

Origin and Evolution of the *Apotomopterus* Ground Beetles (Coleoptera, Carabidae) as Deduced from Mitochondrial ND5 Gene Sequences

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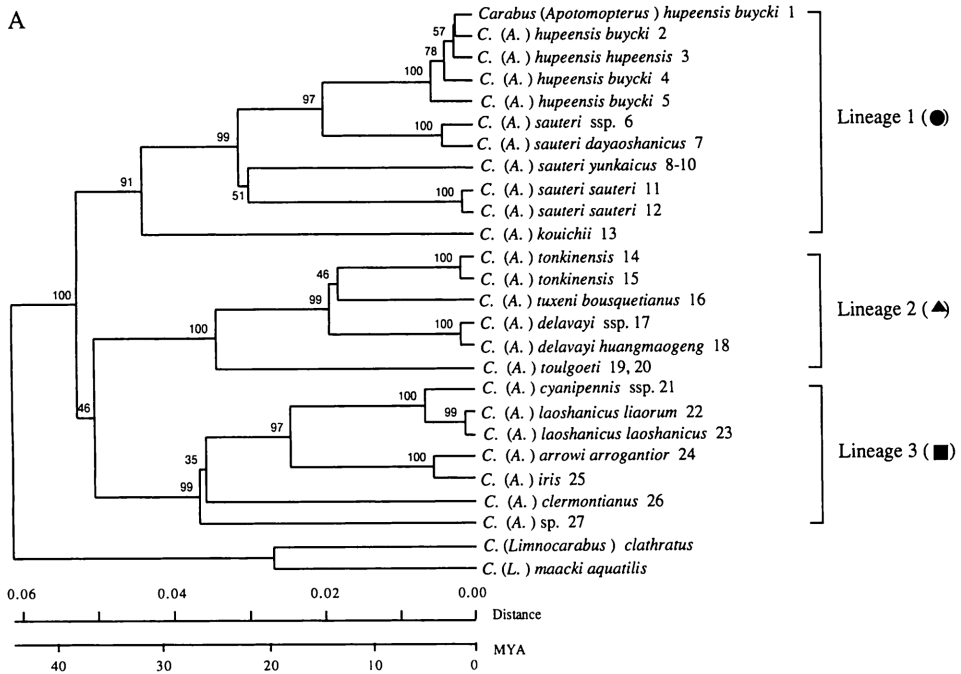
Abstract Phylogenetic trees have been constructed from the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences of 13 species (27 specimens) of *Carabus* (*Apotomopterus*) with *C. (Limnocarabus) clathratus* and *C. (L.) maacki* as outgroup. The ND5 trees indicate that there are three main lineages. One of the species, *C. (A.) sauteri*, is morphologically divided into several subspecies by slight differences. Seven specimens of four *sauteri* subspecies from various localities in mainland China and Taiwan have been classified by the ND5 gene sequences into three clades with their ancient separation ca. 22 MYA. *Carabus (A.) hupeensis*, which shows considerable morphological difference from *C. (A.) sauteri*, branched off from one of the *sauteri* stems.

Introduction

The subgenus *Apotomopterus* of the genus *Carabus* (s. lat.) comprises nearly 100 species distributed in China and the adjacent regions including Taiwan. In the previous paper (IMURA *et al.*, 1998), a phylogenetic tree of the NADH dehydrogenase subunit 5 (ND5) gene sequences from 9 taxa of the subgenus *Apotomopterus* was reported. In the present study, revised phylogenetic trees have been constructed by adding 10 taxa, using two *Limnocarabus* sequences as outgroup. Based on the trees, evolutionary history and diversification pattern within the subgenus *Apotomopterus* are discussed.

Materials and Methods

The species analysed for the ND5 gene sequences including the previously reported ones (IMURA *et al.*, 1998) are listed in Table 1. For the analytical methods and construction of the phylogenetic trees, see SU *et al.* (1996, 1998) and KIM, ZHOU *et al.* (1999). Using the neighbor-joining (NJ) method (SAITOU & NEI, 1987) and unweighted pair-group method with arithmetic mean (UPGMA) (KUMAR *et al.*, 1993), the phylogenetic trees were constructed using the sequences containing a 1069 bp 3'-region of the ND5 gene of 19 taxa of the subgenus *Apotomopterus*. Bootstrap analysis was performed for the trees based on 500 resamplings (FELSENSTEIN, 1985). The evolutionary

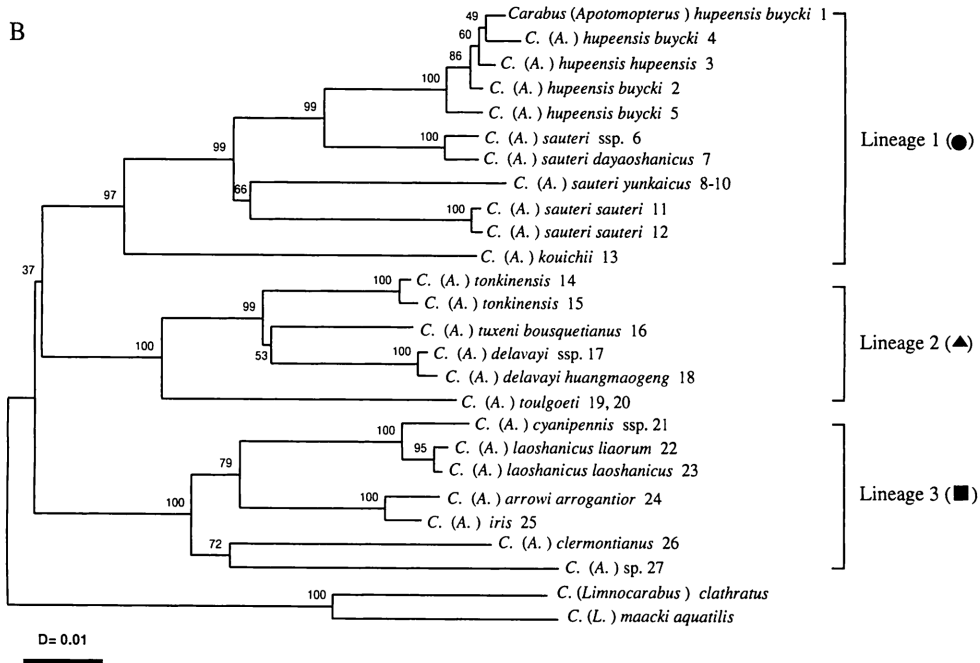


distances (D) were computed by KIMURA's two-parameter method (KIMURA, 1980). For setting the time scale, a 0.01 D unit corresponding to 3.6 million years (MYR) was used (SU *et al.*, 1999).

Results and Discussion

Origin and Phylogeny.

Apotomopterus is the sole subgenus in the division Spinulati of the grand genus *Carabus*, and its members constitute a well-defined monophyletic cluster on the ND5 phylogenetic trees (IMURA *et al.*, 1998). The present phylogenetic trees indicate that there are three main lineages of the *Apotomopterus* species that have been examined (Fig. 1). The first lineage includes *C. (A.) kouichii* from North Vietnam, *C. (A.) sauteri* and *C. (A.) hupeensis* from the Chinese Continent and Taiwan. *Carabus (A.) kouichii* and *C. (A.) sauteri* separated long time ago [ca. 32 million years ago (MYA)]. Following this, three *C. (A.) sauteri* sublineages diverged at about the same time ca. 22 MYA. One of them containing *C. (A.) s. dayaoshanicus* and *C. (A.) s. ssp.* also includes five specimens of *C. (A.) hupeensis*, indicating that *C. (A.) hupeensis* from various localities branched off from one of the *sauteri* stems. The second lineage contains *C. (A.) tonkinensis* from North Vietnam and *C. (A.) toulgoeti*, *C. (A.) delavayi*, and *C. (A.) tuxeni* from Central and South China. *Carabus (A.) toulgoeti* and *C. (A.) tonkinensis* / *C. (A.) tuxeni* / *C. (A.) delavayi* separated ca. 25 MYA, followed by diversifi-



cation of the latter three species ca. 14 MYA. The third lineage consists of *C. (A.) clermontianus* from North Vietnam, and *C. (A.) cyanipennis* ssp., *C. (A.) iris*, *C. (A.) sp.* (allied to *C. breuningianus*; IMURA *et al.*, 1999), *C. (A.) arrowi*, *C. (A.) laoshanicus liaorum* and *C. (A.) l. laoshanicus* from Central and South China. *Carabus (A.) clermontianus* and *C. (A.) sp.* separated from a common ancestor ca. 26 MYA, and the remaining species diverged ca. 18 MYA into two sublineages, i.e., *C. (A.) cyanipennis*/*C. (A.) laoshanicus liaorum*/*C. (A.) l. laoshanicus* and *C. (A.) arrowi*/*C. (A.) iris*. Further diversification into three and two descendant species took place within the respective sublineages less than 5 MYA.

The origin of *Apotomopterus* is old and can be traced back to the time of the explosive radiation of the Carabina 50–40 MYA (see SU *et al.*, 1999). Thus, *Apotomopterus* may be considered as one of the groups originated upon the radiation. Shortly after the radiation, three lineages shown in Fig. 1 and discussed above diverged almost simultaneously presumably somewhere in the southeastern region of the Chi-

Fig. 1 (on pp. 644–645). Phylogenetic trees of the mitochondrial ND5 gene (1,069 bp upstream from 3' terminal stop codon) from the *Apotomopterus* 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 (KIMURA, 1980). The sequences of the specimens 8–10, and those of 19 and 20 were identical, respectively.

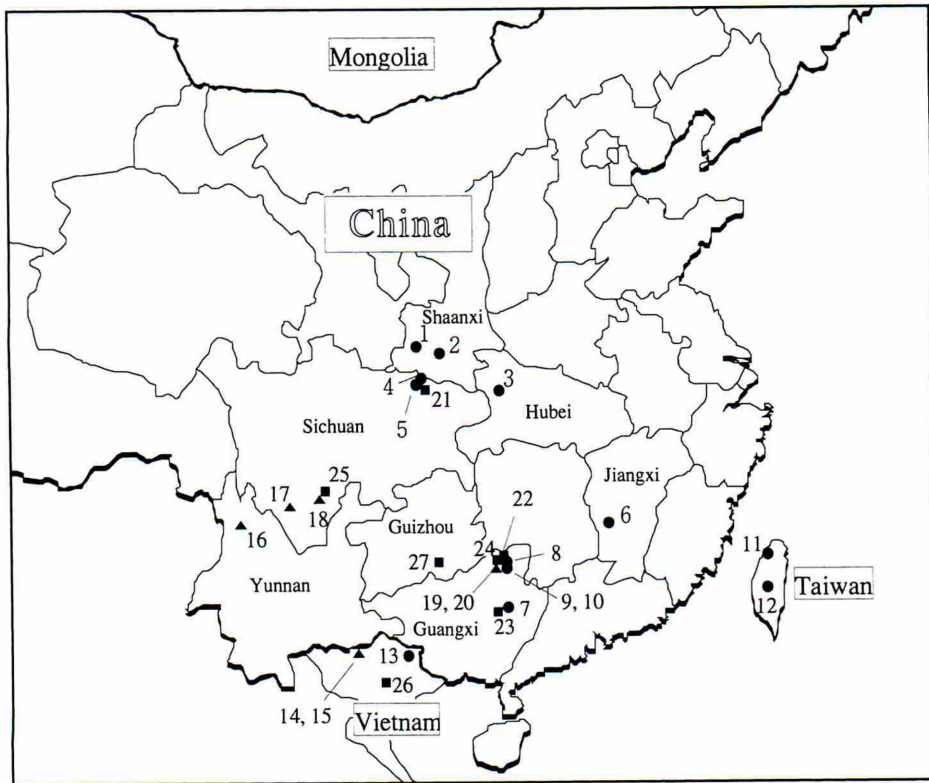


Fig. 2. Map showing the collecting sites of the *Apotomopterus* species used in this study. Numerals correspond to those shown in Table 1 and Fig. 1. ●, lineage 1; ▲, lineage 2; ■, lineage 3.

nese Continent (see fig. 32 of IMURA *et al.*, 1998). Figure 2 and the ND5 trees indicate that the distribution ranges of the three lineages are wide and overlap considerably. No definite distribution boundaries between them can be drawn. This suggests that the *Apotomopterus* ancestor was divided into at least three isolates upon geographic changes of the continent ca. 37 MYA. Then each isolate expanded its distribution to occupy the present habitat.

Pattern of morphological evolution.

Four *sauteri* subspecies, *dayaoshanicus*, *yunkaicus*, *sauteri* and *ssp.* (population from Mt. Jinggang Shan) differ only slightly from one another, even though they have been classified as subspecies within the same species (IMURA, 1994, 1995 & 1999). However, their separation occurred long time ago, ca. 22 MYA (Fig. 1). *Carabus* (*A.*) *clermontianus* and *C.* (*A.*) *sp.* are also similar in morphology, but they show a remote phylogenetic relationship, separated ca. 26 MYA. Thus, even if more than two taxa are treated as the same or allied species because of similar morphology, their time of di-

Table 1. List of the *Apotomopterus* specimens used in this study.

Species	Locality No.	Locality	DDBJ/EMBL/GenBank Accession No.
<i>Carabus (Apotomopterus) hupeensis buycki</i>	1	Feng Xian, SW. Shaanxi, China	AB031483
<i>C. (A.) hupeensis buycki</i>	2	N. Yang Xian, S. Shaanxi, China	AB031484
<i>C. (A.) hupeensis hupeensis</i>	3	Shennongjia, W. Hubei, China	AB031485
<i>C. (A.) hupeensis buycki</i>	4	Micang Shan Mts., NE. Sichuan, China	AB031486
<i>C. (A.) hupeensis buycki</i>	5	Mt. Guangwu Shan, NE. Sichuan, China	AB031487
<i>C. (A.) sauteri ssp.</i>	6	Mt. Jinggang Shan, SW. Jiangxi, China	AB031488
<i>C. (A.) sauteri dayaoshanicus</i>	7*	Mt. Dayao Shan, E. Guangxi, China	AB031489
<i>C. (A.) sauteri yunkaicus</i>	8*	Antangping, Mt. Miao'er Shan, NE. Guangxi, China	AB031490
<i>C. (A.) sauteri yunkaicus</i>	9, 10	Hongjunting, Mt. Miao'er Shan, NE. Guangxi, China	AB031490
<i>C. (A.) sauteri sauteri</i>	11*	Shinchu, NW. Taiwan	AB031491
<i>C. (A.) sauteri sauteri</i>	12	Nanshan, Nantou, C. Taiwan	AB031492
<i>C. (A.) kouichii</i>	13	Mt. Pia Oac, Cao Bang Prov., N. Vietnam	AB031493
<i>C. (A.) tonkinensis</i>	14*	Deo Tram Ton, Lao Cai Prov., N. Vietnam	AB031494
<i>C. (A.) tonkinensis</i>	15	Deo Tram Ton, Lao Cai Prov., N. Vietnam	AB031495
<i>C. (A.) tuxeni bousquetianus</i>	16	Haba Mts., NW. Yunnan, China	AB031496
<i>C. (A.) delavayi ssp.</i>	17	Xichang-Yanyuan, S. Sichuan, China	AB031497
<i>C. (A.) delavayi huangmaogeng</i>	18	Daliang Shan Mts., S. Sichuan, China	AB031498
<i>C. (A.) toulgoeti</i>	19*, 20	Mt. Miao'er Shan, N. Guangxi, China	AB031499
<i>C. (A.) cyanipennis ssp.</i>	21	Mt. Guangwu Shan, NE. Sichuan, China	AB031500
<i>C. (A.) laoshanicus liaorum</i>	22*	Mt. Miao'er Shan, NE. Guangxi, China	AB031501
<i>C. (A.) laoshanicus laoshanicus</i>	23*	Mt. Dayao Shan, E. Guangxi, China	AB031502
<i>C. (A.) arrowi arroganti</i>	24*	Mt. Miao'er Shan, N. Guangxi, China	AB031503
<i>C. (A.) iris</i>	25	Daliang Shan Mts., S. Sichuan, China	AB031504
<i>C. (A.) clermontianus</i>	26*	Mt. Tam Dao, Vinh Phu Prov., N. Vietnam	AB031505
<i>C. (A.) sp.</i>	27	Maolan, Libo Xian, SE. Guizhou, China	AB031506
<i>C. (Limnocarabus) clathratus*</i>		W. Niedersachsen, N. Germany	AB031507
<i>C. (L.) maacki aquatilis**</i>		Nakasato, Aomori, N. Japan	D50358

* From IMURA *et al.* (1998); ** from SU *et al.* (1996).

vergence is not necessarily recent. Such a “silent morphological evolution” can be recognized in other carabids such as *Damaster blaptoides*, *Phricocarabus glabratus*, *Tomocarabus* spp., etc. (SU *et al.*, 1999).

As noted above, *C. (A.) hupeensis* split from one of the *C. (A.) sauteri* stems including *C. (A.) s. dayaoshanicus* and *C. (A.) s. ssp.* This suggests that *C. (A.) hupeensis* emerged with a considerable morphological change from *C. (A.) sauteri* whose morphology has remained almost unchanged for a long time. The speciation of this type resembles the branching of *C. (Euleptocarabus) porrecticollis* from the *C. (Limnocarabus) clathratus* stem (IMURA *et al.*, 1998; KIM, SU *et al.*, 1999; SU *et al.*, 1999). The difference in morphology between *C. (A.) cyanipennis* and *C. (A.) laoshanicus*, and between *C. (A.) arrowi* and *C. (A.) iris* are considerable, and yet their diversification occurred recently. Thus, evolution of *Apotomopterus* has been brought about by having a silent phase and a phase accompanied by a considerable morphological change, as has been widely recognized in the other carabid groups (SU *et al.*, 1999)

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要 約

金 衝坤・蘇 智慧・井村有希・大澤省三：ミトコンドリアND5遺伝子によるトゲオサムシ亜属(*Apotomopterus*)の系統解析。——*Apotomopterus*は、雄の交尾器内袋基部に基棘と呼ばれる硬化片を持つトゲオサムシ群の唯一の構成員で、中国南東部を中心に分布し、100種近くを含む大亜属である。本研究では、IMURAら(1998)の報告に加え、中国とその周辺地域からえられたトゲオサムシ亜属19分類単位(27個体)のミトコンドリアND5遺伝子の塩基配列から系統関係を検討した結果、3系統の存在が認められた。これらの3系統は、オサムシの一斉放散(約5~4千万年前)直後にほぼ同時に分岐し、さらに各系統の中で2~3回にわたって分岐が起きたと推定された。*Carabus (Apotomopterus) sauteri*は、中国南東部と台湾にいくつかの亜種を産するが、形態的にはたがいによく似ているため、その分化は比較的最近に起ったものと想像されがちである。しかし、mtDNAの結果では*sauteri*は3つの系統に分かれ、それぞれ2200万年前の古い時代に分岐したことが示唆された。また、*C. (A.) sp.*(プロイニングトゲオサムシに近縁な未記載種)と*C. (A.) clermontianus*も基本形態はたがいによく似ているが、mtDNAから両者は非常に古い時代に分化していることが示された。一方、*C. (A.) sauteri*の一つの枝から*C. (A.)*

hupeensis が分岐しているが、この事実は、長いあいだ形態変化がほとんどなかった *sauteri* から、形態的に異なる種が出現したことを示唆している。さらに、*C. (A.) cyanipennis* と *C. (A.) laoshanicus*, および *C. (A.) arrowi* と *C. (A.) iris* とのあいだでは、顕著な形態的差異にもかかわらず、分子系統樹からみるとそれぞれの種に分岐した時期は比較的新しいことが判明した。以上の結果から、トゲオサムシ亜属の進化には、形態が長いあいだほとんど変わらなかった時期と、短いあいだに変化した時期のあることが示唆された。

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