

## Radiation of Several Carabina Groups (Coleoptera, Carabidae) Inferred from the Mitochondrial ND5 Gene Sequences

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**Abstract** The DNA sequences of the mitochondrial ND5 gene were determined for 9 French Carabina-species. The molecular phylogenetic trees were constructed from these sequences in conjunction with those from the representative Japanese Carabina-species. The results suggest that a rapid radiation of the major genera examined took place in the initial stage of the Carabina evolution.

**Key words:** ND5; mt DNA; phylogeny; radiation; French and Japanese Carabina beetles.

One of the papers by SU *et al.* (1996 a), in which a phylogenetic tree of the Japanese Carabinae was constructed based on the mitochondrial DN5 (NADH dehydrogenase subunit 5) gene sequences, showed that the Japanese Carabina may be classified into 5 main groups, most of which correspond to the genera [often treated as the subgenera of *Carabus* (s. lat.)] that are recognized in the current classification system based on morphological characters (see fig. 5 of SU *et al.*, 1996 a). These are *Procrustes* (*Megodontus*=*Pachycranion*), *Damaster* (*Damaster*+*Acoptolabrus*+*Coptolabrus*), *Apotomopterus* (*Limnocarabus*+*Euleptocarabus*), *Leptocarabus* (*Leptocarabus*+*Adelocarabus*+*Aulonocarabus*+*Pentacarabus*+*Asthenocarabus*), *Hemicarabus* (*Hemicarabus*+*Homoeocarabus*), and *Carabus* (*Carabus*+*Ohomopterus*). It was also noted that *Carabus* (s. str.) and *Ohomopterus* may be treated as separated groups because of the bootstrap confidence value of the cluster representing these two genera is

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Abbreviations: ND5, NADH dehydrogenase subunit 5; mt, mitochondria; bp, base pair; UPGMA, unweighted pair-group method with arithmetic mean; NJ, neighbor-joining; Myr, million years.

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession numbers D86203–D86211.

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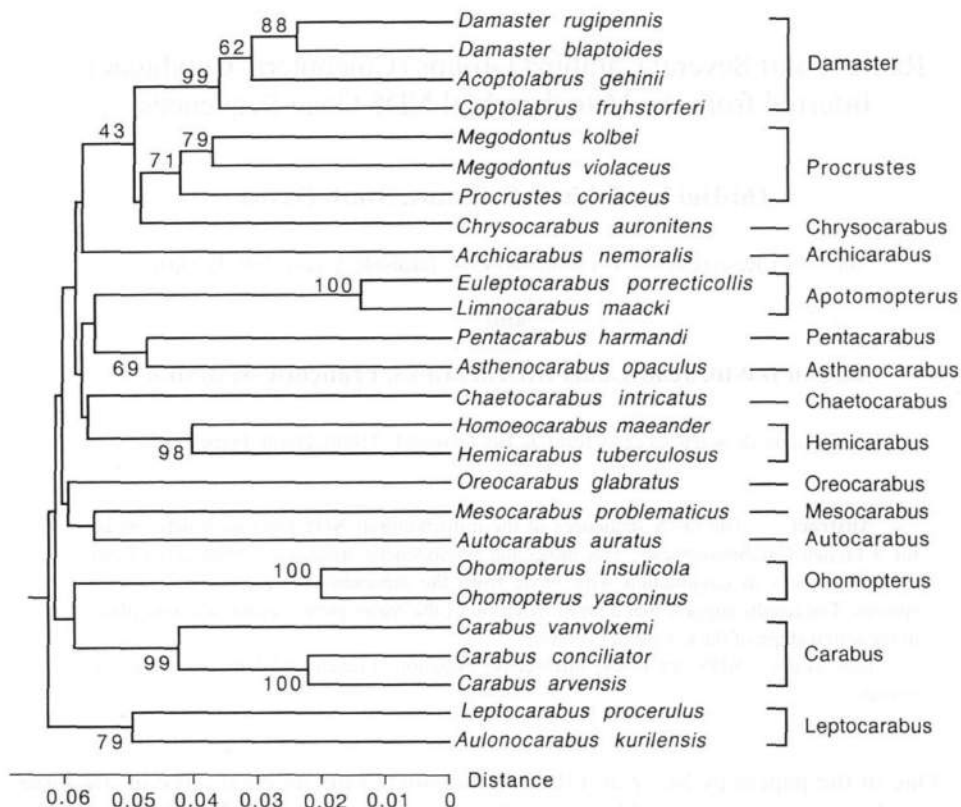


Fig. 1a

not very high. The tree suggests that the above 6 (or 7) main groups radiated more than 10 Myr ago within a short period from their common ancestor.

In the present paper, 9 French Carabina-species, the genera of 7 of which do not occur in Japan, have been examined for ND5 gene sequences to obtain further evidence for radiation of the main groups ( $\approx$ genera) of the Carabina and to know the phylogenetic relationships between the Japanese and French species.

### Materials and Methods

The French specimens used were collected in Burgundy, and analyzed for the 1069 bp. upstream from 3'-terminal stop codon of the mitochondrial ND5 gene sequences. For the preparation of DNA, PCR direct sequencing, and construction of the phylogenetic trees, see SU *et al.* (1996 a) and KUMAR *et al.* (1993). The phylogenetic trees were constructed by the UPGMA (KUMAR *et al.*, 1993), and the neighbor-joining

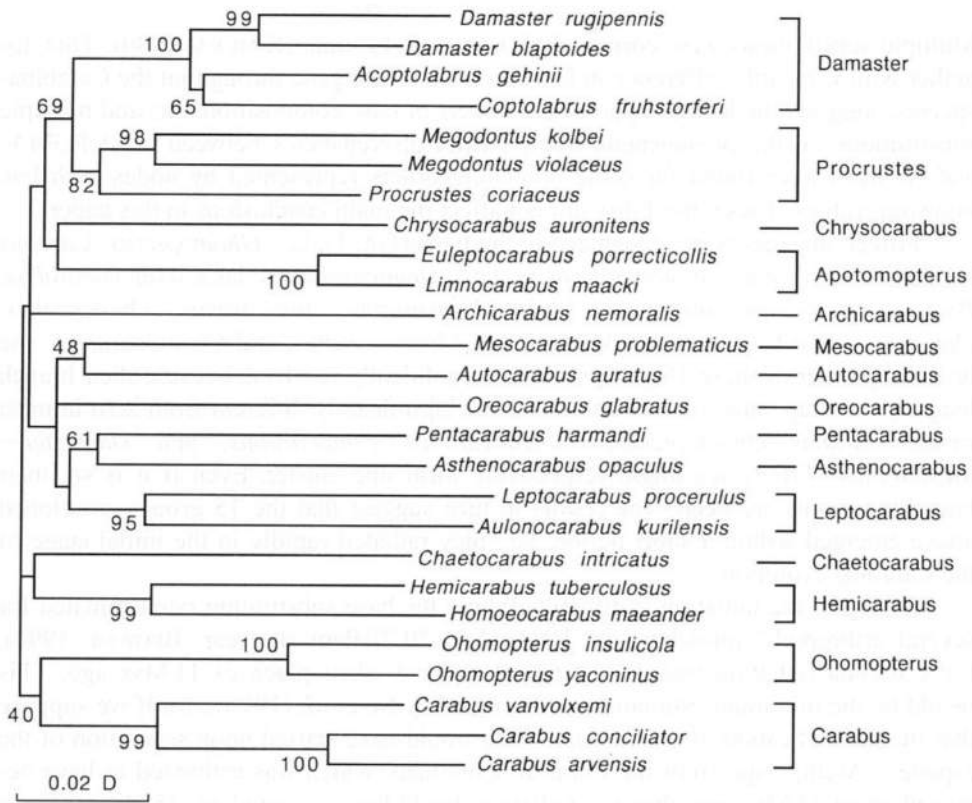


Fig. 1b

Fig. 1. Phylogenetic trees of the mitochondrial ND5 gene of the French and Japanese Carabina-species. a: UPGMA-tree. b: NJ-tree. The bootstrap confidence level (based on 1,000 resamplings) of more than 40% is shown at each branching point. D indicates KIMURA's two-parameter evolutionary distance (KIMURA, 1980).

(NJ) method (SAITOU & NEI, 1987), using the evolutionary distances computed by KIMURA's two-parameter method (KIMURA, 1980). The trees were evaluated using the bootstrap test (FELSENSTEIN, 1985). For these analyses, the ND5 gene sequences from 9 French and 17 representative Japanese Carabina-species (see SU *et al.*, 1996a) were used.

### Results and Discussion

Throughout the ND5 gene sequences used, neither deletions nor insertions were required for multiple alignment and the G+C contents were nearly constant ( $21.0 \pm 1.0\%$ ). The maximum difference percent in all sites, and that at codon third positions in the ND5 gene between all the species examined were 14% and 37%, respectively.

Multiple substitutions were corrected by KIMURA's formula (KIMURA, 1980). This, together with very little difference in G+C content of the gene throughout the Carabina-species, suggests the lack of appreciable effects of base composition bias and multiple substitutions on the phylogenetic trees. Partial discrepancies between the UPGMA- and NJ-trees were found for some branching orders represented by nodes with low bootstrap values. These instability did not affect the main conclusions in this paper.

Fifteen lineages were recognized in the trees (Fig. 1), i.e., *Ohomopterus*, *Carabus* (s. str.), *Archicarabus*, *Apotomopterus* (s. lat.), *Hemicarabus* (s. lat.), *Asthenocarabus*, *Pentacarabus*, *Leptocarabus* (s. lat.), *Mesocarabus*, *Autocarabus*, *Oreocarabus*, *Damaster* (s. lat.), *Procrustes*+*Megodontus*, *Chrysocarabus*, and *Chaetocarabus*. The branching order of these 15 groups was not confidently resolved, because their branch length (bootstrap value of less than 70) is not significantly different from zero in most cases. *Carabus*+*Ohomopterus*, *Asthenocarabus*+*Pentacarabus*, and *Damaster*+*Megodontus*+*Procrustes* might respectively form one cluster. Even if it is so, their branching points are deep. The results in turn suggest that the 15 groups mentioned above emerged within a short period, i.e., they radiated rapidly in the initial stage of the Carabina evolution.

Dating of the radiation is difficult. Taking the base substitution rate estimated for several arthropods' mitochondrial DNA ( $1.2 \times 10^{-8}$ /silent site/year; BROWER, 1994), the Carabina radiation may be estimated to have taken place ca 11 Myr ago. This would be the minimum estimation as discussed by SU *et al.* (1996 a, b). If we suppose that the diversification of *Damaster* (s. str.) would have started upon separation of the Japanese Archipelago from the Eurasian Continent, which was estimated to have occurred about 15 Myr ago, then the radiation would have occurred ca. 35 Myr ago (see SU *et al.*, 1996 b).

Six French (European) genera, *Archicarabus* (*nemoralis*), *Chaetocarabus* (*intricatus*), *Autocarabus* (*auratus*), *Mesocarabus* (*problematicus*), *Oreocarabus* (*glabratus*), and *Chrysocarabus* (*auronitens*) which are absent in Japan, do not show clear direct phylogenetic relations to any of the Japanese Carabina genera. Two *Megodontus*-species, *kolbei* (Hokkaido, Japan) and *violaceus* (France) form a clade as expected. *C. conciliator* is sometimes treated as a subspecies of *C. arvensis*. Indeed, *C. conciliator* (Hokkaido, Japan) is close to *C. arvensis* (France) on the ND5 phylogenetic trees. Suppose that these two species branched off from their common ancestor somewhere in the Eurasian Continent and reached France and Japan, respectively. The branch length from the node that supports these two species roughly represents the relative time elapsed since they split. If we assume the Carabina radiation to have occurred 35 Myr ago (see above), then the branch length of the two *Carabus*-species corresponds to 15 Myr. In other words, ca. 15 Myr were required for the respective species to have arrived at the present localities after their separation. This has also to be related to the time of separation of the Japanese Archipelago from the Eurasian Continent, and may strengthen the idea that the initial Carabina radiation is older than given by BROWER's substitution rate.

## 要 約

蘇 智慧, 岡田節人, 大澤省三, B. DAVID, J.-L. DOMMERGUES, F. MAGNIEZ: ミトコンドリアND5遺伝子のDNA塩基配列に基づくオサムシ類の放散。——フランス産オサムシ亜族の9属(7属は日本にいない)9種について, ミトコンドリアND5遺伝子のDNA塩基配列を決定し, 日本の代表的オサムシの配列とともに分子系統樹を作成した。分類学上の属(ときに亜属とされる)に相当する15の系統の存在が示されたが, これらは, オサムシ亜族の分化初期の短期間に放散したと推定される。

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Additional Records of *Euxestocis bicornutus* (Coleoptera, Ciidae)

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*Euxestocis bicornutus* MIYATAKE, 1954 [Japanese name: Futatsunotsuya-tsutsukinokomushi], is relatively rare among the Japanese ciid species, and it has been considered to be endemic to Shikoku (MIYATAKE, 1985). This ciid is recently collected from rather wide areas in Japan, and KITABATA (1993) recorded it from central Honshu. In this report, I will summarize