

Population Density and Habitat Associations of Endangered Warty Frog (*Callulina shengena*) in Chome Nature Forest Reserve, Tanzania



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Abstract

Population density and habitat associations are critical and fundamental aspects in the conservation and management of wildlife species such as *Callulina shengena*. Being a critically endangered warty frog in Chome Nature Forest Reserve (CNFR), we therefore established vital information previously unavailable and thus demonstrated substandard conservation for this threatened amphibian species. We documented the information on population density estimates, habitat traits, associations, and threats subjected to *C. shengena*. We carried out active searching in four rectangular plots (70m × 50m) established in three different altitudinal zones in both wet and dry seasons in the CNFR. There was a significant variation in *C. shengena* density with altitude ($P < 0.001$). The highest density of *C. shengena* was in the mid altitude, followed by the lower altitudes. We found that the average population density of *C. shengena* was higher in the wet season (22 individuals/ha) than in the dry season (15 individuals/ha), suggesting that rainfall and temperature influenced *C. shengena* abundance. Plots that exhibited higher density of *C. shengena* were largely shady areas covered by closed canopies and located near water sources. Furthermore, we found that *C. shengena* was highly distributed in the mid altitudes (1951 - 2050 m.a.s.l) in the western part of the reserve. We also found that about 55% of *C. shengena*'s population was close to the forest boundary with the neighboring villages, which exposes the species to risks due to ongoing human activities in the reserve. More emphasis on habitat protection is necessary to avoid the extinction of *C. shengena*. Proper management of water sources (rivers and swamps) and natural vegetation in the forest should be prioritized for *C. shengena* conservation.

Keywords: Amphibia; *Callulina shengena*; Conservation; Distribution; Habitats; Population density; Threats

Introduction

Amphibians were the first vertebrates to spend part of their lives out of water [1]. During the first stage they live in water as gilled, larval forms, and during the second stage, they live on land as lung-breathing adults. Despite being the pioneers of life on land, they are still the most susceptible vertebrate taxon as revealed by their rapid global decline due to habitat loss, climate change, and many other factors [2-6]. Amphibians are highly sensitive to environmental changes due to their low mobility and strict physiological constraints [7]. They face disproportionately high risk of extinction, with many species' populations rapidly declining. Amphibians can thus act as useful indicators of environmental changes across space and time [7-9].

Most of the world's amphibian species inhabit the major biodiversity hotspots found in tropics especially in Central and South America and sub-Saharan Africa [10-11]. The Eastern Arc Mountains (EAMs) of Tanzania and Kenya are recognized as biodiversity hotspots and harbour a higher number of amphibian species with a high level of endemism [12,13]. In the EAM, forest

habitats have persevered under stable conditions which ensured the accumulation of brevipitid frog species over a long span of geological time [14]. Within this chain, there are multiple patches of mountains including South Pare Mountains where Chome Nature Forest Reserve (CNFR) is located. The CNFR is home to an endemic frog species, *Callulina shengena* [15], which is a warty frog species of the family Brevicipitidae firstly reported in 2010 during a survey in the rainforests on the northern part of Eastern Arc Mountains [16]. *Callulina* species are distinguished from other brevipitids by their truncate to expanded toe and finger tips [17]. However, *Callulina shengena* lacks prominent glands on the arms and legs, less distinct and less continuous interocular band sometimes present [17-19].

Upon the urge to comprehend the core factors of amphibian ecology, herpetologists realized that the spatial distribution and population density are important matters to work on since species distribution is never random [20]. There is a need to better understand the population density and dynamics of amphibian

populations and the factors that govern the distribution of species in different sites [21,22]. Population size and density estimates are crucial for the determination of the status of species and guides how to utilize resources for the management and conservation of species [23]. Most amphibians do not live-in isolation in a single microhabitat and effective conservation will require an integrated landscape approach [24,25].

One of the focal constraints in understanding amphibians' impulsive decline is that there is no enough and long-term information about their populations which could be useful in creating better ways to respond to their decline. has been reported to be an endemic within the Chome Nature Forest Reserve [26]. Furthermore, the IUCN Red List [26] listed *C. shengena* as Critically Endangered (sub-criteria a & b) because the extent of occurrence was estimated to be less than 100 km², number of locations and declining quality of habitat. There was a lack of information on *C. shengena* population density and trend in its area of occurrence [26]. Baseline information on *C. shengena* population density will enhance the proficiency of conservationists to manage *C. shengena* and avert any odds of any possible threats and extinction risks.

Knowledge of the habitat associations for *C. shengena* and understanding of the factors governing the species' population size is very crucial for its conservation. Characteristics including altitudinal range, distance from water, availability of shade, and disturbance influence the distribution and diversity of amphibian species [27-31]. Furthermore, declines in amphibian populations

have been vastly attributed to habitat alterations [32]. To manage and conserve any species effectively, a proper knowledge on the population size and its habitat association is critical [23,33]. Amphibian species with lower densities are under high extinction risks and therefore understanding and forecasting deviations in populations is a fundamental matter [34]. This study was conducted to estimate the population density of *C. shengena* and examine the influence of habitat characteristics and human disturbance on the *C. shengena*'s distribution in Chome Nature Forest Reserve.

Materials and Methods

Description of the Study Site Area

Our study was conducted in Chome Nature Forest Reserve (CNFR). The CNFR is the largest forest block in the South Pare Mountains situated between the North Pare Mountains and the West Usambaras in north-eastern Tanzania (Figure 1). It is located between 4°10' - 4°25' South and 37° 53' - 38° East and includes the highest ridge of the range and the highest peak of Shengena (2463 m.a.s.l.), but it slopes down to an altitude of 1250 m.a.s.l on its Eastern edge [35]. The forest consists of three main vegetation types; sub-montane forest on the eastern ridge between 1250 - 1600m. Montane forests are found above 1500m with a drier type on the lower slopes and rain-shadow areas, whereas a wetter type covers 60% of the reserve [36]. Elfin forest occurs above 2300m on the highest ridges.

Climate and Geology

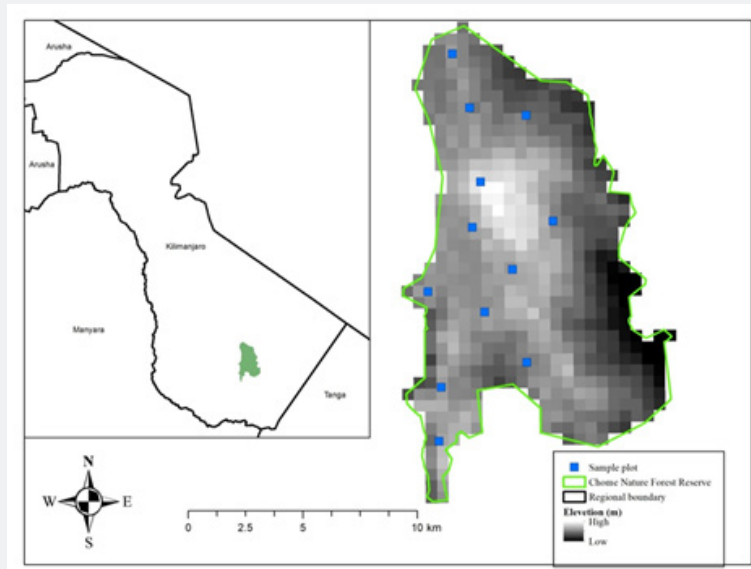


Figure 1: Map of Chome Nature Forest Reserve (CNFR) showing sampling locations.

Weather systems are bi-modal, the long rains moving in from the south-east during October to June. Rainfall is estimated at 3000mm on the wetter, eastern side of the Shengena Mountains,

the dryer western slopes, heath and montane grassland receive an estimated 1500 - 2000mm, with mist effect at higher altitudes [36]. The dry season is between June and September, with light

rainfall occurring at higher altitudes. Temperatures vary with rainfall, from a minimum of 15°C in July to 20°C in February [37]. Soil types vary with topography, acidic lithosols predominate on ridges with ferralitriclatosols on the slopes. On the Chome-suji Plateau (western grasslands), hitosols have developed in depressions under the heath and bog vegetation [36]. The Shengena range was formed by faulting uplift some 25-100 million years ago and consists of gneiss and migmatite precambium crystalline basement rocks [36] (Figure 1).

Data Collection

We systematically established a total of twelve (12) plots in three different altitudinal zones (Figure 1) covering different vegetation types [38]. The three altitudinal zones included low zone (less than 1950 m.a.s.l), mid altitude (between 1951 and 2050m.a.s.l), while the higher altitudinal zone was higher than 2051 m.a.s.l. In each altitudinal zone, four rectangular plots of 70m × 50m (0.35ha) were established while the distance between plots was 300 m apart. Nested plot sampling method was used and dominant tree species were recorded in 50m x 20m quadrats within the permanent plots, and shrubs in 10m x 10m quadrats

while grasses were covered in 1m x 1m quadrats.

We carried out an active search during the dry season in 2019 and wet season in 2020 conducted five (5) times per season. In each plot, seven (7) people conducted a search by walking in parallel lines of 10 m apart each until the whole plot was sufficiently searched following [23]. The area search occurred from 0800HRS to 1600HRS with the same search efforts throughout the survey. Plots were searched during the wet season and dry season to determine the effects of seasonal variations on the population density and distribution of *C. shengena*. We searched thoroughly in the tree branches or trunks of small trees, ground and under the rocks due to the semi-arboreal nature of *C. shengena* (Figure 2). Externally, *Callulina* species are distinguished from other brevicryptids by their truncate to expanded toe and finger tips. We used key to the species of *Callulina* to positively identify the species. *Callulina shengena* has prominent, relatively pale glandular mass on arms and feet. Furthermore, *C. shengena* has less distinct and less continuous interocular band and restricted to South Pare Mountains [17].



Figure 2: Endangered warty frog *Callulina shengena* in Chome Nature forest Reserve, Tanzania (Photo by Simon Loader).

In each plot, we recorded the number of individuals counted, the coordinates of each plot, and habitat variables including altitude measured using an altimeter, air temperature using thermometer, percentage canopy cover by ocular estimation at each plot surveyed [29]. We also recorded the distance proximity to water sources by measuring the distance between the plot and the nearest water sources. Human disturbance levels were estimated by the percentage of logged trees in a plot, size of the bare land left after mining activities and human trails across the plot.

Statistical Analysis

We estimated the population density of *C. shengena* by computing the average number of individuals recorded in a unit area (ha). *C. shengena* population densities are expressed as means ± standard error. We used t-test to determine if there was any significant difference in the *C. shengena* population density between sampling seasons. *C. shengena* population density was compared among different altitudes using analysis of covariance (ANCOVA) in the linear model procedure of SPSS 22.0 (IBM Corp., Chicago, IL, USA). Furthermore, we assessed the influence of the

recorded habitat variables on the population density by running General Linear Model (GLM). A one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD, $P < 0.05$) test was used to compare *C. shengena* population densities between the three altitudes. A general linear model (GLM) in SPSS version 22.0 (IBM Corp., Chicago, IL, USA), was used to determine the effects of habitat variables on *C. shengena* population density whereby disturbance levels were used as covariates while altitude, canopy cover, distance to water, annual rainfall and mean monthly temperature were used as random factors.

Results

Population density and distribution

We encountered 179 individuals that were spotted during an active search in the study site. The total number of individuals

varied significantly between sampling seasons ($t_{11} = 5.9$, $P < 0.001$) with 62 individuals in the dry season and 117 individuals in the wet season. The highest average density of *C. shengena* was recorded during wet seasons (Figure 3). There was a significant variation in density with altitude where the recorded highest density was in the mid altitude followed by the lower altitudes. The average density of *C. shengena* ranged between 15 individuals/ha to 22 individuals/ha during dry and wet seasons, respectively. There was a significant variation in density with altitude where the recorded highest density was in the mid altitude followed by the lower altitudes (ANOVA; $F_{1, 5} = 3.55$, $P < 0.001$). About 60% of the total density was confined in the mid-altitudinal areas compared to the higher or lower altitudes (Figure 3). The higher altitudinal areas had the lowest average density for both wet and dry seasons.

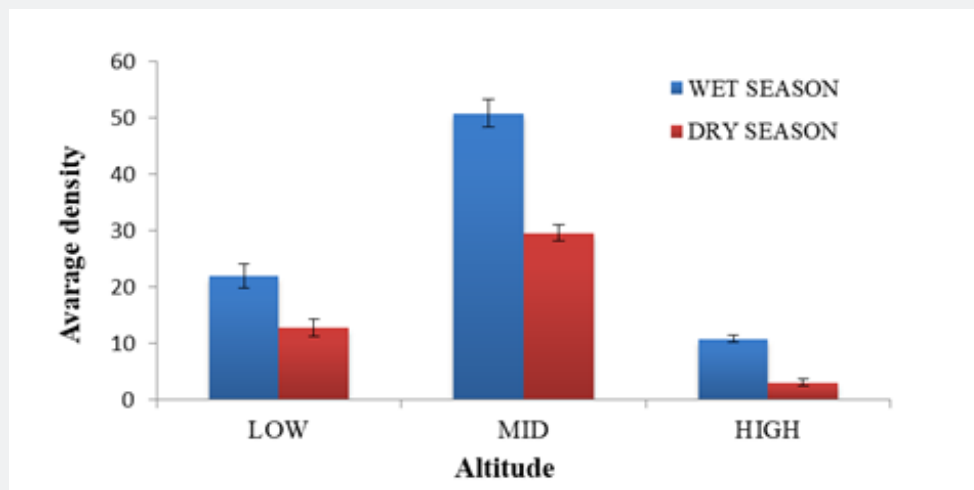


Figure 3: Average density of *C. shengena* within the three altitudinal zones for both wet and dry seasons. Values are means and error bars present standard errors of the means (SEM).

Callulina shengena habitat associations

Habitat attributes that were investigated in this study included altitude, canopy cover, distance from the water sources, and levels of disturbance. There was a significant main effect of the altitude on *C. shengena* population density ($F_{1, 11} = 22.78$, $P = 0.001$). Mid altitude exhibited the highest population density of *C. shengena* compared to the lower or higher altitudinal zones. Canopy cover had a significant influence on *C. shengena* distribution ($F_{1, 11} = 18.1$, $P = 0.002$). About 66% of the plots surveyed had canopy cover greater than 75%. Large trees with broad branches and leaves were more abundant in the plots, which had higher counts of *C. shengena*. Furthermore, the plots were set within various distances from the water sources. Plots located closer to water source (less than 50m) had a higher density of *C. shengena* (57%), while the density decreased as the distance from water source increases ($F_{1, 11} = 69.88$, $P = 0.004$).

The mid altitudes were dominated by *Ocotea* spp, *Macaranga* spp, and *Podocarpus* spp, Fern (*Drynaria volkensii*), Cladonia

usambarensis as the understory. The higher altitude zones were dominated by *Ocotea* spp, *Syzygium* spp, *Podocarpus* spp, and *Rapanea* sp. The lower zones were dominated by *Acacia* spp (exotic), *Albizia* spp, and grass *Sporobolus* spp. Human disturbance in the forest was among the factors assessed for *C. shengena* distribution and population density (Figure 4). Plots located in the lower zone were heavily disturbed by both illegal gold mining activities and logging, which left large bare spaces and holes with natural vegetation cleared. It was observed that disturbances induced by the anthropogenic activities varied within the reserve. This had a statistically significant but yet negative influence on the distribution of *C. shengena* ($F_{1, 11} = 31.1$, $P < 0.001$).

The microclimatic data recorded included surface temperature and rainfall in both wet and dry seasons. Average monthly temperatures were 12.2 ± 1.14 °C and 25.3 ± 1.06 °C for the wet and dry seasons, respectively. The average monthly rainfall was higher for the wet season (268.4 ± 34 mm) than for the dry season (160 ± 18 mm). Throughout the study duration, monthly

temperature showed a significant influence on the population density of *C. shengena* ($F_{1, 1} = 25.94, P = 0.001$). Furthermore, rainfall had a significant effect on *C. shengena*'s density ($F_{1, 11} = 18.13, P = 0.001$). This suggests that *C. shengena*'s density increased with increasing rainfall.

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Figure 4: Open land after human activities in the CNFR.

Discussion

In this study, we found that *C. shengena* had a humped distribution with the highest density within the mid elevations. *C. shengena* is found on the western side of CNFR, mostly on the mid altitudes of the mountains in the reserve. The mid altitude of the mountain provides more ideal environments for the survival of *C. shengena*. Elsewhere, amphibian species have been reported to show their population peak in the mid-altitudinal range [39]. *C. shengena* is categorized as Critically Endangered (sub-criteria a & b) as per the IUCN Red List because its distribution is restricted to an area estimated to be 100 km², number of locations and declining quality of habitat [26]. This range is potentially at risk of shrinkage due to on-going human activities and possible climatic changes [40-42].

C. shengena is known from one locality, where the maximum distribution range and quality of its habitat is expected to decline due to the on-going development of large-scale tourism activities and walking trails in the forest. These activities have been promoted by the management of the reserve and may cause a high risk of shrinkage of the presently small range of *C. shengena*. One of the probable limiting factors that subject *C. shengena* to mid and lower altitudes is the availability of water that decreases as the altitude increases. Similarly, Spesies et al. [43] found that amphibian species richness and distribution varied along an elevational gradient in the Malaysian forests. The mid altitudes of the western part of the CNFR were observed to have several water sources. Moreover, the mid altitudes had high tree density with a large canopy that obstructs most of the Ultra Violet (UV) radiation, fresh water streams, and swamps with warm temperatures slightly higher than the higher altitudes. This had been shown to

be preferable and conducive habitat for *C. shengena*.

Amphibian species depend on multiple habitat characteristics which are necessary for the completion of the life circle and sustainability of the whole population [1]. These include water, shade or canopy cover, rainfall, temperature, and disturbance in the area could either support or suppress a given population. With the increase in altitude, temperature declines monotonically, whereas precipitation normally peaks at mid-altitude [39]. We observed that *C. shengenawas* highly dependent on the proximity of fresh water streams. Distance from the water source has been reported to have an impact on the occurrence of amphibian species throughout the world [40].

Most of the *C. shengena* individuals were closer to water bodies and many would run into the water after being disturbed. The slowly moving waters or swamps had high densities of *C. shengena* (Figure 5). This explains the way *C. shengena* uses calm or slow moving water for reproduction and it is highly beneficial for their oxygen absorption and survival tactics in case of disturbances. The density of *C. shengena* was positively influenced by canopy cover. Plots with the highest percent of canopy cover harbored high *C. shengena* population density. The shade provided by trees has shown to be better refuge for the species to avoid direct and too much sunlight. This is because the shades are preferable to the amphibian physiology and camouflage from predators and disposal of UV-light [44-47].

Canopy cover also acts as a blanket to ground dwelling organisms including *C. shengena* during harsh weather conditions. The mid altitude in the CNFR had more than 75% canopy cover, which allows optimal passage of sun rays and warm temperatures

for reproduction and other normal physiological activities. *Ocotea* spp, *Macaranga* spp, and *Podocarpus* spp were the most abundant trees in the mid altitudes. Highest densities of *C. shengena* were recorded in plots with the highest abundance of tree species. This

suggests that trees are more advantageous to the survival of the frog populations due to their large canopy for shade and moisture retention.



Figure 5: *Callulina shengena* hotspot in the CNFR.

As ectothermic organisms, it is widely recognized that warmer and moderate temperatures and precipitation in low and mid altitude locations usually support more species and individuals [31]. In the Eastern Arc Mountains, rainfall increases with increasing altitude while temperature decreases with increasing altitude [45]. Temperature and precipitation are crucial to the physiological functioning and survival of amphibian species everywhere [28]. Climatic variables, mainly temperature and rainfall affect the survival and existence of amphibians in an area [48]. Climate may also play an indirect role in facilitating epidemics of infectious diseases [49]. During the high rainfall season, more individuals were easily spotted because the weather provided wet conducive environments for species survival, which when integrated with the intrinsic population characteristics, can sustain the individual species.

In this study, the observed rainfall pattern had an influence on the number of occurrences recorded during both the wet and dry seasons. The rainfall significantly increases the volume of swamps and ponds that are highly used by *C. shengena* and it was clearly observed in the dropping of counts during the dry season. Despite the high rainfall at high altitudes in the CNFR, much of the water observed elsewhere settled on mid-altitudes and rest on the foot of the mountain, creating breeding sites for open water breeders [50]. This is one of the reasons for the high density of *C. shengena* observed in the mid elevations because it is the ideal condition that supports amphibian populations.

Changes in rainfall seasons were reported in many parts of Tanzania in 2019 and early 2020. The months that were found to experience low rainfall or drought received extremely high

rainfall in the past 40 years [51]. The longer dry seasons and rainfall variability have been associated with the increase of deadly amphibian fungi that have been sweeping off global amphibian populations [1,5,52,53,54]. These changes in weather in many areas also affect the life circles of amphibians and alter their reproductive rates since their eggs are highly affected by the temperature and humidity of the corresponding habitats. The unexpected scenarios like these are tampering the typical ecosystem behaviour and if the species are not adaptive to the harsh turnover, it could lead to the disruption of the gene flow of the individual species and the whole ecosystem. In this study, we found that frog counts were negatively correlated with human-induced disturbances in the forest. Humans' trails, illegal mining, and logging that have been taking place in the forest are pushing the frogs into a smaller habitat range. Similarly, Behangana et al. [29] found disturbance had a negative impact on amphibian abundance and caused more damage to the species survival.

The previous illegal mining had left the low altitudes of the eastern part of the reserve with multiple holes and despite of introduction and plantation of the trees by the United Nations Development Programme (UNDP) to restore the natural vegetation of the area. Human activities, either legal or illegal reduce the habitat range of species and might subject them to more threats and fuel their population decline. Habitat change can cause amphibian decline and extinction [8,55,56]. Habitat loss has contributed to amphibian declines globally with an estimated 63% of all amphibian species affected, and as much as 87% of the Threatened species affected [57]. Many amphibians require specific microhabitats with appropriate conditions of moisture,

temperature, pH, and sufficient refuge and food resources. These conditions are easily disrupted after habitat modification.

The most common forms of habitat change in and around CNFR include clearance for crops, logging, clear-cutting, and urbanization. The extent of the effects of habitat change can be difficult to determine as many amphibians spend most of their lives in one or two terrestrial environments and seasonally migrate to a different, usually aquatic environment, to breed. To disrupt the breeding, migration would thereby cause a decline [58]. Habitat change may affect one or more of the habitats necessary for completion of the life cycle, for example, the environment in which amphibians spend most of their years (e.g. the water bodies in which they breed and are utilized by their larvae (e.g., ponds, streams).

Tourism activities that are highly encouraged by the government through the Ministry of Natural Resources and Tourism pose a high risk to *C. shengena*'s range. Walking trails across the forest tend to be among the major causes of the introduction and dispersion of exotic tree species. Tree species such as *Acacia mearnsii* and *Eucalyptus* spp were not reported in the forest decades ago, but now the dispersal of their seeds by human movements has spread them into the forest. We noted that native tree species such as *Ocotea usambarensis* and *Podocarpus latifolius* have declined following the introduction of invasive species. Sites dominated by native tree species were found to be more preferred by *C. shengena*. Despite the conservation efforts and banning of commercial logging activities, the habitat preferences of *C. shengena*'s population could be threatened [59].

Conclusion

Our study has provided a clearer insight of the population status as well as the habitat characteristics pertaining to *C. shengena*'s survival. Population density and habitat associations are key features in the conservation and management of *C. shengena*. We estimated an average density of 22 individuals/ha and being in a very small area of occupancy of less than 100 km², it is appropriate to categorize *C. shengena* as critically endangered as per the IUCN ranks. *C. shengena* was most abundant in the mid altitudes of the reserve compared to the higher and lower altitudes. This is highly attributed to the availability of basic requirements for their survival including shade, warm temperature and fresh water to sustain their population.

Mid altitude, fresh waters, high canopy cover, and rainfall were the crucial factors identified to be more preferred by *C. shengena*. More emphasis on habitat protection is necessary to avoid the extinction of *C. shengena*. Given that *C. shengena* are highly sensitive to environmental changes, our findings highlight that the responses of habitat suitability can be critical considerations in future conservation measures for species with weak dispersal abilities. Proper management of water sources (rivers and swamps) and natural vegetation in the forest should be prioritized for amphibian conservation. We also recommend a

review of tourism activities in the reserve to minimize the impact of tourism on suitable habitat for *C. shengena*, which is Critically Endangered and endemic to the CNFR. For example, the presence of undesignated walking trails along the mountain should be avoided to minimize disturbance and habitat destruction. Integrated landscape approach is necessary for effective conservation of *C. shengena* because the species does not live in isolation in a single microhabitat.

Data Availability

All data used to support the findings of this study are available from the corresponding author upon request.

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