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RESEARCH ARTICLE

Rediscovery of the myrmecophilous larvae of *Italoichrysa italica* (Insecta: Neuroptera: Chrysopidae)

DAVIDE BADANO^{1,2}

Abstract

The larvae of *Italoichrysa italica* (Rossi, 1790) were observed in nature for the first time since the original observations by PRINCIPI in the 1940s. Several larvae of this species were found in different nests of the arboreal ant *Crematogaster scutellaris* (Olivier, 1792) (Hymenoptera: Formicidae) in dead oaks in the Italian Apennines. The present observations agree with the few previous studies on the behaviour of *Italoichrysa* Principi, 1946 species and confirm that the larvae of *I. italica* spend most of their time within ant nests. The chrysopid larvae were found in loose aggregations within the nest, sometimes just outside the brood chamber. *Italoichrysa* larvae adopt a static behaviour, avoiding ants and using the packet of debris carried on the body to hide and protect themselves from them. The morphology of the larvae is also discussed.

Key words: ants, green lacewings, myrmecophily, social parasitism, larval morphology, ethology.

Zusammenfassung

Die Larven von *Italoichrysa italica* (Rossi, 1790) wurden zum ersten Mal seit ihrer Beschreibung durch PRINCIPI in den 1940er Jahren in der Natur beobachtet. Mehrere Larven dieser Art wurden im italienischen Apennin an abgestorbenen Eichen in verschiedenen Nestern der arborealen Ameise *Crematogaster scutellaris* (Olivier, 1792) (Hymenoptera: Formicidae) gefunden. Die vorliegenden Beobachtungen stimmen mit den wenigen früheren Verhaltensstudien der Gattung *Italoichrysa* Principi, 1946 überein und bestätigen, dass die Larven von *I. italica* die meiste Zeit ihres Lebens in Ameisennestern verbringen. Die Larven wurden in losen Aggregationen innerhalb des Nestes gefunden, manchmal direkt außerhalb der Brutkammer. Die Larven von *Italoichrysa* besitzen ein statisches Verhalten, mit dem sie Ameisen vermeiden. Sie tragen Debrishaufen auf dem Körper, um sich zu vor Ameisen zu verstecken und sich gegen diese zu schützen. Die Morphologie der Larven wird ebenfalls diskutiert.

Introduction

The larvae of Chrysopidae, or green lacewings, are commonly described as active predators feeding on aphids and other homopterans, readily attacking every suitably-sized insect, including conspecifics. Their active, predatory habits have earned them a prominent role as bio-control agents (NEW 1983; CANARD 2001). However, life histories are only known for a minority of species—usually the more common ones—and most observations were made in artificial settings, using surrogate prey. Most chrysopid species have rather narrow ecological requirements, being associated with specific plant substrates (e.g., SZENTKIRÁLYI 2001; MONSERRAT & MARIN 2001), which might reflect similarly specialized predatory habits. Moreover, little is known about their ethology and aspects of their natural behaviour. For example, the larvae of some species (e.g., *Chrysoperla* Steinmann, 1977) supplement their diet with nectar and other sweet liquids, significantly improving their growth and survival (LIMBURG & ROSENHEIM 2001). The tribe Belonopterygini is the most remark-

able exception to the life history of chrysopids, since all known larvae of this group are myrmecophiles.

Belonopterygini include around 159 species and are widespread in all tropical and warm-temperate regions of the world (BROOKS & BARNARD 1990; BREITKREUZ 2018; TAUBER 2021). Field observations of the larvae of this group are limited to single findings of a handful of species, i.e., the Palaearctic *Italoichrysa italica* (Rossi, 1790), the Australian *I. insignis* (Walker, 1853) and the Neotropical *Nacarina valida* (Erichson in Schomburgk, 1848) (WEBER 1942; PRINCIPI 1943, 1946; TAUBER & WINTER-TON 2014). Moreover, the larvae of the Nearctic species *Abachrysa eureka* (Banks, 1931) were successfully reared on a diet of ant larvae and pupae under artificial conditions (by E. G. MACLEOD, as reported by TAUBER et al. 2020). The larvae of a couple of other genera, i.e., *Calochrysa* Banks, 1943 and *Vieira* Navás, 1913, are only known from specimens hatched from eggs but not reared (NEW 1986; TAUBER et al. 2006). The known larvae of *Italoichrysa* are debris-carriers and associated with arboreal nesting ants. *Italoichrysa italica* was always observed as exclusively

associated with *Crematogaster scutellaris* (Olivier, 1792) (Myrmecinae), one of the most common Mediterranean arboreal ants, while *I. insignis* was found within a nest of *Techomyrmex jocundus* Forel, 1910 (Dolichoderinae) (PRINCIPI 1943, 1946; TAUBER & WINTERTON 2014). The larvae of *I. italica* were discovered in the Italian Apennines by PRINCIPI (1943, 1946), who described in detail their morphology and ethology. PRINCIPI (1943, 1946) observed several larvae, also for a considerable length of time, but always outside the nest, on tree trunks and wooden poles; interactions with the ants were studied during the occasional brood transfer between nests. On these occasions, the chrysopid larvae grabbed the ant larvae carried by the workers and fed on them; occasionally, *I. italica* larvae were also observed catching the ant workers with their jaws but then releasing them (PRINCIPI 1943, 1946). *Italo-chrysa italica* is widespread in the Mediterranean Basin and the Middle East, reaching Central Asia (ASPÖCK et al. 1980, 2001). In Western Europe, this species is present in Mediterranean habitats, where it is widespread but never abundant, and reaches the boundaries of its northern distribution in xerothermic sites along the southern slopes of the Alps (DUELLI 1994; NICOLI ALDINI 1998; CANARD et al. 2007). Nevertheless, the preimaginal stages of *I. italica* had not been reported in the field since the observations of PRINCIPI, although R. A. PANTALEONI managed to obtain first instars of this species from eggs (see TAUBER & WINTERTON 2014).

The present article reports the first finding of larvae of *I. italica* in the field since PRINCIPI (1943, 1946); it also adds a few novel ethological observations.

Material and methods

Field sampling took place in May 2021, in the coastal plain of Lazio (Italy) near Maccarese, Rome (41°52'53.13"N 12°15'43.17"E) (Table 1). The site of investigation is a small Mediterranean wood with prevalence of holm and downy oak growing on sandy substrate and surrounded by extensive dry grasslands and Mediterranean maquis. Three ant nests were examined (Table 1).

Ethological observations in the laboratory were made at room temperature. Larvae (n=3) were placed with the ants and their brood in large Petri dishes with paper as a substrate. Mor-

phological observations and microphotographs were made with a Zeiss© Axio Zoom.

Abbreviations for the larval stages: L1: 1st instar; L2: 2nd instar; L3: 3rd instar.

Results

Morphology

Family Chrysopidae Schneider, 1851
Subfamily Chrysopinae Schneider, 1851
Tribe Belonopterygini Navás, 1913
Italo-chrysa italica (Rossi, 1790)

Diagnostic description of 3rd instar. Body length: 7.50–8.00 mm. Body robust, globose, ventrally flattened (Figs. 1, 2). Head capsule small compared to body, slightly wider than long, thick in lateral view, posterior section withdrawn into cervix and pronotum (Figs. 1, 3–5). Eye with 6 stemmata (Figs. 3, 5). Antenna articulating on prominent cranial ridge, slightly shorter than head capsule with robust setae on anterior half (Figs. 3, 5). Basal antennomere membranous, stocky, subaerial, partly tractable within head capsule (Fig. 3). Antenna with four antennomeres, second antennomere as wide as long, third antennomere much longer than wide, with 4–6 annulations, fourth antennomere short, thin, bearing a robust lateral receptor and an apical bristle (Fig. 3). Jaws stout, shorter than head capsule, curved inward. Maxilla slightly wider than mandible (Figs. 3–5). Labial palp stocky, with three palpomeres; first palpomere as wide as long; second palpomere longer than wide, with a distinct median annulation (resembling a palpomere); third palpomere longer than wide, subconical, slightly curved inward (Fig. 4). Thorax with rounded setiferous tubercles, wider than long, with apically bent setae covered in minute indentations. Legs robust. Abdomen robust. Lateral setiform tubercles with dentellate setae. Last abdominal segments (VIII–X) telescopic (Fig. 6). Body cream coloured, not patterned, head with dark brown markings (Figs. 1–5).

Remarks. *Italo-chrysa* is the only genus of Belonopterygini occurring in the Western Palaearctic, therefore its larva can be distinguished from all other Euro-Medi-

Table 1. Examined nests of *Crematogaster scutellaris* and number of *Italo-chrysa italica* larvae observed within each nest. All observations were made near Maccarese (Italy, Lazio, Rome province).

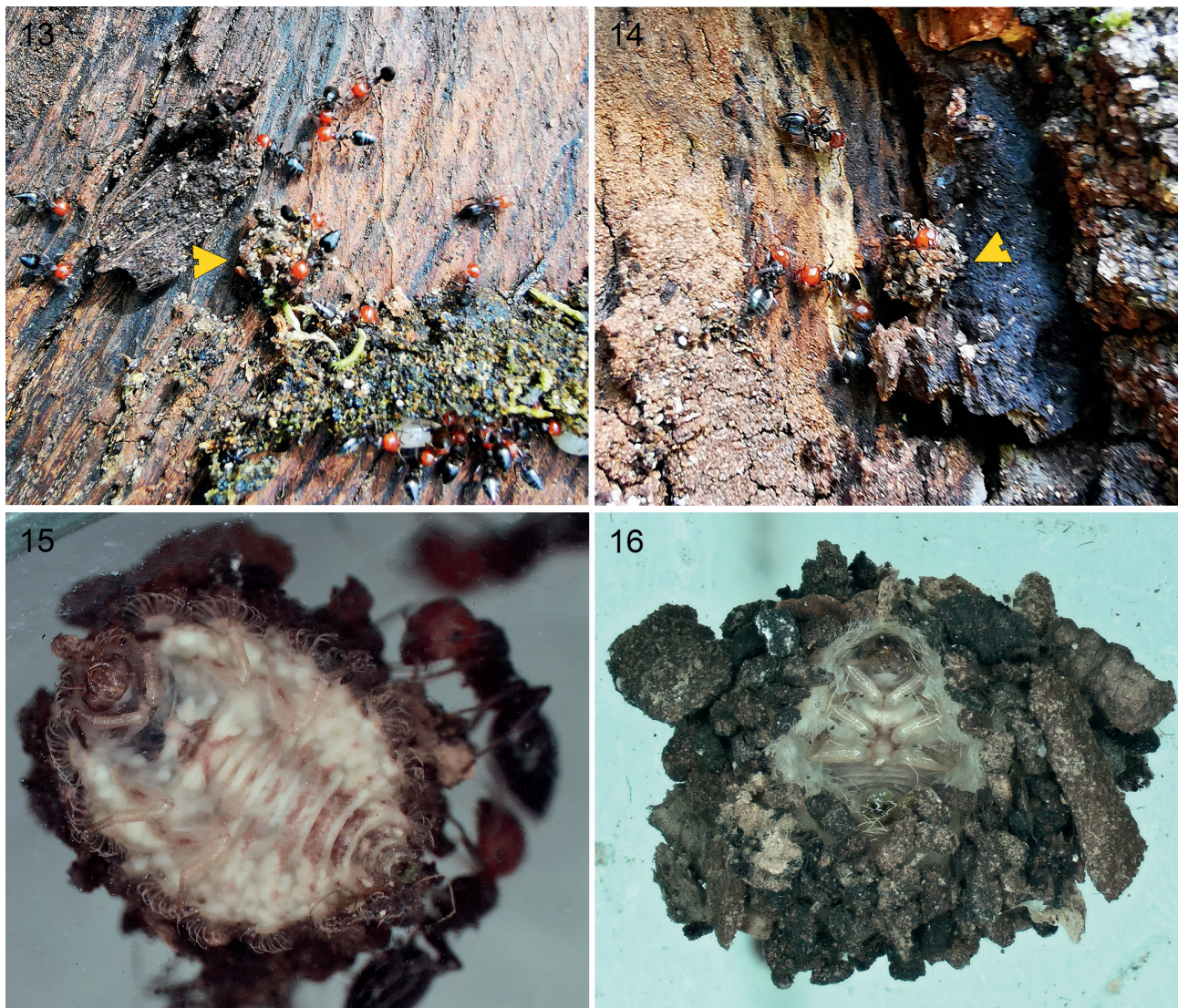
Nest	Observation date	Location	<i>I. italica</i> larvae
1	23.V.2021	Dead standing tree	5 L3
2	30.V.2021	Tree stump	1 L2, 6 L3
3	30.V.2021	Dead standing tree	1 L3 (+ 2 empty pupal cocoons)



Figs. 1–6. *Italochrysa italica* (Rossi), morphology of live 3rd instar. – 1. Habitus, lateral view (with debris packet). 2. Habitus, ventral view. 3. Head capsule, dorsal view. 4. Head capsule, ventral view. 5. Head capsule, lateral view. 6. Pygopodium. Scale bars: 1 mm.



Figs. 7–12. *Italo-chrysa italica* (Rossi) larvae in nature, within nests of *Crematogaster scutellaris* (Olivier). – 7. Brood chamber of ant nest 1. 8. Two larvae in nest 1. 9. Four larvae resting in proximity of nest 2. 10. Larva moving away from ant workers. 11. Larva with debris packet made of wood fragments. 12. Old empty pupal cocoons of *I. italica* in nest 3. Yellow arrows indicate camouflaged chrysopid larvae.



Figs. 13–16. Interactions between *Italochrysa italica* (Rossi) and *Crematogaster scutellaris* (Olivier). – **13–14.** Ants investigating chrysopid larvae in nature. **15.** Laboratory interaction between 3rd instar *I. italica* larva and worker ants, showing larva firmly adhering to petri dish cover while being checked out by ants. **16.** Harassed larva showing its ability to partly roll into a defensive ball. Yellow arrows indicate camouflaged chrysopid larvae.

terranean chrysopids by the synapomorphies of the tribe, i.e., i) antenna robust, partly withdrawable within cranium; ii) L1 with two apical sensilla; iii) mandible robust, shorter than head capsule length; iv) second labial palpomere stout, shorter than first and second palpomeres combined; v) thoracic sclerites reduced; vi) L1 with setae of lateral tubercles rough (see also TAUBER et al. 2021). *Italochrysa stigmatica* (Rambur, 1838) is the only other European species of this genus, being present in the Iberian Peninsula and marginally in southern France (OSWALD 2022). The first instar of this species was

described based on specimens hatched from eggs laid in the laboratory, and does not differ significantly from the *I. italica* L1 (MONSERRAT & DÍAZ ARANDA 2012). The host of *I. stigmatica* remains unknown.

Behavioural observations

Italochrysa italica in *Crematogaster scutellaris* nests. The larvae were collected under bark on dead holm oak trees, both standing and as stumps (Table 1). In ant nests 2 and 3 (Table 1), the wood was strongly tunnelled by sap-

roxylophagous insects and ants. In all observations, the larvae of *Italochrysa* were associated with nests of *C. scutellaris* (Figs. 7–11). With the only exception of ant nest 3, where only a single larva was found, in the other two nests several *I. italica* larvae were found just a few centimetres apart and not interacting with one another (Figs. 8, 9). Ant nest 1 was the only one where the chrysopid larvae were found in proximity to the ant brood. In this nest, the ant workers had built a brood chamber made of chewed wood debris (as it is usual for this species) and containing several ant larvae (Figs. 7, 8). The larvae of *Italochrysa* rested just outside the chamber, in some cases along its external surface; no chrysopid larva was observed among the ant larvae (Fig. 7). In ant nest 2, the ant brood was not observed and several *I. italica* larvae remained immobile under the bark, among the ant workers (Fig. 9). In nest 3, only a few ant workers were observed (Fig. 11). At this location, two empty *I. italica* pupal cocoons, likely dating back to the previous summer, were also found (Fig. 12).

Ant-chrysopid interactions. Within the nests, the camouflaged larvae of *I. italica* were observed resting motionless and firmly anchored to the substrate with their legs. The appendages were kept unexposed under the covering of debris; thus, the larvae were hardly distinguishable from the woody debris and frass present in the nest. The occasional passing ant worker ignored the immobile larva hidden nearby. Removal of the bark and the resulting sunlight did not trigger any reaction in the observed chrysopid larvae. However, the aggressive response of the *C. scutellaris* workers to the intrusion disturbed a few of the chrysopid larvae, which slowly crawled away from the ants with a swaying motion (Fig. 10; Supplementary Video 1). During an interaction with an ant worker, the chrysopid larva froze, withdrawing its head, legs, and the tip of its abdomen below the covering debris. Then, the ant investigated the chrysopid's debris packet, climbing on top of it and inspecting it with its antennae, then quickly losing interest (Figs. 13, 14; Supplementary Video 2; Supplementary Video 3). At the end of the interaction, the larvae of *Italochrysa* slowly crawled away from the spot.

Laboratory observations (n=3). Under artificial conditions, the behaviour of the chrysopid larvae did not significantly differ from the observations in the field. The larvae spent most of their time entirely motionless, ignored by the ants. When the chrysopid larvae began to move and met an ant worker, they quickly adopted the same defensive behaviour shown in the field, hiding their appendages and waiting motionless until the ant lost interest (Fig. 15). Occasionally, the ants unsuccessfully tried to remove debris from the larval dorsal packet. A mechanically stimulated larva showed the ability to roll into an almost complete ball of debris, though this response was likely extreme because of the artificial settings (Fig. 16). Feeding was not observed despite live ant larvae being offered.

Discussion

Several morphological traits characterizing the larvae of Belonopterygini, i.e., small and compact head capsule, stout appendages and bulky body shape, are shared with other myrmecophilous insects, notably beetles (HÖLLDOBLER & WILSON 1990; PARKER 2016). These characters suggest that myrmecophily might characterize the whole tribe, including those genera whose life history is still unknown. The larvae of the genera *Abachrysa* Banks, 1938 and *Italochrysa* (and likely also *Calochrysa* and *Vieira*) are debris-carriers, whereas *Nacarina* Navás, 1915 belongs to the “naked” type—though observations regarding the latter require confirmation—implying that the representatives of this tribe have evolved different strategies to interact with ants (TAUBER et al. 2020). The debris-carrying behaviour was independently evolved and lost in different lineages of Chrysopidae in a rather complex evolutionary scenario, making it difficult to reconstruct the ancestral state of this trait in Belonopterygini and its association with myrmecophily (TAUBER et al. 2014; GARZÓN-ORDUÑA et al. 2019; WINTERTON et al. 2019; TAUBER et al. 2020). The present observations largely agree with the previous studies by PRINCIPI (1943, 1946) and TAUBER & WINTERTON (2014) on *Italochrysa* behaviour. The larvae of *Italochrysa* rely on debris-carrying for both camouflage and mechanical protection from their host ants, but it is still unclear whether the larvae also display active chemical mimicry. However, the camouflage is made of wood and soil debris collected within the ant nest, so the larvae are likely chemically indistinguishable from the surrounding nest through passive chemical mimicry. The investigations by PRINCIPI (1943, 1946) were highly accurate but only involved *I. italica* specimens found outside the nest. The present observations are instead remarkably consistent with those on *I. insignis* (TAUBER & WINTERTON 2014), demonstrating that the larva of *I. italica* penetrates within the ant nest and performs the same behavioural patterns as its Australian congener. Moreover, this study shows that the larvae of *I. italica*, as previously observed in *I. insignis* and apparently also in *N. valida*, often gather in loose groups not showing any intraspecific aggression (WEBER 1942; TAUBER & WINTERTON 2014). This type of passive behaviour is quite unusual among Chrysopidae.

The larvae of *Italochrysa* exemplify the diversity of life histories and specializations evolved by Neuroptera during their long evolutionary history. However, several questions remain to be solved regarding the behaviour of these chrysopids, especially whether they can mimic their host's chemical communication and how they select among available ant nest, since they appear to be rather rare despite the abundance of their host in Mediterranean habitats.

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Supplementary videos:

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Supplementary Video 1: Crawling 3rd instar of *Italoichrysa italica* (Rossi) (Neuroptera: Chrysopidae).

Supplementary Video 2: Interaction between 3rd instar of *Italoichrysa italica* (Rossi) and *Crematogaster scutellaris* (Olivier) workers.

Supplementary Video 3: Interaction between 3rd instar of *Italoichrysa italica* (Rossi) and *Crematogaster scutellaris* (Olivier) workers.

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