

Supplementary Table I : Molecule size for the optical map for *B. MO1*

Optical Molecule #	Length (bp)
1	4,416,462
2	3,532,684
3	2,290,926
4	507,370
5	272,473
6	230,786
15	137,680
7	83,877
SUM	11,472,258

Supplementary Table II. Genome comparison and gene statistics

	<i>Babesia divergens</i> (Rouen-87)	<i>Babesia</i> MO1 (F12 clone)	2018 <i>B. divergens</i> Rouen assembly
Total gene models / annotated	5,274 / 3,558	4,569 / 2,795	4,546 / 3,386
Exon mean / median / mode	472.85 / 185 / 75 & 102	458.065 / 176 / 99	727 / 352 / 65
Intron mean / median / mode	605.89 / 40 / 33	421.24 / 39 / 33	328 / 105 / 36
Average exons per gene	2.65	3.14	1.68

Supplementary Table III. Assembly statistics of *Babesia divergens* Rouen and *Babesia* MO1

	<i>B. divergens</i> (Rouen 87)	<i>Babesia</i> MO1 (clone F12)	<i>Babesia</i> MO1 (clone B12)	2018 <i>B. divergens</i> Rouen (assembly ASM107745v2)
Total length (Mb)	10.78	11.03	10.8	9.73
Total chromosomes	3	3	3	Undetermined # 5 scaffolds > 1Mb
Unplaced contigs	7	14	9	141
Mean contig length (Mb)	1.07	0.787	0.899	0.069
Longest contig (Mb)	4.35	3.98	3.67	2.20
N50/L50 (Mb/contigs)	3.95 / 2	3.65 / 2	3.49 / 2	1.08 / 4
GC content	45	45	45	43
BUSCO v5 (Apicomplexa lineage)	437/446 Complete 1/446 Fragmented 8/446 Missing	434/446 Complete 2/446 Fragmented 10/446 Missing	434/446 Complete 2/446 Fragmented 10/446 Missing	437/446 Complete 1/446 Fragmented 8/446 Missing

Supplementary Table IV. Predicted enzymes of the glycolytic pathway of *B. MO1*

Glycolysis Steps	Enzyme	Gene ID	Protein Length	RNA expression level (TPM)
Glucose				
↓ ←	Hexokinase	BspMO1_0180700.t1	539	573.231018
Glucose-6P				
↓ ←	Phosphoglucose Isomerase	BspMO1_0354600.t1	592	416.748413
Fructose-6P				
↓ ←	6-Phosphofructokinase	BspMO1_0305600.t1	1339	344.636505
Fructose-1,6P2				
↓ ←	Fructose-1,6-bisphosphate aldolase	BspMO1_0017000.t1	357	875.367493
Glyceraldehyde-3P				
↓ ←	Glyceraldehyde-3P dehydrogenase	BspMO1_0213700.t1	336	1282.513428
Glycerate-1,3P2				
↓ ←	Phosphoglycerate Kinase	BspMO1_0381000.t1	412	745.640198
Glycerate-3P				
↓ ←	Phosphoglycerate mutase	BspMO1_0145800.t1	248	1116.424927
Glycerate-2P				
↓ ←	Enolase	BspMO1_0146600.t1	442	1319.670044
Phosphoenolpyruvate				
↓ ←	Phosphoenolpyruvate carboxykinase	BspMO1_0269300.t1	546	253.2034
Pyruvate				
↓ ←	Lactate dehydrogenase	BspMO1_0431300.t1	338	2129.362793
Lactate				

Supplementary Table V. Predicted enzymes of the TCA cycle of *B. MO1*

Krebs Cycle steps	Enzyme	Predicted Gene ID	Protein Length	C/M/S	TM	RNA expression level (TPM)
Citrate						
↓	← Aconitate Hydratase	BspMO1_0041000.t1	914	M	-	263.30365
Isocitrate						
↓	← Isocitrate dehydrogenase	BspMO1_0098400.t1 /BspMO1_0139600.t1	519 / 455	M	-	102.51371 / 595.400269
2-oxaloglutarate						
↓	← 2-Oxoglutarate dehydrogenase	BspMO1_0276100.t1	952	C	-	251.601196
Succinyl-CoA						
↓	← Succinyl-CoA synthetase	BspMO1_0145000.t1 /BspMO1_0407900.t1	461	M	-	324.873474
Succinate						
↓	← Succinate dehydrogenase	BspMO1_0256000.t1 /BspMO1_0324700.t1	273 / 624	M	-	269.766235 / 139.289917
Fumarate						
↓	← Fumarase	BspMO1_0281100.t1	468	M	-	156.482895
Malate						
↓	← Malate dehydrogenase	BspMO1_0431300.t1	338	M	-	2129.362793
Oxaloacetate						
↓	← Citrate synthase	BspMO1_0118500.t1 /BspMO1_0313600.t1	361 / 608	C / M	-	267.057251 / 214.3311

B. MO1 TCA cycle



Supplementary Table VI. Predicted GPI-anchored proteins of *B. MO1*

GPI-AP ID	Protein ID	Signal P5.0	PredGPI		Protein length (aa)	TPM Value
		Score	Specificity Score	Probability		
BMO1GPI1	BspMO1_0016700.t1.1	0.9782	100	Highy Probable	459	102.585358
BMO1GPI2	BspMO1_0018700.t1.1	0.9763	100	Highy Probable	479	763.598877
BMO1GPI2	BspMO1_0001800.t1.1	0.9784	99.9	Highy Probable	134	10.512491
BMO1GPI4	BspMO1_0090300.t1.1	0.9243	100	Highy Probable	555	331.263947
BMO1GPI5	BspMO1_0096800.t1.1	0.6156	100	Highy Probable	619	403.405823
BMO1GPI6	BspMO1_0088800.t1.1	0.0071	99.9	Highy Probable	158	190.414551
BMO1GPI7	BspMO1_0090900.t1.1	0.751	99.9	Highy Probable	512	837.06189
BMO1GPI8	BspMO1_0119500.t1.1	0.972	99.9	Highy Probable	232	1594.880371
BMO1GPI9	BspMO1_0120200.t1.1	0.6151	99.9	Highy Probable	733	239.136703
BMO1GPI10	BspMO1_0126800.t1.1	0.0839	99.9	Highy Probable	357	258.695862
BMO1GPI11	BspMO1_0183500.t1.1	0.9153	100	Highy Probable	483	45.006592
BMO1GPI12	BspMO1_0213300.t1.1	0.7709	99.9	Highy Probable	139	528.32074
BMO1GPI13	BspMO1_0275300.t1.1	0.9899	99.9	Highy Probable	181	569.44812
BMO1GPI14	BspMO1_0277500.t1.1	0.9811	99.9	Highy Probable	579	7.428508
BMO1GPI15	BspMO1_0342900.t1.1	0.9442	100	Highy Probable	178	3517.976807
BMO1GPI16	BspMO1_0343500.t1.1	0.9454	100	Highy Probable	152	3709.012207
BMO1GPI17	BspMO1_0320700.t1.1	0.4404	99.9	Highy Probable	201	381.76651
BMO1GPI18	BspMO1_0407700.t1.1	0.0101	99.9	Highy Probable	448	186.392136
BMO1GPI19	BspMO1_0419800.t1.1	0.0032	99.9	Highy Probable	413	330.82
BMO1GPI20	BspMO1_0431600.t1.1	0.0466	99.9	Highy Probable	345	101.67

Supplementary Table VII. Predicted AP2 proteins of *B. MO1*

Gene Name	Gene ID	Protein length (aa)	RNA Expression (TPM value)	Protein MW (kDa)	Domains
BMO1-AP2-1	BspMO1_0177400.t1	629	4.00421	68.3	AP2
BMO1-AP2-2	BspMO1_0027500.t1	680	52.212086	75.8	AP2
BMO1-AP2-3	BspMO1_0036300.t1	742	384.286835	84.1	AP2, RPT1
BMO1-AP2-4	BspMO1_0063100.t1	197	315.744598	23.3	AP2
BMO1-AP2-5	BspMO1_000584-T1	74	--	8.6	AP2
BMO1-AP2-6	BspMO1_0136500.t1	375	46.351261	41.9	AP2
BMO1-AP2-7	BspMO1_0155600.t1	401	722.822876	45.3	AP2
BMO1-AP2-8	BspMO1_0164600.t1	581	179.783752	65.2	AP2
BMO1-AP2-9	BspMO1_0196700.t1	932	456.914459	104	AP2
BMO1-AP2-10	BspMO1_0206900.t1	513	178.840546	58.7	AP2
BMO1-AP2-11	BspMO1_0279000.t1	488	388.687714	55.9	AP2
BMO1-AP2-12	BspMO1_0297600.t1	475	1053.084351	53.8	AP2
BMO1-AP2-13	BspMO1_0426700.t1	691	186.523087	75.3	AP2
BMO1-AP2-14	BspMO1_0425100.t1	669	56.950947	75.6	AP2, ACDC
BMO1-AP2-15	BspMO1_0423600.t1	459	132.788696	52.2	RPAP2_Rtr1
BMO1-AP2-16	BspMO1_003360-T1	794	--	90.6	AP2
BMO1-AP2-17	BspMO1_0103400.t1	261	159.928848	29.7	AP2
BMO1-AP2-18	BspMO1_0109600.t1	214	107.731911	25.2	AP2
BMO1-AP2-19	BspMO1_0112900.t1	148	20.662474	16.8	AP2
BMO1-AP2-20	BspMO1_0138400.t1	408	84.778854	46.2	PAP2_C
BMO1-AP2-21	BspMO1_0377200.t1	541	77.83445	61.2	AP2

Supplementary Table VIII A: Genome-wide read count Pearson correlations- ChIP-Seq analysis on *B. MO1* clone F12

H3K9me3_rep1	1				
H3K9me3_rep2	0.970557561	1			
H3K9ac_rep1	-0.226618949	-0.221437881	1		
H3K9ac_rep2	-0.225918015	-0.22061916	0.993004902	1	
IgG	0.407501319	0.407465025	0.459774564	0.458195753	1
	H3K9me3_rep1	H3K9me3_rep2	H3K9ac_rep1	H3K9ac_rep2	IgG

Supplementary Table VIII B: Genome-wide read count Pearson correlations- ChIP-Seq analysis on *B. MO1* clone B12

H3K9me3_rep1	1				
H3K9me3_rep2	0.958261066	1			
H3K9ac_rep1	-0.227423493	-0.228878465	1		
H3K9ac_rep2	-0.227801951	-0.229025069	0.992019183	1	
IgG	0.333857894	0.30096601	0.133843448	0.126827877	1
	H3K9me3_rep1	H3K9me3_rep2	H3K9ac_rep1	H3K9ac_rep2	IgG

Supplementary Table IX. Multigene families in different apicomplexan parasites.

Organism	Name of multigene family	No. of members	Associated publication
<i>P. falciparum</i>	<i>var</i>	50-60	PMID : 16790763
<i>P. falciparum</i>	<i>stevor</i>	39	PMID: 21332983
<i>P. falciparum</i>	<i>rifin</i>	150-200	PMID: 18197962
<i>P. falciparum</i>	<i>sera</i>	9	PMID: 32252804
<i>P. knowlesi</i>	<i>kir</i>	~68	PMID: 35677565
<i>P. vivax</i>	<i>vir</i>	~346	PMID: 19036639
<i>P. chabaudi</i>	<i>cir</i>	~200	PMID: 22458863
<i>P. cynomolgi</i>	<i>cyir</i>	~256	PMID: 22863735
<i>P. berghei</i>	<i>bir</i>	~180	PMID: 26996203
<i>P. yoelii</i>	<i>yir</i>	~800	PMID : 12368865
<i>B. bovis</i>	<i>smorf</i>	44	PMID: 22138017
<i>B. bovis</i>	<i>ves</i>	~135	PMID:17953480
<i>B. divergens</i>	<i>vesa</i> <i>ves 1α, ves1β, ves2</i>	134 (359)	This study. (PMID: 24799432)
<i>B. MO1</i>	<i>vesa1</i> <i>vesa2</i>	276 14	This study.
<i>B. duncani</i>	<i>Bdumgf</i> <i>Bdomgf</i>	73 105	PMID: 37055610
<i>B. microti</i>	<i>bmn</i>	10	PMID: 22833609

Supplementary Table X. Comparison of half minimal inhibitory concentration (IC₅₀) of various antiparasitic drugs between clones of *B. MO1* and *B. divergens* Rouen 87.

Antiparasitic drug (Target)	<i>B. MO1</i> B12	<i>B. MO1</i> F12	<i>B. divergens</i> Rouen87 Clone H2	<i>B. divergens</i> Rouen87 Clone H6	Fold difference
Atovaquone (Cyt-b)	11 ± 0.7 nM	10 ± 1.1 nM	4.5 ± 0.9 nM	4.7 ± 0.02 nM	2.4
Azithromycin (RPL6)	30 ± 2 uM	43 ± 1.7 uM	11.6 ± 0.7 uM	24.6 ± 1.7 uM	1.2
Clindamycin	113 ± 8.2 uM	81 ± 4.2 uM	11.6 ± 0.7 uM	24.6 ± 1.7 uM	1.3
Quinine	25 ± 1.4 uM	20 ± 2.6 uM	57 ± 1.9 uM	67 ± 2 uM	2.7
WR99210 (DHFR-TS)	3.1 ± 0.04 nM	0.2 ± 0.01 nM	162 ± 4.9 nM	330 ± 8.2 nM	164
Pyrimethamine(DHFR-TS)	30 ± 2.1 uM	26 ± 1.6 uM	10 ± 0.9 uM	9 ± 0.7 uM	2.9

Supplementary Table XI. RNA -seq TPM values of folate metabolism genes

Gene Name	<i>B. MO1</i> Clone B12	<i>B. MO1</i> Clone F12	<i>B. divergens</i> Rouen 87
Serine hydroxymethyltransferase-1 (SHMT)	290.896484	255.062485	108.65
S-adenosylmethionine synthase-2 (SAMS)	87.162872	81.135635	336.04
Glutathione synthetase (GS)	224.737961	232.131134	22.56
Dihydrofolate reductase thymidylate synthase (DHFR-TS)	297.84668	304.414795	382.95
Adenosyl homocysteinase (AHC)	238.004715	240.822906	328.56
Dihydropteroate synthase (DHPS)	214.073288	199.632187	16.59