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Morphological description and life history of the Philippine Swamp Frog *Limnonectes leytensis* (Boettger, 1893)

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Abstract. *Flores ABA, Diesmos AC, Nuñeza OM. 2022. Morphological description and life history of the Philippine endemic* Limnonectes leytensis *Leyte swamp frog in Rogongon, Iligan City, Philippines. Biodiversitas 23: 133-139.* The Philippine endemic *Limnonectes leytensis* (Leyte Swamp Frog) is a widespread species, however, most studies concerning it only focus on adult stages, except for the study on Negros Island. With this, we conducted a study on the morphology and life history of *L. leytensis* in Rogongon, Iligan City, Philippines. We collected different tadpole stages through opportunistic sampling. Gosner developmental stages 23 to 40 were considered and measured using standard tadpole body morphometrics. Linear regression was used for modeling the relationship between the different morphometrics. Results showed that the tadpoles have a depressed body, dorsolateral eye position and has golden, light to dark brown pigmentation. Apart from this, its mouthpart showed an anteroventral shape, and the labial tooth row formula is 1/2-3, showing a single row in the upper lip and three rows in the lower lip, having the 3rd row half the length of the other two, which is different to that of the results of the past studies which reported that the labial tooth was 1/2:1-1 showing a broken gap in the first tooth row of the lower lip. The analysis revealed a positive relationship between different morphometrics to the total length (body length rs: 0.96; tail length rs: 0.99; musculature height rs: 0.90 and maximum tail height rs: 0.85) with a significance value of <0.001. The data generated from this study will ignite future efforts on tadpole research in the country, given that there are differences found in the tadpoles from different regions.

Keywords: Correlation, development, identification, larvae, morphometrics

INTRODUCTION

Amphibians play an integral part in the ecosystem (Pradhan et al. 2018) as primary consumers of insects, prey for birds, mammals, and other predators (Cortéz-Gómez et al. 2015). Apart from this, they are also considered one of the most effective indicators of ecological health (Guzy et al. 2012). Frogs being amphibians, are among the planet's most threatened taxa, which puts most of the species vulnerable to becoming extinct (Desforges et al. 2022). They are also less mobile, have smaller home ranges, and thus may be affected with a greater impact through alteration of their habitat (Beebee 2013).

Declines affecting anuran population are catastrophic and widespread in the tropics, which include the Philippines (Hocking and Babbitt 2014). So, numerous studies have been conducted in the country in order to address these problems (Alcala et al. 2012; Diesmos et al. 2012; Warguez et al. 2013; Decena et al. 2020). However, most ecological studies only address the adult stage, especially diversity (Almeria and Nuñeza 2013; Mapiot et al. 2015; Aureo and Bande 2017), whereas larval stages are rarely included (Wells 2007), so they received lesser attention than their adult stages (Duellman and Trueb 1986). It is rather surprising considering that the biphasic life cycle of anurans imposes a more vulnerable stage than adults because of the dramatically different selective regimes on the aquatic larval stage, the metamorphic stage, and the terrestrial post-metamorphic stage (Blaustein et al. 2012). Considering that some anuran species have a biphasic life cycle, they breed in different bodies of water and eventually turn into tadpoles (Yaoita 2019). These tadpoles exhibit different structural characteristics depending on their specific life stages (Altig et al. 2007). Despite this, the tadpole stages of anurans are just as important as those of their adult stage for the persistence of the species in the area and for their survival success and distribution. Studying the tadpole's basic morphology, description, and life history is needed, especially in the Philippines, because of its small quantity of studies.

There is a need for quick evaluation of research sites using the identification of anuran amphibian larval stages, regional surveys, habitat inventories, resource use studies, interspecific competition studies, and conservation in order to maximize amphibian species counts and to identify their larvae in the field. This is especially evident in tropical ecosystems such as the Philippines, which are physically complex and have a diverse range of species. Although there has been a recent rise in efforts in tadpole surveys and tadpole fauna inventories, a substantial number of frogs, one of which is *Limnonectes leytensis*, still lack thorough descriptions of its larval stages (Chou and Lin 1997; Leong and Chou 1999; Haas and Das 2011).

Limnonectes leytensis, a species of anuran known as Leyte Swamp Frog, is considered to be a Philippine endemic fanged frog (Inger 1954). This frog is relatively small-sized and has a fang-like odontoid process that can be found in its lower jaw (Siler et al. 2009). It occurs on different islands of the Philippine Islands including Samar, Leyte, Bohol, Camiguin Sur, Cebu, Negros, and Mindanao (Siler et al. 2009; Diesmos et al. 2015; Sanguila et al. 2016). It can also be observed in varied habitat types, such as swamps, streams, and riparian areas in the agricultural, primary, and secondary forest habitats.

Despite this, there are only a few studies on the tadpoles' description, morphology, and life history of *Limnonectes leytensis.* Studies were only carried out on Negros Islands, Philippines (Alcala and Brown 1956; Alcala 1962). Considering the dates of publication which were 1956 and 1962, this means that there is antiquity of the study. Since there are no published accounts and no studies conducted on the tadpoles of *L. leytensis* on Mindanao Island showing a backlog for taxonomic work in its description, this study would likely fill in the gap on the lacking information of the morphology of the *L. leytensis*, especially on Mindanao Island. So, this study was done to describe the tadpoles of *L. leytensis*, their morphology, as well as their life history in Brgy. Rogongon, Iligan City, Philippines.

MATERIALS AND METHODS

Study area

The study was conducted within the vicinity of Brgy. Rogongon, Iligan City, Philippines (Figure 1). Upon conducting the study, a Wildlife Gratuitous Permit (GP) for the legal collection of samples was adhered to the Department of Environment and Natural Resources (DENR) Region X, with GP No. R10 2021-07 in Rogongon, Iligan City. The said Barangay constitutes a total land area of 35,555 hectares and is considered to be the only remaining part of the city to host forest ecosystems. It is rugged, mountainous to hilly semi-rolling topography with an elevation of up to 1020 meters above sea level (Casim et al. 2012). Specifically, the study was conducted at Sitio Libandayan of the said barangay. The sampling site was laid out opportunistically, especially in the bodies of water, following the presence, abundance, and distribution of their adults in the different sites.

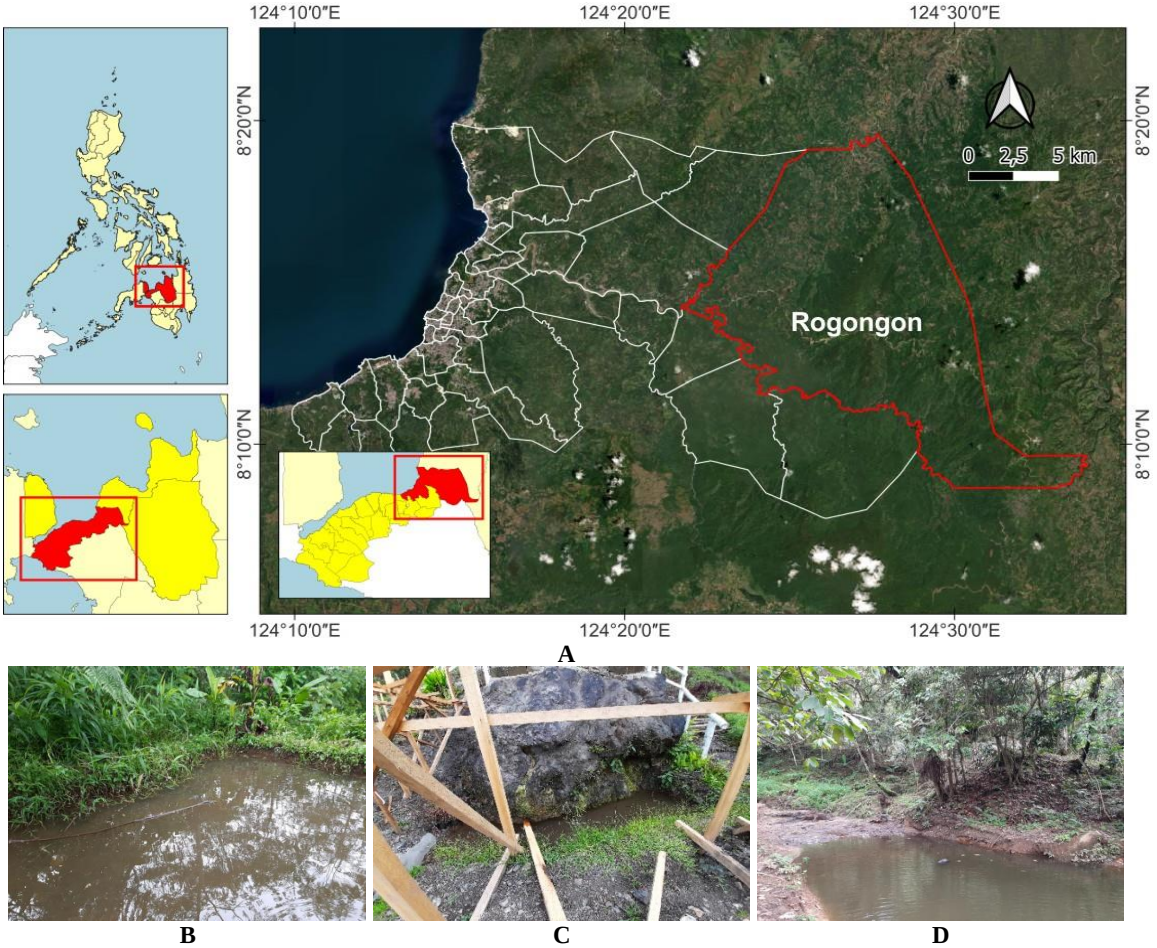


Figure 1. A. Study area and habitat of the *Limnonectes leytensis;* B. Site A: Lower elevation pond where we saw tadpoles of *L. leytensis;* C. Site B: Roadside water ways; D. Site C: Pond

Sampling protocol

Fieldwork was conducted in the different sampling sites from June 21 to July 05, 2021. At least 3 individuals from different embryonic stages of tadpoles and eggs were obtained and collected from the different sampling sites in the area. The field specimens were preserved at 10% formalin, which was mixed equally with 70% ethanol solution (Grosjean 2001). Some of the specimens were put into a container and transferred into an aquarium at the Terrestrial and Freshwater Biodiversity Laboratory in the Premiere Research Institute for Science and Mathematics (PRISM) for rearing identification, observation of life histories, and their larval development. The water was freshened and replaced every after 4 days and mixed with green algae, as this serves as food for the larvae. Meanwhile, to maintain the oxygen supply, we put an aquatic plant such as *Hydrilla* sp. and an electrically-operated aerator in the aquarium. The specimens were then removed from the aquarium and preserved at frequent intervals throughout the developmental period. The measurements were also based on freshly preserved materials.

The specimens that were preserved comprised almost all of the developmental stages of tadpoles based on tadpole staging of Gosner (1960). Lateral and ventral views of the tadpoles were photographed and staged according to their corresponding developmental stages. Specimens were also measured following preservation through standard tadpole body measurement by Altig et al. (2007) and Grosjean (2005), specifically, Body Length (BL), Tail Length (TaL), Musculature Height (MH), Maximum Tail Height and Total Length (TL) depending on developmental phases. Vernier caliper was used to measure the morphometric measurements (Widholzer and Castroviejo-Fisher 2018) and a stereomicroscope was used to identify their labial tooth row formula (Gamal 2014).

Data analysis

The tadpole morphological descriptions, including its oral structure, were studied based on Altig (1970) at Gosner stage 38. This also includes the description of its oral disc and labial tooth row formula (LTRF) (Nokhbatolfoghahai et al. 2020). Spearman's Rho was used to analyze the significant differences between the group means for tested variables and in understanding the strength of the relationship between these two variables (Gogtay and Thatte 2017). Linear regression was used to model the relationship between the tested variables.

RESULTS AND DISCUSSION

External morphology of tadpoles

Limnonectes leytensis showed a high abundance because they breed all year round in the area where eggs and different tadpole stages have been collected. This serves as baseline information for the tadpoles of *L. leytensis* to be present on the different sites. So, we are able to observe some breeding adult frogs and their eggs on the sampling sites. We are also able to collect egg clutches (Figure 2), and tadpoles at various stages were also observed and collected in all of the sites.

During the collection of samples, we observed that the external morphological structure of the tadpoles of *L. leytensis* showed a depressed body morphology that is benthic in nature. The positioning of their eyes also showed a dorsolateral position which is visible in their dorsal head. Their ventral fin is found on the right, wherein the anus creates a short tube on the median position of the vent position of the tadpole. The tail is observed to be pointing backward and creates a blunt-like shape in terms of width. Their tail is also approximately greater than its body length showing three-fourths of the tadpoles' total length. Meanwhile, it was observed that the dorsal fin is broader than its ventral fin (Figure 3).

We have also noted the coloration of the tadpoles of *L. leytensis* in the area. The coloration of tadpoles varies from golden brown, light brown to dark brown and the external morphological description is similar to that of the results given by Alcala and Brown (1956) and Alcala (1962). Their tail fins also showed clear with random brown speckles with small irregular brown spots scattered across their dorsal and ventral tail. However, after preservation, the golden-brown coloration of the tadpoles turned to dark brown and their ventral part and tail became pale grey and the some of their speckles were gone and some others also turned to dark brown in color. The morphometric measurement of tadpoles varies in their developmental stages, wherein the total length is from 8 mm at stage 23 to 40.7 mm at stage 40.

Based on the findings in this study, their external morphological characteristics showing depressed body shape are typical for the genus *Limnonectes* (Yodthong et al. 2021), probably because they are dependent on their benthic feeding behaviors on which they can be observed in the bottom portion of their microhabitat (de Souza et al. 2022). The coloration of the tadpoles of *L. leytensis* is also said to be typical for its genus (Rowley et al. 2014). Apart from this, the morphometric measurement of the tadpole of *L. leytensis* shows a small to medium-sized tadpole depending on their specific developmental stages, showing the growth in the total length of tadpoles as their development stages progress.

Labial Tooth Row Formula (LTRF)

The mouth of the *L. leytensis* tadpole is found in the tip of the ventral head of the tadpole, which shows an anteroventral shape. Except for a tiny central ventral gap, it is surrounded by a marginal row of papillae laterally and ventrally. It was also noted that there is only a single row of teeth in the upper lip of the tadpole. Meanwhile, on the lower lip, we noted at least three rows, 2 long rows, and the lowest row is roughly half the length of the middle row or somewhat longer. Therefore, the Labial Tooth Row Formula based on Altig et al. (2007) is 1/2-3 (Figure 4).

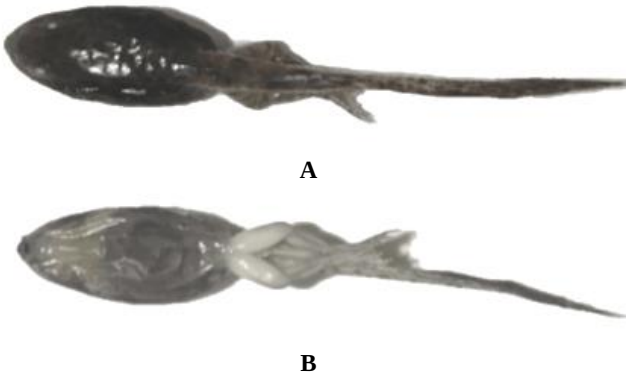
Typically, based on the data collected from the tadpoles in Rogongon, Iligan City, the labial tooth row formula or mouth formula of the tadpoles from this study is slightly different from those of the past studies by Alcala and Brown (1956) and Alcala (1962) because they reported that the labial tooth formula of the *L. leytensis* tadpole show the

upper lip have only one single row which is the same as the tadpoles presented in this study. Meanwhile, the lower portion of the lip also exhibits three rows, but the uppermost portion is broken, and the lowest oral papillae are short. In this study, we found that the first tooth formula in the lower lip showed a completely showing unbroken oral papilla. So, we quantify the labial tooth row formula in this study as 1/2-3, which contradicts the results of Alcala and brown (1956) and Alcala (1962), who stated that the labial tooth was 1/2:1-1. Given this, we still don't know what causes these differences, probably because of the environmental pressures, feeding habits, food availability, geographical differences, or even the evolution and natural history of the species, given the antiquity of the existing studies. Therefore, it is recommended to further scrutinize these issues.

Life history of *Limnonectes leytensis*

Upon rearing, we preserved some of the tadpole specimens and some were grown into an adult stage to confirm the species since adult stages are more likely to be identified. The staging of embryos and larval development was based on Gosner (1960). Out of the forty-six preceding stages of *L. leytensis*, only eighteen tadpole stages were included. These are stages 23 to stage 40 because they are easy to observe and more dominant than the other stages not mentioned (Figure 5). Transitioning of tadpoles was noted at stages 23-25, where it was observed that the operculum and the slow disappearance of gills through time which can be observed in the ventral position of the tadpole. It was also observed that the tadpoles from these stages had formed an initial pattern at their tail. At this stage, we can also see that the labial tooth formula begins to differentiate, which becomes the diagnostic feature for the identification of tadpoles. At about stage 26, the tooth rows begin to develop, and the number and forms of the oral papillae are gradually changing. After this stage, limb development was used to delineate between the different stages of tadpoles from stages 26 to 40. These stages can be easily identified using the length and diameter of the developing limb bud. Upon reaching stage 31, the foot forms paddle-shaped and individual toes begin to emerge at stage 37. Meanwhile, at stages 38-40, it was noted that there are differential changes in the proportion in the length of individual toes. In stage 39, they emerge as bright patches, and lastly, in stage 40, the individual toes become actual tubercles.

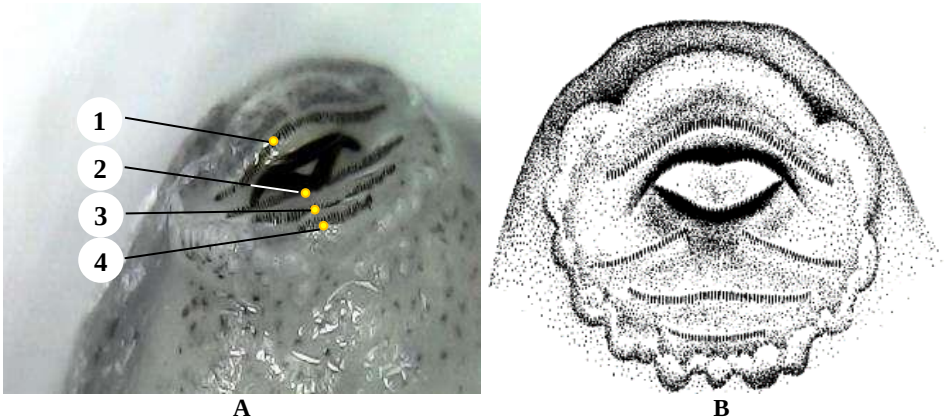
Figure 2. *Limnonectes leytensis* breeding and eggs seen on the site



A

B

Figure 3. Tadpole of *Limnonectes leytensis* in A. Dorsal Position and B. Ventral Position



A **B**

Figure 4. A. Larval mouthpart and labial tooth row formula: 1/2-3, showing the different position of tooth papillae and its corresponding number of teeth of *Limnonectes leytesis* in the current study (1. Upper Labial Tooth Row (1); 2. Lower Labial Tooth Row (1); 3. Lower Labial Tooth Row (2); 4. Lower Labial Tooth Row (3) and; B. Labial toot row formula 1/2-3, showing a single row in the upper lip and three rows in the lower lip having the 3rd row half the length of the other two based on the past studies of Alcala and Brown 1956; Alcala 1962 in Negros Island

As developmental stages progress, it was observed that the tadpole's total length also grows (Figure 6). From stage 23, with two individuals, the mean total length is 8 mm. Then at stage 24, two individuals were used as a representative with a total length of 10.75 mm. At stage 25, it was noted that the total length of the tadpole at this stage is 16.93 mm, with 7 individuals sampled. At stage 26, the total length progresses to 18.1 mm with five individuals sampled. For stage 27, we have at least four individuals with a total mean length of 20.43 mm. As the morphometric progresses, we can see those individuals from different stages are also progressing until they reach up to 40.7 mm at stage 40.

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Table 1. *Limnonectes leytensis* tadpoles morphometrics correlation using Spearman's rho and provided p-value

		Body length	Tail length	Musculature height	Maximum tail height	Total length
Body length	Spearman's rho	—				
	p-value	—				
Tail length	Spearman's rho	0.92***	—			
	p-value	< .001	—			
Musculature height	Spearman's rho	0.89***	0.90***	—		
	p-value	< .001	< .001	—		
Maximum tail height	Spearman's rho	0.84***	0.84***	0.84***	—	
	p-value	< .001	< .001	< .001	—	
Total length	Spearman's rho	0.96***	0.99***	0.90***	0.85***	—
	p-value	< .001	< .001	< .001	< .001	—

Note**:** * p <0.05; ** p <0.01; *** p < 0.001

Figure 5. Life history and developmental stages of tadpole. A. Stage 24; B. Stage 25; C. Stages 26, D. Stage 28; E. Stage 30; F. Stage 32; G. Stage 34; H. Stage 36; I. Stage 38; J. Stage 39; K. Stage 40 (Stages are derived from Gosner 1960). The red arrow indicates the emergence of hind limb bud

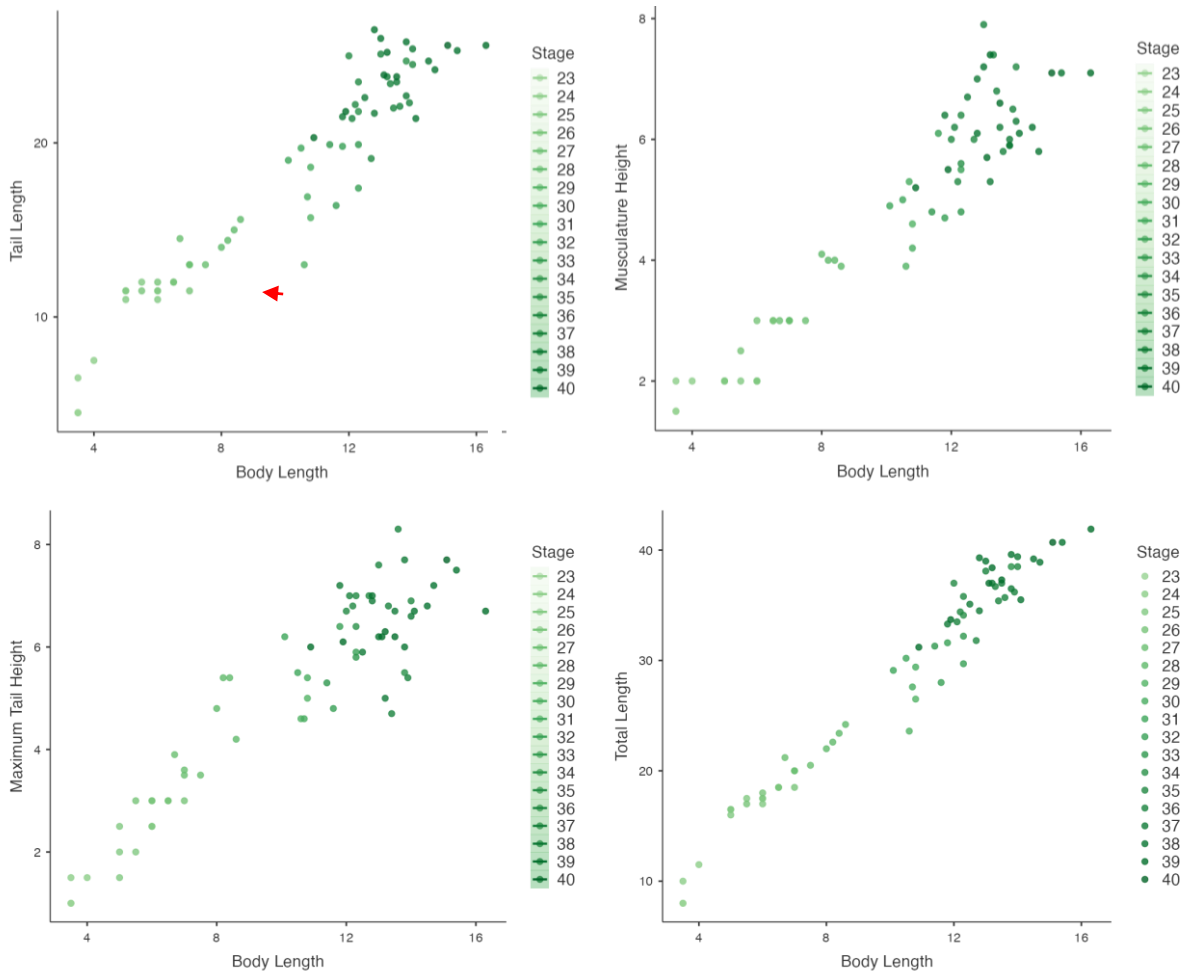

Figure 6. Linear regression modelling of the morphometrics of the tadpoles of *Limnonectes leytensis***,** showing a direct positive relationship between morphometrics in response to different stages

Spearman rho's correlation analysis revealed a positive relationship between different morphometrics to the total length (body length rs: 0.96, tail length rs: 0.99, musculature height rs: 0.90 and maximum tail height rs: 0.85) with a significance value of <0.00, all of which were laid in the data in Table 1. It was also proved by the Linear regression modeling herein growing stages also have a direct relationship to the different morphometrics. The life history and morphology of *L. leytensis* in Mindanao Island remain poorly unknown. A few studies on these tadpoles have only been conducted on Negros Island (Alcala and Brown 1956; Alcala 1962), and no studies have been done in Mindanao. Although tadpole stages of *L. leytensis* were already known in the Visayas region, there is still a large gap in the delineation based on the morphology of this species, specifically because of its antiquity and geographical isolation from the study site. Apart from this, the previous study only sampled fewer individuals from different stages, but in this study, more individuals and life stages were documented.

In conclusion, the tadpoles of *L. leytensis* is a small to medium-sized tadpole exhibiting a depressed body shape and has a dorsolateral eye position and greater length on its dorsal fin than its ventral fin, which is due to their benthic feeding ecology. The coloration also varies from golden brown to light brown to dark brown. However, coloration changes after preservation, which means that the preservative has a direct effect on the tadpole's color. They also possess an anteroventral shape mouth that has a single row of teeth in the upper lip and 3 rows where the 2 rows (1st and 2nd row) are relatively long while the third row is half the length of the 2 rows, showing a labial tooth row formula 1/2-3 which is different from the past findings in a different area. As developmental stages progress, it was observed that the tadpole's total length also grows and there are morphological differences between different life stages of the tadpoles.

This study is a perfect step in further scrutinizing the need for this kind of research in the country, including a description of other species, assessing their developmental biology and their ecology. With the current study, an attempt has been made in order to cover oral and external morphological descriptions of *L. leytensis* tadpoles and provide a more detailed description of their developmental changes following different stages. Consequently, this study will add to the large gap of tadpole studies in the

country, providing baseline information. So further scrutiny is highly recommended in order to provide more conducive research, especially to other species, because this will benefit both freshwater ecosystems and the society.

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