Geographic Differentiation of Colombian *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) haplotypes: evidence for Solanaceae host plant association and Holdridge life zones for genetic differentiation

Jorge Mario Londoño[1], Ana Elizabeth Díaz[2], Gerardo Gallego[1], Wilson Vasquez[3], Harold Suárez[1] and Joe Tohme[1]

[1]Agrobiodiversity and Biotechnology Proyect, Centro Internacional de Agricultura Tropical (CIAT), Palmira, Valle del Cauca - Colombia
[2]Corporación Colombiana de Investigación Agropecuario (CORPOICA), Centro de Investigación La Selva, Rionegro, Antioquía - Colombia
[3]Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP, Ecuador)

g.gallego@cgiar.org

INTRODUCTION

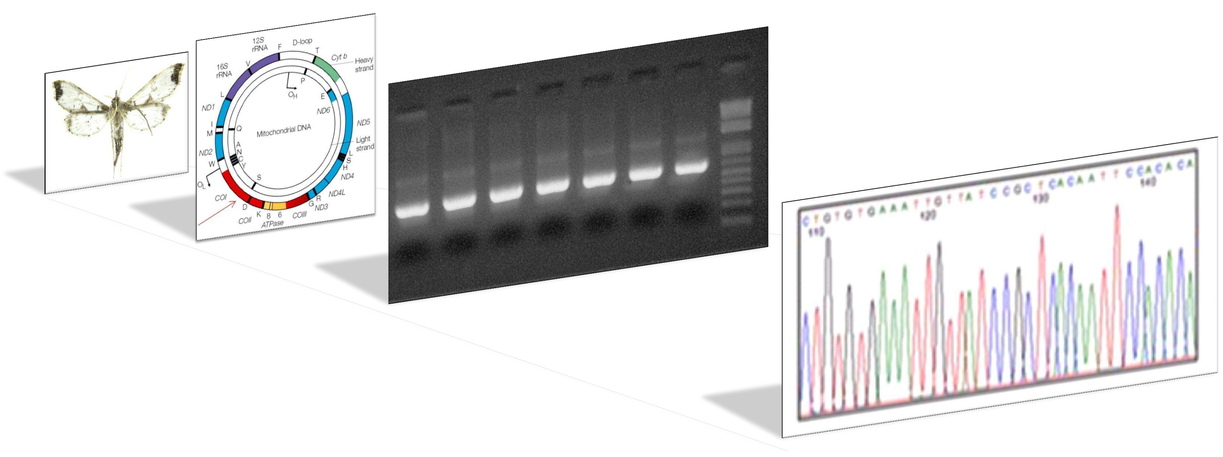
Neoleucinodes elegantalis, known as tomato fruit borer, is an insect of Neotropical origin, widely distributed in Central America and South America. This species is considered one of the most important pests for fruit production in the Solanaceae. *N. elegantalis* is adapted to a wide altitudinal range (0 – 2600 m.a.s.l), a considerable diversity of natural enemies, and exhibits variability in terms of oviposition and pupation habit, besides a wide spectrum of host wild Solanaceae, and a differential behavior to sexual pheromone *Neoelegantol®*. Using the DNA Barcode tool, 272 samples were processed and analyzed through *Cytocrhome oxidase I (COI)* gene, which represents a standard segment of the mitochondrial genome (~ 650 base pairs). The geographical differentiation and population structure in relation to host-plant association and to Holdridge life zones were examined. The mains goals of this study are to evaluate the utility of DNA barcoding in the identification of haplotypes in the populations of *N. elegantalis* from Colombia and Ecuador, and to establish a DNA barcode database for this species.

METHODOLOGY

DNA extraction from 163 individuals collected in 14 departments of Colombia and 95 individuals from Ecuador was performed using the *GF – 1 Nucleic Acid Extraction Kit* (GF1-100-*Vivantis*®). Amplification was carried out using universal primers that flank the *COI* region, with COI-Forward (5'GGTCAACAAATCATAAAGATATTGG3') and COI-Reverse (5' TAAACTTCAGGGTGACCAAAAAATCA 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*) and the assembled with Sequencher 4.6 (*Gene codes corporation Ann Arbor; MI*).

Different bioinformatic tools were used for alignment and verification of sequence quality, nucleotide composition for each sample, genetic distances and heterogeneity among sequences. Additionally, the geographic distribution for each sample was determined, using the software ArcGIS – ArcMap (ERSI 1999 – 2008).



RESULTS

Nucleotide composition and haplotype frequencies of *N. elegantalis*

Amplification of the mitochondrial gene *COI* produced a 658 bp fragment from a total of 272 individuals with average base (nucleotide) frequencies of A=34.06%, T=34.06%, C=15.94%, G=15.94%. The number of haplotypes obtained was 9 in which the most frequent haplotype, with 87 sequences, was H5 representing 32.2% including 90% of the Ecuadorian individuals. H5 was followed by H1 with 82 individuals and corresponds to 50% of Colombian sequences. Moreover, H1 was found in the Magdalena valley watershed while H3 correspond to Inter-Andean slopes and H2 to Cauca valley watershed. The number of substitutions at the nucleotide level varied between 9 and 24 in all haplotypes observed.

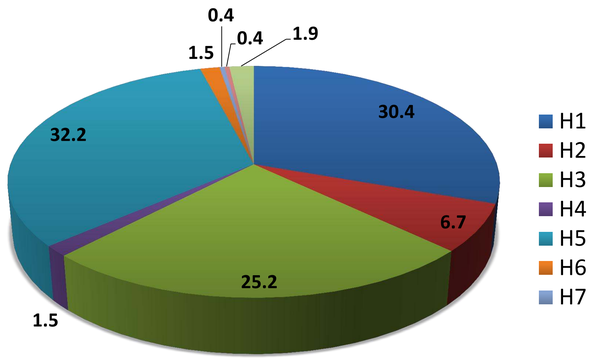


Figure 1. Haplotype frequencies obtained for the evaluation of 272 samples, the most frequent haplotypes are H5 (light blue) and H1 (dark blue).

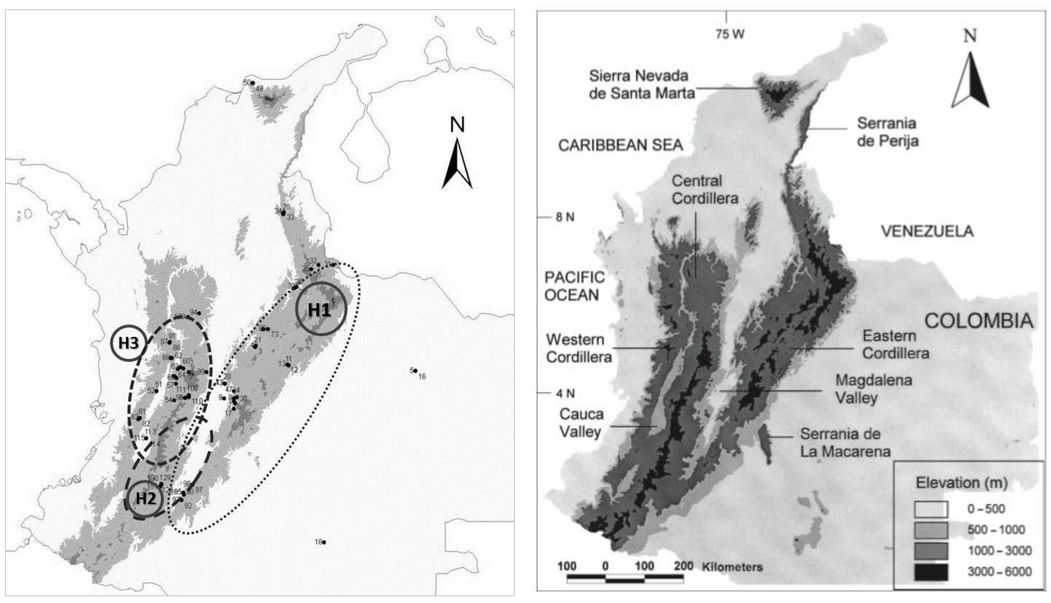


Figure 2. a) Geographical distribution of most common *N. elegantalis* haplotypes in Colombia, b) Clasification of the 5 sub-regions proposed by Kattan *et al.,* (2004), also refered to by the author as *faunistic zones*. Cauca Valley, Central Cordillera and Magdalena Valley are the three sub-regions where all Colombian individuals come from.

The composition and heterogeneity analysis of COI sequences allowed the establishment of variability at the inter and intra specific levels. This variation could identify significative divergence between individuals of *N. elegantalis.*

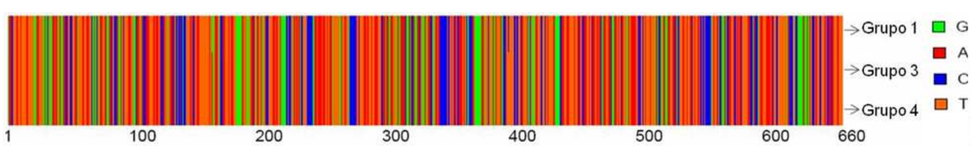


Figure 3. Heterogeneity (He) of the COI in *N. elegantalis.*

The genetic distances between sequences were calculated using Neighbor-Joining/UPGMA algorithm implemented in MEGA 5. These distances yield 5 groups, of which 2 belong to Ecuador and Honduras. Additionally, 14 individuals from Honduras, which were used as the outgroup, formed a separate group; and 3 Colombian groups can be distinguished. The faunistic classification proposed by Kattan *et al* (2004) in order to group the origins of the samples.

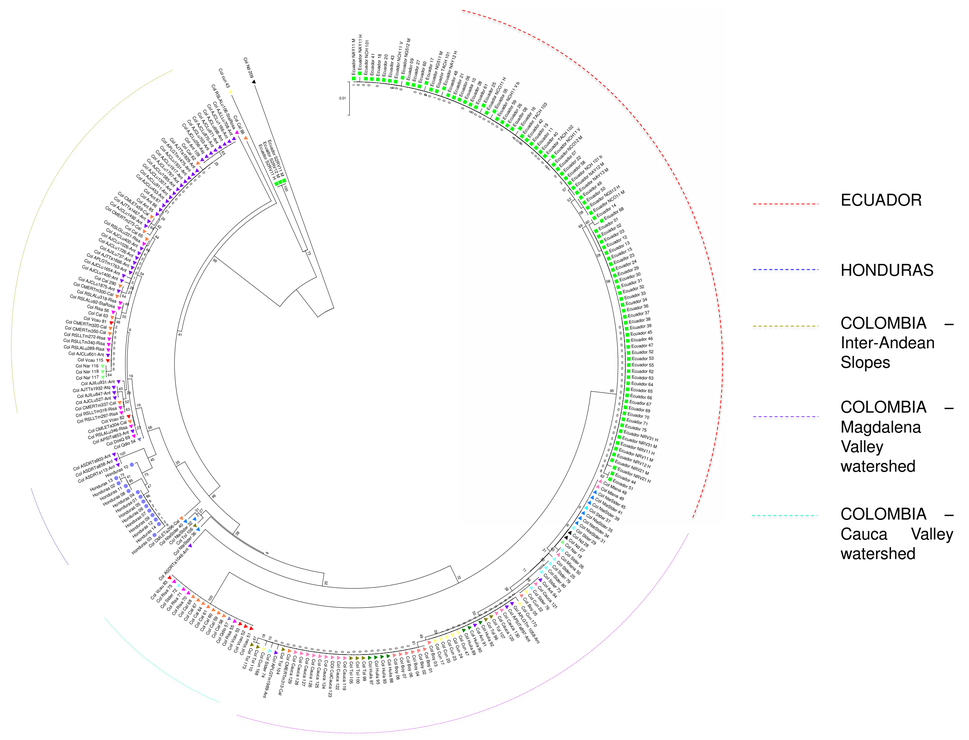


Figure 3. Genetic distances inferred using Neighbor-Joining (NJ). Classification follows the faunistic zones proposed by Kattan *et al* (2004): yellow (Inter-Andean Slopes), blue (Cauca Valley watershed), violet (Magdalena Valley watershed), red (Ecuador), deep blue (Honduras).

DISCUSSION

DNA Barcoding was an accurate tool for the identification of haplotypes as well as discrimination of species (*N. silvane*) reported previously by Díaz & 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.

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.
- ✧ 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 & Solís (2007) with the use of morphological characters.

REFERENCES

DIAZ A E., SOLIS M A 2007. A New species and species distribution records of *Neoleucinodes* (Lepidoptera: Crambidae:Spilomelinae) from Colombia feeding on *Solanum* sp. Proceedings of the Entomological Society of Washington. 109 (4):897–908.

HOLDRIDGE L R 1947. Determination of World Plant Formations from Simple Climatic Data. Science Vol, 105 No. 2727: 367-368.

HEBERT P D N., CYWINSKA A., BALL S L., DE WAARD J R. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B. 270, 313-321.

KATTAN G H., FRANCO P., ROJAS V., & MORALES G. Biological diversification in a complex region: a spatial analysis of faunistic diversity and biogeography of the Andes of Colombia

ACKNOWLEDGMENTS

Special thanks to Fondo Regional de Tecnología Agropecuaria FONTAGRO for financial support.