

## **Area tematica: Sanità Animale**

### **Titolo del progetto: Sviluppo e valutazione di nuovi metodi biomolecolari da applicare nella sorveglianza di *Aethina tumida***

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### **SINTESI**

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### **SUMMARY**

*Aethina tumida*, order Coleoptera, family Nitidulidae is an exotic parasite, known as a small beehive beetle or "Small Hive Beetle" (SHB), responsible for a notifiable disease in the EU, for which specific measures for the import of bees from Third Countries are envisaged (Decision 2003/881 and subsequent amendments and additions).

The first SHB outbreak was in Calabria, Southern Italy, in September 2014 opening a new health emergency throughout Europe.

The official diagnosis of SHB in apiary is carried out through a clinical examination of the hives, by means of appropriate traps in the nest, which means a long time for the evaluation of SHB presence in the apiary. In addition, traps only detect adults not larvae or eggs.

For this reason, there was the need to accelerate the research of *A. tumida* in apiary using molecular methods and especially considering "new matrices" based on the biological cycle of *A. tumida* and on the model of an apiary.

The biological cycle of SHB was taken into consideration for the choice of matrices to sample.

The applied methodology involved three work phases.

In the first, positive controls for *A. tumida* were found and used to artificially contaminate selected negative matrices in such a way as to verify the validity and robustness of the DNA extraction protocols. Specifically, positive controls for SHB were: a) larvae of *A. tumida* preserved in ethanol; b) cloning of the genomic portion detected in Real Time PCR and represented by a 109bp region of Cytochrome Oxidase I (COI) of *A. tumida* within a specific plasmid (PCR® II-TOPO, Invitrogen) - recombinant plasmid-COI.

In the second phase, DNA extraction protocols were developed from the debris of the hive, soil, beetles, larvae, rotten fruit, fermented honey, burlap, swabs, sampled both on the arnia bottom and the frames' surface.

In the third phase a Ring Test was organized for the verification and validation of DNA extraction protocols from all the above cited matrices between the Istituto Zooprofilattico Lazio e Toscana,

Istituto Zooprofilattico Lombardia ed Emilia Romagna, Istituto Zooprofilattico Sperimentale delle Venezie – National Reference Laboratory for Apiculture, Istituto Zooprofilattico Sperimentale Abruzzo e Molise and Istituto Zooprofilattico Sperimentale del Mezzogiorno.

As a result of this Ring Test, amongst the different laboratories, all tested protocols showed a 100% repeatability.

All positive samples detected with molecular methods on the different tested matrices were found in Calabria.

Finally, it should be emphasized that a very important result was achieved with the accreditation of the *Aethina tumida* PCR Real Time test from the beehive bottom debris (POS VIR 030 INT).

Keywords: SHB PCR research, hive, swabs