

## Meta-Transcriptome Profiling of Novel Invasive Pest Spodoptera frugiperda in Yunnan, China

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## Dear Editor,

The fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae), a highly agricultural destructive pest of cereals, is originally rampant in tropical and subtropical America (Kenis et al. 2019). The caterpillars of this moth feed on more than 350 plant species (Harrison et al. 2019), including several economically important cultivated grasses such as maize, rice, sorghum and sugarcane, severely threatening the food security (Cock et al. 2017). Spodoptera frugiperda is recognized to comprise two morphologically identical but genetically distinct strains with different host range. The 'rice strain' (R strain, RS) is thought to preferentially feed on rice and various pasture grasses, while the 'corn strain'(C strain, CS) on maize, cotton and sorghum (Juarez et al. 2012). Unable to tolerate freezing temperature, S. frugiperda is seldom reported outside of America. However, since its first detection in Nigeria in 2016, several congruent reports from African countries about sudden and severe upsurges of S. frugiperda raised the alarm that S. frugiperda is continuously expanding their range to become truly threat (Goergen et al. 2016). Notably, this pest is now spreading to Southeast Asia countries (including Myanmar and Thailand) and China where the environmental requirements for

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this pest to establish itself presented (Early *et al.* 2018). Bordering with Myanmar, Yunnan Province of China is in great danger of *S. frugiperda* invasion while several sporadic reports have documented the identification of *S. frugiperda* in Yunnan Province (https://www.ippc.int) (Guo *et al.* 2018; Zhang *et al.* 2019). Till now (May, 2019), *S. frugiperda* has been reported in nearly the whole south region of China where the diverse host plant sources and favorable climatic environment are provided (Kenis *et al.* 2019). Therefore, it's of great significance to monitor this new pest invasion and take preventive measures to minimize the agricultural damage of it.

Here, we reported the meta-transcriptome profiling of S. frugiperda larva collected in Yunnan Province, China. Ten larvae of S. frugiperda were collected from four localities bordering with Myanmar in Dehong prefecture of Yunnan Province from Feb 27 to Mar 1, 2019. RNA was extracted from combined pool consisting of four whole larvae from each of the regions and subjected to RNA-seq using HiSeq 3000 sequencer. A summary of sequencing and assembly results were listed in Supplementary Table S1 and Fig. 1. Totally,  $\sim 30$  G reads assembled into 531,181 transcripts were obtained and submitted to the NCBI Sequence Read Archive (SRA) under accession number SRR9317345. As shown in Fig. 1A, most of the transcripts are less than 500 nt and the longest one reaches to 71,803 nt. 86.3% of the reads can be mapped to the assembled transcripts with mean depth of  $40 \times$  suggesting a good quality of assembly and single base accuracy (Supplementary Table S1 and Fig. 1B). All the transcripts were annotated via homology-based search against the Genbank nr and trEMBL database via diamond blastx command (Buchfink et al. 2015), and taxonomy was based on the NCBI taxonomy database performed with MEGAN software (Huson et al. 2016). Nearly 38% transcripts can be annotated with a P value cutoff at 1e-5. Among the annotated sequences, bacteria is the most abundant organism with 76% reads assigned and the remaining are

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✓Fig. 1 A Distribution of assembled transcripts with different length. **B** Distribution of sequencing depth. Bar graph representation of sequencing depth versus the percentage of transcripts having the respective depth. C A summary of transcripts annotation. The left pie shows the proportional distribution of transcripts with/without homologous reference in nr/trEMBL database. The right pie shows a detail distribution for taxon of bacteria, plant, insect, fungus, viruses and unassigned. D The top 8 taxons involving bacterias from matagenomic sequencing were presented. E Taxons involving plants from matagenomic sequencing were presented. F PCR confirmation of the detected viruses from RNA-Seq analysis in the sample. G Phylogenetic analysis of S. frugiperda. Maximum-Likelihood phylogenetic tree of S. frugiperda was constructed based on the COX I gene using Busseola spp. as outgroups. Sequences derived from RNA-seq were highlighted with a red solid circle. H Comparison of the portion of the S. frugiperda Tpi gene with Corn and Rice Strain. Strain-specific polymorphic sites were highlighted with red triangle.

plant, insect, virus and fungus with 10%, 9.7%, 0.06% and 0.01% proportion respectively (Fig. 1C).

Lepidopera, as the largest order of insect including many biological and economically important species as well as some most destructive pests, genetic information of them is quite little to be known (Kakumani *et al.* 2014). And reference information of Lepidpopera mainly relies on *Bombyx mori* genome, while most of the genes of *S. frugiperda* remain to be annotated. So it is reasonable that 62% reads are unassigned and in 38% annotated sequences only 9.7% reads are insect-related among which 95% are *S. frugiperda* related. The remaining sequences mainly belong to taxons of *Diptera* and *Hymenoptera*, which contained the majority of over 150 reported parasitoids species of *S. frugiperda* (Molina-Ochoa *et al.* 2003; López *et al.* 2018), and their distribution and diversity varied with host geographical distribution (Molina-Ochoa *et al.* 2003).

Fall armyworm is the prey of over 150 parasitoid and parasite species, and most of which belong to Hymenoptera and Diptera orders (Harrison et al. 2019). Here, to find potential effective biopesticide we investigated the basic natural parasitoids presented in S. frugiperda of China. We found the existence of sequences with 65% or so identity to Trichogramma pretiosum (Hymenoptera: Trichogrammatidae) and Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) which are commonly used in inundative release for S. frugiperda control (Carneiro and Fernandes 2012). Many other ubiquitous parasitoid wasps of Spodoptera spp. including Ceratosolen spp., Trichomalopsis spp., Copidosoma spp., Nasonia spp. in Chalcidoidea family and Glyptapanteles spp., Microplitis spp. in Braconidae family were detected in our pool (Li et al. 2015; Ou-Yang et al. 2018). Taken together, we detected possible parasitoids of S. frugiperda indicating the possible utilization of them instead of introduction of exotic parasitoids for S. frugiperda pest control. As a novel invasive pest, figuring out the parasitoids complex of *S*. *frugiperda* has important implications for the biological control in China. However, whether these species were native parasitoids of *S*. *frugiperda* or just mingled into our pool while sample collection remains further investigation.

As for bacterium community, 2159 kinds of bacteria within 98 families were detected. The top 8 families accounting for 93% of the bacteria profile were showed in Fig. 1D. Bacteria belonging to Flavobacteriaceae and Pseudomonadaceae are significantly enriched, which are universal bacteria in plant and soil. Notably, among the bacterial sequences, 35 contigs were assigned to Bacillus thuringiensis (Bt) with 90% identity or so, and Bt is commonly used as biological insecticides to control fall armyworm (López et al. 2018). Except for bacteria, entomopathogenic fungi within the genera Beauveria and Metarhizum is also an important biopesticide (Cruz-Avalos et al. 2019). In our study, 33 kinds of fungi within 20 families were identified, among which 35 contigs were assigned to Beauveria with 60%-90% identity. This result may have implication for S. frugiperda management via Bt and Beauveria in China.

We also detected a large quantity of sequences of plants involving 9 families (Fig. 1E). And sequences within *Poaceaea*, including rice, maize, sorghum and sugarcane, account for 90% of all the plant among which rice and maize were the most abundant. The diversity of the detected plants reflects the highly polyphagous characteristic of *S. frugiperda*. Such results provide reasonable warnings that the novel invasive pest is most likely jeopardize the production of maize and sugarcane in Yunnan.

Finally, we investigate the viral community of S. frugiperda. Totally, five kinds of viruses were detected based on the homologous search involving alphabaculovirus, betabaculovirus, unclassified rhabdovirus and phage belonging to Siphoviridae (Table 1). One of the alphabaculovirus shares 99% identity with identified Autographa californica multiple nucleopolyhedrovirus (AcMNPV), which is the most-studied baculovirus. And another alphabaculovirus shared 40% identity with Clanis bilineata nucleopolyhedrovirus, which should be considered as a novel species. The betabaculovirus-related contig shares 62% identity with strain Helicoverpa armigera granulovirus (Genbank NC\_010240.1), suggesting a novel granulovirus carried by S. frugiperda. The rhabdovirusrelated contig shares 92% identity with strain Spodoptera frugiperda endogenous virus rhabdovirus L-like EVE 1 also detected in S. frugiperda which remains unclassified. And a phage-related contig shares 87% identity with strain Serratia phage Eta. And the Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV), which is a valuable tool for S. frugiperda control, was absent in our viral community (Bentivenha et al. 2019). We confirmed the presence

Contig No.	Reference virus (mapped gene)	Taxon	Contig Length	Identity with reference (aa level)
TRINITY_DN128371_c8_g2_i1	Autographa californica nucleopolyhedrovirus strain E2 (hypothetical protein)	Baculoviridae, Alphabaculovirus	428	99%
TRINITY_DN128284_c3_g2_i1	<i>Clanis bilineata</i> nucleopolyhedrovirus (P87/VP80)	Baculoviridae, Alphabaculovirus	824	40%
TRINITY_DN128671_c0_g6_i1	Helicoverpa armigera granulovirus (hypothetical protein HaGV_gp053)	<i>Baculoviridae</i> , Betabaculovirus	2345	62%
TRINITY_DN153113_c0_g1_i1	Serratia phage Eta_0034 (hypothetical protein)	Unclassified Siphoviridae	246	87%
TRINITY_DN123344_c0_g1_i2	Spodoptera frugiperda rhabdovirus (L protein)	Unclassified <i>Rhabdoviridae</i>	2344	92%

Table 1 List of BLASTX homology for contigs predicted as viruses corresponding to their closet reference.

of these viral sequences in the same sample for RNA-seq analysis by PCR (Fig. 1F). Primers of each detected virus were designed based on the corresponding contigs (Supplementary Table S2). In conclusion, it seems no pathogenic virus to human or crops has been carried by *S*. *frugiperda* samples so far. However, whether these identified viruses can serve as pathogen to fall armyworm remained to be studied.

To clarify the species identity that invaded into China, sequence TRINITY\_DN117122 (Genbank MN068212) annotated as mitochondrial cytochrome C oxidase subunit I (COI), which is commonly recognized as 'DNA barcode' for classification of S. frugiperda, was used for molecular analysis. Additionally, a 610 bp COI gene fragment was amplified by PCR (primers see Supplementary Table S2). Contig derived from RNA-Seq was identical to the PCR amplification results indicating the reliability of RNA-Seq. Combined with representative specimens of S. frugiperda and other Spodoptera spp., we performed Maximum-Likelihood phylogenetic analysis using Busseola spp. as outgroup (Fig. 1G). Obviously, two separated sub-cluster on S. frugiperda were formed, and our specimen clustered with the R strain. To futher confirm the identity of our fall armyworm sample, another nuclear marker single-nucleotide polymorphisms (SNPs) within the region of the fall armyworm Tpi gene between extron 3 and extron 4 were also used to distinguish strain type of S. frugiperda (Juarez et al. 2012). Contig TRINITY\_DN102113 annotated as Tip gene and 310 bp fragments (Genbank MN066368) amplified (primers see Supplementary Table S2) were compared with referential R strain (Genbank GQ411913) and C strain (Genbank GQ411916). As shown in Fig. 1H, all the 17 sites of SNPs from our sample were identical to the corn-strain specific polymorphism. It seems that our sample of S. frugiperda belongs to COI-RS Tpi-C, which can be generated by matings between ricestrain females and corn-strain males (Nagoshi 1938). Because our sampling pool was a mixture of four fall armyworms from different localities, such discordance whether suggests the presence of both rice and corn strain or the only identity of *COI*-RS *Tpi*-C in China deserves further investigation.

Summarily, this work documented the bio-community of the novel invasive pest *S. frugiperda* in China via metatranscriptome, which presents a high biodiversity, especially concerning bacterial community. And the presence of parasitoids and pathogens of the fall armyworm larvae in China suggests a great capacity to utilize them as biological control agents. Additionally, evidences of Corn strain and Rice strain were simultaneously detected via different molecular marker, suggesting a possible mixed species invasion or interstrain hybrids of *COI*-RS *Tpi*-C invasion.

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## **Compliance with ethical standards**

Conflict of interest The authors have declared no competing interests.

Animal and human rights statement This article does not contain any studies with human or animal subjects performed by any of the authors.

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