

Culicoides species from the subgenus *Culicoides* in Catalonia (NE Spain)

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Identification Background

- Identification of *Culicoides* based on morphology
 - Metric traits
 - Size dependent: individual metric characters
 - Non-size dependent: indexes
 - Non metric traits
 - Presence/absence: sensilla coeloconica, other
 - Number of: sensilla coeloconica, teeth, other
 - Shape: sensory pit, aedeagus, other
 - Colour
 - Wing pattern
 - Spots distribution and intensity
 - Colour
 - Macrotrichia
- Identification of *Culicoides* based on molecular methods
 - Sequencing
 - PCR based diagnostics

Identification troubleshooting

- Morphological troubles →

Intraspecific variability?

Metric traits

Non-metric traits

Wing pattern

Results: Discrepancy in literature

Useful differential characters?
Synonym species?

- Molecular concerns

- Selection and standardization of the target diagnostic region (ITS, COI)
- Lack of morpho-molecular tandem analyses.
- Few *Culicoides* sequences available.
- Few works focussed on design of new PCR based diagnostics for *Culicoides*
- Limitations and errors of PCR based diagnostics → advantages of sequencing.

Identification troubleshooting

- Limitations and errors of PCR based diagnostics

Diagnostic PCR design LIMITATIONS

	Few individuals/specie/region	Many individuals/specie/region	
Regional or small scale analyses	1,2,	2	Done
Large scale analyses	1		Still not done

- 1 Non detection of sibling sympatric species
- 2 Non extrapolable to other regions without previous assessment

Culicoides PCR diagnose ERRORS

	Few individuals/specie/region	Many individuals/specie/region
Regional or small scale analyses	3, 4	3
Large scale analyses	3, 4	

3. Negative diagnostic: intraspecific polymorphism within the target region
4. Positive diagnostic: no interspecific polymorphisms within the target region in non detected sibling species

Aims

Perform:

Analyses species diversity within the subgenus Culicoides

Many individuals

Regional scale

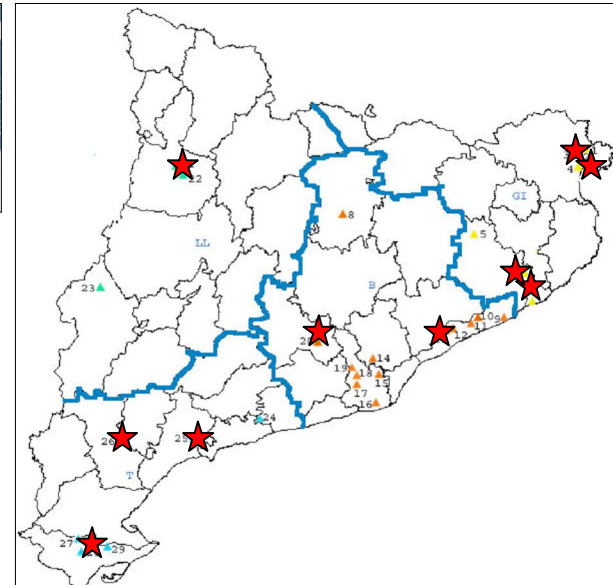
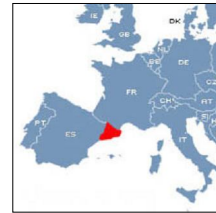
To:

1. Determine the presence of sibling species within the subgenus.
2. Determine useful characters to distinguish them.
3. Develop species-specific diagnostic assays.

Analyses in Catalonia (NE Spain)

Culicoides capture:

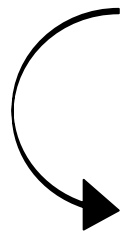
- Surveillance area of 31.114Km²
- Individuals of the subgenus *Culicoides* trapped in 10 sheep/goat farms.
- CDC blacklight traps



Culicoides identification:

- Midge identification according to wing morphology

- *C. punctatus*
- *C. pulicaris*, *C. lupicaris*
- *C. fagineus*
- *C. newsteadi*
- *C. flavipulicaris*



Storage (-20°C)



Dissection

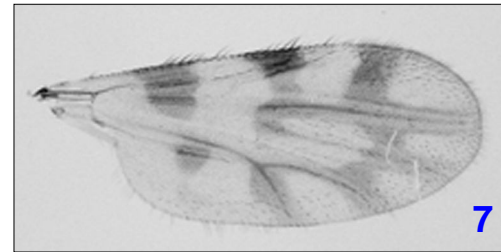
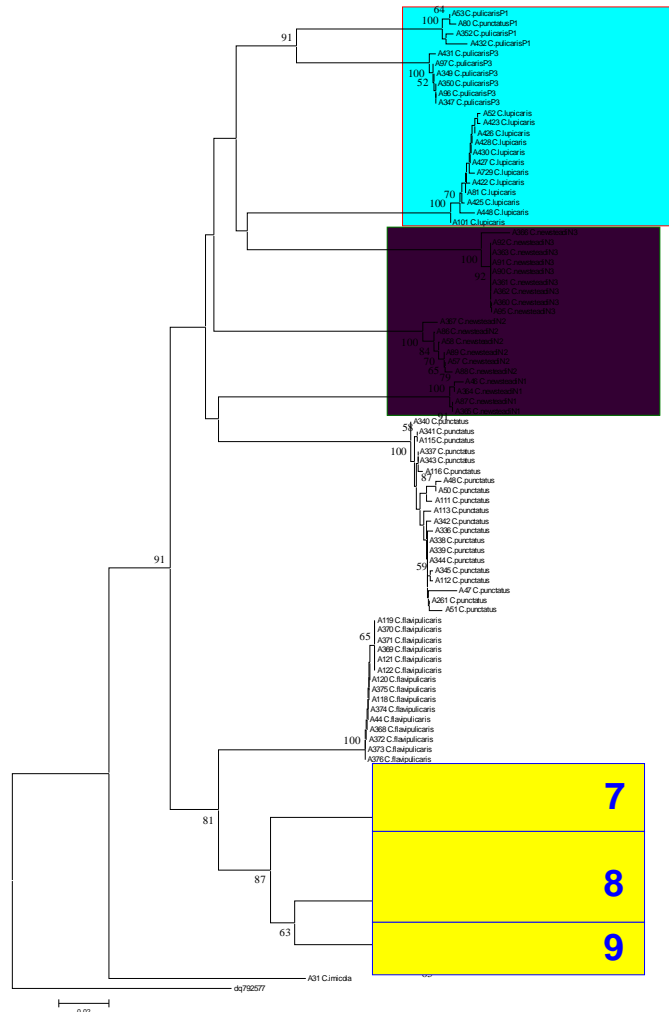


{ Sequencing (COI gene)
Morphological analyses }

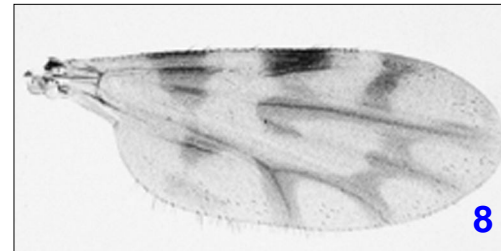
n=118

COI sequencing of *Culicoides*

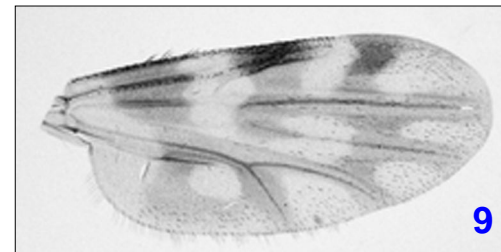
Neighbour-Joining tree



C. subfagineus



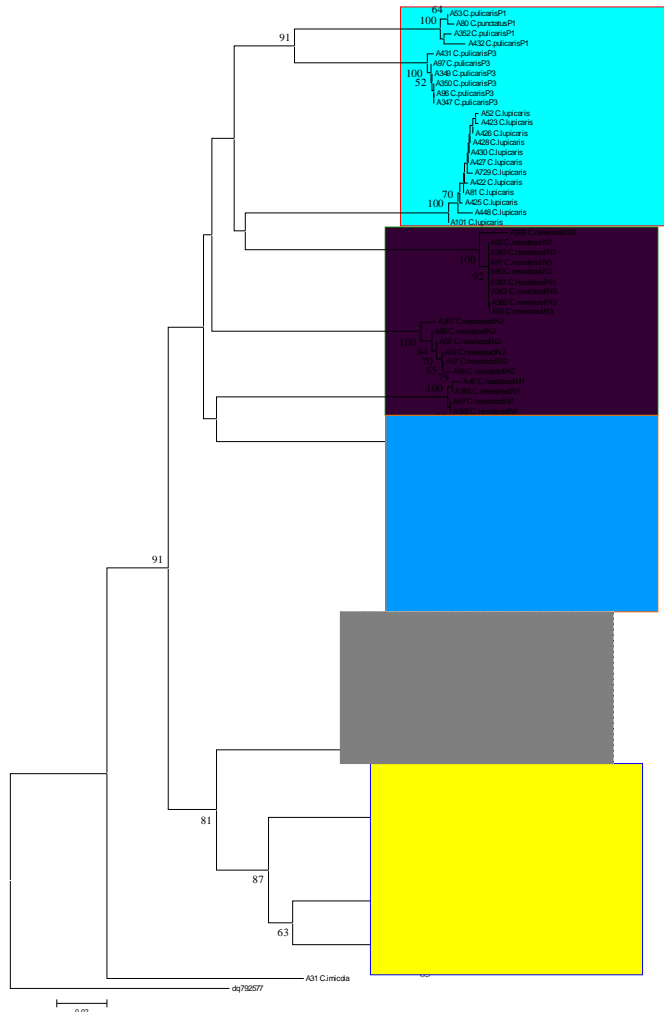
C. fagineus



C. fagineus F1

COI sequencing of *Culicoides*

Neighbour-Joining tree



Culicoides pulicaris group (n=3 spp.)

Culicoides newsteadi group (n=3 spp.)

Culicoides punctatus (n=1 sp.)

Culicoides flavipulicaris (n=1 sp.)

Culicoides fagineus group (n=3 spp.)

Morphological analyses

Morphological data recorded

- Eye data: 3 records
- Mouth parts: 11 records
- Wing metric data: 5 records
- Legs: 7 records
- Spermathecae: 2-6 records
- Antenna: 29 records

Wing pattern analysis

- Few differences found within groups of species
- No differences found between *C. pulicaris* P1 and *C. pulicaris* P3



Statistical analyses grouping individuals according COI sequence data



PCA: principal component analyses



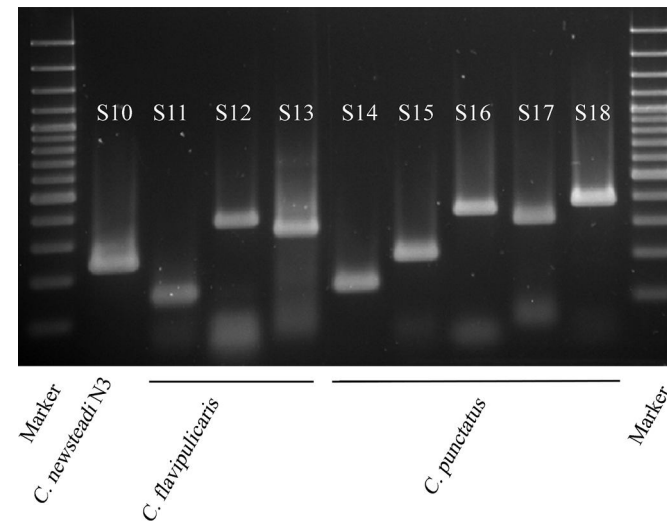
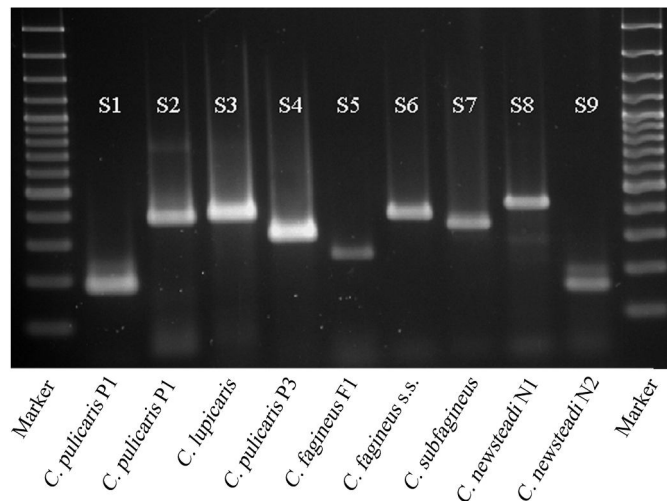
Useful morphological diagnostic characters found for:

- *C. pulicaris* group
- *C. newsteadi* group
- *C. fagineus* group

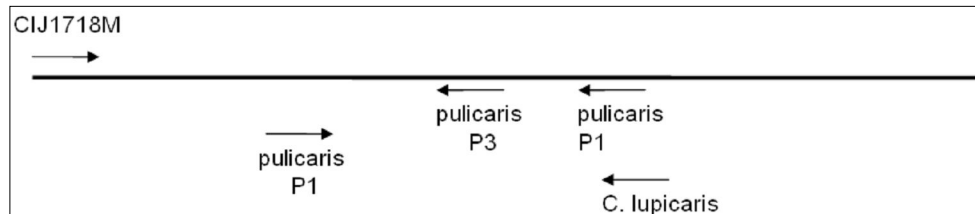
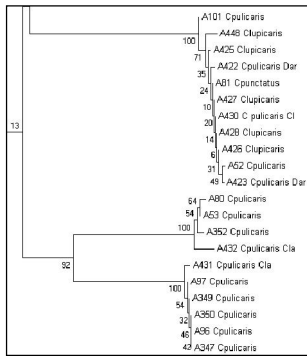
Design of PCR based diagnostic assays.

Single PCRs

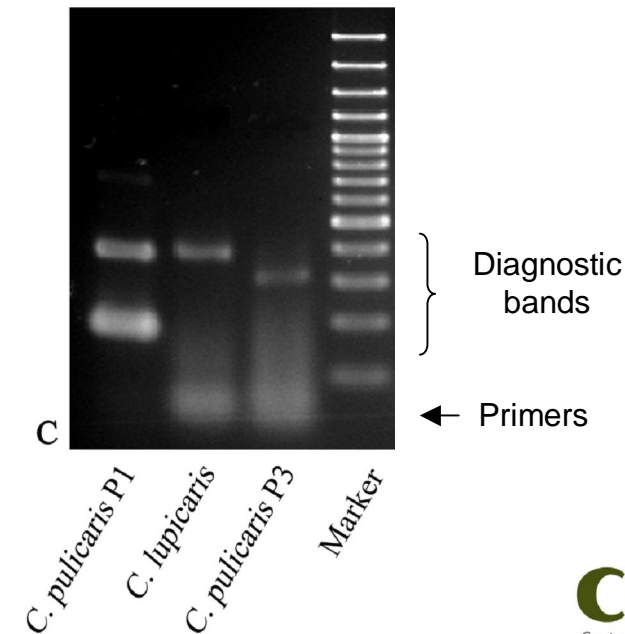
- Species found: *C. pulicaris* P1, *C. pulicaris* P3, *C. lupicaris*, *C. newsteadi* N1, *C. newsteadi* N2, *C. newsteadi* N3, *C. fagineus*, *C. fagineus* F1, *C. subfagineus*, *C. flavipulicaris* and *C. punctatus* (n=11)
- Design of 15 species-specific primers and validation of 18 single diagnostic PCRs



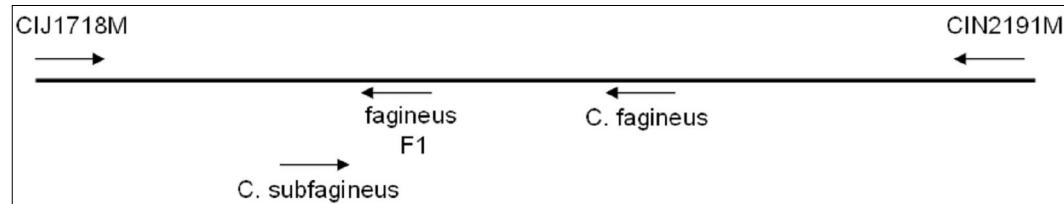
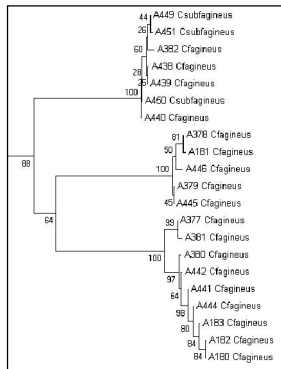
Design of PCR based diagnostic assays. Multiplex PCR for pulicaris group.



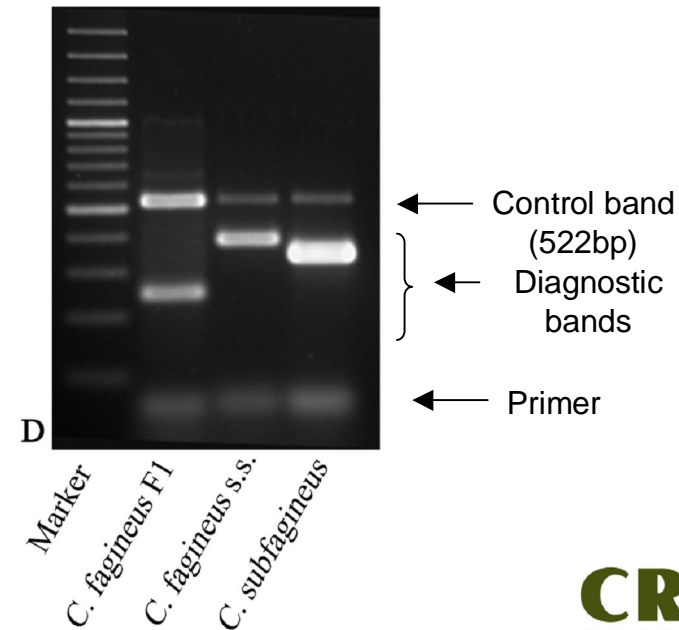
<i>C. pulicaris</i> P1:	197bp 410bp
<i>C. pulicaris</i> P3:	310bp
<i>C. lupicaris</i>:	405bp



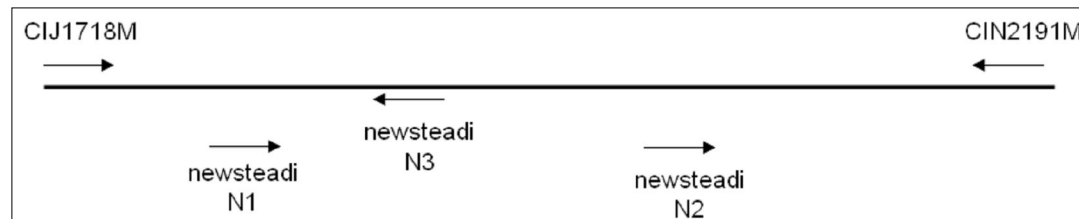
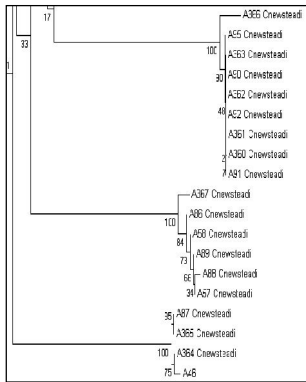
Design of PCR based diagnostic assays. Multiplex PCR for fagineus group.



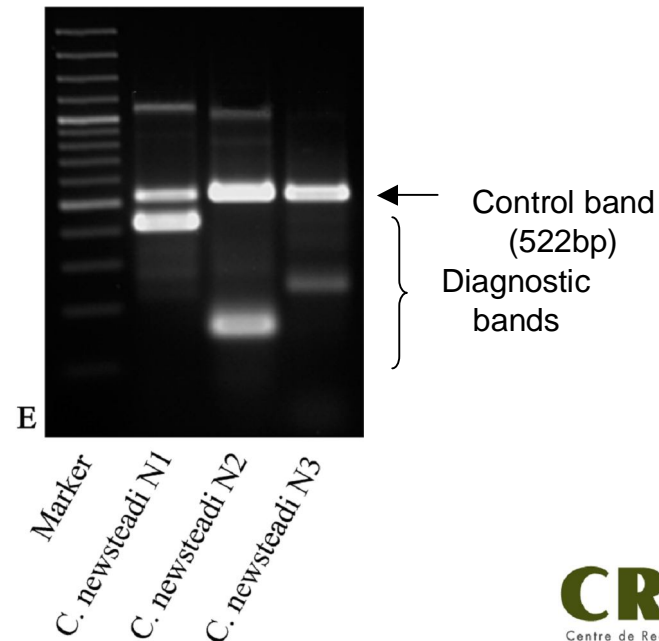
<i>C. fagineus</i> F1:	242bp 522bp
<i>C. fagineus</i>:	415bp 522bp
<i>C. subfagineus</i>:	379bp 522bp



Design of PCR based diagnostic assays. Multiplex PCR for newsteadi group.



<i>C. newsteadi</i> N1:	415bp 522bp
<i>C. newsteadi</i> N2:	153bp 522bp
<i>C. newsteadi</i> N3 :	231bp 522bp



Thanks for your attention