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New Records of Parasitoids Hymenopteran (Hymenoptera: Chalcidoidea) Associated With *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in the State of São Paulo

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Abstract

Survey of hymenoptera parasitoids of pink-hibiscus-mealybug, *Maconellicoccus hirsutus* (Green, 1908) infesting *Hibiscus rosa-sinensis* was conducted in two municipalities of state of São Paulo, Brazil from August 2016 to August 2017. Nine species of chalcidoid wasps were obtained: *Anagyrus kamali* Moursi (1948); *Anagyrus* sp. aff. *mirtesae* Noyes and Menezes (2000); *Anagyrus* sp.; *Bothriocraera bicolor* Compere and Zinna (1955); *Cheiloneurus* sp., *Gyranusoidea deionae* Noyes (2000); *Prochiloneurus* sp. (Encyrtidae); *Aprostocetus* sp. (Eulophidae); and *Chartocerus* sp. (Signiphoridae). *Anagyrus kamali* and *G. deionae* were the most frequent species, corresponding to 67.26% and 22.62% of the parasitoids, respectively. *Bothriocraera bicolor*, *Chartocerus* and *G. deionae* are recorded for the first time associated with *M. hirsutus*. *Gyranusoidea deionae* and *B. bicolor* are recorded for the first time in Brazil. The seasonal study of *M. hirsutus* and its parasitoids was carried out in January to December 2017on 16 hibiscus plants, aligned in the hedge in the experimental area of the FCAV/UNESP in Jaboticabal, SP. For this purpose, ovisacs, nymphs and adults of mealybug were counting on infested plants and the adult parasitoids collected in Möerick traps. *Maconellicoccus hirsutus* was observed during the periods of January-March and October-December, with the highest infestation in January and February. In the traps, a total of 92 specimens of pink-mealybug parasitoids were obtained: 65% belonging to *A. kamali* and 35% to *G. deionae*.

Keywords: *Hibiscus rosa-sinensis*, tritrophic interactions, Encyrtidae

1. Introduction

The pink-hibiscus-mealybug (PHM), *Maconellicoccus hirsutus* (Green, 1908) (Hemiptera: Pseudococcidae), is a polyphagous species reported on more them 300 species of host plants, distributed in 78 families, mainly in the tropical and subtropical regions of the world (García Morales et al., 2018).

In Brazil, this mealybug was first reported in 2010, in Boa Vista, Roraima. Currently, it is spread across seven other states, of which Santa Catarina is the southernmost. Of the 32 species of host plants from 17 families reported in the country, stand out: cocoa, *Theobroma cacao* L.; cupuaçu, *Theobroma grandiflorum* (Willd. Ex Spreng) K. Schum; and okra, *Abelmoschus esculentus* L. Moench; (Malvaceae); sugar-apple, *Annona squamosa* L., and soursop, *Annona muricata* L.; (Anonnaceae), and teak, *Tectona grandis* L.f. (Lamiacea) (Culik et al., 2013, Marsaro-Junior et al., 2013, Alexandre et al., 2014; Broglio et al., 2015, Morais et al., 2015, Peronti et al., 2016; Peres-Filho, 2017, Ramos et al., 2018).

In São Paulo State, *M. hirsutus* was first recorded in 2012, in the municipality of São Carlos on *H. rosa-sinensis* (Morais et al., 2015). In the next year collected in other 11 municipalities infesting *Erythrina* spp. (Fabaceae), *Ficus pumila* L. (Moraceae), and *Eugenia uniflora* L. (Myrtaceae), but mainly on hibiscus species in urban areas; and some teak, *Tectona grandis* L.f. (Lamiales: Lamiaceae), cultivated in the northwest region of State (Peronti & Martinelli, 2014).

The management of *M. hirsutus* might be performed in different ways, including chemical control indicated mainly for high infestations in restricted environments, such as nursery planting, where infested plants do not resist the presence of the pest (Chong et al., 2015). However, the waxy cover, eggs inserted in a filamentous secretion (ovisac), and habit of nymphs and adult females to settle in cryptic parts of the host plant, protects them from contact with insecticidal sprays (Kairo et al., 2000).

Biological control has been extensively used for this species. Worldwide, 85 natural enemies have been reported that are associated with *M. hirsutus*, 39 are hymenopterous parasitoids (Hymenoptera: Encyrtidae) and 46 predators, predominantly distributed in the families of Coccinellidae (Coleoptera) and Chrysopidae (Neuroptera) (Chong et al., 2015, Peronti et al., 2016).

However, the complex of natural enemies associated with an insect pest can be variable between different zoogeographic regions or even in different parts of the same country, demonstrating the importance of regional surveys. Goolsby et al. (2002) found the predators *Cryptolaemus montrouzieri* Mulsant, 1853 (Coleoptera: Coccinellidae), *Cacoxenus perspicax* (Knab, 1914) (Diptera: Drosophilidae) and the parasitoid *Gyranusoidea indica* Shafee, Alam & Agarwal, 1975 (Hymenoptera: Encyrtidae) in Queensland, northeastern Australia. While whereas in western Australia and the northern territory the researchers found the predator *Mataeomera* sp. (Lepidoptera: Erebidae); the parasitoids *Coccophagus* sp. (Hymenoptera: Aphelinidae) and *Coccidoctonus* sp. (Hymenoptera: Encyrtidae), all collected from *M. hirsutus* on a native *Hibiscus* species.

In Brazil, nine species of natural enemies are reported that are associated with PHM. The parasitoids *G. indica* and *Anagyrus kamali* Moursi, 1948 (Hymenoptera: Encyrtidae); and predators *Cycloneda sanguinea* (Linnaeus, 1763), *C. montrouzieri*, *Chilocorus nigrita* (Fabricius, 1798), *Exoplectra* sp., *Harmonia axyridis* (Pallas, 1773) and *Tenuisvalvae notata* (Mulsant, 1850) (Coleoptera: Coccinellidae) and *Ceraeochrysa* sp. (Neuroptera: Chrysopidae) (Marsaro-Júnior et al., 2013, Peronti et al., 2016).

A. kamali and *G. indica* are important in biological control programs of PHM, mainly in the South of North America and the Caribbean region (Kairo et al., 2000). In states of California, Florida, Hawaii (USA) and Mexico, biological control programs using these natural enemies were successfully achieved up to 95% reduction of *M. hirsutus* population (Roltsch et al., 2006).

In Roraima, northern Brazil Negrini et al. (2018) studied the population fluctuations of *M. hirsutus* and its natural enemies in a sugar-apple orchard, *Annona squamosa* L. (Anonnaceae) and verified highest infestation occurred in August and February-March and the average parasitism by *A. kamali* in fruits of the 50%, with highest rates in periods of greatest infestation by *M. hirsutus*.

Therefore, the objective of this work was to increase the knowledge about the hymenopterous parasitoids associated to *M. hirsutus* in the state of São Paulo and study of the seasonality of *M. hirsutus* and its parasitoids on *Hibiscus rosa-sinenses* in an experimental area in the municipality of Jaboticabal, SP.

2. Materials and Methods

2.1 Survey of Natural Enemies

The branches, leaves and flowers of *Hibiscus rosa-sinensis* infested by *M. hirsutus* were collected in public and private gardens in the municipalites of Campinas, Jaboticabal and São Carlos, SP, Brazil and transported to the Laboratory of Biosystematics of Hemipteran (LABHEM) of the Plant Protection Department of the Universidade Estadual Paulista "Julio de Mesquita Filho"-Campus Jaboticabal, from August 2016 to August 2017. Sporadic samples were also obtained in Jales, Ribeirão Preto, São Paulo, Vinhedo and Votuporanga, SP.

The collected part of the specimens of *M. hirsutus* was stored in vials containing 70% alcohol for posterior montage and identification. Other parts were placed in glass tubes sealed with paper film under controlled conditions (25±2 °C, photoperiod of 12 hours and UR 65±5%) in BOD incubator for 25 days, according to methodology adapted from Prado et al. (2015). At each 48 hour intervals, the emerged parasitoids were transferred to 2 ml eppendorf microtubes filled in 70% alcohol.

The mealybugs were slided-mounted following the methodology described by Granara de Willink (1990) and identified according morphological characteristics of the adult female as described by (Miller, 1999; Miller et al., 2011).

Most of hymenopterous parasitoids were prepared in double-mount (Hanson & Gauld, 2006). Species smaller than 0.7 mm were mounted on permanent slides following the technique of Querino and Zucchi (2011). Subsequently, the parasitoids were identified under optical stereoscopic microscope at the genus or species level, according to the following works: Fernández and Sharkey (2006) and Hanson and Gauld (2006) for

identification of families of Chalcidoidea; for genera of Encyrtidae, Noyes (1980); for Eulophidae, Schauff et al., (1997); and for Signiphoridae Rao (1974). The determination of the species was used: Noyes (2000) and Noyes and Hayat (1994).

Specimens "voucher" were deposited in the following institutions: Reference Collection of Insects and Mites (CRIA) of the Department of Plant Protection of FCAV/UNESP-Jaboticabal/SP; and Collection "Oscar Monte" of Entomophagous Insects (IB-CBE) of the Biological Institute, Campinas, SP, for future consultations.

2.2 Seasonality of M. hirsutus and Its Parasitoids

The samples were carried out on a hedge of *Hibiscus rosa-sinensis* L. (Malvaceae), with 16 plants, in an open environment in the experimental area of the Plant Protection Department of FCAV/UNESP, between January and December 2017. Monthly, nymphs and adults of mealybug were collected from an apical branch of each plant.

In the same period, parasitoid hymenoptera were obtained from Möericke traps arranged close to the hibiscus plants and kept for two days. The mealybugs and parasitoids previously filled in 70% alcohol were triaged and counted under optical stereomicroscope; and subsequently mounted on entomological pin or permanent slides, for identification.

3. Results

3.1 Survey of Natural Enemies

A total of 168 specimens of parasitoid hymenoptera (Hymenoptera: Chalcidoidea) were obtained from *M. hirsutus*, distributed in nine species: 7 encyrtidae, 1 Eulophidae and 1 Signiphoridae (Table 1). *Bothriocraera bicolor*, *Chartocerus* and *G. deionae* are registered for the first time associated with this pseudococcid. *Gyranusoidea deionae* are recorded for the first time in Brazil; and *Bothriocraera bicolor* be the first record for the state of São Paulo (Figure 1).

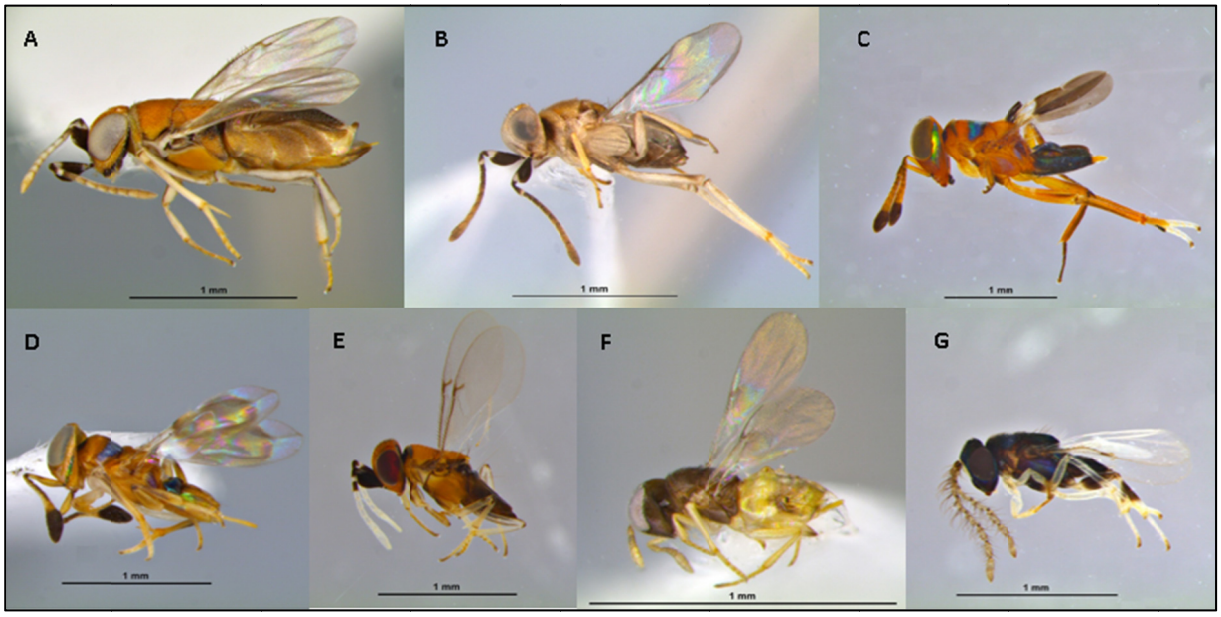

Figure 1. Parasitoids associated with *Maconellicoccus hirsutus* in *Hibiscus-rosa-sinensis* in the state of São Paulo: (A) *Anagyrus kamali*; (B) *Gyranusoidea deionae*; (C) *Cheiloneurus* sp.; (D) *Prochiloneurus* sp.; (E) *Anagyrus* sp. aff. *mirtesae*; (F) *Bothriocraera* sp.; (G) *Anagyrus* sp. Fotos: (A, E, F) Costa V.A.; (B, C. D.G) Siqueira, M.A.

Encyrtidae presented the highest number of specimens obtained from *M. hirsutus*, corresponding to 98.80% of the total. *A. kamali* and *G. deionae* were the most frequent species, with 67.26% and 22.62%, respectively. Sporadic species with a small number of emerged specimens not exceeding 2% of the total (Table 1).

Table 1. Parasitoids associated with *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) collected on *Hibiscus* sp. in the state of São Paulo between August 2016 and August 2017. Number and percentage obtained by species

Parasitoids	Number of individuals and percentage (%)
Encyrtidae	
Anagyrus kamali Moursi, 1948	113 (67.26%)
Anagyrus sp. aff. *mirtesae* Noyes & Menezes, 2000	3 (1.79%)
Anagyrus sp.	4 (2.38%)
Bothriocraera bicolor Compere & Zinna, 1955	5 (2.98%)
Cheiloneurus sp.	2 (1.19%)
***Gyranusoidea deionae* Noyes, 2000	38 (22.62%)
**Prochiloneurus* sp.	1 (0.60%)
Eulophidae	
Aprostocetus sp.	1 (0.60%)
Signiphoridae	
***Chartocerus* sp.	1 (0.60%)

Note. * Species associated for the first time with *Maconellicoccus hirsutus*; ** Species recorded for the first time in Brazil.

3.2 Seasonality of M. hirsutus and Its Parasitoids

The pink-hibiscus-mealybug was observed in the periods of January-March and October-December, with the highest infestation in the months of January and February, reaching the average of 12 adults and 63 nymphs by evaluated branch (Figure 2). From April to September PHM was not observed, probably due to the lower temperatures. The number of nymphs was greater than the number of ovisacs and adults obtained during all experiments.

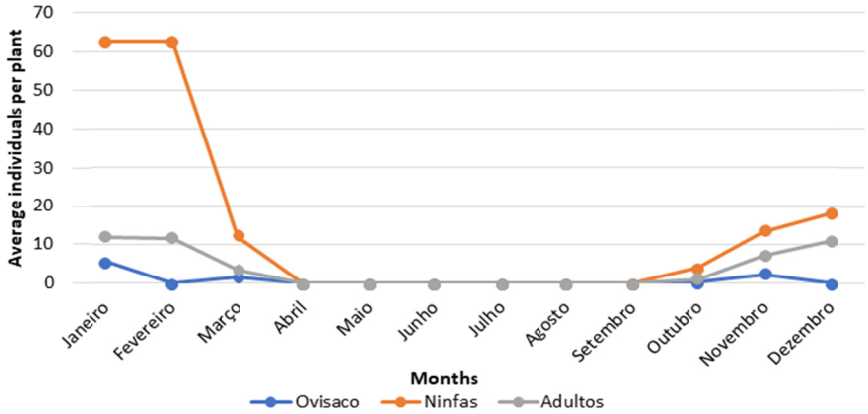

Figure 2. Seasonality of *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) collected in *Hibiscus* sp. in the state of São Paulo between January 2017 and December 2017. Average individuals per plant

In the traps, a total of 92 specimens of hymenopterous known to parasite PHM were obtained within the 12 months of sampling: 65% of *A. kamali* and 35% of *Gyranusoidea deionae* (Hymenoptera: Encyrtidae). Both species had higher incidence in the month of February, coinciding with the final period of high infestation of the mealybug; and, in November, period in which the pest population was re-established. Only 14 specimens of parasitoids were obtained between March and September, with a reduction in the populations of *M. hirsutus*. (Figure 3).

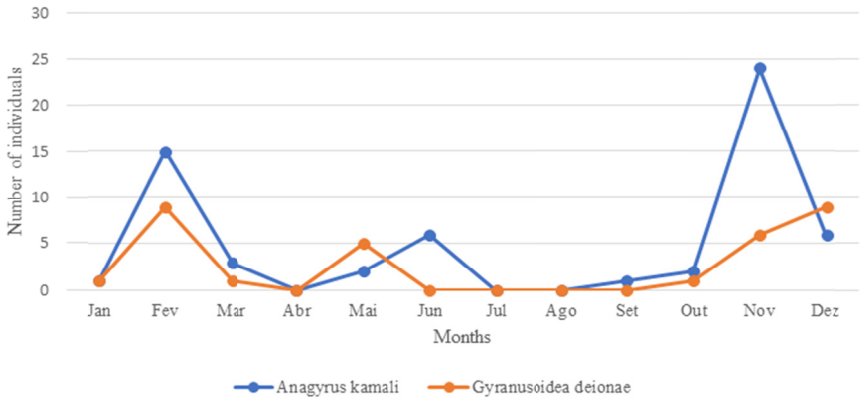

Figure 3. Number of *Anagyrus kamali* and *Gyranusoidea deionae* collected with Morieck traps in *Hibiscus* sp. in the state of São Paulo between January 2017 and December 2017

4. Discussion

4.1 Encyrtidae

Encyrtidae has the most parasitoids used for biological control of species of Hemiptera. Coccidae and Pseudococcidae (Hemiptera: Coccomorpha), corresponding to more than 60% of the already registered hosts (Noyes & Hayat, 1994; Noyes et al., 1997). Noyes and Hayat (1994) summarized information about Encyrtidae imports to biological control programs in all the world since 1992, accounting 94 species in 382 introductions; with a success rate about 90% (Noyes et al., 1997). *Anagyrus aegyptiacus* Moursi, 1948, *Anagyrus dactylopii* (Howard, 1898), *A. kamali* and *Anagyrus pseudococci* (Girault, 1915) have applied to control of pseudococcids (Mani & Shivaraju, 2016).

Anagyrus spp. is frequently reported in the parasitoid complex associated with species of several genus of Pseudococcidae, (Noyes, 1994). *Anagyrus* Howard has about 280 species around the world, of which nine exist in Brazil (Noyes, 2018). In Brazil, the encyrtids *Anagyrus diversicornis* (Howard, 1894) and *Aenasius vexans* Kerrich, 1967 were imported from Colombia and Venezuela, respectively, for the control of mealybug, *Phenacoccus herreni* Cox and Williams (1981), pest of cassava in the northeastern region of Brazil (Bento, 2002).

Anagyrus kamali is solitary endoparasitoid (Pollard, 1995). From Asian (Williams, 1986) this species currently occurs in 23 countries around the world (Noyes, 2018). It was widely used for the biological control of *M. hirsutus* in USA (California and Florida), Egypt, Mexico, India, Hawaii and Caribbean Islands (Kairo, 2000; Roltsch et al., 2006). The first imported specimens were obtained from China, California and Florida (Chong, 2009). Several other pseudococcids of economic importance such as, striped-mealybug *Ferrisia virgata* (Cockerell, 1893), cotton-mealybug *Phenacoccus solenopsis* Tinsley 1898, and root-mealybug *Planococcus citri* (Risso, 1813) are frequently associated with this encyrtid (Sagarra et al., 2001a; Gregory et al., 2012).

Anagyrus mirtesae, is known to Costa Rica (Noyes, 2000) and Mexico (Sánchez-García, 2016), with uncertain origin (Noyes, 2000). The information related to this parasitoid is scarce, having only the description of females (Noyes, 2000). It resembles *Anagyrus cepio* Noyes, 2000 and *Anagyrus remotor* Noyes, 2000. The three species are differentiated by the coloration of the flagellum. In *A. mirtesae*, only the first flagellum has coloration dark brown; *A. remotor* the brown coloration is present in the first and third flagellum; and *A. cepio* shows all flagellum segments dark brown (Noyes, 2000).

Bothriocraera bicolor, with uncertain origin, is known only to the Neartic and African regions (Noyes, 2018). This species has already been used in several regions of the city of Trinidad in California, USA. It was also introduced in Ghana and occidental Africa for the biological control of mealybugs *Ferrisia virgata*, *Planococcus citri* and *Pseudococcus longispinus* on citrus and cacao crops (Trjapitzin et al., 2004).

Cheiloneurus Westwood, 1833 has 150 species distributed around the world. In Brazil, the following are known: *Cheiloneurus inimicus* Compere, 1925, *Cheiloneurus kuisebi* Prinsloo, 1985, *Cheiloneurus nigrescens* Howard, 1897 (Noyes, 2018; De Santis & Fidalgo, 1994; De Santis, 1972). Species of this genus, in general are reported with secondary parasitoids of coccids (Noyes, 2018; Noyes & Hayat, 1984).

Gyranusoidea deione is a Neotropical species of uncertain origin, registered only in Costa Rica (Noyes, 2000). It is very similar to *G. indica*, differing mainly in having an intense dark spot that connects the torulus and eye, the mid coxa is concolorous with the hind coxa, in addition to the length of the gonostylus 0.25x as long as the ovipositor. In *G. indica* the spot between the torulus and the eyes is conspicuous and dark brown; the hind coxae is pale yellow contrasting with brown mid coxa; and the length of the gonostylus 0.25x as less as the ovipositor (Noyes, 2000).

Prochiloneurus Silvestri 1915, is a genus composed of 29 species in the world (Noyes, 2018). In Brazil, for example, only *Prochiloneurus dactylopii* (Howard, 1885) was registered (Löhr et al., 1990; Noyes, 2018). They are known mainly as secondary parasitoids of Coccidae, Pseudococcidae and Coccinellidae (Noyes & Hayat, 1984; Hayat, 2006).

4.2 Eulophidae

Eulophidae are parasitoids of a great diversity of arthropods, being found parasitizing from arachnids, nematodes and distinct species of insects during various stages of development (La Salle, 1994). There are few associations between eulophids with mealybugs. Most species have been associated as primary or secondary parasitoids of coccids and other 41 families of arthropods (La Salle, 1994; Peck, 1963).

Aprostocetus Westwood, 1833 is a very complex genus, with more than 800 species registered around the world (La Salle, 1994). *Aprostocetus minutus* (Howard, 1881) is the species with the highest number of hosts belonging to the family Pseudococcidae, being associated with six genera in the world (La Salle, 2006; Noyes, 2018). The species reported in Brazil are not associated with this family of scale insects, representing 15 species in the country, but the genus *Aprostocetus* has already been cited in the literature associated with Pseudococcidae in Brazil (Lopes et al., 2017; Chong et al., 2015).

4.3 Signiphoridae

Signiphoridae is one of the smallest families of Chalcidoidea, with four genera and 84 species in the world (Hayat, 2004). In Brazil, 16 species were registered, 15 of the genus Signiphora (Noyes, 2018), of wich four were associated with species of mealybugs, *Chartocerus niger* (Ashmead, 1900), *Signiphora bifasciata* Ashmead, 1900, *Signiphora fax* Girauld, 1913 and *Signiphora hyalinipennis* Girauld, 1913 (Löhr et al., 1990).

Chartocerus Motschulsky, 1859, is mostly hyperparasitoids of species of Hymenoptera, presenting preference for other Chalcidoidea (Woolley 1988). This genus is cosmopolitan, with 33 species described around the world (Noyes 2018). In Brazil only, *Chartocerus niger* was associated with *Planococcus citri* (Risso, 1813) (Ashmead, 1900) (De Santis, 1979).

4.4 General Discussion

Nine species of hymenopterous parasitoids were recorded to be associated with M. hirsutus in the state of São Paulo distributed into seven genera. According to the literature, species of *Anagyrus*, *Gyranusoidea*, *Bothriocraera* have been mentioned preferentially as primary parasitoids of mealybugs and those of the genera *Chartocerus* sp., *Cheiloneurus* sp. and *Prochiloneurus* sp. as secondary parasitoids. Species of the genus Aprostocetus have been related as primary and secondary parasitoids (Table 2).

Table 2. Species by family, associations and literature pertinent to their hosts. * numbers in parentheses are corresponding to the number of host families of the parasitoid

Family	Species	Category of parasitism and hosts	Reference
Encyrtidae	*Anagyrus kamali*	Primary parasite of Pseudococcidae nymphs	Noyes, 1994; Moursi, 1948; Miller, 1999.
	Anagyrus sp. aff. *mirtesae*	Unknown	Noyes, 2000; Sánchez-García, 2016.
	Anagyrus sp.	Primary parasite of Pseudococcidae nymphs	Kerrich, 1982, Noyes, 1994.
	Bothriocraera bicolor	Primary parasite of Pseudococcidae nymphs	Bartlett, 1958; Compere, 1955;
	Cheiloneurus sp.	Hyperparasitoids of Chalcidoidea (Encyrtidae and Aphelinidae).	Herting, 1972; Japoshvili & Celik, 2010.
	Gyranusoidea deionae	Unknown	Noyes, 2000.
	Prochiloneurus	Hyperparasitoids of Hemiptera (Coccidae, Pseudococcidae and Coccinelidae) and primary parasitoid of Chalcidoidea (Encyrtidae).	Noyes, 1984; Prinsloo, 1983; Triapitsyn, 2014.
Eulophidae	*Aprostocetus*	Primary parasite of 43 families of the phylum Arthropoda - Díptera (6), Coleoptera (4), Hemiptera (9), Lepidoptera (16), Orthoptera (1) and Orthoptera (2); and Hyperparasitoid of Diptera (2) and Hymenoptera (5) *.	La Salle, 1994; Noyes, 2018.
Signiphoridae	*Chartocerus*	Hyperparasitoid of Diptera (Drosophilidae), Hemiptera (Diaspididae, Pseudococcidae and Psyllidae) and Lepidoptera (Gracillariidae), and primary parasitoid of Chalcidoidea (Encyrtidae and Eulophidae).	Simmonds, 1957; Mani, Thontadarya & Singh, 1987;

Anagyrus was the genus with the largest number of specimens, *A. kamali*, *A.* sp. aff. *mirtesae* and *Anagyrus* sp.; Species of this genus are known for their high specificity to mealybugs. Of the nine registered hosts for *A. kamali*, eight are from pseudococcids (Noyes, 2018). This exotic hymenopteran was probably introduced in Brazil together with the pink-hibiscus-mealybug, both recorded for this country in the same year, in Boa Vista, Roraima (Marsaro-Junior et al., 2013). For *A. mirtesae*, previously known to Costa Rica and Mexico, no host has been known until now (Noyes, 2000; Sánchez-García, 2016).

Several studies related to the biology of *A. kamali* were carried out and verified the great potential of the species for the biological control of *M. hirsutus* (Sagarra et al., 2000a; Sagarra et al., 2000b; Sagarra et al., 2001a; Sagarra et al., 2001b; Persad & Khan, 2002; Serrano & Lapointe, 2002; Persad & Khan, 2007; Montes-Rodríguez, 2012). *Anagyrus kamali*, preferably feeds from adult female of PHM, but it can also parasitize all the nymphal instars (Sagarra & Peterkin, 1999).

For *Gyranusoidea deionae*, the second species with the largest number of specimens was reported as a first host of it in this study. This species is extremely similar to *G. indica* which as obtained from *M. hirsutus* by Peronti et al. (2016) in the state of São Paulo. However, it was not found during this survey. Due to the great similarity of them morphometric and molecular studies would be required to verify the status and distribution of both.

For the Neotropical region, 19 species of parasitoids had previously been associated with *M. hirsutus* (Chong et al., 2015; Culik et al., 2013). Adding the four species here reported for the first time to this region, 23 species are now known. In general, *Anagyrus* and *Gyranusoidea* include species better distributed in the Australian and Oriental regions, except for a few species such as *Anagyrus* sp. aff. *mirtesae* and *G. deionae*, which are only registered in Costa Rica, Mexico and Brazil.

The number of species of hymenopterous parasitoids associated with mealybugs has been increased in recent years, however the records, have been done to isolated species. Studies that include surveys of species in a given region are less frequent (Chong et al., 2015; Culik, et al., 2013; Mani et al., 1987). Surveys of hymenopterous parasitoids associated with *M. hirsutus* were conducted in India by Mani et al. (1987) and Michaud & Evans, (2018) in Puerto Rico. In India: *A. dactylopii*, *Anagyrus mirzai* Agarwal and Alam, 1959 (= *Gyranusoidea mirzai*) and *Alamella flava* Agarwal, 1966 (Encyrtidae), *Allotropa* sp. Near *A. japonica* Ashmead, 1904 (Platygastridae), *Leptopilina* sp. (Figitidae) and *Chartocerus* sp. Near *C. walkeri* Hayat, 1970, (Signiphoridae) were reported and in Puerto Rico, *A. kamali*, *G. indica*, *Acerophagus nubilipennis* Dozier, 1926, *C. inimicus* and *A. minutus*. Of the six species obtained in India, two are included in common genera to those found in this study, *Anagyrus* spp. and

Chartocerus sp. Four of the five species associated with PHM in Puerto Rico belong to genres common to those species found in this study with *A. kamali* representing 70% of the number of parasitoids obtained.

In the municipality of Jaboticabal-SP, the population peaks of *M. hirsutus* in the years 2016-2017 occurred in January and February, where temperatures were higher. These results corroborate with those obtained by Negrini et al. (2018), demonstrating that in the months of May-July there is no presence of mealybug in the field, but in the months that followed, in the periods of spring and summer, February and end of September, respectively, the greater amount of mealybugs occur and their re-establishment in the field, also resulted in the appearance of the natural enemies.

Negrini et al. (2018) evaluated the number of *M. hirsutus* mummies, corresponding to parasitized mealybugs, in *A. squamosa* fruits. The highest pupal average was obtained in September, February and March, being the period corresponding to the highest population peaks of PHM, all of *A. kamali*. In the present study, hymenopteran parasitoid species were collected from Möericke traps and two species known to parasite PHM were captured (*A. kamali* and *G. deionae*). The seasonality of *A. kamali* in relation to population peaks of *M. hirsutus* was similar in both studies.

This survey contributed to report new species of natural enemies associated with *M. hirsutus* in the world and in Brazil particularly in addition, the study demonstrated that this exotic pest is being naturally controlled in the state of São Paulo by a complex of hymenoptera parasitoids especially *A. kamali* corresponding to more than 65% of the parasitoids obtained by manual collection of parasitized mealybugs and traps.

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Appendix

Anagyrus kamali

Material studied. (**602-A**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 3 ♀♀, 07.ix.2016, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., A.L.B.G. Peronti col., M.A. Siqueira det. (UNESP/FCAV). (**681**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′24.33″ S, 48°17′20.64″ W), 1 ♀ and 3 ♂♂, 11.i.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**744**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′24.33″ S, 48°17′20.64″ W), 9 ♀♀ and 8 ♂♂, 14.ii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**747**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′32.19″ S, 48°17′37.03″ W), 1 ♂, 16.ii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**770-A**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 3 ♀♀ and 19 ♂♂, 13.iii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., J.G. Alexandrino col., M.A. Siqueira det. (UNESP/FCAV). (**781-A**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′39.57″ S, 48°18′24.09″ W), 9 ♀♀ and 13 ♂♂, 22.iii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**788**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′39.06″ S, 48°17′17.06″ W), 10 ♀♀ and 11 ♂♂, 22.iii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., A.L.B.G. Peronti col., M.A. Siqueira det. (UNESP/FCAV). (**811-B**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′33.11″ S, 48°18′53.80″ W), 1 ♀ and 1 ♂, 26.iv.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., A.L.B.G. Peronti col., M.A. Siqueira det. (UNESP/FCAV). (**874-B**) BRASIL. **SÃO PAULO**: Jales (20°15′27.53″ S 50°32′47.72″ W), 1 ♀ and 1 ♂, 30.vi.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira, M.A. Siqueira det. (UNESP/FCAV).

Anagyrus sp.* aff. *mirtesae

Material studied. (**874-A**) BRASIL. SÃO PAULO: Jales (20°15′27.53″ S 50°32′47.72″ W), 2 ♀♀ and 1♂, 30.vi.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV).

***Anagyrus* sp.**

Material studied. (708-B) BRASIL. **SÃO PAULO**: Campinas (22°52′21.73″ S, 47°1′34.86″W), 1 ♂, 05.ii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). **Material estudado.** (**770-B**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 2 ♂, 13.iii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., J.G. Alexandrino col., M.A. Siqueira det. (UNESP/FCAV). (**781-D**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′39.57″ S, 48°18′24.09″ W), 2 ♂, 22.iii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV).

Bothriocraera bicolor

Material studied. (602-B) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 5 ♀♀, 07.ix.2016, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., A.L.B.G. Peronti col., M.A. Siqueira det. (UNESP/FCAV).

***Cheiloneurus* sp.**

Material studied. (**681-C**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′24.33″ S, 48°17′20.64″ W), 1♀, 11.i.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**770-E**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 1 ♀, 13.iii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., J.G. Alexandrino col., M.A. Siqueira det. (UNESP/FCAV).

Gyranusoidea deione

Material studied. (**602-E**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 2 ♀♀, 07.ix.2016, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., A.L.B.G. Peronti col., M.A. Siqueira det. (UNESP/FCAV). (**661**) BRASIL. **SÃO PAULO**: Campinas (22°52′4.61″ S, 47° 1′23.39″ W), 2 ♀♀, 23.x.2016, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**681-D**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′24.33″ S, 48°17′20.64″ W), 2 ♀♀ and 3 ♂♂, 11.i.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**708**) BRASIL. **SÃO PAULO**: Campinas (22°52′21.73″ S, 47°1′34.86″ W), 9 ♀♀ and 3 ♂♂, 05.ii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**738**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′24.33″ S, 48°17′20.64″ W), 3 ♀♀ and 1 ♂, 14.ii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**781-B**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′39.57″ S, 48°18′24.09″ W), 7 ♀♀ and 2 ♂♂, 22.iii.2017,

Maconellicoccus hirsutus on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV). (**811-A**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′33.11″ S, 48°18′53.80″ W), 1 ♀ and 2 ♂♂, 26.iv.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV).

***Prochiloneurus* sp.**

Material studied. (**781-C**) BRASIL. **SÃO PAULO**: Jaboticabal (21°14′39.57″ S, 48°18′24.09″ W), 1♀, 22.iii.2017, *Maconellicoccus hirsutus* on *Hibiscus* sp., M.A. Siqueira col., M.A. Siqueira det. (UNESP/FCAV).

***Aprostocetus* sp.**

Material studied. (**770-C**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 1 ♀, 13.iii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., J.G. Alexandrino col., M.A. Siqueira det. (UNESP/FCAV).

***Chartocerus* sp.**

Material studied. (**770-D**) BRASIL. **SÃO PAULO**: Jaboticabal (21°15′29.17″ S, 48°18′53.25″ W), 1 ♀, 13.iii.2017, Ex: *Maconellicoccus hirsutus* on *Hibiscus* sp., J.G. Alexandrino col., M.A. Siqueira det. (UNESP/FCAV).