

Phylogenetic Relationships of Some Chinese Ground Beetles Belonging to the Subgenera *Neoplesius*, *Pagocarabus* and *Aristocarabus* (Coleoptera, Carabidae) Based on Mitochondrial ND5 Gene Sequences

Yûki IMURA

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

Hong-Zhang ZHOU

Institute of Zoology, Chinese Academy of Sciences, 19 Zhongguancun Lu,
Haidian, Beijing, 100080 China,

Munehiro OKAMOTO

The Institute of Experimental Animal Sciences, Osaka University Medical School,
2–2, Yamadaoka, Suita, Osaka, 565–0871 Japan,

Zhi-Hui SU and Syozo OSAWA

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

Abstract Phylogenetic trees are constructed using the mitochondrial ND5 gene sequences for *Carabus* (*Neoplesius*) *sichuanicola*, *C. (N.) tatsienlui*, *C. (Pagocarabus)* *crassesculptus* and *C. (Aristocarabus)* *viridifossulatus* together with some other representative carabine species. These four species form a single cluster together with other *Neoplesius*/*Eocechenus* species. *Damaster* (s. lat.) is the outgroup of this cluster. The tree suggests that radiation of *Neoplesius*/*Eocechenus* species took place a little after separation from *Pagocarabus* from their common ancestor. The male of *C. (N.) sichuanicola* is figured and described for the first time.

Introduction

The subdivision Procrustimorphi (sensu IMURA, 1996) (division Multistriati, subtribe Carabina) contains 54 subgenera and yet their phylogenetic relationships have not been satisfactorily established. Based on the mitochondrial ND5 gene sequence comparisons, IMURA *et al.* (1997) reported that the subgenera *Neoplesius* and *Eocechenus* form a monophyletic cluster that shares a common ancestry with *Damaster* (s. lat.),

with the *Procrustes*–*Megodontus* lineage as their sister group.

We have had an opportunity to analyse the ND5 gene sequences of several Chinese species belonging to the Procrustimorphi. These are *Carabus* (*Neoplesius*) *sichuanicola*, *C. (N.) tatsienlui*, *C. (Pagocarabus) crassesculptus* and *C. (Aristocarabus) viridifossulatus*. The results are described in this paper.

Materials and Methods

The species analysed for the ND5 gene sequences are listed in the following lines. Of these, the specimens Nos. 1, 2 and 3 were collected during the Sino-Japanese Co-operative Expedition to Sichuan, China, in June 1997.

For the analytical methods and construction of the phylogenetic trees, see SU *et al.* (1996) and IMURA *et al.* (1997).

1) *Carabus (Neoplesius) sichuanicola* DEUVE, 1989

(Figs. 1, 6–8)

Carabus (Acoptolabus) sichuanicola DEUVE, 1989, *Nouv. Revue Ent.*, (N. S.), **6**, p. 170; type locality: "Chine, Sichuan, Dong He."

Description of male. Length (including mandibles): 22.3–24.5 mm (23.0–27.7 mm in female). Antennae longer than in female, extending slightly beyond the middle of elytra; terminal segments of palpi almost the same in width as in female; all the four basal segments of foretarsi densely scattered with hairpads on the ventral surface; genitalia as shown in Figs. 6–8, ostium lobe slightly bilobed at the tip, median lobe absent, prepraeputal lobes with the left lobe larger than the right, parpraeputal lobes rather sharply pointed at the tips and the right lobe a little larger than the left.

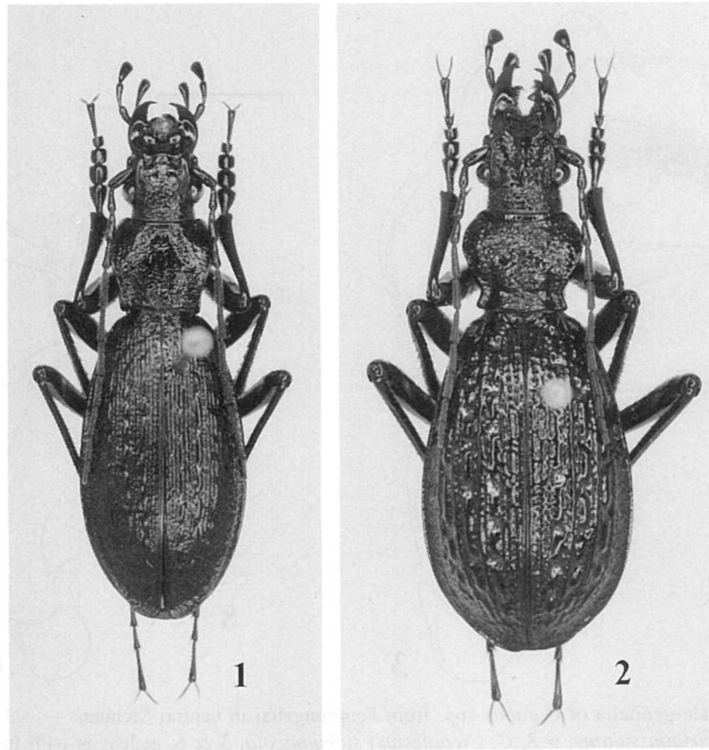
Specimens examined. 25 ♂♂, 16 ♀♀ (1 ♂ for DNA analysis), above Guobaye (so-called "Mt. Baiyu Shan"), 2,700–3,200 m in altitude, on the Qionglai Shan Mts. stretching along the left side of the Riv. Dong He, in the Fengtongzhai Nature Protective Area of Baoxing Xian, Central Sichuan, China, 4–VI–1997, Y. IMURA, Z.-H. SU & M. OKAMOTO leg.

Notes. This species was described by DEUVE on the basis of a single female specimen preserved in the collection of the Institute of Zoology, Chinese Academy of Sciences, Beijing, and only "Dong He" of Sichuan was originally given as its type area. However, the holotype specimen bears a label with more exact collecting data as given below.

四川[Sichuan], 东河[Donghe], 硃磬 [Qiaoqi (=“Yaoqi” according to the pronunciation of native people)], 30083.

Thus, the exact type locality of *Carabus (Neoplesius) sichuanicola* should be restricted to Qiaoqi, a small village of Tibetans. Our locality is situated a few kilometers distant to the east-southeast from there.

In all the specimens examined, dorsal surface is blackish brown with faint bronze



Figs. 1–2. *Carabus* spp. from Fengtongzhai in central Sichuan. — 1, *C. (Neoplesius) sichuanicola*, ♂; 2, *C. (Aristocarabus) viridifossulatus*, ♂.

or red-purplish tinge which is much darker than in the holotype. The coloration of the latter seems to have been faded due to aging or rather inferior preservative condition of the specimen.

2) *Carabus (Neoplesius) tatsienlui* BREUNING, 1934

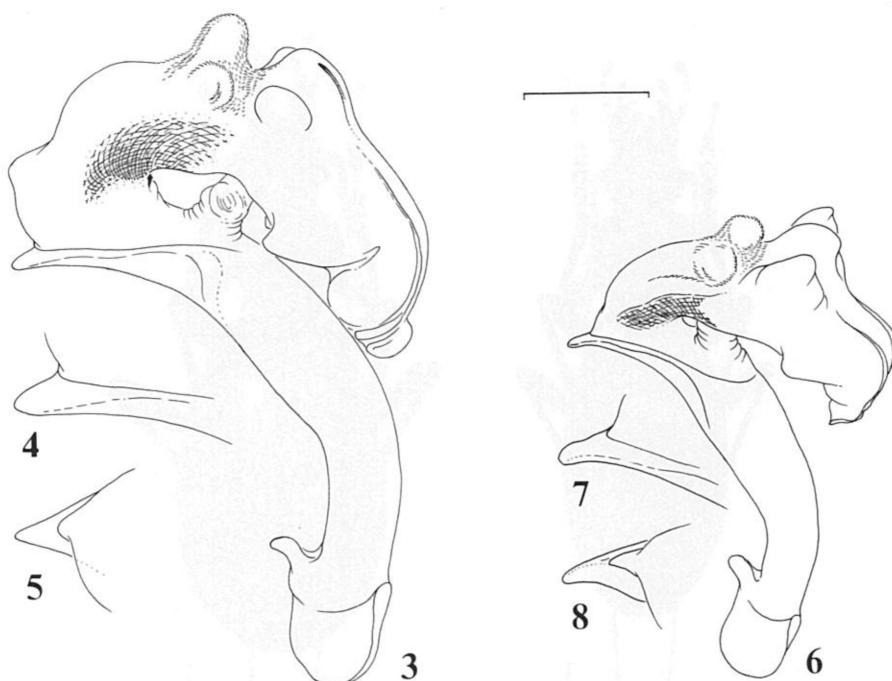
Carabus (Pagocarabus) tatsienlui BREUNING, 1934, Folia zool. hydrobiol., **7**, p. 48; type locality: China, Prov. Szetschuan, Berge bei Tat-sien-lu.

Specimens examined. 2 ♀♀ (1 ♀ for DNA analysis), Mt. Zheduo Shan (ca. 4,000 m in alt.) in Kangding Xian, central Sichuan, China, 7–VI–1997, S. UENO leg.

3) *Carabus (Pagocarabus) crassesculptus* KRAATZ, 1881

Carabus crassesculptus KRAATZ, 1881, Dt. ent. Z., **25**, p. 268; type locality: Gebirgen westlich von Peking.

Specimens examined. 2 ♂♂ (1 ♂ for DNA analysis), Xiaolongmen of Men-



Figs. 3–8. Male genitalia of *Carabus* spp. from Fengtongzhai in central Sichuan. — 3–5, *C. (Aristocarabus) viridifossulatus*; 6–8, *C. (Neoplesius) sichuanicola*; 3 & 6, aedeagus with fully everted endophallus in right lateral view; 4 & 7, aedeagal apex in the same view; 5 & 8, ditto in dorsal view. Scale: 2 mm for 3 & 6, 1 mm for 4, 5, 7 & 8.

tougou, in W Beijing, China, 19~22-VII-1997, Hai-Sheng ZHOU leg.

4) *Carabus (Aristocarabus) viridifossulatus*, FAIRMAIRE, 1887

(Figs. 2–5)

Carabus viridi-fossulatus FAIRMAIRE, 1887, Anns. Soc. ent. Belg., **31**, p. 91; type locality: Moupin (=Baoding).

Specimens examined. 1 ♂, 2 ♀♀ (1 ♀ for DNA analysis), same data as for *C. (Neoplesius) sichuanicola*, though the collected site was a little higher in altitude (ca. 3,200 m).

Notes. It is well-known that this species is polytypic and is discriminated into several subspecies. Our specimens seem to be identical with the nominotypical subspecies judging from the morphology and locality, though much more colourful (head and pronotum light purple, elytra metallic green with the primary foveoles yellowish golden) than in FAIRMAIRE'S holotype now preserved in the Muséum Nationale d'His-

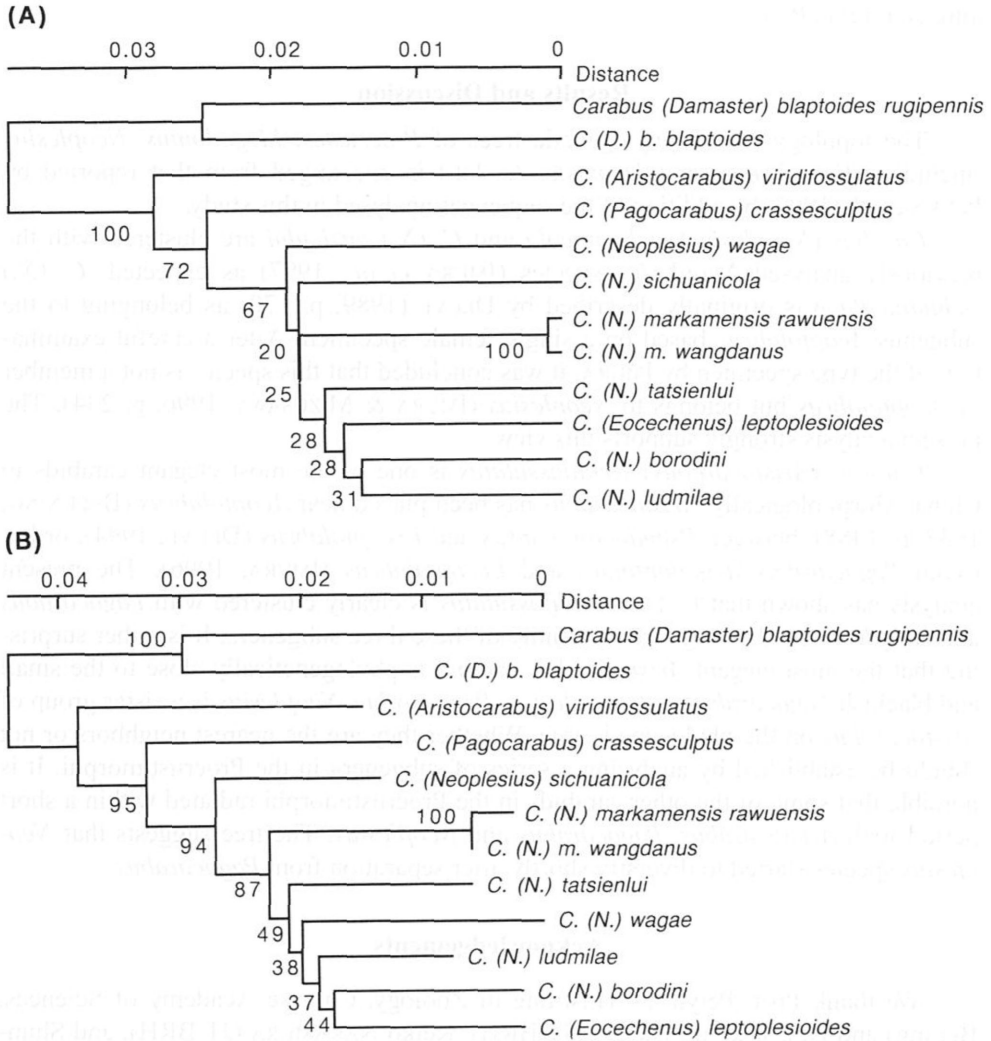


Fig. 9. Phylogenetic trees of some Chinese ground beetles based on 1,069-bp sequences of mitochondrial ND5 gene. The evolutionary distances were computed by KIMURA's two-parameter method (KIMURA, 1980). The trees were constructed by the UPGMA (A) and the neighbor-joining (NJ) method (B) (SAITOU & NEI, 1987) of BIORESEARCH/SINCA ver. 3.0 (Fujitsu System Engineering, Japan). The values at the nodes represent percent of bootstrap confidence level (based on 500 resamplings) (FELSENSTEIN, 1985). The ND5 gene sequences of *Damaster* were used as the outgroup for the tree construction. Accession numbers for DDBJ, EMBL, and GenBank databases are as follows: *Carabus (Damaster) blaptoides rugipennis* (D50351[†]), *C. (D.) b. blaptoides* (D50349[†]), *C. (Aristocarabus) viridifossulatus* (AB010718), *C. (Pagocarabus) crassesculptus* (AB010719), *C. (Neoplesius) sichuanicola* (AB010720), *C. (N.) markamensis rawuensis* (AB001513[†]), *C. (N.) m. wangdanus* (AB001514[†]), *C. (N.) tatsienlui* (AB010721), *C. (N.) wague* (AB001515[†]), *C. (N.) ludmilae* (AB001512[†]), *C. (N.) borodini* (AB001511[†]), *C. (Eoecchenus) leptoplesioides* (AB001510[†]). [†]: analyzed in the previous studies (SU *et al.*, 1996; IMURA *et al.*, 1997).

toire Naturelle, Paris.

Results and Discussion

The topology on the phylogenetic trees of *Procrustes*, *Megodontus*, *Neoplesius* (including *Eocechenus*) and *Damaster* (s. lat.) is unchanged from that reported by IMURA *et al.* (1997) by addition of the sequences analysed in this study.

Carabus (*Neoplesius*) *sichuanicola* and *C. (N.) tatsienlui* are clustered with the previously analysed *Neoplesius* species (IMURA *et al.*, 1997) as expected. *C. (N.) sichuanicola* was originally described by DEUVE (1989, p. 170) as belonging to the subgenus *Acoptolabrus* based on a single female specimen. After a careful examination of the type specimen by IMURA, it was concluded that this species is not a member of *Acoptolabrus* but belongs to *Neoplesius* (IMURA & MIZUSAWA, 1996, p. 234). The present analysis strongly supports this view.

Carabus (*Aristocarabus*) *viridifossulatus* is one of the most elegant carabids in China. Morphologically, *Aristocarabus* has been placed near *Acoptolabrus* (BREUNING, 1937, p. 1488), between *Pseudocoptolabrus* and *Eccoptolabrus* (DEUVE, 1994), or between *Pagocarabus/Megodontoides* and *Eccoptolabrus* (IMURA, 1996). The present analysis has shown that *C. (A.) viridifossulatus* is clearly clustered with *Pagocarabus* and *Neoplesius*, indicating a close affinity of these three subgenera. It is rather surprising that the most elegant *Aristocarabus* carabid is phylogenetically close to the small and blackish *Pagocarabus* and *Neoplesius*. *Pagocarabus/Neoplesius* is a sister group of *Aristocarabus* on the phylogenetic tree. Whether they are the nearest neighbors or not should be established by analysing a series of subgenera in the Procrustimorphi. It is possible that some of the other carabids in the Procrustimorphi radiated within a short period with *Aristocarabus*, *Pagocarabus* and *Neoplesius*. The tree suggests that *Neoplesius* species started to diversify shortly after separation from *Pagocarabus*.

Acknowledgements

We thank Prof. Peiyu YU (Institute of Zoology, Chinese Academy of Sciences, Beijing) and Drs. Tokindo OKADA (JT BRH), Keiko NAKAMURA (JT BRH), and Shun-ichi UENO (National Science Museum, Tokyo) for the arrangement and help in the Sino-Japanese Cooperative Expedition to Sichuan, China, in 1997.

要 約

井村有希・周 紅章・岡本宗裕・蘇 智慧・大澤省三：ミトコンドリアND5遺伝子解析に基づく中国産ヨロイオサムシ類（チベットオサムシ，マンダラオサムシおよびニシキオサムシ各亜属）の系統関係。——ヨロイオサムシ亜群に属する中国産の4種（シセンチベットオサムシ，ターチェンルーチベットオサムシ，マンダラオサムシ，ニシキオサムシ）のミトコンドリアND5遺伝子を解析し，分子系統樹を作成して，その系統関係について検討を加えたところ，こ

これらの4種は、既報のチベットオサムシ-タカネオオズオサムシ群とともに単一のクラスターを構成し、広義のマイマイカブリ群はその外群に位置することがあきらかになった。また、同系統樹は、本クラスター内における分岐順として、共通の祖先種からまずニシキオサムシ、ついでマンダラオサムシが分かれ、しかるのちにチベットオサムシ-タカネオオズオサムシ群が形成されたことを示している。なお、本論文において、これまで未知であったシセンチベットオサムシの♂を図示、記載した。

References

- BREUNING, S., 1932-'37. Monographie der Gattung *Carabus* L. *Best.-Tab. eur. Coleopt.*, (104-110): 1-1610, 41 pls. Reitter, Troppau.
- 1934. Zwei neue Carabini aus Ostasien. *Folia zool. hydrobiol.*, **7**: 48.
- DEUVE, Th., 1989. Nouveaux Carabidae des collections de l'Institut Zoologique de l'Academia Sinica de Pékin (Coleoptera). *Nouv. Revue Ent.*, (N. S.), **6**: 159-171.
- FAIRMAIRE, L., 1887. Coléoptères de l'intérieur de la Chine. *Annls. Soc. ent. Belg.*, **31**: 87-136.
- FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**: 783-791.
- IMURA, Y., 1996. A revised classification of the major divisions and subdivisions of *Carabus* (s. lat.) (Coleoptera, Carabidae). *Elytra, Tokyo*, **24**: 5-12.
- & K. MIZUSAWA, 1996. The *Carabus* of the World. In FUJITA, H. (ed.), *Mushi-Sha's Iconographical Series of Insects*, 2. 261 pp., 84 pls. Mushi-sha, Tokyo. (In Japanese, with English book title and summary.)
- IMURA, Y., Z.-H. SU & S. OSAWA, 1997. Morphology and molecular phylogeny of some Tibetan ground beetles belonging to the subgenera *Neoplesius* and *Eoecchenus* (Coleoptera, Carabidae). *Elytra, Tokyo*, **25**: 231-245.
- KIMURA, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. mol. Evol.*, **16**: 111-120.
- KRAATZ, G., 1881. Fünf neue chinesische *Carabus*. *Dt. ent. Z.*, **25**: 265-269.
- SAITOU, N., & M. NEI, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**: 406-425.
- SU, Z.-H., T. OHAMA, T. S. OKADA, K. NAKAMURA, R. ISHIKAWA & S. OSAWA, 1996. Phylogenetic relationships and evolution of the Japanese Carabinae ground beetles based on mitochondrial ND5 gene sequences. *J. mol. Evol.*, **42**: 124-129.