Analytical method for animal health

REFERENCE: ANSES/SOP/ANA-I1.MOA.1500 - Version 06

August 2024

Identification of the Small Hive Beetle, *Aethina tumida*, by morphological examination (WOAH method)

Sophia-Antipolis Laboratory

National Reference Laboratory – Bee Health

European Union Reference Laboratory – Bee Health

History of the method

A method can be updated in order to take changes into account.

A change is considered major when it involves the analytic process, the scope or critical points of the analysis method, the application of which may modify the performance characteristics of the method and/or the results. A major change requires major adaptations and either total or partial revalidation.

A change is considered minor if it provides useful or practical clarifications, reformulates the text to make it clearer or more accurate, or corrects minor errors. A minor change in the method does not alter its performance characteristics and does not require revalidation.

The table below summarises the version history of this method and provides qualifications for the changes. The changes are highlighted in grey in the document.

Version	Nature of changes (Major / Minor)	Date	Main changes
V04	Minor	01/02/2020	1. Reformatting of the method. 2. Updating of references. 3. Revisions of the protocol taking into account EURL practical feedback and results of the comparative laboratory testing SHBCLT18: - Precisions added on how to carry out the analysis. - Precisions added concerning the morphological identification criteria: reformulation of certain criteria, footnotes to explain entomological terms, precisions for the colour and the tip of the antennae, extension of the adult size range to better take into account the specimens of extreme size, precisions on the "spines" of the larva. - Reorganisation of the order of the criteria in order to better correspond to the course of the analysis. - Addition of details on figures (new figure concerning the morphology of the larvae = Fig. 6; revision of Fig. 8 comparing SHB and wax moth larvae). - Precisions in the "analytical results" section for "uninterpretable" cases and addition of a section on opinions.
V05	Minor	15/09/2023	- « OIE » now « WOAH » (World Organisation for Animal Health). - New Anses logo. - Updates in the introduction (epidemiological and regulatory context) and in the bibliography. - Precision added concerning the criterion n°6 for identifying adults (shape of club antennae). - Inclusion of a note and a figure on the differential diagnosis of *A. tumida* larvae with those of *Urophorus humeralis* (another species of Nitidulidae): § 3.4, Figure 9. - Performance characteristics of the method.

V06	Minor	14/08/2024	- Addition of a flowchart (§ 1) - Minor precisions in the introduction and in the protocol (§ 8.1) - Rewording of certain identification criteria to make them more precise. (§ 8.2 / adult). - Addition of two figures specifying the shape of *A. tumida* antennae (Figure 3 and 4). - Update of the bibliography.

Foreword

This method was developed by:

ANSES - Sophia-Antipolis Laboratory

French National Reference Laboratory for Bee Health

European Reference Laboratory for Bee Health

WOAH Reference Laboratory for Small Hive Beetle Infestation

Address: Les Templiers - 105 route des Chappes - CS 20111 - 06902 Sophia-Antipolis Cedex

Contact: eurl.bee@anses.fr

Contents

Introduction

The small hive beetle, *Aethina tumida* (Murray, 1867) (Coleoptera: Nitidulidae) (SHB) is a parasite and a predator of honey bees, native to South Africa. Part of its biological cycle takes place in honey bee colonies and part in the soil. Female beetles drawn by the smell of the hive enter it and lay masses of eggs in wood crevices. These eggs hatch into larvae. The predatory larvae grow by feeding on bee brood (eggs, larvae), honey and pollen. While feeding on food stores the remaining honey is fermenting and the comb is destroyed. SHB can cause significant damage for beekeeping, depending on the strength of the colony, particularly in areas with warm temperature and high humidity (factors favouring its biological cycle). In severe cases, *A. tumida* infestation can lead to absconding, colony collapse and harvest losses.

The small hive beetle has been introduced in various regions of the world over the past few years (Neumann, 2016; WOAH, 2018). An introduction was reported in Portugal in 2004, following a bee queen importation from Texas, United States. Sanitary measures implemented allowed to avoid the spread of the beetle in this country. In September 2014, the presence of SHB was confirmed in the South of Italy. Sanitary measures were set up immediately, but visits of the apiaries in the same areas showed that the SHB has spread in the regions of Calabria and Sicily. The infestation has since been eradicated in Sicily, but remains enzootic in Calabria[1]. In July 2022, the SHB was also detected on the island of Reunion in France[2].

The infestation by SHB is ruled in European Union (EU)[3] and internationally (it belongs to the disease list of the World Organisation for Animal Health / WOAH).

In case of suspicion, rapid and reliable diagnosis is crucial to implement sanitary measures and avoid dissemination of the coleopteran. This method, based on the WOAH Manual (WOAH, 2018), describes a protocol for the morphological identification of *A. tumida* adults and larvae. It provides a result in a short time with a low cost technique and is therefore indicated for first intention diagnosis.

[1] https://www.izsvenezie.it/aethina-tumida-in-italia/
[2] Veille sanitaire internationale | PLATEFORME ESA (plateforme-esa.fr)
[3] Commission implementing regulation (EU) 2018/1882 of 3 December 2018 on the application of certain disease prevention and control rules to categories of listed diseases and establishing a list of species and groups of species posing a considerable risk for the spread of those listed diseases. In this regulation, infestation with Aethina tumida (small hive beetle) is listed in the categories D (“listed disease for which measures are needed to prevent it from spreading on account of its entry into the Union or movements between Member States”) and E (“listed disease for which there is a need for surveillance within the Union”).

Warnings and safety precautions

The user of this method should be closely familiar with standard laboratory practices. It is the responsibility of the user to establish suitable health and safety practices and ensure compliance with the current regulations.

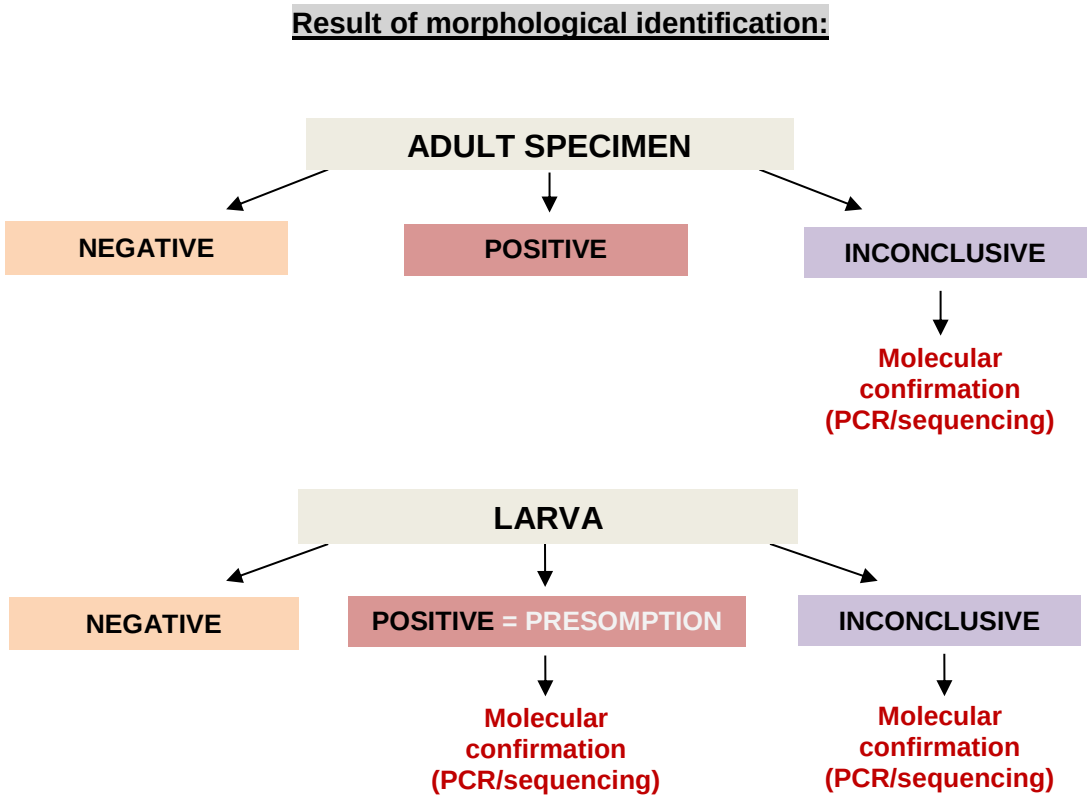
All actions taken in accordance with this method must be performed by employees who have attended relevant training.

1. Purpose and scope

Aethina tumida Murray, 1867 can be identified by examining the external appearance of adults and/or larvae. The identification method described here is based on the method described in WOAH manual (2018). It consists in the visual examination of individuals (adults and/or larvae) with recording of morphological characteristics and, if necessary, comparison of the sample to be identified with a reference sample or detailed photographs.

The insects to be identified are collected in or near honeybee hives (for example, in beekeeping equipment or queen cages).

The morphological identification is confirmed if necessary using a molecular method (e.g. PCR, sequencing). For larvae, if the morphological identification leads to a positive or an inconclusive result, molecular identification is systematically performed (see flowchart bellow).



2. Reference documents

[1] ***WOAH, 2018. Small hive beetle infestation (Aethina tumida). Chapter 2.2.5. In: Manual of standards for diagnostic tests and vaccines for terrestrial animals, Paris.***

3. Terms, abbreviations and definitions

EU: European Union

WOAH: World Organisation for Animal Health

PCR: Polymerase Chain Reaction

SHB: Small Hive Beetle

4. Principle of the method

The identification of *A. tumida* takes into account certain morphological characteristics of the adult insects and larvae.

Particularly, a distinction shall be made with other beetles belonging to the Nitidulidae family, found in honeybee hives in Europe and with not known pathogenic effect on colonies:

- *Cychramus luteus* Fabricius, 1787 (Coleoptera: Nitidulidae), another member of the Nitidulidae family, found in Europe, mainly feeds on pollen (Neumann and Ritter, 2004).
- *Carpophilus lugubris* Murray, 1864 (Coleoptera: Nitidulidae), found in hives in Italy (Marini et al., 2013).

The larvae of *A. tumida* can also be mistaken for larvae of the lesser wax moth, *Achroia grisella* Fabricius, 1794 (Lepidoptera: Pyralidae), as well as for the honeycomb moth, *Galleria mellonella* Linnaeus, 1758 (Lepidoptera: Pyralidae). These lepidopterans are generally found in colonies and beekeeping equipment.

Note: Due to the sanitary risk implied by this exotic parasite, the analysis must be done rapidly after reception of the sample, in order to confirm or not the suspicion and thus apply the official sanitary measures.

5. Reagents

Warning: Trade names or supplier names may be mentioned in the description of the products required to implement this method. This information is provided for users of the method and does not mean that ANSES recommends the exclusive use of these products. Similar products may be used if it has been demonstrated that they achieve the same results.

- Ethanol 70% (not denatured)

Note: Not denatured ethanol shall be used in order to limit the risk of inhibition of the PCR (if performed later).

6. Equipment and materials

Warning: Trade names or supplier names may be mentioned in the description of the equipment and materials required to implement this method. This information is provided for users of the method and does not mean that ANSES recommends the exclusive use of these materials. Similar materials may be used if it has been demonstrated that they achieve the same results.

- Stereomicroscope (and/or magnifier) (minimum 40' magnification)
- Entomological tweezers and spatula
- Glass, plastic, or porcelain evaporating dishes, or Petri dishes
- Capped tubes

7. Samples

7.1 Acceptance conditions for samples

The customer sampling form should mention that every specimen suspected to belong to the species *A. tumida* must be sent dead to the laboratory. In case of doubt, the package must be opened in containment conditions.

If the specimens arrive alive to the laboratory, the sample is placed, for example, for one hour in a freezer at least at -70°C. Afterwards, the specimens are directly placed in a tube with ethanol 70% in order to kill them.

7.2 Sample storage before analysis

The specimens are stored in ethanol 70% in capped tubes at room temperature.

7.3 Storage of samples or residual materials after analysis

The specimens are stored in ethanol 70% in capped tubes at room temperature.

8. Procedure

8.1 Protocol

1. Lay-out of the work area

Clean the work area before the analysis and prepare the material required.

2. Sampling for analysis

- Place the specimens in a dish (Petri dish or similar).

- Count the number of specimens present in the sample with the naked eye or a stereomicroscope or magnifier and record the result. If the number is greater than 100, stop at 100 and note: ">100".

- Carry out the analysis sampling on the basis of the number of specimens counted according to the protocol presented in the Table 1. The specimens are taken at random using entomological twizzers or a spatula.

Table 1 – Analysis sampling strategy

Total number of specimens present in the sample	1-30	31-100	> 100
Number of specimens to analyse	all	30 + 30 % of the remaining specimens (total number of specimens – 30)*	51

** Round up to the next whole number.*

Example: if the total number of specimens in the sample is 58, analyse 30 + 30/100 x (58-30) = 38.4, i.e. *39 specimens to examine.*

Notes:

- *The sampling strategy is inspired by the referential of the French Association for the Study of epidemiology of animal diseases (Toma et al., 2010), and based on the data allowing the detection of an expected prevalence of 5% of positive cases in a population, with a risk of error of 5% (for a population of 1 to 180 units).*

- *It should be noted that the analysis is consistent with an approach of identification of entomological specimens sampled in the apicultural context, further to suspicions. Therefore, the number of specimens to analyse is, in fact, generally low. Nevertheless, it may happen that some larval samples contain a large number of specimens.*

3. Observation with the stereomicroscope

- Allow the specimens to dry until the ethanol has evaporated before making the observation (so that their colour can be correctly assessed).

- Examine the sample using different magnifications in order to appreciate the different criteria for the identification detailed in paragraph 8.2 and annexes 1 and 2. Compare with *A. tumida* reference specimens if necessary (and available).

Note: If at least one of the criteria 1 to 3 is missing, compliance with the other criteria is not achieved.

- Measure the size of the specimens (x1 magnification).

Note: The length of adult beetles is measured from the caudal end to the cranial end without taking into account the antennae and mouthparts which may possibly protrude from the body of the insect. The width of the insect body is measured at the widest part of the pronotum.

- If different species are identified during the examination, sort them by category (sub-samples), and analyse them separately according to the criteria detailed in paragraph 8.2 and annexes 1 and 2.

4. Conclusion of the analysis

- **Adult specimens:**

If the result is inconclusive (non-assessable criteria, or if the physical condition of the specimen makes morphological identification difficult or even impossible), molecular identification will be necessary.

- **Larval specimens:**

If the morphological examination of larvae leads to a negative result, it can be concluded the non-identification of *A. tumida*.

If the morphological examination of larvae leads to a positive result, a molecular test must be performed in order to confirm the identification. If the result is inconclusive, molecular identification will be also necessary.

In case of a positive result, the official sanitary authorities must be informed with no delay.

8.2 Identification of the small hive beetle *A. tumida*

A. tumida belongs to the class of Insects, order of Coleoptera and family of Nitidulidae.

▪ Guidelines for the identification of *A. tumida*, adult form

(Ellis, 2002; Lee et al., 2017; Li et al., 2018 ; Lundie, 1940; Marini et al., 2013; Menier and Jouan, 2003; Neumann and Ritter, 2004; Neumann et al., 2013; Numa-Vergel, 2021; WOAH, 2018)

Annex 1: Figures 1, 2, 3 and 4.

Table 2 - Adult diagnosis criteria

1.	Body divided in three parts: head, thorax and abdomen.
2.	Three pairs of legs.
3.	Presence of elytra[4].
4.	Elytra not covering the entire abdomen: some abdominal segments are apparent in dorsal view.
5.	Overall uniform body colour (no spots), light brown to black when beetles are fully mature. *Note: The colour may change with environmental conditions and conservation of the specimens.*
6.	Antenna tips with compact, almost rounded club ends. The three terminal articles of the antennas, corresponding to the "clubs"[5], are narrowed between them.. The transversal size of the first segment of the clubs (proximal article) is slightly larger than the other two segments (distal articles). (Figure 3 and Figure 4)
7.	Posterio-lateral angles of the pronotum appear sharp (dosarl view).[6]
8.	Dimensions: Length: 4 to 7 mm (+/-1 mm); Width: 3 mm (+/- 1 mm).

Notes:

- Size is one of the indicator criteria that are used to identify *A. tumida* according to our conditions. Under no circumstances shall size be a sole criterion for identifying this beetle.

The size of *A. tumida* can indeed vary if the beetle is in a retracted (defensive) or "extended" position, and according to sex (Ellis, 2002; Menier and Jouan 2003). Factors such as food availability, climate (Ellis, 2002) or soil type could also influence size.

- *A. tumida* has a lighter border around the pronotum and elytra (row of fine yellow bristles). This characteristic is not always observable on dead specimens preserved in ethanol.

- In the case *Cychramus luteus*: (Annex 1: Figure 5; Neumann and Ritter, 2004)
  - Elytra completely cover the abdominal apex;
  - Antennal clubs are looser with detached segments. They do not have a rounded shape;
  - Latero-posterior tips of the pronotum are not sharp;
  - Colour of the body is light brown.

[4] Elytra: sclerotized (= thickened) forewings covering the hind wings at rest in beetles and some other insects.
[5] Clubs: In some beetle families, such as the Nitidulidae, the terminal articles of the antennae are larger and club-shaped.
[6] Pronotum: dorsal part of the first segment of the thorax (the first segment of the thorax is called the prothorax, it never carries wings but the first pair of legs on the ventral side).

- In the case of *Carpophilus lugubris*: (Annex 1: Figure 6 - Marini et al., 2013)
  - Body is brown; elytra have orange regions. Legs and antennae are orange (antennal clubs are dark orange);
  - Body length: 3.3 to 4.5 mm.
  - Elytra don't cover the entire abdomen;
  - Club-shaped antennae have compact segments, but their shape is oval rather than rounded and the proximal article is narrower that the distal ones;
  - Latero-posterior tips of the pronotum are sharp.

▪ Guidelines for the identification of *A. tumida*, larval form

(Marini et al., 2013; Menier and Jouan, 2003; Neumann and Ritter, 2004; WOAH, 2018)

Annex 2: Figures 7, 8, and 9.

Table 3 - Larva diagnosis criteria

1. Three pairs of legs, one on each of the anterior segments, corresponding to the larva thorax.
2. All of the posterior leg segments are bare and have no false legs (also called pseudopods) on their ventral part.
3. From the mesothorax[7], presence on each segment, of two dorsal tubers on either side of the midline. These tubers are finished with a short fine silk. They look like "spines".

The identification of the small hive beetle larva is always confirmed by PCR, except when the results are negative (see paragraph 9. Results).

Notes:

- The larvae of the SHB have generally a creamy light beige body colour. The cephalic capsule (head of the larva) is brown in colour.
- The body length at maturity is about 1 cm (1.2 cm maximum length). The length depends on feeding. The width is about 1.6 mm.
- To distinguish *A. tumida* larvae from Lepidoptera larvae (lesser wax moth, *A. grisella* and honeycomb moth, *G. mellonella*), frequently present in honeybee hives:

The Lepidoptera larvae present pseudopods on the ventral side of the abdominal segments.

[7] Mesothorax: It corresponds to the second thoracic segment of the larva. It has the second pair of legs. The prothorax corresponds to the first thoracic segment; it does not have a tuber, its dorsal part (tergum) is sclerified.

There are two bare segments between the last segment with legs and the first segment with pseudopods (Annex 1: Figure 10).

Besides, the Lepidoptera larvae can make a silky web, cocoons, and dark faeces (these webs and faeces can be observed in the sample containers received by the lab).

- The dorsal tubers are pigmented. They are terminated by a short fine silk and preceded by two short spines. The last segment of the larva abdomen has two dorsal tubers and two urogomphs[8] (Figure 8).

- Some larvae of other species of Nitidulidae are morphologically very similar to *A. tumida*. Figure 11 shows, for example, the larva of the beetle *Urophorus humeralis* (Fabricius, 1798) detected in hives on Reunion Island. These larvae have dorsal tubers on either side of the midline and urogomphs, but no marked "spines", unlike *A. tumida*. If there is any doubt about identification, a confirmatory PCR analysis is recommanded.

9. Results

9.1 Reporting of results

Table 4 – Results: adult forms

Analysis results	Conclusion
Positive: Criteria 1 to 8 confirmed for *A. tumida.*	Positive
Negative: Certain fundamental morphological characteristics of *A. tumida* are not present: • At least one out of the three criteria (1 to 3) not confirmed (in this case, the other observations are not realised). • Or at least one out of the five other criteria (4 to 8) not confirmed.	Negative
Inconclusive: Impossibility to rule on the positive or negative character of the sample: it was not possible to rule on the presence/absence of certain morphological identification criteria (*e.g.* damaged specimen). → Molecular identification systematically realised.	Inconclusive

Table 5 – Results: Larval forms

Analysis results	Conclusion
Positive: all criteria 1 to 3 confirmed.	Positive / Suspicion

[8] Urogomph: An extension, fixed or mobile, attached to one of the last segments of the abdomen of certain larvae.

→ Molecular identification systematically realised.	
Negative: criteria 1, 2 or 3 not confirmed.	Negative
Inconclusive: Impossibility to rule on the positive or negative character of the sample: it was not possible to rule on the presence/absence of certain morphological identification criteria (*e.g.* damaged specimen). → Molecular identification systematically realised.	Inconclusive

9.2 Guidelines for expressing opinions

Opinions, taking into consideration the results of the different analysis performed (morphology and/or molecular diagnosis), can be expressed according to the decisions rules described in Table 6.

Table 6 - Decision rules for giving opinions

		PCR result			
		Analysis not performed	Positive	Negative	Inhibited
Morphological examination result	**ADULT**				
	Positive	(1)	(1)	(3)	(1)
	Negative	(2)	(3)	(2)	(2)
	Inconclusive		(1)	(2)	(4)
	LARVA				
	Suspicion/Positive		(1)	(2)	(4)
	Negative	(2)	(3)	(2)	(2)
	Inconclusive		(1)	(2)	(4)

(1)**:** « Positive identification of the small hive beetle, *Aethina tumida* »
(2): « Negative identification of the small hive beetle, *Aethina tumida* »
(3)**:** « Suspected identification of the small hive beetle, *Aethina tumida.* Further analysis is required to ascertain the identification. »
(4)**:** « Inconclusive result of *Aethina tumida* identification »

10. Performance characteristics of the method

Caracteristic	Parameter	Value	Main information on the methods used for characterisation
Sensitivity (SE) and inclusivity (IN)	SE: Percentage of positive results found among the expected positive results. IN : Ability of the method to detect the target analyte from a wide range of strains, isolates, populations, etc.	**100%**	Characterisation performed using two approaches: ▪ Intra-laboratory: 41 specimens of *A. tumida* (18 larvae and 23 adults) from different geographical areas (South Africa, United States, Mexico, Italy, France/ Réunion Island, Mauritius, England/FERA experimental production). ▪ Inter-laboratory: comparative laboratory testing organised in 2018 (SHBCLT18 campaign) with 9 participating laboratories, panel consisting of 18 samples, 6 of which were positive.
Spécificité (SP)	Percentage of negative results found among expected negatives.	**100%**	Characterisation performed using two approaches: ▪ Intra-laboratory: 82 specimens (67 adults and 15 larvae) corresponding to samples received for analysis (and collected in beehive environment) or to Nitidulidae species collected on plants. ▪ Inter-laboratory: SHBCLT18 CLT, 9 participating laboratories, panel consisting of 18 samples, 12 of which were negative.
Reproducibility	Probability of obtaining two similar results, based on the distribution of observed values.	**100%**	Characterisation performed using two approaches: ▪ Intra-laboratory: 15 specimens (8 adults and 7 larvae) analysed by 3 different operators trained and authorised for the method. ▪ Inter-laboratory: SHBCLT18 CLT, 9 laboratoires participants, 9 participating laboratories, panel consisting of 18 samples (11 adults and 7 larvae).

REFERENCES

- Boecking, O., and T. Wagner. 2005. Der Kleine Beutenkäfer *Aethina tumida*, wichtige morphologische Bestimmungsmerkmale und Lebenszyklus. LAVES, Institut für Bienenkunde, Celle, Germany. Available online at: https://www.laves.niedersachsen.de/download/42553/Kleiner_Beutenkaefer_-_Merkmale_und_Lebenszyklus.pdf. Accessed on 22/07/2024.

- Ellis, J. D., K. S. Delaplane, and W. M. Hood. 2002. Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the southeastern United States. *American Bee Journal* 142:520-522.

- Ellis, J. D. et A. Ellis. 2010. Featured Creatutres, Common name: small hive beetle, Scientific name: *Aethina tumida* Murray (Insecta: Coleoptera: Nitidulidae). University of Florida, Entomology & Nematology (UF/IFAS). Last review: March 2019. https://entnemdept.ufl.edu/creatures/misc/bees/small_hive_beetle.htm. Accessed on 22/07/2024.

- GBIF (Global Core Biodata Resource) Secretariat. 2023. *Aethina concolor* (Macleay, 1872). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei. https://www.gbif.org/species/7760044. Accessed via GBIF.org on 22/07/2024.

- iNaturalist contributors, iNaturalist. 2024. iNaturalist Research-grade Observations. iNaturalist.org. Occurrence dataset https://doi.org/10.15468/ab3s5x. https://www.gbif.org/occurrence/3994171725. Accessed via GBIF.org on 22/07/2024.

- Lee, S., K.-J. Hong, Y. Sang Cho, Y. S. Choi, M.-S. Yoo, and S. Lee. 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 (2):553-558. https://doi.org/10.1016/j.aspen.2017.03.006.

- Li, Dongmei, David W. Waite, Qing-Hai Fan, Sherly George, Linda Semeraro and Mark J. Blacket. 2018. Molecular detection of small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae): DNA barcoding and development of a real-time PCR assay. *Scientific Reports* 8 (1): 9623. https://doi.org/10.1038/s41598-018-27603-x.

- Lundie, A. E. 1940. The small hive beetle *Aethina tumida*. *Science Bulletin*, Department of Agriculture and Forestry: Government Printer; Pretoria, South Africa. 220: 30.

- Manaaki Whenua, Landcare Research. 2024. What is this bug? Hibiscus beetle *Aethina concolor* (Macleay). https://www.landcareresearch.co.nz/tools-and-resources/identification/what-is-this-bug/hibiscus-beetle/. Accessed on 22/07/2024.

- Marini, F., F. Mutinelli, F. Montarsi, A. Richard Cline, E. Gatti, and P. Audisio. 2013. First report in Italy of the dusky sap beetle, C*arpophilus lugubris*, a new potential pest for Europe. *Journal of Pest Science* 86 (2):157-160. https://doi.org/10.1007/s10340-013-0479-9

- Menier, J., and M. Jouan. 2003. Biologie et description d'*Aethina tumida* Murray, 1867, espèce invasive déprédatrice de l'Abeille mellifère (Coleoptera, Nitidulidae). *Bulletin de la Societé Entomologique de France*:323-328.

- Neumann, P., and W. Ritter. 2004. A scientific note on the association of *Cychramus luteus* (Coleoptera: Nitidulidae) with honeybee (*Apis mellifera*) colonies. *Apidologie* 35 (6):665-666. https://doi.org/10.1051/apido:2004051

- Neumann P., and J.D Ellis. 2008. The small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): distribution, biology and control of an invasive species. *Journal of apicultural Research. Bee World*, 47 (3), 181–183. https://doi.org/10.1080/00218839.2008.11101453

- Neumann, P., J. D. Evans, J. S. Pettis, C.W.W. Pirk, M.O. Schäfer, and J.D. Ellis. 2013. Standard methods for small hive beetle research. *Journal of Apicultural Research* 52 (4). https://doi.org/10.3896/IBRA.1.52.4.19

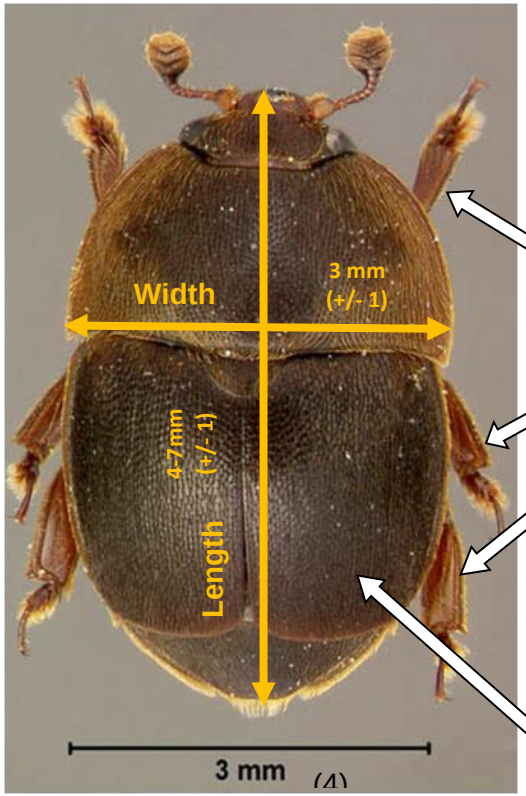
- Neumann, P., J. S. Pettis J. S., and M. O. Schäfer. 2016. Quo vadis *Aethina tumida*? Biology and control of small hive beetles. *Apidologie* 47(3): 427-466. https://doi.org/10.1007/s13592-016-0426-x

- Numa-Vergel, S. J., Y. P. Sandoval-Cáceres, and E. V. Vergara-Navarro. 2021. First Record of *Aethina tumida* Murray (Coleoptera: Nitidulidae) in Colombia. *Journal of Agricultural and Urban Entomology* 37 (1): 33-37. https://doi.org/10.3954/JAUE21-09.

- WOAH (World Organisation for Animal Health). 2018. Small hive beetle infestation (*Aethina tumida*). Chapter 2.2.5. In: *Manual of standards for diagnostic tests and vaccines for terrestrial animals*, Paris.

- Toma, B., B. Dufour, J.J. Bénet, M. Sanaa, A. Shaw, and F. Moutou. 2010. « Épidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures. » 3rd edition. AEEMA, p. 209.

ANNEX 1 - Adult identification



Dorsal view

Light brown to black body (5)

Lighter band around thorax and abdomen

3 pairs of legs (2)

Elytra = hardened anterior wings (4)

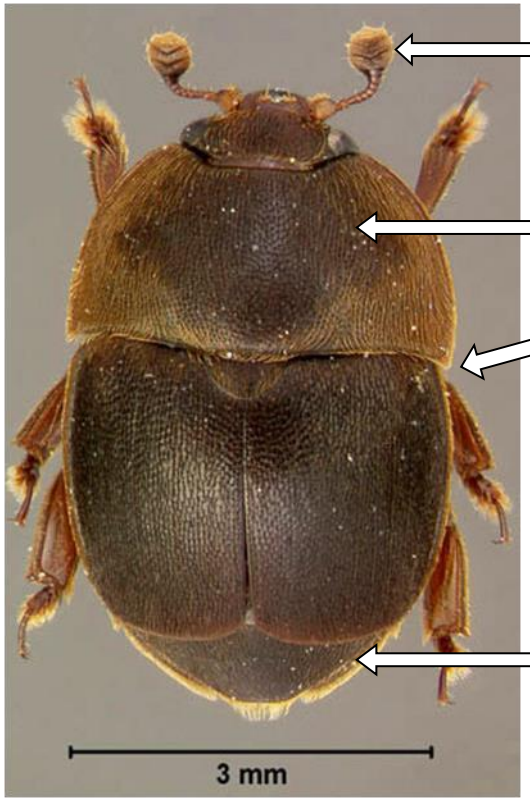
Ventral view

Head (1)

Thorax (1)

Abdomen (1)

Figure 1 - Small hive beetle, *Aethina tumida* Murray
Source: Josephine Ratikan, University of Florida (Ellis, 2010); WOAH, 2018



Dorsal view

Club-shaped antennae, with compact segments, and almost rounded club ends, the transversal size of the proximal article is slightly larger than the other two articles (6)

Pronotum (pro-thorax dorsal side)

Sharp latero-posterior tips of the pronotum (7)

Abdomen partially covered by elytra: visible apex (posterior tip of the abdomen) (4)

Dorsal view
Source: Anses, Sophia Antipolis

Defense position: SHB retracts its appendages beneath its body, leaving nothing extended for bees to grasp.

Figure 2 - Small hive beetle, *Aethina tumida* Murray – Guidelines to distinguish *A. tumida* from other Nitidulidae species present in the hive
Source: Josephine Ratikan, University of Florida (Ellis, 2010); WOAH, 2018

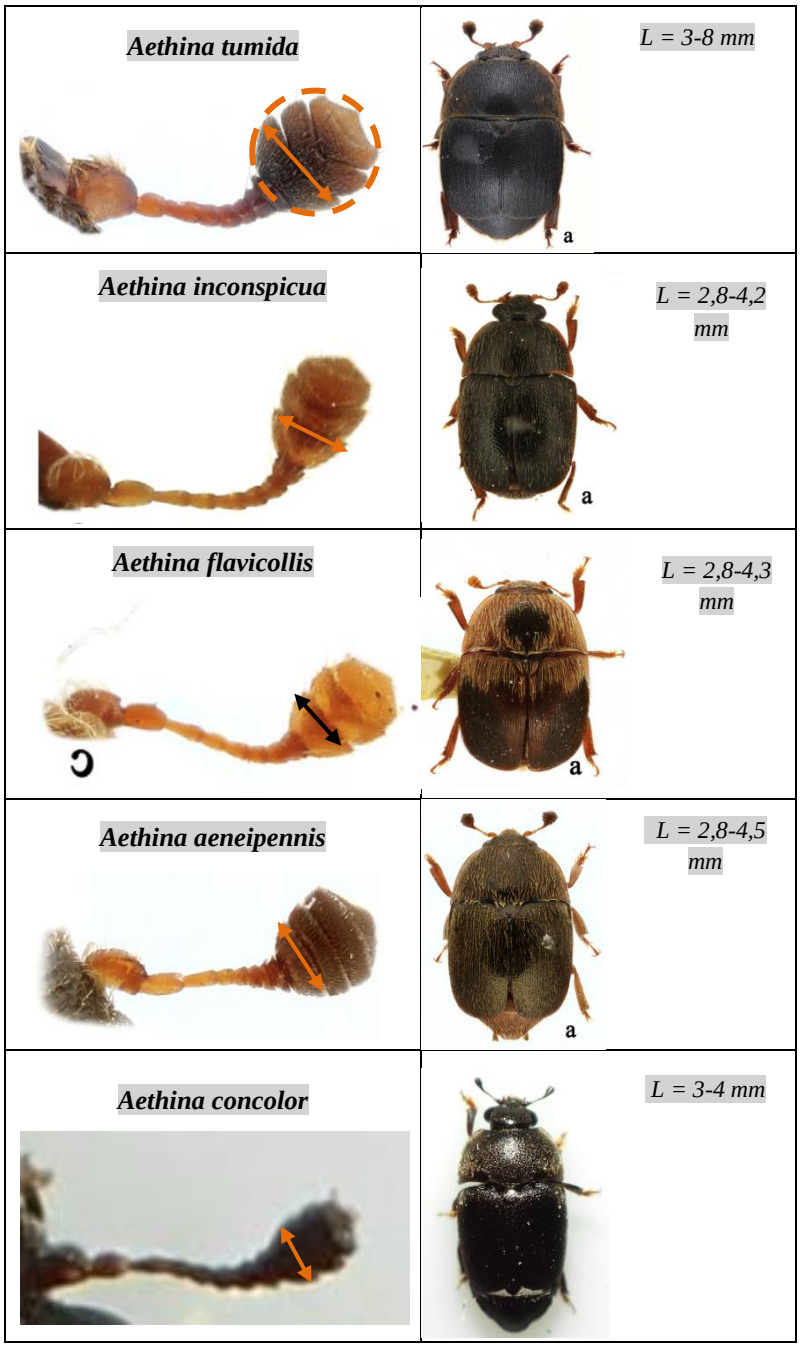
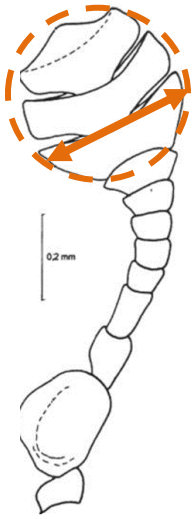
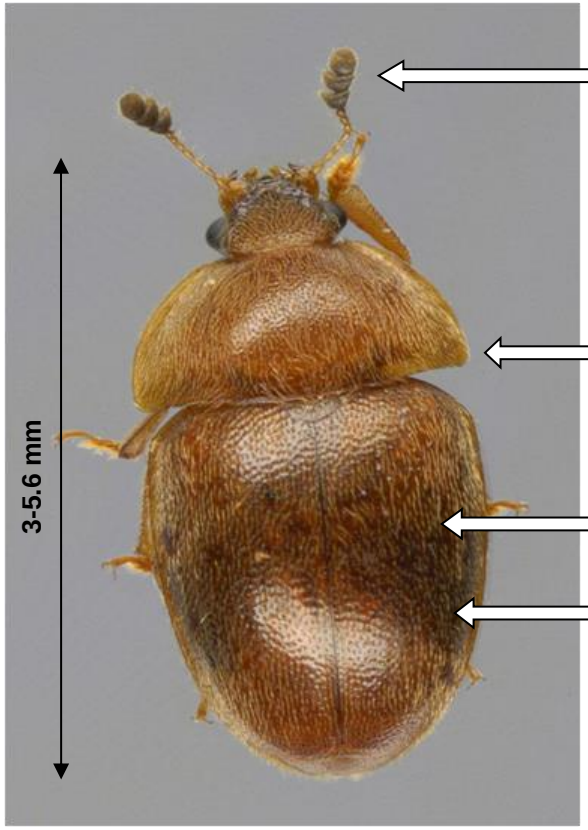


Figure 4 – Antenna shapes of four species of Aethina found in Korea (Lee, 2017) and of *A. concolor*, a species found in New Zealand, Australia, New Caledonia and French Polynesia in particular (iNaturalist contributors, 2024 ; GBIF, 2023 ; Manaaki Whenua Landcare Research, 2024). *Aethina concolor* is the only other species of the genus *Aethina* already detected in beehives (Li, 2018). *(L = length)*



The transversal size of the first segment of the clubs (proximal article) is slightly larger than the other two segments (distal articles) (6)

Figure 3 - A. *tumida* antenna (Menier and Jouan, 2003)



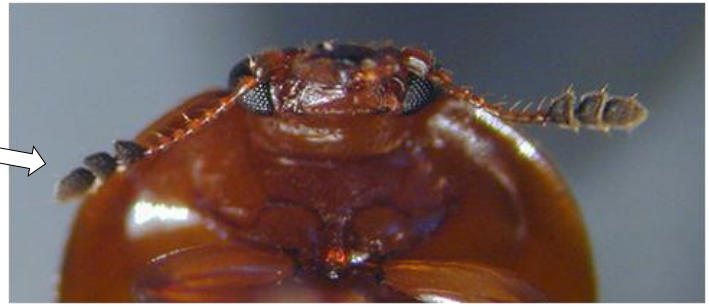
Source: Malcom Storey, 2003, www.bioimages.org.uk
Web page accessed on 01/12/2016

Club-shaped antennae, with loose segments, their shape is not rounded

Round pronotum latero-posterior tips

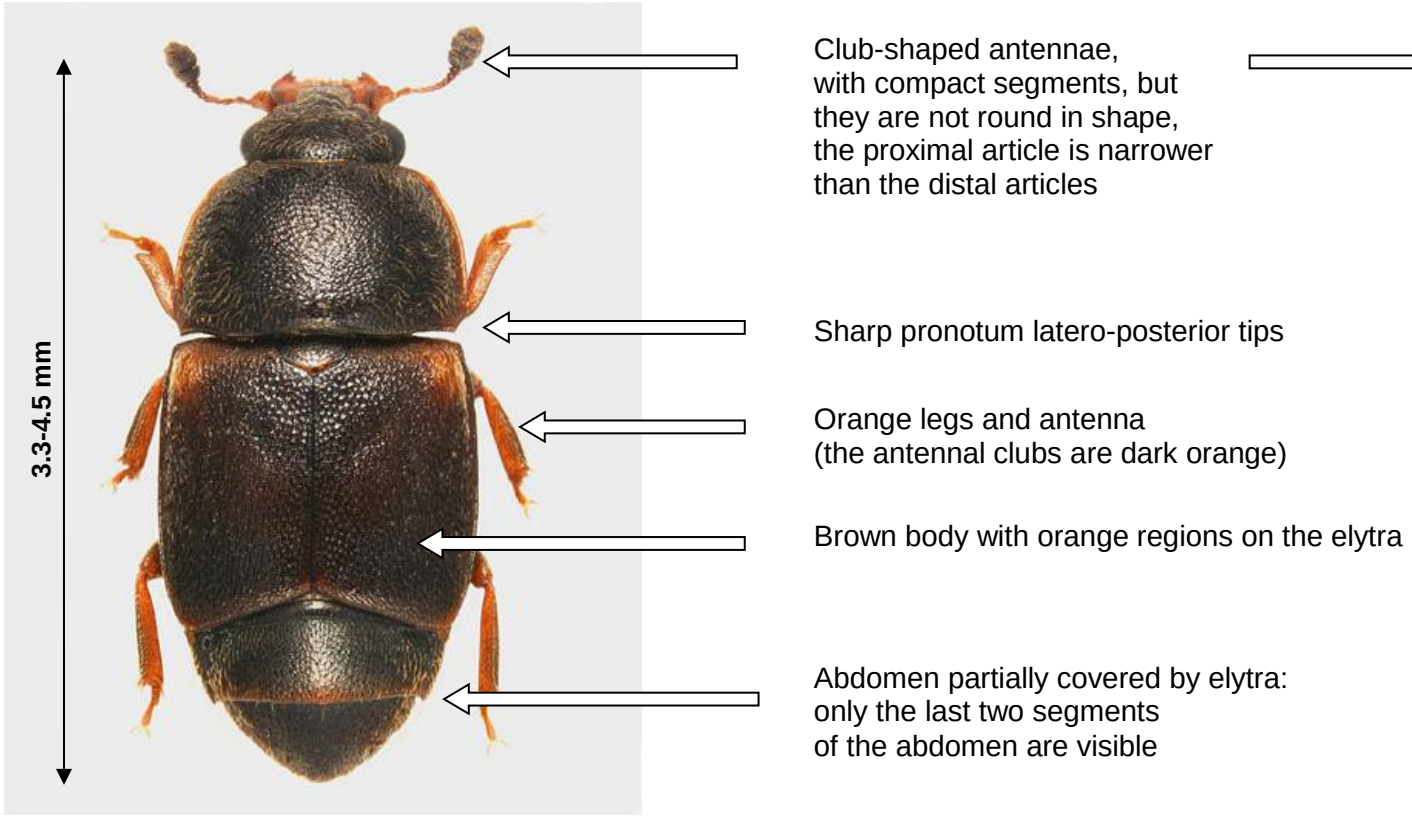
Light-brown body

Abdomen completely covered by elytra



Source: Malcom Storey, 2003, www.bioimages.org.uk
Web page accessed on 01/12/2016

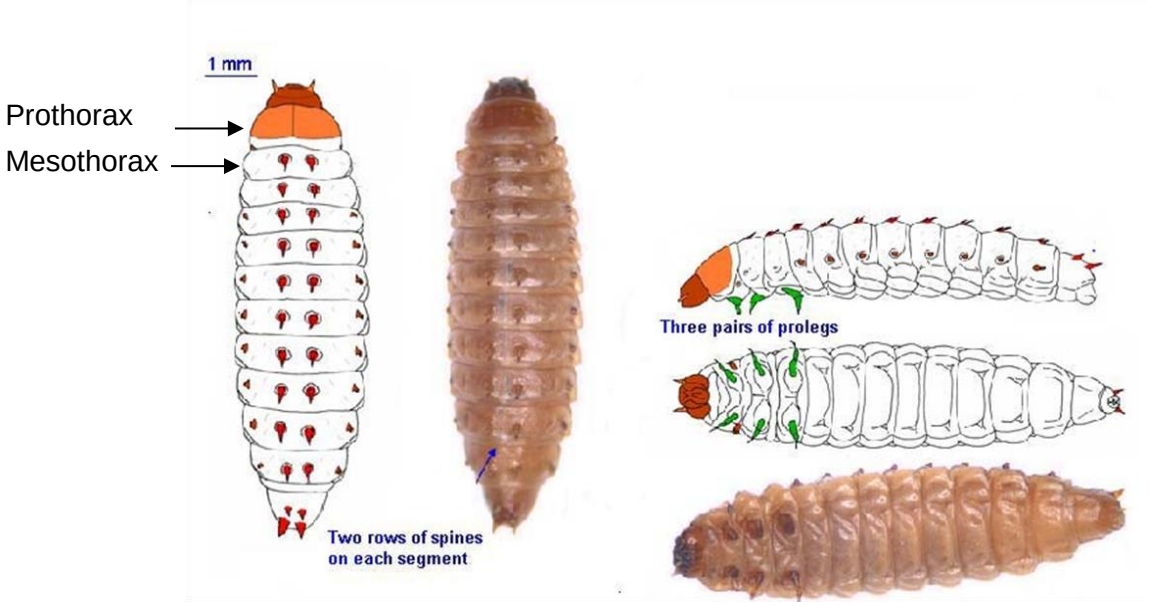
Figure 5 - *Cychramus luteus* Fabricus (Neumann and Ritter, 2004)



Dorsal view
Source: Marini *et al*., 2013

Figure 6 - *Carpophilus lugubris* Murray (Marini *et al*., 2013)

ANNEX 2 - Larva identification



Source: Boeking, 2005

Figure 7 - Larva of *Aethina tumida* Murray

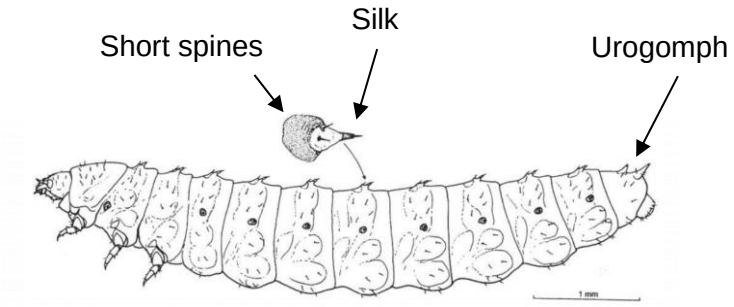
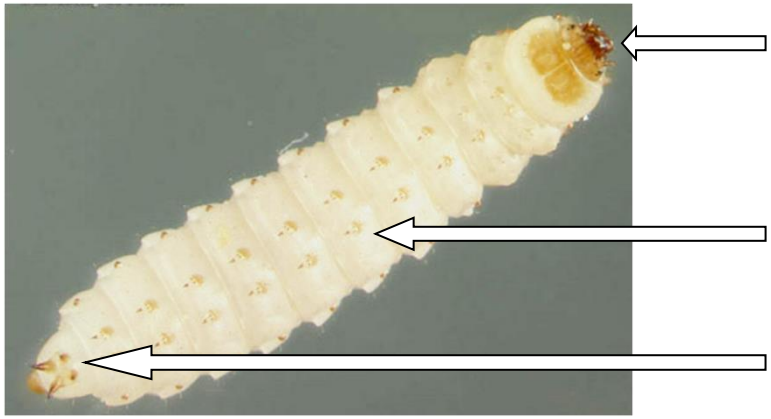


Figure 8 - Detail of a dorsal tuber
Source: Menier and Jouan, 2003



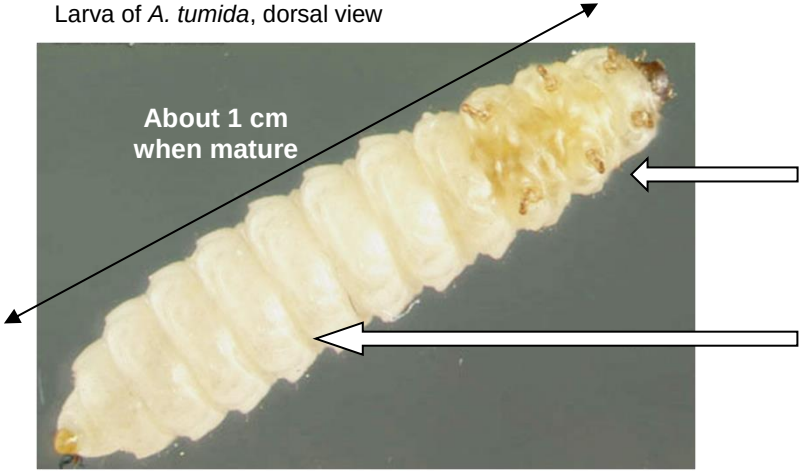
Brown cephalic capsule

Light-beige body

2 rows of dorsal tubers (“spines”) on each segment (starting form the mesothorax) (3)

2 dorsal tubers (“spines”) and two urogomphs on the last segment (3)

Larva of *A. tumida*, dorsal view



3 pairs of legs, one on each of the anterior segments (1)

No pseudopods on the ventral side of the posterior abdominal segments (2)

Larva of *A. tumida*, ventral view

Source: Josephine Ratikan, University of Florida; WOAH, 2018

Figure 9 - Larva of *Aethina tumida* Murray

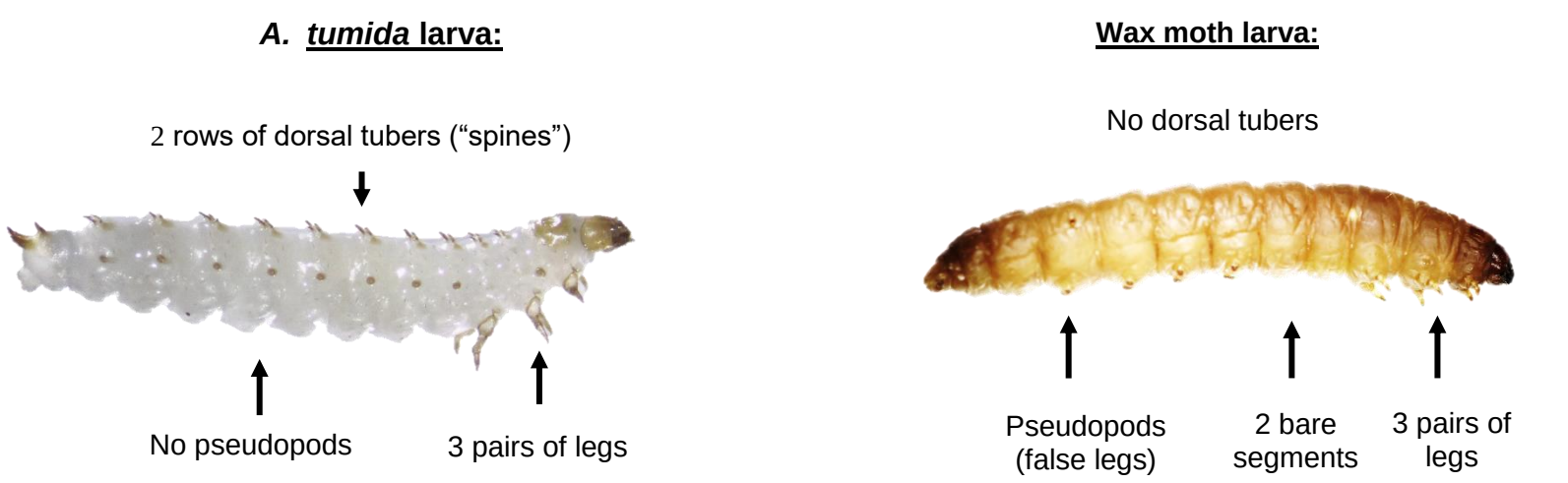


Figure 10 - Differentiation of *A. tumida* from wax moth larvae

Source: Anses, Sophia Antipolis laboratory

3 pairs of legs, one on each of the anterior segments (1)

No pseudopods on the ventral side of the posterior abdominal segments (2)

Light-beige body

Brown cephalic capsule

Presence of two dorsal tubers on each segment on either side of the midline, but no marked "spines", unlike *A. tumida* (3)

Two larger tubers in the posterior segment: presence of urogomphs.

Figure 11 - Larvae of the beetle *Urophorus humeralis* (Fabricius, 1798)

Source: Cirad La Réunion (Gérard LEBRETON)