Discrepancy between Mitochondrial and Nuclear DNA Phylogenies in the Genus *Mesechthistatus* (Coleoptera, Cerambycidae)

Hiroshi Nakamine

Sanda Municipal Arimafuji Nature Study Center, Fukushima 1091–2,
Sanda, Hyôgo, 669–1313 Japan

and

Makio Takeda

Laboratory of Insect Science, Faculty of Agriculture, Kobe University,
Rokko-dai 1–1, Nada, Kobe, Hyôgo, 657–8501 Japan

Abstract   Nucleotide sequences of the mitochondrial cytochrome oxidase subunit I gene (COI) and the nuclear internal transcribed spacer 1 (ITS1) gene are examined for four *Mesechthistatus* species endemic to Japan. Though the mitochondrial gene tree does not recover the monophyly of each species except for *M. binodosus*, the nuclear gene genealogy reveals the monophyly of all four species and sister relationships between *M. binodosus* and *M. fujisanus* and between *M. furciferus* and *M. tanguchii*. Altitudal ditributions of the four species are also examined.

Introduction

The longicorn beetles belonging to the genus *Mesechthistatus* Breuning, 1950, are flightless because of atrophy of the hindwings. This genus includes four species based on external morphology and distributional patterns: *M. binodosus* (Waterhouse, 1881), *M. furciferus* (Bates, 1884), *M. taniguchii* (Seki, 1944), and *M. fujisanus* Hayashi, 1957. Although *M. yamahoi* (Mitono, 1943) was described from Taiwan, its existence is currently in doubt, since no subsequent records have been available since the time of original entry (Hasegawa, 2007). The four *Mesechthistatus* species are endemic to Japan, and are distributed almost parapatrically in Honshu and Sado Island (Fig. 1). Each of the species can be clearly discriminated from the others based on external morphology.

It is true that the molecular phylogenetic analysis is a powerful tool to reveal interspecific relationships, but gene genealogies are sometimes incongruent between mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) as is previously reported

(*e.g.*, Sota & Vogler, 2001; Shaw, 2002; Kiyoshi & Sota, 2006). We already reported molecular phylogenetic relationships of the four *Mesechthistatus* species inferred from mitochondrial COI gene (Nakamine & Takeda, 2008). In this paper, the genealogies of nuclear and mitochondrial DNA will be compared to resolve the non-monophyly of mitochondrial haplotypes and to reconstruct the species relationships. Altitudinal distributions of these species are also investigated to examine the degree of range overlaps which would facilitate interspecific hybridization.

Materials and Methods

Samples and sequence analysis of DNA

The data for 21 specimens analyzed in this study are listed in Table 1, and the localities where they were collected are shown in Fig. 1. The beetles were immediately fixed in 95–99.5% ethanol and preserved in the same solution until dissection. Total genomic DNA was extracted from cephalic and thoracic muscles by using GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, Inc.). Each genomic DNA sample was finally dissolved in 200 μl elution buffer.

Fragment of the mitochondrial DNA containing 1144 bp of the cytochrome oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) with an originally designed primer set (Nakamine & Takeda, 2008) as follows: KobCI1.2 (5′-TAA GAA GAA TTG TAG AAA ATG G-3′) and YhzCI2.2 (5′-TGT AGC GAT TTC TAA AAA AG G-3′). The fragment of the nuclear DNA containing about 1000 bp of the 18S rRNA, internal transcribed spacer 1 (ITS1) and 5.8S rRNA gene was amplified from the total DNA solution by PCR with a newly designed primer set as follows: 18SrRNA (5′-TAG TGA GGT CTT CGG ACT GG-3′) and 5.8SrRNA (5′-AAT GTG CGT TCG AAA TGT CG-3′).

The subcloning of purified PCR products of ITS1 region from agarose gels were carried out into pBluescript vector, infecting competent cells (DH5α, *Escherichia coli*). After cultivation, one colony was picked up from the plate and incubated in cultivation solution. The plasmid was prepared for sequencing by alkaline lysis mimi-prep method. The plasmid containing ITS1 region was amplified by using a BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems) with primer 5.8SrRNA. A partial sequence of the ITS1 gene was determined by a ABI PRISM® 310 Genetic Analyzer or ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Direct sequencing of the COI gene fragment was performed by the same method as above with primer set KobCI1.2 and YhzCI2.2.

Phylogenetic analysis

The sequences of COI gene were aligned without alignment program, because the COI sequences had no indels (insertions/deletions). The 555–585 bp sequences of ITS 1 region were aligned by using CLUSTAL W version 1.83 (Thompson *et al*., 1994) with default setting. The maximum likelihood (ML) trees were constructed using the
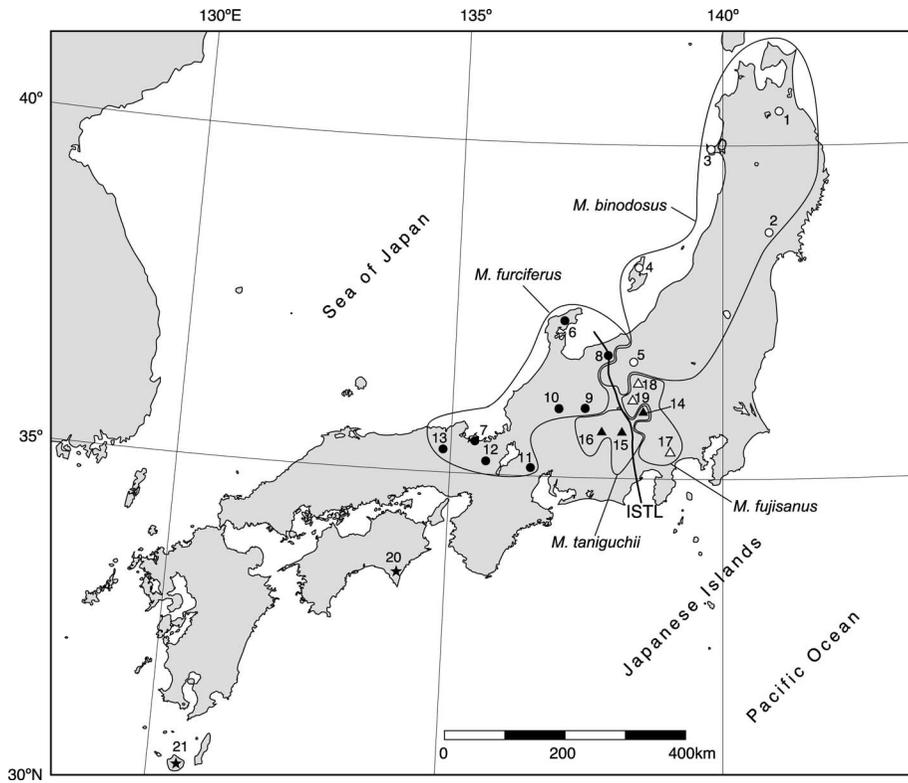
Fig. 1. The distributional areas of four *Mesechthistatus* species enclosed by lines and the localities where specimens of the four species were collected. Open circles, localities of *M. binodosus* specimens collected; closed circles, localities of *M. furciferus*; open triangles, localities of *M. fujisanus*; closed triangles, localities of *M. taniguchii* and closed stars, localities of *Parechthistatus* and *Hayashiechthistatus* used as outgroup specimens for phylogenetic analysis. Locality numbers correspond to the number in Table 1 and Fig. 2. ISTL: Itoigawa-Shizuoka Tectonic Line.

GARLI version 0.96 beta (ZWICKL, 2006). The substitution model used in the ML analysis was the HKY85＋G＋I selected in COI gene and the K80＋G selected in ITS 1 region with hierarchical likelihood ratio tests (hLRTs) using the Modeltest version 3.06 (POSADA & CRANDALL, 1998). The bootstrap test was executed on 1000 replicates with default resample proportion value (1.0). To obtain the bootstrap proportions, we used PAUP* version 4.0b10 (SWOFFORD, 2002). *Parechthistatus gibber* and *Hayashiechthistatus inexpectus* were used as outgroups.

The constructed trees of COI and ITS were examined for homogeneity by Incongruence Length Difference (ILD) test (FARRIS *et al*., 1994, 1995) with the number of ILD replicates at 100, with the number of random taxon addition replicates at 10 per ILD replicate on PAUP* version 4.0b10.

Table 1. The specimens analyzed in this study.

Species and Specimen no. in Map	Isolate Code	Locality	DDBJ/EMBL/GenBank Accession number COI/ITS
Mesechthistatus binodosus			
1	BINher	Mt. Herai, Aomori	AB278237/AB428382
2	BINfgt	Mt. Funagata, Miyagi	AB278243/AB428383
3	BINsiz	Shinzan, Akita	AB278249/AB428384
4	BINddy	Mt. Donden, Sado Is., Niigata	AB278265/AB428385
5	BINayg	Akiyamagô, Nagano	AB278290/AB428387
M. furciferus			
6	FURhrz	Mt. Hôryû, Ishikawa	AB278333/AB428388
7	FURaob	Mt. Aoba, Fukui	AB278349/AB428389
8	FURamk	Amakazari, Nagano	AB278353/AB428390
9	FURhwd	Hiwadakôgen, Gifu	AB278374/AB428391
10	FURkam	Kanmuriyama Pass, Gifu	AB278381/AB428392
11	FURokd	Mt. Oike, Mie	AB278384/AB428393
12	FURasy	Ashû, Kyoto	AB278398/AB428394
13	FURiti	Itoikeikoku, Hyôgo	AB278406/AB428395
M. taniguchii			
14	TANydh	Yadehara rindô, Nagano	AB278413/AB428396
15	TANnkz	Nakazawa Pass, Nagano	AB278418/AB428397
16	TANodr	Ôdaira Pass, Nagano	AB278422/AB428398
M. fujisanus			
17	FUJddr	Dôdaira, Kanagawa	AB278437/AB428399
18	FUJtni	Takinoirisawa, Nagano	AB278445/AB428400
19	FUJsjr	Sanjirô, Nagano	AB278447/AB428401
Out group			
Parechthistatus gibber			
20	GIBnny	Mt. None, Kôchi	AB278516/AB428411
Hayashiechthistatus inexpectus			
21	INEydg	Yodogawa, Yaku Is., Kagoshima	AB278551/AB428415

Altitudinal distributions of the four species

    We have registered 231 data of mitochondrial COI gene sequence of four *Mesechthistatus* species to DDBJ/NCBI/EMBL GenBank (accession numbers AB278221–AB28451), and the latitude and longitude data of collected localities were registered together. The number of collected localities: 46 for *M. binodosus*, 49 for *M. furciferus*, 16 for *M. taniguchii*, and 11 for *M. fujisanus*. To investigate altitudinal distributions of the four species, the altitude and longitudinal data from each collected locality were obtained by using Denshi Kokudo web system (http://cyberjapan.jp/).
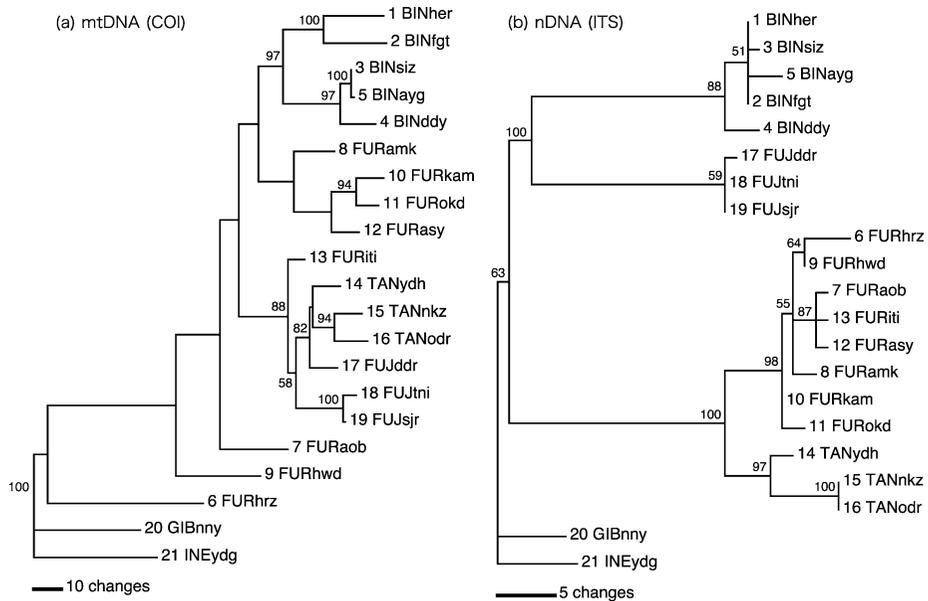
Fig. 2. Maximum likelihood trees based on the mitochondrial COI gene sequences (a) and the nuclear ITS gene sequences (b) of the four *Mesechthistatus* species, *P. gibber* and *H. inexpectus*. The number in each node indicates the bootstrap value (when > 50%). The locality numbers before the specimen identity corresponds to the number for locality in Table 1 and Fig. 1.

Results

Molecular phylogeny

Figure 2 shows the maximum likelihood phylogenetic trees. The mtDNA tree (Fig. 2a) revealed that only the haplotypes from *M. binodosus* (BIN) were monophyletic, whereas monophyly of haplotypes from each of the other three species, i.e., *M. furciferus* (FUR), *M. tanguchii* (TAN), and *M. fujisanus* (FUJ), was not recovered. In contrast, the nDNA tree (Fig. 2b) revealed the monophyly of sequences from each of the four species with the sister species relations between *M. binodosus* (BIN) and *M. fujisanus* (FUJ) and between *M. furciferus* (FUR) and *M. tanguchii* (TAN). The ILD test of mtDNA vs. nDNA sequence data indicated significant incongruence of phylogenetic information contents between these data sets (P=0.001).

Vertical distribution of the four Mesechthistatus *species*

Figure 3 shows altitudinal distributions of four *Mesechthistatus* species. *M. binodosus* and *M. furciferus* showed wide altitudinal ranges between approximately 0 m to 1,800 m, whereas *M. tanguchii* and *M. fujisanus* were more confined to higher altitudes from approximately 1,000 m to 1,800 m. The distributions of *M. binodosus* and
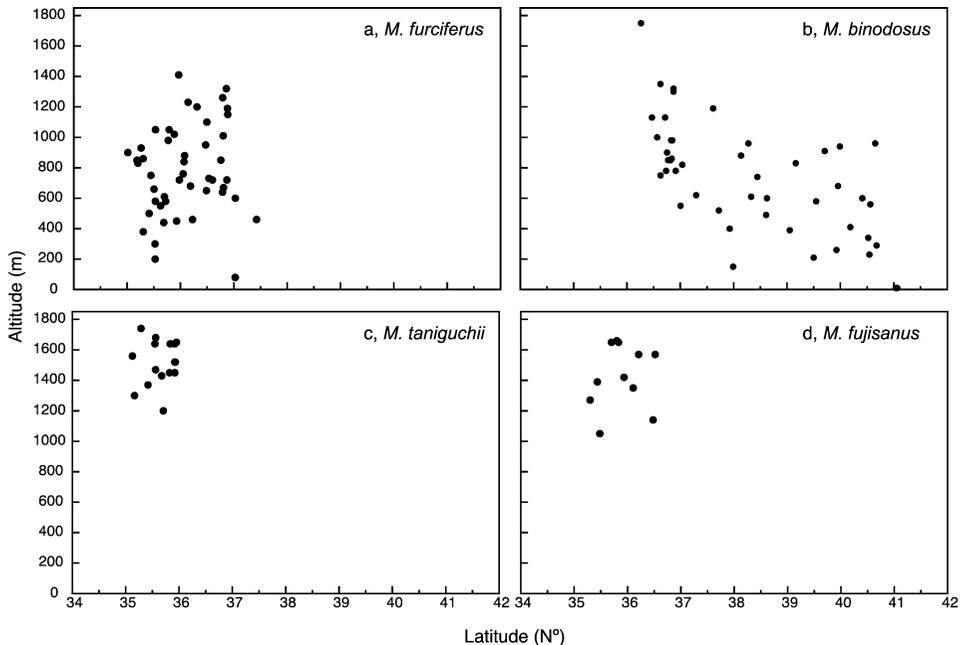
Fig. 3.   Altitudinal distributions of four *Mesechthistatus* species. a, *M. furciferus*; b, *M. binodosus*; c,
     M. taniguchii; d, *M. fujisanus*.

M. fujisanus, and of *M. furciferus* and *M. tanguchii* are vertically overlapping, respectively, at the boundaries of horizontal distribution.

Discussion

The incongruence between a genealogy based on the mitochondrial DNA and that on nuclear genes can be explained by assuming introgressive hybridization and the lineage sorting of ancestral polymorphism (*e.g.*, SOTA & VOGLER, 2001). NAKAMINE & TAKEDA (2008) have already investigated the mitochondrial gene genealogy of *Mesechthistatus* for more samples from wider sampling areas and inferred that some mitochondrial haplotype of *Mesechthistatus* species were derived from introgressive hybridizations. In fact, putative hybrid specimens were collected from the species boundaries of *Mesechthistatus* (*e.g.*, TAKAKUWA, 1988; TAKAKUWA *et al*., 2004).

Mesechthistatus binodosus and *M. fujisanus* are distributed in the area east of the Itoigawa-Shizuoka Tectonic Line (ISTL), whereas *M. furciferus* and *M. taniguchii* are distributed west of this line (Fig. 1). The genealogical tree of the nuclear ITS (Fig. 2) indicated the sister relationships between *M. binodosus* and *M. fujisanus* and between *M. furciferus* and *M. taniguchii*, which are consistent with the distribution patterns. Thus,

ISTL has been the dispersal barrier for *Mesechthistatus* since the initial differentiation of lineages.

Differences have been found in larval feeding habits among the four species that exhibit different altitudinal ranges. Although *M. binodosus* and *M. furciferus* are mainly found in beech forest belts, their habitat ranges from lowlands to the lower belt of conifer forests. *M. taniguchii* and *M. fujisanus* are distributed between the high altitude beech forest belt and conifer forest belt. *Mesechthistatus* larvae feed on the woody part of dead trees, though different species prefer different tree species (HASEGAWA, 2007). *M. binodosus* and *M. furciferus* infest various species of broadleaved trees, whereas *M. taniguchii* and *M. fujisanus* are hosted by *Abies* spp., *Tsuga* spp., *Picea* spp., and other varieties of Pinaceae, as well as dead broadleaved trees. The habitat and host choices of *M. taniguchii* and *M. fujisanus* indicate that they are adapted to high altitudes and cold climate. Their speciation might occur during the glacial periods in the Pleistocene, because the mitochondrial haplotypes of *Mesechthistatus* started to diverge at the end of the Pliocene (NAKAMINE & TAKEDA, 2008). A *Mesechthistatus* ancestor might originally use dead broadleaved trees, but with the arrival of a glacial period, its host range might be broadened to include conifers.

The majority of Cerambycidae feeding dead trees are found in the subcortical tissue (HANKS, 1999). In comparison to sapwood and heartwood containing mainly lignin and cellulose, subcortical tissue is richer in nutrients than the core and surface parts. However, larvae of the four *Mesechthistatus* species are usually found in wood of advanced decomposition stage like dead sapwood and heartwood that has fallen to the ground and rotten roots buried underground (K. MORI & H. NAKAMINE, unpublished observation). Cortical tissue of dead conifer in cold areas tends to peel off easily, presenting problems to cerambycid larvae seeking subcortical tissue to feed. However, *Mesechthistatus* makes use of the woody part, and this might be a "pre-adaptation" that facilitated their use of a new host, i.e., conifers. In order to understand the evolution of species in *Mesechthistatus*, it will be necessary to conduct a more comprehensive molecular phylogenetic analysis and ecological research.

要　　約

中峰　空・竹田真木生: コブヤハズカミキリ属の分子系統の不一致. ―― 日本産コブヤハズカミキリ属4種について，ミトコンドリア DNA の COI 部分配列と核 DNA の ITS1 領域を用いて

分子系統解析を行い，得られた二つの系統樹を比較した．その結果，ミトコンドリア DNA では
コブヤハズのみが単系統性を示したが，核 DNA では 4 種すべてが単系統性を示した．さらに核
DNA の系統解析から，コブヤハズとフジコブヤハズが，マヤサンコブヤハズとタニグチコブヤ
ハズがそれぞれ姉妹種関係にあることが示唆された．

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