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Author(s)	渡部, 友子
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## 博士論文要約

## 博士専攻分野の名称 博士(農学) 氏名 渡部 友子

## 学位論文題名

Taxonomic study on tree-dwelling aphids, the *Tetraneura akinire* species group and the *Tuberculatus quercicola* species group (Insects; Aphididae) in Japan

(日本産樹上性アブラムシ Tetraneura akinire 種群及び Tuberculatus quercicola 種群の分類

学的研究)

In Chapter 1, I taxonomically deal with the genus *Tuberculatus*. Aphids of the genus *Tuberculatus* Mordvilko (Drepanosiphinae) are associated with *Quercus* species, including about 50 species from the world (Blackman & Eastop 1994). Aphids of this genus are non-host-alternating, and the adults are characterized by one or more pairs of tubercular processes on the abdominal dorsum. All viviparae and males are winged. Some species are myrmecophilous, being attended by several species of ants.

Matsumura (1917) first recorded *Acanthocallis quercicola* Matsumura from *Q. crispula* in Sapporo, Hokkaido, northern Japan, and this species was later transferred to *Tuberculatus (Tuberculoides)* by Shinji (1941). On the other hand, Essig and Kuwana (1918) recorded *Myzocallis macrotuberculata* Essig & Kuwana, as new to science, from *Q. dentata* in Tokyo, Japan. Matsumura (1919) synonymized *M. macrotuberculata* with *A. quercicola*, and this taxonomic treatment has been followed by other researchers (Richards 1968; Higuchi 1969; Eastop & Hille Ris Lambers 1976; Blackman & Eastop 1994; Quednau 1999). In his revision of *Tuberculatus*, Higuchi (1969) treated *T. quercicola* as a species associated with several *Quercus* species, including *Q. acutissima, Q. dentata*, and *Q. variabilis*. Since then, several authors have regarded, as *T. quercicola*, a species that is associated with *Q. dentata* and obligatorily attended by ants in Hokkaido, northern Japan (Ito & Higashi 1991; Yao et al. 2000; Yao 2012). Using *T. quercicola* on *Q. dentata* as material, Yao and coworkers have examined aphid adaptation to attending ants and the population genetic characteristics (Yao & Akimoto 2001, 2002, 2009; Yao et al. 2003; Yao 2010; Yao & Kanbe 2012).

Later, Yao (2010, 2011) found that a *T. quercicola*-like species is associated with *Q. crispula* and that this species, called sp. A, is genetically differentiated from "*T. quercicola*" on *Q. dentata* in mitochondrial genes and microsatellites. Yao's genetic study shows that there may be two closely-related species that are associated with *Q. dentata* or *Q. crispula*. Furthermore, Yao (2011) found that a specific morphological form of "*T. quercicola*" is distributed on *Q. dentata* in eastern Hokkaido, and he tentatively referred to it as sp. B. However, there have been no taxonomic studies that attempted to determine the taxonomic status of these species and clarify the

relationship of them with the two specific epithets, *quercicola* and *macrotuberculata*. In the present paper, I refer to the myrmecophilous *Tuberculatus* species associated with *Q. dentata* and *Q. crispula* as the *T. quercicola* group and attempt to revise this species group taxonomically based on information from morphometry and molecular phylogeny.

Our preliminary study indicated that in the *T. quercicola* group, populations on *Q. crispula* and those on *Q. dentata* exhibit consistent morphological differences. This observation motivated our further study on the relationship between morphology, host associations, and molecular phylogeny. Thus, the objects of the present study are to indicate morphological differentiation linked to the host plants, clarify the phylogenetic relationships of the host-plant associated populations, and finally determine the species status in the *T. quercicola* group. In the present study, I will formally redescribe the species of the *T. quercicola* group.

All the specimens used in the present study are preserved in Systematic Entomology, Graduate School of Agriculture, Hokkaido University (SEHU).

I used 22 individuals collected from Hokkaido, Honshu, and Kyushu for molecular phylogenetic analysis. Total DNA was extracted from the entire aphid following the Chelex procedure (Walsh et al. 1991). Mitochondrial cytocrome oxidase subunit I (COI) was amplified separately. Primer sets, C1-J-1718 (5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3') (Simon et al. 1994) + R2191 (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') and TQ-INT-F (5'-CAA GCA CAT TTA TTC TGA TTT TTT GG-3') + TQ-INT-R (5'-GGG AAT CAG TGA ATG AAT CTT GC-3') were used to amplify the two partial COI regions. PCR was performed in 20 ml volumes, which included 2 ml of  $10 \times$  PCR buffer (Takara-Bio, Shiga, Japan), 1.6 ml of dNTP mixture (2.5 mM of each), 1 ml of 2 pM of each primer, 20 ng/ml of genomic DNA, and 0.05 units of Ex-Taq DNA polymerase (Takara-Bio). The reaction cycle parameters were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 sec, 45 °C for 20 sec, and 65 °C for 90 sec.

The entire PCR product was purified using the QIAquick PCR purification kit (QIAGEN, Tokyo). For sequencing reaction, I used a 5 ml volume of solution consisting of 2 ml of Quick Start Mix (Beckman Coulter, Tokyo), 0.5 ml of 10 pM forward or reverse primers, and 2.5 ml of 10 ng/ml template DNA. The reaction cycle parameters were as follows: 33 cycles of 94  $^{\circ}$ C for 30 sec, 50  $^{\circ}$ C for 15 sec, and 65  $^{\circ}$ C for 90 sec. DNA sequencing was performed using CEQ2000XL DNA Analysis System (Beckman Coulter, Brea, USA). A total of 738 bp was aligned for all samples. Alignment was conducted manually using MacClade 4.08 (Maddison & Maddison 2005). Sequences of COI were deposited in the DNA Data Bank of Japan under accession numbers AB900070-AB900094.

Principal component analysis for 33 samples from Hokkaido indicated that PC1 and PC2 explained 53.3% and 25.8% of the variance, respectively). The PC1 loadings were positive with similar absolute values for all the characters except the length of processus terminalis of the antennal segment VI. This result implies that PC1 represents the general body size except for processus terminalis length, which had a weak relationship with PC1. On the other hand, PC2 represented differences in shape among the samples. Of the 11 characters, the length of ultimate rostral segment and the number of secondary rhinaria strongly and negatively contributed to PC2, whereas processus terminalis length and caudal width strongly and positively contributed to PC2. The plots of PC1 and PC2 scores were clustered into three groups without overlaps, suggesting that there may be three distinct morphological groups. In Fig. 2, all samples from *Q. crispula* and

*Q. serrata* formed one group, whereas those from *Q. dentata* in central Hokkaido and those from *Q. dentata* in eastern Hokkaido, each, clustered into one group. These morphological groups were tentatively called the *Q. crispula*-associated population, the central Hokkaido group of the *Q. dentata*-associated population, and the eastern Hokkaido group of the *Q. dentata*-associated population.

The hypothesis of three morphological groups was tested by discriminant analysis. The discriminant function indicated that every sample plot was correctly classified into the original morphological group with the probability of unity; there were no misidentifications. This morphological difference was not correlated with the distance between populations; for example, in Iwamizawa, aphid colonies on one *Q. dentata* tree (the central Hokkaido group) were only 7 m from those on one *Q. crispula* tree and exhibited a large morphological difference. Discriminant analysis was further applied to five alate samples collected from *Q. dentata* in northern Honshu and Kyushu to examine with which group they are affiliated. All of the five alates were classified as the central Hokkaido group of the *Q. dentata*-associated population with probabilities of 0.989 to 1.0.

The central Hokkaido group and the eastern Hokkaido group of the *Q. dentata*-associated population are generally distributed allopatrically. However, in Obihiro City only, the two forms were collected in two localities, 7 km from each other, but in different years. This may suggest that the two forms can coexist parapatrically.

Our observations showed that on Q. serrata, aphid colonies persisted from spring to summer in 2011 and 2012, but became extinct until autumn without producing sexuals. Because aphids on Q. serrata were morphologically included in the Q. crispula-associated population, colonies on Q. serrata may have been temporarily maintained through migration from neighboring populations on Q. crispula. Thus, it is difficult to consider Q. serrata as an original host-plant species.

MP analysis produced six most parsimonious phylogenies for the samples. The ML tree and one of the MP trees agreed in the topology. That phylogenetic tree indicated that aphid populations associated with *Q. crispula* and those associated with *Q. dentata* are clearly separated into different monophyletic groups with high bootstrap supports. A sequence divergence of 3.25% was found in the COI gene between a sample from *Q. crispula* and that from *Q. dentata* at Iwamizawa where the two populations coexist. Collection localities, Hokkaido or Honshu (the Main Island), had a minor effect on the grouping; each of the two monophyletic groups included populations from Hokkaido and Honshu, and also included a few local groups with weak bootstrap supports (54-78). Although morphometric analyses distinguished the eastern Hokkaido group from the other samples of the *Q. dentata*-associated population, the present phylogenetic analysis did not support the presence of the eastern Hokkaido group. Most of the samples of this group had the same sequence as samples of the *Q. dentata*-associated population from other regions of Hokkaido.

In this paper, I revised the taxonomy of the *Tuberculatus quercicola* group, myrmecophilous drepanosiphine aphids that are associated with *Quercus dentata* and *Q. crispula* in Japan, based on morphometry and molecular phylogeny. This species group has been recognized as *T. quercicola* with some junior synonyms. Morphometric analysis of 11 morphological characters divided the group into three clusters; i.e., the *Q. crispula*-associated population, the central

Hokkaido group of the *Q. dentata*-associated population, and the eastern Hokkaido group of the *Q. dentata*-associated population. MP and ML analyses of the mitochondrial COI gene indicated that samples of the *T. quercicola* group are separated, with high bootstrap supports, into two monophyletic groups that are associated with *Q. dentata* or *Q. crispula*. However, no genetic differentiation was detected between the central Hokkaido group and the eastern Hokkaido group of the *Q. dentata*-associated population. These results led me to conclude that populations associated with *Q. dentata* are genetically and morphologically distinct from those associated with *Q. crispula*, and thus they are in a full specific status. On the other hand, I treated the two local groups of the *Q. dentata*-associated population as local races based on morphology. I formally redescribed the *Q. crispula*-associated and *Q. dentata*-associated populations under the names *T. quercicola* and *T. macrotuberculatus* stat. rev., respectively

In chapter 2, I dealt with Tetraneura akinire species group. The genus Tetraneura Hartig (Aphididae, Eriosomatinae), a group of gall-forming aphids associated with Ulmus species, is comprised of three subgenera, Tetraneura, Tetraneurella, and Indotetraneura, including 35 valid species worldwide (Blackman & Eastop 1994; Sano & Akimoto 2011; Favret 2021). Galls of this genus are conspicuous in the shape and color, and sometimes occur densely on the leaves of Ulmus species. Aphids of this genus are typically host-alternating between the primary hosts, Ulmus species, and the secondary hosts, the roots of gramineous species. Sexual generation appears in autumn on the truck of Ulmus species to undertake sexual reproduction. In contrast, asexual (unholocyclic) lineages of some species are distributed widely in association with gramineous species beyond the distributional range of Ulmus species (Eastop 1958, 1966; Heie 1967; Vadivelu et al. 1975; Delfino 1982; Mifsud et al. 2009). Some Tetraneura species are pest insects on the secondary host plants, including upland rice and sugar cane (Tanaka 1961; Singh & Singh 2017). In this genus, although several taxonomic confusions still remain, the difficulty in discriminating species has long hindered taxonomic work. After Hille Ris Lambers (1970) published a taxonomic revision, several species have been added to the genus as new to science (Pal & Raychaudhuri 1978; Chakrabarti & Maity 1982; Zhang et al. 1991; Zhang & Qiao 1997). However, this genus has not been taxonomically revised sufficiently. In particular, morphological simplification of apterous adults associated with their parasitic and sessile life modes, as well as morphological similarity of alate females have made species delimitation difficult. In addition, the coexistence of some species on the same *Ulmus* species, especially the coexistence of galls of different species on the same tree (Akimoto 1995, Muramatsu & Akimoto 2016), has confused the taxonomy.

Despite the similarity in the adult stage, Hille Ris Lambers (1970) stated that critical diagnostic characters of each species emerge in the first instar nymphs of each morph, for example, fundatrix first instars and those deposited by the emigrants. First-instar nymphs have high mobility and specific roles in their life cycle; for example, gall formation by fundatrix first instars, and movement to the underground parts of the secondary host by first instars deposited by emigrants. Such specific activities may have evolved species-specific morphology in the first instars. Mainly based on the characters of the first instar nymphs and wax gland plates, Hille Ris Lambers (1970) founded the taxonomy of the genus *Tetraneura*. Later, Zhang *et al.* (1991) and Zhang & Qiao (1997) described nine species and one subspecies as new to science from China,

indicating that China is the center of the species diversity of *Tetraneura*. Currently, using the morphological characters of first instar nymphs, coupled with molecular techniques, I can discriminate species that are distributed sympatrically and allopatrically. In particular, in the present study, the size and morphology of fundatrix first instar nymphs are emphasized. Since *Tetraneura* galls are closed, it is easy to find and collect the cast-off skins of the fundatrix first instars, the gall formers, from the galls. The skin is blackish, hard and conspicuous.

The present paper attempts to taxonomically deal with a species group, herein called the *akinire* species group, belonging to the subgenus *Tetraneurella*. A species of this group that induces reddish and spindle-shaped galls with a rugged surface on *Ulmus* species has been referred to as *T. nigriabdominalis, T. akinire, T. fusiformis, T. chinensis,* or *T. hirsuta*, with a wide distribution from East Asia to Europe in association with *Ulmus* species (Roberti 1972; Blackman & Eastop 1994; Walczak et al. 2017; Blackman & Eastop 2021). This species is introduced to North America (Hille Ris Lambers 1970; Foottit et al. 2006), where it induces galls on *Ulmus* species native to North America and those introduced from other continents. Currently, according to Blackman & Eastop (1994), this species is treated as *T. nigriabdominalis*. However, the taxonomic position of this species, phylogenetic relationships among allopatric populations, and relationships with closely related species have not been clarified. This paper deals with all of these problems to stabilize the taxonomy of this species group consisting of three species, with description of one new species and redescription of two species. Further, it attempts to resolve a nomenclatural problem.

Aphids used in the present study were collected from leaf galls on *Ulmus* spp., the primary hosts, or the roots of gramineous plants, the secondary hosts. In particular, I collected the cast-off skins of fundatrix first-instar nymphs from the galls by dissecting them. Collected aphids were maintained at room temperature or at -20°C in vials of 80% or 99% ethanol until they were mounted on slides or their DNA was extracted. These aphids were either collected by me or were available through the courtesy of other researchers. For the measurements of fundatrix first instar nymphs (or the skins), I used samples from Spain, France, Italy, South Korea, and Japan. Aphid specimens including cast-off skins were mounted on glass slides using Canada balsam or Hoyer's medium after several processes of chemical treatments of the samples (van Emden 1972). For measurements of body dimensions, the images of the aphids were captured on a computer via a microscope camera (Dino-Eye AM423, AnMo Electronics Corp., Taipei, Taiwan), and the lengths of morphological traits were measured using the software ImageJ version 2.0.0-rc-69/1.52p (Abramoff et al. 2004 available from http://rsbweb.nih.gov/ij/). The length of hind femorotrochanter (hereafter, hind femur length) was used as an index of body size because it is difficult to exactly measure body length in slide-mounted specimens (Akimoto & Yamaguchi 1985). The terminology followed Akimoto (1983, 1985). All specimens used for the description and morphological measurements were preserved at the Hokkaido University Museum.

Total genomic DNA was extracted using a Blood and Tissue Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's protocol. One individual was selected from each gall or each colony on the grass roots, and the DNA was used for analysis. PCR amplification was conducted using the primer sets C1–J–2183 (5-CAACATTTATTTTGATTTTTGG-3) and R2740 (5-CCTAAAAAATGTTGAGGGAAAAA-3) (Lee et al. 2012). PCR reactions were performed in 10 mL reaction volumes using TaKaRa *Ex Taq* (TaKaRa Bio, Shiga, Japan). PCR

amplification of 35 cycles each consisting of 30 s at 94 °C, 30 s at 45 °C, and 1 min at 65 °C, was performed after an initial denaturation step of 3 min at 94 °C. Amplified products were purified using the QIAquick PCR Purification Kit (Qiagen), and then sequenced with a CEQ2000 DNA Analysis System (Beckman Coulter, Fullerton, CA, USA) following the manufacturer's protocols. Sequence alignment and editing were performed using the MEGA X (Kumar et al. 2018). The alignment was unequivocal because the sequences included no indels or repeats. The sequences were trimmed to 511 bp in length. I used a total of 42 DNA samples, including one sample from the outgroup (*Tetraneura (Tetraneura) yezoensis* Matusumura) to construct phylogenetic trees (Table 1, GenBank accession numbers pending).

Phylogenetic analysis of the mitochondrial haplotypes from all samples was conducted using the maximum-likelihood (ML) method. Prior to the ML phylogenetic estimations, the best-fit ML model was searched using MEGA X, and the Tamura-Nei model of evolution was selected as the best-fit ML model. Branch support was evaluated using 1000 bootstrap replicates. I calculated the genetic distances (*p*-distance) between the pairwise combinations of haplotypes using MEGA X

Three types of galls remain distinctive in the subgenus *Tetraneurella* distributed in Japan and South Korea: greenish globular galls, reddish or greenish, spindle-shaped galls, and reddish small globular galls. Greenish globular galls and spindle-shaped galls were collected in Japan and South Korea.

These three types of galls were induced by different-sized fundatrix first-instar nymphs. Figure 2 shows the hind femur lengths of the fundatrix first-instar nymphs as an index of body size. Greenish globular galls were induced by small-sized fundatrix nymphs, spindle-shaped galls were induced by medium-sized fundatrix nymphs, and small-sized globular galls were caused by large-sized fundatrix nymphs. Korean greenish globular galls were inhabited by fundatrix nymphs that are intermediate in size between small-sized and medium-sized nymphs. In the same locality, for example, at Sapporo, Hokkaido, the hind femur lengths of gall formers exhibited clear discontinuity among the three gall types without overlaps, suggesting the presence of at least three species. Because the body size of fundatrix first instar nymphs directly reflects the body size of sexual females in Eriosomatinae (Tong & Akimoto 2019), this difference suggests interspecific discontinuity in the body size of sexual females and the presence of a reproductive isolating mechanism.

Morphological observations of emigrant adults and their progeny collected from the three types of galls indicated that the gall formers of spindle-shaped galls and small-sized globular galls were referable to *T. akinire* sensu nov. and *T. sorini*, respectively. However, I failed to identify the gall formers of greenish globular galls from South Korea and Japan. The lengths of the hind femurs were stable within the same type of galls. In *T. akinire*, fundatrix nymphs collected from Japan, South Korea, Europe, and North America exhibited hind femurs of a similar length, and no significant differences were detected in hind femur length between fundatrix nymphs collected from galls on *U. parvifolia* and those collected from *U. davidiana* var. *japonica*.

Regarding *T. sorini*, the mean and variance of the hind femur length were conspicuously larger than in other species. The means of hind femur length varied largely among the localities. Muramatsu & Akimoto (2016) indicated that the body size of *T. sorini* is evolutionarily affected by the local densities of *T. akinire* sensu nov. and other species. When the densities of other species

are high, *T. sorini* fundatrices easily usurp incipient galls of other species by taking advantage of their large body size, resulting in weak selection pressures for their body size. However, in localities where the densities of other species are lower, *T. sorini* fundatrices more frequently compete with each other, leading to strong selection pressures for larger body size and longer legs.

Of the 42 haplotypes used for the phylogenetic analysis, I obtained 15 unique haplotypes. I detected phylogenetic clusters that corresponded to the three types of galls and gall formers. Aphids from small-sized globular galls constituted a clade in which the fundatrix first instars were characterized by long hind femurs. This clade, with 87% bootstrap support, corresponded to *T. sorini*. Aphids from greenish globular galls also constituted a unique clade with 100% bootstrap support and were characterized by shorter hind femurs. These morphological and molecular information indicate that this clade is a distinct biological species, which will be described in the next section as *T. ovaliformis*, forming an independent clade. This information and morphological evidence that the hind femur lengths are on average longer than those of *T. ovaliformis* suggests that the Korean gall formers are either an undescribed species or a species that has already been described using different morphs. This result also suggests that gall morphology readily evolves convergently.

Gall formers of spindle-shaped galls (*T. akinire*) were separated into two clades, both of which were highly supported by bootstrapping (81% and 99%). This result supported the results of Lee et al. (2012), who indicated that *T. nigriabdominalis* (= *T. akinire* sensu nov.) consisted of two phylogenetic groups (types A and B). In the present study, type A included samples from Japan, Europe, and North America and also included samples from *U. parvifolia* and *U. davidiana* var. *japonica*. This result suggests that members of type A are widely and commonly distributed in Eurasia and are associated with several species of *Ulmus* without genetic differentiation. *U. davidiana* forms a clade together with *U. minor* in Europe and *U. rubra* in eastern North America, whereas *Ulmus parvifolia* and *U. davidiana* are distantly related in *Ulmus* (Bate-Smith & Richens 1973; Wiegrefe et al. 1994). Therefore, the separation of types A and B may be ascribed to vicariance events, not to genetic differentiation related to host shifts among *Ulmus* species.

Hille Ris Lambers' (1970) assumption that *T. akinire* was artificially introduced into North America was supported by this phylogeny, which demonstrated no genetic differentiation between American samples and Asian samples. The three *COI* sequences of *T. chinensis* used by Zhang et al. (2008) completely agreed with the sequences of type A of *T. akinire*. "*T. sorini*" in Zhang et al. (2008) formed a cluster with *T. ovaliformis*, while "*T. akinire*" was placed outside the *T. akinire* species group.

*T. akinire* type B was composed of samples collected in Hokkaido, northern Japan, and those collected from the roots of gramineous plants in tropical-subtropical areas. Gall formers collected outside Hokkaido were not included in type B. At Sapporo, gall formers of both types A and B coexisted. The tropical samples from Okinawa, Japan and Malaysia are most likely members of asexual lineages that reproduce parthenogenetically on the grass roots all year round because no *Ulmus* species are distributed in these regions (Elias 1970; Wiegrefe et al. 1994). A number of reports have indicated that *T. nigriabdominalis* or *T. fusiformis* (= *T. akinire* sensu nov.) is distributed widely on the grass roots (or roots of rice) in regions outside the distributional ranges of *Ulmus* species, including South Asia, Southeast Asia, Oceania, and South America (Villalobos

Muller et al. 2010; Foottit et al. 2012; Simbaqueba-Cortés et al. 2015; Mille et al 2020).

Despite the difference in the mitochondrial *COI* region, types A and B were not distinguished morphologically. In Figure 2, samples 4 (Bibai, Hokkaido) and 5 (Iwamizawa, Hokkaido) belonged to the populations where only type B was collected, whereas samples 7-14 belonged to the populations of type A. However, there was no significant difference in the hind femur length between the two groups. In other morphological characters, I was not able to distinguish between types A and B. The tarsal character of exule first instar nymphs (spinulose or smooth) was examined because it was the critical criterion for discriminating between *T. nigriabdominalis* sensu Hille Ris Lambers (1970) and *T. akinire* sensu Hille Ris Lambers (1970). However, smooth tarsi were found only in two localities, and most of the potential members of type A had spinulose tarsi.

The genetic difference (*p*-distance) in the *COI* region between type A and type B was small (0.0246) compared to the distances between other species (Table 3). Lee & Akimoto (2015) indicated that mean divergence in *COI* among species within the genera of Eriosomatinae is approximately 5%. The genetic difference between *T. akinire* type A and *T. sorini* was also small (0.0265), but they were morphologically distinct.

There are two possibilities for the taxonomic status of *T. akinire* types A and B. First, the two types may represent two distinct species that have recently separated but have attained reproductive isolation. The second possibility is that the two types belong to a single species, which may have originated through the fusion of genetically divergent populations, or incipient species. If two incipient species have weak reproductive barriers, they may have fused into one species after secondary contact, but may have kept the mitochondrial genes unchanged because of adaptation to local environments. To explore which is true, it is necessary to confirm whether genetic divergence is present in some nuclear genes between the two types. In addition, the observation of mating behavior between the members of the two types is inevitable. It is also necessary to investigate the micro-geographical distribution of galls of the two types on host trees in the locality where these types coexist.

An interesting finding is the inclusion of tropical asexual lineages in type B, the gall formers of which are distributed in cool temperate regions. It has been reported that asexual lineages could originate from hybridization between closely related species or incipient species in animals and plants (Simon et al. 2002; Kearney 2005). In aphids, the hybrid origin of asexual lineages has been proposed for *Rhopalosiphum padi* (Delmotte et al. 2003). If females of type B and males of type A hybridized to produce hybrid clones, they may have inherited mitochondrial genes of type B and simultaneously may have lost the ability to produce sexuparae (autumnal migrants) and sexuals.

A gall-forming aphid species called *Tetraneura nigriabdominalis* or *T. fusiformis* and its closely related species were taxonomically revised. By referring to the original descriptions, the name *T. nigriabdominalis* (Sasaki, 1899) was discarded as an erroneous combination, and *T. akinire* Sasaki, 1904 was adopted as a valid name. The *T. akinire* species group was defined as having long claws in the first instar nymphs of the root generation. Of the *T. akinire* species group distributed in Korea and Japan, *T. ovaliformis* sp. nov., which induces globular galls on the leaves of *Ulmus davidiana* var. *japonica*, was described, and *T. akinire* sensu nov. and *T. sorini* Hille Ris Lambers, 1970 were redescribed. Molecular phylogeny based on partial sequences of

mitochondrial cytochrome c oxidase subunit I (*COI*) indicated that *T. akinire* is composed of two clusters, one (type A) of which is distributed widely from Europe to East Asia on *Ulmus* spp., and the other (type B) of which is found in Hokkaido, northern Japan on *U. davidiana* var. *japonica* and in tropical regions as anholocyclic lineages. *T. fusiformis* Matsumura, 1917, which has often been treated as a junior synonym of *T. nigriabdominalis* = *T. akinire*, likely corresponded to type B. We discussed the taxonomic status of *T. fusiformis* and tentatively concluded that it is a junior synonym of *T. akinire* sensu nov.