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The complete mitochondrial genome of the firefly, *Asymmetricata circumdata* (Motschulsky) (Coleoptera: Lampyridae)

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ABSTRACT

We report the complete mitochondrial genome of firefly, *Asymmetricata circumdata* (Motschulsky). The circular genome of 15,967 bp has a base composition of A (42.44%), C (12.83%), G (8.79%) and T (36.16%). Similar to other Metazoa, our sequence contains 13 protein-coding genes. All 13 protein-coding genes were initiated by the ATN (ATT, ATA and ATG) codon. Eight protein-coding genes stopped with TAA or TAG codon and the other 5 genes have an incomplete termination codon, a single T. We sequenced the mitochondrial genome of fireflies to analyze phylogenetic relationships and deduce the evolution of their flashing signals.

ARTICLE HISTORY

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KEYWORDS

Asymmetricata circumdata (Motschulsky); firefly; Lampyridae; mitochondrial genome

Introduction

Fireflies have always been regarded as mysterious because of their bioluminescence. By using morphological characters, the genus *Asymmetricata* was erected in the Luciolinae in 2009 (Ballantyne & Lambkin 2009). *Asymmetricata circumdata* (Motschulsky) is distributed from Myanmar and Thailand to Cambodia (Ballantyne & Lambkin 2009). It is also widely dis-

tributed in adjacent Chinese provinces of Hainan, Guangxi, Jiangxi and Guangdong (Fu 2014).

Mitochondrial genome sequences are essential to a deeper understanding of the evolution of Lampyridae and other luminescent beetles (Ermakov et al. 2006). Here, we elucidate the mtDNA genome of *A. circumdata*.

Specimens were collected from Guangxi Province, China (23°24'N, 108°22'E) and were stored in Natural History

Table 1. Gene encoded by *Asymmetricata circumdata* mitochondrial genome

Gene	Accession Number
12S rRNA	MF000001
16S rRNA	MF000002
COI	MF000003
COII	MF000004
COIII	MF000005
CYTB	MF000006
CYTD	MF000007
CYTE	MF000008
CYTF	MF000009
CYTG	MF000010
CYTH	MF000011
CYTJ	MF000012
CYTK	MF000013
CYTL	MF000014
CYTM	MF000015
CYTN	MF000016
CYTO	MF000017
CYTP	MF000018
CYTQ	MF000019
CYTR	MF000020
CYTS	MF000021
CYTU	MF000022
CYTV	MF000023
CYTW	MF000024
CYTX	MF000025
CYTY	MF000026
CYTZ	MF000027
ND1	MF000028
ND2	MF000029
ND3	MF000030
ND4	MF000031
ND5	MF000032
ND6	MF000033
ND7	MF000034
ND8	MF000035
ND9	MF000036
ND10	MF000037
ND11	MF000038
ND12	MF000039
ND13	MF000040
ND14	MF000041
ND15	MF000042
ND16	MF000043
ND17	MF000044
ND18	MF000045
ND19	MF000046
ND20	MF000047
ND21	MF000048
ND22	MF000049
ND23	MF000050
ND24	MF000051
ND25	MF000052
ND26	MF000053
ND27	MF000054
ND28	MF000055
ND29	MF000056
ND30	MF000057
ND31	MF000058
ND32	MF000059
ND33	MF000060
ND34	MF000061
ND35	MF000062
ND36	MF000063
ND37	MF000064
ND38	MF000065
ND39	MF000066
ND40	MF000067
ND41	MF000068
ND42	MF000069
ND43	MF000070
ND44	MF000071
ND45	MF000072
ND46	MF000073
ND47	MF000074
ND48	MF000075
ND49	MF000076
ND50	MF000077
ND51	MF000078
ND52	MF000079
ND53	MF000080
ND54	MF000081
ND55	MF000082
ND56	MF000083
ND57	MF000084
ND58	MF000085
ND59	MF000086
ND60	MF000087
ND61	MF000088
ND62	MF000089
ND63	MF000090
ND64	MF000091
ND65	MF000092
ND66	MF000093
ND67	MF000094
ND68	MF000095
ND69	MF000096
ND70	MF000097
ND71	MF000098
ND72	MF000099
ND73	MF000100
ND74	MF000101
ND75	MF000102
ND76	MF000103
ND77	MF000104
ND78	MF000105
ND79	MF000106
ND80	MF000107
ND81	MF000108
ND82	MF000109
ND83	MF000110
ND84	MF000111
ND85	MF000112
ND86	MF000113
ND87	MF000114
ND88	MF000115
ND89	MF000116
ND90	MF000117
ND91	MF000118
ND92	MF000119
ND93	MF000120
ND94	MF000121
ND95	MF000122
ND96	MF000123
ND97	MF000124
ND98	MF000125
ND99	MF000126
ND100	MF000127
ND101	MF000128
ND102	MF000129
ND103	MF000130
ND104	MF000131
ND105	MF000132
ND106	MF000133
ND107	MF000134
ND108	MF000135
ND109	MF000136
ND110	MF000137
ND111	MF000138
ND112	MF000139
ND113	MF000140
ND114	MF000141
ND115	MF000142
ND116	MF000143
ND117	MF000144
ND118	MF000145
ND119	MF000146
ND120	MF000147
ND121	MF000148
ND122	MF000149
ND123	MF000150
ND124	MF000151
ND125	MF000152
ND126	MF000153
ND127	MF000154
ND128	MF000155
ND129	MF000156
ND130	MF000157
ND131	MF000158
ND132	MF000159
ND133	MF000160
ND134	MF000161
ND135	MF000162
ND136	MF000163
ND137	MF000164
ND138	MF000165
ND139	MF000166
ND140	MF000167
ND141	MF000168
ND142	MF000169
ND143	MF000170
ND144	MF000171
ND145	MF000172
ND146	MF000173
ND147	MF000174
ND148	MF000175
ND149	MF000176
ND150	MF000177
ND151	MF000178
ND152	MF000179
ND153	MF000180
ND154	MF000181
ND155	MF000182
ND156	MF000183
ND157	MF000184
ND158	MF000185
ND159	MF000186
ND160	MF000187
ND161	MF000188
ND162	MF000189
ND163	MF000190
ND164	MF000191
ND165	MF000192
ND166	MF000193
ND167	MF000194
ND168	MF000195
ND169	MF000196
ND170	MF000197
ND171	MF000198
ND172	MF000199
ND173	MF000200
ND174	MF000201
ND175	MF000202
ND176	MF000203
ND177	MF000204
ND178	MF000205
ND179	MF000206
ND180	MF000207
ND181	MF000208
ND182	MF000209
ND183	MF000210
ND184	MF000211
ND185	MF000212
ND186	MF000213
ND187	MF000214
ND188	MF000215
ND189	MF000216
ND190	MF000217
ND191	MF000218
ND192	MF000219
ND193	MF000220
ND194	MF000221
ND195	MF000222
ND196	MF000223
ND197	MF000224
ND198	MF000225
ND199	MF000226
ND200	MF000227
ND201	MF000228
ND202	MF000229
ND203	MF000230
ND204	MF000231
ND205	MF000232
ND206	MF000233
ND207	MF000234
ND208	MF000235
ND209	MF000236
ND210	MF000237
ND211	MF000238
ND212	MF000239
ND213	MF000240
ND214	MF000241
ND215	MF000242
ND216	MF000243
ND217	MF000244
ND218	MF000245
ND219	MF000246
ND220	MF000247
ND221	MF000248
ND222	MF000249
ND223	MF000250
ND224	MF000251
ND225	MF000252
ND226	MF000253
ND227	MF000254
ND228	MF000255
ND229	MF000256
ND230	MF000257
ND231	MF000258
ND232	MF000259
ND233	MF000260
ND234	MF000261
ND235	MF000262
ND236	MF000263
ND237	MF000264
ND238	MF000265
ND239	MF000266
ND240	MF000267
ND241	MF000268
ND242	MF000269
ND243	MF000270
ND244	MF000271
ND245	MF000272
ND246	MF000273
ND247	MF000274
ND248	MF000275
ND249	MF000276
ND250	MF000277
ND251	MF000278
ND252	MF000279
ND253	MF000280
ND254	MF000281
ND255	MF000282
ND256	MF000283
ND257	MF000284
ND258	MF000285
ND259	MF000286
ND260	MF000287
ND261	MF000288
ND262	MF000289
ND263	MF000290
ND264	MF000291
ND265	MF000292
ND266	MF000293
ND267	MF000294
ND268	MF000295
ND269	MF000296
ND270	MF000297
ND271	MF000298
ND272	MF000299
ND273	MF000300
ND274	MF000301
ND275	MF000302
ND276	MF000303
ND277	MF000304
ND278	MF000305
ND279	MF000306
ND280	MF000307
ND281	MF000308
ND282	MF000309
ND283	MF000310
ND284	MF000311
ND285	MF000312
ND286	MF000313
ND287	MF000314
ND288	MF000315
ND289	MF000316
ND290	MF000317
ND291	MF000318
ND292	MF000319
ND293	MF000320
ND294	MF000321
ND295	MF000322
ND296	MF000323
ND297	MF000324
ND298	MF000325
ND299	MF000326
ND300	MF000327
ND301	MF000328
ND302	MF000329
ND303	MF000330
ND304	MF000331
ND305	MF000332
ND306	MF000333
ND307	MF000334
ND308	MF000335
ND309	MF000336
ND310	MF000337
ND311	MF000338
ND312	MF000339
ND313	MF000340
ND314	MF000341
ND315	MF000342
ND316	MF000343
ND317	MF000344
ND318	MF000345
ND319	MF000346
ND320	MF000347
ND321	MF000348
ND322	MF000349
ND323	MF000350
ND324	MF000351
ND325	MF000352
ND326	MF000353
ND327	MF000354
ND328	MF000355
ND329	MF000356
ND330	MF000357
ND331	MF000358
ND332	MF000359
ND333	MF000360
ND334	MF000361
ND335	MF000362
ND336	MF000363
ND337	MF000364
ND338	MF000365
ND339	MF000366
ND340	MF000367
ND341	MF000368
ND342	MF000369
ND343	MF000370
ND344	MF000371
ND345	MF000372
ND346	MF000373
ND347	MF000374

Table 1. Continued

Gene	Direction	P			Base comp. (%)				Start codon*	Start codon	Stop codon
		F	T	Se (bp)	A	C	G	T			
<i>tRNA^{Asn}</i>	F	6036	6100	65	49.23	10.77	10.77	29.23	0		
<i>tRNA^{Ser}</i>	F	6101	6163	63	39.68	11.11	9.52	39.68	0		
<i>tRNA^{Glu}</i>	F	6164	6227	64	46.88	7.81	3.13	42.19	0		
<i>tRNA^{Phe}</i>	R	6226	6286	61	36.07	13.11	4.92	45.90	-2		
ND5	R	6287	7997	1711	48.45	11.86	8.12	31.56	0	ATA	T+ RNA
<i>tRNA^{His}</i>	R	7995	8057	63	46.03	12.70	3.17	38.10	-3		
ND4	R	8058	9381	1324	50.76	12.92	7.93	28.40	0	ATG	T+ RNA
ND4L	R	9375	9665	291	50.86	13.40	6.53	29.21	-7	ATG	TAA
<i>tRNA^{Thr}</i>	F	9667	9728	62	45.16	6.45	8.06	40.32	1		
<i>tRNA^{Pro}</i>	R	9729	9792	64	39.06	14.06	6.25	40.63	0		
ND6	F	9797	10279	483	39.75	12.84	7.66	39.75	4	ATA	TAA
<i>cytB</i>	F	10279	11412	1134	34.57	14.99	11.55	38.89	-1	ATG	TAG
<i>tRNA^{Ser}</i>	F	11411	11477	67	43.28	7.46	11.94	37.31	-2		
ND1	R	11494	12426	933	50.16	14.26	9.75	25.83	16	ATT	TAG
<i>tRNA^{Lys}</i>	R	12446	12506	61	40.98	18.03	8.20	32.79	19		
16s rRNA	R	12507	13770	1264	46.52	12.10	5.85	35.52	0		
<i>tRNA^{Val}</i>	R	13771	13839	69	42.03	13.04	7.25	37.68	0		
12s rRNA	R	13840	14579	740	44.32	13.51	6.22	35.95	0		
AT-rich region		14580	15967	1388	44.81	7.71	2.74	44.74			

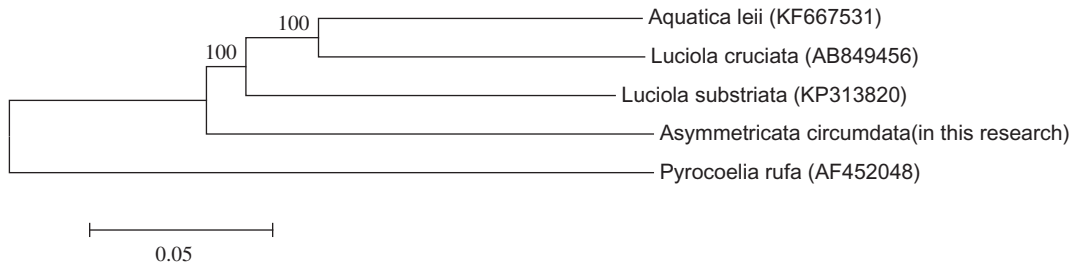


Figure 1. Molecular phylogenetic tree of *Asymmetricata circumdata* and five other firefly species based on the complete mitochondrial genome sequences. The complete mitochondrial genome sequences were aligned in MEGA 5 (Bioinformatics Research Center, Beijing, China) and the phylogenetic tree was constructed by Neighbour-Joining method with 1000 bootstrap replicates. Mitochondrial DNA accession numbers: *Aquatica leii* (KF667531), *Luciola cruciata* (AB849456), *Luciola substriata* (KP313820), *Pyrocoelia rufa* (AF452048).

Museum, Huazhong Agricultural University, Wuhan, Hubei, China (its accession no. is AC2014071301). As a species of steady-state bioluminescence fireflies, its habits, flashing signals and some morphology have been studied in detail (Wattanachaiyingcharoen et al. 2012; Goswami et al. 2015). However, there is no genetic research information about *A. circumdata*.

Specific primers were designed based on these conserved regions sequences. The PCR reaction was carried out with LA Taq polymerase for 35 cycles at 94 °C for 30 s, and annealed at 50 °C for 30 s, followed by extension at 72 °C for 1 min per 1 kb. Sequences were assembled using the software DNASTar v7.1 (Madison, WI) and adjusted manually to generate the complete sequence of mitochondrial DNA.

The complete mitochondrial genome sequence of *A. circumdata* (GenBank KX229747) has 15,967 bp and has a base composition of A (42.44%), C (12.83%), G (8.79%), T (36.16%). Similar to other Metazoa, our sequence contains 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a non-coding AT-rich region, which represents a typical insect mitochondrial genome (Wolstenholme 1992). The open frames of the 13 protein-coding genes were inferred from three other fireflies: *Aquatica leii*, *Luciola substriata* and *Pyrocoelia rufa* (Lee et al. 2004; Jiao et al. 2013; Mu et al. 2015). All 13 PGGs initiated with ATN (ATT, ATA and ATG) codon, while 8 PGGs stopped with TAA or TAG codon, and the other 5 PGGs have an incomplete termination codon, namely, a single T (Table 1). The AT-rich region is 1388 bp,

which is shorter than that of the other fireflies with reported sequences.

The phylogenetic tree among the five species based on mitochondrial genome sequences were aligned in MEGA 5 (Phoenix, AZ) (with 1000 bootstrap replicates) to construct a Neighbour-Joining tree (Figure 1).

The result shows *A. circumdata* is most closely related to *L. substriata*, which belongs to an entirely different genus in the Lampyridae.

In conclusion, the complete mitochondrial genome sequence of *A. circumdata* provides an important molecular framework for further phylogenetic analyses of fireflies. These data are essential for deeper understanding of the role of sexual and natural selection in the evolution of firefly flashing signals.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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