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Prevalence and abundance of the mite *Neomegistus julidicola* (Mesostigmata: Paramegistidae) on *Doratogonus rugifrons* (Diplopoda: Spirostreptidae) are highly male-biased

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ABSTRACT

We have explored the patterns of association between the giant spirostreptid millipede *Doratogonus rugifrons* (Attems, 1922) and a common symbiotic mite *Neomegistus julidicola* Trägårdh, 1906. In our study, mites clearly preferred male millipedes: 97% of the recorded mites occurred on males and 66% were on the anterior part of the millipede body. No mites' feeding on the millipede excretions was observed. We conclude that mites prefer males because those are more mobile during surface activity in the wet summer season when they are searching for mates. The six new records of mites on other species of spirostreptid millipedes (*Cacuminostreptus vumbaensis, Doratogonus flavifilis, Doratogonus rhodesianus, Doratogonus uncinatus, Spirostreptus kruegeri* and *Zinophora* sp.) suggest that such associations are widespread. Our results also support the view that phoretic mites on millipedes are not host specific. Given the highly male-biased association between the mite species and spirostreptids, future studies should explore what attracts the mites specifically to males.

KEYWORDS: Mesostigmata, mites, Diplopoda, Spirostreptida, millipedes, phoronts, hosts, symbiosis, southern Africa.

INTRODUCTION

In Southern Africa, millipedes of the order Spirostreptida Brandt, 1833 become active on the soil surface during the summer rainfall season, which typically begins in November and ends in March. During their surface activity, spirostreptidan millipedes feed and mate (Dangerfield & Telford 1991). Adult mites of the order Mesostigmata are associates of other animals including some myriapods, beetles and vertebrates (Domrow 1978; Hunter & Rosario 1988; Flechtmann & Johnston 1990; Klompen & Austin 2007; Baker & Seeman 2008; Gerdeman & Klompen 2009). On myriapods, mites are most common on larger millipedes (Farfan & Klompen 2012; Mwabvu pers. obs.); however, some of these associations are locality specific rather than host specific (Klompen & Austin 2007; Klompen et al. 2013).

In South Africa and Madagascar, *Neomegistus julidicola* Trägårdh, 1966 has been recorded on spirostreptidan millipedes, whilst in Queensland, Australia, *Neomegistus remus* occurs on a spirobolidan millipede *Proporobolus* sp. (Baker & Seeman 2008). Additionally, mites of the family Paramegistidae Trägårdh, 1946 are also associated with reptiles, millipedes and carabid beetles (Flechtmann & Johnston 1990; Kim & Klompen 2002). The association between mites and diplopods could be phoretic. In some cases, when the phoretic association is obligate, the life cycles of the phoronts and host are synchronized (Hunter & Rosario 1988).

A paucity of data on associations among soil arthropods in general and host preferences of mites in particular serves as a justification for the present endeavour, more so because mites and millipedes are widely distributed and are important players in soil processes in Southern Africa and elsewhere. Furthermore, the reported preference of males of *D. cristulatus* (Lawrence 1939; Mwabvu 2014) suggests that these phenomena may also occur in other species of *Doratogonus*.

Therefore, we sought answers to the following questions:

(1) Are adult mites more abundant on males of *D. rugifrons*?

(2) Are adult mites abundant on the anterior rather than posterior part of the millipede body?

MATERIALS AND METHODS

Study area

A survey of *Neomegistus julidicola* on *Doratogonus rugifrons* (Attems, 1922) was carried out in a 30-ha *Vachellia sieberiana* (DC.) Kyal & Boatwr. primary woodland (25°24.35'S 30°57.28'E) in HL Halls & Sons (Pvt) farm in Mbombela local municipality, Mpumalanga Province, South Africa (Fig. 1, Site 1). *Vachellia sieberiana* was the dominant woody plant at the site, with different grasses forming the undergrowth. The woodland, which is in the savanna, burns annually between July and August.

Sampling technique

Given that the millipedes are active on the surface during the rainfall season, sampling occurred during the 2021-2022 and 2022-2023 rainfall seasons. The latter season had longer dry spells, sometimes exceeding two weeks, during which millipedes were inactive. Doratogonus rugifrons millipedes were dominant in the woodland, and were sampled along eight 100 m long by 2 m wide transects on each sampling day. The sampling was done weekly or fortnightly after a rainfall event by two people, one on each side of the transect line for 10-15 minutes. Active searching along transects was the only method that was used because surface active millipedes only were targeted in this study. Millipedes were picked up to determine their sex and to check for the presence and position of adult mites on the millipedes. The mites were red brown and approximately 2 mm long, thus visible

with the naked eye. Mites on the millipedes were counted and their position on the millipedes (in the anterior or posterior half) was recorded. Millipedes that coiled were uncoiled so that their ventral surface could be checked for mites. The handling time (15– 30 seconds) of each millipede depended on the coiling behaviour of the millipede and the abundance of mites on the millipede. Thereafter, the millipedes were released. Females and juveniles tended to coil immediately after being picked up unlike males, which twisted around to free themselves. In each season, ten mating pairs of millipedes carrying mites were taken to the laboratory to confirm their identity using the keys to the male gonopods. Adult mites were also compared with the previously identified voucher specimens. Identification of the millipedes was based on descriptions by Hamer (2000) and Mwabvu et al. (2009, 2010). All specimens were retained in 96% ethanol to preserve DNA integrity. Voucher specimens will be accessioned in the KwaZulu-Natal Museum, Pietermaritzburg, South Africa.

We also carried out random sampling of millipedemite associations by active searching along transects in other parts of South Africa and in some parts of Zimbabwe, including in Miombo woodland.

Data analyses

In R version 4.1.2, the Shapiro-Wilk test showed that the data (abundance of mites on different sexes (W=0.75, p<0.001), and on different parts of the body (W=0.85, p<0.001)) were not distributed nor-

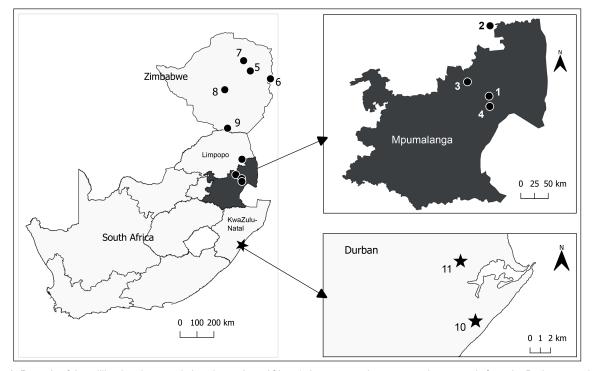


Figure 1: Records of the millipede-mite associations in southern Africa: 1–9, new records; stars, previous records from the Durban area. Localities: 1 – the present study site in Mpumalanga; 2 – Olifant Game Reserve, Hoedspruit; 3 – Sterkspruit Nature Reserve, Lydenburg; 4 – Barberton Nature Reserve, Barberton; 5 – Marondera; 6 – Muterere, Honde Valley; 7 – Harare; 8 – Gweru; 9 – Bube, near Beitbridge; 10 – Bluff, Durban (Lawrence 1939); 11 – Pigeon Valley Nature Reserve, Durban (Mwabvu 2014).

mally. As such, to test if the number of mites was similar between males and females, as well as between the anterior and posterior parts of the body, we used the generalized linear models with the Poisson distribution. The infestation intensity was the average number of mites per infested male millipedes.

RESULTS

Four hundred and seventy-eight individuals (109 males, 249 females and 120 juveniles) of *Dorato-gonus rugifrons* were examined. Of the 109 males, 76 carried mites, five females had mites, and there were no mites on juveniles. Mites were found only on the latero-ventral and ventral surfaces of the millipedes (Fig. 2), including immediately behind the mandibles and around the gonopods in males.



Figure 2: Two mites *Neomegistus julidicola* (arrowed) on the lateroventral surface on the anterior part of the *D. rugifrons* millipede.

Of the 395 mites recorded in both seasons, 97% (n=384) were recorded on 70% of the males, while 3% (n=11) were on 2% of the females. There was significantly higher abundance of mites in males than females (t=12.29, p<0.001, Fig. 3a). Of the mites recorded on males, 66% (n=251) were on the anterior half of the body, while 34% (n=127) of the mites were on the posterior part, mostly on the last six body rings of the millipede. There was a significantly greater abundance of mites on the anterior part of the body compared to the posterior part of the body (t=-6.49, p<0.001, Fig. 3b). In the two rainfall seasons, the incidence rate and infestation intensity of mites on male millipedes were not significantly different (Table 1).

In Zimbabwe, we also found what we presume are *N. julidicola* on males of *Doratogonus uncinatus* (n=19) in Marondera [18°11'S 31°32'E] and in Mu-

Table 1. Incidence (%) and infestation intensity of mites on *D. ru-gifrons* males during two rainfall seasons (n = sample size).

2021-2022			2022-2023		
n	Incidence	Intensity	n	Incidence	Intensity
132	38	1.91	60	33	2.86

terere, Honde Valley $[18^{\circ}43'S 32^{\circ}96'E]$, *Cacumino-streptus vumbaensis* Mwabvu, 2010 (n=4) in Harare $[17^{\circ}30'S 31^{\circ}05'E]$, *Spirostreptus kruegeri* (Attems, 1928) (n=3) in Gweru $[19^{\circ}27'S 29^{\circ}49'E]$ and *Doratogonus rhodesianus* (Chamberlin, 1927) (n=22) at Bube, near Beitbridge $[22^{\circ}02'S 30^{\circ}00'E]$ (Fig. 1). In South Africa, *N. julidicola* was recorded on males of *Doratogonus flavifilis* (Peters, 1855) in Olifant Game Reserve, Hoedspruit $[24^{\circ}07'S 30^{\circ}58'E]$ and in Sterkspruit Nature Reserve in Lydenburg $[25^{\circ}09'S 30^{\circ}33'E]$ (n=5), and on *Zinophora* sp. (Harpagophoridae) (n=4) in Barberton Nature Reserve, Barberton $[25^{\circ}36'S 30^{\circ}58'E]$ (Fig. 1).

DISCUSSION

In Southern Africa, few mite-millipede associations have been recorded (Fig. 1). Among these, Lawrence (1939) reported the mites' preference for males of Doratogonus cristulatus as hosts. Similarly, Mwabvu (2014) observed the preference of males of D. cristulatus and Orthoporoides pyrhocephalus by mites. The mesostigmatid mite N. julidicola appears to have a phoretic association with D. rugifrons. Our results demonstrate that N. julidicola associates predominantly with males of D. rugifrons and other species of spirostreptid millipedes. These results seem to support the notion that mites may feed on seminal fluids, as suggested in Lawrence (1939). However, spermophagy has not been recorded so far (Baker & Seeman 2008). Given their male-biased prevalence, the occasional occurrence of mites on female millipedes could be explained by possible mites' moving from males to females during mating. Baker and Seeman (2008) also reported that there is no evidence to support that mites feed on external secretions that are produced by millipedes, because millipede secretions do not have nutritional value as food. Although there may be other factors that draw mites to male millipedes, our view is that the greater mobility of males (Mwabvu pers. obs.) when they are searching for mates may make them ideal phoretic hosts for mites than the relatively less active conspecific females and juveniles. The fact that mites were recorded more on the anterior part of the body of males, particularly near the gonopods and the head, appears to support the seminal fluid feeding hypothesis. Kleptoparasitism and the potential shelter behind the head and under the collum cannot explain the strong preference for

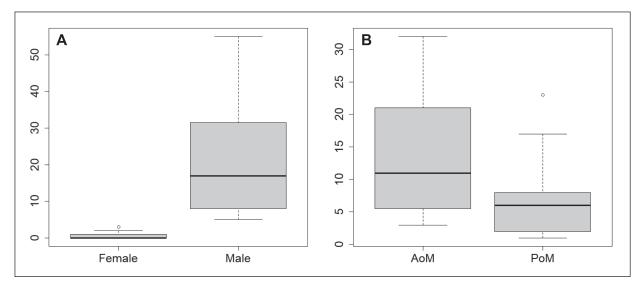


Figure 3: (A) Abundance of mites recorded on female and male millipedes, (B) abundance of mites recorded in the anterior (AoM) and the posterior (PoM) parts of male millipedes.

males because the same opportunities are present in females.

The fact that mites have been recorded only on males of large millipedes (*C. vumbaensis*, *D. flavifilis*, *D. rhodesianus*, *D. uncinatus*, *S. kruegeri* and *Zinophora* sp.) in different habitats is interesting because it demonstrates the mites' preference for males across different species. These observations further support those by Klompen & Austin (2007) and Klompen *et al.* (2013) who reported that phoretic mites were locality rather than host specific. For example, the widely distributed *D. uncinatus*, which was sampled in different habitats in the Miombo woodland carried mites in one area, which further strengthens the argument that the mites are not host specific.

Our observations demonstrate mites' clear preference for males as hosts, and for the anterior part of the millipede body over its posterior part. However, we have not directly observed spermophagy or mites' feeding on external secretions. The new records of associations documented in our study suggest that these associations are widespread and that more could be discovered as further habitats are surveyed. Thus, future studies may clarify this highly malebiased preference by mites.

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