

Phylogeny of *Tomocarabus opaculus* (Coleoptera, Carabidae) as Deduced from Mitochondrial ND5 Gene Sequences

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Abstract *Tomocarabus opaculus* is widely distributed in Hokkaido and its adjacent islands, and also occurs in the restricted mountainous areas of northeastern Honshu. The phylogenetic relationship between the Hokkaido and the Honshu populations has been analysed using mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences. Two major lineages of *T. opaculus* are recognized in the phylogenetic trees. One is exclusively composed of the specimens from Hokkaido, and the other is from all parts of Honshu, and a specimen from Samani-Cho, Hokkaido is also included in the Honshu lineage. The Hokkaido lineage is further divided into three geographically linked sublineages: northeastern, central and southern sublineages, while the specimens of the Honshu lineage are very close to each other. The emergence time of the two major lineages is estimated to be around 20 million years ago, suggesting that *T. opaculus* is the oldest Japanese carabine species.

Introduction

Tomocarabus opaculus (*sensu* IMURA & MIZUSAWA, 1996), is widely distributed in Hokkaido and its adjacent islands (presumably including Sakhalin according to IMURA & MIZUSAWA, 1996). It also occurs in the restricted mountainous areas of northeastern Honshu. This species is the commonest in Hokkaido, while it is rather rare in Honshu. The Honshu population is discriminated from nominotypical *opaculus* as ssp. *shirahatai* (NAKANE, 1963) by minor morphological differences. The specimens from Mt. Daisetsu in Hokkaido are named ssp. *kurosawai* (BREUNING, 1957) which is probably a mere mountainous form of *opaculus opaculus* (NAKANE, 1977).

In the present study, phylogenetic trees have been constructed by analysing a large part of the mitochondrial ND5 gene sequences of 19 examples of *T. opaculus* from various localities in Hokkaido and Honshu to know the relationship between the Hokkaido and the Honshu populations.

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Materials and Methods

The specimens analysed for the ND5 gene sequences are listed in Table 1. DNA extraction, PCR amplification and sequencing were carried out as described (SU *et al.*, 1996, 1998). Phylogenetic trees were constructed by the neighbor-joining (NJ) method (SAITOU & NEI, 1987) and UPGMA (unweighted pair-group method with arithmetic mean) with the evolutionary distance (D) computed by KIMURA's two-parameter method (KIMURA, 1980). To obtain the reliability of the inferred phylogenetic tree, the bootstrap method was applied.

Results

Two major lineages of *T. opaculus* can be recognized in the phylogenetic trees (Fig. 1). One (A in Fig. 1) is exclusively composed of the specimens from Hokkaido, and the other (B in Fig. 1) is from all parts of Honshu, and a specimen from Samani-Cho, Hokkaido. The A lineage is further divided into three sublineages, A1, A2 and A3, while all the sequences in the B lineage are very close to one another even be-

Table 1. List of specimens analysed.

Scientific name by morphology	Locality number	Locality	DDBJ/EMBL/GenBank Accession No.
<i>T. opaculus</i>	1	Nemuro, Hokkaido	AB039794
	2	Kushiro, Hokkaido	AB039795
	3	Saroma, Hokkaido	AB039796
	4	Yūbetsu, Hokkaido	AB039797
	5*	Mt. Daisetsu, Hokkaido	D50340
	6	Mt. Daisetsu, Hokkaido	AB039798
	7	Higashigawa, Hokkaido	AB039799
	8	Niikappu, Hokkaido	AB039800
	9	Imakane, Hokkaido	AB039801
	10	Hakodate, Hokkaido	AB039802
	11	Samani, Hokkaido	AB039803
	12	Mt. Hakkōda, Aomori	AB039804
	13	Hachimantai, Iwate	AB039805
	14	Mt. Hayachine, Iwate	AB039806
	15	Mt. Gassan, Yamagata	AB039807
	16	Mt. Zao, Yamagata	AB039808
	17	Mt. Azuma, Fukushima	AB039809
<i>T. harmandi</i>	18	Nasu, Tochigi	AB039810
	19	Abetōge, Shizuoka	AB039811
	20*	Chino, Nagano	D50364
	21	Myōkō, Niigata	AB039812
	22	Arimine, Toyama	AB039813
	23	Mt. Hakusan, Ishikawa	AB039814

* Taken from SU *et al.* (1996).

tween the specimen from Samani-Cho, Hokkaido and those from Honshu.

The specimens belonging to A1 were all collected from central Hokkaido, A2 from the southern part (Oshima Peninsula) and A3 from northeastern Hokkaido, suggesting geographically linked distribution of the three lineages (Fig. 2).

Discussion

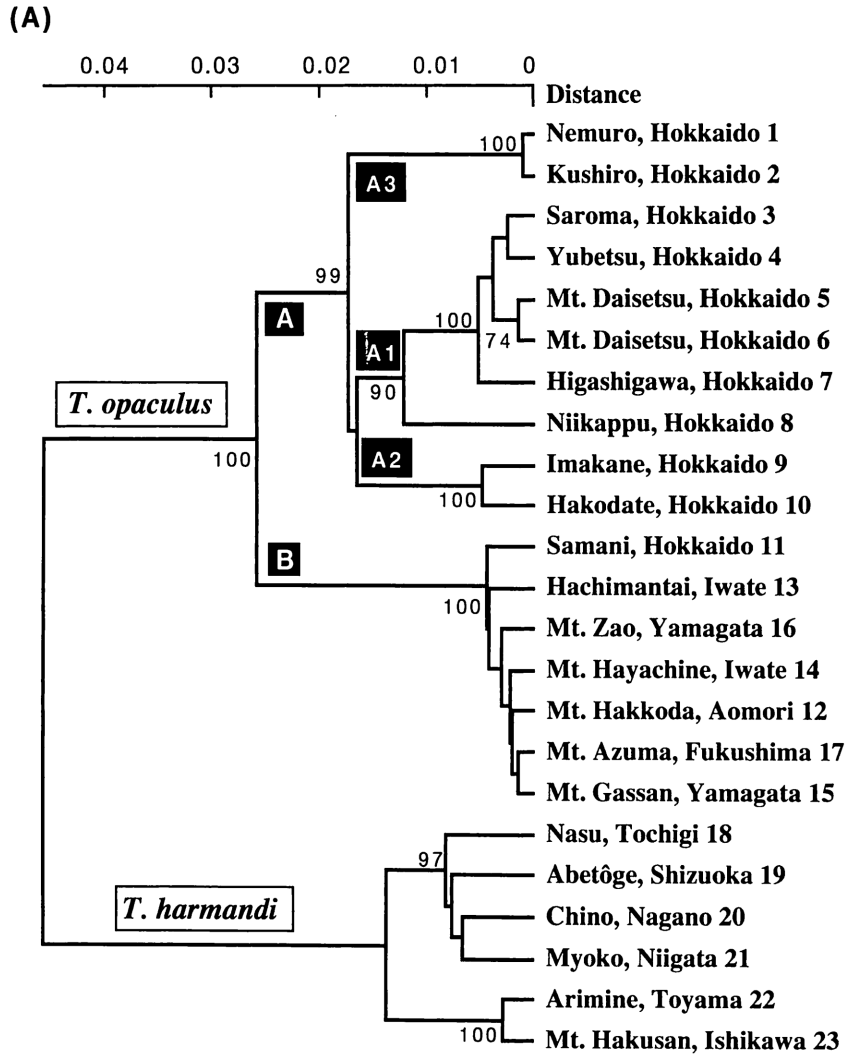
An ND5 phylogenetic tree covering the representative carabid species from the world (to be published) suggests that the major (sub)genera radiated at 50–40 million years ago (MYA), when calculated taking 0.01D of KIMURA's 2-parameter distance to correspond to 3.6 million years (MYR) (SU *et al.*, 1998). The origin of *T. opaculus* can be traced back to about the time of this radiation. No other species that branched off from the *T. opaculus* lineage have been found by examining a number of species belonging to *Tomocarabus* and its allied genera from the world (to be published).

Separation of A and B took place ca. 20 MYA, followed by diversification of A into A1, A2, A3 ca. 13 MYA in Hokkaido, while diversification of B started quite recently, at most 2 MYA.

The formation of the present distribution pattern of *T. opaculus* in the Japanese Islands is now considered. The time of separation of A and B (20 MYA) is much older than the time of split of the Japanese Islands from the Eurasian Continent (15 MYA), suggesting that A and B separated from each other in the continent before the Japanese Islands split from the continent. Since A1, A2 and A3 are clearly separated from one another geographically, each of them would have been isolated during the formation of Hokkaido Island. Concerning the habitat of the ancestor of B in the ancient Japan area in the continent, two possibilities may be considered. One would be that the B ancestor inhabited a part of the ancient Hokkaido area and has been isolated until recently. During the Glacial Era, B immigrated to Honshu via a land bridge and rapidly dispersed over the northeastern half of Honshu. An alternative possibility would be that the habitat of the B ancestor was the ancient northern Honshu area, and it has been geographically isolated in the restricted region of Honshu until recently, and then started to disperse rapidly in northeastern Honshu. At about the same time, B immigrated to the Samani-Cho area. We have no data at present to decide which alternative is correct.

As mentioned above, *T. opaculus* started to diversify at about 20 MYA corresponding to about one half the history of carabine evolution. This would mean that morphology of *T. opaculus* has remained almost unchanged for a long time, as has been observed in *Apotomopterus sauteri* (KIM *et al.*, 1999), *Phricocarabus glabratus* (IMURA *et al.*, 1998), and several others (to be published).

Taxonomic notes. In the present paper, we tentatively adopted the scientific name, *Tomocarabus opaculus*. ISHIKAWA (1991) regarded this species as belonging to *Leptocarabus* (*Asthenocarabus*), while DEUVE (1994), SHILENKOV (1994), and IMURA & MIZUSAWA (1996) used *Tomocarabus*, and BREZINA (1999) adopted *Diocarabus*. Our ND5 phylogenetic analyses indicate that *opaculus* is phylogenetically independent



from *Leptocarabus*. The origin of *opaculus* is as old as other Latitarsi (sub)genera. It would be appropriate to use *Asthenocarabus* as the generic name for *opaculus*.

Appendix

The ND5 sequences of *Tomocarabus harmandi* (*sensu* IMURA & MIZUSAWA, 1996) from several localities were taken as an outgroup in the phylogenetic trees in Fig. 1. This species is distributed in central and northern Honshu. The origin of *T. harmandi* is as old as *T. opaculus*. No other species have been found in the *harmandi* lineage. The trees suggest that there are two major clusters of *T. harmandi*, the distributional

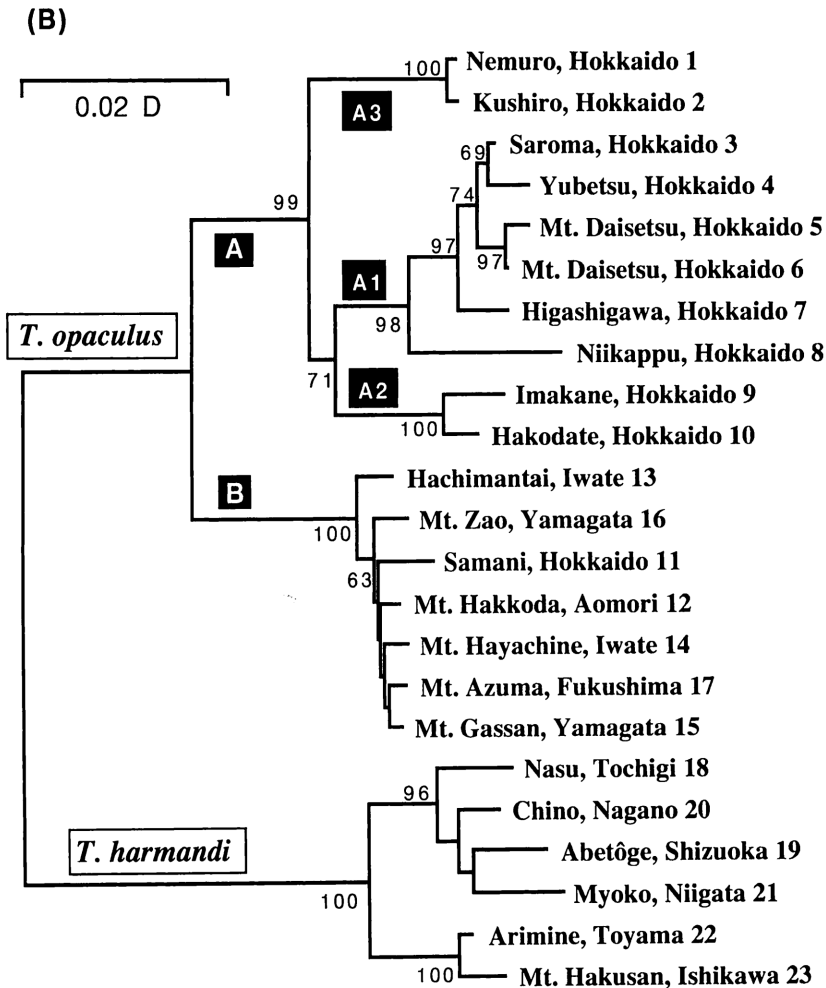


Fig. 1 (on pp. 16–17). Phylogenetic trees of the mitochondrial ND5 gene of *Tomocarabus opaculus* and *T. harmandi*. A 1084 bp sequence including 1069 bp 3'-region of ND5 gene, 8 bp noncoding sequence, and 7 bp 5'-terminus of the phe-tRNA gene was used for molecular phylogenetic analysis. The UPGMA (A) and NJ (B) trees were constructed by SINCA sequence analysis package (Fujitsu System Engineering) using KIMURA's two-parameter distance (KIMURA, 1980). The bootstrap confidence level (%) (based on 500 resamplings) is shown at each branching point, and that less than 60% was omitted.

ranges of which are separated by the Itoigawa–Shizuoka tectonic line. *Tomocarabus harmandi* is independent from *Leptocarabus* for the same reason as *T. opaculus*. *Pentacarabus* may be used for *harmandi* as the (sub)generic name.

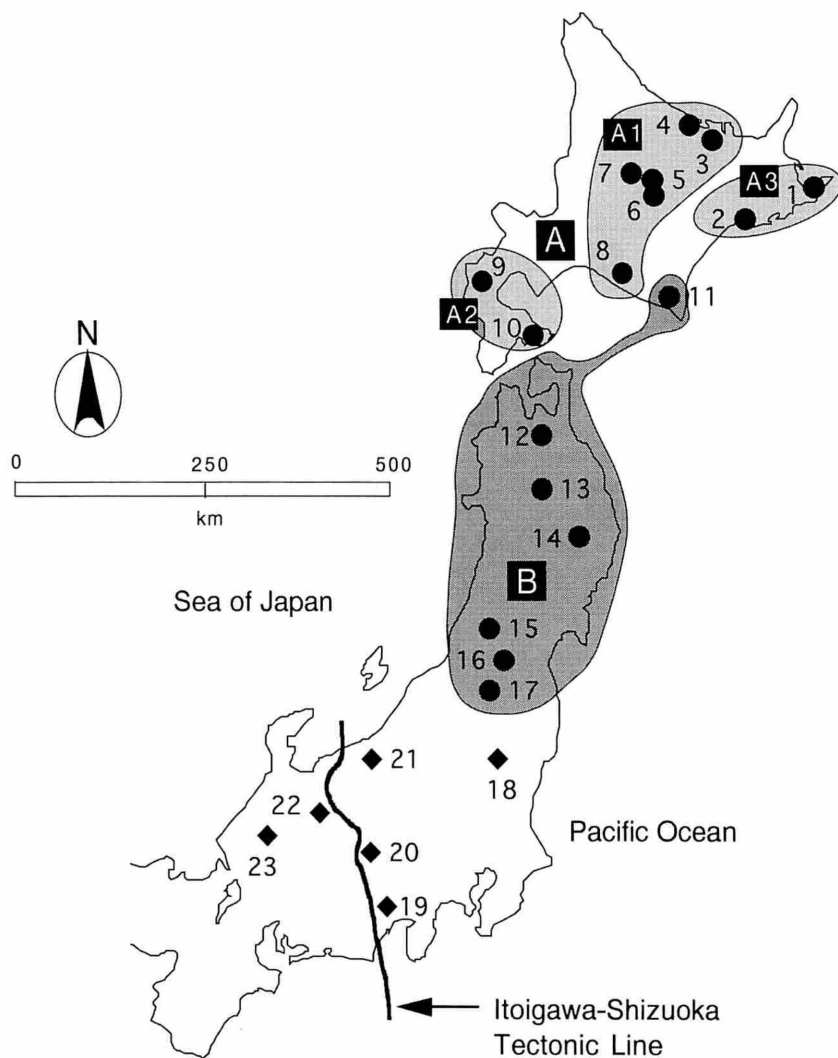


Fig. 2. Locality map of *Tomocarabus* specimens used in this study. Locality numbers correspond to those shown in Table 1 and Fig. 1. A and B indicate the Hokkaido and the Honshu lineages of *T. opaculus*, respectively. A1, A2 and A3 denote the three geographically linked sublineages within the Hokkaido lineage. (●) *T. opaculus*, (◆) *T. harmandi*.

要 約

蘇 智慧・富永 修・斉藤秀生・金 衝坤・大澤省三：ヒメクロオサムシの分子系統。——ヒメクロオサムシは、北海道と本州北部に分布し、本州北部のものは *ssp. shirahatai* NAKANE とされる。日本各地のヒメクロオサムシの ND5 遺伝子を解析し、地域変異を調べたところ、本州のものはたがいに近縁だが、北海道のものは大きく 4 つの系統に分かれた、そのうちのひとつ

(様似町産)は本州のものと同じクラスターに入り、本州のものと区別がつかないほど近縁である。この系統と他の3つの系統が分かれた時期は約2,000万年前と推定されたので、ヒメクロオサムシは日本産オサムシの中でもっとも起源の古いオサムシであると考えられる。本論文では構築した系統樹に基づき、ヒメクロオサムシの分布域形成の歴史を考察した。また、ND5の系統樹から見て、ヒメクロオサムシは長期間(約2千万年)形態変化がほとんどなかったことが示唆された。

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A Third Record of *Curculio amabilis* MORIMOTO (Coleoptera, Curculionidae, Curculioninae)

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The curculionid weevil *Curculio amabilis* was described by MORIMOTO (1962) on the basis of one male collected from Hikosan, Fukuoka Prefecture, Kyushu. This species seems to be very rare, and only one additional record has so far been known from Gifu Prefecture, Chûbu District, Honshu (NOHIRA, 1990). In the middle of May, 1999, the author obtained one male of this species by net-sweeping of *Quercus crispula* BLUME [Mizu-nara in Japanese] (Fagaceae) in Kanagawa Prefecture, Kantô District, Honshu. The collecting data will be shown below as a third record of the species.

Curculio amabilis MORIMOTO, 1962

Curculio amabilis MORIMOTO, 1962, *Mushi*, Fukuoka, **36**: 36–37.

Specimen examined. 1♂, Mt. Daigatake, Hakone, Kanagawa Pref., 17–V–1999, H. YOSHITAKE leg.

Distribution. Japan (Honshu, Kyushu).

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