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Phylogenetic Analysis of the Lamiine Genus *Anoplophora* and its Relatives (Coleoptera, Cerambycidae) Based on the Mitochondrial COI Gene

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Abstract The phylogenetic relationships of the lamiine genus *Anoplophora* and its relatives have been analyzed on the basis of the mitochondrial cytochrome oxidase subunit I (COI) gene sequences of 114 specimens representing 13 species of *Anoplophora,* two species of *Dolichoprosopus* and one species each of *Monochamus*, *Calloplophora* and *Pseudonemophas.* The two species, *Apriona germari* and *Batocera celebiana* are also analyzed as outgroup for phylogenetic analysis. The results show that the specimens analyzed, excepting *Calloplophora* and *Pseudonemophas*, are separated into six lineages within a short time. Four lineages (Lineage 1–4) include only the species of *Anoplophora,* and the *Dolichoprosopus* species also form an independent lineage (Lineage 5), but the last one (Lineage 6) consists of the species of two genera, *Anoplophora* and *Monochamus.* The *Calloplophora* and *Pseudonemophas* species form basal lineages are largely separated from the above-mentioned six lineages. The Lineage 1 further divided into two sub-lineages and some groups. On the basis of these results, the origin of the *Anoplophora* species distributed in the Japanese Islands is discussed.

Introduction

In spite of the fact that the lamiine genus *Anoplophora* includes many economically important species attacking several kinds of fruit trees and also roadside trees, their taxonomic status has not been settled for a long time. In 2002, however, LINGAFELTER and HOEBEKE published an important world revision of the genus *Anoplophora*. It was an excellent work and almost all of the taxonomic problems seemed clarified. On the other hand, their conception of the generic and species ranges seems to be rather wide, and we cannot agree completely with their opinion in some aspects. In this study, we

used mitochondrial DNA sequences, a more objective tool, to elucidate the phylogenetic relationships of the lamiine genus *Anoplophora* and its allied genera. Although we were unable to make complete sampling, essential samples including the type species, *A. stanleyana* and various local specimens from the Japanese Islands were included in this study. We obtained certain conclusion about the genus *Anoplophora* with some relative genera, and would like to discuss the result hereinafter.

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Materials and Methods

1. Specimens examined.

Almost all the specimens examined were newly collected for the purpose of DNA analysis. Collected specimens were immediately dipped and killed in 99.9% ethanol and stored until use. Most of the collecting localities are plotted in the map of Fig. 1. Symbols in the brackets of the specimens examined show the sample numbers in Fig. 2. The nucleotide sequence data reported in this paper have appeared in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession numbers AB439136–AB 439205, AB448738 and AB448739.

1. *Anoplophora chinensis* (FORSTER, 1771)

Cerambyx chinensis FORSTER, 1771, Nov. Spec. Ins., p. 39; type area: China.

Distribution. China, Indochina (?).

Specimens examined. [00-17c], [00-17d]: Botanical garden, Shanghai, China, 17. VII. 2000, N. OHBAYASHI; [02-05]: Wuyishan, Fujian, China, 30. V. 2002, N. OHBAYASHI.

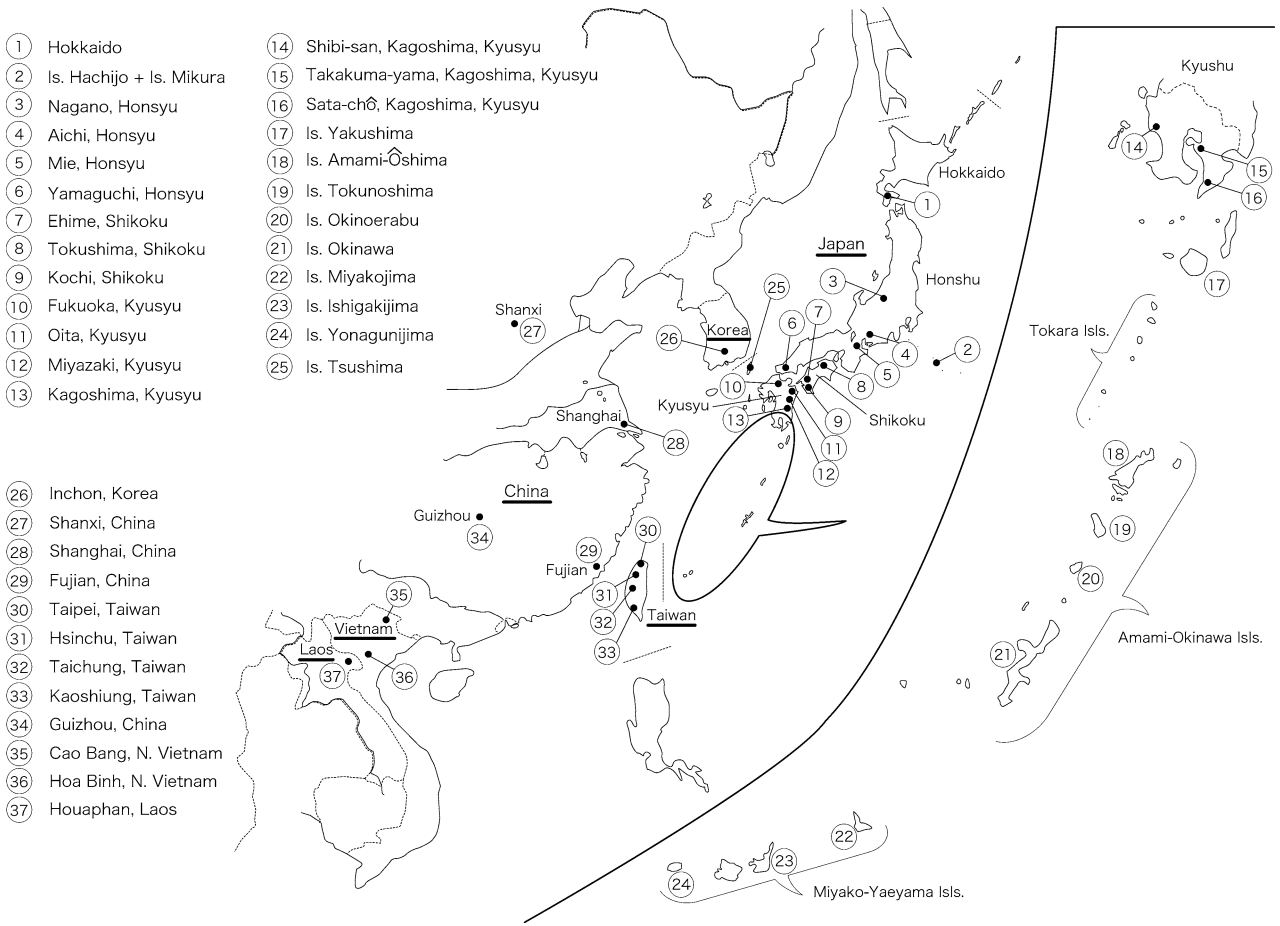

Fig. 1. Map showing the localities of the specimens examined.

2. *Anoplophora malasiaca* (THOMSON, 1865)

Calloplophora malasiaca THOMSON, 1865, Syst. Ceramb., p. 553; type area: Malasia? (Malas).

Distribution. Japan (Hokkaido, Honshu, Shikoku, Izu Isls., Is. Tsushima, Is. Yakushima, Is. Amami-ôshima, Is. Okinawa), Korea, China (?), Malaysia (?).

Specimens examined. [01-03a]: Esashi-chô, Hokkaido, Jpn., 28. VII. 2001, Y. KAMITE; [01-03b]: Kaminokuni-chô, Hokkaido, Jpn., 28. VII. 2001, Y. KAMITE; [01-02a], [01-02b]: Is. Hachijô-jima, Tokyo, Jpn., 22. VII. 2001, K. NAGATA; [02-03]: Is. Mikura-jima, Tokyo, Jpn., 26. V. 2002, N. OHSHIGE; [01-05a], [01-05b]: Komagane-shi, Nagano Pref., Jpn., 9. VIII. 2001, Y. NOTSU; [00-27c]: Kasugai-shi, Aichi Pref., Jpn., 22. VI. 2000, Y. ARITA: [00-27d]: Kasugai-shi, Aichi Pref., Jpn., 7. VII. 2000, Y. ARITA; [00-27f]: Nagakute-chô, Aichi Pref., Jpn., 18. VII. 2000, Y. UTSUNOMIYA; [00-27a]: Matsusaka-shi, Mie Pref., Jpn., 14. VII. 2000, Y. UTSUNOMIYA; [00-27b]: Matsusaka-shi, Mie Pref., Jpn., 20. VI. 2000, Y. UTSUNOMIYA; [00-27e]: Sakauchi-river, Matsusaka-shi, Mie Pref., Jpn., 13. VII. 2000, Y. UTSUNOMIYA; [00-17k], [00-17l]: Hôjô-shi, Ehime-Pref., Jpn., VII. 2000, K. YAMAWAKI; [00-m2]: Hôjô-shi, Ehime Pref., Jpn., 18. VII. 2000, K. YAMAWAKI; [00-m1]: Matsuyama-shi, Ehime Pref., Jpn., 4. VII. 2000, H. ISHIKAWA; [00-17n]: Tokushima-shi, Tokushima Pref., Jpn., 6. VII. 2000, J. OGAWA; [00-17o]: Muroto-shi, Kôchi Pref., Jpn., 6. VII. 2000, J. OGAWA; [02-01]: Toyoura-chô, Shimonoseki-shi, Yamaguchi Pref., Jpn., 3. VII. 2002, Y. UTSUNOMIYA; [03-m1]: Mt. Seburi, Sawara-ku, Fukuoka Pref., Jpn., 22. VIII. 2003, Y. UTSUNOMIYA; [04-m2]: Tsukahara, Yufuin-chô, Ôita Pref., Jpn., 11. VII. 2004, Y. UTSUNOMIYA; [01-6]: Takanabe, Miyazaki Pref., Jpn., 22. VIII. 2001, M. YAMAGUCHI; [01-04b], [01-04a]: Kagoshima-shi, Kagoshima Pref., Jpn., 28. VII. 2001, K. MORI; [04-m1]: Shibushi-chô, Kagoshima Pref., Jpn., 27. VII. 2004, Y. UTSUNOMIYA; [02-02]: Sata-chô, Kanzoku-gun, Kagoshima Pref., Jpn., 8. VIII. 2002, K. MORI; [02-04]: Onoaida, Is. Yakushima, Jpn., 14. IX. 2002, N. OHBAYASHI; [00-07e], [00-07f], [00-g1], [00-g2], [00-g3], [00-g4], [00-g5], [00-g6], [00-g7]: Ôgimi-son, Is. Okinawa, Jpn., 2. VI. 2000, N. OHBAYASHI; [00-17 g], [00-17h]: Is. Tsushima, Nagasaki Pref., Jpn., 12. VII. 2000, Y. SUGIURA; [04-m3]: Tanohama, Is. Tsushima, Nagasaki Pref., Jpn., 11. VII. 2004, M. KIMURA; [01-01]: Inchoen, Korea, 11. VII. 2001, Y. ARITA.

3. *Anoplophora macularia* (THOMSON, 1865)

Calloplophora macularia THOMSON, 1865, Syst. Ceramb., p. 553; type area: China.

Distribution. Japan (Is. Ishigaki-jima, Is. Miyako-jima, Is. Okinawa), Taiwan, China (?).

Specimens examined. [00-07a], [00-07b], [00-t1], [00-t2], [00-t3], [00-t4]: Chinen-son, Is. Okinawa, Jpn., 6. VI. 2000, N. OHBAYASHI; [00-07c], [00-07d]: Gushikawa-shi, Is. Okinawa, Jpn., 1. VI. 2000, N. OHBAYASHI; [00-t5], [00-t6], [00-t7], [00-t8]: Gushikawa-shi, Is. Okinawa, Jpn., 6. VI. 2000, N. OHBAYASHI; [00-t9], [00-t10]:

Nago-shi, Is. Okinawa, Jpn., 1. VI. 2000, N. OHBAYASHI; [01-10a], [01-10b]: Hirara-shi, Is. Miyako-jima, Jpn., 15. VII. 2001, N. OHSHIGE; [01-11a]: Yonehara, Is. Ishigaki-jima, Jpn., 28. VI. 2001, N. OHSHIGE; [01-11b]: Mt. Omoto-dake, Is. Ishigaki-jima, Jpn., 5. VI. 2001, N. SUGIMOTO; [00-27g]: Lushan Spa, Nantou County, Taiwan, 3. VII. 2000, S. INADA; [00-27h]: Tengzi, Kaoshiung County, Taiwan, 8. VIII. 2000, M. SATÔ; [02-06], [02-07], [02-08]: Shin Peitou, Taipei County, Taiwan, 23. VI. 2002, N. OHBAYASHI; [02-09]: Mt. Pahsienshan, Taichung County, Taiwan, 29. VI. 2002, N. OHBAYASHI; [02-10]: Smangus, Hsinchu County, Taiwan, 23. VI. 2002, C.-L. LI.

4. ***Anoplophora ryukyensis*** BREUNING et OHBAYASHI, 1964

Anoplophora oshimana subsp. *ryukyensis* BREUNING et OHBAYASHI, 1964, Bull. Japan ent. Acad., **1**: 15; type locality: Is. Yonaguni, Japan.

Distribution. Japan (Is. Yonaguni-jima).

Specimens examined. [00-07i]: Is. Yonaguni-jima, Jpn., 31. V. 2000, M. KIMURA; [01-12b], [01-12a]: Mt. Urabe-dake, Is. Yonaguni-jima, Jpn., 10. VI. 2001, Y. KIMURA.

5. ***Anoplophora oshimana oshimana*** (FAIRMAIRE, 1895)

Melanauster oshimanus FAIRMAIRE, 1895, Bull. Soc. ent. Fr., **1895**: 390; type locality: Is. Oshima, Ryukyu, Japan.

Distribution. Japan (Is. Amami-ôshima, Is. Uke-jima, Is. Okinoerabu, Is. Okinawa).

Specimens examined. [00-17i], [00-17j]: Is. Amami-ôshima, Jpn., 30. VI. 2000, M. KIMURA; [01-07a], [01-07b]: Akatsuchi-yama, Is. Amami-ôshima, Jpn., 18. VI. 2001, M. SATÔ; [01–09a], [01-09b]: Ôyama, Is. Okinoerabu, Jpn., 27. VI. 2001, N. OHSHIGE; [00-07g], [00-07h], [00-o1], [00-o2], [00-o3], [00-o4], [00-o5], [00-o6], [00-o7]: Nago-shi, Is. Okinawa, Jpn., 1. VI. 2000, N. OHBAYASHI.

6. ***Anoplophora oshimana tokunoshimana*** SAMUELSON, 1965

Anoplophora malasiaca tokunoshimana SAMUELSON, 1965, Pacif. Ins., **7**: 89, fig. 1; type locality: Tokunoshima Is., Ryukyu, Japan.

Distribution. Japan (Is. Tokunoshima).

Specimens examined. [01-08a], [01-08b]: Boma, Is. Tokunoshima, Jpn., 20. VI. 2001, N. OHBAYASHI.

7. ***Anoplophora davidis*** (FAIRMAIRE, 1886)

Melanauster davidis FAIRMAIRE, 1886, Annls. Soc. ent. France, (6), **6**: 355; type locality: Lou-tse Kiang, China.

Distribution. Indochina, China (?).

Specimen examined. [99-7b]: Ban Xa Lenh (890 m), Xa Pa Co, Huyen Mai Chau, Hoa Binh Prov., North Vietnam, 8. VI. 1999, A. SAITO.

8. ***Anoplophora glabripennis*** (MOTSCHULSKY, 1853)

Cerosterna glabripennis MOTSCHULSKY, 1853, Etud. ent., **2**: 48; type area: North China.

Distribution. China, Korea, North America, Europe.

Specimens examined. [00-17a], [00-17b]: Botanical garden, Shanghai, China, 12. VII. 2000, N. OHBAYASHI; [00-17e], [00-17f]: Taiyuan, Shanxi, China, 30. VI. 2000, L-Z. LI.

9. ***Anoplophora elegans*** (GAHAN, 1888)

Cyriocrates elegans GAHAN, 1888, Ann. Mag. nat. Hist., (6), **2**: 450; type locality: Ruby Mines District, Upper Myanmar.

Distribution. Indochina, China.

Specimens examined. [02-14]: Mt. Phu Pan, Houaphan Prov., Laos, V, 2001, H. WAKAHARA; [02-15]: Xiangkhoang, Xiangkhoang Prov., Laos, V, 2001, H. WAKAHARA.

10. ***Anoplophora stanleyana*** HOPE, 1839

Anoplophora stanleyana HOPE, 1839, Proc. linnean. Soc. London., **1**: 43; type locality: Assam, India.

Distribution. India, Bhutan, Indochina, China.

Specimen examined. [08-2]: Mt. Fanjingshan, Tongren city, Guizhou, China. 15～28. IV. 2008, Native collector.

11. ***Anoplophora beryllina*** (HOPE, 1840)

Monochamus beryllinus HOPE, 1840, Proc. linn. Soc., London, **1**: 79; type locality: Assam, India.

Distribution. India, Indochina, China.

Specimens examined. [02-12]: Mt. Phu Pan, Houaphan, Laos, 2. V. 2002, H. WAKAHARA; [02-13]: Mt. Phu Pan, Houaphan, Laos, 29. IV. 2002, N. OHBAYASHI. [99-9h]: Mt. Pia Oac (1,250–1,500 m), Cao Bang Prov., North Vietnam, 23～27. V. 1999, A. SAITO.

12. ***Anoplophora lurida*** (PASCOE, 1856)

Monochamus luridus PASCOE, 1856, Trans. ent. Soc., London, (2), **4**: 47; type area: North China.

Distribution. Taiwan, China?

Specimen examined. [02-11]: Mt. Anmashan, Taichung, Taiwan, 26. VI. 2002, N. OHBAYASHI.

13. ***Anoplophora granata*** HOLZSCHUH, 1993

Anoplophora granata HOLZSCHUH, 1993, FBBA, Wien, **75**: 48; type locality: Chiang Mai, North Thailand.

Distribution. Indochina.

Specimen examined. [02-16]: Mt. Phu Pan, Houaphan, Laos, V. 2001, H. WAKAHARA.

14. ***Calloplophora sollii*** (HOPE, 1839)

Oplophora sollii HOPE, 1839, Proc. linn. Soc., London, **1**: 42; type locality: Assam, India.

Distribution. India, Indochina.

Specimen examined. [02-17]: Mt. Phu Pan, Houaphan Prov., Laos, 29. IV. 2002, N. OHBAYASHI.

15. ***Dolichoprosopus yokoyamai*** (GRESSITT, 1937)

Monochamus yokoyamai GRESSITT, 1937, Kontyû, Tokyo, **11**: 149; type locality: Niigata Pref., Japan.

Distribution. Japan (Honshu, Shikoku, Kyushu).

Specimens examined. [y-4]: Mt. Shibisan, Izumi-gun, Kagoshima Pref., Jpn., 15. VIII. 2004, S. SAMESHIMA; [y-6]: Mt. Seburi, Sawara-ku, Fukuoka Pref., Jpn., 7. VIII. 2004, N. OHBAYASHI.

16. ***Dolichoprosopus sameshimai*** N. OHBAYASHI, 2001

Dolichoprosopus sameshimai N. OHBAYASHI, 2001, Jpn. J. syst. Ent., **7**: 265; type locality: Mt. Hoyoshi-dake, Kôyama-chô, Kagoshima Pref. Japan.

Distribution. Japan (Kagoshima Pref.).

Specimens examined. [02-19]: Ôura, Sata-chô, Kagoshima Pref., Jpn., 9. VIII. 2002, K. MORI; [02-20]: Mt. Takakuma-yama, Tarumizu-shi, Kagoshima Pref., Jpn., 24. VIII. 2002, K. MORI; [y-2]: Summit of Mt. Takakuma-yama, Tarumizu-shi, Kagoshima Pref., Jpn., 28. VII. 2004, S. SAMESHIMA; [y-3]: Foot of Mt. Takakuma-yama, Tarumizu-shi, Kagoshima Pref., Jpn., 28. VII. 2004, S. SAMESHIMA; [y-5]: Sugiyama-dani, Sata-chô, Kagoshima Pref., Jpn., 13. VIII. 2004, S. SAMESHIMA.

17. ***Monochamus guerryi*** PIC, 1902

Monochamus guerryi PIC, 1902, Échange, **18**: 121; type area: Yunnan, China.

Distribution. Indochina, China.

Specimen examined. [02-18]: Mt. Phu Pan, Houaphan Prov., Laos, 2. V. 2002, H. WAKAHARA.

18. *Pseudonemophas versteegii* (RITSEMA, 1881)

Monochamus versteegii RITSEMA, 1881, Not. Leyd. Mus., **3**: 155; type locality: Sumatra, Indonesia.

Distribution. India, Indochina, China, Malaysia, Indonesia.

Specimen examined. [08-1]: 19 miles point, Pahang, Malaysia, 2. IV. 2008, N. OHBAYASHI.

19. *Batocera celebiana* THOMSON, 1858

Batocera celebiana THOMSON, 1858, Arch. Ent., **1**: 453, pl. 20, fig. 1; type area: Celebes, Indonesia.

Distribution. Indonesia (Celebes, Moluccas, Java, Sangihe).

Specimen examined. [00-07j]: Kolaka, SE. Celebes, Indonesia, 31. XII. 1999, A. SAITO.

20. *Apriona germari* HOPE, 1831

Apriona germari HOPE, 1831, Gray, Zool. Misc., **1**: 28; type area: India.

Distribution. India, Indochina, Nepal, China.

Specimen examined. [00-17m]: Shanghai, China, 30. VI. 2000, L-Z. LI.

2. DNA extraction, PCR and sequencing

Isolation of DNA: Total DNA was extracted from thoracic muscle (10–25 mg) of a single individual specimen by using the QIAamp DNA Mini Kit (QIAGEN), and finally dissolved in 200 μl elution buffer. Altogether, 114 specimens from Japan, Taiwan, China, Vietnam, Laos, Malaysia and Indonesia were analyzed.

PCR amplification and DNA sequencing: The total DNA was used as a template for amplification of the mitochondrial cytochrome oxidase subunit I (COI) gene by the Polymerase Chain Reaction (PCR) (SAIKI *et al.*, 1988). About 1,000 bp 5′-region sequence of the COI gene was amplified by the forward primer SKCOI-7 (5′-CGC TCT AGA ACT AGT GGA TCA CAN AYC AYA ARG AYA TYG GNA C-3′) and the reverse primer KSCOI-2 (5′-TCG AGG TCG ACG GTA TCA CRT ART GRA ART GNG CNA CNA CRT ART A-3′) (KIM *et al.*, 2003). The underlined sequences of the two PCR primers were used for sequencing. PCR amplification was carried out in a 50 μl reaction mixture containing 2 μl of the total DNA solution, 5 μl of 10X *Ex Taq* buffer (TaKaRa), 4 μl of dNTP mixture (2.5 mM each), 2.5 μl of each primer (10 pmol/μl), and 2.5 U of *Ex Taq* polymerase (TaKaRa). PCR was performed for 50 cycles of denaturation at 94°C for 30 sec, primer annealing at 50°C for 1 min, and extension at 70°C for 2 min. The final single cycle was performed under the same conditions but with

an extension step at 70°C for 7 min. The PCR product was purified with QIAquick PCR Purification kit (QIAGEN).

Direct sequencing was performed with an automated ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using the dideoxy chain-termination method (SANGER *et al.*, 1977). The reaction mixture for cycle sequencing consisted of 5–20 ng of template DNA, 3.2 pmol of sequencing primer, 4 μl of BigDye Terminator Mix and 2 μl of 5X BigDye Sequencing Buffer (Applied Biosystems), and distilled water to a total volume of 20 μl. The cycle-sequencing conditions were 25 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min, followed by an indefinite hold at 4°C. The DNA products were cleaned with Centri-Sep spin columns (Applied Biosystems). Mostly, the two sequencing primers (underlined parts of the PCR primers) were sufficient to read the DNA fragment of the COI gene.

3. Phylogenetic Analysis

The sequences were aligned and compared by using the multiple-alignment program CLUSTAL W (THOMPSON *et al.*, 1994) and DNASIS, version 3.7 (Hitachi software Engineering, Japan). The evolutionary distances (D) were computed by KIMURA's (1980) two-parameter method, and the phylogenetic trees were constructed by the neighbor-joining (NJ) method (SAITOU & NEI, 1987). All of these processes were performed with the DNA sequence analysis package PAUP (SWOFFORD, 2001). Bootstrap analysis was performed for the trees based on 1000 re-samplings. The COI sequences of *Batocera and Apriona* species were used as the out-groups.

Results

1006 bp sequences of the COI gene were finally used for construction of the phylogenetic tree. Neither deletions nor insertions were required for multiple alignment. The G+C contents of the COI gene sequences from the specimens analyzed in this study were nearly constant (~32%). NJ-phylogenetic tree of the specimens analyzed is shown in Fig. 2–a & b. *Apriona germari* and *Batocera celebiana* were used for out-group. The species of the lamiine genus *Anoplophora* and its allied genera analyzed in this study, excluding *Calloplophora sollii* and *Pseudonemophas versteegii*, constitute six major lineages, the *malasiaca* species-group (Lineage 1), *A. glabripennis* (Lineage 2), *A. elegans* (Lineage 3), *A. stanleyana* (Lineage 4), *Dolichoprosopus* spp. (Lineage 5) and the complex lineage (Lineage 6) which consists of *A. beryllina*, *A. lurida*, *A. granata* and *Monochamus guerryi*. Of these six lineages, the Lineage 6 is likely to be the basal lineage to other five lineages, and the Lineages 1–4 seem to be rather closely related to each other. However, the divergence order of the lineages was supported without high bootstrap values, and thus these lineages might be radiated within a short time. *Calloplophora sollii* and *Pseudonemophas versteegii* were separately placed outside of the above-mentioned six lineages, and formed independent lineages from the species of the genera *Anoplophora*, *Dolichoprosopus* and *Monochamus*.

The *malasiaca* species-group is divided into two sub-lineages. The Sub-lineage A includes *A. davidis* from North Vietnam, *A. chinensis* from China and A. *malasiaca* from Japan and Korea, and the Sub-lineage B includes *A. oshimana* from the Amami-Okinawa Isls., *A. ryukyensis* from Yonaguni Is. and *A. macularia* from Taiwan and the Ryûkyû Archipelago of Japan. Here we provisionally named them the north element for former and the south element for latter. The origin (ancestral species) of the north element is considered to be *A. davidis* of North Vietnam, then *A. chinensis* and *A. malasiaca* speciated from *A. davidis*. *A. malasiaca* is further separated into two groups, of which one is distributed on Hokkaido, Honshu, Shikoku and Korea through Is. Tsushima, and the other is distributed on Kyushu, Is. Yakushima and Is. Okinawa, Japan.

The south element of the *malasiaca* species-group consists of *A. oshimana* (including two subspecies) and *A. macularia*+*A. ryukyensis*, which are clearly separated from each other. The former does not show the geographical separation among islands, but the latter consists of two distinct clusters, *A. ryukyensis* of Is. Yonaguni and *A. macularia* of Taiwan (including recently invaded population of the Ryûkyû Archipelago).

We tentatively calculated the divergence time between the lineages assuming that a 0.01 *D* (KIMURA's two parameter distance) unit of the COI gene sequence corresponds to about 2.7 million years (Myr), which is estimated from the data of Cychrini ground beetles (SU *et al.*, 2004). As shown in Fig. 2, the divergence of the lamiine genus *Anoplophora* including its two allied genera, *Monochamus* and *Dolichoprosopus* started about 27 million years ago (Mya) between the Lineage 6 and the others, and the four lineages (Lineage 1–4) seemed to radiate within a short time about 22 Mya. The separation between the two sub-lineages took place about 9.5 Mya, and that between *Anoplophora chinensis* and *An. malasiaca* was culcalated to occur about 4.5 Mya. However, these divergence times calculated in this study seems to be rather older than the traditional view. We have no any evidence at present to confirm whether the evolutionary rate of COI gene of carabid ground beetles is same to that of the Lamiine genus *Anoplophora* and its relatives or not, thus the calculation of the divergence time may be only a speculation.

Discussion

One of the purposes of this paper is to know the phylogenetic position and relationships of the *chinensis* group of *Anoplophora* based on molecular analysis, a rather objective tool that is completely different from the traditional method based on morphology. Another purpose of this paper is to point out the problems and contradictions of previous system, but not to propose a new taxonomic system.

The *chinensis* species-group included in the Lineage 1 is a well-defined group characterized by the following features: Body black; underside of body, entire part of first and second antennal segments, basal part of third to tenth in male or third to eleventh of female antennal segments, and legs furnished with fine bluish white pubes-

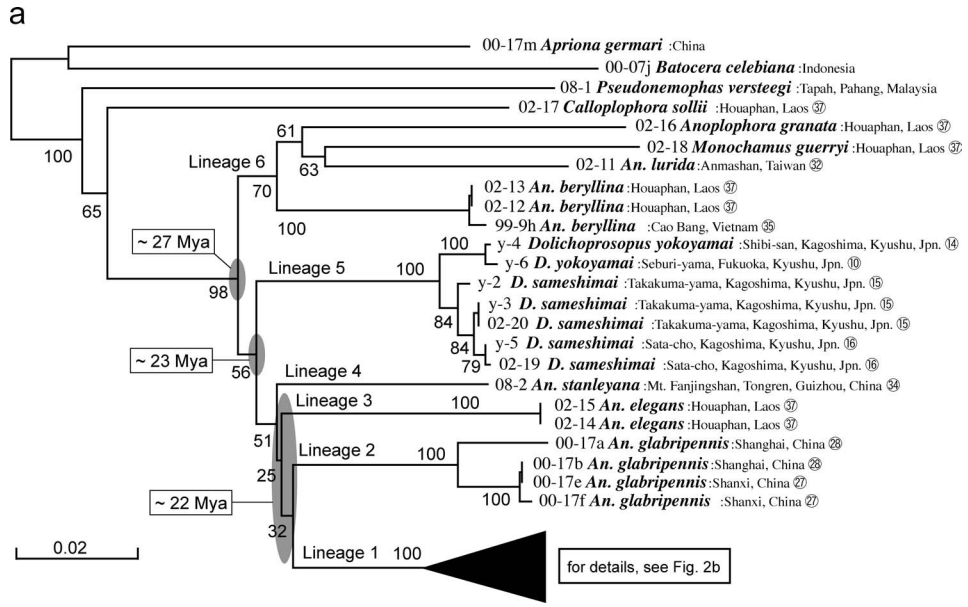

Fig. 2a. NJ-phylogenetic tree of the specimens of the lamiine genus *Anoplophora* and its relatives analyzed. Symbol on the top of scientific name shows the sample number. The circled-number in the end of each specimen name is locality number that corresponds to that shown in Fig. 1. The divergence time is shown at some branching points.

cence; a pair of pronotal maculation (sometimes lacking) and several elytral maculations consisting of white, bluish white or yellowish thick recumbent hairs, and the scutellum covered with the same kind of hairs; head with inferior eye lobe 1.5 times as deep as gena below it; antenna ca 1.7 to 2.0 times as long as body length in male and about 1.2 times in female; pronotum constricted in font and base, provided with a distinct tubercles at the center in front of basal constriction and a pair of well pointed spines on lateral sides; elytra almost parallel-sided in female and slightly narrowed posteriad in male, and then gently rounded to almost right angled sutural apices; elytral disc moderately scattered shallow punctures from which long or moderate-sized suberect black hairs arise, with basal area distinctly covered with node-like granules.

For the classification of the *chinensis* species-group, there are different ideas. LINGAFELTER and HOEBEKE (2002) synonymized *A. malasiaca* with *A. chinensis*, and also *A. oshimana*, *A. oshimana tokunoshimana* and *A. oshimana ryukyensis* with *A. macularia*. On the other hand, N. OHBAYASHI (1992), and MAKIHARA (2007) regarded them as independent species or subspecies, respectively. Here we tentatively distinguish seven species and a subspecies, *A. davidis* (FAIRMAIRE), *A. chinensis* (FORSTER), *A. malasiaca* (THOMSON), *A. macularia* (THOMSON), *A. oshimana* (FAIRMAIRE), *A. oshimana tokunoshimana* SAMUELSON, *A. ryukyensis* BREUNING et

OHBAYASHI and *A. ogasawaraensis* MAKIHARA in this species-group. These seven species basically show allopatric distribution.

Though *A. ogasawaraensis* was not available for our analysis, the result of molecular phylogenetic analysis seems to support the independence of each species. OHBAYASHI (2001) reported sympatric distribution of three *Anoplophora* species, *A. oshimana*, *A. macularia* and *A. malasiaca* on Okinawa Island of the Ryûkyû Archipelago, and the latter two species were suggested to be recent invaders. According to the DNA phylogenetic analysis of these three species collected on Okinawa Island, the independence of each species was also supported in spite of four specimens found ([00-o3], [00-o5], [00-g6], [00-07h]) which probably originated from hybridization between *A. oshimana* and *A. malasiaca*.

Anoplophora chinensis from Shanghai and Fujian, China was clearly clustered with *A. malasiaca* as the sister groups as seen in the Sub-lineage A. However, the separation between the two specimens of *A. chinensis* was rather deep, even older than that between the Shanghai specimen of *A. chinensis* and *A. malasiaca*, suggesting that there are at least two strains in *A. chinensis*, although they cannot be distinguished from each other morphologically. Perhaps, these are two geographical lineages, but we need to analyze more samples to confirm this presumption. This result also implies that *A. malasiaca* might speciate from the Shanghai strain of *A. chinensis* with some rapid morphological changes. *Anoplophora malasiaca* seems to be separated into two geographical groups, the southern group and the northern group, though they are also difficult to distinguish from each other morphologically. The southern group is distributed in Kyushu, Yakushima Island and Okinawa Island, and the northern group is widely distributed in Hokkaido, Honshu, Shikoku and Is. Tsushima. Interestingly, the specimen from Korea was certainly clustered into the northern groups though the Korean Peninsula is geographically separated from Japan, but it does not form a sister group to all Japanese specimens. Additionally, no difference was found in the DNA sequences between the *A. malasiaca* specimens from Korea and Tsushima Island, Japan. These facts indicate that *A. malasiaca* originated from *A. chinensis* in the Asian continent and immigrated into the Japanese Archipelago through the Korean Peninsula at least twice in the past time, probably by some artificial reasons, for example in association with the introduction of citrus trees.

Three *Anoplophora* species are clustered in the Sub-lineage B. *Anoplophora macularia* and *A. ryukyensis* are closely related to each other and *A. oshimana* is their sister group, suggesting that *A. ryukyensis* was separated from *A. macularia.* Because no continental species (Chinese species) was found to cluster into the Sub-lineage B in this analysis, it is difficult to presume the origin of the species in this sub-lineage. However, based on the result that *A. oshimana* and *A. macularia* are rather deeply separated from each other, it could be speculated that the ancestor of these two species had been distributed in continental China, and severally spread their distribution into Taiwan and the Amami-Okinawa Islands of Japan. In fact it is well known that the fauna of Amami-Okinawa Islands includes several species, whose affinity is not to Taiwan but

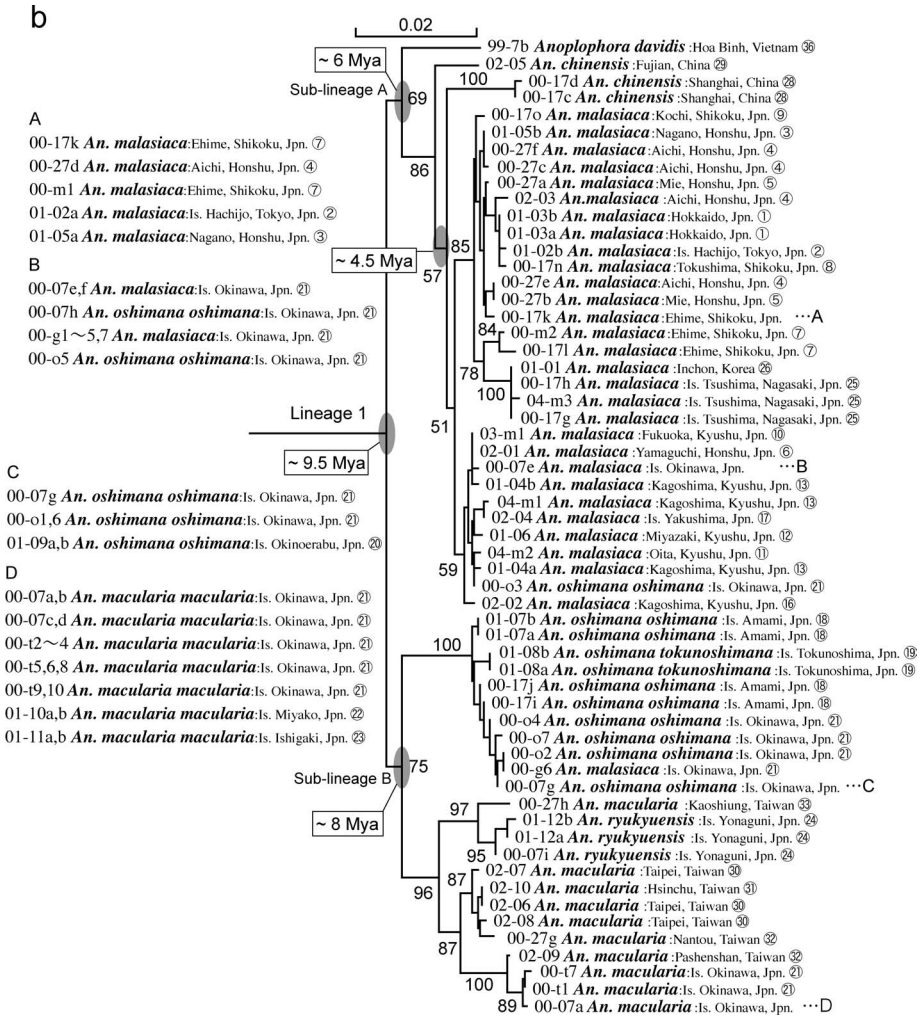

Fig. 2b. NJ-phylogenetic tree of the specimens clustered in the Lineage 1 of Fig. 2a. For details, see Fig. 2a.

directly to South China or Indochina.

Anoplophora oshimana complex distributed in Amami-Okinawa Islands is now separated into two subspecies by the slight morphological characteristics, but there are no clear differences of DNA among the populations of Amami-ôshima Is., Okinoerabu Is., Tokunoshima Is. and Okinawa Is. This species is abundant in Amami-ôshima Is., but the populations of other islands are rather small, respectively. The population of Okinoerabu Is. is considered to be introduced from Amami-ôshima in the 1970s (pers.

comm. from MAKIHARA). *Anoplophora oshimana tokunoshimana* was described in 1965 based on the specimens collected in 1963. The record of this species from Okinawa Island is uncertain, but after 1950 at the earliest. According to these facts, there is a possibility that the native place of *A. oshimana* should be restricted to Amami-ôshima Is. and other populations in the Amami-Okinawa Islands could be established by expansion of its distribution in the 20th century.

Anoplophora macularia group including *A. ryukyensis* is rather complicated. Our result basically supports the separation of the two morphological species, *A. ryukyensis* and *A. macularia*, except one specimen (00-27h) of *A. macularia* from Taiwan, which is phylogenetically independent from other specimens of the same species and clearly clustered with *A. ryukyensis*. The phylogenetic relationships of these two species can be interpreted as follows. Two lineages of *A. macularia*, which may be geographically separated, existed in Taiwan, one of the two lineages invaded Yonaguni Is., Japan, and speciation has taken place with rapid morphological changes, and the other one was introduced into Okinawa Is., Miyako Is. and Ishigaki Is. of the Ryukyu Islands in recent years. OHBAYASHI (2001) also suggested the recent immigration of *A. macularia* from Taiwan to the Ryukyu Archipelago on the basis of morphological viewpoint. Further analyses for additional samples of *A. macularia* from Taiwan are necessary to confirm the present results because only one specimen (00-27h) was found to cluster with *A. ryukyensis* lineage.

The Lineage 2 consists of only one species, *A. glabripennis*. We were unable to examine sufficient samples of this species, but at least two sub-lineages were recognized even from the same population in Shanghai, China. This species attacks more than ten genera of deciduous trees, and causes heavy damages especially on popular, maple and willow. Previously it was separated into two species, *A. glabripennis* and *A. nobilis* GANGLBAUER, but LINGAFELTER and HOEBEKE (2002) synonymized the latter with the former. ISONO *et al.* (1999) suggested that the two species (*A. glabripennis* and *A. nobilis*) rapidly spread their distribution by afforestation project in China and seemed to be mixed especially in Northwest China. To understand whether there are two distinct species or not, it will be expected to conduct further DNA analysis of specimens from various areas.

The Lineage 3 consists of a single species, *A. elegans*, which was once placed in the subgenus *Cyriocrates* of the genus *Anoplophora*. The Lineage 4 also consists of a single species, *A. stanleyana*, which is the type species of the genus *Anoplophora*. The Lineage 5 includes two Japanese species of the genus *Dolichoprosopus*, *D. yokoyamai* and *D. sameshimai*. These two species showed very close DNA sequences and could not be separated from each other on the phylogenetic tree. *Dolichoprosopus yokoyamai* is widely distributed throughout Japan up to 800 m in altitude and its host is limited to *Fagus crenata*. On the other hand, *D. sameshimai* is only distributed in the low altitude range of the southernmost of Kyushu Island, and its host is restricted to *Lithocarpus edulis*. These facts suggest that recent speciation with rapid morphological changes have occurred from *D. yokoyamai* to *D. sameshimai* presumably due to the host switching.

The type species of this genus, *D. maculatus* RITSEMA=*D. lethalis* (PASCOE) is distributed in Indonesia, which is far apart from the distributional ranges of the Japanese species, so that it seems necessary to make an additional analysis of other species including the type species to clarify the phylogenetic position and relationships of this genus.

The Lineage 6 includes three *Anoplophora* species (*A. granata*, *A. lurida* and *A. beryllina*) and one *Monochamus* species (*M. guerryi*). In other words, the three *Anoplophora* species have a common origin with the species of different genus, but are widely separated from other species of the same genus. As the result, their generic status should be comprehensively reviewed in the future together with the Japanese *Dolichoprosopus* species.

Pseudonemophas versteegi was first described as a species of the genus *Monochamus*, and it has been placed in the genus *Anoplophora* since BREUNING (1943), then LINGAFELTER and HOEBEKE (2002) transferred it to the genus *Pseudonemophas*. According to our result, it is reasonable to place this species out of *Anoplophora*. On the other hand, *Calloplophora sollii* was first described as a species of the genus *Oplophora*, but THOMSON (1864) replaced it to the *Calloplophora* because of preoccupied name. LINGAFELTER and HOEBEKE (2002) once synonymized *Calloplophora* with *Anoplophora*, but our result supports that this species also should be placed out of the genus *Anoplophora*.

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