Population fluctuation of predators and sanitary importance mites (Acari) in commercial laying hen: ecological interactions

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**Abstract**

This study aimed to evaluate the mite fauna and the ecological interactions in commercial laying hen farms, in the state of Rio Grande do Sul, Brazil. The evaluations were conducted from August 2013 through August 2014 with two sampling strategies (feathers and traps) in three different production system: automated (*A1,2,3*), semi-automated (*S1,2*) and free-range (*FR*). A total of 38,383 mites collected belonging to 23 families and 33 species were found, being most of which collected in feather (74%) followed by traps (26%). At *S1* (10,774-28.1%) and *S2* (11,023-28.7%) there was higher abundance followed by *FR* (6,972-18.2%), *A1*(1,896-4.9%), *A2* (4,775-12.4%) and *A3* (2,943-7.7%). Higher richness has been noted at *S1*(23 species), *S2* (18 species) and *FR* (19 species). *Megninia ginglymura* (Mégnin) (Analgidae) has been the species of greater health importance, being eudominant on feathers and its populations seems to be related with increase of temperature. *Tuccioglyphus setosus* Horn et al. (Pyroglyphidae) seems to be influenced by relative humidity of the air and temperature. The predators with highest populations were *Cheyletus malaccensis* (Oudemans) (Cheyletidae), *Typhlodromus transvaalensis* (Nesbitt) (Phytoseiidae), *Blattisocius keegani* (Fox) and *Blattisocius dentriticus* (Berlese) (Blattisocidae).

**Key words:** *Megninia ginglymura,* *Tuccioglyphus setosus,* *Cheyletus malaccensis,* biodiversity.

**1. Introduction**

Intensive egg production affects the welfare of laying hens and increases the risk of epidemics, and it can be affected by various complications, such as ectoparasites and commensal mites (Guimarães and Leffer, 2009), decreasing egg production, causing fragility of the eggshell, and in addition, the laying hens become anemic, restless and aggressive towards each other (Sparagano, 2009).

The commercial laying hens are affected by mites of sanitary and economic importance worldwide. *Dermanyssus gallinae* (De Geer) (Dermanyssidae) (poultry red mite), since *Salmonella* vaccination, seems to be the new economic, welfare and epidemiological problem around the world (Sparagano, 2009). In addition to this hematophagous mite, *Ornithonyssus bursa* (Berlese) and *Ornithonyssus sylviarum* (Canestrini and Fanzago)(Macronyssidae) are registered in poultry farms and *O. bursa* seems to have replaced by *O. sylviarum* over time in Brazilian poultry industry and it has recently been reported (Soares et al., 2008).

Among the feathers mites, *Megninia ginglymura* (Mégnin) (Analgidae) is the most reported in commercial laying hen in Brazil (Reis, 1939; Tucci et al., 2005) and in the state of Rio Grande do Sul (Silva et al., 2013; Faleiro et al., 2015; Horn et al., 2016). This species spends its life cycle on its host and oviposits on feathers (Hernández et al., 2007). The ecological studies and its economic influences in poultry farms are scarce worldwide, might not be as abundant as *Dermanyssus* spp. and *Ornithonyssus* spp. and cause less significant damage (Hernández et al*.*, 2006). In Cuba, *M. ginglymura* was the more important sanitary species present in commercial laying hen in all provinces (Hernández et al., 2006). In that country, the population peak of the species indicated relationship with the wet season (Hernández et al., 2007). In Mexico, the presence of two population peaks of *M. ginglymura*, one in July and other in November, suggest that the seasonality affects the population, but the factors that influences the population is yet unknown (Quintero et al*.*, 2010). In the state of Rio Grande do Sul, higher population was related between February to April, reaching a peak in February, with 16.3 mites/hen in free-range and in April with 22.3 mites/hen (Faleiro et al*.*, 2015).

*Tuccioglyphus setosus* Horn and Klimov(Pyroglyphidae) seems to have a strong relationship with poultry farms, since is being observed in all management of laying hens in Rio Grande do Sul but before its description (Horn et al., 2017) it was misidentified as *Pyroglyphus* sp. in Silva et al. (2013) and Horn et al. (2016). This species was observed in laying hen facilities, laying hen feathers as well as in nests of wild birds (*Columbina picui* (Temminck, 1813), *Columbina talpacoti* (Temminck, 1810) and *Zenaida auriculata* (Des Murs, 1847)). Pyroglyphid house dust mites include free-living species that are mostly known as human associates living in house dust, upholstery, pillow and mattress stuffing, and causing allergies (Arlian, 1991; Lloyd, 2009; Tovey et al., 1981) and some species have economic importance as pests in stored food in warehouses and residential homes (Fain, 1990). No ecological and food habit information about *T. setosus* is known.

 Alternative pest control using natural enemies allows the use of cleaner practices and is less environmentally impactful (Lesna et al., 2009). The traditional strategy in the control of pest species in poultry systems, *i.e*., with synthetic chemical pesticides, tends in the long term to cause development of resistance in mites, and the pesticides also have adverse effects on the birds’ nervous system and can be immunosuppressive and carcinogenic as well (Nero et al., 2007; Marangi et al., 2009; Wright et al., 2009). *Androlaelaps casalis* (Berlese) (Laelapidae), *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae) (Lesna et al., 2009) and *Cheyletus eruditus* (Schrank) (Cheyletidae) (Maurer and Hertzberg, 2001) are recognized as natural enemies of *D. gallinae.* The predators *Blattisocius dentriticus* (Berlese) (Blattisocidae) and *Cheyletus malaccensis* (Oudemans) (Cheyletidae)were evaluated in laboratory feeding on *M. ginglymura* (Silva et al., 2016; Granich et al., 2016) and the greatest potential for control was presented by *C. malaccensis.*

Studies on population dynamics and efficient techniques for the control of sanitary mites are scarce in poultry farms in Brazil (Silva et al., 2013). Due to the importance of this activity to the economy of the state of Rio Grande do Sul, it is necessary to know the associated mites, their frequency and damages caused by them. Considering that *M. ginglymura* seems to be influenced by environmental conditions (Quintero et al., 2010) and predatory mites presented potential for biological control of this ectoparasite in laboratory (Silva et al., 2016; Granich et al., 2016), the present study expect (1) that environmental conditions influence *M. ginglymura* populations and *T. setosus* in laying hen systems and (2) that there is an association between the populations of *M. ginglymura* and *T. setosus* with predatory mites present in these systems. Therefore, this study aimed to evaluate the mite fauna and the ecological interactions in commercial laying hen farms, in Rio Grande do Sul, Brazil, and to support future studies using predatory mites as biocontrol strategy applied in laying hen farms.

**2. Material and Methods**

**2.1 Study area**

This study was conducted in different commercial laying hen systems between August 2013 through August 2014 in Lajeado municipality, Vale do Taquari, state of Rio Grande do Sul, Brazil.

Six poultry houses were sampled, where in three of them the laying hen system consisted of an automated vertical battery cages (*A1,2,3*), two semi-automated (*S1,2*) and one free range (*FR*) (Table 1). In automated system, the laying hen were confined in metal cages on six floors with an area of approximately 450 cm2/hen (nine hens/cage), and the cages were placed on top of the other in stacks of four. Hen feed was provided in a metal structure and water in nipple-type drinker, and eggs were collected on an automatic treadmill. In addition, feces were collected at least three times per week by treadmills at the bottom of the floor of cages. In this laying hen system, there are screens throughout the laying hen house to prevent wild bird access.

In the semi-automated laying hen system the cages were arranged in the form of stair steps with two stacks of cages in each poultry house. Feed and water were provided in an automated manner and eggs collected manually. The *S1* system was a wood structure in the style of a “California house,” and *S2* was a “wide-span model” (Axtell, 1986). *S1* did not receive any type of pesticide application during the evaluation period and was considered the semi-automated control.

The other laying hen house evaluated was raised free under a sawdust bed arranged over ground, popularly known as free-range (*FR*). In Brazil, this system is popularly known as "caipira". Feed and water were provided in an automated way and egg collecting was manual. The nests were packed in a wooden structure with sawdust inside for maintenance of eggs. The laying hens were released in the day to sunbathe, ground pecking and wing flapping.

The sampling efforts were different in the laying hen houses due to the absence of laying hen in some periods depending on the pause between the disposal of the old bath and entrance to the new laying hen batch. Cars’ access from other hen houses has been denied throughout the study.

### 2.2 Mite samplings

To collect the mites, we placed 16 traps of 27-cm PVC pipe (50 mm diameter) with 13 holes of 0.8 mm with the ends closed with caps (PVC cap) and attached to the cages with a rubber band in each laying hen house (Tucci et al. 1988). Three lightly crushed paper towel sheets were placed inside the traps, to provide shelter. Attracting substances were not used. Throughout the evaluation period, the traps were maintained at the same point, where they were replaced every 15 days. In *A1*, *A2*, *A3*, *S1* and *S2*, the traps were arranged on the second floor of the cages, while in *FR* they were placed on perches and inside the nests. At each evaluation, the paper towel was collected, packed individually in plastic bags, labeled and taken to the laboratory, where it was kept in a freezer (0°C) for at least 24 hours. For each evaluation, the collected paper towel was placed in Petri plates and observed under a stereomicroscope.

To collect the ectoparasites, it was examined ten laying hen for each laying hen houses, selecting chickens along the length of laying hen house. From each laying hen, it was collected a total of five feathers/hen every 15 days. The feathers were placed in plastic containers with 70% alcohol during a minimum of 24 hours before the screening. The plastic containers were taken to the laboratory in paper box with styrofoam inside. The screening was performed by filtering the alcohol in qualitative filter paper of diameter 12.5 cm and weight of 80 g/m2.

All mites were collected with a fine-tipped paintbrush and mounted with Hoyer's medium on microscope slides (Walter and Krantz, 2009). The slides were kept for up to 10 days at 50-60°C to dry the medium, extension of legs and diaphanization of specimens. Representative specimens of each species were deposited on the mites reference collection of the Museum of Natural Sciences at UNIVATES - University Center (ZAUMCN), Lajeado, Rio Grande do Sul, Brazil.

**2.3 Data analysis**

The data analyzed concerned the mites found in the laying hen houses evaluated sampled in traps and feathers. Several ecological indices were determined using the software DiVes 2.0 (Rodrigues, 2005):

1. Shannon-Wiener index (*H’*) expresses richness and uniformity, giving more weight to the rare species. *H’* is determined by the formula *H’=-∑ pi Log pi,* where *pi* is the proportion of specimens of each species in relation to the total number of specimens found in the assessment performed (Shannon, 1948);
2. Shannon’s evenness index (*J*) expresses the equitability of abundances in a community and allows the assessment of species stability over time. *J* is determined by the formula *J=H’/Hmax’*, where the *H’* is the Shannon-Wiener index and *Hmax’* is given by the following expression: *Hmax’=Log s*, where *s* is the number of species sampled) (Brower and Zar, 1984).
3. Berger-Parker dominance (*BPd)* considers the highest proportion of species with the highest number of individuals. *BPd* is determined by the formula *d*= *Nmax/NT*, where *Nmax* is the number of specimens from the most abundant species and *NT* is the total number of specimens from the sampling (Berger and Parker, 1970).

Species constancy (*C*) was classified as constant when present in more than 50% of the samples (*C* > 50%), accessory when present in 25 – 50% of the samples (25% < *C* < 50%) and accidental when present in less than 25% of the samples (*C* < 25%) (Bodenheimer, 1955). The dominance (*D*) was defined by the formula *D*% = (i/t) x 100, where i = total number of individuals of a species and t = total individuals collected and clustered according to categories: eudominant (≥ 10%), dominant (5 ≤ 10%), subdominant (2 ≤ 5%), eventual (1 ≤ 2%) and rare (*D* < 1%) (Friebe, 1983).

 Climate parameters precipitation (mm), relative humidity of air (%) and temperature (ºC) for the study period were provided by UNIVATES University Center Meteorological Station, Lajeado, state of Rio Grande do Sul (Fig. 1).

A Principal Component Analysis (PCA) was performed with software used to investigate the correlation between abiotic end biotic parameters (PRIMER-E Clarke and Gorley, 2002) version 5.2.9, in traps and feathers. The main species with recognized predatory potential were considered for this analysis, besides the species *M. ginglymura* and *T. setosus.* In order to verify the highest inclinations and amplitudes of each species, rank-abundance curves were performed for traps and feathers.

**3. Results**

A total of 38,383 mites collected in feathers and traps belonging to 23 families and 33 species were found (Table 2). Most mites were sampled in feathers (74%) and the others in traps (26%). In the semi-automated systems there was a great abundance, *S1*(10,774) and *S2* (11,023), followed by *FR* (6,972), while the automated systems (*A1*: 1,896; *A2*: 4,775 and *A3*: 2,943) were observed lower abundance. The richness follows the same logic of abundance, it was greater in *S1* (23) and *S2* (18), followed by *FR* (19) and less richness in *A1*, *A2* (13) and *A3* (12) (Fig. 2). The rank-abundance analysis showed that there was a drastic difference in acarofauna present in the feathers compared to the traps, in terms of abundance and richness, having higher richness associated to traps (32 species) than feathers (13 species) in the evaluated systems.

 **3.1 Ecological Diversity indices**

The automated systems presented greater indices of diversity and evenness (*A1* – *H’*: 0.7018, *J:* 0.6301; *A2* – *H’*: 0.5078, *J:* 0.4558 and *A3* – *H’*: 0.546, *J:* 0.5046) than semi-automated systems (*S1* – *H’:* 0.1977, *J:* 0.1452; *S2* – *H’*: 0.2764, *J:* 0.2202) or free range (*FR* – *H’:* 0.233, *J:* 0.1822) (Table 3). The lowest indices of Berger-Parker dominance were observed in *A1* (*BPd:* 0.0538), *S1* (*BPd:* 0.0644), *S2* (*BPd:* 0.064) and *FR* (*BPd:* 0.0839) and greatest indices in *A2* (*BPd:* 0.128) and *A3* (*BPd:* 0.1927).

**3.2 Biodiversity and mite fauna fluctuation**

The families with the great richness were Cheyletidae with four species (*Chelacheles bipanus* Summers & Price, *Cheletomimus (Hemicheyletia*) *wellsi* (Baker)*, C. eruditus* and *C. malaccensis* followed by Acaridae with three (*Aleuroglyphus ovatus* (Troupeau), *Thyreophagus entomophagus* (Laboulbéne)and *Tyrophagus putrescentiae* (Schrank)).

*Megninia gynglymura* was the species with sanitary importance and presented greater abundance with 29,633 specimens (77.2%). It was present throughout the year in all systems evaluated, excepting *A1* where it was present since the fifth sampling (Fig. 3 and 4 - *A2* with graph different scale). This species was considered constant in feathers and traps in all laying hen houses evaluated, except in *A1* where *M. ginglymura* was accessory in the feathers. In the feathers, it was eudominant in all systems. Besides, it was eudominant in traps of S1 and *FR*, dominant in *A1* and *S2* and subdominant in *A2* and *A3*. In *A1* and *A2*, the population peak of *M. ginglymura* occurred between January and March/2014 with highest average in February/2014, with 1.6 and 11.6 mites/feathers, respectively. The application of synthetic chemical pesticide in September/2013 occurred in the period of low populations. In *A3*, there was a late population peak, in April/2014, with the average of 11.1 mites/feathers. In the semi-automated systems the populations remained in high numbers in most of the period. In *S1*, high population extended from November/2013 to April/2014, averaging 12.8 mites/feathers in December/2013 and January/2014; in *S2* the averages were high between December/2013 and June/2014, with the highest average, 13.4 mites/feather, in April/2014. In *FR*, population with high number were observed during September to November/2013, with the high population peak, 10.9 mites/feathers, in October/2013. The mite populations had increased immediately after the application of synthetic chemical pesticide, with a new population peak between February and June/2014.

Among the predators, *C. malaccensis,* with 3,511 (9.1%) was present in the systems during all the sampling period. This species stood out as constant and eudominant in traps of all systems, except in *S1*, where it was accessory and subdominant. In the feathers, this predator was observed in *A1* and in the other laying hen house was accidental and rare. In *A1*, *C. malaccensis* populations increased after the start of the population peak of *M. ginglymura* in January/2014, but no significant correlation between these populations*.* In *A2*, laying hen house with high abundance of *C. malaccensis*, the population peak occurred between January and March/2014, when coinciding with the population peak of *M. ginglymura*. In *A3*, the population peak of *C. malaccensis* occurred between February and August/2014. In *S2*, the population peak was between December/2013 and February/2014 (4.9 mites/traps in January/2014). In *FR*, this predator population remained high, with various population peaks between September to November/2013, in February and March to May/2014.

The other collected mite, *C. eruditus*,was present only in *FR* in lower population (18 specimens – 0.05%).

The second more abundant predatory mite present in all systems was *Typhlodromus transvaalensis* (Nesbitt),with 309 specimens (0.8%), considered constant in traps of *A1*, *A2* and *S2*, where they were dominant, eudominant and subdominant, respectively. This species was observed in the feathers in *S1* and *S2*, being considered accidental and rare. In *A1* there was a period of population slightly higher between October and December/2013 and in June/2014. In *A3*, the population peak occurred between June and August/2014 with averaging 2.3 mites/traps in July/2014. In *S2*, the high population were registered between October/2013 and January/2014, with 1 mite/traps. In *A2*, *S1* and *FR* the populations were very low.

*Blattisocius dentriticus* and *Blattisocius keegani* (Fox) (Blattisocidae)were the predatory mites with low abundance evaluated for potential biological control. Populations of both predators remained low during the study period. *Blattisocius keegani* was the third predator most abundant present in at least five laying hen houses (*A1*, *A2*, *A3*, *S1* and *S2*), totalizing 187 specimens (0.5%). This predator was constant and subdominant in the traps of *A1* and *A3*; accessory and rare in *A2*; accidental in *S1* and *S2* where it was subdominant and rare, respectively. In the feathers, *B. keegani* was observed also in low numbers and considered accidental and rare, except in *A1* where it was absent. This species was absent in *FR* system. *Blattisocius dentriticus* presented only 93 specimens (0.2%), it was considered accessory and subdominant in the traps of *S1*and accessory and rare in *A2* and *A3*. In the traps of other laying hen houses it was considered accidental and rare. In the feathers, the species was present in *A1* where it was accidental and rare; in *S1*presented a population peak between September and November/2013.

Among the generalist species, *T. setosus,* with 3,294 (8.6%) was the species more abundant and present during the sampled period in the laying hen houses, except in *FR* where the population was low. This species was considered constant and eudominant in the traps, except in *FR* where it was accessory and eventual. In the feathers, was accessory and rare in *A3*; accidental and rare in *A2* and *S2*; accidental and subdominant in *A1*. This species was absent in the feathers of *S1* and *FR*. In *A1*, the synthetic chemical pesticide application in September/2013 did not prevent high populations between December/2013 and February/2014, with higher average in January/2014 (6.3 mites/feathers). In *A2*, population with high average occurred between June and August/2014, with population peak in August/2014 (23.1 mites/traps). Due to this high population average in this system, this graph was presented in different scale of others (Fig. 3). In *A3*, the population remained low and the average oscillate between 0.7 and 4.4 mites/traps with the peak registered in January/2014. In *S1*, the population remained low with any significant population peak. In *S2*, two population peaks were present between September and October/2013 and another from March to August/2014 with high average registered in August/2014 (16.2 mites/traps). In *FR*, the population remained low with high average, 0.13 mites/traps, in December/2013.

With the first two components of the PCA for feathers was explained ca. 55% of the variation between the different laying hen (Fig. 5). The component 1 was most correlated with the total abundance of mites (0.54), abundance of *M. ginglymura* (0.53) and temperature (0.45). The component 2 was most correlated with, relative humidity (0.51), followed by abundance of *M. ginglymura* (0.44) and temperature (-0.44). In general, there is an indication of the relationship between *T. setosus*, relative humidity and richness.The PCA for traps was explained 55% of the variation between the different laying hen (Fig. 6). The component 1 was most correlated with the abundance of *T. setosus* (0.48), followed by the total abundance of mites (0.53) and temperature (-0.41). The component 2 was most correlated with, relative humidity (-0.59), followed by temperature (0.44) and total richness of mites (-0.44). In traps, *M. ginglymura* is more related to free range and automated systems with temperature being a determining factor as well as the increase in temperature is related to greater abundance of this species. In traps, the abundance is not related to *M. ginglymura*.

In a second PCA analysis, to investigate the possible relationships among predatory-prey, we find the following, with the first two components of the PCA for feathers, was explained ca. 62% (Fig. 7). The component 1 was most correlated with the abundance of *M. ginglymura* (0.580) follow by *B. keegani* (0.526), the component 2 was most correlated with *T. transvaalensis* (0.562) and *T. setosus* (-0.536). The PCA for traps was explained ca. 65% of the variation between the different laying hen based on abundance (Fig. 8). The component 1 was most correlated with the abundance of *M. ginglymura* (-0.545) and abundance of *T. transvaalensis* (0.510). The component 2 was most correlated with the abundance of *T. setosus* (-0.762) and *C. malaccensis* (0.367).

**4. Discussion**

Data presented in this study are important as a preliminary for the identification of associated species and evaluation of its population dynamics in different laying hen houses management. The management influences the abundance, richness, and diversity of mites in laying hen systems (Horn et al., 2016). Furthermore, it highlights *M. ginglymura* as the mainly sanitary importance mite associated to laying hen in the region of the study. This species is strongly associated to feathers, but in the laying hen systems with high population densities it seems to leave the hen and move looking for a new host, and could thus be captured by traps designed to catch predatory mites and other ectoparasites as *D. gallinae*,which it rises the hen only to blood feeding. No hematophagous mites were registered. *Megninia ginglymura* feed on skin, fat and parts of feathers, and the mite saliva can cause lesions, allergic reactions, serious scabs, stress, crust formation and secondary bacterial infections (Tucci et al.,2005). Few studies that confirm the percentage of economic loss are known. Therefore, it must be highlighted the importance to improve the knowledge of the management of this species in commercial laying hen. The laying hen in the *FR* system showed improved visual appearance, with no injuries to the skin and practically intact feathers until the discard phase due to senescence and reduced egg productivity and the opposite was observed in all other systems (Horn et al., 2016).

**4.1 Mite fauna**

The highest richness was found in the traps, while highest abundance has been associated to feathers. Automated systems seem to induce a lower abundance of sanitary importance mites than the other systems. Even with more confinement hens, the automated systems had lower or similar to the abundance as other laying hen systems (Horn et al., 2016). While most richness was observed in semi-automated systems (*S1*; *S2*) and *FR*. The highest richness were associated to the laying hen in the absence of synthetic chemical pesticides (*S1*), and no significant higher abundance in total mites was found, when compared with the other laying hen houses (Horn et al*.*, 2016).

This data shows typical pattern of mites stability of the community and ecological balance with the greatest richness and more rare species in traps. This pattern also appears in FR system. In other systems, richness seems to reduce. The greater number of exclusive species and the greater community heterogeneity among the traps in comparison to the feathers, show the mite fauna community has a more equitable distribution with a larger number of rare and less abundant species (Magurran, 1988).

The automated laying hen houses had highest diversity (*H’*) and evenness (*J*) than the other systems. The lowest dominance was observed in semi-automated laying hen houses, *FR* and *A1*.

Comparing the mite fauna associated with each kind of mite collection samplings, it is observed that traps presents greater mites stability of the community while feathers presents an extremely high abundance of one species (*M. ginglymura*). It should be considered that the predators abundance was very low and this data could influence the PCA evaluations on feathers (Figures 7 and 8).

Overall, the mite fauna showed obvious population peaks, and some species seemed to demonstrate resistance to synthetic chemical pesticides used by the farmers with the aim to control *M. ginglymura.*

**4.2 Population dynamics of *M. ginglymura* and *T. setosus***

Quintero et al. (2010) reported the presence of two population peaks of *M. ginglymura* in Mexico, one in July and other in November suggesting that the seasonality affects the population, but the factors that influence the population is yet unknown. Population peaks of *M. ginglymura* in the laying hen systems occurred between September/13 and April/2014, coinciding with periods of high average temperatures. Also corroborate with the period of high population from February to April, reaching a peak in February, with 16.3 mites/hen in free-range and in April with 22.3 mites/hen (Faleiro et al., 2015). These authors associate such differences to the seasonal temperature with the population peaks of free-range in warmer month and in colder months in battery cages.

The application of synthetic chemical pesticide in *A1* e *A2* seems to not have been effective in population control of *M. ginglymura*, because in short period of time after the application there was a population increase. In *A3*, there was a late population peak probably due to introduction of a new batch of laying hen only in January/2014. There was a decrease of *M. ginglymura* populations with the use of pesticides. However, due to the late peak, it is uncertain the real capacity of the pesticides to contain the population, or, actually, the decrease was related to environmental conditions. In *FR*, the populations were influenced by pesticides in December/2013 and January/2014. However, it is noteworthy that immediately after application there was fast increase in population, indicating that there is a tendency to resistance to pesticides. The laying hen houses without use of pesticides (*S1*) act as a blank, aiding elucidate the ecology of this species, who seemed to have population peaks during periods of higher average temperatures. Our data showed more relationship of *M. ginglymura* population with temperature. The population increases of this species in Cuba occurred between the months of May and June and extending until December, indicating that the population is related to the wet season (Hernández et al., 2007).

Regarding the *T. setosus,* although there was no apparent sanitary risk for laying hens due probably to be a dust mite, as are most of the representatives mites of Pyroglyphidae family, it was decided to clarify some information about it*,* since nothing is known about this species described from this environment (Horn et al., 2017). The *T. setosus* has a strong relationship with poultry farms, being observed in all systems of management of laying hens throughout the year and in high populations (second species more abundant in feathers and traps).

*Tuccioglyphus setosus* was collected in feathers and traps. The population peaks were not concomitant in the laying hen systems, but oscillated between September/2013 and February/2014 (*A1*, *A2*, *A3* and *S2*) and an additional peak in *A2* was observed between June and August/2014. The multivariate analyses showed directly relationship with relative humidity of air and the abundance of *T. setosus*.

**4.3 Promising biological control agents**

*Cheyletus malaccensis* seems to be the most important predatory mite observed. The same was reported by Faleiro et al. (2015) that suggested *D. gallinae* as a suitable prey of this predator. This predator was evaluated with the preys *Acarus siro* L. (Acaridae), *A. ovatus*, *Caloglyphus redickorzevi* Zachvatkin (Acaridae), *Caloglyphus rodriguezi* Samšiňák (Acaridae), *T. putrescentiae* and *M. ginglymura* (Pekár and Hubert, 2008; Palyvos and Emmanouel, 2009; 2011; Cebolla et al., 2009; Al-Shammery, 2014; Granich et al., 2016). When the results obtained in the life table of *C. malaccensis* feeding on *M. ginglymura* are compared with studies that evaluate other preys, the best results so far (*Ro =* 135.6; *T =* 41.6; λ = 1.13; *rm =* 0.12)were obtained when feeding on *M. ginglymura* (Granich et al*.*, 2016). The population peaks of this predator seem to coincide with *M. ginglymura* peaks (Figs. 3 and 4). *Cheyletus malaccensis* seems to be the most common predator when there is a decrease in populations of *M. ginglymura*. This species showed higher population when *Chortoglyphus arcuatus* Troupeau was present in large numbers, indicating a predator-prey association between them (Faleiro et al., 2015). In traps, the PCA analyses showed a relationship between this predator and *T. setosus* (Fig. 8). The both abundances of this two mites were so similar. This relationship needs to be clarified, since low information about *T. setosus* is knowed, whereas this species is recently described (Horn et al., 2017). *Cheyletus eruditus* waspresent only in *FR* in lower population. *Cheyletus eruditus* commonly occur in stored foods, feeding on pest mites and reducing pest populations (Pulpan and Verner, 1965).

 *Typhlodromus transvaalensis* was the second more abundant predator in the laying hen systems. Faleiro et al. (2015) found this species in laying hen farms associated to traps and nests of wild birds in low populations. However, the real role of this predator in these environments remains to be elucidate. Laboratory tests showed that it is able to feed on *M. ginglymura* (unpublished results - first author).

The predators *B. dentriticus* and *B. keegani* were the predatory mites with less abundance and low populations during the sampled period. *Blattisocius keegani* is associated to beetles in stored products and shows potential for the control of the navel orangeworm *Amyelois transitella* Walker (Lepidoptera: Pyralidae) (Thomas et al., 2011) and *B. dentriticus* feeds on arthropod eggs and *T. putrescentiae* (Schrank) (Fenilli and Flechtmann, 1990). Three species of this genus were associated to laying hen farms: *B. dentriticus*, *B. keegani* and *Blattisocius tarsalis* (Berlese) where *B. dentriticus* was the most commom in traps, coinciding with population of *D. gallinae* and *M. ginglymura* (Faleiro et al., 2015). *Blattisocius dentriticus* fed on *M. ginglymura* showed lower values (*Ro =* 2.79; *T =* 23.76; λ = 1.04; *rm =* 0.04) than when the prey was *T. putrescentiae,* since the population of this predator increased about 7.53 times every 14.3 days (*Ro =* 7.53; *T =* 14.3; λ= 1.15; *rm =* 0.14) (Silva et al*.*, 2016). *Blattisocius dentriticus* had no relationship with *M. ginglymura* populations in the systems, and only with the traps population of *T. setosus* in *S1*. *Blattisocius keegani* and *M. ginglymura* presents in traps had relationship in *S2* and this predator was not influenced by *T. setosus*.

 The results obtained in the present study are corroborated by the data obtained in Faleiro et al. (2015) that *C. malaccensis* could be considered a natural enemy with potential for future biological control studies of ectoparasites associated with laying hens. In laboratory, this predator proved to be effective in biological control of *M. ginglymura* resulting in a high fertility rate with more than 310 eggs/female (Granich et al., 2016). For cleaner control without the use of synthetic chemical pesticides, inoculative releases would be a better choice to control of poultry pest mites, involving the release of low numbers of this natural enemy several times before periods of infestation (Faleiro et al., 2015). In addition, the management provides greater permanence, and natural proliferation of predatory mites becomes a cleaner strategy, thus, avoiding the use of synthetic chemical pesticides that are harmful to human and animal health, since the sanitary importance mites had already demonstrated resistance to these products.

**5. Conclusions**

In our hypothesis 1 we proposed that environmental conditions influence populations of *M. ginglymura* and *T. setosus*. The multivariate analyses showed directly relationship with temperature and *M. ginglymura*. The species *T. setosus* was related torelative humidity of air.

*Cheyletus malaccensis*, *T. transvaalensis*, *B. keegani* and *B. dentriticus* were the most common and abundant predatory mites, with population variation depending on husbandry systems evaluated, and considered with potential to be evaluated for biological control of sanitary importance mites in the laying hen farms. Besides, *C. eruditus* even with low populations and found only in FR was considered due to its recognized potential in biological control of *D. gallinae* in commercial laying hen farms (Lesna *et al.*, 2009).

The hypothesis 2 was supported by the data that there is a relationship between *M. ginglymura* and the predators *B. keegani* and *T. transvaalensis* presented in multivariate analyses. In addition, *T. setosus* had relationship with the predators *C. malaccensis*.

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**Legends to figures**

**Fig. 1** Meteorological data precipitation (Pp) (mm), temperature (Temp) (ºC) and relative humidity of air (RH) (%) in Lajeado, state of Rio Grande do Sul between August 2013 to August 2014

**Fig. 2** Rank-abundance curves of fern species, comparing edge and interior of the studied sites. *A1*, *A2*, *A3* – automated systems; *S1*, *S2*– semiautomated systems; FR – free range.

**Fig. 3** Population fluctuation of mites in automated (*A1*, *A2*\* and *A3*) laying hen houses between August 2013 to August 2014 in Lajeado municipality, state of Rio Grande do Sul, Brazil

\* the scale of *A2* is different of others due a high population peak of *Tuccioglyphus setosus*.

The arrows represent moment of pesticides application.

**Fig. 4** Population fluctuation of mites in semi-automated (*S1* and *S2*) and free range (*FR*) laying hen houses between August 2013 to August 2014 in Lajeado municipality, state of Rio Grande do Sul, Brazil

The arrows represent moment of pesticides application.

**Fig. 5** Principal component analysis (PCA) of feathers samples on different systems of laying hen – AB: Total Abundance of Mites; Mg: Abundance of *Megninia ginglymura*; T °C: Temperature; P: Pluviosity; S: Richness; RH: Relative humidity; T: *Tuccioglyphus setosus.* *A1* (Δ), *A2*(□), *A3*(■) – automated systems; *S1*(○), *S2*(●) – semiautomated systems; FR (**+**) – free range).

**Fig. 6** Principal component analysis (PCA) of traps samples on different systems of laying hen – AB: Total Abundance of Mites; Mg: Abundance of *Megninia ginglymura*; T °C: Temperature; P: Pluviosity; S: Richness; RH: Relative humidity; T: *Tuccioglyphus setosus.* *A1* (Δ), *A2*(□), *A3*(■) – automated systems; *S1*(○), *S2*(●) – semiautomated systems; FR (**+**) – free range).

**Fig. 7** PCA based on abundance of mites collected from feathers, (A) *Blattisocius dentriticus*, (B) *Blattisocius keegani*, (C) *Megninia ginglymura*, (D) *Tuccioglyphus setosus*, (E) *Cheyletus malaccensis*, (F) *Typhlodromus transvaalensis*. *A1*, *A2*, *A3*– automated systems; *S1*, *S2* – semiautomated systems; FR– free range.

**Fig. 8** PCA based on abundance of mites collected from traps, (A) *Blattisocius dentriticus*, (B) *Blattisocius keegani*, (C) *Megninia ginglymura*, (D) *Tuccioglyphus setosus*, (E) *Cheyletus malaccensis*, (F) *Typhlodromus transvaalensis*, (G) *Cheyletus eruditus*. *A1*, *A2*, *A3*– automated systems; *S1*, *S2* – semiautomated systems; FR– free range.