



Diversity of pholcid spiders (Araneae: Pholcidae) in selected areas of Southeastern Mindanao, Philippines

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Abstract. Pholcidae is one of the species-rich spider families. However, pholcid spiders are poorly studied in Southeast Asian countries like the Philippines. In this study, selected areas of southeastern Mindanao, Philippines were surveyed in order to determine the pholcid fauna in the area. Pholcids were collected using a combination of aerial-hand searching and ground-hand searching methods. Nonparametric richness estimators were used to estimate the species richness of the sampling area using EstimateS version 9.0 software. In addition, biodiversity and similarity indices were calculated using Paleontological Statistics Software Package (PAST) version 2.17c. Seventeen species of pholcid spiders were documented in which 88% of the samples collected were probably new species. Based on richness estimates computed, the true species richness in the surveyed areas might exceed 37 species and probably much more. The highest species diversity among the six sampling areas was documented in Mount Hamiguitan Wildlife Sanctuary located at San Isidro, Davao Oriental. However, Epol falls in Marilog District seems to be the distinct sampling area among the six sites surveyed because of the unique species found in the area. The results of this study clearly showed that the diversity of pholcid spiders is much higher in the country than the 13 species that were previously documented.

Key Words: biodiversity, EstimateS, estimators, fauna, species richness.

Introduction. Spiders comprise a large portion of the invertebrate fauna in any habitat (Coddington & Levi 1991). Currently, more than 44,000 species in 114 families are recognized around the world (Platnick 2014). They play important roles in terrestrial ecosystems as abundant predators which regulate insect pest populations (Barrion et al 2012) and as bio-indicators to evaluate impacts of anthropogenic disturbances (Maelfait & Hendrickx 1998).

Spiders of the family Pholcidae show a high ecological plasticity and can be found everywhere from deserts to humid tropical forests (Huber 2000, 2005b). Pholcid spiders are globally distributed and inhabit a wide range of habitats in different ecosystems, especially in subtropical and tropical regions (Huber 2011a). They prefer dark places such as caves, cracks, and crevices in rocks (Huber 2000, 2005b). In forested areas they can be found in the leaf litter, in webs between buttresses, in small holes in trees or fallen logs, and on the undersides of large leaves (Huber 2005b). This makes them difficult to see and perhaps difficult for their main predators to capture (Bradley 2013). They mostly have long, slender legs which account for one of their common names, "daddy long legs spiders". The family Pholcidae is the ninth largest spider family, currently counting a total of 79 genera and 1458 species (Huber 2015).

Recently, Huber (2000, 2001, 2003a,b, 2005b, 2009, 2011b) reported a large number of new species and revised many genera. The classification of the family has been improved through cladistic analyses of morphological and molecular data (Huber 2011a; Dimitrov et al 2013). In Southeast Asia, few papers on the pholcid fauna have been published recently (Huber 2005a,b, 2011b; Zhang & Peng 2011; Yao et al 2014; Yao et al 2015), but Southeast Asian countries remain among the most poorly studied as to pholcid diversity. Many taxa remain undescribed and probably even uncollected. In the

Philippines, very few pholcid spiders have been described despite the country's extraordinary biodiversity. It was Simon (1892) who first described four species of pholcids from the Philippines; *Calapnita vermiformis*, *Pholcus bicornutus*, *Spermophora estebani* and the type species of the genus *Uthina* (Platnick 2014). In the 1980s, two species were further described, *Calapnita subphylicola* and *Panjange lanthana* (Deeleman-Reinhold & Deeleman 1983; Deeleman-Reinhold 1986). Additionally, six species were designated by Huber (2005b, 2011b) which are *Belisana apo*, *Belisana davao*, *Pholcus arayat*, *Pholcus pagbilao*, *Spermophora luzonica*, including the type species of the genus *Aetana*. Recently, *Pholcus schawalleri* has been added on the list (Yao et al 2014), bringing the total number of species to 13.

Results from the recent biodiversity assessment by Huber & Nuñez (2014) in the Philippines implied that the diversity in the country is much higher. Hence, a great focus must be needed to study the alpha taxonomy to phylogenetic analyses and conservation of pholcid spiders in the Philippines.

The study was conducted in selected areas of southeastern Mindanao, Philippines. It specifically aims to provide fundamental data on species composition, richness and diversity of pholcids in Davao Region that will contribute to the existing information on Pholcidae in the Philippines. The chosen sites are relatively undisturbed areas which contain the most important and significant natural habitats for the conservation of biological diversity. Mt. Hamiguitan Wildlife Sanctuary is of particular interest as it has been recently added to the prestigious United Nations Educational, Scientific and Cultural Organization (UNESCO) World Heritage List (UNESCO 2014). No scientific studies have been conducted on the pholcid spiders neither in this site nor in any of the other sampling areas.

Material and Method. The study was conducted in Davao Region (Region XI) located in Southeastern portion of Mindanao, Philippines (Figure 1). The sampling areas of this particular study are the following: (1) Baganihan, Marilog District, (2) Epol falls, Marilog District, (3) Mount (Mt.) Hamiguitan Wildlife Sanctuary (access at San Isidro), (4) Mt. Hamiguitan Wildlife Sanctuary (access at Governor Generoso), (5) Mt. Candalaga, Maragusan Compostela Valley and (6) Mt. Mayo located at Tarragona, Davao Oriental.

Field sampling was conducted from November 16, 2014 to Feb 13, 2015 within the rainforest of the six sampling areas in which three sites per sampling area were selected for diversity assessment. A 1 km line transect was established in each site for semi-quantitative sampling as suggested by Coddington et al (2009). Each sampling site has been described using the Habitat Description Form of the Haribon Foundation (Mallari et al 2001). Sampling at these three sites was conducted at daytime for one week employing ground hand collecting and aerial hand collecting methods (Sorensen et al 2002). Each collecting method was applied for about two hours active sampling excluding interruptions (e.g. traveling time to different site, personal tasks). Pholcid spiders were searched up to 5 m at both sides of the transect line to provide an extensive area for sampling. For ground hand collecting method, pholcid spiders were searched from ground level to knee height which includes cryptic species found in leaf litter, small holes in trees or fallen logs, bark crevices, within rotting logs or trees, bark, and among rocks. Aerial hand collecting covers the higher strata from knee level to as high as the collector can reach (Sorensen et al 2002). During aerial hand collection, the leaf surface of available plants was gently turned upwards and all pholcid spiders encountered were noted. The sexes of leaf dwelling spiders were noted including the orientation relative to the major leaf veins and the number of juveniles present. Adult male pholcids are characterized by their large and conspicuous pedipalps (Huber 2011a). Female pholcids are easily recognized only when carrying their egg sacs (Huber 2009). Specimens were collected using transparent plastic jars containing 70-80% ethanol. Duplicates were collected into pure ethanol (>96%) in order to allow for future molecular work. For transport to the laboratory, specimens were transferred to narrow tubes in order to avoid damage. Specimens were stored in tubes together with the labels which include date, locality (including coordinates), habitat, concentration of ethanol, and sexes. Specimens collected were examined by Dr. Huber of Alexander Koenig Zoological Research Museum, Bonn

Germany and identified to morphospecies which is a useful means of identifying arthropod groups (Derraik et al 2002). Species codes used were assigned by the same taxonomist indicating the unique species found in the Philippines and will be comparable across different studies.



Figure 1. Map of Mindanao, Philippines showing the sampling areas located in the Southeastern portion of Mindanao (www.maphill.com).

The most widely used and successful richness estimators which are nonparametric estimators were used for biodiversity inventory assessment of pholcid spiders (Colwell & Coddington 1994). Species richness or the number of species in a given community is the most basic and natural measure of diversity (Sorensen et al 2002; Chiarucci et al 2003; Hortal et al 2006; Gotelli & Chao 2013). However, the exact species richness and relative abundance in a given habitat or study area are unknown in most applications (Gotelli & Chao 2013). Thus, species richness must be estimated from samples taken from the habitat or study area (Gotelli & Colwell 2001). In this study, Incidence-based Coverage Estimators (ICE), Chao 1, Chao 2, Jackknife 1, Bootstrap and Michaelis-Menten (MM) estimator nonparametric estimators were used for species richness estimation (Colwell & Coddington 1994). Species richness estimates were computed using EstimateS 9.1 software. Computed values were used to construct species accumulation curve using Paleontological Statistics Software Package version 2.17c (Hammer et al 2001). Species accumulation curve or "collector's curve" is used to test whether the six surveyed sites were adequately sampled based on the number of individuals from an assemblage (x-axis) and observed species richness (y-axis). If the curve flattens out to an asymptote, then slope is calculated using the formula:

$$\text{Slope} = 1/(nS - nS_{\pm 1})$$

where nS = final number of individuals for each curve (corresponding to the total richness) and $nS_{\pm 1}$ = number of individuals corresponding to the point in the curve where the final single species was added or subtracted to S (Cardoso et al 2008).

Inventory completeness and sampling intensity were also computed. Inventory completeness is the ratio of the observed species richness to the Chao1 richness estimated while sampling intensity is a measure of sampling effort (specimens:species) (Scharff et al 2003; Gotelli & Cowell 2001). In addition, the most common biodiversity indices; Shannon diversity (H') and Simpson diversity ($1-D$) which combine richness and evenness in a single measure were also computed. Lastly, species similarity estimator, Morisita-Horn index is used to compare the species composition and diversity within two or more study sites using quantitative data (Magguran 2004).

Results and Discussion

Species composition and richness. A total of 566 individuals of pholcids were recorded in Southeastern Mindanao (Table 1). Out of 566 individuals recorded, only 473 individuals were identified up to morphospecies. The remaining 93 individuals are all juveniles which are usually not identifiable and assigning them to any of the species requires molecular data that will give some indication. Seventeen morphospecies of pholcid spiders were identified in this study. Two of the species found, *Belisana apo* and *Calapnita vermiformis* were previously defined by Simon (1892) and Huber (2005b), respectively. Hence, the remaining 15 morphospecies or approximately 88% of the samples were undescribed which are probably new species. Figure 2a shows the rank-abundance curve for pholcid spiders found in the surveyed area. The rank abundance-curve shows that the area is dominated by the species *Belisana* sp. Phi129 with 167 individuals. Long tail of rarer species has been also noticed in the curve. According to Cowell (2009), an even distribution of individuals among species in a hypothetical community must be flat instead of declining which means that every species has the same abundance.

On the other hand, species accumulation curve was also constructed in order to show if an asymptotic number of species has been reached (Figure 2b). If the surveyed area is sufficiently homogeneous, all of the species will eventually be collected and the curve will flatten out to an asymptote that denotes the true species richness of an area (Gotelli & Chao 2013). In this case, accumulated species was continuously rising which means that not all species present in the sampling sites were found during the surveys, even though the overall inventory completeness was 94.44% and the sampling intensity was 27.82 for the total data.

Nonparametric estimators have been used in order to estimate the asymptote of the species accumulation curve (Colwell & Coddington 1994). Figure 3 shows the estimated species richness for the six estimators which are based on 100 randomized samples for the total data. Thus, we have estimated the overall species richness of the six surveyed areas. Species richness estimates ranged from 18 to 37.66 species. Chao 1 obtained the lowest estimate with 18 species, while Michaelis-Menten estimator got the highest estimate with 37.66 species. Among the nonparametric richness estimators employed in this study, only Michaelis-Menten estimator showed much tendency to asymptote with a slope value of 0.55. The estimate of Michaelis-Menten estimator is more than two times of the observed species richness. However, the continually rising accumulation curves of the majority of the estimators would mean that the inventory is not complete at the end of the sampling. The true species richness in the surveyed areas might exceed 37 species, and probably much more.

Table 1

Species composition of pholcid spiders in selected areas of Southeastern Mindanao

Species	Number of individuals per sampling area						Total	RA (%)
	Baganihan	Epol falls	Governor Generoso	San Isidro	Mount Mayo	Maragusan		
<i>Aetana</i> sp. Phi92	35	18	0	8	0	0	61	12.9
<i>Aetana</i> sp. PSt1055	0	0	0	0	4	0	4	0.85
<i>Belisana</i> sp. Phi129	51	0	0	0	87	29	167	35.31
<i>Belisana</i> apo	5	0	0	4	0	0	9	1.9
<i>Belisana</i> sp. Phi169	0	13	0	0	0	0	13	2.75
<i>Belisana</i> sp. PSt513	0	35	0	0	0	0	35	7.4
<i>Belisana</i> sp. PSt691	0	0	21	19	0	0	40	8.46
<i>Belisana</i> sp. PSt745	0	0	2	6	0	1	9	1.9
<i>Belisana</i> sp. PSt1102	0	0	0	0	1	0	1	0.21
<i>Belisana</i> sp. PSt1158	0	0	0	0	0	2	2	0.43
<i>Calapnita vermiformis</i>	0	0	39	32	20	0	91	19.24
<i>Calapnita</i> sp. PSt1161	0	0	0	0	0	18	18	3.81
<i>Panjange</i> sp. Phi125	10	0	0	0	0	0	10	2.11
<i>Panjange</i> sp. PSt695	0	0	9	0	0	0	9	1.9
<i>Pholcus</i> sp.	0	1	0	0	0	0	0	0.21
<i>Spermophora</i> sp. PSt663	0	1	0	0	0	0	1	0.21
<i>Spermophora</i> sp. PSTt1157	0	0	0	0	0	2	2	0.43
Total number of individuals	101	68	71	69	112	52	473	-
Total number of species	4	5	4	5	4	5	17	-

RA - Relative abundance.

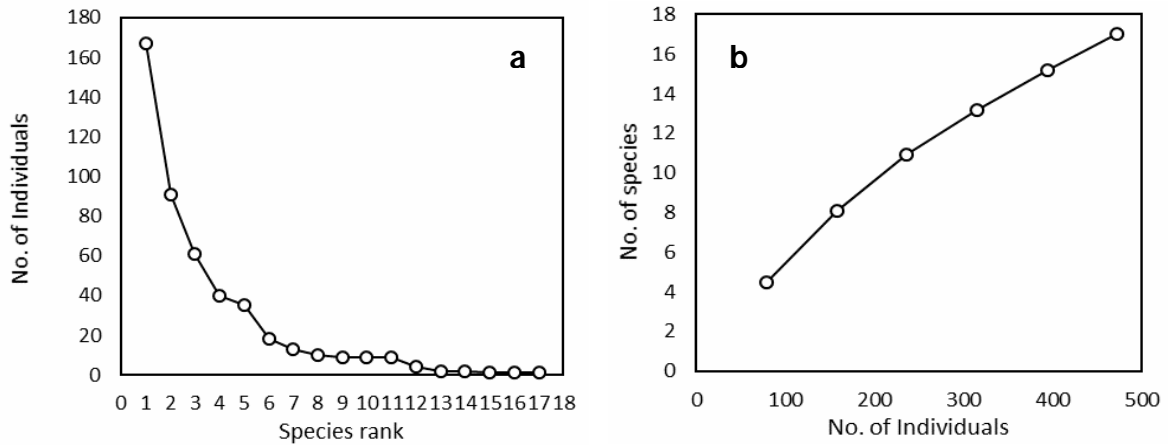


Figure 2. Rank abundance curve (a) and species accumulation curve (b) of pholcid spiders in Southeastern Mindanao.

In 2014, a total of 28 species of pholcid spiders (excluding previously defined 13 species) were found in the country (Huber & Nuñez 2014). This would mean that the 13 currently known species of pholcids in the Philippines are just a tiny portion of the species richness of pholcids that are yet to be discovered. This preliminary data in our present study suggest that the number of species of pholcid spiders in the Philippines is much higher specifically in the Genus *Belisana*, *Panjange* and *Aetana*. In fact, additional seven morphospecies of *Belisana*, were found in the surveyed areas, including unique species of *Panjange*, *Aetana* and *Spermophora*. Hence, this research may serve as a baseline data to the pholcid fauna in the country.

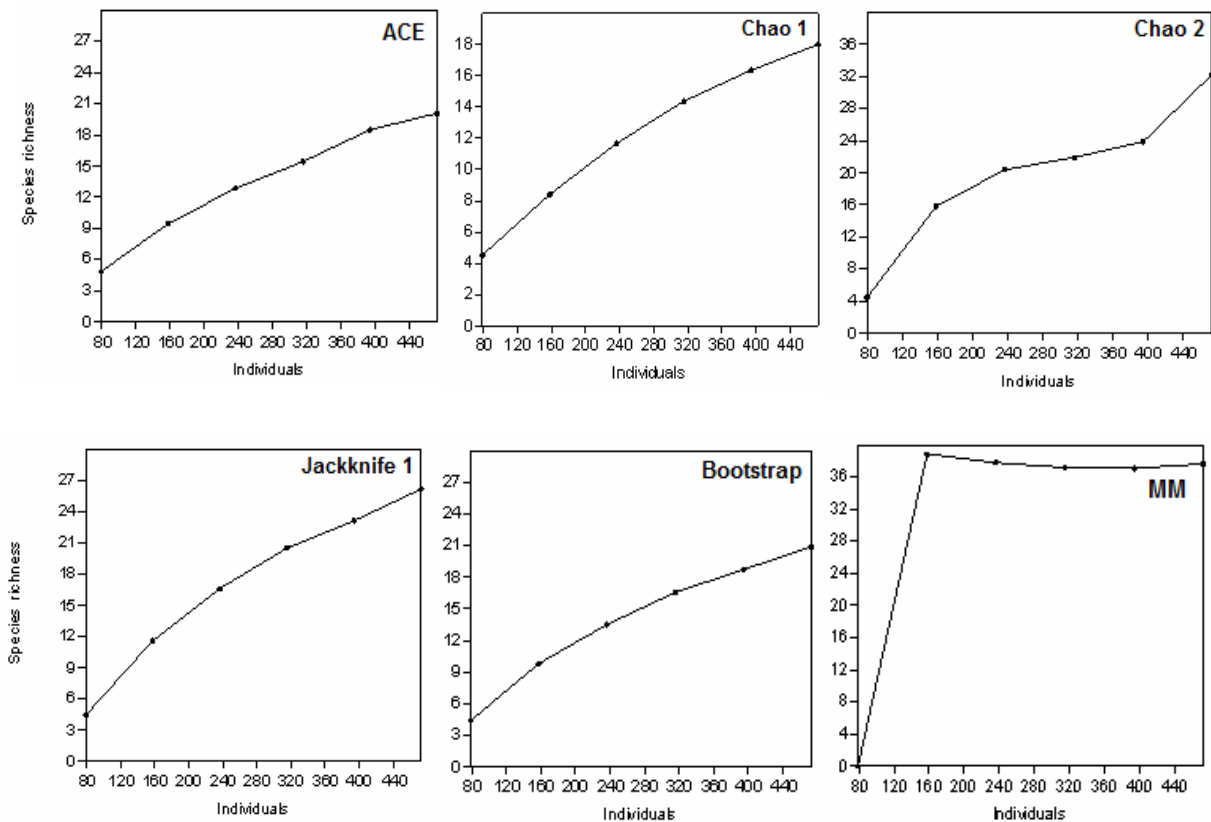


Figure 3. Randomized accumulation curves for richness estimators. Incidence-based Coverage Estimators (ICE); Michaelis-Menten estimator (MM).

Species diversity. Shannon and Simpson diversity indices of the sampling areas are shown in Table 2. Both of these indices are mathematical functions that combine richness and evenness in a single measure (Cowell 2009). For the Shannon diversity, the diversity index increases as both the richness and evenness increases. The typical values are generally between 1 and 2 which means that the diversity is moderate, low diversity if the index value is below 1 and high diversity if the index value is greater than 2. Due to the confusion of richness and evenness in the Shannon index measure, many biodiversity researchers prefer to stick to two numbers for comparative studies, combining a direct estimate of species richness (the total number of species in the community) with some measure of dominance or evenness (Magguran 2004). The most common dominance measure is Simpson's index. Simpson diversity calculates dominance (D), so as D increases, diversity in the sense of evenness decreases. Thus, Simpson's index is usually reported as its complement $1-D$.

The total Shannon diversity index (H') recorded in this study was 2.07 and its corresponding dominance (D) value was 0.1948. All sites have moderate species diversity as shown in the values of Shannon and Simpson indices. Among the six sampling areas, Mt. Hamiguitan Wildlife Sanctuary located at San Isidro, Davao Oriental obtained a higher value of diversity (H' : 1.466; $1-D$: 0.6926), while Mt. Mayo in Tarragona, Davao Oriental was the least diverse site (H' : 1.096; $1-D$: 0.6048). Mt. Hamiguitan Wildlife Sanctuary obtained a higher value of diversity as this site is relatively the least undisturbed sampling area which is protected by way of several protected area regulations. In fact, the Conservation International declared this area as one of the Philippine "hotspots" (Conservation International 2014). Several studies have been conducted in this protected area which reported interesting flora and fauna (Amoroso & Aspiras 2011; Mohagan & Treadaway 2010; Villanueva & Mohagan 2010; Karger et al 2012; Relox et al 2011). On the other hand, Mt. Mayo in Davao Oriental appears to be the least diverse site and dominated by *Belisana* sp. Phi129.

Table 2
Diversity indices and evenness of pholcid spiders in Southeastern Mindanao

<i>Location</i>	<i>Shannon index (H')</i>	<i>Dominance (D)</i>	<i>Simpson index ($1-D$)</i>
Baganihan	1.310	0.3339	0.6661
Epol Falls	1.369	0.3204	0.6796
Governor Generoso	1.311	0.3333	0.6667
San Isidro	1.466	0.3074	0.6926
Mt. Mayo	1.096	0.3952	0.6048
Mt. Candalaga	1.334	0.3285	0.6715
Total	2.007	0.1948	0.8052

Similarity of sampling sites. Figure 4 shows the dendrogram of cluster analysis in the six sampling areas. It shows that the two sites in Mt. Hamiguitan (San Isidro and Governor Generoso) are very similar with 98% similarity. This means that the two entry points in Mt. Mount Hamiguitan share almost the same species. The pholcids found in these areas prefer dark shaded primary and secondary forests surrounded by a variety of shrubs and saplings. Unlike the other sampling areas, sampling points in Mt. Hamiguitan have a dense forest and undisturbed vegetation. Mt. Mayo, Mt. Candalaga, and Baganihan were also the same sites with similarity indices ranging from 91–93%. Sampling points in both Mt. Mayo and Mt. Candalaga are located in lowland areas (below 1000 masl). These sampling areas also have almost the same vegetation which consist of medium trees (20-29.9" in diameter) and the understory plants are commonly surrounded by aroids. Epol falls in Marilog District seems to be the distinct sampling area

among the six sites. One reason for this is the presence of singleton species which are *Pholcus* sp. and *Spermophora* sp. PSt663. In addition, Epol falls is a unique site as it has a dense undisturbed shaded forest located along a natural water body which might be one factor that influences the diversity of pholcids in the area.

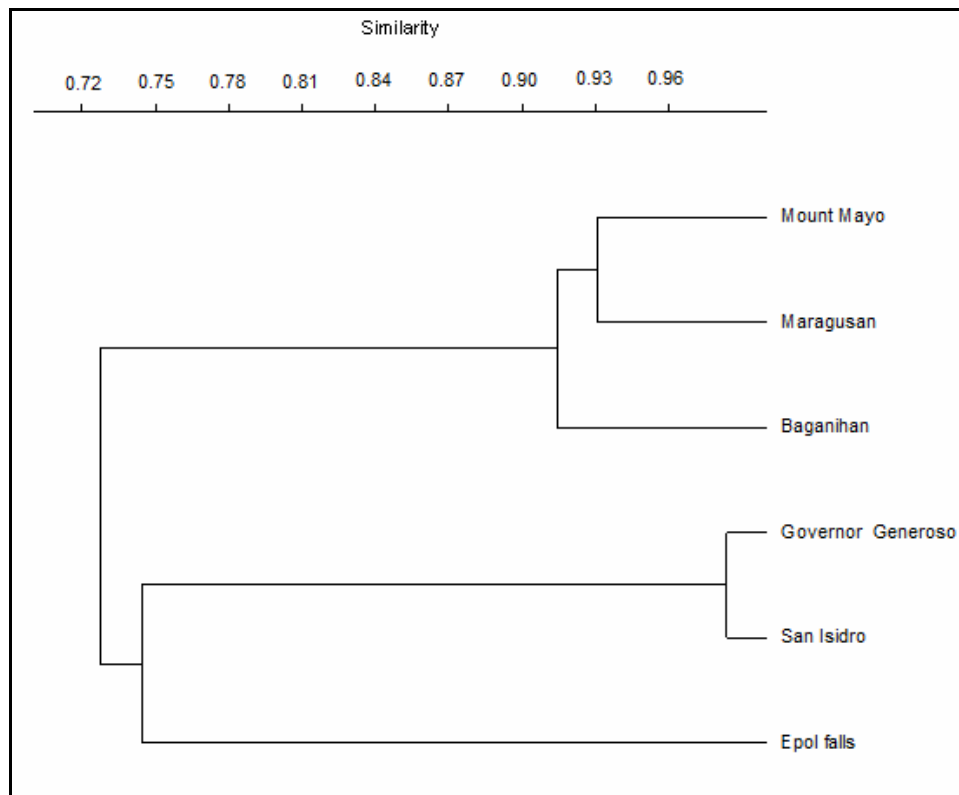


Figure 4. Cluster analysis of six sampling areas in Southeastern Mindanao based on shared species.

Conclusions. Moderate diversity was recorded in this study based on the computed diversity indices. Out of seventeen morphospecies, only two species were previously defined by Simon (1892) and Huber (2005b). This means that the 15 remaining morphospecies are probably new species. For the species richness estimation, species richness estimates were much higher from the observed richness which ranged from 18-37 species and probably much more as the species accumulation curves were continuously rising. The results clearly suggest that the 13 previously known species of pholcids in the Philippines are just tiny portion of the vast diversity of pholcids that are yet to be discovered.

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