Phylogenetic Relationships in the Division Arciferi (Coleoptera, Carabidae)

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Abstract Four subgenera (*Platycarabus*, *Chaetocarabus*, *Heterocarabus* and *Hygrocarabus*) which construct the division Arciferi (IMURA, 1996) of the genus *Carabus* (s. lat.) are examined for the mitochondrial ND5 gene sequences to clarify their phylogenetic relationships. The constructed trees suggest that *Hygrocarabus* may be excluded from the Arciferi.

Introduction

The Arciferi was proposed by IMURA (1996, p. 7) as one of the five subdivisions in the division Multistriati of the genus *Carabus* (s. lat.). It is composed of four subgenera, *Platycarabus*, *Chaetocarabus*, *Heterocarabus* and *Hygrocarabus*, and is morphologically well-defined by bearing a characteristically shaped ligulum, named "arculus", at the base of the endophallus. According to the results drawn by IMURA *et al.* (1998), it has become reasonable to raise this subdivision to a distinct division of the genus *Carabus* (s. lat.).

In the present study, we have determined the ND5 (NADH dehydrogenase subunit 5) gene sequences of five species belonging to the Arciferi which cover the representatives of the above mentioned four subgenera, and phylogenetic relationships among them are discussed.

Materials and Methods

The specimens used in this study for determination of the ND5 gene sequences are listed in Table 1. The methods of DNA sequencing and the construction of the phylogenetic trees are the same as described previously (e.g., SU et al., 1998).

Locality number	Scientific name by morphology	Locality DI	DBJ/EMBL/GenBank Accession No.
1	Hygrocarabus nodulosus	Gunsbach, Haut-Rhin, E. France	AB047195
2	Heterocarabus marietti	W. Giresun, NE. Turkey	AB047196
3-1	Chaetocarabus intricatus	Andrate, Torino, Piemonte, NW. Italy	AB047197
3-2	Ch. intricatus	Cúneo, Piemonte, NW. Italy	AB047197
4*	Ch. intricatus	Bourgogne, CE. France	D86208
5	Platycarabus depressus	Val Sesia, Varallo, Piemonte, NW. Italy	AB047198
6	P. depressus	Andrate, Torino, Piemonte, NW. Italy	AB047199
7-1	P. irregularis	Lamspringe, Hildesheim, Niedersachsen, N. Germa	any AB047200
7-2	P. irregularis	Göttingen, Niedersachsen, N. Germany	AB047200
8	P. irregularis	Jura, E. France	AB047201
9*	Limnocarabus clathratus	Dalums Moor, Lingen, Niedersachsen, N. German	v AB031507
10*	L. maacki aquatilis	Nakasato, Aomori, Honshu, N. Japan	D50358
11*	Euleptocarabus porrecticollis	Nakatsugawa, Gifu, Honshu, C. Japan	AB022564
12*#	Cychrus morawitzi	Daisetsu-zan Mts., Hokkaido, N. Japan	D50347

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

The sequences of 3-1 and 3-2, and those of 7-1 and 7-2 are the same, respectively.

Results and Discussion

In a previous paper, we showed that *Platycarabus irregularis**) and *Chaeto-carabus intricatus* are clustered together with a rather deep branching point on the ND5 phylogenetic tree of the representative Carabina species in the world (IMURA *et al.*, 1998, p. 21).

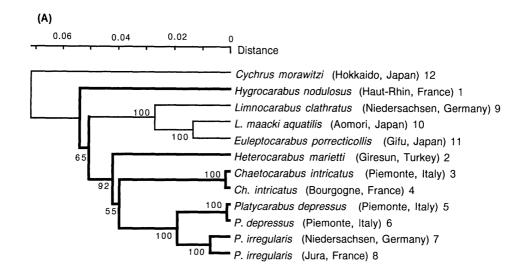
As shown in Fig. 1, four species, *Chaetocarabus intricatus*, *Heterocarabus marietti*, *Platycarabus depressus* and *P. irregularis*, are clustered together on both the NJ-and UPGMA trees, while *Hygrocarabus nodulosus* is not tightly related to the other Arciferi species. Its emergence may be traced back to the time of the radiation of the Carabina (SU *et al.*, 1996 a; IMURA *et al.*, 1998). Since there is no other species that are clustered with *Hygrocarabus*, it should be excluded from the division Arciferi and is considered to form an independent position in the subtribe Carabina. In fact, emergence of *Hygrocarabus* is as early as that of *Limnocarabus* and *Euleptocarabus* which are the members of the division Lepidospinulati (IMURA *et al.*, 1998). This seems to be consistent with the behavior and the larval morphology of this unique carabid; it is well-known that *Hygrocarabus* leads a semi-aquatic habit throughout its life, and the larva bears quadricuspidate epistoma, while the epistoma of the other three are rostrilabral (CASALE *et al.*, 1982).

CASALE et al. (1998) reported a phylogenetic tree of *Platycarabus* and *Chaeto-carabus* based on the mitochondrial ND1 gene sequences (336 bp). Unfortunately, the molecular tree does not contain either *Hygrocarabus* or *Heterocarabus*, but on their tree based on the morphological data, *Hygrocarabus* was positioned outside other *Platycarabus* species.

^{*} Taken from previous studies (SU et al., 1996a; 1996b and KIM et al., 1999a; 1999b).

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

^{*} For convenience, we use hereafter the subgeneric name in place of routinely used generic name, *Carabus*, such as "*Platycarabus irregularis*" = *Carabus* (*Platycarabus*) irregularis".



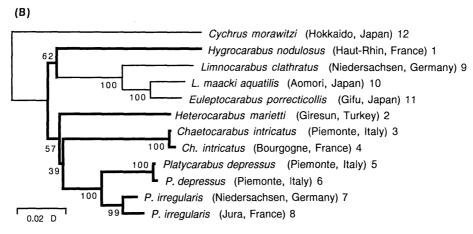


Fig. 1. Phylogenetic trees of the mitochondrial ND5 gene (1,069 bp upstream from 3 terminal stop codon) constructed from the species used in this study. The UPGMA tree (A) and the NJ tree (B). The bootstrap confidence level (%) based on 500 resamplings is shown at each branching point. Distance (D) denotes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

Chaetocarabus, Heterocarabus and Platycarabus are well separated on the trees in accordance with the morphological classification. The separation of these three subgenera appears to have taken place within a short time. The sequences of Chaetocarabus intricatus specimens from Northeast Italy and France are almost the same. Platycarabus depressus and P. irregularis are separated well. Two P. depressus specimens from Northeast Italy carry almost the same ND5 gene sequences, and P. irregularis from North Germany is reasonably close to the same species from France.

In summary, morphological classification in the division Arciferi is consistent with the phylogenetic analyses using the ND5 gene sequences, except that *Hygrocarabus* may be excluded from this division.

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要 約

井村有希・蘇 智慧・大澤省三:ヒラタオサムシ群内の系統関係。—— ヒラタオサムシ群 Arciferi はヒラタオサムシ亜属 Platycarabus,キバナガヒラタオサムシ亜属 Chaetocarabus,ヒメツヤヒラタオサムシ亜属 Heterocarabus,ミズベオサムシ亜属 Hygrocarabusの4 亜属からなるとされ、 δ 交尾器内袋基部に弓状片 arculusとよばれる器官を共有することにより、他の群から形態学的に識別されてきた。これら各亜属から1~2種ずつを選び、ミトコンドリア ND5 遺伝子の塩 基配列を決定して、群内の系統関係を再検討したところ、ミズベオサムシ Hygrocarabus のみは他の3 亜属から類縁が遠く、オサムシ亜族内において独立した地位を与えるべきものであろうと思われた。

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A New *Cychrus* (Coleoptera, Carabidae) from Zayü in Southeast Tibet

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Our knowledge is still very poor on the cychrine fauna of Tibet. Two species of the genus *Cychropsis* have been described from the southeastern part of that region (cf. Deuve, 1992; IMURA, 1999), but nothing has been reported about the genus *Cychrus*.* Through the courtesy of Mr. and Mrs. Roman Businský (Prague), I was given an opportunity to examine a short series of cychrine specimens collected from Zayü near the southeastern periphery of Tibet (Xizang). Though resembling *Cychrus kralianus*, the Zayü race is peculiar in bearing characteristically shaped aedeagal apex. It is therefore introduced to science as follows.

Cychrus businskyianus IMURA, sp. nov.

Length: 13.2–14.3 mm (including mandibles). Entirely black and not so strongly polished. Closely similar to *Cy. kralianus* Deuve (1996, p. 87, figs. 8 & 16) described from the Hengduan Shan Mountains in northwestern Yunnan, but differs from it in the following respects: 1) size a little smaller; 2) frontal margin of labrum more strongly arcuate; 3) vertex more remarkably rugoso-punctate; 4) postero-lateral margins of pronotum less strongly reflexed above; 5) primary callosities of elytra wider. Male genitalia as shown in Fig. 2, with the apical portion of aedeagus much more elongate, less strongly arcuate, obviously sinuate on the ventral margin in lateral view and more obtuse at apex in dorsal view; endophallus rather simple, only bearing a pair of small lateral lobes at the basal portion.

Type series. Holotype: ♂, about 20 km distant to the northeast from Zayü [察隅], 3,800–4,000 m in altitude, on the eastern side of the Riv. Zayü Qu, in Zayü Xian of Nyingchi

^{*} Another new species of this genus is described from Southeast Tibet on page 222 of this volume.