

EPPO STANDARD ON DIAGNOSTICS

PM 7/154 (1) *Agrilus planipennis*

Specific scope: This Standard describes a diagnostic protocol for *Agrilus planipennis*.

This Standard should be used in conjunction with PM 7/76 Use of EPPO diagnostic protocols¹

Specific approval and amendment: First approved in 2023–04.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

The emerald ash borer, *Agrilus planipennis* Fairmaire, 1888 has recently established in the EPPO region and represents a threat to ash forest stands in many European countries. The native distribution range of *A. planipennis* includes East Asia, more specifically China, the Russian Far East, the Korean peninsula and under the assumption that *A. marcopoli ulmi* is synonymous to *A. planipennis*, also parts of Japan (EFSA, 2020; Orlova-Bienkowskaja & Volkovitsh, 2018). Following its recent introduction and detection in 2002 in the United States, *A. planipennis* has quickly established and rapidly spread across the eastern states of the USA and in some of the Canadian provinces. The emerald ash borer was first detected within the EPPO region in the European part of Russia in 2003, initially in Moscow (Orlova-Bienkowskaja et al., 2020; Volkovitsh & Mozolevskaya, 2014). This was then followed by detections in Eastern Ukraine in 2019 (Drovalenko et al., 2019) and also in St Petersburg (Russia) in 2020 (Volkovitsh & Suslov, 2020). Depending on latitude and local temperatures, adult emergence usually begins in May or June, peaks in late May to early July, and adult activity can persist into September. Females lay eggs individually onto or in the crevices of tree bark. Eggs hatch within 2 weeks and larvae chew their way through the outer bark and develop in the nutritious layer between the phloem and the cambium.

In general, *A. planipennis* overwinters as pre-pupa, but in colder regions where development is slower, individuals may overwinter as early instar larvae in the first year and as prepupae in the second year. Emerging adults chew their way through the bark and start a new generation later in the summer. Adults of both sexes are

able to fly over long distances especially in the spring and summer months when searching for mates and host plants. Especially when local hosts and mates are abundant, *A. planipennis* tend to attack trees in the vicinity of their original host and may colonize host trees in groups (EFSA, 2020). Passive dispersal takes place by the transportation of infested timber and firewood.

While primary host plants of the emerald ash borer are *Fraxinus* species, such as *F. mandshurica* in its native range in Asia, other *Fraxinus* species have been attacked in the US. European congeners such as *F. excelsior*, *F. angustifolia*, and *F. ornus* are all suitable hosts with a distribution across the entire European region (Baranchikov et al., 2014). Moreover, native *Fraxinus* stands are often interspersed with North American *Fraxinus* species across North – Eastern European cities, which may facilitate spread and transfer to novel hosts (see also EFSA, 2020; EPPO, 2013). In addition to the preferred *Fraxinus* species, the white fringe tree (*Chionanthus virginicus*), native to the US and an exotic ornamental in the EU, is also noted as a suitable albeit suboptimal host plant. *Olea europaea* has been found to be susceptible but in laboratory trials only (Cipollini et al., 2017). Although *Juglans mandshurica* var. *sieboldiana* as well as *Ulmus davidiana* var. *japonica*, and *Pterocarya rhoifolia* are indicated as hosts for *A. marcopoli ulmi* (Akiyama & Ohmomo, 1997 – see Chamorro et al., 2015; Jendek & Grebennikov, 2011), there is no evidence of successful larval development on these plants so far. Further details on the biology of *A. planipennis* are available in the EPPO Datasheet (EPPO, 2022).

Agrilus is regarded to be the most speciose genera in the animal kingdom with more than 3000 recorded species (Kelnarova et al., 2019). According to Jendek (2016), 87 species are reported in Europe: identification of *Agrilus* species thus is difficult and confirmation by a specialist is highly recommended in case of first identification (see Section 8). A flow diagram describing the diagnostic procedure for *Agrilus planipennis* is presented in Figure 1.

2 | IDENTITY

Name: *Agrilus planipennis* Fairmaire, 1888

¹Use 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.

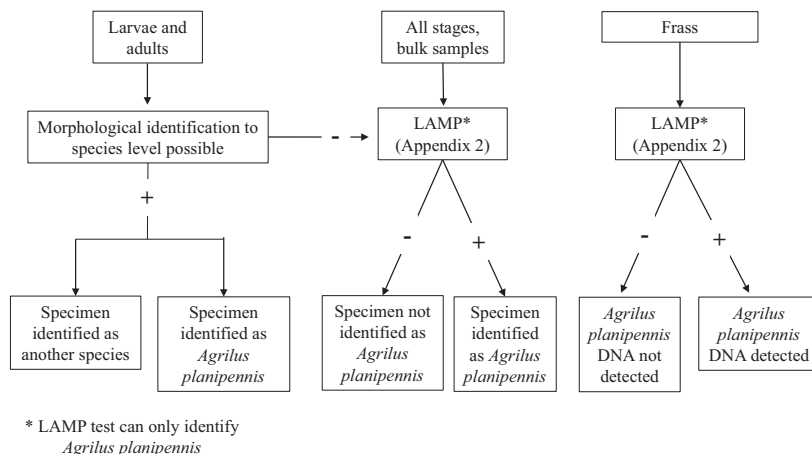


FIGURE 1 Diagnostic procedure for *Agrilus planipennis*. This flow diagram is intended to provide an overview of the diagnostic process and may not cover all possible scenarios.

Other scientific names: *Agrilus marcopoli* Obenberger, 1930; *Agrilus feretrius* Obenberger, 1936; *Agrilus marcopoli ulmi* Kurosawa, 1956

Taxonomic position: Insecta, Coleoptera, Buprestidae

EPPO Code: AGRPL

Phytosanitary categorization: EPPO A2 (2009); EU A1 Quarantine pest (Annex II A)

3 | DETECTION

Different methods are available to detect emerald ash borer such as the use of traps, girdled ‘sentinel’ trees² and molecular tests.

3.1 | Trapping and girdled ‘sentinel’ trees

An overview of the efficiency of trap designs is listed in EFSA (2020). Sticky panel traps come in different designs from simple panels to prism traps and to double decker traps (EPPO, 2013; McCullough & Poland, 2017; Poland et al., 2019). Purple traps are more attractive to females whereas green traps are more attractive to males (EFSA, 2020; EPPO, 2013). Multi-funnel and green prism traps are better placed high in the canopy, whereas purple sticky traps and double decker traps (affixed on a drainpipe) are more effective when placed lower, at the height of trunks. Organic solvents (e.g. citrus oil) can be used to remove the glue from specimens before morphological identification or molecular tests. In addition to sticky traps, multi-funnel traps (most frequently green) are often used, with propylene glycol as a killing/preservative. Different infochemical lures may be added to traps, such as manuka or phoebe oil (EPPO, 2013), or the green – leaf – volatile Z-3-hexenol



FIGURE 2 Adult of emerald ash borer (Courtesy AV Kovalev).

alone or in combination with (3Z)-lactone (Parker et al., 2020).

Since weakened trees emit volatiles which are especially attractive for ovipositing females, girdled ‘sentinel trees’ in combination with purple sticky traps, may be used to detect *A. planipennis* adults (EFSA, 2020; EPPO, 2013), although 3-flat sided purple plastic prism sticky traps baited with a lure were found to be more efficient (EPPO, 2013).

Trapping should be performed during periods when adults (Figure 2) are active.

3.2 | Detection in plants

3.2.1 | Period for detection

Symptoms of damage on trees can be detected throughout the year. Inspection for the detection of

²An EPPO Standard PM 3/91(1) Sentinel woody plants: concepts and application has been approved in 2020.

larvae may be carried out from late summer until mid-autumn and from late autumn and throughout the winter months for pre-pupae. In the colder areas larval development lasts 2 years and larvae can be found throughout the year.

3.2.2 | Signs of presence

Early infestations of emerald ash borer are difficult to detect as symptoms may take years to become visible. Larvae (Figure 3) make extensive serpentine tunnels (galleries) under the bark (Figure 4) which consequently leads to the death of phloem and cambium tissues directly around and above the affected areas. As a result, large patches of loosened bark may be observed. In case of extensive damage, the tunnelling may expand around the circumference of branches and even the trunk leading to girdling and killing of branches, limbs and eventually the entire tree (Figure 5). Early symptoms include the die-back of branches, reduced growth and discoloration (lighter green) of leaves. The growth of new epicormic shoots below attacked parts of the trunk (Figure 6) or signs of woodpecker activity is also typical (Figure 7). When preparing to overwinter, prepupae assume a J-shaped posture. For identification of *A. planipennis* with a high level of



FIGURE 3 Larvae of the emerald ash borer with the characteristic trapezoidal abdominal segments (Courtesy: Pennsylvania Department of Conservation and Natural Resources – Forestry Archive, Bugwood.org).



FIGURE 4 Galleries of the larvae (Courtesy: Mark Volkovitch).



FIGURE 5 Dieback of ash caused by *Agrilus planipennis* (Courtesy: Mark Volkovitch).

certainly the distinguishing morphological features of larvae (see Section 4.1.1) should be used. The exit holes made by adults of all *Agrilus* species have D-shaped cross-sections (Figure 8) although the holes made by *A. planipennis* may be much larger (3–4 mm wide) than those made by native European *Agrilus* species attacking *Fraxinus*. Eggs are light brown, elliptical in shape and 1 mm long (Figure 9) and are laid by females into bark cracks.

3.2.3 | Molecular detection

3.2.3.1 | Emerald ash borer -LAMP test

A rapid Loop-mediated isothermal Amplification test (LAMP) was recently developed to target a diagnostic



FIGURE 6 Epicormic shoots (Courtesy: Gernot Hoch).



FIGURE 8 Exit hole of the emerald ash borer (Courtesy: Mark Volkovitsh).



FIGURE 7 Woodpecker activity (photo by E.G. Mozolevskaya, after Volkovitsh & Mozolevskaya, 2014, with permission).

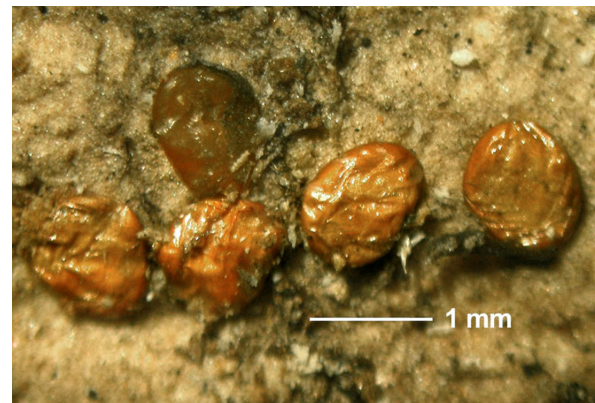


FIGURE 9 Egg cluster (Courtesy: David Cappaert, Bugwood.org).

region of the *COI* gene in *A. planipennis* and was evaluated on all life stages, on frass collected from host *Fraxinus* trees, and on pooled samples from traps containing multiple species (Kyei-Poku et al., 2020). Emerald ash borer -LAMP primers were developed against an in silico molecular dataset containing *Agrilus COI* barcodes from North America, Europe, and Asia. Several European species that feed on *Fraxinus* were not included in this dataset, and the emerald ash borer -LAMP test has thus far only been validated using North American *Agrilus* species

and two *Dendroctonus* species commonly caught in traps with *A. planipennis* in this region. A more recent study by Peterson et al. (2023) including several European *Agrilus* spp. shows that this LAMP test can positively differentiate *A. planipennis* from the species evaluated. The emerald ash borer -LAMP protocol is described in [Appendix 2](#).

3.2.3.2 | Test sample requirements for molecular detection

Collected samples should be frozen at -20°C and/or preserved in 90–100% ethanol to reduce DNA degradation prior to extraction. If this is not possible, upon collection, samples should be kept dry and preserved as soon as possible; excessive sunlight and wet or humid conditions will reduce DNA

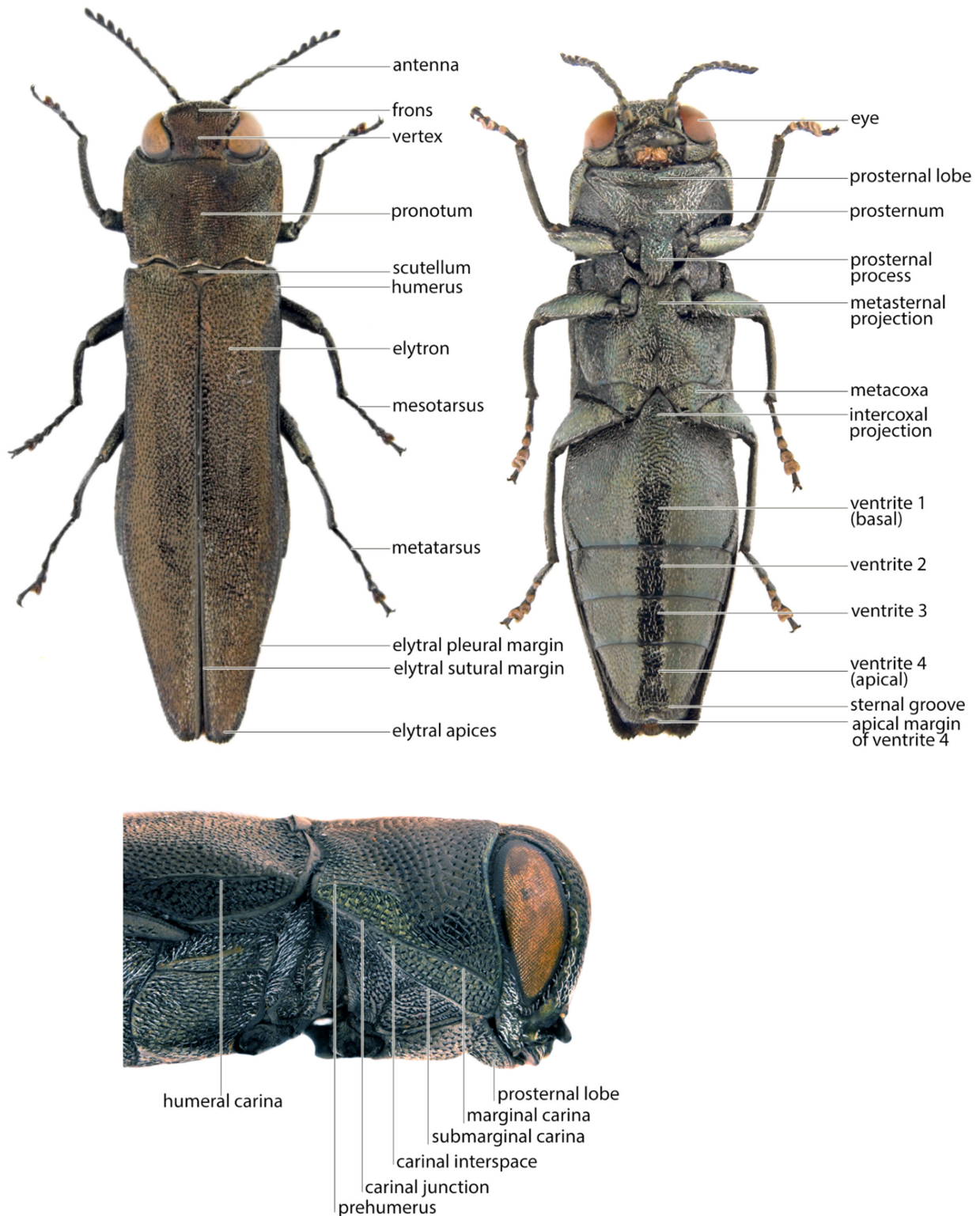


FIGURE 10 Morphological characters of an *Agrilus* adult after Jendek and Grebennikov (2011) (Courtesy: E. Jendek). Note (MV): Ventrite 1 (basal) is actually fused ventrites 1 and 2, while ventrite 4 (apical) corresponds to ventrite 5.

quality. Kyei-Poku et al. (2020) found that the glue from sticky traps did not noticeably impact DNA quality or cross-react with reagents under the conditions of the study.

4 | IDENTIFICATION

Morphological identification is possible on larvae and adults. Molecular tests can be used for all stages.

4.1 | Morphological identification

4.1.1 | Morphological identification of the larva

A key to the genus level for larvae is provided in [Appendix 1](#) (Table A1). The key can be used on live or preserved specimens. A table with distinctive characteristics for the most similar-looking larger *Agrilus* species and species found on *Fraxinus* spp. is provided in [Appendix 1](#), Table A2. The characteristics which are similar to or the same as *A. planipennis* are highlighted in bold.

4.1.1.1 | Larvae description

Chamorro et al. (2012, 2015) describe the larvae of *A. planipennis*. The body of the larva is yellowish white, can be up to 36mm long ([Appendix 1](#), [Figure 35a](#)). The most important characteristic of the larvae of *A. planipennis* is the presence of the bell-shaped abdominal segments. The pronotum has a groove which is posteriorly bifurcated. The terminal processes ([Appendix 1](#), [Figure 34](#)) are narrow, cylindrical, and bearing 1–3 ledges appearing from the first instar, and 2 to 3 internal excretion ducts. The posterior contour of the microsetal area on prementum is zigzag-shaped. The distance between the anterior margin of the prementum and posterior border of the microsetal area is equal to approximately 2/5 of the distance from anterior margin to the bases of the apical setae of corner sclerites of labium ([Appendix 1](#), Table A3, 1). Anterior margin of the labrum is glabrous ([Appendix 1](#), [Figure 36a](#)). The microspinulae on the mala and internal surface of the maxillary stipes and cardo are concentrated subapically (see Chamorro et al., 2012, [Figure 8f, g](#)).

4.1.1.2 | Possible confusion with other buprestid larvae

A key for the identification of larvae of Buprestid beetles to the *Agrilus* genus level for Central Europe on basis of Alexeev (1981), Bily (1999) and Volkovitsh et al. (2020a) is provided in [Appendix 1](#) (Table A1). The larvae of the genus *Agrilus* are very similar. Five other species may also occur in *Fraxinus* spp.: *A. convexicollis*, *A. beauprei*, *A. graminis*, *A. cyanescens*, and *A. roscidus* (Jendek & Poláková, 2014). However, for *A. graminis* and *A. cyanescens* the main hosts are respectively *Quercus* and *Lonicera*, while *A. roscidus* is an extremely polyphagous species, and the records on *Fraxinus* need confirmation.

In [Appendix 1](#) (Table A2) there is a characteristics table for larvae of all above species adapted from Alexeev (1981) and Volkovitsh et al. (2020a) which includes also the species reported from *Fraxinus* spp. The main diagnostic character of *A. planipennis* larva is the bell-shaped abdominal segments 1–7 (Chamorro et al., 2012; Volkovitsh et al., 2020a) in comparison to all European *Agrilus* species (Volkovitsh et al., 2020a). Among the species which may attack *Fraxinus* trees,

A. convexicollis, *A. graminis*, *A. cyanescens*, and *A. roscidus* are much smaller than *A. planipennis* and have a different abdominal segment shape (Volkovitsh et al., 2020a). The larvae of *Agrilus beauprei* is not yet described. However, it is expected to be similar to *A. convexicollis* as they belong to the same subgenus. For confirmation the molecular method described in [Appendix 2](#) can be used.

4.1.2 | Morphological identification of the adult

The determination from family to genus level can be done with Schaefer (1950) (France), Richter and Alexeev (1965) (European part of the USSR), Cobos (1986) (Iberian Peninsula) and Curletti (1994) (Italy). A table with the characteristics of *Agrilus planipennis* and larger *Agrilus* species and species attacking *Fraxinus* is provided in [Appendix 1](#) (Table A4). The characteristics in other species which are similar to or the same as *A. planipennis* are highlighted in bold. Identification to species level is possible based on morphological characters (Chamorro et al., 2015; Jendek & Grebennikov, 2011; Volkovitsh et al., 2020a). A description of the genitalia is available in Jendek and Grebennikov (2011) (page 301) and Chamorro et al. (2015) (page 101).

Morphological features used for identification are presented in [Figure 10](#).

4.1.2.1 | *Agrilus planipennis* adult description

Most of the characteristics are described in detail by Volkovitsh et al. (2020a):

Body ([Appendix 1](#), [Figure 37a](#)): minimum body size is 7.5mm, but it is usually around 12 to 15mm. The body shape is typical of the genus *Agrilus* and is elongate. The colour of the body is metallic, uni- or bicolourous. It has an emerald colour, but the pronotum, head and abdomen are frequently coppery, and the entire beetle is rarely completely violet-blue or green.

Head ([Appendix 1](#), [Figures 38a, 39a](#)): the frons and vertex have a deep longitudinal medial impression, which is clearly visible from above. The vertex from above is about 1.3–1.5 times the transverse diameter of the eye and bearing concentric punctate striae ([Figure 38a](#)).

Pronotum ([Appendix 1](#), [Figures 39a, 40a](#)): the position of the widest point is variable but always in the basal half of the pronotum. The sides are arcuate and converge toward the anterior corners of the disc which has deep medial impressions. The pronotum is covered with strongly curved transverse rugosities. *Agrilus planipennis* has on the pronotum marginal and submarginal carinae which are convergent, and the interspaces are broadest medially ([Appendix 1](#), [Figure 39a](#)). The prehumeral are poorly marked, arcuate, and extending to posterior third of pronotal length.

Elytra (Appendix 1, Figure 37a): the elytra are covered with very short, inconspicuous dark hairs, and poorly visible groups of light, scale-like setae at the beginning of their posterior third but lack distinct tomentose spots. The apices are arcuate with finely denticulate margins.

Legs: the hind tibia has a sinuate posterior margin, and occasionally 1–2 triangular projections.

Abdomen: abdominal ventrites without distinct tomentose spots. The upper side of the abdomen is copper-red. Pygidium bearing apical process extending beyond elytral tips (Appendix 1, Figure 37b).

4.1.2.2 | Possible confusion with adults of other native Buprestidae

Agrilus spp. adults are very characteristic in comparison to other European buprestid species. As mentioned above, the genus is the largest in the animal kingdom (Kelnarova et al., 2019). In Europe 87 species of *Agrilus* occur (Jendek, 2016). Volkovitsh et al. (2020a) present the first illustrated key for the recognition of *Agrilus planipennis* compared to other European *Agrilus* species. The species which are similar to *Agrilus planipennis* are presented in a table describing their characteristics (Appendix 1, Table A4). Most adults of the European *Agrilus* species are much smaller than *A. planipennis* (mostly 12–15 mm). The only species which are similarly large are: *Agrilus ater*, *A. biguttatus*, *A. guerini*, *A. mendax*, *A. sinuatus*, *A. subauratus*, *A. suvorovi* and *A. viridis*. The main characteristics for the identification of *A. planipennis* are the deep impression on the head, copper red upperside of the abdomen (tergites), pronotal sides arcuately converging toward anterior corners and disc with deep anterior and posterior medial impressions, elytra mostly brightly emerald, without distinct tomentose spots, and the pygidium bearing apical process. See Appendix 1 for a characteristic table with the above-mentioned species adapted from Volkovitsh et al. (2020a).

4.2 | Molecular identification of all life stages

The LAMP test from Kyei-Poku et al. (2020) described in Appendix 2 can be used for the identification of all life stages of *A. planipennis*. Kyei-Poku et al. (2020) evaluated the test for the identification of all life stages of *A. planipennis* against a background of US *Agrilus* spp. An additional evaluation was performed by Peterson et al. (2023) on DNA extracted from single specimens of European *Agrilus* species as well as Scolytinae and Cerambycidae and could positively differentiate *A. planipennis* from European *Agrilus* spp.

A barcoding protocol is described in Kelnarova et al. (2019). Although these tests might provide some

useful information on the identity of *Agrilus* species, the authors of the present protocol do not consider that it should be recommended due to the lack of assessment of inclusivity and exclusivity.

5 | REFERENCE MATERIAL

Reference material is available from the EURL for Insects and Mites reference collection 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:

- M. de Groot, Department of Forest Protection, Slovenian Forestry Institute (SI)
- E.O. Campbell, Canadian Food Inspection Agency, Government of Canada (CA)
- T. Bukovinszky, Netherlands Food and Consumer product Safety authority Ministry of Agriculture, Nature and Food quality (NL)
- M.G. Volkovitsh, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

9 | FEEDBACK ON THIS DIAGNOSTIC PROTOCOL

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this

protocol 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 protocols. Protocols 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.

ACKNOWLEDGEMENTS

This protocol was originally drafted by:

- M. de Groot, Department of Forest Protection, Slovenian Forestry Institute (SI)
- E. O. Campbell, Canadian Food Inspection Agency, Government of Canada (CA)
- T. Bukovinszky, Netherlands Food and Consumer product Safety authority Ministry of Agriculture, Nature and Food quality (NL)
- M. G. Volkovitsh, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

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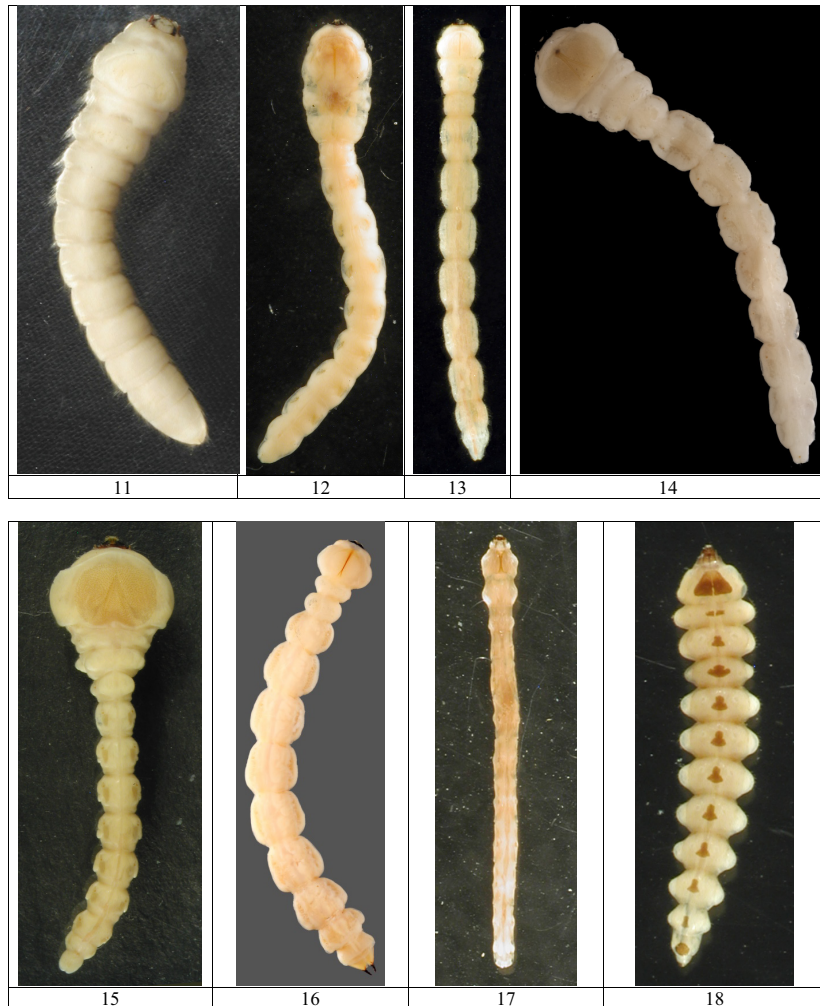
How to cite this article: EPPO (2023) PM 7/154(1) *Agrilus planipennis*. *EPPO Bulletin*, 53, 285–308. Available from: <https://doi.org/10.1111/epp.12926>

APPENDIX 1 - KEYS AND DIAGNOSTIC TABLES TO LARVAL AND ADULT STAGES BASED ON MORPHOLOGY

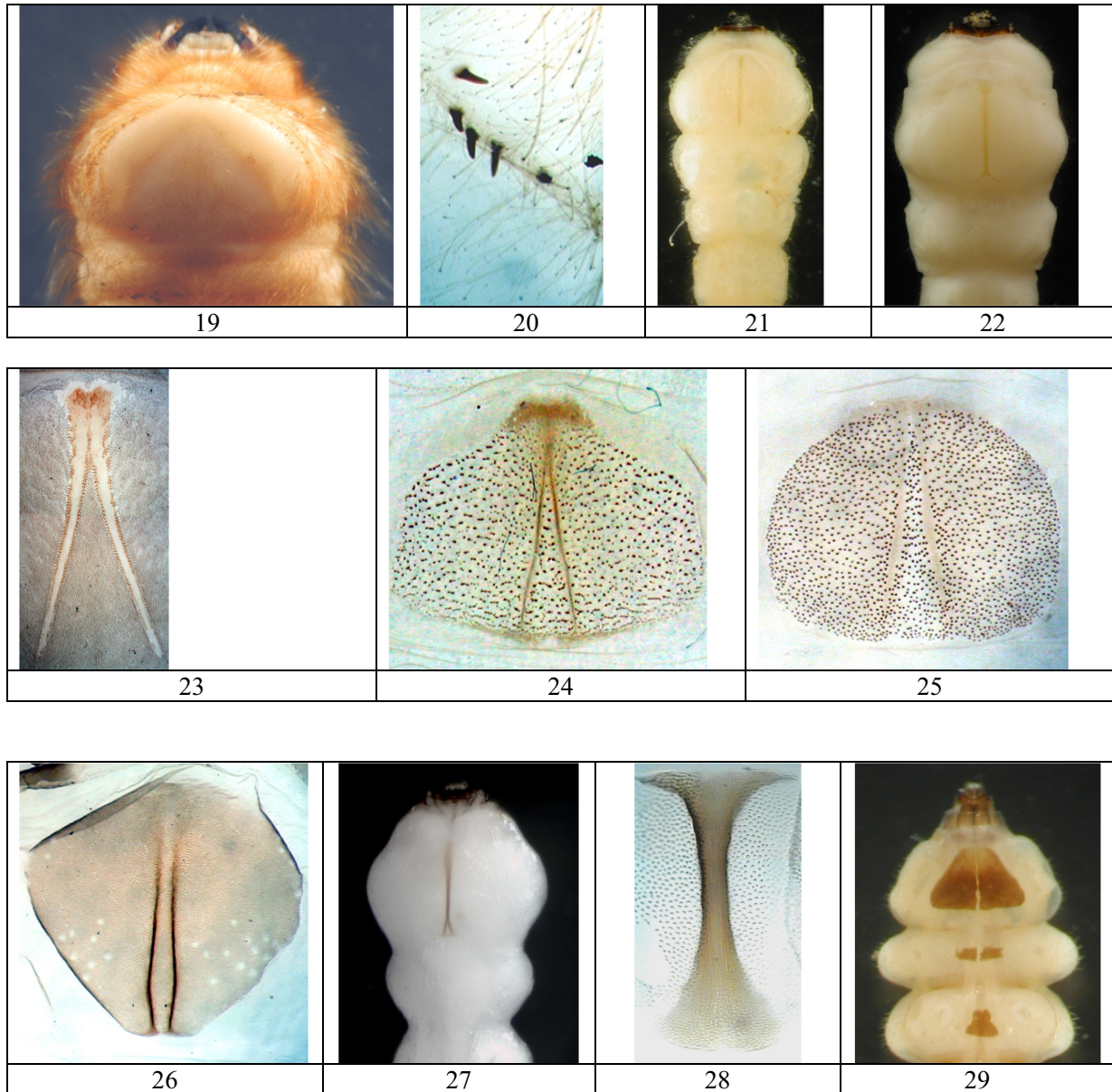
Keys for larvae of the subfamily Agrilinae and genus *Agrilus*.

TABLE A1 Key to distinguish the larvae of Agrilinae and genus *Agrilus* from other European Buprestids larvae.

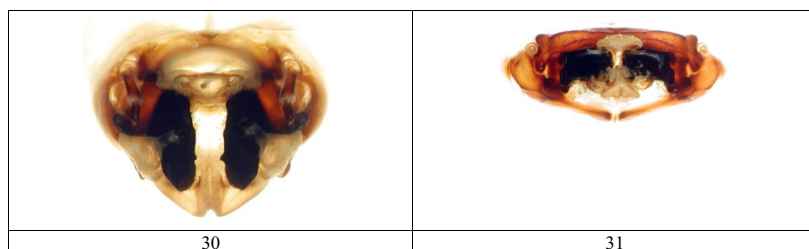
1.	Body covered with short or indistinct pubescence; prothorax usually much wider than other thoracic segments and, except for some Agrilinae, abdominal segments (Figures 12–15); thorax without sclerotized claw-like spines; prothoracic plates with more or less sclerotized medial grooves (Figures 21–29); mandibles without vertical lamina (Figure 31); development within plant tissues	2
	Body covered with long pubescence; thoracic segments only moderately expanded (Figure 11), bearing sclerotized claw-like spines along the periphery (Figure 20); prothoracic plates without medial grooves (Figure 19); mandibles with wide, shovel-shaped, vertical lamina (Figure 30); development in soil	JULODINAE
2.	Last abdominal segment without sclerotized terminal processes (Figures 12–15, 17, 18)	3
	Last abdominal segment with paired, sclerotized, 2- or 3-ledged terminal processes (Figures 16, 32–34)	6
3.	Pronotal medial groove singular, undivided or bifurcated distally; pronotal plates glabrous, covered with microstructures or bearing sclerotized plates (Figures 21, 22, 28, 29)	4
	Pronotal medial groove inverted “Y”- or “V”-shaped; prothoracic plates glabrous, covered with microteeth and/or sclerotized asperities (Figures 23–25)	CHRYSOCHROINAE/ BUPRESTINAE
4.	Mature larvae usually more than 10 mm; prothorax distinctly wider than other thoracic segments; body without sclerotized plates or without unsclerotized paired knolls (apical tubercles) on the last abdominal segment (Figures 12–14); xylophagous, more rarely herbaceous stem borers except Gramineae, Juncaceae and Cyperaceae	POLYCESTINAE/ GALBELLINAE
	Mature larvae <10 mm; prothorax as wide as, equal to or more narrow than other thoracic segments; body with sclerotized plates or with unsclerotized knolls (apical tubercles) on the last abdominal segment (Figures 17, 18); stalk borers on Gramineae, Juncaceae and Cyperaceae or leaf-miners	5
5.	Body long, slender, last abdominal segment bearing paired unsclerotized knolls; entire body without sclerotized plates; abdominal segments not expanded, more narrow than prothorax (Figure 17); stalk borers	AGRILINAE (Aphanisticini)
	Body short, robust; last abdominal segment without paired unsclerotized knolls; entire body or only prothorax with sclerotized plates; abdominal segments expanded, as wide as or wider than prothorax (Figure 18); leaf-miners	AGRILINAE (Tracheini)
6.	Pronotal plate with singular groove, sometimes bifurcated distally (Figures 16, 27)	7
	Pronotal plate with double, subparallel or inverted “Y”-shaped, groove (Figure 26)	AGRILINAE (Coraebini, part)
7.	Terminal processes of mature larvae 2-3-ledged (Figures 33, 34)	AGRILINAE (Agrilini: <i>Agrilus</i>)
	Terminal processes of mature larvae 1-ledged (Figure 32)	AGRILINAE (Coraebini, some <i>Meliboeus</i> [= <i>Nalanda</i>] spp.)



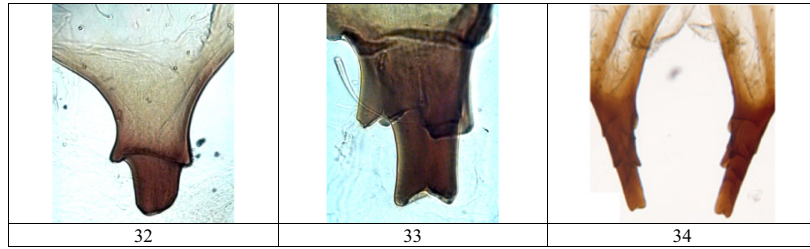
FIGURES 11–18 Larvae of different subfamilies and tribes of Buprestidae: 11 – Julodinae (*Julodis*), 12 – Polycestinae (*Ptosima*), 13 – Galbellinae (*Galbella*), 14 – Chrysochroinae (*Lamprodila* (*Palmar*)), 15 – Buprestinae (*Belionota*), 16–18 – Agrilinae: 16 – Agrilini (*Agrilus*), 17 – Aphanisticini (*Aphanisticus*), 18 – Tracheini (*Trachys*) 11 – after Bilý et al. (2013); 14 – after Volkovitsh et al. (2020b); 16 – after Volkovitsh et al. (2020a); 12, 13, 15, 17, 18 – M. Volkovitsh, original.



FIGURES 19–29 19, 21–29 – Shape of prothoracic plates and pronotal grooves; 20 – claw-like spines of prothorax. 19, 20 – Julodinae (*Julodis*), 21 – Galbellinae (*Galbella*), 22 – Polycestinae (*Polycetis*), 23 – Chalcophorinae (*Lamprodila*), 24 – Chalcophorinae (*Chalcophora*), 25 – Buprestinae (*Trachypterus*), 26–29 – Agrilinae: 26 – Coraebini (*Coraebus*), 27 – Agrilini (*Agrilus*: *A. planipennis*), 28 – Aphanisticini (*Aphanisticus*), 29 – Tracheini (*Trachys*). 19 – after Bílý et al. (2013); 23 – after Volkovitsh et al. (2020b); 20–22, 24–29 – M. Volkovitsh, original.



FIGURES 30, 31 Mouth parts, frontal view: 30 – Julodinae (*Julodis*), 31 – Buprestinae (*Belionota*). 30 – after Bílý et al. (2013); 31 – M. Volkovitsh, original.



FIGURES 32–34 Abdominal terminal processes of Agrilinae: 32 – Coraebini (*Meliboeus*); 33–34 – Agrilini (*Agrilus*): 33 – *A. biguttatus*, 34 – *A. planipennis*. 32, 33 – M. Volkovitsh, original; 34 – after Chamorro et al. (2012).

TABLE A2 Diagnostic table for larvae of *Agrilus* on basis of Alexeev (1981), Bily (1999) and Volkovitch et al. (2020a). The bold text highlights the characteristics that are common between *A. planipennis* and other European *Agrilus* species. The underlined characteristic is unique to *A. planipennis*. *Agrilus beaupret* was omitted from the table because data are not available.

	<i>(Uragrilus)</i>	<i>(Anambus)</i>	<i>(Uragrilus)</i>	<i>(Uragrilus)</i>	<i>(Sinuatiagrilus)</i>	<i>(Sinuatiagrilus)</i>	<i>(Robertus)</i>	<i>(Agrilus)</i>	<i>(Convexagrilus)</i>	<i>(Quercuagrilus)</i>	<i>(Dentagrilus)</i>	<i>(Rosagrilus)</i>
Species group ^b	<i>Cyanconiger</i>	<i>Biguttatus</i>	<i>Spinipennis</i>	<i>Spinipennis</i>	<i>Sinuatus</i>	<i>Sinuatus</i>	Unassigned	<i>Viridis</i>	Lacking	<i>Sulcicollis</i>	<i>Cyanescens</i>	<i>Roscidus</i>
Species	<i>Planipennis</i>	<i>Biguttatus</i>	<i>Guarini</i>	<i>Guarini</i>	<i>Mendax</i>	<i>Mendax</i>	<i>Subauratus</i>	<i>Sovorovivridis</i>	<i>Convexicollis</i>	<i>Graminis</i> ^a	<i>Cyanescens</i> ^a	<i>Roscidus</i>
Larva: abdominal segments 1–7	Bell-shaped, posterolateral angles produced laterad (Figure 35a)	Subquadrate or elongate, with arcuate or subparallel sides (Figure 35b)	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides	Subquadrate or elongate, with arcuate or subparallel sides
Larva: pronotal groove	Bifurcated (Figure 35a)	Bifurcated (Figure 35b)	Bifurcated	Bifurcated	Bifurcated	Entire but expanded and sclerotized distally	Bifurcated	Entire	Entire	Entire	Entire	Entire
Labrum: anterior margin	Glabrous (Figure 36a)	Setose	Setose (Figure 36b)	Triangular (Table A3, 3)	Zigzag shaped (Table A3, 2)	Zigzag shaped (Table A3, 5)	Nearly straight (Table A3, 7)	Triangular (Table A3, 8)	Prementum glabrous (Table A3, 9)	Glabrous	Setose	Glabrous
Prementum: posterior contour of microsetal area along anterior margin (Table A3)	Zigzag shaped (Table A3, 1)	Zigzag shaped (Table A3, 2)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)	Separated medially by glabrous space, with arcuate posterior margin (Table A3, 4)
Host plants	Certain species of Oleaceae: <i>Fraxinus</i>, <i>Chionanthus</i> (US)	<i>Castanea</i> , <i>Fagus</i> , <i>Quercus</i> , <i>Tilia</i> , <i>Populus/Ulmus</i> ?	<i>Populus</i> , <i>Salix</i>	<i>Populus</i> , <i>Salix</i>	<i>Crataegus</i> , <i>Cydonia</i> , <i>Malus</i> , <i>Mespilus</i> , <i>Prunus</i> , <i>Sorbus</i>	<i>Sorbus</i>	<i>Populus</i> , <i>Salix</i>	<i>Populus</i>	<i>Fraxinus</i> , <i>Ligustrum</i> , <i>Syringa</i>	<i>Quercus</i> (possibly <i>Fraxinus</i>)	<i>Lonicera</i> (possibly <i>Fraxinus</i>)	Polyphagous (24 plant families) (possibly <i>Fraxinus</i>)
Size (mature larva)	30–36mm	25–28mm	19–25mm	21–24mm	25–28mm	17–22mm	14–22mm	7–10mm	13–16mm	14–17mm	14–17mm	14–17mm

^aSubgenera after Alexeev (1998).

^bSpecies groups after Jendek & Grebennikov (2011).

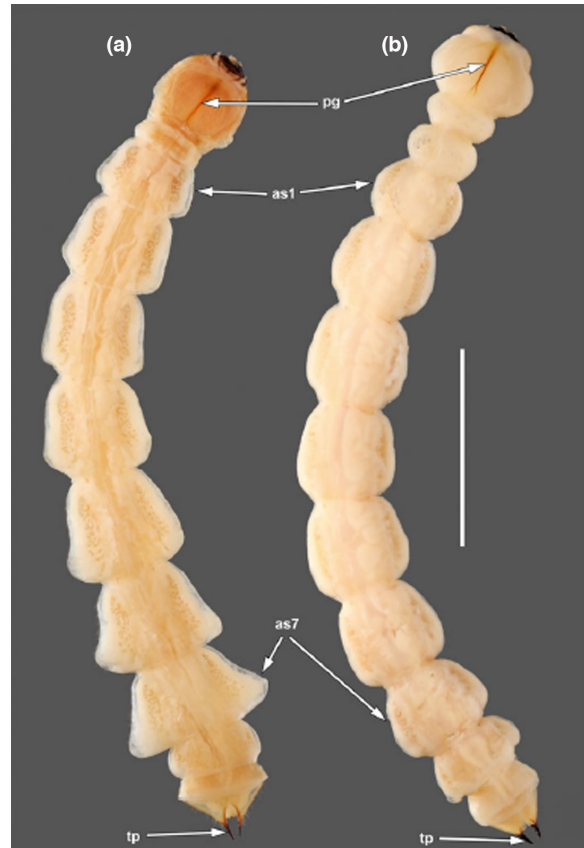


FIGURE 35 *Agrilus planipennis* (a) and *Agrilus biguttatus* (b). as1, as7 – abdominal segments 1 and 7, pg – pronotal groove, tp – terminal process. (after Volkovitsh et al., 2020a).

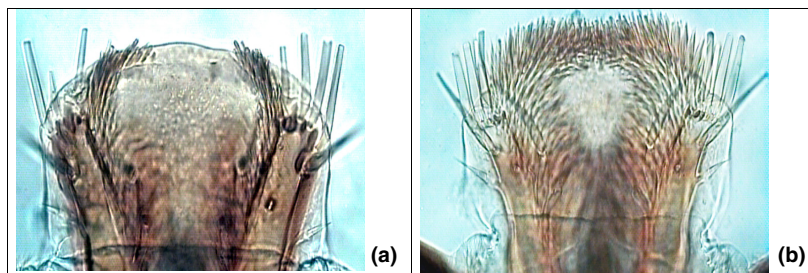


FIGURE 36 (a, b) – Labrum of the 4th instar larvae of *Agrilus*: (a) *A. planipennis*, (b) *A. ater* (Photo: M. Volkovitsh).

TABLE A3 Prementum of the larvae of different species occurring in Europe. After Alexeev (1981) and Chamorro et al. (2012, 2015).

Species	Prementum	Posterior contour of microsetal area along anterior margin
<i>A. planipennis</i>		Zigzag shaped
<i>A. biguttatus</i>		Zigzag shaped
<i>A. ater</i>		Triangular
<i>A. guerini</i>		Separated medially by glabrous space, with arcuate posterior margin
<i>A. sinuatus</i>		Zigzag shaped
<i>A. mendax</i>		Triangular
<i>A. subauratus</i>		Nearly straight

TABLE A3 (Continued)

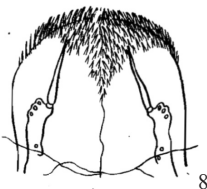


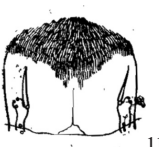
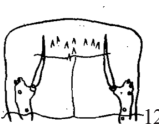
Species	Prementum	Posterior contour of microsetal area along anterior margin
<i>A. viridis</i>	 8	Triangular
<i>A. convexicollis</i>	 9	Prementum glabrous
<i>A. graminis</i>	 10	W-shaped, microsetal area formed by sparse microteeth
<i>A. cyanescens</i>	 11	Triangular or W-shaped, microsetal area formed by dense microsetae
<i>A. roscidus</i>	 12	Prementum nearly glabrous, without marked microsetal area, with few microteeth in the middle

TABLE A 4 Diagnostic table to adults of European *Agrilus* of comparable size. The bold text highlights the characteristics which are the same between *Agrilus planipennis* and other European *Agrilus* species. The underlined characteristic is unique to *A. planipennis*.

Subgenus	(<i>Uragrilus</i>)	(<i>Anambus</i>)	(<i>Uragrilus</i>)	(<i>Uragrilus</i>)	(<i>Sinuatiagrilus</i>)	(<i>Sinuatiagrilus</i>)	(<i>Robertius</i>)	(<i>Agrilus</i>)	(<i>Agrilus</i>)
Species group	<i>Cyanooniger</i>	<i>Biguttatus</i>	<i>Spinipennis</i>	<i>Spinipennis</i>	<i>Sinuatus</i>	<i>Sinuatus</i>	Unassigned	<i>Viridis</i>	<i>Viridis</i>
Species	<i>Planipennis</i>	<i>Biguttatatus</i>	<i>Ater</i>	<i>Guerini</i>	<i>Sinuatus</i>	<i>Mendax</i>	<i>Subauratus</i>	<i>Savorovi</i>	<i>Viridis</i> ^a
Body length	(7.5) 12–15 mm	8–13 mm	8–12 mm	9–12 mm	8–10 mm	10–12 mm	7–10 mm	6.8–10.5 mm	5.3–10.2 mm
Body dorsally: coloration	Emerald, partly cupreous (copper red), rarely violet-blue (Figure 37a)	Green, blue – green, bronzy-green, blue (Figure 37c)	Black-bronze (Figure 37d)	Blue, blackish blue or violet (Figure 37e)	Copper-red, bronze-red or golden bronze (Figure 37f)	Copper-red or copper bronze (Figure 37g)	Pronotum blue, green or orange; elytra golden green, orange or blue (Figure 37h)	Olive-green, bluish, bronzy (Figure 37i)	Olive-green, bluish, bronzy (Figure 37i)
Body: tomentose spots	Poorly visible, on elytra only (Figure 37a)	Well – marked dorsally and ventrally (Figure 37c: ts)	Well – marked dorsally and ventrally (Figure 37d)	Well marked dorsally and ventrally (Figure 37e)	Poorly visible on elytra only (Figure 37f)	Absent (Figure 37g)	Absent (Figure 37h)	Absent (Figure 37i)	Absent (Figure 37i)
Head: medial impression	<u>Deep (Figure 39a)</u>	Shallow (Figure 39b)	Shallow (Figure 39c)	Shallow (Figure 39d)	Absent (Figure 39e)	Shallow (Figure 39f)	Nearly absent (Figure 39g)	Absent (Figure 39h)	Absent (Figure 39h)
Head: vertex, shape	Narrow (Figures 38a and 39a)	Wide (Figures 38b and 39b)	Narrow (Figures 38c and 39c)	Wide (Figures 38d and 39d)	Wide (Figures 38e and 39e)	Wide (Figures 38f and 39f)	Very wide (Figures 38g and 39g)	Wide (Figures 38h and 39h)	Wide (Figures 38h and 39h)
Head: vertex, punctate striae	Concentric (Figures 38a and 39a)	Arcuate (Figures 38b and 39b)	Concentric (Figures 38c and 39c)	Concentric (Figures 38d and 39d)	Arcuate (Figures 38e and 39e)	Arcuate (Figures 38f and 39f)	Concentric (Figures 38g and 39g)	Straight (Figures 38h and 39h)	Straight (Figures 38h and 39h)
Pronotum: sides (dorsal view)	Arcuately converging (Figure 39a)	Nearly arcuate (Figure 39b)	Arcuate or diverging (Figure 39c)	Arcuate or diverging (Figure 39d)	Arcuate or sinuate (Figure 39e)	Arcuate or diverging (Figure 39f)	Arcuate or angulate (Figure 39g)	Diverging or arcuate (Figure 39h)	Diverging or arcuate (Figure 39h)
Pronotum: medial impressions	Deep (Figure 39a)	Shallow (Figure 39b)	Inconspicuous (Figure 39c)	Shallow (Figure 39d)	Shallow (Figure 39e)	Indistinct (Figure 39f)	Indistinct (Figure 39g)	Posterior deep (Figure 39h)	Posterior deep (Figure 39h)
Prehumeri	Poorly defined (Figures 39a and 40a)	Absent (Figures 39b and 40b)	Well defined (Figures 39c and 40c)	Poorly defined (Figures 39d and 40d)	Well defined (Figures 39e and 40e)	Well defined (Figures 39f and 40f)	Poorly defined (Figures 39g and 40g)	Poorly defined (Figures 39h and 40h)	Poorly defined (Figures 39h and 40h)
Elytra: apices	Arcuate (Figure 37a)	Arcuate (Figure 37c)	Angular with tooth (Figure 37d)	Tooth-like (Figure 37e)	Arcuate (Figure 37f)	Arcuate (Figure 37g)	Arcuate (Figure 37h)	Arcuate (Figure 37i)	Arcuate (Figure 37i)
Pygidial process	Present (Figure 37b: pp)	Absent (Figure 37c)	Present (Figure 37d)	Present (Figure 37e)	Absent (Figure 37f)	Absent (Figure 37g)	Absent (Figure 37h)	Absent (Figure 37i)	Absent (Figure 37i)

^a *Agrilus viridis* is very similar to *A. savorovi* although it is smaller. No pictures of this species are available, refer to those of *A. savorovi*.

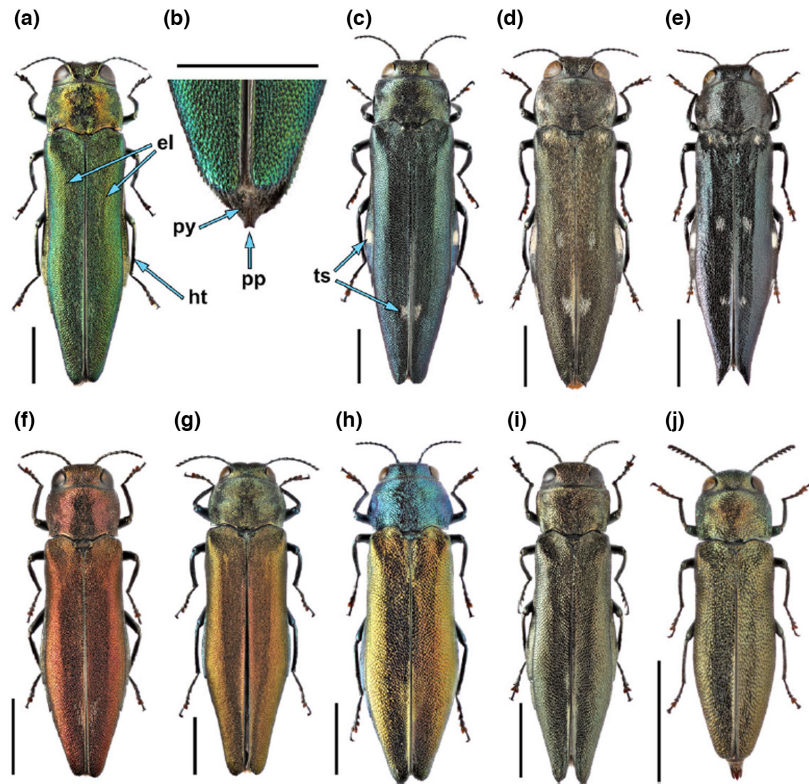


FIGURE 37 Emerald ash borer, *A. planipennis* and other *Agrilus* species from Europe: *A. planipennis*: (a) habitus, (b) pygidial process, (c) *A. biguttatus*, (d) *A. ater*, (e) *A. guerini*, (f) *A. sinuatus*, (g) *A. mendax*, (h) *A. subauratus*, (i) *A. suvorovi*, (j) *A. convexicollis*. el – elytra, ht – hind tibia, pp – pygidial process, py – pygidium, ts – tomentose spots. Scale bars: 2 mm. Photo: A.V. Kovalev. Volkovitsh et al. (2020a).

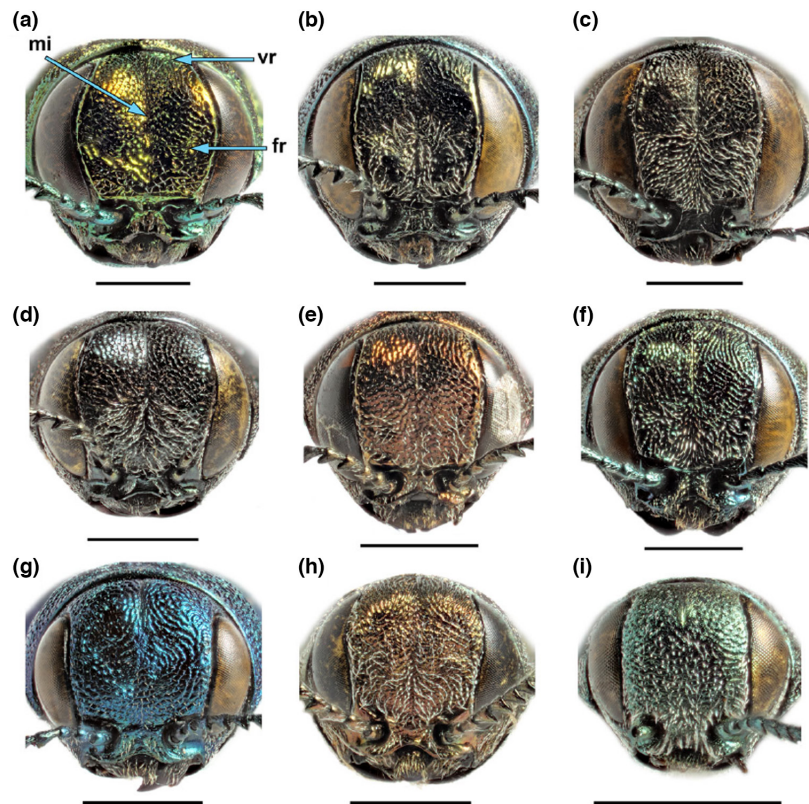


FIGURE 38 Emerald ash borer, *A. planipennis* and other *Agrilus* species from Europe, head, frontal view: (a) *A. planipennis*, (b) *A. biguttatus*, (c) *A. ater*, (d) *A. guerini*, (e) *A. sinuatus*, (f) *A. mendax*, (g) *A. subauratus*, (h) *A. suvorovi*, (i) *A. convexicollis*. fr – frons, mi – medial impression, vr – vertex. Scale bars: 1 mm. Photo: A.V. Kovalev. Volkovitsh, et al. (2020a).

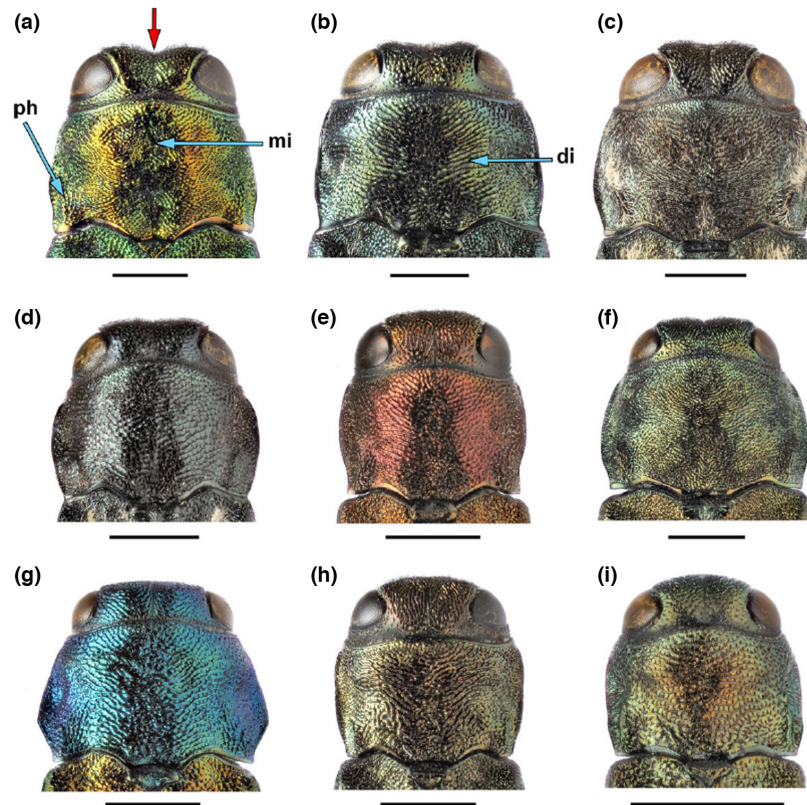


FIGURE 39 Emerald ash borer, *A. planipennis* and other *Agrilus* species from Europe, pronotum and head, dorsal view: (a) *A. planipennis*, (b) *A. biguttatus*, (c) *A. ater*, (d) *A. guerini*, (e) *A. sinuatus*, (f) *A. mendax*, (g) *A. subauratus*, (h) *A. suvorovi*, (i) *A. convexicollis*. di – disc, mi – medial impression of pronotum, ph – prehumeral, arrow shows medial impression of head. Scale bars: 1 mm. Photo: A.V. Kovalev. Volkovitch et al. (2020a).

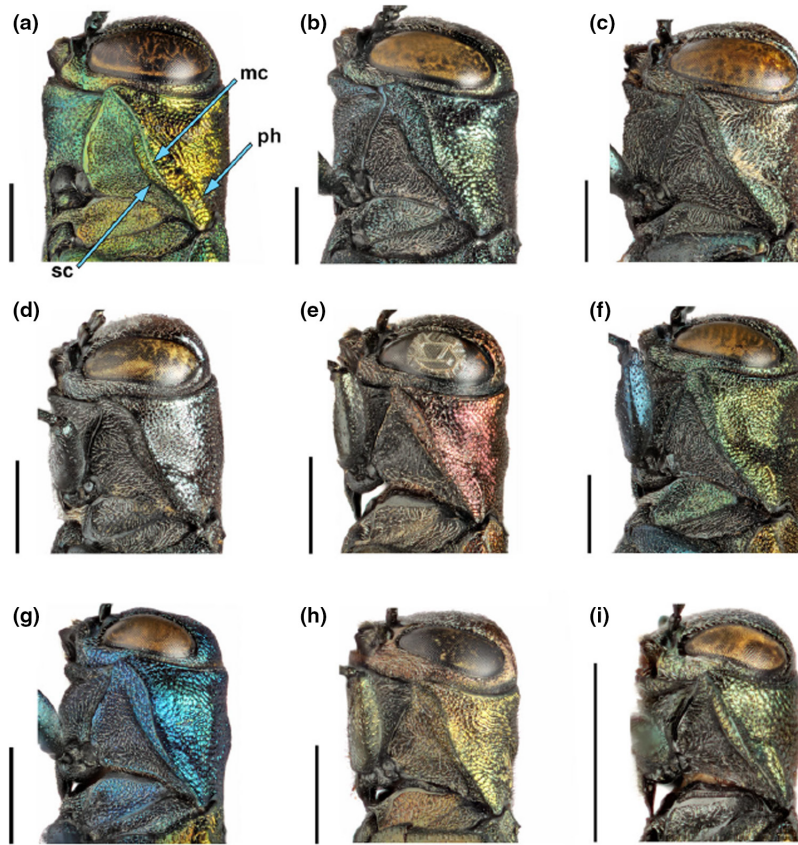


FIGURE 40 Emerald ash borer, *A. planipennis* and other *Agrilus* species from Europe, pronotum and head, lateral view: (a) *A. planipennis*, (b) *A. biguttatus*, (c) *A. ater*, (d) *A. guerini*, (e) *A. sinuatus*, (f) *A. mendax*, (g) *A. subauratus*, (h) *A. suvorovi*, (i) *A. convexicollis*. mc – marginal carina, ph – prehumerus, sc – submarginal carina. Scale bars: 1 mm. Photo: A.V. Kovalev. Volkovitsh et al. (2020a).

APPENDIX 2 - LAMP TEST FOR *AGRILUS PLANIPENNIS* KYEI-POKU ET AL. (2020)

The test below is described as it was 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 test is suitable for the detection and identification of all life stages of *A. planipennis* and its larval frass.
- 1.2. This test was developed by Kyei-Poku et al. (2020).
- 1.3. This test targets a region of the mitochondrial *COI* gene which can distinguish *A. planipennis* from its North American congeners. It requires three primer pairs to conduct loop-mediated isothermal amplification (LAMP):

Primer name	Sequence	Primer description
EABI_F3	5'-CTCCCTCCCTCTTT AACATTAC-3'	External primer set
EABI_B3	5'-GATCAGACTAGTAG AGGTGT-3'	
EABI-FIP ^a	5'-ATATTAGCCGC TAATGGTGG GAATAGTCGAAA GAGGAGCAG-3'	Internal primer set
EABI-BIP ^b	5'-GGCTCTGTTGA CTTAGCAA GGTATTCC TATTGCTCGC-3'	
EABI_LF	5'-ATATACTGTCCAAC CAGTCC-3'	Loop primer set
EABI_LB	5'-CTGGAATCTCCTCA ATTCTAGG-3'	

^a5'-labelled with 6-FAM for lateral flow device (LFD) tests.

^b5'-labelled with biotin for lateral flow device (LFD) tests.

2. Methods

2.1. Nucleic acid extraction and purification

2.1.1. For eggs, larvae, and adults

Genomic DNA (gDNA) can be extracted using a Wizard Genomic DNA purification kit (Promega, Madison, WI) or other standard methods/commercial kits normally suitable for insects following manufacturer's instructions.

2.1.2. For larval frass

Grind frass collected from host trees with a sterile mortar and pestle, and weigh out 50mg samples prior to DNA extraction. DNA extraction can be done using the NucleoSpin Tissue Kit (Macherey-Nagel, Bethlehem, PA) following manufacturer's instructions.

2.1.3. For samples obtained from sticky traps

Glue-coated individuals should be ground in 500µL of lysis buffer (40mM Tris-HCl at pH 6.5, 400mM NaCl, 0.4% SDS). Homogenates are then boiled for 10min, transferred onto ice for 10min, and then incubated at room temperature for 10min. Spin homogenates until a supernatant, which should contain crude DNA, forms. Transfer 200µL of this clarified supernatant to a new tube.

2.1.4. DNA storage

Once DNA is extracted, use immediately in the LAMP test or store at -20°C until use.

2.2. LAMP test

2.2.1. Master mix

Prepare first a 10× primer mix as described below.

	Working primers conc. (µM)	Volume (µL) for 10× Primer mix	Final conc. (µM)
EABI_F3	10	2	0.2
EABI_B3	10	2	0.2
EABI-FIP	10	16	1.6
EABI-BIP	10	16	1.6
EABI_LF	10	4	0.4
EABI_LB	10	4	0.4
Molecular grade water		56	
Total		100	

Prepare the LAMP mix as detailed below.

Reagent	Volume per reaction (µL)
Molecular grade water	To make up to 25
ISO-001 Isothermal Mastermix (OptiGene)	15
10× Primer Mix	5
gDNA extract	2
Total	25

2.2.2. Emerald ash borer-LAMP reaction conditions

Tests can be performed using a Genie II real-time fluorometer (Optigene Ltd., Horsham, UK) with a reaction temperature of 65°C for 30min. The use of a standard thermocycler or hot water bath under the same time and temperature conditions was found by Kyei-Poku et al. (2020) to produce similar results.

2.2.3. Analysis of LAMP product

LAMP product may be analysed using different methods. It is recommended that users confirm positive results using some combination of at least two of the following four assessments:

2.2.3.1. Using the Genie II fluorometer

2.2.3.2. Using SYBR Green I

For direct observation of results via visible colour change, users may add 1 μ L of a 1:10 diluted solution of SYBR Green I fluorescent DNA-intercalating dye (Lonza, Allendale, NJ) to the reaction tube *after* EAB-LAMP incubation (see Section 2.2.2). After the addition SYBR Green I, briefly vortex the sample tubes to mix the dye with the amplified product.

2.2.3.3. Using electrophoresis

Run SYBR Green I-dyed LAMP product on an agarose gel for approximately 80 min.

2.2.3.4. Lateral flow device (LFD) detection

Mix 2 μ L of labelled product with 98 μ L of HybriDetect 2T assay buffer (Milena Biotec GmbH, Giessen, Germany) in a new tube. Immerse an LFD detection strip from the HybriDetect 2T kit into the amplicon-buffer solution at room temperature for 10 min.

3. Essential procedural information

3.1. Controls

The following controls should be included for each series of DNA extraction and amplification:

- Negative isolation control (NIC) to monitor contamination during the DNA extraction process; DNA extraction and subsequent amplification of clean extraction buffer.
- Positive isolation control (PIC) to ensure DNA extracts are of sufficient quality and quantity; DNA extraction and amplification of the target organism or a matrix sample containing the target organism.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix; amplification of the molecular-grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the amplification efficiency of the DNA of the target organism; this control may include DNA from the target organism or a synthetic control (cloned PCR product).

3.2. Interpretation of results

3.2.1. Using the Genie II fluorometer

Verification of the controls

- NIC and NAC should produce no fluorescence (flat line on the Genie II amplification graph).
- PIC and PAC should produce:
 - a sigmoid-shaped amplification curve.
 - a time-to-positive (T_p) value between 10 and 14 min.
 - an anneal derivative value (T_m) of approximately 84°C.

When these conditions are met:

- A test will be considered positive if it produces a positive reaction as defined for PIC and PAC (see above).
- A test will be considered negative, if it produces no fluorescence.
- Tests should be repeated if any contradictory or unclear results are obtained.

3.2.2. Using SYBR Green I

Verification of the controls

- NIC and NAC should produce no colour change.
- PIC and PAC should exhibit a colour change from pale orange to green, or otherwise emit a green fluorescence. This colour change can be viewed with the naked eye or under UV (254 nm) light.

When these conditions are met:

- A test will be considered positive if it produces a positive reaction as defined for PIC and PAC (see above).
- A test will be considered negative, if it produces no turbidity/colour change.
- Tests should be repeated if any contradictory or unclear results are obtained.

3.2.3. Using electrophoresis

Verification of the controls

- NIC and NAC: no band is visualized on the gel.
- PIC and PAC: a ladder-like banding pattern is visualized on the gel.

When these conditions are met:

- A test will be considered positive if a ladder-like banding pattern is visualized on the gel.
- A test will be considered negative, if it produces no band.
- Tests should be repeated if any contradictory or unclear results are obtained

3.2.4. Lateral flow device (LFD) detection

Verification of the controls

- NIC and NAC: a single band in the control region is visualized.
- PIC and PAC: Two red bands should be visualized (one test band and one control band) on the detection region of the strip.

When these conditions are met:

- A test will be considered positive if it produces two red bands.
- A test will be considered negative, if it produces a single band for the control.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance characteristics available

All performance details for this test are from Kyei-Poku et al. (2020).

4.1. Analytical sensitivity data

Frass Samples

The LAMP test successfully detects trace amounts of *A. planipennis* DNA extracted from frass (as low as 100 fg/ μ L). These results also indicated that frass samples dried and stored for up to 3 years had increased detection sensitivity compared to samples collected and stored for 1 month, suggesting that higher moisture content in the more recently collected frass samples may have a minor inhibitory effect on the test.

4.2. Analytical specificity data

Exclusivity: In silico evaluation

Primer specificity was evaluated in silico by using publicly available sequence data for *A. planipennis* against non-target *A. anxius*, *A. bilineatus*, *A. cuprescens*, *A. hastulifer*, *A. kubani*, *A. lacroixi*, *A. liragus*, *A. lubopetri*, *A. olivicolor*, *A. quadrisignatus*, *A. ribbei*, *A. roscidus*, *A. salicis*, *A. sulcicollis*, *A. suvoroviviridis*, *A. tempestivus*, and *A. viridicaerulans*. Cross reaction with these species is not expected.

Exclusivity evaluation on specimens: 100%

Primer specificity was evaluated using the LAMP test and PCR against non-target *A. anxius*, *A. bilineatus*, *A. sulcicollis*, *A. subcinctus*, *D. ponderosae*, and *D. rufipennis* (adults). No cross reactions were observed. However, further validation against a greater number of *Agrilus* species, as well as other beetles commonly caught in traps with *A. planipennis* outside of North America, would expand the taxonomic utility of this method.

4.3. Selectivity: 100%

Primer specificity evaluated using the LAMP test against phloem and/or frass samples from *F. pennsylvanica*, *F. nigra*, *F. americana*, *F. quadrangulata*, and *F. profunda*.

4.4. Repeatability: 100%

Each test was conducted in triplicate on different days using at least two independent DNA samples. The results were found to be repeatable.

4.5. Other information

- Insects collected from traps

Agrilus planipennis and other unsorted insects obtained from 14 sticky traps were used to assess the use of this test for detecting *A. planipennis* in trap samples. The diagnostic sensitivity from these tests was 100%, while the diagnostic specificity was 71.4% (due to two potential false positives); overall, the accuracy of this test was 86%.

Pooled total DNA samples from sticky traps either positive or negative for *A. planipennis* were tested to estimate potential bulk sample sizes suitable for detecting *A. planipennis*. Samples evaluated contained a different ratio of *A. planipennis* positive: negative traps and was performed in triplicate on different days. The results indicate that the Emerald Ash borer-LAMP test successfully detected *A. planipennis* DNA in a maximum pool size of 15 traps (ratio of 1 *A. planipennis* positive trap:14 *A. planipennis* negative traps); however, the number of specimens sampled from each trap was not specified in Kyei-Poku et al. (2020), so these results should be considered exploratory.

- Data from Peterson et al. (2023)

Analytical specificity (exclusivity)

Exclusivity of the test was evaluated on DNA extracted from single specimens of the following species:

Agrilus angustulus, *Agrilus ater*, *Agrilus convexicollis*, *Agrilus curtulus*, *Agrilus graminis*, *Agrilus hastulifer*, *Agrilus laticornis*, *Agrilus obscuricollis*, *Agrilus olivicolor*, *Agrilus roscidus*, *Agrilus sulcicollis*, *Agrilus viridis*, *Anthaxianitidula*, *Chrysobothris affinis*, *Coraebus undatus*, *Lamprodila mirifica*.

Curculionidae, Scolytinae:

Anisandrus dispar, *Xyleborinus saxesenii*

Cerambycidae:

Aegomorphus clavipes, *Exocentrus punctipennis*, *Leiopus nebulosus*, *Saperda punctata*, *Trichoferus pallidus*.

No cross reaction was observed.