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ORIGINAL ARTICLE

Seasonal activity of millipedes (*Diplopoda*) – their economic and medical significance

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Abstract

The millipede *Brachydesmus superus* Latzel, *Polydesmus inconstans* Latzel (Diplopoda: Polydesmida) and *Kryphioiulus occultus* C. L. Koch (Diplopoda: Julida) were collected from compost in gardens in Lublin, eastern Poland. Collections were made by using pitfall traps between April – September 2009 and 2010. *Brachydesmus superus*, *Polydesmus inconstans* and *Kryphioiulus occultus* play a significant role in composting of plant residues. *Cylindroiulus caeruleocinctus* Wood and *Ommatoiulus sabulosus* Linnaeus (Diplopoda: Julida*)* were collected manually in 2009-2011 in fallows and ruderals of Lublin and Kraków. *C. caeruleocinctus* and *O. sabulosus* caused considerable nuisance during mass occurrence and migration in human residences. The sex ratio has been determined for populations of *C. caeruleocinctus*, total sex ratio average 1:1.46. The number of females prevailed. Millipedes of the temperate climate have two peaks in the spring and autumn pattern of activity of the year. Both common species *C. caeruleocinctus* and *O. sabulosus* were analysed bacteriologically. The millipede *Cylindroiulus caeruleocinctus* transmits *Citrobacter freundii, Pantoea agglomerans, Serratia marcescens, Raoultella planticola, Salmonella arizonae.* The millipede *Ommatoiulus sabulosus* transmits *Citrobacter freundii, Pantoea agglomerans, Raoultella planticola* and *Xanthomonas maltophila*.

Key words

Diplopoda, periodical activity, compost, vectors of diseases, urban areas

INTRODUCTION

Most millipedes are detritivores, their role is decomposition of organic matter. Certain species feed on living plants and can be considered as plant pests [1]. Millipedes constitute the main epigeic macrofauna group in temperate forests. Diplopoda feeding activity is specialized on dead organic matter, or on saprophytic organism consumption [2]. Millipedes till the soil, mix it with the leaf litter, draw them inside the soil to the burrows and bring the organic matter from underneath to the top soil, to transform it into proper type of humus [3].

The economic significance of millipedes should be considered as producers of compost in organic farming, and pests for crops, e.g. sugar-beet, Irish and sweet potatoes, maize, strawberries, groundnuts, carrots, tapioca, coffee, sunflowers and corn [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. Some synanthropic species of millipedes live in compost, e.g. *Blaniulus guttulatus, Choneiulus palmatus, Cylindroiulus parisiorum, Cylindroiulus britannicus, Polydesmus denticulatus* and *Kryphioiulus occultus* [15, 16, 17]. Saprophagous fauna play a significant role in the composting of plant residues. The millipede *Arthrosphaera magna* process the plantation litter (Areca leaves, Cocoa leaves and leaves of Acacia) efficiently and the composting of plant residues for organic farming [18]. Millipede compost could play a vital role in soil enrichment in terms of nutrition and soil microflora. Millipede compost derived from various organic substances showed significant enhancement in the physiochemical and biochemical constituents [19]. Diplopoda become noxious for humans in synanthropic areas during their mass occurrence and migrations in urban areas [20, 21, 22, 13, 23, 24]. The striped millipede *Ommatoiulus sabulosus* transmits *Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter sakazaki, Citrobacter freundii* and *Hafnia alvei* [14].

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MATERIAL AND METHODS

The millipede *Cylindroiulus caeruleocinctus* Wood and *Ommatoiulus sabulosus* Linnaeus (Diplopoda:Julida*)* were collected manually in 2009-2011 (May, June and September) in fallows and ruderals of Lublin and Kraków. Both common species *C. caeruleocinctus* and *O. sabulosus* were analysed bacteriologically. *Brachydesmus superus* Latzel, *Polydesmus inconstans* Latzel (*Diplopoda: Polydesmida*) and *Kryphioiulus occultus* C. L. Koch (*Diplopoda:Julida*) were collected from compost in gardens and the Botanical garden of Marie Curie-Sklodowska Universtity (UMCS) in Lublin. Collections were made by using pitfall traps between April – September 2009 and 2010.

In the laboratory, the millipedes were identified to species and determinated to sex using a stereoscopic microscope (Stemi DV-4 Zeiss with the use of literature key by Stojałowska [25] and Blower [26]). Adult specimens of *Cylindroiulus caeruleocinctus* and *Ommatoiulus sabulosus* were placed in a sterile container, 50 ml of distilled sterile water was used and the millipedes were washed. They were then killed by immersion in ether, and their surfaces sterilized with ethanol (70%). The millipedes were cut with a sterile scalpel at the end of the telson, in order to obtain the intestine. The intestinal contents were placed into 2 ml of broth medium. Suspension was precisely mixed and left to precipitation. Supernatant was collected with a sterile syringe and a volume of 0.2 ml inoculated onto the following media: Columbia agar with 5% sheep blood, Chapman medium, McConkey medium with crystal violet. The Petri dishes were placed in a thermostat at a temperature of 35^{0}C for 24 hours. Afterwards, selected colonies underwent biochemical identification with the use of API 20E tests (BioMerieux).

Statistical analysis. Statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL). The differences between groups were evaluated using the Chi-square Test for Independence. In assessing whether the numbers of millipedes in subsequent months of the year are uniformly distributed, the Chi-Square Goodness of Fit Test was used. p-values of less than 0.05 (2-sided) were considered statistically significant.

RESULTS

Cylindroiulus caeruleocinctus and *Ommatoiulus sabulosus* (Julida) caused considerable nuisance during mass occurrence and migration in human residences on walls of houses, surrounding open habitats, especially meadows and fallows in the Lublin and Kraków areas. Distribution of *C. caeruleocinctus* and *O. sabulosus* was connected with a low amount of humus in soil and a high degree of artificiality in both study sites.

C. caeruleocinctus indicated activity during April – September, with a decline in activity in the summer months. The seasonal number of *C. caeruleocinctus* showed two peaks in the spring and autumn pattern of activity of the year. For each season, the average data of *C. caeruleocinctus* activity from the three years (2009-2011) are similar, as differences between years were insignificant. The sex ratio has been determined for populations of *C. caeruleocintus:* sex ratio average 1:1.44 (Tab. 1), sex ratio average 1:1.51 (Tab. 2) sex ratio average 1:1.43 (Tab. 3), respectively. The number of females prevailed (Tab. 1, 2, 3).

Table 1. *Cylindroiulus caeruleocinctus* in Lublin (2009)

Sample	Sex						Total		Sex ratio
	Females		Males		Juveniles				
	n	%	N	%	n	%	n	%	
1	41	47.7	28	32.6	17	19.8	86	100.0	1 : 1.46
2	54	49.5	40	36.7	15	13.8	109	100.0	1 : 1.35
3	39	52.0	25	33.3	11	14.7	75	100.0	1 : 1.56
Total	134	49.6	93	34.4	43	15.9	270	100.0	1 : 1.44

p = 0.8.
Differences are not statistically significant in the case of sex of specimens collected in samples.

Table 2. *Cylindroiulus caeruleocinctus* in Lublin (2010)

Sample	Sex						Total		Sex ratio
	Females		Males		Juveniles				
	n	%	n	%	n	%	n	%	
1	71	49.7	60	42.0	12	8.4	143	100.0	1 : 1.18
2	38	55.1	16	23.2	15	21.7	69	100.0	1 : 1.38
3	42	49.4	24	28.2	19	22.4	85	100.0	1 : 1.75
Total	151	50.8	100	33.7	46	15.5	297	100.0	1 : 1.51

p = 0.004.
Differences are statistically significant in case of sex specimens collected in samples (p = 0.004). In sample 1 a higher percentage of male specimens and lower of juvenile ones has been found than in sample 2 (p=0.003) and 3 (0.006). Whereas, samples 2 and 3 did not differ in terms of sex ratio in collected specimens (p = 0.74).

Table 3. *Cylindroiulus caeruleocinctus* in Lublin (2011)

Sample	Sex						Total		Sex ratio
	Females		Males		Juveniles				
	n	%	n	%	n	%	n	%	
1	32	45.1	25	35.2	14	19.7	71	100.0	1 : 1.28
2	46	49.5	34	36.6	13	14.0	93	100.0	1 : 1.35
3	38	49.4	22	28.6	17	22.1	77	100.0	1 : 1.73
Total	116	48.1	81	33.6	44	18.3	241	100.0	1 : 1.43

p = 0. 61.
Differences are not statistically significant in terms of sex of specimens collected in samples.

Table 4. *Cylindroiulus caeruleocinctus* in Lublin 2009-2011

Year	Sex						Sex ratio
	Females		Males		Juveniles		
	n	%	n	%	N	%	
2009	134	49.6	93	34.4	43	15.9	1 : 1.44
2010	151	50.8	100	33.7	46	15.5	1 : 1.51
2011	116	48.1	81	33.6	44	18.3	1 : 1.43
Total	401	49.6	274	33.9	133	16.5	1 : 1.46

p = 0. 92.
Differences are not statistically significant in case of sex of specimens collected in 2009-2011.

Table 5. Intestinal dominant bacteria species of the millipede *Cylindroiulus caeruleocinctus.*

Dominant bacteria species	% of identification	
	2010	2011
Citrobacter freundii	99.1	99.3
Pantoea agglomerans	99.2	98.9
Serratia marcescens	97.5	98.2
Raoultella planticola	97.3	98.4
Salmonella arizonae	98.5	99.2

Table 6. Intestinal dominant bacteria species of the millipede *Ommatoiulus sabulosus*

Dominant bacteria species	% of identification	
	2010	2011
Citrobacter freundii	99.4	99.1
Pantoea agglomerans	97.8	98.4
Raoultella planticola	98.8	99.4
Xanthomonas maltophila	99.1	98.7

Millipedes *Brachydesmus superus, Polydesmus inconstans* and *Kryphioiulus occultus* take part in soil humification and compost production in gardens. Therefore, *B. superus, P. inconstans* and *K. occultus* preferred humic soil, the sites of its occurrence characterized by a low amount of litter on the soil. During the seasonal activity of abundant millipede species *Brachydesmus superus*, *Polydesmus inconstans* and *Kryphioiulus occultus*, a total of 509 specimens were collected in 2009 and a total of 1130 specimens were collected in 2010 (Tab. 7). In both study years (2009, 2010), the number of *B. superus* and *P. inconstans* showed one peak in spring, and the number of *Kryphioiulus occultus* showed one peak in the autumn pattern of activity of the year.

Table 7. Number of millipede species in gardens of Lublin in individual months[A]

Milipede Species	Months		2009		2010		Significance
			n	%	n	%	
Brachydesmus superus	Month	April	2	1.5	125	17.8	0.000001
		May	54	41.2	250	35.5	
		June	36	27.5	182	25.9	
		July	27	20.6	115	16.3	
		August	2	1.5	30	4.3	
		September	10	7.6	2	0.3	
	Total		131	100.0	704	100.0	
Polydesmus inconstans	Month	April	16	7.3	24	14.0	0.000005
		May	20	9.1	13	7.6	
		June	108	49.1	81	47.4	
		July	60	27.3	32	18.7	
		August	2	0.9	19	11.1	
		September	14	6.4	2	1.2	
	Total		220	100.0	171	100.0	
Kryphioiulus occultus	Month	April	8	5.1	19	7.5	0.0017
		May	12	7.6	31	12.2	
		June	52	32.9	71	27.8	
		July	7	4.4	36	14.1	
		August	6	3.8	17	6.7	
		September	73	46.2	81	31.8	
	Total		158	100.0	255	100.0	

[A] The numbers of millipede species in subsequent months were assumed to be uniformly distributed. In each year of observation and for each species the chi-square value for differences between expected and observed counts of the species in the subsequent months was extremely significant ($p < 0.0000001$).

DISCUSSION

Diplopoda of the temperate climate have bimodal activity during a year. Besides the spring and autumn activity peaks, there is a lower summer activity period [25, 27, 28] as indicated in species of millipedes in the presented study. Specimens of *Brachydesmus superus, Polydesmus inconstans* and *Kryphioiulus occultus* were found in compost. Their decline of activity was observed in August, which can be explained by the summer aestivation of millipedes. *Brachydesmus superus, Polydesmus inconstans* and *Kryphioiulus occultus* are common synanthropic species in gardens, compost heaps and parks of the Lublin area [17], which was confirmed at similar sites in Warsaw [29].

Previous studies confirmed that *B. superus* and *P. inconstans* are eurytopic species occurring in gardens, parks, greenhouses and lawns [15, 29, 30, 31, 32, 33, 34]. *Brachydesmus superus* was found in a shaded and wet clump of trees, under pieces of tree bark lying on wet soil [35], as indicated in areas of Lublin. According to Berg and Hemerik [36] and Voigtländer [37] *B. superus* preferred hygrophilic soil. The Lublin data support this, although its numbers in compost samples vary considerably from place to place. *B. superus* is one of the best known species in cultivated land, where it is sometimes a nuisance, feeding on crops [38]. *Kryphioiulus occultus* is stenotopic species inhabits grassland, meadows, fields and fallows [28, 37]. *Cylindroiulus caeruleocinctus* and *Ommatoiulus sabulosus* are eurytopic species that live in fallows, fields, meadows, gardens and parks, sometimes in natural biotopes [13, 17, 29, 32, 34, 39, 40, 41]. *Ommatoiulus sabulosus* indicated the most activity in May and June [22, 27]. This is a species without ecological preferences [36, 37], confirmed by data from Kraków [23].

In southern Finland specimens of *C. caeruleocinctus* were collected from the walls of a building, in the soil around the house, as well as in the neighbouring oak forest. Samšinak [20] and Lehtinen and Terhivuo [42] also noticed a great population of *C. caeruleocinctus* on the walls of a house. *C. caeruleocinctus* is a xerobiont open land species with preference for fields, abandoned fields, and fallows, with their highest activity in spring and autumn [27, 37, 43], as confirmed by data from Lublin [13, 17]. Distribution of *C. caeruleocinctus* has connected with a low amount of humus in soil and a low amount of litter [44], which is confirmed by present results. In the presented study, the average sex ratio is 1:1.46 in *C. caeruleocinctus,* and for comparision 1:1.25 for females/males in previous data from Lublin [13].

Millipedes are sanitarily important in urban environments. The presence of *Citrobacter freundii* has been seen in the intestine of *C. caeruleocinctus* and *O. sabulosus* (Tab.5, 6), which confirmed previous studies on millipedes *Xenobolus carnifex* [45] and *Ommatoiulus sabulosus* [14]. The *Citrobacter* species cause a wide spectrum of infections in the urinary tract, blood, superficial wounds, skin and peritoneum [46]. *C. freundii* has been associated with neonatal meningitis and brain abscesses. The mortality and morbidity rate of *Citrobacter* meningitis is high [47].

The presence of *Pantoea agglomerans* in the intestines of *C. caeruleocinctus* and *O. sabulosus* (Tab.5, 6) are in accordance with previous studies on millipedes published by Byzov et al. [48] and Jarosz and Kania [49]. *Pantoea agglomerans* is the most commonly isolated species of bacillus in humans. *P. agglomerans* bacteremia has been described in association with the contamination of intravenous fluid, parenteral nutrition and blood products. *P. agglomerans* is the cause of infections in children that involve the bloodstream, soft tissues and bones/joints [50, 51]. *Pantoea agglomerans* septicemia and deaths of newborn infants has also been described [52].

Enterobacter species are associated with wound, intra-abdominal, respiratory, urinary and blood stream infections, representing an increasingly important nosocomial pathogen [53]. *Serratia marcescens* has been isolated in *C. caeruleocinctus* (Tab. 5). *S. marcescens* is well-known as the cause of urinary tract infections, wound infections, meningitis, pneumonia, septicemia, and endocarditis [54, 55]. *S. marcescens* can be responsible for nosocomial infections, mostly in immunocompromised hosts [56]. There have been frequent reports of *S. marcescens* outbreaks in intensive care and neonatal care units [57, 58].

Raoultella planticola has been isolated in *O. sabulosus* and *C. caeruleocinctus* (Tab.5, 6). *R. planticola* is a cause of human infections, e.g. of the soft tissues and severe pancreatitis [59, 60]. *Salmonella arizonae* has been isolated in *O. sabulosus.* A fatal case of *Salmonella enterica* subsp. *arizonae* gastroenteritis has been described in an infant with microcephaly [61]. Human infections of *S. arizonae* have been reported in hosts with impaired immune systems caused by conditions such as collagen vascular diseases requiring immunosuppressive therapy, malignancy, organ transplantation and human immunodeficiency virus (HIV) infection. *Xanthomonas maltophila* has been isolated in *C. caeruleocinctus*. *Stenotrophomonas* (*Xanthomonas*) *maltophilia* has been recognized with necrotizing pancreatitis [62], and cases of early onset neonatal sepsis [63].

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