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## DESCRIPTION OF COCOON, LAST INSTAR LARVA AND PUPA OF CHRYSIS PULCHELLA SPINOLA, 1888 (HYMENOPTERA: CHRYSIDIDAE)

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The cocoons of Chrysididae have never been specially studied previously. The cocoon structure of *Chrysis pulchella* Spinola, 1888 is described and illustrated in this contribution. It unexpectedly appeared to be similar to the cocoons of the species in the genera *Trichrysis* and *Chrysidea*. The studied cocoons of the mentioned chrysidids were attributed to the so called morphological type of thimble-shaped cocoons. Moreover, this similarity may possibly indicate the taxonomical linkage between the genera *Trichrysis* and *Chrysidea* from one hand, and the most rich in the number of species genus *Chrysis* though the *pulchella* species group. Only a small percentage of species in the family Chrysididae have the larval stage described (at any instar). The attempt to analyze the larval characters for these wasps had already been made, but there were many gaps in taxa studied. Present article contributes to fill this gap: the description of last instar larva of *C. pulchella* Spinola, 1888 is given herein. Several character states of this larva differ from these declared for the subfamily Chrysidinae, where *C. pulchella* belongs to. Similarly, the pupae of Chrysididae have never been studied, but the pupa in studied species is shown to have a set of unique structures absent in imago.

Key words: Chrysididae, cocoon, larva, pupa, Chrysis pulchella, Ukraine.

## Опис кокону, личинки останнього віку та лялечки *Chrysis pulchella* Spinola, 1888 (Hymenoptera: Chrysididae) Мартинова К.В.

В роботі наведено опис кокону оси-блискітки *Chrysis pulchella* Spinola, 1888. Виявилось, що за своєю структурою він є схожим на кокони видів з родів *Trichrysis* та *Chrysidea*. Ця риса, можливо, свідчить про наявність таксономічного зв'язку між цими родами та родом *Chrysis* саме через групу видів *pulchella*. Личинки ос-блискіток залишаються слабо вивченими. В цій роботі також описано личинку останнього віку *C. pulchella*, стани деяких ознак якої не вкладаються у список ознак для підродини Chrysidinae, до якої належить даний вид. Для лялечки досліджуваного виду описано особливі структури, що відсутні у імаго.

Ключові слова: Chrysididae, кокон, личинка, лялечка, Chrysis pulchella, Україна.

# Описание кокона, личинки последнего возраста и куколки Chrysis pulchella Spinola, 1888 (Hymenoptera: Chrysididae).

#### Мартынова Е.В.

В работе приведено описание кокона осы-блестянки *Chrysis pulchella* Spinola, 1888. Примечательно, что по своей структуре он оказался схожим с коконами видов из родов *Trichrysis* и *Chrysidea*. Эта черта, возможно, свидетельствует о наличии таксономической связи этих родов и рода *Chrysis* именно через группу видов *pulchella*. Личинки ос-блестянок остаются слабо изученными. В данной работе описана также личинка последнего возраста *C. pulchella*, состояния некоторых признаков у которой не вписываются в список декларируемых для подсемейства Chrysidinae, к которому принадлежит данный вид. Для куколки исследуемого вида описаны особые структуры, не встречающиеся у имаго.

Ключевые слова: Chrysididae, кокон, личинка, куколка, Chrysis pulchella, Украина.

**Introduction.** The cosmopolitan family Chrysididae (Hymenoptera: Chrysidoidea) comprises diverse wasps that exhibit parasitoid life style. These insects, often called cuckoo wasps, have the wide range of hosts known to include the walking sticks (Phasmatodea), sawflies, nonsocial wasps and bees (Hymenoptera), and a few silk moths (Lepidoptera) (Kimsey, Bohart, 1991).

Bionomy of the family Chrysididae remains to be poorly studied, while referring mainly to the host-parasitoid records. The morphology and features of development of the preimaginal stages are the least studied. Among about 2500 recognized species in the family (Mitroiu et al., 2015) the larvae were described only for 28 species (Ferton, 1905; Giordani Soika, 1934; Parker, 1936; Grandi, 1959, 1961; Thorp, 1968; Danks, 1970; Gauss, 1964; Ouayogode, 1979; Asís et al., 1994; Tormos et al., 1996, 1999, 2001, 2005, 2006, 2007, 2009; Tormos, 2009). For each of these species the last instar larva or/and first instar larva were described; a few cuckoo wasps have the second instar larva described (Tormos et al., 2001). The detailed data on the development and feeding mode of larvae of all instars were obtained only for several species; these data are given in the dissertation research of Ouayogode (1979). Generally, the larval characters of Chrysididae were analyzed by Tormos (2009) and Tormos et al. (1996, 1999, 2001, 2005, 2006, 2007, 2009).

The pupae of cuckoo wasps have never been specially studied. Dissertation research of the author revealed that Chrysididae have pupae that do not fully replicate the adult morphology and thus bear special pupal structures (Martynova, 2016), similarly to some bee pupae (Michener, 1954; Yager, Rozen, 1966; Rozen, Yanega, 1999).

Similarly, there were no attempts to study in detail the cocoon structure of cuckoo-wasps, and analyze these data was not minutely studied, though the short descriptions of cocoons were previously published for a number of species. The research of the author showed (Martynova, in press.) that the cocoons of these wasps are diverse and complex structures with characters of taxonomical importance.

*Chrysis pulchella* Spinola, 1888 is a Mediterranean species recorded from warm countries of Europe, North Africa, Asia Minor and West Asia (Berland, Bernard, 1938; Móczár, 1967; Linsenmaier, 1959; Banaszak, 1980). It is localy distributed in Ukraine, and therefore is generally rarely collected. The biology of species remained almost completely unstudied, except for a singe record of host-parasitoid relations with eumenine wasp *Psiliglossa odyneroides* (Saunders, 1850) (Vespidae: Eumeninae) given in unpublished thesis of Kilimnik (1993).

Present research contributes to the data on morphology of the preimaginal stages of *C. pulchella* and presents the descriptions of last instar larvae, pupae and cocoon of the species.

**Material and methods.** We collected different plant stems that could possibly be used by nonsocial wasps and bees as nesting sites at the territory of the National Nature Park "Dniester canyon" at June, 2017. All the stems containing hollows inside, or that with traces of insect activities, were collected and transferred to laboratory. Then the stems were dissected to reveal the nests of hymenopterans.

Among the material collected one stem of walnut tree (*Juglans* sp.) was found containing the single cocoon of chrysidid wasp inside the host nest. This stem was collected at the vicinities of Dzvenyhorod village, Ternopil Region, Ukraine, on June 16, 2017. The host nest and cuckoo wasp cocoon were minutely studied, then the cocoon was dissected to pull out and study the living last instar larva. This larva was consequently transferred to small glass vial with cotton plug for further observations and left under normal conditions. In to days after being studied the specimen successfully tuned into pupa and its cast off skin were obtained for minute study (allows to ascertain minute larval characters). The pupa was carefully studied and left for further development in the glass vial. After the emergence of imago the identification of the species became possible. The obtained cocoon was also dissected and studied.

The method described allowed to obtain the sure data on morphology of the last instar larva, cocoon and pupa of one species (and based on a single specimen).

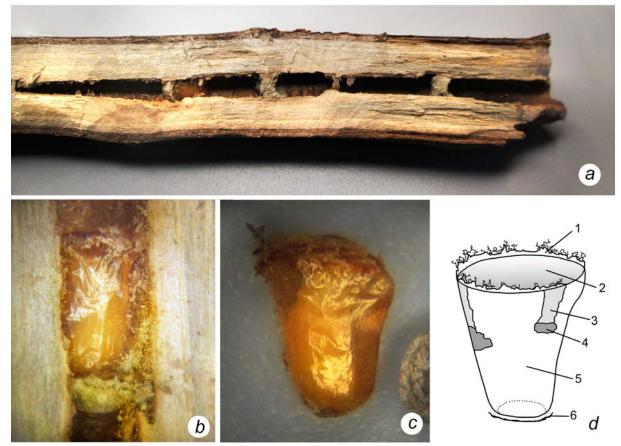
The nest, cocoon, larva, pupa and imago was studied under light binocular microscope MBS-10 and photographed with Canon PowerShot A2300 camera; illustrations were prepared using Adobe Photoshop software.

The terminology of larval morphology and style of description follows that of Tormos et al. (2001, 2006). The nest structure is described according to Krombein (1967).

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## Results

Host nest structure (Fig. 1*a*). The nest of unknown host was constructed in the thick stem of walnut tree with through-cutout hollow of 4 mm in diameter. The nest contained only a single chrysidid cocoon inside, and consisted of following structural elements: the preliminary plug, cell with parasitoid cocoon, cell partition, vestibular cell and closing plug. The plugs and partitions consisted of compressed grey soil and were very thick. The true cell was long, three times longer than wide, and chrysidid cocoon occupied only one third of its length. The vestibular cell was very long, almost twice longer than the true cell.



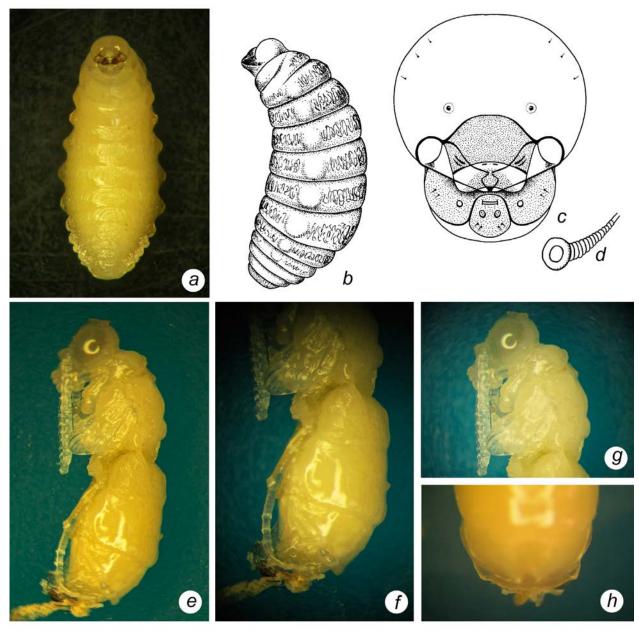
**Fig. 1.** Nest and cocoon of *Chrysis pulchella* Spinola, 1888: *a* – host nest made in stem of walnut tree, *b* – chrysidid cocoon in host cell, *c* – cocoon, lateral view, *d* – cheme of structure of cocoon (1 – collar, 2 – operculum, 3 – internal patch, 4 – external patch, 5 – cocoon wall, 6 – underlying film).

**Description of cocoon** (Fig. 1*b*, *c*, *d*). The cocoon is 6 mm high and 4 mm wide; it is silken, varnished, semitransparent, consists of the thimble-shaped cocoon proper and small underlying film. The cocoon proper has the truncated anterior end (opaque brown smooth operculum) that bears thin whitish collar and rounded tapering posterior end. The cocoon wall bears two small whitish external patches and two longitudinal internal patches.

**Description of last instar larva** (Fig. 2a-d). <u>General aspect</u> (Fig. 2a). Body slender, elongated (length = 5.1 mm, width = 2.2 mm); body segments similar, not divided into annulets defined by shape; segments have transveral central folded stripes: central part of each segment appers to be folded, while anterior and posterior parts of are smooth, polished. Pleural lobes medium-sized, evident. Anus very small, inconspicuous. Integument smooth, without any visible setae. Spiracles without peritreme; atrium simple, naked; diameter of first pair = 53 µm, diameter of remainder spiracles = 40 µm. <u>Cranium</u>

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(Fig. 2c) 0.60 mm wide and 0.70 mm high, with sparse setulae (length = about 10  $\mu$ m) regularly arranged. Coronal suture weakly impressed, chitinized laterally, curved. Parietal bands evident, strong. Antennal orbits (diameter = 20  $\mu$ m) circular. Clypeus trapezoidal, 0.21 mm high and 0.44 mm wide, finely regularly punctured, with fine folds laterally at anterior margin. Labrum 0.13 mm high and 0.33 mm wide, slightly convex, semi-lunar, finely punctured anteriorly and smooth, polished, emarginate posteriorly (see Fig. 2c). <u>Mouthparts</u>. Mandibles 0.20 mm long and 0.12 mm wide, bidentate, rather slender; subapical tooth curved backwards. Labium 0.19 mm high and 0.17 mm wide, with four setae, entire surface of labium extremely finely punctured. Labial palpus (diameter = 19  $\mu$ m) ovoidal, with four sensillae at apex. Salivatory orifice 0.10 mm wide, as a transverse straight slit. Maxillae 0.19 mm wide, with two setae laterally, entire surface of maxillae finely punctured. Maxillary palpus (diameter = 26  $\mu$ m) roundish.



**Fig. 2.** Last instar larva and pupa of *Chrysis pulchella* Spinola, 1888: a – larva, ventral view, b – larva, lateral view, c – head capsule of larva, d - spiracle of larva, e – pupae, total lateral view, f – pupa, metasoma in laterall view, g – pupa, head and mesosoma in lateral view, h – pupa, anal rim of third metasomal tergite.

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**Features of pupa** (Fig. 2*e*-*h*). The pupa obtained and studied was similar to adult morphology, but differed in following characters:

- 1. Scapal basin has two deep foveae with striate surface.
- 2. Antennomeres have tubercles: antennomere 3 with one small tubercle at inner lateral side; antennomere 4 without any tubercles; antennomere 5 with one weak lateral tubercle; antennomeres 6-12 with two conical tuberceles; antennomere 13 rounded, without tubercles, but weakly swollen apically.
- 3. Body has tubercles: at lateral part of pronotum anteriorly; at the center of metasomal tergite 2 (conical tubercle); at the center of metasomal tergite 3.
- 4. Body has carinae: along all midline of propodeum, along midline of metasomal tergite I, at posterior part of midline of metasomal tergite II (below the tubercle at the middle).
- 5. Pronotum has deep large fovea at anterior half and carinate anterolateral angles.
- 6. Posterolateral angles of propodeum very large, rounded, flattened, with depressions at the middle; these angles reach anterior margin of metasomal tergite I.
- 7. Metasomal tergum I with large anterior angles separated by longitudinal foveae.
- 8. Scutellum has two round fields with striate surface.
- 9. Fore femora have carinae along all anterior margin.
- 10. Mesotarsi are crossed below antennae.
- 11. Anal rim of metasomal tergite III has characteristic shape: with six waves and one small middle tooth.

## Discussion

The single cocoon of a chrysidid wasp was found inside the empty cell; no provisions or remains of host larva could be found. That means that the host larva got the chance to consume the stored provisions, but was eaten by the chrysidid larva before it could spin its own cocoon. The nest structure, absence of lining in cell and partitions made of mud suggest that the nest was made by a wasp, quite possible an eumenine wasp (Hymenoptera, Vespidae: Eumenine).

The cocoon of *C. pulchella* belongs to the morphological type of thimble-shaped cocoons (data in press.) and the most similar to that produced by the cuckoo wasps in the genera *Trichrysis* Lichtenstein, 1876 and *Chrysidea* Bischoff, 1913. Such closeness of the cocoon structure appears to be quite unexpected. Furthermore, this closeness of the cocoon structure may indicate the taxonomical linkage (at least this one) between the genera *Trichrysis* and *Chrysidea*, that are sometimes treated as belonging to one genus (Linsenmaier, 1987), and the genus *Chrysis* trough the *pulchella* species group.

The studied last instar larva of *C. pulchella* did not show two characters from the set of features that define the subfamily Chrysidinae according to Tormos et al. (2001): the abdominal segments appear to be undivided into two annulets, but each of the body segments have median stripe of striate microsculpture; labrum has no visible sensillae, but its middle part is finely punctate anteriorly.

The pupa of *C. pulchella* is characterized with a unique set of characters, that may further be used for comparisons and analysis of pupal characters in Chrysididae.

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