

Morphology, ecology, and behaviour of *Hylarana intermedia*, a Western Ghats frog

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Abstract. Despite being common in the Western Ghats-Sri Lanka biodiversity hotspot, Golden-backed frogs (*Hylarana*, Ranidae) remain poorly studied. In this paper, we present some preliminary data on the morphology, behaviour, and habitat use of *Hylarana intermedia*, a member of the *Hylarana aurantiaca* species group. We find evidence for female biased size dimorphism, as well as potential shape differences between the sexes in this species. Additionally, we investigate the relationships between traits that may contribute to male conspicuousness (call rates, dorsal coloration, and body size) in two breeding habitats, a paddy field and a trench. Our results suggest both size-dependent and environment-dependent variation in call rate and colour in this species. Specifically, we find evidence against the adoption of sneaker mating strategies by small males in *H. intermedia*, and instead find variation both within and between populations in traits contributing to male conspicuousness. We conclude by proposing future directions for research on this common frog species.

Keywords. Calling rate; colour; *Hylarana intermedia*; microhabitat; size; Western Ghats.

INTRODUCTION

Golden-backed frogs (*Hylarana*, Ranidae) are widespread in the biodiversity hotspot of the Western Ghats and Sri Lanka, and are commonly encountered in a wide variety of habitats in this region (Daniel, 2002; Purushotham and Tapley, 2011). A recent phylogenetic study has revealed extensive genetic variation among South Asian Golden-backed frogs, prompting the description of seven new species in the genus *Hylarana* (Biju et al., 2014). However, the ecology and behaviour of this diverse group of frogs are almost completely unknown.

Hylarana intermedia is a member of the *Hylarana aurantiaca* species group, and is found north of the Palghat Gap in the Western Ghats of India (Biju et al., 2014). A nocturnal frog, *H. intermedia* begins to breed with the

arrival of the south-west monsoon, from June to August. Large aggregations of calling males are found in pools of water and the surrounding vegetation (Purushotham and Tapley, 2011). In this paper, we present some preliminary data on the morphology, behaviour, and habitat use of *H. intermedia*, and propose future directions for research on this common frog species.

Sexual size dimorphism is widespread in frogs. Females are larger than males in almost 90% of all anuran amphibians (Shine, 1979). However, the presence of male-biased sexual size dimorphism in a frog species could reveal interesting biology, such as the occurrence of physical combat as part of male-male competition (Shine, 1979) or an atypical age-structure of the breeding population (Monnet and Cherry, 2002). In a first step towards characterizing the morphology of this species, we inves-

tigate whether *H. intermedia* conforms to the pattern of female-biased sexual size dimorphism seen in most frogs. We also describe patterns of sexual shape dimorphism to identify potential morphological targets of sex-specific selection in this species.

Across animals, variation in body size is often related to variation in mating strategy (Andersson, 1994). In frogs, a male's mating success is primarily determined by his conspicuousness to females. Phenotypically, male frogs can be conspicuous through both auditory and visual means. Auditory conspicuousness is achieved through male calling behaviour, and abundant evidence indicates that a male frog's vocalization is an important component of his conspicuousness and contributes to his mating success (e.g., Arak, 1988; Passmore et al., 1992; Grafe, 1997; Pröhl, 2003; reviewed in Wells, 2007). Visual conspicuousness in frogs can be influenced by size, coloration, and the performance of visual signals (Wells, 1980; Haddad and Giaretta, 1999; Grafe and Wanger, 2007), even in nocturnal frogs that are active in conditions of low light (e.g., Sheldon et al., 2003; Vasquez and Pfennig, 2007; Gomez et al., 2009).

A common mating strategy for smaller male frogs is to remain inconspicuous and behave as a satellite near a large, calling male, only to sneak matings with females that are attracted to the conspicuous male (Emlen, 1976; Blackwell and Passmore, 1991). Males of *H. intermedia* vary in body size, call rate, and dorsal coloration (Fig. 1) and we test whether smaller male *H. intermedia* are in fact less conspicuous than larger males by examining the relationships of calling rates and dorsal coloration with body size.

A male's choice of microhabitat provides the environmental context in which his conspicuousness is assessed by both females and predators (e.g. Leal and Fleishmann, 2002; Galeotti et al., 2003; Amézquita and Hödl, 2004). The strength and direction of selection and constraints acting on his phenotype thus depend on microhabitat, and different habitats provide different ranges of available microhabitats for males to choose among. The relationships between conspicuousness traits might therefore differ substantially between habitats (Ohmer et al., 2009). In *H. intermedia*, breeding pools can be of different shapes and sizes, and within each pool, breeding males perch at and call from a variety of locations at different distances from the edge of the pool. We ask if *H. intermedia* differ phenotypically between two breeding sites that differ in their spatial structure: a large, disused paddy field and a narrow trench. We also examine if phenotypic variation in the larger, more varied habitat (the paddy field) is structured by an environmental variable (distance from dry land). We use our results to make predictions about how selection might act on the phenotypic traits determining conspicuousness in different breeding habitats of *H. intermedia*.

MATERIALS AND METHODS

Study site and data collection

This study was conducted during the southwest monsoon period, from 5-19 July 2011, at the Agumbe Rainforest Research Station, Agumbe, Karnataka, India (13°50'N, 075°09'E; 560 m above sea level). We observed frogs in two locations: a large, dis-



Fig. 1. Variation in the dorsal coloration of *Hylarana intermedia* from light (left; colour score of one) to dark (right; colour score of five; see Materials and Methods for details of colour scoring).

used paddy field and a narrow trench in an open forest clearing. We divided each site into non-overlapping sections, and only one section was sampled per night and not subsequently resampled, to minimize repeated observations of the same individuals. Up to 11 individuals were observed and caught per section.

Male frogs, which can be differentiated from females by the presence of vocal sacs, were located either visually or by following their calls. Male frogs were observed, without disturbing them, for 5 minutes, during which we counted the number of vocalizations produced. After the observation, a single observer (AK) scored the colour of the frog on a scale of 1 to 5 as follows: 1 = pale golden, 2 = light with intermediate spots, 3 = intermediate, 4 = intermediate with dark spots, 5 = dark (Fig. 1). The same light source was used to illuminate all frogs to ensure a consistent scoring of colour. While dorsal colour can change within minutes (e.g., on being captured), changes in coloration were not noticed during observations.

In the paddy field habitat, we also measured the shortest distance from the frog's position to dry land. Because the centres of ponds in the habitat had less vegetation than the edges (pers. obs.), the distance to dry land is an approximate indicator of habitat openness.

We then caught the frogs to measure the following variables: snout-vent length (SVL), mass, head length, head width, trunk length, femur length, tibia length, foot-to-toe length, humerus length, and radius-to-finger length. All limb measurements were made on only the left side of the frog. After all frogs were measured, they were released into the section of the site in which they were captured. Morphological measurements were also made on additional male and female frogs captured at both sites as well as near trails between the two sites.

Statistical analyses

A Wilcoxon Rank-Sum test was conducted on snout-vent length (SVL) to compare body size between males and females. To compare the morphology of male and female frogs, a principal components analysis was conducted on all morphological variables, and PC1 and PC2 were compared between males and females using Wilcoxon Rank-Sum tests.

To examine if male frogs in the paddy field and trench habitats differed in the traits contributing to conspicuousness, we compared both SVL and calling rates between frogs from the two habitats using Wilcoxon Rank-Sum tests. Dorsal colour scores were compared between the two habitats using a Chi-squared test.

Analysing data separately for the paddy field and trench habitats, we tested whether male body size (SVL) was correlated with calling rate using Spearman Rank Correlations. We tested if frogs of different colours differed in size using Jonckheere-Terpstra tests, which are similar to Kruskal-Wallis tests but apply to populations that are ordered by a factor (in this case, colour score). Again, we considered data from the trench and paddy field habitats separately. Finally, for the paddy field site, we used Spearman Rank Correlations to test if the distance to dry land was correlated with SVL or calling rate, and a Jonckheere-Terpstra test to examine if distance to dry land varied with colour.

We conducted all analyses in R v 3.2.2 (R Core Team, 2015), and used the *clinfun* package (Seshan, 2015) to perform the Jonckheere-Terpstra tests, using 5000 permutations to estimate p-values.

RESULTS

We observed and caught a total of 68 male frogs (38 individuals in the paddy field and 30 individuals in the trench). Additionally, 43 males and seven females were caught for morphological measurements only.

Male and female *H. intermedia* differed significantly in SVL: males were smaller in size than females (mean \pm standard deviation in SVL for males: 40.9 \pm 2.3mm; females: 52.1 \pm 2.4mm; $W_{7,111} = 777$; $P < 0.001$). The first principal component axis (PC1) from the PCA on morphological variables yielded a composite variable reflecting overall body size (i.e., positive loadings of similar magnitudes on all variables; Table 1), and PC1 also differed significantly between the sexes ($W_{7,111} = 777$; $P < 0.001$; Fig. 2).

The second principal component axis loaded positively on humerus length and trunk length, and negatively on head length and width. There was a trend towards higher PC2 in females than males ($W_{7,111} = 552$; $P = 0.06$; Fig. 2), suggesting a pattern of relatively longer trunks and humeri in females and relatively large heads in males.

Male frogs' SVL did not differ between the paddy field and trench habitats (mean \pm standard deviation; paddy field: 41.2 \pm 2.6 mm; trench: 41.4 \pm 3.9 mm; $W_{44,71} = 1590.5$, $P = 0.87$). Calling rates also did not differ between male frogs from the two habitats (mean \pm standard deviation; paddy field: 21.9 \pm 20.7; trench: 20.2 \pm 15.2; $W_{38,30} = 551$, $P = 0.82$). However, frogs were lighter

Table 1. Loadings on the first and second principal component axes (PC1 and PC2) from a principal components analysis on morphological variables from males and females. Bold numbers indicate loadings with a magnitude greater than 0.25.

	PC1	PC2
% Variance Explained	76%	6%
SVL	0.34	-0.14
Head Length	0.39	-0.64
Head Width	0.32	-0.40
Femur	0.32	0.18
Tibia	0.34	0.12
Foot	0.33	0.13
Humerus	0.27	0.42
Ulna+Hand	0.33	0.06
Trunk Length	0.28	0.39
Mass	0.34	-0.06

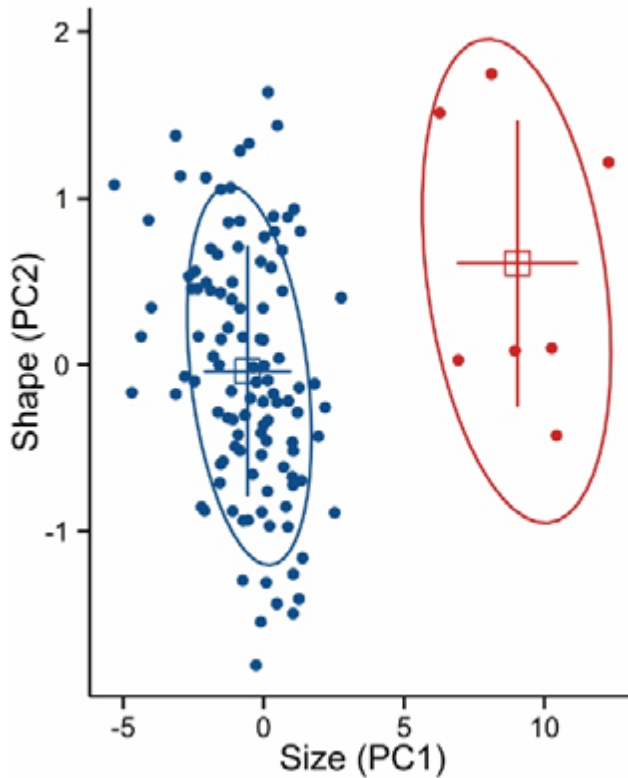


Fig. 2. Plot of the first vs. the second principal component axes (PC1 vs. PC2) from a principal component analysis on the morphology of *Hylarana intermedia*. Blue and red circles represent male and female frogs respectively. Squares represent means of PC1 and PC2 by sex, bars indicate standard error, and ellipses indicate 95% confidence intervals.

in colour in the paddy field than the trench (median colour score; paddy field = 3; trench: 4; $\chi_4 = 17.1$, $P = 0.002$).

Frogs of different colour did not differ in SVL, within either the trench or paddy field habitat (paddy field: $JT_{5, 38} = 262$, $P = 0.73$; trench: $JT_{5, 30} = 177.5$, $P = 0.31$). Calling rate did not vary with SVL (Spearman's Rho = 0.08; $S_{38} = 8427$, $P = 0.64$) in the paddy field. However, in the trench habitat, smaller frogs called more often than larger frogs (Spearman's Rho = -0.47; $S_{30} = 6587$, $P = 0.01$; Fig. 3).

In the paddy field, frogs further from dry land called more often (Spearman's Rho = 0.36; $S_{38} = 5820$, $P = 0.025$), and were darker in colour ($JT_{5, 38} = 330.5$, $P = 0.03$) than frogs closer to dry land. However, SVL did not vary with distance to dry land (Spearman's Rho = -0.08; $S = 9861$, $P = 0.64$).

DISCUSSION

To our knowledge, this paper is among the first on the behaviour and ecology of a South Asian species in

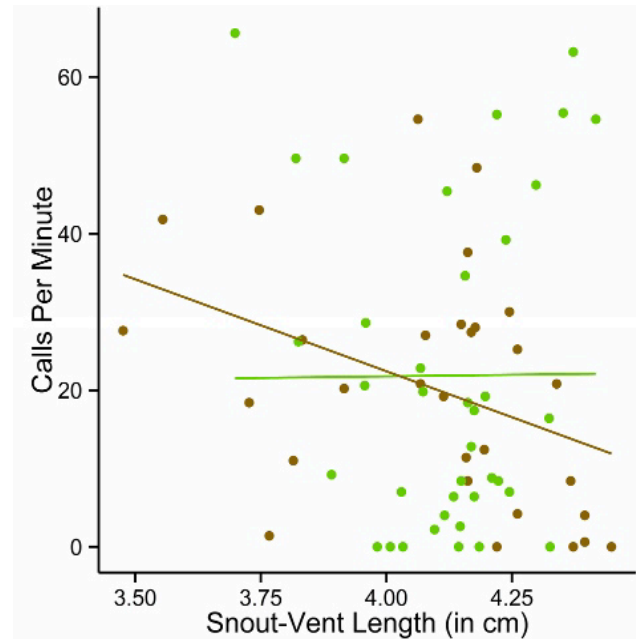


Fig. 3. Correlations between call rate and snout-vent length (SVL) of *Hylarana intermedia* in trench (brown circles) and paddy field (green circles) respectively. Lines represent linear regression lines, and are provided for visualization purposes only.

the genus *Hylarana*. Our conclusions lend insight into the phenotypic variation of these frogs and yield testable hypotheses that can be addressed by measuring the strength of sexual selection and viability selection on phenotypic traits of male *H. intermedia* in different habitats.

The pattern of sexual size dimorphism in *H. intermedia* is similar to a majority of anuran amphibians: males are smaller than females (Shine, 1979). A trend towards shape differences between the sexes could result from male and female reproductive success being limited by mate attraction and fecundity respectively. Relatively larger heads in males might function to accommodate the vocal sac, which plays a crucial role in mate attraction. Relatively longer trunks in females might be necessary for females to accommodate eggs. Though little research been conducted on shape dimorphism in frogs, the patterns seen in *H. intermedia* seem to concur at least partially with other studies. For example, Herrel et al. (2012) found that male *Xenopus tropicalis* have relatively longer heads than females, and Schauble (2004) found that male *Limnodynastes peronii* have relatively wider heads than females. Further comparisons between males and females, with a larger sample size for females, can confirm these patterns in *H. intermedia*.

Though a satellite mating strategy is often adopted by smaller frogs (Emlen, 1976; Blackwell and Passmore, 1991), smaller *H. intermedia* males do not appear to

adopt this tactic. Smaller males neither call less frequently nor are darker in colour than larger male *H. intermedia* in either the paddy field or the trench habitat. In fact, smaller frogs called more frequently than larger frogs in the trench. We also found that frogs differed in colour between the two sampled breeding sites. Interpopulation differences not only in traits contributing to conspicuousness but also in relationships between these traits indicate that male *H. intermedia* do not adopt consistent alternative reproductive strategies.

In the paddy field, frogs far from dry land are both darker and call more frequently than frogs close to dry land. This result is consistent with at least two non-mutually-exclusive predictions based on sexual selection and viability selection, each of which is testable. First, the attention of females, who approach breeding ponds from land, may be attracted differently depending on their distance to the male frog. Different types of signals attenuate differently with increasing distance between the signaller and receiver (Endler, 1992; Shine, 2005; Bradbury and Vehrencamp, 2011). We predict that, in *H. intermedia*, auditory conspicuousness is more effective in attracting females over long distances and visual conspicuousness more effective over short distances. This would lead to frogs in the centre of the paddy field, at large distances from the females found on dry land, to rely more on auditory conspicuousness and therefore call frequently. Second, we predict that predation pressure from visual predators is higher in the more open centre of breeding ponds than at the more vegetated edges, leading frogs to adopt darker, less conspicuous, coloration in the centre.

There is growing evidence that multimodal signals combining visual and auditory cues are important for male frogs attracting females, even in nocturnal species (e.g., Sheldon et al., 2003; Vasquez and Pfennig, 2007; Gomez et al., 2009). Future studies on *H. intermedia* can begin by assessing whether dorsal colour is an indicator of some aspect of male condition or quality (Sheldon et al., 2003; Vasquez and Pfennig, 2007), and whether females are preferentially attracted to lighter males relative to darker males, through either positive phototaxis or active choice (Jaeger and Hailman, 1971). However, signal traits do not evolve in isolation but coevolve in an environmental context that can bias signalling through sensory drive (Endler, 1992). Our results indicate that different phenotypic traits contributing to *H. intermedia* conspicuousness, as well as the correlations among them, can be spatially structured and habitat-dependent. We therefore caution future researchers to account for environmental variation when attempting to understand sexual selection and viability selection on the phenotypic traits contributing to conspicuousness in male *H. intermedia*. The environmental variation to be consid-

ered includes not only spatial components such as those investigated here but also temporally varying factors such as auditory interference from calls of sympatric frogs (e.g. *Zakerana* sp.) and the monsoon rain.

Rapid habitat loss in the Western Ghats (Anand et al., 2010), little previous research on even common taxa in the region, and the continued discovery of novel species imply that ecologists and natural historians are fighting a battle against time to document the biology of species in one of the world's biodiversity hotspots (Aravind et al., 2007). Our aim in this paper is not to reach definite conclusions about the reproductive biology of *H. intermedia*, but instead to provide a framework and preliminary data on which future investigations can be based. We hope that this study stimulates further research on the behavioural ecology of *H. intermedia*, yielding interesting insights into how phenotypic variation is maintained both within and between populations of this common Western Ghats frog.

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Since our study area did not fall within a Protected Area, and *Hylarana intermedia* is not mentioned as a scheduled species in the Wildlife (Protection) Act (1972) and subsequent amendments, we did not require government permits to conduct our study. Our study did not entail collection of individuals and we made all efforts to minimize discomfort to the animal by ensuring careful handling and quick release of each captured individual back into the habitat they were first observed in. We thank Neeti Mahesh, Gautam Ramachandra, Shyam Rao, Katya Saini, Naren Srinivasan, Quentin Fournier, and Francis Crawley for help with data collection. Sidharth Rao helped to formulate the project and sampling methods. Logistical support was provided by the Agumbe Rainforest Research Station, Agumbe, Karnataka. Yoel Stuart, Jonathan Losos, Katie Boronow, and two anonymous reviewers made suggestions that improved the quality of the manuscript.

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