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EPPO STANDARD - DIAGNOSTICS

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

Specific scope: This Standard describes a diagnostic protocol for *Anoplophora glabripennis* and *Anoplophora chinensis*.[1]

This Standard should be used in conjunction with PM 7/76 *Use of EPPO Diagnostic Protocols*.

Specific approval and amendment: First approved in 2020–10.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

The two Asian cerambycid species, *Anoplophora glabripennis* (Asian Longhorn Beetle) and *A. chinensis* (Citrus Longhorn Beetle), are invasive pests that have caused severe damage to a broad range of host plants in the urban environment outside their native range over the last decade. *A. glabripennis* is native to China and the Korean peninsula (Cavey et al., 1998; Lingafelter & Hoebeke, 2002). It was introduced in North America (United States and Canada) and in Europe (Austria, Belgium, Finland, France, Germany, Italy, Montenegro, the Netherlands, Switzerland and the United Kingdom), where some outbreaks have been eradicated in Austria, Belgium, France, Germany, the Netherlands, Switzerland and the United Kingdom. *A. chinensis* also originates from eastern Asia. It is native to China, the Korean peninsula and Japan, and is also occasionally reported in Indonesia, Malaysia, Myanmar, the Philippines, Taiwan and Vietnam (EFSA, 2019a). Like *A. glabripennis*, it was introduced into the United States, where it has been eradicated, and in Europe (Croatia, Denmark, France, Germany, Italy, the Netherlands, Switzerland and Turkey), where some outbreaks have been eradicated in Denmark, France, Germany and the Netherlands. For more detailed information on the pest status and interceptions, see the EPPO Global Database (EPPO, 2020) and Hérard and Maspero (2019), respectively.

Despite their close phylogenetic relationship, the two species spread via different pathways. The main pathway for *A. glabripennis* is wood packaging material, whereas the primary pathway for *A. chinensis* is trade of plants for planting, including bonsais. Both species are polyphagous. *A. glabripennis* is capable of attacking a wide range of broad-leafed tree species, including *Populus* spp. (in particular poplars from the section Aigeiros and hybrids with parental plants belonging to this section (Hu et al., 2009), *Acer* (Gao et al., 1997; Haack et al., 1997; Tomiczek & Hoyer-Tomiczek, 2007; Faccoli & Favaro, 2016), *Salix* spp., *Betula* spp., *Ulmus* spp. and *Aesculus hippocastanum*. *A. chinensis* is known to attack plants from more than 20 families, including *Acer* spp., *Platanus* spp., *Betula* spp., *Fagus* spp., *Corylus* spp., *Rosa* spp., *Malus* spp., *Melia* spp., *Pyrus* spp., *Prunus* spp., *Populus* spp., *Ulmus* spp., *Salix* spp., *Vaccinium* spp. and *Citrus* spp. In comparison to *A. glabripennis*, *A. chinensis* has a wider host range in Asia, which also includes coniferous species in the genera *Cryptomeria* spp. and *Pinus* spp. More information on *A. glabripennis* and *A. chinensis* host tree preferences can be found in EFSA (2019b) and EFSA (2019a), respectively, and EPPO (2021).

Figure 1 shows the diagnostic procedure for *A. glabripennis* and *A. chinensis*.

2 | IDENTITY

Name: *Anoplophora glabripennis* (Motschulsky, 1854).
Other scientific names (Tavakilian & Chevillotte, 2018*): *Cerosterna glabripennis* Motschulsky, 1854; *Cerosterna laevigator* Thomson, 1857; *Melanauster nobilis* Ganglbauer, 1889; *Melanauster angustatus* Pic, 1925; *Melanauster luteonotatus* Pic, 1925; *Melanauster nankineus* Pic, 1926; *Melanauster glabripennis* v. *laglaisei* Pic, 1953.
Taxonomic position: Insecta, Coleoptera, Cerambycidae, Laminae, Monochamini.
EPPO Code: ANOLGL.
Phytosanitary categorization: EPPO A1 List (1999); EU quarantine pest (Annex IIAC9).

Name: *Anoplophora chinensis* (Forster, 1771).
Other scientific names (Tavakilian & Chevillotte, 2018*): *Cerambyx chinensis* Forster, 1771; *Cerambyx farinosus* Houttuyn, 1766; *Lamia punctator* Fabricius, 1776; *Cerambyx pulchricornis* Voet, 1778; *Cerambyx (Stenocorus) sinensis* Gmelin, 1790; *Calloplophora afflicta* Thomson, 1865; *Calloplophora abbreviata* Thomson, 1865; *Calloplophora luctuosa* Thomson, 1865; *Calloplophora sepulcralis* Thomson, 1865.
Taxonomic position: Insecta, Coleoptera, Cerambycidae, Laminae, Monochamini.

[1]Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

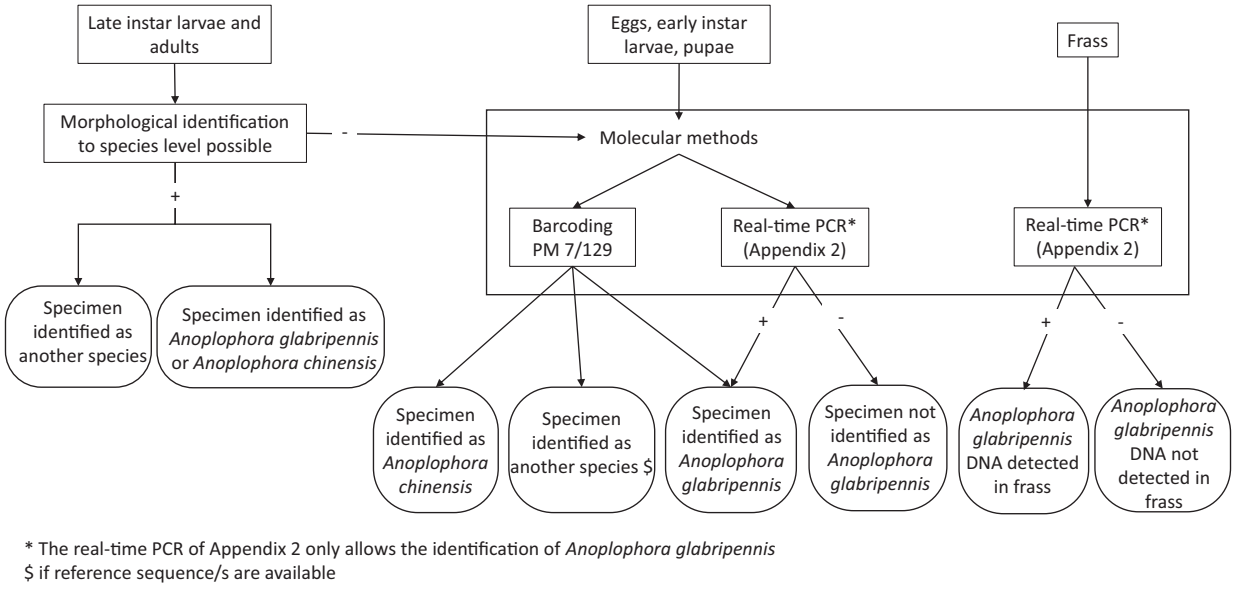

FIGURE 1 Diagnostic procedure for *Anoplophora glabripennis* (from specimen(s) or frass material) and *A. chinensis* (from specimen(s))

EPPO Code: ANOLCN.
Phytosanitary categorization: EPPO A2 List (2007); EU quarantine pest (Annex IIBC2).

* Other names exist (see, e.g., Lingafelter & Hoebecke, 2002).

3 | DETECTION

Additional information on the detection of *A. glabripennis* and *A. chinensis* is included in EPPO Standards PM 3/79 (1) *Consignment inspection for Anoplophora chinensis and Anoplophora glabripennis* (EPPO, 2016), PM 9/15 (1) *Anoplophora glabripennis: procedures for official control* (EPPO, 2013a) and PM 9/16 (1) *Anoplophora glabripennis: procedures for official control* (EPPO, 2013b).

Inspections are usually based on the detection of visual signs, but sniffer dogs trained specifically for the detection of *A. glabripennis* or *A. chinensis* presence in small plants and wood can also be used (EPPO, 2013a,b). Pheromone traps are being used in some EPPO countries to monitor *A. glabripennis* and *A. chinensis* (EFSA, 2019ab; EU, 2017). The efficacy of traps for *A. chinensis* may be low and needs further confirmation (EFSA, 2019a).

3.1 | Anoplophora glabripennis

3.1.1 | Detection in plants

3.1.1.1 | Period for detection

PM 9/15 (1) *Anoplophora glabripennis: procedures for official control* (EPPO, 2013a) recommends that surveys should be carried out at least twice a year, including times of year when (i) the activity of *A. glabripennis* is likely to be high (May–September) and (ii) symptoms can be detected easily because of the absence of leaves on trees (mid-October to March). If it is not possible to survey during the main period of adult activity, inspection of trees can be carried out over the winter months, focusing on trees with signs of *A. glabripennis* activity. In southern Europe, pupae are likely to be found during April/May, young larvae during June/July and larger larvae in September/October. In central and northern Europe, the development of *A. glabripennis* may differ, for example inspectors in Austria and Germany found first-instar larvae even at the end of October.

During the activity period, adults may be seen flying or resting on surfaces.

3.1.1.2 | Signs of presence

Since *A. glabripennis* activity typically occurs in the upper part of the attacked trees, symptoms of infestation and specimens at different development stages should be looked for in the upper part of the tree canopy, upper part of the trunk and main branches (Haack et al., 2010; EFSA 2019b). Experience in Canada and Europe shows that the best way to perform inspections on large trees in urban areas is to use tree climbers.

Detection of potential symptoms due to the tree's response:

- Sap oozing from oviposition sites and where larvae have pushed shavings through the bark (Figure 2a)
- Wilting or loss of foliage
- Tree death or partial death of aerial parts and branches
- Obvious signs of loss in vigour.

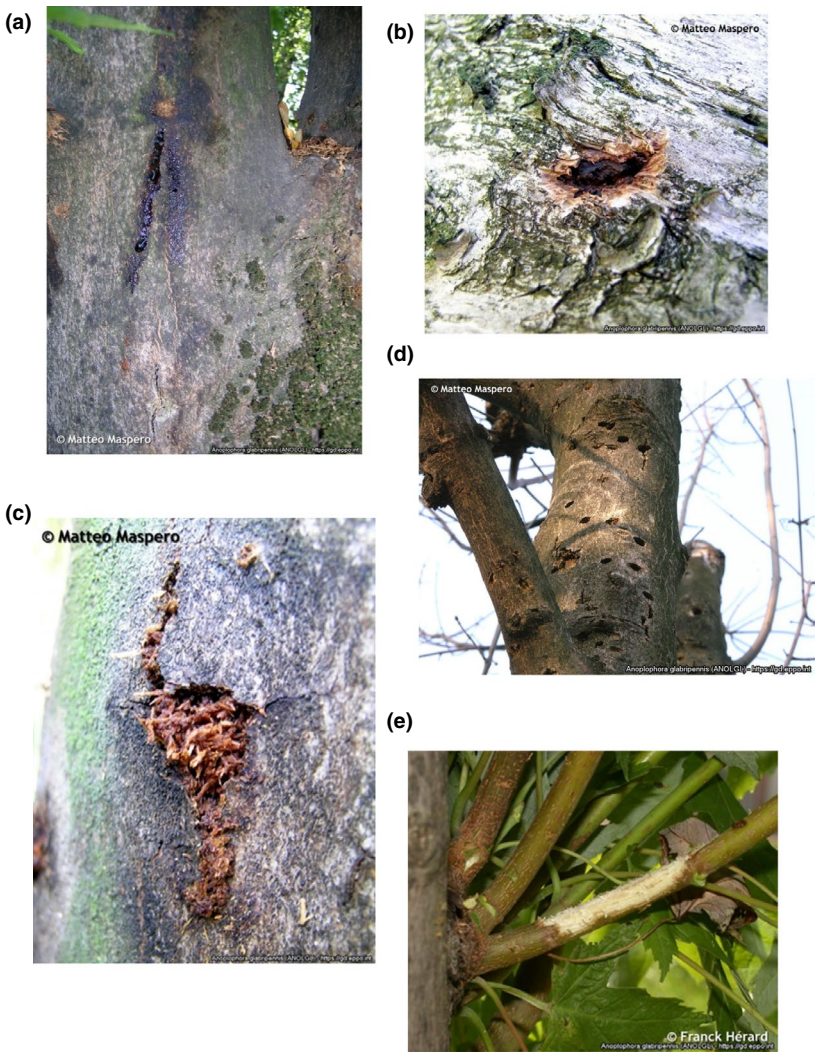

FIGURE 2 Signs of the presence of *Anoplophora glabripennis*. (a) Sap oozing from an oviposition pit of *A. glabripennis*. (b) Oviposition slit of *A. glabripennis* on birch (*Betula*). (c) Frass extruded from the larval tunnel of *A. glabripennis* through cracks in bark. (d) Exit holes and oviposition pits of *A. glabripennis* on maple (*Acer*). (e) Feeding damage caused by adults of *A. glabripennis*. (Courtesy: (a) to (d), Matteo Maspero, Fondazione Minoprio (IT); (e), Franck Herard, European Biological Control Laboratory (FR).)

Visual detection of evidence of *A. glabripennis* presence:

- Oviposition signs (Figure 2b)
- Frass and wood shavings from feeding- and exit-hole boring activity (Figure 2c)
- Discoloration and deformation of bark on plants for planting (including bonsais)
- Larval galleries and grub holes in the upper part of the tree (peeling off bark on wood increases the probability of detection)
- Fresh round exit holes of approximately 10–15 mm diameter (this is the typical size, but they may measure 6–20 mm) in the upper part of the tree (Figure 2d); exit holes will be overgrown with callus tissue over time
- Signs of maturation feeding (Figure 2e)

Damage caused by woodpeckers is an additional sign of the possible presence of *A. glabripennis*.

3.1.2 | Detection in wood packaging material

The most important pathway for the introduction of *A. glabripennis* is the import of wood packaging material from areas where it is native (EPPO, 2013a). In wood packaging material, visual inspection should focus on:

- The existence of mobile insect stages
- Boreholes
- Shavings or frass
- Larval galleries and grub holes (peeling off residual bark increases the chance of detection).

3.1.3 | Molecular detection in frass

A molecular test (real-time PCR) allowing the detection of *A. glabripennis* DNA in frass has been developed (Taddei et al., 2021) (see Section 4 and Appendix 2).

3.2 | Anoplophora chinensis

3.2.1 | Detection in plants

3.2.1.1 | Period for detection

PM 9/16 (1) *Anoplophora chinensis: procedures for official control* (EPPO, 2013b) recommends that surveys should be carried out at least once per year at any time, but preferably in September–October. Inspectors should expect to find pupae during April/May, young larvae during June/July and larger larvae during September/October in the case of 1- and 2-year life cycles. The adults live for approximately 1–3 months, generally between May and August. During the activity period, they may be seen flying in the tree canopy or around the tree, or can be seen on the surface of the soil. Most of the life cycle takes place under ground in roots.

3.2.1.2 | Signs of presence

The lifecycle of *A. chinensis* is very similar to that of *A. glabripennis*. The main differences concern the position and shape of oviposition sites. For *A. chinensis*, signs of infestation are easier to detect and samples easier to collect, since they are sited closer to ground level, and frass piles can be seen around the collar region at the base of the trunks. The most important pathway for the introduction of *A. chinensis* is the import of host plants for planting (including bonsais) from areas where *A. chinensis* is present. Destructive sampling of these plants may be needed to detect *A. chinensis* (EPPO, 2016).

Detection of potential symptoms due to the tree response:

- Sap oozing from the lower section of the tree (Figure 3a)
- Wilting or loss of foliage
- Tree death or dieback of aerial parts and branches (Figure 3b)
- Obvious loss of vigour.

Visual detection of signs of *A. chinensis* activity:

- Damage caused by maturation feeding on young shoots and leaves (Figure 3c)
- *A. chinensis* larval galleries and grub holes bark on wood should be peeled off to increase the probability of detection
- Fresh round exit holes of approximately 10–15 mm diameter (this is the typical size, but they may measure 6–20 mm) in the lower part of the trees (Figure 3d); exit holes will be overgrown with callus tissue over time
- Frass from feeding and exit hole-boring activity.

3.2.2 | Molecular detection in frass

A molecular test (nested PCR) allowing the detection of *A. chinensis* DNA in frass has been developed (Strangi

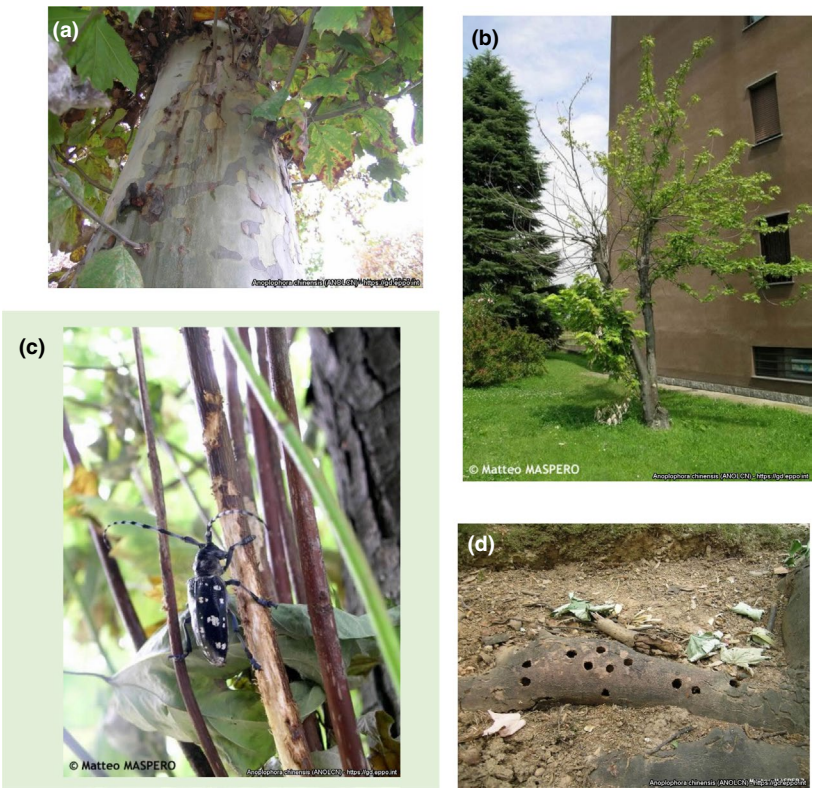

FIGURE 3 Sign of the presence of *Anoplophora chinensis*. (a) Oozing sap on an infested *Platanus*. (b) Tree infested by *A. chinensis*. (c) Damage caused by maturation feeding. (d) Exit holes of *A. chinensis*. (Courtesy: Matteo Maspero, Fondazione Minoprio (IT).)

et al., 2013), but limited validation data are available for this test. The authors reported two false-negative results out of 10 positive samples, suggesting a possible lack of sensitivity of the test (Strangi et al., 2013). In addition, sensitivity was found to be influenced by the environmental conditions in which the frass was present (see Section 3.3), therefore, a negative result using this test should be considered as inconclusive.

3.3 | Test sample requirements for molecular detection in frass

Environmental conditions (exposure to sunlight and weather conditions) can affect the quality of the DNA present in frass. Dry conditions improve DNA stability, while humidity (rain, but also dew) can reduce DNA conservation.

For these reasons, special precautions should be taken during sampling. Experience shows that recovering faecal DNA suitable for PCR is more likely from freshly expelled frass material or from frass collected inside trunks (Strangi et al., 2013). As a consequence, when possible, it is advisable to remove a small bark portion to collect material from inside the gallery. Alternatively, the collection of freshly expelled frass should be preferred. If the frass material is wet or damp, once in the laboratory it must be allowed to dry at room temperature before processing or long-term storage.

4 | IDENTIFICATION

Identification of *A. glabripennis* and *A. chinensis* by morphological examination is quite straightforward for adult specimens. The habitus of adults displays characters that are unique when compared to other longhorn beetle species native to Europe. The morphological identification of larvae is possible, especially in the case of fully developed larvae, but it can be difficult to distinguish between the two species. Moreover, the morphology of the larvae of the majority of *Anoplophora* species has not been described, therefore it is recommended to confirm the identification of larvae with molecular tests.

There are no adequate keys for the identification of eggs, early instar larvae and pupae. Molecular tests (see Section 4.2) can be performed on all life stages, especially on those for which morphological identification to species level is not possible. Additionally, in cases where adult specimens are damaged, molecular tests may provide further relevant information for identification.

4.1 | Morphological identification

4.1.1 | Morphological identification of the larva of *A. glabripennis* and *A. chinensis*

The general aspect of the larvae of *A. glabripennis* and *A. chinensis* is common to the family Cerambycidae (Figures 4 and 5) (Stehr, 1987; Cavey et al., 1998; Lingafelter & Hoebecke, 2002), characterized by a cylindrical, fleshy, pale-yellow body with prothorax always wider than the meso- and metathorax and abdominal segments. The head is usually retracted into the prothorax; pronotum more or less partially sclerotized and often provided with a pronotal pigmented plate (or shield). There are 10 abdominal segments, and a total of nine spiracles, one on the mesothorax and one on each of the abdominal segments I–VIII.

Larval morphology of the two *Anoplophora* species is almost identical, but *A. chinensis* can be distinguished

FIGURE 4 *Anoplophora glabripennis*: (a) 46 mm mature larvae (photo by James Connell, BFW) and (b) mature larvae (lateral view). Note epipleurum protuberant on abdominal segments VII–IX. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

from *A. glabripennis* by slightly different features of the pronotum. These differences are not clearly visible on early instars larvae (Figure 5). Thus, it is recommended to perform the morphological identification on late instars larvae and to use molecular methods for early instars.

For a key to the families of Coleoptera larvae, see Stehr (1987) and Lawrence et al., (1999).

A simplified key for late instars larvae of *A. glabripennis* and *A. chinensis* (after Pennacchio et al., 2012) is provided in Appendix 1.

FIGURE 5 *Anoplophora glabripennis*, early instar larva (dorsal view), scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

4.1.1.1 | Larva description

The body is elongate, cylindrical, fleshy, cream-coloured.

The head is retracted into the prothorax and mouthparts are prognathous; labrum is yellowish, semicircular and dorsally carries dense, long, erect setae. Clypeus is trapezoidal in shape (Figures 6b and 7a,b). Mandibles are stout, heavily sclerotized.

Legs are absent.

In *A. glabripennis*, the pronotum bears a transverse band of rather dense, long, stiff setae along the anterior margin. The anterior area of the pronotum is lightly sclerotized, pale yellow in colour, densely covered with shallow pits or wrinkles and sparse setae. The posterior area of pronotum is more heavily sclerotized, darker yellow to orange in colour, distinctly raised; the anterior margin of this shield is distinctly shaped, symmetrically undulate (Figure 7b) and the surface covered by micro-spiculae, only visible at high magnification. Moderately dense, much lighter in colour, elongate, shallow pits are scattered all over the pronotal shield, together with sparsely fine and short setae. In *A. chinensis*, the pits in the pronotal shield are smaller in size and finer (Figure 7a). This character, together with the presence of a distinct, clearly visible pigmented band anterior to the pronotal shield, which

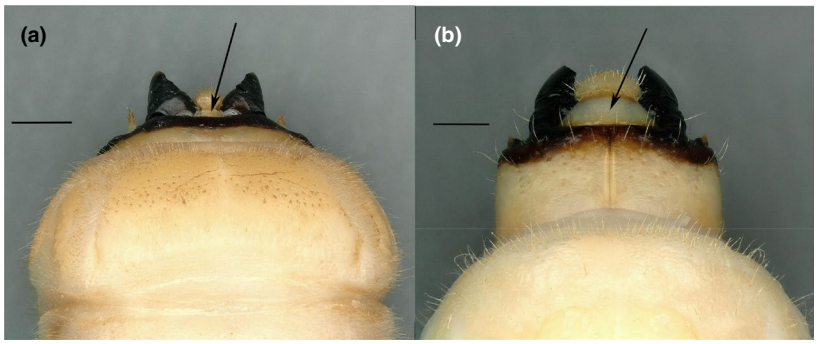

FIGURE 6 (a) Clypeus narrow in subfamily Cerambycinae (*Phoracantha semipunctata*). (b) Clypeus trapezoidal in subfamily Lamiinae (*Anoplophora glabripennis*). Dorsal view, scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

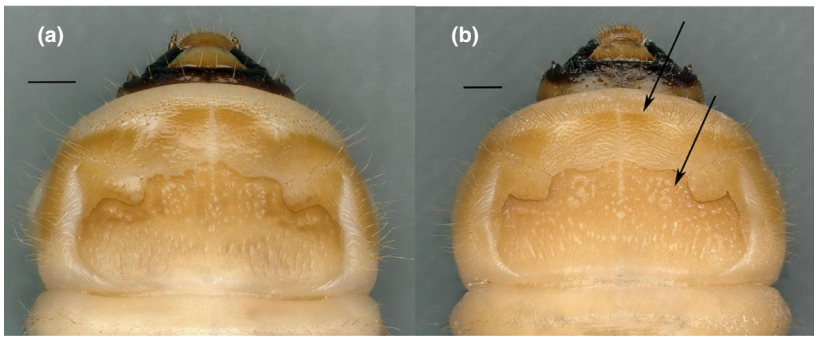

FIGURE 7 Pronotal shield: (a) *Anoplophora chinensis* and (b) *A. glabripennis*. Note larger pits in the pronotal shield and a less pigmented band anterior to pronotal shield in *A. glabripennis*. Dorsal view, scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

FIGURE 8 (a) *Monochamus sartor* larva (48 mm) (photo by James Connell, BFW) and (b) *Monochamus* sp. larva (lateral view). Epipleurum protuberant on abdominal segments III–IX. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

is usually less pigmented in *A. glabripennis*, allows the separation between the two species, even if this is sometimes not obvious.

Larvae at maturity are relatively large, up to 50 mm in body length or slightly more.

4.1.1.2 | Possible confusion with other native and introduced Lamiinae

Species identification of larvae may be difficult, and they can be confused with some other longhorn beetles present in Europe. *A. glabripennis* and *A. chinensis* belong to the tribe Monochamini of the subfamily Lamiinae (Löbl & Smetana, 2010). Among the European fauna, there are about 340 species listed in this subfamily, but only one genus of the native species represents the tribe Monochamini, i.e. *Monochamus* Dejean, 1821. Larvae of *A. glabripennis* and *A. chinensis* can be distinguished from *Monochamus* spp. by the characters of pronotum, prosternum, and dorsal and ventral ampullae (Cavey et al., 1998). Moreover, the ventral ray of the anal pore is distinctly shorter than the two lateral rays (Pennacchio et al., 2012). Last instars larvae of *Monochamus* spp. (Figure 8) can be distinguished from *A. glabripennis* and *A. chinensis* larvae by the presence of protuberant epipleurum on the abdominal segment III–IX, whilst they are limited to abdominal segment VII–IX in *Anoplophora* (Figure 5). It should be noted that larvae of *Monochamus* spp. do not share the same host plants range with *A. glabripennis*, as they develop only in the wood of coniferous trees of the genera *Pinus*, *Picea* and *Abies*.

In the last 20 years, another species of Asian origin belonging to the Monochamini tribe has been introduced into Europe: *Psacothea hilaris* (Pascoe, 1857) (Jucker et al., 2006). Last instar larvae of *Psacothea hilaris*, which develop in the wood of *Ficus* and *Morus* spp., can be distinguished by the presence of protuberant epipleurum on the abdominal segment III–IX and by the shorter length of the ventral ray of the triradiate anal pore (Pennacchio et al., 2012). *Ficus* species are not known to be host plants for *A. glabripennis*. Records are available about the capability of the beetle to lay eggs on *Morus* plants, but full development has never been observed (Van der Gaag & Loomans, 2014).

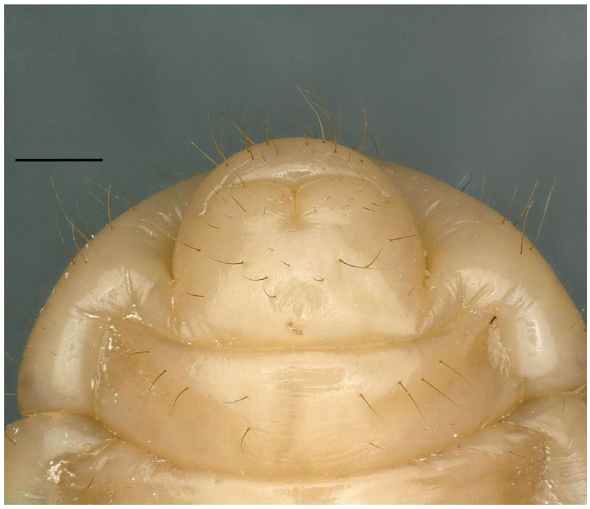

FIGURE 9 Anal pore triradiate in *Anoplophora glabripennis*, postero-ventral view, scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

Among the Lamiini tribe, some species display features and body size similar to those of *A. glabripennis* and *A. chinensis*. The larvae could be mistaken for *Morimus asper* (Sulzer, 1776) and *Lamia textor* (Linnaeus, 1758), which share some host plants with

the two *Anoplophora* species such as *Salix* and *Populus* spp. (Tavakilian & Chevillotte, 2018). However, they can be easily excluded by the presence of a simple, transverse anal pore (whilst in *Anoplophora* it is triradiate) (Figure 9).

Species belonging to the *Saperda* genus (tribe Saperdini) are frequently hosted by broadleaves such as *Populus*, *Ulmus*, *Salix* and *Acer* spp. The tribe is characterized by the presence of dark spicules on the pronotal shield and on dorsal ambulatory ampullae, clearly visible under low magnification (Figures 10B and 11). In *A. glabripennis*, spicules are also present, but they are visible only under high magnification.

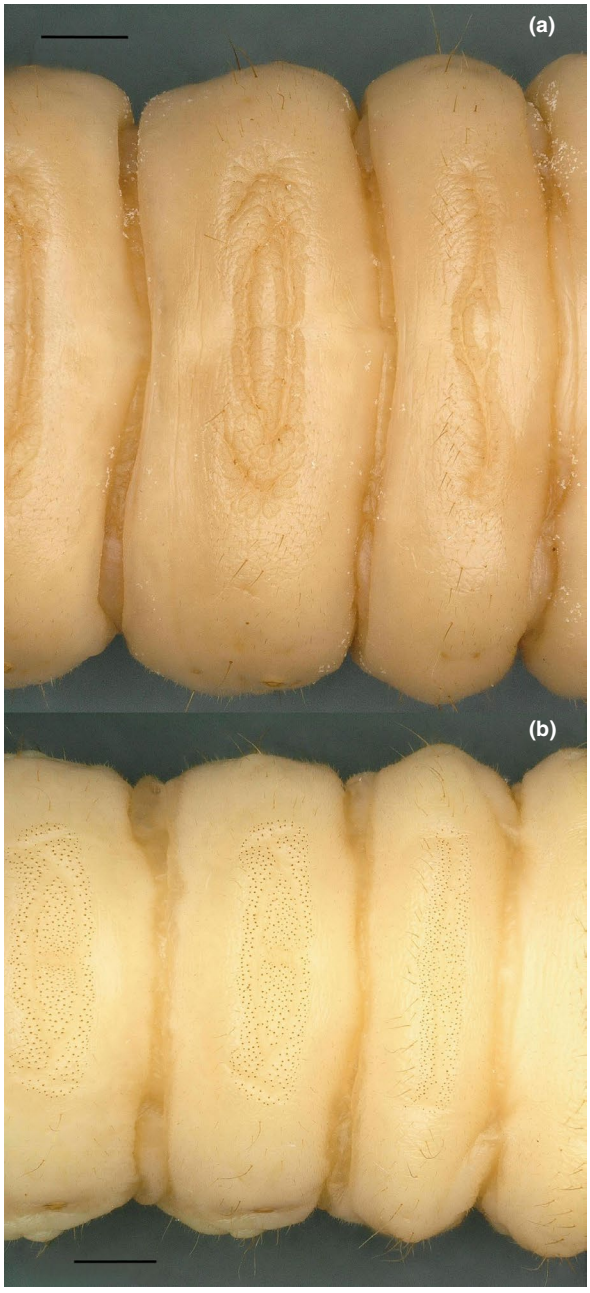

FIGURE 10 Dorsal ambulatory ampullae: (a) *Anoplophora glabripennis* and (b) *Saperda similis* (Lamiinae, Saperdini). Scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

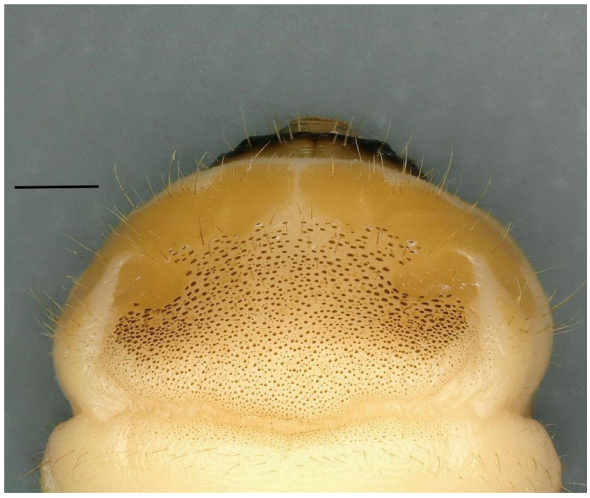

FIGURE 11 Pronotum spiculose in *Saperda similis* (Lamiinae, Saperdini), dorsal view, scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

4.1.2 | Morphological identification of the adult

For a key to the subfamilies and tribes of Cerambycidae in Europe, see Berger and Villiers (2012). A key for the Monochamini genera in Europe is provided in Appendix 1.

A simplified key for adults of *A. glabripennis* and *A. chinensis* is provided in Appendix 1. A comprehensive identification key for adults of 36 *Anoplophora* species is provided by Lingafelter & Hoebecke, 2002. This protocol describes the characteristics for the identification of the two species and the possible confusion with similar species belonging to the same genus (after Lingafelter & Hoebecke, 2002).

Due to the unique habitus and morphological features, a misidentification with other European longhorn beetles is considered very unlikely. The only other taxons representing the Monochamini tribe in Europe are members of the genus *Monochamus* Dejean, 1821, and the species *Psacothea hilaris* (Pascoe, 1857).

4.1.2.1 | *Anoplophora glabripennis* description

Body: Body length can vary in the range of 17–39 mm. However, in most cases, specimens are in the range of 20–35 mm. The lower limit of the range can be reached especially in the case of reared individuals.

Head: Black, generally glabrous, with inconspicuous fine setae around genae and eyes margins. As in most Cerambycidae, antennae have 11 segments (antennomeres); in the male, antennae exceed the apex of elytra by five antennomeres, with third antennomere extending

to mesocoxa. In the female, antennae exceed the apex of elytra by one to two antennomeres, with third antennomere not extending to posterior margin of pronotum. Antennomeres variably annulate in pale blue or white at basal one third or one half (Figure 12a,b); the annulation becomes more conspicuous on distal antennomeres. Some specimens can display inconspicuous annulation until antennomeres 5 or 6.

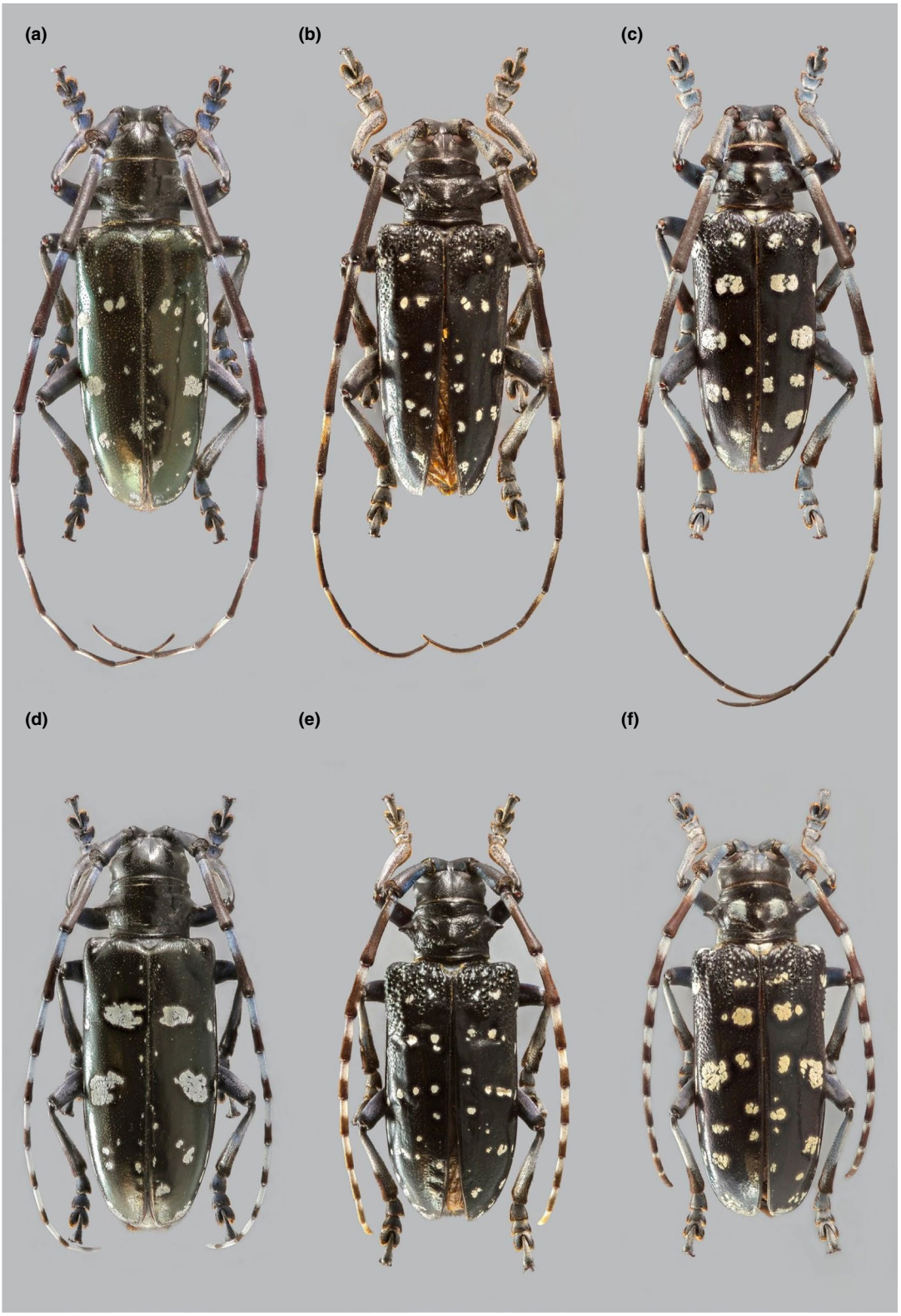

FIGURE 12 Habitus of adults: (a) ♂, (b) ♀ *Anoplophora glabripennis*, (c) ♂, (d) ♀ *A. chinensis*, (e) ♂, and (f) ♀ *A. chinensis* form *malasiaca*. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

Pronotum: Black, generally glabrous but with inconspicuous coating of fine, appressed setae at posterior of lateral pronotal spine and ventrally. Lateral pronotal spine straight, moderate in length and thickened at base, with acute apex.

Scutellum: Black, densely pubescent although pubescence is short, translucent and sometimes inconspicuous to the naked eye; triangular with semi-acute posterior apex (Figure 13a).

Elytra: Either with shiny or matt (more often) surface, weakly iridescent copper, blue or violet sheen, with 10–20 irregularly sized and irregularly distributed patches of white pubescence. In the Chinese province of Gansu, most of the specimens display the yellow colour of pubescent patches; sometimes the patches can be light orange in colour, but this case is very rare. Some extremely rare aberrant specimens display no patches on elytra. Another aberrant rare morph displays scattered spots of white pubescence throughout the elytra. A South Korean morph displays some nearly complete bands on elytra. Regularly distributed, translucent setae are scattered elsewhere on elytra. The elytral base is smooth, lacking granulae (Figure 13a), but very fine microreticulations are present at the base of elytra.

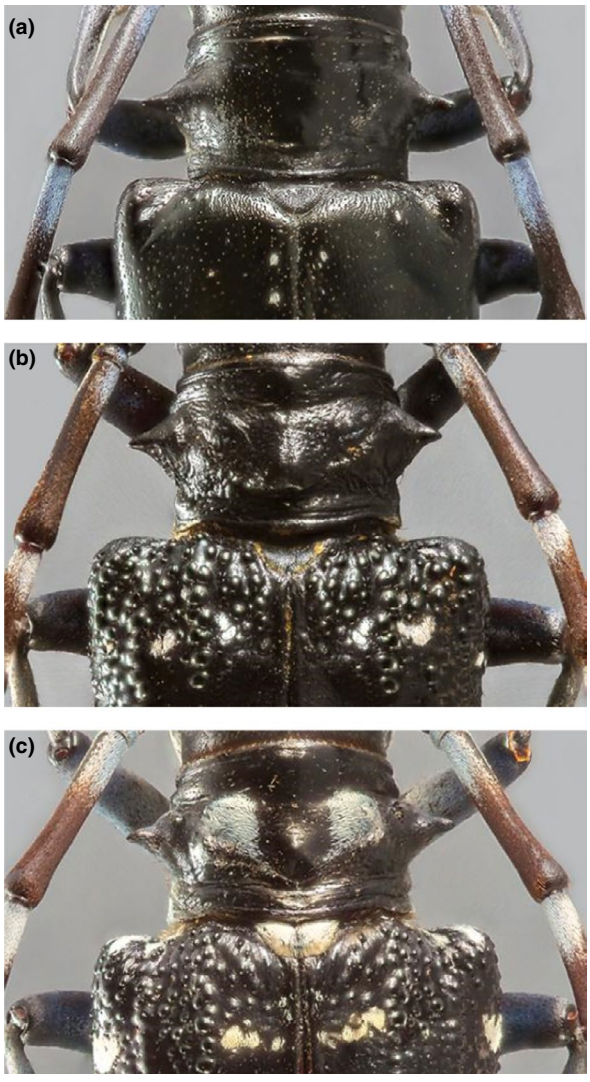

FIGURE 13 Detail of pronotum, elytra and scutellum: (a) *Anoplophora glabripennis*, (b) *A. chinensis*, (c) *A. chinensis* form *malasiaca*. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

Legs: Black, iridescent white to pale blue pubescence on tarsomeres 1–3 and tibiae. Tarsi with brilliant iridescent blue pubescence dorsally on fresh specimens (Figure 14a), white and weakly iridescent in older ones.

Abdomen: Black, covered by white or pale inconspicuous appressed setae. In the female, the apex of the abdomen is bilobed, with a strong notch in the middle and densely fringed with pubescence. In the male, the apex of the abdomen is broad, truncate, with a very slight notch in the middle and short setae at margin.

Possible confusion with other congeneric species.
(taxonomy here follows Lingafelter and Hoebecke (2002)).

A. glabripennis adults are very similar to *A. coeruleoantennata* (Breuning, 1946) (Figure 15a) and *A. freyi* (Breuning, 1946), which are known only from Sichuan and Sichuan/Yunnan (China), respectively. All these species display the characteristic black integument with scattered white spots on the elytra. The elytral base is smooth, lacking granulae and has very indistinct microreticulations. However, *A. glabripennis* can be distinguished from the two similar species taking into account the following characters:

- The tarsi of fresh *A. glabripennis* specimens have bright, iridescent bluish pubescence, which is less conspicuous in *A. freyi* and *A. coeruleoantennata*;
- *A. freyi* has a very shiny elytral integument with strong iridescent sheen, while in *A. glabripennis* and *A. coeruleoantennata* the integument is less shiny, often opaque, and less iridescent.
- *A. coeruleoantennata* has nearly all antennomeres bluish-purple annulated on at least the basal two thirds, while in *A. glabripennis* the annulations are white and restricted to the basal one third or one half.

4.1.2.2 | Anoplophora chinensis *description*.
Body: Body length is in the range of 21–37 mm.

Head: Black, with pubescence variable. Antennae of males exceed apex of elytra by five antennomeres. In the female, antennae exceed the apex of elytra by one to two antennomeres. Most of the antennomeres are strongly white or light blue annulated at basal one-fourth or one-half (Figure 12c–f).

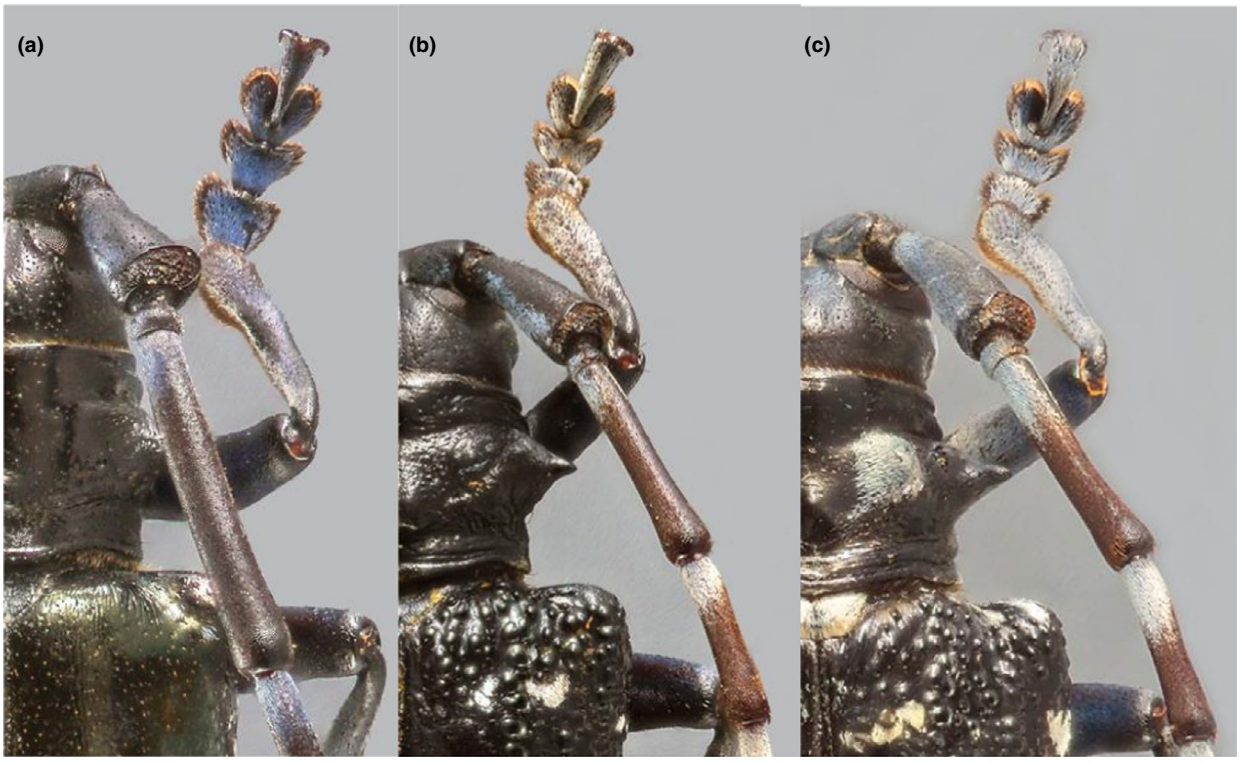

FIGURE 14 Detail of tibia, tarsi, antennal annulation: (a) *Anoplophora glabripennis*, (b) *A. chinensis* and (c) *A. chinensis* form *malasiaca*. Tarsi with brilliant iridescent blue pubescence in *A. glabripennis*. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

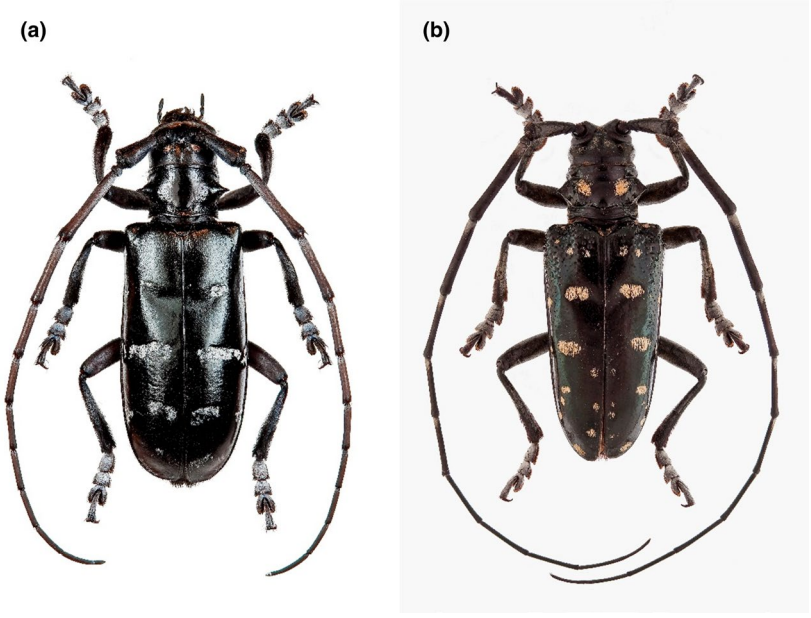

FIGURE 15 Dorsal view of the habitus of male adults of (a) *Anoplophora coeruleoantennata* and (b) *A. macularia*. (Courtesy: Theodoor Heijerman, NVWA (NL).)

Pronotum: Black with three major calli (thickenings of the integument) and anterior and posterior constriction (Figure 13b,c). Pubescence on pronotum is variable: in specimens from China pubescence is usually absent (glabrous), sometimes with very indistinct patches of whitish hairs. In Japan, most of the specimens have large, sometimes bold, patches of whitish scale-like hairs on both sides of the middle (*malasiaca* form; Figure 13c). Lateral pronotal spines are strong, thickened at base, with acute apex.

Scutellum: Moderately pubescent at posterior half, but pubescence is inconspicuous to naked eye.

Elytra: Black, shiny but without iridescent sheen, each displaying 10–20 small patches of white pubescence. Those patches are larger in the *malasiaca* form. Numerous granulae are present at basal one-fourth of elytra, sometimes exceeding posteriorly in rows along costae (Figure 13b,c).

Legs: Black, with bluish or white pubescence on tarsomeres 1–3 and tibiae, more conspicuous in specimens from Japan (*malasiaca* form) (Figure 14b,c).

Abdomen: Black, coated by white or bluish appressed hairs, more conspicuous in the *malasiaca* form. In the female, the apex of the last abdominal segment is roughly V-shaped with fringe of short hair at margin, whereas in the male, apex is truncate, with a slight notch in the middle and short hairs at margin.

Lingafelter and Hoebecke (2002) synonymized *A. malasiaca* (Thomson, 1865) with *A. chinensis* (Forster, 1771) due to the identical morphology of genitalia and many other common features. The *malasiaca* form differs from the *chinensis* by displaying larger maculations on elytra, pubescence patches on pronotum and blue ventral pubescence (white pubescence in the *chinensis* form).

Possible confusion with other congeneric species.
(taxonomy here follows Lingafelter and Hoebecke (2002)).

A. chinensis adults are similar to *A. davidis* and *A. macularia* (Figure 15b). It can be distinguished from the two similar species taking into account the following characters:

- *A. davidis* have blue pubescence ventrally (whereas it is white in the typical *A. chinensis*, but blue in the *malasiaca* form); a small, glabrous region on the lateral middle part of metasternum is lacking (whereas it is present in *A. chinensis*).
- *A. davidis* and *A. macularia* have long, conspicuous, suberect hairs on elytra (whereas in *A. chinensis* they are shorter and less conspicuous).
- In *A. davidis*, the setae on the female's last sternites are shorter than in *A. chinensis*, not or slightly extending beyond the apex of the abdomen.
- *A. macularia* have elytral patches H3 and H4 fused in one large maculation (whereas in *A. chinensi*s they are separated).

4.2 | Molecular identification

Molecular tests can be used for the identification of eggs, early instar larvae or pupae, or to support the morphological identification of late instar larvae or adults of *A. glabripennis* and *A. chinensis*.

For the identification of *A. glabripennis*, a target-specific real-time PCR test based on TaqMan probe chemistry has been developed by Taddei et al. (2021) and is described in Appendix 2. This test can be applied on tissue from specimens.

In contrast, target-specific real-time PCR tests to support the morphological identification of *A. chinensis* are not available yet. The *Chinensis* primer pair used by Strangi et al. (2013) (*ChinensisF* and *ChinensisR*) may be used for the identification of specimens. However, so far there is no validation data on the direct use of those primers on *A. chinensis* specimens for identity confirmation.

A protocol for DNA barcoding based on COI described in Appendix 1 of PM 7/129 (2) *DNA barcoding as an identification tool for a number of regulated pests: DNA barcoding Arthropods* (EPPO, 2021) allows the identification of *A. glabripennis* and *A. chinensis*. Sequences are available in EPPO-Q-bank https://qbank.eppo.int/arthropods/. However, the barcoding approach cannot be reliably applied to frass material due to possible annealing of primers on the DNA of other organisms that rapidly colonize frass material, such as fungi, nematodes and other arthropods.

5 | REFERENCE MATERIAL

Reference material may be available at ANSES – Laboratoire de la santé des végétaux, Unité entomologie et plantes invasives, 755 avenue du Campus Agropolis, 34988 Montferrier-Sur-Lez Cedex, France.

6 | REPORTING AND DOCUMENTATION

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis*.

7 | PERFORMANCE CHARACTERISTICS

When performance characteristics are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8 | FURTHER INFORMATION

Further information on this protocol can be obtained from:

- Andrea Taddei, ANSES – Laboratoire de la santé des végétaux, Unité entomologie et plantes invasives, 755 avenue du Campus Agropolis, 34988 Montferrier-Sur-Lez Cedex, France; andrea.taddei@anses.fr
- Matthias Becker, Julius Kühn-Institut – Bundesforschungsinstitut für Kulturpflanzen Institut für nationale und internationale Angelegenheiten der Pflanzengesundheit Messeweg 11/12, 38104 Braunschweig, Germany; matthias.becker@julius-kuehn.de

9 | FEEDBACK ON THIS DIAGNOSTIC PROTOCOL

If you have any feedback concerning this Diagnostic Standard, or any of the tests included, or if you can provide additional validation data for tests included in this Standard that you wish to share, please contact diagnostics@eppo.int.

10 | PROTOCOL REVISION

An annual review process is in place to identify the need for revision of Diagnostic Standards. Standards identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

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- A. Taddei, European Union Reference Laboratory for pests of plants on insects and mites ANSES – Laboratoire de la santé des végétaux, Unité entomologie et plantes invasives (FR) [previously employed in the Regione Lombardia Plant Health Laboratory c/o Fondazione Minoprio (IT)]
- B. Hoppe, Matthias Becker, Beatrice Berger, Julius Kühn-Institut – Bundesforschungsinstitut für Kulturpflanzen Institut für nationale und internationale Angelegenheiten der Pflanzengesundheit (DE)
- D. Rizzo, Daniele de Lio, Laboratory of Phytopathological Diagnostics and Molecular Biology, Plant Protection Service of Tuscany (IT).

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APPENDIX 1– KEYS TO LATE INSTARS LARVAE AND ADULTS BASED ON MORPHOLOGY

Key for the identification of *A. glabripennis* and *A. chinensis* late instars larvae (after Pennacchio et al., 2012)

1	Legs present, 4 jointed (excluding coxa) (Figure 16)	Subfamily Cerambycinae (pars) and other subfamilies
1′	Legs absent	Subfamily Cerambycinae (pars) and subfamily Lamiinae 2
2	Clypeus very narrow, with only slender basal arms reaching to mandibular articulations (Figure 6a). Mandibular apex and dorsal angle more or less lacking; mandible short, apically rounded, spoon-like (Figure 17).	Subfamily Cerambycinae
2′	Clypeus more or less trapezoidal, filling entire space between dorsal mandibular articulations (Figure 6b). Mandibles not rounded, with distinct apex and more or less distinct dorsal angle (Figure 17b).	Subfamily Lamiinae 3
3	Anal pore transverse.	Tribe Lamiini
3′	Anal pore triradiate (one ventral and two lateral rays) (Figure 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 (Figures 10b and 11).	Tribe Saperdini
4′	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 depigmented rounded areas, more or less joined (Figure 7a,b). 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
5	Dorsal ambulatory ampullae granular, built up by small granules in distinct transverse rows or in elongate oval clusters formed by large joined granules (Figure 10a).	6
5′	Dorsal ambulatory ampullae not granular, but with small spinule.	Tribe Acanthocinini

(Continues)

(Continued)

Key for the identification of *A. glabripennis* and *A. chinensis* late instars larvae (after Pennacchio et al., 2012)		
6	Dorsal ambulatory ampullae medially with large granules in 4 distinct transverse rows (Figure 10a). Body size of the last instars larvae generally more than 40 mm.	Tribe Monochamini 7
6′	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 (Figure 8b). Anal pore with the ventral ray distinctly shorter than the two rays.	Other Monochamini
7′	Abdominal epipleurum protuberant only on the segments VII–IX (Figure 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 Figure 7a	***Anoplophora chinensis***
8′	Anterior to the pronotal shield, the band is less observable due to less pigmentation; typical pronotum as in Figure 7b	***Anoplophora glabripennis***

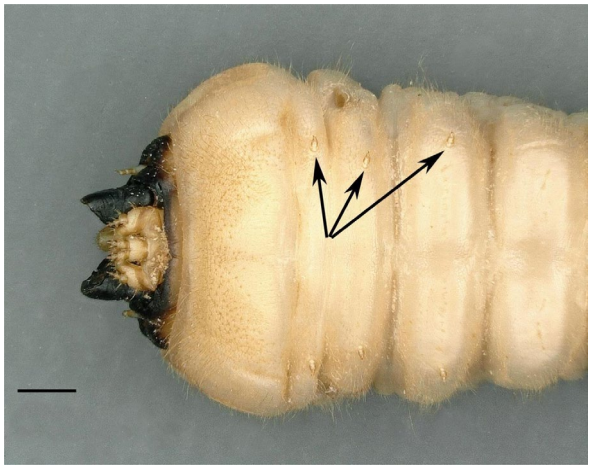

FIGURE 16 Legs, ventral view (*Phoracantha semipunctata*, Cerambycinae), scale bar 1 mm. (Courtesy: Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

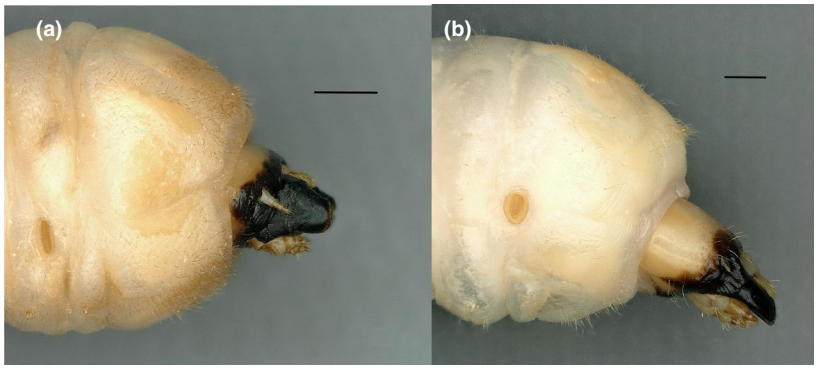

FIGURE 17 (a) Mandibles short and rounded, spoon-like in subfamily Cerambycinae (*Phoracantha semipunctata*). (b) Mandibles not rounded, with distinct apex in subfamily Lamiinae (*Anoplophora glabripennis*). Lateral view, scale bar 1 mm. (Photo by Laurent Soldati, INRAE Montpellier (FR) and Andrea Taddei, ANSES (FR).)

It should be noted that the separation of *A. glabripennis* and *A. chinensis* through larval morphology can be quite difficult, even in the case of fully developed late instars. It is recommended to confirm the identification by molecular analysis if sufficient experience is lacking.

Key for adults of the Monochamini genera in Europe		
1	Elytra from brown to blackish brown, with irregular marmorization, sculptured with numerous confluent punctures. Antennae completely black or brown in the male.	*Monochamus*
1′	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 vertex. Body shiny black. Elytra with irregularly distributed patches of dense, generally white, pubescence. Lateral pronotal spines strong, well developed.	*Anoplophora*
2′	Presence of longitudinal stripes of yellow pubescence on pronotum and head 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*

Simplified key for the identification of *A. glabripennis* and *A. chinensis* adult specimens within the *Anoplophora* genus (after Lingafelter & Hoebecke, 2002)		
1	Antennae with conspicuous pubescent annulations on most antennomeres (Figures 12–14)	2
1′	Antennae without conspicuous pubescent annulations	Other species
2	Antennae with distinct narrow annulation at base and apex of most antennomeres	Other species
2′	Antennae with annulations at basal fourth or more of most antennomeres (Figures 12–14)	3
3	Most of the body covered with dense, uniform blue-grey, blue-green or turquoise pubescence	Other species
3′	Most of the body not uniformly covered with pubescence of different shades of blue (Figure 12)	4
4	Elytra with bands or spots of dense yellow pubescence	*A. horsfieldii*
4′	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
5′	Pronotum with very weak or no mediolateral calli, anterior margin not highly elevated and without pronounced middle depression in front of posteromedial callus (Figure 13a–c)	6
6	Elytra with 4–7 complete or nearly complete transverse bands of pubescence	Other species
6′	Elytra with pubescent maculations in form of numerous irregularly sized spots on disk, most non-forming bands (Figure 12)	7
7	Elytral base with numerous (10 or more) conspicuous granules (Figure 13b,c)	8
7′	Elytral base without granules (or at most 10) (Figure 13a)	11
8	Pubescent maculations on elytra poorly defined, fuzzy margined, numerous (about 30), variably sized and bicoloured	Other species
8′	Pubescent maculations on elytra less numerous, usually well defined, with distinct edges and unicolourous (white, yellow, light orange, light blue) (Figure 12)	9

(Continues)

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Simplified key for the identification of *A. glabripennis* and *A. chinensis* adult specimens within the *Anoplophora* genus (after Lingafelter & Hoebecke, 2002)		
9	Elytra with few, if any, erect or suberect, long black hairs; white, blue or translucent pubescence ventrally	10
9′	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 (Figure 13b,c); antennal annulation light blue or white	***A. chinensis*** (Figure 12c–f)
10′	Elytra with about 10 granules each	*A. variantennatus*
11	Pronotum with conspicuous, dense pubescence dorsally	other species
11′	Pronotum without dense pubescence (Figure 13a)	12
12	Antennomeres with a broad basal purple or deep blue pubescent annulation on at least basal two-thirds of most antennomeres	*A. coeruleoantennata*
12′	Antennomeres with white or pale blue pubescent annulation occupying no more than the basal half of most antennomeres (Figure 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*
13′	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 (Figure 14a); maculations on elytra white or yellow (rarely pale orange)	***A. glabripennis*** (Figure 12a,b)

APPENDIX 2– COI SEQUENCE-BASED REAL-TIME PCR TEST FOR *ANOPLOPHORA GLABRIPENNIS* (TADDEI ET AL.,2021)

The test below is described as carried out to generate the validation data provided in Section 4. *Other equipment, kits or reagents may be used provided that a verification (see PM 7/98) is carried out.*

1. GENERAL INFORMATION

1.1. This TaqMan based real-time PCR is suitable for the identification of *A. glabripennis* eggs, larvae and adults. It can also be used on frass or wood shaving samples produced by *A. glabripennis* larvae.

1.2. This test was developed by Taddei et al. (2021). One primer pair, to be used in combination with an associated TaqMan probe, is available and has been tested.

1.3. The targeted gene is the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene.

1.4. The primers used for those tests are:

Primers/probe	Sequence	Amplicon size
ALB_185_F	5′-CCA ACA GGA ATT AAA GTA TTT AG-3′	163 bp
ALB_347_R		
ALB_320_P	5′-CGT GGA GAA TAA TAT CAA TAG A-3′	
	FAM-TGG CTA AGA CTA CTC CTG TTA ATC CTC-BHQ1	

1.5 The tests were performed on the StepOnePlus Real-Time PCR System (Applied Biosystems).

2. METHODS

2.1. Nucleic acid extraction and purification.

2.1.1. Nucleic acid extraction for insect tissue

DNA from insect tissue can be extracted using the Blood & Tissue kit (Qiagen) following manufacturer instructions as adopted in the EPPO Standard PM 7/129 (2) *DNA barcoding as an identification tool for a number of regulated pests*. DNA extraction by means of Maxwell RSC 48 automatic instrument (Promega), using a CTAB-based protocol (Maxwell RSC PureFood GMO and Authentication kit), is also effective.

2.1.2 Nucleic acid extraction for frass and woody shaving samples

For frass and woody shavings samples, a CTAB-based extraction protocol combined with the Zymo Quick-DNA Fecal/Soil Microbe Miniprep Kit D6010 (including mercaptoethanol) is recommended.

- Grind dry frass or woody shaving sample to a fine powder by means of a homogenizer (e.g. Retsch MM400, Qiagen TissueLyser II, in an iron jar with iron beads, after liquid nitrogen treatment, 3 min at 30.0 frequency, or in a MP FastPrep-24™ 5G Instrument, in plastic tube with iron beads, 3 × 1 min at maximum speed).
- Use 50 mg of the obtained powder for DNA extraction.

- Add 1 mL of CTAB solution (2% CTAB, 0.1 M TrisHCl pH 8, 1.4 M NaCl, 0.02 M EDTA pH 8, 4% PVP40) to the tubes, and vortex.
- Incubate for 30 min at 65°C.
- Centrifuge at maximum speed for 10 min.
- Collect 500 µL of the solution, leaving the debris at the bottom of the tube and transfer to a new 1.5 µL tube.
- Add 500 µL of chloroform/isoamyl alcohol 24:1.
- Centrifuge at maximum speed for 5 min.
- Transfer the supernatant (400 µL) to the Zymo column and perform the DNA isolation according to the manufacturer's instructions. DNA is eluted in 100 µL of elution buffer.

Analytik Jena InnuPREP TCM DNA Extraction Kit is also effective for DNA extraction from frass, but is not commercially available yet.

2.1.3 DNA should be used immediately for the real-time PCR reactions or should be stored at approximately −20°C.

2.2 Real-time PCR.

Target DNA from insect tissue, frass and wood shavings was successfully amplified using the PerfeCTa qPCR ToughMix (QuantaBio), which is particularly suitable for overcoming problems of PCR inhibitors that might be present in environmental or plant samples.

It is recommended to test each sample in duplicate or triplicate, especially for DNA extracted from frass material.

2.2.1 TaqMan real-time PCR.

2.2.1.1 Master mix.

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular-grade water	NA	6.5 µL	NA
PerfeCTa qPCR ToughMix (QuantaBio)	–	10	–
Forward primer (ALB_185-F)	10 µM	1	0.5 µM
Reverse primer (ALB_347-R)	10 µM	1	0.5 µM
TaqMan probe (ALB_320-P)	10 µM	0.5	0.25 µM
Subtotal		19	
DNA dilution		1	
Total		20	

NA, Not applicable.

2.2.1.2 PCR conditions for the TaqMan approach: 2 min at 95°C; then 40 cycles of 10s at 95°C, 15s at 58°C and 30s at 68°C.

3. ESSENTIAL PROCEDURAL INFORMATION

3.1 Controls

For a reliable test result to be obtained, the following (external) controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively:

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification on clean extraction buffer.
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the target.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: amplification of molecular-grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This can include nucleic acid extracted from the target organism, whole-genome amplified DNA or a synthetic control (e.g. cloned PCR product).

Internal positive controls (IPC) can be used to monitor each individual sample separately. Specific amplification of endogenous nucleic acid, using conserved primers that amplify conserved non-pest target nucleic acid that is also present in the sample (e.g. eukaryotic 18S rDNA) is recommended in order to check the quality of the DNA extracted from any animal or plant (frass) sample and to detect false-negative results that could potentially be caused by inhibitors present in the wood or issues occurred during the DNA extraction procedure. During the validation, a universal primer pair (18S uni F/-R) and a dual labelled probe (18S uni-P) are used for this purpose (Ioos et al., 2010).

3.2 Interpretation of results:

To assign results the following criteria should be followed:

Verification of the controls

- The PIC and PAC amplification curves should be exponential.
- NIC and NAC should give no amplification.
- IPC (e.g. eukaryotic 18S rDNA) should give an exponential amplification curve.

When these conditions are met

- A sample will be considered positive if it produces an exponential amplification curve.

- A test will be considered negative if it does not produce an amplification curve or if it produces a curve which is not exponential.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. PERFORMANCE CHARACTERISTICS AVAILABLE

Performance characteristics are provided in detail in Taddei et al. (2021).

4.1 Analytical sensitivity data

Using the TaqMan test, 1.0–2.0 pg of *A. glabripennis* larval DNA could be detected in 100% of the replicates (nine technical replicates (distributed on three plates) for each of the eight *A. glabripennis* samples tested).

Using the TaqMan test, 1.0–2.0 pg of *A. glabripennis* larval DNA could also be detected after spiking a 10^4 dilution of a 10–20 ng/uL DNA solution (measured with Nanodrop ND2000; Thermo Scientific) to wood shavings (as a matrix), meaning (i) that the protocol is almost as sensitive for *A. glabripennis*-DNA from frass as for larval DNA and (ii) that there is no inhibiting effect of woody compounds on *A. glabripennis*-DNA amplification following this approach. Wood shavings from *Acer* sp. and *Salix* sp. were used for this purpose.

4.2 Analytical specificity data

Analytical specificity was evaluated on a panel of target and non-target samples (larval/adult samples and, when available, frass material). The panel of non-target specimens consisted of Coleoptera and Lepidoptera specimens covering 21 xylophagous species (*Anoplophora chinensis* (6), *Aromia bungii* (8), *Aromia moschata* (1), *Morimus asper* (2), *Saperda carcharias* (1), *Saperda punctata* (1), *Aegomorphus* sp. (1), *Penichroa fasciata* (1), *Cerambyx scopolii* (1), *Stictoleptura cordigera* (1), *Rutpela maculata* (1), *Aegosoma scabricorne* (1), *Psacothea hilaris* (1), *Prionus coriarius* (1), *Xylotrechus* sp. (1), *Cossus cossus* (2), *Zeuzera pyrina* (2), *Synanthedon myopaeformis* (1), *Synanthedon spuleri* (1), *Paranthrene tabaniformis* (1), *Sesia* sp. (1)) which can share the same ecological niche as *A. glabripennis*, collected from host plants belonging to genera such as *Acer*, *Populus*, *Ulmus*, *Prunus*, *Betula* and *Malus*. The panel of target specimens consisted of 10 *A. glabripennis* specimens, covering the known genetic variability of the Italian outbreaks and the Magdeburg outbreak (Germany). All the DNA samples were tested in three technical replicates. No cross reactions were observed.

4.3 Data on repeatability

Eight biological replicates analysed in nine technical replicates by the same operator, on the same real-time thermocycler gave repeatable results.

4.4 Data on reproducibility available in Taddei et al., 2021

DOI: 10.1111/epp.12844

ERRATUM

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

This Diagnostic Standard (EPPO, 2021) was published in December 2021 in the *EPPO Bulletin*.

In Figure 12, the letters corresponding to the different species, as described in the figure legend, were moved by mistake during the publication process. This has been corrected in the article on Wiley Online Library, and the updated figure is shown below.

The publisher would like to apologize for this error.

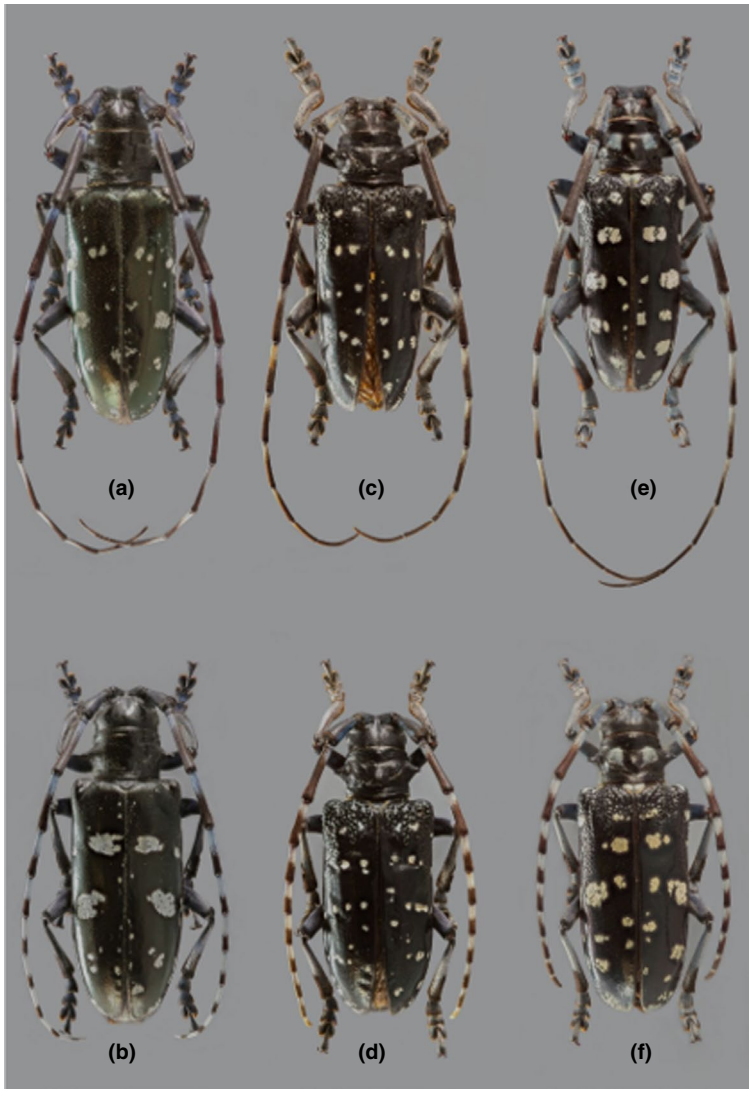

FIGURE 12 Habitus of adults: (a) ♂, (b) ♀ *Anoplophora glabripennis*, (c) ♂, (d) ♀ *A. chinensis*, (e) ♂, and (f) ♀ *A. chinensis* form *malasiaca*. (Courtesy: Bruno Serrate, editing by Laurent Soldati, INRAE Montpellier (FR).)

REFERENCE

EPPO (2021) EPPO Standard PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis*. *Bulletin OEPP/EPPO Bulletin* 51, 568–586.

DOI: 10.1111/epp.12906

CORRIGENDUM

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

Following the publication of Taddei et al., 2021 and the validation study performed by the EURL for insects and mites, the following changes to the Standard PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis* (EPPO, 2021) were proposed and approved by the EPPO Panel on Diagnostics in Entomology.

- **Section 4.1.2.2, subsection 'possible confusion with other congeneric species'.**

In the last bullet point in this subsection, the text below (in bold) and the Figure 18 should be added to illustrate the position of the H3 and H4 patches which are used to differentiate *A. chinensis* from *A. macularia*.

- *A. macularia* have elytral patches H3 and H4 fused in one large maculation (whereas in *A. chinensis* they are separated). **For the location of H3 and H4 on elytra, see Figure 18.**

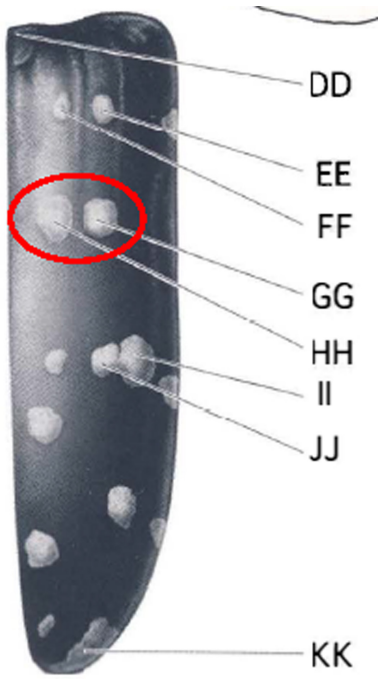

FIGURE 18 Patches pattern on the elytra of *Anoplophora*; HH = H3, GG = H4 (Lingafelter & Hoebeke (2002), modified)

- **Appendix 1**

The following explanatory text in bold should be added regarding the epipleurum (this should link to the word epipleurum in couplet 7 (in 7 and 7′) in the Key for the identification of *A. glabripennis* and *A. chinensis* late instars larvae (after Pennacchio et al., 2012)):

***It is not always easy to see if the epipleurum is protuberant or not. This will depend on the preservation mode used for the larvae: if a larva is placed in ethanol without boiling, it can shrink and the epipleurum can appear as protuberant even on other segments; on the contrary, if a larvae is boiled it can swell and the epipleurum can no longer look protuberant anymore. Therefore, this character should be carefully observed in the light of other characters, before a decision is made concerning how to proceed in the key.**

The simplified key for the identification of *A. glabripennis* and *A. chinensis* adult specimens within the *Anoplophora* genus should be modified as followed (modifications in bold):

1	**Antennae with conspicuous pubescent annulations on most antennomeres (Figures 12–14)**	**2**
1′	Antennae without conspicuous pubescent annulations	Other species
2	Antennae with distinct narrow annulation at base and apex of most antennomeres	Other species
2′	Antennae with annulations at basal fourth or more of most antennomeres (Figures 12–14)	3
3	Most of the body covered with dense, uniform blue-grey, blue-green or turquoise pubescence	Other species
3′	Most of the body not uniformly covered with pubescence of different shades of blue (Figure 12)	4
4	Elytra with **large** bands or spots of dense yellow pubescence	*A. horsfieldii*
4′	Elytra with pubescence otherwise. **If yellow, then in much smaller spots not forming partial or complete bands (Figure 19)**	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
5′	Pronotum with very weak or no mediolateral calli, anterior margin not highly elevated and without pronounced middle depression in front of posteromedial callus (Figure 13A–C)	6
6	Elytra with 4–7 complete or nearly complete transverse bands of pubescence	Other species
6′	Elytra with pubescent maculations in form of numerous irregularly sized spots on disk, most non forming bands (Figure 12)	7
7	Elytral base with numerous (10 or more) conspicuous granules (Figure 13B,C)	8
7′	Elytral base without granules (or at most 10) (Figure 13A)	11
8	Pubescent maculations on elytra poorly defined, fuzzy margined, numerous (about 30), variably sized and bicoloured	Other species
8′	Pubescent maculations on elytra less numerous, usually well defined; with distinct edges and unicolourous (white, yellow, light orange, light blue) (Figure 12)	9
9	Elytra with few, if any, erect or suberect, long black hairs; white, blue or translucent pubescence ventrally	10
9′	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 (Figure 13B,C); antennal annulation light blue or white	***A. chinensis*** (Figure 12C–F)
10′	Elytra with about 10 granules each	*A. viriantennatus*
11	Pronotum with conspicuous, dense pubescence dorsally	Other species
11′	Pronotum without dense pubescence (Figure 13A)	12
12	Antennomeres with a broad basal purple or deep blue pubescent annulation on at least basal two-thirds of most antennomeres	*A. coeruleoantennata*
12′	Antennomeres with white or pale blue pubescent annulation occupying no more than the basal half of **at least the first two** antennomeres **(after scape)** (Figure 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*
13′	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 (Figure 14A); maculations on elytra white or yellow (rarely pale orange)	***A. glabripennis*** (Figure 12A,B)

The new figure below should be added.

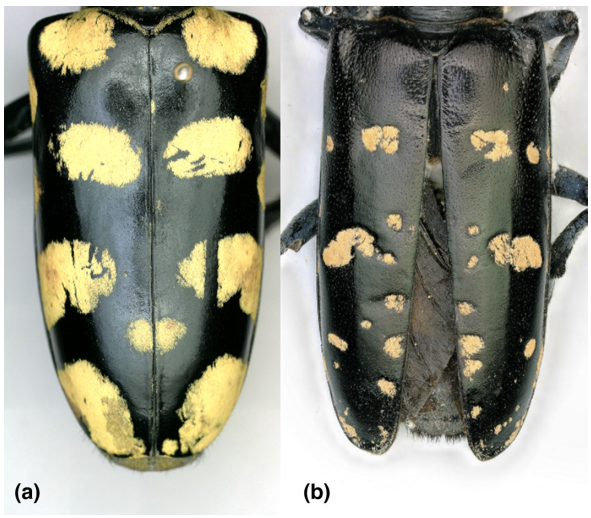

FIGURE 19 Elytra of (a) *A. horsfieldii* and (b) *A. glabripennis* form *nobilis*. Courtesy: Andrea Taddei (ANSES, FR)

- **Appendix 2**

In section 2.1.2 for the 6th bullet point the size of the new tube should be changed. The corrected bullet point is as follows (new text in bold).

- Collect 500 µL of the solution, leaving the debris at the bottom of the tube and transfer to a new 1.5 **mL** tube.

The validation data in this appendix should be updated as followed (new or modified text in bold):

4.1 Analytical sensitivity data

Using the TaqMan test, 1.0 to 2.0 pg of *A. glabripennis* DNA could be detected in 100% of the replicates (9 technical replicates (distributed on 3 plates) for each of the 8 *A. glabripennis* sample tested).

***Anoplophora glabripennis* DNA from frass samples (*Acer saccharinum* and *Aesculus hippocastanum*) could be detected in all replicates until the 100-fold dilution of the initial DNA solution, and in some replicates at the 1000-fold dilution. However, it was not possible to determine the initial concentration of the target organism's DNA in frass samples because the solution contained also plant DNA and, potentially, DNA from other organisms that can colonize the wood shavings.**

4.2 Analytical specificity data

Analytical specificity was evaluated on a panel of target and non-target samples (larval/adult samples and, when available, frass material). The panel of non-target specimens consisted of Coleoptera and Lepidoptera specimens covering **20** xylophagous species (*Anoplophora chinensis* (6), *Aromia bungii* (8), *Aromia moschata* (1), *Morimus asper* (2), *Saperda carcharias* (1), *Saperda punctata* (1), *Aegomorphus* sp. (1), *Penichroa fasciata* (1), *Cerambyx scopolii* (1), *Stictoleptura cordigera* (1), *Rutpela maculata* (1), *Aegosoma scabricorne* (1), *Psacothea hilaris* (1), *Prionus coriarius* (1), *Xylotrechus* sp. (1), *Cossus cossus* (2), *Zeuzera pyrina* (2), *Synanthedon myopaeformis* (1), *Synanthedon spuleri* (1), *Paranthrene tabaniformis* (1)) which can share the same ecological niche as *Anoplophora glabripennis*, collected from host plants belonging to genera such as *Acer*, *Populus*, *Ulmus*, *Prunus*, *Betula* and *Malus*. The panel of target specimens consisted of **16** *Anoplophora glabripennis* specimens, covering the known genetic variability of the Italian outbreaks and the Magdeburg outbreak (Germany). All the DNA samples were tested in 3 technical replicates. No cross reactions were observed.

REFERENCES

Taddei A, Becker M, Berger B, Da Lio D, Feltgen S, König S, Hoppe B & Rizzo D (2021) Hoppe B & Rizzo D (2021) Molecular identification of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and detection from frass samples based on real-time quantitative PCR. *Journal of Plant Diseases and Protection*. https://doi.org/10.1007/s41348-021-00501-7.

EPPO (2021) PM 7/149 (1) *Anoplophora glabripennis* and *Anoplophora chinensis*. *EPPO Bulletin*, 51: 568–586. https://doi.org/10.1111/epp.12797