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Character visualization of millipede (*Cylindroiulus* sp.) from Household Organic Waste Composter in Surabaya

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ABSTRACT

Millipede is an animal in the Diplopoda class. These animals are found as decomposers that eat plant debris and play a major role in increasing nutrients in the soil, so that they are able to supply the needs and fertility of the soil that is beneficial to plants. Although these animals play a large role in most terrestrial ecosystems, there is little information about the morphological diversity of these animals. Millipede morphological characterization is important for identification, but research related to millipede identification is very rare in Indonesia. This study aims to provide information about the visual characterization of local millipedes (*Cylindroiulus* sp.) obtained from household waste composter in Surabaya. Millipede samples were obtained through random hand sorting from household waste. Character visualization was done using a camera. The morphological characters observed such as, body size and color, segment, appendages, and antenna. The information obtained from this study is expected to be able to complement basic data on the diversity of millipedes in Indonesia.

***Key words**: Cylindroiulus sp., Decomposing agent, Morphological characters, Solid waste, Surabaya*

Introduction

Millipede or known as thousand-legged animal is classified into the diplopods class which has the third-largest diversity following Insecta and Arachnids in the Arthropod phylum (Golovatch *et al.,* 1995). According to Shelley (2003) more than 12,000 species of millipede have been described. However, it is estimated that there are more than 80,000 species of millipedes yet to be described (Sierwald and Bond, 2007).

The diversity of millipedes is related to their important role in the environment. Millipedes are known as a terrestrial arthropod that contributes greatly in increasing the structure content of organic matter and soil nutrients (Sridhar and Ashwini, 2011). Most of the millipedes are decomposers that live actively on the leaf litter and rotting wood (Akkari *et al.,* 2009). The millipede can ingest 20-100% of plant residue in the forest and return up to 90% of organic matter in the form of faecal pellets mixing it with microbes to be converted into humus

(Sridhar and Ashwini, 2011). In addition, millipede was also known as an environmental indicator because of their low vagility (Hopkin and Read, 1992) and sensitive to several environmental factors (Sridhar and Ashwini, 2011). The millipede was found on all continents except in Antarctica and widely distributed in a variety of habitats ranging from tropical rainforests to Mediterranean (Sierwald and Bond, 2007). They are also known to be able to live in household composter in Surabaya Indonesia which contain dead plant matter, food scraps, paper, and so on.

In general, millipedes have an elongated roundshape or flattened bodies consisting of a head and trunk which are equipped with appendages. Their bodies are composed of numerous segments and each segment has two pairs of legs. According to Minelli and Golovatch (2007) and Hopkin and Read (1992) the body size of millipedes ranges from 0.2-35 cm length and 0.1-4 cm width. Most studies on millipede were focused in uncovering their metabolite compounds (Wood *et al.,* 2000; Schmitt *et al.,* 2004), the role of millipede as decomposers in the environment (Suzuki *et al.,* 2013; Kitz *et al.,* 2015), an association of millipede with microbes (Knapp *et al.,* 2010; Ramanathan and Alagesan, 2012) and millipede diversity in a region (Akkari *et al.,* 2009; Krubphachaya *et al.,* 2016; Golovatch and Liu, 2020). Although they play an important ecological role, little is known about the morphological visualization of millipede (Cylindroiulus), especially in Indonesia. Meanwhile, research that stated the visualization of millipedes is very important for identification. According to Hilgert *et al.* (2019), studies that revealed a list of species or determination keys of millipedes in Indonesia are still rare. Millipede's research in Indonesia was still limited in revealing its diversity in an area (Hilgert *et al.,* 2019). Therefore, this investigation aims to reveal the morphological characters of local millipedes from household waste composter in Surabaya, Indonesia..

Materials and Methodology

Microcosm and sample acclimatization

This research was conducted in Surabaya Indonesia using organic waste from household composter as the main sample. Household organic waste was put in a 40 x 60 cm glass jar leaving 2/3 airspace. The jar contains household organic waste and all of the decomposing organisms. Acclimatization was carried out in 7 days, kept the sample at 26 to 31 ^{o}C, pH 5 to 6, and moisture 50 to 60% by watering the microcosm twice a day. The microcosm was covered by fabric to prevent animal and moisture loss.

Sample collection and morphological characterization

All specimens were collected by hand sorting randomly then were anaesthetized using chloroform and preserved in 70% ethanol. The identification process was carried out by observing the morphological characters of the millipede directly or with the aid of a light microscope (Olympus CX 23) at minimum magnification. Observations, including colour, body length and diameter, number of body segments, number of legs in one body segment, telson, setae, etc, were documented using a digital camera. All characters that have been recorded then analyzed for the identification process based on the literature.

Results and Discussion

Observations on household waste composter that have been used for the last ten years indicate the existence of various soil fauna populations.

One of the populations found was the millipede population

Based on our observations, there are various species of millipede in the composter. The most often found of millipede species is visualized in Figure 1. The millipede species has the following morphological characters: the dorsal part is brown to black while the ventral part is light brown. On the head there is a mouth, black ocelli, and a pair of antennae that are light brown in color. The width of the head is almost the same as the width of the body. The body is an arrangement of segments. In each segment there are metazonal stripes, and there are circular lines that look like two segments joined together (diplo segment). The number of body segments is between 38 – 43. In each segment there are two pairs of legs which are light brown in color. The leg is shaped like a hook. There are several setae on the legs. On the posterior side there is an epiproct which is rounded and slightly tapered at the ends. Millipede length between 19.0 - 23.5 mm, body width between 1.7-2.3 mm. Based on these morphological characters, it is suspected that the observed millipede was

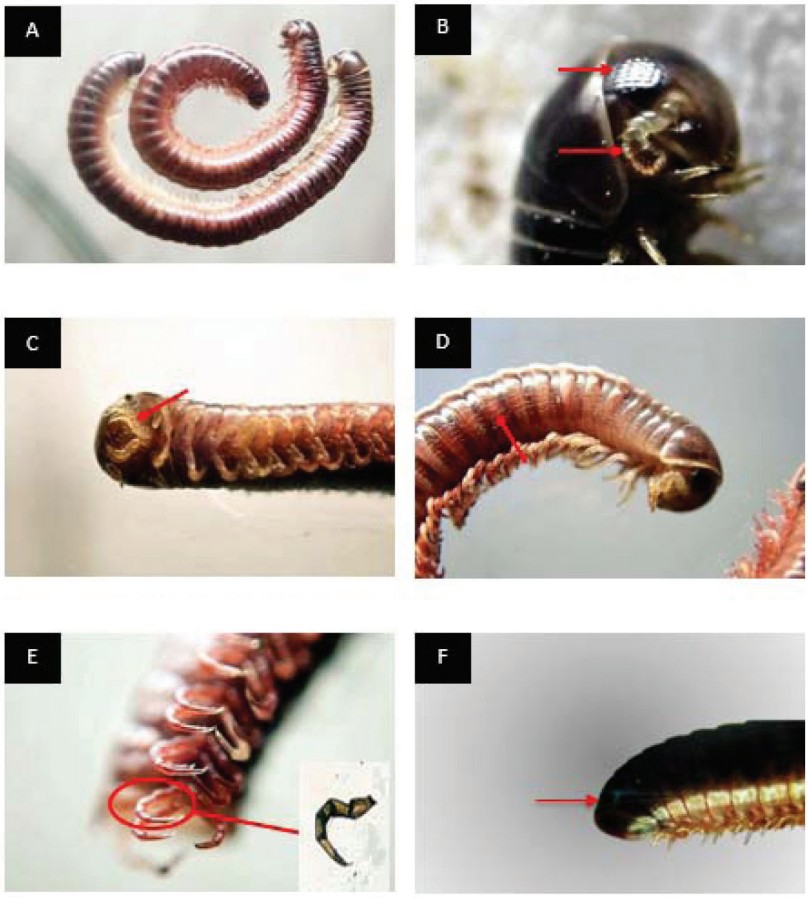

Fig. 1. Character visualization of *Cylindroiulus* sp. including (A) whole body, (B) the head consisting of the ocelli and mouth and a pair of antennas, (C) mouth, (D) the segment of the body with longitudinal striations (metazonal strips), (E) each segment has two pairs of legs and on the legs there are setae, (F) telson.

Cylindroiulus sp.. Visualization of the character of *Cylindroiulus* sp. observed are shown in Figure 2.

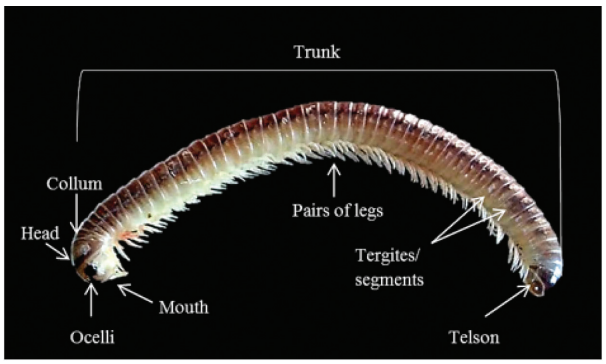

Fig. 2. Habitus lateral view of *Cylindroiulus* sp

The decomposer fauna obtained was identified as *Cylindroiulus* sp. based on the dichotomous key by Henne (2015) with the order of determination keys, 1B, 2B, 4A, 5B, 22B, 23B, 24A, 25B, 26A. The characteristics that support the dichotomous key are as follows:

1. Hard body; with calcified exoskeleton; if there are setae, then in simple form it is not arranged in tufts; adult animals with more than 11 body segments and at least 17 pairs of legs; longer than 3 mm (1B).
2. The head is about the same size as the body, at least half of its width; legs apparent when observed from above; male with 7 pairs of legs in front of the gonopod (2B).
3. Adult body with more than 20 segments; with or without having ocelli; with or without paranota (4A).
4. Epiproct without a pair of spinnerets; sternites, pleurites, and tergites merge into a cylindrical ring, ocelli sometimes in a linear configuration (5B).
5. Body without longitudinal crests; varies in

Table 1. Copmparison of Morphological characters between *Cylindroiulus sp*

Species	Body Length (mm)	Diameter (mm)	Number of Segments	Colour	Telson
Cylindroiulus mitta (Akkari and Enghoff, 2008)	♂ 11.3 – 12.8 ♀ 13.3 – 16.7	♂ 0.90 – 1.02@ ♀ 1.05–1.33	♂ 36–49 @ ♀ 44–53	Body yellowish brown	Telson with no caudal projection, preanal ring and anal scale each with 2 setae, each anal valve with one row of 4–5 setae
Cylindroiulus villumi sp.(Reboleira and Enghoff, 2018)	♂ 11.4 ♀ 13	♂ 0.7 ♀ 0.9	♂ 38 ♀ 42	Body yellowish	Anterior constriction pronounced and pilosity of the telson scarce
Cylindroiulus distinctus (Read, 2005)	♂ 35–41.5 ♀ 43	♂ 2.5–2.6 ♀ 3.5	♀ 50–51 ♀ 47–52	Head chestnut brown, dark brown metazonae on the main trunk	Telson dark chocolate brown. In male usually lacking any dorsal projection but a slight protuberance
Cylindroiulus algerinus (Read, 2005)	♂ 31–37 ♀ 47	♂ 2.2 – 2.8 ♀3.5	♂ 46–51 ♀ 52	Head and first four rings chestnut brown, ventral body ginger/ chestnut with darker suture between pro and metazonae	Telson darker brown. Mature males with slightly protruding dorsal projection
Cylindroiulus attemsi (Read, 2005)	♂ 31 – 39 ♀ 52	♂ 2.2–3.0 ♀ 4.2	♂ 44–48 ♀ 52	Head and first six segments are chestnut brown, trunk is pale chestnut, metazonae brownish, and prozonae bluish	Telson and anal valves are chestnut brown. Slightly protruding dorsal projection found in mature males. 5–14 pairs of setae usually in a single row pre-anal ring
Cylindroiulus djebelensis (Read, 2005)	♂ 29-31.5 ♀ 39	♂ 2.1 ♀ 3.4	♂ 51–53 ♀ 57	Paler than most of the other species, with clearly seen ozopores	Females and juveniles are lacking dorsal projection, in mature males slightly more pronounced.
Cylindroiulus gauthieri (Read, 2005)	♂ 33.5–40.5 ♀ 49	♂ 2.3–2.8 ♀ 3.5	♂ 49–52 ♀ 47–56	Head chestnut brown, Main trunk is dark. Thin black mid-dorsal lines are usually present but not continuous. Anterior portions of metazonae often yellowish	Pointed, in the pre-anal ring usually there are 9–12 pairs of setae.

Table 1. *Continued ...*

Species	Body Length (mm)	Diameter (mm)	Number of Segments	Colour	Telson
Cylindroiulus jijelensis (Read, 2005)	♂ 29 ♀ 24	♂ 2.2 ♀ 2.3-2.0	♂ 55 ♀ 46-51	Much paler than all other species. Head and first three segments pale to mid-brown. Main trunk is pale yellow, mature male has darker ozopores.	Telson brown, lacking any dorsal projection even in male. Preanal ring with 9–17 setae, three or more setae on a ventral scale. 11–15 pairs of setae in anal valves mostly located marginal.
Cylindroiulus ouridae (Read, 2005) 10	♂36.5	♂ 2.5 ♀ 2.9	♂ 52 ♀ 50	Colour body is gingery brown, especially metazonae, with faint mid-dorsal line	In males dorsal projection is slight, 7– pairs setae in preanal ring, 13–25 setae in anal valves, ventral scale with 2-7 pair
Cylindroiulus maurus (Read, 2005)		♂ 1.8	♂ 47	Head and first two rings are brown, rest yellowy brown with thin black mid-dorsal stripe	Slight dorsal projection in males, slightly darker posteriorly. rather more sparsely setose, 6 pairs of setae in preanal ring, anal valves with 8 pairs of setae in almost com plete row, ventral scale with one pair
Cylindroiulus sp. (This work)	19-23.5	1.7-2.3	38-43	Main trunk of the body with the dorsal part is brown to black while the ventral part is light brown. The head is black	Telson is brown to black, rounded and slightly tapered at the ends

color; fifth segment with one or two pairs of legs (22B).

6. Body color varies, but never dark blue-black on the anterior with an orange-red stripe on the posterior; the body has a length of 10-50 mm; sidepieces of gnathochilarium not separated by the mentum; median suture on head not extending up from labrum (23B).
7. Body with longitudinal lines encircling each segment; usually with a caudal epiproct projection but may be indistinct; there is ocelli; in males the first pair of legs is shaped like a hook (24A).
8. Body length is in the range of 7mm-40mm; caudal projection of epiproct (if present) small, non-obvious (25B).
9. Body length 20 mm – 40 mm; with black and brown caudal metazonal stripes; usually without caudal projection on epiproct; generally without setae fringing the metazonite; epiproct and paraprocts with only a few setae; gonopod mesomerite associated with promerited (26A).

Cylindroiulus is a millipede group that is found in various tree bark and leaf litter, as well as rotting wood, for example *C. caeruleocinctus (Mock 2006), C. attemsi Read, C. tunetanus Attems, C. mitta Akkari & Enghoff, and C. truncorum* Silvestri (Akkari *et al.*, 2009). *Cylindroiulus* sp. observed in this study also

came from composter which is often filled with various organic waste, including leaf litter, wood, and twigs. Table 1 shows the comparison of morphological characters between *Cylindroiulus* sp. observed in this study with various species from other genus *Cylindroiulus* which were found from leaf litter and rotting wood.

The frequent species of the genus *Cylindroiulus* found in habitats where decomposition of organic matter is occurring indicates the important role of these organisms in the environment as decomposers. *Cylindroiulus* is known to play a role in the decomposition process of organic matter (Knapp *et al.,* 2009). The interesting thing is that there is a role for decaying fauna which is closely related to the symbiotic bacteria that inhabit their digestive tract. Other research that we have done previously shows that there are bacteria capable of producing various enzymes that are important in the process of decomposition of organic matter, for example amylase, protease, cellulase and lipase which inhabit the digestive tract of decomposer fauna which we found in the household waste composter (Ni'matuzahroh *et al.,* 2020). Research by Knapp *et al.* (2010), reported the presence of various bacteria that inhabit the digestive tract *Cylindroiulus fulviceps*.

Our morphological observations can identify the millipede species found in household composter in Surabaya down to the genus level. Identification down to the species level is very possible, but the limitations of researchers in observing the similarities and differences in the millipede morphology observed with existing databases make this difficult to do. In addition, there are still few libraries that contain visualizations of millipede, especially millipede species in Indonesia. This creates an opportunity for Indonesian researchers to be able to explore biodiversity in Indonesia to be translated into visualization of the organism's character. In addition to providing benefits in the form of science, character visualization can also be a documentation of various biodiversity of Indonesia. In addition, besides using visualization morphological characters, currently molecular approaches are also widely used for identification, for example through the identification of the mitochondrial gene cytochrome c oxidase 1 (CO1) (Likhitrakarn *et al.,* 2020).

Conclusion

Millipede morphological characters that have been observed include brown to black body color, body length 19.0 - 23.5 mm, body width 1.7-2.3 mm, ocelli, a pair of antennae, two pairs of legs in each body segment with several setae, and rounded and slightly tapered at the ends of telson. Millipede has a close resemblance to the genus *Cylindroiulus*. Molecular identification for *Cylindroiulus* sp. should be done considering the potential of *Cylindroiulus* sp. as well as microbes that are symbiotic in the intestines of these organisms so that they can be explored deeper and developed more widely in the decomposition of organic matter that will benefit humans and the environment.

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