Hymenopteran parasitoids associated with scale insects (Hemiptera: Coccoidea) in tropical fruit trees in the eastern Amazon, Brazil

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Abstract

Fifteen species of parasitoids emerged from 6 species of scale insects, all are recorded for the first time in Maranhão State, Brazil. A total of 20 coccoid/parasitoid associations were found, 5 of which are new host records for the parasitoid species: *Coccus hesperidum* (L.) (Hemiptera: Coccidae) with *Arrhenophagus chionaspidis* (Aurivillius) (Hymenoptera: Encyrtidae); and *Praelongorthezia praelonga* (Douglas) (Hemiptera: Ortheziidae) with *Aenasius* sp. (Hymenoptera: Encyrtidae), *Encyrtus aurantii* (Geoffroy) (Hymenoptera: Encyrtidae), *Aprostocetus* sp. (Hymenoptera: Eulophidae) and *Signiphora* sp. (Hymenoptera: Signiphoridae).

Key Words: Aphelinidae; biological control; Encyrtidae; natural enemies; tritrophic interactions

Resumo

Quinze espécies de parasitoides emergiram de seis espécies de cochonilhas, todas são registradas pela primeira vez no Estado do Maranhão, Brasil. Um total de 20 interações cochonilhas/parasitoides foram encontradas, das quais cinco são novos registros de hospedeiros para as espécies de parasitoides: *Coccus hesperidum* (L.) (Hemiptera: Coccidae) com *Arrhenophagus chionaspidis* (Aurivillius) (Hymenoptera: Encyrtidae); e *Praelongorthezia praelonga* (Douglas) (Hemiptera: Ortheziidae) com *Aenasius* sp. (Hymenoptera: Encyrtidae), *Encyrtus aurantii* (Geoffroy) (Hymenoptera: Encyrtidae), *Aprostocetus* sp. (Hymenoptera: Eulophidae) e *Signiphora* sp. (Hymenoptera: Signiphoridae).

Palavras Chave: Aphelinidae; controle biológico; Encyrtidae; inimigos naturais; interações tritróficas

Scale insects (Hemiptera: Coccoidea) are phytophagous insects that infest a great number of economically important plants. These insects damage plants directly by sucking their sap, and indirectly by injecting toxic salivary secretions, attracting ants, transmitting pathogens, and encouraging the development of sooty molds (Zucchi et al. 1993). The sooty molds produced by high infestation of scale insects can inhibit photosynthesis and reduce the tree vigor. They also can stain the fruit, reducing its commercial value about 20 to 30% in highly infested crops (Oliveira et al. 2001; Barbosa & Paranhos 2004).

Parasitoids belonging to the families Aphelinidae, Encyrtidae, Eulophidae, Eupelmidae, Pteromalidae, and Signiphoridae (Hymenoptera: Chalcidoidea) are among the most important groups of natural enemies of scale insects and have been used extensively in biological control (Noyes 2017). In Brazil, the occurrence of primary and secondary parasitoids associated with scale insects were recorded in São Paulo by Toledo (1940) and Peronti et al. (2016); in Alagoas by De Santis (1972); in Brasilia by Murakami & Consenza (1984); in the Rio Grande do Sul by Wolff et al. (2004, 2014) and Silva et al. (2007); in Espírito Santo by Culik et al. (2011); in the Rio de Janeiro by Rodrigues & Cassino (2012); in Roraima by Marsaro-Junior et al. (2013, 2016); and in Minas Gerais by Prado et al. (2015).

It is important to know the parasitoid species that can control scale insects, because they can maintain the populations of harmful insects below the economic injury level if properly managed (Wolff et al. 2004). Correct identification of species is essential for studies of biological control and integrated pest management (Abreu et al. 2015). According to Araújo & Zucchi (2002), for the establishment of an integrated regional management program, the knowledge of the diversity of parasitoids in the area of interest is essential. There are no such surveys in Maranhão State, so we conducted an inventory of the species of parasitoids associated with scale insects of 6 fruit tree species in the Eastern Amazon, Brazil.

Material and Methods

These studies were conducted in 3 areas of fruit production in the municipalities of São José de Ribamar (2.8483°S, 44.0594°W), São Luís (2.5130°S, 44.3024°W), and Paço do Lumiar (2.9083°S, 44.2141°W) in

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Maranhão State, Brazil. Samples of scale insects were collected monthly in the period from Jun 2014 to Aug 2015 on 6 fruit trees species: *Anacardium occidentale* L. var. *nanum* (cashew; Anacardiaceae), *Citrus* spp. (*citrus* trees; Rutaceae), *Cocos nucifera* L. (coconut; Arecaceae), *Malpighia emarginata* L. (acerola; Malpighiaceae), *Mangifera indica* L. (mango; Anacardiaceae), and *Theobroma grandiflorum* (Willd. ex Spreng.) Schum. (cupuassu; Malvaceae).

The scale insects were randomly collected from infested plant tissues (leaves, stems, branches, and fruits) from 8 plants of each species of fruit tree, 5 samples per plant, over a period of 15 mo, which resulted in 3,600 samples per area. The infested samples collected were placed in labeled plastic bags with the date, place of collection and host culture, and transported to the Entomology Laboratory of the Universidade Estadual do Maranhão, Maranhão, Brazil. In the laboratory, infested samples were placed in Petri dishes lined with moist filter paper, which then were covered with plastic film and incubated in an environmental control chamber at 26 ± 1 °C, 60 ± 10% RH, and a 12:12 h (L:D) photophase.

The majority of emerged hymenopterous parasitoids obtained were double-mounted on points; however, the smaller species were slide-mounted following the techniques of Querino & Zucchi (2011). Afterwards, they were identified to genus or species level using De Santis (1964), Annecke & Prinsloo (1974), Noyes (1980), Noyes et al. (1997), and Noyes (2010) for Encyrtidae; Gibson (1995) for Eupelmidae; Schauff et al. (1997) for Eulophidae; Compere (1939), Woolley (1997a), and Myartseva & Evans (2008) for Aphelinidae; and Woolley (1997b) for Signiphoridae.

The adult females of scale insects were mounted on permanent slides following the methodology described by Gullan (1984), and were identified according to the morphological characteristics of the adult female as used by Williams & Granara de Willink (1992) for Pseudococcidae; Granara de Willink (1999) for Coccidae; and Miller & Davidson (2005) for Diaspididae.

Voucher specimens were deposited in the following institutions: hymenopterous parasitoids were deposited in the Collection of Entomophagus Insects "Oscar Monte"» from Instituto Biológico, Campinas, São Paulo State, Brazil; and scale insects in the Reference Collection of Insects and Mites-CRIA from Universidade Estadual Paulista, Jaboticabal, São Paulo State, Brazil. Also, specimens of both groups were deposited in the Entomological Collection "Iraci Paiva Coelho" from Universidade Estadual do Maranhão, São Luís, Maranhão State, Brazil.

Results

A total of 20 coccoid/hymenopteran parasitoid associations were found, 5 of which are new host records for the parasitoid species: *Coccus hesperidum* (L.) (Hemiptera: Coccidae) with *Arrhenophagus chionaspidis* (Aurivillius) (Hymenoptera: Encyrtidae); and *Praelongorthezia praelonga* (Douglas) (Hemiptera: Ortheziidae) with *Aenasius* sp. (Hymenoptera: Encyrtidae), *Encyrtus aurantii* (Geoffroy) (Hymenoptera: Encyrtidae), *Aprostocetus* sp. (Hymenoptera: Eulophidae), and *Signiphora* sp. (Hymenoptera: Signiphoridae) (Table 1). All insects and interactions found in this study were recorded for the first time for the Maranhão State of Brazil.

Table 1. Parasitoids associated with species of scale insects, their host plant, and collection sites (Jun 2014 to Aug 2015) at Maranhão Island (Maranhão, Brazil).

Scale insects	Parasitoid	Host plant	Collection sites
Coccidae			
Coccus hesperidum (L.)	**Arrhenophagus chionaspidis* Aurivillius	*Malpighia emarginata* (acerola)	Paço do Lumiar
	**Coccophagus fallax* Compere	*Anacardium occidentale* (cashew)	São José de Ribamar
	**Coccophagus basalis* Compere	*Malpighia emarginata* (acerola)	Paço do Lumiar
	**Encyrtus aurantii* (Geoffroy)		Paço do Lumiar
	**Metaphycus* sp.	*Mangifera indica* (mango)	São José de Ribamar
***Pulvinaria* sp.	**Encyrtus aurantii* (Geoffroy)	*Malpighia emarginata* (acerola)	Paço do Lumiar
Diaspididae			
Aonidiella aurantii (Maskell)	**Aphytis holoxanthus* DeBach	*Cocos nucifera* (coconut)	Paço do Lumiar
	**Encarsia* sp.		São Luís
	**Metaphycus* sp.		
Aulacaspis tubercularis (Newstead)	**Aphytis* group *lingnanensis*	*Mangifera indica* (mango)	São José de Ribamar
	**Aphytis holoxanthus* DeBach	*Citrus* sp. (citrus trees)	São Luís
	**Arrhenophagus chionaspidis* Aurivillius	*Citrus* sp. (citrus trees)	Paço do Lumiar
			São Luís
		Cocos nucifera (coconut)	São Luís
		Mangifera indica (mango)	Paço do Lumiar
			São José de Ribamar
	**Encarsia citrina* (Craw)	*Citrus* sp. (citrus trees)	Paço do Lumiar
	**Encarsia lounsburyi* (Berlese and Paoli)	*Citrus* sp. (citrus trees)	São Luís
Ortheziidae			
***Praelongorthezia praelonga* (Douglas)	**Aenasius* sp.	*Citrus* sp. (citrus trees)	Paço do Lumiar
	**Aprostocetus* sp.		São José de Ribamar
	**Encyrtus aurantii* (Geoffroy)	*Anacardium occidentale* (cashew)	Paço do Lumiar
	**Signiphora* sp.	*Citrus* sp. (citrus trees)	Paço do Lumiar
Pseudococcidae			
***Nipaecoccus* sp.	**Acerophagus* sp. aff. *Alexte*	*Cocos nucifera* (coconut)	São Luís
	**Acerophagus* sp.	*Cocos nucifera* (coconut)	São Luís

* New record for the Maranhão State (Brazil)
** New record of the interaction between parasitoid and scale insect.

Fifteen species of Hymenoptera parasitoids were found, distributed in 4 families of Chalcidoidea: Aphelinidae - *Aphytis holoxanthus* DeBach and *Aphytis* group *lingnanensis*, *Coccophagus fallax* Compere, *Coccophagus basalis* Compere, *Encarsia* sp., *Encarsia lounsbury* (Berlese and Paoli), and *Encarsia citrina* (Craw); Encyrtidae - *Acerophagus* sp. aff. *alexte*, *Acerophagus* sp., *Aenasius* sp., *Ar. chionaspidis*, *E. aurantii*, and *Metaphycus* sp.; Eulophidae - *Aprostocetus* sp.; and Signiphoridae - *Signiphora* sp. They were reared from 6 species of scale insects: *Aonidiella aurantii* (Maskell) and *Aulacaspis tubercularis* (Newstead) (Diaspididae), *C. hesperidum* and *Pulvinaria* sp. (Coccidae), *Nipaecoccus* sp. (Pseudococcidae), and *P. praelonga* (Ortheziidae) (Table 1, Fig. 1).

Discussion

Aphelinidae and Encyrtidae are the most important families of Chalcidoidea associated with scale insects, and have been associated with 335 and 416 species of scale insects, respectively (García et al. 2016; Noyes 2017). Both families include several species used in biological control programs around the world, especially in warmer climates (Noyes et al. 1997).

Aphytis Howard has been associated with 165 species of scale insects (García et al. 2016; Noyes 2017). This genus is cosmopolitan and comprises the more efficient natural enemies of Diaspididae (Rosen & Rose 1989). In diaspidid scales, *Aphytis* species develop exclusively as primary ectoparasitoids. The adult female inserts its ovipositor through the scale cover and deposits the eggs in the space between the body and the scale cover (Yu & Luck 1988; Rosen & Rose 1989). As natural enemies, several species of this genus have been employed successfully in biological control programs for economically important armored scale insects (Rosen & DeBach 1979; García et al. 2016; Noyes 2017). In Brazil, *A. holoxanthus* was introduced in São Paulo State to control *Chrysomphalus aonidum* (L.) (Diaspididae) (Gonçalves 1962; DeBach & Rosen 1976). Ten species of Diaspididae are associated to *A. holoxanthus* in several countries (Noyes 2017).

Coccophagus Westwood includes 200 described species. Most parasitize species of Coccoidea, mainly Coccidae. Some of these parasitoids have also been associated with species of Diaspididae, Eriococcidae, Kermesidae, Pseudococcidae, and Stictococcidae (Hayat 1997; García et al. 2016; Noyes 2017). In most species of this genus the larvae of female and male of the same species develop on different hosts (Hunter & Wooley 2001). For example, the females of *Coccophagus malthusi* Girault, a secondary parasitoid, has been obtained from Ceroplastinae spp., mainly *Ceroplastes* spp., whereas the males are reared from coccids of other species or genera such as *C. hesperidum*, *Marsipococcus proteae* (Brain) (synonym *Coccus proteae* Brain), *Saissetia* spp. (Coccidae), and *Parasaissetia* spp. (Coccidae). Also, the males almost always occur on different host plants from the females (Flanders 1937; Hunter & Woolley 2001).

In Brazil, *Coccophagus basalis* Compere and *Coccophagus fallax* Compere were found on *Saissetia oleae* Olivier (Coccidae) in São Paulo, Distrito Federal, Bahia, Rio de Janeiro (Compere 1939) and Minas Gerais (Prado et al. 2015).

Encarsia Förster has over 400 species described worldwide, distributed in 26 groups, and 115 species occur in the Neotropics. This is one of the most important genera for biological control, parasitizing

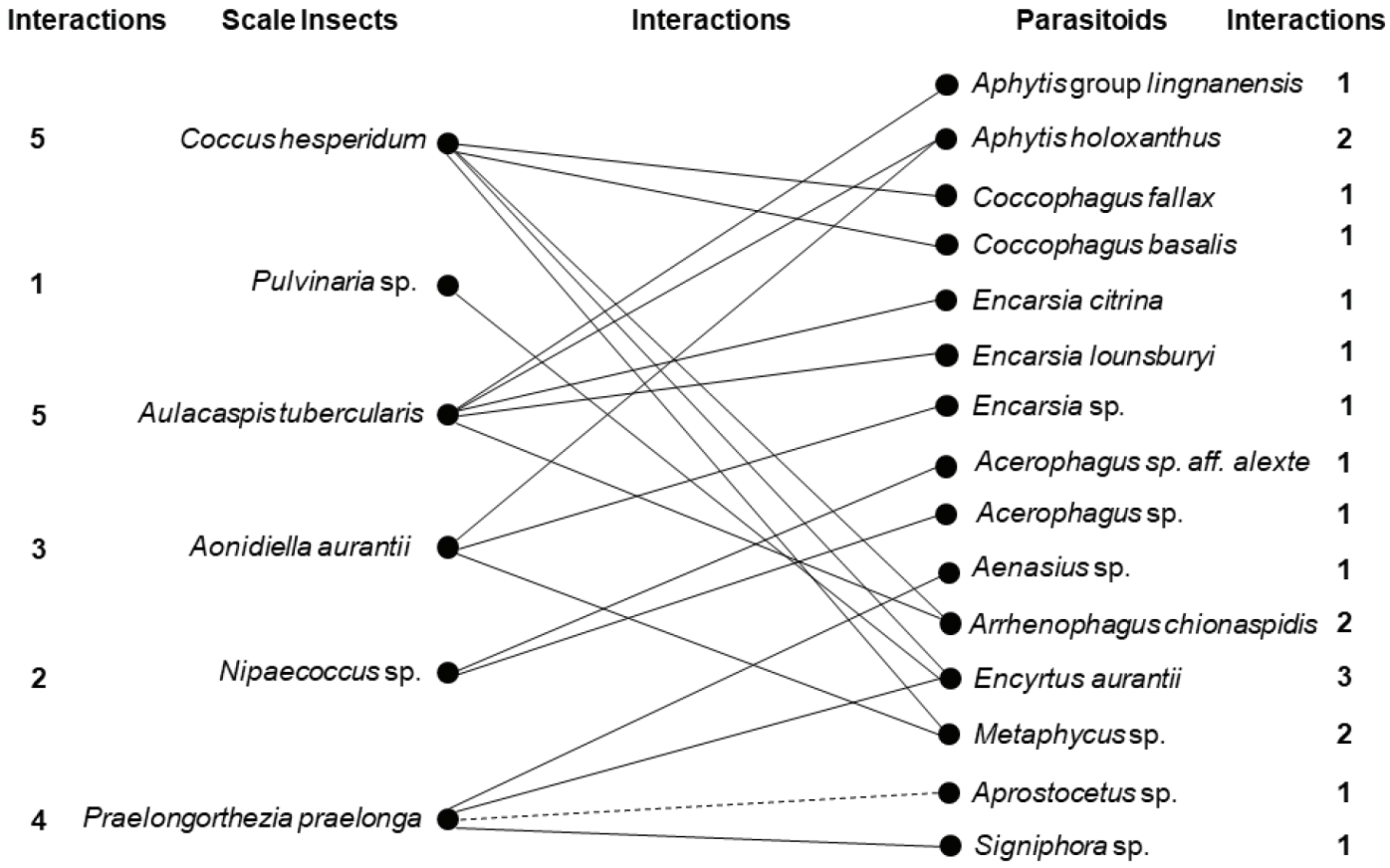

Fig. 1. Interactions between species of scale insects and parasitoids with the total number of interactions with each species of host plant (Jun 2014 to Aug 2015) at Maranhão Island, Maranhão, Brazil.

species of Aleyrodidae and Diaspididae (Evans et al. 1995; Heraty et al. 2007; Myartseva & Evans 2008). In Brazil, *E. lounsburyi* was recorded on *C. aonidum* in the states of São Paulo (Toledo 1940), Rio de Janeiro (Gomes 1942), and in the city of Campo Grande (Parker et al. 1953). It also was recorded on *Hemiberlesia cyanophylli* (Signoret) and *Aonidella aurantii* in the state of Rio de Janeiro (De Santis 1980). On the other hand, *Encarsia citrina* (Craw) occurs on an unknown host in the state of Bahia (Ashmead 1904), on *Chrysomphalus* sp. and *Aspidiotus* sp. (Diaspididae) in the state of São Paulo (Parker et al. 1953; Bergmann et al. 1991), and on *C. aonidium* in the state of Rio de Janeiro (Gomes 1942).

Acerophagus Smith has a wide geographic distribution, and all species have been found to be primary parasitoids of mealybugs (Noyes 2010). This genus is associated with 32 species of scale insects (García et al. 2016; Noyes 2017), of which at least 16 have been widely used in classical control biological programs (Noyes 2010). *Acerophagus papayae* Noyes and Schauff is efficient in control of *Paracoccus marginatus* Williams and Granara de Willink (Pseudococcidae) on papaya in Florida, USA (Amarasekare et al. 2010), and on papaya, cassava, eggplant, jatropha, and hibiscus plants in Malaysia (Mastoi et al. 2011). It was not possible to determine the 2 *Acerophagus* species found in this study, but 1 of them is near *A. alexte* Noyes, a species recorded only from Costa Rica, with no host association known (Noyes 2010). In Brazil, the only known species of the genus is *A. coccois* Smith, a parasitoid used for the control of *Phenacoccus herreni* Cox and Williams (Pseudococcidae) in cassava in Bahia and Pernambuco (Bento et al. 1999).

Aenasius Walker is mainly a New World genus, but also has been recorded from other biogeographic areas. There are more than 42 described species that are primary parasitoids of 24 species of mealybugs (Noyes & Hayat 1994; Garcí*a* et al. 2016; Noyes 2017). In the present study, it was verified the first record of an *Aenasius* species parasitizing *P. praelonga* (Fig. 1), which represents a new host family association. The most effective natural enemy of cotton mealybug, *Phenacoccus solenopsis* Tinsley (Pseudococcidae), is the endoparasitoid *Aenasius bambawalei* Hayat (Hayat 2009; Poorani et al. 2010; Prasad et al. 2011; Shahzad et al. 2016). In Brazil, there are 11 recorded species of *Aenasius*, which are associated with several Pseudococcidae species (Noyes 2017).

Arrhenophagus aurivillius is a cosmopolitan genus and includes only 4 species. *Arrhenophagus chionaspidis* is a cosmopolitan species, parasitizing about 50 species of scale insects of the families Coccidae, Diaspididae, and Eriococcidae (Noyes 2017). In Brazil, this species was found in Pernambuco on unspecified Coccoidea (Annecke & Prinsloo 1974; De Santis 1980).

Encyrtus Latreille is a well-known cosmopolitan genus, with 94 distinct species of which 45 are associated with scale insects, mainly coccids (García et al. 2016; Noyes 2017). Species of *Encyrtus* have been used in biological classical biological control, for example, *E. aurantii* from Israel was employed for biological control of *C. hesperidum* in Texas, USA (Prinsloo 1997). In Brazil, *E. aurantii* was recorded in the Bahia and Santa Catarina states (Noyes 2010).

Metaphycus Mercet, with about 473 described species, plays an important role in natural control of Coccidae and Diaspididae (Guerrieri & Noyes 2000; García et al. 2016; Kapranas & Tena 2015; Noyes 2017). Where their biology is known, all species of this genus are primary endoparasitoids (Guerrieri & Noyes 2000; Kapranas & Tena 2015). These insects are facultative gregarious parasitoids, where the number of offspring they produce per scale is dependent on the size of the scale host (Bernal et al. 1999; Tena & Garcia-Marí 2009; Kapranas et al. 2011b; Kapranas & Tena 2015). *Metaphycus angustifrons* was introduced in California specifically for the control of black scale, *S. oleae* (Compere 1957; Dean & Bailey 1960; Lampson & Morse 1992; Kapranas et al. 2007). However, up until recently they have been the most abundant parasitoids of brown soft scale, *C. hesperidum* (Kapranas et al. 2007; Kapranas et al. 2011a). In Brazil, there are records of 5 *Metaphycus* species: *M. alboclavatus* Compere on *Pseudococcus* sp. (Pseudococcidae) in São Paulo (Compere 1939), *M. brasiliensis* Compere on *Chaetococcus bambusae* (Maskell) (Pseudococcidae) in Rio de Janeiro (Compere & Annecke 1961), and on unknown hosts in São Paulo (De Santis 1980), *M. discolor* (De Santis) on *Ceroplastes* sp. (Coccidae) in Minas Gerais (De Santis 1970), *M. flavus* on *Parthenolecanium perlatum* (Cockerell) (Coccidae) in Rio de Janeiro State (Gomes 1942), and on *Cerococcus parahybensis* Hempel (Cerococcidae), *Coccus viridis* and *Chrysomphalus aonidum* in São Paulo State (De Santis 1980). The fifth species is *M. omega* Noyes, which is associated with *Aleurothrixus floccosus* Maskell (Hemiptera: Aleyrodidae) in Pernambuco State. Also, Bergmann et al. (1991) observed an undetermined *Metaphycus* species associated with *Aspidiotus* sp. (Diaspididae) in São Paulo.

Signiphora Ashmead is represented by 46 species described worldwide, distributed throughout the American continent, India, and Australia, associated with about 51 scale insect species, mainly Coccidae, Diaspididae, and Pseudococcidae (García et al. 2016; Noyes 2017). In Brazil, there are more than 14 *Signiphora* species, distributed in Alagoas, Pará, Amazonas, and in the southeast region (De Santis 1972, 1980).

Aprostocetus Westwood is a cosmopolitan genus, with approximately 800 described species; from these, 33 have been found associated with scale insects (Noyes 2017). This genus has an extremely varied biology (Graham 1987; LaSalle 1993; LaSalle et al. 2006; Hesami et al. 2010). Cave (2006) found *Aprostocetus purpureu*s (Cameron) parasitizing cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Diaspididae), in Southeast Asia, and it is a potential biological control agent in Florida. Japoshivili et al. (2015) recorded 4 *Aprostocetus* species from 5 *Kermes* species (Kermesidae). There are 16 species of *Aprostocetus* in Brazil (Noyes 2017); 2 of them are associated with scale insects, and these are *Aprostocetus chapadae* (Ashmead) and *Aprostocetus zemani* (Brèthes) (Brèthes 1920; Parker et al. 1953; De Santis 1979). From the original description, the *Aprostocetus* species obtained in this study differs from *A. chapadae* by having only 1 row of adnotaular setae, whereas *A. chapadae* has 2 rows of adnotaular setae (Ashmead 1904). It is different from *A. zemani* because specimens have dark brown coloration, with subtle metallic tinges, whereas in *A. zemani* the females are mainly yellow, with dark areas on the head, mesosoma, and gaster (Brèthes 1920).

Of the new interactions between parasitoids and scale insects, only *P. praelonga* had no records of being parasitized by Hymenoptera, except in the case of *Cales noacki* Howard (Aphelinidae) (Gonçalves 1962) and 1 unidentified (or unknown) parasitoid wasp (Garcia-Roa 1995). The only natural enemy recorded for citrus orthezia is *Rhinoleucophenga brasiliensis* Hendel (Drosophilidae); its larva develops on the surface of the ensign scale, and may in turn be parasitized by *Aprostocetus* sp. (Matile-Ferrero & Étienne 2006). This also may have occurred in the present study, with the *P. praelonga* female, but it was not possible to determine if the fly host was present. However, there are no reports of them being used to control the citrus orthezia.

The knowledge of the parasitoid species of scale insects, as well as their distribution, are important to increase the efficiency of these insects as biocontrol agents, and will be useful for management of this group of pests in Maranhão State, Brazil.

Acknowledgments

We extend our appreciation to FAPEMA - Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão

for financial support (PROCESS: BIC-02536/12). Also to Instituto Nacional de Ciência e Tecnologia dos Hymenoptera Parasitoides da Região Sudeste Brasileira for financial support (Process CNPq 573802/2008-4) for one of the authors (VAC). And finally, to Takumasa Kondo for reviewing the manuscript.

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