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Molecular Phylogeny and Evolution of Carabid Ground Beetles

With 119 Figures, Including 63 in Color

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Dedicated to our friend, the former director of
JT Biohistory Research Hall, Takatsuki, Osaka,

Tokindo S. Okada

without whose constant encouragement
this work could not have been undertaken.

Preface

One of the authors, Syozo Osawa, still clearly remembers the afternoon when one of his colleagues, a young embryologist, succeeded in extracting RNA from the optic tissues of a newt. He was so impressed and showed the test tube containing the transparent RNA solution to a well-known professor of morphological embryology. The professor said, "What, nucleic acid? I don't believe what I cannot see with my eyes." This took place about 50 years ago.

DNA is indeed invisible to the naked eye but has become a powerful tool in the study of phylogeny, evolution, and taxonomy. Naturally, without morphology molecular phylogeny alone does not have much meaning. Morphology and molecular phylogeny are like the two wheels of a cart: both are necessary for the vehicle to run smoothly.

Nowadays, there is no one who does not believe that DNA can be useful in determining such things as parent–child relationships or the identity of a criminal.

For entomologists, regardless of whether they are professional or amateur, it is very enjoyable to look at well-arranged insect specimens in a box. A DNA solution in a test tube is prosaic by comparison, yet it is this solution that provides a greater wealth of information to the researchers.

It is true, however, that DNA analysis has not yet been embraced by a large number of insect taxonomists. One reason for this is the assumption that molecular biology is difficult to understand. This may be true to some extent for traditional entomologists and amateur insect lovers, but a detailed knowledge of molecular biology is not necessary to study phylogeny, evolution, and taxonomy with the aid of DNA.

What is required is a fundamental knowledge of the following kind. The first important point to keep in mind is that DNA to be used for phylogenetic study using "a molecular clock" (see below) is unrelated to phenotypes, i.e., morphological structures, various physiological functions, etc. To give an example, if one has examples of three species of carabid ground beetle, *a*, *b*, and *c*, and finds the difference in the nucleotide (A, G, C, and T) sequence between *a* and *b* is 1% and that between *c* and *a* (or *b*) is 5%, these results may be explained in the following way. The percentage of difference in the nucleotide sequence of the species may indicate that *c* and *a*/*b* descended from a common ancestor, after which *a* separated from *b*.

If we assume that a period of 0.4 million years is required to produce a 1% difference in nucleotide sequences, then the separation of *c* and *a*/*b* from a common ancestor took place 2 million years ago. Nucleotide sequence changes, the substitution of A by T for example, takes place at a more or less constant rate, which means that it can be regarded as a molecular (or DNA) clock.

Construction of a phylogenetic tree can be successfully completed by applying this feature of DNA. To put it simply, the smaller the difference in the nucleotide sequence between species, the closer the phylogenetic relationship and vice versa. Of course, there are some complicated technical and theoretical problems associated with this approach, which will be considered in Chapter 4.

As the changes occurring in DNA that are used as a reference for this molecular clock are neutral, i.e., they are neither deleterious nor are they related to morphology and function, they do not cause any phenotypic changes. On the contrary, some non-neutral changes result in morphological and physiological alterations that are

unrelated to the number of neutral changes. This is because changes leading to phenotypic alterations occur on sites different from the neutral site.

Motoo Kimura (1986) stresses the fact that the "phenotype is conventional; molecular evolution is conservative." Morphological character is a part of the phenotype that does not change relative to time, while a molecular clock ticks at a constant rate. Some claim that the use of the mitochondrial DNA molecular clock is equivalent to an examination based on morphology. This is incorrect, however, because it confuses the concept of phenotype and that of molecular clock.

The purpose of molecular phylogeny is to show the order in which species or other taxa in a given group of organisms have diversified against a relative (or absolute) time axis. When molecular phylogenetic methods were not available, one could only construct a phylogenetic tree by means of cladistic analysis using morphological characteristics. This allowed researchers to deduce phylogenetic relationships with some degree of accuracy, because the phenotype, even if it changes in a conventional way, reflects phylogeny to some extent.

It is the case, however, that various researchers choose widely different morphological characteristics when undertaking cladistic analysis, which means that the phylogenetic trees they produce quite often disagree. The "character" or the "character condition" referred to by cladists does not necessarily have a genetic basis and does not therefore necessarily correspond to a "genetic character."

In most cases, it is not possible to know what genetic event(s) created a morphological change that is apparent to the naked eye as a character. It has been known since Mendel's time that one mutation sometimes leads to a change in a morphological character, but sometimes one genetic change is responsible for multiple phenotypic alterations. There is no way to verify which of these is the case using only a traditional cladistic approach. This means that cladistic analysis can be thought of as having played an important role only until molecular phylogenetic analysis techniques became available.

About nine years have passed since we began to study the DNA phylogeny of the carabid ground beetle. In the initial stages of this study, we experienced much resistance to the idea of using DNA analysis in the field of entomology, with its long tradition dating from the time of Carl von Linné. For all of Linné's achievements, about 200 years have passed since he pioneered the field. In the twenty-first century, it is to be expected that new techniques will supplant the old.

When we submitted a paper on our early findings to a journal in the field, one reviewer said, "I don't have faith in the molecular results, because they do not agree with findings based on morphology. Molecular phylogeny has value only when it supports the results of cladistic analysis." Even today, a fraction of entomologists continue to view the results of our studies on molecular phylogeny with doubt.

This reaction is not surprising, as it is always the case that new techniques are met with skepticism. Indeed, when we began molecular phylogenetic studies, only a few researchers around the world were engaged in this kind of study. The validity of molecular phylogeny has, however, gradually been acknowledged as a useful tool by a considerable number of young entomologists. In Japan, molecular phylogenetic studies are now being rapidly extended to cover many groups of insects, such as butterflies, moths, longhorn beetles, lucanid beetles, and dragonflies, in addition to the carabid ground beetles.

We have studied the phylogeny and evolution of the carabid beetle as objectively as possible. It is, however, important to remember that being objective is not necessarily equivalent to being correct. Some erroneous results or false explanations might be involved in our study. These are inevitable, because at present biology, especially morphology, phylogeny, evolution, ecology, and biogeography, are far from being exact sciences and therefore we have not been able to avoid presenting what are still only hypotheses in some places within this book.

This book contains only the results of our own work. It should be emphasized that our work represents only the first step in a long process aimed at giving us a better understanding of the evolutionary principles involved in the mechanisms of mor-

phological differentiation and transdifferentiation. It is our belief that molecular developmental studies are absolutely essential to gain this understanding.

Our study of the molecular phylogeny of carabid ground beetles began in 1995 at JT Biohistory Research Hall (BRH), Takatsuki, Osaka, Japan. The results presented in this book have been performed in collaboration with Osamu Tominaga, Munehiro Okamoto, Choog-Gon Kim, Nobuo Kashiwai, and Takeshi Ohama, in addition to the three authors.

Yui Itani (Kyoto University, Shirahama, Japan), Kazuhiro Masunaga (Biwako Museum, Shiga, Japan), Shusei Saito (Japan Wildlife Research Center, Tokyo, Japan), and Ken Oyama (National University of Mexico, Mexico) contributed a great deal to the study during their short postings with BRH.

We would like to express our appreciation for the assistance offered by those who provided us with invaluable specimens. In particular, we are grateful for the assistance offered by the following individuals in Japan: Azuma Abe (Aomori), Mitsuro Arai (Tokyo), Motoyasu Anzai (Sapporo), Shoichi Imasaka (Kitakyushu), Hiroshi Koike (Niigata), Toshikazu Kosaka (Hiroshima), Shuhei Nomura (Tokyo), and Nobuki Yasuda (Hokkaido). We are also grateful for the help of Klaus Staven (Germany), Walter Heinz (Germany), Bernard Lassalle (France), Pierfranco Cavazzuti (Italy), Boleslav Březina (Czech Republic), Roman Businský and Ludmila Businský (Czech Republic), Igor Belousov (Russia), Dmitry Obydov (Russia), Ilia Kabak (Kazakhstan), Eric Van Den Berghe (USA), and Robert Davidson (USA), among others.

We owe special thanks to Hideko Kanda for her skillful technical assistance from the beginning of this study up to the present. Without the help of all of these individuals, we could not have accomplished our study. We also wish to thank Tokindo S. Okada, former director of BRH, and others there for their hospitality. During the course of this study, valuable suggestions and help have also been provided by Seiichi Kashiwabara (The Asahi Press, Tokyo, Japan), Atuhiro Sibatani (Ibaraki, Osaka, Japan), Kiyohiko Ikeda (Yamanashi, Japan), Yorio Miyatake (Kashiwa, Japan), Takeshi Yoro (Kamakura, Japan), Barry P. Moore (Queensland, Australia), Yutaka Nishijima (Chitose, Japan), Takaaki Matsuda (Himeji, Japan), Yoichiro Otofuji (Kobe, Japan), Hong-Zhang Zhou (Beijing, China), and Jong-Cheol Paik (Sunchon, South Korea). We are very grateful for their kindness.

This book is a revised and enlarged version of a book entitled *Molecular Phylogeny and Evolution of the Carabid Ground Beetles of the World* by the same authors. That book, written in Japanese, was published by Tetsugakushobo, Tokyo, in 2002. We should mention that this English monograph was conceived by the editors of Springer-Verlag, Tokyo, and Masataka Nakano of Tetsugakushobo. We greatly appreciate the expert advice and assistance we received from the two publishers while we were writing this book.

September 2003

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Abbreviations

BRH	JT Biohistory Research Hall
COI	cytochrome oxidase subunit 1
ICZN	International Code of Zoological Nomenclature
ITS	internal transcribed spacer
ML	most likelihood
MP	maximum parsimony
MYA	million years ago
MYR	million years
NCGB	non-Carabinae ground beetles of the family Carabidae
ND5	NADH dehydrogenase subunit 5
NJ	neighbor-joining
PCR	polymerase chain reaction
SEM	scanning electron micrograph
Ts	transition
Tv	transversion
UPGMA	unweighted pair-group method using arithmetic mean

Contents

Chapter 1
Background of the Molecular Phylogenetic Studies of the Carabid Ground Beetles

1.1 Early Days

In 1968, one of the authors, Syozo Osawa (S.O.), and his collaborators found that ribosomal protein composition is different in each bacterial species when analyzed with carboxymethyl-cellulose column chromatography. At that time, he was not aware of the now well-known work done by Zuckerkandl and Pauling (1965) on the molecular evolution of hemoglobin molecules, and the neutral theory of molecular evolution developed by Kimura (1968).

S.O. was engaged in this line of investigation with Hiroshi Hori (now a professor at Nagoya University) and constructed a phylogenetic tree that showed the bigger the difference in ribosomal protein composition, the remoter the relatedness (Hori and Osawa 1978). These results led S.O. and Hori to believe that phylogenetic relationship can be traced by biological macromolecules.

Hori and S.O. then began to analyze the 5S ribosomal RNA and succeeded in constructing a phylogenetic tree covering all the main groups of organisms (Hori and Osawa 1987). This task took about 10 years to complete. DNA sequencing techniques were not yet available, making RNA the focus of the analysis done by Hori and S.O. The sequencing of RNA is considerably more difficult than that of DNA, contributing to the time taken to complete task.

The most interesting result of this study was one showing that archaebacteria are more closely related to the eukaryotes than to the eubacteria. This suggests that the name metabacteria should replace that of archaebacteria.

S.O. and Hori set out the possibility of a field of study that might be called "molecular evolutionary phylogenetics," in which phylogeny and evolution are studied through biological macromolecules. An article laying out these ideas was published in *Shizen* (Osawa and Hori 1980), a scientific journal published in Japan. The word *shizen* means nature in Japanese.

1.2 Early Stages in the Study of Carabid Ground Beetles

Given the experience described in section 1.1, S.O. wished to construct a phylogenetic tree of a certain group of beetle, which he had been fond for more than 50 years. It was about three years before his retirement from Nagoya University when he first thought about the possibility of undertaking a full-fledged study of a beetle group.

At about this time, however, which was the early 1980s, S.O. and his research group were occupied with the implications of an unexpected discovery: the genetic code is not frozen but still evolving. This discovery meant that S.O. had no time to devote to the study of beetles. Just before the retirement from Nagoya University, the project on the genetic code was completed, thus, giving S.O. time to begin analyzing the DNA of various types of beetles.

S.O. first chose lady beetles as the subject of his research, but soon came to the conclusion that the diversification shown among lady beetles and their wide distribution throughout the world would make them an inappropriate subject. It would take hundreds of years to collect even only representative groups of such insects.

S.O. eventually decided to study the carabid ground beetles (subfamily Carabinae in the family Carabidae), because they show limited diversification and have been the subject of morphological studies for more than 100 years, particularly in Europe.

In Japan, Takehiko Nakane began studying the carabid ground beetles in earnest in the early 1960s. Researchers such as Ryosuke Ishikawa, the Keihin Insect Club, and the Kinki Research Group of Carabid Beetles have since added to our knowledge of this insect group in Japan. Studies on carabid beetles represented throughout the world have also moved forward, with contributions coming from young scientists such as Yûki Imura (Y.I.), one of the authors of this book.

Especially noteworthy is the publication *The Carabus of the World*, by Y.I. and Kiyoyuki Mizusawa in 1996. This publication shows most of the world's carabid beetles in color illustrations. This book has played an extremely helpful role in our study to learn morphology and taxonomy of carabid ground beetles.

FIG. 1.1. Munehiro Okamoto

FIG. 1.2. Osamu Tominaga

It has been reported in popular scientific journals that museum specimens can successfully be used for DNA sequencing. In autumn 1991, S.O. repeatedly tried to sequence carabid mitochondrial DNA prepared from dry specimens but was unsuccessful. At about this time, Munehiro Okamoto (Fig. 1.1; then of Osaka University; now at Tottori University) visited S.O. at Nagoya University to discuss his work on the molecular genetics of mammalian parasites, his main field of study. S.O. took the opportunity to tell Okamoto about his interest in analyzing the DNA of carabid beetles.

Okamoto immediately became interested in the subject, because he had been an enthusiastic collector of carabid beetles, such as *Acoptolabrus gehinii* and *Megodontus kolbei*, in days as a student at Hokkaido University. He then promised to collaborate with S.O. as soon as the project got underway on a full-scale basis.

In March 1992, S.O. retired from Nagoya University and began his study of carabid molecular phylogeny in earnest. In the planning stage, S.O. mapped out a rough image of what he thought would be the phylogenetic relationship of Japanese carabid beetles based on his knowledge of their morphology and distribution. DNA research showed that these early speculations were quite far from it.

1.3 Early Work on Japanese Carabid Beetles

In 1993, JT Biohistory Research Hall (BRH) opened. At first, the research team planned to use nuclear DNA, such as the genes for elongation factors, to study carabid phylogeny. It was soon found, however, that nuclear DNA was not suited for research because of the slow rate of nucleotide substitutions, and the mitochondrial *ND5* gene was chosen instead.

Since dry specimens were not useful, analyses were made on ethanol-immersed specimens. These analyses proved successful. In 1994, Takeshi Ohama (currently a professor at Kochi Technical College) and one of the authors of this book, Zu-Hui Su (Z.-H.S.) joined the group, and considerable progress was made. Both Ohama and H.-Z.S. have considerable experience of DNA study.

Two *ND5* gene sequences of *Ohomopterus albrechti* and *Damaster blaptoides rugipennis* were first analyzed, and it was found that the difference between the two was 10%, suggesting that this gene was well suited for a phylogenetic analysis of carabid ground beetles. Soon after this analysis, the sequences of several other species were added, including *Calosoma inquisitor*, *Leptocarabus procerulus*, *Euleptocarabus porrecticollis*, *Homoeocarabus maeander*, *Tomocarabus opaculus*, *Tomocarabus harmandi* and a few species of the genus *Ohomopterus*.

The preliminary phylogenetic tree constructed from these sequences surprised the members of the research team, because it was quite different from the phylogeny predicted from morphological knowledge. The tree seemed counter-intuitive when looked at from a morphological point of view, but we had to learn to view it objectively from the viewpoint of molecular biology.

The most serious problem at this stage was the difficulty of collecting enough samples for analysis. S.O. asked Takehiko Nakane, one of the leading entomologists in Japan, for advice on collecting specimens. Nakane suggested asking amateur insect collectors and museums to offer specimens and introduced S.O. to Yorio Miyatake, director of the Osaka Museum of Natural History.

Miyatake soon paid a visit to BRH with Osamu Tominaga (Fig. 1.2) and other members of the Kinki Research Group of Carabid Beetles, who agreed to collaborate with us. Tominaga, who works for the Osaka Prefectural Government, is a well-known "walking encyclopedia" of the Japanese carabid beetles and supplied us with much useful material.

When we had informed Tominaga of the results of our early research, he made a number of useful comments and sent us much of the material that allowed us to continue our analysis. Tominaga continues to be of great assistance in our research, and we are thankful for his continued support.

In 1997, just after publishing his book *The Carabus of the World*, Y.I. joined the research team, helping us to accelerate the pace of our research. At about this time, S.O. sent letters to about 50 insect collectors asking them to supply us with materials. Many collectors responded enthusiastically, sending specimens immersed in

FIG. 1.3. Nobuo Kashiwai

FIG. 1.4. Choog-Gon Kim

ethanol. To keep these collectors informed of developments, S.O. began publishing a newsletter describing the results of our research. The first of these newsletters was sent out in July 1995 and the last, the 20th, was mailed in March 1999. Mrs. Yayoi Takahashi, S.O.'s secretary, acted as the editor of the newsletters, in which role she contributed a great deal to the team.

In March 1995, Seiichi Kashiwabara, editor of the *Kagaku Asahi* (Asahi Science Journal, the Asahi Press, Tokyo) visited BRH to see our results on *Damaster* and *Ohomopterus*, and introduced the readers of his journal to our project. Not long afterward, S.O. received a telephone call from Takaaki Matsuda of Himeji University of Technology (HUT) to inform him that the research team's estimate of the time of the formation of the Japanese Islands, at 15 MYA, based on the differentiation profile of carabid beetles was in agreement with the paleomagnetic estimation of Matsuda et al.

Later, Professors Matsuda and Yoichiro Otofuji of Kobe University presented a lecture at BRH on paleomagnetic studies of the Japanese Islands. This is a promising development, in that it brings insect biogeograpy and geohistory closer together. Shuya Tanimoto, then a member of the BRH team, and Tominaga were helpful in drawing links between the biogeography of Japanese carabid ground beetles and the results of geohistory studies.

As to the genus *Ohomopterus*, which had been classified into four species-groups based on the shape of the male genital organ, the NADH dehydrogenase subunit 5 (*ND5*) phylogenetic tree showed that they form five geographically dependent clusters with intermingled occurrence of members of different species-groups. Tominaga suggested this phenomenon be called "parallel evolution in radiation." Kazuyuki Mogi, another member of the BRH team at the time and currently an associate professor at Seitoku University, suggested that this parallel evolution might involve discontinuous morphological change which could be called "type-switching," i.e., the discontinuous transformation of one morphological type to another.

The results of our early study of the genus *Ohomopterus* were criticized by a number of entomologists. Though it later became necessary to revise some of these results, type-switching has been found to be a common pattern in the evolution of many varieties of carabid beetles. In the autumn of 1995, S.O. presented a paper on the *ND5* phylogenetic tree to the annual meeting of the Japan Coleopterological Society in Tokyo. The presentation brought a question from Yamanashi University Professor Kiyohiko Ikeda, who asked, "Can you explain the discrepancy between your results and those gathered via a morphology-based taxonomic approach or a traditional Darwinian evolutionary analysis?" S.O. replied with, "Your theory of structuralism will be helpful to understand the discontinuous structural change."

1.4 Towards a Study of Carabid Beetles Around the World

Professor Ikeda eventually helped us to obtain specimens of *Pamborus opacus*, a carabid indigenous to Australia. At the 1995 meeting of the Coleopterological Society referred to in section 1.3, S.O. had an opportunity to meet Y.I., who joined the research team mainly with a view to working toward creating a phylogenetic analyses of the carabid beetles of the world, especially those in China, Europe, and Russia.

This was just before the publication of Y.I.'s book *The Carabus of the World*, a huge iconography based on traditional taxonomic techniques. Y.I. was forced to consider the possibility that this approach might be shown to be inaccurate to some extent as a result of developments in DNA study.

In the autumn of 1995, through the courtesy of Tokindo S. Okada, then the director of BRH, we were able to contact Burno David, Jean-Louis Dommergues, and Francois Magniez of the Centre de Sciences de la Terre of France and had a chance to analyze several European carabid beetles in collaboration with these scientists. The specimens we examined included *Megodontus violaceus*, *Procrustes coriaceus*, *Chrysocarabus auronitens*, *Archicarabus nemoralis*, *Oreocarabus glabratus*, *Chaetocarabus intricatus*, *Carabus conciliator*, and *Carabus arvensis*. We concluded from these examinations that a rapid radiation of the major genera took place in the initial stage of the Carabina evolution (Su et al. 1996b).

Nobuo Kashiwai (Fig. 1.3) of Hosen Gakuen High School in Tokyo and a lepidopterist, has made a number

of trips to Chile to collect butterflies, and brought us many specimens of *Ceroglossus* which are indigenous to Chile and part of Argentina. Our analysis of these samples and specimens of *Pamborus* from Australia made it clear that their phylogenetic position is in the subfamily Carabinae. In addition to this, it was found that *Ceroglossus* reveals a wonderful sympatric convergence of body color (Okamoto et al. 2001).

Y.I., who knows entomologists all over the world, obtained large numbers of samples from his acquaintances. These efforts have considerably accelerated DNA analyses of the world's carabid beetles.

Between 1997 and 1999, Z.-H.S., Y.I., and Okamoto undertook three scientific expeditions to Sichuan, Gansu, and Shaanxi in China, in collaboration with the Chinese Academy of Science with sponsorship from BRH. In 1998, a collecting trip was made to Korea by Z.-H.S., Okamoto, and Choog-Gon Kim (who is currently working at the Japanese National Institute of Genetics; Fig. 1.4), who joined our research group in the spring of 1998. Kim analyzed specimens of the Procrustimorphi species from the world, constructing phylogenetic trees for these insects.

The samples collected and analyzed thus far amount to about 2,000 in number. It has been a challenge to collect this many specimens, and there are many areas from which it is still almost impossible to collect samples, because of political instability or inaccessibility. It is difficult to obtain even dry specimens from these areas, a matter that will be discussed in greater detail in Chapter 2.

During the course of our DNA analyses at BRH, we have discovered many things and gained a better understanding of matters that were previously unclear, including the mode of morphological diversification in carabid beetles (Osawa et al. 1999). In parallel with this, Y.I. has made extensive morphological studies on the specimens collected, and has discovered a considerable number of new species and subspecies.

Chapter 2
Collection of the Specimens from the World

2.1 Collection of Materials for DNA Analysis from All Over the World

When Y.I. joined the carabid research team, BRH members S.O., Z.-H.S., and others had already completed analyses of most of the Japanese Carabinae species with considerable assistance from Osamu Tominaga. The group was hoping to extend their analysis to species found in other countries. To carry out work on a worldwide scale, the greatest challenge was procuring useable specimens.

Up to this time, nearly all the species belonging to the subtribe Carabina were in Y.I.'s private collection. For DNA analyses to be effective, however, we had found that specimens immersed in alcohol were necessary. To collect enough specimens preserved in this way to complete a worldwide survey would have taken us at least a decade if we had been working on our own. We were, however, able to speed up this process with the kind cooperation of colleagues abroad.

Klaus Staven of Germany, a good friend of Y.I.'s for nearly 20 years, sent us specimens, preserved in alcohol, of *Limnocarabus clathratus* from northern Germany. These specimens were indispensable for the completion of one of our representative papers on the molecular phylogeny of *Apotomopterus* and its allied groups (Imura et al. 1998a). Walter Heinz, also of Germany, supplied us with many samples from the Middle East, Iran, and Pakistan. Bernard Lassalle of France provided us with a number of specimens of phylogenetically interesting genera such as *Chrysotribax, Ctenocarabus, Rhabdotocarabus,* and *Hygrocarabus* from western Europe, as well as *Eurycarabus* and *Cathoplius* from North Africa. Pierfranco Cavazzuti of Italy sent us many specimens, mainly *Orinocarabus* species from the European Alps, which were useful in preparing our paper on the reconstruction of the *Oreocarabus* complex (Imura et al. 1998b). He was the first to supply us with samples of the genus *Procerus* from Turkey, one of the largest carabine genera in the world.

Many Chinese carabid specimens were provided by Hong-Zhang Zhou of Academia Sinica, Beijing, who was one of the leaders of the collecting expedition organized by BRH in 1997. It is worth noting that we found a new species in the samples he collected in the Beijing area; it was described under the name *Titanocarabus sui* (Imura and Zhou 1998). An astonishing new genus and species, *Shenocoptolabrus osawai,* was also discovered among specimens collected by Hong-Zhang Zhou in the Shennongjia area of western Hubei Province (Imura et al. 1999). The discovery of *S. osawai* may be regarded as one of the greatest achievements of our project and, in fact, in the whole field of Coleopterous taxonomy. BRH members have made three collecting trips to China, allowing us to gather samples of almost all species belonging to the division Procrustimorphi for molecular phylogenetic analyses.

Close cooperation from Russian colleagues was also helpful in our quest. Igor Belousov of St. Petersburg provided us with many specimens, including rare species from his main area of interest, the Caucasus Mountains and northern Turkey. These samples enriched the molecular phylogenetic studies of species found in the areas bridging Asia and Europe. Ilia Kabak, based in Almaty, provided us with many samples from Central Asia. His assistance enabled us to obtain almost all the species found in this area, including *Leptoplesius* and *Acrocarabus* of the Tianshan Mountains and *Ulocarabus* and *Deroplectes* of Turkmenistan. Dmitry Obydov of Moscow offered us some valuable samples from Central Asia and southern Siberia.

Many North American species were made available through the courtesy of Eric Van Den Berghe of Seattle and Robert Davidson of the Carnegie Museum in Pittsburgh, who provided us with not only the Carabina specimens but also the Cychrini and the Calosomina specimens. These samples widened the scope of our study to include carabids other than the Carabina.

Most specimens were received by Y.I. from colleagues at the International Insect Day held annually in Prague, Czech Republic. Figure 2.1 shows the source of all the materials collected for the present study. They include more than 2,000 specimens from nearly 500 locations in 35 countries and account for more than 90% of supraspecific categories and about half the hitherto known species of the subtribe Carabina.

These specimens allowed us to carry out an extensive study of the phylogeny and evolutionary history

FIG. 2.1. Localities in which carabid ground beetles used for DNA sequencing were collected. The *black dots* represent one locality, each one of which may include up to 20 small regions

of carabid beetles as well as allowing us to consider the appropriate taxonomy and classification of these insects. It is thus thanks to the assistance of specialists from the world that we have been able to extend the scope of our study, making it truly international.

2.2 Collecting Trips to China

The BRH research group organized joint expeditions with Academia Sinica of China to collect specimens of Chinese mountainous carabid species for DNA analysis. The first expedition traveled to Sichuan Province in the early summer of 1997.

2.2.1 Fengtongzhai Nature Reserve (Central Sichuan)

Y.I. was quick to choose Sichuan Province as the first destination of a collecting excursion in China due to the area's wealth of carabid fauna. We focused on the Fengtongzhai Nature Reserve in central Sichuan, a deep valley stretching to the north from Baoxin, where the giant panda was first discovered by a Westerner—a French missionary, H. David.

Baoxin is well known as home to many carabids, especially of *Aristocarabus viridifossulatus*, which is of considerable interest to entomologists. The expedition was carried out by four Japanese and five Chinese: Y.I., Z.-H.S., Munehiro Okamoto, Shun-Ichi Uéno of the National Science Museum, Tokyo—who has considerable experience of Chinese expeditions, Hong-Zhang Zhou of Academia Sinica, Beijing, and four students from the same institute.

The Japanese party left Japan on June 2, traveling via Beijing to Chengdu in Sichuan Province, where they arrived on the evening of June 3. The supervisor of our expedition in Sichuan was Fan Ting, who is responsible for the International Academic Exchange Center of Academia Sinica. The Chinese members arrived in Chengdu and joined us at our hotel.

The next day, we set 500 pitfall traps on Mt. Qingcheng Shan, which is about 60 km northwest of Chengdu, where several *Coptolabrus* and *Apotomopterus* species had been recorded. Unbelievably enough, however, we found no carabid beetles in the traps when we checked them a week later.

On June 5, we arrived in Baoxin, which is 1,100 m above sea level and is situated at the entrance to the Fengtongzhai Valley. On the following day we entered the nature reserve, pleased with the fine weather we were experiencing. It took about an hour by car from Baoxin to the reserve's central control station, which is itself 1,650 m above sea level, where we stayed for five days. Fengtongzhai is well known as a habitat of the giant panda. A few young individuals caught in the nearby mountains were being kept in cages behind the station buildings to receive medical treatment for malnutrition. We set 800 traps along the main forest road and around Qiaoqi, a Tibetan village.

The next morning, we checked all the traps, but rather than finding *Aristocarabus viridifossulatus, Neoplesius, Pagocarabus, Pseudocranion,* and *Coptolabrus*, as we had expected, we had trapped only

FIG. 2.2. Habitat of *Aristocarabus viridifossulatus* (Fengtongzhai Nature Reserve, central Sichuan, China; 3,200 m above sea level)

a single female of *Carabus* (s. str.) *paris* above Qiaoqi. This poor showing impressed upon us the difficulty of collecting carabid samples in China.

On June 8, we ventured into the alpine zone on the left bank of the Tong He River to continue our efforts with what we hoped would be greater success. It was a dangerous trip, because the road was rough and visibility was poor as a result of heavy rain and fog. After a tough trip, we finally reached a wonderful primitive forest preserved at 3,200 m above sea level (Fig. 2.2).

The forest is composed of old-growth fir trees, with the forest floor covered with low-growth bamboo, one of the food plants of the giant panda. The area was resplendent with the large, pink flowers of wild rhododendrons. We felt that the area must be home to *Aristocarabus*.

A total of 800 traps were set along the mountain slopes between the altitudes of 3,200 m and 2,700 m. On June 9, our last day in Fengtongzhai, we succeeded in collecting three specimens of a brilliant *Aristocarabus* (shown later, in Fig. 8.2.6) from cups set out at 3,200 m, together with a medium-sized, dark example of *Neoplesius sichuanicola*, which is a very rare species, only a single female specimen having been known until that time.

We also collected examples of three cychrine species: two of the three were new to science and were described as *Cychrus choui* and *C. okamotoi* after members of the excursion team (Imura et al. 1998c). The remaining one was an example of the little-known species, *Cychropsis draconis* (shown later, in Fig. 5.1.7).

Although the total number of carabid beetles obtained in this expedition was much less than had been expected, we were able to discover two new cychrine species in addition to examples of *Aristocarabus viridifossulatus* and the males of *Neoplesius shicuaniloca* for the first time. These samples were very useful in future phylogenetic and evolutionary studies on the Procrustimorphi and Cychrini that inhabit the Eurasian Continent (Imura et al., 1998c,d).

2.2.2 From Jiuzhaigou (Northern Sichuan) to Wenxian (Southern Gansu)

Early in the summer of 1998, our second expedition to northern Sichuan and southern Gansu was carried out by Y.I. and Z.-H.S. On June 6, the day after our arrival at Chengdu via Shanghai, we made the long trip to Jiuzhaigou, one of the best sightseeing spots in northern Sichuan about 450 km away from Chengdu.

Jiuzhaigou is a valley located at the eastern periphery of the Tibetan Plateau, consisting of high mountains over 5,000 m above sea level and arid inland areas under 1,000 m in altitude. Conditions in this area are of the kind preferred by carabid beetles and various important species have been recorded there.

We felt that this was a good place to collect examples of a number of species quickly during what would be a short trip. For the first two days, we set pitfall traps in several places along the road between the Gonggaling Pass (3,400 m) and Jiuzhaizhen Village (2,000 m). This area is covered in alpine meadow dotted with happily grazing Yaks. A coniferous forest is found on the northern slope of the pass, extending to a mixed forest in the lower zone. Near Jiuzhaizhen, at an elevation of 2,000 m, dry slopes covered by thorny shrubs can be seen on either side of the road.

In Gonggaling, we found *Cychrus stoetzneri* (shown later, in Fig. 5.1.13), *Rhigocarabus pusio* and *Pseudocranion zhanglaense* under stones. We also captured an example of *Aristocarabus viridifossulatus* in the mixed forest. This specimen was one belonging to the local subspecies, *ventrosior*. On the dry slope near Jiuzhaizhen, we discovered *Acathaicus alexandrae*, which was one of our main target species in this expedition. This species has an unusually enlarged head and is one of the most unique Procrustimorphi species found in China.

At the same place, we collected two individuals of the Calosonima species, *Campalita chinense* (shown later, in Fig. 5.6.8) and *Charmosta lugens* (Fig. 5.6.4). Most species of the subtribe Calosomina have well developed hind wings and are more readily able to expand their distribution than the Carabina, so that geographical variation among the Calosomina species is usually less marked than in the Carabina. The mitochondrial DNA sequences of the Chinese and Japanese *C. chinense* are almost the same, suggesting a much lower level of isolation in the Calosomina populations than seen in the Carabina, whose hind wings are usually absent or less developed (Osawa et al. 2001).

On June 12, we moved to Wenxian in southern Gansu. This is dry hill area without large trees (Fig. 2.3), and numerous snails inhabit the shrubs covering the slopes, with the land surface dotted with their empty shells. At

FIG. 2.3. Habitat of *Acathaicus alexandrae* and *Cephalornis potanini* (near Wenxian, southern Gansu, China)

FIG. 2.4. An example of *Acathaicus alexandrae* (*right*) and *Cephalornis potanini* (*left*)

first glance, it did not seem a hospitable environment for carabid beetles.

We stayed in Wenxian for two nights, and succeeded in collecting examples of two remarkable species: *Cephalornis potanini* and *Acathaicus alexandrae idolon* (Fig. 2.4). These are perhaps the most uniquely differentiated of all Chinese carabid beetles, with the *Cephalornis* demonstrating extreme microcephaly and *Acathaicus* displaying advanced macrocephaly. All the *Acathaicus* populations in various localities are morphologically alike, while the branching point as shown by mitochondrial DNA sequence examination is considerably deep between specimens from the Wenxian and Jiuzhaigou regions. Endophallic features are also clearly differentiated in specimens from the two localities, suggesting that *Acathaicus* shows more differentiation in populations isolated geographically than had been understood from an examination of external morphology alone (Imura and Su 1998).

The occurrence of these two peculiar species in the relatively small area of southern Gansu is certainly noteworthy. We collected a few species such as *Carabus* (s. str.) *pseudolatipennis* and *Pagocarabus crassesculptus* (shown later, in Fig. 8.2.8) in the pass between Wenxian and Wudu on the way back to Jiuzhaigou.

In traps set several days before on dry slopes near Jiuzhaizhen, we found *Titanocarabus titanus* (shown later, in Fig. 5.18, U1-1), a large carabid ground beetle of which only a single specimen had heretofore been known, the first having been found in Hubei Province. This specimen has been described by Breuning (1932) and is preserved in the Zoological Museum of Amsterdam. This species was widely regarded as a member of the genus *Oreocarabus*, but mitochondrial DNA phylogeny and the morphology of the male genital organ show that this is not the case (Imura et al. 1998b).

In traps set in Gonggaling, we found *Neoplesius nanschanicus* along with three other species of which we already obtained examples. In the mixed forest in the same area, we caught a number of individuals of *Aristocarabus viridifossulatus,* widely regarded as one of the rarest of carabid species. We also found an example of "*Oreocarabus*" *latro* (shown later, in Fig. 5.23.27). DNA analysis of this specimen showed that it is a member of the *Rhigocarabus* lineage and does not belong to *Oreocarabus*. In addition to these important species, several more species such as *Pseudocranion sackeni, Cychrus furumii* (shown later, in Fig. 5.1.14), and *C. minshanicola* (Fig. 5.1.12) were obtained and their DNA was analyzed, allowing us to regard this excursion as being particularly fruitful in terms of both quality and quantity.

2.2.3 Micang Shan Mountains (Northeast Sichuan) and Southwest Shaanxi

Our third expedition to China, which was undertaken in 1999, was focused on northeastern Sichuan and southwestern Shaanxi, areas in which little work on carabid beetles had been done. The members of the expedition, Y.I. and Z.-H.S. left Japan on May 27.

FIG. 2.5. Habitat of *Shenocoptolabrus osawai* (Micang Shan Mountains in northeast Sichuan, China)

After arriving in Chengdu, we drove 450 km to the northeastern end of Sichuan Province and the town of Nanjiang. After staying in Nanjiang overnight, we visited Mt. Guangwu Shan, which is situated near the border of Shaanxi. The native people told us that this area had once been covered by old-growth forests, and wolves had often appeared even in the suburbs of Nanjiang until a few decades ago. Although many trees had already been cut down, we were able to find small, scattered oak groves on the northwestern slope of Mt. Guangwu Shan, and set a few hundred pitfall traps along the forest roads running through the area.

On May 30, we found two species, *Apotomopterus cyanopterus* and *Carabus* (s.str.) *vigil*, in some of the traps. A close morphological examination revealed that both were newly discovered subspecies (Imura and Su 2000). On the afternoon of the same day, after driving along a deep valley for several hours, we unexpectedly came across an old-growth forest of *Fagus* and oak trees (Fig. 2.5). It is quite rare to see such a well-preserved forest in China outside of a nature reserve.

On the evening of the same day, we moved to Shaanxi Province, staying for a few days in Liuba Town at the southwestern end of the Qinling Mountains. Our main purpose there was to find another species, *Lasiocoptolabrus sunwukong*, that had been described by Y.I. a few years before.

Unfortunately, we failed to find any of this species, and collected only *Carabus* (s.str.) *pseudolatipennis*, *Pagocarabus crassesculptus*, and *Cychrus bispinosus*, which are all widely distributed in the Qinling Mountains. We also found *Qinlingocarabus reitterianus*, *Apotomopterus hupeensis*, *Coptolabrus formosus*, and two Calosomina species, *Calosoma inquisitor* and *C. maximowiczi*. We returned to Sichuan on the afternoon of June 3 with these specimens.

We then examined the traps set on the northwestern slope of Mt. Guangwu Shan. In one oak grove at 1,600 m above sea level, we found nothing in the first 20 cups except a single example of *Apotomopterus hupeensis*. The next trap we picked up proved to hold a large beetle with very strange features, including a slender, purplish head and pronotum that reminded us of the Japanese *Damaster*. The roughly sculptured elytral surface of this specimen was in an intermediary state between that of *Coptolabrus* and *Acoptolabrus*, which proved to be *Shenocoptolabrus osawai*. A female of this astonishing species was first discovered in Shennongjia of Hubei Province by Hong-Zhang Zhou of Academia Sinica, with a manuscript offering the first description of the species having been sent to a publisher just before the trip (Imura et al. 1999) (shown later, in Fig. 8.2.1). This paper had not yet been published when we found this second specimen, making it an as yet officially undescribed species.

We captured a total of three specimens of *S. osawai* in the area, including the first male yet recorded. After a close morphological examination, the Micang Shan population was recognized as a new subspecies, and was named *micangshanus* (Imura and Su, 2000). We also collected *Leptocarabus yokoae* and *Coptolabrus pustulifer* in the same area.

On June 4, we checked the traps set in the central part of the Micang Shan Mountains, finding many *S. osawai* specimens. This meant our third expedition was successful in that we found many interesting carabid beetles that contributed considerably to our studies on DNA phylogeny and taxonomy.

2.3 Expedition to Korea

As described in Section 2.2 of this chapter, we made three expeditions to Sichuan and its neighboring districts in the interior of China beginning in the early summer of 1997. It has been speculated that carabid beetles have their origin somewhere in China, and indeed there is evidence to support this view. However, an understanding of the carabid species found in the Korean Peninsula is indispensable for a full understanding of the origin of Japanese carabids, since several representative Japanese species, such as *Ohomopterus* spp., *Leptocarabus* spp., and *Damaster blaptoides* are believed to share common ancestry with Korean species. In one example, *Ohomopterus* may have been derived from *Isiocarabus fiduciarius* (shown later, in Fig. 5.27.5) of Korea.

FIG. 2.6. Old-growth forest on Mt. Halla-san on Cheju-do Island, South Korea

FIG. 2.7. An example of *Coptolabrus smaragdinus* in a trap

In April 1997, Choong-Gon Kim, a Korean biologist, joined our team, making it possible to undertake an expedition to Korea. Kim made inquiries on the best areas in which to search for carabid beetles, receiving particularly useful information from Jong-Cheol Paik of the Sunchon National University of Korea and Sai-Ho Jung of the Zoology Department of the Cheju Folklore and Natural History Museum.

In addition to this, various information was obtained from Y.I., who has ample experiences in collecting carabid beetles in various parts of Korea. Kim, Okamoto, and Z.-H.S. left Japan on July 3, 1998, and collected a considerable number of carabid beetles between July 3 and 17. They focused their expeditions on Cheju-do Island and the Chiri-san Mountains in south Korea and Mt. Odae-san in mid Korea.

On July 3, the Japanese party met Paik and Jung at the Cheju airport and went straight to Mt. Halla-san, which, at 1,950 m above sea level, is the highest mountain in South Korea and is well-preserved environmentally (Fig. 2.6). It was a sunny, hot day of about 30°C on the northern side of the mountain. It gradually became cloudy at higher altitude and we eventually met with heavy rain.

At about 1,000 m above sea level, about 300 traps were set at four points, and the next day, about 500 traps were set along the road on the southern slope of the mountain. In the ditches along the road on the way back to Cheju City we collected *Campalita chinense, Isiocarabus fiduciarius,* and *Coptolabrus jankowskii.*

On July 5, we went to check the traps set on July 3 and 4. Surprisingly, almost all the traps contained a number of carabid beetles. Among the samples captured were *Coptolabrus smaragdinus* (Fig. 2.7), *C. jankowskii, Hemicarabus tuberculosus, Eucarabus sternbergi* (shown later, in Fig. 5.27.86), and *Isiocarabus fiduciarius.*

Unfortunately, we were unable to obtain *Scambocarabus kruberi* or *Homoeocarabus maeander*, both of which are found only around the top of the mountain, an area that is out of bounds. *Isiocarabus* is found in the southeastern China and Cheju-do Island, but not in mainland Korea. This and other species found on the island are valuable for the study of biogeography.

FIG. 2.8. Environment on the top of Sangwang Bong Mountain in the Odae-san Mountains, South Korea

On the evening of July 7, our party arrived at the Chiri-san Mountains, and about 300 traps were set at the foot of the mountain. On July 8, we climbed up toward the top of the mountain to set traps at higher altitudes. It took more than two hours to reach the top. About 450 traps were set at this higher elevation. To leave the traps as long as possible, collection was postponed until after the trip to Mt. Odae-san.

On July 9, on the way to Taegu airport, two species of *Coptolabrus* and *Eucarabus sternbergi* were captured.

On July 10, we flew to Kangnung via Seoul. During the flight from Taegu to Seoul, there was violent air turbulence so that the aircraft suddenly dropped by nearly 100 meters. From Kangnung, we went to the Odae-san

Mountains by car, spending three days there between July 11 and 13 to collect carabids.

Our main purpose was to collect various carabid species around Sangwang Bong, at 1,594 m above sea level, which is one of the peaks of the Odae-san Mountains and is covered by broad-leaved deciduous trees (Fig. 2.8). It looked to be an excellent habitat for carabids.

About 1,200 traps were set around the top, the side and the foot of the mountain. Two days later, we checked the traps and found *Coptolabrus smaragdinus, C. jankowskii, Acoptolabrus mirabilissimus* (shown later, in Fig. 8.2.3), *A. leechi* (Fig. 8.2.2), *Eucarabus cartereti, Tomocarabus fraterculus, Morphocarabus venustus*, and *Leptocarabus semiopacus* (Fig. 5.20.9).

On July 14, we moved from Kangnung back to the Chiri-san Mountains to check the traps set around the top of the mountain on July 7. Unfortunately, it rained heavily and we had a hard time reaching the top. It took about 2 hours to collect about 450 traps, in which we found *Coptolabrus smaragdinus* of a metallic-green color along with *Acoptolabrus leechi* and several other species.

In total, we found 14 species, 18 subspecies and 850 individuals on this expedition, providing a sample of the full range of South Korean species with the exception of *Scambocarabus kruberi* and *Homoeocarabus maeander,* which inhabit the top of Mt. Halla-san on Cheju-do Island. These specimens have since proved very useful in tracing the origins of the Japanese carabid beetle, a subject that will be discussed in greater detail in Chapter 7.

Chapter 3
Molecular Phylogenetic Tree

3.1 DNA

It is now relatively easy to determine the nucleotide sequence of DNA and construct a phylogenetic tree with the aid of a personal computer using techniques taken from analytical biochemistry. To be able to effectively evaluate the resultant phylogenetic tree, however, one must have a fundamental understanding of the DNA structure, population genetics, and molecular evolution.

DNA is present in both the cell nuclei and in mitochondria, with both being independent of the other. DNA is often thought of as being synonymous with the gene, but this is a misconception. The gene consists of DNA (or RNA in certain viruses), the spacers connecting the genes, the regulatory region, and "junk" DNA with no known function. In the case of mammalian species such as human beings, junk DNA accounts for more than 90% of total DNA.

For the construction of a phylogenetic tree, any DNA region fulfilling the necessary requirements may be used. Fundamental knowledge of the gene regions most frequently used for phylogenetic analysis is, however, necessary. Genes can be roughly divided into two categories: those that encode the amino acid sequence of a protein and those that encode ribosomal RNA (rRNA), transfer RNA (tRNA), and messenger RNA (mRNA).

The process of protein synthesis begins with transcription of the DNA nucleotide sequence to RNA. Amino acids are then arranged on the RNA template, specifically on the ribosome (which is itself composed of rRNAs and ribosomal proteins), with the aid of tRNAs. This is then followed by protein synthesis through peptide-bond formation between amino acids in a process known as translation. The final product of the whole process is a protein, rRNA or tRNA. The latter two are not translated into proteins. Genes determine such complex traits as coloring indirectly, through the production of proteins responsible for color development.

3.2 Genetic Code

A basic knowledge of the genetic code is necessary to construct a phylogenetic tree. Proteins are composed of a combination of a total of 20 amino acids. The role of proteins in determining such things as enzyme production, hormone output, and body architecture is ascribed to the amino acid sequence of the particular protein, as determined by the gene. Amino acid sequences are determined by the nucleotide sequence of the DNA in line with the genetic code. The genetic code consists of a set of 64 possible combinations of four RNA nucleotides, U, C, A, and G in triplet form (Fig. 3.1). Generally, one of a total of 61 codons is assigned to a specific amino acid, while three other codons act to terminate protein synthesis and do not correspond to any particular amino acid.

As the number of amino acids used for protein synthesis is 20, the same amino acid can have more than one codon assigned to it. For example, AAA and AAG are codons for Lys; GUC, GUU, GUA, and GUG are codons for Val. A further six codons—CGU, CGC, CGA, CGG, AGA, and AGG—are synonymous and are translated as Arg.

A box in which four codons are synonymous is called a "family box" or a "4-codon box," of which there are eight in total. A set in which a single amino acid has two codons is called a "2-codon set." There are 13 of these 2-codon sets. Codons for Arg, Leu, and Ser exist both in a family box and in a 2-codon set. There are exceptions to the even-number set rule, however, including 3-codon sets such as AUU, AUC, and AUA, which are assigned to Ile. Met and Trp have only a single codon—AUG and UGG, respectively—because the codon AUA is for Ile and UGA is a stop codon. There are therefore two to six synonymous codons for a single amino acid, with the exception of Met and Trp.

Translation of messenger RNA begins with recognition of the initiation codon, AUG, followed by successive readings of amino acid codons and termination at the site of the stop codon. Any protein gene region, which is a row of codons, is sandwiched by an initiation codon and a stop codon. When the sequence AUG UUU UCC UUG AAA GUU————-AAA UGA is translated, the resultant amino acid sequence of the protein is Phe-Ser-Leu-Lys-Val————-Lys. The initiation and stop codons do not exist in rRNA or tRNA genes.

The genetic code shown in Fig. 3.1 was formerly believed to be common throughout organisms. However, it is now clear that some organisms, as well as the

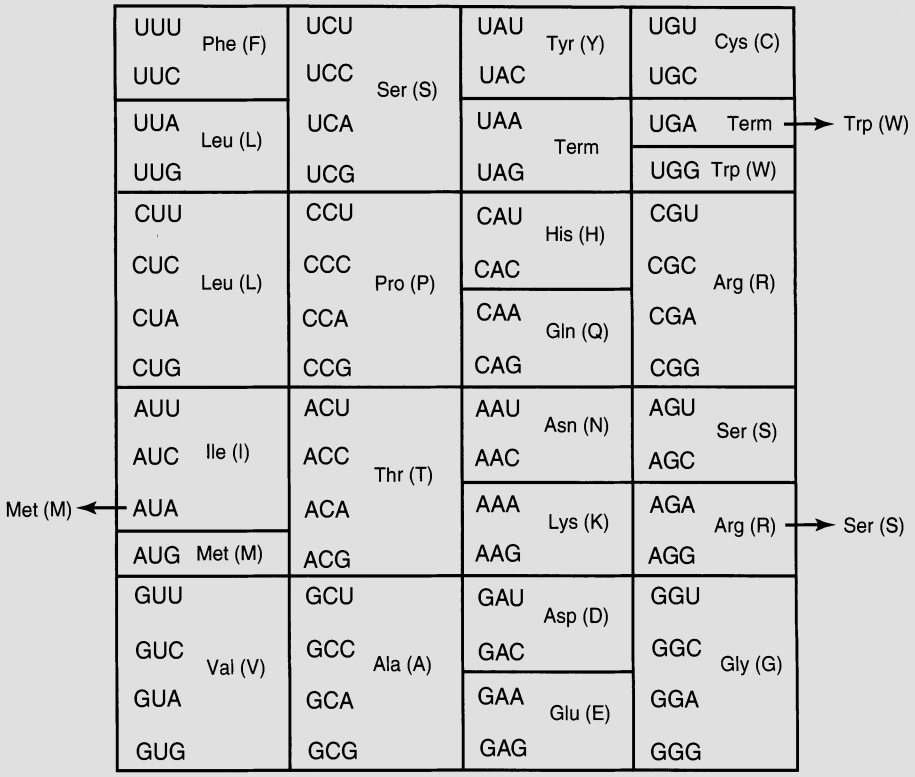


FIG. 3.1. The genetic code table. Codons in this table and in the text are customarily written at the RNA level. U should read as T at the DNA level. One letter code of amino acid is shown in *parentheses* after the abbreviated amino acid name

mitochondria of most eukaryotes, have a deviant genetic code. As shown in the margin of the genetic code table in Fig. 3.1, for example, in insect mitochondria AUA codes for Met instead of Ile, AGA is a Ser codon and not an Arg codon, and UGA, a universal stop codon, is used as a Trp codon.

3.3 Mutations

It is often said that genetic material, DNA, replicates following the Watson-Crick rule, passing on maternal DNA accurately from parent to offspring. If this were always true, however, the characters of a given species would never alter. DNA replication is always accompanied by errors, most of which are classified as a point mutation where a particular nucleotide is replaced by another. In addition, deletion or insertion of one or more nucleotides, replacement of a certain gene region, and duplication of a gene have been known to occur frequently. In constructing a phylogenetic tree, only point mutation plays an important role, so that the other changes should be dealt with only when necessary.

In each set of synonymous codons, the first and the second nucleotides are common, and two to four of the third nucleotides are free to change without altering amino acid assignment. This "free site" area extends to the first nucleotide in the case of Arg and Leu. In family boxes, third nucleotides are all free to change, while in the 2-codon sets, changes are only between A and G, or U and C of the third nucleotide. These sites are often collectively referred to as silent sites. In 2-codon sets, however, these sites are actually only semi-silent, because in codons for Lys, for example, freedom exists only between AAA and AAG, and a change from AAA to AAU or AAC results in a change of amino acid assignment from Lys to Asn. Only transitions (Ts) between A and G and between U and C are silent, and transversions (Tv) between A and U or C and between G and U or C are not silent in 2-codon sets. A site where the change is accompanied by a change of amino acid assignment is called an "(amino acid) replacement site." The second nucleotide in all codons and the first nucleotide in all except Leu and Arg are replacement sites of this type.

Mutations occur at all codon sites randomly. These changes occur at the level of the individual and should not be confused with those at the level of the population as a whole. A fraction of mutations occurring at the silent sites is fixed in a population by random genetic drift; mutations at the replacement sites are removed by negative selection if the resulting amino acid change is deleterious (see below).

We are dealing here with mutations occurring only in the germ cells, because those occurring in the somatic cells are not inherited. Mutation usually begins with an error in copying at the time of DNA replication. A certain fraction of errors is corrected by protein factors responsible for this process, which has been well studied in the nuclear gene. In contrast, mitochondria are said to have no such error-correcting function. This is probably one of the reasons for the higher mutation rate of mitochondrial DNA when compared with nuclear DNA.

Point mutations may be divided into three categories as defined by theories of molecular evolution: lethal or deleterious mutations, advantageous mutations, and neutral mutations. Most mutations belong to the first and third categories, with advantageous mutations being quite rare. The genetic code table provides us with the easiest way to explain the nature of these mutations.

3.3.1 Lethal and Deleterious Mutations

A protein takes on a three-dimensional structure that is primarily determined by its amino acid sequence. As was described in the previous section, a mutation at the replacement site causes a change in the amino acid sequence of a protein. This change is in many cases deleterious or lethal to the organism, affecting the functional structure of the protein. Mutations of this kind almost never spread throughout a population, being quickly removed from the gene pool owing to their deleterious effect on the individual organism carrying them.

3.3.2 Advantageous Mutations

Very rarely, a single amino acid change brought about by mutation of a non-neutral codon site is advantageous and may spread throughout a population over time.

3.3.3 Neutral Mutations

This type of mutation is the most important when constructing a phylogenetic tree, because only neutral mutations function as a molecular clock. The targets of neutral mutation are concentrated at synonymous codon sites (see above). A mutation at a synonymous site is fully silent. In addition to this, a mutation on the replacement site is often neutral if the amino acid change does not affect the functional structure of a protein. Therefore, all mutations at synonymous sites as well as those at replacement sites that do not result in deleterious effects are neutral, while neutral mutations are not always silent.

As noted in the previous section, it is misleading to consider the mutations at the third codon position as being consistently neutral, because transversion in a 2-codon set causes amino acid replacement. It is also important to note that the predominant occurrence of synonymous changes over non-synonymous changes arises simply as a result of the fact that the number of neutral changes in synonymous sites is much greater than that of non-synonymous sites. It is important when constructing a phylogenetic tree for a given group of organisms to take the neutral mutations described above into account. It is also necessary to have a proper understanding of the evolutionary (or genetic) distance that will often appear later.

3.4 Fixation of Mutations

As already pointed out above, observable nucleotide replacements result mostly from neutral mutations, because lethal or deleterious mutations are quickly removed from a population, and advantageous mutations occur only rarely. A large fraction of neutral mutations at the individual level disappears, while only a small fraction spreads throughout a population and finally becomes a fixed character of a given species over a long period of time (Fig. 3.2, top). For example, a mutation in a population that results in the replacement of site G with site A may spread gradually through a species until every member of the population has an A site where once there would have been a G site. Almost all the nucleotide changes that we can recognize in gene sequences that have been determined are those fixed in this way. Sometimes, mutations occurring during the course of fixation or disappearance may be observed in a given DNA sequence. In such cases, the nucleotide sequences have become polymorphic. The effect of polymorphism can be ignored when the fixed mutations considerably exceed mutations at the intermediate states in number. What we actually observe for any codon change in a natural population is mostly a substitution resulting from fixation of a mutant, as noted above (Kimura 1983).

The number of gametes produced in the reproductive process is much greater than that of the individuals in a population, but only a small proportion of these gametes manages to reach maturity successfully, helping to keep population size within a consistent range. The frequency of genetic variations produced in a given population is proportional to that in the gametes. However, as only a small proportion of gametes relative to population size is extracted at random, the frequency with which individuals manifest a genetic change is different with each generation. This means that the rate of genetic change varies by generation in accordance with random genetic drift, with the frequency deviating from that of the nominal original generation to a greater extent with every passing generation, finally disappearing entirely (0%) or becoming fixed (100%) (Fig. 3.2, top). The frequency of mutation is proportional to the frequency of fixation (Fig. 3.2c,

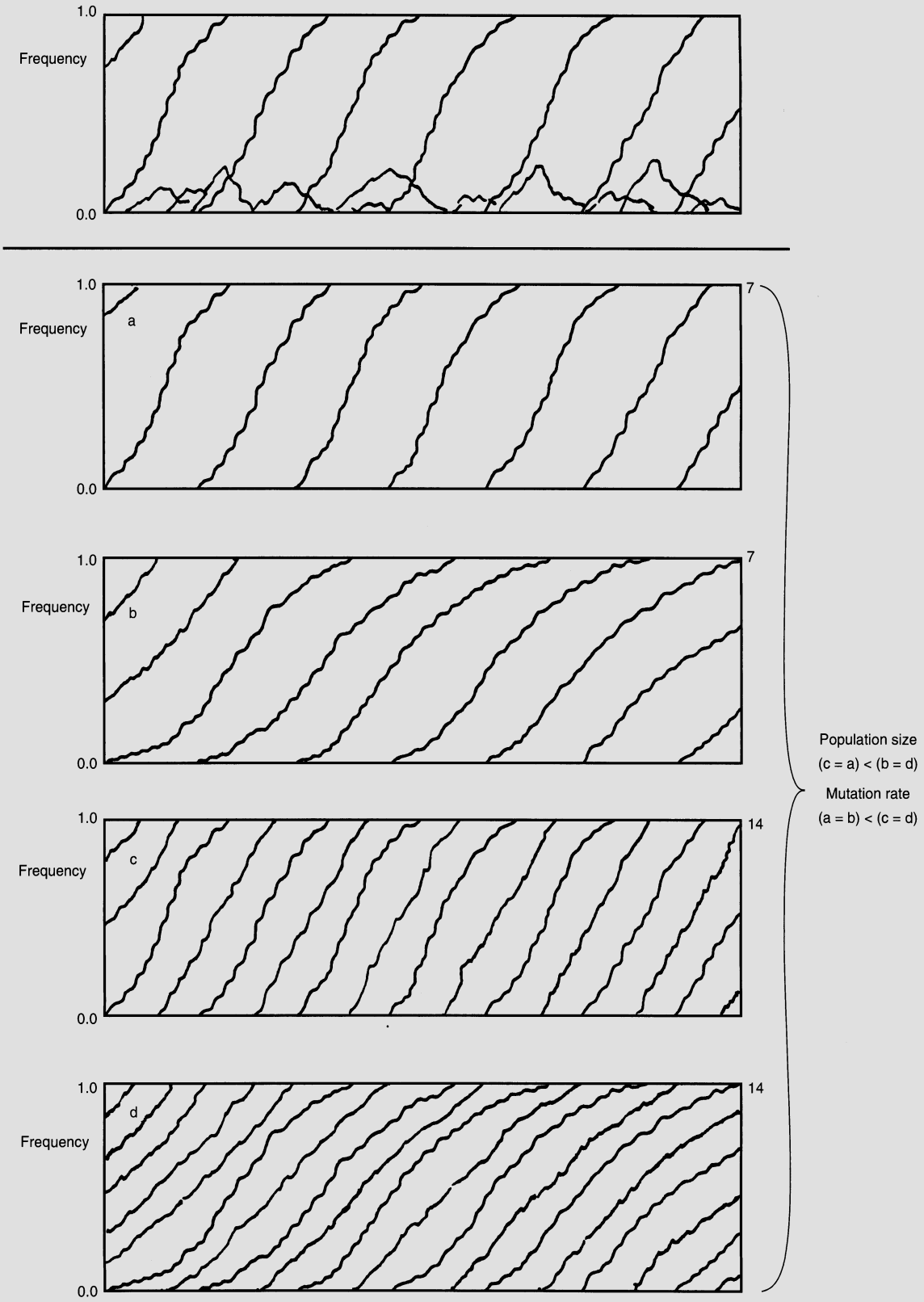


FIG. 3.2. Diffusion and fixation of neutral mutations in a population. Fixation of mutations is proportional to the rate of mutation. Fixation of mutations in a given period of time is the same regardless of the population size, if the rate of mutation remains constant. Population size: (a = c) < (b = d). Mutation rate: (a = b) < (c–d), in this and other figures

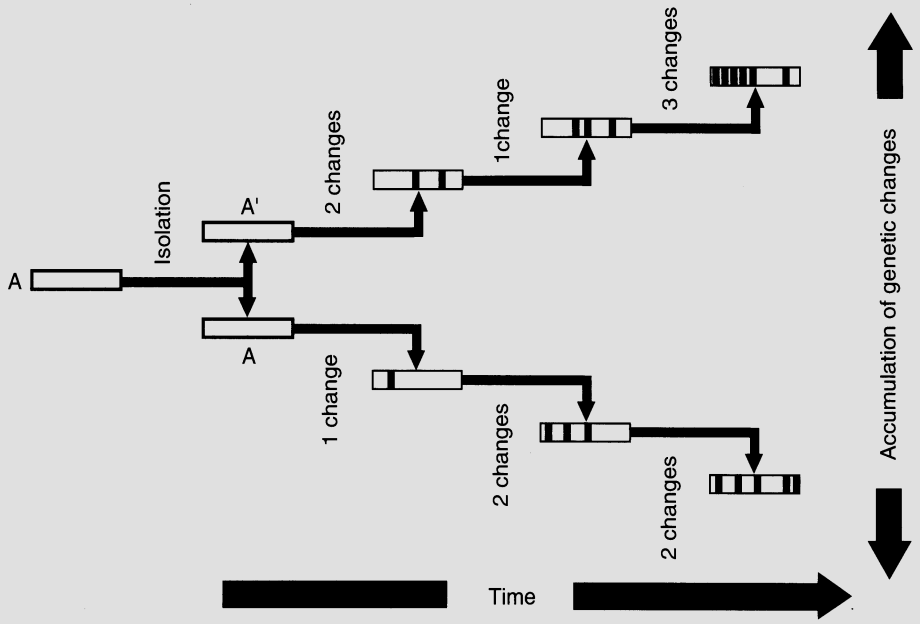


FIG. 3.3. Accumulation of genetic changes. In two lineages formed by speciation or geographic isolation, changed nucleotides accumulate independently, while the ratio of change becomes nearly the same between the two lineages. Nucleotide changes occur 5.5 times on average during the given period of time shown in the figure. The total number of changes for the two lineages is 11 (after Lewin 1997)

d). The larger the population, the longer is the time required for fixation of a mutation, but the frequency of fixation (the number of fixed mutations over time) is independent of the population size.

According to the neutral theory of molecular evolution (Kimura 1983), the average number of generations required for a neutral change to spread over a population is 4N (2N for haploid genomes such as mitochondria and chloroplasts). When the population size is 100 million or more, substitution at one nucleotide site requires 400 million generations on average (200 million for mitochondria). This means that it would take 400 million years for a neutral change to spread throughout a population of 100 million individuals in a species with a lifespan of only one year. One might think that the larger the population, the greater the number of mutations, which raises the question of why the number of substitutions is the same irrespective of population size.

The answer to this question is simple. In a population of diploid organisms consisting of N individuals, the number of alleles is 2N. Assuming that mutation frequency (μ) is per generation, the number of mutations occurring per generation is $2N \times \mu$. As the frequency of these mutations to be fixed in the population is 1/2N, the number of finally fixed mutants is $2N \times (\mu) \times 1/2 = (\mu)$. In other words, the number of mutations to be fixed within a certain period is independent from the population size if the mutation rate is constant. For example, when a population doubles in size, the number of mutations generated in one generation also doubles ($4N \times \mu$). However, the frequency (opportunity) of fixation of these mutations falls by half (1/4N), so that an equal number of mutations will be fixed in these two populations of different size.

This phenomenon is detailed in Fig. 3.2a, b, c and works on two assumptions: the frequency at which mutations become fixed is proportional to the mutation rate; and the number of mutations to be fixed in a population within a certain period is the same regardless of population size if the mutation rate is constant. Figure 3.2 schematically illustrates the fixation of mutations as occurring at equal intervals, which is calculated as a mean over a long period. Of course, the interval is actually more irregular than suggested by this calculation (Fig. 3.4).

Figure 3.3 shows the accumulation of fixed mutations after separation of a species into two lineages by reproductive or geographic isolation. Mutations, which randomly occur on every nucleotide site, are shown by black bars, each located at a different site, because the probability of two or more mutations occurring on one site is quite low. Even if mutations take place at somewhat irregular intervals between the two lineages within a short period, the number of mutations is approximately equal over a long period of time. In other words, the molecular clock is ticking, on average, at the same pitch in both lineages. As already mentioned, such a relationship holds only when the frequency of mutation is the same in both lineages. If, for some reason, the rate of mutation increases in one lineage, the molecular clock principle can no longer be applied.

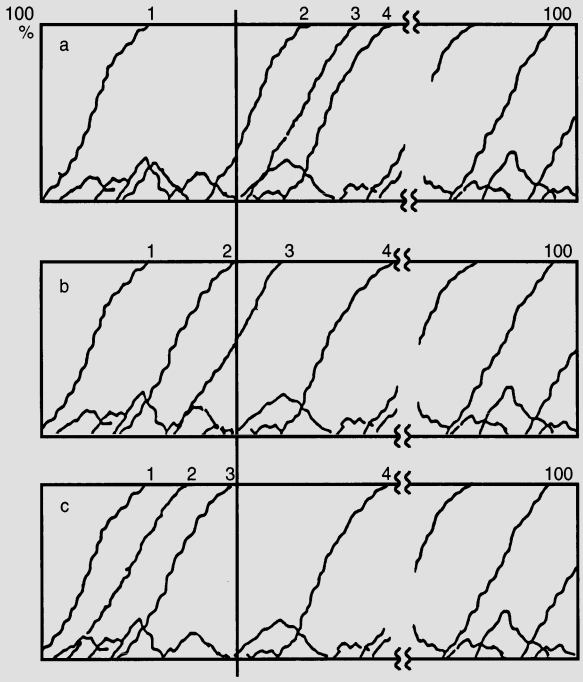


FIG. 3.4. Fixation of mutants showing the initial stage

3.5 Collection and Preservation of Specimens

The first step in undertaking phylogenetic analysis using DNA is the collection of the necessary specimens. In most cases, dry specimens in museums or in private collections cannot be used. This is because the DNA has often, in such cases, been fragmented or become compromised by the combination of inhibitory substances. We intended to sequence the mitochondrial DNA of the holotype specimen of *Damaster blaptoides* preserved in the Natural History Museum of Wien to find its type locality, because the mitochondrial *ND5* DNA sequence is locality-specific (see Chapter 7, pp. 128–137). We hypothesized that the specimen's type locality was somewhere in Kyushu. Despite the fact that the specimen was collected nearly 160 years ago, the muscle tissue from which the DNA was extracted appeared to be in good condition. Our attempt to analyze the DNA sequence was unsuccessful, however.

It is recommended that insect specimens used be killed in ethanol or acetone solution of more than 70% and preserved in this solution in a refrigerator or freezer. It is also wiser to use specimens that have been collected recently. Insect specimens in this state or DNA extracted from such specimens is stable for at least 10 years if kept in ethanol. Insects killed in ethylacetate, which many insect collectors prefer, or those preserved by freezing alone do not provide good results.

DNA already plays a decisive role in the study of insect phylogeny and taxonomy and will become increasingly important in future, leading us to recommend that whole or partial specimens be preserved in ethanol. A dry sample of a particular insect along with a sample of the species DNA will soon come to be regarded as constituting a complete specimen set.

3.6 Some Problems Associated with the Construction of a Phylogenetic Tree

We have chosen not to discuss here the details of the analytical methods and procedures involved in construction of a phylogenetic tree because many manuals describe these techniques at length. We will instead focus on points not raised in the majority of publications covering this subject.

One question that arises concerns the number of gene species necessary for construction of a phylogentic tree. One gene species is sufficient if the gene species used fulfills the requirement for a molecular clock. Even if several gene species are analyzed, the phylogenetic tree resulting from this effort will not be sufficiently sound if at least one of these genes does not meet this requirement. It is, therefore, important to pick an appropriate gene or segment of DNA that will be sufficient when setting out to construct a phylogenetic tree.

The DNA region to be sequenced is first amplified using the PCR method with appropriate primers, and the sequences of the amplified DNA to be referred to are then determined. The DNA sequences obtained in this way from individuals of the same species or those of different species are then aligned so as to adjust the homologous nucleotide site to take on comparable positions in a multiple alignment of sequences. Based on this alignment, an evolutionary distance matrix is then prepared and a phylogenetic tree is constructed using an appropriate method (see below). The bootstrap test (Felsenstein 1988) is then performed to estimate the reliability of the node supporting each branch of the tree (see below). All these processes can be completed simply by following the steps in one of the manuals that are readily available.

While these steps make it possible to produce a phylogenetic tree, many researchers find it difficult to properly analyze the tree produced due to a lack of understanding of some of the fundamental areas of knowledge outlined earlier in this chapter. An outline of the key areas in which understanding is required to properly analyze a phylogenetic tree is provided below. It is also important that the sequence data be registered with an appropriate databank, such as DDBJ, EMBL, or GenBank, so that other researchers may examine the results or use them in other studies. Any scientific paper lacking registered sequences is similar to a description of a new species without a designation of the type specimen.

3.6.1 How to Construct a Sound Phylogenetic Tree

To construct a phylogenetic tree of a certain insect group, a researcher should first take three or more distantly related species (species *a*, *b*, and *c*) and determine the nucleotide sequence of the mitochondrial cytochrome oxidase subunit 1 (*COI*) or *ND5* gene, to provide one example. The longer the gene sequence, the higher the reliability of the phylogenetic tree is likely to be. A sequence of less than 500 bp (base pairs) in length would be too short to obtain a reliable result. About 1,000 bp or more is more likely to provide the kind of results desired. A sequence of about 2,000 bp in length would increase reliability by 10%–20%. Once a nucleotide sequence of sufficient length has been determined for all of the species to be compared, the sequences for species *a*, *b*, and *c* should be aligned and compared for differences. If the difference is less than 1% or more than 15%, the gene or gene region being referred to is not appropriate. If the difference is too small, the possibility of considerable statistical error arises, as does the problem of unfixed nucleotides (sequence polymorphism; see below). If the difference is too great, the frequency of multiple substitutions at a single site increases to the point where it is impossible to correct (see below).

When we look at the fixation of mutants at the initiation stage (over a short period), the fixation intervals are irregular, as shown in Fig. 3.3. Viewed over a longer period, however, the average number of fixed mutations will reach the expected level. An additional problem is presented by the existence of sequence polymorphism arising from unfixed mutations, which create noise that makes accurate analysis of the sequence being referred to difficult.

To give an example, let us assume that the number of fixed mutants will be 1, 2, and 3 in species *a*, *b*, and *c*, respectively. If the difference between *a*, *b*, and *c* (or any two of these species) were to register as 20%, this would be too high a figure for accuracy. As noted above, mutations occur randomly at every nucleotide site, and the probability of mutations occurring at the same site (multiple substitutions) increases over time. In other words, the same nucleotide site will likely undergo mutations more than twice, but such multiple mutations are counted as one nucleotide substitution. A formula to correct these multiple substitutions (e.g., Kimura's method) is available in the form of a computer software package.

However, the formula is limited by the fact that it is unable to correct for too great a number of multiple substitutions. In cases where there is a considerably high number of multiple substitutions, some other DNA regions or genes with a lower substitution rate should be used. The method commonly used for correcting for multiple substitutions is shown in Fig. 3.5 and also is discussed in more detail in the next section.

3.7 Evolutionary Distance

Evolutionary (or genetic) distance refers to the percent of sequence difference between two or more samples taking the directionality of the codon change and multiple substitutions into account. Jukes-Cantor's formula (Jukes and Cantor 1969) or Kimura's two-parameter method (Kimura 1980) are widely used to estimate evolutionary distance. The evolutionary distance obtained with Kimura's formula is abbreviated as *D*. Roughly speaking, 0.01 *D* between two sequences corresponds to a difference of about 1%. The *D* value is not the same between different DNA regions, different gene species, or even between the same gene of distantly related organisms. As shown in Fig. 3.6, it is possible for *D* between *a* and *b* to be 0.04 for gene I, 0.03 for gene II, and 0.02 for gene III. Assuming that the mutation rate (not the fixation frequency) is the same for both *a* and *b*, the differences in *D* between I, II and III can be ascribed to the difference in the number of neutral sites and the magnitude of freedom to change. Remember that family box codons have a greater degree of freedom to change than those of 2-codon sets. This means that the more family box codons used in gene I, the greater is the freedom to change, resulting in a higher *D* value for gene I than for gene II. In other words, gene III as shown in Fig. 3.6, has more nonneutral codon sites that are removed upon the occurrence of deleterious mutations than does gene I or II. Therefore, the *D* value of gene III is below that of the other two, genes I and II having fewer nonneutral sites. It is clear, then, that a comparison of species *a* and *b* using different protein genes, or different regions of the same gene, is meaningless.

It is often said that the higher the *D* value, the faster the rate of mutation. This is somewhat misleading, however. What we have described above is concerned with how many neutral mutations occurring at the individual level are fixed when the mutation rate is constant. In other words, *D* does not necessarily reflect the actual mutation rate at the individual level, because the number of deleterious mutants to be removed is variable from one gene to another.

As *D* denotes the sum of the lengths of the two branches separated from a common ancestor, the scale inserted into a phylogenetic tree is usually expressed as one half of the *D* value.

3.8 Correction of Observed Nucleotide Substitutions

To know the degree of saturation arising from multiple nucleotide substitutions, we plot the observed differences between all pairs as a percentage against *D* for the respective pairs. Figure 3.5a shows that the percentage difference and *D* maintain a linear relationship up to

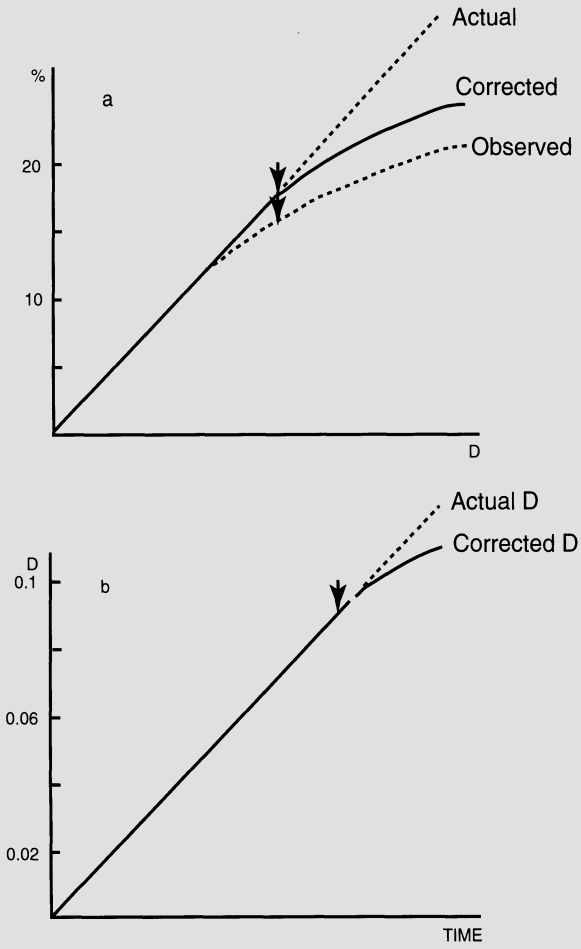


FIG. 3.5. Correction of multiple substitutions

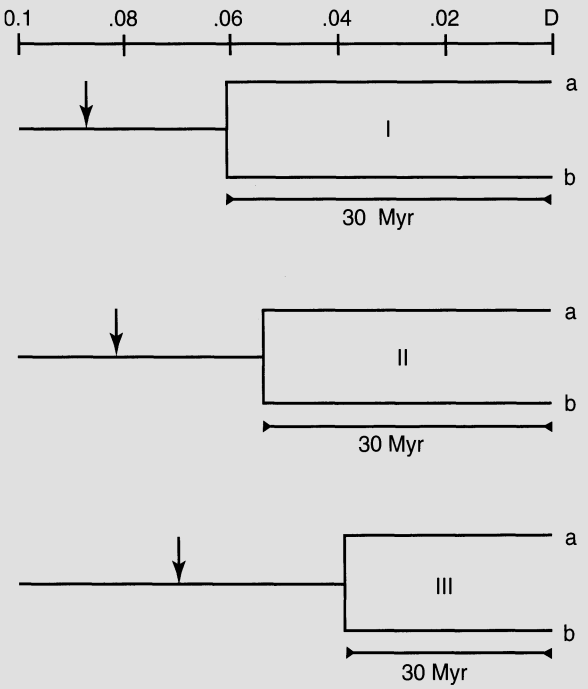


FIG. 3.6. The separation patterns of two species using three genes differing in amino acid composition. *Arrows* indicate the saturation point

the point indicated by the arrow. The linearity after the arrow results from correction for multiple substitutions. Thereafter, linearity no longer holds because it is no longer possible to correct for multiple substitutions. It is preferable to use the linear portion of this comparison when undertaking a phylogenetic analysis. We have noted that the usable maximum nucleotide difference in a group of organisms is about 15%. This value is only approximate, increasing or decreasing depending on the gene used. This value is higher for a gene that has a higher degree of family-box codons than does a gene where 2-codon sets predominate. This procedure is aimed at assessing the correctable range of multiple substitutions, and time scale is not taken into account. If one can plot *D* against time (Fig. 3.5b), chronology can be incorporated into a phylogenetic tree (see below).

When all the genes examined reveal uncorrectable saturation, there are several possible approaches that may be helpful. One approach involves the use of substitutions of non-synonymous codons, i.e., amino acid replacements. If *D* values obtained in this way are proportional to the observed replacements, this approach will work. Amino acid replacements have frequently been used for distantly related organisms but it is not clear whether they are useful for closely related insect groups, such as a certain carabid group, because of the much smaller number of neutral amino acid substitutions as compared with silent nucleotide substitutions.

When gene analysis of a particular organism reveals that the *D* value is too small, or is saturated, it is common to use another gene with a "faster" evolutionary rate, or one with a "slower" rate. This approach is faulty, however, because mutation frequency (not fixation frequency) in all mitochondrial genes are approximately (but not exactly) the same in the same organism. Therefore, the magnitude of "noise" is the same in both a "faster" gene and a "slower" gene, and both these genes reach saturation at the same time.

Figure 3.6, which shows the *D* value of a "faster" gene is larger than that of a "slower" gene, illustrates this. Although the resolution of branching relationships in the tree using a "faster" gene is somewhat better than that in which a "slower" gene has been used, there is no essential difference between them.

The mutation rate of nuclear genes is usually much slower than that of mitochondrial genes, and thus some of the nuclear genes might be used when saturation is observed in mitochondrial genes. However, the process from mutation to fixation is much more complex in nuclear genes than in mitochondrial genes, so that

sometimes the molecular clock does not work well. As noted above, the appropriate range of nucleotide substitutions lies between >1%–15%.

The magnitude of nucleotide substitutions in the *ND5* or *COI* gene is 4%–6% within a genus, 10% between the genera, and 15% between the tribes for the Carabinae ground beetles, indicating that these genes fit the requirements for construction of a phylogenetic tree. The substitution rate of the nuclear genes is much slower than that of mitochondrial genes, and, in addition to the complex fixation process, is generally not appropriate for the phylogenetic analyses of a small group such as the Carabinae. It must be kept in mind that mitochondria are female-inherited, so that the mitochondrial DNA of the hybrid individual is of the maternal type. In such cases, a supplementary analysis using a nuclear gene may be required.

In summary, to construct a reliable phylogenetic tree, one must carefully check whether the difference in nucleotide substitutions is too small, or is beyond saturation level.

3.9 Deletions and Insertions

Once the nucleotide sequences are obtained and aligned, it is desirable not to include any deletion or insertion in the sequences. In most cases, there are no deletions/insertions in the protein genes, because even one deletion/insertion causes a change in the subsequent amino acid sequence of the protein, the in-frame appearance of a stop codon, or the disappearance of the stop codon from the proper site (Fig. 3.7).

Deletions/insertions (gaps) are artificially introduced by hand or by computer to align the sequences, but in reality there are no gaps in the DNA molecules. When one finds deletions/insertions, this is most likely the result of a misreading of the sequences (except in the case of deletions/insertions of a multiple of three). Neither the human eye nor a computer-based DNA sequencer is always 100% reliable.

DNA sequences other than those of the protein genes, such as rRNA genes or spacer regions, often require the introduction of gaps because of the occasional presence of deletions/insertions. In such a case, the sequences should be very carefully aligned to adjust the homologous sites so as to obtain a reasonable phylogenetic tree.

There is no consensus as to the treatment of deletions/insertions; with some regarding a deletion or an insertion as one change, while others do not count this change. Sometimes, there exist two or more consecutive deletions/insertions, which are counted as one change by some researchers. Regardless of this, the fewer the deletions/insertions, the more reliable the phylogenetic tree.

3.10 Nucleotide Composition of DNA

Codon usage is not "symmetrical," in other words, two synonymous codons in a 2-codon set, or four in a family box, are not evenly used. This means that the usage of silent codon sites is uneven, sometimes even extremely uneven in any gene in a single species. The main factor affecting codon usage is the genomic G+C content of the organism.

Many species of insect show wide differences in their G+C content in both nuclear and mitochondrial DNA. It is likely that directional mutation pressure affects the

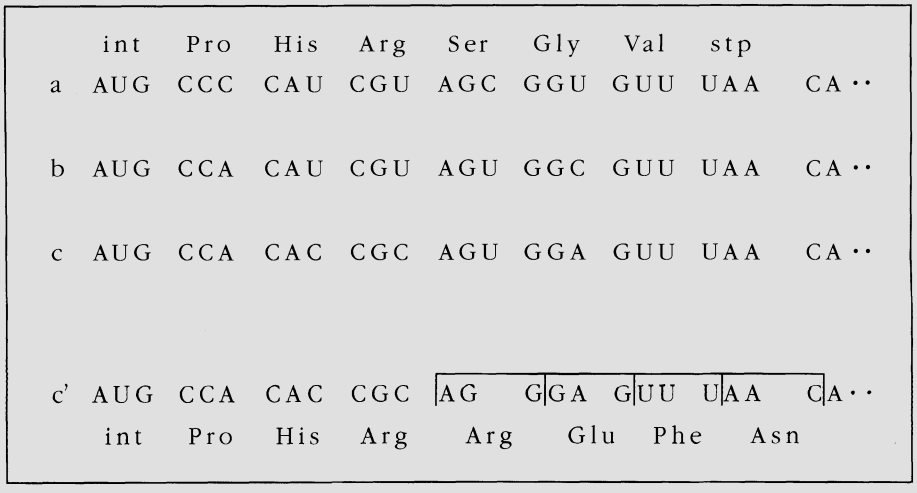


FIG. 3.7. Alignment of nucleotide sequences. Met is an initiation codon when it is at the initiation site, while it is read as Met within the reading frame. The amino acid sequence does not change only through mutations of synonymous nucleotides (a, b, and c). When the 25th U is deleted from c, the amino acid sequence is thereafter altered and a stop codon disappears as shown in c'. **int**, initiation codon; **stp**, stop codon

G+C content and that the magnitude of this pressure varies among various insect groups. An increase or decrease of G+C content results from a change in the mutation rate. If a species (or an insect group) has a relatively high GC or AT content in the DNA, the mutation rate will be at variance with that of other species, or some mechanism(s) maintaining a higher GC or AT is in operation.

The difference in G+C/A+T content includes the change in the mutation rate either at present or in the past. The molecular clock will not work at the same rate in organisms with different G+C/A+T contents. Therefore, to construct a reliable phylogenetic tree, an insect group with a uniform G+C content should be used.

The overall G+C content of the insect mitochondrial *COI* gene is higher than that of the *ND5* gene. This is unrelated to the mutation rate, because the G+C content of the silent sites is the same in both genes. The higher G+C content of the *COI* gene is a result of the predominant use of GC codons (rich in G or C for amino acid replacement sites; CCX (Pro), GCX (Ala), CGX (Arg), and GGX (Gly); X: A, G, C, or U).

The G+C content of the *ND5* gene varies considerably—from 45%–85%—among various groups of beetles. For example, the G+C content is nearly constant in carabid beetles (79 ± 1%), and in some groups of cerambycid beetles (85%). Therefore, "an *ND5* phylogenetic tree" containing carabid beetles and cerambycid beetles together does not have much meaning. In such a case, we do not know whether another nuclear gene might be usable for the purpose. Indeed, there is no ideal gene that meets all the necessary requirements for construction of an absolutely correct phylogenetic tree.

3.11 Phylogenetic Tree

Based on the multiple alignment of the nucleotide sequences of a group of insects, one can construct a phylogenetic tree using the NJ-(neighbor-joining) method (Saitou and Nei 1987), the UPGMA (unweighted pair-group method using an arithmetic mean), the MP-(maximum parsimony) method, or the ML-(most likelihood) method (Kumar et al. 1993). Details on these methods are available in various manuals.

Methods such as UPGMA have been much criticized, with critics claiming that other methods like MP should be used. This argument is shortsighted, however, because factors affecting the construction of a phylogenetic tree are not the same for all organismal groups, so that a certain method fits one group, while it is not always right for other groups.

In the NJ-method, a phylogenetic tree in which the sum total of the evolutionary distances is kept to a minimum is considered the best model. In a tree of this kind, the difference in the evolutionary rate may be estimated by the length of each branch. The tree is rootless, so that a root should be created by taking related organisms into account as outgroups. In trees created using this method, the terminus of each branch (the position of each descendant species) does not form a straight line. A species revealing too long or too short a branch from the terminus includes a considerable difference in the evolutionary rate of DNA, and should be omitted in the interest of avoiding an overall deformation of the tree.

A UPGMA-tree is also constructed using an evolutionary distance matrix, in which two species whose evolutionary distance is the shortest are paired first, followed by the addition of the next-shortest species to the first pair. This procedure is repeated until the full tree is completed, with a root and the termini of all species in a straight line. This method is based on the assumption that the evolutionary rate is constant throughout. There are, however, a number of cases in which the evolutionary rate is not the same. In such cases, the UPGMA approach cannot be used.

The MP-method is useful to estimate the branching order, though the evolutionary distance is missing so that the length of the branches have no meaning. This method as well as the ML-method involve a huge amount of calculation; with the most plausible tree selected from about 200,000 possibilities.

When the trees obtained by all of these methods reveal an essential agreement, this suggests high reliability. Generally speaking, however, the trees produced will not agree in every detail. The UPGMA (as well as all other methods) can be used for the mitochondrial *ND5* gene of the carabid beetle, because of the near-constancy of the evolutionary rate (see Chapter 4, p. 25).

3.12 Bootstrap

To evaluate the reliability of each branching in the phylogenetic tree, the bootstrap method (Felsenstein 1988) is routinely used. Details on this method are available in various manuals. It is generally said that the branching is reliable when the bootstrap percent is above 95%, and is unstable when it is less than 70%. The bootstrap value is generally shown at the position of each branching point. It should be noted, however, that the process of the construction of a tree is not taken into account in a bootstrap analysis; a high bootstrap value is meaningless if there is a misreading of sequences, inadequate treatment of insertions/deletions, and/or alignment errors.

When the branching profile is kept essentially unchanged by replacing the outgroup species, or adding to or removing some species from the tree, reliability may be reasonably high even if the bootstrap value is not very high.

3.13 Dating

If DNA to be used for construction of a phylogenetic tree works as a molecular clock, and the evolutionary distance per year is known, one can set the time scale in the phylogenetic tree. This is a rather difficult task, because the evolutionary rate is not always constant as revealed by the difference in the branch length in the NJ-tree.

Difficulty also lies in the fact that the evolutionary distance per year is in most cases unknown. Fossil records are useful to some extent, if they can be combined with the evolutionary distance. However, there is no guarantee that a fossil species in problem did not inhabit before its discovery. When the time of isolation between two or more species or races divided by a geographic barrier is known, the evolutionary distance between them can be used to set the time scale.

Chapter 4
Phylogeny and Distribution of the Subfamily Carabinae

4.1 DNA of Carabid Beetles Used in this Study

The samples collected and used in this study cover almost the entire distribution range of carabid beetles (Fig. 2.1). Specimens come from more than 500 locations in about 35 countries. The samples represent more than 90% of the carabid genera and include about a half of the entire carabid species so far discovered.

Unless otherwise noted, DNA used in this study is the mitochondrial genome containing 1,069 bp of the *ND5* gene, which was amplified using the primer set shown in Fig. 4.1. In some cases, as in that of the Cychrini species, 1,059 bp of the *COI* gene was used in place of the *ND5* gene. The primers for this are also shown in Fig. 4.1. Because the genetic map of the mitochondrial DNA of carabid beetles has not yet been determined, the map of *Drosophila yakuba* is illustrated in Fig. 4.2 to indicate the locations of the genes used in this study.

In addition to these two mitochondrial genes, nuclear rDNA, internal transcribed spacer (ITS) (Figs. 4.1 and 4.3), and treharase gene (shown later, in Fig. 4.9) were used as supplements when necessary.

4.2 Dating

Because a lack of chronology greatly restricts the researcher's scope to interpret the phylogenetic data, the base substitution rate of the *ND5* gene was calculated in conjunction with several geographical data.

In one example, two races of the carabid *Eulepto-carabus porrecticollis kansaiensis* in the Kinki region of Japan are separated by the Yodogawa River–Biwako Lake line, which took form 3 MYA (Yokoyama 1973; Takemura 1985). This shows that these two races started to diversify once the river was formed. A calculation of the evolutionary distance (*D*) between these two races using Kimura's method produced a value of 0.0076 ± 0.0011.

Another example is seen in paleomagnetic evidence indicating that the ancient Japanese Islands split from the eastern periphery of the Eurasian Continent into the northeast arc and the southwest arc about 15 MYA (Otofuji et al. 1991, 1994). The carabid *Damaster blaptoides* is endemic to Japan and would have begun diversifying from the proto-*Damaster* of the continent upon this separation around 15 MYA.

The diversification of *Damaster* began with separation of the eastern (E) lineage and the western (W) lineage. The *D* value between E and W is 0.042 ± 0.0029 (Su et al. 1998). The diversification of *Ohomopterus*, the other carabid group endemic to Japan, started a little later (*D*/lineage = 0.0394 corresponding to 14.2 million years (MYR); see Chapter 7, p. 103) than that of *Damaster*. A 9-MYR-old fossilized example of *Ohomopterus* (Hiura, 1965) is consistent with the mitochondrial dating, at about 14 MYA.

A third example is provided by the two distinct races of both *Phricocarabus glabratus* and *Tomocarabus convexus*, separated by the Alps, which took form about 20 MYA. The two races of each species have thus been isolated for about 20 MYA. Their *D* values are 0.065 ± 0.0012 and 0.051, respectively.

Another example is offered by *Apotomopterus sauteri*, which is found in both southeastern China and Taiwan. Taiwan split from mainland China 25–20 MYA (Jahn et al. 1976), and the *D* value between the mainland and Taiwanese populations is 0.062. The relationship between chronology and evolutionary distance shows that an accumulation of nucleotide substitutions increases in an almost linear fashion over time (Fig. 4.4), suggesting a near-constancy of the base substitution rate throughout the carabids.

Figure 4.4 shows that a 0.01 *D* unit corresponds to 3.6 MYR for the *ND5* gene. A 0.01 *D* for the *COI* gene was calculated to correspond to about 2.7 MYA by comparing the *D* of the *COI* genes from six Cychirini species with the *D* of the *ND5* gene from the corresponding species (Su et al. 2003c). It should be noted, however, that this value can be applied neither to other insects nor to other genes. The above observations enable us to use the UPGMA for construction of a phylogenetic tree using the *ND5* gene sequence. In fact, analyses using the UPGMA, NJ-, MP-, and ML-methods yielded phylogenetic trees with essentially the same topology.

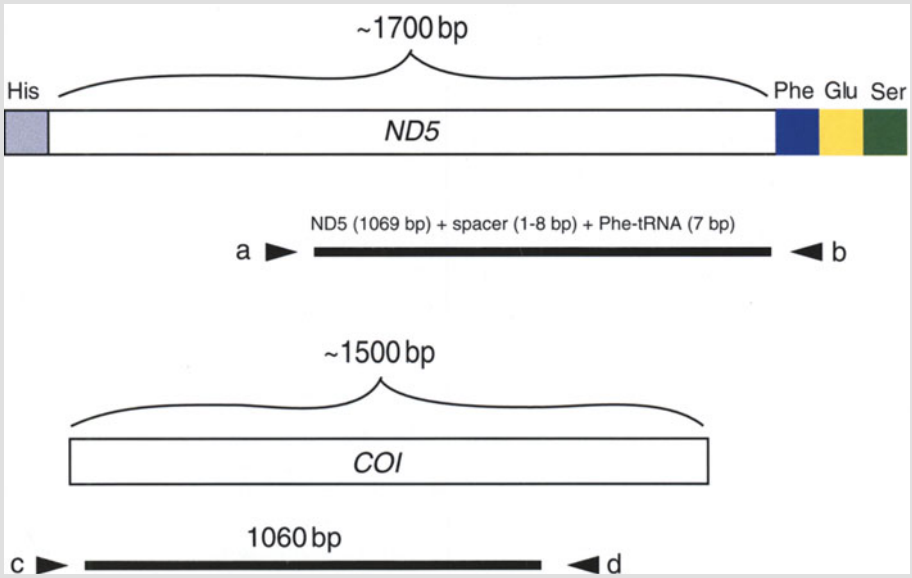


FIG. 4.1. The regions of the *ND5* and *COI* genes used for sequence analyses. *Black bar* indicates regions used for amplification by PCR and for sequencing. **a** V1.61: 5′-CCT GTT TCT GCT TTA GTT CA-3′; **b** V1.04–4: GTC ATA CTC TAA ATA TAA GCT A-3′ (after Su et al. 1996); **c** SKCOI-7: 5′-CGC TCT AGA ACT AGT GGA TCA CNA AYC AYA ARG AYA TYG GNA C-3′; **d** KSCOI-2: 5′-TCG AGG TCG ACG GTA TCA CRT ART GRA ART GNG CNA CNA CRT ART A-3′

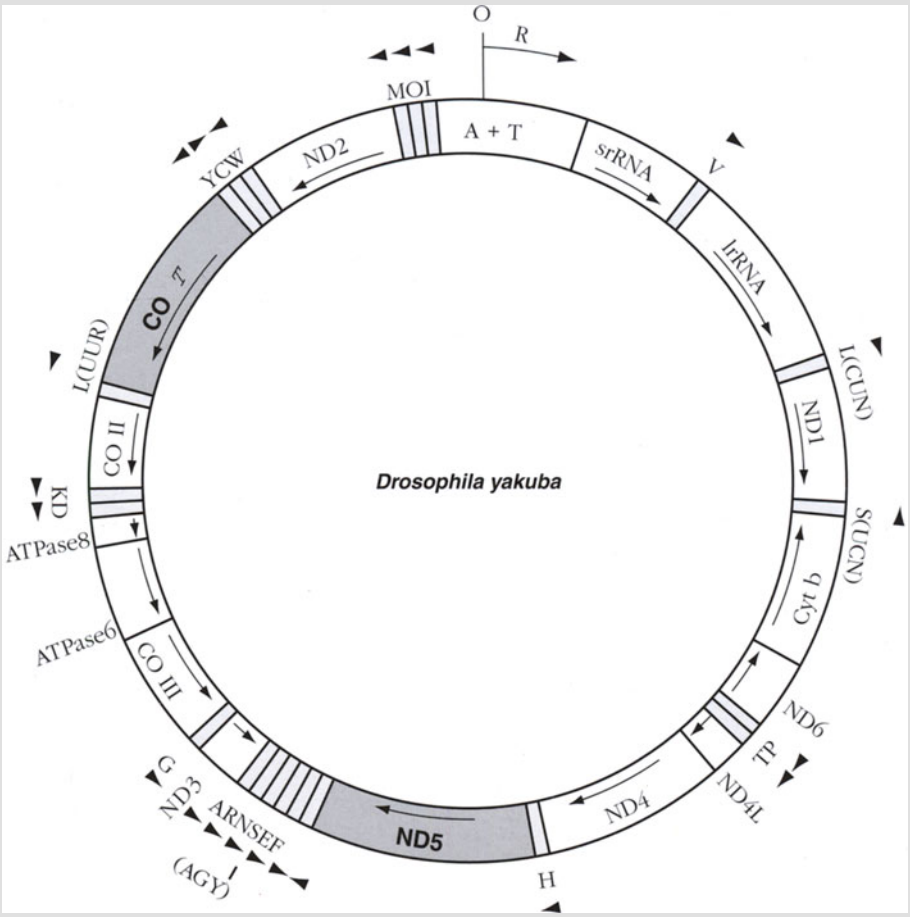


FIG. 4.2. The genetic map of mitochondrial genome of *Drosophila yakuba* (after Clary and Wolstenholme 1985)

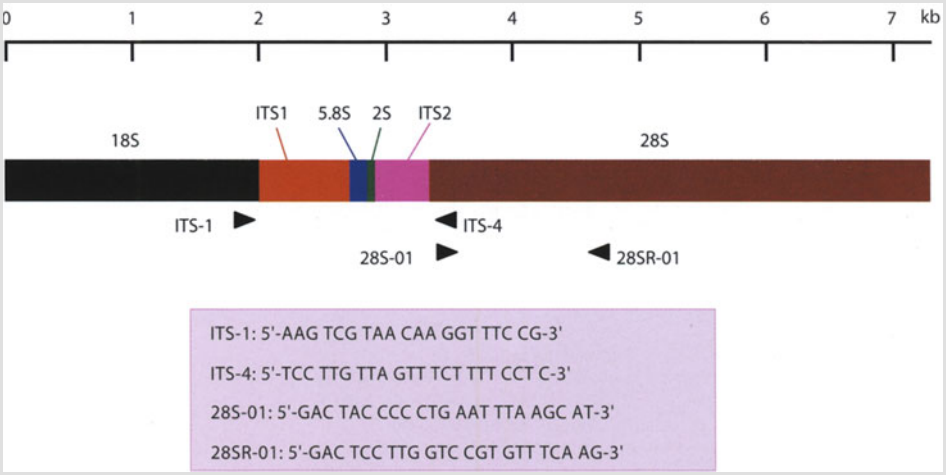


FIG. 4.3. The nuclear ribosomal DNA region of *Drosophila melanogaster* (after Tautz et al. 1988) and the primers used for PCR amplification and sequencing

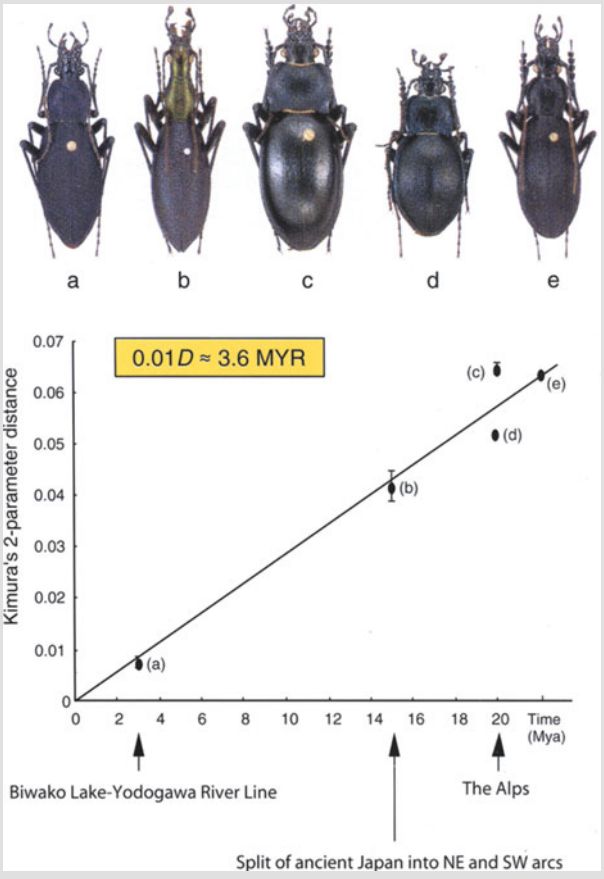


FIG. 4.4. Relationship between evolutionary distance (*D*) and chronology. **a** indicates the split of two populations of *Euleptocarabus porrecticollis kansaiensis* by the Yodogawa River–Biwako Lake line, which was formed 3 MYA. **b** indicates the split of E and W lineages of *Damaster blaptoides*, presumably by the cleavage of ancient Japan into NE and SW arcs ca. 15 MYA. **c** and **d** indicate the split of two populations of *Phricocarabus glabratus* (**c**) and those of *Tomocarabus convexus* (**d**) by the Alps, which took form ca. 20 MYA. **e** indicates the split of two populations of *Apotomopterus sauteri* by separation of Taiwan and mainland China, which occurred 20–30 MYA (not shown by *arrow* in figure). **MYA**, million years ago; **MYR**, million years (after Su et al. 2001, 2003b)

A degree of discrepancy was found in some branching orders of phylogenetic trees represented by nodes with low bootstrap values. These instabilities did not affect the main conclusions, however, because the precise branching order of the carabids on the trees was, in most cases, of only secondary importance. In this book, phylogenetic relationships are presented with either UPGMA-trees or NJ-trees, or both.

4.3 Phylogenetic Position of the Carabinae Ground Beetles in the Family Carabidae

The ground beetles treated in this book belong to the subfamily Carabinae of the family Carabidae in the order Coleoptera (beetles). The Carabidae ground

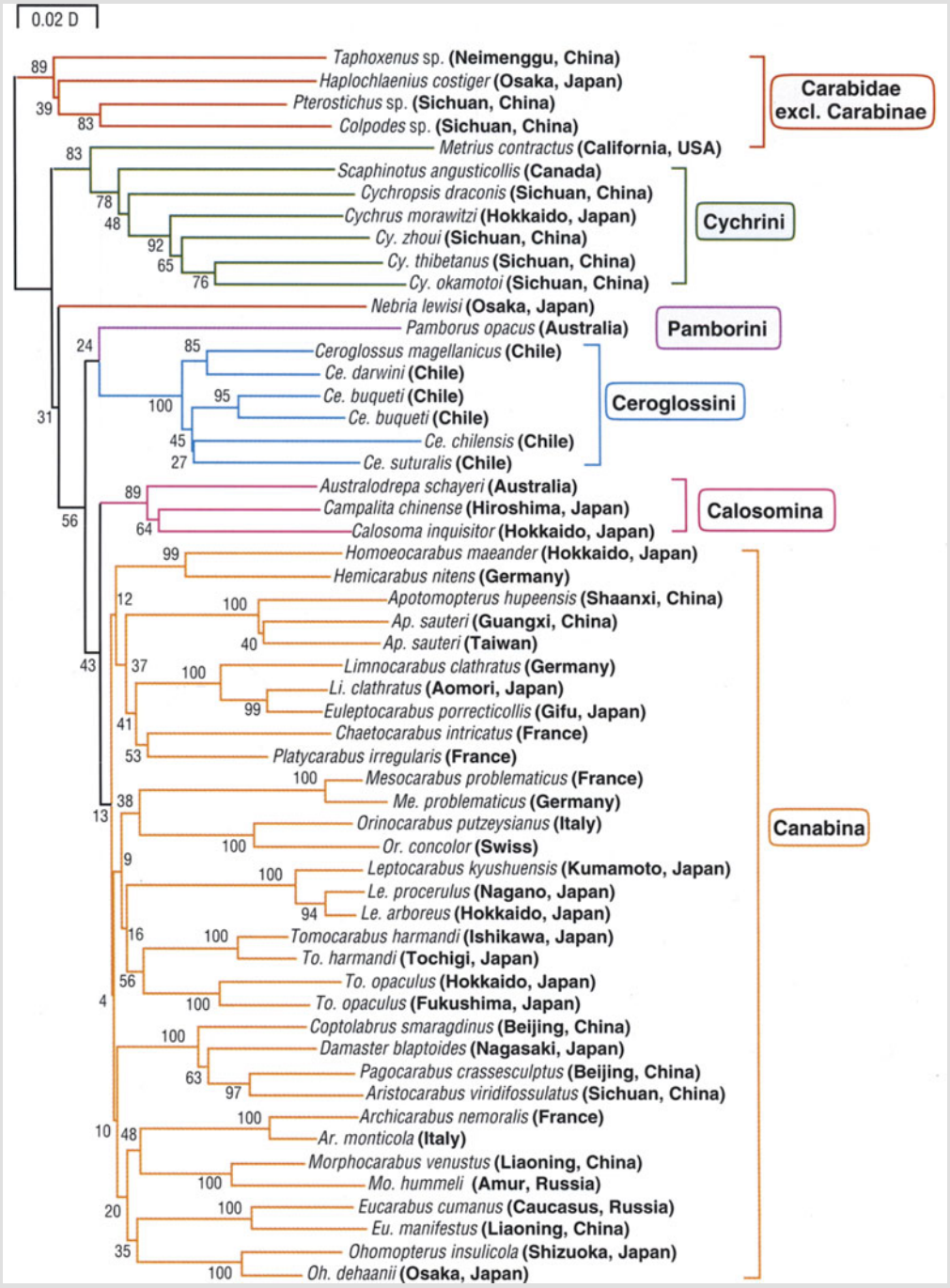


FIG. 4.5. Phylogenetic tree of the mitochondrial *ND5* gene showing the relationship between the subfamily Carabinae and the Carabidae excluding the Carabinae. Constructed using the NJ-method

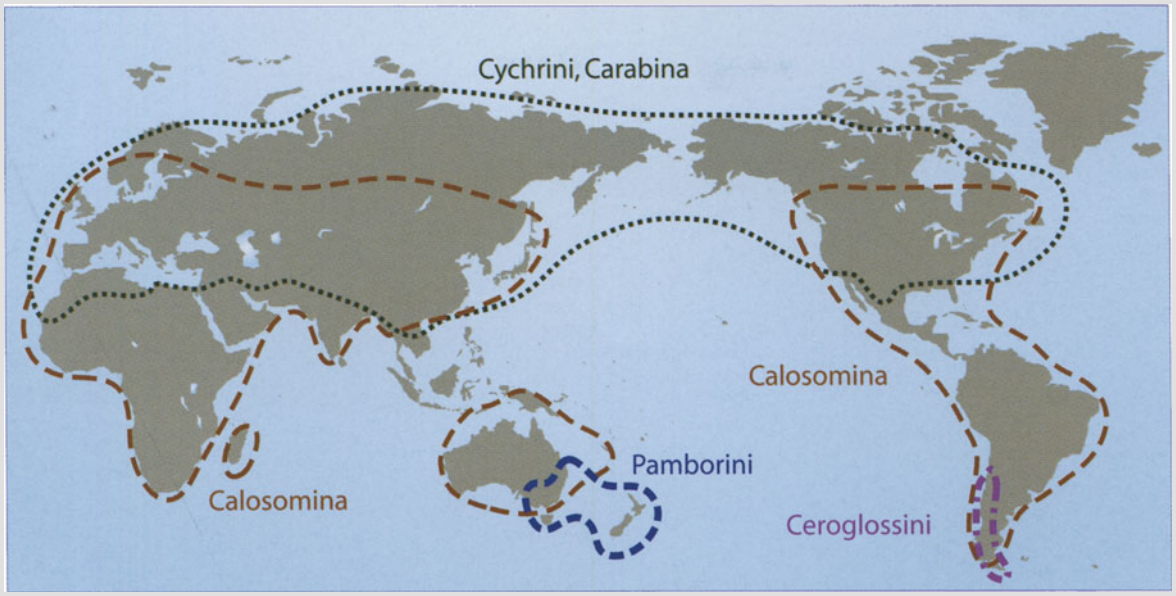


FIG. 4.6. Distribution map of tribes of the subfamily Carabinae

beetles excluding members of the Carabinae are divided into many subfamilies, the classification scheme of which differs widely depending on the taxonomist. Some authors enumerate the family Carabidae beginning with the Carabinae, Omophroninae, Nebriinae, etc. and ending with Harpalinae. Jeannel (1949) speculates that the Carabidae evolved in this order without giving convincing evidence.

The Carabidae beetles, with the exception of the Carabinae (conventionally called non-Carabinae ground beetles, or NCGB) are found almost all over the world. There are known to be about 30,000 species, and this number will likely double or triple in the near future, with many new species discovered every year by specialists. In contrast to this, the Carabinae ground beetles (subfamily Carabinae) are mainly distributed in the Northern Hemisphere.

The number of known Carabinae species is 1,000 to 1,500. Because this group of beetles has been well studied by many professional as well as amateur entomologists for many years, the total number may not greatly exceed 1,500, even if a number of new species are yet to be discovered on the Eurasian Continent, where thorough investigation has not been carried out.

As noted above, the Carabinae beetles are far fewer in number than NCGB and their distribution range is smaller, while NCGB are found throughout the world. It is not unreasonable to speculate that NCGB appeared first and have expanded their range with repeated speciation, while the Carabinae ground beetles emerged relatively recently.

The molecular phylogeny does not provide a convincing answer to this question at present, because DNA analyses of NCGB have thus far been done only for

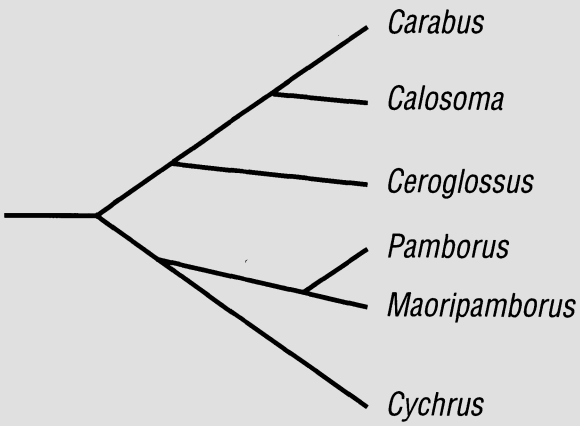


FIG. 4.7. Phylogenetic tree of the subfamily Carabinae constructed according to larval morphology (after Moore 1966)

the purpose of using them as an outgroup of the Carabinae beetles, limiting the number of species examined. The rate of nucleotide substitutions of NCGB is not known and the *D* may be saturated near the root of the tree, so that the emergence of NCGB might predate that shown in Fig. 4.5.

NCGB species examined include *Taphoxenus* from Neimenggu in northern China, *Haplochlaenius costiger* from Japan, *Pterostichus* from Sichuan, China, *Colpodes* sp. from Sichuan, and *Nebria lewisi* from Japan. All the species except *Nebria lewisi* form a single cluster, while *N. lewisi* belongs to a different line. Figure 4.5 shows that it is likely that NCGB and *Nebria* first separated, followed by the emergence of carabine ground beetles.

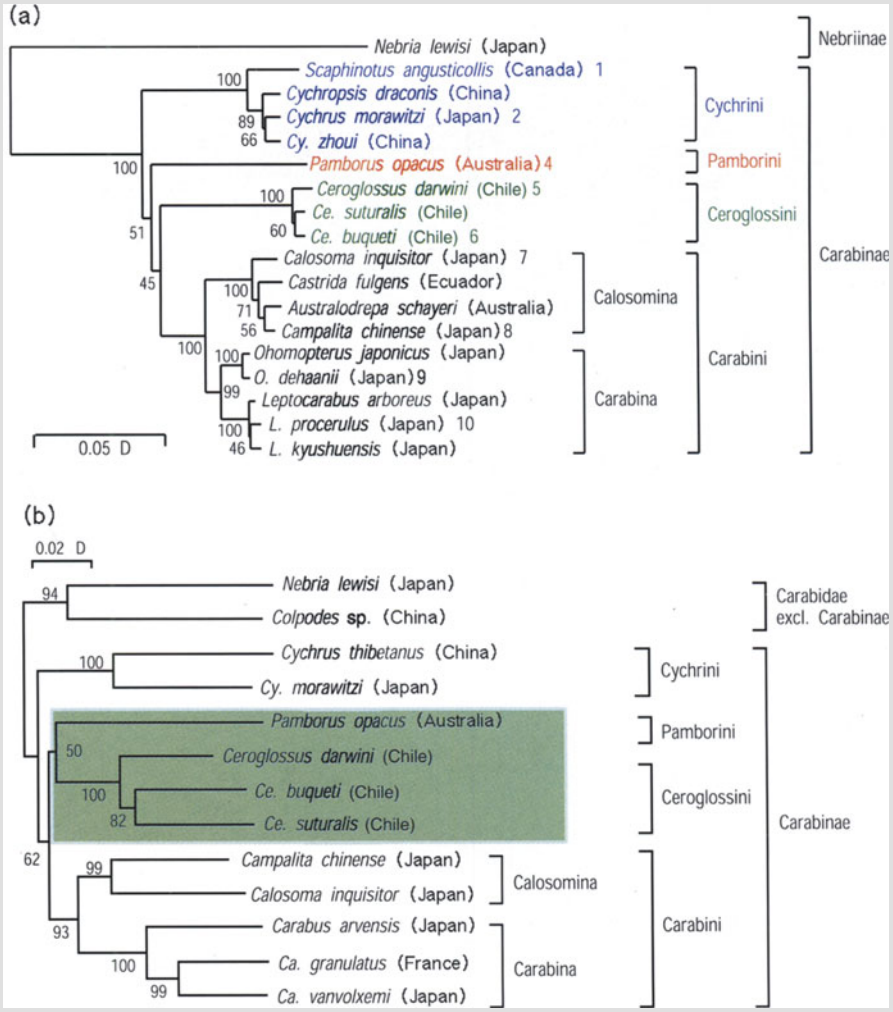


FIG. 4.8. Phylogenetic trees of the nuclear 28S rDNA (**a**) and mitochondrial *ND5* gene (**b**) for the subfamily Carabinae. Constructed using the NJ-method. *Numbers* on the photograph corresponds to those in **a**, except for number 3, which is *Pamborus alternans*, the type species of this genus (not analyzed for DNA) (unpublished)

This profile is consistent with the view that the Nebriini (to which *N. lewisi* belongs) and the Carabini can be categorized in one group. Diversification within NCGB seems to have begun much earlier than within the Carabinae, suggesting that the emergence of NCGB also took place earlier than that of the Carabinae. Thus, the molecular data contradicts Jeannel's opinion.

4.4 Taxonomy of Carabinae Based on Morphology

Taxonomically, the subfamily Carabinae are classified into two tribes, the Cychrini and the Carabini, and the Carabini are further divided into two subtribes, the Carabina and the Calosomina. In addition to the above,

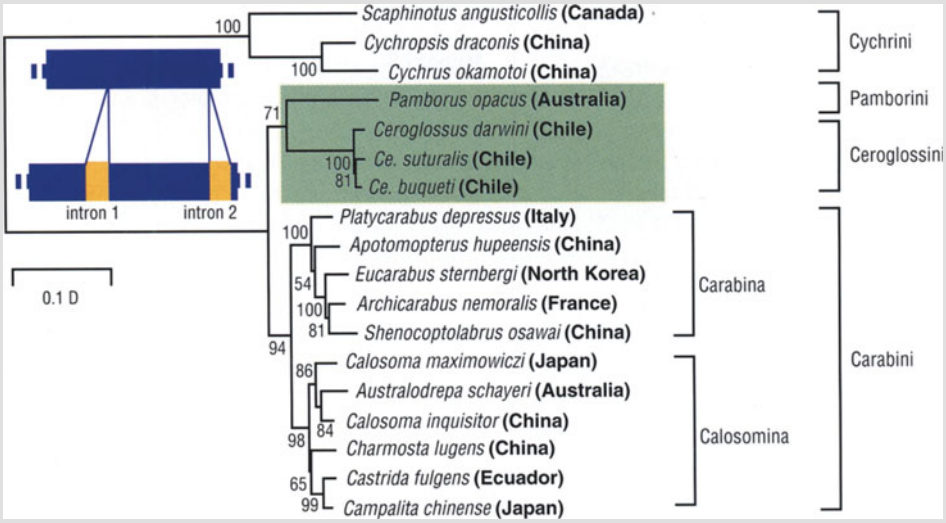


FIG. 4.9. Phylogenetic tree of the nuclear trehalase gene of the subfamily Carabinae. Constructed using the NJ-method. *Upper left figure* shows a lack of two introns within the trehalase gene in the Cychrini species (unpublished)

one carabid group, *Ceroglossus*, is found in Chile and part of Argentina (about 10 species), and other specialized carabids, *Pamborus* and *Maoripamborus* (12 species) inhabit Australia and New Zealand, respectively (Fig. 4.6). The taxonomic positions of these two groups have not been well studied. Moore (1966), from a study of larval morphology, stressed that *Pamborus* is a sister group of Cychrini, and *Ceroglossus* is clustered with the Carabini (Fig. 4.7). Kryzhanovsky (1976) established an independent tribe, known as the Pamborini.

4.5 Molecular Phylogeny of the Carabinae

Figures 4.8 and 4.9 show the phylogenetic trees of all the groups of the subfamily Carabinae inferred from nuclear 28S rDNA, the mitochondrial *ND5* gene, and the nuclear trehalase gene, respectively. From the tree, it is clear that the Cychrini consist of a distinct phylogenetic lineage as separated either from *Pamborus*, *Ceroglossus* or the Carabini when the sequence of *Nebria lewisi* was used as the outgroup. The Cychrini were always the outgroup of all the Carabini groups, *Ceroglossus* and *Pamborus* on all the trees of mitochondrial as well as nuclear genes.

This means that the Cychrini are probably the oldest group in the Carabinae, although the node of the Cychrini was supported by rather low bootstrap scores in the trees with the exception of the trehalase tree. Because diversification within the Cychrini started about 44 MYA (see Chapter 5), the separation of the Cychrini from the other carabid tribes would have taken place more than 44 MYA (Su et al. 2003c).

The trees indicate that *Pamborus*, *Ceroglossus*, and the Carabini separated a long time ago with deep branching points. In the *ND5* and trehalase gene trees, *Pamborus* and *Ceroglossus* are ambiguously clustered together. In the Carabini, the wingless Carabina and the winged Calosomina have a sister relationship. These results support the traditional classification in which the Cychirini and the Carabini are separate tribes and the Carabini are further divided into the Calosomina and the Carabina.

The trees also suggest that both *Pamborus* and *Ceroglossus* may be treated as independent tribes, i.e., the Pamborini and the Ceroglossini are equivalent with the Cychrini and the Carabini from a taxonomic point of view. These molecular data are not consistent with the suggestion by Moore (1966) that *Cychrus* shares a common ancestry with *Pamborus*/*Maoripamborus*, and *Ceroglossus* is a sister to the Carabini when deduced from larval morphologies.

4.6 Establishment of the Distribution of the Carabinae Ground Beetles

According to the chronology created using the *ND5* gene, *Ceroglossus* and *Pamborus* split about 60 MYA, which is a little later than the time at which South America, the Antarctic, and Australia separated (120–65 MYA). The separation of 60 million years between the two tribes is the minimum estimate, because the nucleotide substitutions of the *ND5* gene are more than 15% between the two tribes, suggesting the possible involvement of uncorrectable multiple nucleotide substitutions.

Therefore, the actual separation between *Pamborus* and *Ceroglossus* likely occurred earlier than had been estimated above. *Pamborus* and *Ceroglossus* are exclusive to Australia/New Zealand and Chile, respectively, and are not found anywhere else. Because North and South America split about 70 MYA and were separated by the sea until 3 MYA, it is hard to imagine that these two carabids entered South America via North America.

It is more likely that the common ancestor of these beetles inhabited the land mass consisting of ancient South America, Antarctica, and Australia, followed by isolation and differentiation into the two tribes when these continents separated.

The origins of the Cychrini, Calosomina, and Carabina are still a mystery, because it is unknown what the direct ancestry of the Carabinae is, and where it emerged. It is possible to speculate that the ancestry of the Cychrini differentiated from the *Nebria*-like ancestry somewhere in Gondwanaland, one of the two ancient supercontinents. One line that emerged from this source consists of *Pamborus* and *Ceroglossus*, and another evolved into the Cychrini and the Carabini, which acquired habitats in the southern areas of the Eurasian Continent.

When the Indian Subcontinent attached itself to the Eurasian Continent, creating the Himalayan Mountains, it is likely that the number and variety of carabid ground beetles expanded dramatically. This is a story that remains to be told in detail with convincing evidence.

Chapter 5
Molecular Phylogeny of the Carabinae

5.1 Cychrini

5.1.1 Taxonomy and Phylogeny

There are now known to be about 150 species of Cychrini which are classified into four genera. All these beetles are without hind wings and have elytra fused at suture, so that they cannot fly, having undergone considerable geographically linked speciation. The range of their distribution is restricted to the Northern Hemisphere.

Cychrini ground beetles have undergone morphological differentiation that has created variance in morphological characters between populations on the Eurasian Continent and those in the New World. Representative species of the Cychrini are illustrated in Fig. 5.1.

A phylogenetic tree of the mitochondrial *COI* gene sequence was constructed for 52 individuals representing four genera and 33 species including all the known genera found in the Eurasian Continent, Japan, and North America—areas covering almost the whole distribution range of the Cychrini (Fig. 5.2) (Su et al. 2003c). As noted in Chapter 4, the origin of the Cychrini is venerable. The diversification initiated 44 MYA assuming that 0.01 *D* of the *COI* gene corresponds to 2.7 MYR (Su et al. 2001, 2003c).

On the phylogenetic tree, a considerable number of lineages that emerged within a short time scale in the process of radiation can be identified as judged by short branch lengths with low bootstrap values. As an exception, lineage A is well separated from the others (Fig. 5.2), which are supported by high bootstrap values in the UPGMA-, NJ-, ML-, and MP-trees. The clusters other than lineage A, which are marked as B, C, and D in the trees, are probably of phylogenetically distinct lineages which are almost equivalent with lineage A, although these are supported only by low bootstrap values. This notion may be supported by the facts that, in all the trees, B, C, and D form apparently independent clusters, which are in agreement with their taxonomic classification as well as the distribution ranges (see Table 5.1). For example, lineage D consists of only the member of the genus *Cychrus*, the distribution range of which is well defined. Thus, the examined species have been conventionally classified into four lineages, A, B, C, and D, each of which may be further subdivided into several sublineages. The short internal branches around the root of the tree would not be because of saturation of nucleotide substitutions, since the actual percentage of substitutions was linearly proportional to the evolutionary distance (*D*) (Su et al. 2003c).

Lineage D4 may be further subdivided into various sublineages that emerged at almost the same period. It should be pointed out, however, that these classifications, though tentative, are linked tightly in geographic terms (Table 5.1). There is one exception, i.e., *Cychrus brezinai* which is not clustered with other *Cychrus* species (lineage D). This species forms lineage C with *Cychropsis draconis*, although they separated at a relatively ancient time (see below).

To lineage A, belong the species of the genus *Scaphinotus* from North America, to B the *Sphaeroderus* species from southern parts of North America, to C the *Cychropsis* species from China, and to D the *Cychrus* species from Eurasia, Japan, and part of North America. The diversification of these four lineages took place within a short period. Their common ancestor may have emerged somewhere in the Northern Hemisphere when the Eurasian Continent and North America were still united. The establishment of respective lineages then took place upon the split of these two continents.

5.1.2 Lineage A (Genus *Scaphinotus*)

This lineage consists of the species belonging to the genus *Scaphinotus*, and are found in Canada and the United States. The beetles in this lineage radiated about 35 MYA into five sublineages, A1 to A5. The composition of these sublineages is shown in Table 5.1.

The A1 species (subgenera *Irichroa*, *Nomaretus*, and *Steniridia*) are found in eastern parts of the US in states such as Pennsylvania and Virginia (eastern American-type distribution). The species belonging to the sublineage A2 (subgen. *Brennus*), A3 (subgen. *Neocychrus*), A4 (subgen. *Brennus*), and A5 (subgen. *Stenocantharus*) are found in western parts of Canada and the USA, in areas such as British Columbia, Washington and California (western American-type distribution).

FIG. 5.1. Representative species of the tribe Cychrini. **1** *Scaphinotus (Irichroa) viridis*, **2** *S. (Steniridia) ridingsi*, **3** *S. (Neocychrus) labontei*, **4** *S. (Brennus) interruptus*, **5** *S. (Stenocantharus) velutinus*, **6** *Sphaeroderus lecontei*, **7** *Cychropsis draconis*, **8** *Cychrus tuberculatus*, **9** *C. morawitzi*, **10** *C. aeneus starcki*, **11** *C. caraboides*, **12** *C. minshanicola*, **13** *C. stoetzneri*, **14** *C. furumii*

The morphological classification of the subgenera in lineage A is generally consistent with molecular phylogenetic lineages. The branching in A2 to A5 are older than those within A1.

5.1.3 Lineage B (Genus *Sphaeroderus*)

Lineage B consists of species belonging to a single genus *Sphaeroderus*, which show the eastern American-type distribution patterns.

5.1.4 Lineage C (Genus *Cychropsis*)

The genus *Cychropsis* contains about 20 species, which are found only in the high mountainous areas of the Himalayan Mountains and Southwest China—Xizang, Sichuan and Yunnan (Imura 2001). In this study, only one species, *Cychropsis draconis*, was examined. This lineage seems to be remote from the genus *Cychrus* (lineage D), although their distribution ranges partly overlap.

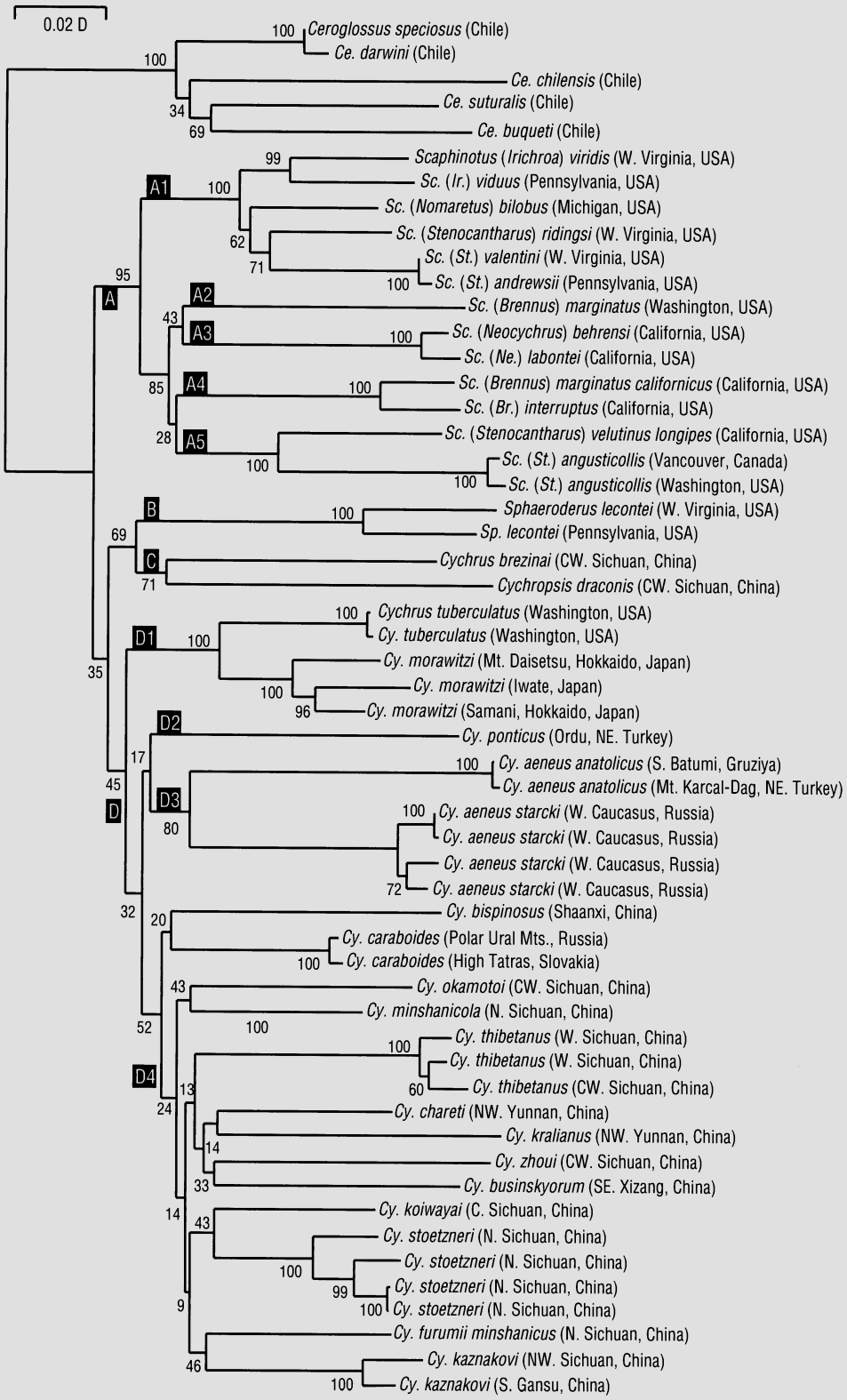


FIG. 5.2. Phylogenetic tree of the mitochondrial *COI* gene for the tribe Cychrini. Constructed using the NJ-method (after Su et al. 2003c)

TABLE 5.1. Geographically linked lineages and sublineages of the Cychrini species examined in this study

Lineage	Genus	Sublineage	Subgenus	Distribution
A	*Scaphinotus*	A1	*Irichroa, Nomaretus, Steniridia*	E. USA
		A2	*Brennus*	W. USA
		A3	*Neocychrus*	W. USA
		A4	*Brennus*	W. USA
		A5	*Stenocantharus*	Br. Columbia & W. USA
B	*Sphaeroderus*	—	—	E. USA
C	*Cychropsis*[a]	—	—	China
D	*Cychrus*	D1	—	Japan, Br. Columbia, & NW. USA
		D2	—	Turkey
		D3	—	Caucasus[b]
		D4	—	Mainly China, some in Europe

[a] See the text

[b] One species is from NE Turkey (after Su et al. 2003c)

"Cychrus" brezinai is clustered with *Cychropsis draconis*. The species *brezinai* would have most probably been misplaced in the genus *Cychrus*, because its morphology resembles *Cychropsis* rather than *Cychrus* as pointed out by Imura (2002c).

5.1.5 Lineage D (Genus *Cychrus*)

As outlined above, lineage D contains at least four sublineages that radiated within a short period about 35 MYA.

The sublineage D1 contains the Japanese species, *Cychrus morawitzi*, and the northwestern American *Cy. tuberculatus*. Interestingly, the Japanese species is more closely related to the American species than to species from the Eurasian Continent (D2 to D4).

The ancestor of the Japanese and the American species likely originated many thousands of years ago somewhere on the northeastern Asian mainland and immigrated to the ancient Japan/North American area.

Separation of the Japanese and the American *Cychrus* species is likely to have occurred by separation of these land masses when the Bering Straits were formed.

The Japanese and the American *Cychrus* are very limited in variety, with only one species having been found in the former region and 1–2 species in the latter. This situation stands in sharp contrast to the very rich *Cychrus* fauna found on the Eurasian Continent, especially in the mountainous areas of China.

The Japanese species *Cychrus morawitzi* is found mainly in Hokkaido, and also in a restricted area of the northeastern part of Honshu (Iwate Prefecture). Specimens from three localities (Mt. Daisetsu, Hokkaido; Samani-cho, Hokkaido; Genbeidaira, Iwate Prefecture, Honshu) have been examined for the *COI* sequence. The branching points of specimens found in these three localities have been calculated to be about 10 MYA, suggesting that they have been isolated geographically for a long time. Indeed, small morphological variations can be recognized among the specimens from various localities.

The Turkish species *Cychrus ponticus* is quite remote from the other species of the lineage D, thus constituting a distinct sublineage D2.

Only a single species *Cychrus aeneus*, which is found in the Caucasus region, belongs to sublineage D3. The lineage D3 consists of two clades, the separation of which took place a considerably long time ago despite the fact that morphological differences are of a merely subspecific level. This may be taken as a good example of silent or near-silent morphological evolution (see below and Chapter 8).

Most species of the sublineage D4 are distributed in the mountainous areas of China, with a few found in Europe. This sublineage is phylogenetically quite heterogeneous, and may be subdivided into at least 13 clusters that emerged in ancient times, i.e., within a short time after the beginning of the Cychrini radiation.

It is remarkable that despite the long history of *Cychrus* evolution in lineage D, fundamental morphology has not changed much (see Fig. 5.1), and each cluster constitutes a single species, without the emergence of any other species as far as can be ascertained. This may be taken as an example of silent evolution (Su et al. 2003c). Such silent phylogenetic diversification (radiation) with only limited morphological change has been reported for some groups in the subtribe Carabina (Su et al. 2001), the subtribe Calosomina (Osawa et al. 2001), and the tribe Ceroglossini (Okamoto et al. 2001). In contrast, considerable morphological diversification took place at the beginning of the Carabina radiation. Thus, the phylogenetic diversification in the Carabinae has occurred sometimes with, and at other times without, morphological differentiation. This matter will be discussed in more detail in Chapter 8.

There exist a considerable number of examples where two or more species of lineage D are sympatrically distributed, and yet their separation took place a long time

ago. In one example, *Cychrus zhoui* and *C. okamotoi* have been found in the same pitfall traps in central Sichuan, China (Imura et al. 1998c). This would imply that these sympatrically occurring species have been reproductively isolated for a long time with only a limited morphological diversification.

As noted above, the *Cychrus* fauna is quite rich in the mountainous areas of China, with more than 100 species having been found, in contrast to the limited variety found in Japan, the New World, and in Europe. This suggests that *Cychrus* may have its origin somewhere in China, from where it spread out westward and eastward about 44 MYA.

Whether the ancestry of the tribe Cychrini emerged in the ancient Eurasian region or in the ancient North American region cannot be estimated from the present phylogenetic trees alone, because the direct precursor of the Cychrini is not known.

5.2 Pamborini

As mentioned in Chapter 4, this tribe consists of the genus *Pamborus* found in Australia and the genus *Maoripamborus* found in New Zealand. Although *Pamborus* contains about 10 species, only one species, *P. opacus*, was examined for molecular phylogeny.

This species inhabits the state of New South Wales in eastern Australia, and is probably closely related to *P. alternans*, the type species of this genus. The other *Pamborus* species are found mainly in the southeastern areas of Australia. Since morphology of the male genitalia is considerably different from one species to another, molecular phylogenetic analysis of this genus is urgently needed. *Maoripamborus*, found in New Zealand, cannot be examined, because its capture is prohibited by law.

5.3 Ceroglossini

5.3.1 Taxonomy

The *Ceroglossus* ground beetles are without hind wings and are beautifully colored; they inhabit Chile and part of western Argentina. As mentioned in Chapter 4, the genus *Ceroglossus* forms the small but distinct tribe of Ceroglossini, which is equivalent to the large tribes of Cychrini and the Carabini, as well as the Pamborini.

As will be discussed later, the Carabina, which is one of the two subtribes in the tribe Carabini, has accomplished manifold morphological differentiation, having radiated widely in the Northern Hemisphere. On the contrary, the number of species belonging to the tribe Ceroglossini is very limited because of scarce variation in fundamental morphology. By contrast, variations in body surface color are conspicuous enough to make classification of this group rather ambiguous.

Recently, Jiroux (1996) proposed a new system of classification, in which *Ceroglossus* was divided into four groups, *C. chilensis*, *C. buqueti*, *C. darwini* (including *C. darwini*, *C. speciosus*, and *C. magellanicus*), and *C. suturalis* (including *C. suturalis*, *C. ochsenii*, and *C. guerini*). This classification was made on the basis of the degree of punctures on the propleuron, mesepisternum, and metepisternum, the location of the thiridium in the male antenna, and the shape of the male genital organ (Fig. 5.3). Jiroux did not adopt body color as a taxonomic criterion for distinguishing the species or the species-group.

5.3.2 Phylogeny

The composition of the *Ceroglossus* species in the respective localities where they are found is detailed in Fig. 5.4. The localities in which the *Ceroglossus* specimens examined in this study were found represent almost the full range of distribution with the exception of *C. suturalis*, the distribution range of which extends to higher latitude districts and reaches Navarino Island and Tierra del Fuego.

Four distinct lineages are clear on a phylogenetic tree (Fig. 5.5)—*C. chilensis*, *C. buqueti*, and *C. suturalis* form independent clades (lineages CHI, BUQ and SUT in Fig. 5.5), respectively. *Ceroglossus magellanicus*, *C. speciosus*, and *C. darwini*, all of which belong to Jiroux's *C. darwini* species-group, are related to each other and form one clade (lineage DAR in Fig. 5.5). This result is perfectly consistent with the classification done by Jiroux, although *C. ochsenii* and *C. guerini* in Jiroux's *C. suturalis* species-group, and *C. suturalis* from the higher latitude regions have not been examined because of the lack of available materials.

Assuming that a 0.01 *D* unit corresponds to 3.6 MYA, diversification into the four lineages started ca. 32 MYA. *Ceroglossus chilensis* would have first diverged from the common ancestral line, followed by almost simultaneous diversification of the remaining three lineages. The above chronological estimations might have been influenced somewhat by a higher G+C content of the *ND5* gene in *C. chilensis*.

Ceroglossus chilensis (Lineage CHI)

Ceroglossus chilensis is found in almost entire regions of Chile. Tome [locality (loc.) no. 1] is located in the northern part of the distribution range of *C. chilensis*, and Villa O'Higgins (loc. no. 32) is nearly at the southern limit. The lineage CHI further separated into four clusters (sublineages) ca. 10–12 MYA (M, N, C, and S in Fig. 5.5; for designations of clusters, see below). Although samples of the group S were collected from distantly separated localities, the genetic variations between the specimens were very small.

Ceroglossus buqueti (Lineage BUQ)

The beetles belonging to *C. buqueti* inhabit almost entire regions of Chile including Chiloe Island. There

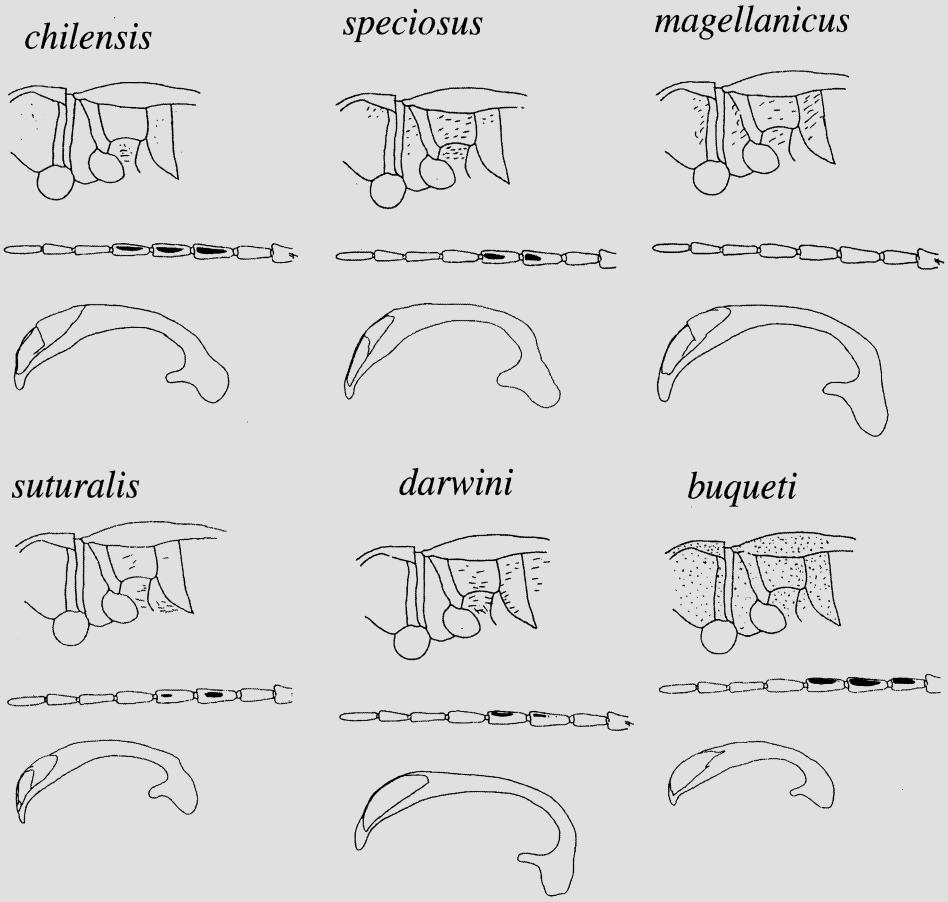


FIG. 5.3. Propleuron, mesepisternum and metepisternum (lateral view), thiridum of antenna, and male genitalia of *Ceroglossus* spp. After Jiroux (1996) modified by Nobuo Kashiwai

are, however, empty regions in the northernmost and southernmost areas. The lineage BUQ, to which all *C. buqueti* specimens belong, is further separated into three sublineages (clusters N, C, and S in Fig. 5.5), which diverged ca. 10–12 MYA. The cluster N diverged first. Samples of the cluster S collected from the southern area (loc. nos. 26–31) were closely related to each other.

Ceroglossus darwini Species-Group (Lineage DAR)

Ceroglossus darwini, *C. magellanicus*, and *C. speciosus* belong to this species-group according to Jiroux (1996). The lineage DAR is further divided into two clusters, N (*C. magellanicus*) and C (*C. speciosus and C. darwini*) (Fig. 5.5). *C. magellanicus* (N) emerged first, ca. 18 MYR, followed by separation of *C. speciosus* and *C. darwini* ca. 5 MYA. Further diversification in the cluster C occurred at about the same time of its separation from the cluster S. Samples of *C. darwini* (cluster S) collected from the southern area (loc. nos. 26–31) form one clear clade among which genetic variations were almost nil.

Ceroglossus suturalis (Lineage SUT)

The lineage SUT is further separated into three clusters. One of them (cluster C), which is distributed on the Chilean mainland (loc. no. 13), diverged ca. 12 MYA. The remainder further diverged into two clusters (C and S) ca. 8 MYA. The genetic variation in the cluster S was very small.

5.3.3 Origin and Distribution of *Ceroglossus*

Taken altogether, it may be inferred that the ancestor of *Ceroglossus* differentiated into four lineages (= species or species-group) between 25 and 32 MYA, and came to occupy their present distribution ranges. This was followed by differentiation into two to four clusters within each lineage.

The tree shows that the distribution of the clusters M, N, C, and S discussed above are clearly linked to geography; the cluster M is restricted to the northernmost region (loc. nos. 1–3), the cluster N to the northern region (loc. nos. 4–9), the cluster C to the central region (loc. nos. 10–25) and the cluster S to the southern region (loc. nos. 26–32). In each lineage, two to three region-specific clusters can be seen. In other words, specimens of a species derived from a certain region form a clade independent from those of the same species from other regions.

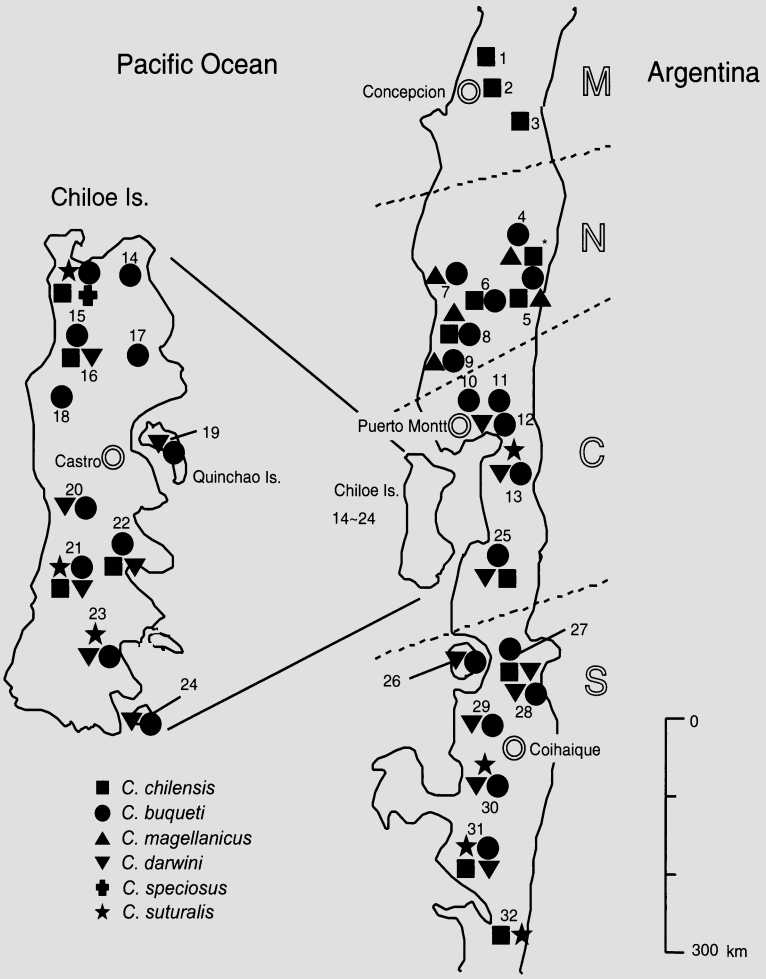


FIG. 5.4. Localities of the *Ceroglossus* spp. specimens used for the phylogenetic analysis of the mitochondrial *ND5* gene. **M** northernmost region, **N** northern region, **C** central region, **S** southern region (after Okamoto et al. 2001)

Thus, it may be inferred that the geographic isolation of each regional cluster within a lineage would have occurred fairly long after the diversification of the four lineages. The above region-specific rule does not hold in Chiloe and its adjacent islands, where the C and S components are intermingled.

In spite of the wide distribution area of the southern region (S), the genetic variations within the same species are very small, suggesting that they suffered from the bottleneck effect during expansion of the distribution range.

As noted above, components in Chiloe and its adjacent islands are a mixture of the lineage CHI–clusters C and S (*C. chilensis*), the lineage BUQ–clusters C and S (*C. buqueti*), the lineage DAR–cluster C (*C. darwini* and *C. speciosus*) and the lineage SUT–clusters C and S (*C. suturalis*). Thus, the Chiloe inhabitants are rich in genetic variations.

These results suggest that the Chiloe inhabitants were derived from two different sources, i.e., the intrinsic components of the central region and invaders from the other regions over land bridges that existed in the past. Alternatively, it is possible that *Ceroglossus* originated in the ancient Chiloe region.

5.3.4 Mode of Morphological Differentiation

The Carabina radiated ca. 40 MYA, and over 800 species are recognized as descendants. This stands in sharp contrast to the situation of *Ceroglossus*; the evolutionary history of *Ceroglossus* is comparable to that of the Carabina, and yet there are only eight species in the former because of poor morphological diversification except in the color of the body surface (see Chapter 8). Thus, the evolutionary history of *Ceroglossus* has largely proceeded "silently." This phenomenon will be discussed in more detail in Chapter 8.

5.4 Carabini

5.4.1 Calosomina

5.4.1.1 Overview of the Calosomina

The tribe Carabini are divided into two subtribes, the Calosomina and the Carabina. The Calosomina is composed of about 100 species. Most of the Calosomina species are hind-winged and can fly well, allowing them

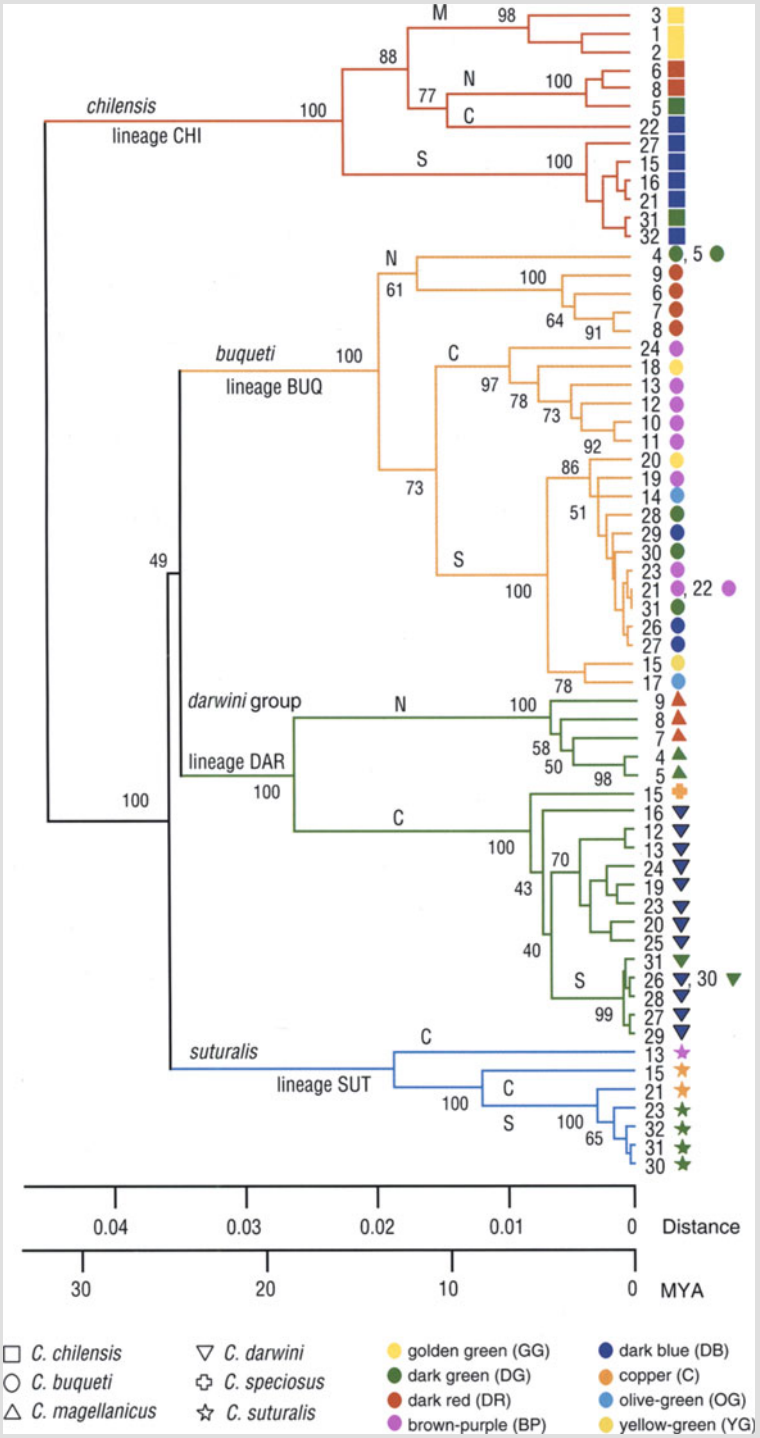


FIG. 5.5. Phylogenetic tree of the mitochondrial *ND5* gene for the genus *Ceroglossus*. Constructed by UPGMA (after Okamoto et al. 2001)

to expand their distribution to almost all parts of the world.

This situation stands in sharp contrast to the Carabina group, which is without hind wings and consists of more than 800 species, most of which are found in the Northern Hemisphere. The Calosomina fauna is rich in the New World as compared with the Eurasian Continent. In Australia, only two species belonging to the Calosomina group have been found (Jeannel 1940). Several species occur in Africa, though it has as yet been impossible to examine any examples of these species due to a lack of material.

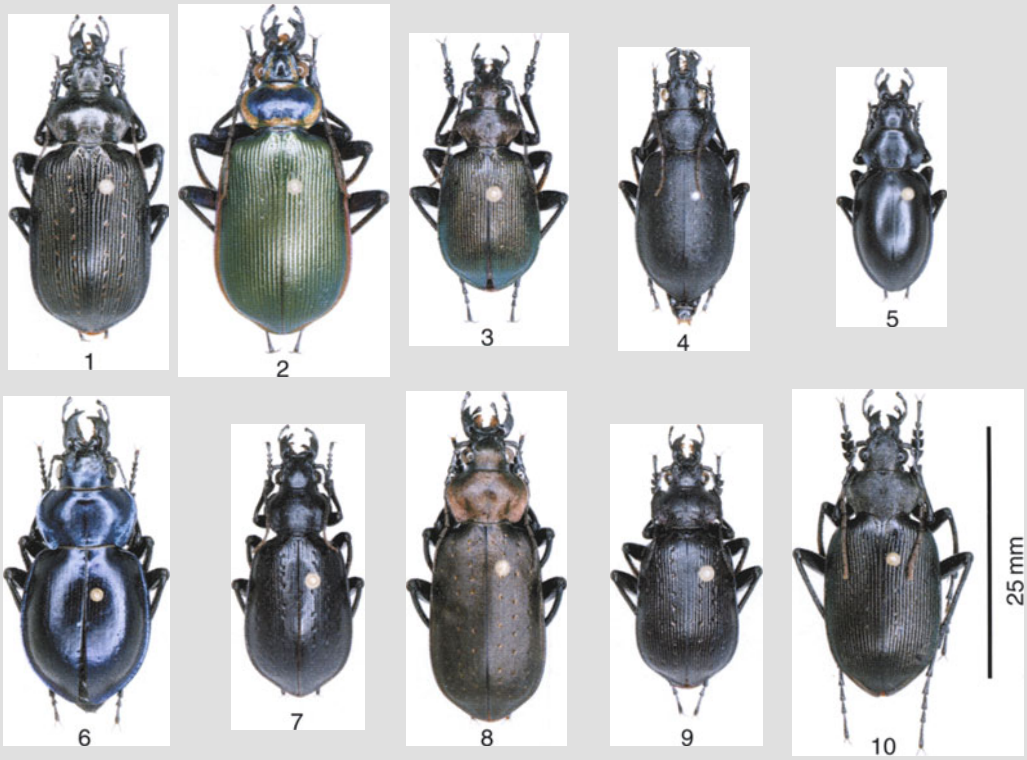


FIG. 5.6. Representative species of the subtribe Calosomina. **1** *Castrida sayi*, **2** *Calodrepa scrutator*, **3** *Calosoma inquisitor cyanescens*, **4** *Charmosta lugens*, **5** *Callitropa (Blaptosoma) chihuahua*, **6** *Callisthenes kuschakewitschi*, **7** *Campalita davidi*, **8** *Cam. chinense*, **9** *Calosoma frigidum*, **10** *Cal. maximowiczi*

There is no consensus on the reasonable classification of the Calosomina. Some authors treat the whole Calosomina as one genus, *Calosoma*, under which many subgenera are included. Others, such as Jeannel (1940), divide the Calosomina into several genera. We have used Jeannel's system in this study, with some reservations. The representative Calosomina species are illustrated in Fig. 5.6.

5.4.1.2 Overview of Molecular Phylogeny

A phylogenetic tree of the mitochondrial *ND5* gene shown in Fig. 5.7 contains 43 Calosomina specimens consisting of 26 species belonging to 12 genera from various regions of the world. They are composed of about one-quarter of known species, found in localities throughout most of the world with the exception of Africa.

Sixteen lineages (A–P as shown in Fig. 5.7) radiated within a relatively short time. At least four lineages are acknowledged to exist on the Eurasian Continent, ten in the New World and two in Australia and Indonesia. Their branching order cannot be estimated because of short branches supporting the lineages with low bootstrap values.

Lineage A includes three species, all found in South America—*Castrida alternans* (Brazil), *C. sayi* (Nicaragua) and *C. fulgens* (Ecuador). This lineage might have separated earlier than the rest of the lineages, as shown by somewhat longer branch lengths. *Castrida alternans* and *C. sayi* are close morphologically as well as phylogenetically. The divergence of *C. fulgens* from the other three species occurred earlier and may be estimated to have occurred about 29 MYA.

5.4.1.3 An Outline of the Composition of the Respective Lineages

Fifteen out of 16 lineages are accounted for by species belonging to the same genus which include in most cases one, and sometimes two to three, species. An exception is lineage H, which includes four genera and five species (see below).

Surprisingly, three species taxonomically classified as belonging to the genus *Calosoma* appear in an independent lineage. These are *C. maximowiczi* from Japan and China (lineage P), *C. inquisitor* from Japan and China (lineage C), and *C. frigidum* from the northwestern United States (lineage L). The former two species are very close in morphology.

Similarly, three species belonging to the genus *Callitropa*, *C. macrum* (Texas, USA), *C. chihuahua* (Mexico), and *C. haydeni* (Mexico) appear in lineages D, H, and I, respectively. *Australodrepa schayeri* from

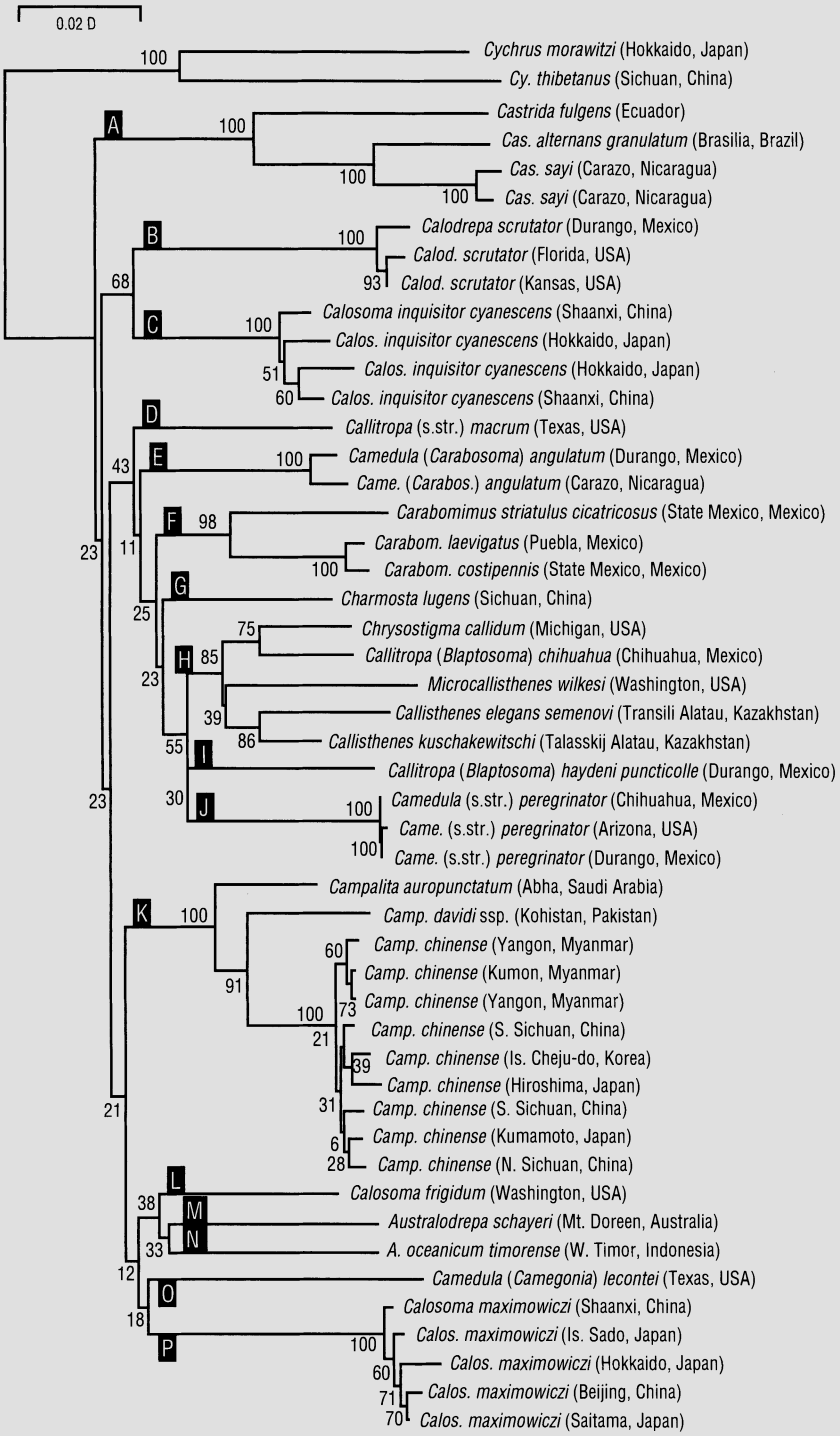


FIG. 5.7. Phylogenetic tree of the mitochondrial *ND5* gene for the subtribe Calosomina. Constructed using the NJ-method (unpublished)

Australia (lineage M) is also remote from *A. timorense* from West Timor, Indonesia (lineage N). In other words, species in the genera *Calosoma*, *Callitropa*, and *Australodrepa* are unlikely to be monophyletic. One of the *Callitropa* species (*chihuahua*) is clustered with the species of other genera such as *Chrysostigma*, *Microcallisthenes*, and *Callisthenes* (lineage H; see below).

5.4.1.4 Pattern of Sequence Divergence

As noted above, many of the phylogenetic lineages that consist of a single genus or species are only remotely related to one another. They separated in the early stages of the Calosomina evolution (in a process of radiation) (Osawa et al. 2001). In lineage K, *Campalita*

chinense (China, Korea and Japan), *C. auropunctatum* (Saudi Arabia) and *C. davidi* (Pakistan) are clustered together with their separation estimated to have taken place about 14 MYA.

Sequence divergence between populations belonging to the same species but inhabiting separate geographical areas is small. This is shown by *Calosoma maximowiczi*, found in the Beijing area of China and in three localities in Japan (lineage P); *C. inquisitor* from Shaanxi, China, and Hokkaido, Japan (lineage C); *Campalita chinense* from Sichuan, China, Cheju, Korea, and two localities in Japan (lineage K). This small sequence divergence in geographically separated populations can also be seen in *Camedula peregrinator* from two localities in Mexico and Arizona, USA (lineage J); *Carabosoma angulatum*, found in Arizona, USA and Nicaragua (lineage E); and in the case of *Calodrepa scrutator*, found in Mexico and Texas, USA (lineage B) (see below).

Sequence divergence between different species within the same genus is variable. The difference between *Castrida alternans* and *Castrida sayi* (lineage A), and *Carabominus laevigatus* and *C. costipennis* (lineage F) is small, while that between *Carabominus striatulus* and the two above-mentioned *Carabominus* species is relatively large.

We interpret the phylogenetic tree in such a way that diversification of the Calosomina occurred about 40–30 MYA, which is somewhat later than the radiation of the Carabina (50–40 MYA) (Osawa et al. 2001). Our studies thus far show that in the early stages of the Calosomina diversification, at least 14 major lineages of the Calosomina seem to have been established within a relatively short period. Perhaps, the ancestors for the respective lineages were reproductively isolated as a result of early radiation. Because of this, their branching order cannot be determined with certainty.

In most cases, the composition of each lineage is simple; it consists of only a single genus and/or species, suggesting a lack of conspicuous morphological changes within the lineage from its emergence. Although the specimens analyzed in this study are limited in number, morphological stability within a given lineage, i.e., silent morphological evolution after radiation, seems to be nearly the general feature of its own, as many of the lineages consist of a single species/genus and no other species belonging to other genera branched off from each lineage. Such silent evolution may often be observed in other carabid groups and is a general feature of carabid beetles (see Chapter 8).

Another point of interest is that a few species in the same genus belong to an independent lineage, as shown by *Calosoma inquisitor*, *C. maximowiczi*, and *C. frigidum*. They separated from one another almost at the beginning of the Calosomina radiation. Indeed, the evolutionary distance between these *Calosoma* species is as remote as that between *Calosoma* and other genera such as *Campalita* and *Charmosta*. In particular, *C. inquisitor* and *C. maximowiczi* are so close in morphology that it is sometimes difficult to distinguish between them without close examination.

There are several possible ways to account for the above phenomenon. One possibility is that a *Calosoma*-like morphology is the ancestral morphology of the Calosomina. Upon radiation the ancestor of *Calosoma* split into reproductively isolated populations to produce the ancestral forms of the present-day *Calosoma* species, such as *C. inquisitor* and *C. maximowiczi* with maintenance of the fundamental ancestral morphology up to the present. Another possibility is that these *Calosoma* species shared common ancestry for some time after radiation, but this possibility cannot be verified at present because of poor resolution at the root of the phylogenetic tree. Still another possibility is that the morphological resemblance between different *Calosoma* species is a result of parallel evolution.

At present, we have no data on which to base a decision as to which of these possibilities is most likely to be correct. It should be emphasized, however, that morphological similarity does not necessarily indicate phylogenetic relatedness in the subtribe Calosomina.

5.4.1.5 Migration Capability of Winged Calosomina

It is worth noting that sequence divergence within the same species that occupy distantly separated geographical areas is very small, as seen in *Campalita chinense*, *Calosoma inquisitor*, *C. maximowiczi*, and *Camedula peregrinator* (see above).

In one example, *Campalita chinense* is found throughout much of East Asia including Japan. The genetic distance as shown by the *ND5* gene is very small or almost nil in samples from various parts of this range (Sichuan, China; Yangon and Kumon, Myanmar; Chejudo Island, Korea; Hiroshima and Kumamoto, Japan). Similarly, the genetic distance between *Calosoma maximowiczi* from various localities (Shaanxi and Beijing, China; Sado Island, Hokkaido, and Saitama, Japan) is almost the same. This phenomenon may be explained by poor geographic isolation resulting from the ability of winged Calosomina insects to migrate.

This stands in sharp contrast to the wingless Carabina species, in that a considerable geographical difference is seen in both morphology and the gene sequence in the Carabina.

5.4.1.6 An Apterous Group

The lineage H includes four genera, *Chrysostigma*, *Callitropa*, *Blaptosoma*, *Microcallisthenes*, and *Callisthenes*, that are of special interest. According to Lindroth (1961), the hind-wings are slightly reduced and probably non-functional in species related to *Chrysostigma callidum*, and in *Microcallisthenes* and *Callisthenes*, which contain species with a *Carabus*-like appearance

that bear extremely reduced hind-wings, thus often inhabiting within the restricted areas.

This would have made diversification within this lineage easier through geographic isolation followed by reproductive isolation. It might be the case that some genetic event leading to a reduction of the hind-wings took place in the common ancestor of this group. The apterous groups such as *Callisthenes* and *Microcallisthenes* have been thought to be phylogenetically remote from other hind-winged Calosomina. However, the present molecular analysis strongly suggests that this is not the case, as it has been shown that they emerged probably later than, or at least in a period near that of other winged Calosomina.

5.4.1.7 Origin and Establishment of Distribution Ranges

It is difficult to ascertain the details of the origin and establishment of the present distribution range of the Calosomina species because of the limited number of species studied, and the ability of this hind-winged group to migrate.

It is of interest to note, however, that the Australian species, *Australodrepa schayeri* (lineage M) and *A. timorense* (lineage N) are included within the Eurasian and North American group without revealing a direct connection to the South American group (lineage A).

5.4.1.8 Taxonomy and Molecular Phylogeny

We have adopted a classification system in which the Calosomina are divided into several genera. Phylogenetically, however, there is no rationale for such a system, because most of the genera form independent clusters, each having an almost equally deep branching point (see Fig. 5.7). How the phylogenetic lineages match taxonomic ranks assigned on the basis of morphology must await further study.

5.4.2 Carabina

5.4.2.1 Overview of the Higher Classification

The Carabina beetles are called "walking jewels," as many of them can move only by walking and boast beautiful, jewel-like colors. Because of these attractive characters, these insects have long been a popular subject of study among European amateur and professional entomologists.

This means that efforts to classify the Carabina beetles taxonomically have a long history, and many higher-order classification systems have been proposed. The current system of classification is based mainly on the endophallic characteristics of the male genital organ, use of which for the purpose of classification was first proposed by Ishikawa (1973, 1978, 1979).

His system classifies the Carabina into three divisions. Deuve (1994) posits five divisions and 114 genera. Imura (1996) proposed a new system, in which the Carabina are first separated into two large divisions. The first of these is composed of three subdivisions containing 14 (sub)genera, while the second includes five subdivisions containing 80 (sub)genera. Several other systems proposed by various researchers are not mentioned here, because the primary purpose of this book is to study the phylogeny and evolution of the carabids, and not to review past taxonomic studies.

All the Carabina species have been routinely treated as belonging to a single grand genus, *Carabus,* within which there are many subgenera. In this book, we use subgeneric names as generic names in place of "*Carabus*" simply to distinguish between the respective groups. The number of species of the Carabina posited by Imura and Mizusawa (1996) is 710–720, while Březina (1999) suggests that there are 802. As several more species have been discovered since these researchers put forward their opinion of the number of species, the total number of known species at this time is about 900.

If the great many "subspecies" that have been described were included in the total number, it would double or triple. In this and the following chapters, the scientific names that were adopted by Imura and Mizusawa (1996) are used unless otherwise specified. It is true, however, that many of these names and the classifications they specify are ambiguous because of a lack of sufficient supporting evidence, and should be reexamined in the light of molecular phylogeny in conjunction with more sophisticated morphological procedures.

Březina (1999) effectively describes the present state of classification of the Carabina, when he notes, "The classification of *Carabus* (= the Carabina) at the infraspecific level is extremely subjective and disputable, burdened by hundreds of forms, described under various status . . . Even in the most recent works, especially in those dealing with the Chinese fauna, almost all newly discovered local populations are being automatically described as new subspecies, with insufficient information on the distributional ranges of the respective species, often with only one specimen in hand . . ."

It is our opinion that this is a well-reasoned opinion, and many subspecies are excluded from discussion in this chapter with the exception of those we thought it worthwhile to consider.

5.4.2.2 Molecular Phylogeny of the Carabina

Su et al. (1996b) first pointed out that the main Carabina groups radiated at about the same time. Figure 5.8 shows a phylogenetic tree completed using the mitochondrial *ND5* gene from the 45 representative species of the Carabina, including all the eight subdivisions (sensu Imura 1996).

In accordance with the view put forward by Su et al. (1996b), the tree suggests that a number of lineages

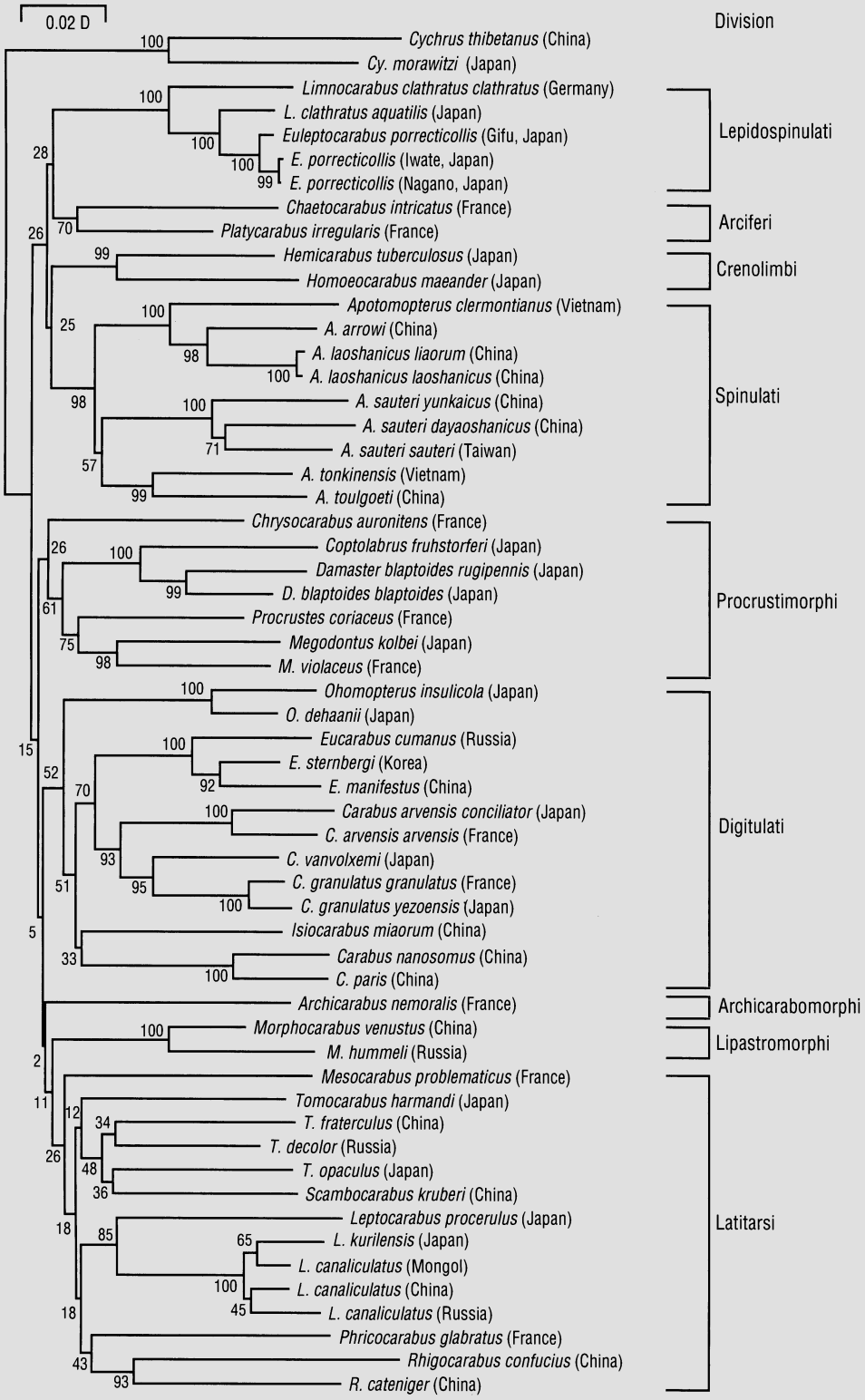


FIG. 5.8. Phylogenetic tree of the mitochondrial ND5 gene for the subtribe Carabina. Constructed using NJ-method. (After Imura et al. 1998a)

emerged at almost the same time. The lineages so recognized are consistent with Imura's subdivisions on all except three points.

In the first of these, as mentioned above, Imura (1996) posited two major divisions, the Carabogenici and the Multistriati, between the genera and the subdivisions. Since the branching order of the lineages in the *ND5* tree cannot be determined, presumably because of their almost simultaneous emergence, they should be treated as "equivalent" taxa without further grouping. This means that it would be appropriate to raise Imura's subdivisions to divisions directly above the genera (Fig. 5.8).

Imura (1996) combined the genus *Apotomopterus* (s. str.) and the genera *Limnocarabus*/*Euleptocarabus* to form a single subdivision known as the Spinulati. However, the species belonging to the "Spinulati" appear on the tree as two distinctly separate clusters. All the species of *Apotomopterus* form a clear single cluster (Fig. 5.8), while the species belonging to *Limnocarabus* and *Euleptocarabus* constitute another monophyletic cluster (named Lepidospinulati by Imura et al. 1998a; see Fig. 5.8). There is no indication of any phylogenetic relatedness between these two lineages.

Imura's subdivision Latitarsi is probably polyphyletic (Fig. 5.8), containing several independent lineages that emerged almost simultaneously. Taxonomically, it would be one of the ways to settle the divisions corresponding to the respective lineages. This subject will be discussed in more detail in Chapter 9.

In the following sections, we give a phylogenetic profile of each division from top to the bottom following the order shown in Fig. 5.8.

Lepidospinulati

This division contains the two genera *Limnocarabus* and *Euleptocarabus*. *Limnocarabus* is rather sporadically but widely distributed in the northern parts of the Eurasian Continent and several adjunctive islands, and is usually treated as monotypical, though the type species, *Limnocarabus clathratus*, shows marked geographical variations and is separated into several subspecies.

Euleptocarabus is composed of a single species, *porrecticollis*, which is endemic to Honshu, the main Japanese island. It is separated into two local races, i.e., nominotypical subspecies and subsp. *kansaiensis*, but the geographical variation of the species is more complicated (see Chapter 7).

The species of this division are characterized by spine- or thorn-shaped basal sclerite (= lepidospinula) situating at the base of the membranous wall, from where they emerge in a nearly vertical fashion; and a dense scale-like microstructure covering over at least part of the surface (Fig. 5.9).

Members of the division Spinulati lack the scale-like microstructure. The distribution ranges of the two divisions are clearly separated and do not overlap (Fig. 5.10).

Su et al. (1996a) reported that two Japanese species, *Limnocarabus clathratus aquatilis* and *Euleptocarabus porrecticollis*, are clustered together on the phylogenetic tree of the mitochondrial *ND5* gene sequences. The evolutionary distance between these two species is rather small, although they are morphologically separated from each other (Fig. 5.11). This will be discussed in greater detail in Chapter 7.

Arciferi

The division Arciferi has been regarded as containing four genera, *Platycarabus*, *Chaetocarabus*, *Heterocarabus*, and *Hygrocarabus* (Imura 1996), and is morphologically well-defined by a characteristically shaped ligulum (= arculus) at the base of the endophallus of the male genitalia. Most of them are distributed in Europe and some in Asia Minor.

As shown in Fig. 5.11, *Chaetocarabus intricatus*, *Heterocarabus marietti*, *Platycarabus depressus* and *P. irregularis* are clustered together on the *ND5* tree, while *Hygrocarabus nodulosus* is not closely related to the other Arciferi species. Its emergence may be traced back to the time of the radiation of the Carabina. Since there is no other species clustered with *Hygrocarabus*, it may be excluded from the division Arciferi and is considered to form an independent position in the Carabina (see Chapter 9). In fact, the emergence of *Hygrocarabus* took place as early as that of Lepidospinulati (see Fig. 5.11). *Hygrocarabus* is semi-aquatic in habit throughout its life, and the larval morphology is different from that of the other three Arciferi genera (Casale et al. 1982).

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

Crenolimbi

The genera *Hemicarabus* and *Homoeocarabus* are morphologically similar to each other, and have been combined into a single category, the Crenolimbi. The Crenolimbi is the smallest division of the Carabina, consisting of only five species (four in *Hemicarabus* and one in *Homoeocarabus*).

They are widely, but rather sporadically, distributed throughout the northern parts of the Eurasian Continent and North America, including several adjunctive islands such as the British Isles, Sakhalin, the Japanese islands, Cheju-do Island (South Korea), Newfoundland,

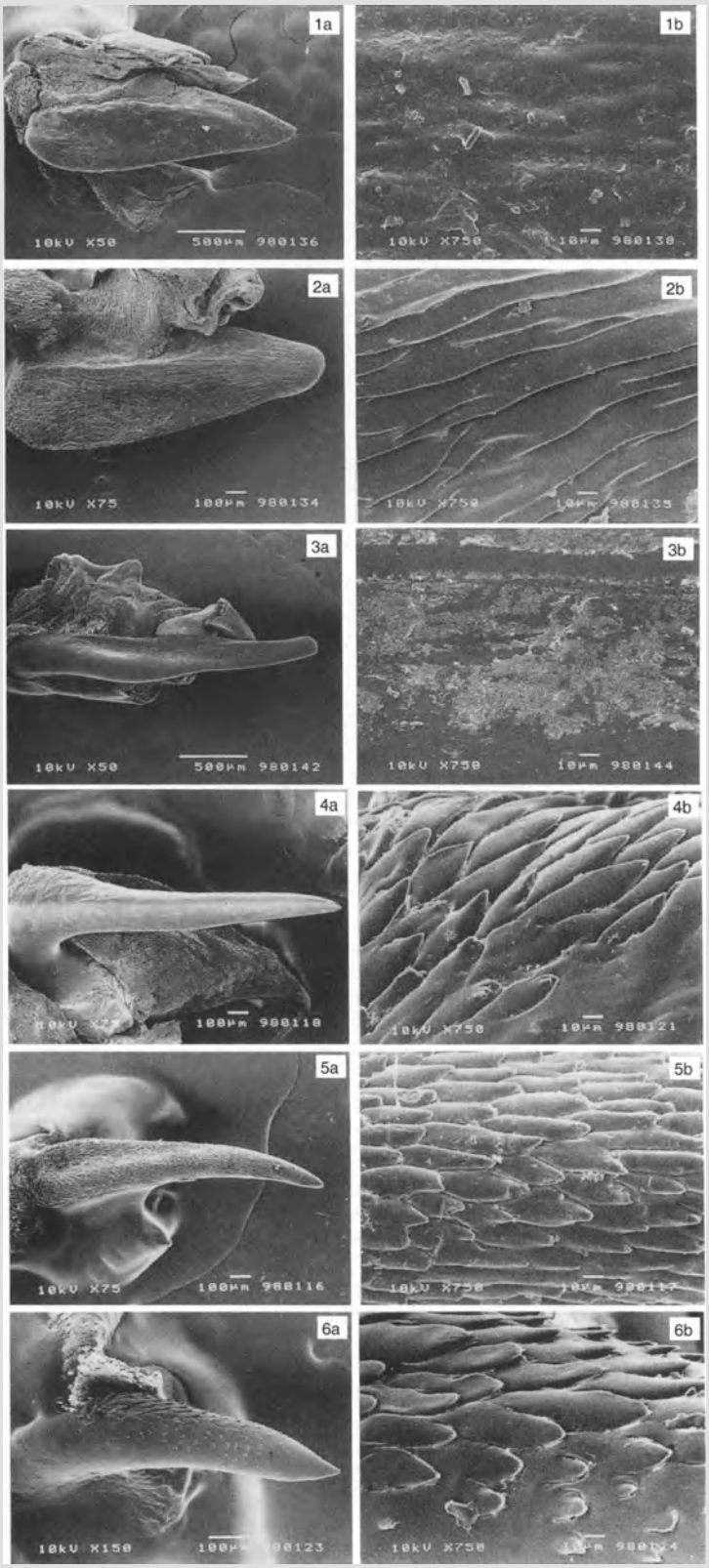


FIG. 5.9. SEM photographs of spinula. **1a,b** *Apotomopterus clermontianus*, **2a,b** *A. laoshanicus liaorum*, **3a,b** *A. sauteri* (1–3: division Spinulati), **4a,b** *Limnocarabus clathratus clathratus* from Germany, **5a,b** *L. c. aquatilis* from Japan, **6a,b** *Euleptocarabus porrecticollis* (4–6: division Lepidospinulati). **a** Total view, **b** surface (high magnification) (after Imura et al. 1998a)

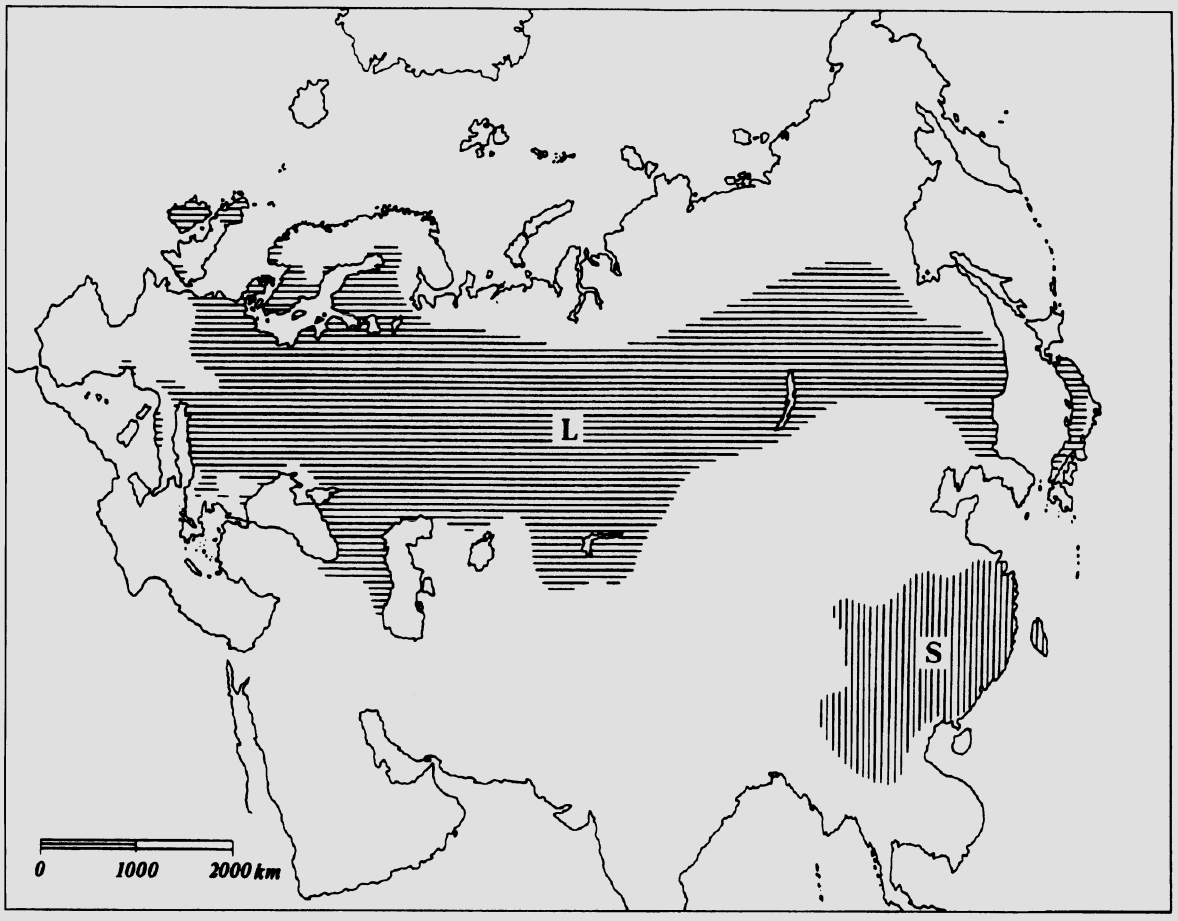


FIG. 5.10. Distribution areas of the division Spinulati (S) and the Lepidospinulati (L) (after Imura et al. 1998a)

FIG. 5.11. Phylogenetic tree of the mitochondrial *ND5* gene for two divisions, the Arciferi (bold branch) and the Lepidospinulati. Constructed using the NJ-method with the use of *Cychrus morawitzi* as outgroup (after Imura et al. 2000b)

St. Pierre, and Miquelon. It is worth noting that most of the Crenolimbi species prefer habitats such as meadowland and/or lowland moor. This tendency seems to be more marked in *Homoeocarabus*.

An *ND5* phylogenetic tree of all the species in this division from various localities (Fig. 5.12) shows that *Homoeocarabus maeander* is sharply separated from four species of *Hemicarabus*. Diversification of these two genera may be estimated to have taken place about 29 MYA as calculated by Su et al. (2000a).

Within the genus *Hemicarabus, H. tuberculosus* from various localities of Japan, Korea, and Primorskij, *H. macleayi* from Kamchatka, and *H. nitens* from Germany are very close in their *ND5* gene sequences. This sug-

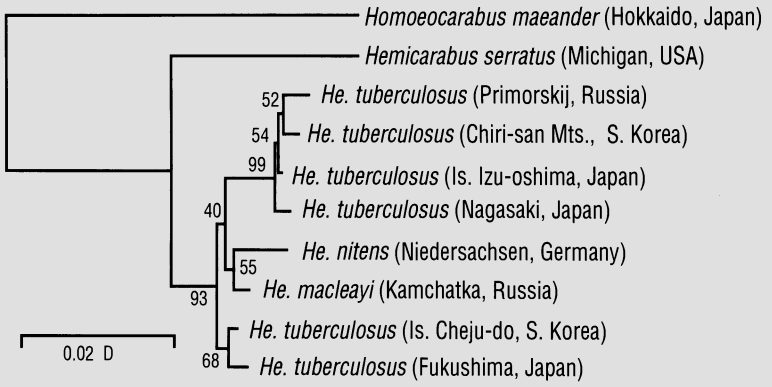


FIG. 5.12. Phylogenetic tree of the mitochondrial *ND5* gene for the division Crenolimbi. Constructed using the NJ-method (after Su et al. 2000a)

gests that the common ancestor of these species was distributed throughout the northern part of the Eurasian Continent, consisting of nearly a single reproductive population until recently.

Speciation occurred recently in the respective distribution ranges presumably as a result of geographic isolation, followed by genetic changes affecting morphology. Alternatively, it is possible that the ancestor inhabited a restricted region of the continent and rapidly spread in distribution only recently, then differentiating into the respective species. It is worth noting that the three species can be clearly separated morphologically, suggesting occurrence of a rapid morphological differentiation between the species. The migration of *H. tuberculosus* to the Japanese Islands as posited by Tominaga et al. (2000) will be discussed in greater detail in Chapter 6.

Hemicarabus serratus from North America (Michigan) separated fairly long ago (about 14 MYA) from the other Eurasian *Hemicarabus* species. Presumably, the common ancestor of all the *Hemicarabus* species was distributed throughout the Eurasian Continent and North America when they were still connected by a land bridge. Upon the formation of the Bering Straits, the Eurasian population and the North American one evolved in different directions, resulting in differentiation of *H. serratus* in North America.

Archicarabomorphi

The Archicarabomorphi is one of the divisions of the subtribe Carabina, consisting of four (sub)genera, namely, *Archicarabus, Ischnocarabus, Gnathocarabus*, and *Acrocarabus* (Deuve 1991,1994; Imura 1996; Imura and Mizusawa 1996). Morphologically, they are characterized mainly by the characteristic structure of the male genital organ, i.e., a narrow preostium lacking the ostium lobe, uniquely developed paraligula and the characteristically shaped membranous wall of the endophallus.

The genus *Archicarabus* comprises nearly ten species distributed over the greater part of Europe and Asia Minor. The type species, *A. nemoralis*, has also been recorded on the eastern shore of North America and in southeast Kazakhstan in Central Asia. These examples of the species were probably introduced from Europe by humans.

The remaining three genera are rather restricted both in terms of the number of species they include and their range of distribution. *Ischnocarabus* includes two species, both endemic to Turkey. *Gnathocarabus* is monotypical with the type species, *G. kuznetzovi*, known only in the mountainous area of northeast Iran. *Acrocarabus* consists of two closely allied species, both found in the eastern part of the Tianshan Mountains.

An *ND5* phylogentic tree of the representative species of all the above genera except for *Ischnocarabus* shows four well-defined lineages (Fig. 5.13). Diversification of the three Archicarabomorphi lineages seems to have occurred within a short time about 28 MYA. The *Acrocarabus* lineage would have emerged considerably earlier than the other three. The first lineage contains two species of *Acrocarabus*, i.e., *Ac. guerini* and *Ac. callisthenoides*, both from the Dzhungarskij Alatau area of eastern Tianshans, in southeast Kazakhstan.

From the evolutionary distance (*D*) between *Ac. guerini* and *Ac. callisthenoides*, it would appear that these two species diversified about 15 MYA. With a high phylogenetic independence together with considerable difference in the fundamental structure of the male genital organ from that of the other three Archicarabomorphi species, *Acrocarabus* may not be appropriate for inclusion in this division.

An *ND5* phylogenetic tree of the representative Carabina species clearly shows that *Acrocarabus* is positioned within the division Digitulati. In fact, 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 (see Fig. 5.28 and pp. 76–77 in the section on the Digitulati).

The second lineage is represented solely by *Gnathocarabus kuznetzovi* from northeast Iran. The third lineage includes two *Archicarabus* species, i.e., *Ar. monticola* and *Ar. nemoralis*. These two taxa are sharply separated from each other on the phylogenetic tree.

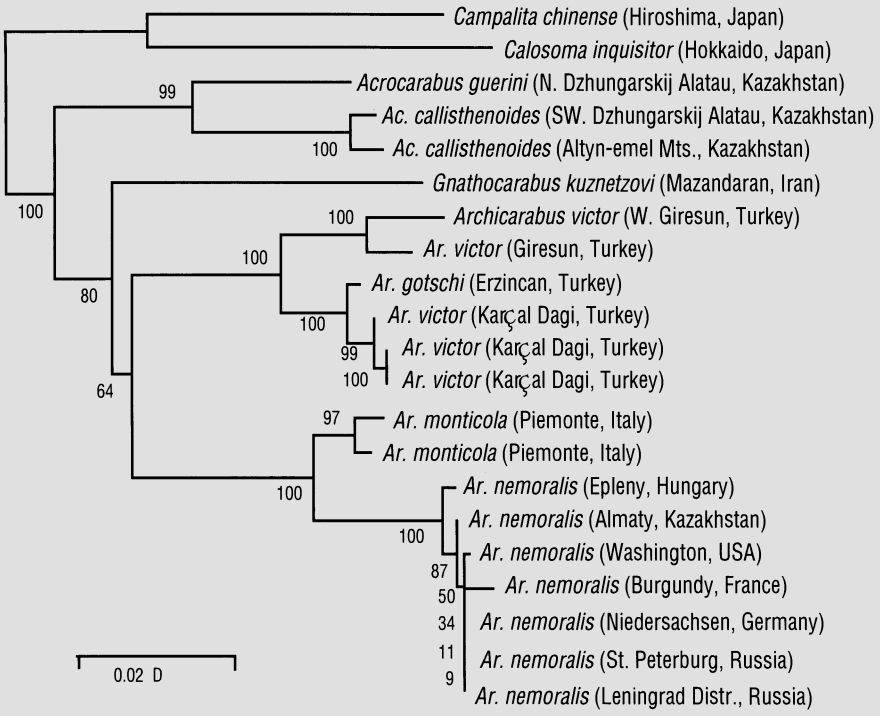


FIG. 5.13. Phylogenetic tree of the mitochondrial *ND5* gene for the division Archicarabomorphi. Constructed using the the NJ-method (after Imura et al. 2000a)

Their separation was calculated to have occurred fairly recently, about 20 MYA. Two examples of *Ar. monticola* from two different localities of northwest Italy show a small difference in their sequences. The sequence difference is very small among all nine examples of *Ar. nemoralis* from seven different localities including North America and Central Asia. Those from Germany, West Russia, Kazakhstan, and USA show almost no difference, while specimens from France and Hungary are a little more remote in their sequences as compared with those mentioned above.

Archicarabus nemoralis is the type species of the genus, and has the widest distribution range, and yet it shows poor geographical variation. The population of *Ar. nemoralis* occurring in North America has been regarded as an introduction from Europe (Lindroth 1961). The same situation may be true for specimens from Kazakhstan, as would seem to be the case from the results of our study.

The fourth lineage includes two Turkish species, *Archicarabus victor* and *Ar. gotschi*. This lineage may be separated into two sublineages, one containing *Ar. victor* and *Ar. gotschi* from Karcal Dagi and Erzincan situated near the northeastern periphery of the country, and the other containing two specimens of *Ar. victor* from Giresun which is about 350 km west of the above localities. 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. Analyses of both molecular phylogeny and morphology need to be done to ascertain whether these two are conspecific or not.

Spinulati

Apotomopterus is the sole constituent genus of the division Spinulati, and is thought to comprise more than 100 species (see below) distributed mostly in southeastern Eurasia (southeast China including Taiwan and the northern mountainous areas of the Indochina Peninsula).

This genus had been united into this division together with the species now placed in the division Lepidospinulati, from which it is made distinct by well-developed spinula without a scale-like microstructure on the surface, and by an apparent independence from the Lepidospinulati on the *ND5* phylogenetic tree (Fig. 5.9; see the section Lepidospinulati).

Figure 5.14a shows a phylogenetic tree based on the mitochondrial *ND5* gene sequence of 38 individuals from 17 species. The tree indicates that there are four main lineages. Lineage 1 includes *A. kouichii* from North Vietnam, *A. sauteri, A. protenes*, and *A. hupeensis* from continental China and Taiwan. *Apotomopterus kouichii* separated from other species in this lineage as long ago as about 32 MYA. Following this, three *A. sauteri* sublineages diverged at about the same time, 20–24 MYA.

We looked at five specimens of *A. hupeensis* from various localities, indicating that *A. hupeensis* branched off from one of the *sauteri* stems. Still another *sauteri*

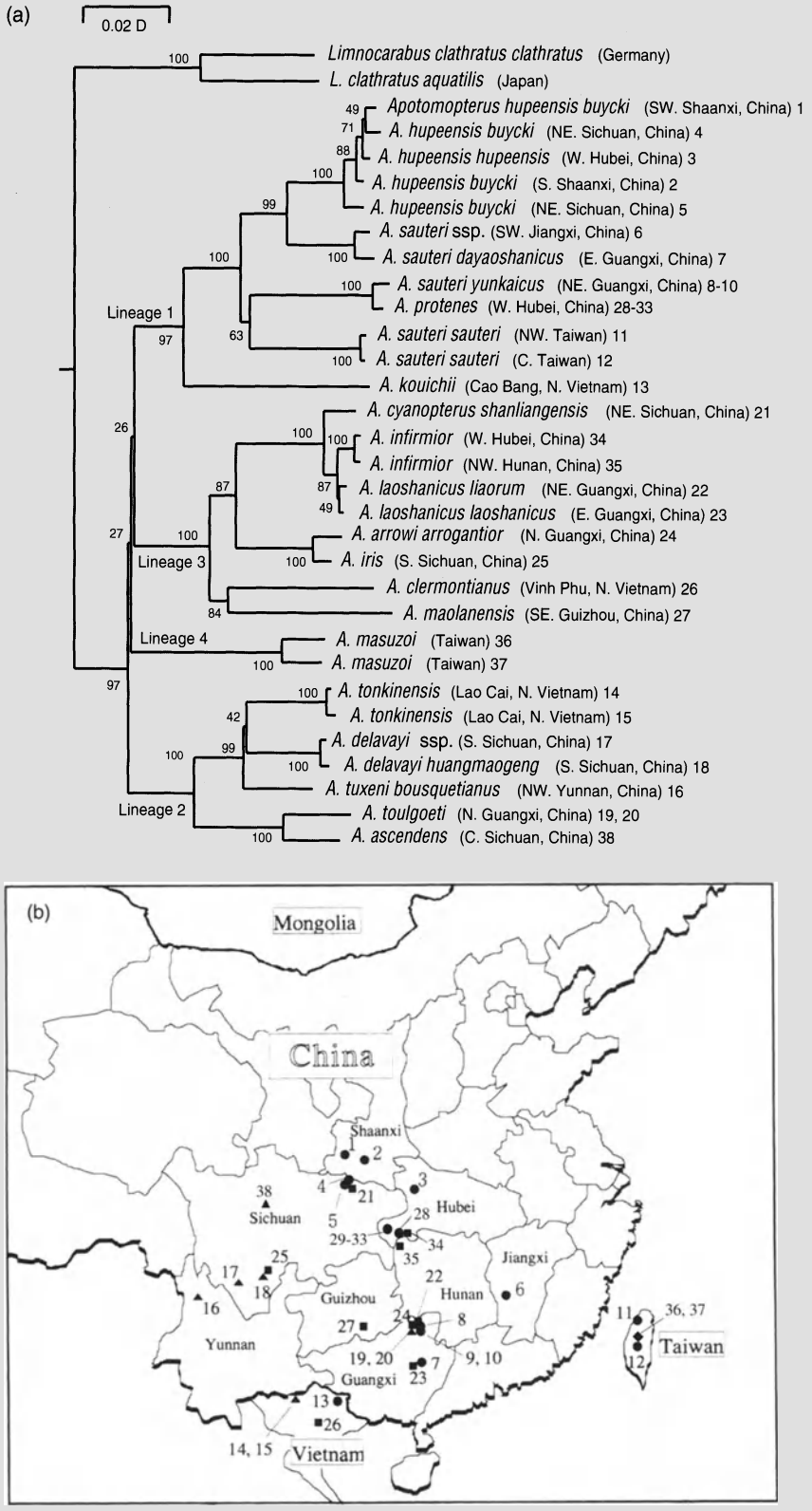


FIG. 5.14. Phylogenetic tree of the mitochondrial *ND5* gene for the division Spinulati constructed using the NJ-method (**a**) and localities of the samples analyzed (**b**) (after Kim et al. 1999b; Su et al. 2003b)

sublineage contains *A. protenes* from western Hubei in China. The sequences of six *A. protenes* specimens were found to be identical, and revealed only 0.56% difference from those of *A. sauteri* from Guangxi in China. Although we conventionally treat them as "two distinct species," they may very well be conspecific, because of their close morphological similarity as pointed out by Imura (2001).

The sequence difference between *A. protenes/A. sauteri* (Guangxi, China) and the two *A. sauteri* races/*A. hupeensis* is 5.2%. There has recently been a tendency to classify *Apotomopterus* by splitting into many species or subspecies based on only small morphological differences. The validity of these classifications, at least in some cases, should be reconsidered in light of molecular phylogeny, morphology and distribution ranges.

The second lineage contains *A. tonkinensis* from northern Vietnam, and *A. toulgoeti, A. delavayi, A. tuxeni*, and *A. ascendens* from central and south China. *A. toulgoeti/A. ascendens* and *A. tonkinensis/A. tuxeni/A. delavayi* separated ca. 25 MYA, followed by diversification of the latter three species ca. 14 MYA.

The third lineage consists of *A. clermontianus* from northern Vietnam, and *A. cyanopterus, A. iris, A. maolanensis, A. arrowi, and A. laoshanicus* from central and southern China. *Apotomopterus clermontianus* and *A. maolanensis* separated ca. 26 MYA, and the remaining species diverged ca. 18 MYA into two sublineages, i.e., *A. cyanopterus/A. laoshanicus/A. infirmior,* and *A. arrowi/A. iris.*

Further apparent diversification into several descendant "species" seems to have taken place within the respective sublineages. It should be pointed out, however, that the sequence difference between the two *A. infirmior* specimens is only 0.28%, and that between *A. infirmior* and *A. laoshanicus* is 0.56%, suggesting that diversification of these three "species" is a very recent event.

The origin of *Apotomopterus* is relatively venerable and is indicated by the fact that the maximum sequence difference among all the *Apotomopterus* species that have been examined is 10.2%, corresponding to an evolutionary distance of about 37 MYA.

The fourth lineage contains only one species, *A. masuzoi.* This species has been found only in the high mountains of central Taiwan and reveals, at first glance, a *Leptocarabus*-like appearance. Because of several morphological characteristics distinct from those of other *Apotomopterus* species, the subgenus *Taiwanocarabus* has been proposed for this species (Imura and Satô 1989).

As to the origin of *A. masuzoi*, two possibilities have been put forward. One possibility is that *A. masuzoi* separated from *A. sauteri* within Taiwan with accompanied morphological transformation. Another possibility is that *A. masuzoi* constitutes a distinct lineage, whose origin is independent from that of *A. sauteri.* A recent molecular analyses show that *A. masuzoi* is phylogenetically quite remote from *A. sauteri*, constituting an independent (fifth) lineage from the other *Apotomopterus* lineages (Su et al. 2003b).

The geohistoric connection between continental China and Taiwan suggests that Taiwan split from the continent 30–20 MYA (Jahn et al. 1976). Phylogenetic analysis suggests that *A. sauteri* first distributed widely and then became isolated in at least three populations (sublineages) about 24–22 MYA in continental China. One of these that inhabited the ancient region of Taiwan was isolated upon the separation of Taiwan from the continent 22 MYA. Note that the time of the separation of the Taiwanese *sauteri* from other races roughly coincides with the time of separation of Taiwan from the continent.

On the other hand, *A. masuzoi* emerged at a much earlier time than did *A. sauteri.* The origin of *A. masuzoi* can be traced back to the time of the radiation of the other three lineages of *Apotomopterus* in continental China about 40 MYA. Because *A. masuzoi* is strictly endemic to the high mountains of Taiwan, the proto-*masuzoi* would have exclusively inhabited the ancient Taiwanese region on the continent and the present-day *masuzoi* would have been carried to Taiwan upon its separation from the mainland.

This situation is analogous to that of the Japanese autochthon carabids, such as *Damaster, Leptocarabus*, and *Ohomopterus* (see Chapter 6, pp. 96–97), which are also thought to have been carried to the ancient Japanese islands when they split from the continent. The Japanese species succeeded in expanding their distribution with considerable differentiation, while *A. masuzoi* inhabits only high mountainous area as a relic presumably because of its failure to adapt to the subtropical environment of Taiwan.

From this it will be clear that *A. masuzoi* is biogeographically an important species that emerged in ancient continental China, and not in Taiwan.

Apotomopterus is monophyletic and its origin may be traced back to almost the time of the explosive radiation of the Carabina. This makes it seem likely that *Apotomopterus* consitutes one of the divisions Spinulati. Shortly after the radiation, four (or five, in case *A. masuzoi* is considered) lineages diverged almost simultaneously, presumably somewhere in the southeastern region of continental China (Fig. 5.14a).

As shown in Fig. 5.14b, the distribution ranges of the four lineages are wide and overlap considerably. Nȯ definite distribution boundaries between them can be drawn. This suggests that the ancestor of *Apotomopterus* was divided into at least four isolates upon geographic changes on the continent ca. 37 MYA. Then each isolate expanded its distribution to occupy its present habitat.

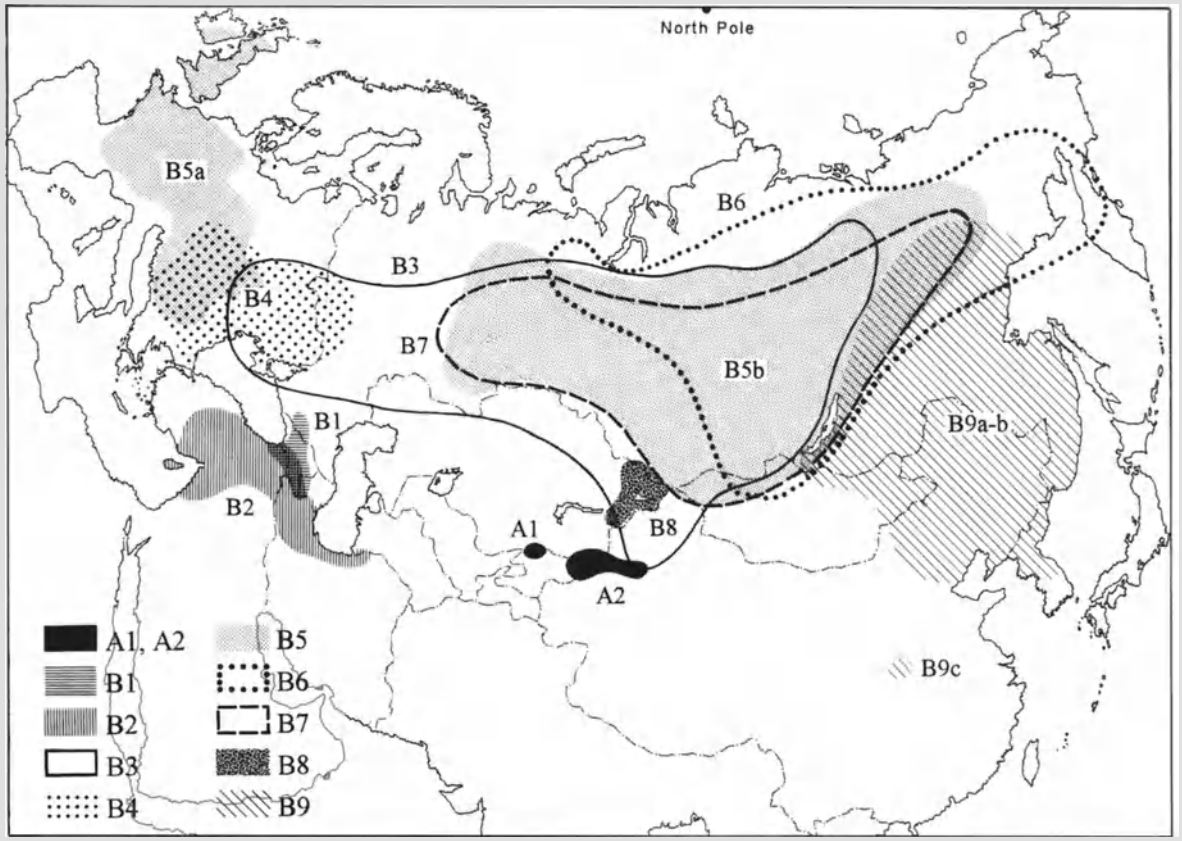


FIG. 5.15. Distribution of the division Lipastromorphi. Distribution area for each lineage in this figure (see Fig. 5.17) is after Breuning (1937) and Battoni et al. (1995). A number of species have not been analyzed for the *ND5* gene sequence (after Su et al. 2003a)

From an evolutionary point of view, *Apotomopterus* has two remarkable features. One is the occurrence of the same species in lines separated long ago, as seen in three *A. sauteri* sublineages (silent evolution). It is also the case in *A. clermontianus* and *A. maolanensis,* which are morphologically similar and yet their separation occurred a long time ago. The second remarkable feature of *Apotomopterus* is that certain species (e.g., *A. hupeensis*) emerged with considerable morphological change from *A. sauteri* (discontinuous evolution), whose morphology has remained almost unchanged for a long period. These phenomena will be discussed in more detail in Chapter 8.

Lipastromorphi

This division is a fairly large group in the subtribe Carabina, the distribution range of which covers a great part of the Eurasian Continent including some adjunctive islands such as the British Isles and Sakhalin, while this group is poorly represented in southern continental China, and none has been recorded in Japan (Fig. 5.15). All the species are without hind wings and can move only by walking as with most other Carabina species.

This division is currently classified into seven (sub)genera and altogether 152 species have been recognized (Březina 1999). Some of the species reveal considerable geographic and individual variations in morphology. This makes the taxonomy of this group rather ambiguous. The representative species are shown in Fig. 5.16.

A phylogenetic tree based on the *ND5* gene sequence from 85 individuals of the Lipastromorphi containing 40 species representing all the known genera of this division is shown in Fig. 5.17. The genera belonging to this division are *Cyclocarabus, Ophiocarabus, Cryptocarabus, Lipaster, Mimocarabus*, and *Morphocarabus*. The tree has made it possible to suggest an evolutionary history and classification for this division that cannot be achieved through morphology alone.

The Lipastromorphi specimens analyzed in this study are divided into two lineages (A and B) (Fig. 5.17). Their separation was estimated to have occurred about 35 MYA.

Lineage A is further divided into two groups, A1 and A2, which respectively include the genus *Cyclocarabus* and a complex of two genera, *Ophiocarabus* and

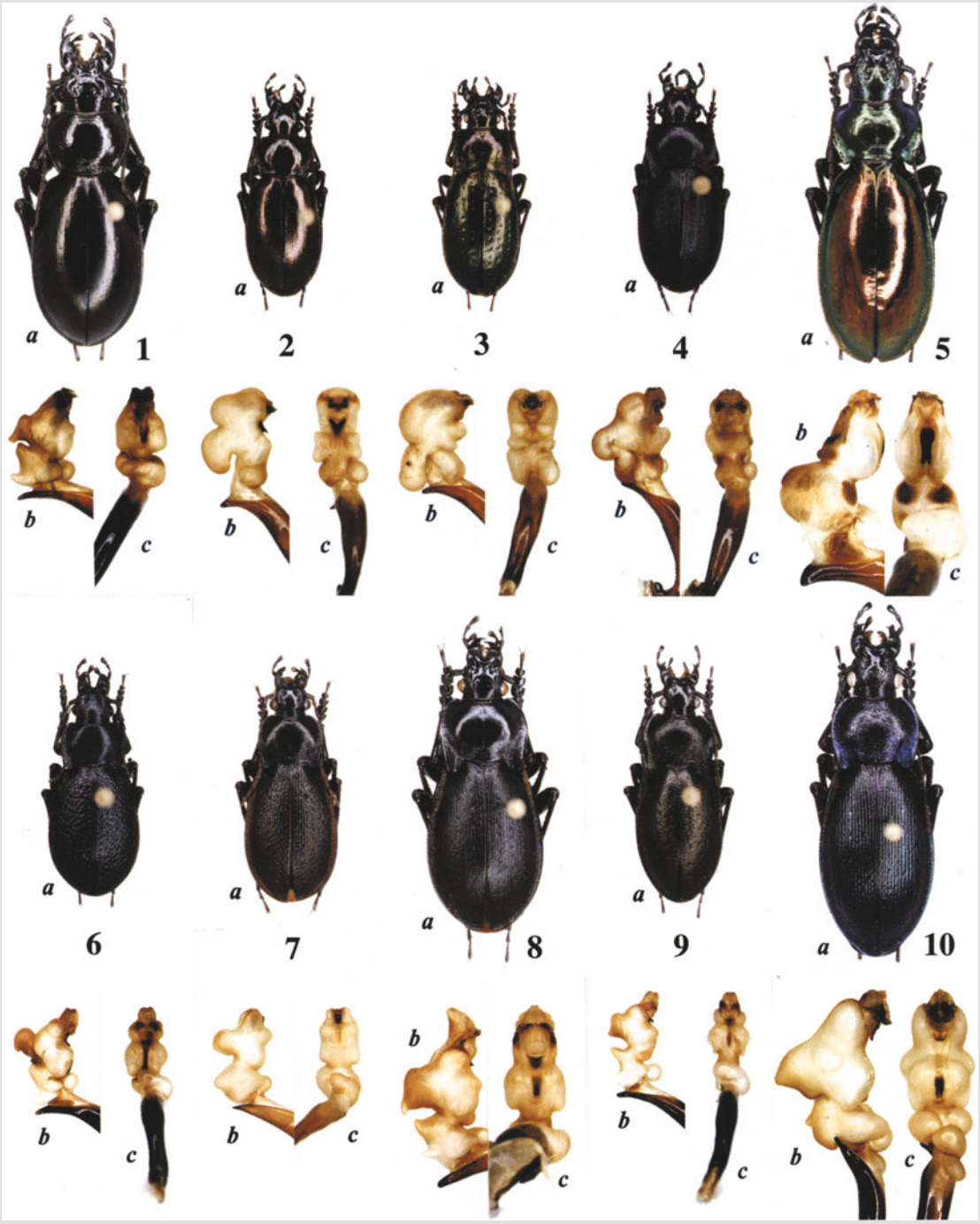


FIG. 5.16. Representative species of the division Lipastromorphi. **1a–c** *Cyclocarabus pseudolamprostus*, **2a–c** *Ophiocarabus striatus*, **3a–c** *Op. aeneolus*, **4a–c** *Cryptocarabus subparallelus*, **5a–c** *Lipaster stjernvalli*, **6a–c** *Mimocarabus maurus hochhuthi*, **7a–c** *Morphocarabus estreicheri*, **8a–c** *Mo. mandibularis buchtarmensis*, **9a–c** *Mo. scabriusculus*, **10a–c** *Mo. monilis scheidleri*

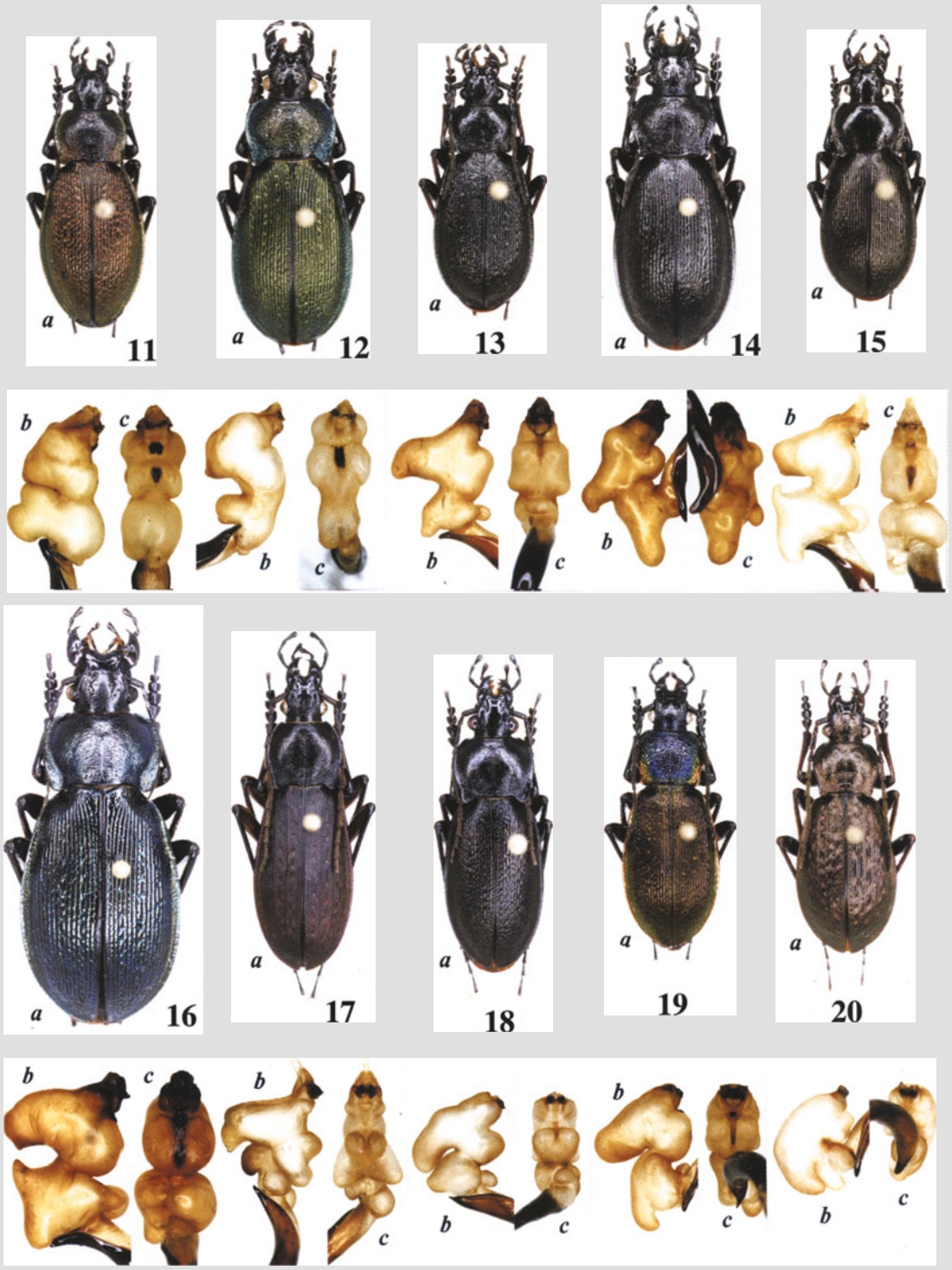


FIG. 5.16. **11a–c** *Morphocarabus henningi*, **12a–c** *Mo. regalis*, **13a–c** *Mo. odoratus krugeri*, **14a–c** *Mo. aeruginosus*, **15a–c** *Mo. tarbagataicus*, **16a–c** *Mo. gebleri ultimus*, **17a–c** *Mo. venustus liaoningensis*, **18a–c** *Mo. latreillei*, **19a–c** *Mo. hummeli*, **20a–c** *Rhigoidocarabus zhubajie*. **a** Habitus, **b** male genital organ in right lateral view, **c** in basal view

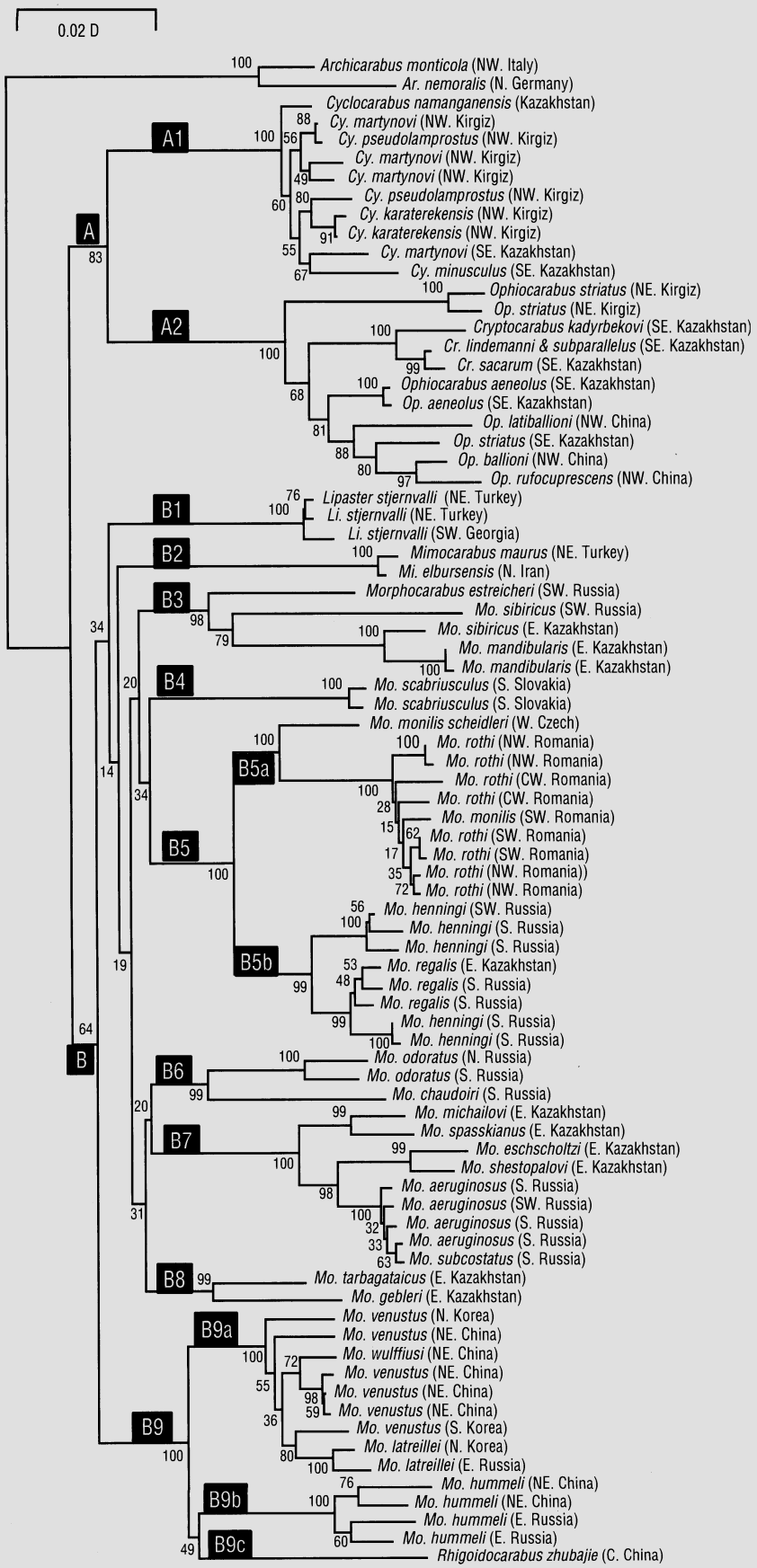


FIG. 5.17. Phylogenetic tree of the mitochondrial *ND5* gene for the division Lipastromorphi. Constructed using the NJ-method (After Su et al. 2003a)

Cryptocarabus, all of which have been found only in the Tianshan Mountains of Kazakhstan in Central Asia. A1 and A2 separated as long ago as 30 MYA.

There are five morphological species belonging to the genus *Cyclocarabus* from the sublineage A1 (*namanganensis, martynovi, pseudolamprostus, karaterekensis* and *minusculus*), and yet the genetic sequences of these were hardly distinguishable from each other. Their independence as distinct species should be carefully reexamined.

The branching points for species in the sublineage A2 are relatively deep. *Ophiocarabus striatus* appears in two distinctly separate clusters. One specimen from the Ketmen Mountains, Kazakhstan, is clustered with four other *Ophiocarabus* species, and two specimens from the Terskei Ala-Too, Kirgiz, fall out in the remotest place from the specimens from the Ketmens. This suggests that the ancestor of *Op. striatus* was isolated into at least two lines, and their morphology has remained almost unchanged. Alternatively, *Op. striatus*-type morphology emerged in parallel in two different lines.

All the four *Cryptocarabus* species examined branched off within the sublineage A2 relatively recently. In other words, the *Cryptocarabus* species are intermingled with the *Ophiocarabus* species on the tree. In the *Ophiocarabus* species, the upper surface usually bears a metallic tint, the legs are more or less reddish and the body is more slender. In the *Cryptocarabus* species, on the other hand, the upper surface is usually black and mat, the legs also black and the body more robust in shape. The endophallic structure of the male genitalia is similar in the two subgenera, with specialization of the lacinia varying considerably among the species (see Fig. 5.16).

The sequence divergence of the four *Cryptocarabus* species examined (*kadyrbekovi, sacarum, lindemanni*, and *subparallelus*) is small. The sequence of *Cr. lindemanni* is identical with that of *Cr. subparallelus* (not shown in the tree). Here again, reexamination of the taxonomic status of each "species" may be required. Note that these two specimens were found in localities near one another (the northwestern foot of the Zailiiskii Alatau and the Kok-Tinbe Mountains near Almaty).

The taxonomy of the lineage A based on morphology is partly consistent with the molecular data at genus level, but the traditional separation of *Ophiocarabus* and *Cryptocarabus* based on morphology should be reconsidered (see above). The taxonomy at species level is considerably confused, and should be reexamined by taking the results of molecular analysis into account. It is possible that some or many groups that have been thought of as distinct species might be individual and/or geographic variants of the same species.

Lineage B contains the species classified as belonging to the genera *Lipaster, Mimocarabus, Morphocarabus*, and *Rhigoidocarabus*, and yet this lineage is separated into the nine sublineages, B1–B9. Their branching order cannot be properly analyzed because of the short branch lengths supporting the respective lineages with low bootstrap values. This suggests that these groups radiated within a short time, ca. 30 MYA.

The sublineage B1 is composed of a sole species, *Lipaster stjernvalli*, from northeastern Turkey and southwestern Georgia. The sequences of the three specimens examined were almost the same.

The sublineage B2 is represented by two species belonging to the genus *Mimocarabus*, namely, *Mi. maurus* from northeastern Turkey and *Mi. elbursensis* from northern Iran. They are morphologically distinct despite revealing almost the same *ND5* gene sequences. No reasonable explanation can be given to this phenomenon until more specimens are analyzed.

The validity of *Mimocarabus* and *Lipaster* as two independent subgenera is supported by the present molecular analysis, because they form independent groups.

The sublineage B3 consists of three dark-colored *Morphocarabus* species, *estreicheri* (southwestern Russia), *sibiricus* (southwestern Russia and eastern Kazakhstan), and *mandibularis* (eastern Kazakhstan). This group is separated into three lines that radiated fairly long ago (ca. 25 MYA). Note that *Mo. sibiricus haeres* from southwestern Russia is quite remote from another subspecies, *obliteratus*, from eastern Kazakhstan, which is clustered with another Kazakhstan species, *Mo. mandibularis*, suggesting that the phylogeny of this species is geographically linked and does not reflect the morphological differences. Molecular phylogeny shows that all the species in the sublineage B3 are only remotely related to other *Morphocarabus* species belonging to groups four to nine (see below).

The sublineage B4 consists of a single species, *Morphocarabus scabriusculus*, found in southern Slovakia, forming most probably an independent clade.

Specimens belonging to the sublineage B5 consist of four *Morphocarabus* species, and are separated into two major subgroups, B5a and B5b. The B5a contains two species, *Mo. monilis* and *Mo. rothi* from southern Bohemia and several parts of western Romania. Since these two species, with the exception of *Mo. monilis scheidleri*, are intermingled in the cluster B5a with only very small sequence differences, they may be the same "phylogenetic" species. The subgroup B5b consists of *Mo. henningi* and *Mo. ragalis* from the southern Ural region and southern Siberia in Russia. These two species are made distinct by their differently shaped endophallus (Fig. 5.16), and yet they are not distinguishable phylogenetically because of their intermingled occurrence in the cluster B5b with only small sequence differences. There is no doubt that these two "species" are phylogenetically close to one another.

The sublineage B6 includes two species, *Morphocarabus chaudoiri* (southern Siberia) and *Mo. odoratus* (the polar Ural region and southern Siberia in Russia), that separated long ago (ca. 20 MYA).

The sublineage B7 contains five species of *Morphocarabus* collected from the southern Ural and southern Siberian regions, and is roughly divided into three well-separated clusters. The first cluster contains two species, *Mo. michailovi* and *Mo. spasskianus,* both from eastern Kazakhstan, which are well-separated. The second one contains two, also well-separated, Kazakhstan species, *Mo. eschscholtzi* and *Mo. shestopalovi.* The third one consists of two allied species, *Mo. aeruginosus* and *Mo. subcostatus*, from southern Ural and southern Siberia, and the evolutionary distance among all the specimens examined was almost nil. Whether these two are distinct species or not should be reexamined.

The sublineage B8 contains two species, *Morphocarabus tarbagataicus* and *Mo. gebleri*, both from the easternmost part of Kazakhstan. The branching point of these two species is rather deep, and yet they may be regarded as belonging to a single category on the *ND5* trees. This is consistent with the similarity in endophallic structures in these two species (Fig. 5.16).

The sublineage B9 is further divided into three lines, B9a, B9b, and B9c, which radiated ca. 20 MYA. The B9a includes three *Morphocarabus* species, *venustus*, *wulffiusi*, and *latreillei*, from northeastern China, the Korean Peninsula, and Amur. The B9b is composed solely of *Mo. hummeli* from northeastern China, Amur, and Sakhalin. In both the B9a and B9b, diversification started relatively recently at ca. 9 MYA. Note that three species are intermingled in the B9a without forming a species-specific cluster, suggesting that the phylogeny does not necessarily reflect the morphology. The B9c is composed of a single species, "*Carabus*" *zhubajie*, from Shaanxi in central China. This species was originally described as a member of the (sub)genus *Rhigocarabus* in the division Latitarsi (Imura 1993). Later, Deuve (1997) placed it in the Lipastromorphi, proposing a new subgenus, *Rhigoidocarabus.* The phylogenetic tree is consistent with Deuve's view. As shown in Fig. 5.16.20, the endophallus of this species is deformed, and yet its basic structure as well as other morphological characteristics is in the range of the *Morphocarabus* groups.

As has already been pointed out, the major carabine divisions radiated explosively 50–40 MYA, followed by occasional radiation events varying in scale. The Lipastromorphi is one of the divisions that emerged upon the initial radiation. Its diversification started about 35 MYA with separation of the lineages A and B, followed by radiation of various sublineages included therein within a short time. Thus, the evolutionary history of each lineage and sublineage is venerable, corresponding to four-fifths to one-half the history of the Carabina evolution.

As shown in Fig. 5.15, the distribution of each sublineage as well as the extant species are geographically linked to a considerable extent, although partial or considerable overlapping is seen in the distribution range of most of the sublineages in the lineage B. Overlapping is especially notable in groups with wide a distribution range, i.e., B3, B5b, B6, and B7.

Sublineages A1 and A2 in the lineage A are strictly endemic to the Tianshan Mountains and nearby regions and appear parapatrically. Sublineages B1 and B2 are composed of species distributed from northeastern Turkey to the Caucasus region and those from Asia Minor to northern Iran, respectively. Of the three species in the group B3, *Mo. sibiricus* has the widest distribution range, from northern Ukraine to central-eastern Siberia, covering the ranges of the other two species, *Mo. estreicheri* and *Mo. mandibularis.*

The sublineage B4 is composed of a single species, *Mo. scabriusculus*, which is distributed mainly in eastern Europe. The sublineage B5a is made up of several races of *Mo. monilis* and *Mo. rothi* from the western Czech Republic and western Romania. The distribution ranges of these two species cover almost all of central Europe, Great Britain, and the eastern part of Ireland. The species in the sublineages B3, B5b, B6, and B7 are distributed very widely in central and eastern Eurasia, and their ranges overlap in central-southern Siberia.

The distribution range of the sublineage B8 is narrowly restricted to the easternmost part of Kazakhstan with partial penetration into Russian territory. The group B9 is rather widely distributed in the easternmost part of the Eurasian Continent including Sakhalin.

It is likely, although by no means certain, that the ancestor of the Lipastromorphi emerged in Central Asia, above all in the Tianshan Mountains and nearby regions, and then split into the two lineages, A and B. This view may be supported by the fact that the species in the lineage A inhabit only this region and five of nine groups in the lineage B occur in southern Siberia, northeast of the Tianshan Mountains (see Fig. 5.15).

Perhaps the prototypes of the lineage B started to expand their distribution westward and eastward from southern Siberia, followed by geographical and/or reproductive isolation to form phylogenetically isolated groups. Occupation of wide distribution ranges for B3, B5b, B6, and B7 might have been resulted from secondary expansion that occurred after the initial isolation. The partial overlapping between the sublineages may also have been caused by secondary migration. In contrast to the lineage B, migration of the species in the lineage A has been limited to the area around the Tianshan Mountains.

During the Lipastromorphi diversification, definite morphological differentiation took place between the sublineages A1, A2, B1, B2, and B3–B9 as deduced from morphologies of the extant species. However, differences in morphological characteristics in the sublineages B3 to B9 do not exceed the differences among the species within a sublineage. In other words, the existence of the definite sublineages B3 to B9 can only be ascertained by means of molecular phylogeny and not by morphology alone. The sublineages B3 to B9 have

not undergone much morphological differentiation in spite of their long evolutionary history, in an example of silent evolution (Osawa et al. 1999; Su et al. 2001), which will be discussed in more detail in Chapter 8.

During the more or less "silent" morphological evolution mentioned above, species with distinct morphological characteristics occasionally branched off from a stem within a certain group. The appearance of the *Cryptocarabus* cluster between two *Ophiocarabus* clusters in the sublineage A2 can be explained by morphological differentiation from the *Ophiocarabus*-type to the *Cryptocarabus*-type.

This will make it clear that there are a number of discrepancies between the mitochondrial DNA phylogeny and the classification as defined by morphological evidence. The reasons for this may be different from one case to another, and more detailed examinations both from the viewpoints of molecular phylogeny and morphology are urgently required. The arrangement of the component genera in the Lipastromorphi will be discussed in more detail in Chapter 9.

The phylogenetic separation of *Morphocarabus* into six independent clusters suggests the necessity of having corresponding "phylogenetic genera," even if they are morphologically indistinguishable.

Latitarsi

The Latitarsi are a large taxonomic division of the Carabina, and are widely distributed throughout the Holarctic region and the northern periphery of North Africa. Morphologically, they have been classified into 17 genera (Imura 1996), which include 168 species (Březina 1999). The representative species of this division are shown in Fig. 5.18.

Phylogenetic trees based on the mitochondrial *ND5* gene sequences for some groups in this division have been reported. Those groups examined in this way include the *Oreocarabus* complex (Imura et al. 1998b), *Leptocarabus* (Kim et al. 2000a,b), and *Tomocarabus*, found in the Japanese Islands (Su et al. 2000c).

The representative genera in this division seem to have radiated within a relatively short time after the radiation of the Carabina 50–40 MYA, as deduced from an *ND5* phylogenetic tree. This division is most probably polyphyletic (Imura et al. 1998b). This situation makes it quite difficult to estimate the phylogenetic position of various Latitarsi groups in a meaningful way.

Figure 5.19a shows an NJ-phylogenetic tree of almost all genera that have been taxonomically classified as belonging to the division Latitarsi, together with a few representative species of other taxonomic divisions. The examined materials include 165 species consisting of 77 species from various divisions including 38 species from the division Latitarsi. On the tree were recognized a considerable number of lineages that emerged within a short period after the radiation of the Carabina as judged by short branch lengths with low bootstrap values, so that it is impossible to determine their branching order with certainty. As for the Latitarsi, at least 16 remotely related lineages may be recognized.

As compared with other divisions, which are mostly monophyletic, the Latitarsi seems to be polyphyletic. *Cavazzutiocarabus latreillei* (=*latreilleanus* under the genus *Carabus* s. lat.) (lineage H) and *Autocarabus cristoforii* (lineage L) are even weakly and indecisively clustered with members of other divisions. In addition, the branching point of *Autocarabus* (lineage A), *Mesocarabus* (lineage B), and *Orinocarabus* (lineage C) are as deep as those of other taxonomic divisions such as Procrustimorphi (lineage D), Archicarabomorphi (lineage E), Lipastromorphi (lineage F), Digitulati (lineage G), Lepidospinulati (lineage I), Crenolimbi (lineage J), Spinulati (lineage K), and Arciferi (lineage M), so that the lineages A, B, and C are not likely to have a direct phylogenetic affinity with other members of the Latitarsi.

In other Latitarsi members, the lineages N to X are weakly clustered as one group, but their topologies on the *ND5* tree are somewhat unstable upon replacement of an outgroup, the use of another method of phylogenetic tree construction such as the UPGMA or MP approach, or the addition/removal of species.

In *Tomocarabus* (except *To. harmandi*), *Tanaocarabus*, *Ulocarabus*, *Carpathophilus* (Imura et al. 1998b), *Scambocarabus*, and *Semnocarabus*, the constituent species in each genus are always clustered together around the root of the lineage X in Fig. 5.19a upon various treatment, having given essentially the same topology. Details of each lineage are described below.

Lineages A and L. There exist two distinct lineages for the *Autocarabus* species, which reveal no direct phylogenetic affinity to each other on the tree (A and L in Fig. 5.19a). Their emergence is estimated to have taken place around the same time as, or somewhat earlier than, the radiation of the Carabina. The lineage A is divided into two sublineages, A1 and A2, with a deep branching point.

The sublineage A1 includes a sole species, *Autocarabus auratus*, found in eastern France, while A2 is comprised of several subspecies of *Au. cancellatus*, found in various localities in Europe and western Russia. In the sublineage A2, subspp. *tuberculatus* (western Russia), *emarginatus* (northwestern Italy) and *carinatus* (eastern France) show affinity with each other and considerable difference from two specimens of subsp. *graniger* found in western Russia. Morphologically, they display a metallic dorsal surface and marked preapical emargination of the female elytra, which are rather exceptional in the Latitarsi.

On the other hand, the endophallic morphology of these two species is similar, and no one has yet pointed out the fact that the *Autocarabus* species in the lineage A occupies such an independent phylogenetic position

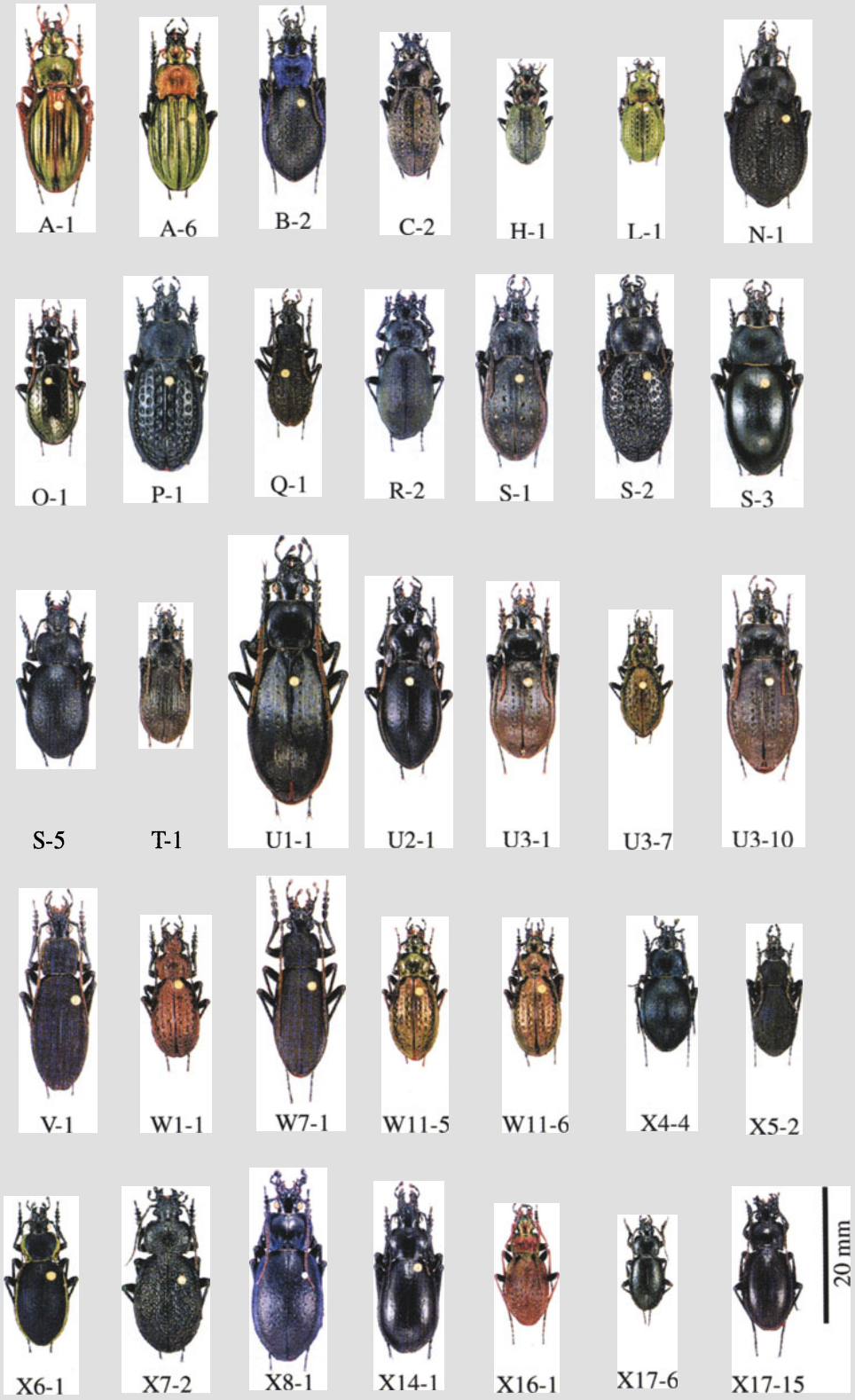


FIG. 5.18. Representative species of the division Latitarsi. Symbols/numbers for the photographs correspond to those shown after scientific names and localities in Fig. 5.19

in the Carabina. Indeed, lineage A can be regarded not only as an independent genus but also as a distinct division on the *ND5* tree.

The lineage L contains a single species *Autocarabus cristoforii*, which is a small-sized species with an external appearance at variance from other *Autocarabus* species only in having a characteristic elytral sculpture, and is endemic to the Pyrenees of southwestern Europe. This species is highly phylogenetic independent from other *Autocarabus* species as evidenced by the present molecular phylogeny.

Lineage B. *Mesocarabus problematicus* is widely found in Europe and its adjacent islands, and is the sole constituent of the lineage B. The evolutionary distance between the specimens from eastern France (subsp. *planiusculus*) and those from northern Germany (subsp. *harcyniae*) is fairly large.

Morphologically, *Mesocarabus* has no distinct features in male genital structures as compared to other members of the Latitarsi. However, the *ND5* tree shows that it belongs to an independent lineage, which may be regarded as a distinct division.

Lineages C, H, P, S, U, W13, and X16. These lineages and sublineages have been taxonomically placed in one genus "*Oreocarabus*" (Imura 1996) consisting of five species-groups (Imura and Mizusawa 1996). Four of them were previously analyzed for the *ND5* sequence in order to understand their phylogenetic relationship (Imura et al. 1998b).

It was recognized that there were eight independent lineages, which were respectively treated as independent genera: *Orinocarabus* (**C**), *Cavazzutiocarabus* (**H**), *Cytilocarabus* (**P**), *Euporocarabus* (**S**), *Phricocarabus* (**S**), *Titanocarabus* (**U1**), *Rhigocarabus* (**W**), and *Carpathophilus* (**X16**) (Imura et al. 1998b). Figure 5.19b shows a tree revealing the polyphyletic nature of the Latitarsi, especially of the so-called *Oreocarabus*. Note that *Phricocarabus glabratus* is clustered with the *Pachystus* species (lineage S; see below).

The "*Oreocarabus*" species found in China were rearranged on the basis of morphological characteristics in the following manner (Imura 1998b) (component species are shown in brackets): *Titanocarabus* [*titanus, sui*]; *Qinlingocarabus* [*kitawakianus, reitterianus, nanwutai, blumenthaliellus*]; *Heptacarabus* [*ohshimaianus*]; *Piocarabus* [*vladimirskyi*]; *Rhigocarabus* [*latro, qinlingensis, laotse, tewoenisis, mikhaili*].

Thereafter, several species were added to *Rhigocarabus*. The Chinese "*Oreocarabus*" species, with the exception of *Rhigocarabus,* form a well-defined cluster on the *ND5* tree (lineage U), which may be divided into three subclusters. To the first one (sublineage U1), *Titanocarabus titanus* and *Ti. sui* belong. The second sublineage (U2) contains two specimens of *Piocarabus vladimirskyi*. The third sublineage (U3) includes all four species of *Qinlingocarabus* (*kitawakianus, reitterianus, nanwutai, blumenthaliellus*) and *Heptacarabus ohshimaianus*.

Overall, there was fairly strong correlation between the morphology and the molecular phylogeny at genus level. Two exceptions are *Heptacarabus*, which is clearly in the third subcluster to which all the *Qinlingocarabus* species belong. *Rhigocarabus choui* is clustered with the *Qinlingocarabus* species and is not included in the lineage W, to which all other *Rhigocarabus* species belong (see below).

The morphological reexamination of *choui* reveals that, in spite of the similarity in external structure to that of *Rhigocarabus*, the endophallus of male genitalia is undoubtedly of the *Qinlingocarabus*-type (see Fig. 8.6).

All the species in the lineage U are distributed in southwestern and western China. At species level, *Titanocarabus titanus* and *Ti. sui* are intermingled on the tree with only small sequence differences. They may be two geographic races of the same species, despite some difference in their male genitalic morphology. The difference between *Qinlingocarabus kitawakianus* and *Qi. nanwutai* is very small. The respective pairs may be conspecific. Thus, the Chinese "*Oreocarabus*" includes a number of taxonomic ambiguities, and should be reexamined.

Two "*Oreocarabus*" species, *cribratus* and *porrectangulus*, should be placed in the genus *Cytilocarabus* (Imura et al. 1998b). The sequence analysis show that *Cy. cribratus* and *Cy. porrectangulus* are not clearly separated on the phylogenetic tree (lineage P). Indeed, these two "species" are morphologically very close with only a small difference in the shape of the aedeagal appex of the male genitalia. These two may very well be conspecific.

"*Oreocarabus*" *gemellatus*, found in Iran, is unambiguously clustered with *cribratus/porrectangulus* with a reasonable evolutionary distance. Thus, this Iranian species should be considered as a member of the lineage P.

Lineages N and O. Of the two species in the genus *Eurycarabus*, *Eu. famini* from North Africa was available for the DNA analysis. They form an independent lineage (lineage N). The genus *Nesaeocarabus* contains three species, all endemic to the Canary Islands. One of them, *Ne. abbreviatus* (the type species of *Nesaeocarabus*), has been analyzed. This species forms lineage O, and appears to be related to the *Eurycarabus* lineage (N).

These results suggest the possibility that two genera were derived from common ancestry, with the two becoming isolated when the Canary Islands separated from the African Continent. Morphologically, *Eurycarabus* is made distinct by its small head, robust body, short antennae lacking hairless ventral depressions in the male, a thick and characteristically sculptured elytral surface and a unique membranous projection on the ventral wall of the endophallus. *Nesaeocarabus* is characterized by a polished body surface and the completely degenerated

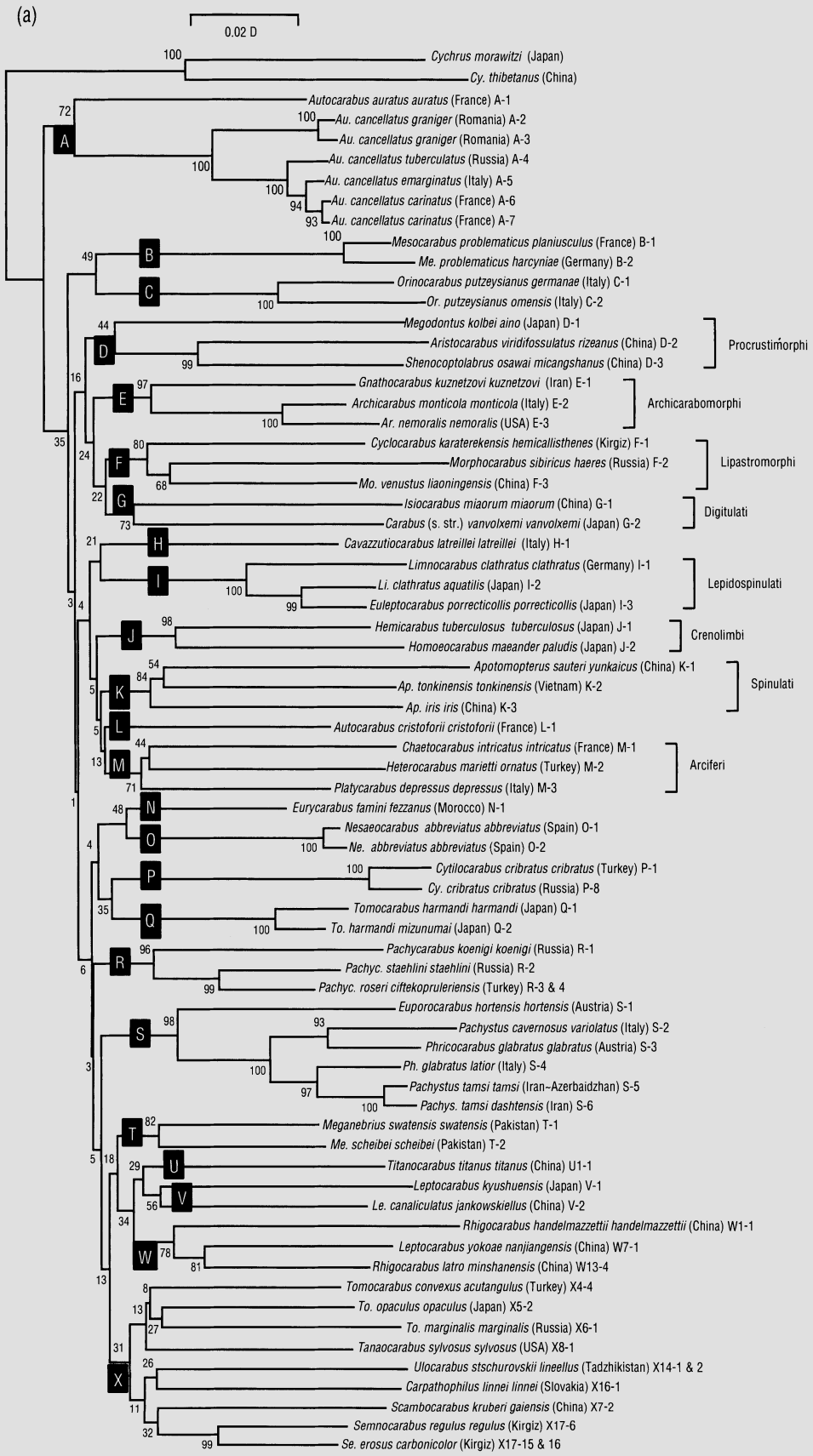


FIG. 5.19 (**a**) Phylogenetic tree of the mitochondrial *ND5* gene for the division Latitarsi showing its relationship to other divisions (lineages **D, E, F, G, I, J, K**, and **M**), constructed using the NJ-method. For symbols for lineages and sublineages, see the text

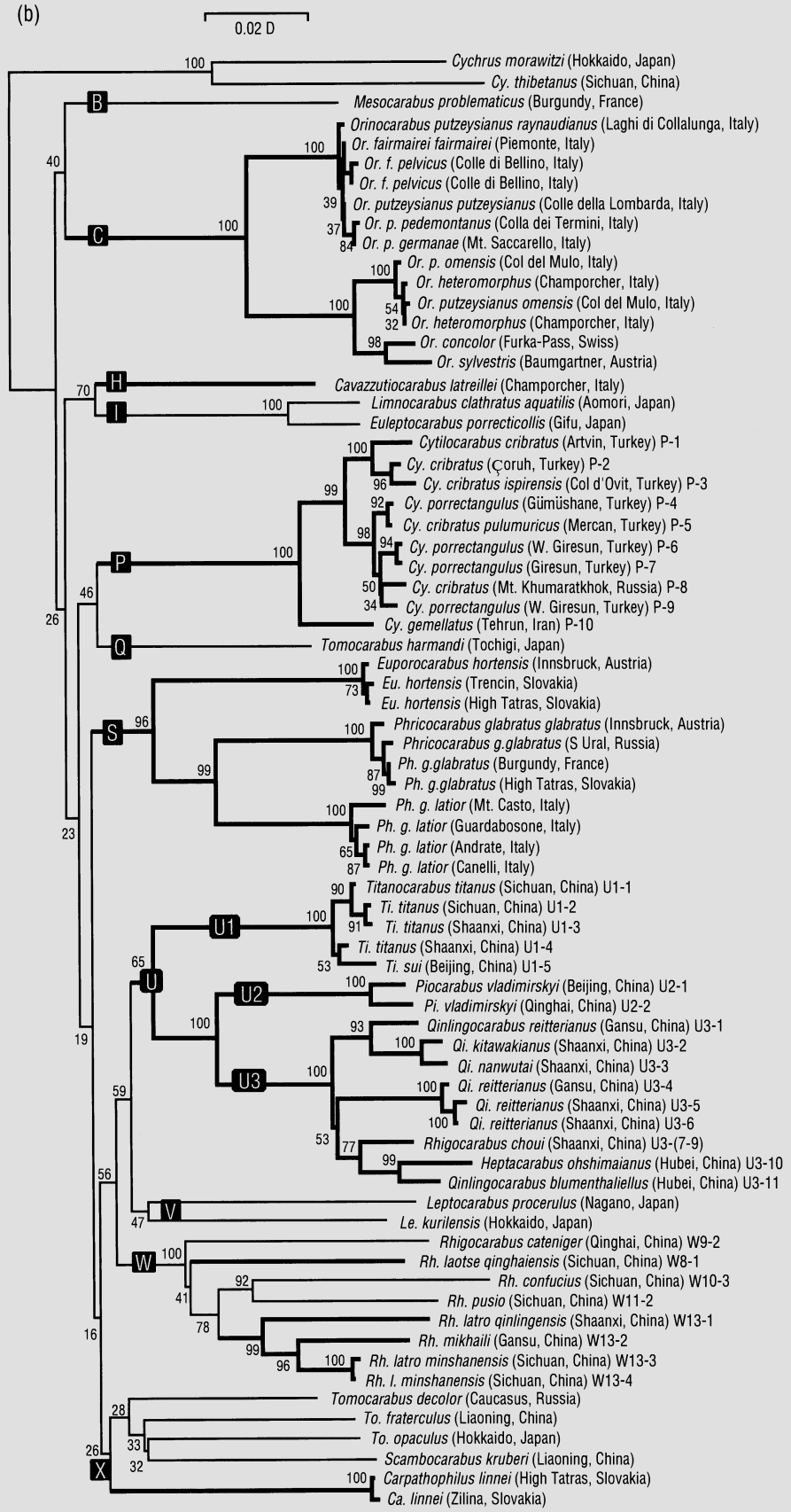


FIG. 5.19 (**b**) Phylogenetic tree of the mitochondrial *ND5* gene for the division Latitarsi showing that polyphyletic nature of the "*Oreocarabus*" group (**bold lines**)

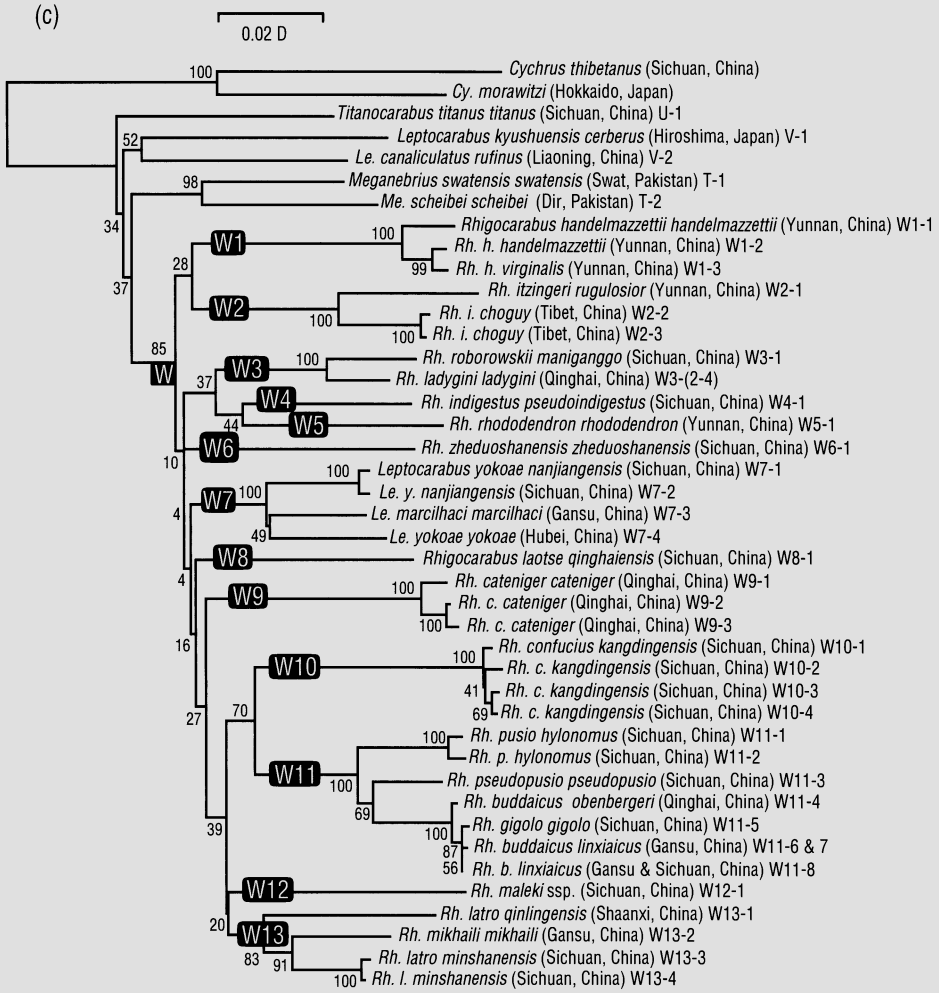


FIG. 5.19 (c) Phylogenetic tree of the mitochondrial *ND5* gene for the lineage W of the division Latitarsi

ostium lobe of the male genitalia. The most characteristic feature of the endophallic morphology of *Nesaeocarabus* is the presence of a strongly sclerotized projection on the ventral wall of the endophallus, which is suggestive of a remote affinity with *Eurycarabus*.

Lineage Q. This lineage contains only a single species, *Tomocarabus harmandi*, which is endemic to eastern Honshu, Japan. Geographic variations within this species have been investigated using the *ND5* gene sequence (see Chapter 7) (Su et al. 2000). Figures 5.19b and 5.19d show that the phylogenetic position of *To. harmandi* is rather remote from other *Tomocarabus* species.

Lineages R and T. Three species of *Pachycarabus* (*koenigi* and *staehrini* from the Caucasus region, and *roseri* from northeastern Turkey) form one cluster (lineage R). They are endemic to the Caucasus region including northeastern Turkey. Two species of the genus *Meganebrius*, *swatensis* and *scheibei*, both from northern Pakistan, form another cluster (lineage T). All the species in the lineages R and T bear a dark, mat body surface and are similar in morphology despite their phylogenetic independence, suggesting that similar morphologies arose in the two lineages in parallel.

Lineage S. This lineage consists of two major sublineages, which are represented by *Euporocarabus* and the *Pachystus-Phricocarabus* complex, respectively. *Pachystus cavernosus* from central and eastern Italy is clearly clustered with *Ph. glabratus* from Austria, and *Pachys. tamsi* from northern Iran is clustered with *Ph. glabratus* from northwestern Italy.

The branching point between the *cavernosus-glabratus* cluster and the *glabratus-tamsi* cluster is rather deep, having emerged about 20 MYA (Su et al. 2001). On the other hand, *Pachystus* and *Phricocarabus* are morphologically classified into two distinct genera. This may be interpreted as meaning that their ancestor divided into two lineages about 20 MYA, followed by a parallel emergence from *Phricocarabus* to *Pachystus* or vice versa in the respective lineages, probably by a discontinuous morphological change.

Lineage V. This lineage is composed of the species belonging to *Leptocarabus* (s. lat.) with the exception of

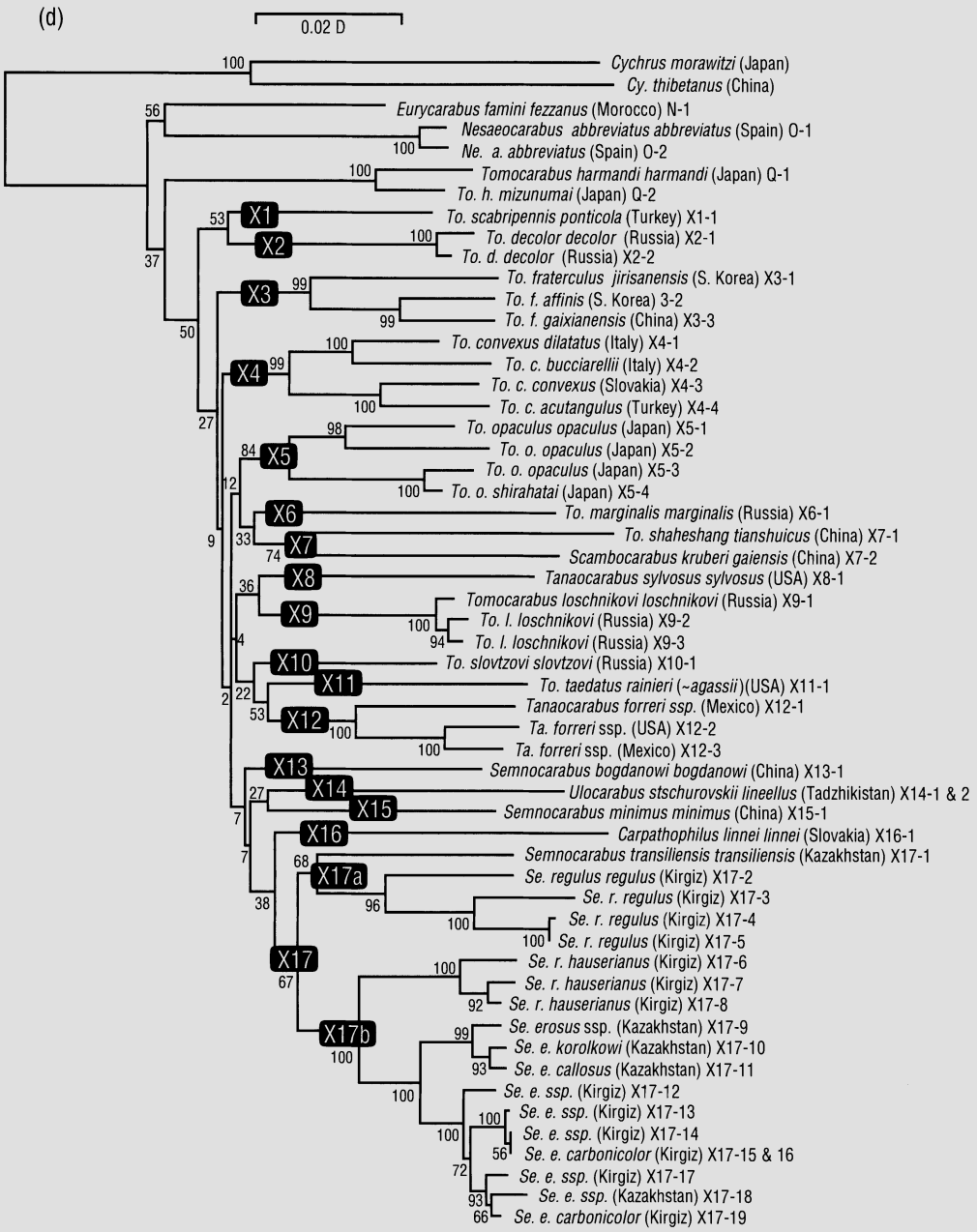


FIG. 5.19 (**d**) Phylogenetic tree of the mitochondrial *ND5* gene for the lineage X of the division Latitarsi (after Su et al. 2003e)

two Chinese "*Leptocarabus*" species, *yokoae* and *marcilhaci*. (Fig. 5.20). Three main groups of *Leptocarabus* become apparent when a molecular phylogenetic tree including the Chinese species is consulted (Fig. 5.21).

The three main groups are the Japanese group, the continental group and the Chinese group. A distribution map is shown in Fig. 5.22 (Kim et al. 2000a,b).

The Chinese group, however, is not included in the lineage V and is a constituent of the lineage W (see below) (Figs. 5.23 and 5.24). These results have been confirmed by analysis using nuclear 28S rDNA (see Fig. 5.24).

Lineage W. The species in the genus *Rhigocarabus* are the main constituents of this lineage, with inclusion of two Chinese "*Leptocarabus*" species (see above) (Su et al. 2001). *Rhigocarabus* contains 28 species and a number of subspecies (Březina 1999). Most of these are found in the high mountains of southwestern China (Fig. 5.25), and reveal similar external morphology so as to make their classification rather difficult.

Thirty-two specimens comprising 19 species from various localities have been examined for the *ND5* sequence (Fig. 5.19c). The *Rhigocarabus* species are clustered well with a high bootstrap value, and the Chinese "*Leptocarabus*" species, *yokoae* and *marcilhaci*, fall into this lineage.

Thirteen sublineages are recognized in this lineage, and eight of these consist of only a single species. It is

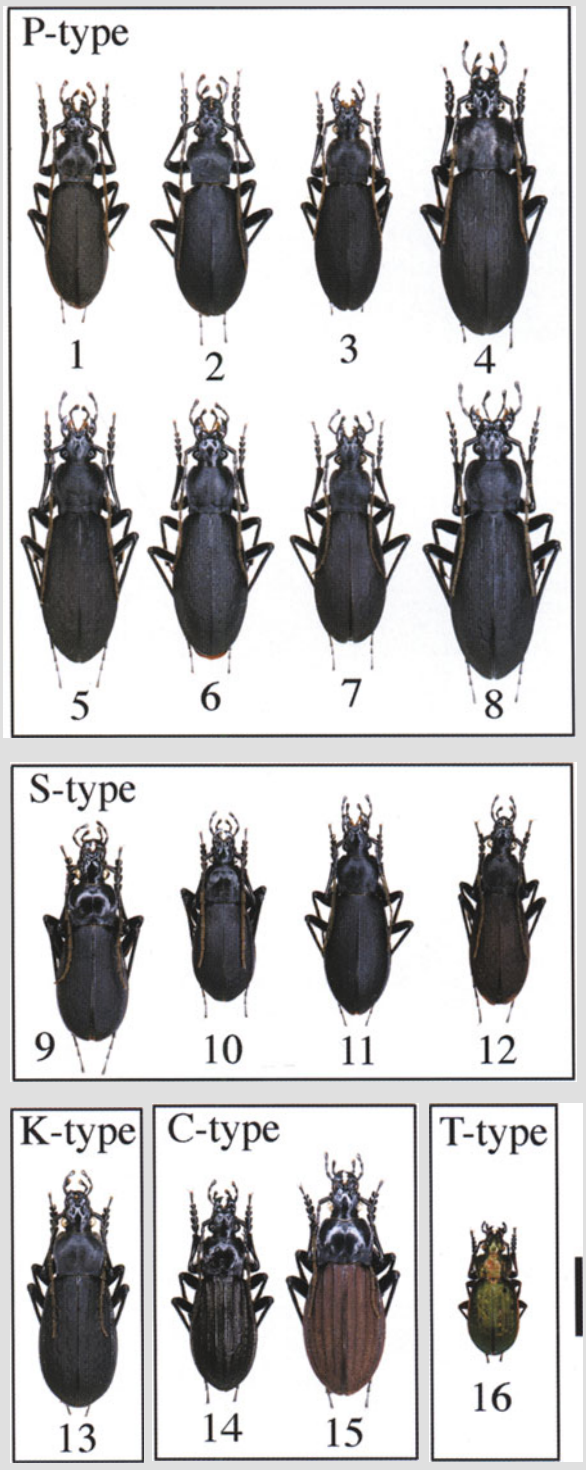


FIG. 5.20. Representative species of the genus *Leptocarabus*. **1** *Leptocarabus marcilhaci*, **2** *L. yokoae*, **3** *L. kyushuensis kyushuensis*, **4** *L. k. cerverus*, **5** *L. hiurai*, **6** *L. procerulus procerulus*, **7** *L. p. miyakei*, **8** *L. kumagaii*, **9** *L. semiopacus*, **10** *L. seishinensis*, **11** *L. arboreus arboreus*, **12** *L. a. gracillimus*, **13** *L. koreanus*, **14** *L. kurilensis rausuanus*, **15** *L. canaliculatus*, **16** *L. truncaticollis* (after Kim et al. 2000b)

difficult to determine the branching order of the sublineages with certainty because of the short branching lengths with low bootstrap values. In other words, a considerable number of the sublineages would have radiated within a short time about 30 MYA. Thirteen sublineages, W1 through W13, require further examination.

The sublineage W1 contains *Rh. handelmazzettii*, found in northwestern Yunnan.

The sublineage W2 includes three specimens of *Rh. itzingeri*, one (subsp. *rugulosior*) from northwestern Yunnan and two (subsp. *choguy*) from southeastern Tibet (Xizang). The evolutionary distance between the Yunnan specimen and the Tibet specimens is considerably large, although these two populations show at most subspecific morphological differentiation. These results suggest that the geographical divergence is not always accompanied by morphological changes.

The sublineage W3 is composed of *Rh. roborowskii maniganggo* from northwestern Sichuan and *Rh. ladygini* from eastern Qinghai with a reasonably deep branching point.

The sublineages W4–W6, W8, and W12 each comprise only a single species. W4 (*Rh. indigestus* from western Sichuan) might be remotely related to W5 (*Rh. rhododendron* from northwestern Yunnan).

The sublineage W7 contains two "*Leptocarabus*" species, *yokoae* and *marcilhaci*, from central China, found near regions in which some *Rhigocarabus* species are found. These two species are morphologically very different from other *Rhigocarabus* species, and are quite similar to some Japanese *Leptocarabus* species (Su et al. 2001). This will be discussed in more detail in Chapter 8.

The sublineage W8 contains only one species, *Rh. laotse* (subsp. *qinghaiensis*), from northwestern Sichuan.

The sublineages W9 and W10 constitute three specimens of *Rh. cateniger* from eastern Qinghai and several specimens of *Rh. confucius* from central-western Sichuan, respectively. The evolutionary distance between the species within the respective sublineages are small or almost nil.

The sublineage W11 is composed of four species, *Rh. pusio* (subsp. *hylonomus* from northern Sichuan), *Rh. pseudopusio* (from northern Sichuan), *Rh. buddaicus* (subspp. *obenbergeri* from eastern Qinghai, *linxiaicus* and *gansuicus* from southern Gansu), and *Rh. gigolo* (southern Gansu). *Rhigocarabus pusio* and *Rh. pseudopusio* form independent lines, and are distinctly separate from *Rh. buddaicus* and *Rh. gigolo* on the tree, while the evolutionary distance between *Rhigocarabus buddaicus* and *Rh. gigolo* is almost nil. Morphologically, *Rh. gigolo* is one of the most specialized species, having a peculiarly sclerotized ostium lobe on the membranous preostium of the male genital organ (Fig. 8.5.1), and is easy to distinguish not only from all the subspecies of *Rh. buddaicus* but also from any other *Rhigocarabus* species.

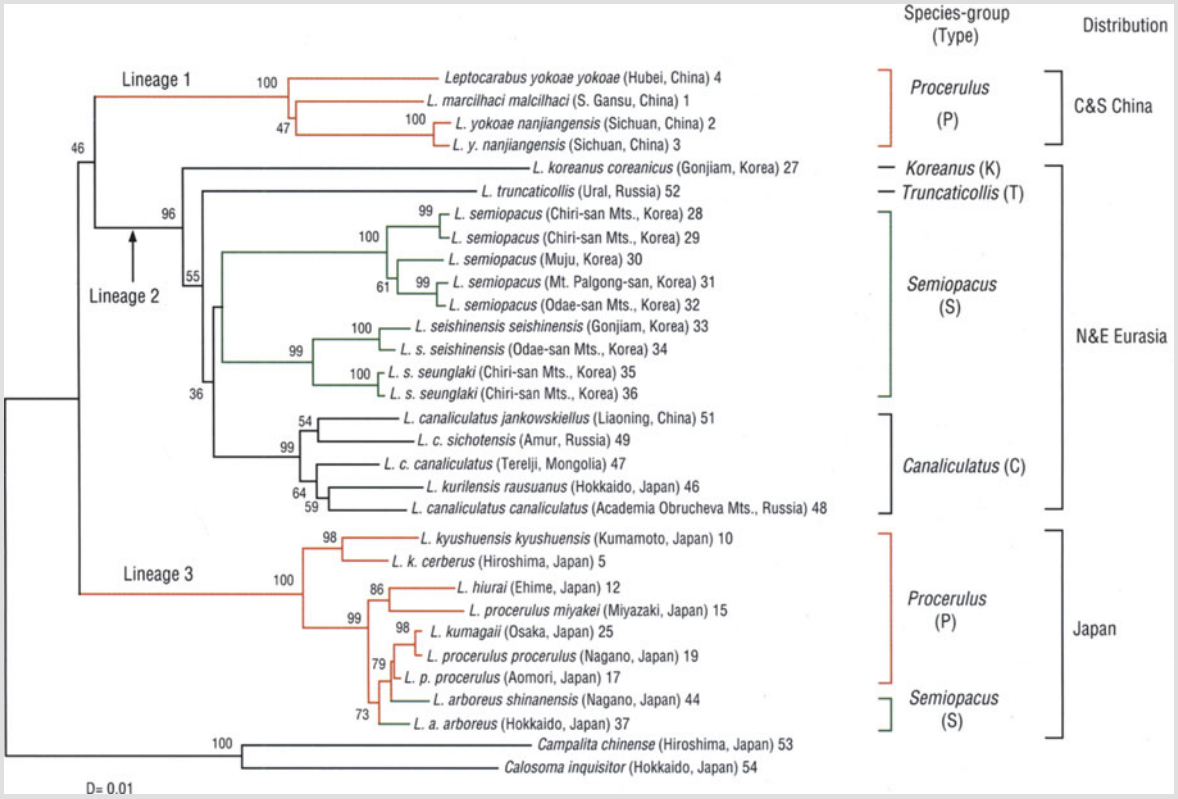


FIG. 5.21. Phylogenetic tree of the mitochondrial *ND5* gene for the genus *Leptocarabus*. Constructed using the NJ-method. Numbers after species name (locality) correspond to the locality numbers in Fig. 5.22 (after Kim et al. 2000b)

These findings suggest that rapid morphological change (discontinuous evolution) took place quite recently. W11 is remotely clustered with W10 (*Rh. confucius*) with a higher bootstrap value, suggesting that W10 and W11 share common ancestry dating from about the time of the *Rhigocarabus* radiation.

The sublineage W12 contains *Rh. maleki* from central-northern Sichuan.

The sublineage W13 is composed of three species, *Rh. qinlingensis* from southern Shaanxi, *Rh. mikhaili* from southern Gansu, and *Rh. latro* from northern Sichuan. *Rh. mikhaili* is remotely related to *Rh. latro*, with *Rh. qinlingensis* as their outgroup. Morphologically, the three species in this sublineage and *Rh. laotse* (W8) were placed in the *latro* species-group of the genus *Oreocarabus* (Imura and Mizusawa 1996). The molecular phylogenetic study of the "*Oreocarabus*" complex makes it clear that *latro* should be placed in the genus *Rhigocarabus* and is not a member of *Oreocarabus* (Imura et al. 1998b) (Fig. 5.19c). The other three species (*laotse*, *qinlingensis*, and *mikhaili*) also fall out in W13 of *Rhigocarabus* (Fig. 5.19c).

As shown in Fig. 5.15, not only is the distribution range of the lineage W narrowly restricted to southwestern China, but each species in the lineage W has its own habitat, strictly isolated from those of the other sublineages in most cases. This suggests, despite the long evolutionary history of this lineage, that the members of each sublineage did not much expand their distribution ranges after isolation, presumably because of their poor migration capability.

Lineage X. The species morphologically classified as belonging to the genera *Tomocarabus*, *Scambocarabus*, *Tanaocarabus*, *Ulocarabus*, *Carpathophilus*, and *Semnocarabus* (all established by Reitter in 1896) fall out in the lineage X with the exception of *Tomocarabus harmandi*, which forms the independent lineage Q (see above). The branching order in X is ambiguous, as they are supported by low bootstrap values.

A detailed phylogenetic tree of the lineage X is shown in Fig. 5.19d. There are considerable inconsistencies between the morphological classification and the molecular phylogeny. The species placed in the same genus do not form a single group, with morphologically defined genera not necessarily being monophyletic. This situation is especially apparent for the species of *Tomocarabus* (Imura and Mizusawa 1996), which appear in many distinct clusters.

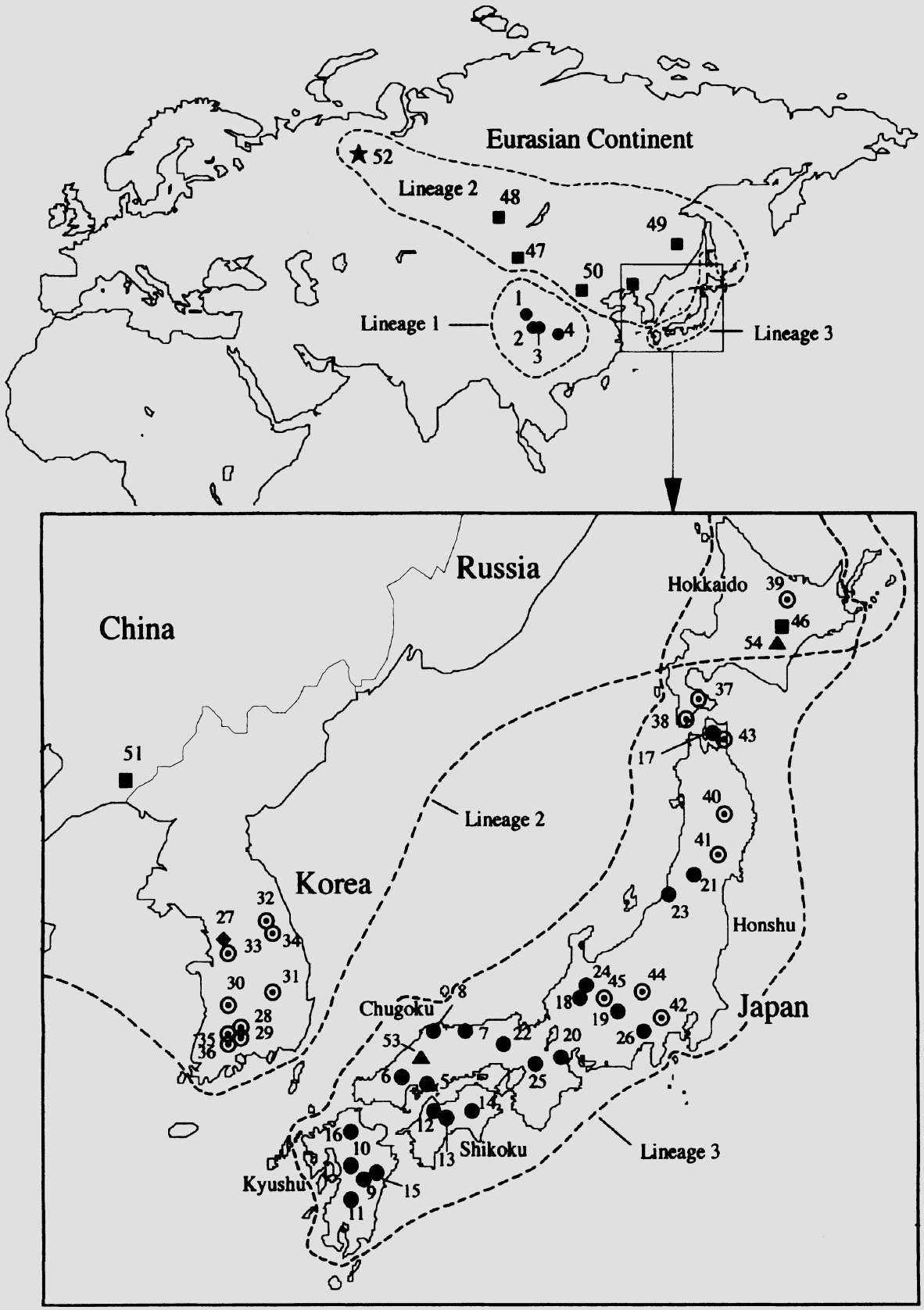
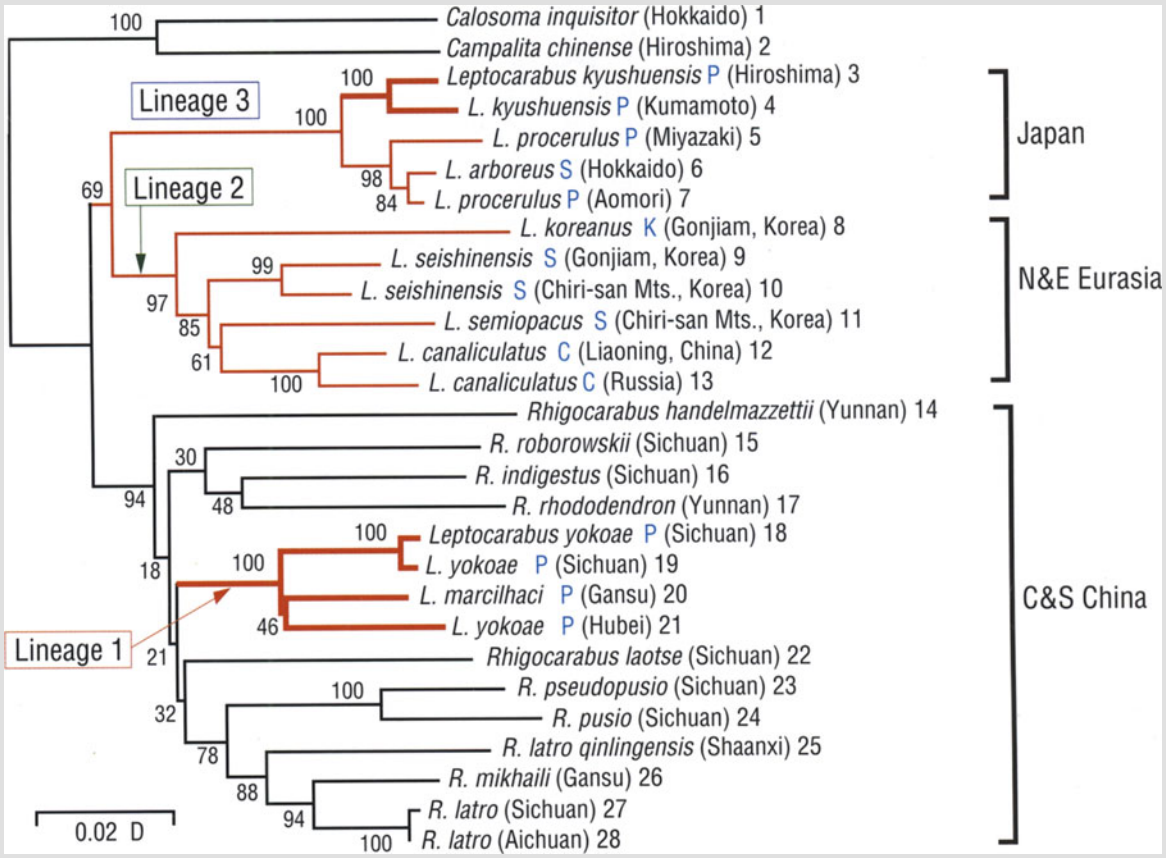


FIG. 5.22. Distribution of the *Leptocarabus* species. **Open circles**, S-type; **closed circles**, P-type; **diamond**, K type; **squares**, C-type; **star**, T-type; **triangles**, outgroup species. For numbers in the figure, see Fig. 5.21 (after Kim et al. 2000b)



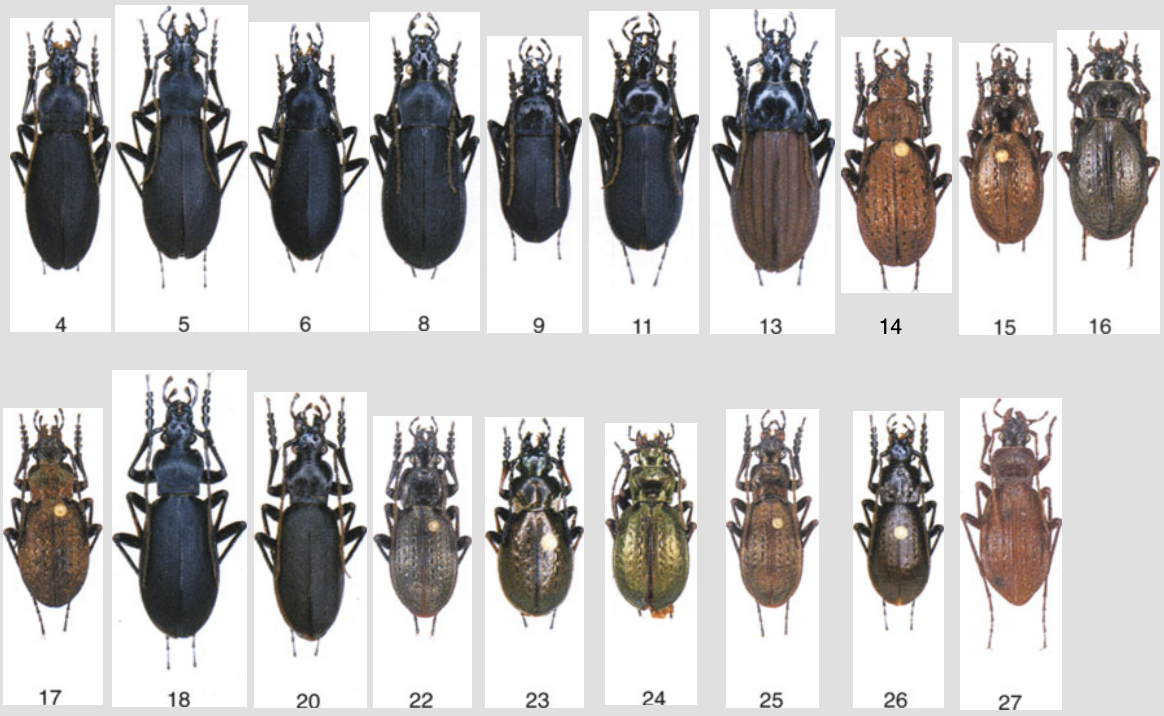


FIG. 5.23. Phylogenetic tree of the mitochondrial *ND5* gene for the genera *Rhigocarabus* and *Leptocarabus*. Constructed using the NJ-method. Numbers of photos correspond to those after species name (locality) in the tree. The blue letter after species name of *Leptocarabus* denotes the type to which the species belongs (after Su et al. 2001)

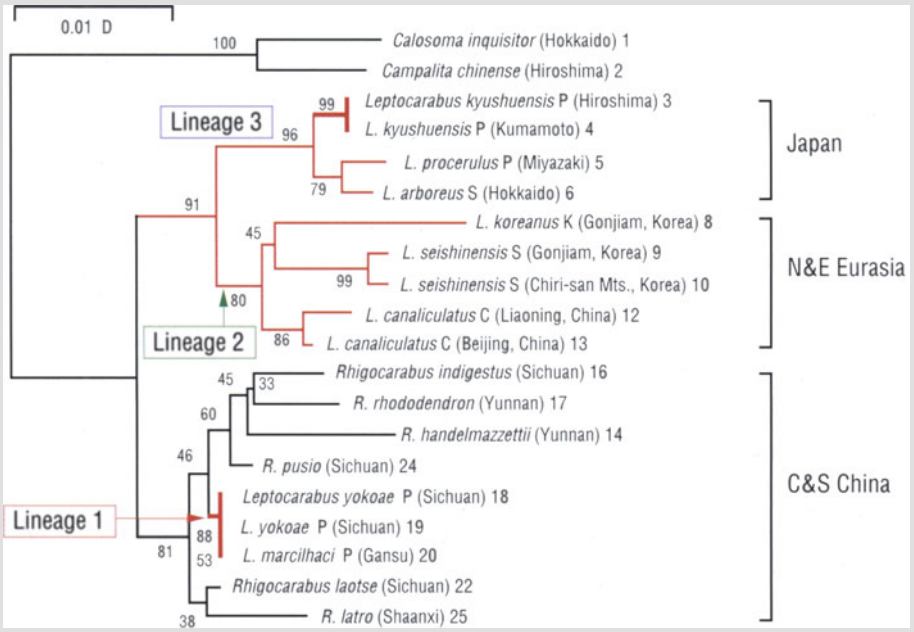


FIG. 5.24. Phylogenetic tree of the nuclear 28S DNA for the genera *Rhigocarabus* and *Leptocarabus*. Constructed using the NJ-method. The specimen number corresponds to that in Fig. 5.23 (after Su et al. 2001)

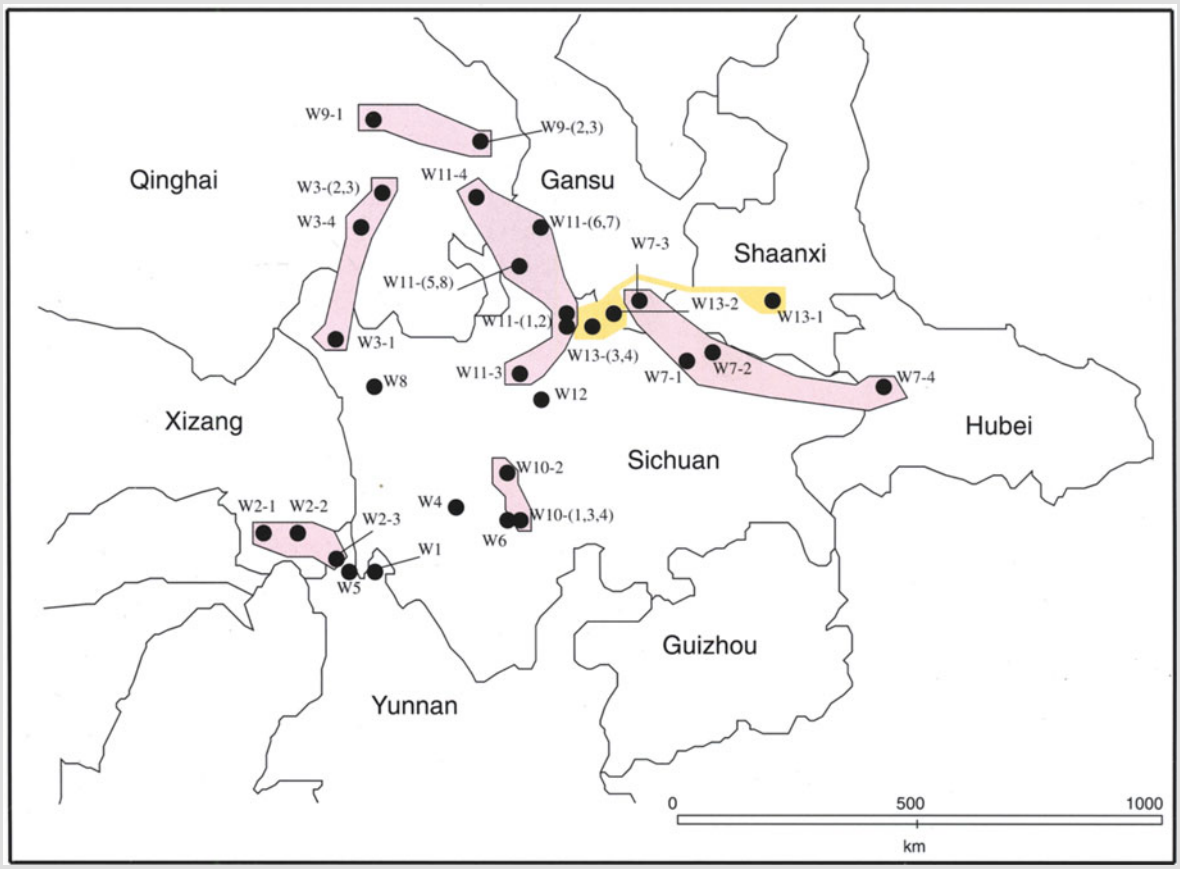


FIG. 5.25. Distribution map of the lineage W in the division Latitarsi. For symbols in the figure, see Fig. 5.19c (after Su et al. 2003e)

Morphologically, *Tomocarabus* is divided into four species-groups, namely the *convexus*-group, the *marginalis*-group, the *loschnikovi*-group, and the *taedatus*-group. Of these, the *marginalis*-group and the *taedatus*-group correspond to X6 and X11, respectively. The members of X6 are distributed in eastern Europe through western Russia, and those of X11 are endemic to North America. The remaining two groups are further divided into three to six sublineages on the *ND5* tree, and are apparently polyphyletic. The species in the *convexus*-group fall out in three different sublineages, X1 (*scabripennis*), X2 (*decolor*), and X4 (*convexus*).

The sublineage X4 contains four subspecies of *To. convexus*, with comparatively large evolutionary distances between them. X4 is further divided into two geographic groups, one containing specimens from Italy (subspp. *dilatatus* and *bucciarellii*) and another consisting of those from Slovakia (nominotypical *convexus*) and northeastern Turkey (subsp. *acutangulus*). The separation of the two groups would have been caused by geographic isolation upon the formation of the Alps about 20 MYA (Su et al. 2001).

Although classified into the same group as that of *To. convexus* morphologically, *To. scabripennis* forms an independent sublineage (X1). *Tomocarabus decolor*, which has a sclerotized projection on the ventral wall of the endophallus, also forms a distinct sublineage (X2).

The species belonging to the *loschnikovi*-group are scattered in the lineage Q and five different sublineages in the lineage X, namely, X3 (*fraterculus*), X5 (*opaculus*), X7 (*shaheshang*), X9 (*loschnikovi*), and X10 (*slovtzovi*). The lineage Q was represented only by *To. harmandi*, which is endemic to Japan (see above). *Tomocarabus opaculus* is the sole component of the sublineage X5, and is found in Japan, Sakhalin and the Kurile Islands (Su et al. 2000c).

The sublineage X2 is composed of three subspecies of *To. fraterculus* from the Korean Peninsula and northeastern China (Liaoning). Morphological differences among these three subspecies are small, and yet their branching points are considerably deep. The sublineage X7 is composed of two Chinese species placed in two different genera, *To. shaheshang* from southern Gansu and *Scambocarabus kruberi* from eastern Liaoning.

The genus *Tanaocarabus* comprises three species endemic to North America, and two of these, *sylvosus* and *forreri*, were analyzed. The basic morphological structure is very similar in both the species, and yet they belong to two independent sublineages (X8 and X12). This suggests that they arose in parallel, or their morphology did not change much after their separation from a common ancestor (silent evolution). In the sublineage X12 (*Ta. forreri*), the evolutionary distance between a specimen from Durango (central-western Mexico) and two specimens from Arizona (southwestern USA) and Chihuahua (northern Mexico) is relatively large, while that of the latter two is small.

The genus *Semnocarabus* is composed of eight species (Březina 1999), all endemic to the Tianshan Mountains of Central Asia. Five species were available for DNA analysis. Morphologically, all the species in this genus are similar to one another, and yet they fall out in three different phylogenetic lines on the *ND5* tree. Three species, *transiliensis*, *regulus*, and *erosus*, are included in the main sublineage X17, and two species, *bogdanowi* and *minimus*, both from Xinjiang in northwestern China, appear to form distinct sublineages, X13 and X15, separated from the main sublineage X17. X17 is further separated into at least two clusters, X17a and X17b. The cluster X17a contains *Se. transiliensis* from the Zailiiskii Alataus area and two subspecies of *Se. regulus* (*lutshniki* and nominotypical *regulus*) from the Terskei Ala-Toos. X17b is composed of *Se. regulus regulus* from the eastern part of the Terskei Ala-Toos and several subspecies of *Se. erosus* from southeastern Kazakhstan and northeastern Kirgiz.

Note that *Se. regulus* appears to form two different subclusters, and one of them is phylogenetically related to *Se. erosus* more than it is to the same species in another cluster. It is possible that the *Se. regulus*-like beetle is the ancestral form of the sublineage X17, from which *Se. erosus* branched off within X17b.

The remaining two genera in the lineage X, *Ulocarabus* and *Carpathophilus*, form two distinct sublineages (X14 and X16), which seem to have little relation to the genus *Semnocarabus*.

The lineages V, X, and W are of special interest from the viewpoint of morphological evolution, which will be discussed to some extent below, and in more detail in Chapter 8.

5.4.2.3 Comparisons of Molecular Phylogeny and Taxonomy

The molecular phylogeny suggests that at least 17 lineages that have been morphologically placed in the division Latitarsi emerged at about the time of the Carabina radiation together with other divisions. Therefore, these "Latitarsi" lineages may be regarded as separate divisions, especially in the case of the lineage A (*Autocarabus*).

The rest of the lineages, including B, C, H, and L, may also be treated as independent divisions. However, it is possible that at least some of these lineages had a common ancestry at their emergence. Since this possibility cannot be verified, we have conventionally treated all the lineages as belonging to the Latitarsi.

There exist considerable discrepancies between classification by morphology and molecular phylogeny for the Latitarsi. A new classification system for the "Latitarsi," in which they are broken into several divisions, will be presented in Chapter 9.

5.4.2.4 Mode of Morphological Evolution

Morphological diversification of not a few species in the Latitarsi is rather poor, and yet they radiated in the early stage of the Carabina evolution. For example, the external morphology of almost all the species is alike throughout *Tomocarabus* and its related genera (lineage X). In contrast to this silent evolution, a remarkable morphological differentiation occasionally took place during the Latitarsi evolution (discontinuous evolution), as is detailed in this section.

Digitulati

The division Digitulati is a large group of ground beetles in the subtribe Carabina, the distribution range of which covers the greater part of the Eurasian Continent, including some adjunctive islands such as the British Isles and Sakhalin (Fig. 5.26). Most of the species are without hind wings and can move only by walking.

Some species, such as *Carabus granulatus* and *C. arvensis,* sometimes have complete hindwings. All the species in this division are characterized by having a chitinized structure called digitulus (or copulatory piece) in the male genital organ.

The division Digitulati has been taxonomically divided into four (sub)genera (Imura 1996), which contain altogether 82 species (Březina 1999): 1) the genus *Carabus* which contains 20–30 species distributed widely in the Holarctic region; 2) the genus *Eucarabus* consisting of about ten species whose distribution range is dipolarized, one in the vicinity of the Korean Peninsula and another in eastern Europe; 3) the genus *Isiocarabus* consisting of about 10 species found in southeastern China and Korean Peninsula; 4) the genus *Ohomopterus* endemic to the Japanese Islands, which contains 16 species.

Morphologically, *Ohomopterus* reveals a considerable resemblance to and has been thought to be derived from *Isiocarabus*. Some of the species reveal either considerable geographic and individual variation or very similar morphology among different species. This makes not only the taxonomy but also the morphology-dependent phylogeny of this group rather ambiguous.

An *ND5* tree covering 87 individuals of the Digitulati representing 32 species (Figs. 5.26 and 5.27) includes all the known genera of this division. Additionally, two species that were believed to belong to the division Archicarabomorphi, *Acrocarabus guerini* and *Ac. callisthenoides*, were found to be members of the division Digitulati (Fig. 5.28a, b; see also p. 49).

The Digitulati specimens analyzed for *ND5* sequences have been divided into six lineages (A trough F) (Fig. 5.27). Their separation occurred within a short period, with this radiation estimated to have occurred about 3.5 MYA (Su et al. 1998, 2001).

Lineage A consists exclusively of members of the genus *Ohomopterus*, all the species of which are endemic to the Japanese Islands (Fig. 5.27). A detailed description of this genus will be presented in Chapters 6 and 7 and only four representative species are included in Fig. 5.27.

Lineage B is also monophylteic and contains only the species belonging to the genus *Isiocarabus* (Fig. 5.27). There is likely to have been some biogeographic connection between southeastern China and Cheju-do Island in South Korea, because *Isiocarabus* is distributed in these two regions, but not in the Korean Peninsula, although a species *I. fiduciarius* has been known from a part of North Korea. The tree strongly suggests that *Ohomopterus* and *Isiocarabus* are phylogenetically independent, and therefore *Isiocarabus* is not a direct ancestor of *Ohomopterus* (see Chapter 6 and also Tominaga et al. 2000).

Lineage C is composed solely of the Chinese species of the genus *Carabus* which is sometimes treated as *Archaeocarabus* separated from *Carabus* (s. str.) (Březina 1999). They radiated into three sublineages, C1, C2, and C3 a long time ago (Fig. 5.27). These Chinese *Carabus* have been said to have undergone considerable speciation mainly in the mountainous areas of central China, although morphological differences among them are not so conspicuous.

Their taxonomy, however, seems to be quite confused and must be a serious subject for reexamination, because in many cases only small sequence differences are recognized between "different species," for example, between *C. nanosomus, C. nestor*, and *C. morphocaraboides* (all in the sublineage C1). In addition to this, *C. pseudolatipennis* appears in two different sublineages, one clustered with *C. latipennis* (?) (sublineage C2) and another with *C. vigil* (sublineage C3). In both cases, the two "paired species" are genetically very close in their sequences.

Lineage D contains a North American species, *Carabus limbatus.*

Lineage E includes the *Carabus* species from the Eurasian Continent and its adjacent islands. The lineage is further divided into three sublineages, E1, E2, and E3 (Fig. 5.29).

E1 contains a widely distributed species, *C. arvensis*, with *C. deyrollei* from Spain as the outgroup. The sequence difference between the specimeus from various localities in the Eurasian Continent, Sakhalin and Japan is small, while that from eastern France is fairly remote from the others.

E2 contains all the specimens of *C. granulatus* from various parts of the Eurasian Continent and its adjacent islands including Japan. *Carabus sculpturatus* is the outgroup. The genetic distance between the specimens from the Eurasian Continent and those from Japan/Sakhalin are very close and yet they are clearly separated from each other. This suggests the recent

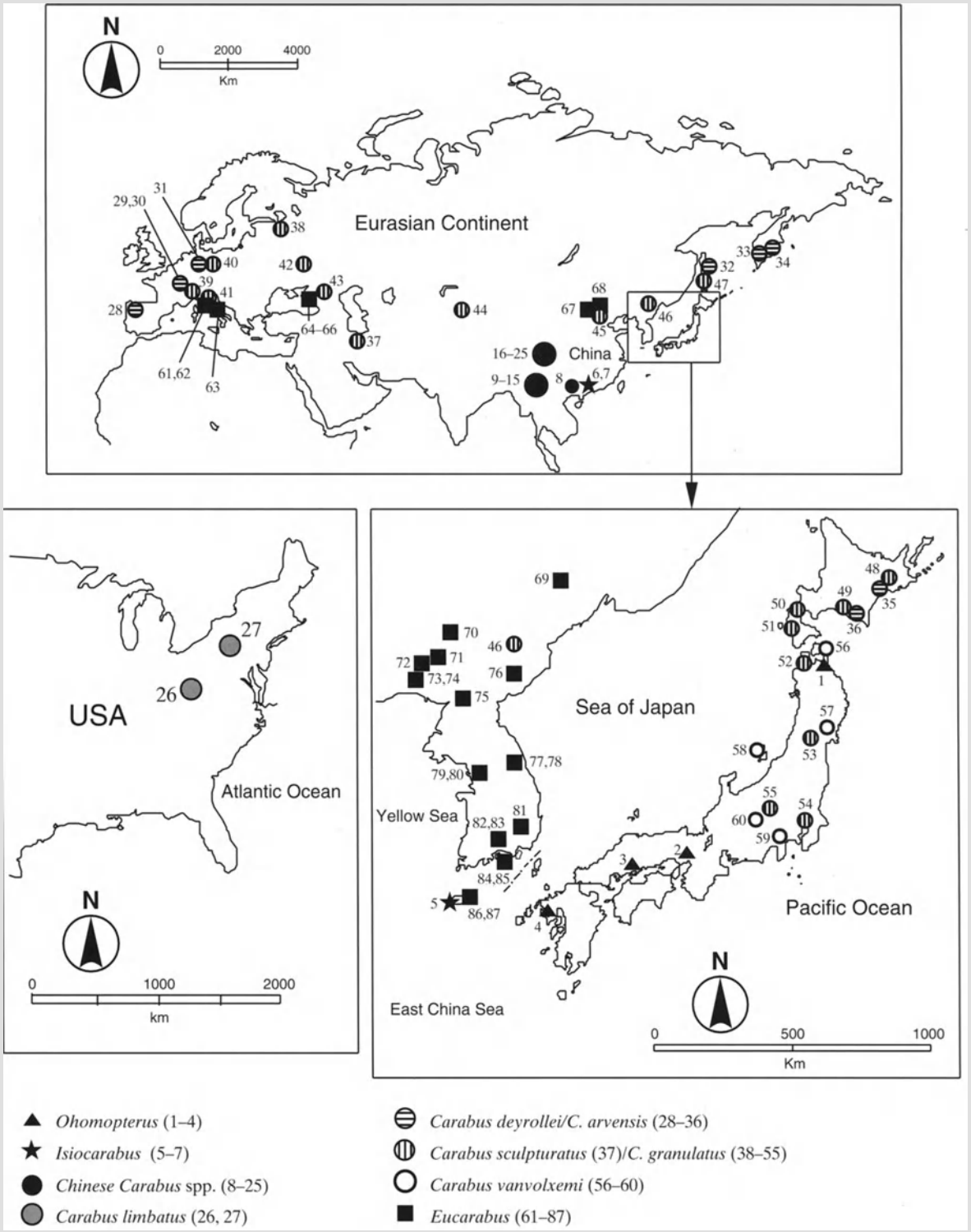


FIG. 5.26. Localities of the samples of the division Digitulati for analyses. Numbers in the figure correspond to those in Figs. 5.27–30 (after Su et al. 2003f)

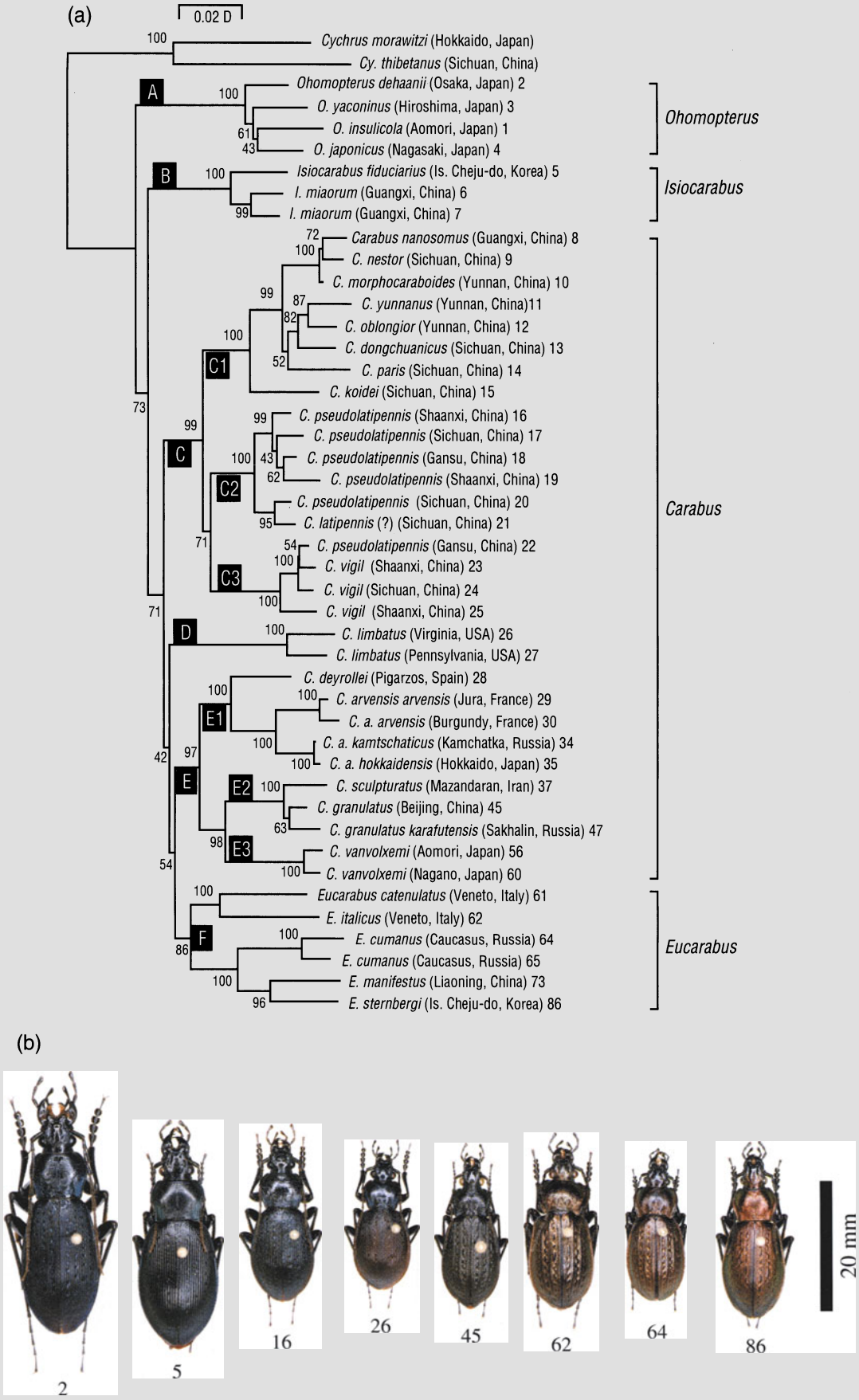
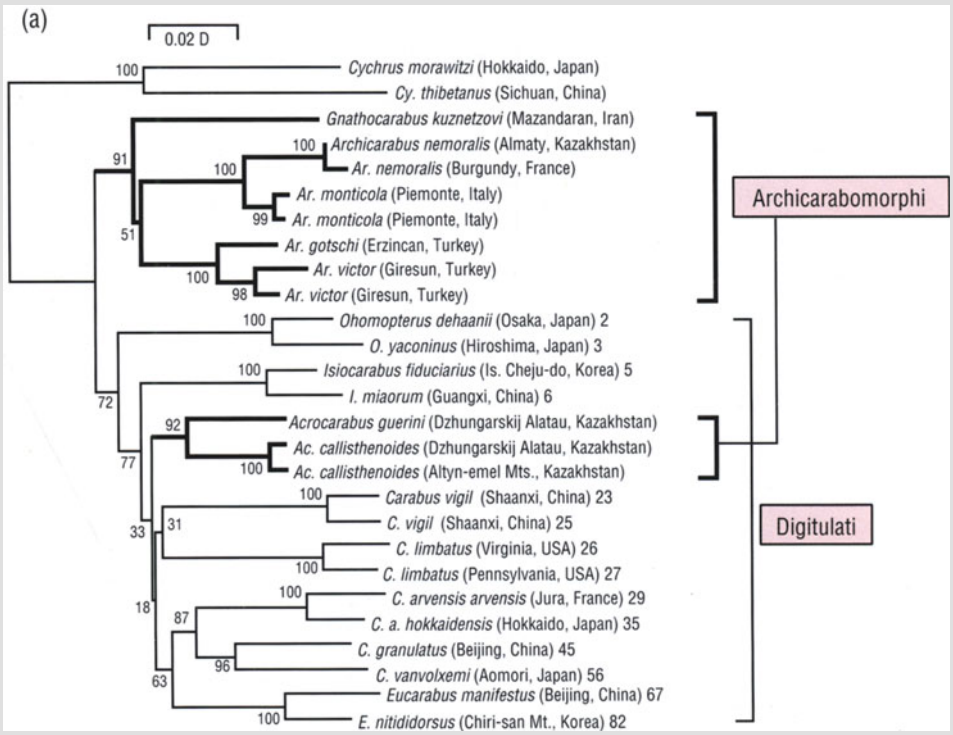


FIG. 5.27. Phylogenetic tree of the mitochondrial *ND5* gene for the division Digitulati (**a**) and the representative species (**b**). Constructed using the NJ-method. Numbers after species names (localities) correspond to locality numbers in Fig. 5.26 (after Su et al. 2003f)



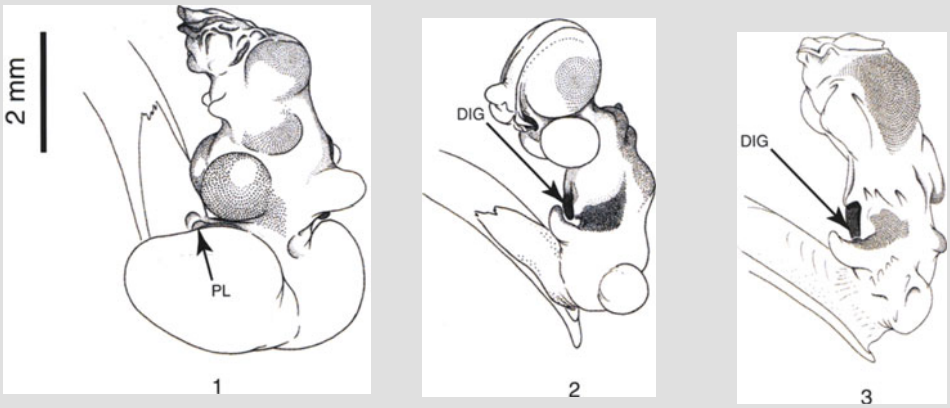


FIG. 5.28. Phylogenetic positions of *Acrocarabus* by means of the mitochondrial *ND5* gene analysis (**a**) and male genital organ (**b**). The phylogenetic tree was constructed using the NJ-method. For number after species name (locality), see Fig. 5.26. **b1** *Archicarabus nemoralis* (division Archicarabomorphi), **b2** *Acrocarabus callisthenoides*, **b3** *Carabus borni-anus* (division Digitulati). **PL**, paraligula; **DIG**, digitulus (after Su et al. 2003e)

immigration of the Eurasian component to Japan, presumably via Sakhalin (see Chapter 6, p. 97).

The close genetic distance in *C. granulatus* or in *C. arvensis* from a wide range of localities is likely a result of their considerable migration capability with less reduced, or even completely developed hindwings. These two species are separated into many subspecies based on rather small morphological differences, but this classification is not made evident by the phylogenetic data.

E3 is composed of a single species, *C. vanvolxemi*, from Japan. This species will be discussed in greater detail in Chapter 7.

Lineage F includes the species of the genus *Eucarabus*. The tree (Fig. 5.30) shows that the sublineage **F1** contains two European species, *E. catenulatus* and *E. italicus*, that separated from two species from the Caucasus region (**F2**) plus several species from eastern Asia (see below). As mentioned above, the members of *Eucarabus* show a dipole distribution, and the above phylogenetic profile is consistent with this.

The origin and the distribution route of *Eucarabus* is still only a matter of speculation. The ancestor of *Eucarabus* is likely to have existed in China. It expanded its range westward and eastward and the ancestral population eventually became extinct in continental China.

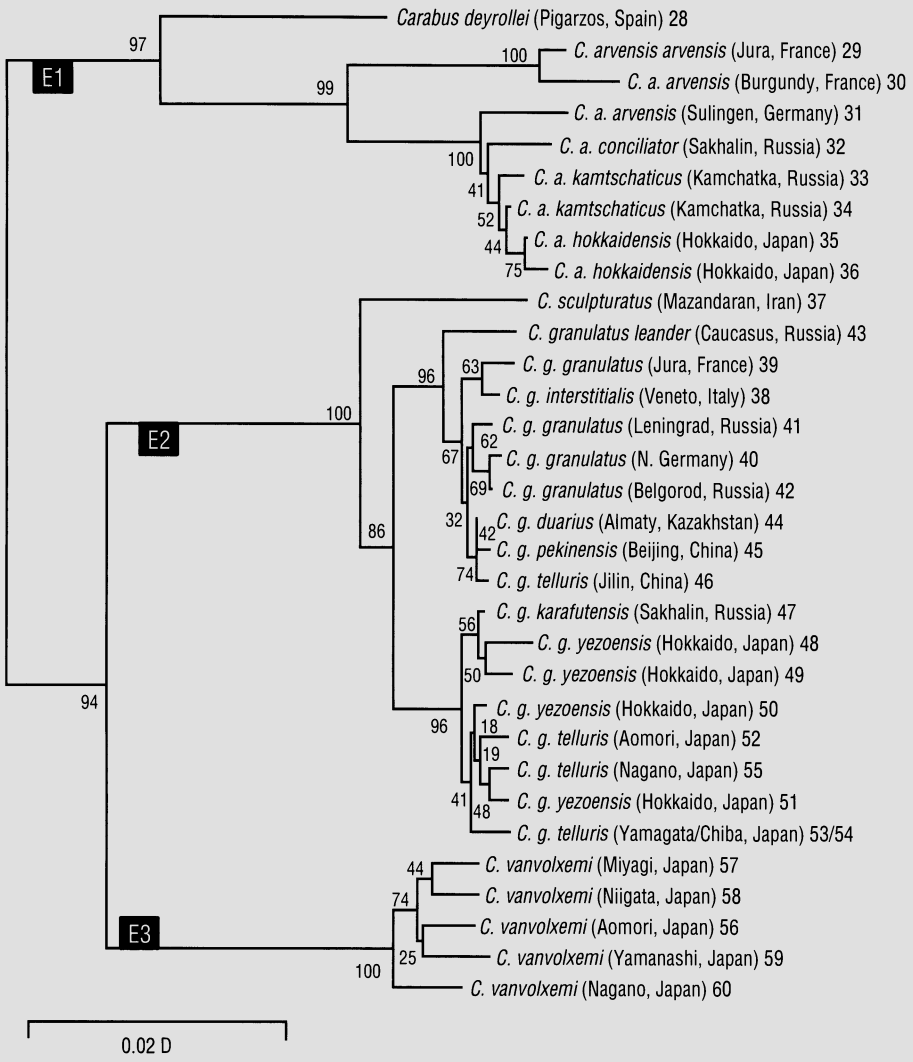


FIG. 5.29. Phylogenetic tree of the mitochondrial *ND5* gene for the lineage E of the division Digitulati. Constructed using the NJ-method (after Su et al. 2003f)

The East Asian members then divided into three sublineages (**F3**, **F4**, and **F5**).

The members of these three sublineages are all inhabitants of eastern China and Korea, and are linked tightly to geography. Members of F3 have been found in northeastern China and North Korea, those of F4 in central Korea and Cheju-do Island, and those of F5 in central-southern Korea. The phylogenetic lineages do not reflect the taxonomy based on morphology. For example, *Eucarabus sternbergi* appears in all the lineages, F3, F4, and F5 along with other species.

A possible explanation for this discrepancy would be that the individuals belonging to each lineage represent one species, and therefore there exist altogether three species that correspond to F3, F4, and F5. This view may be supported by the small genetic distance and minor morphological differences between these "species" in each phylogenetic lineage.

All the species of the East Asian region resemble each other in external appearance with some geographic and individual variations (Imura and Mizusawa 1996). The classification of the species largely depends on the shape of the male genital organ, and yet even this characteristic seems to be less than decisive (Imura and Mizusawa 1996). For example, *Eucarabus nitididorsus* has often been treated as a subspecies of *E. sternbergi*.

The taxonomy of the East Asian *Eucarabus* should surely be subjected to reexamination from a molecular phylogenetic viewpoint, including analysis of nuclear genes, as well as from a morphological viewpoint.

As mentioned in the section on Archicarabomorphi, two species of the genus *Acrocarabus* from Kazakhstan have been found not to be the members of that division. The male genital organ of the *Acrocarabus* species has a chitinized piece (digitulus) on the ventral wall of edophallus, which is characteristic of the division Digitulati.

The phylogenetic tree shown in Fig. 5.28 clearly shows that *Acrocarabus* is a member of the Digitulati,

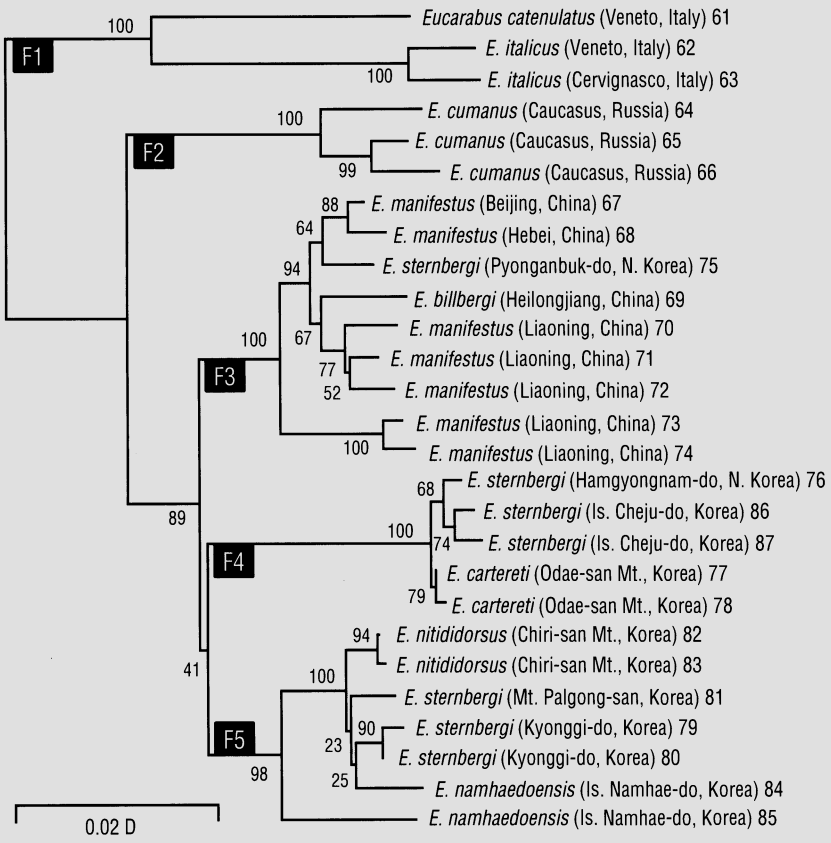


FIG. 5.30. Phylogenetic tree of the mitochondrial *ND5* gene for the lineage F of the Division Digitulati. Constructed using the NJ-method (after Su et al. 2003e)

forming the 7th lineage, G, of this division. *Acrocarabus* emerged at almost the same time as the six other lineages of this division.

Procrustimorphi

The division Procrustimorphi is the largest taxonomic group among the nine divisions of the Carabina ground beetles (Imura 1996; Imura et al. 1998b), containing nearly half of the genera and 35% of the species in the Carabina. The carabids of this division reveal the most remarkable morphological diversification, and have been classified into about 50 genera and many species/subspecies (Imura 1996, 2002b; Imura and Mizusawa 1996; Březina 1999). The complexity of similar external morphology combined with poor differentiation of the male genitalia make it difficult to assess the phylogenetic relationship of the Procrustimorphi by cladistic analysis that depends on morphology alone.

About 500 specimens representing 150 species of the representative genera of this division were gathered from nearly all the distribution ranges around the world for the construction of the *ND5* phylogenetic tree.

The Procrustimorphi as a Distinct Phylogenetic and Taxonomic Group

A phylogenetic tree of the *ND5* gene from the representative divisions of the Carabina reveals that all Procrustimorphi species, except three (see below), are grouped together without any cross contamination by species taxonomically belonging to other divisions, despite a short branch length with a low bootstrap value that supports the Procrustimorphi.

This also holds true when one outgroup species is replaced by another or the addition/removal of a species. We interpret this result as showing that the Procrustimorphi are one distinct phylogenetic and taxonomic group, and various phylogenetic lineages in this division emerged shortly after radiation of the Carabina.

Rhabdotocarabus melancholicus and *Ctenocarabus galicianus*, both found in northwestern Spain, and *Cathoplius asperatus* from Morocco have been treated as members of the Procrustimorphi. However, the *ND5* molecular tree suggests that these three are not clustered with other Procrustimorphi and they most probably form distinct divisions independent of the Procrustimorphi (Fig. 5.31) (see below).

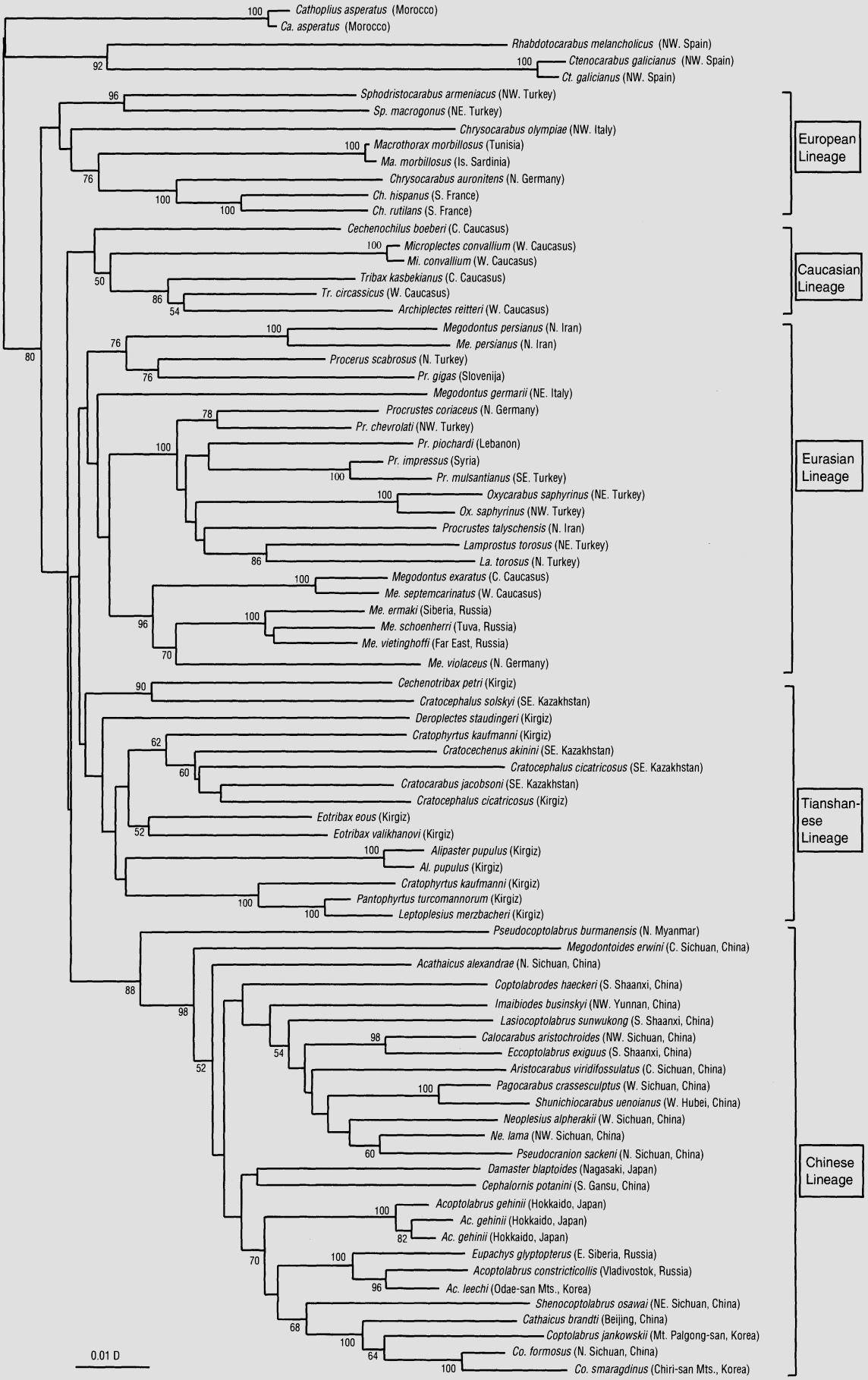


FIG. 5.31. Phylogenetic tree of the mitochondrial *ND5* gene for the division Procrustimorphi. Constructed using the NJ-method (after Kim et al. 2003)

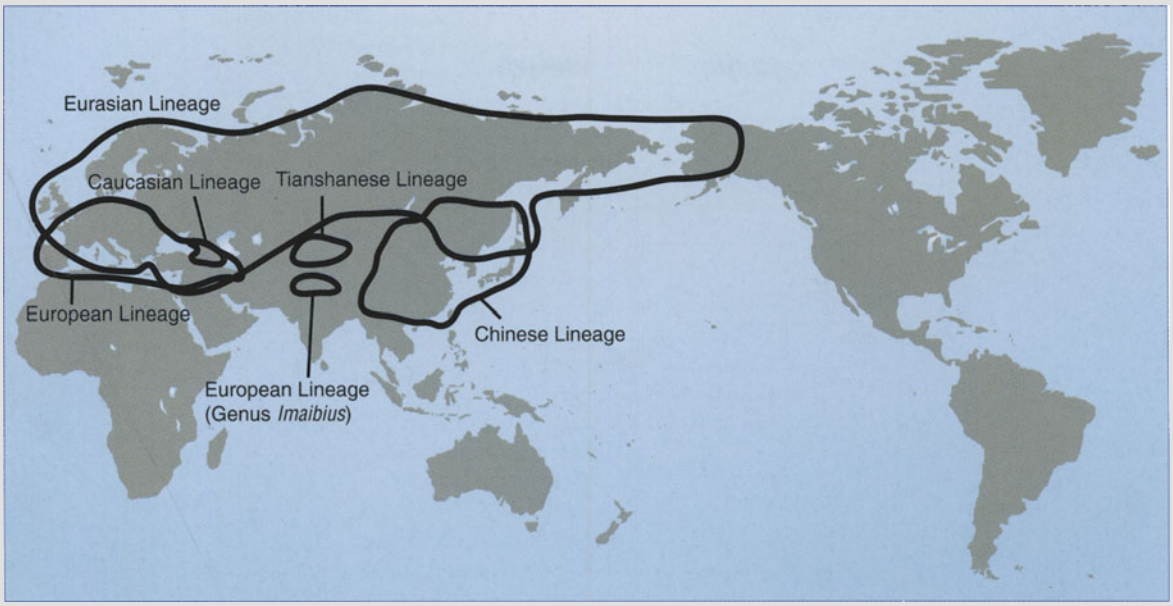


FIG. 5.32. Distribution ranges of the lineages belonging to the division Procrustimorphi (after Kim et al. 2003)

Rhabdotocarabus melancholicus and *Ctenocarabus galicianus* form one cluster, although their divergence occurred a long time ago. The branch length on the tree for *Cathoplius asperatus* is much shorter than those of the others. This might be because of a slower rate of nucleotide substitutions. A reliable phylogenetic position for *Cathoplius asperatus* awaits further study.

Geographically Linked Phylogenetic Lineages

The phylogenetic tree shown in Fig. 5.31 includes only the representative species of the Procrustimorphi. As noted above, *Rhabdotocarabus*, *Ctenocarabus*, and *Cathoplius* form clusters outside the Procrustimorphi, and they are considered to be outgroups. There exist at least five lineages, each of which is further divided into a few sublineages. Here also, the branch length supporting each lineage is short, and this has been interpreted as showing that diversification of different lineages occurred within a short time after the radiation of the Carabina.

It is worth noting that there is no case in which a given genus appears in two or more lineages. In other words, each lineage is composed of genera specific to it, suggesting that the tree is correct overall. Another point of interest is the fact that each lineage is geographically linked as described below (Fig. 5.32).

The European lineage is mainly found in southeastern Europe. The following genera belong to this lineage: *Macrothorax*, *Chrysocarabus*, *Imaibius*, and *Sphodristocarabus*.

The Caucasian lineage is found only in the Caucasus region. The genera *Microplectes*, *Cechenochilus*, *Archiplectes*, and *Tribax* belong to this lineage.

The Tianshanese lineage members inhabit only the Tianshan Mountains of Central Asia. This lineage is composed of the genera *Cratophyrtus*, *Pantophyrtus*, *Eotribax*, *Cratocarabus*, *Cratocechenus*, *Deroplectes*, *Cechenotribax*, *Alipaster*, and *Leptoplesius*.

The Eurasian lineage is found over an exceptionally wide area as compared to other Procrustimorphi. They are distributed mainly in Europe, but their habitat extends to central and western Asia, and includes Japan and Alaska. *Megodontus*, *Procerus*, *Procrustes*, *Lamprostus*, and *Oxycarabus* are included in this lineage.

The members of the Chinese lineage are widely distributed, mostly in continental China, with some found in Sakhalin and Japan. Morphological diversification in this lineage is the most remarkable not only within the Procrustimorphi but also throughout the Carabina.

Because of this diversity, many (sub)genera have been established, including *Damaster*, *Cephalornis*, *Acoptolabrus*, *Eupachys*, *Shenocoptolabrus*, *Cathaicus*, *Acathaicus*, *Cupreocarabus*, *Coptolabrus*, *Coptolabrodes*, *Imaibiodes*, *Lasiocoptolabrus*, *Aristocarabus*, *Shunichiocarabus*, *Pagocarabus*, *Calocarabus*, *Neoplesius*, *Pseudocranion*, *Eccoptolabrus*, and *Eocechenus*.

Pseudocoptolabrus and *Megodontoides* may also belong to this lineage, but this is not certain because of their somewhat unstable topology on the *ND5* phylogenetic tree. The topology of the genus *Imaibius*, found in Pakistan, is also unreliable, sometimes clustering with the Chinese lineage and sometimes with the European lineage. From its distribution range, *Imaibius* is likely to constitute a distinct lineage.

The following section will describe the phylogeny of each lineage in detail.

The European Lineage

The distribution range of this lineage includes southeastern Europe, North Africa, and western Asia. Their

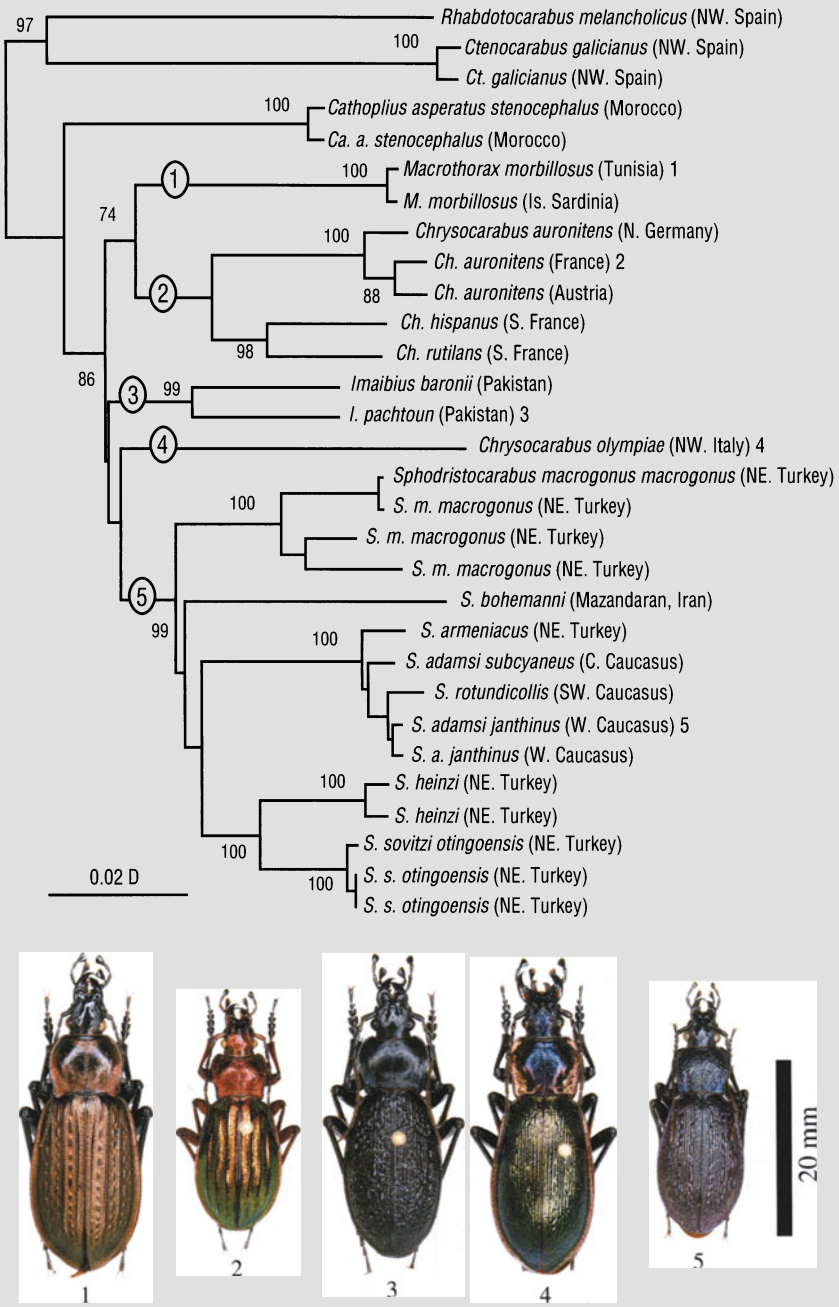


FIG. 5.33. Phylogenetic tree of the mitochondrial *ND5* gene for the European lineage of the division Procrustimorphi. Constructed using the NJ-method. Photographs are shown below the tree, and the numbers correspond to those following scientific names in the tree (after Kim et al. 2003)

phylogeny based on morphology has been quite ambiguous, especially for some of the members. On the other hand, the results of molecular phylogenetic study reveals that the taxonomically established genera are clearly separated from one another on a phylogenetic tree with the exception of *Chrysocarabus olympiae* (Fig. 5.33).

The sequences of *Macrothorax morbillosus* from Tunisia in North Africa and the island of Sardinia in the Mediterranean are almost the same (sublineage 1 in Fig. 5.33). This species seems to be clustered together with the European genus *Chrysocarabus*, to which *C. auronitens*, *C. hispanus*, and *C. rutilans* belong (sublineage 2), although the branching point between *Macrothorax* and *Chrysocarabus* is deep.

This suggests that the common ancestry of these two subgenera moved long ago from Europe to North Africa, where *Macrothorax* differentiated. The three *Chrysocarabus* species are well separated from each other on the tree. Two *Imaibius* species from Pakistan

are well separated, with rather deep branching points, and form the sublineage 3. Unexpectedly, *Chrysocarabus olympiae* from the alpine region in northwestern Italy, which was believed to be close to other *Chrysocarabus* species, appears on the tree at a place (sublineage 4) entirely independent from that of the main *Chrysocarabus* cluster mentioned above. The origin of *C. olympiae* is venerable, suggesting that it has perhaps been isolated within a limited area of northwestern Italy for a long time.

The genus *Sphodristocarabus* from Turkey, Iran, and Caucasus is a rather difficult group for taxonomy. The molecular phylogenetic tree reveals that *Sphodristocarabus* is monophyletic (sublineage 5), and each species examined forms its own cluster separated clearly from the other species, except that *S. armeniacus*, *S. adamsi*, and *S. rotundicollis* form one cluster with only small sequence differences. The sequence divergence within the same species is small, except for *S. macrogonus*. The *S. macrogonus* cluster is divided into two lines. To the first line belong two specimens from Ordu. The second one is composed of two specimens from Trabzon and Giresun.

The emergence point of the sublineage 3, to which the genus *Imaibius* belongs, on the phylogenetic tree is the deepest in this lineage and is sometimes unstable so as to be placed outside the other four sublineages. This fact together with the isolated distribution range of this sublineage (the midwestern part of the Himalayas; Fig. 5.32) suggests that the genus *Imaibius* is a group independent from the European lineage.

The Caucasian Lineage

The members of this lineage are distributed in the Caucasus Mountains and northeastern Turkey, and are composed of three beautiful carabid genera, *Microplectes*, *Archiplectes*, and *Tribax*, and a macrocephalic genus, *Cechenochilus*. Figure 5.34 shows a phylogenetic tree of this lineage. Six sublineages radiated within a short time. The first sublineage consists of solely the members of the macrocephalic genus *Cechenochilus*, which are divided into two subclusters with a deep branching point.

The first one is represented by one species, *C. heydenianus* from the central Caucasus, while the second one contains three specimens of *C. boeberi* from the western Caucasus. Thus, the phylogeny is geographically linked and reflects their taxonomy. The ancient divergence (ca. 21 MYA) of the two subclusters with little morphological change may be taken as an example of silent evolution as seen in the *Microplectes* cluster (see below).

The second sublineage contains two subspecies of *Microplectes convallium*, which are clearly divided into two clusters, the divergence of which occurred about 18 MYA. This suggests that their morphologies have remained almost unchanged for a long time.

The third sublineage is composed of two species of *Archiplectes*, i.e., *A. starcki* and *A. reitteri* from the western Caucasus. The sequence difference between them is very small, although these two are distinguishable in morphology especially through examination of the characteristic male genitalia. This may be taken as an example of recent morpholgical transformation.

The fourth sublineage is composed of a single species, *Tribax osseticus* from the central Caucasus.

To the fifth sublineage also belongs a single species, *Archiplectes starckianus* from the western Caucasus.

The composition of the sixth sublineage is complex; six lines that emerged long ago can be recognized. Each line contains a single species, except for the third one, which has two species. The species of the first to the fourth clusters inhabit the western and central Caucasus, while those of the fifth and sixth clusters inhabit northeastern Turkey. The Turkish *Tribax* species are thus well separated from the Caucasian *Tribax* species.

As can be seen in Fig. 5.34, the genera *Archiplectes* and *Tribax* appear in four sublineages that are remote from one another. The members of *Archiplectes* belong to the third, the fifth, and the sixth sublineages, while the species of *Tribax* belong to the fourth and the sixth sublineages. No cross contamination of these two genera in a given sublineage is observed.

Note that *Archiplectes starcki* appears independently in the third and sixth sublineages. Thus the clustering of the genera on the tree does not correlate with morphological characteristics. In other words, taxonomically the same genus falls out in more than two different places on the tree. This suggests that either parallel morphological evolution took place in different sublineages, or the two genera can be united into a single genus by careful morphological reexamination.

The Eurasian Lineage

The principal constituents of the Eurasian lineage are the genera *Megodontus*, *Procrustes*, and *Procerus*, the distribution range of which is exceptionally wide when compared to other Procrustimorphi and includes Europe, Central Asia, East Asia, Japan, and Alaska. The morphological difference between the genera is remarkable, but is not so within each genus.

On an *ND5* phylogenetic tree (Fig. 5.35), there are at least four sublineages, each of which emerged shortly after the radiation of various Procrustimorphi lineages.

The first sublineage is composed of only the *Megodontus* species, and is further divided into at least three clusters, one made up of two species from the Caucasus region, another comprising six species from Kazakhstan, Russia, and Japan, and the third one species from Europe (Germany, Slovakia, Hungary, and Italy).

The second sublineage contains only one species, *Megodontus germarii savinicus* from northeastern Italy.

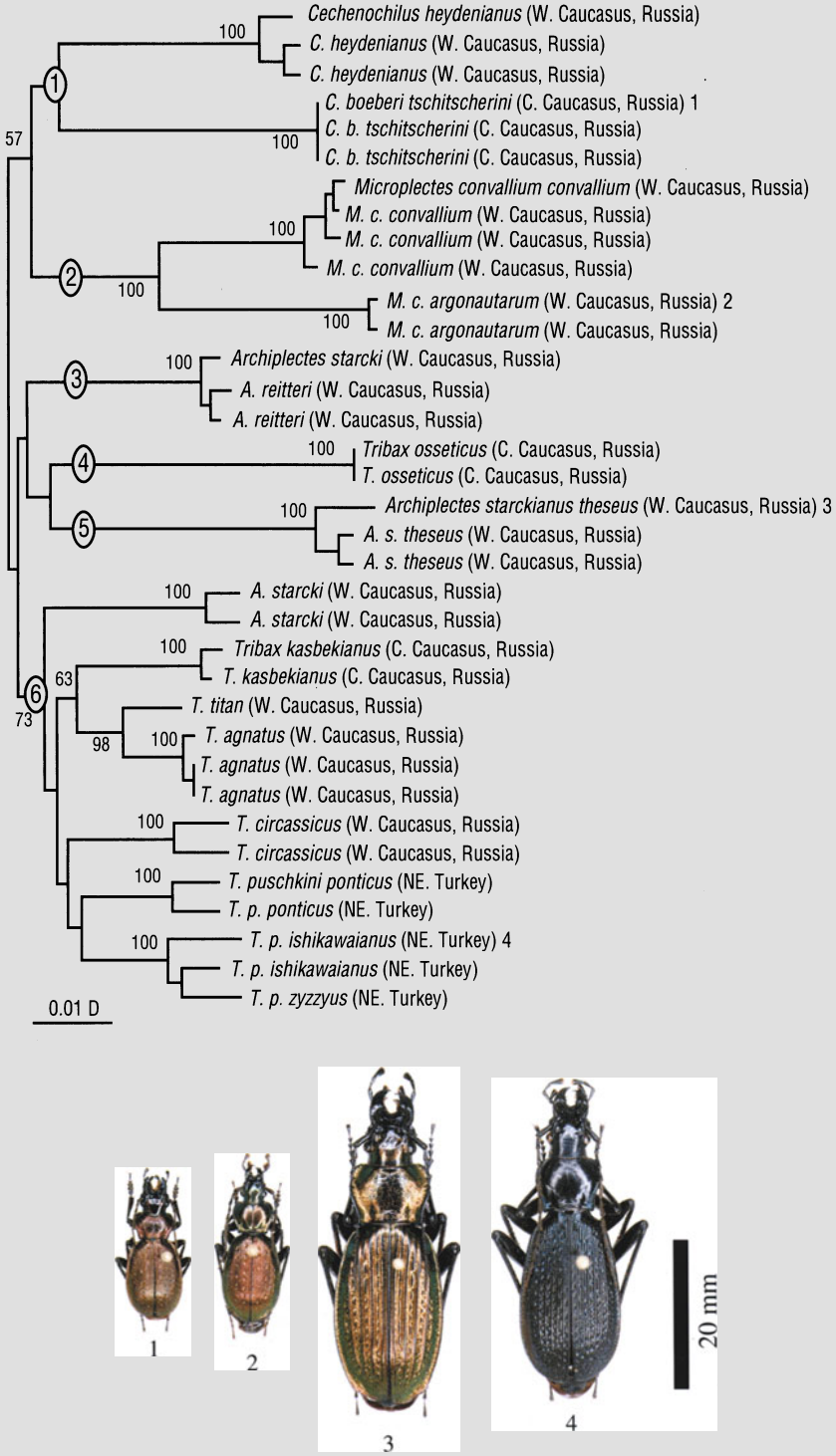


FIG. 5.34. Phylogenetic tree of the mitochondrial *ND5* gene for the Caucasian lineage of the division Procrustimorphi. Constructed using the NJ-method. Photographs are shown below the tree, and the numbers correspond to those following scientific names in the tree (after Kim et al. 2003)

Two specimens showed identical gene sequences. This species is morphologically very close to *M. violaceus* which belongs to the first sublineage, and has sometimes been treated as one of its local races. However, note that these two taxa are phylogenetically quite remote from each other as shown in Fig. 5.35.

The third sublineage is divided into two clusters. The first one is composed solely of the genus *Procerus*. *Procerus scabrosus* from Turkey and the central Caucasus is well separated from *P. gigas*, which inhabits Slovenija. The second cluster is composed of three species of *Megodontus*; *M. bonvouloiri* from Turkey and *M.*

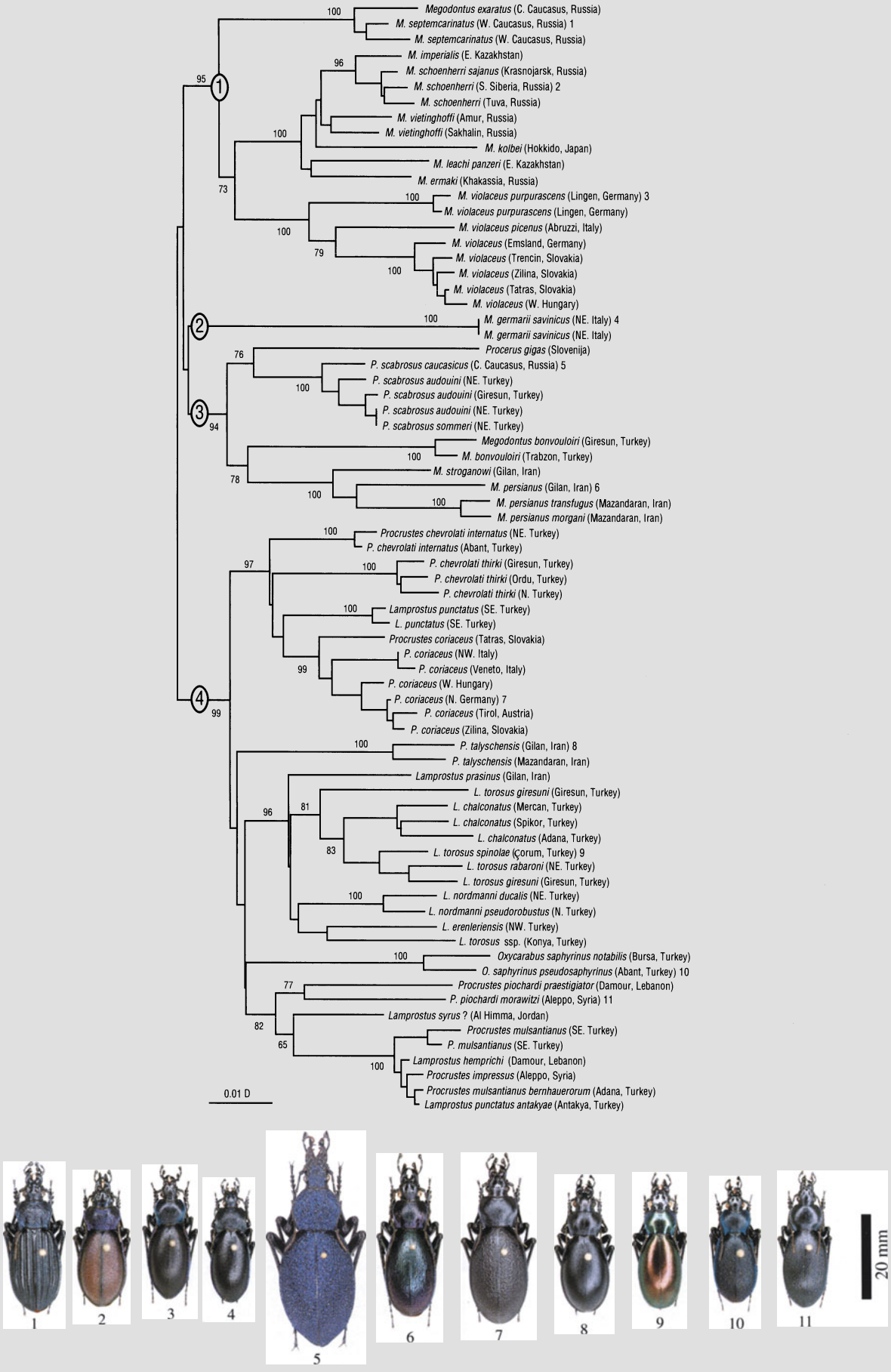


FIG. 5.35. Phylogenetic tree of the mitochondrial *ND5* gene for the Eurasian lineage of the division Procrustimorphi. Constructed using the NJ-method. The numbers of photographs below correspond to those following scientific names in the tree (after Kim et al. 2003)

stroganowi and *M. persianus* from Iran. The Turkish species are well separated from the Iranian species with a deep branching point. It is remarkable that the *Megodontus* species appear in three remote clusters revealing ancient separation and, surprisingly, two morphologically different genera, *Megodontus* and *Procerus*, are clustered together in the same sublineage.

The appearance of *Megodontus* in three distinct sublineages may suggest that a *Megodontus* species such as proto-*M. germarii* is the ancestral form and its morphology has remained unchanged to the present. An alternative and less likely possibility is that *Medogontus*-like morphology emerged in parallel. It is also remarkable that *Procerus* branched off from some ancestral *Megodontus* species accompanied by a remarkable discontinuous morphological transformation.

The fourth sublineage includes three genera, i.e., *Procrustes*, *Lamprostus*, and *Oxycarabus*. The species of *Procrustes* are widely distributed across most of Europe (except the Iberian Peninsula), Asia Minor, the Caucasus, Turkey, and Iran, while those of *Lamprostus* have a narrower distribution range and are not found in Europe.

The *Oxycarabus* species are found only in the mountainous area of northern Turkey. The position of this genus is not yet entirely clear because of the lack of morphological affinity with other groups of Procrustimorphi. The sublineage may be divided into five clusters, emergence of which seems to have started at about the same time.

Since the members of *Procrustes* and *Lamprostus* appear in three distinct clusters and do not form a single cluster of their own, they are clearly polyphyletic. In the first and fourth clusters, the species of *Procrustes* and *Lamprostus* are intermingled. Imura and Mizusawa (1996) state that *Lamprostus* is very close to *Procrustes* in morphology and the two may be connected together. From the molecular tree too, there is no rationale for separating them.

The diversification of the *ND5* gene within the European *Procrustes coriaceus* (cluster 1 of sublineage 4) started considerably later than that of the Turkish *P. chevrolati*, suggesting past migration of a species such as proto-*P. chevrolati* from somewhere in Turkey to Europe where *P. coriaceus* differentiated.

The position of *Oxycarabus* is of special interest. This genus is phylogenetically related to *Procrustes*/*Lamprostus*, suggesting that in the restricted area of Turkey, the morphologically distinct *Oxycarabus* line emerged from the *Procrustes/Lamprostus* cluster (cluster 4 of sublineage 4).

The Tianshanese Lineage

The members of this lineage are mostly macrocephalic and are found in the Tianshan Mountains of Central Asia. Taxonomy based on the morphology of this group is not consistent with the *ND5* phylogeny in many respects (Fig. 5.36). The same species or the same subgenera are scattered in different sublineages on the tree. About ten sublineages are recognizable and appear to have radiated shortly after the radiation of the Carabina, and therefore the origin of each sublineage is venerable 30–40 MYA. The sublineages 2 to 5 are supported by a node with a high bootstrap value (86%) and were probably derived from a common ancestor.

The sublineage 1 contains *Cratophyrtus kaufmanni*, *Pantophyrtus, P. brachypedilus* from Kirgiz and Uzbekistan, and *P. turcomannorum* from Uzbekistan. The composition of this sublineage 1 is almost the same as that of the sublineage 10, which is composed of *Cratophyrtus kaufmanni* and *Pantophyrtus turcomannorum* from Kirgiz together with *Leptoplesius merzbacheri* from Kirgiz, implying the parallel appearance of *Cratophyrtus* and *Pantophyrtus* in two different phylogenetic lines.

Similarly, the members of *Cratocephalus* separately appear in three different sublineages 4, 5, and 8. *Cratocechenus akinini* appears in the sublineages 2 and 3, and *Eotribax* in the sublineages 2 and 6. The sublineage 3 (*Cratocechenus*), 7 (*Deroplectes*), and 9 (*Alipaster*) are each composed of a single genus that does not appear in other sublineages.

The Chinese Lineage

The Chinese lineage consists of more than 120 species and many subspecies, which are widely distributed in southwestern China and eastern Asia including Japan, Sakhalin, and the Kurile Islands. Their morphological diversification is remarkable not only within the Procrustimorphi but also throughout the subfamily Carabinae.

Molecular phylogenetic analysis has made it clear that almost all important principles governing the evolution of the carabid beetles are manifested in this lineage, as will be discussed in Chapter 8.

The branching orders of various groups within this lineage are not definite, because resolution around the root of the *ND5* tree is rather ambiguous, and we tentatively assume the presence of eight sublineages (Fig. 5.37). The sublineages 1 and 2, which consist of *Pseudocoptolabrus* spp. and *Megodontoides erwini*, respectively, always occupy the outgroup positions on the trees, even if they are constructed using the UPGMA, MP-, ML-, or NJ-method or the outgroup is replaced. This suggests that these two (or either one of them) are the ancestral lineages to all other Chinese Procrustimorphi ground beetles.

Five species of *Pseudocoptolabrus* are known and are all found in the high mountainous regions of northern Myanmar, and Yunnan and Sichuan, China. *Megodontoides erwini* is found in central Sichuan.

Emergence of the sublineages 3 to 8 seems to have taken place within a short time. The sublineages 3 and 4 are composed of a single macrocephalic species, *Acathaicus alexandrae* from the southern part of Gansu

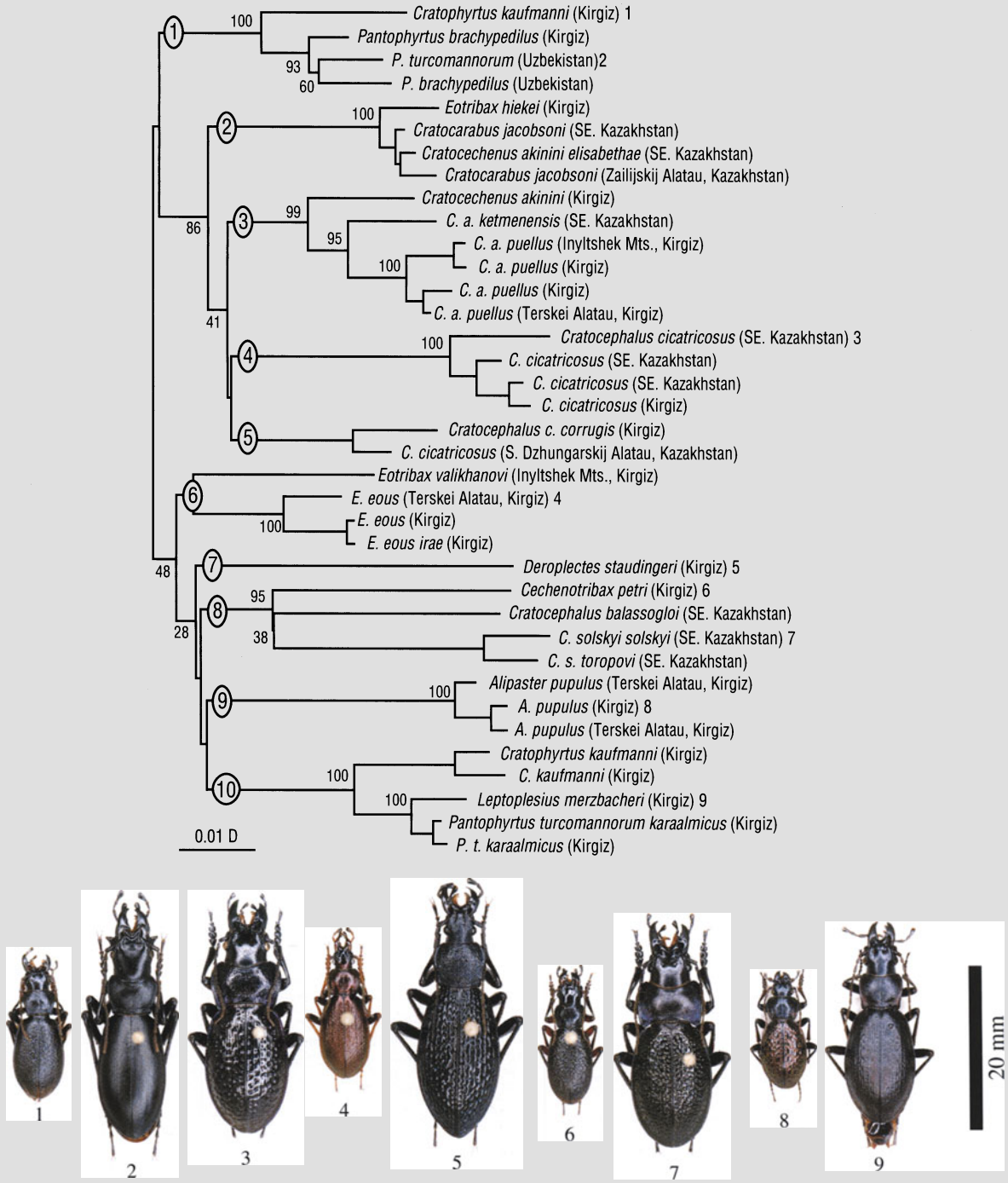


FIG. 5.36. Phylogenetic tree of the mitochondrial *ND5* gene for the Tianshanese lineage of the division Procrustimorphi. Constructed using the NJ-method. Photographs are shown below the tree, and the numbers correspond to those following scientific names in the tree (after Kim et al. 2003)

and northern Sichuan, in China, and a beautifully colored *Acoptolabrus*-like species, *Coptolabrodes haeckeri*, from southern Shaanxi, respectively.

The composition of the sublineage 5 is complex; about ten groups emerged at about the same time. Among them, *Imaibiodes businskyi* from northwestern Yunnan, *Lasiocoptolabrus sunwukong* from southern Shaanxi, and *Aristocarabus viridifossulatus* from various parts of Sichuan and western Hubei, respectively, constitute well-isolated clusters, containing only a single species within each cluster.

In contrast, some species classified into different genera are assembled together in one cluster as seen for *Shunichiocarabus uenoianus* and *Pagocarabus crassesculptus*, *Neoplesius* spp. and *Eocechenus leptoplesioides*, and *Eccoptolabrus exiguus* and *Calocarabus aristochroides*.

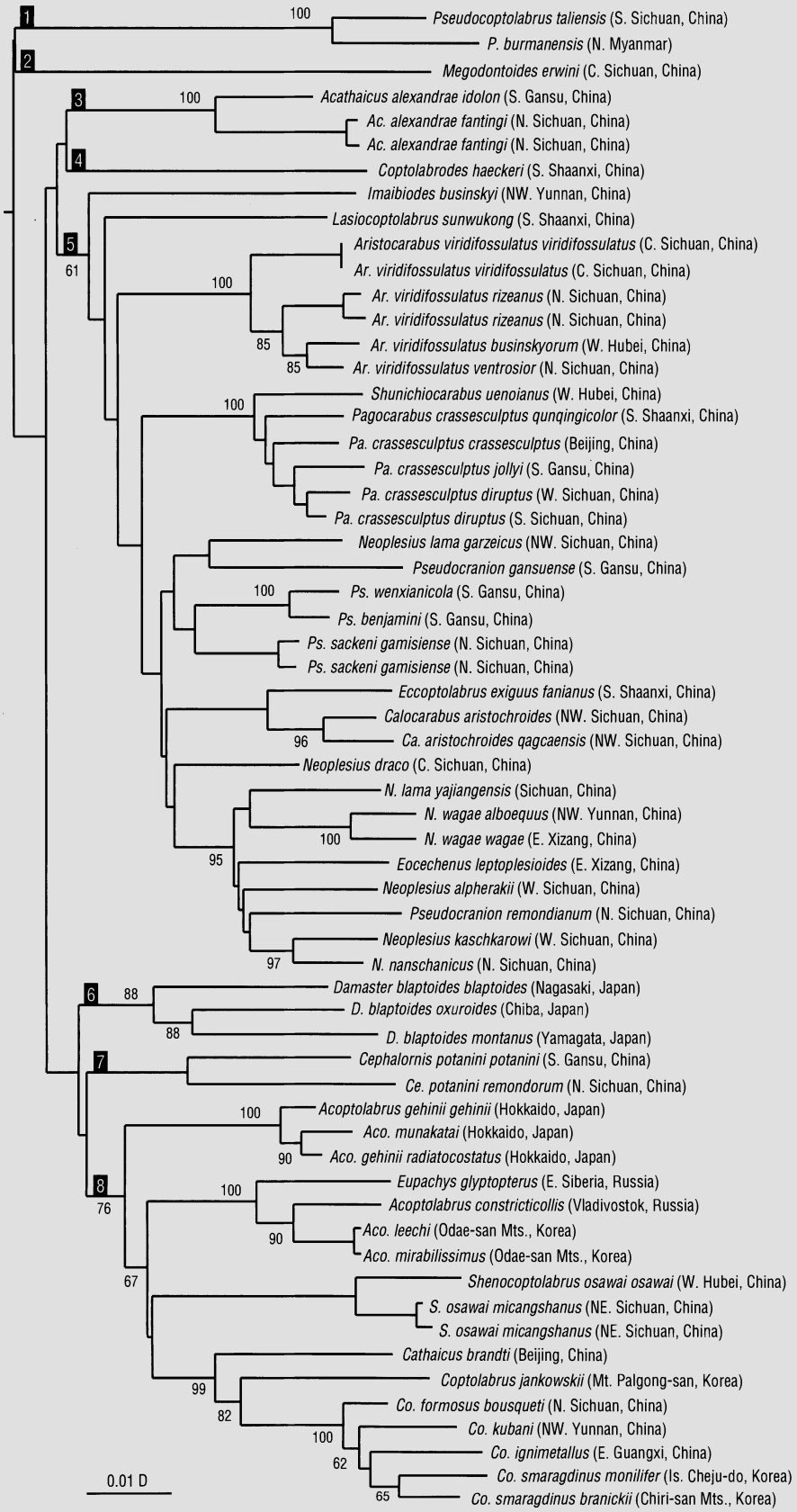


FIG. 5.37. Phylogenetic tree of the mitochondrial *ND5* gene for the Chinese lineage of the division Procrustimorphi. Constructed using the NJ-method (after Kim et al. 2003)

Shunichiocarabus uenoianus is found in Sichuan and Hubei. This species has been considered a relative of *Pseudocranion*, *Eccoptolabrus* (see below), or *Lasiocoptolabrus*, but on the *ND5* tree it is closely related to *Pagocarabus crassesculptus*, which is distributed widely in northern China. Reexamination of morphology indicates that *Shunichiocarabus* and *Pagocarabus* share several common characters such as a hypertrophic anterior tooth on the right mandibular retinaculum and the basic structure of male genitalia (Imura 2002b). It is likely that *Shunichiocarabus* is a specialized local form of *Pagocarabus*.

The phylogenetic relationships among the remainders of the subcluster 5 are quite complex. Three species of *Pseudocranion*, *P. wenxianicola*, *P. benjamini*, and *P. sackeni*, are clustered together, but *P. gansuense* pairs with *Neoplesius lama garzeicus*, and *P. remondianum* appears with some other *Neoplesius* species (see below). *Eccoptolabrus exiguus* is clustered with *Calocarabus aristochroides*, although the external morphology of these two species is quite different (see Fig. 8.2, Nos. 9 and 10).

Many *Neoplesius* species (except for *N. lama garzeicus* (see above) and *N. draco*) mainly found in the high mountainous areas of Sichuan and Xizang, are clustered together with species belonging to other genera, such as *Pseudocranion remondianum* and *Eocechenus leptoplesioides*.

The diversification of most of these populations took place within a short time about 10 MYA. Morphologically, *Neoplesius* and *Pseudocranion* are alike, while *Eocechenus* is quite different from *Neoplesius*, revealing a remarkable macrocephaly (see Chapter 8 and Fig. 8.2, Nos. 11 and 12; Fig. 8.11a). These results greatly shocked us, because such a phylogenetic relationship between *Eocechenus* and other species was far beyond our expectations, and would be a good example of discontinuous morphological differentiation as will be discussed in Chapter 8.

Another point of interest is that the species that have been thought to belong to the same genus appear in different clusters. For example, *Neoplesius lama* and *Pseudocranion gansuense*, form a single line, while as noted above, the other *Neoplesius* species and *Pseudocranion remondianum* are clustered together. *Neoplesius draco* seems to be remote from other *Neoplesius* species. Although more analysis with nuclear DNA should be performed, our results suggest that morphology does not necessarily run in parallel to phylogeny.

The sublineage 6 is composed of only *Damaster blaptoides*, which is endemic to Japan and the Kurile Islands. A detailed description of this species will appear in Chapters 6 and 7.

The sublineage 7 comprises a single species, *Cephalornis potanini*, which is one of the most peculiarly shaped carabine beetles. Its body shape approaches that of the Cychrini characterized by a considerable microcephaly. The genetic distance between a specimen from southern Gansu and one from northern Sichuan was relatively large, and their separation is estimated to have initiated about 15 MYA.

The sublineage 8 contains the rest of the Chinese Procrustimorphi species, containing a number of "star" ground beetles such as *Coptolabrus*, *Acoptolabrus*, and *Shenocoptolabrus*. The resolution of various groups near the root of the tree is poor, so that they are presumed to have radiated within a relatively short period. The genera *Damaster* (s. str.), *Coptolabrus*, and *Acoptolabrus* have often been incorporated into *Damaster* (s. lat.) as a result of their morphological similarity. From cladistic analysis based on morphology, it was assumed that *Damaster* (s. str.) shares common ancestry with *Coptolabrus* (Ishikawa 1986a). In addition to these three, the genera *Coptolabrodes* (a member of the sublineage 4; see above) and *Shenocoptolabrus*, both of which have been recently found in China, have been considered members of *Damaster* (s. lat.).

Coptolabrodes haeckeri is morphologically similar to *Acoptolabrus*, and *Shenocoptolabrus osawai* has morphological characters similar to *Acoptolabrus*, *Coptolabrus*, and *Damaster* combined together. However, the *ND5* tree is not consistent with this classification based on morphology. Rather, the emergence of each of genera is venerable so as to form an independent cluster.

More surprisingly, *Eupachys glyptopterus*, found in eastern Siberia, which has a stout, black body marked by extreme macrocephaly, is clustered with the beautifully colored *Acoptolabrus* spp. from the northeastern Eurasian Continent. Similarly, *Cathaicus brandti* from the Beijing area of China, which resembles *Eupachys glyptopterus* in body shape, has a sister relationship with the brilliantly decorated *Coptolabrus* spp. from the southeastern Eurasian Continent (allopatric parallel evolution; see Chapter 8).

Eupachys, *Cathaicus*, and *Acathaicus* (sublineage 3) are, at first glance, very similar in their morphology, and have been treated as closely related genera. Thus, the molecular phylogeny has uncovered surprising phylogenetic relationships between these three as well as some other genera. It should also be pointed out that considerable genetic differences between individuals from different localities has been revealed in several species, such as *Damaster blaptoides*, *Shenocoptolabrus osawai*, *Cephalornis potanini*, and *Acathaicus alexandrae*, suggesting that fundamental morphology remained unchanged for a long time after geographic isolation. The evolutionary significance of these findings will be discussed in Chapter 8.

The distribution areas of the members of *Acoptolabrus* are the northeastern region of the Eurasian Continent surrounding the Sea of Japan, including Sakhalin and Hokkaido. They were considered to be phylogenetically close because of considerable morphological similarities. However, an *ND5* phylogenetic tree reveals that

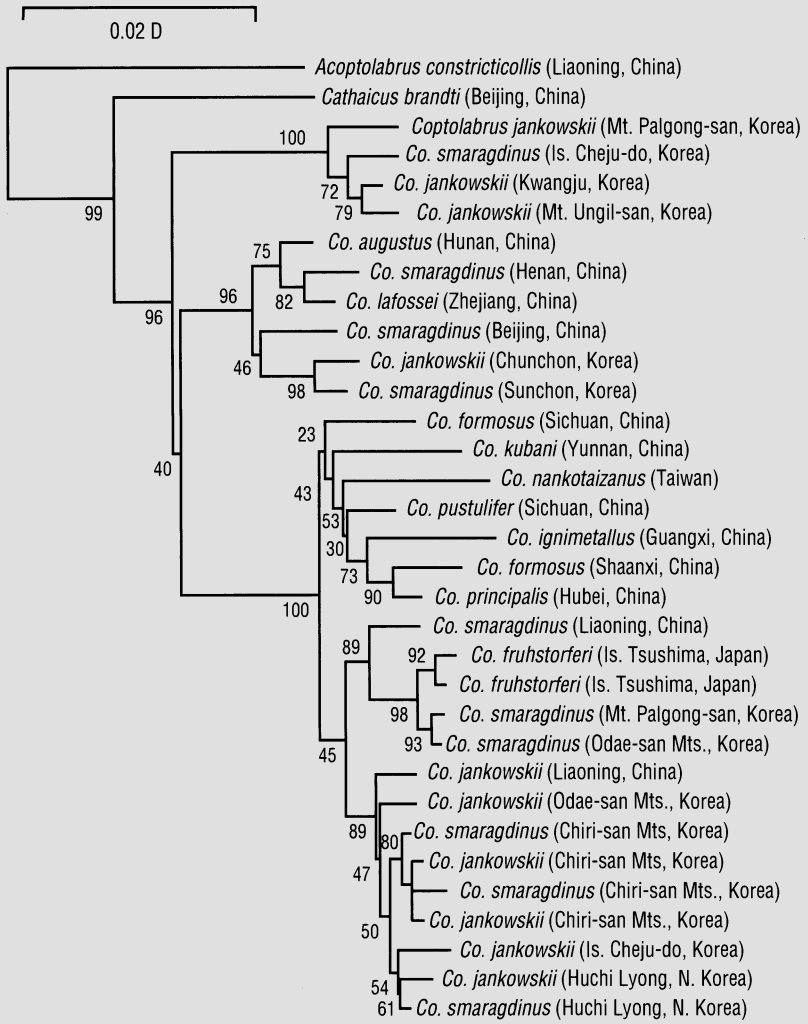


FIG. 5.38. Phylogenetic tree of the mitochondrial *ND5* gene for the genus *Coplolabrus*. Constructed using the NJ-method (after Okamoto et al. unpublished)

A. constricticollis, *A. leechi*, and *A. mirabilissimus*, found in the Eurasian Continent, including the Korean Peninsula, form a cluster clearly separable from another cluster composed of *A. gehinii* from Hokkaido and *A. lopatini* from Sakhalin (see the lineage 8 in Fig. 5.37). Their separation took place 20–30 MYA. It should also be noted that the former cluster contains *Eupachys glyptopterus* (see above). Presumably, the continental species and the *A. gehinii*/*A. lopatini* were derived from a common ancestor, but it is also possible, though not very likely, that they evolved independently with parallel morphological changes. *Acoptolabrus leechi* and *A. mirabilissimus* occur sympatrically in Odae-san, South Korea, and are made morphologically distinct from one another by the considerable macrocephaly of *A. mirabilissimus*, and yet the genetic difference between them is almost nil. It is possible that these two "species" are actually two forms of the same species. The genetic distances between various subspecies of *A. gehinii* (including *A. munakatai*) are also very small. For the origin of *A. gehinii*, see Chapter 7, p. 139.

The genus *Coptolabrus* contains 14 species (Imura and Mizusawa 1996; Březina 1999), all of which have a beautiful metallic color and wonderfully sculpted elytra. The center of distribution of this genus is southeastern China and some of them are also found in eastern Siberia, around Primorskij, the Korean Peninsula, Cheju-do Island, the Tsushima Islands, and Taiwan. Most of the species show considerable geographic variations, so that quite a few subspecies or forms have been described.

An *ND5* tree shows that *Coptolabrus* diversified into three lineages ca. 14 MYA, which is a little later than that of *Damaster* (15 MYA). The composition of each lineage is complex. The first lineage contains *C. jankowskii* and *C. smaragdinus* from three localities on the Korean Peninsula and *C. smaragdinus* from Chenju-do Island. The second lineage includes *C. smaragdinus* from Henan and Beijing in China, and the southern edge of the Korean Peninsula, *C. jankowskii* from the northern part of the Korean Peninsula, *C. augustus* from Hunan, and *C. lafossei* from Zhejiang in China. The third

lineage seems to be further divided into three clusters, though these are not definite because of the small genetic distance between them.

The first cluster contains several *Coptolabrus* species from southern China such as *C. formosus* from Sichuan and Shaanxi, *C. kubani* from Yunnan, *C. nankotaizanus* from Taiwan, *C. pustulifer* from Sichuan, C. *ignimetallus* from Guangxi, and *C. principalis* from Hubei. These six species are considerably different in appearance and are considered as different morphological species. It is of particular interest that this cluster includes *C. kubani* from Yunnan, which is the smallest *Coptolabrus* species with *Cychrus*-like mouth parts. Despite this apparent morphological diversification, their divergence took place relatively recently, less than 7–8 MYA. The second cluster includes *C. fruhstorferi* from the Tsushima Islands, *C. smaragdinus* from the Korean Peninsula and Liaoning in China. The third cluster includes *C. smaragdinus* and *C. jankowskii* from the Korean Peninsula plus Cheju-do Island and Liaoning in China.

This demonstrates that there are a number of apparent disagreements between classification of species according to the traditional morphological approach and those completed using molecular phylogeny. Ignoring such ambiguity for the moment, the establishment of the above three haplotype lineages will be discussed below. The ancestral form of *Coptolabrus* that inhabited some part of the Huabei Plains, China, diversified into three lines.

The ancestor of the first line (sublineage) invaded southern Korea from southeastern China at a relatively early time. This is consistent with the fact that this sublineage does not include the Chinese inhabitants. The members of the second line expanded their distribution to northeastern China and some invaded the whole area of the Korean Peninsula. The third line originated from a part of the second line, and invaded the whole area of the Korean Peninsula and the Tsushima Islands. Since the descendants of the first and the third lines seem to co-inhabit the southern part of the Korean Peninsula and Cheju-do Island, the invasion of these areas would have occurred twice.

As noted above, the "species" defined by morphology are not always consistent with the results of mitochondrial *ND5* phyogeny. *Coptorabrus smaragdinus* and *C. jankowskii* appear in all the three lineages. Especially notable is the fact that the *ND5* sequences of samples of the above two "species" derived from the same locality, such as Chiri-san in the southern part of South Korea, are almost the same. One explanation for this might be that these two "species" are only two morphological types of a single species that can change morphology from one type to another. Another possibility is that *C. smaragdinus/jankowskii* is the ancestral form of *Coptolabrus*, from which various species emerged from the respective lineages.

At present, there is nothing to suggest the horizontal transfer of mitochondrial genes by hybridization, or participation of ancestral polymorphism and random lineage sorting, although such a possibility cannot be avoided. It is hoped that more samples from various localities will be analyzed for not only mitochondrial genes but also nuclear genes.

It is clear that the evolutionary history of *Coptolabrus* is relatively short; all the species are phylogenetically close despite of apparent diversification of bright decoration.

In this chapter we have dealt with the molecular phylogeny of the Carabinae. Although several genera and species have not yet been analyzed, the overall pattern of the Carabinae phylogeny has become clear. One merit of molecular phylogeny may be that it allows us to pinpoint erroneous taxa classifications based on morphology alone. In most cases, a disagreement may be solved by morphological reexamination. However, there exist not a few cases in which the discrepancy cannot be bridged, as has been described in this chapter. This suggests that clear evidence of evolutionary principles in action cannot be reached by morphology alone. The purpose of this book is to cast some light on the principles of morphological evolution rather than to taxonomically arrange the Carabinae. It is hoped that this chapter plays an introductory role for Chapter 8, in which the outline of the Carabinae evolution is discussed in greater detail.

Chapter 6
Formation of the Japanese Carabina Fauna

6.1 Two Aspects of the Establishment of the Japanese Fauna

The Japanese Carabina are currently classified into 12 genera, 36 species, and many subspecies (Imura and Mizusawa 1996) (Fig. 6.1).

It is widely believed that not only all the Japanese Carabina species (Ishikawa 1989, 1991), but also many other Japanese insect species migrated to the Japanese Islands from the Eurasian Continent during the glacial era (<2 MYA) over land bridges, followed by propagation and differentiation within the Japanese Islands.

Those who hold this view speculate that the insects that had inhabited the Japanese Islands at that time failed in many cases to adapt entirely successfully to the abrupt transition from the subtropical climate that prevailed in the late Miocene epoch to the much colder climate of the Pleistocene epoch that began ca. 2 MYA, so that ground beetles such as many of the Carabina species became extinct. According to this hypothesis, the Japanese Islands were likely without the Carabina species (and most other insect species) for some time after the climate change.

In contrast, Hiura (1965) has suggested the occurrence of the Miocene elements that are mostly endemic to the Japanese Islands (the autochthonous or geohistoric type species). Hiura (1971) discovered a late Miocene fossil (ca. 9 MYA) of a species of *Ohomopterus* (reported as *Apotomopterus* sp.) in Tottori Prefecture, Japan. This was, at that time, the most convincing evidence of the presence of autochthonous-type inhabitants in Japan long before the glacial era.

One could argue, however, that though *Ohomopterus*-like species undoubtedly inhabited these areas at one stage, they became extinct due to climate change. This means that the present-day *Ohomopterus* species might be derived from later invaders that migrated to the Japanese Islands from the continent during the glacial era.

6.2 Geohistory of the Japanese Islands

To draw a scenario of the formation of the Japanese Carabina fauna, a brief geohistory of the Japanese Islands is perhaps helpful. Paleomagnetic evidence indicates that the ancient Japan area broke off about 15 MYA from the eastern periphery of the Eurasian Continent, followed by its separation into northeast and southwest arcs as a consequence of the double-door opening of the Sea of Japan (Otofuji et al. 1991, 1994) (Fig. 6.2).

Shortly after this event, the proto-Japanese Islands took on the shape of an archipelago as a result of an extensive submergence, especially of the northeast arc (Fig. 6.2b). Following the formation of the archipelago, an extensive upheaval began (Fig. 6.2c, d), and the proto-form of the Japanese Islands was established. About 2 MYA, the Japanese Islands were subjected to a glacial era, during which the islands connected to (Fig. 6.2e), and separated from, the continent several times.

6.3 Procedures Used to Estimate the Establishment of the Japanese Carabina Fauna

Specimens representing all the Japanese species and a number of subspecies (geographic races) of the Carabina have been examined for the *ND5* gene sequence. A number of species from the Eurasian Continent and Sakhalin that were believed to be the same as, or closely related to, the Japanese species were also examined. The classification at subspecies level has not been considered because of its limited importance to matters under discussion in this section. The phylogenetic tree constructed as a result in conjunction with *ND5* DNA-based dating and the geohistory of the Japanese Islands suggests that the Japanese Carabina species can be roughly classified into two categories with respect to the establishment of their present habitats in the Japanese Islands.

Figure 6.3 shows the phylogenetic relationships of the Japanese Carabina species plus some related species from the Eurasian Continent and its adjacent islands.

FIG. 6.1. Representative Japanese Carabina species. **1** *Limnocarabus clathratus*, **2** *Euleptocarabus porrecticollis*, **3** *Carabus granulatus*, **4** *Carabus arvensis*, **5** *Carabus vanvolxemi*, **6** *Ohomopterus dehaanii*, **7** *Ohomopterus yaconinus*, **8** *Ohomopterus insulicola*, **9** *Hemicarabus tuberculosus*, **10** *Homoeocarabus maeander*, **11** *Leptocarabus procerulus*, **12** *Leptocarabus kurilensis*, **13** *Tomocarabus opaculus*, **14** *Tomocarabus harmandi*,**15** *Megodontus kolbei*, **16** *Coptolabrus fruhstorferi*, **17** *Acoptolabrus gehinii*, **18** *Damaster blaptoides*

If the diversification of a given species as revealed by the *ND5* gene sequence started long before the glacial period, and if a species is endemic to Japan and the same species or a direct ancestor does not inhabit the Eurasian Continent, then the species may be thought of as belonging to the autochthon category. If evidence showing that species A split in Japan long before the glacial era from species B inhabiting the continent and Japan, then both A and B also belong to this category. In other words, the direct ancestry of the species was an

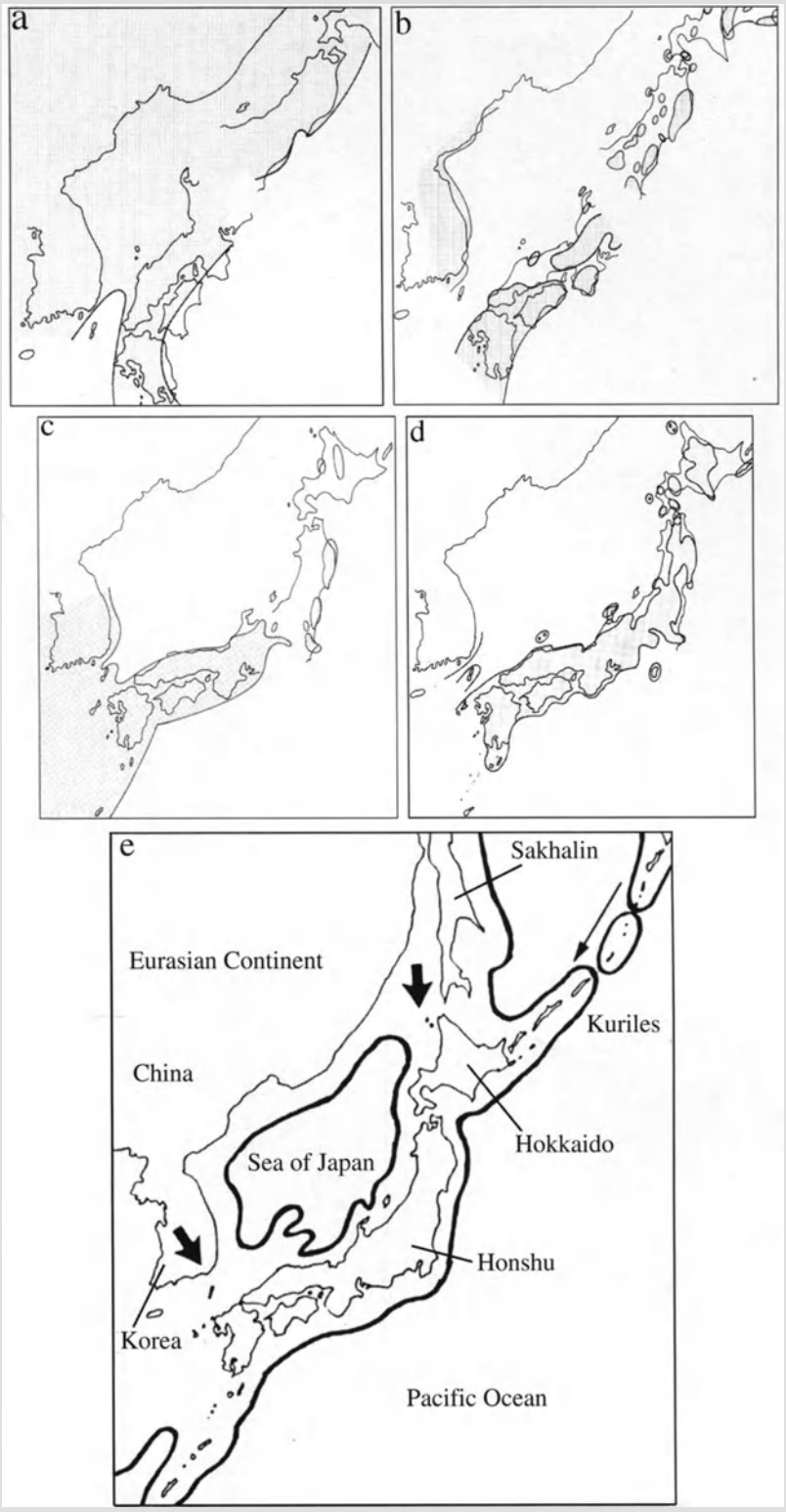


FIG. 6.2. Goehistory of the Japanese Islands. **a** 20–15 MYA, **b** 16 MYA, **c** 13 MYA, **d** 4.5 MYA, and **e** <2 MYA. **Shaded areas** in a-d indicate lands that are superimposed onto the Japanese Islands. **Shadow** for most of the land areas on the continent is omitted in **b–d**. After Editorial Group for Computer Graphics of Geology of the Japanese Islands (1996); modified, **e** Coastlines are shown in bold lines. Arrows indicate invasion [after Baba and Hirashima (1991) modified, and Tominaga et al. (2000)]

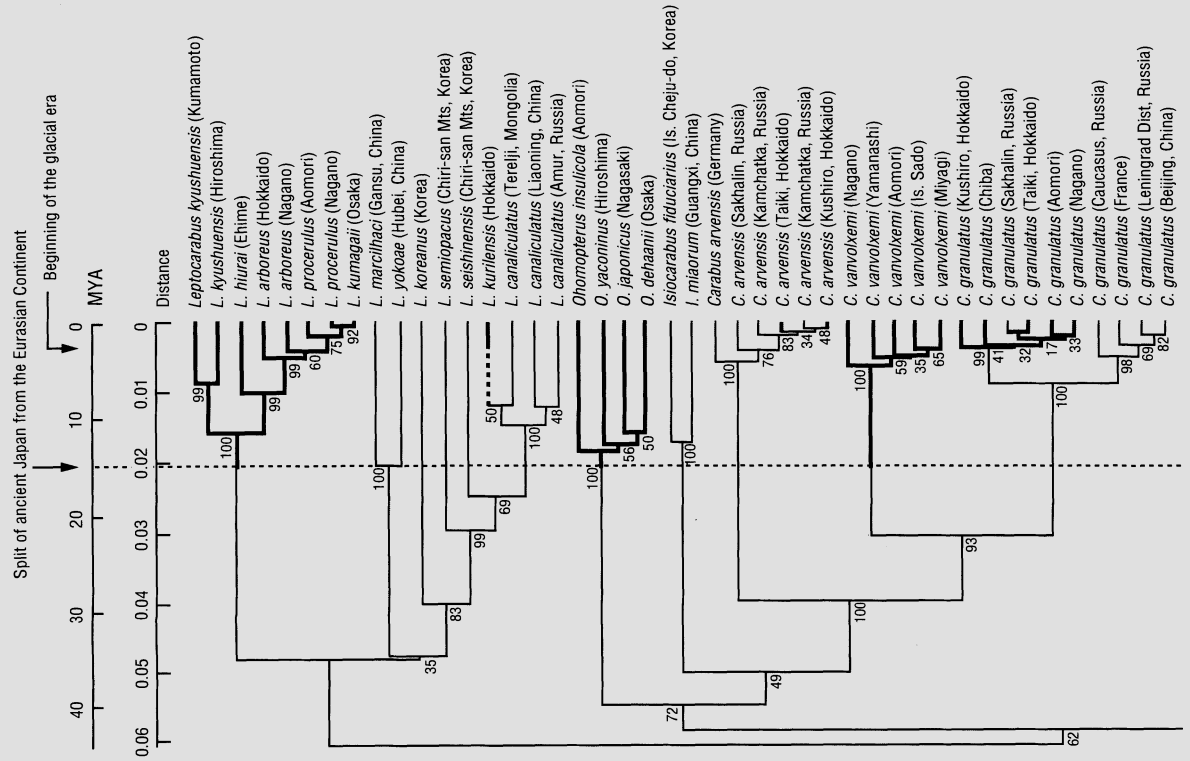
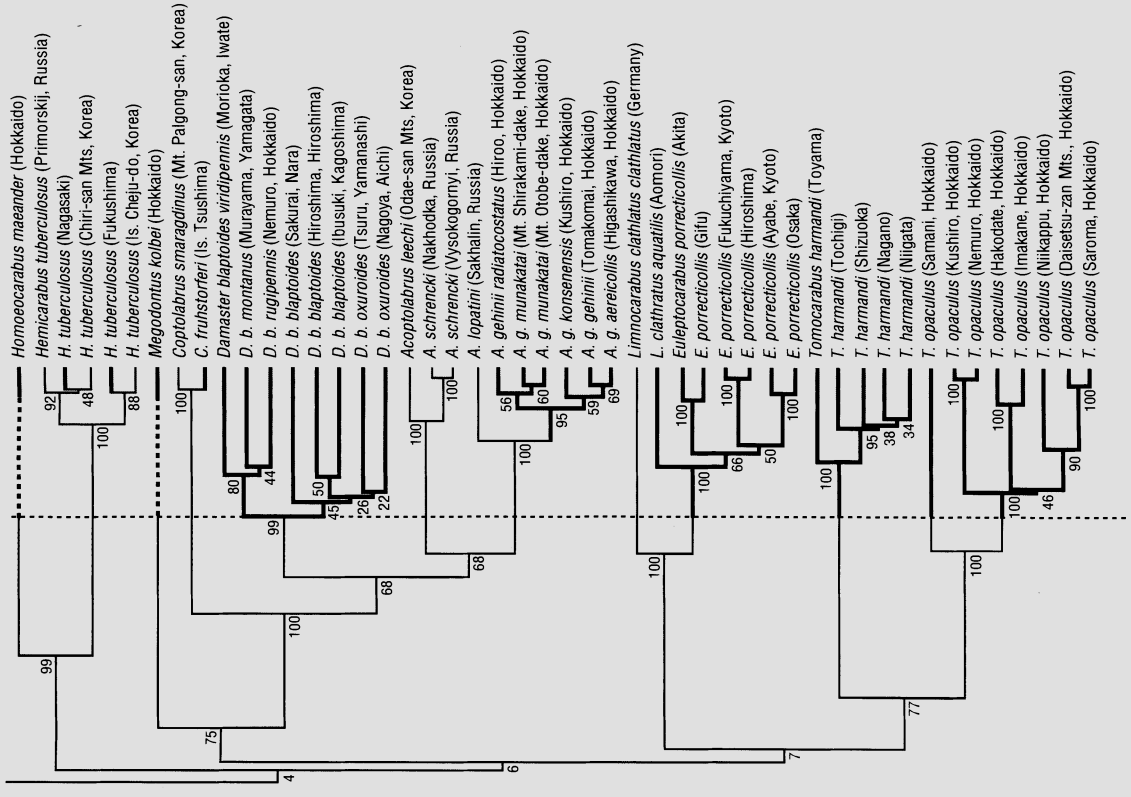


FIG. 6.3. Phylogenetic tree of the mitochondrial *ND5* gene for the Carabina species from Japan and adjacent areas. Only limited numbers of individuals from each species are used for construction of the tree among a considerably larger number of specimens analyzed. Some species outside the Japanese Islands are included in the tree to show their phylogenetic relationship to the Japanese species, branches of which are shown in **bold lines**. Because of a shortage of materials, the diversification point of the Japanese population cannot be determined for *Hemicarabus tuberculosus*, *Megodontus kolbei*, or *Leptocarabus kurilensis*. The branches for these species are shown in **broken lines**. For more detailed phylogenetic trees for the Japanese species, see Chapter 7 (after Tominaga et al. 2000)


Homoecarabus maeander (Hokkaido)
Hemicarabus tuberculosus (Primorskij, Russia)
H. tuberculosus (Nagasaki)
H. tuberculosus (Chiri-san Mts, Korea)
H. tuberculosus (Fukushima)
H. tuberculosus (Is. Cheju-do, Korea)
Megodontus kolbei (Hokkaido)
Coptolabrus smaragdinus (Mt. Palgong-san, Korea)
C. fruhstorferi (Is. Tsushima)
Damaster blaptoides viridipennis (Morioka, Iwate)
D. b. montanus (Murayama, Yamagata)
D. b. rugipennis (Nemuro, Hokkaido)
D. b. blaptoides (Sakurai, Nara)
D. b. blaptoides (Hiroshima, Hiroshima)
D. b. blaptoides (Ibusuki, Kagoshima)
D. b. oxuroides (Tsuru, Yamanashi)
D. b. oxuroides (Nagoya, Aichi)
Acoptolabrus leechi (Odae-san Mts, Korea)
A. schrencki (Nakhodka, Russia)
A. schrencki (Vysokogornyi, Russia)
A. lopatini (Sakhalin, Russia)
A. gehinii radiatocostatus (Hiroo, Hokkaido)
A. g. munakatai (Mt. Shirakami-dake, Hokkaido)
A. g. munakatai (Mt. Otobe-dake, Hokkaido)
A. g. konsenensis (Kushiro, Hokkaido)
A. g. gehinii (Tomakomai, Hokkaido)
A. g. aereicollis (Higashikawa, Hokkaido)
Limnocarabus clathlatus clathlatus (Germany)
L. clathratus aquatilis (Aomori)
Euleptocarabus porrecticollis (Akita)
E. porrecticollis (Gifu)
E. porrecticollis (Fukuchiyama, Kyoto)
E. porrecticollis (Hiroshima)
E. porrecticollis (Ayabe, Kyoto)
E. porrecticollis (Osaka)
Tomocarabus harmandi (Toyama)
T. harmandi (Tochigi)
T. harmandi (Shizuoka)
T. harmandi (Nagano)
T. harmandi (Niigata)
T. opaculus (Samani, Hokkaido)
T. opaculus (Kushiro, Hokkaido)
T. opaculus (Nemuro, Hokkaido)
T. opaculus (Hakodate, Hokkaido)
T. opaculus (Imakane, Hokkaido)
T. opaculus (Niikappu, Hokkaido)
T. opaculus (Daisetsu-zan Mts., Hokkaido)
T. opaculus (Saroma, Hokkaido)

inhabitant of ancient Japan at the time of its separation from the continent (ca. 15 MYA).

The species in the second group are recent invaders from the continent that migrated over land bridges from Sakhalin and/or the Kurile Islands to Hokkaido, or from the Korean Peninsula to western Japan during the glacial era (<2 MYA, except for *Acoptolabrus gehinii*, see below). These hypotheses may be drawn from examining data on the evolutionary distance between a given carabid species found in Japan and the same species or a phylogenetically close species from the continent including the Korean Peninsula and Sakhalin. If the distance is the shortest, for example, between the Sakhalin and Japanese populations, then one is led to the conclusion that the species currently inhabiting Japan were derived from recent immigrants from Sakhalin (the invaders). However, the distance of around 0.005 or less, which corresponds to 2 MYR or less, is not large enough to estimate the precise time at which each species migrated.

In summary, the first category includes species that were directly derived from ancestors that inhabited ancient Japan when it was attached to the eastern periphery of the Eurasian Continent (ca. 15 MYA), followed by their propagation within the Japanese Islands. This category may also include species that differentiated in the Japanese Islands from an ancient immigration from the continent.

The second category contains species that invaded from the Eurasian Continent through Sakhalin and/or the Kurile Islands, or from the Korean Peninsula during the glacial era (< 2 MYA).

6.4 The Autochthons

The genus *Damaster* (division Procrustimorphi), which is endemic to the Japanese Islands, is currently treated as a single species, *blaptoides*, consisting of nine subspecies or geographic races based on morphology.

A phylogenetic tree of the Carabina using the *ND5* gene sequence reveals that *Damaster* comprises a well-defined clade without any other species intermingled with it. Its origin can be traced back to the time of the radiation of the Chinese Procrustimorphi group at ca. 20 MYA (Su et al. 2001). The diversification of the *ND5* gene sequence started ca. 15 MYA, resulting in the emergence of western (W) and eastern (E) lineages, presumably as a consequence of the double-door opening of the Sea of Japan.

The subsequent archipelago formation would have caused the geographic isolation and independent evolution of proto-*Damaster* that had survived on the respective islands. The result would be the differentiation of the present eight *Damaster* races (Su et al. 1998).

The genus *Ohomopterus* (division Digitulati) is also endemic to the Japanese Islands. Diversification into more than ten species and many subspecies seems to have taken place in Japan starting shortly after the separation of ancient Japan from the continent (ca. 15 MYA). The *ND5* tree shows the existence of two major lineages under which several sublineages are organized (Su et al. 1996c).

Unlike *Damaster* (s. str.), the first *Ohomopterus* lineage contains the species inhabiting western Japan and the second one contains those from the Japan Sea islands and eastern Japan. One possibility is that the ancestor of *Ohomopterus* inhabited the southwest arc of the ancient Japan area, and expanded its distribution to Kyushu, western Japan, and central Japan through ca. 9 MYA.

Along with the upheaval of eastern Japan, a certain fraction of the Japan Sea islands population of *Ohomopterus* invaded the new environment (eastern Japan) and propagated. The species in the genus *Isiocarabus*, which inhabit eastern China and the Korean Peninsula, are morphologically quite similar to *Ohomopterus*, so that it has long been believed that *Isiocarabus*, especially *I. fiduciarius* found in the Korean Peninsula, shares a common ancestry with *Ohomopterus*. However, the *ND5* phylogenetic tree clearly shows that *Ohomopterus* belongs to a lineage independent not only from *Isiocarabus fiduciarius* (Korea) and *I. miaorum* (southwest China) but also from the other genera in the division Digitulati from the continent (see Chapter 5, p. 72). No direct sister group of *Ohomopterus* has been found either on the Korean Peninsula or in China.

The Japanese *Leptocarabus* species (division Latitarsi) are classified into three species-groups (or subgenera) based on morphological criteria (Imura and Mizusawa 1996). *Leptocarabus arboreus* belongs to the subgenus *Adelocarabus* and is found on Honshu and Hokkaido, showing considerable local variations. *Leptocarabus procerulus* (Honshu and Kyushu), *L. kumagaii* (Honshu), *L. hiurai* (Shikoku), and *L. kyushuensis* (Kyushu and western Honshu) belong to the subgenus *Leptocarabus* (s. str.), and *L. kurilensis* to the subgenus *Aulonocarabus* (see Chapter 7, pp. 120–125).

It has been speculated that the Japanese *Leptocarabus* (*Adelocarabus*) species imigrated from the Korean Peninsula via land bridges in the glacial era. Morphological features including the male genitalia of the Korean species, *L.* (*Adelocarabus*) *seishinensis*, is very similar to that of *L. arboreus*. Similarly, the Japanese *Leptocarabus* (s. str.) species bear a striking resemblance to two Chinese species, *L.* (s. str.) *yokoae* and *L. marcilhaci*, and reveal a certain morphological affinity to *L.* (*Weolseocarabus*) *koreanus*.

Thus the Japanese *Leptocarabus* (s. lat.) species were thought to share common ancestry with the Korean or the Chinese species (Ishikawa 1991; Imura and Mizusawa 1996). However, phylogenetic analysis using the *ND5* gene and nuclear 28S rDNA (Kim et al. 2000b) show that the *Leptocarabus* (s. lat.) species that

have been examined consist of three distinct lineages (see Chapter 5, pp. 64–66, and Chapter 8, pp. 120–125). The first lineage contains two Chinese species, *L. marcilhaci* and *L. yokoae* from central China. The second one includes all the Korean species (*L. seishinensis*, *L. semiopacus*, and *L. koreanus*) and *L. canaliculatus* (including *L. kurilensis*) from Mongolia, China, Russia, and Hokkaido.

The third lineage consists of all the Japanese *Leptocarabus* species. No direct sister species for the Japanese *Leptocarabus* have been found either in the Korean Peninsula or from China. Diversification of the Japanese *Leptocarabus* as seen from the *ND5* gene sequences began with the separation of *L. kyushuensis* from all the other Japanese *Leptocarabus* species. The *ND5* gene sequences of *L. procerulus* (Honshu), *L. kumagaii* (Honshu), and *L. arboreus* (Honshu) are so close to each other that meaningful distinction was not possible (Kim et al. 2000a).

From these results, it may be inferred that the ancestor of the Japanese *Leptocarabus* inhabited the southwest arc of ancient Japan, and later expanded its distribution to the northeast with differentiation into several species and many geographic races. This situation somewhat resembles that of *Ohomopterus*. The origin and diversification of the Japanese *Leptocarabus* will be discussed in further detail in Chapter 7.

Tomocarabus opaculus (division Latitarsi) is found in greatest numbers in Hokkaido, and is also distributed in the mountainous areas of northern Honshu. *Tomocarabus* and its allied genera radiated into several species 30–35 MYA. The origin of *T. opaculus* can be traced back to the time of the radiation. No other species branched off from the *opaculus* lineage (Su et al. 2001).

Diversification of the *ND5* sequences started ca. 20 MYA, which is significantly before the time that the Japanese Islands separated from the continent. It may be speculated that the ancestors of *T. opaculus* were already divided into at least two isolates in the ancient northeastern Japan area before the islands split from the continent.

Tomocarabus harmandi (division Latitarsi) is distributed rather sporadically in the mountainous areas of central to northern Honshu and is not found in Hokkaido. Like *T. opaculus*, the origin of this species is venerable and no direct sister species have been discovered. The *ND5* gene diversification began ca. 10 MYA. Presumably, the ancestors of *T. harmandi* inhabited the restricted area of ancient central Honshu.

Carabus (s. str.) *vanvolxemi* (division Digitulati) is distributed in eastern Honshu. The Chinese *Carabus* (s. str.) species (e.g., *nanosomus*, *paris*, and *pseudolatipennis*) are morphologically similar to *C. vanvolxemi* and have been thought to share common ancestry with *C. vanvolxemi*. The *ND5* phylogenetic tree, however, revealed that the Chinese species form an independent cluster from that including *C. vanvolxemi*. *Carabus vanvolxemi* shares common ancestry with *C. granulatus* with their ancient separation ca. 20 MYA most probably having taken place in the continent (see Chapter 5, p. 75).

The *ND5* sequences from specimens from various localities, including Sado Island, reveal that diversification started 5–6 MYA. Because of a lack of phylogenetically close relatives, we assume that the ancestors of *C. vanvolxemi* inhabited the ancient area of eastern Honshu in the continent, and was isolated in some restricted area until its diversification.

Euleptocarabus porrecticollis (division Lepidospinulati) is endemic to Japan and is sporadically distributed solely on the island of Honshu, from the Tohoku District (eastern Honshu) through the Chugoku District (western Honshu). This species is phylogenetically most closely related to *Limnocarabus clathratus* (Su et al. 1996a; Imura et al. 1998a; Kim et al. 1999a).

Limnocarabus clathratus is widely distributed in the northern half of the Eurasian Continent and several adjunctive islands including Japan. *Limnocarabus clathratus* on the Eurasian Continent would have immigrated to a restricted region in ancient Japan with its separation from the continent (see Tado Collaborative Research Group [1998] for the fossil record of this species). Following this, *E. porrecticollis* branched off from *L. clathratus* ca. 11 MYA and diverged.

6.5 The Invaders

Two *Carabus* (s. str.) species (division Digitulati), *C. granulatus* and *C. arvensis*, are widely distributed in the Eurasian Continent, Sakhalin, and Hokkaido. The distribution of *C. granulatus* extends to the eastern half of Honshu. The evolutionary distance of *C. granulatus* from all the localities is close (Su et al. 2003e).

The specimens from Japan reveal more affinity with those from Sakhalin than those from the continent. The situation is similar to that in *C. arvensis*, in which two specimens from the Kamchatka Peninsula are also close to the Hokkaido population. Thus, the Japanese populations of these two species were established by immigration first from the continent to Sakhalin and/or the Kuriles, and then to Hokkaido (and eastern Honshu for *C. granulatus*) probably over land bridges in the glacial era.

Hemicarabus tuberculosus (division Crenolimbi) is widely distributed all over Japan, Siberia, Sakhalin, and Korea. Except for Sakhalin specimens that have not been analyzed, all the specimens from the above localities are close in the *ND5* gene sequence. The Japanese population is likely to have been recently established by invader(s) from north (Sakhalin ?) and/or from south (Korea).

Coptolabrus fruhstorferi (division Procrustimorphi) inhabits only the Tsushima Islands. Its *ND5* sequence is

very close to that of *C. smaragdinus* from the southern part of Korea, suggesting that *C. fruhstorferi* was derived from a Korean ancestor through a recent land bridge and then was isolated on the island.

Acoptolabrus gehinii (division Procrustimorphi) is distributed in Hokkaido and divided into many subspecies. Several species morphologically very similar to *A. gehinii* have been found in eastern Asia (Primorskij through Korea) (*A. constricticollis*, *A. schrencki*, *A. leechi*, etc.), and Sakhalin (*A. lopatini*). The *ND5* phylogenetic tree shows that *A. gehinii* from various localities in Hokkaido reveal only a small difference in gene sequence from *A. lopatini*, found in both the central and southern parts of Sakhalin. They are, however, remote from all species in the continent.

The geohistorical relations between the continent, Sakhalin, and Hokkaido, remain unclear, yet the tree suggests that the ancestor of *A. lopatini* was an inhabitant of the ancient Sakhalin area in the continent, and was isolated from the continental species ca. 18 MYA, having differentiated to *A. lopatini* there. The direct ancestor of *A. gehinii* is most probably the Sakhalin ancestor that invaded Hokkaido earlier (7–8 MYA) than *Carabus arvensis/C. granulatus* (<2 MYA), and started to propagate 3–4 MYA. If this is correct, *A. gehinii* most likely belongs to another category different from the typical invader defined above.

Megodontus kolbei (division Procrustimorphi), which inhabits Hokkaido, belongs most probably to the second category, being either a recent immigrant from Sakhalin or from northeastern Asia, although not enough specimens have been analyzed to be certain.

Homoeocarabus maeander (division Crenolimbi) is rather sporadically distributed in Hokkaido, Sakhalin, Cheju-do Island (South Korea), eastern Asia, and North America, and is not found in Honshu, Japan, at present. Recently, a fossil of *H. maeander* was discovered in Nagano Prefecture, Honshu (Hayashi and Tominaga 1995; Hayashi 1998) and estimated to be 1–2 MYA. This indicates that this species inhabited Honshu before the latest glacial era.

The Honshu population became extinct, presumably through loss of the kind of habitat (likely to have been lowland moor) required by this species. Whether this species is an autochthon or an invader would be made certain by examining more specimens from various localities in Hokkaido and the Eurasian Continent. [The above *H. maeander* fossil might be that of *Hemicarabus tuberculosus* (Hayashi 2002)].

Leptocarabus (*Aulonocarabus*) *kurilensis* (division Latitarsi) is found in Hokkaido, Sakhalin, and the Kuriles, and is one of the descendants of the *L. canaliculatus* species-complex which inhabit the northeastern part of the Eurasian Continent (Kim et al. 2000b). *Leptocarabus kurilensis* of Hokkaido is probably a recent invader from Sakhalin and/or the Kuriles, but this is not certain because only one specimen from Mt. Daisetsu, Hokkaido, was analyzed. More specimens, especially those from Sakhalin and the Kuriles, should be examined.

TABLE 6.1. Origins of the Japanese Carabina species

Autochton	Invader
Ohomopterus spp.	*Carabus granulatus*
Carabus vanvolxemi	*Carabus arvensis*
Limnocarabus clathratus	*Hemicarabus tuberculosus*
Euleptocarabus porrecticollis	*? Homoeocarabus maeander*
Leptocarabus spp. (excluding *L. kurilensis*)	*? Leptocarabus kurilensis*
	? Megodontus kolberi
Tomocarabus opaculus	*Coptolabrus fruhstorferi*
Tomocarabus harmandi	*Acoptolabrus gehinii* (including *A. munakatai*)
Damaster blaptoides	

(after Tominaga et al. 2000)

As mentioned in the introductory part of this section, the major Japanese Carabina groups, such as *Damaster*, *Ohomopterus*, and *Leptocarabus* (autochthons) were thought to have invaded Japan during the glacial era from the Korean Peninsula, assuming that direct ancestors existed there. For example, *Damaster* was derived from *Coptolabrus*, the Japanese *Leptocarbus* from *Leptocarabus semiopacus/L. koreanus/ L. seishinensis*, and *Ohomopterus* from *Isiocarabus*, all Korean inhabitants.

What the mitochondrial phylogenetic tree shows is that no direct sister species of the above Japanese Carabina species have been found in either the Korean Peninsula or in continental China. Therefore, invasions of Korean ancestors to the Japanese Islands during the glacial era are highly unlikely for the above species. The most plausible explanation is that the Japanese autochthons were the exclusive inhabitants of the ancient Japan area of the continent. This, together with the geohistory of the Japanese Islands, is consistent with the idea that the history of the autochthons began at the time of the separation of the Japanese Islands from the continent (15 MYA). In conclusion, there are roughly two types of Japanese Carabina species with respect to origin (Table 6.1). A good number of species can be thought of as autochthons, the ancestors of most of which inhabited the ancient Japan area before its separation from the continent, with others being recent invaders from the continent via Sakhalin, and/or the Kuriles, or the Korean Peninsula. These results lend strong support to Hiura's views.

Chapter 7
Detailed Exposition of the Japanese Carabina Species

7.1 *Limnocarabus clathratus* and *Euleptocarabus porrecticollis*

Euleptocarabus porrecticollis is endemic to Japan and is sporadically distributed solely in Honshu, from the Tohoku District (eastern Honshu) to the Chugoku District (western Honshu) (Fig. 7.1). The distribution pattern suggests that this species is well isolated geographically in various places, and yet only two subspecies have been identified, based on minor morphological differences. The two subspecies are *E. p. kansaiensis*, found in the Kinki District of western Honshu, and the nominotypical subspecies found throughout other areas (Nakane 1961).

7.1.1 *ND5* Phylogenetic Tree

An ND5 tree for 38 specimens from nearly all the known localities of this species with *Limnocarabus clathratus* as the outgroup (Fig. 7.2) shows the presence of three major lineages, which are geographically isolated, i.e., in the Tohoku, Kanto, and Chubu Districts of northern, central, and western Honshu, respectively (hereafter referred to as TKC), the Kinki District of central Japan (hereafter referred to as KNS), and the Chugoku District of western Japan (hereafter referred to as CHK).

The TKC and KNS lineages can both be divided into three sublineages. In the TKC lineage, the three sublineages of the Tohoku and Kanto Districts (TKN), the northern Chubu District (NCB), and the southern Chubu District (SCB) can be identified.

The TKN sublineage occupies the widest distribution range, being found from Akita Prefecture through Nagano Prefecture. The gene sequences of the TKN specimens from various localities were very similar to each other. NCB is restricted to the northern Chubu District (loc. nos. 13–15 in Fig. 7.1), while SCB (loc. nos. 10–12 in Fig. 7.1) is clustered with TKN (loc. nos. 1–11 in Fig. 7.1). Part of the TKN sublineage is also found in a restricted area near the boundary of Gifu, Aichi, and Nagano Prefectures in the southern Chubu District (loc. nos. 8, 9 in Fig. 7.1).

The KNS lineage (loc. nos. 16–32 in Fig. 7.1) is composed of three sublineages, all of which are more or less geographically isolated in the Kinki District. One sublineage is found on the west side of the Yodogawa River–Biwako Lake line up to Ishikawa Prefecture along the Japan Sea coast (hereafter referred to as WKN) (loc. nos. 16–23 in Fig. 7.1). Another sublineage is found to the south of the Yodogawa River (hereafter EKN) (loc. nos. 24–28 in Fig. 7.1), and the third to the northeast of the Yodogawa River (hereafter SKN) (loc. nos. 29–32 in Fig. 7.1).

The CHK lineage is found mainly in the northeastern part of the Chugoku District of western Japan (loc. nos. 33–38).

7.1.2 Origin

Euleptocarabus porrecticollis is phylogenetically most closely related to *L. clathratus aquatilis*, from which *E. porrecticollis* would have branched off in the Japanese Islands. Emergence of *E. porrecticollis* may be calculated to have taken place about 11 MYA (see Chapter 6).

Limnocarabus clathratus (s. lat.) is widely distributed in the northern half of the Eurasian Continent and Japan. The Japanese population has been treated as its subspecies, *aquatilis*, which has thus far been found only in northern Kanto and Tohoku. However, it would be misleading to think that *E. porrecticollis* branched off from *L. c. aquatilis* somewhere in northeastern Japan for the following reason. The difference of the *ND5* sequence between specimens from all over the TKN distribution range (Kanto and Tohoku) was very small, suggesting relatively recent migration of individuals from the NCB sublineage, followed by rapid propagation into Kanto and Tohoku, where the TKN population was established.

If *E. porrecticollis* emerged in Tohoku from *L. c. aquatilis*, radiation in TKN must have occurred much earlier than shown in the tree, i.e., the branching point of TKN would have to be much deeper than that seen in the tree. Presumably, the emergence of *E. porrecticollis* took place either in or near the distribution range of the NCB sublineage. *Limnocarabus clathratus aquatilis* was evidently found in this region in the past. The type locality of *L. c. aquatilis* (Shimonosuwa = Simosuwa near Suwako Lake) (Bates 1883) is near the distribution range of NCB, although it is no longer extant in this area.

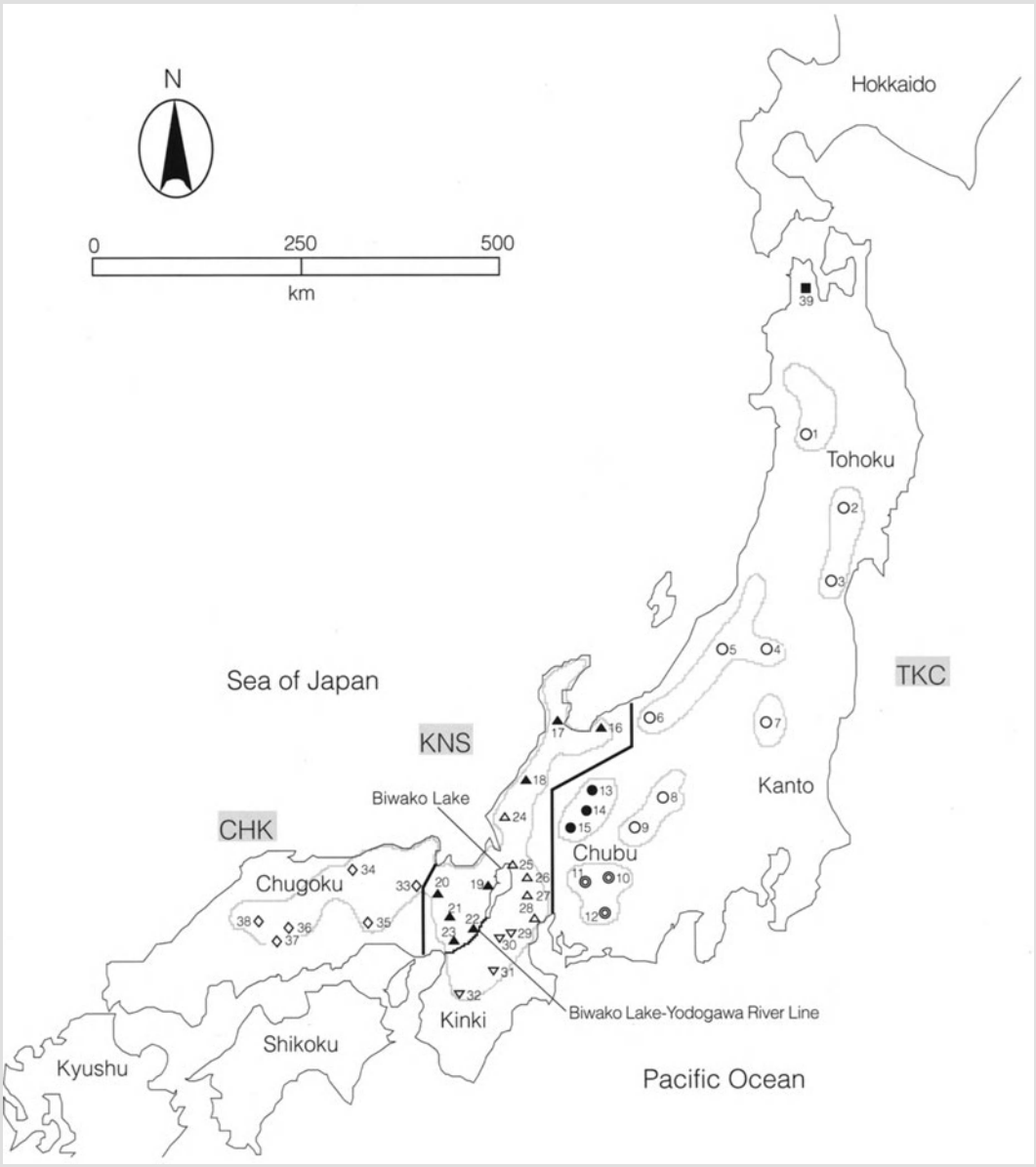


FIG. 7.1. Distribution map of geographic races of *Eulepto-carabus porrecticollis* based on a phylogenetic tree of the mitochondrial *ND5* gene. Localities of the analyzed specimens: **open circles**, TKN; **closed circles**, NCB; **double circles**, SCB; **closed triangles**, WKN; **open triangles**, EKN; **inverted open triangles**, SKN; and **diamonds**, CHK. **Square** indicates *Limnocarabus clathratus aquatilis*. Locality numbers and sublineage symbols correspond to those shown in Fig. 7.2. The known distribution range (·····) is shown using the data from the samples analyzed, referring to the distribution map by the Kinki Research Group of Carabid Beetles (1979). TKC, Tohoku/Kanto/Chubu lineage; KNS, Kinki lineage; CHK: Chugoku lineage (after Kim et al. 1999c)

7.1.3 Diversification

The pairwise sequence comparisons reveal that the maximum difference between the three major lineages of TKC, KNS, and CHK is 3.1%, which is comparable to that between *E. porrecticollis* and *L. c. aquatilis* (3.1%). This suggests that TKC, KNS, and CHK radiated about 10 MYA, shortly after their separation from *L. c. aquatilis*. It is difficult to estimate the branching order of TKC, KNS, and CHK because of a low bootstrap value supporting the KNS/CHK node (Fig. 7.2). Thus, which lineage of *E. porrecticollis* that was directly derived from *L. c. aquatilis* cannot be specified from the phylogenetic tree alone. Presumably, *L. clathratus* (s. lat.) migrated from the Eurasian Continent to a restricted region in ancient Japan such as the northern Chubu region at

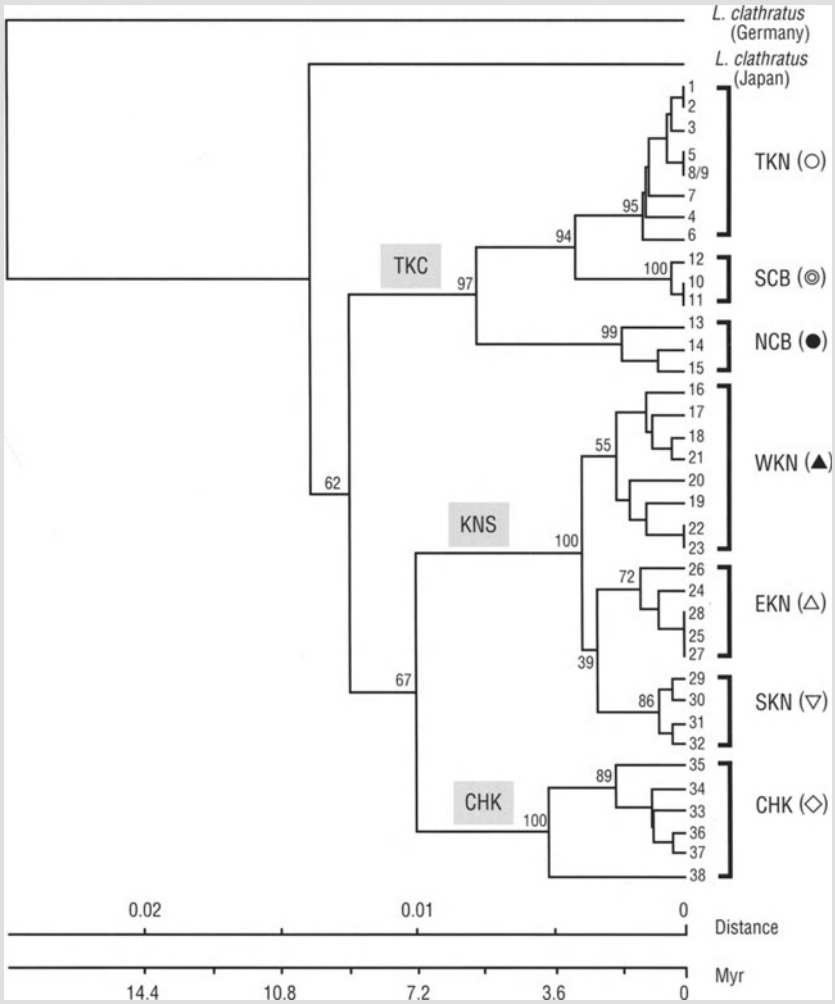


FIG. 7.2. Phylogenetic tree of the mitochondrial *ND5* gene for *Euleptocarabus porrecticollis*. Constructed using the UPGMA. Numbers and symbols correspond to those used in Fig. 7.1. Several sublineages may be identified under TKC and CHK. In TKC, there are the Tohoku/Kanto sublineage (TKN), the southern Chubu sublineage (SCB), and the northern Chubu sublineage (NCB). In KNS, three sublineages are identifiable; the western Kansai sublineage (WKN), the eastern Kansai sublineage (EKN), and the southern Kansai sublineage (SKN) (after Kim et al. 1999c; modified)

about the time the Japanese Islands began to separate from the continent about 15 MYA. Following this, *E. porrecticollis* branched off from *L. clathratus*, and began its own divergence.

7.1.4 Taxonomic Notes

Despite the direct sister relationship between *L. c. aquatilis* and *E. porrecticollis*, it might be reasonable to treat these two species as each belonging to a distinct genus because of considerable morphological differences.

Euleptocarabus contains only one species, *E. porrecticollis*, which has been divided into two subspecies, *E. p. porrecticollis* and *E. p. kansaiensis*. The distribution range of *E. p. kansaiensis* is confined to the Kinki District, but sometimes the Chugoku population (lineage CHK) is described as belonging to this subspecies. The specimens from the rest of the distribution range have been thought of as nominotypical subspecies with the exception of the inconsistent treatment of the Chugoku population, as mentioned above.

Our phylogenetic analysis indicated that KNS specimens roughly correspond to *E. p. kansaiensis*, and TKC specimens evidently belong to *E. p. porrecticollis*. The Chugoku population (CHK) is phylogenetically equivalent to *E. p. kansaiensis* and *E. p. porrecticollis*. Reexamination of morphology needs to be done on this population.

TABLE 7.1. Species and subspecies of the genus *Ohomopterus*

O. japonicus-species group (J-type) (digitulus: small triangle)	*O. dehaanii*-species group (D-type) (digitulus: long triangle)	*O. yaconinus*-species group (Y-type) (digitulus: pentagonal shape)	*O. insulicola*-species group (I-type) (digitulus: hook shape)
O. japonicus [1]Subsp.: *tsushimae*, *chugokuensis*, and others (total 15 subspp.)	*O. dehaanii* Subsp.: *punctatostriatus*, and others (total 7 subspp.)	*O. yaconinus* Subsp.: *blairi*, and others (total 8 subspp.)	*O. insulicola* Subsp.: *nishikawai*, and others (total 9 subspp.)
O. daisen Subsp.: *okianus*	*O. tosanus* Subsp.: *ishizuchianus*, *kawanoi*, and other 1 (total 3 subspp.)	*O. iwawakianus* Subsp.: *kiiensis*, and others (total 5 subspp.)	*O. esakii*
O. yamato			*O. arrowianus* Subsp.: *komiyai*, *nakamurai*, *murakii*, and others (total 6 subspp.)
O. kimurai			
O. lewisanus Subsp.: *awakazusanus*			*O. uenoi*
O. albrechti Subsp.: *tohokuensis*, *freyi*, *esakianus*, *okumurai*, and others (total 12 subspp.)			*O. maiyasanus* Subsp.: *shigaraki*, *takiharensis*, and others (total 7 subspp.)

[1] Only subspecific names that appeared in the text are shown

7.2 Genus *Ohomopterus*

7.2.1 Overview

Members of the genus *Ohomopterus* are endemic to the Japanese Islands. All the species belonging to this genus are similar to each other in both appearance and structure, so that there are a number of opinions on their taxonomy.

In pioneering work, Nakane (1952a, b, c; 1960; 1966) and Nakane and Iga (1955) suggest that the morphology of the chitinized copulatory pieces of the male genitalia is the most reliable character upon which classification is based. Nakane (1963) divides the genus into five species (treated as belonging to the genus *Apotomopterus*), i.e., *dehaanii*, *yaconinus*, *insulicola*, *japonicus*, and *albrechti*, under which several subspecies are recognized.

Later, *O. uenoi* was described by Ishikawa (1960), and *O. albrechti* was downgraded to a subspecies of *O. japonicus* (Nakane 1963). Ishikawa (1989, 1991) reorganized this genus by treating Nakane's species as species-groups, in which some of Nakane's subspecies were raised to species rank. Table 7.1 shows the classification of the *Ohomopterus* species (plus the representative subspecies) according to Ishikawa (1991).

The morphological characteristics of digitulus (copulatory piece) of each species-group are shown in Fig. 7.3. For the sake of simplicity, the name of each species-group is expressed as J-type, D-type, Y-type, and I-type, with the initial letter of the representative species serving as the type letter.

In recent years, a tremendous number of subspecies have been described based on minor locality-dependent

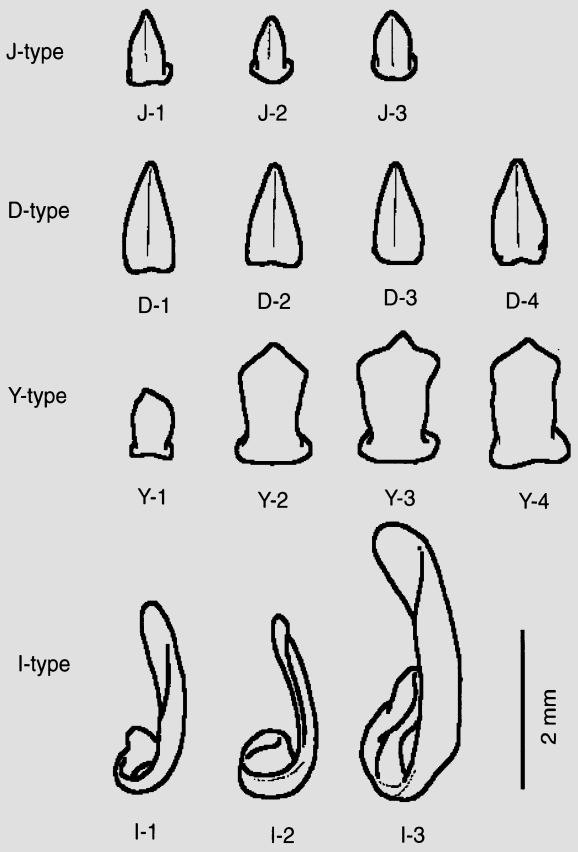


FIG. 7.3. Digitulus (copulatory piece) of male genitalia of each of *Ohomopterus* species-group (type). **J-1** *O. japonicus chugokuensis*, **J-2** *O. japonicus japonicus*, **J-3** *O. lewisianus*, **D-1-4** *O. dehaanii*, **Y-1** *O. iwawakianus kiiensis*, **Y-2–4** *O. yaconinus*, **I-1** *O. maiyasanus*, **I-2** *O. arrowianus*, **I-3** *O. insulicola* (after Su et al. 1996c)

differences, mostly in the male genital organ. This has made it almost impossible to know what subspecies a specimen belongs to without knowing the locality in which it was found, especially in the case of female specimens. Only a few highly skilled specialists can identify a subspecies through examining the male genitalia under a microscope.

It has become fashionable to give subspecific names to "geographic races" differentiated by minor morphological differences without thorough justification being given, as may be seen especially in the case of *Ohomopterus* and other carabid beetles found in Japan. Indeed, in *Ohomopterus*, the number of subspecies so far described is attaining nearly 100, in addition to 16 nominotypical subspecies. There is no guarantee that these "subspecies" will evolve into species.

The race to name subspecies and even sometimes "species" will result only in hopeless confusion and is biologically almost meaningless in many cases. In our opinion, the use of (and "creation" of) subspecies should be undertaken with a much greater degree of caution.

It is of greater importance to gain an insight into the evolutionary history of the species under examination, including the mechanism of its emergence and the establishment of a habitat niche. In this book, therefore, we have chosen to refer only to those subspecies that are relevant to our study.

Morphological indicators suggest that in *Ohomopterus*, evolution proceeded mainly by changes of the copulatory piece from simple/small towards complex/large (Ishikawa 1989).

Figure 7.4 shows the distribution of each species in this genus, with the exception of the two species *O. insulicola* and *O. albrechti*, which are distributed east of the Itoigawa-Shizuoka tectonic line. These two species will be discussed separately below.

7.2.2 Outline of Mitochondrial DNA Phylogeny

The *ND5* phylogenetic tree of the Japanese Carabinae reveals that *Ohomopterus* is clearly monophyletic, as noted in Chapter 6.

Two major haplotype lineages of *Ohomopterus* have been identified, i.e., the lineage I composed of five sublineages, and the lineage II composed of three sublineages (Fig. 7.5a; for localities of the samples, see Fig. 7.5b).

Western Japan Lineage (Lineage I)

This lineage includes the following five geographically linked sublineages. The first is the northern Kyushu/San-in sublineage (KSI) and includes *O. yaconinus* (Y-type), *O. japonicus* (+ *O. j. daisen*) (J-type), and *O. dehaanii* (D-type) (loc. nos. 31–45 in Fig. 7.5).

The second is the San-yo sublineage (SYO), which includes *O. japonicus* (ssp. *chugokuensis*) (J-type) and *O. dehaanii* (D-type) (loc. nos. 27–30 in Fig. 7.5).

The Shikoku sublineage (SHK) also includes *O. japonicus* (J-type), along with *O. dehaanii* (D-type) (+ *O. tosanus*) (D-type) (loc. nos. 20–26).

The Japan Sea Is./eastern Japan (including Hokkaido) sublineage (JSE) shows a rather complicated composition of species and an unexpected distribution range. JSE includes the following J-type species: *O. japonicus* (ssp. *tsushimae*) found in the Tsushima Islands; *O. daisen* (ssp. *okianus*) found in the Oki Islands; *O. albrechti* (*O. a. freyi*) found in Sado Island; *O. yamato* found in west-central Japan; *O. lewisianus* and its related species found in eastern Japan; and many *O. albrechti* races found in eastern and northeastern Japan and Hokkaido. JSE also includes the two I-type species, *O. insulicola* (eastern and northeastern Japan and the southern tip of Hokkaido) and *O. esakii* (found in a restricted region of eastern Japan) (loc. nos. 1–19).

The fifth sublineage (WJP) is found in western Japan and consists exclusively of *O. yaconinus* (Y-type). The distribution range of WJP overlaps with the other four sublineages in western Japan except for Kyushu and a part of Honshu and Shikoku (Fig. 7.4) (loc. nos. 46–55). "*Ohomopterus yaconinus*" is also found in the Kinki District; its origin is discussed below (see pp. 113–120).

Chubu/Kinki Lineage (Lineage II)

The Kinki sublineage (KNK) includes the species belonging to Y-, D-, and I-types, such as *O. maiyasanus* (I-type), *O. dehaanii* (D-type), *O. iwawakianus* (Y-type), *O. yaconinus* (Y-type) and *O. insulicola murakii* (I-type). (loc. nos. 68–84). For the origin of such a complex composition, see below (pp. 113–120).

The Central Japan sublineage (CJP) is composed exclusively of the two I-type species, *O. arrowianus* and *O. uenoi* (loc. nos. 57–67).

The Kii sublineage (KII) comprises only one race, *O. iwawakianus kiiensis* (Y-type), found only in the Kii Peninsula in the Kinki District (loc. no. 56).

7.2.3 Scenario of Formation of *Ohomopterus* Fauna

A comparison of the *ND5* phylogenetic tree with the geohistory of the Japanese Islands makes it possible to speculate on the process of formation of the *Ohomopterus* fauna. This has already been outlined in the previous chapter and the discussion in this chapter will inevitably include some repetition for the purpose of clarity.

Ohomopterus began to diversify about 12 MYA, which is a little after the proto-*Damaster* split into two lineages upon the separation of the Japanese Islands from the Eurasian Continent about 15 MYA (Fig. 7.6; see Chapter 6, p. 96).

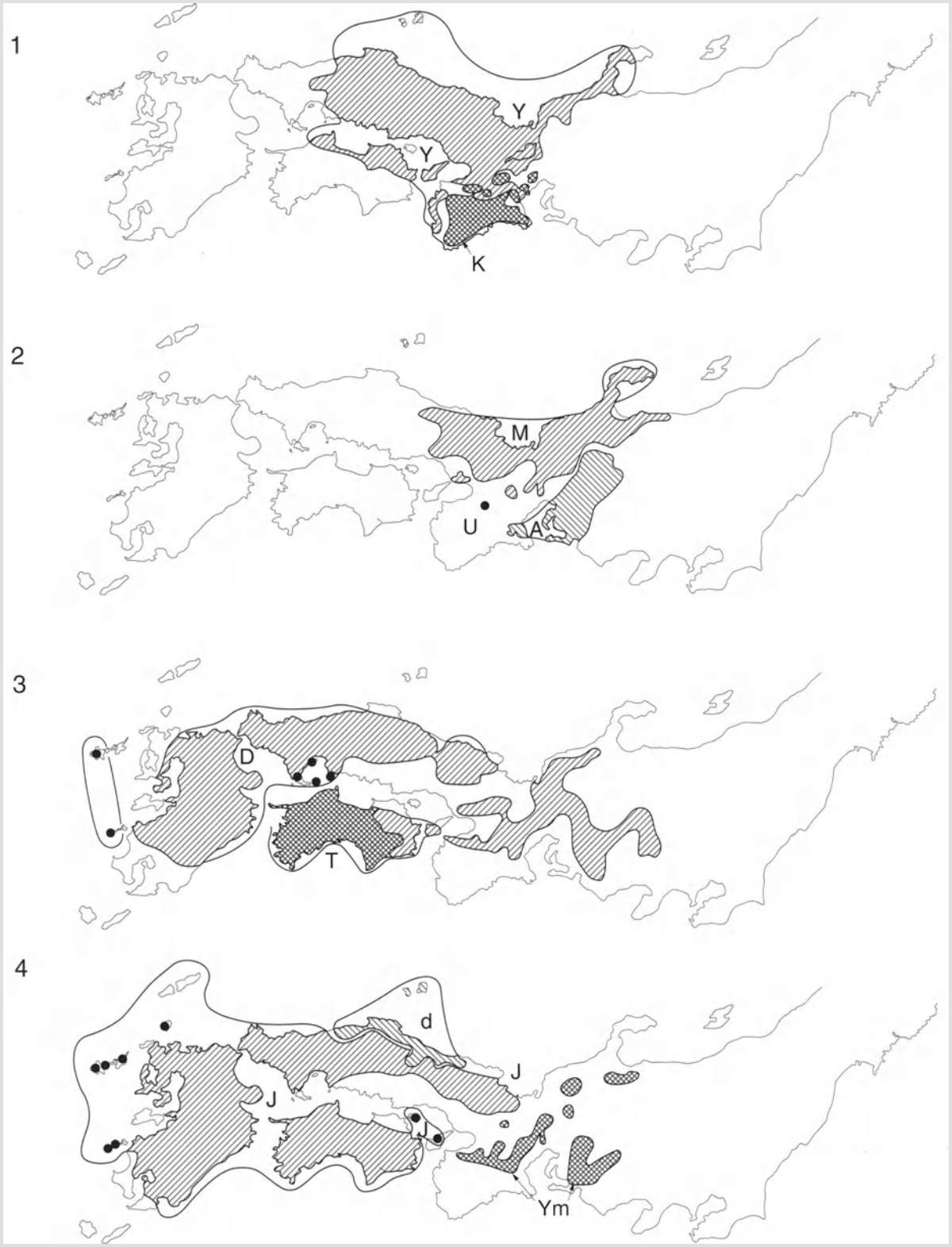


Fig. 7.4. Distribution map of the *Ohomopterus* species. **1Y** *O. yaconinus*, **1K** *O. iwawakianus* including *O. i. kiiensis*. **2M** *O. maiyasanus*, **2A** *O. arrowianus*, **2U** *O. uenoi*. **3D** *O. dehaanii*, **3T** *O. tosanus*. **4J** *O. japonicus*, **4d** *O. daisen*, **4Ym** *O. yamato* (after the Kinki Research Group of Carabid Beetles 1979; modified)

The initiation of diversification in the Japan Sea Is./eastern Japan sublineage (JSE) seems to have taken place at about the time of the connection of the western arc to the eastern arc of Japan (Fig. 7.6), the proto-*Ohomopterus* would have inhabited the southwest half of the ancient Japan region of the continent, in contrast to the wider distribution of proto-*Damaster* in the ancient Japan region. It then follows that only one lineage of proto-*Ohomopterus* was distributed, and this was only in the southwest arc of the ancient Japan region of the continent (see below) (B′ in Fig. 7.6).

Shortly after this event, the proto-Japanese Islands took on the form of an archipelago as a result of extensive submergence, especially of the northeast arc. This would have caused divergence of the proto-*Ohomopterus* into the western (B′1 in Fig. 7.6) and the Kinki/Chubu (B′2 in Fig. 7.6) lineages. An extensive upheaval of the Japanese Islands beginning around 9 MYA saw the southwest and northeast Japan arcs fused upon the disappearance of the Fossa Magna Sea, at which point B′1 is likely to have migrated to the northwestern region of the new world.

This left a niche for B′1a (= JSE). B′1a may have been derived directly from the inhabitants of the ancient islands of the Sea of Japan, or as a result of an invasion of B′1 into central Japan, after which it differentiated to become *O. yamato* and then moved to northeastern Japan.

7.2.4 Details of the *Ohomopterus* Lineages

7.2.4.1 Western Japan Lineage (Lineage I)

The Kyushu/San-in Sublineage (KSI)

The KSI includes *O. japonicus* (J-type) (including part of *O. j. chugokuensis*), *O. daisen* (J-type), and *O. dehaanii* (D-type), members of which are distributed in Kyushu and the San-in region of the Chugoku District (Fig. 7.7). No analysis has been made of *O. j. chugokuensis* of Kyoto Prefecture, so the eastern limit of this lineage is still unclear. *Ohomopterus j. chugokuensis* of central Hyogo Prefecture belongs to this lineage, so that a part of this subspecies appears to have moved southward.

The San-yo Sublineage (SYO)

The SYO includes *O. japonicus chugokuensis* (J-type) and *O. dehaanii* (D-type), examples of which are found in the San-yo region of the Chugoku District, from Yamaguchi Prefecture to Okayama Prefecture.

The Shikoku Sublineage (SHK)

The SHK, as shown in Fig. 7.7, consists of *O. japonicus* (J-type), *O. dehaanii* (D-type), and *O. tosanus*. *Ohomopterus tosanus* (sometimes treated as a subspecies of *O. dehaanii*) and its subspecies, *ishizuchianus*, *kawanoi*, and *O. dehaanii* from eastern Shikoku and Awajishima Island are all included in SHK, together with *O. japonicus*. Diversification of all these species as revealed by the mitochondrial haplotype is a relatively recent event.

There are several possible explanations for the pairwise appearance of D- and J-types in the respective regions, leading to the formation of three independent clusters, but there is as yet no decisive evidence for any of these hypotheses.

The simplest explanation is provided by the idea of a protoform of *O. japonicus* (or *O. dehaanii*) that was isolated geographically on the islands of Kyushu and Shikoku and in the San-yo region so as to have formed distinct sublineages. During independent accumulations of nucleotide substitutions in mitochondrial DNA in the respective sublineages, a type-switching (i.e., morphological transformation) from *O. dehaanii* to *O. japonicus* (or vice versa) took place in parallel within each sublineage (Su et al. 1996c).

The second possibility is that morphological polymorphism (e.g., *O. japonicus*-type and *O. dehaanii*-type) existed in the ancestor. If the polymorphic ancestors were then isolated in Kyushu, Chugoku, and Shikoku, after which they divided into the two species, it would explain the appearance of the *ND5* phylogenetic tree. However, it is not possible to prove the presence of polymorphism in the ancestor. Type-switching must, however, have occurred in the ancestor.

The third possibility is that the speciation may have occurred only once in each case, meaning that the appearance of the tree may be explained as the result of random linear sorting among polymorphic mitochondrial haplotypes into the ancestral population. However, the distribution of the "same species" (in this case, *O. japonicus* or *O. dehaanii*), as represented by mitochondrial sequences, is not random. It is difficult, moreover, to imagine that the protoform of the present-day species had a mitochondrial polymorphism that would affect the appearance of the tree.

Despite this, if we suppose that the ancestor of the two species had polymorphic mitochondria of three types, *a*, *b*, and *c*, and speciation (separation of *O. japonicus* and *O. dehaanii*) occurred with their isolation in three geographic regions, the species tree agrees with the mitochodrial DNA tree if enough time has elapsed from speciation to geographic isolation. This is not, however, the case.

If the interval between speciation and geographic isolation is relatively short, *a*, *b*, and *c* were sorted randomly, and the tree would be considerably affected, although it is unlikely to have occurred, however. If we suppose that *a* was sorted to *O. dehaanii* in Kyushu, *b* to San-yo, and *c* to Shikoku, the probability of the sorting of *a* to *O. dehaanii* in Kyushu, *b* to San-yo, and *c* to Shikoku is 1/27. In other words, the sorting hypothesis cannot explain the geographically dependent pair-wise appearance of the two species.

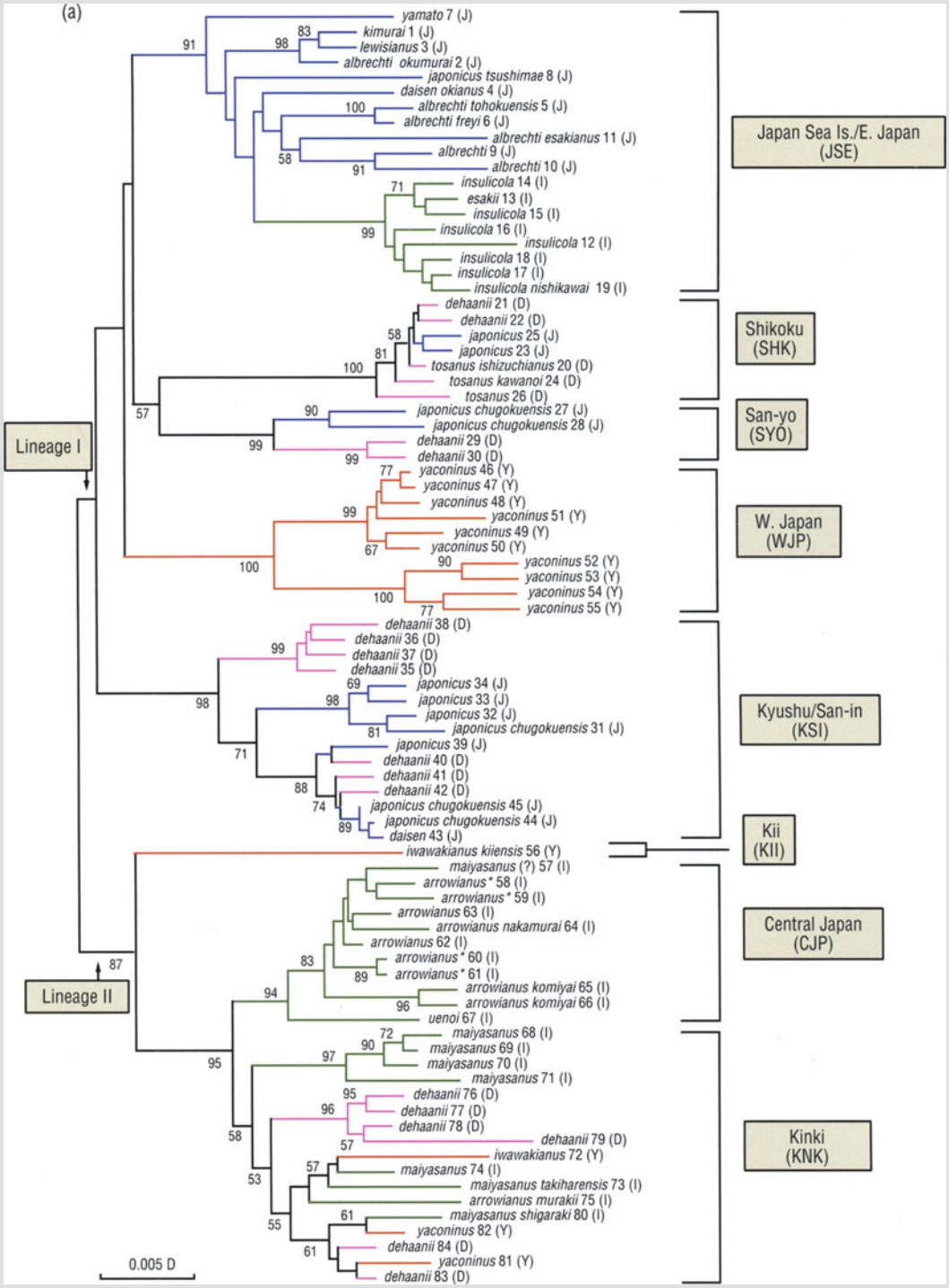


FIG. 7.5. Phylogenetic tree of the mitochondrial *ND5* gene for the genus *Ohomopterus* (**a**)and the localities of specimens analyzed (**b**). Locality numbers correspond to those shown in **a**. Color of branch: violet = J-type; green = I-type; red = D-type; brown = Y-type (Su et al. 1996c)

FIG. 7.5. *Continued*

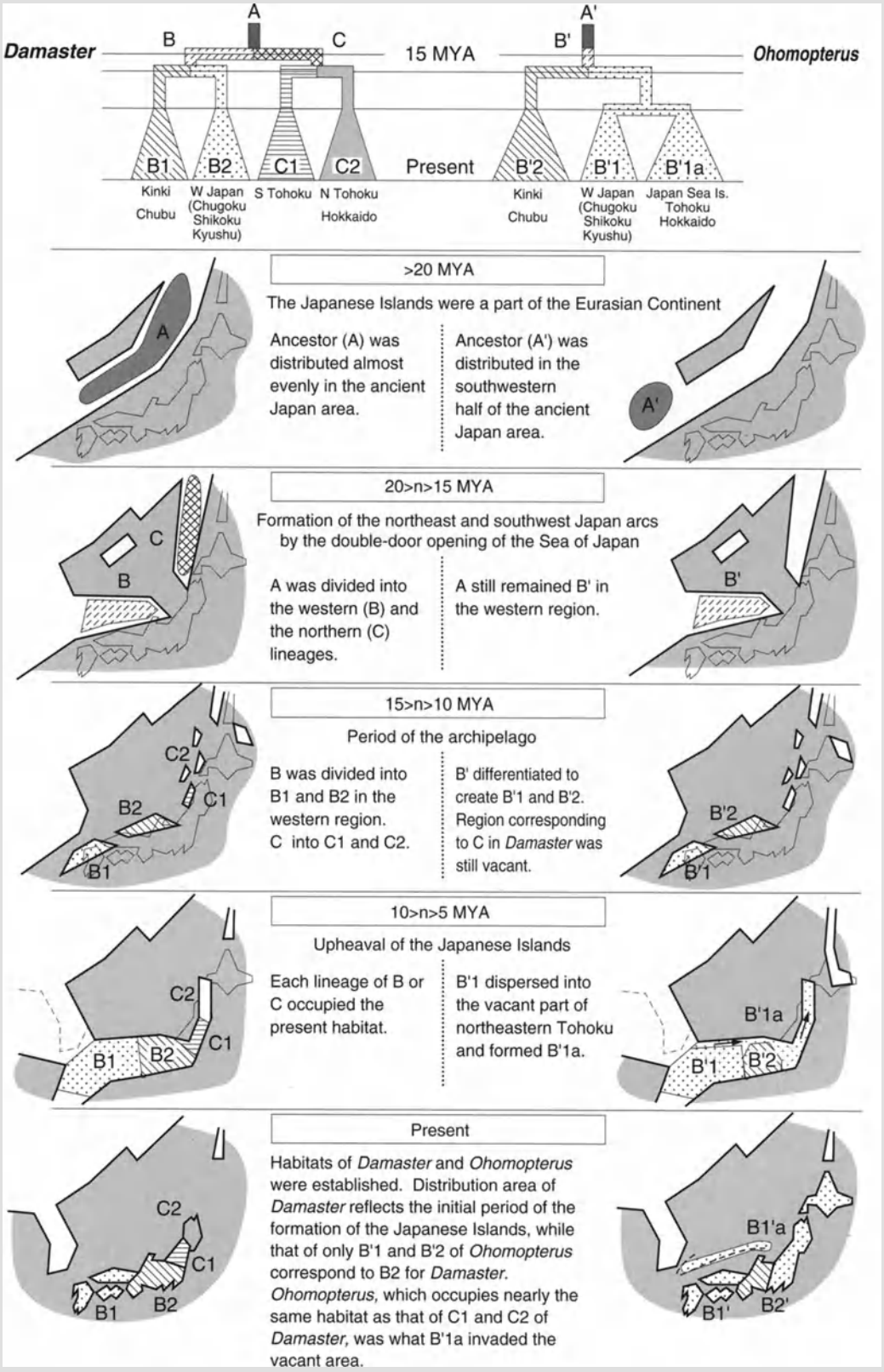


FIG. 7.6. Comparative illustration of establishment of the fauna of the genera *Damaster* and *Ohomopterus* in the Japanese Islands

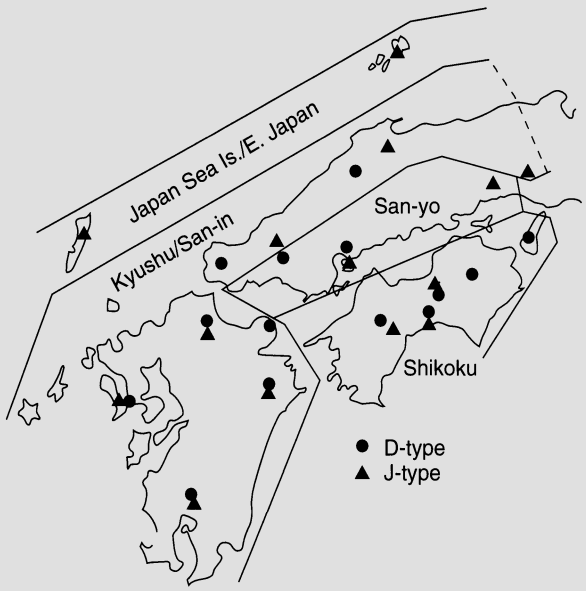


FIG. 7.7. Demarcation of *Ohomopterus* sublineages in western Japan based on the phylogenetic trees of the mitochondrial *ND5* gene. Localities of the samples analyzed are shown in the figure. **Circles**, D-type; **triangles**, J-type

The fourth possibility is that the pairwise occurrence of the two species in the *ND5* phylogenetic tree is the result of horizontal transfer of mitochondria resulting from hybridization of *O. japonicus* and *O. dehaanii*. If we take as an example the two species in Kyushu/San-in, San-yo, and Shikoku, the *ND5* DNA sequence difference between them is small within one region, while the difference is much larger between the region-dependent pair of the two species, i.e., a paired species forms a cluster independent of other pairs. Furthermore, the three clusters each containing the two species emerged at almost the same time.

If the hybrid hypothesis is correct, then the *O. japonicus*- and the *O. dehaani*-type mitochondria should be identifiable within one region. This, however, is not the case.

The hybridization hypothesis is valid only when mitochondria in one species, *O. japonicus* (or *O. dehaanii*) for example, were replaced by those of *O. dehaanii* (or *O. japonicus*) in all the regions (unidirectional replacement). In this case, hybridization should have taken place between *O. dehaanii* (female) and *O. japonicus* (male) (or vice versa) and the authentic *O. japonicus* should have become extinct.

In the early period of *Ohomopterus* diversification, proto-*O. japonicus* and proto-*O. dehaanii* may each have inhabited a geographically isolated region (for example, *O. japonicus* in the ancient Japan Sea region and *O. dehaanii* somewhere in the ancient area of northern Kyushu). Thereafter, *O. dehaanii* could have dispersed into San-in, Chugoku, and Shikoku, followed by the invasion of *O. japonicus* into the respective regions, after which it hybridized with *O. dehaanii*, resulting in the unidirectional replacement of mitochondria mentioned above. This explanation is consistent with the *ND5* phylogenetic tree.

However, the geohistory of western Japan is still not clear enough to provide proof for this hypothesis, and further studies, including analysis of nuclear DNA, are necessary to prove or disprove the hypothesis.

Japan Sea Is./Eastern Japan Sublineage (JSE)

As already mentioned, this sublineage is unique in being distributed widely throughout the Japanese Islands. This sublineage includes the following J-type species: *O. japonicus tsushimae* found in the Tsushima Islands; *O. daisen okianus* from the Oki Islands; *O. yamato*, which is found in the Kinki and Chubu Districts; *O. kimurai* from Shizuoka Prefecture; *O. lewisianus* from the Izu Peninsula and part of the Bôsô Peninsula (*O. l. awakazusanus*); and *O. albrechti*.

The last species is distributed mainly east of the Itoigawa-Shizuoka tectonic line, and has divided into many subspecies according to analysis of morphological features and distribution area. *Ohomopterus a. tohokuensis*, *O. a. freyi*, *O. a. esakianus*, and *O. a. okumurai* may be enumerated among others.

Nakane (1955) treated *O. esakianus*, *O. lewisianus*, and *O. yamato* as subspecies of *O. albrechti*, while later all of these species, including *O. albrechti*, were downgraded to subspecies of *O. japonicus*. The recent tendency is to split them into many species and subspecies as mentioned in p. 103.

Besides the J-type species mentioned above, two I-type species, *O. insulicola* (which is found mainly east of the Itoigawa-Shizuoka tectonic line) and *O. esakii* (Shizuoka Prefecture) are included in JSE.

A phylogenetic tree of the J-type species in JSE using the *ND5* or *COI* gene (Fig. 7.8) suggests that the mitochondrial DNA phylogeny does not coincide, in many respects, with morphological classifications and with a tree constructed based on minor morphological differences (Takami and Ishikawa 1997). This is indicated by the intermingled occurrence of various morphological "subspecies" in different phylogenetic groups.

The *COI* DNA tree (which is essentially the same as the *ND5* tree) shows that the J-type species of JSE are clearly divided into seven groups (I–VII in Figs. 7.8 and 7.9). As these seven groups radiated within a short time, their branching order cannot be determined. The identification of species and subspecies was undertaken by Osamu Tominaga, one of our collaborators. For simplicity, these subspecies are not for the most part taken into consideration in the following discussion.

As may be seen in Fig. 7.9, distribution of the phylogenetic groups do not overlap except for part of Niigata Prefecture, suggesting that each group is geographically linked. The followings are details of our findings on the origin, distribution, and other features of these groups in relation to geohistory (Figs. 7.8 and 7.9).

FIG. 7.8. Phylogenetic tree of the mitochondrial *COI* gene for *Ohomopterus albrechti* and its related species. Constructed using the UPGMA. Symbols at the right of the figure correspond to those in Fig. 7.9. For groups I–VII, see the text. Outgroup: *O. dehaanii* from Awajishima Island (after Saito et al. 2003; modified)

The Group I is the most widely distributed group and includes mostly *O. albrechti* and its various "subspecies" irrespective of the subspecies classification (loc. nos. 1–19 in Figs. 7.8 and 7.9). *Ohomopterus lewisianus awakazusanus*, found in the Bôsô Peninsula (loc. no. 19) is also included in this group, suggesting that this is a form of *O. albrechti* and not a subspecies of *O. lewisianus*. The distribution of this group ranges from the northern part of the Bôsô Peninsula to Hokkaido along the eastern part of the Japanese Islands.

Samples from Hokkaido (loc. nos. 1–7) are very close to each other in their gene sequence and are also akin to samples from the Pacific coast of the Tohoku District (loc. nos. 8–19). The phylogenetic relationships

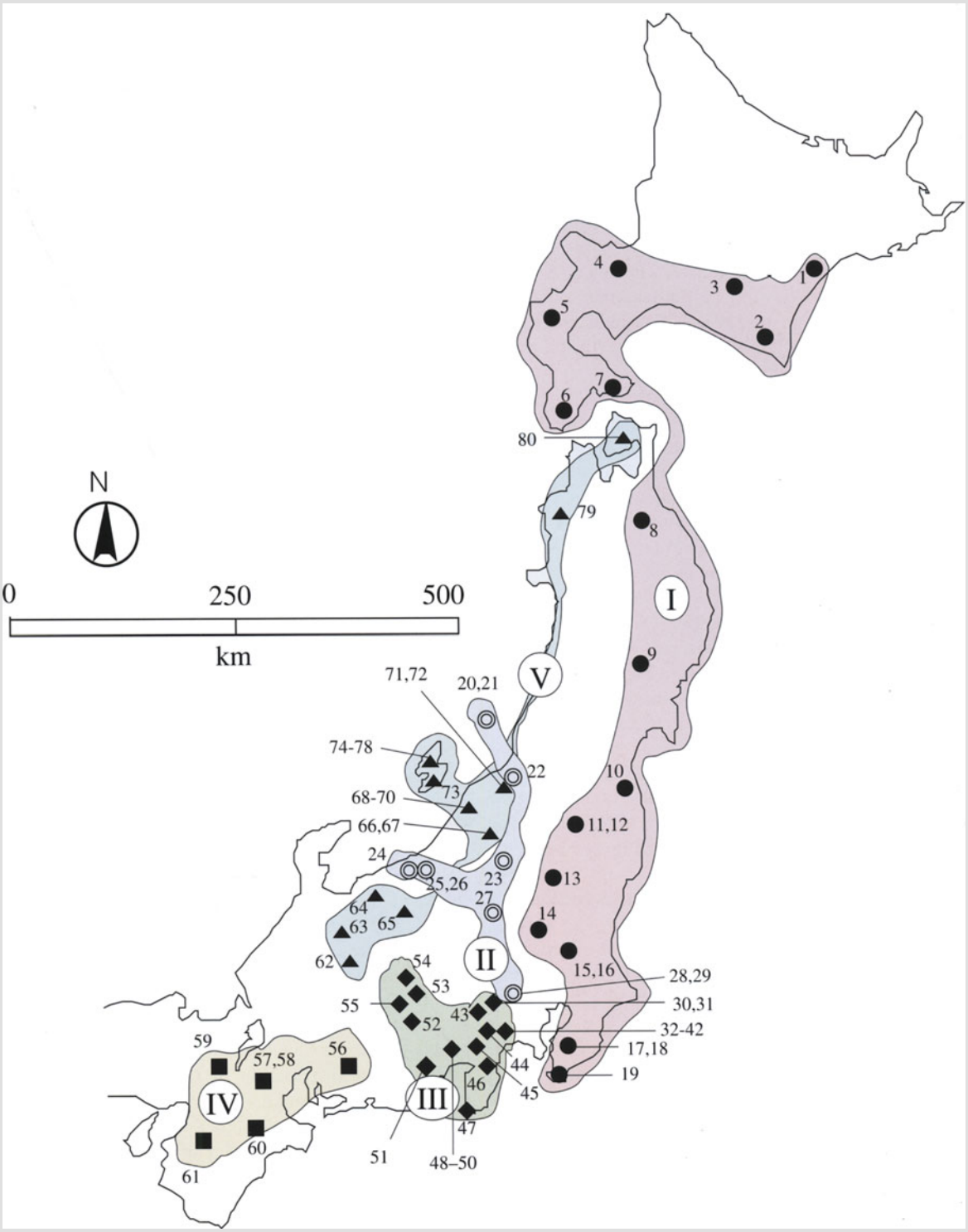


FIG. 7.9. Distribution map and the localities of analyzed samples of *Ohomopterus albrechti* and its related species. Samples collected at the same or nearby localities contain two to 11 specimens and are shown by one symbol. These samples showed almost no sequence differences. Groups I–V appear in the text and in Fig. 7.8. Groups VI (*O. japonicus tsushimae*) and VII (*O. daisen okianus*) are omitted from this figure (after Saito et al. 2003)

within this group, together with other facts (see below), suggest that an inhabitant of the Kanto area migrated along the Pacific coast, reached Hokkaido at a relatively recent time, and quickly expanded its distribution there.

Group II also consists of *O. albrechti* and its subspecies. The various subspecies are intermingled to a considerable extent (loc. nos. 20–29). The northwestern Kanto District is the main distribution range of this group, which extends in two directions, one reaching Awashima Island (loc. nos. 20–21) through Shibata in Niigata Prefecture (loc. no. 22), and another to Itoigawa and its vicinity (loc. nos. 24–26). As will be noted later, the Sado Island population (*O. albrechti freyi*; loc. nos. 73–78) is not included in this group.

The main constituent of Group III is *O. lewisianus*, found in Kanagawa Prefecture and its vicinity. Some examples of *O. albrechti esakianus* (loc. nos. 32–34, 39, and 42) and *O. albrechti okumurai* (loc. nos. 30, 31, 52–55) inhabit the eastern and northwestern parts of the distribution range of this group and are also included in the group. Furthermore, all the examples of *O. kimurai* from Shizuoka Prefecture analyzed (loc. nos. 48–51) belong to this group. Various species and subspecies are intermingled on the tree without forming species- or subspecies-specific clusters (loc. nos. 30–55).

The monophyletic Group IV includes solely *O. yamato*, which is found in the Chubu and the Kinki Districts (loc. nos. 56–61). Near the western edge of the distribution range of Group V, individuals with *O. albrechti*-type mitochondrial DNA and *O. yamato*-type morphology (loc. nos. 62–63) were found. There appears to be a hybrid zone between *O. yamato* and *O. albrechti*, and the examples examined represent such a hybrid (loc. nos. 62–63).

Group V consists of inhabitants distributed along the Japan Sea coast in the Tohoku District, i.e., Toyama Prefecture through Niigata and Akita Prefectures to the Shimokita Peninsula in Aomori Prefecture (loc. nos. 62–80). The population of Sado Island (*O. albrechti freyi*) is also included in this group (loc. nos. 73–78). A probable hybrid zone exists around the western edge of distribution of this group (see above).

Group VI includes only *O. daisen okianus*. On the mitochondrial phylogenetic tree, *O. daisen okianus* belongs to a different cluster from *O. daisen daisen* on the mainland of Honshu (see p. 109).

Group VII consists of only *O. japonicus tsushimae*. The mitochondrial gene sequence of this subspecies is phylogenetically distinct from that of *O. j. japonicus* found in Kyushu, Honshu, and Shikoku.

As discussed here, the specimens shown in Figs. 7.8 and 7.9 likely include some hybrid individuals, especially around the border regions of the two (sub)species. However, the origins of the seven groups discussed above are venerable, and there is no doubt as to their existence regardless of the history of their migration and the occurrence of hybridization.

The diversification of the seven groups started between the time of the upheaval of northeastern Japan (7 MYA) and the formation of the Tanzawa Sea Peak. This estimation is consistent with the assumption that *Ohomopterus* first originated in western Japan and entered eastern Japan upon the disappearance of the Fossa Magna Sea (see above).

However, the route by which each group expanded its range to establish its present habitat is not easy to trace. As noted above, Group IV (*O. yamato*) is found west of the Itoigawa-Shizuoka tectonic line (the western periphery of the Fossa Magna), and the western limit of distribution for groups III and V is only a little west of the tectonic line. Groups IV and V have adjoining distribution ranges around the northern part of the tectonic line. On the other hand, the distribution range of Groups I and II are clearly on the northeastern side of the tectonic line.

These findings, along with the distribution map of each group, allow us to speculate that Group IV (*O. yamato*) is the origin of all the groups except Groups VI and VII. A part of Group IV went up north along the coast of the Sea of Japan to form Group V, and another part of Group IV migrated eastwards and became Group III (mainly *O. lewisianus* and *O. kimurai*). A little later, a part of Group III moved northwards to form Group II, which finally arrived at Is. Awashima. Alternatively, Group II might have been derived from a part of Group V. A rather tight clustering of Group I with Group II on the *COI* phylogenetic tree (Fig. 7.9) suggests that Group I could have originated from a fraction of Group II, then propagated its distribution northeastwards along the Pacific Ocean side of the Kanto and the Tohoku Districts, and finally reached Hokkaido.

It should be pointed out that *Damaster blaptoides*, found in the southern Tohoku District, and on Sado Island and Awashima Island, belongs to the same lineage together, whereas *Ohomopterus albrechti* races from the above three regions differ in their phylogenetic profile. This difference may be attributed to the difference in their evolutionary history, which is linked to the geohistory of the Japanese Islands. The migration and expansion of distribution of *O. albrechti* to eastern Japan began at around the time as the disappearance of the Fossa Magna Sea (<9–6 MYA), while the establishment of the distribution range of *Damaster blaptoides* began much earlier than that of *O. albrechti* (>10 MYA) (Fig. 7.6).

Ohomopterus insulicola (I-type) also belongs to the Japan Sea Is./eastern Japan (JSE) lineage. This species is roughly distributed east of the Itoigawa-Shizuoka tectonic line including large areas of the Kanto and the Tohoku Districts and the southwestern edge of Hokkaido (Hakodate). Examples have been found at the mouth of the Jintûgawa River in Toyama Prefecture (Miyahara 1992), although its origin is not known. *Ohomopterus i. nishikawai*, found in the Bôsô Peninsula, and *O. esakii* (I-type) from Shizuoka are indistinguish-

able from *O. insulicola* according to the mitochondrial phylogenetic tree.

The Western Japan Sublineage (WJP)

The WJP is monophyletic and contains only the species *O. yaconinus* (Y-type) which is distributed across much of the area from the Chugoku District to the Hokuriku District along the Sea of Japan coast, and also found in the western half of Shikoku. The Oki Islands are also a habitat of this species (Fig. 7.10). The WJP is clearly divided into two clusters, the first of which contains inhabitants of the San-in region including the Oki Islands (what we call the San-in group). The second cluster consists of inhabitants of the San-yo, Shikoku, Awajishima Island, and coastal regions facing the Sea of Japan from Kyoto Prefecture to Fukui and Toyama Prefectures (what we call the San-yo group; Fig. 7.10).

It is interesting to note that inhabitants of the Sea of Japan coastal regions belong to the San-yo group and not to the San-in group, although the San-in region faces the Sea of Japan. Such a distribution profile is likely to have resulted from northward migration of the San-yo population. The separation of the population of the Oki Islands from that of the mainland is calculated to have taken place 3.6 MYA, which corresponds to the time at which *Damaster blaptoides* of the Oki Islands and the mainland branched off, suggesting that the Oki Islands were connected to the mainland at one time.

"*Ohomopterus yaconinus*" is also widely distributed in the Kinki District, and is not included in WJP. Its origin is discussed in the next section. Several specimens identified as *O. yaconinus blairi* from the Noto Peninsula carry *O. arrowianus*-type mitochondrial DNA and *O. yaconinus*-type nuclear ITS I, suggesting that these are derivatives of hybrids of *O. arrowianus* (female) and *O. yaconinus* (male) (see below).

7.2.4.2 Chubu/Kinki Lineage (Lineage II)

Three sublineages belong to this lineage, i.e., the Kinki sublineage (KNK), the Central Japan sublineage (CJP) and the Kii sublineage (KII) appear to be closely connected from a phylogenetic point of view, and are therefore described together.

The *ND5* phylogenetic tree of this lineage is quite complex, and yet it provides us with an idea of the biogeohistory of the *Ohomopterus* fauna undetectable from morphology and distributional patterns alone. Figure 7.11 shows an *ND5* phylogenetic tree constructed by adding more sequences than used in the construction of the tree shown in Fig. 7.5. The clusters marked I, II, III, and IV in Fig. 7.11 each contains only one species (except II; see below), i.e., *O. iwawakianus kiiensis* (Y-type) in the cluster I, *O. arrowianus* (I-type) in the cluster II, *O. maiyasanus* (I-type) in the cluster III and *O. dehaanii* (D-type) in cluster IV.

Several specimens (*O. yaconinus blairi*, *O. maiyasanus*, and *O. insulicola*) are derivatives of hybrids (see above). For *O. uenoi* (I-type), see p. 120. On the other hand, the cluster V is quite heterogeneous with respect to the compositions of species-groups, species, and subspecies. These are *O. dehaanii* (D-type), *O. arrowianus murakii* (I-type), a few subspecies of *O. iwawakianus* (Y-type), a few subspecies of *O. maiyasanus* (I-type), and two subspecies of *O. yaconinus* (Y-type). All of them inhabit a restricted area of the central Kinki District with the exception of *O. dehaanii*, which is found in an area extending to the Chubu District (Fig. 7.4).

The mitochondrial DNA of these various "species" or "subspecies" of the Y-, D-, and I-types are intermingled in one (or two) clusters, and their diversification took place relatively recently. It is also of some significance to note that most of them are different in morphology from their nominotypical (authentic) forms to some extent, so that they have been regarded as "subspecies."

All these facts suggest that members belonging to the cluster V may be derived from hybridization between two or more species in the past, which would have involved horizontal transfer of mitochondrial DNA from one species to another. To prove this, nuclear ITS I sequences were analyzed in parallel with *ND5* sequences.

Before going further, some comments as to the horizontal transfer of mitochondrial DNA may be appropriate. When species *a* (female) crosses with species *b* (male), the mitochondrial DNA of all F1 individuals is of the *a*-type, while the nuclear DNA becomes heterozygous of *a* and *b*. In the case of a cross between *a* (male) and *b* (female), the offspring has *b*-type mitochondria, because mitochondria is inherited only through the female line. A back-cross or a cross between the offspring produces homozygotes of *a* (or *b*)-type nuclear DNA, and *a* (or *b*)-type mitochondria are replaced by *b* (or *a*)-type. In other words, mitochondrial DNA of species *a* (or *b*) is eventually replaced by that of species *b* through successive crossings.

Strictly speaking, the DNA in descendants *b* (or *a*) of the hybrid-origin contain a part of the nuclear DNA of *a* (or *b*), because of the occurrence of crossing-over at the time of hybridization. There is, therefore, the possibility that the morphology of the hybrid-derived descendant *b* "species", for example, could be somewhat different from the ancestral homozygous *b* species. Some of the "subspecies" recorded might in fact be descendants of this type with their origins in hybridization.

Nuclear DNA analysis may provide some hint as to the origin of hybrid-derived specimens. One problem with this procedure is that the rate of nucleotide substitutions in nuclear DNA is so slow that a reliable phylogenetic tree of relatively closely related species such as *Ohomopterus* spp. is difficult to construct, even with ITS, which is one of the fastest-mutating regions of nuclear DNA.

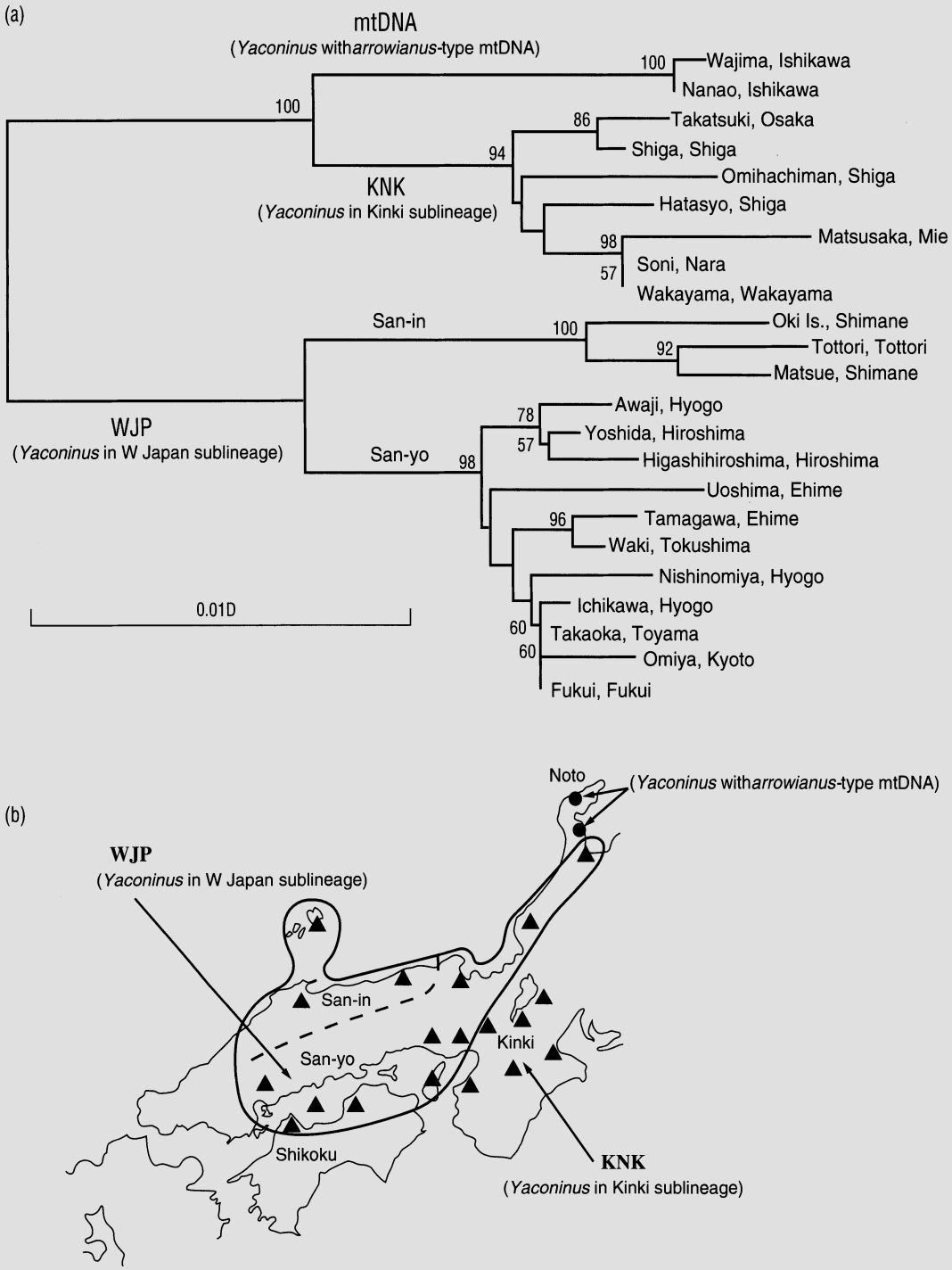


FIG. 7.10. Phylogenetic tree of the mitochondrial *ND5* gene for *Ohomopterus yaconinus* (**a**) and its distribution range (**b**). WJP (Western Japan sublineage) is monophyletic, while KNK (Kinki sublineage) is not (see Figs. 7.5 and 7.11). The *ND5* sequence of *O. y. blairi* is of the *O. arrowianus*-type (see p. 113). Tree constructed using the NJ-method (unpublished)

The ITS sequences have a few deletions/insertions consisting of one to several nucleotides, the positions and lengths of which are specific to the species, so as to make them usable as a marker of species based on nuclear DNA. We will here describe the preliminary results we gained from the ITS I analysis, in comparison with findings gathered from studies based on mitochondrial DNA (Fig. 7.11 and Table 7.2).

The cluster I in Fig. 7.11 is composed solely of *O. iwawakianus kiiensis* (Y-type), specimens of which were collected by Nobuo Kashiwai in various parts

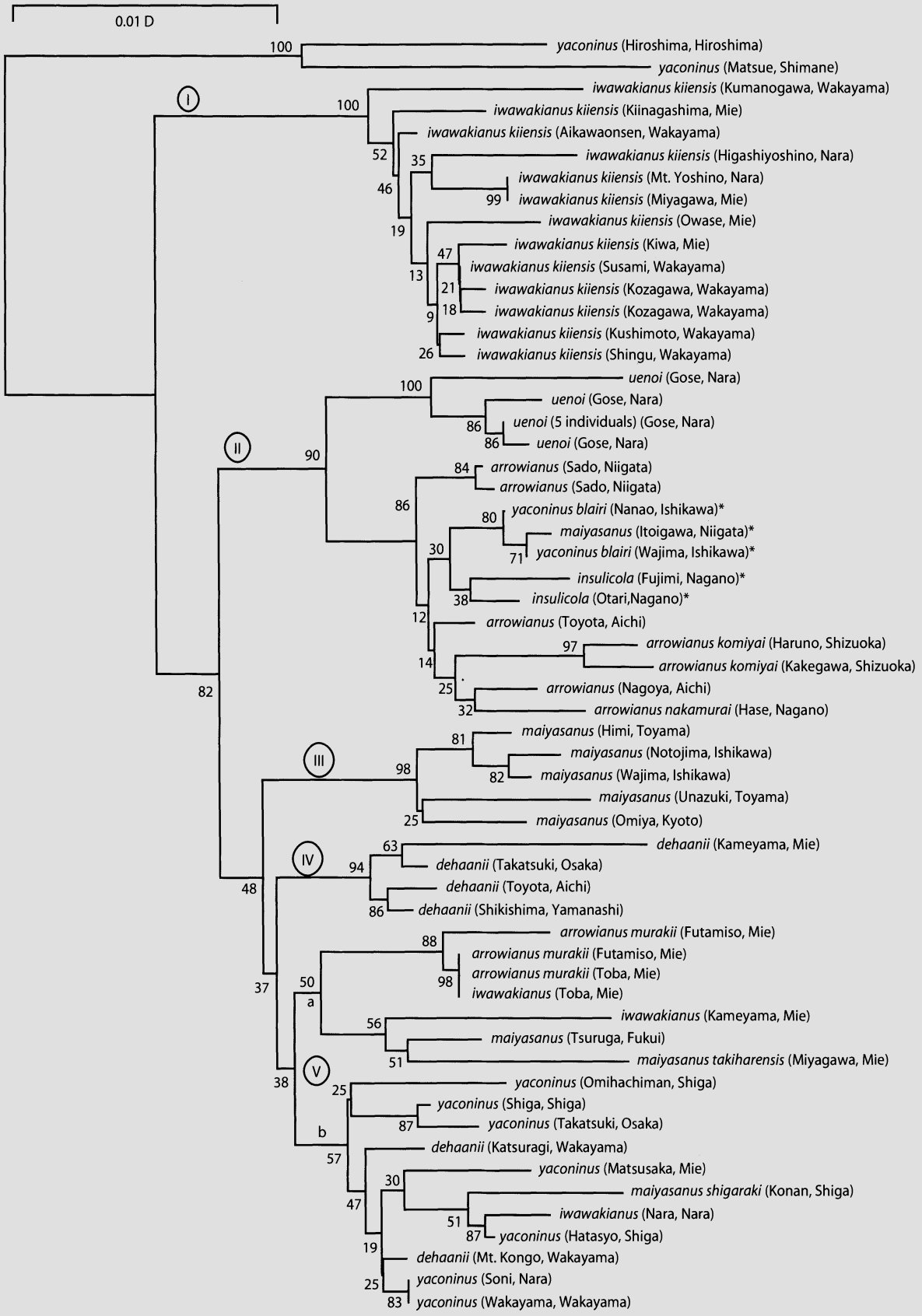


FIG. 7.11. Phylogenetic tree of the mitochondrial *ND5* gene for the Central Japan/Kinki (CJP/KNK) sublineages of the genus *Ohomopterus*. Constructed using the NJ-method. * Hybrid or hybrid-derivative. For details, see the text (unpublished)

TABLE 7.2. Origin of the *Ohomopterus* "species" and "subspecies" in the Chubu and Kinki Districts

(Sub)species	Mt ND5 DNA	Nuclear ITS I	Remarks (female) × (male)
O. iwawakianus kiiensis	k	k	authentic
O. i. iwawakianus	y2	w	K-*yaconinus* × *i.kiiensis*
O. yaconinus	y2	y	*maiyasanus* × *yaconinus*
O. maiyasanus shigaraki	y2	~w	K-*yaconinus* × *.i.iwawakianus*
O. m. takiharensis	y2	~w	K-*yaconinus* × *.i.iwawakianus*
O. dehaanii	d2	d	*maiyasanus* × *dehaanii*
O. dehaanii (Wakayama)	y2	?	?
O. arrowianus (incl. *O. a. nakanurai* and *O. a. komiyai*)	a	a	authentic
O. arrowianus murakii	y2	a	K-*yaconinus* × *arrowianus*
O. uenoi	a	y	*arrowianus* × *K (?) yaconinus*
O. maiyasanus	m	m	authentic
O. yaconinus blairi	a	y	*arrowianus* × *yaconinus*
O. yaconinus (WJP)	y	y	authentic
O. dehaanii (SYO)	d	d	authentic

k, *kiiensis*-type; y, *yaconinus*-type; d, *dehaanii*-type; a, *arrowianus*-type; m, *maiyasanus*-type; y2, Kinki (K)-*yaconinus*-type derived from *maiyasanus*; d2, Kinki (K)-*dehaanii*-type derived from *maiyasanus*; w, related to *kiiensis*-type; ~w, related to w; ?, not examined

of the Kii Peninsula covering almost the entire distribution area of this carabid (the areas marked K in 1 and 2 of Fig. 7.13). The ITS sequence of all the samples are characteristic to this group [k(*kiiensis*)-type ITS], indicating a congruence of the results gathered by mitochondrial DNA and nuclear ITS analysis.

Ohomopterus iwawakianus kiiensis was first described as a subspecies of *O. yaconinus* and later came to be treated as a subspecies of *O. iwawakianus*. As *O. yaconinus* is phylogenetically independent from *kiiensis*, *O. iwawakianus iwawakianus* and some other subspecies are probably hybrid-descendants (see below). This means that *kiiensis* may better be treated as a "pure" or "authentic" independent species.

The cluster II is composed of mainly *O. arrowianus* and its subspecies, *O. a. komiyai* and *O. a. nakamurai*. All of these are found in the central to southern part of the Chubu District, west of the Itoigawa-Shizuoka tectonic line (Fig. 7.4; area marked A in 3 of Fig. 7.13). Sado Island is also a habitat of this species (see p. 120). There exist hybrid zones between this species and *O. maiyasanus* (I-type), *O. insulicola* (I-type), and *O. yaconinus* (Y-type) within their distribution boundaries (asterisks in Fig. 7.11). All of them, except hybrid specimens, have characteristic *a* (*arrowianus*)-type ITS. *Ohomopterus uenoi* is also in this cluster (see below).

The cluster III contains only *O. maiyasanus*, which is found in the northern half of the Chubu and Kinki Districts (see area marked M in 2 of Fig. 7.4; M in 3 of Fig. 7.13). The ITS sequence of all the specimens (except one from Fukui; see below) is characteristic to this species [*m* (*maiyasanus*)-type ITS].

The cluster IV contains only *O. dehaanii* (D-type) from some localities of the Kinki and Chubu Districts. The same "species" from Wakayama belongs to the cluster V and not to this cluster. As already discussed above, this species is also widely distributed in western Japan, and yet *O. dehaanii* from the Kinki and Chubu Districts belongs to a lineage distinct from that of the "same" species from western Japan on the mitochondrial DNA tree.

On the other hand, the ITS sequences of this species in cluster IV are quite close to those from western Japan [*d* (*dehaanii*)-type ITS], i.e., there is no ITS specific to the Kinki/Chubu population. On the mitochondrial phylogenetic tree, cluster III of *O. maiyasanus* (I-type) always forms the outgroup of the cluster IV of *O. dehaanii* (Figs 7.5 and 7.11). These facts suggest that *O. dehaanii* in the cluster IV is most probably a result of past hybridization between *O. dehnaaii* (male) and *O. maiyasanus* (female).

The composition of the cluster V is quite complex, as noted above. The constituents of this cluster all inhabit the Kinki District excluding the areas K (distribution area of *O. iwawakianus kiiensis* in Fig. 7.13) and M (distribution area of *O. maiyasanus* in Fig. 7.13). On the mitochondrial tree, "*O. yaconinus*," which belongs to a lineage independent form *O. yaconinus* of western Japan (see p. 113), is the main member of the lineage V with intermingled occurrence of other species. All the mitochondrial DNA of the cluster V specimens was what we call "y2-type mtDNA" (see Table 7.2).

From these facts, it may be said that "*O. yaconinus*" (Y-type), *O. iwawakianus* and its subspecies (Y-type) (excluding *kiiensis*), *O. maiyasanus takiharensis* (I-

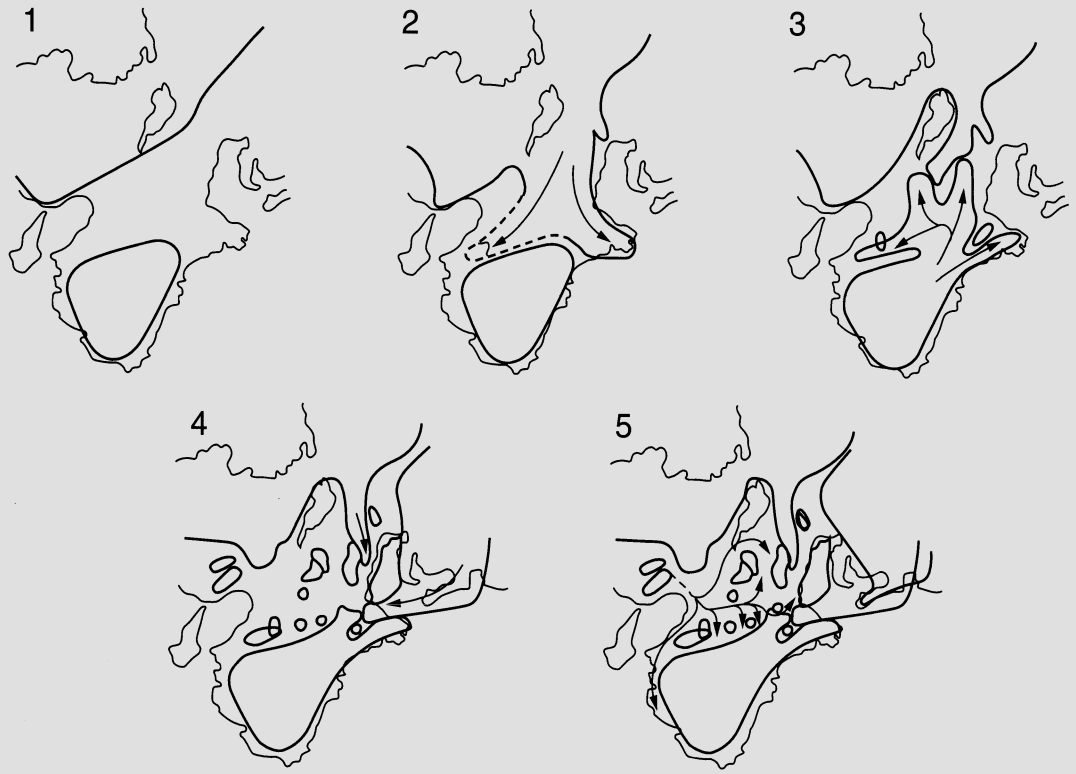


FIG. 7.12. A hypothesis on the formation of the *Ohomopterus* fauna in the Kinki District. **1** *O. maiyasanus* (ancestor) in the northern part and *O. iwawakianus kiiensis* in the southern part of the Kinki District. **2** Southward migration of *O. maiyasanus* and differentiation of its subspecies. **3** Northward migration of *O. iwawakianus*. **4** Secondary southward migration of *O. maiyasanus* and invasion of *O. arrowianus*. **5** Invasion of *O. yaconinus* into the Kinki Triangle (after Katsura et al. 1978)

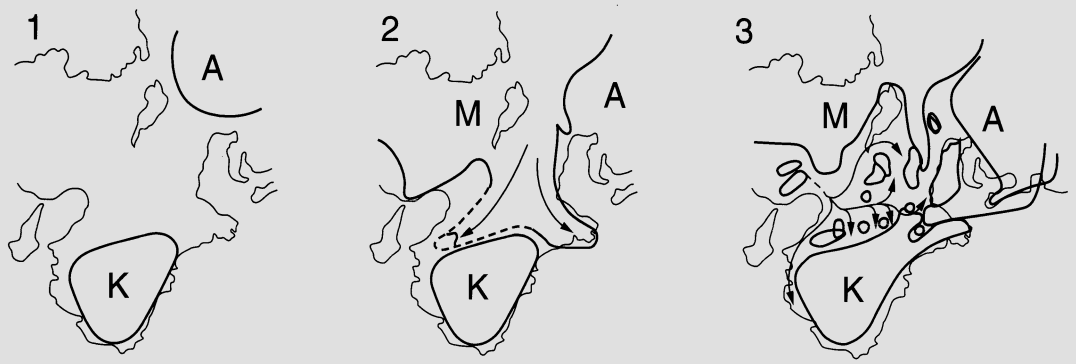


FIG. 7.13. Formation of the *Ohomopterus* fauna in the Kinki District as deduced by the phylogenetic tree of the mitochondrial *ND5* gene. **1** *O. arrowianus* (A) in the eastern part, *O. iwawakianus kiiensis* (K) in the southern part. **2** *O. maiyasanus* (M) in the northern part (derived from *O. arrowianus*) and *O. i. kiiensis* in the southern part. **3** Invasion of *O. yaconinus* and *O. dehaanii* into the Kinki Triangle and their hybridization with *O. maiyasanus*, having resulted in replacement of the mitochondrial DNA of the species whose territory had been invaded by that of *O. maiyasanus* (after Tominaga 1999; modified)

type), *O. maiyasanus shigaraki* (I-type), *O. arrowianus murakii* (I-type), and *O. dehaanii* from Wakayama (D-type), all carry the y2-type mtDNA. This y2-type mtDNA in each species in the cluster V belongs to an independent lineage when compared with the mitochondrial DNA of the corresponding authentic species. As *O. maiyasanus* of the cluster III is the outgroup of the clusters IV and V on the phylogenetic tree, the y2-type mtDNA of all "species" in the cluster V were presumably derived from that of *O. maiyasanus*.

The most likely explanation for this is that the mitochondrial DNA of *O. yaconinus* inhabiting the Kinki District was replaced by that of *O. maiyasanus*. The resultant mitochondrial DNA is the y2-type mtDNA,

which was transferred from *O. maiyasanus*. The mitochondrial DNA of other species in this cluster was then replaced by the y2-type mtDNA of *O. yaconinus* in the Kinki District (hereafter referred to as Kinki-*O. yaconinus*). This assumption is consistent with the fact that the ITS I sequence of *O. yaconinus* in the Kinki District cannot be differentiated from that of the same species in western Japan, in spite of the phylogenetic independence of the mitochondrial DNA between these two *O. yaconinus* populations. In other words, ITS specific to Kinki-*O. yaconinus* does not exist. These facts suggest that *O. yaconinus* in the Kinki District is a descendant of a hybrid between the authentic *O. yaconinus* (male) and *O. maiyasanus* (female). Since no examples of *O. maiyasanus* having *O. yaconinus*-type mitochondrial DNA, nor the authentic *O. yaconinus* have been discovered, the mitochondrial replacement was unidirectional and the authentic strain of *O. yaconinus* became extinct in the Kinki District.

The ITS sequence in other species in the cluster V is various and corresponds to that of each authentic species (Table 7.2), i.e., the cluster V-specific ITS does not exist. For example, *O. arrowianus murakii* (I-type) is distributed around the Shima Peninsula and has been considered a descendant of *O. arrowianus*, which migrated from the Atsumi Peninsula in Aichi Prefecture when these two peninsulas were connected. As can be seen in Fig. 7.11, all the three specimens of *O. a. murakii* that we examined had y2-type mtDNA, whereas their ITS sequences were all *a* (*arrowianus*)-type, suggesting that *O. a. murakii* was derived from a hybrid of *O. arrowianus* (male) and the Kinki-*O. yaconinus* (female). The ITS of *O. dehaanii* (D-type) from Wakayama has not been analyzed. We suspect that it was derived from past hybridization between *O. dehaanii* (male) and *O. yaconinus* (female).

The ITS sequence of *O. iwawakianus* and some of its subspecies shows a considerable resemblance to that of *O. i. kiiensis*, suggesting that *O. iwawakianus* is an offspring of hybridization between *O. i. kiiensis* (male) and Kinki-*O. yaconinus* (female). The ITS sequences of *O. maiyasanus takiharensis* (I-type) and *O. m. shigaraki* (I-type) are close to the sequence of *O. iwawakianus*, while their mitochondrial DNA is y2-type and is different from that of *O. maiyasanus*. These facts suggest that both subspecies of *O. maiyasanus* are the result of hybridization of *O. iwawakianus* (male) and Kinki-*O. yaconinus* (female).

The findings remain tentative, however, because of the shortage of samples analyzed. Nevertheless, our results suggest that the only "pure or authentic" *Ohomopterus* species in the Chubu/Kinki District are likely to be the following three species: *O. arrowianus* (I-type), *O. maiyasanus* (I-type), and *O. iwawakianus kiiensis* (Ẏ-type; this should be called *O. kiiensis* and not of a subspecies of *iwawakianus*). All others including *O. uenoi* are of hybrid origin (see below).

7.2.5 Origins of *Ohomopterus* Species Inhabiting the Chubu and Kinki Districts

How did the complex *Ohomopterus* fauna of the Kinki District, as described in the previous section, take form? Can this be deduced from the results of DNA analysis? The Kinki District is composed of the Kii Peninsula in the south, the Hida Mountains in the northeast, the Tanba Highlands of the northwest and a roughly triangular lowland area including Biwako Lake.

This triangular area contains low mountains, hills, and lowlands, and is called the Kinki Triangle on the basis of the structure of the earth's crust in the area. Differentiation in the carabid fauna in this region is well known. Katsura et al. (1978) tried to delineate the relationships between the carabid fauna and geohistory of this area. These researchers assumed that the *Ohomopterus* species found in the Kinki District originated from proto-*O. maiyasanus* and proto-*O. iwawakianus kiiensis*, which contrast each other in terms of morphology, ecology, and distribution (Fig. 7.12).

The diversification sequence may be expressed as follows: 1) *O. maiyasanus* and *O. iwawakianus kiiensis* inhabited the northern area and the southern areas of the Kinki District, respectively. 2) *O. maiyasanus* migrated southwards with differentiation of *O. m. shigaraki* and *O. m. takiharensis*. 3) *O. iwawakianus kiiensis* expanded its distribution northwards, followed by differentiation of *O. iwawakianus iwawakiianus*. 4) *O. maiyasanus* again migrated southwards, and *O. arrowianus* invaded the Shima Peninsula from the Chubu District. 5) Migration of *O. yaconinus* to the Kinki District from west.

Tominaga (1999) interpreted the *ND5* mitochondrial phylogenetic tree created in 1996 (Fig. 7.5) based on the hypothesis of Katsura et al. (1978) (Fig. 7.13). *O. yaconinus* and *O. dehaanii* are found over a wide area from western Japan to the Chubu District (see Fig. 7.4), with the Kinki District population situated between the western and the Chubu populations.

On the other hand, *O. maiyasanus* is limited to the Kinki and Chubu Districts, *O. iwawakianus kiiensis* to the Kii Peninsula, and *O. arrowianus* to the Chubu District. Each of them forms a well defined cluster on the *ND5* phylogenetic tree (clusters I, II, and III in Fig. 7.11).

The other subspecies of *O. maiyasanus*, i.e., *O. m. takiharensis*, and *O. m. shigaraki*, together with Kinki-*O. yaconinus* and Kinki-*O. dehaanii*, which are all inhabitants of the Kinki Triangle and its adjacent regions, are intermingled on the tree, and do not form (sub)species-specific clusters (Fig. 8.11; see also the preceding section).

From these facts, a scenario as to the formation of the *Ohomopterus* fauna of the Kinki and Chubu Districts may be deduced (Fig. 7.13). 1) The species of *Oho-*

mopterus in the Kinki and Chubu Districts were originally composed solely of *O. iwawakianus kiiensis* (in Kinki) and *O. arrowianus* (in Chubu). 2) *O. maiyasanus* differentiated allopatrically from *O. arrowianus* and expanded its distribution from the northern part of the Hokuriku area to the northern Kinki District. 3) Following this, *O. yaconinus* and *O. dehaanii* invaded the Kinki District from the west and hybridized with *O. maiyasanus*, thus resulting in the unidirectional replacement of mitochondrial DNA in the two species whose territory had been invaded by that of *O. maiyasanus*.

The *ND5* phylogenetic tree (Fig.7.11) makes it appear that hybridization occurred around the time of the split of the clusters IV and V from the cluster III (*O. maiyasanus*) (6–5 MYA). The lowland area around the Yurakawa River and the Kakogawa River (the western limit of the Kinki district) to that around the Yodogawa River and Biwako Lake were formed by the upheaval of the First Inland Sea of Japan. We may assume that *O. yaconinus* and *O. dehaanii* did not inhabit the Kinki District at the time of the upheaval of the First Inland Sea, which separated western Japan and the Kinki District.

Upon the connection of the western Japan to the Kinki District by the disappearance of the First Inland Sea (>5 MYA), migration of these two species to the Kinki District and hybridization with *O. maiyasanus* took place 6–5 MYA, as mentioned above. These events would have occurred before the formation of the Yodogawa River–Biwako Lake line, which took form about 3 MYA, because these two species are distributed to the east of the Yodogawa River.

The main area where hybridization occurred came to be occupied by *O. dehaanii* and *O. yaconinus*, whose mitochondrial DNA had been replaced by that of *O. maiyasanus*, and *O. maiyasanus* converged to its mother population in the northeastern Kinki District. As *O. yaconinus* from the western Japan sublineage (WJP) inhabit the Hokuriku area along with *O. maiyasanus* without formation of hybrids, the former species would have invaded much later than the first migration of *O. yaconinus* to the Kinki District.

The hybrid-derived *O. dehaanii* in the cluster IV in Fig. 7.11 also expanded its range into the Chubu District later than the first invasion of *O. dehaanii* from the west to the Kinki District. The above scenario by Tominaga (1999) is consistent with the results from both mitochondrial and nuclear ITS DNA analyses described in the previous section.

Tominaga (1999) did not discuss *O. iwawakianus iwawakianus* and *O. arrowianus murakii*. *Ohomopterus i. iwawakinaus* (Y-type) would have been derived from a hybrid of Kinki-*O. yaconinus* (Y-type; female) and *O. i. kiiensis* (Y-type; male) at the distribution periphery of the latter. *O. arrowianus murakii* (I-type) was established by hybridization between Kinki-*O. yaconinus* (Y-type; female) and *O. arrowianus* (male) that had immigrated from the Chubu District to the Shima Peninsula.

The ITS I sequences of *O. maiyasanus shigaraki* and *O. m. takiharensis* (both I-type) are close to *O. iwawakianus iwawakianus* (Y-type), it is likely that these two subspecies of *O. maiyasanus* (I-type) emerged from a hybrid of *O. iwawakianus iwawakianus* (Y-type) and Kinki-*O. yaconinus* (Y-type).

Upon emergence of these two subspecies, transformation, i.e., type-switching, of the male digitulus (copulatory piece) from the Y-type to the I-type would have taken place. It is clear then that, whatever the model by which these changes took place, the Kinki Triangle may be regarded as a crucible of hybrid-derived *Ohomopterus* ground beetles.

It may be of interest to note that the "authentic" *O. yaconinus* (WJP sublineage; see Fig. 7.4); *O. arrowianus* (CJP sublieage), *O. dehaanii* (SYO sublineage), and *O. iwawakianus kiiensis* (KII sublineage) that participated in the emergence of Kinki-*O. yaconinus*, *O. arrowianus murakii*, and Kinki-*O. dehaanii* are all thought to have been male. Whether this is related to physical hardiness or ease of copulation is not known.

Another point worth noting is that the mitochondria of the invader species (e.g., *O. yaconinus*) were all replaced by those of the female of the authentic species (e.g., *O. maiyasanus*) and the authentic mitochondria (e.g., of *O. yaconinus*) disappeared. Which type is finally selected and fixed is thought to be determined by random genetic drift. In the case of *Ohomopterus*, however, hybrid-derived individuals might be more advantageous, because all the authentic ones do not survive. It is also worth noting that the formation of new species accompanied by a considerable morphological change does not seem to be created by hybridization; the above mentioned hybrid populations are in many cases distinguished as "subspecies" of the authentic species. Taxonomically recognizable morphological characters for "subspecies" are likely to have resulted from not only geographic isolation but also hybridization through which some nuclear DNA of other species is introduced.

The quite complex pattern of the fauna found in the area would have been produced by the appearance of new habitat or disappearance of old habitat caused by geographic changes such as the submergence or upheaval of land. Whether this complex pattern is an extreme or exceptional one in the evolution of the carabid ground beetle deserves further consideration.

Ohomopterus uenoi is one of the most peculiar species in the genus *Ohomopterus* in several respects. Firstly, the distribution of this species is restricted sharply to the upper parts of Mt. Kongo and Mt. Katsuragi of the Kongo Mountains in the Kinki District (Ishikawa 1991; Kinki Research Group of Carabid Beetles 1979). No species other than *O. uenoi* inhabiting such an isolated region has been known among the

TABLE 7.3. Japanese *Leptocarabus* species and subspecies

Subgenus *Leptocarabus* (s. str.)	Subgenus *Adelocarabus*	Subgenus *Aulonocarabus*
L. kyushuensis [1]Subsp.: *cerberus, nakatomii* *L. hiurai* *L. kumagaii* *L. procerulus* Subsp.: *miyakei*	*L. arboreus* Subsp.: *hakusanus, ohminensis*, and others (total 21 subspecies)	*L. kurilensis* Subsp.: *daisetsuzanus*, and others (total 5 subspecies)

[1] Only subspecific names that appeared in the text are shown

Japanese carabid beetles. Secondly, the male of *O. uenoi* has an exceptionally large I-type copulatory piece. Except for this peculiarity, the general appearance of *O. uenoi* is almost indistinguishable from that of *O. yaconinus* having the Y-type copulatory piece.

We must ask, as a result of these oddities, where *O. uenoi* comes from. The mitochondrial *ND5* gene of *O. uenoi* shares common ancestry with *O. arrowianus* (Fig. 7.11). The separation of the *ND5* gene sequence in *O. arrowianus* and *O. uenoi* is calculated to have occurred about 5.4 million MYA.

There are at least two ways with which the origin of *O. uenoi* might be explained. One possibility is that there was a period when the ancestor of *O. arrowianus/O. uenoi* was distributed in central Japan through the Kongo Mountains. The ancestor of *O. uenoi* underwent a convergence to *O. yaconinus*-type appearance with hypertrophy of the male copulatory piece. Another possibility is that an ancestor of *O. arrowianus* (female) inhabiting the Kongo Mountains hybridized with *O. yaconinus* (male), resulting in the emergence of the ancestor of the present-day *O. uenoi*.

The individuals of *O. arrowianus* that participated in this hybridization must have been female (unidirectional hybridization), because the *ND5* gene of *O. uenoi* reveals an *O. arrowianus*-type sequence. In either case, most of the *O. arrowianus* fraction of the intermediate regions between central Japan and the Kongo Mountains became extinct. It is, however, worth noting that *O. arrowianus murakii* inhabiting the mid-eastern part of the Kinki District is a derivative of a hybrid of *O. arrowianus* (female) and *O. yaconinus* (male) (see above).

The Kongo Mountains are situated only about 60 km west of the habitat of *O. arrowianus murakii*, suggesting that *O. arrowianus* once inhabited the intermediate region between the mid-eastern part of the Kinki District and the Kongo Mountains. According to a preliminary analysis, the ITS I sequence of *O. uenoi* is of the *O. yaconinus*-type, suggesting that *O. uenoi* was derived from the hybrid ancestor of *O. arrowianus* (female) and *O. yaconinus* (male).

7.2.6 Distribution Boundary between *Ohomopterus arrowianus* and *O. insulicola*

As is shown in Fig. 7.5, on the mitochondrial *ND5* phylogenetic tree *O. insulicola* belongs to the Japan Sea Is./eastern Japan (JSE) sublineage of the western Japan lineage (Lineage I) and is found mainly east of the Itoigawa-Shizuoka tectonic line. The tree also shows that *O. arrowianus* is a member of the central Japan (CJP) sublineage of the Kinki/Chubu lineage and is found in central Japan, west of the tectonic line.

Analysis of the *ND5* DNA and nuclear ITS I DNA, revealed the presence of hybrid individuals in Mima, Matsumoto, and Fujimi-Cho, all in Nagano Prefecture along the tectonic line. Independent clones of ITS I from a single specimen from these localities include both *O. arrowianus*- and *O. insulicola*-ITS sequence at the ratio of one to one, so that they may be considered F1 between these two species. These hybrids all reveal an *O. insulicola*-like phenotype.

As the localities where the hybrids were found are on the Itoigawa-Shizuoka tectonic line, there may be a hybrid zone along the tectonic line. Sado Island is also included in the hybrid zone. Further study is required in order to establish a more detailed picture of these two species and any hybrid zone.

7.3 Genus *Leptocarabus*

The genus *Leptocarabus* of Japan has been well studied taxonomically and is classified into the following species: *L.* (s. str.) *procerulus*, *L.* (s. str.) *kumagaii*, *L.* (s. str.) *hiurai*, *L.* (s. str.) *kyushuensis*, and *L.* (*Adelocarabus*) *arboreus* (Nakane 1961; Ishikawa 1991).

All the species, with the exception of *L. kumagaii* and *L. hiurai*, are further separated into a number of subspecies (local races). In one extreme example, *L. arboreus* is divided into 21 subspecies based on minor morphological and distributional differences (Ishikawa 1992).

A distribution map of the *Leptocarabus* species is shown in Fig. 7.14 (after Tominaga and Hiura 1979), where the subspecific rank is not considered except in a few cases. Similarly, subspecific rank will have little

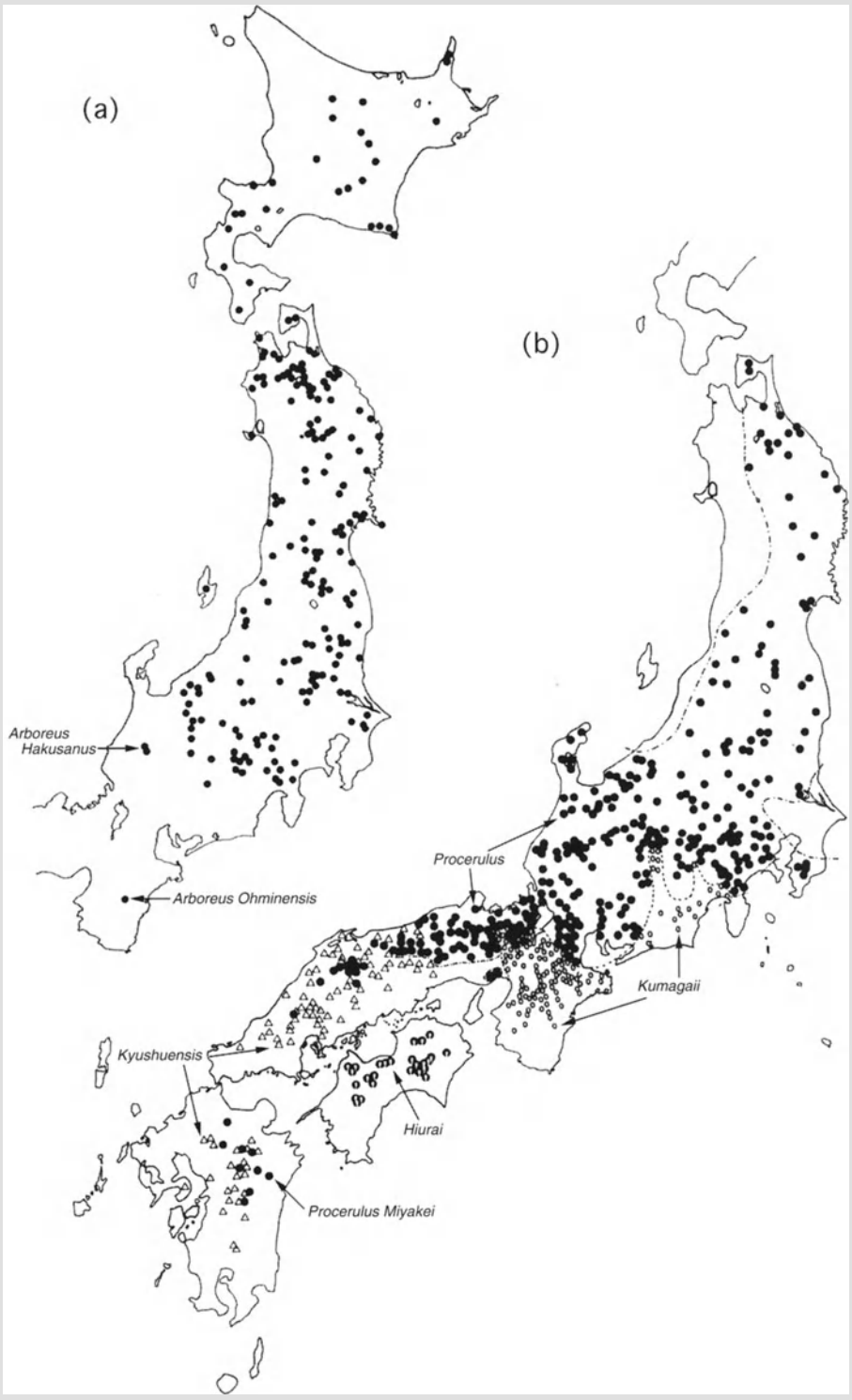


FIG. 7.14. Distribution ranges of *Leptocarabus arboreus* (**a**) and those of other *Leptocarabus* species (**b**) in the Japanese Islands. Dots and other symbols denote the locality of specimens identified by morphological characteristics and not those classified by means of DNA analysis (after Tominaga and Hiura 1979; modified)

influence on our discussion in this section. Indeed, there is little guarantee that such subspecies will evolve into species.

Despite detailed taxonomic studies, little had been known of the origins and phylogenetic relationships among the Japanese *Leptocarabus* species and subspecies until phylogenetic trees based on the *ND5* gene and of 28S rDNA from the Japanese and continental *Leptocarabus* species were constructed (Fig. 5.21) (Kim et al. 2000). As mentioned in Chapter 6, the Japanese *Leptocarabus* species have no direct ancestry on the Eurasian Continent at present, and the ancestor of the Japanese species is likely to have inhabited the ancient Japan area of the continent, followed by its diversification into several species after separation of the Japanese Islands from the continent.

Leptocarabus (*Aulonocarabus*) *kurilensis* has been excluded from this section due to the lack of a direct phylogenetic relationship between this species and the other Japanese *Leptocarabus* species (Fig. 5.21) (Kim et al. 2000b; Tominaga et al. 2000). *Tomocarabus opaculus* and *T. harmandi* are sometimes treated as belonging to *Leptocarabus* but are not dealt with here, because each of them constitutes a lineage independent from *Leptocarabus* (Figs. 5.19d and 7.17) (Su et al. 2000c).

7.3.1 Molecular Phylogeny

Figure 7.15 shows an *ND5* phylogenetic tree constructed using 101 specimens of *Leptocarabus* collected at various spots throughout almost the entire range of this genus in the Japanese Islands.

Two major lineages of the mitochondrial haplotype have been identified. Their separation is calculated to have taken place about 11 MYA. The lineage 1 includes *L. kyushuensis* (with an important exception, see below), which is further divided into two sublineages. The first one (KYU1 in Fig. 7.15; loc. nos. 97–101 in Fig. 7.16) contains *L. kyushuensis* from Kyushu, and the second one (CHG; loc. nos. 91–96) contains all the specimens of *L. kyushuensis* from the Chugoku District in Honshu and one specimen identifiable as *L. procerulus* from Kuchiwa in Hiroshima Prefecture, Honshu (loc. no. 71). The separation of the sublineages 1 and 2 may be estimated to have occurred about 5–7 MYA. The sequence diversification within each sublineage is rather small.

The lineage 2 is composed of at least six sublineages. The first one (UNZ; loc. no. 86–90) is represented solely by *L. kyushuensis* from the Shimabara Peninsula in Nagasaki Prefecture, Kyushu. The second one (KYU2; loc. nos. 72–73) contains *L. procerulus miyakei* from Kyushu and one specimen from Fukue in Yamaguchi Prefecture, Honshu (loc. no. 85).

The third sublineage (SKU; loc. nos. 81–84) is composed of *L. hiurai* from Shikoku and, surprisingly, *L. arboreus ohminensis* from Mt. Ohmine in Nara Prefecture, Honshu (loc. no. 45). Diversification of the *ND5* sequences in this sublineage is fairly old. The fourth sublineage (HON; loc. nos. 20–80) is composed of a mixture of three different species, *L. procerulus*, *L. kumagaii*, and *L. arboreus*, which are widely distributed from the Kinki District up to northernmost Honshu. *Leptocarabus procerulus* is found throughout the distribution range of HON, while *L. kumagaii* occupies the Kinki and Chubu Districts, and *L. arboreus* inhabits mostly the northern half of the HON range as has been shown by taxonomic studies. The sequence divergence is very small among all the specimens in this sublineage, and the sequences of many of them are identical.

The mitochondrial haplotypes of the three species are intermingled on the trees and there are no species-specific clusters. The fifth sublineage, THK (loc. nos. 13–19), consists of only *L. arboreus*, the distribution range of which is embedded within HON in the Tohoku district of northern Honshu. The sixth sublineage, HKD, is composed of *L. arboreus* from Hokkaido and is further divided into HKD1 (loc. nos. 1–5) and HKD2 (loc. nos. 6–12), which inhabits eastern and southwestern Hokkaido, respectively.

There are a number of cases in which different species appear in the same mitochondrial haplotype lineage. In the CHG sublineage of the lineage 1, *L. procerulus* sympatrically occurs among *L. kyushuensis*. In the KYU2 sublineage of the lineage 2, *L. kyushuensis* appears allopatrically with *L. procerulus*. *Leptocarabus arboreus ohminensis* from Mt. Ohmine in Nara Prefecture belongs to the same SKU sublineage of the lineage 2 as does *L. hiurai*. These two are distributed allopatrically.

An extreme case of intermingled occurrence of more than two species is seen in the sublineage HON of the lineage 2, where *L. procerulus*, *L. kumagaii*, and *L. arboreus* appear either sympatrically (e.g., *L. procerulus* and *L. arboreus hakusanus* on Mt. Hakusan, Gifu Prefecture, Honshu; loc. nos. 66 and 44 in Fig. 7.15) or allopatrically (e.g., *L. procerulus* and *L. kumagaii* in the Kinki District). These Japanese *Leptocarabus* species have been taxonomically separated mainly by morphology of the male genital organ.

Based on morphological characters, *L. arboreus* is often treated as being separate from the other *Leptocarabus* species and belonging to the distinct subgenus, *Adelocarabus*. How can this phenomenon be interpreted? One explanation is that the similar morphologies in different lineages in the mitochondrial DNA might result from hybridization between two or more species. This might be the case for sympatrically or parapatrically distributed species, but it is hard to imagine the occurrence of hybridization between two allopatrically distributed species such as *L. hiurai* in Shikoku and *L. arboreus ohminensis* from Mt. Ohmine, Honshu. The Kitan Strait separates these two and *L. arboreus ohminensis* is sharply isolated geographically

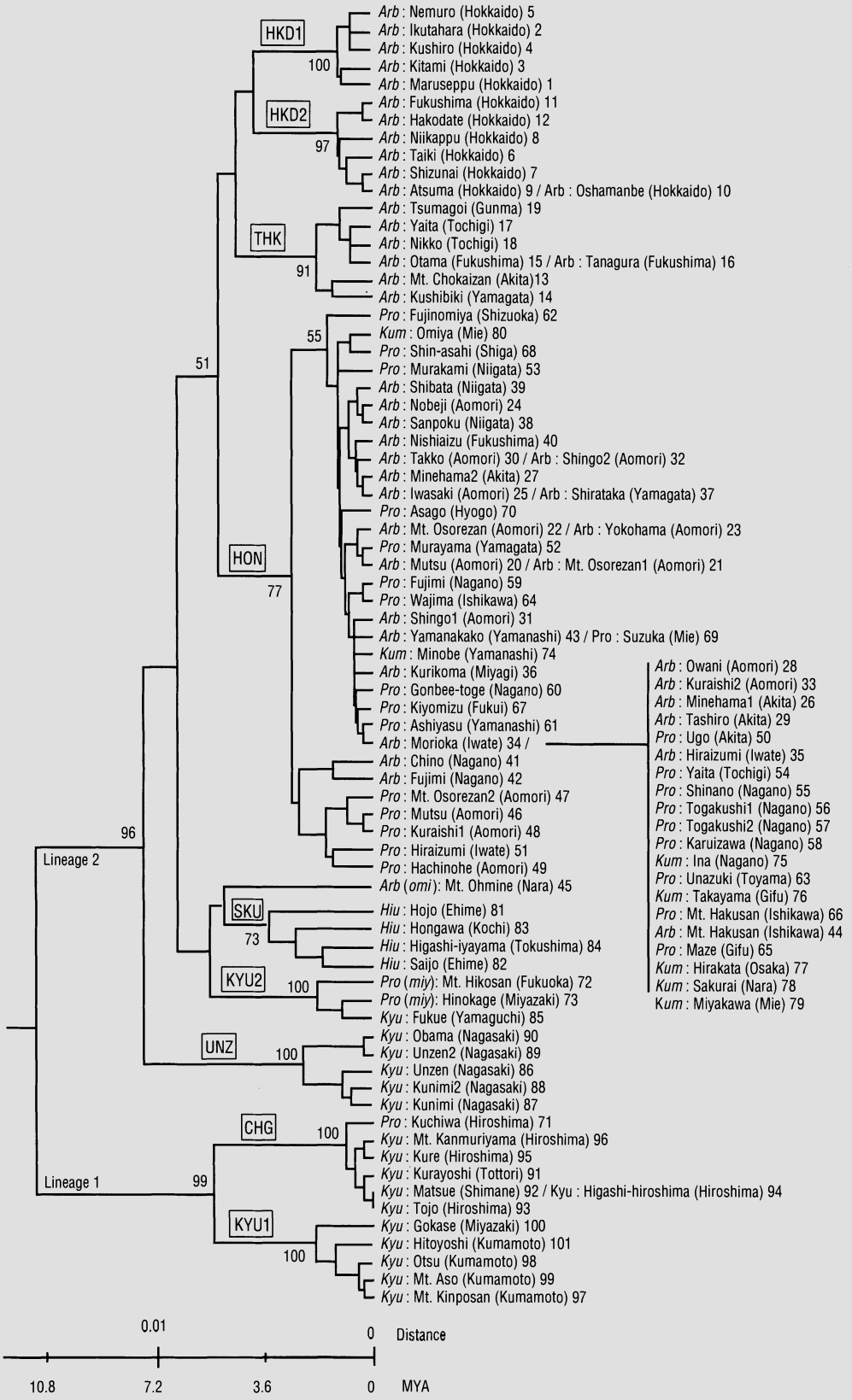


FIG. 7.15. Phylogenetic tree of the mitochondrial *ND5* gene for the Japanese *Leptocarabus* species. Constructed using the UPGMA. **Arb** *Leptocarabus arboreus*, **Pro** *L. procerurus*, **Kum** *L. kumagaii*, **Hiu** *H. hiurai*, **Kyu** *L. kyushuensis* (after Kim et al. 2000a)

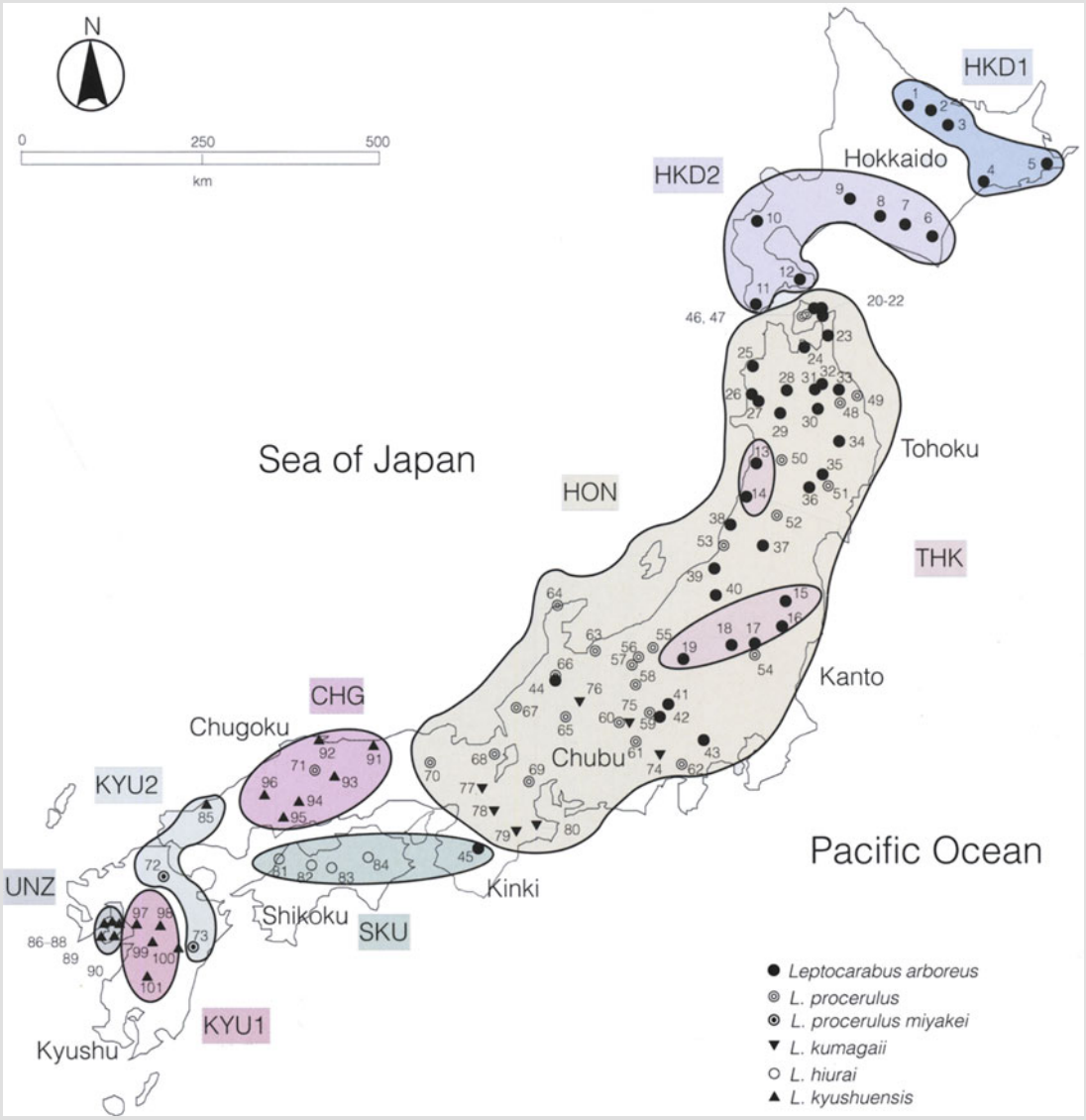


FIG. 7.16. Distribution map of the lineages and sublineages of the Japanese *Leptocarabus* species based on the phylogenetic tree of the mitochondrial *ND5* gene. Locality numbers correspond to those shown in Fig. 7.15 (after Kim et al. 2000a)

from the other *L. arboreus* subspecies, the distribution range of which is far distant from that of *L. a. ohminensis*.

In the sublineage HON in the lineage 2, the haplotype DNA sequence of many specimens of *L. procerulus*, *L. kumagaii*, and *L. arboreus*, especially those inhabiting the Kinki, Chubu, and part of the Tohoku District, are identical or very close, and no species-specific DNA sequences have been found (Fig. 7.15). These facts are not in accord with the hybridization hypothesis. The very limited divergence in the mitochondrial haplotype sequences, as mentioned above, suggests a recent emergence of at least two species out of three.

An alternative explanation is that discontinuous transformation from one type to another took place in different phylogenetic lines in various points in the evolutionary process. Most examples of the intermingled occurrence of more than two morphological species in the same lineage may be explained by this hypothesis. This kind of morphological transformation, which we call "type switching," has been observed in a number of carabid lines (see Chapter 8).

7.3.2 Formation of the Japanese *Leptocarabus* Fauna

As noted above, the Japanese *Leptocarabus* species started to diversify much later (ca. 12–10 MYA) than the continental species (ca. 28–25 MYA)(Fig. 5.21). From the phylogenetic trees (Fig. 7.15) and the distribution

map of the lineages and sublineages (Fig. 7.16), it can be inferred that the ancestor of all the Japanese *Leptocarabus* species inhabited the ancient Japan area (presumably somewhere in the ancient northern Kyushu area), and had a morphology similar to that of the *L. kyushuensis*-type, and separated into two lineages, the lineage 1 and the lineage 2, about 11 MYA after separation of the Japanese Islands from the Eurasian Continent.

The lineage 1 then split into the two sublineages KYU1 and CHG, and the latter invaded the Chugoku District of Honshu, followed by geographic isolation from KYU1. In both of these two sublineages, *L. kyushuensis*-type morphology has been maintained except in some specimens in Hiroshima Prefecture, Honshu, which can be identified as *L. procerulus*.

In the lineage 2, the sublineage UNZ separated from the rest of the sublineages and became isolated on the Shimabara Peninsula (Kim et al. 2001), having maintained *L. kyushuensis*-type morphology. From the other branch of the lineage 2, five sublineages i.e., KYUZ, SKU, HON, THK, and HKD emerged. Since these have either *L. procerulus*- or *L. arboreus*-type morphology, a morphological transformation is assumed to have occurred after the split from the UNZ sublineage.

The ancestor of these sublineages rapidly spread northeast and southeast, forming the sublineages in different areas in the Japanese Islands, presumably as a result of geographic and/or ecological isolation, followed by expansion or restriction of their distribution ranges.

The first sublineage KYU2 consists of *L. procerulus miyakei* from northern Kyushu of which we examined one specimen from Yamaguchi Prefecture, Honshu, with *L. kyushuensis*-type morphology. The second sublineage is SKU, found in Shikoku (*L. hiurai*) and in the area of Mt. Ohmine, Nara Prefecture, Honshu (*L. arboreus ohminensis*). KYU2 and SKU have some affinity on the phylogenetic trees we constructed. Because of the low bootstrap value between KYU2 and SKU, these two sublineages may not be entirely independent.

The third sublineage, HON, is found from the Kinki District to the northern tip of Honshu and contains *L. procerulus*, *L. kumagaii*, and *L. arboreus*, The fourth, or THK, sublineage is found in a restricted area of the Tohoku District of northern Honshu (*L. arboreus*). The fifth sublineage, HKD, is found in Hokkaido (*L. arboreus*).

The ancestor of these five sublineages would have had *L. procerulus*-type morphology, because *L. procerulus* and its congeners are the most widely distributed species in the distribution range of the lineage 2. Note that *L. hiurai* was first described as a subspecies of *L. procerulus*, and *L. kumagaii* was not distinguished from *L. procerulus*. Perhaps, during expansion of the distribution range of each sublineage, morphological transformation often occurred as mentioned in the previous section. *Leptocarabus arboreus* seems to be adapted to the alpine/subalpine environments in northeastern Japan, while *L. procerulus* and *L. kumagaii* seem to prefer the more moderate habitats of central Japan and the Kinki District, although *L. procerulus* inhabits the same area as *L. arboreus*. Transformation from *L. procerulus*-type morphology to *L. arboreus* might have taken place in the northeastern areas for ecological reasons.

Finally, it is of interest to note that the nuclear ITS I and II of all the *L. kyushuensis* specimens examined, including those from the Shimabara Peninsula, was 3.0 kbp long, while that of all others was 2.0–2.3 kbp long. This suggests that some changes leading to elimination of the ITS sequence (and therefore some other nuclear genes?) may have taken place in the nuclear genome during emergence of the *Leptocarabus* species.

7.4 *Tomocarabus opaculus* and *T. harmandi*

Tomocarabus opaculus is widely distributed in Hokkaido and its adjacent islands. It also occurs in the restricted mountainous area of northeastern Honshu. This species is the commonest in Hokkaido, while it is rather rare in Honshu.

The Honshu population is discriminated from the nominotypical *opaculus* as ssp. *shirahatai* (Nakane 1961) by minor morphological differences. Specimens from Mt. Daisetsu in Hokkaido have been classified

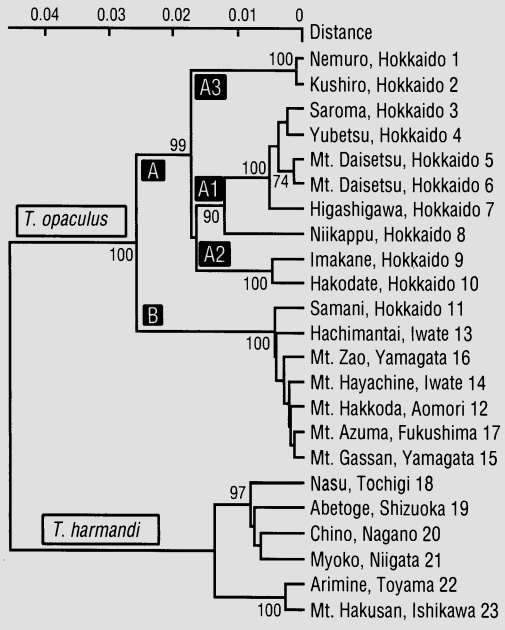


FIG. 7.17. Phylogenetic tree of the mitochondrial *ND5* gene for *Tomocarabus opaculus* and *T. harmandi*. Constructed using the UPGMA (after Su et al. 2000c)

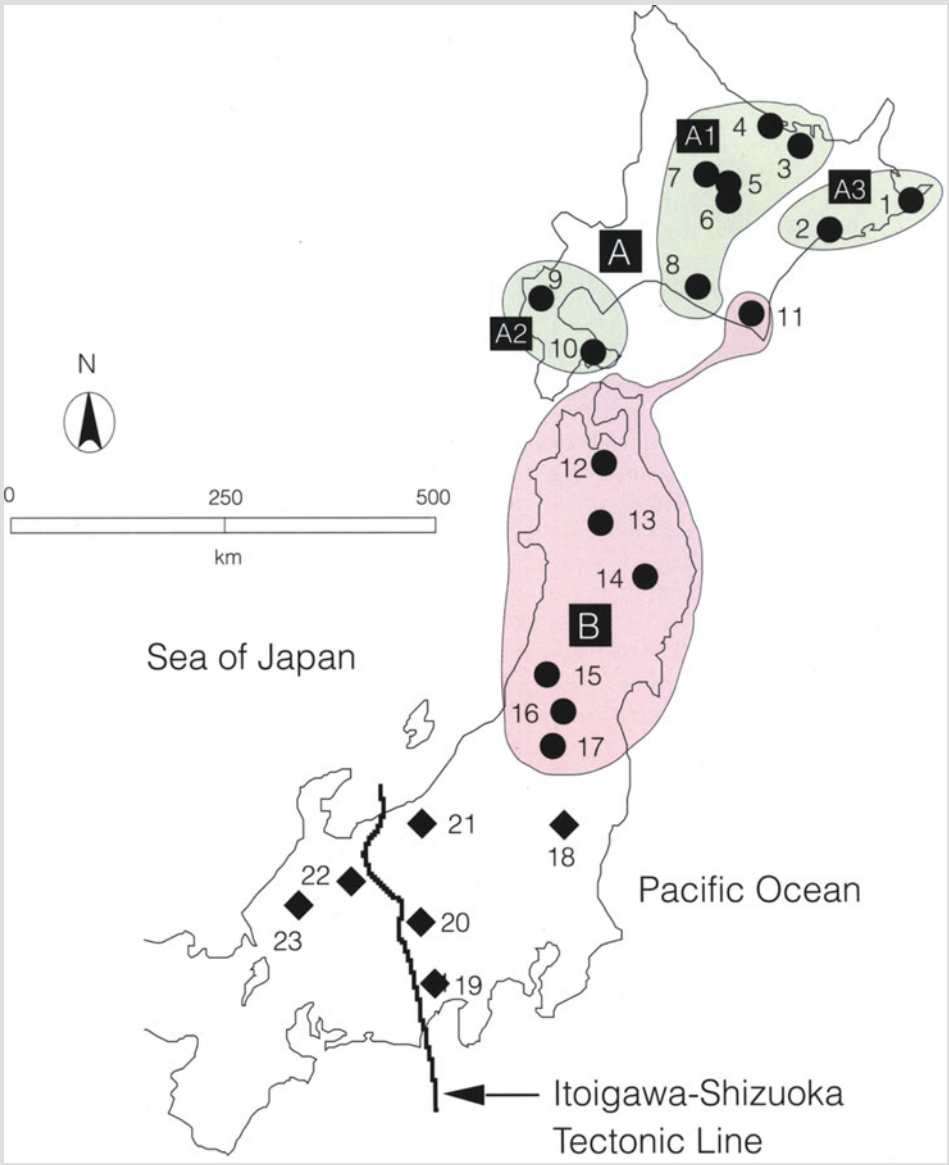


FIG. 7.18. Locality map of *Tomocarabus* specimens. Locality numbers correspond to those shown in Fig. 7.17. **A** The Hokkaido lineage and **B** the Honshu lineage of *T. opaculus*, respectively. **A1**, **A2**, and **A3** denote the three geographically linked sublineages within the Hokkaido lineage. **Circles** *T. opaculus*, **diamonds** *T. harmandi* (after Su et al. 2000c)

as ssp. *kurosawai* (Breuning 1957), which is probably a mountainous form of the nominotypical *opaculus* (Nakane 1977).

An *ND5* phylogenetic tree was constructed for 19 examples of *T. opaculus* from various localities in Hokkaido and Honshu with a view to gaining a better understanding of the relationship between the Hokkaido and the Honshu populations.

Two major lineages of *T. opaculus* can be identified on the phylogenetic tree (Fig. 7.17). One (lineage A in Figs. 7.17 and 7.18; loc. nos. 1–10) is exclusively composed of specimens from Hokkaido, and the other (lineage B in Figs. 7.17 and 7.18; loc. nos. 11–17) is found throughout Honshu, with inclusion of a specimen from Samani-Cho, Hokkaido (loc. no. 11).

The lineage A is further divided into three sublineages, A1 (loc. nos. 3–8), A2 (loc. nos. 9–10), and A3 (loc. nos. 1–2), while all the sequences in the lineage B were very close to one another even in the specimens from Samani-Cho and Honshu.

Specimens belonging to A1 were all collected from central Hokkaido, A2 from the southern part of Hokkaido (Oshima Peninsula), and A3 from northeastern Hokkaido, suggesting geographically linked distribution of the three lineages (Figs. 7.17 and 7.18). An *ND5* phylogenetic tree of the Carabina around the world (see Chapters 4 and 5) suggests that the major genera radiated at 50–40 MYA. The origin of *T. opaculus* can be traced back to a little after this radiation. No other species that branched off from the *T. opaculus*

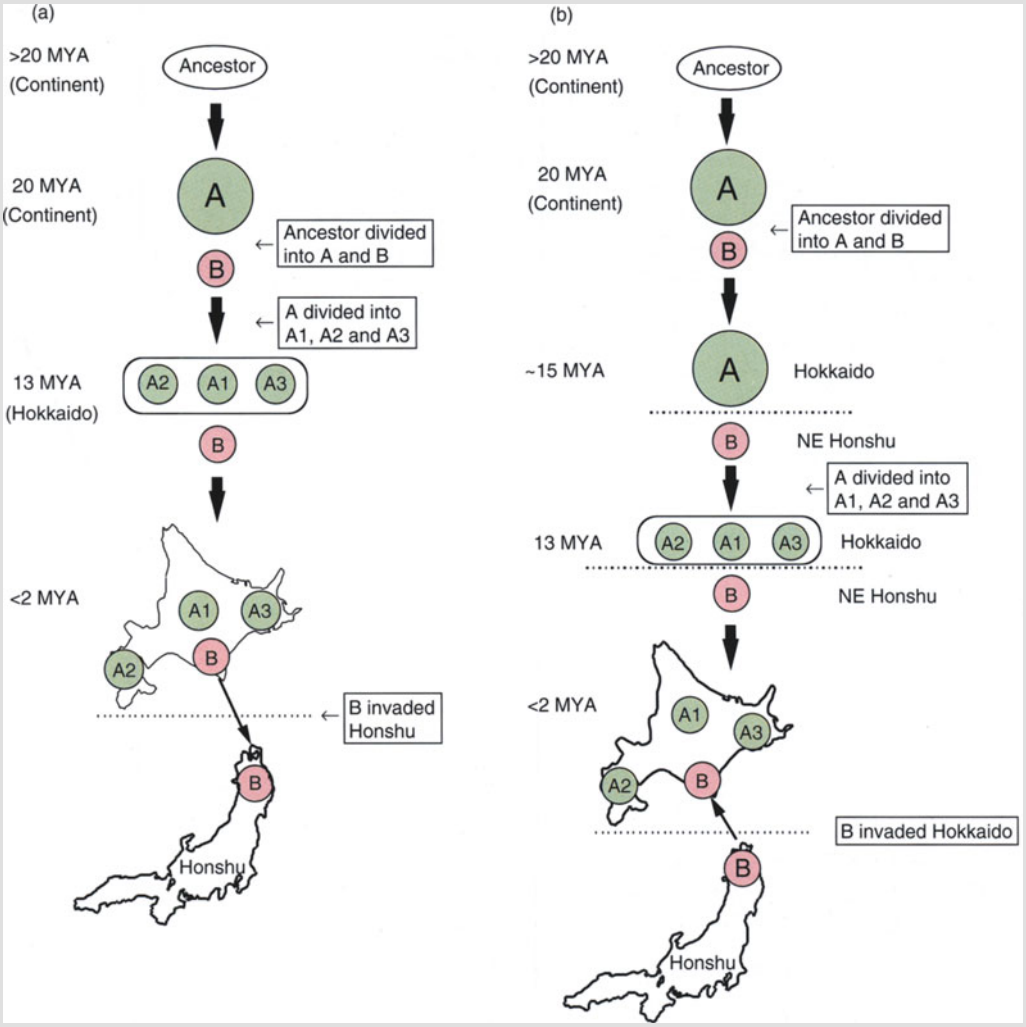


FIG. 7.19. Origin of *Tomocarabus opaculus*

lineage have been found in examinations of a number of species belonging to *Tomocarabus* and its allied genera in the world (see Chapter 8).

Separation of A and B took place ca. 20 MYA, followed by diversification of A into A1, A2, and A3 ca. 13 MYA in Hokkaido, while diversification of B started as recently as 2 MYA.

The present distribution pattern of *T. opaculus* in the Japanese Islands can be explained in several ways (Fig. 7.19). The first possibility (Fig. 7.19a) is that the separation of A and B (20 MYA) occurred considerably earlier than the split of the Japanese Islands from the Eurasian Continent (15 MYA), suggesting A and B separated from each other on the continent before the Japanese Islands separated from the continent.

Since A1, A2, and A3 are clearly separated from one another geographically, each of them would have been isolated during the formation of Hokkaido. Concerning the habitat of the ancestor of B in the ancient Japan area on the continent, two possibilities may be considered. One is that the ancestor of B inhabited a part of the ancient Hokkaido area and has been isolated until recently. During the glacial era, B migrated to Honshu via a land bridge and rapidly spread over the northeastern half of Honshu.

An alternative possibility (Fig. 7.19b) is that the habitat of the ancestor of B was the ancient northern Honshu area, and it has been geographically isolated in a restricted region of Honshu until recently, and then started to propagate rapidly in northeastern Honshu. At about the same time, B migrated to the Samani-Cho area. There is not enough data available at present to allow us to choose between these two alternatives.

As mentioned above, *T. opaculus* started to diversify about 20 MYA, corresponding to about one half the history of carabine evolution. This would mean that the morphology of *T. opaculus* has remained almost unchanged for a long time (silent evolution; see Chapter 8).

The *ND5* sequences of *Tomocarabus harmandi* from several localities were taken as an outgroup in the phy-

logenetic tree in Fig. 7.17. This species is found in central and northern Honshu and is classified into 13 subspecies (Ishikawa 1986; Imura and Mizusawa 2002). The origin of *T. harmandi* is as venerable as *T. opaculus*. No other species have been found in the *T. harmandi* lineage. The tree suggests that there are two major clusters of *T. harmandi*, the distribution ranges of which are separated by the Itoigawa-Shizuoka tectonic line (loc. nos. 22–23 for the western lineage and loc. nos. 18–21 for the eastern lineage). *Tomocarabus harmandi* is independent from *Leptocarabus* for the same reason as in *T. opaculus*.

Since the evolutionary history of both *T. opaculus* and *T. harmandi* are venerable and independent from each other, their taxonomic treatment should be reconsidered (see Fig. 5.19 and Chapter 9).

7.5 *Damaster blaptoides*

7.5.1 Overview

Damaster blaptoides is one of the most peculiarly shaped carabine beetles, (see Fig. 6.1, no. 18 on p. 92) characterized by an elongate body and slender mouth parts similar in appearance to those of the genus *Cychrus*. It is devoid of hind-wings and has a fused elytra so that it cannot fly. These beetles are admired by amateur entomologists and collectors because of their appearance and often-beautiful color.

Damaster is endemic to the Japanese and Kurile Islands. This species has been found throughout the Japanese Islands and the Kurile Islands, and is distributed from Brat Chirpoyev Island of northern Kuriles to Tanegashima and Yakushima of the Ohsumi Islands, south of Kyushu.

There is a long history of study of this species by many researchers. The *Damaster* beetles vary geographically in color and form and were initially classified into seven independent species. Because of the occurrence of intermediate forms or "supposed hybrids" between the "species" at their distribution boundaries, it has become generally accepted that they belong to a single species, *blaptoides*, under which eight to nine subspecies are arranged (see Fig. 7.22b).

The subspecies include *rugipennis* from Hokkaido, *viridipennis* from the northern area of the Tohoku District, *fortunei* from Awashima Island, *montanus* (=*babaianus*) from the southern area of the Tohoku District and Niigata Prefecture, *capito* from Sado Island. Other subspecies are *cyanostola* from the southern periphery of the distribution range of *montanus*, *oxuroides* from the Kanto and Chubu Districts, *brevicaudus* from the Oki Islands, and *blaptoides* from the Kinki, Chugoku, Shikoku, and Kyushu Districts.

The outline of the above classification was set out by Nakane (1960–1963). Following this, Nishikawa and Okumura (1971) proposed five subspecies (*blaptoides*, *oxuroides*, *fortunei*, *capito*, and *rugipennis*), by including *cyanostola* in *oxuroides*, and *viridipennis* and *montanus* (= *babaianus*) in *fortunei*. In addition, many geographic races and mountainous forms were recognized in *blaptoides, oxuroides,* and *fortunei*. Ishikawa (1985, 1988, 1991) recognized *cyanostola* as a good subspecies, and Imura and Mizusawa (1995) added subsp. *brevicaudus* from the Oki Islands. In addition to the above, ssp. *hanae* was described from Taiwan, probably on an individual accidentally introduced to that island. *Damaster* is not distributed in the Ryukyu Islands.

Generally speaking, the northern subspecies are characterized by a greenish or violet metallic tint of body above, presence of ventral hair-pads on male protarsi, and a rudimental protrusion of elytral apical edges (mucrones). These characteristics change gradually, i.e., from bright to black in color, from the presence to absence of protarsi hair-pads, and from rudimental to well-developed mucrones, along with the distribution ranges of the subspecies from north to south.

This rule has some exceptions, however, especially for the inhabitants of smaller islands such as Sado and Oki, which have rudimentary or poorly developed mucrones irrespective of the place their habitat takes in the north-south axis. The classification and phylogeny of *Damaster* based on morphology and distribution still involve considerable ambiguity.

7.5.2 Speculations on the Origin and Formation of the Present Habitat

The origin of the *Damaster* beetles is not yet satisfactorily understood. According to Sakaguti (1980), there exist several hypotheses (speculations in our opinion) that suggest *Damaster* originated from *Acoptolabrus*-type and/or *Coptolabrus*-type ancestors. The predominant opinion is that *Coptolabrus* (found in Korea and mid-southern China) could be the ancestor of *Damaster* (Nakane 1960; Ishikawa 1986a, 1989), as inferred from cladistic analyses using morphological characters (Ishikawa 1986a).

The proto-*Damaster* has been presumed to have migrated into Japan across a land-bridge from Korea before the last glacial era and to have arrived in northern part of Japan (<2 MYA), where the direct ancestor of *Damaster* took shape. It then spread southwest until the present distribution ranges of the respective subspecies were established (Ishikawa 1989).

This view is based on the assumption that the opportunity that enabled the continental carabids to migrate to Japan must have existed during the glacial era, when Japan was connected to the continent, and, according to Ishikawa (1989), the northern subspecies reveal more "ancestral" characters as compared with the southern subspecies.

Assuming a *Coptolabrus*-origin for *Damaster*, the proto-*Damaster* moved northeastward from the Korean Peninsula or nearby regions, and invaded Hokkaido <20 MYA. This "ancestral" form then spread southwest and finally reached Kyushu.

Another possibility is that *Damaster* was derived from *Acoptolabrus* in Sakhalin or in the northwest periphery of the Eurasian Continent. Still another possibility is that the northern *Damaster* subspecies emerged during the glacial era from a particular northern *Acoptolabrus* species, while the southwestern ones came from *Coptolabrus* inhabiting the Korean Peninsula or nearby regions including the Tsushima Islands (Tamanuki 1972).

Looking at this from an entirely different viewpoint, Nakane (1993) opines that since the Japanese Islands constituted part of the eastern periphery of the Eurasian Continent >15 MYA, it may be assumed that the proto-*Damaster* migrated to Japan at that time from the ancient Japan area of the continent, followed by establishment of various subspecies by geographic isolation.

It is impossible to draw a clear conclusion as to the origin of *Damaster*, because of the lack of substantive evidence.

7.5.3 Origin, Distribution, and Classification Based on Mitochondrial DNA Phylogeny

Specimens of *Damaster* (s. str.) were collected from various parts of the Japanese Islands (Fig. 7.22a) for construction of an *ND5* phylogenetic tree, showing the presence of two major lineages, the eastern (E) and western (W) (Figs. 7.20 and 7.21).

The lineage E includes three sublineages, found in Hokkaido (hereafter HKD), northern Tohoku (hereafter NTK), and southern Tohoku (hereafter STK). This view is supported by high bootstrap values in the phylogenetic tree. HKD is found throughout Hokkaido and the northern edge of the Tohoku District, Honshu.

The specimens from Hokkaido formed a subclade (HKD1; loc. nos. 1–7 in Figs. 7.20 and 7.22), and the sequences of eight specimens from various localities in Hokkaido and the Kurile Islands were seen to be very close to one another (0.0–1.0%). The specimens from the northern edge of Honshu (HKD2; loc. nos. 8–11) are rather remote from each other when compared with those belonging to HKD1, and are not clustered into a single group (Figs. 7.20 and 7.22). This suggests that the ancestor of HKD1 had diverged from HKD2, and then migrated to Hokkaido, but the opposite is not likely to have occurred, i.e., there is no evidence of a migration from Hokkaido to Honshu.

With a view to gaining a clearer understanding of the distribution boundary between HKD and NTK, the *ND5* gene sequences from specimens from the Tohoku District were analyzed (Fig. 7.23). The results point to the existence of a boundary between HKD (loc. nos. 8–23 in Fig. 7.23b) and NTK (loc. nos. 23–30), running along the Yoneshirogawa River in northern Akita Prefecture and the Mabechigawa River in southern Aomori Prefecture (Y-M line).

The existence of the Y-M line does not, however, necessarily mean that a common ancestor once inhabiting one region was divided into two races by the river barrier. Perhaps the archipelago formation of the proto-Japanese Islands about 13 MYA resulted in isolation of proto-HKD and proto-NTK in separate isolates. Following this, the two races expanded their distribution upon an extensive upheaval of the Tohoku District until the river barrier divided them.

The tree shows that the diversification of HKD into several clades occurred at a relatively early time. The subspecies *rugipennis* belongs to one of the clades (Fig. 7.23). It is notable that two specimens of *viridipennis* we examined from Kodomari in the Tsugaru Peninsula (loc. nos. 8 and 9 in Fig. 7.23) are closely related to *rugipennis* on the tree, suggesting that an ancestor inhabiting the northern edge of the Tsugaru Peninsula migrated to Hokkaido and then the Kurile Islands via a land bridge, followed by isolation of the Hokkaido/Kurile populations from the Tsugaru population upon formation of the Tsugaru Straits.

The population of the Shimokita Peninsula would not have directly participated in the migration as shown by the presence of *rugipennis* from Hokkaido on the tree. The specimens from there (loc. nos. 10–11 in Fig. 7.23) are rather remote from *rugipennis* from Hokkaido on the tree.

In STK, the populations of Awashima Island and Sado Island have been treated as belonging to ssp. *fortunei* and ssp. *capito*, respectively, because of their morphological differentiation, particularly in *capito*, compared with examples of *montanus* inhabiting southern Tohoku in mainland Honshu. According to the molecular phylogenetic data, *fortunei* and *capito* fall out in the STK clade and are indistinguishable from *montanus*.

The specimens from the northern part of the Tohoku District (except the range of HKD2) belong to the NTK race (loc. nos. 12–17 in Fig. 7.22). The STK race occupies the southern Tohoku District (loc. nos. 18–23) including Awashima Island (loc. no. 24) and Sado Island (loc. no. 25) as mentioned above.

The lineage W includes five sublineages (races), one inhabiting the Kanto District (hereafter KTO), one found in the Chubu District (hereafter CBU), one in the Kii Peninsula, south of Kinki District (hereafter KII), one in western Japan (WJN), and one in Kyushu (KYU). These findings are supported by bootstrap values of more than 94% in all the phylogenetic analyses undertaken.

The sublineage KTO (loc. nos. 26–41) is found in the south of the STK range with a western limit roughly set by the Itoigawa-Shizuoka tectonic line. The distribution

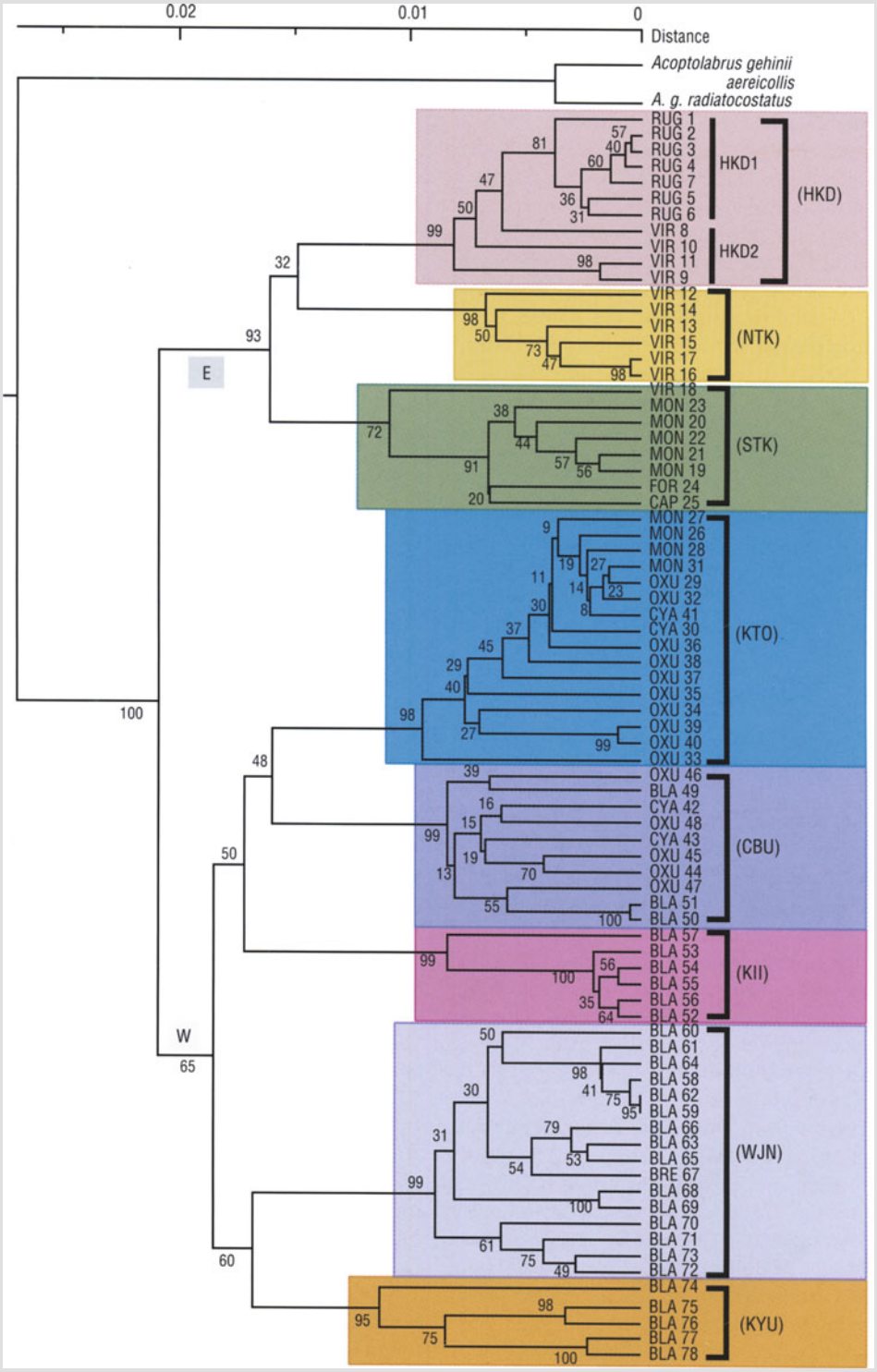


FIG. 7.20. Phylogenetic tree of the mitochondral *ND5* gene for the genus *Damaster*. Constructed using the UPGMA. The three-letter code and the number that follows correspond to the scientific name (by morphology) and the locality number in Fig. 8.22. **RUG**, *rugipennis*; **VIR**, *viridipennis*; **MON**, *montanus*; **FOR**, *fortunei*; **CAP**, *capito*; **BLA**, *blaptoides*; **BRE**, *brevicaudus*. Sublineage symbols are shown at the right of the tree (after Su et al. 1998; modified)

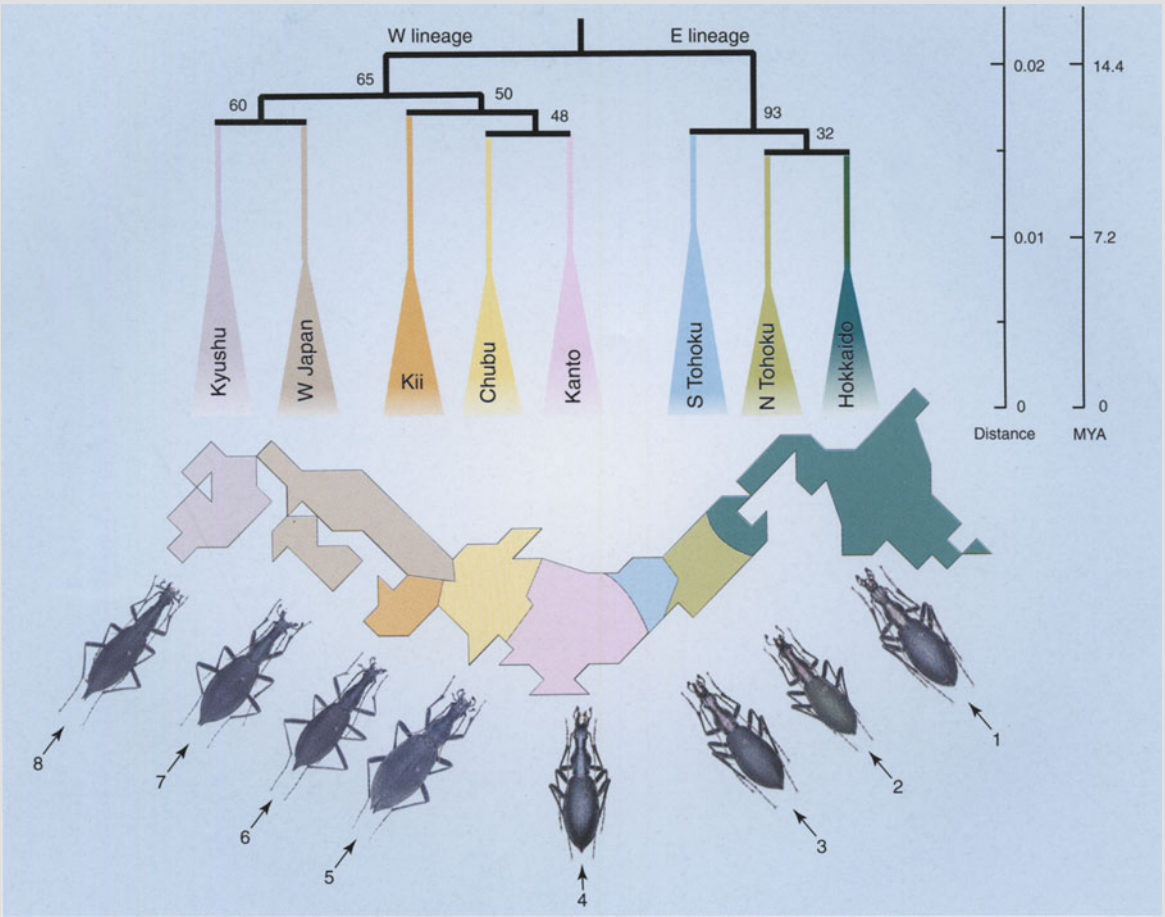


FIG. 7.21. Schematic illustration of Fig. 7.20

range of CBU (loc. nos. 42–51) is sandwiched by KTO and two other sublineages, KII and WJN. The sublineage KII (loc. nos. 52–57) is found in the Kii Peninsula and is sharply isolated from WJN (loc. nos. 58–73) by the Yodogawa River–Biwako Lake line, with the northern limit being south of Biwako Lake.

The inhabitants of the Chugoku District including the Oki Islands (loc. no. 67), and Shikoku (loc. nos. 71–73) belong to WJN, separated by the sea from those in the Kyushu District (KYU; loc. nos. 74–78), which include specimens from mainland Kyushu (loc. nos. 74–77), the Goto Islands (loc. no. 76), and Yakushima Island (loc. no. 78).

7.5.4 Hypothesis on the Origin and Diversification of *Damaster*

A definite dating of the *Damaster* emergence is difficult, because the rate of base substitution of the *Damaster ND5* gene is not definitely known. The paleomagnetic evidence indicates that ancient Japan separated from the Eurasian Continent about 15 MYA, followed by separation of the northeast and southwest Japan arcs as a consequence of the double-door opening of the Sea of Japan (Fig. 7.6; see also p. 25) (Otofuji et al. 1991, 1994).

Since *Damaster* is almost strictly endemic to the Japanese Islands, it is not unreasonable to assume that the proto-*Damaster* that inhabited the ancient Japan area of the continent was divided into two races that became geographically isolated, i.e., one in the east and another in the west upon the double-door opening of the Sea of Japan, resulting in the W and E *Damaster* lineages.

This suggests that the diversification of *Damaster* began about 15 MYA. The diversification of *Ohomopterus*, the other carabid group endemic to the Japanese Islands, started a little later than that of *Damaster* (Fig. 7.6) (Su et al. 1996c), and a 9 MYR-old *Ohomopterus* fossil was discovered (Hiura 1965). Thus, the notion that *Damaster* emerged 15 MYA may be close to the truth.

Shortly after the double-door opening of the Sea of Japan, the proto-Japanese Islands became an archipelago as a result of an extensive submergence, especially of the northeast arc. This would have caused the geographic isolation and independent evolution of proto-*Damaster* that had survived on the respective islands. This may have led to the differentiation into the eight

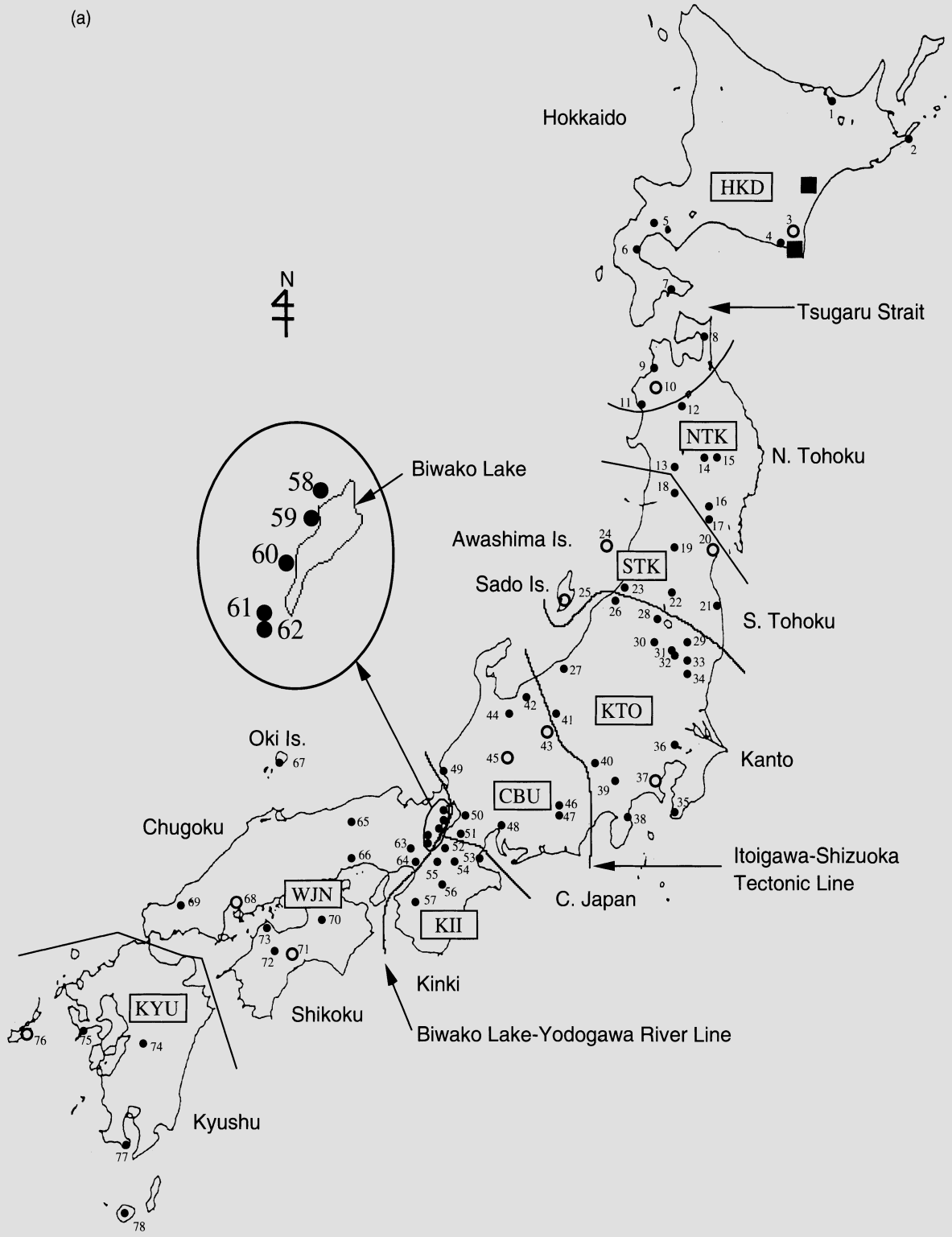


FIG. 7.22 (a). Specimen locations and distribution map of the *Damaster* races based on the phylogenetic tree of the mitochondrial *ND5* gene. **Open circles**, localities of specimens analyzed. **Squares**, localities of two *Acoptolabrus gehinii* subspecies used as an outgroup for phylogenetic analysis. Locality numbers correspond to those in Fig. 7.20. The boxed three-letter code represents the race based on the tree. See the text (after Su et al. 1998).

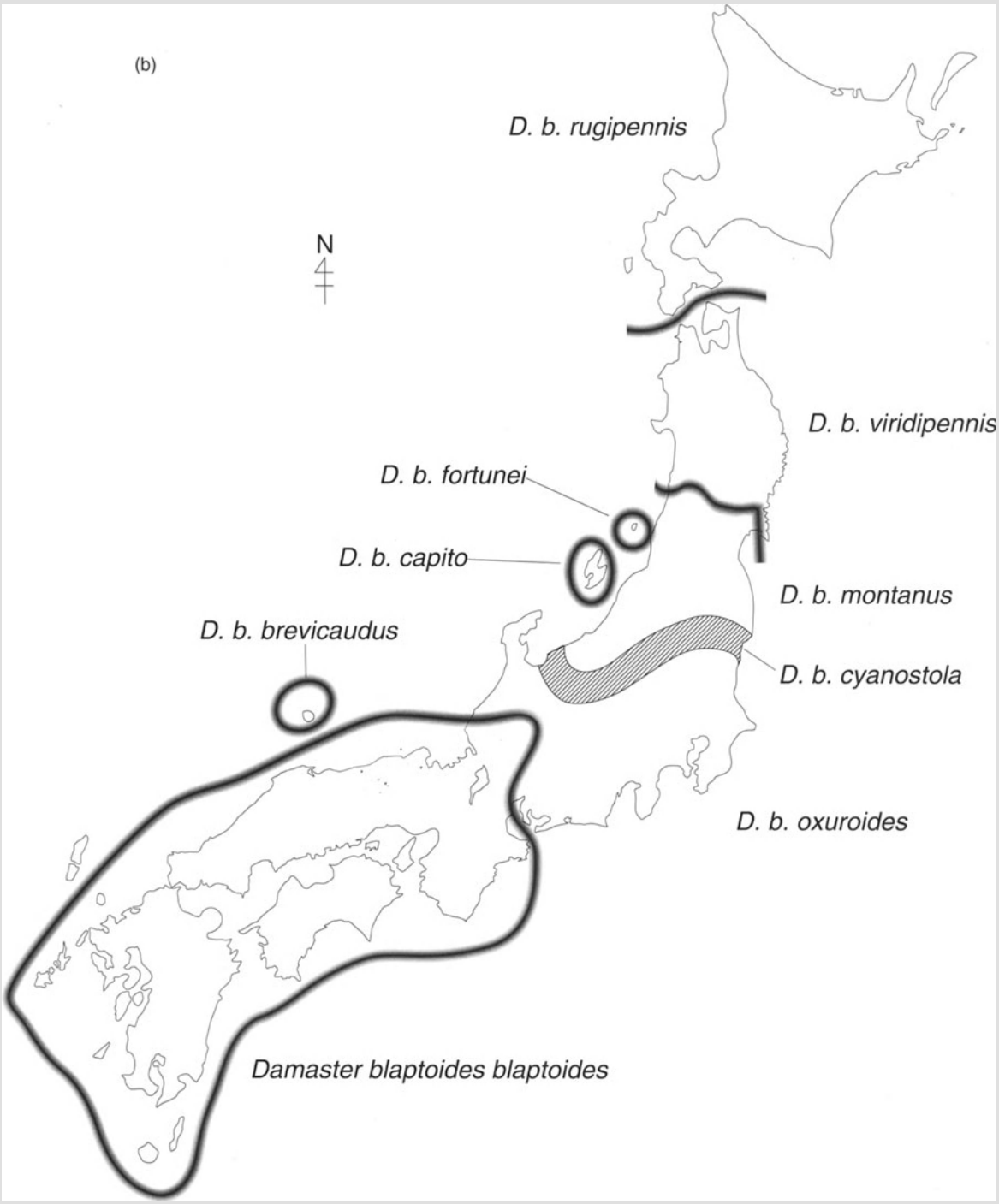


FIG. 7.22 (**b**). Subspecies classification of *Damaster* by morphology. The subspecies names are indicated (after Su et al. 1998; modified based on Ishikawa 1991)

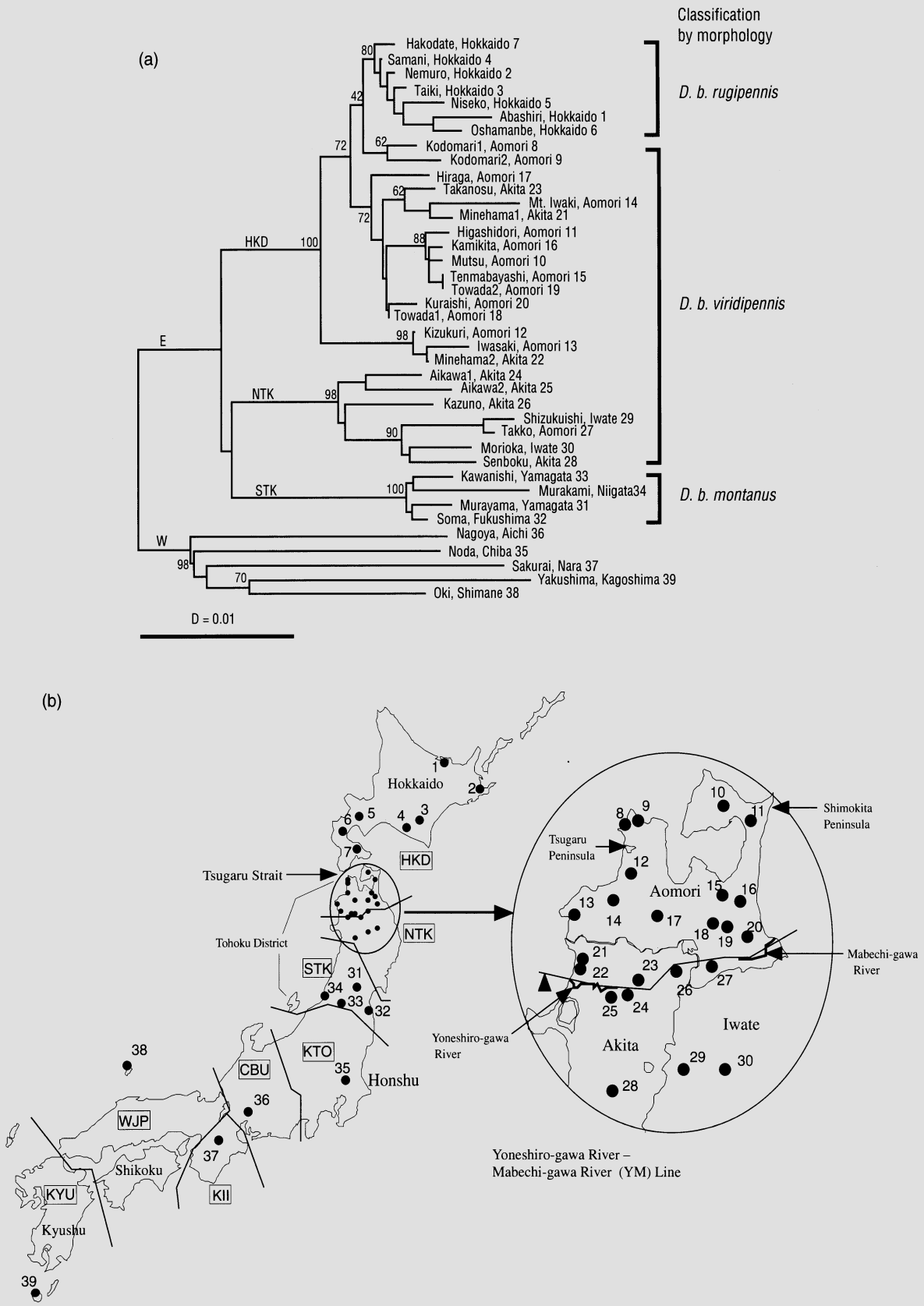


FIG. 7.23. Phylogenetic tree of the mitochondrial *ND5* gene for the eastern Japan lineage (E) of *Damaster* with some species of the western Japan lineage (W), constructed using the NJ-method (**a**). Locality map of the *Damaster* specimens (**b**). Locality numbers correspond to those shown in (**a**). For boxed three-letter codes showing the geographic races (sub-lineages) and the distribution boundaries of the races, see Fig. 7.22a (after Kim et al. 1999a)

Damaster races that currently exist in Japan. The exact identification of the location inhabited by each race prototype is difficult, because of the still somewhat ambiguous geohistorical record of the Japanese Islands.

Figure 7.24 presents a map of what the Japanese Islands may have looked like about 15 MYA, and includes a hypothetical placement of each of the race prototypes (in some other maps, the habitats of KYU and WJN are completely fused, and that of HKD is in the sea).

Following the formation of an archipelago, an extensive upheaval started about 9–6 MYA, and the present form of the Japanese Islands was finally established. The upheaval enabled the respective races to spread to new habitats. The spread of each race may be estimated to have initiated about 8–5 MYA, which is not far from the time of the upheaval initiation. This expansion of the distribution range continued until it came to halt by various barriers such as straits, tectonic lines, rivers, and mountains, etc.

Reproductive barriers might also have been generated between two or more races when they came into contact with each other. The present geographically linked distribution of the *Damaster* races could be a result of such events. The scenario presented above should be regarded as only one of several possibilities, but agrees in principle with the ideas put forward by Nakane (1993).

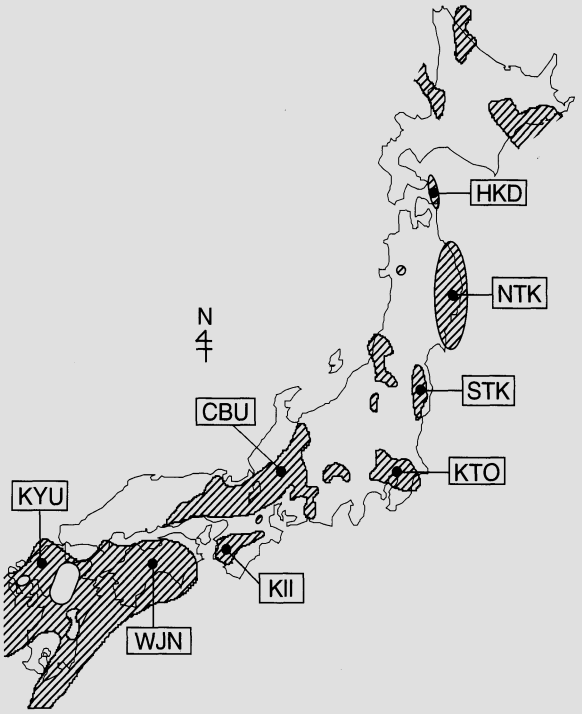


FIG. 7.24. A map of the ancient Japanese Islands (archipelago) on which the presumed habitat of each race is indicated (after Su et al. 1998)

7.5.5 Comparison of *ND5* Genealogical Tree and Morphological Subspecies Classifications

The subspecies classification of *Damaster* (s. str.) based on morphology is shown in Fig. 7.22b (Ishikawa 1991, with modifications). As noted above, the classification of subspecies is based mainly on the presence or absence of ventral hair-pads on the male protarsi, the extent of protrusion of the elytral apical edges, and body coloration.

On an *ND5* tree, there exist eight easily recognizable and geographically linked clades (Fig. 7.22a). Comparisons of Fig. 7.22a and b make it clear that the morphological subspecies deviate from the races indicated by molecular phylogenetic analysis, and the distribution boundaries settled for the subspecies do not coincide with those presented by phylogenetic examination.

The HKD2 and NTK (Figs. 7.20 and 7.23) have been collectively treated as *viridipennis*, while HKD1, which corresponds to *rugipennis* in Hokkaido, is separated from *viridipennis* by coloration, rudimentary mucrones, the elytral shape and some other minor morphological characters. The molecular data indicates that HKD1 and HKD2 belong to the same clade, whereas NTK is in an independent line from HKD. Perhaps, an ancestor in northern Tohoku migrated to Hokkaido via a land bridge, followed by geographic isolation of HKD1 and HKD2 upon formation of the Tsugaru Straits.

It is notable that the population of *viridipennis* in Aomori Prefecture (the distribution range of HKD2) is morphologically similar to *rugipennis* (= HKD1). Indeed, Nakane (1960) pointed out "The difference between subsp. *rugipennis* from Hokkaido and *viridipennis* from Aomori or Iwate is not particularly remarkable." Yamazaki et al. (1989) also pointed out that *rugipennis* and *viridipennis* (particularly from Aomori Prefecture) are morphologically similar. The very close *ND5* gene sequences of HKD1 from various localities in Hokkaido suggest a fairly recent and rapid expansion of distribution of HKD1.

In STK, the populations of Awashima Island and Sado Island have been treated as ssp. *fortunei* and ssp. *capito*, respectively, because of the morphological differentiation, above all in *capito* compared with *montanus* (=*babaianus*) inhabiting southern Tohoku. The difference between *fortuni* and *montanus* is not remarkable, however. In terms of molecular phylogeny, *fortunei* and *capito* fall out in the STK clade and are indistinguishable from *montanus*. Presumably, a rapid morphological differentiation has taken place in these small, isolated populations, especially on Sado Island.

The distribution boundary between STK and KTO shifts to the north to a considerable extent compared with that between *montanus* and *oxuroides* based on morphology. This means that KTO includes a part of so-called *montanus* and *oxuroides*. *Oxuroides* is said to show some morphological variations within central

Japan. The present analysis shows that what taxonomists call *oxuroides* can be clearly separated into two clades, KTO (partly including *montanus* as noted above), and CBU. *Cyanostola*, which is distributed along the boundary of *montanus* and *oxuroides* (Fig. 8.22), has been treated as a hybrid between the two subspecies. Since specimens identified as *cyanostola* appear in the mountainous area of both KTO and CBU, and their distribution range does not correspond to the boundary between either STK and KTO, or KTO and CBU, the hybrid hypothesis is not likely to be correct. The so-called *cyanostola* may very well be simply a mountainous form of both KTO and CBU.

KII, WJN, and KYU have been treated as a single subspecies, *blaptoides* (except *brevicaudus*, see below), because of the limited morphological difference among them. This suggests that, in spite of the long independent evolutionary histories of KII, WJN, and KYU, no conspicuous morphological differentiation took place since they separated from a common ancestor. *Brevicaudus* in the Oki Islands is differentiated from the mainland population by its smaller size, more robust pronotum, shorter and more robust elytra with shorter mucrones, etc. (Imura and Mizusawa 1995). *Brevicaudus* is phylogenetically indistinguishable from WJN in mainland Honshu. This is another good example of rapid morphological differentiation in a small, isolated population, as seen in the inhabitants of Sado Island (*capito*) and Awashima Island (*fortunei*).

One might argue that the morphological subspecies are real entities and that the *ND5* sequence of, for example, a part of the KTO population (morphologically identifiable as *montanus* and not *oxuroides*) may be nothing but the result of horizontal transfer of *oxuroides* mitochondria into *montanus* as a consequence of hybridization.

A similar possibility exists for WJN, KII and CBU, or STK and NTK. This possibility is highly unlikely, however, because of the deep and almost identical branching points of the respective races that have been identified by *ND5* genealogy, and the rather sharp distribution boundaries separating the races.

The classification of *Damaster* relying on morphology and distribution range is based on several rather vague characteristics that results in the classification of several groups as subspecies, and not as species. Indeed, individual and regional variations within some morphological subspecies are fairly large, so that distribution boundaries cannot be clearly established in some cases. Furthermore, the rate of morphological change is affected by population size and other factors, changes that would have been considerable in apterous beetles such as *Damaster* and are such that they cannot be estimated by morphology alone.

This means that subspecies classification of *Damaster* from the phylogenetic point of view is hard to realize though the use of morphology alone. Instead, the mitochondrial DNA genealogy as expressed in a phylogenetic tree of the races (Fig. 7.22) indicates the existence of eight geographically linked races, the origin of each of which is relatively venerable, predating many other Japanese carabid species (Su et al. 1996a).

7.5.6 Taxonomy

How do we classify *Damaster* from the molecular phylogeny, distribution pattern, and morphology? The primary purpose of molecular phylogeny based on a DNA clock is to trace the routes through which the present-day organisms took form, not to settle taxonomic ranks such as genus, species, and subspecies.

The approach to taxonomy taken since the time of Linné has been based primarily on morphological characters, a technique that is prone to subjectivity because the "important" characters adopted as reference points are quite often in disagreement depending on taxonomists making the classification.

Compared with morphological classifications, population genetics presents the idea that "a species is defined as a population which owns the genetic pool (biological species)." In most cases, however, it is almost impossible to perform experiments over many generations under near-natural conditions for thousands of "species". Moreover, even under natural conditions, offspring derived from hybrids may become extinct through hybrid sterility.

Even if experiments covering successive generations are completed, the taxonomic status of the respective *Damaster* "subspecies" may not be made entirely clear (see below). There are several reasons for treating *Damaster* as a single species, including the morphological characters discussed in Section 7.5.1, which are apparently consistent with the existence of an intermediate zone between two "subspecies." This does not necessarily mean that *Damaster* consists of a single species, because the existence of the hybrid zone between two species is a well-known phenomenon, with occurrence of hybrids having intermediate morphological characters.

Another reason that *Damaster* is treated as a single species is the marked similarity of the male genital organ across all the "subspecies." More justification for the one-species approach is provided by the fact that offspring can be produced between individuals of nearby districts, and not between individuals from widely separated populations (Baba 1938). For example, a beetle from Hokkaido cannot copulate with one from Kyushu (note, however, that at least F1 can be produced even by the crossing of *D. blaptoides rugipennis* and *Acoptolabrus gehinii*, both from Hokkaido!).

Shirôzu (1981) states that if one assumes that Honshu and Shikoku were subjected to abrupt submergence by some geographic event, the Honshu/Shikoku popula-

tions of *Damaster* would have become extinct, leaving only the Hokkaido and Kyushu populations. Since mating between these two populations is not possible, the Japanese *Damaster* would be properly classified as belonging to two species (Shirôzu 1981). This discussion shows that there is no firm evidence that the Japanese *Damaster* represent a single species.

With the development of molecular phylogenetic procedures, discrepancies between the results of molecular phylogeny and those of morphological taxonomy have often resulted. Under these circumstances, a concept of "phylogenetic species" has been proposed by several researchers (Davis and Nixon 1992; Vogler and Desalle, 1994; Kim et al. 1999c). Even if two or more species are almost indistinguishable morphologically from each other, and yet belong to distinct phylogenetic lines, they may be regarded as "phylogenetic species". This is no doubt much more objective than the findings of morphological taxonomy.

There are, however, no generally acceptable criteria for defining the rank of species or genus by the magnitude of genetic distance. Moreover, it is in most cases impossible to relate the phylogenetic species with the biological species, unless appropriate and extensive crossing experiments are performed. This concept also affects our proposal of "silent evolution." How can we treat two or more lineages separated in the past from a common ancestor with morphology that has remained almost unchanged? (see Chapter 8)

Taking these limitations into account, we have undertaken classification of *Damaster* using the phylogenetic species concept. Should the descendants of the two lineages or eight sublineages of *Damaster* be treated as species or subspecies? Since the eight sublineages emerged at about the same time with their distinct distribution boundaries at present, we prefer to treat them as species rather than subspecies. Naturally, such a treatment is conventional and there is no definite evidence correlating these "phylogenetic species" with "biological species." Then, how do we treat "*capito*" from Sado Island, which is phylogenetically indistinguishable from "*montanus*" from Honshu, but are clearly different morphologically? It may be assumed that "*capito*" emerged in Sado Island as an outgrowth of "*montanus*" (see above). If this is the case, the Sado population may be regarded as a subspecies of "*montanus.*"

7.5.7 Phylogenetic Classification of *Damaster*

A summary of this subject is given in Table 7.4.

The Hokkaido sublineage (HKD) of the *Damaster* population is presumed to have originated from the northernmost population on Honshu. Despite this biological event, *rugipennis* in Hokkaido was described earlier (1861) than *viridipennis* from northern Honshu (1880), so that *rugipennis* should be used for the HKD lineage. When *Damaster* in Hokkaido is separated from the northernmost Honshu population, ssp. *rugipennis* for the former and ssp. *viridipennis* for the latter should be used, although such a treatment seems to have little significance.

The northern Tohoku sublineage (NTK) of *Damaster* is definitely different phylogenetically from HKD in which the northernmost population is included. The type-locality of *viridipennis* is "Awomori," presumably the areas of Aoni and the Tsugaru Peninsula, which are in the distribution range of HKD. Therefore, there is no available scientific name for NTK at present.

The southern Tohoku sublineage (STK) has a much narrower range than that of the morphologically defined subspecies, "*montanus.*" About 50% of the range for "*montanus*" belongs to that of the Kanto sublineage (KTO).

The *Damaster* populations in Awashima Island and Sado Island belong to STK. Thus, the specific name for STK should be *fortunei*, with *montanus* providing a synonym. As mentioned above, the Sado population is morphologically considerably different from the specimens of STK found in mainland Honshu, and may be discriminated from the nominotypical *fortunei* as ssp. *capito*. If the phylogenetic species concept is strictly applied, *capito* might be better treated as a form or morpha of *fortunei.*

The Kanto sublineage (KTO) includes mainly the *Damaster* population of the Kanto District. Considerable parts of what have in the past been referred to as

TABLE 7.4. A tentative plan of the classication of the genus *Damaster* based on the *ND5* gene sequence

Eastern Japan (E) lineage	Western Japan (W) lineage
1. *Damaster rugipennis* (Hokkaido sublineage; HKD) Hokkaido and Northern edge of Honshu	4. *Damaster oxuroides* (Kanto sublineage; KTO) Kanto District and Southern Tohoku District
2. *Damaster* sp. (Northern Tohoku sublineage; NTK) Northern Tohoku District	5. *Damaster paraoxuroides* (Chubu sublineage; CBU) Chubu District
3. *Damaster fortunei* (Southern Tohoku sublineage; STK) Southern Tohoku District including Awashima Island Subsp. *capito* Sado Island	6. *Damaster* sp. (Kii sublineage; KII) Southern Kinki District (Kii Peninsula)
	7. *Damaster lewisii* (Western Japan sublineage; WJN) Northwestern Kinki District, Chugoku District including the Oki Islands, and Shikoku
	8. *Damaster blaptoides* (Kyushu sublineage; KYU) Kyushu including the Goto Islands and Yakushima Island

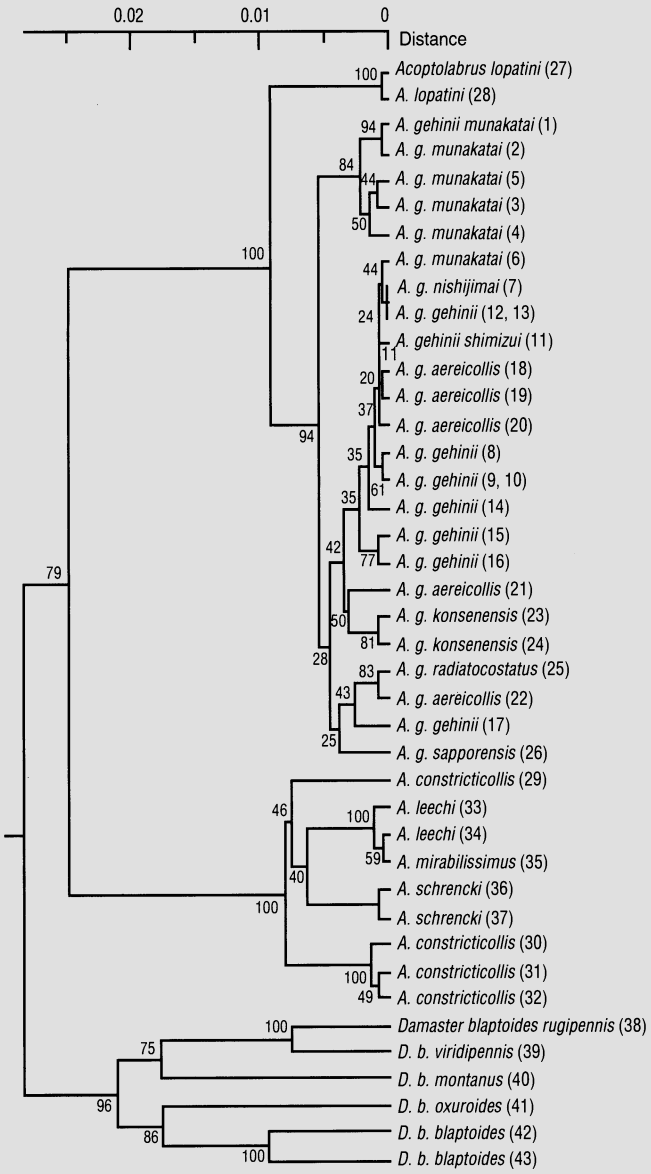


FIG. 7.25. Phylogenetic tree of the mitochondrial *ND5* gene for the genus *Acoptolabrus*. Constructed using the UPGMA (after Okamoto 1999)

"*montanus*" from northern Kanto, and a fraction of "*cyanostola*" also belong to KTO. The distribution range of "*cyanostola*" which was believed to occupy a hybrid zone between "*montanus*" and "*oxuroides*" is situated at the center of the KTO range, suggesting that "*cyanostola*" could be a mountainous form of KTO (see above), rather than a hybrid. "*D. oxuroides*" from the Chubu District belongs to the Chubu lineage (CBU; see below) and not to KTO. It may be appropriate therefore to use the specific name *oxuroides* to refer to KTO.

The Chubu sublineage (CBU) of *Damaster* have in the past been identified as "*oxuroides.*" However, the type locality of *oxuroides* is assumed near Yokohama, which belongs to KTO. Therefore, *paraoxuroides* named for specimens in the vicinity of Nagoya should be used for CBU, which includes part of what has been identified as *blaptoides blaptoides* and *cyanostola.*

The Kii sublineage (KII) constitutes of a part of what has been included in *blaptoides blaptoides.* One might argue that the KII individuals are a hybrid of *blaptoides blaptoides* and "*oxuroides*" found in the Chubu District (= CBU). This is, however, unlikely because the branching between CBU, KII, and WJN (see below) is old and occurred almost simultaneously. If KII were of hybrid origin, it would have branched off from CBU, or WJN, much later than the observed branching of CBU, KII, and WJN. There is no specific name available for KII at present.

The western Japanese sublineage (WJN) includes populations from the Chugoku District including the Oki Islands (= *brevicaudus*) and Shikoku. For the specific name, *lewisii* may be applied tentatively, because the name "*lewisii*" was given to the specimens from both Hyogo Prefecture and Shimabara (Kyushu) and we

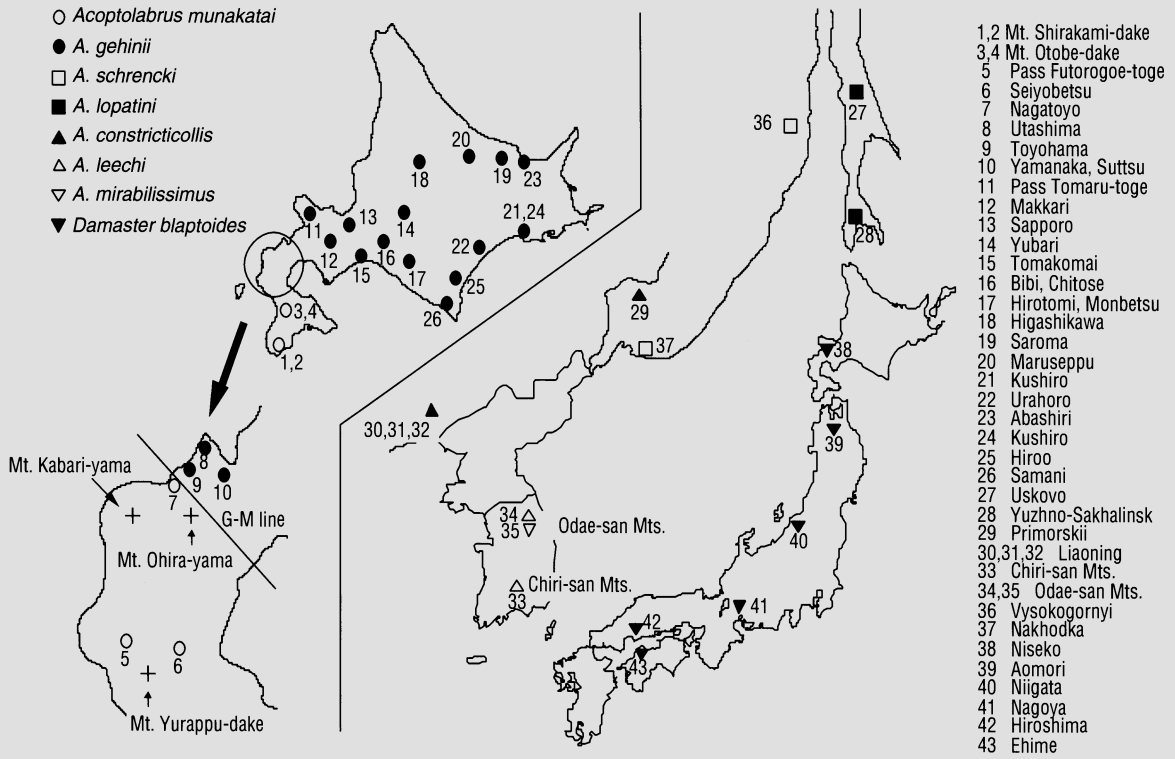


FIG. 7.26. Location map of the specimens used for Fig. 7.25 (after Okamoto 1999)

do not know the locality of the specimen to be designated as the lectotype. WJN seems to be somewhat related to KYU from Kyushu (see below) on the tree, but this is not definite.

The Kyushu sublineage (KYU) is found all over Kyushu and the adjacent islands including the Goto Islands and Yakushima Island. The type locality of *Damaster blaptoides* is probably somewhere in Kyushu, and therefore *blaptoides* may be given as the specific name for KYU.

7.6 *Acoptolabrus gehinii*

Acoptolabrus gehinii and *A. munakatai*, found in Hokkaido, are the most beautiful carabids in Japan and have generally been treated as two distinct species, although the latter is sometimes considered a subspecies of *A. gehinii*.

Because of the considerable geographic variations revealed by these two "species," many subspecies have been described for them. As was discussed in Chapter 5, *Acoptolabrus* from Hokkaido is much more closely related to *A. lopatini* from Sakhalin (loc. nos. 27–28 in Fig. 8.26) than to the morphologically similar *Acoptolabrus* species from the Eurasian Continent (loc. nos. 29–36) on an *ND5* phylogenetic tree (Fig. 7.25) (Okamoto 1999).

Despite considerable morphological diversity in such features as color pattern, the difference in the *ND5* gene sequence of many individuals from a wide variety of localities in Hokkaido (loc. nos. 1–26 in Fig. 7.26) is very small at less than 1% and in most cases 0–0.3% (Fig. 7.25). There are almost no differences in their ITS sequence (Okamoto 1999).

These results suggest that diversification started quite recently in Hokkaido with rapid morphological diversification. We were unable to ascertain whether the respective "subspecies" reflect the phylogeny, because of the small genetic difference between them. Whether *A. munakatai* is a distinct species or a subspecies of *A. gehinii* cannot be answered with the *ND5* tree, but it should be mentioned that diversification of these two took place very recently.

For details on *Carabus arvensis*, *C. granulatus*, *Hemicarabus tuberculosus*, and *Megodontus kolbei*, see Chapter 6.

Chapter 8
Pattern of Diversification: Evolutionary Discontinuity

8.1 Diversity of the Carabid Ground Beetles

As described in Section 5.4.2, Imura (1996) divided about 1,000 species belonging to the subtribe Carabina of the world into eight (sub)divisions and 94 (sub)genera, and Březina (1999) recognized 114 (sub)genera. These researchers reasoned that there was such a large number of genera primarily on the basis of the considerable morphological diversity of this group. Examining phylogenetic relationships by means of the mitochondrial gene sequence indicates, however, that there are a number of examples where morphologically similar carabids are phylogenetically quite remote from each other, while there exist also many cases in which phylogenetically close species differ in morphology to a considerable extent. The present chapter will deal with the origins of the morphological as well as phylogenetic diversity of the carabid beetles.

8.2 Radiation (Big Bang)

Figure 8.1 shows an *ND5* phylogenetic tree of the representative Carabina species. The branching order of various lineages is obscure because of short branch lengths with low bootstrap values. Unresolved branching orders near the root of the tree would not be the result of saturation of the base substitutions, because actual substitution percentages have a linear correlation to Kimura's evolutionary distance (*D*), the value corrected for multiple substitutions (Kimura 1980).

One plausible explanation is that the major Carabina groups radiated explosively over a short time, in something like a big bang, resulting in considerable morphological diversification among the descendant species. The radiation has been calculated to have taken place 40–50 MYA. Such a dramatic radiation may also be inferred in the evolution of the other ground beetles in the tribes, Cychrini and Ceroglossini, and the subtribe Calosomina.

As has been shown in Fig. 5.2, the Cychrini first separated into two lineages, followed by an extensive radiation into more than ten sublineages. In the Ceroglossini, three out of four lineages diversified about 25 MYA almost at the same time; they are *Ceroglossus buqueti*, the *C. darwini* species-group, and *C. suturalis* (Fig. 5.5). The hind-winged Calosomina from the Eurasian Continent, North and South America, and even from Australia and Indonesia (Timor) radiated into at least 16 lineages about 30 MYA (Fig. 5.7). The radiation of the Carabina, Calosomina, and Cychrini seems to have occurred between 30–50 MYA, roughly coinciding with the time at which the Himalayan Mountains took form as a result of the attachment of the Indian Subcontinent to the Eurasian Continent.

The magnitude of the morphological changes upon radiation is variable depending on the carabid group. It is most conspicuous in the Carabina, especially in the division Procrustimorphi.

Successive radiation after the big bang of the Carabina is seen in most of the divisions. This is best represented by the division Procrustimorphi. Shortly after the Carabina big bang, the Procrustimorphi again radiated into six geographically linked groups about 30 MYA (see Chapter 5). Among these groups, the Chinese group reveals the most remarkable array of successive radiations. Shortly after the first Procrustimorphi radiation, the Chinese group radiated into at least six clusters, followed by further radiation on a smaller scale (Fig. 8.2).

These radiations seem to have been accompanied by a considerable morphological diversification, as can be inferred from an examination of the descendant species. However, sometimes radiation was represented only by diversification of lineages without remarkable morphological changes. For example, eight lineages of *Damaster blaptoides*, which is endemic to the Japanese Islands, were geographically isolated and diversified without considerable morphological variation (see Chapter 7).

Neoplesius spp., which is found in mountainous areas of mid-western China, radiated into many species about 20 MYA with only minor morphological diversification (Fig. 5.37). Note, however, that such a species as *Eocechenus leptoplesioides*, which is different morphologically from *Neoplesius*, emerged upon the radiation with *Neoplesius* (see below). Thus, the radiation is represented by a series of lineage diversifications,

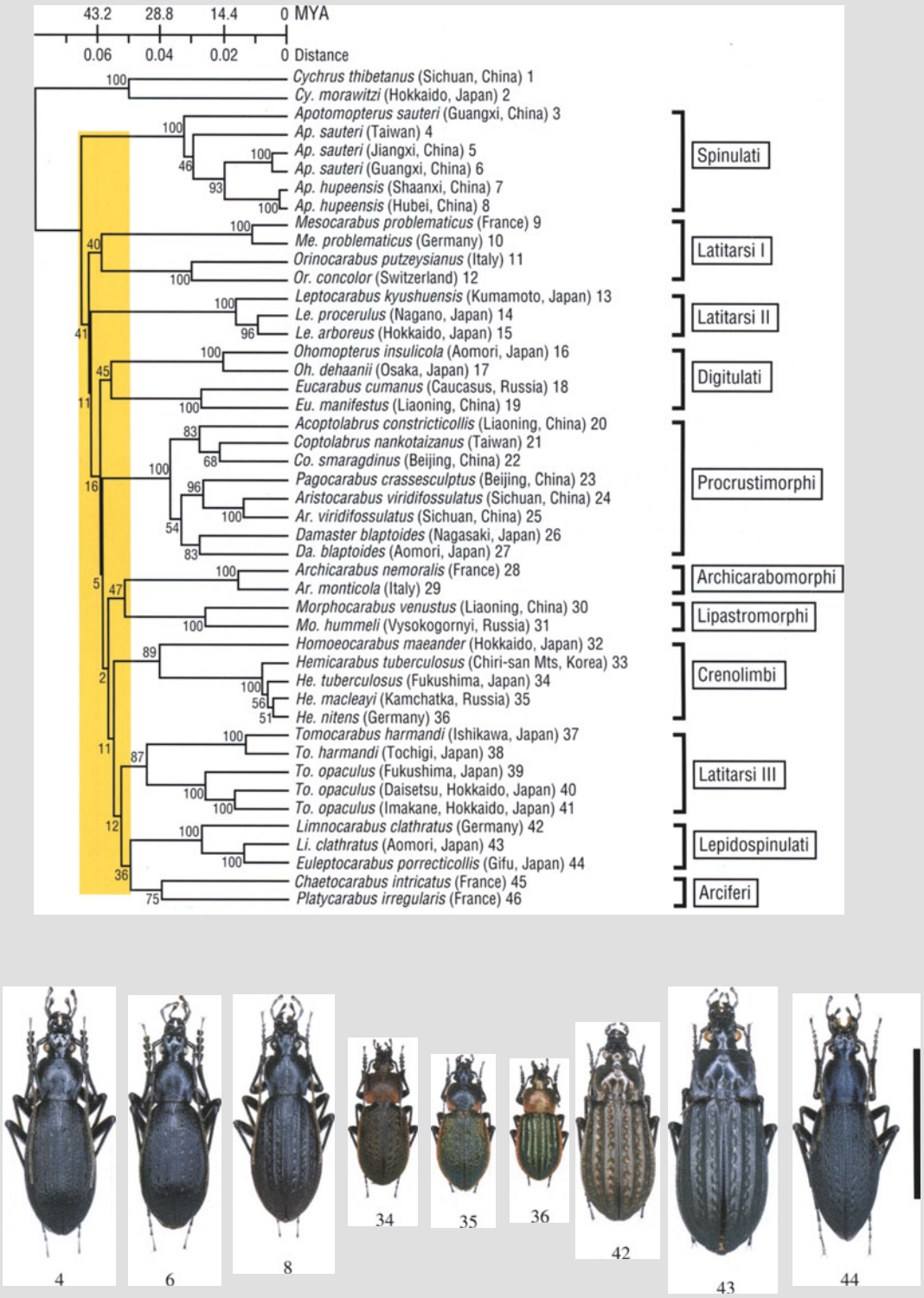


FIG. 8.1. Phylogenetic tree of the miotochondrial *ND5* gene for representative species of the Carabina. The taxonomic divisions are indicated on the tree. Two *Cychrus* species (tribe Cychrini) were taken as an outgroup. Constructed using the UPGMA. Taxonomic divisions roughly coincide with the lineages on the *ND5* tree, except that the Latitarsi are heterogeneous and separated into several lineages (see Chapter 9). The yellow shaded area indicates the radiation of the major groups. Photographs of the specimens are shown below the tree, and the numbers correspond to those following the scientific names on the tree. Scale bar = 20 mm (after Su et al. 2001)

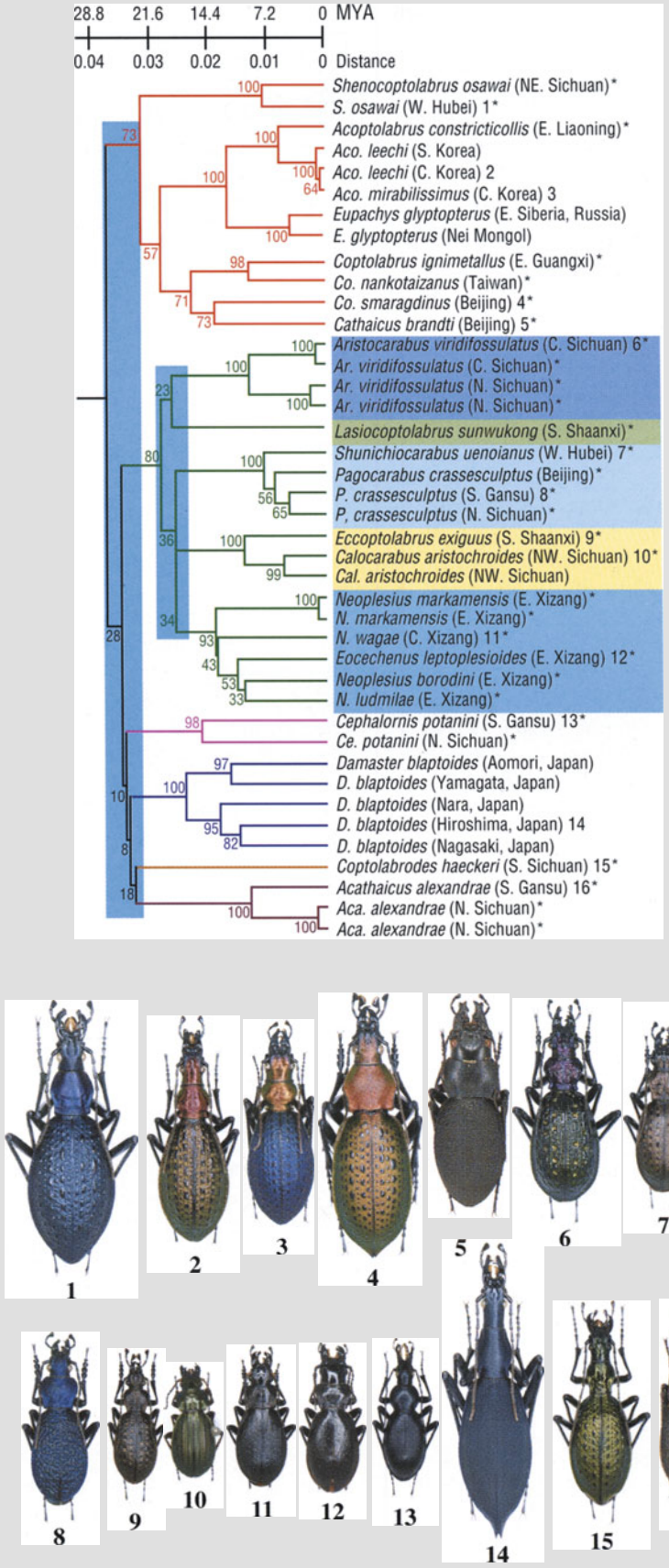


FIG. 8.2. Phylogenetic tree of the mitochondrial *ND5* gene for representative species of the Chinese group in the division Procrustimorphi. **Asterisks**, specimens from China. **Shadows** indicate radiation, which occurred twice in this group. Photographs of 16 species are shown below the tree. Scale bar = 20 mm (after Su et al. 2001)

sometimes with and sometimes without marked morphological transformation.

Many other examples of radiation have been recognized for various Carabina divisions. In the division Spinulati, four lineages emerged almost simultaneously about 35 MYA after the big bang, followed by occurrence of a smaller sized radiation in each lineage (Fig. 5.14). It is interesting to note that *Apotomopterus sauteri*, which consists of one of the lineages of the Spinulati, radiated about 20 MYA into three sublineages, from one of which a morphologically distinguishable species, *A. hupeensis,* branched off recently (see below).

In the division Lipastromorphi, one of the lineages radiated into at least nine groups after the initial cleavage of the ancestor into two lineages (Fig. 5.17). As was noted in Chapter 5, the division Latitarsi is most probably polyphyletic, and may be taxonomically divided into several divisions (see Chapter 9). The radiation of this division into many lineages may be traced back to the time of the big bang (Fig. 5.19a).

Diversification of most of the lineages in the Latitarsi took place in the form of radiation. For example, lineage W, which consists of most of the *Rhigocarabus* species, radiated into 13 sublineages about 30 MYA (Fig. 5.19c), and lineage X, containing several genera, began to diversify into 17 sublineages about 28 MYA. The genus *Tomocarabus* in lineage X radiated into ten species, and each of them corresponds to one sublineage (Fig. 5.19d).

In the division Digitulati, 6 lineages consisting of *Ohomopterus, Isiocarabus, Carabus,* and *Eucarabus* emerged at about the same time ca. 30 MYA.

All the examples mentioned above imply that radiations of various scale took place quite often in most of the carabid groups, suggesting that radiation is one of the general principles governing evolution of the carabid ground beetle.

Explosive radiation is known to have occurred during the evolutionary cycle of various biota, the Cambrian explosion of various animal phyla and the extensive diversification of cichlids in Victoria Lake and its satellite lakes in Africa (Stanley 1981) being well-known examples.

8.3 Discontinuous Evolution (Type-switching)

Radiation involves the occurrence of multiple lineage diversifications within a short time, presumably accompanied, in many cases, by morphological diversification. However, this can only be deduced by the morphology of the descendant species at the termini of branches on the phylogenetic tree. Strictly speaking, it is not logical to say that lineage radiation accompanied morphological change because it is possible that morphological change has gradually proceeded in each lineage, finally reaching its present state.

However, many examples presented here suggest that the observed morphological changes follow the divergence of the lineage, and each morphological change resulted from discontinuous transformation and not from gradual accumulation of small changes. Hereafter, we refer to discontinuous morphological transformation as "type-switching." Some attention will be given to the causes of radiation and discontinuous morphological changes at the end of this chapter.

An apparently discontinuous but singular emergence of a morphologically different species or lineage is occasionally identified in particular carabine groups. Following are some examples.

The diversification of three species in the Tianshanese sublineage of the division Procrustimorphi, two macrocephalic species, *Cratocechenus akinini*, *Cratocarabus jacobsoni*, and a normal-headed *Eotribax hiekei*, took place very recently as seen by the small difference in the *ND5* gene sequences (Fig. 8.3).

Hemicarabus tuberculosus (division Crenolimbi) is widely distributed throughout northeastern Asia including Japan, Korea, and Primorskij in Russia. Two other *Hemicarabus* species, *H. nitens* and *H. macleayi,* inhabit Europe and East Asia, respectively (Fig. 8.4). These three species, which are clearly distinguishable from one another morphologically, are very close in their *ND5* gene sequences (Fig. 5.12), suggesting their recent (rapid) morphological differentiation.

It is also the case for a macrocephalic Korean species, *Acoptolabrus mirabilissimus*, which is sympatric with *A. leechi,* with the latter having a normal head and almost the same *ND5* DNA sequence as *A. mirabilissimus* (Fig. 7.25).

As described in Chapter 5, many species of *Rhigocarabus* have been found in the mountains of western China. Their external morphologies are quite similar to one another. Among them, the male genitalia of *R. gigolo* is very different from that of other *Rhigocarabus* species despite a close similarity in other external morphology, and yet the *ND5* gene sequence of *R. gigolo* is identical to that of *R. buddaicus* (Figs. 8.5 and 5.19c).

"Rhigocarabus" choui inhabits the Qinling Mountains of Shaanxi, China, which are remote from the major distribution range of other *Rhigocarabus* species. In the *ND5* phylogenetic tree, this species does not belong to the *Rhigocarabus* cluster, and is closely related to the members of *Qinlingocarabus* in the Qinling Mountains, above all to *Q. blumenthaliellus* (Fig. 5.19b).

Most of the *Qinlingocarabus* species are black in color, have fine linear stripes on the elytra and are immediately distinguishable from the *Rhigocarabus* species. Despite an apparent similarity in the external structure to other *Rhigocarabus* species, the male genitalia of *choui* is definitely of the *Qinlingocarabus*-type (Fig. 8.6), suggesting the recent occurrence of

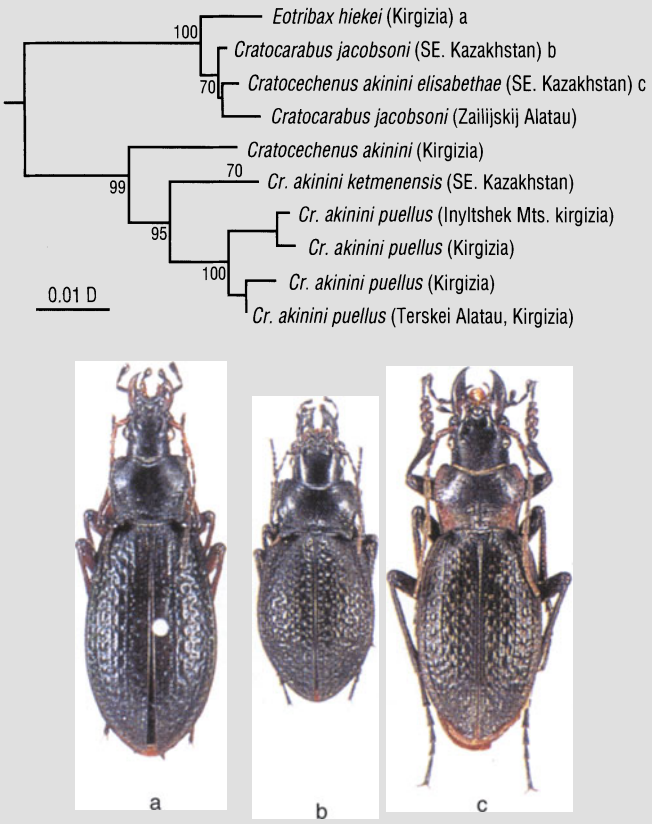


FIG. 8.3. Phylogenetic tree of the mitochondrial *ND5* gene for some members of the Tianshanese lineage of the division Procrustimorphi, showing recent separations of hypercephalic *Cratocarabus jacobsoni* (**b**) and *Cratocechenus akinini elisabethae* (**c**) from *Eotribax hiekei* (**a**) with normal-sized head. Constructed using the NJ-method

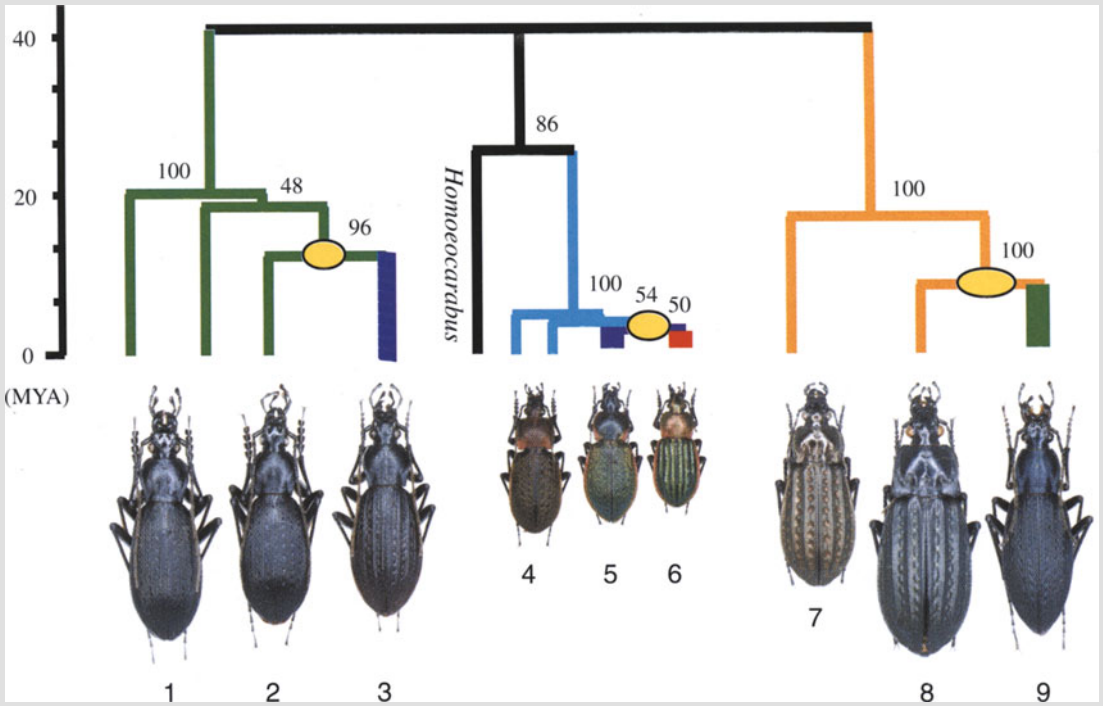


FIG. 8.4. Several examples of type-switching as shown by a phylogenetic tree of the mitochondrial *ND5* gene constructed using the UPGMA. **1** and **2** *Apotomopterus sauteri*, **3** *A. hupeensis*, **4** *Hemicarabus tuberculosus*, **5** *H. macleayi*, **6** *H. nitens*, **7** *Limnocarabus clathratus* (Germany), **8** *L. clathratus* (Japan), **9** *Euleptocarabus porrecticollis*. For a detailed explanation, see the text (after Su et al. 2001)

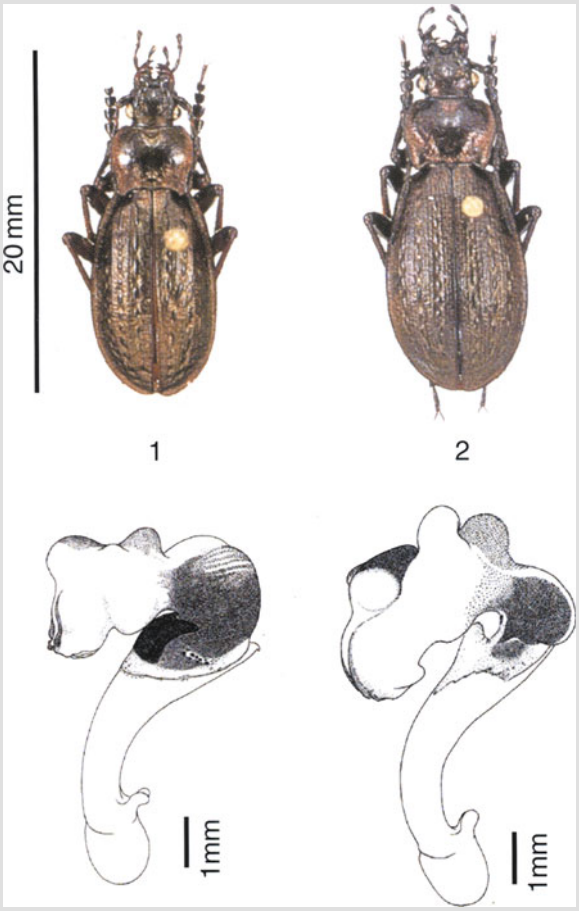


FIG. 8.5. Habitus of *Rhigocarabus* (1) *gigolo* and *R. buddaicus* (2). Male genitalia are shown directly below each species

type-switching from the *Qinlingocarabus*-type to the *Rhigocarabus*-type, with the structure of the male genital organ of the *Qinlingocarabus*-type having been retained.

These examples strongly suggest that structural transformation, or type-switching, can occur within a short time.

Followings are more examples of what we regard as type-switching.

Euleptocarabus porrecticollis (division Lepidospinulati) is strictly endemic to Japan, while another species in the same division, *Limnocarabus clathratus*, are distributed in the northern parts of the Eurasian Continent and Japan. The much closer phylogenetic relatedness of the Japanese *L. clathratus* with *E. porrecticollis* compared with that between the Japanese and continental *L. clathratus* (Fig. 5.8) suggests that *E. porrecticollis* branched off from the Japanese *L. clathratus*, accompanied by a rapid morphological differentiation long after separation of the two *L. clathratus* races (Fig. 8.4) (Kim et al. 1999c).

Apotomopterus sauteri (division Spinulati) radiated into three lineages ca. 20 MYA. *Apotomopterus hupeensis*, which is clearly distinguishable from *A. sauteri*, emerged recently from one of the *A. sauteri* stems (Fig. 8.4, see above).

In some cases, the species of more than two different genera are intermingled in the same cluster. Two Chinese carabids, *Eccoptolabrus exiguus* and *Calocarabus aristochroides*, are placed in the same cluster despite considerable morphological differences between them. These two species diverged about 10 MYA (Fig. 8.2).

As noted above, the Tibetan carabids, *Neoplesius* spp., radiated ca. 14 MYA into several species and subspecies whose morphologies are very close to one another (Imura et al. 1997; see Section 8.2). *Eocechenus leptoplesioides*, another Tibetan carabid, which is characterized by macrocephaly and is dissimilar to *Neoplesius*, sympatrically occurs with some *Neoplesius* species and is included in the *Neoplesius* cluster (Figs. 8.2 and 5.37). In addition to *Eocechenus*, and *Pseudocranion remondianum* is also in this cluster (Fig. 5.37). This suggests that the morphological change of *Eocechenus* and *Pseudocranion* took place rapidly after branching from the *Neoplesius* stem.

Cathaicus brandti and *Eupachys glyptopterus* (division Procrustimorphi) have been treated as being phylogenetically close to each other because of their morphological similarity, both having a black, stout body and displaying remarkable macrocephaly. However, on the *ND5* phylogenetic tree (Figs. 8.7 and 8.10) as well as a nuclear 28S rDNA tree (not shown), *Cathaicus* and *Eupachys* are clustered with the morphologically dissimilar, beautifully decorated *Coptolabrus* and *Acoptolabrus*, respectively. These results suggest that these two macrocephalic carabids evolved from independent ancestors; *Cathaicus* from the *Coptolabrus* lineage, and *Eupachys* from the *Acoptolabrus* lineage. The most plausible explanation for these phenomena is that morphologically very different carabid species emerged by type-switching.

One of the most astonishing findings of the present study was the origin of *Procerus*. This genus consists of two species, *P. scabrosus* and *P. gigas*, which are well-known as the largest carabids attaining nearly 8 cm long (see No. 5 in Fig. 5.35 in Chapter 5). The genus *Megodontus* (division Procrustimorphi) forms several lineages on the *ND5* tree, and *Procerus* shows a clear sister relationship with one of the *Megodontus* lineages (Fig. 5.35), the members of which show entirely different morphology from that of *Procerus*. This suggests that *Procerus* branched off from the *Megodontus* stem.

Many more examples of probable type-switching, which are not mentioned here, can be seen in the *ND5* phylogenetic trees shown in Chapter 5. As type-switching denotes the occurrence of a remarkable morphological change upon the emergence of a species, most of the examples described in this section may be

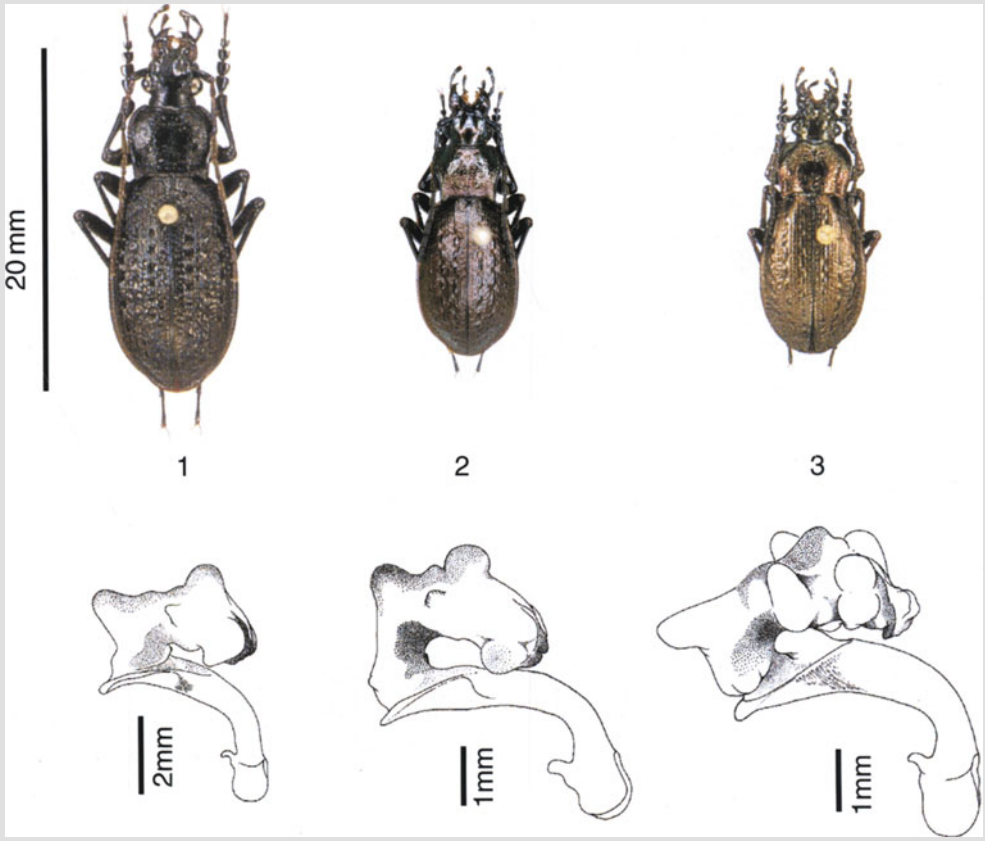


FIG. 8.6. Habitus of *Qinlingocarabus blumentaliellus* (1), *"Rhigocarabus" choui* (2), and *Rhigocarabus confucius* (3). Male genitalia are shown directly below each species

included in this category. This in turn suggests that a remarkable morphological change took place within a short time and did not proceed gradually. Examples of gradual change in morphology are discussed at the end of this chapter.

8.4 Silent Evolution

If rapid, presumably discontinuous, morphological differentiation is a general phenomenon in evolution, there must exist silent periods with little morphological change in various carabid lineages. The representative examples are described below.

8.4.1 The Tribe Cychrini

Morphological differences between four lineages (genera) of the tribe Cychrini are clearly recognizable and yet the difference within a given lineage is generally small (see Imura 2001). For example, lineage diversifications in the Chinese *Cychrus* are tremendous and indicate that the emergence of each took place at an ancient time. However, their morphological diversification is small, although they have been treated as independent species (Fig. 5.2). This would imply that the radiation of the *Cychrus* species was not accompanied by marked morphological differentiation, i.e., the ancient morphology has not changed much presumably even after geographic isolation within very mountainous regions in continental China.

It is noteworthy that some *Cychrus* species occur sympatrically as well, suggesting that an apparent speciation took place not only by means of geographic isolation but also through reproductive isolation.

8.4.2 The Tribe Ceroglossini

All the species of *Ceroglossus* from Chile diversified ca. 20 MYA and reveal considerable color variation, and yet the fundamental structure has almost remained unchanged (Figs. 5.3 and 8.22).

8.4.3 The Subtribe Calosomina

The Calosomina radiated into more than 16 lineages ca. 30 MYA. Like *Tomocarabus* and its related genera (Fig. 5.19d), and the *Cychrus* species (see above), each lineage contains one or two species, although there are some exceptional lineages (Fig. 5.7). This would imply that

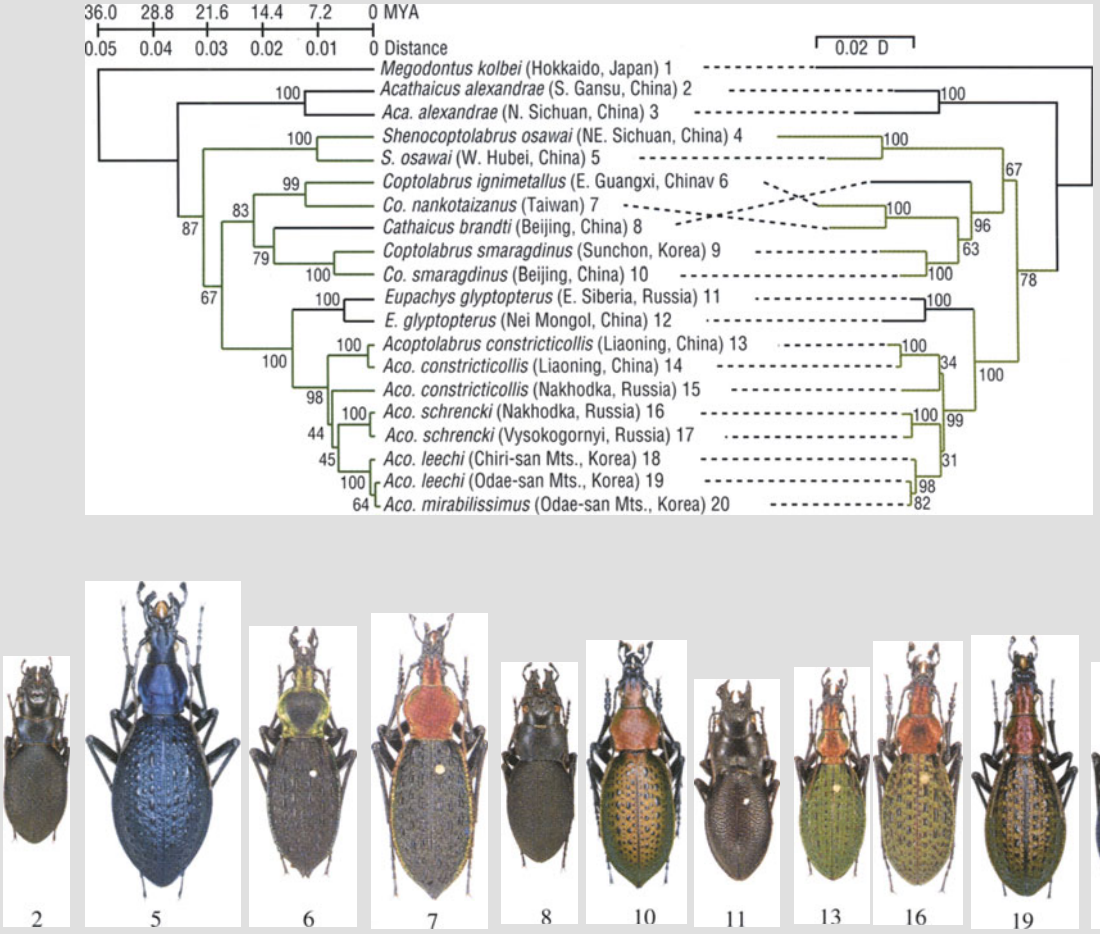


FIG. 8.7. Phylogenetic positions of *Acathaicus*, *Cathaicus*, and *Eupachys*. Phylogenetic trees of the mitochondrial *ND5* gene were constructed using the UPGMA (left) and the NJ-method (right). *Megodontus kolbei* was taken as an outgroup. Photographs of 11 species including *Acathaicus*, *Cathaicus,* and *Eupachys* are shown below the trees. Numbers under photographs correspond to those shown after the scientific names (localities) (after Su et al. 2001)

the species in a lineage has neither undergone a conspicuous morphological differentiation nor allowed the separation into other species.

It is interesting to note that *Calosoma inquisitor* and *C. maximowiczi* are morphologically very similar, so that these have been believed to be phylogenetically very close to one another. According to the *ND5* phylogenetic tree, however, the two species separated almost at the same time as the Calosomina emergence. Thus, these two *Calosoma* species might have kept the ancestral morphological characters of the Calosomina.

8.4.4 The Subtribe Carabina

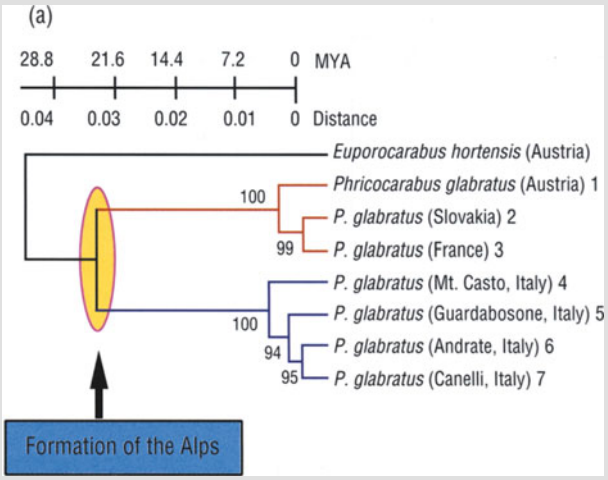
8.4.4.1 Division Latitarsi

Two *Phricocarabus glabratus* races were isolated ca. 20 MYA, corresponding to the time of the formation of the Alps, and yet they can hardly be distinguished from each other by morphology (Figs. 8.8 and 4.4) (Imura et al. 1998b; Su et al. 2001).

The genus *Tomocarabus* and its related genera, such as *Semnocarabus,* may be taken as the most remarkable example of the silent evolution. They radiated ca. 28 MYA into more than ten well-defined lineages (Fig. 5.19d), in all of which almost no morphological changes have been recognized. In other words, each lineage contains only one species without any other species intermingled with it, despite the ancient initiation of the *ND5* sequence diversification.

To give an example, *Tomocarabus convexus* mainly distributed in Europe, *T. fraterculus* found in the Korean Peninsula and its adjacent districts, and *T. opaculus* which is found in northern Japan and its adjacent regions, belong to independent lineages. The *ND5* sequence of each species began to diversify about 28 MYA with little morphological changes (Fig. 8.9) (Su et al. 2000c; Su et al. 2001).

The genus *Tanaocarabus*, which is endemic to North America, includes three morphologically allied species, *T. sylvosus, T. forreri,* and *T. hendrichsi.* Surprisingly, *T. sylvosus* and *T. forrei* belong to entirely different lineages. Furthermore, the genetic difference between three individuals of *T. forrei* from various localities examined were as large as in the *Tomocarabus* species cases mentioned above.



(b)

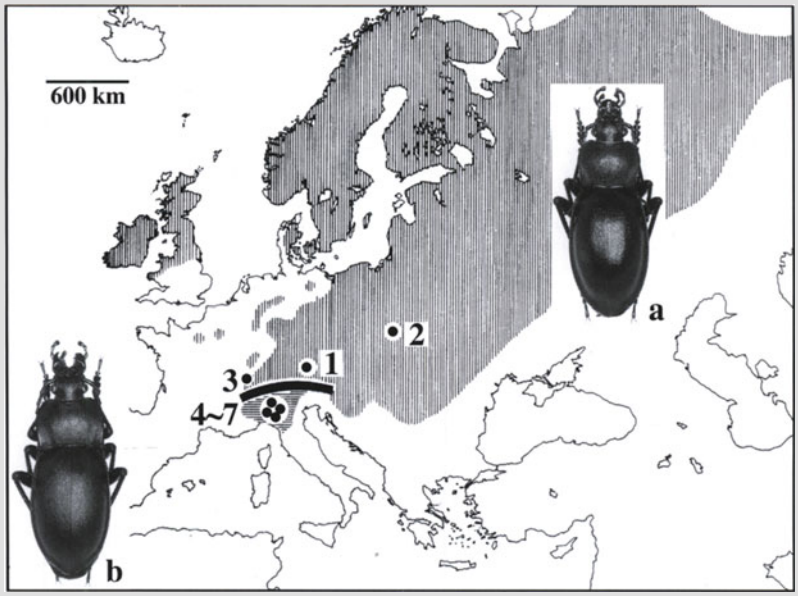


FIG. 8.8. Phylogenetic tree of the mitochondrial *ND5* gene for two geographic races of *Phricocarabus glabratus* (**a**) and a map showing their distribution range (**b**). The tree was constructed using the UPGMA taking *Euporocarabus hortensis* as an outgroup. **Black bar** indicates the Alps by which the two races are separated (after Imura et al. 1998b; modified)

Rhigocarabus spp. consists of many species and reveals very similar morphology among almost all the species, which diversified ca. 30 MYA. Most of the species are geographically well-isolated in the mountainous areas of western China (Figs. 5.19c and 5.25).

8.4.4.2 Division Spinulati

Apotomopterus sauteri (division Spinulati) is divided into several subspecies by slight morphological differences (see Chapter 5). Specimens from various localities in China and Taiwan have been placed in three geographically linked lineages by the *ND5* gene sequences, with their separation having taken place as long ago as ca. 20 MYA (Fig. 8.4) (Imura et al. 1998a; Kim et al. 1996b; Su et al. 2003b). Note that a different species, *A. hupeensis*, branched off from one of the *A. sauteri* stems (Fig. 5.14).

8.4.4.3 Division Digitulati

The members of the genus *Carabus* (s. str.) are widely distributed in the Eurasian Continent and North America. According to the *ND5* phylogenetic tree, however, they are divided into three independent

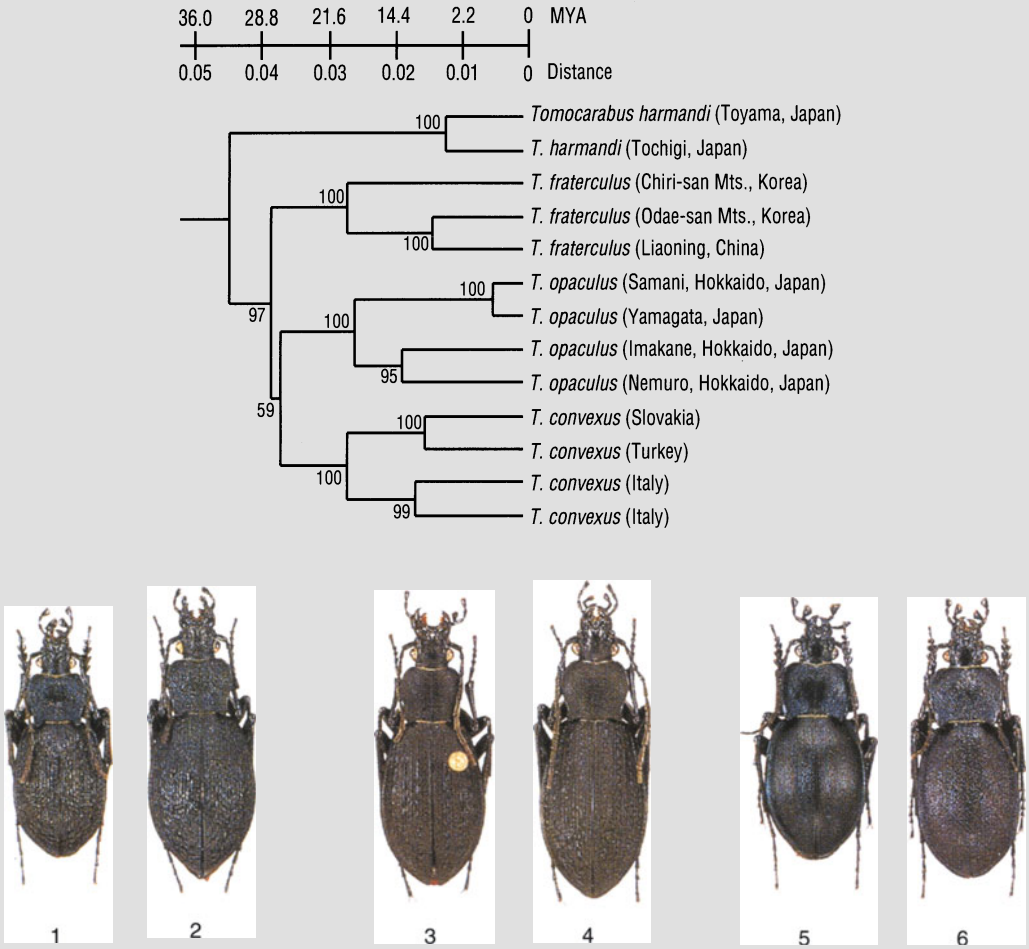


FIG. 8.9. Phylogenetic tree of the mitochondrial *ND5* gene for three species of the genus *Tomocarabus*. Constructed using the UPGMA. *Tomocarabus harmandi* was taken as an outgroup. Photographs: **1**, **2** *T. fraterculus* (1 from Chiri-san Mts. of Korea and 2 from Liaoning, China); **3**, **4** *T. opaculus* (3 Akita, Japan and 4 Hokkaido, Japan); **5**, **6** *T. convexus* (5 Germany and 6 Turkey) (after Su et al. 2001)

clusters that emerged in ancient times (Fig. 5.27). Among them, the members in continental China (= *Archaeocarabus*; see p. 168) includes many species with very similar morphology. There are at least three groups whose separation took place a long time ago.

8.4.4.4 Division Procrustimorphi

Damaster blaptoides has been divided into several subspecies by minor morphological differences. In particular, inhabitants west of the Kinki District through Kyushu in Japan exhibit almost the same morphology, so that they have been treated as a single subspecies, *D. blaptoides blaptoides* (Ishikawa 1991; see Chapter 7). However, the *ND5* trees reveal that the populations of the Kinki District, the Chugoku/Shikoku area, and Kyushu each belong to a distinct lineage. Their diversification took place ca. 13 MYA, shortly after the Japanese Islands separated from the continent (Figs. 7.20 and 7.21) (Su et al. 1998).

Acoptolabrus gehinii (including *munakatai*), a beautiful species found in Hokkaido, shows a remarkable morphological resemblance to *A. schrencki, A. leechi,* and *A. constricticollis* from the eastern periphery of the Eurasian Continent and the Korean Peninsula, and yet there separation took place long ago at ca.15 MYA (Figs. 7.25 and 7.26) (Okamoto 1999).

A European *Megodontus* species, *violaceus*, is divided into many subspecies (geographic races) due to small morphological differences. *M. violaceus violaceus/M. v. purpurascens* and *M. germarii* (sometimes treated as a subspecies of *M. violaceus*; see Fig. 5.35) were isolated from each other by the Alps ca. 20 MYA, and yet their morphologies are alike.

Microplectes convallium, found in the Caucasus region, is divided into two subspecies by minor morphological differences, and yet their separation took place 20 MYA. (Fig. 5.34).

The relationship between the morphology and phylogeny of the Tibetan *Neoplesius wagae/N. mark-*

amensis species-complex is worth mentioning. Morphologically, specimens in all the populations of these species may be unified into a single species, because there is only very little difference. One population (*N. wagae*) and two others (*N. markamensis*) are separated by a deep branching point on the *ND5* tree at ca. 12 MYA (Fig. 5.37). The locality of *N. wagae* is 400–600 km apart from that of *N. markamensis,* and they are well isolated geographically. They can be regarded as two distinct groups, and are conventionally treated as two species (Imura et al. 1997).

As mentioned in the previous section, the morphologically very different species *Eocechenus leptoplesioides* appears in the *Neoplesius* cluster and occurs sympatrically with *N. markamensis* in Xizang, China.

Cephalornis potanini from two different localities (southern Gansu and northern Sichuan, both in China) separated 15 MYA, but show little morphological change. *Acathaicus alexandrae, Aristocarabus viridifossulatus,* and *Shenocoptolabrus osawai*, all are inhabitants of mountainous areas of southwest China, reveal considerable *ND5* gene sequence differences within the same species from different localities (Fig. 5.37).

The examples mentioned above, together with many more that are not mentioned here, suggest that geographic isolation *per se* does not necessarily bring about any conspicuous morphological change for a long time, corresponding to at least one-third to two-thirds the history of carabid evolution. This phenomenon may be called silent evolution, in which the molecular clock works but with little morphological change. Of course, the "silent" in silent evolution does not mean "absolutely silent." Minor morphological differentiation must be included in the process.

This is often observable when a species is completely or incompletely geographically isolated. Most of what one calls forms, subspecies, allied species, or species-groups may be included in this category. Silent evolution results from a simple non-deleterious random mutation or an accumulation of such mutations. Thus, minor changes during the period of silent evolution would have no directionality, except those of hybrid or hybrid-derived individuals. In most cases, the idea of "morphological continuity" supported by many taxonomists is not supported by substantive evidence.

Silent evolution is not an exceptional phenomenon and is one of the principal mechanisms in evolution. Quite often, organisms that have undergone little morphological change over a long period are called "living fossils" (see Stanley 1981).

For the kind of conspicuous morphological changes described in Sections 8.2 and 8.3, it is likely that some other drastic genetic changes are required (see the last section of this chapter).

8.5 Parallel Evolution

8.5.1 Allopatric Parallel Evolution

Still another phenomenon of carabid evolution is the occurrence of more than two species morphologically similar in structure, but each belonging to a phylogenetically independent lineage. Many of these cases also include discontinuous morphological changes.

Cathaicus brandti (found in the basin along the Huang He River, from Shandong to Gansu, China), *Eupachys glyptopterus* (found from southeastern Siberia to northeastern China), and *Acathaicus alexandrae* (found from a restricted area in southern Gansu to northern Sichuan, China) have been considered to be phylogenetically close to one another. This thinking is based on their morphological similarity, all having a black, stout body and a remarkable macrocephaly (Fig. 8.10).

As noted on p. 146, *Cathaicus* is clustered with *Coptolabrus*, and *Eupachys* with *Acoptolabrus*. *Acathaicus* is not clustered with any other species examined as part of this study. *Cratocephalus cicatricosus* (Fig. 8.10) found on the Tianshan Mountains of Central Asia may be included in this category. These results indicate that morphologically similar species arose in parallel in different lineages whose distribution ranges are at a distance from one another.

Hereafter, this phenomenon is referred to as "allopatric parallel evolution". Parallel evolution may be conveniently divided into two categories: one involving the occurrence of total morphological resemblance in more than two species; the other the development of partially similar morphology in more than two species.

The *Cathaicus-Eupachys-Acathaicus* relationship is an example of the former category. The latter category is best exemplified by macrocephaly, which can be seen in many carabid groups as well as other groups of the order Coleoptera.

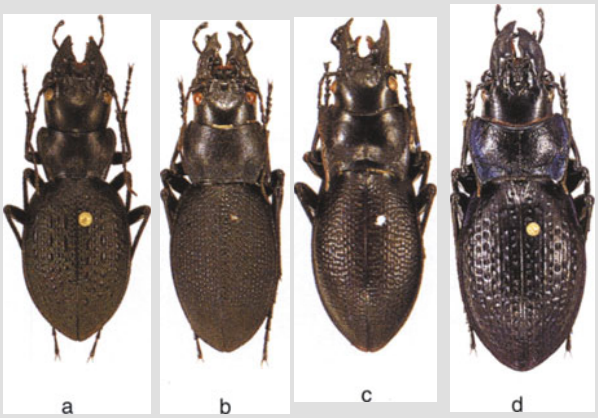


FIG. 8.10. Four macrocephalic species in the division Procrustimorphi. **a** *Acathaicus alexandrae*, **b** *Cathaicus brandti*, **c** *Eupachys glyptopterus*, **d** *Cratocephalus cicatricosus*

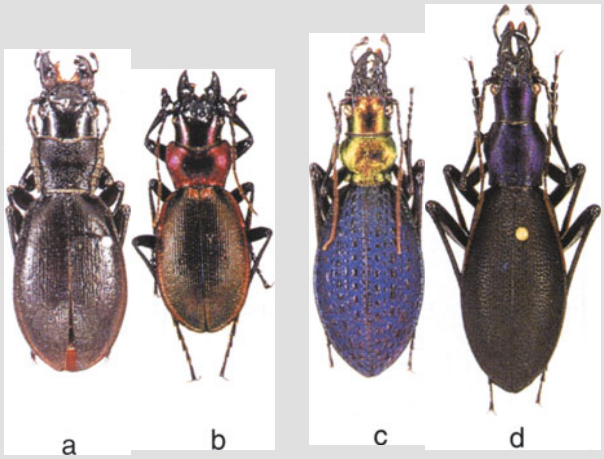


FIG. 8.11. Four examples of macrocephalic carabid species. **a** *Eocechenus leptoplesioides*, **b** *Cechenochilus boeberi*, **c** *Acoptolabrus mirabilissimus*, **d** *Damaster blaptoides capito*

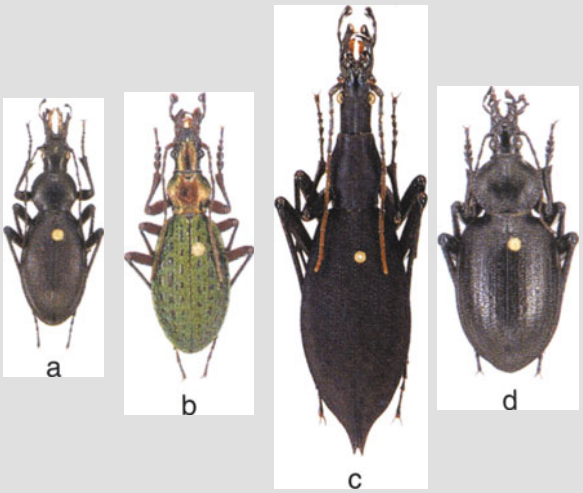


FIG. 8.12. Four examples of microcephalic carabid species. **a** *Cephalornis potanini*, **b** *Acoptolabrus constricticollis*, **c** *Damaster blaptoides*, **d** *Cathoplius asperatus*

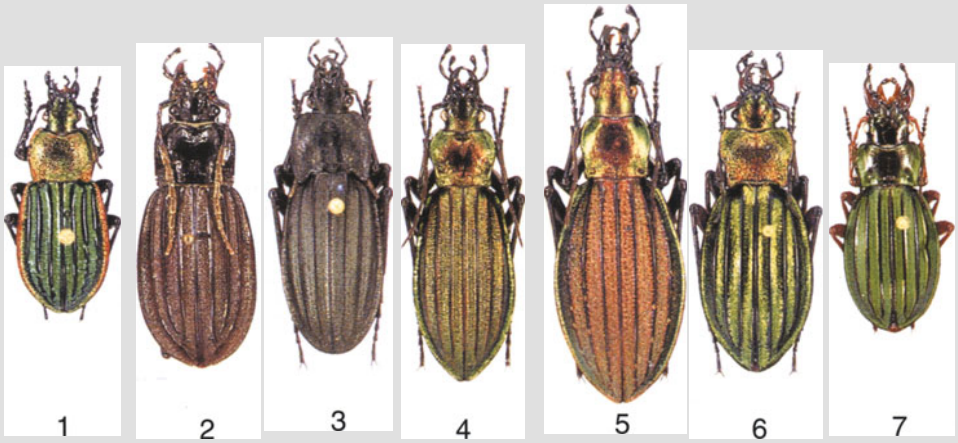


FIG. 8.13. Examples of several carabid species having elytra with remarkably elevated intervals. **1** *Hemicarabus nitens*, **2** *Leptocarabus canaliculatus*, **3** *Rhabdotocarabus melancholicus*, **4** *Megodontus kolbei*, **5** *Acoptolabrus gehinii*, **6** *Chrysocarabus auronitens*, **7** *Calocarabus gratus*

Examples that demonstrate macrocephaly include *Eocechenus* from mountainous areas of western China, a considerable number of the Procrustimorphi species from the Tianshan Mountains (*Cratocechenus*, etc.), *Cechenochilus* from the Caucasus region, *Acoptolabrus mirabilissimus* from the Korean Peninsula, and *Damaster blaptoides capito* from Sado Island, Japan (Fig. 8.11). *Taphoxenus* sp. also reveals a remarkable macrocephaly (Fig. 8.15; see next section).

The species of the tribe Cychrini display morphological characteristics at the opposite end of the scale from the macrocephalic species and are characterized by a narrow, slender head including the mouthparts. Characteristics of this type often appear in parallel in various groups of the Carabina. There are a particularly large number of examples in the division Procrustimorphi, including *Cephalornis potanini* from Gansu and Sichuan, China, *Acoptolabrus constricticolis* from Primorskij, Russia, *Damaster blaptoides* from Japan, and *Cathoplius asperatus* from North Africa (Fig. 8.12).

There are quite a few carabid beetles in various groups in which the elytral primary intervals are well elevated to form costae (Fig. 8.13). *Hemicarabus nitens*, *Leptocarabus canaliculatus*, *Rhabdotocarabus melancholicus*, *Megodontus kolbei*, *Acoptolabrus gehinii*, *Chrysocarabus auronitens*, and *Calocarabus gratus* may be such examples.

As discussed in Chapter 5, species of the divisions Spinulati and Lepidospinulati both have a well-developed spine (spinula) at the base of the endophallus of male genitalia (see Fig. 5.9). Despite apparent morphological similarity in this structure, there is

probably no direct phylogenetic relationship between these two divisions. The distribution ranges of these two divisions are clearly different (Fig. 5.10), and would have emerged at the time of the radiation, or big bang. This suggests that the unique morphology of the male genital organ developed in parallel allopatrically in these two independent phylogenetic lines.

8.5.2 Allopatric Parallel Evolution in the Genus *Leptocarabus*

Apparently allopatric parallel evolution, possibly involving type-switching, can be recognized in the genus *Leptocarabus* (division Latitarsi). Although the details of *Leptocarbus* phylogeny have already been presented in Chapters 5 and 6, an outline is provided here for easier understanding of the complex nature of the parallel evolution in this group.

The *Leptocarabus* species are morphologically classified into five discrete types, P, S, K, C, and T (Fig. 5.20), while both the phylogenetic trees of the *ND5* gene and the nuclear 28S rDNA show the existence of three geographically linked lineages. One of these lineages is made up of two P-type species found in China, another consists of several species of the K-, T-, S-, and C-types found in northeast Asia, and still another is made up of three P-type species and one S-type species found in the Japanese Islands.

The Chinese *Leptocarabus* species, *yokoae* and *marcilhaci*, are quite similar to the Japanese *Leptocarabus* in morphology. It is rather surprising that morphologically close but phylogenetically remote species occur in Japan and continental China, and not on the Korean Peninsula. Nevertheless, the ancestry that led to the present-day Chinese and the Japanese P-type *Leptocarabus* species must have inhabited the ancient Chinese Continent, but we do not know what it was.

If the Chinese *Leptocarabus* is the ancestral form of all *Leptocarabus* species, then both the north Asian and the Japanese species were derived from a Chinese ancestor via two routes. One route might have been seen in the north Asian species differentiating from a portion of this ancestor population. In this case, some changes leading to the K-, T-, C-, or S-type morphology from the P-type would have taken place during differentiation of the northeast Asian species. Another fraction of the ancestor population, having inhabited the ancient Japan region of the continent, immigrated to the Japanese Islands upon their separation from the continent and diversified into different species in Japan. This view is consistent with the close similarity of the Chinese *Leptocarabus* species, *yokoae* and *marchilhaci*, to *L. kyushuensis* in Japan, which is thought to be the ancestral form of all other Japanese *Leptocarabus* species, including the S-type species (see pp. 124–125). Perhaps, morphology of the Chinese proto-*Leptocarabus* (P-type) remained almost unchanged until *L. kyshuensis* differentiated.

If this explanation is correct, the overall phylogenetic profile points to the possibility that the S-type Korean *Leptocarabus* and the S-type Japanese *Leptocarabus* evolved allopatrically in parallel. Another possibility, which is less likely, is that the ancestry of all the *Leptocarabus* species, including the Chinese P-type species, became extinct in the past, and the present P(K)-type *Leptocarabus* in China, Korea, and Japan, and the S-type in Korea and Japan respectively arose allopatrically in parallel.

8.5.3 *Leptocarabus* and *Rhigocarabus* in Continental China

As noted in Chapter 5, about 30 *Rhigocarabus* species (division Latitarsi) inhabit mountainous areas of Sichuan, Gansu, Qinghai, and Xizang, China (Fig. 5.25). They are all small in size, round in shape and cuprous in color. Emergence of most of the species, as seen by the DNA phylogeny, is venerable, and their classification as distinct species is supported by the fact that they consist of several different lineages (Fig. 5.19e).

Quite unexpectedly, the Chinese *Leptocarabus* species, *yokoae* and *marcilhaci*, are not directly clustered with other *Leptocarabus* species, and are clearly positioned in the *Rhigocarabus* cluster on the phylogenetic tree of the whole Latitarsi as described in Chapter 5 (Figs. 5.23 and 5.24). Such a relationship is schematically presented in Fig. 8.14 with the rough distribution ranges of the carabid groups concerned.

There are at least two possible ways to account for this astonishing phenomenon. One is that the ancestral form is the Chinese *Leptocarabus* from which *Rhigocarabus* branched off, involving type-switching from *Leptocarabus*-like to the *Rhigocarabus*-like morphology. Another possibility is that *Rhigocarabus* is the ancestral form, from which *Leptocarabus* emerged by type-switching. The consequent evolutionary processes of various *Leptocarabus* groups are as described in the previous section.

Leptocarabus truncaticollis found in the Ural region of Russia is a member of the northeast Asian lineage. Its morphology is, at first glance, very different from other *Leptocrabus*, and rather resembles certain species of *Rhigocarabus*. This may also be considered an example of allopatric parallel evolution between this species and *Rhigocarabus*.

8.5.4 Some More Examples of Possible Allopatric Parallel Evolution

In the western parts of the Japanese Islands, *Ohomopterus dehaanii* (D-type) and *O. japonicus* and its related species (J-type) form a cluster in pairs in Kyushu/San-in (Honshu), San-yo (Honshu), and Shikoku (see Chapter 7, and Figs. 7.5 and 7.7), suggesting the occurrence of allopatric parallel evolution by

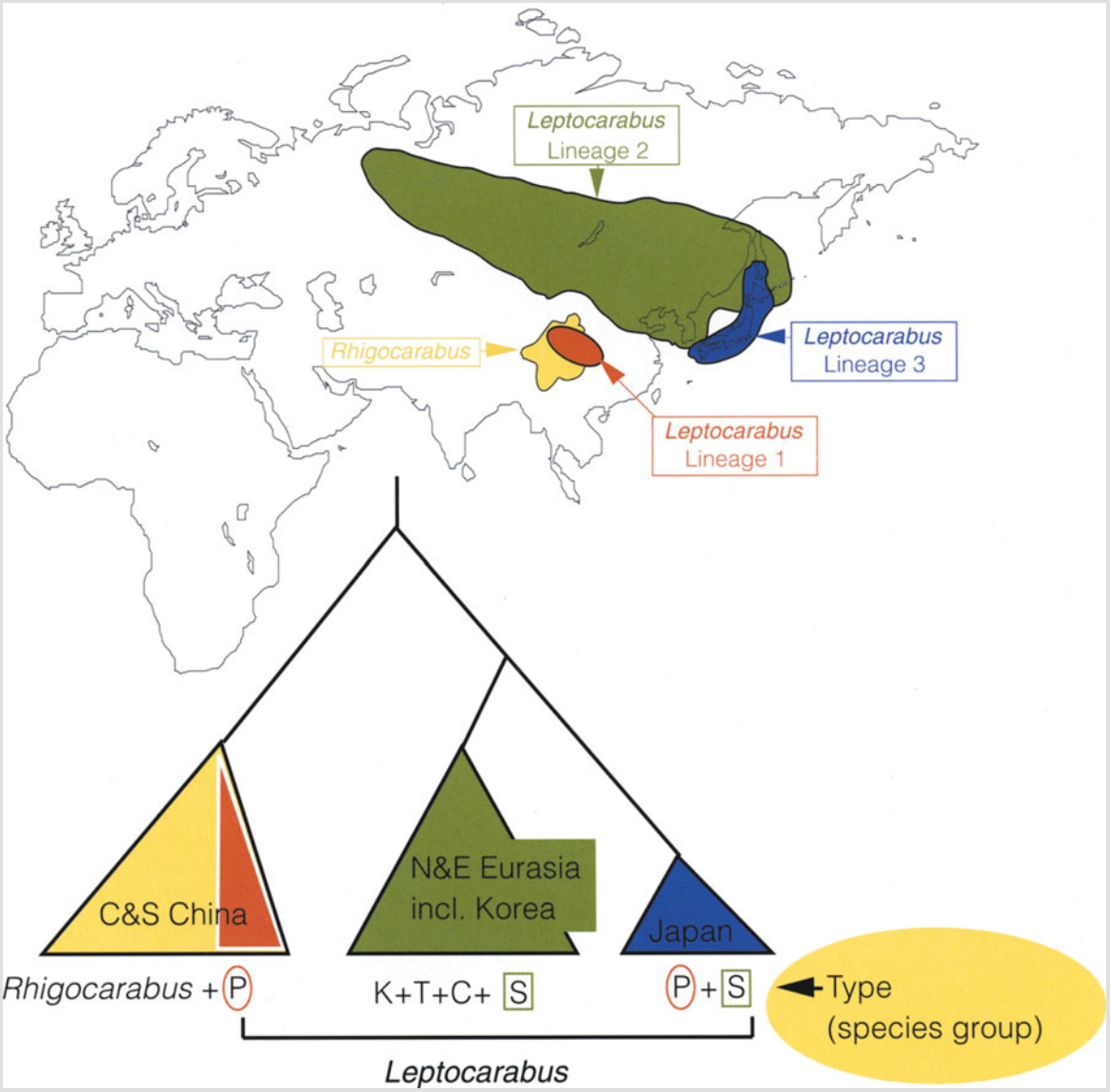


FIG. 8.14. Distribution of the genus *Rhigocarabus* and the genus *Leptocarabus*. For a detailed explanation, see the text, and also Figs. 5.23, 5.24, and 5.25 (after Su et al. 2001)

type-switching in the respective regions, although other possibilities exist in this case (see p. 105).

Phricocarabus glabratus from northwestern Italy is clustered with *Pachystus tamsi* from Iran, while *Ph. glabratus* from Austria forms a cluster with *Pa. cavernosus* from east-central Italy (Fig. 5.19a). Separation of the two clusters, and that of *Phricocarabus* and *Pachystus* in each cluster occurred about 20 MYA and 15 MYA, respectively, although which genus is the ancestor of the paired genera cannot be determined.

In the Tianshanese group of the division Procrustimorphi, allopatric parallel evolution seems to have taken place occasionally. For example, the morphologically similar species of the genus *Cratocephalus* appear in three independent lineages (Fig. 5.36). The genus *Cratophyrtus* pairs with the genus *Pantophyrtus*, and this pair appears in two entirely different lineages (Fig. 5.36). The genus *Tribax* and the genus *Archiplectes*, both found in the Caucasus region, appear in two and three independent lineages, respectively. Whether these are examples of parallel evolution that occurred allopatrically or sympatrically cannot be deduced until more specimens from different localities are analyzed.

We have discussed on the assumption that, except for several cases where the nuclear genes have been examined together, the *ND5* phylogeny can be equated to the species phylogeny, without considering a possible horizontal transfer of mitochondria by hybridization, ancestral polymorphism or random lineage sorting. These factors should ultimately be subjects for examination with a view to reaching a final conclusion for each case.

8.5.5 Allopatric Parallel Evolution or Lineage Sorting?

It is misleading to consider the occurrence of similar morphology in different lineages as necessarily representing allopatric parallel evolution. When more than two lineages branch off with little morphological change, it is possible that the descendant species in each lineage also reveal similar morphology, as seen in many examples of "silent evolution."

For example, there exist several geographically isolated *Tomocarabus opaculus* populations that have undergone little morphological differentiation over a long period, and yet no one thinks of this as allopatric parallel evolution. This is only a result of lineage sorting of the ancestral morphologies to the descendants.

This phenomenon can be understood only by means of molecular phylogenetic examination in conjunction with morphology, and never by morphology alone. In some cases, it is not possible to decide whether the phylogenetic data obtained represents parallel evolution or lineage sorting.

In one example, outlined above, the Chinese *Leptocarabus* and the Japanese *Leptocarabus* are morphologically very similar and yet their branching point is very deep and a considerable number of other *Leptocarabus* species have emerged between them. In a case such as this, it is possible to speculate that the Chinese and the Japanese *Leptocarabus* emerged in parallel, and at the same time it is also possible that these two resulted from lineage sorting of the ancestral morphologies (see p. 153).

One European *Megodontus* species, *germarii*, is closely similar in morphology to *M. violaceus* and is often treated as one of the subspecies of *violaceus*. However, as is shown in Fig. 5.35, these two species are phylogenetically quite remote, and the emergence point of *M. germarii* is almost at the root of the phylogenetic tree, and many different species, even one different genus, *Procerus*, emerged after *M. violaceus* and *M. germarii* separated. Can the appearance of these two species, *M. violaceus* and *M. germarii*, be considered a case of parallel evolution? No unambiguous answer may be given to this question, because it is possible that *M. germarii* is the ancestral species to the other species including *M. violaceus* (see p. 83 and p. 164).

Another example of this kind may be seen in the *Acoptolabrus* species. Morphologically, the continental and Japanese *Acoptolabrus* species are very similar, and yet their separation occurred long ago at ca. 20 MYA (Fig. 7.25) (Okamoto 1999). A phylogenetic tree containing several other genera, which are together sometimes called the *Damaster*-group, contains *Coptolabrus* and *Shenocoptolabrus* and others (Fig. 5.37), which are phylogenetically remote from one another. These facts suggest that the species in this group containing both continental and Japanese *Acoptolabrus* diversified within a short time, and their branching order cannot be determined. It is therefore by no means clear whether the continental and the Japanese *Acoptolabrus* have a strictly sister relationship, or these two populations arose in parallel.

8.6 Sympatric Parallel Evolution (Convergence)

Sympatric parallel evolution, often called convergence, is a phenomenon in which similar morphologies appear sympatrically in different phylogenetic lines. Convergence is as common as allopatric parallel evolution throughout the various carabid groups.

8.6.1 Convergence at the Subfamily Level

In August 1999, we received several ethanol-immersed carabid specimens from Hong-Zhang Zhou of the Chinese Academy of Science, Beijing, for DNA analysis. These specimens were captured in Inner Mongolia by Hong-Zhang Zhou himself. The collection contained two macrocephalic carabids that were very similar to each other in appearance (Figs. 8.15 and 8.16).

One of these specimens was an example of *Eupachys glyptopterus* (see the previous section). At first glance, the other specimen was thought to be a member of the subfamily Carabinae. Surprisingly, this curious beetle was later identified by Noboru Ito as a species of the subfamily Harpalinae known as *Taphoxenus* sp. (subtribe Sphodrina, tribe Platynini), which lacks hind wings. An *ND5* phylogenetic tree shows that *Taphoxenus* is clustered with other Carabidae beetles except those of the Carabinae, while *Eupachys glyptopterus* is surely a member of the division Procrustimorphi of the subtribe Carabina. This is a remarkable example of convergence at the subfamily level.

8.6.2 Convergence at the Division Level

Morphocarabus hummeli (division Lipastromorphi), which is widely distributed in the Far East, reveals considerable locality-dependent color variation. *Megodontus vietinghoffi* (divison Procrustimorphi), *Hemicarabus macleayi* (divison Crenolimbi), and *Morphocarabus hummeli*, all found in the same locality, show similarity of color and appearance. In fact, the similar appearance of *Morphocarabus hummeli* and *Megodontus vietinghoffi* found in the same area was so marked that the distinction between them, each immersed in ethanol required careful examination (Fig. 8.17). As might be expected, a phylogenetic tree including these three species shows that they belong to distinct lineages (Fig. 8.18).

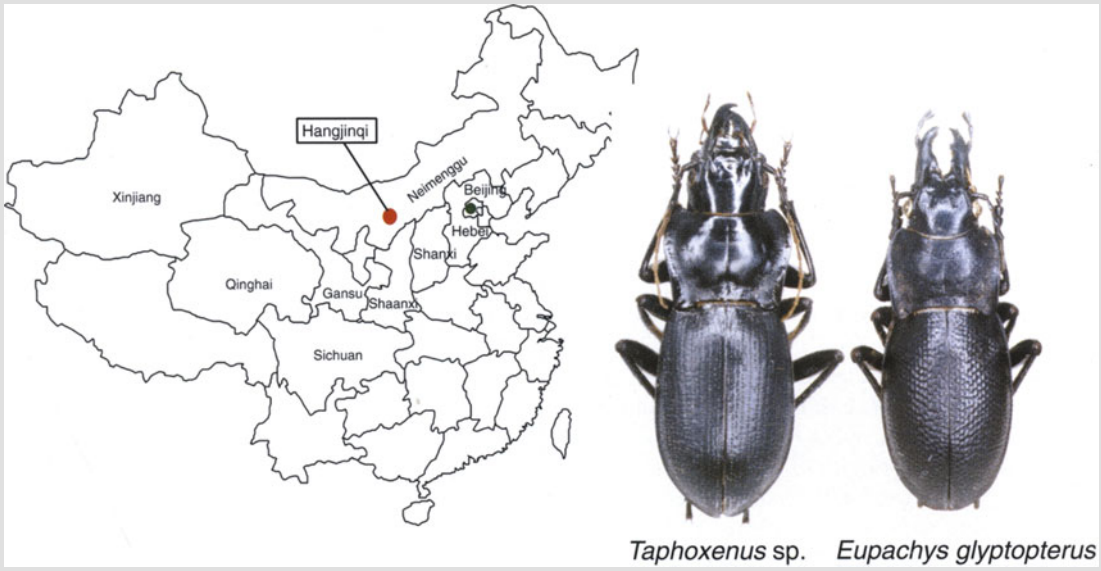


FIG. 8.15. *Taphoxenus* sp. (**left**) and *Eupachys glyptopterus* (**right**) and their habitat (**red point** on the map)

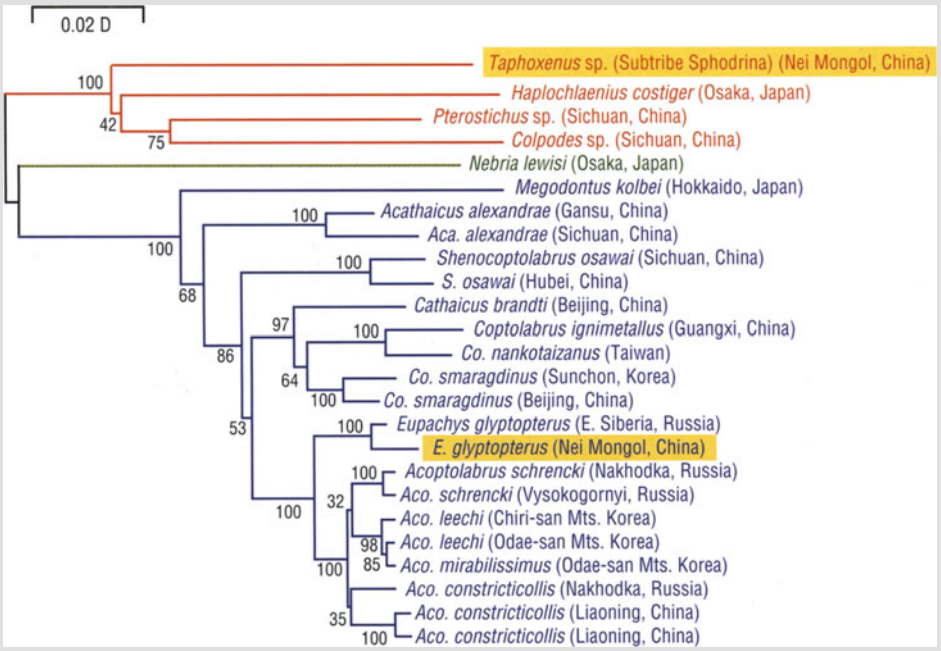


FIG. 8.16. Phylogenetic tree of the mitochondrial *ND5* gene showing relationship between *Taphoxenus* sp. and *Eupachys glyptopterus*. Constructed using the NJ-method

8.6.3 Convergence at the Genus Level

Following is a description of convergence displayed in the genera *Acoptolabrus* and *Megodontus* (both in the division Procrustimorphi) in Hokkaido and Sakhalin in Russia. As shown in Fig. 8.19, *Acoptolabrus lopatini* is very similar to *Megodontus avinovi*, both of which are found in Sakhalin.

In various places of Hokkaido, the color and physical structure of *Acoptolabrus gehinii* (including *A. munakatai*) are very nearly in parallel with those of *Megodontus kolbei*. The beetles shown in Fig. 8.19 are likely to appear to be examples of the same species to the untrained eye, though they are specimens of two distinct species belonging to the different genera. The phylogenetic tree (Fig. 8.20) shows a clear distinction

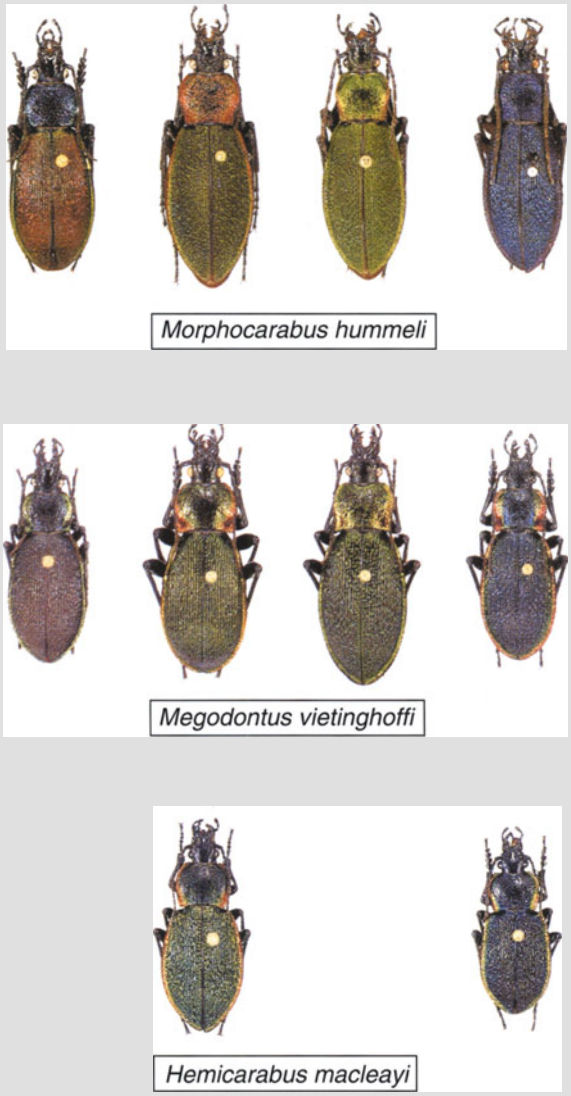


FIG. 8.17. Convergence of *Morphocarabus hummeli*, *Megodontus vietinghoffi*, and *Hemicarabus macleayi*. For a detailed explanation, see the text

between *Acoptolabrus* and *Megodontus*. It is worth noting that variations in *Acoptolabrus gehinii* emerged quite recently (see Fig. 7.25) (Okamoto 1999).

8.6.4 Convergence at the Species Level

Coloration of the body surface of Chilean species belonging to the genus *Ceroglossus* varies considerably. The color pattern of inhabitants of the continental mainland may be classified into the following five types: the golden-green type (hereafter GG) with a golden-green elytra and dark blue pronotum; the dark green type (DG) with a dark green elytra and pronotum; the dark-red type (DR) with a dark red elytra and a yellow-green pronotum; the brown-purple type (BP) with a brown-purple elytra and a dark olive-green pronotum; and the dark-blue type (DB) with a dark blue elytra and pronotum (Figs. 8.21 and 8.22) (Okamoto et al. 2001).

The color pattern of inhabitants on Chiloe Island is more complex. In addition to GG, DG, BP, and DB, three additional color types are present. These are the copper type (C) with a copper-colored elytra and pronotum, the olive-green type (OG) with an olive-green elytra and a dark blue pronotum, and the yellow-green type (YG) with a yellow-green elytra and a dark green pronotum. It is also worth noting that examples of the DR type have not been found on this island (Fig. 8.21).

As seen in Figs. 5.4, 8.21, and 8.22, the color pattern is similar among different species inhabiting the same area (with the exception of Chiloe Island; see below) with only a few exceptions, while that of the same species inhabiting distant areas is different despite their close phylogenetic relationship. In short, color type is linked to geography and not to the phylogeny of the species (= the lineage). As a result, two or more distinct species exhibit the same color pattern (Figs. 8.21 and

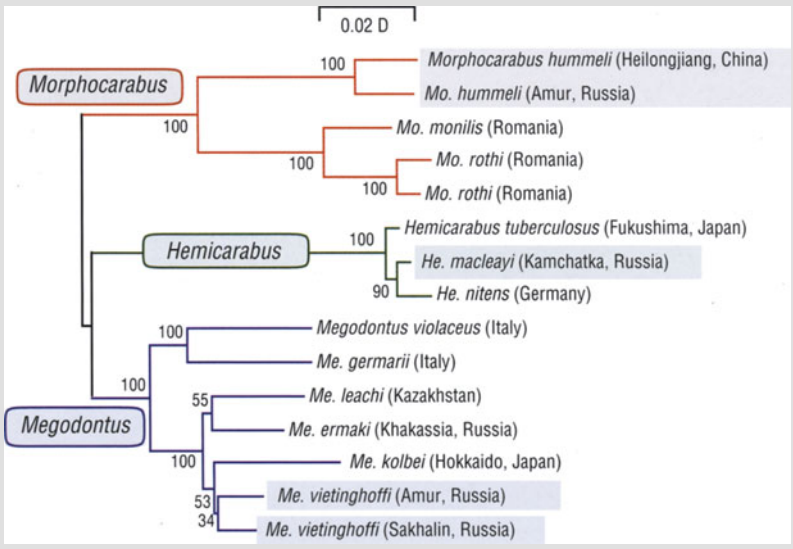


FIG. 8.18. Phylogenetic tree of the mitochondrial *ND5* gene for *Morphocarabus* spp., *Hemicarabus* spp., and *Megodontus* spp. Constructed using the NJ-method

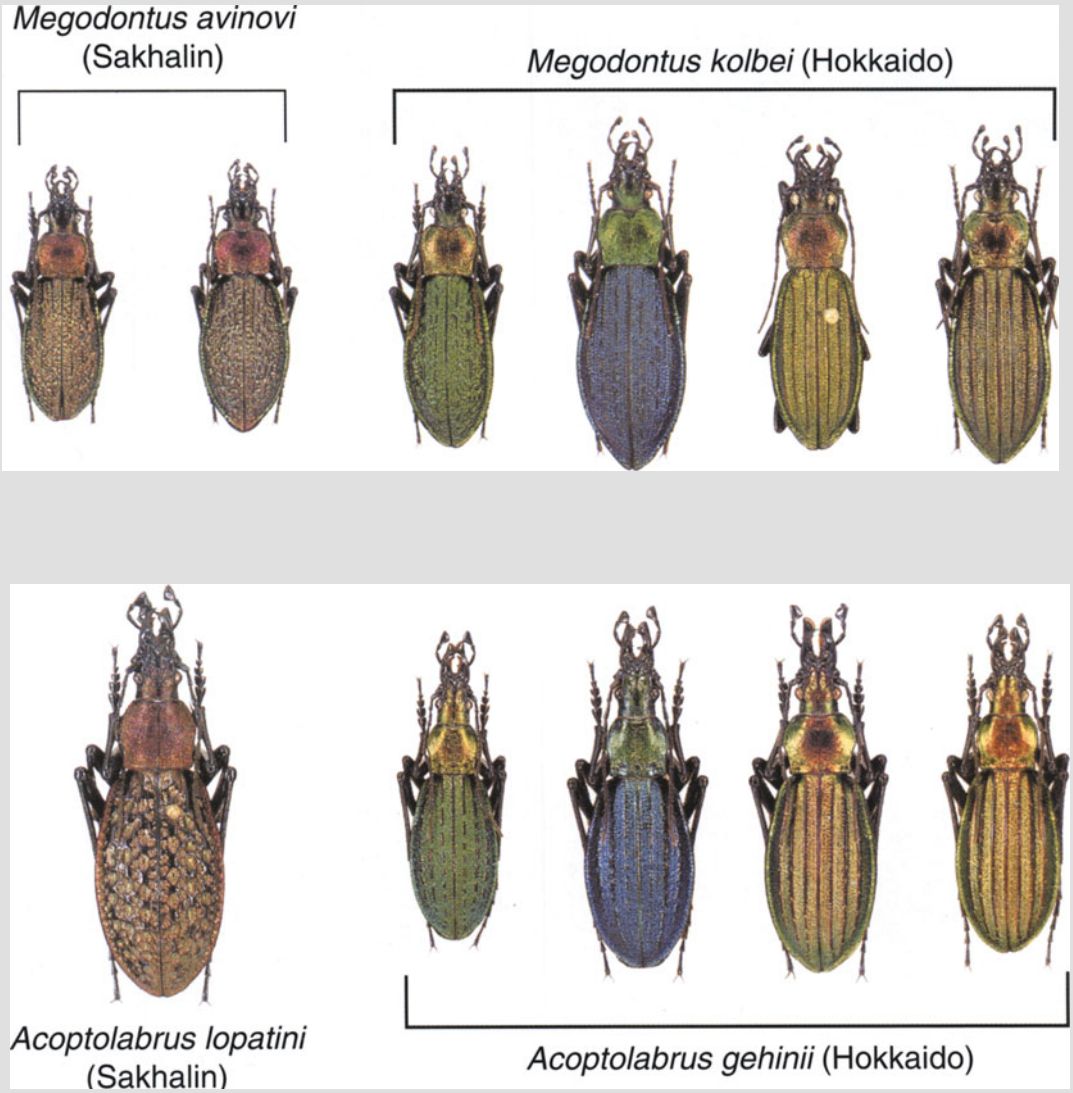


FIG. 8.19. Convergence of *Megodontus* (**upper row**) and *Acoptolabrus* (**lower row**)

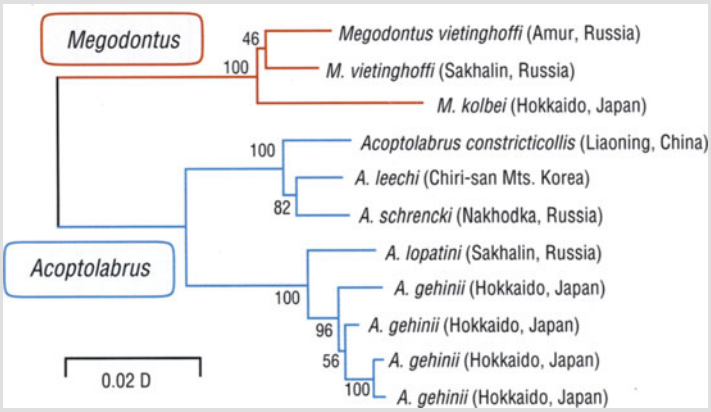


FIG. 8.20. Phylogenetic tree of the mitochondrial *ND5* gene for *Megodontus* spp. and *Acoptolabrus* spp. constructed using the NJ-method

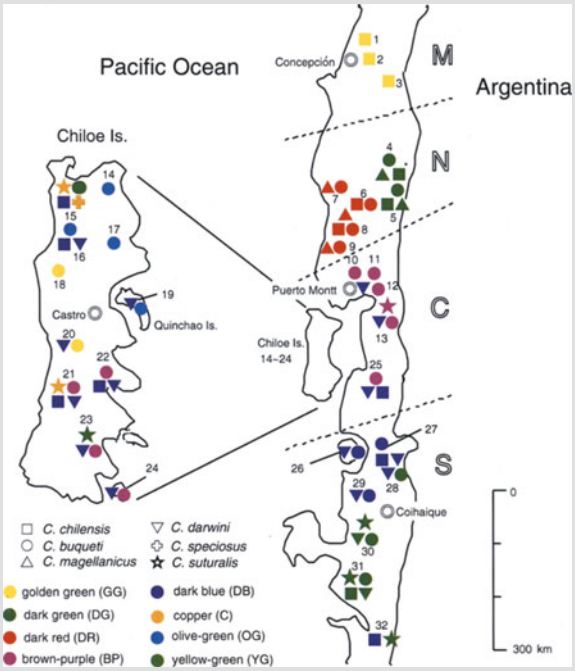


FIG. 8.21. Localities of the *Ceroglossus* specimens analyzed. Locality numbers correspond to those shown in the phylogenetic tree shown in Fig. 5.5. The colored symbol denotes the color type of the sample shown in Table 8.1. M northernmost region, N northern region, C central region, S southern region (after Okamoto et al. 2001)

TABLE 8.1. Convergence of color pattern in *Ceroglossus*

Locality No.	Species	Color
4,5,6 (N)	*chilensis, buqueti, magellanicus*[1]	green
6,7,8,9 (N)	*chilensis, buqueti, magellanicus*[1]	red
26,27,28,29 (S)	*chilensis, buqueti, darwini*	deep blue
30,31 (S)	*chilensis, buqueti, darwini, suturalis*	green

N, northern region; S, southern region

[1] *Magellanicus* belongs to the *darwini*-group. For locality numbers and symbols, see Fig. 8.21 (after Okamoto et al. 2001)

8.22). For localities and symbols of the species, see Fig. 8.21 and Table 8.1.

In the M region indicated in Fig. 8.21, only golden-green (GG) specimens of *C. chilensis* (lineage CHI-group M) have been found.

In the N region shown in Fig. 8.21, three species—*C. buqueti* (lineage BUQ), *C. magellanicus* (lineage DAR), and *C. chilensis* (lineage CHI)—have been found. Examples of these three species found in the western district of this region all exhibit dark red (DR) coloration, while those of the eastern district are of the dark green (DG)-type. In all these localities, two to three species of a single color type occur sympatrically, and have often trapped in the same bait traps (loc. nos. 4–5 for the DG-type, loc. nos. 6–9 for the DR-type).

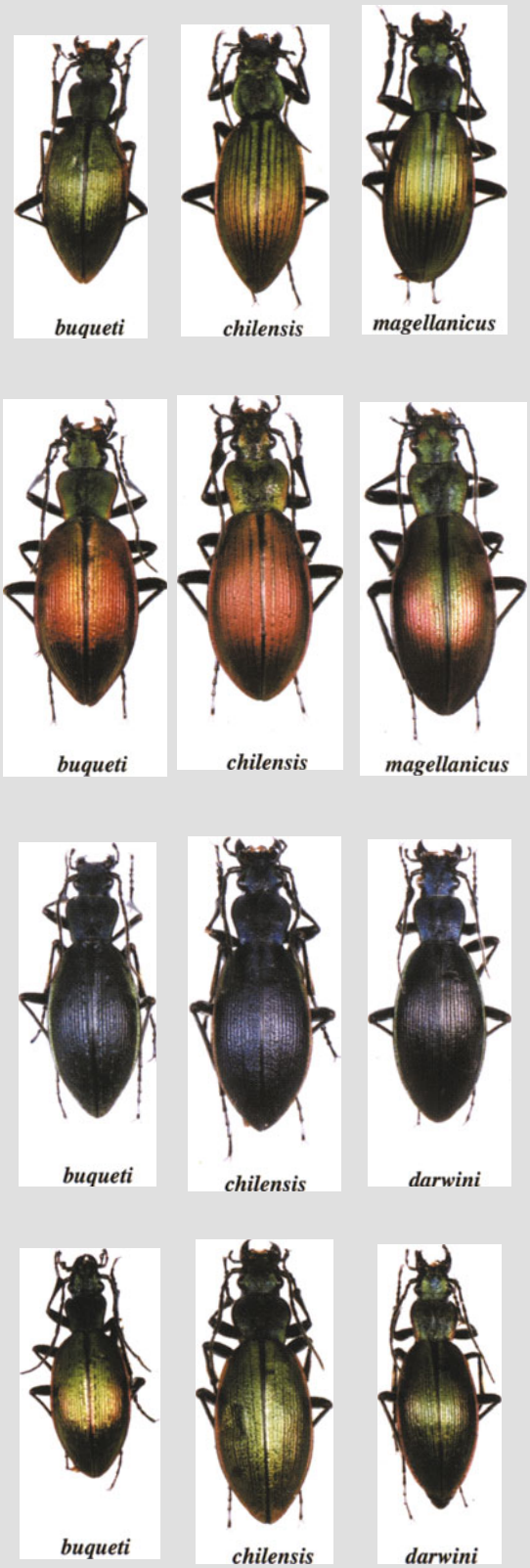


FIG. 8.22. Color convergence of *Ceroglossus* species. Specimens from loc. nos. 5, 8, 27, and 31 appear from top row to bottom row (after Okamoto et al. 2001; modified)

In the C region shown in Fig. 8.21 (except for Chiloe Island in Chile, see below), the four species *C. buqueti* (lineage BUQ; color type BP), *C. chilensis* (lineage CHI; color type DB), *C. darwini* (lineage DAR; color type DB), and *C. suturalis* (lineage SUT; color type BP) have been found. The BP color type of *C. buqueti* and *C. suturalis* occurs in locality 13 indicated in Fig. 8.21 together with the DB-type of *C. darwini*. Similarly, locality 25 shown in Fig. 8.21 is inhabited by the DB-color type of *C. chilensis* and *C. darwini* and the BP-color type of *C. buqueti*. These findings demonstrate the sympatric convergence of coloration in the C region of the mainland, though convergence is less marked here than in the N region.

In Chiloe Island and its adjacent islands, all the species (except *C. magellanicus*) are present. *Ceroglossus buqueti* (lineage BUQ) reveals various color types, i.e., GG, BP, OG, and YG (Fig. 8.21). The OG- and YG-types appear only in *C. buqueti*. The C-type appears in *C. sutularis* and *C. speciosus*. The OG-, YG-, and C-types have not been found in the C region on the mainland.

The BP-type of BUQ 24 is likely to have the same origin as the mainland inhabitants of BUQ 10–13 in the C region. All the *C. darwini* (lineage DAR) and *C. chilensis* (CHI) specimens from various parts of the islands (SAR 16, 19–24; CHI 15, 16, 21, 22) show the DB-type coloration, suggesting that these two species in the islands would have an origin in common with the respective species of the same color type in the S region of the mainland (DAR 12, 13, 25; CHI 27, 31, 32), because of their close phylogenetic relationship. The GG color type of BUQ 18 and 20 is likely to have emerged independently from the same type of CHI 1-3 because of their different phylogenetic origins.

The complex coloration pattern shown by species on Chiloe Island is likely to have resulted from what we can assume were the multiple origins of these species. In several localities, two different species of the same color type appear in conjunction with other species of different color types. This is seen in localities 15 (C-type *C. suturalis* and *C. speciosus)*, 16 (DB-type *C. chilensis* and *C. darwini*), and 21 and 22 (same as locality 16).

In the S region, four species occur, i.e., *C. buqueti* (lineage BUQ), *C. darwini* (lineage DAR), *C. chilensis* (lineage CHI), and *C. suturalis* (lineage SUT). Dark blue (DB) specimens are found mainly in the northern part of this region, while examples of the dark green (DG)-type are distributed in the southern part. As in the N region, the same color type appears in different species in the same locality. One example is given by appearance of the DB-type *C. buqueti*, *C. darwini*, and *C. chilensis* in localities 26–29 (with exception of the DG-type of *C. buqueti* in locality 28). The DG-type of the three species also appears in localities 30 and 3l.

This suggests that convergence of coloration took place sympatrically in parallel for more than two species in more than two regions.

In addition to examples of sympatric convergence, the same color type appears in more than two species allopatrically, as exemplified by the occurrence of the DG-color type in the N region and the S region (Fig. 8.21 and Table 8.1).

As seen from the phylogenetic tree (Fig. 5.5), the various color types are distributed across the different lineages. It is, however, difficult to deduce when and how these color patterns came into being. Whatever the coloration of the common ancestor of *Ceroglossus* might have been, the present color patterns were likely to have been established after geographic isolation of each lineage (species or species-group), as deduced from the phylogenetic tree.

At least some of these geographically linked color changes are likely to have occurred relatively recently. For example, in the N region, *C. buqueti* of the DG-color type (loc. nos. 4–5 in the eastern part of the region) separated from the DR-type of the same species (loc. nos. 6–9 in the western part of the region) 11 MYA at most, while *C. chilensis* with the DG-type coloration (loc. no. 5) branched off from the DR-type nearly 3 MYA.

In the S region, DB-type *C. darwini* (loc. nos. 26–29 in the northern part of the region) branched off from the same species with DG-type coloration (loc. nos. 30–31 in the southern part of the region) ca. l MYA. The separation of the DB-type of *C. chilensis* (loc. no. 27 in the northern part of the region) from the DG-type of the same species occurred 2–3 MYA.

This leads us to ask what factors have brought about the sympatric convergence of color patterns. Obviously, the involvement of certain ecological or environmental factors must be postulated as the cause, though this is as yet a matter of speculation and is not discussed here.

As for genetic events, it may be assumed that some region-specific environmental conditions have affected the genome so as to express certain gene(s) among the preexisting gene family determining coloration existing in all the species. Alternatively, mutation(s) that led to specific color patterns would have been selected by environmental factors. In either case, geographic isolation would have prevented a mixing of different color types.

Still another possibility is that color polymorphism existed in the common ancestors of the *Ceroglossus* species and a particular color type was sorted to their descendants inhabiting the localities examined in this study through selection affected by locality-dependent environmental factors.

One might argue that the specific genes responsible for a particular color pattern spread selectively over different species in geographically isolated areas as a consequence of crossing between two or more species. This is not very likely to have occurred, however, because mitochondrial phylogeny reveals a long history for each

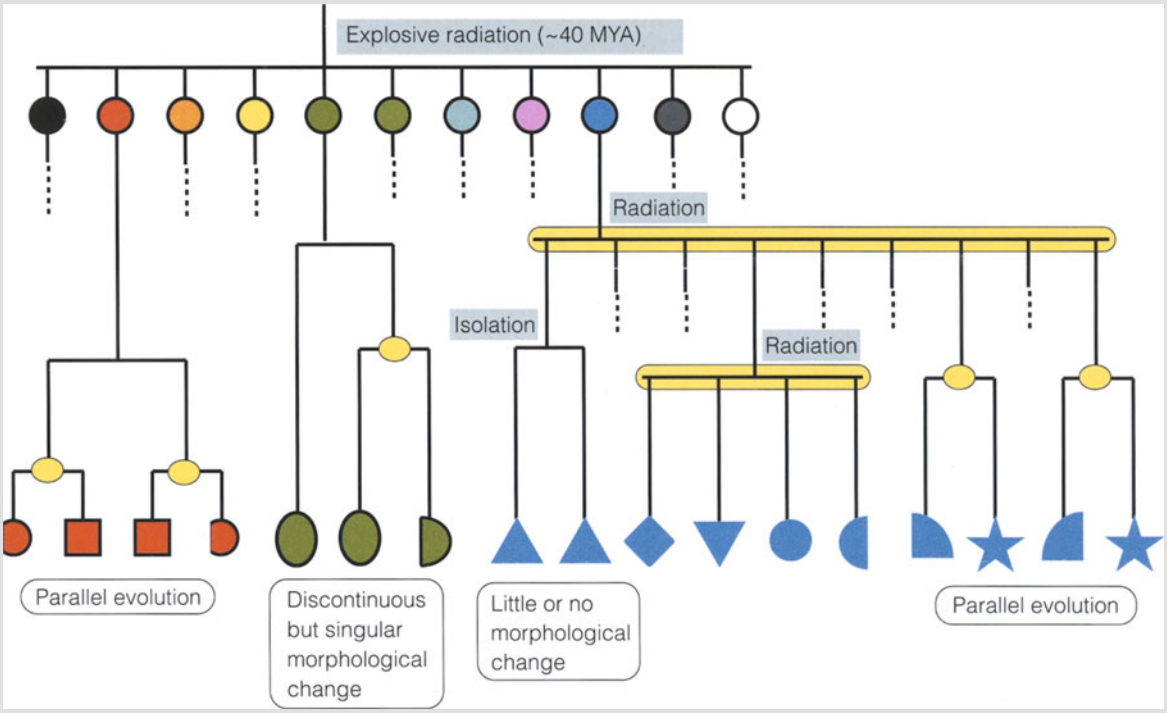


FIG. 8.23. A model of discontinuous evolution of the carabid ground beetles. Shape and color of symbols indicate morphological difference. For a detailed explanation, see the text (after Osawa et al. 1999; modified)

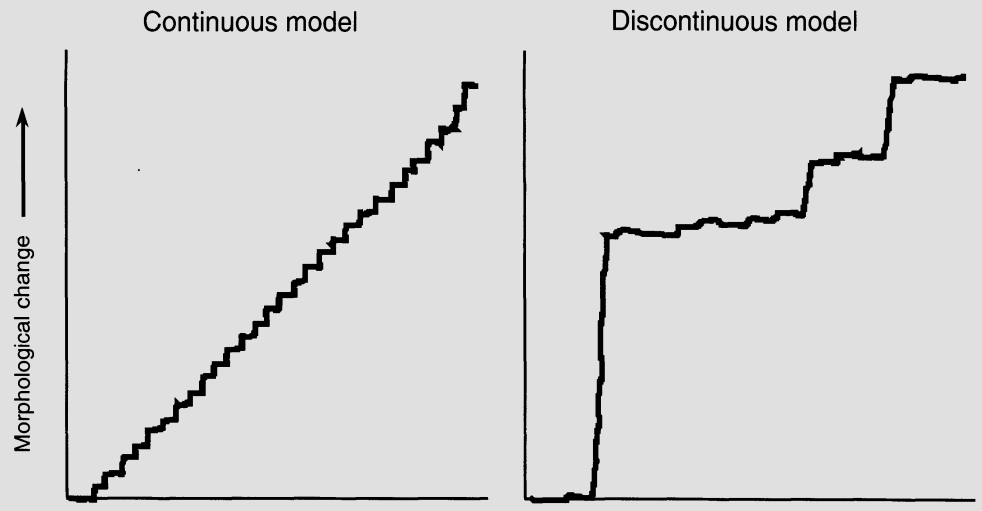


FIG. 8.24. Continuous (**left**) and discontinuous (**right**) models of morphological evolution. For a detailed explanation, see the text

species in accordance with the taxonomic classification, and yet acquisition of the particular color pattern is a relatively recent event.

The examples of convergence described in this section are superficially alike, but the underlying mechanisms whereby the convergence was brought about is not necessarily the same in each case. It is, however, worth noting that convergence in *Acoptolabrus/Megodontus*, or in various species of *Ceroglossus*, took place within a short time corresponding to considerably less than one-tenth of the entire history of Carabinae evolution.

8.7 Concluding Remarks: Discontinuous Evolution Model

Carabid diversification took place 40–50 MYA as an explosive radiation of the major groups. During evolu-

tion, occasional radiation explosions of various sizes also took place, sometimes accompanied by parallel morphological evolution in phylogenetically remote as well as close lineages.

The existence of silent periods, in which few morphological changes took place, has often been recognized during evolution (Fig. 8.23). Figure 8.24 provides a schematic illustration of the morphological changes in one carabid species from its emergence to the present constructed using the widely accepted (neo)Darwinian model (gradual evolution model) (left) and the discontinuous evolution model (right).

Overall, carabid evolution is generally discontinuous, showing alternative phases of rapid morphological change of various scales and silent phases of various lengths. These instances of discontinuous evolution (explosive radiation) could have occurred allopatrically as a result of geographic or reproductive isolation but, more importantly, these instances of radiation seem to have occurred sympatrically as well.

As noted repeatedly, it should be emphasized that isolation *per se* does not necessarily cause morphological change. Instances of explosive radiation could be brought about by relaxation of environmental or ecological constraints acting as the cause of negative selection. Some deleterious genetic changes, which include various mutations of the genetic cascade system affecting morphogenesis, or some drastic changes such as those caused by the gene transposition, would have been tolerated and became neutral. This would have accelerated morphological diversification. Some other explanations are possible, but we will leave further discussion to the future because of the lack of substantive evidence available at the present time.

Whatever the underlying mechanisms were, the process of morphological differentiation is not parallel with evolutionary distance, i.e., approximate time elapsed after emergence of a given species. This is because "phenotype is conventional, and molecular evolution is conservative" as Kimura has noted.

Discontinuous evolution has been adequately discussed by Hiura (1970), who opines that, "According to paleontological evidence, emergence of new types of organisms from its ancestor occur explosively within a short period and is not a process of gradual changes. After the elapse of comparatively silent period for some time, a new bang again occurs. . . . Explosive radiation would have been induced by emergence of a new environment that occurred in a global scale." Discontinuous evolution is also well documented in the theory of "punctuated equilibrium" put forward by Gould and Eldredge (1977; see also Gould 1989 and Stanley 1981) on the basis of paleontological evidence.

Chapter 9
Phylogeny and Taxonomy: Reorganization of the Subfamily Carabinae Based on Molecular Phylogeny

9.1 Phylogenetic Taxa Versus Morphological Taxa

The purpose of phylogenetic studies based on the principle of the molecular clock is primarily to trace the evolutionary history of the organisms under examination, rather than taxonomic classification. However, molecular phylogenetic data are quite often useful in taxonomic classification, although the findings produced by phylogenetic studies in many cases produce results at great variance from those of traditional taxonomic studies based on morphology alone.

As discussed in Chapter 7, *Damaster blaptoides* has been considered a single morphological species, and yet it is clearly divided into eight geographically isolated lineages that diversified a long time ago.

In one striking example, the lack of morphological differences in inhabitants of such districts of Japan as Kyushu, Chugoku/Shikoku, and Kinki means that these beetles have been treated as a single subspecies, *blaptoides blaptoides*, in spite of the fact that each has a long, independent evolutionary history. This is also the case for *Tomocarabus opaculus* which split into several lineages at a more ancient time than that at which the diversification of the *Damaster blaptoides* lineages took place.

This raises the question of whether it is reasonable to regard each of these phylogenetically distinguishable lineages with a long evolutionary history as a distinct species. Several researchers have proposed the concept of "phylogenetic species," in an attempt to provide an answer to this question.

In classifying organisms using the phylogenetic species concept to those populations in which the branching between two or more lineages on a phylogenetic tree takes place far enough back in time, descendants in these lineages can be defined as distinct species even if they are considered morphologically conspecific.

In Chapter 7, *Damaster blaptoides* was tentatively classified as belonging to several phylogenetic species using the phylogenetic species approach. It should be emphasized, however, that this approach is subject to considerable debate because "old branching" is an ambiguous term, and there is as yet no persuasive criteria for defining phylogenetic species according to the length of the history of a particular lineage.

A similar situation is observed in *Tomocarabus opaculus* and some other *Tomocarabus* species (Figs. 5.19d and 8.9).

Another question arises with regard to the way to treat clearly distinguishable morphological species that demonstrate only a small evolutionary distance from one another. For example, three *Hemicarabus* species, *H. tuberculosus*, *H. nitens*, and *H. macleayi* are clearly distinguishable from one another as three distinct species (Fig. 8.4), and yet they diversified much more recently than *Damaster blaptoides* (Figs. 7.20 and 7.21) or *Tomocarabus opaculus* (Figs. 5.19d and 8.9).

If we adopt the phylogenetic species concept alone, these three morphological species should be treated as belonging to the same species. It is not therefore possible to define the species by either evolutionary distance or morphology alone. Long-range crossing experiments are useful in solving this problem (though not always practical).

We now turn to slightly more complicated cases. *Apotomopterus sauteri* is found in Southwest China and Taiwan, and radiated into at least three geographically isolated lineages (Fig. 5.14). This situation resembles that of *Damaster blaptoides* or *Tomocarabus opaculus*. In the case of the genus *Apotomopterus*, one of the *A. sauteri* stems (tentatively called X; Fig. 5.14) gave rise to the morphologically distinguishable species, *A. hupeensis*. This suggests that *sauteri* in X is phylogenetically more related to *A. hupeensis* than to *A. sauteri* belonging to two other phylogenetic lines.

If only the phylogenetic distance is taken into account in taxonomy, *A. sauteri* cannot be discriminated from *A. hupeensis*, because *A. sauteri* in X is positioned much nearer to *A. hupeensis* than to the *A. sauteri* in other two lines. It is obvious that no taxonomist would accept such a treatment.

One European species, *Phricocarabus glabratus*, is divided into two lineages that separated long ago (see Figs. 4.4 and 8.8). From each branch, to which the same species (*glabratus*) belongs, two different species, *Pachystus tamsi* and *P. cavernosus*, branched off (or vice versa; *P. glabratus* may have derived from *Pachystus*;

Fig. 5.19a, lineage S). If these are classified by morphology alone, two *Pachystus* species must be placed nearer together than either is in relation to *Phricocarabus glabratus*, while the situation is reversed if phylogenetic relationships are adopted as the only criterion for classification. This is a typical example of the gap between classification done using molecular phylogeny and that using morphological features.

Megodontus violaceus is widely distributed in Europe and shares common ancestry with other *Megodontus* species from the Caucasian region, Central Asia, and the Far East. Surprisingly, *Megodontus germarii* in Italy, which has been regarded as the closest relative of *M. violaceus*, is the remotest of all the *Megodontus* species.

The Iranian and Turkish *Megodontus* species are also remote from the European species and, unexpectedly, share common ancestry with all *Procerus* species (see Fig. 5.35). The mitochondrial DNA tree suggests that *M. germarii* was the ancestor of all the *Megodontus* and *Procerus* species. Using morphological classification, it seems more than reasonable to place *M. germarii* near *M. violaceus* with the addition of other *Megodontus* species, and to put *Procerus* species on the fringe of *Megodontus* species. Here again, the results of molecular phylogeny are not consistent with those of morphology.

The relationship between *Leptocarabus* and *Rhigocarabus* is another remarkable example of inconsistency between phylogeny and morphology (Figs. 5.23 and 5.24). Morphologically, *Leptocarabus* (s. str.) *yokoae* and *L. marcilhaci* from China are most similar to *L.* (s. str.) *kyushuensis* from Japan and the two may be regarded as having the closest relationship. *Leptocarabus* (s. str.) *procerulus*, *L.* (s. str.) *kumagaii*, and *L.* (s. str.) *hiurai* from Japan and *L.* (*Weolseocarabus*) *koreanus* from the Korean Peninsula come next, followed by other *Leptocarabus* species such as *L.* (*Adelocarabus*) spp. and *L.* (*Aulonocarabus)* spp. found in Japan and in the Far East and the Korean Peninsula.

All the known *Rhigocarabus* species are morphologically entirely different from *Leptocarabus* and they can be easily distinguished. Therefore, *Rhigocarabus* has been regarded as an entirely independent genus from *Leptocarabus*. Contrary to this view, two Chinese "*Leptocarabus*" species are definitely included in the *Rhigocarabus* group, separated from the rest of the *Leptocarabus* species, including the morphologically very similar *Leptocarabus kyushuensis*.

The direct ancestry of *Leptocarabus* and *Rhigocarabus* is unknown. If the two Chinese *Leptocarabus* species are tentatively assumed to be the closest to the prototype of these two genera, then *Rhigocarabus* is likely to have derived from an ancestor similar to the Chinese *Leptocarabus* during an early stage of *Leptocarabus* evolution.

The results of molecular phylogenetic analysis show that the Chinese *Leptocarabus* is much nearer to *Rhigocarabus* than to other *Leptocarabus* species. This raises the question of what approach to take to the taxonomic classification done along the lines of traditional Linnean Hierarchy. No doubt most taxonomists would resist the placement of the Chinese *Leptocarabus* within the *Rhigocarabus* group at a far remove from other *Leptocarabus* groups.

The apparent morphological affinity between *Acathaicus alexandrae*, *Cathaicus brandti* and *Eupachys glyptopterus* (Fig. 8.10) is not supported by their molecular phylogeny. Phylogenetic analysis shows *Cathaicus* and *Eupachys* clustered with *Coptolabrus* and *Acoptolabrus*, respectively. No phylogenetic relatives have been found for *Acathaicus*. Therefore, these three species are phylogenetically independent (Fig. 8.2).

Autocarabus auratus and *A. cancellatus* have been classified as members of the Latitarsi, and both are widely distributed in Europe. These two species are clustered on a mitochondrial phylogenetic tree and yet they have only a remote affinity to other Latitarsi groups including *Autocarabus cristoforii* from the Pyrenees.

Chrysocarabus auronitens, *C. rutilans*, and *C. hispanus* from Europe are clustered together on a molecular phylogenetic tree, while *Chrysocarabus olympiae* from northwestern Italy does not show any phylogenetic affinity to other *Chrysocarabus* species mentioned above, forming an independent lineage.

As discussed on pp. 67–71, the genus *Tomocarabus* is not monophyletic despite the morphological similarity between the component species. A mitochondrial phylogenetic tree suggests that they split into several independent lineages at the time of the explosive radiation of the Carabina with only a little morphological differentiation. From the phylogenetic point of view, each lineage may be discriminated from the others not only at the generic level but even at the divisional level (see below).

The "genus *Morphocarabus*" of the division Lipastromorphi is also not monophyletic in terms of molecular phylogenetic analysis, and is split into several distinct lineages (Fig. 5.17), although these lineages are hardly distinguishable from each other by the morphological criteria.

It might be useful to incorporate the phylogenetic genus or division into the taxonomic breakdown in a way analogous to the phylogenetic species concept. The so-called genus *Oreocarabus* (division Laitarsi) is quite heterogeneous from the molecular phylogenetic point of view, and Imura et al. (1998b) attempted to reorganize "*Oreocarabus*" by taking phylogenetic aspects into account (Fig. 5.19b).

The examples enumerated above together with many others described in this book indicate that morphological differentiation is unrelated to the lapse of time. When silent evolution continues for a long time, little morphologically recognizable taxa are formed, while occurrence

of type-switching produces morphological taxa considerably different from the maternal line. This is especially remarkable at the time of radiation, where many different taxa appear within a short period.

There are, therefore, a number of examples of phylogenetic relationships that could never be inferred by morphology. Sometimes taxonomists use the term "phylogenetic taxonomy" (based on morphology including cladistics) in the firm belief in that taxonomy should reflect only phylogeny. This is, however, a misguided view, obviated by the fact that phylogenetic relationships cannot be understood entirely accurately by morphological characters alone.

This then raises the question of the best approach to take in setting out a hierarchical arrangement of a given selection of taxa. We have tentatively used molecular phylogenetic taxa in conjunction with traditional morphological data to lay out what we hope will be a first step in creating a sounder taxonomy of the carabid beetles (see Sections 9.3 and 9.4).

9.2 Morphological Reexamination of Taxa Considering Molecular Phylogenic Analysis

There are many examples in which the assessment of taxa based on morphology has created erroneous results that can be corrected with the use of results from molecular phylogenetic analysis. Two examples are given here.

"*Rhigocarabus*" *choui* has been regarded as a member of the genus *Rhigocarabus*, while this species falls into the cluster of *Qinlingocarabus* and does not belong to *Rhigocarabus* on the basis of molecular phylogeny. A morphological reexamination of the male genital organ has made it clear that *choui* is surely a member of *Qinlingocarabus* (Fig. 8.6).

Acrocarabus guerini has been placed in the division Archicarabomorphi. A molecular phylogenetic analysis shows clearly that this species is a member of the division Digitulati. Indeed, the male genital organ of this species carries the digitulus, which is the most important morphological character of the division Digitulati and is not present in the Archicarabomorphi (Imura et al. 2000a) (Fig. 5.28).

In the cases outlined above, the discrepancy between morphology and phylogeny may be easily resolved by careful reexamination of morphological characters. However, the examples described in Section 9.1 of this chapter have nothing to do with morphology alone, so that we will have to leave a full discussion of this matter for some point in the future (see also Section 9.3). In taxonomy, there are many more important problems such as the species or subspecies concept. In this book, we do not go into the details of this problem in order to avoid fruitless discussions at the present.

9.3 Classification of the Subfamily Carabinae Based on Molecular Phylogenetic Trees

Following the construction of a number of phylogenetic trees making use of mitochondrial and nuclear DNA, we undertook the following classification of the subfamily Carabinae.

The genus *Haplothorax* from St. Helena Island in the South Atlantic Ocean, which has been placed in the subfamily Carabinae, is excluded from this system because of a lack of specimens available for analysis. The figure number of the phylogenetic tree, on which the classification system outlined below is based, is shown in parentheses in this and the following sections.

Subfamily Carabinae (Figs. 4.8 and 4.9)
- Tribe Cychrini (Fig. 5.2)
  - (Genera *Cychropsis*, *Cychrus*, *Scaphinotus*, *Sphaeroderus*, etc.)
- Tribe Pamborini (Fig. 4.8)
  - (Genera *Pamborus*, *Maoripamborus*)
- Tribe Ceroglossini (Fig. 5.4)
  - (Genus *Ceroglossus*)
- Tribe Carabini (Fig. 4.8)
  - Subtribe Calosomina (Fig. 5.7)
    - (Genera *Calosoma*, *Campalita*, etc.)
  - Subtribe Carabina (Fig. 5.8)
    - (Genera *Carabus*, *Limnocarabus*, *Damaster*, etc.)

9.4 Higher Classification of the Subtribe Carabina Based Mainly on Phylogenetic Trees Using the Mitochondrial *ND5* Gene

In this section we present a new classification system down to the generic level of the subtribe Carabina. The system is mainly based on phylogenetic trees based on analysis of the mitochondrial *ND5* gene, having been arranged according to the Linnean Hierarchy along the lines of the conventional criteria. This is still a tentative system and alternative systems are, of course, possible.

(1) The genus *Carabus* has been used in the broad sense, equivalent to the subtribe Carabina. In this system, Carabina (= genus *Carabus* s. lat.) is divided into a certain number of divisions and genera, and the genus *Carabus* is used only in a strict sense.

(2) Highly independent groups, or even lineages that contain only a single species, are treated as divisions whenever they appear to have emerged at the beginning of the explosive radiation of the Carabina. Each division is further divided into more than two subdivisions where necessary.

(3) A well-defined group within a division is regarded as a genus. Each genus is further divided into two or more subgenera if necessary.

(4) If morphological differences in two or more taxa are large enough in a given division, they are distinguished as distinct genera even though they belong to a single cluster on the phylogenetic tree (e.g., *Acathaicus* and *Acoptolabrus*). This is based on the assumption that a morphologically distinguishable genus (or sometimes genera) emerged from the maternal stem by type-switching.

(5) If two or more taxa that are recognized as being morphologically congeneric fall into different lineages on the tree, they are treated as distinct genera (e.g., the Chinese *Leptocarabus* species and those in other regions). This is really a conventional treatment. As discussed in Section 9.2, it is possible that the Chinese *Leptocarabus* have kept their ancestral form in all the present-day *Leptocarabus* species, and that *Rhigocarabus*, which is clustered with the Chinese *Leptocarabus*, was derived from the ancient Chinese proto-*Leptocarabus*. If this is the case, it is not necessary to create a distinct genus for the Chinese *Leptocarabus*.

A similar situation exists for *Megodontus germarii* and other *Megodontus* species. If *M. germarii* is the ancestral form of all the *Megodontus* species, there is no rationale for giving a distinct generic name to *M. germarii*. *Procerus* was derived from one of the *Megodontus* stems, and because of its considerable morphological difference from *Megodontus*, *Procerus* may be considered a distinct genus as defined in (4). This means that the treatment of the Chinese *Leptocarabus* and *Megodontus germarii* might be better considered operational than biological.

Since it was impossible to analyze the DNA of several genera, the following list does not cover all the taxa belonging to the Carabina.

A comparison of the morphological classification proposed by Imura in 1996 with the new system to which molecular phylogenetic data are introduced makes it clear that the two fundamentally agree. There are differences in some areas, however, exemplified by the fact that in the new system the Latitarsi was divided into many independent divisions. The Spinulati, meanwhile, was separated into two independent divisions, the Spinulati and the Lepidospinulati (Imura et al. 1998a) and a few genera in the Procrustimorphi were separated into distinct divisions.

Terminology for the supraspecific categories of the Carabina except for those properly described as genus or subgenus is confused. Some higher names with the rank of "division," "subdivision," "section," or "group" have been proposed by previous authors. In this book, we tentatively used such divisional names as the Lipastromorphi, the Latitarsi, and the Procrustimorphi, etc., most of which were originally settled between the genus *Carabus* (s. lat.) and its subgenera. According to Article 10.4 of the International Code of Zoological Nomenclature (ICZN), however, these names are deemed to be subgeneric names and destined to be the synonyms of certain subgenera described previously, even if they become available by satisfying the provisions of Article 10. In cases where the higher names were settled between subtribe and genus (the Spinulati, for example) they are not regulated by ICZN. However, their adoption and rejection are, if anything, complicated, since no type genus was designated.

To avoid further confusion, a new system was proposed by Imura (2002b); he once revoked all these synonymous- or non-regulated names and proposed to give applying the new divisional names between the subtribe Carabina and its components genera, under the concept of the "type genus." According to him, the new divisional names are indicated by compound words with the stem from that of the type genus and the suffix spelled "genici". For example, the division composed of the three genera, *Heterocarabus* Morawitz, 1886, *Chaetocarabus* Thomson, 1875, and *Platycarabus* Morawitz, 1886, is automatically named the "Chaetocarabigenici," because *Chaetocarabus* is the oldest name among the above three genera.

The benefit of this principle is that we can cope with the alternation of the component genera when it is necessary, and in view of the ICZN regulation the Imura's new system sounds more logical than the system we used in the text.

In the following list, we primarily enumerate the divisional names defined by Imura (2002b), showing those used in the text of this book in parentheses to avoid confusion.

Classification

Subtribe Carabina

Division Limnocarabigenici (= Lepidospinulati) (Fig. 5.8)
- Genus *Limnocarabus*
  - Subgenus *Limnocarabus* (northern Eurasia and adjacent islands; 1 or 2 species)
    - Type species: *L. (L.) clathratus*
  - Subgenus *Euleptocarabus* (Honshu, Japan; 1 species)
    - Type species: *L. (E.) porrecticollis*

Division Chaetocarabigenici (= Arciferi [partim]) (Fig. 5.11)
- Genus *Heterocarabus* (Asia Minor; 1 species)
  - Type species: *H. marietti*
- Genus *Chaetocarabus* (Europe; 2 species)
  - Type species: *C. intricatus*
- Genus *Platycarabus* (Europe; 5 species)
  - Type species: *P. depressus*

Division Hemicarabigenici (= Crenolimbi) (Fig. 5.12)
Genus *Hemicarabus* (Eurasia and North America; 4 species)
Type species: *H. nitens*
Genus *Homoeocarabus* (eastern Eurasia and North America; 1 species)
Type species: *H. maeander*

Division Ischnocarabigenici (= Archicarabomorphi [partim]) (Fig. 5.13)
Genus *Archicarabus* (Europe and North America; introduced species?; about 10 species)
Type species: *A. nemoralis*
Genus *Gnathocarabus* (northeast Iran; 1 species)
Type species: *G. kusnetzovi*

Division Apotomopterigenici (= Spinulati) (Figs. 5.8 and 5.14)
Genus *Apotomopterus* (China and adjacent regions; over 50 species)
Type species: *A. prodigus*
Genus (undescribed) (China and Taiwan; over 10 species)
Representative species: *A. sauteri*
Genus *Dolichocarabus* (China and adjacent regions; over 10 species)
Type species: *D. delavayi*
Genus *Taiwanocarabus* (Taiwan; 1 species)
Type species: *T. masuzoi*

Division Lipastrigenici (= Lipastromorphi) (Fig. 5.17)

Subdivision A (group of *Cyclocarabus*)
Genus *Cyclocarabus* (northwestern Tianshan Mountains; over 10 species)
Type species: *C. namanganensis*
Genus *Ophiocarabus* (Tianshan Mountains and neighboring areas; over 20 species)
Type species: *O. striatus*

Subdivision B (group of *Lipaster*)
Genus *Lipaster* (northeastern Turkey to Caucasian region; 1 species)
Type species: *L. stjernvalli*
Genus *Mimocarabus* (northeastern Turkey to southern Turkmenistan; 5 species)
Type species: *M. maurus*
Genus *Lyperocarabus* (Ukraine and the adjacent areas; 1 species)
Type species: *L. estreicheri*
Genus *Trachycarabus* (eastern Europe, southwestern Russia, and northwestern Turkey; 1 or more species)
Type species: *T. scabriusculus*
Genus *Morphocarabus* (Eurasia; 5–10 species)
Type species: *M. monilis*
Genus *Apostocarabus* (Eurasia; 1 or more species)
Type species: *A. odoratus*
Genus *Pancarabus* (Eurasia; a few species)
Type species: *P. aeruginosus*
Genus *Ancylocarabus* (Central Asia; 2 species)
Type species: *A. tarbagataicus*
Genus *Leptinocarabus* (eastern Eurasia; a few species)
Type species: *L. venustus*

Division Tachypigenici (= Latitarsi [partim]) (Fig. 5.19a)
Genus *Tachypus* (= *Autocarabus*) (Europe; 3 species)
Type species: *T. auratus*

Division Mesocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
Genus *Mesocarabus* (Europe; 3 species)
Type species: *M. problematicus*

Division Orinocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
Genus *Orinocarabus* (Europe; about 10 species)
Type species: *O. sylvestris*

Division Cavazzutiocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
Genus *Cavazzutiocarabus* (western part of the Alps; 1 species)
Type species: *C. latreillei*

Division Tmesicarabigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
Genus *Tmesicarabus* (the Pyrenees; 1 species)
Type species: *T. cristoforii*

Division Eurycarabigenici (= Latitarsi [partim]) (Fig. 5.19a)
Genus *Eurycarabus* (North Africa; 2 species)
Type species: *E. famini*

Division Nesaeocarabigenici (= Latitarsi [partim]) (Fig. 5.19a)
Genus *Nesaeocarabus* (Canary Islands; 3 species)
Type species: *N. interruptus*

Division Cytilocarabigenici (= Latitarsi [partim]) (Fig. 5.19a)
Genus *Cytilocarabus* (Asia Minor to the Caucasian region; a few species)
Type species: *C. cribratus*

Division Pentacarabigenici (= Latitarsi [partim]) (Fig. 5.19a and d)
Genus *Pentacarabus* (Honshu, Japan; 1 species)
Type species: *P. harmandi*

Division Pachycarabigenici (= Latitarsi [partim]) (Fig. 5.10a)
Genus *Pachycarabus* (Caucasia; 5 species)
Type species: *P. staehlini*

Division Pachystigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
- Genus *Euporocarabus* (Europe; 1 species)
  - Type species: *E. hortensis*
- Genus *Pachystus* (eastern Europe to Asia Minor; 5 species)
  - Type species: *P. hungaricus*
- Genus *Phricocarabus* (Europe; 1 species)
  - Type species: *P. glabratus*

Division Meganebriigenici (= Latitarsi [partim]) (Fig. 5.19a)
- Genus *Meganebrius* (Himalaya Mountains and the neighboring areas; about 20 species)
  - Type species: *M. indicus*

Division Piocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and b)
- Genus *Titanocarabus* (China; 2 species)
  - Type species: *T. titanus*
- Genus *Qinlingocarabus* (China; 5 species)
  - Type species: *Q. kitawakianus*
- Genus *Piocarabus* (southern Siberia to northern China; 1 species)
  - Type species: *P. vladimirskyi*

Division Leptocarabigenici (= Latitarsi [partim]) (Figs. 5.19 and 5.21)
- Genus *Aulonocarabus*
  - Subgenus *Weolseocarabus* (Korean Peninsula; 1 species)
    - Type species: *A. (W.) koreanus*
  - Subgenus *Adelocarabus* (Korean Peninsula and adjacent areas; a few species)
    - Type species: *A. (A.) semiopacus*
  - Subgenus *Aulonocarabus* (eastern Eurasia and adjacent islands; a few species)
    - Type species: *A. (A.) canaliculatus*
  - Subgenus *Baptaulonocarabus* (northern and eastern Eurasia, Alaska; a few species)
    - Type species: *A. (B.) truncaticollis*
- Genus *Leptocarabus* (Japan and adjacent islands; 5 species)
  - Type species: *L. (L.) procerulus*

Division Rhigocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and c)
- Genus *Zhongdianocarabus* (China; 1 or more species)
  - Type species: *Z. handelmazzettii*
- Genus *Batangocarabus* (China to northern Myanmar; 1 or more species)
  - Type species: *B. itzingeri*
- Genus *Araeocarabus* (China; 1 or more species)
  - Type species: *A. roborowskii*
- Genus *Litangocarabus* (China; 1 or more species)
  - Type species: *L. indigestus*
- Genus *Deqenocarabus* (China; 1 or more species)
  - Type species: *D. rhododendron*
- Genus *Zheduocarabus* (China; 1 species)
  - Type species: *Z. zheduoshanensis*
- Genus *Sinoleptocarabus* (China; 2 species)
  - Type species: *S. yokoae*
- Genus *Tibetorinocarabus* (China; 1 species)
  - Type species: *T. laotse*
- Genus *Syzygocarabus* (China; 1 or more species)
  - Type species: *S. cateniger*
- Genus *Mianningocarabus* (China; 1 or more species)
  - Type species: *M. confucius*
- Genus *Tachycarabus* (China; 1 or more species)
  - Type species: *T. pusio*
- Genus *Sangocarabus* (China; 1 species)
  - Type species: *S. maleki*
- Genus *Hypsocarabus* (China; 4–5 species)
  - Type species: *H. latro*

Division Tomocarabigenici (= Latitarsi [partim]) (Fig. 5.19a and d)
- Genus *Rhytidocarabus* (Asia Minor and adjacent areas; 1 or more species)
  - Type species: *R. scabripennis*
- Genus *Glossocarabus* (Caucasian region; 1 species)
  - Type species: *G. decolor*
- Genus *Coreocarabus* (Korean Peninsula and adjacent areas; 1 species)
  - Type species: *C. fraterculus*
- Genus *Tomocarabus* (western Eurasia; 1 or more species)
  - Type species: *T. convexus*
- Genus *Asthenocarabus* (northern Japan; 1 species)
  - Type species: *A. opaculus*
- Genus *Callistocarabus* (eastern Europe; 1 species)
  - Type species: *C. marginalis*
- Genus *Scambocarabus* (eastern Eurasia; 4–5 species)
  - Type species: *S. kruberi*
- Genus *Tanaocarabus* (North America; 1–2 species)
  - Type species: *T. sylvosus*
- Genus *Diocarabus* (Eurasia; 1 or more species)
  - Type species: *D. loschnikovi*
- Genus *Watanabeocarabus* (southern Siberia; 1 or more species)
  - Type species: *W. slovtzovi*
- Genus *Neocarabus* (North America to the Aleutians; 1 species)
  - Type species: *N. taedatus*
- Genus *Durangocarabus* (southwestern North America; 1 species)
  - Type species: *D. forreri*
- Genus *Zoocarabus* (Tianshan Mountains; 1 or more species)
  - Type species: *Z. bogdanowi*
- Genus *Ulocarabus* (Tianshan Mountains; 2 species)
  - Type species: *U. stschurovskii*

Genus *Coccocarabus* (Tianshan Mountains; 1 species)
Type species: *C. minimus*
Genus *Carpathophilus* (eastern Europe; 1 species)
Type species: *C. linnei*
Genus *Semnocarabus* (Tianshan Mountains; a few species)
Type species: *S. regulus*

Division Carabigenici (= Digitulati) (Figs. 5.27–5.30)
Genus *Carabus* (Eurasia and adjacent islands; 7–8 species)
Type species: *C. granulatus*
Genus *Archaeocarabus* (China; about 50 species)
Type species: *A. relictus*
Genus *Lichnocarabus* (North America; 2 species)
Type species: *L. vinctus*
Genus *Eucarabus* (western and eastern Eurasia; about 20 species)
Type species: *E. ullrichi*
Genus *Isiocarabus* (eastern China, Korean Peninsula, and Cheju-do Island; about 10 species)
Type species: *I. fiduciarius*
Genus *Ohomopterus* (Japan; 16 species)
Type species: *O. dehaanii*
Genus *Acrocarabus* (Tianshan Mountains; 2 species)
Type species: *A. guerini*

Division Ctenocarabigenici (= Procrustimorphi [partim]) (Fig. 5.31)
Genus *Rhabdotocarabus* (North Africa and Iberian Peninsula; 1 species)
Type species: *R. melancholicus*
Genus *Ctenocarabus* (Iberian Peninsula; 1 species)
Type species: *C. galicianus*

Division Hygrocarabigenici (= Arciferi [partim]) (Fig. 5.11)
Genus *Hygrocarabus* (Europe; 2 species)
Type species: *H. nodulosus*

Division Cathopliigenici (= Procrustimorphi [partim]) (Fig. 5.31)
Genus *Cathoplius* (North Africa; 1–2 species)
Type species: *C. asperatus*

Division Procrustigenici (= Procrustimorphi) (Fig. 5.31)
Subvidision A (European group or *Macrothorax* group) (Fig. 5.33)
Genus *Macrothorax* (areas around the Mediterranean Sea; 3 species)
Type species: *M. morbillosus*
Genus *Chrysocarabus*
Subgenus *Chrysocarabus* (Europe; 3–4 species)
Type species: *C. (C.) auronitens*
Subgenus *Chrysotribax* (southwestern Europe; 2 species)
Type species: *C. (C.) hispanus*
Genus *Imaibius* (Kashmir to the Himalayas; about 20 species)
Type species: *I. barysomus*
Genus *Sellaecarabus* (northwestern Italy; 2 species)
Type species: *S. olympiae*
Genus *Sphodristocarabus* (Asia Minor to northern Iran; about 20 species)
Type species: *S. adamsi*

Subdivision B (Caucasian group or *Tribax* group) (Fig. 5.34)
Genus *Microplectes* (western to central Caucasian region; 2 species)
Type species: *M. riedeli*
Genus *Cechenochilus*
Subgenus *Cechenochilus* (Caucasian region; 1 species)
Type species: *C. boeberi*
Subgenus *Procechenochilus* (Caucasian region; 1 species)
Type species: *C. heydenianus*
Genus *Tribax* (Caucasian region to northeastern Turkey; about 50 species)
Type species: *T. puschkini*

Subdivision C (Eurasian group or *Procrustes* group) (Fig. 5.35)
Genus *Pachycranion*
Subgenus *Aulacocarabus* (Caucasian region to northeastern Turkey; 2 species)
Type species: *P. (A.) septemcarinatum*
Subgenus *Pachycranion* (Eurasia; 10 or more species)
Type species: *P. (P.) schoenherri*
Subgenus *Proteocarabus* (western to central Eurasia; 1 species)
Type species: *P. (P.) violaceum*
Genus *Protomegodontus* (south-central Europe; 1 species)
Type species: *P. germarii*
Genus *Procerus* (Balkan Peninsula to Asia Minor and the Caucasian region; 2 or more species)
Type species: *P. gigas*
Genus *Megodontus* (areas around the Adriatic Sea to northern Iran; a few species)
Type species: *M. caelatus*
Genus *Procrustes*
Subgenus *Procrustes* (Europe to Asia Minor; nearly 10 species)
Type species: *P. (P.) coriaceus*
Subgenus *Creprostus* (southwest of the Caspian Sea; 1 species)
Type species: *P. (C.) talyschensis*

Subgenus *Lamprostus* (Balkan Peninsula to Asia Minor; nearly 10 species)
Type species: *P. (L.) torosus*
Subgenus *Oxycarabus* (Turkey; one species)
Type species: *P. (O.) saphyrinus*
Subgenus *Chaetomelas* (Asia Minor to the Middle East; about 5 species)
Type species: *P. (C.) ehrenbergi*

Subdivision D (Tianshanese group or *Cratocephalus* group) (Fig. 5.36)
Genus *Cratophyrtus* (western Tianshan Mountains; about 5 species)
Type species: *C. kaufmanni*
Genus *Pantophyrtus* (western Tianshans; about 5 species)
Type species: *P. turcomannorum*
Genus *Cratocarabus* (central Tianshans; about 5 species)
Type species: *C. puer*
Genus *Cratocechenus* (northwestern to eastern Tianshans; a few species)
Type species: *C. akinini*
Genus *Cratocephalus* (central to northeastern Tianshans; 5–6 species)
Type species: *C. cicatricosus*
Genus *Eotribax* (central Tianshans; about 10 species)
Type species: *E. eous*
Genus *Deroplectes* (Pamir Plateau and adjacent regions; about 5 species)
Type species: *D. sphynx*
Genus *Cechenotribax* (central to northeastern Tianshans; 1 species)
Type species: *C. petri*
Genus *Alipaster* (Tianshan Mountains, China; 5–6 species)
Type species: *A. pupulus*
Genus *Leptoplesius* (eastern Tianshans; about ten species)
Type species: *L. marquardti*

Subdivision E (Chinese group or *Damaster* group) (Fig. 5.37)
Genus *Pseudocoptolabrus* (southwestern China to northern Myanmar; 5 species)
Type species: *P. taliensis*
Genus *Megodontoides* (Sichuan, China; one species)
Type species: *M. erwini*
Genus *Acathaicus* (Gansu and Sichuan, China; 1 species)
Type species: *A. alexandrae*
Genus *Coptolabrodes* (Shaanxi, China; 1 species)
Type species: *C. haeckeli*
Genus *Imaibiodes* (Yunnan, China; 1 species)
Type species: *I. businskyi*
Genus *Lasiocoptolabrus* (Shaanxi, China; 1 species)
Type species: *L. sunwukong*
Genus *Aristocarabus* (Sichuan to Hubei, China; 1 species)
Type species: *A. viridifossulatus*
Genus *Pagocarabus* (northern China; 1 or more species)
Type species: *P. crassesculptus*
Genus *Shunichiocarabus* (Sichuan and Hubei, China; 1 species)
Type species: *S. uenoianus*
Genus *Pseudocranion* (Sichuan and adjacent regions, China; about 20 species)
Type species: *P. gansuense*
Genus *Neoplesius* (Xizang to Qinghai, Sichuan, and Yunnan, China; 20 or more species)
Type species: *N. wagae*
Genus *Eocechenus* (Xizang to Qinghai and Sichuan, China; about 10 species)
Type species: *E. kaznakovi*
Genus *Eccoptolabrus* (Gansu and Sichuan, China; 1 species)
Type species: *E. exiguus*
Genus *Calocarabus* (Qinghai, Sichuan, and Gansu, China; about 10 species)
Type species: *C. gratus*
Genus *Damaster* (Japan to southern Kuril Islands; 1 species)
Type species: *D. blaptoides*
Genus *Cephalornis* (Gansu and Sichuan, China; 1 species)
Type species: *C. potanini*
Genus *Shenocoptolabrus* (Hubei and Sichuan, China; 1 species)
Type species: *S. osawai*
Genus *Eupachys* (northern China to southern Siberia; 1 or 2 species)
Type species: *E. glyptopterus*
Genus *Acoptolabrus* (areas around the Sea of Japan; about 7 species)
Type species: *A. schrencki*
Genus *Cathaicus* (northern China; 1 species)
Type species: *C. brandti*
Genus *Coptolabrus* (China and adjacent areas; 14 species)
Type species: *C. smaragdinus*

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Further Reading for Chapter 3

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