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Discovery of the first *Aximopsis* (Hymenoptera: Eurytomidae) parasitoid of Lepidoptera in Brazil and notes on its biology

Wagner de Souza Tavares¹, Carlos Frederico Wilcken², Francisco de Sousa Ramalho³, Marcus Alvarenga Soares⁴, Flávio Lemes Fernandes⁵, José Eduardo Serrão⁶, and José Cola Zanuncio^{7,*}

Abstract

Eucalyptus cloeziana F. Muell. (Myrtales: Myrtaceae) and the tropical almond *Terminalia catappa* L. (Myrtales: Combretaceae) are widely cultivated in urban and forest areas of many countries where biological control is the most-preferred method to control insects. *Aximopsis* sp. (Hymenoptera: Eurytomidae) is reported for the first time in Brazil in a new group of lepidopteran hosts. Individuals of this species emerged from the pupae of *Thyrintheina arnobia arnobia* Stoll (Lepidoptera: Geometridae) and *Thagona tibialis* Walker (Lepidoptera: Lymantriidae) that developed from larvae defoliating *E. cloeziana* and *T. catappa* plants on the campus of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais, Brazil. *Aximopsis* sp. was identified by comparing it with species of this group as described for the Neotropical region. Voucher specimens were deposited in the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France. Twenty new pupae each of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) were held individually in test tubes with a drop of honey as food and 3 mated *Aximopsis* sp. females for 2 d. *Aximopsis* sp. parasitized 20% of the *T. molitor* pupae but none of the *A. gemmatalis* pupae. The duration of the life cycle (egg to adult), parasitism and emergence rates, total individuals emerged from each pupa, sex ratio, length of the body and width of the head capsule, and the longevities of *Aximopsis* sp. males and females that emerged from parasitized *T. molitor* pupae were evaluated. The duration of the life cycle of *Aximopsis* sp. was 14 ± 2 d. An average of 62 ± 5 *Aximopsis* sp. individuals emerged from each *T. molitor* pupa, and their sex ratio was 0.96 ± 0.02 . The total number of parasitoids that emerged was 248 individuals. Measurements of characters of progeny *Aximopsis* sp. females ($n = 10$) and males ($n = 5$), respectively, were as follows: body length: 3.50 mm (2.40–3.80 mm) and 1.99 mm (1.97–2.02 mm); head capsule width: 0.63 mm (0.58–0.72 mm) and 0.48 mm (0.46–0.51 mm), and longevity: 6 ± 1 d and 4 ± 1 d. These results open prospects for investigations of biological control of pests with this natural enemy.

Key Words: Chalcidoidea; Combretaceae; Myrtaceae; urban area

Resumo

Eucalyptus cloeziana F. Muell. (Myrtales: Myrtaceae) e amendoeira-da-praia, *Terminalia catappa* L. (Myrtales: Combretaceae), são cultivados em áreas urbanas e florestais de muitos países onde o controle biológico é o método preferido para controlar insetos. *Aximopsis* sp. (Hymenoptera: Eurytomidae) é relatado pela primeira vez no Brasil em um novo grupo de Lepidoptera hospedeiros. Indivíduos desta espécie emergiram de pupas de *Thyrintheina arnobia arnobia* Stoll (Lepidoptera: Geometridae) e *Thagona tibialis* Walker (Lepidoptera: Lymantriidae) que se desenvolveram de lagartas desfolhando plantas de *E. cloeziana* e *T. catappa* no campus da Universidade Federal de Viçosa (UFV) em Viçosa, Minas Gerais, Brasil. *Aximopsis* sp. foi identificado por comparação com espécies desse grupo descritas para a região Neotropical. Espécimes desse inseto foram depositados no Centre de Coopération Internationale en Recherche Agronomique (CIRAD), França. Vinte pupas de *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) e vinte de *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) foram individualizadas em tubos de ensaio com uma gota de mel como alimento e 3 fêmeas de *Aximopsis* sp. por 2 dias. *Aximopsis* sp. parasitou 20% das pupas de *T. molitor*, mas nenhuma de *A. gemmatalis*. A duração do ciclo de vida (ovo a adulto), taxas de parasitismo e emergência, total de indivíduos emergidos por pupa, razão sexual, comprimento do corpo e a largura da cápsula cefálica e longevidades de machos e fêmeas de *Aximopsis* sp. que emergiram de pupas parasitadas de *T. molitor* foram avaliados. A duração do ciclo de vida de *Aximopsis* sp. foi de 14 ± 2 dias. Uma média de 62 ± 5 indivíduos de *Aximopsis* sp. emergiu por pupa de *T. molitor* com razão sexual de $0,96 \pm 0,02$. O número total de parasitoides emergidos foi de 248. Medidas da progênie de *Aximopsis* sp. fêmeas ($n =$

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10) e machos ($n = 5$) foram, respectivamente, de: comprimento do corpo: 3,50 mm (2,40–3,80 mm) e 1,99 mm (1,97–2,02 mm), largura da cápsula cefálica: 0,63 mm (0,58–0,72 mm) e 0,48 mm (0,46–0,51 mm) e longevidade: 6 ± 1 e 4 ± 1 dias. Estes resultados abrem perspectivas para investigações de controle biológico de pragas com este inimigo natural.

Palavras Chave: Chalcidoidea; Combretaceae; Myrtaceae; área urbana

Eucalyptus cloeziana F. Muell. (Myrtales: Myrtaceae) is cultivated in urban and forest areas to supply raw material for blacksmithing, props, structures, sleepers, and, especially, poles (Dall'Oglio et al. 2013). However, this plant does not thrive in regions with frost, soils with low fertility, altitudes above 1,600 m, fires, and severe water deficits, and it has reduced sprouting (Marques et al. 1996; Okino et al. 2004). *Eucalyptus cloeziana* can be infested by *Ctenarytaina spatulata* Taylor (Hemiptera: Aphalaridae), although other species of eucalyptus and other Myrtaceae are preferred by this aphalarid for feeding and oviposition (Queiroz et al. 2010). Furthermore, *E. cloeziana* is susceptible to defoliation by *Hylesia paulex* Dognin (Pereira et al. 2009) and *Dirphia moderata* Bouvier (Lepidoptera: Saturniidae) (Zanuncio et al. 1998; Pereira et al. 2008a) and suffers extensive damage from *Thyriniteina arnobia arnobia* Stoll and *Thyriniteina leucoceraea* Rindge (Lepidoptera: Geometridae) (Lemos et al. 1999; Pereira et al. 2008b).

Lepidopteran defoliators of *Eucalyptus* species can be controlled with chemical insecticides (Elek et al. 2003; Mansfield et al. 2006), but biological control with pupal parasitoids can reduce applications of these expensive and sometimes problematic products (Tavares et al. 2014a). The identification of natural enemies is the first step to develop biological control techniques in integrated management of a forest pest (Dall'Oglio et al. 2013). Thus, it is significant that *Palmistichus elaeisis* Delvare & LaSalle and *Trichospilus diatraeae* Cherian & Margabandhu (Hymenoptera: Eulophidae) were found to parasitize *T. arnobia arnobia* pupae on *E. cloeziana* plants (Pereira et al. 2008b,c), and the development and reproduction of *P. elaeisis* in *T. arnobia arnobia* pupae were studied in the laboratory (Pereira et al. 2010).

The number of natural enemies associated with *T. arnobia arnobia* may be great than currently is known, because *Paropsis atomaria* Olivier (Coleoptera: Chrysomelidae), the main pest of *E. cloeziana* in Australia, has a large number of egg and larval parasitoids and hyperparasitoids (Nahrung et al. 2008). The main natural enemy of this pest is *Neopolycystus* sp. (Hymenoptera: Pteromalidae), which was found to parasitize 45% of this beetle's eggs (Duffy et al. 2008). The aim of this study was to identify parasitoids of *T. arnobia arnobia* pupae on *E. cloeziana* plants in an urban area of Brazil and determine their parasitism rate on alternative hosts in order to develop mass production methods for use in biological control programs.

Materials and Methods

EXPERIMENTAL SITE

Thyriniteina arnobia arnobia pupae of various ages were collected on *E. cloeziana* plants at the campus of the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais, Brazil (20°44'S, 42°50'W; 650 m asl) in 2007. In 2011, *Thagona tibialis* Walker (Lepidoptera: Lymantiriidae) pupae of various ages were obtained from larvae defoliating *Terminalia catappa* L. (Myrtales: Combretaceae) trees, which are cultivated in urban areas and on the campus of UFV (20°45'S, 42°51'W, 651 m asl). These pupae were brought to the Laboratory of Biological Control of Insects (LCBI) of UFV and kept in a room at 25 ± 1 °C, a 12:12 h L:D photoperiod, and $70 \pm 10\%$ RH. Each pupa was placed in a test tube (14.0 cm long \times 2.2 cm diameter), which was sealed with a cotton swab until emergence of the lepidopteran or parasitoids. The collec-

tion areas were subjected to human impact and presented a flat terrain near a fragment of secondary forest with a diversified flora and fauna (Pereira et al. 2009; Tavares et al. 2011b, 2012c).

IDENTIFICATION OF INSECTS

Seventy-three individuals of an undescribed gregarious endoparasitoid species belonging to the genus *Aximopsis* (Hymenoptera: Eurytomidae), emerged from 1 *T. arnobia arnobia* pupa collected in Nov 2007 on *E. cloeziana* plants. In addition, 73 and 79 *Aximopsis* individuals emerged from 2 *T. tibialis* pupae sampled on a *T. catappa* tree in May and Jun 2011, respectively. This parasitoid was identified by Gerard Delvare of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France, from initial comparison with the types of Neotropical Eurytomidae housed in the U.S. National Museum of Natural History, Washington D.C., USA, and the Natural History Museum, London, United Kingdom. Individuals of this species were deposited in the CIRAD and in the Regional Museum of Entomology of the Department of Entomology at UFV.

BIOLOGICAL PROCEDURES

Twenty recent *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) (271.7 \pm 6.8 mg) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (118.0 \pm 5.6 mg) pupae were obtained from cultures of these species reared at UFV (Zanuncio et al. 2008; Pereira et al. 2010). Larvae of *A. gemmatalis* had been fed with a solid artificial diet composed of 125.0 g bean grains, 62.4 g yeast, 100.0 g wheat germs, 100.0 g soy protein, 50.0 g casein, 35.0 g agar, 5.0 g Nipagin®, 6.0 g ascorbic acid, 3.0 g sorbic acid, 6.0 mL formaldehyde at 40.0%, and 10.0 mL vitamin solution (Pereira et al. 2010). Larvae of *T. molitor* had been fed ad libitum with wheat bran (12% protein, 2% lipids, 75% carbohydrates, and 11% minerals/sugar) and pieces of *Saccharum officinarum* L. (Poales: Poaceae) and *Sechium edule* (Jacq.) Swartz (Cucurbitales: Cucurbitaceae). Sheets of paper were placed on top of the substrate to reduce the intensity of the light on the insects (Zanuncio et al. 2008).

Each pupa was individualized in a test tube with a drop of honey and 3 mated *Aximopsis* sp. females that had emerged from *T. tibialis* pupae. These insects remained in the test tubes for 2 d according to the method proposed for parasitism of *A. gemmatalis* and *T. molitor* pupae by *P. elaeisis* in the laboratory (Zanuncio et al. 2008; Pereira et al. 2010). Parasitism and emergence rates of parasitoids emerged from *A. gemmatalis* and *T. molitor* pupae were evaluated according to the number of parasitized pupae presenting caramel color. Unviable pupae became black, hollow, and died, whereas adult lepidopterans or coleopterans emerged from the non-parasitized ones (Zanuncio et al. 2008; Pereira et al. 2010).

EXPERIMENTAL DESIGN

The design was completely randomized with 2 treatments represented by *A. gemmatalis* and *T. molitor* pupae. Each treatment had 20 replications (1 pupa each). The duration of the life cycle (egg to adult, in d), parasitism and emergence rates (%), number of emerged individuals from each pupa, sex ratio, length of the body and width of the head capsule, and the longevity of *Aximopsis* sp. females and males that emerged from host pupae were determined according to

the method proposed for parasitism of *Heraclides anchisiades capys* Hübner (Lepidoptera: Papilionidae) pupae by *P. elaeisis* in the laboratory (Tavares et al. 2013c).

Results

Aximopsis sp. did not parasitize *A. gemmatalis* pupae, but it parasitized *T. molitor* pupae, which resulted in parasitism and emergence rates of 20% and 62 ± 5 individuals per pupa of this latter host (Table 1). The duration of the life cycle (egg to adult) of *Aximopsis* sp. was 14 ± 2 d. The sex ratio was 0.96 ± 0.02 . The total number of parasitoids that emerged was 248 individuals. The longevity of *Aximopsis* sp. females was 6 ± 1 d and that of males was 4 ± 1 d. The body length and the width of the head capsule of *Aximopsis* sp. progeny were 3.50 mm (2.40–3.80 mm) and 0.63 mm (0.58–0.72 mm), respectively, in females ($n = 10$) and 1.99 mm (1.97–2.02 mm) and 0.48 mm (0.46–0.51 mm), respectively, in males ($n = 5$).

This is the first report of an *Aximopsis* sp. parasitizing lepidopteran pupae, i.e., *T. arnobia arnobia* and *T. tibialis*. Individuals of *Aximopsis* sp. that emerged from pupae of these 2 hosts in Brazil differed in their morphology from other species of this genus. Prior to this report, *Aximopsis* was known to parasitize only hymenopteran hosts. Thus, *Aximopsis* sp. that emerged from *T. arnobia arnobia* and *T. tibialis* pupae appear to be representatives of a new group that specializes on lepidopteran host species.

Discussion

The identification of *Aximopsis* sp. parasitizing *T. arnobia arnobia* pupae is important, because the pupal stage has no defense, whereas the larvae can reduce the impact of natural enemies by camouflage, by attacking natural enemies with their jaws, and by hanging from silk threads (Soares et al. 2009).

The number of individuals of *Aximopsis* sp. that emerged per *T. molitor* pupa (62 ± 5) was smaller than the numbers of *P. elaeisis* obtained in the following cases: (i) 4 *P. elaeisis* females per *T. molitor* pupae resulted in 100% parasitism, 90.8% emergence, and 70 ± 3 individuals per pupa (Zanuncio et al. 2008), (ii) 6 *P. elaeisis* females per *A. gemmatalis* pupa resulted in 100% parasitism, 100% emergence, and 110 ± 19 individuals per pupa (Pereira et al. 2010), (iii) 10 *P. elaeisis* females per *H. anchisiades capys* pupa resulted in 40% parasitism, 40% emergence, and 323 ± 38 individuals per pupa (Tavares et al. 2013c), and (iv) 10 *Trichospilus pupivorus* Ferrière (Hy-

menoptera: Eulophidae) females per *A. gemmatalis* pupa resulted in 35% parasitism, 35% emergence, and 242 ± 12 individuals per pupa (Tavares et al. 2011a).

This lower number of individuals of *Aximopsis* sp. that emerged per *T. molitor* pupa compared with other cases may be because of the greater body length and the greater width of the head capsule of *Aximopsis* sp. female and male progeny than those of *P. elaeisis* (2.00 ± 0.03 mm and 0.58 ± 0.01 mm, respectively, in females and 1.34 ± 0.02 mm and 0.45 ± 0.01 mm, respectively, in males) that emerged from *T. molitor* pupae (Zanuncio et al. 2008) and the width of the head capsule of female and male *T. diatraeae* (0.71 ± 0.01 mm and 0.55 ± 0.02 mm, respectively) that emerged from *T. arnobia arnobia* pupae (Pastori et al. 2012).

This first report of *Aximopsis* sp. parasitizing pupae of *T. arnobia arnobia* and *T. tibialis* in Brazil opens possibilities for biological control of these pests in *Eucalyptus* plantations and on *T. catappa* plants in urban and forest areas by producing and releasing this natural enemy. The initial infestation of *T. tibialis* (population peak in May) on *T. catappa* plants (Tavares et al. 2013b, 2014b) near *E. cloeziana* plantations may have contributed to the dispersion, parasitism, and occurrence of *Aximopsis* sp. in Nov in *T. arnobia arnobia* pupae. *Terminalia catappa* is cultivated along the Atlantic coast of Brazil and in municipalities of Amapá, Pará, and Roraima States, which border French Guiana, Guyana, and Suriname (Tavares et al. 2011a, 2012a). This area may be the region of entrance and distribution of *Aximopsis* sp. to central Brazil. *Terminalia catappa* plants are grown near *Eucalyptus* crops, and those plants can be a refuge for natural enemies of forest pests, just as has been reported for agricultural crops where *Crotalaria juncea* L. (Fabales: Fabaceae) shelters natural enemies of pests of *Zea mays* L. (Poales: Poaceae) (Tavares et al. 2011c). *Thagana tibialis* pupae are also hosts of the eulophid parasitoids *P. elaeisis* and *T. pupivorus*, which are promising biological control agents of agricultural pests such as *A. gemmatalis*, a major defoliator of *Glycine max* (L.) Merr. (Fabales: Fabaceae) (Tavares et al. 2011a, 2012a,b, 2013a).

This newly discovered Brazilian species of *Aximopsis* is most similar to one that parasitizes *Sibine* sp. (Lepidoptera: Limacodidae) pupae in the Neotropical region, and which is deposited in the collection of CIRAD (G. Delvare, pers. com.). Probably, the Brazilian species is native to South America, although the Australasia fauna of this genus is largely unknown. The phylogenetic position of the genus *Aximopsis* Ashmead in the Eurytominae has been redefined (Gates et al. 2006). Additional phylogenetic studies of the species of this genus are needed, because it is extremely diverse in the Neotropical region. The DNA of some Eurytominae species was sequenced in order to verify a phylogenetic analysis based on morphological characters (Lotfalizadeh et al. 2007).

The *Aximopsis* sp. that emerged from *T. arnobia arnobia* and *T. tibialis* pupae appears to be representative of a new group of *Aximopsis* that specializes on host species differing from those parasitized by *Aximopsis masneri* Gates (Hymenoptera: Eurytomidae). The latter species parasitizes *Euglossa variabilis* Friese and *Euglossa cybelia* Moure (Hymenoptera: Apidae) in nests of these wasps in the Neotropical region (Gates 2009).

This is the first report of a species of the genus *Aximopsis* in Brazil. In addition, *Aximopsis* sp. parasitized Lepidoptera pupae of forest pests of the families Geometridae and Lymantriidae, which constitute a host group not previously known to be parasitized by species of *Aximopsis*. *Tenebrio molitor*, an adequate alternative host for some forest parasitoids in Brazil, has the potential to be used for mass rearing *Aximopsis* sp. in the laboratory. This study opens prospects for programs of biological control of pests with this natural enemy in urban and forest plantation areas of *E. cloeziana* and *T. catappa*.

Table 1. Parameters (mean \pm SD, or range) of *Aximopsis* sp. that parasitized pupae of *Tenebrio molitor* in the laboratory.

Parameter	Value
Duration of the life cycle (egg to adult) (d)	14 ± 2
Parasitism rate (%)	20
Emergence rate (%)	20
Number of emerged individuals from each pupa	62 ± 5
Total number of parasitoids that emerged	248
Sex ratio	0.96 ± 0.02
Length of the body (female) (mm) ($n = 10$)	3.50 (2.40–3.80)
Length of the body (male) (mm) ($n = 5$)	1.99 (1.97–2.02)
Width of the head capsule (female) (mm) ($n = 10$)	0.63 (0.58–0.72)
Width of the head capsule (male) (mm) ($n = 5$)	0.48 (0.46–0.51)
Longevity (female) (d)	6 ± 1
Longevity (male) (d)	4 ± 1

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