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1 **High inter-species and low intra-species variation in 16S-23S rDNA spacer**
2 **sequences of pathogenic avian mycoplasmas offers potential use as a**
3 **diagnostic tool**

4

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27 **Abstract**

28 In order to investigate its value for phylogenetic analysis, species
29 characterisation and diagnosis, the 16S-23S rDNA intergenic spacer regions (ISRs) of
30 the type strain of 23 avian *Mycoplasma* species were amplified and the sequences
31 determined. Also sequenced were the reference strains of *Mycoplasma iowae* serotypes
32 J, K, N, Q and R and a number of field strains of *M. synoviae*, *M. gallisepticum*, *M.*
33 *meleagridis* and *M. iowae*. The ISRs demonstrated a high level of size variation (178-
34 2488 bp) between species and did not include tRNA genes. Phylogenetic analysis
35 performed using the information conflicted with that based on the 16S rDNA and was
36 therefore not helpful for phylogenetic studies. However the ISR did appear to be of
37 value for determining species since there was high inter-species variation between all 23
38 avian *Mycoplasma* species, and in addition there was low intra-species variation, at least
39 in the four pathogenic species. It could also be very useful as additional information in
40 the description of a new species and as a target for species-specific PCRs.

41

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44

45 *Keywords* Mollicutes, *Mycoplasma*, intergenic spacer region, phylogeny,

46 **1. Introduction**

47 There are 23 recognised avian *Mycoplasma* species of which four, *Mycoplasma*
48 *gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae*
49 are recognised as economically important poultry pathogens. They belong to the class
50 *Mollicutes* and are characterized by a lack of cell wall and for being the smallest self-
51 replicating prokaryotes (Bradbury, 2001).

52 All self-replicating cells contain ribosomes, consisting of ribosomal proteins and
53 ribosomal RNA (rRNA). Bacteria have between one and 15 rRNA operons, the
54 common organization being 16S rRNA-23S rRNA-5S rRNA with an intergenic spacer
55 region (ISR) separating each rRNA gene. Sequence variation in 16S-23S ISRs among
56 relatively close bacterial taxa is high for some bacterial genera (Gütler, 1999).

57 In the genus *Mycoplasma* knowledge of the number and organization of the
58 rRNA operons is limited but the most common number is one or two (Johansson et al.,
59 1998; Razin et al., 1998) and unlike other bacteria no tRNAs have been found in the
60 16S-23S rRNA ISR (Harasawa et al., 1992; Johansson et al., 1998, Razin et al., 1998;
61 Gütler, 1999). ISRs from many *Mycoplasma* spp. have been sequenced (Harasawa et al.,
62 1992, 2000, 2004; Harasawa, 1999; Volokov et al., 2006) and although the region was
63 not useful for differentiating between the ‘mycoides cluster’ mycoplasmas (Harasawa et
64 al., 2000), it was valuable in supporting separation of two biovars of *Ureaplasma*
65 *urealyticum* into distinct species: *U. urealyticum* and *Ureaplasma parvum* (Harasawa
66 and Kanamoto, 1999; Robertson et al., 2002).

67 Our goal was to explore the value of the ISR of avian mycoplasmas in
68 phylogenetic studies and for its possible diagnostic use. A similar study was reported by
69 Chalker and Brownlie (2004) for canine mycoplasmas. We designed a primer set to
70 obtain PCR products containing the entire ISR of all the avian *Mycoplasma* type strains.

71 The ISRs were sequenced and a phylogenetic tree constructed. Inter-species
72 differentiation of avian mycoplasmas using the ISR was evaluated and compared with
73 results obtained using 16S rDNA sequences.

74

75 2. Materials and methods

76

77 2.1. Organisms and culture conditions

78 The 23 avian *Mycoplasma* species (type strains) used are named in Table 1.
79 They were cultivated in mycoplasma broth and on mycoplasma agar plates (Bradbury,
80 1977) at 37°C for between one and 5 days, in a CO₂ rich atmosphere. They had been
81 filter-cloned through membranes of pore size 0.45 µm (Tully, 1983) and their identity
82 and purity confirmed by the indirect immunofluorescence (Rosendal and Black, 1972).
83 In addition to the type strains five other *M. iowae* serotypes (reference strains J, K, N, Q
84 and R), 21 field strains of *M. synoviae*, 11 of *M. gallisepticum*, 16 of *M. meleagridis* and
85 16 of *M. iowae* were examined.

86

87 2.2. DNA preparation and PCR

88 DNA was extracted from broth cultures using Chelex resin as described
89 elsewhere (Harasawa et al., 2004). To obtain the complete ISR sequence the PCR
90 protocol described by Harasawa et al. (2004) was used in a DNA thermal cycler
91 (Perkin-Elmer) but with different primers. In the present study the forward primer was
92 5'- CGT TCT CGG GTC TTG TAC AC-3' and the reverse primer was 5'-CGC AGG
93 TTT GCA CGT CCT TCA TCG-3'. DNA amplification was accomplished with 5
94 cycles of denaturation at 94 °C for 15 s, renaturation at 60 °C for 30 s, and elongation at

95 72 °C for 2 min, followed by 30 cycles with the same parameters, except for an
96 extension of 2 s per cycle in the elongation step.

97

98 2.3. *Sequencing and sequence analysis*

99 The ISRs of the type strains of 21 species were sequenced and analysed, the type
100 strains of *M. gallisepticum* and *M. imitans* having been sequenced in previous work
101 (Harasawa et al., 2004). The relevant accession numbers are in Table 1.

102 Sequencing was conducted by Imperial College, London (UK) and was
103 determined for each strand of DNA. The resulting chromatograms were examined in
104 Chromas (version 1.45; School of Health Science, Griffith University, Australia). The
105 forward sequence and reverse complimented sequences of all the type strains were
106 compared to produce a consensus sequence using Generunner (version 3.05; Hastings
107 Software, Inc.). All the sequences were then aligned automatically with Clustal W and
108 then the alignment was completed manually using Bioedit version 7.0.0 (Hall, 1999).

109

110 2.4. *Cloning*

111 Since the sequence of the *M. synoviae* DNA was impossible to elucidate due to
112 the presence of heterogenous sequences this mycoplasma was amplified using *Pfu*
113 polymerase (Stratagene) for cloning. The blunt ended ISR fragments were ligated into
114 *Sma* I digested PuC 18 (modified to add an extra *Eco*R1 site for screening purposes) in
115 the presence of *Sma* I (Invitrogen), using *T4* DNA ligase (Fermentans). The resultant
116 mixture was transformed into Library Efficiency Competent *E. coli* DH5 α (Invitrogen),
117 and the recombinants were selected according to the standard blue-white cloning
118 procedure (Sambrook et al., 1989). The selected clones were grown in LB broth
119 containing ampicillin (100 μ g ml⁻¹), and the plasmids were purified with DNA Spin

120 MiniPrep Kit (Qiagen) according to the manufacturer's instructions. The size of the
121 inserts was confirmed by 1.5% agarose gel electrophoresis after *EcoR*I enzyme (Gibco
122 BRL) digestion. At least five selected clones were then sequenced and analysed.

123

124 2.5. *Percent DNA homology*

125 The percent homology between the ISRs of all 23 species and also of their 16S
126 rDNA sequences were determined using the program Bioedit version 7.0.0 (Hall, 1999).
127 The accession numbers of the 16S rDNA sequences used are shown in Table 1. The
128 putative transposase gene sequence inserted in the ISR of *M. imitans* (Harasawa et al.,
129 2004) was not included in this analysis. In addition, the other *M. iowae* serotypes and all
130 the field strains of *M. synoviae*, *M. gallisepticum*, *M. meleagridis* and *M. iowae*
131 sequenced in this study, were compared for percent homology within species for
132 checking the intra-species variability.

133

134 2.6. *Statistical analysis*

135 Statistical analysis was performed to compare the inter-species sequence
136 homology measures obtained from the ISR with the 16S rDNA using Minitab (version
137 13.20; Minitab Inc, State Collage, Pennsylvania. USA). Pearson (r^2) correlation
138 coefficient (range from -1 to +1) was calculated to quantify the accuracy of linear
139 correlation. A p value of $p < 0.05$ was considered statistically significant.

140

141 2.7. *Phylogeny*

142 A phylogenetic tree was built using the type strains based on the ISR sequence
143 information and compared with that based on the 16S rRNA gene. Evolutionary
144 distances were calculated by using the model of Jukes and Cantor, and the neighbour-

145 joining method was used for constructing a phylogenetic tree. The robustness for
146 individual branches was estimated by bootstrapping with 1,000 replications. All these
147 programs are included in the phylogenetic program package PHYLIP version 3.4
148 (Felsenstein, 1989).

149

150 **3. Results**

151

152 *3.1. PCR of the 16S-23S rDNA ISR*

153 The amplification with the newly-designed primers yielded a single product in
154 each case (Fig. 1). The amplicon comprised the entire ISR and approximately 164 bp of
155 the 3' end of the 16S rRNA gene and approximately 71 bp of the 5' end of the 23S
156 rRNA gene. The PCR products generated from the avian *Mycoplasma* species were
157 between 400-600 bp with the exception of *M. gallisepticum* (~1000 bp) and *M. imitans*
158 (~3000 bp).

159

160 *3.2. Sequence analysis*

161 ISR sequences of the 21 avian *Mycoplasma* type strains analysed ranged from
162 178 bp to 341 bp (Table 1) with 16 of them being between 250 and 350 bp. The
163 Genbank/EMBL accession numbers for the ISR sequences of the 21 avian mycoplasma
164 species determined here are AJ780982 to AJ781002.

165 The alignment of the ISR sequences from the 21 species demonstrated a marked
166 inter-species variation in sequence as well as in length (supplementary data 1). *M.*
167 *gallisepticum* and *M. imitans* were not included due to the length of their ISRs (Table 1
168 and Fig. 1). The ISRs contained conserved and non-conserved regions, comprising five
169 regions in the following order: a 5' hypervariable region, box B, an interbox

170 hypervariable region, box A, and a 3' hypervariable region (supplementary data 1). Box
171 A- and box B-like sequences were found in all the ISRs analysed (Figure 2). No tRNA
172 genes were found in this region.

173 The locus of the ISRs examined in this study, were not assigned. However, some
174 polymorphisms were detected in *M. columbinasale*, *M. columbinum*, *M. columborale*,
175 *M. corogypsi*, *M. gallinaceum*, *M. pullorum* and *M. synoviae* and insertion/deletions in
176 *M. columborale*, *M. pullorum* and *M. synoviae* (Table 1).

177

178 3.3. Percent DNA homology

179 ISR and 16S rDNA sequence homology for the avian *Mycoplasma* species can
180 be seen as supplementary data 2. High levels of homology ($\geq 80\%$ in ISR and $> 95\%$ in
181 the 16S rDNA) were seen in both regions with *M. anseris* and *M. falconis* and *M.*
182 *cloacale*, with *M. columbinum* and *M. iners* and with *M. buteonis*, *M. glycyphilum* and
183 *M. gallopavonis*.

184 Intra-species variation was not seen in *M. iowae*. All the serovars and also the
185 16 field strains were identical. The identity between field strains within the species *M.*
186 *synoviae*, *M. gallisepticum* and *M. meleagridis* strains was at least 96.1%, 94 % and
187 97.2% respectively (data not shown).

188

189 3.4. Statistical analysis

190 There was a significant strong positive linear relationship ($r^2 = 0.708$, $p < 0.05$)
191 between the sequence homology measures of the ISR and the 16S RNA gene.

192

193 3.5. Phylogenetic trees

194 The resulting trees from the ISR and the 16S rDNA sequences are seen in Figs. 3 and 4.
195 The named clusters and groups shown on both figures are those derived for the 16S
196 rRNA gene (Pettersson et al., 2000 and 2001; Weisburg et al., 1989). The tree based on
197 the ISR sequences did not support the 16S phylogeny.

198

199 **4. Discussion**

200 Avian *Mycoplasma* species yielded a PCR product between 400 and 3000 bp.
201 Most of the species were between 400-600 bp with the exception of *M. gallisepticum*
202 and *M. imitans*, confirming the earlier observations of Harasawa et al. (2004) where a
203 putative transposase gene was identified inserted into the ISR of *M. imitans*.

204 Box A- and box B-like structures have been identified previously for
205 mycoplasmas by Harasawa and co-workers (Harasawa et al., 1992; Harasawa 1999;
206 Harasawa and Kanamoto, 1999; Harasawa et al., 2000; Harasawa et al., 2004). These
207 sites were found in all the type strains analysed in the present study, although box B was
208 absent in *M. imitans* and *M. gallisepticum* (Harasawa et al., 2004). Box A is an
209 antiterminator site, localized in the ISR, which prevents premature termination of
210 transcription in the rRNA operon. For this reason, its sequence is relatively well
211 conserved in bacteria. Box B (with more variable sequence) is another antiterminator
212 site and upstream of box A, but may not be essential for the control of transcription
213 (Condon et al., 1995). The function of these conserved regions is also thought to be the
214 formation of double-stranded processing stems involved in the maturation of 16S and
215 23S rRNA (Pérez Luz et al., 1998).

216 The fact that no tRNA genes were found in this region in any of the analysed
217 avian strains, confirms earlier observations by others on mammalian *Mycoplasma*

218 species (Harasawa et al., 1992; Johansson et al., 1998; Razin et al., 1998; Harasawa et
219 al., 1999).

220 When there is more than one operon they are often not identical (intercistronic
221 heterogeneity), particularly in relation to the ISR (Condon et al., 1995). When
222 mycoplasmas have more than one rRNA operon, polymorphism (sequence differences)
223 or sequence length variation in poly A regions can appear if the primers have amplified
224 more than one operon (Johansson et al., 1998). In our study, we assumed no primer
225 mismatch within the operons, since universal primers were used. Therefore, the locus of
226 the spacer sequence determined could not be identified. Polymorphisms and sequence
227 length variations in the ISR between the 16S and 23S rRNA genes were found in some
228 of the avian *Mycoplasma* species. Polymorphisms were detected in seven of the species
229 analysed and insertion/deletions in three (Table 1). In this situation, the sequences were
230 easy to determine as both DNA strands were analyzed, only in the case of *M. synoviae*
231 was cloning needed to obtain the sequences. This suggests that, at least in these
232 mycoplasmas, there is more than one copy (operon) of the ISR but further studies are
233 needed to confirm this. Allen et al. (2005) have recently shown that *M. synoviae* has
234 two rRNA operons in the same orientation. *M. gallisepticum*, on the other hand,
235 contains a single ISR between the 16S and the 23S rRNA genes, because, despite
236 having two sets of rRNA genes in its genome, only one of them is organized in an
237 operon cluster (Papazisi et al., 2003).

238 Variability of the ISR among field strains within a single *Mycoplasma* species
239 has not been well documented. Eight isolates of *M. pulmonis* were found to be identical
240 (Harasawa, 1999) and complete homology was found among 21 isolates of *M.*
241 *pneumoniae* (Dorigo Zetsma, et al., 2000) and strains of *U. urealyticum* and of *U.*
242 *parvum* were found to be identical (Harasawa and Kanamoto, 1999; Robertson et al.,

243 2002). Other authors (Vолоkov et al., 2006) analysing sequences of *M. hominis*, *M.*
244 *arginini*, *M. bovis*, *M. genitalium*, *M. pneumoniae*, *M. hyorhinis* and *M. felis* obtained
245 from GenBank found 98-100% homology within species. In our study all 22 *M. iowae*
246 strains examined were identical, while the homology of strains within the species *M.*
247 *synoviae*, *M. gallisepticum* and *M. meleagridis* strains was at least 96.1%, 94 % and a
248 97.2% respectively. Despite some intra-species variability the information coded in the
249 ISR appears to be useful in differentiating between the avian *Mycoplasma* species,
250 because the maximum and minimum percent homology found between the 23 species
251 studied were 90.5% and 7.9% respectively, having a mean of 50.1%, while the
252 equivalent data obtained from the 16S rRNA gene were 99.8% and 74.2 with a mean of
253 86.5%. There was a significant strong positive linear relationship between percent
254 homology of ISR sequences and those of the 16S RNA gene, indicating a strong
255 correlation. The sign of the coefficient (+) indicates the direction of the relationship. In
256 this case both variables tend to increase together, so the coefficient is positive.

257 Using the ISR as a phylogenetic marker in this study and comparing the results
258 with the 16S rDNA data (Fig. 3-4), we see that the phylogenies are not identical, as was
259 previously observed by Harasawa (1999) using other *Mycoplasma* species. According to
260 the 16S rRNA-based phylogeny, members of the hominis and pneumonia groups form
261 two distinct monophyletic clusters (Pettersson et al., 2000 and 2001; Weisburg et al.,
262 1989) but this is not the case in the ISR-based tree. However Chalker and Brownlie, in
263 their comparison of canine mycoplasmas by 16S rDNA and ISR-based phylogeny,
264 reported that the latter gave a 'good indication of taxonomic positioning in most cases'.

265 As expected, percent homologies between avian *Mycoplasma* species in the ISR
266 were lower than those of the 16S rDNA. The great variability in sequences and length
267 of the ISR make it difficult to elucidate phylogenetic relationships between distantly

268 related organisms and therefore the value of ISR sequences for database purposes is
269 reduced together with their value for identification or phylogenetic analysis of unknown
270 organisms (Yoon et al., 1997). However when dealing with more closely related
271 organisms the same features could make them good markers for bacterial classification
272 (Pérez-Luz et al., 1998). Indeed Harasawa et al. (1999, 2000) suggested that the spacer
273 region could be more useful in the comparison of closely related species or subspecies,
274 whilst 16S rRNA gene is more useful as a chronometer for phylogenetic analysis.

275 Recently some PCRs based on the ISR have been described. It has been stated to
276 minimize the possibility of cross-reaction, improving diagnostic accuracy over PCRs
277 based on the 16S rRNA gene (Takahashi-Omoe et al., 2004). The ISR has been the
278 target of PCRs to detect mycoplasmas in cell cultures (Harasawa et al., 1993), *M.*
279 *pulmonis* in clinical isolates (Takahashi-Omoe et al., 2004) and *M. felis* in clinical
280 samples (Chalker et al., 2004). It has also been used to identify phytoplasmas in infected
281 plants (Smart et al., 1996) and recently, a diagnostic *M. synoviae* PCR has given very
282 good results (Ramírez et al., 2006).

283 From the present study it can be concluded that the ISR of avian mycoplasmas is
284 not helpful for phylogenetic studies between species at least between avian *Mycoplasma*
285 species. However, it could be very useful in providing additional information in the
286 description of a new species and as a target for species-specific PCRs.

287

288 **Nucleotide sequence accession numbers.** The Genbank/EMBL accession numbers for
289 the 16S-23S rDNA ISR sequences of the 21 avian mycoplasma species determined here
290 are AJ780982 to AJ781002 (Table 1).

291

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295

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297

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396 Table 1: Avian *Mycoplasma* species, the length of the intergenic spacer region (ISR),
 397 the polymorphisms found and the GeneBank accession numbers for the ISR and 16S
 398 rDNA
 399

Species	Strain ^a	ISR (n)	Position of polymorphic sites ^b	GenBank accession no (ISR) ^c	GenBank accession no (16S rDNA)
<i>M. anatis</i>	1340 ^T	269	-	AJ780982	AF412970
<i>M. anseris</i>	1219 ^T	178	-	AJ780983	AF125584
<i>M. buteonis</i>	BbT2g ^T	294	-	AJ780984	AF412971
<i>M. cloacale</i>	383 ^T	191	-	AJ780985	AF125592
<i>M. columbinasale</i>	694 ^T	341	293 _R	AJ780986	AF221112
<i>M. columbinum</i>	MMP1 ^T	330	271 _S	AJ780987	AF221113
<i>M. columborale</i>	MMP4 ^T	282	41 _t , 67 _R	AJ780988	AF412975
<i>M. corogypsi</i>	Bv1 ^T	302	1 _Y , 5 _R , 64 _W , 120 _R	AJ780989	L08054
<i>M. falconis</i>	H/T1 ^T	191	-	AJ780990	AF125591
<i>M. gallinaceum</i>	DD ^T	285	36 _Y	AJ780991	L24104
<i>M. gallinarum</i>	PG16 ^T	305	-	AJ780992	L24105
<i>M. gallisepticum</i>	PG31 ^T	645	-	AB098504	M22441
<i>M. gallopavonis</i>	WR1 ^T	292	-	AJ780993	AF064062
<i>M. glycyphilum</i>	486 ^T	299	-	AJ780994	AF412981
<i>M. gypis</i>	B1/T1 ^T	226	-	AJ780995	AF125589
<i>M. imitans</i>	4229 ^T	2488	-	AB098503	L24103
<i>M. iners</i>	PG30 ^T	308	-	AJ780996	AF221114
<i>M. iowae</i>	695 ^T	224	-	AJ780997	U29676
<i>M. lipofaciens</i>	R171 ^T	303	-	AJ780998	AF221115
<i>M. meleagridis</i>	17529 ^T	289	-	AJ780999	L24106
<i>M. pullorum</i>	CKK ^T	298	17 _a , 34 _Y , 65 _Y , 248 _R	AJ781000	U58504
<i>M. sturni</i>	UCMF ^T	264	-	AJ781001	U22013
<i>M. synoviae</i>	WVU1853 ^T	308	17 _W , 58 _{KWWaaa} , 282 _Y	AJ781002	X52083

400
 401 ^aT = type strains

402 ^b Nucleotide positions of the ISR are given according to the numbering of the sequences
 403 in GeneBank and the designation of the polymorphisms used the IUB letter code. A
 404 lower-case letter indicates a nucleotide present in only one of the operons.

405 ^c Bold accession numbers were sequenced for this study.

406
 407

408

409 Figure Legends:

410

411 **Figure 1:** Gel electrophoresis showing PCR products of the ISR regions of 23 avian

412 *Mycoplasma* species.

413

414 **Figure 2:** Alignment of a detail of ISR avian mycoplasma sequences. Identities are

415 indicated by solid box. Positions where nucleotide differences exist in one sequence are

416 denoted with the ambiguity setter code according to IUB.

417

418 **Figure 3:** Phylogenetic tree based on 16S/23S rRNA ISR sequences. *M. mycoides*

419 subsp. *mycoides* SC type served as the out-group; bootstrap percentage values from

420 1000 resamplings are located at nodes of the tree. GeneBank accession numbers of

421 sequences are given in parentheses. The tree is compared with phylogenetic groups and

422 clusters (shown on the right hand side) obtained by analyzing the primary structures of

423 the 16S rRNA molecule (Petersson et al., 2000 and 2001; Weisburg et al., 1989).

424

425 **Figure 4:** Phylogenetic tree based on 16S rRNA gene sequences. *M. mycoides* subsp.

426 *mycoides* SC type served as the out-group; bootstrap percentage values from 1000

427 resamplings are located at nodes of the tree. Phylogenetic groups and clusters are also

428 shown on the right hand side (Petersson et al., 2000 and 2001; Weisburg et al., 1989).

429

430

431 **Supplementary data 1:** Alignment of ISR avian mycoplasma sequences. Identities are

432 indicated by solid boxes. Positions where nucleotide differences exist in one sequence

433 are denoted with the ambiguity letter code according to IUB. The sequence length

434 variation of *M. columborale*, and *M. pullorum* is indicated in low case. Differences in

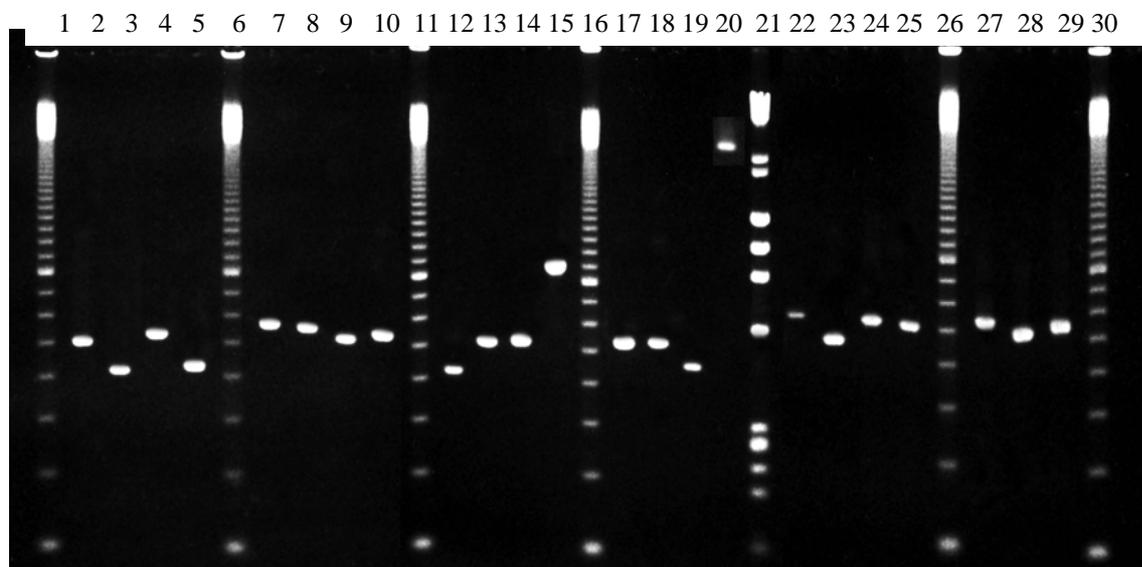
435 the sequence of the two copies of the ISR in *M. synoviae* are shown in a box.

436

437 **Supplementary data 2:** Levels of ISR DNA sequence homology and 16S rDNA

438 sequence homology for the type strains of the avian *Mycoplasma* species.

439



Caption: Lanes: 1, 6, 11, 16, 26 and 30. Ladder (100 bp) the arrow indicates 800 bp; 2. *M. anatis* 1340^T; 3. *M. anseris* 1219^T; 4. *M. buteonis* Bb/T2g^T; 5. *M. cloacale* 383^T; 7. *M. columbinasale* 694^T; 8. *M. columbinum* MMP1^T; 9. *M. columborale* MMP4^T; 10. *M. corogypsi* BV1^T; 12. *M. falconis* H/T1^T; 13. *M. gallinaceum* DD^T; 14. *M. gallinarum* PG16^T; 15. *M. gallisepticum* PG31^T; 17. *M. gallopavonis* WR1^T; 18. *M. glycophilum* 486^T; 19. *M. gypis* B1/T1^T; 20. *M. imitans* 4229^T; 21. Ladder (mixture of λ DNA/*Hind*III and ϕ X174 RF DNA/*Hae*I fragments), the arrow indicates 2,300 bp; 22. *M. iners* PG30^T; 23. *M. iowae* 695^T; 24. *M. lipofaciens* R171^T; 25. *M. meleagridis* 17529^T; 27. *M. pullorum* CKK^T; 28. *M. sturni* UCMF^T; 29. *M. synoviae* WVU1853^T.

	150	160	Box B	190	Box A	210			
<i>M. anatis</i>	ACTAGC-GT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCA	-----T	GATCTTTGAA	AACTGAATAG	T-----T	ATACA
<i>M. anseris</i>	AA---T-TT	GTATCCAGTT	TTGAGAGAAC	TTT-CTCTCA	AT---T---	GTTCTTTGAA	AACTGAATAT	AG-----	ACATT
<i>M. buteonis</i>	AT-AG--AT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCT	-----T--A	GTTCTTTGAA	AACTGAATAG	TAAAG----	ATATT
<i>M. cloacale</i>	A----T-TT	GTATCCAGTT	TTGAGAGAAC	TTT-CTCTCA	AT---TT--T	GTTCTTTGAA	AACTGAATAT	AG-----	ACATT
<i>M. columbinasale</i>	AAGCAAAAT	ATATCTAGTT	TTGAGAGAAC	ATTCTCTCA	CAATGAAAT	GTTCTTTGAA	AACTGAATAG	TAAG-----	ATATT
<i>M. columbinum</i>	AAGCAT--T	ATCTCTAGTT	TTGAGAGAAC	ATT-CTCTCA	-----AT	GTTCTTTGAA	AACTGAATAG	TAAA-----	TTATT
<i>M. columborale</i>	AA-TG--AT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCA	TT---A---T	GTTCTTTGAA	AACTGAATAG	TAAAG-----	ATAA-
<i>M. corogypsi</i>	TT-TG--AT	ATATCTAGTT	TTGAGAGARC	TTT-CTCTCT	TA---A---T	GTTCTTTGAA	AACTGAATAG	TAAAG-----	ATAT
<i>M. falconis</i>	A----T-TT	GTATCCAGTT	TTGAGAGTAC	TAA-CTCTCT	TT---TT---	GTTCTTTGAA	AACTGAATAT	CG-----	ACATT
<i>M. gallinaceum</i>	ATGTC--AT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCT	-----T--T	GTTCTTTGAA	AACTGAATAG	TAAAGTAAAG	ATATT
<i>M. gallinarum</i>	AAGCTT--T	ATATCTAGTT	TTGAGAGAAC	ATT-CTCTCA	-----AT	GTTCTTTGAA	AACTGAATAG	TAAG-----	ATATT
<i>M. gallopavonis</i>	AT-AG--AT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCT	-----T	GTTCTTTGAA	AACTGAATAG	TAAAG-----	ATATT
<i>M. glycyphilum</i>	AT-AG--AT	ATATCTAGTT	TTGAGAGAAC	TTT-CTCTCT	AA---T---T	GTTCTTTGAA	AACTGAATAG	TAAAG-----	ATATT
<i>M. gypis</i>	AA---T-TT	ATATCCAGTT	TTGAGAGTAT	TAT-CTCTCT	AG---TT--A	GTTCTTTGAA	AACTGAATAG	AA-----	AAGAC
<i>M. iners</i>	AAGCAA--T	ATATCTAGTT	TTGAGAGAAC	ATT-CTCTCA	-----AAT	GTTCTTTGAA	AACTGAATAG	TAAA-----	TTATT
<i>M. iowae</i>	GA-----TT	CTATTTAGTT	TTGAGAGATA	TATTCCTCTCA	TG----ATA	GTTCTTTGAA	AACTGAATAT	T-----	----
<i>M. lipofaciens</i>	AAGCTT--T	ATATCTAGTT	TTGAGAGAAC	AAAACTCTCA	-----AAAT	GTTCTTTGAA	AACTGAATAG	TAAA-----	TTATT
<i>M. meleagridis</i>	GAGTTA--T	ATATCTAGTT	TTGAGAGAAC	ATT-CTCTCA	-----AT	GTTCTTTGAA	AACTGAATAG	TAAA-----	TTATT
<i>M. pullorum</i>	AATAGT-GT	ATATCTAGTT	TTGAGAGAAC	AAT-CTCTCA	AA---A---T	GTTCTTTGAA	AACTGAATAG	TATAT-----	AAATA
<i>M. sturni</i>	AA-TG--GT	ATATCTAGTT	TTGAGAGAAC	TT--CTCTCA	A---A---T	GTTCTTTGAA	AACTGAATAG	TAAAG-----	ATATT
<i>M. synoviae</i>	GGGCTA-TT	GTATTTAGTT	TTGAGAGAAC	AAC-CTCTCT	TA---AAAT	GTTCTTTGAA	AACTGAATAG	TAAATA--AAG	ATATT

G: Guanine; A: Adenine; T; Thymine; C: Cytosine; R: A/G

