

The evolution of anuran locomotion and skeletal anatomy

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Introduction

This paper explores the anuran anatomical specializations of limbs and pelvis for different movement modes. Locomotor performance is critical for fitness as it determines the efficiency of predators escaping [1]. It is suggested that jumping plays a key role in evolutionary success[2]. Nevertheless, Anurans acquired a wide range of skeletal structures, habitats and locomotor modes (e.g. jumping and hopping, which differ in relative jumping distance[3]). The aim of this work is to examine how skeletal anatomy in anurans is affected by locomotor and phylogenetic demands.

Methods and Results

Data analysis was performed using R-4.2.1 and RStudio 2022.12.0 for Windows. Additional packages include ***tidyverse*** (*ggplot2*, *ggfortify* libraries) for data visualization. Package ***fields*** was installed to produce heatmaps (*fields* library). Finally, ***kableExtra*** package with the homonymous library to create summary tables.

Data

Data provided by UCL GEE Department includes 6 skeletal measurements from 164 anuran species across four clades (Basal/Hyloidea/Neobatrachia/Ranoidea). Snout-vent length(SVL), expansion of the sacral diapophyses(ESD), calcaneus(calc), femur(fem), tibiofibular(tib), and foot length were measured. Additionally, one of five locomotor modes was assigned to each species: arboreal jumper(AJ), terrestrial jumper(TJ), burrower-walker-hopper(BWH), walker-hopper(WH), or aquatic/swimmer(AQ).

Data transformations and preliminary analysis

First, skeletal measurements were corrected for body size by dividing each variable by corresponding SVL. Additionally, it was shown in previous studies that body size has no evolutionary association with locomotion[4], so we did not include it in our analysis.

Preliminary data investigation involved plotting each skeletal measurement grouped by clade and locomotor_mode to compare their descriptive statistics. Figure1a shows a strong variation in ESD across clades. We saw that Basals exhibit larger ESD, increasing variance and affecting ESD distribution. Figure1b depicts the general tendency of jumper frogs (AJ/TJ) to have greater lengths of calcaneus/femur/tibiofibular.

Since the experimental design is complex, outliers were not excluded because it would reduce variation within phylogenetic groups. Normality assumptions for skeletal measurements were checked using histograms and Shapiro-Wilk test($p > 0.05$) for further statistical methods .

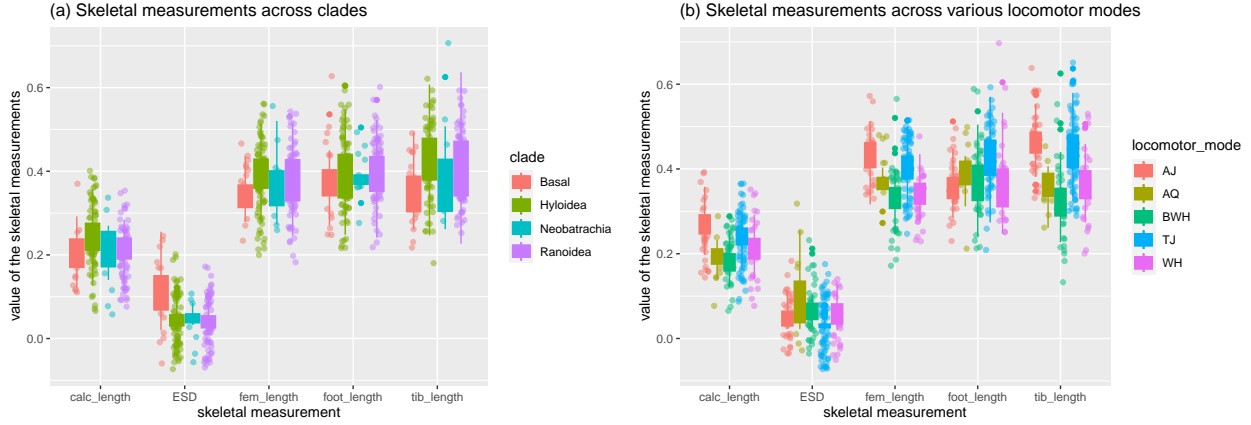


Figure 1: Skeletal measurements across (a) Clades and (b) Locomotor modes

Clade and locomotor mode

To assess the significant association between clades and locomotor modes Chi-squared test was used since variables are categorical. Additionally, a mosaic plot was used to visualize types of locomotion across phylogenetic groups (Figure2).

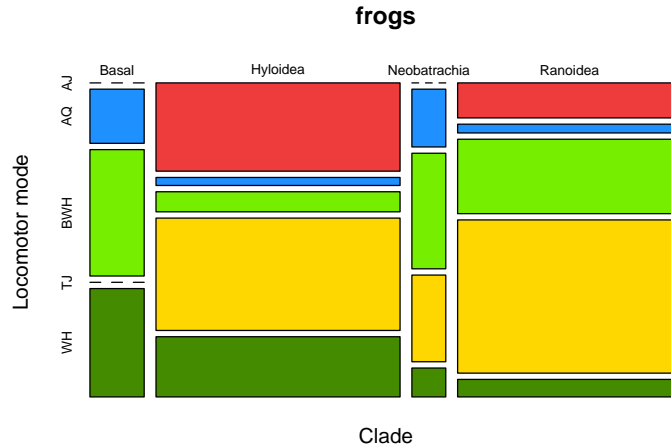


Figure 2: Association between clades and locomotor modes

Results show that clades differ significantly in the acquired locomotor modes (Chi-squared: $p < 0.05$). Figure 2 supports this and shows that Basal has no jumping mode but is associated with BWH/WH/AQ. On contrary, Hyloidea/Ranoidea are associated with AT/TJ and rarely AQ/WH/BWH.

Linear models for skeletal measurements

To identify any associations between skeletal anatomy, clade and/or locomotor mode *lm()*, *summary()*, and *anova()* functions were used. Each measurement was a response variable for a linear regression model (*lm*) with clade and/or locomotor_mode predictors. Models were designed without intercept.

Deviance ratio test was performed with *logLik()* to compare and fit each of four models (clade or locomotor_mode, clade+locomotor_mode, and clade*locomotor_mode). While difference between two best-

performed models was significant (Chi-squared: $p<0.05$), better fitted model was chosen. Otherwise, the simpler model was used. The summaries of final linear models were presented in Table1.

- a. ESD: ANOVA (model:ESD~clade*locomotor_mode-1) confirmed that all terms explained significant variation (ANOVA: $p<0.05$ for each term). Basal is associated with larger ESD, whereas Ranoidea and Hyloidea clades are associated with smaller ESD. Anurans with AQ also associate significantly with larger ESD. However, Hyloidea/Neobatrachia/Ranoidea with AQ show decrease in ESD. Hyloidea with TJ is also associated with decrease in ESD.
- b. calcaneus: ANOVA (model:calc_length~locomotor_mode*clade-1) confirmed that effect of locomotor_mode on calcaneus_length was the same between different clades(clade:locomotor_mode: $p>0.05$). Estimates (model:calc_length~locomotor_mode+clade-1) reflect that jumping modes are associated with larger calcaneus, whereas BWH/WH/AQ have smaller calcaneus (Table 1; note: Basal row for calc column relates to AJ). Meanwhile, Neobatrachia is associated with longer calcaneus.
- c. femur: ANOVA (model:fem_length~clade*locomotor_mode-1) revealed significance of all terms (ANOVA: $p<0.05$). The summary for lm illustrates that BWH/WH modes are associated with shorter femur. Similarly, aquatic frogs tend to have smaller femur.
- d. tibiofibular: There was no significant difference in effect of locomotor_mode across clades (clade:locomotor_mode: $p>0.05$). Therefore, a simpler model was chosen (tib_length~clade+locomotor_mode-1). We found that BWH/WH/AQ are associated with smaller tibiofibular length. Neobatrachia is associated with larger tibiofibular length.
- e. foot: ANOVA (model:foot_length~clade*locomotor_mode-1) revealed that clade did not explain significant variation (ANOVA: $p>0.05$). Estimates show that Neobatrachia is associated with longer foot length, whereas Basal has an association with smaller foot length.

Table 1: Summary of lm estimates for each skeletal measurement (3s.f.) (red: $\Pr(>|t|)<0.05$, blue: $\Pr(>|t|)>0.05$)

	ESD	calc	fem	tib	foot
cladeBasal	0.07320	0.279	0.436000	0.472	0.33500
cladeHyloidea	0.05790	-0.00441	0.427000	-0.13	0.35600
cladeNeobatrachia	0.02140	0.000988	0.422000	-0.0148	0.39900
cladeRanoidea	0.02880	-0.0196	0.432000	-0.102	0.37800
locomotor_modeAQ	0.12200	0.198	-0.071900	0.466	0.02970
locomotor_modeBWH	0.03810	0.195	-0.084400	0.477	0.07830
locomotor_modeTJ	0.00378	0.25	-0.021400	0.461	0.04750
locomotor_modeWH	0.01150	0.216	-0.107000	-0.116	-0.00694
cladeHyloidea:locomotor_modeAQ	-0.12900	-	-0.012900	-	0.00476
cladeNeobatrachia:locomotor_modeAQ	-0.08210	-	-0.031000	-	-0.04530
cladeRanoidea:locomotor_modeAQ	-0.12400	-	0.028300	-	0.01210
cladeHyloidea:locomotor_modeBWH	-0.02730	-	-0.024300	-	-0.09350
cladeNeobatrachia:locomotor_modeBWH	-0.01670	-	0.064000	-	-0.06230
cladeRanoidea:locomotor_modeBWH	-0.00272	-	-0.027400	-	-0.11000
cladeHyloidea:locomotor_modeTJ	-0.03020	-	0.000389	-	0.02570
cladeNeobatrachia:locomotor_modeTJ	0.04000	-	-0.022500	-	-0.09660
cladeHyloidea:locomotor_modeWH	-0.01320	-	0.038600	-	0.03580

PCA

Principal component analysis (PCA) was performed on 5 preliminarily scaled skeletal measurements (excluding SVL), using *prcomp()* function.

a. variance and loadings

Variance explained by each principle component (PC) show that PC1+PC2 explain over 81%(Figure3a). Figure3b illustrates that PC1(61.84%) shows strong negative correlation with tibiofibular/femur/calcaneus length (loadings: -0.5550, -0.5386, and -0.4711, respectively). PC2(19.36%) has strong positive correlation with ESD (0.8230). Therefore, these skeletal measurements are the strongest drivers of morphological variation.

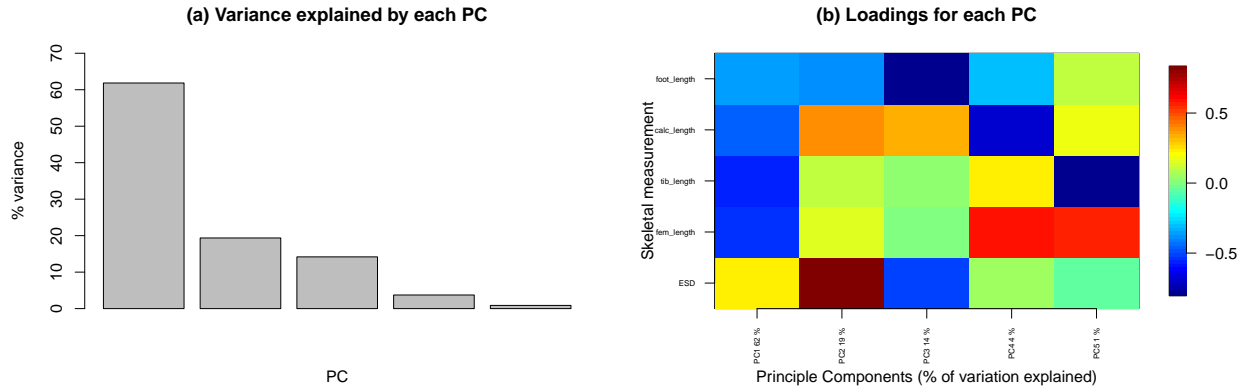


Figure 3: PCA (a) Variation and (b) loadings

b. biplots

Using PCA biplots colour-coded by (a) locomotor_mode and (b) clade, we compared species in phylomorphospace(Figure4). Loadings for each variable are shown with the respective vectors on a biplot. By analyzing their length we found that ESD contains the most and foot_length the least variation. Acute angles between fem_length and tib_length vectors indicate correlation between these variables. We confirmed a correlation between the calcaneus and tibiofibular by looking at the changes in loadings for PC4 and PC5 (Figure3b).

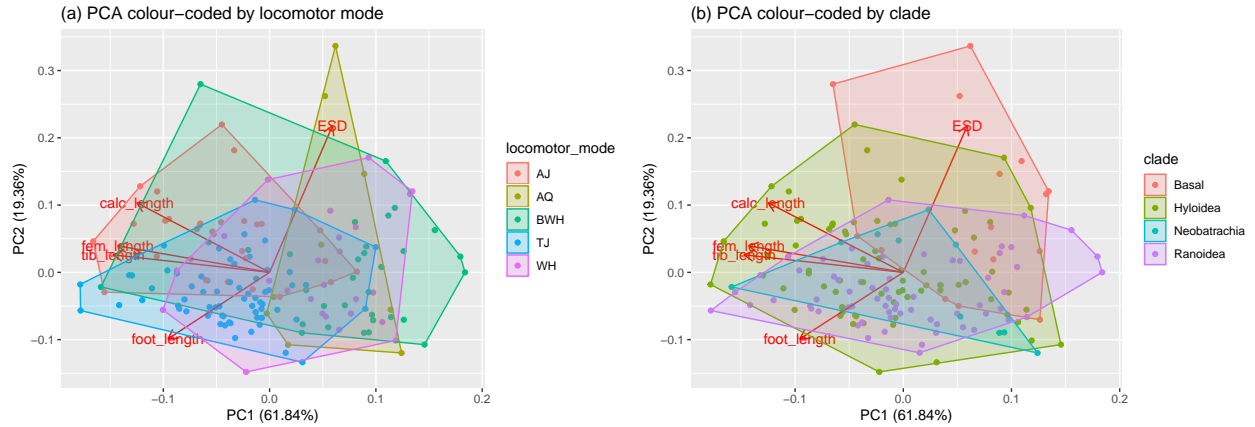


Figure 4: PCA biplots color-coded by (a) locomotor mode and (b) clade

There were no distinct clusters, but general trends in data distribution can be obtained. AQ is located in the area with smaller hindlimb compartments (fem_length, tib_length, calc_length), and greater ESD (Figure4a). AJ/TJ are shifted towards longer hindlimbs and smaller ESD. BWH/WH clusters are shifted to larger ESD and smaller hindlimbs. BWH mode shows the greatest variation.

Basal clustered around greater ESD and smaller hindlimbs(Figure4b). Neobatrachia and Ranoidea are both clustered around the origin, and prone to have smaller ESD. Hyloidea is shifted to greater hindlimbs, and shows the greatest variation.

c. linear models for PCs

Models designed for each PC(response) detected association with clade/locomotor_mode(predictors). Estimates for each model in Table 2 show that PC1 (negative correlation with tib_length/fem_length) is associated with negative values for Hyloidea/Ranoidea, and with greater values for BWH/WH. PC2 (positive correlation with ESD) is associated with greater values for Basal clade and AQ mode. The rest of PCs explained very minor variation. None of the terms for PC5 explained significant variation(ANOVA:p>0.05)

Table 2: Summary of lm for PCs (3s.f.) (red: $\Pr(>|t|)<0.05$, blue: $\Pr(>|t|)>0.05$)

	pc1	pc2	pc3	pc4	pc5
frogs\$cladeBasal	-0.8930	1.2400	0.57700	0.5840	-0.263000
frogs\$cladeHyloidea	-1.0700	0.9410	0.70800	-0.0734	0.079400
frogs\$cladeNeobatrachia	-1.2400	-0.3170	0.55100	0.0360	-0.012600
frogs\$cladeRanoidea	-1.0900	-0.0872	0.63100	0.3540	-0.000636
frogs\$locomotor_modeAQ	2.4200	1.8900	-2.36000	-0.3940	0.141000
frogs\$locomotor_modeBWH	2.2200	-0.5090	-1.93000	-0.5530	0.362000
frogs\$locomotor_modeTJ	0.2580	-0.3830	-0.71600	-0.2260	-0.032800
frogs\$locomotor_modeWH	2.4300	-0.5110	-0.44700	-0.7410	0.061100
frogs\$cladeHyloidea:frogs\$locomotor_modeAQ	-0.0184	-3.4100	1.33000	0.4950	-0.057400
frogs\$cladeNeobatrachia:frogs\$locomotor_modeAQ	0.7700	-2.0600	1.34000	0.2700	0.042100
frogs\$cladeRanoidea:frogs\$locomotor_modeAQ	-1.1600	-2.8600	1.46000	0.4870	-0.056700
frogs\$cladeHyloidea:frogs\$locomotor_modeBWH	0.9940	-0.4990	1.17000	0.6870	-0.389000
frogs\$cladeNeobatrachia:frogs\$locomotor_modeBWH	-1.1600	0.3340	1.05000	1.0400	-0.414000
frogs\$cladeRanoidea:frogs\$locomotor_modeBWH	0.8570	0.4410	1.28000	0.2250	-0.371000
frogs\$cladeHyloidea:frogs\$locomotor_modeTJ	-0.1750	-0.9560	-0.00314	0.1520	-0.063400
frogs\$cladeNeobatrachia:frogs\$locomotor_modeTJ	0.9520	1.5300	0.72200	-0.2740	0.112000
frogs\$cladeHyloidea:frogs\$locomotor_modeWH	-0.7300	-0.5250	-0.37000	0.7210	-0.167000

Discussion

This work examined the relationship between anatomical features, ecological factors and evolutionary history. Using regression models and PCA we determined which morphological features contribute to the variation the most. Thus, we found to what extent locomotor mode and phylogenetic demands impact anuran skeletal anatomy.

ESD is the strongest driver of morphological variation[4] and it changed the most when modern frogs emerged. Larger sacral diapophyses expansion associates with Basal frogs, which include walkers-hoppers/swimmers[3,4], so we suggest that ESD serves as an attachment point for muscles involved in walking and swimming. Modern frogs that acquired a new jumping mode have decreased ESD. This finding echoes the idea that jumping is crucial in anuran evolutionary success[2] and is supported by the emergence of arboreal jumpers only in later phylogenetic groups[5,6].

Length of calcaneus/femur/tibiofibular correlated with each other and together contributed to the hindlimb length, greater values of which are associated with jumper modes. This finding suggests that longer hindlimbs improve jumping performance, whereas frogs with shorter hindlimbs tend to perform better at burrowing/walking/hopping[7,8]. This is possible due to elevated take-off velocity by stronger propulsive force via increasing the distance through which it acts[9]. Recent studies suggest that elongation of the hindlimbs is connected to the high locomotion demand via an increment of bone formation[10].

In addition, our results show that the most diverse group in terms of anatomical structure is Hyloidea, also known as tree frogs, suggesting ESD/hindlimb weak conservation. Consequently, such anatomical variation enables specific adaptation to new microhabitats/behaviours[1]. This way, we suggest that long hindlimbs may improve climbing ability, as well as larger ESD allowing fore-aft translation of the iliac shafts during climbing[11].

In conclusion, our work complies with previous studies and illustrates that despite general trends between associated movement modes, we cannot predict locomotor function or phylogenetic position using morphology alone. Additionally, the diversity of habitats and interactions between clade and locomotion complicate their prediction made using skeletal anatomy.

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