Bulletin of Insectology **74** (1): 79-85, 2021
ISSN 1721-8861 eISSN 2283-0332

Identification of odorant-binding proteins (OBPs) in *Aethina tumida*

Giovanni Cilia[1,2], Filippo Marzoli[1,3], Ilaria Cardaio[2], James D. Ellis[4], Antonio Nanetti[2]
[1]*Department of Veterinary Sciences, University of Pisa, Italy*
[2]*CREA Research Centre for Agriculture and Environment, Bologna, Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD) Italy*
[4]*Entomology and Nematology Department, University of Florida, Gainesville, USA*

Abstract

Aethina tumida Murray (small hive beetle, SHB) is an invasive pest of *Apis mellifera* L. colonies, and is attracted to honey bee colony odors and pheromones. This work aims to investigate *A. tumida* odorant binding proteins (Atum_OBPs) from adult individuals, to improve the knowledge on the molecular basis of olfaction and, thus, contribute to the development of sustainable, science-based pest control strategies. RNA was extracted and sequenced from dissected SHB heads and antennae. Complete and partial Odorant-binding proteins (OBPs) were identified in downstream analysis. Overall, four classical OBP from heads and one Minus-C from antennae were recognized as complete OBPs. A phylogenetic analysis showed high sequence homology with previously predicted Atum_OBPs and OBPs described in other coleopteran species. Notably, the antennal OBP showed high similarity with one of the OBPs found in the head. Moreover, partial OBP 29 was detected for the first time in SHB head. This investigation provides an additional knowledge about the OBPs in *A. tumida.* The molecular mechanisms of olfactory perception include compounds other than OBPs. A more complete vision of the involved functions, structures and pathways is necessary to develop attractants for the SHB control in the field.

Key words: small hive beetle, transcriptomic, Illumina, OBPs, sequencing, invasive pest, Nitidulidae.

Introduction

The small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera Nitidulidae) (Murray, 1867) is native to Sub-Saharan Africa (Lundie, 1940; Schmoke, 1974). This beetle is a destructive and invasive pest of *Apis mellifera* L. (Hymenoptera Apidae) colonies, and is now present in all continents except Antarctica (Neumann *et al*., 2016; Al Toufailia *et al*., 2017; Lee *et al*., 2017; Schäfer *et al*., 2019), having reached North America initially and some countries in Europe, South America and Asia more recently (Murilhas, 2004; da Silva, 2014; Palmeri *et al*., 2015; Granato *et al*., 2017).

The adult SHBs invade colonies, where they can feed, reproduce and act as potential mechanical transmitters of honey bee pathogens (Eyer *et al*., 2009; Schäfer et *al*., 2010; Cilia *et al.*, 2018). Invasion mechanisms and interaction patterns with the host have not been fully elucidated to date. However, SHB imagoes are generally believed to be attracted by pheromones and other volatiles produced by the honey bee colony (Elzen *et al*., 1999; Suazo *et al*., 2003; Torto *et al*., 2005). This behaviour has promoted the development of control measures based on odorant-baited traps (Arbogast *et al*., 2007; Torto *et al*., 2010).

Olfaction is essential for insect detection and location of mates and hosts (Pelosi *et al*., 2014). The olfactory system of insects is based on four main protein groups: the odorant binding proteins (OBPs), the odorant receptors (ORs), the odorant-degrading esterases (ODEs), and the chemosensory-proteins (CSPs) (Justice *et al*., 2003). The OBPs are present in the chemosensory organs of insects, where they act as the first step in odorant perception. They bind the hydrophobic odorant molecules present in the environment and, as water-soluble proteins, carry them via the haemolymph to the ORs located in the dendrite membrane of the antennal olfactory sensory neurons, where the signal transduction occurs (Vogt *et al*., 1999; Nakagawa *et al*., 2005). Within insect species, various OBPs are expressed in different organs, including the antennae and others in the head, where they maintain the same function (Pelosi *et al*., 2006; 2014).

There is little understanding of the role of the individual OBPs in the olfactory perception of SHB. Insects carrying a deletion for a gene encoding an OBP (called LUSH) show an uncommon behavior. Specifically, the gene *OBP lush*, encodes for a crucial OBP and its deletion is known to result in abnormal attraction of *Drosophila melanogaster* Meigen (Diptera Drosophilidae) to food sources with high ethanol concentrations (Kim *et al*., 1998; Kim and Smith, 2001).

In general, OBPs contain six highly conserved cysteine residues, which allow their genome-wide identification and annotation in a range of insect species (Liu *et al.*, 2010; Zhou *et al*., 2010). Based on the number of contained cysteine residues, the OBPs are presently sorted in the following groups: "Classic", "Minus-C", "Plus-C", "Dimer", and "Atypical" (Hekmat-Scafe *et al*., 2002; Xu *et al*., 2003; Zhou *et al*., 2004). Insect OBPs often consist of 120-150 amino acids (aa) in the whole, included of the signal peptides, processed after the translational process. Thanks to genome annotation and transcriptome sequencing, OBPs have been widely identified in many insect orders, including Diptera (Zhou *et al*., 2004; Mitaka *et al*., 2011; Zheng *et al*., 2013), Lepidoptera (Gong *et al*., 2009; Grosse-Wilde *et al*., 2011), Hymenoptera (Gotzek *et al*., 2011), Hemiptera (Gu *et al*., 2011) and Coleoptera (Richards *et al*., 2008; Sánchez-Gracia *et al*., 2009; Andersson *et al*., 2013; Liu *et al*., 2015).

Herein, we aim to characterize the OBP sequences from head and antennal adult SHB isolates. This knowledge is needed to better understand the molecular basis of olfaction in SHB and may facilitate the development of science-based lures that can be used in the field to control this honey bee pest.

Materials and methods

Samples collection and RNA extraction

In summer 2017, twenty SHB adults were randomly sampled alive from combs and hive floors of free-flying colonies belonging to an experimental apiary of the University of Florida, Gainesville, Florida, USA. The SHBs were euthanized with dry ice and stored until use in a deep freezer at −80 °C.

In the laboratory, the head of each SHB was dissected and separated from the antennae. The antennae and remaining parts of the heads were pooled separately in 1.5 ml tubes containing 500 µl sterile and DNA/RNA-free water. Then, the RNA was extracted from each of the two pools with the High Pure RNA Isolation Kit (Roche, Basel, Switzerland) following the manufacturer's protocol.

Transcriptome analysis and sequencing

Sample quality control and RNA sequencing were performed by Genomix4Life Srl (Baronissi, Salerno, Italy). The RNA was quantified using a NanoDrop 2000c spectrophotometer (ThermoScientific), and its integrity was assessed by measuring the RNA integrity number (RIN) using a 2100 Bioanalyzer (Agilent Technologies). Indexed libraries were prepared from 2 µg of each purified RNA with TruSeq RNA Sample Prep Kit (Illumina) according to the manufacturer's instructions. Libraries were quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies) and pooled to have each index-tagged sample present in equimolar amounts, with a final concentration of 2 nM. The pooled samples were subjected to cluster generation and sequencing using an Illumina HiSeq 2500 System (Illumina) in a 2 × 150 paired-end formats at a final concentration of 8 pM. The raw sequence files generated underwent quality control analysis using FastQC Version 0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The Illumina paired-end reads were preprocessed for both quality and adapter trimming with fqtrim Version 0.94 with default settings (-A -125 parameters) in order to avoid any read data loss due to false positive (https://ccb.jhu.edu/software/fqtrim/index.shtml).

Sequence analysis

Reads were assembled by Genomix4Life Srl using Trinity *de novo* assembly 3.0 (Grabherr *et al*., 2011). Functional annotations were assigned based on Blast2GO (e-value 10^{-3}) (Conesa *et al*., 2005). After OBP sequence annotation, a set of 'non-redundant' representative sequences was obtained through CD-HIT-EST 1.3 (Li and Godzik, 2006; Fu *et al*., 2012). OBP genes were translated in Expert Protein Analysis System (Artimo *et al*., 2012). Protein structure homology-modeling of the obtained proteins was further analysed through SWISS-MODEL (Waterhouse *et al*., 2018) and further identified through BLASTP. The signal peptide in N terminus was predicted by the SignalP-5.0 program (Bendtsen *et al.*, 2004) (http://www.cbs.dtu.dk/services/SignalP/). The mature form of protein was predicted using MatureP (Orfanoudaki *et al.* 2017). ClustalW Multiple alignment of complete proteins (> 120 aa) was conducted using Bioedit (Hall, 1999). The presence of highly conserved cysteine residues was manually checked and conserved C-pattern of complete OBP was determined.

In order to confirm OBP never detected in SHB, protein family validation through conserved cysteines pattern analysis was employed (Xu *et al*., 2009).

Phylogenetic analysis

Complete amino acid sequences of putative OBP proteins of SHB along with putative OBP sequences of other Coleoptera deposited in GenBank were used to construct a phylogenetic tree. The neighbor joining tree was constructed using Mega-X (Kumar *et al*., 2018) and the evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965). Other OBPs belonging to Coleoptera species of the same infraorder (Cucujiformia) were selected for phylogenetic analysis. The Coleoptera species included were: *A. tumida*, *Tribolium castaneum* (Herbst), *Dendroctonus ponderosae* (Hopkins) and *Anoplophora glabripennis* (Motschulsky).

Results

Illumina sequencing and analysis

The SHB heads and antennae transcriptome was generated starting from high quality total RNA extracted (head RIN = 8.6; antennae RID = 8.2). The cDNA libraries were sequenced, resulting in ~40 million PE 150-bp reads of good quality (Phred quality score ≥ 33).

Analysis by protein structure homology-modelling, allowed to identify seven and eight putative OBPs in the SHB antennae and heads, respectively (Supplemental material tables S1 and S2). Of them, six OBPs from antennae and three from heads resulted as partial proteins. All the partials OBPs completely lacked the signal peptide. Moreover, the MatureP software predicted the proteins as incomplete due to the partial sequence of mature form. The six partial OBP from antennae were identified as follow through BLASTP analysis: two sequences belonging to 83a-like, two sequences belonging to 72-like and other two sequences belonging to OBPs 72 and 70. The three partials OBP detected in head were: one 72-like sequences, one 19-d like sequence and one OBP 29 sequence never detected in SHB. The OBP 29 lacked the first conserved cistein in N terminus and, starting from the second conserved cistein, it showed the following C-pattern: $C2\text{-}X_3\text{-}C3\text{-}X_{38}\text{-}C4\text{-}X_9\text{-}C5\text{-}X_8\text{-}C6$.

The complete OBP aa sequences (120-150 aa) carrying signal peptide were present in four proteins detected in the heads (Atum_OBP1, Atum_OBP2, Atum_OBP3, Atum_OBP4) and one protein detected in the antennae (Atum_OBP5). The features of the five sequences are listed in table 1. Such sequences were employed for OBP

Table 1. Odorant-binding proteins (OBPs) found in heads and antennae of *A. tumida* adults.

Gene name	ORF	Length	Source	Accession number
Atum_OBP1	444	148	Head	MT211982
Atum_OBP2	444	148	Head	MT211983
Atum_OBP3	417	139	Head	MT211984
Atum_OBP4	525	175	Head	MT211985
Atum_OBP5	375	125	Antennae	MT211986

Legend: Gene name, name of genes identified from SHB; ORF, open reading frame; Length, number of amino acids including signal peptide region; Source, head or antennae; Accession number, nucleotide sequence available in GenBank.

downstream analysis. The analysis of the translated proteins showed that the five *A. tumida* OBPs (Atum_OBP) belong to two distinct subgroups: Classical OBPs and Minus-C OBP (figure 1). The classical OBPs were Atum_OBP1, Atum_OBP2, Atum_OBP3 and Atum_OBP4. The remaining Atum_OBP5 sequence refers to Minus-C OBPs.

The conserved C-pattern of complete OBP was C1-X_{25-40}-C2-X_3-C3-X_{38-43}-C4-X_{10-8}-C5-X_8-C6.

Phylogenetic analysis

A phylogenetic tree was generated to study the relationship between the five detected OBPs (Atum_OBP) and OBP aa sequences of the abovementioned Coleopteran species: *A. tumida* (11), *T. castanuem* (30), *D. ponderosae* (21) and *A. glabripennis* (16) (figure 2). In this comparison, the five Atum_OBPs showed to belong to three different groups of orthologous proteins.

Atum_OBP1, Atum_OBP2 and Atum_OBP5 clustered together in the 19d-like OBP phylogenetic group. Moreover, the Atum_OBP2 and Atum_OBP5 also clustered in a branch together with a previously identified *A. tumida* classical-OBP.

After BLASTP alignment, the antennal Atum_OBP5 showed 96.8% similarity with Atum_OBP2 detected in the head. Moreover, also Atum_OBP1 is a 19d-like protein but it showed a similarity of 43.24% and 41.60% with Atum_OBP2 and Atum_OBP5, respectively. The classical OBP Atum_OBP3 clustered in the 56d-like OBP phylogenetic group with OBPs found in other Coleopterans and displayed high homology to another classical OBP from *A. tumida*.

The Atum_OBP4 clustered in the 70 OBP phylogenetic group with OBPs detected in other Coleopterans and showed high homology to another classical OBP detected in *A. tumida*.

Discussion

This investigation aimed to identify OBPs in SHB by sequencing the RNA extracted from heads and antennae of adult individuals, elucidating the OBP sequences in the SHB transcriptome.

Before, whole genome sequencing and transcriptomic analysis were performed on SHB (Tarver *et al*., 2016; Evans *et al*., 2018). Although the aims of such studies (Tarver *et al*., 2016; Evans *et al*., 2018) were not mainly based on OBP research and identification due to the focus on whole genome of SHB, these molecular approaches discovered 14 possible OBPs, which were deposited in GenBank as "predicted". Overall, 14 OBPs, including partial and complete ones, were found in analysed SHB specimens. The number of OBPs detected is very close to previously predicted OBPs (Tarver *et al*., 2016; Evans *et al*., 2018). Indeed, in previously studies performed by Evans and colleagues and Tarver and colleagues, 14 predicted possible OBPs were found, while the same number of proteins were reported in this investigation. The analytical approach employed in this investigation proved the presence of the already predicted OBPs in other SHB specimens, also confirming them *via* protein structure homology-modelling and the C-pattern analysis of Coleoptera.

First, seven OBPs were identified in the SHBs' antennae and heads, respectively. After, translation, protein length and the presence of signal peptide were employed to identify the complete Atum_OBPs.

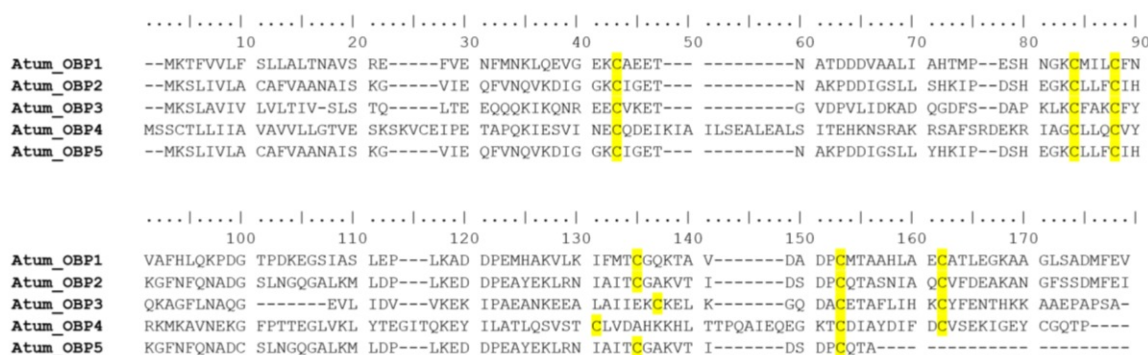

Figure 1. Alignment of the detected sequences of *Aethina tumida* OBPs. Full-length amino acid sequences of Atum_OBPs are aligned by BioEdit. Yellow boxes show conserved cysteine residues. Accession numbers of the 12 Classical OBPs are listed in table 1. The *A. tumida* OBPs include classical OBPs (Atum_OBP1, Atum_OBP2, Atum_OBP3 and Atum_OBP4) and Minus-C OBPs (Atum_OBP5).

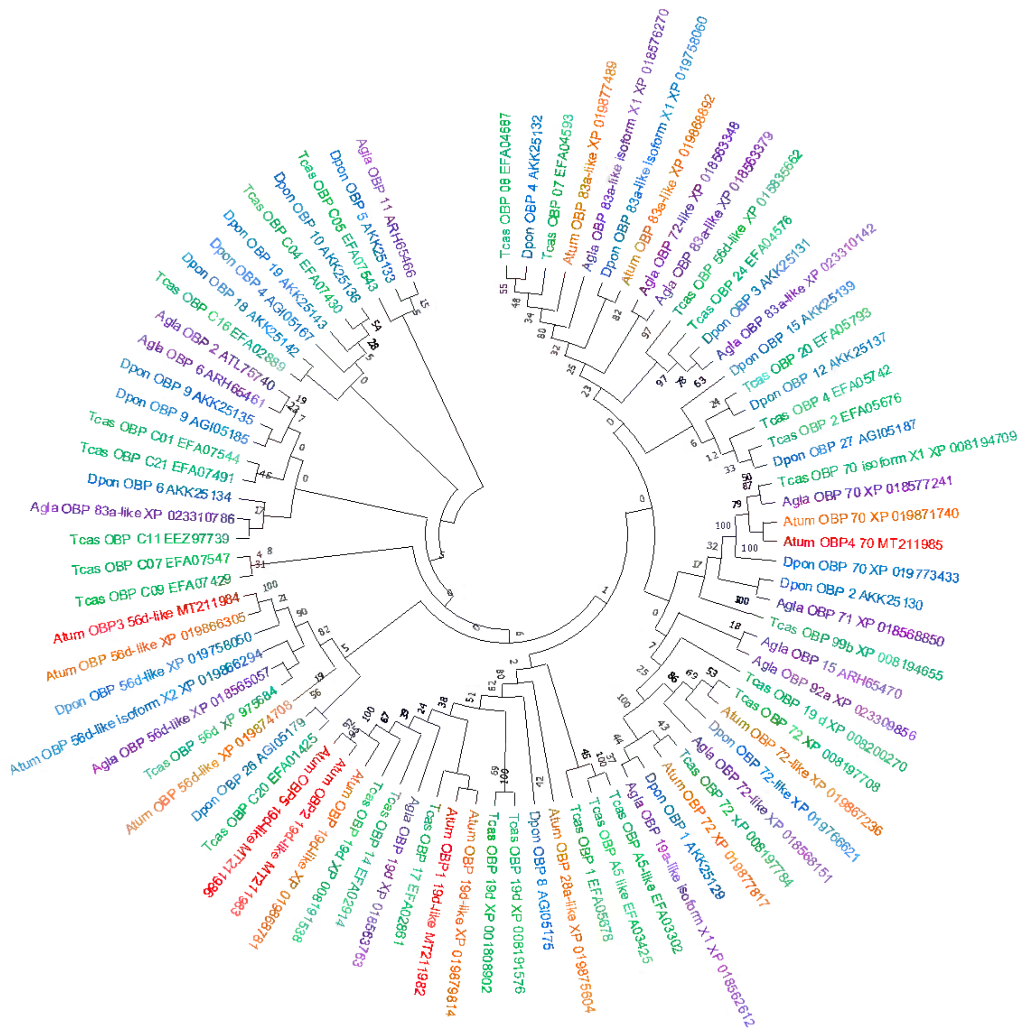

Figure 2. Phylogenetic analysis of Atum_OBPs detected in this investigation (in red). The sequences used for the analysis included *Aethina tumida* (in orange) and three other Coleoptera species with full OBP amino acid sequence: *Tribolium castaneum* (Tcas, in green), *Dendroctonus ponderosae* (Dpon; in blue) and *Anoplophora glabripennis* (Agla; in purple). The GenBank accession numbers are included after the OBP names. The neighbor joining tree was constructed using Mega-X and the evolutionary distances were computed using the Poisson correction method. The analysis involved 83 amino acid sequences. There were 222 positions in the final dataset.

Overall, only four sequences from the SHB head and one from antennae were recognized as complete OBPs. Among them, *A. tumida* OBPs (Atum_OBPs) include classical OBPs (Atum_OBP1, Atum_OBP2, Atum_OBP3 and Atum_OBP4) and Minus-C OBPs (Atum_OBP5) (Hekmat-Scafe *et al*., 2002; Xu *et al*., 2003; Zhou *et al*., 2004). All of the identified Atum_OBPs share high sequence homology (from 98.4% to 100% identity) with predicted *A. tumida* OBPs deposited in GenBank (XP_019879814, XP_019868781, XP_019866305 and XP_019871740). The conserved C-pattern obtained for complete OBP resulted suitable to the general one for Coleoptera (Xu *et al*., 2009).

The Atum_OBP 29 is a partial protein detected for the first time in SHB head in present investigation. It showed 33.33% identity to one OBP 29 found in *Pyrrhalta aenescens* (Fairmaire) (Accession number: APC94285.1), a Coleoptera belonging to Cucujiformia infraorder. In order to confirm protein family validation, C-pattern analysis obtained for partial Atum_OBP 29 (Accession number: MT383114) was compliant with those proposed for Coleoptera (Xu *et al*., 2009).

Notably, the phylogenetic analysis highlighted that all the detected Atum_OBPs clustered with OBPs described in coleopteran species, which may be indicative of conserved functions in species belonging to this order. Among discovered Atum_OBPs, only the antennal Atum_OBP5 displayed high similarity (96.8%), with Atum_OBP2 detected in the head, while other Atum_OBPs did not show relevant similarity. The high similarity between Atum_OBP5 in antennae and Atum_OBP2 in head is suggestive of similar functions in the same individual, as previously reported in other insects (Pelosi, 1994; Pelosi *et al*., 2014).

The results of this investigation showed higher complete Atum_OBPs abundance in the heads (4 OBPs) than in the antennae (1 OBP). As well as in antennae, olfactory sensilla are present in head, thorax and leg (Yan *et al*.,

2016). For these reasons, OBPs expressed in head could be involved in the non-volatile host chemicals perception and other olfactory function (Mitaka *et al*., 2011; Yan *et al*., 2016). As reported for other insects, including coleopteran, OBPs may perform their functions mainly in non-antennae tissues, which could be involved in olfactory-independent functions (Li *et al*., 2015a; 2015b; Yan *et al*., 2016).

Conclusions

Science-based control of SHB invasion requires both behavioural studies involving candidate attractants and knowledge of the molecular mechanisms behind the olfactory perception. Laboratory and field assays are improving the knowledge on the range of volatiles that may find an application in the field (Dekebo and Jung, 2020; Stuhl, 2020). Despite this, there are few information about the molecular perception of volatile cues followed by SHB adults to reach mates, food sources and honey bee colonies. This investigation is an attempt to cover this gap. In this regard, a new OBP was for the first time detected in SHB. Then, of the five complete sequences attributable to OBPs, four were detected in the heads and one in the antennae of examined SHB samples. The high molecular similarity between the antennal OBP and one of the groups found in the head deserves further investigation as it may be suggestive of functional similarity and particular sensitivity to specific volatiles.

This investigation provides a new knowledge about the OBPs in *A. tumida*. However, further studies are required to understand OBP functions, structures and pathways in relation to the other proteins involved in SHB olfactory detection.

Acknowledgements

The Authors are grateful to Brandi Simmons and Branden Stanford of the Entomology and Nematology Department, University of Florida, for their valuable technical support.

This research was funded by Italian Ministry of Agricultural, Food and Forestry Policies, grant number AETHINET ("Monitoraggio e tecniche innovative di diagnosi e di controllo del piccolo coleottero dell'alveare, *Aethina tumida*") (A.N.) and by a cooperative agreement provided by the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-ARS), AP17PPQS&T00C079 (J.D.E.).

G.C. and F.M. equally contribute to this work; conceptualization, G.C. and A.N.; methodology, G.C., F.M., I.C., J.D.E. and A.N.; investigation, G.C., F.M. and I.C.; in-filed activity, I.C. and J.D.E; laboratory activity, G.C.; data curation, G.C. and F.M.; writing original draft preparation, G.C., F.M. and A.N.; writing review and editing, G.C., F.M., I.C., J.D.E. and A.N.; supervision, J.D.E. and A.N.; funding acquisition, A.N. and J.D.E. All authors have read and agreed to the published version of the manuscript. The authors declare no conflict of interest.

References

AL TOUFAILIA H., ALVES D. A., BENÁ D. DE C., BENTO J. M. S., IWANICKI N. S. A., CLINE A. R., ELLIS J. D., RATNIEKS F. L. W., 2017.- First record of small hive beetle, *Aethina tumida* Murray, in South America.- *Journal of Apicultural Research*, 56: 76-80.

ANDERSSON M. N., GROSSE-WILDE E., KEELING C. I., BENGTSSON J. M., YUEN M. M. S., LI M., HILLBUR Y., BOHLMANN J., HANSSON B. S., SCHLYTER F., 2013.- Antennal transcriptome analysis of the chemosensory gene families in the tree killing bark beetles, *Ips typographus* and *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae).- *BMC Genomics*, 14: 198.

ARBOGAST R. T., TORTO B., VAN ENGELSDORP D., TEAL P. E. A., 2007.- An effective bait and trap combination for monitoring the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae).- *Florida Entomolist*, 90: 404-406.

ARTIMO P., JONNALAGEDDA M., ARNOLD K., BARATIN D., CSARDI G., DE CASTRO E., DUVAUD S., FLEGEL V., FORTIER A., GASTEIGER E., GROSDIDIER A., HERNANDEZ C., IOANNIDIS V., KUZNETSOV D., LIECHTI R., MORETTI S., MOSTAGUIR K., REDASCHI N., ROSSIER G., XENARIOS I., STOCKINGER H., 2012.- ExPASy: SIB bioinformatics resource portal.- *Nucleic Acids Research*, 40: W597-W603.

BENDTSEN J. D., NIELSEN H., VON HEIJNE G., BRUNAK S., 2004.- Improved prediction of signal peptides: SignalP 3.0.- *Journal of Molecular Biology*, 340: 783-795.

CILIA G., CARDAIO I., DOS SANTOS P. E. J., ELLIS J. D., NANETTI A., 2018.- The first detection of *Nosema ceranae* (Microsporidia) in the small hive beetle, *Aethina tumid*a Murray (Coleoptera: Nitidulidae).- *Apidologie*, 49: 619-624.

CONESA A., GÖTZ S., GARCÍA-GÓMEZ J. M., TEROL J., TALÓN M., ROBLES M., 2005.- Blast2GO: a universal annotation and visualization tool in functional genomics research. Application note.- *Bioinformatics*, 21: 3674-3676.

DA SILVA M. J. V., 2014.- The first report of *Aethina tumida* in the European Union, Portugal, 2004.- *Bee World*, 91: 90-91.

DEKEBO A., JUNG C., 2020.- Olfactory responses of *Aethina tumida* Murray (Coleoptera: Nitidulidae) to some major volatile compounds from hive materials and workers of *Apis mellifera*.- *Journal of Asian-Pacific Entomology*, 23: 504-508.

ELZEN P., BAXTER J., WESTERVELT D., RANDALL C., CUTTS L., WILSON W., EISCHEN F., DELAPLANE K., HOPKINS D., 1999.- Status of the small hive beetle in the US.- *Bee Culture*, 127: 28-29.

EVANS J. D., MCKENNA D., SCULLY E., COOK S. C., DAINAT B., EGEKWU N., GRUBBS N., LOPEZ D., LORENZEN M. D., REYNA S. M., RINKEVICH F. D., NEUMANN P., HUANG Q., 2018.- Genome of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae), a worldwide parasite of social bee colonies, provides insights into detoxification and herbivory.- *GigaScience*, 7 (12): giy138.

EYER M., CHEN Y. P., SCHÄFER M. O., PETTIS J., NEUMANN P., 2009.- Small hive beetle, *Aethina tumida* , as a potential biological vector of honeybee viruses.- *Apidologie*, 40: 419-428.

FU L., NIU B., ZHU Z., WU S., LI W., 2012.- Sequence analysis CD-HIT: accelerated for clustering the next-generation sequencing data.- *Bioinformatics*, 28: 3150-3152.

GONG D. P., ZHANG H. J., ZHAO P., XIA Q. Y., XIANG Z. H., 2009.- The odorant binding protein gene family from the genome of silkworm, *Bombyx mori*.- *BMC Genomics*, 10: 332.

GOTZEK D., ROBERTSON H. M., WURM Y., SHOEMAKER D. W., 2011.- Odorant binding proteins of the red imported fire ant, *Solenopsis invicta*: an example of the problems facing the analysis of widely divergent proteins.- *PLoS ONE*, 6 (1): e16289.

GRABHERR M. G., HAAS B. J., YASSOUR M., LEVIN J. Z., THOMPSON D. A., AMIT I., ADICONIS X., FAN L., RAYCHOWDHURY R., ZENG Q., CHEN Z., MAUCELI E., HACOHEN N., GNIRKE A., RHIND N., DI PALMA F., BIRREN B. W., NUSBAUM C., LINDBLAD-TOH K., FRIEDMAN N., REGEV A., 2011.- Full-length transcriptome assembly from RNA-Seq data without a reference genome.- *Nature Biotechnology*, 29: 644-652.

GRANATO A., ZECCHIN B., BARATTO C., DUQUESNE V., NEGRISOLO E., CHAUZAT M.-P., RIBIÈRE-CHABERT M., CATTOLI G., MUTINELLI F., 2017.- Introduction of *Aethina tumida* (Coleoptera: Nitidulidae) in the regions of Calabria and Sicily (southern Italy).- *Apidologie*, 48: 194-203.

GROSSE-WILDE E., KUEBLER L. S., BUCKS S., VOGEL H., WICHER D., HANSSON B. S., 2011.- Antennal transcriptome of *Manduca sexta*.- *Proceedings of the National Academy of Sciences of the United States of America*, 108: 7449-7454.

GU S. H., WANG S. P., ZHANG X. Y., WU K. M., GUO Y. Y., ZHOU J. J., ZHANG Y. J., 2011.- Identification and tissue distribution of odorant binding protein genes in the lucerne plant bug *Adelphocoris lineolatus* (Goeze).- *Insect Biochemistry and Moleclar Biology*, 41: 254-263.

HALL T. A., 1999.- BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.- *Nucleic Acids Symposium Series*, 41: 95-98.

HEKMAT-SCAFE D. S., SCAFE C. R., MCKINNEY A. J., TANOUYE M. A., 2002.- Genome-Wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*.- *Genome Research*, 12: 1357-1369.

JUSTICE R. W., BIESSMANN H., WALTER M. F., DIMITRATOS S. D., WOODS D. F., 2003.- Genomics spawns novel approaches to mosquito control.- *BioEssays*, 25: 1011-1020.

KIM M.-S., SMITH D. P., 2001.- The invertebrate odorant-binding protein LUSH is required for normal olfactory behavior in *Drosophila*.- *Chemical Senses*, 26: 195-199.

KIM M.-S., REPP A., SMITH D. P., 1998.- LUSH odorant-binding protein mediates chemosensory responses to alcohols in *Drosophila melanogaster*.- *Genetics*, 150: 711-721.

KUMAR S., STECHER G., LI M., KNYAZ C., TAMURA K., 2018.- MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms.- *Molecular Biology and Evolution*, 35: 1547-1549.

LEE S., HONG K.-J., CHO Y. S., CHOI Y. S., YOO M.-S., LEE S., 2017.- Review of the subgenus *Aethina* Erichson s. str. (Coleoptera: Nitidulidae: Nitidulinae) in Korea, reporting recent invasion of small hive beetle, *Aethina tumida*.- *Journal of Asia-Pacific Entomology*, 20 553-558.

LI W., GODZIK A., 2006.- CD-HIT: a fast program for clustering and comparing large sets of protein or nucleotide sequences.- *Bioinformatics*, 22: 1658-1659.

LI X., JU Q., JIE W., LI F., JIANG X., HU J., QU M., 2015a.- Chemosensory gene families in adult antennae of *Anomala corpulenta* Motschulsky (Coleoptera: Scarabaeidae: Rutelinae).- *PLoS ONE*, 10: e0121504.

LI X. M., ZHU X. Y., WANG Z. Q., WANG Y., HE P., CHEN G., SUN L., DENG D. G., ZHANG Y. N., 2015b.- Candidate chemosensory genes identified in *Colaphellus bowringi* by antennal transcriptome analysis.- *BMC Genomics*, 16: 1028.

LIU R., LEHANE S., HE X., LEHANE M., HERTZ-FOWLER C., BERRIMAN M., PICKETT J. A., FIELD L. M., ZHOU J. J., 2010.- Characterisations of odorant-binding proteins in the tsetse fly *Glossina morsitans morsitans*.- *Cellular and Molecular Life Science*, 67: 919-929.

LIU S., RAO X. J., LI M. Y., FENG M. F., HE M. Z., LI S. G., 2015.- Identification of candidate chemosensory genes in the antennal transcriptome of *Tenebrio molitor* (Coleoptera: Tenebrionidae).- *Comparative Biochemistry and Physiology, Part D*, 13: 44-51.

LUNDIE A. E., 1940.- The small hive beetle, *Aethina túmida*.- *Science Bulletin*, 220: 1-30.

MITAKA H., MATSUO T., MIURA N., ISHIKAWA Y., 2011.- Identification of odorant-binding protein genes from antennal expressed sequence tags of the onion fly, *Delia antiqua*.- *Molecular Biology Report*, 38: 1787-1792.

MURILHAS A. M., 2004.- *Aethina tumida* arrives in Portugal. Will it be eradicated?- *EurBee Newsletters*, 2: 7-9.

MURRAY A., 1867.- List of Coleoptera received from Old Calabar, on the west coast of Africa.- *Annals and Magazine of Natural History*, 19: 167-179.

NAKAGAWA T., SAKURAI T., NISHIOKA T., TOUHARA K., 2005.- Insect sex-pheromone signals mediated by specific combinations of olfactory receptors.- *Science*, 307 (5715): 1638-1642.

NEUMANN P., PETTIS J. S., SCHÄFER M. O., 2016.- Quo vadis *Aethina tumida*? Biology and control of small hive beetles.- *Apidologie*, 47: 427-466.

ORFANOUDAKI G., MARKAKI M., CHATZI K., TSAMARDINOS I., ECONOMOU A., 2017.- MatureP: prediction of secreted proteins with exclusive information from their mature regions.- *Scientific Reports*, 7: 3263.

PALMERI V., SCIRTÒ G., MALACRINÒ A., LAUDANI F., CAMPOLO O., 2015.- A scientific note on a new pest for European honeybees: first report of small hive beetle *Aethina tumida*, (Coleoptera: Nitidulidae) in Italy.- *Apidologie*, 46: 527-529.

PELOSI P., 1994.- Odorant-binding proteins.- *Critical Reviews in Biochemistry and Molecular Biology*, 29: 199-228.

PELOSI P., ZHOU J. J., BAN L. P., CALVELLO M., 2006.- Soluble proteins in insect chemical communication.- *Cellular Molecular Life Science*, 63: 1658-1676.

PELOSI P., IOVINELLA I., FELICIOLI A., DANI F. R., 2014.- Soluble proteins of chemical communication: an overview across arthropods.- *Frontiers in Physiology*, 5: 320.

RICHARDS S., THE *TRIBOLIUM* GENOME SEQUENCING CONSORTIUM, 2008.- The genome of the model beetle and pest *Tribolium castaneum*.- *Nature*, 452: 949-955.

SÁNCHEZ-GRACIA A., VIEIRA F. G., ROZAS J., 2009.- Molecular evolution of the major chemosensory gene families in insects.- *Heredity*, 103: 208-216.

SCHÄFER M. O., RITTER W., PETTIS J., NEUMANN P., 2010.- Small hive beetles, *Aethina tumida*, are vectors of *Paenibacillus larvae*.- *Apidologie*, 41: 14-20.

SCHÄFER M. O., CARDAIO I., CILIA G., CORNELISSEN B., CRAILSHEIM K., FORMATO G., LAWRENCE A. K., LE CONTE Y., MUTINELLI F., NANETTI A., RIVERA-GOMIS J., TEEPE A., NEUMANN P., 2019.- How to slow the global spread of small hive beetles, *Aethina tumida*.- *Biological Invasions*, 21: 1451-1459.

SCHMOKE M. D., 1974.- *A study of* Aethina tumida*: the small hive beetle*.- Project Report, University of Rhodesia, Salisbury, Zimbabwe.

STUHL C. J., 2020.- The development of an attract-and-kill bait for controlling the small hive beetle (Coleoptera: Nitidulidae).- *Apidologie*, 51: 428-435.

SUAZO A., TORTO B., TEAL P. E. A., TUMLINSON J. H., 2003.- Response of the small hive beetle (*Aethina tumida*) to honey bee (*Apis mellifera*) and beehive-produced volatiles.- *Apidologie*, 34: 525-533.

TARVER M. R., HUANG Q., DE GUZMAN L., RINDERER T., HOLLOWAY B., REESE J., WEAVER D., EVANS J. D., 2016.- Transcriptomic and functional resources for the small hive beetle *Aethina tumida*, a worldwide parasite of honey bees.- *Genomics Data*, 9: 97-99.

TORTO B., SUAZO A., ALBORN H., TUMLINSON J. H., TEAL P. E. A., 2005.- Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified from honeybee (*Apis mellifera*) volatiles.- *Apidologie*, 36: 523-532.

TORTO B., FOMBONG A. T., ARBOGAST R. T., TEAL P. E. A., 2010.- Monitoring *Aethina tumida* (Coleoptera: Nitidulidae) with baited bottom board traps: occurrence and seasonal abundance in honey bee colonies in Kenya.- *Environmental Entomology*, 39: 1731-1736.

VOGT R. G., CALLAHAN F. E., ROGERS M. E., DICKENS J. C., 1999.- Odorant binding protein diversity and distribution among the insect orders, as indicated by LAP, an OBP-related protein of the true bug *Lygus lineolaris* (Hemiptera, Heteroptera).- *Chemical Senses*, 24: 481-495.

WATERHOUSE A., BERTONI M., BIENERT S., STUDER G., TAURIELLO G., GUMIENNY R., HEER F. T., DE BEER T. A. P., REMPFER C., BORDOLI L., LEPORE R., SCHWEDE T., 2018.- SWISS-MODEL: homology modelling of protein structures and complexes.- *Nucleic Acids Reseach*, 46: W296-W303.

XU P. X., ZWIEBELT L. J., SMITH D. P., 2003.- Identification of a distinct family of genes encoding atypical odorant-binding proteins in the malaria vector mosquito, *Anopheles gambiae*.- *Insect Molecular Biology*, 12: 549-560.

XU Y. L., HE P., ZHANG L., FANG S. Q., DONG S. L., ZHANG Y. J., LI F., 2009.- Large-scale identification of odorant-binding proteins and chemosensory proteins from expressed sequence tags in insects.- *BMC Genomics*, 10: 632.

YAN W., LIU L., QIN W., LUO Y., MA X., HAIDER N., INAYEH M., 2016.- Identification and tissue expression profiling of odorant binding protein genes in the red palm weevil, *Rhynchophorus ferrugineus*.- *SpringerPlus*, 5: 1542.

ZHENG W., PENG W., ZHU C., ZHANG Q., SACCONE G., ZHANG H., 2013.- Identification and expression profile analysis of odorant binding proteins in the oriental fruit fly *Bactrocera dorsalis*.- *International Journal of Molecular Science*, 14 14936-14949.

ZHOU J. J., HUANG W., ZHANG G. A., PICKETT J. A., FIELD L. M., 2004.- "Plus-C" odorant-binding protein genes in two *Drosophila* species and the malaria mosquito *Anopheles gambiae*.- *Gene*, 327: 117-129.

ZHOU J. J., VIEIRA F. G., HE X. L., SMADJA C., LIU R., ROZAS J., FIELD L. M., 2010.- Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid *Acyrthosiphon pisum*.- *Insect Molecular Biology*, 19: 113-122.

ZUCKERKANDL E., PAULING L., 1965.- Evolutionary divergence and convergence in proteins, pp. 97-166. In: *Evolving genes and proteins* (BRYSON V., VOGEL H. J., Eds).- Academic Press, New York, USA.

Authors' addresses: Giovanni CILIA (corresponding author: giovannicilia23@gmail.com), Filippo MARZOLI, Department of Veterinary Sciences, University of Pisa, viale delle Piagge 2, 56124, Pisa, Italy; Ilaria CARDAIO, Antonio NANETTI, CREA Research Centre for Agriculture and Environment, via di Saliceto 80, 40128, Bologna, Italy; James D. ELLIS, Entomology and Nematology Department, University of Florida, Bldg. 970 Natural Area Dr., P.O. Box 110620, Gainesville, FL 32607-0620, USA.

Received July 8, 2020. Accepted January 19, 2021.

(Supplemental material available at http://www.bulletinofinsectology.org/Suppl/vol74-2021-079-085cilia-suppl.pdf)