

RING TRIAL FOR MOLECULAR IDENTIFICATION OF PALAEARCTIC SPECIES OF THE SUBGENUS *AVARITIA*

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■ VECTOR SURVEILLANCE

A ring trial was conducted for molecular identification of Palaearctic species of the subgenus *Avaritia*, and especially the following four species: *Culicoides chiopterus*, *C. dewulfi*, *C. obsoletus* and *C. scoticus*. It was based on multiplex polymerase chain reaction (PCR) on the molecular markers cytochrome oxidase type 1 (CO1), internal transcribed spacer 2 (ITS-2), and ITS-1. Each of the 13 participating laboratories (from seven different countries) received on the 4th of August 2008 a panel of 38 samples of 11 µL of a phosphate-buffered saline (PBS) solution containing parts of a single specimen of insect ground up into 200 µL of PBS, as well as four tubes, identified and mentioned in the accompanying letter, for which deoxyribonucleic acid (DNA) had already been extracted.

The panel was coded with a letter followed by different numbers. The laboratories had two months from the date of arrival

of the samples to give back the results by sending an Excel file containing the coding. The 38 samples used for the trial were exchanged for identification between two international experts (Drs J.C. Delécolle and R. Meiswinkel). Only one identification differed between the two experts: *scoticus* vs. *obsoletus*, and sequencing revealed it to be a *C. scoticus* specimen.

Only one laboratory used molecular marker ITS-2, five laboratories used ITS-1, and four used CO1. Only two found the expected results. The eight remaining laboratories found some false positive or false negative results. Five out of ten correctly identified the species from the DNA samples. Seven out of ten laboratories had 100% sensitivity.

KEYWORDS: *CULICOIDES* OBSOLETUS COMPLEX – TRIAL METHOD – MOLECULAR GENETICS – IDENTIFICATION – PCR.

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