Species Composition of Freshwater Zooplankton Fauna from Selected Groundwater-Dependent Ecosystems in Bulacan Province (Philippines) with Taxonomic Notes of New Locality Record of Harpacticoid Species in the Philippines

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ABSTRACT

In the Philippines, the taxonomy of freshwater zooplankton fauna from ground waters remain poorly understood, yet knowledge on this group is important to know patterns in groundwater biodiversity to develop sound conservation policies. In this study, zooplankton were studied by collecting samples from selected groundwater-dependent ecosystems in Bulacan Province. Results indicate the presence of 13 species belonging to 10 families from Rotifera, Cladocera, and Copepoda. Large portion of the samples included the copepods *Mesocyclops* and *Thermocyclops* spp. together with cladocerans *Ceriodaphnia cornuta* and *Moinodaphnia macleayi.* Observation of these taxa in groundwater-dependent ecosystems suggests high surface and sub-surface hydrological connectivity in the Province. Lastly, new locality record of *Elaphoidella bidens* in the Philippines was established.

INTRODUCTION

Primary source of freshwater in the hydrological cycle comes from the groundwater. Groundwater is important in providing good quality water for human consumption and many groundwater-dependent ecosystems. Aside from this, groundwater and its dependent ecosystems harbor diverse group of organisms (Brancelj et al., 2013), which are dominated by freshwater zooplankton including rotifers, cladocerans, and copepods (Galassi et al., 2009). Zooplankton are important in freshwater ecosystems as they serve as link between primary producers and higher level consumers. In addition, zooplankton are good bio-indicators (Papa et al., 2012; Papa & Briones, 2014) due to their sensitivity to their habitat making them suitable indicator for environmental changes, which may be utilized in knowing current environmental health status of most freshwater ecosystems.

Freshwater zooplankton research in the Philippines is mainly limited to surface waters such as rivers and lakes and completely disregarding groundwater and groundwater-dependent ecosystems including caves, open-wells, springs, and piped groundwater pumps. The diversification of freshwater zooplankton in surface waters is said to be parallel to those found in groundwater ecosystems, especially the copepods (Galassi et al., 2009). Like surface water, groundwater diversity studies are essential as these can contribute information needed to maintain a sustainable biodiversity for this type of ecosystems, as well as to provide useful biological indicators of subsurface-surface water connectivity.

In Southeast Asia, the lack of interest and scarcity in available literature regarding groundwater fauna made it one of the "cold spots", where there is poor knowledge about groundwater biodiversity in the region (Brancelj et al., 2013). In the Philippines, a total of 47 rotifers, 82 cladocerans, 20 cyclopids, and 15 calanoid copepods species were documented from different surface waters like lakes, rivers, and temporary water pools (Mamaril 1986; Mamaril 2001; Papa & Mamaril 2011; Papa & Zafaralla 2011, Papa & Hołyńska 2013; Pascual et al., 2014, Dela Paz et al., 2016), but there are limited literature concerning groundwater species especially harpacticoid copepods. Overall, only 6 groundwater copepod species

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Table 1: List of the different sampling sites included in this study.

Sampling Site	Ecosystem Type	Coordinates		Municipality
		Latitude	Longitude	
Aguinaldo Cave	Cave	121.0676	15.1009	San Miguel
Bahay Paniki Cave	Cave	121.0520	15.1009	San Miguel
Bayabas Well	Open-Well	121.0887	14.9549	Meycauayan
Biak Na Bato Poso	Piped groundwater pump	121.0843	15.1378	San Miguel
Lumot Spring	Spring	121.0646	15.1156	San Miguel
Tungkod na Bato Spring	Spring	121.0643	15.1153	San Miguel
DTR Spring	Spring	121.2231	14.9603	Dona Remedios Trinidad
NHV Bitungol Well	Open-Well	121.0495	14.8763	Norzagaray
NHV Bitungol Phase 2 Well	Open-Well	121.0662	14.8929	Norzagaray
Pinagrealan Cave	Cave	121.0915	14.8643	Norzagaray
Pinagrealan Open Well	Open-Well	121.0915	14.8640	Norzagaray
Pinagrealan Poso	Piped groundwater pump	121.0847	14.8523	Norzagaray
Puning Cave	Cave	121.0921	14.9623	Dona Remedios Trinidad
Tanggapan Cave	Cave	121.0689	15.1202	San Miguel
Yungib 1 Cave	Cave	121.0763	15.1149	San Miguel
Yungib 2 Cave	Cave	121.0563	15.1649	San Miguel

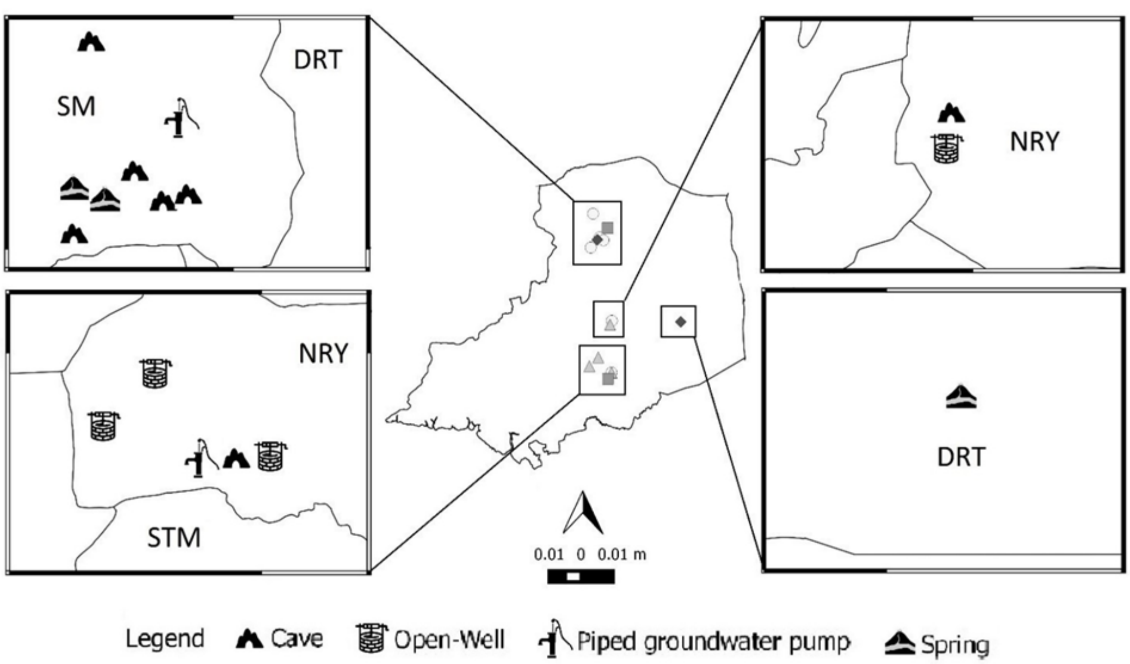

Fig. 1: Selected groundwater-dependent ecosystems in Bulacan province included in the study. SM, San Miguel; DRT, Dona Remedios Trinidad; NRY, Norzagaray; STM, Sta. Maria.

were documented in the country (Rouch, 1972; Fosshagen & Iliffe, 1989; Bruno & Cottarelli, 1999; Bruno & Cottarelli, 2006; Brancelj et al., 2013), which includes endemic harpacticoid species collected from hyporheic waters in Bohol and Cebu. Strengthening research on groundwater fauna could lead to the increase in the total number of stygobionts known in the Philippines. With this background, this study aims to provide baseline data on the species composition of zooplankton in selected groundwater-dependent ecosystems (caves, open-wells, piped groundwater pumps and springs) in Bulacan, Philippines.

Materials and Methods

Sampling Sites: Bulacan covers a total land area of 2,796.10 km^2 that occupies the southeastern section of the Central Luzon region. In term of groundwater resources, Bulacan contributes largely to the total groundwater resource in the country, wherein around 74.23 million m^3 amounting to 207, 794.65 million pesos were being extracted per annum (Ebarvia, 1997) from open-wells, piped groundwater pumps, artesian springs, and caves. In this study, the following selected groundwater-dependent ecosystems presented in Table 1 and Figure 1 were visited for the collection of zooplankton.

Collection of Sample: All collections of zooplankton from each selected sampling sites were done in triplicates using conical plankton nets with mesh size of 45 µm and 60 µm. Cave puddles was disturbed to incorporate the sediments with water and then filtered. Vertical and oblique methods were executed in larger puddles in the caves (Pipan & Brancelj, 2004; Camacho, 2006; Meleg et al., 2011). As for open-wells, plankton net was dipped up and down from the bottom of the well with constant shaking to disturb the water. This was done for six times per sampling (Culver & Sket, 2000; Eberhard et al., 2009). Furthermore, water samples from piped groundwater pumps were collected by pumping 100L of water and passed through 45 µm and 60 µm conical plankton net (Sorensen et al., 2013). Lastly, spring water was sieved using plankton net (45 µm and 60 µm mesh size) placed at the outlet of the spring at the beginning of the spate. One meter long net with a large filtering area was used to minimize clogging by fine sediment particles (Külköylüoğlu & Yılmaz, 2006).

Processing of Collected Samples: Water samples were strained using 33 µm filter and the resulting residues were stained with rose-bengal dye with 70% (v/v) ethanol (Papa, 2012). Zooplankton were sorted using storkbill forceps and enumerated by following this sorting scheme: (1) adult cyclopid copepods, (2) adult calanoid copepods, (3) adult harpacticoid copepods, (4) cladocerans, and (5) rotifers (Papa, 2012). Sorted individuals were kept in 10mL vial with 70% ethanol.

Dissection and Identification of Samples: Specimens, mostly copepods, were dissected for thorough examination of the morphological characteristics to delineate each zooplankton species. This was done using dissecting (Olympus SZ61, Japan) and compound microscopes (Olympus CX21, Japan) guided by the techniques proposed by Papa (2012). Glycerine was used as the mounting medium which was then sealed with a clear nail polish. Existing taxonomic keys by Cheng & Clemente (1954), Lai & Fernando (1980), Petersen & Carlos (1984), Mamaril (1986), Wells (2007), Papa & Holynska (2013), Pascual et al. (2014), and Dela Paz et al. (2016) were used for identification. The initial identifications of the specimens were verified by Dr. Rey Donne S. Papa (University of Santo Tomas, Philippines) and Dr. Tomislav Karanovic (Sungkyunkwan University, South Korea).

Results and Discussion

Species Composition of Zooplankton Fauna: A total of 13 species of zooplankton consisting of 4 rotifer, 4 cladoceran, and 5 copepod species were observed in this study (Table 2). For rotifers, *Asplanchna sieboldi*, *Branchionus plicatilis*, *Lecane leontina*, and *Epiphanes senta* of Asplanchnidae, Brachionidae, Lecanidae, and Epiphanidae, respectively were noted in this study. Cladocerans were represented by four families, namely: Sididae (*Diaphanosoma sarsi*); Chydoridae (*Chydorus sphaericus*); Moinidae (*Moinodaphnia macleayi*); and Daphniidae (*Ceriodaphnia cornuta*). Lastly, copepods species under two families, Cyclopidae (*Thermocyclops crassus*, *Mesocyclops thermocyclopoides, Mesocyclops aspericornis*, and the endemic species *Mesocyclops microlasius*) and Canthocamptidae (*Elaphoidella* bidens). This study documented new distribution record of *Elaphoidella bidens* in the Philippines, which was found in a piped groundwater pump in Biak na Bato, San Miguel, Bulacan.

In terms of relative abundance (Figure 2), 48% of collected zooplankton individuals belong to Cyclopidae represented by a total of four species (*Mesocyclops* and *Thermocyclops*). This was followed by the cladocerans coming from Daphniidae and Moinidae with relative abundance of 13% and 12%, respectively. Lastly, rotifers that has the least number of collected specimens from the selected groundwater-dependent ecosystems in Bulacan Province were represented by Epiphanidae (13%) and Asplanchnidae (11%).

At present, there are limited published studies regarding the taxonomy and ecology of zooplankton species found in groundwater ecosystems in the Philippines. Specifically, no studies focus on zooplankton fauna from groundwater ecosystems in Bulacan. However, some researches have

Table 2: List of species observed from selected groundwater ecosystems in Bulacan Province*.

Taxa	Species
Rotifera	
Family Asplanchnidae	*Asplanchna sieboldin* (Leydig, 1854)
Family Brachionidae	*Branchionus plicatilis* (Müller, 1786)
Family Lecanidae	*Lecane leontina* (Turner, 1892)
Family Epiphanidae	*Epiphanes senta* (Müller, 1773)
Cladocera	
Family Sididae	*Diaphanosoma sarsi* (Richard, 1894)
Family Chydoridae	*Chydorus sphaericus* (Müller,1776)
Family Moinidae	*Moinodaphnia macleayi* (King, 1853)
Family Daphniidae	*Ceriodaphnia cornuta* (Sars, 1885)
Copepoda	
Family Cyclopidae	*Thermocyclops crassus* (Fischer, 1853)
	Mesocyclops thermocyclopoides (Harada, 1931)
	Mesocyclops aspericornis (Daday, 1906)
	Mesocyclops microlasius (Kiefer, 1981)
Family Canthocamptidae	*Elaphoidella bidens* (Schmeil, 1894)

*All species listed are surface water species except for *Elaphoidella bidens,* which can be a facultative groundwater species.

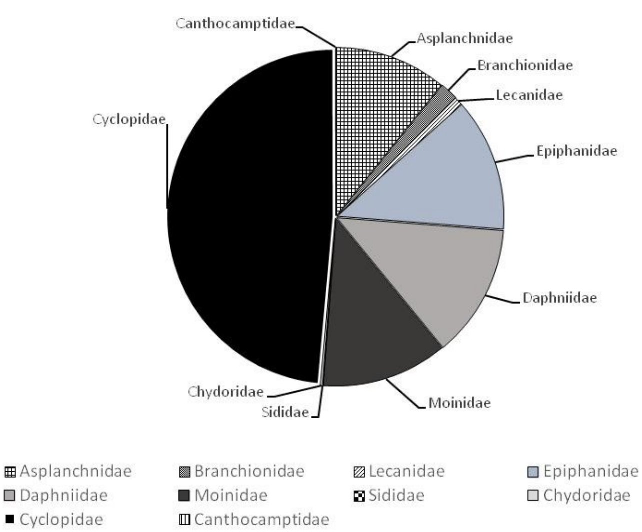

Fig. 2: Species composition of zooplankton fauna from selected groundwater-dependent ecosystems in Bulacan, Province.

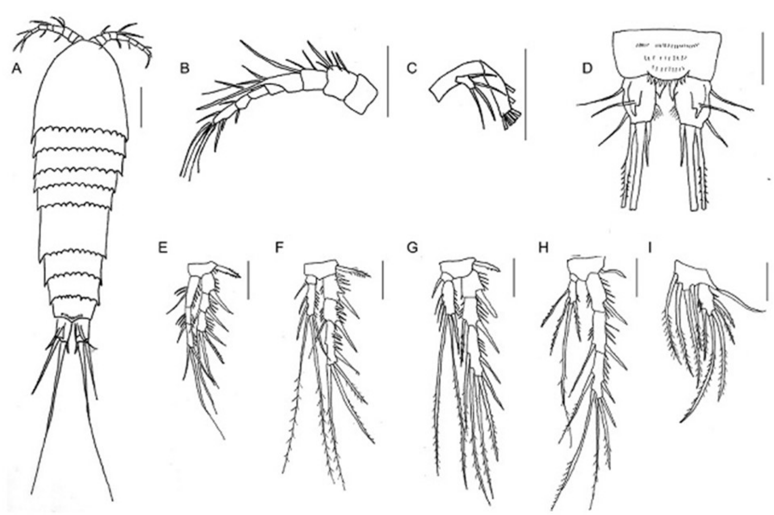

Fig. 3: *Elaphoidella bidens* (Schmeil, 1894) A, habitus; B, antennule; C, antenna; D, caudal rami; E, P1; F, P2; G, P3; H, P4; and I, P5. Scale bar = 50 µm.

listed surface water zooplankton species from the province. Some of these were the works done by Mamaril (1986). The presence of high numbers of non-stygobiotic taxa or surface water species (12 out of 13 species, Table 2) indicates high interconnectivity and relationship between surface-subsurface systems among the sampling sites in Bulacan Province. All cladoceran species found in this study were previously noted in lakes and rivers in Luzon, which included Moinidae, Sididae and Chydoridae (Korovchinsky, 2013). This supports previous records that Sididae and Chydoridae are considered as the most diverse and well-distributed among families inhabiting Luzon (Pascual et al., 2014). Another common component of the groundwater fauna collected in this study are the copepods, which are thought to be responsible in increasing groundwater biodiversity (Brancelj et al., 2013). Cyclopidae species were mostly collected from wells, but include non-stygobiotic species. Other studies have shown that *Mesocyclops* spp. and *Thermocyclops* spp. are occasionally present in subterranean habitats (Brancelj et al., 2013, Van Damme et al., 2013). Species from these two genera are widely distributed in major lakes, rivers, and freshwater temporary pools throughout the Philippines (Papa & Holynska, 2013). Of these, only *M. microlasius* was previously collected from a concrete well (in the City of Manila) (Kiefer, 1981). Our results confirm the previous observation by Kiefer (1981) that *M. microlasius* may be found in artesian wells in Luzon Island. In addition, the frequent occurrence of *Mesocyclops* spp. in artesian wells may be attributed to the high occurrence of mosquito larvae since *Mesocyclops spp.* species which naturally prey on mosquito larvae thus making them potential biological control agents of Dengue carrying mosquitoes (Papa & Holynska 2013).

Lastly, for the harpacticoid copepods, observation of *Elaphoidella bidens* in this study serve as the first distribution record of this species in the Philippines. Harpacticoid copepods are the least studies group of microcrustacean zooplankton not only in the Philippines but in Southeast Asia (Brancelj et al., 2013). The scarcity of studies on the systematics of these taxa in the Philippines and Southeast Asia is the main reason for the difficulties encountered by the researchers in knowing species identities of the collected samples. Comprehensive examination of morphological details in the future may help broaden existing knowledge about the distribution and ecology of harpacticoid copepods since previous studies have been limited to phreatic and hyporheic waters.

Taxonomic Notes on *Elaphoidella bidens* (Schmeil, 1894):
Class: Maxillopoda Dahl, 1956
Subclass: Copepoda Milne-Edwards, 1840
Order: Harpacticoida Sars, 1903
Family: Canthocamptidae Sars, 1906

Materials Examined: Two adult females from a piped groundwater pump in Biak na Bato, San Miguel, Bulacan were dissected and mounted. Two specimens were stored in UST Zooplankton Reference Collection with the reference number of 1088 and 1100, respectively.

Description: Body length ranges from 0.45-0.50mm (Figure 3). Segments of the posterior dorsal margins serrated, abdominal ventral margins spinose. Small anal operculum, rounded with 12 teeth. Rectangular caudal rami, with numerous hairs on the inner margins (Figure 3D). Antennules 8-segmented (Figure 3B). First segment of the antennal exopodite with 4 setae. P1-P4 exopodites 3-segmented, last segments bear a total of 5, 6, 6 spines and setae, respectively. P1 endopodite 3-segmented. P2-P4 endopodites 2-segmented; terminal segments with a total of 5, 6, 4 spines and setae, respectively (Figure 3E-H). Basal expansion of the 5th leg with four plumose setae; exopodite segment oval, spinose on the interior and exterior margins and armed with 5 setae (Figure 3I).

Distribution: Its distribution covers almost all continents except Antartica. Records for the species were noted in Slovenia (Mori & Brancelj, 2013), New Zealand (Lewis, 2010), Brazil (Reid, 1990), Nepal (Ishida, 1994), and Thailand (Boonyanusith & Athibai, 2014).

Conclusions

From the visited groundwater-dependent ecosystems in Bulacan Province, 13 species were recorded and identified. A big majority of the samples collected contain surface water species suggesting high hydrological connectivity between the surface and sub-surface water in the Province. In addition, new locality record has been established for the facultative groundwater harpacticoid *Elaphoidella bidens* in the Philippines. Additional efforts in surveying groundwater habitats in the Philippines is highly needed. These would be useful in establishing knowledge regarding groundwater biodiversity in the country.

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