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PREDICTING LIZARD GENDER: SEXUAL DIMORPHISM IN *CALOTES ROUXII* (REPTILIA: AGAMIDAE) FROM AGUMBE, KARNATAKA, INDIA

RACHAKONDA SREEKAR[1,3]**, *KATYA SAINI***[1]**, *SHYAM N. RAO***[1,2] **AND *CHETANA B. PURUSHOTHAM***[1]

[1]*Agumbe Rainforest Research Station, Agumbe, Karnataka, India-57741*
[2]*Colorado State University, 109 Wagar Building, Campus delivery 1474, Fort Collins, Colorado 80523, USA*
[3]*Corresponding author e-mail: sreekar1988@gmail.com*

Abstract.**—Roux's Forest Lizard, *Calotes rouxii* (Reptilia: Agamidae), does not exhibit distinct dimorphism characters outside the breeding season. Ornamentation and the swelling around the cloaca in males are the primary characters in determining sex and detectable only during the breeding season. We used univariate and multivariate analyses to determine if other morphological characters could be used to determine the sex of an adult *Calotes rouxii* outside the breeding season. We analyzed seven morphological features of 33 adult lizards from an *Areca catechu* plantation in Agumbe, Karnataka, India. Male snout-vent length (SVL) exceeded that of females and male head size (head length and head width) was greater than that of females with the same SVL. Males exhibited greater tail length and tail width, and females exhibited greater trunk size (trunk length and body width). All seven characters were used to correctly classify males and females with 100% accuracy. However, tail length and tail width were found to be significant morphological characters with very little overlap in values that could help in predicting the sex 96% of the time.**

Key Words.—Agamidae; *Calotes rouxii;* fecundity selection; morphology; sexual selection; size dimorphism

INTRODUCTION

In some lizards, though the sexes look alike outside the breeding season, male and female lizards can differ in many traits including body shape and size. Within sexes, body shape and size are important life-history traits that influence physiology, behavior, ecology and reproductive success (Shine 1989; Cox et al. 2003). In the majority of species males are larger than females, although female-skewed sexual size dimorphism (SSD) occurs in nearly every family (Cox et al. 2003). Mechanisms such as sexual selection, fecundity selection and ecological divergence offer alternative explanations for SSD (Aghili et al. 2010).

Calotes rouxii Duméril and Bibron, 1837 is a medium-sized diurnal agamid found in Tamil Nadu, Kerala, Karnataka, Goa, Maharashtra, Andhra Pradesh, Madhya Pradesh and Gujarat in India (Smith 1935; Sharma 2002; Vyas 2004; Chandra and Gajbe 2005; Srinivasulu and Das 2008). The body coloration of the two sexes of this little known agamid varies largely with the breeding cycle. In the breeding season, between April and September (Daniel 2002; Das 2002), adult males develop a brilliant red head and crest with black coloration dorso-laterally and ventrally (Fig. 1*a*); adult females develop a slaty-black coloration with reddish orange throat. Outside the breeding season both sexes look alike (olive brown; Fig. 1*b* and *c*).

The sex of *Calotes rouxii* can be readily determined during the breeding season when the area around the cloaca swells in adult males and the presence of eggs

FIGURE 1. *a:* Adult male *Calotes rouxii* in the breeding season. *b* and *c;* Adult male *Calotes rouxii* in the post-breeding season (Photographed by Rachakonda Sreekar [a], Chetana B. Purushotham [b], and Sumaithangi R. Ganesh [c]).

TABLE 1. Allometric relationships of body characters to snout-vent length (SVL) for male (M) and female (F) adult *Calotes rouxii* from Agumbe, Karnataka, India. *$p < 0.001$.

Measurement	Sex	Mean ± SD (range)	df	F
Tail Length (TL)	M	183.88 ± 9.13 (165–196)	3,25	37.70*
	F	156.90 ± 6.02 (150–168)		
Tail Width (TW)	M	7.25 ± 1.22 (5.0–9.6)	3,27	20.88*
	F	4.41 ± 0.83 (3.3–6.5)		
Trunk Length (AG)	M	38.85 ± 3.64 (32.6–44.7)	3,29	6.74
	F	41.89 ± 2.26 (37.0–45.8)		
Body Width (BW)	M	10.23 ± 2.34 (6.5–15.1)	3,29	3.88
	F	12.91 ± 2.86 (7.9–20.2)		
Head Length (HL)	M	21.94 ± 1.86 (19.6–25.7)	3,29	9.81*
	F	18.54 ± 1.83 (13.0–21.0)		
Head Width (HW)	M	13.21 ± 1.12 (11.4–16.0)	3,29	9.98*
	F	11.76 ± 1.02 (9.8–13.0)		

is obvious in adult females. However, no notable differences in these features or body coloration occur between the sexes after the breeding season, thus, morphometric variations may prove to be useful characters in sexing this species. Ultrasound imaging could be useful in determining sex, but it is impractical to use in the field (Morris et al. 1996; Makowsky et al. 2010).

Though the sexes in lizards often differ in body shape (Andersson 1994), it has not been determined if such differences can be used to predict sex. We considered two primary dimorphic traits that involve SSD to evaluate the success of correctly identifying the sex: (1) Large head size in males, which confers an advantage in male-male rivalry (intrasexual selection hypothesis) and (2) Larger interlimb lengths in females, which confers fecundity advantage (fecundity selection hypothesis). We tested these predictions by measuring the ways in which morphological variation is useful for identification of sex.

METHODS AND MATERIALS

We caught adult *Calotes rouxii* by hand or with a noose during the breeding season in an *Areca catechu* plantation in the Agumbe Rainforest Research Station, Agumbe, Karnataka, India (13˚ 31’ N, 75˚ 05’ E; 557 m above sea level). Thereafter, we measured seven characters using callipers accurate to 0.1mm. We did not include individuals < 63 mm in snout-vent length (SVL) in the study. For each individual, we determined sex by coloration and the presence of a cloacal bulge, and all measurements were taken using a slide vernier calliper reported to the nearest 0.1 mm: SVL, tail length (TL), maximum tail width (TW), head length (HL, from posterior axis of snout to the tip of the jaw), head width (HW, at the widest point of head), maximum body width (BW) and trunk length (AG, axilla to groin distance).

We $\log_{10}$ transformed all numerical variables prior to analysis to meet assumptions of normality and homeoscedasticity. We evaluated differences in body shape between males and females of *C. rouxii* using an analysis of covariance (ANCOVA) for each character, with $\log_{10}$SVL as the covariate and sex as the factor. We used the Welch two sample t-test to evaluate sexual differences in body size (SVL). Differences in body shape were expressed using the sexual dimorphism index (SDI) after making slight changes to Lovich-Gibbons two step ratio (Lovich and Gibbons 1992):

$$SDI = \frac{\dfrac{\text{Mean character size of larger sex}}{\text{Mean SVL size of larger sex}}}{\dfrac{\text{Mean character size of smaller sex}}{\text{Mean SVL size of smaller sex}}} - 1$$

Negative values by convention occur when male morphometric characters are larger than female characters and positive when female morphometric characters are larger.

We used Principal Component Analysis (PCA) to explore patterns of sexual dimorphism in the multivariate level and we used Linear Discriminant Analysis (LDA) to calculate the probability of correctly classifying each lizard to its predetermined sex. All measurements are presented as mean ± standard deviation (SD), and we analyzed all data using the programming and statistical language R 2.11.1 (R Development Core team 2008). For all tests, $\alpha = 0.05$

RESULTS

In our limited sample, the adult males of *C. rouxii* averaged 8% longer than adult females (SVL, males = 75.90 ± 3.06 mm, range = 70.80–82.40 mm, n = 18; females = 70.01 ± 3.53 mm, range = 63.85–74.90 mm, n=15; $t = 5.06$, df = 28, $P < 0.001$). In this study the longest specimen was a male with a SVL of 82.40 mm, 5.4 mm larger than the largest confirmed record of SVL, and we also recorded a TL of 196 mm, 26 mm larger than the largest confirmed record of TL (Smith 1935; Sharma 2002). Males of this species also had larger head size (HL and HW), TL and TW when compared to the females of the same size (Table 1).

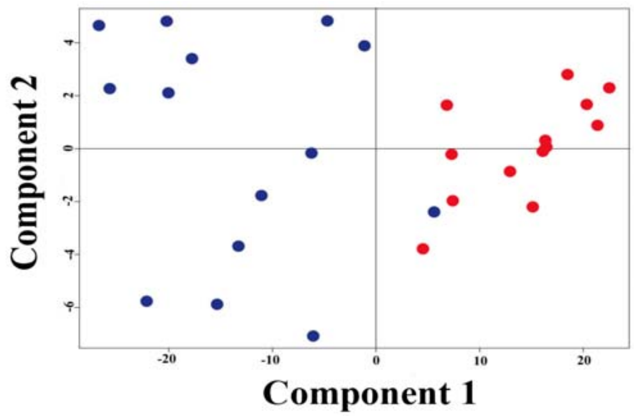

FIGURE 2. Scatter plot using principal component analysis (PCA) showing the degree of sexual dimorphism in male (blue) and female (red) *Calotes rouxii***.**

Although trunk size (AG and BW) did not show great differences between sexes, females exhibited larger AG (F = 6.74, df = 3,29, P = 0.001) and BW (F = 3.88, df = 3,29, P = 0.018) when compared to males of the same size (Table 1).

Cross validation results of LDA predicted sex correctly 100% of the time for males and females. PCA also showed very little overlap between the sexes on component 1 and 2 (Fig. 2 and Table 2). However, two characters were found to differ significantly between the sexes, with little overlap in the recorded values: TL and TW (Fig. 3*a* and *b*). Using these two morphological characters LDA was able to predict sex 93% of the time for adult males and 100% of time for adult females.

DISCUSSION

We recorded dimorphisms in body shape between sexes in *Calotes rouxii*, and, as hypothesized, males had significantly larger heads (HL and HW) and females had significantly greater trunk size (AG and BW). This may be attributed to both sexual and fecundity selection. All these characters, including TL and TW attribute sex of *C. rouxii* outside their breeding season in a quantitative framework. However, one should consider TL and TW first, due to very little overlap in values between sexes (Table 1). Males had longer and thicker tails as compared to females of the same SVL.

We observed male-male rivalry in *C. rouxii* during field work. Territorial males produce an array of displays toward their conspecifics, which include push-ups, head-bobs and extension of the gular region (*pers. obs.*). If the conspecific male continued to approach, the display often ended in combat. We observed several instances of male combat in late April and early May usually in the vicinity of one or two females perched on the same or adjacent tree. Male-male competition is often considered a product of sexual selection, which is responsible for the evolution of male-skewed SSD (Andersson 1994). However, Lappin and Husak (2005) revealed that male reproductive success is not correlated with the body size, but rather with the bite force that indicates fitness in male-male rivalry. Studies that have related male-male rivalry with male-skewed head-size dimorphism (Carothers 1984; Vitt and Cooper 1985; LeBas 2001; Kratochvı΄l and Frynta 2002; Huyghe et al. 2005) showed a positive relationship between bite force and head size (Herrel et al. 2001). Thus, it appears that it is not body size but head size that determines fitness in male lizards.

Evolutionary increases in clutch or litter sizes are associated with a shift towards larger trunk-size (AG and BW) in females to provide space for more

TABLE 2. Factor loadings on the first two principal component (PC) axes on the six variables used to distinguish male and female adult *Calotes rouxii* from Agumbe, Karnataka, India.

Variable	PC1	PC2
Tail length (TL)	-0.958	-0.166
Tail width (TW)	-0.100	0.156
Trunk length (AG)	0.112	-0.746
Body width (BW)	0.046	0.472
Head length (HL)	-0.120	0.363
Head width (HW)	-0.060	0.121
% of cumulative variance	90.8	94.7

TABLE 3. Comparisons of Sexual Dimorphism Index scores using different measures of body shape for adult *Calotes rouxii* from Agumbe, Karnataka, India.

Measurement	Male	Female	SDI
Tail length (TL)	183.88	156.9	-0.08
Tail width (TW)	7.25	4.41	-0.51
Trunk length (AG)	38.85	41.89	0.17
Body width (BW)	10.23	12.91	0.37
Head length (HL)	21.94	18.54	-0.09
Head width (HW)	13.21	11.76	-0.03

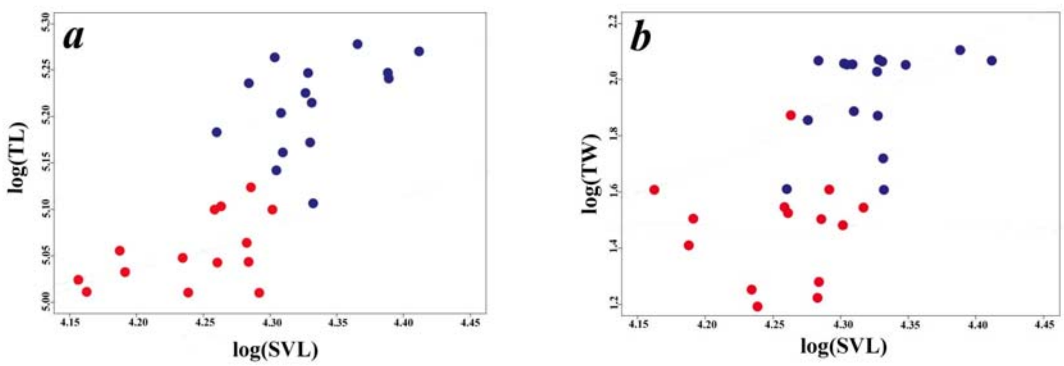

FIGURE 3. Scatter plots showing the relationship between *a*, snout-vent length (SVL) and tail length (TL) and *b*, snout-vent length (SVL) and tail width (TW) in male (blue) and female (red) *Calotes rouxii***.**

eggs (Olsson et al. 2002). Females of *C. rouxii* have developed greater trunk size in comparison to males of the same SVL (Table 3 and Fig. 4). Studies show that unlike males, female abdomen exhibits positive allometric growth (Brana 1996; Olsson et al. 2002).

So, selection for increased fecundity could favor large female trunk size. On the other hand shorter tails in female lizards could be the consequence of fecundity selection for increased reproductive capacity (King 1989) and greater tail width in males accommodate the paired hemipenes and protective muscles (Radder et al. 2006).

In conclusion, we have determined a practical method to ascertain sex of adult *Calotes rouxii* throughout the year and across seasons. This is useful not only in morphometric studies but also forms an imperative aspect of numerous ecological and ethological studies. Considering that the species is commonly found along the forest edges along the Western Ghats of India (Venugopal 2010), little is known about the ecology and behavior of *C. rouxii*. Attempting to study agamids without ascertaining sexes confidently could lead to misleading results. This technique will aid long-term monitoring of population trends especially across seasons and varying climatic conditions, which is critical in assessing the status of breeding populations, adult sex ratios and patterns in turnover. Needless to say, the importance of such monitoring and its influence on their conservation management will only heighten in light of the gradual yet unprecedented threats to their microclimate and habitat.

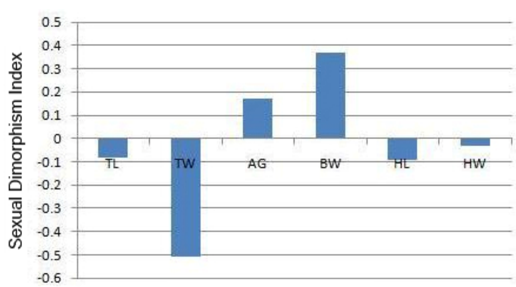

FIGURE 4. Distribution of sexual dimorphism index (SDI) in major characters of *Calotes rouxii*: TL = tail length, TW = tail width, AG = trunk length, BW = body width, HL = head length, and HW = head width. Negative SDI indicates a male dimorphic trait and a positive SDI a female dimorphic trait.

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RACHAKONDA SREEKAR received his Bachelor of Technology in Mechanical Engineering from the Jawaharlal Nehru Technological University, located in Hyderabad, India. After that he went on to work with Dr. Suhel Quader at the National Centre for Biological Sciences, and later joined the Agumbe Rainforest Research Station as a Research Associate. His interests lie in the ecological and evolutionary connections between carbon pools, land-use history, population biology, and animal behavior. (Photographed by Imesh N. Bandara)

SHYAM N. RAO is an undergraduate student from Colorado State University, Fort Collins, Colorado, USA. He worked with the Agumbe Rainforest Research Station for a short period estimating the population of *Calotes rouxii* in an *Areca catechu* plantation. His interests lie in herpetology and natural history. (Photographed by anonymous)

KATYA SAINI received her Bachelor of Science in Environmental Science from the Bangalore University, India, after which she worked at the Agumbe Rainforest Research Station. Her interest in studying the ecology of endemic species of the tropical forests grew with research on the Western Ghats Gliding Lizard (*Draco dussumieri*). She currently works with the Nature Conservation Foundation in Mysore. Her interests include behavioural ecology and understanding the effects of human impact and climate change on tropical environments. (Photographed by Madhushree)

CHETANA B. PURUSHOTHAM recieved her Bachelors in Environmental Science from the Bangalore University in India, she went on to work at the Agumbe Rainforest Research Station. Here she works as the Communication Officer. She is currently initiating long-term avian and phenology monitoring programs that can be carried out by students and volunteers who visit the research station. Her main interests lie in the ecology of amphibian and avian communities in the evergreen forests of India. (Photographed by Damini Kaushik)