

Phylogeny in the Division Archicarabomorphi (Coleoptera, Carabidae) as Viewed from Mitochondrial ND5 Gene Sequences

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Abstract The phylogenetic trees for seven species belonging to the subgenera *Archicarabus*, *Gnathocarabus* and *Acrocarabus* in the division Archicarabomorphi of the genus *Carabus* (s. lat.) are constructed using the mitochondrial ND5 gene sequences. The phylogenetic relationships in and among each taxon are discussed.

Introduction

The Archicarabomorphi is one of the nine major divisions of the genus *Carabus* (s. lat.) (sensu IMURA *et al.*, 1998), consisting of four subgenera, namely, *Archicarabus*, *Ischnocarabus*, *Gnathocarabus* and *Acrocarabus* (cf. DEUVE, 1991 & '94; IMURA, 1996; IMURA & MIZUSAWA, 1996, etc.). Morphologically, they are characterized mainly by the basic structure of the male genital organ, *e.g.*, narrow preostium lacking the ostium lobe, uniquely developed paraligula, apparently projected peripheral rim of the gonopore, etc. In *Archicarabus*, each lobe of the endophallus is remarkably inflated, above all at the basal portion (Fig. 6). *Acrocarabus* is peculiar in having a digitulus-like sclerite on the ventral wall of the endophallus (Fig. 2), though this fact has never been pointed out by previous taxonomists.

The subgenus *Archicarabus* comprises nearly ten species distributed over the greater part of Europe and Asia Minor. The type species, *nemoralis*, is also recorded from North America and Southeast Kazakhstan of Central Asia, most probably as an introduction from Europe. The remaining three subgenera are rather restricted both in the number of species and the range of distribution. *Ischnocarabus* includes two species, both endemic to Turkey. *Gnathocarabus* is monotypical, and the type species, *kuznetzovi*, has been known only from the mountainous area of Northeast Iran. *Acrocarabus* consists of two species distributed on and around the Dzhungarskij Alatau of

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

Locality number	Scientific name by morphology	Locality	DDBJ/EMBL/GenBank Accession No.
1	<i>Acrocarabus guerini</i>	Uighentas, N. Dzhungarskij Alatau, SE. Kazakhstan	AB047276
2	<i>Ac. callisthenoides</i>	Tekeli-Koksu, SW. Dzhungarskij Alatau, SE. Kazakhstan	AB047251
3	<i>Ac. callisthenoides</i>	Altyn-emel Mts., SE. Kazakhstan	AB047252
4	<i>Gnathocarabus kuznetzovi</i>	Karim Ishan, NE. Gombad-e Kavus, Mazandaran, NE. Iran	AB047253
5	<i>Archicarabus victor</i>	Gönderic Tepesi, W. Giresun Mts., NE. Turkey	AB047277
6	<i>Ar. victor</i>	Karagöl Ridge, Giresun Mts., NE. Turkey	AB047254
7	<i>Ar. gotschi</i>	Mercan Mts., Erzincan, NE. Turkey	AB047255
8	<i>Ar. victor</i>	Karçal Dağı, Coruh, NE. Turkey	AB047256
9	<i>Ar. victor</i>	Karçal Dağı, Coruh, NE. Turkey	AB047257
10	<i>Ar. victor</i>	Karçal Dağı, Coruh, NE. Turkey	AB047258
11	<i>Ar. monticola</i>	Brondello, Cúneo, Piemonte, NW. Italy	AB047259
12	<i>Ar. monticola</i>	Tavigliano, Biella, Piemonte, NW. Italy	AB047260
13	<i>Ar. nemoralis</i>	Epleny, Zirc Veszprem, W. Hungary	AB047261
14*	<i>Ar. nemoralis</i>	Bourgogne, E. France	D86209
15	<i>Ar. nemoralis</i>	W. Index, Snohomish, Washington Stat., NW. USA	AB047262
16	<i>Ar. nemoralis</i>	Kok-tinbe Mt., Almaty, SE. Kazakhstan	AB047263
17	<i>Ar. nemoralis</i>	Liebiazhie, Leningrad Distr., W. Russia	AB047264
18	<i>Ar. nemoralis</i>	Lamspringe, Hildesheim, Niedersachsen, N. Germany	AB047265
19	<i>Ar. nemoralis</i>	St. Peterburg, W. Russia	AB047266
20*#	<i>Campalita chinense</i>	Oasa, Hiroshima, Honshu, SW. Japan	D50343
21*#	<i>Calosoma inquisitor</i>	Tokachi, Hokkaido, N. Japan	D50342

* Taken from SU *et al.* (1996a; 1996b). # As an outgroup for constructing the trees (see Fig. 1).

the northeastern Tianshans in Central Asia.

In the present study, we have examined the mitochondrial ND5 gene sequences from the representative species of all the above subgenera except *Ischnocarabus* to clarify the phylogenetic relationships among them. The result shows overall consistency between the molecular phylogeny and the morphological classification.

Following the same manner as in the other articles of ours recently published or those now in press, we conventionally use the subgeneric name in place of the generic name, i.e., "*Archicarabus*" means "*Carabus (Archicarabus)*".

Materials and Methods

The specimens used in this study are listed in Table 1. The analytical methods and the construction of the phylogenetic trees are the same as those described by SU *et al.* (1998).

Results and Discussion

On both the NJ- and the UPGMA trees, there exist four well-defined lineages. The two trees yielded the same topology of the species examined. Diversification of the four Archicarabomorphi lineages seems to have started within a short time about 28 million years ago.

The first lineage on the trees contains two species of *Acrocarabus*, i.e., *Ac. guerini* and *Ac. callisthenoides*, both from the northeastern Tianshans in Southeast Kazakhstan. The evolutionary distance (D) between the two species is large, suggesting that

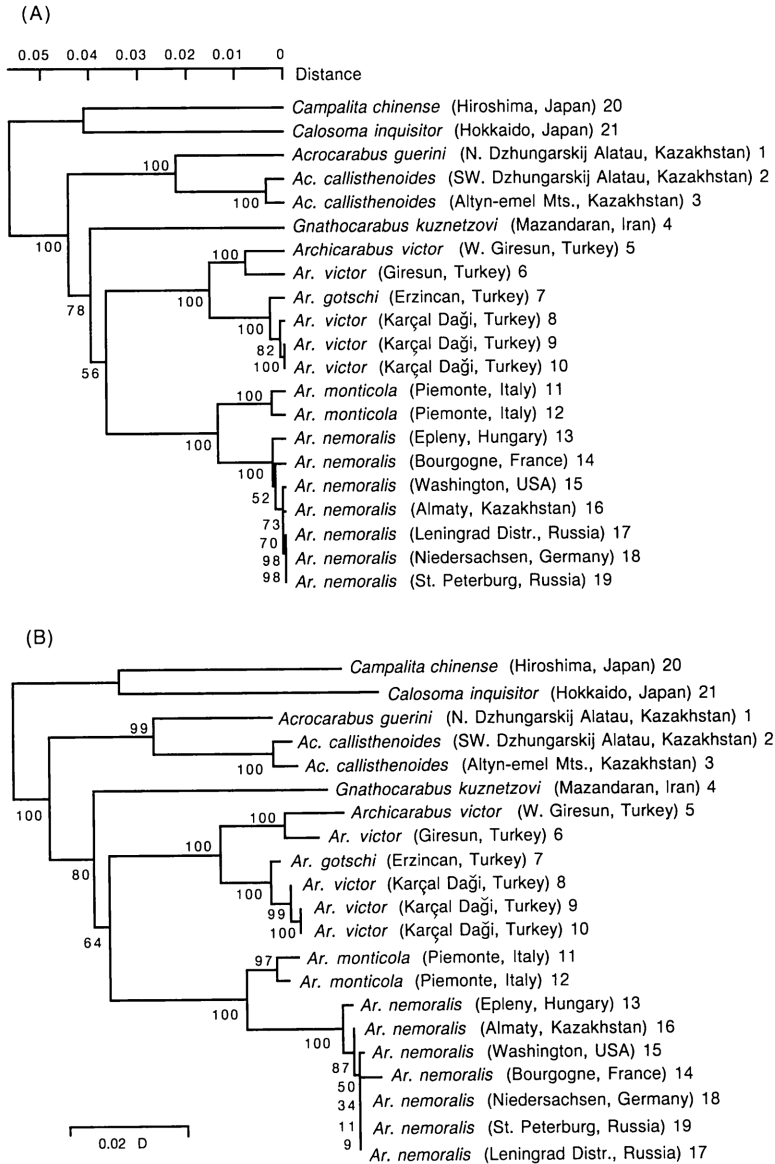
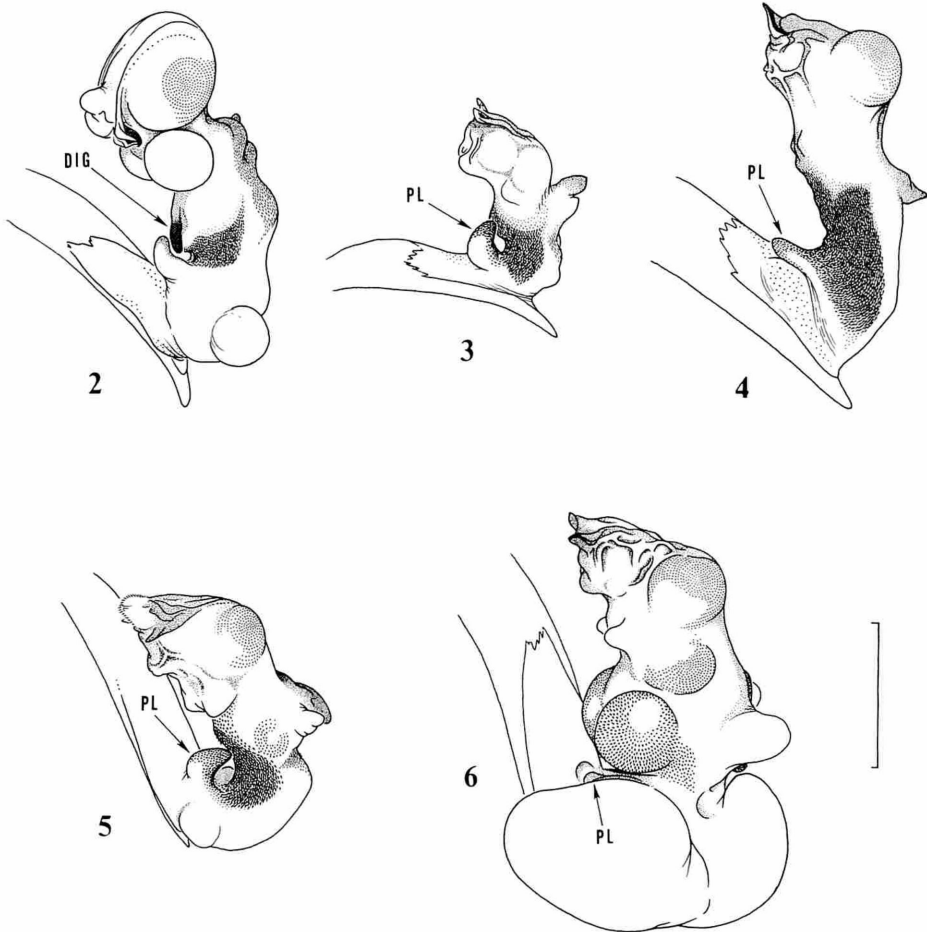


Fig. 1. Phylogenetic trees of the mitochondrial ND5 gene (1,069 bp upstream from 3 terminal stop codon) constructed from the species used in this study. The UPGMA tree (A) and the NJ tree (B). The bootstrap confidence level (%) based on 500 resamplings is shown at each branching point. Distance (D) denotes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



Figs. 2–6. Completely everted endophallus (left lateral view) of the subgenera *Acrocarabus*, *Gnathocarabus*, *Ischnocarabus* and *Archicarabus*. — 2, *Acrocarabus callisthenoides* (SW. Dzhungarskij Alatau, SE. Kazakhstan); 3, *Gnathocarabus kuznetzovi* (Karim Ishan, Mazandaran, NE. Iran); 4, *Ischnocarabus tenuitarsis* (Corum, Jskilip, Turkey); 5, *Archicarabus victor* (Karçkal-Otingo, NE. Turkey); 6, *Ar. nemoralis* (Hesel, Niedersachsen, N. Germany); DIG, digitulus; PL, paralingula. Scale: 2 mm.

despite morphological similarity their separation took place long time ago. The *Acrocarabus* lineage would have emerged earlier than the other three. Since the fundamental structure of the male genital organ of this subgenus is considerably different from that of the other three Archicarbomorphi-subgenera, *Acrocarabus* may not be appropriate to be included in this division. As shown in Fig. 2, the male genital organ of the *Acrocarabus* species is characterized by the presence of a chitinized piece on the ventral wall of the endophallus, which seems to be homologous with the digitulus of the

division Digitulati.

The second lineage is represented solely by *Gnathocarabus kuznetzovi* from Northeast Iran.

The third lineage includes two Turkish species, *Archicarabus victor* and *Ar. gotchi*. This lineage may be separated into two sublineages; one containing two specimens of *Ar. victor* from Giresun, and the other containing *Ar. gotchi* from the Mercan Mts. of Erzincan and *Ar. victor* from Karçal Dağı near the northeastern periphery of the country. Note that the same species, *Ar. victor*, appears in two different sublineages. These facts suggest a geography-linked phylogeny, and do not seem to reflect the morphology-based classification. It may be adequate to apply the subgenus *Deutero-carabus* REITTER to this lineage, if the type species (*montivagus*, distributed in the Balkan Peninsula) is also clustered here.

The fourth lineage includes two *Archicarabus* species, i.e., *Ar. monticola* and *Ar. nemoralis*. These two taxa are sharply separated from each other on the phylogenetic trees. Their separation was calculated to have occurred about 20 million years ago, assuming that a 0.01 D unit corresponds to 3.6 million years (SU *et al.*, 1998). Two examples of *Ar. monticola* from two different localities of Northwest Italy show a small difference in their sequences. The sequence difference is also small among all nine examples of *Ar. nemoralis* from seven different localities including North America and Central Asia. The difference is almost null among the specimens from the USA, Kazakhstan, West Russia and Germany, while the specimens from Hungary and France are a little remote in their sequences from the others mentioned above. The population of *Ar. nemoralis* occurring in North America has been considered to be a European introduction (*e.g.*, LINDROTH, 1961). The same situation may be applied to the specimen from Kazakhstan. The present result is consistent with this view.

Unfortunately, none of the *Ischnocarabus*-species were available for the DNA analysis. Judging from the similarity in the basic structure of the endophallus, this subgenus seems to be most closely allied to “*Deutero-carabus*” (*cf.* Figs. 4 & 5)

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要 約

井村有希・蘇 智慧・大澤省三：ミトコンドリアND5遺伝子の塩基配列からみたマルオサム

シ群内の系統。——マルオサムシ群 Archicarabomorphi を構成する4亜属のうち、3亜属から代表的な種を選び、ミトコンドリアND5遺伝子の塩基配列を決定して、群内の系統関係について検討した。分析した3亜属のうち、天山山脈のキンマルオサムシ *Acrocarabus* はもっとも分岐が古く、♂交尾器内袋に指状片を有するなど、形態学的にもかなり特異なので、マルオサムシ群から除外して骨片オサムシ群に分類するべきものと思われる。北西イランに孤立分布するキバヒョウタンオサムシ *Gnathocarabus* もかなり独立性の高いクラスターを形成したが、分子・形態双方の所見から、マルオサムシ群の一員と考えてよいだろう。狭義のマルオサムシ *Archicarabus* は大きく2サブクラスターに分かれ、一方はトルコ産の2種、他方はヨーロッパ産の2種によって構成された。前者に対しては *Deutero-carabus* 亜属を適用するべきかもしれない。*Archicarabus* の基準種である *nemoralis* の塩基配列には産地による相違がほとんどみられず、北米や中央アジアに分布する集団は、従来の指摘どおり、移入種である可能性の高いことが示唆された。

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