Phylogeny and Evolution of the Tribe Platycerini (Coleoptera, Lucanidae) of the World Inferred from Mitochondrial 16S rRNA and COI Gene Sequences

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Abstract   The tribe Platycerini is a small group in the family Lucanidae, which is divided into three genera, *Platycerus*, *Platyceroides* and *Platyceropsis* (Benesh, 1946). We have analyzed the mitochondrial 16S rRNA and COI gene sequences of approximately 300 specimens consisting of 37 species of this tribe of the world. The molecular genealogical trees suggest that the Platycerini is divided into two major lineages, the *Platyceropsis* – *Platyceroides* lineage and the *Platycerus* lineage, and the branching point between these two is considerably deep. The *Platycerus* lineage is further divided into four geographically linked lines, namely, the Japanese, North American, West Eurasian, and East Eurasian sublineages. Based on the molecular phylogenetical data, a probable evolutionary history of the platycerine lucanid beetles is discussed.

Introduction

The tribe Platycerini Mulsant, 1842 (*sensu* Benesh, 1946) is a unique group of lucanid beetles, characterized by small body usually less than 15 mm in length, short eye canthus less than one-quarter of the eye, partially geniculate antennae, and arcuately curved lateral margin of the pronotum (Howden & Lawrence, 1974). This tribe has been considered to include three genera, namely, *Platycerus* Geoffroy, 1762, *Platyceropsis* Benesh, 1946, and *Platyceroides* Benesh, 1946. Of these, the genus *Platycerus* contains more than 40 species widely distributed in the Holarctic Region, and particularly diversified in China and Japan where approximately 30 species have been recognized, while the other two genera are endemic to western North America, containing only a single (*Platyceropsis*) and seven (*Platyceroides*) species, respectively.

In this study, we have analyzed the mitochondrial gene sequences of 16S rRNA and

Table 1.   List of the specimens used in this study

No.	Scientific name	Locality	DDBJ Accession No. (16S)
[*Platyceroides*]			
1	*Platyceropsis keeni* (Casey, 1895)	Curry Co., Oregon, NW. USA	AB490781
[*Platyceropsis*]			
2	*Platyceroides aeneus* (Van Dyke, 1928)	Del Norte Co., California, W. USA	AB490780
[*Platycerus*]			
Japanese lineage			
3	*P. acuticollis* Y. Kurosawa, 1967	Hôshi, Gunma, C. Japan	AB490400
4	*P. albisomni* Kubota *et al*., 2008	Mt. Gassan, Yamagata, NE.Japan	AB490401
5	*P. takakuwai* Fujita, 1987	Daibosatsu Mts., Yamanashi, C. Japan	AB490406
6	*P. viridicuprus* Kubota *et al*., 2008	Mt. Hyôno-sen, Hyogo, SW. Japan	AB490408
7	*P. sue* Imura, 2007	Ishizuchi Mts., Ehime, SW. Japan	AB490407
8	*P. sugitai* Okuda et Fujita, 1987	Ishizuchi Mts., Ehime, SW. Japan	AB490404
9	*P. urushiyamai* Imura, 2007	Kujû Mts., Oita, SW. Japan	AB490409
10	*P. akitaorum* Imura, 2007	Odaigahara, Nara, SW. Japan	AB490405
11	*P. kawadai* Fujita et Ichikawa, 1982	Misakubo, Shizuoka, C. Japan	AB490403
12	*P. delicatulus* Lewis, 1883	Asahi Mts., Yamagata, NE. Japan	AB490402
North American lineage			
13	*P. oregonensis* Westwood, 1844	Santa Clara Co., California, W. USA	AB490170
14	*P. virescens* (Fabricius, 1775)	Palos Forest Reserve, Chicago, NE. USA	AB490171
West Eurasian lineage			
15	*P. caprea* (De Geer, 1774)	near Mt. Grebeni, S. Uralskiy Mts., Russia	AB490165
16	*P. caraboides* (Linné, 1758)	Dokutchaeva Vil., Kharkov, N. Ukraine	AB490166
17	*P. caraboides* (Linné, 1758)	Fontainebleau, Paris, France	AB490168
18	*P. caraboides* (Linné, 1758)	Banska-Bystrica, C. Slovakia	AB490167
19	*P. caraboides* (Linné, 1758)	Zlata Idka, Kosice, E. Slovakia	AB490169
20	*P. caucasicus* Parry, 1864	Mt. Lysaja, Krasnodar, NW. Caucasus, Russia	AB490172
21	*P. primigenius* E. Weise, 1960	Mt. Azish-Tau, Krasnodar, NW. Caucasus, Russia	AB490779
East Eurasian lineage			
22	*P. tangi* Imura, 2008	Mt. Jiuding Shan, NC. Sichuan, SW. China	AB490298
23	*P. hiurai hiurai* Tanikado et Tabana, 1997	Baoxing Xian, C. Sichuan, SW. China	AB489987
24	*P. tieguanzi* Imura, 2007	Mt. Erlang Shan, C. Sichuan, SW. China	AB490174
25	*P. cyanidraconis* Imura, 2008	Mt. Jiuding Shan, NC. Sichuan, SW. China	AB490299
26	*P. cupreimicans* Imura, 2006	Yunling Mts., NW. Yunnan, SW. China	AB489984
27	*P. tabanai tabanai* Tanikado et Okuda, 1994	Qinling Mts., S. Shaanxi, C. China	AB489982
28	*P. dundai* Imura et Bartolozzi, 1994	Hailuo Gou Valley, C. Sichuan, SW. China	AB489983
29	*P. ladyae* Imura, 2005	Mt. Erlang Shan, C. Sichuan, SW. China	AB490301
30	*P. feminatus* Tanikado et Tabana, 1997	Meigu Xian, SC. Sichuan, SW. China	AB490696
31	*P. feminatus* Tanikado et Tabana, 1997	Heizhu Gou, SC. Sichuan, SW. China	AB489986
32	*P. hongwonpyoi hongwonpyoi* Imura et Choe, 1989	Mt. Gaji-san, Gyeongsangnam-do, S. Korea	AB490699
33	*P. h. hongwonpyoi* Imura et Choe, 1989	Mt. Jiri-san, Gyeongsangnam-do, S. Korea	AB490698
34	*P. h. hongwonpyoi* Imura et Choe, 1989	Mt. Odae-san, Gangwon-do, S. Korea	AB490700
35	*P. h. shennongjianus* Imura, 2008	Shennongjia, W. Hubei, C. China	AB490704
36	*P. h. qinlingensis* Imura, 1993	Qinling Mts., S. Shaanxi, C. China	AB489979
37	*P. h. qinlingensis* Imura, 1993	Ningshan Xian, S. Shaanxi, C. China	AB489985
38	*P. rugosus* Okuda, 1997	Mt. Guangtou Shan, N. Chongqing, C. China	AB489980
39	*P. rugosus* Okuda, 1997	Shennongjia, W. Hubei, C. China	AB490703

Table 1. List of the specimens used in this study

No. Scientific name	Locality	DDBJ Accession No. (16S)
40 *P. rugosus* OKUDA, 1997	Micang Shan Mts., NE. Sichuan, SW. China	AB490701∼490702
41 *P. businskyi* IMURA, 1996	Ningshan Xian, S. Shaanxi, C. China	AB489981
42 *P. turnai sichuanus* IMURA, 2008	Wolong, C. Sichuan, SW. China	AB490300
43 *P. turnai turnai* IMURA, 2001	Shennongjia, W. Hubei, C. China	AB490697
44 *P. xiongmao* IMURA, 2008	Baoxing Xian, C. Sichuan, SW. China	AB489988
45 *P. consimilis phagophilus* IMURA, 2005	Micang Shan Mts., NE. Sichuan, SW. China	AB490694
46 *P. consimilis consimilis* TANIKADO et TABANA, 1998	Miyaluo, NC. Sichuan, SW. China	AB490695
47 *P. consimilis consimilis* TANIKADO et TABANA, 1998	Bipeng Gou, NC. Sichuan, SW. China	AB490173
48 *P. nagahatai* IMURA, 2008	Qinling Mts., S. Shaanxi, C. China	AB490293
49 *P. bashanicus* IMURA et TANIKADO, 1998	Guangtou Shan, N. Chongqing, C. China	AB490296∼490297
50 *P. yeren* IMURA, 2008	Shennongjia, W. Hubei, C. China	AB490294
51 *P. kitawakii* IMURA et TANIKADO, 1998	Daba Shan Mts., N. Chongqing, C. China	AB490295

*Numerals correspond to those shown in Figs. 1–3.

the cytochrome *C* oxidase subunit I (COI) of approximately 300 specimens consisting of 37 species of all the above three genera from various regions of the world. The examined species consist of more than 70% of all the known species, and their localities cover most of the overall distributional ranges of the tribe. On the molecular genealogical trees are recognized two major lineages each corresponding to *Platyceropsis* plus *Platyceroides* from western North America and *Platycerus* from the whole range of the Holarctic Region. In the latter lineage are recognized four geographically linked phylogenetic lines, namely, the Japanese, North American, West Eurasian, and East Eurasian sublineages. No cross contaminations of any species within a given lineage to other lineages have been found. Based on the results obtained, a probable evolutionary history of the platycerine lucanid beetles is discussed.

Materials and Methods

Sampling

The specimens analyzed for mitochondrial 16S rRNA gene (512–514 bp) are listed in Table 1, and the localities where the samples analyzed were collected are shown in Figure 1. The representative species used in this study are shown in Figure 2. The gene sequences of most species were also analyzed for mitochondrial COI (552 bp). The scientific names used herein are according to those routinely adopted by the taxonomists without considering any molecular data. To prevent DNA degradation, the beetles were immediately killed in 95% ethanol and sorted in the same solution until use. Thorax muscles from adult individuals were used for DNA extraction. For several examples (some of the European and North American species and *P. kitawakii* from China), dried specimens were used for DNA extraction.
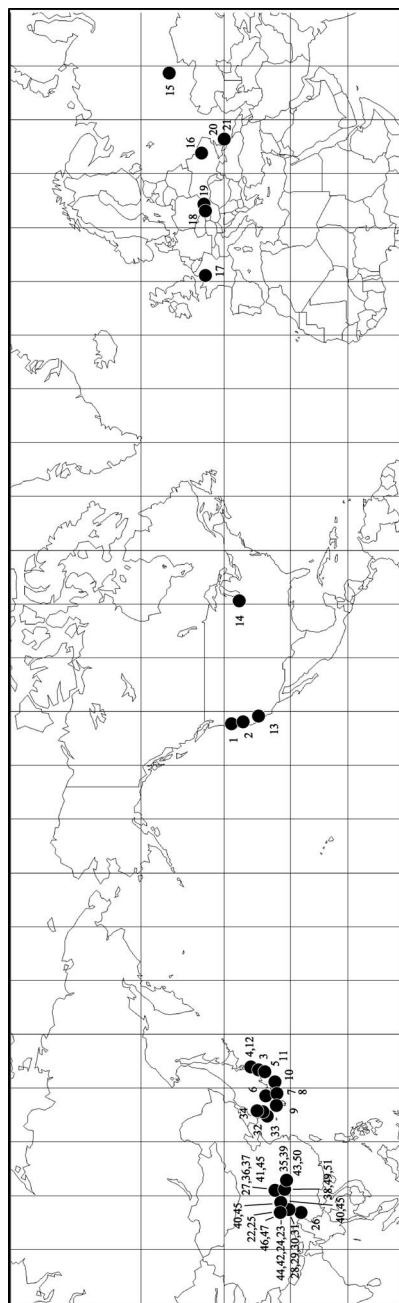
Fig. 1. Map showing the collecting sites of the specimens used in this study. Numerals correspond to those shown in Figs. 2, 3 and Tab. 1.
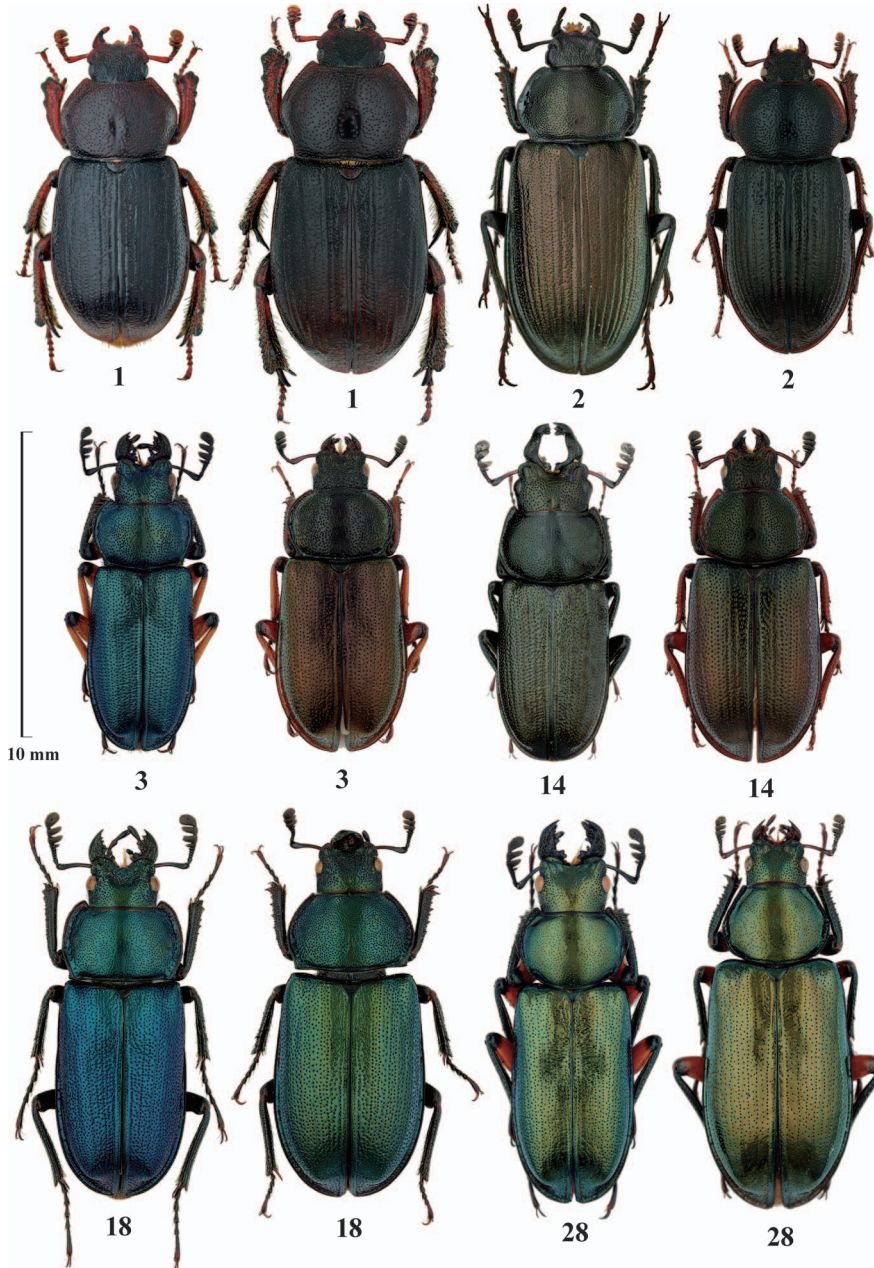
Fig. 2. Habitus of the representative species of the tribe Platycerini. Numerals correspond to those used in Figs. 1, 3, and Tab.1. Left – male; right – female. Identification of Specimen No. 2 (*Platyceroides aeneus*) is tentative, since we were unable to make a direct comparative study between the type specimen.

DNA extraction, amplification (PCR) and sequencing

Total DNA was prepared using DNA Extraction FM Kit (Wako, Osaka, Japan). DNA for each specimen was finally dissolved in 12 μl reaction system, and ca. 50 ng of solution was used as template for amplification of DNA fragments by polymerase chain reaction (PCR). The fragment of the mitochondrial 16S rRNA DNA containing 512–514 bp 3′-region was amplified using a primer pair (forward 5′-A ATG ATT TTT AGG ATT GGA AGT GTC-3′; reverse 5′TTT AAT CCA ACA TCG AGG TCG-3′). Direct sequencing was performed with Big Dye Terminator v1.1 Cycle Sequence Kit (ABI, Foster, CA). The following primers were used for amplification of the COI gene (552 bp): LCO1490 and HCO2198 (HEBERT *et al*., 2003). Details for the PCR amplification and direct sequencing methods were described in a series of papers on the phylogenetic studies of carabid beetles (*e.g.*, SU *et al*., 2005).

Phylogenetic analyses

The 16S rRNA gene region (512–514 bp upstream from the 3′-terminal), and the COI gene region (552 bp upstream from the 3′-terminal stop codon) were used for phylogenetic analyses. Sequence alignments were carried out using the multiple alignment program MEGA 4.0. The following analyses were made: neighbor-joining (NJ) (SAITOU & NEI, 1987), unweighted pair-group method with arithmetic mean (SOKAL & MICHENER, 1958) and maximum parsimony (MP) (FARRIS, 1970). Construction of the NJ- and UPGMA trees was made using evolutionary distance (D) computed by KIMURA's two-parameter method (KIMURA, 1980). A heuristic search to determine the MP tree was performed using tree-bisection-reconnection (TBR) branch-swapping. The string tree was obtained via stepwise addition. All the trees were evaluated using the bootstrap test (FELSENSTEIN, 1985) based on 1,000 replicates. The 16S rRNA tree and the COI tree were outgroup-rooted using the gene sequences of six lucanid species and two lucanid species, respectively, taken from the database of the DDBJ. The nucleotide sequence data reported in this paper appear in the DDBJ, EMBL, and GenBank nucleotide sequence database (Table 1).

Results

The number of insertion-delition of the 16S rRNA was 0 to16 (three in average) in pairwise sequence comparisons between two species. The G＋C contents were nearly constant (22.3±1.1％ for the 16S rRNA gene and 33.3±1.5％ for the COI gene). The 16S rRNA tree showed essentially the same topology with the COI tree. Some minor differences between the 16S and COI trees were found in the branching order within some subclusters. Since this did not affect the main conclusion and discussion, the 16S rRNA tree was mainly used in the following results and discussions.

Molecular phylogeny of Platycerini based on the 16S rRNA gene sequences

Figure 3 shows the NJ-phylogenetic tree of the three genera treated in this study, together with six representative species of other genera belonging to the family Lucanidae as the outgroup. From the tree were recognized two major lineages, the *Platyceropsis – Platyceroides* lineage and the *Platycerus* lineage. The branching point between these two lineages was considerably deep. Their differentiation seems to have taken place long ago, and would have been traced back to almost the same period of differentiation of several other major genera belonging to the family Lucanidae, *e.g.*, *Dorcus*, *Lucanus* and *Prismognathus*, etc. On the tree, the *Platycerus* lineage was further divided into four sublineages.

The Platyceropsis – Platyceroides *lineage*

This lineage contained two North American genera, and was divided into two branches, each composed of *Platyceropsis keeni* CASEY, 1895, from Oregon of north-western USA, which is a single component of the genus *Platyceropsis*, and *Platyceroides aeneus* VAN DYKE, 1928, from California of western USA, which is one of the seven species belonging to the genus *Platyceroides*. The branching point between the two branches was very deep, which means that their differentiation have taken place long ago.

The Platycerus *lineage*

All the species in this lineage belong to the genus *Platycerus*, and was radiated into four sublineages within relatively short time. It is worth noting that each sublineage was apparently linked with the geography. The precise branching order among the sublineages could not be determined presumably because of their almost simultaneous emergence, though the diversification of the Japanese species seems to have taken place a little earlier than that of the species distributed in other regions. This was suggested from both the 16S rRNA and COI trees.

The first sublineage was represented by the species distributed in Japan alone, and we call it the Japanese sublineage. Only selected examples were included in this tree among much more specimens analyzed, including all the species and subspecies distributed in Japan. This sublineage was further divided into two clusters; one was composed of *P. acuticollis* and its allied species, and the other was composed of all the remaining species containing both the species-group of *P. sugitai* and the *P. delicatulus*-complex. In the latter cluster, greater parts of the species were differentiated within very short time, and the result of the present phylogenetic analysis did not reflect the morphological classification.

The second one, called the North American sublineage, was composed of two out of four North American species, *P. oregonensis* and *P. virescens*. The former is from California of western USA and the latter is from Chicago of northeastern USA.

The third sublineage consisted of four out of nine (according to BARTOLOZZI & SPRECHER-UEBERSAX, 2006) species distributed in the western part of the Eurasian

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Nicagus japonicus (AOMORI, JAPAN)

Ceruchus lignarius (AOMORI, JAPAN)

Aesalus asiaticus (KYOTO, JAPAN)

79 *Dorcus rectus* (AOMORI, JAPAN)

87 *Lucanus maculifemoratus* (AOMORI, JAPAN)

Prismognathus angularis (FUKUOKA, JAPAN)

92 *Platyceropsis keeni* (CURRY, CALIFORNIA, USA) 1 **Platyceropsis-Platyceroides**

Platyceroides aeneus (DEL NORTE, CALIFORNIA, USA) 2

81

96 *P. acuticollis* (HOSHI, GUNMA, JAPAN) 3
66 *P. albisomni* (MT.GASSAN, YAMAGATA,JAPAN) 4
P. takakuwai (DAIBOSATSU, YAMANASHI, JAPAN) 5
P. viridicuprus (MT.HYONO-SEN, HYOGO, JAPAN) 6
96 *P. sue* (ISHIZUCHI MTS., EHIME, JAPAN) 7 **JAPAN**
P. sugitai (MT.TSURUGI-SAN, TOKUSHIMA, JAPAN) 8
99 *P. urushiyamai* (MT.HIKO-SAN, FUKUOKA, JAPAN) 9
P. akitaorum (ODAIGAHARA, NARA, JAPAN) 10
44 *P. kawadai* (DAIBOSATSU, YAMANASHI, JAPAN) 11
47 20 *P. delicatulus* (ASAHI MTS., YAMAGATA, JAPAN) 12

97 *P. oregonensis* (SANTA CLARA, CALIFORNIA, USA) 13 **NORTH AMERICA**
P. virescens (PALOS, CHICAGO, USA) 14

99 38 81 *P. caprea* (MT.GREBENI, Uralskiy, Russia) 15
94 *P. caraboides* (DOKUTCHAEVA, KHARKOV, UKRAINE) 16
P. caraboides (FONTAINEBLEAU, PARIS, FRANCE) 17
98 *P. caraboides* (BANSKA-BYSTRICA, SLOVAKIA) 18 **WEST EURASIA**
54 *P. caraboides* (ZLATA IDKA, KOSICE, SLOVAKIA) 19
P. caucasicus (MT.LYSAJA, KRASNODAR, CAUCASUS, RUSSIA) 20
97 *P. primigenius* (MT.AZISH-TAU, KRASNODAR, CAUCASUS, RUSSIA) 21

52 99 *P. tangi* (MT.JIUDING SHAN, SICHUAN, CHINA) 22
P. hiurai hiurai (BAOXING XIAN, SICHUAN, CHINA) 23
28 *P. tieguanzi* (MT.ERLANG SHAN, SICHUAN, CHINA) 24
53 *P. cyanidraconis* (MT.JIUDING SHAN, SICHUAN, CHINA) 25

97 *P. cupreimicans* (YUNLING MTS., YUNNAN, CHINA) 26
99 *P. tabanai tabanai* (QINLING MTS., SHAANXI, CHINA) 27
7 *P. dundai* (HAILUO GOU, SICHUAN, CHINA) 28
97 *P. ladyae* (MT.ERLANG SHAN, SICHUAN, CHINA) 29
1 *P. feminatus* (MEIGU XIAN, SICHUAN, CHINA) 30
98 *P. feminatus* (HEIZHU GOU, SICHUAN, CHINA) 31

18 42 88 *P. hongwonpyoi hongwonpyoi* (MT.GAJI-SAN, S.KOREA) 32
15 *P. hongwonpyoi hongwonpyoi* (MT.JIRI-SAN, S.KOREA) 33
P. hongwonpyoi hongwonpyoi (MT.ODAE-SAN, S.KOREA) 34
93 *P. hongwonpyoi shennongjianus* (SHENNONGJIA, HUBEI, CHINA) 35
96 *P. hongwonpyoi qinlingensis* (QINLING MTS., SHAANXI, CHINA) 36
83 *P. hongwonpyoi qinlingensis* (NINGSHAN, SHAANXI, CHINA) 37
49 *P. rugosus* (MT.GUANGTOU SHAN, CHONGQING, CHINA) 38
92 *P. rugosus* (SHENNONGJIA, HUBEI, CHINA) 39
84 *P. rugosus* (MICANG SHAN MTS., SICHUAN, CHINA) 40

P. businskyi (NINGSHAN, SHAANXI, CHINA) 41 **EAST EURASIA**
96 *P. turnai sichuanus* (WOLONG, SICHUAN, CHINA) 42
P. turnai turnai (SHENNONGJIA, HUBEI, CHINA) 43
96 69 *P. xiongmao* (BAOXING XIAN, SICHUAN, CHINA) 44
65 *P. consimilis phagophilus* (MICANG SHAN MTS., SICHUAN, CHINA) 45
91 83 *P. consimilis consimilis* (MIYALUO, SICHUAN, CHINA) 46
94 *P. consimilis consimilis* (BIPENG GOU, SICHUAN, CHINA) 47
P. nagahatai (QINLING MTS., SHAANXI, CHINA) 48
22 *P. bashanicus* (MT.GUANGTOU SHAN, CHONGQING, CHINA) 49
P. yeren (SHENNONGJIA, HUBEI, CHINA) 50
83 *P. bashanicus* (MT.GUANGTOU SHAN, CHONGQING, CHINA) 49
17 *P. kitawakii* (DABA SHAN MTS., CHONGQING, CHINA) 51

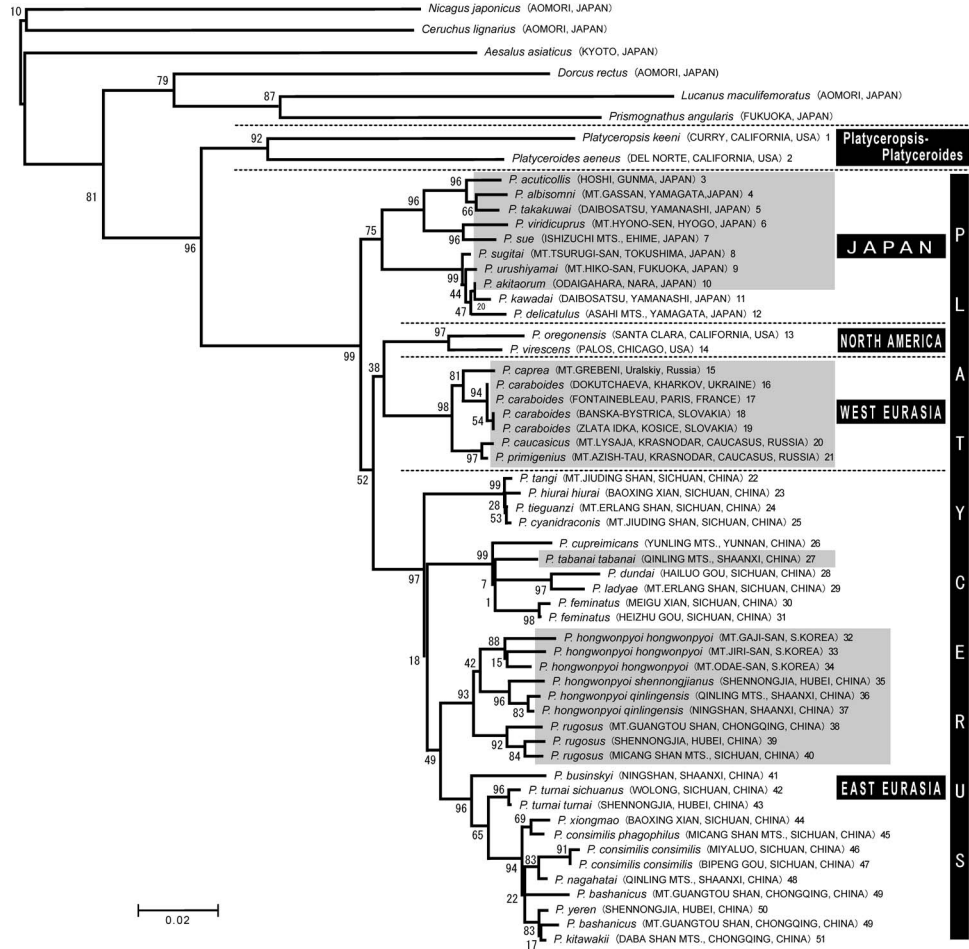**P L A T Y C E R U S**

0.02

Fig. 3 Neighbor-joining (NJ) phylogenetic tree of the Platycerini of the world constructed from mitochondrial 16S rRNA gene sequences. Numerals at each branching point indicate bootstrap percentage. Those shown after the scientific names correspond to those used in Figs. 1, 2, and Table 1. The species currently regarded as belonging to the *P. acuticollis*-complex (bearing sharply pointed pronotal hind angles) are boxed in gray shadow. Scale bar represents Kimura's 2-parameter evolutionary distance.

Continent, and we call it the West Eurasian sublineage. This sublineage was further subdivided into two clusters. One cluster included two species, *P. caraboides* from Slovakia, Ukraine and France, and *P. caprea* from Russia. Another cluster included the two species, *P. primigenius* and *P. caucasicus*, both from Caucasus.

The fourth one, here called the East Eurasian sublineage, consisted of all the known species from the eastern part of the Eurasian Continent, and was further divided into

four clusters. The first cluster consisted of four species from central Sichuan of Southwest China. Externally, all the species belonging to this cluster are characterized by small male mandibles and characteristically shaped endophallus of the male genital organ. It is interesting that *P. hiurai* belonged to this cluster. This species is peculiar in having entirely black legs and can be distinguished from any of other East Eurasian species at a glance. The second cluster contained five species mainly distributed in the southwestern part of the distributional range of the genus *Platycerus* in China. It is remarkable that *P. tabanai* of southern Shaanxi belonged to this lineage. This species bears sharply pointed hind angles of the pronotum, and has been regarded, together with *P. hongwonpyoi* and *P. rugosus*, as belonging to the *P. acuticollis*-complex. The third cluster contained two species from Korea and China, namely, *P. hongwonpyoi* distributed from the Korean Peninsula to Central China and *P. rugosus* which are distributed from western Hubei to northeastern Sichuan of Central China. Both of these species are characterized by having sharply pointed hind angles of the pronotum, and, as mentioned above, have been regarded as belonging to the *P. acuticollis*-complex to which all the Japanese species with pointed pronotal hind angles also belong. The fourth cluster was represented by eight species from western Hubei to central Sichuan of China. Of these, *P. businskyi* from southern Shaanxi, *P. turnai* from western Hubei and central Sichuan, and *P. kitawakii* from northern Chongqing are unique in having peculiarly shaped male genital organ, and readily distinguished from each other. All the remaining five species belong to the species-group of *P. bashanicus*, which are characterized by small mandibles and uniquely featured male genital organ.

Discussion

Morphologically, the two North American genera, *Platyceropsis* and *Platyceroides*, have been placed, together with the Holarctic genus *Platycerus*, in a single tribe Platycerini (BENESH, 1946). However, the present genealogical tree shows that they are phylogenetically remote from each other. The genus *Platyceropsis* is monotypical, represented by a flightless species, *P. keeni*, distributed from British Columbia to northern California along the Pacific coast (BENESH, 1946), where this species is found under the decayed driftwood on sandy beach. The genus *Platyceroides* contains seven species, the distributional range of which are from British Columbia to California throughout the Cascade, Sierra Nevada, and the Coast Mountains. The male of this genus is fully-winged and can fly, but the female is flightless (BENESH, 1946). According to Taro ELDREDGE of New York (pers. comm.), they are found from relatively matured mixed- or broadleaved forest and the larvae prefer to feed on white-rotten branches. Since we have been unable to analyze any of the six other species of this genus, discussion as to the phylogenetic relationship among them will have to be left until these species become available for analyses. Very recently, PAULSEN and HAWKS (2008) established a new tribe, Platyceroidini for these two genera (type genus: *Platyceroides*) mainly based on the peculiar morphological characters. The two Ameri-

can authors' view is questionable, because these two genera are phylogenetically quite remote from each other. However, it is possible to regard these two higher taxa as two subtribes of the tribe Platycerini.

On the mitochondrial 16S rRNA as well as the COI trees, all the species belonging to the genus *Platycerus* consist of a single, distinct phylogenetic lineage which is supported by a high bootstrap value of 99%. The most noticeable is that the *Platycerus* lineage is differentiated into four geographically well-linked phylogenetic lines, namely, the Japanese, North American, West Eurasian, and East Eurasian sublineages. This genus has been currently classified into two species-complexes according to the shape of hind angles of the pronotum; one is the *P. delicatulus*-complex with the hind angles of the pronotum rounded, and the other is the *P. acuticollis*-complex with the same angles sharply pointed. On the present 16S rRNA as well as the COI trees, however, the morphology of pronotal hind angles and molecular phylogeny does not always run parallel. For example, eight of the 10 Japanese species, all the European species, and three of the 19 Chinese species are morphologically classified into the *P. acuticollis*-complex, and yet they appear in three of the four different sublineages on the molecular genealogical trees (see gray-shaded parts in Figure 3). The species morphologically classified into the *P. delicatulus*-complex also appear in three of the four different sublineages. All the North American species, two of the 10 Japanese species and a greater part (16 of 19) of the Chinese species are of this type. So far as the morphology of the pronotal hind angles is concerned, the two types of character states, namely, either rounded or pointed, appear randomly in each sublineage, and therefore cannot be regarded as synapomorphy. Rather, it should be regarded as homoplasy most probably depending on the living environment, mating strategy, and ovipositional behavior of each species.

To discuss about the origin and establishment of the present distributional ranges of the platycerine lucanid beetles, the phylogenetic position and distributional range of the two genera, *Platyceropsis* and *Platyceroides*, must be important. Both of them are considered to be plesiomorphic in both the external and male genitalic morphologies, and rather narrowly extant in the western part of North America. One possible scenario on the origin and process of dispersal of the genus *Platycerus* as deduced from the 16S rRNA (as well as COI) phylogenetic profile is that this genus emerged in North America from the common ancestry of *Platyceropsis* and *Platyceroides* and expanded its distributional range to the Holarctic region. They radiated into four geographically liked sublineages within a short time, although their branching order cannot be determined with certainty. The speciation would have initiated shortly after establishment of each sublineage. Above all, an explosive diversification of the species seems to have taken place in the Chinese Continent. Our knowledge of the platycerine fauna of this region is still insufficient, and yet more than 20 species have been known up to the present (including a new species described from Guizhou by IMURA in 2009). This situation is comparable with the evolutionary history of the tribe Cychrini in the family Carabidae (SU, pers. comm.). As to the Chinese sublineage, we have already finished molecular

and morphological analyses for all the known taxa, the details of which will be introduced in our next paper.

Acknowledgements

要　　約

井村有希・永幡嘉之： ミトコンドリア 16S rRNA および COI 遺伝子の塩基配列からみた世界のルリクワガタ族の系統と進化.　── ルリクワガタ族 Platycerini はルリクワガタ属 Platycerus, ムカシルリクワガタ属 Platyceropsis, ニセルリクワガタ属 Platyceroides の3属によって構成される. 本研究では，世界各地から収集した，同族の構成種の7割以上に相当する計37種，約300個体について，ミトコンドリア 16S rRNA および COI 遺伝子の塩基配列を決定して分子系統樹を作製し，検討をくわえた. その結果，ムカシルリクワガタ属・ニセルリクワガタ属の二者とルリクワガタ属との分岐は古く，その時期はクワガタムシ科の他の主要な属が分化した時期にまで遡りうることが判明した. 一方，世界のルリクワガタ属は分布域と密接に関連した4系統（日本系，北米系，西ユーラシア系，東ユーラシア系）に分かれ，これまで属内の系統を論じる上で重視されてきた前胸背板後角の形状は，分子系統とは無関係に各系統にランダムに出現することが判明した. 本属はおそらく，北米大陸においてムカシルリクワガタ属・ニセルリクワガタ属と共通の祖先種から分化し，比較的急速に全北区へと分布を広げ，現在の4主要分布域において種分化を起こしたものであろうと考えられる.

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Food Plants of a Supralittoral Hydrophilid Beetle, *Cercyon* (*Cercyon*) *dux* (Coleoptera, Hydrophilidae)

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Cercyon (*Cercyon*) *dux* Sharp, 1873 is a supralittoral Hydrophilid beetle that is widely distributed along the shorelines from the southern Kuril Isls. to Kyushu Is., and it is commonly present along the cobble and shingle beaches of Japan (Ôhara & Jia, 2006; Satô, 1981, 1989; Sharp, 1873; Shatrovskiy, 1989, 1992). Its adults feed on detritus such as seaweeds and sea grasses that drift ashore (Hansen, 1999; Ôhara & Jia, 2006). However, it is still poorly known for the food plant utilization of *C.* (*C.*) *dux* to each seaweed and sea grass, although they would be frequently observed under the decomposed sea tangle more than the other seaweeds and sea grass in field condition (Ôhara & Kobayashi, personal observations). In this paper, I examine the food acceptance tests of *C.* (*C.*) *dux* for the commonly observed seaweeds (sea tangle, sea

lettuce, and gulfweed) and sea grass (tape grass) along the seaside shorelines of Hokkaido.

Materials and Methods

Non-choice feeding tests were carried out to detect the acceptability for seaweeds and sea grass. A total of 31 adult individuals of *Cercyon* (*Cercyon*) *dux* were sampled from four localities in Hokkaido as follows: Onishika, Obira, Rumoi (N 44°03′ 14″, E 141°39′ 14″, 4 indiv.); Bikuni, Furubira (N 43°17′ 44″, E 140°36′ 47″, 8 indiv.); Nishikioka, Tomakomai (N 42°35′ 20″, E 141°27′ 09″, 3 indiv.); Ayoro cape, Noboribetsu (N 42°27′ 11″, E 141°12′ 21″, 16 indiv.). In this experiment, three species of seaweeds, sea lettuce (Ulvaceae: *Ulva* sp.), gulfweed (Sargassaceae: *Sargassum* sp.), sea tangle (Laminariaceae: *Laminaria* sp.), and one species of sea grass, tape grass (Zosteraceae: *Phyllospadix* sp.) were examined because these four plant species are commonly found along the beaches of Hokkaido. All plant species were sampled from Shioya, Otaru (N 43°12′ 54″, E 140°55′ 16″). Seven to nine individuals were tested for each plant species.

Pieces of plants (about 0.1 g) were each placed in a transparent polystyrene case (65×55× 20 mm), the bottom of which was covered with moist filter paper soaked in marine water. A beetle was released into the case and was allowed to feed on one of four plant species during 48 hours. Prior to the examination, beetles were settled on starvation for 48 hours. The experiments were performed at 20℃ in dark conditions. Since it is impossible to assess the amount of the feeding trace, I checked the acceptance of each plant by the number of beetles' fecal pellets after 48 hours. I pooled the data of different sex and different locality samples, because each sample size is very small. I examined the number of fecal pellets among the treatments using ANOVA. And, for pair-wise comparison between treatments, I used the Turkey-Krammer method.

Results and Discussion

Observed numbers of fecal pellets of *Cercyon* (*Cercyon*) *dux* among treatments were different in food acceptance tests (F=29.834, d.f.=3, p<0.0001, one-way ANOVA). And, in pair-wise comparisons, the number of fecal pellets of *C*. (*C*.) *dux* between treatments was significantly different, except for that between *Sargassum* sp. and *Laminaria* sp. (Table 1).

Fecal pellets of *C*. (*C*.) *dux* were observed in the seaweeds treatments, but they were not observed in the sea grass' (*Phyllospadix* sp.) treatment. Adults of *C*. (*C*.) *dux* would not use

Table 1. Number of fecal pellets of adult individuals of *Cercyon* (*Cercyon*) *dux* for four plant species during 48 hours in non-choice feeding test.

Plant species	(*N*)	Number of fecal pellets in each beetle	Mean±SD
Ulva sp. (Ulvaceae)	(8)	10, 10, 9, 11, 6, 9, 18, 12	10.63±3.46[a]
Sargassum sp. (Sargassaceae)	(7)	2, 8, 8, 6, 3, 2, 3	4.57±2.70[b]
Laminaria sp. (Laminariaceae)	(7)	6, 4, 5, 7, 3, 8, 6	5.57±1.72[b]
Phyllospadix sp. (Zosteraceae)	(9)	0, 0, 0, 0, 0, 0, 0, 0, 0	0±0[c]

N means the examined number of beetles. Values with different letters are significantly different (Turkey-Krammer method, *P*<0.01).

Phyllospadix sp. as their food plants. In the comparisons of three species of seaweed, the average number of fecal pellets for *Ulva* sp. was significantly higher than those for the rest. The present study suggests that *Ulva* sp. presents a suitable food plant for *C.* (*C.*) *dux*, more than *Sargassum* sp. or *Laminaria* sp. Surprisingly, this result is not consistent with the abundance of *C.* (*C.*) *dux* in the field observation. As mentioned above, adults of this species frequently occur under *Laminaria* sp. more than other seaweeds. Although this inconsistency has not been satisfactorily clarified yet, some other factors could be also influential for the food selection of *C.* (*C.*) *dux* in field conditions, for example, richness of seaweeds at the shoreline, degree of decomposition of seaweeds, etc. Further examinations are highly required.

Acknowledgements

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