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Two new species of the genus *Mystilus* Distant (Hemiptera: Miridae: Mirinae) from Vietnam, with discussion on morphological variation based on molecular data, and a revised key for *Mystilus* species

Junggon Kim[1] & Sunghoon Jung[2,3*]

Abstract. Two new species, *Mystilus frederici* Kim et Jung, and *M. carvalhoi* Kim et Jung, are described from Vietnam. Morphological data including diagnoses and descriptions of male genitalia are presented. A revised key to the *Mystilus* species with corrections is also presented. The variation of key characters of *Mystilus* is discussed in correlation to the molecular data.

Key words. Miridae, Mecistoscelini, *Mystilus*, new species, variation, DNA Barcoding

INTRODUCTION

The family Miridae (Hemiptera: Heteroptera), commonly referred to as plant bugs, is the most diverse group in the Heteroptera suborder. Miridae has the widest geographical distribution in the suborder, inhabiting all continents except for Antarctica and other extreme geographic regions (Schuh, 1995; Menard et al., 2014; Kim & Jung, 2019). Studies on mirids in the Oriental Region are severely lacking. Early pioneers conducted some studies in the early 1900s. However, there continues to be undiscovered, and therefore undocumented, biodiversity in the region. Recently, investigations to discover new mirid taxa have been conducted by heteropterists (Yasunaga et al., 2016; Duwal et al., 2017; Roca-Cusachs et al., 2018; Kim et al., 2019a).

The tribe Mecistoscelini is one of the smallest groups in the plant bug family, with four genera and 11 described species, and was recently recovered as a monophyletic group based on phylogenetic analyses (Chérot, 2003; Kim & Jung, 2019). This group has a distribution ranging from the Oriental to Pacific Regions (Schuh, 2002–2013; Kim et al., 2018; Yeshwanth & Chérot, 2019), and all genera (*Erimiris*, *Mecistoscelis*, *Mystilus*, and *Mecistoceloides*) have been reported to have specific host relationships with bamboo plants (Miyamoto & Hasegawa, 1967; Kim et al., 2018; Yeshwanth & Chérot, 2019). Among the Mecistoscelini groups, the genus *Mystilus* is the largest. However, morphological and biological information concerning *Mystilus* is lacking, despite the comprehensive review of the genus by Lansbury (1963). Lansbury (1963) used the colouration of the pronotum to identify all members of the genus, except for one species. However, the colouration can be inconsistent, especially in reference to other genera of the subfamily Mirinae, which could lead to misidentification. In addition, in a recent study, *Mystilus* was briefly referenced, and the variation of one species was discussed (Yeshwanth & Chérot, 2019). However, it is necessary to evaluate the variation in colouration using additional data to validate its usefulness as a characteristic to identify taxa. From this, the taxonomic key for distinguishing between the species of this highly variable group can be revised to reflect this information.

Molecular techniques have been developed in various fields in biology, and molecular data is also useful in the taxonomy and systematics of insect groups. Among these techniques, DNA barcoding using the sequence of mitochondrial cytochrome oxidase subunit I (COI) is used as an identification tool in some insect groups, especially in tiny or morphologically cryptic groups (Jung & Lee, 2011; Kim et al., 2019b).

In this study, two new *Mystilus* species from Vietnam are described, and their morphological information, such as their description and identification, are presented with photographs and illustrations. A revised key for *Mystilus* based on updated morphological findings resulting from additional molecular information is included.

MATERIAL AND METHODS

Morphology. Examined specimens were photographed using Leica DMC 2900 camera in combination with Leica M165C

Accepted by: Ang Yuchen

[1]Gyebaek-ro, Jung-gu, Daejeon, Korea; Email: thesv12@gmail.com

[2]Laboratory of Systematic Entomology, Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Korea

[3]Department of Smart Agriculture Systems, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Korea; Tel.: +82-42-821-5767; Fax: +82-42-823-8679; Email: jung@cnu.ac.kr (*corresponding author)

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Table 1. Information of samples used in this study.

Species	Collecting site	GenBank Accession Number	References
Adelphocoris reichelii	Korea	KY367048	Kim & Jung (2018)
Adelphocoris suturalis	Korea	KY367050	Kim & Jung (2018)
Adelphocoris piceosetosus	Korea	KY367042	Kim & Jung (2018)
Adelphocoris lineolatus	Korea	KY367039	Kim & Jung (2018)
Adelphocoris lineolatus	Germany	KM022151	Kim & Jung (2018)
Capsodes flavomarginatus	Spain	MF669002	Kim & Jung (2019)
Capsodes gothicus	Korea	KY367097	Kim & Jung (2018)
Apolygus pulchellus	Korea	KY367072	Kim & Jung (2018)
Apolygus subhilaris	Korea	KY367083	Kim & Jung (2018)
Apolygus lucorum	Korea	KY367070	Kim & Jung (2018)
Erimiris tenuicornis	Korea	MF669007	Kim & Jung (2019)
Eurystylis coelestialium	Korea	KY367119	Kim & Jung (2018)
Mystilus frederici, new species	Vietnam	MF669016	Kim & Jung (2019)
Mystilus frederici, new species	Vietnam	MT084551	sequenced for this study
Mystilus frederici, new species	Vietnam	MT084552	sequenced for this study
Mystilus carvalhoi, new species	Vietnam	MF669015	Kim & Jung (2019)
Mystilus carvalhoi, new species	Vietnam	MT084549	sequenced for this study
Mystilus carvalhoi, new species	Vietnam	MT084550	sequenced for this study
Polymerias opacipennis	Korea	KY367162	Kim & Jung (2018)
Polymerias opacipennis	Korea	KY367163	Kim & Jung (2018)
Stenodema rubrinervis	Korea	KY367176	Kim & Jung (2018)
Stenodema rubrinervis	Korea	KY367177	Kim & Jung (2018)
Stenodema rubrinervis	Korea	KY367178	Kim & Jung (2018)
Stenodema sibirica	Korea	KY367179	Kim & Jung (2018)
Stenodema sibirica	Korea	KY367180	Kim & Jung (2018)

microscope. Measurements were made using software (LAS Interactive Measurements) on the same microscope. All measurements are given in millimetres (mm). For examination of male genitalia, the entire abdomen was detached from the body, and was heated in 10% KOH solution at 70°C for approximately 10 minutes until it became transparent. The abdomen was washed in distilled water and was dissected to examine male genital structures. Terminology mainly follows Kim & Jung (2019). All the type specimens are deposited in the Laboratory of Systematic Entomology, Chungnam National University (CNU), Daejeon, Korea.

Molecular protocols. In order to evaluate genetic distances of individuals and finally test the variation of morphological characters, and to incorporate constructed sequence data in a public database (NCBI), we re-generated a simply modified neighbor-joining tree (NJ) (Saitou & Nei, 1987) as the generally used tool, using cytochrome oxidase subunit I (COI; 511 bp) sequences. The taxa for the tree were chosen based on their close relation to the genus *Mystilus*, and the genera with some species sharing the same host plant, based on the phylogenetic result of the subfamily Mirinae (Kim & Jung, 2019). Some sequences were used from previous molecular studies by the same authors (Kim & Jung, 2018, 2019) (Table 1), whereas others were generated for this study (Table 1). The other individuals including each *Mystilus* species were also sequenced after the examination of external and genital morphology according to the same protocol (Kim & Jung, 2018) with the COI primer set LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2190 (TAA ACT TCA GGG TGA CAA AAA ATC A) (Folmer et al., 1994). *Eurystylus coelestialium* was used as the outgroup. All samples are deposited in CNU as the vouchers and sequences used here are deposited in NCBI.

TAXONOMY

Mystilus Distant, 1904

Mystilus Distant, 1904: 420.

Type species. *Mystilus priamus* Distant, 1904.

Diagnosis. This genus can be distinguished from other genera of Mecistoscelini by the following characters: body normally elongate, not narrow; head dull, vertex width distinctly longer than width of compound eye; calli region with vestiture; apex of labium surpassing hindcoxae; pronotum with bluish colouration; ostiolar peritreme dark brown; left paramere with short setae; right paramere two types, rod-shape with swollen apical part and with thin and small hypophysis (Fig. 3B) or expanded form, with large hypophysis (Fig. 3F); endosoma membranous with sclerite and without spicules.

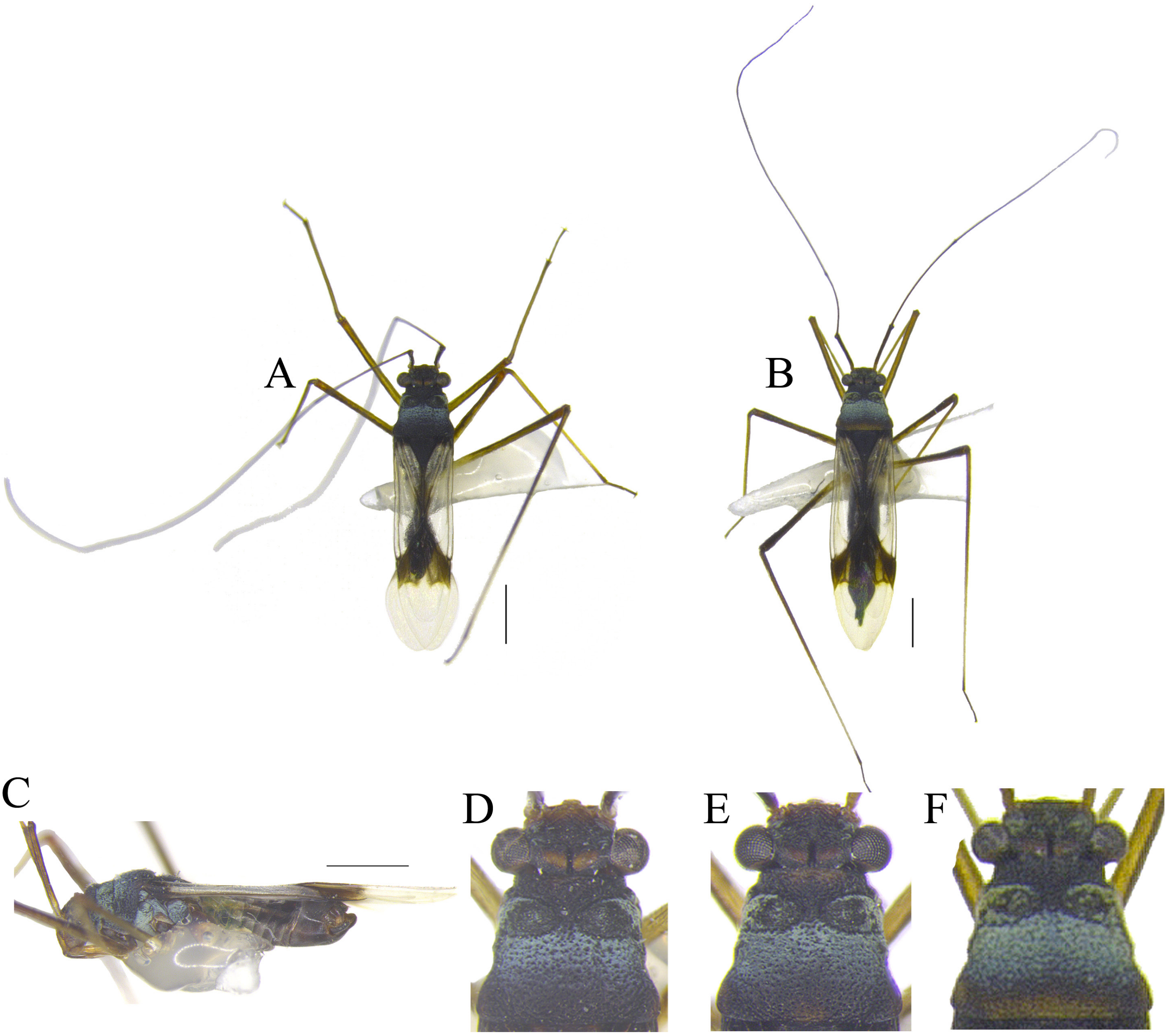

Fig. 1. Dorsal and lateral habitus with variation of *Mystilus carvalhoi*, new species. A, holotype, male; B, paratype, female; C, lateral view of male; D–F, variation of pronotum colouration. Scale bar = 1 mm.

Updated key to the species of *Mystilus*

1. Hemelytra opaque, not transparent, or slightly translucent depending on the area .... *M. alboviridescens*
– Hemelytra distinctly transparent. 2
2. Scutellum with a distinct longitudinal keel medially.............. ....*M. carinatus*
– Scutellum without a longitudinal keel...................................3
3. Cuneus not hyaline, entirely dark brown 4
– Cuneus hyaline or transparent, partly (outer margin only) dark brown 5
4. Body small, less than 4 mm; second antennal segment shorter than ½ third antennal segment..............................*M. priamus*
– Body not small, more than 4.5 mm; second antennal segment longer than ½ third antennal segment ....*M. carvalhoi*, new species
5. Right paramere rod-shape and swollen apically, with small hypophysis .... *M. antrami*
– Right paramere not rod-shape, expanded form with large hypophysis 6
6. Right paramere not angled, with blunt and thick process on sensory lobe ....*M. manipurensis*
– Right paramere angled vertically, without any process on sensory lob.......................................*M. frederici*, new species

Mystilus carvalhoi Kim et Jung, new species
(Figs. 1, 3A–D)

Material examined. Holotype male (CNU), VIETNAM: Cuc Phuong National Park, 20°19′07″N 105°36′18″E, Ninh Binh Prov., on *Gigantochloa* sp., 10 May 2017, J. Kim. Paratypes: 2 males and 1 female (CNU), VIETNAM: same data as for holotype.

Diagnosis. Recognised by the following combination of characters: frons dark brown; first antennal segment almost dark brown; margin of compound eye reaching margin of head laterally; collar almost dark brown; second antennal segment longer than ½ third segment; scutellum dark brown, slightly tumid laterally; cuneus dark brown, slightly transparent; hypophysis of left paramere blunt, upper margin of hypophysis convex; right paramere rod-shaped, slightly

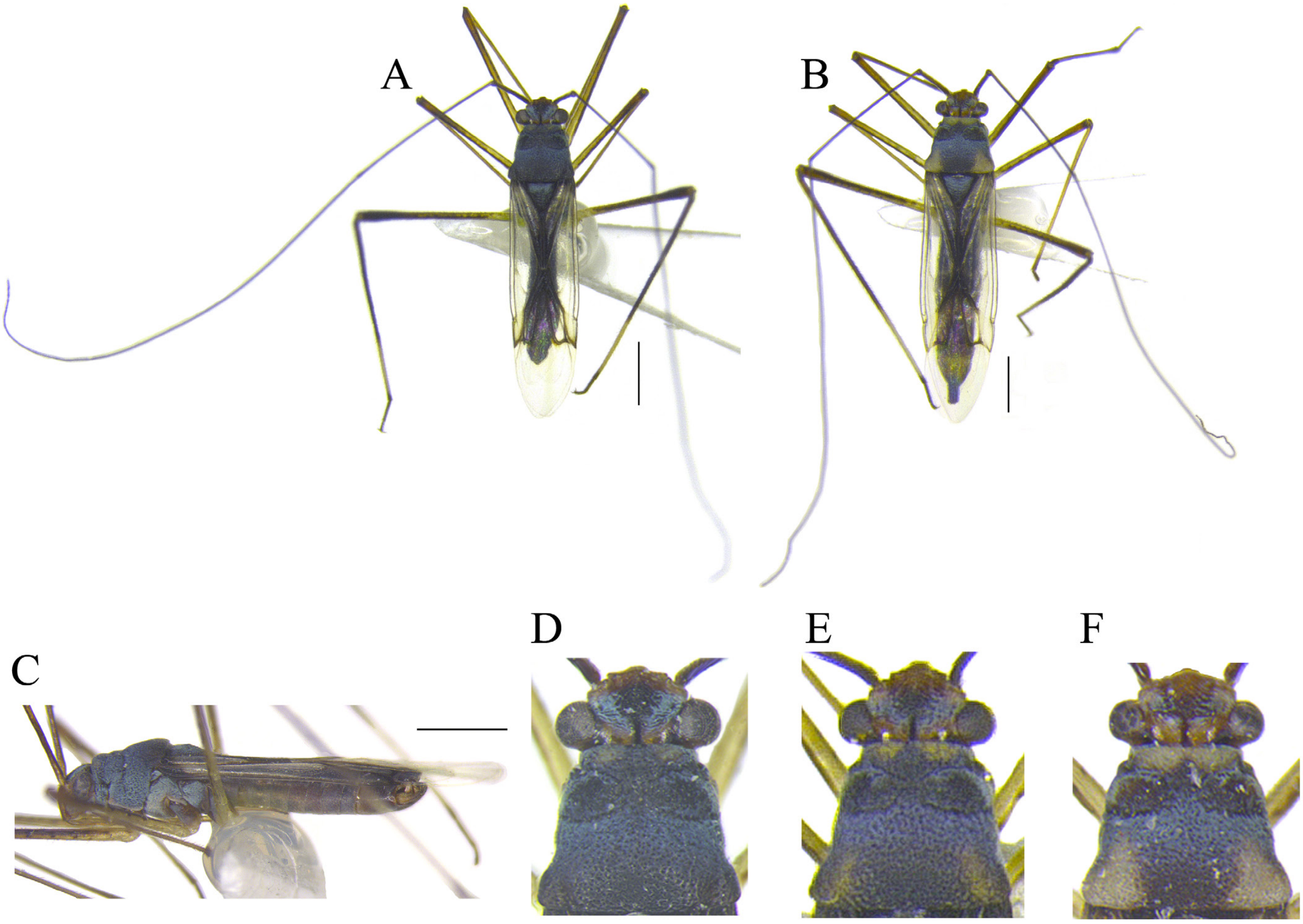

Fig. 2. Dorsal and lateral habitus with variation of *Mystilus frederici*, new species. A, holotype, male; B, paratype, female; C, lateral view of male; D–F, variation of pronotum colouration. Scale bar = 1 mm.

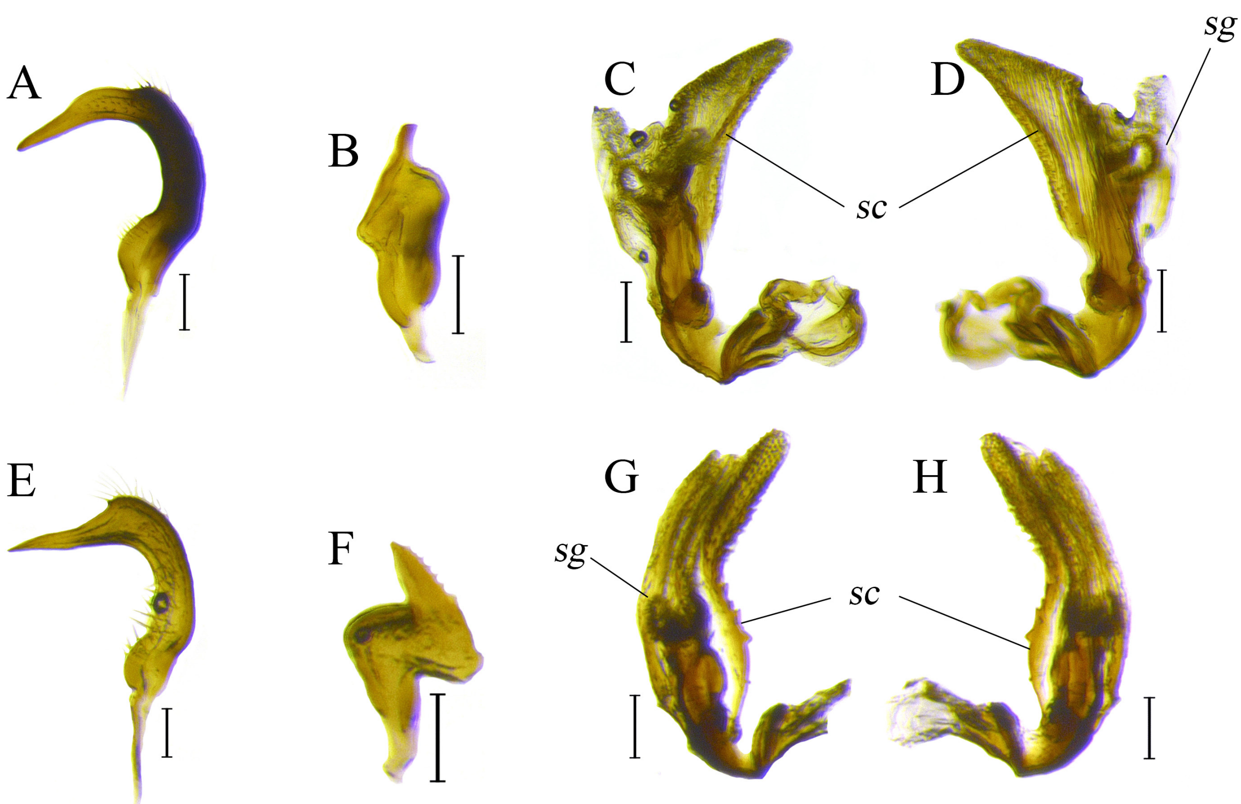

Fig. 3. Parameres and endosome of *Mystilus* spp. A–D, *M. carvalhoi*, new species; E–H, *M. frederici*, new species. A, E, left paramere; B, F, right paramere; C, D, G, H, endosoma; *sc*, sclerite; *sg*, secondary gonopore. Scale bar = 0.1 mm.

swollen apically, hypophysis short and narrow; endosomal sclerite with densely tiny tooth (Fig. 3C, D).

Description. Male. Body elongate, length 4.55–4.68. COLOURATION: generally bluish brown with hyaline hemelytra (Fig. 1A–C). **Head:** almost dark brown; vertex brown; frons dark brown; clypeus brown; antennae almost dark brown; first antennal segment generally dark brown except for pale base. **Thorax:** pronotum bluish brown, variable in range of bluish region (Fig. 1D–F); collar dark brown; sometimes posterior part of pronotum with variable yellowish marking; scutellum distinct dark brown; hemelytra almost hyaline, base of hemelytra dark brown; commissure and posterior margin of corium dark brown; cuneus dark brown, slightly transparent; vein dark brown; femur almost pale brown except for dark apical part, with dark medial line and dark markings, inner part of femur entirely pale brown; tibia almost dark brown except for pale subapical part; tarsus dark brown. **Abdomen:** dark brown. SURFACE AND VESTITURE: not glabrous, dull; head with transverse wrinkle; pronotum deeply punctate; hemelytra glabrous with weakly punctate. STRUCTURE: **Head:** distinctly prognathous; vertex width wider than compound eye; antennae much longer than length of body, linear except for first segment; first antennal segment thicker than second antennal segment, apex of first antennal segment swollen; third antennal segment longest; proportion of first to fourth antennal segments 1.1 : 2.8 : 5.3 : 2.3; labrum slightly shorter than first labial segment; labium exceeding hindcoxae. **Thorax:** pronotum trapezoid, longitudinal length longer than ½ basal maximal width; suture of collar obsolete; calli distinct swollen; scutellum slightly tumid laterally, not perfectly flat, anterior width shorter than ½ basal pronotal width, longitudinal length shorter than commissure; lateral margin of hemelytra almost straight; cuneus elongate; legs extremely long and slender; femur apically swollen. **Abdomen:** rounded, reaching to apex of cuneus. GENITALIA: left paramere with setae partly, hypophysis not sharp, blunt, upper margin of hypophysis convex (Fig. 3A); right paramere rod shaped, without setae, slightly swollen apically, hypophysis short and narrow (Fig. 3B); endosoma membranous with sclerites, sclerite oriented from ductus seminis, sclerite with densely tiny tooth (Fig. 3C, D).

Female. Body elongate, length 5.67. COLOURATION: as in male. SURFACE AND VESTITURE: as in male. STRUCTURE: as in male except for possessing genital tube at last abdominal segment.

Measurements (in mm). Male (n=3)/Female (n=1). Body length, clypeus–apex of membrane: 4.55–4.68/5.67; head length, excluding collar: 0.31–0.33/0.38; head width, including compound eyes: 0.83–0.85/0.92; vertex width: 0.45–0.46/0.47; 1st antennal segment length: 1.15–1.17/1.22; 2nd antennal segment length: 2.79–2.89/2.94; 3rd antennal segment length: 5.33–5.46/5.88; 4th antennal segment length: 2.31–2.40/2.99; total antennal length: 11.58–11.92/13.03; labial length: 2.14–2.16/2.38; anterior pronotal maximal width (straight): 0.75–0.76/0.79; mesal pronotal length: 0.81–0.83/0.87; posterior pronotal maximal width (straight): 1.05–1.10/1.22; anterior scutellar width: 0.51–0.53/0.69; mesal scutellar length: 0.43–0.49/0.65; commissure length: 0.82–0.91/1.10; outer embolial margin length (straight): 2.01–2.19/2.72; outer cuneal margin length (straight): 0.50–0.56/0.65; maximal width across hemelytron: 0.57–0.59/0.67.

Fig. 4. Host plant, *Gigantochloa* sp.

Etymology. Named after J.C.M. Carvalho, a taxonomist for Miridae who studied Mecistoscelini; a noun in genitive case.

Host. *Gigantochloa* sp. (Poaceae: Bambusoideae), the same plant as the host of *M. fredrici*, new species, above (Fig. 4).

Distribution. Vietnam (Northern).

Remarks. This species is similar in general appearance to the species *M. priamus*, but it can be distinguished by the larger body more than 4.5 mm and the length of second antennal segment longer than ½ length of third antennal segment. According to the previous studies on *M. priamus* (Distant, 1904; Lansbury, 1963), *M. priamus* is not more than 4 mm, making it the smallest species in this genus.

Mystilus frederici Kim et Jung, new species
(Figs. 2, 3E–H)

Material examined. Holotype male (CNU), VIETNAM: Cuc Phuong National Park, 20°19′07″N 105°36′18″E, Ninh Binh Prov., on *Gigantochloa* sp., 10 May 2017, J. Kim. Paratypes: 2 males and 2 females (CNU), VIETNAM: same data as for holotype.

Diagnosis. Recognised by the following combination of characters: frons bluish and dark brown medially; first antennal segment pale brown with dark lateral longitudinal stripe; second antennal segment as long as ½ third segment; margin of compound eye reaching margin of head laterally; scutellum bluish brown somewhat tumid laterally; cuneus hyaline, sometimes slightly tinged with greyish; left paramere covered with dense setae mostly, hypophysis long and sharp, upper margin of hypophysis concave; right paramere angled vertically, with serrate finger-like and thick hypophysis, sensory lobe without any process; endosomal sclerite with sparsely large tooth (Fig. 3E–H).

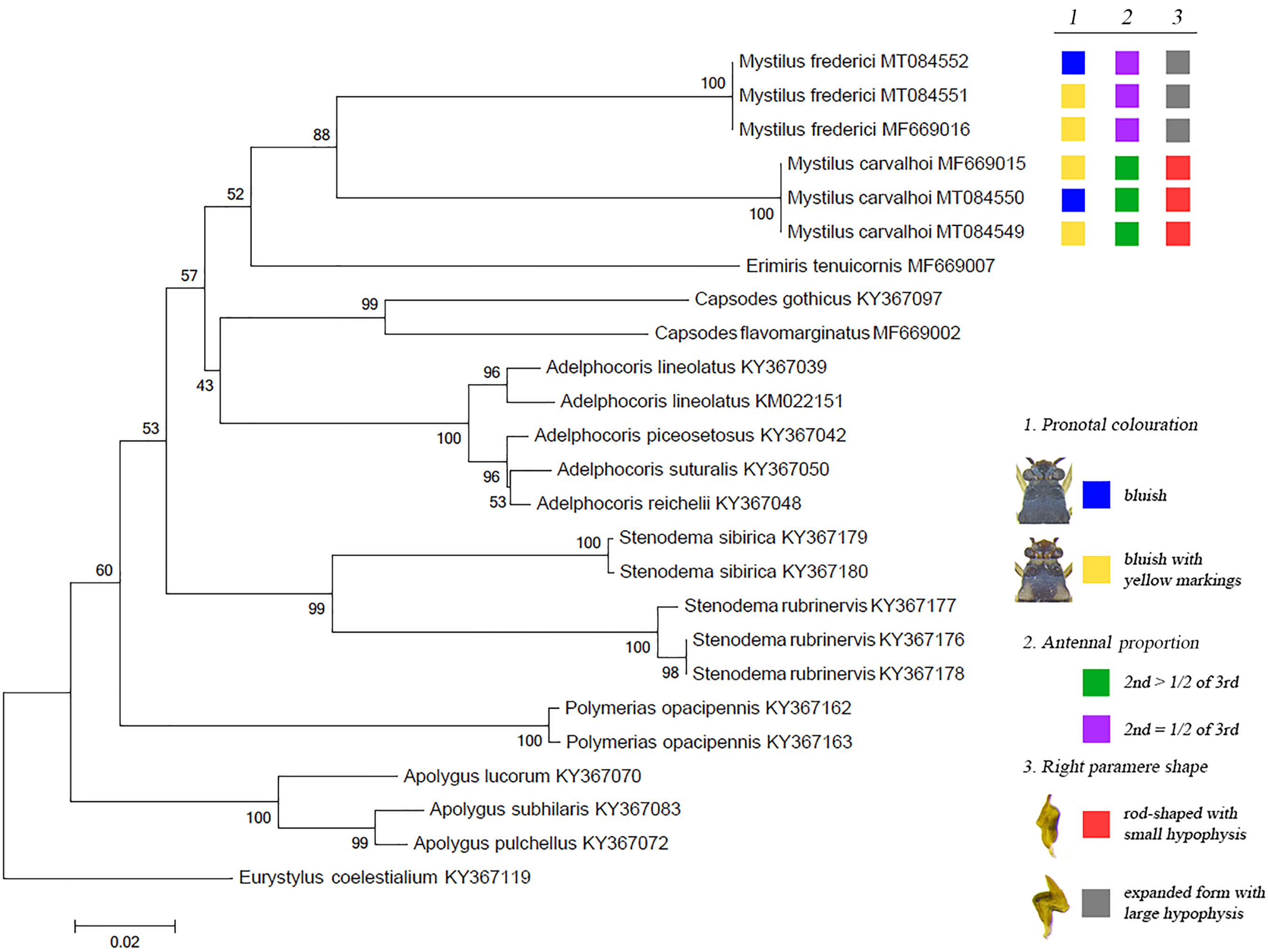

Fig. 5. Modified Neighbor-Joining tree based on a total of 25 COI sequences of six *Mystilus* species and other 19 species in the subfamily Mirinae in this study. Coloured squares next to the *Mystilus* species names indicate character states for three characters.

Description. Male. Body elongate, length 5.25–5.33. COLOURATION: generally bluish brown with hyaline hemelytra (Fig. 2A–C). **Head:** almost brown partly tinged with bluish brown; vertex brown; frons bluish brown; clypeus brown; antennae almost dark brown; first antennal segment generally pale brown. **Thorax:** pronotum almost bluish brown; sometimes collar with variable yellowish marking; posterior part of pronotum dark brown, with variable yellowish marking (Fig. 2D–F); scutellum distinct bluish brown; hemelytra almost hyaline, base of hemelytra dark brown; commissure and posterior margin of corium dark brown; cuneus hyaline, sometimes tinged with grayish; vein dark brown; femur almost pale brown except for dark apical part, with dark medial line and dark markings, inner part of femur entirely pale brown; tibia almost dark brown except for pale subapical part; tarsus dark brown. **Abdomen:** dark brown. SURFACE AND VESTITURE: not glabrous, dull; head with transverse wrinkle; pronotum deeply punctate; hemelytra glabrous and weakly punctate. STRUCTURE: **Head:** distinctly prognathous; vertex width wider than compound eye; antennae much longer than length of body, linear except for first segment; first antennal segment thicker than second antennal segment, apex of first antennal segment swollen; third antennal segment longest; proportion of first to fourth antennal segments 1.1 : 2.9 : 5.8 : 2.8; labrum slightly shorter than first labial segment; labium exceeding hindcoxae. **Thorax:** pronotum trapezoid, longitudinal length longer than ½ basal maximal width; suture of collar obsolete; calli distinct swollen; scutellum somewhat tumid laterally, anterior width shorter than ½ basal pronotal width, longitudinal length shorter than commissure; lateral margin of hemelytra almost straight; cuneus elongate; legs extremely long and slender; femur apically swollen. **Abdomen:** rounded, reaching to apex of cuneus. GENITALIA: left paramere with dense setae mostly, hypophysis long and sharp, narrow in frontal view, upper margin of hypophysis concave (Fig. 3E); right paramere expanded and angled vertically, without setae, hypophysis serrate finger-like and thick (Fig. 3F); endosoma membranous with sclerites, sclerite oriented from ductus seminis, sclerite with some distinct tooth (Fig. 3G, H).

Female. Body elongate, length 5.79–5.92. COLOURATION: as in male. SURFACE AND VESTITURE: as in male. STRUCTURE: as in male except for possessing genital tube at last abdominal segment.

Measurements (in mm). Male (n=2)/Female (n=2). Body length, clypeus–apex of membrane: 5.25–5.33/5.79–5.92; head length, excluding collar: 0.46–0.47/0.48–0.49; head width, including compound eyes: 0.87–0.88/0.91–0.93; vertex width: 0.40–0.43/0.47–0.48; 1st antennal segment length: 1.16–1.18/1.29–1.33; 2nd antennal segment length: 2.92–2.98/3.14–3.32; 3rd antennal segment length: 5.77–5.81/5.92–6.07; 4th antennal segment length: 2.81–2.86/3.22–3.35; total antennal length: 12.66–12.83/13.57–14.07; labial length: 2.83–2.89/2.95–2.98; anterior pronotal maximal width (straight): 0.60–0.62/0.64–0.65; mesal pronotal length: 0.94–0.96/0.98–0.99; posterior pronotal maximal width (straight): 1.08–1.11/1.25–1.30; anterior scutellar width: 0.54–0.56/0.59–0.60; mesal scutellar length: 0.58–0.59/0.60–0.61; commissure length: 0.90–0.97/1.06–1.11; outer embolial margin length (straight): 2.32–2.45/2.65–2.72; outer cuneal margin length (straight): 0.49–0.51/0.51–0.53; maximal width across hemelytron: 0.56–0.58/0.64–0.65.

Etymology. Named after Frédéric Chérot, who studied Mecistoscelini including this genus; a noun in genitive case.

Host. *Gigantochloa* sp. (Poaceae: Bambusoideae) (Fig. 4).

Distribution. Vietnam (Northern).

Remarks. This new species is similar in general appearance to the species *M. antrami* and *M. manipurensis*, but it can be easily distinguished by the right paramere angled vertically and the length of second antennal segment as long as ½ length of third antennal segment. The yellowish colouration of anterior and posterior parts of pronotum is variable.

DISCUSSION

The two species described herein have unique morphological characteristics that can be used to distinguish them from other species, in addition to their molecular differences. In a simple NJ tree analysis, molecular sequences from each species formed a separate clade (Fig. 5), and the individual sequences with morphological variation in each species were identical, which confirms that each species has a variable pronotum colouration. The interspecific distance of the two species is 16.8%, which is a sufficiently separated value at the independent species level, and is higher than previous barcode studies for other Heteroptera species, including the family Miridae (Jung et al., 2011; Park et al., 2011; Raupach et al., 2014; Kim & Jung, 2018).

According to Lansbury's (1963) comprehensive work, the colouration of the anterior or anterior and posterior parts of the pronotum were important in diagnosing all species in the genus *Mystilus*. Recently, variations in the anterior part of the pronotum in another species (*M. antrami*) and the paramere structures of the other *Mystilus* species were also mentioned as the diagnostic characters (Yeshwanth & Chérot, 2019). However, in the present study, it was revealed that the pronotal colouration is not the diagnostic character. The analysis of the molecular data also confirmed that the pronotal colouration was too variable across taxa to be the sole character, and could lead to misidentification. Meanwhile, the cuneal morphology and paramere structures are consistent within each species, and correspond with the molecular information, suggesting that these characters are more suitable diagnostic characters.

The evidence also suggests the importance of structural characters, such as the proportion of antennal segments and/or paramere structures, in correctly identifying the species within this group, because the general appearance of the *Mystilus* species in colouration is very similar (see the other species in Yeshwanth & Chérot, 2019).

When collecting both new species, *M. carvalhoi* and *M. frederici*, in Vietnam, it was thought that the two were conspecific with variation, because they were interestingly found on the same bamboo plant (Fig. 4; *Gigantochloa* sp.) and Mecistoscelini bugs are known as specialists of specific bamboo plant species (Kim et al., 2018; Yeshwanth & Chérot, 2019). However, the morphological and molecular data revealed that the two are obviously different species. It is rare for species in the same genus, especially in such a small genus within the Mirinae subfamily, to share the same host plant in the same season, which might be simply understood as to be in the sympatric speciation from the same host plant. However, the genetic distance between the two *Mystilus* species described here (16.8%) is much higher than those of several species that share the same host plant within all large Mirinae genera (e.g., *Apolygus* and *Adelphocoris*) (< 2%), and the average genetic congener distance at the genus level is 11.3% (Kim & Jung, 2018). This means that the two species were more genetically separated than species in other mirid groups. This unusual sharing of two congeners might not be simple speciation, but rather re-competition for the same host after previous speciation.

The plant bug family, Miridae, is the largest group in Heteroptera and is associated with various host plants, despite several species of the mirid group not being phytophagous. However, no mirid group has been known to have a special host relationship with bamboo plants, except for the tribe Mecistoscelini, which is one of the smallest groups within Miridae. Among the Mecistoscelini genera, *Mystilus* is the largest. The bamboo plant is broadly distributed across the Oriental Region, and contains more than 1,400 species (Kelchner et al., 2013). Given the high potential diversity from the richness of bamboo species and interesting biological issues, there is still room to learn more about this interesting groups of bugs.

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