
Original Article

Faunal and genetic studies of mosquitoes on Chichi-jima and Haha-jima, the Ogasawara (Bonin) Islands, Japan

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Abstract: Thirty years have passed since the last surveillance of the mosquito fauna on the entire Chichi-jima, Ogasawara Islands, Japan. Herein, we report the results of a mosquito study conducted on the Ogasawara Islands in 2015 and 2017. In this study, 2,371 individuals belonging to seven species in three genera were collected, including 262 mosquitoes belonging to five species in three genera collected in 2015 and 2,109 specimens representing six species in three genera obtained in 2017. The dominant species in dry-ice trap collections were *Culex boninensis* and *Cx. quinquefasciatus*, and the latter was dominant in yeast trap collections. *Aedes albopictus*, *Ae. savoryi*, and *Cx. boninensis* were dominant in sweep net collections, while *Ae. albopictus* and *Cx. quinquefasciatus* were dominant in the larval collection. *Culex boninensis* and *Ae. albopictus* were widely distributed on the islands. Gene sequences of cytochrome c oxidase subunit I from 55 specimens belonging to the seven species in the three genera, including four endemic species (*Ae. wadai*, *Ae. savoryi*, *Cx. boninensis*, and *Lutzia shinonagai*), were registered in GenBank. Genetic distance analysis showed low intraspecific variations and low mean genetic distances (between 0.0% and 0.8%), indicating significant homogeneity among mosquito populations on the Ogasawara Islands.

Key words: *Aedes wadai*, *Aedes savoryi*, *Culex boninensis*, *Lutzia shinonagai*, cytochrome c oxidase subunit I, Bonin Islands

INTRODUCTION

The Ogasawara Islands are oceanic islands located 1,000 km south of Tokyo, Japan. The chain consists of over 30 large and small islands spanning 400 km from north to south. These islands have never been connected to other islands, except their respective nearest island (Ogasawara Islands Branch Office, 2020). The Ogasawara Islands are important and precious sites for studying island biology, with a variety of endemic flora and fauna (Ohbayashi et al., 2004; Karube et al., 2012). Eleven mosquito species in three genera have been found on the Ogasawara Islands, including four endemic species [*Aedes wadai* Tanaka, Mizusawa and Saugstad, *Ae. savoryi* Tanaka, Mizusawa and Saugstad, *Culex boninensis* Bohart, and *Lutzia shinonagai* (Tanaka, Mizusawa and Saugstad)]. These species have been reported on the Chichi-jima, Haha-jima, Ani-jima, Ototo-jima, and Muko-jima Islands (Wada et al., 1969; Takahashi, 1973; Tanaka et al., 1979; Kusui et al., 2004; Toma and Miyagi, 2005).

Six of the 11 species have been reported as vectors of infectious diseases (Tanaka et al., 1979; Tsuda, 2019), but there have been no recent reports of mosquito-borne infectious diseases on the Ogasawara Islands. Although inhabitation of *Ae. aegypti* (Linnaeus) and cases of dengue fever were previously reported on Chichi-jima (Yamada, 1917, 1932; Hsiao and Bohart, 1946), there have been no recent reports. *Aedes aegypti* is a known important vector of yellow fever and dengue fever (Yamada, 1917, 1932) and is considered the vector mosquito for dengue fever on Chichi-jima. *Aedes albopictus* (Skuse), despite being a very common species on Honshu, Shikoku, and Kyushu, Japan (Yamada, 1921, 1932; La Casse and Yamaguti, 1947), was not reported on the Ogasawara Islands before 1949. The earliest collection record of *Ae. albopictus* on Chichi-jima and Haha-jima is believed to be in 1949 by Mead, and 1951 by Bohart, respectively (Bohart, 1956). *Aedes albopictus* was shown to be the vector of dengue fever when outbreaks occurred in the Yoyogi Park and its vicinity in Tokyo, Japan, in the summer of

2014 (Tsuda et al., 2016). Thus, the Ogasawara Islands are a likely area for outbreaks of mosquito-borne infectious diseases (Yamanouchi and Kasai, 2016), and it is important to understand their mosquito fauna.

The most recent mosquito surveillance on the islands was conducted in port facilities and surrounding residential areas on Chichi-jima and Haha-jima in 2002 (Kusui et al., 2004), and port sanitary investigations were conducted on Chichi-jima in 2015 and 2016 by the quarantine station (Yamanouchi and Kasai, 2016, 2017). However, extensive mosquito fauna surveillance of the entire island was conducted on Chichi-jima in 1990 (Toma and Miyagi, 2005), which was almost 30 years ago, while no recent information on the mosquito fauna and vector mosquitoes on the Ogasawara Islands is available. The environmental conditions of the Ogasawara Islands have been rapidly changing. Their World Heritage registration has increased the number of tourists (Kawanami, 2016), and ocean cruise ships stop at the Futami port on Chichi-jima, adding to the anthropogenic impacts on local ecology. Currently, the transportation of people and supplies is restricted to ships and freight shipping services that provide regular service between Tokyo and the Futami port. Human pathogens and vector animals such as mosquitoes, can be carried to Chichi-jima by ships from the main islands and/or abroad, similar to the situations reported globally (Yamada, 1917; Hawley, 1988; Kurihara, 2003; Derraik, 2004; Chapple et al., 2012; Medlock et al., 2012). Therefore, information on the mosquito fauna and vectors across the island should be updated. Such information for both inhabited islands, Chichi-jima and Haha-jima, is essential for the prevention and control of mosquito-borne infectious diseases.

Maekawa et al. (2016a) reported mosquito identification using molecular techniques that allow accurate identification of morphologically similar species, such as sibling species and subspecies. The use of the cytochrome c oxidase subunit I (COI) gene region in the mitochondrial DNA (mtDNA) is especially convenient for Japanese mosquito studies because genetic sequences of various species have been registered in GenBank. As it is important to evaluate geographical genetic diversity to establish COI barcoding, the information on isolated populations (such as those on the Ogasawara Islands) could provide significant contributions.

In this study, we surveyed mosquito populations on Chichi-jima and Haha-jima and determined the COI sequences of mosquitoes collected on the Ogasawara Islands to examine their intraspecific variations, as well as genetic divergence of common species among the Ogasawara Islands, main islands of Japan, and the Ryukyu Archipelago. Subsequently, we constructed a phylogenetic tree of the collected mosquitoes and

related species based on the COI sequences. Four endemic species, *Ae. wadai*, *Ae. savoryi*, *Cx. boninensis*, and *Lt. shinonagai*, have been morphologically classified, but there is limited molecular evidence. In particular, the morphology of *Cx. (Sirivanakarnius) boninensis* and *Lt. (Insulalutzia) shinonagai*, is clearly different from that of the other species of the same genus, and they are classified as the only species of the monotypic subgenus (Tanaka et al., 2005). Thus, we confirmed the lineage relationship among the four endemic species and related species based on the COI phylogenetic tree. Finally, we discussed the possibility of introducing mosquito-borne infectious diseases from outside the islands to prevent and control such diseases in the Ogasawara Islands.

MATERIALS AND METHODS

Study sites

The islands of Chichi-jima and Haha-jima are inhabited, with a total population of approximately 2,600 (Ogasawara Islands Branch Office, 2020). Chichi-jima has a subtropical climate with an annual rainfall of 940 mm and an average annual temperature of 24.1°C (Ogasawara Islands Branch Office, 2020). Mosquito surveillance was conducted on Chichi-jima from February 28 to March 14, and from July 30 to August 20, 2015, and from October 5 to 10, 2017. On Haha-jima, mosquito surveillance was conducted from October 12 to 14, 2017.

Mosquito collection

Collection of adult mosquitoes was conducted on Chichi-jima in 2015, and adult and larval collections were carried out on Chichi-jima and Haha-jima in 2017 (Fig. 1a and b). The study area was divided into the following three sub-study areas based on the natural environment and altitude: a coastal area, residential area, and hilly area. The following four methods were employed to collect adult mosquitoes: (1) a sweep net (15–30 min/collection between 10:00 and 16:00) at 10 sites (February and March) and 6 sites (July and August) in 2015, and 1 site in 2017. The collections were carried out 1–6 times at each site (Tables 1 and 2). We sought mosquitoes attracted to the collector for feeding or those that rested in nearby grass and shrubby vegetation; (2) an 8-min human-baited sweep net (8 min/collection between 10:00 and 16:00), whereby we stayed in a certain place while collecting mosquitoes attracted to the collector. This collection was performed at Kitakou shuraku ato, an area uninhabited for 70 years on Haha-jima, to determine the density of *Ae. albopictus* in an isolated area; (3) a yeast trap (1 trap/site/collection) at 13 sites on Chichi-jima, whereby the traps were operated for 1–6 collections at each site (Table 1) from 15:00 to 08:00 in July and August 2015, with each trap fixed on a tree approximately 1–2 m above the ground; and

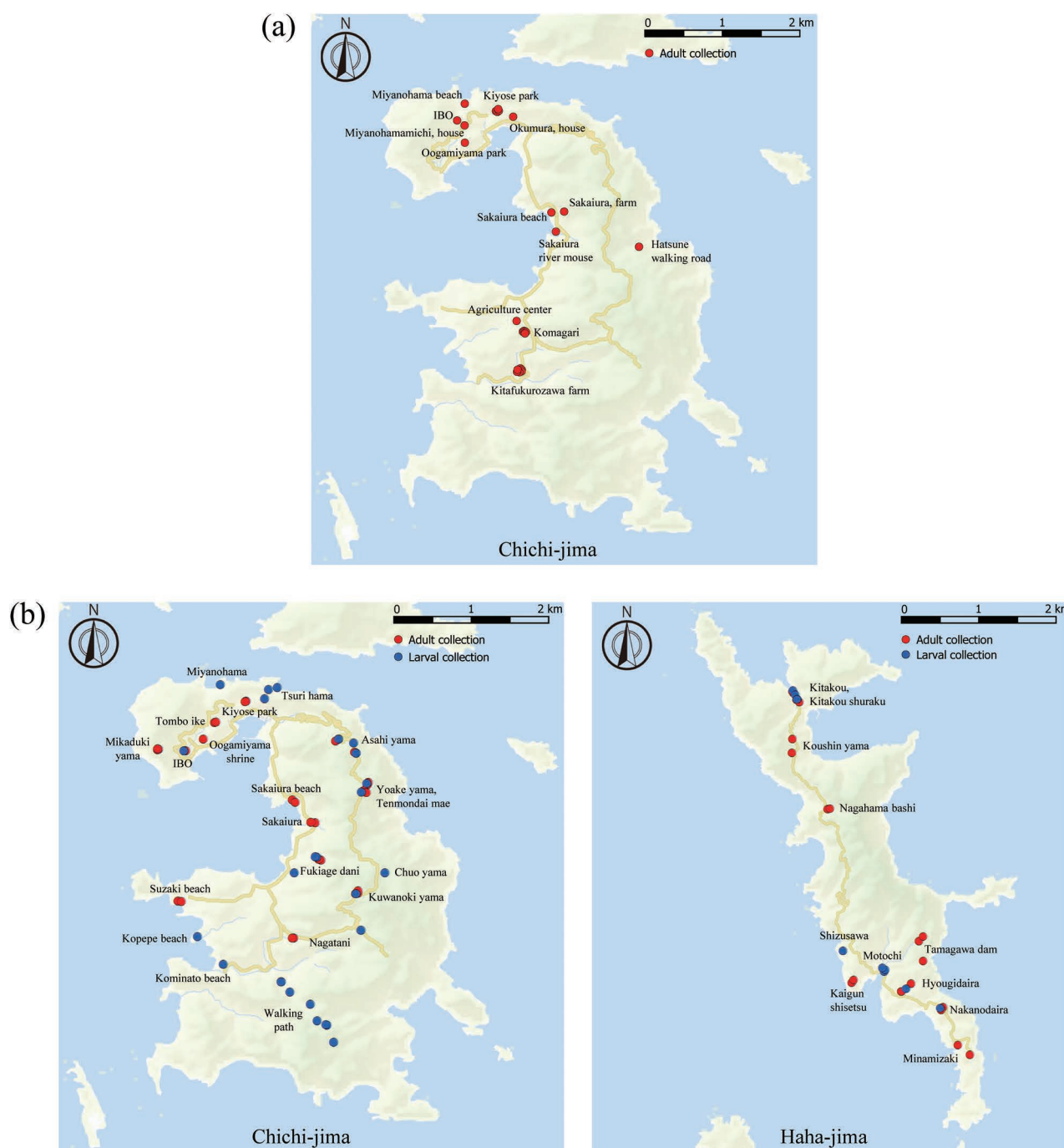


Fig. 1. A map showing the adult collection sites on Chichi-jima and Haha-jima, Ogasawara Islands. (a) A map showing the adult collection sites on Chichi-jima in 2015. Some of the collection sites at Komagari, Kiyose, and Kitafukurozawa are overlapped. (b) A map showing the adult and larval collection sites on Chichi-jima and Haha-jima in 2017. Some collection sites of adult and larva were the same or very close, and these sites are shown to overlap.

(4) a dry-ice trap (1 trap/site/collection) at 30 and 20 sites on Chichi-jima and Haha-jima, respectively. In October 2017, the collections were carried out for two consecutive days at each site (Table 2) from 16:00 to 07:00. The traps were hung from a tree approximately 1.5 m from the ground.

Obtaining dry ice or liquid CO₂ on the remote islands of Ogasawara was difficult. We used yeast-generated CO₂ as an attractant during the 2015 mosquito survey, but dry ice was available for

mosquito collection in 2017, *via* shipments from Tokyo and could secure a site where dry ice could be stored frozen on Chichi-jima. For yeast trap and dry-ice trap collections, we used the methods of Tsuda et al. (2009) and Maekawa et al. (2016b), respectively. Collectors wore long-sleeved shirts, long trousers, and a cap and applied a repellent to the bare skin of their hands and face to avoid mosquito bites while conducting the sweep net collection and 8-min human-baited sweep net collection.

Table 1. Total number of mosquitoes collected by yeast trap (upper) and sweep-net collection (lower) on Chichi-jima, Ogasawara Islands in 2015.

Methods	Periods	Collection		Location	Aedes		Culex		Total		
		sites	No.*		albopictus	savoryi	quinquefasciatus	boninensis	sp.	Lutzia	shinonagai
yeast trap	Jul.–Aug.	Komagari, farm, palm tree	6		Hilly area, some farms and buildings	31	1		32		
		Komagari, farm 1	6		Hilly area, some farms and buildings	19			19		
		Kiyose park, work shed	3	1	Residential area, young plants	9			10		
		Kitafukurozawa, farm, mango tree	4	1	Coastal area, some farms and buildings	4			5		
		Kitafukurozawa, farm, hen house	4		Coastal area, some farms and buildings	4			4		
		Kitafukurozawa, farm, bush	3		Coastal area, some farms and buildings	3			3		
		Kiyose park, bush	1		Residential area, young plants		3		3		
		Okumura, house	1	1	Residential area				1		
		Komagari, farm 2	1		Hilly area, some farms and buildings	1			1		
		Komagari, farm 3	1		Hilly area, some farms and buildings	1			1		
		Kitafukurozawa, farm, work shed	1		Coastal area, some farms and buildings	1			1		
		Kitafukurozawa, farm, river side	1		Coastal area, some farms and buildings	1			1		
		Kiyose park, garden	1		Residential area, young plants	1			1		
		Total				33	3	75	4		82
sweep-net collection	Feb.–Mar.	Kitafukurozawa, farm	3		Coastal area, some farms and buildings		1		44		
		Komagari, farm	1	1	Hilly area, some farms and buildings	9			10		
		Okumura, house	1	3	Residential area	1			4		
		IBO	3		Residential area	3			3		
		Kiyose park	2	1	Residential area, young plants		1		1		
		Sakaiura beach	1	1	Coastal area	1			2		
		Agriculture center	1		Hilly area, young plants		2		2		
		Miyanoama beach	1		Coastal area		1		1		
		Oogamiyama park	1	1	Residential area				1		
		Miyanohamamichi, house	1		Residential area			1	1		
		Sub total				15	7	57	5	1	71
		Jul.–Aug.	Kiyose park	6	41	Residential area, young plants	1		1	43	
			Sakaiura, river mouse	3	4	Coastal area	1		23	28	
			Sakaiura, farm	1	14	Hilly area, some residences			4	18	
Sakaiura beach	1		8	Coastal area			7	15			
Hatsune walking road	1		2	Hilly area			1	4	1		
Kitafukurozawa, farm	1		1	Coastal area, some farms and buildings				1			
Sub total				13	70	2	36	1	109		
Total				28	77	57	41	1	1	180	
Ground total				61	80	57	77	45	1	262	

* Total number of collections at each collection site.

Table 2. Total number of mosquitoes collected by dry-ice trap, sweep net collection, and 8-min human-baited sweep net on Chichi-jima and Haha-jima, Ogasawara Islands in 2017.

Island	Methods	Collection		Location	No.*	Culex		Aedes		Lutzia		Total
		Site				<i>boninensis</i>	<i>quinquefasciatus</i>	<i>albopictus</i>	<i>shinonagai</i>			
Chichi-jima	dry-ice trap	Kuwanoki yama, farm 1		Hilly area, vegetable field	3	190	2		1			193
		Kuwanoki yama, farm 2		Hilly area, vegetable field	3	112	1					113
		Oogamiyama shrine		Residential area	2	2	75					77
		Tenmondai mae 1		Hilly area, bird sanctuary	2	64			2			66
		Yoake yama 2		Hilly area	2	62						62
		Kiyose park 1		Residential area, young plants	2	22	15		6 (2)			43 (2)
		Yoake yama 1		Hilly area	2	32	2		2			36
		Yoake yama 3		Hilly area	1	29			1			30
		Sakaiura beach 2		Coastal area	2	18	10					28
		Sakaiura 1		Hilly area, some residences	2	19			2 (1)			21 (1)
		Tombo ike 1**		Residential area	1		22					22
		Yoake yama 4		Hilly area	1	20			1			21
		Tombo ike 2		Residential area	2	2	18					20
		Mikaduki yama 1		Hilly area, artificial structures	2	7	3		10			20
		Asahi yama 1		Hilly area	2	19			1			20
		Nagatani, farm 1		Hilly area, some residences	2	10	9		1			20
		Nagatani, farm 2		Hilly area, some residences	2	6	14					20
		Sakaiura beach 1		Coastal area	2	15	4					19
		Kiyose park 2		Residential area, young plants	2	4	9		4 (1)			17 (1)
		Tenmondai mae 2		Hilly area, bird sanctuary	2	17						17
		Mikaduki yama 3		Hilly area, artificial structures	2		2			14		16
		IBO		Residential area	2	10	1		5			16
		Sakaiura 2		Hilly area, some residences	2	10	5					15
		Suzaki beach 2		Coastal area	2	6	9					15
		Fukiage dani 1		Hilly area, and some residences	2	9	2		2 (1)			13 (1)
		Fukiage dani 2		Hilly area, some residences	2	8	1		2			11
		Suzaki beach 1		Coastal area	2	1	6					7
		Mikaduki yama 2		Hilly area, artificial structures	2					5		5
		Asahi yama 2		Hilly area	2	3						3
		Kuwanoki yama, farm 3		Hilly area	2	1						1
		Total	59	698	210	59 (5)				967 (5)		
sweep-net collection		Mikaduki yama	2	7 (2)						(2)	7 (4)	

Table 2. Continued.

Collection			Culex			Aedes		Lutzia		Total
Island	Methods	Site	Location	No.*	boninensis	quinquefasciatus	albopictus	shinonagaei		
Haha-jima	dry-ice trap	Hyougi daira 1	Residential area	1	6	176			182	
		Tamagawa dam 1	Hilly area	1	76	3	1		80	
		Minamizaki 1	Hilly area	1	57	5	1		63	
		Koushin yama 1	Hilly area	1	57	1			58	
		Tamagawa dam 3	Hilly area	1	57				57	
		Kitakou shuuraku ato 1	Hilly area	1	39	2	1		42	
		Kitakou shuuraku ato 2	Hilly area	1	38				38	
		Koushin yama 2	Hilly area	1	37				37	
		Hyougi daira	Hilly area	1	28	8			36	
		Nakano daira 1	Hilly area	1	30	2	1		33	
		Kitakou 1	Hilly area	1	30		1		31	
		Nagahama bashi 1	Hilly area	1	30				30	
		Tamagawa dam 2	Hilly area	1	28				28	
		Hyougi daira 2	Residential area	1	5	22			27	
		Minamizaki 2	Hilly area	1	23				23	
		Nagahama bashi 2	Hilly area	1	20				20	
		Nakano daira 2	Hilly area	1	17		2		19	
		Kitakou 2	Hilly area	1	7				7	
		Kaigun shisetsu 1	Hilly area	1	5				5	
		Kaigun shisetsu 2	Hilly area	1	3		(1)		3 (1)	
Total				20	593	219	7 (1)	819 (1)		
8-min human-baited sweep net				1			3	3		
Ground total					1298 (2)	429	69 (6)	(2)	1796 (10)	

* Total number of collections at each collection site.

** Set a trap for 2 days, but machine trouble occurred on the first day.

The number of male mosquitoes is shown in parentheses.

During larval collection in October 2017, we used a dipper and a pipette in various larval habitats and collected larvae from 26 and 11 sites on Chichi-jima and Haha-jima, respectively. Samples were collected in possible larval habitats around adult mosquito collection sites or along a road and walking path in different environments, such as the residential area, coastal area, and hilly area on both islands. Larvae were kept in lidded plastic bottles (diameter, 3.2 cm; height, 8 cm), and the collection date, collection site, habitat type, and GPS location were recorded on the bottle label. The collected larvae and pupae were reared in their collection bottles at the researchers' accommodations. Natural water was used for rearing, and larvae were fed a mixture of dry yeast (Ebios; Asahi Group Foods, Ltd., Tokyo, Japan) and fish food. Larvae of the genus *Lutzia* were fed with collected *Culex* sp. larvae. Some of the larvae were individually reared from late instars until adult emergence. The location information of adult and larval collection sites in 2017 was recorded by GPS (GPSMAP64; Garmin, USA).

For species identification, field collected and emerged adult mosquitoes were killed by freezing and morphologically identified using the identification key by Tanaka et al. (1979). Adult mosquito specimens that were in good condition, including endemic species, were preserved as dried pin specimens and as frozen samples for DNA analysis. Samples were placed in specimen boxes for future morphological observation and stored at the Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan.

Molecular analysis

For molecular analysis of mitochondrial *COI* gene sequences, DNA from the midleg of dried mosquito pin specimens was used, as previously described (Maekawa et al., 2016a). DNA was amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994) and the Takara ExTaq Hot Start version (Takara Bio, Shiga, Japan). Amplification products were purified using an ExoSAP-IT kit (Affymetrix, Santa Clara, CA, USA), and samples for sequencing were prepared using BigDye Terminator ver. 1.1 (Life Technologies, Carlsbad, CA, USA). Base sequences were decoded using an ABI PRISM 3100-Avant genetic analyzer (Life Technologies) and edited using ATGC ver. 7 for Windows (GENETYX Corp., Tokyo, Japan). The sequences of a fragment of the *COI* gene (658 bp) were registered in GenBank. Sequences were aligned using Clustal W in MEGA ver. 5.2 to construct a phylogenetic tree. A neighbor-joining tree was constructed using the Kimura two-parameter (K2P) distance model, and the reliability of the branches was examined by a bootstrap test with 1,000 replicates. The *COI* gene sequences of *Culex* (*Culex*) spp., *Culex* (*Oculeomyia*) spp., *Culex* (*Culiciomyia*) spp., *Lutzia* spp., *Aedes* (*Stegomyia*) spp., and *Aedes* (*Tanakaius*)

spp. were obtained from GenBank to verify the lineage relationships between the four endemic species from the Ogasawara Islands and their related species in the phylogenetic tree. *Chironomus riparius* Meigen (Diptera: Chironomidae; GenBank accession No. HM137925 and HM137890) was used as the outgroup.

The mean nucleotide sequence divergence was calculated based on the K2P model to investigate intraspecific variations among the collected specimens. For *Ae. albopictus*, *Cx. quinquefasciatus*, and *Lt. vorax* Edwards, the mean nucleotide sequence divergences were calculated including sequences from the main islands of Japan and the Ryukyu Islands.

RESULTS

In total, 2,371 individuals belonging to seven species in three genera were collected in this study, including 262 mosquitoes belonging to five species in three genera collected in 2015 and 2,109 specimens representing six species in three genera obtained in 2017. *Aedes savoryi* and *Ae. wadai* were collected only via adult and larval collection, respectively.

Adult collection

In 2015, *Cx. quinquefasciatus*, *Cx. boninensis*, and *Ae. albopictus* were collected in yeast traps; the most abundant species was *Cx. quinquefasciatus*, accounting for 91.5% of all collected specimens (Table 1, upper). Using sweep net collection, three species were collected, except *Culex* sp. and *Lutzia* sp., in the early spring collection periods (February and March) (Table 1, lower). *Aedes savoryi* was the most abundant species, accounting for 80.3% of all collected specimens. This species was obtained by sweep-net collection only in early spring but was not collected in summer collection periods (July and August). *Aedes albopictus* and *Cx. boninensis* accounted for only 10.1% and 7.2% of all collected specimens, respectively. Four species were collected in the summer. *Aedes albopictus* and *Cx. boninensis* were the dominant species, accounting for 64.2% and 33.0%, respectively. The remaining two species, *Cx. quinquefasciatus* and *Lt. shinonagai*, which accounted for only 2.8% of all collected specimens.

In 2017, three species in two genera were collected by dry-ice trap collection on Chichi-jima and Haha-jima (Table 2). *Culex boninensis* and *Cx. quinquefasciatus* were the dominant species and accounted for 72.0% and 23.9% of all collected species on both islands, respectively. The remaining species, *Ae. albopictus* accounted for only 4.0% of all collected specimens. The mosquito density for dry-ice traps was 16.5 individuals/trap/collection on Chichi-jima and 41.0 individuals/trap/collection on Haha-jima; thus, Haha-jima had a higher mosquito density than Chichi-jima. *Culex boninensis* was widely distributed on both islands and was collected at almost all (47/50) collection sites. Many *Cx. boninensis* were

collected at collection sites with rich vegetation in hilly areas, such as Kuwanoki yama farms 1 and 2 on Chichi-jima, as well as Tamagawa dams 1 and 3, Minamizaki 1, and Koushin yama 1 on Haha-jima, but *Cx. quinquefasciatus* was obtained in small numbers (Table 2). By contrast, in the residential areas, a large number of *Cx. quinquefasciatus* were collected in the Oogamiyama shrine and Tombo ike 1 and 2 on Chichi-jima and Hyougi daira 1 and 2 on Haha-jima while there were few *Cx. boninensis*.

Using sweep nets inside an air raid shelter (Bokugo) on Mt. Mikaduki-yama, Chichi-jima, both *Cx. boninensis* and *Lt. shinonagai* were collected. When we walked close to Bokugo, these mosquitoes immediately flew in our direction, which suggested that they rested on the clay walls near the entrance of Bokugo. Three *Ae. albopictus* were collected in an uninhabited area on Haha-jima by 8-min human-baited sweep net collection.

Larval collection

Adults belonging to six species in three genera emerged from the collected larvae, including three endemic species, *Cx. boninensis*, *Ae. wadai*, and *Lt. shinonagai* (Table 3). *Aedes albopictus* was the most abundant and widely distributed species on both Chichi-jima and Haha-jima. Of the two *Culex* spp., *Cx. boninensis* was relatively more abundant on Chichi-jima and *Cx. quinquefasciatus* on Haha-jima.

Various aquatic habitats exist on Chichi-jima, and larvae were most abundant in tree holes, were mainly found along walking paths through the forest in hilly areas and in a windbreak (Boufuri) in the coastal area. Larvae of *Ae. albopictus* were predominant in tree holes, whereas only few larvae of *Ae. wadai* and *Lt. shinonagai* were collected on Chichi-jima. Additionally, some *Ae. albopictus* larvae were collected from ground pools and a water tank inside Bokugo which has no sunlight. Larvae of *Cx. boninensis* were collected from a variety of larval habitats, including ground pools, artificial containers, swamps, and catch basins. On Haha-jima, most of the larvae were collected from artificial containers, and a few larvae were collected from a ground pool and river bed (Table 3). The artificial containers comprised various types and sizes of buckets, bathtubs, polystyrene trays and boxes, stone bowls, rain gutters, and rain bowls. Larvae of *Ae. albopictus* were detected in all artificial containers found on Haha-jima while those of *Cx. quinquefasciatus* and *Cx. boninensis* were collected from large-size buckets and polystyrene boxes and from bathtubs. Larvae of *Lt. vorax* were collected from buckets and polystyrene boxes with *Ae. albopictus* or *Cx. quinquefasciatus*. These findings imply that artificial containers are important habitats for the collected species. A few younger larvae were collected from some ground pools and riverbeds, but died during rearing, and their species could not be confirmed.

Table 3. Mosquito species and number of emerged adults by larval habitats found on Chichi-jima and Haha-jima, Ogasawara Islands in October, 2017.

Island	Species	artificial container	tree hole	ground pool	bamboo stump	air-raid shelter	swamp	catch basin	well	river bed	Total
Chichi-jima	<i>Aedes albopictus</i>	16	62	7	12	4					101
	<i>Culex boninensis</i>			14			4	1		1	20
	<i>Cx. quinquefasciatus</i>	1	4	1			1				7
	<i>Lutzia shinonagai</i>	1	3								4
	<i>Ae. wadai</i>		2								2
	<i>Lt. vorax</i>					1			1		2
	<i>Aedes</i> sp.		2								2
	Total No. of emerged adults	18	73	22	12	5	5	1	1	1	138
	Total No. of habitats	11	23	4	4	7	2	1	3	2	57
Haha-jima	<i>Ae. albopictus</i>	96									96
	<i>Cx. quinquefasciatus</i>	48									48
	<i>Lt. vorax</i>	10									10
	<i>Cx. boninensis</i>	9									9
	<i>Culex</i> sp.	1									1
	<i>Lutzia</i> sp.	1									1
	Total No. of emerged adults	165	0	0	0	0	0	0	0	0	165
	Total No. of habitats	25	0	2*	0	0	0	0	0	1*	27
Ground total No. of emerged adults		183	73	22	12	5	5	1	1	1	303
Total No. of collected species		5	4	3	1	2	2	1	1	1	6

Artificial container contained buckets, bathtub, polystyrene trays and boxes, stone bowls, rain gutters, rain bowls.

There were 6 ground pools and one water-concrete tank in boukugo (air-raid shelter).

* Larvae were dead

Molecular analysis

Fifty-five specimens, comprising seven species in three genera, were analyzed, and the sequences of the *COI* gene were registered in GenBank (Appendix 1). The mean intraspecific variations were calculated for the seven species collected on Chichi-jima and Haha-jima and were found to be low (0.0% to 0.8%), which indicated genetic homogeneity of the populations (Appendix 1). The mean pairwise divergences were calculated for *Ae. albopictus*, *Cx. quinquefasciatus*, and *Lt. vorax* species using the *COI* gene sequences (650bp) from our specimens from both islands, as well as respective sequences of mosquitoes from main islands of Japan and the Ryukyu Archipelago. The mean pairwise divergence for each species was small, at 0.1% (0.0% to 0.3%; $n=37$) for *Ae. albopictus*, 0.1% (0.0% to 0.3%; $n=22$) for *Cx. quinquefasciatus*, and 0.4% (0.0% to 1.2%; $n=15$) for *Lt. vorax*. *Aedes albopictus* and *Cx. quinquefasciatus* collected on Chichi-jima and Haha-jima did not show genetic divergence and thus, represented homogeneous genetic populations.

Among the four endemic species, *Cx. boninensis* was highly diverse and had eight haplotypes (bon1–bon8) on Chichi-jima and Haha-jima. *Aedes savoryi* had three haplotypes (sav1–sav3), and *Ae. wadai* and *Lt. shinonagai* had only one haplotype each (wad1 and shi1, respectively) on Chichi-jima (Appendix 1). We confirmed six haplotypes in *Ae. albopictus*, including one haplotype (alb1) on Chichi-jima and Haha-jima, four haplotypes (alb1–alb4) from the Ryukyu Archipelago, and two haplotypes (alb5 and alb6) from the main islands of Japan (Appendices 1 and 2). Among the six haplotypes of *Ae. albopictus* occurring in the Ryukyu Archipelago, one (alb1) was common with that of individuals from Chichi-jima and Haha-jima. No common haplotype was found when comparing *Ae. albopictus* from main islands of Japan with those from the Ogasawara Islands and the Ryukyu Archipelago. *Culex quinquefasciatus* had two haplotypes (qui1 and qui2) on Chichi-jima and Haha-jima, and qui1 was also found among 10 *Cx. quinquefasciatus* haplotypes occurring in the Ryukyu Archipelago (Appendices 1 and 2). Nine haplotypes (vor1–vor9) were identified in the *Lt. vorax* populations from Chichi-jima, Haha-jima, and main islands of Japan. Among these, six haplotypes were detected in the populations from Chichi-jima and Haha-jima, while a specimen collected in Ehime by Maekawa et al. (2016a) had a haplotype (vor8) with six substitutions relative to vor1 (Appendices 1 and 2). Three specimens of *Lt. vorax* (LC544036: Haha-jima, LC054506: Wakayama, and LC054510: Miyazaki) had the same nucleotide sequences (vor1). The most commonly substituted nucleotide in *Lt. vorax* collected on Chichi-jima and Haha-jima was at position 614 (A→G) (Appendix 1), in specimens from

the main islands of Japan, a substitution occurred at position 311 (G→A) (Appendix 2). Thus, there are two groups of haplotypes, which are distinguished by the substitution sites, and the dominant group differed between the Ogasawara Islands and the main islands of Japan.

Phylogenetic analysis based on the *COI* gene sequences (650bp) of the four endemic species and related species registered in GenBank showed specific clades (Fig. 2a and b). *Aedes wadai* belongs to the subgenus *Stegomyia* and was clustered with *Ae. galloisi* Yamada. *Aedes savoryi* was found in the same clade as the subgenus *Tanakaius*, which includes *Ae. togoi* (Theobald). *Culex boninensis* is derived from the paraphyly of the *Cx. mimeticus* group. *Lutzia shinonagai* is derived from the subgenus *Metalutzia* and formed a clade with *Lt. tigripes* (de Grandpré and de Charmoy). Hence, we could not confirm a distinct solo subgenus clade of *Cx. boninensis* and *Lt. shinonagai*, respectively.

DISCUSSION

In this study, we obtained seven mosquito species belonging to three genera that included four endemic species (*Ae. wadai*, *Ae. savoryi*, *Cx. boninensis*, and *Lt. shinonagai*). However, we did not find *Ae. aegypti*, *Ae. togoi*, *Cx. tritaeniorhynchus* Giles, and *Cx. mimeticus* Noé, which were previously reported to be present on the Ogasawara Islands. Yamada (1917) reported a collection record of *Ae. aegypti* on Chichi-jima in 1916. Since then, several mosquito surveys, including this study, have been conducted on the Ogasawara Islands, but there have been no reports of the occurrence of *Ae. aegypti* (Wada et al., 1969; Takahashi, 1973; Kusui et al., 2004; Toma and Miyagi, 2005). Moreover, it has been reported that *Ae. aegypti* became extinct on Kyushu, the southern island of the main islands of Japan, and in the Ryukyu Archipelago where this species inhabited before and after World War II (Kurihara, 2003). Apparently, *Ae. aegypti* is extinct on the Ogasawara Islands, as mentioned by Tanaka et al. (1979). Collection records of the other three species were reported 30–50 years ago (Wada et al., 1969; Toma and Miyagi, 2005). *Aedes togoi* was collected in October 1968 by Wada et al. (1969), but we did not collect this species in this study. Sota et al. (2015) reported that the distribution of *Ae. togoi* was expanded to Southeast Asia and the Pacific coast of Canada and the USA by shipments from Japan. *Aedes togoi* breed in small water bodies, including artificial containers and used tires, along the coast of Japan and are transported by ships as eggs or larvae in water tanks or used tires. Hence, it is considered that they have been continuously invading Chichi-jima from Tokyo by ships and freight shipping services. Additional research will be necessary to confirm the inhabitation by adults and larvae of *Ae. togoi* along

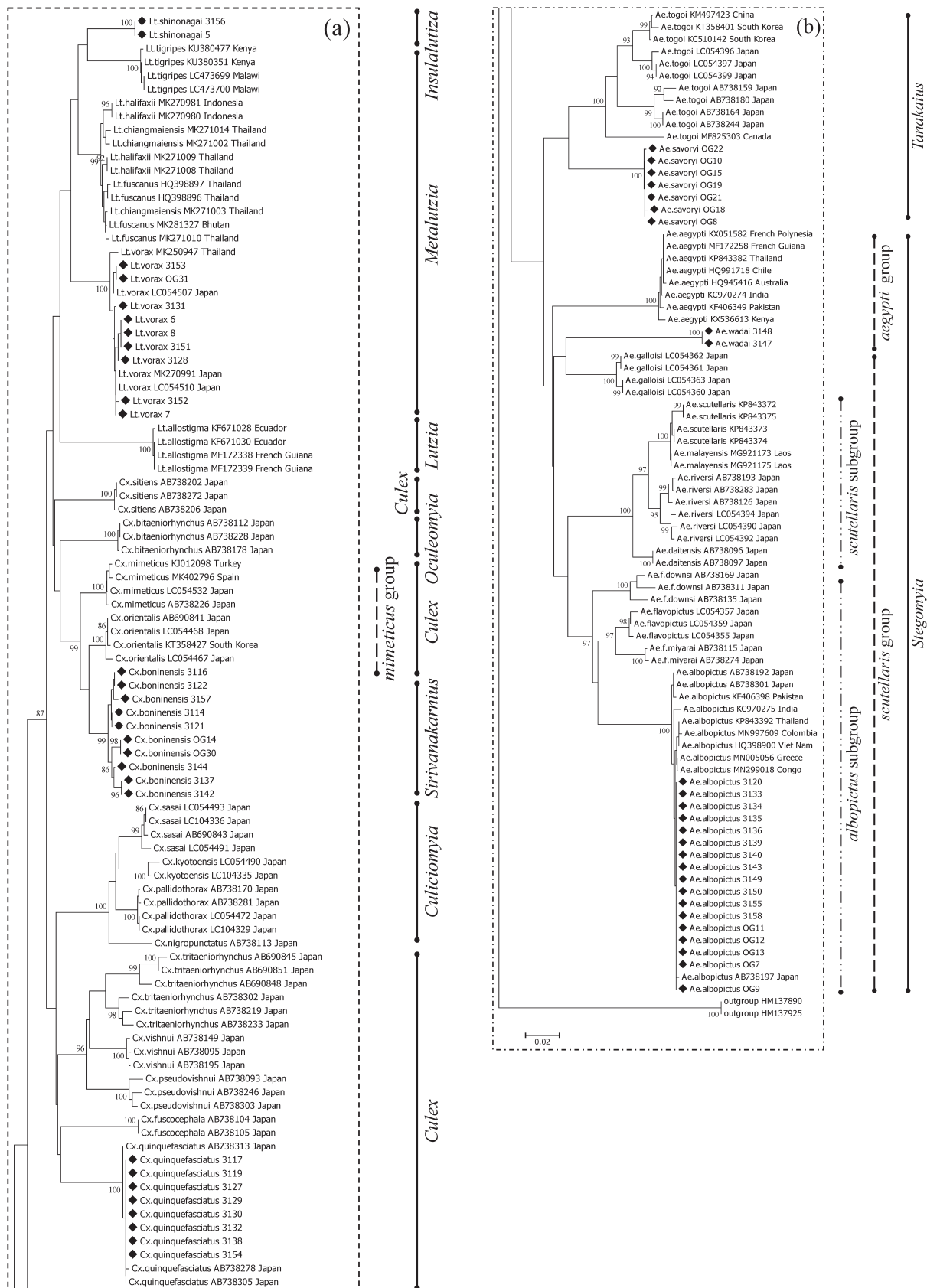


Fig. 2. A neighbor-joining tree with 1,000 bootstrap replicates constructed using the Kimura 2-parameter (K2P) calculated from the COI sequences (650bp) of 55 mosquitoes collected in this study (◆) and GenBank registered specimens of *Cx. (Culex) spp.*, *Cx. (Oculeomyia) spp.*, *Cx. (Culiciomyia) spp.*, *Lutzia spp.*, *Ae. (Stegomyia) spp.*, *Ae. (Tanakiaius) spp.*, and 2 outgroups, *Chironomus riparius* Meigen (Diptera: Chironomidae). The specimens collected in this study are labeled with the species name and specimen code listed in Appendix 1. Specimens found in GenBank are labeled with species names, GenBank accession numbers, and countries. (a) Subgenus (solid line) and species group (long dashed line) are shown. (b) Subgenus (solid line), species group (long dashed line), and species subgroup (two-dot dashed line) are shown.

the coastline of Chichi-jima in different seasons. Wind-borne transoceanic flights of mosquitoes over sea water have been reported by several authors (Asahina, 1970). Transoceanic flights have been demonstrated as a possible method of colonization for *Culex* mosquitoes (Asahina and Tsuruoka, 1969, 1970; Miyagi, 1973; Hayashi et al., 1979), and *Cx. tritaeniorhynchus* and *Cx. mimeticus* may have thus reached the Ogasawara Islands. Animals as a blood source and larval habitats are necessary for the persistence of colonizing mosquito populations. The main larval habitats of *Cx. tritaeniorhynchus* and *Cx. mimeticus* are large water bodies, such as rice fields and swamps, which are rare on Chichi-jima. Therefore, the maintenance of *Cx. tritaeniorhynchus* and *Cx. mimeticus* populations on the Ogasawara Islands appears to be difficult.

Adult collection

Little is known about the habits of *Cx. boninensis* in the field, as it is an endemic species to the Ogasawara Islands. In this study, we obtained interesting results for this species as follows: 1) sweep-net collection showed a higher density of *Cx. boninensis* than that of *Cx. quinquefasciatus*; 2) dry-ice trap collection showed that some collection areas where *Cx. boninensis* was abundant tended to have a low density of *Cx. quinquefasciatus*; and 3) *Cx. boninensis* was more abundantly collected in rich vegetation and hilly areas than in residential areas. *Culex boninensis* was previously reported to be a night feeder, feeding on chickens and Mongolian gerbils, and to often bite humans at night in the laboratory (Tanaka et al., 1979; Toma and Miyagi, 2005). *Culex quinquefasciatus* feeds on birds, as well as on humans, at night (Tanaka et al., 1979). Both *Cx. boninensis* and *Cx. quinquefasciatus* have similar feeding preference and feed on humans and animals. Considering that *Cx. quinquefasciatus* is known to be a vector mosquito for various infectious diseases in humans and animals (Sirivanakarn, 1976; Tanaka et al., 1979; Valkiunas, 2005), *Cx. boninensis* is possibly a vector mosquito and a bridge vector mosquito for some infectious diseases. An intensive study should be conducted in the future to understand the vector capacity of *Cx. boninensis*.

According to Toma et al. (2007), yeast traps might be valuable for studying mosquito fauna in remote areas. Our results support this notion because the same three species were collected using dry-ice traps and yeast traps on Chichi-jima. Yeast traps were useful for investigating the mosquito fauna on Chichi-jima; however, the collection density of yeast traps was lower than that of dry-ice traps. Consequently, year-round surveillance of the mosquito density using dry-ice traps will be required in the future to determine the high-risk season for mosquito-borne infectious disease outbreaks on the Ogasawara Islands.

On Haha-jima, a small number of *Ae. albopictus* was collected via 8-min human-baited sweep net collection in an uninhabited area, Kitakou shuraku ato in this study. Takeuchi et al. (2018) also performed 8-min human-baited sweep net collections at six sites in inhabited areas, including the Motochi and Oki ports on Haha-jima, in the same periods, but no *Ae. albopictus* were collected. In this study, the presence of *Ae. albopictus* larvae was observed in various types of artificial containers in inhabited areas, including Motochi, Nakanodaira, Kitakou, and Kitakou shuraku. Thus, it is considered that *Ae. albopictus* is distributed widely and sparsely on Haha-jima.

Larval collection

The low incidence of *Ae. wadai* (1 out of 23 tree holes) observed on Chichi-jima in this study is consistent with the results of Tanaka et al. (1979) and Toma and Miyagi (2005). This species is endemic and rare on the Ogasawara Islands, and ecological and genetic studies on *Ae. wadai* are valuable to understand the local adaptation and island biogeography of mosquitoes.

Three of the seven ground pools used as larval habitats of *Ae. albopictus* on Chichi-jima were unique. The ground pools varied in size and were generally 2 m in length and 5–40 cm in depth. The larval habitats of *Ae. albopictus* in the main islands of Japan are mainly small-sized containers, such as cans, flowerpot saucers, tree holes, tires, and various artificial containers. Larger larval habitats in the main islands of Japan include stone basins, catch basins, bathtubs, and tarpaulins. There are no reports of *Ae. albopictus* larvae collected from ground pools. Ground pools at historical battle sites on Chichi-jima were considerably larger than larval habitats of *Ae. albopictus* in main islands of Japan. Notably, we collected *Ae. albopictus* larvae from large ground pools in completely dark places that have never been reported as larval habitats of *Ae. albopictus*, which potentially indicates behavioral changes in ovipositing females on Chichi-jima populations. Ecological studies on ovipositing females and on the development and survival of larvae in ground pools will be required to understand the local adaptation of the island population of *Ae. albopictus*.

Lutzia shinonagai ecologically differs from *Lutzia* spp. distributed in the main islands of Japan and the Ryukyu Archipelago and uses only tree holes as larval habitats (Tanaka, 2005). In the present study, we found that *Lt. shinonagai* also inhabited artificial containers (buckets) on Chichi-jima. *Lutzia vorax* was collected on both Chichi-jima and Haha-jima, but *Lt. shinonagai* was not collected on Haha-jima. In a previous study, *Lt. shinonagai* was collected from Oki-mura and the port facility area on Haha-jima (Kusui et al., 2004). Extensive larval collections in these areas will be required to confirm the presence of this species on Haha-jima.

Molecular analysis

Consistent with previously reported data (Kumar et al., 2007; Taira et al., 2012; Maekawa et al., 2016a), the mean intraspecific divergences of the seven species collected on Chichi-jima and Haha-jima was lower than 2%, indicating homogeneous mosquito species. However, the variety of haplotypes obtained for *Cx. boninensis* and *Lt. vorax* showed high genetic diversity. Some individuals of *Lt. vorax* inhabiting Haha-jima and the main islands of Japan showed the same haplotype; however, it is difficult to understand how these populations could have frequent genetic exchanges, considering their geographical isolation from each other. To resolve the uncertainties surrounding the haplotypes of *Cx. boninensis* and *Lt. vorax*, additional entomological surveys and molecular analyses should be conducted on these and related species collected on other islands of the Ogasawara Islands and on the main islands of Japan.

Tanaka et al. (1979) and Tanaka (2005) described the taxonomic positions of the four endemic species and related species based on morphological classification. *Culex* (*Sirivanakarnius*) *boninensis* and *Lt. (Insulalutzia) shinonagai*, show a unique lineage without closely related species and are classified as solo species of the subgenus, and *Ae. wadai* and *Ae. savoryi* belong to the subgenera *Stegomyia* and *Tanakaius*, respectively. The results of the molecular analysis of the four endemic mosquito species on the Ogasawara Islands partly supported their phylogenetic relationships suggested by morphological taxonomy. Phylogenetic analysis showed that *Ae. savoryi* clustered next to *Ae. togoi*, indicating a systematically close relationship, in agreement with the morphological taxonomy. *Culex boninensis*, another endemic species in the monotypic subgenus *Sirivanakarnius*, formed a clade with *Cx. orientalis*, a member of the *Cx. mimeticus* group (Fig. 2a). The phylogenetic relationship between *Cx. boninensis* and *Cx. orientalis* did not support the unique taxonomic position of *Cx. boninensis*. However, additional genetic analyses, focused on different DNA regions, will be required to confirm our results.

In Japan, the genus *Lutzia* is represented by three species in two subgenera (*Lt. shinonagai* in *Insulalutzia* and *Lt. fuscus* and *Lt. vorax* in *Metalutzia*) (Tanaka, 2003; Phanitchakun et al., 2019; Somboon and Harbach, 2019). *Lutzia shinonagai* is classified in the monotypic subgenus *Insulalutzia* and is conspicuously different from other *Lutzia* spp. based on the morphological taxonomy of whole stages (Tanaka, 2003, 2005). The characteristic features of *Lt. shinonagai* are dark-scaled wing veins and tarsi and a non-hairy male palpus, whereas other *Lutzia* spp. in main islands of Japan and the Ryukyu Archipelago exhibit different characteristics. However, the phylogenetic tree showed that *Lt. shinonagai*

clustered next to *Lt. tigris* belonging to the subgenus *Metalutzia* (Fig. 2a). *Lutzia tigris* is the only *Lutzia* species found in the Afrotropical region, and its morphological characteristics closely resemble those of *Lt. fuscus* and *Lt. vorax* (Tanaka, 2003). The phylogenetic analysis of *Lt. shinonagai* performed in this study is not conclusive, and additional genetic studies will be necessary to compare DNA sequences of different genetic regions.

In Japan, the subgenus *Stegomyia* of the genus *Aedes* is classified into two species groups, *aegypti* and *scutellaris*, with *Ae. wadai* classified in the former group (Tanaka et al., 1979). In our phylogenetic tree, *Ae. wadai* clustered next to *Ae. galloisi*; these species appear to belong to a distinct lineage, different from either the *aegypti* or *scutellaris* groups (Fig. 2b). The COI region (658bp) obtained using the primer set LCO1490 and HCO2198 in this study is used for DNA barcoding to confirm species and sibling species worldwide, and genetic information has progressively accumulated. The use of the COI region for lineage relationship analysis between genera and subgenera is under investigation (Toju, 2016). Sota and Mogi (2006) examined the evolutionary relationships of 11 species in the subgenus *Stegomyia* based on the sequence data of the COI gene in mtDNA and 16S and 28S rRNA genes and showed that *Ae. wadai* was appropriately placed in the *aegypti* group. It is suggested that for DNA barcoding and phylogenetic analysis of subgenera and subspecies, longer sequences should be used than those used in this study and by Sota and Mogi (2006). We recommend continuing the accumulation of DNA sequence data for the COI gene and the development of advanced analytical methods based on the whole COI gene sequence.

Risk of the introduction of mosquito-borne infectious diseases from outside of the islands

Three of the seven species collected in this study are vectors of mosquito-borne infectious diseases, including *Ae. albopictus* (dengue fever, chikungunya fever, Zika fever, West Nile fever, avian malaria, and heartworm disease), *Cx. quinquefasciatus* (Japanese encephalitis, West Nile fever, lymphatic filariasis and avian malaria), and *Lt. vorax* (avian malaria) (Tsuda, 2019). Native mosquito species might transmit pathogens to humans if these pathogens are introduced to the Chichi-jima or Haha-jima. The introduction routes for human pathogens are likely restricted to ships that provide regular service between Tokyo and Chichi-jima and to infected patients from domestic and international ships navigating around the Ogasawara Islands. A dengue outbreak occurred in Tokyo during summer in 2014; the virus was spread by humans, and dengue transmission occurred between native *Ae. albopictus* and humans in each area (Kobayashi et al., 2018). If the dengue

virus is introduced by tourists to Chichi-jima, native *Ae. albopictus*, which is widely distributed across the islands, might transmit the virus and cause an outbreak. To be prepared for the transmission of *Ae. albopictus*-borne infectious diseases during a summer season, it is necessary to establish regular mosquito surveillance on Chichi-jima and Haha-jima. Furthermore, the potential of migratory birds to carry and disseminate certain pathogenic microorganisms is of concern (Hubálek, 2004). Several studies have shown that migratory birds carry pathogenic organisms that cause human and zoonotic diseases and bird-borne diseases, such as West Nile fever, St. Louis encephalitis, eastern and western equine encephalitis, type A influenza, Newcastle disease and avian hematozoa (Hubálek and Halouzka, 1999; Reed et al., 2003; Hubálek, 2004; Foti et al., 2011). In Japan, migratory birds carry avian hemosporidia to the main islands and surrounding remote islands from overseas and transmit these pathogens to domestic birds *via* mosquitoes at stopover sites (Murata, 2007; Ejiri et al., 2008; Tanigawa et al., 2013). On Chichi-jima, the avian malaria parasite (*Plasmodium elongatum*) has been detected in wild birds and has been suggested to have invaded Chichi-jima *via* migratory birds (Kimura, personal communication). Many rare endemic birds are inhabiting the Ogasawara Islands. The protection of these birds from mosquito-transmitted bird-borne pathogens will require the accumulation of information on vector mosquitoes and pathogens to evaluate the risk of disease outbreaks. Finally, we recommend the development of a system to accumulate data on the mosquito fauna and migratory birds to prevent the spread of mosquito-borne infectious diseases in humans and rare endemic birds on the Ogasawara Islands.

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REFERENCES

- Asahina, S. 1970. Transoceanic flight of mosquitoes on the Northwest Pacific. *Jpn. J. Med. Sci. Biol.*, 23: 255–258.
- Asahina, S. and Turuoka, Y. 1969. Records of the insects visited a weather-ship located at the ocean weather station “Tango” on the Pacific, III. *Kontyu*, 37: 290–304 (in Japanese with English summary).
- Asahina, S. and Turuoka, Y. 1970. Records of the insects visited a weather-ship located at the ocean weather station “Tango” on the Pacific, V. Insects captured during 1968. *Kontyu*, 38: 318–330 (in Japanese with English summary).
- Bohart, R. M. 1956. Diptera: Culicidae. *Insects of Micronesia*, 12: 1–85. Bernice P. Bishop Museum, Honolulu.
- Chapple, D. G., Simmonds, S. M. and Wong, B. B. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? *Trends Ecol. Evol.*, 27: 57–64.
- Derraik, J. G. B. 2004. Exotic mosquitoes in New Zealand: a review of species intercepted, their pathways and ports of entry. *Aust. N. Z. J. Public Health*, 28: 433–444.
- Ejiri, H., Sato, Y., Sasaki, E., Sumiyama, D., Tsuda, Y., Sawabe, K., Matsui, S., Horie, S., Akatani, K., Takagi, M., Omori, S., Murata, K. and Yukawa, M. 2008. Detection of avian *Plasmodium* spp. DNA sequences from mosquitoes captured in Minami Daito Island of Japan. *J. Vet. Med. Sci.*, 70: 1205–1210.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, 3: 294–299.
- Foti, M., Rinaldo, D., Guercio, A., Giacobello, C., Aleo, A., De Leo, F., Fisichella, V. and Mammina, C. 2011. Pathogenic microorganisms carried by migratory birds passing through the territory of the island of Ustica, Sicily (Italy). *Avian Pathol.*, 40: 405–409.
- Hawley, W. A. 1988. The biology of *Aedes albopictus*. *J. Am. Mosq. Control Assoc.*, 1: 1–39.
- Hayashi, K., Suzuki, H., Makino, Y. and Asahina, S. 1979. Notes on the transoceanic insects-captured on East China Sea in 1976, 1977 and 1978. *Trop. Med.*, 21: 1–10.
- Hsiao, T. Y. and Bohart, R. M. 1946. The mosquitoes of Japan and their medical importance. 47 pp. Bureau of Medicine and Surgery, Navy Department, Washington, D. C.
- Hubálek, Z. 2004. An annotated checklist of pathogenic microorganisms associated with migratory birds. *J. Wildl. Dis.*, 40: 639–659.
- Hubálek, Z. and Halouzka, J. 1999. West Nile fever—a reemerging mosquito-borne viral disease in Europe. *Emerg. Infect. Dis.*, 5: 643–650.
- Karube, H., Matsumoto, K., Kishimoto, T. and Ozono, A. 2012. Records of notable insect species from the Ogasawara Islands. *Ogasawara Research*, 38: 1–15 (In Japanese with English abstract).
- Kawanami, T. 2016. Changes in tourists in the Ogasawara Islands after registration as a World Heritage Site. *Geogr. Rev. Jpn. Ser. A*, 89: 118–135 (In Japanese with English abstract).
- Kobayashi, D., Murota, K., Fujita, R., Itokawa, K., Kotaki, A., Moi, M. L., Ejiri, H., Maekawa, Y., Ogawa, K., Tsuda, Y., Sasaki, T., Kobayashi, M., Takasaki, T., Isawa, H. and Sawabe, K. 2018. Dengue Virus Infection in *Aedes albopictus* during the 2014 Autochthonous Dengue Outbreak in Tokyo Metropolis, Japan. *Am. J. Trop. Med. Hyg.*, 98: 1460–1468.
- Kumar, N. P., Rajavel, A. R., Natarajan, R. and Jambulingam, P. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). *J. Med. Entomol.*, 44: 1–7.
- Kurihara, T. 2003. Review of dengue vector mosquitoes in Japan. *Med. Entomol. Zool.*, 54: 135–154.
- Kusui, Y., Shimamura, H., Oomatu, T., Savory, N. Jr., Suzuki, S.,

- Iizuka, S. and Aoki, H. 2004. Surveys on the mosquitoes and cockroaches in the Bonin (Ogasawara) islands, Southern Japan. *J. Jap. Quarant. Med. Assoc.*, 6: 62–68 (In Japanese).
- La Casse, W. J. and Yamaguti, S. 1947. Mosquitoes of Japan, Part I. Mosquito survey data on Japan and their application in the control of mosquito-borne diseases. 142 pp. Off. Surg., HQ. I Corps, APO 301, U.S. Army.
- Maekawa, Y., Ogawa, K., Komagata, O., Tsuda, Y. and Sawabe, K. 2016a. DNA barcoding for molecular identification of Japanese mosquitoes. *Med. Entomol. Zool.*, 67: 183–198 (In Japanese with English abstract).
- Maekawa, Y., Tsuda, Y. and Sawabe, K. 2016b. A nationwide survey on distribution of mosquitoes in Japan. *Med. Entomol. Zool.*, 67: 1–12 (In Japanese with English abstract).
- Medlock, J. M., Hansford, K. M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. and Van Bortel, W. 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. *Vector Borne Zoonotic Dis.*, 12: 435–447.
- Miyagi, I. 1973. On the medically important insects in the Danjo Islands, especially on the larval habitats, the blood-sucking habits and transoceanic flight of the blood sucking insects. *Trop. Med.*, 15: 1–10.
- Murata, K. 2007. Study on avian haemosporidan parasites in Japanese wild birds. *J. Anim. Protozoo.*, 22: 1–8 (In Japanese).
- Ogasawara Islands Branch Office. Information on branch office. Tokyo Metropolitan Ogasawara Island branch office. [accessed March. 3, 2020]. Available from <https://www.soumu.metro.tokyo.lg.jp/07ogawara/guidance/summary.html>
- Ohbayashi, T., Inaba, M., Suzuki, H. and Kato, M. 2004. List of insects in the Ogasawara Islands, Japan (2002). *Ogasawara Research*, 29: 17–74.
- Phanitchakun, T., Namgay, R., Miyagi, I., Tsuda, Y., Walton, C., Harbach, R. E. and Somboon, P. 2019. Morphological and molecular evidence for a new species of *Lutzia* (Diptera: Culicidae: *Culicini*) from Thailand. *Acta Trop.*, 191: 77–86.
- Reed, K. D., Meece, J. K., Henkel, J. S. and Shukla, S. K. 2003. Birds, migration and emerging zoonoses: west nile virus, lyme disease, influenza A and enteropathogens. *Clin. Med. Res.*, 1: 5–12.
- Sirivanakarn, S. 1976. Medical entomology studies III: a revision of the subgenus *Culex* in the oriental region (Diptera: Culicidae). *Contrib. Am. Entomol. Inst.*, 12: 1–272.
- Somboon, P. and Harbach, R. E. 2019. *Lutzia* (*Metalutzia*) *chiangmaiensis* n. sp. (Diptera: Culicidae), formal name for the Chiang Mai (CM) form of the genus *Lutzia* in Thailand. *J. Med. Entomol.*, 56: 1270–1274.
- Sota, T., Belton, P., Tseng, M., Yong, H. S. and Mogi, M. 2015. Phylogeography of the coastal mosquito *Aedes togoi* across climatic zones: testing an anthropogenic dispersal hypothesis. *PLoS One*, 10: e0131230.
- Sota, T. and Mogi, M. 2006. Origin of pitcher plant mosquitoes in *Aedes* (*Stegomyia*): a molecular phylogenetic analysis using mitochondrial and nuclear gene sequences. *J. Med. Entomol.*, 43: 795–800.
- Taira, K., Toma, T., Tamashiro, M. and Miyagi, I. 2012. DNA barcoding for identification of mosquitoes (Diptera: Culicidae) from the Ryukyu Archipelago, Japan. *Med. Entomol. Zool.*, 63: 289–306.
- Takahashi, S. 1973. Insects of medical importance in Ogasawara (Bonin) Islands. *Med. Entomol. Zool.*, 24: 143–148 (In Japanese with English summary).
- Takeuchi, M., Kasai, A. and Yokoduka, Y. 2018. The vectors surveillance of rodent and mosquito-borne diseases on Chichijima and Haha-jima, Ogasawara Islands. *Ogasawara Kenkyu Nenpou*, 41: 111–124 (In Japanese).
- Tanaka, K. 2003. Studies on the pupal mosquitoes of Japan (9), Genus *Lutzia*, with establishment of two new subgenera, *Metalutzia* and *Insulalutzia* (Diptera, Culicidae). *Jpn. J. Syst. Enty.*, 9: 19–169.
- Tanaka, K. 2005. Culicidae. In: Aquatic insects of Japan: manual with keys and illustrations, (eds. Kawai, T. and Tanida, K.), pp. 757–1006, Tokai University Press, Kanagawa (In Japanese).
- Tanaka, K., Mizusawa, K. and Saugstad, E. S. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu archipelago and the Ogasawara islands) and Korea (Diptera: Culicidae). *Contr. Am. Entomol. Inst.*, 16: 1–987.
- Tanigawa, M., Sato, Y., Ejiri, H., Imura, T., Chiba, R., Yamamoto, H., Kawaguchi, M., Tsuda, Y., Murata, K. and Yukawa, M. 2013. Molecular identification of avian haemosporidia in wild birds and mosquitoes on Tsushima Island, Japan. *J. Vet. Med. Sci.*, 75: 319–326.
- Toju, H. 2016. Exploring ecosystems with DNA information: environmental DNA, large scale community analysis, and ecological networks. xiii+201 pp., Kyoritsu shuppan, Tokyo. (In Japanese).
- Toma, T., Higa, Y., Okazawa, T. and Miyagi, I. 2007. Comparison of four light-trap methods for collecting mosquitoes in Iriomote Island, Ryukyu Archipelago, Japan. *Ryukyu. Med. J.*, 26: 39–45.
- Toma, T. and Miyagi, I. 2005. Notes on mosquitoes in Chihijima, Ogasawara Archipelago, Japan and biology of *Culex* (*Sirivanakarnius*) *boninensis* (Diptera: Culicidae). *Med. Entomol. Zool.*, 56: 237–241.
- Tsuda, Y. 2019. An illustrated book of the mosquitoes of Japan: adult identification, geographic distribution and ecological note. 127 pp., Hokuryukan, Tokyo.
- Tsuda, Y., Maekawa, Y., Ogawa, K., Itokawa, K., Komagata, O., Sasaki, T., Isawa, H., Tomita, T. and Sawabe, K. 2016. Biting density and distribution of *Aedes albopictus* during the September 2014 outbreak of dengue fever in Yoyogi Park and the vicinity in Tokyo Metropolis, Japan. *Jpn. J. Infect. Dis.*, 69: 1–5.
- Tsuda, Y., Matsui, S., Saito, A., Akatani, K., Sato, Y., Takagi, M. and Murata, K. 2009. Ecological study of avian malaria vectors on the island of Minami-Daito, Japan. *J. Am. Mosq. Control. Assoc.*, 25: 279–284.
- Valkiunas, G. 2005. Avian malaria parasites and other haemosporidia. 946 pp., CRC Press, New York.
- Wada, Y., Fujita, K., Sasa, M., Ishii, A. and Shirasaka, A. 1969. Survey of sanitary pests in Ogasawara. *Nettai*, 13: 57–61 (In Japanese).
- Yamada, S. 1917. Distribution of yellow fever vector *Stegomyia fasciata* in the Japanese Empire. *J. Hyg. Dis. Tokyo*, 12: 386–403 (In Japanese).
- Yamada, S. 1921. Description of ten new species of *Aedes* found in Japan, with notes on the relation between some of these mosquitoes and the larva of *Filaria bancrofti* Cobbold. *Annot. Zool. Japon.*, 10: 45–81.
- Yamada, S. 1932. Diptera: Culicidae. In: Nippon Konchu Zukan: Iconographia insectorum Japonicorum (ed. Uchida, S.), pp. 210–235. Hokuryukan, Tokyo (In Japanese).
- Yamanouchi, S. and Kasai, A. 2016. The necessity of regular surveillance for vector-borne diseases pathogens and vectors at Futami port, Ogasawara Islands. *Ogasawara Kenkyu Nenpou*, 39: 45–56 (In Japanese).
- Yamanouchi, S. and Kasai, A. 2017. An experimental methods of adult mosquito collection for vector-borne diseases pathogens and vectors surveillance at Futami port, Ogasawara Islands. *Ogasawara Kenkyu Nenpou*, 40: 73–87 (In Japanese).

Appendix 1. The mosquito specimens used in the genetic study, with the details of their collection sites, specimen code, GenBank accession number, intraspecific divergences, haplotype number, and single nucleotide polymorphism.

Serial no.	Species	Details of collection sites					Specimen code	GenBank accession no.	No. of specimens	No. of haplotypes	Mean intraspecific divergences (%)	position and different substituted nucleotide**	Name of haplotypes***
		Island	Place	GPS coordinates	Date	Collection method and environment							
1	<i>Aedes albopictus</i>	Chichi-jima	Sakaura beach	N 27.0825, E 142.2097	March 2015	SC/bushes inside coastal forest	OG7	LC543988	17	1	0	—	alb1
2	<i>Ae. albopictus</i>	Chichi-jima	Ogamiyama park	N 27.0943, E 142.1936	March 2015	SC/bushes inside park	OG9	LC543989					
3	<i>Ae. albopictus</i>	Chichi-jima	Okumura	N 27.1003, E 142.2022	March 2015	SC/garden of private house	OG11	LC543990					
4	<i>Ae. albopictus</i>	Chichi-jima	Okumura	N 27.1003, E 142.2022	March 2015	SC/garden of private house	OG12	LC543991					
5	<i>Ae. albopictus</i>	Chichi-jima	Okumura	N 27.1003, E 142.2022	March 2015	SC/garden of private house	OG13	LC543992					
6	<i>Ae. albopictus</i>	Haha-jima	Tamagawa dam	N 26.6464, E 142.1687	October 2017	DT/bushes in car parking	3120	LC544003					
7	<i>Ae. albopictus</i>	Chichi-jima	Asahi yama	N 27.0951, E 142.2167	October 2017	LC/ground pool inside the air-raid shelter	3133	LC544004					
8	<i>Ae. albopictus</i>	Chichi-jima	Kominato beach	N 27.0604, E 142.1963	October 2017	LC/tree hole in coastal forest	3134	LC544005					
9	<i>Ae. albopictus</i>	Chichi-jima	Denshin yama	N 27.102, E 142.2028	October 2017	LC/ground pool inside the air-raid shelter	3135	LC544006					
10	<i>Ae. albopictus</i>	Chichi-jima	Kitafukurozawa	N 27.051, E 142.2124	October 2017	LC/tree hole in hilly area	3136	LC544007					
11	<i>Ae. albopictus</i>	Chichi-jima	Nishi machi	N 27.0939, E 142.1901	October 2017	LC/bucket in private house	3139	LC544008					
12	<i>Ae. albopictus</i>	Chichi-jima	Kitafukurozawa	N 27.0577, E 142.2054	October 2017	LC/bamboo stump in hilly area	3140	LC544009					
13	<i>Ae. albopictus</i>	Chichi-jima	Kuwanoki yama	N 27.0715, E 142.2171	October 2017	LC/bucket in vegetables & fruits farm	3143	LC544010					
14	<i>Ae. albopictus</i>	Haha-jima	Motochi	N 26.6404, E 142.1618	October 2017	LC/artificial container in private house	3149	LC544011					
15	<i>Ae. albopictus</i>	Haha-jima	Motochi	N 26.6404, E 142.1618	October 2017	LC/artificial container in private house	3150	LC544012					
16	<i>Ae. albopictus</i>	Chichi-jima	Denshin yama	N 27.102, E 142.2028	October 2017	LC/ground pool inside the air-raid shelter	3155	LC544013					
17	<i>Ae. albopictus</i>	Chichi-jima	Denshin yama	N 27.102, E 142.2028	October 2017	LC/ground pool inside the air-raid shelter	3158	LC544014					
18	<i>Ae. savoyi</i>	Chichi-jima	IBO	N 27.1000, E 142.1935	March 2015	SC/garden of private house	OG8	LC543993	7	3	0.1	—	sav1
19	<i>Ae. savoyi</i>	Chichi-jima	Okumura	N 27.1003, E 142.2022	March 2015	SC/garden of private house	OG10	LC543994				—	sav1
20	<i>Ae. savoyi</i>	Chichi-jima	IBO	N 27.1000, E 142.1935	March 2015	SC/garden of private house	OG15	LC543995				—	sav1
21	<i>Ae. savoyi</i>	Chichi-jima	Komagari	N 27.0667, E 142.2046	March 2015	SC/vegetables & fruits farm	OG18	LC543996				208:A→G	sav2
22	<i>Ae. savoyi</i>	Chichi-jima	Komagari	N 27.0667, E 142.2046	March 2015	SC/vegetables & fruits farm	OG19	LC543997				—	sav1
23	<i>Ae. savoyi</i>	Chichi-jima	Komagari	N 27.0667, E 142.2046	March 2015	SC/vegetables & fruits farm	OG21	LC543998				—	sav1
24	<i>Ae. savoyi</i>	Chichi-jima	Komagari	N 27.0667, E 142.2046	March 2015	SC/vegetables & fruits farm	OG22	LC543999				22:A→G	sav3
25	<i>Ae. wadai</i>	Chichi-jima	Fukiage dani	N 27.0772, E 142.2109	October 2017	LC/tree hole in hilly area	3147	LC544015	2	1	0	—	wad1
26	<i>Ae. wadai</i>	Chichi-jima	Fukiage dani	N 27.0772, E 142.2109	October 2017	LC/tree hole in hilly area	3148	LC544016				—	
27	<i>Culex boninensis</i>	Chichi-jima	Miyanohama	N 27.1034, E 142.1942	March 2015	SC/bushes in coastal area	OG14	LC544000	10	8	0.8	—	bon1
28	<i>Cx. boninensis</i>	Chichi-jima	FPRI	N 27.1015, E 142.1998	March 2015	SC/bushes inside experimental station	OG30	LC544001				57: A→G, 220: C→T, 433: A→T, 437: A→G, 646: C→T, 652: C→T	bon1
29	<i>Cx. boninensis</i>	Haha-jima	Tamagawa dam	N 26.6422, E 142.1696	October 2017	DT/bushes in hilly area	3114	LC544017				57: A→G, 220: C→T, 433: A→T, 437: A→G, 641: T→C, 646: C→T, 652: C→T	bon2
30	<i>Cx. boninensis</i>	Haha-jima	Tamagawa dam	N 26.6422, E 142.1696	October 2017	DT/bushes in hilly area	3116	LC544018				437: A→G, 641: T→C, 646: C→T, 652: C→T	bon3
31	<i>Cx. boninensis</i>	Haha-jima	Tamagawa dam	N 26.6464, E 142.1687	October 2017	DT/bushes in car parking	3121	LC544019				57: A→G, 220: C→T, 433: A→T, 437: A→G, 646: C→T, 652: C→T, 658: T→C	bon4
32	<i>Cx. boninensis</i>	Haha-jima	Tamagawa dam	N 26.6464, E 142.1687	October 2017	DT/bushes in car parking	3122	LC544020				57: A→G, 220: C→T, 433: A→T, 437: A→G, 641: T→C, 646: C→T, 652: C→T, 658: T→C	bon5
33	<i>Cx. boninensis</i>	Haha-jima	Shizuka sawa	N 26.6443, E 142.1531	October 2017	LC/bucket in vegetables & fruits farm	3137	LC544021				57: A→G, 79: T→C, 278: T→C, 437: A→G, 474: C→T, 496: A→G, 652: C→T	bon6
34	<i>Cx. boninensis</i>	Chichi-jima	Yoake yama	N 27.0935, E 142.2171	October 2017	LC/ground pool in hilly area	3142	LC544022				57: A→G, 79: T→C, 278: T→C, 437: A→G, 474: C→T, 496: A→G, 652: C→T	bon6
35	<i>Cx. boninensis</i>	Chichi-jima	Nagatani	N 27.0658, E 142.2178	October 2017	LC/swamp in hilly area	3144	LC544023				57: A→G, 437: A→G, 496: A→G, 652: C→T	bon7
36	<i>Cx. boninensis</i>	Chichi-jima	Kitafukurozawa	N 27.0516, E 142.211	October 2017	LC/river bed in hilly area	3157	LC544024				57: A→G, 400: T→G, 433: A→T, 437: A→G, 641: T→C, 646: C→T, 652: C→T, 658: T→C	bon8

Appendix 1. Continued.

Serial no.	Species	Details of collection sites					Specimen code	GenBank accession no.	No. of specimens	No. of haplotypes	Mean intraspecific divergences (%)*	position and different substituted nucleotide**	Name of haplotypes***
		Island	Place	GPS coordinates	Date	Collection method and environment							
37	<i>Cx. quinquefasciatus</i>	Haha-jima	Tamagawa dam	N 26.6464, E 142.1687	October 2017	DT/bushes in car parking	3117	LC544025	8	1	0	—	quil
38	<i>Cx. quinquefasciatus</i>	Haha-jima	Tamagawa dam	N 26.6464, E 142.1687	October 2017	DT/bushes in car parking	3119	LC544026					
39	<i>Cx. quinquefasciatus</i>	Haha-jima	Hyougi daira	N 26.636, E 142.1651	October 2017	DT/outside of cow shed	3127	LC544027					
40	<i>Cx. quinquefasciatus</i>	Haha-jima	Motochi	N 26.6409, E 142.1612	October 2017	LC/bath tub on the road	3129	LC544028					
41	<i>Cx. quinquefasciatus</i>	Chichi-jima	Nagatani	N 27.0658, E 142.2178	October 2017	LC/swamp in hilly area	3130	LC544029					
42	<i>Cx. quinquefasciatus</i>	Chichi-jima	Fukiage dani	N 27.0772, E 142.2109	October 2017	LC/tree hole in hilly area	3132	LC544030					
43	<i>Cx. quinquefasciatus</i>	Chichi-jima	Nishi machi	N 27.0939, E 142.1901	October 2017	LC/bucket in private house	3138	LC544031					
44	<i>Cx. quinquefasciatus</i>	Chichi-jima	Kominato beach	N 27.0604, E 142.1963	October 2017	LC/tree hole in coastal forest	3154	LC544032					
45	<i>Lutzia shinonagai</i>	Chichi-jima	Kuwanoki yama	N 27.0715, E 142.2171	October 2017	LC/bucket in vegetables & fruits farm	5	LC544033	2	1	0	—	shil
46	<i>Lt. shinonagai</i>	Chichi-jima	Kuwanoki yama	N 27.0715, E 142.2171	October 2017	LC/bucket in vegetables & fruits farm	3156	LC544034					
47	<i>Lt. vorax</i>	Chichi-jima	FPRI	N 27.1015, E 142.1998	March 2015	SC/bushes inside experimental station	OG31	LC544002	9	6	0.3	101: T→C, 311: G→A 312: G→A, 614: A→G	vor2 vor3
48	<i>Lt. vorax</i>	Haha-jima	Nakano taira	N 26.6326, E 142.1731	October 2017	LC/bucket inside cow shed	6	LC544035					
49	<i>Lt. vorax</i>	Haha-jima	Nakano taira	N 26.6326, E 142.1731	October 2017	LC/bucket inside cow shed	7	LC544036					
50	<i>Lt. vorax</i>	Chichi-jima	Denshin yama	N 27.102, E 142.2028	October 2017	LC/water tank inside the air-raid shelter	8	LC544037					
51	<i>Lt. vorax</i>	Haha-jima	Nakano taira	N 26.6326, E 142.1731	October 2017	LC/bucket inside cow shed	3128	LC544038				312: G→A, 614: A→G 614: A→G	vor3 vor4
52	<i>Lt. vorax</i>	Haha-jima	Nakano taira	N 26.6326, E 142.1731	October 2017	LC/bucket inside cow shed	3131	LC544039				557: G→A	vor5
53	<i>Lt. vorax</i>	Haha-jima	Motochi	N 26.6404, E 142.1618	October 2017	LC/artificial container in private house	3151	LC544040				312: G→A, 614: A→G	vor3
54	<i>Lt. vorax</i>	Haha-jima	Kitakou shuraku ato	N 26.6963, E 142.1437	October 2017	LC/artificial container in bushes	3152	LC544041				290: A→T	vor6
55	<i>Lt. vorax</i>	Chichi-jima	Kitafukurozawa	N 27.0483, E 142.2136	October 2017	LC/well in mountainous area	3153	LC544042				101: T→C, 311: G→A	vor2

DT: dry-ice trap, SC: sweep-net collection, LC: Larval collection

IBO: Institute of Boninology

FPRI: Kiyose Experimental Station of the Forestry and Forest Products Research Institute

*Intraspecific divergences calculated for each species using the K2P model. Means were calculated for specimens for which the sequences were examined in more than two individuals.

**The nucleotide showed single nucleotide polymorphism at some position in the COI region (the position number of the substituted nucleotide, original and different nucleotide).

***Assigned haplotype name for each haplotype of *Ae. albopictus*, *Cx. quinquefasciatus*, and *Lt. vorax*.

Appendix 2. The list of specimens registered in GenBank that was used in the genetic study and the single-nucleotide polymorphism at position compared with Chichi-jima and Haha-jima specimens.

Serial no.	Species	Collection locality	Region	GenBank accession no.	Reference	position and different substituted nucleotide*	Name of haplotypes**
1	<i>Aedes albopictus</i>	Ishigakijima	Ryukyu archipelago	AB738090	Taira et al., 2012	323: G→A	alb2
2	<i>Ae. albopictus</i>	Tokunoshima		AB738121		—	alb1
3	<i>Ae. albopictus</i>	Tokunoshima		AB738122		—	alb1
4	<i>Ae. albopictus</i>	Miyakojima		AB738143		323: G→A	alb2
5	<i>Ae. albopictus</i>	Miyakojima		AB738161		359: T→A	alb3
6	<i>Ae. albopictus</i>	Iriomotejima		AB738179		323: G→A	alb2
7	<i>Ae. albopictus</i>	Iriomotejima		AB738192		323: G→A	alb2
8	<i>Ae. albopictus</i>	Yonagunijima		AB738197		—	alb1
9	<i>Ae. albopictus</i>	Okinawajima		AB738203		359: T→A	alb3
10	<i>Ae. albopictus</i>	Okinawajima		AB738211		—	alb1
11	<i>Ae. albopictus</i>	Amamioshima	Mainland	AB738240	Maekawa et al., 2016a	359: T→A	alb3
12	<i>Ae. albopictus</i>	Amamioshima		AB738241		323: G→A	alb2
13	<i>Ae. albopictus</i>	Yonagunijima		AB738294		47: A→G	alb4
14	<i>Ae. albopictus</i>	Ishigakijima		AB738301		323: G→A	alb2
15	<i>Ae. albopictus</i>	Kumejima		AB738308		—	alb1
16	<i>Ae. albopictus</i>	Kumejima		AB738310		—	alb1
17	<i>Ae. albopictus</i>	Wakayama		LC054323		57: A→G	alb5
18	<i>Ae. albopictus</i>	Kumamoto		LC054324		57: A→G	alb5
19	<i>Ae. albopictus</i>	Kumamoto		LC054325		57: A→G	alb5
20	<i>Ae. albopictus</i>	Kumamoto		LC054326		513: G→A	alb6
21	<i>Culex quinquefasciatus</i>	Miyakojima	Ryukyu archipelago	AB738158	Taira et al., 2012	—	qui1
22	<i>Cx. quinquefasciatus</i>	Miyakojima		AB738162		—	qui1
23	<i>Cx. quinquefasciatus</i>	Okinawajima		AB738200		—	qui1
24	<i>Cx. quinquefasciatus</i>	Okinawajima		AB738204		—	qui1
25	<i>Cx. quinquefasciatus</i>	Amamioshima		AB738238		—	qui1
26	<i>Cx. quinquefasciatus</i>	Amamioshima		AB738239		—	qui1
27	<i>Cx. quinquefasciatus</i>	Ishigakijima		AB738257		18: G→A	qui2
28	<i>Cx. quinquefasciatus</i>	Ishigakijima		AB738258		18: G→A	qui2
29	<i>Cx. quinquefasciatus</i>	Yonagunijima		AB738278		18: G→A	qui2
30	<i>Cx. quinquefasciatus</i>	Yonagunijima		AB738279		18: G→A	qui2
31	<i>Cx. quinquefasciatus</i>	Iriomotejima		AB738304		—	qui1
32	<i>Cx. quinquefasciatus</i>	Iriomotejima		AB738305		—	qui1
33	<i>Cx. quinquefasciatus</i>	Kumejima		AB738306		—	qui1
34	<i>Cx. quinquefasciatus</i>	Kumejima		AB738313		—	qui1
35	<i>Lutzia vorax</i>	Wakayama	Mainland	LC054506	Maekawa et al., 2016a	—	vor1
36	<i>Lt. vorax</i>	Gifu		LC054508		311: G→A	vor7
37	<i>Lt. vorax</i>	Ehime		LC054505		311: G→A, 380: T→C, 381: T→C, 479: T→C, 557: G→A, 641: T→C	vor8
38	<i>Lt. vorax</i>	Kumamoto	Kyushu	LC054507		311: G→A	vor7
39	<i>Lt. vorax</i>	Kumamoto		LC054509		311: G→A, 557: G→A	vor9
40	<i>Lt. vorax</i>	Miyazaki		LC054510		—	vor1

*The nucleotide showed single nucleotide polymorphism at some position in the *COI* region (the position number of the substituted nucleotide, original and different nucleotide).

**Assigned haplotype name for each haplotype of *Ae. albopictus*, *Cx. quinquefasciatus*, and *Lt. vorax*. Bold letter indicates haplotypes not shared to Chichi-jima and Haha-jima islands population.