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INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

ISPM 27 DIAGNOSTIC PROTOCOLS

DP 3: *Trogoderma granarium* Events

(2012)

CONTENTS

1. Pest Information

Trogoderma granarium Everts (Coleoptera: Dermestidae) is a stored product pest of great importance. Its economic importance lies not only in the serious damage it can cause to stored dry commodities but also in the export restrictions faced by countries when they have established populations of this pest. Live populations can stay in uncleaned containers, packaging material and cargo holds for extended periods of time, infesting non-host material. *Trogoderma granarium* may also increase the likelihood of contamination by *Aspergillus flavus* (Sinha and Sinha, 1990).

Trogoderma granarium may have originated from the Indian subcontinent and it is now present in some areas of Asia, the Middle East, Africa and a few countries in Europe. It is one of the very few stored products pests with a limited distribution. It is found from 35° north latitude to 35° south latitude, but occurs mainly in regions near the equator in dry and hot environments. However, viable populations should be able to survive in almost any country in a closed storage environment. *T. granarium* has very limited ability to spread without human aid because it is unable to fly, so international movement of host commodities appears to be the only means of spreading the pest. It is very important to distinguish between records that relate to interceptions of the pest in imported commodities (i.e. its finding in the commodity during the border phytosanitary control without further spread) and those of established infestations (EPPO, 2011).

T. granarium usually occurs in various dry stored products of primarily plant origin. Primary hosts are cereals, buckwheat, cereal products, pulses, alfalfa, various vegetable seeds, herbs, spices and various nuts. It can also successfully complete its life cycle in copra, dried fruits and various gums, as well as many different dried products wholly or partially of animal origin such as milk powder, skins, dried dog food, dried blood, dead insects and dried animal carcasses. As a pest it is most prevalent under hot dry conditions, where very heavy infestations can develop. In cooler and also in hot and humid conditions it tends to be out-competed as a pest by other species such as *Sitophilus* spp. and *Rhyzopertha dominica* (Fabricius). Commodities stored in bags in traditional warehouses are more at risk from this pest than commodities that are stored at bulk.

There are important features of *T. granarium* biology that enable the pest to survive in harsh conditions.

T. granarium may have from one to more than ten generations per year depending on food availability and quality, temperature and humidity. The complete life cycle may be as short as 26 days (temperature 32–35° C) or as long as 220 days or more in a suboptimal environment. In temperate climates larvae become inactive at temperatures below 5° C, so the pest is able to survive and breed only in protected environments. There are two general variations of larvae: those that are able to undergo facultative diapause and those that are unable to do so. Larvae of the first type are stimulated into diapause by adverse conditions such as low or high temperatures and/or lack of food. During diapause their respiration drops to an extremely low level leading to tolerance to fumigation. Diapausing larvae are also cold hardy and may survive temperatures below −10° C. When favourable conditions return, the pest is able to multiply rapidly and cause serious damage to the commodity (EPPO/CABI, 1997).

Trogoderma species other than *T. granarium* may also be found in stored products, but only some of these feed on such products. Among these species the biggest economic losses are caused by *T. variabile* Ballion, which may cause significant economic damage and is recognized as a quarantine pest in some countries. However, most *Trogoderma* species occurring in stored products appear to be scavengers, feeding on dead bodies of other insects. During a 12-year survey conducted in California, eight species of *Trogoderma* were found in stored seeds, animal feed and grocery commodities (Strong and Okumura, 1966). Mordkovich and Sokolov (1999) mention other *Trogoderma* species that may be found in stored products. Among them, *T. longisetosum* Chao and Lee has been noted as a stored product pest in China. It is very similar to *T. glabrum* (Herbst). Some tropical *Trogoderma* species may also be present in stored products (Delobel and Tran, 1993). One of such species is *T. cavum* Beal, which was described by Beal (1982) after examination of specimens infesting stored rice in Bolivia. Some species occurring in stored products closely resemble *T. granarium*.

For more general information on *T. granarium*, see the EPPO PQR database (EPPO, 2011) as well as Hinton (1945), Lindgren *et al*. (1955), Varshalovich (1963), Bousquet (1990) Kingsolver (1991), EPPO/CABI (1997), Pasek (1998), OIRSA (1999a), PaDIL (2011) and CABI (2011).

Diagnostic protocols for *T. granarium* have been published by two regional plant protection organizations – OIRSA (1999a) and EPPO (2002). The initial point for preparation of this protocol was the document issued by EPPO (2002).

2. Taxonomic Information

Name: *Trogoderma granarium* Everts, 1898

Synonyms: *Trogoderma khapra* Arrow, 1917
Trogoderma koningsbergeri Pic, 1933
Trogoderma afrum Priesner, 1951
Trogoderma granarium ssp. *afrum* Attia and Kamal, 1965

Common names: khapra beetle (English)
Trogoderme (dermeste) du grain, dermeste des grains (French)
Trogoderma de los granos, escarabajo khapra, gorgojo khapra (Spanish)
الشـــعرية الحبـــوب خنفســـاء (Arabic)

Taxonomic position: Insecta: Coleoptera: Dermestidae

3. Detection

Trogoderma granarium has the following life development stages: eggs on the surface of grain and other stored products; larvae (5–11 instars) in stored products (larvae may be found in packing material or within storage structures); pupae in stored products, in the last larval exuviae (cast skins); adults in stored products.

Methods to detect *T. granarium* infestations include inspection, physical search, use of food baits and pheromone traps. Often the infested material contains only larvae because (1) adult longevity is usually between 12 and 25 days (it can be as long as 147 days in unfavourable conditions), whereas larval longevity is usually 19–190 days (and can be up to six years in diapausing larvae); (2) most of the dermestid larvae occurring in stored products will partially or wholly consume dead adults; and (3) adults are most prevalent when conditions are favourable for population growth. Larval exuviae are usually not consumed so their presence is a clear indication of a possible active infestation. Larvae are extremely cryptic by nature, particularly diapausing larvae that may stay inactive for long periods in cracks and crevices where they are very difficult or nearly impossible to locate.

Many other dermestid species belonging to genera other than *Trogoderma* may occur in stored products. Members of *Dermestes* and *Attagenus* genera are frequently found feeding on materials of animal origin such as dog biscuits, dried meat and dried blood. They also feed on rat, mice and bird carcasses. *Anthrenus* and *Anthrenocerus* species can be serious pests of wool and woollen products. In stored products heavily infested with other stored products pests, non-pest *Trogoderma, Anthrenus* and *Anthrenocerus* are usually found feeding on carcasses of these pests.

T. granarium infestations are usually recognized by (1) the presence of the pest (especially feeding larvae and exuviae) and (2) symptoms of infestation. The short-lived adults are sometimes not seen. Damage to the commodities can be a warning sign, but often it is a result of the feeding of other common stored product pests. Larvae usually feed first on the germ portion of cereal seeds and then on the endosperm. The seed coat is eaten in an irregular manner. In bulk commodities infestations usually concentrate in the surface layers, where numerous larval exuviae, broken setae and frass (excrement) are present (Figure 1). However, larvae can occasionally be found as deep as 3–6 m in bulk grain. It is therefore important to consider biased sampling when inspecting for these types of pests.

Samples of suspect products have to be visually inspected in a well-lit area, using a 10× magnification hand lens. If appropriate, samples should be passed over sieves with aperture sizes relevant to the particle size of the products. Usually sets of sieves of aperture sizes 1, 2 and 3 mm are used. The sifted material collected on particular sieves should be placed in Petri dishes and examined under at least 10× to 25× magnification through a stereoscopic microscope to detect the pest. This screening technique allows the detection of various developmental stages of the pest. However, some larvae feeding within grains may remain undetected. Therefore, it may become necessary to heat samples to 40° C to drive pests out of the grains with an extractor tool such as a Berlese funnel, especially in case of heavy infestation. Visual inspection is preferable to sieving because the latter can easily destroy or seriously damage dead adults and larval exuviae rendering the morphological identification very difficult or impossible.

Inspections for this pest are particularly difficult in cases of low-level infestation. The larvae of *Trogoderma* species are most active at dawn and dusk. Populations can persist in small quantities of residues that may occur within a structure or mode of transport. Larvae in diapause can survive long periods without food. For diapausing larvae it is important to search under pieces of dirt, flaking paint and rust and also in empty packaging materials such as hessian bags, tarpaulins and corrugated cardboard. Larvae are often hiding behind wall panelling, under internal lining, between floorboards, under insulation, on dry ledges, electrical cable trays and conduits, switch boxes etc. Because larval exuviae become airborne very easily, window sills, grilles of ventilators, ledges and spider webs must be checked. Rodent traps containing baits should be always inspected.

Additionally to initial inspections, it is possible to monitor the presence of *T. granarium* using various traps. Food-baited traps (containing oil seeds, peanuts, wheat germ) or attractant traps (containing wheat germ oil) can be used to attract larvae. Simple traps offering hiding places for the larvae, such as pieces of corrugated cardboard or hessian bag, can be placed on the floor. After monitoring, all the traps should be destroyed. Adults may be detected with the use of pheromone traps where the pheromone capsule is combined with a non-drying sticky trap. However, the *Trogoderma* pheromone traps are not species-specific and attract many species of dermestid beetles (Saplina, 1984; Barak, 1989; Barak *et al.*, 1990; Mordkovich and Kolov, 2000). Traps baited both with pheromone and food bait are commercially available.

Insects found should be picked out carefully with small forceps or collected using an aspirator. It is important to collect multiple specimens of the pest. Identification of larvae is difficult; if the dissection of a single specimen is not successful and serious damage occurs to the mouthparts, exact identification is impossible. Specimens should be placed in 70% ethyl alcohol for preservation and safe shipping if the identification is not done immediately at the same locality.

4. Identification

The genus *Trogoderma* in recent years has been reported to include 117 species (Mroczkowski, 1968), 115 species (Beal, 1982), 130 species (Háva, 2003) and 134 species (Háva, 2011). There are many other species of *Trogoderma* yet to be described. Great caution needs to be exercised with the synonymies established because few of them are based on detailed comparison of the type specimens.

Identification of *Trogoderma* eggs and pupae based on external features is currently not possible. Insect eggs and pupae possess very few external features and therefore are poorly studied. Larval identification is difficult. It requires experience in identification and also good skills in dissection of small insects. Pupation takes place in the last larval cast. The larval exuviae can be used for identification, but one needs to be more cautious because the material is brittle. Adults are the easiest to identify, though misidentification is still common, so training in preparation, mounting and determination of *Trogoderma* specimens is required.

Adults in good condition can be identified by experienced staff using a stereomicroscope at 10× to 100× magnification. However, for reliable identification it is recommended that the genitalia are always examined. Movement of the stored product, particularly cereals, will damage the dead adults.

In most cases the legs and antennae will break off and also the setae on the elytra and pronotum will be rubbed off. In the case of a damaged specimen with missing body parts or morphological features not visible, identification should always be based on examination of the genitalia. Genitalia should be removed (section 4.2) and mounted temporarily on a cavity microscope slide using glycerol, Hoyer's medium (50 ml water, 30 g gum arabic, 200 g chloral hydrate, 20 ml glycerine[1]) or similar mounting media.

For larval identifications the mouthparts should be dissected out (section 4.1). The larval exuviae and dissected mouthparts should be mounted on a cavity microscope slide using Hoyer's medium (Beal, 1960) or other mounting media, such as polyvinyl alcohol (PVA). Details of mounting procedures are included in section 4.1.

Adult and larval dissection can be performed under 10× to 40× magnification using a stereomicroscope. For the examination of genitalia and larval mouthparts, particularly the papillae of the epipharynx, a good-quality compound microscope is necessary and must be capable of 400× to 800× magnification in bright field and phase contrast. Use of higher magnifications (1000×) may be necessary to achieve a more satisfactory resolution.

Methods have been developed for the identification of a limited number of pest *Trogoderma* species, using both immunological (ELISA test) and molecular techniques for specific purposes. As these methods still do not allow for a reliable and unequivocal distinction between *T. variabile* and other *Trogoderma* species that are likely to occur in stored products, they still cannot be used as quarantine diagnostic techniques for the determination of insect specimens found during inspection of stores and consignments of plant material in trade. Currently, research is being carried out this area in the USA and Australia.

4.1 Procedure for preparation of larvae and larval exuviae

Before dissection the larva should be examined under a stereomicroscope. Size, body colour, arrangement and colour of setae should be recorded. Use of microscope photography provides a record of material prior to disturbance via manipulation and handling and so allows for its independent interpretation.

For identification the larvae should be mounted in Hoyer's medium or other mounting media such as PVA on a microscope slide using the following method:

(1) First, place the specimen on a microscope slide; it is best done ventral side up in order to preserve the diagnostic characters.

(2) Cut open the whole body along the mid-line from under the head capsule to the last abdominal segment using eye surgery scissors.

(3) Next put the larva in a test-tube containing 10% potassium hydroxide (KOH) solution and put in a water bath until larval tissues loosen and begin to separate from the cuticle.

(4) Rinse thoroughly in warm distilled water.

(5) Remove all internal tissues using a very fine, short hair brush or the convex surface of a hooked tip of a no. 1 insect pin, or a loop formed from a micropin. All setae should be removed from one side of the 7th and 8th abdominal segment; stains such as acid fuchsin or chlorazol black may be used to make the analysed structures more visible.

(6) Remove the head capsule and put it back in the hot KOH solution for 5 minutes. Rinse the head capsule in warm distilled water. Dissection of the head can be performed in a few drops of Hoyer's mounting medium or glycerol on a microscope slide or in water in an excavated glass block. Turn the head ventral side up and hold it to the glass with a blunt no. 1 insect pin.

(7) Remove the mandibles, maxillae and labial palpi using jeweller's forceps and micropins. Remove the epipharynx and antennae, which may be additionally stained with a stain such as

[1] Some experts prefer Hoyer's mounting medium containing 16 ml of glycerine.

acid fuchsin or chlorazol black. Mount the head capsule and the mandibles in the cavity of the slide using Hoyer's medium or another mounting media. Mount the cleared skin, fully opened on the flat part of the microscope slide, next to the cavity. It is usually best done ventral side up. Epipharynx, antennae, maxillae and labial palpi should be mounted with the skin under the same cover slip. Mount all body parts on the same microscope slide.

(8) In the case of larval exuviae, before proceeding with the dissection soak the specimen in a 5% solution of any laboratory detergent for about two hours and rinse thoroughly in distilled water. Cut the specimen open anteriorly and dissect out the mouthparts. They can be mounted directly in Hoyer's medium without clearing.

(9) Label slides immediately after mounting specimens and place them in an oven for at least three days at 40 °C to improve their quality (the best slides are obtained after 2–4 weeks). After drying, ring the slides using any lacquer recommended for sealing of microscopic slides (e.g. Glyptal, Brunseal), or at least two layers of nail polish in order to prevent the Hoyer's medium from drying and possibly damaging the specimen. However, microscopic slides may be examined immediately after preparing.

Permanent slides can be made using Euparal or Canada balsam for mounting, but these require a laborious dehydration process.

4.2 Procedure for preparation of adults

Adult *Trogoderma* specimens may need to be cleaned before identification, with any laboratory detergent or using an ultrasonic cleaner. If the specimen was caught in a sticky trap the glue can be dissolved using a number of solvents (e.g. kerosene). These solvents can be removed from the specimen with any laboratory detergent.

Before beginning the preparation, soak the adult in warm distilled water for about an hour. Perform the preparation in the following way:

(1) First remove abdomen while the specimen is still in the water using fine forceps. Dry the specimen (minus abdomen) and mount it on a cardboard rectangle, preferably laterally. The specimen will be less exposed to damage and accessible for both dorsal and ventral examination if it is glued on the side.

(2) Next cut the abdomen laterally open, leaving the last abdominal segment untouched. Place it in a 10% KOH or sodium hydroxide (NaOH) solution in a hot water bath for about 10 minutes.

(3) Rinse the specimen in water and carefully remove the genitalia using hooked micropins. After removing the genitalia the abdomen should be glued onto the same cardboard rectangle with the insect's ventral side facing up.

(4) The genitalia need to be macerated further in the caustic solution. Separate the aedeagus from the peripallic tergum and the 9th abdominal segment using micropins. They may be stained with a small quantity of acid fuchsin or chlorazol black to make them more visible.

Genitalia can be mounted on a microscope slide using Hoyer's medium or other mounting media such as PVA. The aedeagus should be mounted on a cavity microscope slide to keep its shape. Female genitalia can be mounted on a flat microscope slide.

Slides and pinned insects should be labelled immediately after mounting the specimens. The slides should be placed in an oven for at least three days at 40 ºC (the best slides are obtained after 2–4 weeks). After drying, all slides should be ringed (see section 4.1.(9)).

If there is no need for mounting the genitalia using a permanent or semi-permanent mounting agent, they can be examined in a drop of glycerol on a microscope slide. After the identification the organs can be placed in a microvial in a drop of glycerol or glued onto the cardboard rectangle next to the abdomen.

4.3 Genera of the family Dermestidae frequently occurring in stored commodities

Besides *Trogoderma*, other dermestid genera may also be found in stored products, such as *Anthrenus*, *Anthrenocerus*, *Attagenus* and *Dermestes*. The first step of diagnosis of collected specimens is identification to genus. Adults of these beetles, and in some cases larvae, can be identified using at least one of the keys of Mound (1989), Haines (1991), Kingsolver (1991), Banks (1994), Háva (2004) and Rees (2004). Genera of the North American Dermestidae can be identified using the key of Kingsolver (2002).

The simple keys below (Key 1 and Key 3) quickly enable *Trogoderma* to be distinguished from four other dermestid genera commonly occurring in stored commodities. Distinguishing characters are illustrated in section 9, Figures 2 to 23. It should be mentioned that other genera of dermestid beetles may also be found in stores. These genera include *Thaumaglossa, Orphinus* and *Phradonoma* (Delobel and Tran, 1993). However, stores are not typical habitats for them, so they are not included in above-mentioned keys.

4.3.1 Differentiation of dermestid larvae

Dermestid larvae may be differentiated using a simple key (Key 1). Larval or pupal specimens identified to *Trogoderma* genus with this key are very likely to belong to a species from this genus and therefore it is warranted to check the detailed list of their features listed in section 4.4.1.

If the diagnostic key being used was not specifically written to include the area of origin (and interception) of the specimens, the key should be used with caution as there are many undescribed species of Dermestidae worldwide.

Key 1: Simple key for differentiation of dermestid larvae

1. Urogomphi present on 9th abdominal segment, 10th segment sclerotized, cylindrical ..... ***Dermestes* spp.**

Urogomphi absent, 10th abdominal segment not sclerotized **2**

2. Dorsal surface without hastisetae, maxillary palp 4-segmented ..... ***Attagenus* spp.**

Dorsal surface with hastisetae (Figure 18(a)), maxillary palp 3-segmented **3**

3. Posterior margins of abdominal terga sinuate, or emarginate, tufts of hastisetae placed on posterior membranous parts of terga, 8th abdominal tergum without tufts of hastisetae ..... ***Anthrenus* spp.**

Posterior margins of terga not sinuate or emarginate, tufts of hastisetae placed on sclerotized tergal plates, 8th tergum with tufts of hastisetae **4**

4. Second antennal segment about twice as long as last segment, head of hastisetae at least three times as long as wide at the widest point ..... ***Anthrenocerus* spp.**

Second and last antennal segments subequal, head of hastisetae less than three times as long as wide at widest point ..... ***Trogoderma* spp.**

4.4 Identification of *Trogoderma* larvae

There is no published key that covers all *Trogoderma* species. In part this is because there are still many undescribed species. Several keys have been published for the economically important species. Banks (1994) published a key to adults and larvae of the genus *Trogoderma* associated with stored products, as well as keys to larvae and adults of some species found in warehouses. Beal (1960) constructed an identification key to larvae of 14 species of *Trogoderma* from different parts of the world, including stored products pests. Mitsui (1967) published illustrated keys for identification of larvae and adults of some Japanese *Trogoderma* species. Kingsolver (1991) and Barak (1995) published keys to adults and larvae of some dermestid beetles, including a few *Trogoderma* species.

Zhang *et al.* (2007) published a key for identification of eight economically important species in the genus *Trogoderma.*

4.4.1 Discriminating features of *Trogoderma* larvae

Discriminating features of *Trogoderma* larvae below are adapted from Rees (1943), Hinton (1945), Beal (1954, 1960), Okumura and Blanc (1955), Haines (1991), Kingsolver (1991), Lawrence (1991), Peacock (1993), Banks (1994) and Lawrence *et al.* (1999a):

(1) body elongated, cylindrical, somewhat flattened, roughly six times as long as wide, nearly parallel-sided but gradually tapering toward rear part

(2) head well developed, sclerotized, and hypognathous

(3) three pairs of jointed legs present

(4) pretarsal setae on the ventral side of claws unequal

(5) very hairy, being covered with different types of setae: hastisetae, spicisetae and/or fiscisetae (Figures 18 and 20)

(6) head of hastisetae not more than three times longer than wide (Figure 20)

(7) numerous hastisetae on all nota and terga, with prominent tufts of erect hastisetae inserted on the posterolateral part of the tergal plates of abdominal segments 6–8 (in Anthrenus genus the tufts of hastisetae are inserted on the membrane behind the sclerotized part of terga 5, 6 and 7)

(8) urogomphi absent.

4.4.2 Identification of *Trogoderma* last instar larva

Larvae of *T. granarium* (Figures 2(C), 2(D) and 21) may be separated from other *Trogoderma* species occurring in stores using the following short key (Key 2). This key does not allow for identification of all *Trogoderma* species known to occur in stores. So, if necessary, larvae of other pest and a few non-pest species can be identified, or at least separated, with reasonable confidence using the keys of Beal (1956, 1960), Banks (1994) and Peacock (1993). Features of larval specimens identified to *Trogoderma granarium* species with this key should next be compared with the detailed list of this species' features in section 4.4.3 and larval description in section 4.4.4.

Key 2: Identification key for *Trogoderma granarium* larvae

1. Epipharynx with 6 distal papillae, usually in a single sensory cup (Figure 23(A)).....**2**

Epipharynx with 6 distal papillae in a distal sensory cup; sometimes one or two papillae outside of the sensory cup (Figure 23(B), (C)).....**3**

2. Terga uniformly yellowish-brown, without greyish pigmentation at base of large spicisetae; acrotergites weakly sclerotized; antecostal suture on 8th abdominal segment almost always absent (if present, faint and usually broken); setae occupying 50 to 75% of the basal antennal segment, second segment usually with a single seta or no seta, apical segment with sensory pores in basal quarter; hastisetae morphology as in Figure 20(A), (B) .....***Trogoderma granarium* Everts**

Terga usually dark greyish-brown, at least at base of major spicisetae; acrotergites brownish, sclerotized; antecostal suture on 8th abdominal segment distinct; second antennal segment without setae; hastisetae morphology as in Figure 20(C), (D) ..... ***Trogoderma glabrum* (Herbst)**

3. Setae on basal antennal segment grouped on inner and inner-dorsal side leaving the outer and outer-ventral side glabrous; on fully extended antenna setae on basal segment not reaching apex of the second segment, sensory pore(s) on apical antennal segments not in basal quarter; median small spicisetae on acrotergites not long enough to extend over the antecostal suture (Figure 19(C); compare with Figure 19(D)); hastisetae (Figure 20(E), (F)) very sparse on thoracic and anterior abdominal terga (Figure 19(A)); terga with single row of large spicisetae (Figure 19(B)) . ***Trogoderma variabile* Ballion**

Specimen without above combination of characters .....**other *Trogoderma* spp.**

Larval identification should be considered unreliable if it is based only on one specimen, or exuviae or worn specimens. This is because in many species the intraspecific variation is such that in individual specimens features considered specific to the species may not be seen, while features specific to other species may be. In addition, large numbers of non-pest *Trogoderma* species occur in stored commodities and many of their characteristics are not well studied.

4.4.3 Discriminating features of *Trogoderma granarium* larvae

Discriminating features of *T. granarium* larvae are as follows:

(1) antennal segments subequal

(2) setae of basal antennal segment occupying 50–75% of the circumference of the segment, reaching or surpassing apex of second segment, at least three-fourths as long as the second antennal segment

(3) second antennal segment of last instar usually with one seta or sometimes no seta

(4) last antennal segment with at least one sensory pore in basal quarter

(5) epipharynx (Figure 22) with four papillae in distal sensory cup, usually in a single unit (Figure 23(A))

(6) fiscisetae absent

(7) mesally directed tergal setae absent

(8) at least six small spicisetae on first abdominal tergum posterior to antecostal suture, anterior to large spicisetae

(9) anterior-median small spicisetae anterior to antecostal suture long enough to reach over the suture

(10) large median spicisetae on first abdominal segment smooth or covered with inconspicuous scales with tips smooth for at least four times the diameter of seta

(11) antecostal suture of 8th abdominal tergum almost always absent, but if present, faint and interrupted

(12) antecostal suture on 7th abdominal tergum absent or interrupted

(13) no greyish pigmentation on sides of thoracic and other segments, not even at the base of large lateral spicisetae.

4.4.4 Description of *Trogoderma granarium* larvae

The first-instar larva (Figure 2(C)) is 1.6–1.8 mm long and 0.25–0.3 mm wide. Body is uniformly yellowish-white, head and hairs are reddish-brown. The mature larva (Figure 2(D)) is 4.5–6 mm long and 1.5 mm wide and body is reddish-brown. The larval body is covered with two kinds of hairs: spicisetae (Figure 18(B)), in which the shaft is covered with tiny, stiff, upwardly directed, pointed scales, and hastisetae (Figure 18(A)), in which the shaft is multi-segmented with spear-headed apex. Spicisetae are scattered over the dorsal surface of the head and body segments. Two groups of long spicisetae on the 9th abdominal segment form the tail. Hastisetae are found on all notal and abdominal segments, but on the last three or four segments they form distinctive, paired, erect tufts (Beal, 1960, 1991; EPPO/CABI, 1997).

4.5 Identification of *Trogoderma* adults

4.5.1 Differentiation of dermestid adults

Dermestid adults may be differentiated using a simple key (Key 3). Adult insect specimens identified to *Trogoderma* genus with this key are very likely to belong to a species from this genus and therefore it is warranted to check the detailed list of their features in section 4.5.2.

Key 3: Simple key for differentiation of dermestid adults

1. Median ocellus absent ..... ***Dermestes* spp.** (Figure 15)
Median ocellus present **2**

2. Body covered with scale-like setae; antennal cavity filled by antennae, fully visible from anterior view (Figure 14(A)) ..... ***Anthrenus* spp.** (Figure 17)
Body covered with simple setae, some of them whitish, flattened (ensiform) but never scale-like **3**

3. Antennal cavity completely closed behind, antennal club 3-segmented and well defined ..... ***Anthrenocerus* spp.**
Antennal cavity open behind or partially delimited by a posterior carina, antennal cavity much wider than antennae, not visible in anterior view **4**

4. Antennal cavity open behind, posterior margin of hind coxa angulate, first segment of posterior tarsus shorter than second segment ..... ***Attagenus* spp.** (Figure 16)
Antennal cavity carinate posteriorly, posterior margin of hind coxa straight, arcuate or sinuate, first segment of posterior tarsus longer than second segment ..... ***Trogoderma* spp.** (Figures 2(A), 4(A), 14(B)).

4.5.2 Discriminating features of *Trogoderma* adults

The features below are adapted from Hinton (1945), Beal (1954, 1960), Okumura and Blanc (1955), Haines (1991), Kingsolver (1991), Lawrence and Britton (1991), Peacock (1993), Banks (1994), Lawrence *et al.* (1999b) and Háva (2004):

(1) body ovate, densely setose, setae simple, usually 2–3 different types, recumbent, yellowish-white slightly flattened, sword-shaped setae

(2) presence of median ocellus

(3) pronotum without lateral carina

(4) antennal cavity of anteroventral surface flat, or only slightly visible in anterior view (Figure 14(B))

(5) antennal cavity carinate posteriorly at least to half of length and open laterally

(6) prosternum forming a "collar" anteriorly

(7) mesosternum deeply divided by sulcus

(8) posterior margin of hind coxal plate curved or sinuate, never angulate

(9) first segment of hind tarsus longer than second segment

(10) antennae short, 9–11-segmented, with a 3–8-segmented club, antennal outline usually smooth or barely flattened, terminal segment never disproportionately enlarged

(11) tarsi of all legs 5-segmented.

4.5.3 Identification of *Trogoderma* adults

The following short key (Key 4) should be used to distinguish adult *T. granarium* from some other *Trogoderma* species frequently occurring in stored commodities. This key does not allow for identification of all *Trogoderma* species known to occur in stores. So, if necessary, other species, not included in the key, can be identified with the keys of Beal (1954, 1956), Kingsolver (1991), Banks (1994), and Mordkovich and Sokolov (1999). These keys include species occurring in stored products and therefore may be used for identification of *Trogoderma* adults. It should be noted that identification of adult sex of various *Trogoderma* species is practically possible only after dissecting their genitalia (for morphology of male and female genitalia, see Figures 11 and 12). Checking of external distinguishing features as antennal club morphology should be performed on specimens surely identified to sex.

Features of adult specimens identified to *Trogoderma granarium* species with this key should be next compared with the detailed list of this species' discriminating features in section 4.5.4 and adult description in section 4.5.5.

Key 4: Identification key to *Trogoderma granarium* adults

1. Dorsal pubescence unicolorous ..... **non-pest *Trogoderma* spp.**

Dorsal pubescence not unicolorous but with pattern or pubescence completely rubbed off; (ensiform setae in addition to yellowish- and reddish-brown setae) **2**

2. Elytra without well-defined pattern, unicolorous or vaguely mottled **3**

Elytra with well-defined lighter and darker areas (Figure 3) **4**

3. Integument black, rarely with vague brownish maculation, basal loop, submedian and subapical bands formed by yellowish and whitish, ensiform setae; antennae always 11-segmented, male antennal club 5–7-segmented, female 4–5-segmented; 5th sternite of male with uniform, recumbent setae ..... ***Trogoderma glabrum* (Herbst)** (Figure 6(B))

Integument light reddish-brown, often with indistinct lighter maculation, scattered ensiform setae rarely forming 2–3 indistinct bands; antennae usually 11-, rarely 9- 10-segmented, male antennal club 4–5-segmented, female 3–4-segmented; 5th sternite of male with apical patch of dense, coarse setae ..... ***Trogoderma granarium* Everts**

4. Elytral integument with distinct light basal loop **5**

Elytral integument with distinct bands and spots only **7**

5. Anterior margin of eyes distinctly emarginated ..... ***Trogoderma inclusum* LeConte** (Figure 6(D))

Anterior margin of eyes straight or slightly sinuate **6**

6. Basal loop never connected to the antemedian band ..... ***Trogoderma variabile* Ballion** (Figures 4(A)–4(C), 5, 6(H))

Basal loop of elytral maculation connected to the antemedian band by a longitudinal band or bands (*T. inclusum* with less obvious emargination of eyes may key out here) ..... ***Trogoderma ornatum* (Say)** (Figure 6(E)), ***T. simplex* Jayne** (Figure 6(F)), ***T. sternale* Jayne** (Figure 6(G)), ***T. versicolor* (Creutzer)** (Figure 6(I))

7. Elytral integument with three well-defined (basal, submedian and apical) fasciae, setae on fasciae large, white, ensiform with very sparse yellowish recumbent setae ..... ***Trogoderma angustum* (Solier)** (Figure 6(A))

Elytral integument with well-defined basal band and median or posterior spot (Figure 5, left) ..... ***Trogoderma variabile* (reduced pattern)**

In general, elytral fasciae of *Trogoderma* species usually form a more or less complete basal loop, antemedian and median bands and apical spots. Some specimens have a reduced elytral pattern where the basal loop is indicated by curved anterior band, antemedian and/or median bands by small spots, and apical spots are usually missing.

For positive identification, all (especially in the case of damaged specimens) of the discriminating features should be observed (section 4.5.4).

Genital dissections should be carried out because there is a large number of undescribed *Trogoderma* species; by examining the genitalia, the chances of misidentifications are significantly reduced.

Maximova (2001) provides additional features for separating of adults of *Trogoderma granarium* from *T. variabile* and *T. glabrum*. Size and morphology of hind wings can be useful for identifying damaged specimens and although considering these two characteristics is not mandatory, it helps to

increase the certainty of identification based on other features (Figures 9, 10). During dissection hind wings must be removed and mounted in glycerol or Hoyer's medium.

Hind wings of *T. granarium* are smaller (mean length is 1.9 mm as compared with 2.5 mm for *T. variabile* and *T. glabrum*); they are paler in colour with less visible venation; number of setae S1 on costal vein (mean = 10) is half that on *T. variabile* and *T. glabrum* (mean = 20–23); number of small setae S2 between costal vein and pterostigma (mean = 2, sometimes absent) is less than that for *T. variabile* and *T. glabrum* (mean = 8) (Figures 9, 10).

4.5.4 Discriminating features of *Trogoderma granarium* adults

Adults of *T. granarium* are oblong-oval beetles, 1.4–3.4 mm long and 0.75–1.9 mm wide. The head is deflexed, head and pronotum darker than elytra, legs and abdomen are brownish. The elytra are brown. Females are slightly larger than males and lighter in colour.

To identify the adult stages of *T. granarium* correctly, specimens should correspond to the characters used to identify the family Dermestidae, the genus *Trogoderma* and the species *granarium*. These characters are as follows:

(1) elytral cuticle unicoloured, usually light brown or reddish-brown, or vaguely mottled without a clearly defined pattern

(2) elytral setae predominantly brown (yellowish or white setae forming no clearly defined banded pattern may also be present; these setae are gradually abraded off as the beetle moves around and the adult thus develops a shiny appearance)

(3) antennae with 9–11 segments; male antennal club with 3–5 segments; female antennal club with 3–4 segments (Figures 7, 8).

(4) inner eye margin straight or sinuate

(5) male abdominal tergum 8 more or less evenly sclerotized, with setae along its margin sometimes tending to be grouped medially; tergum 9 with proximal margin of broader section almost U-shaped; tergum 10 with many long setae

(6) serrate sclerites of bursa copulatrix of female small, not longer than corrugated part of spermatheca, with 10–15 teeth (Figures 12, 13(A))

(7) male genitalia with bridge straight and evenly wide, broader at connections to the parameres (Figure 11(A), (B)).

4.5.5 Description of *Trogoderma granarium* adults

The adult stage of *T. granarium* is illustrated in Figure 2(A), (B).

Adult male

Body length 1.7–2.3 mm (mean 1.99 mm), width 0.75–1.1 mm (mean 0.95 mm), ratio of length to width about 2.1:1.1. Head and pronotum dark reddish-brown; elytra reddish-brown, usually with indistinct lighter reddish-brown fasciae. Venter of thorax and abdomen reddish-brown; legs yellowish-brown.

Setae: Dorsal surface with evenly distributed, coarse, semi-erect, yellowish-brown and few, scattered, dark reddish-brown setae, with the colour of setae corresponding to the colour of the cuticle beneath; pronotum medially and laterally with indistinct patches of yellowish-white, ensiform setae, elytra with two or three indistinct bands of yellowish-white, ensiform setae. Ventral surface with dense, simple setiferous punctures, which are denser on ventrites, setae fine, short, recumbent, yellowish-brown.

Head: Punctures large, largest anteriorly, ocellate, separated by a distance of about the diameter of one to five punctures, surface between them shiny. Antennae yellowish-brown, 9-, 10- or 11-segmented with 4- or 5-segmented club. Antennal fossa shallow, loosely filled in by antenna. Eyes medially straight, or sometimes slightly sinuate.

Thorax: Anterior margin of pronotum with row of yellowish-brown, coarse setae pointing to middle of anterior margin, setae on anterior half of disc pointing backward, on posterior half pointing to the scutellum. Punctures slightly larger and more dense along anterior and lateral margins, and medially, otherwise small, simple on disc and separated by about 2–4 diameters.

Posterolateral end smooth, shining, otherwise very finely and densely punctured. Prosternum densely punctured, sides of posterior process straight and gradually tapering to apex.

Elytra densely punctured by setiferous punctures, punctures small, denser laterally, on disc separated by 2–4 diameters, laterally by 1–2 diameters.

Hind wings with vague venation; mean number of larger setae S1 on costal vein is 10, mean number of small setae S2 between costal vein and pterostigma is 2, but sometimes these are missing (for additional details see Figure 9).

Tibiae with small spines along outer edge. Proximal segment of hind tarsi about same length as second; distal segment about twice as long as fourth segment.

Abdomen: First ventrite with or without weak femoral lines. Ventrites covered by fine, yellowish-brown, recumbent setae, posterior half of penultimate ventrite with very dense, coarser, semi-erect, dark yellowish-brown setae.

Genitalia: Distal end of median lobe of aedeagus shorter than apices of parameres. Parameres wide, with sparse, short setae on inner and outer margins, setae extending to half the length of aedeagus. Paramere bridge is located at about one third of the total length from distal end, straight distally and proximally, bridge is as wide as or wider than aedeagus at crossing, basal process is tapered.

Adult female

Body: Length 2.1–3.4 mm (mean 2.81 mm); width 1.7–1.[illegible] mm (mean 1.84 mm); ratio of length to width about 1.6:1.

Antenna sometimes less than 11-segmented, club 3-segmented.

Posterior half of penultimate ventrite without a dense fringe of semi-erect, yellowish-brown, coarse setae.

Other external morphological characters as in male above.

Genitalia: Bursa copulatrix with two small dentate sclerites, length of sclerites equal to or shorter than the length of the corrugated part of spermatheca.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27.

In cases where other contracting parties may be adversely affected by results of the diagnosis, the records and evidence (in particular, preserved larvae and adults, slide-mounted specimens, photographs) should be kept for at least one year.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Department of Agriculture and Food Western Australia, Biosecurity & Research Division, Plant Biosecurity Branch, Entomology Unit, 3 Baron-Hay Court, South Perth, WA 6151, Australia (tel: +61 8 9368 3248, +61 8 9368 3965; fax: +61 8 9368 3223, +61 8 9474 2840; e-mail: aszito@agric.wa.gov.au).

Main Inspectorate of Plant Health and Seed Service, Central Laboratory, Żwirki i Wigury 73, 87-100 Toruń, Poland (tel: +48 56 639 1111, +48 56 639 1115; fax: +48 56 639 1115; e-mail: w.karnkowski@piorin.gov.pl).

Laboratorio de Plagas y Enfermedades de las Plantas. Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Av. Ing. Huergo 1001, C1107AOK Buenos Aires, Argentina (tel:

+54 11 4362 1177, extns 117, 118, 129 and 132; fax: +54 11 4362 1177, extn 171; e-mail: abriano@senasa.gov.ar, albabriano@hotmail.com).

Disinfection Department of All-Russian Plant Quarantine Centre, 32 Pogranichnaya street, Bykovo-2, Ramensky area, Moscow region, Russian Federation (tel: +7 499 2713824, fax: +7 4952237241, e-mail: artshamilov@mail.ru).

7. Acknowledgements

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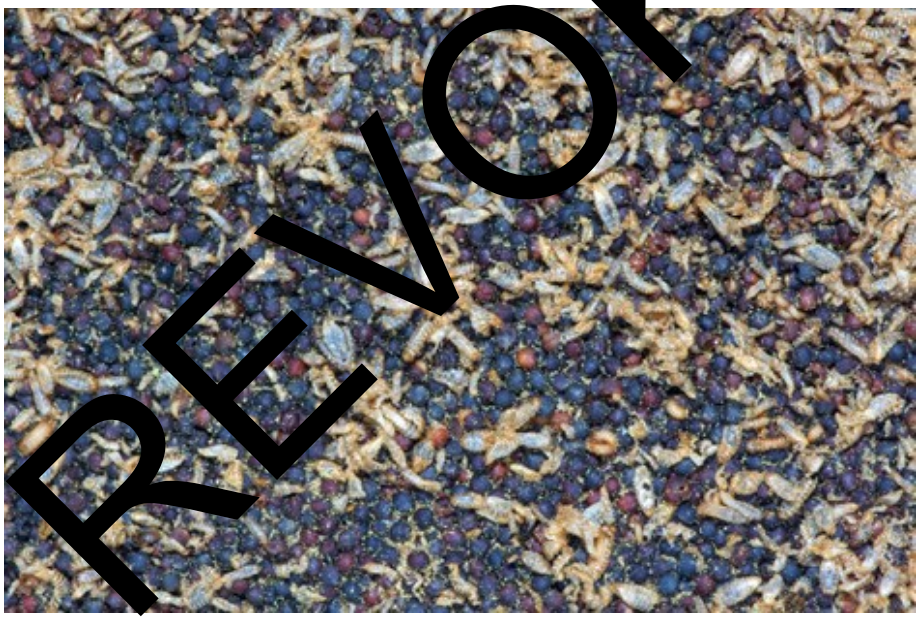
9. Figures

(A)

(B)



(C)

(D)

Figure 1: Symptoms of infestation of stored products with *Trogoderma granarium*: (A) damaged wheat grain; (B) infested rape seeds; (C) totally destroyed wheat grain (dust and remains of grains); (D) larval exuviae (cast skins) contaminating stored product (Paweł Olejarski, Instytut Ochrony Roślin - Państwowy Instytut Badawczy, Poznań, Poland)

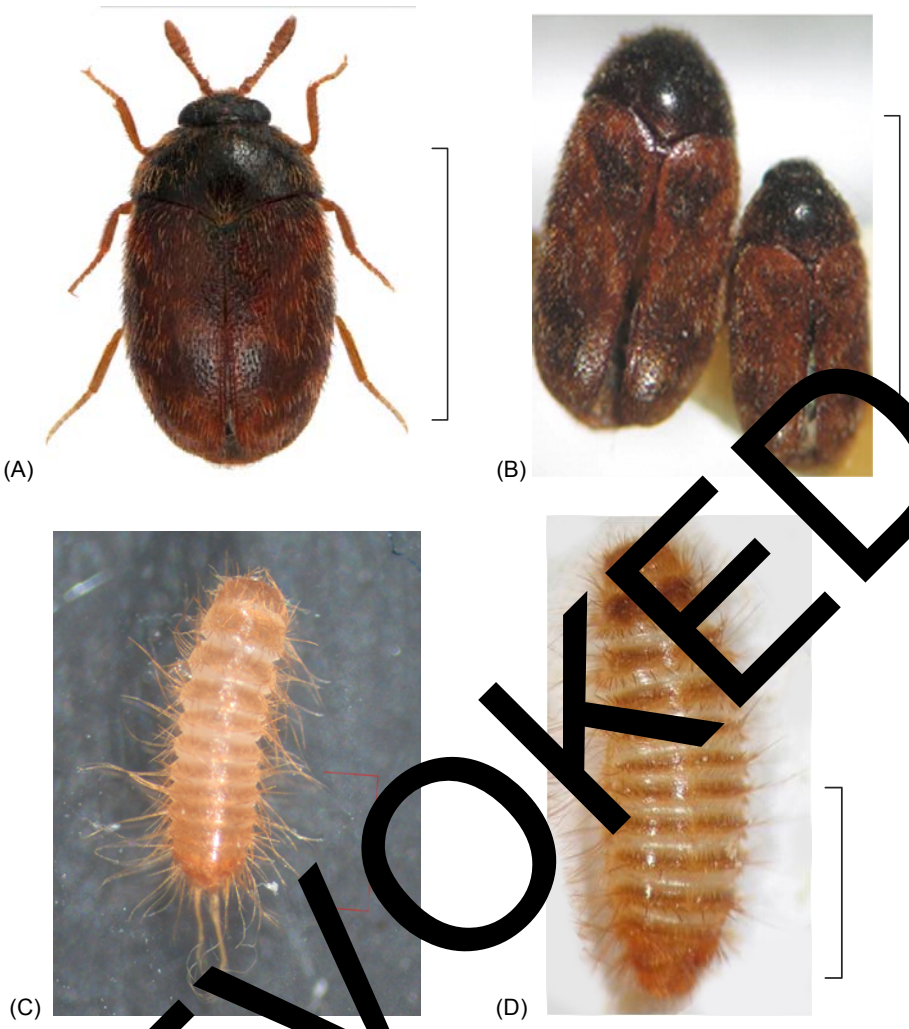


Figure 2: ***Trogoderma granarium*****:** (A) adult, female; (B) comparison of shape of female (left) and male (right); (C) young larva; (D) mature larva. Scale bar: (A), (B), (D) = 2 mm; (C) = 1 mm. ((A), Tomasz Klejdysz, Instytut Ochrony Roślin - Państwowy Instytut Badawczy, Poznań, Poland; (B), (D), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo Russia); (C), Cornel Adler, Julius Kűhn-Institut; (JKI) Germany))

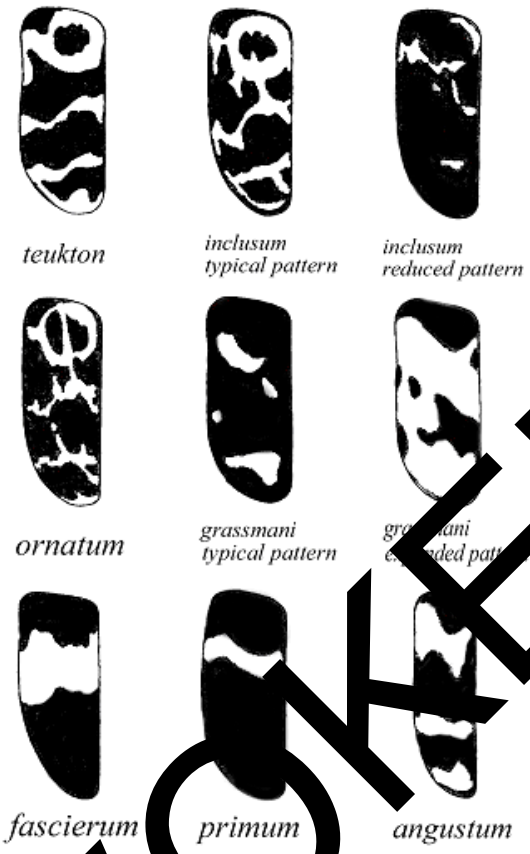


Figure 3: *Trogoderma* spp. elytral patte (Bea 954)

Figure 4: *Trogoderma [illegible]bile*: (A) [illegible]ematic drawing of the adult; (B) male; (C) female; (D) larva. Scale bar = 2 mm. ((A) [illegible]RSA (1999b); (B), (C), (D), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, B[illegible]ovo, R[illegible]sia)

Figure 5: Elytral pattern of *Trogoderma variabile*: *left*, reduced pattern; *centre*, typical; *right*, expanded (Beal, 1954)

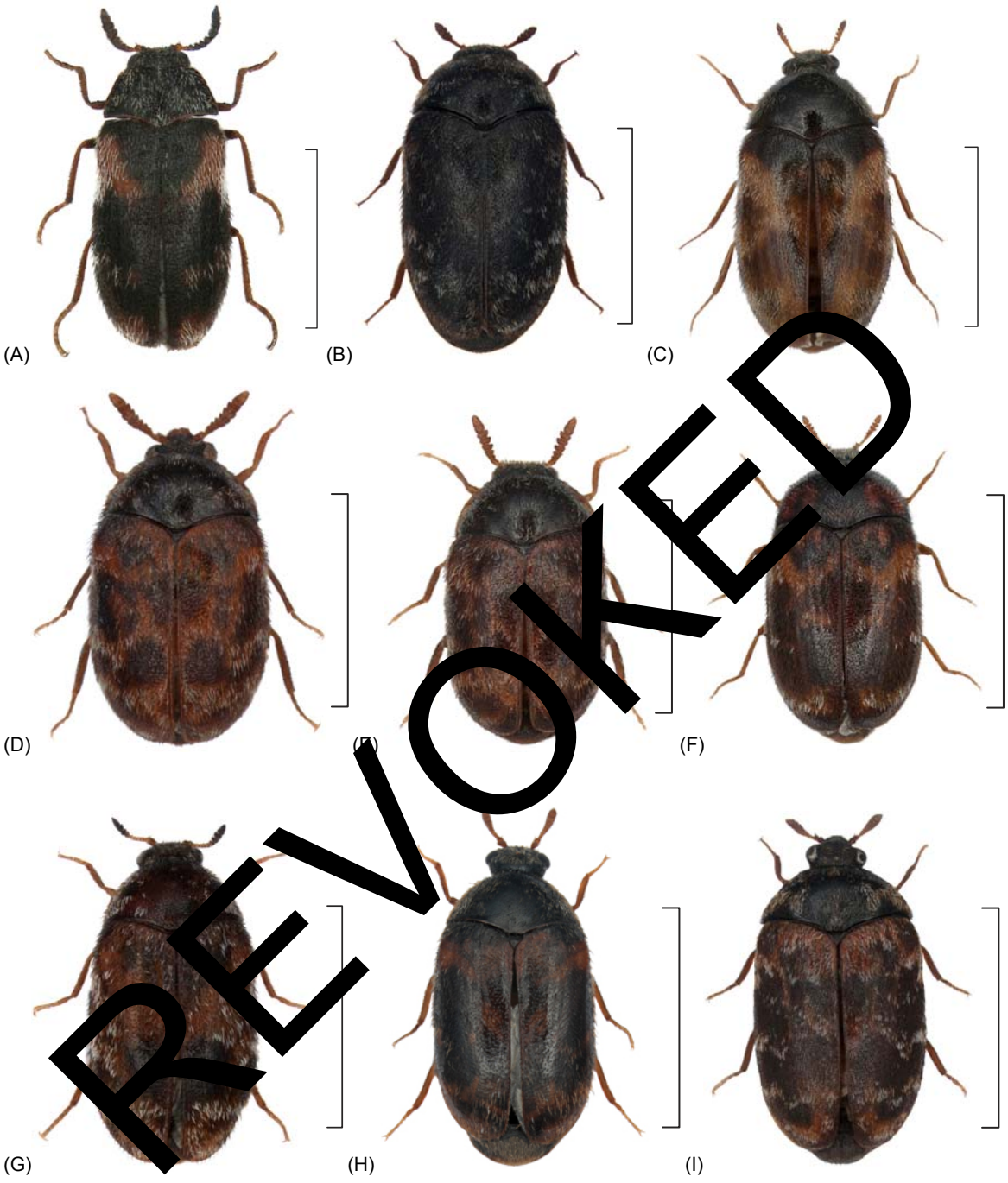


Figure 6: Comparison of females of some Trogoderma non-granarium species: (A) *T. angustum*; (B) *T. glabrum*; (C) *T. grassmani*; (D) *T. inclusum*; (E) *T. ornatum*; (F) *T. simplex*; (G) *T. sternale*; (H) *T. variabile*; (I) *T. versicolor*. Scale bar = 2 mm. (Tomasz Klejdysz, Instytut Ochrony Roślin - Państwowy Instytut Badawczy, Poznań, Poland)

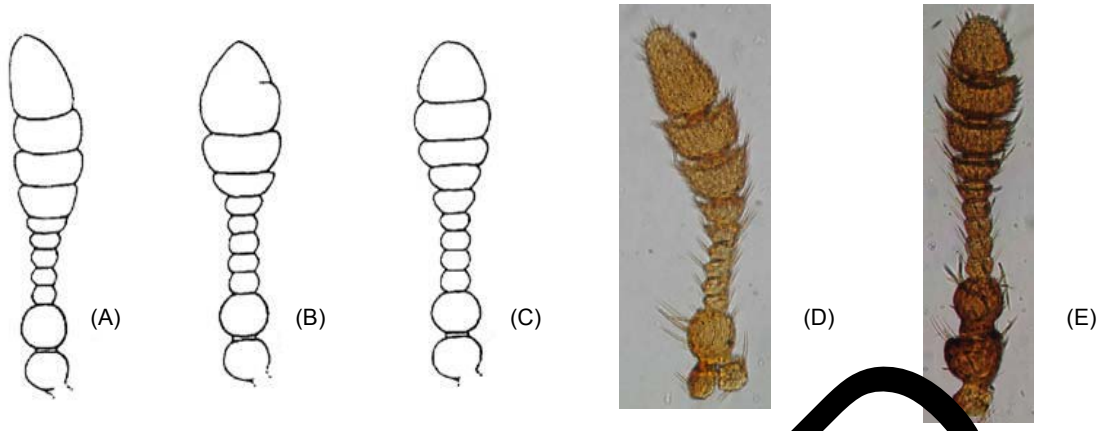


Figure 7: Antennae of *Trogoderma granarium*: (A), (D) male antenna with normal number of segments; (B) female antenna with reduced number of segments; (C), (E) female antenna with normal number of segments ((A)–(C), Beal (1956); (D), (E), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)

Figure 8: Antennae of some *Trogoderma* species: (A) *T. variabile*; (B) *T. glabrum*; (C) *T. teukton*; 1, male antenna with normal number of segments; 2, female antenna with normal number of segments (Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)

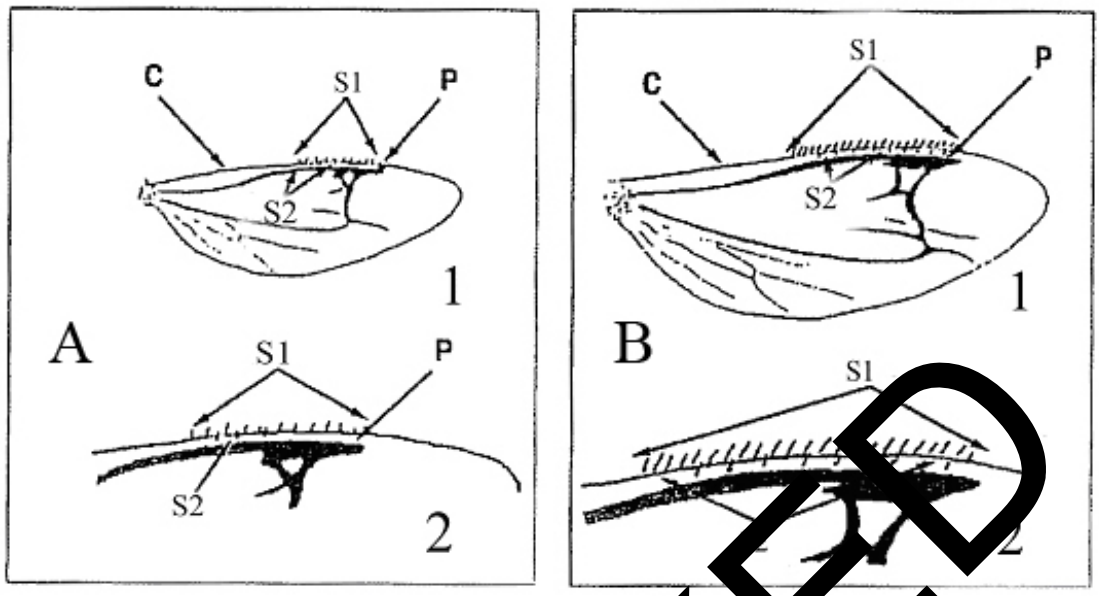


Figure 9: Schematic representation of the morphology of the hind wing: (A) *Trogoderma granarium* (Maximova, 2001), with up to 14 S1 setae on costal vein (mean = 1 S1), and 2–5 S2 setae, or with no S2 setae, between costal vein and pterostigma (mean = 2 S2); (B) *Trogoderma variabile* and *T. glabrum* with 16 or more than 16 S1 setae.
Details: 1, general morphology of the wing; 2, enlarged anterior part of the wing (C, costal vein; P, pterostigma; S1, setae on costal vein; S2, small setae between costal vein and pterostigma). The number of S2 setae is not used for the diagnosis because this character is not known for other species.

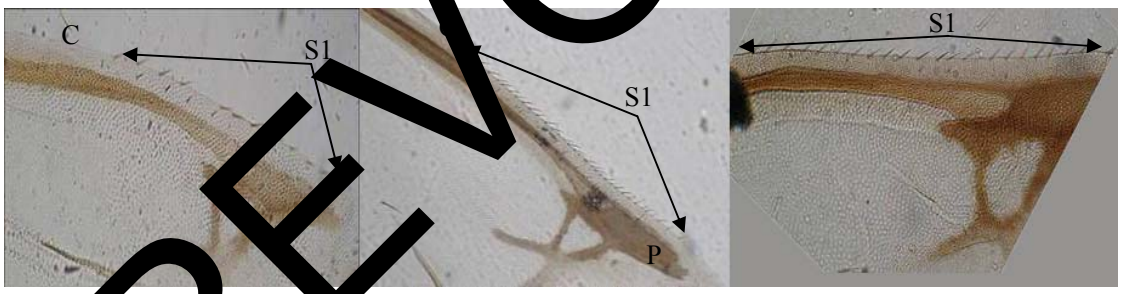


Figure 10: Morphology of hind wings: (A) *T. granarium*; (B) *T. glabrum*; (C) *T. variabile* (Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)

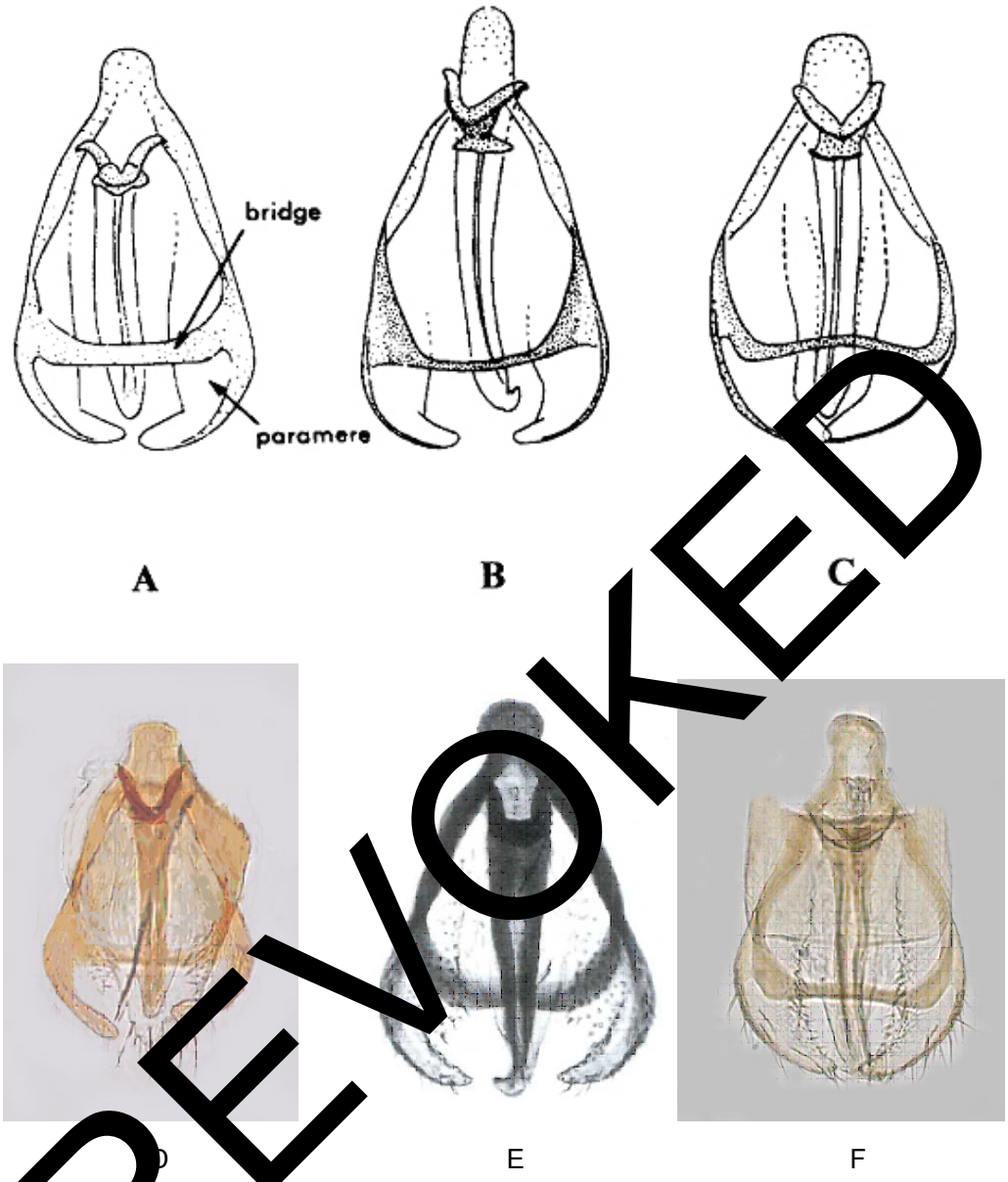


Figure 11: Male genitalia: (A), (D) *Trogoderma granarium*; (B) *T. inclusum*; (C), (F) *T. variabile*; (E) *T. glabrum* ((A)–(C), Gilbert (1979); (D)–(F), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)

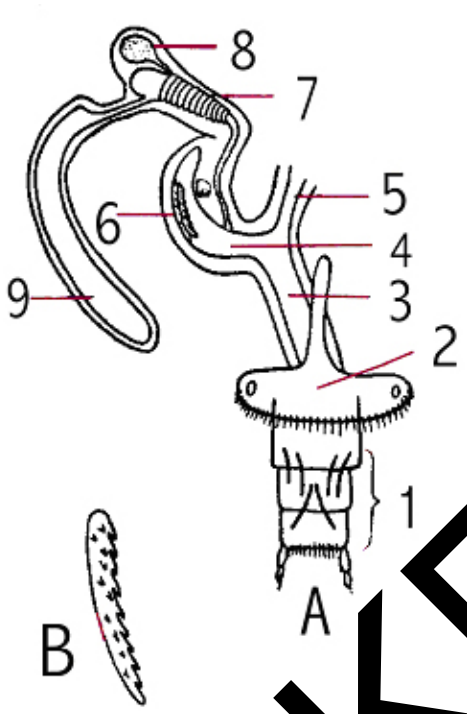


Figure 12: Female genitalia of *Trogoderma granarium*: (A) general view of genitalia; (B) one of the serrate sclerites from the bursa copulatrix (Varshalovich, 1963). Details: 1, ovipositor; 2, 7th abdominal sclerite; 3, vagina; 4, bursa copulatrix; 5, oviduct; 6, two serrate sclerites of bursa copulatrix; 7, corrugated part of spermatheca; 8, spermatheca; 9, accessory glands.

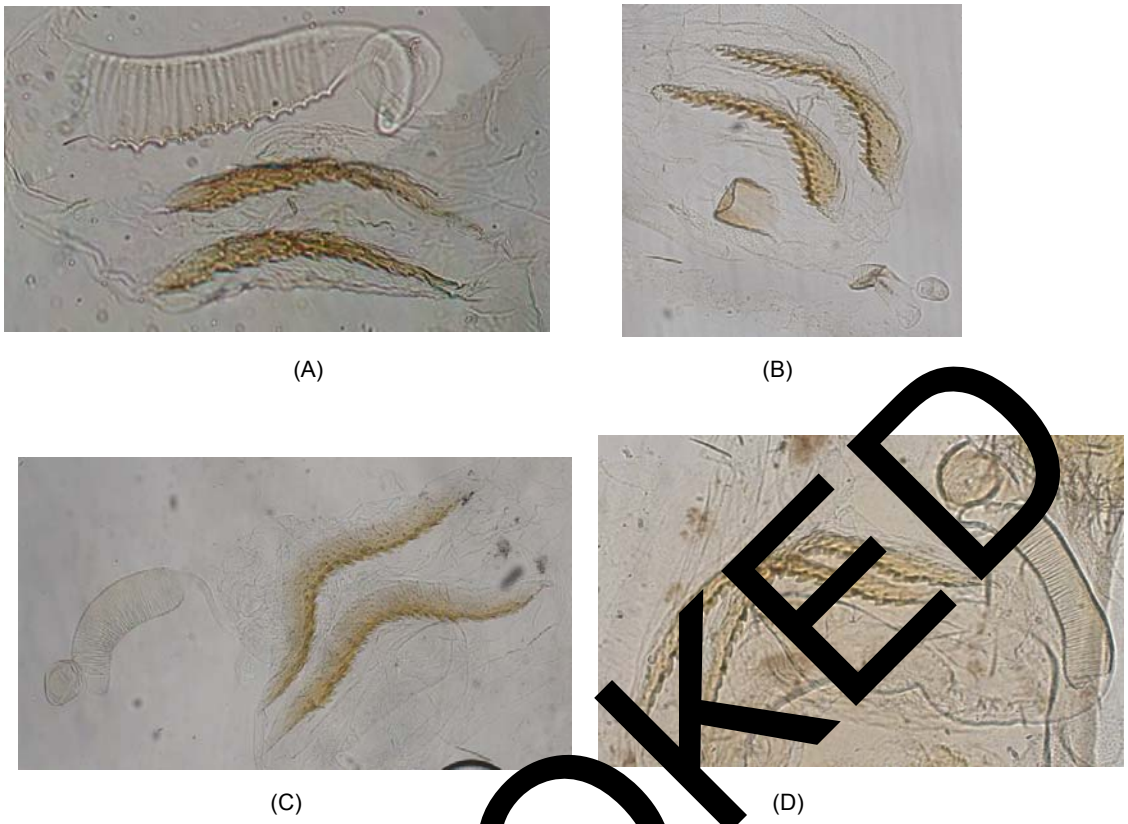
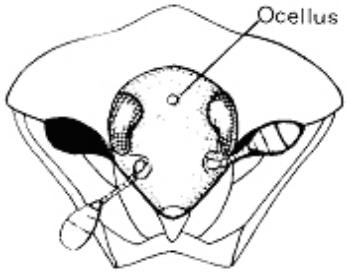


Figure 13: Serrate sclerites from the bursa copulatrix of female genitalia of various *Trogoderma* species: (A) *T. granarium*; (B) *T. variabile*; (C) *T. glabrum*; (D) *T. teukton* (Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)



(A)

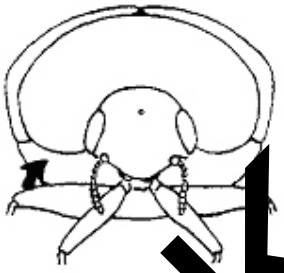
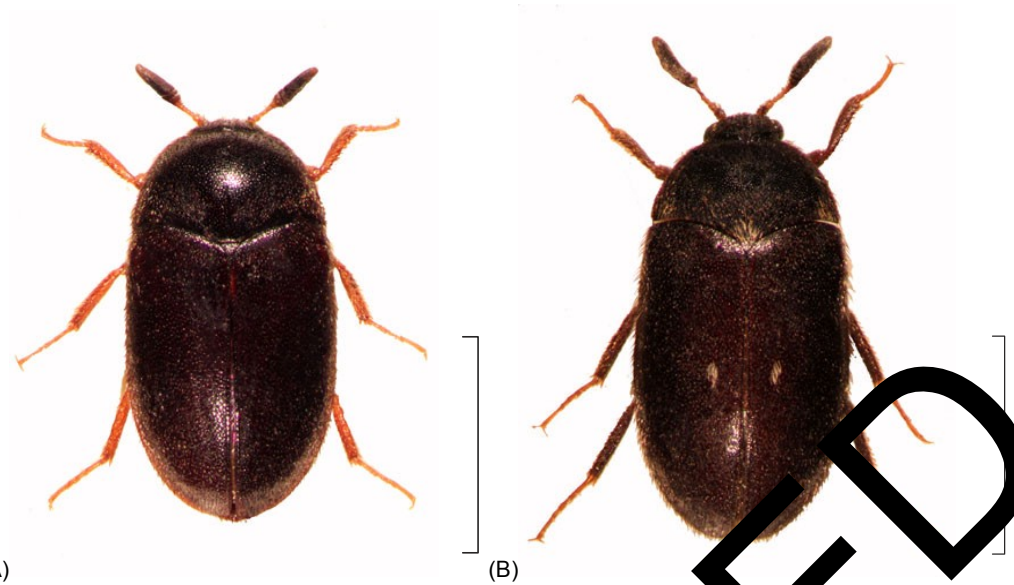


Figure 14: Antennal cavity: (A) antennal cavity clearly visible in anterior view (*Anthrenus*), antennae fully filling the cavity; (B) antennal cavity not visible in anterior view (*Trogoderma*), antennae loosely fit in the cavity ((A), Mound (1989), copyright: Natural History Museum, London, UK; (B), Kingsolver (1991))

(A) (B)

Figure 15: Adults of *Dermestes* species: (A) *D. lardarius*; (B) *D. maculates. S*cale bar = 2 mm. (Marcin Kadej, Instytut Zoologiczny, Uniwersytet Wrocławski, Wrocław, Poland)



(A) (B)

Figure 16: Adults of *Attagenus* species: (A) *A. unicolor*; (B) *A. pellio*. Scale bar = 2 mm. (Marcin Kadej, Instytut Zoologiczny, Uniwersytet Wrocławski, Wrocław, Poland)

Figure 17: Adult of *Anthrenus verbasci*: Scale bar = 2 mm. (Marcin Kadej, Instytut Zoologiczny, Uniwersytet Wrocławski, Wrocław, Poland)

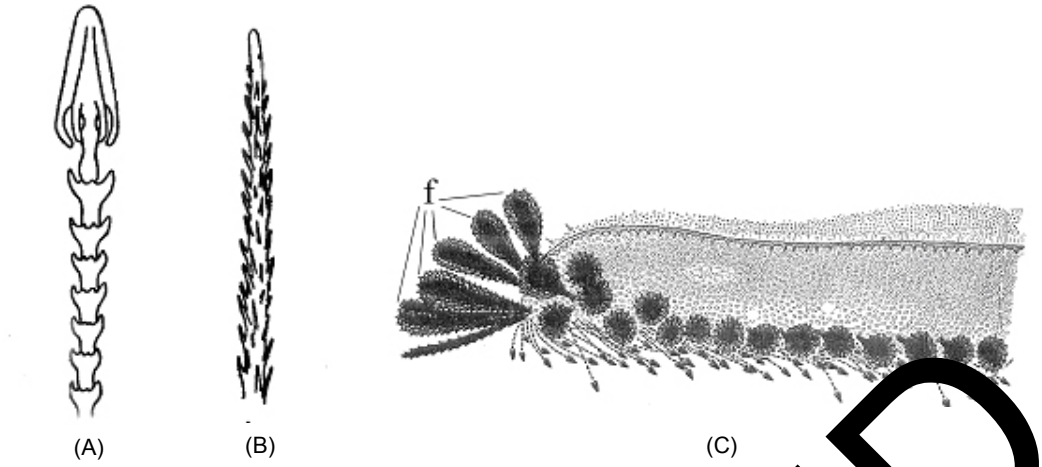


Figure 18: Larval setae: (A) hastiseta; (B) spiciseta; (C) fiscisetae (f) on first abdominal tergum of *Trogoderma carteri* larva ((A), (B), Varshalovich (1963); (C), Beal (1960))

Figure 19: Abdominal tergite and setae: (A) abdominal tergite of *Trogoderma variabile* larva with enlarged hastiseta; (B) first abdominal tergite of *T. variabile* larva; (C) setae of the anterior portion of first abdominal tergite not long enough to extend caudally over the antecostal suture (*T. variabile*); (D) the same setae long enough to extend caudally through the antecostal suture (*T.* non-*variabile*) ((A), Kingsolver (1991); (B), Beal (1954); (C), (D), OIRSA (1998a))

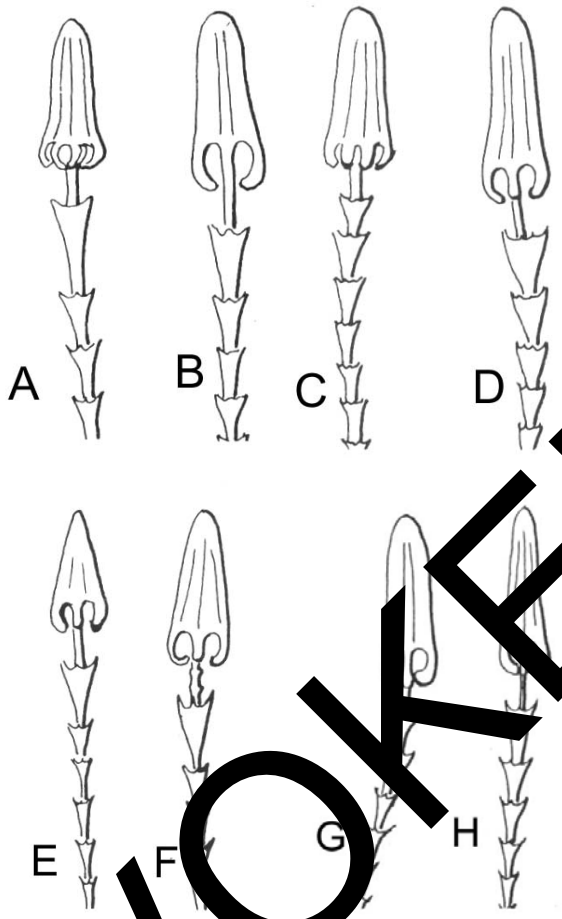


Figure 20: Comparison of hastisetae morphology of various *Trogoderma* larvae: (A), (B) *T. granarium*; (C), (D) *T. glabrum*; (E), (F) *T. variabile*; (G), (H) *T. inclusum*; copyright: Natural History Museum, London, UK (Peacock, 1993)

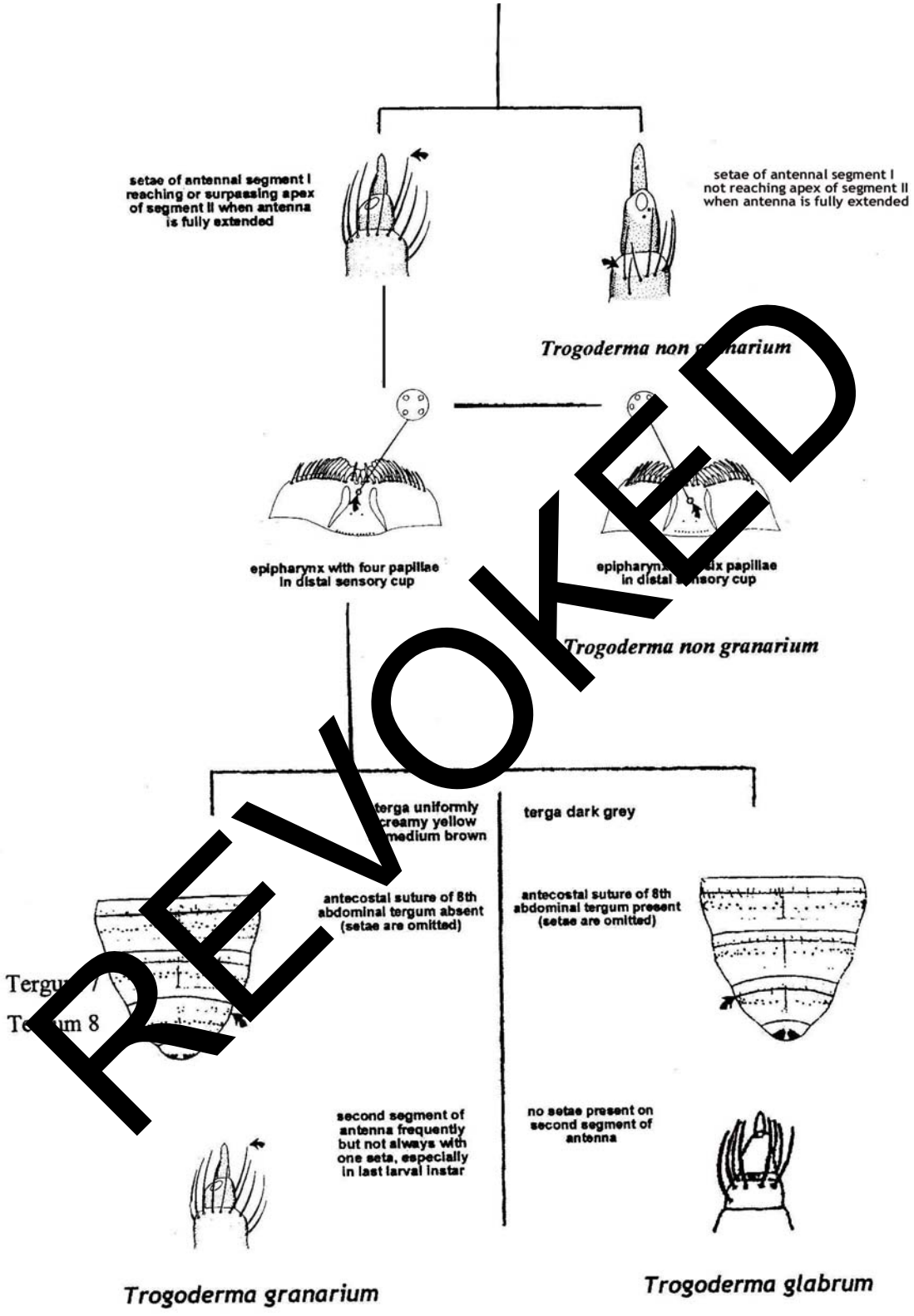


Figure 21: Pictorial key for distinguishing larvae of *Trogoderma granarium* from other species of *Trogoderma* (Kingsolver, 1991; OIRSA, 1999a)

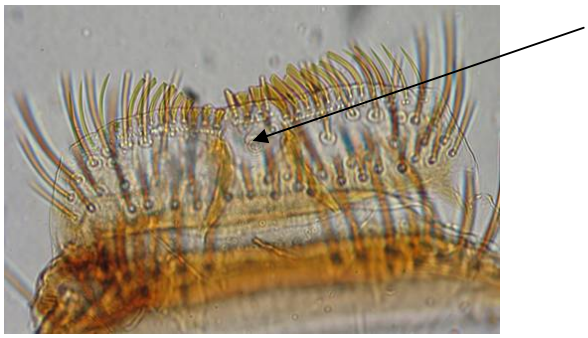
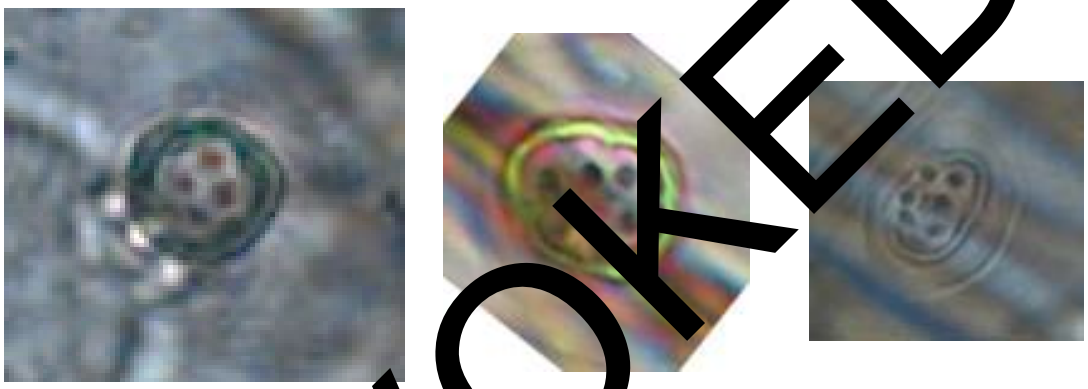


Figure 22: Epipharynx of *Trogoderma* sp. larva with a distal sensory cup marked [illegible] arrow (Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)



(A) (B) (C)

Figure 23. Distal papillae: (A) four distal papillae in sensory cup of *T. granarium* larva; (B) six distal papillae in *T. variabile*; (C) six distal papillae in *T. glabrum*. (Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo, Russia)

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2008-09 SC approved for member consultation via e-mail

2011-06 member consultation

2012-03 CPM-7 adopted Annex 3 to ISPM 27

ISPM 27. 2006: **Annex 3** *Trogoderma granarium* Everts (2012)

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