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MITOCHONDRIAL DNA MARKER ANALYSIS IN LABORATORY COLONIES OF THE *CERATITIS* FAR COMPLEX

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The *Ceratitis* FAR species complex currently consists of the African species *C. anonae, C. fasciventris*, *C. quilicii* and *C. rosa*. All members of the complex are economic important pests since they infest and destroy a variety of fruits and vegetables [1]. The discrimination of the complex's species is challenging due to the absence of robust morphological differences and the ongoing gene flow among them, causing problems to their management and control efforts [1,2]. Therefore, there is great interest for the exploration of their phylogenetic relationships and the development of robust molecular markers for their identification. Nowadays, it has become evident that resolution of species complexes requires multidisciplinary approaches, ideally applied on the same specimens, in the context of integrative taxonomy [3]. Here, we present the analysis of three mitochondrial sequences (COI-5', COI-3' and ND6) in specimens of five well-characterized *Ceratitis* FAR colonies.

•Colonies (*Insect Pest Control Laboratory, Seibersdorf, Austria)*

Ceratitis fasciventris *(F2)*-Kenya
Ceratitis rosa-Kenya (K)
Ceratitis rosa- South Africa (SA)
Ceratitis quilicii-Kenya (K)
Ceratitis quilicii-South Africa (SA)

•PCR amplification primers

COI5'	F: CTAAACTTCAGCCATTTAATCGC	670bp
	R: GGTATAAAATAGGGTCTCCTCC	
COI3'	F: ACGTCATTCTTTGACCCAGC	730 bp
	R: AATCCATTGCACTAATCGCC	
ND6	F: TAAAAACATTGGTCTTGTAAATC	520bp
	R: TTTTACTACAGCAATTAAAGTAA	

Polymorphism analysis

- Alignment by ClustalOmega revealed:
  - 8 nucleotide positions that consistently differed in *C. fasciventris* (Table 1).
  - 18 polymorphisms differentiating the *C. quilicii* SA from and *C. quilicii K* and the *C. rosa* (K and SA) specimens (Table 2).
- Haplotype analysis showed common haplotypes between *C. rosa* K and *C. quilicii* K.

Table 1. Nucleotide polymorphisms differentiating the *C. fasciventris* from the *C. rosa* and *C. quilicii* specimens analyzed. Positions in reference to the *C. fasciventris* mitogenome sequence KY436396.

Gene fragment	*COI 5'*			*COI 3'*			*ND6*	
Position	1.668	2.031	2.151	2.244	2.302	2.544	10.111	10.435
C. fasciventris	C	G	C	C	G/T	G	A	G
C. rosa; C. quilicii	T	A	T	T	A	A	T	A

Table 2. Nucleotide polymorphisms differentiating the *C. quilicii SA* specimens from and *C. quilicii K and* the *C. rosa* specimens analyzed. Positions in reference to the *C. fasciventris* mitogenome sequence KY436396.

Gene fragmant	COI 5'								COI 3'						ND6				
Position	1.746	1.833	1.875	1.980	2.028	2.070	2.101	2.133	2.472	2.622	2.805	2.829	2.838	2.850	10.072	10.435	10.450	10.453	
C. quilicii SA	A	C	T	T	A	T	T	C	A	T	T	T	A	T	T	T	T	C	
C. rosa K; SA; *C. quilicii* K	G	T	C	C	G	C	C	T	G	C	C	C	T	C	C	C	C	T	

Phylogenetic analysis

- Maximum likelihood analysis in MEGA 7 grouped the sequences of the five colonies into four clades (Figure 1).
- *C. rosa* K specimens were grouped either in the *C. rosa* SA or the *C. quilicii* K clades.

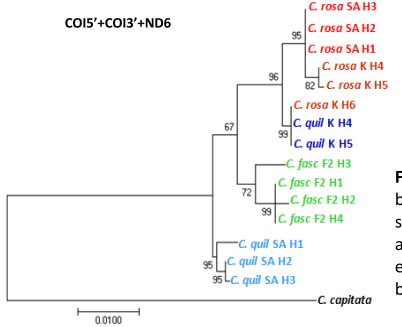

Figure 1. Phylogenetic tree based on the concatenated sequences of the fragments analyzed. H1-6: haplotypes for each analyzed species. Scale bar: 0.01 substitutions per site.

Conclusions

- ✓ Several SNPs that could be potential markers for discriminating among species and/or populations of the *Ceratitis* FAR complex were identified. However, further analysis is required for their evaluation.
- ✓ The mitochondrial sequences analyzed failed to clearly distinguish among *C. rosa* and *C. quilicii* colonies.
- ✓ Our results could contribute to multidisciplinary approaches towards the resolution of the *Ceratitis* FAR complex.

References

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Acknowledgements

We would like to thank Drs. Sunday Ekesi and Fathiya Khamis, as well as Dr Aruna Manrakhan for providing the biological material from Kenya and South Africa, respectively, for the establishment of the colonies in the Insect Pest Control Laboratory (Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria).