Eur. J. Entomol. **104**: 165–172, 2007
http://www.eje.cz/scripts/viewabstract.php?abstract=1214
ISSN 1210-5759

Geographic variation in body and ovipositor sizes in the leaf beetle *Plateumaris constricticollis* (Coleoptera: Chrysomelidae) and its association with climatic conditions and host plants

TEIJI SOTA[1], MASAKAZU HAYASHI[2] and TSUYOSHI YAGI[3]

[1]Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto, 606-8502, Japan;
e-mail: sota@terra.zool.kyoto-u.ac.jp

[2]Hoshizaki Green Foundation, Izumo, Shimane, 691-0076, Japan

[3]The Museum of Nature and Human Activities, Hyogo, Sanda, Hyogo, 669-1546, Japan

Keywords. Chrysomelidae, life history, molecular phylogeny, wetland, 28S rRNA gene

Abstract. *Plateumaris constricticollis* is a donaciine leaf beetle endemic to Japan, which lives in wetlands and uses Cyperaceae and Poaceae as larval hosts. We analyzed geographic variation in body size and ovipositor dimensions in three subspecies (*constricticollis*, *babai*, and *toyamensis*) in different climatic conditions and on different host plants. In addition, the genetic differentiation among subspecies was assessed using nuclear 28S rRNA gene sequences. The body size of subspecies *toyamensis* is smaller than that of the other subspecies; mean body size tended to increase towards the northeast. Ovipositor length and width are smaller in subspecies *toyamensis* than in the other subspecies. Although these dimensions depend on body size, ovipositor length still differed significantly between *toyamensis* and *constricticollis-babai* after the effect of body size was removed. Multiple regression analyses revealed that body size and ovipositor size are significantly correlated with the depth of snow, but not temperature or rainfall; sizes were larger in places where the snowfall was greatest. Haplotypes of the 28S rRNA gene sequence were not shared among the subspecies. Subspecies *constricticollis* and *babai* each had a unique haplotype, whereas subspecies *toyamensis* had four haplotypes, indicating differentiation among local populations within *toyamensis*. The evolution of body and ovipositor size in relation to habitat conditions and host plants is discussed.

INTRODUCTION

Geographic variation in insect body size reflects the differential adaptation of local populations to local environmental conditions such as climatic factors, food availability, and the presence of related species (Masaki, 1967, 1978; Roff, 1980; Mousseau & Roff, 1989; Blanckenhorn & Fairbairn, 1995; Sota et al., 2000a, b). As body parts are particularly important in the adaptation to habitat and food conditions, the ovipositor and associated structures also show marked geographic variation in response to abiotic conditions (Masaki, 1979, 1986; Bradford et al., 1993; Mousseau & Roff, 1995) and host plant morphology (e.g., Toju & Sota, 2006a, b). Analyses of geographic variation in body size and the size of other functional parts are important for understanding locally variable adaptations, which produce morphological diversity within insect species and potentially lead to speciation.

Here, we analyze the geographic variation in body and ovipositor sizes of a wetland leaf beetle, *Plateumaris constricticollis* (Coleoptera, Chrysomelidae), a Donaciinae species endemic to the Japanese archipelago the larvae of which feed on the roots of monocotyledonus plants. Fossils of this species are known from the late Pliocene, along with another endemic species *P. akiensis*, although few extant species occur in late Pliocene and early Pleistocene deposits (Hayashi, 2002b, 2004; Hayashi & Shiyake, 2002). Thus, *P. constricticollis* represents one of the few ancient insect species persisting since the late Pliocene. Whereas *P. akiensis* is confined to a small area in western Honshu, *P. constricticollis* is distributed widely from western Honshu to Hokkaido and is polytypic with three subspecies (Hayashi, 2004; Hayashi & Shiyake, 2004) characterized by external and internal (ovipositor) characters. The two northern subspecies, *constricticollis* and *babai*, have equally long ovipositors with an acutely angled apex (Fig. 1), but differ in body coloration. The southwestern subspecies, *toyamensis*, has a shorter ovipositor with a right-angled apex and a smaller body than the other two subspecies (Fig. 1). The different morphological characters of the *P. constricticollis* subspecies may reflect local adaptations to different host plants or climatic conditions; the length of its ovipositor appears to match the stem thickness of its host plants (T. Yagi, unpubl. observ.), and larger body may have resulted from an adaptation to a climate with higher snowfall (Ego et al., 1988; Tominaga, 1988).

Here, we describe the variation in body and ovipositor size among local populations and analyze the effects of environmental variables on morphological variation. Further, we examine the relationship between morphological (subspecies) differentiation and genetic differentiation using partial sequence data from the nuclear 28S rRNA gene.

MATERIAL AND METHODS

Morphological analysis

A total of 235 female beetles were collected from 20 sites for morphological analysis (Table 1, Fig. 1). To measure pronotum

TABLE 1. Sampling localities and number of specimens of *Plateumaris constricticollis* used in the morphological analysis of female beetles and molecular phylogenetic analysis.

Locality	Latitude, °N	Longitude, °E	Host plants available[a]	Sample size	
				Morphology	DNA
Subspecies *constricticollis*					
1. Toyotomi, Hokkaido	45°13′00″	141°41′59″	C	8	1
2. Kuromatsunai, Hokkaido	42°38′14″	140°20′18″	C, P	14	1
3. Nanae, Hokkaido	41°59′42″	140°39′53″	C, P	–	1
4. Ohma, Aomori	41°32′42″	140°54′44″	?	8	1
5. Takizawa, Iwate	39°47′01″	141°01′49″	?	8	–
6. Onoda, Miyagi	38°30′07″	140°39′25″	C, P	20	1
7. Goshikinuma, Yamagata	38°29′38″	140°00′02″	?	–	1
8. Sekikawa, Niigata	38°03′42″	139°37′25″	P	3	1
9. Oguni, Yamagata	38°00′05″	139°51′23″	P	8	–
Subspecies *babai*					
10. Shiobara, Fukushima	37°37′5″	140°00′27″		20	1
11. Hanawa, Fukushima	36°57′13″	140°25′28″	C, P	5	1
12. Fujihara, Tochigi	36°54′53″	139°44′10″	C, E	10	1
13. Numata, Gunma	36°46′31″	139°04′13″	C, P	22	1
14. Funabashi, Chiba	35°43′33″	140°02′20″	C	6	1
15. Shitada, Niigata	37°26′38″	139°07′42″	C, P	–	1
16. Yamakoshi, Niigata	37°20′09″	138°54′27″	P	6	–
17. Tohkaichi, Niigata	37°06′21″	138°46′29″	P	16	–
18. Itoigawa, Niigata	36°57′35″	137°54′34″	P	–	1
19. Shinano, Nagano	36°48′56″	138°12′48″	C, P	20	1
Subspecies *toyamensis*					
20. Miyagawa, Gifu	36°21′43″	137°14′22″	C, P	20	1
21. Tugu, Aichi	35°11′15″	137°35′28″	C	9	1
22. Ohkawachi, Hyogo	35°09′10″	134°41′24″	E	20	1
23. Kamisaibara, Okayama	35°17′46″	133°54′08″	C, P	5	1
24. Tessei, Okayama	34°55′00″	133°21′06″	C, P	7	1

[a] C – *Carex* (Cyperaceae); E – *Eleocharis* (Cyperaceae); P – *Phragmites* (Poaceae).

width, right elytral length, and ovipositor length and width, a dorsal image of the whole body and dorsal and lateral images of the ovipositor were taken using a CCD camera mounted on a microscope. The software ImageJ 1.34s (National Institute of Health, USA) was used to determine the dimensions from the images (see Fig. 2). Ovipositor length was measured along the curved line from the apex to the base on the lateral image of the chitinized ovipositor. The maximum width on the dorsal image was used as ovipositor width.

In the statistical analysis, all millimeter dimensions were $\log_{10}$ transformed to ensure normality and homoscedasticity. All statistical analyses of the morphological data were performed using JMP 5.0.1J (SAS Institute Inc.). To reveal the climatic factors affecting body size variation, annual mean temperature, annual rainfall, and snowfall data from Mesh Climatic Data 2000 (Japan Meteorological Agency, 2002) were used in a multiple regressions with stepwise model selection. For snowfall, the maximum depth of snow in February, among others (maximum depth of snow in December–March, and the average and maximum of these monthly data), because the *F*-values were highest for the effect of this variable on body and ovipositor sizes. In the multiple regression, subspecies (0 = *toyamensis*, 1 = *babai* + *constricticollis*) were included as an independent variable.

DNA sequencing and phylogenetic analysis

To determine the genetic relationship among the subspecies, partial sequences of the nuclear 28S rRNA gene (28S) were analyzed. A total of 20 ethanol-fixed specimens from 20 localities were used. In addition, samples of the four other Japanese *Plateumaris* species were used: *P. weisei* (*n* = 1 from Higashikawa, Hokkaido), *P. akiensis* (*n* = 1 from Geihoku, Hiroshima), *P. sericea* (*n* = 1 from Shimane) and *P. shirahatai* (*n* = 1 from Iwaizumi, Iwate).

Total genomic DNA was extracted from muscles using an AquaPure Genomic DNA Kit (Bio-Rad Laboratories, Hercules, CA, USA). An 804-bp fragment of 28S was PCR-amplified using the primer pair 28S-01/28SR-01 (Kim et al., 2000). For direct sequencing of the PCR products, a dye terminator cycle-sequencing reaction was performed using an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit, followed by electrophoresis on an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA).

Sequence alignment was done manually without gaps. A maximum-likelihood tree was obtained using PHYML version 2.4.4 (Guindon & Gascuel, 2003) with a GTR+I+G substitution model. Node support was obtained using 1000 replicates of non-parametric bootstrapping. A statistical parsimony network was

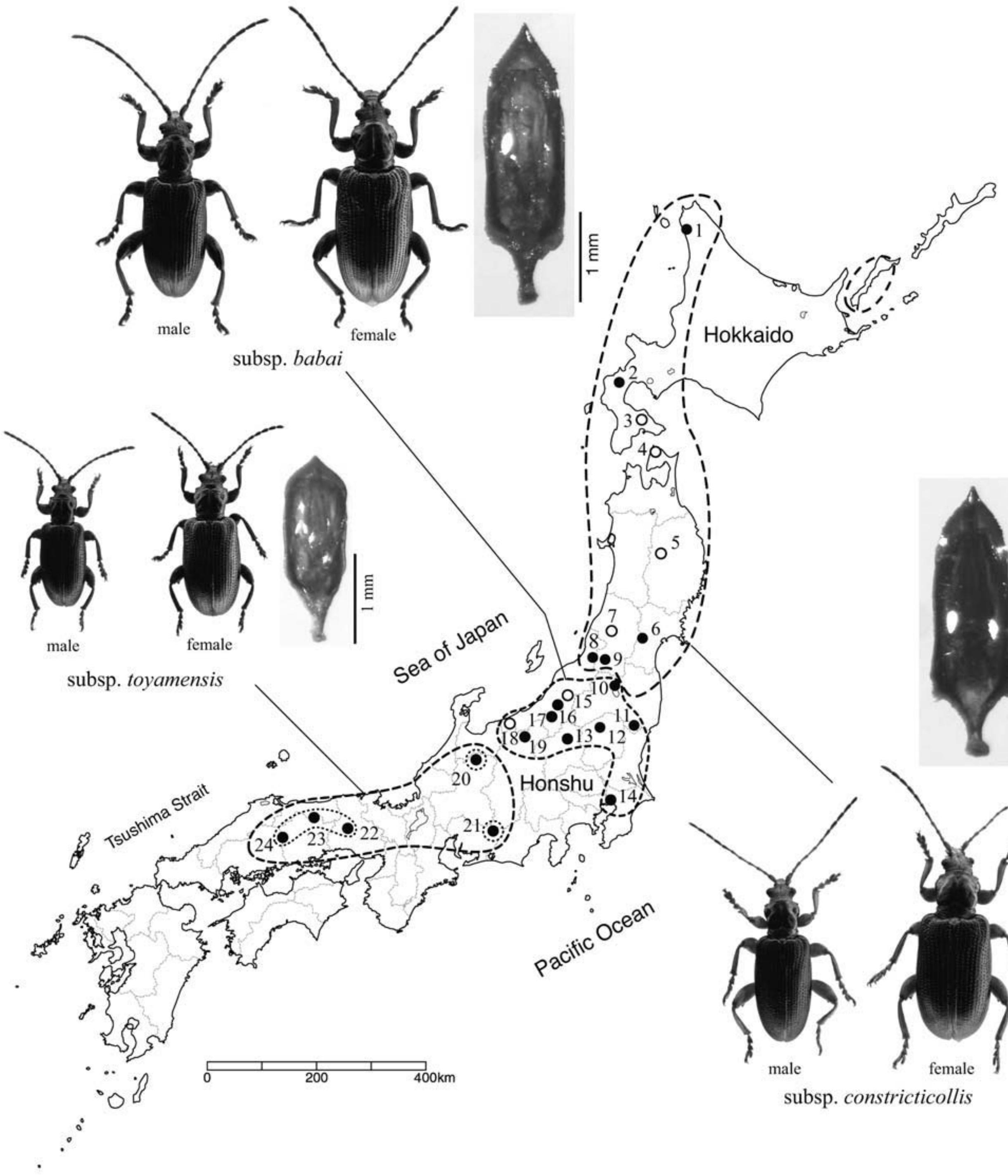

Fig. 1. Sampling localities of *Plateumaris constricticollis* (closed and open circles). Open circles represent localities where DNA samples only were collected. See Table 1 for locality numbers beside the circles. Photographs are of adult beetles and the ovipositors of three subspecies.

constructed using TCS version 1.21 (Clement et al., 2000) and a 95% parsimony connection limit.

RESULTS

The elytral length (EL) and pronotum width (PW) differed among subspecies (nested ANOVA; Table 2 a), and were smaller for subspecies *toyamensis* than for the other two subspecies (least-squares means ± standard error [SE], in mm; log$_{10}$-transformed EL: ssp. *babai*, 0.81 ± 0.0026; ssp. *constricticollis*, 0.80 ± 0.0032; ssp. *toyamensis*, 0.74 ± 0.0038; log$_{10}$-transformed PW: ssp. *babai*, 0.34 ± 0.0029; ssp. *constricticollis*, 0.33 ± 0.0035; ssp. *toyamensis*, 0.27 ± 0.0041). The geographical clines of mean EL (Fig. 3a, b) and PW with latitude or latitude were not simple. Both size dimensions had positive coefficients for longitude, but not for latitude (multiple regression; EL: latitude effect, df = 1, F = 0.3, P = 0.587; longitude effect, df = 1, F = 6.2, P = 0.024; PW: latitude

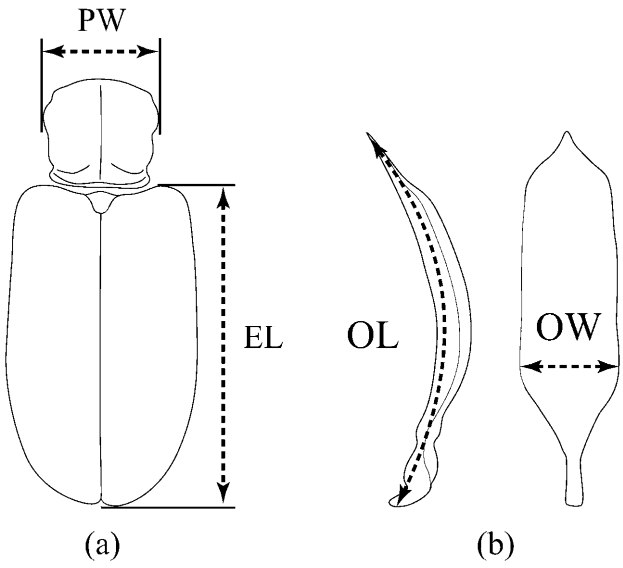

Fig. 2. Measurements of body size dimensions (a: pronotum width, PW; right elytral length, EL) and ovipositor size dimensions (b: length in lateral view, OL; width in dorsal view, OW).

effect, df = 1, $F = 0.2$, $P = 0.633$; longitude effect, df = 1, $F = 7.0$, $P = 0.0167$). These size dimensions increased with latitude from about 35°N to 37°N, and were relatively constant between 37°N and 45°N; body size was regressed significantly on latitude when the northern subspecies *constricticollis* populations were excluded (EL: df = 11, $F = 43.7$, $P < 0.0001$; PW: df = 11, $F = 55.6$, $P < 0.0001$).

Both ovipositor length (OL) and width (OW) were correlated with body size (OL–EL: $r^2 = 0.86$; OL–PW: $r^2 = 0.85$; OW–EL, $r^2 = 0.79$; OW–PW: $r^2 = 0.84$; all $P < 0.0001$; Fig. 4a). Both OL and OW differed among subspecies (nested ANCOVA; Table 2 b, c). The population-mean OL was correlated with mean EL (and PW). The interaction effect of EL and subspecies was not significant (ANOVA; $P > 0.05$). After removing the interaction term, subspecies had a significant effect on OL (ANCOVA; EL effect: df = 1, $F = 41.7$, $P < 0.0001$; subspecies effect: df = 2, $F = 12.0$, $P = 0.0007$). OL was much shorter in subspecies *toyamensis* (least square mean ± SE of $\log_{10}$[OL × 10, mm]: 1.47 ± 0.013) than in subspecies *babai* (1.55 ± 0.008) and subspecies *constricticollis* (1.54 ± 0.008).

The population-mean OW was correlated with mean OL (Fig. 4b). The interaction effect of OL and subspecies was not significant (ANOVA; $P > 0.05$). After removing the interaction term, subspecies did not have a significant effect on OW (ANCOVA; OL effect: df = 1, $F = 29.1$, $P < 0.0001$; subspecies effect: df = 2, $F = 3.4$, $P = 0.0598$).

Stepwise multiple regression analysis of body and ovipositor dimensions with three climatic variables commonly resulted in models with maximum depth of snow in February, together with the effect of subspecies difference (*toyamensis* vs. *babai* + *constricticollis*) in body size and ovipositor dimensions (Table 3). Only for OL was a model selected that included rainfall, but the effect of rainfall was not significant. Body and ovipositor size increased with depth of snow (Fig. 3c, d).

The 28S sequences were aligned unambiguously without requiring gaps. The 28S haplotype (sequence) was monomorphic within subspecies *constricticollis* ($n = 7$) and subspecies *babai* ($n = 8$), but four haplotypes were found for subspecies *toyamensis* ($n = 5$; Fig. 5). The statistical parsimony network revealed that the haplotypes from different subspecies were connected via missing haplotypes. In particular, subspecies *constricticollis* was separated by three or more steps from subspecies *babai*

Table 2. Nested analysis of variances for the female body and ovipositor sizes.

(a) Elytral length (EL) and pronotum width (PW)

Dependent variable:	$\log_{10}$ EL		$\log_{10}$ PW	
Source variable	df	*F*	df	*F*
Locality (subspecies)	17	18.5***	17	16.3***
Subspecies	2	141.1***	2	114.5***

(b) Ovipositor length (OL) and width (OW)

Dependent variable:	$\log_{10}$ OL		$\log_{10}$ OW	
Source variable	df	*F*	df	*F*
Log_{10} EL	1	110.5***	1	129.7***
Locality (subspecies)	17	14.0***	17	13.1***
Subspecies	2	305.1***	2	8.7***

(c) Ovipositor length (OL) and width (OW)

Dependent variable:	$\log_{10}$ OL		$\log_{10}$ OW	
Source variable	df	*F*	df	*F*
Log_{10} PW	1	83.8***	1	129.9***
Locality (subspecies)	17	13.8***	17	11.0***
Subspecies	2	266.8***	2	12.6***

***, $P < 0.0001$.

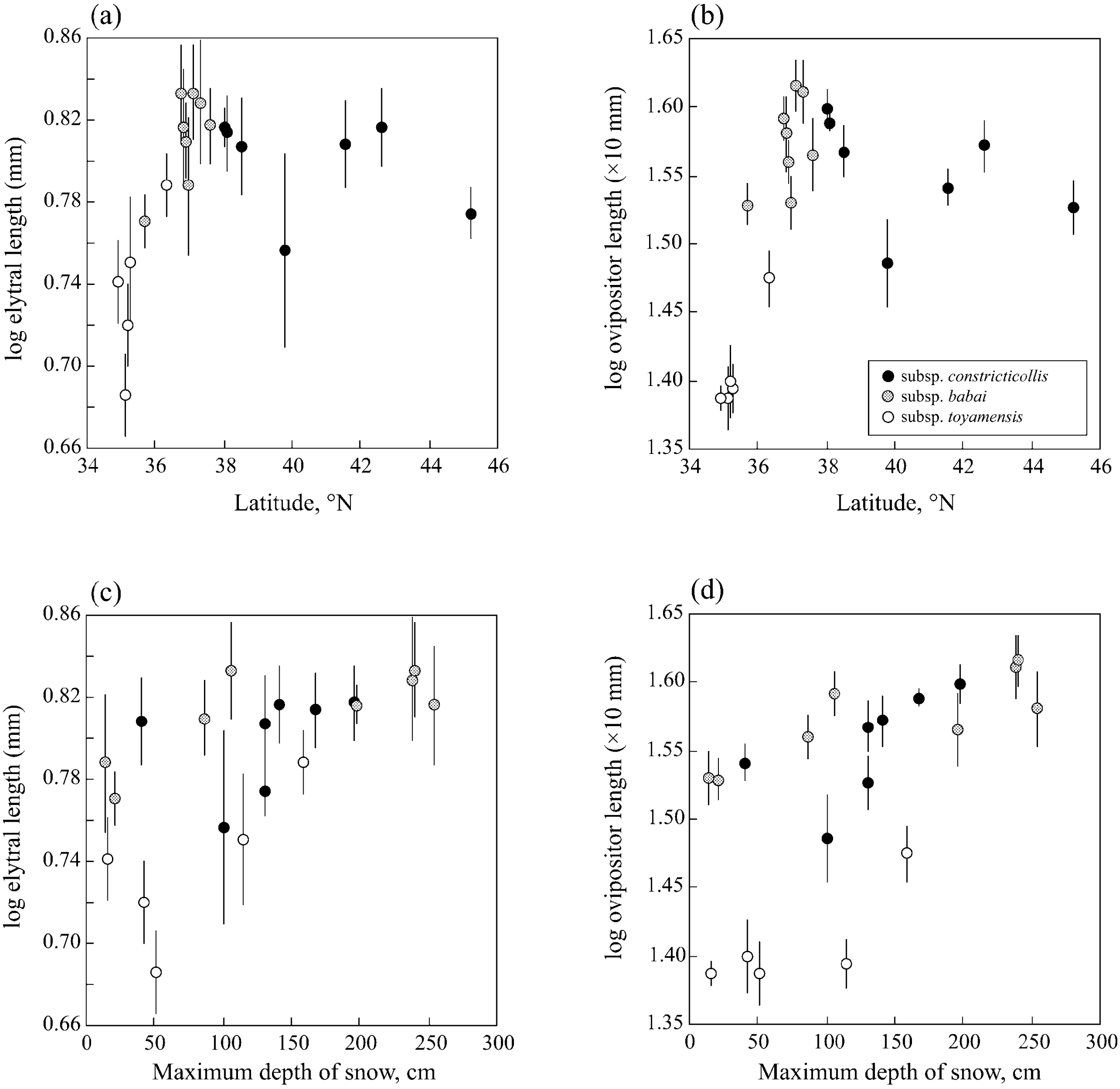

Fig. 3. Variation in elytral length (a, c) and ovipositor length (b, d) among local populations of *Plateumaris constricticollis*, relative to latitude (a, b) and maximum depth of snow in February (c, d). Values are mean ± SD.

and *toyamensis*, indicating a deep coalescence between these subspecies.

DISCUSSION

The three subspecies of *P. constricticollis* were distinguished by their nuclear 28S haplotypes. The 28S sequence was monotypic for both *constricticollis* and *babai* subspecies, whereas four distinct haplotypes were found for *toyamensis*. Although the sample size was small, the 28S gene sequences generally showed very little variation within or between populations. Indeed, the sequences of *P. weisei*, *P. sericea* and *P. shirahatai* are shared by continental populations of the same species (T. Sota, unpubl. data). Thus, differentiation among the subspecies of *P. constricticollis* has a substantial historical basis. Subspecies *constricticollis* exhibited a rather large genetic differentiation from subspecies *babai*, despite their small morphological differences (body colour), indicating a long period of differentiation between the two subspecies without strong divergent selection on morphology. The monotypic haplotypes of these subspecies may be attributed to bottleneck events following range contraction. In contrast, the *toyamensis* subspecies exhibited haplotype divergence among localities, suggesting the relatively long persistence of segregated local populations. A more detailed analysis of *P. constricticollis*'s historical biogeography using mitochondrial cytochrome oxidase subunit I gene sequences will be reported elsewhere (Sota & Hayashi, unpblished data).

Fossils of *P. constricticollis* are recorded from the late Pliocene to the late Pleistocene (e.g., Fossil Insect Research Group for Nojiri-ko Excavation, 1987, 1990; Hayashi, 1999a, b, 2004b) and provide insight into the evolution of body size in subspecies *babai*. The ovi-

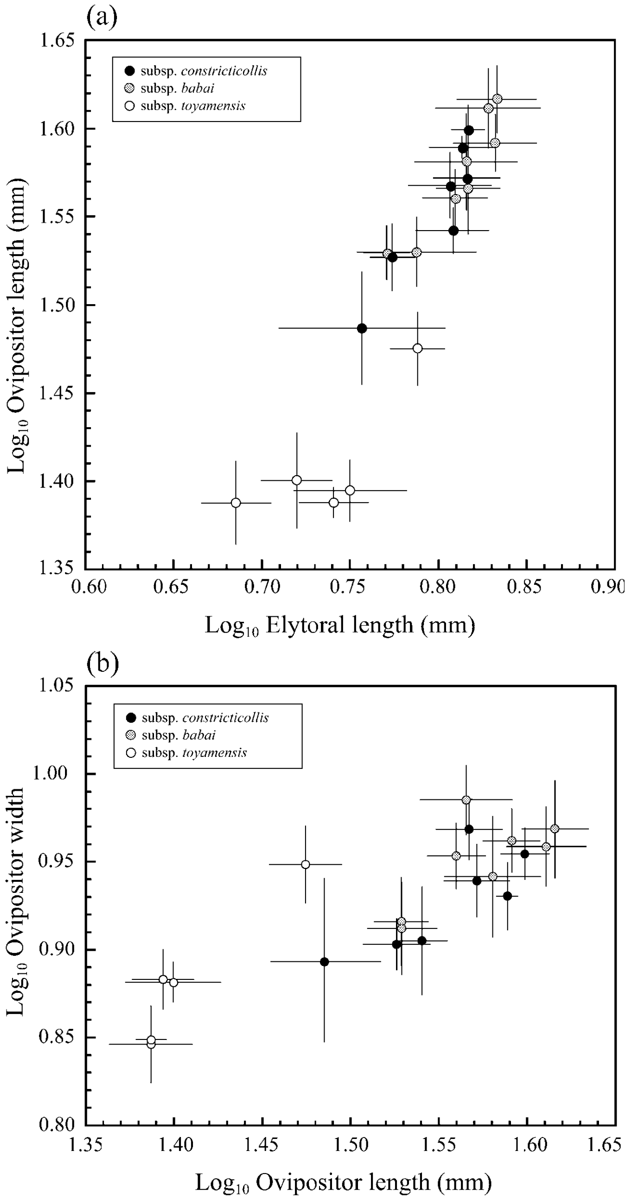

Fig. 4. Relationship between ovipositor length and elytral length (a), and between ovipositor width and ovipositor length (b) in *Plateumaris constricticollis*. Values are population mean ± SD.

positor shape of early Pleistocene fossils of *P. constricticollis* from the Uonuma Formation in Niigata, Honshu (within the present distribution of subspecies *babai*) is similar to that of the current subspecies *babai*, but the body size is small like that of subspecies *toyamensis*, suggesting that the ancestral body size of subspecies *babai* may have been smaller than it is today (Hayashi, 1999a). Similarly, *P. constricticollis* fossils obtained from Plio-Pleistocene strata in Saitama resemble subspecies *babai* and *constricticollis*, but the body size is smaller than in the present subspecies *babai* (Hayashi, 1999b). In addition, fossils of *P. constricticollis* found in strata from the last glacial period at Nojiriko, Nagano, Honshu, identified as subspecies *babai*, are smaller than beetles in a nearby extant population, suggesting an increase in body size during the postglacial period (Fossil Insect Research Group for Nojiri-ko Excavation 1987, 1990). Thus, the body sizes of subspecies *babai* and *constricticollis* may have changed along with climatic changes. Fossils identified as subspecies *toyamensis*, based on ovipositor shape and external morphology, occur in the mid-Pleistocene (0.3 Ma) deposits in Gifu, central Honshu (Ego et al., 1988); the body size of these fossils is comparable to the extant *toyamensis* in the same region, indicating there has not been a body size change in this subspecies.

The body size of *P. constricticollis* did not show simple clinal variation, as is expected based on local adaptation to temperature conditions and a fixed life cycle (voltinism; Masaki, 1967; Roff, 1980). Because Tominaga (1988) mentioned the possibility of selection for large body size in areas with deep snow (see also Ego et al., 1988), we tested the effect of climatic factors, including temperature, rainfall, and snowfall, on body and ovipositor dimensions, and found that only depth of snow had a consistent positive effect on these dimensions. Heavy snowfall in the central northern region of Honshu, facing the Sea of Japan, is associated with the inflow of the warm Tsushima Current from the western Tsushima Channel. The Tsushima Channel was closed until the beginning of the Pleistocene and has been open since, except during glacial periods (Kitamura et al., 2001; Kitamura & Kimoto, 2004). The correlation of body size with snowfall and the probable change in snowfall pattern after

Table 3. Model selected by the stepwise multiple regression analysis. Explanatory variables were annual mean temperature, mean annual rainfall, maximum depth of snow in February and subspecies (0 = *toyamensis*; 1 = *babai* + *constricticollis*).

Dependent variable[a]	log EL	log PW	log OL	log OW
Model				
R^2	0.72	0.68	0.91	0.65
df	2	2	3	2
F	21.8***	17.8***	53.8***	15.6***
Regression coefficient:				
Independent variable				
Subspecies (df = 1)	–0.028***	–0.028***	–0.074***	–0.021**
Snowfall (df = 1)	2.1×10^{-4} *	2.0×10^{-4} **	2.6×10^{-4} *	2.7×10^{-4} **
Rainfall (df = 1)	—	—	1.9×10^{-5} ^{NS}	—

[a] EL – elytral length; PW – pronotum width; OL – ovipositor length; OW – ovipositor width NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

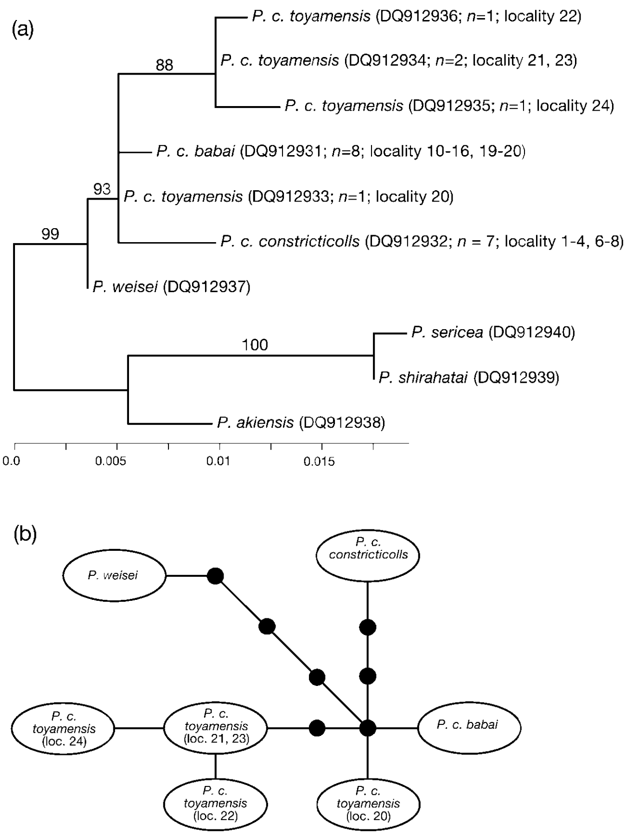

Fig. 5. Phylogram resulting from a maximum-likelihood analysis of the nuclear 28S rRNA gene (upper) and a statistical parsimony network for the same sequence data (lower). GenBank accession numbers for the sequence data are shown in the upper panel.

the early Pleistocene may explain the aforementioned change in the body size of *P. constricticollis* detected by comparing fossil and extant specimens. Thus, with the inflow of the Tsushima Current, the body size of *P. constricticollis* in the north, on the Sea of Japan, would have increased in response to changes in habitat conditions due to heavy snowfall.

The differences in body and ovipositor sizes among the subspecies can be associated with differences in life-history traits. The two northern subspecies, *constricticollis* and *babai*, emerge as adults in June and early July. The adult beetles mate on *Carex* spp. and other plants, but have never been observed feeding. Therefore, females are thought to deposit eggs immediately after emergence and mating, without feeding (Fossil Insect Research Group for the Nojiriko Excavation, 1985). In contrast, adult beetles of subspecies *toyamensis* emerge in late May and June, and feed on pollen and leaves of *Carex* and *Scirpus* spp. Ego et al. (1988) and Tominaga (1988) hypothesized an association between large body size and a climate with heavy snowfall, and suspected that the longer larval period under snow cover results in delayed adult emergence and asynchrony with host plant phenology, favouring both the enlargement of body size and gonad maturation without adult feeding. In the region occupied by subspecies *toyamensis*, the relatively mild climate allows for a short larval period, and natural selection may favour early adult emergence at a smaller body size and active feeding for gonad maturation. In the region of subspecies *babai* and *constricticollis*, however, the cool climate would delay larval development and the start of adult activity, and selection would favour adult emergence at a larger size and immediate oviposition without feeding.

Although ovipositor length largely depended on body size, the difference in ovipositor shape between subspecies *toyamensis* and *constricticollis-babai* can be related to differences in their host plants. Some of the larval host plants of *P. constricticollis* are known: *Carex* sp. for subspecies *constricticollis* (Narita, 2003); *Carex thunbergii*, *Carex ampliata* and *Phragmites australis* for subspecies *babai* (Hayashi, 2002a; Narita, 2003); and *Carex* sp. and *Eleocharis* sp. for subspecies *toyamensis* [Narita, 2003; Hayashi, 2005; Narita (2003) records *Scirpus* sp., but it was actually *Eleocharis* sp.; Y. Narita, pers. comm.]. At some of the locations where subspecies *constricticollis* and *babai* were collected, only reeds of *Phragmites australis* were available and likely to be the sole host plant (Table 1). Ovipositor morphology may represent an adaptation to different host plants because the importance of matching ovipositor length and host plant stem diameter is indicated by laboratory observations (T. Yagi, unpubl. data). Females of subspecies *toyamensis* with short ovipositors laid eggs in the thin stems of *Eleocharis* and *Carex*, whereas females of subspecies *babai* from Niigata and Nagano, which have long ovipositors, could not use these hosts and laid eggs in the thick stems of reeds. Interestingly, the long ovipositor of subspecies *babai* went through the stem of *Eleocharis* and the eggs were laid on the outside of the stem. Because reeds are more common in the habitats of subspecies *babai* and *constricticollis* than in the habitats of *toyamensis* (Table 1), the elongated ovipositor in the former subspecies may an adaptation allowing the use of this plant.

In conclusion, the differentiation in body size and ovipositor size and shape among the subspecies of *P. constricticollis* can be attributed to differential adaptation to host plant and climatic conditions. However, the evolutionary processes remain to be explored further, based on the subspecies' life histories in the field. In particular, more information is needed on host plant use and the life cycle, especially during the larval period.

ACKNOWLEDGEMENTS. We thank K. Katsura and Y. Nagahata for samples, and Y. Narita for information on host plants. Thanks are also due to anonymous reviewers for their helpful comments. Supported by Grants-in-Aid from Japan Society for the Promotion of Science (No. 17405007) and from Ministry of Education, Culture, Sports, Science and Technology, Japan (21st Century COE Research A14).

REFERENCES

Blanckenhorn W.U. & Fairbairn D.J. 1995: Life history adaptation along a latitudinal cline in the water strider Aquarius remigis (Heteroptera: Gerridae). *J. Evol. Biol.* **8**: 21–41.

Bradford M.J., Guerette P.A. & Roff D.A. 1993: Testing hypotheses of adaptive variation in cricket ovipositor length. *Oecologia* **93**: 263–267.

Clement M., Posada D. & Crandall K.A. 2000: TCS, a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657–1660.

Ego M., Katsura K., Kanazawa I., Kinugasa N., Tominaga O., Harusawa K., Hosoi T. & Miyatake Y. 1988: Distribuion process and phylogeny of donaciine beetles inferred from fossil data. In Sato M. (ed.): *Japanes Beetles, Their Origin and Speciaiton*. Tokai University Press, Tokyo, pp. 165–179.

Fossil Insect Research Group for the Nojiriko Excavation 1985: *Atlas of the Japanese Donaciinae: Guide for Identification of the Fossil Donaciine Beetles.* Fossil Insect Research Group for the Nojiriko Excavation, Osaka, 182 pp. [in Japanese].

Fossil Insect Research Group for the Nojiri-ko Excavation 1987: Fossil insects obtained from the Nojiri-ko Formation in the 9th Nojiri-ko excavation and the 4th hill site excavation. *Monograph Assoc. Geol. Collab. Japan* **37**: 93–110 [in Japanese].

Fossil Insect Research Group for the Nojiri-ko Excavation 1990: Fossil insects obtained from the Nojiri-ko Formation during the 10th Nojiri-ko and the 5th hill site excavation. *Monograph Assoc. Geol. Collab. Japan* **32**: 117–136 [in Japanese].

Guindon S. & Gascuel O. 2003: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**: 696–704.

Hayashi M. 1999a: Late Pliocene to Early Pleistocene donaciinae fossils from the Uonuma Formation in Higashikubiki hills, Niigata Prefecture, Japan, with description of a new species (Coleoptera: Chrysomelidae: Donaciinae). *Bull. Osaka Mus. Nat. Hist.* **53**: 1–22.

Hayashi M. 1999b: A revisional study on fossil Donaciinae from the Plio-Pleistocene Kazusa Group in Saitama Prefecture, Japan, with reference to phylogeny of the extinct Plateumaris dorsata Hayashi (Coleoptera, Chrysomelidae). *Earth Sci.* **53**: 38–52.

Hayashi M. 2002a: Host plants of Plateumaris constricticollis babai Chûjô (Coleoptera: Chrysomelidae: Donaciinae). *Entomol. Rev. Japan* **57**: 203–204.

Hayashi M. 2002b: Late Cenozoic Coleoptera of Japan (Part 4): Donaciinae. *Gekkan-Mushi* **374**: 36–38 [in Japanese].

Hayashi M. 2004a: Revisional study on Japanese members of Donaciinae (Coleoptera: Chrysomelidae). *Bull. Hoshizaki Green Foundation* **7**: 29–126.

Hayashi M. 2004b: Faunal changes in Donaciinae during the Quaternary in central Japan (Coleoptera, Chrysomelidae). In Jolivet P., Santiago-Blay J.A. & Schmitt M. (eds): *New Development in the Biology of Chrysomelidae*. SPB Academic Publishing, The Hague, pp. 263–274.

Hayashi M. 2005: Illustrated manual of Japanese Donaciinae. *Gekkan-mushi* **408**: 2–18.

Hayashi M. & Shiyake S. 2002: Late Pliocene Donaciinae (Coleoptera, Chrysomelidae) from the Koka Formation, Kobiwako Group in Shiga Prefecture, Japan. *Elytra (Tokyo)* **30**: 207–213.

Hayashi M. & Shiyake S. 2004: A check-list of the Japanese members of Donaciinae (Coleoptera: Chrysomelidae). *Entomol. Rev. Japan* **59**: 113–125.

Kim C.G., Zhou H.Z., Imura Y., Tominaga O., Su Z.H. & Osawa S. 2000: Pattern of morphological diversification in the the Leptocarabus ground beetles Coleoptera, Carabidae as deduced from mitochondrial ND5 gene and nuclear 28S rDNA sequences. *Mol. Biol. Ecol*. **17**: 137–145.

Japan Meteorological Agency 2002: *Mesh Climatic Data 2000.* Japan Meteorological business support center, Tokyo.

Kitamura A. & Kimoto K. 2004: Reconstruction of the southern channel of the Japan Sea at 3.9–1.0 Ma. *Quat. Res.* (*Daiyonki-Kenkyu*) **43**: 417–434 [in Japanese with English abstr.].

Kitamura A., Takano O., Takata H. & Omote H. 2001: Late Pliocene-early Pleistocene paleoceanographic evolution of the Sea of Japan. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **172**: 81–98

Masaki S. 1967: Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution* **21**: 725–741.

Masaki S. 1978: Climatic adaptation and species status in the lawn ground crickets. II. Body size. *Oecologia* **35**: 343–356.

Masaki S 1979: Climatic adaptation and species status in the lawn ground crickets. III. Ovipositor length. *Oecologia* **43**: 207–219.

Masaki S 1986: Significance of ovipositor length in life cycle adaptations of insects. In Taylor F. & Karban R. (eds): *Insect Life Cycles.* Springer, Berlin, pp. 20–34.

Mousseau T.A. & Roff D.A. 1989: Adaptation to seasonality in a cricket: patters of phenotypic and genotypic variation in body size and diapuse expression along a cline in season length. *Evolution* **43**: 1483–1496.

Mousseau T.A. & Roff D.A. 1995: Genetic and environmental contributions to geographic variation in the ovipositor length of a cricket. *Ecology* **76**: 1473–1382.

Narita Y. 2003. Descriptions of Donaciinae larvae (Coleoptera, Chrysomelidae) from Japan. *Elytra (Tokyo)* **31**: 1–30.

Roff D.A. 1980: Optimizing development time in a seasonal environment: the "ups and downs" of clinal variation. *Oecologia* **45**: 202–208.

Sota T., Takami Y., Kubota K. & Ishikawa R. 2000a: Geographic variation in the body size of some Japanese Leptocarabus species (Coleoptera, Carabidae): The "toppled-domino pattern" in species along a geographic cline. *Entomol. Sci.* **3**: 309–320.

Sota T., Takami Y., Kubota K., Ujiie M. & Ishikawa R. 2000b: Interspecific body size differentiation in species assemblages of the carabid subgenus Ohomopterus in Japan. *Popul. Ecol.* **42**: 279–291.

Toju H. & Sota T. 2006a: Imbalance of predator and prey armament: geographic clines in phenotypic interface and natural selection. *Am. Nat.* **167**: 105–117.

Toju H. & Sota T. 2006b: Phylogeography and the geographical cline in the armament of a seed-predatory weevil: effects of historical events vs. natural selection from the host plant. *Mol. Ecol.* **15**: 4161–4173.

Tominaga O. 1988: When did Plateumaris constricticollis babai become large? *Tohoku-no-shizen* **41**: 5–7 [in Japanese].

Received September 1, 2006; revised and accepted October 27, 2006