Use of DNA sequences for identification of possible biotypes of the fruit borer *Neoleucinodes elegantalis* (Lepidoptera: Crambidae), an important pest of Andean solanaceous fruits

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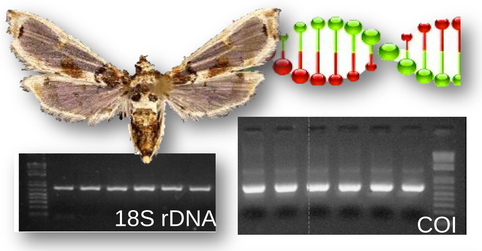
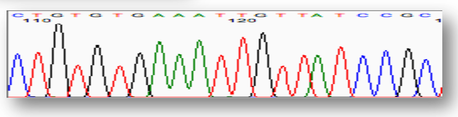
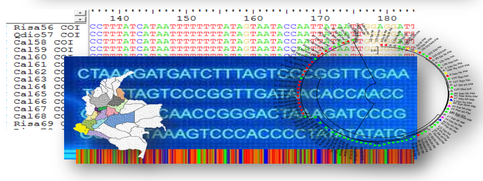
INTRODUCTION

In Colombia, Venezuela, Ecuador, Brazil and Honduras, the tomato borer, *Neoleucinodes elegantalis*, is the most important fruit-related plague of the Solanaceae family. A suitable molecular characterization using a DNA barcoding system is necessary to clarify different issues inside the taxonomy of *Neoleucinodes* genus. Additionally, other DNA sequences used for molecular identification and phylogenetics studies, can be implemented to obtain a better understanding of the genetic variability across different animal groups and allows to acquire a enhanced description of the population s genetic variation. The main objectives of this study are: 1. Evaluate the performance of DNA barcoding sequences (COI gen and 18S rDNA gene), in the genetic characterization of populations of N. elegantalis, collected in different wild and cultivated solanaceous plants in Colombia and Ecuador. 2. Determination of possible haplotypes related with each population belonging to this species. 3.Identification of geographical patterns associated with the distribution of this insect.

MATERIALS AND METHODS







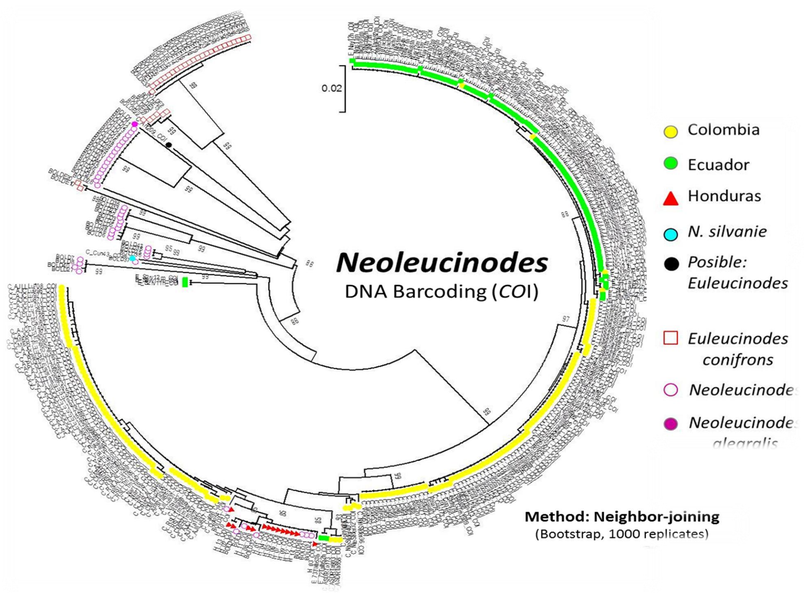
DNA extraction from 292 individuals collected in Colombia, Ecuador and Honduras was performed using the GF – 1 Nucleic Acid Extraction Kit (GF1-100-Vivantis) and the protocol proposed by Gilbertson *et al.* (1991).

Amplifications were carried out using universal primers that flank the COI region, with COI-Forward (5'-GGTCAACAAATCATAAAGATATTGG-3') and COI-Reverse (5'-TAAACTTCAGGGTGACCAAAAAATCA 3') (Folmer *et al.* 1994).Additionaly a new set of primers was developed to amplify the 18S rDNA gene: Neol_CIAT-18S-01-For (5'-AAAGCGGGCTCAAAATGCTG-3') and Neol_CIAT-18S-05-Rev(5'-CGGTCCGAAGACCTCACTAA-3'). Purification of the PCR product was performed using the PCR Clean up system (Promega).

The sequences were obtained using an automatic sequencer *ABI 3730* (Perkin Elmer/Applied Biosystem - Foster City, CA) from MACROGEN squencing service and then were assembled using the software Sequencher 4.6 (Gene Codes Corporation Ann Arbor; MI).

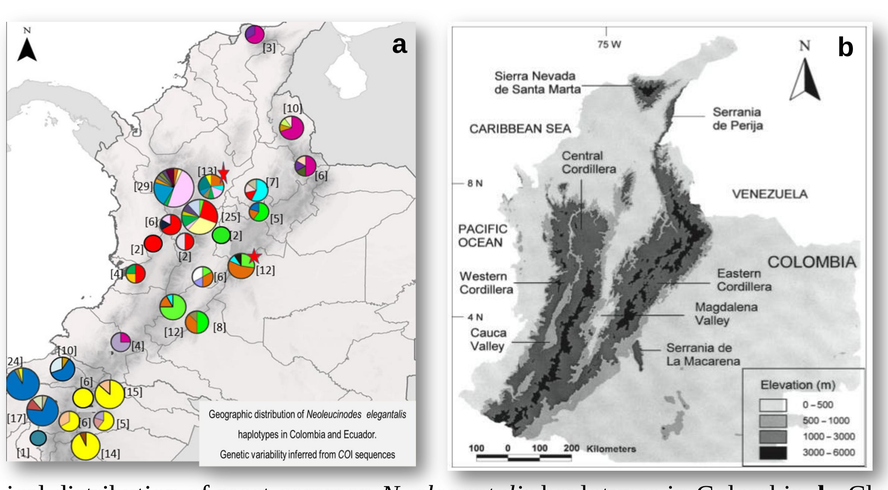
Different bioinformatic tools were used for alignment and verification of quality (Sequencher 4.6, BioEdit7.0.9.0). The nucleotide composition for each sample, genetic distances and heterogeneity among sequences (MEGA5). The haploypes determination was obtained from DnaSP v5. Additionally we performed an analysis to establish the geographic distribution for each sample was determined, using the software ArcGIS – ArcMap vs.10 (ERSI 1999 – 2008).

RESULTS



The genetic distances between sequences were calculated using Neighbor-Joining/UPGMA algorithm implemented in MEGA 5. These distances yield 5 principal groups, of which 2 belong to Colombia, the other groups represents to Ecuador, Honduras and Costa Rica.

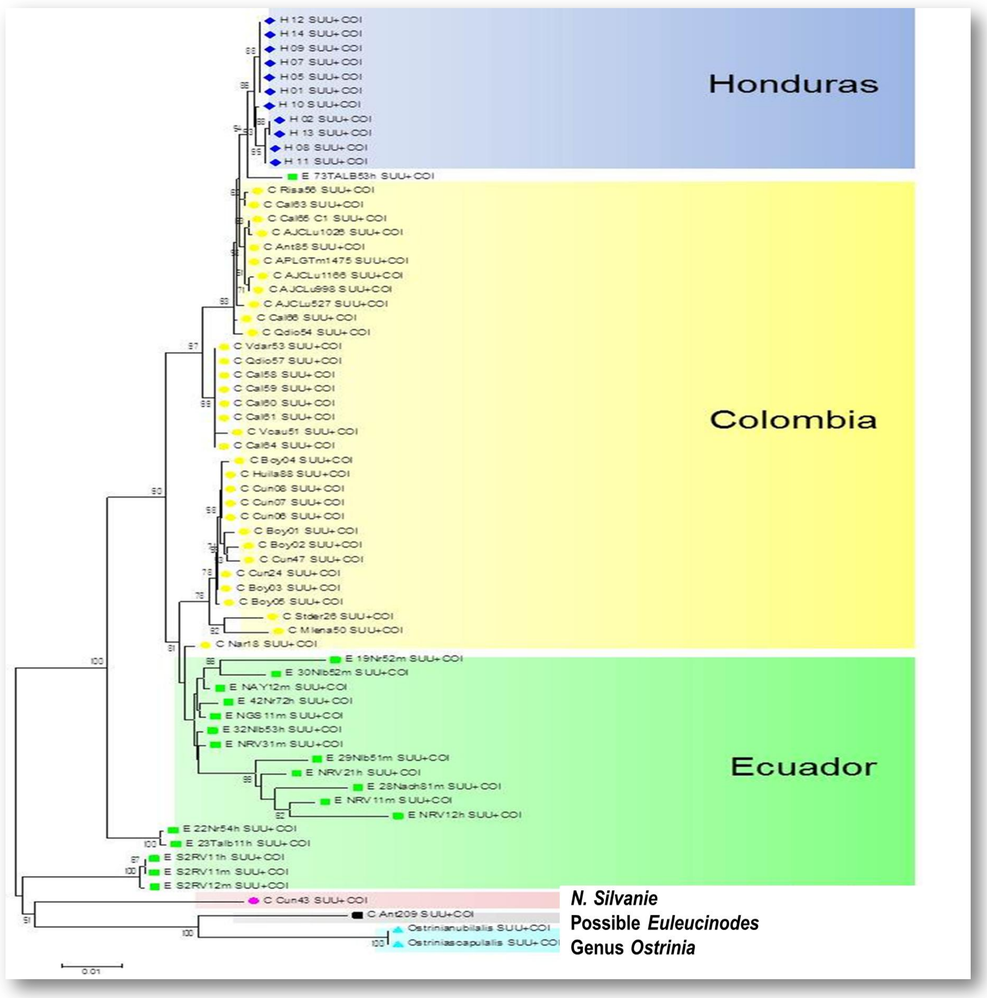
Divergence between groups was very high and the greatest were found in one specific group from Colombia, which was distributed all along the Western Cordillera, and was also apparently geographically isolated from the rest of the populations of *N. elegantalis*.



a. Geographical distribution of most common *N. elegantalis* haplotypes in Colombia, **b**. Clasification of the 5 sub-regions proposed by Kattan *et al.*, (2004), also refered to by the author as *faunistic zones*. Cauca Valley, Central Cordillera and Magdalena Valley are the three sub-regions where all Colombian individuals come from.

The analysis of the *COI* showed good sensitivity, achieving an initial differentiation of 49 possible haplotypes, with some association to specific life zones, but without apparent relation to the host type.

Genetic distances were inferred using the Maximum Likelihood (ML) method and including the evolutive model GTR+G. The sequences used to construct the dendrogram corresponds to *Cytocrome c oxidase* I (*COI*) and18S rDNA sequences respectively. The evaluation of both sequences in a combined way allows a better characterization of samples according their geographical origin. Besides it was possible to improve the resolution in term of genetic differentiation into samples distributed across Colombia and Ecuador. This distribution was associated with the *faunistic zones* proposed by Kattan *et al* (2004), for the specific case of Colombia.



Additionally, our possible new species samples are clustered with individuals included in the group associated with samples from *Euleucinodes* genus.

DISCUSSION

DNA Barcoding was an accurate tool for the identification of haplotypes as well as discrimination of species (*N. silvane*) reported previously by Díaz and Solís (2007) using geometric morphology. The number of haplotypes obtained revealed a possible role of biogeographic isolation between valleys and of possible human pressure through the use of pesticides inducing divergent selection in *N. elegantalis*. The NJ analysis shows a wide distribution of the species along the Magdalena valley watershed, this sub-region is located between the Central and Eastern Cordilleras. The distribution of *N. elegantalis* in this region could explain the wide range of altitudinal adaptation of the species that could facilitate dispersal. With regard to the Cauca Valley, diversity centers on the southwestern slope of the Central Cordillera and on the East of Western Cordillera. The classification of regions proposed by Kattan *et al* (2004), are highly correlated to the grouping of haplotypes recovered in the species. The evaluation of sequences of *Cytocrome c oxidase* I (*COI*) and18S rDNA gene, as a one single sequences reveals a powerful tool in haplotypes characterization, showing a strong differentiation in relation with the geographic origin of each sample.

CONCLUSIONS

- The DNA Barcoding tool shows high sensitivity in *N. elegantalis* haplotype identification, and to correlation with the sub-regions of colombia which suggest at least 3 different geographic groups, related to both biogeographic separation, and human intervention through the use of pesticides.
- The evaluation of sequences of *Cytocrome c oxidase* I (*COI*) and18S rDNA gene, as a one single sequences reveals a powerful tool for *N. elegantalis* haplotypes characterization
- This is the first genetic analysis of *N. elegantalis* and the first attempt to obtain a molecular characterization of the species.
- Genetic differentiation could mean that there is partial reproductive isolation in *N. elegantalis*; further research could center on resolving this issue.
- Through this methodology we confirm the existance of a new species of *Neoleucinodes* genera (*N. silvanie*) previously proposed by Díaz and Solís (2007) with the use of morphological characters.
- Thus, for *N. elegantalis*,both mitochondrialDNAdifferentiation and morphological divergence provide evidence for the existence of populations that are going through ecological speciation

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