

# Incidence of *Anagrus obscurus* (Hymenoptera: Mymaridae) egg parasitism on *Calopteryx haemorrhoidalis* and *Platycnemis pennipes* (Odonata: Calopterygidae, Platycnemididae) in Italy

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## ABSTRACT

Very little is known about the incidence of egg parasitoids in odonates, perhaps because Odonata eggs are well protected in stems or leaves, sometimes below water. In Central Italy (Pontecorvo, Frosinone province) two damselflies, *Calopteryx haemorrhoidalis* and *Platycnemis pennipes* occur at high densities. In August 2007 we collected 30 stems of the aquatic plant *Potamogeton* sp. used as substrate for oviposition and incubated eggs in the laboratory. Most stems (24 for *C. haemorrhoidalis* and 23 for *P. pennipes*) contained odonata eggs. Parasitoids emerged from 12 stems, with a mean parasitism of 2% for *C. haemorrhoidalis* and 6% for *P. pennipes*, and a maximum of 14% and 50%, respectively. Furthermore, we observed egg-laying of 19 females of *C. haemorrhoidalis* and 11 of *P. pennipes*, and marked the stems where oviposition was observed. Clutches remained in the river for 5 days and were then collected and incubated. Parasitoids emerged from 11 stems out of 30, with an average parasitism of 8% for *C. haemorrhoidalis* and 3% for *P. pennipes* (maximum of 50% and 29%, respectively). All parasitoids belonged to the family Mymaridae, and were identified as *Anagrus* (*Anagrus*) *obscurus* Förster, 1861, sensu Soyka, 1955. This is the first time that such species is described as an egg parasitoid of odonates, and that an egg parasitoid of *C. haemorrhoidalis* and *P. pennipes* is identified. Our data suggest that egg parasitism might be a significant selective factor for both odonates in the studied locality, affecting female oviposition behaviour.

## KEYWORDS

Damselflies, parasitism rate, egg parasitoid, endophytic oviposition

It is widely known that most insect species are hosts of one or more parasitoids, either in the egg, larval, pupal or adult stage (Askew 1971). Egg parasitoids are hymenopterous species specialized in searching, locating and parasitizing recently laid eggs (Godfray 1994). They are idiobiont organisms (i.e. prevent further development of the host after initial parasitization) whose larvae develop inside the host egg, consuming the yolk, which greatly limits their adult size. Given that the immune system of insect embryos is still not developed, eggs are especially vulnerable to parasitoid attack (Beckage 1985). For this reason, insect eggs are commonly protected from parasitoids by oothecae, by protecting coverings such as scales, setae, feces, silk or spumaline, by increasing the hardness and thickness of egg chorion, or by introducing eggs into plant tissues (Gross 1993).

Literature on Odonata egg parasitism is scarce and data on parasitism rate is just occasionally presented, perhaps because odonate eggs are commonly laid in stems, leaves or other rigid structures, sometimes below water, making them well protected against egg parasitoids. Predation of endophytic odonate eggs by other animals is rarely reported, with the exception of predation by nymphs of water mites or the eggs observed in the stomach of insectivorous fishes (Corbet 1999). The only detailed information on egg mortality of endophytic dragonflies is associated with parasitism by micro-hymenopterans belonging to the superfamily Chalcidoidea, in which four families (Eulophidae, Mymaridae, Scelionidae and Trichogrammatidae) and ten genera are represented (Corbet 1999). In the current study, we identified the parasitoid of the eggs of *Calopteryx haemorrhoidalis* Vander Linden (Odonata) and *Platycnemis pennipes* Pallas (Odonata), and evaluated the parasitism rate achieved.

The study was conducted in August 2007 in central Italy, at 120 km from Rome (Pontecorvo, Frosinone province), in the stream Forma Quesa (UTM coordinate: 33TUF 878873), where both *C. haemorrhoidalis* and *P. pennipes* have large populations. The stream had a rocky bottom, with muddy and sandy areas, and with a well developed riparian forest dominated by *Populus alba* (Salicaceae), *Alnus glutinosa* (Betulaceae), *Acer monspessulanus* (Sapindaceae) and *Salix* sp. (Salicaceae). In order to study egg parasitism of *C. haemorrhoidalis* and *P. pennipes*, we collected 30 stems of the aquatic plant *Potamogeton* sp. (Potamogetonaceae), the main egg-laying substrate in the studied locality, with signs of having been used for oviposition by odonates. Such stems were floating or up to 5 cm below the water surface. While some *C. haemorrhoidalis* females submerge to lay eggs (Fig.1), *P. pennipes* females only introduce the abdomen under the water. The collected stems were maintained in plastic boxes with river water and kept under room temperature (approx. 26°C) until either the parasitoid emerged or the larvae of the odonates hatched. Because eggs of *Calopteryx* and *Platycnemis* greatly differ in length (1.3 versus 0.9 mm), emerged parasitoids were easily assigned to each odonate depending on their size. Unhatched eggs were opened under a dissecting microscope. Emerged parasitoids were fixed in 90% ethanol for taxonomic identification. Parasitism rate was calculated as the ratio between parasitized eggs and the total amount of eggs of each odonate.

Furthermore, to investigate the window of vulnerability of fresh eggs, we observed egg-laying of 19 females of *C. haemorrhoidalis* and 11 of *P. pennipes*, and marked the stems where oviposition was observed. Clutches remained in the river for 5 days and were then collected and incubated. We chose this time interval because older eggs would be unsuitable for parasitism, considering that a significant negative correlation exists

between the duration of the host development (approximately 2 weeks for both odonate embryos) and the percentage of host eggs successfully parasitized (Strand 1986).

Most stems collected for the descriptive study (24 for *C. haemorrhoidalis* and 23 for *P. pennipes*) contained odonata eggs. To estimate parasitism rate, we checked 1,587 eggs of *C. haemorrhoidalis* (with a mean of  $69.1 \pm 16.8$  SE per stem) and 387 eggs of *P. pennipes* (with a mean of  $43 \pm 13.7$  SE eggs per stem). Parasitoids emerged from 12 stems, with a mean parasitism of  $2\% \pm 0.01$  SE for *C. haemorrhoidalis* and  $6\% \pm 0.03$  SE for *P. pennipes*, and a maximum of 14% and 50%, respectively. In the second study, 551 eggs of *C. haemorrhoidalis* (with a mean of  $26.24 \pm 6.7$  SE eggs per stem) and 207 eggs of *P. pennipes* (with a mean of  $34.5 \pm 12.4$  SE eggs per stem) were checked.

Parasitoids emerged from 11 stems over a total of 30, with an average parasitism of  $8\% \pm 0.03$  SE for *C. haemorrhoidalis* and  $3\% \pm 0.02$  SE for *P. pennipes* (maximum of 50% and 29%, respectively). There was no significant difference between the overall parasitism rate suffered by both odonates (Mann-Whitney U test:  $U = 1136.5$ ,  $P = 0.919$ ).

All parasitoids belonged to the family Mymaridae, and were identified as *Anagrus* (*Anagrus*) *obscurus* Förster, 1861, sensu Soyka, 1955 (for anatomical description see Förster 1981, Chiappini 1989, Chiappini et al. 1996, Baquero & Jordana 1999), a solitary, idiobiont egg parasitoid (Fig 2). The Mymaridae is a moderately large cosmopolitan family containing nearly 1300 species in about 95 genera. All species are minute (wing length varying from 0.35 to 1.8 mm) solitary (rarely gregarious) endoparasitoids of eggs of Hemiptera, Coleoptera, Odonata and Psocoptera (Gauld & Bolton 1996). They usually parasitize eggs in concealed situations such as those embedded in plant tissues, placed under scales, bracts or in soil (Huber 1986). Adults of some mymarid species spend most of their life under water, being small enough to respire through the general body surface (Askew 1971).

The genus *Anagrus* Haliday is very common in the Palearctic region and well known due to its use in biological control programs (Triapitsyn & Berezovskiy 2004). According to Chiappini (1989), in Europe there are 18 species of *Anagrus*, all of them egg parasitoids of Cercopidae, Cicadellidae, Delphacidae, Miridae, Tingidae and Odonata (Huber 1986). *Anagrus obscurus* was known from Italy as egg parasitoid of leafhoppers such as *Cicadella viridis* L. (Homoptera, Cicadellidae), which lay eggs along the venation of *Carex* sp. (Cyperaceae) leaves (Chiappini 1989). As far as we know, this is the first time that *A. obscurus* is reported to be parasitoid of odonate eggs, and that a parasitoid of *C. haemorrhoidalis* and *P. pennipes* is described. It has been previously reported the existence of a micro-hymenopterous parasitoid of *P. pennipes* although the insect remains unidentified (Robert 1958, Martens 1996). In the genus *Calopteryx*, it has been reported parasitism of *Calopteryx* spp. clutches by *Aprostocetus* (*Tetrastichus*) *natans* Kostyukov & Fursov (Hymenoptera, Eulophidae) (Girault 1914), of *C. virgo* L. by *Anagrus incarnatus* Haliday (Hymenoptera, Mymaridae) (Henriksen 1919), and of *C. atrata* Selys by *Anaphes* sp. (Hymenoptera, Mymaridae) (Asahina 1948).

In the laboratory, emerged parasitoids were observed to submerge and walk on *Potamogeton* stems, apparently searching for host eggs. Some egg parasitoids such as *Polynema* (Mymaridae) and *Tiphodytes* (Scelionidae) are known to swim beneath the

water to find their hemipteran hosts (Hirashima et al. 1999). The behaviour of chalcidoids that locate Odonata hosts above and under water was described by Jarry (1960), who reported that female of *A. incarnatus* moved over the woody plants where its host, *Lestes viridis* Vander Linden, had oviposited, repeatedly tapping the bark with the clubbed tips of the antennae to detect host egg presence. *Anagrus* oviposition occurs only if the host egg is surrounded by living and turgid plant tissues and if dorsal closure of the host embryo is not complete (Witsack 1973).

Parasitism rates suffered by host eggs of both odonates exposed ad libitum (first study) to parasitoid attack or during 5 days (second study) are quite similar, although the overall number of eggs of *C. haemorrhoidalis* is almost three-fold higher in the first study, as a consequence of the longer exposition of the oviposition plants. This fact suggests that 5 days may represent the optimal window of vulnerability of host eggs for *A. obscurus*. The mechanism by which *A. obscurus* detects host age is actually unknown, but it is probably related to antennal drumming and sting probing, as other mymarids do (Godfray 1994). Further investigations on host discrimination behaviour and host age preference of *A. obscurus* are needed in the future. Age dependence in the association between parasitoids and hosts, including age dependence in the susceptibility of host to attack, is of potentially great importance in the dynamics of host-parasitoid populations, because host eggs that escape parasitism may represent a demographic refuge that promote stability of the host-parasitoid association (Sait et al. 1995). Although the mean parasitism rate detected is not high, our data suggest that egg parasitoids might be a significant selective factor in the studied locality, and might affect oviposition substrate selection and behaviour of egg-laying females of odonates, as it has been shown for the water strider *Aquarius paludum insularis* (Heteroptera: Gerridae) which avoids a high rate of egg parasitism through submerged oviposition on deeper substrates (Amano et al. 2008).

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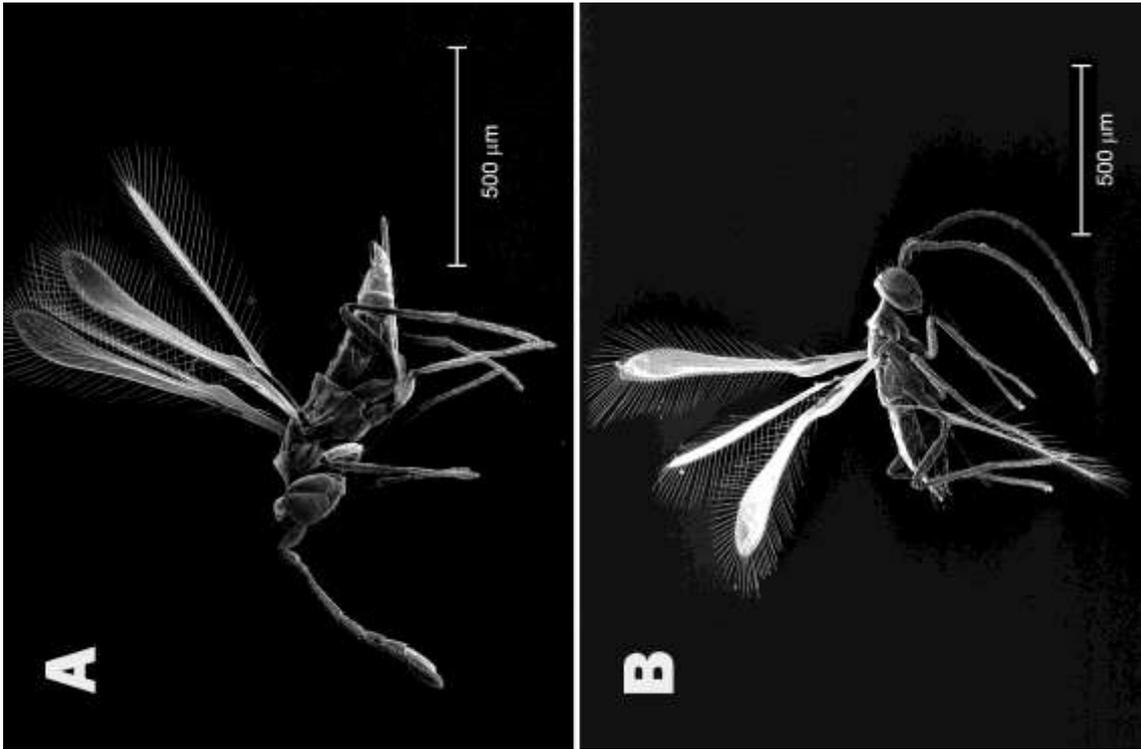
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**Figure 1.** Underwater oviposition by *Calopteryx haemorrhoidalis* on *Potamogeton* sp. plants.



**Figure 2.** Adults of *Anagrus obscurus*. A, female; B, male.