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Taxonomy and DNA barcoding of *Cyamophila* (Hemiptera: Psyllidae) from Japan, with the description of a new species

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Abstract

The jumping plant-lice of the genus *Cyamophila* from Japan are reviewed. Three species are recognized. The new species, *Cyamophila burckhardti* **sp. nov.** is described. One new synonym is proposed: *Cyamophila hexastigma* (Horváth) = *Cyamophila floribundae* Cho & Burckhardt **syn. nov.** *Cyamophila hexastigma* and *C. willieti* (Wu) are redescribed, and the latter is newly recorded from Japan. Keys to species for adults and immatures, diagnoses, biological information, and DNA barcoding results are provided.

Key words: psyllids, jumping plant-lice, Psylloidea, new record, DNA barcode, COI, Fabaceae

Introduction

Psyllids, or jumping plant-lice, are small phloem-feeding hemipterans that are highly host-specific. Closely related psyllid species develop on closely related host plants (Burckhardt *et al.* 2014).

The genus *Cyamophila* Loginova (Psyllidae: Psyllinae) was originally erected for *Psylla fabra* Loginova, and is associated with Fabaceae (Fabales) (Loginova 1977). More than 60 species of *Cyamophila* have been described so far, all of which occur in the Palaearctic region. More than 50 of these species have been found in China, Mongolia, and Central Asia. Among other East Asian countries, three species are known to occur in South Korea (Cho *et al.* 2022).

Only one species, *Cyamophila hexastigma* (Horváth), is known to occur in Japan (Inoue 2016). This psyllid species is known to damage *Maackia amurensis* (Fabaceae), which is indigenous and widely distributed in mountainous areas in Japan and is planted as garden trees. In addition to *C. hexastigma*, a new and previously unrecorded *Cyamophila* species was confirmed by morphological and DNA barcoding identification based on samples from field surveys conducted by the author over the last two decades throughout Japan. In this study, the genus *Cyamophila* in Japan is reviewed. Identification keys, diagnoses, descriptions, illustrations, and biological information are provided. The phylogeny and genetic diversity based on the sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene fragment are also discussed.

Materials and Methods

Collection of specimens and morphological study. Over 1300 specimens of *Cyamophila* collected in Japan from 1998 to 2022, mostly from plants and a few from light traps, were examined. For morphological studies, adult specimens were dry-mounted and immature specimens were kept in 70% ethanol. Some adult and immature specimens were slide-mounted in Canada balsam following the procedure described by Inoue (2004) to conduct more detailed morphological observations under a phase-contrast microscope. All illustrations were drawn from the slide-mounted specimens using a drawing tube. Photographs of dry-mounted adult specimens were captured using a Nikon SMZ25 stereomicroscope (Nikon, Tokyo, Japan) with an attached Nikon Digital Sight 10 digital

camera, and serial images were automatically stacked using the NIS-Elements BR software version 5.42.00 (Nikon, Japan). Photographs of slide-mounted immature specimens were captured using a Nikon ECLIPSE Ni-U phase-contrast microscope equipped with a Nikon DS-Fi3 digital camera. Morphological terminology follows mainly Ossiannilsson (1992). The nomenclature of the plants is based on World Flora Online (WFO 2023). Abbreviations of measurements are as follows. Adults: BL, total body length measured from the anterior margin of the head to the tip of folded wings; WL, forewing length; WW, forewing width; HW, head width; AL, antennal length; VW, vertex width; VL, vertex length along median suture; GL, genal cone length; MP, male proctiger length; PL, paramere length; FP, female proctiger length. Immatures: BL, body length; BW, body width; AL, antennal length; WPL, forewing pad length; CRW, outer circumanal ring width. In the forewing illustrations, the dashed and dashed-dotted lines represent the limits of areas of surface spinules and radular spinules, respectively. The holotype of the new species is deposited at the Insect Museum, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan. Other materials are preserved in the author's collection, which is presently located at the Institute for Plant Protection, NARO, Hiroshima, Japan (HIC), and partly at the Osaka Museum of Natural History (OMNH), Osaka, Japan.

Molecular analysis. Specimens for DNA barcoding analysis were stored in 99.5% ethanol at -30°C until DNA extraction. Genomic DNA was extracted from the whole body of each individual using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) by a non-destructive method based on Johnson et al. (2004), with initial incubation for 24-48 h. The final elution volume was 200 µL. The voucher specimens were slide-mounted in Canada balsam and stored at HIC. Polymerase chain reaction (PCR) was conducted to amplify the COI gene of mitochondrial DNA using the primers CACF and CACR (Kuznetzova et al. 2002), and for Cyamophila willieti, the primers mCACF2 (5'-GTTCCTTGCCTTTATCAGCC-3') and mCACR4 (5'-GGTAAAATTAAAATTAAAACTTC-3'). PCR reactions were conducted using a PCR Thermal Cycler MP TP3000 (Takara Bio, Shiga, Japan) in 50 μL volumes containing 1 μL of DNA template, 1.5 μL of each primer at 10 μM, 1 unit of Tks Gflex PCR polymerase (Takara Bio, Shiga, Japan), and 25 μL of 2x PCR buffer including MgCl₂. The thermal cycling conditions were as follows: an initial denaturation stage at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and an extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were cleaned using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) and directly sequenced using an ABI3730 genetic analyzer (Applied Biosystems) at Eurofins Genomics Inc. (Tokyo, Japan). The sequences were aligned using ClustalW in the MEGA11 software (Tamura et al. 2021). Among the 723 bp of obtained sequences, the first 9 bp were cropped as they belonged to the tRNA-Tyr gene, and the remaining 714 bp of COI gene sequences were used for phylogenetic analysis. The sampling information and accession numbers in the DNA Data Bank of Japan (DDBJ)/European Molecular Biology Laboratory (EMBL)/GenBank for the DNA sequences determined in this study are listed in Table 1. Pairwise distances were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA11. A neighbour-joining (NJ) tree was constructed using a K2P model with 1000 bootstrap replications in MEGA11. The phylogenetic analysis included the sequences of Cyamophila willieti from Yinchuan, Ningxia of China (Song et al. 2019; accession number MN364946), and as out-group, Anomoneura mori Kuwayama from Shimane, Honshû of Japan (accession number LC784157).

Taxonomy

Genus Cyamophila Loginova, 1976

Cyamophila Loginova, 1976: 596. Type species: Psylla fabra Loginova, 1964, by original designation.

Keys to the species of Cyamophila from Japan

Adults

Fifth instar immatures

Cyamophila hexastigma (Horváth, 1899)

(Japanese name: Mutsuboshi-kijirami) (Figs 1–6, 7–10, 26, 29, 32, 35–36, 41–42)

Psylla hexastigma Horváth, 1899: 373. Type locality: Japan (Hokkaidô).

Cyamophila hexastigma: Loginova (1977: 582).

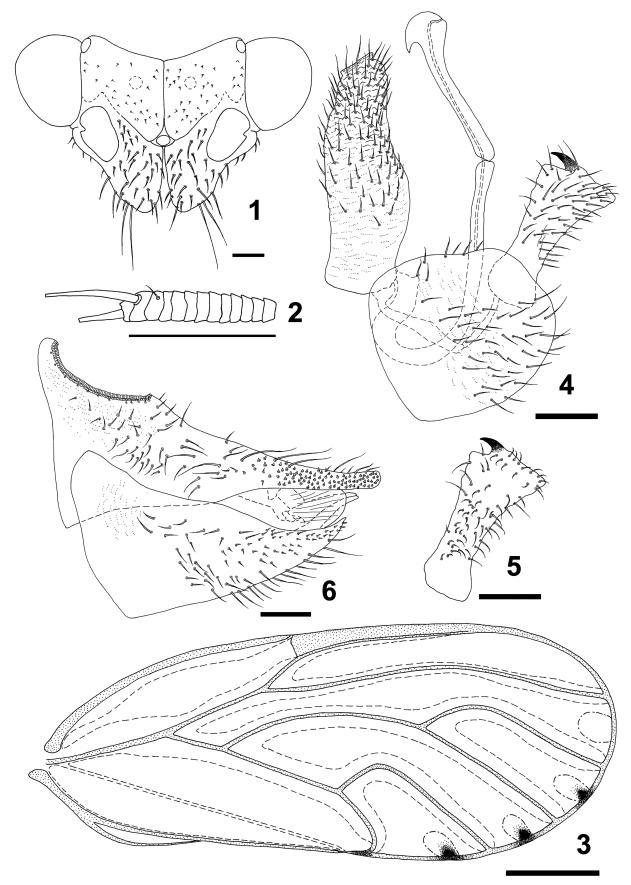
Cyamophila floribundae Cho & Burckhardt in Cho et al. (2017: 551), syn. nov.

Description. Adult. Colouration. General colour (Figs 35, 41) light yellowish green with obscure light yellowish brown markings and longitudinal stripes on thorax; in overwintered individuals (Fig. 36), body colour chestnut brown to dark brown in general, with many cream-yellow coloured fine markings and stripes on head and thorax. Antenna yellow; apices of segments IV–VI and most of segment VII dark brown; segments VIII–X entirely dark brown to black. Forewing membrane transparent, colourless in general but faintly yellowish along veins in the apical half of forewing in fully matured adults, with a prominent dark brown spots at the location of the radular spinules in cells m₁, m₂, and cu₁ (Fig. 3); veins yellow in summer adults, becoming uniformly dark brown in overwintered adults. Apical tooth of paramere black.

Structure. Head (Fig. 1) strongly inclined downwards, 85–90° from longitudinal body axis in profile, slightly wider than thorax. Vertex nearly half as long as wide or slightly shorter. Genal processes conical, about 0.8 times as long as vertex, slightly divergent and subacute apically. Antenna long, 2.2–2.4 times as long as head width; longer terminal seta of segment X about twice as long as shorter seta, about 0.6 times as long as segment X (Fig. 2).

Forewing (Fig. 3) oblong oval, 2.3–2.4 times as long as wide, widest at around 2/3 from the base; membrane with dense surface spinules in all cells; spinule-free bands along veins rather broad; fields of radular spinules as in Fig. 3; pterostigma well developed, more than 1/3 of the forewing length; Rs slightly sinuate, slightly curved towards costal margin apically; M_{1+2} rather strongly arched at around the basal 1/3 and nearly straight thereafter, very slightly curved towards costal margin apically; Cu_{1a} strongly arched around the basal 1/4 and nearly straight thereafter. Meracanthus moderate in size, subacute and slightly curved downwards apically; metatibia with prominent genual spine, with five apical sclerotised spurs arranged in 1+3+1; basal segment of metatarsus with a pair of sclerotised lateral spurs.

Male terminalia (Fig. 4) moderate in size. Proctiger slender, slightly curved caudad apically. Paramere stout, 0.7 times as long as proctiger, strongly constricted in the middle, strongly widened towards the tip, weakly produced cephalad at anteroapical corner, and strongly produced caudad at posteroapical corner; inner surface (Fig. 5) with many retrorse setae; inner apical tooth prominent, acute, and projected cephalad. Distal aedeagal segment slightly longer than paramere; apex round and thickened, strongly hooked.



FIGURES 1–6. Cyamophila hexastigma, adult. 1. Head, dorsal view. 2. Terminal flagellomere. 3. Right forewing. 4. Male terminalia, lateral view. 5. Paramere, inner face. 6. Female terminalia, lateral view. Scale bars: 0.1 mm for 1–2, 4–6; 0.5 mm for 3.

Female terminalia (Fig. 6) rather stout. Proctiger curved and slightly sinuate at dorsal margin, slightly upturned apically, and slightly truncated at apex (Fig. 32). Subgenital plate with sparse setae, acute at apex.

Fifth instar immature. Body (Fig. 42) light green, gently swollen dorsally. Antenna slender, 2.1–2.2 times as long as forewing pad, seven-segmented, with one apical rhinarium each on segments III and V, and two on the middle of segment VII, with rather long capitate setae on the middle of segment III and apices of segments III–V (Fig. 26). Forewing pad oblong oval; outer margin with 8–9 long capitate setae and short simple setae present in between (Fig. 29). Hindwing pad with two long capitate setae apically. Legs long, hairy, with many short simple setae and long capitate setae. Abdomen rounded apically, with many long capitate setae dorsally and many long simple setae ventrally. Caudal plate with many long capitate setae on dorsum and margin, with 4+4 truncated sectasetae on posterior margin. Anus located on ventral side. Outer circumanal pore ring relatively small, heart-shaped, strongly curved in front, comprising a single row of elongated pores. Inner circumanal pore ring comprising a single row of small elongated pores.

Measurements (in mm): Adult (n = 5 males, 5 females): BL: 3.13–3.80; WL: 2.59–3.15; WW: 1.06–1.35; AL: 1.90–2.33; HW: 0.82–0.96; VW: 0.46–0.58; VL: 0.22–0.27; GL: 0.18–0.23; MP: 0.36–0.43; PL: 0.27–0.31; FP: 0.68–0.83. Fifth instar immature (n = 5): BL: 2.28–2.60; BW: 1.23–1.50; AL: 1.38–1.63; WPL: 0.65–0.78; and CRW: 0.09–0.10.

Material examined. Hokkaidô: 5 males, 3 females, 2 immatures, Sapporo-shi, Minami-ku, Jôzankei, 300 m, 9.vii.2001, on Maackia amurensis, H. Inoue (dry- and slide-mounted; HIC); 13 males, 12 females, Sapporo-shi, Minami-ku, Jôzankei, Jôzankei Dam, 42.982° N, 141.159° E, 300 m, 19-20.vii.2012, on M. amurensis, H. Inoue (dry- and slide-mounted; HIC); 18 males, 24 females, same data, 12.vi.2013 (dry- and slide-mounted; HIC); 9 males, 8 females, 23 immatures, Kamikawa-gun, Shimizu-chô, Haobi, 42.957° N, 142.905° E, 190 m, 30.vi.2015, on M. amurensis, H. Inoue (dry- and slide-mounted, 99.5% and 70% ethanol; HIC); 3 males, 1 females, Sarugun, Hidaka-chô, Chiroro-rindô, 2.vii.1998, N. Takahashi (dry-mounted; HIC); 1 female, Akan-gun, Akan-chô, Meakan-dake, 29.vi.1998, N. Takahashi (dry-mounted; HIC); Honshû: 8 males, 8 females, 4 immatures, Aomoriken, Sannohe-gun, Shingô-mura, Herai, Kosaka, 40.444° N, 141.131° E, 170 m, 2.vii.2015, on M. amurensis, H. Inoue (dry- and slide-mounted, 99.5% ethanol; HIC); 3 males, 5 females, Aomori-ken, Sannohe-gun, Shingô-mura, Herai, Sawaguchi, 40.454° N, 141.149° E, 160 m, 2.vii.2015, on M. amurensis, H. Inoue (dry- and slide-mounted; HIC); Kyûshû: 2 females, Fukuoka-ken, Miyako-gun, Miyako-machi, Saigawahobashira, near Notôge, 33.502° N, 130.970° E, 750–930 m, 9.vi.2014, H. Inoue (slide-mounted; HIC); 19 males, 26 females, 10 immatures, Nagasakiken, Unzen-shi, Obama-chô, Unzen, Ikenohara, 32.745° N, 130.270° E, 750 m, 27.v.2010, on Maackia floribunda, H. Inoue (dry- and slide-mounted; HIC); 136 males, 140 females, 4 immatures, same data, 4.vi.2010 (dry- and slide-mounted, 99.5% ethanol; HIC); 2 males, 5 females, same data, 14.iv.2013 (dry-mounted; HIC); 38 males, 50 females, same data, 13.vi.2014 (dry-mounted, 99.5% ethanol; HIC).

Distribution. Japan (Hokkaidô, Honshû, Shikoku, Kyûshû, Tsushima); China (Beijing, Jilin, Liaoning, Shanxi), South Korea, Russian Far East (Khabarovsk Territory, Primorsky Territory) (Wu 1932, *as P. hexastigma*; Sasaki 1954, as *P. hexastigma*; Miyatake 1963, as *P. hexastigma*; Kuwayama & Miyatake 1971, as *P. hexastigma*; Miyatake 1976, as *P. hexastigma*; Park *et al.* 1979, as *P. hexastigma*; Konovalova 1988; Li 2011; Cho *et al.* 2017, as *C. floribundae*; Kwon & Kwon 2020).

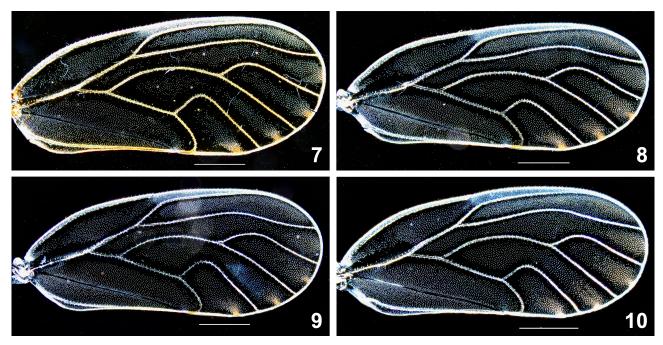
Host plant. *Maackia amurensis* Rupr. (Miyatake 1976); *Maackia floribunda* (Miq.) Takeda (Fabales: Fabaceae) (new host plant record). These host plants were confirmed by the presence of immatures.

Styphnolobium japonicum (= Sophora japonica) (Fabaceae) recorded by Kwon (1983) is the host for Cyamophila willieti (Cho et al. 2022). Kuwayama (1932) referred to Hydrangea (Cornales: Hydrangeaceae) as follows: 'In Hokkaidô, adults of Psylla hexastigma are abundant on Saxifragaceae plants, such as Hydrangea paniculata and Hydrangea serrata, around August. This species likely feeds on these plants'; however, these plants are not hosts on which the immatures develop. Hydrangea has been cited as a host for this psyllid species in subsequent studies, such as in Sasaki (1954), Miyatake (1963), Klimaszewski (1973), Kwon (1983), and Kwon & Kwon (2020), and is therefore formally eliminated here as the host of C. hexastigma.

Biology. Univoltine. Adults emerge from late May (Kyûshû in southwest Japan) to early July (Hokkaidô in northern Japan) and leave the host plant within a short period. Dispersed adults are thought to stay on the evergreen shelter plants until spring.

Comments. Cyamophila floribundae Cho & Burckhardt, which feeds on Maackia floribunda, was described from Jeju Island, in the southernmost part of South Korea (Cho et al. 2017). In my field survey, a Cyamophila population feeding on M. floribunda was confirmed from Kyûshû, Japan; this population cannot be distinguished

from *C. hexastigma* populations feeding on *M. amurensis* from Hokkaidô, from which this psyllid species was originally described, through morphology as well as DNA barcoding (Fig. 48). According to Cho *et al.* (2017), *C. floribundae* differs from *C. hexastigma* in the following aspects: 1) denser surface spinules of forewing membrane; 2) slightly widened parameres towards the apex; and 3) host plant. However, 1) some variation was observed in the density of the forewing surface spinules in *C. hexastigma*, ranging from sparse (Figs 7, 9) to dense (Figs 8, 10), even within the same locality, although the spinules in *M. floribunda*-feeders tended to be somewhat denser. 2) No stable morphological differences were observed in paramere between the populations of different host plants. These slight differences were likely within the range of intraspecific variation. 3) Notably, Japanese *C. hexastigma* also feeds on *M. floribundae*, implying that these two nominal psyllid species cannot be distinguished from each other by their host plants. Additionally, for the Korean *C. hexastigma* and *C. floribundae* molecular data on the GenBank database used in the analysis of Cho *et al.* (2019), no divergence was observed in the 575 bp of the COI-tRNA leu-COII region, and only 1.45% (*p*-distance) or 1.46% (K2P distance) divergence was observed in >690 bp of the mitochondrial 12S and 16S ribosomal RNA data. Therefore, I conclude that these two taxa are conspecific and can be synonymised as *Cyamophila hexastigma* (Horváth, 1899) = *Cyamophila floribundae* Cho & Burckhardt, 2017, **syn. nov.**



FIGURES 7–10. Right forewings of *Cyamophila hexastigma*. **7–8.** Male collected from Hokkaidô on *Maackia amurensis*; **9–10.** Male collected from Kyûshû on *Maackia floribunda*. Scale bars: 0.5 mm.

Cyamophila burckhardti sp. nov.

urn:lsid:zoobank.org:act:AD760C92-FC32-4DD8-8413-EED0415FA7FF (Japanese name: Usuiro-mutsuboshi-kijirami)

(Figs 11–19, 27, 30, 33, 37–38, 43–44)

Cyamophila hexastigma sensu Inoue & Yamauchi (2001: 55), nec Horváth (1899: 373). Cyamophila sp.: Hayashi et al. (2011: 218); Hayashi & Miyatake (2012: 28); Nozawa (2022: 90).

Description. Adult. Colouration. General colour (Figs 37, 43) light yellowish green with obscure light yellowish brown markings and longitudinal stripes on thorax; in overwintered individuals (Fig. 38), body colour chestnut brown to dark brown in general, with many cream-yellow coloured fine markings and stripes on head and thorax. Antenna yellow; apices of segments IV–VI and most of segment VII dark brown; segments VIII–X dark brown to black. Forewing membrane transparent in general, faintly yellowish along veins in the apical half of forewing in younger adults, strongly yellowish throughout the apical half of forewing in fully matured adults, with reduced and obscure pale brown spots at the location of the radular spinules in cells m₁, m₂, and cu₁ (Fig. 13); veins yellow in summer adults, becoming uniformly dark brown in overwintered adults. Apical tooth of paramere black.

Structure. Head (Fig. 11) strongly inclined downwards, 85–90° from longitudinal body axis in profile, slightly wider than thorax. Vertex nearly half as long as wide or slightly shorter. Genal processes conical, about 0.7 times as long as vertex, slightly divergent and subacute apically. Antenna long, 2.3–2.6 times as long as head width; longer terminal seta of segment X about 1.8–2.0 times as long as shorter seta, 0.5–0.6 times as long as segment X (Fig. 12).

Forewing (Fig. 13) oblong oval, about 2.2–2.3 times as long as wide, widest at around 2/3 from the base; membrane with dense surface spinules in all cells; spinule-free bands along veins rather narrow, gradually becoming narrower apically; fields of radular spinules as in Fig. 13; pterostigma well developed, more than 1/3 of the forewing length; Rs slightly sinuate, not curved towards costal margin apically; M₁₊₂ rather strongly arched at around the basal 1/3 and nearly straight thereafter, mostly very slightly curved towards costal margin apically; Cu_{1a} strongly arched around the basal 1/3–1/4 and nearly straight thereafter. Meracanthus moderate in size, subacute and slightly curved downwards apically; metatibia with prominent genual spine, with five apical sclerotised spurs arranged in 1+3+1; basal segment of metatarsus with a pair of sclerotised lateral spurs.

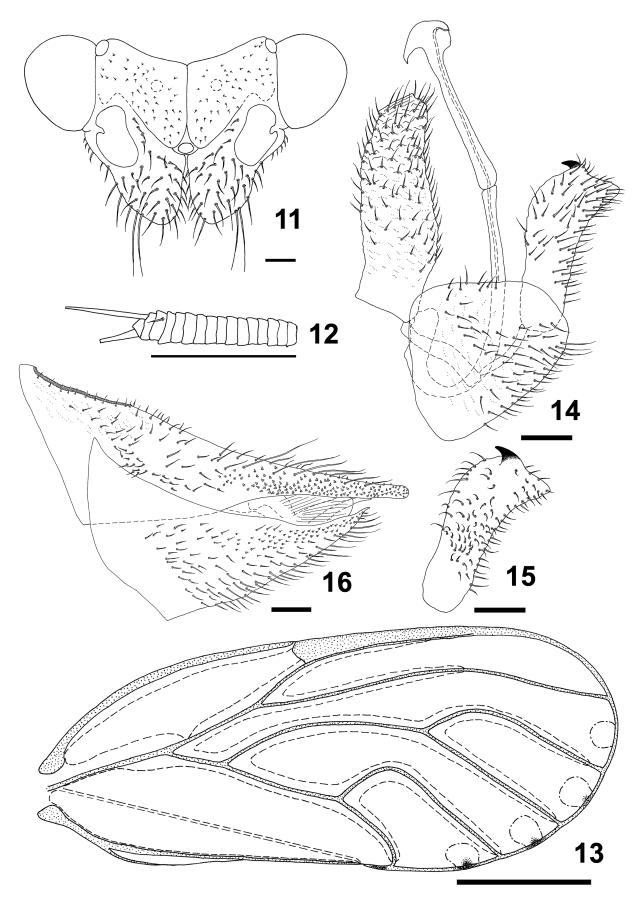
Male terminalia (Fig. 14) moderate in size. Proctiger slender, slightly curved caudad apically. Paramere stout, 0.8 times as long as proctiger, narrowest at base, gradually becoming wider and curved caudad towards the tip, truncated apically; anterior margin roundly projected cephalad around the apical 1/3; posteroapical corner strongly projected caudad; inner surface (Fig. 15) with many retrorse setae; inner apical tooth prominent, acute and projected cephalad. Distal aedeagal segment slightly longer than paramere; apex round and thickened, strongly hooked.

Female terminalia (Fig. 16) rather slender. Proctiger almost looks straight and very slightly curved at dorsal margin, not upturned apically, round at apex (Fig. 33). Subgenital plate with rather dense setae, acute at apex.

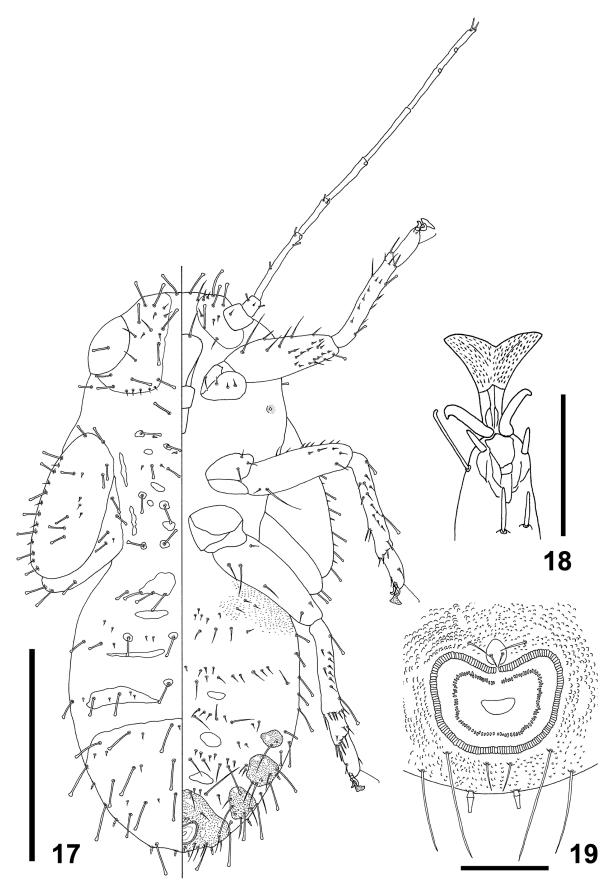
Fifth instar immature. Body (Figs 17, 44) light green, gently swollen dorsally. Antenna slender, 2.1–2.6 times as long as forewing pad, seven-segmented, with one apical rhinarium each on segments III and V, two on the middle of segment VII, with rather short capitate setae on the middle of segment III and apices of segments III–V (Fig. 27). Forewing pad oblong oval; outer margin with 8–9 long capitate setae and short simple setae in between (Fig. 30). Hindwing pad with two long capitate setae apically. Legs long, hairy, with many short simple setae and long capitate setae; tarsal arolia petiolate and fan-shaped (Fig. 18). Abdomen rounded apically, with many long capitate setae dorsally and many long simple setae ventrally. Caudal plate with many long capitate setae on dorsum and margin, with 4+4 truncated sectasetae on posterior margin. Anus located on ventral side. Outer circumanal pore ring (Fig. 19) relatively small, heart-shaped, strongly curved in front, comprising a single row of elongated pores; caudal margin of outer circumanal pore ring away from abdominal margin. Inner circumanal pore ring comprising a single row of small elongated pores.

Measurements (in mm): Adult (n = 5 males, 5 females): BL: 3.62–3.92; WL: 3.02–3.29; WW: 1.19–1.37; AL: 2.18–2.68; HW: 0.96–1.05; VW: 0.56–0.61; VL: 0.26–0.28; GL: 0.22–0.27; MP: 0.40–0.47; PL: 0.34–0.38; FP: 0.85–0.99. Fifth instar immature (n = 5): BL: 2.50–2.83; BW: 1.28–1.50; AL: 1.78–1.85; WPL: 0.70–0.85; CRW: 0.08–0.09.

Material examined. HOLOTYPE: male (dry-mounted; NARO), Japan, Honshû, Hiroshima-ken, Hatsukaichishi, Yoshiwa, Nakatsuya, 34.492° N, 132.083° E, 750 m, 3.vi.2022, on Cladrastis shikokiana, H. Inoue. PARATYPES: Honshû: 2 males, 1 female, Gunma-ken, Tone-gun, Kawaba-mura, Kawaba-yubara, Akakura-keikoku, 6.v.2004, N. Takahashi (dry-mounted; HIC); 7 males, 7 females, Nagano-ken, Matsumoto-shi, Azumi, Shimashimadani, 4.vi.2009, N. Takahashi (dry-mounted; HIC); 9 males, 7 females, Nagano-ken, Matsumoto-shi, Azuminagawado, 36.145° N, 137.744° E, 934 m, 26.vi.2021, on C. shikokiana, T. Matsuda (dry- and slide-mounted; HIC); 1 male, 6 females, 1 immature, Nagano-ken, Shimominochi-gun, Sakae-mura, Shiratori, 36.978° N, 138.499° E, 287 m, 13.vi.2021, on Platyosprion platycarpum, T. Matsuda (dry- and slide-mounted; HIC); 1 male, Hiroshima-ken, Shôbara-shi, Takano-chô, Kenashi-yama, 950 m, 7.viii.2004, at light trap, T. Yamauchi (dry-mounted; HIC); 1 male, 1 female, Hiroshima-ken, Fukuyama-shi, Ryûzu-kyô, 13.v.2009, N. Takahashi (dry-mounted; HIC); 77 males, 87 females, 97 immatures, same data as holotype (dry- and slide-mounted, 99.5% and 70% ethanol; HIC, OMNH); 10 males, 11 females, same locality, 22.vii.2000, light trap, T. Yamauchi (dry- and slide-mounted; HIC); Kyûshû: 1 female, Fukuoka-ken, Miyako-gun, Miyako-machi, Saigawahobashira, near Notôge, 33.502° N, 130.970° E, 750–930 m, 18.xi.2000, H. Inoue, on Tsuga sieboldii (dry-mounted; HIC); 13 males, 12 females, same locality, 1.vi.2001, H. Inoue, on Aria japonica (dry- and slide-mounted; HIC); 5 males, 3 females, same data, 3.vi.2001 (dry-mounted; HIC); 2 males, 3 females, same data, 12.vi.2001 (dry- and slide-mounted, 99.5% ethanol; HIC); 1 male, same data, 21.vi.2001 (dry-mounted; HIC); 1 female, same locality, 7.iv.2012, N. Takahashi (dry-mounted;



FIGURES 11–16. Cyamophila burckhardti sp. nov., adult. 11. Head, dorsal view. 12. Terminal flagellomere. 13. Right forewing. 14. Male terminalia, lateral view. 15. Paramere, inner face. 16. Female terminalia, lateral view. Scale bars: 0.1 mm for 11–12, 14–16; 0.5 mm for 13.



FIGURES 17–19. *Cyamophila burckhardti* **sp. nov.**, fifth instar. **17.** Habitus, dorsal view (left half), ventral view (right half). **18.** Apex of tarsus. **19.** Anal pore field and apex of abdomen, ventral view. Scale bars: 0.1 mm for **18–19**; 1 mm for **17**.

HIC); 3 females, same data, 7.v.2012 (dry-mounted; HIC); 1 female, same data, 4.vi.2012 (dry-mounted; HIC); 45 males, 47 females, same locality, 9.vi.2014, H. Inoue (dry- and slide-mounted, 99.5% ethanol; HIC); 1 female, same data, 20.v.2015 (dry-mounted; HIC); 80 males, 77 females, 4 immatures, Kumamoto-ken, Kamimashiki-gun, Yabe-machi, Naidaijin-rindô, 7.vi.2001, H. Inoue (dry- and slide-mounted, 99.5% ethanol; HIC); 4 males, 5 females, Miyazaki-ken, Higashiusuki-gun, Shiiba-son, Shiiya-tôge, 7.vi.2001, H. Inoue (dry-mounted; HIC).

Distribution. Japan (Honshû, Kyûshû).

Host plant. Cladrastis shikokiana (Makino) Makino; Platyosprion platycarpum (Maxim.) Maxim. (= Cladrastis platycarpa (Maxim.) Makino) (Fabales: Fabaceae). These host plants were confirmed by the presence of immatures.

Etymology. This species is dedicated to and named after our most famous psyllidologist, Daniel Burckhardt, who is still very active at over 70 years of age.

Biology. Univoltine. The adults emerge in early June and leave the host plant within a short period. In summer and autumn, the adults are often observed on non-host plants, such as *Aria japonica* Decne. (Rosales: Rosaceae) and *Tsuga sieboldii* Carrière (Pinales: Pinaceae), which grow on the ridges of mountainous areas. Presumably, adults overwinter on evergreen shelter plants, such as *T. sieboldii*.

Comments. The diagnoses for the identification of Japanese *Cyamophila* species are presented in Table 2. The population corresponding to the new species was first collected using a light trap at the type locality (Fig. 47), as reported by Inoue & Yamauchi (2001). The material was provisionally recorded as 'C. hexastigma', but the absence of prominent spots on the forewing posterior margin required reconsideration for identification. This species was therefore subsequently reported by several authors as 'Cyamophila' sp.' (see synonymic list).

Whereas *C. shikokiana* is endemic to Japan, *P. platycarpum* is found also in China (eFloras 2008). Therefore, *C. burckhardti* may also occur in China; however, psyllids corresponding to or similar to this species have not yet been recorded.

Cyamophila willieti (Wu, 1932)

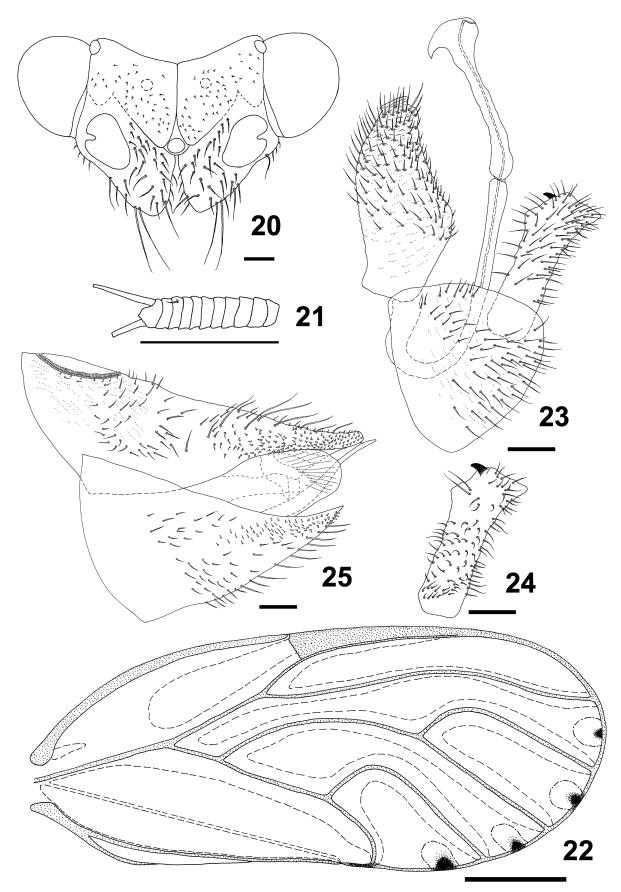
(Japanese name: Yatsuboshi-kijirami) (Figs 20–25, 28, 31, 34, 39–40, 45–46)

Psylla willieti Wu, 1932: 71. Type locality: China (Beijing). Cyamophila willieti; Conci & Tamanini (1988: 171).

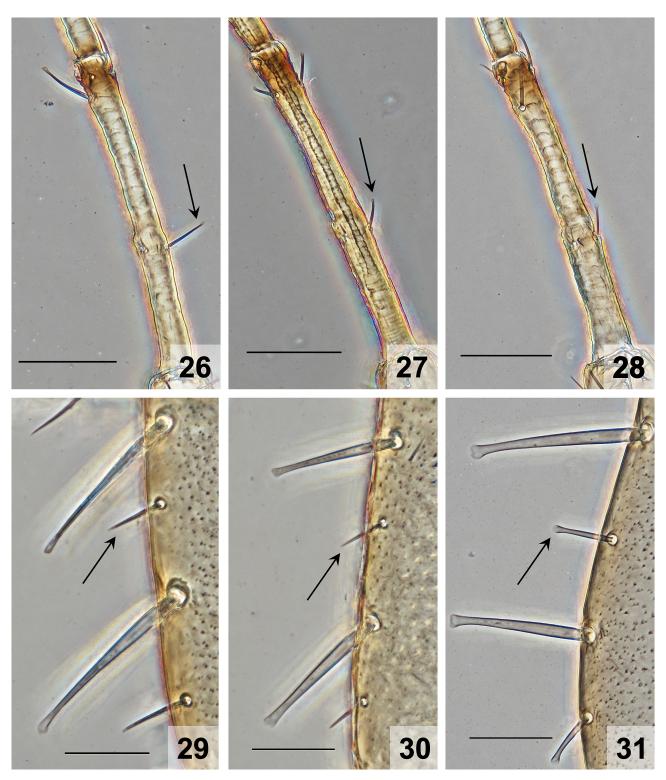
Description. Adult. Colouration. General colour (Figs 39, 45) light yellowish green with obscure light yellowish brown markings on thorax; in overwintered individuals (Fig. 40), body colour chestnut brown to dark brown in general, with many cream-yellow coloured fine markings and stripes on head and thorax. Antenna yellow; apices of segments IV and V, apical half of segment VI and most of segment VII dark brown; segments VIII—X entirely dark brown to black. Forewing membrane transparent, colourless, with prominent dark brown spots at the location of the radular spinules in cells r_2 , m_1 , m_2 , and cu_1 (Fig. 22); veins yellow in summer adults, becoming uniformly dark brown in overwintered adults. Apical tooth of paramere black.

Structure. Head (Fig. 20) strongly inclined downwards, 85–90° from longitudinal body axis in profile, slightly wider than thorax. Vertex shorter than the half width. Genal processes conical, about 0.8 times as long as vertex, slightly divergent, subacute or slightly rounded apically. Antenna long, 2.1–2.2 times as long as head width; longer terminal seta of segment X 1.8–2.2 times as long as shorter seta, about half as long as segment X (Fig. 21).

Forewing (Fig. 22) oblong oval, about 2.2–2.3 times as long as wide, widest at around 2/3 from the base; membrane with dense surface spinules in all cells; spinule-free bands along veins broad; fields of radular spinules as in Fig. 22; pterostigma broad, more than 1/3 of the forewing length; Rs rather strongly sinuate, slightly curved towards costal margin apically; M₁₊₂ strongly arched at around the basal 1/3 and slightly or rather strongly curved towards costal margin apically; Cu_{1a} strongly arched at approximately basal 1/4 and nearly straight thereafter. Meracanthus moderate in size, subacute and slightly curved downwards apically; metatibia with prominent genual spine, with five apical sclerotised spurs arranged in 1+3+1; basal segment of metatarsus with a pair of sclerotised lateral spurs.



FIGURES 20–25. *Cyamophila willieti*, adult. 20. Head, dorsal view. 21. Terminal flagellomere; 22. Right forewing. 23. Male terminalia, lateral view. 24. Paramere, inner face. 25 Female terminalia, lateral view. Scale bars: 0.1 mm for 20–21, 23–25; 0.5 mm for 22.

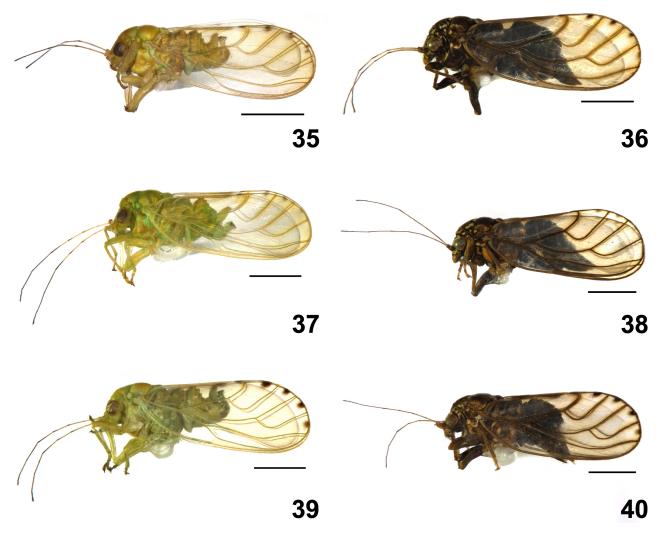


FIGURES 26–31. Comparison between *Cyamophila* spp., fifth instar immatures. 26–28. Capitate seta (arrow) on the middle of antennal segment III. 29–31. Shorter seta (arrow) on the left forewing pad margin. 26, 29. *Cyamophila hexastigma*. 27, 30. *Cyamophila burckhardti* sp. nov.; 28, 31. *Cyamophila. willieti*. Scale bar: 0.05 mm.

Male terminalia (Fig. 23) moderate in size. Proctiger slender, slightly curved caudad apically. Paramere slender, 0.8 times as long as proctiger, almost constant in width throughout, truncated apically; anterior margin angularly projected cephalad around the apical 1/4; posteroapical corner weakly projected caudad; inner surface (Fig. 24) with many retrorse setae; inner apical tooth rather prominent, acute and projected cephalad. Distal aedeagal segment slightly longer than paramere; apex round and thickened, strongly hooked.



FIGURES 32–34. Apices of the female proctigers of *Cyamophila* spp. **32.** *Cyamophila. hexastigma*. **33.** *Cyamophila burckhardti* sp. nov. **34.** *Cyamophila willieti*. Scale bars: 0.05 mm.



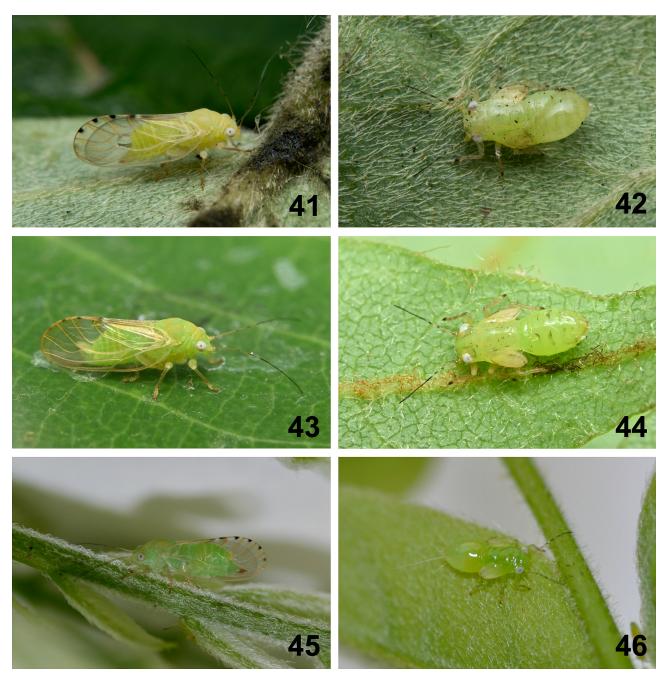
FIGURES 35–40. Dry-mounted specimens of *Cyamophila* spp. from Japan. 35–36. *Cyamophila hexastigma*. 37–38. *Cyamophila burckhardti* sp. nov. 39–40. *Cyamophila willieti*. 35, 37, 39. Male (in summer). 36, 38, 40. Female (overwintered). Scale bars: 1 mm.

Female terminalia (Fig. 25) stout. Proctiger stout, gently curved and slightly sinuate at dorsal margin, very slightly upturned apically, round at apex (Fig. 34). Subgenital plate with sparse setae, acute at apex.

Fifth instar immature. Body (Fig. 46) light green, gently swollen dorsally. Antenna slender, 2.0–2.2 times as long as forewing pad, seven-segmented, with one apical rhinarium on each of segments III and V, two on the middle of segment VII, with rather short capitate setae on the middle of segment III and apices of segments III–V (Fig.

28). Forewing pad oblong oval; outer margin with 8–9 long capitate setae and short capitate setae in between (Fig. 31). Hindwing pad with two long capitate setae apically. Legs long, hairy, with many short simple setae and long capitate setae. Abdomen rounded apically, with many long capitate setae dorsally and many long simple setae ventrally. Caudal plate with many long capitate setae on dorsum and margin, with 4+4 truncated sectasetae on posterior margin. Anus located on ventral side. Outer circumanal pore ring relatively small, heart-shaped, strongly curved in front, comprising a single row of elongated pores. Inner circumanal pore ring comprising a single row of small elongated pores.

Measurements (in mm): Adult (n = 5 males, 5 females): BL: 3.62–4.16; WL: 2.99–3.40; WW: 1.24–1.44; AL: 2.10–2.90; HW: 0.97–1.06; VW: 0.58–0.63; VL: 0.27–0.28; GL: 0.19–0.23; MP: 0.43–0.45; PL: 0.33–0.36; FP: 0.83–0.93. Fifth instar immature (n = 5): BL: 2.05–2.90; BW: 1.30–1.60; AL: 1.43–1.63; WPL: 0.73–0.83; CRW: 0.10–0.11.



FIGURES 41–46. Live *Cyamophila* spp. from Japan. 41–42. *Cyamophila hexastigma*. 43–44. *Cyamophila burckhardti* sp. nov. 45–46. *Cyamophila willieti*. 41, 43, 45. Adult. 42, 44, 46. Fifth instar immature.



FIGURE 47. Habitat (type locality) of *C. burckhardti* **sp. nov.**, Japan, Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya, 34.492°N, 132.083°E, altitude of 750 m.

Material examined. Honshû: 3 males, 5 females, Ibaraki-ken, Tsukuba-shi, Fujimoto, 36.051° N, 140.100° E, 20 m, 30.v.2003, on *Styphnolobium japonicum*, H. Inoue (slide-mounted, 99.5% ethanol; HIC); 25 males, 36 females, same data, 2.vi.2003 (dry-mounted; HIC, OMNH); 2 females, same data, 3.vi.2003 (dry-mounted; HIC); 4 males, 9 females, same data, 15.vi.2003 (dry-mounted; HIC); 3 males, 9 females, 10 immatures, same data, 15.iv.2004 (dry- and slide-mounted; HIC).

Distribution. Japan (Honshû; new distributional record); China, Korea (Wu 1932, *as P. willieti*; Kwon 1983, as *C. hexastigma*)

Host plant. *Styphnolobium japonicum* (L.) Schott (= *Sophora japonica* L.) (Fabales: Fabaceae) (Conci & Tamanini 1988). The host plant was confirmed by the presence of immatures.

Biology. Probably univoltine. In April, overwintered adults gather on host plants to mate and lay eggs on their shoots. The new adults emerge in June. Adults are thought to leave the host plant after the emergence and hibernate on evergreen shelter plants; however, the details of where they stay throughout the winter are unclear.

Comments. This species is newly recorded from Japan. The host plant, the Japanese pagoda tree, *S. japonicum* is native to China and was introduced into Japan, where it has been planted as a garden and roadside tree (Ohashi 2016). Therefore, *C. willieti* may be a pest of *S. japonicum* in Japan. No other species of *Styphnolobium* are native to Japan, *C. willieti* is also likely an introduced species.

Results of molecular analysis

A total of 47 COI sequences (714 bp) were obtained from three Japanese *Cyamophila* species: 16, 25, and 6 from *C. hexastigma*, *C. burckhardti*, and *C. willieti*, respectively (Table 1). Consequently, a neighbour-joining tree (Fig. 48) was constructed. All three *Cyamophila* species were supported with bootstrap values of 100% each. Notably, among the three species, *C. burckhardti* and *C. willieti* are sister taxa, which was supported by a high bootstrap value (99%). These two species share an important morphological feature of the paramere, which is not prominently extended immediately below the sclerotized apical tooth, although they differ in the distinctness of the posterior marginal spots of the forewings. The genetic distances within and between the three *Cyamophila* species are presented in Table 3. Similar to many other insect groups, an uncorrected *p*-distance of 3% is considered to be the threshold for separating species for psyllids (Martoni *et al.* 2018; Wonglersak *et al.* 2017). Hence, the three *Cyamophila* species

TABLE 1. Samples of Cyamophila from Japan used in the molecular analysis.

Species	Voucher	Sex/stage	Locality	Latitude	Longitude	Altitude	Collection	Host plant	*Accession
	ID			(°N)	(°E)	(m)	date		No.
Cyamophila hexastigma	JPsy0095	Male	Hokkaidô, Sapporo-shi, Jôzankei	42.974	141.166	300	9.vii.2001	Maackia amurensis	LC784110
Cyamophila hexastigma	JPsy0096	Male	Hokkaidô, Sapporo-shi, Jôzankei Dam	42.983	141.157	300	19.vii.2012	Maackia amurensis	LC784111
Cyamophila hexastigma	JPsy0097	Male	Hokkaidô, Sapporo-shi, Jôzankei Dam	42.983	141.157	300	19.vii.2012	Maackia amurensis	LC784112
Cyamophila hexastigma	JPsy0098	Female	Hokkaidô, Sapporo-shi, Jôzankei Dam	42.983	141.157	300	19.vii.2012	Maackia amurensis	LC784113
Cyamophila hexastigma	JPsy0099	Male	Hokkaidô, Sapporo-shi, Jôzankei Dam	42.983	141.157	300	12.vi.2013	Maackia amurensis	LC784114
Cyamophila hexastigma	JPsy1859	Male	Honshû, Aomori-ken, Shingô-mura, Herai, Sawaguchi	40.454	141.149	160	2.vii.2015	Maackia amurensis	LC784115
Cyamophila hexastigma	JPsy1860	Female	Honshû, Aomori-ken, Shingô-mura, Herai, Sawaguchi	40.454	141.149	160	2.vii.2015	Maackia amurensis	LC784116
Cyamophila hexastigma	JPsy1862	Female	Honshû, Aomori-ken, Shingô-mura, Herai, Sawaguchi	40.454	141.149	160	2.vii.2015	Maackia amurensis	LC784117
Cyamophila hexastigma	JPsy0980	Female	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784118
Cyamophila hexastigma	JPsy0981	Female	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784119
Cyamophila hexastigma	JPsy0107	Male	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784120
Cyamophila hexastigma	JPsy0108	Male	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784121
Cyamophila hexastigma	JPsy0109	Male	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784122
Cyamophila hexastigma	JPsy0110	Female	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784123
Cyamophila hexastigma	JPsy0111	Female	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784124
Cyamophila hexastigma	JPsy0112	Male	Kyûshû, Nagasaki-ken, Unzen-shi, Ikenohara	32.745	130.270	750	4.vi.2010	Maackia floribunda	LC784125
Cyamophila burckhardti sp. nov.	JPsy2069	Female	Honshû, Nagano-ken, Sakae-mura, Shiratori	36.978	138.499	287	13.vi.2021	Platyosprion platycarpum	LC784126

TABLE 1. (Continued)									
Species	Voucher	Sex/stage	Locality	Latitude	Longitude	Altitude	Collection	Host plant	*Accession
	an l			(² N)	(^E)	(m)	date		No.
Cyamophila burckhardti sp. nov.	JPsy2070	Female	Honshû, Nagano-ken, Sakae-mura, Shiratori	36.978	138.499	287	13.vi.2021	Platyosprion platycarpum	LC784127
Cyamophila burckhardti sp. nov.	JPsy2071	Immature	Honshû, Nagano-ken, Sakae-mura, Shiratori	36.978	138.499	287	13.vi.2021	Platyosprion platycarpum	LC784128
Cyamophila burckhardti sp. nov.	JPsy2072	Male	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784129
Cyamophila burckhardti sp. nov.	JPsy2073	Male	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784130
Cyamophila burckhardti sp. nov.	JPsy2074	Male	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784131
Cyamophila burckhardti sp. nov.	JPsy2075	Female	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784132
Cyamophila burckhardti sp. nov.	JPsy2076	Female	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784133
Cyamophila burckhardti sp. nov.	JPsy2077	Female	Honshû, Nagano-ken, Matsumoto-shi, Azuminagawado	36.145	137.744	934	26.vi.2021	Cladrastis shikokiana	LC784134
Cyamophila burckhardti sp. nov.	JPsy2078	Male	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784135
Cyamophila burckhardti sp. nov.	JPsy2079	Male	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784136
Cyamophila burckhardti sp. nov.	JPsy2080	Male	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784137
Cyamophila burckhardti sp. nov.	JPsy2081	Female	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784138
Cyamophila burckhardti sp. nov.	JPsy2082	Female	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784139
Cyamophila burckhardti sp. nov.	JPsy2083	Female	Honshû, Hiroshima-ken, Hatsukaichi-shi, Yoshiwa, Nakatsuya	34.492	132.083	750	3.vi.2022	Cladrastis shikokiana	LC784140
Cyamophila burckhardti sp. nov.	JPsy0982	Male	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784141

TABLE 1. (Continued)									
Species	Voucher	Sex/stage	Locality	Latitude	Longitude	Altitude	Collection	Host plant	*Accession
	ID			(°N)	(°E)	(m)	date		No.
Cyamophila burckhardti	JPsy0983	Male	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784142
Cyamophila burckhardti	JPsy0985	Female	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784143
Cyamophila burckhardti	JPsy0986	Female	Kyûshû, Fukuoka-ken, Miyako-machni, Notôge	33.502	130.970	750–930	9.vi.2014	Not determined	LC784144
Cyamophila burckhardti	JPsy0113	Male	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaiiin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784145
Cyamophila burckhardti sp. nov.	JPsy0114	Male	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaijin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784146
Cyamophila burckhardti sp. nov.	JPsy0115	Male	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaijin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784147
Cyamophila burckhardti sp. nov.	JPsy0116	Female	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaijin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784148
Cyamophila burckhardti sp. nov.	JPsy0117	Female	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaijin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784149
Cyamophila burckhardti sp. nov.	JPsy0118	Female	Kyûshû, Kumamoto-ken, Yabe-machi, Naidaijin-rindô	32.569	131.029	1000	7.vi.2001	Not determined	LC784150
Cyamophila willieti	JPsy0101	Male	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784151
Cyamophila willieti	JPsy0102	Male	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784152
Cyamophila willieti	JPsy0103	Male	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784153
Cyamophila willieti	JPsy0104	Female	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784154
Cyamophila willieti	JPsy0105	Female	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784155
Cyamophila willieti	JPsy0106	Female	Honshû, Ibaraki-ken, Tsukuba-shi, Fujimoto	36.051	140.100	20	30.v.2003	Styphnolobium japonicum	LC784156
* DDBJ/EMBL/GenBank accession numbers	ccession nun	nbers							

* DDBJ/EMBL/GenBank accession numbers

were considered to be sufficiently differentiated (> 8% interspecific divergences; Table 3). In the case of two East Asian species of *Anomoneura* (Psyllidae: Psyllinae), the intraspecific genetic divergence of the mitochondrial COI-tRNA^{leu}-COII gene for *Anomoneura mori* from South Korea and Japan was 1.8% (*p*- and K2P distances), whereas the intraspecific distance between *A. mori* and Taiwanese *Anomoneura taiwanica* Cho & Liao was 9.0% (*p*-distance) or 9.7% (K2P distance) (Cho *et al.* 2020).

In *C. hexastigma*, the intraspecific genetic distance was only 0.18% (*p*- and K2P) between populations of Hokkaidô and Kyûshû, which are nearly 1500 km apart. Furthermore, the genetic distance between populations from different host plants (*M. amurensis* and *M. floribunda*) was similar at 0.19% (*p*- and K2P). Moreover, in *C. burckhardti*, the genetic distances were only 0.06% (*p*- and K2P) between populations from different host plants

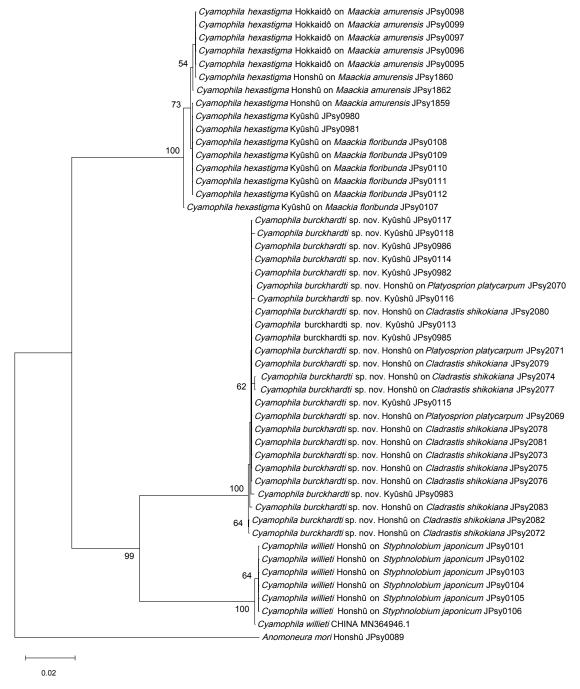


FIGURE 48. Neighbor-joining tree based on the 714 bp of the mitochondrial COI sequence data of three *Cyamophila* species. On each branch, the bootstrap support values of > 50% are shown. Each taxon is followed by the name of the Japanese main island where the specimen was obtained, host plant name (if determined), and voucher ID (GenBank accession number for *C. willieti* from China). *Anomoneura mori* was used as an outgroup. The scale bar indicates Kimura 2-parameter genetic distance of 0.02.

(*C. shikokiana* and *P. platycarpum*), and 0.09% (*p*- and K2P) between populations of central Honshu and southwest Japan (western Honshû and Kyûshû), which are 500–800 km apart. In *C. willieti*, the genetic distance was only 0.14% (*p*- and K2P) between the Chinese and Japanese populations, indicating no greater differentiation than that found within Japan. This suggests that *C. willieti* has not been isolated in Japan for a long time.

Among *Cyamophila* species, the upstream 5' end sequence of the mitochondrial COI gene as a standard barcoding region is currently unknown for species other than the three species studied here. To discuss the diversity and phylogeny of *Cyamophila* in detail, more genetic information is required for a large number of species of this Palaearctic genus, especially from China, where nearly half of the species have been known to occur.

TABLE 2. Diagnoses of the characters among *Cyamophila* spp. from Japan.

Character	C. hexastigma	C. burckhardti sp. nov.	C. willieti
Adult			
Forewing posterior	With 3 prominent dark	With 2–3 obscure pale brown	With 4 prominent dark
margin	brown spots	spots	brown spots
Antenna	Often longer than 2.4 times head width	Shorter than 2.4 times head width	Shorter than 2.4 times head width
Paramere	Narrowest at middle, strongly wider towards the tip	Narrowest at base, roundly projected cephalad around the apical 1/3	Constant in width throughout, angularly projected cephalad around the apical 1/3
Female proctiger	Rather stout, curved and sinuate dorsally, slightly upturned apically, rather truncated at apex	Slender, almost straight dorsally, not upturned apically, round at apex	Stout, gently curved dorsally, very slightly upturned apically, Round at apex
Immature			
Length of capitate seta on the middle of antennal segment III	Longer than the thickness of antennal segment III	As long as or shorter than the thickness of antennal segment III	As long as or shorter than the thickness of antennal segment III
Shorter setae on forewing pad margin	Simple hair-like	Simple hair-like	Capitate

TABLE 3. Mean genetic distances (%) within and between *Cyamophila* species (K2P pairwise; in parenthesis, uncorrected p-distance). N = number of specimens.

	G	N T	W/141.1	Between species	
	Species	IV	Within species	1	2
1	Cyamophila hexastigma	16	0.13 (0.13)		
2	Cyamophila burckhardti sp. nov.	25	0.09 (0.09)	12.29 (11.13)	
3	Cyamophila willieti	7	0.04 (0.04)	11.52 (10.52)	9.36 (8.67)

Cyamophila willieti included one individual from China (MN364946). All other samples were obtained from Japan (Table 1).

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