Morphometric Discrimination of *Culicoides obsoletus* and *Culicoides scoticus*

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Abstract

- Bluetongue was introduced into northern Europe in 2006, and members of the Obsoletus Group of *Culicoides* biting midges were implicated as vectors of the disease;
- Identification of two of the four members of this group is considered difficult, if not impossible, when undertaken morphologically. Previous studies have attempted to determine morphological techniques to differentiate these species, yet have not investigated the effect of seasonality, or geographical location on midge morphology;
- Here, midges were collected from two sampling locations the UK, France and Spain. Morphometric measurements were compared between the start, middle and end of the vector season;
- While both geographical and seasonal variation in midge morphology was identified, identification techniques, based on the morphology of the abdomen, were also determined.

Introduction

- Bluetongue (BT), transmitted by *Culicoides* biting midges, has been causing high economic losses of cattle, sheep and goats throughout Europe, since 2006;
- Since BT's arrival in northern Europe, four Palaearctic midge species (collectively called the Obsoletus Group) have been implicated in transmission within the region;
- Identification of *Culicoides* is primarily based on wing morphology using a light microscope, but two of the species (*C. obsoletus* and *C. scoticus*) are considered difficult, if not impossible to discriminate this way, hindering work on the ecology of these newly implicated vectors ;
- Previous studies disagree on whether these species can be differentiated morphologically or whether molecular methods are necessary. Such studies have also only examined small populations of *Culicoides* from within one region, while seasonality and geographical location are known to affect species morphology throughout Europe.

Aim

- The aim was to investigate whether *C. obsoletus* and *C. scoticus* midges collected from different regions of Europe, and during differing time-points in the vector season, can indeed be discriminated using morphological techniques.

Methods

Trapping of Midges

- Light traps (Fig. 1) were run overnight on farms;
- Catches stored in 70% ethanol.

Figure 1. An Onderstepoort type down draught black light trap, fitted with UV bulb.

Identification & Slide Mounting

- *Culicoides* identified to group level based on wing patterns (Fig. 2);
- The head, wings and posterior part of abdomen were dissected and slide mounted using Canada Balsam.

Figure 2. Wings of *Culicoides* species implicated as BT vectors in northern Europe. The Obsoletus Group members include, a) *C. chiopterus*, b) *C. obsoletus*, c) *C. scoticus*, d) *C. dewulfi*

Morphometric Calculations

- 15 measurements taken from the head, wings and abdomen of slide mounted midges (Fig.3) , using a compound light microscope with camera attachment and Image-Pro Plus.

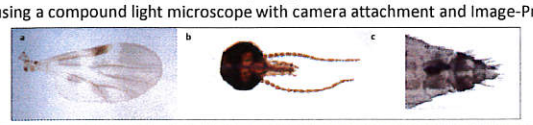

Figure 3. Orientation of slide-mounted *Culicoides*. a) wing; b) head positioned dorsal-side upmost with antennae extended to the right; and c) posterior end of the abdomen situated ventral-side upmost.

Molecular Identification

- Primers and PCR amplification conditions (cytochrome oxidase I gene [COI]) were as described by Nolan *et al.*[1], with results visualised via electrophoresis with gel red staining.

Statistical Analyses

- Eight additional variables were calculated as ratios of the head, wing and abdominal measurements.
- Statistical differences between *C. obsoletus* and *C. scoticus* measurements were determined using the Mann Whitney test and adjusted for multiple comparisons with the Bonferroni correction. Mayr's coefficient of difference[2] (CD) was also established.
- Principal component analysis (PCA) was used to explore the correlation structure between variables.

Results

Molecular Analyses

- 819 midges identified using the COI gene (Fig. 4), of those 410 were *C. obsoletus* and 348 were *C. scoticus*.

Figure 4. Gel image of 27 *Culicoides* Obsoletus Group members. Lanes 1 to 27 contain samples with the species indicated by o=*C. obsoletus*, s=*C. scoticus*, c=*C. chiopterus*, and d=*C. dewulfi*. Lanes 28 to 31 contain the positive controls for each of the species and are labeled using the same key as the sample lanes.

Descriptive Statistics

- High degree of correlation between the 15 morphometric characteristics;
- Four variables (length and width of larger and smaller spermathecae) exhibited CD values over 1.28, the critical threshold over which subspecies can be distinguished (Fig. 5).

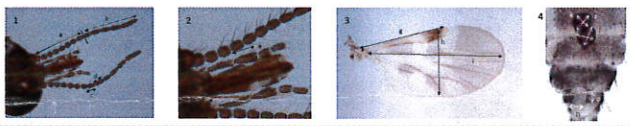

Figure 5. Morphometric measurements of: **1 & 2) head of *Culicoides***, where a) is length of eight basal flagella segments of the antenna; b) is the length of the five apical flagella segments of the antenna; c) is the length of flagellomere 10; d) is the length of flagellomere 11; e) is the length of the 3rd palpal segment; and f) is the width of the 3rd palpal segment. **3) wing**; where g) is the costa length; h) is the width of the wing; and i) is the length of the wing (arculus to tip). **4) abdomen**; where j) is the length of spermatheca 1; k) is the width of spermatheca 1; l) is the length of spermatheca 2; m) is the width of spermatheca 2; n) is the length between the chitinous plates; and o) is the width of the chitinous plates.

Geographical Variation

- Overall, 9 of the measurements were significantly smaller in the Spanish midges (Fig. 6) compared to the UK or French midges.

Figure 6. The locations of the trapped *Culicoides* in the UK, France and Spain

Seasonal Variation

- Variation was observed in 7 of the measurements between the start, middle and end of the season, with measurements in the middle of the season being smaller than those at the start or end. Such variation was only observed for head and wing, but not abdominal measurements.

Principal Component Analyses

- The PCA scatter plot of morphometric measurements (Fig. 7a) unambiguously separated *C. obsoletus* and *C. scoticus*, with the lengths and widths of both spermathecae demonstrating the best diagnostic abilities out of the measurements;
- The ratios were unable to separate *C. obsoletus* and *C. scoticus* (Fig. 7b).

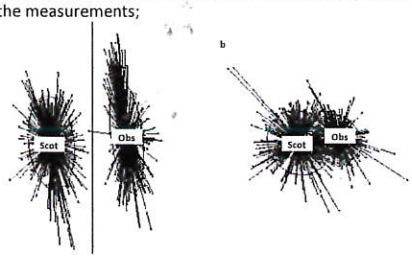

Figure 7. Principal component analysis plots of a) morphometric measurements; and b) ratios of measurements of *C. obsoletus* (Obs) and *C. scoticus* (Scot).

Overall

- CD results suggest that *C. obsoletus* and *C. scoticus* could be differentiated based on a combination of the length and width of their spermathecae. These results were confirmed in the principal component analyses.

Conclusions

- Geographic variation in the size of *Culicoides* was observed and may be related to temperature at trapping sites, with small *Culicoides* trapped further south.
- Seasonal variation was observed between time-points for both head and wing measurements, but not for the abdominal measurements.
- The length and width of the spermathecae can be used to discriminate between *C. obsoletus* and *C. scoticus*, but this is a time-consuming process and we recommend only undertaking this on a sub-sample of individuals from a catch.

References:

- [1] Nolan DV, *et al.* (2007) Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. Veterinary Microbiology 124: 82–94.
- [2] Mayr E. 1953. Methods and Principles of Systematic Zoology. McGraw-Hill, New York, USA., Pages: 328.