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.

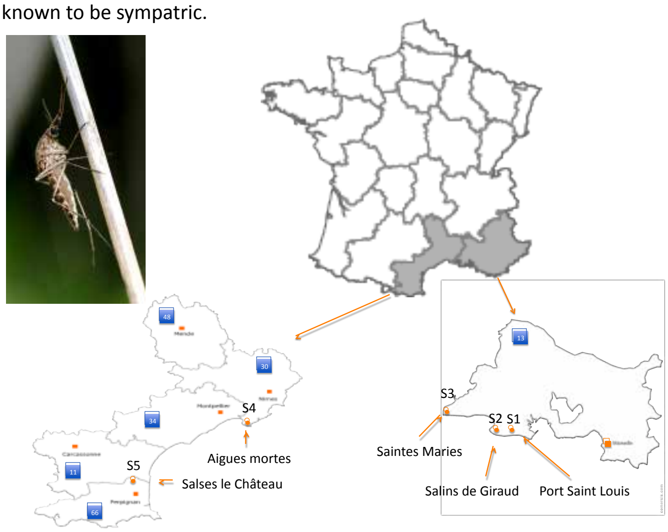
Both species can be found in France, occurring in some littoral regions (e.g., Mediterranean and Atlantic shores) where they are a heavy nuisance to humans and/or animals.

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	*Ae. coluzzii* (1) / *Ae. detritus* (5)
S2	Salins-de-Giraud (13)	21/04/2011	6	*Ae. coluzzii* (3) / *Ae. detritus* (3)
S2	Salins-de-Giraud (13)	06/05/2011	6	*Ae. coluzzii* (4) / *Ae. detritus* (2)
S2	Salins-de-Giraud (13)	17/06/2011	6	*Ae. coluzzii* (5) / *Ae. detritus* (1)
S3	Saintes-Maries-de-la-Mer (13)	03/05/2011	6	*Ae. coluzzii* (3) / *Ae. detritus* (3)
S3	Saintes-Maries-de-la-Mer (13)	10/08/2011	6	*Ae. coluzzii* (6)
S4	Aigues-Mortes (30)	28/04/2011	6	*Ae. coluzzii* (5) / *Ae. detritus* (1)
S4	Aigues-Mortes (30)	21/06/2011	6	*Ae. coluzzii* (5) / *Ae. detritus* (1)
S5	Salses le Château (66)	11/05/2011	6	*Ae. detritus* (6)

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

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                      ——— CoDeUN ———>
Ae_coluzzii CAGTACGAGGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTATACGTGCATGCGCGTTTC
            ..................................................................
Ae_detritus CAGTACGAGGTACACATTTTTGAGTGCCTATATTTATCTATTCAACTATACGTGCATGCGCGTTTC

Ae_coluzzii ACTTCGGGTGGACAGGCGCATGGCCCAGGAAGCACGTATGCGTAGTGACGTTTTCCCGCCTTCGGT
            ..................................................................
Ae_detritus ACTTCGGGTGGACAGGCGCATGGCCCAGGAAGCACGTATGCGTAGTGACGTTTTCCCGCCTTCGGT

Ae_coluzzii GCGCGGTAAAACGTTCAAGATAGTCAGGCGCGTCCCACGACGTGGTTGATGAATACATCCCATACA
            ..................................................................
Ae_detritus GCGCGGTAAAACGTTCAAGATAGTCAGGCGCGTCCCACGACGTGGTTGATGAATACATCCCATACA
                                                         <——— AeCo ———
Ae_coluzzii CC-ATAGAATAGGGTTTATGTTGTATTCCATCACACCGGATCACCCCGGTCCACCATA--ATTCCA
            ..*.*.**.*....*..............................**.*........***......
Ae_detritus CCCACACCAGAGGGGTTATGTTGTATTCCATCACACCGGATCACCG-GATCCACCATTCCATTCCA
                  <—— AeDe ———
Ae_coluzzii TTCCATTCCAGTAGGCCTCAAATAATGTGTGACTACCCCCTGAATTTAAGCATAA
            .......................................................
Ae_detritus TTCCATTCCAGTAGGCCTCAAATAATGTGTGACTACCCCCTGAATTTAAGCATAA
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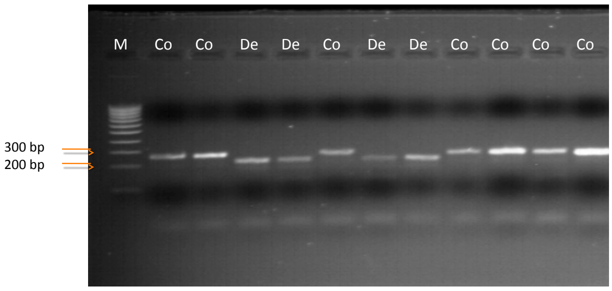
Primers were designed based on fixed sequence differences between haplotypes to develop a multiplex PCR for identification of each species within the complex.

Species	Primer code	Primer nucleotide sequence (5'-3')	Tm (°C)	Lenght of specific PCR product (bp)
Universal primer	CoDeUN	AGTACGAGGTACACATTTTTGAG	46	_
Ae. coluzzii	AeCo	TGGAATTATGGTGGACCG	54	255
Ae. detritus	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.