
Distribution of harpagophorid millipedes in different tropical forest types

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The distributions of harpagophorid millipedes were examined in different forest types in Sakaerat Environmental Research Station (SERS), NakhonRatchasima, Thailand. All millipedes were collected between June 2010 to May 2011 from each permanent plot of 20 m x 20 m (400 m²). For each plot, the millipedes were collected in a small plot of 30x30x30 cm³ within four forest types: dry evergreen forest (DEF); dry dipterocarp forest (DDF); plantation forest (PTF); and ecotone (ECO). The results revealed that the highest index of diversity (Shannon - Wiener index) was 1.67 and the highest species richness (6) was found in the ecotone of dry evergreen forest and the dry dipterocarp forest (ECO). The most abundance of this family was found in DEF during raining season ($p \leq 0.05$). This work suggested that the DEF had a good factor for supporting the distribution of harpagophorid millipedes in Sakaerat Environmental Research Station. Harpagophorid millipedes diversity was negatively correlated with light intensity, soil pH, soil temperature, soil moisture and litter moisture, while organic matter showed the highest positive correlation ($p \leq 0.05$).

Keywords: Harpagophorid millipedes, ecological factors, forest types

Introduction

Soil macroinvertebrates are very important in improving the structure, content of organic matter and nutrient elements of soil (Loranger *et al.*, 2007; Seeber *et al.*, 2008). Millipedes are one of the most diverse groups of terrestrial animals and arthropods with more than 12,000 described species worldwide and an estimated diversity of about 80,000 species (Marek and Shelley, 2005). The Diplopoda (millipedes) is the third largest class of terrestrial Arthropoda following Insecta and Arachnida. Millipedes are major component

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of terrestrial ecosystems throughout the temperate, subtropical and tropical zones of the world. They are ecologically important as detritivores and are prominent biogeographical indicators because of their profound diversity and geological age, as well as low vagility (Hopkin and Read, 1992). The family Harpagophoridae is “probably the most characteristic and conspicuous element in the millipede fauna of the Oriental Region” (Hoffman, 1975). They comprise of mostly large to gigantic species (up to 25 cm long). The family includes 38 genera and 214 described species, distributed in tropical Africa and mainly the Indian subregion and Southeast Asia (Jeekel, 2006; Pimvichai *et al.*, 2010).

The Sakaerat Environmental Research Station (SERS), Nakhon Ratchasima, is one of the four UNESCO designated biosphere reserves of Thailand (Hanboonsong, 2000). SERS is covered by two major forest types; dry evergreen forest and dry dipterocarp forest. Millipedes are major invertebrate decomposers and distribute in every habitat of SERS (Sukteea *et al.*, 2011).

Objectives: This study aimed to compare the distribution, abundance of harpagophorid millipedes in different forest types (dry evergreen forest; DEF, ecotone; ECO, dry dipterocarp forest; DDF and plantation forest; PTF) in SERS and to investigate the environmental factors influencing this distribution.

Materials and methods

Sampling sites

Three sampling sites in each of four forests: dry evergreen forest (DEF); dry dipterocarp forest (DDF); plantation forest (PTF); and ecotone (ECO) were surveyed during June 2010 to May 2011. The sampling sites located at altitude 500-910 m. These plots were chosen as a representative of the major forest areas in the least disturbed area. The area includes good stands of each all forests (Fig. 1).

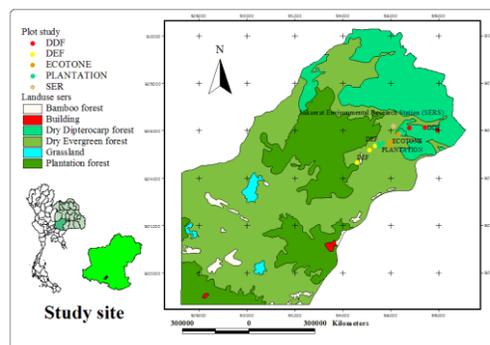


Fig. 1. Study site at Sakaerat Environmental Research Station

Population Density

Three replicates of soil and leaf litter were collected from study sites once a month in June 2010-May 2011 to determine millipede distribution in these areas. Three sampling sites in each forest were selected to study the distribution of millipedes in relationship to environmental factors. The sampling method involved the selection of a good stand sampling area and establishment of the permanent plot of 20 m x 20 m (400 m²). Millipedes were sampled by forcing steel frames (30 × 30 × 30 cm²) into the soil and excavated soil along with litter was transferred to trays.

Ecological Factors

Ecological factors were measured and analysed in this study included light intensity, soil temperature, litter moisture, soil moisture, air temperature, relative humidity, phosphorus, potassium, total nitrogen, organic carbon, organic matter, C:N ratio and soil pH. These data and results were used to evaluate the abundance, distribution of the harpagophorid millipedes and relationships between millipedes and their habitats.

Data Analysis

Differences in millipede number and species abundance among forest types were analyzed with ANOVA. The Pearson correlation was employed to investigate relationships among soil parameters, environmental factors and millipede distributions.

Results

Harpagophorid millipedes in four forest types

In all four selected forests, a total of 152 individuals in seven millipede species were found in family Harpagophoridae. The results of species are listed in Table 1.

Table 1 Occurrence of harpagophorid millipedes collected in Sakaerat Environmental Research Station

Family	Species	Forests type				Total
		DEF	DDF	ECO	PTF	
Harpagophoridae	Harpagophoridae 1	2	-	1	-	3
	Harpagophoridae 2	-	17	1	3	21
	<i>Thyropygusallevatus</i>	8	-	-	3	11
	<i>Thyropygusinduratus</i>	-	1	2	-	3
	<i>Thyropygus</i> sp1.	3	-	2	1	6
	<i>Thyropygus</i> sp2.	17	-	3	13	33
	<i>Anurostreptussculptus</i>	74	-	1	-	75
Total		104	18	10	20	152

DEF = dry evergreen forest
ECO = ecotone

DDF = dry dipterocarp forest
PTF = plantation forest

A total of 152 millipedes were collected in this study. The highest millipede density was *Anurostreptussculptus* (average 6.25 ind/m²) followed by *Thyropygus* sp2. (average 2.75 ind/m²), and Harpagophoridae 2 (average 1.75 ind/m²). The lowest density millipedes were Harpagophoridae 1 and *Thyropygusinduratus* (average 0.25 ind/m²). The *Anurostreptussculptus* showed significant difference in density among all millipedes ($p \leq 0.05$). The density of millipede species in each forest type is shown in Table 2.

The highest number of specimens was *Anurostreptussculptus* (Fig. 2) followed by *Thyropygus* sp2., but the lowest number of specimens was Harpagophoridae 1 (Fig. 3) and *Thyropygusinduratus* (Fig. 4). Interestingly, the Harpagophoridae 1 and *Anurostreptussculptus* distributed in DEF and ECO, as well as *Thyropygusinduratus* distributed in DDF and ECO but *Thyropygusallevatus* was found in DEF and PTF. Whereas, two species (*Thyropygus* sp1. and *Thyropygus* sp2.) were presented in three forests (DEF, ECO and PTF) but Harpagophoridae 2 was found in DDF, ECO and PTF (Table 2).



Fig. 2.
Anurostreptus sculptus



Fig. 3. Harpagophoridae 1



Fig. 4.
Thyropygus induratus

Millipedes abundance

Average of adult millipede abundance in a year (June 2010-May 2011) was 12.77 ind/m² (Table 2). The abundance of millipedes was minimum (0.93 ind/m²) in ECO and maximum (8.68 ind/m²) in DEF.

Table 2 The density (individual per m²) of adult harpagophorid millipede species in DEF, DDF, ECO and PTF

Millipede Species	Forest type				Total
	DEF	DDF	ECO	PTF	
Harpagophoridae 1	0.17	-	0.08	-	0.25
Harpagophoridae 2	-	1.42	0.08	0.25	1.75
<i>Thyropygus allevatus</i>	0.67	-	-	0.25	0.92
<i>Thyropygus induratus</i>	-	0.08	0.17	-	0.25
<i>Thyropygus</i> sp1.	0.25	-	0.17	0.08	0.50
<i>Thyropygus</i> sp2.	1.42	-	0.25	1.08	2.75
<i>Anurostreptus sculptus</i>	6.17	-	0.08	-	6.25
	8.68	1.5	0.93	1.66	12.77

DEF = dry evergreen forest
ECO = ecotone

DDF = dry dipterocarp forest
PTF = plantation forest

Species richness

The species richness of each forest types is shown in Table 3. The results showed that the highest species richness was 6 in ECO and decreased to 5, and 4 in DEF and PTF, respectively. The lowest species richness was 2 in DDF. Species richness of ECO was higher than DEF, PTF, and DDF.

Table 3 Species diversity index and evenness index of harpagophorid millipedes in forest types

Index	Forest type			
	DEF	DDF	ECO	PTF
Species richness	5	2	6	4
Evenness	0.57	0.31	0.95	0.72
Species Diversity (H')	0.91	0.21	1.67	0.99

DEF = dry evergreen forest DDF = dry dipterocarp forest
ECO = ecotone PTF = plantation forest

Shannon - Wiener index and Evenness

Species diversity was investigated by Shannon-Wiener index (H'). There was different between each forest type (Table 3). The results showed that the highest diversity index was 1.67 in ECO followed by in DEF (0.99) and in PTF (0.91). The lowest species diversity index was 0.21 in DDF. The species evenness was calculated from species diversity index and the results are also shown in Table 3. The greatest species evenness was 0.95 in ECO, and declined to 0.72 in PTF, 0.57 in DEF and 0.31 in DDF. The highest species diversity index and evenness index in DEF showed that ECO had more millipede species than other forest types. However, value of the index usually lies between 0.21-1.67, thus the Shannon - Wiener index of all habitat types at the SERS indicated a high diversity of millipede species.

Climatic factors

Climatic factors are composed of air temperature, relative humidity (RH), light intensity and rainfall. The results indicated that mean of temperature was the highest (27.56 ± 1.05 °C) in DDF, and the lowest (24.25 ± 0.56 °C) in DEF. Mean of relative humidity was the highest ($87.09 \pm 2.25\%$) in DEF, followed closely by PTF ($84.51 \pm 1.64\%$) and ECO ($72.68 \pm 2.10\%$) respectively, and the lowest ($70.53 \pm 1.39\%$) was in DDF. Regarding light intensity, DDF had the highest of 1999.39 ± 244.82 lux while DEF had the lowest of 649.28 ± 57.24 lux.

Generally, the temperature of all forest types varies in place and time with significant variation in plants cover. The mean temperature of all forest types was not significantly different. The lowest recorded mean temperature was 24.25 ± 0.56 °C in DEF, while the highest mean temperature of 27.56 ± 1.05 °C was recorded in DDF (Table 4). This might be caused by plant cover. Because DEF has high density of crown canopy and moisture content, it can reduce light and radiation from the sun. The modification of temperature by plant cover is both significant and complex. Shaded ground is cooler during the day than open

area. Vegetation interrupts the laminar flow of air, impeding heat exchange by convection.

Table 4 Mean (\pm SE) of climatic factors in four forest types

Forest type	Temperature (°C)	Relative humidity (%)	Light intensity (lux)
DEF	24.25 \pm 0.56	87.09 \pm 2.25	649.28 \pm 57.24
DDF	27.56 \pm 1.05	70.53 \pm 1.39	1999.39 \pm 244.82
ECO	27.10 \pm 0.84	72.68 \pm 2.10	902.88 \pm 111.57
PTF	25.12 \pm 0.82	84.51 \pm 1.64	657.53 \pm 89.98

DEF = dry evergreen forest
 ECO = ecotone

DDF = dry dipterocarp forest
 PTF = plantation forest

The mean relative humidity of all forest types varied and it was significantly different ($p \leq 0.05$). DEF of this study had higher relative humidity (87.09 \pm 2.25%) than PTF (84.51 \pm 1.64), ECO (72.68 \pm 2.10) and DDF (70.53 \pm 1.39) because this forest type had higher tree density and more crown cover than the others.

The average of light intensity of all forest types had significant differences. Light intensity of DDF was the highest (1999.39 \pm 244.82 lux), while that of DEF was the lowest (649.28 \pm 57.24 lux). This might be caused by crown density, stands density and canopy gap.

The ordination of Principal Components Analysis (PCA) was analysed, and provided the diagram of radiating line of joint plot diagram to identify the relationship between ecological factors and species composition. The angle and length of the line indicate the direction and strength of the relationship. Thus, the result of joint plot diagram can be identified plot composition and the result is shown in dimension order. The result indicated that the habitat types were three separate groups (Fig. 5).

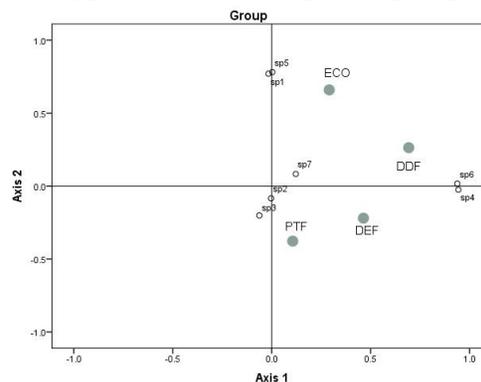


Fig. 5. PCA ordination of ecological factors

The PCA plot is consistent with the cluster analysis results in showing how the DEF, PTF, ECO and DDF are separated widely in space. The output from PCA analysis were also utilized to identify the relationship of millipedes community and ecological factors. The Pearson and Kendall correlation with ordination axes are shown in Table 5.

Table 5 The Pearson and Kendall correlation with ordination axes

Axis	1		2		3	
Factors	r	r-sq	r	r-sq	r	r-sq
Light intensity (lux)	-0.616	0.539	-0.260	0.404	0.208	0.425
Soil temperature (°C)	-0.490	0.545	0.440	0.572	0.672	0.563
Litter moisture (%)	-0.211	0.719	0.786	0.566	0.342	0.563
Soil moisture (%)	0.211	0.873	0.871	0.480	0.272	0.574
Air temperature (°C)	-0.326	0.659	0.235	0.467	0.255	0.025
Relative humidity (%)	-0.309	0.934	0.895	0.826	0.536	0.435
Phosphorus (ppm)	0.044	0.731	0.057	0.301	0.259	0.704
Potassium (ppm)	0.023	0.713	0.024	0.227	0.308	0.238
Total nitrogen (%)	0.475	0.833	-0.215	0.418	0.418	0.411
Organic carbon (%)	0.929	0.950	-0.267	0.47	-0.459	0.336
Organic matter (%)	0.958	0.949	-0.319	0.115	-0.460	0.469
C:N ratio	0.329	0.695	-0.123	0.253	-0.151	0.476
Soil pH	-0.598	0.705	-0.389	0.294	0.456	0.273

The ordination diagram is shown in Fig. 6. It can be explained as following. The plots related to organic carbon are included DEF and PTF ($r = 0.929$ in axis 1 and $r = -0.0215$ in axis 2). The plots related to organic matter are included DEF and PTF ($r = -0.958$ in axis 1 and $r = -0.0319$ in axis 2). The angle and length of the line indicate the direction and strength of the relationship. Thus, the result of joint plot diagram in Fig. 6 can be identified plot composition as follows:

On axis 1, temperature, relative humidity, soil moisture and water content of litter were the most significant factors determining in millipedes

composition, followed by pH, potassium and magnesium. On axis 2, organic carbon and organic matter were the most significant factors.

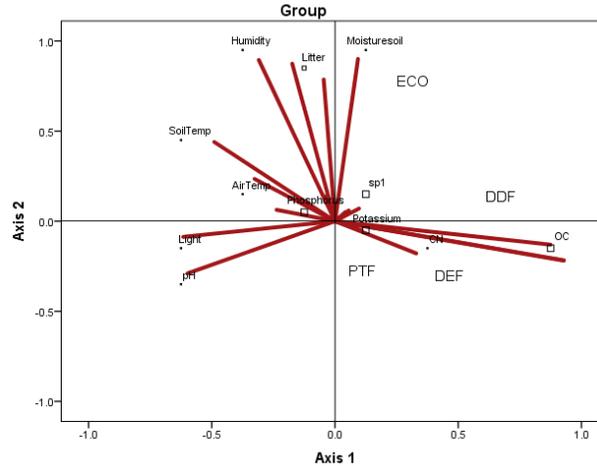


Fig. 6. The joint plot diagram showing the relationship between a set of ecological factors and millipedes abundance

Discussions

Harpagophorid millipedes are certainly very prominent members of the oriental fauna, reaching up to 25 cm in length. The genus *Thyropygus* Pocock, 1894, is the largest genus of Harpagophoridae in Southeast Asia. It had a complicated history but, mainly due to the work of Hoffman (1975), the genus is now quite well circumscribed. The genus is broadly distributed in Southeast Asia: Thailand, Myanmar, Vietnam, Laos, Cambodia, continental Malaysia, Sumatra, Java, and Borneo (Hoffman, 1975; Enghoff, 2005). The family Harpagophoridae has a narrow distribution range depends on climatic factors. The seasonal fluctuations in soil moisture responding to rainfall events may affect the millipede species and millipede abundance. When the soil moisture levels decrease during December to February, most millipedes' burrows are deeper into the soil. This result is supported by Karamaouna and Geoffroy (1985) and Karamaouna (1987) who reported that the activity periods of some Mediterranean species were very pronounced and only active in wet period (winter and spring). No millipedes were found between May and October when it was very dry. During this period, they burrowed into the soil.

The results showed that the highest species richness was 6 in ECO and decreased to 5, and 4 in DEF and PTF, respectively. The lowest species richness was 2 in DDF. Species richness of ECO was higher than DEF, PTF, and DDF. It may be due to humidity, tree species and density of trees.

In addition, the correlation of diversity and evenness had the same tendency. As a result, ECO, DEF and PTF had higher diversity indices than DDF. It can be explained that the overall of millipedes increased due to the dominance of millipede species. Furthermore, many factors such as soil moisture, depth of litter, density of tree and soil type determine the spawning, survival and feeding behavior. DEF and ECO had close index of diversity. It can be explained that ecological factors of them were similar.

The millipede distributions were positively significantly correlated ($p \leq 0.05$) with total nitrogen, soil moisture, phosphorus, potassium, organic carbon (OC) and organic matter (OM). However, they were negatively correlated with light intensity, soil temperature, air temperature, relative humidity and soil pH. These results supported by Zimmer *et al.*(2000) they showed that the distribution of diplopod species was mainly influenced by temperature. However, moisture conditions also influenced the distribution pattern of many diplopods. For example, *Polyzoniumbermanicum* can be found in high abundances in thick litter layers which contain a high humidity around the year (David and Vannier, 1995). It can be argued that the relative humidity is relevant to water vapor content in the air. Water vapor gets into the air by evaporation from moist surfaces and from evapotranspiration by plants. This supported the results studied by (Dajoz, 2000) who reported that relative humidity is generally higher in forest than open area, especially in summer when transpiration from trees is at its height. Furthermore, temperatures also influence relative humidity. Relative humidity is generally higher at night and early morning when the air temperature is lower; it is lower during the day when temperature increases. Thus, DEF had higher relative humidity than PTF, ECO, and DDF because it had lower temperature than them.

The availability of organic matter is one of the most important factors influencing millipede abundance. A significant correlation was found between biomass of *Arthrosphaera* and soil organic carbon among several edaphic features (organic carbon, pH, phosphate, calcium and magnesium) of the above biomes (Ashwini and Sridhar, 2008). Moreover, Lorangeret *al*(2007) showed that the quality of organic matter was an important factor determining millipede distributions and thus, accounting for local variations of population abundance and species richness.

The PCA analysis provided that harpagophorid millipedes diversity was negatively correlated with light intensity, soil pH, soil temperature, soil moisture and litter moisture, while organic matter showed the highest positive correlation ($p \leq 0.05$).

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