

Morphological and Morphometric Analysis of *Euphlyctis adolfi* (Anura: Dicroglossidae) from Rawalpindi-Islamabad Area

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ABSTRACT

The frogs of the family Dicroglossidae are commonly known as fork-tongued frogs. The dicroglossid genus *Euphlyctis* Fitzinger, 1843, also known as skittering frogs or skipper frogs is a highly aquatic and littoral frog. Common Skittering Frog *Euphlyctis adolfi* appears to be more polymorphic than previously assumed which causes frequent misidentification during field surveys. We aimed to examine morphological and morphometric characters of *E. adolfi* (n= 97) found in Rawalpindi-Islamabad area, Pakistan. We recorded four morphotypes A (20), B (24), C (36), and D (17) of *E. adolfi* based on their morphology and morphometry. We found that except for eye-tympanum distance, distance between nares and inter-orbital distance, all measurements for type D had smaller means. Principal component analysis revealed that first 3 principal components were responsible for 92% variability in the data. In the first component six variables: snout-vent length, head length, head width, thigh length, shank length and foot length were found significant ($r > 0.2$). Five variables were found significant in the second principal component ($r > 0.2$), the variables being snout-vent length, intra-orbital distance, anterior intra-orbital distance, foot length and tarsus length. In the third principal component 5 variables were found significant, which included snout-vent length, inter-narial distance, thigh length, foot length, and shank length, respectively. The Pillai's Trace test statistics revealed that the means of 10 morphometric measurements, obtained through PCA, are statistically significant with means of the type D showing the difference. Our samples exhibited difference in a few measurements when compared with samples from Bangladesh and Punjab (Pakistan).

Article Information

Received 09 July 2024

Revised 20 April 2025

Accepted 09 May 2025

Available online 22 October 2025
(early access)

Authors' Contribution

AA gave the idea and supervised the study. HN collected samples, implemented the methodology and interpreted the results, wrote the manuscript. MR performed the statistical analysis and reviewed the manuscript. SG helped in sample collection. MK helped in mapping of study area.

Key words

Common skittering frog, Morphotypes, Principal component analysis, *Adolfi*

INTRODUCTION

The dicroglossid genus *Euphlyctis* Fitzinger, 1843, also known as skittering frogs or skipper frogs has a widespread distribution range from the Arabian Peninsula, Iran, Afghanistan, India, Pakistan to tropical Myanmar and Sri Lanka (Wagner *et al.*, 2016; Akram *et al.*, 2021; Dinesh *et al.*, 2021; Khatiwada *et al.*, 2021; Zug, 2022). The genus *Euphlyctis* (Fitzinger, 1843) was previously treated as *Rana* by Dubois (1981). The taxon named *Euphlyctis adolfi*, originally described as *adolphi* (Günther, 1860), was synonymized by Boulenger (1882) as *Rana cyanophlyctis* (Schneider, 1799). The species name *Euphlyctis adolfi*

has long been obliterated, and *Euphlyctis cyanophlyctis* was being used in literature (Khan, 2006; Baig *et al.*, 2008; Masroor, 2011). After description of a type series from Bangladesh by Howlader *et al.* (2015), the taxon was reassigned as *E. kalasgramensis*. Subsequently Ali *et al.* (2020) and Akram *et al.* (2021) reported the same from Punjab, Pakistan based on morphology and mtDNA analysis. Dufresnes *et al.* (2022) formally preferred the use of oldest valid name *Euphlyctis adolfi* based on genetic resemblance, and declared *E. kalasgramensis* as a junior synonym of *E. adolfi*.

Euphlyctis, being a wide spread genus poses an inherent challenge to identify morphologically similar populations (Joshy *et al.*, 2009). The species are now recognized to exhibit greater polymorphism than previously assumed, leading to frequent misidentification (Dinesh *et al.*, 2021). As anuran surveys typically rely on phenotypic identification in the field, the extent of morphological variations needs to be addressed when more than one morphological form is expected, which casts doubts on the identity of populations. We aimed to examine morphological and morphometric characters of

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0030-9923/2025/0001-0001 \$ 9.00/0



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E. adolfi found in the Rawalpindi, Islamabad areas. Our findings will be beneficial for herpetologists to conduct field identification and monitoring surveys in a more effective way.

MATERIALS AND METHODS

Study area

Randomly selected 27 sampling sites were located in Rawalpindi District 72.9933° E, 33.4095° N and Islamabad Capital Territory 33.7294° N, 73.0931° E. Rawalpindi district dominated by rocks and scrub flora covers an area of 5286 km² with an elevation of 508m. The climate of this region is humid subtropical with extreme hot and long summers, winters are mild wet and monsoon is short (Chaudhry and Rasul, 2004). Islamabad Capital Territory covers an area of 906.50 km², and elevation ranges from 457 to 610 m (Sheikh and Hafeez, 2001). Its climate is classified as humid subtropical, characterized by a mean minimum temperature of 24.4 °C and a maximum temperature of 34 °C.

Study design

Adult specimens (n=97) of common skittering frog were collected from August to November, 2022 by conducting 27 field visits. Surveys were conducted during morning and evening hours. All major habitats such as water pools, agriculture canals and surrounding vegetation were thoroughly searched. Samples were collected by using dip nets, after capturing, adult frogs were shifted into jars and brought to the laboratory. Specimens were initially examined based on the morphological characters (Khan, 2006; Howlader et al., 2015; Ali et al., 2020; Rais et al., 2021). Description of these morphometric characters. The samples were categorized into 4 types on the basis of their morphology (spots present on their body, both ventral and dorsal side) as A 20, B 24, C 36, and D 17. Frogs were euthanized with chloroform and then fixed and preserved in 10% formalin solution. Morphometric measurements of all samples were recorded with the help of a digital vernier caliper (Insize, 1112, 0.01mm) (Ali et al., 2020). Twenty three morphometric measurements: as snout vent length, head length, head width, eye length, snout length, tympanum diameter, hand length, foot length, tarsus length, upper eye lid, intra-orbital distance, anterior intra-orbital distance, posterior intra-orbital distance, distance between nares, distance from posterior of the mandible to the nostrils, distance from posterior of the mandible to the anterior of the eyes, distance from posterior of the mandible to the posterior of the eyes, snout-nostril distance, snout length, eye-nostrils distance, tympanum diameter, eye-tympanum distance, forearm length, thigh length, and shank length, were taken.

Statistical analysis

For morphometric measurements, the descriptive statistics such as mean, standard error, and maximum and minimum values were calculated. Principal components analysis (PCA) was performed on the 23 morphometric measurements of 4 types of *E. adolfi* in program R 4.3.1 to scale down an examined measurement into the less noteworthy variable ($r > 0.20$) which was then compared using MANOVA (Pillai's trace test). For post-hoc analysis, we performed linear discriminant analysis (LDA) and plotted the results as a scatter plot between LD 1 and LD2. The intention is to lower the largest set of variables into smaller set of the artificial variables, referred to as principal components, in the original variables these account for maximum of the variance.

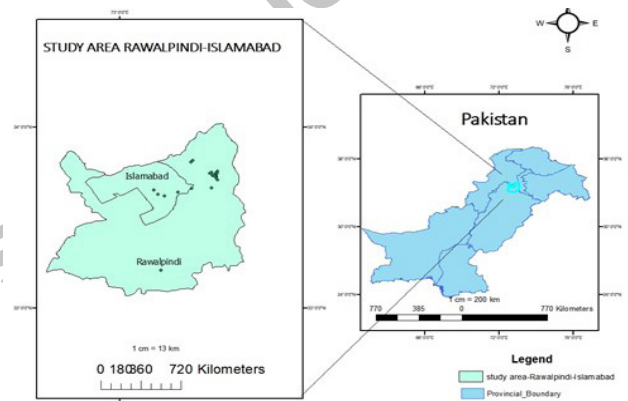


Fig. 1. Map of the study area showing sampling locations in Rawalpindi-Islamabad, Pakistan.

RESULTS AND DISCUSSION

Morphology of *E. adolfi*

The following description is based upon the 97 adult frogs of *E. adolfi* and photographs of different types of *E. adolfi* shown in Figure 2. These are classified on the basis of spots present on the dorsal and ventral surface of frogs. However, the morphological differences observed among these types are mentioned in Figure 2.

The samples of *E. adolfi* had different dorsal coloration such as brown, light brown, grey, and olive green. Among all types A, B, C, D, out of total 97 samples, 19 were light grey, 18 exhibited light brown and brown colors and remaining 60 samples were olive green in color. Dark rounded spots were present on dorsal surface of type B and type C. Limbs had dark bands (type A and B). Ventral coloration of samples was white and dark dots were present on the type A and type C. The samples became grey or greyish-black in the preservative.



Fig. 2. Dorsal (a) and ventral sides (b) of four morphotypes of *Euphlyctis adolfi* recorded from the study area. Body dorsum in type B and C bears irregular black spots while type A and D lack any spots. The ventral side in type A and C bears many black dots while these are absent in type B and D.

Morphometric measurements of *E. adolfi*

Table I shows 23 morphometric measurements of 4 types of *E. adolfi*. Except for eye-tympanum distance, distance between nares and inter orbital distance, all measurements for type D had smaller means. Principal component analysis resulted into 3 principal components, which accounted for most of the variation. Standard deviation, variability (%), cumulative variability (%) and factor loading of the 23 morphometric measurements are given in Table II. The first 3 principal components were responsible for 92% variability in the data. The first principal component showed 88.50%, second showed 2.7% and third showed 1.5% of total variation. In first component six variables: snout-vent length, head length, head width, thigh length, shank length and foot length were found significant ($r > 0.2$). Five variables were found

Table I. Morphometric measurements (Mean $\pm$ SE) of four morphotypes (A-D) of *Euphlyctis adolfi* collected from Rawalpindi and Islamabad area.

	Type A	Type B	Type C	Type D
Number of samples	20	24	36	17
Snout vent length	46.40 $\pm$ 1.69	47.76 $\pm$ 2.28	47.69 $\pm$ 1.26	40.42 $\pm$ 2.06
Head length	18.49 $\pm$ 0.70	18.91 $\pm$ 0.99	18.62 $\pm$ 0.50	15.81 $\pm$ 0.67
Head width	18.01 $\pm$ 0.67	18.32 $\pm$ 0.82	18.08 $\pm$ 0.51	15.37 $\pm$ 0.77
Eye length	5.41 $\pm$ 0.19	5.10 $\pm$ 0.21	5.20 $\pm$ 0.13	4.55 $\pm$ 0.29
Upper eye lid	3.63 $\pm$ 0.15	3.64 $\pm$ 0.19	3.38 $\pm$ 0.12	3.23 $\pm$ 0.19
Inter-orbital distance	2.64 $\pm$ 0.22	2.98 $\pm$ 0.20	2.30 $\pm$ 0.12	2.59 $\pm$ 0.20
Anterior intra-orbital distance	3.32 $\pm$ 0.21	3.69 $\pm$ 0.24	3.23 $\pm$ 0.13	3.20 $\pm$ 0.28
Posterior inter-orbital distance	4.01 $\pm$ 0.23	4.19 $\pm$ 0.26	3.85 $\pm$ 0.16	3.69 $\pm$ 0.26
Distance between nares	3.48 $\pm$ 0.27	4.12 $\pm$ 0.30	3.69 $\pm$ 0.20	3.53 $\pm$ 0.28
Distance from posterior of the mandible to the nostrils	12.89 $\pm$ 0.46	13.04 $\pm$ 0.65	13.11 $\pm$ 0.38	11.38 $\pm$ 0.48
Distance from posterior of the mandible to the anterior of the eyes	9.95 $\pm$ 0.36	10.24 $\pm$ 0.52	10.16 $\pm$ 0.24	8.68 $\pm$ 0.43
Distance from posterior of the mandible to the posterior of the eyes	5.77 $\pm$ 0.25	6.02 $\pm$ 0.39	6.07 $\pm$ 0.20	5.01 $\pm$ 0.26
Snout-nostril distance	4.37 $\pm$ 0.24	4.80 $\pm$ 0.33	4.22 $\pm$ 0.14	3.9 $\pm$ 0.29
Snout length	7.53 $\pm$ 0.31	7.98 $\pm$ 0.41	7.72 $\pm$ 0.25	6.8 $\pm$ 0.39
Eye-nostrils distance	2.90 $\pm$ 0.12	3.35 $\pm$ 0.14	3.19 $\pm$ 0.10	2.72 $\pm$ 0.15
Tympanum diameter	3.62 $\pm$ 0.14	4.12 $\pm$ 0.33	3.72 $\pm$ 0.16	3.28 $\pm$ 0.27
Eye-tympanum distance	1.72 $\pm$ 0.11	2.03 $\pm$ 0.17	1.74 $\pm$ 0.10	1.78 $\pm$ 0.17
Forearm length	8.60 $\pm$ 0.33	9.35 $\pm$ 0.47	8.99 $\pm$ 0.24	7.71 $\pm$ 0.48
Hand length	9.23 $\pm$ 0.46	9.01 $\pm$ 0.48	9.33 $\pm$ 0.30	8.30 $\pm$ 0.39
Thigh length	21.32 $\pm$ 0.80	22.15 $\pm$ 1.27	20.96 $\pm$ 0.59	18.39 $\pm$ 1.29
Shank length	22.38 $\pm$ 0.90	22.73 $\pm$ 1.20	22.67 $\pm$ 0.62	19.23 $\pm$ 1.20
Foot length	23.37 $\pm$ 0.86	23.75 $\pm$ 0.93	24.50 $\pm$ 0.73	20.13 $\pm$ 0.93
Tarsus length	10.76 $\pm$ 0.44	11.75 $\pm$ 0.77	11.04 $\pm$ 0.33	9.5 $\pm$ 0.67

Table II. Standard deviation, proportion of variance, cumulative proportion and factor loading of the 23 morphometric measurements of four morphotypes (A-D) of *Euphlyctis adolfi* collected from Rawalpindi and Islamabad area.

	PC1	PC2	PC3
Standard deviation	13.3007	2.35217	1.77092
Proportion of Variance	0.88508	0.02768	0.01569
Cumulative Proportion	0.88508	0.91276	0.92845
Factor loading			
Snout-vent length (SVL)	0.65229	0.31216	0.46118
Head length (HL)	0.24950	-0.0666	-0.0379
Head width (HW)	0.24696	-0.041	0.09934
Eye length (EL)	0.05374	0.00999	0.07853
Width of upper eyelid (UEW)	0.04635	-0.0597	0.08503
Inter-orbital distance (IOD)	0.03829	-0.2278	0.13787
Anterior Inter-orbital distance (AIOD)	0.05004	-0.2084	0.14598
Posterior Inter-orbital distance (PIOD)	0.05771	-0.1864	0.19124
Distance between nares (IND)	0.06391	-0.1984	0.24719
Distance from the posterior of the mandible to the nostrils (MN)	0.165	0.04753	0.05143
Distance from posterior of the mandible to the anterior of the eyes (MFE)	0.12563	0.01755	0.09025
Distance from posterior of the mandible to the posterior of the eyes (MBE)	0.08437	0.0447	-0.0608
Snout-nostril distance (SND)	0.07489	-0.1642	0.10975
Snout length (SL)	0.10783	-0.0978	0.17507
Eye-nostrils distance (END)	0.03517	-0.0254	0.02971
Tympanum diameter (TD)	0.05738	-0.1106	-0.0955
Eye-tympanum distance (ETD)	0.02904	-0.12	0.04426
Forearm length (FAL)	0.11433	-0.1019	-0.14
Hand length (HAL)	0.12321	0.07334	0.14125
Thigh length (THIGHL)	0.32387	-0.3582	-0.3423
Shank length (SHL)	0.32746	-0.3721	-0.3764
Foot length (FOL)	0.30551	0.55704	-0.4957
Tarsus length (TAL)	0.16855	-0.2492	-0.1065

The factor loadings with absolute correlation values greater than 0.2 were considered significant (in bold).

significant in the second principal component ($r > 0.2$), which were snout-vent length, intra-orbital distance, anterior intra-orbital distance, foot length and tarsus length. In the third principal component five variables were found significant, which included snout-vent length, inter-narial distance, thigh length, foot length, and shank length, respectively (Table II).

The Pillai's trace test statistics revealed that the means of 10 morphometric measurements, obtained through PCA, were statistically significant [Pillai's trace = 0.48, $F(30, 258) = 1.64$, $p = 0.02$] with means of the type D showing the difference (Fig. 3). Common skittering frog or skipper frog has been identified as *E. kalasgramensis* and *E. cyanophlyctis*. However, Dufresnes *et al.* (2022) suggested the use of valid old name *Euphlyctis adolfi*. The following discussion treats *Euphlyctis kalasgramensis*, *E. cyanophlyctis* and *E. adolfi* as the same species, and

the comparison shows differences due to the geographic distribution, not due to the species characteristics.

The coloration of dorsal surface of our samples varies from light to dark shades of brown, grey and olive green. The same variation was also reported for *E. cyanophlyctis* and *E. kalasgramensis* from Pakistan and India (Howlader *et al.*, 2015; Ali *et al.*, 2020; Khatiwada *et al.*, 2021). The morphotype B and C had irregular black spots and smooth tubercles, as reported for *E. cyanophlyctis* from South India (Schneider, 1799), *E. kalasgramensis* from Kalasgram, Bangladesh (Howlader *et al.*, 2015), from Punjab, Pakistan (Ali *et al.*, 2020) and from Rawalpindi-Islamabad, Pakistan (Akram *et al.*, 2021), and *E. cyanophlyctis* from Sindh, Pakistan (Shaikh *et al.*, 2012). The ventral surface of the morphotypes A and C have numerous black dots as reported for *E. kalasgramensis* from Kalasgram (Howlader *et al.*, 2015), and from Punjab,

Pakistan (Ali *et al.*, 2020). On the other hand, the type B and D have whitish ventral surface without any spots or dots as reported for *E. kalasgramensis* (Howlader *et al.*, 2015; Ali *et al.*, 2020). Our data suggest that first and second fingers in all four studied morphotypes were equal as reported for *E. kalasgramensis* (Howlader *et al.*, 2015), but different from *E. cyanophlyctis microspinulata* (Khan, 1997) which have second finger smaller than the first finger.

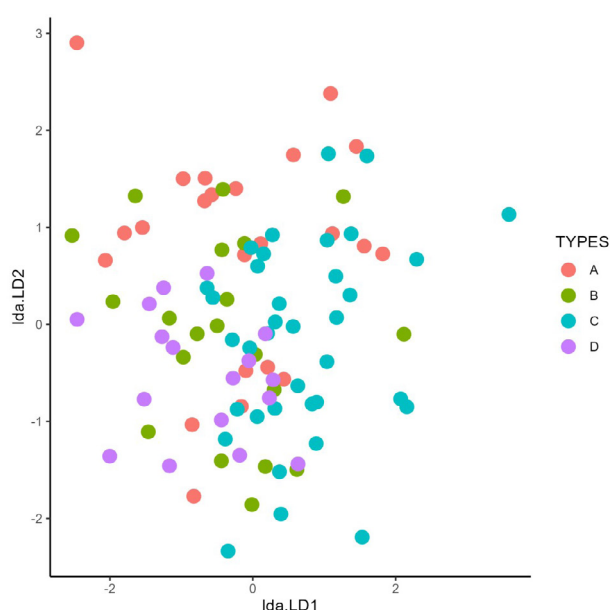


Fig. 3. Visualizing four morphotypes (A-D) of *Euphlyctis adolfi* separation based on post-hoc MANOVA using Linear Discriminant Analysis (LDA).

The studied characters such as longer than wider head, proximity of nares to snout than eyes and greater width of upper eyelid than inter orbital distance are similar to *E. kalasgramensis* from Kalasgram, Bangladesh (Howlader *et al.*, 2015) and from Punjab, Pakistan (Ali *et al.*, 2020). The snout-vent length (SVL) of our samples was greater than *E. kalasgramensis* described by Howlader *et al.* (2015) and Ali *et al.* (2020). This also attributes difference in some other measurements. For instance, the length of foot is 50% of the length of SVL in type A, B and D but 51% in type C of our samples while it is 55% and 54% of SVL for *E. kalasgramensis* described by Howlader *et al.* (2015) and Ali *et al.* (2020), respectively. Likewise, the length of head is 39% of length of SVL in our samples, and 32% for *E. kalasgramensis* (Howlader *et al.*, 2015; Ali *et al.*, 2020).

DECLARATIONS

Acknowledgement

We would like to extend our sincere gratitude to Prof. Dr Shamim Akhter Chairperson of Department of Zoology, Wildlife and Fisheries, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan for providing support and guidance throughout this research.

Funding

This research was conducted without external funding support and was entirely self-funded.

Ethical statement

The research was conducted with the approval of ethical committee (PMAS-AAUR/IEC/230) of Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi (PMAS-AAUR), Pakistan.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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