Studies on differentiation of species and distribution of surinam cockroaches inhabiting in Japan

日本に生息するオガサワラゴキブリの種の鑑別および分布に関する研究

小松謙之

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The so-called “Ogasawara cockroaches”, which hitherto has also been synonymously called “Surinam cockroaches”, are widely distributed throughout the world in the tropical and sub-tropical areas. In Japan, their distribution is limited to Kagoshima in Kyushu and the island chain of Nansei and Ogasawara island chain.

In recent years, this cockroach has invaded into urban buildings and abode, resulting in the report of their capture or eradication attempts. The problem of urban buildings invasion by this cockroach has also been seen in other countries. From an epidemiological perspective, Kim and Erko (2008) reportedly detected the potentially zoonotic helminth ascarid and taeniid eggs on the body surface of this species of cockroach in Ethiopia. Furthermore, they also reported the presence of trichurid eggs and *Entamoeba coli* cyst in the digestive tract of the cockroach. These findings has put the spotlight on the role of the cockroach as a potential mechanical transmitter zoonotic infectious diseases.

Regarding the constituent species of the so-called “Ogasawara cockroaches” or “Surinam cockroaches”, it seem that there were two apparently morphologically similar species, namely *Pycnoscelus indicus* and *Pycnoscelus surinamensis*, with the former showing bisexual reproduction and the latter, parthenogenesis. For the identification of these two species, Roth (1967) proposed that for the adults, 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.

The male of *P. indicus* are relatively more susceptible to dryness than the female, and easily died off under non-optimal environmental condition, leading to reduced chances of mating. However, since *P. surinamensis* is parthenogenetic and does not need male to reproduce, they can even proliferate under a harsh environmental condition if a single female is able to invade and survive under that condition.

Asahina (1991) reported that both male and female individuals could always be found among the “Ogasawara cockroaches”, without noting that the female were

parthenogenetic. This led him to suspect that the Japanese “Ogasawara cockroaches” might include *P. indicus.* However, he proposed that until the detailed breeding experiments had been carried out, the “Ogasawara cockroaches” should all be tentatively be identified as *P. surinamensis.*
Thus, despite that the Ogasawara cockroaches has been recognized as a pest, their species identification and distribution has not been clearly elucidated.

To clarify the species involved, first of all, we need to establish a criteria be able to identify the sex of the cockroach at the larval stage before they mature into set out to establish a method to differentiate the sex of male and female cockroach from the larval stage to the adult stage. Sexual differentiation of the cockroach nymph for all the instar stages of *Blatta orientalis*, *Supella longipalpa* and *Periplaneta fuliginosa* has been established, based on the morphological observation of the abdominal segments of the in star.

In our study, cockroaches were collected in Ishigaki island, Taketomi-cho, district, Okinawa prefecture, Japan. They were then reared and passaged for several generations in the laboratory. Only those groups that produced offspring, which matured into male and female adults were used in the following experiment. The instar that hatched from the eggs were immediately isolated and separated according to their morphoplogical characteristic of ventral segments. Changes on the ventral segment were also noted for each instar stages and ultimately, the sex of the mature adult for each group was determined. Using the aforementioned method, the female of the 1st to 6th stage instar nymph were found to possess a V-shaped notch at the middle of the posterior edge of the 9th sternite. This notch was not seen in the male nymph. In the female 7th stage (final stage) instar nymph, the styli were not apparent and, the 8th and 9th sternites became degenerated and were covered over by the profoundly developed 7th sternite. In contrast, all stages of the male nymph until the 7th stage nymph showed the presence of the 8th and 9th sternitesas well as styli. Based on these observations, our study has demonstrated that it is possible to differentiate the sex of the Indian cockroach, *P. indicus,* at different developmental stages.

Next, to identify the various specimens as to which stage the nymph instar belong to, we counted the number of cercal segments from the dorsal and ventral view. It was observed that the number of cercal segments from the dorsal view in 2nd and 3rd stage nymph were the same, that is 4 and thus could not be used to identify the nymph stage. However, when viewed ventrally, it was observed that the number of cercal segments on the1st stage nymph was 3; 2nd stage, 4; and in the

subsequent stages, an increase of one extra segment for each stage. The number of cercal segments of all the stages of the female, right up to the 7th stage nymph, when viewed ventrally were the same as that of the male nymph. Therefore, the developmental stage of the nymph could be identified by examining the number of segments from the ventral view.

Based on the above results, we set out to confirm species and distribution of Surinam cockroaches inhabiting Japan. We collected cockroaches from Ogasawara island chain (Iwoto, Hahajima, Chichijima, Nishijima, Nakodojima), Amami island chain (Tokunoshioma, Amamiooshima), Okinawa island chain (Okinawato, Miyakojima, Ishigakijima) and Hawaii, kept the female in solitary rearing for use in later mating experiments. Cross breeding experiments that were carried out showed that there were those cockroaches from Iwoto, Tokunoshima, and Okinawato that produced only female offspring, and also that produced both male and female offspring. The former group was designated A group and the latter, B group. Both groups comprises of 14 isolates from 11 areas. The cockroaches from both groups were then used for subsequent cross breeding experiments.

For the cross breeding experiments, those specimens that produced only female offspring were mated with those male from Hawaii that had produced both male and female offspring and had been identified a *P. indicus* as previously reported by Roth (1967). From the results of our study, the area that contain the two different characteristic types of the cockroaches were Iwoto, Tokunoshima and Okinawato, whereas an individuals 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.* On the contrary, 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*. 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 thus be 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 be 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.

Futhermore, all the various specimen from our study were examined for morphologic differences between the 2 species. 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, we could not find any female adult cockroach of *P. surinamensis,* whose ocelli were in contact with the compound eye, that is, 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 > Nakodojima, 0.13 mm > Nishijima, Tokunoshima-A & Okinawato-A, 0.12 mm > Iwoto-A, 0.10 mm, respectively, with an average distance of 0.13 mm. For the groups that produce both male and female offspring, identified as *P. indicus,* 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, with an average distance of

0.15 mm.

There was no significant difference in the distance between the ocelli and compound eye between the two species. Thus, this morphological criterion is not applicable for species identification.

The length of tegmina has been used as a criterion for species identification in many insects. Thus, we proceed to measure the length of the tegmina of the adult female cockroaches in our study. For the groups that do not produce any male offspring, identified as *P. surinamensis,* 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, identified as *P. indicus,* 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. It was observed that there was not much difference in the tegmina length among the specimens from different localities and also between the two species, thereby excluding the used of this criterion for species identification.

Moreover, to differentiate the species without using the cross breeding experiment, we tried the method of solitary rearing of the unmated female adult cockroaches obtained from our previous experiments. Our results showed that all individuals identified as *P. surinamensis* produced offspring, and based on our previous sexual differentiation method of the nymph, all the offspring nymph were found to be female. On the contrary, individuals identified as *P. indicus* did not produce any offspring. From this observation, we can identify the species of the adult female cockroach collected from the wild by solitary rearing and examining the sex of the offspring produced. Thus, those female adult that produce only female offspring can be identified as *P. surinamensis* and that that produce both male and female offspring or those that did not produce any offspring can be identified as *P. indicus*.

Based on the results of the above studies, the so-called “Ogasawara cockroaches” which has until now been thought to consist of only *P. surinamensis,* actually also comprises of *P. indicus,* which are also distributed in the same area. From our study, we have also added one more species of cockroach that inhabit Japan, that is to 58 species. Until recently, *P. indicus* and *P. surinamensis* were thought to be distributed in different areas but our study shows that there are areas in which both species co-

habitat together and there are also areas in which either only one of the two species can be found. This finding has deep implication for future studies. In addition, we also found that the criterion of using the distance between the ocelli and the compound eye for species identification, as proposed by Roth (1967), is applicable nor reliable. We propose an alternative method in the form of solitary rearing of wild female adult and determining the sex of the offspring for species identification. Those that produce only female offspring be identified as *P. surinamensis,* while those that produce both male and female offspring, as well as that that failed to produce, should be regarded as *P. indicus*. Since the report by Asahina (1991) that species identification of "Ogasawara cockroaches", needs further clarification, the results of our present study has provided the answer to his question through our morphological observation, cross breeding and solitary rearing experiments of those cockroaches.

日本に生息するオガサワラゴキブリの種の鑑別および分布に関する研究

オガサワラゴキブリ（Common name：Surinam cockroach）は、世界の熱帯、亜熱帯に広く分布する害虫であり、国内では鹿児島から南西諸島、小笠原諸島などに分布している。近年、このゴキブリが、我が国の都市部の建築物内にも侵入し、捕獲や駆除が報告されはじめている。また海外においても、同様に都市部の家屋内への侵入が問題となっている。疫学的には、エチアピアにおいて、Kinfu and Erko (2008) は、このゴキブリの体表から、ヒトに感染性をもつ蠕虫類の回虫卵やテニア科条虫卵を検出し、さらに腸管からは、これらに加え、鞭虫卵や原虫類である大腸アメーバのシストの検出を報告しており、感染症を媒介する衛生害虫としても注目されている。

オガサワラゴキブリに関しては、形態的に良く似た 2 種が知られており、両性生殖を行う *Pycnoscelus indicus* と、単為生殖を行う *Pycnoscelus surinamensis* が存在する。*P. indicus* と *P. surinamensis* の種の分類方法については、Roth (1967) が成虫を用いた交配実験を行い、受精嚢内に精子があるにもかかわらず、雌のみを産出した個体を *P. surinamensis*、雌雄を産出した個体を *P. indicus* としている。また、形態的な違いとして *P. indicus* は複眼と単眼点の距離が離れているのに対して、*P. surinamensis* は両眼が接していることで区別できるとしている。

P. indicus の雄は雌に比べて乾燥に弱く、生育環境が適切でないと雄が死んでしまうため交尾ができず、繁殖することはできない。ところが、*P. surinamensis* は繁殖に雄は必要ないため、雌が 1 匹でも生息すれば多少環境が悪くても侵入した場所で繁殖を繰り返すことが可能である。

朝比奈 (1991) は、日本産の個体は、雌雄が常に同時に採集されており、雌だけの単為生殖の系統は観察されないことから、日本に生息する個体は *P. indicus* である可能性を示唆した。しかしながら、日本産のオガサワラコギブリを用いた詳細な実験による証明が行なわれるまでは、従来の *P. surinamensis* とすることを提唱し、今日に至っている。このように、オガサワラゴキブリは衛生害虫として重要なゴキブリでありながら、種の鑑別と分布がまったく不明なのが現状である。

そこで本研究では、成虫にまで発育する前に雌雄の区別ができるように、まず幼虫期における雌雄の鑑別方法の確立を行った。ゴキブリ類の幼虫期における雌雄の鑑別については、トウヨウゴキブリ *Blatta orientalis*、チャオビゴキブリ *Supella longipalpa*、クロゴキブリ *Periplaneta fuliginosa* で確立されており、幼虫期の腹板の形態により鑑別可能であることが報告されている。そこで、沖縄県八重山郡竹富町石垣島で採集し、予備実験により産仔幼虫が雌雄の成虫に発育することを確認した個体群を使用して実験を行い、孵化直後の幼虫

を腹板の形態ごとにグループに分け、成長段階における腹板の形状を記録し、最終的に各グループの個体が雌雄のどちらになるかを調べた。その結果、雌では 1～6 齢期の幼虫において第 9 腹板の後縁中央部に雄では見られない notch (V 字型) を有し、7 齢 (終齢) 期では、第 7 腹板が発達して第 8～9 腹板および尾突起を覆い隠した。これに対して雄では 7 齢期まで第 8～9 腹板、尾突起がみられた。したがって、この特徴を観察することによって幼虫期の雌雄鑑別が可能であることが判明した。

次に幼虫期の齢期を判定するため、尾肢の腹面および背面の環節数を計測した。その結果、背面の環節数は 2、3 齢で同数となり判定できないが、腹面の環節数は 1 齢幼虫で 3 節、2 齢幼虫で 4 節と加齢するごとに 1 節ずつ増加することが分かり、この部位の環節数を調べることにより幼虫の齢期の判定が可能となった。

これらの結果をもとに、日本に生息するオガサワラゴキブリの種と分布を明らかにするため、小笠原諸島 (硫黄島・母島・父島・西島・媒島)、奄美諸島 (徳之島・奄美大島)、沖縄諸島 (沖縄島・宮古島・石垣島)、ハワイ島から採集した雌成虫を単独で飼育し、実験に用いる個体の繁殖を行った。その結果、硫黄島・徳之島・沖縄島は、雌のみを産出する個体と、雌雄を産出する個体が見られたため、前者を A グループ、後者を B グループとして 11 地域 14 系統の個体群を使用して交配実験を行った。

交配実験は、雌のみを産出する個体群には、Roth (1967) と同様に *P. indicus* と判明しているハワイ産の雄を使用し、雌雄産出する個体群は、その個体群内の雄を使用した。その結果、1 地域で 2 系統見られた硫黄島、徳之島、沖縄島では、硫黄島 A グループの個体での産出数は、雄 0 個体、雌 478 個体で、産出後のすべての各個体における受精嚢内に精子を保有していたことより *P. surinamensis* であった。硫黄島 B グループは、雄 162 個体、雌 157 個体を産出し、1 回の平均産仔数の雄雌比は、10.8 : 10.5 ($p>0.05$) で、性比に有意差は認められず *P. indicus* であった。徳之島 A グループは、雄 0 個体、雌 221 個体を産出し、すべて受精嚢内に精子を保有していたことより *P. surinamensis* であった。徳之島 B グループは、雄 242、雌 207 を産出し、1 回の平均産仔数の雄雌比は 12.1 : 10.4 ($p>0.05$) で、性比の有意差は認められず *P. indicus* であった。沖縄島 A グループは、雄 0 個体、雌 724 個体を産出し、すべての受精嚢に精子が保有されていたことより *P. surinamensi* であった。沖縄島 B グループは、雄 322、雌 312 を産出し、1 回の平均産仔数の雄雌比は 16.1 : 15.6 ($p>0.05$)で、性比の有意差は認められず *P. indicus* であった。以上の結果より、硫黄島、徳之島、沖縄島は、*P. surinamensis* と *P. indicus* の 2 種が同時に生息していることが初めて明らかとなった。

雌のみが産出された母島・父島・西島・媒島の個体のうち、母島の受精嚢に精子が確認された個体での産出数は、雌 248 個体、父島の精子が確認された個体での産出数は、雌 59 個体、西島の精子が確認された個体の産仔数は、雌 663 個体、媒島の精子が確認された個体での産出数は、雌 143 個体であったことから、以上 4 島の個体はすべて *P. surinamensis* であることが明らかとなった。

雌雄産出した個体のみであった奄美大島・宮古島・石垣島では、奄美大島の個体は、雄 260、雌 260 で、1 回の平均産仔数が 14.4 : 14.4 (p＞0.05) であった。宮古島の個体は、雄 230、雌 267 で、1 回の平均産仔数が 16.4 : 19.1 (p＞0.05) であった。石垣島の個体は、雄 281、雌 266 で、1 回の平均産仔数が 16.5 : 15.6 (p＞0.05) であった。ハワイ島の個体は、雄 199、雌 189 で、1 回の平均産仔数が 11.7 : 11.1 (p＞0.05) であり、以上 4 島の個体はすべて *P. indicus* であることが明らかとなった。

以上の結果より、日本には *P. indicus* と *P. surinamensis* の 2 種類が生息しており、これらが混生している地域、および *P. indicus* のみ、あるいは *P. surinamensis* のみが生息する地域があることが明らかとなった。

次に、実験により種が判明した個体を使い、この 2 種類の形態的な違いを調べた。Roth (1967) は、複眼と単眼点が接していれば *P. surinamensis*、離れていれば *P. indicus* であるとしたが、本実験では *P. surinamensis* の雌成虫の複眼と単眼点は接しておらず、その距離は、母島 0.16 ㎜＞父島 0.14 ㎜＞媒島 0.13 ㎜＞西島・徳之島・沖縄島 0.12 ㎜＞硫黄島 0.10 ㎜となり、平均 0.13 ㎜であった。一方、*P. indicus* 雌成虫の複眼と単眼点の距離は、硫黄島 0.18 ㎜＞宮古島 0.16 ㎜＞奄美大島・沖縄島 0.13 ㎜＞徳之島・石垣島 0.12 ㎜で、平均 0.15 ㎜となり、どちらの種も接しておらず、両種の複眼と単眼点の距離による鑑別は不可能であった。

また、昆虫類の種の違いとして前翅長の違いが広く利用されているため、雌成虫の平均前翅長を計測した。その結果、*P. surinamensis* は、沖縄島 15.82 ㎜＞母島 15.26 ㎜＞西島 15.07 ㎜＞媒島 14.16 ㎜＞父島 13.81 ㎜＞徳之島 13.57 ㎜＞硫黄島 12.87 ㎜、*P. indicus* 雌成虫の平均前翅長は、沖縄 14.72 ㎜＞硫黄島 14.35 ㎜＞石垣島 13.81 ㎜＞徳之島 13.54 ㎜＞奄美大島 13.53 ㎜＞宮古島 12.96 ㎜と、地域的な差異が大きく、両種を鑑別することはできなかった。

一方、交配実験を行わなくても種を鑑別できる方法を検討するため、本実験で得られた各雌成虫の未交尾個体による飼育実験を行った。その結果、*P. surinamensis* はすべての個体が幼虫を産出し、前述した幼虫期の雌雄鑑別法により、すべてが雌であることがわかった。一方、*P. indicus* はすべての個体で幼虫は産出しなかった。このことより、野外で採集した雌成虫の同定法として、産仔幼虫がすべて雌であった場合は *P. surinamensis*、また雌雄を産出、あるいはまったく産出しない場合は *P. indicus* と同定できることが判明した。

以上、本研究により、これまで日本に生息するオガサワラゴキブリは *P. surinamensis* のみであると考えられていたが、*P. indicus* も同時に生息していることが明らかとなり、これらの結果から、日本に生息するゴキブリは 1 種増えて 58 種となった。さらに、現在まで *P. indicus* と *P. surinamensis* は生息地域が違うと考えられてきたが、2 種類が同一地域に混生している事実が明らかになり、今後の研究の方向性を再検討する必要がある。また、Roth (1967) が提唱している複眼と単眼点の距離による形態をもとにした鑑別方法は利用できないことがわかった。これに替わる新たな鑑別方法として、交配実験を行わなくとも未交尾の

雌個体であれば、そのまま飼育して産仔すれば *P. surinamensis*、産仔しなければ *P. indicus* と判断でき、野外採集個体であれば、産出された幼虫が雌のみであれば *P. surinamensis*、雌雄産出すれば *P. indicus* と判断できることがわかった。朝比奈（1991）の報告では、我が国におけるオガサワラゴキブリの種に関する知見が明確ではなかったが、本研究における種々の交配実験や形態的な観察により、その詳細が明らかとなった。

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Sexual differentiation and developmental stage identification of the Indian Cockroach, *Pycnoscelus indicus* (Blattodea: Blaberidae)

Noriyuki Komatsu[1,2], Hong-Kean Ooi[3*] and Akihiko Uchida[4]
[1]Civil International Corporation, 1-10-14 Kitaueno, Taito, Tokyo 110-0014 Japan
[2]Laboratory of Parasitology, School of Life and Environmental Science, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 252-5201 Japan
[3]Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 252-5201 Japan
[4]Yamazaki Gakuen University, 4-7-2 Minami-osawa, Hachioji, Tokyo 192-0364 Japan
*Corresponding author e-mail: hkooi@azabu-u.ac.jp
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Abstract. We found that sexual differentiation of all the nymphal stages of *Pycnoscelus indicus* (Fabricius) was possible by observing the developmental features of their posterior abdominal segments. Using this observation, the sex of even the 1st stage instar nymph could be determined. The female of the 1st to 6th stage instar nymph possess a V-shaped notch at the middle of the posterior edge of the 9th sternite. This notch is not seen in the male nymph. In the female 7th stage (final stage) instar nymph, the styli were not apparent and, the 8th and 9th sternites became degenerated and were covered over by the profoundly developed 7th sternite. In contrast, all stages of the male nymph showed the presence of styli. Thus, it is possible to differentiate the sex of all the stages, from 1st to 7th, of the nymph of *P. indicus* taxonomically. Moreover, it is also possible to identify the various specimens as to which stage the nymphal instar belong to, by counting the number of cercal segments from the ventral view.

INTRODUCTION

It is known that age differentiation of cockroach nymphs has been conducted based on the length and breadth of the head, thorax and abdomen, the number of segments on the antenna as well as on the number of sensory organs (Funaki, 1958; Wigglesworth, 1964; Sugimoto, 1967; Makiya, 1969; Mackay, 1978; Takeda, 1993; Gullan, 2000).

Sexual differentiation of the cockroach nymph for all the instar stages of *Blatta orientalis* Linnaeus (Qadri, 1938), *Supella longipalpa* (Fabricius) (Hafez & Afifi, 1956) and *Periplaneta fuliginosa* (Serville) (Saito & Hayashi, 1973) has been reported. Furthermore, sexual differentiation of *Blattella germanica* (Linne), *Periplaneta americana* (Linne) and *Periplaneta japonica* Karny were also reported to be possible, despite the observation of only the 1st stage instar nymph.

For the identification of the various developmental stages of *B. germanica*, it was reported that it can be done by counting the number of segments in the antennae, which were observed to increase correspondingly with age (Ishii, 1971). However, the antennae of the nymph are usually broken and thus this organ is not reliable for age determination. Since the number of cercal segments were observed to be different among the 1st to 5th stage instar male nymph, this can be used to identify their developmental stages. However, this criterion cannot be used to determine the age of the 5th and 6th stage female nymph because of overlapping number of segments in those age groups (Hasegawa, 1977; Saito, 1986). Thus, for determining the developmental stage of

the cockroach nymph, a combination of taxonomical features such as the number of segments on the antennae as well as on the cerca, coupled with the characteristic morphology of the sternite is required.

We report herein the criteria for the sexual differentiation of all the different nymphal stages of the Indian cockroach or Burrowing cockroach, *Pycnoscelus indicus*, based on our laboratory-reared specimens.

MATERIALS AND METHODS

Pycnoscelus indicus was originally collected in Ishigaki island, Taketomi-cho, Yaeyama district, Okinawa prefecture, Japan. The cockroaches were reared in the laboratory and had been passaged for more than 10 generations. The cockroaches used in our experiments were reared in a plastic container of diameter 90mm and a height of 50 mm. The beddings were made up of 10mm 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 10mm 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 hatched nymphs were immediately segregated into those that have a V-shaped notch at the ventral abdominal caudal region and those that do not possess such structure. They were then reared separately in two groups. Immediately after hatching and every 4 days, the nymphs were anaesthesized with carbon dioxide gas, placed in a small transparent plastic bag and their ventral abdominal caudal region examined under a dissection microscope.

RESULTS

Morphology of the ventral abdominal caudal region of the male and female nymphs at different stages

1st stage nymph: Female has a notch at the 9th sternite posterior edge (A type; Fig. 2-A). No notch was seen in male (A type; Fig. 1-A). Besides the aforementioned features, no other differences were noted between the male and the female.

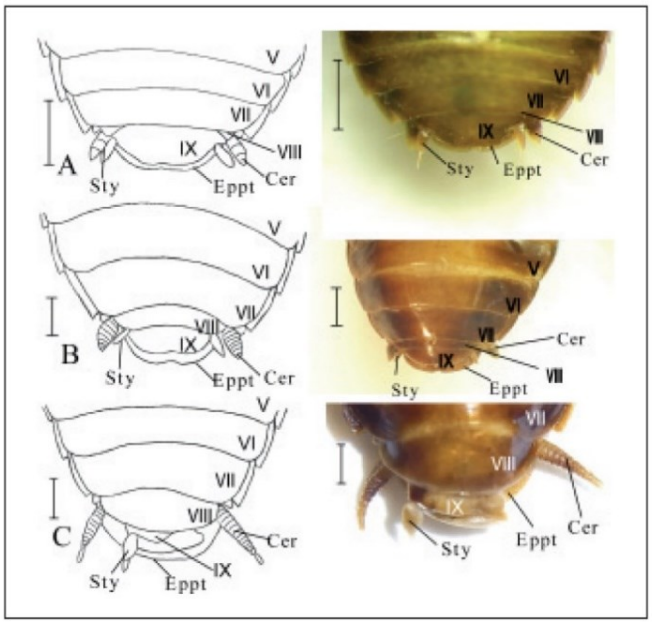

Figure 1. Development of the terminal abdominal sternite of male nymphs of *Pycnoscelus indicus* (Ventral view).
A: First instar nymph. B: Penultimate instar nymphs. C: Adult.
Cer: Cercus. Sty: Stylus. Eppt: Epiproct.
Scale bars. 0.5 mm for A. 1.0 mm for B-C.

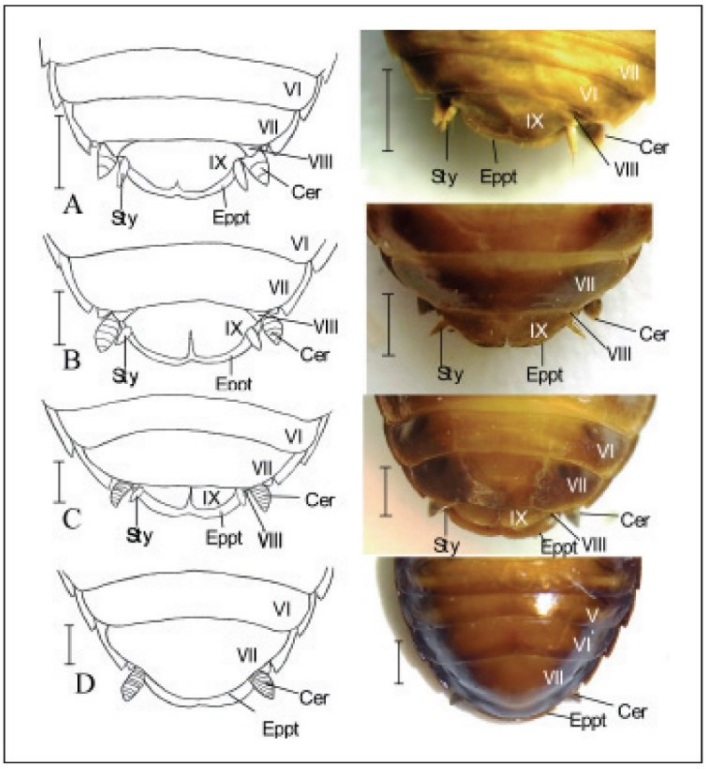

Figure 2. Development of the terminal abdominal sternite of female nymphs of *Pycnoscelus indicus* (Ventral view).
A: First instar nymph. B: Early age instar nymphs. C: Middle instar nymphs. D: 7th instar nymphs and adult. Cer: Cercus. Sty: Stylus. Eppt: Epiproct.
Scale bars. 0.5 mm for A; 1.0 mm for B-D.

2nd stage nymph: Just like in the 1st stage nymph, female has a notch at the 9th sternite posterior edge (A type; Fig. 2-A). No notch was seen in male (A type; Fig. 1-A).

3rd stage nymph: The notch in the female has become deeper and more conspicuous (B type; Fig. 2-B). Male was morphologically similar to 1st and 2nd stage (A type; Fig. 1-A).

4th stage nymph: The female nymph morphology was essentially the same as the 3rd stage (B type; Fig. 2-B). The 8th sternite of the male nymph was completely visible (B type; Fig. 1-B).

5th stage nymph: The notch in the female nymph, which could be seen on only the 9th sternite during the 4th stage, has become deeper and more conspicuous, reaching into the 8th sternite, as though splitting the sternal plate into two halves. (B, C type; Fig. 2-B, C). The male nymph did not show any changes with that of the 4th stage (B type; Fig. 1-B).

6th stage nymph: Both the female and the male nymph did not show any changes with that of the 5th stage (Female: B, C type; Fig. 2-B, C; Male: B type; Fig. 1-B).

7th stage nymph: The 8th and 9th sternite were being covered over by the 7th sternite and were no longer visible, and the styli had also disappeared in the female nymph (D type; Fig. 2-B). The male nymph show almost the same morphology as the 6th stage (B type; Fig. 1-B).

Adult: The 8th and 9th sternite, as well as the styli were no longer visible (D type; Fig. 2-B). The pair of styli, which was still apparent in the 7th stage male nymph, changed to become only one, with only the left stylus remaining on the male adult cockroach (C type; Fig. 1-C).

Cercal segments in all the stages of both female and male nymphs, and adults

The number of cercal segments from the dorsal view in male nymph are as follows: 1st stage, 3; 2nd & 3rd stage, 4; 4th stage, 5; 5th stage, 6; 6th stage, 7; and 7th stage (final stage)

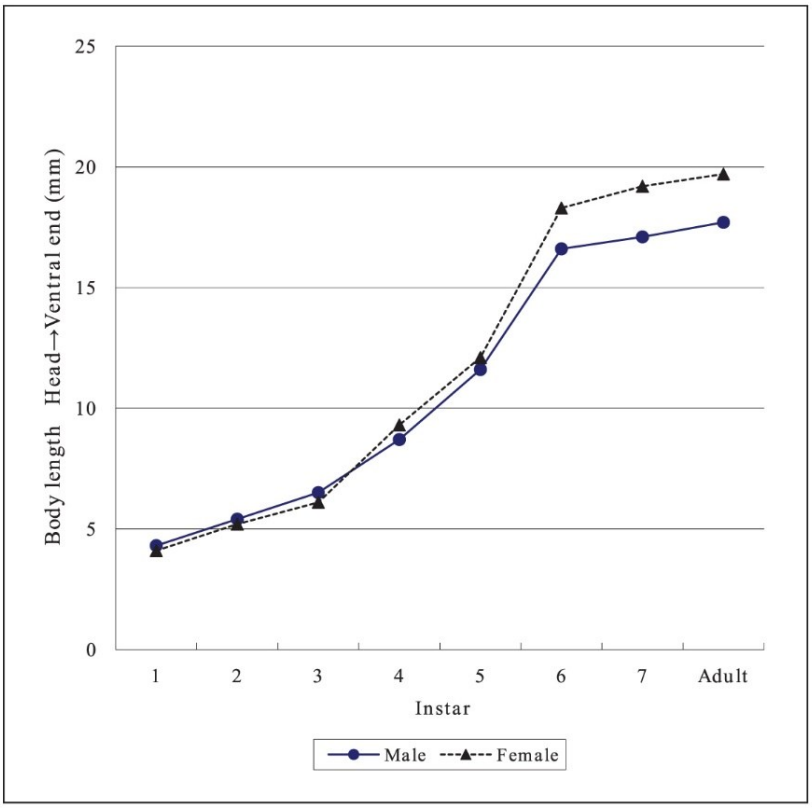

Figure 3. Body length in relation to each age term and sex of *Pycnoscelus indicus*.

8. The adult male has 11 segments on its cerca when viewed dorsally. There was no change in the number of cercal segments from 2nd to 3rd stage male nymph, remaining at 4.

On the contrary, the number of cercal segments from the ventral view in male nymph are as follows: 1st stage, 3; 2nd stage, 4; 3rd stage, 5; 4th stage, 6; 5th stage, 7; 6th stage, 8; and 7th stage (final stage) 9. The adult male has 12 segments on its cerca when viewed ventrally. Thus, there is a variation in the number of cercal segments among the different developmental stages (Table 1).

The number of cercal segments from the dorsal view for all the stages of the female, right up to the 7th stage nymph, were the same as that of the male nymph. The adult female has 9 segments on its cerca when viewed dorsally. Furthermore, the number of cercal segments of all the stages of the female, right up to the 7th stage nymph, when viewed ventrally were the same as that of the male nymph. The adult female has 10 cercal segments when viewed ventrally (Table 2).

Body length and type of sternites in all stages of both female and male nymphs, and adults

The sternite of male nymph of 1st to 3rd stages showed A type, 4th and 5th stage showing A, B type, 6th and 7th stage showing B type, while that of the adult showed the C type.

The sternite of female nymph of 1st and 2nd stages showed A type, 3rd and 4th stages showing B type, 5th stage showing B, C type, 6th stage showing C, D type, 7th stage showing D type, while that of the female adult also showed the D type (Table 3).

DISCUSSION

In recent years, the Indian cockroaches has been reported to invade into the dwellings of humans (Harada & Tsuji, 1995; Tawatsin *et al.*, 2001; Sriwichai *et al.*, 2002; Harunari *et al.*, 2009; Yamauchi & Kato, 2009; Komatsu *et al.*, 2013). From a public health view of point, this warrants a further detailed study of their developmental stages and morphology.

Table 1. The number of cercal segments in male *Pycnoscelus indicus*

Instar		No. of insects	Number of cercal segments											
			1	2	3	4	5	6	7	8	9	10	11	12
Dorsal view	1	15			15									
	2	15				15								
	3	15				15								
	4	15					15							
	5	15						15						
	6	15							15					
	7	15								15				
	Adult	15											15	
Ventral view	1	15			15									
	2	15				15								
	3	15					15							
	4	15						15						
	5	15							15					
	6	15								15				
	7	15									15			
	Adult	15												15

Definition of instar age.
First instar : 3.5-5.5 mm (4.3±0.623), Second instar: 4.5-6.5 mm (5.4±0.767), Third instar: 5.5-7.5 mm (6.5±0.598), Fourth instar: 7.5-13.0 mm (8.7±1.613), Fifth instar: 7.0-16.5 mm (11.6±2.941), Sixth instar: 15.0-19.0 mm (16.6±1.271), Seventh instar: 16.0-18.0 mm (17.1±0.617), Adult: 16.5-19.0 mm (17.5±0.862).

Table 2. The number of cercal segments in female *Pycnoscelus indicus*

Instar		No, of insects	Number of cercal segments											
			1	2	3	4	5	6	7	8	9	10	11	12
Dorsal view	1	15			15									
	2	15				15								
	3	15				15								
	4	15					15							
	5	15						15						
	6	15							14	1				
	7	15								15				
	Adult	15									13	2		
Ventral view	1	15			15									
	2	15				15								
	3	15					15							
	4	15						15						
	5	15							15					
	6	15								15				
	7	15									15			
	Adult	15										13	2	

Definition of instar age.
First instar : 3.5-4.5 mm (4.1±0.530), Second instar: 4.5-6.0 mm (5.2±0.649), Third instar: 5.5-7.0 mm (6.1±0.471), Fourth instar: 7.5-15.0 mm (9.3±1.971), Fifth instar: 9.0-16.0 mm (12.1±2.271), Sixth instar: 16.5-20.5 mm (18.3±1.318), Seventh instar: 17.0-22.0 mm (19.2±1.646), Adult: 17.5-21.5 mm (19.7±0.902).

Table 3. The relation of the instar age, body length and sex to the type of sternite forms in the growth of *Pycnoscelus indicus*

Instar	Male				Female			
	Type of sternites*	No. of insects	Length (mm)	Average± S.D. (mm)	Type of sternites**	No. of insects	Langth (mm)	Average± S.D. (mm)
1	A	15	3.5-5.5	4.3±0.623	A	15	3.5-4.5	4.1±0.530
2	A	15	4.5-6.5	5.4±0.767	A	15	4.5-6.0	5.2±0.649
3	A	15	5.5-7.5	6.5±0.598	B	15	5.5-7.0	6.1±0.471
4	A-B	15	7.5-13.0	8.7±1.613	B	15	7.5-15.0	9.3±1.971
5	A-B	15	7.0-16.5	11.6±2.941	B-C	15	9.0-16.0	12.1±2.271
6	B	15	15.0-19.0	16.6±1.271	C-D	15	16.5-20.5	18.3±1.318
7	B	15	16.0-18.0	17.1±0.617	D	15	17.0-22.0	19.2±1.646
Adult	C	15	16.5-19.0	17.7±0.862	D	15	17.5-21.5	19.7±0.902

*: With reference to Fig. 1.
**: With reference to Fig. 2.

In our study on *P. indicus*, it was observed that among the 1st to 6th stage instar nymph, only the female has a notch at the central posterior edge of the 9th sternite. This notch is not seen in the male nymph. In the female after the 7th stage instar nymph, the 8th and 9th sternites had degenerated but these sternites were still conspicuous in the male. Thus, male and female adult sexual differentiation could be performed based on the number of sternite. This observation conform to that of *B. orientalis*, *S. longipalpa* and *P. fuliginosa* (Qadri, 1938; Hafez & Afifi, 1956; Saito & Hayashi, 1973)

In the 1st to last stage male nymph of *P. fuliginosa*, the 7th, 8th and 9th sternite were wholly conspicuous, but in the 1st to 3rd stage male nymph of *P. indicus*, the 8th sternite was covered over by the 7th sternite, with only a small portion of its tip could be seen.

On the contrary, in the 1st stage female nymph of *P. fuliginosa*, the 7th, 8th and 9th sternite were wholly conspicuous, but in the 1st stage female nymph of *P. indicus*, the 8th sternite was covered over by the 7th sternite, with only a small portion of its tip could be seen. In the mid-stage till the last stage female nymph of *P. fuliginosa*, the 9th sternite posterior periphery was covered over by the 7th sternite, and could not be observed from the outside. Similarly, the 8th sternite was also covered over by the 7th sternite and thus could not be seen.

In the mid stage (3rd and 4th stage) female nymph of *P. indicus*, no difference was noted between that of the 1st stage nymph. However, in the 6th stage female nymph, the 9th sternite central posterior edge notch became narrower and deeper, reaching to near the base of the 7th sternite, as though cutting through 8th sternite into the left and right halves. Furthermore, in the 7th stage female nymph, the 8th and 9th sternites were covered over by the 7th sternite, resulting in the hinderance of the observation (Saito & Hayashi. 1973). Moreover, Asahina (1991) reported that male adult of *P. indicus*, can be differentiated from the female by identifying the former as having 7 abdominal segments and the latter with 9 segments. Thus, our results supported and provided the reason for that report.

It has been reported that there were 3 cercal segments, as viewed from the dorsal and ventral aspects, in the 1st stage male and female nymph of both species of *B. germanica* and *P. indicus* (Hasegawa, 1977; Saito 1986). However, in the 2nd stage nymph of *B. germanica*, there were 6 cercal segments, while in that of *P. indicus*, there were only 4.

In the 3rd stage nymph of *B. germanica*, 7 cercal segments could be seen both dorsally and ventrally, while in that of *P. indicus*, 4 cercal segments could be seen dorsally but 5 could from the ventral view.

From the 4th to the final stage nymph of both species of *B. germanica* and *P. indicus*, the cercal segment increased by one for each of the stages (Hasegawa, 1977; Saito 1986). For *B. germanica*, since the dorsal view of the cercal segment number was found to be constant, it could be used for determining the stage of the nymph. However, this criterion on the cercal segment cannot be applied for the ventral view because of the greater variation in the number of the cercal segments (Hasegawa, 1977; Saito 1986). Nevertheless, the number of cercal segments from the nymph to the adult in *P. indicus*, were constant and can be used for determining the developmental stages. This criterion cannot be used for the female nymph of *B. germanica* because the number of cercal segments of the 5th and 6th stage nymph overlap with each other.

No difference in the body length of 1st to 5th stage male and female nymphs of *P. indicus*, were observed (t test; $p>0.05$. However, from 6th stage nymph onwards, the female nymph became larger than the male nymph ($p<0.05$). This indicates that this parameter can be considered for use in sexual differentiation from the 6th stage nymph onwards. Such observation has not been reported for *P. fuliginosa* and *B. germanica*. Thus, our study has demonstrated that it is possible to differentiate the sex of the Indian cockroach, *P. indicus*, at different developmental stages.

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Species clarification of Ogasawara cockroaches which inhabit Japan

Komatsu, N.[1,2], Kawakami, Y.[2], Banzai, A.[2], Ooi, H.K.[3*] and Uchida, A.[4]
[1]Civil International Corporation, 1-10-14 Kitaueno, Taito, Tokyo 110-0014 Japan
[2]Laboratory of Parasitology, School of Life and Environmental Science, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 252-5201 Japan
[3]Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 252-5201 Japan
[4]Yamazaki Gakuen University, 4-7-2 Minami-osawa, Hachioji, Tokyo 192-0364 Japan
[*]Corresponding author email: hkooi@azabu-u.ac.jp
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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

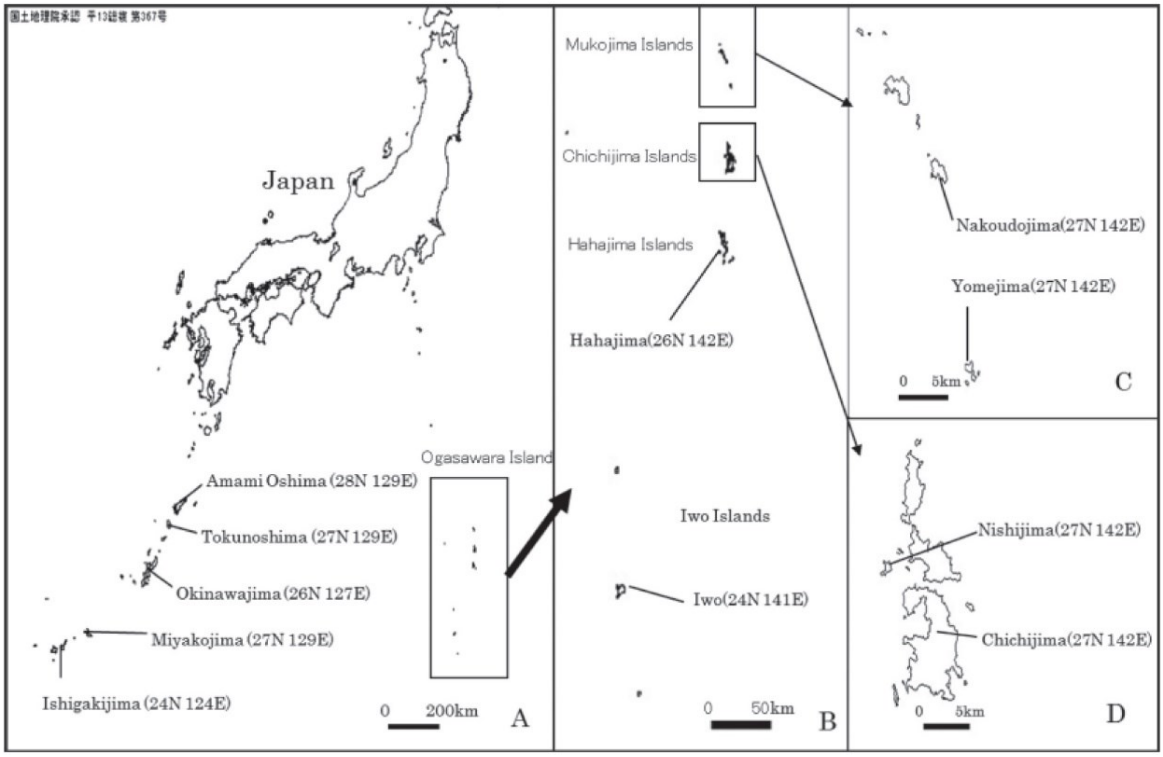

Figure 1 A: Map showing the location of the Nansei Islands and Ogasawara chain islands which is 1,000 km south of Tokyo. B: Map showing the 4 islands which are inhabited by humans, namely, Mukojima Island, Chichijima Island, Hahajima Island and Iwo Island. C: Map showing the location of the Nakoudojima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

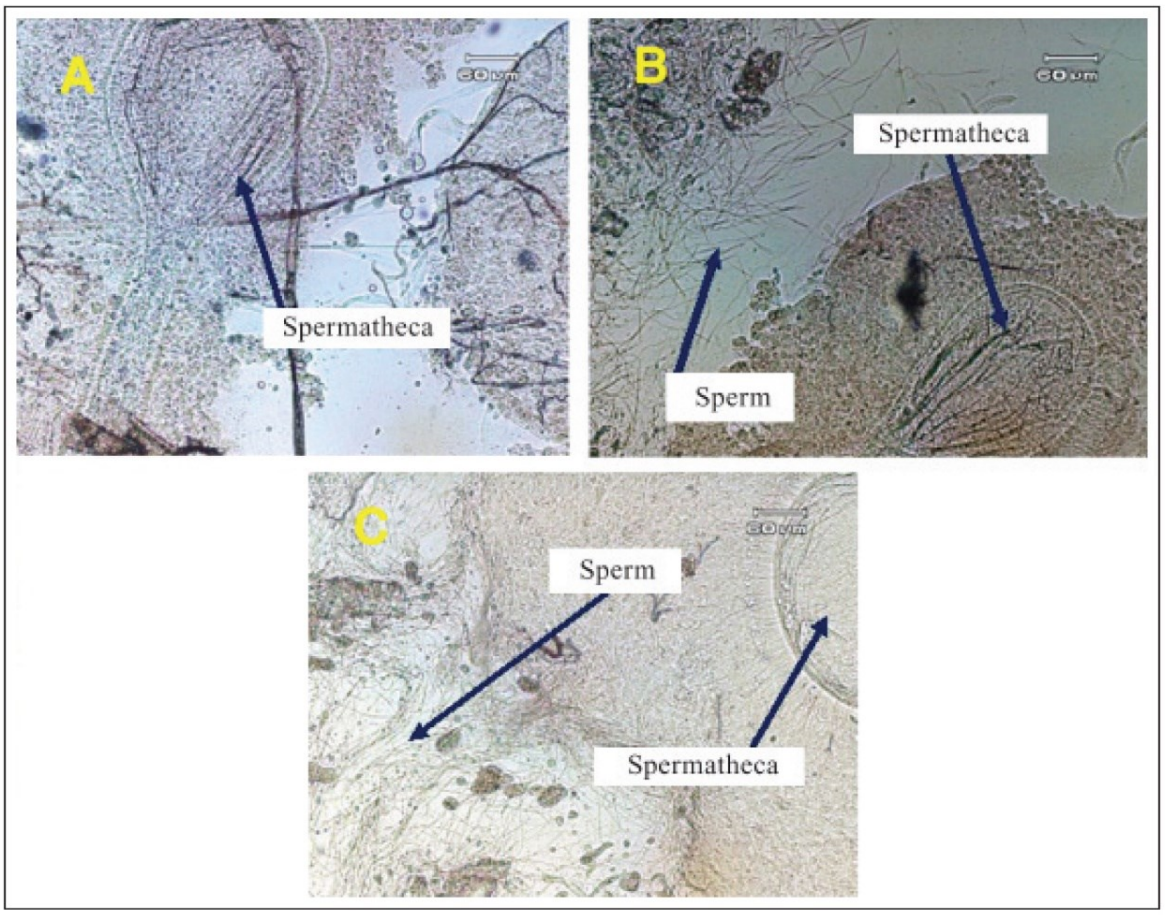

Figure 2. Female spermatheca of the *Pycnoscelus* spp. A: Parthenogenetic of Okinawato-A, B: Parthenogenetic of Iwoto-A, C: Bisexual of Okinawato-B, Spt: Spermatheca.

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).

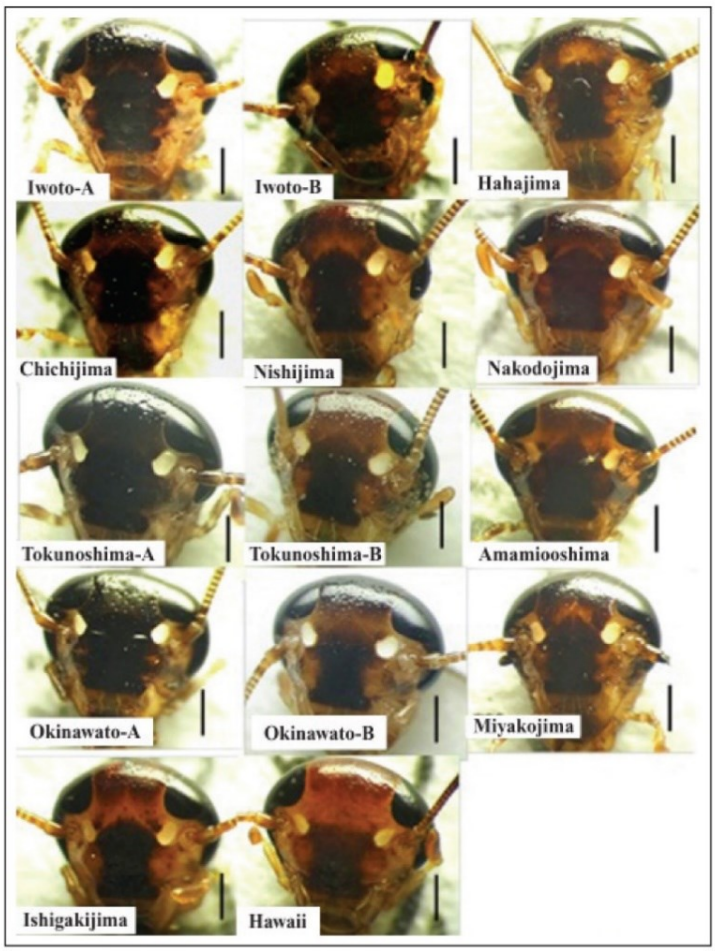

Figure 3. Enface view of *Pycnoscelus* spp. from various localities (Immediately after being sacrificed). Scale bar = 1 mm for all.

> 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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