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A multiplex PCR to differentiate sibling species within the *Aedes (Ochlerotatus) detritus* complex

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Introduction

Aedes (Ochlerotatus) detritus s.l. is a complex of two genetically distinct sibling species which are morphologically indistinguishable : *Aedes detritus* (Haliday, 1833) and *Aedes coluzzii* (Rioux, Guilvard and Pasteur, 1998).

Aedes detritus (former sp B) is anautogenous, eurygame and mainly distributed in Northern Europe. Its larvae develop in brackish water with low salinity. *Aedes coluzzii* (former sp A) is autogenous, stenogame and mainly distributed in the Mediterranean region where it thrives in hypersaline environments such as salt marshes.

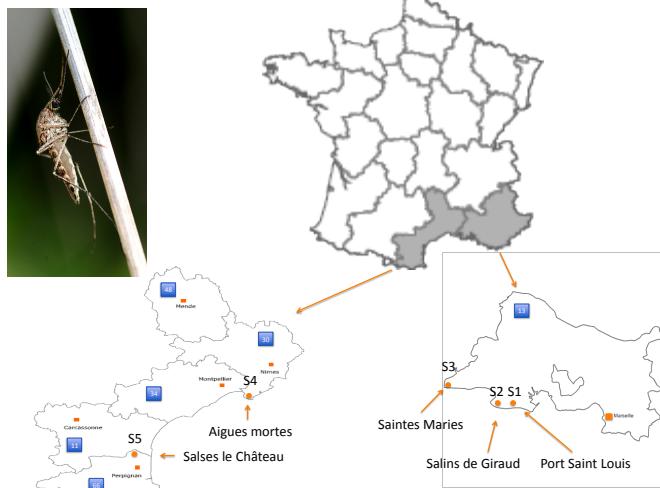
Both species can be found in France, occurring in some littoral regions (e.g., Mediterranean and Atlantic shores) where they are a heavy nuisance to humans and/or animals.

To date, species identification within the complex was only possible through iso-enzymes techniques.

Here, we present a straightforward multiplex PCR for distinguishing *Ae. detritus* and *Ae. coluzzii* in a single assay.

Methods and Findings

We explored molecular polymorphism in the Internal Transcribed Spacer 2 (ITS2) region of nuclear ribosomal DNA in wild mosquito samples collected from littoral areas of Southern France (Camargue and Pyrénées Orientales) where *Ae. detritus* and *Ae. coluzzii* are known to be sympatric.



Sequences analysis revealed fixed nucleotides differences defining two distinct haplotypes tentatively identified as specific of *Ae. detritus* and *Ae. coluzzii*, respectively.

CoDeUN →
Ae_coluzzii CAGTACGAGGTACACATTGAGTGCTATATTATCTATTCAACTATACTGCATGCCGTTTC
Ae_detritus CAGTACGAGGTACACATTGAGTGCTATATTATCTATTCAACTATACTGCATGCCGTTTC

Ae_coluzzii ACTTCGGGTGACAGCCGATGGCCAGGAAGCAGTATGCGTAGTGACGTTTCCGCCCTTCGGT
Ae_detritus ACTTCGGGTGACAGCCGATGGCCAGGAAGCAGTATGCGTAGTGACGTTTCCGCCCTTCGGT

Ae_coluzzii GCGCGGTAACACGTTAAGATAGTCAGCCGCTTCCACGACGGTTGATGAATACATCCCATA
Ae_detritus GCGCGGTAACACGTTAAGATAGTCAGCCGCTTCCACGACGGTTGATGAATACATCCCATA

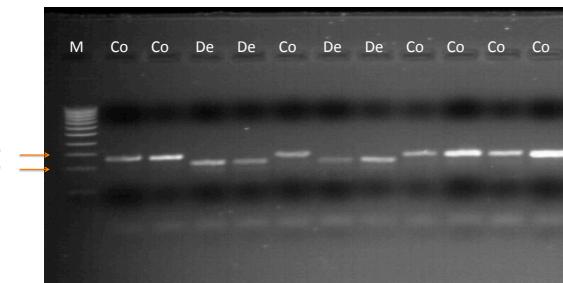
Ae_coluzzii CC-ATAGAATAGGGTTATGTTGATTCACACCGGATCACCCCGCCATA--ATTCCA ← AeCo
Ae_detritus CCCACACAGAGGGTTATGTTGATTCACACCGGATCACCCCGCCATA--ATTCCA

Ae_coluzzii TTCCATTCCAGTAGGCCCTCAAAATATGTGACTACCCCTGAATTAAAGCATAA ← AeDe
Ae_detritus TTCCATTCCAGTAGGCCCTCAAAATATGTGACTACCCCTGAATTAAAGCATAA

Primers were designed based on fixed sequence differences between haplotypes to develop a multiplex PCR for identification of each species within the complex.

Species	Primer code	Primer nucleotide sequence (5'-3')	Tm (°C)	Lenght of specific PCR product (bp)
Universal primer	CoDeUN	AGTACGAGGTACACATTGAG	46	—
<i>Ae. coluzzii</i>	AeCo	TGGATTATGGTGGACCG	54	255
<i>Ae. detritus</i>	AeDe	ACAACATAACCCCTCTGG	54	210

PCR reactions using the three primers produced the expected pattern of size variation in field specimens identified as *Ae. detritus* and *Ae. coluzzii*.



Concluding remarks

- ✓ The multiplex PCR that we have developed is simple, robust and sensitive. Potentially, it can be used on any developmental stage of the mosquito and with both sexes.
- ✓ This new tool prompts for bio-ecological studies to refine basic knowledge on the bionomics of this complex system, towards a better control and prevention of ensuing nuisances.
- ✓ Validation tests are currently carried out using strains of various geographic origins.