

Danish *Culicoides* species of the Obsoletus – group identified by morphological methods

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According to Campbell and Pelham-Clinton (1960), Kremer (1965) and other authors is it difficult to separate females of the subgenus *Avarita* (Obsoletus group) especially the females of *C. obsoletus* and *C. scoticus* .

In a quotation from Baldet et al., 2008 “For some species, like *C. obsoletus* and *C. scoticus* both belongig to the Obsoletus group, females cannot be separated morphologically”.

The aim of our study is to show that it in fact is possible to separate the four species of the Obsoletus group by combining a few morphological characters.

Material and Methods

Field sampling

The biting midges were collected (light traps) at two localities in Middle and Southern Sweden and from more localities on Zealand, Denmark

The insect material was transferred to 70% ethanol. Prior to the DNA measurements the *Culicoides* specimens were morphologically identified under a stereomicroscope.

Identification

➤ Males

- Hypopygium
- One wing

➤ Females

- Head
- One wing
- Posterior abdominal segments

Slide mounts were made.

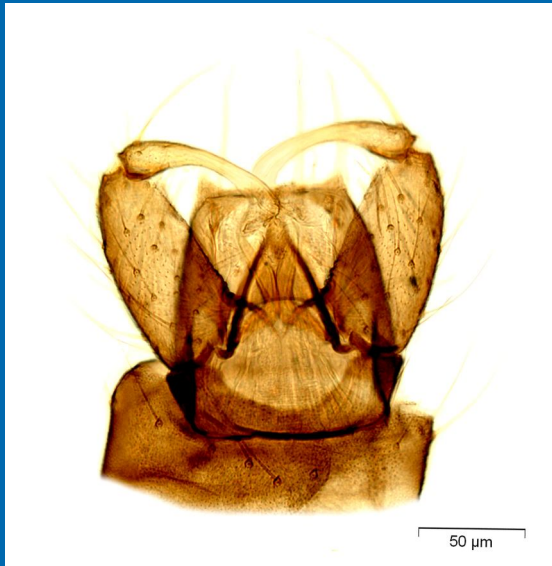
The remaining parts were transferred to 96 % ethanol in a test tubes for DNA analysis.

Measurements

- Lengths of every palpal segments
- Lengths of flagellar segments
- Lengths of wings (from arculus to tip)
- Lengths of spermatheka

- Antennae ratio (AR: 11-15 antennal segments divided with segments 3-10)
- Palpal ratio (PR: length of segment 3 divided with greatest breadth)

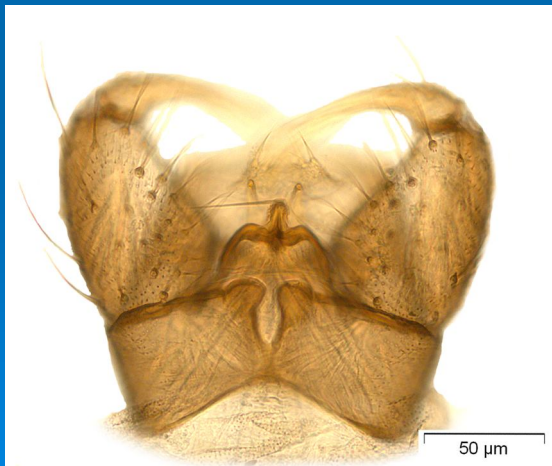
Hypopygium



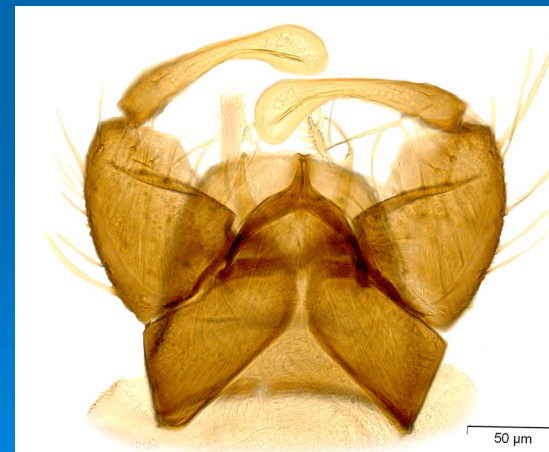
C. chiopterus



C. dewulfi

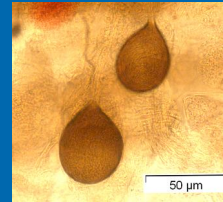
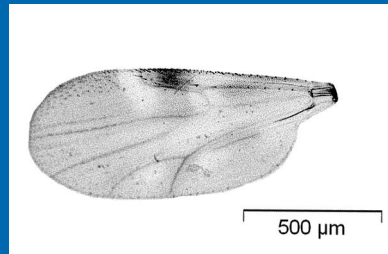
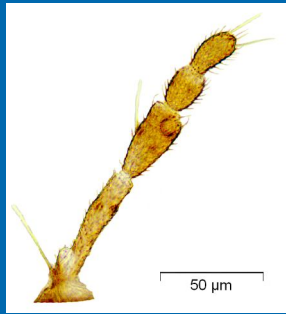


C. obsoletus

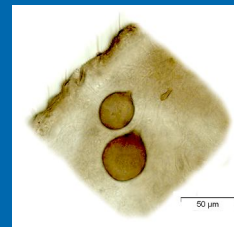
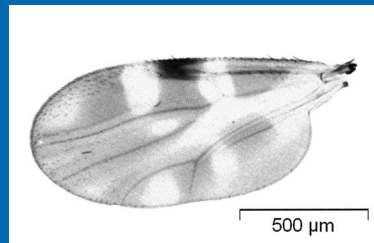
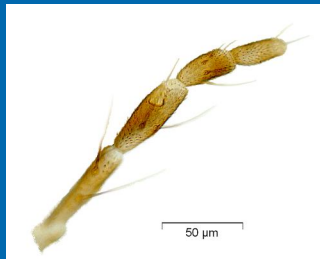


C. scoticus

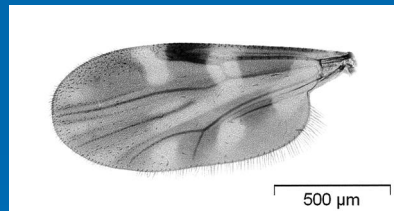
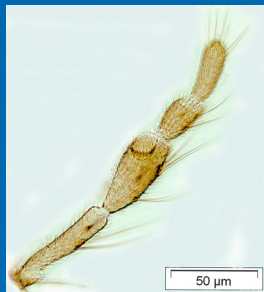
Females – Obsoletus group



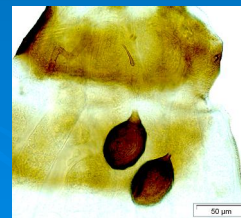
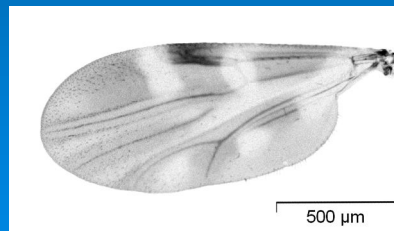
C. chiopterus



C. dewulfi



C. obsoletus



C. scoticus

Female palpal measurements

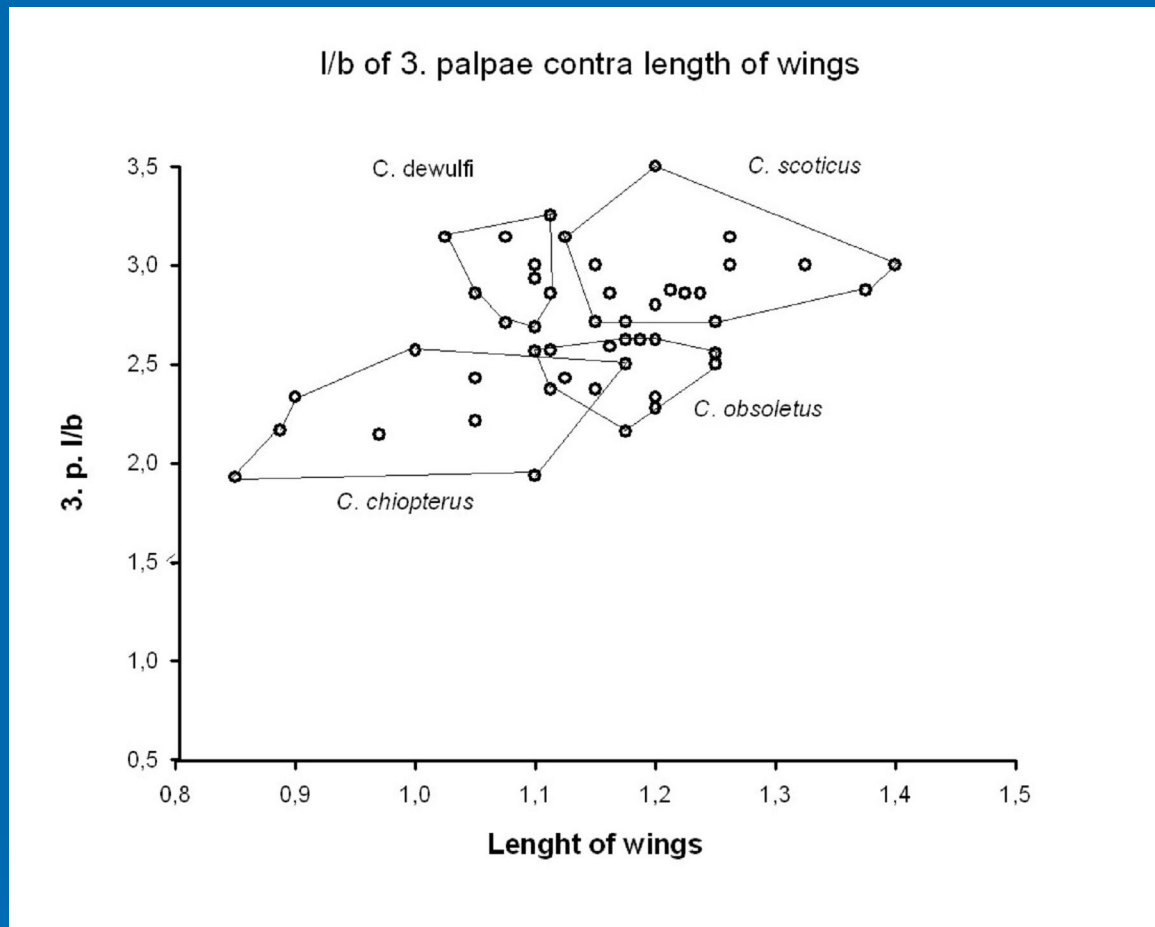
Species	N	2	3	4	5	Total	3 rd l/w
<i>C. obsoletus</i>	20	58	51	24	29	161.9±9.9 ^A	2.4±0.4 ^A
<i>C. scoticus</i>	20	61	53	26	31	168.4±12,3 ^A	3.0±0.2 ^B
<i>C. dewulfi</i>	10	58	54	30	29	171.3±5.6 ^A	3.0±0.2 ^B
<i>C. chiopterus</i>	10	46	40	22	25	133.1±13.2 ^B	2.3±0.2 ^A

3.l/b length of third maxillary segment to the width. Means with the same letters are not significant different (Multiple comparison of Kruskal-Wallis).

Female measurements

Species	N	Antenne (length)	AR	Wing (length)	Spermathek		
					1	2	Ratio 1/2
<i>C. obsoletus</i>	20	543±24 ^A	1.15±0.05 ^B	1.19±0.05 ^A	52	49	1.06
<i>C. scoticus</i>	20	541±29 ^A	1.21±0.03 ^C	1.24±0.08 ^C	60	57	1.05
<i>C. dewulfi</i>	10	475±20 ^B	1.16±0.03 ^{BC}	1.09±0.03 ^B	52	43	1.21
<i>C. chiopterus</i>	10	423±33 ^B	1.06±0.04 ^A	1.01±0.11 ^B	49	44	1.11

Antennal ratio (AR) of the female is the ratio of the sum of the lengths of the apical five segments of the the flagellum (11-15) to the sum of basal eight. Means with the same letters are not significant different (Multiple comparison of Kruskal-Wallis).



There is none or a very little overlap between the size to 3. l/b relationships between the four species.

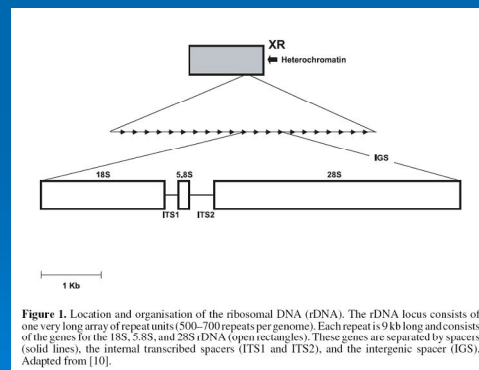
Danish *Culicoides* species
identified by molecular
methods





The molecular marker used is ITS1
(Ribosomal Internal Transcribed Region I).

Method adapted and basic reference sequences
from Perrin *et al* 2006 (Montpellier-
Strasbourg).



Cêtre-Sossah *et al.* 2004. *Veterinary Research* 35: 325-337.

Perrin *et al.* 2006. *Medical and Veterinary Entomology* 20: 219-228.



Molecular test

- 158 *Culicoides* specimens in ethanol.
- From *obsoletus* or *pulicaris* group.
- Morphological determination by S. A. Nielsen.
- DNA purification by DNAzol.
- PCR + sequence of ITS1.
- Sequence analysis (CLCbio; www.clcbio.com)



Molecular results

<i>Culicoides</i>	N
- <i>obsoletus</i>	52
- <i>scoticus</i>	60
- <i>dewulfi</i>	10
- <i>chiopterus</i>	9
- <i>pulicaris</i>	10
- <i>punctatus</i>	10

- 151 *Culicoides* specimens identified.
- 100% agreement between morphology and molecular data in both male and female *Culicoides*.
- Other species identified in Danish samples by molecular methods: *circumscriptus*, *duddingstoni*, *festivipennis*, *newsteadi* more to come.



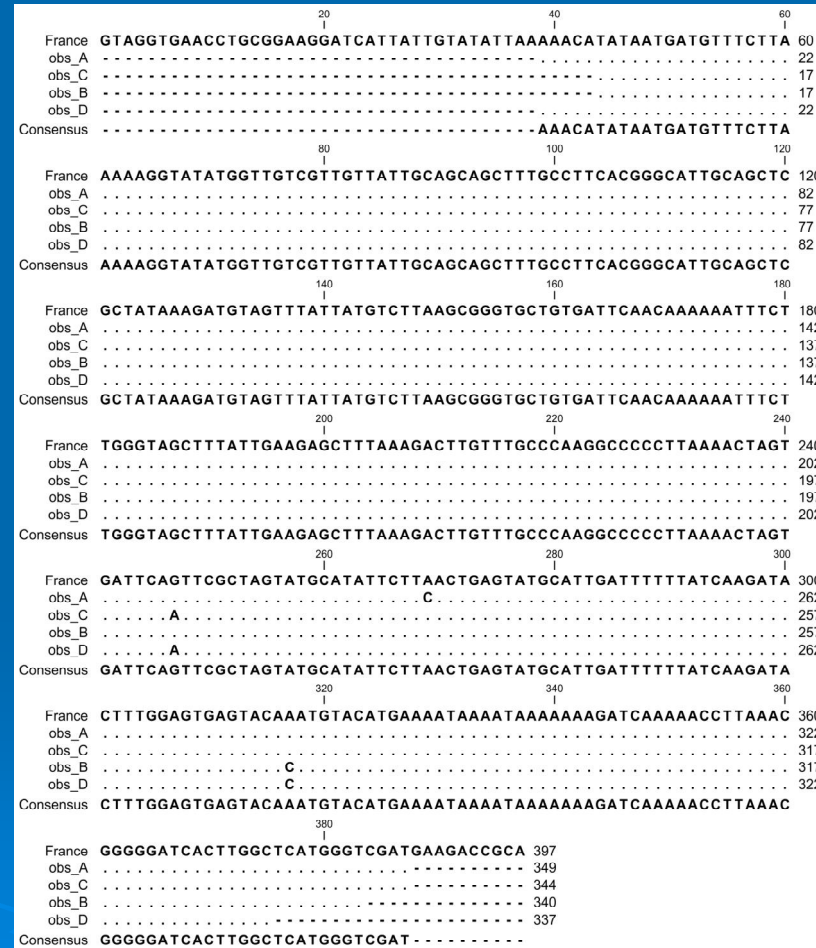
C. obsoletus s.s.

➤ Intraspecies difference is <1%; in *obsoletus* s.s. polymorfism is found at 3 of 350 bases (N=52)

➤ Four types identified.

- obs A
- obs B
- obs C
- obs D

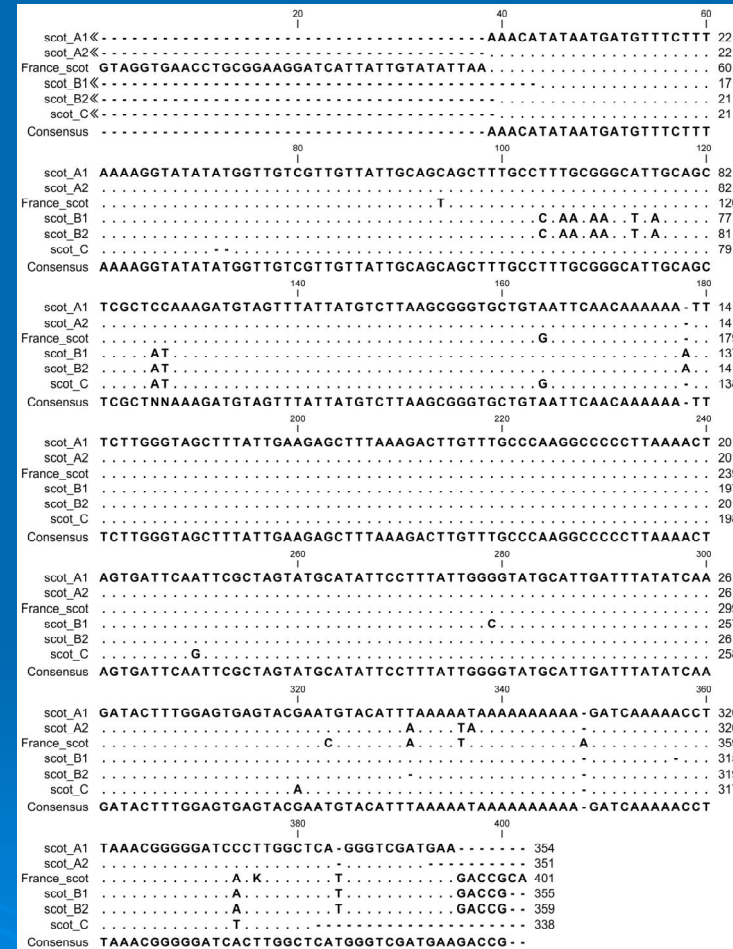
➤ All found as homo- and heterozygotes.





C. scoticus

- Intraspecies difference is ~3%; in *scoticus* polymorphism is found at 17 positions (N=60).
- Five types identified
 - scot A1 and A2 (<1%)
 - scot B1 and B2 (<1%)
 - scot C
- All found as homo-and heterozygotes.





C. obsoletus s.s. - *C. scoticus*

	20	40	60	
scot_A1	AAACATATAATGATGTTTCTTTAAAGGTATATATGTTTGC	CGTTGTTATTGCAGCAGCT	60	
scot_B1	55	
scot_C	57	
obs_A	58	
Consensus	AAACATATAATGATGTTTCTTTAAAGGTATATATGTTTGC	CGTTGTTATTGCAGCAGCT		
	80	100	120	
scot_A1	TTGCCTTTNGCGGCATTGCAGCTCGCTCCAAAGATGTAGTTTATTATGTCTTAAGCGGGT	120		
scot_B1	115	
scot_C	117	
obs_A	118	
Consensus	TTGCCTTTNGCGGCATTGCAGCTCGCTCCAAAGATGTAGTTTATTATGTCTTAAGCGGGT			
	140	160	180	
scot_A1	GCTGTAATTC AACAAAAA - TTTCTTGGGTAGCTTTATTGAAGAGCTTTAAAGACTTGTT	179		
scot_B1	175	
scot_C	176	
obs_A	177	
Consensus	GCTGTAATTC AACAAAAA - TTTCTTGGGTAGCTTTATTGAAGAGCTTTAAAGACTTGTT			
	200	220	240	
scot_A1	TGCCCAAGGCCCCCTTAAAACTAGTGATTCAATTCGCTAGTATGCATATTCCTTTATTGG	239		
scot_B1	235	
scot_C	236	
obs_A	235	
Consensus	TGCCCAAGGCCCCCTTAAAACTAGTGATTCAATTCGCTAGTATGCATATTCCTTTATTGG			
	260	280	300	
scot_A1	GGTATGCATTGATTTAT - ATCAAGTACTTTGGAGTGAGTACGAATGTACATTTAAAAAT	298		
scot_B1	294	
scot_C	295	
obs_A	295	
Consensus	GGTATGCATTGATTTAT - ATCAAGTACTTTGGAGTGAGTACGAATGTACATTTAAAAAT			
	320	340	360	
scot_A1	AAAAAAAAAAGATCAAAAACCTTAAACGGGGGATCCCTTGGCTCA - GGGTCGATGAA - - -	354		
scot_B1	353	
scot_C	338	
obs_A	349	
Consensus	AAAAAAAAAAGATCAAAAACCTTAAACGGGGGATCCCTTGGCTCATGGGTCGATGAA - - -			
scot_A1	- -	354		
scot_B1	CG	355		
scot_C	- -	338		
obs_A	- -	349		
Consensus	- -			

- Morphology and molecular data agree on 60 *scoticus* (male and female).
- Morphology and molecular data agree on 52 *obsoletus* s.s. (male and female).
- Difference between *obsoletus* s.s. and *scoticus* is 5-7%