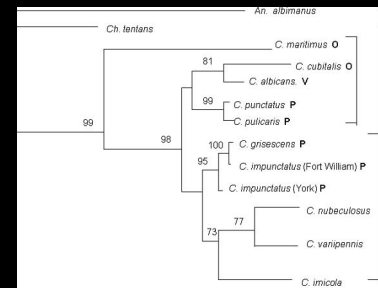
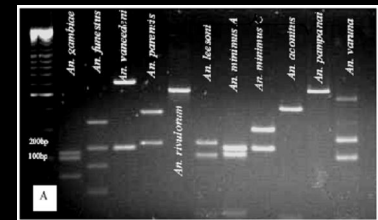
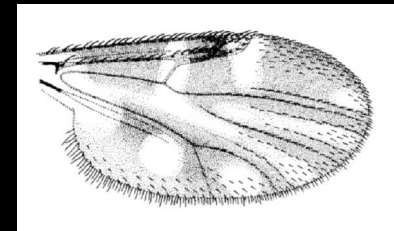
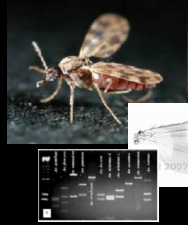


Systematics and taxonomy of the genus *Culicoides* what is coming next?

Claire Garros¹, Bruno Mathieu², Thomas Balenghien¹,
Jean-Claude Delécolle²

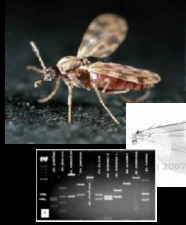
¹CIRAD, Montpellier, France
²IPPTS, Strasbourg, France





Molecular approach for an integrative taxonomy

- Morphology-based taxonomy of the genus *Culicoides* has reached its apogee (roughly 1250 species described in 36 subgenera) however, morphological or behavioral similarities do not reflect phylogenetic relationships
- The internal classification of *Culicoides* is problematic with four main problems
 - phylogenetic relationships between and within subgenera are unknown
 - lack of uniformity in taxonomic categories
 - various species-groups not yet investigated on a worldwide basis
 - the entire classification has not been published in its entirety
- The *Culicoides* classification remains untested for its accurate representation of the *Culicoides* phylogeny. This makes the classification difficult to use as a model for systematic and phylogenetic studies and hypotheses, or biogeographical scenarios

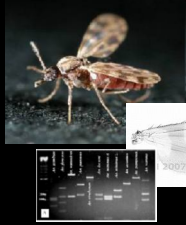


Molecular approach for an integrative taxonomy

- Molecular approach in insect systematics and taxonomy is now recognized as essential to
 - overcome taxonomic impediments
 - propose adequate phylogenetic hypotheses
 - validate systematic schemes
 - produce DNA barcoding to help species identification
 - develop reliable molecular identification assays

Molecular approach must be integrative into taxonomy practice without supplanting classical taxonomy

Morphological and molecular approaches together hold promise for elucidating relationships within species complexes and between closely related species



The systematic imbroglio of the genus *Culicoides*

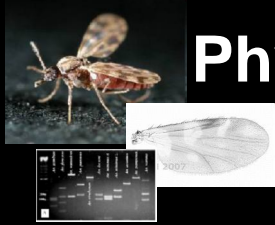
Imbroglio in

Phylogenetic relationships

Relationships between and within sugenera are unknown

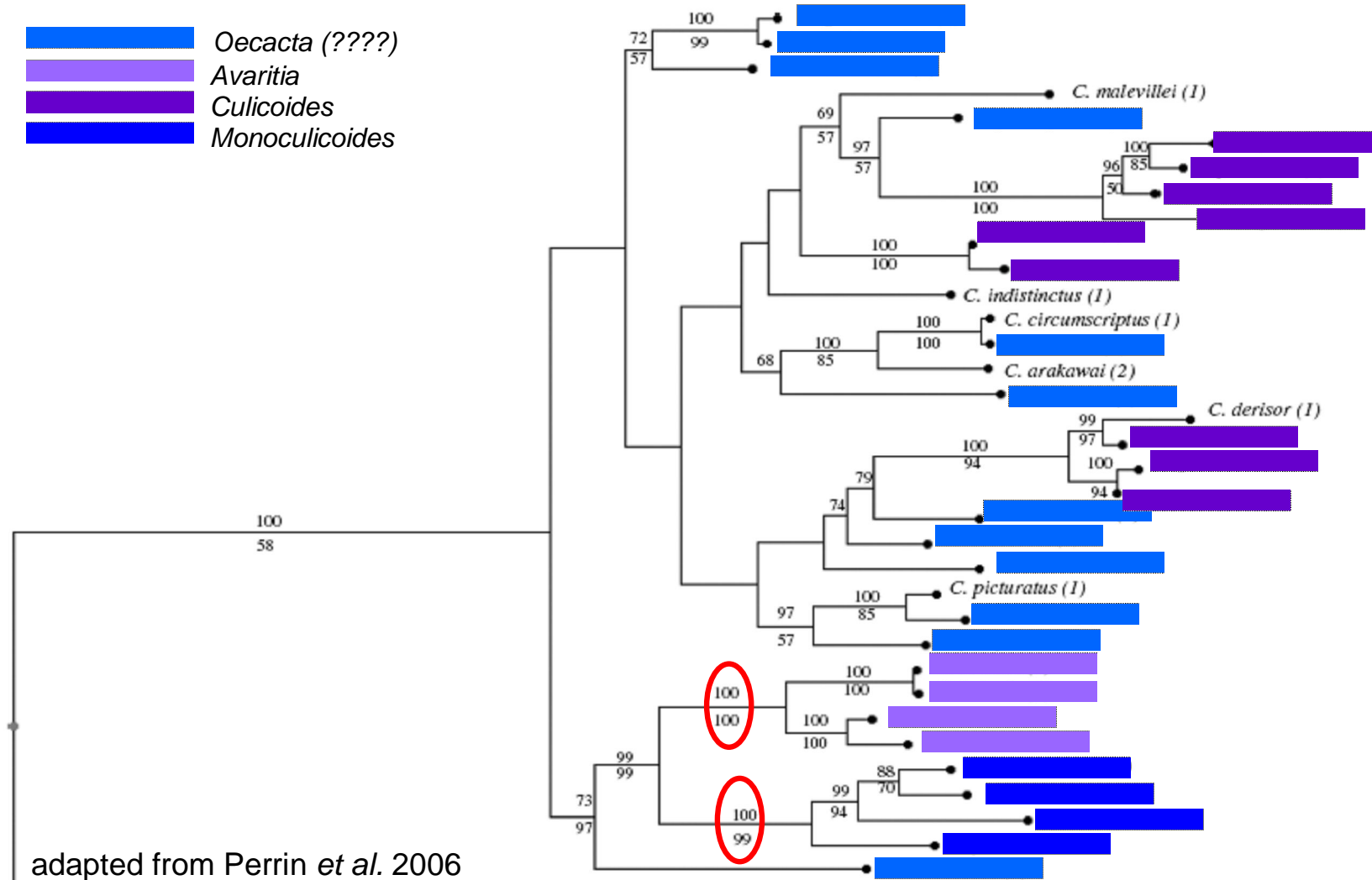
Subgenera are not monophyletic

Unknown relationships within groups/complexes

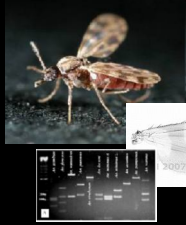


Phylogenetic relationships between subgenera

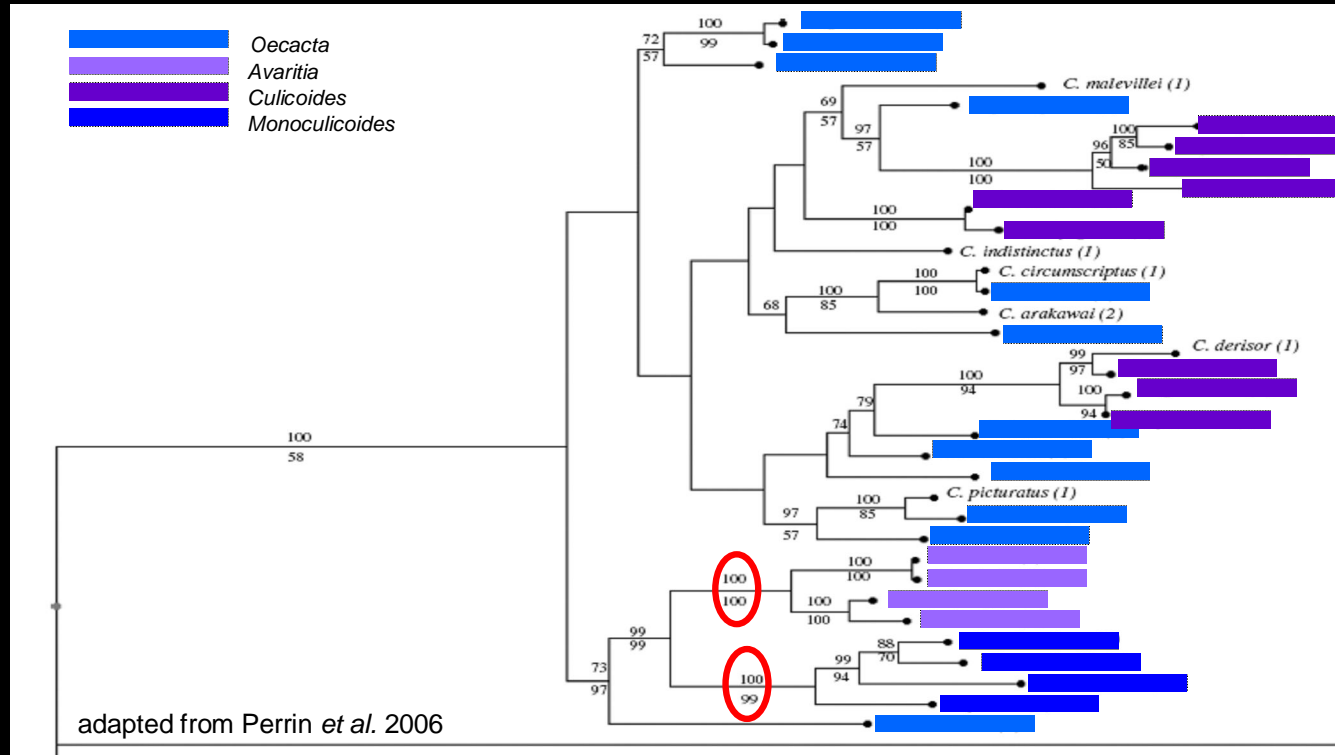
- Oecacta* (????)
- Avaritia*
- Culicoides*
- Monoculicoides*



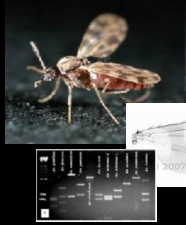
adapted from Perrin *et al.* 2006



Phylogenetic relationships between subgenera









- subgenus *Oecacta*: clearly paraphyletic subgenus
- subgenus *Culicoides*: clearly paraphyletic subgenus
- subgenera *Avaritia* and *Monoculicoides*: limited sampling representation but it seems monophyletic



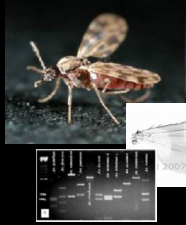
Phylogenetic relationships within subgenus

i.e. within the subgenus *Avaritia*

6 species groups (and 1 species subgroup) (from Meinswinkel 2004)

	group	Andicola	
		Grahamii	
Itoua & Cornuet 1986		Imicola	 
Gomulski et al. 2005		Orientalis	 
		Obsoletus	
		Pusillus	
	subgroup	Pseudopallidipennis	

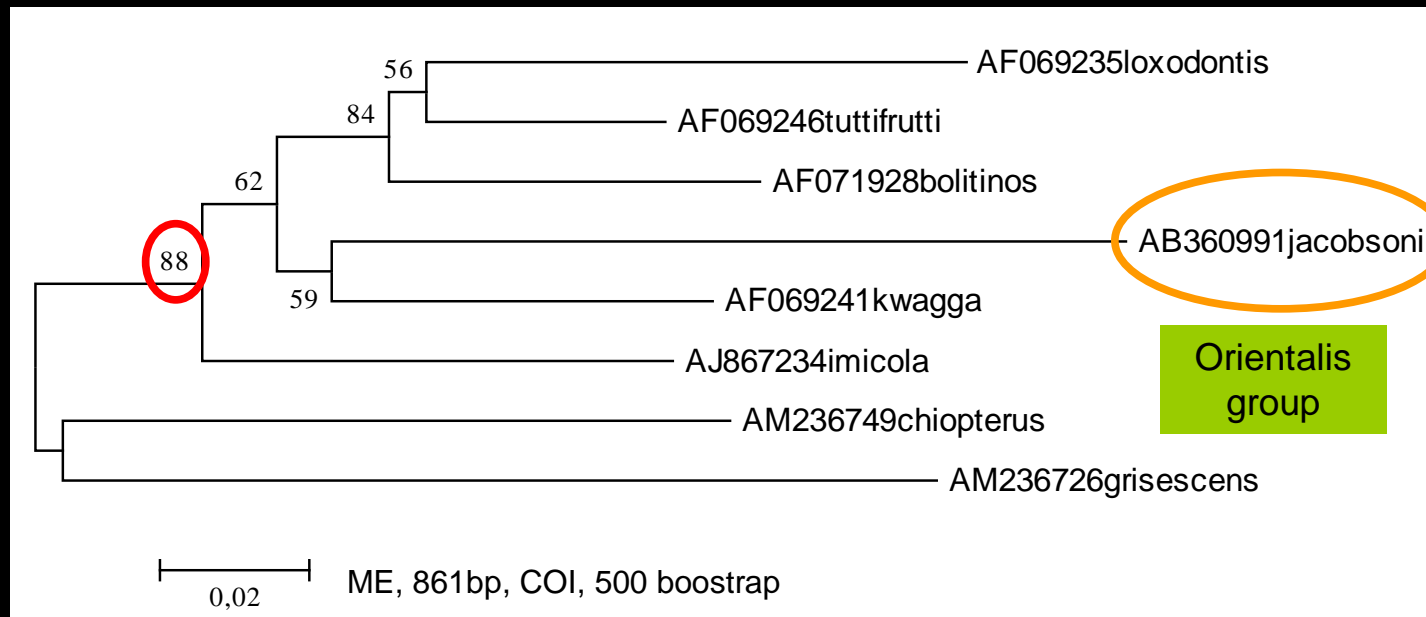
Taxonomic categories are basically subjective groupings. There is a need to confirm the monophyletic status of these groupings and their relationships

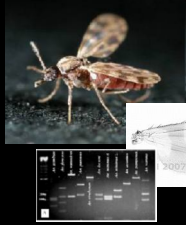


Phylogenetic relationships within subgenus

i.e. within the subgenus *Avaritia*

Relationships between *Imicola* and *Orientalis* Group (Meinswinkel 2004)





Phylogenetic relationships within groups

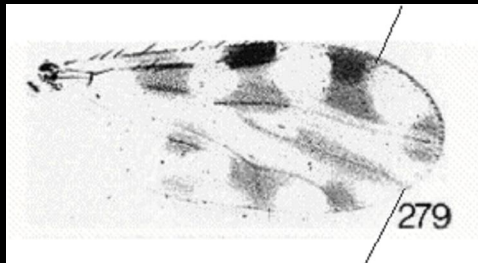
i.e. within Imicola group

Relationships between the 9 species of the Imicola group

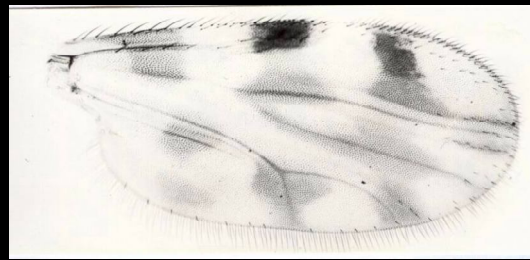
C. bolitinos, *C. brevitarsis* and *C. imicola*

C. imicola and *C. nudipalpis*

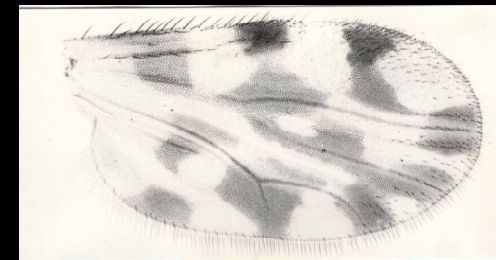
Confirmation of the presence of *C. tuttifrutti* and *C. kwagga*



C. brevitarsis



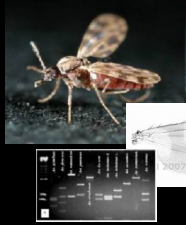
C. bolitinos



C. imicola

	<i>jacobsoni</i>	<i>brevitarsis</i>	<i>bolitinos</i>	<i>imicola</i>
<i>jacobsoni</i>	x			
<i>brevitarsis</i>	82.9	x		
<i>bolitinos</i>	83.1	89.2	x	
<i>imicola</i>	82.5	87.7	86.9	x

Sequence identity matrix (COI)



Phylogenetic relationships within groups

Within Imicola group

Relationships between the 9 species of the Imicola Group

C. bolitinos, *C. brevitarsis* and *C. imicola*

C. imicola and *C. nudipalpis*

Confirmation of the presence of *C. tuttifrutti* and *C. kwagga*

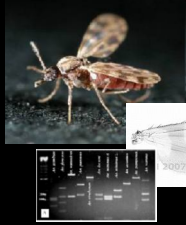
Within Obsoletus group

Relationships between *C. obsoletus*, *C. montanus* and *C. scoticus*
and the other putative related species

Within Pulicaris group

Relationships between *C. newsteadi*, *C. lupicaris*, and *C. pulicaris*

Not on limited taxonomic sampling but on wide-ranging taxonomic sampling



The systematic imbroglio of the genus *Culicoides*

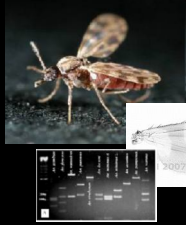
Imbroglio

Phylogenetic relationships

Relationships between and within sugenera are unknown
Subgenera are not monophyletic
Unknown relationships within groups/complexes

Systematic scheme

Taxonomic categories are not well defined
Regional approaches with limited taxonomic sampling

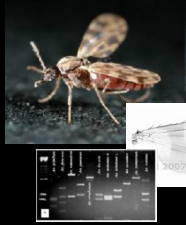


Systematic scheme and taxonomic categories

- The International Code of Zoological Nomenclature recognizes 11 ranks in which
 - genus
 - subgenus
 - species group
- **A nice example from mosquitoes**
 - A systematic review was first published in 1994 by Ralph Harbach, and then updated in 2004
 - Informal categories are used but with a clear intended meaning of each category (genus, subgenus, group, subgroup, complex) and a printing rule

Ralph Harbach. 1994. Review of the internal classification of the genus *Anopheles* (Diptera: Culicidae): the foundation for comparative systematics and phylogenetic research. Bull Entomol Res 84: 331-342

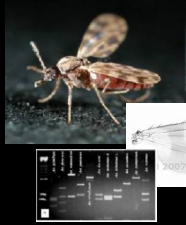
Ralph Harbach. 2004. The classification of the genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. Bull Entomol Res 94: 537-553



Systematic scheme and taxonomic categories

- Recommendations have already been made
 - Meiswinkel (2004)
 - Complex should be used instead of group
 - Species complex is employed to group closely related species showing synapomorphies and phylogenetically related

However, it is not satisfactory as it does not allow to distinguish species that speciate recently from the ones that are less related



Systematic scheme and taxonomic categories

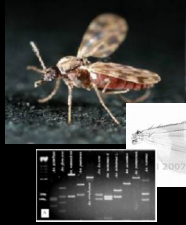
New recommendation

Species group is a grouping of phylogenetically closely related species. Female diagnose is possible on wing pattern for example

Species complex is a grouping of phylogenetically closely related species for which no morphological characters are known to differentiate them or for which it is difficult to differentiate them in one adult stage.

=> **speciation event probably very recent**

The species belonging to a complex are called **sibling species** or cryptic species



Systematic scheme and taxonomic categories

Printing rule for informal group names

Informal group names are treated as vernacular names.

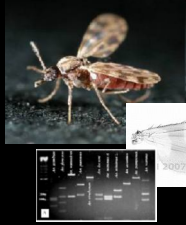
Names are printed in roman type with the first letter capitalized even though the name of a nominal species is used in the combination.

The oldest species gives its name to the group or complex species.

Obsoletus Group rather than *obsoletus* Group or *C. obsoletus* Group

or

Obsoletus group rather than *obsoletus* group or *C. obsoletus* group



Systematic scheme and taxonomic categories

Example

Subgenus *Avaritia*

C. sinanoensis

C. sanguisuga

C. gornostaevae

C. montanus

C. obsoletus

C. scoticus

} Obsoletus complex

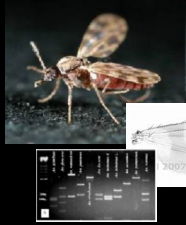
} Obsoletus group

C. chiopterus

C. dobyi

} Chiopterus group

C. dewulfi



Systematic scheme and taxonomic categories

Australasian/Oriental species

Afrotropical species

Applications to the genus *Culicoides*

Imicola group

C. brevitarsis

C. bolitinos

C. imicola

C. kwagga

C. loxodontis

C. miombo

C. nudipalis

C. pseudopallidipennis

C. tuttifrutti



Obsoletus group

C. chiopterus

C. gornostaevae

C. montanus

C. sanguisuga

C. sinanoensis

Obsoletus complex

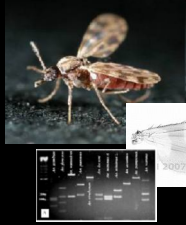
C. obsoletus

C. scoticus

Dewulfi group

C. dewulfi





The systematic imbroglio of the *Culicoides* genus

Imbroglio

Phylogenetic relationships

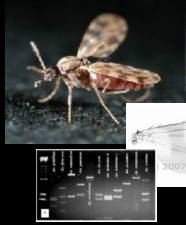
Relationships between and within sugenera are unknown
Subgenera are not monophyletic

Systematic scheme

Taxonomic categories are not well defined
Regional approaches with limited taxonomic sampling

Taxonomy

Existence of sibling species/synonymies/new species
Difficulties in morphological identification
Few molecular identification assays



Hypotheses of new species

- Description of new species ?
 - *C. newsteadi* form A and form B



C. newsteadi
typical wing

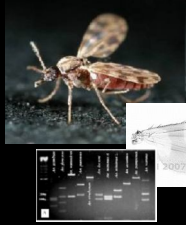


form A



form B

- *C. pulicaris* and *C. pulicaris* dark

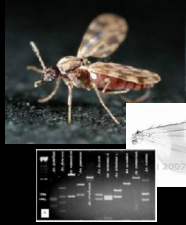


Molecular identification assays

- **Molecular identification assays**

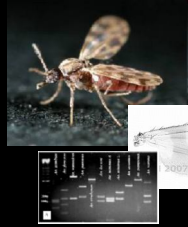
- *C. imicola* RT quantitative PCR (Cêtre-sossah et al. 2008)
- Obsoletus Group Multiplex Allele-Specific PCR (Mathieu et al. 2007)
Multiplex Allele-Specific PCR (Nolan et al. 2007)
- Pulicaris Group Allele-Specific PCR (Nolan et al. 2007)
- and more ...

A barcoding database based on accurate and reliable identification of voucher specimens is needed



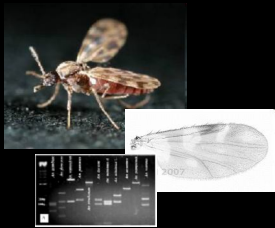
Conclusions

- **Update of the internal classification of the genus *Culicoides***
 - Need for a review of the internal classification of the genus with a special interest for the main subgenera (*Avaritia*, *Culicoides*, *Monoculicoides*)
- **Molecular phylogenetic analyses**
 - Use of different type of markers to produce robust phylogenetic hypotheses
- **Development of morphological tools to help identification**
 - Multi-access online keys for both wing pattern and microscopic characters are on-going



Conclusions

- **Development and validation of molecular identification assays**
 - The second ring trial will help in validating molecular assays
 - Barcoding database with voucher specimens accurately identified
- **Sequencing of new molecular markers to infer intraspecific and interspecific species variations**
 - rDNA
 - mtDNA
- **Large population sampling to investigate specific status of putative species**
 - Exchange of biological material is mandatory
 - Combination of morphological analyses and molecular work



Thank you for your attention



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