DETECTION OF *Helicoverpa armigera* POPULATIONS IN NORTH AND NORTHEAST OF BRAZIL

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INTRODUCTION

Brazilian producers have reported reduced yields up to 35% on major crops during the 2012/2013 harvest due to the recently introduced moth *Helicoverpa armigera* (Hübner). The economic losses reached US$ 1 billion only in Western Bahia, triggering a phytosanitary crisis. The deficiencies in existing taxonomic keys to deal with the morphologically indistinct larvae of *H. armigera* and the native *Helicoverpa zea* (Boddie) have constraint the detection of new incursions of this heliothine invader. From March to July 2013, heliothine larvae were found infesting crops in Roraima, Piauí and Bahia states. The aim of this study was, therefore, to identify these larvae through sequences of the mitochondrial *cytochrome c oxidase* subunit I gene (COI).

RESULTS and DISCUSSION

All specimens yielded high-quality DNA and were successfully sequenced for the COI fragment (658 bp). The four phylogenetic analyses (NJ under *p*-distance and K2P models, BI and ML under MrAIC 1.4.4 favored substitution model) gave nearly identical tree topologies. The 65 heliothine specimens from Roraima, Piauí and Bahia joined by more than 95% BS and 0.9 PP values, forming a monophyletic group with the 16 *H. armigera* sequences reported by Li et al.(2011). The *H. armigera* group formed a robustly supported monophyletic clade with *H.zea*, and this same phylogenetic pattern had been observed in previous studies, which have suggested that the American *H. zea* populations were established via founder event from *H. armigera* (or from their joint common ancestor) no more than 1.5 million years ago, what is further supported by the high similar morphology and the possibility of mating compatibility between the two species. The other defined species (*S. frugiperda*, *Heliothis virescens*, and the other 5 *Helicoverpa* species) were recovered in distinct positions or clades on strongly supported branches.

MATERIAL and METHODS

Larvae were collected from host plants in the states of Roraima, Piauí and Bahia, and preserved in 100% ethanol at -20 ºC. Total DNA was extracted from the last three abdominal segments of the larvae through the phenol: chloroform method. The partial sequences of the COI gene were amplified by using the primers (COI*F* and COI*R*) and PCR conditions described by Li et al.(2011). Amplicons were purified with the Illustra™GFX™ kit and sequenced by the ABI3730xl DNA Analyzer sequencer. The 65 COI fragments from the heliothine specimens from Roraima (n=14), Piauí (n=39), and Bahia (n=12) were used to perform the phylogenetic analyses along with sequences of 3 *Spodoptera frugiperda*, one *Heliothis virescens*, one *Helicoverpa punctigera*, one *Helicoverpa gelotopoeon*, one *Helicoverpa hawaiiensis*, 3 *Helicoverpa assulta*, 5 *Helicoverpa zea*, and the 16 *H. armigera* haplotypes reported by Li et al.(2011) (***arm*01 – *arm*16**). Neighbor-joining (NJ) analysis was calculated in MEGA 5.1 software with Kimura two-parameters (K2P) and uncorrected sequence divergences (*p*-distances) models. The Bayesian inference (BI) and Maximum-likelihood (ML) methods were also performed.
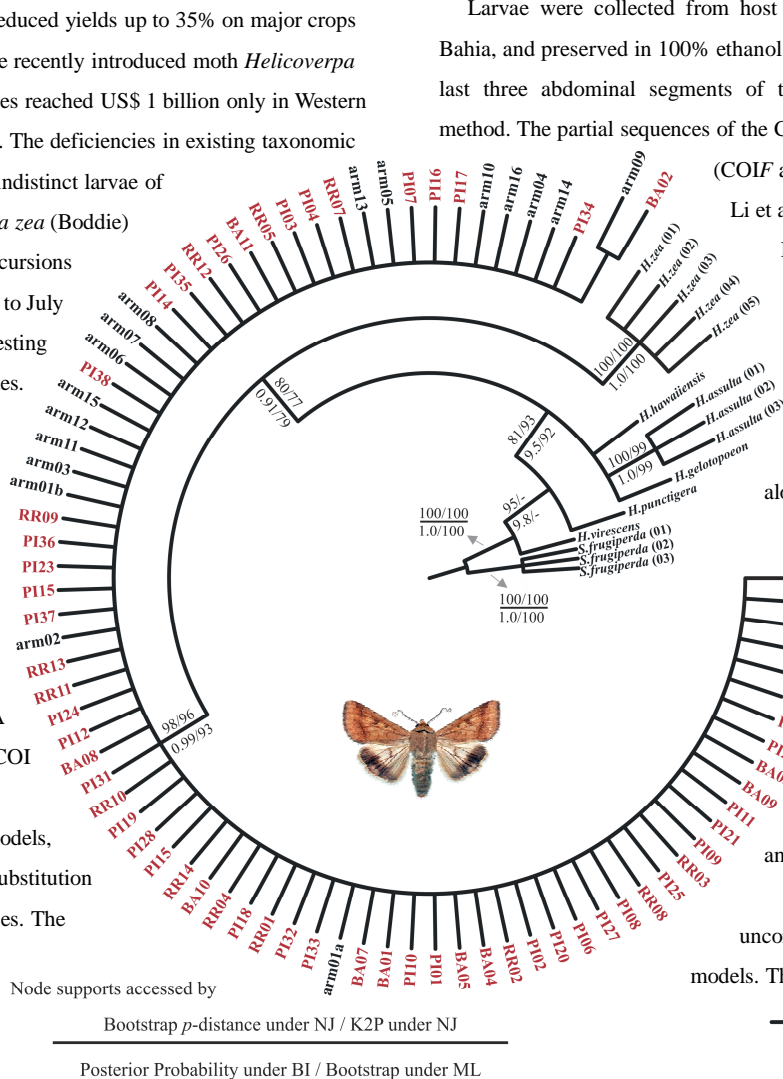

Node supports accessed by

Bootstrap *p*-distance under NJ / K2P under NJ

Posterior Probability under BI / Bootstrap under ML

Figure 1. Neighbor-Joining strict consensus tree (topology under *p*-distance model) inferred using COI complete dataset for Heliothine species. The 65 specimens from Roraima (RR01-RR14), Piauí (PI01-PI39) and Bahia (BA01-BA12) are in **red**. Sequences of *Spodoptera frugiperda*, *Heliothis virenses* and *Helicoverpa punctigera* were used as outgroups. Numbers above branches refer to Neighbor-joining (NJ) bootstrap (BS) proportions among 5.000 replicates, while numbers below branches refers to Bayesian inference (BI) posterior probability (PP) and Maximum-likelihood (ML) BS proportions among 1.000 replicates. The four analyses gave nearly identical tree topologies, and node supports values below 70% (BS) and/or 0.7 (PP) were not recorded in the tree (-).

CONCLUSION

The information content of the COI fragment selected in this study was sufficient to enable recognition of the divergences among the lepidopterans analyzed. The *mt*DNA COI phylogeny strongly supported that the 65 heliothine larvae from Roraima, Piauí and Bahia are conspecific with *H. armigera*, proving that this exotic pest is widely spread in North and Northeast of Brazil.