Phylogeny of the subgenus *Ohomopterus*  
(Coleoptera, Carabidae, genus *Carabus*):  
A morphological aspect

by

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Abstract: The phylogenetic hypothesis of ground beetles (genus *Carabus*) belonging to the subgenus *Ohomopterus* endemic to Japan is proposed on the basis of the analysis of morphological characters using cladistic methods. Twenty-three characters are extracted from adults of 35 species or subspecies. Two species of the subgenus *Isiocarabus*, *Carabus fiduciarious saishutoicicus* and *C. kiukiangensis*, are used as outgroups. In cladistic analyses, three character weighting methods, equally weighting, successive weighting and Goloboff fitting, are applied. Of these, the latter two down-weight homoplastic characters. The comparisons between the results of analyses with or without outgroups indicate that the position of outgroups changes among weightings but ingroup trees are stable. The analysis of the most parsimonious attachment of outgroups on ingroup branches reveals the serious ambiguity of outgroup rooting. Thus, an ingroup tree is rooted by the evidence from the study of molecular phylogeny. In this case, an appropriate ingroup topology is chosen among equally parsimonious trees on the basis of the direction of character evolution and topological resolution. As a result, the obtained tree is different from existing morphological taxonomy and molecular phylogeny. Two major clades appear, in which one consists of the *japonicus*, *deaunii*, *albrechti* species groups and *C. yacominus* and the other of the *insulicola* species group and *C. iwawakianus*. In the former, each of the morphologically recognized *japonicus* and *deaunii* species groups is monophyletic and they are close to each other. The *albrechti* species group is paraphyletic because *C. yamato* is closer to the *japonicus* + *deaunii* group. *C. yacominus* is placed at the most ancestral position of the former major clade. In the latter clade, the *insulicola* species group is monophyletic but seriously polytomous, and the subspecies of *C. iwawakianus* are paraphyletically positioned at the ancestral branch of the *insulicola* species group. Therefore, the *yacominus* species group may be divided into two clades. The establishing process of distribution pattern of *Ohomopterus* within the Japanese Archipelago is briefly discussed.

Key words: character weighting, cladistics, Carabidae, Japan, morphology, phylogeny.

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Introduction

Diversity of ground beetles within the Japanese Archipelago is mainly characterized by the endemic subgenus *Ohomopterus*. The subgenus *Ohomopterus* belonging to the genus *Carabus* consists of fifteen species and many subspecies. Taxonomists have often proposed phylogenetic hypotheses of them as classification systems. Nakane (1962) recognized four species and many subspecies in this subgenus on the basis of the morphology of the chiton tooth on the male genitalia (=copulatory piece sensu Ishikawa, 1973). Subsequently, Ishikawa (1985) recognized four species groups, and later five species groups, in this subgenus (Ishikawa, 1991). In his treatment, Nakane’s subspecies were raised to specific status from the viewpoint of the biological species concept. Ishikawa (1989, 1991) proposed phylogenetic relationships among four or five species groups based on a morphcline of the copulatory piece (see also Kubota and Sota, 1998). These phylogenetic hypotheses were, however, inferred by traditional taxonomy but were not the results of modern cladistic analyses.

Su et al. (1996) proposed a phylogeny of *Ohomopterus* species based on the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences. The molecular tree of these sequences suggested radiative divergence of this group, and the topology disagreed with the morphological taxonomy. In their result, taxa traditionally treated as one species or one subspecies were divided polyphyletically into several distinct lineages. Moreover, taxa which were distributed sympatrically or parapatrically formed a monophyletic group within each geographical area, though they had been assumed to be distinct lineages on the basis of morphological characters. They explained this disagreement between morphology and molecular phylogeny by the type-switching hypothesis. Their hypothesis insists that morphology is easily changeable by a few genetic mutations, and morphologically identical form (= morphospecies) can arise in different lineages in parallel because of similar genetic mutations (Su et al., 1996, Osawa et al., 1999). Kubota and Sota (1998) pointed out the impact of natural hybridization on the divergence of *Ohomopterus*. They showed many evidences which indicated that introgressive hybridization between species frequently occurred in *Ohomopterus*. According to them, the mitochondrial gene tree may not agree with the history of divergence of this group because of the introgression of mitochondria between distinct lineages.

Discordance between morphological and molecular trees is often found in phylogenetic studies (Patterson et al., 1993). In phylogenetic reconstruction, molecular sequence is gen-
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Generally superior to morphology in the number and independency of characters, stochastic mode of evolution, and no phenotypic variance. However, even in molecular phylogeny, some causes mislead wrong results.

If taxa in question have diverged at a moderate tempo and are completely reproductively isolated, molecular phylogeny will correctly reflect their evolutionary history. However, molecular phylogeny may provide seriously misleading results in an analysis of radiation caused by rapid divergence of species, or in the analysis of species incompletely reproductively isolated from each other (Maddison, 1997). The former situation may lead to random lineage sorting resulted from ancestral polymorphism (Avise *et al.*, 1983; Tajima, 1983; Takahata and Nei, 1985; Neigel and Avise, 1986; Nei, 1987; Pamilo and Nei, 1988; Wu, 1991). In frequently diverging lineages in which speciation continually occurs, evolutionary time between speciation events is not enough to diminish allele polymorphism, and alleles with their origins mismatched with the speciation event are retained and sampled in terminal species. As a result, the tree of such alleles can not be overlaid on the species tree. In the latter situation, molecular phylogeny may mistake because of interspecific hybridization (Doyle, 1992). Natural and/or sexual selection causes adaptation which often accelerates speciation. Selection pressure leads to morphological and ecological differentiation between species. Such phenotypic differentiation may work as prezygotic isolation even if postzygotic isolation is less established (Schluter, 1998). Furthermore, such species incidentally hybridize and exchange their genes (Arnold, 1997). In such situations, the reliability of molecular data decreases.

Recently, Sota (2000) and Sota and Vogler (in press) analyzed nuclear gene sequences of this group. Their results showed significant incongruence between mitochondrial and nuclear gene sequences, suggesting introgressive hybridization. However, their nuclear trees were weakly resolved. At present, there has been no reasonable hypothesis presented on the phylogenetic relationships of *Ohomopterus* species, because a cladistic analysis of morphological characters has not been carried out, there is a possible incongruence of the mitochondrial gene tree with the nuclear tree, and the nuclear gene tree is not resolved enough to be determinate. Under these condition, the analysis of morphological characters may provide valuable information on phylogenetic relationships of species (Lanyon, 1988). For a comprehensive understanding of the evolutionary history of the species of the subgenus *Ohomopterus*, this paper gives a phylogenetic hypothesis of the ground beetles of this subgenus that is proposed based on morphological characters using cladistic methods with parsimony criteria.

**Taxa examined**

Thirty-five species or subspecies of the subgenus *Ohomopterus* were used in the analysis (Table 1), based on 23 morphological characters of the adult. On the species that were divided into distinct lineages by the analysis of mitochondrial DNA sequences (Su *et al.*, 1996), those distinct lineages were treated as distinct OTUs. Two species of the subgenus *Isiocarabus*, *C. (I.) fiduciaruis saishutoicus* and *C. (I.) kiukiangensis*, were adopted as
Isiocarabus is thought to be the sister group of Ohomopterus (Ishikawa, 1989), because it is the only taxon sharing morphological characters which can be traced their homologies with Ohomopterus.

The majority of materials for this study were basically obtained from the collection of Department of Natural History, Tokyo Metropolitan University. Additional materials were collected by the author, and will be deposited in the collection of NHTMU.

Character evaluation and coding

Variations of morphological characters were evaluated and discretely coded as follows (Table 2). Terminology of each character was based on Ishikawa (1973, 1978 and 1979), Deuve (1994) and Marciniak (1995), or was newly proposed in this study.

Male genitalia (Figs. 1-26)

1. **Left basal lobe**: absent (0); present (1). Most Ohomopterus species have a lobe at the left base of the endophallus except C. uenoi, and the shape varies among species.

2. **Right basal lobe**: absent or little swelling (0); strongly lobate (1). This lobe is posi-
tioned on another side of the left basal lobe, i.e., the right base of the endophallus.

3. **Hind lobe**: absent (0); present (1). This small lobe is positioned at the ventral part of the left basal lobe in *C. kimurai* and the several subspecies of *C. japonicus*.

4. **Ligula**: absent (0); present (1). This structure is strongly developed in Spinulati (sensu Ishikawa, 1978). In *Ohomopterus*, however, the ligula is absent or found as a weakly sclerotized spot.

5. **Paraligula**: absent (0); present (1). This structure is a membranous projection at the dorsal base of the endophallus beside the ligula. Its surface is usually covered with micro-structural scales.

6. **Median swelling**: slightly swollen (0); strongly lobate (1). The endophallus is often swollen or lobate at both sides of the base of the copulatory piece.

7. **Praeputial lobe**: absent (0); present but fused with the praeputial pad (1); present and

![Fig. 1. Representation of characters on the endophallus: a) Carabus (Ohomopterus) japonicus ikiensis, dorsal view; b) the same species, ventral view; c) C. (O.) esakii, dorsal view, partly omitted. 1-10, characters used in the phylogenetic analysis: 1) left basal lobe; 2) right basal lobe; 3) hind lobe; 4) ligula; 5) paraligula; 6) median swelling; 7) praeputial lobe; 8) preapical lobes; 9) aggonoporius; 10) copulatory piece.](image-url)
Figs. 2-12. Endophalli of the *albrechti* and *japonicus* species groups: 2) *Carabus* (*Ohomopterus*) *albrechti*; 3) *C. (O.) lewesianus*; 4) *C. (O.) kimurai*; 5) *C. (O.) yamato*; 6) *C. (O.) japonicus* [KYUSHU]; 7) *C. (O.) j.* [SHIKOKU]; 8) *C. (O.) j. tsushimae*; 9) *C. (O.) j. chagokuenis* [SAN’IN]; 10) *C. (O.) j. c.* [SAN’YO]; 11) *C. (O.) daisen*; 12) *C. (O.) d. okianus*. Scale bar = 0.5 mm.
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There is a small lobe beside the praeputial pad on the ventral side of the endophallus. This lobe is assumed to be a derivative from the outermost lobe of the praeputial pad.

8. **Preapical lobes**: absent (0); slightly swollen (1); strongly lobate (2). A pair of small lobes at both sides of the apical portion of the endophallus.

9. **Aggonoporus**: less developed without sclerotization (0); developed as a membranous wall without sclerotization (1); both sides of the wall elongate with pointed and partly sclerotized apices (2). The gonopore is surrounded by a membranous structure, namely aggonoporus, which is variously developed.

10. **Shape of copulatory piece**: weakly developed and digitate (0) (Fig. 26); triangular 1 (1) (Figs. 5-16); triangular 2 (2) (Figs. 2-4); pentagonal 1 (3) (Figs. 17 and 18); pentagonal 2 (4) (Fig. 19); short hook (5) (Figs. 20 and 21); long hook twisted to the right (6) (Fig. 22); long hook twisted to the left (7) (Fig. 23-25). There is a sclerotized part on the dorsal side of

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**Figs. 13-19.** Endophalli of the *dehaanii* and *yaconinus* species groups: 13) *Carabus* (*Ohomopterus*) *tosanus kawanoi*; 14) *C.* (*O.*) *t. ishizuchianus*; 15) *C.* (*O.*) *t. tosanus*; 16) *C.* (*O.*) *dehaanii* [KINKI]; 17) *C.* (*O.*) *iwawakanus kiensis*; 18) *C.* (*O.*) *t. narakawa*; 19) *C.* (*O.*) *yaconinus cupidicornis*. Scale bar = 0.5 mm.
the endophallus in Carabogenici (sensu Ishikawa, 1978), which is strongly developed and digitate in the subgenus *Ohomopterus* and is called the copulatory piece (Ishikawa, 1973), although Deuve (1994) named it a digitulus. The various forms of the copulatory piece have been used by taxonomists for a long time. Nakane (1952, 1953, 1962 and 1963) and Ishikawa (1985, 1989 and 1991) recognized three categories of shapes of the copulatory piece, *i.e.*, triangular, pentagonal and hook-shaped. In this study, the triangular state was subdivided into two distinct states, triangular 1 and 2. The former is found in *C. dehaanii*, *C. tosanus*, *C. japonicus*, *C. daisen* and *C. yamato*, and the latter in *C. albrechti*, *C. lewisianus* and *C. kimurai*. Triangular state 1 is less raised from the membranous wall of the endophallus and looks flatter in lateral view than triangular state 2; there is a ridged median line in the former, but not in the latter. Pentagonal state was also subdivided into two distinct states, pentago-
nal 1 and 2. The former is found in *C. iwawakianus* and the latter in *C. yaconinus*. Pentagonal state 1 is less robust than pentagonal state 2; both sides of the former are evenly arched, whereas those of the latter are strongly broadened near the apex. The hook-shaped state was also subdivided into three distinct states, short hook, long hook twisted to the right, and long hook twisted to the left. Short hook is found in *C. esakii* and *C. arrowianus komiyai*, long hook twisted to the right in *C. insulicola*, and long hook twisted to the left in *C. arrowianus* and *C. maiyasanus* except *C. arrowianus komiyai*. *C. ueno* has the extraordinarily enlarged and elongated copulatory piece. It is assumed to be the long hook twisted to the left, because the weakly sclerotized part of the copulatory piece is shared with *C. maiyasanus*. The outgroup species of the subgenus *Isiocarabus* has the weakly developed and digitate copulatory piece.

11. **Position of copulatory piece**: middle (0); subapical (1). The base of the copulatory piece is positioned at the middle or subapical part of the endophallus.

12. **Apex of aedeagus**: simple (unmodified) (0); grooved on dorsal margin (1); swollen (2); strongly bent (3). The apex of the aedeagus is gradually narrowed and evenly arched in many species. However, in some species, it is modified.

### Female genitalia

13. **Vaginal appendix**: absent (0); present (1). All the species of *Ohomopterus* have a membranous sack called the vaginal appendix at the ventral wall of the vagina, posterior to the inner plate of the vaginal apophysis. It functions as the receptacle of the male copulatory piece in copulation (Ishikawa, 1987). In the outgroup species, there is a similar receptacle structure but it does not elongate posteriorly.
14. **Inner plate of vaginal apophysis:** K-type (0) (Fig. 34); A-type (1) (Fig. 27); Y-type (2) (Fig. 28); I-type (3) (Figs. 35-38); D-type (4) (Figs. 29 and 30); J-type (5) (Figs. 31-33).

The vaginal apophysis is a strongly sclerotized structure at the ventral wall of the vagina. The inner plate is the dorsal half of the vaginal apophysis facing the vaginal chamber. The shape of the inner plate is very complex and varies among species. In *C. iwawakianus* *kiensis* and the outgroup species of *Isiocarabus*, the inner plate is thin and shallow, cup-shaped without the anterior rim or median groove (K-type). In *C. insulicola*, *C. uenoi*, *C. arrowianus*, *C. maiyasannus*, *C. yaconinus* and *C. iwawakianus* except the subspecies *kiensis*, the inner plate is thick and flat with the median groove (I-type). In *C. dehaanii*, *C. tosanus* and *C. daisen*, the median groove is strongly concaved and outlined by fine carinae (D-type). In *C. japonicus*, the median groove is covered with the anterior rim raised at the anterior half; the bottom of the inner plate is strongly wrinkled at both sides of the median...
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groove (J-type). In *C. yamato*, the bottom of the inner plate is smooth; the median groove is weakly developed with the weakly raised anterior rim (Y-type). In *C. albrechti*, *C. lewisiatus* and *C. kimurai*, the bottom is smooth; the median groove is weakly or not developed; the anterior rim is strongly raised and covering the anterior half of the inner plate (A-type).

15. **Hindvaginal plate**: no sclerotization (0); weakly sclerotized (1); strongly sclerotized (2). The membranous wall posterior to the opening of the vaginal appendix is often sclerotized. Kamiyoshi (1963) called this structure the “semi chitin case”, but it is a plate rather than a case. Thus, this structure should be called the “hindvaginal plate”.

16. **Outer plate of vaginal apophysis**: narrow (0); triangular (1); rectangular (2). The ventral half of the vaginal apophysis functions as an attachment for muscles. The structure is simpler than the inner plate and its outer margin is membranous. In most species of *Ohomopterus* and outgroup species, the outer plate is weakly developed and narrow in width. In *C. dehaanii*, *C. tosanus*, *C. daisen*, *C. yamato*, *C. albrechti* and several subspecies of *C. japonicus*, it is broadened at the anterior half, becoming triangular. In *C. lewisiatus* and *C.
kimurai, it is evenly broadened, becoming rectangular.

**External structures**

17. **Male antennal depression**: large (0); small (1); absent (2). Undersides of the 5-7th antennal segments are depressed and hairless in the male. The extent of the hairless portion is varied.

18. **Gular setae**: absent (0); present (1). In *Ohomopterus*, there are two setae on the submentum namely gular setae. However, the outgroup species do not have them.

19. **Pronotal setae**: two setae (0); variable with two or three setae (1); always three setae (2); more than three setae (3). There are some setae on both sides of the pronotum.

20. **Anterior hindcoxal seta**: absent (0); present (1). Most *Ohomopterus* species have three setae on the hindcoxa. However, in *C. uenoi*, *C. yaconinus* in western Japan and *C. dehaanii* in the Chūgoku District, the anterior one of them is usually absent.

21. **Inner hindcoxal seta**: absent (0); present (1). In *C. japonicus* and *C. daisen*, the
inner seta is absent. The outgroup species do not have both of the anterior and inner setae on the hindcoxa.

22. **Fourth primary intervals**: absent (0); present (1). In most carabid species, there are three primary intervals on each elytron. *C. dehaanii* and *C. tosanus* have the 4th primary intervals on the outside.

23. **Inner margin of male foretibia**: straight (0) (Fig. 39); weakly convex (1) (Fig. 40); strongly, angulately convex (2) (Fig. 41). In most *Ohomopterus* species, the male foretibia is strongly and angulately or weakly convex, whereas in *C. dehaanii*, *C. tosanus* and the outgroup species, it is not convex.

**Phylogenetic analyses**

All multistate characters were treated as unordered in all analyses of this study, because evolutionary polarity and order of morphological characters were not known *a priori*. To assess the effect of homoplastic characters on phylogenetic reconstruction, three kinds of character weighting methods, equally weighting, successive weighting (Farris, 1969) and Goloboff fitting (Goloboff, 1993), were attempted. In successive weighting, each character was weighted by rescaled consistency index (RC; Farris, 1989), which was calculated on the tree of equally weighted analysis, and then reanalyzed. Reweighting and reanalysis were repeated until the result became stable. Weighting by RC decreases the cost of evolution in characters with more homoplasies. Consequently, successive weighting extracts the hierarchic correlation of given set of characters, and reduces the correlation caused by homoplasies (Farris, 1969). In Goloboff fitting, characters were weighted on the basis of their

**Figs. 39-41.** Inner margins of the male foretibia: 39) *Carabus (Ohomopterus) dehaanii*, showing inner margin straight; 40) *C. (O.) japonicus*, inner margin weakly convex; 41) *C. (O.) maiyasanus*, inner margin strongly, angulately convex. Scale bar = 1 mm.
Table 2. Character matrix used in cladistic analyses. Thirty five species, subspecies or local populations of the subgenus *Ohomopterus* and two outgroup species of the subgenus *Isiocarabus* are shown. Twenty three morphological characters are treated as unordered.

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<th>Female genitalia</th>
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<td>1 2 3 4 5 6 7 8</td>
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<td>Carabus (I.) kiukiangensis</td>
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<td>Carabus (O.) maiyasanus shigaraki</td>
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<td>Carabus (O.) lewisianus</td>
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extra steps on a given tree (Goloboff, 1993). The sum of the weights of all characters is defined as tree fitness. The maximization of tree fitness means the minimization of extra steps of weighted characters. Thus, the tree with maximum tree fitness gives the most parsimonious solution in topology as well as the most appropriate weighting on each character. Steepness of the weighting function in Goloboff fitting depends on the parameter $k$ (Goloboff, 1993), in which the smaller $k$ value causes homoplasic characters less weighted. In this study, the values of $k = 2, 4, 8$ and $16$ were given. In successive weighting and Goloboff fitting, homoplasic characters are down-weighted and the effect of homoplasy on phylogenetic reconstruction is reduced. The results obtained by analyses with three weighting methods were compared.

All analyses for tree searching were performed by PAUP® ver. 4.0b2a (Swofford, 1999). Heuristic searching was started from 10 replicates of random trees with tree bisection-
reconnection (TBR) branch swapping. Character evolution, tree length, and some indices for tree and character evaluation were studied by PAUP® and MacClade ver. 3.08a (Maddison and Maddison, 1992-1999). Statistical support for branches were evaluated by decay index (Bremer, 1988, 1994) and bootstrap probability (Felsenstein, 1985).

Results

 Twenty-three variable characters consisted of 11 binary, 8 three-state, 2 four-state, 1 six-state and 1 eight-state characters. Twenty-two of 23 variable characters were informative, but only one character (No. 1) was uninformative.

Analyses with all taxa

In the analysis using equally weighted characters, 10 equally parsimonious trees were obtained, which were minimal in tree length calculated with equally weighted characters (TLa, Fig. 42a). Two morphologically recognizable taxa, the dehaanii and japonicus species groups, are monophyletic. However, the morphologically recognized species C. dehaanii and C. tosanus are not monophyletic, whereas morphologically recognized species C. daisen and C. japonicus are monophyletic. The albrechti species group except C. yamato is monophyletic. C. yamato is the sister species of the dehaanii + japonicus group. The yaconinus and insulicola species groups are entirely paraphyletic, although three monophyletic groups, two subspecies of C. yaconinus, two subspecies of C. insulicola and two subspecies of C. maiyasanus (subspecies maiyasanus and shigaraki), appeared. As a result, three species proposed by morphological evidence, C. iwawakianus, C. arrowianus and C. maiyasanus, are not monophyletic. C. uenoi having extraordinarily specialized genitalia is placed at the most ancestral branch.

In the analysis with successive weighting, five equally parsimonious trees were obtained, which were minimal in weighted tree length calculated with characters multiplied by RC (TLb, Fig. 42b), although they had two more steps than the trees with equally weighted characters. Two major clades appeared, and were separated by basal dichotomy. One clade consists of the albrechti, insulicola and yaconinus species groups, and the other consists of the dehaanii and japonicus species groups. In the former, each of the insulicola species group, the albrechti species group except C. yamato, and the yaconinus species group except C. iwawakianus kiiensis are monophyletic. The insulicola species group is seriously polytomous except C. esakii that is placed at the ancestral branch of the group. The yaconinus species group except C. iwawakianus kiiensis is the sister group of the insulicola species group, and C. iwawakianus kiiensis is placed at the ancestral branch of the insulicola + yaconinus group. The albrechti species group except C. yamato is the sister group of the insulicola + yaconinus group, and C. yamato is placed at the most ancestral position of the former major clade. In the latter, each of the dehaanii and japonicus species groups is monophyletic. In the dehaanii species group, each of the morphologically recognized species C. dehaanii and C. tosanus is not monophyletic, where C. tosanus kawanoi is placed at their ancestral position. In the japonicus species group, C. japonicus is monophyletic, whereas
two subspecies of *C. daisen* are paraphyletic and placed at the ancestral branch of *C. japonicus*.

In the analysis with Goloboff fitting, only one tree was obtained, which was maximal in Goloboff’s tree fitness (TF, Fig. 42c), although it had also two more steps than the trees with equally weighted characters. The tree was similar to the result of the successive weighting analysis, and the polytomy of the *insulicola* species group was resolved better than that of

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**Fig. 42.** Strict consensus trees obtained by analyses with all taxa: a) analysis with equally weighted characters; b) successive weighting; c) Goloboff fitting. N: number of equally parsimonious trees; TLa: tree length with equal weight as in (a); TLb: tree length with characters weighted by mean RC on (b) tree; RC: rescaled consistency index; TF: tree fitness calculated with Goloboff’s fitting criterion. d) Representation of outgroup attachments (filled circles) in (a), (b) and (c) trees. The unrooted ingroup tree is obtained by the analysis with equal weighted characters, whose topology does not completely match with (b) and (c).
successive weighting. In that group, C. uenoii, two subspecies of C. arrowianus (subspecies arrowianus and murakii), two subspecies of C. maiyasanus (subspecies maiyasanus and shigaraki) are monophyletic. The other two subspecies of C. maiyasanus (subspecies takiharenisis and ohkawai) are placed at the ancestral position of the C. uenoii + C. arrowianus + C. maiyasanus branch, and C. arrowianus komiyai is at a more ancestral position. Thus, each of the morphologically recognized species C. arrowianus and C. maiyasanus is not monophyletic. The monophyletic species C. insulicola is the sister group of the C. uenoii + C. arrowianus + C. maiyasanus branch, and C. esakii is at the most ancestral position of the insulicola species group. The yaconinus species group is entirely paraphyletic and placed at the ancestral branch of the insulicola species group. The subtree consisted of the dehaanii and japonicus species groups is identical to the outcome of the successive weighting analysis (Fig. 42b).

Trees (a), (b) and (c) in Fig. 42 may look very different from one another, but the topologies of ingroup trees are similar as in Fig. 42d, in which solid circles indicate the position of outgroups on the unrooted ingroup tree. Thus, the position of outgroups may be unstable. To infer the stability of ingroup topology as well as the reliability of outgroup rooting, the relationships among ingroup species except outgroups and the most parsimonious attachment of outgroup species (e.g., Maddison et al., 1999) were analyzed (given below).

**Analyses without outgroups**

In the analyses with only ingroup species, three weighting methods, equal weighting, successive weighting and Goloboff fitting, were attempted. The resulting unrooted ingroup trees were very similar to one another (Fig. 43).

In the analysis with equally weighted characters, 10 equally parsimonious trees were obtained, which were minimal in TLa (Fig. 43a). Each of the insulicola, dehaanii and japonicus species groups is clustered and shared each stem branch, whereas the albrechti and yaconinus species groups are scattered in the middle part of the tree among the clusters of those species groups (Fig. 43a). In the cluster of the insulicola species group, C. esakii is placed at the outermost branch, and other species are seriously polytomous. Two subspecies of C. insulicola and two subspecies of C. maiyasanus (subspecies maiyasanus and shigaraki) share each stem branch even in the polytomy. The cluster of the dehaanii species group is also polytomous, and the morphologically recognized subspecies of C. dehaanii and C. tosanus do not share each common stem branch. Instead, three subspecies distributed in Shikoku, C. tosanus tosanus, C. tosanus ishizuchianus and C. dehaanii katsumai, are clustered. The local populations of C. dehaanii distributed in the Chūgoku District, dehaanii [SAN’YO] and dehaanii [SAN’IN], are also clustered. In the cluster of the japonicus species group, two subspecies of C. daisen share the common stem branch at the outermost part of the cluster. The subspecies of C. japonicus are also clustered and share the common stem branch, in which two populations of the subspecies chugokuensis are placed at the distal part of the cluster. In the albrechti species group, C. albrechti, C. lewissianus and C. kimurai are clustered and share the common stem branch, but C. yamato is placed between this cluster and the cluster consisting of the dehaanii and japonicus species groups. In the
Yaconinus species group, two subspecies of *C. yaconinus* are clustered, whereas two subspecies of *C. iwawakianus* do not share the common stem branch. The species *yaconinus* and *iwawakianus* are placed between the *insulicola* and *albrechtii* species groups.

In both of analyses with successive weighting and Goloboff fitting (*k* = 4, 8 and 16), 10 equally parsimonious trees were obtained. They were entirely identical in both analyses.

**Fig. 43.** Strict consensus trees obtained by analyses of the ingroup species with three types of character weightings. N: number of equally parsimonious trees; TLa: tree length with equal weight as in (a); TLb: tree length with characters weighted by mean RC on (b) tree; RC: rescaled consistency index; TF: tree fitness calculated with Goloboff’s fitting criterion.
and minimal in TLb, although they had one more step than the trees of equally weighted analysis. The topologies of obtained trees were similar to those by equally weighted analysis, although two subspecies of C. daisen and two local populations of C. japonicus chugokuensis became paraphyletic within the japonicus species group (Fig. 43b).

In the analyses with Goloboff fitting \((k = 2)\), seven equally parsimonious trees were obtained, which were maximal in Goloboff’s tree fitness (TF), although they had two more steps than the trees with equally weighted characters. The topology obtained in this analysis was similar to those of successive weighting and Goloboff fitting \((k = 4, 8 \text{ and } 16)\), and the polytomies of the insulicola and dehaanii species groups which appeared in those analyses were more highly resolved, but the branch between C. yaconinus and two subspecies of C. iwawakianus, and the stem branch of C. albrechti and its relatives were collapsed (Fig. 43c).

Twenty-seven trees \((i.e., 10 + 10 + 7)\) were obtained from analyses of ingroup species with different weighting methods. They were similar to one another, but there are no identical trees in them.

Analysis of outgroup attachment
In phylogenetic analyses which include outgroup species, it was shown that the outgroup

Fig. 44. Analyses of the most parsimonious attachment of outgroups. Target tree used in the analysis (left, unrooted tree), which is 50 % majority rule consensus tree of 27 unrooted trees obtained by analyses of the ingroup species with three different weighting methods shown in Fig. 43. Branches are identified with numbers (1-60). Right panels a-c, variation of the most parsimonious attachment of outgroups among different character weighting methods, a) All characters were equally weighted; b) weighted by mean RC, which is calculated on the tree of Fig. 42b; c) weighted by mean RC, which is calculated on the target tree. Height of columns shows the shortness of tree length, calculated as (maximum [worst] tree length obtained by outgroup insertion – tree length for attachment of the outgroup at that branch). Solid column indicates the most parsimonious attachment of the outgroup in each weighting method.
species changed their position among different character weightings contrary to a stable ingroup tree. To infer how much ambiguity was included in outgroup rooting, the variations of tree length caused by the insertion of outgroups into each ingroup branch were calculated under different character weightings. The 50 percent majority rule consensus tree of 27 trees obtained by the analysis of ingroup species was used as the target tree (Fig. 44). As a result, changes of tree length made by attachments of outgroups varied among weightings (Fig. 44, Friedman test, $P < 0.0001$). When characters were equally weighted, it was most parsimonious when outgroups were attached to branch No. 4, the terminal branch connected to *C. uenoii* (Fig. 44a). When weighting was by rescaled consistency indices (RC) calculated on the tree of Fig. 42b including outgroups, it was most parsimonious when outgroups were attached to branch No. 19, the terminal branch connected to *C. iwawakianus kiensis* (Fig. 44b). When characters were weighted by RC calculated on the target tree without outgroups, it was most parsimonious when outgroups were attached to branch No. 51, the stem branch of the subspecies of *C. japonicus* (Fig. 44c). As in Fig. 44, the landscape of shortness were similar over weightings, but the highest peaks were different among them. A nearly-highest peak appeared commonly in branches No. 32 - 34, but it did not provide the most parsimonious attachment.

## Analyses of character evolution

Each character evolution was reconstructed on the basis of ACCTRAN optimization other than on the subtree of the *insulicola* species group, in which five equally parsimonious topology were obtained. Character reconstruction on this subtree was determined from the viewpoint of direction of character evolution and topological resolution (Fig. 45, see Discussion in detail).

Informativeness, or homoplasy, of characters was evaluated on the ingroup tree by number of steps and four indices, *i.e.*, consistency index (CI), retention index (RI), rescaled consistency index (RC) and Goloboff’s fitness (GF) (Table 3). The RI and RC of four characters, the left basal lobe and the ligula on the male genitalia, the vaginal appendix of the female genitalia, and the gular setae (Nos. 1, 4, 13 and 18 respectively), could not be defined because they were constant or uninformative within the ingroup tree. Among the remained 19 characters, number of steps was significantly negatively correlated with other four indices (Spearman’s rank correlation: vs CI, $r_s = -0.456$, $P = 0.0261$; vs RI, $r_s = -0.421$, $P = 0.0437$; vs RC, $r_s = -0.421$, $P = 0.0438$; vs GF, $r_s = -0.589$, $P = 0.0046$). On the other hand, four indices, CI, RI, RC and GF, were significantly positively correlated among each other (Spearman’s rank correlation, CI vs RI, $r_s = 0.942$, $P < 0.0001$; CI vs RC, $r_s = 0.9981$, $P < 0.0001$; CI vs GF, $r_s = 0.9456$, $P < 0.0001$; RI vs RC, $r_s = 0.973$, $P < 0.0001$; RI vs GF, $r_s = 0.894$, $P = 0.0002$; RC vs GF, $r_s = 0.911$, $P = 0.0001$).

Informativeness of the characters measured by CI, RI, RC and GF significantly differed among the characters (Friedman test, $P < 0.0001$). However, there was no significant difference among characters of male and female genitalia and external structures (Kruskal-Wallis test, $P = 0.9324$ in CI, $P = 0.8113$ in RI, $P = 0.9193$ in RC, and $P = 0.9902$ in GF). Five characters, the shape and position of the copulatory piece of the male genitalia, the inner
Table 3. Indices of characters used in cladistic analyses, calculated on the ingroup tree shown in Fig. 46. Characters 13 and 18 are omitted because they are constant among ingroup species. Characters 1 and 4 are phylogenetically uninformative within the ingroup.

<table>
<thead>
<tr>
<th>No.</th>
<th>Character</th>
<th>Steps</th>
<th>Consistency index</th>
<th>Retention index</th>
<th>Rescaled consistency index</th>
<th>Goloboff fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left basal lobe</td>
<td>1</td>
<td>1.000</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>Right basal lobe</td>
<td>5</td>
<td>0.400</td>
<td>0.800</td>
<td>0.320</td>
<td>0.500</td>
</tr>
<tr>
<td>3</td>
<td>Hind lobe</td>
<td>3</td>
<td>0.333</td>
<td>0.500</td>
<td>0.167</td>
<td>0.600</td>
</tr>
<tr>
<td>4</td>
<td>Ligula</td>
<td>1</td>
<td>1.000</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>1.000</td>
</tr>
<tr>
<td>5</td>
<td>Paraligula</td>
<td>5</td>
<td>0.200</td>
<td>0.667</td>
<td>0.133</td>
<td>0.429</td>
</tr>
<tr>
<td>6</td>
<td>Median swelling</td>
<td>2</td>
<td>0.500</td>
<td>0.923</td>
<td>0.462</td>
<td>0.750</td>
</tr>
<tr>
<td>7</td>
<td>Praepatral lobe</td>
<td>6</td>
<td>0.333</td>
<td>0.750</td>
<td>0.250</td>
<td>0.429</td>
</tr>
<tr>
<td>8</td>
<td>Preapical lobes</td>
<td>3</td>
<td>0.667</td>
<td>0.933</td>
<td>0.622</td>
<td>0.750</td>
</tr>
<tr>
<td>9</td>
<td>Aggonoporitus</td>
<td>3</td>
<td>0.667</td>
<td>0.929</td>
<td>0.619</td>
<td>0.750</td>
</tr>
<tr>
<td>10</td>
<td>Shape of copulatory piece</td>
<td>6</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>11</td>
<td>Position of copulatory piece</td>
<td>1</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>12</td>
<td>Apex of aedeagus</td>
<td>5</td>
<td>0.600</td>
<td>0.778</td>
<td>0.467</td>
<td>0.600</td>
</tr>
<tr>
<td>13</td>
<td>Shape of copulatory piece</td>
<td>6</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>14</td>
<td>Inner plate of vaginal apophysis</td>
<td>5</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>15</td>
<td>Hindvaginal plate</td>
<td>9</td>
<td>0.222</td>
<td>0.632</td>
<td>0.140</td>
<td>0.300</td>
</tr>
<tr>
<td>16</td>
<td>Outer plate of vaginal apophysis</td>
<td>3</td>
<td>0.667</td>
<td>0.933</td>
<td>0.622</td>
<td>0.750</td>
</tr>
<tr>
<td>17</td>
<td>Male antennal depression</td>
<td>5</td>
<td>0.400</td>
<td>0.667</td>
<td>0.267</td>
<td>0.500</td>
</tr>
<tr>
<td>18</td>
<td>Pronotal setae</td>
<td>9</td>
<td>0.333</td>
<td>0.455</td>
<td>0.152</td>
<td>0.333</td>
</tr>
<tr>
<td>19</td>
<td>Anterior hindcoxal seta</td>
<td>3</td>
<td>0.333</td>
<td>0.333</td>
<td>0.111</td>
<td>0.600</td>
</tr>
<tr>
<td>20</td>
<td>Inner hindcoxal seta</td>
<td>1</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>21</td>
<td>Fourth primary intervals</td>
<td>1</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>22</td>
<td>Inner margin of male foretibia</td>
<td>3</td>
<td>0.667</td>
<td>0.929</td>
<td>0.619</td>
<td>0.750</td>
</tr>
</tbody>
</table>

Phylogeny of Ohomopterus

Table 3. Indices of characters used in cladistic analyses, calculated on the ingroup tree shown in Fig. 46. Characters 13 and 18 are omitted because they are constant among ingroup species. Characters 1 and 4 are phylogenetically uninformative within the ingroup.

Plate of the vaginal appendix of the female genitalia, the inner hindcoxal seta and the fourth primary intervals (Nos. 10, 11, 14, 21 and 22, respectively), were scored 1.000 in all indices, suggesting no homoplasy, while relatively low scores indicating more homoplasious were observed in five characters, the hind lobe and the paraligula of the male genitalia, the hindvaginal plate of the female genitalia, the pronotal and anterior hindcoxal setae (Nos. 3, 5, 15, 19 and 20, respectively).

Discussion

Phylogenetic analyses

1) Ambiguity of outgroup rooting

Comparison of cladograms obtained by phylogenetic analyses with and without outgroups exhibited the unstable positioning of outgroups within a stable ingroup cladogram. The analysis of the most parsimonious attachment of outgroups revealed the ambiguity of outgroup rooting, in which multiple candidate branches (“peaks”) for outgroup connection appeared and the highest peak of them changed among weightings. Such ambiguity may be due to long branch attraction, in which long branches are likely to be connected with each other because homoplasies accumulated independently in each branch are misleadingly recog-
nized as synapomorphies (Felsenstein, 1978). For example, *C. uenoi* has the extraordinarily
equilibrated copulatory piece and consequently some other structures degenerate secondarily.
Branch No. 4, connected to *C. uenoi*, is chosen as the most parsimonious attachment of
groups with equally weighted characters, because *C. uenoi* and the outgroup species
share a common condition appearing degeneration in those characters although they are
homoplasiastically gained. The most parsimonious outgroup attachment moves to branch No.
51, when characters are weighted by RC calculated on the ingroup tree (target tree shown in
Fig. 44). Moreover, outgroups move to branch No. 19, when the RC are calculated on the
tree including outgroups (shown in Fig. 42b). Character weighting by RC causes down-
weighting of homoplastic characters. The degree of down-weighting increases when the
outgroups are included in the calculation of RC, because the outgroups contain many ho-
mplasies in this case. Thus, the change of the most parsimonious attachment of outgroups
according to different weightings shows the alternation of the magnitude of long branch
attraction.

2) Choice among equally parsimonious ingroup trees

Contrary to ambiguous outgroup position, ingroup cladograms are relatively stable among
character weightings. However, ingroup cladograms still varies, because there are equally
parsimonious trees within each analysis, and analyses with different weighting methods
gave different results (Fig. 43). Character weighting is often used for choosing among
equally parsimonious trees (Carpenter, 1988). When a large number of equally parsimoni-
ous trees are obtained by analysis with unweighted characters, analysis with weighted char-
acters can usually select a small number of trees from them. However, in this study, differ-
ent weighting methods produced no identical tree. This means that character weighting did
not work for tree choice.

Ingroup trees differ only in the topology of the *japonicus* species group among analyses
with three weighting methods, *i.e.*, equally weighting, successive weighting and Goloboff
fitting (*k* = 4, 8 and 16) (Fig. 43a, b). Here, trees obtained by Goloboff fitting with the
parameter *k* = 2 (Fig. 43c) are not taken into account, because some branches are collapsed.
The parameter *k* = 2 in Goloboff fitting may be too small to estimate the weights of char-
acters because loss of phylogenetic information may be too much to reconstruct cladograms.
In the clade of the *japonicus* species group, two subspecies of *C. daisen* and two local
populations of *C. japonicus chugokuensis* became monophyletic under equally weighted
analysis, but not under other weighted analyses. Moreover, trees of weighted analyses have
one or two more steps than those of unweighted analyses, although they optimize other
criteria. Thus, equally weighted analysis is preferred to other two weighted analyses.

One optimal tree should be chosen from 10 trees obtained by equally weighted analysis
rather than consensus, because a consensus tree is reduced in branch resolution and not
appropriate for proposal of the phylogenetic hypothesis (Carpenter, 1988). In this study, the
choice of the optimal tree is conducted on the basis of topology and character evolution.
The phylogenetic position of *C. insulicola* varies among these 10 trees, and five patterns are
recognized in its position (Fig. 45). Only *C. insulicola* has the male copulatory piece that is
elongated and twisted to the right (character No. 10, state 6). It is assumed that the elon-
gated copulatory piece twisted to the right can not be derived from the elongated one twisted to the left (state 7) from the viewpoint of genital mechanics, and vice versa. This assumption means that topologies assuming the change of the copulatory piece from the state 7 to 6 are little supported. Trees a and b in Fig. 45 satisfy this criterion, although the tree b is supported only when the evolution from 5 to 7 is placed on the branch shared by C. uenoi, C. arrowianus except the subspecies komiyai and C. maiyasanus. In this case, trees a and b become identical, because the branch in the tree b shared by C. insulicola, C. uenoi, C. arrowianus except the subspecies komiyai and C. maiyasanus has no supporting characters and is collapsed as in tree a. Thus, the position of C. insulicola is determined as in Fig. 45a. It must be noticed that this situation occurs only when the branch within the insulicola species group is not rooted. The other variation of tree is found in the topology of the subspecies of C. japonicus, in which the clade consisted of C. japonicus [SHIKOKU], C. japonicus ikiensis and the stem branch shared by C. japonicus chugokuensis and C. japonicus [KYUSHU] is or is not polytomous. In this case, the more highly resolved tree is selected.

**Fig. 45.** Equally parsimonious trees of the insulicola species group, which are the subtrees of unrooted ingroup trees obtained by the analysis with equally weighted characters. Solid bars refer to the most parsimonious reconstructions (MPRs) of the evolution of the shape of the copulatory piece (character No. 10); changes of the states are shown on bars. The arrow in b shows a pair of exchangeable MPRs.
because both of character evolution on these trees are acceptable. Consequently, the unrooted tree shown in Fig. 46 is chosen as the most appropriate phylogenetic hypothesis of the subgenus \textit{Ohomopterus} in character evolution and branch resolution.

3) Attempt of rooting by molecular evidence

Outgroup rooting is a major method in phylogenetic analysis. In this study, however, it may not be available because of the ambiguity of outgroup positioning. Thus, another criterion is necessary to determine the root of the ingroup tree. One possibility is a comparison with molecular phylogenetic trees. Mitochondrial ND5 gene phylogeny of the subgenus \textit{Ohomopterus} was proposed by Su \textit{et al.} (1996). According to them, two major lineages were recognized. Lineage I includes four species of the \textit{albrechi} species group, two species of the \textit{japonicus} species group, \textit{C. tosanus} and most local populations of \textit{C. yaconinus}. Lineage II includes \textit{C. iwawakianus}, \textit{C. arrowianus}, \textit{C. maiyasanus} and \textit{C. uenoi}. \textit{C. dehaanii} and \textit{C. insulicola} are divided into two lineages possibly due to introgressive hybridization between both of the lineages (Kubota and Sota, 1998). In the tree of the present study, two similar lineages are established when the branch arrowed in Fig. 46 is rooted. The clade consisting of the \textit{albrechi}, \textit{japonicus} and \textit{dehaanii} species groups and \textit{C. yaconinus} roughly corresponds to lineage I, and the clade consisting of the \textit{insulicola} species group and \textit{C. iwawakianus} to lineage II (Fig. 46). The reliability of the mitochondrial DNA tree may be decreased by introgressive hybridization as mentioned in Introduction, but hybridization between these major lineages divided by deep bifurcation is not likely to occur. Thus, it is concluded that hypothetical root can be attached to that branch.

Recently, Sota (2000) and Sota and Vogler (in press) analyzed the phylogeny of this group from the combined nuclear sequences in a study of comparisons between three nuclear and two mitochondrial gene sequences. It shows results similar to those of the present study in the topology of the ingroup tree, although the position of outgroups was discordant with the result of this study. In relation to this, they suggested that outgroup species used in their study, \textit{C. granulatus}, might be inappropriate for rooting.

Character evolution

Four indices of character evolution were used to examine the informativeness or homoplasiousness of characters, and different indices were strongly correlated with each other. This suggests that character informativeness was reasonably evaluated by them. Significant difference of informativeness among characters indicates that phylogenetic importance varies among characters used in this analysis and character weighting may be effective to extract phylogenetic information.

Morphology of the genitalia have been used for taxonomy because most taxonomists have believed that genitalic characters reflect the identity of species. In this study, there is no difference among the homoplasiousness of characters of the male and female genitalia and external structures. However, most of variable characters were found in the male genitalia (52.2\%). Thus, it is concluded that genitalic characters are useful not in its quality but in quantity.

The characters with no homoplasy, \textit{i.e.}, the shape and position of the copulatory piece,
the inner plate of the vaginal apophysis, the inner hindcoxal seta and the fourth primary intervals of the elytra, were mapped on higher branches of the tree which refer to the common ancestors of each species or species group (Fig. 47). This indicates that the branches higher than specific level tend to be supported by these reliable characters. These characters have also been used for the definition of species groups in the taxonomy of *Ohomopterus* (Ishikawa, 1989, 1991). This suggests that his evaluation and treatment of characters were reasonable even from the viewpoint of cladistics.

**Relationships of particular taxa**

The phylogeny of *Ohomopterus* inferred from morphological characters is different from that of morphological taxonomy (Ishikawa, 1991) or molecular phylogeny based on mitochondrial DNA sequences (Su *et al.*, 1996) (Figs. 47 and 48). Taxonomically defined species groups, *dehaanii, japonicus* and *insulicola*, are supported as monophyletic groups in the present study, although they were not in the study of molecular phylogeny based on mitochondrial DNA. However, two other species groups, *albrechti* and *yaconinus*, are not monophyletic even in the present study.

1) The *japonicus* and *dehaanii* species groups

Close relationships between the *japonicus* and *dehaanii* species groups were suggested.
by Ishikawa (1991) on the basis of the shape of the inner plate of the vaginal apophysis. His suggestions on these species groups are well supported in this study. Monophyly of each species group is supported by the moderate value of bootstrap probability.

In the japonicus species group, the monophyly of C. japonicus is strongly supported by a large decay index and high bootstrap probability. Within the species, branches among subspecies or local populations are weakly supported, although the monophyly of the subspecies chugokuensis is moderately well supported. C. daisen becomes the sister species of C. japonicus, but its monophyly is only weakly supported.

In the dehaanii species group, branches are mostly collapsed, in which two species of this group, C. dehaanii and C. tosanus, are not divided into distinct clades. This means that the species of this group are too uniform in morphology to resolve their branching order.

2) The albrechti species group

Ishikawa (1991) recognized close relationships among C. albrechti, C. lewisiyanus, C. kimurai and C. yamato and arranged them the albrechti species group on the basis of the shape of the copulatory piece (simple and triangular) and the inner plate of the vaginal apophysis (cup-shaped). However, in this study, this group become paraphyletic, where C. yamato is closer to the japonicus and dehaanii species groups. It is supported by moderate bootstrap probability. The phylogenetic position of C. yamato should be investigated further, because a recent study of nuclear gene sequences supports the monophyly of the albrechti species group (Sota, 2000, Sota and Vogler, in press). On the other hand, the monophyly of the other three species, C. albrechti, C. lewisiyanus and C. kimurai, is supported by moderate bootstrap probability, where C. lewisiyanus and C. kimurai are closer than C. albrechti.

Ishikawa (1991) also suggested that the albrechti species group was most ancestral and shared a common ancestor with other four species groups of Ohomopterus. His hypothesis is rejected, however, by the result of the present study. The members of this group are paraphyletically positioned at the outside of the japonicus and dehaanii species groups and share a common ancestor with them.

3) The yaconinus species group

The yaconinus species group have been recognized on the basis of the shape of the copulatory piece (broad and pentagonal) (Ishikawa, 1989, 1991). In the present study, however, the monophyly of this group is not supported, and two species, C. yaconinus and C. iwawakianus, are polyphyletically divided into distinct clades. Even if the root of the ingroup tree is moved to the other branch, this group can not be considered monophyletic.

The yaconinus species group was believed to be closely related to the insulicola species group (Ishikawa, 1989, 1991). However, this study reveals that C. yaconinus is closely related to the albrechti, japonicus and dehaanii species groups, and shares a common ancestor with them. The monophyly of the subspecies of C. yaconinus is moderately supported. On the other hand, C. iwawakianus is paraphyletically positioned at the outside of the insulicola species group, but the shared branch is very weakly supported. These conclusions mostly depend on the rooting, which is inferred from the result of a molecular phylogenetic study (Su et al. 1996). Thus, phylogenetic relationships of this species group should be considered with much attention.
Fig. 47. Proposed phylogeny of the subgenus Ohomopterus (see “Discussion” in detail). Ingroup topology of this tree is identical to that in Fig. 46, being chosen on the basis of the evolution of the copulatory piece (character 10) and branch resolution. Branch length represents the number of steps occurring there. Character evolution was reconstructed with ACCTRAN optimization (except character 17 on branches of the subspecies of C. japonicus). Solid bars refer to synapomorphies, simple bars to homoplasies and crossing bars to reversals. Characters are identified with numbers (1-23) assigned above bars. Bootstrap probabilities (shown only on branches appearing in bootstrap consensus of 100 replicates, bold numbers) and decay indices (italic numbers) are shown beside branches. Solid brackets show the ranges of species groups recognized by morphological taxonomy.
4) **The *insulicola* species group**

The *insulicola* species group is the most divergent group in *Ohomopterus* and is defined by the elongate, hook-like copulatory piece. This group is monophyletic, but it is weakly supported. Within the group, species with the relatively short copulatory piece, *C. esakii* and *C. arrowianus komiyai*, are placed at the ancestral position. Two subspecies of *C. insulicola* (subspecies *insulicola* and *shinano*) are monophyletic with weak branch support. Sota (2000) and Sota and Vogler (in press) gave a similar result for this species based on analyses of nuclear DNA, although it was divided into distinct lineages in the study of molecular phylogeny based on mitochondrial DNA (Su *et al.*, 1996). Subspecies of *C. arrowianus* (except the subspecies *komiyai*) and *C. maiyasanus* become monophyletic together with *C. uenoii*, but branches between them are less resolved and weakly supported. The monophyly of *C. arrowianus* and *C. maiyasanus* is not supported. Low resolution and
weak support of the branches in the *insulicola* species group may be due to overall uniformity in the morphology of this group other than the shape of the copulatory piece.

**Establishment of distribution within the Japanese Archipelago**

It is inferred that the divergence of *Ohomopterus* initiated in the Kinki District, where the ancestors of two major clades were divided by the valleys on the Median Tectonic Line, because the most ancestral species of the two clades, *C. yaconinus* and *C. iwawakianus*, are distributed in the northern and southern sides of the Line, respectively. Some subspecies of these species which inhabit the other sides of the Line are secondarily invading populations, because they are assumed to be derived subspecies (Ishikawa, 1991, Ishikawa and Kubota, 1994, 1995).

In the northern side of the Kinki District, the ancestor of *C. yaconinus* may have occurred in the coast of the Japan Sea where the most ancestral subspecies of this species, *C. y. blairi* and *C. y. sotai*, were distributed. The ancestor of the *albrechti* species group derived in its eastern margin, which corresponds to the Chubu District, and completed further divergence there (Takami and Ishikawa, 1997). On the other hand, the ancestor of the *japonicus* and *dehaanii* species groups dispersed into the western Japan, and was divided into two species groups. The ancestor of the *japonicus* species group was established in the Chugoku District, where the ancestral species of this group, *C. daisen*, occurred, and that of the *dehaanii* species group was originated in Shikoku, where the ancestral subspecies, *C. tosanus kawanoi*, was distributed.

In the southern side of the Kinki District, the ancestor of the *insulicola* species group and *C. iwawakianus* may have been originated in the mountains of the Kii Peninsula, where *C. iwawakianus kiiensis* which had some ancestral characteristics was distributed. *C. iwawakianus* diverged within the Kii Peninsula, and the ancestor of the *insulicola* species group invaded eastward along the coast of the Pacific Ocean, and further diverged into several species. *C. insulicola* was established in the central part of the Chugoku District and invaded the Kanto and Tohoku districts (Ishikawa and Ujiie, 2000). *C. maiyasamus*, *C. arrowianus* (subspecies *murakii*) and *C. ueno* may have recolonized from the Chubu District to the Kinki District.

**Conclusion**

This paper proposed a new phylogenetic hypothesis of the subgenus *Ohomopterus* based on morphological characters analyzed by cladistic methods. The result is different from the existing morphological taxonomy and molecular phylogeny inferred from mitochondrial DNA sequences. In the course of this study, several character weighting methods were attempted and the ambiguity of outgroup rooting was revealed. The root of the relatively stable ingroup tree was determined by the comparison with the tree inferred from mitochondrial DNA sequences. Brief discussion about distributional process was also given.

Morphological characters as well as molecular data provide phylogenetic information, from which we can extract hierarchic relationships of organisms using cladistic methods
(Hennig, 1966). Morphological taxonomy is likely to recognize paraphyletic or polyphyletic groups based on symplesiomorphy, but cladistics can distinguish them from monophyletic groups. Such hidden para- or polyphyly was revealed in the albrechti and yaconinus species groups by the cladistic analysis of morphological characters. This indicates that morphological characters are useful enough to infer phylogenetic relationships when modern analytical methods are carefully used.

The use of morphological characters is often restricted when homologies can not be traced between taxa. Such situation tends to result from rapid evolution of morphology. In this study, outgroup rooting was not possible, because of lack of information for determining the position of outgroups. This problem may result from the deep bifurcation between the species of Ohomopterus and outgroup species. In such a situation, we may be able to use information other than morphology as to infer the root of the present study, because our aim is thorough understanding of evolutionary history of organisms in question. However, it is likely to include circularity, when the result obtained by combined evidence is compared with the hypothesis whose information is used for combined evidence. Hence, such results must be carefully discussed in tree comparison.

For a comprehensive understanding of the evolutionary history of the subgenus Ohomopterus, we must collect many kinds of information, e.g., various gene sequences inherited maternally, paternally or parentally, and various levels of morphology from macro- to micro-structure observed by scanning microscope. Finally, phylogenetic hypotheses based on the different kinds of information should be compared.

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References

Phylogeny of *Ohomopterus*


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