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Molecular evidence resolving the confusion of two species of *Spilopteron* (Hymenoptera: Ichneumonidae) caused by marked geographical colour variation

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Key words. Hymenoptera, Ichneumonidae, *Spilopteron*, systematics, Japan, latitudinal gradient, mtCOI, nuclear 28S, parasitoid, thermal melanism

Abstract. The delimitation of two ichneumonid species, *Spilopteron apicale* (Matsumura) and *S. tosaense* (Uchida), was investigated using DNA sequences of the mitochondrial COI and nuclear 28S rRNA genes, as well as adult morphology. The two species have long been confused and were until recently speculated to be the same species with continuous colour variation. Our molecular and morphological studies reveal that there are two distinct species: a dark species confined to northern or high latitude localities (*S. apicale*) and a widely distributed species with a marked geographical gradient in body colour (*S. tosaense*) across the Japanese Archipelago. In the latter species, female body colour became significantly darker with latitude and altitude. A lectotype of *Chorischizus apicalis* Matsumura, 1912 is designated.

INTRODUCTION

The classification of parasitoid wasps has long been based on morphological characteristics such as body proportions, surface structures, wing venation, and hair distribution. The use of body colour has also been common in identification, because of the ease of using this characteristic and the fact that it is genuinely reliable in some instances (Quicke et al., 2006). However, there is often large intraspecific variation in body colour due to genetic and environmental factors (Abe et al., 2013). Because of recent developments in molecular taxonomy, mitochondrial and nuclear DNA sequences have increasingly been used for the species-level classification of parasitoid wasps (e.g., Quicke et al., 2006; Rugman-Jones et al., 2009; Stigenberg et al., 2011). Accurate biological classification using both morphological and molecular data is sometimes possible, but many parasitoid groups are still far from seeing the benefit of molecular taxonomy.

Spilopteron Townes is a medium-sized genus of the ichneumonid subfamily Acaenitinae. It contains 30 species worldwide, eleven of which are known from the Palaearctic region (Yu et al., 2012). They are all large species, 10.0–20.0 mm in body length, and primarily parasitic on cerambycid larvae (Kusigemati, 1981; Yu et al., 2012). Since Shaw & Wahl (1989) reported that a related species, *Acaenitus dubitator* (Panzer, 1800), is a koinobiont endoparasitoid of beetle larvae, it is possible that *Spilopteron* utilizes the same strategy of parasitism.

In Japan, five species of *Spilopteron*, *S. apicale* (Matsumura, 1912) [= *S. apicalis*], *S. tosaense* (Uchida, 1934) [= *S. tosensis*], *S. pyrrhonae* Kusigemati, 1981, *S. mucronatum* Lee, 2008, and *S. luteum* (Uchida, 1930), have been recorded (Kusigemati, 1981; Ito et al., 2012; Yu et al., 2012). According to Kusigemati (1981), his dark-coloured species "*S. apicalis*" from the northern islands of Japan (Hokkaido and Honshu) was clearly distinguished from his light-coloured species "*S. tosensis*" from the southern islands (Shikoku, Kyushu, and Yakushima Is.) (cf. Fig. 6). However, many additional specimens of intermediate colouration have since been found on Honshu and Shikoku, making the delimitation of these species unclear. Thus, it has been speculated that they all belong to a single species with a continuous gradient of colour variation.

Geographical colour variation across the Japanese Archipelago has been studied in various insects. For example, the elytral colour polymorphism of ladybirds shows that the frequency of dark forms is higher in high latitude areas (Kawakami et al., 2013). Similarly, there is a positive latitudinal gradient in the frequency of the black-mark morph of male pygmy grasshoppers *Tetrix japonica* (Bolívar) (Tsurui & Nishida, 2010). Although some Japanese species of ichneumonids show marked geographical colour variation (Ito & Maeto, 2014; Ito et al., 2014), there have been no quantitative analyses of the effect of geographical factors on the colour variation of the parasitoid wasps of the archipelago.

In this study, we first describe the genetic structure of the so-called "*S. apicalis*" and "*S. tosensis*" species by using DNA sequences of the mitochondrial COI and nuclear 28S rRNA genes. Second, to delimit the two species in question, we examine the morphological characteristics of adult wasps, in the context of the results of the DNA sequence

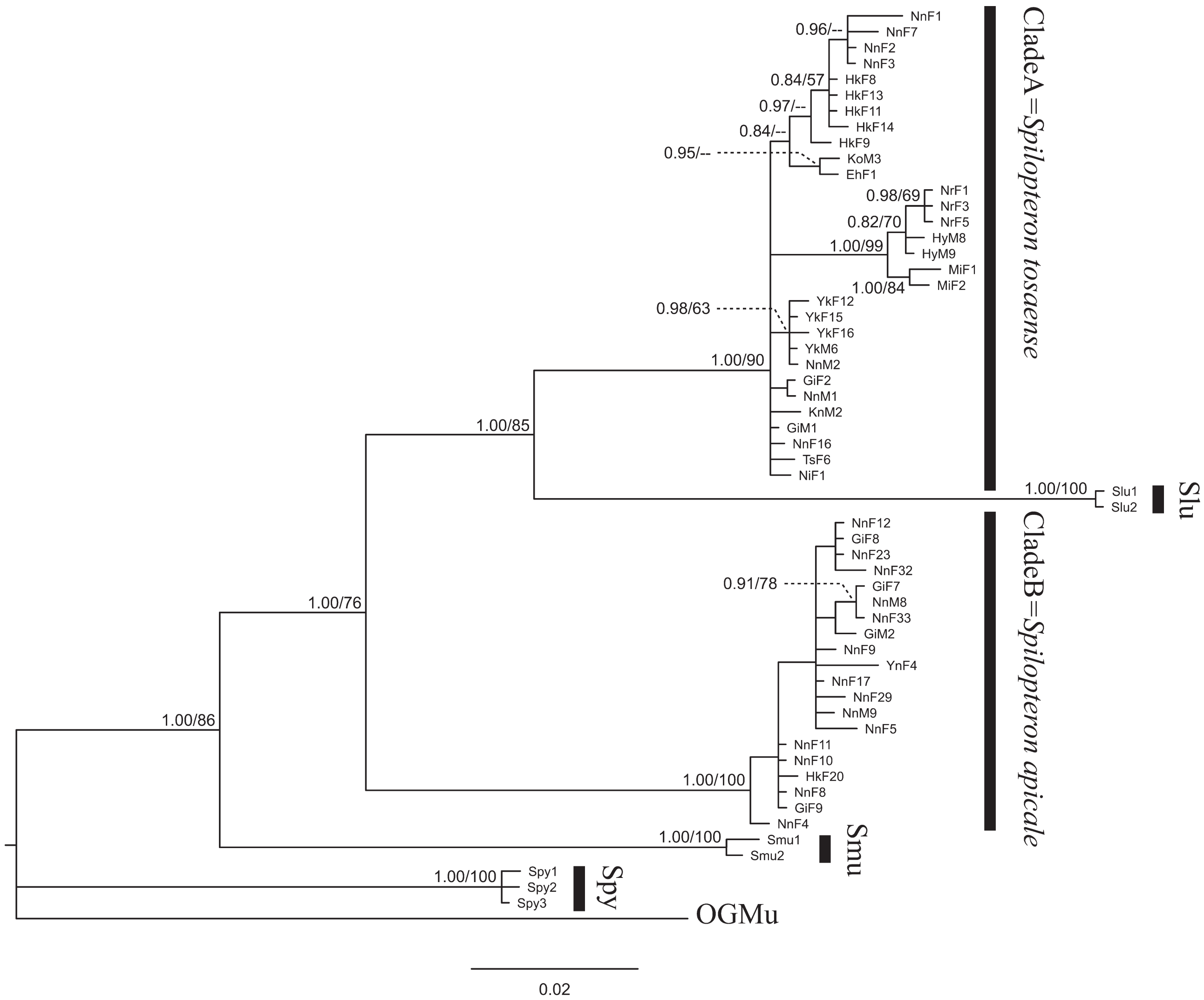

Fig. 1. BI tree of *Spilopteron* species based on mtCOI sequences. BI posterior probabilities (> 0.8) and ML bootstrap values (> 50%) are indicated at the nodes. Slu – *S. luteum*; Smu – *S. mucronatum*; Spy – *S. pyrrhonae*; OGMu – *M. unicolor*.

analyses, including type material of *S. apicale* and *S. tosaense*. Third, the intraspecific color variation is analyzed to determine whether it is influenced by any geographical factors.

MATERIAL AND METHODS

Mitochondrial and nuclear DNA analyses

We sequenced 52 Japanese individuals from the species complex (*apicalis* and *tosensis*), seven individuals of three other species of *Spilopteron* (*luteum*, *mucronatum*, *pyrrhonae*), and one individual of *Metachorischizus unicolor* Uchida as an outgroup species (Table S3).

A middle tarsus of each individual was removed and preserved in 99.5% ethanol. After drying, the tissue was ground in 20 µl of 50 mM NaOH and digested for 15 min at 95°C. After that, samples were neutralized using 20 µl of 200 mM Tris-HCl.

The mtCOI primers designed by Folmer et al. (1994) (LCO1490: 5′ GGT CAA CAA ATC ATA AAG ATA TTG G3′; HCO2198: 5′ TAA ACT TCA GGG TGA CCA AAA AAT CA3′) (648 bp), and the nuclear 28S D2 primers designed by Campbell et al. (1993) (fwd: 5′ AGT CGT GTT GCT TGA TAG TGC AG3′; rev: 5′ TTG GTC CGT GTT TCA AGA CGG G3′) (ca. 650 bp) were used for the polymerase chain reaction (PCR). PCR was conducted for COI and 28S by using the KOD FX NEO kit (Toyobo), with an initial 2-min denaturation at 94°C, followed by 35 cycles at 98°C for 10 s, 48°C for 30 s, and 68°C for 15 s. PCR products were purified using the illustra GFX kit (GE Healthcare Life Sciences). Gene regions were sequenced with the same primers used in the PCRs, and using the BigDye™ Terminator ver. 3.1 Cycle Sequencing kit (Applied Biosystems). The cycle sequencing programs both for COI and 28S consisted of 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Cycle sequencing reactions were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

For assembly and viewing of the sequence data, the DNA Dynamo Sequence Analyze Software program (Blue Tractor Software) was used. The sequences were aligned using ClustalW (http://www.genome.jp/tools/clustalw/). Bayesian inference (BI) analyses were performed for COI and 28S using MrBayes ver. 3.2.3 (Ronquist et al., 2012). The model selection for BI was performed according to the Akaike information criterion (AIC) in MrModeltest 2.3 (Nylander, 2004). The best-fit substitution model was HKY, with rate heterogeneity among sites modeled using a proportion of invariable sites (+I) and a gamma distribution (+G) for the COI dataset and GTR + G for the 28S dataset. Maximum likelihood (ML) trees were constructed for both COI and 28S using raxml GUI ver. 1.3.1 (Silvestro & Michalak,

Fig. 2. BI tree of *Spilopteron* species based on 28S sequences. BI posterior probabilities (> 0.8) and ML bootstrap values (> 50%) are indicated at the nodes. Slu – *S. luteum*; Spy – *S. pyrrhonae*; OGMu – *M. unicolor*.

2012), and based on the GTR + G model, with 1000 bootstrap replications. Pairwise p-distances between individuals and clades were calculated using MEGA6.0 (Tamura et al., 2013). All the DNA sequences obtained were deposited in the DDBJ/EMBL GenBank database.

Morphological examination

We examined 345 dried adult specimens deposited in the following institutes: the Systematic Entomology, Hokkaido University, Sapporo (SEHU); the National Institute of Agro-Environmental Science, Tsukuba (NIAES); the National Museum of Nature and Science, Tsukuba (NSMT); the Kanagawa Prefectural Museum of Natural History, Odawara (KPMNH); the Entomological Laboratory of Meijo University, Nagoya (MU); the Osaka Museum of Natural History, Osaka (OMNH); the Ehime University, Matsuyama (EUM); and the Entomological Laboratory of Kagoshima University, Kagoshima (KU). The type series of *S. apicale* and *S. tosaense* (including the synonymous *yakushimensis* Uchida) deposited in SEHU were also examined.

Specimens were observed using a stereoscopic microscope (Nikon SMZ660). Photos (Figs 3, 6, 9) were taken using a digital camera (Nikon D60) or a digital microscope (Keyence Digital Microscope VHX-600). Scanning electron micrographs (Figs 4, 5) were taken using a JEOL JSM-6010LV SEM. General morphological terminology follows Gauld (1991, 2002) and terminology for surface sculpture follows Eady (1968).

Geographical analysis of body colour variation in female *S. tosaense*

Three body parts (face, mesopleuron and metasomal tergite 1) were coded as light (0) or dark (1), as shown in Fig. 8. The face was considered to be light (0) when it was entirely yellowish brown inside the rectangle below the antennal sockets or dark (1) when it was black, or at least partly so. The mesopleuron (excluding the dorsal region below the tegula) was light (0) when it was yellowish brown at least in part or dark (1) when it was entirely black. Tergite 1 was light (0) when the inside of the basal rectangle anterior of the spiracles was yellowish brown at least in part or dark (1) when it was entirely black. The effects of two geographical variables, altitude (40–1570 m) and latitude (30–44°N), on the darkness indices were analyzed with Generalized Estimating Equations (GEE), using IBM SPSS Statistics for Windows, Version 19.0. The observed values (0 or 1) of the darkness indices were specified to have a binomial distribution and a logit link function. The three body parts were treated as within-individual variables. After the analysis was performed using the data from 187 females, the overall predicted value (probability) of the darkness of each body part was calculated.

The observed darkness values and collection localities of the female specimens were positioned on the BI tree based on the mtCOI sequences, to show the geographical distribution of mitochondrial lineages and color forms within Japan.

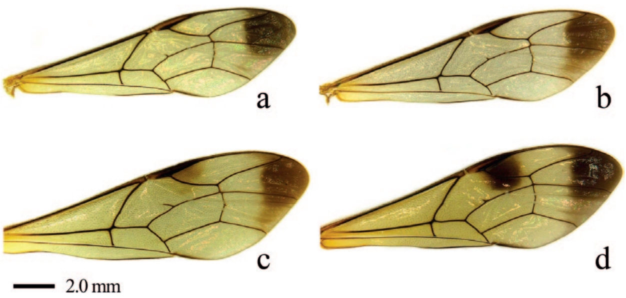

Fig. 3. Female fore wing. *S. apicale* (a) from Nagano Pref. and *S. tosaense* (b–d) from Hokkaido (b), Ishikawa Pref. (c), and Yakushima Is. (d).

RESULTS

Molecular evidence

Figs 1 and 2 show the BI trees based on the mitochondrial COI partial region (590 alignable bp) and the nuclear 28S D2 region (587 alignable bp), respectively, including the associated ML bootstrap values. The result of mtCOI analyses suggested the existence of two distinct clades, one of which contained specimens that could have been identified using existing characters as "*S. apicalis*" and "*S. tosensis*" (Clade A), and another which contained some "*S. apicalis*" specimens only (Clade B) (Fig. 1). The pair-wise p-distances within Clade A and Clade B were less than 0.037 and 0.017, respectively, and the mean distance between them was 0.099. Analyses of nuclear 28S sequences also provided two groups corresponding to those obtained from the mtCOI analyses (Fig. 2). Both mtCOI and 28S analyses supported the monophyly of Clade A + Clade B + *S. luteum*; however, the relationships between them were not determined (Figs 1, 2).

Morphological discrimination and taxonomy

Clades A and B could be morphologically discriminated from each other. The apical dark mark on the fore wing was extended downwards in Clade A (Fig. 3b–d), while it was rounded and not extended downwards in Clade B (Fig. 3a). A depression between the eye and antennal socket on the frons was absent in Clade A (Fig. 4b), while it was present in Clade B (Fig. 4a). In addition, the apical margin of the clypeus had weak lateral projections in Clade A (Fig. 5b), while it had two strong lateral projections in Clade B (Fig. 5a). Based on the examination of type specimens of *S. apicale* and *S. tosaense*, it has been confirmed that Clade A belongs to *S. tosaense* and Clade B to *S. apicale* (Fig. 6).

As our molecular data suggested, *S. luteum* from Japan and Taiwan was similar to *S. apicale* and *S. tosaense*. They could be distinguished from two other Japanese *Spilopteron* species, *S. mucronatum* and *S. pyrrhonae*, by the combination of the following character states: slenderness of the hind femur (without a ventral convexity), S1 with a round projection (without a sharp projection), and area superomedia confluent with area petiolaris. However, *S. luteum* was distinct from *S. apicale* and *S. tosaense* by the presence of a median longitudinal carina on the mesoscutum, a long lateral carina on the scutellum, and a strong lateral longitudinal carina on the propodeum (cf. Ito et al., 2012). The diagnosis, distribution, and other taxonomic information of *S. apicale* and *S. tosaense* is summarized below.

Spilopteron apicale (Matsumura, 1912)

Chorischizus apicalis Matsumura, 1912: 149.
Spilopteron apicalis: Townes et al., 1965: 391.
Spilopteron apicale: Yu et al., 2005, 2012.

Diagnosis. Fore wing with an apical dark mark rounded and not extended downward, without a dark mark below the pterostigma (Fig. 3a); frons with a depression between eye and antennal socket (Fig. 4a); clypeus with a very weak median projection and two strong lateral projections (Fig. 5a); body black; antennal flagellum with a white band; inner margin of eye, clypeus, basal area of mandible, subalar prominence, scutellum, postscutellum, apex of propodeum, front and middle legs, hind trochanter, hind trochantellus, apex of hind tarsus, and apices of T1 and T2 yellowish brown. Males same as females but face entirely yellow.

Distribution. Japan (Hokkaido, Honshu, Shikoku, and Kyushu) (Fig. 7a), and Far East Russia (Sakhalin). In central and southern Honshu, Shikoku and Kyushu occurring only at high altitudes (Fig. 8a). A record from China (Sheng et al., 2013) needs confirmation.

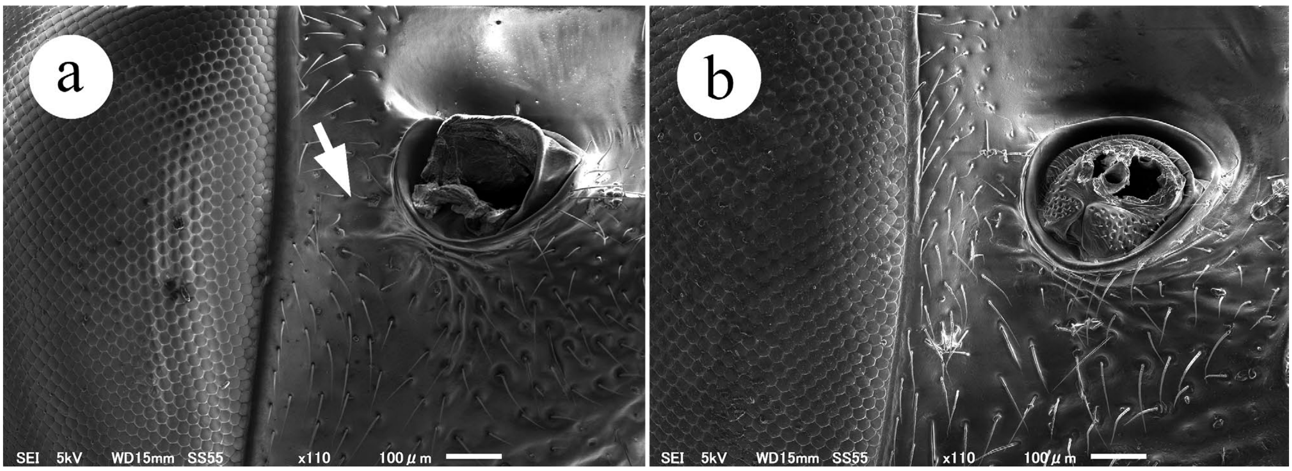

Fig. 4. Female frons. *S. apicale* (a) from Nagano Pref. and *S. tosaense* (b) from Yakushima Is. Arrow indicates a depression.

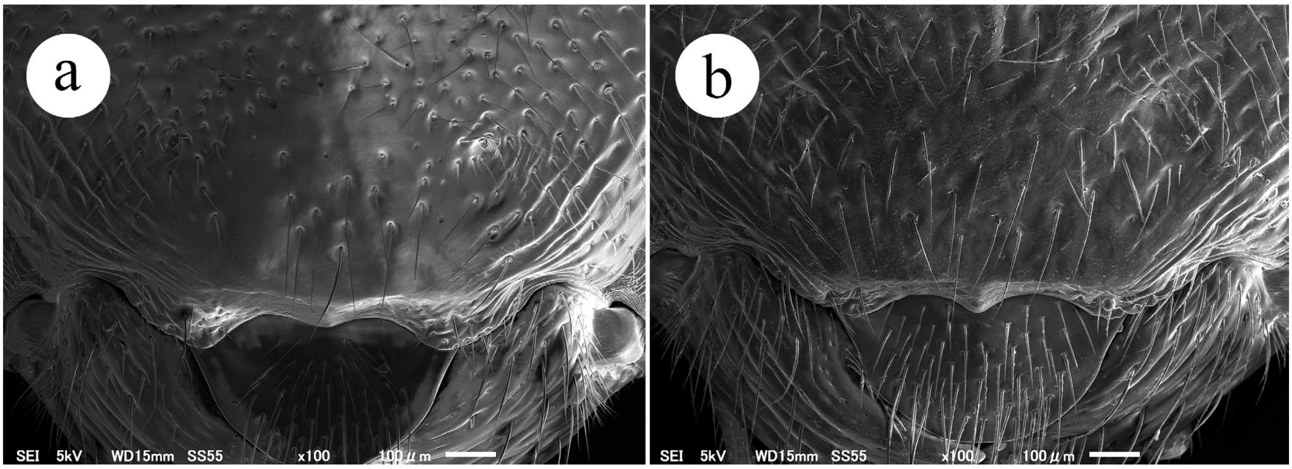

Fig. 5. Female clypeus. *S. apicale* (a) from Nagano Pref. and *S. tosaense* (b) from Yakushima Is.

Bionomics. Host unknown. Adults fly in July and August, visit flowers of *Angelica* spp. (Apiaceae), and oviposit on coniferous trunks in Japan (MI, pers. observ.).

Specimens examined. Lectotype: ♀, "Jyozan-kei, Sapporo City, Hokkaido, End of August 1907, S. Matsumura" (SEHU); paralectotype: ♀, "Sapporo City, Hokkaido, July 1907, S. Matsumura" (SEHU), both designated here. For additional specimens see Table S1.

Spilopteron tosaense (Uchida, 1934)

Siphimedia apicalis f. *tosaensis* Uchida, 1934: 53.
Siphimedia apicalis f. *yakushimensis* Uchida, 1934: 53. Synonymized by Townes et al. (1965).
Spilopteron tosensis: Townes et al., 1965: 391 (misspelling).
Spilopteron tosaense: Yu et al., 2005, 2012.

Diagnosis. Fore wing with an apical dark mark extended downward, sometimes with an additional dark mark below the pterostigma (Figs 3b–d); frons lacking a depression between eye and antennal socket (Fig. 4b); clypeus with a median and two lateral projections of equal size (Fig. 5b); body black to yellowish brown; antennal flagellum with a white band; inner margin of eye, clypeus, basal part of mandible, legs and basal part of metasomal tergite 1 yellowish brown to black; apical parts of metasomal tergites 1 and 2 yellowish brown (Fig. 9). Male same as female but body invariably yellow with dark stripes.

Distribution. Japan (Hokkaido*, Honshu, Sadogashima Is.*, Shikoku, Kyushu, Tsushima Is.*, and Yakushima Is.) (Figs 7b, 8b). (* – new record). A record from China (Sheng & Sun, 2010) needs confirmation.

Bionomics. Host unknown. Adults fly in July and August, visit flowers of *Angelica* spp. (Apiaceae), and oviposit on coniferous trunks in Japan (MI, pers. observ.).

Specimens examined. Holotype of *S. a*. f. *tosaensis*: ♀, "Mt. Koeda, Prov. Tosa (Kochi Pref.), 12. July 1933, Y. Sugihara" (SEHU). Holotype of *S. a*. f. *yakushimensis*: ♀, "Hananoegawa, Kuriu, Yakushima Is. (Kagoshima Pref.), 31. July 1929, H. Hori" (SEHU). For additional specimens see Table S2.

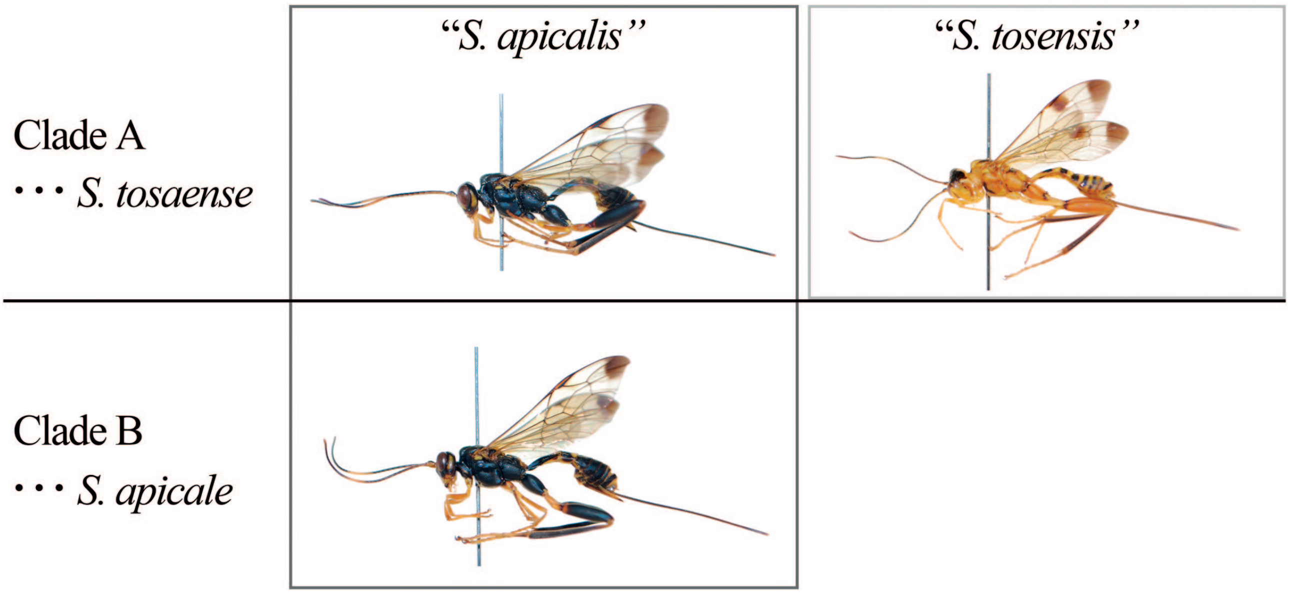

Fig. 6. Previous (in squares) and present classifications of *S. apicale* and *S. tosaense*.

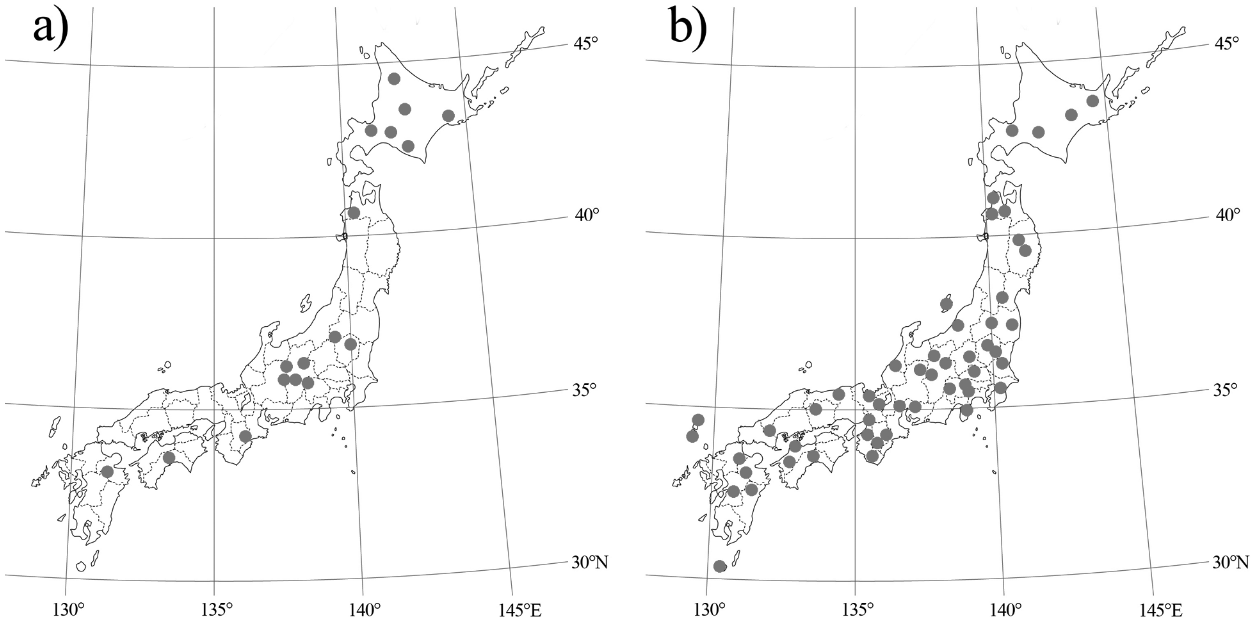

Fig. 7. Distribution of *S. apicale* (a) and *S. tosaense* (b) in Japan.

Remarks. The holotypes of *S. a*. f. *tosaensis* and *S. a*. f. *yakushimensis* differ in the colour of the mesosoma (mostly black in *S. a*. f. *tosaensis* but mostly yellowish brown in *S. a*. f. *yakushimensis*) and hind legs (dark brown in *S. a*. f. *tosaensis* but mostly yellowish brown in *S. a*. f. *yakushimensis*), but no other morphological differences were found between them. Therefore, we accept their synonymy proposed by Townes et al. (1965).

A geographical gradient of color variation in *S. tosaense*

The result of GEE showed that the effects of altitude and latitude on the body darkness of female *S. tosaense* were both significant and positive (Table 1). The darkness indices of all three body parts (Fig. 9) were predicted to increase with the degree of latitude, indicating a distinct gradient of body color variation along the latitudes of 30–40°N (Fig. 10).

As shown in Fig. 11, there seems to be a mitochondrial lineage of dark specimens (1/1/1) (Fig. 9) distributed on Hokkaido and the highlands of Honshu and Shikoku, a lineage of light specimens (0/0/0) (Fig. 9) on the southernmost island Yakushima, and a lineage of intermediate color forms (0/1/1, 1/1/0) on the Kii Peninsula of Honshu, but several other basal lineages of dark or light specimens were present in central Honshu and Tsushima Is.

DISCUSSION

Our molecular analyses and morphological examination have revealed that *S. apicale* is a monotypic, dark species found in northern or high altitude localities in Japan, while *S. tosaense* is a variously coloured species widely distributed from southern to northern Japan. Body color of female *S. tosaense* showed a marked geographical gradient, with individuals being increasingly dark with increasing latitude from Yakushima Is. to Hokkaido (Fig. 10).

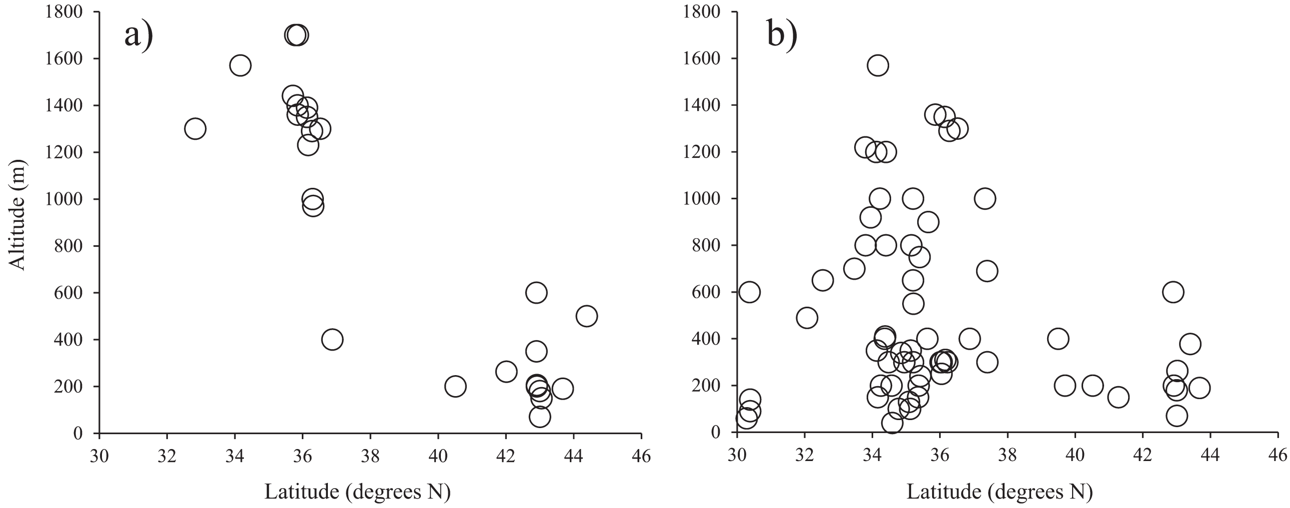

Fig. 8. Relationships of latitude and altitude in *S. apicale* (a) and *S. tosaense* (b).

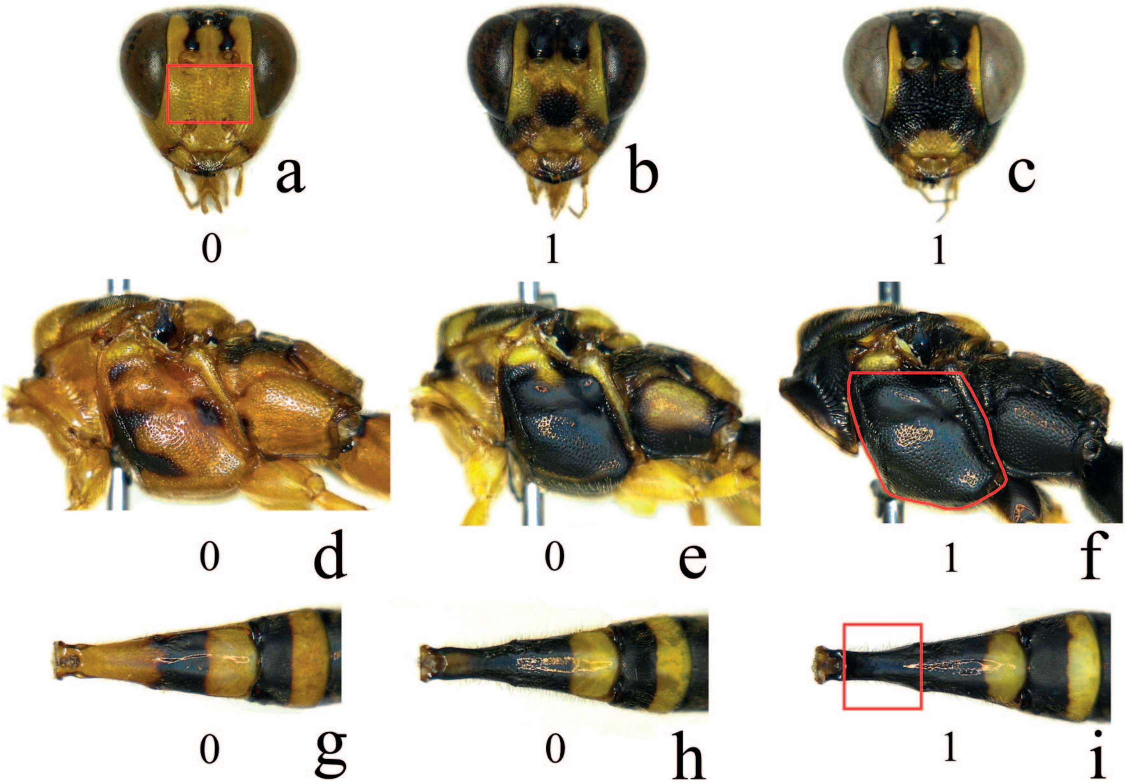

Fig. 9. Darkness indices (0 or 1) of the face (a–c), mesopleuron (d–f), and metasomal tergite 1 (g–i) of female *S. tosaense* from Hokkaido (c, f, i), Nara Pref. (b, h), Tsushima Is. (e), and Yakushima Is. (a, d, g). Red enclosures indicate the parts examined.

Within the Japanese Archipelago, some insects show a similar gradual increase in the frequency of dark forms with latitude (e.g., Tsurui & Nishida, 2010; Kawakami et al., 2013). Such geographical gradients of insect body color are considered to be an adaptation to thermal conditions (Brakefield & Willmer, 1985). The high frequencies of dark color morphs in the ladybird *Adalia bipunctata* (Linnaeus) associated with higher latitudes could be interpreted as being a thermal adaptation for absorbing more solar radiation. In contrast, the higher frequencies of light color morphs associated with a decrease in latitude may be an adaptation for reducing heat stress (Brakefield & Willmer, 1985). In addition, the study on thermoregulatory function of a braconid parasitoid *Meteorus pulchricornis* (Wesmael) demonstrated that body color variation markedly affected the body temperature of adult wasps in sunlight (Abe et al., 2013).

One of other conceivable hypotheses is the different pathway of entry into Japan between two color forms of *S. tosaense*. According to this hypothesis, the dark-colored population may have entered northern Japan, while the light-colored population entered southern Japan separately, subsequently leading to the current latitudinal gradient of color variations. However, the results of the mitochondrial COI analyses seem to contradict this hypothesis, because the lineages of northern dark individuals and southern light individuals are not clearly defined. In fact, this indicates the occurrence of multiple introduction events, each establishing various color forms on the southern islands (Fig. 11).

Last but not least, it is also noteworthy that *S. tosaense* males lack any geographical color variation, and are always light colored. Female wasps must stay on tree trunks for long periods of time during oviposition, irrespective of the strength of solar radiation and the benefits provided by body color variation may be more significant for thermal regulation. Another possibility is that the color patterns of females are a form of camouflage or mimicry designed to avoid predation, most likely by birds, during oviposition. Such protection is not necessary for the males, which are free to escape predation by fleeing the predator. The body color patterns of certain groups of parasitoid wasps have been implicated in mimicry (Mason, 1964; Quicke et al., 1992), and predation pressure has the potential to limit the fitness of parasitoids in the field (Heimpel et al., 1997). Nevertheless, there is no available data that demonstrates the existence of a corresponding geographical gradient in camouflage backgrounds or mimicry models, and further research will be required to fully test this hypothesis.

Table 1. Result of a Generalized Estimating Equations (GEE) analysis on darkness indices.

Effect	Beta estimate	Wald Chi-Square	df	P
Intercept	–47.560	73.43	1	<0.001
Altitude (m)	0.001	10.25	1	0.001
Latitude (degrees N)	1.340	72.21	1	<0.001
Part (face)	1.065	15.46	1	<0.001
Part (mesopleuron)	2.280	42.88	1	<0.001
Part (tergite 1) [a]	0.000	–	–	–

[a] Beta estimate is set to zero as a redundant parameter.

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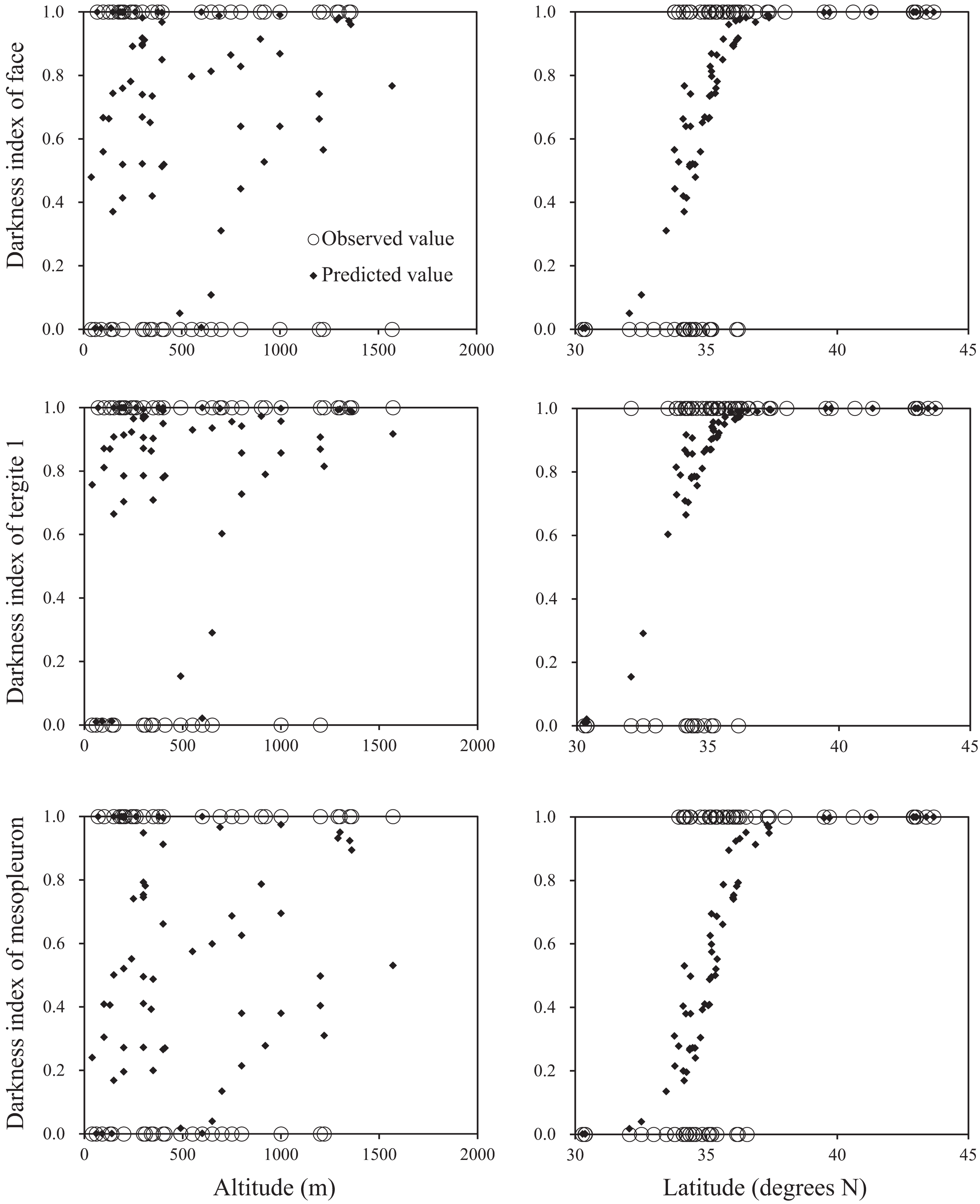

Fig. 10. Observed (0 or 1) and GEE predicted values (0–1) of the darkness indices on the three body parts (face, mesopleuron, and metasomal tergite 1) of female *S. tosaense*, in relation to altitude and latitude.

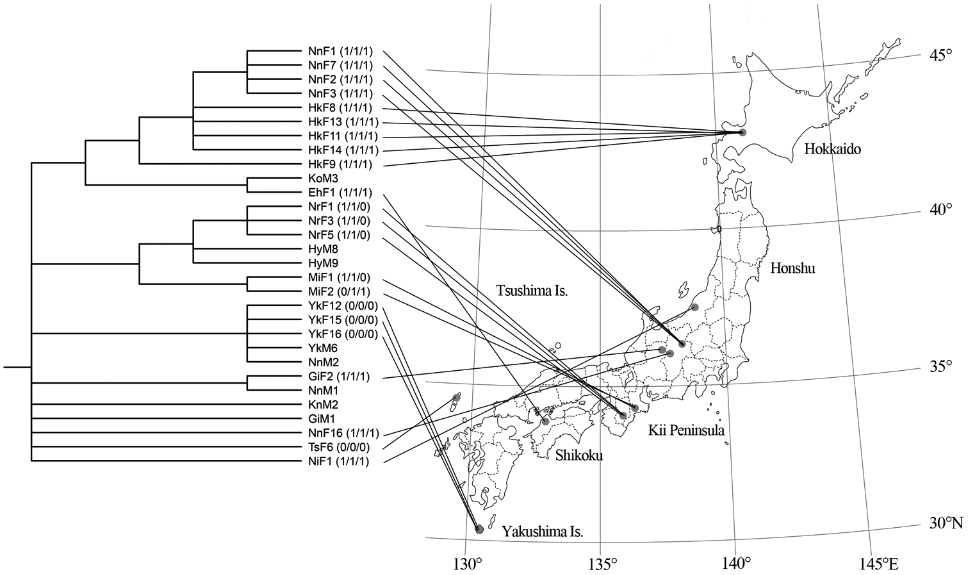

Fig. 11. Darkness indices (face/mesopleuron/metasomal tergite 1) and collection places of *S. tosaense* females positioned on the BI tree based on mtCOI (see Fig. 1).

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Table S1. Specimens of *S. apicale* used in the analysis.

Locality		Collection date	Code[1]	Alt.	Lat. N	Lon. E	Accession number		Depository
							COI	28S	
Hokkaido	Sapporo City	1–8. Aug., 2011	HkF20	70	43.0	141.4	LC041250	LC041946	OMNH
	Sapporo City	27. Jul.–21. Aug., 2007	HkF31	600	42.9	141.9	–	–	OMNH
	Sapporo City	4–27. Jul., 2007	HkF32	600	42.9	141.9	–	–	OMNH
	Sapporo City	27. Jul.–21. Aug., 2007	HkF33	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF34	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF35	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF36	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF37	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF38	600	42.9	141.9	–	–	EUM
	Sapporo City	27. Jul.–21. Aug., 2007	HkF39	600	42.9	141.9	–	–	EUM
	Sapporo City	24. Jul.–24. Aug., 2006	HkF28	206	42.9	141.9	–	–	OMNH
	Sapporo City	24. Jul.–24. Aug., 2006	HkF29	206	42.9	141.9	–	–	OMNH
	Sapporo City	3–17. Aug., 2007	HkF24	180	43.0	142.1	–	–	OMNH
	Sapporo City	24. Jul.–24. Aug., 2006	HkF25	263	42.0	142.1	–	–	OMNH
	Sapporo City	24. Jul.–24. Aug., 2006	HkF26	263	42.0	142.1	–	–	OMNH
	Sapporo City	19. Aug., 1966	HkF47	150	43.0	141.3	–	–	OMNH
	Kuriyama Town	16. Jul.–5. Aug., 2009	HkF42	200	42.9	141.9	–	–	OMNH
	Teshikaga Town	5. Aug., 2004	HkF45	190	43.7	144.4	–	–	OMNH
	Sapporo City	21–29. Jul., 1989	HkF48	350	42.9	141.1	–	–	NIAES
	Horokanai Town	9. Aug., 1981	HkF40	500	44.4	142.3	–	–	NIAES
	Horokanai Town	9. Aug., 1981	HkF41	500	44.4	142.3	–	–	NIAES
	Mt. Petegariyama	28 Jul., 1970	HkF49	–	42.5	142.9	–	–	NSMT
	Samani Town	8. Aug., 1970	HkF50	–	42.1	143.0	–	–	NSMT
	Kushiro City	6. Aug., 1947	HkM1	–	–	–	–	–	SEHU
Aomori	Nishimeya Vill.	6–14. Aug., 2013	AoF6	200	40.5	140.2	–	–	EUM
Fukushima	Hinoemata Vill.	18. Aug., 1981	FsF3	–	37.0	139.3	–	–	NIAES
	Hinoemata Vill.	28–29. Jul., 1990	FsF4	–	37.0	139.3	–	–	NIAES
Tochigi	Yaita City	11–22. Aug., 1989	TtF6	400	36.9	139.9	–	–	NIAES
Nagano	Ueda City	8. Aug.–3. Sep., 2014	NnF4	1300	36.5	138.3	LC041251	LC041960	KPMNH
	Ueda City	3. Sep.–26. Sep., 2014	NnF5	1300	36.5	138.3	LC041252	LC041961	KPMNH
	Nagawa Town	25. Aug., 2011	NnF8	1350	36.1	138.2	LC041253	–	OMNH
	Nagawa Town	25. Aug., 2011	NnF9	1350	36.1	138.2	LC041254	–	OMNH
	Nagawa Town	25. Aug., 2011	NnF10	1350	36.1	138.2	LC041255	LC041944	OMNH
	Nagawa Town	22. Aug., 2012	NnF11	1350	36.1	138.2	LC041256	LC041943	OMNH
	Nagawa Town	23. Aug., 2012	NnF12	1350	36.1	138.2	LC041257	LC041945	OMNH
	Nagawa Town	22. Aug., 2012	NnF13	1350	36.1	138.2	–	–	OMNH
	Nagawa Town	26. Aug., 2011	NnF14	1350	36.1	138.2	–	–	OMNH
	Nagawa Town	27. Aug., 2011	NnF15	1390	36.1	138.2	–	–	OMNH
	Outaki Vill.	28. Jul., 2013	NnF17	1360	35.9	137.6	LC041258	LC041955	SEHU
	Outaki Vill.	31. Jul., 2013	NnF18	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	31. Jul., 2013	NnF19	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	31. Jul., 2013	NnF20	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	31. Jul., 2013	NnF21	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	31. Jul., 2013	NnF22	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	28. Jul., 2013	NnF23	1360	35.9	137.6	LC041259	–	SEHU
	Outaki Vill.	31. Jul., 2013	NnF24	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	31. Jul., 2013	NnF25	1360	35.9	137.6	–	–	KPMNH
	Outaki Vill.	28. Jul., 2013	NnF26	1700	35.9	137.5	–	–	SEHU
	Outaki Vill.	28. Jul., 2013	NnF27	1700	35.9	137.5	–	–	SEHU
	Outaki Vill.	28. Jul., 2013	NnF28	1700	35.9	137.5	–	–	SEHU
	Outaki Vill.	28. Jul., 2013	NnF29	1700	35.9	137.5	LC041260	LC041956	SEHU
	Outaki Vill.	28. Jul., 2013	NnF30	1700	35.9	137.5	–	–	SEHU
	Ina City	30. Jul., 2013	NnF31	1700	35.8	137.6	–	–	KPMNH
	Ina City	30. Jul., 2013	NnF32	1700	35.8	137.6	LC041261	LC041958	KPMNH
	Ina City	30. Jul., 2013	NnF33	1700	35.8	137.6	LC041262	LC041959	KPMNH
	Azumino City	24. Aug., 2012	NnF34	970	36.3	138.8	–	–	OMNH
	Karuizawa Town	8. Sep., 1962	NnF35	1000	36.3	138.5	–	–	NSMT
	Mt. Hakkaisan	28. Jul., 2013	NnM3	–	–	–	–	–	SEHU
	Mt. Hakkaisan	28. Jul., 2013	NnM4	–	–	–	–	LC041949	KPMNH
	Mt. Hakkaisan	6. Aug., 2007	NnM5	–	–	–	–	–	KPMNH
	Mt. Hakkaisan	7. Aug., 2010	NnM6	–	–	–	–	–	KPMNH

TABLE S1 (continued).

Locality		Collection date	Code[1]	Alt.	Lat. N	Lon. E	Accession number COI	28S	Depository
Nagano	Mt. Asamayama	1. Aug., 1978	NnM7	–	–	–	–	–	OMNH
	Ina City	30. Jul., 2013	NnM8	1300	–	–	LC041264	LC041950	OMNH
	Mt. Hakkaisan	28. Jul., 2013	NnM9	–	–	–	LC041265	LC041957	OMNH
Gifu	Takayama City	13. Aug., 2013	GiF3	1290	36.3	137.6	–	–	NSMT
	Takayama City	13. Aug., 2013	GiF4	1290	36.3	137.6	–	–	NSMT
	Takayama City	3. Aug., 2013	GiF5	1290	36.3	137.6	–	–	NIAES
	Takayama City	13. Aug., 2013	GiF6	1290	36.3	137.6	–	–	NSMT
	Takayama City	13. Aug., 2013	GiF7	1290	36.3	137.6	LC041266	LC041948	NSMT
	Takayama City	13. Aug., 2013	GiF8	1290	36.3	137.6	LC041267	LC041953	NSMT
	Takayama City	4. Aug., 2013	GiF9	1230	36.2	137.5	LC041268	LC041952	NIAES
	Takayama City	4. Aug., 2013	GiM2	–	36.2	–	LC041269	LC041951	NIAES
Yamanashi	Hokuto City	7. Aug., 2007	YnF1	1400	35.9	138.6	–	–	OMNH
	Hokuto City	8. Aug., 2008	YnF2	1400	35.9	138.6	–	–	OMNH
	Hokuto City	28. Jul.–7. Aug., 2007	YnF3	1400	35.9	138.6	–	–	OMNH
	Hokuto City	28. Jul.–7. Aug., 2007	YnF4	1400	35.9	138.6	LC041270	LC041947	OMNH
	Hokuto City	28. Jul.–7. Aug., 2007	YnF5	1400	35.9	138.6	–	–	OMNH
	Hokuto City	8. Aug., 2008	YnF6	1400	35.9	138.6	–	–	OMNH
	Shioyama City	5. Aug., 2008	YnF7	1440	35.7	138.8	–	–	KPMNH
	Sagashino	14. Aug., 1974	YnF8	–	–	–	–	–	NSMT
	Owa Town	5. Aug., 2008	YnM4	900	35.7	138.8	–	–	KPMNH
	Akeno Vill.	7. Aug., 2007	YnM5	–	–	–	–	–	KPMNH
Nara	Kamikitayama Vill.	15–22. Aug., 2005	NrF9	1570	34.2	136.1	–	–	OMNH
Oita	Mt.Sobo–san	28. Jul., 1978	OiF2	1300	32.8	131.3	–	–	NIAES

[1] F – female, M – male.

TABLE S2. Specimens of *S. tosaense* used in the analysis.

Locality		Collection date	Code[1]	Alt.	Lat. N	Lon. E	Darkness index Face	Meso-pleuron	T1	Accession number COI	28S	Depository
Hokkaido	Sapporo City	25. Jul.–1. Aug., 2012	HkF1	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2012	HkF2	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2012	HkF3	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2012	HkF4	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	4–11. Aug., 2008	HkF5	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2011	HkF6	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2011	HkF7	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	26. Jul.–2. Aug., 2012	HkF8	70	43.0	141.4	1	1	1	LC041271	–	EUM
	Sapporo City	20–27. Jul., 2010	HkF9	70	43.0	141.4	1	1	1	LC041272	LC041962	EUM
	Sapporo City	18–25. Jul., 2012	HkF10	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	4–11. Aug., 2008	HkF11	70	43.0	141.4	1	1	1	LC041273	–	EUM
	Sapporo City	25. Jul.–1. Aug., 2011	HkF12	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	20–27. Jul., 2010	HkF13	70	43.0	141.4	1	1	1	LC041274	LC041963	EUM
	Sapporo City	1–8. Aug., 2011	HkF14	70	43.0	141.4	1	1	1	LC041275	–	EUM
	Sapporo City	1–8. Aug., 2012	HkF15	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	1–8. Aug., 2012	HkF16	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	1–8. Aug., 2012	HkF17	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	1–8. Aug., 2011	HkF18	70	43.0	141.4	1	1	1	–	–	EUM
	Sapporo City	22–29. Aug., 2011	HkF19	70	43.0	141.4	1	1	1	–	–	EUM
	Yubari City	3–17. Aug., 2007	HkF21	180	43.0	142.1	1	1	1	–	–	OMNH
	Yubari City	24. Jul.–24. Aug., 2006	HkF22	263	43.0	142.1	1	1	1	–	–	OMNH
	Yubari City	24. Jul.–24. Aug., 2006	HkF23	263	43.0	142.1	1	1	1	–	–	OMNH
	Kyriyama Town	24. Jul–24. Aug., 2006	HkF27	206	42.9	141.9	1	1	1	–	–	OMNH
	Ashoro Town	17. Jul–7. Aug., 2008	HkF46	378	43.4	143.9	1	1	1	–	–	OMNH
	Kyriyama Town	16. Jul.–5. Aug., 2009	HkF43	200	42.9	141.9	1	1	1	–	–	OMNH
	Sapporo City	27. Jul.–21. Aug., 2007	HkF30	600	42.9	141.9	1	1	1	–	–	OMNH
	Teshikaga Town	5. Aug., 2004	HkF44	190	43.7	144.4	1	1	1	–	–	OMNH
Aomori	Mt. Hakkodasan	15. Aug., 1927	AoF1		40.6	140.8	1	1	1	–	–	SEHU
	Nakadomari Town	28. Jul., 2013	AoF2	150	41.3	140.3	1	1	1	–	–	KPMNH
	Nishimeya Vill.	25. Jul.–6. Aug., 2013	AoF3	200	40.5	140.2	1	1	1	–	–	EUM
	Nishimeya Vill.	4–19. Aug., 2011	AoF4	200	40.5	140.2	1	1	1	–	–	EUM
	Nishimeya Vill.	4–19. Aug., 2011	AoF5	200	40.5	140.2	1	1	1	–	–	EUM
Iwate	Shizukuishi Town	9. Aug., 1975	IwF1	200	39.7	141.0	1	1	1	–	–	NIAES
	Mt. Hayachinesan	2–8. Aug., 1989	IwF2	400	39.5	141.4	1	1	1	–	–	NIAES
Miyagi	Zao Town	8. Aug., 1953	MgF1		38.0	140.4	1	1	1	–	–	NIAES
Fukushima	Mt. Hakaseyama	29. Jun.–26. Jul., 1998	FsF1	1000	37.3	139.7	1	1	1	–	–	MU
	Namie Town	em.19.Jun.1995	FsF2	300	37.4	140.9	1	1	1	–	–	NIAES
Tochigi	Yaita City	11–22. Aug., 1989	TtF1	400	36.9	139.9	1	1	1	–	–	NIAES
	Yaita City	11–22. Aug., 1989	TtF2	400	36.9	139.9	1	1	1	–	–	NIAES
	Yaita City	11–22. Aug., 1989	TtF3	400	36.9	139.9	1	1	1	–	–	NIAES
	Yaita City	11–22. Aug., 1989	TtF4	400	36.9	139.9	1	1	1	–	–	NIAES
	Yaita City	11–22. Aug., 1989	TtF5	400	36.9	139.9	1	1	1	–	–	NIAES
	Mogi Town	23. Jul., 2011	TtM1	–	–	–	–	–	–	–	–	KPMNH
Gumma	Mt. Akagisan	15–20. Aug., 1993	GuF1		36.6	139.2	1	1	0	–	–	NSMT
Ibaraki	Mt. Houkyousan	28. Jun.–2. Aug., 2013	IbF1	310	36.2	140.1	0	0	0	–	–	NSMT
	Tsuchiura City	1. Jul., 2014	IbM1	310	36.2	140.1	–	–	–	–	LC041979	NSMT
Niigata	Mt. Nokogiriyama	21. Jul.–21. Aug., 2014	NiF1	690	37.4	138.9	1	1	1	LC041276	LC041976	KPMNH
Nagano	Ueda City	10. Aug., 2012	NnF1	1300	36.5	138.3	1	1	1	LC041277	LC041980	KPMNH
	Ueda City	8. Aug.–3. Sep., 2014	NnF2	1300	36.5	138.3	1	1	1	LC041278	LC041977	KPMNH
	Ueda City	8. Aug.–3. Sep., 2014	NnF3	1300	36.5	138.3	1	1	1	LC041279	LC041978	KPMNH
	Nagawa Town	26. Aug., 2011	NnF6	1350	36.1	138.2	1	1	1	–	–	OMNH
	Nagawa Town	22. Aug., 2012	NnF7	1350	36.1	138.2	1	1	1	LC041280	LC041981	OMNH
	Outaki Vill.	31. Jul., 2013	NnF16	1360	35.9	137.6	1	1	1	LC041281	–	NIAES
	Outaki Vill.	31. Jul., 2013	NnM1	1360	35.9	137.6	–	–	–	LC041282	LC041969	NIAES
	Ueda City	18. Aug., 2012	NnM2	1300	36.5	138.3	–	–	–	LC041283	–	KPMNH

Table S2 (continued).

Locality		Collection date	Code [1]	Alt.	Lat. N	Lon. E	Darkness index			Accession number		Depository
							Face	Meso-pleuron	T1	COI	28S	
Gifu	Takayama City	13. Aug., 2013	GiF1	1290	36.3	137.6	1	1	1	–	–	NSMT
	Takayama City	13. Aug., 2013	GiF2	1290	36.3	137.6	1	1	1	LC041284	LC041968	NSMT
	Takayama City	4. Aug., 2013	GiM1	–	36.3	137.5	–	–	–	LC041285	LC041975	NSMT
Saitama	Ogawa Town	12. Sep., 1992	SiF1	300	36.0	139.2	1	1	1	–	–	NIAES
	Ogawa Town	23. Aug., 1992	SiF2	300	36.0	139.2	1	1	1	–	–	NIAES
	Ogawa Town	23. Aug., 1992	SiF3	300	36.0	139.2	1	1	1	–	–	NIAES
	Yorii Town	20. Jul., 1980	SiF4	300	36.1	139.2	1	1	1	–	–	NIAES
	Yorii Town	25. Jul., 1980	SiF5	300	36.1	139.2	1	1	1	–	–	NIAES
	Otaki Vill.	31. Jul., 1973	SiF6		35.8	138.9	1	1	1	–	–	NIAES
	Mt. Sengenyama	3. Aug., 1995	SiF7	250	36.0	139.3	1	1	1	–	–	NIAES
	Ootaki Town	13–14. Jul., 2002	SiM1	–	–	–	–	–	–	–	–	KPMNH
	Chichibu City	20–21. Jul., 2013	SiM2	–	–	–	–	–	–	–	–	NSMT
	Higashichichibu Vill.	20. Jul., 2013	SiM3	–	–	–	–	–	–	–	–	NSMT
	Higashichichibu Vill.	20. Jul., 2013	SiM4	–	–	–	–	–	–	–	–	NSMT
Yamanashi	Koushu City	5. Aug., 2008	YnF9	900	35.7	138.8	1	1	1	–	–	KPMNH
	Maruno Town	1. Aug.–10. Oct., 2007	YnM1	–	–	–	–	–	–	–	–	OMNH
	Maruno Town	1. Aug.–10. Oct., 2007	YnM2	–	–	–	–	–	–	–	–	OMNH
	Hokuto City	8. Aug., 2008	YnM3	1360	35.9	138.6	–	–	–	–	–	KPMNH
Tokyo	Mt. Takaosan	21. Jul., 1968	ToF1	400	35.6	139.2	1	1	1	–	–	NIAES
	Mt. Takaosan	10. Aug., 1964	ToF2	400	35.6	139.2	1	1	1	–	–	NIAES
Kanagawa	Mt. Koboyama	28. Jun., 2008	KnF1	200	35.4	139.3	1	1	1	–	–	KPMNH
	Odawara City	11. Jul., 1987	KnF2	300	35.2	139.1	1	1	1	–	–	NIAES
	Odawara City	16. Jul., 1987	KnF3	300	35.2	139.1	1	1	1	–	–	NIAES
	Odawara City	25. Jul., 1987	KnF4	300	35.2	139.1	1	1	1	–	–	NIAES
	Hakone Town	27. Jul., 1999	KnM1	–	–	–	–	–	–	–	–	KPMNH
	Manaduru Town	30. Jun., 2013	KnM2	40	35.1	139.2	–	–	–	LC041286	LC041974	KPMNH
Chiba	Kimitsu City	18. Jul., 2010	TiM1	–	–	–	–	–	–	–	–	KPMNH
Shizuoka	Mishima City	23. Jun.–8. Dec., 2012	SzF1	100	35.1	138.9	1	1	1	–	–	KPMNH
	Higashiizu Town	7. Jul., 2009	SzF2	100	34.8	139.0	1	1	1	–	–	KPMNH
Ishikawa	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF1	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–18. Jul., 2002	IsF2	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF3	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF4	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	19–31. Jul., 2002	IsF5	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF6	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF7	300	36.2	136.3	0	1	0	–	–	NIAES
	Mt. Kariyasuyama	19–31. Jul., 2002	IsF8	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF9	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsF10	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	19–31. Jul., 2002	IsF11	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	18–30. Jul., 2002	IsF12	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	18–30. Jul., 2002	IsF13	300	36.2	136.3	1	1	0	–	–	NIAES
	Mt. Kariyasuyama	19–31. Jul., 2002	IsF14	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	18–30. Jul., 2002	IsF15	300	36.2	136.3	1	1	1	–	–	NIAES
	Mt. Kariyasuyama	28. Jun.–19. Jul., 2002	IsM1	300	36.2	136.3	–	–	–	–	–	NIAES
Hyogo	Kami Town	16. Jun.–14. Jul., 2013	HyF1	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF2	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	26. Jun.–18. Jul., 2011	HyF3	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF4	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF5	750	35.4	134.5	1	1	0	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF6	750	35.4	134.5	1	1	0	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF7	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF8	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF9	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF10	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF11	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF12	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF13	750	35.4	134.5	1	1	1	–	–	OMNH
	Kami Town	16. Jun.–14. Jul., 2013	HyF14	750	35.4	134.5	1	1	0	–	–	OMNH
	Haga Town	4. Aug., 2003	HyF15	550	35.2	134.5	0	0	0	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM1	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM2	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM3	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM4	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM5	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM6	750	35.4	134.5	–	–	–	–	–	OMNH
	Kami Town	16. Jun–14. Jul., 2013	HyM7	750	35.4	134.5	–	–	–	–	–	OMNH
	Mt. Hyonosen	13. Jul., 2013	HyM8	500	35.3	134.6	–	–	–	LC041287	LC041972	KPMNH
	Mt. Hyonosen	13. Jul., 2013	HyM9	500	35.3	134.6	–	–	–	LC041288	LC041973	KPMNH
	Kami Town	17. Jul., 2011	HyM10	750	35.4	134.5	–	–	–	–	–	OMNH
Kyoto	Kita Ward	7. Aug., 2011	KyF1	130	35.1	135.8	1	1	0	–	–	OMNH
	Kyoto City	30. Jul., 2008	KyF2	800	35.2	135.8	0	1	1	–	–	OMNH
	Maizuru City	1–15. Jul., 2008	KyF3	240	35.4	134.3	1	1	1	–	–	OMNH
	Hanase-toge	22. Jul., 2004	KyM1	–	–	–	–	–	–	–	–	OMNH
Shiga	Otsu City	16. Aug., 2008	SgF1	1000	35.2	135.9	1	1	0	–	–	SEHU
	Otsu City	29. Jul., 2008	SgM1	–	–	–	–	–	–	–	–	SEHU
Aichi	Toyota City	18–28. Jul., 1998	AiF1	650	35.2	137.4	1	1	0	–	–	MU
	Toyota City	18–28. Jul., 1998	AiF2	650	35.2	137.4	0	1	0	–	–	MU
	Toyota City	18–28. Jul., 1998	AiF3	650	35.2	137.4	1	1	0	–	–	MU
	Toyota City	18–28. Jul., 1998	AiF4	650	35.2	137.4	1	1	0	–	–	MU
	Toyota City	18–28. Jul., 1998	AiF5	–	–	–	1	1	0	–	–	NIAES
	Asuke Town	28. Jun.–5. Jul., 2005	AiM1	–	–	–	–	–	–	–	–	MU
	Asuke Town	28. Jun.–5. Jul., 2005	AiM2	–	–	–	–	–	–	–	–	MU
	Yawata Town	17–26. Jun., 1998	AiM3	650	–	–	–	–	–	–	–	MU
	Yawata Town	17–26. Jun., 1998	AiM4	650	–	–	–	–	–	–	–	MU
	Yawata Town	17–26. Jun., 1998	AiM5	650	–	–	–	–	–	–	–	MU

Table S2 (continued).

Locality		Collection date	Code [1]	Alt.	Lat. N	Lon. E	Darkness index			Accession number		Depository
							Face	Meso-pleuron	T1	COI	28S	
Mie	Matsusaka City	2. Sep., 2012	MiF1	400	34.4	136.2	1	1	0	LC041289	LC041964	OMNH
	Matsusaka City	2. Sep., 2012	MiF2	400	34.4	136.2	0	1	1	LC041290	LC041965	OMNH
	Matsusaka City	7. Aug., 2011	MiF3	400	34.4	136.2	0	1	1	–	–	OMNH
	Matsusaka City	7. Aug., 2011	MiF4	400	34.4	136.2	0	1	0	–	–	OMNH
	Matsusaka City	13. Aug., 2011	MiF5	400	34.4	136.2	1	1	0	–	–	OMNH
	Matsusaka City	13. Aug., 2011	MiF6	400	34.4	136.2	0	1	1	–	–	OMNH
	Matsusaka City	13. Aug., 2011	MiF7	400	34.4	136.2	1	1	1	–	–	OMNH
	Kuwana City	12. Jul., 2009	MiF8	350	35.1	136.6	0	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF9	350	35.1	136.6	1	0	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF10	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF11	350	35.1	136.6	0	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF12	350	35.1	136.6	0	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF13	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF14	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF15	350	35.1	136.6	0	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF16	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF17	350	35.1	136.6	0	1	0	–	–	EUM
	Kuwana City	12. Jul., 2009	MiF18	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	11. Jun., 2009	MiF19	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	11. Jun., 2009	MiF20	350	35.1	136.6	1	1	0	–	–	EUM
	Kuwana City	11. Jun., 2009	MiF21	350	35.1	136.6	0	0	0	–	–	EUM
	Kuwana City	11. Jun., 2009	MiF22	350	35.1	136.6	1	1	1	–	–	EUM
	Kuwana City	11. Jun., 2009	MiF23	350	35.1	136.6	1	1	1	–	–	EUM
	Misugi Vill.	24. Jul., 2011	MiM1	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM2	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM3	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM4	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM5	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM6	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM7	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM8	–	–	–	–	–	–	–	–	OMNH
	Misugi Vill.	24. Jul., 2011	MiM9	–	–	–	–	–	–	–	–	OMNH
Osaka	Kawachinagano City	18. Jul., 2004	OsF1	410	34.3	135.6	0	0	0	–	–	OMNH
	Minoo City	21. Jul.–16. Aug., 2006	OsF2	340	34.9	135.5	0	0	0	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF3	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF4	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF5	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF6	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF7	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF8	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF9	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF10	300	34.9	135.6	1	1	1	–	–	OMNH
	Takatsuki City	30. Jun.–4. Aug., 2013	OsF11	300	34.9	135.6	1	1	1	–	–	OMNH
	Kaizuka City	20–30. Jun., 2003	OsM1	–	–	–	–	–	–	–	–	OMNH
Nara	Totsukawa Vill.	10. Aug., 2013	NrF1	1200	34.1	135.9	1	1	0	LC041291	LC041970	SEHU
	Totsukawa Vill.	20. Jul.–11. Aug., 2013	NrF2	1200	34.1	135.9	1	1	0	–	–	SEHU
	Totsukawa Vill.	20. Jul.–11. Aug., 2013	NrF3	1200	34.1	135.9	1	1	0	LC041292	–	SEHU
	Totsukawa Vill.	20. Jul.–11. Aug., 2013	NrF4	1200	34.1	135.9	0	1	1	–	–	SEHU
	Totsukawa Vill.	20. Jul.–11. Aug., 2013	NrF5	1200	34.1	135.9	1	1	0	LC041293	LC041971	SEHU
	Kamikitayama Vill.	26. Jul., 2010	NrF6	1000	34.2	136.0	0	1	0	–	–	SEHU
	Kamikitayama Vill.	15. Jul., 1999	NrF7	1000	34.2	136.0	0	0	1	–	–	OMNH
	Kamikitayama Vill.	8–15. Aug., 2005	NrF8	1570	34.2	136.1	0	1	1	–	–	OMNH
	Mt. Azumidake	6. Aug., 2010	NrF10	150	35.4	135.1	1	1	1	–	–	OMNH
	Mt. Azumidake	6. Aug., 2010	NrF11	150	35.4	135.1	1	1	1	–	–	OMNH
Wakayama	Mt. Koyasan	20. Jul., 1999	WkM1	–	–	–	–	–	–	–	–	OMNH
	Mt. Otousan	13. Jun., 1999	WkM2	–	–	–	–	–	–	–	–	OMNH
	Mt. Hansarei	14. Jun., 1999	WkM3	–	–	–	–	–	–	–	–	OMNH
	Mt. Hansarei	14. Jun., 1999	WkM4	–	–	–	–	–	–	–	–	OMNH
	Oishi–kogen	15. Jul., 1994	WkM5	–	–	–	–	–	–	–	–	OMNH
Tottori	Mt. Hyonosen	6. Aug., 2011	ToM1	880	35.3	134.5	–	–	–	–	–	KPMNH
Hiroshima	Mt. Kakezuyama	18. Jul., 1998	HiM1	–	–	–	–	–	–	–	–	OMNH
Ehime	Mt. Takanawasan	11. Aug., 2013	EhF1	920	33.9	132.9	1	1	1	LC041294	LC041967	OMNH
	Oda Town	17. Jul., 1995	EhF2	–	–	–	1	0	0	–	–	NIAES
	Mt. Odamiyama	16. Jun., 1998	EhM1	–	–	–	–	–	–	–	–	OMNH
	Oda Town	5. Jul., 1997	EhM2	–	–	–	–	–	–	–	–	EMU
	Oda Town	5. Jul., 1997	EhM3	–	–	–	–	–	–	–	–	EMU
Kochi	Mt. Kanpuzan	31. Jul., 2006	KoF1	1220	33.8	133.3	1	1	0	–	–	OMNH
	Mt. Kanpuzan	31. Jul., 2006	KoF2	1220	33.8	133.3	1	1	0	–	–	OMNH
	Mt. Kanpuzan	31. Jul., 2006	KoF3	1220	33.8	133.3	0	1	0	–	–	OMNH
	Okawa Vill.	21. Aug., 2000	KoF4	800	33.8	133.4	1	1	0	–	–	OMNH
	Mt. Kanpuzan	31. Jul., 2006	KoM1	1220	33.8	133.3	–	–	–	–	–	OMNH
	Otoyo Town	22–25. Jul., 2012	KoM2	–	–	–	–	–	–	–	–	OMNH
	Otoyo Town	22–25. Jul., 2012	KoM3	–	–	–	–	–	–	LC041295	LC041966	OMNH
Yamaguchi	Mt. Jakuchisan	23. Aug., 1992	YcF1	1200	34.4	132.0	0	0	1	–	–	OMNH
	Mt. Jakuchisan	21. Jun., 1999	YcF2	800	34.4	132.0	1	1	1	–	–	NIAES
Fukuoka	Mt. Hikosan	28–29. Jul., 2007	FoF1	700	33.5	130.9	0	1	0	–	–	KPMNH
Oita	Mt. Kujusan	23. Jun., 1980	OiF1	–	33.0	131.2	0	0	0	–	–	NIAES
Kumamoto	Yatsushiro City	12. Aug., 2011	KmF1	650	32.5	130.9	0	0	0	–	–	SEHU
	Yatsushiro City	1. Aug., 1976	KmF2	650	32.5	130.9	0	0	0	–	–	SEHU
Nagasaki	Tsushima Is.	12. Aug., 2001	TsF1	350	34.1	129.2	0	1	0	–	–	OMNH
	Tsushima Is.	25. Jul., 1994	TsF2	200	34.3	129.2	1	1	0	–	–	OMNH
	Tsushima Is.	14. Aug., 1993	TsF3	200	34.6	129.4	0	1	0	–	–	OMNH
	Tsushima Is.	7. Jul., 1984	TsF4	300	34.5	129.4	0	0	0	–	–	NIAES
	Tsushima Is.	28. Aug., 1970	TsF5	150	34.2	129.2	0	0	1	–	–	NSMT
	Tsushima Is.	5. Jul., 2014	TsF6	40	34.6	129.4	0	0	0	LC041296	LC041984	OMNH
	Tsushima Is.	5. Jul., 2014	TsF7	40	34.6	129.4	0	0	0	–	LC041985	OMNH
	Tsushima Is.	14. Jul., 1992	TsM1	–	–	–	–	–	–	–	–	OMNH

Table S2 (continued).

Locality		Collection date	Code [1]	Alt.	Lat. N	Lon. E	Darkness index			Accession number		Depository
							Face	Meso-pleuron	T1	COI	28S	
Miyazaki	Kobayashi City	16. Jul., 2004	MzF1	490	32.1	131.0	0	0	0	–	–	OMNH
	Kobayashi City	28. Jul., 2005	MzF2	490	32.1	131.0	0	1	0	–	–	OMNH
	Kitagata Town	21. Jul., 2008	MzM1	1000	–	–	–	–	–	–	–	OMNH
Kagoshima	Yakushima Is.	14. Jul., 1994	YkF1	60	30.3	130.4	0	0	0	–	–	OMNH
	Yakushima Is.	9. Aug.–2. Sep., 2000	YkF2	600	30.4	130.6	0	0	0	–	–	MU
	Yakushima Is.	28. Jun., 2012	YkF3	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	3. Jul., 2012	YkF4	90	30.4	130.6	0	0	0	–	–	OMNH
	Yakushima Is.	28. Jun., 2012	YkF5	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	28. Jun., 2012	YkF6	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	3. Jul., 2012	YkF7	90	30.4	130.6	0	0	0	–	–	OMNH
	Yakushima Is.	28. Jun., 2012	YkF8	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	28. Jun., 2012	YkF9	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	28. Jun., 2012	YkF10	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	28. Jun., 2012	YkF11	90	30.4	130.6	0	0	0	–	–	KPMNH
	Yakushima Is.	28. Jun., 2012	YkF12	90	30.4	130.6	0	0	0	LC041297	LC041982	KPMNH
	Yakushima Is.	29. Jun., 2012	YkF13	90	30.4	130.6	0	0	0	–	–	SEHU
	Yakushima Is.	3. Jul., 2012	YkF14	90	30.4	130.6	0	0	0	–	–	OMNH
	Yakushima Is.	29. Jun., 2012	YkF15	140	30.4	130.4	0	0	0	LC041298	LC041983	SEHU
	Yakushima Is.	29. Jun., 2012	YkF16	140	30.4	130.4	0	0	0	LC041299	–	SEHU
	Yakushima Is.	28. Jun., 2012	YkF17	140	30.4	130.4	0	0	0	–	–	KPMNH
	Yakushima Is.	2. Jul., 2012	YkF18	140	30.4	130.4	0	0	0	–	–	OMNH
	Yakushima Is.	2. Jul., 2012	YkF19	140	30.4	130.4	0	0	0	–	–	OMNH
	Yakushima Is.	29. Jun., 2012	YkF20	140	30.4	130.4	0	0	0	–	–	SEHU
	Yakushima Is.	29. Jun., 2012	YkF21	140	30.4	130.4	0	0	0	–	–	SEHU
	Yakushima Is.	29. Jun., 2012	YkF22	140	30.4	130.4	0	0	0	–	–	SEHU
	Yakushima Is.	8. Jul., 2010	YkM1	–	–	–	–	–	–	–	–	KPMNH
	Yakushima Is.	21. Jun.–9. Jul., 2000	YkM2	600	–	–	–	–	–	–	–	MU
	Yakushima Is.	21. Jun.–9. Jul., 2000	YkM3	600	–	–	–	–	–	–	–	MU
	Yakushima Is.	21. Jun., 2012	YkM4	–	–	–	–	–	–	–	–	KPMNH
	Yakushima Is.	2. Jul., 2012	YkM5	140	30.4	130.4	–	–	–	–	–	KPMNH
	Yakushima Is.	29. Jun., 2012	YkM6	140	30.4	130.4	–	–	–	LC041300	–	KPMNH
	Yakushima Is.	29. Jun., 2012	YkM7	140	30.4	130.4	–	–	–	–	–	KPMNH
	Yakushima Is.	29. Jun., 2012	YkM8	140	30.4	130.4	–	–	–	–	–	KPMNH

[1] F – female, M – male.

Table S3. Specimens of out-groups used in the analysis.

Species	Locality	Collection date	Sex	Code	Accession number		Depository
					COI	28S	
M. unicolor	Yakushima Is.	2. Jul., 2012	Male	OgMu	LC008221	LC041991	NSMT
S. pyrrhonae	Shizuoka Pref.	27. Apr., 2013	Male	Spy1	LC041305	LC041988	OMNH
S. pyrrhonae	Shizuoka Pref.	27. Apr., 2013	Male	Spy2	LC041306	LC041989	OMNH
S. pyrrhonae	Shizuoka Pref.	27. Apr., 2013	Female	Spy3	LC041307	LC041990	NIAES
S. luteum	Amami-oshima Is.	27. Jun., 2011	Male	Slu1	LC041303	LC041986	OMNH
S. luteum	Amami-oshima Is.	28. Jun., 2011	Female	Slu2	LC041304	LC041987	OMNH
S. mucronatum	Kyoto Pref.	23. Jun., 2012	Female	Smu1	LC041301	–	NIAES
S. mucronatum	Hokkaido	13. Jul., 2012	Male	Smu2	LC041302	–	NIAES