

Supplementary information

Table S1. Primers used in this study

Purpose	Primer name	Sequence (5'-3')*	Region
Identification of <i>Spiroplasma</i> isolated in this study	BF1	AGAGTTTGATCMTGGCTC	16S rRNA
	BF2	ACTCCTACGGGAGGCAGCAGTRGGG	
	BR2	CCCCGTCAATTCMTTGTGATTT	
	BR1	GACGGGCGGTGWGTRCA	
Detection of <i>Spiroplasma</i> from mosquitoes for prevalence	MF1	ACACCATGGGAGCTGGTAAT	16S-23S
	MR1	CTTCWTCGACTTYCAGACCCAAGGCAT	Spacer

*M: A or C, R: A or G, W: A or T, Y: C or T

Table S2. Detailed information on bacteria in the phylogenetic tree

Species, strain, (accession no.)	Serotype ^a	Host
<i>S. diabroticae</i> strain DU-1 (NR_104751.1)	[XII]	<i>Diabrotica undecimpunctata</i> beetles ^a
<i>S. diabroticae</i> (GU908490.1)	[XII]	
<i>E. melaleucae</i> strain M1 (NR_042854.1)		<i>Melaleuca quinquenervia</i> ^b
<i>S. floricola</i> strain 23-6 (NR_025703.1)	[III]	Insects, flower ^a
<i>S. floricola</i> 23-6 (CP025057.1)	[III]	
<i>Spiroplasma</i> sp. W115 (AY189317.1)	[XXI]	<i>Prunus</i> sp. flowers ^a
<i>Spiroplasma</i> sp. BIUS-1 (AY189319.1)	[XXX]	
<i>Spiroplasma</i> sp. BIUS-1 (CP048386.1)	[XXX]	<i>Bidens</i> sp. ^b
<i>S. diminutum</i> CUAS-1 (NR_121702.1)	[XXV]	<i>Culex</i> mosquito ^a
<i>S. diminutum</i> strain CUAS-1 (NR_025702.1)	[XXV]	
<i>S. diminutum</i> CUAS-1 (CP005076.1)	[XXV]	
<i>Spiroplasma</i> sp. CB-1 (AY189315.1)	[XVI-2]	Cantharid beetle ^a
<i>S. cantharicola</i> strain CC-1	[XVI-1]	

(CP012622.1)			
<i>S. cantharicola</i> strain Q-6 (DQ861915.1)			
<i>S. cantharicola</i> strain CC-1 (NR_125516.1)	[XVI-1]		Cantharid beetle ^a
<i>S. cantharicola</i> strain CC-1 (DQ861914.1)	[XVI-1]		
<i>Spiroplasma</i> sp. Ar-1357 (AY189316.1)	[XVI-3]		Mosquito ^a
<i>S. cantharicola</i> strain AR1357 (DQ861916.1)			
<i>S. monobiae</i> MQ-1 (CP025543.1)	[VII]	<i>Monobia quadridens</i> ^b	<i>Monobia</i> wasps ^a
<i>S. monobiae</i> strain MQ-1 (NR_104854.1)	[VII]		
<i>S. citri</i> strain R8A2HP (NR_036849.2)	[I-1]		Dicots, leafhoppers ^a

^aInformation on serotype and host was retrieved from Regassa L.B. *et al* [2].

^bInformation on host was retrieved from Genbank site because it was not available in the reference [2].

Table S3. Detailed information of samples

Mosquito Pool ID	Species	Sex	Number of Mosquitoes	Collection site	Collection date	PCR	BLAST search result
1	<i>Aedes togoi</i>	Male	1	Otaru aquarium	11th Aug 2016	-	
2	<i>Aedes togoi</i>	Male	3	Otaru aquarium	11th Aug 2016	-	
3	<i>Aedes togoi</i>	Male	5	Otaru aquarium	25th Aug 2016	-	
4	<i>Aedes togoi</i>	Male	20	Otaru aquarium	25th Aug 2016	-	
5	<i>Aedes togoi</i>	Male	8	Otaru aquarium	25th Aug 2016	-	
6	<i>Aedes togoi</i>	Male	1	Otaru aquarium	12th Sep 2016	-	
7	<i>Aedes togoi</i>	Male	9	Otaru aquarium	12th Sep 2016	-	
8	<i>Aedes togoi</i>	Male	20	Otaru aquarium	12th Sep 2016	-	
9	<i>Aedes togoi</i>	Male	2	Otaru aquarium	12th Sep 2016	-	
10	<i>Aedes togoi</i>	Male	8	Otaru aquarium	27th Sep 2016	-	
11	<i>Aedes togoi</i>	Female	5	Otaru aquarium	20th Jul 2016	-	
12	<i>Aedes togoi</i>	Female	1	Otaru aquarium	20th Jul 2016	-	
13	<i>Aedes togoi</i>	Female	4	Otaru aquarium	20th Jul 2016	-	
14	<i>Aedes togoi</i>	Female	6	Otaru aquarium	11th Aug 2016	+	nonspecific reaction
15	<i>Aedes togoi</i>	Female	1	Otaru aquarium	25th Aug 2016	-	
16	<i>Aedes togoi</i>	Female	17	Otaru aquarium	25th Aug 2016	-	
17	<i>Aedes togoi</i>	Female	2	Otaru aquarium	12th Sep 2016	+	nonspecific reaction
18	<i>Aedes togoi</i>	Female	1	Otaru aquarium	12th Sep 2016	-	
19	<i>Aedes togoi</i>	Female	3	Otaru aquarium	12th Sep 2016	-	

20	<i>Aedes togoi</i>	Female	7	Otaru aquarium	12th Sep 2016	-	
21	<i>Aedes togoi</i>	Female	14	Otaru aquarium	12th Sep 2016	-	
22	<i>Aedes togoi</i>	Female	1	Otaru aquarium	27th Sep 2016	-	
23	<i>Aedes togoi</i>	Female	1	Otaru aquarium	27th Sep 2016	-	
24	<i>Aedes togoi</i>	Female	15	Otaru aquarium	27th Sep 2016	-	
25	<i>Aedes japonicus</i>	Male	8	Otaru aquarium	20th Jul 2016	+	nonspecific reaction
26	<i>Aedes japonicus</i>	Male	20	Otaru aquarium	20th Jul 2016	-	
27	<i>Aedes japonicus</i>	Male	20	Otaru aquarium	20th Jul 2016	-	
28	<i>Aedes japonicus</i>	Male	20	Otaru aquarium	20th Jul 2016	-	
29	<i>Aedes japonicus</i>	Male	7	Otaru aquarium	25th Aug 2016	-	
30	<i>Aedes japonicus</i>	Male	3	Otaru aquarium	12th Sep 2016	-	
31	<i>Aedes japonicus</i>	Female	1	Otaru aquarium	6th Jul 2016	-	
32	<i>Aedes japonicus</i>	Female	7	Otaru aquarium	20th Jul 2016	-	
33	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	11th Aug 2016	-	
34	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	11th Aug 2016	-	
35	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	11th Aug 2016	-	
36	<i>Aedes japonicus</i>	Female	1	Otaru aquarium	11th Aug 2016	-	
37	<i>Aedes japonicus</i>	Female	15	Otaru aquarium	12th Sep 2016	-	
38	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	12th Sep 2016	-	
39	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	27th Sep 2016	-	
40	<i>Aedes japonicus</i>	Female	20	Otaru aquarium	27th Sep 2016	-	
41	<i>Aedes japonicus</i>	Male	1	Rakuno Gakuen Univ.	15th Jul 2020	-	

42	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	24th Jun 2020	-
43	<i>Aedes japonicus</i>	Female	8	Rakuno Gakuen Univ.	24th Jun 2020	-
44	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	14th Jul 2020	-
45	<i>Aedes japonicus</i>	Female	1	Rakuno Gakuen Univ.	14th Jul 2020	-
46	<i>Aedes japonicus</i>	Female	8	Rakuno Gakuen Univ.	15th Jul 2020	-
47	<i>Aedes japonicus</i>	Female	1	Rakuno Gakuen Univ.	15th Jul 2020	-
48	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	20th Aug 2020	-
49	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	20th Aug 2020	-
50	<i>Aedes japonicus</i>	Female	8	Rakuno Gakuen Univ.	20th Aug 2020	-
51	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	15th Jul 2020	-
52	<i>Aedes japonicus</i>	Female	10	Rakuno Gakuen Univ.	15th Jul 2020	-
53	<i>Aedes japonicus</i>	Female	9	Rakuno Gakuen Univ.	15th Jul 2020	-
54	<i>Aedes japonicus</i>	Female	1	Rakuno Gakuen Univ.	15th Jul 2020	-
55	<i>Aedes japonicus</i>	Male	4	Nopporo Forest Park	20th Aug 2020	-
56	<i>Aedes japonicus</i>	Female	4	Nopporo Forest Park	20th Aug 2020	-
57	<i>Aedes japonicus</i>	Female	6	Nopporo Forest Park	8th Sep 2020	-
58	<i>Aedes japonicus</i>	Female	3	Nopporo Forest Park	20th Aug 2020	-
59	<i>Aedes hokkaidensis</i>	Female	1	Rakuno Gakuen Univ.	24th Jun 2020	-
60	<i>Aedes hokkaidensis</i>	Female	1	Rakuno Gakuen Univ.	24th Jun 2020	-
61	<i>Aedes hokkaidensis</i>	Female	1	Rakuno Gakuen Univ.	24th Jun 2020	-
62	<i>Aedes hokkaidensis</i>	Female	2	Rakuno Gakuen Univ.	14th Jul 2020	-
63	<i>Aedes galloisi</i>	Male	3	Nopporo Forest Park	20th Aug 2020	-

64	<i>Aedes galloisi</i>	Male	3	Nopporo Forest Park	20th Aug 2020	-	
65	<i>Aedes galloisi</i>	Male	4	Nopporo Forest Park	20th Aug 2020	-	
66	<i>Aedes galloisi</i>	Male	1	Nopporo Forest Park	8th Sep 2020	-	
67	<i>Aedes galloisi</i>	Female	3	Nopporo Forest Park	20th Aug 2020	-	
68	<i>Aedes galloisi</i>	Female	2	Nopporo Forest Park	20th Aug 2020	-	
69	<i>Aedes galloisi</i>	Female	2	Nopporo Forest Park	20th Aug 2020	-	
70	<i>Aedes galloisi</i>	Female	1	Nopporo Forest Park	20th Aug 2020	-	
71	<i>Aedes galloisi</i>	Female	2	Nopporo Forest Park	8th Sep 2020	-	
72	<i>Aedes galloisi</i>	Female	3	Nopporo Forest Park	8th Sep 2020	-	
73	<i>Aedes galloisi</i>	Female	1	Nopporo Forest Park	8th Sep 2020	-	
74	<i>Aedes galloisi</i>	Female	2	Nopporo Forest Park	20th Aug 2020	+	nonspecific reaction*
75	<i>Aedes albopictus</i>	Female	2	laboratory-colony	30th Sep 2020	-	
76	<i>Aedes albopictus</i>	Female	2	laboratory-colony	30th Sep 2020	-	
77	<i>Aedes albopictus</i>	Female	2	laboratory-colony	30th Sep 2020	-	
78	<i>Aedes albopictus</i>	Female	2	laboratory-colony	30th Sep 2020	-	
79	<i>Aedes albopictus</i>	Female	2	laboratory-colony	30th Sep 2020	-	
80	<i>Aedes aegypti</i>	Female	2	laboratory-colony	30th Sep 2020	-	
81	<i>Aedes aegypti</i>	Female	2	laboratory-colony	30th Sep 2020	-	
82	<i>Aedes aegypti</i>	Female	2	laboratory-colony	30th Sep 2020	-	
83	<i>Aedes aegypti</i>	Female	2	laboratory-colony	30th Sep 2020	-	
84	<i>Aedes aegypti</i>	Female	2	laboratory-colony	30th Sep 2020	-	
85	<i>Culex pipiens</i> group	Male	1	Rakuno Gakuen Univ.	Sep to Oct 2020	-	

86	<i>Culex pipiens</i> group	Male	1	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
87	<i>Culex pipiens</i> group	Male	1	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
88	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
89	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
90	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
91	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
92	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
93	<i>Culex pipiens</i> group	Female	10	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction
94	<i>Culex pipiens</i> group	Female	4	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
95	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
96	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
97	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction*
98	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
99	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
100	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	-	
101	<i>Culex orientalis</i>	Female	3	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction*
102	<i>Culex orientalis</i>	Female	2	Rakuno Gakuen Univ.	Sep to Oct 2020	+	nonspecific reaction*
103	<i>Anopheles sineroides</i>	Female	1	Rakuno Gakuen Univ.	Sep to Oct 2020	-	

*Possibly a reaction against mosquito DNA.