

## Natural Hybridization and Mitochondrial Gene Flow at the Distributional Boundary between Two Closely Related Species in the Genus *Megalopaederus* SCHEERPELTZ (Coleoptera, Staphylinidae)

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**Abstract** Natural hybridization between two staphylinid beetles *Megalopaederus lewisi* and *Megalopaederus kurosawai* was examined at their distributional boundary based on variation in male genital morphology and mitochondrial cytochrome oxidase subunit I (*COI*) gene. The results suggest that these two species form a hybrid zone at their distributional boundary, where mitochondrial introgression occurs rarely. Thus, reproductive isolation between the species may be strong enough to maintain parapatry but incomplete to allow mitochondrial gene flow between the species.

**Keywords:** *Megalopaederus lewisi*, *Megalopaederus kurosawai*, genitalia, *COI*, introgressive hybridization.

### Introduction

Quaternary climate change may have altered the distributional ranges of organisms (e.g. BLANT & ORTÍ, 2003; CONROY & COOK, 2000; HEWITT, 2000, 2004). Being restricted to narrow climate zones, populations were fragmented to separate geographical regions. Geographical isolation can promote genetic differentiation between the populations, possibly leading to incipient speciation. After expansion from such fragmented ranges, populations may meet again and construct secondary contact zones (HEWITT, 2001). The amount of gene flow at the contact zones is expected to depend on the levels of reproductive isolation between these populations (BARTON & HEWITT, 1985; ARNOLD, 1997). Hence, secondary contact zones can be arenas to evaluate the levels of reproductive isolation between incipient species (BARTON & HEWITT, 1985; KUBOTA & KUBOTA, 2011; KUBOTA *et al.*, 2013).

Wingless staphylinid beetles of the genus *Megalopaederus* SCHEERPELTZ, 1957 is distributed in the Japanese Archipelago as far south as Taiwan Island (SMETANA, 2004). They are found on shrubs and bamboo leaves in cool temperate mountainous area (WATANABE, 1986). Four externally similar species occur in Japan: *M. poweri* SHARP, 1874; *M. lewisi* CAMERON, 1930; *M. wadai* SCHEERPELTZ, 1957; and *M. kurosawai* WATANABE, 1986. These four species are allopatrically or parapatrically distributed and have been treated as separate species because of clearly distinguishable male genital morphology. Additionally, ASSING (2015) described *M. bucculentus* from Nagano Prefecture, central Japan. Since its genital morphology resembles that of *M. wadai*, the further study is necessary on its species status. However, it is unclear whether morphological difference in genital morphology is maintained at contact zones between species. Elucidating morphological and genetic variation at the contact zones is crucial to reveal the degree of reproductive isolation between parapatric species.

Here we examine variations in male genital morphology and mitochondrial gene sequences in the contact zone between *M. lewisi* and *M. kurosawai*, to evaluate the level of reproductive isolation between the two species. *Megalopaederus lewisi* is distributed widely across Honshu mainland, Japan, from the eastern area of the Tokai District to the northern area of Tohoku District. *Megalopa-*

*ederus kurosawai* parapatrically occurs in the southern region to the range of *M. lewisi*, from the eastern area of Tokai District to a part of Kanto District (Fig. 1). Thus, the ranges of the two species contact at the eastern part of the Tokai District of Honshu mainland (WATANABE, 1986).

## Materials and Methods

### *Study sites and sample collection*

We constructed a transect across the putative contact zone between the two species (Locality 1 in Table 1), which consisted of five study sites (A to E) that were placed from the range of *Megalopaederus lewisi* to that of *M. kurosawai* (Fig. 2). We collected adult beetles in the sites A to E from 10th July 2010 to 16th October 2011. We examined 40 males (morphology: 38; mitochondria: 37; both: 35) and 40 females at sites A–E (Table 2). Additionally, we collected adults from 31 and seven localities that are situated within the distributional ranges of *M. lewisi* and *M. kurosawai*, respectively (Fig. 1, Table 1).

For the phylogenetic analysis, we included samples of congeneric species: *M. poweri* and *M. wadai* obtained from one and four localities in Japan, respectively, and *M. formosanus* ADACHI, 1939 and *M. kosempoensis fulvocaudatus* ADACHI, 1939 from Taiwan (Table 1). We also added *Paederus gottschei* KOLBE, 1886 from Republic of Korea (Table 1). We thought that phylogenetic relationships within the genus are a subject of clarification, rather than a priori assumption. Thus we treated *M. formosanus*, and *M. kosempoensis* as outgroups of Japanese *Megalopaederus* species, and treated *Paederus gottschei* as an outgroup of all *Megalopaederus* species.

### *Morphological analysis*

In some cases, secondary contact results in hybrid individuals, with genital morphologies intermediate between those of parental populations (KUBOTA & SOTA, 1998; SASABE *et al.*, 2007). Therefore, we classified the genital morphological types of samples in the study sites into *lewisi*, *kurosawai*, and intermediate types. We used 38 male individuals from the study sites and comparable samples of 81 male of *M. lewisi*, 39 male of *M. kurosawai* respectively from other monotypic localities.

From their descriptions (WATANABE, 1986), these species could be effectively characterized from the contours of the male genital organs viewed ventrally (Fig. 3). That is, we considered that the variation in the organs of the two species can be described in two dimensions. Therefore, we applied an elliptic Fourier analysis (EFA), a mathematical method for reducing complex curves into their component spatial frequencies (MCLELLAN & ENDLER, 1998). The EFA is based on elliptic Fourier descriptors (EFDs) that can delineate any type of shape with a closed two-dimensional outline, which can then be analyzed statistically (KUHL & GIARDINA, 1982).

First, the male genital organs were extracted and pasted on paperboard. Spermatic ducts were removed and the organs were positioned horizontally on the visual field of a microscope. The ventral views of the organs were photographed with a charge coupled device (CCD) camera mounted on a stereoscopic microscope. For the analysis, the package SHAPE v1.3 (IWATA & UKAI, 2002) was used. The raw images were transformed to black-and-white bitmap images using Adobe illustrator CS2 (Adobe) and Microsoft Paint (Microsoft), accordingly. The images were first converted into chain codes, a coding system describing geometrical information (FREEMAN, 1974) from which normalized EFDs were calculated with a procedure based on the first harmonic ellipse that corresponds to the first Fourier approximation to the contour information (reviewed in LESTREL, 1997). Thus, size, orientation and starting position of the contours were standardized according to the size and alignment of the major axes of the first ellipse, leading to representations of the outlines (i.e., shape) (KUHL & GIARDINA,

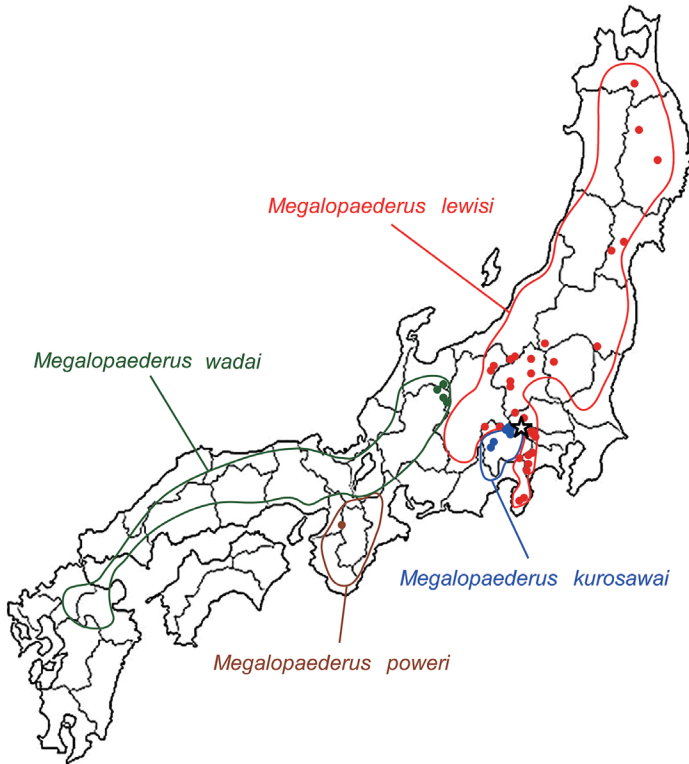


Fig. 1. Distribution map of four *Megalopaederus* species in Japan (tentatively described based on data in WATANABE, 1986) and collection locality. — Star, Locality 1; circle, other collection localities.

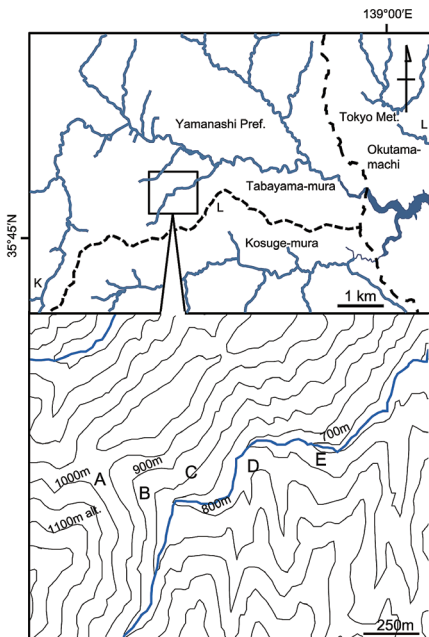


Fig. 2. Study sites A–E located near River Marikogawa, Tabayama-mura, Yamanashi Prefecture. L and K indicate the sites where *Megalopaederus lewisi* and *Megalopaederus kurosawai* were found.

Table 1. Samples examined.

Species	Locality No.	Locality	Number examined	
			Morphology (male)	COI (male and female)
Individuals from the contact zone between <i>Megalopaederus lewisi</i> and <i>M. kurosawai</i> including putative hybrids	1	Near Riv. Marikogawa, Tabayama-mura, Yamanashi Pref.	38	77
<i>Megalopaederus lewisi</i>	2	Ouse, Towada-shi, Aomori Pref.	1	-
	3	Genbédaira, Miyako-shi, Iwate Pref.	1	-
	4	Mt. Shimokurayama, Hachimantai-shi, Iwate Pref.	1	2
	5	Yoshidamasuzawa, Taiwa-chô, Miyagi Pref.	1	-
	6	Mt. Daitôdake, Sendai-shi, Miyagi Pref.	1	-
	7	Miike, Hinoemata-mura, Fukushima Pref.	24	21
	8	Mt. Yamizosan, Tanagura-chô, Fukushima Pref.	1	-
	9	Mt. Akagiyama, Shôwa-mura, Gumma Pref.	3	-
	10	Kirizumi Spa, Annaka-shi, Gumma Pref.	3	-
	11	Mt. Hotakayama, Katashina-mura, Gumma Pref.	3	-
	12	Mt. Tunôti, Takasaki-shi, Gumma Pref.	1	-
	13	Yumoto Spa, Nikkô-shi, Tochigi Pref.	3	-
	14	Mt. Ryokamisan, Chichibu-shi, Saitama Pref.	1	-
	15	Arakawa, Chichibu-shi, Saitama Pref.	2	-
	16	Akigawa-Keikoku, Hinohara-mura, Tokyo Met.	2	-
	17	Nippara, Okutama-chô, Tokyo Met.	1	-
	18	Mizunozawa, Okutama-chô, Tokyo Met.	2	-
	19	Ogawadani, Okutama-chô, Tokyo Met.	1	-
	20	Shiraishizawa, Yamakita-chô, Kanagawa Pref.	1	-
	21	Sengokuhara, Hakone-chô, Kanagawa Pref.	2	-
	22	Mt. Kamiyama, Hakone-chô, Kanagawa Pref.	2	1
	23	Tanzawa, Kanagawa Pref.	1	-
	24	Mt. Tairappyôyama, Yuzawa-chô, Niigata Pref.	1	-
	25	Kouchisawa, Yuzawa-chô, Niigata Pref.	5	-
	26	Kanayama-daira, Hokuto-shi, Yamanashi Pref.	3	-
	27	Mikuni-tôge Pass, Yamanakako-mura, Yamanashi Pref.	1	1
	28	Mt. Manjiroudake, Izu-shi, Shizuoka Pref.	1	-
	29	Amagi-tôge Pass, Izu-shi, Shizuoka Pref.	2	-
	30	Near Riv. Naragawa, Suzaka-shi, Shizuoka Pref.	5	-
	31	Hara-Mura, Nagano Pref.	2	-
	32	Takayama-mura, Nagano Pref.	3	-
<i>M. kurosawai</i>	33	Ichinose-kôgen, Kôshû-shi, Yamanashi Pref.	7	10
	34	Sannose, Kôshû-shi, Yamanashi Pref.	4	6
	35	Mt. Daibosatsurei, Tabayama-mura, Yamanashi Pref.	12	18
	36	Mt. Genjiyama, Fujikawa-chô, Yamanashi Pref.	5	-
	37	Nishizawa-keikoku, Yamanashi-shi, Yamanashi Pref.	8	-
	38	Mt. Amariyama, Nirasaki-shi, Yamanashi Pref.	2	-
	39	Karisaka-tôge, Chichibu-shi, Saitama Pref.	1	-
<i>M. poweri</i>	40	Mt. Kongosan, Chihayaakasaka-mura, Osaka Pref.	-	16
<i>M. wadai</i>	41	Abôtôge, Takayama-shi, Gifu Pref.	-	7
	42	Kamitakarachô, Takayama-shi, Gifu Pref.	-	4
	43	Kamiokachô, Hida-shi, Gifu Pref.	-	10
	44	Arimine, Toyama-shi, Toyama Pref.	-	8
<i>M. kosempoensis fulvocaudatus</i>	45	Sinyi Township, Nantou County, Taiwan	-	1
<i>M. formosanus</i>	46	Fuhshing Township, Taoyuan Country, Taiwan	-	1
<i>Paederus gottschei</i>	47	Jrisan Mts., Gyeongsangnam-do, Republic of Korea	-	1
		Total	158	184

-, not determined.

Table 2. Morphological and mitochondrial type at the study sites.

Morphological type	Mitochondrial type	Site				
		Site A	Site B	Site C	Site D	Site E
Male						
<i>lewisi</i>	<i>lewisi</i>	0	0	1	1	4
<i>lewisi</i>	<i>kurosawai</i>	0	1 <sup>†</sup>	0	0	1 <sup>†</sup>
Intermediate	<i>lewisi</i>	0	0	4	3	2
Intermediate	<i>kurosawai</i>	0	0	3	2	0
<i>kurosawai</i>	<i>lewisi</i>	1 <sup>†</sup>	0	1 <sup>†</sup>	0	0
<i>kurosawai</i>	<i>kurosawai</i>	1	7	3	0	0
<i>lewisi</i>	Unknown	0	0	0	0	1
Intermediate	Unknown	0	0	0	0	1
<i>kurosawai</i>	Unknown	0	0	1	0	0
Unknown*	<i>lewisi</i>	0	0	0	0	0
Unknown*	<i>kurosawai</i>	0	1	1	0	0
Female						
	<i>lewisi</i>	0	1	4	6	12
	<i>kurosawai</i>	5	1	10	1	0
Total number of individuals		7	11	27	13	19

<sup>†</sup>, Incongruence between morphological and mitochondrial types.

\*, due to adult teneral.

1982). The contour shape was described in the first 20 harmonics, and consequently 80 Fourier coefficients (four per harmonic). The 80 coefficients were used as input in principal component analysis, which allowed us to summarize the information assessed in the coefficients and to reduce the dimensionality of the variables (ROHLF & ARCHIE, 1984) in a lower number of principal components (PC). Calculated PC scores of each individual can be considered as quantitative shape variables.

#### Mitochondrial gene analysis

For gene analysis, the thoracic muscle was extracted from adults. Tissue samples were preserved in absolute ethanol. After digestion by proteinase K, total DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Fitchburg, WI, USA). Partial sequence of the mitochondrial cytochrome oxidase subunit I (*COI*) gene (831 bp fragment; primers C1-J-2183 and L2-N-3014; LOXDALE & LUSHAI, 1998; KUBOTA *et al.*, 2011) was amplified by polymerase chain reaction (PCR) under the condition as follows: 94°C for 3 min at beginning followed by 30 cycles of 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products were purified with Exosap-IT (usb, Cleveland, OH, USA). Dye terminator cycle sequencing reaction was performed with an ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster, CA, USA). Products of sequencing reaction were analyzed using an ABI 377 or 3130xl sequencer (Applied Biosystems).

We determined 784 bp of nucleotide sequences in all the samples. All haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB899015–AB899067). For the haplotypes of each gene, maximum likelihood (ML) analysis was conducted using PAUP 4.0 (SWOFFORD, 2002) under the best-fit model selected by Modeltest 3.06 (POSADA & CRANDALL, 1998) based on Akaike's information criterion. Starting trees for branch-swapping were got by stepwise addition, and TBR was used as swapping algorithm. Ten replicates were conducted for the tree search. Confi-



Fig. 3. Ventral view of male genital organs of *Megalopaederus lewisi* (L), putative hybrid (H), and *Megalopaederus kurosawai* (K). Scale: 1.00 mm.

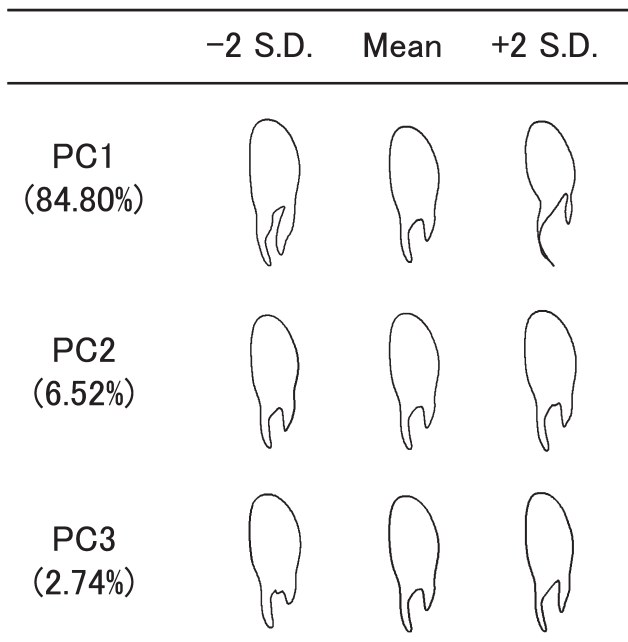


Fig. 4. Reconstructed contours of shape variation described by the first three principal components from the elliptic Fourier analysis (EFA). The percentages indicate how much each principal component explains the shape variation in shape. PC1 contributed a higher percentage, and was a good measure of the difference between the genital morphologies of *Megalopaederus lewisi* and *Megalopaederus kurosawai* (see also Fig. 3L, K).

dence at each node in all analyses was assessed by 100 bootstrap replications. We classified the mitochondrial types at the study sites into *lewisi* and *kurosawai* types, as *M. lewisi* and *M. kurosawai* were clearly distinguishable except for the samples from the contact zone.

#### *Statistical analysis on the geographical clines and relationship of morphological and mitochondrial types*

At the study sites, the geographical clines of male morphological and mitochondrial types were evaluated using the median test, regarding the site numbers as ranks. The relationship between male

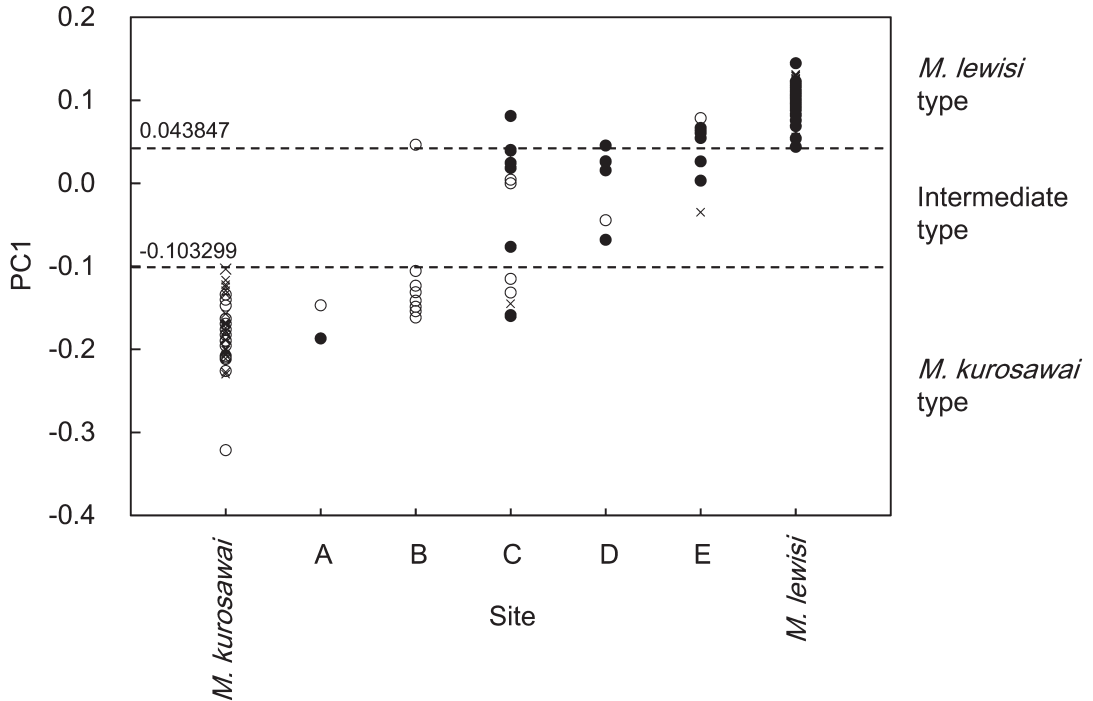


Fig. 5. The first principal components from the elliptic Fourier analysis (EFA) in male genital organs of *Megalopaederus lewisi*, *Megalopaederus kurosawai*, and individuals from the contact zone (Sites A-E). Plot styles indicate mitochondrial types (closed circle, lewisi type; open circle, kurosawai type; cross, not examined).

morphological and mitochondrial types of the individual samples was evaluated using a Fisher's exact test.

## Results

### Morphological analysis

The variation in the Fourier coefficients was reduced to three effective PCs, which cumulatively explained more than 94% (PC1: 84.80%; PC2: 6.52%; PC3: 2.74%) of the total variation. We used the first two PCs to describe the shape variation. Of the two PCs, PC1 contributed higher percentage, and was a good measure of difference between the genital morphologies of *Megalopaederus lewisi* and *M. kurosawai* based on the reconstructed contours (Fig. 4). PC1 completely discriminated

among the morphologies of all individuals of *M. lewisi* (range: 0.043847 to 0.1447444) and all individuals of *M. kurosawai* (range: -0.3214839 to -0.1032999) except for the samples from the contact zone. Therefore, we classified the genital morphological type of each individual collected from the study sites based on PC1 scores into *lewisi* (PC1 score  $\geq$  0.043847), *kurosawai* (PC1 score  $\leq$  -0.1032999), and intermediate ( $-0.1032999 <$  PC1 score  $<$  0.043847) types. Of 38 individuals from the contact zone, nine (23.7%), 14 (36.8%), and 15 (39.5%) individuals were classified into *M. lewisi*, *M. kurosawai*, and intermediate types, respectively (Fig. 5).

The *lewisi* and *kurosawai* types were frequently found in the eastern and western sites of the contact zone, respectively (Table 2; Fig. 5). In addition, the intermediate type was found in the middle



Table 3. Haplotypes determined in this study.

Haplotype	DDBJ Accetion No.	Locality												
		1					4	7	22	27	33	34	35	
		A	B	C	D	E								
TL1	AB899015, AB899036	1		6	4	7	2	21						
TL2	AB899016					3	1							
TL3	AB899017		1											
TL4	AB899018			1										
TL5	AB899019					4								
TL6	AB899020					3								
TL7	AB899021				2									
TL8	AB899022			2										
TL9	AB899023				1									
TL10	AB899024			1		1								
TL11	AB899025					2								
TL12	AB899037										1			
TL13	AB899038								1					
TK1	AB899026, AB899039	3	7	13		1							6	
TK2	AB899027, AB899040	1											1	
TK3	AB899028	1	1		1									
TK4	AB899029, AB899041			1									1	
TK5	AB899030			1										
TK6	AB899042										10	6	4	
TK7	AB899031, AB899043			1	1								1	
TK8	AB899032	1												
TK9	AB899033, AB899044		1		1								4	
TK10	AB899045												1	
TK11	AB899034			1										
TK12	AB899035		1											
		7	11	27	13	19	2	21	1	1	10	6	18	

and eastern sites. The three types were sympatric at site C, and the *lewisi* and *kurosawai* types were sympatric at sites B and C, whereas the distributions of these types were significantly parapatric (median test  $P < 0.001$ ).

#### Mitochondrial gene analysis

We determined 47 haplotypes as shown in Table 2. At the contact zone (sites A–E), 21 haplotypes (TL1–11, TK1–5, 7–9, 11, 12) were found from 75 individuals. From other sites, a total of three haplotypes (TL1, 12, 13) from 25 individuals of *M. lewisi* and seven haplotypes (TK1, 2, 4, 6, 7, 9, 10) from 34 individuals of *M. kurosawai* were determined. As the outgroup, nine haplotypes from 16 individuals of *M. poweri*, 10 haplotypes from 29 individuals of *M. wadai*, and one of each haplotype from individuals of the species from outside of Japan were also determined. The HKY (Hasegawa-Kishino-Yano) + I + G model was selected as the best-fit model of DNA substitution for the ML analysis (AIC = 5806.5518). Based on this model, a single ML tree was constructed (Fig. 6). Japanese species were monophyletic, and four large and distinct clades were recognized. All haplotypes from *M. lewisi* and *M. kurosawai* collected from their distribution ranges except for the contact zone be-



Table 3. Continued.

Haplotype	DDBJ Accetion No.	Locality								
		40	41	42	43	44	45	46	47	
TP1	AB899046	1								
TP2	AB899047	4								
TP3	AB899048	3								
TP4	AB899049	1								
TP5	AB899050	1								
TP6	AB899051	1								
TP7	AB899052	1								
TP8	AB899053	1								
TP9	AB899054	3								
TW1	AB899055		7	2						
TW2	AB899056			1						
TW3	AB899057			1						
TW4	AB899058				1					
TW5	AB899059				2					
TW6	AB899060				5	5				
TW7	AB899061				1					
TW8	AB899062				1					
TW9	AB899063					2				
TW10	AB899064					1				
KE	AB899065						1			
FM	AB899066							1		
GC	AB899067								1	
			16	7	4	10	8	1	1	1

longed to Clades I and II, respectively. Therefore, we considered that Clades I and II originated from *M. lewisi* and *M. kurosawai*, respectively. Clade III and IV seemed to correspond to *M. poweri* and *M. wadai*, respectively. Consequently, we classified the mitochondrial types at the contact zone into the *lewisi* and *kurosawai* types.

The *lewisi* and *kurosawai* types were frequently found at the eastern and western sites of the contact zone, respectively (Fig. 1). Both mitochondrial types were sympatric at sites A–E, but showed strongly uneven distributions (median test  $P < 0.001$ ; Table 2; Fig. 5).

For males, incongruence between the morphological and mitochondrial types in the contact zone was recognized in four of 35 individuals analyzed according to both morphological and mitochondrial types. Two males were identical with *M. lewisi* morphologically, but with *M. kurosawai* from mitochondrial DNA, and the two males were identical as the opposite combination. However, morphological and mitochondrial types were significantly linked (Fisher's exact test,  $P = 0.0176$ ) except for individuals with intermediate morphology. Morphologically intermediate individuals included both *lewisi* and *kurosawai* of mitochondrial types (Table 2).

## Discussion

Except for the contact zone, four species identified by genital morphology formed distinct clades in the mitochondrial tree. It suggested that the taxonomy by WATANABE (1986) is appropriate. However, the phylogenetic relationship among species still remained ambiguous because of the low bootstrap probabilities (Fig. 6).

The genital morphologies of hybrid individuals derived from contact zones are likely to be intermediate between parental populations (KUBOTA & SOTA, 1998; SASABE *et al.*, 2007; MASLY, 2012). In this regard, in the present study, 15 individuals morphologically classified into an intermediate type were assumed to originate from hybridization between *M. lewisi* and *M. kurosawai*. This also supported that the contact zone in this study was a hybrid zone. The family Staphylinidae including the genus *Megalopaederus* is the most species-rich family in the order Coleoptera and in the whole Animal Kingdom (GREBENNIKOV & NEWTON, 2009). The family currently contains more than 55,440 described species placed in about 3,300 genera and 32 subfamilies (GREBENNIKOV & NEWTON, 2009). Despite such large numbers of species, only PILON (1993) has reported hybridization between the species in this family, whereas it should be discovered more in future.

The genital morphology and the frequency of mitochondrial haplotypes changed within a relatively short distance along transect. The genital morphological variation was significantly associated with mitochondrial haplotypes, but there were four individuals showing incongruence between morphology and mitochondrial haplotypes, which suggests that gene flow between the two species occurs in both directions. The interspecific hybridization and subsequent backcrossing results in introgression of mitochondrial genes, as seen in other taxa (ARNOLD, 1997). However, the hybridization needs cost and gene flow seemed to occur only rarely.

The disequilibrium between the genital morphology and mitochondrial haplotypes may suggest two following possibilities. First, *M. lewisi* and *M. kurosawai* have just met at the contact zone and the gene flow between them has not occurred enough yet there. Second, reproductive isolation prevent these two species from genetic mixture. Theory predicts that incomplete premating isolation and negative selection on hybridization between two species results in the maintenance of a parapatric distribution or extinction of one species (REBEIRO & SPIELMAN, 1986; KUNO, 1992). In addition, *M. lewisi* and *M. kurosawai* are continuously and parapatrically distributed in the cool-temperate forests of eastern Honshu (Fig. 1). Thus, the first possibility seems to be unlikely. Alternatively, it is possible that the incomplete but relatively strong reproductive isolation seems to separate their distribution ranges in the case of *M. lewisi* and *M. kurosawai* like as some carabid species combinations (KUBOTA, 1988; SOTA & KUBOTA, 1998; SOTA *et al.* 2000; KUBOTA *et al.* 2013).

In summary, reproductive isolation between *M. lewisi* and *M. kurosawai* is inferred to be relatively strong, although introgressive hybridization occurs rarely. Their taxonomic status as two distinct species is appropriate based on the restricted interspecific gene flow. In addition, artificial hybridization experiments are useful to assess the process of reproductive isolation between species. However, how to raise *Megalopaederus* species has not been established, and is an issue to be explored in future studies.

In this study, the mitochondrial *COI* gene was used alone for a genetic analysis. Since the *COI* gene has been well diversified corresponding to four morphological species of *Megalopaederus*, the hybridization was detected genetically. However, an analysis based on a nuclear gene in the future should help understanding the hybridization of *Megalopaederus* species.

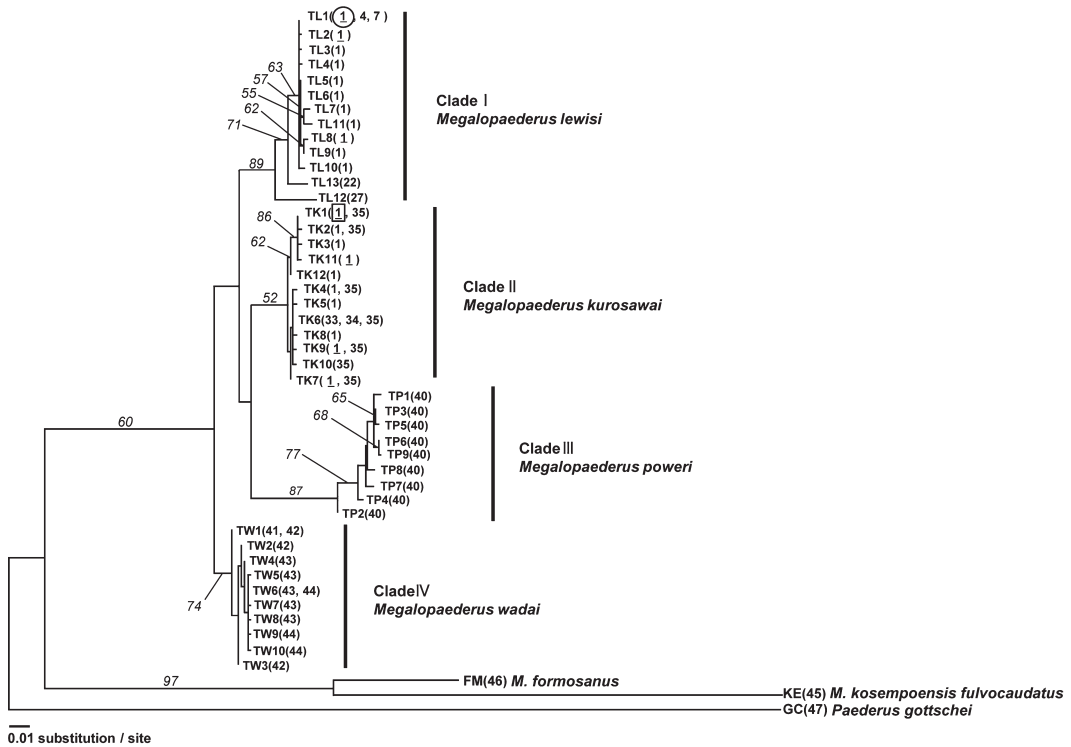


Fig. 6. Maximum likelihood (ML) tree of *COI* gene haplotypes of *Megalopaederus* species. Numbers above the branches indicate bootstrap probability (100 replicates). Numbers following OTUs indicate the site numbers. Species names indicate the species to which the clades essentially corresponded. Site numbers with rectangle, circle, and underline indicate inclusion of individuals morphologically identified as *M. lewisi*, *M. kurosawai*, and intermediate type, respectively. *M. kosempoensis fulvocaudatus* (45), *M. formosanus* (46), and *Paederus gottschei* (47) were treated as the outgroup.

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## 要 約

伏見速雄・岸本年郎・久保田耕平：オオアリガタハネカクシ属 *Megalopaederus* (コウチュウ目ハネカクシ科) 近縁 2 種間の分布境界における自然雑種形成とミトコンドリア遺伝子流動。——ハネカクシ科 2 種, ルイスオオアリガタハネカクシ *Megalopaederus lewisi* とクロサワオオアリガタハネカクシ *M. kurosawai* の分布境界における自然雑種形成を, 雄交尾器の形態とミトコンドリア遺伝子シクロームオキシダーゼ・サブユニット I (*COI*) 遺伝子の変異にもとづいて調査した。結果からはこれらの 2 種が分布境界で交雑帯を形成していることを示唆する。これらの種の生殖隔離は側所分布を維持するほどに強く, 種間のミトコンドリア遺伝子の流動をおこすほどに不完全だと考えられる。

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