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## **Description of the larval stages of *Dryokosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), with notes on their phenology**

**Abstract** - The larval stages of *Dryokosmus kuriphilus* Yasumatsu are described and illustrated. The terminal-instar larva shows marked variation in the mandibular asymmetry and in number and position of the respiratory spiracles. Notes on the larval phenology are given.

**Riassunto** - *Descrizione degli stadi larvali di Dryokosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), con note sulla loro fenologia. Vengono descritti gli stadi larvali di *Dryokosmus kuriphilus* Yasumatsu. La larva matura mostra marcate variazioni nell'asimmetria mandibolare, nonché nel numero e posizione degli spiracoli tracheali. Sono date notizie sulla fenologia larvale.

**Key words:** variations, respiratory spiracle, asymmetric mandibles.

### INTRODUCTION

The gall wasp *Dryokosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is a worldwide chestnut pest, which reproduces by parthenogenesis and develops one generation a year. In recent years the species was recorded also in Italy, where it quickly spread from Piedmont to the other main areas where chestnut is grown (Graziosi & Santi, 2008). Several studies have been carried out on the bio-ecology, natural enemies and control of the cynipid (Itô, 1967; Aebi *et al.*, 2007; Ôtake, 1980; Rieske, 2007, Viggiani & Voto, 2009), but the detailed morphology and phenology of the larva still remain unknown.

### MATERIALS AND METHODS

During 2008-2009 the life-cycle of *D. kuriphilus* was studied in two Campania locations (Calvanico, SA; Teano, CE). Samples of 100 galls were taken weekly from June 2008 to September 2009. All types of larvae in the galls were recorded and, for each sample, 20 larvae of *D. kuriphilus* were mounted on slide using a mixture of water and glycerine. For each larval type 5 permanent slides were made using balsam-phenol.

Observations and images of morphological and anatomical larval traits were taken by Axiophot microscope equipped with camera lucida and photcamera. For SEM microscopy the traditional method of dehydration in alcohol, critical-point drying and gold-sputter coating was utilized. Before making preparations, all larvae were measured. Fifty couples of left and right mandibles were dissected and permanent slides were made in order to study their anatomical variation. For the same purpose mandibles of the terminal-instar larval skin were removed from the pupal site and examined. The variation in number and position of the respiratory spiracles was recorded in 127 terminal-instar larvae mounted in a mixture of water and glycerin. At present, at least in Italy, no inquiline cynipid was recorded in the galls of *D. kuriphilus*. The terminology follows Nieves-Aldrey *et al.* (2005).

## RESULTS

### LARVAL INSTARS

The present study shows that *D. kuriphilus* undergoes three instars in the larval development.

*First-instar larva* (Fig. 1). Subglobular, with very small mandibles (Fig. 2) which are subtriangular and apparently unidentate. No tracheal system was detected by the external and internal examination of the larva. Length: 0.2-0.6 mm. This shape derives from the typical cynipid egg, characterized by a suboval body and a long and thin stalk (Fig. 3). The first-instar larva develops slowly from the summer (July-August) to the late March-April of the next year.

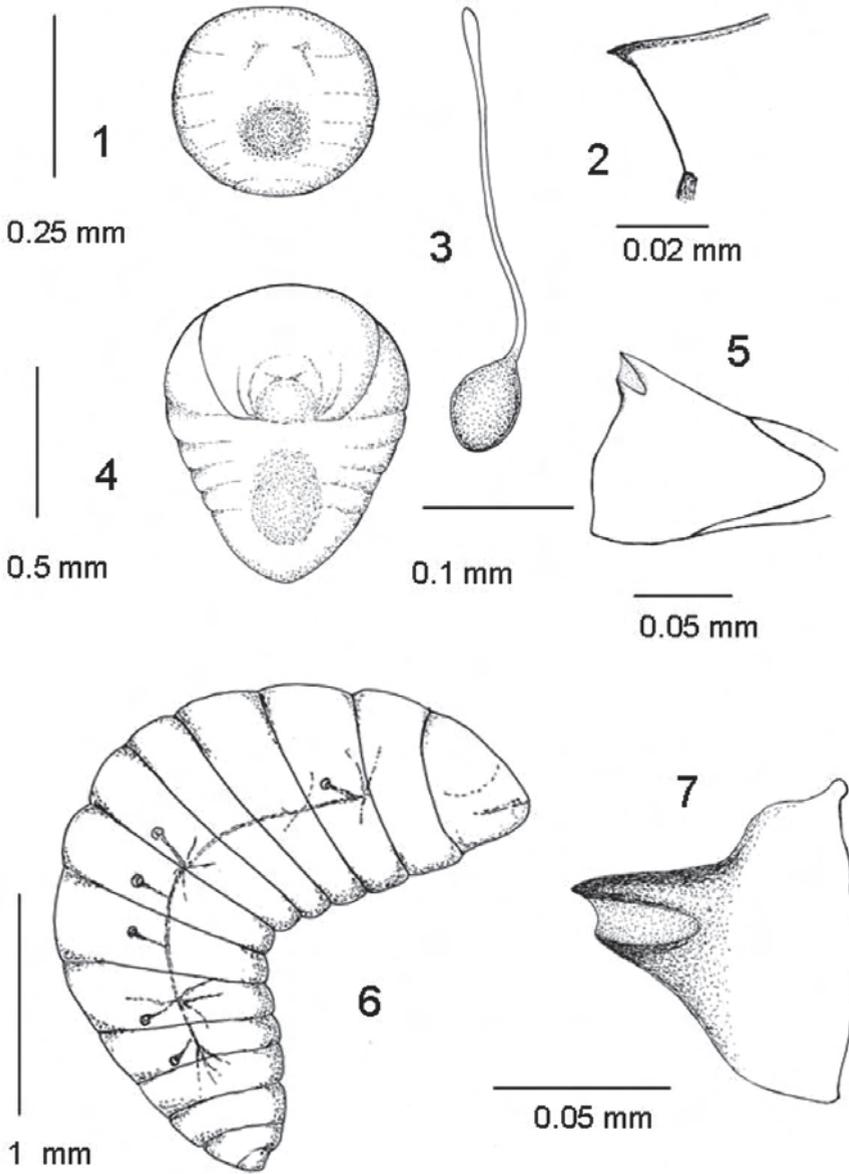
*Intermediate instar larva* (Fig. 4). Hymenopteriform, mandibles (Fig. 5) distally with two teeth, without tracheal system. Length: 0.8-1.5 mm. The intermediate larva stage appears in April-May and lasts less than one month.

*Terminal-instar larva* (Fig. 6). Hymenopteriform, with asymmetric mandibles and a number of variable teeth (Fig. 7). Length: in average 2.3 mm ( $n = 127$ ;  $SD = 0.4$ ). In frontal view the well-developed head capsule (Fig. 8) shows the antennal areas (Fig. 8, AA), a subtriangular clypeus (Fig. 8, C), followed by a transverse labrum. The mandibles (Fig. 8, M; fig. 9) are only partially exposed from the mouth opening. Maxillae subtriangular (Fig. 8, MX) with vestigial palpi (Fig. 8, MP), as on labium, which shows a marked salivary opening (Fig. 8, LA). Respiratory system with 4-6 pairs of spiracles.

The terminal-instar is the only larval stage present in the *D. kuriphilus* galls from late April to end of May.

*Variation.* Remarkable variation in the morphology of mandibles and respiratory system is recorded within the terminal-instar.

*Mandibles.* The distal parts of the left and right mandible of 50 specimens were examined. The most common type (Figs. 10-13) shows a strong external tooth, ventrally concave, followed by an internal and laminar structure. This structure is differently developed and a toothed margin is sometimes present. In other types (Fig. 14-15) the dental part is represented by 2 sclerotized teeth.



Figs. 1-7. *Dryokosmus kuriphilus*: first-instar larva, dorsal view (fig. 1), mandible (fig. 2), egg (fig. 3), intermediate larva, ventral view (fig. 4), mandible (fig. 5), terminal-instar larva, lateral view (fig. 6), mandible (fig.7).

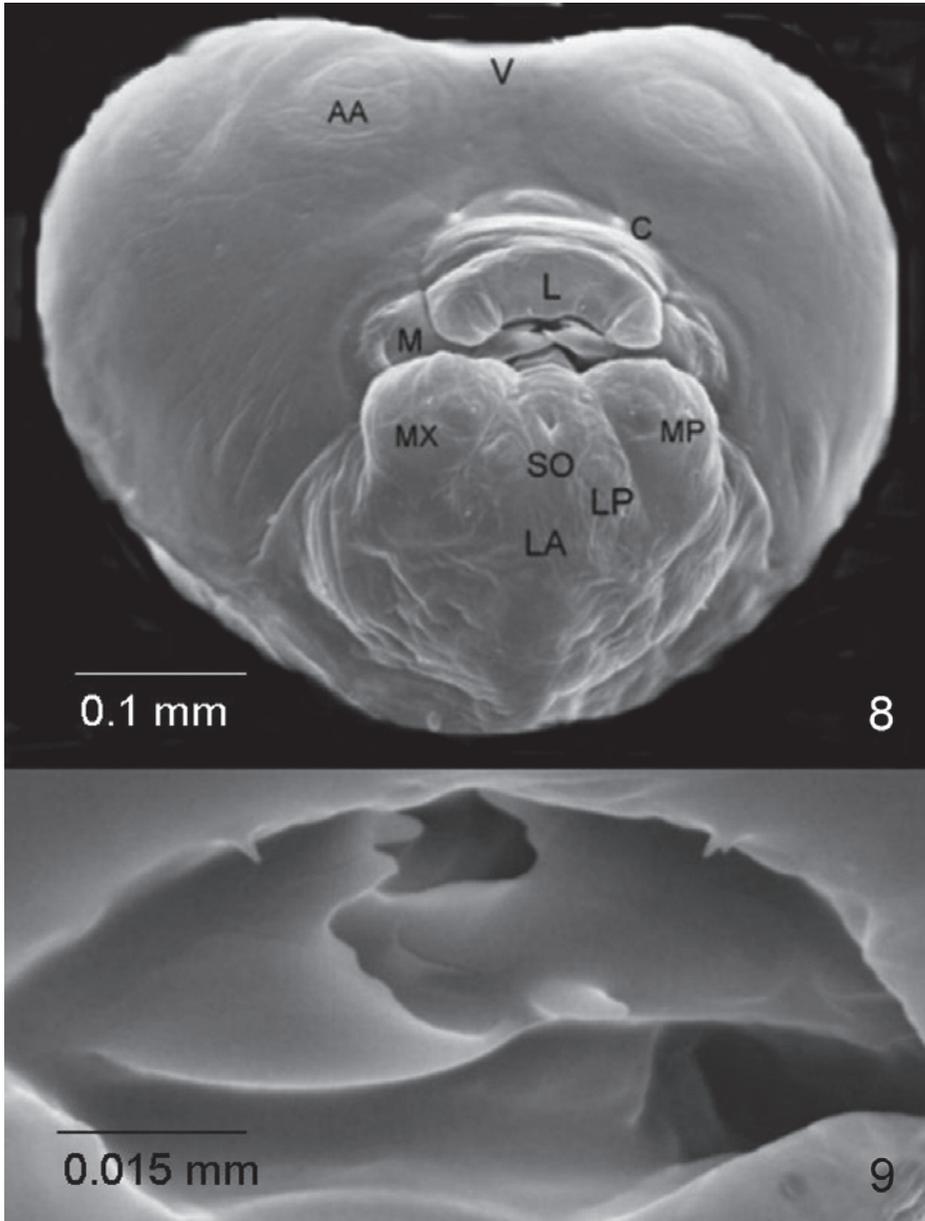


Fig. 8-9. *Dryokosmus kuriphilus*: terminal-instar larva. Head capsule, frontal view (fig. 8), mouth opening and mandible (fig. 9). AA = antennal areas; C = clypeus; L = labrum; LA = labium; LP = labial palpus; M = mandible; MP = maxillary palpus; Mx = maxilla; SO = salivary opening; V = vertex.

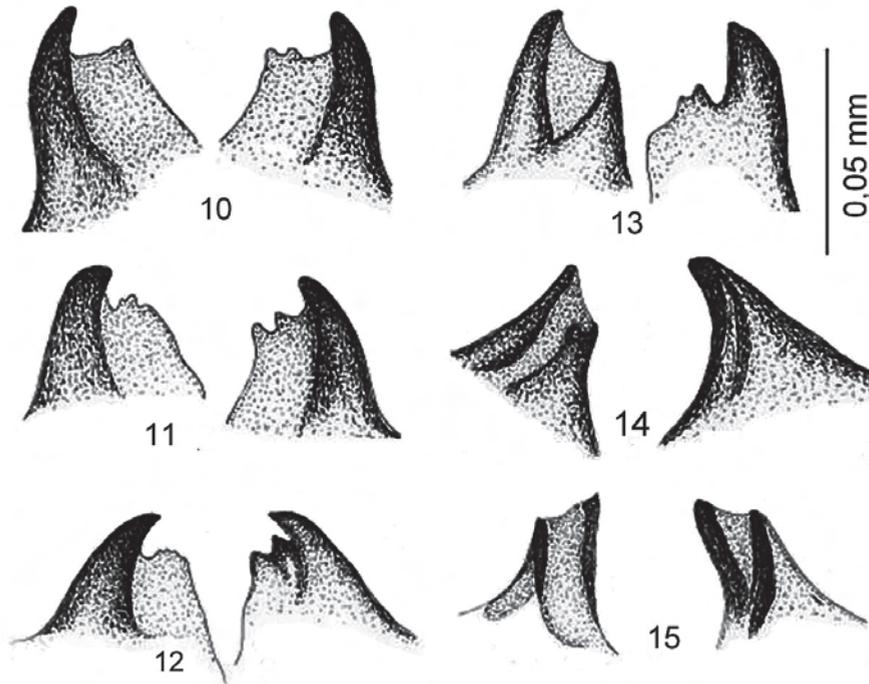


Fig. 10-15. Terminal-instar larva of *Dryokosmus kuriphilus*. Main types of dental parts of the mandibles (dorsal view).

*Respiratory system.* The variation in number and position of the respiratory spiracles have been studied in 127 larvae. The observations are reported in table 1.

Most of the larvae have 4 (44.9%) or 5 (33.8) pairs of spiracles, the first situated on the mesothorax and the others starting from the second abdominal segment. The extreme number of respiratory spiracles found were 3 and 6.

#### DISCUSSION AND CONCLUSION

The Cynipoidea (Hymenoptera) include endoparasitoids and phytophagous species; most of the latter group are gall makers. Although they are distributed worldwide, and have been subject to years of investigation, our knowledge of pre-imaginal morphology is fragmentary and far from complete (Stojanova & Draganov, 2008). Particularly lacking is the knowledge on the youngest larval stages. The shape of the first-instar larva follows that of the egg. The egg of *D. kuriphilus* falls in the category with a long and thin stalk and a stout body, typical of the Cynipidae (Vårdal *et al.*, 2003). The first-instar larva of this species has a globular shape and is macroscopically indistinguishable

Table 1. Variation in number and position of the respiratory spiracles in the terminal-instar larva of *Dryokosmos kuriphilus*.

N. spiracles	Position	Percentage
3	T2 A2 A3	0.8
3	T2 A2 A6	0.8
4	T2 A2 A3 A6	3.9
4	T2 A3 A5 A6	0.8
4	T2 A2 A3 A4	2.4
4	T2 A2 A4 A6	44.8
4	T2 A2 A4 A7	0.8
5	T2 A2 A3 A4 A6	33.8
5	T2 A2 A3 A5 A6	0.8
5	T2 A2 A4 A5 A6	6.3
5	T2 A2 A3 A4 A5	1.6
5	T3 A1 A2 A5 A6	0.8
6	T2 A2 A3 A4 A5 A6	2.4

Legend: A1 = first abdominal segment; A2 = second abdominal segment; A3 = third abdominal segment; A4 = fourth abdominal segment; A5 = fifth abdominal segment; A6 = sixth abdominal segment; seventh abdominal segment; T2 = mesothorax; T3 = metathorax.

from the body of the egg, after the loss of the peduncle, as reported by earlier authors (Itö, 1967). A very slight body segmentation and the presence of very small mandibles characterize this instar.

The variation of the terminal-instar larva in the Cynipoid is remarkable, even between sexes (Stojanova & Draganov, 2008). Most of them concern the shape and the asymmetry of the mandibles. In a comparative study of the terminal-instar larvae of Cynipoidea, Nieves-Aldrey *et al.* (2005) pointed out that head sclerites and mandibles offer many characters of phylogenetic value. The general features of the terminal-instar larva of *D. kuriphilus* are similar to those of other Cynipini (Cynipidae), but the notable variation shown by the dental part of the mandibles, suggests a more extended study of this trait in other species.

The intraspecific variation in number and position of the respiratory spiracles found in the terminal-instar larva of *D. kuriphilus* is certainly surprising and apparently unrecorded for other species. Probably this trait is connected to the fluid content in each loculus of the gall. In general, the maximum number of tracheal spiracles is linked to a dry larval habitat. Some terminal-instar larvae of parasitic Hymenoptera in a host containing body fluid show a reduced number of respiratory openings (Viggiani, 1973; Viggiani, 1984).

#### ACKNOWLEDGMENT

The Author thanks Dr. Hege Vårdal, Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University, Sweden, for reviewing the manuscript.

#### REFERENCES

- AEBI A., SCHÖNROGGE K., MELIKA G., QUACCHIA A., ALMA A., STONE G. N., 2007 - Native and introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*.- Bull. OEPP/EPPO, 37: 166-171.
- GRAZIOSI I., SANTI F., 2008 - Chestnut wasp (*Dryocosmus kuriphilus*): spreading in Italy and new records in Bologna province. - Bulletin of Insectology, 61 (2): 343-348.
- ITÔ Y., 1967 - Population dynamics of the chestnut gall-wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) IV. Further analyses of the distribution of eggs and young larvae in buds using the truncated negative binomial series. - Res. Popul. Ecol., 9: 177-191.
- NIEVES-ALDREY J. L., VÅRDAL H., RONQUIST F., 2005 - Comparative morphology of terminal-instar larvae of Cynipoidea: phylogenetic implications. - Zoologica Scripta, 34 (1): 15-36.
- ÔTAKE A., 1980 - Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae): A preliminary study on the trend of adult emergence and some other ecological aspects related to the final stage of its life cycle. - Appl. Ent. Zool., 15(1): 96-105.
- RIESKE J. K., 2007 - Success of an exotic gallmaker, *Dryocosmus kuriphilus*, on chestnut in the USA: a historical account. - EPPO Bulletin, 37: 172-174.
- STOJANOVA A. M., DRAGANOV M. M., 2008. - Life cycle of *Aylax hypaei* (Insecta: Hymenoptera: Cynipidae), a gall inducer on *Hypocoum* ssp. (*Papaveraceae*). - Cent. Eur. J. Biol., 3(2): 199-204.
- VÅRDAL H., SAHLEN G., RONQUIST F., 2003 - Morphology and evolution of the cynipoid egg (Hymenoptera). - Zool. J. Linn. Soc. Lond., 139: 247-260.
- VIGGIANI G., 1973 - Osservazioni morfo-biologiche sull'*Azotus pulcherrimus* Merc. (Hymenoptera: Aphelinidae). Ricerche sugli Hymenoptera Chalcidoidea. XL. - Boll. Lab. Ent. agr. Filippo Silvestri 30: 300-311.
- VIGGIANI G., 1984 - Bionomics of the Aphelinidae. - Ann. Rev. Entomol. 29: 257-276.
- VIGGIANI G., VOTO A., 2009 - Preliminary data on the phenology of *Dryocosmus kuriphilus* Yasumatsu and the beneficial arthropodfauna of the chestnut in Campania. - Castanea 2009 - 1<sup>st</sup> European Congress on Chestnut - 5<sup>o</sup> Convegno Nazionale Castagno. Cuneo, Italy, 13-16 October. Abstracts: 67.

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Accepted 31 March 2010