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Altitudinal morphometric variation of *Polypedates leucomystax* (Gravenhorst, 1829) in Yogyakarta, Indonesia

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Abstract

Indonesia is one of the world's megabiodiverse countries due to its tropical climate and diverse geographic landscape. One of the species widely distributed in Indonesia is *Polypedates leucomystax*. Imogiri Nature Reserve and Merapi Valley represent two locations with contrasting altitudes, which ultimately drive adaptive responses and cause morphometric variation among *P. leucomystax* populations inhabiting the two areas. The aim of this study was to analyze the morphometric variations of *P. leucomystax* frogs in the Imogiri Nature Reserve and Merapi Valley and also to analyze the relationship between abiotic factors and differentiated morphometric characters. Morphometric character data were tested using the Mann-Whitney test ($p \leq 0.05$), followed by the Spearman correlation test was conducted with abiotic factors. The morphometric characters that differed significantly in males were STL, NS, EN, MBE, THIGHL, and MTI, whereas in females the significant characters were NS, MFE, MBE, IOD, UEW, and 1TL. In addition, temperature, humidity and altitude also have a correlation with the differentiated characters. In male frogs, altitude correlated with the characters EN, MBE, THIGHL, MTI and temperature correlated with MBE, THIGHL, MTI, while in female frogs altitude correlated with the characters NS, MFE, MBE, IOD, UEW, 1TL and temperature correlated with NS, MFE, MBE, IOD. Then humidity correlated with UEW. The conclusion of this study is that there are 6 variations in morphometric characters in male frogs and 6 characters in female frogs found in Merapi Valley and the Imogiri Nature Reserve. Furthermore, abiotic factors are also associated with differentiated morphometric traits.

Introduction

Indonesia is one of the countries with high biodiversity. This is due to its tropical climate and diverse regional characteristics, resulting in an abundance of flora and fauna (Mustofa et al., 2021). One of the abundant diversities in Indonesia is amphibians. Amphibians are divided into three orders: Gymnophiona, Caudata and Anura. However, in Indonesia there are only two orders: Gymnophiona and Anura. The order Anura, commonly known as frogs or toads, is widespread throughout Indonesia (Pranata et al., 2023). One abundant amphibian species in Indonesia is *Polypedates leucomystax*, or the striped tree frog, which lives in lowlands to highlands (Addaha et al., 2015). This frog has a high ability to adapt and tolerate environmental changes, so this frog is often found around human settlements or in extreme places (Wea et al., 2024). *Polypedates leucomystax* frog has a wide population distribution in various habitats due to its high adaptability. These habitat differences can lead to variations in morphology and morphometry even within the same species.

Geographical conditions greatly influence the morphological characteristics of an organism, including amphibians. Differences in altitude, temperature and latitude are factors that can play a role in shaping the morphological adaptation of amphibians for survival. According to Liu et al. (2025), Bergmann's rule shows that geographical factors, such as altitude and temperature, can influence morphological variation in a population of organism. Animals that live in environments with lower temperatures tend to have larger body sizes than animals in warmer environments. Then, Liu et al. (2025) also added that Allen's rule theory supports Bergmann's rule where the size of body parts can be influenced by environmental climate. Animals that live in cold environments tend to have shorter limbs than animals that live in warmer environments. However, whether Bergmann's and Allen's rules apply to amphibians remains debated, necessitating further research (Hsu et al., 2014). In addition, research on variations in altitude of a region on the morphometry of amphibians, especially *Polypedates leucomystax* frogs, is still limited, so further investigation is therefore needed.

Imogiri Nature Reserve and Merapi Valley are two locations in the Special Region of Yogyakarta that represent different geographical conditions and altitudes. Merapi Valley is at an altitude of 803 meters above sea level and is an area formed by the volcanic activity of Mount Merapi. Currently, Merapi Valley currently functions as an educational tourism area, residential area, and community land. Imogiri Nature Reserve is a conservation area located at an altitude of 90–250 meters above sea level with dense and relatively homogeneous vegetation. The differences in habitat characteristics of these two locations have the potential to influence the morphological variation of *P. leucomystax*. The selection of the research location is supported by reports of the presence of *P. leucomystax* in areas adjacent to the research location. Dani et al. (2025) reported this species is found in Wukirsari Village, Imogiri. Then Kuswantoro et al. (2011) also reported in Plawangan Hill, Sleman, which has environmental characteristics similar to Merapi Valley. One method to study the variation of a species is morphometry, namely the measurement of body characteristics which are then compared between populations to identify morphological variations (Fauzan, 2019).

Morphometric research on *Polypedates leucomystax* frog is still very limited and has never been conducted in the Special Region of Yogyakarta. A study on morphometric variation of *P. leucomystax* in Indonesia was conducted by Addaha et al. (2015) in West Sumatra, which found morphometric variation between populations. Therefore, this research is necessary to add scientific data references regarding morphometric variation in the *P. leucomystax* frog, especially in the Special Region of Yogyakarta at different altitudes and locations. The purpose of this study is to analyze morphometric variation in *Polypedates leucomystax* frog in the Imogiri Nature Reserve and Merapi Valley. In addition, this study also aims to analyze the relationship between abiotic factors and significantly different morphometric variations. This research is expected to become a basis for understanding the morphological adaptation of the *Polypedates leucomystax* frog to the differences in environmental topography in the Special Region of Yogyakarta. In addition, data regarding variations in morphometric characters of *P. leucomystax* frogs in the Special Region of Yogyakarta can be used as a comparative reference between characters in *P. leucomystax* frogs from other regions.

Materials and methods

1. Study Area

This research was conducted from November 2025 to January 2026. The location of data collection for *Polypedates leucomystax* frogs was conducted in two locations, namely Imogiri Nature Reserve, Imogiri District, Bantul Regency, Special Region of Yogyakarta (-7.920383, 110.394533) and Merapi Valley, Nganggring Village, Turi District, Sleman Regency, Special Region of Yogyakarta (-7.600066, 110.397250). Frog identification, morphometric character measurements, and abiotic data collection were carried out directly on site.

2. Material and Tools

The materials used in this research are stationery, tally sheet and specimen bags. The tools used in this study include a work identification guidebook by Kusrini (2013) entitled "Panduan Bergambar dan Identifikasi Amfibi Jawa Barat", a work identification guidebook by Alhadi et al. (2021) entitled "Panduan Bergambar dan Identifikasi Amfibi Pulau Jawa", an HTC-1 thermohygrometer with a temperature range of -10 to 50°C and humidity of 10–99% (Dr. Grey), a 0-150 mm digital caliper with an accuracy of 0.01 mm (Mitutoyo), a 15 inch sweep net , an 80 W headlamp (Aoki), a Global Positioning System (GPS) Essential application, a smartphone, and the SPSS ver.30 application.

3. Research Methods and Design

Before conducting the research, a survey and pre-sampling were conducted with the aim of determining the scope of the area and distribution points of *Polypedates leucomystax* frogs. The survey was conducted day and night using the Virtual Encounter Survey (VES) method. After the research location was determined, data collection was conducted using a purposive sampling technique, where data was collected at predetermined points in the area. Locations that serve as habitats for *Polypedates leucomystax* (identified through surveys conducted in the field) were found at water reservoirs and areas with moist plant vegetation.

Data collection was conducted at night from 7:00 PM to 12:00 AM Western Indonesian Time (WIB) (Fauzan & Widya, 2022), when frogs are active during this time period. If frogs were found outside of data collection hours, they were still collected and measured. The frogs observed consisted of 20 adult frogs, namely 10 individuals from the Imogiri Nature Reserve and 10 individuals from Merapi Valley. Each frog location measured consisted of 5 male individuals and 5 female individuals. The number of individuals used in this study refers to previous research conducted by Aini et al. (2022) using 5 male individuals, and Pramesti & Suwartiningsih (2025) using only 3 individuals to each gender. The relatively limited number of individuals is due to the lack of individuals that meet the research criteria in the field, especially in female frogs which are generally found in fewer than male frogs. Similar conditions are also found in various other frog morphometric studies, so that in general the ones that are often used are male frogs. The study by Nesty et al. (2013) also noted that if individuals do not meet the specified number, measurements and analysis will continue according to the number found.

Adult frogs are characterized by the completion of tail absorption (complete metamorphosis) (Primiani, 2021), while male and female frogs are distinguished by their body length, with female frogs being larger than male frogs (Bery, 1975; Inger & Stueing, 2005). The differences between male and female frogs can be seen in Figure 1. Frogs are caught using a sweep net or with bare hands. Identification of the frog *P. leucomystax* the sampling was conducted using a field guidebook, after confirmation, and the frogs collected according to the specified number. The morphometric characteristics of each individual frog were measured twice using a digital caliper directly in the field, so that the measured individuals could be immediately released. To prevent duplicate measurements, measurements were carried out after the specified number of samples had been collected. If the number of samples was not met in one day, the measured individuals were photographed in detail and stored in Google Drive with a clear description. This documentation was used to ensure that the same individuals were not measured again during data collection on the following day. The number of morphometric characters measured was 32 characters from the head to the cloaca (Figure 2). The abiotic factors of altitude, temperature, and humidity were measured three times: at the beginning, middle, and end of data collection. All data obtained was recorded in a tally sheet.

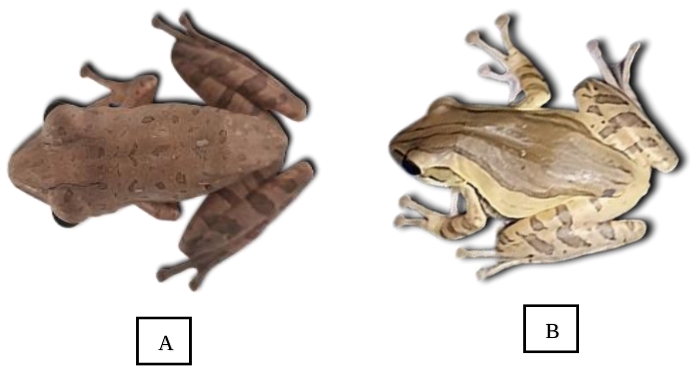

Figure 1. Size Differences of *P. leucomystax* (A) Male and (B) Female

4. Data Analysis

Morphometric character measurement data were standardized as ratios relative to the snout-vent length (SVL) using Microsoft Excel to generate morphometric indices. Furthermore, the morphometric character measurement data were analyzed using the Mann-Whitney test because the data obtained were not normally distributed, to determine the variation between two different populations. Then, if there were morphometric characters that were significantly different between populations, a Spearman correlation test was conducted because the data obtained were not normally distributed, to determine the relationship between abiotic factors (altitude, temperature and humidity) with morphometric characters that were significantly different between the populations.

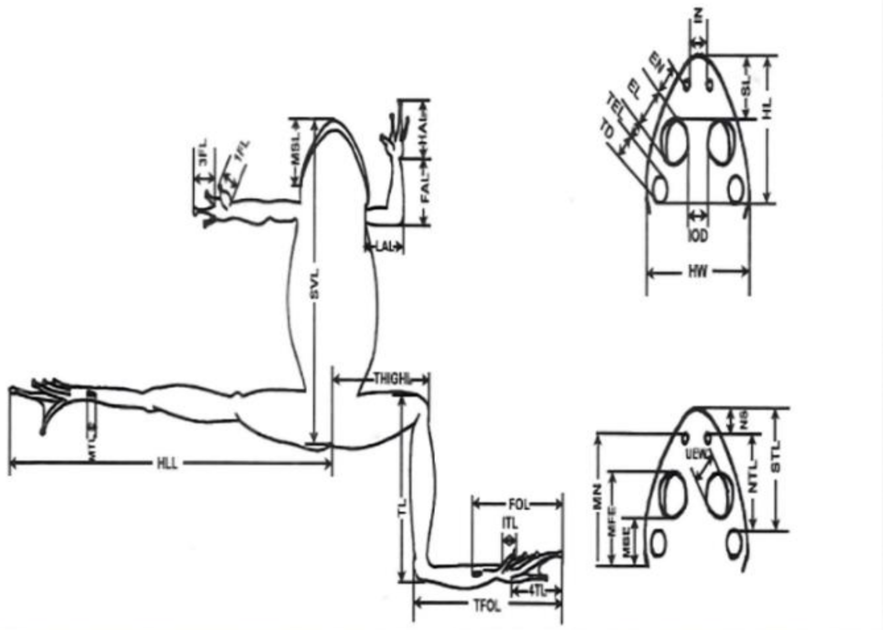

Figure 2. Measured Morphological Characters of Frogs (Aini et al., 2022)

Note : SVL: Body length from tip of snout to cloaca, HL : Head length, HW: Head width, STL : Distance from snout to front of tympanum, MSL : Snout length, NS : Distance from nose to snout, SL : Distance from front eye to snout, NTL : Distance from nose to front of tympanum, EN : Distance from front eye to nose, TEL : Distance from back eye to front of tympanum, DT : Tympanum diameter, MN : Distance from mandible to nose, MFE : Distance from mandible to front eye, MBE : Distance from mandible to back eye, 1FL : Length of first toe of front leg, 4TL : Length of fourth toe of rear leg, IN : Distance between noses, EL : Eye diameter, IOD : Distance between eyes, UEW : Eyelid width, HAL : Length of third toe to front leg, LAL : Distance from shoulder to elbow of front leg, FAL : Distance from elbow to ankle of front leg, HLL : Length of third toe to the cloaca of the hind leg, THIGHL : Distance between the cloaca and the knee of the hind leg, UEL : Length of the eyelid, TL : Length of the tibia , TFOL : Length from the metatarsus to the tip of the fourth toe of the hind leg, FOL : Length from the tarsus to the fourth toe of the hind leg, F3L : Length of the third toe of the fore leg, MTI : Length of the ankle of the hind leg, 1TL : Length of the first toe of the hind leg.

Results and discussion

1. Morphometric Variation in the Entire Population of *Polypedates leucomystax* in Merapi Valley and Imogiri Nature Reserve

Based on the data from the results of morphometric character measurements that have been tested using Mann-Whitney in Tables 1 and 2 below, it is known that there are several morphometric characters that are significantly different.

Table 1. Results of Mann-Whitney Analysis of Morphometric Characters in the Entire Population of Male *Polypedates leucomystax* in Merapi Valley and Imogiri Nature Reserve.

Grouping Character	Morphometric Characters of Males	Merapi Valley		Imogiri Nature Reserve		Sign
		Average	STD	Average	STD	
Overall	SVL/SVL	1.000	0.000	1.000	0.000	1.000
Head	HL/SVL	0.325	0.020	0.345	0.019	0.076
	HW/SVL	0.288	0.021	0.286	0.012	0.754
	STL/SVL	0.270	0.017	0.244	0.021	**0.047***
	MSL / SVL	0.323	0.013	0.325	0.019	0.754
	NS/SVL	0.052	0.005	0.045	0.003	**0.047***
	SL/SVL	0.154	0.017	0.145	0.007	0.602
	NTL/SVL	0.216	0.015	0.214	0.007	0.917
	EN/SVL	0.109	0.005	0.087	0.012	**0.016***
	TEL/SVL	0.023	0.004	0.023	0.006	0.917
	DT/SVL	0.085	0.005	0.079	0.001	0.076
	MN/SVL	0.302	0.030	0.287	0.018	0.602
	MFE/SVL	0.207	0.012	0.208	0.014	0.917
	MBE/SVL	0.114	0.007	0.097	0.009	**0.016***
	IN/SVL	0.089	0.005	0.087	0.003	0.347
	EL/SVL	0.106	0.008	0.114	0.006	0.175
	IOD/SVL	0.103	0.011	0.102	0.010	0.602
	UEW/SVL	0.090	0.011	0.086	0.005	0.465
	UEL/SVL	0.118	0.006	0.130	0.009	0.076
Forelimb	1 FL/SVL	0.119	0.023	0.116	0.014	0.754
	3FL/SVL	0.212	0.028	0.223	0.017	0.251
	HAL/SVL	0.311	0.027	0.290	0.042	0.465
	LAL/SVL	0.168	0.009	0.179	0.008	0.117
	FAL/SVL	0.212	0.027	0.206	0.021	0.602
Hindlimb	4TL/SVL	0.353	0.054	0.302	0.045	0.175
	HLL/SVL	1.610	0.102	1.678	0.045	0.251
	THIGHL/SVL	0.473	0.018	0.538	0.018	**0.009***
	TL/SVL	0.535	0.028	0.564	0.013	0.117
	TFOL/SVL	0.695	0.025	0.699	0.018	0.602
	FOL/SVL	0.442	0.049	0.409	0.023	0.251
	MTI/SVL	0.061	0.008	0.043	0.003	**0.009***
	1TL/SVL	0.111	0.010	0.123	0.003	0.117

Note: *: Indicates significant results ($p \leq 0.05$)

Table 2. Results of Mann-Whitney Analysis of Morphometric Characters in the Entire Population of Female *Polypedates leucomystax* in Merapi Valley and Imogiri Nature Reserve.

Grouping Character	Morphometric Characters of Females	Merapi Valley		Imogiri Nature Reserve		Sign
		Average	STD	Average	STD	
Overall	SVL/SVL	1.000	0.000	1.000	0.000	1.000
Head	HL/SVL	0.272	0.044	0.298	0.016	0.251
	HW/SVL	0.296	0.029	0.317	0.028	0.251
	STL/SVL	0.244	0.010	0.233	0.018	0.347
	MSL/SVL	0.319	0.025	0.298	0.022	0.175
	NS/SVL	0.042	0.005	0.029	0.005	**0.009***
	SL/SVL	0.141	0.009	0.120	0.013	0.076
	NTL/SVL	0.213	0.016	0.192	0.017	0.076
	EN/SVL	0.090	0.005	0.089	0.009	0.754
	TEL/SVL	0.029	0.004	0.026	0.002	0.117
	DT/SVL	0.079	0.003	0.073	0.008	0.251
	MN/SVL	0.289	0.015	0.269	0.025	0.251
	MFE/SVL	0.201	0.009	0.171	0.022	**0.009***

Grouping Character	Morphometric Characters of Females	Merapi Valley		Imogiri Nature Reserve		Sign
		Average	STD	Average	STD	
	MBE/SVL	0.110	0.007	0.086	0.011	**0.009***
	IN/SVL	0.089	0.003	0.087	0.003	0.347
	EL/SVL	0.097	0.006	0.097	0.011	0.917
	IOD/SVL	0.106	0.006	0.127	0.008	**0.009***
	UEW/SVL	0.074	0.007	0.063	0.006	**0.047***
	UEL/SVL	0.107	0.011	0.109	0.010	0.347
Forelimb	1 FL/SVL	0.127	0.010	0.112	0.013	0.117
	3FL/SVL	0.227	0.027	0.203	0.025	0.175
	HAL/SVL	0.279	0.016	0.283	0.037	0.917
	LAL/SVL	0.178	0.010	0.170	0.010	0.251
	FAL/SVL	0.186	0.013	0.209	0.019	0.076
	4TL/SVL	0.333	0.038	0.316	0.024	0.602
Hindlimb	HLL/SVL	1.610	0.118	1.618	0.087	0.917
	THIGHL/SVL	0.529	0.043	0.508	0.040	0.465
	TL/SVL	0.538	0.039	0.513	0.037	0.347
	TFOL/SVL	0.699	0.034	0.670	0.033	0.175
	FOL/SVL	0.423	0.030	0.477	0.013	0.175
	MTI/SVL	0.052	0.004	0.046	0.005	0.076
	1TL/SVL	0.142	0.008	0.117	0.015	**0.028***

Note: *: Indicates significant results ($p \leq 0.05$)

Based on Tables 1 and 2, it can be seen that there is morphometric differentiation in all populations of *Polypedates leucomystax* frogs in Merapi Valley and Imogiri Nature Reserve. Table 1 shows six morphometric characters that differ significantly in male frogs on the head and hind legs. These characters are the distance from the snout to the front of the tympanum (STL), the distance from the nose to the snout (NS), the distance from the front eye to the nose (EN), the distance from the mandible to the rear eye (MBE), the distance from the cloaca to the knee of the hind leg (THIGHL), and the length of the wrist of the hind leg (MTI). Then in the *P. leucomystax* frog population females (Table 2), there are also 6 morphometric characters that are significantly different in the head and hind legs, namely the distance from the nose to the snout (NS), the distance from the mandible to the front eye (MFE), the distance from the mandible to the rear eye (MBE), the distance between the eyes (IOD), the width of the eyelid (UEW) and the length of the finger to one hind leg (1TL). The number of morphometric characters that are significantly different indicates that there has been a high differentiation of morphometric characters in male and female *P. leucomystax* frogs in Merapi Valley and the Imogiri Nature Reserve. The more morphometric characters that differ significantly from the total population tested, the higher the level of variation of the species (Inger & Voris, 2001).

Based on the variation in morphometric characters in male and female frogs in Merapi Valley and Imogiri Nature Reserve, it can be seen that there are similarities in head characters that differentiate between male and female frogs. These characters are the distance from the nose to the snout (NS) and the distance from the mandible to the posterior eye (MBE). This indicates that altitude can affect the morphometric variation of head characters in both male and female frogs. These results are in line with the statement by Wibowo et al. (2006) which show that differences in altitude can cause differentiation of morphometric characters between populations. In addition, research from Addaha et al. (2015) also found that male and female *Polypedates leucomystax* frogs in the highlands of Sumatra have a higher distance from the mandible to the posterior eye (MBE) character compared to frogs living in the lowlands of Sumatra. In addition to altitude factors, morphometric variations that occur in *P. leucomystax* frogs, both male and female individuals, can also be influenced by differences in geographical conditions and the long distance between the locations of Merapi Valley and the Imogiri Nature Reserve. Merapi Valley is a highland with open vegetation, while the Imogiri Nature Reserve is a lowland with closed vegetation. The distance between the two locations is also very large 40 km, because they are located in different areas and separated by various barriers such as hills, residential areas, and highways. This is supported by the statement by Futuyma (1986) that differences in distance between populations can affect phenotypic characteristics.

In addition to having similarities in a number of significantly different characters, there are also variations in morphometric characters that differentiate between male and female frog populations in Merapi Valley and Imogiri Nature Reserve. These characters in male frogs are the distance from the snout to the front of the tympanum (STL), the distance from the front eye to the nose (EN), the distance between the cloaca to the knee of the hind leg (THIGHL) and the length of the wrist of the hind leg (MTI), while in female frogs the different morphometric characters are the distance from the mandible to the front eye (MFE), the distance between the eyes (IOD), the width of the eyelid (UEW) and the length of the finger to one hind leg (1TL). In general, male *P. leucomystax* frogs also have a smaller size than the size of female *P. leucomystax* frogs. According to Alhadi et al. (2021) *P. leucomystax* frogs, males have a size of around 50 mm and females 80 mm. This difference in size can be caused by sexual dimorphism and different activity patterns between male and female frogs. Male frogs tend to be more active in searching for food and mates, thus expending more energy, while female frogs tend to be less active because they minimize the use of energy that will be allocated to the reproductive process in producing eggs (Jin et al., 2017). Morphometric variations in male and female frogs cannot be compared, because sexual dimorphism and differences in activity patterns can affect morphometric differentiation.

2. Morphometric Variation in the Entire Population of Male *Polypedates leucomystax* in Merapi Valley and Imogiri Nature Reserve

Based on the morphometric characters that showed significant differences (Table 1), there were differences in morphometric characters between male *P. leucomystax* frogs found in Merapi Valley and Imogiri Nature Reserve. The distance from the snout to the front of the tympanum (STL), the distance from the nose to the snout (NS), the distance from the front eye to the nose (EN), the distance from the mandible to the back of the eye (MBE), and the length of the hind leg wrist (MTI) of male frogs in Merapi Valley were higher than the morphometric characters of male frogs in Imogiri Nature Reserve, while the distance between the cloaca to the knee of the hind leg (THIGHL) showed the opposite. The results of this study indicate that the location differences between Merapi Valley which higher (800 masl) compared to the lower Imogiri Nature Reserve area (130 masl) can cause differentiation of morphometric characters. This result is in line with the finding Fauzan & Widya (2022) that the frog *Fejervarya cancrivora* males in the Singgalang highlands have longer morphometric characteristics (NS, STL, EN, MTI, MBE) than male *F. cancrivora* frogs in the Padang Ganting lowlands. Furthermore, the findings Zhang et al. (2023) also support that male *Duttaphrynus melanostictus* frogs living in the highlands have larger body sizes than male *D. melanostictus* frogs in the lowlands. This is in accordance with the statement Miguel Á & Miguel Á (2007) where individuals living at higher altitudes with lower temperatures experience a later age of sexual maturity and a longer lifespan than individuals living at lower altitudes with higher temperatures. Frogs living at higher altitudes achieve slower development because they conserve their energy reserves for growth, resulting in larger sizes. This is in line with the statement Liu et al. (2025) that animals living at higher altitudes have larger body sizes than animals living at lower altitudes, this is because animals living at higher altitudes minimize their energy use to maintain metabolism.

The higher distance between the cloaca and the knee of the hind leg (THIGHL) in male frogs in the Imogiri Nature Reserve can also be caused by differences in altitude between populations. In accordance with Allen's rule, where the ratio of limb length to body size tends to decrease with increasing altitude as a form of adaptation to the environment (Leung et al., 2021). Frogs that live in higher altitudes have shorter body extremities than frogs that live in lowlands as a heat insulation adaptation. Research Castellano & Giacom (1998), states that male frogs that live in higher areas have significantly different characters compared to male frogs that live in lower areas. In addition, research from Tarkhnishvili et al. (1999) also supports that frogs in highland habitats have significantly different body sizes than frogs in lowland habitats. Other factors that can influence morphometric variations in frogs are the long distance between populations and the presence of ecological barriers (Riyanto et al., 2011). The distance between Merapi Valley and the Imogiri Nature Reserve is 40 km with various ecological barriers.

Morphometric variations in male frogs also show significant differences in specific head (STL, NS, EN, MBE) and hind leg (THIGHL, MTI). Characteristics head and hind leg characteristics are morphometric characteristics that commonly experience differentiation due to environmental factors such as temperature and altitude. Head and hind leg characteristics are crucial for male frogs to adapt to environmental pressures by enhancing sensory abilities and foraging activities needed for survival (Sun et al., 2025). Geographical conditions of the Merapi Valley*,* the vegetation is more open and close to residential areas, while the Imogiri Nature Reserve has closed vegetation surrounded by trees. This condition results in significantly different food availability and predators in the two locations, leading to morphometric variations. These differences in geographic conditions, such as altitude and vegetation, can influence morphometric variations in male *P. leucomystax* in Merapi Valley and Imogiri Nature Reserve.

3. Morphometric Variation in the Entire Population of Female *Polypedates leucomystax* in Merapi Valley and Imogiri Nature Reserve

Based on the morphometric characters that show significant differences (Table 2), between female *P. leucomystax* frogs found in Merapi Valley and Imogiri Nature Reserve. The distance from the nose to the snout (NS), the distance from the mandible to the front eye (MFE), the distance from the mandible to the back eye (MBE), the width of the eyelid (UEW) and the length of the finger to one hind leg (1TL) of female frogs in Merapi Valley are higher than the morphometric characters of female frogs in Imogiri Nature Reserve, while the distance between the eyes (IOD) shows the opposite. These results have similarities with the male population where male frogs in Merapi Valley have larger body sizes than male frogs in the Imogiri Nature Reserve. This research is in line with the findings Fauzan (2019) that the frog *Fejervarya limnocharis* females have longer morphometric characters (NS, MFE, MBE, 1TL) in the Sumatran highlands compared to *F. limnocharis* females in the lowlands of Sumatra. Furthermore, the findings Hou et al. (2023) also revealed that female *Nanorana parkeri* frogs living in the highlands have larger morphological characteristics than female *N. parkeri* frogs in the lowlands. Female frogs in the highlands adapt to environmental stress by minimizing activity to conserve as much energy as possible for reproduction. Another factor that can influence morphometric variation is food availability (Chahyadi et al., 2016). Female frogs in the Merapi Valley have larger morphological characteristics because they may be influenced by the abundance of insects around settlements, resulting in their larger size.

The higher inter-eye distance (IOD) character in the Imogiri Nature Reserve is thought to be influenced by denser vegetation, minimal light and higher organism activity. This environmental pressure affects the frog's adaptation in the sensory organs to increase the field of view and detect threats quickly (Jiang et al., 2022; Thomas et al., 2020). This is in consistent with research by Nesty et al. (2013) which found that female *Duttaphrynus melanostictus* frogs have different inter-eye distance characters with *D. melanostictus* frogs at two locations with different altitudes.

Morphometric variations in female frogs show significant differences in the characteristics of the head (NS, MFE, MBE, IOD, UEW) and hind limbs (1TL). Female frog populations living in higher altitudes generally have larger body sizes than female frogs in lowlands (Baraquet et al., 2018). Colder temperatures in highlands cause the metabolic rate to slow down but increase the growth period so that female frogs can live longer to produce eggs (Baraquet et al., 2018; Ma et al., 2009). It can be said that the growth rate can make the body size larger. A larger body is more efficient in maintaining body temperature in colder environments because the surface to volume ratio is smaller (Castro et al., 2021). The larger head character of female frogs in highlands allows the mouth opening to be wider, thus providing an advantage for consuming prey of a wider variety of sizes to meet the high energy needs to produce eggs and survive in extreme environments (Chen et al., 2023). Furthermore, the IOD and UEW characters are needed for navigation and detection of predators, where in the Merapi Valley highlands, the vegetation structure is more open, making it easy to be detected by predators (Li et al., 2023). The final character is the 1TL, which functions as a locomotory organ. In highlands, this limb is necessary for support on steep terrain and for supporting the female frog's heavy body weight while producing eggs (Leung et al., 2021). These differences in geographic conditions and altitude may influence morphometric variations in female *P. leucomystax* frogs in Merapi Valley and the Imogiri Nature Reserve.

4. The Relationship between Significant Morphometric Characters and Abiotic Factors (Altitude, Temperature, Humidity)

Altitude, temperature, and humidity play a crucial role in the survival of the *Polypedates leucomystax* frog. As an ectotherm, the frog's body temperature is strongly influenced by the temperature of its surroundings, which influences its metabolic processes and biological activity (Idora et al., 2023). Results of abiotic factor measurements in Merapi Valley and Imogiri Nature Reserve (Table 3) indicate that the environmental conditions in the two locations are suitable for the habitat of the *P. leucomystax* frog, although there are differences in the values of abiotic factors that cause differentiation of morphometric characters between populations.

Table 3. Abiotic Factors

Parameter	Average	
	Merapi Valley	Imogiri Nature Reserve
Altitude (masl)	803.6 ± 5.12	134.5 ± 34.88
Temperature (℃)	21.32 ± 0.89	24.48 ± 0.58
Humidity (%)	90.44 ± 8.85	91.6 ± 7.31

Based on the results of measurements conducted in Merapi Valley and Imogiri Nature Reserve (Table 3), *Polypedates leucomystax* frog was found at an altitude of 803 and 134 meters above sea level. *P. leucomystax* frog can live in lowlands to highlands ranging from an altitude of 50-1000 meters above sea level (Buddhachat & Suwannapoom, 2018; Nazarudin & Sumarni, 2024). Temperature measurements in Merapi Valley and Imogiri Nature Reserve show that the temperature in both locations is in the range of 21-24 ℃. According to Idora et al. (2023) the optimal temperature for frog habitat ranges between 20-35 ℃. Relatively low temperature conditions in the highlands are very necessary for frogs to maintain body temperature stability and minimize the rate of evaporation of body fluids (Simon et al., 2022). In addition, humidity in Merapi Valley and Imogiri Nature Reserve produces humidity at 90-91%. According to Idora et al. (2023) the optimal humidity for frogs is in the range of 89-98%. According to Riddell et al. (2024) high humidity plays a role in maintaining the moisture of the frog's skin surface which is essential for cutaneous respiration. As an arboreal species, *P. leucomystax* frog requires high humidity to prevent the risk of dehydration (Simon et al., 2022). The measurement results show that the altitude, temperature, and humidity factors in Merapi Valley and Imogiri Nature Reserve are still ideal for the habitat and breeding of *P. leucomystax* frogs. This is indicated by the discovery of *P. leucomystax* frogs in Merapi Valley and Imogiri Nature Reserve. Furthermore, the abiotic measurement data were analyzed using the Spearman correlation test to determine whether there is a significant relationship between morphometric characters in male and female frogs with abiotic factors, which can be seen in Tables 4 and 5 below.

Table 4. Results of Spearman Correlation Test between Morphometric Characters of Male *Polypedates leucomystax* that are Significantly Different with Abiotic Factors

Morphometric Characters	Correlation Test	Altitude	Air temperature	Humidity
STL	Sign	0.075	0.128	0.614
	Correlation Coefficient	0.585	-0.515	0.182
EN	Sign	**0.034***	0.060	0.700
	Correlation Coefficient	0.671	-0.612	0.140
NS	Sign	0.197	0.108	0.973
	Correlation Coefficient	0.445	-0.539	-0.012
THIGHL	Sign	**0.015***	**0.013***	0.881
	Correlation Coefficient	-0.738	0.745	0.055
MBE	Sign	**0.019***	**0.029***	0.763
	Correlation Coefficient	0.720	-0.685	-0.109
MTI	Sign	**0.022***	**0.009***	0.987
	Correlation Coefficient	0.707	-0.770	-0.006

Note: *: Indicates a significant correlation ($p \leq 0.05$)

Based on the results of the correlation test in Table 4, it can be seen that abiotic factors correlate with four significantly different characters in male frogs. The correlated abiotic factors are altitude and temperature, while humidity is not correlated with any morphometric character. Altitude correlates with the characters of the distance from the front eye to the nose (EN), the distance between the cloaca to the knee of the hind leg (THIGHL), the distance from the mandible to the rear eye (MBE) and the length of the wrist of the hind leg (MTI) and temperature correlates with the characters of the distance between the cloaca to the knee of the hind leg (THIGHL), the distance from the mandible to the rear eye (MBE) and the length of the wrist of the hind leg (MTI). Ecologically, altitude has an inverse relationship with air temperature, the higher a location, the lower the temperature at that location (Baraquet et al., 2018). Lower temperatures in the highlands cause frogs to have larger sizes than in the lowlands (Baraquet et al., 2018; Ma et al., 2009). This is in accordance with the statement Ashton (2002) that individuals living in environments with lower temperatures experience a slow metabolism so that their development period to sexual maturity is hampered and provides a longer growth period until adulthood. The higher location of Merapi Valley and its lower temperature compared to Imogiri Nature Reserve causes the morphometric characters of the distance from the mandible to the rear eye (MBE) and the length of the hind limb wrist (MTI) in male frogs in Merapi Valley to be higher than those in Imogiri Nature Reserve. This is supported by research Fauzan (2023) which found that male frogs *Fejervarya limnocharis* in the highlands have longer head characters compared to *F. limnocharis* frogs in the lowlands. In addition, research Abdul Aziz et al. (2021) also found that the population of male frogs in Malaysian reserve forests showed that environmental temperature has a significant influence on almost all morphometric characters including head characters and the length of the hind limb wrist, meaning that at higher temperatures the body size tends to shrink as a form of adaptation to environmental changes.

The distance from the front of the eye to the nose (EN) only correlates with altitude. This is thought to be because the distance from the eye to the nose is more related to sensory adaptation and respiratory efficiency at different air pressures in the highlands. At high altitudes, the EN character tends to be longer in line with the increase in overall head dimensions to support sensory organs (Li et al., 2023). The THIGHL character in Merapi Valley and Imogiri Nature Reserve also showed a negative correlation with altitude, meaning that the higher a location, the shorter the distance from the cloaca to the knee of the hind limb. These results are consistent with the finding Leung et al. (2021) that the ratio of limb length to body size of male frogs *Rana kukunoris* tends to decrease with increasing altitude. The morphometric characters of the distance from the snout to the front of the tympanum (STL) and the distance from the nose to the snout (NS) were not correlated at all with altitude, temperature, and humidity. This indicates that differentiation in the STL and NS characters may be influenced by other factors. According to Li et al. (2023) snout length, it is often more correlated with the size of prey available in a specific habitat than macroclimatic parameters. In addition, Leung et al. (2021) it was added that head and snout shape can also be influenced by self-defense mechanisms strategies in each habitat.

The results of this study indicate that altitude and temperature have a relationship to the morphometric variation of male frogs, while humidity does not show any significant effect (Inger & Voris, 2001) stated that differences in temperature and altitude can affect the occurrence of morphometric differentiation due to adaptation to differences in environmental conditions. This is supported by the finding Pramesti & Suwartiningsih (2025) that male frogs of *Odorana hoshii* found in 3 areas of Jatimulyo which have different temperatures, altitudes and geographical conditions show morphometric variations in characters in the head.

Table 5. Results of Spearman Correlation Test between Morphometric Characters of Female *Polypedates leucomystax* that are Significantly Different with Abiotic Factors

Morphometric Characters	Correlation Test	Altitude	Temperature	Humidity
MFE	Sign	**0.000***	**0.022***	0.148
	Correlation Coefficient	0.945	-0.709	-0.492
MBE	Sign	**0.005***	**0.008***	0.532
	Correlation Coefficient	0.805	-0.782	-0.225
IOD	Sign	**0.021***	**0.043***	0.434
	Correlation Coefficient	-0.713	0.648	0.280
NS	Sign	**0.016***	**0.033***	0.393

Morphometric Characters	Correlation Test	Altitude	Temperature	Humidity
	Correlation Coefficient	0.732	-0.673	-0.304
	Sign	**0.002***	0.108	**0.050***
UEW	Correlation Coefficient	0.854	-0.539	-0.632
	Sign	**0.028***	0.229	0.137
1TL	Correlation Coefficient	0.689	-0.418	-0.505

Note: *: Indicates a significant correlation ($p \leq 0.05$)

Based on the results of the correlation test in Table 5, it can be seen that all abiotic factors correlate with several characters that are significantly different in female frogs. Altitude correlates with the characters of the distance from the mandible to the front eye (MFE), the distance from the mandible to the rear eye (MBE), the distance from the nose to the snout (NS), the width of the eyelid (UEW), the distance between the eyes (IOD) and the length of the finger to one hind limb (1TL) and temperature correlates with the characters of the distance from the mandible to the front eye (MFE), the distance from the mandible to the rear eye (MBE) and the distance between the eyes (IOD). The characters of MFE, MBE and NS have a correlation with altitude and temperature, meaning that colder temperatures in the highlands may contribute to larger head dimensions. This is in accordance with the heat balance hypothesis, where a larger body size in cold habitats helps in thermoregulation (Gouveia et al., 2013; Trochet et al., 2019). The location of Merapi Valley is higher and has a lower temperature than Imogiri Nature Reserve, thus causing the characters of MFE, MBE and NS in female frogs in Merapi Valley to be higher than Imogiri Nature Reserve. This aligns with findings that female Fu et al. (2022) *Feirana taihangnica* frogs exhibit significantly different head characteristics between locations, with higher summer temperatures and lower winter temperatures. This morphometric variation represents an adaptation to foraging in extreme environments.

Single-toe length (1TL) trait correlates with only one abiotic factor, namely altitude. This suggests that the larger 1TL trait in the highlands supports stronger locomotion on steep or rocky terrain (Leung et al., 2021). This hypothesis aligns with the topographical conditions of Merapi Valley, which has a steeper elevation and slope than Imogiri Nature Reserve. The intereye distance (IOD) trait also correlates with two abiotic factors: temperature and altitude. This indicates that IOD is related to changes in environmental temperature, which can affect visual acuity in searching for prey and detecting predators (Gul et al., 2024). This aligns with the results of previous research, which found that lower temperatures in Merapi Valley compared to Imogiri Nature Reserve are associated with differences in intereye distance at both locations. These findings are supported by research Nesty et al. (2013) who found that the IOD character of female *Duttaphrynus melanostictus* frogs in the highlands of Sumatra has morphometric variations with female *D. melanostictus* frogs in the lowlands. In contrast to other morphometric characters, eyelid width (UEW) showed a significant correlation with two abiotic factors, namely altitude and humidity. Ecologically, an increase in elevation is directly proportional to a decrease in environmental temperature which then triggers an increase in relative humidity (Pabontongan et al., 2024). Therefore, the relationship between UEW and this humidity parameter indicates the existence of a morphological adaptation mechanism to maintain eye hydration in response to fluctuations in air humidity at a certain altitude gradient (Peng et al., 2025). The results of this study indicate that temperature and altitude are more dominant in their relationship in the morphometric variations of female frogs than humidity. According to Liu et al. (2012) temperature and altitude are more related factors in morphometric differentiation in female frogs than humidity because temperature and altitude are directly correlated to faster frog growth. In addition, findings from Liao et al. (2011) also supports that the female *Fejervarya limnocharis* frog in the highlands has a larger size than the female *F. limnocharis* frog in the lowlands.

Conclusion

There are morphometric variations in male and female *Polypedates leucomystax* frogs found in Merapi Valley and Imogiri Nature Reserve. Male frogs exhibit six differentiated traits, four in the head and two in the hind legs: STL, NS, EN, MBE, THIGHL, and MTI. Female frogs also exhibit six differentiated traits: five in the head and one in the hind legs: NS, MFE, MBE, IOD, UEW, and 1TL. These results indicate that differences in altitude and location in the Merapi Valley and the Imogiri Nature Reserve can influence the differentiation of morphometric characters in male and female *P. leucomystax* frogs.

The results of the Spearman correlation test also represent that abiotic factors have a significant correlation with morphometric characters in male and female *Polypedates leucomystax* frogs. In male frogs, altitude has a correlation with 4 characters, namely EN, MBE, THIGHL and MTI. Then temperature has a correlation with 3 characters, namely MBE, THIGHL and MTI, while humidity is not correlated with any character. In female frogs, altitude has a correlation with 6 characters (MFE, MBE, NS, UEW, IOD and 1TL). Then temperature has a correlation with 4 characters, namely MFE, MBE, IOD and NS. Finally, humidity has a correlation with 1 character, namely UEW. These results explain that abiotic factors have a relationship with the occurrence of morphometric variations. Research that has been conducted shows that differences in habitat altitude affect morphometric variations in *Polypedates leucomystax*. These findings provide information regarding the phenotypic plasticity of amphibians and can support future research and conservation efforts.

Differences in altitude, temperature and humidity in Merapi Valley and Imogiri Nature Reserve are related to morphometric variations in *Polypedates leucomystax* frogs, both in male and female individuals. Abiotic factors such as altitude, temperature and humidity also show a significant relationship to several morphometric characters, so it is suspected that they play a role in the process of morphological adaptation of frogs to different habitats. Considering that this phenotypic variation is strongly influenced by environmental factors, further studies are recommended to use a molecular approach through genetic marker analysis (e.g., DNA Barcoding or microsatellites) to determine whether these physical differences are the result of genetic isolation or simply phenotypic plasticity.

Future studies should include to use a larger number of frog individuals, in order to represent the entire population in a region and strengthen the results obtained.

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Generative AI: Generative artificial intelligence (AI) tools are used to facilitate the search for reference articles that support research and develop sentence structure ideas. AI tools are also used in the language editing process.

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