First report of *Ovomermis sinensis* (Nematoda: Mermithidae) parasitizing fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in China

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This paper was edited by Raquel Campos-Herrera.

Received for publication December 24, 2019.

**Abstract**

*Spodoptera frugiperda* invaded China in the end of 2018 and has caused severe damage to maize and other crops. Several *S. frugiperda* naturally parasitized by nematodes were observed in Hainan Province, China. The morphological characteristics based on the results of scanning electron microscopy indicated that the nematode belongs to the family Mermithidae. Additionally, coding sequences for the 18S and 28S rDNA were amplified from the nematode genome, and phylogenetic analysis revealed that the nematode belongs to *Ovomermis sinensis*, a known entomoparasitic nematode. Our finding is the first record that *S. frugiperda* was naturally parasitized by *O. sinensis*. The results of this study are of great significance for potential biological control of *S. frugiperda* by indigenous natural beneficial organisms, i.e. *O. sinensis* within an integrated pest management system.

**Keywords**

Biological control, Monoxenous obligate parasites, Fall armyworm, Mermithidae, Insect parasitism.

*Spodoptera frugiperda* (J. E. Smith), fall armyworm (FAW) is a pest native to tropical and subtropical regions of the America and widely distributed throughout the American continents (Todd and Poole, 1998). The pest, which is indigenous, is highly polyphagous, causing economic damage in various crops such as maize, beans, cotton, and sorghum (Day et al., 2017). Due to its voracity, high dispersal ability, wide host range, and high fecundity, it has already invaded many countries in Africa and Asia and is causing substantial yield losses (Baudron et al., 2019). FAW causes up to $16 billion in crop losses across Africa annually (Harrison et al., 2019). FAW invaded Yunnan province, China in late 2018. Since then FAW has dramatically spread across the country (Zhang et al., 2019; Li et al., 2019), and was recorded in 1 million hectares from 26 provinces in China by December 2019.

Due to intensive pesticide application, FAW has developed resistance to a variety of chemical pesticides in several countries (Yu et al., 2003; Zhao et al., 2019), i.e. substantially high levels of resistance to omethoate was observed in FAW captured in China (Zhao et al., 2019). Based on the adverse effects of some chemicals on human health, the environment and living organisms, researchers are focusing on potential biological control agents (Harrison et al., 2019). FAW are attacked by various natural biocontrol agents such as parasitoids (López et al., 2018), bacteria (del Valle Loto et al., 2019),
fungi (Shylesha et al., 2018), nematodes (Ruiz-Nájera et al., 2013; Viteri et al., 2018), and virus (Souza et al., 2019). There is a dearth of information on natural enemies of FAW, in China, particularly because the insect only invaded the region within the past year. It is high time to develop biological control methods for FAW in China. Although assessing the virulence of commercial biological control agents is a very important way to control FAW from the perspective of environmental protection, there is also a great need to understand, promote, and maximize the effectiveness of indigenous populations of natural enemies. Nematodes are the most abundant metazoan on earth. Nematodes in several families are able to kill insects, known to be entomoparasitic nematodes. Of them, Mermithidae, Steinernematidae, and Heterorhabditidae have been more studied and some are considered as biological control agents for FAW (Huot et al., 2019; Ruiz-Nájera et al., 2013; Tarla et al., 2015; Viteri et al., 2018).

In general, mermithids nematode parasites can infect various hosts, such as spiders, mosquitoes, grasshoppers, or cockroaches (Košulič and Mašová, 2019; Kobylinski et al., 2012; Tarla et al., 2015). In addition, mermithids are also fatal to the insect host (Nikdel et al., 2011). Mermithid parasites have a great degree of species-specificity, so they are more promising to control target pests (Sáringer-Kenyeres et al., 2017). For example, in China the mermithid Ovomermis sinensis is the key mortality factors for Mythimna separata (Walker) (Sharma et al., 2002). Recently, we found FAW naturally parasitized by a mermithid nematode in a field located in Hainan Province, China. This is the first report of O. sinensis (Nematoda: Mermithidae) parasitizing FAW S. frugiperda (Lepidoptera: Noctuidae) in the world. Our discovery can provide urgent and useful information on policy making for the control of FAW in China and Asia.

Materials and methods

Field survey

From May 8 to July 24, 2019, the collection of FAW was carried out in the corn fields of Qiongzhong County, Ledong County, Yazhou District, Danzhou City, and Qionghai City, Hainan Province. Large numbers of larvae were collected by a chessboard sampling method in maize fields in the above areas. The instar and number of FAW larvae obtained were recorded and all insects were brought back to the laboratory for feeding and observation. Digital images were obtained using Olympus BX63. For the molecular description, the nematodes were removed from the parasitized FAW.

DNA extraction, PCR amplification, sequencing, and alignment

Genomic DNA was extracted from the nematodes using a Universal Genomic DNA Kit (CWBIO, China) according to the manufacturer’s protocol. Amplifications of 18S and partial 28S ribosomal DNA (D3 region) were performed according to Kobylinski et al. (2012) and Wang et al. (2007). As for 18S, the following primers were used: 18S-F: 5'-CAAGGAC GAAAGTTAGAGGTTC-3' (forward) and 18S-R: 5'-GG AAACCTTGTTACGACTTTTA-3' (reverse) (Kobylinski et al., 2012). A pair of primers of 28S, 28S-5F: 5'-ACCC GTCTTGAAACACCGGA-3' (forward) and 28S-9R: 5'-TCGGAAGGAAACCAGCTACTA-3' (reverse) adopted from Wang et al. (2007) were used in D3 region study. Each polymerase chain reaction (PCR) was made in a total volume of 25 μl containing 12.5 μl × Es Taq MasterMix (CWBIO, China) 0.5 μl 10 μM of each primer, 2 μl template DNA and 9.5 μl ddH2O. PCR products were electrophoresed in 1.5% agarose gel, purified and sequenced in both directions with ABI 3730 (Suzhou Genewiz Biotechnology Co., Ltd., Tianjin, China). Sequences were aligned using Clustalw with the default settings in MEGA X software package (Kumar et al., 2018). Sequences were visually proofread, edited, and assembled into contigs in Bioedit v7.1.7 (Hall, 2012). The resulting sequences were submitted to GenBank. Sequences of mermithid from Genbank were searched and involved into the phylogenetic analysis. Pairwise distances and neighbor-joining (NJ) (Saitou and Nei, 1987) phylogenetic analysis were done using MEGA X software package (Kumar et al., 2018) under a Kimura 2-parameter (Kimura, 1980) model. Bootstrap analysis was computed with 500 replicates.

Results

The prevalence of parasitic nematodes in parts of Hainan province is shown in Table 1. According to the survey data, the parasitic rate of the nematodes in FAW is about 2%. During the cultivation process, FAW was found and the survey distribution of nematode infection rate is shown in Table 1.

Diagnostic characters

According to morphological characteristics, these nematodes obtained from Hainan province belong to the family Mermithidae. This nematode has a stylet on the anterior portion of post-parasitic juvenile (Fig. 1A) and post-parasitic tail appendage (0.62 mm) on posterior end of post-parasitic juvenile (Fig. 1B). The mermithids are white and 228.5 mm in length.
Table 1. Location, date, coordinates, and number of larvae, parasitic nematodes of fall armyworm (FAW) Spodoptera frugiperda and conditions of plants at three surveyed sites in Hainan province, China.

| Date       | Location                                                                 | Coordinates                  | Total FAW (No.) | Parasitized FAW (No.) | Plant height (cm) | Plant status           | Planting gap (cm) | Spray insecticide situation |
|------------|--------------------------------------------------------------------------|------------------------------|-----------------|-----------------------|--------------------|------------------------|-------------------|----------------------------|
| May 08, 2019 | Wuna road, Qiongzhong Li and Miao autonomous county, Hainan Province, China | N 19°22’20” E 109°50’40”   | 43              | 1                     | 600                | The late seedling     | 50                | No-spray insecticide       |
| June 13, 2019 | Qionghai X356, Hainan Province, China                                   | N 19°15’51” E 110°28’49”   | 52              | 1                     | 2300               | Jointing and booting stage | 40                | No-spray insecticide       |
| June 11, 2019 | Foluo town, Ledong Li autonomous county, Hainan Province, China         | N 18°61’37” E 108°7’33”    | 105             | 2                     | 990                | Mature stage           | 45                | No-spray insecticide       |

Figure 1: Microscopic photo of *Ovomermis sinensis*. A: Stylet on the anterior portion of post-parasitic juvenile (arrow); B: Tail appendage on post-parasitic juvenile (arrow).

Figure 2: Post-parasitic juvenile *Ovomermis sinensis* nematode (scale bar: 1 cm).

Figure 3: Post-parasitic juvenile *Ovomermis sinensis* nematode emerging from *Spodoptera frugiperda*. 
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(Fig. 2). *S. frugiperda* parasitized by the nematodes is shown in Figure 3.

**Molecular analyses**

In the molecular analyses, the two individuals analyzed showed no polymorphism in the 18S rDNA gene fragment detected (accession number MN367956 and MN367957). The D3 fragment of 28S rDNA gene was uploaded with accession numbers MN367954 and MN367955. Based on the NJ trees of 18S and D3 region in 28S (Figs. 4 and 5), respectively, we confirmed that the nematodes isolated from *S. frugiperda* in Hainan province are

![Figure 4: Neighbor-joining tree of the Merithidae family. The tree was based on 18S rDNA data and Kimura 2-parameter model; numbers on branches represent bootstrap support (>70%) based on 500 replicates; scale represents K2P genetic distance.](image)
Figure 5: Neighbor-joining tree of the Mermithidae family. The tree was based on 28S rDNA data and Kimura 2-parameter model; numbers on branches represent bootstrap support (>70%) based on 500 replicates; scale represents K2P genetic distance.

O. sinensis and form an OTU with very small genetic distance.

The 18S rDNA sequences of HN-1 and HN-2 nematode specimens from Hainan Province were analyzed by Blast, and the similarity with O. sinensis (KU177046) was 99.35%. There are five base differences between them and the difference sites as shown in Figure S1. The corresponding genetic distance between the two tested samples (HN-1 and HN-2) and the known species O. sinensis from GenBank was 0.0040 and 0.0080 based on 18S rDNA gene, 0.0836 and 0.0825 based on D3 region in 28S rDNA gene, respectively. This genetic distance is significantly smaller than that between any two species, which is enough to suggest that the nematodes found in Hainan province belong to the species O. sinensis.

Discussion

To our knowledge, this is the first report that S. frugiperda was parasitized by O. sinensis in a natural context, an indigenous mermithid nematode in China. In a survey conducted in Mexico, mermithid nematode species from another genus, Hexamermis sp., was found to be parasitic on FAW, causing a mortality rate of 8.42% (Ruiz-Nájera et al., 2013). The present study confirms new associations of mermithid nematodes O. sinensis as natural enemies of S. frugiperda.

O. sinensis had been efficiently used to control pest insects from family Noctuidae, i.e. Spodoptera litura and Helicoverpa armigera (Li et al., 2003) as well as M. separata (Walker) (Chen et al., 1991; Zhang et al., 1992). S. frugiperda, also from Noctuidae, was found naturally parasitized by O. sinensis in the present study. All these findings confirmed the great potential of O. sinensis for control against the invasive pest insect S. frugiperda and other pest insects from Noctuidae. An intriguing finding in this study is that we can exploit the great potential to use O. sinensis to control FAW. Information on the occurrence and rates of parasitism of indigenous natural enemies is of paramount importance in designing a biological control program for S. frugiperda, either through conservation of native natural enemies or the introduction of new species for augmentative release. Major field releases of mermithids have been conducted for Anopheles population control (Kobylinski et al., 2012). Therefore, further study is needed to understand the biological and ecological relationship between O. sinensis nematodes and their hosts, and to explore the approach to artificially rear this nematode for potential field application in future.

Acknowledgments

The authors are indebted to Dr. Yuanyuan Mei for her help in taking the photos. This work was jointly...
supported by financial support of Hainan University KYQD(ZR)1963 and KYQD(ZR)1951, and the National Key R&D Program of China (2017YFE0130400 and 2018YFD00201002), China National Tobacco Company (110202001034-LS 03) and Yunnan Provincial Company of National Tobacco Corporation (2017YN15 and 2014YN21).

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**Figure S1:** Alignment of 18S sequences for comparative purposes of *Ovomermis sinensis* from Hainan province showed nucleotide identical to NCBI. Dashes are inferred insertion-deletion and dots indicate identity with the first sequence.