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First record of *Epipompilus excelsus* (Bradley, 1944) (Hymenoptera, Pompilidae) as a koinobiont ectoparasitoid of *Ariadna mollis* (Holmberg, 1876) (Araneae, Segestriidae)

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Abstract

Epipompilus Kohl comprises 52 species of wasps that are parasitoids of spiders; 16 species occur in the Neotropical region and 36 species occur in the Australian region. The biological knowledge of this genus is limited and its interactions and host spiders are still incipient. Here, we report some behavioural and biological characteristics of *E. excelsus*, a parasitoid of the tube-dwelling spider *Ariadna mollis*. We observed an *E. excelsus* female attacking an adult female of *A. mollis* in São Paulo, Brazil. We photographed daily the larval development of the wasp, from the egg stage to adult emergence. The entire developmental cycle of the wasp took 24 days. This period was shorter than the developmental periods of wasps belonging to other genera of Pompilidae. Although all species within Pompilidae use spiders as host, they present great behavioural diversity, characterized by different ethological sequences. In conclusion, this study demonstrated that the Neotropical species of *Epipompilus* exhibit biological characteristics similar to the Australian species, acting as a koinobiont ectoparasitoid, but displays differences in larval morphology. Studies on other species could elucidate the extent of these differences and similarities, contributing to our understanding of the evolutionary history of *Epipompilus*, and consequently of Pompilidae.

Keywords

Egg-wasp development, Neotropical, Parasitoid, Tube-dwelling spider

Introduction

Pompilidae is a megadiverse family of ectoparasitoid wasps, with more than 5,000 valid species distributed worldwide and approximately 1,000 species inhabiting the Neotropical region (Shimizu 1994, Pitts et al. 2006). Although some pompilid species show primitive levels of sociality, most species are solitary (Shimizu 2004, Shimizu et al. 2010, Santos et al. 2016). These species are known as “spider wasps”, since adult females usually uses spiders as food resource for their larva (Brothers and Finnamore 1993, Wasbauer 1995). Pompilids presents a wide diversity of behavioural strategies to parasitize their hosts, with species nesting in the ground, in pre-existing cavities, mud nests they have built, or using as hosts active spiders that are posteriorly killed by the wasp’s larvae (El-Hennawy 1996, Souza et al. 2015). Most species are solitary, but some show primitive levels of sociality (Shimizu 2004, Shimizu et al. 2010, Santos et al. 2016).

The genus *Epipompilus* Kohl comprises 52 species of spider wasps, 16 of which occur in the Neotropical region, one in the Nearctic region, and 36 species in the Australian region (Roig-Alsina and Barneche 2017). The current biological knowledge of this genus is based on three species from the Australian region, *E. insularis* Kohl, 1884, *E. exleyae* Evans, *E. reiki* Evans (Evans 1972, Pollard 1982, Harris 1987), and one Neotropical species, *E. platensis* (Roig-Alsina and Barneche 2017). Evans (1972) reported *E. exleyae* as an ectoparasitoid of immature Sparassidae, and *E. rieki* as an ectoparasitoid of an unidentified spider. Pollard (1982) and Harris (1987) reported *E. insularis* as a koinobiont ectoparasitoid of multiple cursorial spiders, including the clubionid *Clubiona cambridgei* Koch, 1873, and the salticids *Salticus* sp., *Trite auricomma* Urquhart, 1885 and *T. planiceps* Simon, 1899.

Segestriidae (Simon, 1893) consists of haplogyne spiders, small-to-medium in size (total body length between 2.8 and 20.25 mm) (Grismado 2008, Giroti and Brescovit 2018), usually found in loose barks, cracks in tree trunks, under rocks, or even in anthropomorphized places (Roig-Alsina and Barneche 2017). These spiders are nocturnal, sedentary, construct funnel-like webs and have been reported as a host of *Epipompilus platensis* (Roig-Alsina and Barneche 2017). Currently, Segestriidae comprises 130 species divided into four genera (World Spider Catalog 2018), among them *Segestria* Latreille, 1804 and *Ariadna* Audouin, 1826 occurs in South America (Grismado 2008, Giroti and Brescovit 2011, World Spider Catalog 2018).

The genus *Ariadna* currently consists of 105 of the 130 species included in Segestriidae (Giroti and Brescovit 2018). This genus is distributed across all continents, but the greatest number of species is found in tropical and subtropical regions. The knowledge of biology and ecology of *Ariadna* is quite incipient, based on a single study that reported *A. maxima* as host of the koinobiont ectoparasitoid *Sphaerops appendiculata* Philippi, 1865 (Diptera, Acroceridae). This acrocerid is the only external koinobiont

parasitoid of spiders known in the family, since other species develops inside the opistosoma of spiders (Schlinger 1987).

Here, we report *E. excelsus* (Bradley, 1944) as a koinobiont ectoparasitoid of the tube-dwelling spider *Ariadna mollis* (Holmberg, 1876), with behavioural and biological notes of this wasp-spider interaction.

Methods

We observed *E. excelsus* attacking *A. mollis* on May 5, 2017 at Serra do Japi (23°13'53.60"S, 46°52'47.01"W), a tropical forest located in the municipality of Jundiaí, São Paulo state, Brazil. After the wasp attack, we collected the parasitized spider and transported it to the Laboratório Especial de Coleções Zoológicas (LECZ) - Instituto Butantan, São Paulo, Brazil, where we recorded the larval development. We kept the spider inside a terrarium (15×15×13 cm) and provided cotton balls with water, a piece of loose bark from the tree that we found the spider (used as a shelter by the spider), and we fed the spider every two days with *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae). To access the wasp development (i.e. from the egg stage to adult emergence), we photographed the parasitized spider daily upon a mm scale. We used the software Adobe Photoshop CS6 to analyze the photographs and measure the length and width of the larvae throughout its development. We deposited the remains of the spider carcass and the adult parasitoid in the Arachnida collection of Instituto Butantan, São Paulo, Brazil (Curator: A. D. Brescovit).

Results and discussion

We found an adult female of *E. excelsus* in an inspection flight near a trunk of a *Plinia cauliflora* Mart. (Myrtaceae). The wasp found an adult female of *A. mollis* and flew around the spider for few (six minutes approximately) minutes, remaining at a distance of approximately 6 cm from the spider. The wasp then landed on the spider dorsum, stung and paralyzed the spider. Thereafter, the wasp laid an egg dorsally on the spider abdomen; oviposition took approximately three minutes. Then the wasp drummed the spider (Fig. 1A), and after five minutes, flew off leaving the parasitized spider paralyzed. After approximately two more minutes, the individual of *A. mollis* began to move slowly and continued its normal course on the tree trunk (Fig. 1B).

In laboratory, the parasitized spider constructed a tube-shaped shelter in the space between the terrarium wall and the piece of bark. The spider continued to feed on the flies normally during our observations. The egg developed in two days. Once it hatched, the first instar larva emerged and stuck to the spider abdomen. The larval development included four instars distinguished by abrupt increases of the larvae size between each larval stage. The first instar measured 0.3 mm in width and 2.2 mm in length (Fig. 1C). The larvae reached the second instar (1.1 mm wide and 4.1 mm long,

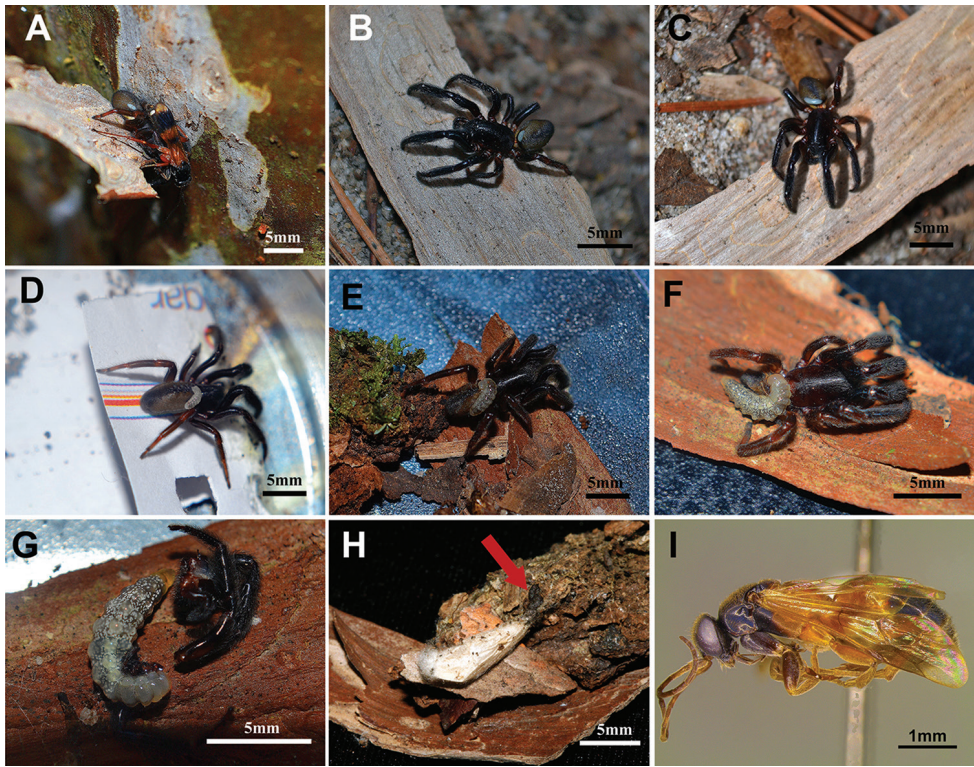


Figure 1. Adult female of *Ariadna mollis* (Segestriidae) parasitized by *Epipompilus excelsus* (Pompilidae). A) Wasp on the back of the spider (the arrow indicates the position of the egg in the abdomen). B) *A. mollis* moving slowly minutes after being paralyzed. C) Larval I instar of the wasp adhered to the abdomen of the spider. D) Larval II instar. E) Larval III instar. F) Larval IV instar feeding from the abdomen of the spider. G) Larva eating the remains of the legs and abdomen of the spider. H) The cocoon containing the meconium (red arrow). I) Adult female of *E. excelsus*.

Fig. 1D) five days later. After three days, the larvae reached the third instar and measured 1.7 mm wide and 6.1 mm long (Fig. 1E). Finally, the fourth instar was observed five days later, measuring 2.2 mm wide and 7.8 mm long, and presented 11 retractile dorsal tubercles (Fig. 1F). The fourth instar larvae killed the host and slowly consumed more than 50% of its body. Only the legs, the part of the ventral abdomen containing the genitalia, and the anterior part of the cephalothorax were not consumed by the larvae (Fig. 1G). On the next day, the larva began to construct a cocoon in the space between the bark and the wall of the terrarium, and fixed the apical portion of the cocoon in the bark (Fig. 1H). Emergence of the adult wasp occurred by the apical region of the cocoon five days after cocoon construction. The entire developmental cycle from oviposition until the emergence of adult wasp, took 24 days. This period is shorter than the developmental periods of wasps of other Pompilidae species. *Paracyphononyx scapulatus*, for example, develops in 54 days (Souza et al. 2015), *P. ruficus* in 30 days (El-Hennawy 1996), and *Notocyphus tyrannicus* Smith in 46 days (Martins et al. 2016).

Studies on congeneric species do not present a detailed description of the individual's life cycle (Roig-Alsina and Barneche 2017). Studies on the interaction between the parasitoid wasp *Epipompilus insularis* and the spiders *Clubiona cambridgei*, *Trite auricoma* or *T. planiceps* (Harris 1987) and the interaction between *E. insularis* and the spiders *C. cambridgei* or *Salticus* sp. (Pollard 1982) showed similar results to those of the present study. Harris (1987) and Pollard (1982) suggested that this genus of diurnal parasitoid wasps usually parasitize nocturnal non-weaver spiders because these spiders rest in shelters during the day, since this behavior of inactivity during daytime decreases the risk of attacks to the spiders, and thereby provides protection for the wasp egg and larvae. It is not possible to predict whether the parasitized spiders return to its shelters, but notably, our study specimen built a shelter and returned to it every day after goes out at night to capture the preys we offered. The spider was active during almost the entire larval development of *E. excelsus*, sheltering only in the final phase of development (fourth instar), before the wasp kill and consume the host. Harris (1987) reported a similar life cycle development time for *E. insularis* (22 days total) when compared to *E. excelsus* (24 days). The time of development time of each larval instar was also similar. However, the larval development of *E. insularis* involves five instars, while our study species presented four instars. Moreover, the final larval instar of *E. insularis* presents 12 lateral tubercles, while *E. excelsus* has 11 tubercles at the last instar.

Although all Pompilidae species use spiders as hosts, there are a high diversity of behavioural strategies characterized by different ethological sequences according to Evans (1953). This author classified the behaviour of *E. insularis* as VPO (*Venari* = to hunt; *Pungere* = to sting the prey; *Ovum parere* = to lay the egg) or VO (*Venari*= to hunt; *Ovum parere*= to lay the egg), and discussed that such ethological sequences represent primitive conditions within Aculeata, but derived conditions within Pompilidae. Species of other genera, such as *Notocyphus* Smith, 1855, *Paracyphononyx* Gribodo, 1884 and *Homonotus* Dahlbom, 1843 present the same ethological sequence (Evans 1953, Souza et al. 2015).

In conclusion, this study showed that the Neotropical species *E. excelsus* presents similar biological characteristics (e.g. koinobiont ectoparasitoidism) when compared to the Australian species. On the other hand, there are differences in larval morphology (e.g. number of tubercles) and development (e.g. number of larval instars). Studies on other koinobiont ectoparasitoid species would elucidate the extent of these differences and similarities, contributing to our understanding of the evolutionary history of *Epipompilus*, and consequently of Pompilidae.

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