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MITOCHONDRIAL DNA MARKERS IN POPULATIONS OF *DACUS PUNCTATIFRONS* (DIPTERA: TEPHRITIDAE)

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Some of the true fruit flies (Diptera: Tephritidae) are major economic agricultural pests in several African countries (White & Elson-Harris 1992). *Dacus punctatifrons* (Karsch) (Subfamily Dacinae; Tribe Dacini) (Caroll et al. 2002) is a major pest of many cultivated and wild cucurbits as well as tomato (*Lycopersicum esculentum*). Even though, It is widely distributed in Sub-saharan Africa (Benin, Cameroon, Democratic Republic of Congo, and Equatorial Guinea) (Tindo & Tamo 1999), no information has been reported on its genetic make up. Surveys for *Dacinae* in Sub-Saharan Africa and identification of species including *D. punctatifrons* often were based on morphological and ecological characters (Mwatawala et al. 2006), but these methods have limitations, especially when the specimen is damaged or the adult stage is not available (Segura et al. 2006). Molecular taxonomy based on mitochondrial DNA has proved to be an efficient alternative to taxonomic identification (Muraji & Nakahara 2001). In fact, mitochondrial markers have been used with a number of insects for systematic and identification purposes (Barr & McPheron 2006; Segura et al. 2006; Virgilio et al. 2008).We have amplified and sequenced here a portion of the mitochondrial COII gene from various populations of *D. punctatifrons*. The specimens were collected at the adult stage and carefully identified basedon morphological features. Genomic DNA was extracted from individuals by the Lifton rapid fly genomic DNA isolation protocol as described in Anleitner & Haymer (1992). The polymerase chain reaction was used to amplify the mitochondrial COII sequences from each specimen with the following primers:

C2KD-(Forward): CAAATTCGAATTTTAG-TAACAGC

C2KD-(Reverse): TTAGTTTGACAWAC-TAATGTTAT

The PCR mix included 9.5 µL of ddH_2O, 1.5µL $MgCl_2$, 0.5 µL primers, 12.5 µL of Amplitaq Gold PCR Master mix (Applied Biosystems, Inc., Carlsbad, CA) and 1 µL of DNA template for a total volume of 25 µL. It has an initial denaturation step of 2 min at 95°C, followed by 35 cycles of 45 s at 94°C, 30 s at 55°C and 45 s at 72°C, and a final extension of 7 min at 72°C. The amplification products were analyzed by electrophoresis in 1% agarose gels in TBE buffer with the 2-Log DNA ladder (0.1-10.0 kb) (New England Biolabs, Beverly, MA) as a molecular weight marker. PCR products from individual specimens were isolated with the "Gene clean" method (Qbiogene, Solon, OH, USA) as described by the manufacturer and resuspended in a total volume of 10 µL (Vogelstein & Gillespie 1979). Depending on the recovery of the cleaned product, 1 or 2 µL of template DNA from each individual for sequencing reactions was carried out with BigDye terminator chemistry (Applied Biosystems, Inc., Carlsbad, CA) on an ABI 3730XL capillary-based automated DNA sequencer. Sequences obtained from the PCR products were 266 bp in length. They were checked for

TABLE 1. FREQUENCY AND DISTRIBUTION OF COII MITOCHONDRIAL DNA HAPLOTYPES DETECTED IN POPULATIONS OF *D. PUNCTATIFRONS* (KARSCH).

	Base pair positions of nucleotide changes in COII sequences							Haplotype distribution			
								Benin	Cameroon*		
Code	43	44	46	47	48	84	85	Cotonou (IITA)	Mbalmayo	Foumbot	Nkometou II
I	A	A	A	A	A	A	A	10	8	11	4
II	C	C	T	T	T	C	C			1	
III	A	C	A	A	A	A	A	2	3		
IV	A	C	T	T	T	A	A		2		
V	C	A	A	A	A	A	A	1			

(*)Distances between localities: Mbalmayo-Foumbot: 211.34 Km (131.32 miles)
Mbalmayo-Nkometou II: 181.4 Km (112.72 miles)
Foumbot-Nkometou II: 187.42 Km(116.46 miles)

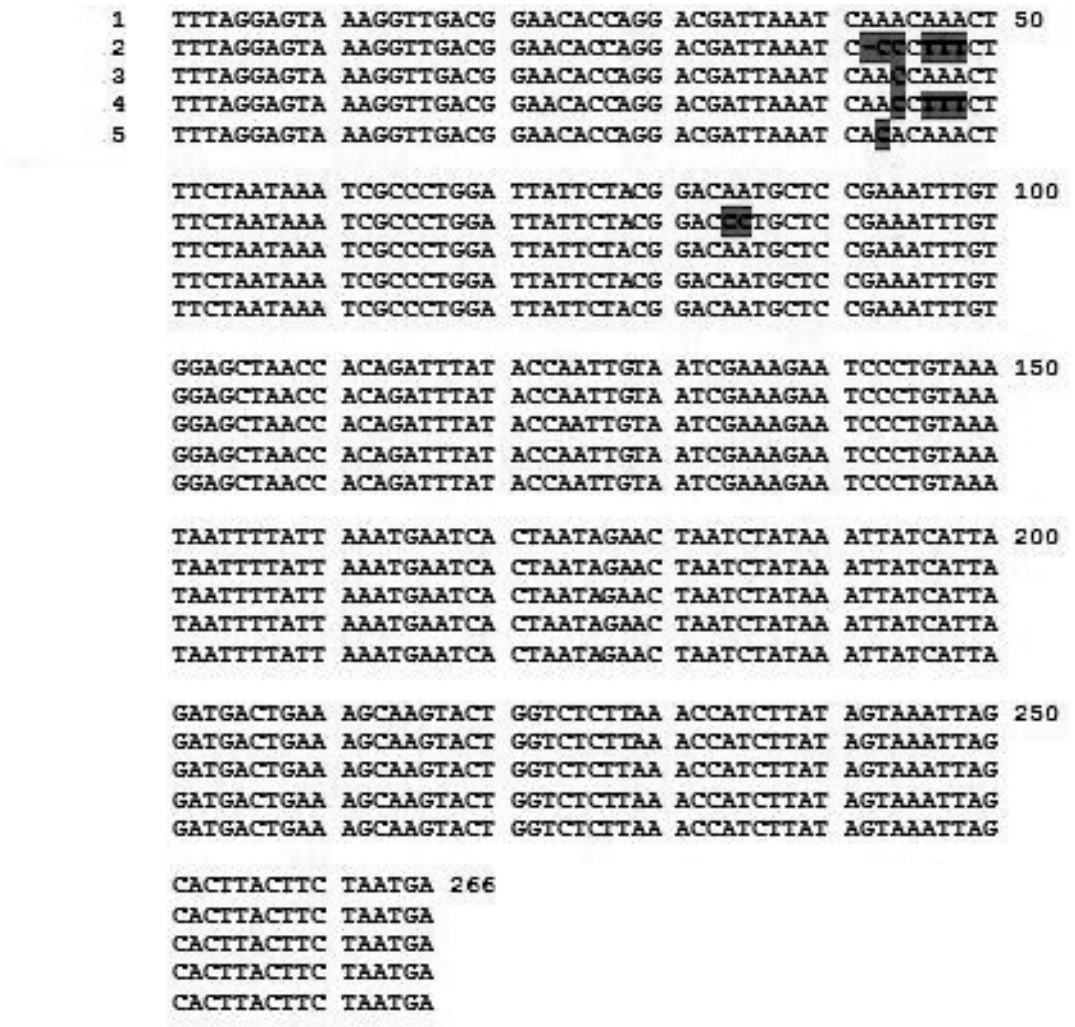

Fig. 1. Alignment of representative DNA sequences from the COII region of mitochondrial DNA of *D. punctatifrons* individual specimens. Nucleotide changes are highlighted and numbers in left margin refer to haplotypes I to V in Table 1.

eventual disruption of open reading frames with the ORF finder function from the NCBI website and aligned by CLUSTALW in the program DS Gene 2.0 (ACCELERYS Inc., San Diego, CA). The alignment revealed the presence of genetic variability between populations (Fig. 1). Five haplotypes were detected among 42 individual specimens collected from Benin (Cotonou) and Cameroon (Mbalmayo, Foumbot, and Nkometou II). The percentage of variability among these populations is around 11%. The nucleotide changes for each haplotype are listed in Table 1, which shows the distribution and frequency of each variant by country and locality. Table 1 also reveals the presence of a dominant haplotype (Haplotype I) in East subsaharan African countries Cameroon and Benin, and the representative sequence of this haplotype is available (GenBank Accession code # EU836643). Some other variants are unique to 1 locality (haplotypes II, IV, and V). These encoded variants detected in populations of *D. punctatifrons* show the presence of intraspecific variability around 11%, whereas Virgilio et al. (2009) reported that *D. punctatifrons* specimens sampled in Kenya, Benin, Cameroon, R.D. Congo, Uganda, and Zimbabwe showed very low levels of intraspecific variability (<0.3%) based on analysis of 16S and COI sequences. This is mainly due to the limited number of specimens analyzed. Our study suggests that the identification of COII-haplotypes would serve as DNA barcodes for species identification (Hebert et al. 2004). These COII segments can be considered as a reference database for *D. punctatifrons* identifica-

tion at quarantine port of entry especially when the specimens are at immature stages and are very useful for detection and monitoring of future infestations and bioinvasions of this pest in others regions worldwide. A more extensive and elaborate cross range sampling of *D. punctatifrons* and generating longer sequences of both COI-COII fragment would lead to a more in-depth evaluation of the genetic diversity of this pest within the Subsaharan African region.

Summary

Mitochondrial DNA sequences from the COII gene were generated from individual specimens of the tephritid fruit fly *D. punctatifrons* collected from localities in Benin and Cameroon. The sequences alignment allowed us to make inferences towards intraspecific genetic diversity of this insect pest and to provide haplotypic variants that can be useful for quarantine applications.

References Cited

Anleitner, J. E., and Haymer, D. S. 1992. Y enriched and Y specific DNA sequences from the genome of the Mediterranean fruit fly, *Ceratitis capitata*. Chromosoma 101: 271-278.

Barr, N. B., and Mcpheron, B. A. 2006. Molecular phylogenetics of the genus *Ceratitis* (Diptera: Tephritidae). Mol. Phyl. and Evol. 38: 216-230.

Carroll, L. E., White, I. M., Freidberg, A., Norrbom, A. L., Dallwitz, M. J., and Thompson, F. C. 2002. Pest Fruit Flies of the World. Version: 15 July 2005, http://delta-intkey.com.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. N., and Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101(41): 14812-14817.

Muraji, M., and Nakahara, S. 2001. Phylogenetic relationships among fruit flies, *Bactrocera* (Diptera: Tehritidae), based on the mitochondrial rDNA sequences. Insect Mol. Biol. 10(6): 549-559.

Mwatawala, M. W., De Meyer, M., Makundi, R. H., And Maerere, A. P. 2006. Biodiversity of fruit flies (Diptera: Tephritidae) in orchards in different agroecological zones of the Morogoro region, Tanzania, Fruits 61(5): 321-332.

Segura, M. D., Callejas, C., Fernández, M. P., and Ochando, M. D. 2006. New contributions towards the understanding of the phylogenetic relationships among economically important fruit flies (Diptera: Tephritidae), Bull. Entomol. Res. 96: 279-288.

Tindo, M., and Tamó, M. 1999. Une mouche des fruits (*Dacus punctatifrons* Karsch: Diptera Tephritidae) comme probleme de production de la tomate dans la region de la Lekie (Sud-Cameroun). Ann. Entomol. Soc. Fr. 35: 525-527.

Virgilio, M., Backeljau, T., Barr, N., and De Meyer, M. 2008. Molecular evaluation of nominal species in the *Ceratitis fasciventris*, *C. anonae*, *C. rosa* complex (Diptera: Tephritidae). Mol. Phyl. Evol. 48(1): 270-280.

Virgilio, M., De Meyer, M., White, I. M., and Backeljau, T. 2009. African *Dacus* (Diptera: Tephritidae): Molecular data and host plant associations do not corroborate morphology based classifications, Mol. Phyl. Evol. 51(3): 531-539.

Vogelstein, B., and Gillespie, D. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

White, I. M., and Elson-Harris, M. M. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International, Wallingford and ACIAR, Camberra.