University of São Paulo "Luiz de Queiroz" College of Agriculture

# Potential use of edaphic predatory mites for the control of hematophagous mites (Acari)

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Dissertation presented to obtain the degree of Master in Science. Area: Entomology

Piracicaba 2015 Renan Venancio da Silva Agronomist

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Hellen Galisteu and Renato Venancio,

my sister

## Isabela Venancio

my brother

## Alan Galisteu

For their love and unfailing support,

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## SUMMARY

#### RESUMO

## Potencial do uso de ácaros predadores edáficos para controle de ácaros hematófagos (Acari)

Uma das principais preocupações ao longo do processo de produção animal é a ocorrência de organismos hematófagos, como carrapatos e piolhinhos. O controle destes organismos usualmente é feito com produtos químicos. Porém, esta técnica de controle vem sendo desencorajada em diversos países. Uma forma alternativa é o controle biológico. Sabese que a maioria dos ácaros do grupo Gamasina (ordem Mesostigmata) são de hábito predador, porém sua diversidade e prevalência nos locais em que carrapatos e piolhinhos causam problemas são pouco conhecidas. Um dos primeiros passos recomendados num programa de controle biológico é a determinação da fauna no local onde deseja-se o controle dos organismos praga. Os objetivos deste estudo foram a determinação dos Gamasina que coocorrem com Ixodes ricninus (L.) em pastagens na Noruega e a busca por possíveis ácaros predadores edáficos daquele grupo no Brasil, para controle de carrapatos e piolhinhos. No presente estudo, 2900 Gamasina afiliados à 12 famílias foram coletados na Noruega, coocorrendo com I. ricinus. As famílias mais numerosas foram Parasitidae (46,9%) e Veigaiidae (25,9%), enquanto que as mais diversas foram Laelapidae, Macrochelidae, Parasitidae e Zerconidae, cada uma com cinco espécies. Dentre estes ácaros, um novo laelapídeo do gênero Cosmolaelaps é descrito da Noruega. No Brasil, foram testados 551 Gamasina afiliados a 11 famílias, incluindo Laelapidae, Macrochelidae, Parasitidae e Veigaiidae, os quais foram testados quanto ao seu potencial em alimentar-se de larvas e ovos dos carrapatos Amblyomma sculptum Berlese e Rhipicephalus microplus (Canestrini), e de todos os estágios do piolhinhode-galinha, Dermanyssus gallinae (De Geer). As famílias mais abundantes foram Ologamasidae (25,4 %) e Parasitidae (21,1%), enquanto as mais diversas (em termos de gênero) foram Ologamasidae e Laelapidae, com cinco e quatro gêneros, respectivamente. Dos predadores avaliados, apenas Stratiolaelaps scimitus (Womersley) alimentou-se das larvas de ambas espécies de carrapatos e de todos os estágios de desenvolvimento de D. gallinae.

Palavras-chave: Controle biológico; Diversidade; Ecologia; Taxonomia

#### ABSTRACT

#### Potential use of edaphic predatory mites for the control of hematophagous mites (Acari)

A major concern in animal production is the occurrence of hematophagous organisms, as ticks and poultry mites. The control of these organisms is usually done with chemicals. However, this technique is being discouraged in several countries. An alternative measure is biological control. It is known that most mites of the cohort Gamasina (order Mesostigmata) are predators and their diversity and prevalence in places where ticks and poultry mites cause problems are poorly understood. One of the first recommended steps in a biological control program is to determine the fauna where the pest organisms are to be controlled. The objectives of this study were to determine the Gamasina co-occurring with Ixodes ricinus (L.) in pastures in Norway and prospect possible edaphic predatory mites of that group in Brazil to control ticks and poultry mites. In this study, 2,900 Gamasina of 12 families were collected in Norway, co-occurring with I. ricinus. The most abundant families were Parasitidae (46.9%) and Veigaiidae (25.9%), while the most diverse were Laelapidae, Macrochelidae, Parasitidae and Zerconidae, each with five species. Among these mites, a new laelapid species of Cosmolaelaps was found and is here described. In Brazil, 551 gamasines assigned to 11 families were collected and tested for their potential to feed on larvae and eggs of two tick species, Amblyomma sculptum Berlese and Rhipicephalus microplus (Canestrini), and all stages of the red poultry mite, Dermanyssus gallinae (De Geer). The most abundant families were Ologamasidae (25.4%) and Parasitidae (21.1%), while the most diverse (in terms of genera) were Ologamasidae and Laelapidae, with five and four genera, respectively. Of the evaluated predators, only Stratiolaelaps scimitus (Womersley) fed on the larvae of both tick species and all stages of *D. gallinae*.

Keywords: Biological control; Diversity; Ecology; Taxonomy

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## **1 INTRODUCTION**

Primary livestock production is an important source of income in Brazil. In 2013, Brazilian milk production was 34 million tons, making this country the fifth world producer (FAO, 2015). Dairy cattle are injured by ticks, a group of hematophagous mites that comprises almost 900 species described around the world (KEIRANS, 1992; GUGLIELMONE et al., 2010) of which 65 have been reported from Brazil (MARTINS et al., 2014).

Ticks cause injuries to animals of economic interest by stressing them, consuming their blood and inoculating them with infectious agents (BARROS-BATTESTI; ARZUA; BECHARA, 2006). The cattle-tick, *Rhipicephalus microplus* (Canestrini), is a species of great economic importance in Brazil for transmitting the protozoa *Babesia bovis* Babes and *Babesia bigemina* (Smith & Kilborne) as well as the bacterium *Anaplasma marginale* Theiler, all of which cause a disease complex called "Tristeza-parasitária-bovina". Norwegian sheep breeders face similar problems. In Norway, a disease called tick-borne fever (TBF) is common in sheep flock (JORE et al., 2011), being responsible for the death of 30% of the herd. TBF is caused by *Anaplasma phagocitophylum* (Foggie), transmitted by the tick *Ixodes ricinus* (L.).

In addition to animals of economic interest, ticks also cause injuries to humans. *Amblyomma cajennense* (Fabricius) was mentioned by HORTA et al. (2004) as the main tick species infesting humans in southeastern Brazil. This species has been mentioned as a vector of the bacterium *Rickettsia rickettsii* (Wolbach), causal agent of the Rocky-Mountain-Spotted-Fever (RMSF), known in Brazil as "febre maculosa". Nava et al. (2014) stated that the name *A. cajennense* has been applied to a complex of parapatric species, consisting of *A. cajennense* s. stricto, *Amblyomma interandinum* Beati, Nava & Cáceres, *Amblyomma mixtum* Koch, *Amblyomma patinoi* Labruna, Nava, Beati, *Amblyomma sculptum* Berlese and *Amblyomma tonelliae* Nava, Beati & Labruna. According to those authors, the main tick species infesting humans in southeastern Brazil is *A. sculptum*.

Ticks have been controlled on and off the host by the use of different techniques, especially by the use of chemical acaricides (GEORGE; POUND; DAVEY, 2008). The discouragement of this control technique in different parts of the world, especially in Europe, for health and environmental reasons (KUNZ; KEMP, 1994; THULLNER; WILLADSEN;

KEMP, 2007; GEORGE et al., 2008), has led to the search for alternative and more sustainable control measures (SAMISH; GINSBERG; GLAZER, 2008).

Raising chickens for egg production is another important activity in Brazil, where about 40 billion eggs are produced yearly, making this country the eighth world egg producer (FAO, 2015; PARANÁ, 2013). Laying hen growers face several sanitary problems, including the occurrence of blood feeding ectoparasites. The poultry red mite, *Dermanyssus gallinae* (De Geer) has been considered the most important ectoparasite of laying hens, causing anemia, reduced production and egg downgrading for the present of blood spots (CHAUVE, 1998; SPARAGANO et al., 2009; GEORGE et al., 2015). That parasite inhabits nests of several birds and small mammals, being responsible for severe damage to laying hens around the world (MAURER et al., 1993; TUCCI et al., 1997; EMOUS et al., 2005). It was first reported in Brazil by Fonseca (1938) and Reis (1939), on canaries and hens in the State of São Paulo. Its control is usually done with the use of chemicals, spraying laying hen cages and housings with acaricides. However, this is not always effective due to acaricide resistance (BEUGNET et al., 1997; NORDENFORS; CHIRICO, 2001), prompting the search for alternative control techniques.

Within the subclass Acari, the cohort Gamasina of the order Mesostigmata is mostly composed by mites that prey on other small arthropods, including mites. For this reason, members of that order have been studied as predators of edaphic pests (INSERRA; DAVIS, 1983; WALTER, 1986, LESNA et al., 2000; ALI; DUNE; BRENNAN, 1997; FREIRE et al., 2007). In a survey conducted in Switzerland by Maurer et al. (1993), *Androlaelaps casalis* (Berlese) (Mesostigmata: Laelapidae) was reported in poultry houses. This species was mentioned to prey on *D. gallinae* (McKINLEY, 1963). In a survey conducted in poultry houses in The Netherlands by Lesna et al. (2009), 12 mesostgmatic species were reported; among them, *A. casalis* and *Hypoaspis aculeifer* (Canestrini) (Laelapidae) were reported feeding on *D. gallinae*. Silva et al. (2013) reported seven predatory mite species from poultry houses in the state of Rio Grande do Sul, Brazil; including *A. casalis* and *H. aculeifer*. The predatory mite *Stratiolaelaps scimitus* (Womersley) (Laelapidae) was reported in poultry houses of small farmers in the state of Minas Gerais, Brazil (LESNA et al., 2012). This species is known to feed on *D. gallinae* (TUOVINEN, 2008) and it is currently used to control *D. gallinae* and other blood feeding mites on pet animals (LESNA et al., 2012).

This could suggest that they can also feed on ticks, considering the similar food of both *D. gallinae* and ticks. Some edaphic predatory mite species have been commercialized for the control of pests that spend their life cycle (or part of it) in the soil (FREIRE et al.,

2007; GERSON; WEINTRAUB, 2007; CASTILHO et al., 2009a, 2009b), including *D. gallinae* (LESNA et al., 2009, 2012). However, apparently no work has been published about Gamasina tick biological control agents. The hypothesis of the present work is that there are Gamasina species that can feed on tick eggs and larvae, and on all developmental stages of *D. gallinae*. One of the first recommended steps in a biological control project of a pest organism usually involves the determination of the fauna where the control of the pest is envisioned. The present study was related to a collaboration between Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), Brazil and the Norwegian Institute for Bioeconomy Research (NIBIO), former BIOFORSK, Norway, whose objectives were:

- To identify the Gamasina fauna from pasture soil in western Norway, where *I. ricinus* is found, in order to find possible biological control agents of that tick species.
- To describe at least one of the new species found in Norway.
- To identify the Gamasina from soil of different places in the states of Minas Gerais and São Paulo, evaluating their potential for controlling eggs and larvae of *A. sculptum*, *R. microplus* and different stages of *D. gallinae* under laboratory condition.

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## 2 DIVERSITY OF EDAPHIC GAMASINE MITES CO-OCCURRING WITH THE TICK *Ixodes ricinus* IN PASTURES OF WESTERN NORWAY

#### Abstract

Tick-borne diseases are of major concern for Norwegian sheep farmers. Ticks are commonly controlled with chemicals. Fungal pathogens, predatory mites and ants are thought to be important tick killers in nature. However, the prevalence and diversity of predatory mites in tick habitats has barely been evaluated. It is known that most edaphic mite species of the cohort Gamasina (order Mesostigmata) are predators. Until now, 220 mesostigmatid species have been reported from Norway, most of them belonging to the Gamasina. One of the first recommended steps in a biological control program involves the determination of the fauna in the pest habitat. The objective of this study was to determine the groups of gamasines co-occurring with I. ricinus in sheep grazing areas in Isfjorden and Tingvoll, Western Norway. A total of 2,900 gamasines of 12 families was collected. The most numerous families were Parasitide (46.9%) and Veigaiidae (25.7%), whereas the most diverse families were Laelapidae, Macrochelidae, Parasitidae and Zerconidae. Our results showed that the Gamasina abundance is strongly related to locality, elevation, tick abundance, temperature and rainfall. Differences in the prevailing environmental conditions resulted in more outstanding differences between Gamasina abundances than diversities. Based on our present knowledge of the potential of different gamasine groups as biological control agents, the results suggested that laelapid mites should be among the priority groups to be further evaluated as to their role in the natural control of *I. ricinus* in Norway.

Keywords: Biological control; Diversity index; Ecology; Predatory mites

## **2.1 Introduction**

Sheep industry is an important source of income to Norway (VATN, 2009; AUSTRHEIM et al., 2008), where sheep population reaches about 2.1 million animals raised on over 16,000 farms (STATISTCS NORWAY, 2015) commonly close to mountainous areas or along the coast (JOANNESEN et al., 2013). These animals account for about 19% of the livestock of the country (EUROSTAT, 2012). Sheep diseases affect the sustainability of sheep farming in the country (STEIGEDAL, 2012; MEJLON, 2000). One of the main diseases in the country, tick-borne-fever (TBF), is caused by the bacterium *Anaplasma phagocytophilum* and can cause reduced growth or death of infected animals (GRØVA et al., 2011; STUEN et al., 2002; STUEN, 2012). The bacterium is transmitted by the sheep tick, *Ixodes ricinus* (L.) (Acari: Ixodidae), which is also a vector of several other pathogens infective to humans and animals.

Ticks have been controlled on and off the host by the use of different techniques, especially chemical acaricides (GEORGE et al., 2008). However, the use of chemical control

has been discouraged in different parts of the world, especially in Europe, for health and environmental reasons (KUNZ; KEMP, 1994; THULLNER et al., 2007; GEORGE et al., 2008). This has led to the search for alternative and sustainable control measures (SAMISH et al., 2008).

After each blood meal, in the beginning of each post-embryonic stage, *I. ricinus* drops from the host to the litter layer for moulting or ovipositing (RANDOLPH, 2009). Considering that over 90% of its life cycle is spent off the host (NEEDHAM, TEEL, 1991), efforts to discover effective methods to control it in the litter seem warranted. Biological control is one of the evaluated possibilities. Many authors have suggested that different biotic and abiotic factors affect the activity and distribution of *I. ricinus* (QVILLER et al., 2014 and references therein) but few have studied the prevalence and effect of *I. ricinus* natural enemies on questing tick density patterns. Fungal pathogens, predatory mites and ants are all thought to be important tick killers in nature (CHANDLER et al., 2000; SAMISH; REHACEK, 1999).

Predatory mites are prevalent in the soil and litter, especially those of the cohort Gamasina of the order Mesostigmata (LINDQUIST et al., 2009). These have been reported as important predators of nematodes, springtails, mites and insect larvae (CALVO et al., 2011; CASTILHO; VENANCIO; NARITA, 2015; KOEHLER, 1999; RUF; BECK, 2005;). Most predatory mites commercialized around the world for the control of edaphic organisms are gamasines of the family Laelapidae. Four species of this family have been used commercially for the control of soil pests such as fungus gnats (Sciaridae), thrips (Thysanoptera) and mites of the cohort Astigmatina of the order Sarcoptiformes (CASTILHO et al., 2009; GERSON et al., 2003; MOREIRA; MORAES, 2015). To our knowledge, predatory mites have not being used for tick control. Only a few laboratory studies have evaluated the possible predation on ticks by mites of the suborder Prostigmata of the order Trombidiformes and of the cohort Astigmatina, as summarized by Samish and Rehacek (1999) and Samish and Alekseev (2001). Apparently, nothing has been published about the possible relation between ticks and Gamasina. However, this does not necessarily mean that these two groups do not interact. Studies about the effect of soil Gamasina on other organisms have referred mainly to arthropods of agricultural importance and, only recently, on important mesostigmatid parasites of laying hens (LESNA et al., 2009, 2012).

One of the first recommended steps when evaluating the effect of naturally occurring enemies, or when developing a biological control strategy of a pest organism, is the determination of the fauna in the area where control of the pest is envisioned. The main objective of the present study was to determine the groups of gamasines co-occurring with *I*.

*ricinus* in sheep pastures of the mid-western coast of Norway and to relate their densities with the density of *I. ricinus*, in order to identify possible natural enemies and support further biological control efforts.

## 2.2 Material and Methods

#### 2.2.1 Study area and sampling procedure

Litter samples were collected from two geographically close localities in Møre and Romsdal county, namely Isfjorden ( $62^{\circ}34'37''N$ ,  $7^{\circ}42'5''E$ ) and Tingvoll ( $62^{\circ}54'49''N$ ,  $8^{\circ}12'17''E$ ). The collecting sites were located in a mountainous region, where the local climate is characterized by relatively cool summers and mild winters. A total of six transects were established in each locality, three at low elevations [100 m above sea level (m.a.s.l.)] and three at high elevations (400 m.a.s.l.). Average distance between the areas of low and high elevations within each locality was 1.2 - 1.5 km, whereas distance between the study areas of Isfjorden (low and high elevations considered together) and Tingvoll was about 50 km.

Historically, monthly mean temperatures range between -1.3 and 13.5 °C in Isfjorden and between -0.5 and 13.8 °C in Tingvoll, whereas annual mean precipitation levels are 1211 mm in Isfjorden and 1160 mm in Tingvoll (EKLIMA, 2015). In the present study, temperature was determined by locally installed weather loggers, and rainfall was determined by the closest weather station. A total of four loggers were used, one for each locality and elevation. The loggers were placed approximately one meter above ground to avoid extreme local conditions and variation at ground level. Further details about the collecting sites are given in Qviller et al. (2014), a sister study conducted to evaluate the population dynamics of *I. ricinus*.

*Ixodes ricinus* and predatory mites were sampled at 14-day intervals from May to November 2011 and May to October 2012. At each sampling date, a litter sample (approximately one L) was collected to evaluate the presence of predatory mites from each of 12 sites spaced at 20 - 50 m along each transect. Samples were placed in plastic bags to prevent dehydration and kept cool until processed in the laboratory.

#### 2.2.2 Sample processing and mite identification

Mites were extracted from the samples with the use of Berlese funnels (HUTCHINS, 1994). Each sample was placed on a metal sieve (3 x 3 mm mesh) in a funnel provisioned with a 40 W incandescent light bulb positioned 10 cm above the surface of the sample. The droping mites were collected from each funnel in a container containing 20 mL of 80% ethanol. Extraction lasted 7 days. Extracted mites were mounted in Hoyer's medium for later identification under phase and interference contrast microscopes using the world literature about the groups collected, available at Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Brazil.

#### 2.2.3 Statistical Analysis

A quasi-Poisson model was fitted to the data with a log link function (McCULLAGH; NELDER, 1989; DEMÉTRIO; HINDE; MORAL, 2014) evaluating the effects of temperature, rainfall and the three-way interaction between gamasina counts, city and elevation on the tick population dynamics. F-tests were used to assess significance of the effects, given that the dispersion parameter was estimated via a quasi-likelihood approach. The proportions of the most abundant gamasine families from both localities were compared by chi-square tests.

The ecological indexes of species richness and of Shannon for species diversity were calculated for sites within elevation at each locality, and Morisita-Horn similarity index was used to relate the gamasine fauna at the different elevations of both localities. Statistical analyses were done using the R software (R CORE TEAM, 2014).

#### 2.3 Results

## 2.3.1 Gamasina diversity and prevalence

A total of 2,900 gamasines assigned to 34 species of 12 families were collected (Table 2.1). Considering both localities together, the most numerous families were Parasitidae, Veigaiidae and Zerconidae, which together accounted for 83.2% of the Gamasina. The frequency of each of the other families was at most 6.0%, with the laelapids accounting for 4.4% (respectively 6.9% and 2.4% of the gamasines from Isfjorden and Tingvoll).

| Local           | Isfjorden |            | Tir                   | ngvoll | Total  |            |  |  |
|-----------------|-----------|------------|-----------------------|--------|--------|------------|--|--|
| Family          | Number    | Proportion | portion Number Propor |        | Number | Proportion |  |  |
| Ascidae         | 3         | 0.2        | 0                     | 0.0    | 3      | 0.1        |  |  |
| Blattisociidae  | 6         | 0.5        | 0                     | 0.0    | 6      | 0.2        |  |  |
| Epicriidae      | 1         | 0.1        | 10                    | 0.6    | 11     | 0.4        |  |  |
| Eviphididae     | 45        | 3.6        | 43                    | 2.6    | 88     | 3.0        |  |  |
| Laelapidae      | 87        | 6.9        | 40                    | 2.4    | 127    | 4.4        |  |  |
| Macrochelidae   | 80        | 6.3        | 93                    | 5.7    | 173    | 6.0        |  |  |
| Ologamasidae    | 17        | 1.3        | 40                    | 2.4    | 57     | 2.0        |  |  |
| Pachylaelapidae | 1         | 0.1        | 7                     | 0.4    | 8      | 0.3        |  |  |
| Parasitidae     | 588       | 46.7       | 773                   | 47.1   | 1361   | 46.9       |  |  |
| Phytoseiidae    | 10        | 0.8        | 5                     | 0.3    | 15     | 0.5        |  |  |
| Veigaiidae      | 325       | 25.8       | 422                   | 25.7   | 747    | 25.8       |  |  |
| Zerconidae      | 97        | 7.7        | 207                   | 12.6   | 304    | 10.5       |  |  |
| Total           | 1260      | 100        | 1640                  | 100    | 2900   | 100        |  |  |

Table 2.1 – Total numbers and proportions (%) of Gamasina families collected in Isfjorden and Tingvoll in 2011 (ca. 504 L of litter) and 2012 (ca. 432 L of litter)

Pooling the data from both altitudes (Table 2.1), the number of specimens collected in Tingvoll was significantly higher than in Isfjorden (p<0.001), corresponding to about 56.4% of all mites collected. Epicriidae, Ologamasidae, Parasitidae, Veigaiidae and Zerconidae were found in significantly larger proportions in Tingvoll than in Isfjorden (p<0.001). The first two families were collected in Tingvoll in relatively low numbers ( $\leq$ 40), while the others were found in relatively high numbers ( $\geq$  207). Laelapidae were found in significant larger proportions in Tingvoll (p<0.001). Ascidae and Blattisociidae were also found in larger proportions in Isfjorden than in Tingvoll; however, it was not possible to compute the chi-square test due to the low expected values.

The most diverse families were Laelapidae, Macrochelidae, Parasitidae and Zerconidae, each with five species of 2 - 4 genera (Table 2.2). Other families were

represented by at most two species of at most two genera, except Veigaiidae, represented by four species of a single genus.

| Region - site<br>Species        | Isfjo | rden - | Isfjo | Isfjorden - |     | voll - | Ting | gvoll - | (continued |     |
|---------------------------------|-------|--------|-------|-------------|-----|--------|------|---------|------------|-----|
|                                 | low   |        | high  |             | low |        | high |         | Total      |     |
|                                 | N     | %      | N     | %           | N   | %      | N    | %       | N          | %   |
| Ascidae                         | 2     | 0.3    | 1     | 0.1         | 0   | 0      | 0    | 0       | 3          | 0.1 |
| Arctoseius<br>magnanalis        | 2     | 0.3    | 0     | 0.0         | 0   | 0      | 0    | 0       | 2          | 0   |
| <i>Neojordensia</i> sp.<br>nov. | 0     | 0.0    | 1     | 0.1         | 0   | 0      | 0    | 0       | 1          | 0   |
| Immatures                       | 0     | 0      | 0     | 0           | 0   | 0      | 0    | 0       | 0          | 0   |
| Blattisociidae                  | 4     | 0.5    | 2     | 0.4         | 0   | 0      | 0    | 0       | 6          | 0,2 |
| Cheiroseiusserratus             | 1     | 0.1    | 1     | 0,2         | 0   | 0      | 0    | 0       | 2          | 0   |
| Lasioseius<br>muricatus         | 1     | 0.1    | 0     | 0           | 0   | 0      | 0    | 0       | 1          | 0   |
| Platyseius italicus             | 0     | 0      | 1     | 0.2         | 0   | 0      | 0    | 0       | 1          | 0   |
| Immatures                       | 2     | -      | 0     | -           | 0   | -      | 0    | -       | 2          | -   |
| Epicriidae                      | 1     | 0,1    | 0     | 0           | 10  | 0,8    | 0    | 0       | 11         | 0,4 |
| <i>Epicrius</i> sp.             | 1     | 0,2    | 0     | 0           | 9   | 0,9    | 0    | 0       | 10         | 0,3 |
| Immatures                       | 0     | -      | 0     | -           | 1   | -      | 0    | -       | 1          | 0   |
| Eviphididae                     | 21    | 2,9    | 24    | 4,5         | 38  | 2,9    | 5    | 1,5     | 88         | 3   |
| Eviphis sp.                     | 20    | 3,7    | 24    | 5,8         | 33  | 3,1    | 2    | 0,8     | 79         | 2,7 |
| Immatures                       | 1     | -      | 0     | -           | 5   | -      | 3    | -       | 9          | -   |

Table 2.2 – Total numbers (N) and proportions (%) of Gamasina mites collected in Isfjorden and Tingvoll in 2011 (ca. 504 L of litter) and 2012 (ca. 432 L of litter)

|                                 | Isfjorden - |      | Isfjo | rden - | Ting | voll - | Ting | gvoll - | (continued |       |  |
|---------------------------------|-------------|------|-------|--------|------|--------|------|---------|------------|-------|--|
| Region - site                   | 1           | low  |       | high   |      | low    |      | high    |            | Total |  |
| Species                         | N           | %    | N     | %      | N    | %      | N    | %       | N          | %     |  |
| Laelapidae                      | 83          | 11,5 | 4     | 0,7    | 23   | 1,8    | 17   | 5       | 127        | 4,4   |  |
| Alloparasitus sp.               | 1           | 0,2  | 0     | 0      | 0    | 0      | 0    | 0       | 1          | 0     |  |
| <i>Cosmolaelaps</i> sp.<br>nov. | 5           | 0,9  | 0     | 0      | 0    | 0      | 0    | 0       | 5          | 0,2   |  |
| Cosmolaelaps<br>vacuus          | 13          | 2,4  | 0     | 0      | 1    | 0,1    | 2    | 0,8     | 16         | 0,6   |  |
| Gaeolaelaps<br>aculeifer        | 1           | 0,2  | 0     | 0      | 0    | 0      | 0    | 0       | 1          | 0     |  |
| Ololaelaps veneta               | 61          | 11,2 | 4     | 1      | 22   | 2,1    | 15   | 5,7     | 102        | 3,5   |  |
| Immatures                       | 2           | -    | 0     | -      | 0    | -      | 0    | -       | 2          | -     |  |
| Macrochelidae                   | 44          | 6,1  | 36    | 6,7    | 68   | 5,2    | 25   | 7,3     | 173        | 6     |  |
| Macrocheles sp1                 | 20          | 3,5  | 12    | 2,9    | 29   | 2,8    | 19   | 7,3     | 80         | 2,8   |  |
| Macrocheles sp2                 | 3           | 0,5  | 8     | 1,9    | 11   | 1      | 0    | 0       | 22         | 0,8   |  |
| Geholaspis sp1                  | 13          | 2,3  | 6     | 1,5    | 9    | 0,9    | 5    | 1,9     | 33         | 1,1   |  |
| Geholaspis sp2                  | 2           | 0,4  | 6     | 1,5    | 16   | 1,5    | 1    | 0,4     | 25         | 0,9   |  |
| <i>Glyptholaspis</i> sp         | 0           | 0    | 0     | 0      | 1    | 0,1    | 0    | 0       | 1          | 0     |  |
| Immatures                       | 6           | -    | 4     | -      | 2    | -      | 0    | -       | 12         | -     |  |
| Ologamasidae                    | 9           | 1,2  | 8     | 1,5    | 21   | 1,6    | 19   | 5,5     | 57         | 2     |  |
| <i>Gamasellus</i> sp.<br>nov.   | 9           | 1,6  | 8     | 1,9    | 21   | 2      | 19   | 7,3     | 57         | 2     |  |

Table 2.2 – Total numbers (N) and proportions (%) of Gamasina mites collected in Isfjorden and Tingvoll in 2011 (ca. 504 L of litter) and 2012 (ca. 432 L of litter)

|                          | Isfjo | Isfjorden - |     | Isfjorden -<br>high |     | voll - | Ting | Tingvoll - |      | (continued |  |
|--------------------------|-------|-------------|-----|---------------------|-----|--------|------|------------|------|------------|--|
| Region - site<br>Species | low   |             | h   |                     |     | low    |      | high       |      | Total      |  |
|                          | N     | %           | N   | %                   | N   | %      | N    | %          | N    | %          |  |
| Immatures                | 0     | -           | 0   | -                   | 0   | -      | 0    | -          | 0    | -          |  |
| Pachylaelapidae          | 0     | 0           | 1   | 0,2                 | 5   | 0,4    | 2    | 0,6        | 8    | 0,3        |  |
| Pachylaelaps sp          | 0     | 0           | 1   | 0,2                 | 3   | 0,3    | 1    | 0,4        | 5    | 0,2        |  |
| Immatures                | 0     | -           | 0   | -                   | 2   | -      | 1    | -          | 3    | -          |  |
| Parasitidae              | 279   | 38,6        | 309 | 57,4                | 616 | 47,5   | 157  | 45,8       | 1361 | 46,9       |  |
| Amblygamasus sp          | 35    | 6,4         | 49  | 11,9                | 50  | 4,7    | 4    | 1,5        | 138  | 4,8        |  |
| Holoparasitus sp         | 19    | 3,5         | 30  | 7,3                 | 68  | 6,5    | 5    | 1,9        | 122  | 4,2        |  |
| Paragamasus              | 110   | 20.7        | 105 | 22.7                | 077 |        | 70   | 07.0       | 500  | 20         |  |
| schweizeri               | 113   | 20,7        | 135 | 32,7                | 277 | 26,3   | 73   | 27,9       | 598  | 20,6       |  |
| Paragamasus aff.         | 1.5   |             |     |                     | 60  |        | _    |            |      |            |  |
| robustus                 | 15    | 2,7         | 15  | 3,6                 | 60  | 5,7    | 7    | 2,7        | 97   | 3,3        |  |
| Vulgarogamasus           |       |             |     | <b>.</b> .          |     |        | _    |            |      |            |  |
| sp1                      | 14    | 2,6         | 14  | 3,4                 | 22  | 2,1    | 3    | 1,1        | 53   | 1,8        |  |
| Immatures                | 83    | -           | 66  | -                   | 139 | -      | 65   | -          | 353  | -          |  |
| Phytoseiidae             | 6     | 0,8         | 4   | 0,7                 | 0   | 0      | 5    | 1,5        | 15   | 0,5        |  |
| Amblyseius obtusus       | 6     | 1,1         | 4   | 1                   | 0   | 0      | 5    | 1,9        | 15   | 0,5        |  |
| Immatures                | 0     | -           | 0   | -                   | 0   | -      | 0    | -          | 0    | -          |  |
| Veigaiidae               | 209   | 28,9        | 116 | 21,6                | 333 | 25,7   | 89   | 25,9       | 747  | 25,8       |  |
| Veigaia nemorensis       | 103   | 18,9        | 40  | 9,7                 | 175 | 16,6   | 33   | 12,6       | 351  | 12,1       |  |
| Veigaia transisalae      | 9     | 1,6         | 20  | 4,8                 | 33  | 3,1    | 28   | 10,7       | 90   | 3,1        |  |

Table 2.2 – Total numbers (N) and proportions (%) of Gamasina mites collected in Isfjorden and Tingvoll in 2011 (ca. 504 L of litter) and 2012 (ca. 432 L of litter)

|                        | Isfjo | rden - | Isfjo | rden - | Ting | Tingvoll - |     | Tingvoll - |      | (conclusion) |  |
|------------------------|-------|--------|-------|--------|------|------------|-----|------------|------|--------------|--|
| Region - site          | 10    | )W     | hi    | high   |      | low        |     | high       |      | otal         |  |
| Species                | N     | %      | N     | %      | N    | %          | N   | %          | N    | %            |  |
| Veigaia kochi          | 0     | 0      | 1     | 0,2    | 3    | 0,3        | 1   | 0,4        | 5    | 0,2          |  |
| Veigaia cervus         | 27    | 4,9    | 7     | 1,7    | 37   | 3,5        | 17  | 6,5        | 88   | 3            |  |
| Immatures              | 70    | -      | 48    | -      | 85   | -          | 10  | -          | 213  | -            |  |
| Zerconidae             | 64    | 8,9    | 33    | 6,1    | 183  | 14,1       | 24  | 7          | 304  | 10,5         |  |
| Zercon<br>zelawaiensis | 1     | 0,2    | 8     | 1,9    | 10   | 0,9        | 15  | 5,7        | 34   | 1,2          |  |
| Zercon suecicoides     | 38    | 7      | 14    | 3,4    | 121  | 11,5       | 5   | 1,9        | 178  | 6,1          |  |
| Zercon triangularis    | 11    | 2      | 0     | 0      | 17   | 1,6        | 0   | 0          | 28   | 1            |  |
| Parazercon<br>radiatus | 2     | 0,4    | 0     | 0      | 0    | 0          | 1   | 0,4        | 3    | 0,1          |  |
| Prozercon kochi        | 0     | 0      | 4     | 1      | 25   | 2,4        | 1   | 0,4        | 30   | 1            |  |
| Immatures              | 12    | -      | 7     | -      | 10   | -          | 2   | -          | 31   | -            |  |
| TOTAL                  | 722   | -      | 538   | -      | 1297 | -          | 343 | -          | 2900 | -            |  |

Table 2.2 – Total numbers (N) and proportions (%) of Gamasina mites collected in Isfjorden and Tingvoll in 2011 (ca. 504 L of litter) and 2012 (ca. 432 L of litter)

Notes. The proportions of species were calculated based only on the number of adults

The most numerous genera were *Paragamasus* (25.5%), *Veigaia* (19.2%) and *Zercon* (8.4%), each corresponding to one of the three most numerous families (Table 2). In all sites, *Paragamasus schweizeri* (Battacharyya) was the species found in highest proportions (20.7 – 32.7%), followed by *Veigaia nemorensis* (Koch) (16.6 – 18.9%), except in Isfjorden at 400 m.a.s.l., where the second most numerous species was the parasitid *Amblygamasus* sp. (11.9%), followed closely by *V. nemorensis* (9.7%).

The numbers of species collected as well as Shannon's diversity indexes were higher in Isfjorden than in Tingvoll (Table 2.3). In both localities these parameters were higher at low than at high elevation sites.

Table 2.3 – Species richness, and Shannon diversity index for mites of the cohort Gamasina collected in Isfjorden and Tingvoll at low and high elevation sites (respectively 100 and 400 m.a.s.l.)

| Region - Site    | Richness | Shannon |
|------------------|----------|---------|
| Isfjorden        | 33       | 2.61    |
| Isfjorden - Low  | 29       | 2.59    |
| Isfjorden - High | 23       | 2.41    |
| Tingvoll         | 25       | 2.55    |
| Tingvoll - Low   | 24       | 2.49    |
| Tingvoll - High  | 22       | 2.43    |

Integrating the information on the species found and their respective numbers, Morisita-Horn similarity index showed greater similarities between the low elevation sites of both localities to each other than to any of the high elevation sites and vice-versa (Figure 2.1). Additionally, higher similarity was observed between the low than between the high elevation sites.

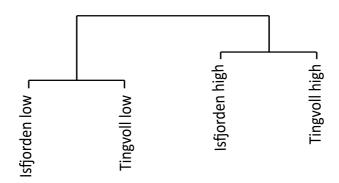


Figure 2.1 – Similarity in terms of presence/absence and proportion of species between four sites (low and high elevations) of Isfjorden and Tingvoll, grouped by the Morisita-Horn similarity index

#### 2.3.2 Climatic data and population dynamics

Considering the period in which the study was conducted, average temperature  $\pm$  standard error in Isfjorden in 2011 was 11.7  $\pm$  0.05 °C in the low elevation sites and 11.3  $\pm$  0.05 °C in the high elevation sites (Figure 2.2). In 2012, average temperature was 9.7  $\pm$  0.06 °C in the low elevation sites and 9.7  $\pm$  0.06 °C in the high elevation. Total rainfall during the experimental period was 584.8 and 355.5 mm respectively in 2011 and 2012.

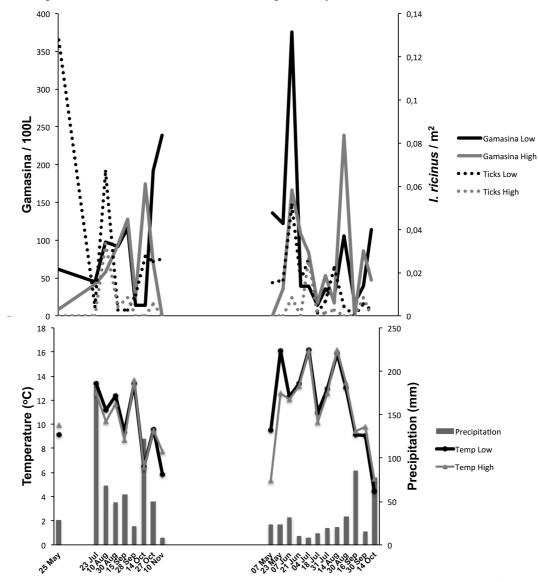


Figure 2.2 – Numbers of Gamasina per 100 L of litter and *Ixodes ricinus* per 20 m<sup>2</sup>of flagging for each transect at low and high sites of Isfjorden, as well as total precipitation and average temperature throughout the collection period in 2011 and 2012

Average temperature  $\pm$  standard error in Tingvoll in 2011 was  $11.3 \pm 0.05$  °C in the low elevation sites and  $10.6 \pm 0.05$  °C in the high elevation sites (Figure 2.3). In 2012,

average temperature was  $9.6 \pm 0.06$  °C in the low elevation sites and  $8.8 \pm 0.06$  °C in the high elevation sites. Total rainfall during the experimental period was 750.2 and 442.6 mm respectively in 2011 and 2012.

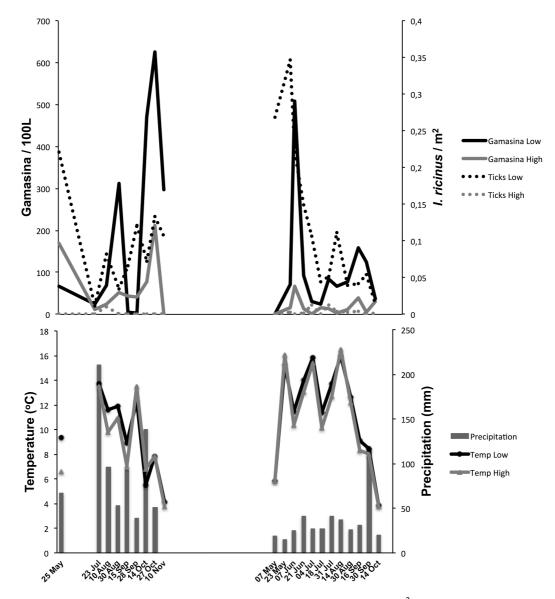


Figure 2.3 – Numbers of Gamasina per 100 L of litter and *Ixodes ricinus* per 20 m<sup>2</sup>of flagging for each transect at low and high sites of Tingvoll, as well as total precipitation and average temperature throughout the collection period in 2011 and 2012

The tick density was significantly related only to locality (p<0.001), elevation (p<0.001) and rainfall (p<0.001). Gamasina density (p=0.277), temperature (p=0.953) and interactions between factors (p $\ge$ 0.169) were not significantly related to tick density. The model that best explained tick density was Log (Ticks)= 1.430\*Locality + 2.911\*Elevation –

0.009\*Rainfall + 0.348, with locality being 1 for Tingvoll and 0 for Isfjorden; elevation, 1 for low and 0 for high; and rainfall in mm.

### 2.4 Discussion

The high proportion of Parasitidae in Isfjorden and Tingvoll was expected, given the dominance of mites of this family, along with the Veigaiidae, within the Gamasina in humus and litter in temperate regions (MICHERDZINSKI, 1969; KARG, 1993; LINDQUIST et al., 2009). The predominance of parasitids was also reported in a previous study of soil Gamasina in other localities of western Norway (SLOMIAN et al., 2005). Three of the four parasitid genera found in this work (*Amblygamasus, Holoparasitus* and *Paragamasus*) belong to the subfamily Pergamasinae, which has been mentioned to be favored by stable habitats, particularly forest and grassland soils (JUVARA-BALS, 1972; HYATT, 1980). Our survey was conducted in stable grassland habitats as well and corresponds well with these reports.

The other two most abundant families in the present study, Veigaiidae (25.7-25.8%) and Zerconidae (7.7-12.6%), were found in considerably lower proportions in the study of Slomian et al. (2005), in which the proportion of Veigaiidae was 3.2-10.0% and the proportion of Zerconidae was 0.0-1.3 %. In that study, Macrochelidae (4.5-6.7 %) and Pachylaelapidae (0.0-17.3 %) were found at a higher proportion than Veigaiidae and Zerconidae. The work by Slomian et al. (2005) was conducted in wooded meadows and wooded pastures in Sogn og Fjordane, located 150-200 km south of the two localities where the present study was conducted. No information was given about the elevation of the sites where that study was conducted. The reason for the difference between the findings of the present and that study is not clear.

Parasitidae and Macrochelidae are often found in temporary habitats, where they have been mentioned to prey on nematodes and insect larvae (AZEVEDO et al., 2015; CASTILHO; VENANCIO; NARITA, 2015). It has been observed that usually a new ephemeral microhabitat, as for example mammal's dung, is first inhabited by parasitids and subsequently by macrochelids (AXTELL, 1963, 1969). The high abundance and/ or diversity of those families in this study is probably due to the fact that it was conducted in rangelands, where sheep and a native ungulate (the red deer, *Cervus elaphus* L.) are frequent, making available the type of microhabitat favored by those mites.

Although not outstanding in terms of abundance or diversity in relation to several other families, the occurrence of laelapid species suggests the suitability of the region where

the study was conducted to mites of this family, especially in the low elevation sites in Isfjorden. One of the laelapid species found at these sites, *Gaeolaelaps aculeifer* (Canestrini), has been commercialized for the control of fungus gnats, thrips and mites (MOREIRA; MORAES, 2015). Species of the laelapid genus *Cosmolaelaps*, represented in this study by a described and a new species, have been less extensively studied, but in a recent work conducted in Brazil, Moreira et al. (2015) reported *Cosmolaelaps jaboticabalensis* Moreira, Klompen & Moraes to be a promising biocontrol agent of the edaphic phases of the thrips *Frankliniella occidentalis* (Pergande).

Differences in the prevailing environmental conditions between the four regions (combinations of locality and elevation) resulted in more outstanding differences between Gamasina abundances than diversities. It was not possible to determine the reason for the considerably higher Gamasina abundance at low altitude in Tingvoll and higher levels of diversity at low altitude in Isfjorden. In agroecosystems, higher total abundance of members of a mite community (usually a phytophagous species) is expected to occur when diversity is reduced as a consequence of a disturbing environmental factor (KARG, 1967; PERDUE; CROSSLEY, 1989; MARIBE et al., 2011). However, the proportions of the major families in this study were not markedly different between the low elevation sites in Isfjorden and Tingvoll. The major difference observed under those conditions refers to the laelapids, found in considerably higher proportions in Isfjorden than in Tingvoll.

The phoretic behavior of some mite groups might facilitate their dispersal among the regions in which the study was conducted. Among the mite groups found, phoresy has been reported for some Eviphididae, Macrochelidae, Pachylaelapidae and Parasitidae (LINDQUIST et al., 2009). However, the eviphidid genus found in this study (*Eviphis*), one of the three macrochelid genera (*Geholaspis*) and three of the four parasitid genera (*Amblygamasus, Holoparasitus* and *Paragamasus*) are typically non-phoretic (LINDQUIST et al., 2009; MAŠÁN; HALLIDAY, 2010; AZEVEDO et al., 2015; CASTILHO; VENANCIO; NARITA, 2015). Despite that, the highest Morisita-Horn's similarity indexes between low elevation sites of both localities on one hand and the high elevation sites of both localities on the other hand suggests that altitude plays a more important role than the geographic closeness of the sites, given that the low and high sites of each locality were much closer than sites of the same altitude between localities. Similarly, the higher similarity between low than high elevation sites reflects the apparently closer environmental characteristics between the low than between the high sites of those localities.

The results of the statistical analysis did not suggest a relation between the densities of *I. ricinus* and the associated soil Gamasina. Thus, the role of the latter in controlling the former could not be determined in the present study. The model shows that ticks are more abundant in Tingvoll than in Isfjorden and, corroborating the findings of Qviller et al. (2014), the model obtained in the present work also express the higher tick abundance in the low than the high elevations. Additionally, the negative relation of rainfall with *I. ricinus* density suggests a detrimental direct or indirect impact of rain on this tick species.

## 2.4.1 Final remarks

Although the effect of *I. ricinus* on sheep raised in Norway is of concern at the moment, important predatory relation between the tick *I. ricinus* and Gamasina in Norway has probably evolved over a long time, given the conceivable long relation between *I. ricinus* and its native hosts such as the red deer. Thus, the conduction of this study allowed the determination of the Gamasina groups naturally occurring in areas of western Norway where *I. ricinus* has probably been present for a very long time.

In estimating the natural effect of Gamasina as predators of *I. ricinus* in that region, it seems that a next logical step should involve an evaluation of the relation between the tick and each of the most common Gamasina collected, under controlled laboratory conditions. Among the groups of Gamasina found in this study, it seems worthy to dedicate special emphasis to the laelapid mites, given their demonstrated potential and actual use for the control of soil insects and mites as well as of hematophagous mites (FREIRE et al., 2007; CASTILHO et al., 2009; LESNA et al., 2009, 2012; MOREIRA et al., 2015). Our unpublished pilot laboratory studies have indicate that the laelapid *Stratiolaelaps scimitus* (Womersley) may prey on larvae of the ticks *Amblyomma sculptum* Berlese and *Rhipicephalus (Boophilus) microplus* (Canestrini), as well as on all stages of the red-poultry-mite, *Dermanyssus gallinae* (De Geer), which similarly to ticks are also hematophagous, although of a different group, the Mesostigmata.

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# **3** DESCRIPTION OF A NEW SPECIES OF *Cosmolaelaps* (ACARI: MESOSTIGMATA: LAELAPIDAE) FROM PASTURE LITTER IN WESTERN NORWAY

#### Abstract

A new species of *Cosmolaelaps* is described based on adult females collected from pasture litter from western Norway. This is the second species of *Cosmolaelaps* described from that country. This new species is most similar to *Cosmolaelaps dendrophilus* Davydova, *Cosmolaelaps liae* Bai & Gu and *Cosmolaelaps paravacua* Nasr & Nawar, described respectively from Russia, China and Egypt.

Keywords Acari; Cosmolaelaps; Taxonomy

### **3.1 Introduction**

More than 11.500 species are assigned to the order Mesostigmata (BEAULIEU et al., 2011; ZHANG, 2013). According to the checklist of the mesostigmatids from Norway, 220 species have been reported from that country (GWIAZDOWICZ; GULVIK, 2005), 56 of which in soil samples from western Norway (SLOMIAN et al., 2005). Gwiazdowicz; Gulvik (2005) reported 36 laelapid species from that country, of which only one belonged to *Cosmolaelaps* Berlese. In a recent appraisal, 108 species were worldwide assigned to that genus by MOREIRA et al. (2014), who also updated the diagnosis of the genus. In the scope of chapter two of this dissertation, a new species of this genus was found in low population levels in litter of a sheep pasture in western Norway. The objective of this study is to describe this new species based on adult females, the only stage collected.

# **3.2 Material and Methods**

Measurements were made with a graded eyepiece, and illustrations were made with the software Adobe Illustrator <sup>TM</sup>, using photos of the specimens taken with a camera attached to an interference contrast microscope (Nikon; Eclipse, 80i). Measurements are given in micrometers and represent the average measurements for the specimens observed, followed (in parentheses, for variable setae) by the corresponding ranges.

## **3.3 Results**

## Cosmolaelaps n. sp.

**Material examined:** holotype female and two paratype females collected on June 2012 by NIBIO Team in litter of sheep pasture at Isfjorden, More og Romsdal county, western Norway (62°34′37″N, 7°42′5″E); holotype and paratypes deposited in the mite reference collection of Departamento de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, state of São Paulo, Brazil.

**Diagnosis:** female dorsal shield with 39 pairs of setae and two unpaired setae (slightly posterior to J2 and slightly posterior to px3), all tricarenate, except z1, setiform. Unsclerotized lateral cuticle with eight pairs of setae. Presternal region sclerotized and fused with sternal shield. Genital shield bearing *st5*. Ten pairs of opisthogastric setae on unsclerotized cuticle, all setiform, except Jv4 and Jv5, tricarinate. Epistome convex, denticulate. Fixed cheliceral digit with five teeth in addition to the apical tooth.

# Female

**Dorsal idiosoma (Figure 3.1):** dorsal shield 498 (475 – 525) long and 327 (307 – 350) wide (at level of *r*6), reticulate, with most reticules transversely elongate, with 39 pairs of setae, including two pairs of extra setae and two unpaired setae. Setal length: *j1* 13 (12–15), *j2* 31 (27–34), *j3* 41 (40–43), *j4* 44 (42–45), *j5* 44 (42–45), *j6* 44 (42–45), *J1* 44 (42–45), *J2* 41 (40–43), *J3* 41 (40–42), *J4* 35 (35–36), *J5* 32 (30–34), *z1* 12 (10–15), *z2* 39 (37–41), *z3* 41 (40–43), *z4* 45 (45–45), *z5* 44 (42–46), *z6* 44 (42–45), *Z1* 47, *Z2* 46 (45–47), *Z3* 46 (42–50), *Z4* 42 (40–45), *Z5* 24 (22–26), *s1* 26 (25–27), *s2* 36 (35–37), *s3* 41 (40–42), *s4* 45 (45–46), *s5* 42 (37–46), *s6* 47 (45–50), *S1* 42 (40–45), *S2* 42, *S3* 41 (38–45), *S4* 41 (39–42), *S5* 39 (37–40), *r2* 35 (37–42), *r3* 38 (37–40), *r4* 36 (32–40), *r5* 45 (40–50), *px2* 40 (posterolaterad of *J2*), *px3* 40 (37–43) (posterolad of *J3*), unpaired *Jx2* 37 (unpaired, slightly posterior to *J2*) and *Jx3* 41 (38–45) (unpaired, slightly posterior to *px3*). Dorsal shield setae tricarenate, except *z1*, setiform. Unsclerotised lateroventral cuticle with eight pairs of setae *r6* 20, *R1* 18 (17–20), *R2* 20, *R4* 17 (15–19), *R5* 21 (20–22), *R6* 20 (19–22), *UR1* 17 (15–20) and *UR2* 18 (17–20).

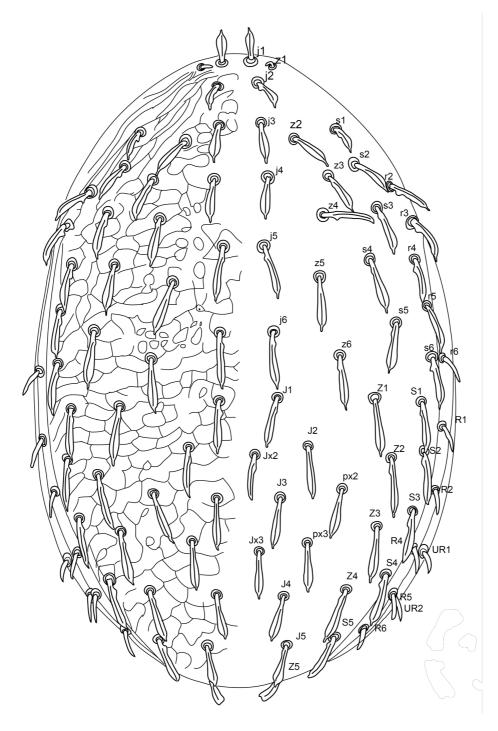


Figure 3.1 – Dorsum of female Cosmolaelaps n. sp. female

**Peritreme and peritrematic plate (Figure 3.2):** Peritreme extending anteriorly to level between s1 and z1. Peritrematic plate fused anteriorly to dorsal shield near z1, with a pair of lyrifissures and a pore behind the stigma; not fused with exopodal plate.



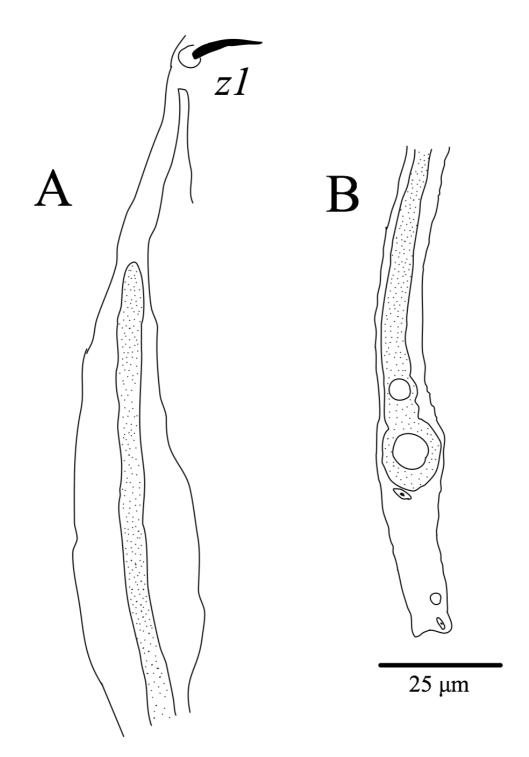


Figure 3.2 – Peritreme and peritrematic shield of *Cosmolaelaps* n. sp. female. A: Peritreme extending anteriorly to level between s1 and z1. Peritrematic plate fused anteriorly to dorsal shield near z1; B: detail of the pair of lyrifissures and a pore behind the stigma

**Ventral idiosoma (Figure 3.3):** presternal region sclerotized and fused with sternal shield, which is lightly sclerotized, longer than wider, with three pairs of setae, st1 37 (35–40), st2 29 (27–31), st3 31 (27–35), and two pairs of lyrifissures (iv1, iv2); posterior margin of sternal shield about straight; distance between st1 and the posterior margin of sternal shield

113 (112 – 115), st1–st1 66 (65 – 67), st2–st2 82, st3–st3 103 (102 – 105). Seta st4 31 (30– 32) on unsclerotized, longitudinal striated cuticle, posterolaterad to iv3, also on cuticle. Genital shield not fused with anal shield, flask-shaped, with three V-shaped, subparallel striae; 225 (225 – 225) long, 102 (100 – 105) wide at widest level, with one pair of setae st530; distance st5–st5 88 (85 – 90). Genital lyrifissure (iv5) on unsclerotized cuticle, posterolaterad of st5. Anterior section of endopodal shield distinctly more sclerotized than sternal shield, fused with it; section behind sternal shield sclerotized, extending untill the level of st5. Exopodal shield indistinct. Anal shield subtriangular, wider section anterior, reticulate, 74 (70 – 77) long and 77 (75 – 80) wide at widest level, with a pair of marginal pores posterolaterad of para-anal setae. With ten pair of setiform opisthogastric setae on unsclerotized cuticle: Jv1 27 (26–28), Jv2 25 (25–26), Jv3 24 (23–25), Jv4 27 (25–30), Jv5 23 (22–25), Zv1 28 (27–30), Zv2 26 (25–27), Zv3 18 (17–20), Zv4 20, Zv5 21 (20–23), all setiform, except Jv4 and Jv5, tricarinate.

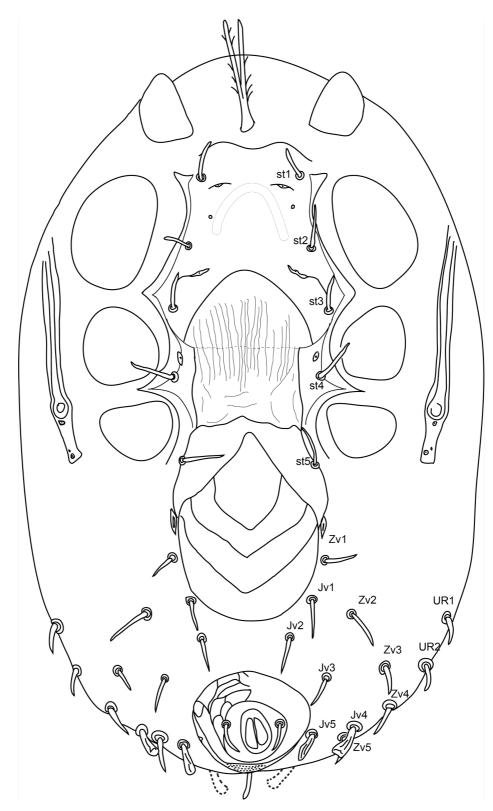


Figure 3.3 - Venter of female Cosmolaelaps n. sp. female

**Gnathosoma (Figure 3.4):** epistome convex, denticulate. Deutosternum with a smooth, transverse distal line followed by six transverse denticulate lines; number of teeth decreasing from distal (20) to proximal row (10); with a pair of smooth, transverse and curved

lines external to deutosternum, between second and third most proximal lines of denticles. Corniculi acuminate, parallel to each other,  $35 (32 - 37) \log$  and 7 (6 - 7) wide basally, reaching 2/3 of palp femur. Palp length 132 (112 - 160), apotele two-tined. Movable cheliceral digit 43 (42 - 45) long, with two teeth in addition to apical tooth; fixed digit 47 (45 - 49) long, with five teeth in addition to apical tooth and a setiform *pilus dentilis*. Cheliceral arthrodial process as a coronet-like fringe.

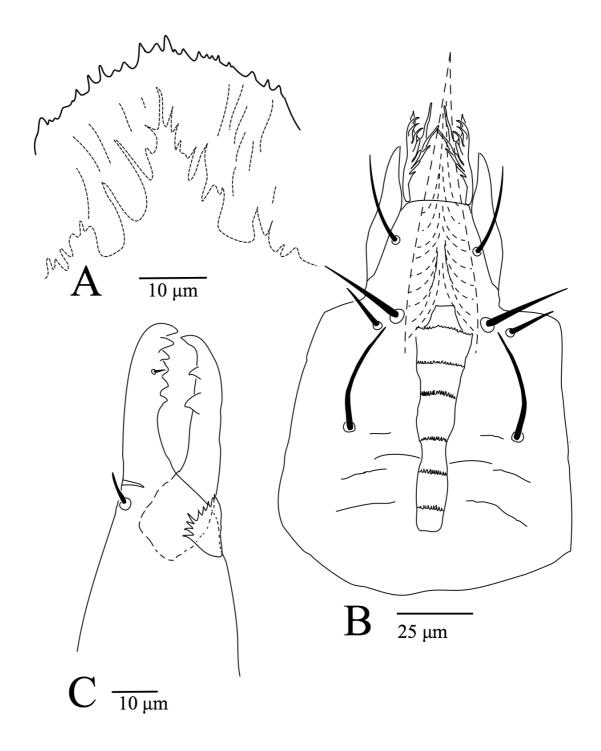


Figure 3.4 - Gnathosoma of Cosmolaelaps n. sp. female. A: epistome; B: hypostome; C: chelicerae

Legs: Chaetotaxy: I—coxa 0-0/2, 0/0-0, trochanter 1-0/2, 1/0-1, femur 1-2/1, 3/3-1, genu 2-3/2, 3/1-2, tibia 2-3/2, 3/1-2; II—coxa 0-0/1, 0/1-0, trochanter 1-0/1, 0/1-1, femur 2-3/1, 2/2-1, genu 2-3/1, 2/1-2, tibia 2-2/1, 2/1-2; III—coxa 0-0/1, 0/1-0, trochanter 1-1/1, 0/1-1, femur 1-2/1, 2/0-0, genu 2-2/1, 2/1-1, tibia 2-1/1, 2/1-1; IV- coxa 0- 0/1, 0/0-0, trochanter 2-1/1, 0/1-0, femur 1-2/1, 1/0-1, genu 1-2/1, 3/1-1, tibia 2-1/0, 2/2-2; tarsi II—IV: 17 setae each. Pre-tarsi with short claws, pulvillus with three distally rounded lobes. Setae uniform in shape; no macrosetae.

**Remarks.** Cosmolaelaps n. sp. is closely related to Cosmolaelaps dendrophilus Davydova, Cosmolaelaps liae Bai & Gu and Cosmolaelaps paravacua Nasr & Nawar, described respectively from Russia, China and Egypt. Cosmolaelaps dendrophilus differs by having setae of the dorsal shield spear-shaped; C. liae differs by having the anterior margin of the peritreme denticulate; C. paravacua differs by having setae of the dorsal shield scimitar shaped, tectum smooth, and more than five teeth on fixed cheliceral digit.

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# 4 PROSPECTION FOR EDAPHIC PREDATORY MITES TO CONTROL HEMATOPHAGOUS MITES IN BRAZIL (ACARI)

### Abstract

Some mites have evolved adaptations to an ectoparasitic mode of life, as for example the poultry red mite, Dermanyssus gallinae (De Geer), and the ticks Amblyomma sculptum Berlese and Rhipicephalus microplus (Canestrini). These cause injuries to animals of economic interest, stressing them by blood loss and transmitting infectious agents. Their control is usually made with chemicals, but this control technique has been discouraged in several countries for health and environmental reasons. This has led to the search for alternative control techniques, as biological control. The order Mesostigmata is mostly composed of mites that feed on other small arthropods, including mites. Some edaphic predatory mites are commercialized for the control of pests that spend their life cycle, or part of it, in the soil, including the poultry red mite. However, apparently there is no published information regarding the use of Mesostigmata for the biological control of ticks. The objective of this study was to evaluate the ability of gamasine mites from soil of different places in the states of Minas Gerais and São Paulo to consume eggs and larvae of A. sculptum and R. microplus, and different stages of D. gallinae. In total, 551 gamasines of 18 genera were evaluated under laboratory conditions. The most abundant families were Ologamasidae (25.4%) and Parasitidae (21.1%), while the most diverse (in terms of genera) were Ologamasidae and Laelapidae, with five and four genera, respectively. Of the evaluated predators, only Stratiolaelaps scimitus (Womersley) fed on larvae of both tick species and all stages of D. gallinae. The results of this work was barely sufficient to support the initial hypothesis, in which only three mesostigmatid species were observed as potential predators of the evaluated prey in the preliminary predation tests, with only one of the predators (S. scimitus) substantiated as promising in the subsequent detailed tests, on larvae of R. microplus and all developmental stages of D. gallinae, with the release of predators at high rates. However, this does not mean necessarily that the other evaluated species are not able to feed on ticks. It is possible that the predatory mites from the field used in the preliminary tests fed on other small arthropods (or on another food source) immediately before the tests, and for this reason they did not feed on ticks. The methodology used in this study must be updated in order to improve further predation tests to identify possible biological control agents of ticks.

Keywords: Amblyomma; Biological control; Stratiolaelaps scimitus; Ticks

### 4.1 Introduction

Over 55 thousand species have been affiliated to the subclass Acari (LINDQUIST, 1984; WALTER; PROCTOR, 1999; KRANTZ; WALTER, 2009). Several morphological and physiological traits of those organisms were naturally selected along time, allowing mites to inhabit different ecosystems, especially adaptations related to feeding habits. Mite species have been reported as saprophagous, mycophagous, phytophagous, predators or parasites. The term parasitic mite has been used to refer to species deriving their food from other animals,

without causing their immediate death but damaging them at some degree (ZUMPT, 1961). The parasitic mode of life evolved in several mite groups. Some mites of the order Mesostigmata (for classification see Krantz; Walter, 2009 and Beaulieu et al., 2011) have evolved adaptation to an ectoparasitic mode of life, with modified mouthparts and reduced body sclerotization, which allow them to pierce their hosts effectively and also to intake large amounts of food. One example is the poultry red mite, *Dermanyssus gallinae* (De Geer), whose chelicerae are elongate, allowing the mite to penetrate them deeper in the host skin than other mites. This species is found in nests of several birds and small mammals, being responsible for severe damage to laying hens around the world (MAURER et al., 1993; TUCCI et al., 1997; EMOUS et al., 2005). That species was reported in Brazil for the first time by Fonseca (1938) and Reis (1939), on canaries and hens in the State of São Paulo.

Ticks constitute another group of hematophagous mites that worldwide comprises almost 900 species (KEIRANS, 1992; GUGLIELMONE et al., 2010), 65 of which have been reported from Brazil (MARTINS et al., 2014). Amblyomma Koch is the most diverse tick genus and some of its member species are of major medical and veterinarian importance. Amblyomma sculptum Berlese is the main tick species infesting humans in southeastern Brazil (HORTA et al., 2004; NAVA et al. 2014), being the vector of the bacterium Rickettsia rickettsii (Wolbach), causal agent of the Rocky Mountain spotter fever (RMSF), which is called "febre maculosa" in Brazil. Amblyomma sculptum is a three-host-tick that undergoes only one generation a year under natural conditions (LABRUNA et al., 2003), with dominance of the larval stage between late April and June, nymphal stage between July and October, and adult stage between November and March, in southeastern Brazil. Studies conducted in the state of Minas Gerais by Oliveira et al. (2000) and Guedes; Leite (2008) showed the same pattern of seasonal distribution for a species identified as Amblyomma cajennense (Fabricius). Knowledge about the pattern of seasonal distribution is of extreme importance in tick control programs because of the difference in susceptibility of different tick stages to some acaricides (BARROS-BATTESTI; ARZUA; BECHARA, 2006).

In addition to *A. sculptum, Amblyomma aureolatum* (Pallas) was also reported as a vector of *R. rickettsii* in Brazil (LABRUNA, 2013). Among the species of this genus, *A. sculptum* is the predominant species at Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), along with *Amblyomma dubitatum* Neumann and *Amblyomma nodosum* Neumann found in much lower proportions (PEREZ, 2007).

The presence of *A. sculptum* and the incidence of "febre maculosa" has increased in the last decade at ESALQ and other places in the state of São Paulo, demanding efforts to

improve the control techniques. An experimental program has been implemented at ESALQ, using pesticides and entomopathogenic fungi, for the control of *A. sculptum* (PEREZ, 2013). To complement the measures adopted in that program, efforts have been dedicated in the search for effective predatory mites that could also be used for tick control.

In addition to humans, ticks also cause injuries to animals of economic interest, stressing them, consuming their blood and inoculating them with infectious agents (BARROS-BATTESTI; ARZUA; BECHARA, 2006). The cattle-tick, *Rhipicephalus microplus* (Canestrini), is of great economic importance in Brazil, vectoring the protozoa *Babesia bovis* Babes and *Babesia bigemina* (Smith & Kilborne), as well as the bacterium *Anaplasma marginale* Theiler, all of which cause a disease complex called "tristeza-parasitária-bovina".

Samish and Rehacek (1999) summarized the literature about pathogens and predators of ticks and their potential use as biological control agents. Within the Acari, members of the cohorts Anystina and Parasitengonina (both of the order Trombidiformes) and the cohort Astigmatina (order Sarcoptiformes) were reported to feed on ticks (HOLM; WALLACE, 1989; OLIVER et al., 1986; PETROVA, 1959). However, the existing information on arthropods as predators of ticks is based mainly on sporadic observations and their role in reducing tick populations is still not clear. Samish and Alekseev (2001) summarized the information about arthropods as predators of ticks. In their work, no reference was made to the Mesostigmata, a group mostly composed of predatory mites that feed on other small arthropods, including other mites.

In a survey conducted in Switzerland by Maurer et al. (1993), *Androlaelaps casalis* (Berlese) (Mesostigmata: Laelapidae) was reported in poultry houses. This species had been mentioned by McKinley (1963) to prey on *D. gallinae*. In poultry houses in The Netherlands, Lesna et al. (2009) reported 12 mesostgmatic species, including *A. casalis* and *Hypoaspis aculeifer* (Canestrini) (Laelapidae), reported by the authors to feed on *D. gallinae*. In Brazilian poultry houses, Silva et al. (2013) reported nine gamasine species in the state of Rio Grande do Sul, while Horn et al. (2015) reported seven gamasine species in the same state; either including *A. casalis* and *H. aculeifer*, and Lesna et al. (2012) reported the predatory mite *Stratiolaelaps scimitus* (Womersley) (Laelapidae) in the state of Minas Gerais. The latter species is known to feed on *D. gallinae* (TUOVINEN, 2008) and it is currently used to control *D. gallinae* and other blood feeding mites on pet animals in Europe (LESNA et al., 2012).

Details about the feeding behavior of predatory mites have been reported in the literature, especially for species of Phytoseiidae. Flechtmann; McMurtry (1992) reported how phytoseiids feed on spider mites, determining that they not only consume the tissues of the prey, but also its gut content. Thus, the studies reported in the previous paragraph suggest that gamasid mites could also prey tick larvae, which similarly to *D. gallinae* ingest vertebrate blood.

Some edaphic predatory mites have been commercialized for the control of pests that spend their life cycle (or part of it) in the soil (FREIRE et al., 2007; GERSON; WEINTRAUB, 2007; CASTILHO et al., 2009a, 2009b), including the poultry red mite (LESNA et al., 2012). Recently, Castilho, Venancio and Narita (2015) summarized the information about the Mesostigmata as biological control agents of harmful organisms. However, there is no information regarding their use as biological control agents of ticks. The hypothesis of the present work is that some Gamasina species can feed on tick eggs and larvae, and on all developmental stages of *D. gallinae*.

Usually, one of the first recommended steps in a biological control project of a pest organism involves the determination of the fauna in the area where the pest is envisioned to be controlled. In chapter two of this dissertation the mite fauna co-occurring with *Ixodes ricinus* (L.) in sheep pastures of western Norway was identified. The objectives of the present study were to identify the Gamasina from soil of different places in the states of Minas Gerais and São Paulo, evaluating their potential to feed on eggs and larvae of *A. sculptum* and *R. microplus*, and on different stages of *D. gallinae*, under laboratory condition.

## 4.2 Material and Methods

## 4.2.1 Origin of the mites used in the study

### 4.2.1.1 Predators

Samples of litter and soil were collected from a municipality in the state of Minas Gerais (Bom Repouso; 22° 28' 04" S 46° 09' 01" W) and seven municipalities in the state of São Paulo (Cananéia, 24° 59' 51" S 47° 56' 06" W; Iperó, 23° 25' 48" S 47° 35' 51" W; Jaboticabal, 21° 15' 19" S 48° 18' 35" W; Nova Granada, 20° 32' 09" S 49° 18' 39" W; Piracicaba, 23° 26' 00" S 47° 35' 51" W; Registro 24° 30' 00" S 47° 50' 41" W; Tapiraí, 24° 03' 30" S 47° 34' 24" W). Mites were extracted from the samples using Berlese funnels, during seven days. The droping mites were collected alive in a container whose bottom was

covered with a layer (about 1.0 cm high) of a dried paste of a mixture of plaster and charcoal (ABBATIELLO, 1965) kept moistened by daily addition of distilled water. In addition to the predatory mites collected in the field, *S. scimitus*, not found in the samples, was selected to be tested because of its commercial availability in Brazil. This species was supplied by the company Promip – Manejo Integrado de Pragas.

## 4.2.1.1 Prey

Three prey species were considered in this study, two species of ticks, *A. sculptum* and *R. microplus*, and the poultry red mite. *Amblyomma sculptum* were provided by the "Departamento de Medicina Veterinária Preventiva e Saúde Animal Faculdade de Medicina Veterinária e Zootecniada Universidade de São Paulo (FMVZ/USP)". *Rhipicephalus microplus* were provided by the "Laboratório de Controle Microbiano de Artrópodes de Importância Médico Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ)". The poultry red mites were collected at the company "Korin - Agricultura Orgânica, Ipeúna, São Paulo".

#### 4.2.2 Predation tests

These consisted of a series here designated as preliminary tests and a subsequent series designated as detailed tests. Both series were conducted in experimental units comprised each of a Petri dish (9 cm of diameter) whose bottom was filled with a layer (0.5 cm high) of the same mixture of plaster and charcoal mentioned in item 3.2.1 (Figure 4.1). The edge of the units was covered with a thin layer of entomological glue to prevent the mites from escaping in the process of transferring them to the experimental units.

The prey and the predators to be tested were then transferred to the units with the use of a thin brush, the number of specimens varying according to the series, as subsequently detailed (4.2.2.1 and 4.2.2.3). Approximately 10 mL of distilled water were then added to the base of each unit and the units were sealed with a plastic film, in order to maintain the air humidity inside next to 80%, as determined in preliminary evaluations.

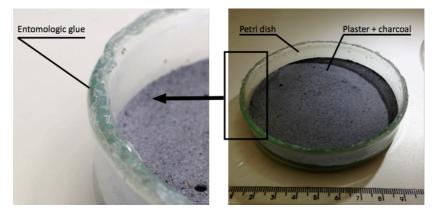


Figure 4.1 – Left: Detail of the thin layer of entomologic glue on top of the borders, used to prevent organisms to escape. Right: Experimental unit composed of a Petri dish with a mixture of plaster + charcoal in the bottom

#### 4.2.2.1 Preliminary predation tests

For the ticks, 50 eggs and 50 larvae of each tick species and a mixture of about 50 collected mesostigmatid mites of different species (or 50 *S. scimitus*) were subsequently released in each unit, starting the observations immediately afterward. Eighteen replicates were evaluated.

For the poultry red mite, a mixture of 100 specimens (about 25 specimens of each of the following developmental stages: eggs, larvae, nymphs and adults) and 50 *S. scimitus* were released in each unit, also starting the observations immediately afterward. Two replicates were evaluated.

After the addition of the predatory mites, the units were observed during 5 h under a stereomicroscope with indirect light. When a predator was observed feeding on any of the ticks, it was separated in order to start a laboratory colony for detailed predation tests (item 4.2.2.3).

## 4.2.2.2 Identification of the predatory mites

After the laboratory tests, mites were mounted in Hoyer's medium for identification under phase and interference contrast microscopes. Initially, identification was done at family level, using mainly Krantz and Walter (2009). Within each family, identification at the genus level was done mainly by using unpublished keys distributed at The Acarology Summer Programm, The Ohio State University, USA. Identification at the species level was done using the world literature about the groups collected, available at Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Brazil

## 4.2.2.3 Detailed predation test

For the ticks, 50 larvae of each tick species were transferred to an experimental unit, and 50 adults of either predator species observed feeding on any of the tick species in the preliminary tests were also released. One of these predators (*Cosmolaelaps barbatus* AUTR) was evaluated in three replicates (each corresponding to an experimental unit), using another unit as control. The second predator (*S. scimitus*) was evaluated in six replicates, using four other units as control. In the control replicates, no predatory mites were released.

For the red poultry mite, 100 specimens of either developmental stage (egg, larva, nymph and adult) were placed in each experimental unit, each stage in a different unit. In total, one unit was used for each immature stage and six units were used for the adult stage. Fifty adults of *S. scimitus* were released in each unit.

The experimental units were kept in a rearing chamber at 28°C, 80% RH and in the dark. After 48 h, the numbers of dead and live prey were counted.

## 4.3 Results

#### **4.3.1 Preliminary predation tests**

A total of 551 gamasines assigned to 18 genera of 11 families were collected and evaluated as possible biological control agents of ticks (Table 4.1). The most abundant families were Ologamasidae (25.4%), Parasitidae (21.1%), Laelapidae (15.8%) and Veigaiidae (11.6%). The majority of the gamasines were not observed to feed on ticks; they were seen touching the eggs and larvae with their first pair of legs and leaving. Only three species were observed feeding on those organisms. For two of them, this was observed very rarely; *C. barbatus*, collected in Nova Granada, was observed once feeding on a *A. sculptum* larva, whereas *Onchodellus* sp. (Pachylaelapidae), collected in Piracicaba, was observed also once feeding on larvae of both tick species (Figure 4.3). Although some predatory mites probed the eggs, none of them were observed to feed on these eggs. A colony of the *C. barbatus* was kept in the laboratory for the detailed tests, but it was not possible to keep a colony of the *Onchodellus* sp., in part because only a few specimens were collected.

Table 4.1 – Total numbers of Gamasina families and genera collected in soils of Bom Repouso (BR), Cananéia (CA), Iperó (IP), Jaboticabal (JA), Nova Granada (NG), Piracicaba (PI), Registro (RG) and Tapiraí (TP) in 2014 and 2015.

| Taxa                               | BR | CA | IP | JA | NG | PI | RG | TP | Total |
|------------------------------------|----|----|----|----|----|----|----|----|-------|
| Blattisociidae                     | 0  | 0  | 0  | 0  | 2  | 20 | 0  | 0  | 22    |
| Lasioseius                         | 0  | 0  | 0  | 0  | 2  | 20 | 0  | 0  | 22    |
| Laelapidae                         | 0  | 2  | 4  | 19 | 4  | 55 | 3  | 0  | 87    |
| Cosmolaelaps                       | 0  | 0  | 2  | 4  | 2  | 46 | 0  | 0  | 54    |
| Gaeolaelaps                        | 0  | 0  | 0  | 0  | 1  | 2  | 0  | 0  | 3     |
| Pseudoparasitus                    | 0  | 0  | 0  | 12 | 1  | 6  | 1  | 0  | 20    |
| Stratiolaelaps                     | 0  | 2  | 0  | 3  | 0  | 0  | 0  | 0  | 5     |
| Unidentified laelapids             | 0  | 0  | 2  | 0  | 0  | 1  | 2  | 0  | 5     |
| Macrochelidae                      | 3  | 0  | 0  | 6  | 0  | 28 | 0  | 0  | 37    |
| Glyptholaspis                      | 1  | 0  | 0  | 1  | 0  | 21 | 0  | 0  | 23    |
| <i>Macrocheles</i><br>Unidentified | 0  | 0  | 0  | 0  | 0  | 6  | 0  | 0  | 6     |
| macrochelids                       | 2  | 0  | 0  | 5  | 0  | 1  | 0  | 0  | 8     |
| Melicharidae                       | 0  | 0  | 1  | 6  | 11 | 5  | 0  | 0  | 23    |
| Proctolaelaps                      | 0  | 0  | 1  | 6  | 11 | 5  | 0  | 0  | 23    |
| Ologamasidae                       | 15 | 3  | 38 | 26 | 22 | 31 | 5  | 0  | 140   |
| Athiasella                         | 1  | 0  | 5  | 0  | 0  | 1  | 0  | 0  | 7     |
| Heydeniella                        | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1     |
| Gamasiphis                         | 7  | 0  | 1  | 21 | 22 | 22 | 1  | 0  | 74    |
| Neogamasellevans                   | 0  | 1  | 14 | 0  | 0  | 2  | 1  | 0  | 18    |
| <i>Ologamasus</i><br>Unidentified  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1     |
| ologamasids                        | 7  | 2  | 18 | 5  | 0  | 4  | 3  | 0  | 39    |
| Pachylaelapidae                    | 0  | 0  | 0  | 0  | 0  | 9  | 0  | 0  | 9     |
| Onchodellus                        | 0  | 0  | 0  | 0  | 0  | 9  | 0  | 0  | 9     |
| Parasitidae                        | 7  | 1  | 22 | 17 | 0  | 49 | 0  | 20 | 116   |
| Parasitus                          | 4  | 0  | 0  | 0  | 0  | 32 | 0  | 0  | 36    |
| Porrhostaspis                      | 0  | 0  | 0  | 0  | 0  | 13 | 0  | 0  | 13    |
| Vulgarogamasus                     | 1  | 0  | 10 | 9  | 0  | 2  | 0  | 5  | 27    |
| Unidentified parasitids            | 2  | 1  | 12 | 8  | 0  | 2  | 0  | 15 | 40    |
| Parholaspididae<br>Unidentified    | 0  | 0  | 0  | 0  | 0  | 7  | 3  | 0  | 10    |
| parholaspidids                     | 0  | 0  | 0  | 0  | 0  | 7  | 3  | 0  | 10    |
| Phytoseiidae                       | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1     |
| Amblyseius                         | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 1     |
| Podocinidae                        | 1  | 0  | 3  | 5  | 0  | 4  | 0  | 0  | 13    |
| Unidentified podocinids            | 1  | 0  | 3  | 5  | 0  | 4  | 0  | 0  | 13    |
| Veigaiidae                         | 36 | 10 | 7  | 3  | 0  | 0  | 8  | 0  | 64    |
| Gamasolaelaps                      | 1  | 0  | 6  | 2  | 0  | 0  | 6  | 0  | 15    |

(continued)

Table 4.1 – Total numbers of Gamasina families and genera collected in soils of Bom Repouso (BR), Cananéia (CA), Iperó (IP), Jaboticabal (JA), Nova Granada (NG), Piracicaba (PI), Registro (RG) and Tapiraí (TP) in 2014 and 2015

|    |                       |                      |                          |                                |                                      |   | (conclusion)  |  |
|----|-----------------------|----------------------|--------------------------|--------------------------------|--------------------------------------|---|---|--|
| BR | CA                    | IP                   | JA                       | NG                             | PI                                   | RG  | TP  | Total  |
| 25 | 5                     | 0                    | 1                        | 0                              | 0                                    | 0   | 0   | 31   |
| 10 | 5                     | 1                    | 0                        | 0                              | 0                                    | 2   | 0   | 18   |
| 11 | 0                     | 8                    | 3                        | 0                              | 7                                    | 0   | 0   | 29   |
| 73 | 16                    | 83                   | 85                       | 39                             | 216                                  | 19  | 20  | 551  |
|    | 25<br>10<br><b>11</b> | 25 5<br>10 5<br>11 0 | 25 5 0   10 5 1   11 0 8 | 25 5 0 1   10 5 1 0   11 0 8 3 | 25 5 0 1 0   10 5 1 0 0   11 0 8 3 0 | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ | BR   CA   IP   JA   NG   PI   RG   TP     25   5   0   1   0   0   0   0     10   5   1   0   0   0   2   0     11   0   8   3   0   7   0   0 |



Figure 4.2 - Stratiolaelaps scimitus feeding on a larva of Amblyomma sculptum

In 20 occasions *S. scimitus* was observed feeding on the different developmental stages of *D. gallinae*. The modification of the color of the predator indicated that it not only fed on tissues of the prey, but also on its gut content, becoming dark brown when the prey was engorged (Figure 4.4).

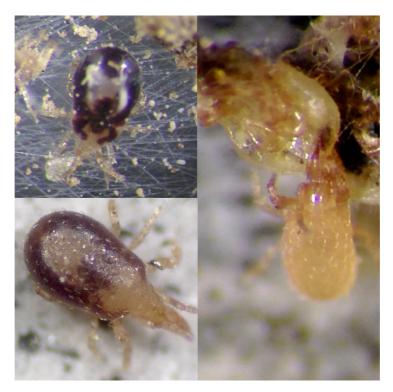


Figure 4.3 – Left up: ingurgitated *Dermanyssus gallinae*. Left down: *Stratiolaelaps scimitus* showing a dark brown color by feeding on ingurgitated *D. gallinae*. Right: *S. scimitus* feeding on an adult of *D. gallinae* 

#### 4.3.2 Detailed predation tests

In the experimental units containing the predator *C. barbatus*, 3 - 4 dead *R. microplus* were observed in each experimental unit at the end of the observation period, while in the control unit, four dead *R. microplus* were observed. In the units containing *S. scimitus*, 18 - 33 dead *R. microplus* and 2 - 11 dead *A. sculptum* were observed in each unit, while in control units 4 - 14 dead *R. microplus* and 0 - 5 dead *A. sculptum* were found. Thus, on the average, about 52 and 15% of the larvae of *R. microplus* and *A. sculptum* died in the units with *S. scimitus* against about 18 and 6% of the larvae of *R. microplus* and *A. sculptum* in the control units.

*Stratiolaelaps scimitus* fed on 63% of the eggs, 99% of the larvae, 100% of nymphs and 39.5% of the adults of *D. gallinae*.

# 4.4 Discussion

The absolute numbers of mites collected in this study cannot be compared with the numbers reported in Chapter 2, due to the different sampling procedures. The proportions at

which each family was found in this and in that Chapter were rather different, as expected, given the differences in climatic conditions between the corresponding sampling sites, in a subtropical region in Brazil and in a temperate region in Norway.

The ologamasids, the most abundant family found in this study, were expected to be among the dominant groups given that these are some of the most common Mesostigmata in soils of the state of São Paulo, Brazil (MINEIRO; MORAES, 2001; SILVA; MORAES; KRANTZ, 2004).

Yet, as in Norway, Parasitidae and Veigaiidae were also in Brazil among the dominant groups, corroborating the reports of Mineiro; Moraes (2001), who found a parasitid species as the most abundant in soils of rubber tree plantations. Although in other surveys of edaphic mites of the state of São Paulo these families were absent (SILVA; MORAES; KRANTZ, 2004). This could be due to the fact that differently from the present study, samples in those works were never taken from pasture. Parasitidae has been reported in the literature to prey mostly on nematodes as well as larvae of Coleoptera and Diptera, as summarized by Castilho; Venancio; Narita (2015). Thus, the relatively high proportions of these mites in the present study might be related to the common occurrence of those prospective prey in pasture areas, where dung of raised animals should serve as adequate substrate for the latter. Conversely, the relatively high proportion of Veigaiidae in this study could have a different explanation. In the present study, most of these mites were collected from an agricultural area at Bom Repouso, Minas Gerais, which is ecologically rather different from the areas where previous studies on edaphic mites in São Paulo state have been conducted. Bom Repouso is located in a mountainous area, at about 1,400 m above sea level, with average winter temperature of about 12 °C.

The report of a species of Pachylaelapidae (an unidentified species of *Onchodellus* Berlese) seems to be the first in Brazil. Members of this family have been described from over all climatic zones of the world, being most diverse in Europe (MAŠÁN; HALLIDAY, 2014). They have been collected from decomposing organic matter, litter, as well as from nests of mammals, birds and social insects.

The results of this work was barely sufficient to support the initial hypothesis, in which only three mesostigmatid species were observed as potential predators of the evaluated prey in the preliminary predation tests, with only one of the predators (*S. scimitus*) substantiated as promising in the subsequent detailed tests, on larvae of *R. microplus* and all developmental stages of *D. gallinae*, with the release of predators at high rates. The commercial population of *S. scimitus* used in this study originated from specimens collected

about 10 years ago in an organic substrate of a greenhouse at "Luiz de Queiroz" campus, in Piracicaba, state of São Paulo.

However, poor performance of other predators does not mean necessarily that they are not able to feed on ticks. It is possible that the predatory mites from the field used in the preliminary tests fed on other small arthropods (or on another food source) immediately before the tests, and for this reason they did not feed on ticks. The developmental stages of the field collected predatory mites were not standardized, and this may have affected the results, because in some cases the immature predators of a given species apparently require softer prey than adults of the same species. This type of feeding behavior has been reported, for example, for some macrochelid (AZEVEDO et al., 2015) and parasitid mites (AL-AMIDI; DOWNES, 1990; CASTILHO; VENANCIO; NARITA, 2015). Immature macrochelids usually feed on nematodes while adults feed mostly on fly eggs and larvae (AZEVEDO et al., 2015). Thus, some of the predator species collected in the present work might not have fed on the offered prey because the predators were not in the adult stage.

Additionally, the results could be different if the predation tests had been conducted with recently laid eggs or recently hatched larvae. The exact ages of the eggs and larvae used in this study are not known, but both were at least several days old. Several studies have shown a preference for predators for recently molted prey (CHAPMAN, 1998; CROMARTY; COBB; KASS-SIMON, 1991; STEGER; CALDWELL, 1983; WITT; DILL, 1996), when the chorion or the exoskeleton is less sclerotized.

Disregarding the age of the prey eggs, it is remarkable that no predation on eggs was observed in this study. Tick eggs have been known to have toxic substances called "ixovotoxins", which have been investigated and identified as serine protease inhibitors, as summarized by Mans, Gothe and Neitz (2008). The name ixovotoxin is particularly apt since egg toxins seem to be limited to "hard ticks" (Ixodidae). Laboratory studies conducted by Aquino et al. (2015) showed that extracts from tick egg wax presented antiviral, antibacterial and antifungal activity. The protective capacity of tick eggs seems essential for their survival, given that they usually remain exposed in the environment for several months (BOWMAN; NUTTAL, 2008). Some of those protective factors may also affect predation of the eggs.

The results obtained so far warrants further investigations of *S. scimitus*. This predator should be further evaluated under laboratory conditions, with more treatment and control replicates in order to make the preliminary observations of this study more solid. With their confirmation, studies in small plots should also be conducted under more natural conditions,

with releases of predators at different densities following the release of known numbers of *R*. *microplus* in the field or *D. gallinae* in chiken houses.

The observation of *S. scimitus* feeding on all developmental stages of *D. gallinae* corroborate the observation of other authors (LESNA et al., 2012; TUOVINEN, 2008). However, in this study, the predator/ prey rate was very high, and lower rates should be considered.

A question to be asked is how feasible is the use of predatory mites for the control of *R. microplus* and *D. gallinae*. Granting the availability of an efficient predator, the possibility of use will certainly depend on the cost of production of the predator, the density of predators necessary to release and the size of the area to be treated. The production cost is amenable to research directed to the determination of adequate methods. The density necessary to release is directly related to the efficiency of the predator. However, the size of the area to be treated seems potentially more limiting to the use of biological control of those preys. Considering the present use of predatory mites for pest control around the globe, it seems that at the present scientific status, biological control of *R. microplus* with edaphic predatory mites should be considered as potentially feasible in areas next to stables or confinement areas, where cattle are densely concentrated and where this technique could complement tick control done (usually by chemicals) on the animal host. This seems especially promising in Brazil, where chemicals for the control of *R. microplus* off the host are not available.

In relation to *D. gallinae*, given the dense concentration of laying hens, the use of biological control with edaphic mites seems more promising in terms of total area to be treated. On the average, 6 hens are kept per square meter in commercial chicken houses. However, until now, laying hens are usually kept individually in wire cages maintained at some distance from the ground, and this may limit the effective use of edaphic mites for the control of the poultry red mites. But there is presently a trend towards the maintenance of chicken on the ground, a technique that while favoring the development of the poultry red mite may also favor the performance of release predatory mites for their control. This trend is presently mostly considered for use in Europe. However, this technique is alreay also used in Brazil. Some organic growers, as the company "Korin - Agricultura Orgânica" already adopt a similar technique in this country. Lesna et al. (2012) reported a bio control tests with *S. scimitus* in small cages specially designed to accommodate few laying hens. In that study, predators and *D. gallinae* were differentially distributed within cages, with *D. gallinae* higher up in the cage structure and predatory mites on the cage floor. At Korin, the highest concentrations of the poultry red mite were also observed on the top of the woden nest boxes,

mainly in the gaps between the boards (VENANCIO, pers. obs). Thus, to improve bio control with *S. scimitus* under those conditions, it would be necessary to develop an artificial environment in which predatory mites could feel comfortable on the roof of the nest boxes.

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## **5 FINAL CONSIDERATIONS**

The present work represents one of the first efforts to determine the possible role of mites of the order Mesostigmata as tick predators, also contributing to the knowledge about the possibility of using mesotigmatid predators as control agents of the poultry red mite, *Dermanyssus gallinae* (De Geer). Field evaluations in western Norway and in southeast Brazil showed Parasitidae and Veigaiidae to be among the dominant families, although in Brazil Ologamasidae was more numerous than those two families. Laelapid mites were also represented in the surveys conducted in both countries.

The results of this study suggest the tick *Amblyomma sculptum* Berlese to be difficult to control with the use of mesostigmatid mites. None of the evaluated predators showed any good perspective for the control. However better results were obtained with the tick *Rhipicephalus microplus* (Canestrini) and with the poultry red mite.

Determining the ability of the laelapid *Stratiolaelaps scimitus* (Womersley) to feed on the larval stage of *R. microplus* and on all developmental stages of the poultry red mite, under laboratory conditions warrants further evaluations of this predator, envisioning its possible use as biological control agents of those ectoparasites. Emphasis should be given to the discovery and testing of new laelapid species, given their demonstrated potential to control of unwanted edaphic organisms.