

Phylogeny in the Crenolimbi Ground-beetles (Coleoptera, Carabidae) as Deduced from Mitochondrial ND5 Gene Sequences

Zhi-Hui SU

JT Biohistory Research Hall,
1–1 Murasaki-Cho, Takatsuki, Osaka,
569–1125 Japan,

Yûki IMURA

Department of Gynecology,
Tôkyû General Hospital, Kita-senzoku,
1–45–6, Ôta-ku, Tokyo, 145–0062 Japan,

Osamu TOMINAGA

and

Syozo OSAWA

Shibatsuji-cho, Nara, 630–8114 Japan

JT Biohistory Research Hall,
1–1 Murasaki-Cho, Takatsuki, Osaka,
569–1125 Japan

Abstract All the five species belonging to the division Crenolimbi (= *Hemicarabus* + *Homoeocarabus*) of the genus *Carabus* (s. lat.) are examined for the mitochondrial ND5 gene sequences to clarify their phylogenetic relationships.

Introduction

The subgenera *Hemicarabus* and *Homoeocarabus* of the grand genus *Carabus* are morphologically similar to each other, and are combined to a single category, the Crenolimbi. This name was first proposed by REITTER (1896) as one of the ranks between the genus and the subgenus, but has been ignored thereafter by most authors for nearly a century. In his revisional work for the classification of *Carabus*, IMURA (1996) readopted the Crenolimbi as a subdivisional name. His view was supported by a molecular phylogenetic analysis of the representative groups of *Carabus* (s. lat.), in which the species classified into the Crenolimbi form a well-defined cluster (IMURA *et al.*, 1998). Several taxonomists also have used the Crenolimbi as a divisional name in their own system (DEUVE, 1997; KLEINFELD & SCHÛTZE, 1999; BŘEZINA, 1999; etc.). It is thus reasonable to regard the Crenolimbi as one of the nine major divisions under the genus *Carabus* (s. lat.).

The Crenolimbi is the smallest division in the genus *Carabus* (s. lat.), consisting of only five species (four in the subgenus *Hemicarabus* and one in *Homoeocarabus*). They are widely, but rather sporadically distributed throughout the northern part of the Eurasian Continent and of North America, including several adjunctive islands such as Great Britain, Ireland, Sakhalin, the Japanese Islands, Cheju-do, Newfoundland, St.

Table 1. List of the Crenolimbi specimens used in this study.

| Locality number | Scientific name by morphology | Locality | DDBJ/EMBL/GenBank Accession No. |
|-----------------|-------------------------------|--|---------------------------------|
| 1 | <i>Hemicarabus serratus</i> | Newaygo, Michigan, CN. USA | AB047149 |
| 2 | <i>He. tuberculosis</i> | Lukashovka, Primorskij, E. Russia | AB047202 |
| 3 | <i>He. tuberculosis</i> | Chiri-san Mts., S. Korea | AB047203 |
| 4 | <i>He. tuberculosis</i> | Kunimi, Nagasaki, Kyushu, SW. Japan | AB047204 |
| 5 | <i>He. tuberculosis</i> | Is. Izu-oshima, Tokyo, C. Japan | AB047205 |
| 6 | <i>He. nitens</i> | Uchte Moor, S. Sulingen, Niedersachsen, N. Germany | AB047206 |
| 7 | <i>He. macleayi</i> | Mt. Vachkazhets, Kamchatka, E. Russia | AB047207 |
| 8 | <i>He. tuberculosis</i> | Is. Cheju-do, S. Korea | AB047208 |
| 9* | <i>He. tuberculosis</i> | Shirasaka, Fukushima, Honshu, C. Japan | D50353 |
| 10*# | <i>Homoeocarabus maeander</i> | Sarobetsu, Hokkaido, N. Japan | D50354 |

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

As an outgroup for constructing the trees (see Fig. 1).

Pierre and Miquelon, etc. It should be worth noting that most of the Crenolimbi species prefer such biotopes as meadows and/or lowland moors. This trend seems to be more evident in the subgenus *Homoeocarabus*.

In the present study, we have sequenced the mitochondrial ND5 (NADH dehydrogenase subunit 5) gene sequences of all the species in this division from various localities, and constructed the phylogenetic trees using the UPGMA- and the NJ method.

Hereafter we conventionally use the subgeneric names in place of the routinely used generic name, *Carabus*. For instance, "*Hemicarabus*" means "*Carabus (Hemicarabus)*".

Materials and Methods

The specimens used in this study are listed in Table 1. The methods employed here are the same as described previously (*e.g.*, Su *et al.*, 1998).

Results and Discussion

On both the NJ- and the UPGMA trees, *Homoeocarabus maeander* is sharply separated from four species of *Hemicarabus*. Diversification of these two subgenera may be estimated to have taken place about 29 million years ago as calculated according to Su *et al.* (1998).

Within the subgenus *Hemicarabus*, *H. tuberculosis* from various localities of Japan, Korea and Primorskij, *H. macleayi* from Kamchatka, and *H. nitens* from Germany are very close in their ND5 gene sequences. This suggests that the common ancestor of these species was distributed throughout the northern part of the Eurasian Continent, consisting of nearly a single reproductive population until recently. Then, speciation occurred in the respective distributional ranges presumably by geographic isolation, followed by genetic changes affecting morphology. Alternatively, it is possible that the ancestor had inhabited certain restricted region of the continent, rapidly

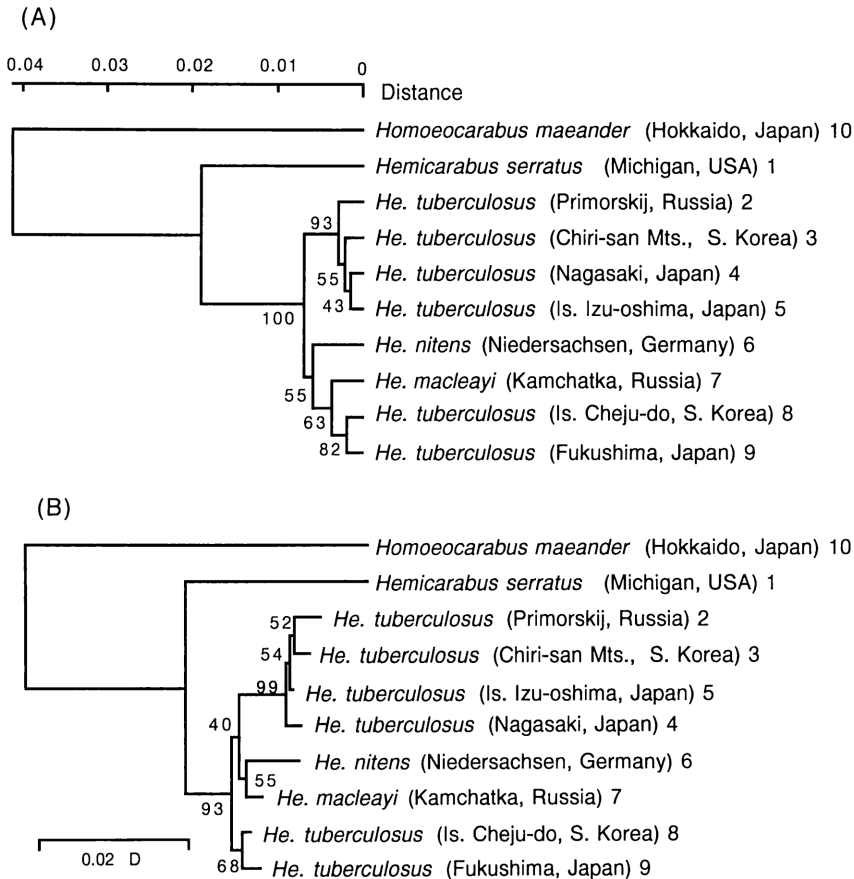


Fig. 1. Phylogenetic trees of the mitochondrial ND5 gene (1,069 bp upstream from 3 terminal stop codon) from the Crenolimbi-species. The UPGMA tree (A) and the NJ tree (B). The bootstrap confidence level (%) (based on 500 resamplings) is shown at each branching point. Distance (D) denotes KIMURA's two-parameter evolutionary distance. For details, see SU *et al.* (1998).

propagated its distributional range recently, and became differentiated into the respective species. It should be worth noting that the three species can be clearly separable morphologically, suggesting occurrence of a rapid morphological differentiation characteristic to each species. As to the immigration of *H. tuberculosus* into the Japanese Islands, see TOMINAGA *et al.* (2000).

Hemicarabus serratus from North America (Michigan) separated fairly long ago (about 14 million years ago) from other Eurasian *Hemicarabus* species. Presumably, the common ancestor of all the *Hemicarabus* species was distributed throughout the Eurasian Continent to North America when they were connected by land. Upon establishment of the Bering Straits, the Eurasian and the North American populations evolved to different directions. As the result, *H. serratus* emerged in North America.

In summary, the subgenus *Homoecarabus* can be sharply separated from the subgenus *Hemicarabus*. All the *Hemicarabus* species in the Eurasian Continent are phylogenetically very close, while *H. serratus* from North America is rather remote from the Eurasian species.

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要 約

蘇 智慧・井村有希・富永 修・大澤省三：ミトコンドリアND5 遺伝子の塩基配列からみたセアカオサムシ群内の系統。——セアカオサムシ群 *Crenolimbi* は、セアカオサムシ亜属 *Hemicarabus* とセアジアカガネオサムシ亜属 *Homoecarabus* の2亜属のみからなる、広義のオサムシ属のなかで最小の群だが、その分布は広く、全北区にわたる。本論文では、本群に属する5種すべてを用いて、ミトコンドリアND5 遺伝子の塩基配列を決定し、群内における系統関係を再検討した。その結果、*Homoecarabus* は *Hemicarabus* から明確に区別されること、*Hemicarabus* 内においては、ユーラシアに分布する3種はたがいに近縁で、北米の *serratus* のみ類縁が遠いことなどが判明した。

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Damaster blaptoides (Coleoptera, Carabidae) from
Brat Chirpoyev Island of the Kurils, Russia

Zhi-Hui SU¹⁾, Masahiro ÔHARA²⁾, Yûki IMURA³⁾ and Syozo OSAWA¹⁾

1) JT Biohistory Research Hall, 1–1 Murasaki-Cho,
Takatsuki, Osaka, 569–1125 Japan

2) The Hokkaido University Museum, Sapporo, 060–0810 Japan

3) Department of Gynecology, Tôkyû General Hospital, Kita-senzoku,
1–45–6, Ôta-ku, Tokyo, 145–0062 Japan

Damaster blaptoides has been known as a peculiarly shaped ground beetle widely distributed in Japan and the southwestern part of the Kurils. The Hokkaido population is discriminated from several other forms distributed in Honshu, Shikoku and Kyushu as subsp. *rugipennis*, although the specimens from northernmost Honshu belong to the same lineage as the Hokkaido population on a phylogenetic tree of the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene (SU *et al.*, 1998; KIM *et al.*, 1999).

During the expedition performed under the International Kuril Islands Project in 1997, two specimens identified with *Damaster blaptoides* were captured at the Cape Garovnikova of Brat Chirpoyev Island (the central part of the Kuril Islands, Russia; 46°28.405'N/150°48.160'E), about 500 km off the eastern edge of Hokkaido. One of the specimens (female, 37.5 mm in length including mandibles, 20–VIII–1997, Yasuhiro KUWAHARA leg.) was analysed for the ND5 gene sequence. The 1,069 bp sequence examined was identical with those from Nemuro, Hokkaido. Also, only 1, 1 and 2 base changes were detected between the Kuril specimen and the specimens from Samani, Taiki and Hakodate in Hokkaido (0.094%, 0.094% and 0.19% difference), while the differences of the Kuril specimen from other parts of Hokkaido such as Niseko, Oshamanbe and Abashiri were 0.56%, 0.56% and 0.66% (6, 6 and 7 base changes), respectively. Incidentally, the average sequence difference between the eastern and the western lineage of the Japanese *Damaster* is 4.2% (45 base changes) corresponding to their divergence about 15 million years ago (SU *et al.*, 1998). Thus, it may be assumed that a population that had