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First report of the Chinese leafhopper, *Membranacea stenoprocessa*, on kiwi-fruit

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Abstract

The present study reports for the first time the leafhopper *Membranacea stenoprocessa* Yu et Yang (Hemiptera: Cicadellidae, Typhlocybinae, Empoascini), feeding on kiwi-fruit, in an orchard located in Xiuwen, Guizhou, southwestern China. During our investigation, we observed large numbers of the leafhoppers feeding on leaves and producing chlorosis. For the first time, we also report on the female of *M. stenoprocessa* and detail both colour polymorphism and sexual dimorphism in both sexes. Habitus and male genitalia are provided herein to aid in identification and the complete mitochondrial genome of the insect was sequenced for the first time.

Key words: kiwi-fruit, chlorosis, pest, leafhopper, Empoascini, Guizhou, mitochondrial genome.

Introduction

Kiwi-fruit (*Actinidia chinensis* Planch) is grown in more than 30 countries around the World (Zhai, 2015), including China, where it is known as Chinese gooseberry. Several insects are reported on kiwi-fruit worldwide of which 11 species are recorded from China (see discussion). Among these, the cell sap sucking typhlocybine leafhoppers are the main pests of kiwi-fruit (Gou, 2013; Yu *et al*., 2016), causing various degrees of damage to its terminal parts during different growth periods. In addition, these insects can complete several generations per year thereby not only building large populations but also prolonging their infestation. Previously, the leafhoppers in Xiuwen kiwi-fruit orchard (Guizhou, southwestern China) were investigated by Long *et al*. (2012) and six species were commonly found. During our studies in the same orchard, we also found several different leafhopper species including the first record of *Membranacea stenoprocessa* Yu et Yang (Cicadellidae Typhlocybinae) on kiwi-fruit in China. Here we record two colour forms of this species for the first time and give habitus and male genitalia photos to aid in its identification. We also extract and sequence mitochondrial DNA for the first time.

Materials and methods

Large numbers of *M. stenoprocessa* adults were collected by sweep net from kiwi-fruit in the Xiuwen kiwi orchard and kept in anhydrous ethanol. Destructive sampling using the entire body (without abdomen) was used for extraction of total genomic DNA by the Qiagen DNeasy kit (Venlo, the Netherlands). DNA was sequenced using Illumina NovaSeq6000 platform (Berry Genomics, Beijing, China) and the readings were assembled and annotated by using Generous Prime ver. 2019.1.3. All tRNA genes were identified by ARWEN ver. 1.2 (Laslett and Canbäck, 2008). The mitogenome has been registered on GenBank and can be accessed via https://www.ncbi.nlm.nih.gov/genbank and searching for accession number: MW426467 or from the following link: https://www.ncbi.nlm.nih.gov/nuccore/MW426467. For identification purposes, the male genitalia of specimens of *M. stenoprocessa* were examined in the following way. Under the microscope the abdomen was removed, placed in a dish containing 10% potassium hydroxide and heated. After the abdomen was cleared it was removed, washed in water and placed in a dish of glycerine and examined under the microscope. Habitus photos were taken using a VHX-6000 digital microscope and male genitalia photos were taken using Nikon Ni-E. Voucher specimens are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (GUGC).

Results

Occurrence and identification of *M. stenoprocessa*

Adults and nymphs of a typhlocybine leafhopper were found in large numbers from May to October, mainly on kiwi-fruit (Guichang cultivar), in the Xiuwen kiwi-fruit orchard. The identity of the specimens was confirmed as *M. stenoprocessa* by dissection of the male genitalia in the laboratory. Field collected samples of males and females showed two colour forms and all males had identical genitalia (figure 1); the species was originally described based on males of one colour form only. In its original description (Yu and Yang, 2013), the species was described from specimens collected from Hubei and Guizhou Provinces but without biological data. It was tentatively separated from *Membranacea plana* Qin et Zhang based on its more pointed male pygofer apex (figure 1F) and in the key by Yu and Yang (2013) by the

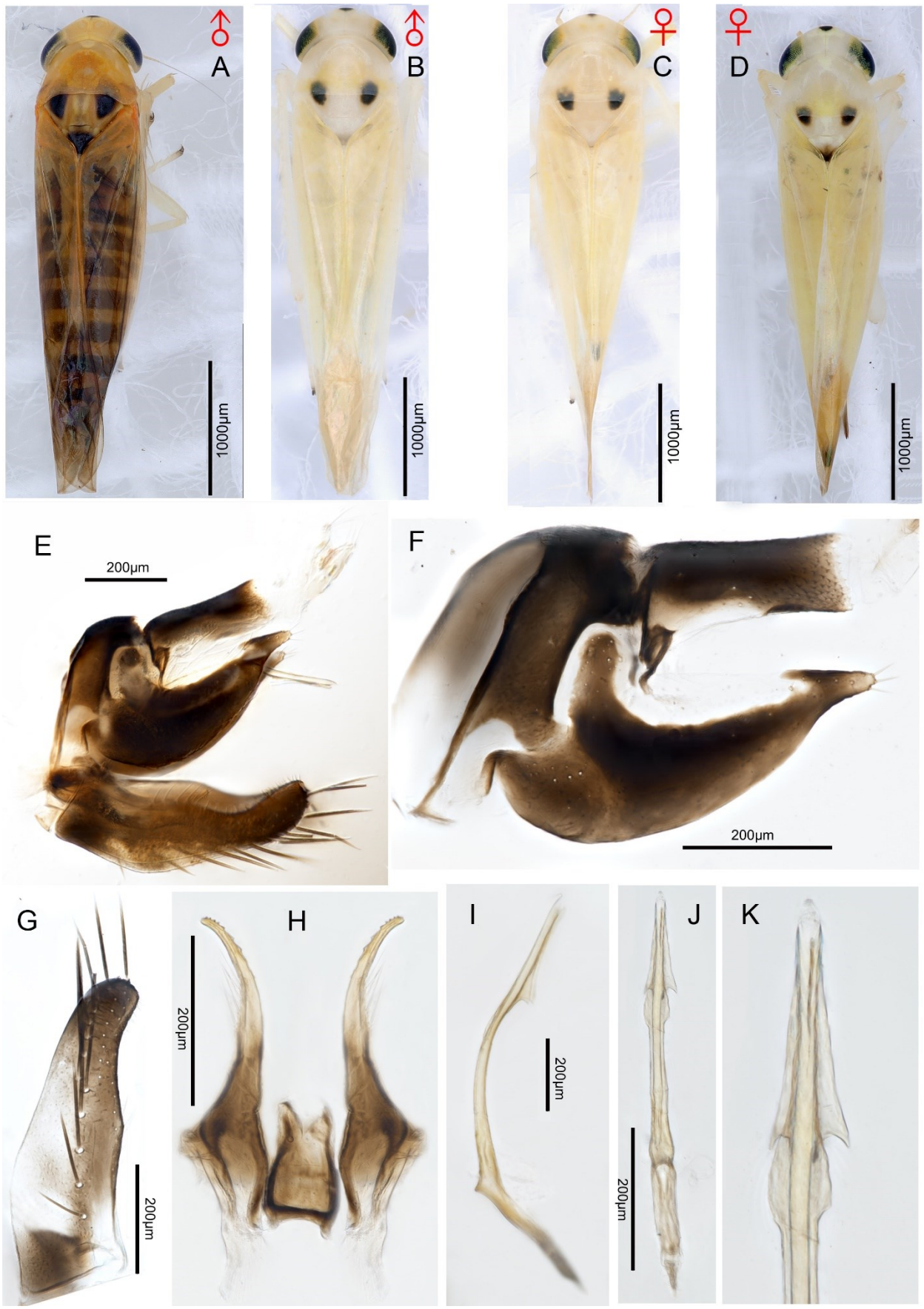

Figure 1. Adult morphological characters of *M. stenoprocessa*. **A**-**B**: male adult, dorsal view; **C**-**D**: female adult, dorsal view; **E**: male terminalia, lateral view; **F**: pygofer and anal tube, lateral view; **G**: right subgenital plate, ventral view; **H**: styles and connective, dorsal view; **I**: aedeagus, lateral view; **J**: aedeagus, dorsal view; **K**: apical half of aedeagus, dorsal view.

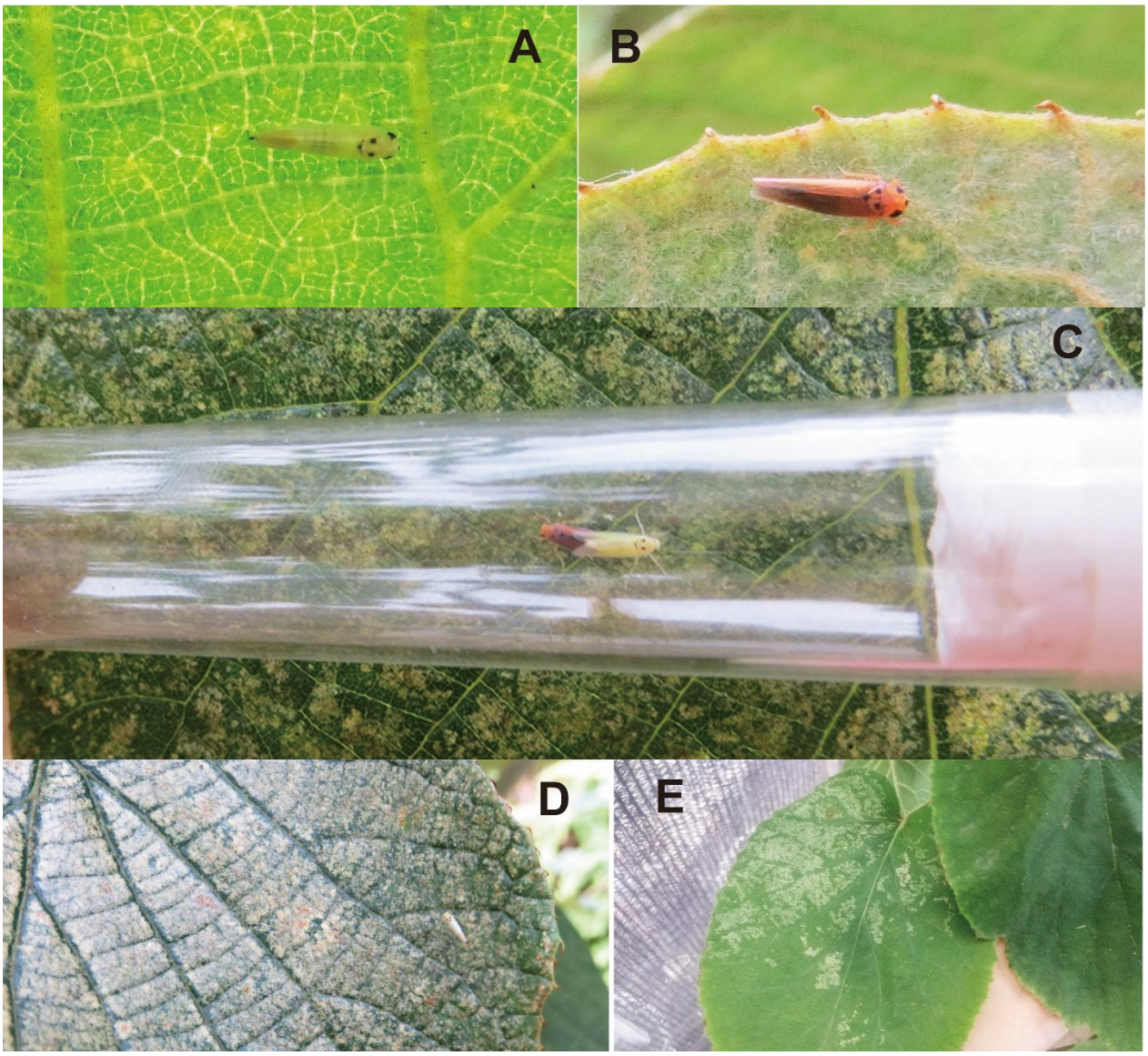

Figure 2. Field photos of *M. stenoprocessa* on kiwi-fruit leaves. **A**: female adult; **B**: male adult; **C**: sexually dimorphic male and female mating; **D**: seriously damaged leaf; **E**: initial damaged leaves.

subapical aedeagal flanges being slightly broader than the more ventral flanges (figure 1K), while the reverse is true in *M. plana*. As variation in the aedeagus was known in another congener (*Membranacea unijugata* Qin et Zhang), the possibility that *M. plana* and *M. stenoprocessa* were the same variable species was considered but the consistency of the differences in the available material suggested otherwise. Similarly, all our specimens examined matched the original aedeagal figures of *M. stenoprocessa*, suggesting that the separation of the two species is correct but a comparison with the DNA of both species could help resolve this issue. Similarly, examination of the female genitalia of *M. stenoprocessa* and a comparison made with other congeners might prove of interest but this is beyond the scope of the current work. Therefore, at present, identification of the species is reliant on male genitalia and the DNA sequence.

DNA sequencing

The circular mitogenome of *M. stenoprocessa* is 14,717 bp in size (GenBank accession number: MW426467). Complete mitogenome of *M. stenoprocessa* contained 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes and one A+T-rich region. The A+T content of *M. stenoprocessa* mitochondrial genome is 77.5% (A: 37.0%, T: 40.5%, C: 11.8%, G: 10.7%). The total length of the 13 PCGs is 10,885 bp, encoding 3,628 amino acids. All PCGs start with ATN codon, except Adenosine Triphosphate 8 (ATP8) and NADH dehydrogenase 5 (ND5) using tissue transglutaminase (TTG). Nine PCGs have TAN as the stop codon, while the cytochrome oxidase 2 and 3 (COX2, COX3) and ND3 and ND5 have an incomplete stop codon (T). The lengths of 16S rRNA and 12S rRNA are 1,159 bp and 784 bp, respectively. A recent phylogenetical analysis of Typhlocybinae using DNA sequencing and a large data set provided strong support for the monophyly of the subfamily and five of the previously recognized tribes but challenged the traditional tribal system based solely on wing venation (Lu *et al*., 2021).

Dimorphism and polymorphism in *M. stenoprocessa*

During our studies in the Xiuwen kiwi-fruit orchard, we observed both colour forms of male and female *M. stenoprocessa* mating on the leaves (figure 2C) indicating that this species was both sexually dimorphic and polymorphic. A total of 3,118 specimens (1,339 ♂♂ and 1,779 ♀♀) of *M. stenoprocessa* were examined and colour polymorphism was found in both male and female as follows: male (1,216 specimens), colour reddish to yellowish orange (figure 1A); male (123 specimens), yellowish (figure 1B); female (178 specimens), yellowish (figure 1C); female (1601 specimens), yellowish with apex of scutellum black (figure 1D).

Discussion

Insect pests on kiwi-fruit in China

In China, eleven species of insect pests have been reported on kiwi-fruit in addition to the leafhopper recorded here. *Proagopertha lucidula* Faldermann (Coleoptera Scarabaeidae), *Porthesia xanthocampa* Dyar (Lepidoptera Lymantriidae); Hemiptera, *Lycorma delicatula* (White) (Fulgoridae), *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Diaspididae), *Drosicha corpulenta* (Kuwana) (Margarodidae), *Cicadella viridis* (L.) (Cicadellidae Cicadellinae) and *Edwardsiana flavescens* (F.), *Empoasca vitis* (Gothe), *Baguoidea rufa* (Melichar), *Erythroneura apicalis* Nawa and *Erythroneura sudra* (Distant) (Cicadellidae Typhlocybinae) (Wu, 2012; Long *et al*., 2012; Hu, 2019).

Conclusion

In the Xiuwen kiwi-fruit orchard a new pest of kiwi-fruit was recorded, the leafhopper *M. stenoprocessa* previously described from elsewhere in China without hostplant data. This is also the first report of this leafhopper species occurring in two colour forms. As identification of Typhlocybinae can be problematical due to their frequent small size and reliance on examining the male genitalia confirmation of identity is therefore best performed by a taxonomist. In this case, identification of the pest species was carried out by the first author at the College of Agriculture, Guizhou University under the guidance of senior researchers. With respect to pest status, some members of Typhlocybinae are important crop pests and can complete several generations per year thereby prolonging their infestation, some species of Empoascini complete 10-13 generations a year in Jianhe, Guizhou (Long *et al*., 2017). As with most other Typhlocybinae, specimens of *M. stenoprocessa* feed by piercing mesophyll leaf cells and sucking the contents. Such activity causes dense pale spots to appear on the leaf surface (figure 2) as the chloroplasts are removed along with cell sap (chlorosis). A heavy infestation by large numbers of individuals results in browning of leaf tips referred to as hopper-burn, in which the intensity of feeding causes leaves to wither. Further studies are required to study the biology of *M. stenoprocessa* and to assess its possible effect on the yield and quality of the kiwi-fruit. It would also be of interest to find out whether this species occurs in other countries where Kiwi-fruit is grown extensively.

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The authors declare that they have no conflict of interest.

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