

## Stygobiotic isopod *Stenasellus* sp. in Sarongge Jompong cave, Tasikmalaya karst area, Indonesia

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**Abstract.** Kurniawan ID, Rahmadi C, Akbar RTM, Prakarsa TBP. 2022. Stygobiotic isopod *Stenasellus* sp. in Sarongge Jompong cave, Tasikmalaya karst area, Indonesia. *Biodiversitas* 23: 1495-1504. *Stenasellus*, a stygobiotic isopod, was first reported in Tasikmalaya karst by local cavers in 2018 in Sarongge Jompong Cave. This study aimed to investigate the population, activities, and habitat characteristics of *Stenasellus* sp. in Sarongge Jompong, Tasikmalaya karst area, Indonesia. Population and activities were recorded through direct intuitive search. We measured several important physicochemical parameters to study habitat characteristics. Statistical analyses-namely Non-metric Multi-dimensional Scaling and Detrended Correspondence Analysis-were conducted to understand the difference in water characteristics among microhabitats and the relation between water characteristics and population size. The results showed that the population was distributed in 4 microhabitats with the maximum observed individuals of 8. Several activities were successfully monitored, including crawling, resting, hiding in crevices, and moving to different locations through the water. All microhabitats were wet gours connected to small water currents located in the dark zone of the cave passage. Water characteristics of microhabitat site 1 were more similar to site 2 and the numbers of individuals in these sites were larger than sites 3 and 4. Among water parameters, resistivity showed the most robust relation with *Stenasellus* population in which both variables were positively correlated. This correlation indicated that *Stenasellus* sp. preferred microhabitats with less contamination. Intrinsic and extrinsic factors may potentially threaten the population. The potential threats and conservation challenges need to be identified and mitigated to reduce the risk of biodiversity loss to these isopods, mainly due to their small population size, specific microhabitat, and susceptibility to disturbance. Considering these factors, research on taxonomy will be essential for conservation efforts.

**Keywords:** Conservation, microhabitat, physicochemical parameter, population, stygobiont

### INTRODUCTION

The cave environment has unique ecosystem characteristics. Most of this environment lacks light which limits photoautotrophic taxa to survive. Furthermore, it also has a relatively stable microclimate condition, saturated humidity, and usually less O<sub>2</sub> concentration compared to surface environments (Prous et al. 2015; Simões et al. 2015). Caves in temperate regions belong to the oligotrophic system, which is well-known for its limited nutrients (Mammola et al. 2019). Meanwhile, tropical caves are commonly eutrophic but lack in quality (Kurniawan et al. 2020). These harsh environmental conditions act as limiting factors for several taxa to occupy this realm (Kosznik-kwaśnicka et al. 2022).

Despite the extreme conditions, caves are essential habitats for diverse groups of organisms, particularly animals. Cave animals can be found in both terrestrial and aquatic habitats. Water in caves is inhabited by numerous different taxa of animals (Knight et al. 2015; Decu et al. 2019). According to their degree of adaptation, aquatic cave-dwelling animals are classified into 3 groups:

stygophile, stygoxene, and stygobite (Trajano and de Carvalho 2017; Kurniawan and Rahmadi 2019). Among these groups, stygobites are the most captivating since they are cave-obligate species that spend their whole life-cycle entirely in groundwater (Gutjahr et al. 2014). As such, they display unique morphological adaptations (troglomorphy), such as appendage elongation, visual organ reduction, and pigmentation loss (Mammola 2019). In addition, they also commonly have a low metabolic rate, small population sizes, and are highly sensitive to environmental disturbance (Howarth and Moldovan 2018; Lunghi and Manenti 2020).

The genus *Stenasellus* Dollfus, 1897, is a stygobiotic isopod that has been extensively studied worldwide (Khalaji-pirbalouty et al. 2018). This genus belongs to suborder Asellota and family Stenasellidae (Bakhshi et al. 2018). All members of *Stenasellus* occur in groundwater and can colonize various microhabitats, including free-water biotopes (e.g., clayey pools, gours, underground rivers) and phreatic water as they are able to live in the interstitial water of coarse alluvium. *Stenasellus* is a widely distributed genus, ranging from Southeast Asia, Central Asia to East Africa and South Europe (Messana et al. 2019). In Indonesia, the genus is distributed on the three

islands of Sumatra, Borneo, and Java (Magniez and Rahmadi 2006; Beron 2015; Puspita et al. 2019). *Stenasellus* has a Thetydean distribution reflecting its ancient origin, so it has been used as a palaeogeographic indicator (Malard et al. 2014; Messana et al. 2019).

*Stenasellus* in Indonesian caves is an important subject to study, not just in its taxonomy but also in its phylogeny, evolution, biogeography, and ecology. The gap of knowledge on *Stenasellus* in Indonesia needs further efforts to be resolved. To date, most research on *Stenasellus* in Indonesia has only focused on their taxonomy (Magniez 1982, 2001; Magniez and Rahmadi 2006). Meanwhile, their ecological aspect is still poorly studied.

The vast majority of cave occur in the karst area, a landscape originating from the dissolution of soluble rock (e.g., calcite, dolomite, gypsum, and rock salt) (Lauritzen 2018; Sun et al. 2018). The Indonesian archipelago hosts extensive karst areas with plentiful caves rich in biodiversity (Rahmadi 2008). One of those karst areas is located in Tasikmalaya reGENCY, West Java. This karst area covers 158.301 ha with more than 500 registered caves (Kurniawan et al. 2020). At the time of publication, studies on the biology of caves in this karst area are still very limited, so its subterranean biodiversity and ecology are poorly understood.

According to a local cavers' report in 2018, they found several individuals of *Stenasellus* living in a single cave (Sarongge Jompong) located in Tasikmalaya karst area. This finding is a new record since the distribution of *Stenasellus* in Java was previously only reported in two karst areas which are geographically situated far from Tasikmalaya, namely Klapanunggal (Magniez and Rahmadi 2006) and Sukabumi (Rahmadi 2008). The only species and the first record of *Stenasellus* described from Java is *Stenasellus javanicus* (Magniez and Rahmadi 2006). This species occurs in several caves located in Klapanunggal karst area situated in Bogor reGENCY (Rahmadi 2008). This karst area is about 175 km from Tasikmalaya and historically formed by different karst formations: Klapanunggal and Karangpucung formations. Considering the existing geographic isolation, the *Stenasellus* that occurs in Tasikmalaya is likely a different species.

The discovery of the *Stenasellus* population in Tasikmalaya karst area is an important record that should get more attention because it is the first-ever cave-obligate species unearthed from Tasikmalaya karst area. However, there was no scientific publication related to the finding. Along with the taxonomical study, research on the ecological aspect of stygobitic species is crucial. This study aimed to investigate the population, activity, and habitat characteristics of *Stenasellus* sp. in Sarongge Jompong cave to provide the first scientific information about the existence of *Stenasellus* in Tasikmalaya karst area. The results of this study are expected to be a reference in the arrangement of management and conservation policies for Tasikmalaya karst area.

## MATERIALS AND METHODS

### Study area

Sarongge Jompong is a karstic cave located in Tasikmalaya District, West Java, Indonesia, at the geographic coordinate 7°33'25.8" S 108°06'24.7" E (Figure 1). This cave has horizontal passages with a small entrance that is situated in a hillside where elevation is 314 m above sea level. An underground river streams down along the main passage of this cave, in which water flows all year round. The river has a rocky riverbed with high water velocity. In the branch passage, which is located on the upper side of the main passage, there is a pool and a wet gour generated by percolating water. According to the local cavers' expedition, the *Stenasellus* population was initially found in this site in late 2018 (Caves Society Tasikmalaya 2020, pers.com).

The cave has been used by the local community as a water source for irrigation, fishery, and daily needs. In addition, it also has been periodically explored by spelunkers and speleological activists, particularly for adventure purposes. At the same time, the local community practices traditional phosphate mining because the soil in the branch passage is rich in guano. The passage is inhabited by insectivorous bat populations, mostly from the genus *Rhinolophus*, which provide guano deposits on the cave floor.

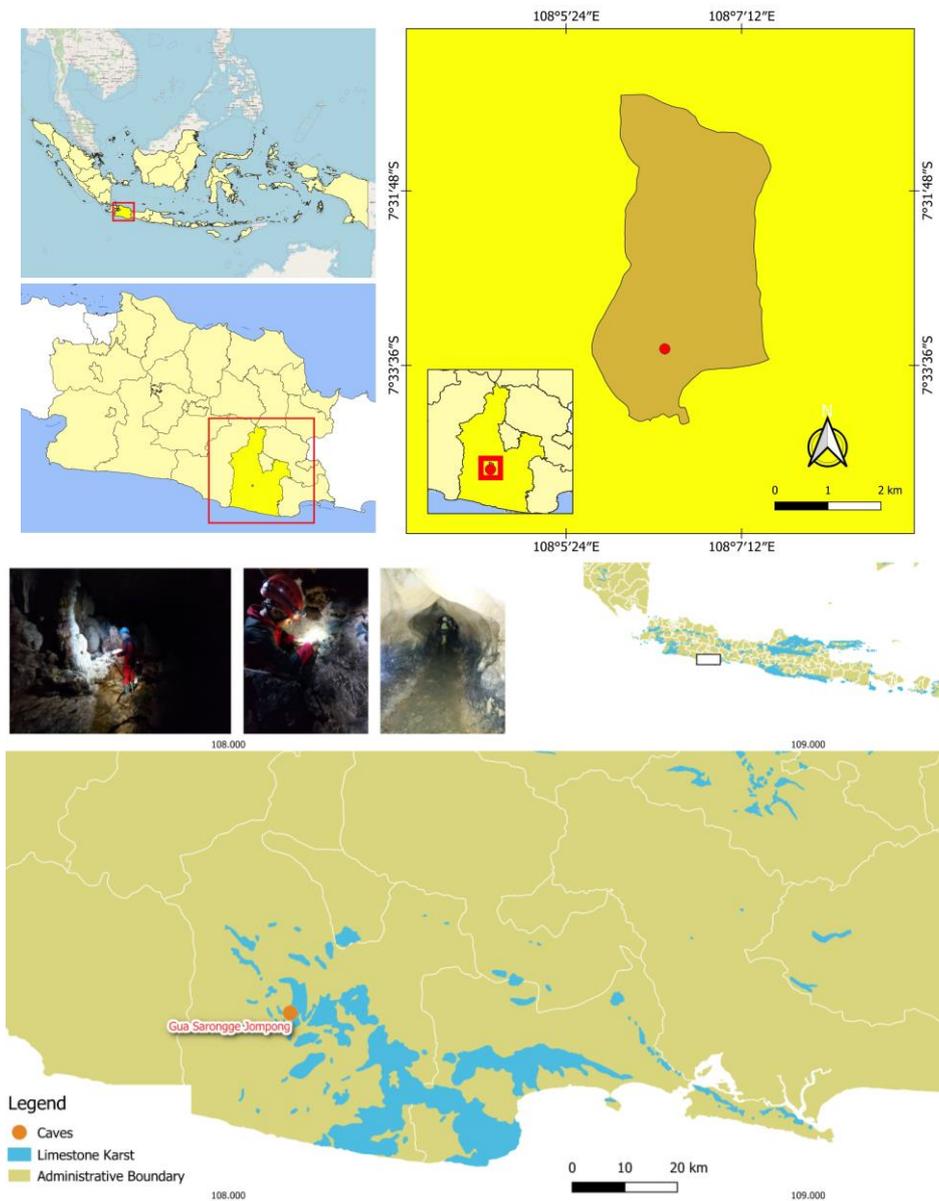
### Procedures

#### *Microhabitat discovery and cave mapping*

All passages of the cave were explored and observed thoroughly to find *Stenasellus* microhabitats. The observation was conducted through direct intuitive sampling (Wynne et al. 2019), focusing on the locations where water occurs (e.g., river, puddle, pool, wet gour). Microhabitat finding was conducted during daylight with 3 repetitions representing different timings (07.00-09.00 am, 11.00 am-01.00 pm, and 03.00-05.00 pm). Observations were performed by 3 observers who have good experiences in sampling cave-dwelling fauna. Each water body was observed for as long as 20 minutes to check the presence or absence of *Stenasellus* individuals. After the specific sites of microhabitats were recorded, cave mapping was then carried out. A cave map is needed to spot the particular locations of microhabitats in the cave passage. Cave mapping was carried out through a magnetic survey method and processed using Caves Survey and Corel Draw software to produce a cave map in plans view. All microhabitat sites were then overlaid onto the cave map.

#### *Field observations and measurements*

After specific sites of microhabitats were chosen, further observations were only conducted in those sites. Population size was estimated by calculating the numbers of *Stenasellus* individuals through direct observation with 3 repetitions for each microhabitat. The repetitions were carried out on different observation days. Each site was observed for 30 minutes to count the number of individuals present. Observations were performed carefully without disturbing the water in the microhabitats. At the same time, all activities performed by *Stenasellus* were also recorded.



**Figure 1.** The geographical position of Sarongge Jompong cave, Tasikmalaya District, West Java, Indonesia

Abiotic factors, including physicochemical parameters, were measured to study the characteristics of *Stenasellus* microhabitat. The measurements were conducted immediately after the observations were complete to prevent disturbance on the animal. The physical parameters comprised light intensity, air temperature, relative humidity (RH), water temperature, and microhabitat area, whereas chemical parameters included pH, salinity, hardness, conductivity, resistivity, total dissolved solid (TDS), dissolved oxygen (DO), and total oxygen. Light intensity was measured using lux meter (Lutron LX-113S), while air temperature and RH used thermo-hygrometer (HTC-1). Water temperature and all chemical parameters were quantified using a water tester (Lutron WAC-2019SD). The measurements were conducted directly in the study cave along with population and activity observations with 3 repetitions for each parameter. In addition, the other

physical attributes which visually reflect discrepancy among microhabitats - e.g., number of crevices and guano abundance - were also documented in each microhabitat. The number of crevices was demonstrated as limited ( $5 <$ ), moderate (5-10), and many ( $>10$ ). Meanwhile, guano abundance was classified into 3 categories: low (+), medium (++), and great quantity (+++).

#### Data analysis

Non-metric Multidimensional Scaling (NMDS) was performed to analyze the disparity of water parameters among microhabitats. In addition, Detrended Correspondence Analysis (DCA) was also carried out to clarify the relationship between *Stenasellus* population and chemical parameters of water. The analyses were executed with Rstudio Software under the Vegan package (Oksanen et al. 2020).

## RESULTS AND DISCUSSION

The genus *Stenasellus* is a groundwater-restricted stygobiotic taxon that evolved from marine to freshwater habitat, particularly subterranean waters (Ketmaier et al. 2003). Its capability to adapt to specific microhabitats of groundwater is an exciting subject to be investigated further. Its distribution and relation to geologic history need further phylogenetic and biogeographic studies. Based on current distribution data in Indonesian caves, *Stenasellus* distribution corresponds to the geologic setting of the Sundaland Core. The genus only occurs in Sundaland especially in Sumatra, Java and Borneo with the eastern limit of the distribution in Borneo in Central Kalimantan (Tumbang Topus) and Tasikmalaya in Java. The genus has not been recorded in the eastern part of Java, East Kalimantan, Sulawesi, the Lesser Sunda Islands, the Moluccas, and Papua. Extensive studies of cave invertebrates conducted by Indonesian Institute of Science (Currently National Research and Innovation Agency) in Sangkulirang karst (East Kalimantan), Maros Karst (South Sulawesi), Muna Karst (Southeast Sulawesi), Gombong Selatan (Central Java), Menoreh Karst (Central Java, Yogyakarta), Gunungsewu Karst (Central Java, Yogyakarta, East Java), and Tuban Karst (East Java) provide convincing evidence that the genus is absent from these karst areas. The distribution in Java and Borneo is presumed to be limited by the Luk Ulo-Meratus Sutures as the southeast border of the Cretaceous Arc (Zahirovic et al. 2014) or by the Meso-Tethys Suture clustered as the Neo-Tethys Suture (Metcalf 2021). Hypothetical groundwater colonization of *Stenasellus* from a stenassellid ancestor of "Mesogean Stock" in the Neo-Tethys Sea (Malard et al. 2014), and it is confirmed that the distribution pattern of *Stenasellus* is related to the Tethyan Orogenic Belt (Metcalf 2021). The records of *Stenasellus* in Tasikmalaya (West Java) and Tumbang Topus (Central Kalimantan) could be the easternmost distribution limit of Stenassellidae. This distribution pattern is similar to the charontid whip spider genus *Catageus* (formerly genus *Stygophrynus*) (Rahmadi et al. 2011).

### Population of *Stenasellus* sp.

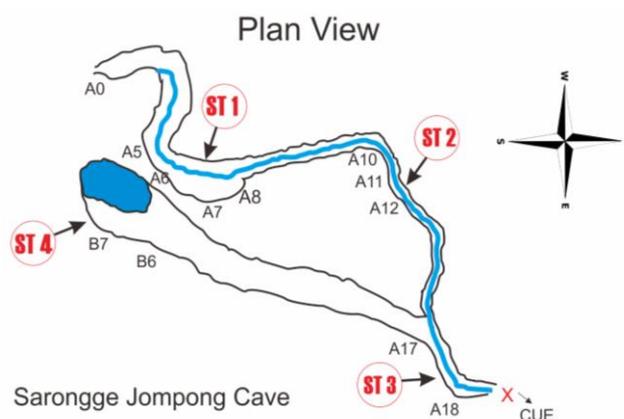
Extensive observations conducted in the recent study have successfully confirmed the existence of a *Stenasellus* population in Sarongge Jompong cave. Our exploration results showed that the population of *Stenasellus* sp. in the study cave was distributed in 4 separate microhabitat sites (Figure 2). These findings constitute new records since the previous notes by speleological activists only recognized 2 sites, corresponding to our site 1 and site 4.

All microhabitats of *Stenasellus* sp. in Sarongge Jompong were situated in the dark zone of the cave passage. This finding supports previous studies which mentioned that all members of the genus *Stenasellus* occur exclusively in subterranean aquatic habitats where sunlight is absent (Magniez 1981; Faille and Deharveng 2021).

Hence, they were classified as stygobites which means aquatic cave-obligate species (Bruno et al. 2020). Stygobiotic species are commonly sensitive to environmental fluctuation, so their distributions are commonly restricted in the dark zone where the microclimate and other abiotic parameters are relatively stable compared to entrance and twilight zones (Kurniawan and Rahmadi 2019; Mokany et al. 2019).

The distribution of the *Stenasellus* population, separated into 4 microhabitats, is an interesting circumstance that should get more attention. Magniez (1981) stated that the genus *Stenasellus* has local migration ability from one site to another in a single cave. However, the migration will only happen if the sites are hydrologically connected. Thus, further studies focused on hydrologic connectivity among microhabitats are needed.

There are 3 types of aquatic habitats in the dark zone of Sarongge Jompong, including an underground river (stream), pools, and wet gours. However, not all these aquatic habitats were inhabited by *Stenasellus*. Although the population was distributed in 4 different sites, all *Stenasellus* individuals were found in a similar type of microhabitat, that is, wet gours generated by percolating water. Meanwhile, there were no individuals observed in the river and pool. Gour, also known as rimstone, is a type of cave ornament (speleothem) formed as rings or stone dams (Pérez et al. 2021). Each ring of a gour has a basin that can be potentially filled by water. In addition, this cave ornament also commonly has crevices that provide shelters for *Stenasellus*. All gours occupied by *Stenasellus* sp. in Sarongge Jompong were relatively small at approximately 1-20 cm in diameter so that they can be classified as microgours. These gours had a part submerged in water because they were passed by small water currents generated by percolating water.



**Figure 2.** Population distribution of *Stenasellus* sp. in Sarongge Jompong cave, Tasikmalaya District, West Java, Indonesia

As the population only occurred in wet gours, the presence of percolating water can be suggested as a limiting factor for the population since it is the source of water (Figure 3). This water originates from surface water that enters the subterranean realm through the soil and permeable rock (Guo et al. 2019). The existence of percolating water in caves is highly dependent on the vegetation structure of the surrounding area and the nature of rock porosity. Vegetation may increase percolation rates because root systems can capture surface water and provide channels that connect water to soil and rock (Duan et al. 2015), whereas rock pores provide penetration pathways for water toward cave passages (Stroj et al. 2020). Hence, surrounding vegetation and rock porosity should be urgently maintained to conserve *Stenasellus* habitat in Sarongge Jompong.

*Stenasellus* sp. in Sarongge Jompong had a small population size (Table 1). According to the observations, it can be estimated that there were only around 8 individuals of *Stenasellus* sp. in 4 microhabitats. This number was the highest record by far since the previous notes by speleological activists stated a maximum count of only 5 individuals (Hidayaturrohmah 2020, pers.com).

Each microhabitat had different numbers of individuals of *Stenasellus* sp. The highest number of individuals was recorded in site 2 with a maximum of 4 individuals, whereas the other sites were relatively lower with 1-2 individuals. In a single microhabitat, the individuals were separated from one another and never found aggregated in a single dip of a gour. The closest distance from one individual to others was approximately 5 cm, whereas the furthest distance was more than 3 m.

Cave-dwelling stygobite faunas are well-known to have small population sizes. Most stygobiotic animals adopt the K-adapted life history, e.g., few eggs and low reproductive rates, which consequently results in a smaller population size compared to their epigeal relatives (Howarth and Moldovan 2018; Kurniawan and Rahmadi 2019). Reproductive events are rare in the genus *Stenasellus*. They generally reproduce only once every two years in suitable conditions, but it can be much longer with lower food availability (Howarth and Moldovan 2018). In addition, they also have a strong cannibalistic tendency that may control their population size (Malard et al. 2014). To date, little information has been available regarding the population size of *Stenasellus*. Several experts reported having collected hundreds of individuals in a single visit (Messana 2021, pers.com), but Rahmadi (2008) only found a few individuals. One monitoring experiment that had been conducted for Javanese species was on *Stenasellus javanicus* in Cikarae cave situated in Klapanunggal karst area. Based on the study, it was reported that only 5 individuals were successfully observed. Furthermore, the population could only be found in a single microhabitat site (Rahmadi 2008).

Along with small population size, many groups of stygobiotic fauna also typically lack aggregation behavior. Although it provides advantages in foraging, reproduction, and defense against predators in most epigeal species, this behavior is significantly reduced in cave obligate fauna. This reduction is believed to be a typical cave adaptation as a response to the lack of visual orientation and predator threats (Howarth and Moldovan 2018).

**Table 1.** The number of individuals in each microhabitat of Sarongge Jompong cave, Tasikmalaya District, West Java, Indonesia

Microhabitat	Number of individuals					
	Observation 1	Observation 2	Observation 3	Min	Max	Mean
Site 1	2	2	0	0	2	1.3
Site 2	0	4	2	0	4	2
Site 3	0	1	1	0	1	0.67
Site 4	0	1	0	0	1	0.33
Total	2	8	3	2	8	4.33



**Figure 3.** A. Wet gour, the main *Stenasellus* sp. habitat in Sarongge Jompong, Tasikmalaya District, West Java, Indonesia; B. *Stenasellus* sp. resting on the surface of a gour

### Activities of *Stenasellus* sp.

During the observations, several activities of *Stenasellus* sp. were successfully observed, including crawling on the surface of gour, resting, hiding in crevices, and moving to different locations through water media. These observed activities are similar to what was illustrated by previous studies (Magniez and Rahmadi 2006; Khalaji-pirbalouty et al. 2018; Messana et al. 2019). In contrast, many sources stated that *Stenasellus* like burrowing in mud and soil substrate, but this activity was absent in the observed individuals because all the microhabitats were in the solid form of gour with rock substrate.

The level of locomotor activity of *Stenasellus* sp. in Sarongge Jompong was very high. Among the observed activities, crawling on the surface of gour was the most frequently performed by *Stenasellus*. On several occasions, they were also observed moving from one location to different locations through water media. Besides moving downward and horizontally, they also could move upward against the current of percolating water (positive rheotaxis). The activities of *Stenasellus* are strongly influenced by water availability. The experiment conducted by Messana et al. (1999) reveals that locomotor activity of *Stenasellus* is dramatically reduced in the absence of water. *Stenasellus* will be immobile during drought periods and only move slowly in the wet clay. This study was conducted in the rainy season when water in all microhabitats was abundant, so *Stenasellus* could move actively.

Another type of activity that was quite often exhibited by *Stenasellus* sp. was hiding in crevices. In general, hiding is common in the members of genus *Stenasellus*. This activity is supported by their dorso-ventrally slender body shape, which helps them enter rock crevices easily (Magniez and Rahmadi 2006). Interestingly, this activity mainly occurred at the end of the observation when the cave microclimate may change due to humans' visits. In addition, it also happened when the *Stenasellus* sp. acquired a disturbance stimulus in the form of water being touched by the observers. According to these facts, it is strongly suspected that hiding activity is related to defense

mechanisms to protect themselves from threats and/or environmental alterations.

### Physicochemical characteristic of microhabitats

Until recently, scientific information related to physicochemical characteristics of *Stenasellus* habitat is still very limited. The vast majority of previous studies focused on species descriptions, while habitat observations were commonly taken at a glance, particularly on a few parameters which can be observed visually. Thus, the chemical parameters of habitat were rarely measured. Members of the genus *Stenasellus* are reported to have a wide distribution globally (Bakhshi et al. 2018). The physical and chemical characteristics of inhabited caves will vary greatly depending on the geographical location and specific conditions of each cave (Lauritzen 2018).

Measured physical parameters were relatively similar among microhabitats. All microhabitats were situated in the dark zone where light intensity was 0 lux. Air temperature ranged from 27.1-27.9°C while water temperature from 25.6-26.6°C. The highest temperature difference among microhabitats was only 1°C. In addition, the relative humidity of all microhabitats was also high, ranging from 85.7-89%. The physical difference among microhabitats was merely in the form of microhabitat size, guano abundance, and other specific features, including the number of crevices and whether or not a pool was present (Table 2).

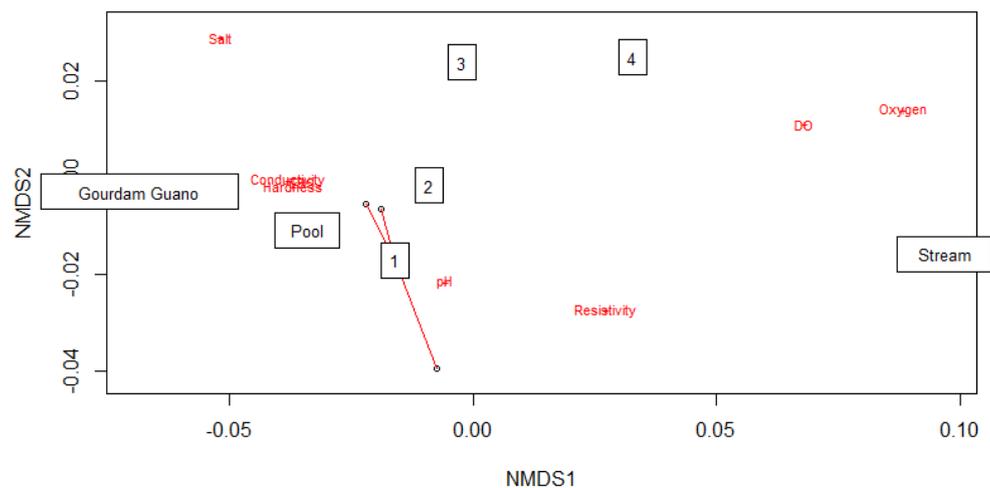
Table 3 shows the chemical characteristics of microhabitats. Several other types of aquatic habitats occurred in Sarongge Jompong, which were not inhabited by *Stenasellus* sp. including the river (stream), big pool, and gour with abundant guano were also presented for comparison purposes. In general, the population of *Stenasellus* sp. could be found in water with the following ranges of chemical characteristics: pH 6.45-7.12, conductivity 0.61-0.66 µS, TDS 409.33-436.67 ppm, salt content 0.03%, hardness 310.33-328 ppm, resistivity 1520.33-1612.33 Ω, DO 7.70-10.27 mg/L, and total oxygen 18.53 -27.5%.

**Table 2.** Physical characteristics of microhabitats

Microhabitat	Light intensity (lux)	Air temperature (°C)	Relative humidity (%)	Water temperature (°C)	Area (m <sup>2</sup> )	Guano abundance	Other features
Site 1	0	27.2	85.7	26.6	5.16	++	Diameter of gour small, many crevices
Site 2	0	27.9	89.0	25.6	1.40	+	Diameter of gour small, many crevices, a small pool
Site 3	0	27.1	88.0	25.7	3.36	++	Diameter of gour small, limited crevices
Site 4	0	27.1	89.0	26.4	1.80	+++	Diameter of gour small, limited crevices, a big pool

**Table 3.** Chemical characteristics of microhabitats

Habitat types	pH	Conductivity ( $\mu\text{S}$ )	TDS (ppm)	Salinity (%)	Hardness (ppm)	Resistivity ( $\Omega$ )	DO (mg/L)	Total oxygen (%)
Site 1	7.10	0.61	410.67	0.03	314.33	1597.37	7.70	18.53
Site 2	6.65	0.62	409.33	0.03	310.33	1612.33	8.03	19.63
Site 3	6.45	0.66	436.67	0.03	328.00	1520.33	8.80	23.73
Site 4	7.12	0.63	418.67	0.03	315.00	1583.00	10.27	27.57
Gour Guano	7.08	0.72	462.67	0.03	349.67	1414.33	6.53	16.93
Stream	7.02	0.53	354.00	0.02	263.67	1888.90	10.80	31.53
Pool	7.12	0.64	427.00	0.03	320.00	1562.00	6.90	18.01

**Figure 4.** The disparity of water parameters among microhabitats

Based on NMDS analysis results visualized in Figure 4, the stream had extremely high DO and total oxygen compared to *Stenasellus* microhabitats. In contrast, the pool and gours with abundant guano had relatively lower DO and total oxygen. Moreover, gours with abundant guano had higher values of conductivity, TDS, and hardness compared to *Stenasellus* microhabitats. Among *Stenasellus* microhabitats, site 1 was chemically more similar to site 2 compared to sites 3 and 4.

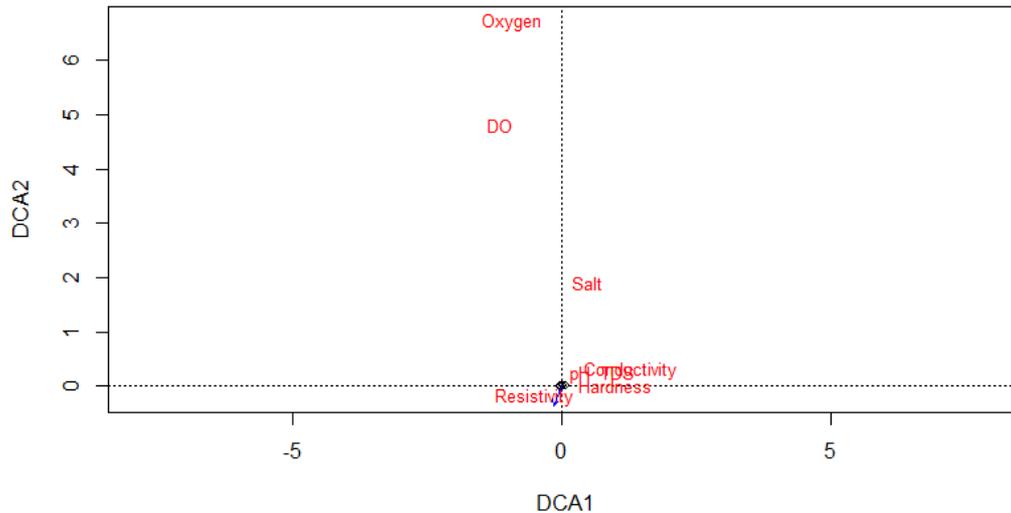
The chemical characteristics of *Stenasellus* microhabitats are relatively distinct from the other types of aquatic habitats which were not inhabited by *Stenasellus*. As a lotic system, the stream has a high  $\text{O}_2$  level due to intense water movement, generating turbulence that supports oxygen diffusion from the air into the water (Atapaththu et al. 2017). Additionally, bat guano is not accumulated in the stream due to water current, so oxygen consumption to decompose this organic material tends to be lower. In contrast, the pool, which is categorized as a lentic system, has relatively low DO and total oxygen (Priti and Sahu 2018). These two chemical parameters were also significantly lower in gours with abundant bat guano compared to *Stenasellus* microhabitats. Massive organic matter deposits are believed to cause the high values of conductivity, TDS, and hardness in gours with abundant guano. These three parameters are known to have strong positive correlations (Dirisu et al. 2018).

Among *Stenasellus* microhabitats, site 1 was chemically more similar to site 2 compared to sites 3 and 4. This result is strongly correlated with the number of *Stenasellus* individuals found in each site which is presented in Table 1. Most *Stenasellus* individuals were observed in sites 1 and 2, where water parameters were very much alike. Larger *Stenasellus* individuals in sites 1 and 2 reflect habitat preference in which chemical characteristics of water may act as deciding factors.

#### Relationship between *Stenasellus* population and chemical characteristics of water

In order to know which chemical parameters of water have a strong influence on habitat preference, DCA was conducted. The analysis was carried out by considering the chemical character of gours with abundant guano, which is physically similar to the *Stenasellus* microhabitats but chemically different.

The result presented in Figure 5 illustrates the relationship between *Stenasellus* population and the chemical characteristics of water. It can be seen that among water parameters, resistivity has a strong relationship with the *Stenasellus* population. The parameter is positively correlated with population, which is indicated by the blue arrow.



**Figure 5.** Relationship between *Stenasellus* population and chemical parameters of water

*Stenasellus* individuals in Sarongge Jompong were more abundant in sites where resistivity was higher. Resistivity is the ability of water to withstand the flow of electricity. The parameter can be an important indicator of water pollution (Jiang et al. 2013). The higher resistivity value of water indicates a lower contamination level in the water (Pierwoła 2013). Among observed microhabitats, site 1 and site 2 were preferred by *Stenasellus*. This condition was in line with the higher resistivity values in both sites compared to other microhabitats. In addition, site 1 and site 2 also had lower TDS and conductivity values, indicating lower pollutant concentration in both sites (Pal et al. 2015).

The main pollutant occurring in every microhabitat was bat guano. Bat guano is actually one of the most important food sources for cave-dwelling fauna since it is rich in organic and other essential matters (Wurster et al. 2015). However, our observations revealed that *Stenasellus* was never seen in the gours with abundant bat guano, where the water visually looked darker and contained a lot of particulate matter. This is a substantial indication that *Stenasellus* prefers clean waters with a low concentration of organic contaminants. In line with this result, previous studies also revealed that troglotibiotic and/or stygobiotic species tend to avoid guano deposits, particularly large fresh guano piles (Ferreira 2019).

### Threat and conservation challenges

Intrinsic and extrinsic factors may cause disturbance in the *Stenasellus* population in Sarongge Jompong. An intrinsic threat that potentially influences the population is the dynamic of bat populations roosting above microhabitat areas. Bats can produce large guano deposits, which may affect the population of *Stenasellus* due to its physicochemical characteristics. Guano can significantly influence *Stenasellus* microhabitats, especially the water, which is vital for *Stenasellus* suitability. Future conservation steps should incorporate further investigation into the influences of guano and bats on *Stenasellus* microhabitats.

An extrinsic factor that can cause disturbance is human visits. Anthropogenic disturbance is considered the main threat for the *Stenasellus* population in Sarongge Jompong. As mentioned previously, the cave has been frequently visited by the local community, spelunkers, and speleological activists. Human visits can disturb the *Stenasellus* population, either directly or indirectly. Direct disturbance can be in the form of stepping and touching that can incidentally hit *Stenasellus* bodies or microhabitats. Furthermore, microhabitat sites 1 and 2, where most *Stenasellus* individuals occur, are both located in the main passage that is very accessible to humans, making them potentially more vulnerable to disturbance.

Human visits may also induce microclimate alteration, indirectly disturbing the *Stenasellus* population. As stygobitic fauna, this species is susceptible to environmental changes caused by anthropogenic activities. Many studies have proven that human visits to caves - particularly with large numbers of people and extensive duration of exploration - can significantly increase air temperature and CO<sub>2</sub> levels (Šebela and Turk 2014; Kurniawan et al. 2018). To date, environmental changes induced by human visits have been widely reported as one of the leading causes of cave-dwelling fauna decline, particularly those belonging to stygobites and troglotibites (Macud and Nuneza 2014; Kurniawan and Rahmadi 2019; Pacheco et al. 2020; Constantin et al. 2021). In addition, other potential threats are land-use changes around the caves which are currently covered by forests that significantly contribute to provisioning percolating water into cave environments. The cave is not only crucial for the future of *Stenasellus* but also for the local community for provisioning water resources for their daily needs, fishery, and agriculture.

### Future directions

This study implies the importance of further scrutiny regarding the *Stenasellus* population in Sarongge Jompong cave. The number of *Stenasellus* individuals recorded in

this study was surprisingly low. Therefore, this result can be an initial indicator of the small population size of this species in Sarongge Jompong. However, several factors of this study may cause underestimation from the actual population size, including limitation of the sampling efforts and lack of sampling technique variation. Hence, further extensive monitoring needs to be carried out to confirm this hypothesis. A continuous observation covering both rainy and dry seasons will provide more robust data since the number of individuals may fluctuate due to differences in water availability.

Furthermore, combining multiple sampling techniques may be more fruitful, e.g., applying bait sampling along with direct intuitive sampling. The source of percolating water is highly inaccessible, making direct observation extremely difficult. As such, bait can beneficially attract *Stenasellus* that lurk in crevices and or the source habitat. In addition, hydrological connectivity among microhabitats needs to be investigated to explain the local migratory pattern of this species within the cave and between caves.

On the other hand, research on taxonomy is urgently needed to determine the taxonomic status of the *Stenasellus*. The taxonomic status will be an essential foundation for a deeper understanding of this species, such as phylogeny, evolution, ecology, physiology, and conservation, including its conservation status.

Phylogenetic relationship between the Sarongge Jompong population and the other *Stenasellus* populations distributed in Java is also an exciting topic to be examined. *Stenasellus* is well-known to have limited dispersal capacity. Consequently, their distribution is exclusively determined by paleo tectonic events (Ketmaier et al. 2003). Molecular data may reveal genetic variability that can indicate species divergence and show their evolutionary history.

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