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Taxonomic study and population variation of scale insects (Hemiptera: Coccidae and Diaspididae) and associated parasitoids (Hymenoptera: Chalcidoidea) in an olive grove at Rio Grande do Sul, Brazil

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Taxonomic study and population variation of scale insects (Hemiptera: Coccidae and Diaspididae) and associated parasitoids (Hymenoptera: Chalcidoidea) in an olive grove at Rio Grande do Sul, Brazil

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Taxonomic study and population variation of scale insects (Hemiptera: Coccidae and Diaspididae) and associated parasitoids (Hymenoptera: Chalcidoidea) in an olive grove at Rio Grande do Sul, Brazil

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Abstract. This study evaluated the population variation of *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) and associated parasitoids in *Olea europaea* L. (Oleaceae) groves located in the municipality of Barra do Ribeiro (30°30′54.95″S; 51°30′20.84″W), Rio Grande do Sul, Brazil. Eight samples were collected during the four seasons from November 2015 until October 2017; four branches with approximately 20 leaves were taken from 12 olive trees (four each from Arbequina, Arbosana and Koroneiki varieties). The collected material was placed in plastic bags and taken to the laboratory for further study. Scale insects were slide-mounted and were identified according to morphological characteristics of the adult female. Parts of two branches and 40 leaves of each variety infested with scale insects were sectioned and placed inside glass tubes, labeled, capped with cotton, kept in the laboratory at room temperature and examined daily to verify the emergence of parasitoids. Parasitoid specimens studied were critical point dried from ethanol and point-mounted. Some specimens were slide mounted in Canada balsam for more detailed study. In all samples, the presence of *H. lataniae*, as well as six associated parasitoid species, were identified. *Metaphycus flavus* (Howard) is recorded for the first time as a parasitoid of *H. lataniae*. Two other scale insects were also identified: *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Diaspididae) and *Saissetia coffeae* (Walker) (Coccidae), but the population data of these did not permit statistical analysis. A key to identify the scale insect species recorded on olive trees in Brazil, based on adult females, is provided.

Key words. *Hemiberlesia lataniae*, *Olea europaea*, hymenopteran parasitoids.

Introduction

Olive tree cultivation in Brazil is becoming increasingly important due to the growing internal consumption of olive oil and table olives, and Rio Grande do Sul is emerging as one of the states that has invested the most in this crop and in the production of olive oil. Despite this, scientific research is still in its beginning stages and could provide important support for commercial development of this crop in the country.

One of the problems that can affect the production of olive trees is the incidence of phytophagous insects, including scale insects (Hemiptera: Coccomorpha). Approximately 80 scale insect species in six families are known to be associated with *Olea europaea* L. (Oleaceae), more than 70% of which belong to the family Diaspididae (García Morales et al. 2016).

In Brazil, Prado and Silva (2006) reported the occurrence of 13 species of scale insects associated with olive trees: *Russellaspis pustulans* (Cockerell) (Asterolecaniidae); *Saissetia coffeae* (Walker) and *Saissetia oleae* (Olivier) (Coccidae); *Acutaspis paulista* (Hempel), *Acutaspis scutiformis* (Cockerell), *Aspidiotus nerii* Bouché, *Chrysomphalus aonidum* (Linnaeus), *Hemiberlesia rapax* (Comstock), *Parlatoria oleae* (Colvée), *Parlatoria proteus* (Curtis), *Pinnaspis aspidistrae* (Signoret), *Pseudaonidia trilobitiformis* (Green) and *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Diaspididae).

The most abundant species in the olive groves of Minas Gerais is *S. oleae*, which was designated as a primary pest (Prado et al. 2012; Santa-Cecília et al. 2014). In Rio Grande do Sul, the soft scales *S. oleae* and *S. coffeae* (Hemiptera: Coccidae) are considered the main phytophagous insects present in olive groves with the potential to reach pest status (Ricalde et al. 2015). In an olive grove with a large infestation of *S. oleae*, in Caçapava do Sul (30°30′43″S, 53°29′27″W), Rio Grande do Sul, the authors of a scientific study concluded that the scale insects were more abundant in spring and summer, and that the temperature was probably the meteorological factor that had the greatest effect on population size; they also found an average parasitism rate of 3.4% (Souza et al. 2015).

In recent studies in areas of commercial cultivation of olive trees in the center and south of Rio Grande do Sul, new locality records were discovered for the following species: *Aonidiella aurantii* (Maskell), *Hemiberlesia cyanophylli* (Signoret), *Hemiberlesia lataniae* (Signoret), *Melanaspis obscura* (Comstock), *Pinnaspis strachani* (Cooley) (Wolff 2014) and *Comstockaspis perniciosa* **(**Comstock) (Ricalde et al. 2015).

Parasitoids, mainly Chalcidoidea (Hymenoptera), are important, often specific biological control agents of scale insects, thus the identification of scale insects and their associated parasitoids is necessary (Rosen 1990). Several species of Chalcidoidea families such as Aphelinidae and Encyrtidae are important for the biological control of phytophagous insects in agricultural and natural ecosystems have been used against pests in several crops.

This research was conducted to verify the seasonal variation of scale insect populations and associated parasitoids. Such data can contribute to the decision-making process in pest management with the hope of developing more sustainable methods to protect the environment and provide for healthier food, free of pesticides. Additionally, a dichotomous key for the identification of the scale insects occurring on *O. europaea* in Brazil is provided.

Materials and Methods

The study was carried out in an olive grove located in the municipality of Barra do Ribeiro (30°30′54.95″S, 51°30′20.84″W), Rio Grande do Sul, Brazil, that was planted in 2011.

Eight samples of scale insects and their parasitoids were collected from November 2015 until October 2017, in all four seasons of the year. On each occasion, four branches with about 20 leaves were randomly collected from 12 trees, four from each of three olive varieties (Arbequina, Arbosana and Koroneiki). The samples were stored in plastic bags identified with data labels (locality, date of collection, collector, host plant, and variety) and immediately transported to the Entomology Laboratory of the Diagnostic and Agricultural Research Department (DDPA/SEAPI) and stored in the refrigerator until sorting.

Parts of two branches and 40 leaves of each of the 12 infested trees were sectioned with the aid of a stereomicroscope and placed in glass tubes with identified and isolated scale insects. The glass tubes were labeled, covered with cotton, kept in the laboratory at room temperature and examined daily to verify the possible emergence of parasitoids.

The collected parasitoids were preserved in 70% ethyl alcohol, then dried at critical point and assembled, and labeled for taxonomic study. Selected specimens were dissected and mounted in Canada balsam following Noyes (1990) and examined under a stereomicroscope. They were later identified using species keys and compared with species descriptions from the literature (De Santis 1948, 1964; Rosen and DeBach 1979; Noyes et al. 1997; Woolley 1997a, b; Gerrieri and Noyes 2000; Myartseva and Ruíz-Cancino 2004; Noyes 2004; Myartseva et al. 2010; Ramírez-Ahuja et al. 2015; Woolley and Dal Molin 2017).

The percentages of total parasitism in each variety, in the adult phase of the female scale insects, were calculated, counting the emergence of adult parasitoids, as well as the presence of perforations in the scale insects' shields.

In the other two branches with leaves of each olive tree variety, the scales were detached from the host. Information on the stage of development was recorded, along with the following additional information: whether the scales were alive or dead, whether they laid eggs, whether they were parasitized, whether they had a perforation in the shield caused by the emergence of parasitoids, and whether they were dried.

Some scale insect adult female specimens were mounted on slides for identification, according to a technique adapted by Wolff et al. (2014). The slides were examined under an optical microscope, and identified using dichotomous keys and morphological descriptions of the species (Ferris 1937, 1938, 1941, 1942; Granara de Willink 1999; Claps and Wolff 2003). The slides were catalogued and included in the entomological collection of the Ramiro Gomes Costa Museum (MRGC), Porto Alegre, Brazil.

Statistical analyses (ANOVA) were performed to compare the occurrences of scale insects and parasitoids across the three olive varieties.

Results

In the leaves and branches of *O. europaea*, in the varieties Arbequina, Arbosana and Koroneiki, the scale insects *S. coffeae*, *P. pentagona* and *H. lataniae* were identified.

Saissetia coffeae adult females were found in small numbers in the samples taken in autumn 2016 (one in Arbequina, one in Arbosana), autumn 2017 (one in Koroneiki, five in Arbosana and five in Arbequina), and winter 2017 (four in Arbosana). The female of *S. coffeae* is parthenogenetic; the body is quite convex in the adult stage and reddish-brown in color, standing out easily among the leaves of the olive tree.

Only one shield of a *P. pentagona* male was found in the autumn 2016 collections, and one adult female in Koroneiki and 29 in Arbosana were found in the winter 2017 collections. These armored scale insects preferentially settle in branches and trunks, more rarely in the leaves, with the white colored shields of the males standing out. Males are generally found very close to each other with the adult females often found underneath the shields of the males, the coloration of which does not stand out from the color of the olive tree.

Data from *S. coffeae* and *P. pentagona* populations were scarce, and there was insufficient sample size for a statistical analysis.

Females of *H. lataniae* were found during all sampling periods, preferentially located on the branches, sometimes in the leaf petiole, with a similar color to the host plant. The highest numbers of live females with and without eggs were obtained in spring 2015 and winter 2016, of ambulatory nymphs in spring 2015 and autumn 2016, of perforated shells in spring 2015 and autumn 2017, and parasitized scales in autumn 2017 (Fig. 1).

The highest population peaks of *H. lataniae* occurred in autumn (2016 and 2017) in the Arbequina variety; there were three population peaks (autumn and spring 2016, autumn 2017) in the Arbosana variety, and two population peaks in the Koroneiki variety (autumn 2016 and autumn 2017) (Fig. 2). The lowest population variation of *H. lataniae* occurred in Koroneiki (Fig. 2). No statistically significant differences were found for *H. lataniae* ($F=0.0339$; $P=0.9672$) among the three olive varieties studied.

Six species of parasitoids associated with *H. lataniae* were identified: *Encarsia citrina* (Crawford), *Aphytis diaspidis* (Howard) (Aphelinidae), *Signiphora flavella* Girault and *Signiphora merceti* Malenotti (Signiphoridae), *Metaphycus flavus* (Howard) (Encyrtidae) and an unidentified species of *Coccophagoides* Girault (Hymenoptera: Chalcidoidea).

Discussion

Hemibelesia lataniae is a cosmopolitan species, distributed in all zoological regions, with recorded host plants in more than 100 families. It is considered a pest of some agricultural crops, including *O. europaea* trees in the Mediterranean region and in Brazil (Wolff 2014; García Morales et al. 2016).

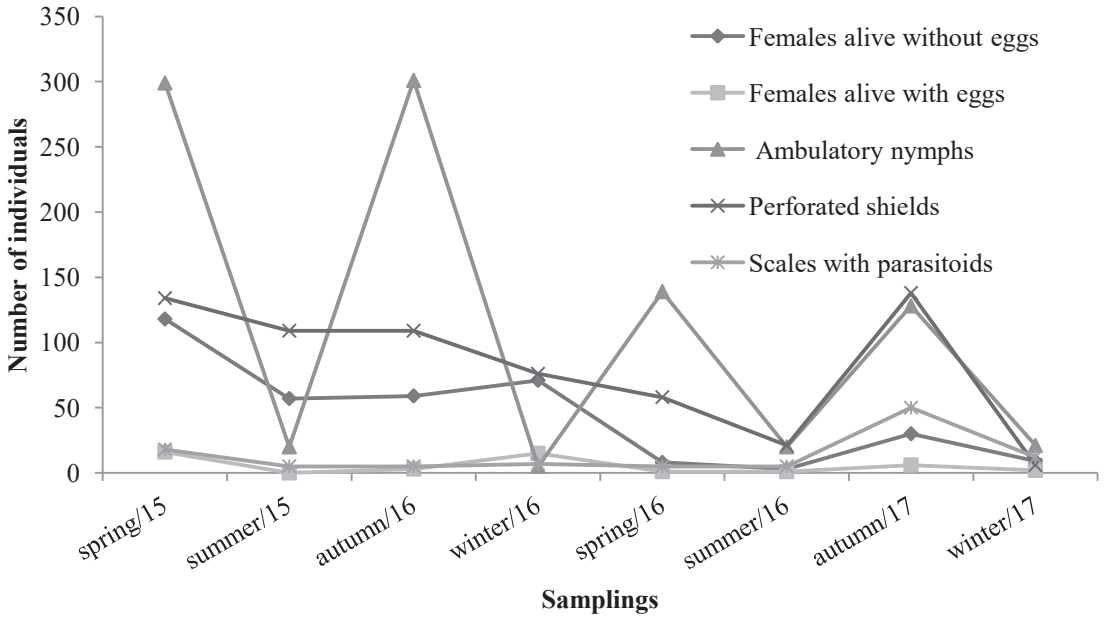

Figure 1. Population variation of *Hemiberlesia lataniae* (Hemiptera: Diaspididae) in an *Olea europaea* multivarietal olive grove (Arbequina, Arbosana and Koroneiki), at different sampling times, considering different phases and stage of development in Barra do Ribeiro (30°30′54.95″S, 51°30′20.84″W), Rio Grande do Sul, Brazil.

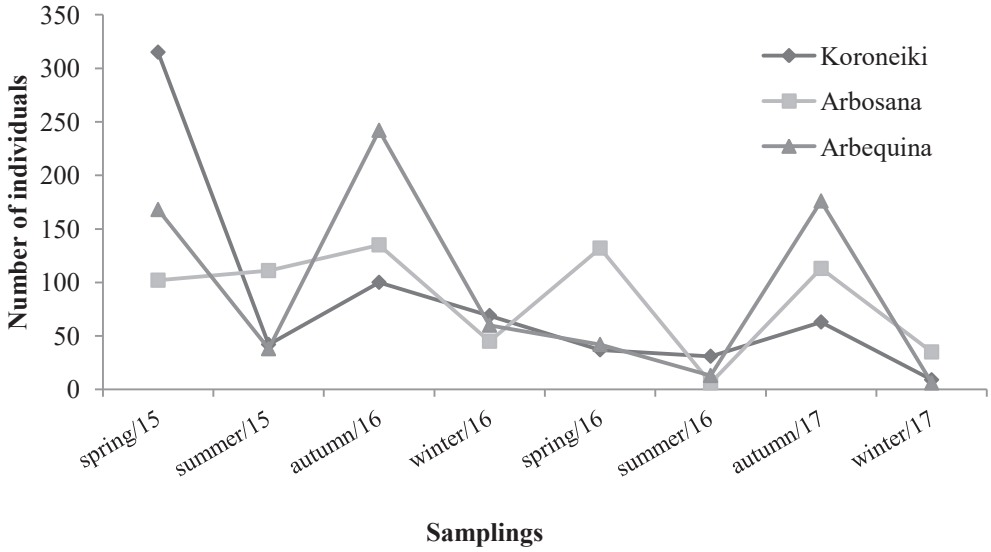

Figure 2. Population variation of *Hemiberlesia lataniae* (Hemiptera: Diaspididae) on different varieties of *Olea europaea* (Arbequina, Arbosana and Koroneiki), at different times of sampling, in Barra do Ribeiro (30°30′54.95″S, 51°30′20.84″W), Rio Grande do Sul, Brazil.

In the present study, six species of parasitoids associated with *H. lataniae* were identified. According to the scale insect database ScaleNet (García Morales et al. 2016), *E. citrina* and *S. merceti* are associated with this armored scale. *Saissetia flavella* is commonly reared from these scales in southern California and elsewhere, often in sympatry with *S. merceti*. The species appears to be a common cosmopolitan parasitoid of armored scales (Woolley and Dal Molin 2017). *Metaphycus flavus* has been recorded as a parasitoid of numerous species of soft scales and a few species of armored scales, cerococcids, eriococcids and whiteflies (Noyes 2004). The present study contains the first record of *M. flavus* as parasitoid of *H. lataniae*. In addition to these, Signiphoridae is a small family of Chalcidoidea, closely related to Azotidae and Aphelinidae (Woolley 1988; Heraty et al. 2013). Signiphorids are mostly primary parasitoids and hyperparasitoids of Hemiptera and Diptera. The species of these families perform a natural control, attacking phytophagous insects present in diverse herbaceous plants, shrubs and trees.

Regarding the number of scale insect parasitoids, no differences were found for populations among olive varieties (F=0.0095; P=0.9913). The total parasitism in *H. lataniae* was 36.3% (37.7% Koroneiki, 38.4% Arbosana, 33.0% Arbequina), indicating the importance of microhymenopteran parasitoids in the population regulation of scale insects. For the development of biological control programs, it is very important to know the native and introduced fauna of beneficial wasps (Myartseva et al. 2009). In several cultures, parasitoids have been successfully used to control scale insects (Greathead 1986; Kennett et al. 1999; Myartseva et al. 2009).

Key to the adult females of scale insect species recorded on *O. europaea* in Brazil. Adapted from Ferris (1937, 1938, 1941, 1942), Gill (1988), Granara de Willink (1999), and Claps and Wolff (2003).

1. With developed legs (Coccidae) **2**
— Without developed legs **3**

2(1). Insect body at maturity convex, with dark brown or almost black coloration; on posterior region with a characteristic elevation in the form of a transversal letter "H"; ventral tubular duct band composed entirely of ducts with filamentous inner ductlets ***Saissetia oleae* (Olivier)**
— Insect body at maturity hemispherical, with red-brown coloration; on posterior region without a characteristic elevation in the form of transversal letter "H"; ventral tubular duct band composed of 2 kinds of ducts, one with inner ductlets as wide or wider than the outer duct, and one with filamentous or thin inner ductlets . . . . . ***Saissetia coffeae* (Walker)**

3(1). Body margin bearing large 8-shaped pores; with an anal ring; body covered by an almost transparent scale cover ***Russellaspis pustulans* (Cockerell) (Asterolecaniidae)**
— Body without 8-shaped pores; without anal ring; covered by a waxy scale test (Diaspididae) **4**

4(3). Macroducts of the "two-barred" type; antennae commonly with two or more setae; anterior spiracles normally with associated disc pores **5**
— Macroducts of the "one-barred" type; antennae rarely with more than one seta; anterior spiracles normally without associated disc pores **9**

5(4). Male test white, felted, very different from the female's scale; second pygidial lobe bilobed; third pair of lobes not well developed; gland spines normally present and usually not fringed **6**
— Male test not as above; at least with three pairs of pygidial lobes present, of subequal size; marginal ducts rather small and with quite heavy oral scleroses; second pygidial lobe never bilobed; gland spines apically very fringed **8**

6(5). Body form broadly oval; median lobes large and prominent, strongly zygotic, with a pair of small setae but without gland spines or a dorsal pore between their bases; some gland spines of the pygidium with two ducts and consequently fringed at the apex . . . . . ***Pseudaulacaspis pentagona* (Targioni-Tozzetti)**
— Body form fusiform; median lobes separate, but their mesal margins very tightly appressed, without setae or gland spines between them; with a sclerosis that forms a yoke at the base of the median lobes; simple gland spines present **7**

7(6). Dorsum of the pygidium with a pair of crescent shaped folds accompanied by a sclerosis, these situated anterior to the anal opening; usually more or less reduced second lobes . . . . . ***Pinnaspis strachani*** **(Cooley)**

— Without a dorsal sclerosis as described above; second lobes more developed . . . . . ***Pinnaspis aspidistrae*** **(Signoret)**

8(5). Dorsal ducts small, scattered, quite numerous in the submarginal region of all abdominal segments; marginal ducts confined to the pygidium, all with strong oral scleroses; perivulvar pores with a tendency to form five groups; anal opening apparently somewhat closer to the apex of the pygidium . . . . . ***Parlatoria oleae*** **(Colvée)**

— Dorsal ducts few, confined to the submarginal areas of the pygidium and the prepygidial abdominal segments, slightly smaller than those of the margins; marginal ducts confined to the pygidium, all with rather weak oral scleroses; perivulvar pores in four groups; anus located at about the center of the pygidium . . . . . ***Parlatoria proteus*** **(Curtis)**

9(4). Dorsomedial area of pygidium with conspicuous areolated pattern . . . . . ***Pseudaonidia trilobitiformis*** **(Green)**

— Dorsomedial area of pygidium without an areolated pattern **10**

10(9). Median pygidial lobes well developed, the second pygidial lobes may or not be developed, third lobes represented merely by a slight point **11**

— Three pairs of developed pygidial lobes **13**

11(10). Median and second pygidial lobes developed and appearing to converge slightly; anal opening small; without perivulvar pores . . . . . ***Comstockaspis perniciosa*** **(Comstock)**

— Second pygidial lobes represented merely by a slight point; anal opening conspicuously large and located close to the apex of the pygidium; with or without perivulvar pores **12**

12(11). Perivulvar pores present in four groups . . . . . ***Hemiberlesia lataniae*** **(Signoret)**

— Perivulvar pores lacking . . . . . ***Hemilberlesia rapax*** **(Comstock)**

13(10). Median, second and third pygidial lobes with different shapes **14**

— Median, second and third pygidial lobes all about the same size and round shaped **19**

14(13). Second pygidial lobes similar in form to the median lobes, third lobe quite small but distinct and sclerotized **15**

— Second and third lobes low, broad, sloping and serrate **16**

15(13). Median pygidial lobes with a preapical notch on each side, parallel axes, with a pair of plates between them well developed, second pygidial lobes rather similar in form to median lobes, but small and slender; third lobe quite small and distinct . . . . . ***Hemiberlesia cyanophylli*** **(Signoret)**

— Median pygidial lobes with a preapical notch on the outer side, with a pair of slender plates between them, separated and straight, second and third pairs similar in form and only slightly smaller; median lobes with a distinct basal prolongation at the pygidium . . . . . ***Aspidiotus nerii*** **Bouché**

16(14). Pygidium apically acute, broadening very rapidly anteriorly **17**

— Pygidium apically rounded **18**

17(16). Median lobes extremely small, apically rounded, plates confined to the interlobular spaces, all extremely small, margin beyond the site of the fourth lobe for some distance slightly sclerotized and with small paraphyses . . . . . ***Acutaspis scutiformis*** **(Cockerell)**

— Pygidium with three pairs of lobes, wider than long, of subequal sizes, median pair larger than the others and with finely toothed margins . . . . . ***Acutaspis paulista*** **(Hempel)**

18(16). Median lobes relatively small, without plates between them, with a notch on each side and with the apex rounded; second and third lobes very short and quite broad, with outer margins sloping and minutely toothed, with strong paraphyses between the pygidial lobes . . . . . ***Melanaspis obscura*** **(Comstock)**

19(13). Prosoma much swollen and strongly sclerotized, varying in form, ranging from slightly to strongly reniform, the lateral prosomatic lobes more or less enclosing the pygidium, with two pairs of small prevulvar scleroses present, with sclerotized spots in addition to the pair of V-shaped apophyses ***Aonidiella aurantii*** **(Maskell)**

— With thoracic spur well developed, acute, sclerotized, pygidium with conspicuous branched plates between the third and fourth lobes, these exceeding the lobes in length; fourth lobe present as a sclerotized point; marginal paraphyses beyond the fourth lobe usually lacking or only weakly developed . . . . . ***Chrysomphalus aonidum*** **(Linnaeus)**

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