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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.

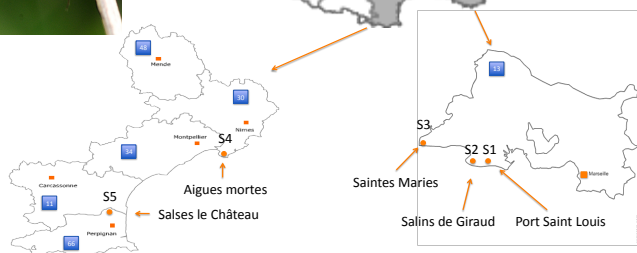
Both species can be found in France, occurring in some littoral regions (e.g., Mediterranean and Atlantic shores) where they are a heavy nuisance to humans and/or animals.

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.



Sites	Locality (N° Department)	Collection date	N. mos	Species
S1	Port-Saint-Louis-du-Rhône (13)	06/05/2011	6	<i>Ae. coluzzii</i> (1) / <i>Ae. detritus</i> (5)
S2	Salins-de-Giraud (13)	21/04/2011	6	<i>Ae. coluzzii</i> (3) / <i>Ae. detritus</i> (3)
S2	Salins-de-Giraud (13)	06/05/2011	6	<i>Ae. coluzzii</i> (4) / <i>Ae. detritus</i> (2)
S2	Salins-de-Giraud (13)	17/06/2011	6	<i>Ae. coluzzii</i> (5) / <i>Ae. detritus</i> (1)
S3	Saintes-Maries-de-la-Mer (13)	03/05/2011	6	<i>Ae. coluzzii</i> (3) / <i>Ae. detritus</i> (3)
S3	Saintes-Maries-de-la-Mer (13)	10/08/2011	6	<i>Ae. coluzzii</i> (6)
S4	Aigues-Mortes (30)	28/04/2011	6	<i>Ae. coluzzii</i> (5) / <i>Ae. detritus</i> (1)
S4	Aigues-Mortes (30)	21/06/2011	6	<i>Ae. coluzzii</i> (5) / <i>Ae. detritus</i> (1)
S5	Salses le Château (66)	11/05/2011	6	<i>Ae. detritus</i> (6)

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

```

CoDeUN
Ae_coluzzii CAGTACGAGGTACACATTTTGGAGTGCCTATATTTATCTATCAACTATACGTGATGGCGGTTTC
Ae_detritus CAGTACGAGGTACACATTTTGGAGTGCCTATATTTATCTATCAACTATACGTGATGGCGGTTTC

Ae_coluzzii ACTTCGGGTGGACAGGCGCATGGCCAGGAAGCAGTATGCGTAGTGACGTTTTCCCGCCTTCGGT
Ae_detritus ACTTCGGGTGGACAGGCGCATGGCCAGGAAGCAGTATGCGTAGTGACGTTTTCCCGCCTTCGGT

Ae_coluzzii GCGCGGTAAAACGTTCAAGATAGTCAGGCGGTCGCCACGACGTGGTGAATACATCCCATACA
Ae_detritus GCGCGGTAAAACGTTCAAGATAGTCAGGCGGTCGCCACGACGTGGTGAATACATCCCATACA

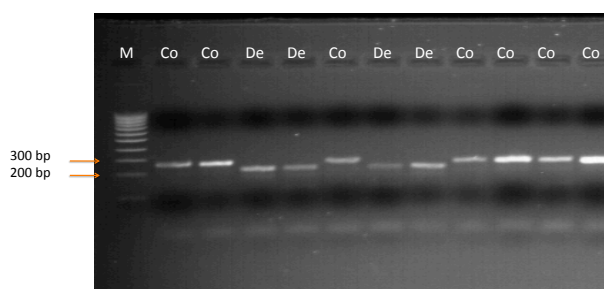
Ae_coluzzii CC-ATAGAATAGGGTTTATGTTGATTCATCACACCGGATCACCCCGGTCCACCATA--ATTCCA
Ae_detritus CCCACACAGAGGGTTATGTTGATTCATCACACCGGATCACCG-GATCCACCATCCCATCCA

Ae_coluzzii TTCCATTCCAGTAGGCGCTCAATAATGTGTGACTACCCCTGAATTTAAGCATAA
Ae_detritus TTCCATTCCAGTAGGCGCTCAATAATGTGTGACTACCCCTGAATTTAAGCATAA
    
```

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)	Length of specific PCR product (bp)
Universal primer	CoDeUN	AGTACGAGGTACACATTTTGGAG	46	-
<i>Ae. coluzzii</i>	AeCo	TGGAATTATGGTGGACCG	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.