Myxoma virus jumps species to the Iberian hare

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Abstract

The study of myxoma virus (MYXV) infections in the European rabbit (*Oryctolagus cuniculus*) has produced one of the most accepted host-pathogen evolutionary models. To date, myxomatosis has been limited to the European rabbit with sporadic reports in hares. However, reports of widespread mortalities in the Iberian hare (*Lepus granatensis*) with myxomatosis-like clinical signs indicate a potential species jump hasoccurred. The presence of MYXV DNA was confirmed by PCR in 244 samples received from regional veterinary services, animal health

laboratories, hunters or rangers over a 5-month period. PCR analysis of 4 MYXV positive hare samples revealed a 2.8 kb insertion located within the M009 gene with respect to MYXV. The presence of this insertion was subsequently confirmed in 20 samples from 18 Spanish provinces. Sanger sequencing and subsequent analysis show that the insert contained 4 ORFs which are phylogenetically related to MYXV genes M060, M061, M064 and M065. The complete MYXV genome from hare tissue was sequenced using Ion torrent next-generation technology and a summary of the data presented here. With the exception of the inserted region, the virus genome had no large scale modifications and 110 mutations with respect to the MYXV reference strain Lausanne were observed. The next phase in the evolution of MYXV has taken place as a host species jump from the European rabbit to the Iberian hare an occurrence which could have important effects on this naive population.

1. Introduction

Virus species jumps are potential threats to humans and wildlife and a fundamental source of emerging infectious diseases. Environmental, host and viral factors determine the success of species jumps. Due to similarities in receptor molecules, intracellular environment and host immune system interactions closely related species are more likely to suffer virus host species jumps (Longdon, Brockhurst, Russell, Welch, & Jiggins, 2014).

Giuseppe Sanarelli was first to observe myxomatosis (Sanarelli, 1898), a lethal systemic viral infection of European rabbits, following a species jump of myxoma virus (MYXV) from its natural host *Sylvilagus brasiiiensis* (Aragão, 1927), the Brazilian cottontail rabbit. Humans facilitated this well-documented jump by bringing the European rabbit into proximity with the natural MYXV reservoir. The use of MYXV as a biological control agent in Australia and Europe contributed to its widespread distribution, and the MYXV/rabbit system has become a model for host-virus evolution (Fenner, 1965; Di Giallonardo & Holmes, 2015; Kerr, 2012; Kerr, Cattadori, Liu, et al., 2017; Kerr, Cattadori, Rogers, et al., 2017). MYXV is a poxvirus with a double-stranded DNA genome of 161.8 kb for the reference strain Lausanne.

Most poxviruses are species-specific, and it is likely that genetic constraints favouring replication in the original host limit putative species jumps to "spillover" events (McFadden, 2005). Although genetically similar and sympatric with the European rabbit, reports of myxomatosis in hares are scarce and limited in duration. Early cases were reported in the European brown hare (*Lepus Europaeus*) in France and more recently in Great Britain (Barlow et al., 2014). However, widespread hare deaths from myxomatosis, compatible with a true species jump event, were not observed until recently (Garcia-Bocanegra et al., submitted).

Here, we report a real-time observation of a potential host species jump of MYXV from the European rabbit to the Iberian hare (*Lepus granatensis*) and identify the naturally occurring genomic modifications that may have allowed this jump to occur.

2. MATERIALS AND METHODS

2.1. Samples

Samples from the carcasses of hares with clinical signs of myxomatosis were received at the national Central Veterinary Laboratory (Laboratorio Central de Veterinaria, Algete, Madrid, Spain) from Regional passive wildlife vigilance campaigns, official regional veterinary, animal health laboratories, gamekeepers, hunters and including a specific emergency health programme in Andalucia. Samples originated from 8 Autonomic communities (including 22 different provinces), Andaluda, Aragon, Castilla La Mancha, Castilla y Leon, Comunidad Valenciana, Extremadura, Madrid and Murcia (Figure 1a).

2.1.1. Cells

RK-13 (Rabbit Kidney) cells (ATCC-CCL-37, lote 3993687) were used for virus isolation.

2.2. DNA extraction

Total DNA was extracted from hare tissue using the QIAamp DNA mini and blood kit as per the manufacturer's instructions (Qiagen, GmbH, Dusseldorf, Germany). Briefly, tissue 25¬50 mg) was diced and resuspended in lysis (ATL) buffer with proteinase K and incubated for 3 hr at 56°C followed by overnight at 37°C. RNAse A (100 mg/ml) and buffer AL were added prior to the addition of 96%-100% ethanol. Samples were applied to the provided spin columns, centrifuged (6,000x g) for 1 min and washed with the supplied buffers AW1, AW2 before drying and elution in sterile nuclease-free water.

2.3. PCR

Initial diagnosis was carried out using a PCR designed to detect a 471 bp region of the M071L gene as described by Cavadini, Botti, Barbieri, Lavazza, & Capucci, 2010. Reactions were carried out using GoTaq hot start green in a volume of 25 ^l with both primers at a concentration 20 μM.

Subsequent PCR reactions were carried out using LA Taq (Takara). The names and sequences of oligonucleotides used for PCR analysis and verification of mutations detected by next-generation sequencing are available on request.

2.4. Genome analysis

Full-genome sequencing was carried out using the Ion Torrent sequencing platform at the University of Oviedo's sequencing facilities (Servicios científicos tecnicos, Universidad de Oviedo). Total genomic DNA extracted from eyelid tissue was used directly for library preparation using standard protocols. DNA was fragmented, size checked (Bioanalyzer, Agilent) and used for adapter ligation and library preparation (Ion Plus Fragment Library Kit for AB Library Builder™ System and Ion Xpress™ Barcode Adapters 1-96 Kit, Thermofisher). The prepared library was subjected to emulsion PCR and amplified using Ion PGM™ Hi-Q™ View OT2 Kit and the Ion PGM™ Hi-Q™ View Sequencing Kit was used for sequencing.

A pool of four samples was used for sequencing on 318 chip (Ion 318™ Chip Kit v2 BC, Thermofisher). A summary output indicated that average read length was 213 bp and the mode length 309 bp, with 5,794,582 usable reads.

Two strategies were followed to analyse the output: scaffold-based variant calling and de novo assembly. For de novo assembly sequenced reads were trimmed and quality filtered by the IonTorrent device. High-quality reads were used to perform de novo assemblies using Unicycler and SPAdes methods through the Galaxy online server (www.usegalaxy.org). In general, the parameters used were those selected by default in the normal bridging mode, which creates moderate-sized contigs and a misassembly rate. However, we reduced the minimum contig size from 100 to 20 bp and avoided the circular options due to the linear genome and presence of repetitions. For scaffold-based variant calling the genome sequence of Lausanne or a manually constructed ha-MYXV (hare myxoma virus) genome were used as reference. The insert Ins-H1 was sequenced using Sanger sequencing, and the contig generated inserted into the Lausanne at site 12,236.

The genomic regions corresponding to regions of de novo contigs containing ambiguous reads were amplified using relevant oligonucleotides and sequenced using the same primers and Sanger sequencing. The ha-MYXV genome accession number is MK340973.

2.5. Bioinformatic analysis

Blastn and Blastp local alignment tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to analyse nucleotide and predicted amino acid sequences against the (nr/nt) nucleotide collection and non-redundant protein sequence databases, respectively. ClustalOmega and Kalign (https://www.ebi.ac.uk/Tools/msa/) multiple alignment tools were used to align individual virus genes or de novo assembled contigs, respectively; output was saved in clustal format. Phylogenetic analysis of multiple sequence alignments was carried out using MEGAX to infer genetic relatedness.

Virus genomes were annotated manually and using the genome annotation transfer utility (GATU) (https://virology.uvic.ca/virology-ca-tools/gatu) and MYXV Lausanne as reference.

3. RESULTS AND DISCUSSION

Between July and November 2018, samples of dead wild hares that demonstrated myxomatosis-like signs were received from official regional veterinary services, animal health laboratories, hunters or rangers. To determine if the hare samples contained MYXV DNA, genomic DNA was extracted from infected tissue and a MYXV specific diagnostic conventional PCR targeting the M071L region (Cavadini et al., 2010) was carried out. The presence of MYXV DNA was detected in 244 hare samples, during this period 3 hare samples resulted negative for the presence of MYXV DNA. Sanger sequencing of the M071 gene confirmed 100% identity with the reference MYXV strain Lausanne. Virus isolation in RK13 cells was carried out on 22 samples from 21 provinces. The large numbers of hares infected indicated a significant change from "spillover" events to a potential host species jump.

Five regions (M002, M008.1, M009, M069 and M135) of the virus genome from 4 hare samples were further analysed by PCR (Figure 1c). DNA extracted from infected tissue was directly

145 analysed to prevent the accumulation of mutations during culture on rabbit cells. Agarose gel
146 electrophoresis and Sanger sequencing of the amplified products identified an insertion
147 (termed Ins-H1, Figure 1b, Lane M009 H) of 2,863 nt located within the MYXV gene M009L,
148 between genome positions 12,336 and 12,337 (MYXV Accession numbers AF170726 (Cameron
149 et al., 1999) and KY548791 (Kerr, Cattadori, Liu, et al., 2017; Kerr, Cattadori, Rogers, et al.,
150 2017). To determine if the insertion was present in further samples, 20 hare samples
151 (originating from 18 provinces) were analysed in the M009L region. All 20 hare DNA MYXV
152 samples were positive for the insertion by PCR and gel electrophoresis. Rabbit samples (n = 8)
153 positive for MYXV DNA received throughout 2018 were positive for MYXV but negative for the
154 insertion (with the exception of 1 rabbit which was positive for the insertion by PCR and gel
155 electrophoresis) (data not shown).

156 In silico analyses of the Ins-H1 sequence predicted it to contain 4 potential ORFs. Phylogenetic
157 analysis of the nucleotide (data not shown) and predicted amino acid sequences (Figure 2) of
158 these ORFs demonstrated relationship with myxoma virus genes/ORFs M060R, M061R, M064R
159 and M065R. The genes within the Ins-H1 insertion were in an inverted orientation with respect
160 to the homologous regions in MYXV genome (Figure 3).

161 The entire genome of the virus was subsequently sequenced using Ion Torrent technology
162 (Genbank Accession Number MK340973) and the genome termed ha-MYXV, to differentiate it
163 from MYXV of rabbits. The genome showed 110 mutations with respect to MYXV Lausanne
164 (Table 1 is a summary of the genome sequencing data). Results demonstrated that all assigned
165 MYXV ORFs (Cameron et al., 1999) were present, the ORFs showed high identity with MYXV
166 Lausanne and, with the exception of the Ins-H1 insertion, no large scale changes were
167 observed. There were 86 mutations affecting codons of which 42 were synonymous.
168 Furthermore, there were nine indels that affect ORFs and 15 mutations occurred in intergenic
169 (IG) regions. In addition to the 2.8 kb insertion in M009, this ORF was interrupted upstream of
170 the insertion by an additional 4nts leading to a potential frameshift from amino acid 121 and
171 subsequent truncation of M009 from 509 to 147 amino acids. No boundary site degradation
172 was observed with respect to the reference sequence M009L. The insertion (Ins- H1) and
173 flanking regions show no homology (data not shown), and this appears to rule out homologous

recombination as a mechanism for insertion. Therefore, the mechanism of insertion remains to be determined. In addition and of particular interest are the truncation of M152R (Serp-3) (Guerin et al., 2001) from 266 to 60 amino acids and the addition of 82 nt in the telomeric DNA region believed to be involved in DNA replication during cruciform formation and concatemer resolution (DeLange, Reddy, Scraba, Upton, & McFadden, 1986). If these mutations may be involved in virulence in the Iberian hare or European rabbit remains to be determined but their presence should be monitored.

MYXV is spreading rapidly in the Iberian hare a phenomenon not seen to date indicating a potential species jump has occurred. This may have important consequences on naive Iberian hare populations. The identification of a 2.8 kb insertion and the complete genome sequence analysis carried out here identifies all the mutations necessary for the species jump to have occurred. Of these mutations, the most striking is the potential duplication and subsequent divergence of host range genes. It is likely this change has contributed to this species jump although the other mutations present cannot be overlooked and all should be investigated further.

Studies of MYXV genomes suggest that M009L, the site of the insertion, is a redundant gene. M009 is one of 5 putative E3 Ub-ligases encoded in the MYXV genome (Cameron et al., 1999; Kerr, Cattadori, Liu, et al., 2017; Kerr, Cattadori, Rogers, et al., 2017) and several naturally occurring MYXV strains contain indels in M009L (Dalton et al., 2015; Kerr et al., 2012; Kerr, Hone, Perrin, French, & Williams, 2010; Morales et al., 2009). Duplication events that interrupt ORF M009 have been observed in the Californian MSW and several Australian strains (Kerr et al., 2013, 2013).

The inserted region contains 4 genes encoding proteins potentially orthologous to the MYXV counterparts which are predicted as a virion protein dimer (M060) (ha-M060 shares 68.7% identity with MYXV M060 at the amino acid level), thymidine kinase (M061) (71.8% identity), a poly A regulatory subunit (M065) (82.9% identity) and notably the host range/virulence factor M064 (Liu, Rothenburg, Rothenburg, & McFadden, 2012; Liu, Wennier, et al., 2012) (36.0% identity). M064 is a homolog of the vaccinia virus C7L host range protein (Adams, Leeuwen, McFadden, & Kerr, 2008; Liu, Rothenburg, et al., 2012; Liu, Wennier, et al., 2012). C7L

moderates host cell specificity and functions in antagonizing the host antiviral defence mechanism (Liu, Rothenburg, et al., 2012; Liu, Wennier, et al., 2012). If the insertion occurred through a duplication event, the resultant virus has subsequently lost the M062 and M063 counterparts. The homology that exists between the promoter regions of M062 and M064 may have facilitated a recombination event to remove these genes. However, until a potential precursor or parental virus is detected, this remains highly speculative.

Blastn analysis of the entire insert (data not shown) suggested that another possible explanation could be a gene capture event that occurred through recombination with a capri- or cervi-poxvirus. However, the geographic distribution of these viruses makes recombination events with MYXV on the Iberian Peninsula seem unlikely. In addition, in the phylogenetic analysis, all individual amino acid sequences cluster with their MYXV counterparts (Figure 2). Subsequent phylogenetic analysis and the detection of precursor ha-MYXV viruses should solve this conundrum. Hare fibroma virus was reported in Italy (Grilli et al., 2003) in farmed hares (*Lepus europaeus*), although DNA sequences were not obtained and therefore cannot be compared to the sequence described here, this may be a plausible candidate and should be investigated further. Evidence of previous recombination events between leporipoxviruses (Shope fibroma virus and myxoma virus) have been described (Upton, Macen, Maranchuk, DeLange, & McFadden, 1988). Future analysis of ha- MYXV genomes will determine genome stability within the inserted region and whether the outbreak is caused by a single variant.

The next phase in the evolution of MYXV has taken place as a host species jump from the European rabbit to the Iberian hare an occurrence which could have devastating effects on this naive population.

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CONFLICT OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT

Ethical Statement is not applicable as samples were collected from dead animals.

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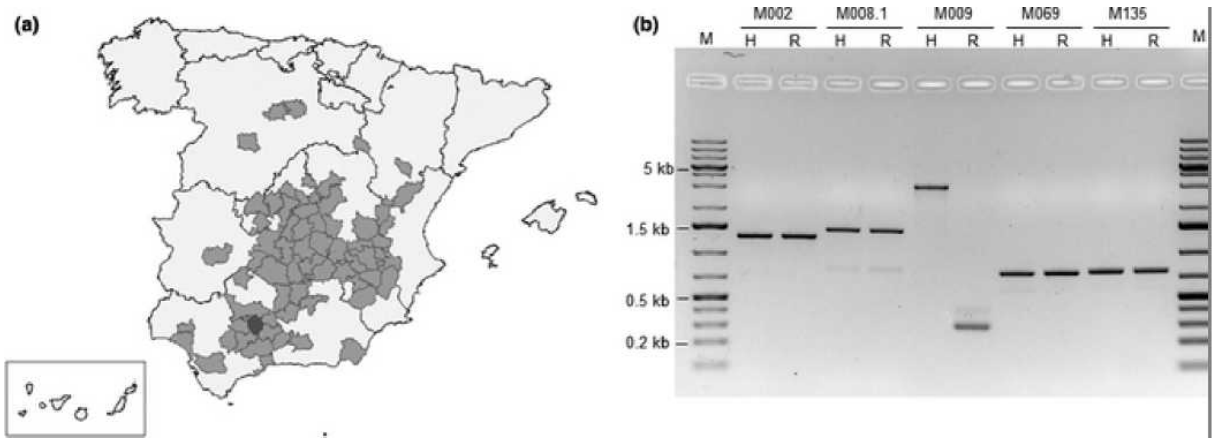
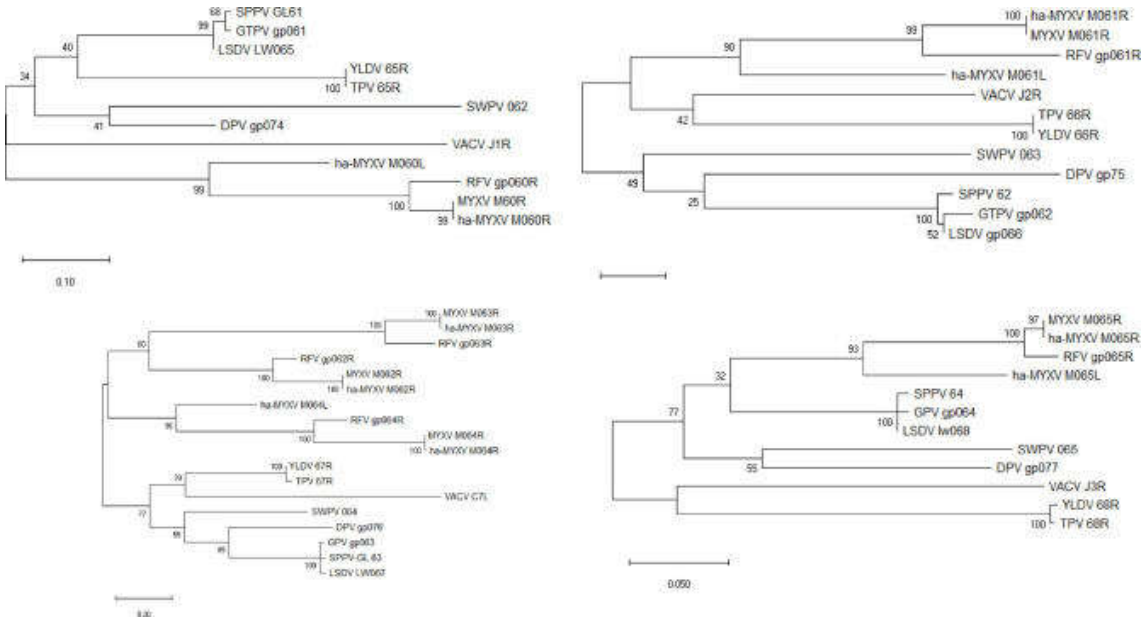
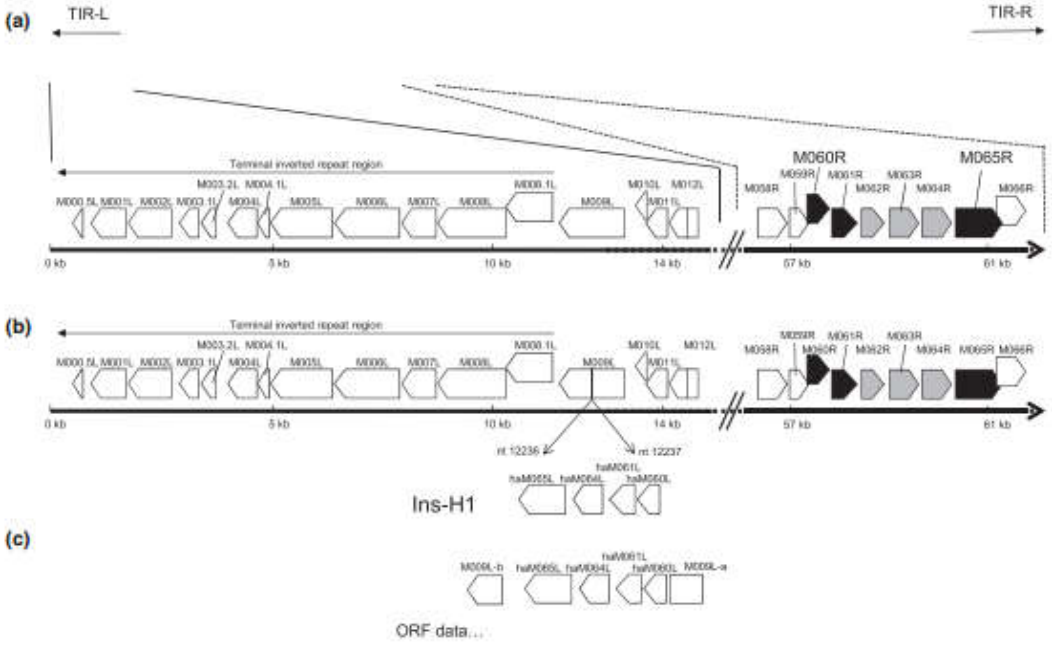


Fig. 1 Detection and geographic distribution of myxomatosis cases in Iberian hares. (a) Map of Spain indicating the distribution of myxomatosis cases found in Iberian hares from July to November 2018. The lines indicated the borders between the 22 Spanish autonomous regions which are further subdivided into a number of livestock regions (LR). Dark grey shading is used to indicate the LR where at least one case of myxomatosis has been confirmed in Iberian hares. The darker shaded area, within the autonomous region of Andalucia, indicates de LR where the first confirmed myxomatosis case in Iberian was described in July 2018. (b) Agarose gel electrophoresis analyses of the PCR amplicons obtained using primers listed in Table S1. In the selected genomic regions. (H) hare sample, (R) positive control of a MYXV field isolate Gran05/09 from rabbits



341 Fig. 2 Evolutionary analysis by the maximum likelihood method. The maximum likelihood
342 method and JTT matrix-based model (Jones, Taylor, & Thornton, 1992) were used to analysis
343 predicted amino acid sequences of genes contained in the Ins-H1 region of ha-MYXV genome.
344 Evolutionary analyses were conducted in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).
345 Trees corresponding to analysis of genes M060R, M061 R, M062-M064R and M065R are
346 shown

347



348 Fig. 3 Linear genomic organization of reference MYXV (Lausanne) strain and relevant regions
349 from ha-MYXV isolated from Iberian hares. a. Genomic map of MYXV reference strain depicting
350 the coding regions (vertical dark grey bars and arrows). Left and right terminal inverted
351 repeated sequences (TIR) are indicated. The first 15 kb and 57-61 kb regions of the MYXV
352 genome are expanded below the genome map indicating the TIR-L ORFs, unique genes up to
353 *M012L,* highlighting (grey dotted boxes) the redundant homologous genes *M006L, M008L* and
354 *M009L* within this region. The expanded internal region (57-61 kb) includes genes putatively
355 involved in the duplication event *M060R, M061R, M065R* (highlighted in black) and
356 virulence/tropism genes *M062R, M063R* and *M064R* (grey shaded). (b) Linear map of first 15
357 kb and 57-61 kb regions of ha-MYXV genome isolated from an Iberian hare that died of
358 myxomatosis. Coding regions and genomic details are indicated as for the above Lausanne
359 MYXV diagram. Ins-H1 indicates the 2.8 kb fragment inserted between residues 12,236 and
360 12,237 of the *M009L* gene in ha- MYXV isolated from Iberian hares. The location and
361 orientations of ORFs *ha-M060L, ha-M061L, ha-M064L* and *ha-M065L* within this insertion are
362 indicated

363

364 Table 1 Summary of mutations in ha-MYXV with respect to MYXV Lausanne

Position Lausanne (nt)	Position_L3_31	Ref	Variant	Gene/IG	Codon	AA position	AA mutation
1	83	-	82 NT INS				
200	200	-	TAGAAG	IG			
498	504	G	A	M.005L/R	TGC-TGT	28	C silent
698	704	A	-	IG 0.005-001			
2.759	2.764		Δ27 NT	IG 002-003			
2.913	2.890	A	G	m003.1L/R	TTG-CTG	119	L silent
2.917	2.894	C	T	m003.1L/R	ATG-ATA	121	M-I
2.985	2.962	G	A	m003.1L/R	TTG-CGG	99	R-W
4.010	3.987	G	C	M004L/R	GAC-GAG	209	D-E
4.937	4.914	C	T	M005L/R	GCG-ACG	483	A-T
6.082	6.059	G	A	M005L/R	GCG-GTG	101	A-V
8.394	8.371	T	C	M007L/R	CAG-CGG	128	Q-R
9.321	9.298	C	T	M008L/R	GAC-AAC	352	D-N
9.324	9.301	A	G	M008L/R	TAC-CAC	351	Y-H
9.579	9.556	C	T	M008L/R	GAA-AAA	266	E-K
11.151	11.128	C	A	M008.1L/R	CGC-CTC	104	R-L
11.253	11.230	T	C	M008.1L/R	GAC-GGC	70	D-G
11.471	11.448	A	-	IG008.1-009			
12.236	12,213-15,079	C	2,867 NT INS	M009L disrupted		Contains novel genes	
12,350-12,370	15.188		Δ21 NT	M009L disrupted	7 aa deletion "VTYIRKR" aa 254-260 MYXV M009		
12.766	15.583	-	TATA	M009L disrupted	aa 121-510 (end)		
13.863	16.684	G	A	M011L	GCG-GTG	88	A-V
17.845	20.666	G	A	M016L	CCC-TCC	2	P-S
17.878	20.699	C	T	M017L	GAA-AAA	71	E-K
18.210	21,031-21,046	-	16 NT INS	IG 017-018			
21.577	24.398	G	A	M021L	ACA-ATA	318	T-I
23.294	26.111	C	T	M022L	CGG-CAG	128	R-Q
24.054	26.871	G	A	M024L	CGT-TGT	79	R-C
27.617	30.434	G	A	M028L	CAC-CAT	436	H silent
28.287	31.108	A	C	M028L	GGA-GTA	213	V-G
29.071	31.892	G	A	M029L	AGC-AGT	80	S silent
29.259	32.080	C	T	M029L	GCG-ACG	18	A-T
29.460	32.281	C	T	M030L	AGA-AAA	194	R-K
30.332	33.153	G	T	M031R	CTG-CTT	64	L silent
31.103	33.924	A	G	M031R	GGG-GGA	321	G silent
31.713	34.534	C	T	M032R	CGG-TGG	128	R-W
34.063	36.884	G	A	M034L	TGG-TGC	935	C silent
38.825	41.646	T	C	M036L	AAG-GAG	46	K-E
39.107	41.928	C	T	M036L	GAC-AAC	IG in Ha-MYXV	D-N in M036L MYXV
39.164	41.986	-	TTTT	M036L	Δ aa1-98	583 aa (from 681 aa)	
41.062	43.887	G	A	M040L	GCG-GTG	106	A-V
41.802	44.627	T	G	M042L	AAA-AAC	357	K-N
41.925	44.750	G	A	M042L	ATC-ATT	316	I silent
44.592	47.417	G	T	M044R	GTT-TTT	145	V-F
44.920	47.745	A	G	M044R	CAC-CGC	254	H-R
47.303	50.128	C	T	M045L	GTG-GTA	221	V silent
48.527	51.352	A	G	M047R	TTA-TTG	79	L silent

49.807	52.632	G	A	M049R	GAC-AAC	165	D-N
51.053	53.878	G	A	M051R	ACG-ACA	82	T silent
51.218	54.043	A	G	M051R	TTA-TTG	137	L silent
52.321	55.146	C	T	M052L	GGG-GGA	11	G silent
53.048	55.873	G	T	M053R	CGG-CGT	222	R silent
53.761	56.586	A	G	M054R	ACA-ACR	192	T silent
56.734	59.559	G	A	M058R	GCG-GCA	177	A silent
58.327	61.152	-	T	M061R		179-181	Ins LKY at C-terminal
59.518	62.344	A	G	M063R	AGC-GGC	193	S-G
59.605	62,432-62,434	-	AAT	IG 063-064			
59.854	62.683	G	A	M064R	CGC-CAC	74	R-H
60.120	62.949	GAA	-	M064R	AGAA	163	AE
60.574	63.400	A	G	M065R	GGA-GGG		G silent
62.164	64.990	-	T	IG 067-068			
62.169	64.995	A	-	IG 067-068			
62.416	65.242	T	A	M068R	TAC-TTC	60	F-Y
64.298	67.124	C	T	M068R	GAC-GAT	687	D silent
65.021	67.847	C	T	M068R	TCC-TCT	928	S silent
68.250	71.076	C	T	M072L	GTG-GTA	775	V silent
71.294	74.120	T	-	IG 073-074			
74.625	77.450	C	T	M076R	TAC-TAY	640	Y silent
75.687	78.512	A	C	M078R	AGC-CGC	26	S-R
81.368	84.193	C	T	M082R	CAC-CAT	17	H silent
87.929	90.754	G	A	M088L	TTC-TTY	207	F silent
91.406	94.231	G	A	M092L	CTC-CTT	174	L silent
91.589	94.414	C	T	M092L	GCG-GCA	113	A silent
91.676	94.501	C	T	M092L	GCG-GCA	84	A silent
92.034	94.859	C	T	M093L	GCT-ACT	137	A-T
96.473	99.298	A	G	M097R	AGA-AGR	56	R silent
97.031	99.856	G	A	M097R	ACG-ACA	242	T silent
98.958	101.783	C	T	M099L	GAG-GAA	382	E silent
99.429	102.254	G	A	M099L	TTC-TTT	225	F silent
103.444	106.269	G	A	M106L	CGG-TGG	84	R-W
104.874	107.699	C	A	M108R	CGC-CGA	183	R silent
107.311	110.136	C	T	M111R	GCG-GTG	336	A-V
114.737	117.562	G	A	M118L	GCC-GCT	31	A silent
115.096	117.921	C	T	M120L	GCG-ACG	225	A-T
115.858	118.683	G	A	M121R	TCG-TCA	2	S silent
117.802	120.627	C	T	M124R	CTA-YTA	96	L silent
118,783-118,800	121.608		Δ18 NT	M125R	ΔDDEGSF	132	ΔDDEGSF
120.886	123.693	A	G	M127L	TCG-CCG	58	S-P
121.272	124.079	C	T	M128L	GCC-ACC	212	A-T
125.416	128.223	G	A	M133R	ACG-ACA	509	T silent
127.311	130.118	C	T	M134R	ATC-ATT	538	I silent
128.601	131.408	G	A	M134R	GCG-GCA	968	A silent
129.675	132.482	G	A	M134R	GGG-GGR	1.326	R silent
129.793	132.600	G	A	M134R	GCC-ACC	1.366	A-T
134.766	137.573	T	-	IG 138-139			
135.541	138.347	C	T	M140R	CAC-TAC	55	H-Y
136.368	139.174	T	C	M140R	TGT-TGY	330	C silent
137.670	140.476	T	C	M141R	TTA-CTA	200	L silent
138.725	141.531	G	A	M143	CTG-CTR	19	L silent
139.412	142.218	G	A	IG 143-144			
141.751	144.557	G	A	M148R	CGA-CAA	41	R-Q

142.282	145.088	A	G	M148R	TAC-TGC	218	Y-C
142.609	145.415	C	T	M148R	GCC-GTC	327	A-V
142.706	145.512	C	T	M148R	GTC-GTT	359	V silent
143.109	147.555	G	A	M148R	GCC-ACC	494	A-T
144.749	147.555	C	T	M149R	GCG-GTG	364	A-V
146.642	149.448	-	T	M150R	changes frame	Δ485-494(end)	FLNENKVEYNV*-FFK*
147.864	150.669	-	C	M152R	Truncates protein	Δ60-266(end)	
149.193	152.000	-	T	IG 153-154			
149.913	152.720	T	-	IG 154-156			

Abbreviations: IG, Intergenic region; Ref, reference sequence Lausanne (AF170726).

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