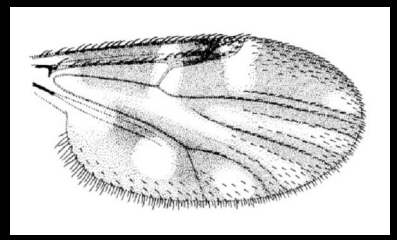
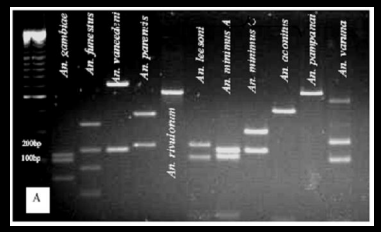
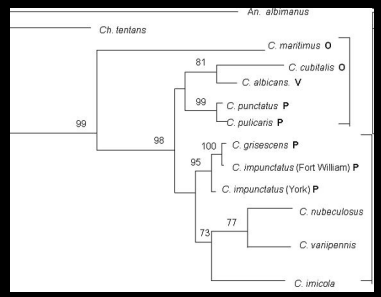
Systematics and taxonomy of the genus *Culicoides* what is coming next?



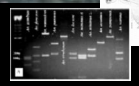




Claire Garros[1], Bruno Mathieu[2], Thomas Balenghien[1], Jean-Claude Delécolle[2]

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

MedReoNet, taxonomy meeting, 17-19th March, Strasbourg, France

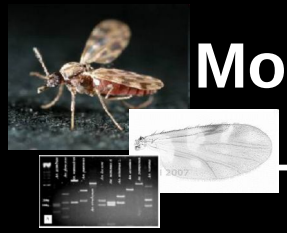


Molecular approach for an integrative taxonomy

- 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 this 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 scenarii

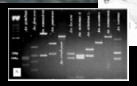


Molecular approach for an integrative taxonomy

- 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

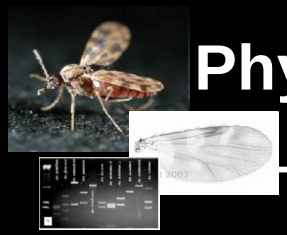


The systematic imbroglio of the genus *Culicoides*

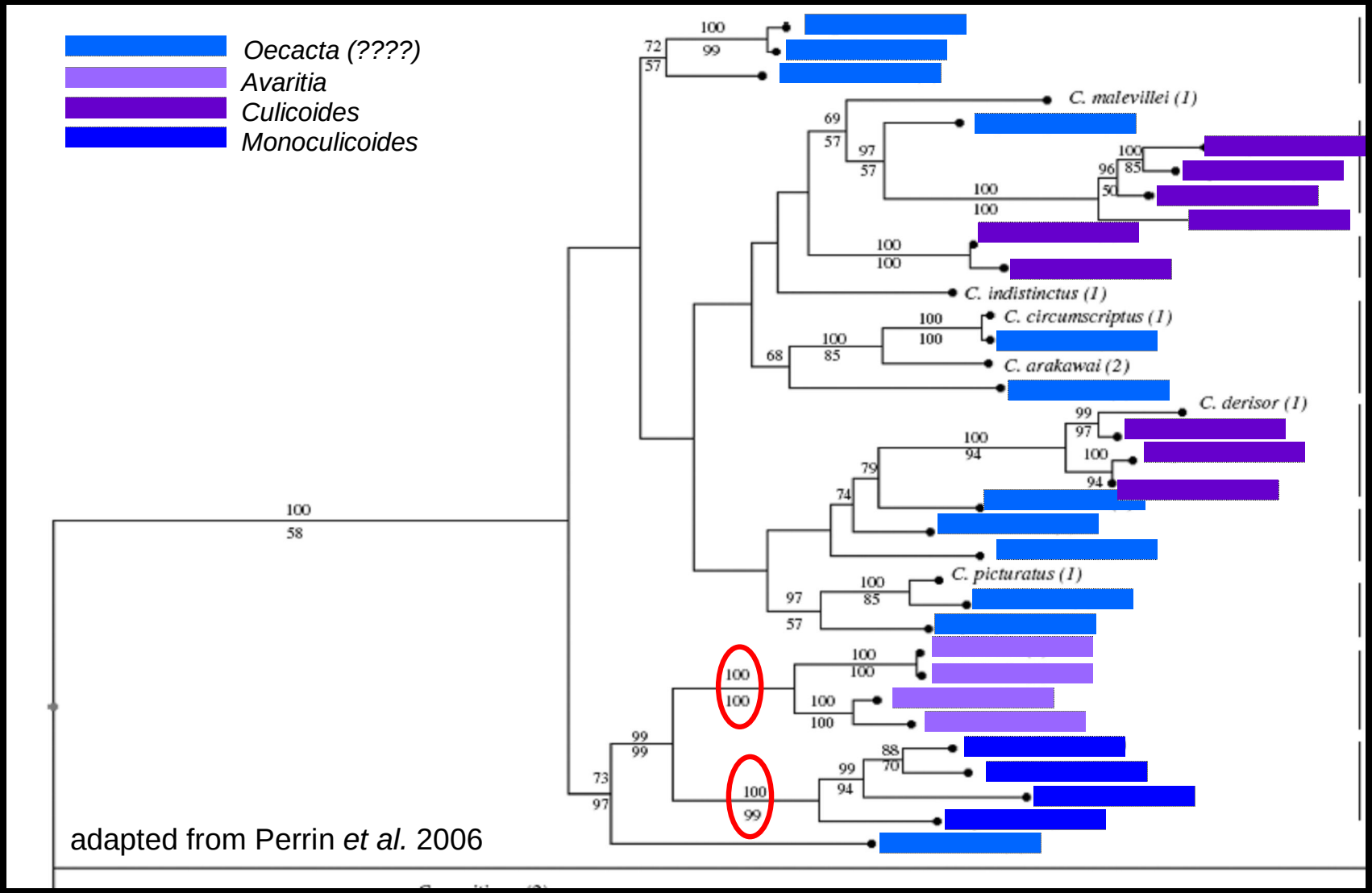
Imbroglio in

Phylogenetic relationships

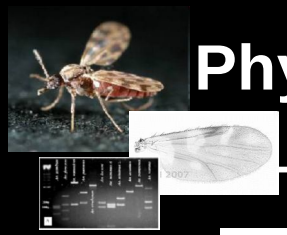
Relationships between and within sugenera are unknown
Subgenera are not monophyletic
Unknown relationships within groups/complexes



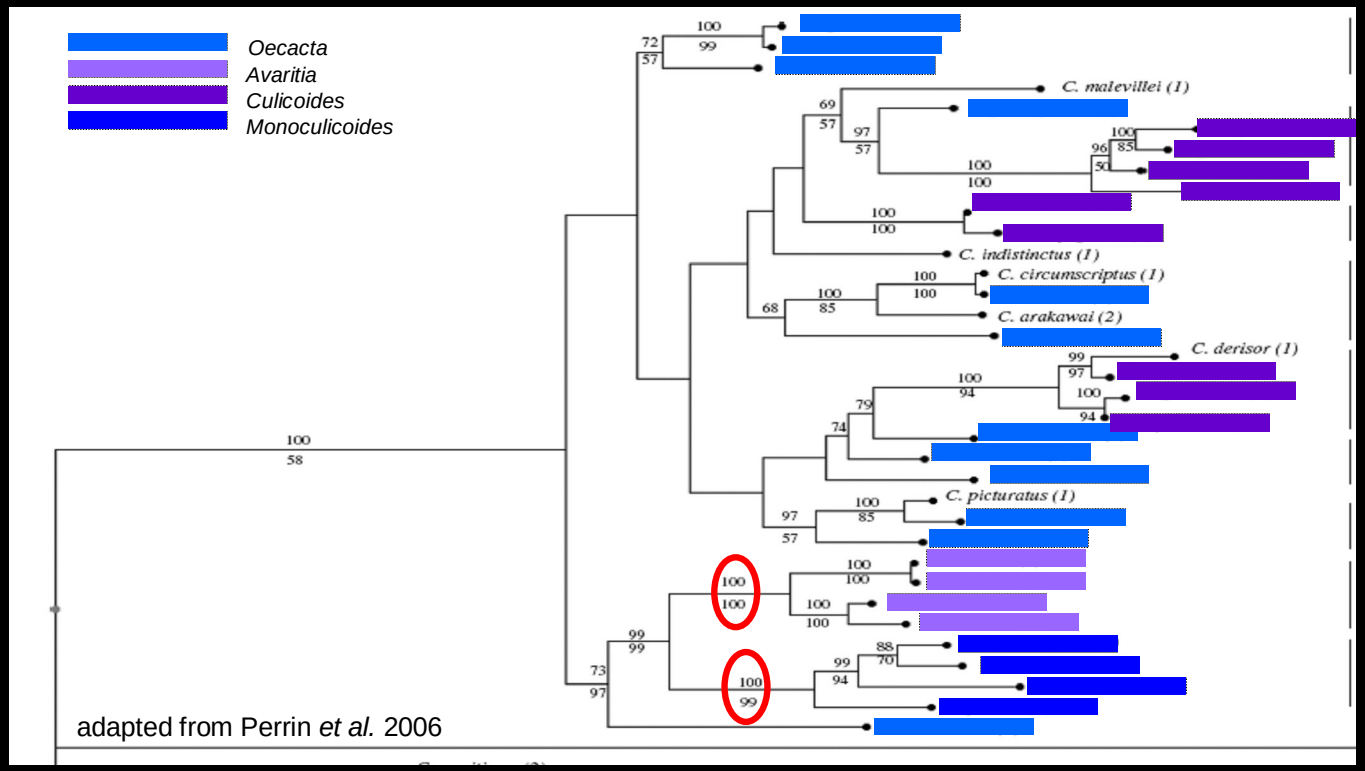
Phylogenetic relationships between subgenera



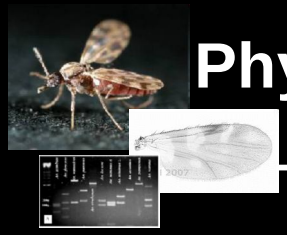
adapted from Perrin *et al.* 2006



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

Imicola

Orientalis

Obsoletus

Pusillus

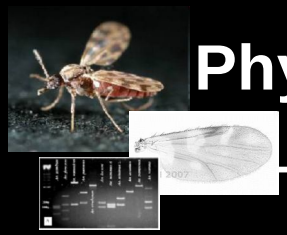
subgroup

Pseudopallidipennis

Itoua & Cornuet 1986

Gomulski et al. 2005

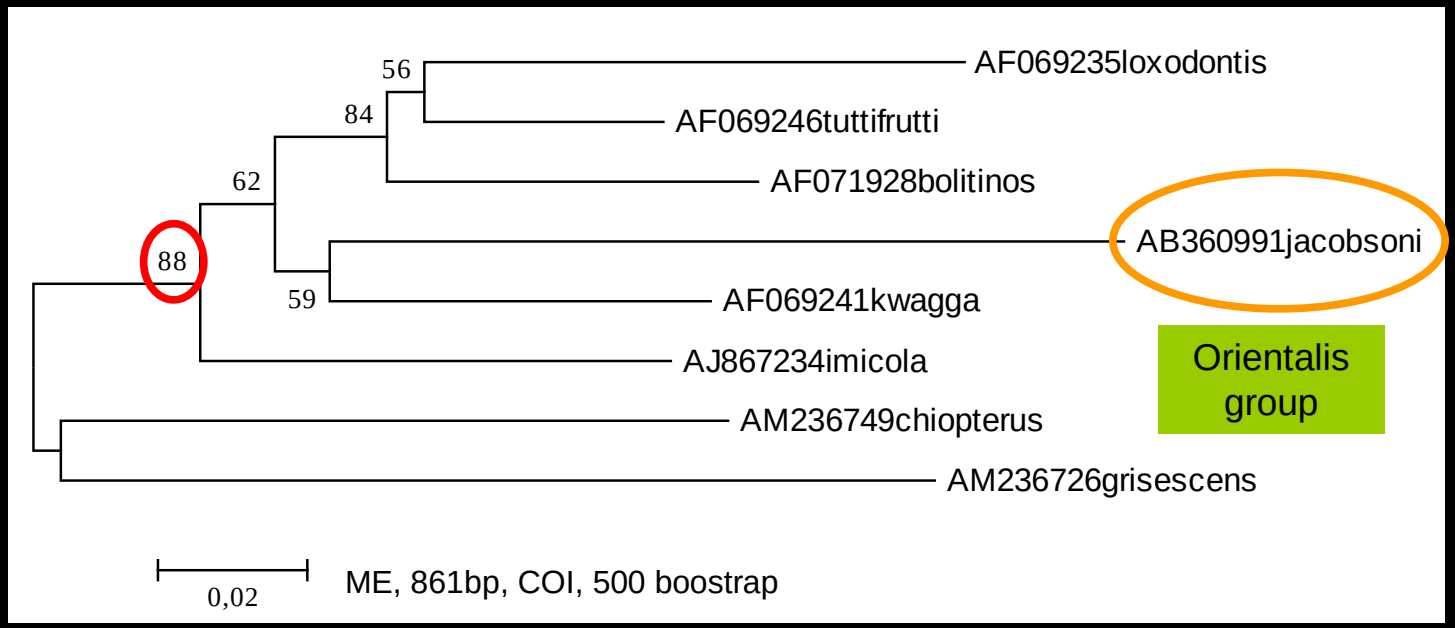
Taxonomic categories are basically subjective groupings. There is a need to confirm the monophyletic status of these groupings and their relationships

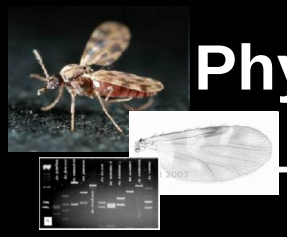


Phylogenetic relationships within subgenus

i.e. within the subgenus *Avaritia*

Relationships between Imicola and Orientalis Group (Meinswinkel 2004)





Phylogenetic relationships within groups

i.e. within Imicola group

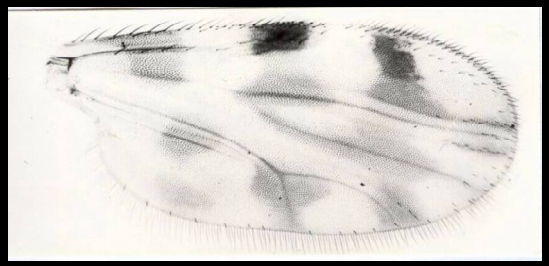
Relationships between the 9 species of the Imicola group

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

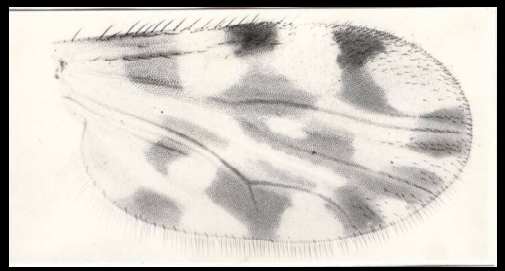
C. imicola and *C. nudipalpis*

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

C. brevitarsis



C. bolitinos

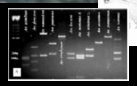


C. imicola

	jacobsoni	*brevitarsis*	*bolitinos*	*imicola*
jacobsoni	x			
brevitarsis	82.9	x		
bolitinos	83.1	**89.2**	x	
imicola	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*

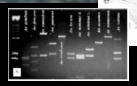
Within Obsoletus group

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*

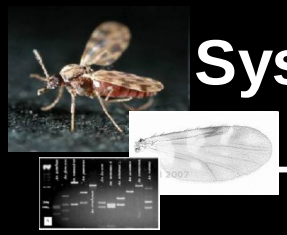
Imbroglio

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



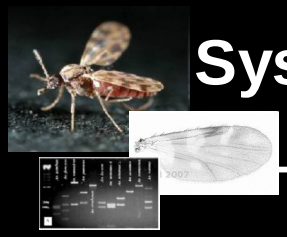
Systematic scheme and taxonomic categories

- The Internationl 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



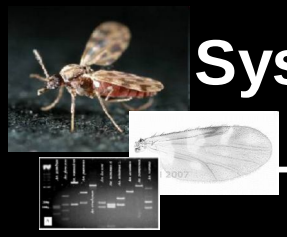
Systematic scheme and taxonomic categories

- 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

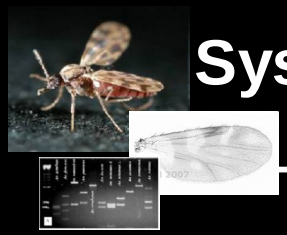
New recommendation

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 caracters are known to differentiate them or for which it is difficult to differenciate 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.

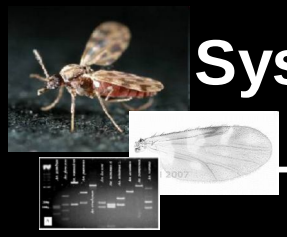
Names are printed in roman type with the first letter capitalized even though the name of a nominal species is used in the combination.

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

<u>Example</u>

Subgenus *Avaritia*

C. sinanoensis
C. sanguisuga
C. gornostaevae
C. montanus
C. obsoletus
C. scoticus

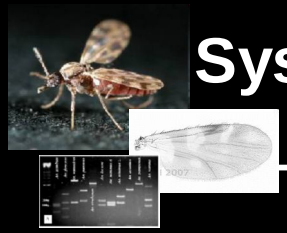
Obsoletus complex

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

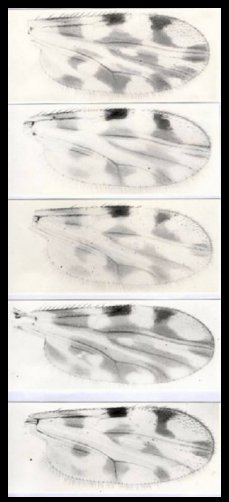
C. loxodontis

C. miombo

C. nudipalis

C. pseudopallidipennis

C. tuttifrutti



Obsoletus group

C. chiopterus

C. gornostaevae

C. montanus

C. sanguisuga

C. sinanoensis

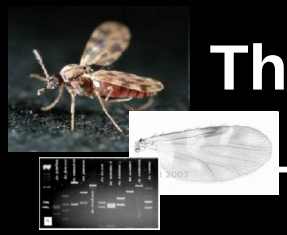
Obsoletus complex

C. obsoletus

C. scoticus

Dewulfi group

C. dewulfi



The systematic imbroglio of the *Culicoides* genus

Imbroglio

Phylogenetic relationships

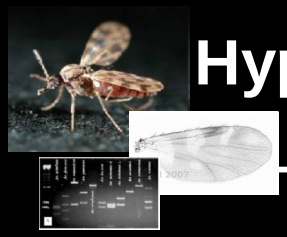
Relationships between and within sugenera are unknown
Subgenera are not monophyletic

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

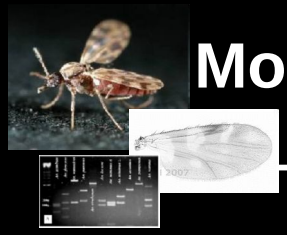
- Description of new species ?
  - *C. newsteadi* form A and form B

C. newsteadi
typical wing

form A

form B

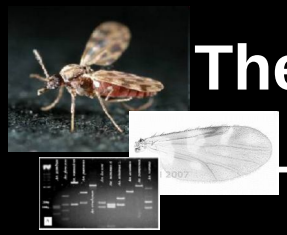
  - *C. pulicaris* and *C. pulicaris* dark



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



The GenBank … be very cautious

NCBI

Entrez PubMed

Search for as c

Display 3 levels using filter: none

Nucleotide Nucleotide EST Nucleotide GSS Protein

Popset SNP 3D Domains Domai

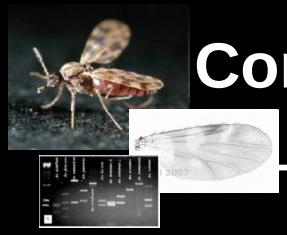
UniSTS PubMed Central Gene Homol

LinkOut BLAST TRACE Taxon

Lineage (full): root; cellular organisms; Eukaryota; Fungi/

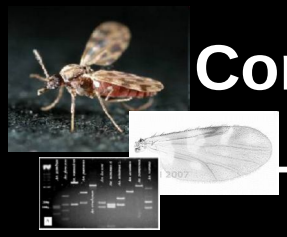
Diptera; Nematocera; Culicimorpha; Chironomoidea; Cer

- Culicoides (punkies) *Click on organism name to get more*
  - Avaritia
    - Culicoides actoni
    - Culicoides brevipalpis
    - Culicoides brevitarsis
    - Culicoides chiopterus
    - Culicoides dewulfi
    - Culicoides jacobsoni
    - Culicoides maculatus
    - Culicoides montanus
    - Culicoides obsoletus
    - Culicoides scoticus
    - Culicoides wadai
    - imicola species complex
      - Culicoides bolitinos
      - Culicoides imicola
      - Culicoides kwagga
      - Culicoides loxodontis
      - Culicoides pseudopallidipennis
      - Culicoides tuttifrutti
    - Culicoides sp. LMG-2004A
    - Culicoides sp. LMG-2004B
  - Beltranmyia
    - Culicoides arakawae (biting midge)
    - Culicoides circumscriptus
    - Culicoides japonicus
  - Culicoides
    - Culicoides aterinervis
    - Culicoides cylindratus
    - Culicoides deltus
    - Culicoides dubius
    - Culicoides fagineus
      - Culicoides fagineus A
      - Culicoides fagineus B
      - Culicoides fagineus C
    - Culicoides flavipulicaris
    - Culicoides grisescens
    - Culicoides impunctatus
    - Culicoides lungchiensis
    - Culicoides lupicaris
    - Culicoides newsteadi
      - Culicoides newsteadi A
      - Culicoides newsteadi B
    - Culicoides nipponensis
    - Culicoides ohmorii
    - Culicoides peregrinus
    - Culicoides pulicaris
      - Culicoides pulicaris 'dark'
    - Culicoides punctatus
    - Culicoides remmi
    - Culicoides subfagineus
    - Culicoides sumatrae
  - Monoculicoides
    - Culicoides parroti
    - Culicoides puncticollis
    - Culicoides riethi
  - Oecacta
    - Culicoides albicans
    - Culicoides cataneii
    - Culicoides charadraeus
    - Culicoides erairai
    - Culicoides festivipennis
    - Culicoides heteroclitus
    - Culicoides kibunensis
    - Culicoides kurensis
    - Culicoides malevillei
    - Culicoides maritimus
    - Culicoides oxystoma
    - Culicoides pictipennis
    - Culicoides sahariensis
    - Culicoides verbosus
    - Culicoides vexans
  - Silvaticulicoides
    - Culicoides picturatus
  - Trithecoides
    - Culicoides humeralis
    - Culicoides matsuzawai
    - Culicoides paraflavescens
  - unclassified Culicoides
    - Culicoides barbosai
    - Culicoides cubitalis
    - Culicoides derisor
    - Culicoides filariferus
    - Culicoides griseidorsum
    - Culicoides indistinctus
    - Culicoides nubeculosus
    - Culicoides saundersi
    - Culicoides submaritimus
    - Culicoides sp. LJH-2002



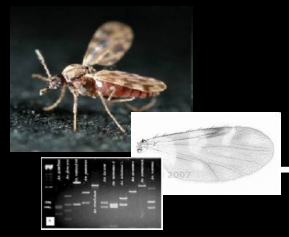
Conclusions

- 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 phylogenic 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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