

## Genetic and morphological differentiation in the Sakura shrimp (*Sergia lucens*) between Japanese and Taiwanese populations

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### Abstract

The Sakura shrimp *Sergia lucens* is a remarkable meso-pelagic crustacean species, which is harvested for human consumption in restricted geographical areas of Taiwan and Japan in the northwestern Pacific. Nucleotide sequence analysis of the mitochondrial DNA control region was conducted to investigate the levels of genetic variability and differentiation between Japanese and Taiwanese populations of *S. lucens*. The latter half of the control region, which contained 589 nucleotide sites, was sequenced using DNA extracted from 178 individuals from the two geographical regions. Analyses yielded 162 haplotypes, and the amount of genetic variability within the populations as shown by the levels of haplotype and nucleotide diversity revealed that the two populations included in this study have higher levels of diversity than intraspecific variation previously reported in other crustaceans. Our AMOVA and pairwise *Fst* results indicated the existence of significant genetic differences between the Japanese and Taiwanese populations. Morphological analyses also revealed minor but notable differences between the two groups. These findings suggest that postulated gene flow of *S. lucens* is unlikely to occur across the isolated Japanese and Taiwanese populations, and that each population completes their life history in a restricted geographical area.

### Contents

Introduction .....	123
Material and methods .....	124
<i>Genetic analysis</i> .....	124
<i>Morphological analysis</i> .....	125
Results .....	125
Discussion .....	127
Acknowledgements .....	128
References .....	128

### Introduction

The genetic population structures of aquatic animals are often correlated with varying levels of dispersal in their species, which are closely associated with swimming ability, type/duration of larval stages, or water currents. In the north Pacific region, from tropical to temperate areas, the Kuroshio Current plays an important role in the distribution of marine organisms. For example, the Japanese eel *Anguilla japonica* Temmink and Schlegel, 1847 does not show any genetic differentiation among Taiwanese, Chinese and Japanese populations (Taniguchi and Numachi, 1978; Minegishi *et al.*, 2012). On the other hand, some species, such as the Bowed fiddler crab *Uca arcuata* (De Haan, 1833), Big-fin reef squid *Sepioteuthis* spp. and Golden rabbitfish *Siganus guttatus* (Bloch, 1787), show genetic differences among populations (Aoki *et al.*, 2008a, b; Imai and Aoki, 2012; Iwamoto *et al.*, 2012).

The Sakura shrimp *Sergia lucens* (Hansen, 1922) is a commercially important species that is found in the same Kuroshio region. In the past, based on limited numbers of specimens, genetic differences were reported between Japanese and Taiwanese populations (Omori *et al.*, 1988), despite the two populations supposedly being connected by the Kuroshio Current. Recently, the number of Sakura shrimp caught in Japan has decreased. It is important to understand this species' population structure for appropriate resource management.

Further compounding potential management problems of this species, the species name for the Sakura

shrimp has not yet been definitively decided. Article 15.1 of the International Code of Zoological Nomenclature (ICZN, 2000; see also Article 11.5.1 concerning “conditional proposals” made before 1961) gives priority to the designation *Sergia kishinouyei* (Nakazawa and Terao, 1915) over *S. lucens*, although the latter name has been popularly used by researchers since Gordon (1935), who provided a detailed account of the species (cf. Omori, 1969). *Sergestes phosphoreus* Kishinouye, 1928 has also been used to refer to the Sakura shrimp, but the validity of this name is questioned (see Vereshchaka, 2000). To avoid taxonomic confusion, herein we use *S. lucens* until the nomenclature issues of the Sakura shrimp are formally resolved.

*Sergia lucens* is found from Suruga Bay, Sagami Bay, and the mouth of Tokyo Bay in Japan, and the coastal waters of Tungkang as well as off the east coast of Taiwan (Omori, 1969, 1995; Holthuis, 1980; Omori *et al.*, 1988; Isshiki and Tajima, 1992; Lee *et al.*, 1996). Furthermore, Vereshchaka (2000) recorded this species from off Borneo and New Guinea. This shrimp has been commercially exploited in Suruga Bay and Tungkang, where the annual yields average around 2,000 and 100 tons, respectively (Omori, 1989; Fukui *et al.*, 2004). Several biological aspects of this shrimp, including the spawning activity, egg abundances, larval development, swimming behaviour, and population size fluctuation, have been reported in Suruga Bay (Huzita, 1959; Kosaka *et al.*, 1969; Omori, 1971; Omori and Ohta, 1981; Tsukui, 1987; Bishop *et al.*, 1989; Muranaka and Jo, 1990; Hirai *et al.*, 1992; Omori and Seino, 1993; Isshiki, 1996). In fact, more than 200 research articles, most of them in Japanese, dealing with this shrimp have been published over the last century (Kubota *et al.*, 1995). Omori *et al.* (1988) conducted a comparative taxonomic study on individuals collected from Suruga Bay and Tungkang waters. These authors did not find morphological differences discriminating the two populations, suggesting that they could be a single species, although they observed differences in spawning season and isoelectrophoretic patterns of water-soluble proteins. However, as this study examined only five individuals per population, no definitive conclusions could be reached in this study. Since this study, no further information regarding the population structure of *S. lucens* has become available.

Genetic markers such as the mitochondrial DNA control region sequence have been shown to be effective in identifying population structures, and therefore genetic data can contribute to resource management and conservation. As the validity of the research results

of Omori *et al.* (1988) are under question, in this study, the mitochondrial DNA of *S. lucens* collected from the Japanese and Taiwanese coastal waters were analyzed together with comparative morphology to more clearly understand the population structure of this commercially important species.

## Material and methods

### Genetic analysis

Samples were obtained by trawl fishing at three locations in Japan and Taiwan, which represent the main fishing grounds of *S. lucens* (Fig. 1). In total, 178 specimens were examined for sequence analysis of mitochondrial control region: 70 from Suruga Bay (35°06'N, 138°38'E) (collected in 1999), 61 from Tungkang (22°20'N, 120°20'E) and 47 from Gueishan Island waters (24°51'N, 121°55'E), Taiwan (collected in 2003 and 2010). All samples were stored at -40°C until use.

Total crude DNA was extracted from the pereopod muscle of the frozen specimens using proteinase K, phenol-chloroform, and TNES-8 M urea buffer (Imai *et al.*, 2004). The mitochondrial (mt) DNA control region was then amplified by polymerase chain reaction (PCR) using the designed primers shrimp-sPCR-f

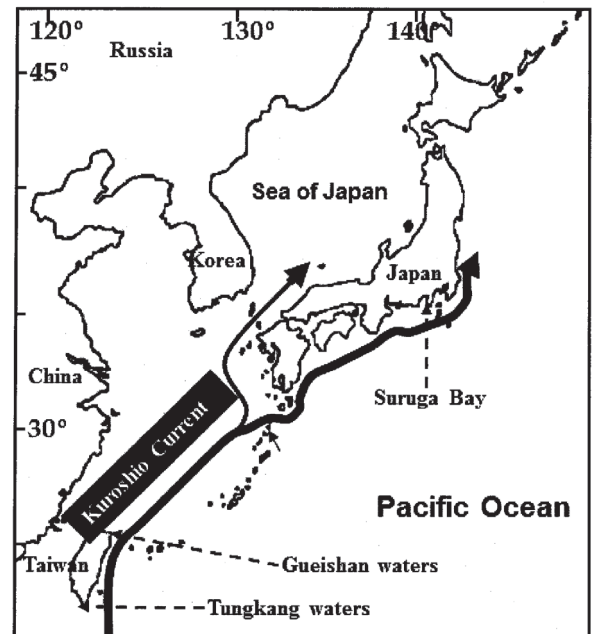


Fig. 1. Sampling sites of *Sergia lucens* in Japan and Taiwan.

(5'-GAAATAGTAACTGTAAAGTT-3') and sakura-tRNA (5'-TCTATCCTATCAAGATAGCCC-3'). All reactions were carried out in a 50  $\mu$ l total volume containing 1  $\mu$ l of total crude DNA, 25 pM each primer, 2.5 U of ExTaq™ DNA polymerase (Takara Bio), 5  $\mu$ l of each dNTP, 1  $\times$  buffer, and dH<sub>2</sub>O. A GeneAmp 9700 thermal cycler (Applied Biosystems) was used with the following parameters: 94°C (60 s) followed by 30 cycles of denaturation at 94.5°C (30 s), annealing at 40.5°C (30 s), and extension at 72°C (60 s). PCR products were purified with Exo-Sap-IT (Usb) and cycle-sequenced using a BigDye Terminator Kit and an ABI 3700 genetic analyzer (Applied Biosystems). The sequences obtained in this research were deposited in the DNA Data Bank of Japan (DDBJ) under Accession Numbers AB846666 to AB846827.

All sequences were initially aligned using ClustalX ver.1.83.1 (Thompson *et al.*, 1997). A median-joining network of haplotypes was constructed using the Network ver. 4.6.1.1 (Bandelt *et al.*, 1999) including all individuals. All calculations [haplotype diversity ( $h$ ; Nei, 1987), nucleotide diversity ( $\pi$ ; Tajima, 1983),  $F_{st}$  value and 10,000 times  $F_{st}$  permutation test (Reynolds *et al.*, 1983), an exact test based on 10,000 steps in a Markov chain (Raymond and Rousset, 1995), and the analysis of the molecular variance (AMOVA) (Excoffier *et al.*, 1992) were processed with Arlequin (Excoffier *et al.*, 2005). A sequential Bonferroni test (Rice, 1989) was used to correct multiple tests of the hypothesis, for which the pairwise  $F_{st}$  statistics did not differ from zero. Gene flow ( $N_e m$ ) was estimated using the relationship  $N_e m = [(1/F_{st}) - 1]/2$  (Hudson *et al.*, 1992). To investigate the population history of *S. lucens*, a mismatch distribution based on pairwise differences in the mtDNA control region sequences was conducted. In addition, to check for deviations from neutrality, two different  $D$  tests (Tajima, 1989; Fu and Li, 1993) were performed using Arlequin.

### Morphological analysis

Thirty-seven specimens of both sexes (8.4–10.4 mm in carapace length) from the two aforementioned sites in Taiwan (Feb. 2003; July 2010) and 18 specimens (10.0–13.0 mm) from Suruga Bay (May 2003) were used for the examination of a relative distance of the photophores on the antennal scale (Fig. 2). The measurements were made using an ocular-micrometer mounted on a bi-ocular microscope. In addition, the structure of the petasma was examined for male specimens (16 individuals from Japan and 42 from Taiwan).

### Results

In total, 589 base pairs (bp) from the control region were sequenced for 178 individuals from the three sampling locations. One hundred and sixty-two haplotypes and 149 variable sites were identified (On-line Supplementary Material, Table S1), and nearly all of the sequenced individuals had a unique haplotype (60 haplotypes from Suruga Bay, 40 haplotypes from Gueishan and 59 haplotypes from Tungkang). However, shared haplotypes were detected in four cases: haplotype 1, seven individuals from Suruga Bay and two from Gueishan; haplotype 2, one from Suruga Bay, three from Gueishan and one from Tungkang; haplotype 92, one from Suruga Bay and one from Gueishan and haplotype 122, one from Gueishan and one from Tungkang. Relationships between haplotypes were represented on a network tree, which did not show any geographical structuring (Fig. 3).

The haplotype and nucleotide diversities of the three geographical groups ranged from 0.991 to 1.000 and from 1.083% to 1.131%, respectively (Table 1). AMOVA in the three populations also showed a significant  $\Phi_{st}$  value of 0.0126 ( $P < 0.001$ ), indicating significant heterogeneity in at least one of the pairwise comparisons. The pairwise  $F_{st}$  value ranged from 0.0179 to 0.0205, differing significantly between Japan and Taiwan ( $P < 0.001$ ); however, the values between Gueishan and Tungkang indicated no significant genetic differences. Significant population subdivisions were detected between the Japanese and Taiwanese groups (Suruga Bay vs. Gueishan, and Suruga Bay vs. Tungkang;  $P < 0.05$ , Bonferroni adjustment), while the  $N_e m$  value between pairwise comparisons was 22.96 (Suruga Bay vs. Gueishan + Tungkang waters). Tajima's  $D$  and Fu and Li's  $D$  tests were also performed to determine neutrality. Significant negative values were obtained from all sampling sites by both tests (Table 1). The distribution of the pairwise number of differences in the control region haplotypes fits an expansion model, with a smooth wave predicted for each location that had undergone a demographic expansion (Fig. 4). The isolation time between the Japanese and Taiwanese populations was estimated to be 305,000 years, based on the observed nucleotide divergence between them and a mutation rate of 19%/MY, taken from the mtDNA control region of penaeid shrimps (McMillen-Jackson and Bert, 2003).

To support the differences observed in genetic analyses, some morphological features of *S. lucens* collected from Japan and Taiwan were examined (Fig. 5). These analyses showed that the relative distance of the three

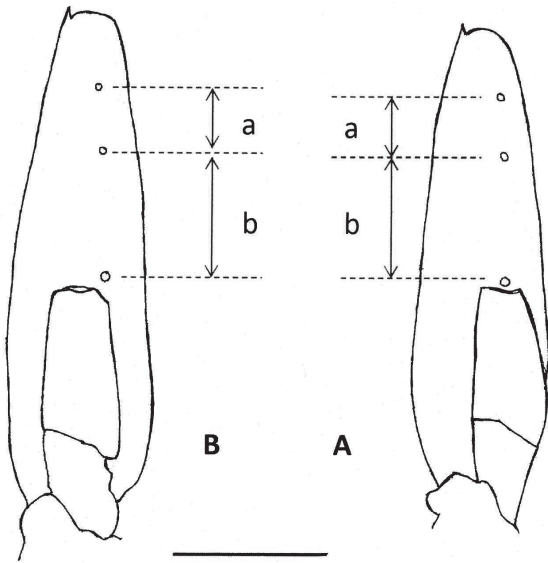


Fig. 2. Antennal scale of *Sergia lucens* and measurement of photophore distance: male 10.3 mm in carapace length from Suruga Bay, Japan (A) and male 10.3 mm from off Gueishan, Taiwan (B).

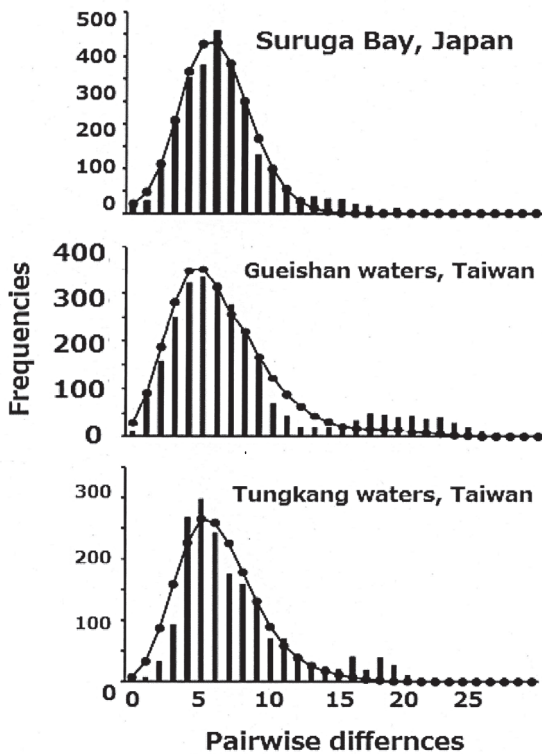


Fig. 4. Observed pairwise differences (bars) and the expected mismatch distributions under the sudden expansion model (line) of haplotypes detected in the Japanese and Taiwanese populations of *Sergia lucens*.

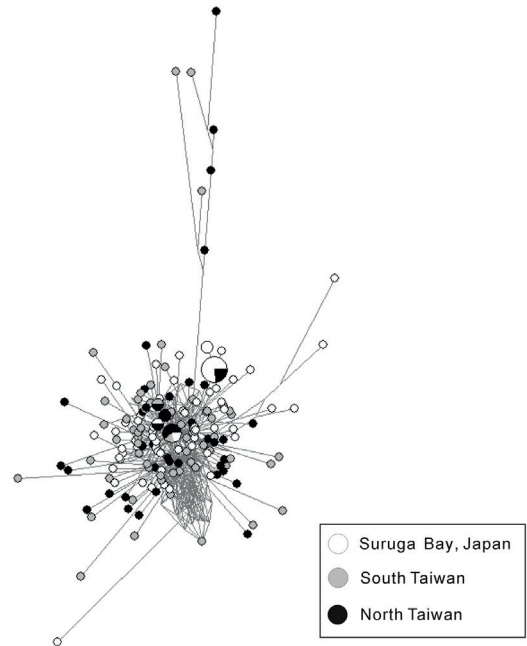


Fig. 3. Haplotype network for 162 haplotypes of *Sergia lucens*. White, dark and black circles indicated haplotypes among localities. The size of the circles is proportional to the haplotype frequencies. Lengths of the line showed relative to the number of mutations between haplotypes.

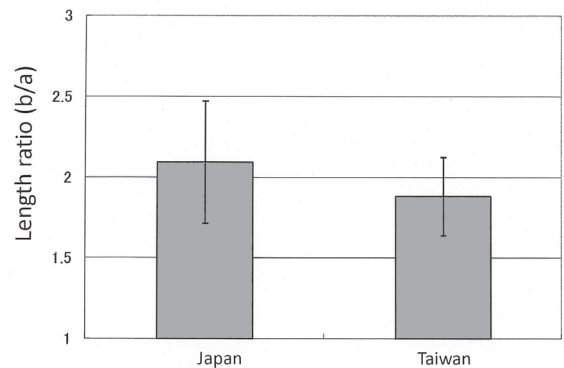


Fig. 5. Comparison in relative distance between the photophores on the antennal scale of *Sergia lucens* in specimens collected from Japanese and Taiwanese waters.

photophores on the antennal scale differed significantly between the two populations ( $t$ -test = 2.414,  $p < 0.05$ ), with the Japanese specimens having a mid-photophore placed more posteriorly than the Taiwanese ones.

The male petasma also showed minor variations in its form between the two groups, with approximately 21% of Taiwanese specimens having a small but distinct spine-like projection at the base of the lobus ter-



minalis as compared to less than 7% in the Japanese specimens (Fig. 6).

## Discussion

Genetic diversity, as shown by haplotype diversity, was found to be very high in *S. lucens*. The values generated in this study are comparable with those derived from the mtDNA control region sequences for the crustaceans *Farfantepenaeus aztecus* (Ives, 1891), *Litopenaeus setiferus* (Linnaeus, 1767) and *Panulirus penicillatus* (Olivier, 1791) (McMillen-Jackson and Bert, 2003; Abdullah *et al.*, in press). The reported value for haplotype diversity of penaeid shrimp and spiny lobster were 0.996 and 1.000, respectively (Tzeng, 2007; Abdullah *et al.*, in press), which is almost same to our study (ranging from 0.991 to 1.000). The nucleotide diversity of penaeid shrimp (McMillen-Jackson and Bert, 2003) was higher than the value of 5.4% obtained in this study. A high level of genetic diversity in the mtDNA control region could be due to a large population size.

Omori *et al.* (1988) conducted a comparative genetic study of *S. lucens* for individuals collected from Suruga Bay, Japan and Tungkang waters, Taiwan, and found that the two populations differed in isoelectrophoretic patterns of water-soluble proteins, implying limited gene flow between them. The present study, based on the significance of isolation indicated by pairwise *Fst* statistical analysis by AMOVA, also suggested a clear isolation between the Japanese and Taiwanese populations.

A similar disjunctive distribution has been observed in other crustaceans: *i.e.*, *Portunus trituberculatus* (Miers, 1876) by Imai *et al.* (1999) and Imai and Numachi (2002), *Farfantepenaeus aztecus* by McMillen-Jackson and Bert (2003), *Alpheus djeddensis* (Coutiere, 1897) by Thompson *et al.* (2005), *Parapenaeopsis hardwickii* (Miers, 1878) by Tzeng (2007), and *Uca arcuata* by Aoki *et al.* (2008b). The Kuroshio Current runs along the eastern coasts of Taiwan and Japan at a rate of nearly 50 million m<sup>3</sup>/s (Taira, 1997; Ujiie *et al.*, 2003). This strong northward current seemingly enhances the larval transportation. However, for some previously studied species (e.g., *Uca arcuata*, *Sepioteuthis* spp. and *Siganus guttatus*), no ongoing gene flow was found between the Japanese and Taiwanese populations (Aoki *et al.*, 2008a, b; Imai and Aoki, 2012; Iwamoto *et al.*, 2012). Additionally, the mtDNA data of this study clearly indi-

cate a lack of ongoing gene flow in *S. lucens*. Although Omori *et al.* (1988) mentioned that Japanese and Taiwanese *S. lucens* were morphologically indistinguishable, in our examination minor but appreciable differences were found in the relative distance of photophores on the antennal scale. The shape of the petasma also slightly differed between the two populations as the males from Taiwan had a tendency to have a developed projection at the base of the lobus terminalis of the petasma (Fig. 6; Table 2).

Following these findings, we presume the isolation time between the Japanese and Taiwanese populations to be 305,000 years (middle of the Pleistocene) based on nucleotide divergence estimation time (19%/MY) for penaeid shrimps (Kimura, 1996; McMillen-Jackson and Bert, 2003). The edge of the Chinese continent, which carries several large rivers, was once much closer to the Okinawa Trough. *Sergia lucens* may require large rivers and ridges on the deep sea floor to sustain a large-sized population (Omori *et al.*, 1988). Thus, it may be that there used to be several small populations along the continental shelf, among which gene flow most likely was maintained. However, now the main populations are distributed in central Japan and Taiwan at the edge of the continental shelf in isolation to each other.

The neutrality of mutations in the mtDNA control region was rejected on the basis of Tajima's *D* and Fu and Li's *D* tests. The significant negative values obtained using these tests suggest that the Japanese and Taiwanese populations have experienced a population expansion during their geographic history. The observed unimodal mismatched frequency distribution pattern also matched the predicted distribution under a model of population expansion. This unimodal pattern has also been observed in other crustaceans, including *Euphausia superba* Dana, 1850, *F. aztecus*, *F. duorarum* (Burkenroad, 1939), and *P. hardwickii* (see Zane *et al.*, 1998; McMillen-Jackson and Bert, 2003; Tzeng, 2007).

Recently, Vereshchaka (2000) recorded a small number of *S. lucens* from off Borneo and New Guinea, which notably expanded the known geographical range of this species. Due to the disjunctive distribution of this species, coupled with the genetic differences observed in this study between the Japanese and Taiwanese populations, *S. lucens* is likely to be formed by several small breeding units implying high genetic variability. Further research into the relationships between these units is needed for effective conservation of this commercially important species.

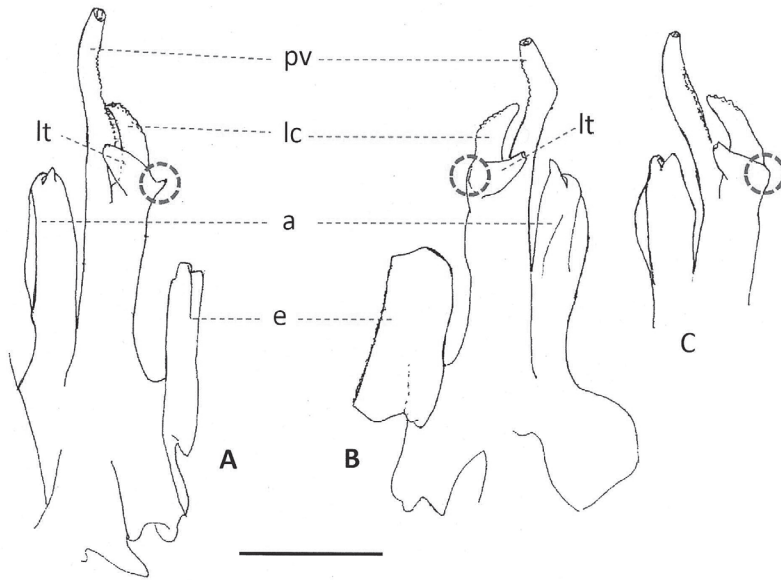


Fig. 6. Variation in form of petasma of *Sergia lucens* as indicated in the part of circle: male (10.3 mm in carapace length) from Suruga Bay, Japan, with no additional projection at the basal part of the lobus terminalis showing a typical form (entire part of left petasma, posterior view) (A); and male (10.0 mm) with a carinate swelling (distal part of right petasma, posterior view) (B); male from Taiwan (10.3 mm) with a distinct projection (entire part of right petasma, posterior view) (C): a, pars astringens; e, pars externa; lc, lobus connectens; pv, processus ventralis. Male with each form of petasma (A, B, C) was categorized as to III, I, and II in Table 2.

Table 1. Sampling locality, haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ ), and Tajima's  $D$  and Fu and Li's  $D$  test results for the Japanese and Taiwanese populations. \* $P < 0.005$ .

sample locality	sampling size	$h \pm SD$	$\pi \pm SD$	Tajima's $D$	Fu and Li's $D$
Suruga Bay, Japan	70	$0.991 \pm 0.01$	$1.096 \pm 0.58$	-2.40*	-11.30*
Gueishan waters, N Taiwan	47	$0.995 \pm 0.01$	$1.331 \pm 0.70$	-2.03*	-2.38*
Tungkang waters, S Taiwan	61	$1.000 \pm 0.00$	$1.299 \pm 0.68$	-2.31*	-11.32*

Table 2. Percentage occurrence of males with an additional projection at the base of the lobus terminals of the petasma. Basal part of lobus terminals (encircled part): I, smooth (typical form); II, carinate swelling; III, distinct projection.

category	Suruga Bay	Taiwan
I	50.0	39.4
II	43.7	39.4
III	6.3	21.2
total N	16	33

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### On-line Supplementary Information (SI)

SI. Variable sites in the 162 haplotypes found in mtDNA control region of 178 *Sergia lucens*. The numbers above sequences correspond to the positions of the polymorphic sites. Dots indicate an identical nucleotide at the position relative to haplotype 1.



