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Hyperparasitic showdown: *Sclerodermus cereicollis*, a non-aggressive but surprisingly secondary hyperparasitoid

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Abstract

This study investigates the dynamics of hyperparasitism within the Bethylidae family, focusing on the interaction between *Sclerodermus cereicollis* and *Goniozus legneri*, two parasitoid wasp species characterized by different sociality and aggressivity towards conspecifics. Experimental trials were conducted using *Corcyra cephalonica* larvae as hosts, with different setups to stimulate competition. The results revealed unexpected behaviors, especially by *S. cereicollis*, including aggressive interactions, cannibalism, and hyperparasitism. In contrast, despite the typically aggressive nature of *G. legneri*, no particular hostility was observed toward *S. cereicollis*. The study highlights the complex dynamics of competition for resources among parasitoids, shedding light on the adaptive strategies and fitness costs associated with hyperparasitism. These findings contribute to a deeper understanding of the ecological interactions within parasitoid wasp communities.

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Introduction

Hyperparasitism is one of the most intricate animal interactions. Hyperparasitoids develop at the expense of other parasitoids, which are known as primary hosts and that are hosted by victims (typically phytophagous insects) known as secondary hosts (Sullivan, 1987, 1988; Poelman *et al.,* 2022). Hyperparasitoids can be defined as 'obligate' if their progeny can only develop in or on a primary parasitoid (Hawkins, 1994), or 'facultative' if their offsprings can develop either on a primary host or on a secondary one (Sullivan & Völkl, 1999). They can also be classified into 'true' hyperparasitoids when they develop directly on the larvae of the primary parasitoid or 'pseudo' hyperparasitoids when they develop on primary parasitoid pupae or prepupae (Poelman *et al*., 2022).

The most important element in the evolution from primary parasitism to hyperparasitism was the competition for hosts (Godfray, 1994), as facultative hyperparasitism can be highly beneficial to competing parasitoids by allowing their access to greater host resources (Grandgirard *et al*., 2002; Poelman *et al*., 2022). Parasitoids sharing the same host species may encounter hosts that have already been parasitized, with a low probability of offspring survival if oviposition occurs on the already parasitized host. For this reason, parasitoids generally avoid oviposition on a host already parasitized by the same species (superparasitism) or a different species (multi-parasitism) (Haye *et al*., 2021). However, in situations of limited host availability, facultative hyperparasitism may allow oviposition and increase the probability of reproductive success (Hubbard *et al*., 1987; van Baaren *et al.,* 1995; Harvey *et al*., 2013). Given that hyperparasitism is generally associated with fitness costs that include a reduced number of offspring, smaller females, and longer developmental times (Cusumano *et al*., 2011; 2016), facultative hyperparasitoids chose this option as an extreme choice.

Only three insect orders have evolved hyperparasitism: most species are found in 17 Hymenoptera families, while a few belong to Diptera and Coleoptera (Gordh, 1981; Godfray, 1994;), and, more specifically, facultative hyperparasitism is more frequent in Hymenoptera (Brodeur, 2000).

Although Brodeur (2000) in his investigation of the taxonomic affiliation of hyperparasitoids did not include any Hymenoptera Bethylidae as hyperparasites, Pérez-Lachaud *et al*. (2002; 2004) observed that C*ephalonomia hyalinipennis* Ashmead (Hymenoptera, Bethylidae) can behave as facultative hyperparasitoids.

Bethylidae are idiobiont ectoparasitoids of larvae, and occasionally pupae, of Coleoptera and Lepidoptera (Stringer *et al.,* 2012). Few bethylid species appear to be monophagous [*e.g.*, *Cephalonomia waterstoni* (Gahan) (Finlayson, 1950; Howard & Flinn, 1990)], but the majority of them are oligophagous (*e.g.*, Finlayson, 1950; David & Cook, 1994; Amante *et al.,* 2017). In addition, even species reported as monophagous or oligophagous can be reared in the laboratory on alternative factitious hosts not included in their natural range [*e.g.*, *Cephalonomia stephanoderis* Betrem (Perez-Lachaud & Hardy, 2001) and *Sclerodermus cereicollis* Kieffer (Abdi *et al*., 2021; Malabusini *et al.,* 2023)].

Bethylidae include species with a wide range of social behavior: from solitary to subsocial and quasi-social. Females of both subsocial and quasi-social species exhibit maternal care by remaining on the host during the period before and after oviposition (Griffiths & Godfray, 1988; Azevedo *et al.,* 2018). During this period, to ensure the development of their offspring, they may behave aggressively toward intruders, even if they are conspecifics (as in subsocial species such as those in the genus *Goniozus* Förster) (Petersen & Hardy, 1996), or they may cooperate with conspecifics by helping each other in paralyzing large hosts, sharing oviposition sites, and showing maternal care (as in quasi-social species such as those in the genus *Sclerodermus* Latreille) (Abdi *et al.,* 2020a; 2020b; Jucker *et al.,* 2020).

In Europe, the species *S. cereicollis*, misidentified in the past as S. brevicornis Kieffer (Masini *et al*., 2024), is a parasitoid of European long-horned beetle larvae (Coleoptera: Cerambycidae), also found in association with non-native cerambycids [*e.g.*, *Psacothea hilaris hilaris* (Pascoe) (Lupi *et al.,* 2017)]. This species has been extensively studied in laboratory studies on both the natural host and the factitious host, the rice moth, *Corcyra cephalonica* (Stainton) (Abdi *et al*., 2020a; 2021; Malabusini *et al.,* 2023).

Despite the social and cooperative aspect of all species in the genus *Sclerodermus*, recent studies have shown that competition and even killing between females can occur (Malabusini *et al.,* 2022; 2023; Guo *et al*., 2023), with even the possibility of dominant behavior by one or a few females (Lehtonen *et al.,* 2023).

The main objective of this work was to evaluate whether intraspecific competition occurs between a cooperative, generally non-aggressive species and a more aggressive one. For this reason, the Bethylidae *Sclerodermus cereicollis* was chosen as representative of the first group, and *Goniozus legneri* Gordh was chosen for the second, due to its ability to be aggressive towards intruders when its territory is invaded by a conspecific (Goubault *et al.,* 2006; Lizé *at al.,* 2012; Hardy *et al.,* 2013). Despite the unlikely occurrence of natural encounters between *Sclerodermus cereicollis* and *Goniozus legneri*, both species have gained prominence as model organisms in this specific study. Their well-documented behaviors and interactions make them valuable subjects for research that provide insights into the broader dynamics of parasitoid wasp ecology. The main objective of this work was to evaluate whether intraspecific competition or aggressive behavior occurs when *S. cereicollis* is challenged by a more aggressive species. Finally, the occurrence of hyperparasitism was assessed.

Materials and Methods

Parasitoid rearing system

The rearing system of *S. cereicollis* and *G. legneri* involved the use of *C. cephalonica* larvae as hosts. For *S. cereicollis*, four females (one week old) were placed in a 10 ml glass vial with a base of 1.4 mm into which a single host larva had previously been introduced. The same type of glass vial was used for rearing the parasitoid *G. legneri*, with individual females presented with single host larvae.

Each glass vial was kept in a climate chamber (23±1°C, 16L:8D and RH 60±5%) and checked regularly, and once the new adult offspring were present, they were stored in a refrigerator (4±1°C) to keep them available for a longer period (Jucker *et al.,* 2020). The new adults were used weekly to maintain the rearing system and were placed in new vials with new hosts or used for subsequent tests. In addition, to maintain the stock rearing system of *S. bevicornis* another rearing system of *S. cereicollis* was maintained on its natural host *P. h. hilaris* according to Malabusini *et al.* (2022).

Corcyra cephalonica rearing system

A stock culture of *C. cephalonica* was maintained in plexiglas cages (36´26´25 cm) in which adults could fly and mate. Females were confined in small containers to allow oviposition. Following the methodology described by Limonta *et al.* (2009), eggs were collected and transferred to Petri dishes (15 cm diameter, 2 cm depth) filled with a diet specifically designed for the rearing of pyralid moths, where the larvae could develop until they could be either used for research purposes or allowed to pupate, resulting in the emergence of fresh adults to maintain the stock culture.

Experiments

The experimental design was set to test the behavior of *S. cereicollis* in the presence of the more aggressive species *G. legneri.* Each trial consisted of one host larva of *C. cephalonica* (mean weight 0.0205±0.0011g), one female of *G. legneri*, and one female of *S. cereicollis* placed in the same 10 ml glass vial (8 cm height, closed with cotton wool and a gauze). A total of 41 replicates were prepared, in which parasitoids and hosts were left together for the entire observation period. In further 44 replicates the female of *G. legneri* was removed just after oviposition, while in 48 replicates it was removed after hatching. Daily monitoring of each replicate included noting the time of oviposition and the developmental stages of the brood of *G. legneri* and, where applicable, that of *S. cereicollis*. More detailed observations (twice per day) were made when particular behavior was noticed (*e.g.*, aggression between females). The decision to remove the *G. legneri* females at different times was aimed at simulating hyperparasitism.

Statistical analyses

Data were analyzed using the statistical software R (version 4.1.0). Before analyzing, all data were examined with Levene's test for homogeneous distribution, and the Shapiro-Wilk test for normal distribution. Data meeting these requirements were analyzed using one-way analysis of variance (ANOVA), and Tukey's tests were used to compare the differences among different treatments. Values are reported as mean ± standard error or percentage.

Results and Discussion

Out of the 133 replicates, *G. legneri* did not lay eggs on the host in only 4.51% (n=6). *G. legneri* exhibited on average a time to oviposition of 4.39±0.23 days, significantly shorter than *S. cereicollis*, which took 9.31±0.60 days to oviposit ($F_{1,147}$=65.18, P<0.001), confirming that *S. cereicollis* needs more time to paralyze the victim and oviposit on them (Abdi *et al.,* 2020a; 2021) in comparison to *Goniozus* sp. (Abdi *et al.,* 2020c).

Although *G. legneri* usually shows aggression towards conspecific intruders (Brodeur, 2000; Lizé *et al.,* 2012), in this experiment, it did not show any aggressive behavior towards *S. cereicollis* during the initial phase of host acceptance and paralysis. This could be because *G. legneri* is known to attack and oviposit the victim immediately when alone (less than 24 hours) (Bentley *et al*., 2009; Lizé *et al*., 2012), whereas *Sclerodermus* sp. takes longer (Gao *et al.,* 2016; Abdi *et al*., 2020c).

In addition, *S. cereicollis* abstained from laying eggs on *C. cephalonica* if the larva was already accepted and/or paralyzed by *G. legneri*, and was also observed assisting in the care of the brood, suggesting potential recognition of the dominant status of *G. legneri* females and thus focussing on brood. In some cases [30.7% of cases (n=39 of 127)] *S. cereicollis* females were observed to exhibit predatory behavior, consuming eggs or larvae of *G. legneri*, both in the presence and absence of *G. legneri.* This behavior was not significantly influenced by the time *G. legneri* female was removed ($F_{2,130}$=156.67, P=0.12). In addition, in the 7.87% (n=10 of 127) of cases, *S. cereicollis* females were observed perforating the cocoon spun by *G. legneri* larvae, entering it to *hyperparasitize G. legneri* larvae, or alternatively, to destroy/eat them (Figure 1).

In nine replicates *S. cereicollis* oviposited on larvae of *G. legneri* (Figure 2). In detail, a maximum of four eggs per replicate and three eggs per *G. legneri* larva were observed. With a total of 21 eggs of *S. cereicollis* oviposited, only one egg reached the adult stage. This value was not influenced by the stage in which *G. legneri* female was removed ($F_{2,130}$=75.27, P=0.75).

Additionally, while conducting rearing observations of *S. cereicollis* on a larva of *P. h. hilaris* within a cage containing four foundresses and mature larvae on the host, hyperparasitization of eight larvae of *S. cereicollis* by 14 eggs of *S. cereicollis* was documented. A maximum of three eggs per larva was observed (Figure 3a). Subsequently, after a period of four days, the majority of these eggs hatched and developed (n=11) (Figure 3b). However, it was not feasible to track their development until pupation.

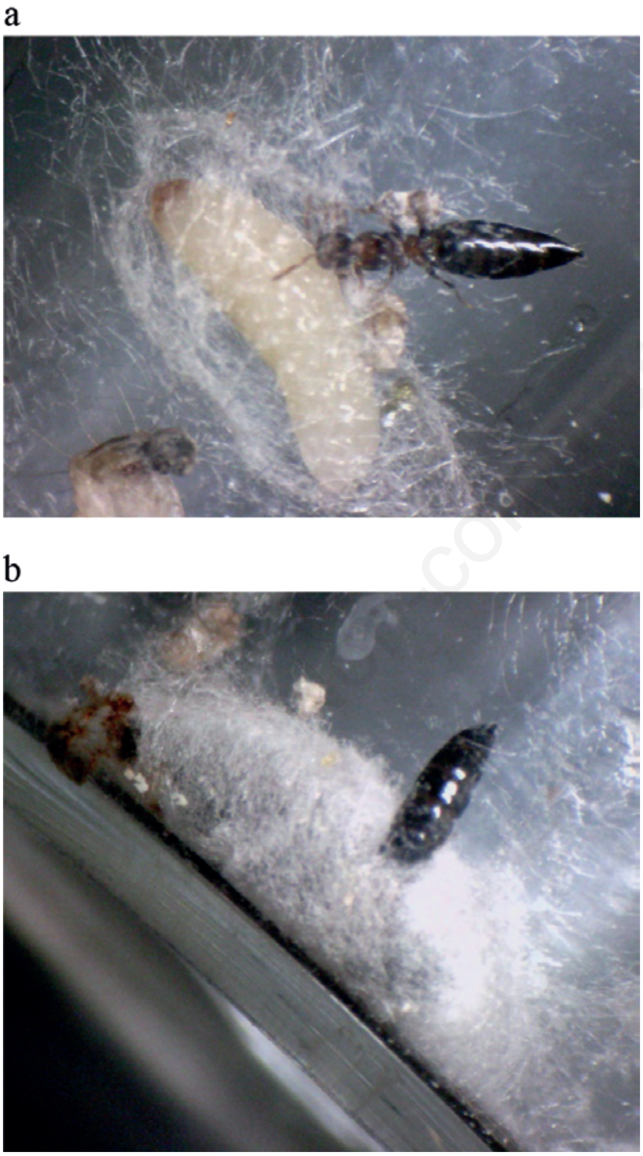

Figure 1. a) *S. cereicollis* female trying to create a hole inside *G. legneri* larvae cocoon; b) *S. cereicollis* females going inside *G. legneri* cocoon.

Conclusions

The observed patterns in the reproductive behavior of *G. legneri* and *S. cereicollis* offer intriguing insights into the dynamics of host selection, oviposition, and interactions between these parasitoids.

Pérez-Lachaud *et al*. (2004) reported cases of hyperparasitism between *Cephalonomia hyalinipennis* and other Bethylidae species, including *Goniozus legneri*. This competitive nature between female betylids sheds light on the complexities underlying the hyperparasitism observed in *S. cereicollis.*

The study by Grandgirard *et al.* (2002) addresses the costs associated with hyperparasitism and highlights the potential advantages of this strategy in accessing additional host resources. However, the quality of these resources may vary, introducing fitness costs that

Figure 2. Two eggs of *S. cereicollis* layed on a larva of *G. legneri* (on *C. cephalonica*).

depend on factors such as host species and size. For *Sclerodermus* spp. it is known that smaller hosts can influence the probability of having only female brood (Lupi *et al.,* 2017; Abdi *et a*l.,2020c) and, also, the size of future offspring (Liu *et al.*, 2011).

The observed hyperparasitic behavior of *S. cereicollis*, particularly to the detriment of the bethylid species *Goniozus legneri*, thus fits into the broader context of interactions within the family Bethylidae, characterized by competitive interactions, different host preferences, and potential benefits and costs associated with hyperparasitism.

In summary, the multifaceted nature of these interactions highlights the complex web of relationships among parasitoid wasps and their hosts in natural ecosystems.

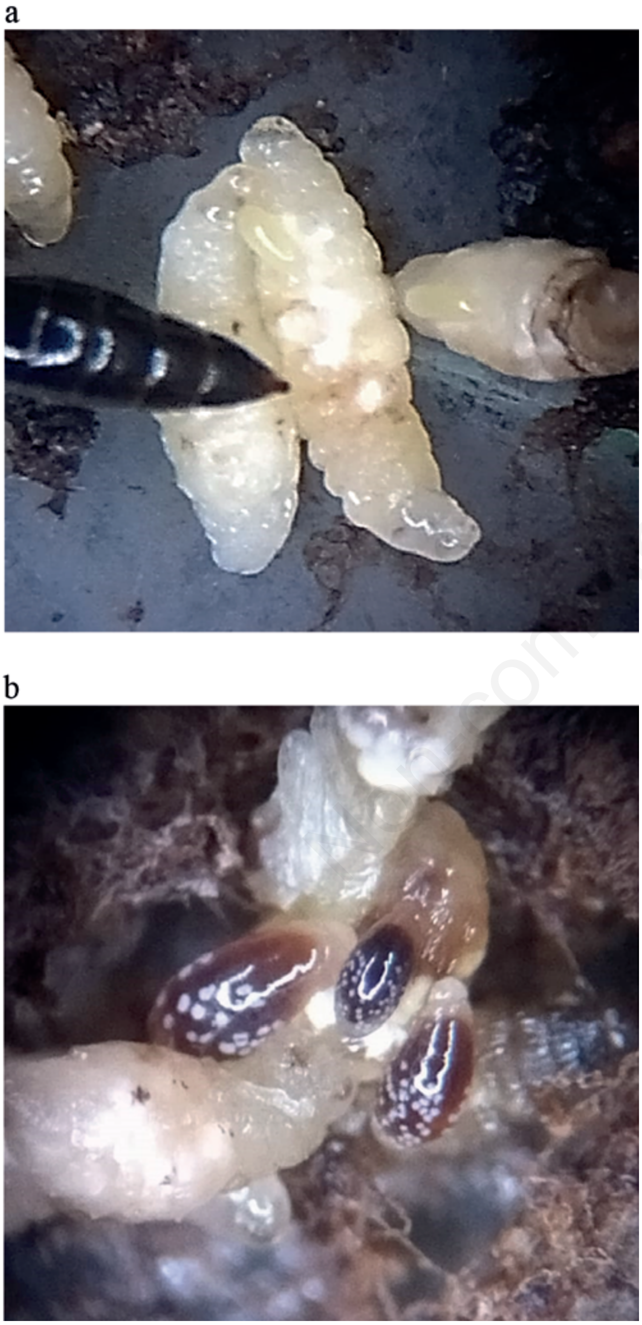

Figure 3. a) Eggs of *S. cereicollis* on *S. cereicollis* larvae; b) larvae of *S. cereicollis* on *S. cereicollis* larvae.

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