Ring Trials on Obsoletus Complex Species

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Culicoides species are difficult to identify but, recently, molecular identification tools based on cytochrome oxidase subunit 1 (CO1), internal transcribed spacer 1 or 2 (ITS-1 or ITS-2) have been developed for Palaearctic species including vector species. In the framework of Medreonet network, the workpackage on "Regional surveillance of vectors" includes guidelines on Palaearctic and Mediterranean *Culicoides* species identification using molecular tools. Three ring trials were carried out to test whether the methods and tools used in the main laboratories of Europe were adequate and able to identify species correctly. Moreover, a questionnaire was sent to each laboratory to record the protocols and tools used for molecular identification. The four species of the Obsoletus complex, *C. obsoletus, C. scoticus, C. chiopterus,* and *C. dewulfi* were selected for the ring trial. All specimens were first morphologically identified by experts.

The first ring trial containing eight specimens (four males and four females) of the four species was sent on the 4th of August 2008 to 13 different participating laboratories from seven countries. Two types of material were sent: extracted deoxyribonucleic acid (DNA) (four samples/species) and phosphate-buffered saline (PBS)-ground biting midges (34 samples/species). Eventually, two laboratories did not participate. Only two out of the 11 participating laboratories correctly identified the species. The molecular identification used was based on ITS-1 and ITS-2 markers. A second ring trial was sent in April 2009 with only extracted DNA samples to avoid the different extraction steps which could interfere with tool sensitivity. A total of 20 extracted DNA samples were sent to 10 participating laboratories. Four out of the 10 correctly identified the species. Three laboratories used methods based on ITS-2 or ITS-1 markers, and one laboratory used CO1. Finally, a third ring trial with 20 PBS-ground samples was sent in September 2009 to 10 laboratories. Only one laboratory correctly identified the species. Sensitivity and specificity of the molecular tools used are discussed.

Keywords: *Culicoides* – Molecular genetics – Identification – Obsoletus complex – PCR – Laboratory diagnosis.

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