Mitochondrial DNA variation within the ND2-COI tRNA Region for the members of the *Anastrepha fraterculus* group

Robert Mier[1], Raul Ruiz-Arce[2], W. Evan Braswell[2], Alexis Racelis[1]

[1] University of Texas Rio Grande Valley, [2] USDA APHIS PPQ Mission, TX

Abstract

Fruit flies belonging to the genus *Anastrepha* are notorious for their impact, as pests, on subtropical agriculture. The interspecific and intraspecific relationship for some species within the *fraterculus* group of *Anastrepha* is poorly understood due to overlapping geographical distribution and probable hybridization. Commonly utilized molecular markers have proved to distinguish the relationship among other Tephritids. However, these same markers have shown limited resolution for some *Anastrepha* species. This research examines the utility of the ND2/COI tRNA mitochondrial DNA region for distinguishing members in the fraterculus group, as well as other *Anastrepha* species. Preliminary results suggest this mitochondrial marker may be effective for differentiating closely related species and could have the capacity for shedding light on the unresolved species boundaries for some members from the fraterculus group.

Background/Introduction

Anastrepha fruit flies consist of more than 200 species (Norbom 1999). The *fraterculus* taxonomic group is composed of agricultural pests which are difficult to distinguish at larval stage. The complexity of the *fraterculus* group increases as each species is examined individually (Vaníčková L. et al. 2015). Previous molecular studies on the fraterculus group indicate complicated relationships between and within species (McPheron, et.al.,1999, Smith-Caldas et.al., 2001, Barr, et.al., 2005, Vaníčková, et. al., 2015). Morphological methods for ID are innefective and not all molecular markers discriminate well among some species. However, further research expanding on the mitochondrial genome is helping clarify the Fraterculus species complex (Barr et al., in-preparation).The correct identification of species and populations is critical for conducting international safe trade. We examine the ND2-COI mtDNA region for it's capacity in distinguishing among these species. Our objectives for the study include: 1) Establish a working protocol for the ND2-COI marker; 2) Examine performance of ND2/COI marker with *Anastrepha* species; 3) Examine performance of marker with *Anastrepha* spp. collections

Methods

Table 1. Primers used for amplification

ND2for_a	5'-YCTACGTYTRTGTTTTGCWGCWTT-3
COIrev_a	5'- GCTCCTGGATGTCCTAATTCA- 3

PCR Parameters

- 80°C for 5 minutes
- Denaturation temperature at 94°C for 45 seconds
- Annealing temperature of 55-58°C for 30 seconds
- Extension period of 72°C for 5 minutes.

Sequencing

- PCR product was purified and sent to Genewiz for sequencing.

Editing and Interpreting Sequences

- Sequences were edited using Sequencher v5.0 and MEGA v7.

Phylogenetic Analysis

- Constructed using MEGA 7
- Tamura 3-parameter model was tested to be the best model for analysis of evolutionary history using Maximum Likelihood.

Results

- 751 bp alignment
- 333 variable and 331 parsimony informative sites among the 31 samples.
- 21 haplotypes, 21 species specific

Table 2: Samples of Anastrepha fruit flies

Species	Location	n	Haplotypes(s) ND2CO1-
A. fraterculus	Peru, Veracruz MX, Nuevo Leon MX, Belize, Guatemala	5	AF001, AF002, AF003
A. ludens	Guatemala	7	AL001, AL002, AL003, AL004, AL005, AL006, AL007
A. suspensa	Puerto Rico	10	AS001, AS002, AS003
A. distincta	Guatemala, Panama	3	AD001, AD002, AD003
A. obliqua	Peru, Columbia, Brazil	5	AO001, AO002, AO003, AO004
A. acris	Nicaragua	1	AA001

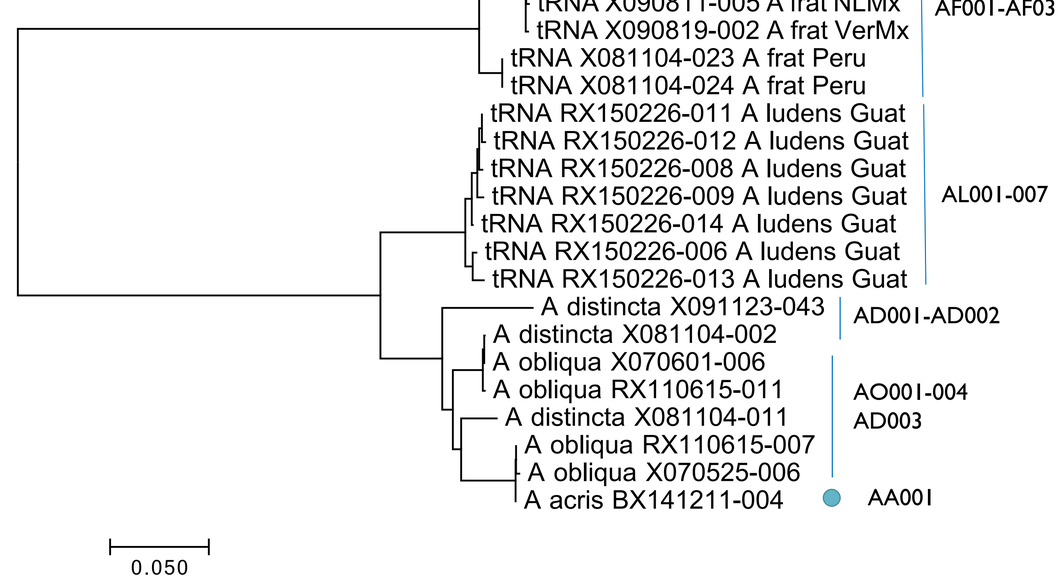

FIGURE 1. Phylogenetic reconstructing using neighbor joining methods.

Conclusions/Recommendations

The complexity of the Fraterculus species complex is yet to be resolved. The molecular variation observed from our sampling suggests the sampling must be increased in order to confirm differences at species and population level. *Anastrepha fraterculus* populations from Peru show to be divergent from Central American populations in our study. Such divergence is consistent from other publications exclusively studying *A. fraterculus* (Sutton, et al. 2015). Previous studies produced a polytomy, suggesting there isn't sufficient variation/data to produce a stern relationship within this species complex (Barr, et al. 2005, Smith Caldas, et al. 2001). These preliminary results suggests this mitochondrial marker shows potential in distinguishing among some *Anastrepha* pest species at the species and population-level. Additional analyses of specimens from the *fraterculus* species complex as well as among other species is required in order to further examine the diagnostic capacity of this marker.

References

Barr, Norman B., Liwang Cui, and Bruce A. McPheron. "Molecular systematics of nuclear gene period in genus Anastrepha (Tephritidae)." *Annals of the Entomological Society of America* 98.2 (2005): 173-180.

Barr, N. B.. et al (in preparation). Diagnosing seven *Anastrepha* (Diptera; Tephritidae) species using DNA Barcodes

McPheron, B. A. et al. (1999). In *Fruit flies (Tephritidae): Phylogeny and evolution of behavior*, 343.

Norrbom, A. (1999). PHYLOGENY OF ANASTREPHA AND TOXOTRYPANA BASED MORPHOLOGY. *CRC Press*.

Smith-Caldas, Martha RB, et al. "Phylogenetic relationships among species of the fraterculus group (Anastrepha: Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase I." *Neotropical Entomology* 30.4 (2001): 565-573.

Sutton, Bruce D., et al. "Nuclear ribosomal internal transcribed spacer 1 (ITS1) variation in the Anastrepha fraterculus cryptic species complex (Diptera, Tephritidae) of the Andean region." *ZooKeys* 540 (2015): 175.

Vaníčková, Lucie, et al. "Current knowledge of the species complex Anastrepha fraterculus (Diptera, Tephritidae) in Brazil." *ZooKeys* 540 (2015): 211.

Acknowledgement

Photos of the larvae infested fruit are from the U.S Customs and A Guide for Diagnosis & Detection Of Quarantine Pests, Ministry of Jihad-e-Agriculture Plant Protection Organization, Islamic Republic Of Iran

We thank staff at the Mission Lab at Moorefield Air Base, Edinburg, Texas.
Thank you for taking me under your wing, building me from the ground up and thank you for your patience!
A special thank you for Dr. Alexis Racelis for the opportunity to conduct research.