

## PAST AND FUTURE: MORPHOLOGICAL AND MOLECULAR BIOLOGY STUDIES OF *CULICOIDES*

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*Culicoides* are the biological vectors of bluetongue virus, an arbovirus transmitted by females after the ingestion of blood from viraemic animals. Conventional microscopy is usually used for taxonomic characterization and recently molecular characterization became a very useful tool to differentiate species with similar morphological characteristics, such as those belonging to *Culicoides* *Obsoletus* complex and *C. dewulfi*. On the other hand, scanning electronic microscopy (SEM) allows the visualization of very small details, which can also be used in *Culicoides* species identification as a complement of other methods. In this work we performed both techniques in order to detect simultaneously subtle features.

Female and male specimens of *Culicoides* *obsoletus sensu stricto*, *C. scoticus* and *C. dewulfi* were washed in ethylic alcohol in gradients until 100%, followed by immersion in acetone. The insects were dried using the point drying method, mounted in stubs and coated with gold palladium. Specimens were observed under electronic microscope. Deoxyribonucleic acid (DNA) was extracted individually from 360 specimens of the *C. Obsoletus*

complex (*C. obsoletus s.s.*, *C. scoticus*) and 18 specimens of *C. dewulfi*, then analysed by PCR Multiplex. Several images of heads including details of the eyes (contiguous and bare), sensilla, flagellomeres, absence of teeth in the cibarial arch and pharynx, maxillary palps and mouth parts, thorax, abdomen and legs (in both sexes), genitalia, hind tibial comb, and fifth tarsi with empodium and claws are presented. PCR Multiplex results are shown.

In this work, the SEM technique was used to show specific morphological details not obtained by optical microscopy of *C. obsoletus*. The SEM technique may be helpful to differentiate members of *Culicoides* species complexes and can be complementary to other classification methods of *Culicoides*. Samples of *Culicoides* analysed by PCR Multiplex and examined in parallel with SEM might improve the taxonomic classification of these insects.

**KEYWORDS:** *CULICOIDES* – SCANNING ELECTRONIC MICROSCOPY – PCR.

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