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EPPO STANDARD ON DIAGNOSTICS

PM 7/157 (1) *Dendrolimus sibiricus*

Specific scope: This Standard describes a diagnostic protocol for *Dendrolimus sibiricus*.[1] This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.
Specific approval and amendment: Approved in 2024-03.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Dendrolimus sibiricus Chetverikov is an insect of the order Lepidoptera, family Lasiocampidae. In its native range, *D. sibiricus* develops on practically all coniferous species of the Pinaceae family: *Abies*, *Pinus*, *Larix*, and *Picea* (Kirichenko & Baranchikov, 2007; Rozhkov, 1963). Major hosts are *Abies nephrolepis*, *Abies sibirica*, *Larix gmelinii*, *Larix sibirica*, and five-needle pines such as *Pinus koraiensis* and *Pinus sibirica* (EPPO, 2023a). For more information on other hosts (e.g. experimental hosts), see EPPO (2023b).

The taxonomy and nomenclature of the genus *Dendrolimus* are not entirely established and for some species the taxonomic position is still controversial. Most experts distinguish around 30 species in the genus, of which 16 species are found in the Palearctic region (Jeong et al., 2018; Mikkola & Ståhls, 2008; Tanaka et al., 2020). Morphologically, *D. sibiricus* has many similarities to the related species, *D. superans*, that co-occurs in the Russian Far East and China (Rozhkov, 1963). Rozhkov (1963) considered *D. superans* as a single species, with two subspecies: *D. superans sibiricus* Chetverikov and *D. superans albolineatus* Butler. In the current taxonomy, they are considered as separate species respectively, *D. sibiricus* and *D. superans* (EFSA, 2023). A second closely-related species is *D. pini*, which is native to Europe and Asia and whose distribution range partially overlaps with *D. sibiricus* in Central Russia (Hardin & Suazo, 2012; Kononov et al., 2016).

For an updated geographical distribution of *D. sibiricus* consult EPPO Global Database (EPPO, 2023a).

A datasheet providing more information on the biology is also available (EPPO, 2023b).

2 | IDENTITY

Name: *Dendrolimus sibiricus* Chetverikov, 1908.
Other scientific names: *Dendrolimus laricis* Chetverikov, 1903, *Dendrolimus superans sibiricus* Chetverikov, 1903.
Common name: Larch caterpillar, Siberian conifer silk moth, Siberian moth, Siberian silk moth.
Taxonomic position: Lepidoptera, Lasiocampidae, *Dendrolimus*.
EPPO Code: DENDSI.
Phytosanitary categorization: EPPO A2 list n° 308, EU Annex II A.

3 | DETECTION

Larvae cause massive defoliation on coniferous hosts (Figures 1 and 2) (EPPO, 2005; Rozhkov, 1963); during outbreaks large amounts of frass are produced that make them easy to detect (Hardin & Suazo, 2012). Larvae can be found in autumn in the leaf litter under infested trees, where they overwinter. Pupae in silky cocoons are difficult to detect and pupation takes place on branches and twigs of hosts (Rozhkov, 1963). Adult males and females can be detected with light traps and adult males can also be caught in pheromone traps (Mikkola & Ståhls, 2008; Pletniev et al., 1999). *Dendrolimus sibiricus* and *D. pini* respond to the same synthetic sex pheromones, such as Z5E7-12Ald, Z5E7-12Ac, Z5E7-12OH, Z5-12Ald, E7-12Ald, E6-12Ald, E6-12OH and E7-12OH (Baranchikov et al., 2006; Klun et al., 2000; Pet'ko et al., 2004).

[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 Severely defoliated *Larix sibirica* (Siberian larch) stand, June 2006, Altai Krai, Russia. Courtesy: N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

FIGURE 2 Totally defoliated twig of *Larix sibirica* with late instar larvae of *Dendrolimus sibiricus* spreading around foraging. June 2006, Altai Krai, Russia. Courtesy: N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

3.1 | Eggs

The eggs of *D. sibiricus* are oval and on average 2.2 mm long and 1.9 mm wide. The colour of eggs changes after a few days from light green to creamy white and then becoming greyish, with a grainy texture of the chorion (Figure 3) (Rozhkov, 1963).

FIGURE 3 Eggs of *Dendrolimus sibiricus*: (a) freshly laid eggs on larch needles. Courtesy: N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU); (b) matured egg clusters on larch twig, Tuva Republic (RU). Courtesy: V Pet'ko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU); (c) mature eggs on needles. Courtesy: JH Ghent, USDA Forest Service (US).

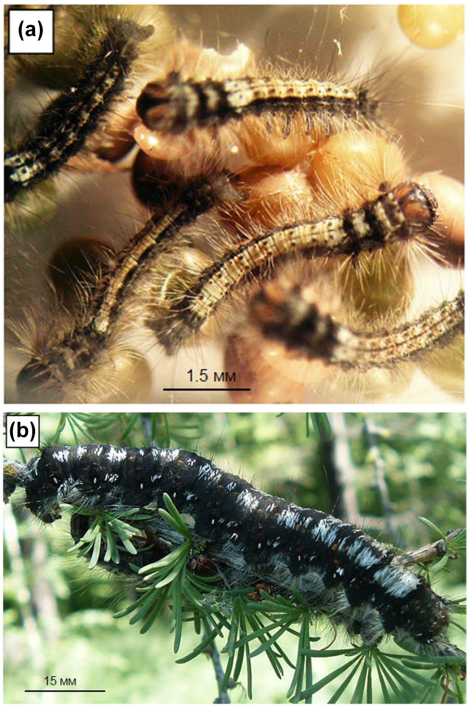

FIGURE 4 (a) Neonate larvae hatched from the eggs and (b) mature larvae, the 6th instar, of *Dendrolimus sibiricus*. Altai Krai, (RU), August 2006. Courtesy: N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

The eggs of *D. pini* are slightly bigger (2.6–2.8 mm long and 2 mm wide), indistinguishable by texture from those of *D. sibiricus*. Their colour changes from green or blue to grey after a few days (Hardin & Suazo, 2012).

Bunches, clusters or chains of 3–100 eggs and up to 200 per cluster (Rozhkov, 1963) are laid by *D. sibiricus* on needles, twigs and rarely on bark (Figure 3). During outbreaks, eggs are laid in different parts of the tree crown and on the ground under the tree (EPPO, 2023b).

3.2 | Larvae

Young larvae are 5.0–5.5 mm long and the full-grown larvae are 50–80 mm long (Figure 4). They are mainly black, dark-brown or grey dorsally and laterally and rusty or red-brown ventrally, with numerous spots and long hairs.

3.3 | Pupae

The pupae are in thick silky cocoons (Figures 5–7); the pupae are brown (Figure 5), 33–39 mm long in females, 28–34 mm in males (EPPO, 2005).

FIGURE 5 Pupa dissected from the cocoon. Courtesy N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

FIGURE 6 Pupae in silky cocoons on *Larix* spp. Courtesy: Y Baranchikov, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

FIGURE 7 Pupae in silky cocoons. Courtesy: J H. Ghent, USDA Forest Service (US).

3.4 | Adults

Dendrolimus sibiricus is a relatively large moth. The wingspans in the populations of *D. sibiricus* developed on Siberian larch (*Larix sibirica*) and Siberian 5-needle pine (*Pinus sibirica*) have the following size ranges (Rozhkov, 1963):

males (larch): 61–83 mm.
females (larch): 73–104 mm.
males (5-needle pine): 59–80 mm.
females (5-needle pine): 72–94 mm.

Females have larger bodies than males.

4 | IDENTIFICATION

Due to significant polymorphism of most *Dendrolimus*-species, identification to species level using only external characters and appearance, cannot be done reliably. Species identification should be based on the examination of male genitalia (Baranchikov et al., 2006; EFSA, 2023; Mikkola & Ståhls, 2008; Rozhkov, 1963) or with molecular methods (DNA barcoding) (Jeong et al., 2018; Kononov et al., 2016; Mikkola & Ståhls, 2008).

Large moths can rot from the inside out (fungi) in humid conditions. This can affect molecular identification by barcoding. It is recommended to preserve material meant to be used for molecular analysis in 96% alcohol (undenatured).

4.1 | Morphological identification

Although species identification based on morphology is only possible based on male genitalia, descriptions of eggs, larvae and adults are also given for reference.

4.1.1 | Eggs

The micropylar area is sculptured and comprised of 7–8 rows of polygonal cells. The remaining surface is smoothed in the form of small, densely intertwined fibres. The micropylar rosette is 47.8–52.7 µm in diameter, with 10 cells, 1.5–2.0 times longer than wide, joined along 2/3–3/4 of their length. The central portion of the rosette is deeply depressed, and there are 10 micropylar openings in it. The remaining egg surface has hardly noticeable cells with poorly expressed and narrow cell edges, and very clearly expressed aeropyles at the junctions of cell edges. The cells are 11.5–36.6 µm in diameter, the cell edges are 0.6–1.1 µm wide, and the aeropyles 4.0–5.4 µm in diameter (Dolinskaya, 2000).

4.1.2 | Larvae

Neonate larvae are variable in colour from yellowish to blackish, with a pronounced black lateral stripe, largely covered with long setae (Figure 4a). In first instar larvae, the body is 5–7 mm long, and head capsule is 0.9–1.2 mm wide. Larvae pass 5–6 instars growing significantly in size. In the last instar, the body is 62–88 mm long, and the head capsule is 5.2–6.3 mm wide (Rozhkov, 1963).

Late (5th–6th) instar larvae are also variable in colours: from greyish and light brown to dark brown and black, the 2nd and 3rd segments crossed by a blue-black stripe. Head is reddish brown, maculated, opaque, at the occiput with two obsolete short fuscous longitudinal stripes. Clypeus is in the middle with a fuscous spot, the labrum is shiny, in the middle shallowly incised. The abdomen shows numerous minute fuscous spots, the shield plate of the first segment is reddish brown, the sides are marmorated with red, the 2nd and 3rd segments are dorsally covered with silvery scales, the 4th to 12th segments each show dorsally 2 large silvery scaly spots and a hexagonal marking, the latter being larger at 6th to 8th segment. Silvery scales reflect a light shade of gold, so that in fresh specimens the dorsal hexagonal markings are not distinct. The stigma is yellowish, with black periphery. The thoracic legs except the bases are black, the abdominal legs are yellowish, each on the outer side with a broad fuscous stripe, that of the anal proleg with 2 whitish stripes in it. The venter shows a series of fuscous spots, which become smaller towards both ends (Bushmelev & Yurchenko, 1989; Matsumura, 1926; Rozhkov, 1963).

4.1.3 | Characteristics of Lasiocampidae and *Dendrolimus* adults

Characteristic features of adults in the family Lasiocampidae and genus *Dendrolimus* are described below.

4.1.3.1 | *Morphological identification*

4.1.3.1.1 | *Morphological characters for Lasiocampidae, from Lemaire and Minet (1999) and Hydén et al. (2006).* Labrum without bristles.

Forewing vein R1 distally distinctly approximated to Sc (see Figure 8).

Areoles (secondary cells on wings) (see Figure 9) absent.

Cu2 (CuA2) of forewing arising closer to the base of the discal cell (DC) than to the origin of M3.

Forewing veins R2 and R3 joined together (branched).

Forewing veins M1 and M2 are originated far from each other from the discal cell.

Absence of frenulum on hindwing of both sexes (Aberlenc, 2020).

Antennae are bipectinate (comb-like) in both sexes.

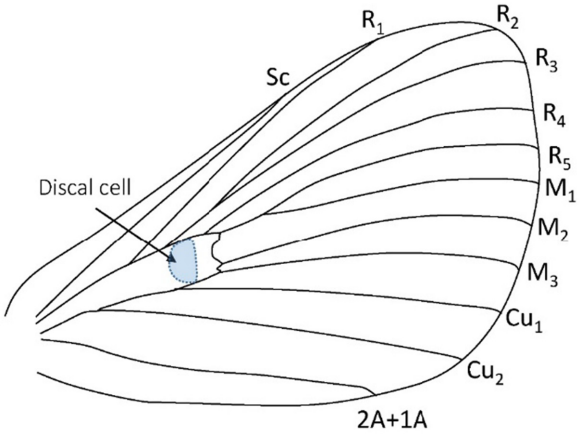

FIGURE 8 Example of forewing venation of a Lasiocampid moth (after Hydén et al., 2006), with a schematic indication of the discal cell position as per Rozhkov (1963). Sketch by N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

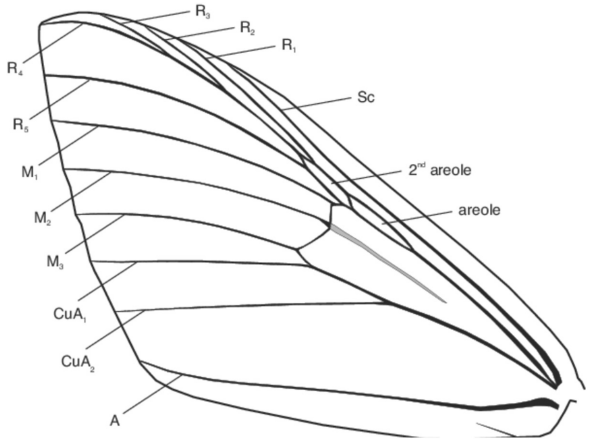

FIGURE 9 Examples of areoles in a wing. (Trusch & Hausmann, 2007).

4.1.4 | *Dendrolimus sibiricus* adults

Extensive colour variation exists in the adults, associated with their geographic origin. The most common are the dark greyish forms found mostly in the western part of Russia. The unicolorous brown forms are generally found in Eastern Siberia and the Russian Far East; the melanic forms are mainly found in the Buryatia and the Altai region in Russia (Mikkola & Ståhls, 2008).

The forewings have 3 distinctive black transverse lines and a distinctive white spot within or along the antemedial line (from the thorax outward these lines are named antemedial, postmedial and subterminal) (see Figure 10a–e). In the holotype of *D. sibiricus*, it looks like the median transverse line is doubled (Figure 10d). However, this character is very variable within the species, i.e. obvious in some specimens but not present in others, thus it cannot be used as a robust character for distinguishing *D. sibiricus* from the related species, in particular from *D. pini* (Figure 10f).

Compared to similar and related species, *D. sibiricus* is usually larger and the male forewings are mostly rusty brown in colour. *D. pini* male forewing colour is usually ash-grey which is in strong contrast with the nut-brown subterminal field. A pale brown female is displayed in Figure 10c. Adults of *D. sibiricus* and *D. pini* cannot be distinguished based on their habitus. Dimorphism of the antennas allows for discrimination of sexes, the bipectinate structures are more pronounced in males than in females (see Figure 10).

4.1.4.1 | *Genitalia*

The genital capsule is dissected from the abdomen. Classical dissection techniques are described for example by Clarke (1941) and in moth dissection website (mothdissection.co.uk).

4.1.4.1.1 | *Male genitalia*. Key characters of male genitalia are adapted from Mikkola and Ståhls (2008), Chetverikov (1908), Matsumura (1926) and Lajonquière (1973). Permanent mounted slides from the Finnish Museum of Natural History were used to prepare Figures 12–14. Size and shape of the harpe, i.e. a process parallel to valva (Figure 11), are the key characters to distinguish *D. sibiricus* from *D. superans* and *D. pini*. These parts of male genitalia are shown in Figures 12 and 13. The order and number of spines in the apical part of cubiles (Figure 11) shows intra- and interspecific variation that seems taxonomically nonsignificant and consequently not useful for diagnostic purposes. Size and abundance of cornuti (spines arming, the diverticula of vesica) on the vesica, better seen when everted, show a variability of diagnostic importance (Figure 14 D, H, L) (Lajonquière, 1973; Mikkola & Ståhls, 2008). According to Mikkola and Ståhls (2008), in *D. sibiricus*, the vesica is equipped with relatively weak cornuti, whereas they are very strong and large in *D. superans* and of medium size in *D. pini*. This seems confirmed by the observation of a few specimens from the collection of the EU Reference Laboratory for Insects and Mites (A. Taddei, ANSES, FR, personal communication) although no significant difference was observed in the size of cornuti between *D. superans* and *D. pini*. To observe cornuti, it is recommended to evert the vesica using ethanol or isopropanol injections in the opening at the base of aedeagus, to be done with a syringe and hypodermic needle.

Harpe of *D. sibiricus* is long, dagger-like, almost parallel on both sides, tapering only towards the end (Figure 13).

Comparison to similar and related species:

Harpe of *D. superans* is long, like a thin dagger, basally wide, clearly tapering gradually (Figure 13). Harpe of *D. pini* is short, almost triangular, therefore

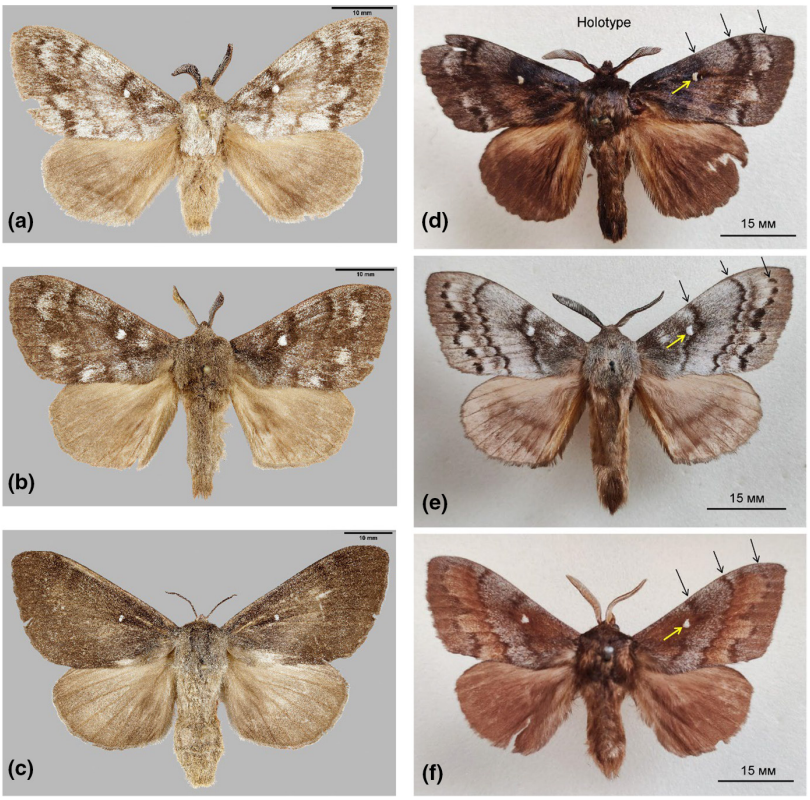

FIGURE 10 Adults of *Dendrolimus sibiricus*. (a, b) adult male (c) adult female (d) holotype, male, brown form (e) male, grey form; (f) adult male of *D. pini*. Black arrows indicate transverse lines; yellow arrow shows white spot on forewings. Localities: (a, c) Russia, Buryatia Barguzin valley, Djirga station; (b) Russia, Altai Republic, Katun valley, 10 km W Katanda; (d) Russia, Orenburgskaya Oblast, Troitsk, Turgoyak, Sushkin G. coll. Zolotuhin V. holotype re-established; (e) Russia, Primorskiy Krai, Shkotovskiy District, Anisimovka village; (f) Russia, Voronezhskaya Oblast, Bitug. Courtesy: P Malinen, Finnish Museum of Natural History (FI) and N Kirichenko, Sukachev Institute of Forest SB RAS, Krasnoyarsk (RU).

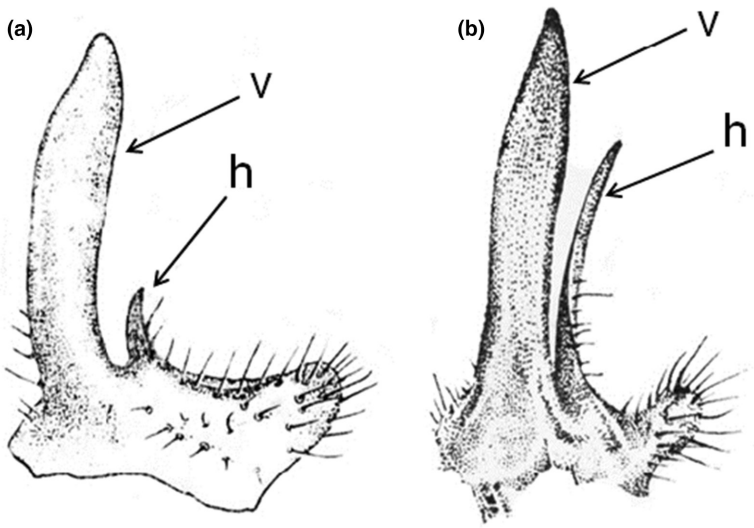

FIGURE 11 *Dendrolimus pini* (a) and *D. sibiricus* (b) detail of male genitalia. The arrows indicate the diagnostic characters: v – valva, h – harpe. Adopted from Baranchikov et al. (2006) (after Rozhkov, 1963).

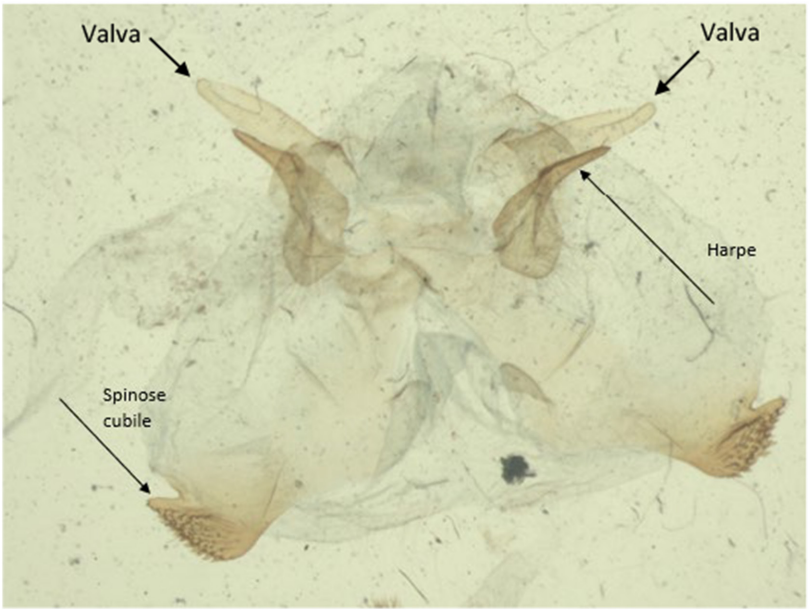

FIGURE 12 Opened male genitalia of *D. sibiricus* in posterior view. Courtesy: V Welling, Finnish Food Authority (FI).

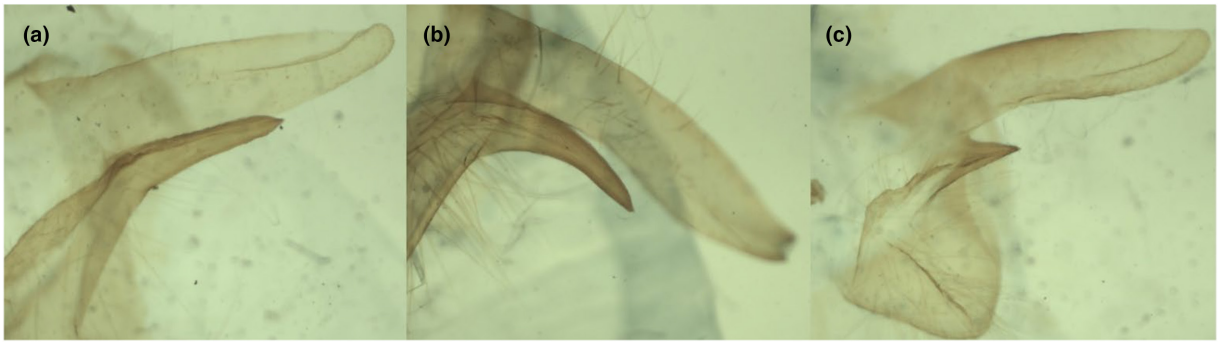

FIGURE 13 Harpe of (a) *Dendrolimus sibiricus*, (b) *D. superans* and (c) *Dendrolimus pini*. Courtesy: V Welling, Finnish Food Authority (FI).

easily distinguished from *D. sibiricus* and *D. superans* (Figure 13). It must be noted that the shape and length of the harpe, which is a tridimensional structure, strongly depends on the view adopted.

Apical cornuti on the arms of everted vesica are relatively short and weak in *D. sibiricus*, large and strong in *D. superans* and in *D. pini* (Figure 14).

4.1.4.1.2 | *Female genitalia*. Size and shape of lamella antevaginalis can be used to differentiate between *D. sibiricus* and *D. pini* but not between *D. sibiricus* and *D. superans* (Rozhkov, 1963). In *D. sibiricus* the lateral plates of lamella antevaginalis are large, with trapezoidal shape and deep folds (Figure 15). In *D. pini*, the lateral plates are smaller in size, with a triangular shape. Mikkola and Ståhls (2008) indicated that in *D. pini* the lateral plates of lamella antevaginalis are less deeply folded than in *D. sibiricus*.

4.2 | Molecular methods

A TaqMan real-time PCR test has been developed for the identification of *Dendrolimus sibiricus* (Stewart et al., 2023). There is no experience in the EPPO region on this test.

A protocol for DNA barcoding based on COI is described in appendix 1 of PM 7/129(2) *DNA barcoding as an identification tool for a number of regulated pests* (EPPO, 2021) and can also support the identification of *Dendrolimus* spp. Sequences are available in different databases; however, DNA barcoding identification results should be interpreted carefully because based on the COI barcode *D. sibiricus*, *D. superans* and *D. pini* cannot be properly resolved. It is recommended to sequence the ITS 2 region (Mikkola & Ståhls, 2008) to differentiate the abovementioned species (Jeong et al., 2018; Kononov et al., 2016).

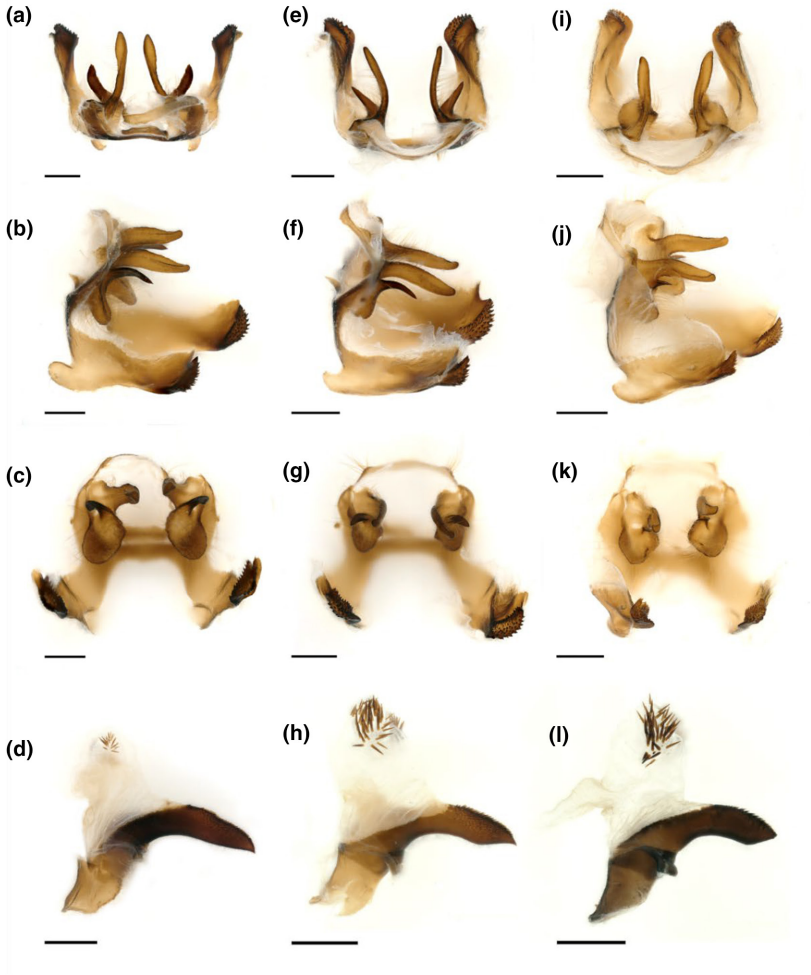

FIGURE 14 Male genital structures of three *Dendrolimus* species after dissection and removal of the aedeagus: *D. sibiricus* genital capsule in (a) dorsal view, (b) lateral view, (c) posterior view and (d) aedeagus with everted vesica in lateral view and cornuti; *D. superans* genital capsule in (e) dorsal view, (f) lateral view, (g) posterior view and (h) aedeagus with everted vesica lateral view and cornuti; *D. pini* genital capsule in (i) dorsal view, (j) lateral view, (k) posterior view and (l) aedeagus with everted vesica lateral view and cornuti. Dorsal view of the genital capsule allows a better appreciation of the shape of the harpe of *D. sibiricus* and *D. superans*. Note that the harpe of *D. pini* is barely visible in dorsal view due to its small size. Scale bar: 1 mm. Courtesy: A. Taddei, ANSES (FR).

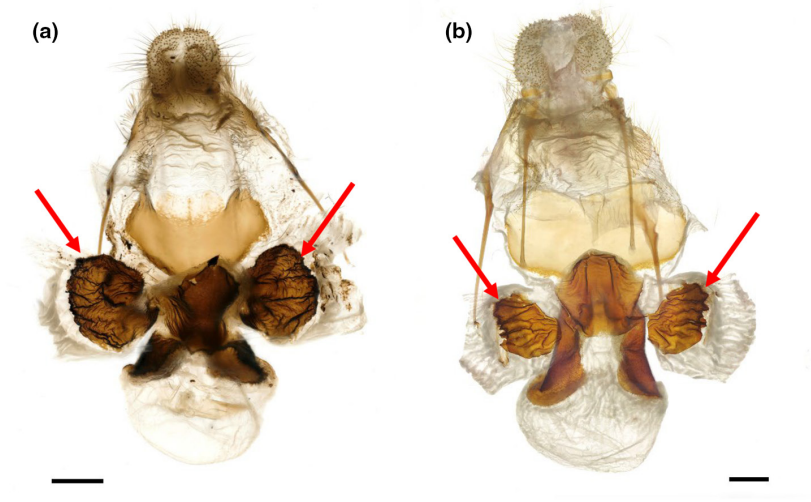

FIGURE 15 Female genital structures of (a) *Dendrolimus sibiricus* and (b) *Dendrolimus pini* with lamella antevaginalis wide open (indicated by arrows) to better see the shape and folds. Scale bar: 1 mm. Courtesy: A. Taddei, ANSES (FR).

5 | REFERENCE MATERIAL

Reference material of *D. sibiricus* is available at

- the Finnish Museum of Natural History, Entomological collections, Pohjoinen Rautatiekatu 13, 00100 Helsinki, Finland,
- the National Plant Protection Organization, Netherlands Food and Consumers Authority, P.O. Box 9102, Wageningen, The Netherlands,
- the Zoological Institute of the Russian Academy of Sciences, Universitetskaya emb., 1, Saint Petersburg, 199034, Russia.

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 organism can be obtained from:

Taddei A., EURL for Insects and mites, ANSES - Laboratoire de la santé des végétaux, Unité entomologie et botanique, 755 avenue du Campus Agropolis, 34988 Montferrier-Sur-Lez Cedex, France.

Kirichenko N., Laboratory of Forest Zoology, Sukachev Institute of Forest SB RAS, Akademgorodok 50/28, Krasnoyarsk, 660036, Russia and All-Russian Plant Quarantine Center, Krasnoyarsk branch, Zhelyabova str. 6/6, Krasnoyarsk, 660020, Russia.

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 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

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Morphological descriptions were reviewed by L. Kaila, Finnish Museum of Natural History, Zoology Unit, Pohjoinen Rautatiekatu 13, 00100 Helsinki, Finland.

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