensus sequences of strains that clustered together in the 16S phylogenetic tree. Most noticeable, however, were the different genome sizes, ranging from 823,238 (005V^T) to 941,164 bp (1449^T), which were attributable to variations in the occurrence of mobile genetic elements (i.e., Mycoplasma Integrative and Conjugative Elements, prophages, Insertion Sequence (IS) transposases) as well as to variable numbers of IS transposases in their genomes.

For genome comparison, ANIb and ANIm values were calculated between each genome pair of the five proposed type strains (1449^T, 654^T, VS42A^T, Z331B^T, 005V^T), *M. seminis* 2200 and *M. verecundum* 107^T, all resulting in values below the proposed species delineation threshold of 95–96 % (Table 3) (Richter and Rosselló-Móra, 2009). However, ANIm values of two genome pairs were shown to fall within an intermediate zone of 93 to 96 % ANIm (1449^T vs. VS42^T: 93.74 %; VS42A^T vs. Z331B^T: 94.55 %), in which measures have been previously interpreted either as intra- or inter-species values (Rosselló-Móra and Amann, 2015). Therefore, ANIm was calculated between and within these strain groups, yielding values of > 98 % ANIm for strains within a group, distanced from the other groups by approximately 4 % ANIm, strongly indicating that i) strains within a group are members of the same species and ii) classification of the three strain groups as distinct taxa is justified (Table S4, S5). In addition, TETRA correlation coefficients were far below the proposed value of ≥ 0.999 for definite species circumscription (Table 3) (Richter and Rosselló-Móra, 2009), and below (≤ 0.991) the range of TETRA values (0.994–0.999) calculated for strains within strain groups shown to be most closely related according to ANIm calculations (VS42A^T vs. Z331B^T) (Table S6). Moreover, ANI and TETRA values were supported by dDDH estimates, all of which were far below the proposed 70 % threshold generally used for species definition (Table 3). In addition, two-way AAI values were below the 95 % threshold used for defining species (Nouioui and Sangal, 2022), demonstrating differences between the organisms at the genome-wide proteome level. In summary, genomic and proteomic relatedness indices (ANIb, ANIm, TETRA, dDDH, AAI) clearly demonstrate the distinctiveness of the proposed type strains 1449^T, 654^T, VS42A^T, Z331B^T, and 005V^T, thus representing five new taxa within the genus *Mycoplasma*.

A phylogenomic tree inferred from comparison of 271 single-copy coding genes (Table S7), resulting in alignment of 100,286 amino acids and 300,858 corresponding nucleotides, corroborates the results obtained from genome coherence studies as well as from phylogenetic analyses of 16S rRNA genes and partial *rpoB* sequences, reinforcing the classification of the 42 investigated strains as five distinct species at the genomic level (Fig. 3).

Functional and comparative genomics

Genome annotation of proposed type strains revealed that they lack genes encoding enzymes of the upper Embden-Meyerhof-Parnas (EMP) pathway (i.e., glucose-6-phosphate isomerase, 6-phosphofructokinase) and the oxidative branch of the pentose phosphate pathway (PPP). In addition, they do not seem to possess a complete phosphoenolpyruvate phosphotransferase system (PTS), as only the components A and B of the enzyme II complex were predicted in their genomes. In contrast, all strains were fully equipped with genes for glycerol-3-phosphate metabolism indicating that this metabolite of glycerol metabolism is preferably catabolized to pyruvate via the lower part of the EMP pathway. Moreover, pyruvate might also be produced via the non-oxidative

Table 3
Average nucleotide identity based on BLAST (ANIb) or MUMmer (ANIm), tetranucleotide signature correlation index (TETRA), digital DNA-DNA hybridization (dDDH), and average amino acid identity (AAI) between genomes of proposed type strains (1449^T, 654^T, VS42A^T, Z331B^T, 005V^T), and their closest relatives, *M. seminis* (2200) and *M. verecundum* (107^T).

ANIb in % [aligned nt in %]	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1449^T (1)	*	90.71 [75.87]	91.59 [75.98]	90.64 [69.50]	83.47 [63.73]	76.54 [53.96]	76.77 [52.47]
654^T (2)	90.86 [79.20]	*	86.89 [77.51]	86.37 [73.72]	83.03 [68.92]	76.84 [52.97]	77.27 [52.80]
VS42A^T (3)	91.85 [80.19]	86.79 [79.23]	*	93.04 [77.03]	83.27 [69.89]	76.80 [54.77]	76.87 [55.09]
Z331B^T (4)	90.97 [75.04]	86.38 [76.80]	93.04 [78.59]	*	83.10 [71.59]	77.04 [56.28]	77.20 [55.82]
005V^T (5)	83.09 [72.78]	82.97 [74.45]	83.29 [74.47]	82.80 [74.49]	*	77.53 [55.10]	77.42 [57.30]
M. seminis 2200 (6)	77.18 [45.22]	77.20 [43.38]	76.92 [44.86]	77.49 [43.38]	77.76 [41.23]	*	76.67 [44.33]
M. verecundum 107^T (7)	76.86 [51.24]	77.53 [48.71]	77.05 [50.37]	77.37 [49.35]	77.67 [48.35]	76.94 [51.09]	*
ANIm in % [aligned nt in %]	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1449^T (1)	*	92.19 [78.60]	93.74 [77.29]	92.69 [69.96]	87.82 [52.48]	85.69 [24.57]	85.47 [22.72]
654^T (2)	92.19 [82.41]	*	89.28 [75.27]	88.84 [70.78]	87.36 [57.37]	85.55 [24.51]	85.40 [24.02]
VS42A^T (3)	93.74 [81.37]	89.30 [75.24]	*	94.55 [77.96]	87.99 [57.63]	85.61 [27.40]	85.62 [23.81]
Z331B^T (4)	92.69 [77.54]	88.84 [74.18]	94.55 [81.39]	*	87.75 [59.13]	85.93 [26.22]	85.39 [26.83]
005V^T (5)	87.82 [60.12]	87.33 [62.12]	87.99 [62.11]	87.75 [61.03]	*	85.47 [29.69]	85.43 [28.00]
M. seminis 2200 (6)	85.69 [21.78]	85.55 [20.49]	85.61 [22.92]	85.93 [20.90]	85.47 [22.97]	*	85.16 [19.32]
M. verecundum 107^T (7)	85.47 [22.59]	85.40 [22.47]	85.62 [22.29]	85.39 [23.99]	85.43 [24.38]	85.16 [21.71]	*
TETRA	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1449^T (1)	*	0.983	0.986	0.988	0.971	0.880	0.904
654^T (2)	0.983	*	0.963	0.979	0.979	0.880	0.932
VS42A^T (3)	0.986	0.963	*	0.990	0.965	0.870	0.882
Z331B^T (4)	0.988	0.979	0.990	*	0.977	0.886	0.903
005V^T (5)	0.971	0.979	0.965	0.977	*	0.874	0.909
M. seminis 2200 (6)	0.880	0.880	0.870	0.886	0.874	*	0.907
M. verecundum 107^T (7)	0.904	0.932	0.882	0.903	0.909	0.907	*
dDDH in %	(1)	(2)	(3)	(4)	(5)	(6)	(7)
1449^T (1)	*	43.3	48.9	44.5	28.3	22.7	21.9
654^T (2)	43.3	*	34.3	32.3	27.8	22.3	22.2
VS42A^T (3)	48.9	34.3	*	53.9	28.3	22.2	21.7
Z331B^T (4)	44.5	32.3	53.9	*	27.8	22	21.8
005V^T (5)	28.3	27.8	28.3	27.8	*	22.1	21.7
M. seminis 2200 (6)	22.7	22.3	22.2	22	22.1	*	21.6
M. verecundum 107^T (7)	21.9	22.2	21.7	21.8	21.7	21.6	*
Two-way AAI in % [number of proteins]	(1)	(2)	(3)	(4)	(5)		
1449^T (1)	*	93.10 [645]	93.13 [657]	92.86 [612]	86.89 [587]		
654^T (2)	93.10 [645]	*	90.64 [659]	90.15 [616]	86.87 [596]		
VS42A^T (3)	93.13 [657]	90.64 [659]	*	94.51 [626]	86.72 [596]		
Z331B^T (4)	92.86 [612]	90.15 [616]	94.51 [626]	*	86.34 [597]		
005V^T (5)	86.89 [587]	86.87 [596]	86.72 [596]	86.34 [597]	*		

branch of PPP through conversion of xylulose-5-phosphate to glyceraldehyde-3-phosphate by a phosphoketolase, leading to entry into glycolysis. In addition, D-lactate dehydrogenase was present in all finished genomes, which is known to enable interconversion of lactate and pyruvate. Further downstream, however, energy is unlikely generated by the oxidation of pyruvate to acetyl-CoA, as most of the genes encoding the pyruvate dehydrogenase complex are missing. Furthermore, the reversible acetate kinase - phosphotransacetylase pathway was found to be incomplete suggesting that the ATP-generating conversion of acetate into acetylphosphate does not ultimately lead to the production of acetyl-CoA.

Comparative genomics also revealed the presence of various mobile genetic elements in the completed genomes, known to play a significant role in genomic plasticity. IS-family transposases were discernible in all completed genomes except the genome of 005V^T. They belonged to the IS1634-, IS256-, IS30- and IS3-family transposases and were most abundantly detected in 1449^T (n = 50 including 17 pseudogenes), frequently in VS42A^T (n = 18 including 7 pseudogenes) and Z331B^T (n = 11), and less commonly in 654^T (n = 5).

Furthermore, Mycoplasma Integrative and Conjugative Elements

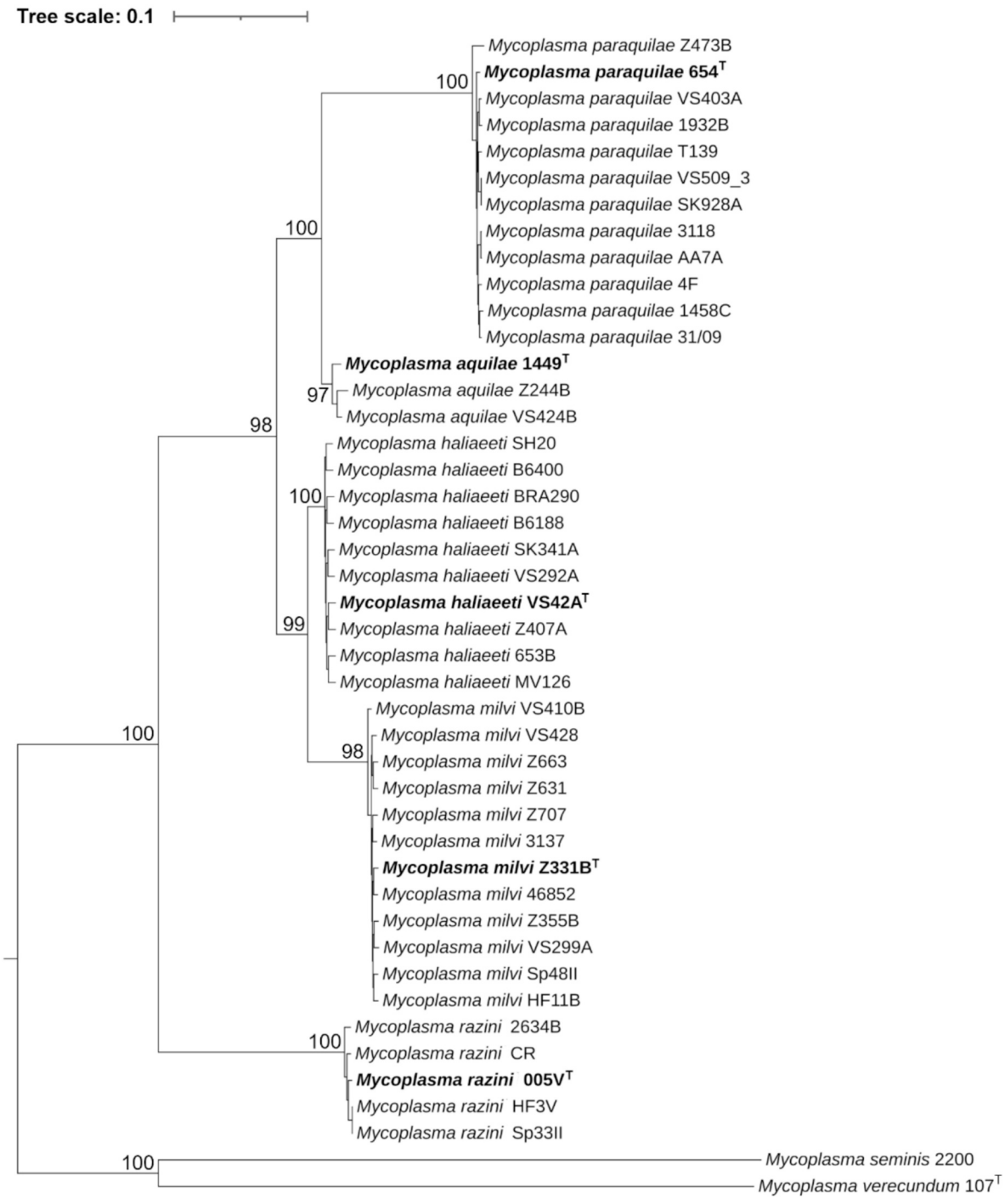

Fig. 3. Phylogenomic tree inferred from genome comparison of the 42 strains, *M. seminis* 2200 (CP132191) and *M. verecundum* 107^T (CP137850). The phylogenomic tree was constructed using RAxML within the codon tree pipeline at BV-BRC by analysing 271 single-copy coding sequences. Numbers at nodes represent confidence values of 100 rounds of fast bootstrapping. Tree scale, number of substitutions per site.

(MICEs) were present as single copies in the genomes of 1449^T, 654^T, and VS42A^T (designated ICEAq$_{1449}$, ICEPaq$_{654}$, and ICEHA$_{VS42A}$), respectively. These MICEs were of 31–33 kb lengths, composed of approximately 30 similarly orientated genes and shared a backbone of structural genes conserved across MICEs (Citti et al., 2018). Based on aa identity between backbone CDSs, the highly similar ICEAq$_{1449}$ and ICEPaq$_{654}$, and the related ICEHA$_{VS42A}$ (distinguishable from ICEAq$_{1449}$/ICEPaq$_{654}$ by the degree of aa identity between CDS3, CDS19, and CDS22) appeared to be unique as blastp search only rendered significant hits (53–83 % aa identity) to backbone CDSs of a MICE present in the genome of *M. seminis* strain 2200 (ICESe$_{2200}$) and low degrees of aa identities (< 40 %) with backbone CDS in documented MICEs (Fig. S3). Screening for MICEs in contig-level genomes disclosed the presence of ICEAq$_{1449}$/ICEPaq$_{654}$ in both strains related to 1449^T

and in few strains (4 out of 11) of the 654^T strain group. In contrast, ICEHA$_{VS42A}$ appeared to be restricted to VS42A^T and Z331B^T strain groups and was commonly present in strains related to VS42A^T (6 out of 9) and less frequent in the Z331B^T strain group (4 out of 11) (Table S8).

Further mobile genetic elements identified include a 15 kb putative prophage in both the 654^T and VS42A^T genomes, presenting a gene content and organisation structure comparable to those of the *M. arthritidis* MAV1 prophage group (Citti et al., 2020), yet exhibiting low overall aa identity (< 40 %) to MAV1 phage proteins, but significant hits (45–78 % aa identity) to a similar prophage detected in the genome of *M. seminis* strain 2200 (Fig. S4). In addition, a large (34 kb) prophage was identified in the genome of Z331B^T, displaying a gene content architecture similar to those of the *M. agalactiae* MAgV1 phage and MAgV1-like sequences of other mycoplasmas belonging to the Hominis phylogenetic group. Both the protein and gene arrangement showed highest similarities with the MAgV1-like prophage found in the canine *Mycoplasma* species *M. molare* (aa identity 45–58 % between core conserved phage CDS) and a MAgV1-like prophage detected in *M. seminis* strain 2200 (aa identities 49–79 %) (Fig. S5). Screening of draft genome datasets revealed MAV1- and MAgV1-like prophages in further 11 strains, suggesting a rather moderate frequency of viral infection in the strains investigated.

Complete type II-A CRISPR/Cas9 systems, which are known to provide defence against invading foreign DNA in mycoplasmas (Ipoutcha et al., 2019) were detected in the genomes of 654^T, Z331B^T and 005V^T. A further search for this defence mechanism in all strains included in this study revealed a frequent occurrence of the type II-A CRISPR/Cas9 system in strains related to 654^T, Z331B^T and 005V^T. In addition, the system was also detected in five of the nine strains related to VS42A^T whose genome appeared to lack a CRISPR/Cas system (Table S8). Interestingly, a phylogenetic tree inferred from Cas9 proteins of CRISPR-harbouring strains clearly separated Cas9 into four phylogroups, which was in accordance with the strains' phylogenetic positioning resulting from 16S rRNA gene, partial *rpoB* and phylogenomic analyses (Fig. S6). In contrast, no CRISPR/Cas systems were found in 1449^T and its two related strains. However, these strains were equipped with restriction-modification systems (type I and II) which appear to be widespread defence mechanisms against invading DNA in the strains studied (Table S8).

CDSs potentially related to virulence and pathogenicity were also found in the genomes of the proposed type strains including several proteins that are regularly identified as cytadhesins in other mycoplasmas (e.g., P60, P68, P80, OppA), a pair of the *Mycoplasma* Ig binding (MIB)/*Mycoplasma* Ig protease (MIP) immune escape system (Nottelet et al., 2021) interrupted by a ATP synthase gene cluster, and a panel of orphan MIP-family Ig-specific serine endopeptidases, suggesting that the strains investigated are adapted to host-associated lifestyles and may possess traits that, under certain circumstances, could contribute to opportunistic behaviour.

Screening metagenomic datasets

To study the geographic and ecological distribution of the newly characterised *Mycoplasma* species, a screening of publicly available shotgun metagenomic and 16S rRNA amplicon sequencing datasets, focusing on samples derived from bird-associated microbiomes, as well as environmental datasets in which members of the *Mycoplasmataceae* family were previously detected (e.g., predicted in Sandpiper tool) was performed (Table S9). Despite employing both metagenomic read recruitment to genome sequences and alignment against 16S rRNA gene markers, we were unable to detect signatures of the new species in any of the datasets analysed. This suggests that these taxa may belong to the rare biosphere (Pedrós-Alió, 2006), comprising microbial species that persist at extremely low abundance or in highly specific ecological niches.

Table 4
Description of *Mycoplasma aquilae* sp. nov.

Genus name	*Mycoplasma*
Species name	*Mycoplasma aquilae*
Species epithet	*aquilae*
Species status	sp. nov.
Species etymology	a.qui'lae. N.L. gen. Fem. n. *aquilae*, of an eagle of genus *Aquila*
Description of the new taxon and diagnostic traits	Cells lack a cell wall and are mostly spherical or oval-shaped. Typical fried egg colonies produced within 3–4 days after incubation at 37 °C under 5 % CO_2 atmosphere. Growth is observed at 25–45 °C with an optimum growth temperature of 37 °C. Non-motile. Chemo-organotroph. It does not hydrolyse arginine or urea. No acid is produced from sugar carbon sources. It shows alkaline phosphatase activity. α-haemolysis is observed on agar with 5 % sheep blood. MALDI-ToF mass spectra distinct from those of other species except from those of three closely related species. 16S rRNA gene and 16S—23S intergenic spacer sequences are ambiguously distinct from closely related species. Partial *rpoB* gene sequences are unique and allow differentiation from closely related species. Genome similarity metrics and phylogenomic analysis demonstrate distinctness at the species level.
Country of origin	Spain
Region of origin	Castilla – La Mancha
Date of isolation	30/06/2002
Source of isolation	Lung of a Spanish imperial eagle (*Aquila adalberti*) nestling
Sampling date	15/06/2002
Latitude	39°36′54"N
Longitude	4°30′00"E
Altitude (meters above sea level)	843
16S rRNA gene accession no.	FM196532
Genome accession number	CP182208
Genome status	Complete
Genome size (bp)	941,164
G + C (%)	29.7
Number of strains in study	3
Source of isolation of non-type strains	Z244B – trachea of an Eastern imperial eagle (*Aquila heliaca*), Austria (2021); VS424B – trachea of an Eastern imperial eagle (*Aquila heliaca*), Austria (2023)
Designation of the type strain	1449^T
Strain collection numbers	ATCC BAA-1896^T, DSM 22458^T
Description of *Mycoplasma paraquilae* sp. nov.	
Genus name	*Mycoplasma*
Species name	*Mycoplasma paraquilae*
Species epithet	*paraquilae*
Species status	sp. nov.
Species etymology	pa.ra.qui'lae. Gr. prep. *Para*, like; N.L. gen. Fem. n. *aquilae*, of an eagle of genus *Aquila* and specific epithet of *Mycoplasma aquilae*; N.L. gen. Fem. n. *paraquilae*, like *Mycoplasma aquilae*
Description of the new taxon and diagnostic traits	Cells lack a cell wall and are mostly spherical or oval-shaped. Typical fried egg colonies produced within 3–4 days after incubation at 37 °C under 5 % CO_2 atmosphere. Growth is observed at 25–45 °C with an optimum growth temperature of 37 °C. Non-motile. Chemo-organotroph. It does not hydrolyse arginine or urea. No acid is produced from sugar carbon sources. It shows alkaline phosphatase activity. α-haemolysis is observed on agar with 5 % sheep blood. MALDI-ToF mass spectra distinct from those of other species except from those of three closely related species. 16S rRNA gene and 16S—23S intergenic spacer sequences are ambiguously distinct from closely related species. Partial *rpoB* gene sequences are unique and allow differentiation from closely related species. Genome similarity metrics and phylogenomic analysis clearly demonstrate distinctness at the species level.

(*continued on next page*)

Table 4 (*continued*)

Genus name	*Mycoplasma*
Country of origin	Austria
Region of origin	Vienna
Date of isolation	06/02/2008
Source of isolation	Lung of an Eastern imperial eagle (*Aquila heliaca*)
Sampling date	02/02/2008
Latitude	48°12′36″N
Longitude	16°21′48″E
Altitude (meters above sea level)	367
16S rRNA gene accession no.	PP693540
Genome accession number	CP182209
Genome status	Complete
Genome size (bp)	885,996
G + C (%)	29.2
Number of strains in study	12
Source of isolation of non-type strains	1458C – lung of a Spanish imperial eagle (*Aquila adalberti*), Spain (2002); 3118 – trachea of a Spanish imperial eagle (*Aquila adalberti*), Spain (2003); 31_09 – choana of a Spanish imperial eagle (*Aquila adalberti*), Spain (2006); 4F – choana of a Spanish imperial eagle (*Aquila adalberti*), Spain (2006); AA7A – oesophagus of a Spanish imperial eagle (*Aquila adalberti*), Spain (2007); T193 – trachea of a hooded vulture (*Necrosyrtes monachus*), Spain (2007); Z473B – trachea of a golden eagle (*Aquila chrysaetos*), Austria (2019); VS403A – trachea of an Eastern imperial eagle (*Aquila heliaca*), Austria (2020); VS509_3 – trachea of an Eastern imperial eagle (*Aquila heliaca*), Austria (2021); 1932B – mouth of an Eastern imperial eagle (*Aquila heliaca*), Austria (2021); SK928A – trachea of an Eastern imperial eagle (*Aquila heliaca*), Austria (2022)
Designation of the type strain	654^T
Strain collection numbers	DSM 113738^T, NCTC 14855^T
Description of *Mycoplasma haliaeeti* sp. nov.	
Genus name	*Mycoplasma*
Species name	*Mycoplasma haliaeeti*
Species epithet	*haliaeeti*
Species status	sp. nov.
Species etymology	ha.li.ae'eti. N.L. gen. Masc. n. *haliaeeti*, of a sea eagle of genus *Haliaeetus*
Description of the new taxon and diagnostic traits	Cells lack a cell wall and are mostly spherical or oval-shaped. Typical fried egg colonies produced within 3–4 days after incubation at 37 °C under 5 % CO_2 atmosphere. Growth is observed at 25–45 °C with an optimum growth temperature of 37 °C. Non-motile. Chemo-organotroph. It does not hydrolyse arginine or urea. No acid is produced from sugar carbon sources. It shows alkaline phosphatase activity. α-haemolysis is observed on agar with 5 % sheep blood. MALDI-ToF mass spectra distinct from those of other species except from those of three closely related species. 16S rRNA gene and 16S—23S intergenic spacer sequences are ambiguously distinct from closely related species. Partial *rpoB* gene sequences are unique and allow differentiation from closely related species. Genome similarity metrics and phylogenomic analysis demonstrate distinctness at the species level.
Country of origin	Austria
Region of origin	Vienna
Date of isolation	14/04/2020
Source of isolation	Trachea of a white-tailed eagle (*Haliaeetus albicilla*)
Sampling date	04/04/2020
Latitude	48°12′36″N
Longitude	16°21′48″E
Altitude (meters above sea level)	367
16S rRNA gene accession no.	PP693552
Genome accession number	CP182210
Genome status	Complete
Genome size (bp)	887,399
G + C (%)	30.2
Number of strains in study	10
Source of isolation of non-type strains	SH20 – choana of a white-tailed eagle (*Haliaeetus albicilla*), Germany (2009); B6188 – choana of a white-tailed eagle (*Haliaeetus albicilla*), Germany (2009); B6400 – choana of a white-tailed eagle (*Haliaeetus albicilla*), Germany (2009); BRA290 – choana of a white-tailed eagle (*Haliaeetus albicilla*), Germany (2009); MV126 – choana of a white-tailed eagle (*Haliaeetus albicilla*), Germany (2009); 653B – lung of a white-tailed eagle (*Haliaeetus albicilla*), Austria (2019); Z407A – trachea of a white-tailed eagle (*Haliaeetus albicilla*), Austria (2020); SK341A – trachea of a white-tailed eagle (*Haliaeetus albicilla*), Austria (2023); VS292A – trachea of a white-tailed eagle (*Haliaeetus albicilla*), Austria (2023)
Designation of the type strain	VS42A^T
Strain collection numbers	DSM 113741^T, NCTC 14856^T
Description of *Mycoplasma milvi* sp. nov.	
Genus name	*Mycoplasma*
Species name	*Mycoplasma milvi*
Species epithet	*milvi*
Species status	sp. nov.
Species etymology	mil.vi. N.L. gen. Masc. n. *milvi*, of a kite of genus *Milvus*
Description of the new taxon and diagnostic traits	Cells lack a cell wall and are mostly spherical or oval-shaped. Typical fried egg colonies produced within 3–4 days after incubation at 37 °C under 5 % CO_2 atmosphere. Growth is observed at 25–45 °C with an optimum growth temperature of 37 °C. Non-motile. Chemo-organotroph. It does not hydrolyse arginine or urea. No acid is produced from sugar carbon sources. It shows alkaline phosphatase activity. α-haemolysis is observed on agar with 5 % sheep blood. MALDI-ToF mass spectra distinct from those of other species except from those of three closely related species. 16S rRNA gene and 16S—23S intergenic spacer sequences are ambiguously distinct from closely related species. Partial *rpoB* gene sequences are unique and allow differentiation from closely related species. Genome similarity metrics and phylogenomic analysis demonstrate distinctness at the species level.
Country of origin	Austria
Region of origin	Vienna
Date of isolation	28/08/2020
Source of isolation	Trachea of a red kite (*Milvus milvus*)
Sampling date	20/08/2020
Latitude	48°12′36″N
Longitude	16°21′48″E
Altitude (meters above sea level)	367
16S rRNA gene accession no.	PP693561
Genome accession number	CP182211
Genome status	Complete
Genome size (bp)	844,529
G + C (%)	29.9
Number of strains in study	12
Source of isolation of non-type strains	HF11B – trachea of a Bonelli's eagle (*Aquila fasciata*), Spain (2005); Sp48II – trachea of a Bonelli's eagle (*Aquila fasciata*), Spain (2006); 46,852 – choana of a common buzzard (*Buteo buteo*), Germany (2009); VS428 – trachea of a red kite (*Milvus milvus*), Austria (2020); Z631 – trachea of a red kite (*Milvus milvus*), Austria (2020); Z663 – trachea of a red kite (*Milvus milvus*), Austria (2020); 3137 – lung of a red kite (*Milvus milvus*), Austria (2021); VS410B – trachea of an Eurasian goshawk (*Accipiter gentilis*), Austria (2022); Z355B – trachea of a red kite (*Milvus milvus*), Austria (2022); VS299A – trachea of a red kite (*Milvus milvus*), Austria (2023); Z707 – trachea of a red kite (*Milvus milvus*), Austria (2023)
Designation of the type strain	Z331B^T
Strain collection numbers	DSM 113740^T, NCTC 14858^T
Description of *Mycoplasma razini* sp. nov.	

(*continued on next page*)

Table 4 (*continued*)

Genus name	*Mycoplasma*
Genus name	*Mycoplasma*
Species name	*Mycoplasma razini*
Species epithet	*razini*
Species status	sp. nov.
Species etymology	ra.zin'i. N.L. gen. Masc. n. *razini*, of Razin, named after Prof. Shmuel Razin (1929–2021) in honour of his pioneering and groundbreaking contributions to mycoplasma research
Description of the new taxon and diagnostic traits	Cells lack a cell wall and are mostly spherical or oval-shaped. Typical fried egg colonies produced within 3–4 days after incubation at 37 °C under 5 % CO_2 atmosphere. Growth is observed at 25–45 °C with an optimum growth temperature of 37 °C. Non-motile. Chemo-organotroph. It does not hydrolyse arginine or urea. No acid is produced from sugar carbon sources. It shows alkaline phosphatase activity. α-haemolysis is observed on agar with 5 % sheep blood. MALDI-ToF mass spectra distinct allowing differentiation from other species. 16S rRNA gene and 16S—23S intergenic spacer sequences are ambiguously distinct from closely related species. Partial *rpoB* gene sequences are unique and allow unequivocally differentiation from closely related species. Genome similarity metrics and phylogenomic analysis demonstrate distinctness at the species level.
Country of origin	Spain
Region of origin	Castilla – La Mancha
Date of isolation	30/09/2005
Source of isolation	Trachea of a booted eagle (*Hieraaetus pennatus*)
Sampling date	16/09/2005
Latitude	38°59′43"N
Longitude	3°55′36"W
Altitude (meters above sea level)	628
16S rRNA gene accession no.	MK615070
Genome accession number	CP182212
Genome status	Complete
Genome size (bp)	823,238
G + C (%)	29.2
Number of strains in study	5
Source of isolation of non-type strains	2634B – choana of a cinereous vulture (*Aegypius monachus*), Spain (2006); CR – trachea of a short-toed snake eagle (*Circaetus gallicus*), Spain (2006); HF3V – choana of a Bonelli's eagle (*Aquila fasciata*), Spain (2006); Sp33II – choana of a Bonelli's eagle (*Aquila fasciata*), Spain (2006)
Designation of the type strain	$005V^T$
Strain collection numbers	(DSM 113739^T = NCTC 14838^T),

Conclusion

In conclusion, the overall results obtained from comprehensive characterisation of the 42 strains studied provide solid evidence that these strains represent five hitherto unclassified species within genus *Mycoplasma*. Four strain groups represented by 1449^T, 654^T, $VS42A^T$, and $Z331B^T$ form a complex of closely related genospecies for which the names *Mycoplasma aquilae* sp. nov., *Mycoplasma paraquilae* sp. nov., *Mycoplasma haliaeeti* sp. nov., and *Mycoplasma milvi* sp. nov. are proposed, corresponding to their preference for colonising a particular avian host genus. Although phenotypically indistinguishable, we propose recognizing them as individual species within a newly defined *Mycoplasma aquilae* complex, emphasizing both the evolutionary independence and host adaptation of its members, while acknowledging their phenotypic similarity. For diagnostic purposes, a two-step workflow to differentiate species within the newly defined *Mycoplasma aquilae* complex is recommended. Initial screening by MALDI-ToF MS provides rapid complex-level assignment, while subsequent sequencing of the partial *rpoB* gene allows reliable discrimination of individual species within the *Mycoplasma aquilae* complex. For the remaining strain group (represented by $005V^T$), which is phenotypically and genetically clearly distinguishable from the species of the *Mycoplasma aquilae* complex, the name *Mycoplasma razini* sp. nov. is proposed, named after Prof. Shmuel Razin (1929–2021) in honour of his pioneering and groundbreaking contributions to mycoplasma research (Table 4).

CRediT authorship contribution statement

Joachim Spergser: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sarah Kugler:** Writing – review & editing, Investigation. **Anna Kübber-Heiss:** Writing – review & editing, Supervision, Resources. **Ursula Höfle:** Writing – review & editing, Resources. **Nora Dinhopl:** Writing – review & editing, Investigation. **Michael P. Szostak:** Writing – review & editing, Investigation. **Igor Loncaric:** Writing – review & editing, Resources. **Tomeu Viver:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Ana S. Ramírez:** Writing – review & editing, Resources, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.syapm.2025.126663.

Data availability

Data will be made available on request.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Aluotto, B.B., Wittler, R.G., Williams, C.O., Faber, J.E., 1970. Standardized bacteriologic techniques for the characterization of *Mycoplasma* species. Int. J. Syst. Bacteriol. 20, 35–58. https://doi.org/10.1099/00207713-201-35.

Balish, M.F., Chopra-Dewasthaly, R., Pereyre, S., Ramírez, A.S., Viver, T., Spergser, J., 2024. 2024. Mycoplasma. in: Bergey's Manual of Systematics of Archaea and Bacteria; in Association with Bergey's Manual Trust, Bergey's. John Wiley & Sons, Inc., Hoboken, NJ, USA https://doi.org/10.1002/9781118960608.gbm01263.pub3.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Boratyn, G.M., Thierry-Mieg, J., Thierry-Mieg, D., Busby, B., Madden, T.L., 2019. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. BMC Bioinform. 20, 1–19. https://doi.org/10.1186/s12859-019-2996-x.

Bradbury, J.M., 1983. Phosphatase activity. In: Razin, S., Tully, J.G. (Eds.), Methods in Mycoplasmology, vol. 1. Academic Press, New York, pp. 363–366.
Bradbury, J.M., 2005. Poultry mycoplasmas: sophisticated pathogens in simple guise. Br. Poult. Sci. 46, 125–136. https://doi.org/10.1080/00071660500066282.
Brown, D.R., Whitcomb, R.F., Bradbury, J.M., 2007. Revised minimal standards for description of new species of the class *Mollicutes* (division *Tenericutes*). Int. J. Syst. Evol. Microbiol. 57, 2703–2719. https://doi.org/10.1099/ijs.0.64722-0.
Citti, C., Dordet-Frisoni, E., Nouvel, L.X., Kuo, C.H., Baranowski, E., 2018. Horizontal gene transfers in mycoplasmas (mollicutes). Curr. Issues Mol. Biol. 29, 3–22. https://doi.org/10.21775/cimb.029.003.
Citti, C., Baranowski, E., Dordet-Frisoni, E., Faucher, M., Nouvel, L.X., 2020. Genomic islands in mycoplasmas. Genes (Basel) 11, 836. https://doi.org/10.3390/genes11080836.
Conrad, R.E., Viver, T., Gago, J.F., Hatt, J.K., Venter, S.N., Rossello-Mora, R., Konstantinidis, K.T., 2022. Toward quantifying the adaptive role of bacterial pangenomes during environmental perturbations. ISME J. 16, 1222–1234. https://doi.org/10.1038/s41396-021-01149-9.
Darling, A.C., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 14, 1394–1403. https://doi.org/10.1101/gr.2289704.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.
Firrao, G., Brown, D.R., 2013. International committee on systematics of prokaryotes subcommittee on the taxonomy of *Mollicutes*. Minutes of the meetings, July 15th and 19th 2012, Toulouse. France. Int. J. Syst. Evol. Microbiol. 63, 2361–2364. https://doi.org/10.1099/ijs.0.052886-0.
Fischer, L., Möller Palau-Ribes, F., Enderlein, D., Fischer, D., Herbst, W., Baudler, L., Hafez, H.M., Lierz, M., 2020. Description, occurrence and significance of *Mycoplasma seminis* sp. nov. isolated from semen of a gyrfalcon (*Falco rusticolus*). Vet. Microbiol. 247, 108789. https://doi.org/10.1016/j.vetmic.2020.108789.
Freundt, E.A., 1983. Film and spot production. In: Razin, S., Tully, J.G. (Eds.), Methods in Mycoplasmology, vol. 1. Academic Press, New York, pp. 373–374.
Gardella, R.S., Del Giudice, R.A., 1983. Haemagglutination, haemadsorption and haemolysis. In: Razin, S., Tully, J.G. (Eds.), Methods in Mycoplasmology, vol. 1. Academic Press, New York, pp. 379–384.
Grózner, D., Sulyok, K.M., Kreizinger, Z., Rónai, Z., Jánosi, S., Turcsányi, I., Károlyi, H.F., Kovács, Á.B., Kiss, M.J., Volokhov, D., Gyuranecz, M., 2019. Detection of *Mycoplasma anatis, M. anseris, M. cloacale* and *Mycoplasma* sp. 1220 in waterfowl using species-specific PCR assays. PLoS One 14, e0219071. https://doi.org/10.1371/journal.pone.0219071.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Harasawa, R., Pitcher, D.G., Ramírez, A.S., Bradbury, J.M., 2004. A putative transposase gene in the 16S–23S rRNA intergenic spacer region of *Mycoplasma imitans*. Microbiology 150, 1023–1029. https://doi.org/10.1099/mic.0.26629-0.
Indikova, I., Vronka, M., Szostak, M.P., 2014. First identification of proteins involved in motility of *Mycoplasma gallisepticum*. Vet. Res. 45, 99. https://doi.org/10.1186/s13567-014-0099-2.
Ipoutcha, T., Tsarmpopoulos, I., Talenton, V., Gaspin, C., Moisan, A., Walker, C.A., Brownlie, J., Blanchard, A., Thebault, P., Sirand-Pugnet, P., 2019. Multiple origins and specific evolution of CRISPR/Cas9 systems in minimal bacteria (*Mollicutes*). Front. Microbiol. 10, 2701. https://doi.org/10.3389/fmicb.2019.02701.
Konstantinidis, K.T., Tiedje, J.M., 2005. Towards a genome-based taxonomy for prokaryotes. J. Bacteriol. 187, 6258–6264. https://doi.org/10.1128/JB.187.18.6258-6264.2005.
Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. Wiley, Chichester, pp. 115–175.
Lecis, R., Chessa, B., Cacciotto, C., Addis, M.F., Coradduzza, E., Berlinguer, F., Muzzeddu, M., Lierz, M., Carcangiu, L., Pittau, M., Alberti, A., 2010. Identification and characterization of novel *Mycoplasma* spp. belonging to the hominis group from griffon vultures. Res. Vet. Sci. 89, 58–64. https://doi.org/10.1016/j.rvsc.2009.12.016.
Lecis, R., Secci, F., Mandas, L., Muzzeddu, M., Pittau, M., Alberti, A., 2016. Molecular identification and sequence characterization of mycoplasmas in free-living birds of prey. J. Zoo Wildl. Med. 47, 917–922. https://doi.org/10.1638/2015-0259.1.
Letunic, I., Bork, P., 2024. Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. 52, W78–W82. https://doi.org/10.1093/nar/gkae268.
Lierz, M., Hafez, H.M., 2008. Occurrence of mycoplasmas in semen samples of birds of prey. Avian Pathol. 37, 495–497. https://doi.org/10.1080/03079450802356961.
Lierz, M., Hagen, N., Harcourt-Brown, N., Hernandez-Divers, S.J., Lüschow, D., Hafez, H.M., 2007a. Prevalence of mycoplasmas in eggs from birds of prey using culture and a genus-specific mycoplasma polymerase chain reaction. Avian Pathol. 36, 145–150. https://doi.org/10.1080/03079450701213347.
Lierz, M., Stark, R., Brokat, S., Hafez, H.M., 2007b. Pathogenicity of *Mycoplasma lipofaciens* strain ML64, isolated from an egg of a northern goshawk (*Accipiter gentilis*), for chicken embryos. Avian Pathol. 36, 151–153. https://doi.org/10.1080/03079450701213354.
Lierz, M., Hagen, N., Lueschow, D., Hafez, H.M., 2008a. Species-specific polymerase chain reactions for the detection of *Mycoplasma buteonis*, *Mycoplasma falconis*, *Mycoplasma gypis*, and *Mycoplasma corogypsi* in captive birds of prey. Avian Dis. 52, 94–99. https://doi.org/10.1637/8094-082107-Reg.
Lierz, M., Obon, E., Schink, B., Carbonell, F., Hafez, H.M., 2008b. The role of mycoplasmas in a conservation project of the lesser kestrel (*Falco naumanni*). Avian Dis. 52, 641–645. https://doi.org/10.1637/8378-061608-Reg.1.
Lierz, M., Hagen, N., Hernadez-Divers, S.J., Hafez, H.M., 2008c. Occurrence of mycoplasmas in free-ranging birds of prey in Germany. J. Wildl. Dis. 44, 845–850. https://doi.org/10.7589/0090-3558-44.4.845.
Loria, G.R., Ferrantelli, E., Giardina, G., Li Vecchi, L., Sparacino, L., Oliveri, F., McAuliffe, L., Nicholas, R.A., 2008. Isolation and characterization of unusual *Mycoplasma* spp. from captive Eurasian griffon (*Gyps fulvus*) in Sicily. J. Wildl. Dis. 44, 159–163. https://doi.org/10.7589/0090-3558-44.1.159.
Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A.W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüssmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., Schleifer, K.H., 2004. ARB: a software environment for sequence data. Nucleic Acids Res. 32, 1363–1371. https://doi.org/10.1093/nar/gkh293.
May, M., Brown, D.R., 2019. International committee on systematics of prokaryotes subcommittee on the taxonomy of *Mollicutes*: minutes of the closed meeting, 8 July 2018, Portsmouth, New Hampshire. USA. Int. J. Syst. Evol. Microbiol. 69, 2169–2171. https://doi.org/10.1099/ijsem.0.003342.
Meier-Kolthoff, J.P., Sardà Carbasse, J., Peinado-Olarte, R.L., Göker, M., 2022. TYGS and LPSN: a database tandem for fast and reliable genome-base classification and nomenclature of prokaryotes. Nucleic Acids Res. 50, D801–D807. https://doi.org/10.1093/nar/gkab902.
Nottelet, P., Bataille, L., Gourgues, G., Anger, R., Lartigue, C., Sirand-Pugnet, P., Marza, E., Fronzes, R., Arfi, Y., 2021. The mycoplasma surface proteins MIB and MIP promote the dissociation of the antibody-antigen interaction. Sci. Adv. 7, eabf2403. https://doi.org/10.1126/sciadv.abf2403.
Nouioui, I., Sangal, V., 2022. Advanced prokaryotic systematics: the modern face of an ancient science. New Microbes New Infect. 49-50, 101036. https://doi.org/10.1016/j.nmni.2022.101036.
Oaks, J.L., Donahoe, S.L., Rurangirwa, F.R., Rideout, B.A., Gilbert, M., Virani, M.Z., 2004. Identification of a novel mycoplasma species from an oriental white-backed vulture (*Gyps bengalensis*). J. Clin. Microbiol. 42, 5909–5912. https://doi.org/10.1128/JCM.42.12.5909-5912.2004.
Panangala, V.S., Stringfellow, J.S., Dybvig, K., Woodard, A., Sun, F., Rose, D.L., Gresham, M.M., 1993. Mycoplasma corogypsi sp. nov., a new species from the footpad abscess of a black vulture *Coragyps atratus*. Int. J. Syst. Bacteriol. 43, 585–590. https://doi.org/10.1099/00207713-43-3-585.
Pedrós-Alió, C., 2006. Marine microbial diversity: can it be determined? Trends Microbiol. 14, 257–263. https://doi.org/10.1016/j.tim.2006.04.007.
Poveda, J.B., Carranza, J., Miranda, A., Garrido, A., Hermoso, M., Fernandez, A., Domenech, J., 1990. An epizootiological study of avian mycoplasmas in southern Spain. Avian Pathol. 19, 627–633. https://doi.org/10.1080/03079459008418718.
Poveda, J.B., Giebel, J., Flossdorf, J., Meier, J., Kirchhoff, H., 1994. *Mycoplasma buteonis* sp. nov., *Mycoplasma falconis* sp. nov., and *Mycoplasma gypis* sp. nov., three species from birds of prey. Int. J. Syst. Bacteriol. 44, 94–98. https://doi.org/10.1099/00207713-44-1-94.
Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Razin, S., Yogev, D., Naot, Y., 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. 62, 1094–1156. https://doi.org/10.1128/MMBR.62.4.1094-1156.1998.
Richter, M., Rosselló-Móra, R., 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. USA 106, 19126–19131. https://doi.org/10.1073/pnas.0906412106.
Richter, M., Rosselló-Móra, R., Glöckner, F.O., Peplies, J., 2015. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32, 929–931. https://doi.org/10.1093/bioinformatics/btv681.
Rosselló-Móra, R., Amann, R., 2015. Past and future species definitions for *Bacteria* and *Archaea*. Syst. Appl. Microbiol. 38, 209–216. https://doi.org/10.1016/j.syapm.2015.02.001.
Ruder, M.G., Feldman, S.H., Wünschmann, A., McRuer, D.L., 2009. Association of *Mycoplasma corogypsi* and polyarthritis in a black vulture (*Coragyps atratus*) in Virginia. J. Wildl. Dis. 45, 808–816. https://doi.org/10.7589/0090-3558-45.3.808.
Sawicka-Durkalec, A., Kursa, O., Bednarz, Ł., Tomczyk, G., 2021. Occurrence of *Mycoplasma* spp. in wild birds: phylogenetic analysis and potential factors affecting distribution. Sci. Rep. 11, 17065. https://doi.org/10.1038/s41598-021-96577-0.
Spergser, J., Hess, C., Loncaric, I., Ramírez, A.S., 2019. Matrix-assisted laser desorption ionization-time of flight mass spectrometry is a superior diagnostic tool for the identification and differentiation of mycoplasmas isolated from animals. J. Clin. Microbiol. 57, e00316–e00319. https://doi.org/10.1128/JCM.00316-19.
Spergser, J., Botes, A., Nel, T., Ruppitsch, W., Lepuschitz, S., Langer, S., Ries, S., Dinhopl, N., Szostak, M., Loncaric, I., Busse, H.J., 2020. *Mycoplasma nasistruthionis* sp. nov. and *Mycoplasma struthionis* sp. nov. isolated from ostriches with respiratory disease. Syst. Appl. Microbiol. 43, 126047. https://doi.org/10.1016/j.syapm.2019.126047.
Spergser, J., DeSoye, P., Ruppitsch, W., Cabal Rosel, A., Dinhopl, N., Szostak, M.P., Loncaric, I., Chopra-Dewasthaly, R., Busse, H.J., 2022. *Mycoplasma tauri* sp. nov. isolated from the bovine genital tract. Syst. Appl. Microbiol. 45, 126292. https://doi.org/10.1016/j.syapm.2021.126292.

Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Suárez-Pérez, A., Ramírez, A.S., Rosales, R.S., Calabuig, P., Poveda, C., Rosselló-Móra, R., Nicholas, R.A.J., Poveda, J.B., 2012. *Mycoplasma neophronis* sp. nov., isolated from the upper respiratory tract of Canarian Egyptian vultures (*Neophron percnopterus majorensis*). Int. J. Syst. Evol. Microbiol. 62, 1321–1325. https://doi.org/10.1099/ijs.0.033803-0.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882. https://doi.org/10.1093/nar/25.24.4876.

Tully, J.G., 1983. Cloning and filtration techniques for mycoplasmas. In: Razin, S., Tully, J.G. (Eds.), Methods in Mycoplasmology, 1. Academic Press, New York, pp. 173–177.

Tully, J.G., 1995. Culture medium formulation for primary isolation and maintenance of mollicutes. In: Razin, S., Tully, J.G. (Eds.), Molecular and Diagnostic Procedures in Mycoplasmology, vol. 1. Academic Press, San Diego, pp. 33–39.

Van Wettere, A.J., Ley, D.H., Scott, D.E., Buckanoff, H.D., Degernes, L.A., 2013. *Mycoplasma corogypsi*-associated polyarthritis and tenosynovitis in black vultures (*Coragyps atratus*). Vet. Pathol. 50, 291–298. https://doi.org/10.1177/0300985812457791.

Volokhov, D.V., Simonyan, V., Davidson, M.K., Chizhikov, V.E., 2012. RNA polymerase beta subunit (rpoB) gene and the 16S-23S rRNA intergenic transcribed spacer region (ITS) as complementary molecular marker in addition to the 16S rRNA gene for phylogenetic analysis and identification of the species of the family *Mycoplasmataceae*. Mol. Phylogenet. Evol. 62, 515–528. https://doi.org/10.1016/j.ympev.2011.11.002.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595. https://doi.org/10.1371/journal.pcbi.1005595.

Woodcroft, B.J., Aroney, S.T.N., Zhao, R., Cunningham, M., Mitchell, J.A.M., Blackall, L., Tyson, G.W., 2024. SingleM and Sandpiper: Robust microbial taxonomic profiles from metagenomic data. bioRxiv. https://doi.org/10.1101/2024.01.30.578060, 2024.01.30.578060.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617. https://doi.org/10.1099/ijsem.0.001755.

Ziegler, L., Möller Palau-Ribes, F., Enderlein, D., Herbst, W., Schmidt, L., Lierz, M., 2019. *Mycoplasma hafezii* sp. nov., isolated from the trachea of a peregrine falcon (*Falco peregrinus*). Int. J. Syst. Evol. Microbiol. 69, 773–777. https://doi.org/10.1099/ijsem.0.003235.