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NEW RECORD OF *Chirixalus trilaksonoi* (RIYANTO & KURNIATI, 2014) IN JATILAWANG, BANYUMAS, CENTRAL JAVA: UPDATED INSIGHTS FROM MORPHOLOGICAL AND VOCAL CHARACTERIZATION

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ABSTRACT

Genus *Chirixalus* is a group of small tree frogs within the family Rhacophoridae, distributed across Asia, including northeastern India, southern China, mainland Southeast Asia, and islands such as Sumatra and Java in Indonesia. Ongoing taxonomic research continues to uncover new species within this genus. With the assistance of citizen science, the discovery of new species and the documentation of species' geographic distribution have been greatly supported, expanding our knowledge of biodiversity and conservation processes. In our study, we recorded the tree frog genus *Chirixalus* from Jatilawang District, Banyumas Regency, Central Java. Based on our examination of morphological traits and call characteristics, these specimens resemble *Chirixalus trilaksonoi*. This finding represents the first record extending the known distribution range of *Chirixalus trilaksonoi* into Central Java. Variation in dorsal morphology and vocal characteristics is the most notable difference observed in this study. This finding can assist field researchers, and citizen scientists particularly those working in taxonomy. Further research and intensive surveys are needed to evaluate its conservation status and enhance our understanding of its taxonomic classification.

Key words: *Chirixalus*, citizen science, taxonomy

INTRODUCTION

Rhacophorids consists of 23 genera and 459 species. This family's members are mostly located in Asia, while some species can also be found in Madagascar and Africa (Frost et al., 2024). Rhacophoridae, also referred to as bush frogs or shrub frogs, are a diverse family of frogs that are especially prevalent in Southeast Asia. Many species in this family find perfect habitat in Java's lowland forests, which are distinguished by their thick vegetation and warm, humid environment (Iskandar, 1998). Rhacophoridae, which lives in the forest canopy, bushes, and undergrowth, is ideally suited to this habitat. Their varied reproductive tactics, such as laying eggs on leaves above water sources so that the tadpoles hatch and fall straight into the water, are thought to be responsible for their capacity to flourish in such environments (Ellepola et al., 2022). This adaptation helps them exploit the vertical structure of the forest, reducing predation risks and increasing survival rates in the dynamic lowland forest ecosystems of Java (Ellepola et al., 2022).

The relationship between genera within the family was redefined by recent taxonomic revisions, which significantly refined our understanding of Rhacophoridae diversity and environmental adaptability. A major review was carried out by Chen et al. (2020), who restored *Chirixalus* as a distinct genus from *Chiromantis*. Their investigation, which included data from two African and two Asian species, showed that *Chirixalus* is a separate sister branch of *Chiromantis*. This classification was better reflected in the true evolutionary relationships between them than the previous inconsistent merger.

Chirixalus was first recorded in Java as *Chirixalus trilaksonoi* described by Riyanto and Kurniati in 2014. The description was based on samples from paddy fields in Bogor, West Java (Riyanto & Kurniati, 2014). Herlambang et al. (2023) identified a new population of *Chirixalus trilaksonoi* in Sumatra and reevaluated its conservation status. Munir et al. (2021) described *Chirixalus pantaiselatan* from samples collected in the Leuweung Sancang Nature Reserve, West Java.

Chirixalus encounters in Java have grown as a result of the development of citizen science through the Amfibi Reptil Kita (ARK) program on the iNaturalist (2024). By allowing researchers and observers to share their observations, this program helps to increase our understanding of the habitat and distribution of the well-known species *Chirixalus pantaiselatan* and *Chirixalus trilaksonoi*. This has led to the discovery of new habitats for these species in different parts of Java, improving knowledge of their conservation status (Maharani et al., 2023). A team in GO ARK comprising Hafizh, Rama, Averroes, Kevin, Azib, Fakhri, and Saktyari discovered *Chirixalus trilaksonoi* during the GO ARK (Gerakan Observasi Amphibi-Reptil Kita) event in 2021 (Ramadhan, 2021).

This paper aims to evaluate and compare the *Chirixalus* individuals found to determine whether they belong to *Chirixalus trilaksonoi*, *Chirixalus pantaiselatan*, or potentially a different species. The publication will also provide thorough descriptions of the environment and physical traits of the species found at the study site.

MATERIALS AND METHODS

Sampling

Administratively, the site is located in Tunjung Lor Village, Jatilawang Subdistrict, Banyumas Regency, Central Java with exact coordinates 7°31'22"S−109° 06' 17"E (Fig. 1). It is located 23 km from the nearest coastline (Cilacap Regency), 198 km from the nearest *Chirixalus pantaiselatan* site (Leuweung Sancang Nature Reserve), and 195 km from the nearest *Chirixalus trilaksonoi* site in Legokbarong Subdistrict, Purwakarta Regency (Ramdanimam, 2022).

Following the initial discovery in November 2021 and November 2022, this study was conducted in February 2024. We collected four male specimens of the genus *Chirixalus* for further identification. The samples were preserved in 70% ethanol after being fixed in 10% formalin.

Bioacoustic analysis advertisement calls were recorded using phone digital camera, and 87% humidity and 22°C temperature were recorded. Raven Pro (version 1.5; Cornell Lab of Ornithology) software, with a Hanning window and a discrete Fourier transform window size of 256 points, was used to analyze advertisement calls (Bioacoustics Research Program, 2014). The definitions and standards provided by Koehler et al. (2017) are adhered to by the terminology, which includes oscillogram and spectrogram.

Figure 1. Location of *Chirixalus* in Java, including this research. Data collected from iNaturalist (2024).

Morphometric

We collected four male specimens of the genus *Chirixalus* for morphometric measurement. Morphometric measurements were precisely recorded to the nearest 0.1 mm with a dial caliper following the methods outlined by Grismer et al. (2007) and Matsui et al. (2014). Meristic observations were conducted using a binocular stereo microscope with 20 variables. Watters et al. (2016) described measurements were included several parameters: the snout–vent length (SVL), which is the distance from the snout tip to the rear of the vent; the head length (HL), extending from the snout tip to the back corner of the jaw; and the head width (HW), measured between the back corners of the eyes.

There were several details of morphometric measurements on the head, such as head length, head width, and eye to nostril distance. Length of the nose was measured from the tip of the nose to the eye's front corner. Eye diameter (ED) was measured from the front to the rear borders of the eye, and eye to nose distance (EN) was measured from the rear edge of the nostril to the front corner of the eye. Tympanum diameter (TD) was measured across the front and back borders of the tympanum, and eye to tympanum distance (ETD) was measured from the rear corner of the eye to the front edge of the tympanum. The interorbital distance (IOD) was measured across the top of the skull between the nearest points of the eye margins, whereas the internarial distance (IND) was the distance between the nostrils.

On the body, we measured several parameters such as lower arm length (LAL). LAL was measured from the elbow to the tip of the third finger, and the length of the third finger (Fin3L) was recorded from the base of the first subarticular tubercle to the tip of the third finger. The disk width of the third finger (Fin3DW) was measured at its widest point, and the length of the inner palmar tubercle (IPTL) was the maximum length of this structure. Thigh length (THL) was recorded from the center of the hind limb insertion to the knee, and tibia length (TL) was measured from the knee to the heel. Foot length (FL) extended from the proximal edge of the inner metatarsal tubercle to the tip of the fourth toe, and the length of the inner metatarsal tubercle (IMTL) was noted as its greatest length. Finally, fourth toe length (Toe4L) was measured from the base of the first subarticular tubercle to the tip of the fourth toe, with fourth toe disk width (Toe4DW) recorded at its widest point.

To correct for allometric bias due to ontogeny, the morphometric data were adjusted using the formula: [X adj=log(X)−ß[log(SVL)−log(SVLmean)], where X adj is adjusted value, ß is the unstandardized regression coefficient, and SVLmean is the overall mean SVL (Thorpe, 1983; Lleonart et al., 2000). Principal Component Analysis (PCA) was performed using PAST v4.03 software (Hammer et al.,2001). To assess interpopulation variation, each morphometric character was tested for normality and subsequently analyzed using one-way analysis of variance (ANOVA) (Vieites et al., 2009).

Measurement comparison data obtained from *C. pantaiselatan* by Munir et al. (2021) and *C. trilaksonoi* by Riyanto & Kurniati (2014). The morphometric data are documented in Table 1, while Table 2 outlines the meristic differences among the populations.

RESULTS

Morphological Characteristics:

The specimens assigned to *C. trilaksonoi* with characteristics: a horizontal pupil, a free and deeply notched tongue, a distinct tympanum, opposed fingers, webbed toes, finger and toe tips dilated into disks, the outer metatarsal separated by a web, and obtuse terminal phalanges (Boulenger, 1893) (Fig. 2 & 3).

The following traits combined can be used to identify this species: SVL length of 23.9–26.11 mm in males; a truncate-shaped snout; a white, yellowish patch on the upper jaw that starts below the middle part of the eye and extends posteriorly to the shoulder region; a consistent coloration of the dorsal body without a light lateral stripe from below the orbital to the mid-body or a light dorsolateral stripe from the canthus rostralis across the upper orbital; yellow to yellowish-brown dorsal coloration, with dark brown blotches or dark brown lines (or resembling linear stripes) running longitudinally from the head to the posterior; a unique advertisement call that consists of short calls with 11 notes split into three parts, lasting roughly 0.792 s.

Figure 2. Live specimen of *Chirixalus trilaksonoi* from Jatilawang. Photographed by Elizabeth Gresia Marbun

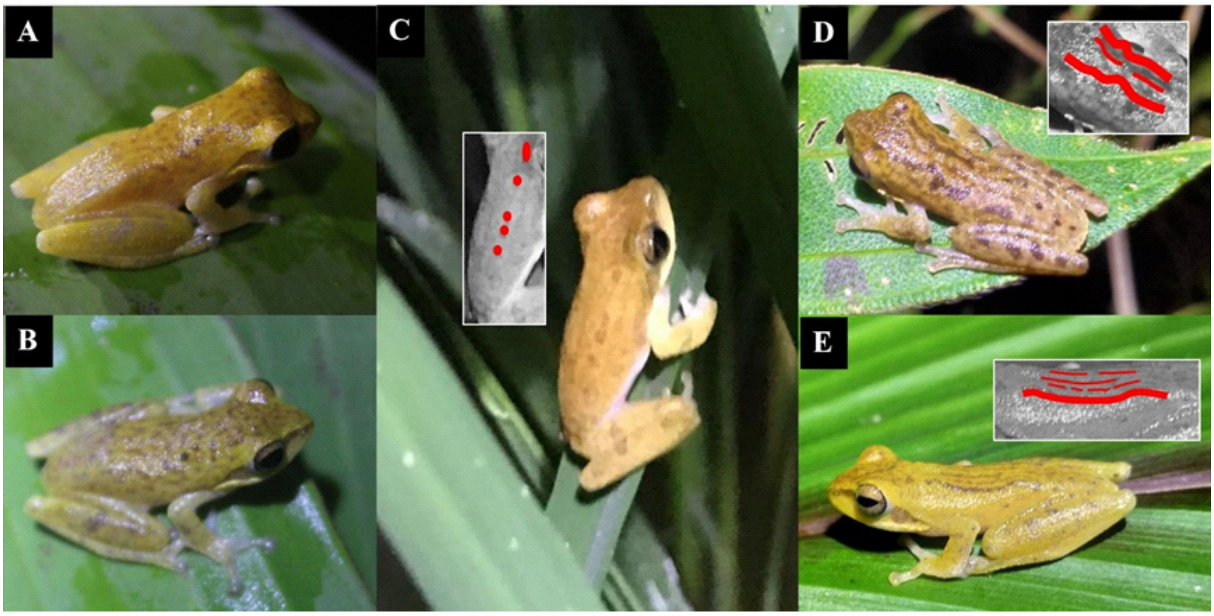

Figure 3. Dorsal coloration patterns of the populations. These include: (A). Brown blotches scattered across the entire dorsal surface with lighter coloration; (B). Brown blotches scattered across the entire dorsal surface with darker coloration; (**C**). Brown blotches with some parallel patterns; (**D**). Two linear stripes (or resembling linear stripes) with thinner and interrupted stripes in the middle, extending from the back of the head to the posterior, with a brown blotch at the posterior area. Dorsal with darker coloration; (**E**). Two linear stripes (or resembling linear stripes) with thinner and interrupted stripes in the middle, extending from the back of the head to the posterior, with a brown blotch at the posterior area. Dorsal with lighter coloration.

Morphological description based on four referred adult male specimens: SVL 23.9 – 26.11 mm; head relatively flat and wider (HW = 30.32% of SVL) compared to its length (HL = 27.66% of SVL); snout trapezoidal in lateral view, longer (SL = 12.36% of SVL) than eye diameter (ED = 10.85% of SVL); nostrils located laterally, closer to the tip of the snout than to the eye (NEL = 51.87% of SL); internarial distance (IND = 7.20% of SVL) is slightly more than half of the interorbital distance (IOD = 18.05% of SVL); eyes relatively large (ED = 10.85% of SVL), twice the size of the tympanum diameter (TD = 4.58% of SVL), over one-third of the head length (ED = 39.33% of HL), prominent, with a horizontally elliptical pupil; tympanum nearly twice as large as the distance from the eye to the tympanum (ETD = 2.27% of SVL); lacking vomerine teeth. Dorsal skin surface smooth; a distinct supra tympanic fold, extending as a glandular fold to the shoulder; a skin fold at the corner of the jaws, forming a short fold to the anterior base of the upper arm; the ventral skin surface is generally smooth.

The forelimbs are of moderate length, with the length of the lower arm less than half of the snout-vent length (LAL = 38.93% of SVL); there is a small, rounded inner palmar tubercle (IPTL = 3.01% of SVL), about one-quarter the length of the third finger (Fin3L = 13.65% of

SVL); finger length formula is I < II < IV < III; the inner two fingers are widely separated from the outer two fingers (opposable); fingertips have rounded disks; the diameter of the third finger disk is approximately half the diameter of the tympanum (Fin3DW = 61.14% of TD).

The hindlimbs are relatively long and slender. The tibiotarsal articulation extends past the tip of the snout when the leg is fully extended and pressed against the body; the thigh (THL = 40.45% of SVL) is slightly shorter than the tibia (TL = 47.63% of SVL), approximately one-fifth the length of the foot (FL = 33.62% of SVL; THL = 120.25% of FL); there is a well-developed, small, oval inner metatarsal (IMTL = 2.87% of SVL), almost one-sixth the length of the fourth toe (Toe4L = 17.87% of SVL), with no outer metatarsal present; the toe length formula is I < II < III < V < IV; webbing formula is (I 0-1 II 1/2-1(1/2) III 0-1 IV 1-0 V); the toes have rounded disks at their tips, with expanded disks featuring marginal and transverse ventral grooves; the disk of the fourth toe (Toe4DW = 2.09% of SVL) is narrower than the disk of the third finger (Fin3DW =2.77% of SVL).

Habitat and Characteristics:

Frogs are frequently observed in the paddy fields during the post-planting period. These frogs, however, are found in surrounding vegetation, such as banana trees and other tall plants, rather than in the rice paddies when the fields are not actively cultivated. The Paddy fields in the region are nearly always flooded throughout the year (Fig. 4), and serve as the primary water source influencing the frogs' distribution.

Breeding Characteristics:

The nests are foam structures constructed on downward-facing leaves above the water body. Foam nest may be positioned more than ten meters above the ground.

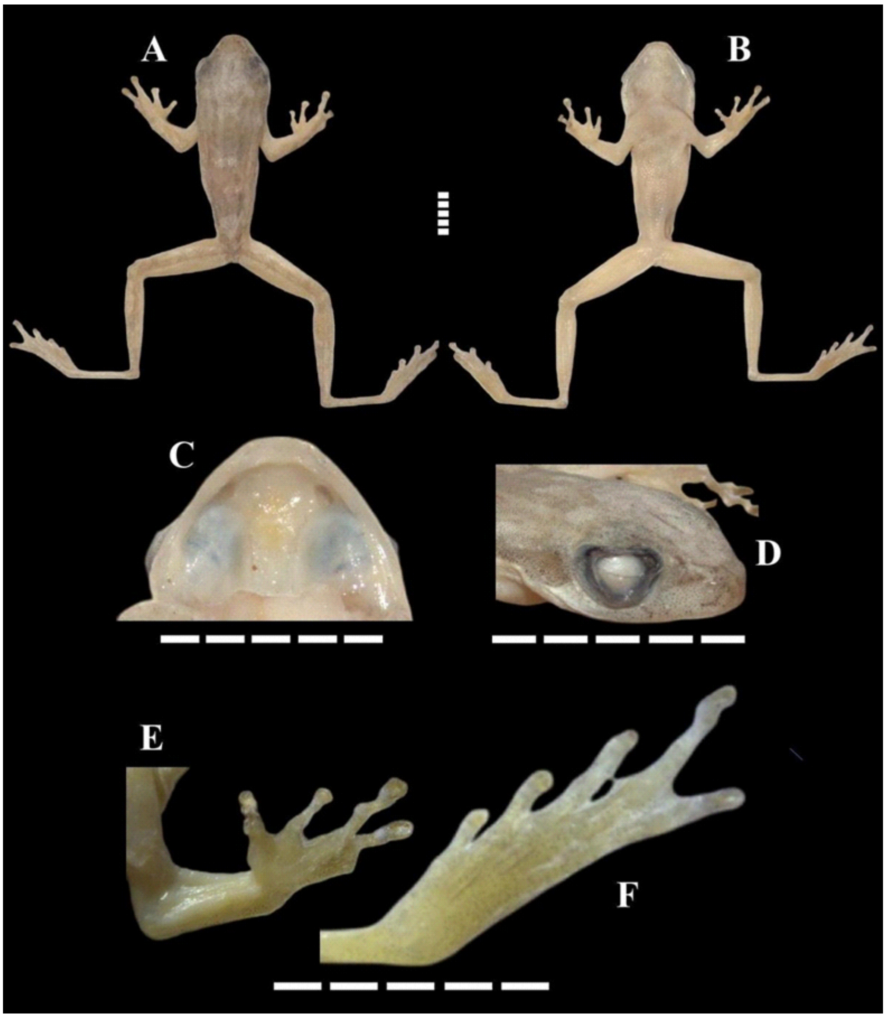

Figure 4. Type specimens of *Chirixalus trilaksonoi* (A). Dorsal view, (B). Ventral view, (C). Palatum (D). Lateral view from head, (**E**). Ventral view of hand, (**F**). Ventral view of foot. Scale bar = 5mm.

Morphometric analysis:

Measurement comparison data obtained from *C. pantaiselatan* by Munir et al. (2021) and *C. trilaksonoi* by Riyanto & Kurniati (2014). The morphometric data are documented in Table 2, while Table 3 outlines the meristic differences among the populations.

Morphological comparisons of male individuals of the three populations from *C. trilaksonoi* in Jatilawang (n = 4), *C. pantaiselatan* (n = 5), and *C. trilaksonoi* (n = 6), showed significant differences as visualized in the PCA (Fig. 5). The Principal Component Analysis (PCA) results further highlight these morphometric differences among the three populations. The PCA plot, which visualizes the first two principal components, shows distinct clustering of each population, with *Chirixalus* sp. represented by orange triangles in the lower-left quadrant, *C. pantaiselatan* by blue squares in the upper-right quadrant, and *C. trilaksonoi* by violet circles in the upper-left quadrant. The eigenvalue table indicates that the first principal component

(PC1) explains 71.76% of the variance, while the second principal component (PC2) accounts for 17.52%, together capturing a significant portion of the data's variation. The loading plots reveal that variables such as "ETD," "TD," "IMTL," “Toe4DW,” and "IPTL" contribute strongly to PC1, while “ETD,” “IOD,” "Toe4DW," "Fin3DW," and "NEL” are major contributors to PC2. This result underscores the unique morphological characteristics of each *Chirixalus* population, with each occupying a distinct region in the morphological space, as reflected in the PCA results (Fig. 6).

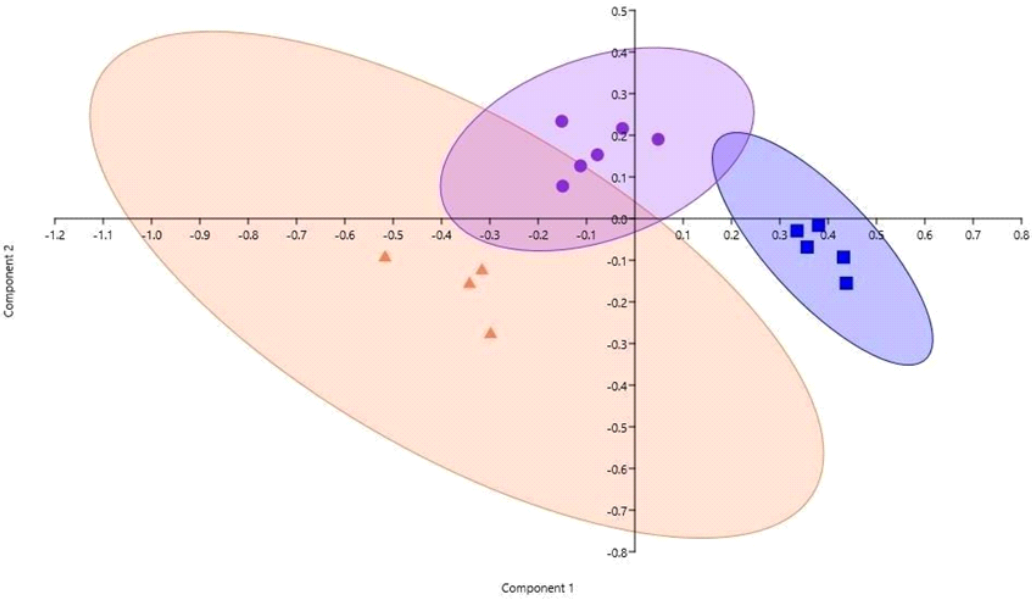

Figure 5. PCA Plot. The plot of the first two principal components with 95% confidence of ellipses within three populations of adult male *Chirixalus* sp. (orange), *C. pantaiselatan* (blue), & *C. trilaksonoi* (violet) based on the size-corrected morphometric variables.

Morphometric analyses revealed significant interpopulation variation in most measured characters (Table 1). Snout–vent length (SVL) did not differ significantly among populations (ANOVA, $p > 0.05$), indicating comparable overall body size. Similarly, Fin3L showed no significant differences among populations. In contrast, head measurements exhibited pronounced divergence. Head length (HL) and head width (HW) differed significantly among populations ($p < 0.05$–0.001). *C. pantaiselatan* population possessed significantly larger HL and HW than *Chirixalus* sp., whereas *C. trilaksonoi* were generally intermediate and did not differ significantly from *C. pantaiselatan* in several characters, as indicated by Tukey HSD groupings. Characters associated with the snout and orbital region, including EN–NEL, ED, ETD, and TD, showed highly significant differences among populations ($p < 0.01$–0.001). *C. pantaiselatan* consistently exhibited the largest values, *C. trilaksonoi* in Jatilawang the smallest, and *C. trilaksonoi* formed a distinct intermediate group or differed significantly from both populations depending on the variable.

Table 1. Size-adjusted morphometric variation among populations

Variabel	*C. pantaiselatan*	*C. trilaksonoi in Jatilawang*	*C. trilaksonoi*	ANOVA
SVL	26.72 ± 1.37	25.29 ± 1.22	26.70 ± 1.73	ns
HL	8.94 ± 0.38^{a}	6.98 ± 0.30^{b}	8.30 ± 0.60^{a}	***
HW	9.14 ± 0.60^{a}	7.65 ± 0.18^{b}	8.04 ± 0.84^{ab}	*
SL	3.82 ± 0.19^{a}	3.13 ± 0.26^{b}	3.43 ± 0.19^{ab}	*
EN-NEL	2.42 ± 0.19^{a}	1.61 ± 0.20^{c}	2.21 ± 0.21^{b}	***
ED	4.48 ± 0.19^{a}	2.74 ± 0.15^{c}	3.54 ± 0.39^{b}	***
ETD	1.24 ± 0.11^{a}	0.58 ± 0.08^{b}	0.57 ± 0.10^{b}	**
TD	2.14 ± 0.17^{a}	1.16 ± 0.15^{c}	1.57 ± 0.17^{b}	***
IND	2.38 ± 0.04^{a}	1.82 ± 0.21^{b}	2.04 ± 0.15^{ab}	**
IOD	3.62 ± 0.28^{b}	4.57 ± 0.24^{a}	3.07 ± 0.28^{b}	***
LAL	12.08 ± 0.61^{a}	9.83 ± 0.63^{b}	10.81 ± 1.13^{ab}	*
Fin3L	3.72 ± 0.24	3.46 ± 0.42	3.73 ± 0.36	ns
Fin3DW	1.16 ± 0.11^{a}	0.70 ± 0.10^{c}	1.04 ± 0.17^{b}	**
IPTL	1.22 ± 0.13^{a}	0.76 ± 0.17^{b}	0.79 ± 0.08^{b}	*
THL	13.62 ± 1.14^{a}	10.21 ± 0.48^{c}	12.39 ± 1.14^{b}	**
TL	14.12 ± 0.95^{a}	12.05 ± 0.91^{b}	13.43 ± 1.08^{ab}	*
FL	10.56 ± 0.51^{a}	8.40 ± 0.20^{c}	9.71 ± 0.87^{b}	***
IMTL	1.28 ± 0.13^{a}	0.73 ± 0.08^{b}	0.81 ± 0.07^{b}	**
Toe4L	5.54 ± 0.28^{a}	4.51 ± 0.19^{b}	5.23 ± 0.59^{ab}	**
Toe4DW	1.02 ± 0.04^{a}	0.53 ± 0.04^{c}	0.91 ± 0.07^{b}	***

Different letters indicate significant differences among populations based on Tukey’s HSD test ($p < 0.05$). Significance levels: ns = not significant ($p \geq 0.05$), *$p < 0.05$, **$p < 0.01$, **$p < 0.001$.

Comparison:

Chirixalus sp. (from Jatilawang) differs from *C. pantaiselatan* by lacking vomerine teeth (present in *C. pantaiselatan*). *Chirixalus* sp. exhibits a faint strip pattern or blotches on the dorsal side, with some having dark markings, whereas *C. pantaiselatan* only has dark markings on the dorsum. *Chirixalus* sp. has a relatively smaller size compared to *C. pantaiselatan* (SVL average on male adult sample is 25.29 mm vs. 26.72 mm). Head size smaller compared to *C. pantaiselatan* (HW/SVL ratio 30.32% vs. 34.23%), a shorter head length (HL/SVL ratio 27.66% vs. 33.48%), and a relatively shorter snout (SL/SVL ratio 12.36% vs. 14.34%). Additionally, the eye diameter in *Chirixalus* sp. is smaller (ED/SVL ratio 10.85% vs. 16.80%), the nostril-eye distance is shorter (NEL/SL ratio 51.87% vs. 63.65%), the eye-tympanum distance is shorter (ETD/SVL ratio 2.27% vs. 4.66%), and the tympanum diameter is smaller (TD/SVL ratio 4.58% vs. 8.02%). The internarial distance in *Chirixalus* sp. is slightly smaller

(IND/SVL ratio 7.20% vs. 8.93%), but the interorbital distance is wider (IOD/SVL ratio 18.85% vs. 13.58%). Furthermore, the lower arm of *Chirixalus* sp. is shorter (LAL/SVL ratio 38.39% vs. 45.26%), with hindlimb length shorter (THL/SVL ratio 40.45% vs. 42.26%), tibia length longer (TL/SVL ratio 47.63% vs. 43.52%), and foot length shorter (FL/SVL ratio 33.62% vs. 39.58%). The webbing formulae are I 0-1 II 1/2-1(1/2) III 0-1 IV 1-0 V (vs. I 1–1 /2 II 0–1(1 /2) III 0–1 IV 1 /2–0 V). The tibiotarsal articulation of the hind limb in *Chirixalus* sp. reaching tip of the snout, (vs. *C. pantaiselatan* it reaches the posterior of the nostril).

Chirixalus trilaksonoi in Jatilawang exhibits several differences in meristic features where it has a truncate snout (vs. an obtusely pointed snout in *Chirixalus trilaksonoi*). *Chirixalus* sp. displays a faint strip pattern or blotches on the dorsal side, with some having dark markings, whereas *Chirixalus trilaksonoi* has distinct dorsum line patterns.

Chirixalus trilaksonoi in Jatilawang is relatively smaller (average SVL for male adults is 25.29 mm vs. 26.1 mm). The head size is larger than *C. trilaksonoi* (HW/SVL ratio 30.32% vs. 29.92%), the head length is shorter (HL/SVL ratio 27.66% vs. 31.45%). The snout length of *C. trilaksonoi* in Jatilawang is also slightly shorter (SL/SVL ratio 12.36% vs. 13.03%), with a smaller eye diameter (ED/SVL ratio 10.85% vs. 13.11%). The nostril-eye distance is shorter in *Chirixalus* sp. (NEL/SL ratio 51.87% vs. 63.26%), while the eye-tympanum distance is slightly longer (ETD/SVL ratio 2.27% vs. 2.11%). The tympanum diameter is slightly smaller (TD/SVL ratio 4.58% vs. 5.82%), and the internarial distance is also slightly smaller (IND/SVL ratio 7.20% vs. 7.85%). However, the interorbital distance is wider in *C. trilaksonoi* in Jatilawang (IOD/SVL ratio 18.85% vs. 11.43%). Additionally, the lower arm of *C. trilaksonoi* in Jatilawang is shorter (LAL/SVL ratio 38.39% vs. 40.15%), with differences in hindlimb length is shorter (THL/SVL ratio 40.45% vs. 46.18%), tibia length shorter (TL/SVL ratio 47.63% vs. 50.01%), and foot length shorter (FL/SVL ratio 33.62% vs. 36.25%). The webbing formulae are I 0-1 II 1/2-1(1/2) III 0-1 IV 1-0 V (vs. I 1–1(1 /2) II 1 /2–1(1 /2) III 0–1 IV 0–0 V).

Table 2. Measurement of adult *Chirixalus* sp., *Chirixalus pantaiselatan* (Munir *et al.,* 2021), and *Chirixalus trilaksonoi* (Riyanto & Kurniati, 2014)

	Chirixalus trilaksonoi in Jatilawang				*Chirixalus pantaiselatan*					*Chirixalus trilaksonoi*						
	Sample	Sample	Sample	Sample	MZB. Amph 30547	MZB. Amph 30548	MZB. Amph 30549	MZB. Amph 30550	MZB. Amph 30551	MZB. Amph 17932	MZB. Amph 17933	MZB. Amph 17934	MZB. Amph 28441	MZB. Amph 28440	MZB. Amph 29165	MZB. Amph 27237
	1	2	3	4	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	-	-	-	-
	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male	Adult male
SVL	26.5	23.9	26.11	24.65	28.9	25.3	26.6	26.9	25.9	25.4	26.6	26	26.8	25	26.8	30.3
HL	6.7	6.75	7.2	7.28	9.4	8.4	9.2	8.9	8.8	7.5	8.4	8.2	8.9	7.5	8.8	8.8
HW	7.8	7.8	7.43	7.58	10	9.3	8.4	8.8	9.2	7.1	7.8	7.7	8.8	7.2	8.3	9.4
SL	3.05	3.04	3.5	2.91	3.8	3.9	3.7	3.6	4.1	3.4	3.1	3.3	3.6	3.4	3.6	3.6
NEL	1.89	1.6	1.41	1.53	2.4	2.5	2.5	2.6	2.1	2.2	2.1	1.9	2.3	2.2	2.2	2.6
ED	2.8	2.65	2.93	2.59	4.5	4.4	4.4	4.3	4.8	3.5	3.5	3.1	3.5	3.7	3.2	4.3
ETD	0.6	0.45	0.63	0.62	1.1	1.2	1.3	1.2	1.4	0.6	0.6	0.6	0.6	0.4	0.5	0.7
TD	1.3	1.05	1.27	1.02	2.1	2	2.2	2.4	2	1.6	1.4	1.4	1.6	1.6	1.5	1.9
IND	1.75	1.58	2.09	1.87	2.3	2.4	2.4	2.4	2.4	2.1	2.1	1.8	2.2	1.9	2.2	2
IOD	4.76	4.3	4.78	4.42	3.5	3.4	3.6	3.5	4.1	2.7	3.1	3.1	3.1	2.9	3	3.6
LAL	10.07	9.38	9.26	10.6	12.7	11.6	11.4	12	12.7	10.6	9.9	9.9	11.5	9.7	11.3	12.8
Fin3L	3.84	3.01	3.92	3.08	4	3.6	3.4	3.7	3.9	3.9	3.3	3.7	4.1	3.2	3.8	4.1
Fin3DW	0.76	0.68	0.57	0.78	1.3	1.2	1.1	1	1.2	0.9	1	0.8	1.3	1.1	1.2	1
IPTL	0.74	0.54	0.92	0.85	1.2	1.1	1.4	1.1	1.3	0.7	0.7	0.8	0.9	0.7	0.9	0.8
THL	9.91	9.8	10.26	10.87	15.3	12.6	12.9	13	14.3	11.9	10.8	12.4	12.8	11.6	12.8	14.4
TL	12.25	10.83	13.03	12.1	15	13.2	13.2	14	15.2	12.8	13.1	12.9	13	12.6	13.9	15.7
FL	8.75	8.29	8.53	8.41	11.1	10.8	10.8	9.8	10.3	9.3	9.5	9.1	10.1	8.7	10.1	11.2
IMTL	0.7	0.72	0.84	0.64	1.2	1.1	1.4	1.3	1.4	0.8	0.8	0.7	0.8	0.9	0.8	0.9
Toe4L	4.4	4.36	4.5	4.78	6	5.4	5.6	5.3	5.4	4.8	4.4	5.1	5.4	5.2	5.4	6.3
Toe4DW	0.57	0.48	0.5	0.56	1	1	1.1	1	1	0.9	0.9	0.9	1	0.8	1	0.9

Table 3. Data matrix of the diagnostic characteristic separating the Javan *Chirixalus* species

Morphological characters	*Chirixalus trilaksonoi* in Jatilawang.	*Chirixalus pantaiselatan*	*Chirixalus trilaksonoi*
Sample size	4	5	7
Snout obtusely pointed (1) or truncate (0)	0	0	1
Vomerine teeth present (1) or absent (0)	0	1	0
Canthus rostralis distinct (1) or indistinct (0)	1	1	1
Dark postorbital stripe present (1) or absent (0)	0	0	0
White patch on side of the upper jaw (1) or not (0)	1	1	1
Tympanum distinct (1) or indistinct (0)	1	1	1
Glandular fold between eye and shoulder distinct (1) or faint (0)	1	1	1
External vocal sac present (1) or absent (0)	1	1	1
Dark stripes on dorsum present (1) or absent (0) either stripe or blotch (#)	#	0	1
Dark markings on dorsum (1) or not (0)	1	1	0
Skin of dorsum smooth (1) with small tubercles (0), or finely granular (x)	1	1	1
Banding on dorsal aspect of thighs (1) or not (0)	1	1	1
Third and fourth fingers onefour webbed (1) or less (0)	1	1	1
Disk on third finger as large as tympanum (1) or not (0)	0	0	0
Inner metatarsal tubercle present (1) or absent (0)	1	1	1
More (1) or less (0) than ⅓ webbing on toes	1	1	1
Webbing Formulae	I 0-1 II 1/2-1(1/2) III 0-1 IV 1-0 V	I 1–1 /2 II 0–1(1 /2) III 0–1 IV 1 /2–0 V	I 1–1(1 /2) II 1 /2–1(1/2) III 0–1 IV 0–0 V
Tibiotarsal articulation of hindlimb	Reaching tip of the snout	Reaching posterior of nostril	Reaching tip of the snout

Vocalization comparison

Vocalization comparison data obtained from *C. pantaiselatan* by Munir et al. (2021) and *C. trilaksonoi* by Riyanto & Kurniati (2014) and Herlambang et al. (2023), with the following results:

The advertisement short calls of *Chirixalus* sp. exhibit distinct differences (Fig. 6). *Chirixalus* sp. produces short calls lasting approximately 0.792 s with 11 notes, where note durations range from 5 – 15 ms and internote intervals vary between 17 – 202 ms. Frequency analysis shows *Chirixalus* sp. covers a wide range up to nearly 16.65 kHz with dominant frequencies around 4.48 kHz.

The advertisement short calls of *C. pantaiselatan* span a broader duration, from 1.27 – 7.57 s, with 2 to 9 notes per call. Note durations for *C. pantaiselatan* range from 4.14–18.03 ms, and internote intervals range from 77.33 – 394.33 ms. Chirixalus *pantaiselatan* has lower dominant frequencies (compared to *Chirixalus* sp.) ranging between 2.34 and 3.94 kHz.

The advertisement short calls of *C. trilaksonoi* are shorter and have fewer notes. Consist of 2 to 3 notes with a call period 0.14 – 0.18 s in Javan population and 0.37 – 0.42 s in Sumatran population. Note durations ranging from 20 – 120 ms in Javan population and 20 – 230 ms in Sumatran population. Shorter internote intervals of 11 – 15 ms. *Chirixalus trilaksonoi* has similar dominant frequency range (compared to *Chirixalus* sp.) from 4.45 to 4.53 kHz, averaging 4.5 kHz.

Chirixalus trilaksonoi in Jatilawang demonstrates distinct morphological and vocalization differences compared to both *C. pantaiselatan* and *C. trilaksonoi*. Although it exhibits more similar traits to *C. trilaksonoi* than to *C. pantaiselatan,* this might be intraspecific variation. The species still displays unique phenotypic characteristics.

DISCUSSION

This study confirms that the *Chirixalus* population recorded from Jatilawang, Central Java, is referable to *C. trilaksonoi*, based on a combined evaluation of morphometric, multivariate, and bioacoustic evidence. Although the Jatilawang population exhibits measurable variation in several characters, its overall morphological configuration and advertisement call structure are more consistent with *C. trilaksonoi* than with *C. pantaiselatan*. These findings support the identification of the Central Java population as *C. trilaksonoi* and represent an additional locality record for the species.

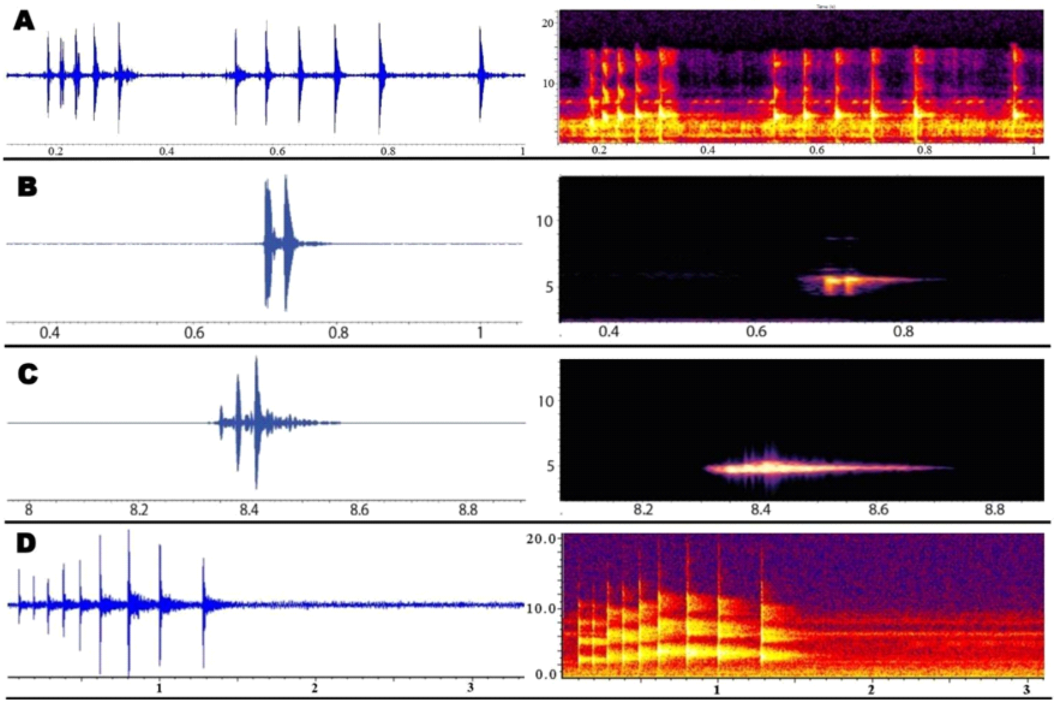

Figure 6. Oscillogram (left) and Spectrogram (Right) illustration of short call types: (**A**). *Chirixalus trilaksonoi* in Jatilawang, (**B**). *Chirixalus trilaksonoi* from Java (Herlambang et al., 2023), (**C**). *Chirixalus trilaksonoi* from Sumatra (Herlambang et al., 2023), (**D**). *Chirixalus pantaiselatan* (Munir et al., 2021). Horizontal scale bar at the bottom represent second. Vertical bar at spectrogram represents kilohertz.

Multivariate analyses revealed clear separation among populations, indicating both interspecific and intraspecific variation within the genus *Chirixalus*. Principal Component Analysis (PCA) showed distinct clustering of *C. pantaiselatan* and *C. trilaksonoi*, reflecting interspecific differentiation driven primarily by head and snout–orbital characters. At the same time, *C. trilaksonoi* from Jatilawang clustered closely with other *C. trilaksonoi* populations, but with partial separation, suggesting intraspecific variation within the species. Such a pattern, where populations of the same species show recognizable morphological shifts while retaining overall diagnostic similarity, is commonly reported in anuran taxa with broad or disjunct distributions.

Univariate morphometric analyses further clarify this pattern. The lack of significant differences in snout–vent length (SVL) among populations indicates comparable overall body size and suggests that observed variation is not attributable to size scaling. In contrast, significant differences in head length, head width, and snout–orbital characters highlight the importance of cranial morphology in both interspecific discrimination and intraspecific structuring. While *C. pantaiselatan* consistently exhibited larger cranial and snout–orbital measurements, *C. trilaksonoi* from Central Java showed smaller or intermediate values that remain within the known morphological range of the species. This indicates proportional variation rather than taxonomically diagnostic divergence.

Bioacoustic data provide additional support for this interpretation. Advertisement calls of *C. trilaksonoi* from Central Java share fundamental call structure and dominant frequency characteristics with previously described *C. trilaksonoi*, while differing from *C. pantaiselatan* in call duration and frequency range. At the same time, subtle differences in note number, call length, and frequency bandwidth among *C. trilaksonoi* populations reflect intraspecific acoustic variation. Such geographic variation in advertisement calls has been widely documented in frogs and is often associated with local ecological conditions, population isolation, or sexual selection rather than species-level divergence.

The morphological and acoustic variation observed in the Central Java population is therefore best interpreted as population-level differentiation influenced by local biotic and environmental factors. *Chirixalus trilaksonoi* is known to inhabit a range of disturbed and semi-natural habitats, including paddy fields, suburban areas, and artificial water bodies. Variation in vegetation structure, microclimate, ambient noise, and breeding site characteristics may influence both cranial morphology and call properties. Similar patterns of geographically structured intraspecific variation have been reported in other anuran species, where local adaptation and phenotypic plasticity contribute to measurable differences without obscuring species identity.

Overall, our results demonstrate that *C. trilaksonoi* exhibits both interspecific differentiation from *C. pantaiselatan* and intraspecific variation across its range. The Central Java population represents a morphologically and acoustically differentiated, yet conspecific, population of *C. trilaksonoi*. These findings highlight the importance of integrating morphometric, multivariate, and bioacoustic data when assessing geographic variation and species limits in anurans. Future studies incorporating molecular data and broader sampling across Java and Sumatra will be essential to further elucidate the evolutionary processes shaping variation within *C. trilaksonoi*.

Chirixalus trilaksonoi, sympatric with *Occidozyga* sp., *Fejervarya cancrivora*, and *Kaloula baleata*, was discovered close to a suburban area in Central Java, roughly 1.2 km from the provincial road and 2.2 km from the Jatilawang District Office in Banyumas Regency. In Palembang, this species also lives in suburban environments; individuals have been seen on palm leaves close to man-made ponds, along with other suburban-adapted frogs (Herlambang et al., 2023). Based on Riyanto & Kurniati (2014) research, *C. trilaksonoi* from Bogor was found at a paddy field close to residential areas. These results imply that *C. trilaksonoi* can adjust to changes in its habitat. Similar to the breeding behavior of *C. trilaksonoi* in Central Java. They also construct nests under leaves close to bodies of water during the rainy season (Alhadi et al., 2021).

Despite their similarities, the physical and vocal traits of each *C. trilaksonoi* population are unique. According to our research, the populations of *C. trilaksonoi* in Central Java and Sumatra reported by Riyanto & Kurniati (2014) and Herlambang et al. (2023) have different characteristics. The study found that the most noticeable variance was in dorsal morphology and voice characteristics. Field researchers can benefit from this discovery, especially in the area of taxonomy. Schäuble (2004) asserted that environmental and regional factors can affect the variances in frog morphology. Another factor might be the lack of genetic variety in their environments (Hu et al., 2019). The small number of specimens employed in morphometric analysis may potentially lead to significant variances (Grabowski & Porto, 2016). Our findings highlight notable differences in morphology and vocal traits linked to geographical and habitat conditions. Further investigation into other factors affecting these differences based on geographic location is warranted.

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