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Species clarification of Ogasawara cockroaches which inhabit Japan

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Abstract. The so-called "Ogasawara cockroaches" were examined by morphological observations and by breeding experiments to elucidate their actual taxonomical status. Fourteen groups (isolate) of "Ogasawara cockroaches" collected from Iwoto-A, Iwoto-B, Hahajima, Chichijima, Nishijima, Nakodojima, Tokunoshima-A, Tokunoshima-B, Okinawato-A, Okinawa-B, Amamiooshima, Miyakojima, Ishigakijima and Hawaii, were bred and passaged in our laboratory. Cockroaches collected from the field were first reared individually and the sexes of their offspring examined. Cockroaches collected from Iwoto, Tokushima and Okinawa, were found to consist of two groups; those whose offspring were all female and the other whose offspring consist of both male and female. Cross-breeding experiments showed that individuals from the group that did not produce any male but only female offspring were parthenogenetic. On the contrary, the group that have bisexual individuals produced both male and female offspring in a ratio of 1:1. Our results show that the so-called "Ogasawara cockroaches" consist of 2 species, namely, *Pycnoscelus surinamensis* and *Pycnoscelus indicus*. There are areas in which both species co-habitated together and there are also areas in which either only one of the two species can be found. The group that reproduces only female offspring and only through parthenogenesis was identified as *P. surinamensis*. The group that reproduces heterosexually and produce male and female offspring was identified as *P. indicus*. Thus, the so-called "Ogasawara cockroaches" found in Japan actually consist of 2 species, namely, *P. surinamensis* and *P. indicus*, which can be differentiated using the solitary breeding method to demonstrate parthenogenesis in the former and the need for sexual reproduction in the latter.

INTRODUCTION

The so-called "Ogasawara cockroaches", which hitherto has also been synonymously called "Surinam cockroaches", are widely distributed in the tropical and sub-tropical areas. In Japan, their distribution is limited to Kyushu and the island chain of Nansei and Ogasawara island chain (Asahina, 1991; Komatsu *et al.*, 2013).

Regarding the constituent species of the so-called "Ogasawara cockroaches" or "Surinam cockroaches", Roth (1967) proposed that irrespective of the presence or absence of sperms in the spermatheca, those which produce only female offspring should be identified as *Pycnoscelus surinamensis*, while those that produce both male and female offspring should be relegated as *Pycnoscelus indicus*. Moreover, he also reported that there were morphological differences between the two aforementioned species, based on the distance between the compound eye and ocelli. In *P. indicus*,

the ocelli and the compound eyes were separated, whereas in *P. surinamensis*, the two eyes were in contact.

On the other hand, Asahina (1991) reported that both male and female individuals could be found among the "Ogasawara cockroaches", without noting that the female were parthenogenetic. Moreover, Furukawa (1930), Azuma (1987), Kawamura (1990), had all reported that the Japanese "Ogasawara cockroaches" were all identified as *P. surinamensis*.

In this paper, we set out to clarify the true identity of the "Ogasawara cockroaches", whether it is actually *P. indicus* or *P. surinamensis* by examining the various colonies of cockroaches collected from different localities using solitary breeding experiment.

MATERIALS AND METHODS

Cockroaches collected from 14 localities were used in this study (Table 1) (Figure 1). Before conducting the breeding experiment in the laboratory, all the female adult cockroaches collected from the field were first reared individually and their offspring were examined.

A group of cockroaches collected from Iwoto, Tokushima and Okinawa, were found to produce only female offspring and no male offspring were observed. This group of cockroaches was designated as Group A. The other group, also from those same areas, that produce both male and female offspring, were designated as Group B.

Breeding experiments

(1) Immediately after the hatching of the egg, each of the female from the group of cockroaches that does not produce any male offspring, which were collected from Iwoto-A, Hahajima, Chichijima, Nishijima, Nakodojima, Tokunoshima-A and Okinawato-A, was kept with a male adult cockroach from Hawaii, and reared together.

(2) For each of the individuals from the group of cockroaches that produce both the male and female offspring, which were collected from Iwoto-B, Tokunoshima-B, Amamiooshima, Okinawato-B, Miyakojima, Ishigakijima and Hawaii, they were kept as male and female pair, and reared together.

(3) Ten pairs of each of the group in (1) and (2) were reared in the breeding experiments described in (1) and (2) above. The female offspring produced in experiments (1) and (2) were each kept and reared individually. Five individuals from each of the groups were reared and observed.

Table 1. Laboratory colonies of the *Pycnoscelus* spp. used in the present study

	Abbreviation	Collection	
		Locality	Date
Parthenogenetic	Iwoto -A	Iwo-To, Ogasawara-mura, Tokyo Japan	Dec., 2009
	Hahajima	Haha-jima, Ogasawara-mura, Tokyo Japan	Jun., 2010
	Chichijima	Chichi-jima, Ogasawara-mura, Tokyo Japan	Feb., 2006
	Nishijima	Nishijima, Ogasawara-mura, Tokyo Japan	Mar., 2007
	Nakodojima	Nakodo-jima, Ogasawara-mura, Tokyo Japan	Jun., 2010
	Tokunoshima -A	Tokunoshima-cho, Kagoshima Pref. Japan	Mar., 2007
	Okinawato -A	Naha-shi, Okinawa Pref. Japan	Feb., 2001
Bisexual	Iwoto -B	Iwo-To, Ogasawara-mura, Tokyo Japan	Dec., 2009
	Tokunoshima -B	Tokunoshima-cho, Kagoshima Pref. Japan	Oct., 2011
	Amamiooshima	Amami-shi, Kagoshima Pref. Japan	Oct., 2011
	Okinawato -B	Nago-shi, Okinawa Pref. Japan	Nov., 2009
	Miyakojima	Miyakojima-shi, Okinawa Pref. Japan	Oct., 2011
	Ishigakijima	Kuro-shima, Yaeyama-gun, Taketomi-cho, Okinawa Pref. Japan	Nov., 2009
	Hawaii	Oahu, Hawaii, USA.	Oct., 2010

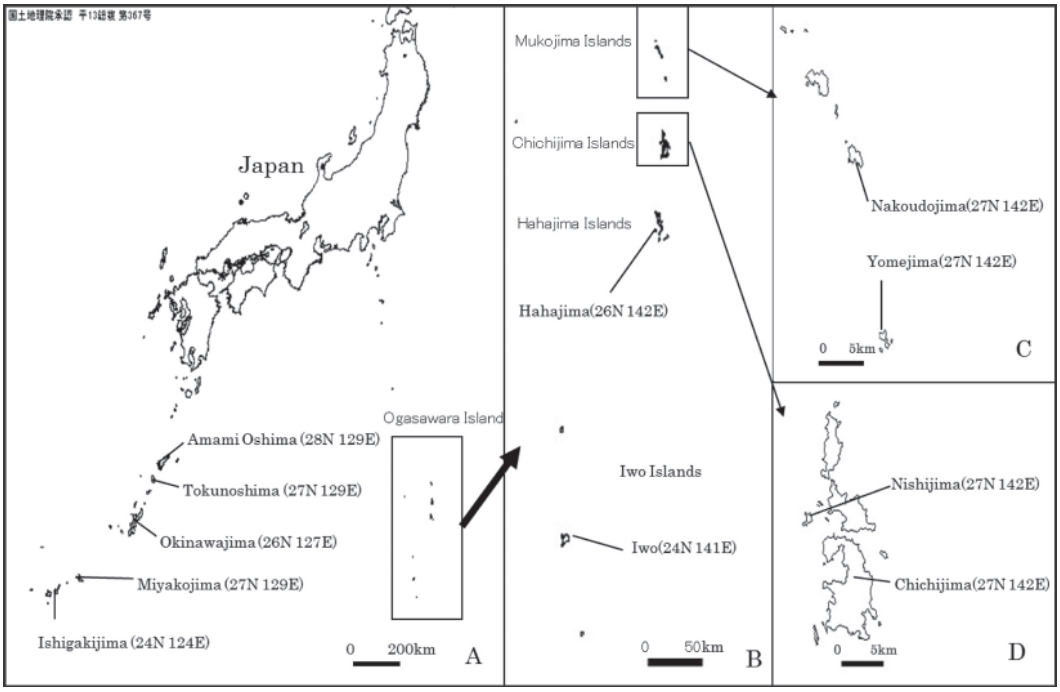

Figure 1 A: Map showing the location of the Nansei Islands and Ogasawara chain islands which is 1,000 km south of Tokyo. B: Map showing the 4 islands which are inhabited by humans, namely, Mukojima Island, Chichijima Island, Hahajima Island and Iwo Island. C: Map showing the location of the Nakoudojima (27N 142E) and Yomejima (27N 142E). D: Map showing the location of the Chichijima (27N 142E) and Nishijima (27N 142E).

The cockroaches in the above experiments were observed for their number of days needed to lay egg, number of offspring produced and the sexes of the offspring. The number of offspring produced was observed up to 2 litter. Those that gave birth to the first litter only once were reared until their natural demise.

Furthermore, the female were observed for the presence or absence of sperms in their spermatheca. Moreover, the offspring were reared until maturity and then euthanized, followed immediately by taking the measurement of their tegmina length as well as the distance between ocelli and compound eyes.

The cockroaches were reared in a plastic container of diameter 90 mm and a height of 50 mm. The bedding were made up of 10 mm thick hydrated insect-rearing mat (Fujicon Co., Japan) and holes were made in the container lid for aeration. The cockroaches were fed slices of carrot cut to 10 mm thickness. The whole container was placed in an incubator (Sanyo Co., Japan), with temperature set at 26-28°C, humidity at 50-70% and left in natural light condition. The rearing-mat and the carrot were replaced at appropriate time to prevent the growth of fungus.

The sex of the hatched nymph were examined under a dissection microscope to determine for the presence or absence of V-shaped notch at the ventral abdominal caudal region, with those having the notch identified as the male and those without the notch as female (Komatsu *et al.*, 2014).

Moreover, for the analysis of the resulting copulation ratio obtained through the mating experiments, the following formula was used for deriving isolating index, *I*.

$$I = \frac{\text{Percentage of copulation between similar strain} - \text{Percentage of copulation between different strain}}{\text{Percentage of copulation between similar strain} + \text{Percentage of copulation between different strain}}$$

For the percentage of copulation between individual of similar strain, the copulation percentage of the male and female of the Hawaiian strain was used. For the percentage of copulation between individual of different strains, the copulation percentage of the male Hawaiian strain with the female of Iwoto-A, Hahajima, Chichijima, Nishijima, Nakodojima, Tokunoshima-A and Okinawato-A was used. The average length of the female tegmina as well as the average distance between the ocelli and compound eyes, were subjected to *t* test for the presence of significant differences.

RESULTS

Mating experiments were carried using 14 strains of cockroaches obtained from 11 locations, namely, Iwoto, Hahajima, Chichijima, Nishijima, Nakodojima, Tokunoshima, Amamiooshima, Okinawato, Miyakojima, Ishigakijima and Hawaii.

Mating experiment (Table 2)
After mating, all females were necropsy to determine the presence or absence of sperm in their spermatheca by observing for the motile sperms under the light microscope (Figure 2). As shown in Figure 2, although both the females of Okinawato-A and Iwoto-A were parthenogenic, the former did not have sperms in their spermatheca but the latter had (Figure 2A, 2B). However, all the females of the bisexual group, as shown in the females of Okinawato-B, had sperms in their spermatheca (Figure 2C).

Group that does not produce male offspring

① Iwoto-A♀X Hawaii ♂: Sperms could be observed in the spermatheca of all the females, and a total of 478 offspring were produced from the 10 females that produced offspring. All the offspring were female. Each female produced between 17-33 offspring, with an average of 23.9.

Table 2. Comparison of the number and sex of offspring as well as the presence or absence of the sperm in the spermatheca of "Ogasawara cockroaches"

Reproduction	Exp. No	No. of Insects ♀(10) × ♂(10)	No. ♀ Fertile	No. Mated or Virgin (%)	No. of offpsings produced ♂	♀	Total	Total litter	Av. Offsprings /litter	Isolating index
Parthenogenetic	①	Iowto-A × Hawaii	10	M 10 (100.0)	0 (0-0)	478 (17-33)	478	20	23.9	+1
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	②	Hahajima × Hawaii	9	M 6 (66.7)	0 (0-0)	248 (4-28)	248	12	20.7	+1
				V 3 (33.3)	0 (0-0)	115 (8-26)	115	6	19.2	
	③	Chichijima × Hawaii	8	M 3 (37.5)	0 (0-0)	59 (2-24)	59	4 [1)]	14.8	+1
				V 5 (62.5)	0 (0-0)	150 (1-25)	150	9 [1)]	16.7	
	④	Nishijima × Hawaii	10	M 9 (90.0)	0 (0-0)	663 (26-48)	663	18	36.8	+1
				V 1 (10.0)	0 (0-0)	62 (30-32)	62	2	31.0	
	⑤	Nakodojima × Hawaii	7	M 5 (71.4)	0 (0-0)	143 (1-32)	143	8 [1)]	17.9	+1
				V 2 (28.6)	0 (0-0)	96 (28-35)	96	3 [1)]	32.0	
	⑥	Tokunoshima-A × Hawaii	10	M 5 (50.0)	0 (0-0)	221 (11-35)	221	10	22.1	+1
				V 5 (50.0)	0 (0-0)	161 (5-28)	161	10	16.1	
	⑦	Okinawato-A × Hawaii	10	M 1 (10.0)	0 (0-0)	57 (21-36)	57	2	28.5	+1
				V 9 (90.0)	0 (0-0)	667 (26-47)	667	18	37.1	
Bisexual	⑧	Iowto-B × Iowto-b	8	M 8 (100.0)	162 (4-16)	157 (9-15)	319	15 [1)]	21.3	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑨	Tokunoshima-B × Tokunoshima-b	10	M 10 (100.0)	242 (3-20)	207 (0-19)	449	20	22.5	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑩	Amamiooshuma × Amamiooshuma	9	M 9 (100.0)	260 (4-19)	260 (4-25)	520	18	28.9	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑪	Okinawato-B × Okinawato-b	10	M 10 (100.0)	322 (9-23)	312 (13-24)	634	20	31.7	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑫	Miyakojima × Miyakojima	8	M 8 (100.0)	230 (9-23)	267 (13-24)	497	14 [1)]	35.5	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑬	Ishigakijima × Ishigakijima	9	M 9 (100.0)	281 (10-22)	266 (2-24)	547	17 [1)]	32.2	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	
	⑭	Hawaii × Hawaii	9	M 9 (100.0)	199 (5-20)	189 (1-24)	388	16 [1)]	24.3	–
				V 0 (0.0)	0 (0-0)	0 (0-0)	0	0	0.0	

[1)] Including those that produced only 1 litter

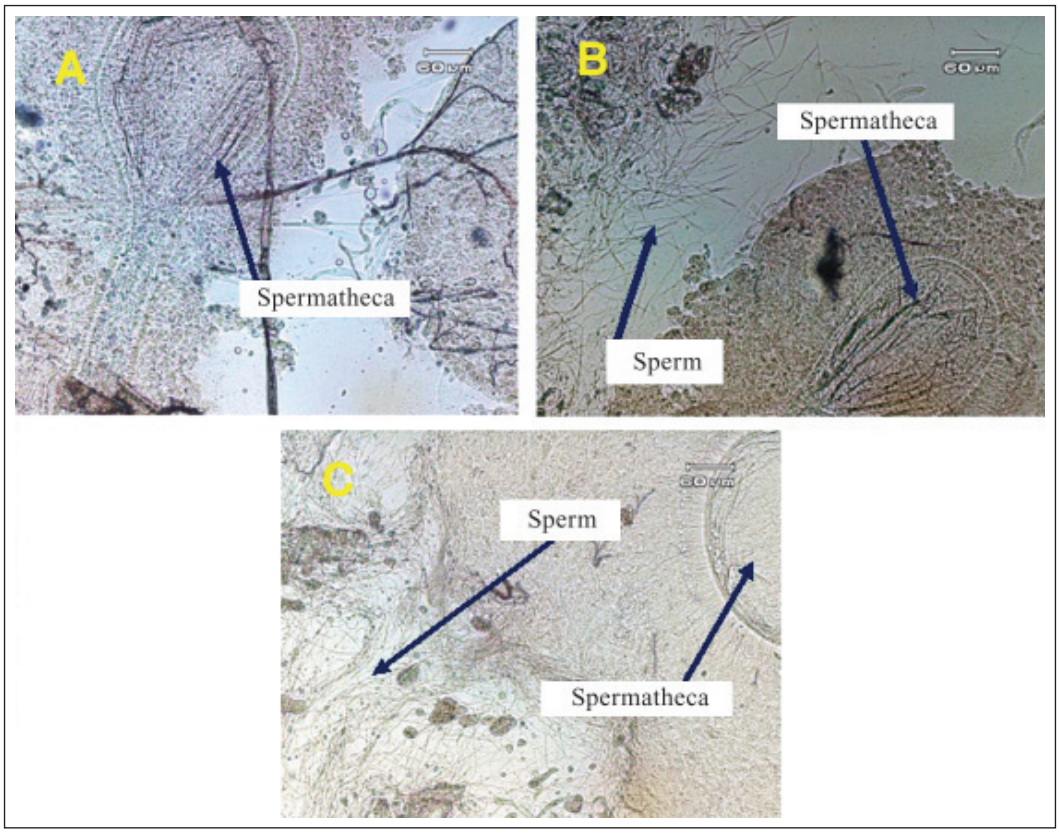

Figure 2. Female spermatheca of the *Pycnoscelus* spp. A: Parthenogenetic of Okinawato-A, B: Parthenogenetic of Iwoto-A, C: Bisexual of Okinawato-B, Spt: Spermatheca.

② Hahajima♀X Hawaii ♂: Sperms could be observed in the spermatheca of only 6 of the 9 females, and they all gave birth to two litter. Those that have sperms in their spermatheca produced a total of 252 offspring (four male, (0-3, average of 0.3); 248 females, (4-28, av. 21.0)). Those three females that do not have sperm in their spermatheca produced 115 offspring (8-26, av. 19.2), all of them female and not a single male.

③ Chichijima♀X Hawaii ♂: Sperms could be observed in the spermatheca of only 3 of the 8 females. Of those three females, one gave birth twice and the other two gave birth only once. A total of 59 female offspring (2-24, av. 14.8) and no male offspring was produced. Of the five females that do not have sperm in their spermatheca, four gave birth to two litter and one to one litter, making a total of 150 female offspring (1-25, av. 16.7), with no male offspring.

④ Nishijima♀X Hawaii ♂: Sperms could be observed in the spermatheca of 9 of the 10 females, and all of them gave birth to two litter. A total of 664 offspring (male 1 (0-1, av. 0.1); female 663 (26-48, av. 36.8)) were produced. The single female that did not have sperm in its spermatheca gave birth to a total of 62 female offspring (30-32, av. 31.0) with no male offspring.

⑤ Nakodojima♀X Hawaii ♂: Sperms could be observed in the spermatheca of five of the seven females, with three giving birth to two litter and two to one litter, making a total of 143 female offspring (1-32, av. 17.9) and no male offspring. For the remaining two females that have no sperm in their spematheca, one gave birth to two litter and the other to one litter,

with a total of 96 female offspring (28-35, av. 32.0) and no male offspring.

⑥ Tokunoshima-A ♀ X Hawaii ♂: Sperms could be observed in the spermatheca of only five of the 10 females, and they all gave birth to two litter. Those that have sperms in their spermatheca produced a total of 221 female offspring (11-35, av. 22.1) and no male. Those 5 females that do not have sperm in their spermatheca produced 161 female offspring (5-28, av. 16.1) and not a single male.

⑦ Okinawato-A ♀ X Hawaii ♂: Sperms were observed in the spermatheca of only one of the 10 females, which gave birth to two litter, producing a total of 57 female offspring (21-36, av. 28.5) and no male offspring. Those nine females that have no sperm in their spermatheca also gave birth to two litter, producing a total of 667 female offspring (26-47, av. 37.1) and not a single male.

Group that produce both male and female offspring

⑧ Iwoto-B ♀ X Iwoto-B ♂: Of the 10 females used, only eight succeeded in giving birth and sperms were observed in their spermatheca, with seven giving birth to two litter and one to one litter. The 8 females produced a total of 319 offspring (male 162 (4-16, av. 10.8); female 157 (9-15, av. 10.5), with an average of 21.3 per litter.

⑨ Tokunoshima-B ♀ X Tokunoshima-B ♂: All 10 females reproduced and sperms were observed in their spermatheca, with all giving birth to two litter. The 10 females produced a total of 449 offspring (male 242 (3-20, av. 12.1); female 207 (0-19, av. 10.4), with an average of 22.5 per litter.

⑩ Amamiooshima ♀ X Amamiooshima ♂: Of the 10 females used, only nine reproduced and sperms were observed in their spermatheca, with all giving birth to two litter. The nine females produced a total of 520 offspring (male 260 (4-19, av. 14.4); female 260 (4-25, av. 14.4), with an average of 28.9 per litter.

⑪ Okinawato-B ♀ X Okinawato-B ♂: All 10 females reproduced and sperms were observed in their spermatheca, with all giving birth to two litter. The 10 females produced a total of 634 offspring (male 322 (9-23, av. 16.1); female 312 (13-24, av. 15.6), with an average of 31.7 per litter.

⑫ Miyakojima ♀ X Miyakojima ♂: Only eight of the 10 females reproduced and sperms were observed in their spermatheca, with six giving birth to two litter and to to one litter. The eight females produced a total of 497 offspring (male 230 (9-23, av. 16.4); female 267 (13-24, av. 19.1), with an average of 35.5 per litter.

⑬ Ishigakijima ♀ X Ishigakijima ♂: Only eight of the 10 females reproduced and sperms were observed in their spermatheca, with seven giving birth to two litter and one to one litter. The eight females produced a total of 547 offspring (male 281 (10-22, av. 16.5); female 266 (2-24, av. 15.6), with an average of 32.2 per litter.

⑭ Hawaii ♀ X Hawaii ♂: nine out of 10 females reproduced and sperms were observed in their spermatheca, with seven giving birth to two litter and two to one litter. The nine females produced a total of 388 offspring (male 199 (2-20, av. 12.4); female 189 (1-24, av. 11.8), with an average of 24.3 per litter.

Rearing result of F1 female (Table 3)

1) Group that does not produce any male offspring

① Iwoto-A ♀: 4 F1 females gave birth to two litter and one to one litter, with all the offspring being female. The five females produced a total of 254 offspring (10-43 offspring per litter, av. 28.2).

② Hahajima ♀: All 5 F1 females gave birth to two litter of all female offspring, producing a total of 225 offspring (14-35 offspring per litter, av. 22.5).

③ Chichijima ♀: All five F1 females gave birth to two litter of all female offspring, producing a total of 208 offspring (16-26 offspring per litter, av. 20.8).

Table 3. Parthenogenesis of the various colonies of cockroaches collected from different localities followed by being reared individually

	Exp. No	♀	N	No. of offsprings produced			No. litter	Av. Offsprings/ litter	Av. Life span
				♂	♀	Total			
Parthenogenetic	①	Iwoto-A	5	0	254 (10-43)	254	9	28.2	—
	②	Hahajima	5	0	225 (14-35)	225	10	22.5	—
	③	Chichijima	5	0	208 (16-26)	208	10	20.8	—
	④	Nishujima	5	0	404 (30-48)	404	10	40.4	—
	⑤	Nakodojima	5	0	230 (13-35)	230	10	23.0	—
	⑥	Tokunoshima-A	5	0	300 (21-38)	300	10	30.0	—
	⑦	Okinawato-A	5	0	324 (24-46)	324	10	32.4	—
Bisexual	⑧	Iwoto-B	5	—	—	0	0	0.0	283.6 (261-312)
	⑨	Tokunoshima-B	5	—	—	0	0	0.0	198.0 (179-237)
	⑩	Amamiooshima	5	—	—	0	0	0.0	227.6 (164-275)
	⑪	Okinawato-B	5	—	—	0	0	0.0	243.8 (165-312)
	⑫	Miyakojima	5	—	—	0	0	0.0	230.2 (139-297)
	⑬	Ishigakijima	5	—	—	0	0	0.0	245.4 (211-315)
	⑭	Hawaii	5	—	—	0	0	0.0	309.4 (278-347)

④ Nishijima♀: All five F1 females gave birth to two litter of all female offspring, producing a total of 404 offspring (30-48 offspring per litter, av. 40.4).

⑤ Nakodojima♀: All five F1 females gave birth to two litter of all female offspring, producing a total of 230 offspring (13-35 offspring per litter, av. 23.0).

⑥ Tokunoshima-A♀: All five F1 females gave birth to two litter of all female offspring, producing a total of 300 offspring (21-38 offspring per litter, av. 30.0).

⑦ Okinawato-A♀: All five F1 females gave birth to two litter of all female offspring, producing a total of 324 offspring (24-46 offspring per litter, av. 32.4).

Groups that produce both male and female F1 offspring

All the F1 from the Iwoto-B, Tokunoshima-B, Amamiooshima, Okinawato-B, Miyakojima, Ishigakijima and Hawaii group did not produce any offspring in their whole life span. The average life span of the F1 from Iwoto-B group was 283.6 days (261-312 days), Tokunoshima-B, 198.0 days (179-237 days), Amamiooshima, 227.6 days (164-275 days), Okinawato-B, 243.8 days (165-312 days), Miyakojima, 230.2 days (139-297 days), Ishigakijima, 245.4 days (211-315 days), and Hawaii 309.4 days (278-347 days), respectively.

Measurement of the tegmina length (Table 4)

For the groups that do not produce any male offspring, the average tegmina length of the adult female from the various localities are as follows: Okinawato-A, 15.82 mm > Hahajima, 15.26 mm > Nishijima, 15.07 mm > Nakodojima, 14.16 mm > Chichijima, 13.81 mm > Tokunoshima-A, 13.57 mm > Iwoto-A, 12.87 mm, respectively.

For the groups that produce both male and female offspring, the average tegmina length of the adult female from the various localities are as follows: Okinawato-B, 14.72 mm > Hawaii, 14.64 mm > Iwoto-B, 14.35 mm > Ishigakijima, 13.81 mm > Tokunoshima-B, 13.54 mm> Amamiooshima, 13.53 mm > Miyakojima, 12.96 mm, respectively.

Measurement of the distance between ocelli and compound eye (Table 4, Figure 3)

For the groups that do not produce any male offspring, the distance between ocelli and compound eye in the adult female from the various localities are as follows: Hahajima, 0.16 mm > Chichijima, 0.14 mm

Table 4. Distance between the ocelli and the compound eye, as well as the length of the fore-wing (tegmina) of the female adult cockroaches collected from different localities

	Exp. No	Locations	N	Length of wings Av. of L&R	Dist. ocelli to compd. eye Av. of L&R
Parthenogenetic	①	Iwoto-A	15	12.87	0.10
	②	Hahajima	15	15.26	0.16
	③	Chichijima	15	13.81	0.14
	④	Nishujima	15	15.07	0.12
	⑤	Nakodojima	15	14.16	0.13
	⑥	Tokunoshima-A	15	13.57	0.12
	⑦	Okinawato-A	15	15.82	0.12
				14.37 *	0.13 *
Bisexual	⑧	Iwoto-B	15	14.35	0.18
	⑨	Tokunoshima-B	15	13.53	0.13
	⑩	Amamiooshima	15	13.54	0.12
	⑪	Okinawato-B	15	14.72	0.13
	⑫	Miyakojima	15	12.96	0.16
	⑬	Ishigakijima	15	13.81	0.12
	⑭	Hawaii	15	14.64	0.21
				14.26 *	0.15 *

* : P > 0.01 No significant difference in distances from ocelli to compound eye as well as in tegmina length between the parthenogenetic and bisexual groups (Student's t test).

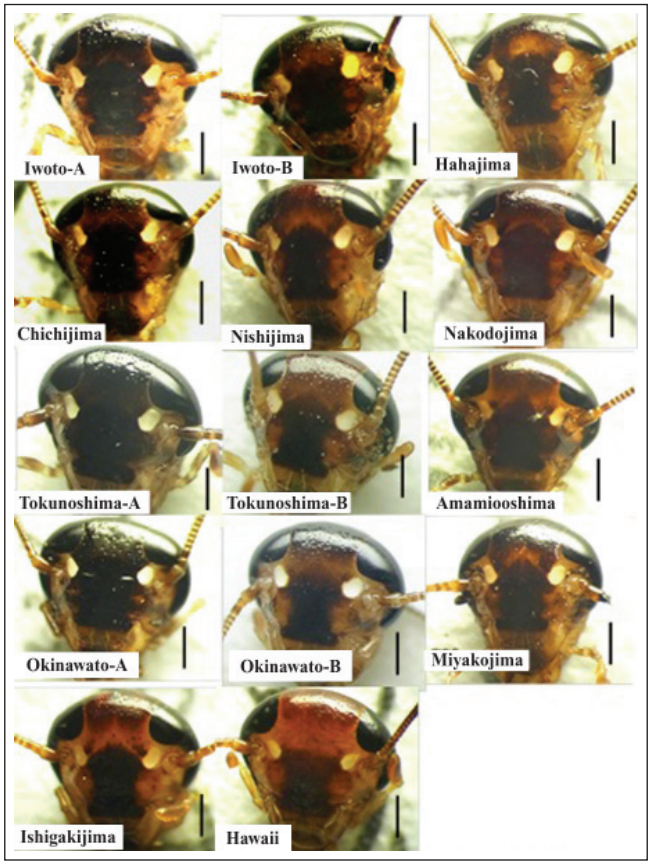

Figure 3. Enface view of *Pycnoscelus* spp. from various localities (Immediately after being sacrificed). Scale bar = 1 mm for all.

> Nakodojima, 0.13 mm > Nishijima, Tokunoshima-A & Okinawato-A, 0.12 mm > Iwoto-A, 0.10 mm, respectively.

For the groups that also produce both male and female offspring, the distance between ocelli and compound eye in the adult female from the various localities are as follows: Hawaii, 0.21mm > Iwoto-B, 0.18 mm > Miyakojima, 0.16 mm > Amamiooshima & Okinawato-B, 0.13 mm > Tokunoshima-B & Ishigakijima, 0.12 mm, respectively.

DISCUSSION

Matsumura (1931), Azuma (1987) and Kawamura (1990) reported that the species of the so-called "Ogasawara cockroaches" in Japan are those of *P. surinamensis*. However, Asahina (1991) reportedly collected both male and female specimens of "Ogasawara cockroaches" from the field but did not proceed to determine whether there is any parthenogenecity among the females or not. Based on the results of some breeding experiments, Roth (1967) reported that despite the presence or absence of sperm in the spermatheca, those that produced only female offspring should be identified as *P. surinamensis*, while those that produced both male and female offspring as *P. indicus*. Moreover, he also suggested that those specimens whose ocelli were separated from the compound eye belong to those of *P. surinamensis*, while those ocelli and compound eye were in contact belong to *P. indicus*. Thus, the actual status or species of the so-called "Ogasawara cockroaches" in Japan needs further clarification.

From the results of our present study, we observed that female cockroaches of Iwoto-A group, which produced a total of 478 female and no male offspring, despite having sperms in their spematheca, can be identified as *P. surinamensis*, while those of Iwoto-B group with all having sperms in their spermatheca, produced a total of 168 male and 157 female offspring in an average ratio of 10.8 to 10.5 (p>0.05) with no significant difference in the sexual ratio, can identified as *P. indicus*.

Similarly, female cockroaches of Tokunoshima-A group that produced a total of 221 female and no male offspring, despite having sperms in their spematheca, can be identified as *P. surinamensis*, while those of Tokunoshima-B group with all having sperms in their spermatheca produced a total of 242 male and 207 female offspring in an average ratio of 12.1 to10.4 (p>0.05) with no significant difference in the sexual ratio, can identified as *P. indicus*.

On the same note, female cockroaches of Okinawato-A group that produced a total of 724 female and no male offspring, despite having sperms in their spematheca, can be identified as *P. surinamensis*, while those of Okinawato-B group with all having sperms in their spermatheca produced a total of 322 male and 312 female offspring in an average sexual ratio of 16.1 to 15.6 (p>0.05) with no significant difference in the sexual ratio, can identified as *P. indicus*.

Thus, both species of *P. surinamensis* and *P. indicus* were found to be distributed on the three islands of Iwoto, Tokunishima and Okinawa, with their habitat overlapping with each other.

The group of five F1 female cockroaches from Hahajima, Chichijima, Nishijima and Nakodojima, produced a total of only 248, 59, 663 and 143 female offspring, respectively and no male offspring, despite having sperms in their spematheca. These cockroaches were identified as *P. surinamensis*. Thus, there is a possibility that only *P. surinamensis* and not *P. indicus* are distributed on these four islands.

The group of five F1 female cockroaches from Amamiooshima, Miyakojima, Ishigakijima and Hawaii, produced a total of 260 male (M) and 260 female (F) offspring with an average ratio of M:F at 14.4:14.4 (p>0.05) per litter, 230M, 267F, av. 16.4:19.1 (p>0.05) per litter, 281M, 266F, av. 16.5:15.6 (p>0.05) per litter and 199M, 189F, av. 11.7:11.1 (p>0.05) per litter, respectively. This probably indicates that only *P. indicus* and not *P. surinamensis* were inhabiting the four islands.

From the above results, we can conclude that there are areas in Japan where the distribution of *P. surinamensis* and *P. indicus* overlap with each other, and there are also areas in which either only one or the other could be found.

Roth (1967) stated that *P. surinamensis* could be morphologically distinguished from *P. indicus* based on the distance between the ocelli and compound eye, in which the former species show contact between the ocelli and the compound eye, while in the latter species, they are separated. However, in our experiments, the distance between the ocelli and compound eye of those identified as *P. surinamensis* based on the breeding experiment were found to be 0.10-0.16mm (average 0.13) and those as *P. indicus* were 0.12–0.21mm (average 0.15), respectively. There was no significant difference between the two species. Thus, this morphological criterion is not applicable for species identification. From our observation, in certain specimens, some translucent materials, probably secretions of some sort, could be found in the area between the compound eye and ocelli sometime after the death of the cockroaches. Since the ocelli were also translucent, the presence of these translucent materials might have led Roth to conclude that the ocelli were in contact with the compound eye. Moreover, we did not observe any specimen whose ocelli was in contact with the compound eye.

Generally, in insects, the body length and tegmina length had been used for species identification (Inoue *et al.*, 1963; Nakane *et al.*, 1963) but these criteria could not be used for the species identification among *Pycnoscelus* species because they are unreliable.

We have shown through our experiments and observation that it is very difficult to distinguish *P. surinamensis* from *P. indicus* morphologically, but can be done through cross-breeding experiments to obtain the isolating index, which is always more than +1. Moreover, the use of solitary breeding of individual female to observe for parthenogenicity can be a criterion for differentiating between *P. surinamensis* and *P. indicus*, which was seen in the former but not in the latter. Thus, we have clarified that the so-called "Ogasawara cockroaches" in Japan consist of *P. surinamensis* and *P. indicus*.

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