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Millipedes diversity and their relationship with soil properties in Nakhon Ratchasima province, Thailand

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The species diversity of millipedes was studied at Suranaree University of Technology in Nakhon Ratchasima, Thailand. The millipedes and soil samples were collected in the same 20 x 20 m sampling sites from three forest types i.e. degraded forest, plantation forest and farm land. The hand sorting method was employed to collect the millipedes by simple random sampling during September 2014 – August 2015. Identification of millipedes was based on external morphology. Six species of five families were found in the studied areas. *Zephronia siamensis*, the only member of the Zephroniidae had the highest population density followed, by *Cylindroiulus* sp. and *Lithostrophus segregatus*. The highest population density was found in farm land (17.67 individuals/m^2) followed by plantation forest (13.83 individuals /m^2) whilst lowest population density was found in degraded forest (9.83 individuals/m^2). The highest index of diversity (Shannon - Wiener index) was 1.49 found in farm land but the highest species richness (6) was found in plantation forest and degraded forest. The present study demonstrated that the millipede densities were positively significantly correlated with phosphorus, organic matter, soil pH, silt and clay. However, they were negatively correlated with sand. *Thyropygus cuisinieri* was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.371$), *Lithostrophus segregatus* was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.423$), soil pH ($p < 0.05$; $r = 0.373$), silt ($p < 0.05$; $r = 0.401$) and clay ($p < 0.05$; $r = 0.388$) *Cylindroiulus* sp. was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.377$).

Keywords: Millipedes diversity, soil properties, Suranaree University of Technology

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Introduction

Millipedes are classified in the Kingdom Animalia, Phylum Arthropoda, Class Diplopoda, with the most legs of any terrestrial animals. They are secretive and nocturnal detritivores that subsist on decaying vegetation. Negative phototactic, they avoid light and live beneath leaves, stone, bark and logs. Millipedes are common in soil and leaf litter and some millipedes are cave dwellers. They vary from 2 mm to almost 30 cm in length. The distribution is cosmopolitan but they are especially abundant in the tropics (Ruppert *et al.*, 2004).

Millipedes are the major saprophagous macroarthropods in temperate and tropical ecosystems (Lawrence, 1984; Blower, 1985; Lawrence and Samways, 2003). Their abundance and diversity facilitate soil mineralization through mechanical fragmentation of plant litter and release of essential elements (Wallwork, 1976; Hopkin and Read, 1992; Dangereld and Milner, 1996). Even though only 10% of total decomposition of plant litter within an ecosystem takes place through millipedes. Their feeding enhances microbial activities, resulting in breaking down of litter up to 90% (Anderson and Bignall, 1980).

Thailand locates in the tropical region which encompasses diverse kinds of natural ecosystems. These natural habitats are homes to some of the world's richest and unique plants and animals, resulting in a high diversity of millipedes. However, the studies in biology and ecology dealing with millipedes in Thailand have been lagging behind due to the limited knowledge of their morphology, taxonomy and the role in ecosystems. This investigation provides information of millipedes on species diversity, distribution, and their relationship with soil properties at Suranaree University of Technology (SUT) in Nakhon Ratchasima, Province, Thailand.

Objectives: This study aimed to compare the species diversity, distribution of millipedes in different forest types (degraded forest; DF, plantation forest; PF and farm land; FL) at Suranaree University of Technology, Nakhon Ratchasima, Thailand and their relationship with soil properties.

Materials and methods

Sampling sites

The research was conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand. The study site, (as shown in Fig 1) consists of three types different of forest, namely degraded forest (DF), plantation forest (PF) and farm land (FL).

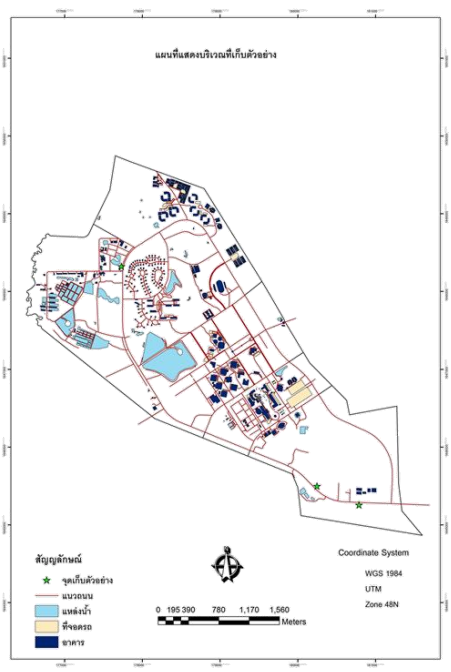


Fig. 1 Study site at Suranaree University of Technology.

Millipedes density

Millipedes and soil samples were collected monthly by hand sorting from degraded forest, plantation forest and farm land during September 2014 – August 2015 to determine millipedes diversity in these areas. Three sampling sites in each forest type of SUT were used to investigate the species diversity and distribution of millipedes, and soil properties. The sampling method involved the selection of a good stand sampling area and establishment of the permanent plot of 20 m x 20 m. The area was divided into 10 rows and each row further divided into 5 sample plots of 2 m x 2 m for each month study (Fig 2). Soil samples (12 cm x 12 cm x 12 cm) were removed from each quadrat and handsorted to collect millipedes.

The millipedes were anesthetized with 10% ethanol and put into containers of 70% ethanol for preservation pending identification at the laboratory of Centre for Scientific and Technological Equiment, Suranaree University of Technology. Millipedes were identified to species level, or in some cases, even genus level as for comparing with the collections of Museum of Zoology, Chulalongkorn University, Bangkok, Thailand (CUMZ). For

identification, a Nikon SM-800 stereo microscope, and the relevant publications (Blower, 1985; Schubart, 1934) were used.

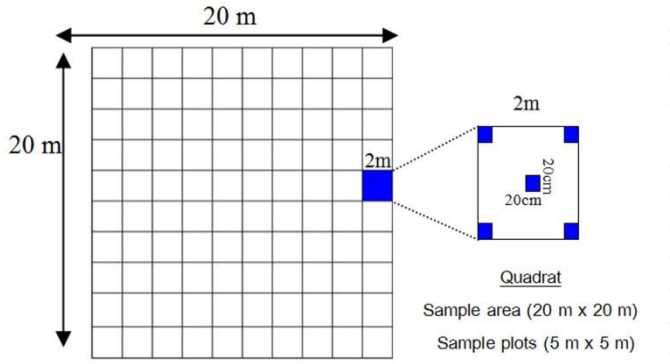


Fig 2 Sampling grid design used for sampling the milipedes within each quadrat.

Soil properties

Soil properties were measured and analysed in this study included soil type, soil pH, organic matter, potassium, sand, silt clay and phosphorus. These data and results were used to evaluate the abundance and distribution of the millipedes and relationships between millipedes and soil properties.

Soil sampling and analysis

In each forest type, soil samples of 0-12 cm depth and 500 g, were collected from five quadrats (where millipedes were sampled) and then these 3 soil samples were mixed together in a plastic bag to make one sample for subsequent analyses. After extracting millipedes from all soil samples, the soil samples were carried to Center for Scienetific and Technological Equipment, Suranaree University of Technology Laboratory, where various analyses were conducted. The soil samples were dried indoor under laboratory conditions for 24 hours. The soil were crushed using a pestle and mortar and filter-tipped with a 2 mm sieve, rejecting roots and stones to give the fine earth fraction.

Data Analysis

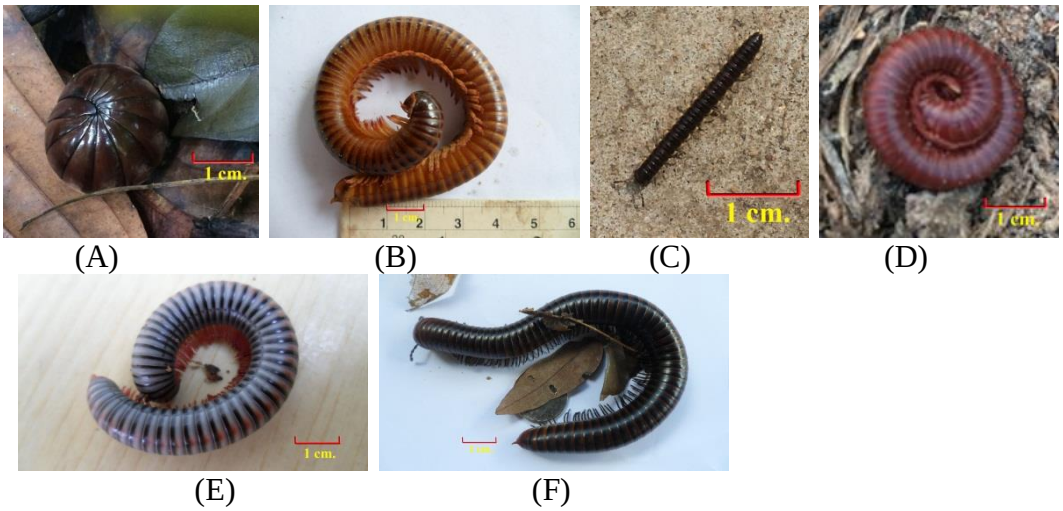
Evenness, richness and Shannon-Wiener index were used to compare the diversity of millipedes among studied sites. Difference in millipede number and species abundance among forest types were analyzed with ANOVA. The

Pearson correlation was employed to find relationship among soil properties and millipede species.

Results

Species diversity and distribution

Total six species belonging to five genera, within five families (Zephroniidae, Harpagophoridae, Pachybolidae, Paradoxosomatidae and Julidae) were collected (Table 1). The *Zephronia siamensis* (Fig 3A) had the highest number of individuals and distributed all over the areas. All millipede species were found in degraded forest and plantation forest. The greatest species evenness was 0.15 in farm land. The highest Shannon-Wiener index (H') was 1.49 in farm land and the lowest was 1.21 in plantation forest. The species richness of all area types ranged from five to six species (Table 2).



(A) (B) (C) (D)

(E) (F)

Fig 3 The millipedes at the Suranaree University of Technology (SUT)
(A) *Zephronia siamensis* (B) *Thyropygus allevatus*
(C) *Orthomorpha coarctata* (D) *Cylindroiulus* sp.
(E) *Lithostrophus segregatus* (F) *Thyropygus cuisinieri*

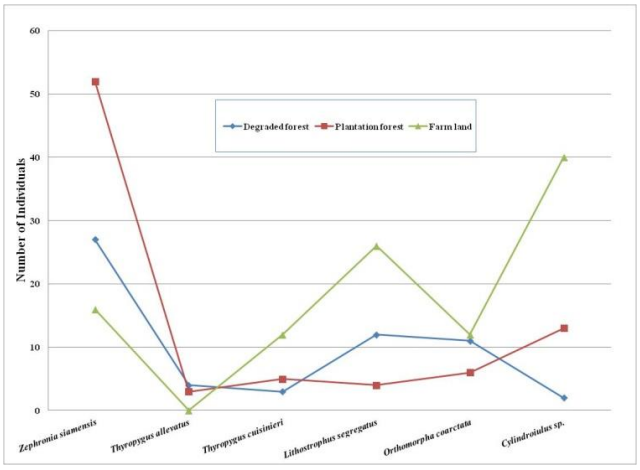


Fig 4 The millipedes at the Suranaree University of Technology (SUT).

Table 1. Distribution and diversity of millipedes in different forest types.

Millipedes	Forest types			Average
Family/species	DF	FL	PF	
Zephroniidae				
Zephronia siamensis	27	16	52	31.67
Harpagophoridae				
Thyropygus allevatus	4	-	3	2.33
Thyropygus cuisinieri	3	12	5	6.67
Pachybolidae				
Lithostrophus segregatus	12	26	4	14.00
Paradoxosomatidae				
Orthomorpha coarctata	11	12	6	9.67
Julidae				
Cylindroiulus sp.	2	40	13	18.33
Average	9.83	17.67	13.83	13.78

DF = degraded forest FL = farm land PF = plantation forest

Table 2. Species diversity and evenness of millipedes in different forest types.

Index	Forest types		
	DF	FL	PF
Species richness	6	5	6
Evenness	0.12	0.15	0.10
Species Diversity (H)	1.44	1.49	1.21

DF = degraded forest FL = farm land PF = plantation forest

Millipede density

A total of 248 millipedes were collected in all studied areas. The highest millipede density was *Zephronia siamensis* (average 31.67 individuals/m^2) followed by *Cylindroiulus* sp. (average 18.33 individuals/m^2) and *Lithostrophus segregatus* (average 14.00 individuals/m^2). The lowest was *Thyropygus allevatus* (average 2.33 individuals/m^2). These results are shown inTable 3.

Millipede densities found at the SUT were particularly high, reaching 13.78 individuals per square metre. Generally more species of millipedes were found in farm land than in the other forests.

Table 3. The mean (±SE) of soil properties in tree forest types.

Factor-Parameter	Forest types			Total
	DF	FL	PF	
potassium	163.55±29.44[c]	383.97±95.02[c]	61.16±6.56	202.89±40.67
phosphorus	22.92±4.67	78.14±19.27[ac]	16.41±5.42	39.16±8.38
organic matter	2.91±0.47	2.45±0.49	1.70±0.36	2.35±0.26
soil pH	7.12±0.16[c]	7.69±0.20[c]	5.94±0.18	6.92±0.17
sand	87.64±2.87	77.14±3.36	92.54±1.49[b]	85.77±1.92
silt	7.00±1.03	11.60±1.38[ac]	6.40±1.12	8.33±0.79
clay	5.36±1.89	11.26±2.13[c]	1.06±0.53	5.89±1.21
soil type	1.80±0.29	2.60±0.43	1.90±0.10	2.10±0.18

Remark: Significant difference is indicated by different small letter at P<0.05 for Oneway ANOVA
DF = degraded forest FL = farm land PF = plantation forest

Relationship of millipedes and soil properties

The millipede densities were positively significantly correlated ($p< 0.05$) with phosphorus, organic matter, soil pH, silt and clay. However, they were negatively correlated with sand (Table 4). The *Thyropygus cuisinieri* was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.371$), *Lithostrophus segregatus* was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.423$), soil pH ($p < 0.05$; $r = 0.373$), silt ($p < 0.05$; $r = 0.401$) and clay ($p < 0.05$; $r = 0.388$) The *Cylindroiulus* sp was also exhibited positively significant correlation with phosphorus ($p < 0.05$; $r = 0.377$). However, *Zephronia siamensis* was negatively correlated with potassium (Table 5).

Tables 4. The correlations among millipede densities and their relationships with soil properties.

	millipedes density	potassium	phosphorus	organic matter	soil pH	sand	silt	clay
potassium	-.090							
phosphorus	.275	.707**						
organic matter	-.051	.450*	.240					
soil pH	.074	.412*	.201	.201				
sand	-.117	-.606**	-.290	-.419*	-.590**			
silt	.137	.509**	.259	.390*	.509**	-.933**		
clay	.095	.626**	.289	.408*	.601**	-.972**	.823**	
soil type	-.124	.454*	.181	.170	.051	-.511**	.385*	.557**

Pearson correlation coefficient: *, ** significant at $p < 0.05$ and $p<0.01$, respectively

Table 5. The correlations among millipede species and soil properties.

	k	p	om	pH	sand	silt	clay	Soil type
Zephronia siamensis	-0.244	-0.052	0.019	-0.230	0.182	-0.082	-0.235	-0.372*
Thyropygus allevatus	-0.132	-0.169	0.081	-0.039	0.023	0.011	-0.043	-0.323
Thyropygus cuisinieri	0.021	0.371*	-0.172	0.247	-0.140	0.048	0.190	-0.078
Lithostrophus segregatus	0.128	0.423*	0.005	0.373*	-0.411*	0.401*	0.388*	-0.004
Orthomorpha coarctata	-0.025	0.239	0.157	0.160	-0.157	0.114	0.174	-0.140
Cylindroiulus sp.	-0.003	0.377*	-0.189	0.132	-0.203	0.186	0.200	0.236

Pearson correlation coefficient: *, ** significant at $p < 0.05$ and $p < 0.01$, respectively

Discussion

Millipede densities found at the SUT were particularly high, reaching 13.78 individuals per square metre. The highest millipede density was *Zephronia siamensis* (average 31.67 individuals/m2). Generally more species of millipedes were found in farm land than in the other forests. The millipede densities were positively significantly correlated ($p< 0.05$) with phosphorus, organic matter, soil pH, silt and clay. However, they were negatively correlated with sand. These results supported by Ashwini and Sridhar (2006) who reported that millipede abundance as well as biomass of millipedes in plantation were

positively correlated (P = 0.001) with soil moisture and soil temperature. Abundance was positively correlated with soil phosphate (P = 0.01) and biomass with calcium (P = 0.01). Biomass of millipedes in plantation was negatively correlated with soil pH (P = 0.01) and potassium (P = 0.05). The most detailed work relating distribution of millipedes to their environment has been conducted by Kime and co-workers in Belgium. They concluded that distribution of soil-dwelling millipedes were a function of edaphic and climatic factors (Kime et al., 1991). Moreover, the important factors influence millipedes distribution were soil texture, soil water content, temperature, mineral content (especially calcium and magnesium), humidity and humus type (Kime, 1991).

Conclusion

Six millipede species were found in this study. They belonged to the following five families: Zephroniidae, Harpagophoridae, Pachybolidae, Paradoxosomatidae and Julidae. The most abundant was *Zephronia siamensis* which presented in all study sites. The rarest species was *Thyropygus allevatus* which found in degraded forest and plantation forest. All millipede species were presented in degraded forest and plantation forest. The greatest species evenness was 0.15 in farm land. The highest Shannon-Wiener index (H') was 1.49 in farm land and the lowest was 1.21 in plantation forest. The species richness of all area types ranged from five to six species. The *Thyropygus cuisinieri* was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.371$), *Lithostrophus segregatus* was also positively significantly correlated with phosphorus ($p < 0.05$; $r = 0.423$), soil pH ($p < 0.05$; $r = 0.373$), silt ($p < 0.05$; $r = 0.401$) and clay ($p < 0.05$; $r = 0.388$). The *Cylindroiulus* sp. was positively significant correlated with phosphorus ($p < 0.05$; $r = 0.377$). However, *Zephronia siamensis* was negatively correlated with potassium.

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