Molecular Detection of *Chilo infuscatellus*

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Abstract

The *Chilo infuscatellus* (Snellen; Lepidoptera: Pyralidae) is the main pest of sugarcane in China. The shortage of easily distinguishable morphological characters especially in early stage make it challenging to diagnosed and promptly take steps for pest management. In the present study, we described a PCR method for the molecular identification of based on barcode region of COI sequences between *C. infuscatellus* and four other sugarcane borer species. A 285bp fragment was successfully amplified from all life stages and different geographical populations. Sensitivity tests revealed that diagnostic bands were generated as low as the DNA template concentration of 5 ng/µl. Our work demonstrated a rapid and accurate way for the molecular diagnosis of *C. infuscatellus*.

Key words: *Chilo infuscatellus*, sugarcane, molecular detection, mitochondrial DNA

Sugarcane (*Saccharum officinarum* L.) is the most important sugar crop worldwide which is widely cultivated in the tropical and subtropical regions (Raza 2010). Besides, sugarcane is also used for ethanol production in Brazil and United States (Macedo et al. 2008, Ometto et al. 2009), and regarded as a source of sustainable energy (Hofsetz and Silva 2012). However, the existence of different sugarcane borers is a threat to the sugarcane yield since it causes damage throughout sugarcane growing season (Goebel et al. 2010, De et al. 2013, Li and Yang 2015). There are two main sugarcane borers in Chinese sugarcane fields: *Chilo infuscatellus* (Snellen; Lepidoptera: Pyralidae) and *Chilo sacchariphagus* which belong to the genus *Chilo* (Lepidoptera: Crambidae) with similar morphology and damages. Damage on sugarcane by these species results in dead heart of seedlings, stem wind-breakage in the adult-plant stage and reduced juice quality and quantity. Several attempts have been tried to control these pests, including chemical pesticides and biological control (Beevor et al. 1990, Conlong and Goebel 2002).

The accurate identification of pest species is the first and most fundamental step to develop suitable pest management strategies (Szalanski and Owens 2003). The way to efficiently control these pests is based on the accurate prediction of occurrence period and appropriate measures (Nibouche et al. 2012). Several methods have been used to manage these moths, including spraying pesticide, mating disruption or trapping by species-specific sex pheromones (Beevor et al. 1990). Evaluation of pheromone effects requires correct identification of individual moths (Chen et al. 2014). The morphological characteristics of *C. infuscatellus* have been used to differentiate with *C. sacchariphagus*: there are two spots in the discal cell of an adult *C. infuscatellus*, while only one spot in the discal cell of *C. sacchariphagus*. Besides, there are five longitudinal lines on the larva of *C. infuscatellus* while only four longitudinal lines are found on *C. sacchariphagus* larva. However, it is extremely difficult to observe longitudinal line during the early instars. During collection of sugarcane borers, we first collected the eggs or early larvae from the damaged sugarcane and raised them until the clear morphological structures could be observed under stereo microscope for identification. The need for rapid and accurate detection without sophisticated instruments or morphological expertise is not only urgent for laboratory experiment but also practical for monitoring of *C. infuscatellus* in the field.

In this study, a species-specific primer was used to identify *C. infuscatellus*. The species-specific primer set was designed according to variations in COI barcodes among five sugarcane borers. Our aim was to develop a PCR-based assay for rapid and accurate detection of *C. infuscatellus* in the field.

Materials and Methods

Insect Collections

The species of *C. infuscatellus* were collected from Guangxi, Yunnan, and Guangdong provinces. Other sugarcane borers tested here were collected as listed in Table 1.

DNA Extractions and PCR Amplification

Genomic DNA was extracted from individual larval using the Qiagen DNeasy Blood and Tissue Kit (Valencia, CA).
The universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTTG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCTAAAAATCA-3′) reported by Folmer et al. (1994) were used to amplify COI sequence for all insects. The primers (F: TTACCCTTCCTTTAACCGGAGG and R: GGTAAGAAAAGGAGGAGGAGG) were used for molecular detection of *C. infuscetellus* with the following conditions: 94°C for 3 min, 35 cycles of (94°C for 30 s, 50°C for 30 s, 72°C for 1 min), a final extension step was done at 72°C for 10 min. The sequences were visualized on an ABI 3100 Genetic Analyzer (Invitrogen Co., Shanghai, China).

**Specificity of *C. infuscetellus*-Specific Primers**

To test the specificity of *C. infuscetellus* specific primers, PCR were conducted on *C. infuscetellus* individuals from different locations and different life stages. Besides, the other four sugarcane borers were also used to test the specificity of our primers.

**Results**

Mitochondrial DNA COI Barcode Sequences

We used the specific primers LCO1490/HCO2198 to amplify 709bp COI sequence from 44 individual sugarcane borers (Table 1). Products from all amplifications were cloned and sequenced. The alignment of the COI sequence for the five common sugarcane borer species were shown on Fig. 1 using DNAMAN (Woffelman 2004) with default setting. The specific primer was designed on the variations in COI sequence among five sugarcane borers as listed in the Fig. 1 with red color.

**Table 1.** Collection localities for different sugarcane borers

| Species                  | Individuals | Location         | Collecting date |
|--------------------------|-------------|------------------|-----------------|
| *Chilo infuscetellus*    | 5           | Zhanjiang Guangdong | Apr 2016      |
|                          | 5           | Laibing Guangxi   | Apr 2016       |
|                          | 6           | Kaiyuan Yunnan    | Aug 2016       |
|                          | 7           | Dehong Yunnan     | Aug 2016       |
| *Chilo sacchariphagus*   | 5           | Nanning Guangxi   | Apr 2016       |
| *Sesamia inferens*       | 5           | Fuzhou Fujian     | Aug 2016       |
| *Argyroploce schistaceana* | 5       | Dehong Yunnan     | Aug 2016       |
| *Scirpophaga excerptalis* | 5           | Zhanjiang Guangdong | Apr 2017      |

![Fig. 1. The alignment of the COI sequence for the five common sugarcane borer species. The specific primer was marked as the red on the top of sequence location.](image-url)
Specificity of the PCR Primers
The specificity of the PCR primer was assessed using five isolates of sugarcane borers. As the Fig. 2a showed, the universal primer was sufficient to amplify 709bp COI fragment from all individuals. In contrast, a 285bp fragment was amplified in all 23 C. infuscetallus using the specific primers, while no fragment was amplified in the other four species (Fig. 2b). The plasmid of 709bp COI sequence from C. infuscetallus and C. sacchariphagus were used as positive control and negative control, respectively.

Sensitivity of the PCR Primers
To confirm the usage and fidelity of our specific PCR primers, we extracted DNA of C. infuscetallus at different life stages. As Fig. 3 showed, the PCR primers could successfully amplify the fragment of COI from larvae, pupae, male moth, and female moth. The sensitivity of the C. infuscetallus-specific primer pair was tested with a serial dilution ranging from 500 ng/µl to 5.0 × 10⁻² ng/µl, our result indicating the detection limit with the primer pair was 5 ng/µl (Fig. 4a). In addition, to confirm the primers are in fact useful for those DNA concentrations at the lower end of the range of their extracts (around 15 ng/µl), the DNA concentration between 50 ng/µl to 1 ng/µl were used for further test. As shown in Fig. 4b, the primer was sufficient to amply the specific 285bp band when the DNA concentration was as low as 5 ng/µl.

Discussion
The sugarcane borers cause great damages to the sugarcane industries throughout the world (Lange et al. 2004). C. infuscetallus is one of the most widely distributed pests in China sugarcane producing areas. With the increasing demand of environmental protection, the biology control has been widely used for pest management in sugarcane field. However, the effect of sex pheromone was specific, thus it required precise identification of moths (Chang et al. 2015). Identification of adult moths requires considerable skills and taxonomic expertise. Beside, early larva is even more difficult for recognition morphologic. Therefore, it is necessary to provide a more rapid and accurate diagnostic tool to identify C. infuscetallus for quarantine so as to assist in sugarcane field pest management of China.

The use of molecular method provides an accurate and easy way to diagnose C. infuscetallus. We designed specific primers based on the COI barcode sequences of five sugarcane borer species which
were widely distributed in China. Those borers shared high sequence similarity (>92.95%) with C. infuscatellus. Besides, we also put geographical variation and life stages into consideration. In addition, the detection limit with our primer was 5 ng/µl which is exceeded the concentration of the DNA we extracted (15.8–736.9 ng/µl). Thus, our result demonstrated the robust specificity of the PCR primers.

In conclusion, our study provided a PCR method using the specific primer for diagnosing the presence of C. infuscatellus, which is the first simple and robust detection tool for the serious sugarcane borer C. infuscatellus in quarantine applications. According to the tests performed, our specific primer set was confirmed to be sensitive and specific for the detection of C. infuscatellus.

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