

IIKC: An Interactive Identification Key for female *Culicoides* (Diptera: Ceratopogonidae) from the West Palearctic region

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Abstract — In 2006, bluetongue virus (BTV) outbreaks appeared surprisingly in northern Europe and widely affected most of the European countries. Correct identification of *Culicoides* species (Diptera: Ceratopogonidae), known as BTV vectors, is a key component of all studies intending to understand vector dynamics and to develop vector control strategies. A computer-based system, Xper², was used to develop an Interactive Identification Key (IIKC) for female *Culicoides* from the West Palearctic region. The current version of IIKC includes 108 taxa, 61 descriptors and 837 pictures and schemes. IIKC is a powerful tool for routinely identifying *Culicoides* species and for training young specialized taxonomists.

Index Terms — *Culicoides* species, identification key, interactive key, bluetongue.



1 INTRODUCTION

Bluetongue is an arboviral disease affecting ruminants, mainly ovines. Vectors of bluetongue virus (BTV) are small biting midges belonging to the genus *Culicoides* (Diptera: Ceratopogonidae). Excluding the 46 fossils, a total of 1308 *Culicoides* species are distributed on every large land mass with the exception of Antarctica and New Zealand, ranging from the tropics to the tundra and from sea level to 4000 m [1]. Worldwide, around 60 biting midges species are suspected or proved to transmit viruses, protozoa or filaria worms

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[2]. Several of these viruses are of major international significance for animal health (African horse sickness virus, bluetongue virus, epizootic hemorrhagic disease virus).

Up to 2010, five autochthonous biting midges species are suspected to transmit BTV in western and central Europe: *Culicoides chiopterus* (Meigen, 1830) [3], *C. dewulfi* Goetghebuer, 1935 [4], *C. obsoletus* (Meigen, 1919) [5], *C. scoticus* Downes and Kettle, 1952 [6] and *C. pulicaris* (Linnaeus 1758) [7]. In addition, along the Mediterranean basin, the main afro-tropical vector *Culicoides imicola* Kieffer, 1913 is present and is in charge of BTV transmission. The emergence and spread of bluetongue disease in Western Europe has highlighted the taxonomic impediment concerning biting midges. Identification of *Culicoides* species is highly important for a clear understanding of virus transmission and biting midges population dynamics and for surveillance activities as well. Because of their small size and of their highly specific diversity, morphological identification of biting midges requires time and expertise.

Despite of the fact that monitoring activities are compulsory, most of the European countries affected by bluetongue virus have very few taxonomists for biting midges. They rely on two main identification keys: Campbell and Pelham-Clinton's key, published in 1960 [8], and Delécolle's key, published in 1985 [9]. These two dichotomous keys are remarkable, but they have not been updated for years and generally, they are not well adapted for non-expert researchers. Therefore, when using the keys, newly described species or synonymies can be missed, and scientists face difficulties with diagnostic and described characters.

Since a decade, the development of computer-aided systems marks a turning point in taxonomy [10; 11]. Interactive identification keys based on multi-entries are easy to use for experts and non-experts. They allow quick updates and are easily released to the scientific community through the web. Today, interactive identification keys have been developed for several arthropods: phlebotomine sandflies [12], *Glossina* flies [13], or mosquitoes [14].

The aim of this work is to present the newly developed interactive identification key for female *Culicoides* for the West Palearctic region (IHKC). Information on availability and some recommendations are given.

2 MATERIAL AND METHODS

2.1 MORPHOLOGICAL CHARACTERS

60 morphological descriptors coded in 164 morphological states are observed from wings, abdomen, head and leg.

The wings descriptors are related to the presence or absence of spots, position, size and shape.

Abdominal descriptors concern the number, size and shapes of spermathecae, the presence and shape of a sclerotized ring, and some special features like abdominal sclerites.

The head descriptors gather those of 4 body parts: antennae, eyes, palps and mouthparts. Antennae are observed for the sensilli distribution (coeloconica,

short and long trichodea) and the antennal ratio (length of the first elongated segment divided by the last short one). On eyes, inter-ocular space and the interfacetal hairs are observed. Data related to the shape of the palpal segments and the sensory pits are collected. Teeth are observed on mandibles and maxilles.

The morphological characters were discussed and validated by 27 entomologists from 14 countries at the taxonomy meeting of the MedReoNet network at Strasbourg in 2009 (<http://medreonet.cirad.fr/>).

2.2 SOFTWARE AND COLLECTIONS

The morphological database of female *Culicoides* was edited with Xper² version 2.0 [15] which allows the creation of the interactive key.

Slide-mounted specimens observed are from the Callot, Kremer and Delécolle collections (IPPTS, Strasbourg, France). Pictures were taken with a Zeiss® microscope equipped with a Motic® camera. All pictures were individually cleaned up using the software Gimp version 2.6.2.

2.3 VALIDATION

A trial has been proposed to 5 non expert scientists on *Culicoides* taxonomy. 37 slide-mounted female specimens belonging to the genus *Culicoides* are anonymously coded. These selected species span a wide range of morphological diversity of *Culicoides*. The identification order of the slides has been randomly determined for each participant. Correct or false status of identification, estimates of user's confidence and the use of definitions and illustrations were also gathered.

3 RESULTS

In total, 60 morphological descriptors have been observed: 27 on the wing, 14 on the abdomen, 16 on the head and 3 on the legs. An additional geographical descriptor has been added, which allows users to limit the taxa list to one country. The 60 morphological descriptors are divided into 164 morphological states illustrated with 403 pictures and schemes. Morphological data of 22 species were collected from stocked species at IPPTS, Strasbourg, France and, 86 others species were from Callot, Kremer and Delécolle collections. In total, the current version of IIKC includes 108 taxa. Among them, 8 species with important morphological variations have been coded as taxa with polymorphic characters. A total of 76 taxa were illustrated with drawings sheets. 73 species were illustrated with 434 pictures (mean of 5.9 pictures per taxon): 24 with only pictures and 49 with both pictures and drawings. Only 8 taxa have not yet been illustrated. IIKC includes a total of 837 pictures and schemes.

IIKC is still in a validation step at the submission date of this communication thus, results could not be shown.

4 DISCUSSION

4.1 AVAILABILITY AND UPDATES

IIKC will be freely available on a CD-Rom upon request to the authors. Moreover, a dedicated website under construction will allow the interactive key as well as updates to be downloaded. To help users with limited computer capacity, IIKC will also be available on-line without local installation.

A scientific committee has been proposed, and annual meetings will be organized to validate updates, discuss new species or synonymies, evaluate new systematic or taxonomic changes. IIKC users are encouraged to contact the authors and the scientific committee for feedbacks and to inform for new taxonomic information.

4.2 RECOMMENDATIONS

IIKC helps in identifying adult female *Culicoides* species. Identification of *Culicoides* genus among other genus of the Ceratopogonidae family is not included.

IIKC helps in identifying slide-mounted specimens and users are recommended to use a microscope for good morphological observations. Stereomicroscope observations of biting midges preserved in alcohol limit the observation on wing patterns only.

IIKC is a multi-entry key. Compared to a dichotomous key, this key allows to choose the descriptors the user wants to observe. If the specimen has damaged parts, identification can go on with others ones. Users can also select a group of descriptors (wing, abdomen, leg, head, or geography). Three optimized list of characters classifying descriptors according to their discriminating power, are available as an option and leads to quick identification. Users are strongly recommended to use the option "Xper original sort". When activated, a number into brackets appears for each descriptor (from 0 to 1) representing the discriminating power. The highest numbers are the most powerful descriptors (i.e. the ones that will best discriminate the taxa).

Users are strongly recommended to use reference collections and national experts to confirm their identification when dealing with new recorded or observed species. Authors strongly encouraged future users to build regional reference collections and to help in exchanging material between collections to improve our systematic and taxonomic knowledge of the genus *Culicoides*.

5 CONCLUSION

IIKC is a newly developed morphological identification key allowing the identification of 108 taxa of *Culicoides* (Diptera: Ceratopogonidae). Largely illustrated with 837 pictures, drawings and schemes, this interactive identification key is based on a multi-entry system, with optimized list of characters (including geographical distribution). The richness of illustrations is a great advantage to train taxonomists. The development of identification tools for *Culicoides* and more

generally for arthropods involved in pathogen transmission will help scientists in identifying species and therefore will give better insights into the bioecology and dynamics of these groups, helping in designing more appropriate vector control strategies.

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