Table 1. Precision on protein estimation of groundnut samples and relationship between the results obtained by NIRS and Skalar methods

Parameter	NIRS-protein (%)	Skalar-protein (%)	Correlation coefficient (r)	p-value
Range	15.30–30.81	16.30–32.48	0.94	<0.00001
Mean	25.55	26.24		
SD	2.69	2.76		
CV (%)	10.55	10.54		

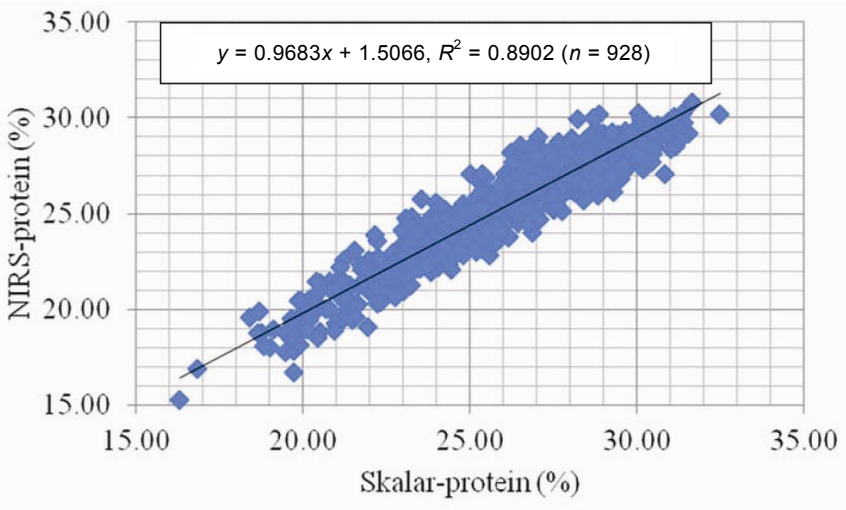

Figure 1. Relationship between Skalar-protein and NIRS-protein in 928 groundnut samples.

comparable, as judged by statistical analysis. The range, mean, standard deviation and CV(%) data are presented in Table 1. There was a positive correlation between protein data determined by the NIRS method and the Skalar colorimetric method; the combined correlation coefficient (r) for the 928 groundnut samples was 0.94.

The relationship between NIRS-protein and Skalar-protein was significant ($P < 0.00001$) and positively correlated ($R^2 = 0.8902$) for groundnut samples (Figure 1); and the relationship represented by the following regression equation

$$\text{NIRS-protein (\%)} = 1.5066 + 0.9683 \times \text{Skalar-protein (\%)},$$

$$R^2 = 0.8902 \ (n = 928).$$

The results on the analysis of protein content in groundnut samples by NIRS method suggest that it could be a useful tool to analyse proteins in groundnuts.

NIRS is an ideal method for quantitative estimation of protein in groundnut. The method based on NIRS provides an alternative cost-effective analytical tool for simple, accurate and rapid determination of protein with small sample size compared to the standard analytical procedures.

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Advertisement calls of Amboli leaping frog *Indirana chiravasi* (Anura: Ranixalidae) from northern Western Ghats, India

The anuran amphibians are one of the most actively vocalizing animal groups. Acoustic characteristics of anurans are species-specific and can be used for their identification, description of new species, understanding phylogenetic relationships among species, resolving cryptic speciation and in the conservation of species[1–3]. Although the Western Ghats of India is rich in anuran diversity, with new species continuously being described, limited information is available on calls of endemic and threatened species. Because call structures could help in designing non-invasive methods for identification

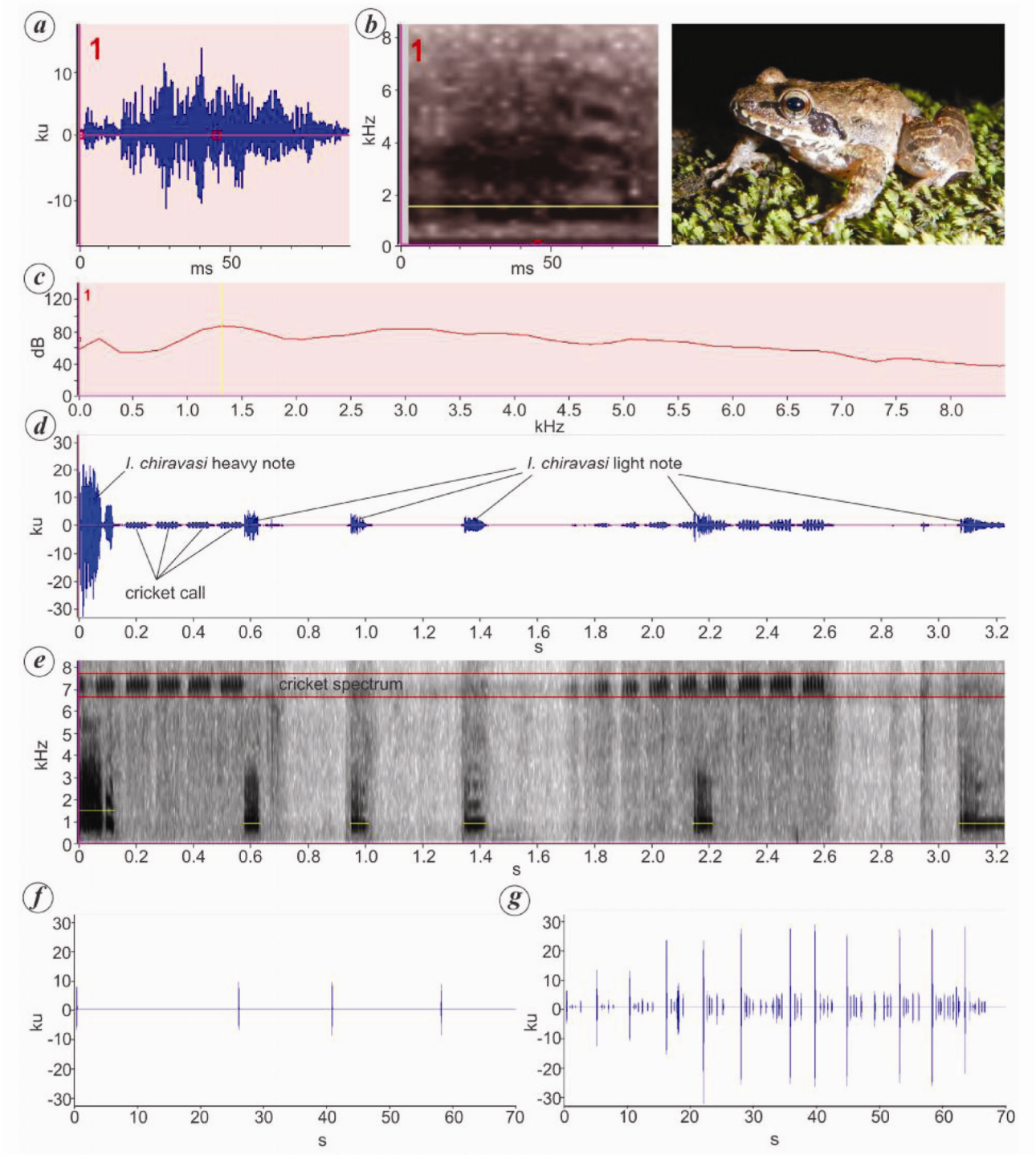

Figure 1. ***a–e***, Oscillogram (***a***); spectrogram (***b***) and selection spectrum (***c***) for call AC1, and oscillogram (***d***) and spectrogram (***e***) for call group 5 of AC2, where yellow line represents the dominant frequency. ***f***, ***g***, Representative call structure for AC1 and AC2 in 70 sec.

of anuran species[4], especially for those endemic groups that are highly threatened and morphologically cryptic[5], studies with detailed call analysis are important.

Genus *Indirana* is endemic to the Western Ghats of India with 12 known species[6], several of which are morphologically cryptic[7,8]. While describing the species *Indirana chiravasi*, Padhye *et al.*[9] provided a video of advertising male from type locality. Further, Gaitonde and Giri[10] provided a brief description of breeding call from type locality. However, analysis for any advertisement call for the species was pending. Here, we provide analysis of two different advertisement calls of *I. chiravasi*, and compare them with calls of other *Indirana* species to know the similarities and differences between their spectral characteristics.

Table 1. Call characteristics for advertisement calls 1 (AC1) and 2 (AC2) from Amboli and Koyna respectively. Range as minimum–maximum is followed by (mean ± standard deviation)

	Amboli	Koyna	
Parameter	AC1	AC2 (high note)	AC2 (low note)
Number of notes	19	12	71
Note rate per minute	2.67	10.83	64.09
Note duration (ms)	0.09–0.13 (0.10 ± 0.01)	0.10–0.13 (0.12 ± 0.01)	0.01–0.40 (0.08 ± 0.05)
Inter-note duration (ms)	14.00–42.40 (23.60 ± 9.60)	3.80–8.30 (5.60 ± 1.30)	0.33–0.82 (0.49 ± 0.13)
Dominant frequency (kHz)	1.13–3.56 (1.74 ± 0.72)	1.13–2.81 (1.55 ± 0.76)	0.75–2.63 (0.98 ± 0.22)
Bandwidth 90% (kHz)	2.06–3.75 (2.80 ± 0.30)	1.88–2.06 (1.92 ± 0.09)	1.88–4.69 (1.26 ± 1.03)
Energy (dB)	102.20–111.00 (107.10 ± 3.00)	100.10–115.70 (112.40 ± 4.90)	68.70–110.90 (93.90 ± 7.10)
Maximum amplitude (ku)	7.60–19.80 (13.30 ± 3.60)	5.40–27.90 (21.60 ± 7.40)	0.30–8.00 (3.10 ± 1.60)

We recorded two types of advertisement calls, AC1 and AC2, at a sampling frequency of 48.0 kHz. AC1 was recorded from the type locality of the species at Amboli (15°57′33″N, 73°59′50″E, 734 m asl, 94% RH and 22.5°C) on 16 August 2015, and AC2 from Koyna (17°23′46″N, 73°44′41″E, 635 m amsl, 89% RH and 23.7°C) on 12 June 2015. Both calls were recorded as .wav format in the wild by keeping the recording device at about 15 cm and using the PCM Recorder Android application on a smartphone, without disturbing the individuals. The analysis of calls was done with the help of Raven Pro 1.4 (ref. 11). We analysed the oscillogram for duration of call and that between the two calls or notes. Further evaluation of each call was done using spectrogram analysis. The spectrogram was analysed using Hamming window, with 3 dB filter bandwidth at 224 Hz, overlap 50% and grid spacing 172 Hz. As the calls had large variations in frequency, we used third quartile frequency, third quartile time, aggregate entropy, average power, 90% bandwidth frequency and its duration, centre frequency, centre time, energy, frequency and time 95%, dominant frequency and maximum power as defined in the Raven Pro 1.4 manual (ref. 12). Although we also recorded advertisement call types AC2 and AC1 from Amboli and Koyna respectively, they were in .avi format and could not be analysed for spectral characteristics. Both calls, AC1 and AC2, are available for download from figshare (http://dx.doi.org/10.6084/m9.figshare.2063460).

Figure 1 shows the call structure for AC1 and AC2, and Table 1 lists the call characteristics. Call AC1 of *I. chiravasi* had 19 single note calls (Figure 1 *e*), with call duration of 0.10 ± 0.01 sec. The average dominant frequency was 1.74 ± 0.72 kHz, with the maximum power ranging from 88.70 to 99.80 dB (94.90 ± 3.20 dB). Average third quartile frequency ranged from 1.90 to 3.60 kHz (2.80 ± 0.40 kHz), with the duration of ≈0.10 sec. The aggregate entropy ranged from 3.50 to 4.10 units (3.70 ± 0.20 units), which suggested that the call was not a pure tone and had varying energy. Average power for each call ranged from 68.6 to 77.2 dB (73.30 ± 3.10 dB). For all 19 calls, 90% bandwidth was between 2.10 and 3.80 kHz (2.80 ± 0.30 kHz), with a duration of 0.10 ± 0.04 sec. Figure 1 *a–c* shows the representative oscillogram, spectrogram and selection spectrum for AC1 respectively.

AC2 had multiple call groups, each call group starting with one heavy note followed by several light notes (3–12 light notes for each call group, Figure 1 *g*). The heavy notes in each call group had maximum amplitude, maximum average power and maximum duration within that call group. Average power of all the heavy notes was 83.9 ± 5.1 dB. The light notes in each call group had a duration of 0.08 ± 0.05 sec (except the third light note in call group-4: 0.40 sec). The average power of all the light notes ranged from 49.60 to 77.20 dB (67.80 ± 6.60 dB). Figure 1 *d* and *e* shows the oscillogram and spectrogram of a call group 5 from AC2 respectively; note the differences between heavy note and light notes. For all the light notes from this call group, the dominant frequency is identical, 0.9 kHz (937.5 Hz).

AC1 of *I. chiravasi* has spectral characteristics similar to those of other species of *Indirana*, when compared with the published data for the calls of *I. beddomii*[13], *I. semipalmata*[14] and *I. gundia*[15]. It has the peculiar feature of single note call repeated after a long interval. The mean dominant frequency of AC1 of *I. chiravasi* is 1.70 (± 0.70) kHz, which is similar to *I. semipalmata* (1.59 kHz), *I. gundia* (1.40 kHz) and *I. beddomii* (0.8–1.8 kHz).

Call AC2 analysed in our study does not match the description of previously analysed calls[13–15] of any of the species of *Indirana*. However, it has higher tempo (higher note rate) than call AC1 (Figure 1 *e* and *f*; Table 1). Further, it has patterned call structure and higher energy expenditure per unit time, compared to call AC1. The average energy input for AC1 is 15 dB/min while that for AC2 is 102 dB/min, which is almost seven times higher. According to Gerhardt and Huber[16], the main driving force behind the evolution of anuran acoustic is sexual selection. According to Wells[17], and Wells and Schwartz[18], many features of anuran calls such as call duration, calling rate, call intensity, call pitch, etc. are

shaped by the mate selection. Therefore, such high average energy input in AC2 is likely to be an indication that it is a breeding call. While low average energy input per unit time in AC1 is likely to be for a function with lower priority, such as territory call.

Interestingly, AC2 matches with the description of breeding call of *I. chiravasi* given by Gaitonde and Giri[10], for which they have assigned functions to this call as associated behaviour such as approach of female leading to amplexus and egg laying. Whereas they have mentioned that the calling males exhibit calls similar to AC1 with associated behaviour like vicinity of another male. However, they have not provided any analysis for the calls. Although we could not assign functions to calls AC1 and AC2 based on our field observations, we suggest that AC1 is a territorial call, whereas AC2 is a breeding call based on the energy expenditure and suggestions made by Gaitonde and Giri[10].

Call analysis for *Indirana* species provided by earlier workers is either qualitative[10] or with limited analysis[13–15], making it difficult to use them for compilations. Nevertheless, superficially, the spectral characteristics of the territory calls of various species of *Indirana* are similar, as also suggested by Kuramoto and Dubois[15], making territory calls of limited value for taxonomy and identification. However, the pattern of breeding calls and energy input may be different for different species. Further studies on the breeding calls of other species of *Indirana* could provide important insight into the ecology and evolution of species belonging to this endemic genus.

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Ground foraging behaviour of Malayan giant squirrel (*Ratufa bicolor*)

Giant squirrels are considered an important component of forested ecosystems, and are advocated as indicators of forest health[1]. The Malayan giant squirrel (MGS; *Ratufa bicolor*), one of the four giant tree squirrels in the Oriental region (the other three being *R. affinis*, *R. indica* and *R. macroura*), is found in the Malayan region, North East India and Myanmar. It is listed as Near Threatened (NT) by IUCN, in Appendix II of CITES and Schedule II of Indian Wildlife (Protection) Act 1972. Some ecological information on the MGS exists from few studies[2,3].

There has been unanimity about the obligate arboreal nature of giant squirrels (genus *Ratufa*) that occupy an ecological niche in the highest levels of primary rainforest. Moore[4] stressed the need of detailed observation and reporting of any ground foraging behaviour of Oriental giant squirrels. Of late, recent squirrel studies in the tropics report some incidents of giant squirrels coming down to the ground across their distributional range[5–13]. We describe here ground