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## Reproductive biology of *Sclerodermus brevicornis*, a European parasitoid developing on three species of invasive longhorn beetles



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#### HIGHLIGHTS

- Invasive longhorn beetle species have recently invaded Europe from Asia.
- As larvae lives inside trunks control is difficult.
- Sclerodermus species can be deployed effectively against longhorn pests in China.
- A European Sclerodermus can attack and develop on all three invader species.
- Sclerodermus brevicornis could be used in mass-rearing and release programs.

#### ARTICLE INFO

# Article history: Received 27 June 2016 Revised 11 November 2016 Accepted 19 November 2016 Available online 21 November 2016

Keywords:
Bethylid wasp
Anoplophora spp.
Psacothea hilaris hilaris
Exotic alien species
Biological control
Rearing techniques
Maternal care

#### ABSTRACT

The reproductive performance of *Sclerodermus brevicornis* (Kieffer), a bethylid wasp native to Europe, was evaluated on three species of factitious hosts. These are longhorn beetles which have recently invaded Europe from Asia: *Anoplophora glabripennis* (Motschulsky), *Anoplophora chinensis* (Forster) and *Psacothea hilaris hilaris* (Pascoe) (Coleoptera: Cerambycidae). *Sclerodermus brevicornis* attacked all three species, but offspring only developed to maturity on medium and large sized host larvae. Host species influenced the duration of parasitoid development and the number of offspring maturing, both were greatest on *A. glabripennis*, with up to 373 adult parasitoids emerging from a single host. The sex ratios of *S. brevicornis* broods were strongly female biased (*ca.* 9% males). We conclude that *S. brevicornis* has the potential to be efficiently mass-reared and actively deployed in the biological control of invasive longhorn beetles. Further progress should be encouraged by the successful use of other species of *Sclerodermus* against beetle pests in China.

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#### 1. Introduction

Many insect species have spread from their geographical origins to become invasive pests in new habitats (Pimentel et al., 2005; Pellizzari et al., 2005; Roques et al., 2009; Jucker and Lupi, 2011). Wood-boring beetles provide good examples of anthropogenic spread as their immature stages are often inside wood imported for building houses or furniture, or in wood packaging materials and timber imported for pulp (Haack, 2006; Liebhold et al., 2006; Cocquempot, 2007; Liebhold and Tobin, 2008; Gandhi and Herms, 2010; Marini et al., 2011; Lupi et al., 2013; Rassati et al.,

2014, 2015). However, they can also arrive in living plants brought in from overseas (Roques, 2010; EPPO, 2012; Liebhold et al., 2012).

Many invasive wood-boring beetle species belong to the family Cerambycidae (Cocquempot and Lindelöw, 2010; Jucker and Lupi, 2011) with species in the genus *Anoplophora* considered some of the most dangerous invasive pests of woody plants worldwide (Haack et al., 2010). For instance, *Anoplophora chinensis* (Forster), the Citrus Longhorn Beetle (CLB), and *Anoplophora glabripennis* (Motschulsky), the Asian Longhorn Beetle (ALB), both native to the far east of Asia and considered quarantine pests, were detected in Italy in 2000 and in 2007, respectively (Colombo and Limonta, 2001; Maspero et al., 2007; Favaro et al., 2015). Specific eradication programs were subsequently implemented, at great social, environmental and economic cost (Faccoli and Gatto, 2016). CLB is currently restricted to several provinces in Lombardy (northern Italy)

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and some areas in central Italy, where active measures are being taken to eradicate it (EPPO, 2015a). The ALB is considered transient, targeted by current eradication actions, but with small isolated occurrences in several localities in the north and the center of the Italian peninsula (Hérard et al., 2009; EPPO, 2015b; Faccoli et al., 2015). Not included among quarantine pests (being officially controlled) or notifiable pests (a species that should be reported to plant health authorities if observed) but considered as a potential threat, is the Yellow Longhorn Beetle (YLB), *Psacothea hilaris hilaris* (Pascoe) (Coleoptera: Cerambycidae), a pest of Fig. and mulberry trees. The Asian YLB was first detected in southern Europe (Italy) in 2005, where it can kill infested plants. It is considered a potential threat for all Mediterranean countries where Figs. and mulberries are common trees (Jucker et al., 2006; Lupi et al., 2013, 2015a).

The containment of longhorn beetle populations is challenging both because these species are highly polyphagous on broad-leaf plants and because their larvae develop within the protection of woody trunks, protecting them from insecticidal spraying. While chemical control is an unrealistic and ineffective solution, biological control, via natural enemies, offers an environmentally sustainable approach to combating these species (Brabbs et al., 2015; Duan et al., 2016) and it can also be successfully combined with other tools, such as the use of lures and light-traps, in integrated pest management programs (Yang et al., 2014). There are a number of parasitoids that are natural enemies of cerambycids and the majority attack the larval and pupal stages (Hanks et al., 1995; Delvare et al., 2004; Zhang et al., 2005; Azevedo and Waichert, 2006; Loni et al., 2015).

The main parasitoids of ALB and CLB in Asia are the beetle Dastarcus helophoroides (Fairmaire) (Coleoptera: Bothrideridae) (Li et al., 2009; Wei and Niu, 2011) and wasp species in the genus Sclerodermus (Hymenoptera: Bethylidae) (Yao and Yang, 2008). In Europe, Dastarcus is represented by just one species, D. cyprianus Dajoz, which appears to occur only in Cyprus (Dajoz, 1989), while 17 species of Sclerodermus are recorded from Mediterranean areas (Andrè et al., 1904; Kieffer, 1914) some of which are associated with invasive exotic longhorn beetles (Hérard et al., 2007; Lupi et al., 2014). Members of the genus Sclerodermus (comprising at least 81 described species worldwide, Gordh and Móczár, 1990; Lanes and Azevedo, 2008), are idiobiont ectoparasitoids (Li and Sun, 2011; Hu et al., 2012) that mainly attack the larvae of wood-boring beetles. The wasps find hosts by entering preexisting galleries bored in the wood, aided by their morphological adaptations (e.g. small size, flattened body, prognathous head) are specifically selected for seeking hosts in enclosed spaces (Kühne and Becker, 1974; Evans, 1978; Yang et al., 2012; Baena and Zuzarte, 2013; Jiang et al., 2015).

The hosts of *Sclerodermus* range from small to large species (Mendel, 1986; Hsu and Wu, 1989; Yang et al., 2014). Within a *Sclerodermus* species, females may be most effective attacking younger (relatively small) hosts (Yang et al., 2014) but multiple females can cooperate in host attack, allowing them to suppress host defense and to lay eggs on larger hosts (Tang et al., 2014). After laying eggs, mothers remain at the host, tending the offspring until pupation into cocoons and emergence of adults (Hu et al., 2012; Yang et al., 2012; Tang et al., 2014). Multiple-female reproduction on a single host appears to be cooperative, without division of labor, and as such may be termed quasi-social (Li and Sun, 2011; Liu et al., 2011; Wang et al., 2016), an unusual life-history character among parasitoid hymenopterans.

Here we report empirical studies on the reproductive biology of *Sclerodermus brevicornis* (Kieffer) a native European species. *Sclerodermus brevicornis* was reported to be associated with the longhorn beetle *Oxypleurus nodieri* Mulsant in Europe approximately a century ago (Kieffer, 1914) and in 2011 it was recorded in association with YLB in northern Italy (Lupi et al., 2014).

Subsequently, in 2013, more than 1,000 adult *S. brevicornis* emerged from a Fig. branch (100 cm long, 32 cm diameter) heavily infested by YLB in the same locality (D. Lupi, pers. obs.) confirming the new host-parasitoid association. We discuss the potential of *S. brevicornis* for the suppression of exotic and invasive longhorn beetle populations. In China, mass rearing and release of Asian species of *Sclerodermus* has formed an integral part of successful forest pest management programs (Chen and Cheng, 2000; Du et al., 2006; Tang et al., 2012; Yang et al., 2014; Jiang et al., 2015), suggesting the possible employment of similar measures within Europe.

#### 2. Materials and methods

#### 2.1. Host rearing

**ALB:** In the spring of 2013, logs infested with ALB larvae were collected from Cornuda in North East Italy (45°49′ N, 12°00′ E) and stored under ambient conditions outdoors in metallic mesh cages ( $2 \times 1 \times 1$  m) until adult ALB emergence (Faccoli et al., 2016; Faccoli and Favaro, 2016). Newly emerged adults were then moved into maturation metallic mesh cages ( $2 \times 1.5 \times 1.5$  m) and fed for around two weeks with fresh maple twigs (*Acer campestre* L.) to allow adult sexual maturation and mating. Fresh willow logs (*Salix alba* L.) (10 cm diameter  $\times$  30 cm long) were also placed in the cages for egg laying.

*CLB*: Several adults of CLB were collected in an urban park of Milan (North Italy,  $45^{\circ}29'$  N,  $9^{\circ}05'$  E) in July 2013 placed in plastic cages ( $30 \times 20 \times 18$  cm) under outdoor conditions and fed with fresh maple twigs. After a few days, maple branches (2-3 cm diameter  $\times$  10-15 cm long) were added to the cages for egg laying.

YLB: A laboratory rearing system for YLB was set up in summer 2013 with adult beetles emerged from infested fig logs collected in the infestation area of the Como province, near to Lake Como in northern Italy (45°49′ N, 9°13′ E) (Lupi et al., 2013, 2015a). Fourto-ten pairs of newly emerged YLB adults were placed in plastic cages (30  $\times$  20  $\times$  18 cm) and kept in a climate room (temperature 25 ± 1 °C, photoperiod L16:D8, relative humidity 60 ± 5%). Three times each week the adult beetles were provided with fresh fig twigs (10–15 cm length  $\times$  1.5–2.5 cm diameter) for feeding and laying eggs on.

Infested material was debarked once a week by a cutter, and freshly laid eggs were moved individually onto an artificial diet known to be suitable for longhorn beetles (Lupi et al., 2015b). Eggs were randomly collected from at least ten different logs/twigs infested by different females. Newly hatched larvae were reared singly in 10 cm-diameter petri dishes in a climate chamber (25  $\pm$  1 °C, 16L:8D, RH 60  $\pm$  5%). Every week each larva was moved into a new petri dish with fresh diet (Lupi et al., 2015b), until they reached a suitable size for presentation to parasitoids.

#### 2.2. Parasitoid rearing

A laboratory stock culture of *Sclerodermus brevicornis* was founded by rearing individuals collected in September 2011 from fig trees infested by YLB in Ponte Lambro (Como province), northern Italy ( $45^{\circ}49'40''$  N;  $9^{\circ}13'07''$  E). In 2013, the stock culture was enriched with specimens coming from a new finding in field. The parasitoid was reared for more than 30 generations on YLB infested branches kept inside ventilated containers in a climate chamber ( $25 \pm 1$  °C, 16L:8D RH,  $60 \pm 5\%$ ). Every 15 days new YLB-infested branches were added to the rearing containers, thus providing new hosts to the newly emerged parasitoids. Some of the newly emerged female *S. brevicornis* were collected from the rearing stock and used in the experiments.

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