Unveiling genome plasticity and a novel phage in *Mycoplasma felis*: genomic investigations of four feline isolates

Sara M. Klose[1,2*], Alistair R. Legione[1*], Rhys N. Bushell[3], Glenn F. Browning[1] and Paola K. Vaz[1]

*S.M.K. and A.R.L. contributed equally to this work.

Affiliation:

1. Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, VIC, Australia

2. Institute of Molecular Medicine and Experimental Immunology, Faculty of Medicine, University of Bonn, NRW, Germany

3. Department of Veterinary Clinical Sciences, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, VIC, Australia

Corresponding Author: Alistair Legione, legionea@unimelb.edu.au

Keywords: *Mycoplasma felis*, whole genome sequencing, genome plasticity, evolution, phage

Repositories: All sequence data and assemblies for data associated with this project can be found under the GenBank Bioproject: PRJNA906261

Abstract:

Mycoplasma felis has been isolated from diseased cats and horses, but to date only a single fully assembled genome of this species, of an isolate from a horse, has been characterised. This study aimed to characterise and compare the completely assembled genomes of four clinical isolates of *M. felis* from three domestic cats, assembled with the aid of short and long read sequencing methods. The completed genomes encoded a median of 759 open reading frames (min, 743, max 777) and had a median average nucleotide identity (ANI) of 98.2% with the genome of the available equid origin reference strain. Comparative genomic analysis revealed the occurrence of multiple horizontal gene transfer (HGT) events and significant genome reassortment. This had resulted in the acquisition or loss of numerous genes within the Australian felid isolate genomes, encoding putative proteins involved in DNA transfer, metabolism, DNA replication, host cell interaction, and restriction modification systems. Additionally, a novel mycoplasma phage was detected in one Australian felid *M. felis* isolate by genomic analysis and visualised using cryo-transmission electron microscopy. This study has highlighted the complex genomic dynamics in different host environments. Furthermore, the sequences obtained in this work will enable the development of new diagnostic tools, and identification of future infection control and treatment options for the respiratory disease complex in cats.

Data summary:

All genome data for this study have been deposited in GenBank under BioProject PRJNA906261. Genome assemblies, as well as Illumina and Oxford Nanopore sequence reads for each isolate, can be found under their respective BioSamples: SAMN32182834 (isolate 047), SAMN32182835 (isolate 219), SAMN32182836 (isolate 329), and SAMN32182837 (isolate 632). The authors confirm all supporting data and protocols have been provided within the article.

Impact statement:

Mycoplasma felis is commonly associated with clinical cases of conjunctivitis and feline respiratory disease complex in cats, the leading cause of euthanasia in animal shelters. In the absence of vaccines, infection control is currently limited to the prolonged treatment with antimicrobials. Prior to this study there was only one complete genome assembly of an isolate of *M. felis*, which had been obtained from a horse. This study has provided the first high quality hybrid assembled genomes of

50 *M. felis* isolates from cats. This work adds four new genomes from clinical cases, as well as the
51 identification and validation of the presence of a novel phage that utilises the mycoplasma
52 translation code. The genomic data presented here can assist future projects investigating improved
53 diagnostics and development of new treatment options for this significant feline pathogen.
54

55 ## Introduction:

56 Mycoplasmas are the smallest free-living bacteria and have some of the smallest bacterial genomes
57 (0.58 – 1.38 Mb). They are thought to approach the minimal gene sets essential for independent life,
58 making them ideal model organisms for studies on the fundamental requirements of a living cell [1].
59 They evolved from Gram positive bacterial ancestors 600 million years ago [2] and have undergone
60 considerable genome reduction during their evolution [3, 4]. They lack a cell wall, are pleomorphic
61 and can infect and cause acute and chronic disease in a diverse range of animal and plant species [5].
62
63 Some mycoplasmas have been found to exhibit significant genetic plasticity and to evolve rapidly,
64 mainly driven by intra-species spontaneous mutation and recombination or horizontal gene transfer
65 (HGT) between and within species, facilitating swift adaptation to environmental changes, enhancing
66 survival and/or virulence [6-9]. Studies examining the genomic stability of *Mycoplasma synoviae* have
67 revealed that mutations occur frequently during infection in birds [10], and that rapid
68 thermoadaptive evolution can occur *in vitro* and *in vivo* [11], presumably to regain fitness and
69 pathogenicity [12, 13]. HGT appears to occur more frequently between species that share an
70 ecological niche, irrespective of their phylogenetic divergence. Examples of significant HGT include
71 the transfer of the genes encoding the phase variable VlhA cell surface lipoproteins between the
72 poultry pathogens *Mycoplasma gallisepticum* and *M. synoviae* [14], and the transfer of several cell
73 surface lipoproteins involved in host colonisation between the ruminant pathogens *Mycoplasma*
74 *capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *Mycoides*, and *Mycoplasma*
75 *agalactiae* and *Mycoplasma bovis* [15].
76
77 At least four species of mycoplasmas have been isolated from cats (*Mycoplasma felis*, *Mycoplasma*
78 *gateae*, *Mycoplasma arginini*, and *Mycoplasma feliminutum*), in most cases as commensals, but they
79 have been associated with conjunctivitis and, increasingly, feline respiratory disease [16-19]. Feline
80 respiratory disease complex (FRDC) is the leading cause of euthanasia of cats in animal shelters [20]

81 and is commonly associated with several viral (feline herpesvirus 1 and feline calicivirus) and bacterial
82 pathogens, including *M. felis* [21, 22]. *M. felis* infections are common in most cat populations and the
83 ability of this organism to persist in an animal enhances opportunities for recurrent opportunistic
84 infections. Vaccines against *M. felis* are not currently available and treatment is limited to the
85 prolonged treatment with tetracyclines or fluoroquinolones [23, 24]. Currently full genomes of three
86 isolates of this species have been published: strain Myco-2 (GenBank accession number:
87 AP022325.1), an isolate from a case of respiratory disease in a horse in Japan [25], which has been
88 completely assembled, and multiple assemblies of isolates 16-040612-4 (CP104192, CP104193,
89 CP104194, CP11027) and 16-057065-4 (CP103988, CP103993, CP110269), which were each isolated
90 from feline bronchoalveolar lavages in Canada [26]. Additionally, there is an incomplete genome of
91 ATCC strain 23391, assembled into scaffolds (GenBank Biosample: SAMN02841218).
92
93 The aim of this study was to sequence and assemble four high quality, complete *M. felis* genomes,
94 using hybrid assembly methods. These genomes would represent the first complete assemblies of
95 feline isolates of this species. The objective was to a significantly expand on our limited
96 understanding of the genomic structure and plasticity of *M. felis*, which may lead to enhanced
97 development of diagnostic tests and vaccines, as has been seen in other mycoplasma species.
98

99 # Methods:

100 *Mycoplasma isolation*

101 Swabs were collected for bacteriological culture from clinical cases of respiratory disease (3 cases)
102 and an unresolved post-surgical infection (1 case) (Table 1). Swabs were used to inoculate sheep
103 blood agar (SBA) plates, which were incubated aerobically at 37°C. Following observation and
104 presumptive identification of small mycoplasma-like colonies, single colonies were transferred to
105 Mycoplasma Broth (MB) containing 10% swine serum (Sigma-Australia) and 0.01% nicotinamide
106 adenine dinucleotide (NAD) (Sigma-Australia), based on the formulation of Frey's medium, with
107 minor modifications [27]. Acidification of the MB medium was used to confirm the growth of
108 *Mycoplasma* spp. and the cultures were stored at -80°C for future investigation.
109

110 *DNA extraction, sequencing, and genome assembly*

111 Mycoplasma isolates were cultured axenically in MB for 18 h at 37°C prior to DNA extraction. Bacteria
112 were pelleted and the medium removed, and then resuspended in the appropriate extraction kit lysis
113 buffer. The DNA for short read sequencing was extracted using the DNeasy Blood and Tissue Kit
114 (QIAGEN), following the manufacturer's protocol, and 100 ng of the extracted DNA was used to
115 prepare sequencing libraries using Illumina's Nextera Flex DNA Library Prep Kit. Sequencing was
116 performed on the NovaSeq platform at Charles River Laboratories, Victoria, Australia, to generate
117 150 bp paired-end reads. Two rounds of Oxford Nanopore Technologies (ONT) long read sequencing
118 were performed. The DNA was extracted using the Promega Wizard DNA Purification Kit (Round 1)
119 or the Promega Wizard High Molecular Weight DNA Purification Kit (Round 2). DNA libraries were
120 generated using the ONT Rapid Barcoding (SQK-RBK004) Kit. Sequencing was performed on a MinION
121 Mk1b device fitted with a FLO-MIN106D Flow Cell (R9.4.1 chemistry). Base calling, de-multiplexing,
122 and adapter removal were performed using the Guppy (version 6.2.1+6588110a6) high accuracy base
123 caller with a minimum quality score of 9 (default settings). The Illumina reads were trimmed and
124 adaptors removed with fastp v0.20.1 [28] using the default settings. The ONT reads from the two
125 rounds were merged for each isolate and processed using Filtlong v0.2.0 [29] to remove all reads
126 shorter than 1000 bp, retaining 90% of input data (after removal of short and low-quality reads).
127 Subsequently, the processed short and long reads were used as input into Unicycler v0.5.0 [30] in
128 'normal' mode, with a modified start gene database that included *M. felis*. The assemblies where
129 then polished using Polypolish v0.5.0 [31], incorporating BWA-MEM v0.7.17-r1188 [32]. Full genome
130 alignments were generated using the progressive MAUVE algorithm [33] to identify regions of
131 reassortment and gene loss or gain.
132
133 *Genome annotation and sequencing analysis*
134 The genomes were annotated using the Prokaryotic Genome Annotation Pipeline v2022-12-
135 13.build6494 [34] for primary analysis. A *Tenericutes* specific protein database, containing both
136 Swiss-Prot (4464 sequences) and TrEMBL (343,598 sequences) annotations, was downloaded from
137 UniProt and converted to a Diamond format protein database [35]. This database was used as input
138 to Pydeel (https://github.com/alegione/pydeel) to determine the coding ratio of our annotated
139 genomes in comparison with *M. felis* genomes on GenBank. This tool identifies the coding ratio of
140 annotated genes compared to their best hit within the provided database to identify the proportion
141 of annotated genes that may have erroneous insertions or deletions as a by-product of the use of
142 long read sequencing. From here, annotated genomes were processed through Panaroo v1.3.0 [36],

143 along with previously sequenced *M. felis* genomes (Myco-2 and ATCC23391), to identify the core
144 genome. The core genome results were visualised using Pagoo [37]. The annotated genomes from
145 this study were investigated for pathogenicity islands and integrative conjugative elements using
146 Island Viewer 4 [38] and ICE finder [39], respectively. Antimicrobial resistance genes and virulence
147 factors were detected by generating a Diamond database of the CARD [40] and full virulence factor
148 databases [41], and using Diamond BLASTp to identify best hits with a cut-off bit score of 50. The
149 UniProt webserver was used to identify homologs of specific open reading frames (ORFs) in other
150 genomes (https://www.uniprot.org).
151
152 *Phage isolation and visualisation*
153 A phage-like contig was identified in the annotation results of one of the assemblies (MF329). In
154 order to confirm whether the detection of a bacteriophage genomic sequence corresponded to the
155 presence of an integrated prophage or to replicative virions, bioinformatic methods were first
156 utilised. The probable terminal ends were investigated using short and long read mapping to the
157 contig with BWA-MEM and minimap2 v2.26-r1175 [42], respectively, and the contig's starting point
158 adjusted based on the depth of coverage. The tool PhageTerm [43] was also used to investigate the
159 potential phage start and end points. To investigate whether this contig was a prophage, the long
160 reads were mapped, using minimap2, to the updated phage contig. Any reads that had overhangs at
161 the 5' and 3' ends of the contig were extracted and subsequently mapped to the assembled MF329
162 genome to determine whether there was a common point of overlap. To detect the presence of
163 bacteriophage virions, transmission electron microscopy was performed on a culture of MF329.
164 Isolate MF329 was cultured in 50 mL of Mycoplasma Broth, as described above, for 20 hours. The
165 culture was centrifuged for 15 mins at 10,000 x g at 4°C and the medium fraction separated from the
166 bacterial cell pellet. The bacterial cells were gently lysed by resuspension in 150 µL of sterile water,
167 followed by centrifugation at 10,000 x g at 4°C for 5 mins to remove cellular debris. This was repeated
168 twice, with the supernatant fraction captured each time for ultracentrifugation. The culture medium
169 and the cell lysate supernatant fractions were separately ultra-centrifugated at 100,000 x g for 90
170 min at 4°C, and the pellets resuspended in 0.2 M HEPES buffer, pH 7.7. Negativeky stained samples
171 were examined at the Ian Holmes Imaging Center (Melbourne, Australia) using a FEI Talos L120C
172 cryoTEM (Talos L120C) microscope.
173

174 # Results:

175 *Characterisation and comparisons of genomes*

176 All four isolates were successfully assembled into circularised, complete genomes. In addition to
177 these four genomes, a novel *M. felis* phage genome was identified that utilised the mycoplasma
178 translation code. Metrics for long and short read sequencing output are provided in Table 2. The
179 assembly statistics for the four isolates are provided in Table 3. Briefly, the circularised isolates were
180 significantly larger than the reference genome (Myco-2; 841,695 bp), with a median length of
181 940,935 bp (range: 905,741 – 948,716) and a median of 759 coding domain sequences (CDSs) (range:
182 743 – 777), compared to the reference genome which has 740 CDSs. The NCBI Prokaryotic Genome
183 Annotation Pipeline incorporates a CheckM [44] completeness analysis and an average nucleotide
184 identity taxonomic classification step to identify any potential issues with an assembled genome. For
185 all four genomes the CheckM completeness mirrored that of the reference Myco-2 genome (99.21%
186 completeness), and all assemblies were classified as *Mycoplasma felis* with high confidence (Table
187 3). The coding ratios of annotated assemblies were investigated to determine the frequency of
188 potential indel errors. The two published *M. felis* genomes (Myco-2 and ATCC23391) had mean
189 coding ratios of 1.0 (standard deviation ±0.02) and 0.96 (±0.32), respectively. Our completed
190 genomes, MF47, MF219, MF329, and MF632, had mean coding ratios of 1.0 (±0.28), 0.99 (±0.30), 1.0
191 (±0.27), and 1.0 (±0.26), respectively. Seven recently sequenced *M. felis* isolates that utilised only
192 Oxford Nanopore sequencing had a substantial number of indel errors in their annotations based on
193 coding ratio analysis (mean coding ratios between 0.44 and 0.47) and were not used in further
194 comparisons for this reason (Figure 1).

195

196 The genomes of two isolates, MF047 and MF632, had an additional tRNA compared to the other
197 isolates, for tRNA-Trp and an unknown tRNA respectively. The genomes of the newly sequenced
198 isolates had 1-3 secretory systems (in all cases these were annotated as Type IV secretory systems),
199 whilst that of Myco-2 has no annotated secretory systems. The newly annotated genomes also had
200 an increased number of transposases, typically annotated as IS1634 or IS256, or in a few cases as IS3.
201 In comparison, the Myco-2 genome only had IS3 and IS1634 family transposase annotations.

202

203 A progressive MAUVE alignment of the four genome assemblies in comparison to that of Myco-2
204 (Figure 2) identified two significant genome inversions in the reference sequence compared to the

205 isolates assembled here, as well as more localised inversions within each of the isolates. These
206 inversions, and the genomic regions present in individual isolates, were typically flanked by
207 transposases. Comparison of the average nucleotide identities (ANIs) of the four new assemblies,
208 and with previously published *M. felis* genomes (Table 4) found that the best match for each of the
209 four Australian isolates was another isolate from this study, with the exception of MF329 (MF047
210 and MF219, 99.8%; MF632 and MF329, 98.3%, MF329 and ATCC23391 98.4%), noting that MF047
211 and MF219 were obtained from the same animal two months apart.
212
213 The core genome of *M. felis*, as determined by Panaroo (genes present in >99% of genomes)
214 contained 595 genes, with 301 shell genes (genes present in 15% - 95% of genomes). Collapsing
215 paralogues increased the number of core genes by one and reduced the number of shell genes to
216 219. Principal component analysis (PCA) highlighted clustering of the two published genomes, as well
217 as clustering of isolates that were obtained from the same individual (Figure 3). All genes, except
218 those annotated as hypothetical proteins, identified in the newly sequenced isolates but not present
219 in the reference sequence are listed in Table 5, with the homologs found in other genomes. The
220 variations between the genomes were mostly observed in sequences encoding putative proteins
221 involved in DNA transfer, metabolism, DNA replication, mycoplasma-host cell interactions, and
222 restriction modification systems (Figure 4).
223
224 *Genomic islands, virulence genes and antimicrobial resistance genes*
225 Genomic islands were detected in three of the four newly sequenced genomes (Table 7). The average
226 length of these islands was 22,145 bp (± 5818 bp) and each contained an average of 16 (±5) annotated
227 genes. In comparison, the Myco-2 strain of *M. felis* had two predicted genomic islands of 3052 bp
228 and 10,027 bp. Further inspection of these predicted genomic islands identified matching regions
229 present in isolates in alternative locations. For example, MF329 had a predicted genomic island of 24
230 kb (nucleotide position [nt] 311,409 – 335,810) containing 19 genes, including a type IV secretion
231 system and an insertion sequence (IS1634) at its midpoint. Extraction and remapping of the sequence
232 of this genomic island showed that it was repeated three times within a wider 122 kb region (nt
233 213,995 – 335,810) of the MF329 genome. This same genomic region occurred, without the IS
234 element present, in the genomes of MF047 (twice), MF219 (once), and MF632 (once). In the case of
235 MF632, the region was also a predicted to be a genomic island, but in this instance was flanked with
236 restriction endonuclease genes and either predicted transposases (3') or an IS256 element (5').

237 Diamond BLAST searches for antimicrobial resistance genes and virulence genes identified a few high
238 identity hits (

239 Table **6** and Supplementary Tables 2 and 3). Only one hit with the virulence factor database had an
240 amino acid sequence identity of greater than 90%, and this was to the gene for the elongation factor
241 Tu in *Mycoplasma synoviae* (90.1% average identity), which has been found to be an adherence
242 factor and is present in all genomes. Only five genes had hits with >50% amino acid sequence identity.
243 The closest hits to these genes were *oppF* (59.9% identity; *Mycoplasma mycoides* subsp. *mycoides*;
244 immune modulation), *eno* (58.9%; *Streptococcus pneumoniae*; exoenzyme), *gap* (57.9%;
245 *Mycoplasma genitalium*; adherence), *dnaK* (53.9%; *Chlamydia trachomatis*; adherence), and *sugC*
246 (50.4%; *Mycobacterium marinum*; nutritional/metabolic factor). No genes had hits with >50% amino
247 acid sequence identity with antimicrobial resistance genes in the CARD database.
248
249 *Phage/prophage detection and identification*
250 A 33.6 kb contig (vB Mfe PM329; *Mycoplasma felis* strain PM329) was assembled and identified as a
251 likely phage or prophage in the sequenced *M. felis* MF329 isolate. It had an average depth of coverage
252 of 40,847.7 reads, with 82.6% of the MF329 Illumina reads mapping to the phage genome.
253 Interrogation of the nanopore read dataset to determine its genome integration status detected only
254 63/18134 (0.35%) reads that mapped to both the phage and the bacterial genome. Of these, most
255 were found to map across the bacterial genome at mostly single-read depth, with the exception of
256 five reads that clustered mid-genome in the region of nt 456,500. If this was indicative of a true
257 prophage insertion site it would disrupt ORF MF329_000403, which encodes a putative 16S rRNA
258 pseudouridine synthase gene. The phage genome had a 27.1% G+C content and encoded 36
259 predicted ORFs using the mycoplasma translation code. It shared 94.8% global pairwise nucleotide
260 sequence identity with two nanopore contigs in the publicly available unassembled *M. felis* contig
261 dataset (NCBI Biosample: SAMN30346842), and there was 96.8% and 97.4% pairwise amino acid
262 sequence identity between each of their encoded putative recombinase and portal proteins,
263 respectively. Interrogation of the read datasets of the other three isolates sequenced in this study
264 with the phage genome identified only 4/9515 (0.04%; MF047), 19/19128 (0.1%; MF632) and
265 22/65383 (0.03%; MF219) mapped nanopore reads, within the range of barcode error. No Illumina
266 reads mapped to the phage genome, indicating that the isolates did not carry the phage. Phylogenetic
267 analyses using VipTree [45] and vContact2 [46] was not able to assign vB Mfe PM329 to any known
268 phage genus or family, but its gene arrangement was similar to those of the MAgV1 phage from *M.*
269 *agalactiae* and MAgV1-like prophage sequences detected in mycoplasma species in the Hominis
270 phylogenetic group. In particular, both the protein and gene arrangement similarity were greatest to

271 those of prophages found in *Mycoplasma mustelae* and *Mycoplasma molare*, including the central
272 position of the recombinase gene. BLASTX analysis of predicted ORFs detected pairwise protein
273 sequence identities of 48% to 69% between core conserved ORFs encoded within the *M. mustelae*
274 and *M. molare* prophage sequences [47] (Supplementary Table 1).
275
276 Confirmation of productive virus formation was achieved using cryoTEM on medium and cell lysate
277 supernatant fractions, which revealed the presence of non-enveloped contractile-tailed polyhedral
278 viral particles approximately 100 nm in length, with a 50 nm diameter head and tails of varying
279 lengths. Higher numbers of virions were observed within the cell lysate supernatant fraction than in
280 the culture medium fraction, with clusters of virions observed seemingly embedded within bacterial
281 membrane debris (Figure 5). These long tailed polyhedral viral structures were consistent with those
282 seen in siphoviruses and myoviruses within the recently ratified *Caudoviricetes* class of
283 bacteriophage. The contractile tail and polyhedral head structure is consistent across all visualised
284 mycoplasma phages, with the main differences being the head diameter and tail lengths. *M. pulmonis*
285 Phage P1 has an isometric head with a diameter of 28 nm and a short tail, Br1 has head diameter of
286 77 nm and a long tail of 104 nm [48], and the *M. hyorhinis* Hr1 phage is a short-tailed (14 nM) phage
287 with a head diameter of 34 nm [49].
288

289 Discussion

290 In this study, the genomes of four new *M. felis* isolates were characterised and compared; three of
291 the isolates were derived from clinical cases of respiratory disease and the remaining one from an
292 unresolved post-surgical infection. Comparisons between these genomes and with several published
293 datasets derived from other isolates of *M. felis* detected genomic variations and inversions between
294 the Australian felid isolates and the only assembled *M. felis* reference isolate, from a horse. These
295 variations and inversions were flanked by transposases, which are commonly involved in HGT and
296 genome reassortment in other mycoplasmas. There were differences in the number of copies of
297 putative transposases from several families, including the IS1634, IS256 and IS3 (Figure 4). Insertion
298 sequences (ISs) are transposable elements that are often present in multiple copies in a genome and
299 involved in chromosomal rearrangements by deletion, insertion, or inversion. The substantial
300 similarity between various isoforms of ISs enables them to facilitate homologous recombination [50].
301 IS families encoded in mycoplasma genomes play an important role in genomic plasticity with several

302 reported instances of IS exchange between strains or species [15, 51-54]. This is in contrast to early
303 research that suggested that some ISs, such as IS1634, which was found in abundance in the
304 sequenced genomes of our isolates, were unique to specific mycoplasma species [55]. The
305 differences between our Australian *M. felis* genomes, which were obtained from three different cats,
306 based on gene presence/absence and the number of coding sequences, underscores the genetic
307 diversity present amongst the sequenced felid *M. felis* isolates (Figure 3). Multiple gene insertions,
308 relative to the equid *M. felis* reference genome, were detected within the cluster of Australian field
309 isolates (Table 5). These included genes involved in DNA transfer, replication, metabolism, and
310 restriction modification (RM) systems, as well as a number of proteins of unknown function (Figure
311 4).
312
313 *Gene gains compared to the equid M. felis reference strain*
314 Of particular interest are the gene acquisitions involved in DNA transfer, such as the multiple copies
315 of genes encoding putative type IV secretory system (T4SS) conjugative DNA transfer family proteins
316 within the Australian felid genomes, which were not detected in the publicly available equid and felid
317 *M. felis* genomes. T4SS conjugation systems translocate DNA through a process dependent on cell to
318 cell contact and have been identified as one of the core genes within mycoplasma Integrative and
319 Conjugative Elements (ICEs), which play a significant role in genomic plasticity in the species in which
320 they have been found [56, 57]. ICEs have been shown to be self-transmissible in *M. agalactiae*,
321 providing conjugative properties from ICE-positive cells to ICE-negative cells, while concurrent
322 chromosomal transfers (CTs) occur in the opposite direction [58]. Protein sequence analysis of the
323 putative T4SS identified in the Australian felid *M. felis* isolates detected 43.2% amino acid sequence
324 identity with ORF5 of the ICE in *Mycoplasma cynos*. Whilst *M. cynos* predominantly infects the
325 respiratory tract of dogs, it has also been detected in cats in association with conjunctivitis and upper
326 respiratory tract disease [59]. Taken together, it is plausible that a potential HGT event may have
327 resulted in transfer of the T4SS conjugative element of an ICE from a *M. cynos*-like species to *M. felis*
328 in a shared feline host. This T4SS was part of a defined region of genes that, along with 15
329 hypothetical proteins and a DUF87 domain-containing protein, was repeated three times in the
330 genome of isolate MF329. DUF87 domain-containing proteins are associated with conjugative
331 elements in other organisms, and include TraJ in the virulence plasmid of *Salmonella enterica* serovar
332 Typhimurium [60], adding further weight to the likelihood that this region is an ICE, even though the
333 bioinformatics tools used here did not detect it.

334

335 Several genes involved in metabolism were newly detected in the felid *M. felis* isolates sequenced in
336 this study. A type 2 glycerol-3-phosphate oxidase (GlpO) was identified within the Australian *M. felis*
337 genomes, while in the equid *M. felis* isolate, only its FAD-dependent oxidoreductase domain was
338 detected, accompanied by two transposases, located upstream. GlpO converts glycerol-3-phosphate
339 to dihydroxyacetone phosphate (DHAP), releasing hydrogen peroxide as a by-product, which has
340 been shown to play a role in cytotoxicity *in vitro* in *M. gallisepticum* and *Mycoplasma pneumoniae*
341 [61, 62], and in the virulence of *M. mycoides* subsp. *mycoides* small colony type and mosquito-
342 associated *Spiroplasma* species [63, 64]. The significant reduction or complete loss of H_2O_2
343 production in highly passaged *M. bovis*, *M. agalactiae* and *M. gallisepticum* strains, and in attenuated
344 vaccine strains of *M. gallisepticum*, compared to wild type strains suggests that glycerol metabolism
345 can rapidly become redundant in some mycoplasmas [61, 65, 66]. It is possible that the sequences
346 coding for GlpO were lost in the equid *M. felis* isolate, possibly because of differing levels of available
347 glycerol in horses compared to cats. Likewise, variations were seen in the counts of genes coding for
348 nutrient transport systems, including ATP-binding cassette (ABC) transporters and the PEP-
349 dependent phosphotransferase transport system (PTS), as well as numerous other metabolic
350 enzymes. A number of these nutrient transport systems, and many cell surface enzymes, are
351 multifunctional in mycoplasmas, playing critical roles in pathogenesis and survival in the host [13, 67-
352 72]. The differences observed in the PTS and ABC transporters of the Australian felid *M. felis* isolates,
353 in contrast to the equid *M. felis* isolate, may also be indicative of rapid metabolic gene gains and
354 losses in response to the varying nutrient availabilities within felid and equid ecological niches.

355

356 In the Australian felid *M. felis* genomes, five new coding sequences related to replication were
357 identified that were absent in the equid origin *M. felis* genome, including genes encoding the putative
358 anti-termination protein NusB, a Hsp70 family protein, a C5 methylase, the 16S rRNA
359 methyltransferase RsmG, and an HNH endonuclease. The presence of these additional proteins
360 suggests potential differences in bacterial growth within the feline and equine hosts, potentially due
361 to distinct replication strategies. Future experiments comparing growth patterns of these isolates in
362 felid or equid derived cells are indicated. The potential origins of these additional sequences have
363 been detected in mycoplasmas isolated from mink, pigs, and dogs, which share a common ecological
364 environment with cats, or pigeons, which are a common prey of cats. The HGT events resulting in the
365 acquisition of these genes by the Australian felid *M. felis* isolates may have been facilitated by the

366 close ecological proximity or may have occurred within the feline gastrointestinal tract (GI) following
367 the ingestion of infected prey. HGT is frequently observed between diverse microorganisms within
368 the mammalian gastrointestinal tract, possibly due to the high population density in this niche, which
369 presumably facilitates genetic exchange [73]. Furthermore, the conjugation mechanism favoured for
370 HGT within the GI tract protects DNA from nucleases and heavy metals, and thus is more likely to
371 result in a successful genetic exchange [74].
372
373 Two additional restriction endonucleases (Eco47II and Sau3AI) were annotated in the Australian felid
374 *M. felis* genomes, and they lost a DpnII family type II restriction endonuclease, compared to the equid
375 isolate. Restriction modification (RM) systems play a pivotal role in regulating bacterial HGT by
376 protecting the genome against incoming mobile genetic elements, or by facilitating recombination
377 with the introduced DNA [75]. A strong correlation has been reported between the presence of active
378 Type II RM systems and the presence of mobile genetic elements and ICEs within *M. agalactiae*
379 genomes, indicating the facilitative role of Type II RM systems in HGT in mycoplasmas [76]. The
380 diversification of RM target recognition sites can regulate HGT by promoting genetic exchanges
381 among those organisms with RM systems that recognise the same target motifs, and by reducing
382 genetic transfer between lineages that possess distinct RM systems [77]. Taken together, it is
383 plausible that following a host switch into horses, *M. felis* may have diversified its RM systems to
384 enhance the level of genetic exchange from phylogenetically distant bacteria.
385
386 *Gene losses compared to the equid M. felis reference strain*
387 A sequence encoding a putative ComEC/Rec2 family competence protein was absent in the
388 Australian felid *M. felis* isolates. This protein is one of the components of DNA translocation via
389 transformation, enabling DNA transport by forming a channel across the cytoplasmic membrane [78].
390 While the ComEC component has been reported to be essential for DNA transport in Gram positive
391 bacteria, deletion of homologous genes did not affect DNA uptake in bacteria without a cell wall [79].
392 Furthermore, the competence domain seems to be missing in many mycoplasmas, possibly due to
393 the reductive evolution of these bacteria [80]. Characterisation of two mycoplasma genomes
394 recovered from gut microbiota of a deep-sea isopod detected the *comEC* gene adjacent to genes
395 encoding enzymes producing dTMP from thymidine or dUMP, suggesting that the role of the ComEC
396 protein might be importing extracellular DNA as a nutrient source [81]. The presence of ComEC in
397 the equid *M. felis* isolate, and its absence in the Australian felid *M. felis* isolates suggests that during

398 evolutionary adaptation DNA translocation via a membrane channel might have become redundant
399 and perhaps was replaced with T4SS conjugation-mediated DNA transfer, as discussed above.
400
401 The Australian felid *M. felis* genomes exhibited a total absence of four coding sequences associated
402 with host cell interactions, those encoding a putative variable surface lipoprotein, a fibronectin type
403 III domain-containing protein, a FIVAR domain-containing protein, and a GA module-containing
404 protein, in contrast to the equid *M. felis* genome. The FIVAR (Found In Various Architectures Region)
405 and GA domains are essential constituents of extracellular matrix-binding proteins (Embp),
406 facilitating biofilm formation and attachment to fibronectin in some bacteria such as *Staphylococcus*
407 *epidermidis* and *S. aureus* [82, 83]. The presence of more than 30% amino acid sequence identity
408 between the FIVAR and GA domains in the equid *M. felis* reference isolate Myco-2 and these domains
409 in Embp of *S. aureus* raises the possibility that the equid *M. felis* isolate might have potential for
410 biofilm formation and/or fibronectin attachment. Conversely, the absence of these proteins, and of
411 the surface lipoprotein and fibronectin type III domain-containing protein, suggests that these
412 capacities may have become obsolete in the Australian felid *M. felis* isolates, possibly indicating
413 different modes of host cell interaction in the two hosts.
414
415 *Rapid evolution within two months in one host:*
416 Two of the Australian *M. felis* isolates sequenced in this study, MF047 and MF219, were obtained
417 from separate bronchoalveolar lavages (BAL) of a feline patient with clinical signs consistent with
418 pneumonia. Isolate MF047 was obtained from a BAL collected during the initial consultation,
419 whereafter the cat was treated with 25 mg of doxycycline twice a day orally for approximately two
420 months. Because the response to treatment was unsatisfactory, a second BAL was collected and
421 isolate MF219 was obtained. While no genome reassortment was detected between the MF047 and
422 MF219 genomes, minor differences were observed in the counts of some genes encoding proteins
423 involved in RM systems, DNA transfer, and methylation (Figure 4). These variations may suggest rapid
424 host adaptation of *M. felis* after only two months. The growing utilisation of whole genome
425 sequencing in mycoplasma diagnostics is revealing links between genetic mutations and
426 antimicrobial resistance. Genomic comparisons of MF047 and MF219 revealed the presence of five
427 single nucleotide polymorphisms (SNPs) within the gene encoding the ribosomal RNA small subunit
428 methyltransferase A (RsmA/KsgA), along with one SNP in the gene encoding the 16S rRNA and
429 another SNP in the gene encoding the 23S rRNA. In other studies, mutations within the 16S RNA

430 methyltransferase family have been reported to confer aminoglycoside resistance in *M. bovis* and *M.*
431 *gallisepticum* [84, 85], while mutations in the 16S rRNA or 23S rRNA genes have been shown to be
432 associated with resistance to tetracyclines in *M. pneumoniae*, *M. hominis*, *M. genitalium*, and *M.*
433 *bovis* [84, 86-88]. MF047 and MF219 both contained five protein coding regions with low confidence
434 matches to tetracycline resistance genes, with the highest bit score of the five associated with a
435 predicted elongation factor G protein with amino acid sequence identity/similarity to *tet*(T) from
436 *Steptococcus pyogenes* of 25.8%/47.7%. It is important to emphasise that the application of novel
437 genome sequencing techniques for mycoplasma diagnostics, particularly in the context of
438 antimicrobial resistance, is still in its early stages, and the availability of data for comprehensive
439 analysis of all mycoplasma species (and particularly animal mycoplasmas) is currently limited. Future
440 research determining the phenotypic susceptibility of isolates MF047 and MF219 to tetracyclines is
441 necessary to determine whether there is a correlation between the genomic variations observed and
442 tetracycline resistance.
443
444 *Phage in mycoplasmas*
445 In other bacteria, phages have been demonstrated to provide critical virulence factors to their hosts,
446 and play a key role in the pathogenesis of diseases associated with a diverse range of bacteria (e.g.
447 cholera, diphtheria, botulism, and haemolytic-uremic syndrome). Approximately 20 mycoplasma
448 phages/prophages have been reported across diverse ruminant, avian, canine, pig, human and
449 rodent mycoplasmas [47, 89]. Of these, most are unclassified, only three have had their virion
450 structure visualised (P1, Hr1 and Br1), and only one has been classified by the International
451 Committee for the Taxonomy of Viruses (ICTV), the temperate *M. pulmonis* phage P1 (12 kb genome),
452 as the sole member of the floating genus *Delislevirus* [90]. The well-described temperate MAV1
453 phage found in *M. arthritidis* (16 kb genome) has not yet (at the time of publication) been classified
454 within the new system. In other mycoplasmas, phages have demonstrated dynamic mobility within
455 the bacterial chromosome, with loss of integrated prophages seen with clonal passage (MFV1 in *M.*
456 *fermentans*), and variable expression of unique phage-encoded membrane surface proteins (MFV1
457 and MAV1 in *M. fermentans* and *M. arthritidis*, respectively) [91], and may have a role in bacterial
458 adaptation and survival. Detection of a similar contig within *M. felis* 16-057065-4 (NCBI Biosample:
459 SAMN30346842) from Canada indicates that this phage is probably carried by *M. felis* strains globally,
460 although in that study it was not identified as a phage/prophage, but rather as *M. felis* bacterial
461 genomic DNA. No known selective advantage or disadvantage of the presence of this phage within

462 *M. felis* was determined in this study because of the low number of *M. felis* genomes available. Future
463 epidemiological studies to investigate the potential role of phage carriage in feline mycoplasma-
464 associated disease cases is indicated.
465
466 Conclusions
467 This study has provided a comprehensive analysis of the first high-quality, complete genomes of *M.*
468 *felis* isolated from cats, and characterised a novel mycoplasma phage. It provides valuable insights
469 into the intricate genomic dynamics of *M. felis*, showcasing a complex interplay of gene gains and
470 losses, horizontal gene transfer events, and adaptations to new host environments. These findings
471 suggest divergent evolutionary paths in different hosts and offer compelling avenues for further
472 research into the evolutionary mechanisms and ecological factors that influence the genomic
473 diversity of mycoplasmas.
474
475

476 **Conflicts of interest:**

477 The author(s) declare that there are no conflicts of interest.
478

479 **Funding information:**

480 This study was supported by the Feline Health Research Fund. P.K.V. was supported by a University
481 of Melbourne, Melbourne Postdoctoral Fellowship.
482

483 **Author contributions:**

484 R.N.B. performed the bacterial isolation and initial classification; A.R.L., S.M.K. and P.K.V. prepared
485 the DNA samples, performed the sequencing and analysis; P.K.V. isolated the phage for TEM; P.K.V.,
486 S.M.K., G.F.B. and A.R.L. conceptualised the study design; P.K.V., S.M.K. and A.R.L. acquired the
487 funding; S.M.K., A.R.L. and P.K.V. drafted the manuscript, and R.N.B. and G.F.B. revised the
488 manuscript.
489

490 **Acknowledgments:**

The authors would like to gratefully acknowledge the small animal medicine team of the U-Vet Werribee Animal Hospital at The University of Melbourne for providing the samples taken from clinical cases, and Eric Hanssen at the Ian Holmes Imaging Centre for the cryoTEM imaging of the vB Mfe PM329 phage.

495 **Table 1.** *Mycoplasma felis* isolates sequenced in this study.

Isolate	Month/Year	Animal ID	Clinical case	Isolation site	Other notes	Genbank #
047	3/2015	Cat A	Pneumonia	Respiratory, BAL	Heavy growth from BAL, treated with doxycycline	CP114890
219	5/2015	Cat A	Pneumonia	Respiratory, BAL	Follow-up isolation from Cat A	CP114889
329	5/2014	Cat B	FRDC	Respiratory, BAL	Chronic bronchitis. PCR negative for herpesvirus, calicivirus and chlamydia	CP115656 OR753717 (phage)
632	12/2010	Cat C	Surgery	Fat tissue	Isolated from non-healing surgical wound	CP114888

496 BAL, bronchioalveolar lavage; PCR, polymerase chain reaction; FRDC, feline respiratory disease complex

497

498 **Table 2.** Summary of DNA sequencing results after trimming and quality filtering

	MF047	MF219	MF329	MF632
Illumina reads				
Total paired reads	8,977,712	6,647,214	6,481,506	6,807,888
Median length	151	151	151	151
>Q20 (%)	96.93	95.7	96.83	96.99
>Q30 (%)	91.31	88.43	91.12	91.41
GC (%)	24.64	24.67	26.35	24.85
Nanopore reads‡				
Total reads	65,383	9,515	103,977	19,128
Median length	7,049	6,563	3,717	5,058.5
N50	16,885	22,105	12,331	16,399
>Q20 (%)	48.38	51.69	41.64	49.27
>Q30 (%)	15.12	16.77	11.64	15.74
GC (%)	24.9	24.79	27.19	25.08

499 ‡Results presented are from combining two nanopore sequencing runs

500

501 **Table 3.** Summary of assemblies and annotations of four isolates from this study, compared to the complete reference sequence for *Mycoplasma felis* (Myco-2)

Isolate	**Myco-2**	**MF047**	**MF219**	**MF329**	**MF632**
Accession	NZAP022325	CP114890	CP114889	CP115656	CP114888
Assembly information					
Length (bp)	841,695	948,716	945,056	936,813	905,741
CheckM completeness (%)	99.21	99.21	99.21	99.21	99.21
PGAP ANI*	98.4%	98.5%	98.6%	98.6%	98.5%
Median sequencing depth					
Short reads	-	1762	1372	467	1499
Long reads	-	695	110	88	181
Genomic features					
CDS	740	759	777	759	743
ncRNA	2	2	2	2	2
rRNA	9	9	9	9	9
Regulatory	2	2	2	2	2
tRNA	29	30	29	29	30
tmRNA	1	1	1	1	1
Genes of interest					
Secretory Systems	0	2	1	3	1
Transposase	22	40	52	27	38

502 * average nucleotide identity to *Mycoplasma felis* ATCC 23991 according to the internal PGAP ANI taxonomic classification

503

504 **Figure 1.** Coding ratio frequency counts across the *Mycoplasma felis* genomes from this study and those currently
505 available on GenBank. Coding ratios were determined by dividing the amino acid sequence length of annotated
506 proteins by the best hit match in a custom UniProt Mycoplasma database. The coding ratio axis is log transformed for
507 visualisation. Title names for NCBI origin data include the accession number and strain name (e.g. NZ_CP104194, and
508 16-040612-4). For Oxford Nanopore data from NCBI, the depth of coverage is added from NCBI (e.g. 58 x).

509

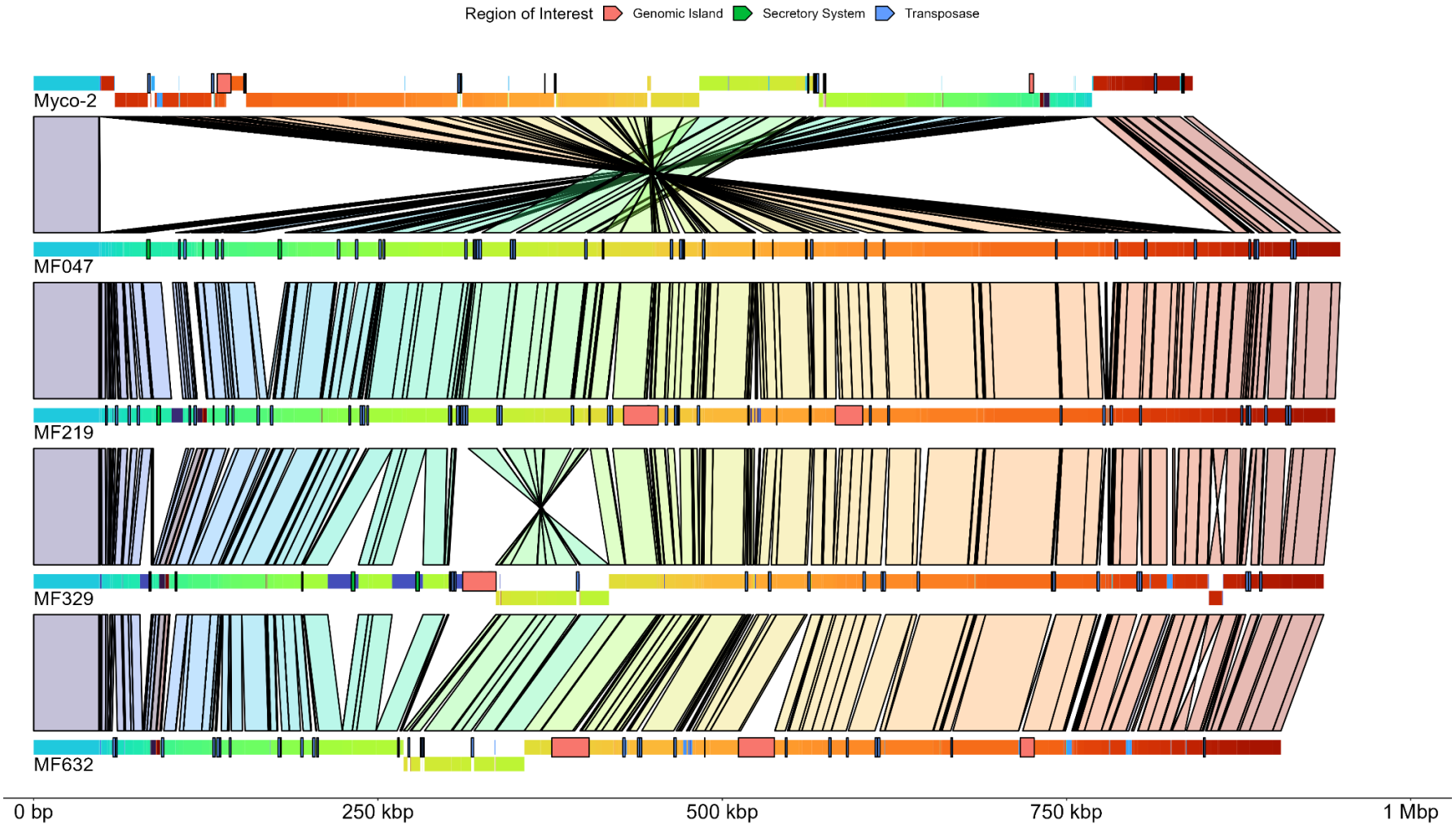


Figure 2. A progressive Mauve alignment of the *Mycoplasma felis* reference genome (Myco-2) and the genomes sequenced in this project. Locally co-linear blocks (LCBs) are separated by colour within genomes, and are the same colour in each genome, with links to identify placement and inversion differences. Solid blocks overlaying the genomes represent the presence of transposases, type IV secretion systems, and genomic islands, whilst solid blocks of blue that break up the colour gradient within the genomes are representative of LCBs not present in linked genomes

516 **Table 4.** Average nucleotide identities (ANI) of Australian *M. felis* isolate genomes, the genome of the equid *M. felis* reference strain (Myco-2) and felid *M. felis* contig
517 datasets (Genbank accessions: MF047: CP114890; MF219: CP114889; MF329: CP115656; MF632: CP114888; Myco-2: AP022325; 16-057065-4: CP103988; 16-040612-
518 4:CP104192; ATCC23391: JNKA01000000).

	MF047	**MF219**	**MF329**	**MF632**	**Myco-2**	**16-057065-4**	**16-040612-4**
MF219	99.79	-	-	-	-	-	-
MF329	98.21	98.16	-	-	-	-	-
MF632	98.21	98.17	98.33	-	-	-	-
Myco-2	98.14	98.06	98.18	98.17	-	-	-
16-057065-4	97.77	97.80	98.25	97.77	97.77	-	-
16-040612-4	97.65	97.65	97.84	97.80	97.72	97.65	-
ATCC23391	98.31	98.29	98.39	98.27	98.33	97.95	97.95

519

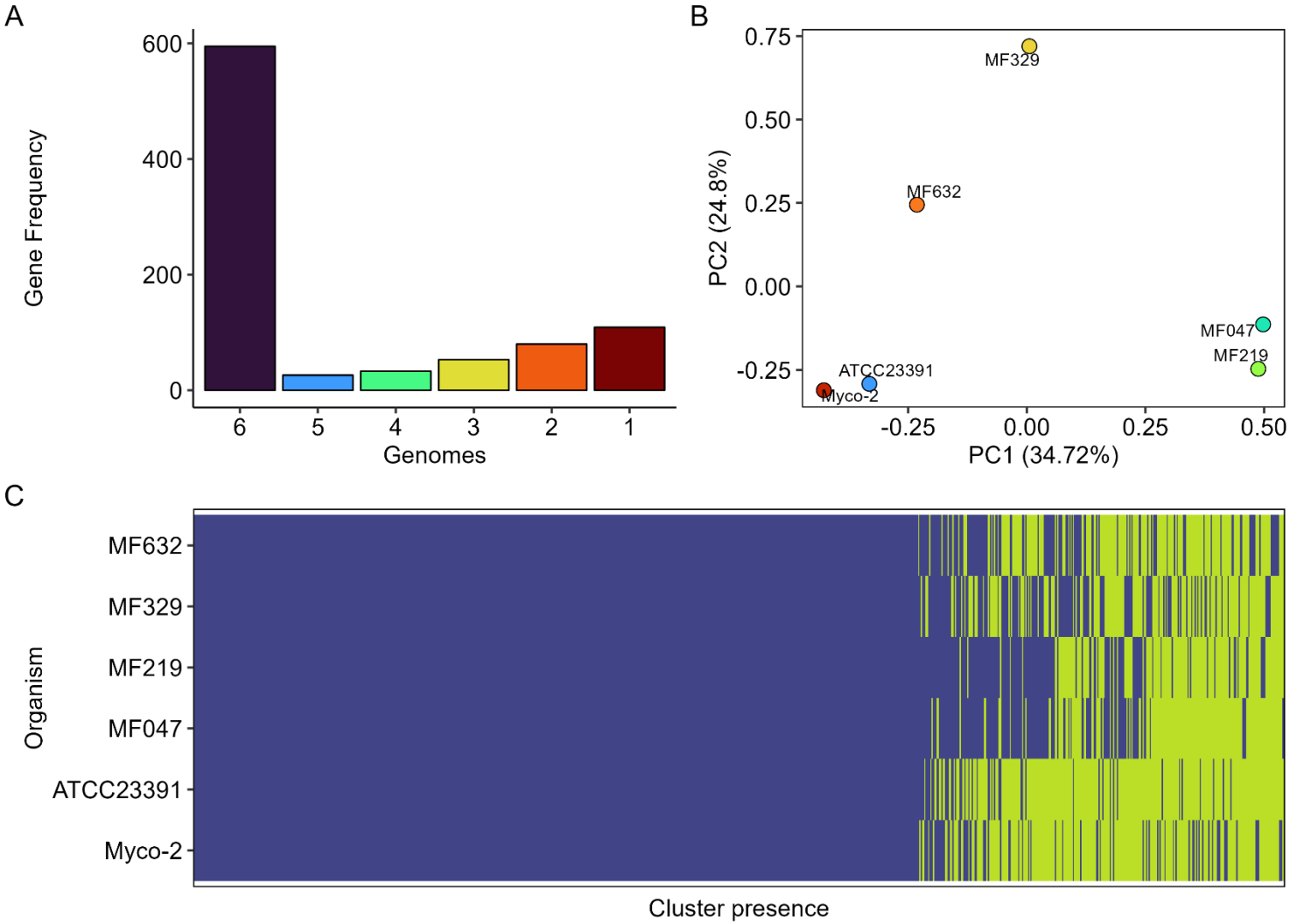


Figure 3. Analysis of the *Mycoplasma felis* core genome and gene presence/absence using Panaroo and plotted using Pagoo. A) the frequency of clustered genes within the six genomes used in the analysis; B) principal component analysis of gene presence/absence using Bray Curtis dissimilarity identifies clustering of the previously published genome sequences; C) clusters of genes present (blue) or absent (yellow) in the six genomes included in analysis.

524 **Table 5.** Coding sequences detected in the genomes of Australian *M. felis* isolates but absent in the equid *M. felis* reference strain (Myco2), and their possible species of
525 origin based on amino acid sequence identity.

Coding sequence	Possible species of origin	% Amino acid pairwise sequence identity	Predominant host
Type IV secretory system conjugative DNA transfer family protein	*M. cynos*	43.2	Dog
IS256 family transposase	*M. penetrans*	50.9	Human
Type 2 glycerol-3-phosphate oxidase (GlpO)	*M. canis*	87.2	Dog
PTS fructose transporter subunit IIA	*M. flocculare*	52.6	Swine
DHHA1 domain-containing protein	*M. canis*	84.8	Dog
M13 family peptidase	*M. canis*	65.4	Dog
1-phosphofructokinase family	*M. canis*	52.6	Dog
Anti-termination protein NusB	*M. mustelae*	61.6	Mink
Hsp70 family protein	*M. flocculare*	85.4	Pig
C5 methylase (MAV1virus-like) protein	*M. columboralis*	70.2	Pigeon
16S rRNA methyltransferase (RsmG)	*M. mustelae*	63.3	Mink
HNH endonuclease	*M. columbina*	38.0	Pigeon
Eco47II family restriction endonuclease	*M. mycoides* subsp. *mycoides* SC	49.6	Cattle
Sau3AI family type II restriction endonuclease	*M. crocodyli*	52.7	Crocodile

526

527

528 **Table 6.** Quantity and range of amino acid sequence identities and similarities of putative virulence and antimicrobial resistance genes (AMR) based on significant BLASTP
529 hits (bit scores >50) compared to the virulence factor database and CARD database, respectively.

Genome	Putative virulence genes			Putative AMR genes		
	Total	Median identity (min – max)	Median similarity (min – max)	Total	Median identity (min – max)	Median similarity (min – max)
Myco-2	48	31.7 (24.4 - 90.1)	52.6 (44.4 - 95.9)	24	29.8 (22.5 - 40.1)	51.7 (39.1 - 69.6)
MF047	49	31.6 (24.6 - 90.1)	52.7 (41.8 - 95.9)	25	29.4 (22.6 - 40.3)	50.6 (39.2 - 69.6)
MF219	49	31.6 (24.6 - 90.1)	52.4 (41.8 - 95.9)	25	29.6 (22.6 - 40.3)	50.6 (39.2 - 69.6)
MF329	50	32 (24.4 - 90.1)	52.2 (41.5 - 95.9)	25	29.7 (22.5 - 40.2)	50.6 (39.1 - 69.6)
MF632	51	31.6 (24.4 - 90.1)	52.4 (41.5 - 95.9)	26	29.7 (22.4 - 40.2)	51 (38.9 - 69.6)

530
531

532 **Table 7.** The location of predicted genomic islands in the genomes of *M. felis* isolates characterised in this study, as well as the reference genome Myco-2.

Strain	Genomic island	Start – End (relative to genome)	Length	Total number of genes (non-hypothetical)
Myco-2	1[b,d,e]	133250 - 143277	10027	11 (5)
	2[b,c,d,e]	723039 - 726091	3052	8 (6)
MF047	-	-	-	-
MF219	1[a,b,d,*]	428340 - 453515	25175	15 (7)
	2[a,b,d,e]	582071 - 602042	19971	11 (6)
MF329	1[b,c,#]	311409 - 335810	24401	19 (15)
MF632	1[a,b,d,*]	376254 - 403316	27062	19 (8)
	2[b,#]	511813 - 537933	26120	22 (15)
	3[a,b,c,d]	716455 - 726594	10139	8 (1)

533 [a, b, c, d, e] – Predicted genomic island is present with some variation, such as the presence/absence of a transposase, in (a) Myco-2, (b) MF047, (c) MF219, (d) MF329, or (e)
534 MF632, but not predicted to be a genomic island in those cases.
535 [*, #] - Predicted genomic islands with structural similarity across different genomes
536 Genomic islands determined with the Island Finder 4 web tool. None were detected in the MF047 isolate.

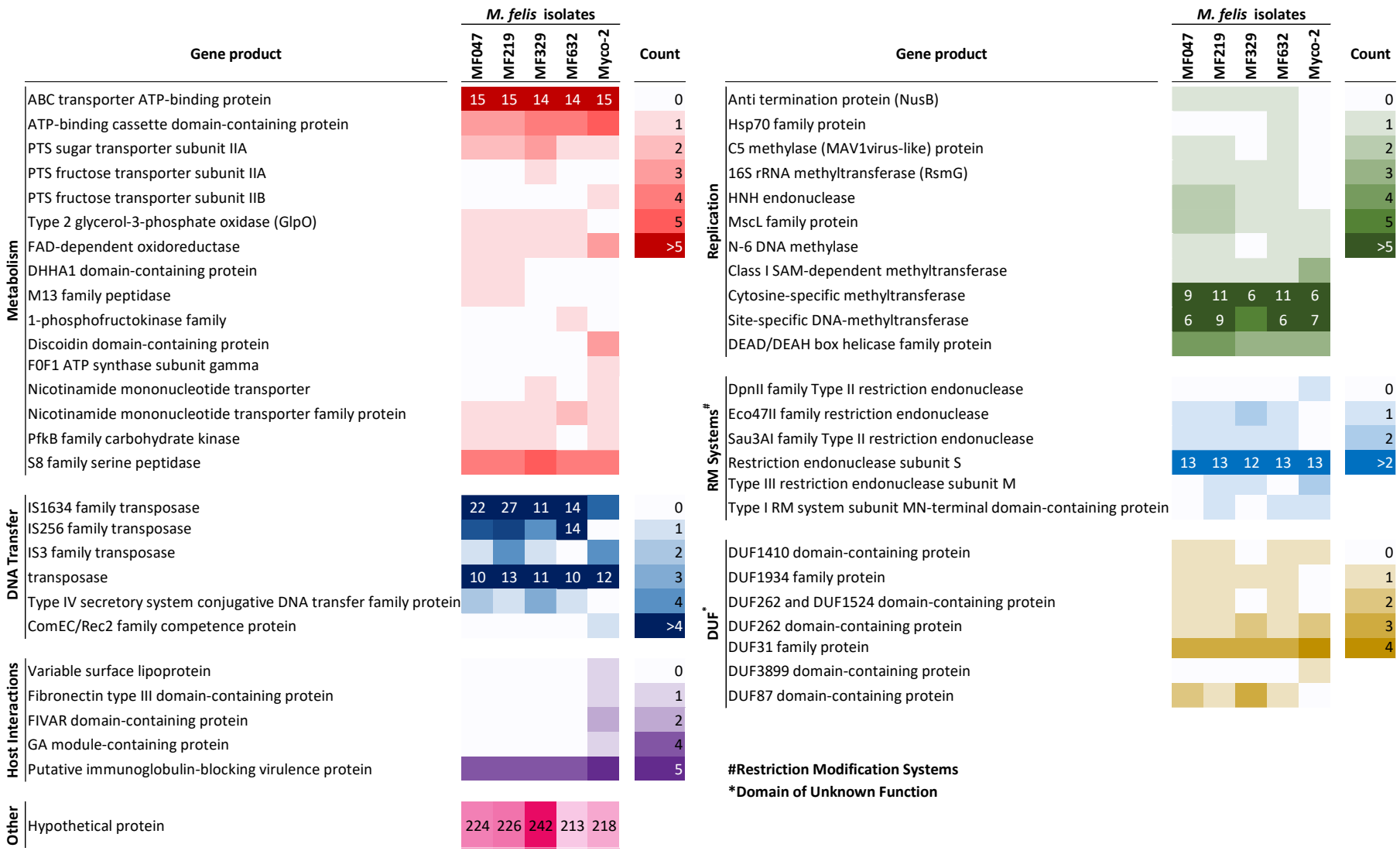
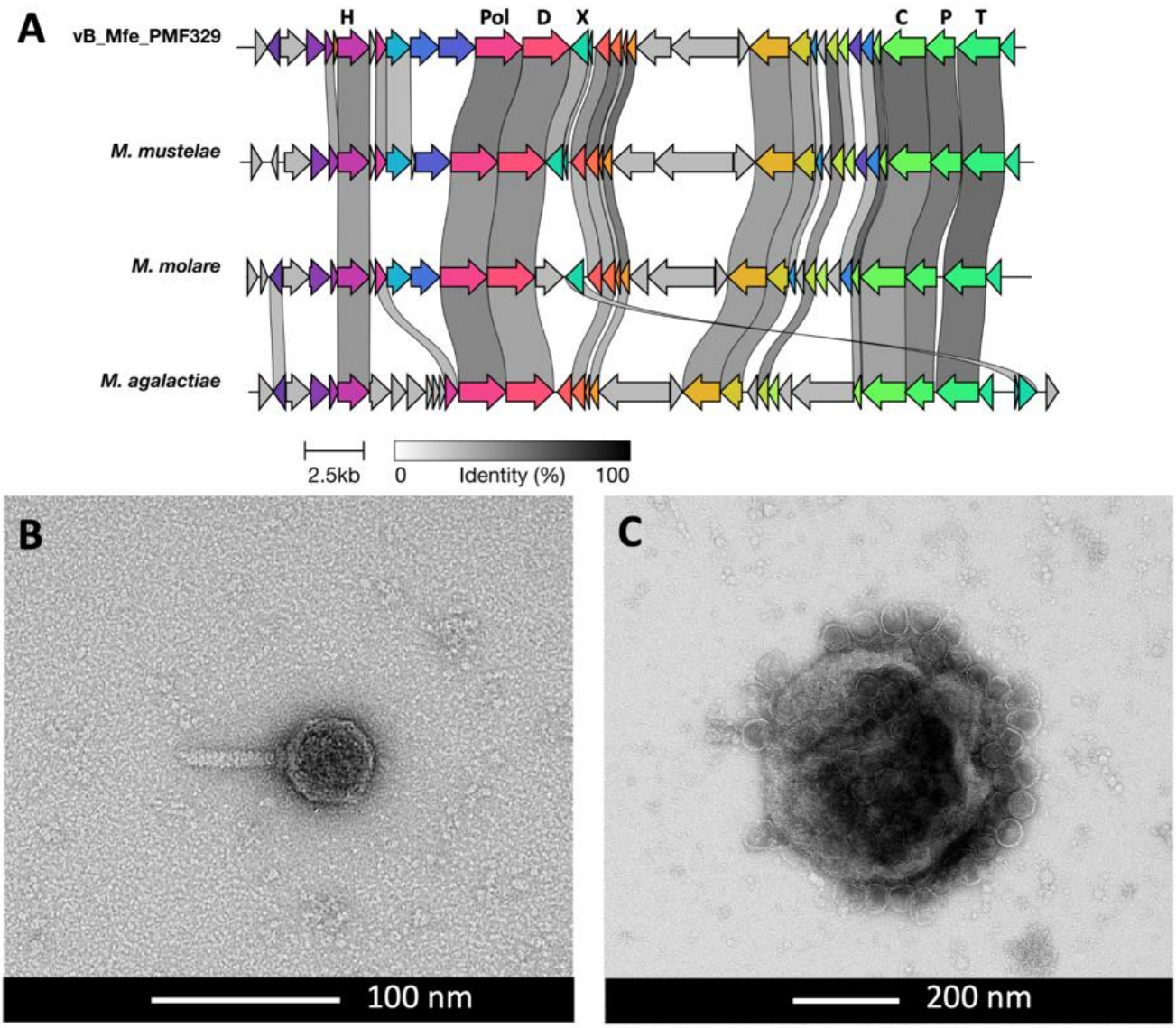


Figure 4. Presence and absence of genes within the genomes of Australian felid *M. felis* isolates compared to the genome of the Myco-2 equid *M. felis* reference strain, clustered by putative protein functions. Only genes that differ between isolates or differ from the reference are listed. Counts indicate the number of gene copies detected within each isolate.

541



542

543 **Figure 5.** A) Genome of the bacteriophage identified in *Mycoplasma felis* strain MF329 compared to MAgV1-like
544 prophage sequences found in *M. mustelae*, *M. molare* and *M. agalactiae*. Genome annotations depicting conserved
545 phage/prophage gene sequences are indicated in black. H = helicase; Pol = DNA polymerase; D = DNA primase; X = Xer
546 recombinase; C = prohead protein; P = portal protein; T = terminase; HP = hypothetical protein. B) and C) cryo-
547 transmission electron photomicrographs of bacteriophages detected in a cell lysate of *M. felis* isolate MF329, showing
548 that the phage structure consists of a contractile tail and polyhedral head.

549

550 ## References:

551 1. **Glass JI, Assad-Garcia N, Alperovich N, Yooseph S, Lewis MR et al.** Essential genes of a
552 minimal bacterium. *Proceedings of the National Academy of Sciences of the United States of America*
553 2006;103(2):425-430.
554 2. **Woese CR, Stackebrandt E, Ludwig W**. What are mycoplasmas: the relationship of tempo and
555 mode in bacterial evolution. *Journal of Molecular Evolution* 1985;21(4):305-316.
556 3. **Fadiel A, Eichenbaum KD, El Semary N, Epperson B**. Mycoplasma genomics: tailoring the
557 genome for minimal life requirements through reductive evolution. *Frontiers in Bioscience-Landmark*
558 2007;12(6):2020-2028.
559 4. **Rocha EPC, Blanchard A**. Genomic repeats, genome plasticity and the dynamics of
560 mycoplasma evolution. *Nucleic Acids Research* 2002;30(9):2031-2042.
561 5. **Shil PK, Wawegama NK, Browning GF, Noormohammadi AH, Marenda MS**. Mycoplasmas.
562 In: J.F. Prescott, A.N. Rycroft, J.D. Boyce, J.I. MacInnes, Immerseel FV et al. (editors). *Pathogenesis of*
563 *Bacterial Infections in Animals* 2022. pp. 667-700.
564 6. **Krause DC, Leith DK, Wilson RM, Baseman JB**. Identification of *Mycoplasma pneumoniae*
565 proteins associated with hemadsorption and virulence. *Infect Immun* 1982;35(3):809-817.
566 7. **Delaney NF, Balenger S, Bonneaud C, Marx CJ, Hill GE et al.** Ultrafast evolution and loss of
567 CRISPRs following a host shift in a novel wildlife pathogen, *Mycoplasma gallisepticum*. *PLOS Genetics*
568 2012;8(2):e1002511.
569 8. **Momynaliev KT, Govorun VM**. Mechanisms of genetic instability in mollicutes
570 (mycoplasmas). *Russian Journal of Genetics* 2001;37(9):979-992.
571 9. **Citti C, Dordet-Frisoni E, Nouvel LX, Kuo CH, Baranowski E**. Horizontal gene transfers in
572 mycoplasmas (mollicutes). *Current Issues in Molecular Biology* 2018;29(1):3-22.
573 10. **Klose SM, Olaogun OM, Disint JF, Shil P, Gyuranecz M et al.** Genomic diversity of a globally
574 used, live attenuated mycoplasma vaccine. *Microbiology Spectrum* 2022;10(6):e02845-02822.
575 11. **Klose SM, Shil P, Underwood GJ, Morrow CJ, Marenda MS et al.** Obg plays a significant role
576 in temperature sensitivity of *Mycoplasma synoviae* live attenuated vaccine strain MS-H. *Veterinary*
577 *Microbiology* 2023:109818.
578 12. **Klose SM, Omotainse OS, Zare S, Vaz PK, Armat P et al.** Virulence factors of *Mycoplasma*
579 *synoviae*: Three genes influencing colonization, immunogenicity, and transmissibility. *Frontiers in*
580 *Microbiology* 2022;13:1042212.
581 13. **Klose SM, De Souza DP, Disint JF, Andrews DM, Underwood GJ et al.** Reversion of mutations
582 in a live mycoplasma vaccine alters its metabolism. *Vaccine* 2023;41(21):3358-3366.
583 14. **Noormohammadi A, Markham P, Duffy M, Whithear K, Browning G**. Multigene families
584 encoding the major hemagglutinins in phylogenetically distinct mycoplasmas. *Infection and Immunity*
585 1998;66(7):3470-3475.
586 15. **Sirand-Pugnet P, Lartigue C, Marenda M, Jacob D, Barré A et al.** Being pathogenic, plastic,
587 and sexual while living with a nearly minimal bacterial genome. *PLOS Genetics* 2007;3(5):e75.
588 16. **Randolph J, Moise N, Scarlett J, Shin S, Blue J et al.** Prevalence of mycoplasmal and
589 ureaplasmal recovery from tracheobronchial lavages and of mycoplasmal recovery from pharyngeal
590 swab specimens in cats with or without pulmonary disease. *American Journal of Veterinary Research*
591 1993;54(6):897-900.
592 17. **Foster S, Barrs V, Martin P, Malik R**. Pneumonia associated with *Mycoplasma* spp in three
593 cats. *Australian Veterinary Journal* 1998;76(7):460-464.
594 18. **Sykes JE**. Feline hemotropic mycoplasmas. *Journal of Veterinary Emergency and Critical Care*
595 2010;20(1):62-69.

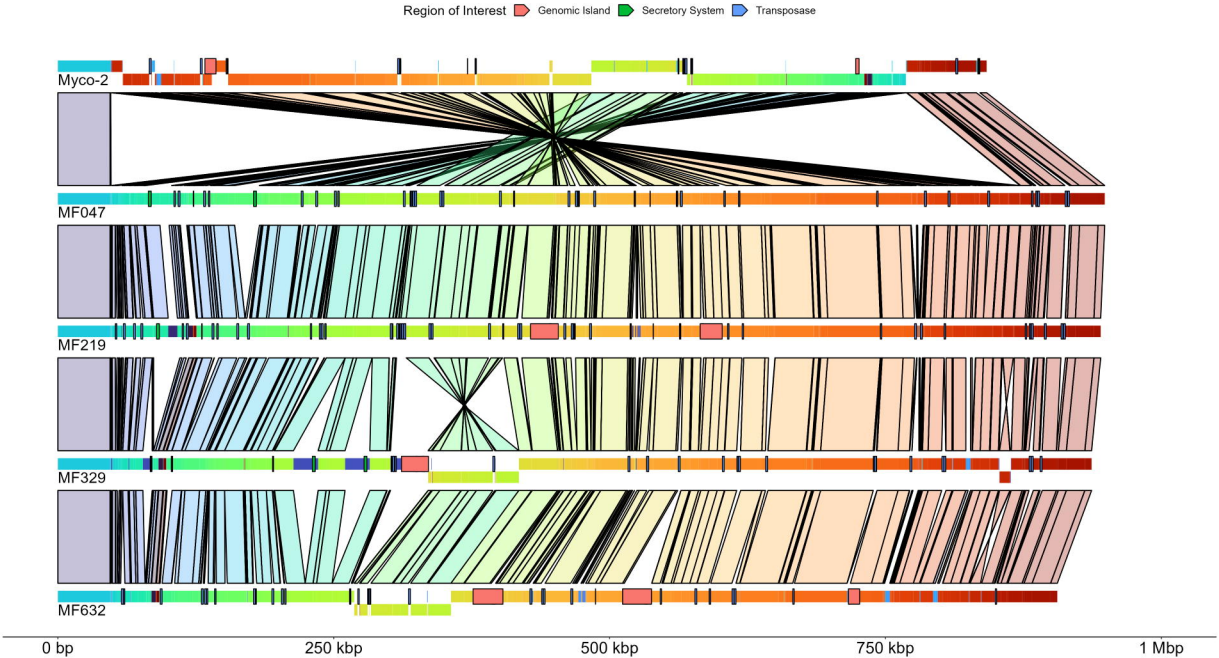
596 19. **Le Boedec K**. A systematic review and meta-analysis of the association between *Mycoplasma*
597 spp and upper and lower respiratory tract disease in cats. *Journal of the American Veterinary Medical*
598 *Association* 2017;250(4):397-407.
599 20. **Cohn LA**. Feline respiratory disease complex. *Veterinary Clinics: Small Animal Practice*
600 2011;41(6):1273-1289.
601 21. **Nguyen D, Barrs VR, Kelman M, Ward MP**. Feline upper respiratory tract infection and
602 disease in Australia. *Journal of Feline Medicine and Surgery* 2019;21(10):973-978.
603 22. **Fernandez M, Manzanilla EG, Lloret A, León M, Thibault J-C**. Prevalence of feline herpesvirus-
604 1, feline calicivirus, *Chlamydophila felis* and *Mycoplasma felis* DNA and associated risk factors in cats
605 in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis. *Journal of Feline*
606 *Medicine and Surgery* 2017;19(4):461-469.
607 23. **Lee-Fowler T**. Feline respiratory disease: what is the role of *Mycoplasma* species? *Journal of*
608 *Feline Medicine and Surgery* 2014;16(7):563-571.
609 24. **Tonozzi CC**. *Feline respiratory disease complex*. MSD Veterinary Manual 2022.
610 25. **Kinoshita Y, Niwa H, Uchida-Fujii E, Nukada T**. Complete genome sequence of mycoplasma
611 felis Strain Myco-2, isolated from an equine tracheal wash sample in Japan. *Microbiology Resource*
612 *Announcements* 2020;9(9):10.1128.
613 26. **Framst I, D`Andrea C, Baquero M, Maboni G**. Development of a long-read next generation
614 sequencing workflow for improved characterization of fastidious respiratory mycoplasmas.
615 *Microbiology* 2022;168(11).
616 27. **Frey M**. A medium for the isolation of avian mycoplasmas. *American Journal of Veterinary*
617 *Research* 1968;29:2163-2171.
618 28. **Chen S, Zhou Y, Chen Y, Gu J**. fastp: an ultra-fast all-in-one FASTQ preprocessor.
619 *Bioinformatics* 2018;34(17):i884-i890.
620 29. **Wick R**. *Filtlong v. 0.2. 0*. 2021.
621 30. **Wick RR, Judd LM, Gorrie CL, Holt KE**. Unicycler: Resolving bacterial genome assemblies from
622 short and long sequencing reads. *PLOS Computational Biology* 2017;13(6):e1005595.
623 31. **Wick RR, Holt KE**. Polypolish: Short-read polishing of long-read bacterial genome assemblies.
624 *PLOS Computational Biology* 2022;18(1):e1009802.
625 32. **Li H**. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*
626 *preprint arXiv:13033997* 2013.
627 33. **Darling AE, Mau B, Perna NT**. Progressive Mauve: multiple alignment of genomes with gene
628 flux and rearrangement. *arXiv preprint arXiv:09105780* 2009.
629 34. **Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP et al.** NCBI prokaryotic
630 genome annotation pipeline. *Nucleic Acids Research* 2016;44(14):6614-6624.
631 35. **Buchfink B, Reuter K, Drost H-G**. Sensitive protein alignments at tree-of-life scale using
632 DIAMOND. *Nature Methods* 2021;18(4):366-368.
633 36. **Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G et al.** Producing polished
634 prokaryotic pangenomes with the Panaroo pipeline. *Genome Biology* 2020;21(1):180.
635 37. **Ferrés I, Iraola G**. An object-oriented framework for evolutionary pangenome analysis. *Cell*
636 *Reports Methods* 2021;1(5):100085.
637 38. **Dhillon BK, Chiu TA, Laird MR, Langille MG, Brinkman FS**. IslandViewer update: improved
638 genomic island discovery and visualization. *Nucleic Acids Research* 2013;41(W1):W129-W132.
639 39. **Liu M, Li X, Xie Y, Bi D, Sun J et al.** ICEberg 2.0: an updated database of bacterial integrative
640 and conjugative elements. *Nucleic Acids Research* 2018;47(D1):D660-D665.

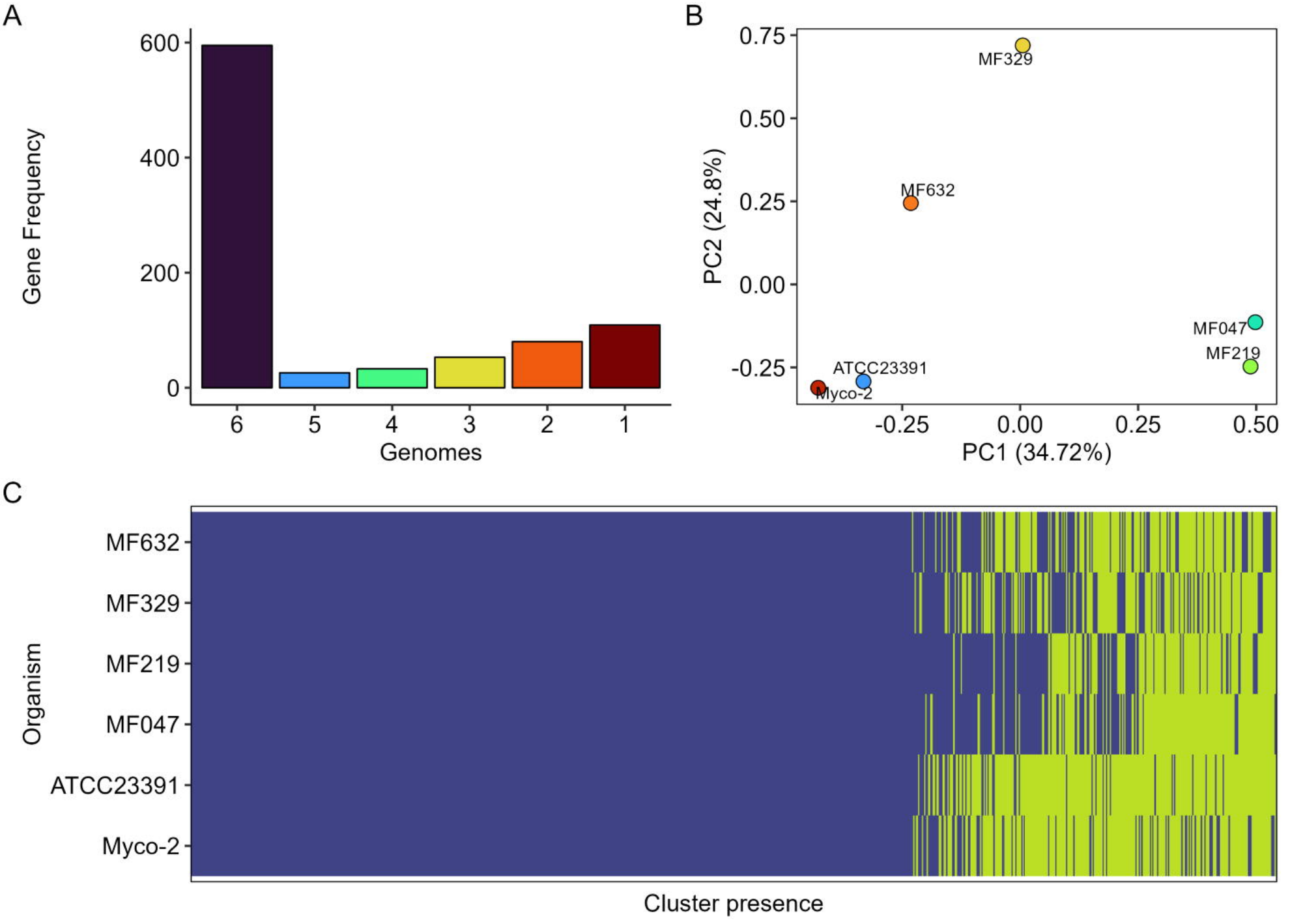
641 40. **Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR et al.** CARD 2023: expanded curation,
642 support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance
643 Database. *Nucleic Acids Research* 2023;51(D1):D690-D699.
644 41. **Liu B, Zheng D, Zhou S, Chen L, Yang J**. VFDB 2022: a general classification scheme for
645 bacterial virulence factors. *Nucleic Acids Research* 2022;50(D1):D912-D917.
646 42. **Li H**. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*
647 2018;34(18):3094-3100.
648 43. **Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M**. PhageTerm: a tool for fast and
649 accurate determination of phage termini and packaging mechanism using next-generation
650 sequencing data. *Scientific Reports* 2017;7(1):8292.
651 44. **Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW**. CheckM: assessing the
652 quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome*
653 *Research* 2015;25(7):1043-1055.
654 45. **Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H et al.** ViPTree: the viral proteomic
655 tree server. *Bioinformatics* 2017;33(15):2379-2380.
656 46. **Bin Jang H, Bolduc B, Zablocki O, Kuhn JH, Roux S et al.** Taxonomic assignment of
657 uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. *Nature Biotechnology*
658 2019;37(6):632-639.
659 47. **Citti C, Baranowski E, Dordet-Frisoni E, Faucher M, Nouvel L-X**. Genomic islands in
660 mycoplasmas. *Genes* 2020;11(8):836.
661 48. **Gourlay RN, Wyld SG, Garwes DJ**. Some properties of mycoplasma virus Br1. *Archives of*
662 *Virology* 1983;75(1):1-15.
663 49. **Gourlay RN, Wyld SG, Poulton ME**. Some characteristics of Mycoplasma virus Hr1, isolated
664 from and infectingMycoplasma hyorhinis. *Archives of Virology* 1983;77(1):81-85.
665 50. **Mahillon J, Chandler M**. Insertion sequences. *Microbiology and Molecular Biology Reviews*
666 1998;62(3):725-774.
667 51. **Frey J**. Insertion sequence analysis. In: Miles R, Nicholas R (editors). *Mycoplasma Protocols*.
668 Totowa, NJ: Humana Press; 1998. pp. 197-205.
669 52. **Lysnyansky I, Calcutt MJ, Ben-Barak I, Ron Y, Levisohn S et al.** Molecular characterization of
670 newly identified IS 3, IS 4 and IS 30 insertion sequence-like elements in *Mycoplasma bovis* and their
671 possible roles in genome plasticity. *FEMS Microbiology Letters* 2009;294(2):172-182.
672 53. **Thomas A, Linden A, Mainil J, Bischof DF, Frey J et al.** *Mycoplasma bovis* shares insertion
673 sequences with *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* SC: evolutionary
674 and developmental aspects. *FEMS Microbiology Letters* 2005;245(2):249-255.
675 54. **Loreto ELS, Ortiz MF, Porto JIR**. Insertion sequences as variability generators in the
676 *Mycoplasma hyopneumoniae* and *M. synoviae* genomes. *Genetics and Molecular Biology*
677 2007;30:283-289.
678 55. **Vilei EM, Nicolet J, Frey J**. IS*1634*, a novel insertion element creating long, variable-length
679 direct repeats which is specific for *Mycoplasma mycoides* subsp. *mycoides* small-colony type. *Journal*
680 *of Bacteriology* 1999;181(4):1319-1323.
681 56. **Baranowski E, Dordet-Frisoni E, Sagné E, Hygonenq M-C, Pretre G et al.** The integrative
682 conjugative element (ICE) of *Mycoplasma agalactiae*: key elements involved in horizontal
683 dissemination and influence of coresident ices. *mBio* 2018;9(4):10.1128.
684 57. **Alvarez-Martinez CE, Christie PJ**. Biological diversity of prokaryotic type IV secretion systems.
685 *Microbiology and Molecular Biology Reviews* 2009;73(4):775-808.
686 58. **Dordet-Frisoni E, Sagné E, Baranowski E, Breton M, Nouvel LX et al.** Chromosomal transfers
687 in mycoplasmas: when minimal genomes go mobile. *MBio* 2014;5(6):10.1128/mbio. 01958-01914.

688 59. **Hartmann AD, Hawley J, Werckenthin C, Lappin MR, Hartmann K**. Detection of bacterial and
689 viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease.
690 *Journal of Feline Medicine & Surgery* 2010;12(10):775-782.
691 60. **Ahmer BMM, Tran M, Heffron F**. The virulence plasmid of *Salmonella typhimurium* is self-
692 transmissible. *Journal of Bacteriology* 1999;181(4):1364-1368.
693 61. **Szczepanek SM, Boccaccio M, Pflaum K, Liao X, Geary SJ**. Hydrogen peroxide production from
694 glycerol metabolism is dispensable for virulence of *Mycoplasma gallisepticum* in the tracheas of
695 chickens. *Infection and Immunity* 2014;82(12):4915-4920.
696 62. **Hames C, Halbedel S, Hoppert M, Frey J, Stülke J**. Glycerol metabolism is important for
697 cytotoxicity of *Mycoplasma pneumoniae*. *Journal of Bacteriology* 2009;191(3):747-753.
698 63. **Chang T-H, Lo W-S, Ku C, Chen L-L, Kuo C-H**. Molecular evolution of the substrate utilization
699 strategies and putative virulence factors in mosquito-associated Spiroplasma species. *Genome*
700 *Biology and Evolution* 2014;6(3):500-509.
701 64. **Pilo P, Vilei EM, Peterhans E, Bonvin-Klotz L, Stoffel MH et al.** A metabolic enzyme as a
702 primary virulence factor of *Mycoplasma mycoides* subsp. *mycoides* small colony. *Journal of*
703 *Bacteriology* 2005;187(19):6824-6831.
704 65. **Khan LA, Miles RJ, Nicholas RAJ**. Hydrogen peroxide production by *Mycoplasma bovis* and
705 *Mycoplasma agalactiae* and effect of *in vitro* passage on a *Mycoplasma bovis* strain producing high
706 levels of H_2O_2. *Veterinary Research Communications* 2005;29(3):181-188.
707 66. **Szczepanek S, Tulman E, Gorton T, Liao X, Lu Z et al.** Comparative genomic analyses of
708 attenuated strains of *Mycoplasma gallisepticum*. *Infection and immunity* 2010;78(4):1760-1771.
709 67. **Tseng C-W, Chiu C-J, Kanci A, Citti C, Rosengarten R et al.** The *oppD* gene and putative
710 peptidase genes may be required for virulence in *Mycoplasma gallisepticum*. *Infection and Immunity*
711 2017;85(6):10.1128.
712 68. **Masukagami Y, Nijagal B, Mahdizadeh S, Tseng C-W, Dayalan S et al.** A combined
713 metabolomic and bioinformatic approach to investigate the function of transport proteins of the
714 important pathogen *Mycoplasma bovis*. *Veterinary Microbiology* 2019;234:8-16.
715 69. **Masukagami Y, De Souza D, Dayalan S, Bowen C, O'callaghan S et al.** Comparative
716 metabolomics of *Mycoplasma bovis* and *Mycoplasma gallisepticum* reveals fundamental differences
717 in active metabolic pathways and suggests novel gene annotations. *MSystems*
718 2017;2(5):10.1128/msystems. 00055-00017.
719 70. **Mahdizadeh S, Masukagami Y, Tseng C-W, Markham PF, De Souza DP et al.** A *Mycoplasma*
720 *gallisepticum* glycerol ABC transporter involved in pathogenicity. *Applied and Environmental*
721 *Microbiology* 2021;87(11):e03112-03120.
722 71. **Chen H, Yu S, Shen X, Chen D, Qiu X et al.** The *Mycoplasma gallisepticum* α-enolase is cell
723 surface-exposed and mediates adherence by binding to chicken plasminogen. *Microbial*
724 *Pathogenesis* 2011;51(4):285-290.
725 72. **Vilei EM, Frey J**. Genetic and biochemical characterization of glycerol uptake in *Mycoplasma*
726 *mycoides* subsp. *mycoides* SC: Its impact on H_2O_2 production and virulence. *Clinical Diagnostic*
727 *Laboratory Immunology* 2001;8(1):85-92.
728 73. **Shterzer N, Mizrahi I**. The animal gut as a melting pot for horizontal gene transfer. *Canadian*
729 *Journal of Microbiology* 2015;61(9):603-605.
730 74. **Norman A, Hansen LH, Sørensen SJ**. Conjugative plasmids: vessels of the communal gene
731 pool. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2009;364(1527):2275-
732 2289.

733 75. **Oliveira PH, Touchon M, Rocha EP**. The interplay of restriction-modification systems with
734 mobile genetic elements and their prokaryotic hosts. *Nucleic Acids Research* 2014;42(16):10618-
735 10631.
736 76. **Dordet-Frisoni E, Vandecasteele C, Contarin R, Sagné E, Baranowski E et al.** Impacts of
737 *Mycoplasma agalactiae* restriction-modification systems on pan-epigenome dynamics and genome
738 plasticity. *Microbial Genomics* 2022;8(5).
739 77. **Oliveira PH, Touchon M, Rocha EPC**. Regulation of genetic flux between bacteria by
740 restriction–modification systems. *Proceedings of the National Academy of Sciences*
741 2016;113(20):5658-5663.
742 78. **Chen I, Dubnau D**. DNA uptake during bacterial transformation. *Nature Reviews Microbiology*
743 2004;2(3):241-249.
744 79. **Kapteijn R, Shitut S, Aschmann D, Zhang L, de Beer M et al.** Endocytosis-like DNA uptake by
745 cell wall-deficient bacteria. *Nature Communications* 2022;13(1):5524.
746 80. **Pimentel ZT, Zhang Y**. Evolution of the natural transformation protein, ComEC, in bacteria.
747 *Frontiers in Microbiology* 2018:2980.
748 81. **Wang Y, Huang JM, Wang SL, Gao ZM, Zhang AQ et al.** Genomic characterization of symbiotic
749 mycoplasmas from the stomach of deep-sea isopod *Bathynomus* sp. *Environmental Microbiology*
750 2016;18(8):2646-2659.
751 82. **Christner M, Franke GC, Schommer NN, Wendt U, Wegert K et al.** The giant extracellular
752 matrix-binding protein of *Staphylococcus epidermidis* mediates biofilm accumulation and attachment
753 to fibronectin. *Molecular Microbiology* 2010;75(1):187-207.
754 83. **Speziale P, Pietrocola G, Foster TJ, Geoghegan JA**. Protein-based biofilm matrices in
755 staphylococci. *Frontiers in cellular and infection microbiology* 2014;4:171.
756 84. **Ledger L, Eidt J, Cai HY**. Identification of antimicrobial resistance-associated genes through
757 whole genome sequencing of *Mycoplasma bovis* isolates with different antimicrobial resistances.
758 *Pathogens* 2020;9(7):588.
759 85. **Mahdizadeh S, Sansom FM, Lee S-W, Browning GF, Marenda MS**. Targeted mutagenesis of
760 *Mycoplasma gallisepticum* using its endogenous CRISPR/Cas system. *Veterinary Microbiology*
761 2020;250:108868.
762 86. **Pereyre S, Goret J, Bébéar C**. *Mycoplasma pneumoniae*: current knowledge on macrolide
763 resistance and treatment. *Frontiers in Microbiology* 2016;7:974.
764 87. **Dégrange S, Renaudin H, Charron A, Pereyre S, Bébéar C et al.** Reduced susceptibility to
765 tetracyclines is associated *in vitro* with the presence of 16S rRNA mutations in *Mycoplasma hominis*
766 and *Mycoplasma pneumoniae*. *Journal of Antimicrobial Chemotherapy* 2008;61(6):1390-1392.
767 88. **Shimada Y, Deguchi T, Nakane K, Yasuda M, Yokoi S et al.** Macrolide resistance–associated
768 23S rRNA mutation in *Mycoplasma genitalium*, Japan. *Emerging Infectious Diseases* 2011;17(6):1148.
769 89. **Kovács ÁB, Wehmann E, Sváb D, Bekő K, Grózner D et al.** Novel prophage-like sequences in
770 *Mycoplasma anserisalpingitidis*. *Infection, Genetics and Evolution* 2021;92:104886.
771 90. **Turner D, Shkoporov AN, Lood C, Millard AD, Dutilh BE et al.** Abolishment of morphology-
772 based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial
773 viruses subcommittee. *Archives of Virology* 2023;168(2):74.
774 91. **Röske K, Calcutt MJ, Wise KS**. The *Mycoplasma fermentan*s prophage ϕMFV1: genome
775 organization, mobility and variable expression of an encoded surface protein. *Molecular*
776 *Microbiology* 2004;52(6):1703-1720.
777

NZ_AP022325-Myco-2
NZ_JNKA00000000-ATCC_23391
MF047
MF219
MF329
MF632
NZ_CP103993-16-057065-4_34x
NZ_CP103988-16-057065-4_105x
NZ_CP110269-16-057065-4_268x
NZ_CP104194-16-040612-4_58x
NZ_CP104193-16-040612-4_153x
NZ_CP110270-16-040612-4_167x
NZ_CP104192-16-040612-4_295x
Frequency
0
200
400
600
0.01
0.10
1.00
Coding Ratio

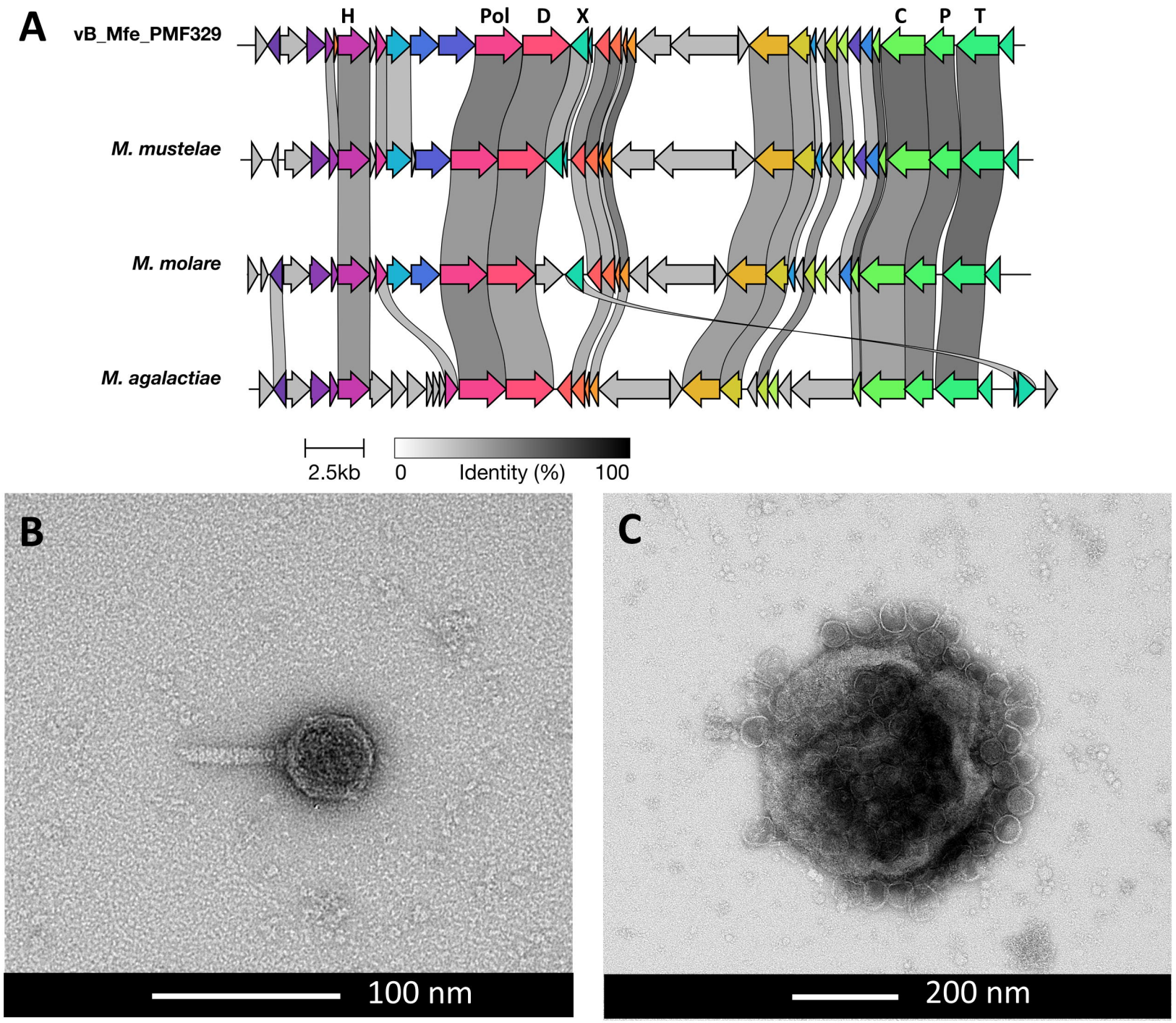

Region of Interest
Genomic Island
Secretory System
Transposase
Myco-2
MF047
MF219
MF329
MF632
0 bp
250 kbp
500 kbp
750 kbp
1 Mbp


A
Gene Frequency
600
400
200
0
6
5
4
3
2
1
Genomes
B
PC2 (24.8%)
0.75
0.50
0.25
0.00
-0.25
MF329
MF632
MF047
MF219
ATCC23391
Myco-2
-0.25
0.00
0.25
0.50
PC1 (34.72%)
C
Organism
MF632
MF329
MF219
MF047
ATCC23391
Myco-2
Cluster presence

	Gene product	*M. felis* isolates					Count
		MF047	MF219	MF329	MF632	Myco-2	
Metabolism	ABC transporter ATP-binding protein	15	15	14	14	15	0
	ATP-binding cassette domain-containing protein						1
	PTS sugar transporter subunit IIA						2
	PTS fructose transporter subunit IIA						3
	PTS fructose transporter subunit IIB						4
	Type 2 glycerol-3-phosphate oxidase (GlpO)						5
	FAD-dependent oxidoreductase						>5
	DHHA1 domain-containing protein						
	M13 family peptidase						
	1-phosphofructokinase family						
	Discoidin domain-containing protein						
	F0F1 ATP synthase subunit gamma						
	Nicotinamide mononucleotide transporter						
	Nicotinamide mononucleotide transporter family protein						
	PfkB family carbohydrate kinase						
	S8 family serine peptidase						
DNA Transfer	IS1634 family transposase	22	27	11	14		0
	IS256 family transposase				14		1
	IS3 family transposase						2
	transposase	10	13	11	10	12	3
	Type IV secretory system conjugative DNA transfer family protein						4
	ComEC/Rec2 family competence protein						>4
Host Interactions	Variable surface lipoprotein						0
	Fibronectin type III domain-containing protein						1
	FIVAR domain-containing protein						2
	GA module-containing protein						4
	Putative immunoglobulin-blocking virulence protein						5
Other	Hypothetical protein	224	226	242	213	218	

	Gene product	*M. felis* isolates					Count
		MF047	MF219	MF329	MF632	Myco-2	
Replication	Anti termination protein (NusB)						0
	Hsp70 family protein						1
	C5 methylase (MAV1virus-like) protein						2
	16S rRNA methyltransferase (RsmG)						3
	HNH endonuclease						4
	MscL family protein						5
	N-6 DNA methylase						>5
	Class I SAM-dependent methyltransferase						
	Cytosine-specific methyltransferase	9	11	6	11	6	
	Site-specific DNA-methyltransferase	6	9		6	7	
	DEAD/DEAH box helicase family protein						
RM Systems#	DpnII family Type II restriction endonuclease						0
	Eco47II family restriction endonuclease						1
	Sau3AI family Type II restriction endonuclease						2
	Restriction endonuclease subunit S	13	13	12	13	13	>2
	Type III restriction endonuclease subunit M						
	Type I RM system subunit MN-terminal domain-containing protein						
DUF*	DUF1410 domain-containing protein						0
	DUF1934 family protein						1
	DUF262 and DUF1524 domain-containing protein						2
	DUF262 domain-containing protein						3
	DUF31 family protein						4
	DUF3899 domain-containing protein						
	DUF87 domain-containing protein						

#Restriction Modification Systems
***Domain of Unknown Function**


A
vB_Mfe_PMF329
M. mustelae
M. molare
M. agalactiae
H
Pol
D
X
C
P
T
2.5kb
0
Identity (%)
100
B
100 nm
C
200 nm