# STUDIES ON THE REMNANTS OF THE PARASITOID LARVAE OF *DRYOCOSMUS KURIPHILUS* YASUMATSU (HYMENOPTERA: CYNIPIDAE), IN THE GALLS COLLECTED IN TWO PLACES IN ITALY FOR DETERMINING PARASITISM LEVEL AND TYPE OF PARASITOIDS

**Mikdat Doğanlar***

* Honorary Professor, Biological Control Research Station, Adana, TURKEY. E-mail: mikdoganlar@yahoo.com.tr

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ABSTRACT: In several parts of the world almost 69 species of parasitoids/hyperparasitoids) of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), were obtained by several works conducted in the galls collected in the spring. Aims of the studies on remnants of larvae in the galls of *D. kuriphilus*, collected in autumn, 2014 sent from two places, Italy were: (1) for determining parasitism level of *Torymus sinensis*; (2) parasitism type of parasitoids in the galls. As study materials 135 galls collected in September, 2014 from Trehago (VR) Italy Se Hembre, and 185 galls, collected October, 2014 from Crespano del Grappa (TV) were send by Drs. Andrea Battisti and Fernanda Colombori, Padova University, Italy. From each group 10 galls were taken and dissecting for determining parasitism level of *T. sinensis* and parasitism type of parasitoids in the galls. For obtaining parasitism level, larvae of *D. kuriphilus* (dead ones) and *T. sinensis* (alive ones) were taken off from the galls, boiled in 10% KOH for 5 minutes, dissected, cleaned from fat-body and slide-mounted in Canada balsam for determining the shape of head structures, mainly mandibles, and spiracles. The other groups of galls were put into vials separately, and kept in room conditions, about 20-25$^{o}$C and 50-70% relative humidity. If galls have holes, the holes were signed by red pencil, in order to identify the new holes on the galls from which any hymenopterous adult will come out in the future. When adult comes out from gall, it was identified by following keys of several works. The gallery following hole of the adult was dissected for reaching to growing-chamber of adult(s) developed. The remnant of adults were taken off, and slide-mounted in Canada balsam, and figured them by using of Leica DM 500 microscopes with a digital Leica ICC 50 camera attached to it. The mandibles and spiracles of the species were identified. Types of parasitism were determined by the following ways: if one type of mandible was found in the gall, it would be *D. kuriphilus*, having two-teethed mandible; if two different mandibles were found in galls, the adult come out is the parasitoid and the other one is its host. By this way, In Trehago (VR) Italy Se Hembre the parasitism by *T. sinensis* was about 65.3% and 3.3% dead adult/lar. of ACGW, and 25.7% of its associates+ACGW (larvae+adults), 1.0% *E. annulatus* and 5.0% of *Pyemotes*, and in Crespano del Grappa (TV) the parasitism by *T. sinensis* was about 62.6% and from the galls 14.4% of dead adult/lar. of ACGW, and 23.0% of its associates +ACGW (larvae /adults),and 4.8% of *Pyemotes* (2.4 on *D. kuriphilus* and 2.4 on *T. sinensis*) were obtained from galls collected in Autumn. The characters of the mandibles and spiracles of *D. kuriphilus*, *T. sinensis*, *Eupelmus annulatus* Nees, *Eurytoma pistacina* Rondani were figured and discussed.

KEY WORDS: Cynipidae, parasitoid, galls, gall wasp

The Asian chestnut gall wasp (ACGW) is a potentially devastating exotic insect that causes globular twig, shoot, and leaf galls on actively growing shoots of all *Castanea* species. Galling reduces fruiting and nut yield, suppresses shoot elongation and twig growth, reduces tree vigor and wood production, and can kill

trees and galling also prevents infested shoots from producing new shoot growth and reproductive flowers, thereby reducing or eliminating nut production (Payne et al., 1975; Yasumatsu & Kamijo, 1979; Dixon et al., 1986; Anagnostakis & Payne 1993; Kato & Hijii, 1997).

Parasitoid complex of the chestnut gall wasp has been worked mainly on the galls collected in Spring and summer populations. In all over the world about 69 species of chalcidoids were recorded as primary parasitoids, or primary/associate and associate ones in the galls of ACGW (Murakami, 1981; Ôtake, 1989; Santi & Maini 2011; Jurc et al., 2013; Melika et al., 2013; Noyes, 2015). In Italy from the galls of ACGW around 27 indigenous parasitoids, all of them antagonists of oak gall wasps, have been recorded in different chestnut areas in Italy (Aebi et al., 2006, 2007; Speranza et al., 2009; Guerrieri et al., 2011; Santi & Maini, 2011; Boriani et al., 2013; Matošević & Melika, 2013; Panzavolta et al., 2013; Quacchia et al., 2013; Palmeri et al., 2014; Fracanti et al., 2015; Noyes, 2015).

Some works on cephalic structure of the final instars larvae and biological evidence of parasitic complex of some pests were conducted in several countries (Finlayson, 1960, 1967; Capek, 1961, 1970; Doğanlar, 1978; Doğanlar et al., 2009).

Viggiani & Nugnes (2010) described of the larval stages of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), and gave some notes on their phonology. Gómez et al. (2011) studied on comparative morphology and biology of terminal-instar larvae of some *Eurytoma* (Hymenoptera, Eurytomidae) species, parasitoids of gall wasps (Hymenoptera, Cynipidae) in Western Europe.

Aim of this work, the characters of the associates of ACGW were explained as parasitoid or hyperparasitoids, and parasitism level of the species were calculated in two regions in Italy for future programs of biological control of chestnut gall wasp.

## MATERIAL AND METHODS

The study based on the materials taken from Turkey for obtaining the alive larvae of *D. kuriphilus* and on the materials receiving from Italy for dissecting and obtaining the results. The galls of ACGW from Italy were collected in September, 2014 from Trehago (VR), Se Hembre (135 galls), and in October, 2014 from Crespano del Grappa (TV) (185 galls), and were send to Turkey by Drs. Andrea Battisti and Fernanda Colombori, Padova University, Italy.

The larvae of ACGW were taken off from galls (Figs. 1a,b) by dissecting them and its head and body were figured. From each group of Italian galls, 10 combined galls were taken and dissecting for determining parasitism level of *T. sinensis* and parasitism type of parasitoids in the galls. For finding parasitism level, larvae of *D. kuriphilus* (dead ones) and of *T. sinensis* (alive ones) were taken off from the galls, boiled in 10% KOH for 5 minutes, dissected, cleaned from fat-body and slide-mounted in Canada balsam for determining the shape of head structures, mainly mandibles, and spiracles.

The other groups of galls were put into vials separately, and kept in room conditions, about 20-25° C and 50-70% relative humidity. If the galls have holes before placing into the vials, the holes were signed by red pencil, in order to identify the new hole on the galls from which any hymenopterous adult will come out in the future. When the adult comes out from the gall, it was identified by following the keys of several works (Graham, 1969; Doğanlar, 1991a,b, 2011, 2014; Doğanlar & Çam, 1991; Graham & Giswijt, 1998; Gibson & Fusu, 2016). Its gallery following hole of the adult was dissected for reaching to growing-chamber of the adult(s) developed (Fig. 1d). Remnants of the adults were taken off, and slide-

mounted in Canada balsam, and figured them by using of Leica DM 500 microscopes with a digital Leica ICC 50 camera attached to it.

The mandibles and spiracles of each species were determined. Types of parasitism were characterized by the following ways: if one type of mandible was found in the gall, it would be *D. kuriphilus*, having two-teethed mandible; if two different mandibles were found in a gall, the adult come out is the parasitoid and the other one is its host. By working the figures, the characters of the associates of ACGW were explained as parasitoid or hyperparasitoids and parasitism level of the species were calculated.

## RESULT AND DISCUSSIONS

The galls of ACGW were developed as simple (having 3-5 gall chambers) and combined galls (having 6-20 gall chambers) (Figs. 1a,b). From the Spring-galls many larvae and prepupae of ACGW were taken off, and figured (Figs. 2a,b). In some chambers with two larvae and one of which was reached prepupal stage.

From the Autumn-galls the head structure of ACGW larvae (mandibles and spiracles) were figured as seen in (Figs. 2c,d). The head structure have distinct mandibular and labial sclerites, and tip of mandibles double-toothed (Fig. 2c). The spiracles are short, with 3-ringed, having circular opening (Fig. 2d).

By dissecting the autumn galls the larva of *T. sinensis* were separately found in each gall chamber together with the remnant of larva of *D. kuriphilus*. The larva of *T. sinensis* (Figs. 3a,b) has many fine long setae each segments, and with 7 crenulate lines (Figs. 3a,b,e,i), first four of which are thickened, pale brown, the others yellow, and fine. Head with clypeus with 6 teeth, and only havng mandibular sclerites (Figs. 3a,f). Mandibles (Figs. 3c,f,j) are with short base, and with long tooth. Larval spiracle with 6 rings, broadening towards the thicker opening (Fig. 3h), and a blunt end.

The larvae of *Eupelmus annulatus* (Fig. 4a) has very long, thicker setae, with 5-ringed thoracic spiracles (Fig. 4d), and 3-ringed abdominal spiracles (Fig. 4e). Mandibles (Fig. 4b) are simple, one toothed. Clypeus (Fig. 4c) sclerotized, with 6 distinct teeth.

The larvae of *Eupelmus* sp. (Fig. 5a) has very long, thinner setae, with 16-ringed thoracic spiracles (Fig. 4d), and 6-ringed abdominal spiracles (Fig. 4e). Mandibles (Fig. 4b) are simple, one toothed. Clypeus (Fig. 4c) sclerotized, with 10 distinct teeth.

The larvae of *Eurytoma pistacina* (Fig. 6a) has very long, finer setae, with 6-ringed thoracic (Fig. 5c) spiracles, and 4-ringed abdominal spiracles (Fig. 5d). Mandibles (Fig. 5b) are three-teethed, apical one of which is long, and others short. The mandibulae of *E. pistacina* is similar to that of *Eurytoma aspila* (Walker) as Fig. 13A of Gomez et al. (2011).

In some dissected chambers of the combined galls with some larvae of ACGW and *T. sinensis* were infected by *Pyemotes* sp. (Acarina: Pyemotidae) (Figs. 7a,b).

Identification results of the contents of the 10 combined galls taken from two regions of Italy were seen in table 1.

In Trehago (VR) Italy Se Hembre the parasitism by *T. sinensis* was about 65.3% and 3.3% dead adult/lar. of ACGW, and 25.7% of its associates+ACGW (larvae+adults), 1.0% *E. annulatus* and 5.0% of *Pyemotes* were obtained from galls collected in Autumn (Table 1).

In Crespano del Grappa (TV) the parasitism by *T. sinensis* was about 62.6% and from the galls 14.4% dead adult/lar. of ACGW, and 23.0% of its associates

+ACGW (larvae /adults),and 4.8% of *Pyemotes* (2.4 on *D. kuriphilus* and 2.4 on *T. sinensis*) were found (Table 1).

From the galls kept in vials none of the alive *D. kuriphilus* was found. Many female and males of *T. sinensis*, 8 females and 2 males of *Eupelmus annulatus* Nees, and 2 females and 2 males of *Eurytoma pistacina* Rondani were reared.

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Table 1. Parasitism by *T. sinensis* and percentages of ACGW and its associates in two places of Italy in 2014 depend on the numbers of materials present in the chambers of ACGW.

| Trehago (VR) Italy Se Hembre ix. 2014 (135 galls) | | | | | |
|---|---|---|---|---|---|
| Gall | Nr chambers | holes | *D. kuriphilus* dead adult/lar | *T. sinensis* larvae | others |
| 1 | 7 | - | - | 6 | 1 *E. annulatus* dead larva |
| 2 | 6 | - | - | 5 | 1 Dk larva with *Pyemotes* sp. |
| 3 | 19 | 1 | 1 | 13 + 1 pupa | 3 Dk larvae with *Pyemotes* sp |
| 4 | 13 | 5 | - | 7 | 1 Dk larva with *Pyemotes* sp. |
| 5 | 8 | 4 | 2 | 2 | - |
| 6 | 11 | 4 | - | 7 | - |
| 7 | 11 | 6 | - | 5 | - |
| 8 | 10 | 3 | - | 7 | - |
| 9 | 8 | 3 | - | 5 | - |
| 10 | 8 | - | - | 8 | - |
| Total | 101 | 26 | 3 | 66 | 1 *E.annulatus* + 5 Dk with *Pyemotes* sp. |
| % | | 25.7 | 3.0 | 65.3% | 1.0% by *E.annulatus* + 5.0% by *Pyemotes* |
| Crespano del Grappa (TV) 02.X.2014 (185 galls) | | | | | |
| 1 | 16 | 7 | 4 | 5 | - |
| 2 | 15 | 5 | 2 | 7 | 1 Dk larva with *Pyemotes* sp. |
| 3 | 7 | 2 | - | 4 | 1 Dk larva with *Pyemotes* sp. |
| 4 | 8 | 3 | - | 5 | - |
| 5 | 7 | - | 1 | 6 | - |
| 6 | 3 | - | 1 | 2 | - |
| 7 | 4 | - | - | 4 | - |
| 8 | 5 | - | - | 4 | 1Ts larva with *Pyemotes* sp. |
| 9 | 11 | 2 | 1 | 8 | - |
| 10 | 7 | - | 1 | 5 | 1Ts larva with *Pyemotes* sp. |
| Total | 83 | 19 | 10 | 50 | 2 Dk+ 2 Ts with *Pyemotes* sp |
| % | | 23.0 | 14.4 | 62.6 | 4.8% by *Pyemotes* |

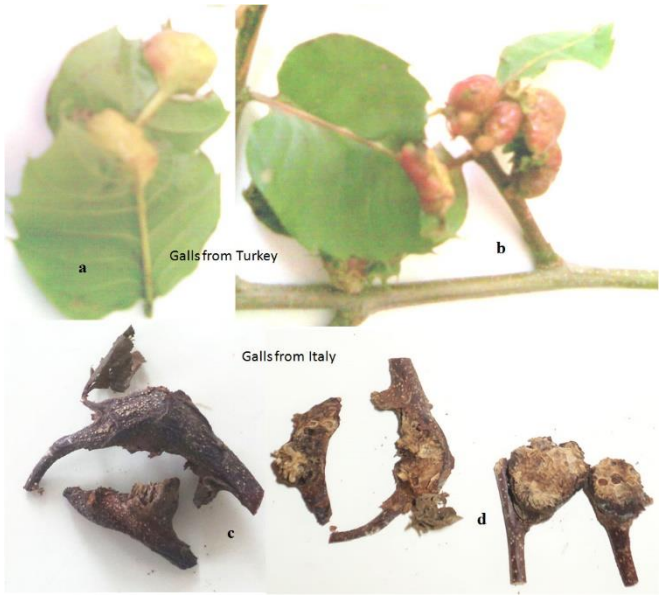


Figure 1. *Dryocosmus kuriphilus* Yasumatsu. a, b. spring galls from Turkey; a. simple; b. combined; c. d. autumn galls from Italy; c. un-dissected galls; d. dissected galls.

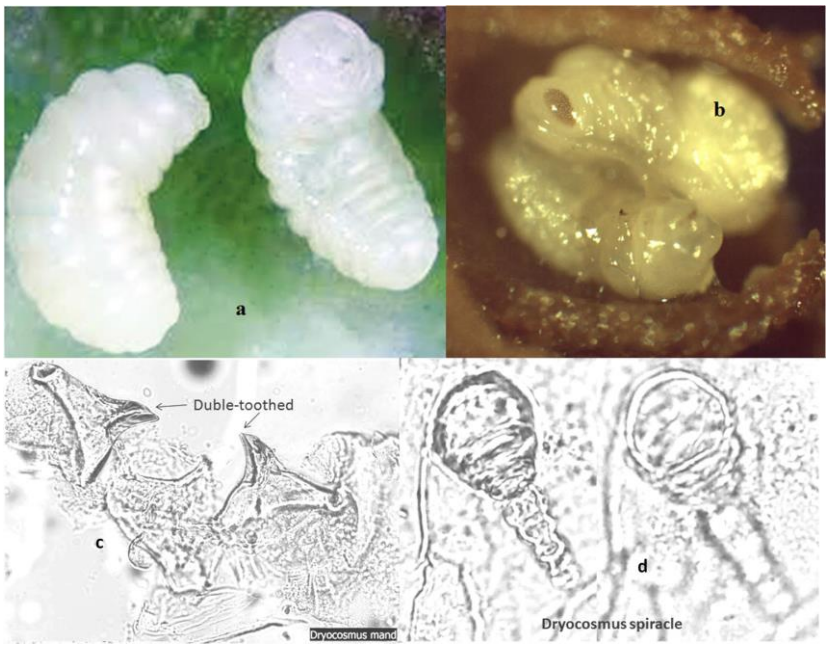


Figure 2. *Dryocosmus kuriphilus* Yasumatsu. a, larvae, in lateral and ventral view; b. prepupa and last instar larva in a gall chamber; c. mandibles of larvae, d. spiracles.

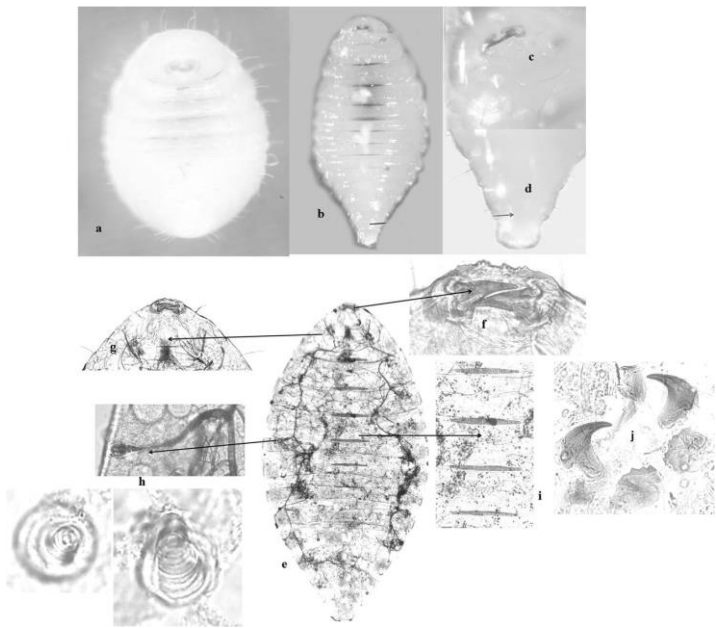


Figure 3. *Torymus sinensis* Kamijo. a, b. larvae, taken from galls; c. head with mandibles; d. caudal end of larva; e. cleared larva, in ventral view; f. mandibles; in ventral view; g. head skeleton; h. abdominal spiracles; i. crenulate area on segments; j. mandibles, in dorsal view, and clypeus.

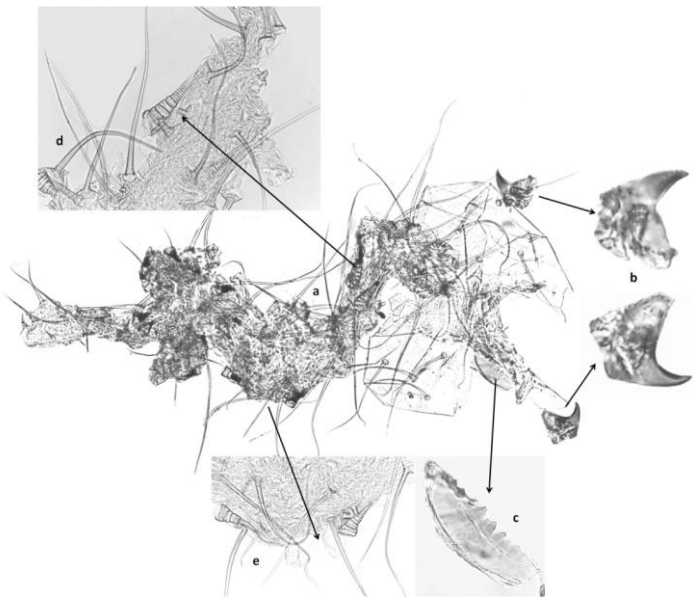


Figure 4. *Eupelmus annulatus* Nees. a. larval remnant; b. mandibles; c. clypeus; d. thoracic spiracle; e. abdominal spiracle.

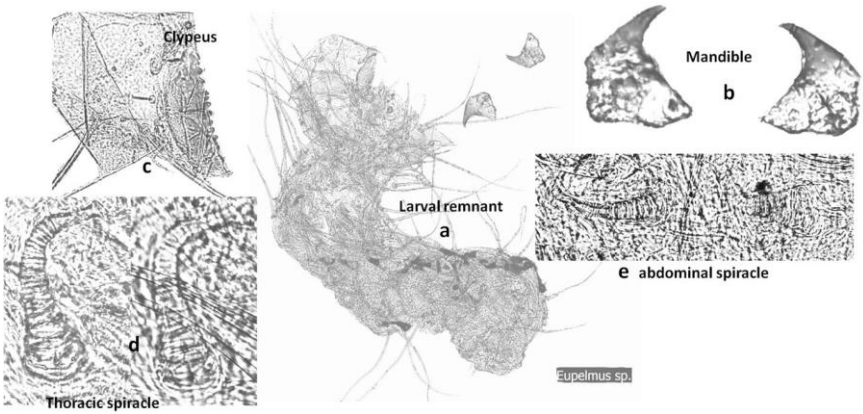


Figure 5. *Eupelmus* sp. a. larval remnant; b. mandibles; c. clypeus; d. thoracic spiracle; e. abdominal spiracle.

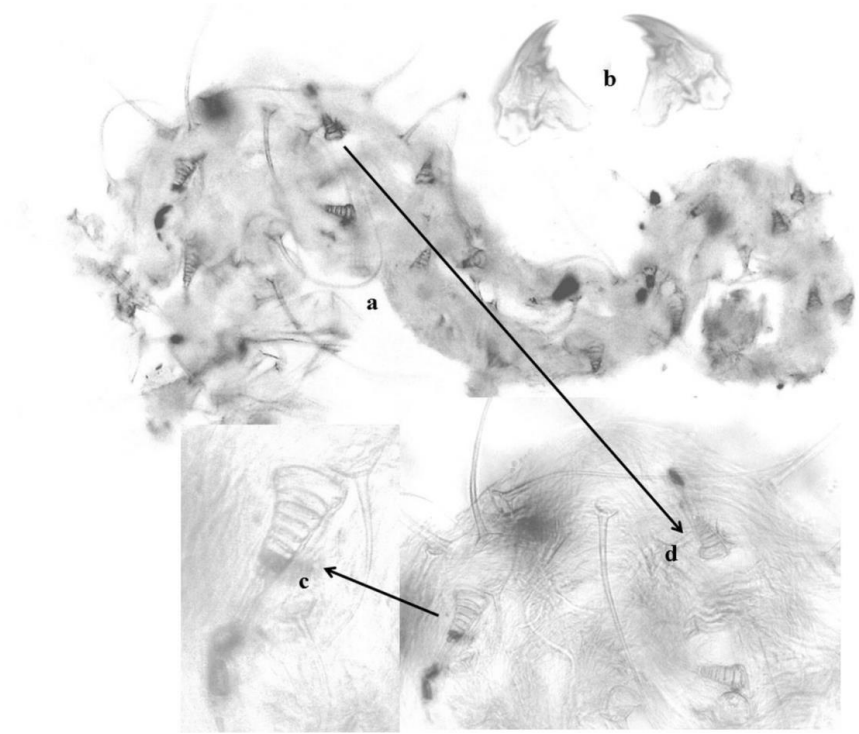


Figure 6. *Eurytoma pistacina* Rondani. a. larval remnant; b. mandibles; c. thoracic spiracle; d. abdominal spiracle.

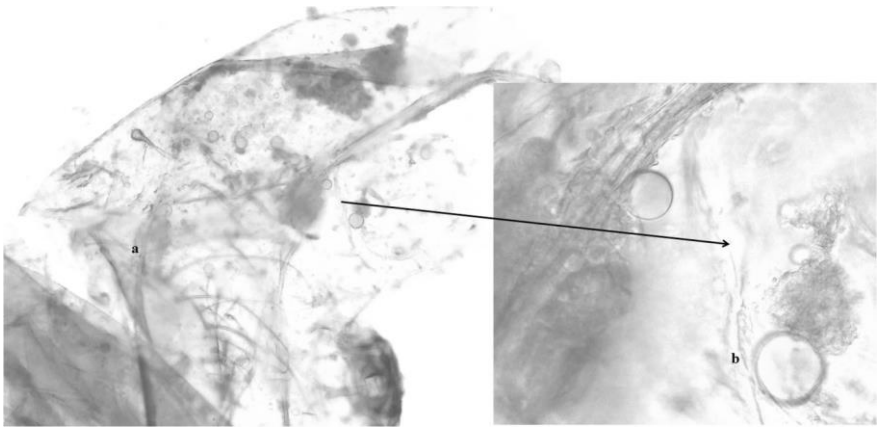


Figure 7. *Pyemotes* sp. on the larva of *Torymus sinensis* Kamijo. a. Tip of larva with adults of *Pyemotes* sp.; b. adults of *Pyemotes* sp.