EURL
European Union Reference Laboratory for
INSECTS AND MITES

FINAL REPORT

Validation of the morphological diagnostic protocol for *Anoplophora glabripennis* (Motschulsky, 1854) and *Anoplophora chinensis* (Forster, 1771)

EPPO PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis*

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Anoplophora glabripennis ***Anoplophora chinensis***

1. Introduction

The European Reference Laboratory for Insects and Mites has to select, adapt or develop reliable protocols/tests for the identification of insect and mite species that are relevant for the European Union (included in the Commission Delegated Regulation (EU) 2019/1702 and in the EURL for Insects and Mites working programmes). One of the tasks of the EURL is to validate available diagnostic protocols before recommending their use to the National Reference Laboratories of the European Union.

The Entomology and Invasive Plants Unit of Anses Plant Health Laboratory (Montpellier, France) and the Institute for Sustainable Plant Production of AGES (Vienna, Austria) are in charge of the activities of the EURL for Insects and Mites. The consortium performs validation studies for morphological and molecular identification tests.

According to the ISO/IEC 17025 standard, the validation of a test is defined as the "confirmation by examination and the provision of objective evidence that the particular requirements for a given intended use are met". In fact, this confirmation consists of comparing the values of the performance criteria determined during the test characterization study with those expected or assigned beforehand (limits of acceptability, objectives to be achieved), then declaring the analytical test valid or invalid. In the field of entomology, identification is qualitative, meaning that diagnostic protocols allow the identification at a given taxonomic level providing a response in terms of presence/absence.

The EURL for Insects and Mites focuses on the validation of tests published in international or regional standards, such as those issued by the International Plant Protection Convention (IPPC) or the European and Mediterranean Plant Protection Organization (EPPO).

Anoplophora glabripennis (MOTSCHULSKY, 1854) and *Anoplophora chinensis* (FORSTER, 1771) (Coleoptera: Cerambycidae: Lamiinae: Monochamini) are both native to Asia, mainly occurring in China and Korea (*A. glabripennis*) respectively in China, Korea and Japan (*A. chinensis*). Due to international trade on wood packaging materials they were introduced to new areas – not only to other Asian countries but also to other continents as North America and Europe. In Europe the first record for *A. glabripennis* was in Austria in 2001, since that time additional local outbreaks and new interceptions have also been reported from other European countries (EPPO, 2021a). The first European report for *A. chinensis* was in the Netherlands in 1980 and since 2000 several outbreaks have occurred in other European countries (EPPO, 2021a) too.
Both species are very polyphagous wood boring beetles on deciduous trees and shrubs including many fruit and ornamental trees. Great damage is caused by the feeding of the larvae into the wood and therefore their occurrence has a high economic impact and represents a great economic loss.

Anoplophora glabripennis and *Anoplophora chinensis* are European Union regulated species, listed among the EU quarantine pests (Annex II of the Commission Implementing Regulation (EU) 2019/2072, amended by the Commission Implementing Regulation (EU) 2021/2285) and among the EU priority pests (Commission Delegated Regulation (EU) 2019/1702).

2. Scope of validation and tests

2.1 Scope

The scope of this validation study is to provide objective evidence that the selected diagnostic protocol is suitable to perform routine identification of *Anoplophora glabripennis* (Motschulsky, 1854) and *Anoplophora chinensis* (Forster, 1771) by the staff of the EU National Reference Laboratories.

2.2 Description of the protocol under validation

This validation study is focused on the evaluation of a diagnostic protocol for the morphological identification of *Anoplophora glabripennis* and *Anoplophora chinensis*, i.e. respectively:

- EPPO PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis* (EPPO, 2021b)

Validation is conducted according to the EPPO PM7/98 (4) Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (EPPO, 2019).

2.2.1 Morphological identification of larvae

Protocol: EPPO PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis* (EPPO, 2021b)

The morphological identification of larvae of *Anoplophora glabripennis* and *Anoplophora chinensis* at the species level is possible for late instar larvae, but it can be difficult to distinguish between the two species. The use of a stereomicroscope is needed. There are no adequate keys for the identification of eggs, early instar larvae and pupae. Therefore, it is recommended to use molecular methods for early instars.

The protocol provides guidance for the identification of *A. glabripennis* and *A. chinensis* late instar larvae:

- **Appendix 1 – Key for the identification of *A. glabripennis* and *A. chinensis* late instar larvae**: a simplified key is given for the morphological identification of late instar larvae of *A. glabripennis* and *A. chinensis* after Pennacchio et al. (2012)
- **paragraph 4.1.1.1. Description of larvae morphology** (head, pronotum, abdomen)
- **paragraph 4.1.1.2. Description of some other similar native and introduced Lamiinae larvae**

2.2.2 Morphological identification of adults

Protocol: EPPO PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis* (EPPO, 2021b)

The identification at the species level for *A. glabripennis* and *A. chinensis* requires morphological examination of adult beetles. The identification is possible both on male and female specimens. The use of a stereomicroscope is needed.

The protocol provides guidance for the identification at species level of *A. glabripennis* and *A. chinensis* adults:

- **Appendix 1 – Key for identification of adult *A. glabripennis* and *A. chinensis*:** a simplified key is given for the morphological identification of *A. glabripennis* and *A. chinensis* adult specimens within the *Anoplophora* genus (after Lingafelter & Hoebecke, 2002)
- **Appendix 1 – Key for adults of the Monochamini genera in Europe:** a key is given for the morphological differentiation of the European Monochamini genera
- **paragraph 4.1.2. Description of adult morphology** for both *Anoplophora* species (body, head, pronotum, scutellum, elytra, legs, abdomen)

2.3 *Composition of the sample sets*

Two sample sets were used, one for larvae and another one for adults, each with 25 samples. The sample set for larvae consisted of specimens belonging to target (2 taxa) and non-target (14 taxa) species, the sample set for adults consisted of target (2 taxa) and non-target (15 taxa) species. Target specimens had different geographic origin: from France and Italy for larvae and from France, Italy, China and Korea for adults. Non-target specimens belonged all to the family Cerambycidae and were selected primarily based on the close morphological similarity and biology with the target species and the availability in the EURL reference collection. The non-target specimens were selected mainly among the European fauna. Table 1 provides a summary of the sample set. For the detailed composition of the sample set, see Appendix 1 of this document.
Each sample was re-labelled with a number from 1 to 25 by supervisors, after randomization. Original codification of samples was available only to supervisors. Larval samples were preserved in single tubes, filled with ethanol and adults were pinned specimen.
The composition of the sets was chosen to allow the evaluation of sensitivity, specificity, repeatability, reproducibility and accuracy of the protocol.

Table 1: Summary of the composition of the sample set for larvae (left) and adults (right).

Identification	total number
Aegosoma scabricorne	1
Anoplophora chinensis	**5**
Anoplophora glabripennis	**5**
Aromia bungii	1
Aromia moschata	1
Cerambyx cerdo	1
Hylotrupes bajulus	1
Lamia textor	1
Monochamus galloprovincialis	2
Niphona picticornis	1
Phoracantha semipunctata	1
Prionus coriarus	1
Saperda punctata	1
Aegomorphus clavipes	1
Xylotrechus chinensis	1
Xylotrechus stebbingi	1

Identification	total number
Aegosoma scabricorne	**1**
Anoplophora chinensis	**4**
Anoplophora davidis	**1**
Anoplophora glabripennis	**5**
Aromia moschata	**1**
Batocera rubus	**1**
Cerambyx cerdo	**1**
Lamia textor	**1**
Monochamus galloprovincialis	**1**
Monochamus sutor	**1**
Morimus asper	**1**
Niphona picticornis	**1**
Plagionotus arcuatus	**1**
Psacothea hilaris	**2**
Rosalia alpina	**1**
Rusticoclytus rusticus	**1**
Saperda carcharias	**1**

3. Validation of the protocol

3.1 Performance characteristics assessed

According to the guidance given in PM 7/98 (4) (EPPO, 2019) and the definitions given in PM 7/76 (5) (EPPO, 2018), PM 7/122 (1) (EPPO, 2014) and EPPO PM 7/129 (2) (EPPO, 2021c), validation of diagnostic tests relies on the evaluation of the following performance characteristics: sensitivity, specificity, reproducibility, repeatability and accuracy.

Table 2 shows the criteria that are used to calculate the performance characteristics of the test.

Table 2: Definition and calculation of performance characteristics

Performance criteria	Definition	Calculation
Diagnostic specificity	The proportion of non-target samples (true negatives) testing negative compared with results from an alternative test (or combination of tests) *Comments: as far as possible, the evaluation of specificity must include samples from non-target organisms that can be confused with the target species*	Diagnostic specificity = true negatives/(true negatives + false positives)
Analytical specificity	Inclusivity: The performance of a test with a range of target organisms covering genetic diversity, different geographical origin and hosts	-
	Exclusivity: The performance of a test with regard to cross-reaction with a range of non-targets (e.g. closely related organisms)	-
Diagnostic sensitivity	The proportion of target samples (true positives) testing positive compared with results from an alternative test (or combination of tests)	Diagnostic sensitivity = true positives/(true positives + false negatives)
Analytical sensitivity	The smallest amount of target that can be detected reliably.	-

Performance criteria	Definition	Calculation
Repeatability	The level of agreement between replicates of a sample tested under the same conditions.	% level of agreement
Reproducibility	The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, persons, equipment, location).	% level of agreement
Accuracy	The proportion of target samples (true positives) testing positive and non-target samples (true negatives) testing negative compared with the total number of samples. It is worth noting that the accuracy is a global criterion, which can be subdivided to refine the analysis into three other criteria: sensitivity, specificity and repeatability.	Accuracy = (true positives + true negatives)/(true positives + false negatives + true negatives + false positives)

3.2 Validation protocol

3.2.1 Morphological test for larvae

The set of 25 specimen was analysed by three operators, belonging to the two different institutions (AGES and Anses). The set composition was prepared by the supervisors, was not known to the operators and it was subject of a separate document. Supervisors provided operators with the Check Lists and Summary Results sheet in Appendix 2, but did not provide operators with origin and host plants data. During the analysis, to be carried out with a stereomicroscope, operators filled in the Check List for each sample and recorded the identification results on the Summary Results sheet. The results of the identification were expressed as:
- POSITIVE, if **all** the characters of the specimen match with those of *A. glabripennis* / *A. chinensis*
- NEGATIVE, if **not all** the characters of the specimen match with those of *A. glabripennis* / *A. chinensis*
For the positive results, operators were required to specify in the column "Notes" either *A. glabripennis* or *A. chinensis*.

After the analysis, the Summary Results sheet was retrieved by the supervisors. In case of deviations of the results from the expected ones, the Check List allowed the supervisors to precisely identify any critical issues within the protocol.
Performance characteristics were assessed according to the following plan:

- Diagnostic sensitivity, specificity and accuracy were assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES).
- Repeatability was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES) (three repetitions of analysis).
- Reproducibility was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (first of the three repetitions of analysis), 2 (AGES) and 3 (Anses).

Figure 1 provides a scheme of the activity.

3.2.2 Morphological test for adults

The set of 25 specimen was analysed by three operators, belonging to the two different institutions (AGES and Anses). The set composition was prepared by the supervisors, was not known to the operators and it was subject of a separate document. Supervisors provided operators with the Check Lists and Summary Results sheet in Appendix 3, but did not provide operators with origin and host plants data. During the analysis, to be carried out with a stereomicroscope, operators filled in the Check List for each sample and recorded the identification results on the Summary Results sheet. The results of the identification were expressed as:
- POSITIVE, if **all** the characters of the specimens match with those of *A. glabripennis* / *A. chinensis*
- NEGATIVE, if **not all** the characters of the specimens match with those of *A. glabripennis* / *A. chinensis*
For the positive results, operators were required to specify in the column "Notes" either *A. glabripennis* or *A. chinensis*.

After the analysis, the Summary Results sheet was retrieved by the supervisors. In case of deviations of the results from the expected ones, the Check List allowed the supervisors to precisely identify any critical issues within the protocol.
Performance characteristics were assessed according to the following plan:

- Diagnostic sensitivity, specificity and accuracy were assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES).
- Repeatability was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES) (three repetitions of analysis).

- Reproducibility was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (first of the three repetitions of analysis), 2 (AGES) and 3 (Anses).

Figure 1 provides a scheme of the activity.

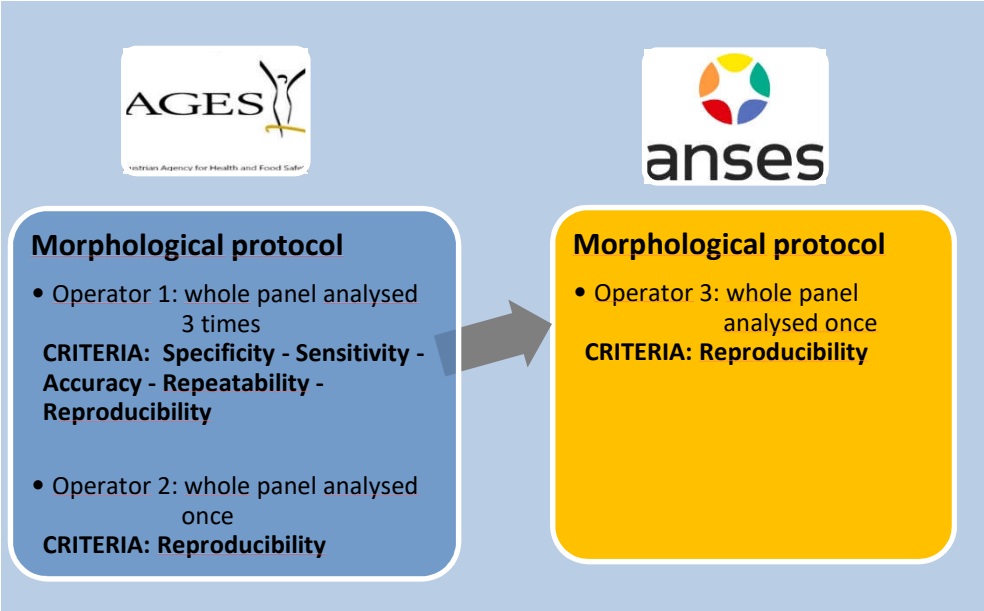

Fig. 1: Outline of the activities conducted by AGES and Anses.

4. Performance adequacy and validation

The performance values obtained by the diagnostic protocol were compared with the predetermined, expected performance characteristics.
The adequate expected performance characteristics are shown in Table 3. They are also referred to as "limits of acceptability" of the protocol. If the obtained performance characteristics will not reach the expected values, a cause analysis will be carried out to identify the critical steps in the protocol that led to the unexpected results (i.e. false negatives, false positives, not determined).

Table 3: Expected performance characteristics (limits of acceptability).

Performance criteria	EPPO PM 7/149 (1) *A. glabripennis* and *A. chinensis* – morphological identification of larvae	EPPO PM 7/149 (1) *A. glabripennis* and *A. chinensis* – morphological identification of adults
Diagnostic specificity	100%	100%
Analytical specificity (Inclusivity)	-	-
Diagnostic sensitivity	100%	100%
Analytical sensitivity	1 larval specimen	1 adult specimen
Repeatability	100%	100%
Reproducibility	100%	100%
Accuracy	100%	100%

5. Time schedule and staff

The testing period was carried out from March 2021 to the beginning of May 2021 and involved staff from the EURL for Insects and Mites.

Participating staff:

- for morphological tests:
  Experts/Supervisors: Sylvia Blümel, Andrea Taddei
  Role: definition, randomization and blind-codification of sample sets, preparation of check-lists, collection and analyses of results, drafting of final report

  Technical staff/Operators: Christa Lethmayer, Gudrun Strauß, Raphaëlle Mouttet
  Role: performance of analyses, help to supervisor in the preparation of check-lists and in the interpretation and analysis of results, drafting of final report

6. Results of the validation analysis

Protocol: EPPO PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis* (EPPO, 2021b)

6.1 Results for larvae

For larvae, the values obtained for diagnostic specificity, diagnostic sensitivity, accuracy and repeatability met the expected value of 100% (Table 4). The value obtained for reproducibility did not meet the expected value of 100%, but reached a value of 98,7%. The cause was found in only one divergent result obtained for 1 specimen by 1 operator. The check lists compiled by operators during the performance of the analyses allowed to track back the critical steps in the protocol that led to the deviation from the expected result.
The test for larvae was found to be inclusive for target specimens from France and Italy. It was exclusive for a range of non-target specimens belonging to other species of the subfamily Lamiinae (*Monochamus galloprovincialis*, *Saperda punctata*, *Lamia textor*, *Niphona picticornis*) and to the subfamilies Cerambycinae (*Phoracantha semipunctata*, *Xylotrechus chinensis*, *Xylotrechus stebbingi*, *Aromia bungii*, *Aromia moschata*, *Cerambyx cerdo*, *Hylotrupes bajulus, Aegomorphus clavipes*) and Prioninae (*Aegosoma scabricorne*, *Prionus coriarius*).

Appendix 4 of this document shows the results for larvae obtained by the three operators.
Appendix 6 shows the calculations for the performance characteristics for larvae.

Table 4: Summary of the results **for larvae** obtained for the morphological protocol.

Performance criteria	Definition	Calculation	Expected performance characteristics	Obtained performance characteristics
Diagnostic specificity	The proportion of non-target samples (true negatives) testing negative compared with results from an alternative test (or combination of tests)	Diagnostic specificity = true negatives/(true negatives + false positives)	**100%**	**100%**
Analytical specificity	Inclusivity: The performance of a test with a range of target organisms covering genetic diversity, different geographical origin and hosts	-	-	**France** **Italy**
	Exclusivity: The performance of a test with regards to cross-reaction with a range of non-targets (e.g. closely related organisms)	-	-	***Lamia textor*** ***Phoracantha semipunctata*** ***Xylotrechus chinensis*** ***Xylotrechus stebbingi*** ***Aromia bungii*** ***Aromia moschata*** ***Cerambyx cerdo*** ***Monochamus galloprovincialis*** ***Saperda punctata*** ***Aegomorphus clavipes*** ***Niphona picticornis*** ***Hylotrupes bajulus*** ***Aegosoma scabricorne*** ***Prionus coriarius***
Diagnostic sensitivity	The proportion of target samples (true positives) testing positive compared with results from an alternative test (or combination of tests)	Diagnostic sensitivity = true positives/(true positives + false negatives)	**100%**	**100%**
Analytical sensitivity	The smallest amount of target that can be detected reliably	-	**1 larval specimen**	**1 larval specimen**
Repeatability	The level of agreement between replicates of a sample tested under the same conditions	% level of agreement	**100%**	**100%**
Reproducibility	The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, persons, equipment, location)	% level of agreement	**100%**	**98,7%**
Accuracy	The proportion of target samples (true positives) testing positive and non-target samples (true negatives) testing negative compared with the total number of samples	Accuracy = (true positives + true negatives)/(true positives + false negatives + true negatives + false positives)	**100%**	**100%**

Analysis of critical steps in the protocol

When performing the analyses, the operators found only one divergent result obtained for 1 larval specimen which concerns the existence of protuberant abdominal segments. The operator has chosen the character "Abdominal epipleurum of the segments III-IX protuberant" (Fig. 2) because the epipleurum of the segments V and VI also look protuberant (see Fig. 4). For this larva, however, the other character ("Abdominal epipleurum protuberant only on the segments VII-IX") (Fig. 3) should have been chosen in order to then obtain the correct determination.

Fig. 2: Larva with protuberant epipleurum on abdominal segments III-IX, from EPPO PM 7/149 (1) modified (© J. Connell, BFW (Austria))

Fig. 3: Larva with protuberant epipleurum on abdominal segments VII-IX, from EPPO PM 7/149 (1) modified (© B. Serrate, editing by L. Soldati, INRAE Montpellier (France))

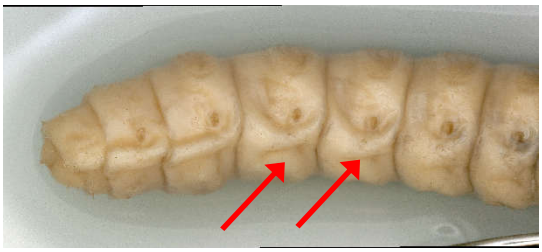

Fig. 4: Abdominal segments displaying a protuberant-like epipleurum in the specimen for which the divergent result was obtained (© A. Taddei, Anses)

A possible explanation is that in some larvae the protuberant epipleurum is not clearly visible. This could depend on the preservation mode of larvae – if a larva is put in ethanol without boiling it can shrink and epipleurum can look like protuberant even on other segments or the larva can swell and epipleurum does not look like protuberant anymore if it is boiled before putting in ethanol. Therefore, this character should be carefully observed in the light of other characters too, before a decision is made how to proceed in the key.

6.2 Results for adults

For adults, the values for all performance criteria (diagnostic specificity, diagnostic sensitivity, accuracy, repeatability and reproducibility) achieved the expected value of 100% (Table 5).
The test for adults was found to be inclusive for target specimens from France, Italy, China and Korea. It was exclusive for one specimen belonging to the *Anoplophora* genus (*Anoplophora davidis*), other species of the subfamiliy Lamiinae (*Psacothea hilaris*, *Monochamus galloprovincialis*, *Monochamus sutor*, *Saperda carcharias*, *Morimus asper*, *Lamia textor*, *Niphona picticornis*, *Batocera rubus*) and to the subfamilies Cerambycinae (*Rusticoclytus rusticus*, *Cerambyx cerdo*, *Aromia moschata*, *Rosalia alpina*, *Plagionotus arcuatus*) and Prioninae (*Aegosoma scabricorne*).

Appendix 5 of this document shows the results for adults obtained by the three operators.
Appendix 7 shows the calculations for the performance characteristics for adults.

Table 5: Summary of the results **for adults** obtained for the morphological protocol.

Performance criteria	Definition	Calculation	Expected performance characteristics	Obtained performance characteristics
Diagnostic specificity	The proportion of non-target samples (true negatives) testing negative compared with results from an alternative test (or combination of tests)	Diagnostic specificity = true negatives/(true negatives + false positives)	**100%**	**100%**
Analytical specificity	Inclusivity: The performance of a test with a range of target organisms covering genetic diversity, different geographical origin and hosts	-	-	**China** **Korea** **France** **Italy**
	Exclusivity: The performance of a test with regards to cross-reaction with a range of non-targets (e.g. closely related organisms)	-	-	***Psacothea hilaris*** ***Rusticoclytus rusticus*** ***Niphona picticornis*** ***Cerambyx cerdo*** ***Batocera rubus*** ***Aromia moschata*** ***Morimus asper*** ***Monochamus galloprovincialis*** ***Monochamus sutor*** ***Rosalia alpina*** ***Lamia textor*** ***Aegosoma scabricorne*** ***Saperda carcharias*** ***Anoplophora davidis*** ***Plagionotus arcuatus***
Diagnostic sensitivity	The proportion of target samples (true positives) testing positive compared with results from an alternative test (or combination of tests)	Diagnostic sensitivity = true positives/(true positives + false negatives)	**100%**	**100%**
Analytical sensitivity	The smallest amount of target that can be detected reliably	-	**1 adult specimen**	**1 adult specimen**
Repeatability	The level of agreement between replicates of a sample tested under the same conditions	% level of agreement	**100%**	**100%**
Reproducibility	The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, persons, equipment, location)	% level of agreement	**100%**	**100%**
Accuracy	The proportion of target samples (true positives) testing positive and non-target samples (true negatives) testing negative compared with the total number of samples	Accuracy = (true positives + true negatives)/(true positives + false negatives + true negatives + false positives)	**100%**	**100%**

Analysis of critical steps in the protocol

Concerning the identification protocol for adults, the operators did not identify any weaknesses in the protocol that could lead to a risk of misidentification of the target species when performing the analyses. However, the operators did recognise the need for minor corrections and improvements of the simplified key (Appendix 1, page 583-584).
These suggestions for improvement are listed below:

- at **couplet 4**, the word "large" should be added when referring to the "bands or spots of dense yellow pubescence", otherwise *A. glabripennis* form *nobilis* might not be excluded here (see Fig. 6 and 7)

Fig. 6: Elytrae from *A. horsfieldii* (© A. Taddei, Anses)

- at **couplet 4'**, the following part should be added: "if yellow, then in much smaller spots not forming partial or complete bands"

Fig. 7: Elytrae from *A. glabripennis* form *nobilis* (© A. Taddei, Anses)

- at **couplet 12'**, the white-blue pubescent annulation seems to occupy more than the basal half in most antennomeres, except the first 2 antennomeres (after scape) (see Fig. 8)

Fig. 8: Antenna of *A. glabripennis* (© A. Taddei, Anses)

- **elytral patches H3 and H4:** (page 579, in description 4.1.2.2.)
"*A. macularia* have elytral patches H3 and H4 fused in one large maculation (whereas in *A. chinensis* they are separated)."
→ these elytral patches H3 and H4 are mentioned without any explanation or illustration (figure missing); adding figures could be useful – as it is shown e.g. in Lingafelter & Hoebeke (2002), see Figure 9.

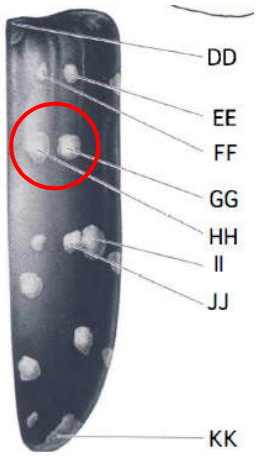

Fig. 9: Elytra with patches: H3 = HH, H4 = GG (© Lingafelter & Hoebeke (2002), modified)

7. Discussion and conclusions

This study aimed at the validation of the EPPO diagnostic protocol PM 7/149 (1) for the morphological identification of larvae and adults of *Anoplophora glabripennis* and *Anoplophora chinensis*. The study has involved staff of the EURL for Insects and Mites from Anses and AGES and the analytical activities have been carried out from March to May 2021. Two sample sets, each of 25 specimens including target and non-target species of the family Cerambycidae, has been used.

Morphological identification of larvae

The morphological identification of larvae obtained the expected value of 100% for the criteria diagnostic specificity, diagnostic sensitivity, accuracy and repeatability. However, the reproducibility obtained a value of 98,7% due to one divergent result for one specimen from one operator. Critical steps in the protocol were tracked back with the check lists of the operators and the divergence was found for the description of the epipleurum on the abdominal segments. It turned out that it was less a "weakness" of the protocol but much more a quality problem of the prepared specimen. Thus, it is recommended to consider carefully also additional characters before a decision is made how to proceed in the key.
It is worth recalling that discrimination between *A. glabripennis* and *A. chinensis* larvae can be challenging even for experienced operators, should be done on mature larvae and confirmed through molecular tests in doubtful cases.

Morphological identification of adults

The morphological identification of adults achieved the expected value of 100% for all performance criteria (diagnostic specificity, diagnostic sensitivity, accuracy, repeatability and reproducibility). The analysis of the results showed that there are no "weaknesses" identified in the diagnostic protocol for the correct determination of adults.
However, following minor improvements are suggested:

- at **couplet 4**, the word "large" should be added, otherwise *A*. *glabripennis* form *nobilis* might not be excluded here
- at **couplet 4'**, the following part should be added: "if yellow, then in much smaller spots not forming partial or complete bands"
- at **couplet 12'**, the white-blue pubescent annulation seems to occupy more than the basal half in most antennomeres, except the first 2 antennomeres (after scape)
- **elytral patches H3 and H4** are mentioned without any explanation or illustration (figure); adding figures could be useful.

Regarding the annulation of antennomeres, the remark that the white-blue pubescent annulation seems to occupy more than the basal half in most antennomeres, in contrast to what is written in the diagnostic protocol and in Lingafelter & Hoebeke (2002), is based on the limited number of specimens used in this study. Therefore, more specimens of *Anoplophora glabripennis* and *Anoplophora freyi* should be observed to confirm it. However, even in the suggested reformulation of this character description, the distinction between *A. glabripennis/A. freyi* and *A. coeruleoantennata* in couplet 12 would still be possible. In fact, even if the annulation exceeds the basal two-thirds of most antennomeres, it is not purple or deep blue, as in *A. coeruleoantennata*.

Based on these results, the EURL recommends the use of the diagnostic protocol EPPO PM 7/149 (1) to National Reference Laboratories performing the morphological identification of larvae and adults of *Anoplophora glabripennis* and *Anoplophora chinensis* with the advice to consider the following points:

- **for larvae:**
  attention should be paid to the quality of larvae concerning the abdominal epipleurum because this character could not be clearly visible depending on the preservation method used;
- **for adults:**
  the following suggested descriptions of diagnostic characters appear to be clearer for a proper identification of adults:
  - **couplet 4/4':**
    - **4** Elytra with **large** bands or spots of dense yellow pubescence
    - **4'** Elytra with pubescence otherwise **- if yellow, then in much smaller spots not forming partial or complete bands**
  - **couplet 12':**
    - annulation occupying no more than the basal half of **at least the first two antennomeres (after scape)**
  - **elytral patches H3 and H4:**
    - explanation and **addition of a figure** for patches H3 and H4 on elytra

Date: 01/04/2022

Philippe Reynaud
EURL Director

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APPENDIX 1 – Composition of the sample sets and codification

Sample set of LARVAE:

Identification	Sample codification	New codification	country of collection
Lamia textor	2100180	**1**	France
Anoplophora chinensis	2100026_4	**2**	Italy
Phoracantha semipunctata	700529	**3**	France
Xylotrechus chinensis	1901653	**4**	France
Aromia moschata	1500226	**5**	France
Cerambyx cerdo	600311	**6**	France
Monochamus galloprovincialis	700459	**7**	Portugal
Anoplophora chinensis	2100027_2	**8**	Italy
Saperda punctata	2001455	**9**	France
Niphona picticornis	1901160	**10**	France
Anoplophora glabripennis	1001399_2	**11**	France
Hylotrupes bajulus	1901134	**12**	Tunisia
Anoplophora glabripennis	1601402_1 ou 1601401_5(1)	**13**	France
Aromia bungii	2100124	**14**	Italy
Anoplophora chinensis	2100171	**15**	Italy
Aegosoma scabricorne	1200135 ou 1001580	**16**	France
Anoplophora chinensis	2100026_8	**17**	Italy
Prionus coriarius	300616	**18**	France
Anoplophora chinensis	2100172	**19**	Italy
Anoplophora glabripennis	1601273_1	**20**	France
Anoplophora glabripennis	1601400(1)	**21**	France
Xylotrechus stebbingi	2001113	**22**	France
Anoplophora glabripennis	/	**23**	Italy
Aegomorphus clavipes	2000923	**24**	France
Monochamus galloprovincialis	700459	**25**	Portugal

Sample set of ADULTS:

Identification	Sample codification	New codification	country of collection
Psacothea hilaris	2100121	**1**	Italy
Rusticoclytus rusticus	2100235	**2**	France
Niphona picticornis	2100236	**3**	France
Anoplophora glabripennis	802960	**4**	China
Anoplophora chinensis	2100015	**5**	Italy
Anoplophora chinensis	1800973	**6**	France
Cerambyx cerdo	501134	**7**	France
Anoplophora chinensis	2100175	**8**	Korea
Batocera rubus	1100506	**9**	n.a.
Aromia moschata	2100237	**10**	France
Morimus asper	2100238	**11**	France
Anoplophora glabripennis	2100147	**12**	China
Monochamus galloprovincialis	2100239	**13**	France
Rosalia alpina	2100240	**14**	France
Anoplophora glabripennis	2100010_9	**15**	Italy
Lamia textor	2100241	**16**	France
Anoplophora glabripennis	802841	**17**	France
Anoplophora glabripennis	2100010_15	**18**	Italy
Aegosoma scabricorne	2100242	**19**	France
Psacothea hilaris	2100120	**20**	Italy
Saperda carcharias	2100243	**21**	France
Anoplophora davidis	2100244	**22**	Vietnam
Anoplophora chinensis	2100011_8	**23**	Italy
Monochamus sutor	2100245	**24**	France
Plagionotus arcuatus	2100246	**25**	France

APPENDIX 2 - Check lists for the morphological identification of LARVAE

Operator		Date	

Key for identification of late instar larvae of *A. glabripennis* and *A. chinensis* (modified from key on page 26, EPPO PM 7/149 (1) *Anoplophora glabripennis* and *A. chinensis*)

Key for identification of late instar larvae of *A. glabripennis* and *A. chinensis*		go to (mark the decision with Y (yes) or N (no); note any comments)										
Morphological character		**Sample code**										
1	**Legs present,** 4 jointed (excluding coxa) (Fig. 16)	…	…	…	…	…	…	…	…	…	…	**sub-fam. Cerambycinae (pars) and other subfamilies**
	Legs absent	…	…	…	…	…	…	…	…	…	…	**2**
2	**Clypeus very narrow**, with only slender basal arms reaching to mandibular articulations (Fig. 6A). Mandibular apex and dorsal angle more or less lacking. **Mandible short, apically rounded, spoon-like** (Fig. 17A)	…	…	…	…	…	…	…	…	…	…	**sub-fam. Cerambycinae**
	Clypeus more or less trapezoidal, **filling entire space** between dorsal mandibular articulations (Fig. 6B). **Mandibles not rounded**, with distinct apex and more or less distinct dorsal angle (Fig. 17B)	…	…	…	…	…	…	…	…	…	…	**3 (sub-fam. Lamiinae)**
3	**Anal pore transverse**	…	…	…	…	…	…	…	…	…	…	**tribe Lamiini**
	Anal pore triradiate (one ventral and two lateral rays) (Fig. 9); the ventral ray can be shorter in some species	…	…	…	…	…	…	…	…	…	…	**4**
4	**Pronotal shield and dorsal ambulatory ampullae with dark spinule** visible under a low magnification (Fig. 11, Fig. 10B)	…	…	…	…	…	…	…	…	…	…	**tribe Saperdini**
	Pronotal shield and dorsal ambulatory ampullae with very minute spinule visible under high magnification. In some tribes (Lamiini, Monochamini, etc.) the pronotal shield under low magnification appears as a dark uniform plate, provided with small	…	…	…	…	…	…	…	…	…	…	**5**

	depigmented rounded areas, more or less joined (Fig. 7A, 7B). Dorsal ambulatory ampullae with different features and never provided with visible spinule under low magnification. In some tribes a distinct pronotal shield is lacking											
5	**Dorsal ambulatory ampullae granular**, built up by small granules in distinct transverse rows or in elongate oval clusters formed by large joined granules (Fig. 10A)	…	…	…	…	…	…	…	…	…	…	**6**
	Dorsal ambulatory ampullae not granular, but with small spinule	…	…	…	…	…	…	…	…	…	…	**tribe Acanthocinini**
6	**Dorsal ambulatory ampullae medially with large granules in 4 distinct transverse rows** (Fig. 10A). Body size of the **last instars larvae** generally **more than 40 mm**	…	…	…	…	…	…	…	…	…	…	**7** **tribe Monochamini**
	Dorsal ambulatory ampullae with different aspect, granules in less than 4 rows or in elongated oval clusters formed by large joined granules. **Last instars larvae smaller than 35 mm**	…	…	…	…	…	…	…	…	…	…	**tribes other than Monochamini**
7	**Abdominal epipleurum of the segments III-IX protuberant** (Fig. 8B). **Anal pore** with the **ventral ray distinctly shorter** than the two rays	…	…	…	…	…	…	…	…	…	…	**other Monochamini**
	Abdominal epipleurum protuberant only on the segments VII-IX (Fig. 4B). **Anal pore** with the **ventral and two lateral rays of the same length**; in some cases, the ventral ray is slightly shorter	…	…	…	…	…	…	…	…	…	…	**8**
8	**A distinct pigmented band** is present anterior to the pronotal shield; typical pronotum as in Fig. 7A	…	…	…	…	…	…	…	…	…	…	***Anoplophora chinensis***
	Anterior to the pronotal shield, the **band** is less observable due to **less pigmentation**; typical pronotum as in Fig. 7B	…	…	…	…	…	…	…	…	…	…	***Anoplophora glabripennis***
	Comments / Results	…	…	…	…	…	…	…	…	…	…	

Summary Results sheet for the morphological test EPPO PM7/149 (1)
***Anoplophora glabripennis* and *A. chinensis* – LARVAE**

Operator	
Stereomicroscope	

Sample code	Identification result	Date of analysis	Notes
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APPENDIX 3 – Check lists for the morphological identification of ADULTS

Operator		Date	

Key for adults of the Monochamini genera in Europe (modified from key on page 27, EPPO PM 7/149 (1) *Anoplophora glabripennis* and *A. chinensis*)

Key for identification of Monochamini genera in Europe		go to (mark the decision with Y (yes) or N (no); note any comments)										
Morphological character		**Sample code**										
1	**Elytra** from brown to blackish brown, **with irregular marmorization**, sculptured with **numerous confluent punctures**. **Antennae completely black or brown** in the **male**	…	…	…	…	…	…	…	…	…	…	***Monochamus* spp.**
	Elytra with white or pale yellow maculations. **Antennae with annulations** both in **male and female**	…	…	…	…	…	…	…	…	…	…	**2**
2	**Absence of longitudinal stripes** on pronotum and head's vertex. **Body shiny black.** **Elytra with** irregularly distributed patches of dense, generally **white pubescence**. **Lateral pronotal spines strong, well developed**	…	…	…	…	…	…	…	…	…	…	***Anoplophora* spp.**
	Presence of longitudinal stripes of yellow pubescence on pronotum and head's vertex. **Body** entirely covered by a fine dense **green-greyish pubescence**. **Elytra with** irregularly distributed patches of dense, **pale yellow pubescence**. **Lateral pronotal spines short and poorly developed.** Represented in Europe by a single, introduced species, *P. hilaris* (Pascoe, 1857)	…	…	…	…	…	…	…	…	…	…	***Psacothea* spp.**
	Comments / Results	…	…	…	…	…	…	…	…	…	…	

Simplified key for the identification of *A. glabripennis* and *A. chinensis* adult specimen within the *Anoplophora* group (modified from key on page 27, EPPO PM 7/149 (1) *Anoplophora glabripennis* and *A. chinensis*)

Key for identification of *A. glabripennis* and *A. chinensis* adults within *Anoplophora* group		go to (mark the decision with Y (yes) or N (no); note any comments)										
Morphological character		**Sample code**										
1	**Antennae with** conspicuous pubescent **annulations** on most antennomeres (Fig. 12-14)	…	…	…	…	…	…	…	…	…	…	**2**
	Antennae without conspicuous pubescent **annulations**	…	…	…	…	…	…	…	…	…	…	**other species**
2	**Antennae** with distinct narrow **annulation at base and apex** of most antennomeres	…	…	…	…	…	…	…	…	…	…	**other species**
	Antennae with **annulations at basal fourth or more** of most antennomeres (Fig. 12-14)	…	…	…	…	…	…	…	…	…	…	**3**
3	Most of the **body covered with** dense, uniform blue-grey, blue-green or turquoise **pubescence**	…	…	…	…	…	…	…	…	…	…	**other species**
	Most of the **body not uniformly covered with pubescence** of different shades of blue (Fig. 12)	…	…	…	…	…	…	…	…	…	…	**4**
4	**Elytra** with **bands or spots** of dense **yellow pubescence**	…	…	…	…	…	…	…	…	…	…	***A. horsfieldii***
	Elytra with **pubescence otherwise**	…	…	…	…	…	…	…	…	…	…	**5**
5	**Pronotum heavily sculptured** with large posteromedial and two mediolateral thickenings of the integument (= **calli**), a **deep middle impression** and **anterior region strongly elevated**	…	…	…	…	…	…	…	…	…	…	**other species**
	Pronotum with very weak or no mediolateral calli, **anterior margin not highly elevated** and **without** pronounced **middle depression** in front of posteromedial callus (Fig. 13A, 13B, 13C)	…	…	…	…	…	…	…	…	…	…	**6**

6	**Elytra with** 4-7 complete or nearly complete **transverse bands of pubescence**	…	…	…	…	…	…	…	…	…	…	**other species**
	Elytra with pubescent maculations in form of numerous irregularly sized spots on disk, most non forming bands (Fig. 12)	…	…	…	…	…	…	…	…	…	…	**7**
7	**Elytral base with numerous** (10 or more) conspicuous **granules** (Fig. 13B, 13C)	…	…	…	…	…	…	…	…	…	…	**8**
	Elytral base without granules (or at most 10) (Fig. 13A)	…	…	…	…	…	…	…	…	…	…	**11**
8	**Pubescent maculations on elytra poorly defined**, fuzzy margined, numerous (about 30), variably sized and **bicoloured**	…	…	…	…	…	…	…	…	…	…	**other species**
	Pubescent maculations on elytra less numerous, usually **well defined**; with distinct edges and **unicolourous** (white, yellow, light orange, light blue) (Fig. 12)	…	…	…	…	…	…	…	…	…	…	**9**
9	**Elytra with few**, if any, erect or suberect, **long black hairs**; white, blue or translucent pubescence ventrally	…	…	…	…	…	…	…	…	…	…	**10**
	Elytra with many, erect or suberect, **long black hairs**; light to bold blue pubescence ventrally	…	…	…	…	…	…	…	…	…	…	***A. davidis* and *A. macularia***
10	**Elytra with 20-40 or more granules each**, occupying basal one-fifth (Fig. 13B, 13C); **antennal annulation light blue or white**	…	…	…	…	…	…	…	…	…	…	***A. chinensis*** (Fig. 12C, 12D, 12E, 12F)
	Elytra with about 10 granules each	…	…	…	…	…	…	…	…	…	…	***A. variantennatus***
11	**Pronotum with** conspicuous, dense **pubescence** dorsally	…	…	…	…	…	…	…	…	…	…	**other species**
	Pronotum without dense **pubescence** (Fig. 13A)	…	…	…	…	…	…	…	…	…	…	**12**

12	**Antennomeres with** a broad basal purple or deep blue **pubescent annulation on at least basal two-thirds of most antennomeres**	…	…	…	…	…	…	…	…	…	…	***A. coeruleo-antennata***
	Antennomeres with white or pale blue **pubescent annulation** occupying **no more than the basal half of most antennomeres** (Fig. 12)	…	…	…	…	…	…	…	…	…	…	**13**
13	**Elytra** shiny, very strong metallic copper, green or violet sheen; surface of elytra without very short, fine, translucent hairs; **tarsi** with **blue pubescence** usually **neither very bright nor iridescent** dorsally	…	…	…	…	…	…	…	…	…	…	***A. freyi***
	Elytra shiny or matte, with weak iridescence; surface of elytra with regularly distributed, sparse, very short, fine, translucent hairs along with dense patches of white or off-white pubescence; **tarsi** of fresh specimens usually **with very bright, iridescent blue pubescence** dorsally (Fig. 14A); maculations on elytra white or yellow (rarely pale orange)	…	…	…	…	…	…	…	…	…	…	***A. glabripennis*** (Fig. 12A, 12B)
	Comments / Results	…	…	…	…	…	…	…	…	…	…	…

Summary Results sheet for the morphological test EPPO PM7/149 (1)
***Anoplophora glabripennis* and *A. chinensis* – ADULTS**

Operator	
Stereomicroscope	

Sample code	Identification result	Date of analysis	Notes
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APPENDIX 4 – Summary Results sheets for LARVAE with the results from the 3 operators

Operator 1	
Instrument	Zeiss Stemi 508
Date of analysis/ identification	19/03/2021 26/03/2021 01/04/2021

Sample number	Analysis/ Identification 1	Analysis/ Identification 2	Analysis/ Identification 3	Notes	Expected result	Species
1	Negative	Negative	Negative		Negative	*Lamia textor*
2	Positive	Positive	Positive	*A. chinensis*; *A. chinensis* (?); *A. chinensis*	Positive	*Anoplophora chinensis*
3	Negative	Negative	Negative		Negative	*Phoracantha semipunctata*
4	Negative	Negative	Negative		Negative	*Xylotrechus chinensis*
5	Negative	Negative	Negative		Negative	*Aromia moschata*
6	Negative	Negative	Negative		Negative	*Cerambyx cerdo*
7	Negative	Negative	Negative		Negative	*Monochamus galloprovincialis*
8	Positive	Positive	Positive	*A. chinensis*	Positive	*Anoplophora chinensis*
9	Negative	Negative	Negative		Negative	*Saperda punctata*
10	Negative	Negative	Negative		Negative	*Niphona picticornis*
11	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
12	Negative	Negative	Negative		Negative	*Hylotrupes bajulus*
13	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
14	Negative	Negative	Negative		Negative	*Aromia bungii*
15	Positive	Positive	Positive	*A. chinensis;* *A. chinensis* (?)	Positive	*Anoplophora chinensis*
16	Negative	Negative	Negative		Negative	*Aegosoma scabricorne*
17	Positive	Positive	Positive	*A. chinensis*	Positive	*Anoplophora chinensis*
18	Negative	Negative	Negative		Negative	*Prionus coriarius*
19	Positive	Positive	Positive	*A. chinensis* (?); *A. chinensis* (?); *A. glabripennis* (?)	Positive	*Anoplophora chinensis*
20	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
21	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
22	Negative	Negative	Negative		Negative	*Xylotrechus stebbingi*
23	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
24	Negative	Negative	Negative		Negative	*Aegomorphus clavipes*
25	Negative	Negative	Negative		Negative	*Monochamus galloprovincialis*

Operator 2	
Instrument	Zeiss Stemi 508
Date of analysis/ identification	22.03.2021

Sample number	Analysis/Identification	Notes	Expected result	Species
1	Negative		Negative	*Lamia textor*
2	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
3	Negative		Negative	*Phoracantha semipunctata*
4	Negative		Negative	*Xylotrechus chinensis*
5	Negative		Negative	*Aromia moschata*
6	Negative		Negative	*Cerambyx cerdo*
7	Negative		Negative	*Monochamus galloprovincialis*
8	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
9	Negative		Negative	*Saperda punctata*
10	Negative		Negative	*Niphona picticornis*
11	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
12	Negative		Negative	*Hylotrupes bajulus*
13	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
14	Negative		Negative	*Aromia bungii*
15	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
16	Negative		Negative	*Aegosoma scabricorne*
17	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
18	Negative		Negative	*Prionus coriarius*
19	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
20	Negative		Positive	*Anoplophora glabripennis*
21	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
22	Negative		Negative	*Xylotrechus stebbingi*
23	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
24	Negative		Negative	*Aegomorphus clavipes*
25	Negative		Negative	*Monochamus galloprovincialis*

Operator 3	
Instrument	Leica M165 C
Date of analysis/ identification	03.05.2021

Sample number	Analysis/Identification	Notes	Expected result	Species
1	Negative		Negative	*Lamia textor*
2	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
3	Negative		Negative	*Phoracantha semipunctata*
4	Negative		Negative	*Xylotrechus chinensis*
5	Negative		Negative	*Aromia moschata*
6	Negative		Negative	*Cerambyx cerdo*
7	Negative		Negative	*Monochamus galloprovincialis*
8	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
9	Negative		Negative	*Saperda punctata*
10	Negative		Negative	*Niphona picticornis*
11	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
12	Negative		Negative	*Hylotrupes bajulus*
13	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
14	Negative		Negative	*Aromia bungii*
15	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
16	Negative		Negative	*Aegosoma scabricorne*
17	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
18	Negative		Negative	*Prionus coriarius*
19	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
20	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
21	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
22	Negative		Negative	*Xylotrechus stebbingi*
23	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
24	Negative		Negative	*Aegomorphus clavipes*
25	Negative		Negative	*Monochamus galloprovincialis*

APPENDIX 5 – Summary Results sheets for ADULTS with the results from the 3 operators

Operator 1	
Instrument	Zeiss Stemi 508
Date of analysis/ identification	16/03/21; 19/03/21 25/03/21 01/04/21

Sample number	Analysis/ Identification 1	Analysis/ Identification 2	Analysis/ Identification 3	Notes	Expected result	Species
1	Negative	Negative	Negative	*Psacothea hilaris*	Negative	*Psacothea hilaris*
2	Negative	Negative	Negative	*Monochamus* sp. (?)	Negative	*Rusticoclytus rusticus*
3	Negative	Negative	Negative	*Monochamus* sp.	Negative	*Niphona picticornis*
4	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
5	Positive	Positive	Positive	*A. chinensis malasiaca*	Positive	*Anoplophora chinensis*
6	Positive	Positive	Positive	*A. chinensis*	Positive	*Anoplophora chinensis*
7	Negative	Negative	Negative	no genus of this key	Negative	*Cerambyx cerdo*
8	Positive	Positive	Positive	*A. chinensis malasiaca*	Positive	*Anoplophora chinensis*
9	Negative	Negative	Negative	no genus of this key	Negative	*Batocera rubus*
10	Negative	Negative	Negative	no genus of this key	Negative	*Aromia moschata*
11	Negative	Negative	Negative	no genus of this key	Negative	*Morimus asper*
12	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
13	Negative	Negative	Negative	*Monochamus* sp.	Negative	*Monochamus galloprovincialis*
14	Negative	Negative	Negative	no genus of this key	Negative	*Rosalia alpina*
15	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
16	Negative	Negative	Negative	no genus of this key	Negative	*Lamia textor*
17	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
18	Positive	Positive	Positive	*A. glabripennis*	Positive	*Anoplophora glabripennis*
19	Negative	Negative	Negative	no genus of this key	Negative	*Aegosoma scabricorne*
20	Negative	Negative	Negative	*Psacothea hilaris*	Negative	*Psacothea hilaris*
21	Negative	Negative	Negative	no genus of this key	Negative	*Saperda carcharias*
22	Negative	Negative	Negative	*A. davidis*	Negative	*Anoplophora davidis*
23	Positive	Positive	Positive	*A. chinensis malasiaca*	Positive	*Anoplophora chinensis*
24	Negative	Negative	Negative	*Monochamus* sp.	Negative	*Monochamus sutor*
25	Negative	Negative	Negative	no genus of this key	Negative	*Plagionotus arcuatus*

Operator 2	
Instrument	Zeiss Stemi 508
Date of analysis/ identification	18.03.2021

Sample number	Analysis/Identification	Notes	Expected result	Species
1	Negative	*Psacothea hilaris*	Negative	*Psacothea hilaris*
2	Negative		Negative	*Rusticoclytus rusticus*
3	Negative		Negative	*Niphona picticornis*
4	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
5	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
6	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
7	Negative		Negative	*Cerambyx cerdo*
8	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
9	Negative		Negative	*Batocera rubus*
10	Negative		Negative	*Aromia moschata*
11	Negative		Negative	*Morimus asper*
12	Positive	*Anoplophora glabripennis*, aberrant morph	Positive	*Anoplophora glabripennis*
13	Negative		Negative	*Monochamus galloprovincialis*
14	Negative	*Rosalia alpina*	Negative	*Rosalia alpina*
15	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
16	Negative		Negative	*Lamia textor*
17	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
18	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
19	Negative		Negative	*Aegosoma scabricorne*
20	Negative	*Psacothea hilaris*	Negative	*Psacothea hilaris*
21	Negative	*Saperda* sp.	Negative	*Saperda carcharias*
22	Negative	*A. davidis* or *A. macularia*	Negative	*Anoplophora davidis*
23	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
24	Negative		Negative	*Monochamus sutor*
25	Negative		Negative	*Plagionotus arcuatus*

Operator 3	
Instrument	Leica M165 C
Date of analysis/ identification	30.04.2021

Sample number	Analysis/Identification	Notes	Expected result	Species
1	Negative	*Psacothea* sp.	Negative	*Psacothea hilaris*
2	Negative		Negative	*Rusticoclytus rusticus*
3	Negative		Negative	*Niphona picticornis*
4	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
5	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
6	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
7	Negative		Negative	*Cerambyx cerdo*
8	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
9	Negative		Negative	*Batocera rubus*
10	Negative		Negative	*Aromia moschata*
11	Negative		Negative	*Morimus asper*
12	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
13	Negative	*Monochamus* sp.	Negative	*Monochamus galloprovincialis*
14	Negative		Negative	*Rosalia alpina*
15	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
16	Negative		Negative	*Lamia textor*
17	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
18	Positive	*Anoplophora glabripennis*	Positive	*Anoplophora glabripennis*
19	Negative		Negative	*Aegosoma scabricorne*
20	Negative	*Psacothea* sp.	Negative	*Psacothea hilaris*
21	Negative		Negative	*Saperda carcharias*
22	Negative	*A. davidis* or *A. macularia*	Negative	*Anoplophora davidis*
23	Positive	*Anoplophora chinensis*	Positive	*Anoplophora chinensis*
24	Negative	*Monochamus* sp.	Negative	*Monochamus sutor*
25	Negative		Negative	*Plagionotus arcuatus*

APPENDIX 6 – Calculation of performance characteristics for LARVAE

Sensitivity, specificity, accuracy:

Diagnostic sensitivity, specificity and accuracy were assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES).

Operator_1_R1

Diagnostic sensitivity = true positives/(true positives + false negatives)
Diagnostic specificity = true negatives/(true negatives + false positives)

		Expected result	
		positive	negative
Operator result	positive	10	0
	negative	0	15

Sensitivity: 100%
Specificity: 100%
Accuracy: 100%

Repeatability:

Repeatability was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES) (three repetitions of analysis).

Operator_1_R1, Operator_1_R2, Operator_1_R3

Expressed as % level of agreement among repetitions by Operator 1

Operator1_R1	Operator1_R2	Operator1_R3	Agreement	Disagreement	Level of agreement in %
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100

Repeatability: 100%

Reproducibility:

Reproducibility was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (first of the three repetitions of analysis), 2 (AGES) and 3 (Anses).

Operator_1_R1, Operator_2, Operator_3

Expressed as % level of agreement

Operator1_R1	Operator2	Operator3	Agreement	Disagreement	Level of agreement in %
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Negative	Positive	2	1	66,7
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100

Reproducibility: 98,7%

APPENDIX 7 – Calculation of performance characteristics for ADULTS

Sensitivity, specificity, accuracy:

Diagnostic sensitivity, specificity and accuracy were assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES).

Operator_1_R1

Diagnostic sensitivity = true positives/(true positives + false negatives)
Diagnostic specificity = true negatives/(true negatives + false positives)

		Expected result	
		positive	negative
Operator result	positive	9	0
	negative	0	16

Sensitivity: 100%
Specificity: 100%
Accuracy: 100%

Repeatability:

Repeatability was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (AGES) (three repetitions of analysis).

Operator_1_R1, Operator_1_R2, Operator_1_R3

Expressed as % level of agreement among repetitions by Operator 1

Operator1_R1	Operator1_R2	Operator1_R3	Agreement	Disagreement	Level of agreement in %
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100

Repeatability: 100%

Reproducibility:

Reproducibility was assessed on the basis of the analysis of the whole sample set carried out by operator 1 (first of the three repetitions of analysis), 2 (AGES) and 3 (Anses).

Operator_1_R1, Operator_2, Operator_3

Expressed as % level of agreement

Operator1_R1	Operator2	Operator3	Agreement	Disagreement	Level of agreement in %
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100
Positive	Positive	Positive	3	0	100
Negative	Negative	Negative	3	0	100
Negative	Negative	Negative	3	0	100

Reproducibility: 100%