SOIL ORGANISMS **86** (2) · August 2014 pp. 147–151

Surface-active millipedes (Diplopoda) and associated mites (Acari, Mesostigmata) in Pigeon Valley Nature Reserve in Durban, South Africa

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Received 10 May 2014 | Accepted 16 July 2014
Published online at www.soil-organisms.de 1 August 2014 | Printed version 15 August 2014

Abstract

Surface-active millipedes and associated mites were surveyed during two rainfall seasons in Pigeon Valley Nature Reserve (PVNR) in Durban, South Africa. Four millipede species, *Doratogonus cristulatus* (Porat, 1872) and *Orthoporoides pyrhocephalus* (C. L. Koch, 1865) (Spirostreptida, Spirostreptidae), *Centrobolus anulatus* (Attems 1934) (Spirobolida, Pachybolidae) and *Sphaerotherium giganteum* Porat, 1872 (Sphaerotheriida, Sphaerotheriidae) were recorded. All the species, except *D. cristulatus*, were arboreal. The sex ratio in *D. cristulatus* was strongly male-biased compared to *O. pyrhocephalus* and *Centrobolus anulatus*. Adult *Neomegistus julidicola* Trägårdh 1906 (Acari, Mesostigmata) were recorded only on males of *D. cristulatus* and *O. pyrhocephalus*. Incidence of mites on *D. cristulatus* and *O. pyrhocephalus* was 50 % and 6 %, respectively. The abundance, incidence and infestation intensity of mites on millipedes were higher at the beginning of the rainfall season when millipedes emerged than at any other time during the season. Surface-active females of *D. cristulatus* were uncommon and *N. julidicola* was found only on males. Most (50 %) of the mites were found on the anterior third of the male millipede body. The association between *O. pyrhocephalus* and *N. julidicola* is a new record. Although the results may not reflect the diversity of millipedes in PVNR because the sampling strategy did not include searching in the soil, they highlight the importance of carrying out surveys in urban reserves to provide data to inform biodiversity and conservation research.

Keywords incidence | abundance | phoresy | biodiversity | conservation

1. Introduction

Soil macroinvertebrates are probably under threat in many biomes because of climate change, increasing habitat modification, and anthropogenic habitat loss and habitat fragmentation. This is particularly the case in millipedes (Hamer & Slotow 2009) and indigenous earthworm species (Horn et al. 2007), which have a narrow distribution and occur in forests. As such, studying soil invertebrates needs to be prioritised because the lack of basic data or checklists impedes the conservation and monitoring of populations.

Although some millipedes are tree-dwellers (Minelli & Golovatch 2013), millipedes are a major part of soil macrofauna (Bueno-Villegas et al. 2008) in terms of the numbers of species and biomass (Dangerfield 1990). As detritivores, millipedes affect plant litter fragmentation (Golovatch & Kime 2009) and interact with other soil invertebrates. In southern Africa, millipedes are surface active during the summer rainfall season between October and March when they feed and mate (Dangerfield & Telford 1989, 1991). The high densities of surface-active millipedes and their large body size relative to other soil invertebrates (Dangerfield & Telford 1989) suggest a much greater ecological importance of millipedes in the tropics.

Inter-taxonomic associations among invertebrates are under-studied. Millipede-mite associations highlight

ISSN 1864-6417

the important roles millipedes play in ecosystems. Hunter & Rosario (1988) reported that the most common association is phoresy, in which the longest lived life stage is transported by the host and the life cycles of the host and phoretic are synchronised. According to Hunter & Rosario (1988) mites belonging to the suborder Mesostigmata have the highest number of associations with arthropods. Adult mite species representing 15 families are known to be in phoretic or parasitic association with millipedes (Gerdeman et al. 2000, Klompen et al. 2013). Mites are associated with the Spirobolida (Baker & Seeman 2008, Gerdeman et al. 2000), the Polydesmida (Kim & Klompen 2001) and the Spirostreptida (Lawrence 1939, Farfan & Klompen 2012). Although several studies have reported mite-millipede associations, millipedes are often not identified to genus or species (Farfan & Klompen 2012), probably because of the lack of taxonomic expertise (Hamer 1997, 1999). In addition, surveys of these associations in African millipedes are rare.

Urban nature reserves that are under the control of local governments are important in maintaining biodiversity. Thus green spaces, such as PVNR, one of eleven reserves in Ethekwini Municipality Area (EMA), are highly important for biodiversity studies. There are few checklists/records of soil invertebrates and associations in urban reserves, yet, baseline data provide valuable information for monitoring biodiversity. In view of this, the objectives of the study were to identify the millipedes, investigate surface activity periods of spirostreptid millipedes and record millipede-mite associations in PVNR throughout the rainfall season.

2. Materials and methods

The survey was carried out in Pigeon Valley Nature Reserve (PVNR) (29°51'52"S; 30°59'19"E) which covers 10 ha in Durban, Ethekwini Municipality Area, South Africa. The vegetation in PVNR is coastal climax forest dominated by *Ficus lutea* Vahl, *F. natalensis* Hochst., *Albizia adianthifolia* (Schumach.) W. Wight, *Chrysophylum viridifolium* J. M. Wood & Franks, *Chaetacme aristata* Planch. and *Celtis mildbraendii* Engl. Undergrowth is predominantly *Isoglossa ciliata* (Nees) Lindau. A thick plant litter layer (> 5 cm) occurs in most parts of the reserve.

Sampling of surface-active millipedes was along four 100 m long by 2 m wide transects on each sampling day during the 2012–2013 and 2013–2014 rainfall seasons between September and March. Searching along transects was the only sampling technique used because the local authority considered it less destructive in the reserve. Sampling was carried out a day after a rainfall event. Each transect was searched by two people, one on each side of the transect line for 30 minutes. Millipedes that were in the litter and walking on the surface and on tree trunks were picked up to determine the sex and record the presence of mites on the body. Each millipede was handled for about 2 minutes. Because of volvation in pill millipedes we could not look for mites on the ventral side or determine the sex of specimens that were encountered. A ladder was used to reach millipedes that were in the trees. Five males of each species were taken to the laboratory as voucher specimens for identification.

The presence of mites, their position and number on the millipede were recorded. In spirostreptids, the anterior third of the body was taken to be from the head to the 20th body ring, the midbody region was from the 21st to the 40th body ring and the posterior third was from the 41st to the anal ring. A magnifying glass was used to look for mites along the body. A toothbrush wetted with 99.9% ethanol was used to dislodge voucher mite specimens from the millipedes. All voucher specimens (millipedes and mites) were preserved in 99.9% ethanol to maintain DNA integrity. After each observation millipedes that were not taken as voucher specimens were returned to approximately the same place from where they had been picked.

Identification of the Spirostreptida was based on descriptions in Krabbe (1982) and Hamer (2000). Other millipedes were identified using dichotomous keys and guides. Mites were identified at Ohio University's Acarology Laboratory, USA.

The incidence (representing occurrence) and infestation intensity (average number of mites per infested millipede) of mites on millipedes were calculated.

3. Results

Adults of *Doratogonus cristulatus* (Porat 1872) and *Orthoporoides pyrhocephalus* (C. L. Koch 1865) (Spirostreptida, Spirostreptidae), *Centrobolus anulatus* (Attems 1934) (Spirobolida, Pachybolidae) and *Sphaerotherium giganteum* Porat 1872 (Sphaerotheriida, Sphaerotheriidae) were recorded in PVNR. All except *D. cristulatus* were arboreal. *Orthoporoides pyrhocephalus* was found mainly on *Ficus lutea*, *F. natalensis* and *Chrysophylum viridifolium*, the tree species that support several microhabitats in the form of phytotelmata and cavities in the trunks. *Centrobolus anulatus* and *S. giganteum* were found on more tree species than *O. pyrhocephalus*.

Unlike in *O. pyrhocephalus*, the sex ratio of surface-active *D. cristulatus* in the 2012–2013 season was male-biased. However, in the 2013–2014 season the sex ratios in *D. cristulatus* and *O. pyrhocephalus* were male-biased (Table 1). The sex ratio in *C. anulatus* was 1:1 in both seasons.

Adult mites, *Neomegistus julidicola* Trägårdh 1906 (Acari, Mesostigmata), were only recorded on *D. cristulatus* and *O. pyrhocephalus*. The association between *O. pyrhocephalus* and *N. julidicola* is a new record. The mite loads and the intensity of infestation on millipedes in PVNR were low (Table 1). The abundance, incidence and infestation intensity of mites on *D. cristulatus* peaked at the beginning of the rainfall season, followed by a steep decline. The peak abundance of mites followed that of *D. cristulatus* (Fig. 1). The number of mites found on each infested *D. cristulatus* male ranged between one and six. Mites were only found on the lateral side of the millipede body between the line of ozopores and the legs.

Approximately 50 % of the mites were located on the anterior third of the body, 17 % in the midbody region and 33 % on the posterior third of the body. The incidence of *N. julidicola* on *D. cristulatus* during the 2012–2013 season was 41 %. It was higher (45 %) in males (n = 33). No mites were recorded on females (n = 4) of *D. cristulatus*. In the 2013–2014 season the incidence of mites on all *D. cristulatus* specimens (n = 42) and *D. cristulatus* males (n = 41) was 50 % and 51 %, respectively. As in the previous season, no mites were found on females (n = 1). In *O. pyrhocephalus*, one male out of 17 was infested (Table 1).

Table 1. Incidence and infestation intensity of mites on *D. cristulatus* and *O. pyrhocephalus* in two rainfall seasons (n= sample size; SR=sex ratio).

	2012–2013				2013–2014			
	n	SR	Incidence(%)	Intensity	n	SR	Incidence (%)	Intensity
D. cristulatus	44	7:1	41	1.13	42	41:1	50	1.79
O. pyrhocephalus	29	0.8:1	0	0	17	1.5:1	6	2

Incidence = occurrence, Intensity= average number of mites per infested millipede

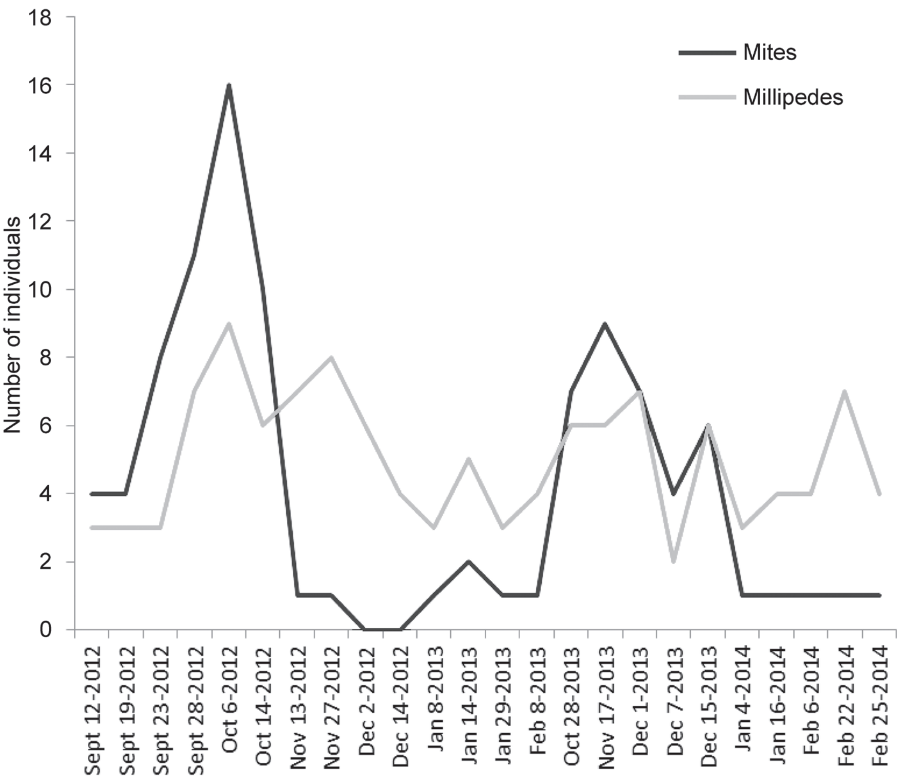

Figure 1. Within-season changes in abundance (per day of sampling) of *D. cristulatus* and associated *N. julidicola*.

4. Discussion

According to Hamer (2000) *D. cristulatus* occurs in small areas in KwaZulu-Natal province, South Africa where it has been collected in coastal forests, adjacent grasslands and suburban gardens. It is thus not surprising that *D. cristulatus* is the most abundant millipede in PVNR which has a pristine coastal forest. Unlike *O. pyrhocephalus*, *C. anulatus* and *S. giganteum*, which were never collected on the ground in the present study, individuals of *D. cristulatus* were not found in trees, thus contradicting Lawrence's (1939) observations in the Bluff. PVNR is approximately 10 km inland from the Bluff area. One possible explanation for the difference in observation may relate to the different times of sampling.

The sex ratio of *D. cristulatus* was strongly male-biased in the 2013–2014 rainfall season than in the one preceding. This could be explained by a longer 2012–2013 season which was six weeks earlier than the 2013–2014 season. The strongly male-biased sex ratio in *D. uncinatus* is likely due to differences in activity patterns of the sexes. Unlike males, females frequently burrow back into the soil to lay eggs whilst males remain on the surface searching for potential mates. Furthermore, because no soil sampling was done millipedes that could have been in the soil were not recorded. The sex ratios in the arboreal *O. pyrhocephalus* and *C. anulatus* were not biased towards males, which is possibly an adaptation to enhance frequent sexual encounters in patchy habitats. For *O. pyrhocephalus* the same marked individuals were recorded in the same tree at different times in the rainfall season. However, it is unknown whether arboreal species move between trees or food patches. Furthermore, *O. pyrhocephalus* was surface-active for a shorter period probably because of their vulnerability to changes in physical conditions in the phytotelmata towards the end of the season. Therefore, understanding the activity patterns of millipedes warrants further studies, since these data may be useful in following responses to habitat or climate change and understanding implications on inter-taxonomic interactions.

Lawrence (1939) reported associations involving two millipede and two mite species in the Bluff. Trägårdh (1907) also reported an association of *N. julidicola* and *Paramegistus confrater* Trägårdh 1906 mites with millipedes. In PVNR, only *N. julidicola* was recorded, whereas *P. confrater* which was present in the Bluff area was not found in PVNR on any of the millipedes. The high incidence of *N. julidicola* on *D. uncinatus* males suggests host preference and specificity. That the association is phoresy and host-specific is supported by more males of *D. uncinatus* being infested. Low incidence of *N. julidicola* on *O. pyrhocephalus* suggests that the association may be accidental. Although present throughout the rainfall season, the peak abundance of *N. julidicola* and *D. uncinatus* was in the first month of the rainfall season (Fig. 1). Presumably the activity of *N. julidicola* adults is synchronized with the surface activity of *D. uncinatus*. This is consistent with Hunter and Rosario (1988) who reported that the life cycle of mites is synchronised with that of the host if the association is close.

The high incidence and abundance of *P. confrater* and *N. julidicola* on males of *D. uncinatus* in the Bluff (Lawrence 1939) and in PVNR further support the view that males are preferred hosts. However, it can be argued that the high incidence (particularly in PVNR) may be the result of a male-biased sex ratio in *D. uncinatus* which inevitably increases the probability of encounters with mites rather than evidence for preference. Given that *P. confrater* was found on females (Lawrence 1939), one can speculate that the absence of *P. confrater* may be linked to the very low numbers of *D. uncinatus* females recorded in PVNR. Although the mite load and abundance on millipedes in PVNR is low, the high incidence of mites is comparable to figures reported by Klompen et al. (2013).

That mites were found only on males and the majority (50%) of mites occurred on the anterior third of the body seems to support Lawrence's (1939) extraordinary suggestion that mites feed on seminal fluids produced when millipedes mate. However, no spermophagy has been observed in mites (Baker & Seeman 2008). The position (between the line of ozopores and legs) where mites were found suggests that mites may feed on defensive secretions of millipedes, as reported by Trägårdh (1907), a possibility rejected by Lawrence (1939) because the secretions are not nutritious.

Although one sampling method was used in this survey, which may have resulted in some species/individuals being missed, this study has highlighted the importance of nature reserves in urban areas where biological diversity is threatened by urban sprawl and other human activities. Populations of fauna may be small and isolated, but they represent taxonomic diversity that would otherwise be lost in the absence of protected areas. As such, this study is an important contribution to data on the species composition and associations among soil invertebrates. These urban reserves provide sites that can be used to monitor changes in species composition and dominance over time.

5. Acknowledgments

Ethekwini Municipality gave permission to carry out the survey in Pigeon Valley Nature Reserve. Ntokozo Sishi, Tawanda Tinago and Nokukhanya Mhlongo assisted during field work. The University of KwaZulu-Natal is thanked for logistical and financial support. Sergei Golovatch and Owen Seeman are thanked for their suggestions and comments on an earlier draft of this manuscript.

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