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Article

Aquatic larval of the genus *Arrenurus* (Trombidiformes: Parasitengonina: Arrenuridae) associated with Odonata species from Pampa Biome, Brazil

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Abstract: Many studies have reported that the interaction between water mite larvae and their Odonata hosts affects mating success, flight, and longevity. Males and females of Odonata species collected in the steppes and coastal plains (Pampa Biome) of Rio Grande do Sul were analyzed. Mites were removed when present and the prevalence and intensity of parasites was calculated. The aim of this study was to search and report new Odonata hosts species that are parasitized by water mite larvae and also to evaluate the prevalence and intensity rates; the differences in mite occurrence and frequency between males and females, and between thorax and abdomen of the dragonflies and damselflies in the southern Pampa biome located in Rio Grande do Sul. A total of 162 larval mites were found associated to two Odonata families: Coenagrionidae (*Acanthagrion lancea* Selys, 1876, *Ischnura capreolus* Hagen, 1861 and *Ischnura fluviatilis* Selys, 1876) and Libelullidae (*Micrathyria ocellata* Martin, 1897 and *Perithemis mooma* Kirby, 1889). All mites were identified as *Arrenurus* (*Arrenurus*) sp. (Arrenuridae) and showed high numbers when attached to *I. capreolus* (55.5%), *I. fluviatilis* (33.3%), followed by low numbers on *M. ocellata* (6.1%), *A. lancea* (3.7%), and *P. mooma* (1.2%). Mites were found on males and females of *I. capreolus* and *I. fluviatilis*, females of *A. lancea* and *P. mooma* and in *M. ocellata* only in males. As the parasitized Odonata species are generalist and abundant in all water body types, traits associated with mating and oviposition or larval behavior are believed to explain the frequency of parasitism in these species.
Keywords: *water mites; lentic systems; dragonfly; damselfly; parasitism*

Larvas aquáticas do gênero *Arrenurus* (Trombidiformes: Parasitengonina: Arrenuridae) associada a espécies de Odonata do bioma Pampa, Brasil

Resumo: Muitos estudos relatam que a interação entre as larvas parasitas e seus hospedeiros Odonata afetam o sucesso do acasalamento, o voo e a longevidade. Foram analisados machos e fêmeas de espécies de Odonata coletados nas estepes e planícies costeiras do bioma Pampa do Rio Grande do Sul. Os ácaros foram removidos quando presentes e a prevalência e intensidade dos parasitas foram calculadas. O objetivo deste estudo foi pesquisar e relatar novas espécies hospedeiras de Odonata que estão parasitadas por larvas de ácaros aquáticos, avaliar as taxas de prevalência e intensidade; avaliar a diferença na ocorrência e frequência de ácaros em fêmeas e machos e entre o tórax e abdômen de libélulas e libelulinhas no bioma Pampa meridional localizado no Rio Grande do Sul. Um total de 162 ácaros foram encontrados associados a duas famílias de Odonata: Coenagrionidae (*Acanthagrion lancea Selys*, 1876, *Ischnura capreolus* Hagen, 1861 e *Ischnura fluviatilis* Selys, 1876) e Libelullidae (*Micrathyria ocellata* Martin, 1897 e *Perithemis mooma*, 1889) Kirby. Todos os ácaros foram identificados como *Arrenurus* (*Arrenurus*) sp. (Arrenuridae) e apresentaram números elevados quando anexados a *I. capreolus* (55,5%), *I. fluviatilis* (33,3%), seguido por números baixos em *M. ocellata* (6,1%), *A. lancea* (3,7%) e *P. mooma* (1,2%). Os ácaros foram encontrados em machos e fêmeas de *I. capreolus* e *I. fluviatilis*, fêmeas de *A. lancea* e *P. mooma* e em *M. ocellata* apenas em machos. Como as espécies de Odonata parasitadas são generalistas e abundantes em todos os tipos de corpos d'água, acredita-se que características associadas ao acasalamento e oviposição ou comportamento larval explicam a frequência de parasitismo nessas espécies.
Palavras-chave: *ácaros aquáticos, sistema lêntico, libélulas, libelulinhas, parasitismo*

Introduction

The hyporder Parasitengonina is characterized by mites that have parasitic larva and predatory nymphs and adults; the resting stages provide an adaptation to avoid unfavorable conditions in unstable environments, and larval parasitism on flying insects provides substantial advantages, ensuring dispersal and rapid exploitation of new habitats (Smith et al. 2001, Smith et al. 2010, Proctor et al. 2015). Hydrachnidiae, or water mites, are a highly diverse group of Parasitengonina, comprising over 6,000 described species and reported in all regions of the world, except for Antarctica (Cook 1974, Viets 1987). They are found in lotic and lentic habitats, as well as in springs, interstitial waters, wetlands, temporary pools, marine habitats, torrential waterfalls, ponds, streams, and lakes (Smith & Cook 1991, Smith et al. 2001, Goldschmidt 2016).

A very ordinary family of Hydrachnidiae is Arrenuridae, and in the preparasitic phase they are very fast and active, searching out their hosts under the water surface walking on the substrate or swimming. The survival period for free-swimming larvae in the preparasitic phase ranges from 4 days to 6 weeks (Smith & Oliver 1986, Smith 1988, Smith et al. 2010). Their larvae parasitize species of Diptera, Coleoptera, and Odonata orders, all groups with the final instar active in the water or flying (Smith & Cook 1991, Smith et al. 2001, Zawal 2008, Smith et al. 2010, Gerecke et al. 2016). The predominant water mite genus that uses Odonata larvae and adults as hosts is *Arrenurus* Dugès (*stricto sensu*) (Arrenuridae) (Andrew et al. 2012), and at least 55 species have been described as Odonata ectoparasites (Davids 1997, Zawal & Dyatlova 2006, Baker et al. 2007, Zawal 2008). The genus *Arrenurus* is one of the most species-rich of the Hydrachnidiae, with about 1000 species present in most of the zoogeographic regions (Smit 2020). However, the genus lacks cosmopolitan species, and each region supports its own set of species (Zawal 2008). A list of South American water mites species was published by Rosso de Ferradás and Fernández (2005) who listed 150 *Arrenurus* species. The number of reported South America *Arrenurus* reaches now 153 species (Smit 2020). Some species listed for Brazil are *A. clavipes* Lundblad, 1941, *A. corniger* Koenike, 1894, *A. epimerosus* Marshall, 1919, *A. ludificator* Koenike, 1905, *A. nitidus* K. Viets, 1937, *A. quadrituberculatus* K. Viets, 1937, *A. triconicus* Marshall, 1919 and *A. undulatus* Lundblad, 1937 (Smit 2020).

Arrenurus s. str. has not been precisely determined, mainly due to the lack of possibility to identify mite larvae (Smith,1988, Zawal 2008, Smith et al. 2010, Zawal & Buczyński 2013). Contemporary taxonomical knowledge on water mites is based primarily on males, but the description of the female should also be provided as they show significant morphological differences as well; the larval and nymphal stages are considerably less known (Zawal 2008, Smith et al. 2010, Smit 2020).

Larvae of many subfamilies of water mites exhibit strong selectivity in their attachment to particular parts of the host body (Smith & Oliver 1986). Larvae of *Arrenurus* s. str species have preferences for either thoracic or abdominal sites and are less seen in the head or wings of odonate hosts (Smith et al. 2010). Studies indicate that mite parasitism can affect longevity, flight, and fecundity (Åbro 1979, Åbro 1982, Forbes 1991, Forbes & Baker 1991).

Many odonate species respond to attached mites by aggregating their haemocytes at the sites of puncture and by producing melanotic encapsulation of feeding tubes, but *Arrenurus* genus have a developed powerful mouthpart specialized in anchor to the host's body; the pedipalps have a well-developed claw that fixes in the cuticle and then with its chelicerae saber pierces the body till finds the hemolymph; then the larva produces a feeding device characterized by a narrow gelatinous resilient blind sac called stylostome, which seems to inject cytotoxins into the wound of the damselfly, thus, producing a paralysis which allows sufficient time to develop a stylostome to absorb nutrients, and this makes the damselfly's defensive apparatus ineffective to cope with the stylostome (Åbro 1979).

Heavy mite infestation brings several wounds in close proximity, accompanied by loss of more or less extensive areas of the epidermis. Despite Odonata wound repair by congregating hemocytes, local lack of epidermis seems to enfeeble the host, presumably owing to desiccation, thus, the infestation contributes to reduced longevity (Âbro 1982).

Through this form of feeding, Reinhardt (1996) observed that ectoparasitic mites have a negative influence on flight ability one meter was the longest distance flown by 35.2 % of the infested adults immediately after release while none flew farther than five meters; in the group of the non-parasitized damselflies, 75% flew more than one meter, and this pattern was consistent in both sexes. Oviposition injuries were observed by Rolff (1999) which tested *Arrenurus cuspidator* (O. F. Müller, 1776) on *Coenagrion puella* Linnaeus, 1758 and found that the number of eggs laid by the damselfly decreased with increased ectoparasite abundance.

Rodrigues et al. (2013) were the first to report larval of the *Arrenurus* genus parasites on Odonata species *Ischnura fluviatilis* Selys, 1876 (Coenagrionidae) and *Miathyria marcella* Selys, 1857 (Libellulidae) in Brazil.

The present study had the main objective to search and report new Odonata hosts for parasitic larvae of Arrenurus genus and also to evaluate the prevalence and intensity rates; the differences in mite occurrence and frequency between males and females, and between thorax and abdomen of the dragonflies and damselflies in the Pampa biome located in the Rio Grande do Sul state, Brazil.

Material and Methods

The present study was the first one performed in the Pampa biome. This biome covers the southern half of the state of Rio Grande do Sul and extends to Argentina and Uruguay. This biome constitutes the Brazilian portion of the South American Pampas, which are classified as steppes by the international phytogeographic system. The Pampa is limited by the Atlantic Forest biome to the north, and by the Chaco and the Patagonian steppes to the west. The steppes in the Pampa region have no dry season. However, they undergo high thermal amplitude and intense drying cold fronts, which increase evapotranspiration, and consequently, cause occasional droughts. This factor limits arboreal flora and riverbanks, valley bottoms, and protects lands from cold fronts; in other areas, grassy-woody species predominate. Steppes have been undergoing an intense anthropization process, due to cattle raising, grain cultivation, and fires (IBGE 2019). The coastal area (Coastal Plains) comprises sedimentary land of both fluvial and marine origin, flattened or depressed areas, generally with sandy soils. Pioneer Formations are predominant in this area. This vegetation occupies unstable land and is in constant ecological succession (IBGE 2019).

1. *Study area*

Samplings were conducted between 2016/17 in the municipalities of Caçapava do Sul, Manoel Viana, Mata, Quaraí, Rosário do Sul, Santa Margarida do Sul, Santana da Boa Vista, São Francisco de Assis, São Gabriel, São Pedro do Sul, São Sepé, São Vicente do Sul, and Uruguaiana (steppe) in temporary waters, rivers, streams lakes and wetlands. Samplings in coastal plains were conducted only in wetlands (the most common water body type in that phytophysiognomy) in the municipalities of Arroio Teixeira, Capão da Canoa, Cidreira, Curumin, Pinhal, Torres, Tramandaí and Xangri-lá between 2016/18.

2. *Sampling methods*

The material referred to Renner et al. (2017) and Renner et al. (2018) was used in the present study. Odonata specimens were preserved in 96% alcohol in glass pots with lids and identification labels. Specimens are deposited at the Natural Science Museum (MCNU) of Univates and the collection authorization process was issued by IBAMA, via the SISBio system under the number 50624-1.

3. *Laboratory activities and identification*

Odonata specimens were observed using a Zeiss 435063-9010-100 Stemi 305 Stereo Microscope and photographed using Zen software. Mites were removed from dragonflies and damselflies with the help of histological needle and tweezers, and stored in Eppendorf tubes in Koenike's fluid (10mL acetic acid; 40mL distilled water; 50mL glycerin) (Walter & Krantz 2009).

Subsequently, mites were mounted on microscopic slides in Hoyer's medium and dried at 60-70ºC for seven days. After this period, slides were sealed with crystal varnish to prevent contamination, and then, they were stored at the Laboratory of Acarology - Univates collection, where air humidity is controlled for proper storage of the material. Larval mites were analyzed using a Zeiss Imager Z2 optical microscope with phase contrast and were photographed using the Zen software. Mites were identified to the genus level using the most recent key provided by Smith et al. (2010). Odonata specimens were identified according to Garrison et al. (2006; 2010) and Lencioni (2006).

4. *Data analysis*

Two indices were calculated: 1. Prevalence (number of parasitized individuals/total number of analyzed individuals X 100), 2. Intensity (total number of parasite/number of parasitized individuals).

In order to analyze the differences in mite occurrence between: (1) thorax and abdomen and (2) females and males was performed a G-test ($p \leq 0.05$), using Bioestat 5.0 software (Ayres et al. 2007).

Results

A total of 3134 specimens divided of 100 species were analyzed (Suplementary material) but only 44 specimens of five species had larval mites attached; they were found in lakes, rivers, and temporary waters of São Francisco de Assis, and in wetlands of São Pedro do Sul, Mata, Caçapava do Sul and Santa Margarida do Sul (steppe); in the coastal area, mites were found in wetlands of Tramandaí, Pinhal, and Cidreira (Figure 1). Four new Odonata species are reported to the host list for *Arrenurus* s.str: *Ischnura capreolus* Hagen, 1861*, Acanthagrion lancea* Selys, 1876*, Perithemis mooma* Kirby, 1889 and *Micrathyria ocellata* Martin, 1897. (Table 1).

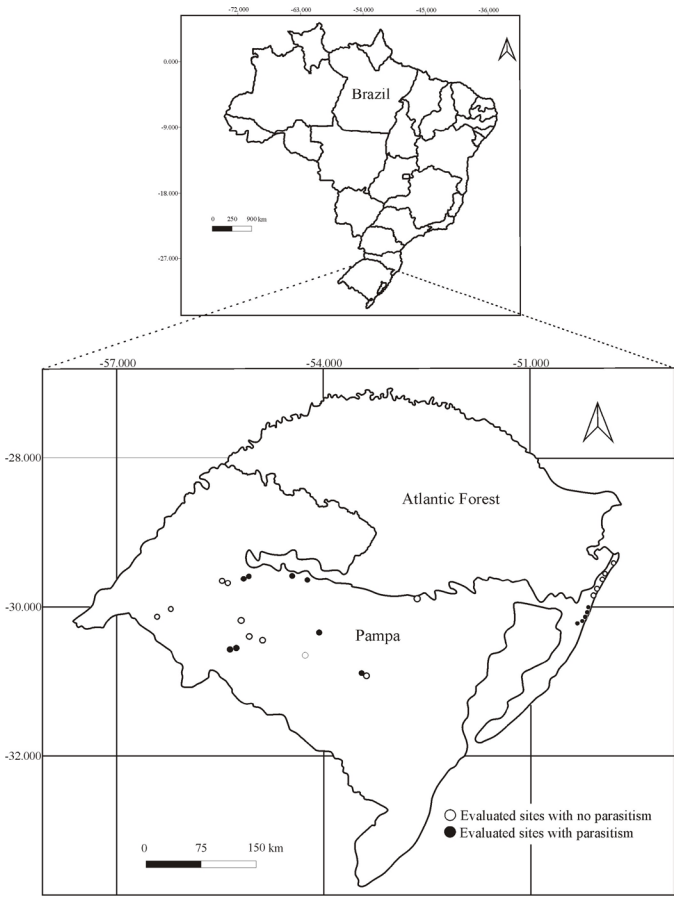

Figure 1. Map with collection points in Rio Grande do Sul state where *Arrenurus* sp. larvae were and were not present.

A total of 162 *Arrenurus (Arrenurus)* sp. larvae (Table 2; Figure 2 A-B) were found to parasitizing five Odonata species: *M. ocellata* (Figure 3 A-B) and *P. mooma* (Figure 3 C-D) (Libellulidae) and *A. lancea* (Figure 4 A-B), *I. capreolus* and *I. fluviatilis* (Coenagrionidae).

A high number of mites occurred when they were attached to *I. capreolus* (55.5%), *I. fluviatilis* (33.3%), followed by low numbers when attached to *M. ocellata* (6.1%), *A. lancea* (3.7%), and *P. mooma* (1.2%). Mites found in the steppes were associated to the five Odonata species listed above; whereas only *I. fluviatilis* and *I. capreolus* were found in the coastal area. The collection points of the steppe did not had urbanization, that results in a higher diversity of Odonata species, and also more hosts for the parasitic mites. In the Coast, higher levels of urbanization were found around the collection points, thus, decreasing the number and diversity of species.

Mites attached to *A. lancea*, *M. ocellata*, and *P. mooma* were only found on the thorax, while mites attached to *I. capreolus* and *I. fluviatilis* were found both on the thorax and abdomen (Table 3). Significant difference was observed in the body's part where water mite larvae was found in the species from steppe ($p = 0.0005$), but not for the coastal species ($p = 0.8776$).

Larvae attached to *A. lanceae* and *P. mooma* were found only in females; when attached to *M. ocellata*, they were found only in males, and when attached to *I. capreolus* and *I. fluviatilis*, they were found in both females and males. No significant differences between host's sex preferences were found in *I. capreolus* and *I. fluviatilis* ($p=0.1413$).

Table 1. Municipalities and coordinates where *Arrenurus* (*A.*) sp. was found along with the water body and Odonata species collected at each site.

	Municipality	W	S	Water body	Species
Steppe	São Pedro do Sul	54°50'22.2"	29°65'96"	Wetland	*Acanthagrion lancea*
	Mata	54°42'85.8"	29°65'29"	Wetland	*Ischnura capreolus*
				Wetland	*Ischnura fluviatilis*
	Caçapava do Sul	53°27'7.1"	30°53'35.4"	Wetland	*Ischnura capreolus*
	Santa Margarida do Sul	53°84'94.4"	30°34'32"	Wetland	*Acanthagrion lancea*
				Wetland	*Perithemis mooma*
				Wetland	*Ischnura fluviatilis*
	São Francisco de Assis	55°17'07.6"	30°34'32"	Lake	*Ischnura capreolus*
				Lake	*Ischnura fluviatilis*
				Lake	*Micrathyria ocellata*
				Lake	*Perithemis mooma*
	São Francisco de Assis	55°19'20.4"	30°34'32"	Lake	*Ischnura capreolus*
				Lake	*Ischnura fluviatilis*
				Lake	*Micrathyria ocellata*
				Lake	*Perithemis mooma*
	São Francisco de Assis	55°08'54.7"	29°36'28.2"	River	*Micrathyria ocellata*
				River	*Perithemis mooma*
	São Francisco de Assis	55°07'7.3"	29°35'43,1"	Temporary waters	*Ischnura fluviatilis*
Coast	Tramandaí	50°10'26"	30°05'37"	Wetland*	*Ischnura fluviatilis*
				Wetland*	*Ischnura capreolus*
	Cidreira	50°12'03"	30°09'26"	Wetland*	*Ischnura fluviatilis*
				Wetland*	*Ischnura capreolus*
	Cidreira	50°13'14"	30°10'57"	Wetland*	*Ischnura fluviatilis*
				Wetland*	*Ischnura capreolus*
	Pinhal	50°17'31.7"	30°12'50.1"	Wetland	*Ischnura fluviatilis*
				Wetland	*Ischnura capreolus*
	Tramandaí	50°09'01"	30°01'16"	Wetland*	*Ischnura fluviatilis*
				Wetland*	*Ischnura capreolus*

* = modified environment (high level of urbanization)

Table 2. Occurrence of *Arrenurus* (*A.*) sp. larvae in Odonata species from the Pampa Biome, Brazil.

Ecosystem	Odonata species	Analyzed individuals	Parasitized individuals	Prevalence of infestation (%)	Total of parasites found	Intensity of infestation
Steppe	*I. capreolus*	19	9	47.4	38	4.2
	I. fluviatilis	53	12	22.6	15	1.3
	A. lancea	11	4	36.4	6	1.5
	M. ocellata	11	1	9.1	10	10
	P. mooma	12	1	8.3	2	2
Coast	*I. capreolus*	37	7	18.9	52	7.4
	I. fluviatilis	49	10	20.4	39	3.9

Discussion

The present study demonstrates that despite having a range of Odonata species for colonization (about 130 different species evaluated) only five of them were found with parasites. The species that were found being parasitized are very common and generalist in our state, they can be found either in lentic and lotic environments, with presence or absence of luminosity, and saturated O_2 or not. The parasitized species in the steppe areas of the Pampa were found in all types of water bodies present in the region: lakes, rivers, wetland and temporary waters, and in the coastal region only in wetlands (which occur most frequently in this area), indicating that there is no preferred location for infestation to

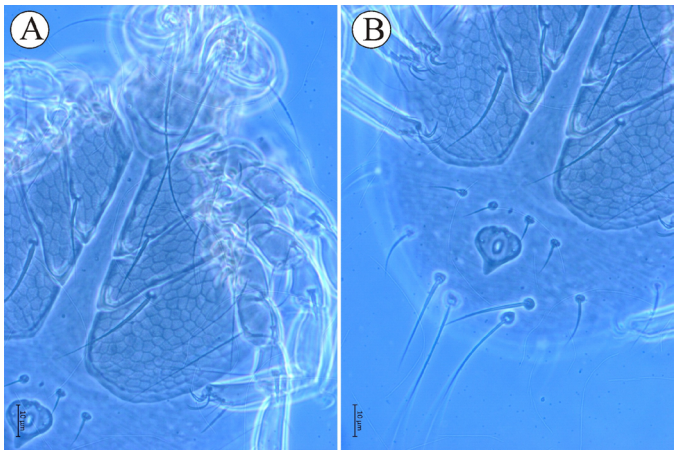

Figure 2. Ventral view of *Arrenurus* (*A.*) sp. parasitic larvae. **A** Pedipalps, Coxal Plates and Legs; **B** Excretory Pore

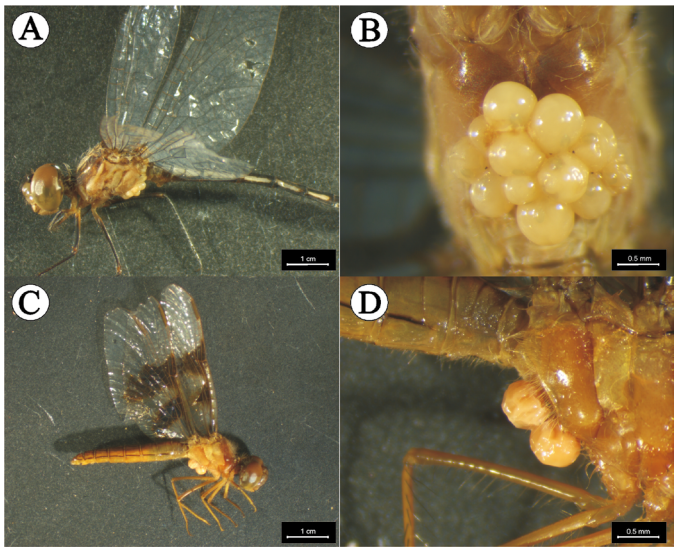

Figure 3. A *Micrathyria ocellata* male (in lateral view) with larvae on thorax; **B** *Micrathyria ocellata* close up of thorax, ventral view; **C** *Perithemis mooma* female (in lateral view); **D** *Perithemis mooma* close up of thorax, lateral view.

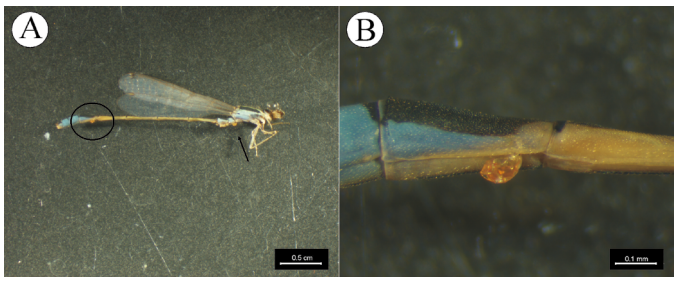

Figure 4. A. *Acanthagrion lancea* male with larvae attached on abdomen (in the circle) and arrow pointing to larvae attached in the thorax; **B**. Close up in the circle.

occur. The mites did not seem to have any preferences either for thoracic or abdominal attachment on the host's body, and either for females or males, and that may indicate that attachment happens by chance.

One important aspect from the analyzed species is that they lay eggs directly in the water, and sometimes in plants and small pieces of wood floating in surface; those species can also mate with more than one partner.

Table 3. Mites found in each part of the body and Odonata individuals parasitized by sex from the Pampa Biome, Brazil.

	Parasites on host´s body		Specimens parasitized by sex	
Steppe	**Abdomen**	**Thorax**	♀	♂
A. lancea	0	6	4	0
I. capreolus	22	16	2	7
I. fluviatilis	10	5	4	8
M. ocellata	0	10	0	1
P. mooma	0	2	0	1
Coast	**Abdomen**	**Thorax**	♀	♂
I. capreolus	31	21	3	4
I. fluviatilis	23	16	2	8

In traits and general behavior shown in Corbet (1999), Córdoba-Aguilar (2008) and Dalzochio et al. (2018), *A. lancea* exhibit larval climbing behavior and their oviposition is epiphytic, which means that egg laying occurs on leaves, woods, or rocks, whether or not submerged in water. *I. capreolus* and *I. fluviatilis* are also climbers but they exhibit endophytic oviposition pattern, i.e. damselflies lay eggs inside plant tissue in the waters surface. On the other hand, Libelullidae larvae, e.g. *P. mooma* and *M. ocellata*, are sprawlers and exophytic, which means egg laying occurs directly into the water. All these species spend a long period near the water, and it is easier for larval mites to attach to the host than those in Aeshnidae and Gomphidae, which never climb, are very active, and difficult to collect. Thus, species with terrestrial behavior are less prone to being parasitized (Smith et al. 2010). A general rule in Coenagrionidae is that adult males spend more time near water bodies searching for mating while adult females disperse in the vicinity and return to the water to breed (Corbet 1999, Córdoba-Aguilar 2008). This may explain the numbers of mites found on *Ischnura* spp. males, as these individuals spend a long time in the water and are very abundant in all kinds of water bodies, and that agrees with some of the Ilvonen et al. 2016, Ilvonen & Suhonen, 2016 found, where many Coeanagrionidae are reported being parasitized by Arrenuridae mites.

Ilvonen et al. (2016) found no differences in infestation by water mites between damselfly males and females, which conflicts with the findings of Rob and Forbes (2005), who observed a higher infestation by water mites on *Lestes disjunctus* Selys, 1862 (Lestidae) females. Ilvonen & Suhonen 2016 tested Odonata immune responses to water mites; mass was significantly different between sexes, females being heavier than males; between species, the encapsulation response was different, but not between sexes; it was also found considerable differences in the encapsulation response between different odonate species, e.g *I. elegans* (Coenagrionidae) had the lowest encapsulation rate, whereas dragonfly *Leucorrhinia dubia* Vander Linden, 1825 (Libellulidae) had the highest. These defense mechanisms add up to an effective immune system, capable of defending against parasites and thus prolonging the host's lifespan and reproductive success. In their study, also found that damselflies had also much higher water mite prevalence than dragonflies.

In Brazil, studies on parasitism of aquatic mites on odonates and other host species are scarce, and many species can only be identified as morphospecies of specific genera due to the lack of taxonomic studies for the Neotropics. It is important to collect adults and conduct oviposition studies with females in order to correctly associate the larvae with their parents. Also, full descriptions of Hydrachnidiae must have males, females

and larvae. In addition, molecular tools (e.g. barcoding) can be useful for creating a database for the identification of species and even larvae. Thus, further studies must be carried out in lotic and lentic environments in order to find and describe larvae, and adults either females and males to report the existing and describe the new species in Brazil; studies on the damage caused on dragonflies and damselflies by mites should be performed in order to discover whether flight, longevity and oviposition are really affected. This type of analysis was already performed by Reinhardt (1996) who observed that ectoparasitic mites have a negative influence on flight ability of *Nehalennia speciosa* Charpentier, 1840 (Coenagrionidae); one meter was the longest distance flown by 35.2 % of the infested adults immediately after release, whereas none flew farther than five meters; in the group of the non-parasitized damselflies, 75% flew more than one meter and this pattern was consistent in both sexes. Advanced adults of *Coenagrion hastulatum* Charpentier, 1825 and *Enallagma cyathigerum* Charpentier, 1840 heavily loaded with parasites had often lost the typical agility to move and could be easily catched (Åbro 1981), this flight injuries is due to the mites attachment in the thoracic region where there is more hemolymph stream and consequently more consumed energy due to the time that Odonata spends flapping their wings (Corbet 1999). Oviposition test was performed by Rolff (1999) which tested *Arrenurus cuspidator* (O. F. Müller, 1776) on *C. puella* Linnaeus, 1758 and found that the number of eggs laid by the damselfly decreased with increased ectoparasite abundance. New hosts should also be sought to report new host-parasite interactions and continue to build knowledge on aquatic mite fauna for Rio Grande do Sul state.

Supplementary Material

The following online material is available for this article:
Table S1 - Analyzed species

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Author Contributions

Gabriel Lima Bizarro: Substantial contribution in the concept, design of the study and to manuscript preparation.
Marina Dalzochio: Contribution to data collection.
Eduardo Périco: Contribution to data analysis and interpretation.
Guilherme Liberato: Contribution to data analysis and interpretation.
Marina Dalzochio: Contribution to data analysis and interpretation.
Liana Johann: Contribution to data analysis and interpretation.
Noeli Juarez Ferla: Contribution to data analysis and interpretation.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of the manuscript.

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