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## Morphological and molecular analysis of the genus *Culicoides* (Diptera: Ceratopogonidae) in Slovakia with five new records

ADELA SARVAŠOVÁ<sup>1</sup>, ALICA KOČIŠOVÁ<sup>1</sup>, MILOŠ HALÁN<sup>1</sup>, JEAN-CLAUDE DELÉCOLLE<sup>2</sup> & BRUNO MATHIEU<sup>2,3</sup>

<sup>1</sup>University of Veterinary Medicine and Pharmacy in Košice, Dept. of Parasitology, Komenského 73, SK-04181 Košice, Slovak Republic. E-mail: [Alica.Kocisova@uvlf.sk](mailto:Alica.Kocisova@uvlf.sk)

<sup>2</sup>Institute of Parasitology and Tropical Pathology (IPPTS), Medicine faculty, EA7292, 3 rue Koeberlé, F-67000 Strasbourg, France. E-mail: [bmathieu@unistra.fr](mailto:bmathieu@unistra.fr)

<sup>3</sup>EID Méditerranée, 165 av Paul Rimbaud, F-34184 Montpellier, France

### Abstract

The biodiversity of *Culicoides* from eastern Slovakia was investigated by light trapping. An integrative taxonomy approach combining DNA barcode sequence and morphological analyses was used to accurately identify specimens. Five species were newly recorded from Slovakia: *Culicoides picturatus* Kremer & Deduit, *C. gejjelensis* Dzhafarov, *C. clastrieri* Callot *et al.*, *C. griseidorsum* Kieffer and *C. odiatus* Austen. The checklist of the *Culicoides* species recorded from SK has been updated to 63 species and barcode sequence data is provided for 8 species not previously available on GenBank. Conflict between results from molecular and morphological analyses resulted in the discovery of some potentially new cryptic species and the inability of DNA barcodes to distinguish *C. festivipennis* Kieffer from *C. clastrieri*, *C. salinarius* Kieffer from *C. manchuriensis* Tokunaga and *C. pallidicornis* Kieffer from *C. subfasciipennis* Kieffer. These conflicts suggest further study is required to clarify the status of these species.

**Key words:** *Culicoides picturatus*, *Culicoides gejjelensis*, *Culicoides clastrieri*, *Culicoides griseidorsum*, *Culicoides odiatus*, DNA barcodes, *Silvaticulicoides*

### Introduction

Biting midges of the genus *Culicoides* are relatively well studied in Europe due to their ability to transmit Bluetongue and Schmallenberg viruses and parasites of domestic and wild animals (Carpenter *et al.* 2013; Mellor *et al.* 2000). Although bluetongue virus (BTV) serotype 8 infections reached countries bordering Slovak Republic, such as Czech Republic, Hungary, and Austria in 2007 and 2008 (Carpenter *et al.* 2009), no outbreaks of bluetongue have been confirmed in Slovakia so far despite the presence of bluetongue antibodies in Holstein heifers transported from France in August 2008 (Lacková *et al.* 2012). In 2011, Schmallenberg virus (SBV), a novel Orthobunyavirus was described as the cause of cattle disease in Germany and the Netherlands (Hoffmann *et al.* 2012) and to date, *Culicoides* are the only identified vectors of SBV (Carpenter *et al.* 2013). SBV has been present in Europe since summer 2011 and was detected for the first time in Slovakia in late autumn 2012 (Bíreš, personal information).

Since the 1960s, several studies of the biting midge fauna of Slovakia have been published (Mráz & Országh 1998; Országh & Chalupský 1987; Országh & Mráz 1996; Országh & Trpiš 1970; Országh 1968; Országh *et al.* 1997; Országh 1969a, 1970, 1971, 1972, 1973, 1974, 1980; Paclt 1962; Paclt *et al.* 1970; Trpiš & Országh 1971). These studies were focused on the diversity, biology and abundance of biting midges but did not include all regions of Slovakia. Twenty-five species were recorded from the eastern part of Slovakia by Trpiš & Országh (1971) and the checklist for Slovakia was increased to 58 species following studies in Western Slovakia (Mráz & Országh 1998; Mráz 1999). More recent surveys of the fauna of Slovakia, particularly the eastern regions, are, however, lacking and the fauna of this region is probably underestimated.

In contrast to these examples of conflict between results of morphological and molecular analyses where pairs of morphologically separable species have little genetic differences, this study has also identified several potentially cryptic species which are morphologically inseparable but different genetically. Intra-specific distances for *C. kibunensis* of 6.6% were found between two specimens from two localities in eastern Slovakia suggesting the presence of at least two cryptic species. The existence of a group of species similar to *C. kibunensis* was suggested previously by Glukhova (1989, 2005) based on morphological observations of specimens from Belarus, Ukraine, Turkmenia, Tajikistan and Kazakhstan. The present study provides molecular support for the existence of a Kibunensis complex of species sensu Glukhova (1989, 2005).

Similarly, two specimens belonging to *C. subg. Culicoides* with morphology similar to *C. pulicaris* and *C. lupicaris* respectively proved to have unique DNA barcodes suggesting these specimens may represent new species. The taxonomy of *C. subg. Culicoides*, particularly the Pulicaris group within this subgenus, is complex and has already been shown to contain some cryptic species (Pagès *et al.* 2009) and a new species was recently described (Ramilo *et al.* 2013). Further study using an integrative taxonomy approach including the use of other gene regions is required from more specimens, particularly males, to evaluate the species status of *speA* and *speB*.

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