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

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Contents

Chapter 1

Taxonomic revision of the *Tuberculatus quercicola* group (Hemiptera: Aphididae: Drepanosiphinae), myrmecophilous aphids associated with *Quercus* species, based on morphometric and molecular phylogenetic studies

Abstract

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.

Key words: COI, description, molecular phylogeny, principal component, *Quercus crispula, Quercus dentata, Tuberculatus macrotuberculatus*.

Introduction

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).

Materials and methods

Aphid samples for morphometry and molecular phylogeny

Aphids were collected from leaves of *Q. crispula, Q. serrata,* and *Q. dentata* in the islands of Hokkaido, Honshu, and Kyushu (Fig. 1, Table S1 in Supporting Information). Collected aphids were preserved in vials of 80% ethanol, and then slide-mounted specimens, which are deposited in Systematic Entomology, Hokkaido University (SEHU), were prepared by using Canada balsam or Hoyer medium. Slide-mounted specimens in the Takahashi and Higuchi collections, which are preserved in SEHU, were also used for morphological analysis. Aphid specimens for molecular experiments were collected separately from each plant and preserved in vials of 99% ethanol at -20ºC until the experiment.

113

114 **Multivariate analysis**

115 The images of adult viviparae were captured via a microscope eyepiece camera (Dino-
116 Eye, AnMo Electronics Corporation, Taipei, Taiwan) based on the mounted specimens,
117 and the length and/or width of morphological characters were measured by using ImageJ
118 (http://rsbweb.nih.gov/ij/). In this analysis, 38 specimens of alates collected from three
119 *Quercus* species in Hokkaido, Honshu, and Kyushu were used. I measured the lengths of
120 the basal part and processus terminalis of the antennal segment VI, ultimate segment of
121 rostrum, the first to third rows of abdominal tubercles (spine 1 to spine 3), hind femur,
122 hind tarsus, and the length and width of cauda. In addition, I counted the number of
123 secondary rhinaria on antennal segments III. Measurement for each morphological
124 character is shown in Appendix S1 in Supporting Information. Principal component
125 analysis was applied to these 11 characters for visual demonstration of morphological
126 differentiation. To demonstrate which character contribute most to the differentiation,
127 eigenvectors and eigen values were calculated based on the correlation matrix. When
128 principal component analysis detected a large morphological differentiation among the
129 samples and found morphological clusters, discriminant analysis was performed to
130 quantify the extent of morphological differentiation between the clusters. Based on the
131 assumption of multivariate normality, the linear discriminant function is constructed to
132 determine at which probability each individual is discriminated into a priori clusters. If
133 the probability of erroneous discrimination is high, then I can understand that the
134 morphological differentiation between the a priori clusters is not clear.

135 I first attempted to apply principal component analysis to 33 specimens collected
136 in Hokkaido to find the pattern of morphological differentiation. If I detected definite
137 clusters, then I examined into which cluster five specimens collected in Honshu and

Kyushu are classified by using discriminant analysis. All statistical tests were conducted with JMP ver. 9.0.2(SAS Institute Inc., Cary, NC, USA).

DNA extraction, PCR, and sequencing

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 × 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 ℃ for 3 min; 30 cycles of 94 ℃ for 30 sec, 45 ℃ for 20 sec, and 65 ℃ 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 ℃ for 30 sec, 50 ℃ for 15 sec, and 65 ℃ 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 (Table S1).

164

165 **Phylogenetic analysis**

166 Most parsimony (MP) and maximum likelihood (ML) analyses were performed using
167 PAUP* 4.0b10 PPC (Swofford 2002). For MP analysis, all characters were equally
168 weighed. MP trees were searched heuristically with 1,000 random addition replication
169 using TBR branch swapping. To assess confidence for branches, non-parametric
170 bootstrap tests were performed for MP trees using full heuristic search and 10,000
171 replicates with TBR (Tree Bisection and Reconnection) branch swapping.

172 Parameters for ML analysis were chosen based on the Akaike Information Criterion
173 as implemented in Modeltest ver 3.7 (Posada & Crandall 1998). The GTR + I + G model
174 was selected for the combined mitochondrial sequences. The ML trees were searched
175 heuristically with TBR branch swapping using a stepwise addition as a starting option.
176 For the ML tree, a branch-and-bound search and 1,000 replicates with the NNI (Nearest-
177 Neighbor Interchange) branch swapping were used to save time.

178

179 **Results**

180 **Multivariate analysis**

181 Principal component analysis for 33 samples from Hokkaido indicated that PC1 and PC2
182 explained 53.3% and 25.8% of the variance, respectively (Table 1). The PC1 loadings
183 were positive with similar absolute values for all the characters except the length of
184 processus terminalis of the antennal segment VI. This result implies that PC1 represents
185 the general body size except for processus terminalis length, which had a weak
186 relationship with PC1. On the other hand, PC2 represented differences in shape among
187 the samples. Of the 11 characters, the length of ultimate rostral segment and the number
188 of secondary rhinaria strongly and negatively contributed to PC2, whereas processus
189 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 (Nos.36, 37 in Fig. 1), 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 (Nos.13, 26 in Fig. 1) 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 (Nos.7, 33 in Fig. 1), 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.

Phylogenetic analysis

MP analysis produced six most parsimonious phylogenies for the samples. The ML tree and one of the MP trees agreed in the topology (Fig. 3). 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.

Discussion

Species in the *T. quercicola* group

Phylogenetic analysis indicates that aphid populations on *Q. dentata* and those on *Q. crispula* are definitely separated and respectively constitute a monophyletic group with a high bootstrap support. This result is consistent with morphometric analysis that shows morphological differentiation between populations on *Q. dentata* and those on *Q. crispula*.

These findings indicate that populations associated with *Q. crispula* should be treated as a distinct species from those associated with *Q. dentata*. Although there is no information about reproductive isolating barriers between populations on *Q. crispula* and *Q. dentata*, I confirmed that they lived on *Q. crispula* and *Q. dentata* trees at an interval of 7 m at Iwamizawa, Hokkaido. This parapatric coexistence suggests that populations on *Q. crispula* and those on *Q. dentata* are at a full specific status with isolating mechanisms. For these reasons, I treat *Q. crispula*-associated populations and *Q. dentata*-associated populations as distinct species and formally describe them.

On the other hand, the taxonomic status of the central Hokkaido and the eastern Hokkaido groups of the *Q. dentata*-associated population is not clear. These two groups are morphologically distinct, so that Yao (2011) tentatively regarded the eastern Hokkaido group as a distinct species, sp. B. However, phylogenetic study implies that there is no genetic divergence between the two groups. I can assume two possibilities for this discrepancy between morphology and phylogeny. First, the two populations may have recently been separated geographically and have rapidly accumulated genetic differences due to strong selective pressures. Specific selective pressures in eastern Hokkaido may have led to rapid morphological changes, while resulting in the lack of genetic divergence in mitochondrial genes. Second, geographic separation of the two populations may have been traced back to an old time, say, a few million years ago, but later the two populations may have contacted with each other recently. Secondary contacts of populations may result in introgression, and in this case, introgression of mitochondrial genes from the central population to the eastern population may have led to the lack of genetic divergence in the COI gene between the populations. Several reports from some animal taxa indicate that because of extensive introgression, two species that are distributed parapatrically share the same sequence of mtDNA, though exhibiting definite divergence in the sequence of nuclear genes and morphologies (Sota & Vogler

2001; Shaw 2002; Melo-Ferreira et al. 2005; Bachtrog et al. 2006). This type of introgressed mtDNA may have been maintained if the density of the eastern population was low at the time of population contact and if the mitochondrial gene of the central type was selectively advantageous.

In the future study, it is necessary to compare the sequences of nuclear genes between the populations to test if there is any genetic divergence between the populations. At the same time, it is required to access whether any isolating mechanism is associated with the difference in morphology.

The morphological differentiation between the central and eastern Hokkaido groups may be linked with the geohistorical changes in the distributions of *Quercus* species. At present, *Q. crispula* constitutes the main element of deciduous hardwood forests in Hokkaido with continuous distributions, while *Q. dentata* is widely distributed along coastal regions of Hokkaido (Horikawa 1972). Pollen analysis of Pleistocene sediments reveals that during the Last Glacial Maximum (LGM) *Quercus* species were very rare in most regions of Hokkaido (Igarashi et al. 2011), but that they rapidly expanded their distributional ranges in the last 8,000 years ago with the recovery of warm and humid climates (Igarashi 1994). Throughout the Pleistocene, the distributions of *Quercus* species were in the cycle of expansion and retrogression depending on the fluctuations of climate conditions (Igarashi 1994). With increase in the average annual temperature and precipitation after the LGM, *Quercus* populations expanding from different refugia may have fused into a single population. Throughout the Pleistocene, retrogression of populations into refugia and fusion of expanding populations may have repeatedly occurred. Such phylogeographic changes in host plants may have had large impacts on the genetic differentiation and speciation of *Tuberculatus* species. I suppose that the morphological differentiation between the central and eastern Hokkaido groups of the *Q. dentata*-associated population may be due to isolation of their populations into

different refugia in the past and subsequent population fusions, although at present I have no palaeobiological evidence for this scenario.

At this time, I tentatively treat the central Hokkaido and the eastern Hokkaido groups of the *Q. dentata*-associated population as taxonomically informal races because phylogenetic analysis indicated the absence of genetic differentiation between the two groups with distinct morphologies. This taxonomic treatment may be revised in the future when I acquire more information on reproductive barriers and genetic divergence in nuclear genes.

Relationship with the specific epithets, *quercicola* and *macrotuberculatus*

Matsumura (1917) mentioned that *A. quercicola* (=*T. quercicola*) was collected from *Q. grosserrata* (=*Q. crispula*) and from *Alnus incana* (=*Alnus japonica*), and characterized by four secondary rhinaria on the antennal segment III and by the short rostrum that does not reach the mid coxae. The host record and morphological characteristics completely correspond to those in the *Q. crispula*-associated population but not to those in the *Q. dentata*-associated population. I think that the collection record from *A. incana* may merely be an error, or the accidental landing of the alates on the plant. Therefore, I conclude that the *Q. crispula*-associated population is *T. quercicola*.

By contrast, *Myzocallis macrotuberculata* (=*T. macrotuberculatus*) is reported to have been collected from *Q. dentata* in Tokyo (Essig & Kuwana 1918), and the lengths of the basal part and processus terminalis of the antennal segment VI are reported to be 0.13 mm and 0.12 mm, respectively. These characteristics completely accord with those in the *Q. dentata*-associated population (0.150 mm and 0.135 mm long, respectively, in this study) and not to those in the *Q. crispula*-associated population (0.149 mm and 0.168 mm long, respectively); the *Q. crispula*-associated population has longer processus terminalis than the basal part. The descriptions of the rostrum and secondary rhinaria on

the antennal segment III also agree with the characteristics of the *Q. dentata*-associated population. Thus, I conclude that the *Q. dentata*-associated population is attributable to *T. macrotuberculatus*.

A problem in this naming is that the usage of *T. quercicola* radically changes: the species associated with *Q. dentata*, which has been referred to as "*T. quercicola*" in several studies, is now called "*T. macrotuberculatus*". Furthermore, Higuchi (1972) mentioned that "*T. quercicola*" was recorded from *Q. acutissima* and *Q. variabilis* as well. Despite our long-term search for *Tuberculatus,* no aphids of the *T. quercicola* group have been collected from *Q. acutissima* or *Q. variabilis*. Thus, *Q. acutissima* and *Q. variabilis* should be removed from the host record of the *T. quercicola* species group.

Description

Tuberculatus macrotuberculatus (Essig & Kuwana, 1918) **stat. rev.**

Myzocallis macrotuberculata Essig & Kuwana (1918), Proc. Calif. Acad. Sci. 8: 90-92.

Tuberculatus quercicola (Matsumura), Higuchi (1969) (in part), Ins. Mats. 32: 117-118; Quednau (1999) (in part), Amer. Ent. Inst. 31(1): 245; Yao & Akimoto (2009), J. Ins. Sci. 9: 1-9; Yao (2010), Bio. Let. 6: 282-286; Yao (2011), Can. Entomol. 143: 35-43.

Tuberculatus (Acanthocallis) macrotuberculata Essig & Kuwana (1918) as a junior synonym of *T. quercicola* (Matsumura, 1917), Eastop & Hille Ris Lambers (1976), *Survey of the World's Aphids*. p. 440.

Alate viviparous female. Descriptions are based on 27 individuals. Body 2.0-3.2 mm long from the vertex to the tip of cauda. Median tubercles on head not developed; vertex nearly flat. Compound eyes rather large, 0.13-0.20 mm in diameter. One frontal cephalic seta present on either side of frontal ocellus, pointed, 1.5 times as long as first antennal

346 segment. Other cephalic setae anterior to the compound eyes approximately equal to
347 frontal setae in length and shape; 6-16 (on average 14.3) setae arranged in a transverse
348 row between compound eyes, a little shorter than frontal setae. Antennae 0.5-0.8 times as
349 long as body (Fig. 4A). Third antennal segment with a row of 4-9 (on average 7.0)
350 secondary rhinaria over the entire segment. Fourth and fifth antennal segments with fine
351 setae, of which the longest one is, in length, 3 times the width of fourth antennal segment
352 at the middle position. The base and processus terminalis of sixth antennal segment, on
353 average, 0.150 mm and 0.135 mm long, respectively. Rostrum surpassing mid coxae.
354 Ultimate rostral segment 1.2-1.4 times as long as the second segment of hind tarsus, with
355 14-20 secondary setae (Fig. 4C). Pronotum with 2 pairs of spinal tubercles; posterior pair
356 larger than anterior pair. Mesonotum with 1 pair of spinal tubercles posteriorly.
357 Metanotum with 1 pair of spinal tubercles. First to seventh abdominal tergites each with
358 1 pair of spinal tubercles imbricated. Tubercles on first to third abdominal tergites
359 projecting long, pigmented conspicuously from the tip to the base. First segment of hind
360 tarsus with 6 setae ventrally and 2 dorsally. Cauda knobbed with many long setae. Anal
361 plate bilobed, with many long setae. Cornicles present on sixth abdominal segment,
362 longer than wide, fringed at the apex.
363
364 Morphologically, *T. macrotuberculatus* is divided into two races that are distributed
365 parapatrically in Hokkaido. Thus, the morphological characteristics and distribution
366 range of the races are described below.
367
368 **The central Hokkaido race of *Tuberculatus macrotuberculatus*** (Fig. 4E)
369 **Alate viviparous female.** Descriptions are based on 14 individuals. Body 2.0-2.6 mm
370 long from the vertex to the tip of cauda. Pointed setae present dorsally on antennal
371 segments and on head to abdomen. Compound eyes 0.13-0.16 mm in diameter. Antennae

1.3-1.6 mm long. First and second antennal segments, respectively, with 4-8 and 2-4 setae, which are shorter than frontal setae. Third antennal segment with 13-18 setae, of which the longest one is in length 1.2 times the width of first antennal segment, with a row of 4-9 (on average 6.5) secondary rhinaria. The average lengths of antennal segments as follows: I 0.073 mm, II 0.062 mm, III 0.472 mm, IV 0.299 mm, V 0.284 mm, and VI 0.264 mm. Pronotum with 3-6 (on average 4.0) pleural setae anteriorly; with 2-5 (on average 4.2) lateral setae; with 3-9 (on average 5.7) small smooth tubercles posteriorly on either side (Fig. 5G); anterior and posterior spinal tubercles each with 2-4 (on average 2.7), 4-8 (on average 5.2) pointed setae, respectively. Spinal tubercle of mesonotum and metanotum, respectively, with 3-10 (on average 5.9) and 3-6 (on average 4.6) pointed setae. Femora with many pointed setae, which are 0.8-1.2 times the width of femur at the middle point. Hind femur 0.50-0.77 mm long (on average 0.58 mm), pigmented strongly. Mid femur pigmented less strongly than hind femur. Second segment of hind tarsus 0.12-0.16 mm long, 1.0-1.2 times as long as processus terminalis of sixth antennal segment. First to seventh abdominal spinal tubercle with 2-9 pointed thick setae. Tubercle on first to third abdominal segments with 1 thick seta, which is present at the apex, and with 1-2 thick setae on lateral inside of the tubercle (Fig. 5A). Tubercles on the second abdominal tergite pigmented more widely than those on the first or third abdominal tergite. Tubercles on the third abdominal tergite pigmented as strongly as hind femur; tubercles on second tergite pigmented as strongly as those on third tergite or sometimes less intensely; those on first tergite pigmented as strongly as those on second tergite or less intensely. The average lengths of spinal tubercules of first to third abdominal tergites as follows: I 0.083 mm, II 0.114 mm, III 0.131 mm. Lateral tubercles present on first to seventh abdominal tergites, inconspicuous, approximately equal in length to or shorter than cornicles, pigmented slightly. Ventral abdominal setae numerous, shorter than dorsal setae. Cauda

0.089 mm long, 0.076 mm wide, laterally with 4-5 setae, which are 1.0-1.3 times as long as cauda, ventrally with 8-15 setae, which are 0.7-1.1 times as long as cauda (Fig. 5D).

Alatoid larvae. Pale green in anterior half of body and blackish in posterior half, with a longitudinal, median white band in life. This white band consists of spinal tubercles, which look like white spots.

Host plant: *Quercus dentata*.

Specimens examined. Alate viviparous female: 7 exs, Sapporo, Hokkaido, 2. VIII.1960, on *Quercus dentata*, R. Takahashi; 2 exs, Chitose, Hokkaido, 12.VII.1973, on *Quercus dentata*, H. Higuchi; 2 exs, Iwamizawa, Hokkaido, 24. VII.2011, on *Quercus dentata*, S. Akimoto; 3 exs, Iwamizawa, Hokkaido, 25.IX.2011, on *Quercus dentata*, S. Akimoto; 5 exs, Syariki, Aomori, 23.VIII.2005, on *Quercus dentata*, I. Yao; 7 exs, Kokonoe, Oita, 23.VI.2007, on *Quercus dentata*, I. Yao.

Phylogenetic and morphometric analysis shows that this race is also distributed in the following localities; Bansei-onsen, Erimo, Esan, Ishikari, Mukawa, Obihiro, Osyoro, Teshio and Tomamae, Hokkaido, Japan; Tsugaru, Aomori; Kisakata and Nyudozaki, Akita; Iwagasaki, Iwamuro and Kashiwazaki, Niigata; Kashiwa, Chiba; Matsumoto, Nagano; Houdatsushimizu, Ishikawa; Aoya and Daisen, Tottori; Hiruzen, Okayama; Yufudake, Oita.

The eastern Hokkaido race of *Tuberculatus macrotuberculatus*

Alate viviparous female. Descriptions are based on 13 individuals. Body 2.8-3.2 mm long from the vertex to the tip of cauda. Capitated setae present dorsally on antennal segments and on head to abdomen (Fig. 5H). Compound eyes 0.15-0.20 mm in diameter. Antennae 1.5-2.2 mm long. Third antennal segment with a row of 6-9 (on average 7.8)

secondary rhinaria. The average lengths of antennal segments as follows: I 0.080 mm, II 0.081 mm, III 0.634 mm, IV 0.425 mm, V 0.380 mm, and VI 0.360 mm. Hind femur 0.65-0.95 mm long (on average 0.83 mm). Second segment of hind tarsus 0.15-0.19 mm long. The average lengths of spinal tubercules of first to third abdominal tergites as follows: I 0.144 mm, II 0.182 mm, III 0.172 mm (Fig. 5B). Cauda 0.106 mm long, 0.106 mm wide (Fig. 5E).

Alatoid larvae. Stout in appearance. Pale green to pale yellow. A longitudinal, median white band not conspicuous.

Host plant: *Quercus dentata*.

Specimens examined. Alate viviparous female: 2 exs, Lake Saroma, Hokkaido, 22.VI.1965, on *Quercus dentata*, H. Higuchi; 3 exs, Lake Saroma, Hokkaido, 11.VII.2003, on *Quercus dentata*, I. Yao; 2 exs, Lake Notoro, Hokkaido, 11.VII.2003, on *Quercus dentata*, I. Yao; 4 exs, Shikaoi, Hokkaido, 21.VI.2005, on *Quercus dentata*, T. Hironaga; 2 exs, Lake Notoro, Hokkaido, 20.VII.2005, on *Quercus dentata*, I. Yao.

This race is also distributed in the following locality; Kawanishi and Shari, Hokkaido.

Remarks to two races of *T. macrotuberculatus*

The eastern Hokkaido race is characterized by capitated setae on antennal segments and on the dorsal side of head to abdomen, whereas the central Hokkaido race always has pointed setae on the same positions. In the eastern Hokkaido race, the proportions of capitated and pointed setae on the dorsum vary among the collection localities. The percentage of capitated setae on spinal tubercules of 1st to 3rd thoracic segments is as follows: in the Lake Saroma 95%-100%, in Shikaoi 75%-100%, and in the Lake Notoro 0%-22%. The percentage of capitated setae on the spinal tubercules of 1st to 7th

449 abdominal segments is as follows; in the Lake Saroma 96%-100%, in Shikaoi 25%-100%,
450 and in the Lake Notoro 6%-17%. The eastern Hokkaido race has longer abdominal
451 tubercules than does the central Hokkaido race. The mean length of spinal tubercules on
452 the second abdominal tergite is 0.114 mm in the central Hokkaido race and 0.182 mm in
453 the eastern Hokkaido race. The two races are clearly distinguished by the combination of
454 larval body color and the above-mentioned adult characteristics.
455
456 ***Tuberculatus quercicola*** (Matsumura, 1917)
457 *Acanthocallis quercicola* Matsumura (1917), Jour. Coll. Agr. 7: 368-369.
458 *Ptychodes quercicola* Matsumura (1919), Tran. Sapp. Nat. Hist. Soc. 7: 101-102.
459 *Tuberculatus quercicola* (Matsumura), Higuchi (1969) (in part), Ins. Mats. 32: 117-118;
460 Eastop & Hille Ris Lambers (1976) (in part), *Survey of the World's Aphids*. p. 440.;
461 Quednau (1999) (in part), Amer. Ent. Inst. 31(1): 245.
462
463 **Alate viviparous female.** Descriptions are based on 21 individuals. Body 2.0-2.7 mm
464 long from the vertex to the tip of cauda. Median tubercles on head not developed; vertex
465 nearly flat. Compound eyes rather large, 0.12-0.15 mm in diameter. One frontal cephalic
466 seta present on either side of frontal ocellus, pointed, 1.5 times as long as first antennal
467 segment. Other cephalic setae anterior to the compound eyes approximately equal to
468 frontal setae in length and shape; 4-10 (on average 8.7) setae arranged in a transverse row
469 between compound eyes, a little shorter than frontal setae. Antennae 1.1-1.5 mm, 0.5-0.6
470 times as long as body (Fig. 4B). First and second antennal segments, respectively, with
471 3-4 and 2-3 setae, which are shorter than frontal setae. Third antennal segment with 8-13
472 setae, of which the longest one is equal to width of first antennal segment, with a row of
473 3-6 (on average 4.1) secondary rhinaria over the entire segment. Fourth and fifth segments
474 with fine setae, of which the longest one is 3 times the width of fourth antennal segment

475 at the middle position. The average lengths of antennal segments as follows: I 0.088 mm;
476 II 0.068 mm; III 0.401 mm; IV 0.241 mm; V 0.245 mm; VI 0.315 mm. The base and
477 processus terminalis of sixth segment, on average, 0.149 mm and 0.168 mm long,
478 respectively. Rostrum just reaching mid coxae (do not surpassing mid coxae). Ultimate
479 rostral segment 0.8-0.9 times as long as the second segment of hind tarsus, with 8-16
480 secondary setae (Fig. 4D). Pronotum with 3-5 (on average 3.6) pleural setae anteriorly;
481 with 2-4 (on average 3.3) lateral setae; with 1-7 (on average 3.8) small smooth tubercles
482 posteriorly on either side; with 2 pairs of spinal tubercles; posterior pair larger than
483 anterior pair; anterior and posterior spinal tubercles each with 1-3 (on average 1.9), 2-5
484 (on average 3.3) pointed setae, respectively. Mesonotum with 1 pair of spinal tubercles
485 posteriorly; each tubercle with 2-5 (on average 3.0) pointed setae. Metanotum with 1 pair
486 of spinal tubercles; each tubercle with 1-3 (on average 2.0) pointed setae. Femora with
487 many pointed setae, which are 0.8-1.2 times the width of hind femur at the middle point.
488 Hind femur 0.45-0.62 mm long (on average 0.54 mm), pigmented strongly. Mid femur
489 pigmented less strongly than hind femur. First tarsal segment with 6 setae ventrally and
490 2 dorsally; second segment of hind tarsus 0.14-0.17 mm long, 0.8-1.0 times as long as
491 processus terminalis of sixth antennal segment. First to seventh abdominal tergites each
492 with 1 pair of spinal tubercles imbricated; each tubercle with 1-6 pointed thick setae.
493 Tubercles on first to third abdominal tergites projecting long, pigmented conspicuously
494 from the tip to the base, each with 1 thick seta, which is present at the apex, with 1-2 thick
495 setae on lateral inside of the tubercle (Fig. 5C). Tubercles on the second abdominal tergite
496 pigmented more widely than those on the first or third abdominal tergite. Tubercles on
497 the third abdominal tergite pigmented most strongly; pigmentation as strong as hind
498 femur; tubercles on second tergite pigmented less intensely than those on third tergite;
499 those on first tergite pigmented less intensely than those on second tergite. The average
500 lengths of spinal tubercules of first to third abdominal tergites as follows: I 0.066 mm, II

0.105 mm, III 0.147 mm. Lateral tubercles present on first to seventh abdominal tergites, inconspicuous, approximately equal to or shorter than cornicles in length, pigmented slightly. Ventral abdominal setae numerous, shorter than dorsal setae. Cornicles present on sixth abdominal segment, longer than wide, fringed at the apex. Cauda knobbed, 0.085 mm long, 0.101 mm wide, laterally with 3-5 setae, which are 1.2-1.3 times as long as cauda, ventrally with 7-14 setae, which are 0.8-1.0 times as long as cauda (Fig. 5F). Anal plate bilobed, with many long setae.

All the type specimens of aphid species described by S. Matsumura should be preserved in SEHU. However, I was not able to find the type specimens of *T. quercicola* in the collection. Higuchi (1969) did not refer to the type specimen, either. Thus, I think that the syntypes of *T. quercicola* are lost from the collection.

Alatoid larvae. Green to pale green with a longitudinal, median white band in life. This white band consists of spinal tubercles, which look like white spots. Sometimes dark green along the white band.

Host plants: *Quercus crispula*, and *Quercus serrata* (temporary host).

Specimens examined. Alate viviparous female: 3 exs, Iwamizawa, Hokkaido, 24.VII.2011, on *Quercus crispula,* S. Akimoto; 2 exs, Iwamizawa, Hokkaido, 24.VII.2011, on *Quercus serrata*, S. Akimoto; 3 exs, Teinehoshioki, Hokkaido, 9.VIII.2011, on *Quercus crispula*, K. Masaya; 4 exs, Iwamizawa, Hokkaido, 20.VIII.2011, on *Quercus crispula*, S. Akimoto; 2 exs, Hakkenzan, Hokkaido, 21.VIII.2011, on *Quercus crispula*, T. Kanbe; 2 exs, Utoro, Hokkaido, 23.IX.2011, on *Quercus crispula*, S. Akimoto; 2 exs, Iwamizawa, Hokkaido, 25.IX.2011, on *Quercus crispula*, S. Akimoto; 2 exs, Iwamizawa, Hokkaido, 13.IX.2012, on *Quercus crispula*, S. Akimoto; 1 ex., Iwamizawa, Hokkaido, 13.XI.2012, on *Quercus serrata*, S. Akimoto.

This species is also distributed in the following localities; Ebetsu, Hitsujigaoka, Poroshiri, Shikabe, Shikaoi, Shari, and Tomakomai, Hokkaido; Towada, Aomori; Omoshiroyama-kogen, Yamagata.

Remarks

Tuberculatus quercicola closely resembles *T. macrotuberculatus* in the general morphology of alates, i.e., body length, body color and the shape of abdominal tubercles. However, the results of measurements indicate consistent differences in metrical characters between *T. quercicola* and *T. macrotuberculatus*.

First, there are three distinct differences in antennal characters. The 6th antennal segment of *T. quercicola* is longer than that of *T. macrotuberculatus*. The ratio of the 6th antennal segment to the 4th (6th/4th) is 1.3 (1.1-1.6) in *T. quercicola*, and 0.9 (0.7-1.1) in *T. macrotuberculatus*. The ratio of the processus terminalis to the base of the 6th antennal segment also differs as follows (pt/base): 1.1 (0.9-1.2) in *T. quercicola*, and 0.9 (0.7-1.2) in *T. macrotuberculatus*. The number of secondary rhinaria on the 3rd antennal segment is 4.1 (3-6) for *T. quercicola*, 6.5 (4-9) for the central Hokkaido race of *T. macrotuberculatus*, and 7.8 (6-9) for the eastern Hokkaido race. Secondly, *T. macrotuberculatus* has a longer rostrum than *T. quercicola*. The ratio of the length to the basal width of ultimate rostral segment (length/basal width) is 1.4 (1.2-1.7) in *T. quercicola*, and 2.3 (1.7-3.0) in *T. macrotuberculatus*. Lastly, *T. quercicola* can be discriminated from *T. macrotuberculatus* in two abdominal characters. The two species have conspicuous spinal tubercles dorsally on the 1st to 3rd abdominal tergites in common, but have different lengths of the tubercles; the relative lengths on the 1st-3rd tergites are 0.6:1:1.4 for *T. quercicola*, 0.7:1:1.2 for the central Hokkaido race of *T. macrotuberculatus*, and 0.8:1:1.0 for the eastern Hokkaido race. *Tuberculatus macrotuberculatus* has a slender, oval cauda, whereas *T. quercicola* has a broadly oval

553 cauda. The ratio of the width to the length of cauda (width/length) is 1.2 (1.0-1.5) for *T.*
554 *quercicola*, and 0.9 (0.7-1.2) for *T. macrotuberculatus*. *T. macrotuberculatus* from the
555 Lake Saroma, Shikaoi, and Lake Notoro (the eastern Hokkaido race) has capitated setae
556 on the antennal segments, head, and abdomen, and is larger in the lengths of body,
557 antennae, legs and cauda than the central Hokkaido race of *T. macrotuberculatus*.
558 Throughout the entire body, *T. macrotuberculatus* exhibits stronger pigmentation,
559 with more numerous setae (see Table S2 in Supporting Information) and longer tubercles
560 than does *T. quercicola*.
561
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568
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Table 1 Loadings on each morphological character in principal component analysis for 11 characters of the *Tuberculatus quercicola* group

Morphological character	PC1 loadings	PC2 loadings
Basal part of ant. seg. VI length [1)]	0.331	0.236
Processus terminalis of ant. seg. VI length [2)]	-0.018	0.551
Ultimate rostrum seg. length [3)]	0.330	-0.325
Spine1 length [4)]	0.355	-0.162
Spine2 length	0.377	-0.016
Spine3 length	0.240	0.208
Hind femur length	0.400	-0.057
Hind tarsus length	0.321	0.317
Cauda length	0.274	-0.058
Cauda width	0.200	0.467
No. secondary rhinaria	0.278	-0.374

[1)] -[4)], See 'AB', 'APT', 'URSW', and 'STI' in Appendix S2 of Supporting Information.

Figure legends

Figure 1 Distribution of the *Tuberculatus quercicola* group in Japan. Closed circles indicate the collection localities of the central Hokkaido race of *T. macrotuberculatus* associated with *Quercus dentata*, gray circles: the eastern Hokkaido race of *T. macrotuberculatus* associated with *Quercus dentata*, and open circles: *T. quercicola* associated with *Quercus crispula*. See Appendix S2 in Supporting Information for localities 1-47.

Figure 2 Principal component analysis for 11 morphological characters of alates in the *Tuberculatus quercicola* group. PC1 and PC2 scores are indicated. Open circles: *T. quercicola* from *Quercus crispula*, closed squares: the eastern Hokkaido race of *T. macrotuberculatus* from *Quercus dentata*, closed triangles: the central Hokkaido race of *T. macrotuberculatus* from *Quercus dentata*, open triangles: *T.* sp. from *Quercus serrata*, and cross: *T.* sp. from *Quercus dentata* in Honshu and Kyushu, Japan.

Figure 3 A phylogenetic tree based on most parsimony (MP) and maximum likelihood (ML) methods for 22 aphids of the *Tuberculatus quercicola* group with 3 aphids of other *Tuberculatus* groups as outgroups. Only ML tree is shown because one of the 6 MP trees is congruent with the ML tree. Bootstrap percentages in ML and MP (ML/MP) greater than 50% are indicated below or near the branches in a tree. Closed bars represent the populations of the central Hokkaido race of *T. macrotuberculatus* from *Quercus dentata*, a gray bar the eastern Hokkaido race of *T. macrotuberculatus* from *Quercus dentata*, and an open bar the populations of *T. quercicola* from *Quercus crispula*. The numbers in species names correspond to those in Figure 1.

Figure 4 Antennae, ultimate rostral segment, and the entire dorsal view of viviparous alates. (A, C, and E) the central Hokkaido race of *T. macrotuberculatus*, and (B, D) *T. quercicola*. Scale bars, 0.1mm for antenna, 0.05mm for ultimate rostral segment, and 0.1 mm for the entire sketch.

Figure 5 Morphological characters of the *T. quercicola* group. (A-C) Spinal tubercules on the 1st-3rd abdominal tergites; (D-F) Cauda; (G) Lateral region of pronotum on a slide-mounted specimen with nine tubercles are in a circle; and (H) Capitated setae on the

696 pronotum. A and D; the central Hokkaido race of *T. macrotuberculatus*, B, E, G, and H;
697 the eastern Hokkaido race of *T. macrotuberculatus*, C and F; *T. quercicola*. Scale bars,
698 0.2mm for A-C, 0.1mm for D-F, H, and 0.03mm for G.
699
700
701 **Table 2** Host plants and collection locality of the *Tuberculatus quercicola* group. The
702 numbers correspond to those on Figure 1.
703 *Qd, Quercus dentata; Qc, Quercus crispula; Qs, Quercus serrata; Tm*(central)*,* The
704 central Hokkaido race of *Tuberculatus macrotuberculata; Tm*(eastern)*,* The eastern
705 Hokkaido race of *Tuberculatus macrotuberculata; Tq, Tuberculatus quercicola.*
706 KM, Kouhei Masaya; HH, Hiromichi Higuchi; IO, Issei Ohshima; IY, Izumi Yao; MS,
707 Masakazu Sano; RT, Ryoichi Takahashi; SA, Shin-ichi Akimoto; SS, Shun'ichiro
708 Sugimoto; TH, Teruhiko Hironaga; TK, Takashi Kanbe.

	Host plant	Collection site	Date	Collector	Species (race)	**Accession number**
1	*Qd*	Teshio, Hokkaido	13.VII.2003	IY	*Tm* (central)	AB900071
2	*Qd*	Tomamae, Hokkaido	20.VII.2006	IY	*Tm* (central)	
3	*Qd*	Iwamizawa, Hokkaido	24.VII.2011, 25.IX.2011	SA	*Tm* (central)	AB900073
4	*Qd*	Ishikari, Hokkaido	8.VII.2005	IY	*Tm* (central)	AB900072
5	*Qd*	Osyoro, Hokkaido	23.VII.2002	IY	*Tm* (central)	

6	*Qd*	Sapporo, Hokkaido	2.VIII.1960	RT	*Tm* (central)	
7	*Qd*	Obihiro, Hokkaido	28.VIII.2007	MS	*Tm* (central)	
8	*Qd*	Chitose, Hokkaido	12.VII.1973	HH	*Tm* (central)	
9	*Qd*	Banseionsen, Hokkaido	14.VII.2010	IY	*Tm* (central)	
10	*Qd*	Mukawa, Hokkaido	14.VII.2010	IY	*Tm* (central)	
11	*Qd*	Erimo, Hokkaido	14.VII.2010	IY	*Tm* (central)	
12	*Qd*	Esan, Hokkaido	17.VII.2007	IY	*Tm* (central)	
13	*Qd*	Syariki, Aomori	25.VI.2010	IY	*Tm* (central)	AB900070
14	*Qd*	Tsugaru, Aomori	25.VI.2010	IY	*Tm* (central)	
15	*Qd*	Nyudozaki, Akita	18.IX.2009	IY	*Tm* (central)	
16	*Qd*	Kisakata, Akita	17.IX.2009	IY	*Tm* (central)	
17	*Qd*	Iwagasaki, Niigata	16.IX.2009	IY	*Tm* (central)	
18	*Qd*	Kashiwazaki,	15.IX.2009	IY	*Tm* (central)	

		Niigata				
19	*Qd*	Iwamuro, Niigata	15.IX.2009	IY	*Tm* (central)	
20	*Qd*	Kashiwa, Chiba	21.VI.2006	IY	*Tm* (central)	AB900074
21	*Qd*	Matsumoto, Nagano	6.IX.2007	IY	*Tm* (central)	
22	*Qd*	Houdatsushimizu, Ishikawa	9.VII.2007	IY	*Tm* (central)	AB900075
23	*Qd*	Aoya, Tottori	22.VI.2011	IY	*Tm* (central)	
24	*Qd*	Daisen, Tottori	21.VI.2011	IY	*Tm* (central)	AB900076
25	*Qd*	Hiruzen, Okayama	21.VI.2011	IY	*Tm* (central)	
26	*Qd*	Kokonoe, Oita	23.VI.2007	IY	*Tm* (central)	
27	*Qd*	Yufudake, Oita	21.VI.2007	IY	*Tm* (central)	AB900077
28	*Qd*	Yufudake, Oita	21.VI.2007	IY	*Tm* (central)	AB900078
29	*Qd*	Lake saroma, Hokkaido	22.VI.1965, 11.VII.2003	IY, HH	*Tm* (eastern)	AB900080
30	*Qd*	Lake notoro, Hokkaido	11.VII.2003, 20.VII.2005	IY	*Tm* (eastern)	

31	*Qd*	Shari, Hokkaido	19.VII.2005	IY	*Tm* (eastern)	AB900081
32	*Qd*	Shikaoi, Hokkaido	21.VI.2005	TH	*Tm* (eastern)	AB900082
33	*Qd*	Kawanishi, Hokkaido	24.VII.2007	IY	*Tm* (eastern)	AB900079
34	*Qc*	Utoro, Hokkaido	23.IX.2011	SA	*Tq*	
35	*Qc*	Shari, Hokkaido	25.VII.2007	IY	*Tq*	AB900089
36	*Qc*	Iwamizawa, Hokkaido	24.VII.2011, 20.VIII.2011, 25.IX.2011, 13.XI.2012	SA	*Tq*	
37	*Qs*	Iwamizawa, Hokkaido	24.VII.2011, 13.XI.2012	SA	*Tq*	AB900085
38	*Qc*	Shikaoi, Hokkaido	21.VI.2005	IY	*Tq*	AB900088
39	*Qc*	Teinehoshioki, Hokkaido	9.VII.2011	KM	*Tq*	
40	*Qc*	Ebetsu, Hokkaido	22.VI.2008	IY	*Tq*	AB900086
41	*Qc*	Hitsujigaoka, Hokkaido	5.X.2007	IY	*Tq*	
42	*Qc*	Hakkenzan,	21.VIII.2011	TK	*Tq*	

		Hokkaido				
43	*Qc*	Poroshiri, Hokkaido	24.VII.2007	IY	*Tq*	AB900087
44	*Qc*	Tomakomai, Hokkaido	19.VII.2007	IY	*Tq*	AB900083
45	*Qc*	Shikabe, Hokkaido	10.VII.2006	IY	*Tq*	AB900084
46	*Qc*	Towada, Aomori	10.VIII.2011	SS	*Tq*	AB900090
47	*Qc*	Omoshiroyama-kogen, Yamagata	31.VIII.2012	IO	*Tq*	AB900091
48	*Qs*	Honmoku, Kanagawa	23.IX.2009	SS	*T. fulviabdominalis*	AB900092
49	*Qs*	Hakone, Kanagawa	20.VIII.2006	SS	*T. pilosulus*	AB900093
50	*Qs*	Nagamine, Hyogo	26.IX.2009	SS	*T. indicus*	AB900094

Table 3

The comparisons between the central Hokkaido race of *Tuberculatus macrotuberculata*, the eastern Hokkaido race of *T. macrotuberculata,* and *T. quercicola* for the number of setae on each part of dorsal side.

The central Hokkaido race of *Tuberculatus macrotuberculata* (N=14)

	head	pronotum		spinal tubercle of thorax				spinal tubercle of abdomen						
	posterior	pleural anterior	lateral posterior	pronotum anterior	pronotum posterior	mesonotum	metanotum	1st tergite	2nd tergite	3rd tergite	4th tergite	5th tergite	6th tergite	7th tergite
avg.	5.1	4.0	4.2	2.7	5.2	5.9	4.6	5.7	5.3	5.5	3.7	3.0	2.8	2.8
mini.	4	3	2	2	4	3	3	4	3	4	2	2	2	2
max.	8	6	5	4	8	10	6	9	8	7	6	5	5	4

717

718 **The eastern Hokkaido race of *Tuberculatus macrotuberculata* (N=13)**

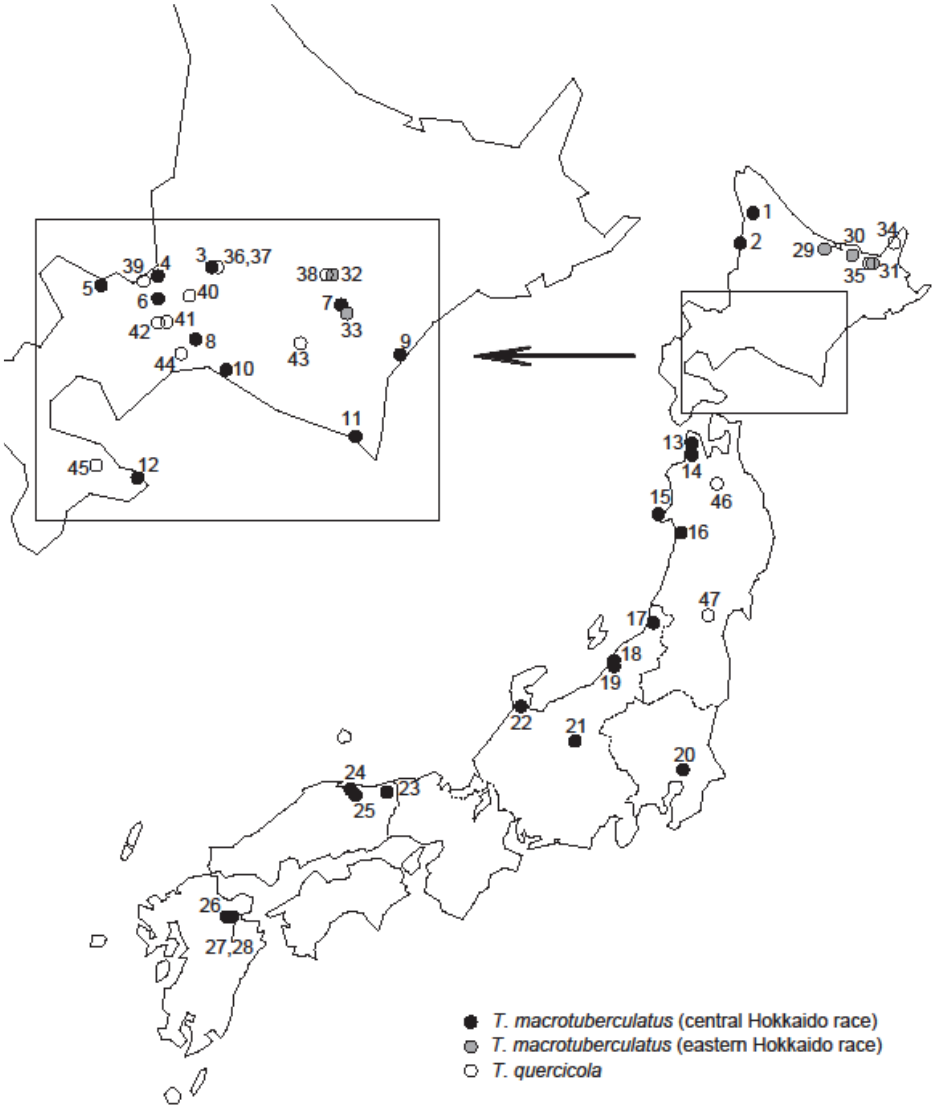
	head	pronotum		spinal tubercle of thorax				spinal tubercle of abdomen						
	posterior	pleural anterior	lateral posterior	Pronotum anterior	pronotum posterior	mesonotum	metanotum	1st tergite	2nd tergite	3rd tergite	4th tergite	5th tergite	6th tergite	7th tergite
avg.	5.2	4.3	3.9	2.7	5.2	5.3	4.3	6.8	5.9	5.6	4.3	3.2	3.2	3.6
mini.	3	3	2	2	3	3	3	4	4	4	3	2	2	2
max.	7	6	6	4	8	8	6	9	9	8	6	4	5	6

719

720 ***T. quercicola* (N=21)**

	head	pronotum		spinal tubercle of thorax				spinal tubercle of abdomen						
	posterior	pleural anterior	lateral posterior	pronotum anterior	pronotum posterior	mesonotum	metanotum	1st tergite	2nd tergite	3rd tergite	4th tergite	5th tergite	6th tergite	7th tergite
avg.	4.4	3.6	3.3	1.9	3.3	3.0	2.0	3.9	3.8	3.7	3.3	2.3	2.1	2.0
mini.	2	3	2	1	2	2	1	3	2	2	2	1	2	2
max.	5	5	4	3	5	5	3	6	6	5	4	4	3	2

721



722

723 **Figure 1**

724

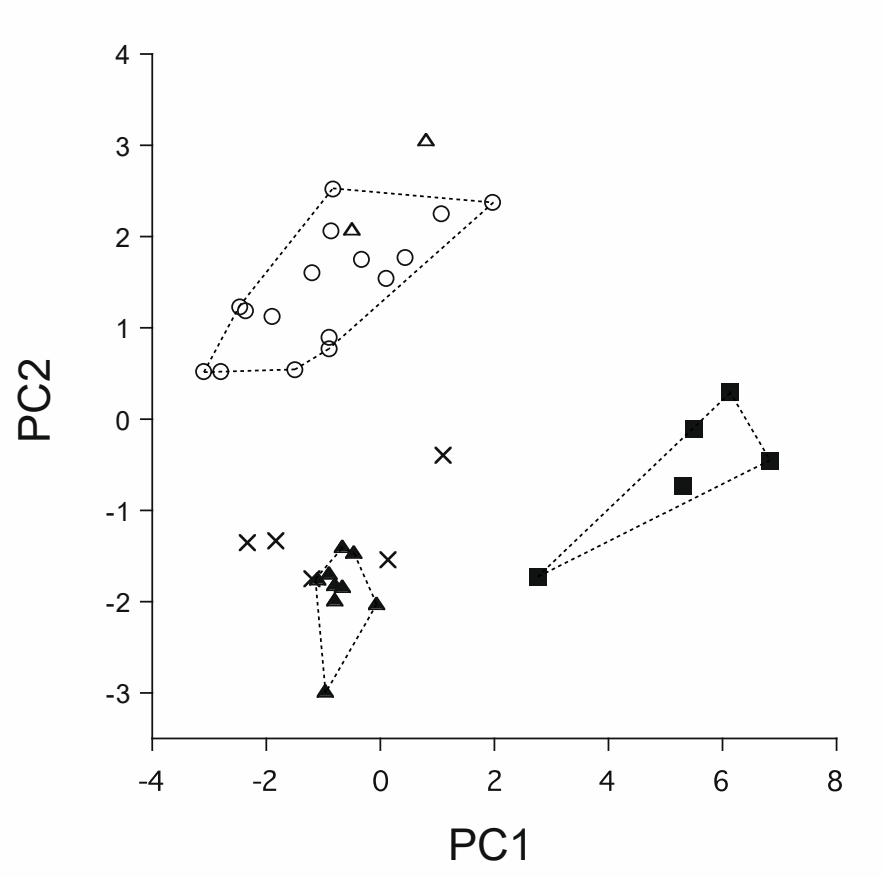
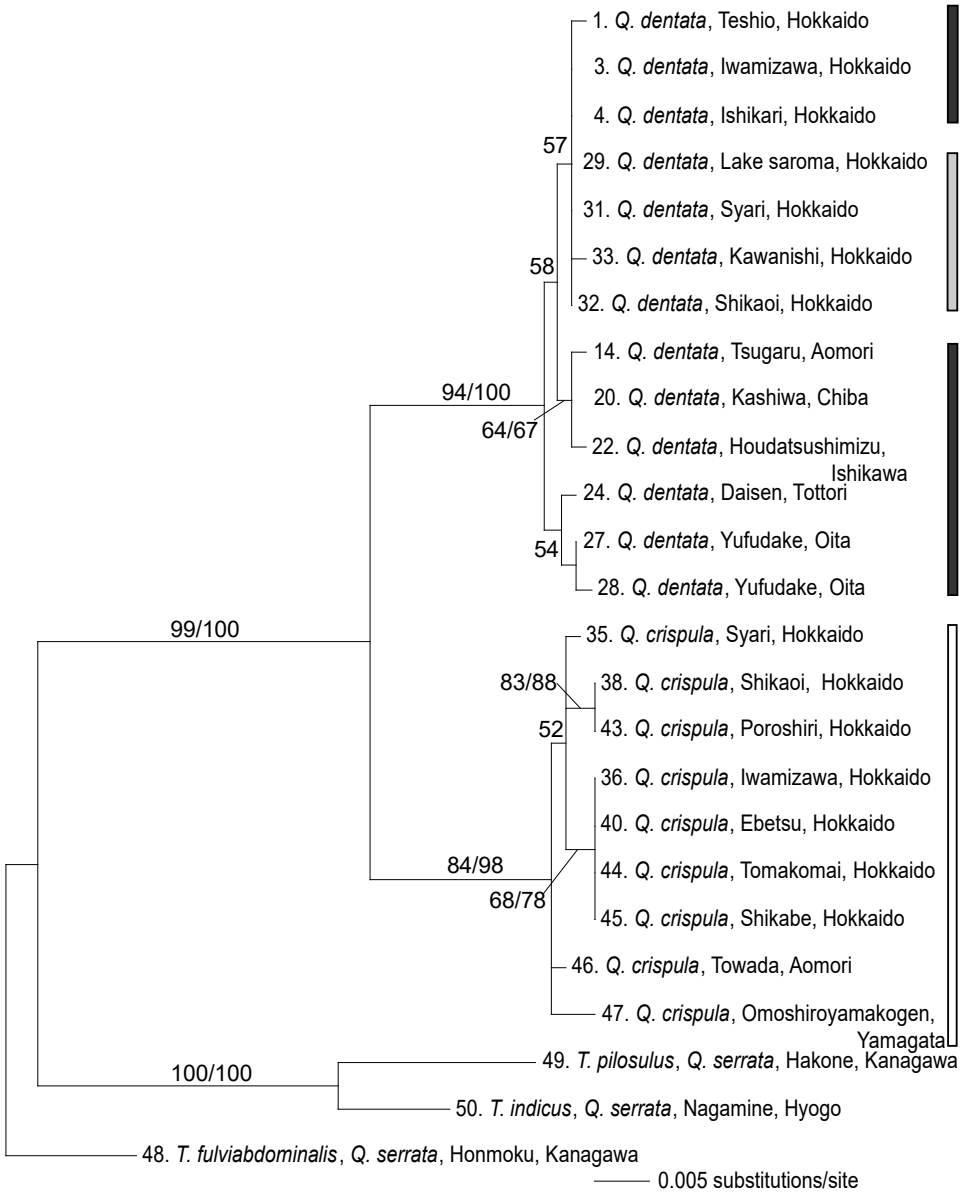


Figure 2



737

738 **Figure 3**

739

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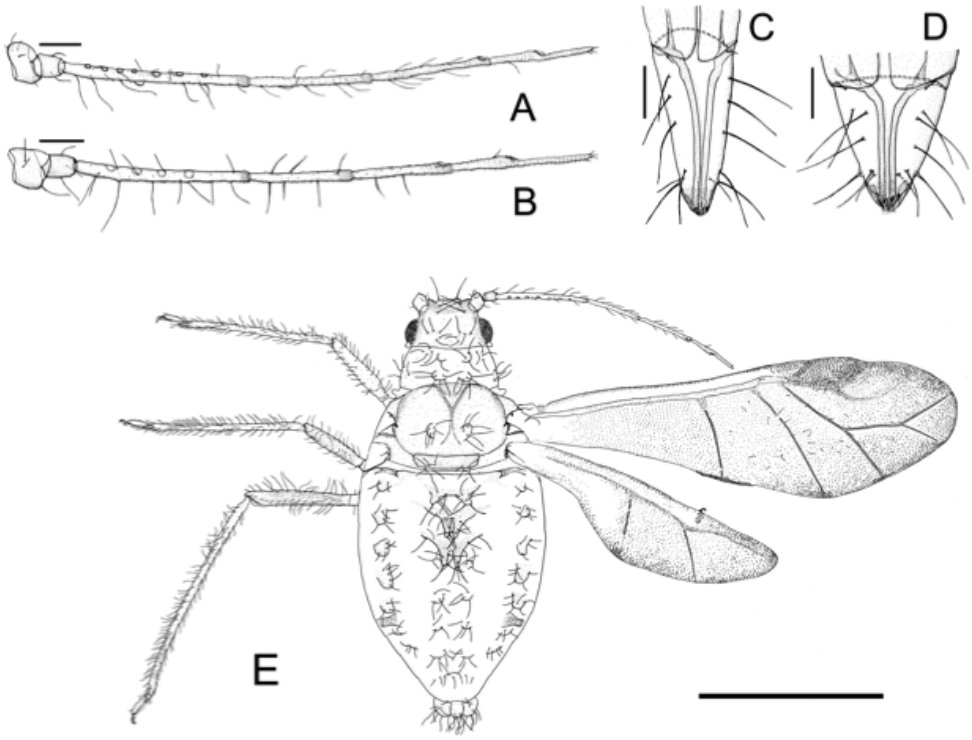

744

745 **Figure 4**

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748

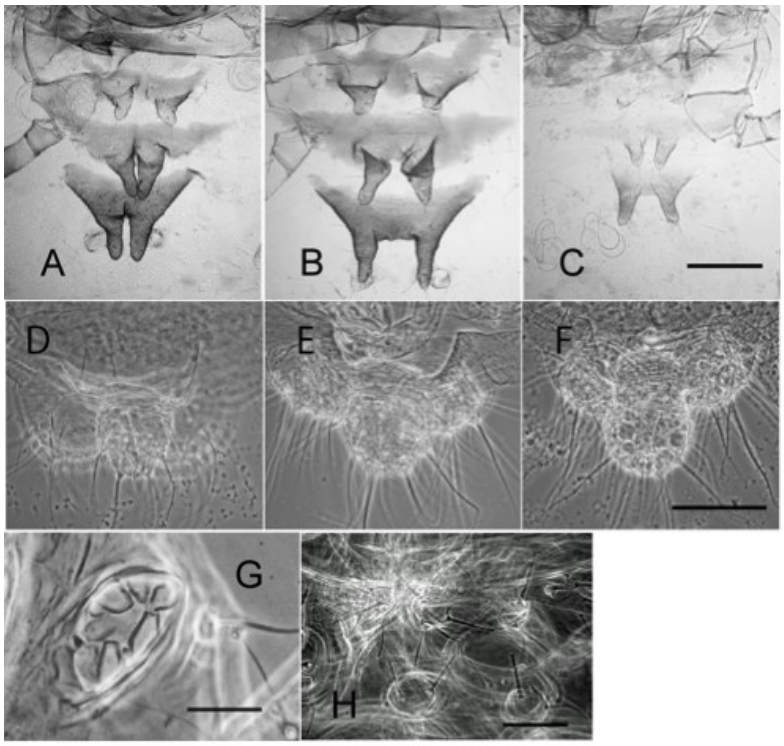

Figure 5

Chapter 2

Taxonomic revision of the *Tetraneura akinire* species group (Insecta, Aphididae, Eriosomatinae), with description of a new species and a correction of a nomenclatural confusion

Running title: Revision of the *Tetraneura akinire* group

772 **Abstract**

773 Gall-forming aphid species called *Tetraneura nigriabdominalis* and *T. fusiformis* and its
774 closely related species were taxonomically revised. By referring to the original
775 descriptions, the name *T. nigriabdominalis* (Sasaki, 1899) was discarded as an erroneous
776 combination, and *T. akinire* Sasaki, 1904 was adopted as a valid name. The *T. akinire*
777 species group was defined as having long claws in the first instar nymphs of the root
778 generation. Of the *T. akinire* species group distributed in Korea and Japan, *T. ovaliformis*
779 sp. nov., which induces globular galls on the leaves of *Ulmus davidiana* var. *japonica*,
780 was described, and *T. akinire* sensu nov. and *T. sorini* Hille Ris Lambers, 1970 were
781 redescribed. Molecular phylogeny based on partial sequences of mitochondrial
782 cytochrome c oxidase subunit I (*COI*) indicated that *T. akinire* is composed of two
783 clusters, one (type A) of which is distributed widely from Europe to East Asia on *Ulmus*
784 spp., and the other (type B) of which is found in Hokkaido, northern Japan on *U.*
785 *davidiana* var. *japonica* and in tropical regions as anholocyclic lineages. *T. fusiformis*
786 Matsumura, 1917, which has been treated as a junior synonym of *T. nigriabdominalis* =
787 *T. akinire*, likely corresponded to type B. I discussed the taxonomic status of *T. fusiformis*
788 and tentatively supported the conclusion that it is a junior synonym of *T. akinire* sensu
789 nov.

790

791 **Key words:** Gall, *Ulmus, Tetraneura nigriabdominalis, T. fusiformis,* primary host,
792 secondary host, asexual

793

794 **Introduction**

795 The genus *Tetraneura* Hartig (Aphididae, Eriosomatinae), a group of gall-forming aphids
796 associated with *Ulmus* species, is comprised of three subgenera, *Tetraneura*,
797 *Tetraneurella*, and *Indotetraneura*, including 35 valid species worldwide (Blackman &

798 Eastop 1994; Sano & Akimoto 2011; Favret 2021). Galls of this genus are conspicuous
799 in the shape and color, and sometimes occur densely on the leaves of *Ulmus* species.
800 Aphids of this genus are typically host-alternating between the primary hosts, *Ulmus*
801 species, and the secondary hosts, the roots of gramineous species. Sexual generation
802 appears in autumn on *Ulmus* species to undertake sexual reproduction. In contrast,
803 asexual (unholocyclic) lineages of some species are distributed widely in association with
804 gramineous species beyond the distributional range of *Ulmus* species (Eastop 1958, 1966;
805 Heie 1967; Vadivelu *et al.* 1975; Delfino 1982; Mifsud *et al.* 2009). Some *Tetraneura*
806 species are pest insects on the secondary host plants, including upland rice and sugar cane
807 (Tanaka 1961; Singh & Singh 2017). In this genus, although several taxonomic
808 confusions still remain, the difficulty in discriminating species has long hindered
809 taxonomic work. After Hille Ris Lambers (1970) published a taxonomic revision, several
810 species have been added to the genus as new to science (Pal & Raychaudhuri 1978;
811 Chakrabarti & Maity 1982; Zhang et al. 1991; Zhang & Qiao 1997). However, this genus
812 has not been taxonomically revised sufficiently. In particular, morphological
813 simplification of apterous adults associated with their parasitic and sessile life modes, as
814 well as morphological similarity of alate females have made species delimitation difficult.
815 In addition, the coexistence of some species on the same *Ulmus* species, especially the
816 coexistence of galls of different species on the same tree (Akimoto 1995, Muramatsu &
817 Akimoto 2016), has confused the taxonomy.
818 Despite the similarity in the adult stage, Hille Ris Lambers (1970) stated that critical
819 diagnostic characters of each species emerge in the first instar nymphs of each morph, for
820 example, fundatrix first instars and those deposited by the emigrants. First-instar nymphs
821 have high mobility and specific roles in their life cycle; for example, gall formation by
822 fundatrix first instars, and movement to the underground parts of the secondary host by
823 first instars deposited by emigrants. Such specific activities may have evolved species-

824 specific morphology in the first instars. Mainly based on the characters of the first instar
825 nymphs and wax gland plates, Hille Ris Lambers (1970) founded the taxonomy of the
826 genus *Tetraneura*. Later, Zhang *et al.* (1991) and Zhang & Qiao (1997) described nine
827 species and one subspecies as new to science from China, indicating that China is the
828 center of the species diversity of *Tetraneura*. Currently, using the morphological
829 characters of first instar nymphs, coupled with molecular techniques, I can discriminate
830 species that are distributed sympatrically and allopatrically. In particular, in the present
831 study, the size and morphology of fundatrix first instar nymphs are emphasized. Since
832 *Tetraneura* galls are closed, it is easy to find and collect the cast-off skins of the fundatrix
833 first instars, the gall formers, from the galls. The skin is blackish, hard and conspicuous.
834 The present paper attempts to taxonomically deal with a species group, herein
835 called the *akinire* species group, belonging to the subgenus *Tetraneurella*. A species of
836 this group that induces reddish and spindle-shaped galls with a rugged surface on *Ulmus*
837 species has been referred to as *T. nigriabdominalis, T. akinire, T. fusiformis, T. chinensis,*
838 or *T. hirsuta*, with a wide distribution from East Asia to Europe in association with *Ulmus*
839 species (Roberti 1972; Blackman & Eastop 1994; Walczak et al. 2017; Blackman &
840 Eastop 2021). This species is introduced to North America (Hille Ris Lambers 1970;
841 Foottit et al. 2006), where it induces galls on *Ulmus* species native to North America and
842 those introduced from other continents. Currently, according to Blackman & Eastop
843 (1994), this species is treated as *T. nigriabdominalis*. However, the taxonomic position
844 of this species, phylogenetic relationships among allopatric populations, and relationships
845 with closely related species have not been clarified. This paper deals with all of these
846 problems to stabilize the taxonomy of this species group consisting of four species, with
847 description of one new species and redescription of two species. The remaining species
848 collected in South Korea remains to be examined. Further, it attempts to resolve an
849 ongoing nomenclatural problem.

The present study comprises three parts; the first part is a bibliographic study on the taxonomic treatments of the elements of the *Tetraneura akinire* species group, the second part deals with species delimitation based on cross-referencing among gall morphology, gall formers' morphology and phylogenetic relationships, and the third part is the formal descriptions (or redescriptions) of the species group.

Taxonomic history of the *akinire* species group

T. nigriabdominalis (Sasaki, 1899) and *T. akinire* Sasaki, 1904

An aphid species attacking upland rice was described under the name of *Schizoneura nigriabdominalis* by Chujiro Sasaki (1899), who reported that this species forms large colonies, including apterous adults, in early July on the roots of upland rice in Tokyo. Alate adults were observed on the roots in early August. Upland rice attacked by this species was reported to show decreased growth. The life cycle and the primary host plant were not mentioned. No type specimens or specimens used for the description are left. The morphology of the apterous and alate adults is described with figures. Later, Tanaka (1961) transferred this species to *Tetraneura*, resulting in a combination of *T. nigriabdominalis*. In the revision of *Tetraneura* by Hille Ris Lambers (1970), *T. nigriabdominalis* was redescribed with the neotype specified based on a specimen collected by Dr. M. Inaizumi from the roots of *Oryza sativa* in Tochigi Prefecture, Japan. In the revision, Hille Ris Lambers divided this species into four types: first, the gall generation on *Ulmus davidiana* var. *japonica*; second, the root generations collected from the roots of upland rice in Japan; third, the root generations collected from grass roots in India and Indonesia; and fourth, the root generations collected from grass roots in Africa and America. Eastop & Blackman (2005) pointed out that all African records of *T. nigriabdominalis* as well as many of those from Asia should be referred to *T. fusiformis*,

875 although a *Tetraneura* species collected on upland rice in East Asia was of the true
876 *nigriabdominalis.*

877 Later, Sasaki (1904) described an aphid species that induces galls on the leaves of
878 *U. parvifolia* under the name of *T. akinire* ("*akinire"* refers to *U. parvifolia* in Japanese).
879 The locality is not mentioned, but Tokyo is most likely. The type specimens are not left,
880 either. Therefore, *nigriabdominalis* is a specific epithet of root generation, whereas
881 *akinire* is for gall generation. Hille Ris Lambers (1970) treated *T. akinire* as a distinct
882 species from *T. nigriabodominalis* based on tarsal characters of first instar nymphs of the
883 root generations, and specified the neotype for *T. akinire* based on a specimen collected
884 by Dr. M. Sorin from a gall on *U. parvifolia* in Osaka. Hille Ris Lambers (1970) indicated
885 that it is very difficult to distinguish *T. nigriabdominalis* from *T. akinire*, and that the only
886 distinction is whether the tarsi of first instar nymphs of the root generations are either
887 spinulose (in *nigriabdominalis*) or smooth (in *akinire*). However, the tarsal character is
888 variable and does not constitute a diagnostic character (see Results). *T. akinire* is currently
889 recognized as a junior synonym of *T. nigriabdominalis* (Blackman & Eastop 2021; Favret
890 2021).

891 Although the name *T. nigriabdominalis* has been used by many authors for a long
892 time when referring to gall and root generations, the original description has not been
893 checked by researchers. In this study, I carefully examined the original description written
894 in Japanese, and concluded that *Schizoneura nigriabdominalis* Sasaki, 1899 does not
895 belong to *Tetraneura,* but probably to *Anoecia.*

896 The original description of *S. nigriabdominalis* indicates that nymphs produced by
897 apterous adults have red compound eyes and 6-segmented antennae, and that the alate
898 adults have the third "oblique veins" forked in the fore wings. None of these characters
899 were consistent with those of *Tetraneura* spp. (Hille Ris Lambers 1970; Heie 1980;
900 Foottit & Richards 1993). In addition, the proportions of the antennal segments in

apterous and alate adults in the figure are different from those of *Tetraneura* spp. An alate female depicted in the figure has a blackish posteriodorsal abdominal patch, which is usually seen in the alate females of *Anoecia* spp (probably, "*nigriabdominalis*" is named after the blackish abdominal patch). The figure shows that the apterous adult has a pleural transverse dark band on each thoracic and abdominal segment, which is not seen in exule adults of *Tetraneura* spp. Furthermore, in *Tetraneura* species, it is difficult to collect a number of alate females from the roots of the secondary host in August. These lines of information clearly indicate that *Schizoneura nigriabdominalis* Sasaki, 1899 does not belong to *Tetraneura*, so the name *T. nigriabdominalis* should be discarded as an incorrect combination. Matsumura (1917) treated *Schizoneura nigriabdominalis* Sasaki, 1899 and *S. fulviabdominalis* Sasaki, 1899 as synonyms of *Anoecia corni*, which was later treated as *A. fulviabdominalis*. Tanaka (1957), however, stated that *Schizoneura nigriabdominalis* Sasaki, 1899 appears to be the same species as *Byrsocrypta ulmi* L., and then in Tanaka (1961) *nigriabdominalis* was erroneously transferred to *Tetraneura.* In contrast, *T. akinire* Sasaki, 1904 is a valid name and a senior synonym of *T. hirsuta* (Baker, 1921), *T. fusiformis* Matsumura, 1917, and *T. chinensis* Mordvilko, 1924.

T. hirsuta Baker, 1921 was described based on specimens from the roots of rice in the Philippine Islands, while *T. chinensis* Mordvilko, 1924 was described from galls on an *Ulmus* species in China. Moldvilko (1935) treated *T. chinensis* Mordvilko, 1924 as a synonym of *T. hirsuta,* which was later treated as a synonym of *T. nigriabdominalis* (= *T. akinire* sensu nov.) in Eastop & Hille Ris Lambers (1976).

T. sorini Hille Ris Lambers, 1970

Another species in the species group is *T. sorini*, which was described based on a specimen collected from a gall on a leaf of *Ulmus* sp. by Dr. S. Takagi in Sapporo, Hokkaido. The host plant is most likely *Ulmus davidiana* var. *japonica,* which is the most

927 common elm species in Hokkaido. Hille Ris Lambers (1970) reported that this species is
928 also distributed in Korea. *T. sorini* is sometimes confused with *T. akinire* sensu nov.
929 because the fundatrix first instars of *T. sorini* coexist with those of *T. akinire* and other
930 *Tetraneura* species on the same elm tree. The fundatrix first instar of this species is very
931 large in body size and parasitic to other *Tetraneura* species, usurping incipient galls of *T.*
932 *akinire* or other *Tetraneura* species (Akimoto & Yamaguchi 1997; Muramatsu &
933 Akimoto 2016); thus, *T. sorini* galls frequently coexist with those of other species. I
934 redescribed this species based on the fundatrix first instar, emigrant and sexupara.
935
936 *T. fusiformis* Matsumura, 1917
937 This specific name was described based on gall generations of *U. davidiana* var. *japonica*
938 collected from Sapporo, Hokkaido, Japan. The gall was reported to be rosy-red and
939 spindle-shaped. Rosy-red galls are common in *U. davidiana* var. *japonica* in Central and
940 Northern Hokkaido, Japan (Figure 1I). No type specimens are left. Hille Ris Lambers
941 (1970) treated this specific name as a junior synonym of *T. nigriabdominalis* sensu Hille
942 Ris Lambers (1970). However, Blackman & Eastop (2021) and Favret (2021) treated *T.*
943 *fusiformis* as a valid name. Therefore, currently, the taxonomic and phylogenetic
944 relationships between *T. akinire* sensu nov. in Honshu and *T. fusiformis* in Hokkaido have
945 not been evaluated. Lee *et al.* (2012) showed that *T. nigriabdominalis* (= *T. akinire*) + *T.*
946 *fusiformis* is phylogenetically divided into two groups (types A and B) in terms of the
947 mitochondrial *COI* sequence, with ca. 2% divergence between the two types. Type A *T.*
948 *akinire* is widely distributed in Japan, whereas type B is distributed only in Hokkaido,
949 with the two types coexisting at Sapporo. Thus, it is necessary to determine whether the
950 two types can be regarded as the same species, and how *T. fusiformis* should be
951 taxonomically dealt with. The present study examines this problem based on
952 morphological and molecular evidence. To examine the phylogenetic relationships

953 between *T. akinire* sensu nov. and *T. fusiformis*, I compared the mitochondrial *COI*
954 sequences of samples collected widely from Europe, North America, Japan (Honshu,
955 Okinawa, and Hokkaido), Korea, and Malaysia.
956
957 Undescribed species, *T.* sp. O
958 Greenish and globular galls of a *Tetraneura* species sometimes coexist with reddish and
959 spindle-shaped galls of *T. akinire* sensu nov. on the same tree of *U. davidiana* var.
960 *japonica* in Hokkaido, northern Japan. This species has been treated as *T.* sp. O (Akimoto
961 1995; Akimoto & Yamaguchi 1994, 1997; Tomisawa & Akimoto 2004; Muramatsu &
962 Akimoto 2014, 2016). The former species is reported to be smaller in the first instar
963 fundatrix (Akimoto & Yamaguchi 1997), with completely smooth tarsi in the first instars
964 of the root generation. According to the criteria of Hille Ris Lambers (1970), this species
965 is classified as *T. akinire* sensu Hille Ris Lambers (1970); however, in addition to the
966 smaller body size of fundatrix first instars, molecular analyses indicated that the species
967 has distinct sequences from *T. akinire* in the mitochondrial *COI*, but is closely related to
968 *T. akinire* and *T. sorini*, forming the *T. akinire* species group (see Results). This species
969 is widely distributed in Hokkaido, and also collected in Hirosaki, northern Honshu, the
970 main island of Japan. I describe this species as new to science under the name of "*T.*
971 *ovaliformis*". Similar globular galls were collected from *U. davidiana* in South Korea.
972 The specific status of the Korean gall formers will be discussed based on morphological
973 and molecular information.
974
975 **Material and methods**
976 *Mounted specimens*
977 Aphids used in the present study were collected from leaf galls on *Ulmus* spp., the primary
978 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℃ 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). Appendix S1 in supporting information (Muramatu & Akimoto 2016) was used for the measurements of the hind femur lengths of *T. sorini*. The terminology followed Akimoto (1983, 1985). All specimens used for the description and morphological measurements were preserved at the Hokkaido University Museum.

Phylogeny

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-CAACATTTATTTTGATTTTTTGG-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.

Results and Discussion

Morphology of galls and the gall formers

Although it is difficult to identify aphid species based on gall morphology, three types of galls remain distinctive in the subgenus *Tetraneurella* distributed in Japan and South Korea: greenish globular galls (Figure 1A-E), reddish or greenish, spindle-shaped galls (F-I), and reddish small globular galls (J-L). 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 (Figure 2, 1-2, Figure 4A), spindle-shaped galls were induced by medium-sized fundatrix nymphs (Figure 2, 4-14, Figure 4B), and small-sized globular galls were caused by large-sized fundatrix nymphs (Figure 2, 15-20, Figure 4C). Korean greenish globular galls were inhabited by fundatrix nymphs that are intermediate in size between small-sized and medium-sized nymphs (Figure 2, 3). 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 (Figure 2, 1, 6, and 18), 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 (Figure 2, 4-8), South Korea (9), Europe (10-12), and North America (13-14) 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* (7-8) and those collected from *U. davidiana* var. *japonica* (4-6 and 9) (ANOVA, df = 1,92, $F = 2.37$, $P = 0.13$).

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.

Phylogeny and morphology

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 (Figure 3, 30-38) in which the fundatrix first instars were characterized by long hind femurs (Figure 2, 15-20). This clade, with 87% bootstrap support, corresponded to *T. sorini*. Aphids from greenish globular galls also constituted a unique clade (Figure 3, 39-41) with 100% bootstrap support and were characterized by shorter hind femurs (Figure 1, 1-2). 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*. Aphids from greenish globular galls collected in South Korea were separated from *T. ovaliformis,* forming an independent clade (Figure 3, 28-29). 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 a vicariance event, 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 (Figure 3, 1-2) and Asian samples (3-6). The three *COI* sequences of "*T. chinensis"* used by Zhang et al. (2008, accession numbers EF534368.1, EF534367.1, and EF534366.1) completely agreed with the sequences of type A of *T. akinire* (Figure 3, 1-6). "*T. sorini*" in Zhang et al. (2008, accession number EF534364.1) formed a cluster with *T. ovaliformis,* while "*T. akinire*" (accession number EF534363.1) 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 (Figure 3, 16-17). Gall formers collected outside Hokkaido were not included in type B. At Sapporo, gall formers of both types A and B (10-12 and 27) 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.)

1108 is distributed widely on the grass roots (or roots of rice) in regions outside the
1109 distributional ranges of *Ulmus* species, including South Asia, Southeast Asia, Oceania,
1110 and South America (Villalobos Muller et al. 2010; Foottit et al. 2012; Simbaqueba-Cortés
1111 et al. 2015; Mille et al 2020).

1112 Despite the difference in the mitochondrial *COI* region, types A and B were not
1113 distinguished morphologically. In Figure 2, samples 4 (Bibai, Hokkaido) and 5
1114 (Iwamizawa, Hokkaido) belonged to the populations where only type B was collected,
1115 whereas samples 7-14 belonged to the populations of type A. However, there was no
1116 significant difference in the hind femur length between the two groups (ANOVA, df
1117 =1,155, F = 2.71, P = 0.102). In other morphological characters, I was not able to
1118 distinguish between types A and B. The tarsal character of exule first instar nymphs
1119 (spinulose or smooth) was examined because it was the critical criterion for
1120 discriminating between *T. nigriabdominalis* sensu Hille Ris Lambers (1970) and *T.*
1121 *akinire* sensu Hille Ris Lambers (1970). However, smooth tarsi were found only in two
1122 localities, and most of the potential members of type A had spinulose tarsi (Table 2).

1123 The genetic difference (*p*-distance) in the *COI* region between type A and type B
1124 was small (0.0246) compared to the distances between other species (Table 3). Lee &
1125 Akimoto (2015) indicated that mean divergence in *COI* among species within the genera
1126 of Eriosomatinae is approximately 5%. The genetic difference between *T. akinire* type A
1127 and *T. sorini* was also small (0.0265), but they were morphologically distinct.

1128 There are two possibilities for the taxonomic status of *T. akinire* types A and B.
1129 First, the two types may represent two distinct species that have recently separated but
1130 have attained reproductive isolation. The second possibility is that the two types belong
1131 to a single species, which may have originated through the fusion of genetically divergent
1132 populations, or incipient species. If two incipient species have weak reproductive barriers,
1133 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.

Conclusion

Through morphological and phylogenetic investigations, *T. ovaliformis* sp. nov. and *T. sorini* were unequivocally identified, and thus they were formally described or redescribed in the next section. However, some problems remain regarding *T. akinire* sensu nov. because it is comprised of two genetic groups with different geographic distributions but without morphological differences. Therefore, I tentatively treat types A and B as two genetic groups within *T. akinire* sensu nov. until more information about the reproductive status of types A and B is available.

Our results suggest that integrating information about the morphology of galls and gall formers and information about DNA sequences is inevitable for constructing the taxonomy of *Tetraneura* species.

Description

***Tetraneura (Tetraneurella) ovaliformis* Watanabe, Sano & Akimoto, New Species**

Tetraneura sp. O.: Akimoto 1995; Akimoto & Yamaguchi 1994, 1997: Tomisawa & Akimoto 2004; Muramatsu & Akimoto 2014, 2016

Fundatrix first instar nymph: Body elliptical, becoming thinner posteriorly, 0.621−0.707 (on average 0.654) mm long, 0.257−0.336 (0.296) mm wide on abdominal segment II, 0.39−0.46 (0.43) times as wide as long (Fig 1A). All tergites sclerotized strongly, except for the posterior part of each segment. No wax gland plates present. Eyes each with 3 ommatidia. Capitated setae present on antennae, legs, and whole body dorsally.

Antennae short, 5-segmented, smooth and not imbricated, 0.123−0.139 (0.131) mm long, 0.196−0.203 (0.199) times as long as body, 0.75−0.89 (0.81) times hind femorotrochanter length (Fig 1D). Antennal segment V as long as or slightly shorter than segment IV; processus terminalis indistinguishable. Antennal segment IV square-shaped, rather wider to the apex. Antennal segment III short, 1/5−1/4 length of segment IV. Primary rhinarium projecting as a horn with the tip not pointed, 0.009−0.016 (0.012) mm long on segment IV and 0.010−0.015 (0.012) mm long on segment V; that on segment V with 2−4 circular accessory rhinaria on the base. Segment V with 6−7 setae, of which the basal one is the longest with a conspicuously capitate tip, 0.031−0.059 (0.048) mm long. Antennal segment IV with 4−5 setae.

Head and prothorax completely fused. Head and prothorax with 5−7 pairs of setae dorsally, 1 pair on the vertex, 1−2 pair(s) ventrally and 2 pairs laterally. Meso- and

metanotum each with 1 spinal pair, 1 pleural pair and 2 lateral pairs of setae. Dorsal and lateral setae on head and thorax capitated. Rostrum reaching hind coxae; ultimate rostral segment rather slender, 0.090−0.105 (0.098) mm long, 0.50−0.64 (0.57) times as long as hind femorotrochanter, with 12 setae. Legs smooth; fore femorotrochanter 0.113−0.141 (0.127) mm long, fore tibia 0.083−0.107 (0.097) mm long, hind femorotrochanter 0.145−0.193 (0.172) mm long and hind tibia 0.130−0.179 (0.158) mm long. Tarsal segment I completely fused with segment II, with an unsclerotized spot basally. One pair of dorsoapical setae on tarsus thick and capitate, 0.065−0.087 (0.074) mm long on hind legs. One pair of ventrobasal setae on tarsus tapering but not pointed, 0.058−0.076 (0.064) mm long on hind legs. One pair of empodial setae capitate, slightly longer than the claws. Spiracles with round rims, not projecting, 0.008−0.011 (0.010) mm in diameter.

Abdominal tergites I−VI each with 1 spinal and 1 lateral pair of setae; tergite I with 1 pleural pair additionally; tergites II−IV sometimes with 1 pleural pair additionally. Tergite VII with 1 spinal and 1 lateral pair of setae; tergite Ⅷ and cauda each with 1 pair of spinal setae. Cauda with 4 setae ventrally. Spinal setae on tergite V 0.011−0.015 (0.013) mm long. Lateral setae on tergite II 0.015−0.024 (0.020) mm long, on VI 0.021−0.035 (0.029) mm long, on VII 0.033−0.043 (0.037) mm long. Dorsal and lateral setae on abdominal segments capitate.

First instar nymph produced by emigrants: Body rather elongate, posteriorly broadly rounded, mostly membranous with wax grand plates on the whole body except posterior abdominal segments, 0.835−0.884 (on average 0.866) mm long and 0.401−0.460 (0.431) mm wide, 0.47−0.52 (0.50) times as wide as long; eyes indistinct, with 3 ommatidia (Fig 2A).

Antennae short, 5-segmented, 0.316−0.324 (0.321) mm long, 0.37−0.38 (0.37) times as long as body, 1.31−1.38 (1.35) times as long as hind femorotrochanter. The length of

antennal segment I 0.047−0.049 (0.048) mm, II 0.056−0.061 (0.059), III 0.024−0.038 (0.030), IV 0.108−0.130 (0.119), and V 0.045−0.054 (0.049). Antennal segments I−III not imbricated and smooth, but segments IV and V imbricated with transverse rows of spinules. Segment I with 3−4 setae, II with 3−5, III with 0−2, IV with 10−18 and V with 6−7. Segment II cylindrical. Segment III thicker apically. Segment IV imbricated, cylindrical, slightly thicker apically, with 19−21 transverse rows of spinules, which are dense on the apical half. Primary rhinarium on segment IV with 1 oval opening, the outer circumference of which is ciliated with 2 tongue-like projections. Segment V wholly with transverse rows of spinules, with an undeveloped processus terminalis, the apex of which is truncated obliquely. Primary rhinarium on segment V with 2 openings, the outer circumferences are ciliated with 1−3 tongue-like projections.

Suture on head visible; antennal tubercles not developed; vertex nearly flat. Head with 5 pairs of setae dorsally, 1 pair on the vertex, 2−3 pairs ventrally. Rostrum reaching over the hind coxae; ultimate segment convergent almost straightly, 0.105−0.118 (0.110) mm long, 0.43−0.50 (0.45) times as long as hind femorotrochanter, with 14−16 setae.

Femorotrochanters, tibiae and tarsi smooth (Figs 2D, 2G). Fore femorotrochanters 0.196−0.208 (0.200) mm long and fore tibiae 0.178−0.187 (0.181) mm long. Hind femorotrochanters 0.235−0.256 (0.242) mm long, 0.34−0.42 (0.37) times as wide as long. Hind tibiae 0.238−0.259 (0.244) mm long. Tarsal segments I and II completely united without a slit. Hind tarsi 0.053−0.060 (0.056) mm long; hind claws 0.074−0.082 (0.079) mm long, 1.34−1.52 (1.41) times as long as hind tarsal length.

Body setae acute. Each thoracic segment pleurally with 2 pairs of setae. Each of abdominal segments I−VII pleurally with 1 pairs of setae. Mesonotum, metanotum and abdominal tergites I−VI spinally with 3 pairs of setae. Tergite VII with 1 or 2 pairs of spinal setae. Lengths of spinal setae on abdominal tergite III 0.034−0.047 (0.040) mm long. Wax gland plates present on head, thoracic segments, and abdominal segments

I−VII; head ventrally with 2 pairs of wax gland plates, which are nearly circular; prothorax with 1 pair pleurally; mesothorax and metathorax respectively with 2−3 small and inconspicuous pairs; abdominal segments I−VII respectively with 2−4 pairs, which are inconspicuous and circular or long oval (Fig 2K). Abdominal sternites III−VII respectively with 2−3 setae. Cauda dorsally with 1 pair of setae spinally, 1 pair laterally, and 1 longer pair ventrally.

Emigrant adult: Body elongated oval, 1.75−2.00 (1.84) mm long, without wax gland plates (Fig 3A). Head and thorax dark brown; antennae and legs brown. Wings wholly shaded in brown, slightly darker along veins.

Antennae 6-segmented, 0.65−0.72 (0.68) mm long, 0.36−0.38 (0.37) times as long as body, 0.98−1.08 (1.04) times as long as hind tibia (Fig 3D). Antennal segments III−VI pigmented thinly; segment V and VI imbricated with numerous transverse rows of spinules. Segment IV sparsely with spinules between secondary rhinaria in some individuals. Segments I−III not imbricated and smooth. Antennal segment III variable in length, 0.23−0.29 (0.25) mm long, 0.72−0.81 (0.75) times the length of IV, V and VI combined. Segment IV rather oval in profile, 0.43−0.46 (0.44) times as wide as long. Segment V 0.16−0.19 (0.17) mm long, 0.61−0.78 (0.69) times as long as segment III, 0.17−0.22 (0.19) times as wide as long at the middle point. Segment VI 0.38−0.47 (0.42) times as wide as long, thickest at the middle point, with a depression at the primary rhinarium, which is elongated transversely; segment VI wholly with spinules arranged in 14−15 transverse rows, without secondary rhinaria (Fig 3G). Secondary rhinaria present on segments III−V, narrow, slightly projecting, microscopically represented as blight lines on dark pigmented background, covering usually 2/3 or the whole circle of the segments from the ventral side. Antennal setae on segments III−V very short and scarce, present on the dorsal side; the longest seta on segment V 0.013−0.017 (0.015) mm long;

segment III with 1−2, IV with 0−2, V with 3−4 and VI with 4−5 setae, of which 3 are present apically and 1−2 on the basal half.

Suture on head invisible; antennal tubercles not developed; vertex nearly flat. Head dorsally with 4−5 and ventrally with 6−11 pairs of setae, respectively; of them, 3−7 pairs of ventral setae situated near the base of clypeus. Rostrum short, not reaching the middle point between the coxae of fore and middle legs. Ultimate rostral segment short, with gently convex margins and 6 pairs of short setae, 0.078−0.103 (0.086) mm long, 0.50−0.58 (0.53) times as long as the second segment of hind tarsi (Fig 3J). Femorotrochanter not imbricated and smooth; hind femorotrochanter 0.427−0.543 (0.475) mm long, 0.69−0.76 (0.72) times as long as hind tibia. Tibiae slightly spinulose at the terminal; hind tibia 0.598−0.735 (0.654) mm long. Tarsi with numerous spinules neatly arranged in transverse rows; first tarsal chaetotaxy 3:3:3; second segment of hind tarsus 0.152−0.178 (0.164) mm long; empodial setae almost the same length as claws (Fig 3M). Abdomen wholly membranous, with short setae. Cornicles absent. Genital plate slightly brown-pigmented, with 30−44 (35.4) setae (Fig 3P). Cauda semicircular, with 2 setae. Fore wings with unbranched media; the veins rather broad, not conspicuously bordered. Hind wings only with 1 inconspicuous oblique vein.

Sexupara adult: Since the sexupara is morphologically close to the emigrant in many respects, the characters that differ between the two morphs and are indispensable for identification will be referred to.

Body 1.80–2.07 (1.95) mm long. Wax gland plates present on the whole body except the posterior segments of abdomen (Fig 4A).

Antennae 6-segmented, 0.78–0.94 (0.86) mm long, 0.43–0.46 (0.44) times as long as body, 1.12–1.13 (1.12) times as long as hind tibia (Fig 4D). Antennal segments III–VI pigmented thinly; segments V and VI imbricated with numerous transverse rows of

spinules; segment IV sparsely with spinules between secondary rhinaria; segments I–III not imbricated and smooth; segment III 0.28–0.39 (0.33) mm long, 1.32 times as long as that of the emigrant, 0.70–0.93 (0.84) times as long as segments IV, V and VI combined; segment IV rather oval in outline, 0.45–0.59 (0.51) times as wide as long; segment V cylindrical, 0.20–0.25 (0.23) mm long, 0.14–0.20 (0.16) times as wide as long, 0.60–0.80 (0.69) times as long as segment III; segment VI 0.063–0.076 (0.071) mm long, 0.39–0.48 (0.44) times as wide as long, with the middle thickest; segment VI with spinules arranged in 15–16 transverse rows, without secondary rhinaria. Primary rhinarium present on segment VI, tongue-like, elongate transversely with ciliated rims (Fig 4G). Secondary rhinaria present on the segments III–V, 0.0015–0.0048 (0.0027) mm wide, slightly projecting, microscopically represented as the blight lines on dark pigmented background, covering usually 1/2 to the whole circumference from the ventral side, more numerous than in emigrant. Antennal setae short and scarce, present on the dorsal surface, 0.014–0.019 (0.016) mm long on segment V.

Suture on head invisible; antennal tubercles not developed; vertex nearly flat. Head dorsally and ventrally with 6 pairs of setae, respectively; of them, 4 pairs of ventral setae situated near the base of clypeus. Median ocellus and 1 pair of wax gland plates situated posteriorly to the ocellus, forming a triangle (Fig 4J). Ultimate rostral segment 0.095–0.111 (0.103) mm long with 7 pairs of setae, convergent with almost straight margins, 0.60–0.67 (0.64) times as long as the second segment of hind tarsus, 1.20 times as long as that of the emigrant (Fig 4M). Pronotum with 2 pairs of wax plates spinally and 1 pair of them pleurally; metanotum and mesonotum each with 1 pair of them spinally. Femorotrochanter and tibia slightly longer than those of the emigrant. First tarsal chaetotaxy 3:3:3. The second segment of hind tarsus slightly shorter than that of the emigrant; second segment of hind tarsus 0.21–0.22 (0.21) times as long as hind tibia (in emigrant, the proportion is 0.24–0.27 (0.25)). Abdominal tergites membranous without

sclerites (Fig 4P). Wax gland plates present on the first to seventh abdominal tergites, 1 pair spinally, 1 pair marginally, and sometimes 1 pair of small ones pleurally; round or oval in the outline, with variation in the size, consisting of 1–50 (23) minute round facets (Figs 4S, 4V). Cornicles present on the pleural and posterior positions of the fifth abdominal tergite, 0.039–0.053 (0.045) mm in diameter, with slightly projecting rim, which is sclerotized slightly (Fig 4Y). The first to sixth abdominal tergites with 6–10 short setae, respectively; the seventh tergite with 6–8 long setae. The second to sixth abdominal sternites with 16–20 short setae, respectively. Dorsal, abdominal setae, 0.017–0.034 (0.023) mm long on segment III spinally, 0.074–0.109 (0.087) mm long on the segment VIII pleurally. Genital plates slightly brown-pigmented, with 13–23 (16.7) setae on each of the right and left sides (Fig 4b).

Host plant: *Ulmus davidiana* var. *japonica* and *Setaria* spp. (secondary host).

Specimens examined: Fundatrix first instar larva: 30exs, Sapporo, Hokkaido, V.1994, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 4exs, Teshikaga, Hokkaido, 27.VII.1985, *Ulmus davidiana* var. *japonica*, S. Akimoto. Emigrant adult: 8exs, Sapporo, Hokkaido, 20.VI.1984, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 12exs, Sapporo, Hokkaido, 17.VI.2000, on *Ulmus davidiana* var. *japonica*, M. Sano. First instar nymph produced emigrant: 1ex., Sapporo, Hokkaido, 14.IX.1985, on grass roots, S. Akimoto; 4exs, Hirosaki, Aomori, 30.IX.1983, on grass roots, S. Akimoto; 1ex., Hirosaki, Aomori, 29.IX.1983, on grass roots, S. Akimoto. Sexupara adult: 10exs, Sapporo, Hokkaido, 15.X.1985, on *Ulmus davidiana* var. *japonica*, S. Akimoto.

Holotype: Emigrant adult: Hokkaido Univ., Sapporo, Japan, 20.VI.1984, on *Ulmus davidiana* var. *japonica*, S. Akimoto leg.

Etymology: from globular shape of the gall.

***Tetraneura akinire* Sasaki, 1904**

Fundatrix first instar nymph: Body elliptical, becoming thinner posteriorly, 0.632−0.826 (on average 0.745) mm long, 0.265−0.366 (0.313) mm wide on abdominal segment II, 0.39−0.47 (0.42) times as wide as long (Fig 1B). All tergites sclerotized strongly, except for the posterior part of each segment. No wax gland plates present. Eyes each with 3 ommatidia. Capitated setae present on antennae, legs, and whole body dorsally.

Antennae short, 5-segmented, smooth and not imbricated, 0.154−0.179 (0.163) mm long, 0.186−0.249 (0.219) times as long as body, 0.581−0.748 (0.683) times hind femorotrochanter length (Fig 1E). Antennal segment V almost equal to or slightly shorter than segment IV; processus terminalis indistinguishable. Antennal segment II square-shaped. Antennal segment IV slightly oval-shaped and wider to the apex. Antennal segment III short, 1/4−1/6 length of segment IV. Primary rhinarium projecting as a horn with the tip not pointed, 0.010−0.013 (0.012) mm long on segment IV and 0.012−0.015 (0.013) mm long on segment V; that on segment V with 3−4 circular accessory rhinaria on the base. Segment V with 6−8 setae, of which the basal one is the longest with a conspicuously capitate tip, 0.054−0.075 (0.064) mm long. Antennal segment IV with 4−7 setae.

Head and prothorax with 5–7 pairs of setae dorsally, 1 pair on the vertex, 1−2 pair(s) ventrally and 2 pairs laterally. Meso- and metanotum each with 1 spinal pair, 1 pleural pair and 2 lateral pairs of setae. Dorsal and lateral setae on head and thorax capitated. Rostrum reaching hind coxae; ultimate rostral segment rather slender, 0.100−0.124 (0.111) mm long, 0.440−0.522 (0.470) times as long as hind femorotrochanter, with 12−18 (12.8) setae. Legs smooth; fore femorotrochanter 0.127−0.173 (0.151) mm long,

fore tibia 0.101−0.130 (0.116) mm long, hind femorotrochanter 0.180−0.265 (0.234) mm long and hind tibia 0.163−0.241 (0.210) mm long. Tarsal segment I completely fused with segment II, with an unsclerotized spot basally. One pair of dorsoapical setae on tarsus thick and capitate, 0.083−0.104 (0.098) mm long on hind legs. One pair of ventrobasal setae on tarsus tapering but not pointed, 0.068−0.097 (0.083) mm long on hind legs. One pair of empodial setae slightly longer than the claws. Spiracles with round rims, not projecting, 0.012−0.015 (0.013) mm in diameter.

Abdominal tergites I−VI each with 1 spinal and 1 lateral pair of setae; tergite I with 1 pleural pair additionally; tergites II−IV sometimes with 1 pleural pair additionally. Tergite VII with 1 spinal and 1 lateral pair of setae; tergite Ⅷ and cauda each with 1 pair of spinal setae. Cauda with 4 setae ventrally. Spinal setae on tergite V 0.014−0.028 (0.019) mm long. Lateral setae on tergite II 0.031−0.047 (0.037) mm long, on VI 0.041−0.050 (0.045) mm long, on VII 0.045−0.056 (0.051) mm long. Dorsal and lateral setae on abdominal segments capitated.

First instar nymph produced by emigrants: Body rather elongate, posteriorly broadly rounded, mostly membranous with wax grand plates on the whole body except posterior abdominal segments, 0.707−0.940 (0.807) mm long and 0.326−0.535 (0.387) mm wide, 0.43−0.57 (0.48) times as wide as long; eyes indistinct, with 3 ommatidia (Fig 2B).

Antennae short, 5-segmented, 0.264−0.302 (0.285) mm long, 0.32−0.38 (0.35) times as long as body, 1.22−1.28 (1.25) times as long as hind femorotrochanter. The length of antennal segment I 0.037−0.050 (0.044) mm, II 0.045−0.055 (0.050), III 0.015−0.029 (0.022), IV 0.088−0.119 (0.104), and V 0.047−0.051 (0.049). Antennal segments I−III not imbricated and smooth, segments IV and V imbricated with transverse rows of spinules. Segment I with 4 setae, II with 3−4, III with 0−1, IV with 11−22 and V with 6. Segment II cylindrical. Segment III thicker apically. Segment IV imbricated, cylindrical,

slightly thicker apically, with 17−25 transverse rows of spinules, which are dense on the apical half. Primary rhinarium on segment IV with 1 oval opening, the outer circumference of which is ciliated with 2 tongue-like projections. Segment V wholly with transverse rows of spinules, with an undeveloped processus terminalis. Primary rhinarium on segment V with 2 openings, the outer circumferences are ciliated with 1−3 tongue-like projections.

Suture on head visible; antennal tubercles not developed; vertex nearly flat. Head with 4−5 pairs of setae dorsally, 1 pair on the vertex, 2−4 pairs ventrally. Rostrum reaching over the hind coxae; ultimate segment convergent almost straightly, 0.107−0.121 (0.115) mm long, 0.48−0.57 (0.51) times as long as hind femorotrochanter, with 14−16 setae.

Femorotrochanters smooth. Tibiae imbricated with transverse rows of spinules apically (Fig 2E). Tarsi not smooth and imbricated with 6−10 transverse rows of spinules (Fig 3H). Spinules in apical tibiae and tarsi absent in 23.8% of the individuals examined (n=84) (Fig 2I). Fore femorotrochanters 0.170−0.206 (0.192) mm long and fore tibiae 0.151−0.188 (0.176) mm long. Hind femorotrochanters 0.192−0.247 (0.226) mm long, 0.35−0.42 (0.39) times as wide as long. Hind tibiae 0.199−0.259 (0.232) mm long. Tarsal segments I and II completely united without a slit. Hind tarsi 0.056−0.068 (0.063) mm long; hind claws 0.060−0.078 (0.071) mm long, 1.02−1.28 (1.12) times as long as hind tarsal length.

Body setae acute. Each thoracic segment pleurally with 2 pairs of setae. Each of abdominal segments I−VII pleurally with 1 pairs of setae. Mesonotum, metanotum and abdominal tergites I−VI spinally with 3 pairs of setae. Tergite VII with 2−4 spinal setae. Lengths of spinal setae on abdominal tergite III 0.039−0.053 (0.045) mm long. Wax gland plates present on head, thoracic segments, and abdominal segments I−VII; head ventrally with 2 pairs of wax gland plates, which are nearly circular; prothorax with 1 pair pleurally;

mesothorax and metathorax respectively with 2–3 small and inconspicuous pairs; abdominal segments I−VII respectively with 2−4 pairs, which are inconspicuous and circular or long oval (Fig 2L). Abdominal sternites III−VII respectively with 2−6 setae. Cauda dorsally with 1 pair of setae spinally, 1 pair laterally, and 1 longer pair ventrally.

Emigrant adult: Body elongated oval, 1.79−2.84 (2.14) mm long, without wax gland plates (Fig 3B). Head and thorax dark brown; antennae and legs brown. Wings wholly shaded in brown, slightly darker along veins.

Antennae 6-segmented, 0.60−0.80 (0.68) mm long, 0.28−0.37 (0.33) times as long as body, 0.96−1.10 (1.02) times as long as hind tibia (Fig 3E). Antennal segments V and VI pigmented thinly; segment V and VI imbricated with numerous transverse rows of spinules. Segment IV depending on the individual with spinules sparsely between secondary rhinaria. Segments I−III not imbricated and smooth. Antennal segment III variable in length, 0.18−0.25 (0.22) mm long, 0.59−0.71 (0.62) times the length of IV, V and VI combined. Segment IV rather oval in profile, 0.44−0.74 (0.53) times as wide as long. Segment V 0.16−0.24 (0.19) mm long, 0.73−0.99 (0.87) times as long as segment III, 0.14−0.21 (0.17) times as wide as long at the middle point. Segment VI 0.41−0.55 (0.46) times as wide as long, thickest at the middle point, with a depression at the primary rhinarium, which is elongated transversely; segment VI wholly with spinules arranged in 14−17 transverse rows, without secondary rhinaria (Fig 3H). Secondary rhinaria present on segments III−V, narrow, slightly projecting, microscopically represented as blight lines on dark pigmented background, covering usually 1/4−2/3 circumference of the segments from the ventral side. Antennal setae on segments III−V short and scarce, present on the dorsal side; the longest seta on segment V 0.011−0.016 (0.014) mm long; segment III with 8−14, IV with 1−4, V with 8−21 and VI with 5−7 setae, of which 3−4 are present apically and 1−3 on the basal half.

Suture on head invisible; antennal tubercles not developed; vertex nearly flat. Head dorsally with 5−7 and ventrally with 6−9 pairs of setae, respectively; of them, 5−7 pairs of ventral setae situated near the base of clypeus. Rostrum short, not reaching the middle point between the coxae of fore and middle legs. Ultimate rostral segment short, with gently convex margins and 6−7 pairs of short setae, 0.077−0.095 (0.087) mm long, 0.45−0.60 (0.51) times as long as the second segment of hind tarsi (Fig 3K). Femorotrochanter not imbricated and smooth; hind femorotrochanter 0.419−0.578 (0.507) mm long, 0.73−0.79 (0.76) times as long as hind tibia. Tibia slightly spinulose at the terminal; hind tibia 0.556−0.760 (0.669) mm long. Tarsi with numerous spinules neatly arranged in numerous transverse rows; second segment of hind tarsus 0.156−0.178 (0.170) mm long; first tarsal chaetotaxy 3:2−3:2; empodial setae slightly shorter than claws (Fig 3N). Abdomen membranous except genital plate, anal plate and cauda, which are slightly pigmented. Cornicles absent. Genital plate, slightly brown-pigmented, with 32−46 (39.1) setae (Fig 3Q). Cauda semicircular, with 2 setae. Fore wings with unbranched media; the veins rather broad, not conspicuously bordered. Hind wings only with 1 inconspicuous oblique vein.

Sexupara adult: Since the sexupara is morphologically close to the emigrant in many respects, the characters that differ between the two morphs and are indispensable for identification will be referred to.

Body 1.77–2.44 (2.20) mm long. Wax gland plates present on the whole body except the posterior segments of abdomen (Fig 4B).

Antennae 6-segmented, 0.76–0.90 (0.83) mm long, 0.365−0.432 (0.381) times as long as body, 0.93–1.03 (0.99) times as long as hind tibia (Fig 4E). Antennal segments III–VI pigmented thinly; segments V, VI imbricated with numerous transverse rows of spinules; segment IV sparsely with spinules between secondary rhinaria; segments I－III

not imbricated and smooth; segment III 0.27–0.37 (0.33) mm long, 1.54 times as long as that of the emigrant, 0.71–0.96 (0.89) times as long as segments IV, V and VI combined; segment IV rather oval in outline, 0.50–0.75 (0.57) times as wide as long; segment V cylindrical, 0.21–0.25 (0.23) mm long, 0.13–0.17 (0.16) times as wide as long, 0.63–0.82 (0.69) times as long as segment III; segment VI 0.059–0.068 (0.064) mm long, 0.46–0.54 (0.49) times as wide as long, with the middle thickest; segment VI with spinules arranged in 12–15 transverse rows, without secondary rhinaria. Primary rhinarium present on segment VI, tongue-like, elongate transversely with ciliated rims (Fig 4H). Secondary rhinaria present on the segments III–V, 0.0017–0.0093 (0.0025) mm wide, slightly projecting, microscopically represented as the blight lines on dark pigmented background, covering usually 1/2 to the whole circumference from the ventral side, more numerous than in emigrant. Antennal setae short and scarce, present on the dorsal surface, 0.017–0.030 (0.022) mm long on segment V.

Suture on head invisible; antennal tubercles not developed; vertex nearly flat. Median ocellus and 1 pair of wax gland plates situated posteriorly to the ocellus, forming a triangle (Fig 4K). Head dorsally with 6−8 pairs setae, ventrally with 6−12 pairs setae; of them, 5−11 pairs of ventral setae situated around the wax gland plates. Ultimate rostral segment 0.100–0.117 (0.107) mm long with 7 pairs of setae, convergent with almost straight margins, 0.59–0.74 (0.68) times as long as the second segment of hind tarsus, 1.34 times as long as that of the emigrant (Fig 4N). Pronotum with 2 pairs of wax gland plates spinally, metanotum and mesonotum each with 1 pair of them spinally. Femorotrochanter and tibia slightly longer than those of the emigrant. First tarsal chaetotaxy 3:2−3:2. The second segment of hind tarsus slightly shorter than that of the emigrant; second segment of hind tarsus 0.17–0.21 (0.19) times as long as hind tibia (in emigrant, the proportion is 0.23–0.32 (0.25)) (Fig 4Q). Abdominal tergites membranous without sclerites. Wax gland plates present on II−VII abdominal tergites, 1 pair spinally,

1 pair marginally, and sometimes 1−2 pair(s) of small ones pleurally; round or oval in the outline, with variation in the size, consisting of 1–44 (20) minute round facets (Figs 4T, 4W). Cornicles present on the pleural and posterior positions of the fifth abdominal tergite, 0.041–0.049 (0.044) mm in diameter, with slightly projecting rim, which is sclerotized slightly (Fig 4Z). The second to sixth abdominal tergites with fine setae sparsely; the seventh tergite with 7–8 slightly long setae. The second to sixth abdominal sternites with 20–29 short setae spinally, respectively. Genital plates slightly brown-pigmented, with 12–26 (15.9) setae on each of the right and left sides (Fig 4c).

Host plant: *Ulmus davidiana* var. *japonica*, *Ulmus parvifolia, Ulmus carpinifolia, Ulmus rubra, Ulmus minor, Ulmus campestris, Ulmus laciniata, Ulmus americana, Setaria* spp. (secondary host), and *Eleusine indica* (secondary host).

Specimens examined: Fundatrix first instar larva: 20exs, Kyoto, Kyoto, 25.IV.1981, on *Ulmus parvifolia*, S. Akimoto; 24exs, Beltsville, MD, USA, 3.V.1982, on *Ulmus carpinifolia*, D. R. Miller; 20exs, Mandavit, Gradignan Gironde, France, 2.V.2010, on *Ulmus* sp., T. Yoshida; 10exs, JC Raulston Arboretum, Raleigh, North Cardina, USA, 1.V.1999, on *Ulmus* ×cathedral, S. Aoki; 10exs, Schenk Forest, North Carolina, USA, 2.V.1999, on *Ulmus rubra*, S. Aoki; 2exs, San Martin del Agostedo, León, Spain, 11.VI.2003, *Ulmus minor*, Nicolás Pérez Hidalgo; 2exs, Bronte, Sicily, Italy, 10.VI.2009, on *Ulmus minor*, S. Akimoto; 23exs, Jeong-seon, Korea, 25.V.2012, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 3exs, Gangwon-do, Korea, 25–27.V.2012, on *Ulmus davidiana*, S. Akimoto; 7exs, Aokizawa, Minakami, Gunma, 3.VI.1983, on *Ulmus* sp., Y. Matsumoto; 20exs, Kashiwa, Chiba, 29.IV.2015, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 20exs, Bibai, Hokkaido, 5.VI.1981, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 20exs, Ukiha, Fukuoka, 8.IV.1985, on *Ulmus parvifolia*, S. Akimoto; 5exs, Sapporo, Hokkaido, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 6exs, Iwamizawa, Hokkaido, 15.VI.2004, on *Ulmus davidiana* var. *japonica*, S. Akimoto. Emigrant adult:

10exs, Sapporo, Hokkaido, 17.VI.2000, on *Ulmus davidiana* var. *japonica*, M. Sano; 10exs, Sapporo, Hokkaido, 20.VI.1984, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 2exs, Miami-Dade County Homestead, Florida, USA, via CHINA, 15.III.2000, ex *Zelkova serrata* (probably misidentification of *Ulmus parvifolia*), Duraid Hanna. First instar nymph produced by emigrants: 2exs, Sapporo, Hokkaido, 6.X.1983, on grass roots, S. Akimoto; 3exs, Sapporo, Hokkaido, 4.X.1983, on grass roots, S. Akimoto; 4exs, Bibai, Hokkaido, 1.X.1983, on grass roots, Y. Yamaguchi; 3exs, Hirosaki, Aomori, 29.IX.1983, on grass roots, S. Akimoto; 6exs, Madang Province, Papua New Guinea, VII. 2014, on grass roots, M. Kanamoto; 6exs, Utunomiya, Tochigi, 5.IX.1982, on grass roots, S. Akimoto; 6exs, Utunomiya, Tochigi, 5.IX.1982, on grass roots, S. Akimoto; 5exs, Kashiwa, Chiba, 6.X.1982, on grass roots, S. Akimoto; 4exs, Kyoto, Kyoto, 31.Ⅷ.1982, on grass roots, S. Akimoto; 6exs, Kuroyama, Osaka, 1.IX.1982 on grass roots, S. Akimoto; 6exs, Ochide, Ehime, 22.V.1974, on *Ulmus parvifolia*, S. Aoki; 3exs, Chiba, Chiba, 16.Ⅷ.1990, ex grass roots, Y. Matsumoto; 14exs, Kashiwa, Chiba, 26.X.1984, ex grass roots, S. Akimoto. Embryos in an emigrant adult: 5exs, Ochide, Ehime, 22.V.1974, on *Ulmus parvifolia*, S. Aoki; 2exs, Yoshi-machi, Fukuoka, 14.V.1980, on *Ulmus parvifolia*, S. Aoki. Sexupara adult: 1ex., Moftituck, New York, IX.1999, ex *Ulmus americana*, Daniel Gilrein, Coll.; 1ex., Adams Co., Bendersville, Bear Mtn. Orchards, Pennsylvania, USA, 28.IX.2000, J. Stimmel, Collector vacuumed from grasses in orchard ground cover; 10exs, Sapporo, Hokkaido, 30.IX.1989, on *Setaria viridis*, S. Akimoto.

***Tetraneura sorini* Hille Ris Lambers, 1970**

Fundatrix first instar larva: Body elliptical, becoming thinner posteriorly, 0.761−1.191 (on average 0.931) mm long, 0.311−0.527 (0.415) mm wide on abdominal segment II, 0.38−0.45 (0.41) times as wide as long (Fig 1C). Antennae short, 5-segmented, smooth and not imbricated, 0.179−0.199 (0.184) mm long, 0.54−0.59 (0.57) times the length of

hind femorotorochanter (Fig 1F). Fore femorotrochanter 0.146−0.249 (0.197) mm long, fore tibia 0.113−0.201 (0.151) mm long, hind femorotrochanter 0.216−0.429 (0.330) mm long, hind tibia 0.193−0.384 (0.299) mm long (Fig 2F). Rostrum reaching hind coxae; ultimate rostral segment rather slender, 0.104−0.167 (0.136) mm long, 0.28−0.55 (0.41) times as long as the length of hind femorotrochanter.

Emigrant adult: Body elongated oval, 1.95−2.13 (2.08) mm long, without wax gland plates (Fig 3C). Antennae 6-segmented, 0.63−0.70 (0.66) mm long, 0.30−0.35 (0.32) times as long as body, 0.97−1.07 (1.02) times as long as hind tibia (Figs 3F, 3I). Antennal segment III 0.19−0.23 (0.21) mm long, 1.15−1.28 (1.19) times as long as segment V. Antennal segment IV 0.076−0.091 (0.085) mm long, segment V 0.168−0.180 (0.174) mm long. Secondary rhinaria present on segments III−V, covering usually 1/3−2/3 circumference of the segments from the ventral side. Ultimate rostral segment 0.095−0.110 (0.103) mm long, 0.52−0.70 (0.62) times as long as the second segment of hind tarsus (Fig 3L). Hind femorotrochanter 0.45−0.53 (0.494) mm long. Second segment of hind tarsus 0.154−0.181 (0.168) mm long (Fig 3O). First tarsal chaetotaxy 3:2:2. Cornicles absent.

Sexupara adult: Body 1.719−2.942 (2.264) mm long (Fig 4C). Wax gland plates present on the whole body except the posterior segments of abdomen. Antennae 6-segmented, 0.741−0.902 (0.841) mm long, 0.29−0.44 (0.38) times as long as body, 0.96−0.89 (0.94) times as long as the length of hind tibia (Figs 4F, 4I). Antennal segment III 0.241−0.323 (0.295) mm long, 1.1−1.2 (1.2) times as long as segment V. Antennal segment IV 0.070−0.095 (0.079) mm long, segment V 0.226−0.268 (0.245) mm long. Secondary rhinaria present on segments III−V, covering usually 1/2−3/4 circumference of the segments from the ventral side. Ultimate rostral segment 0.122−0.166 (0.147) mm long,

0.73−0.84 (0.80) times as long as the second segment of hind tarsus (Fig 4O). Hind femorotrochanter 0.565−0.699 (0.641) mm long, hind tibia 0.775−1.013 (0.894) mm long. Second segment of hind tarsus 0.168−0.197 (0.184) mm long (Fig 4R). First tarsal chaetotaxy 3:2:2. Wax gland plates on marginal abdomen with two types of facets, larger ones of which are present in the center of plate, and small ones present peripherally (Figs 4U, 4X). Cornicles 0.057−0.080 (0.070) mm in diameter (Fig 4a).

Host plant: *Ulmus davidiana* var. *japonica,* and *Miscanthus sinensis* (secondary host).

Specimens examined: Fundatrix first instar larva: 5exs, Ukiha, Fukuoka, 8.IV.1985, on *Ulmus parvifolia*, S. Akimoto; 10exs, Bibai, Hokkaido, 5.VI.1981, on *Ulmus davidiana* var. *japonica*, S. Akimoto; 7exs, Aokizawa, Minakami, Gunma, 3.VI.1983, *Ulmus* sp. Emigrant adult: 4exs, Iwamizawa, Hokkaido, Japan, 15.VI.2009, ex. *Ulmus davidiana* var. *japonica*, S. Akimoto leg.; 8exs, Hokkaido Univ. Sapporo, Japan, 7.VI.1991, *Ulmus davidiana* var. *japonica*, S. Akimoto; 4exs, Iitate, Fukushima, 4.VI.2014, *Ulmus davidiana* var. *japonica*, S. Akimoto leg. Sexupara adult: 12exs, Yoichi, Hokkaido, 7.X.2017, ex *Ulmus davidiana* var. *japonica*, S. Akimoto. Other samples from the Supplementary materials in Muramatsu & Akimoto (2016).

Diagnoses of *Tetraneura ovaliformis* sp. nov., *T. akinire*, and *T. sorini*

I. Fundatrix first instar nymph

T. ovaliformis **sp. nov.**: Body 0.62−0.71 (0.65) mm long, antenna 0.12−0.14 (0.13) mm long, and hind femorotrochanter 0.15−0.19 (0.17) mm long. Antennae 0.75−0.89 (0.81) times as long as hind femorotrochanter. Ultimate rostral segment 0.09−0.11 (0.10) mm long and 0.50−0.64 (0.57) times as long as hind femorotrochanter.

T. akinire: Body 0.63−0.83 (0.75) mm long, antenna 0.15−0.18 (0.16) mm long, and hind femorotrochanter 0.18−0.27 (0.23) mm long. Antennae 0.58−0.75 (0.68) times

as long as hind femorotrochanter. Ultimate rostral segment 0.10−0.12 (0.11) mm long and 0.44−0.52 (0.47) times as long as hind femorotrochanter.

T. sorini: Body 0.76−1.19 (0.93) mm long, antenna 0.18−0.20 (0.18) mm long, and hind femorotrochanter 0.22−0.43 (0.33) mm long. Antennae 0.54−0.59 (0.57) times as long as hind femorotrochanter. Ultimate rostral segment 0.10−0.17 (0.14) mm long and 0.28−0.55 (0.41) times as long as hind femorotrochanter. Tergites sclerotized strongly.

II. Emigrant adult

T. ovaliformis **sp. nov.**: First tarsal chaetotaxy 3:3:3. Antenna 0.36−0.38 (0.37) times as long as body. Antennal segment III 0.23−0.29 (0.25) mm long.

T. akinire: First tarsal chaetotaxy 3:2−3:2. Antenna 0.28−0.37 (0.33) times as long as body. Antennal segment III 0.18−0.25 (0.22) mm long.

T. sorini: First tarsal chaetotaxy 3:2:2. Antenna 0.34−0.38 (0.36) times as long as body. Antennal segment III 0.19−0.23 (0.21) mm long.

III. Sexupara adult

T. ovaliformis **sp. nov.**: First tarsal chaetotaxy 3:3:3. Antenna 0.43−0.46 (0.44) times as long as body. Antennal segment III 0.28−0.39 (0.33) mm long. Cornicle 0.039−0.053 (0.045) mm in diameter. Wax gland plates on marginal abdomen with minute round facets of similar size.

T. akinire: First tarsal chaetotaxy 3:2−3:2. Antenna 0.37−0.43 (0.38) times as long as body. Antennal segment III 0.27−0.37 (0.33) mm long. Cornicle 0.41−0.49(0.44) mm in diameter. Wax gland plates on marginal abdomen with minute round facets of similar size.

T. sorini: First tarsal chaetotaxy 3:2:2. Antenna 0.28−0.44 (0.38) times as long as body. Antennal segment III 0.24−0.32 (0.30) mm long. Cornicle 0.057−0.080 (0.070) mm in

diameter. Wax gland plates on marginal abdomen with two types of facets, larger ones of which are present in the center of plate, and small ones present peripherally.

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1773 **Table 1** List of specimens used for phylogenetic analyses.

ID no.	Species	Collection date	Collection region	Generation
1	*T. akinire* A	2.V.1999	Raleigh, North Carolina, USA	gall, *Ulmus rubra*
2	*T. akinire* A	1.V.1999	Raleigh, North Carolina, USA	gall, *Ulmus* x *cathedral*
3	*T. akinire* A	26.V.2002	Kashiwa, Chiba, Japan	gall, *Ulmus parvifolia*
4	*T. akinire* A	26.V.2002	Kashiwa, Chiba, Japan	gall, *Ulmus davidiana*
5	*T. akinire* A	17.V.2004	Shirahama, Wakayama, Japan	gall, *Ulmus parvifolia*
6	*T. akinire* A	25.IV.2021	Okazaki, Aichi, Japan	gall, *Ulmus parvifolia*
7	*T. akinire* A	2.VI.2006	Bordeaux, France	gall, *Ulmus minor*
8	*T. akinire* A	2.VI.2006	Bordeaux, France	gall, *Ulmus minor*
9	*T. akinire* A	11.VI.2003	San Martin del Agostedo, León, Spain	gall, *Ulmus minor*
10	*T. akinire* A	5.VI.2006	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
11	*T. akinire* A	5.VI.2006	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
12	*T. akinire* A	30.VI.2014	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
13	*T. akinire* A	25.V.2002	Kashiwa, Chiba, Japan	gall, *Ulmus parvifolia*
14	*T. akinire* A	29.IX.2006	Sapporo, Hokkaido, Japan	roots, *Setaria viridis*
15	*T. akinire* A	22.X.2007	Sapporo, Hokkaido, Japan	roots, *Setaria pumila*
16	*T. akinire* B	22.II.2004	Naha, Okinawa, Japan	roots, Gramineae
17	*T. akinire* B	9.III.2004	Ulu Gombak, Malaysia	roots, Gramineae
18	*T. akinire* B	15.VI.2004	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
19	*T. akinire* B	15.VI.2004	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
20	*T. akinire* B	27.V.2002	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
21	*T. akinire* B	28.IX.2006	Iwamizawa, Hokkaido, Japan	roots, *Setaria viridis*
22	*T. akinire* B	29.IX.2006	Sapporo, Hokkaido, Japan	roots, *Setaria viridis*

23	*T. akinire* B	29.IX.2006	Sapporo, Hokkaido, Japan	roots, *Setaria viridis*
24	*T. akinire* B	15.VI.2004	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
25	*T. akinire* B	15.VI.2004	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
26	*T. akinire* B	15.VI.2004	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
27	*T. akinire* B	3.VI.2002	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
28	*T.* sp.	26.V.2012	Jeongseon, Gangwon-do, South Korea	gall, *Ulmus davidiana*
29	*T.* sp.	26.V.2012	Jeongseon, Gangwon-do, South Korea	gall, *Ulmus davidiana*
30	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
31	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
32	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
33	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
34	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
35	*T. sorini*	13.XI.2007	Kashiwa, Chiba, Japan	sexupara, *Ulmus davidiana*
36	*T. sorini*	5.VI.2014	Yonezawa, Yamagata, Japan	gall, *Ulmus davidiana*
37	*T. sorini*	31.V.2020	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
38	*T. sorini*	27.V.2002	Iwamizawa, Hokkaido, Japan	gall, *Ulmus davidiana*
39	*T. ovaliformis*	10.VI.2005	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
40	*T. ovaliformis*	15.VI.2005	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
41	*T. ovaliformis*	15.VI.2005	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*
42	*T. yezoensis*	5.VI.2006	Sapporo, Hokkaido, Japan	gall, *Ulmus davidiana*

Table 2 The number of exule first instar nymphs whose tarsi are spinulose in *Tetraneura akinire* sensu nov. Sample size in parentheses. Types indicate the potential mitochondrial gene type in each locality.

*locality of the neotype of *T. akinire* sensu Hille Ris Lambers.

**locality of the neotype of *T. nigriabdominalis* sensu Hille Ris Lambers.

locality	type	number	collection date	morph	host plant
Madang, Papua New Guinea	?	6(6)	VII, 2014	exule first instars	upland rice roots
Ochide, Ehime, Japan	A	0(6)	22.Ⅴ.1974	progeny of emigrants	gall, *Ulmus parvifolia*
Kyoto, Japan	A	4(4)	31.VIII.1982	exule first instars	grass roots
Kuroyama, Osaka, Japan*	A	6(6)	1.IX.1982	exule first instars	grass roots
Utsunomiya, Japan**	A	12(12)	5.IX.1982	exule first instars	grass roots
Kashiwa, Japan	A	5(5)	6.IX.1982	exule first instars	grass roots
Kashiwa, Japan	A	0(14)	26.X.1984	exule first instars	grass roots
Chiba, Japan	A	3(3)	16.VIII.1990	exule first instars	grass roots
Hirosaki, Japan	A	3(3)	29.IX.1983	exule first instars	grass roots
Sapporio, Japan	A or B	4(4)	4.X.1983	exule first instars	grass roots
Sapporio, Japan	A or B	2(2)	6.X.1983	exule first instars	grass roots
Bibai, Japan	B	15(15)	1.X.1983	exule first instars	grass roots

Table 3 Mean genetic distances (*p*-distances) in COI among *Tetraneura* species or clades detected in the present study. *T.* sp. indicates globular gall formers collected in Jeongseon, Gangwon-do, South Korea.

	T. akinire-A	*T. akinire*-B	*T.* sp.	*T. sorini*
T. akinire-B	0.0246			
T. sp.	0.0387	0.0446		
T. sorini	0.0265	0.0356	0.0405	
T. ovaliformis	0.0504	0.0475	0.0601	0.0560

Figure legends

Figure 1 Galls induced by aphids of the *Tetraneura akinire* species group. *T. ovaliformis* (A, B, and C), unidentified Korean species (D and E), *T. akinire* sensu nov. (F, G, H, and I), and *T. sorini* (J, K, and L). collection data: A, 10.VI.2015, Sapporo, Japan, *Ulmus davidiana*; B, 11.VI.2015, Sapporo, Japan, *Ulmus davidiana*; C, 18.VI.2011, Sapporo, Japan, *Ulmus davidiana*; D and E, 26.V.2012, Jeongseon, Gangwon-do, South Korea, *Ulmus davidiana*; F, 28.IV.2012, Kashiwa, Japan, *Ulmus parvifolia*; G and H, 10.VI.2015, Sapporo, Japan, *Ulmus davidiana*; I and J, 30.V.2015, Iwamizawa, Japan, *Ulmus davidiana*; K, 31.V.2020, Iwamizawa, Japan, *Ulmus davidiana*; L, 7.VI.2020, Iwamizawa, Japan, *Ulmus davidiana*.

Figure 2 The length of the hind femorotrochanter in the *Tetraneura akinire* species group. The mean and SD are indicated. *T. ovaliformis* (1 and 2), unidentified Korean species (3), *T. akinire* sensu nov. (4-14), and *T. sorini* (15-20).

Figure 3 Phylogenetic relationships among the *Tetraneura akinire* species group. The maximum-likelihood (ML) tree based on the partial sequence of COI gene. Circles after collection information indicates host plants; blue circles, *Ulmus davidiana* and the closely related species (Wiegrefe et al. 1994), red circles, *Ulmus parvifolia*, and yellow circles, grass roots.

Figure 4 Fundatrix first instar nymphs: *T. ovaliformis* **sp. nov.** (A), *T. akinire* (B), *T. sorini* (C). Antennae: *T. ovaliformis* **sp. nov.** (D), *T. akinire* (E), *T. sorini* (F).

Figure 5 First instar nymphs produced by emigrants: *T. ovaliformis* **sp. nov.** (A), *T. akinire* (B), *T. sorini* (C). Hind tibiae and tarsi: *T. ovaliformis* **sp. nov.** (D), *T. akinire*

(E), *T. sorini* (F). Setae on hind tarsi and tibiae: *T. ovaliformis* **sp. nov.** (G), *T. akinire* (H), hairless type *T. akinire* (I), *T. sorini* (J). Abdominal wax gland plates: *T. ovaliformis* **sp. nov.** (K), *T. akinire* (L), *T. sorini* (M).

Figure 6 Emigrants: *T. ovaliformis* **sp. nov.** (A), *T. akinire* (B), *T. sorini* (C). Antennae: *T. ovaliformis* **sp. nov.** (D), *T. akinire* (E), *T. sorini* (F). Apex of antennae: *T. ovaliformis* **sp. nov.** (G), *T. akinire* (H), *T. sorini* (I). Ultimate rostral segments: *T. ovaliformis* **sp. nov.** (J), *T. akinire* (K), *T. sorini* (L). Hind tarsus: *T. ovaliformis* **sp. nov.** (M), *T. akinire* (N), *T. sorini* (O). Genital plates: *T. ovaliformis* **sp. nov.** (P), *T. akinire* (Q), *T. sorini* (R).

Figure 7 Sexuparae: *T. ovaliformis* **sp. nov.** (A), *T. akinire* (B), *T. sorini* (C). Antennae: *T. ovaliformis* **sp. nov.** (D), *T. akinire* (E), *T. sorini* (F). Apex of antennae: *T. ovaliformis* **sp. nov.** (G), *T. akinire,* (H), *T. sorini* (I). Median ocelli and wax gland plates on heads: *T. ovaliformis* **sp. nov.** (J), *T. akinire* (K), *T. sorini* (L).

Figure 8 Sexuparae: Ultimate rostral segments: *T. ovaliformis* **sp. nov.** (A), *T. akinire* (B), *T. sorini* (C). Hind tarsus: *T. ovaliformis* **sp. nov.** (D), *T. akinire* (E), *T. sorini* (F). Wax gland plates around the cornicles: *T. ovaliformis* **sp. nov.** (G), *T. akinire* (H), *T. sorini* (I). Wax gland plates on abdomen: *T. ovaliformis* **sp. nov.** (J), *T. akinire* (K), *T. sorini* (L). Cornicles: *T. ovaliformis* **sp. nov.** (M), *T. akinire* (N), *T. sorini* (O). Genital plates: *T. ovaliformis* **sp. nov.** (P), *T. akinire* (Q), *T. sorini* (R).

Figure 1

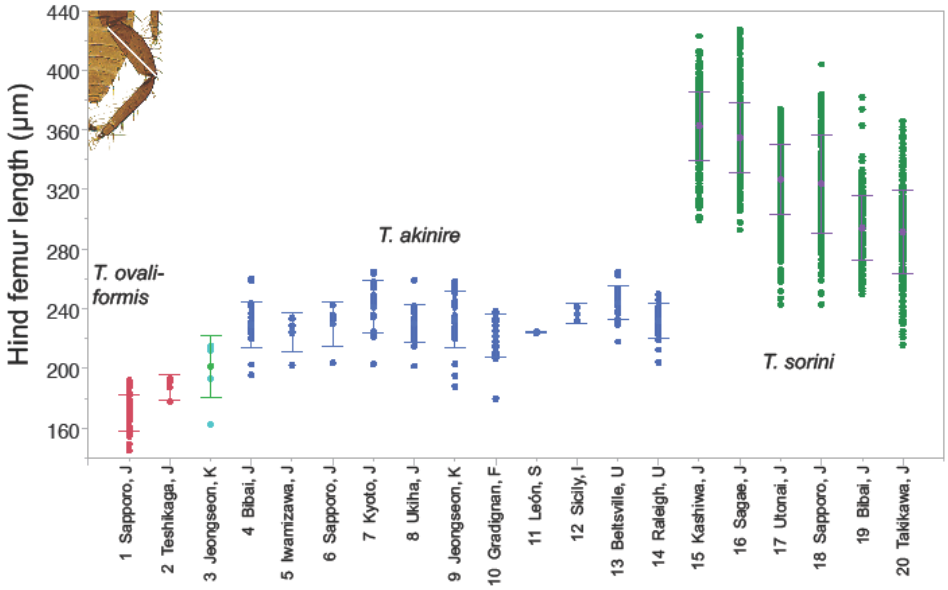
Hind femur length (µm)
440
400
360
320
280
240
200
160
T. ovali-formis
T. akinire
T. sorini
1 Sapporo, J
2 Teshikaga, J
3 Jeongseon, K
4 Bibai, J
5 Iwamizawa, J
6 Sapporo, J
7 Kyoto, J
8 Ukiha, J
9 Jeongseon, K
10 Gradignan, F
11 León, S
12 Sicily, I
13 Beltsville, U
14 Raleigh, U
15 Kashiwa, J
16 Sagae, J
17 Utonai, J
18 Sapporo, J
19 Bibai, J
20 Takikawa, J

Figure 2

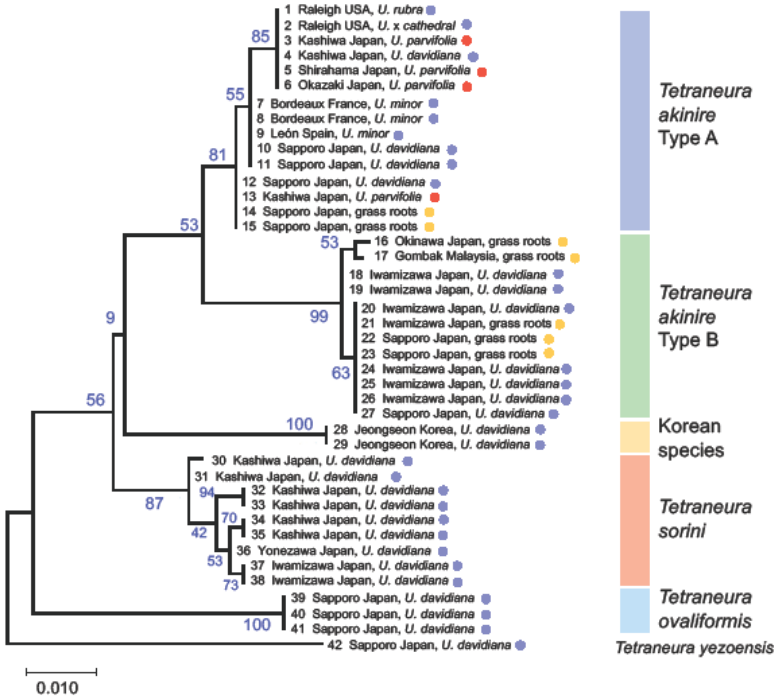
1 Raleigh USA, U. rubra
2 Raleigh USA, U. x cathedral
3 Kashiwa Japan, U. parvifolia
4 Kashiwa Japan, U. davidiana
5 Shirahama Japan, U. parvifolia
6 Okazaki Japan, U. parvifolia
7 Bordeaux France, U. minor
8 Bordeaux France, U. minor
9 León Spain, U. minor
10 Sapporo Japan, U. davidiana
11 Sapporo Japan, U. davidiana
12 Sapporo Japan, U. davidiana
13 Kashiwa Japan, U. parvifolia
14 Sapporo Japan, grass roots
15 Sapporo Japan, grass roots
16 Okinawa Japan, grass roots
17 Gombak Malaysia, grass roots
18 Iwamizawa Japan, U. davidiana
19 Iwamizawa Japan, U. davidiana
20 Iwamizawa Japan, U. davidiana
21 Iwamizawa Japan, grass roots
22 Sapporo Japan, grass roots
23 Sapporo Japan, grass roots
24 Iwamizawa Japan, U. davidiana
25 Iwamizawa Japan, U. davidiana
26 Iwamizawa Japan, U. davidiana
27 Sapporo Japan, U. davidiana
28 Jeongseon Korea, U. davidiana
29 Jeongseon Korea, U. davidiana
30 Kashiwa Japan, U. davidiana
31 Kashiwa Japan, U. davidiana
32 Kashiwa Japan, U. davidiana
33 Kashiwa Japan, U. davidiana
34 Kashiwa Japan, U. davidiana
35 Kashiwa Japan, U. davidiana
36 Yonezawa Japan, U. davidiana
37 Iwamizawa Japan, U. davidiana
38 Iwamizawa Japan, U. davidiana
39 Sapporo Japan, U. davidiana
40 Sapporo Japan, U. davidiana
41 Sapporo Japan, U. davidiana
42 Sapporo Japan, U. davidiana
85
55
81
53
53
99
9
63
56
100
94
87
70
42
53
73
100
0.010
Tetraneura akinire Type A
Tetraneura akinire Type B
Korean species
Tetraneura sorini
Tetraneura ovaliformis
Tetraneura yezoensis

Figure 3

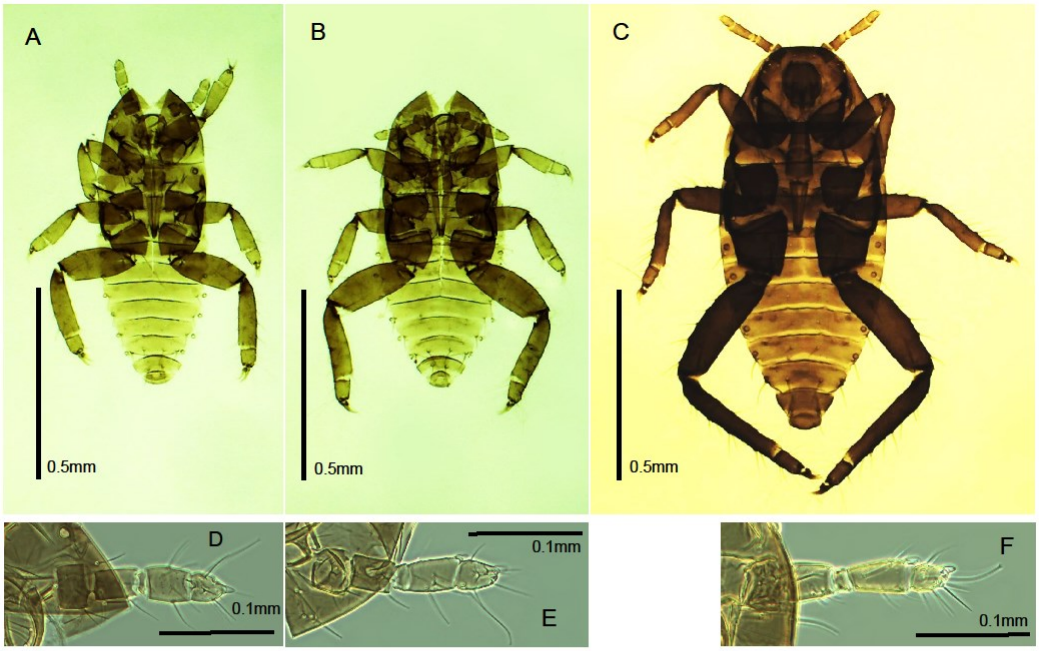

Figure 4

1881

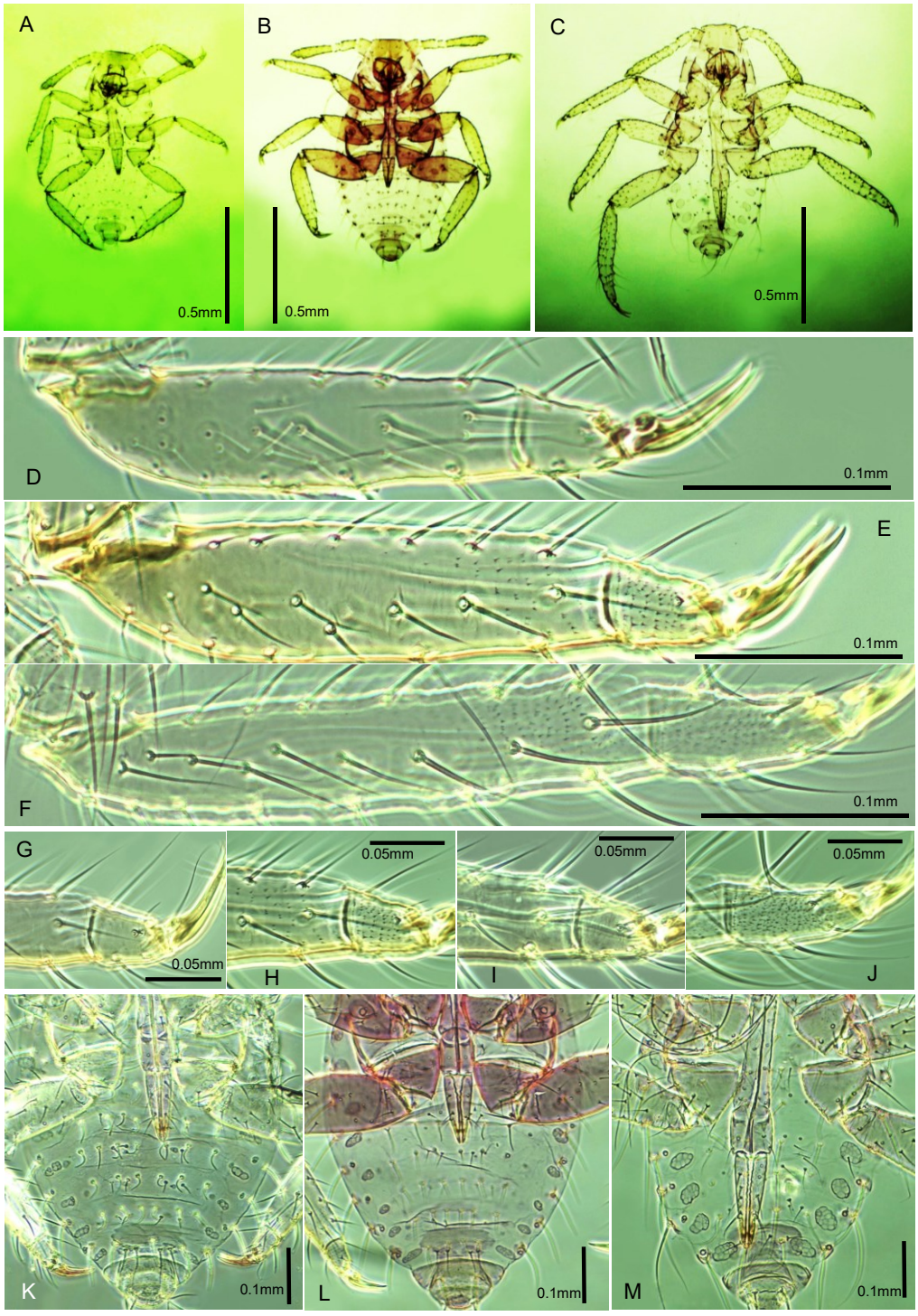

Figure 5

1882

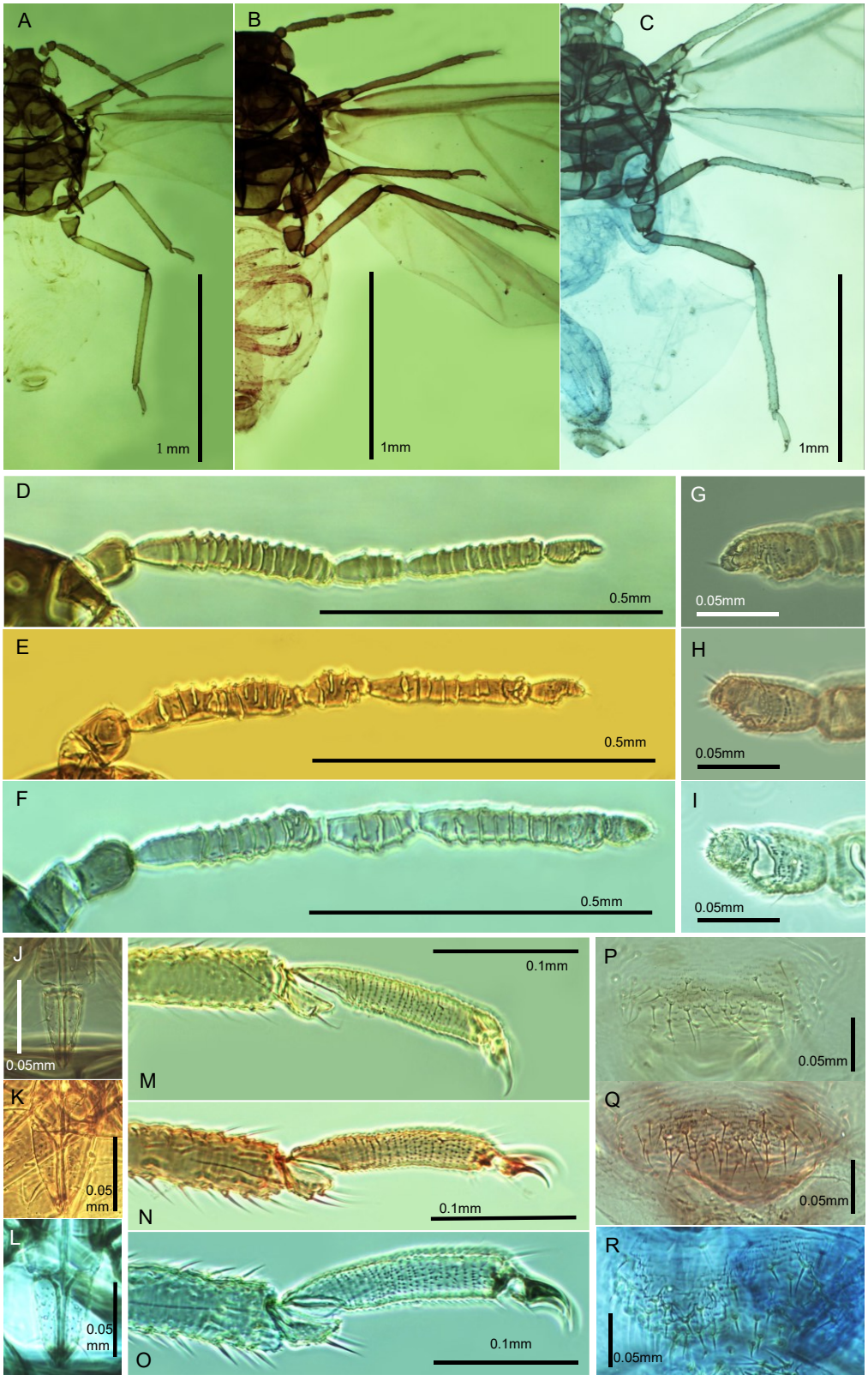

Figure 6

1883

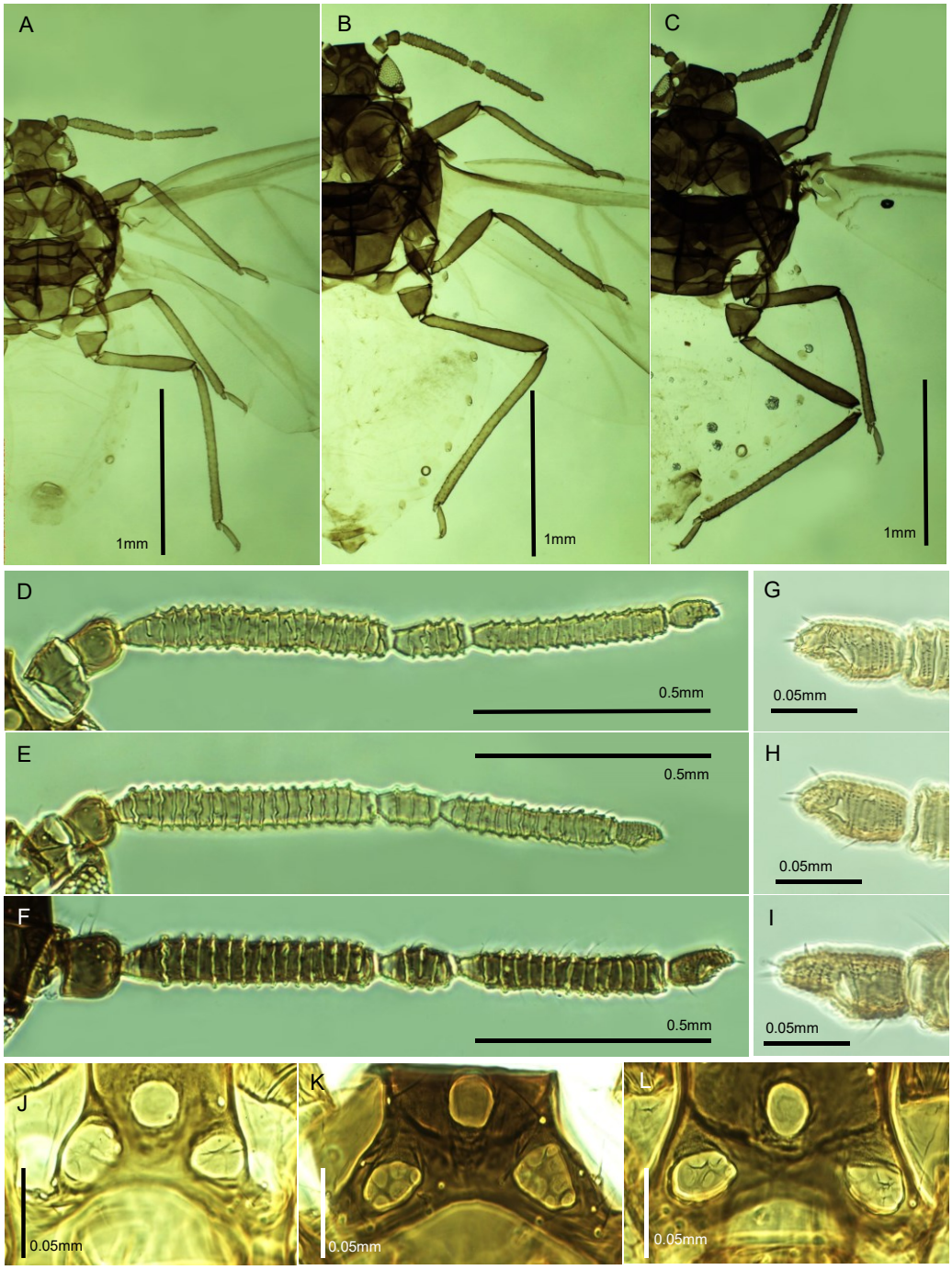

Figure 7

1884

Figure 8

1885