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Bioacoustics, breeding ecology and range of the Kerala warty frog *Minervarya* cf. *keralensis* from north of the Palghat gap, central Western Ghats

AMIT HEGDE & GIRISH KADADEVARU*

Breeding Behaviour and Bioacoustics Lab, Department of Zoology, Karnatak University, Dharwad 580003, India

*Corresponding author e-mail: kadadevarug@gmail.com

ABSTRACT – *Minervarya* cf. *keralensis* has been detected for the first time north of the Palghat gap in the central Western Ghats, a range extension of about 85 km. This species calls and breeds in the post-monsoon period (Jan–March) and exhibits axillary amplexus. Male call characteristics, natural history observations, and distributional complexities are described. Currently, the *Minervarya nilagirica* group that includes *M. keralensis*, show a complex distribution pattern in peninsular India that is worthy of further phylogeographic study.

INTRODUCTION

The genus *Minervarya* includes 31 species that are distributed in South Asia where the majority of species are known to occur in the Western Ghats (Frost, 2022); most of the species are widespread while a few are range restricted (Garg & Biju, 2021). They occupy a wide diversity of habitats including those close to human habitation, agricultural landscapes, open lands, and mud puddles. For several decades this genus was known to be an "unresolved long standing taxonomic enigma" due to its deeply conserved morphological characters and crypticity (Garg & Biju, 2021; Sanchez et al., 2018; Dinesh et al., 2015).

Frogs of the genus *Minervarya* have received more attention from taxonomists than other anurans (Garg & Biju, 2021; Dinesh et al., 2015). Several attempts have been made to classify minevaryans into different groups based on morphological traits, size and phylogenetic analyses (Garg & Biju, 2017; Phuge et al., 2019; Hegde et al., 2020). In peninsular India, the genus *Minervarya* is currently classified into eight groups (Garg & Biju, 2021). The *Minervarya nilagirica* group is endemic to peninsular India and currently contains three species - *M. nilgarica*, *M. kalinga* and *M. keralensis* which all occur in the Western Ghats. This group is known to show complex distributional patterns (Hegde et al., 2020; Garg & Biju, 2021) where *M. keralensis* has been reported from the southern portion of the Western Ghats with a northern limit at Poomala (Biju & Garg 2021).

A previous study (Kuramoto & Joshy, 2001) claimed to have described the advertisement call characteristics of *M.* cf. *keralensis* (*Limnonectes* cf. *keralensis*) from two sites in Karnataka (Talagini and Kudremukh) in the central Western Ghats, but subsequently the subject of their study was revealed to be *M. kudremukhensis* (*Fejervarya kudremukhensis*) (Kuramoto et al., 2007) which is currently synonymised as *M. mysorensis* (Garg & Biju, 2021). Consequently, there are no studies on the breeding behaviour, bioacoustics or natural history of *M. keralensis*. Hence the present study reports the vocal repertoire, breeding ecology and a range extension of *M. keralensis* from central Western Ghats. However, although the morphology of our study species is consistent with *M. keralensis*, we have not undertaken the molecular sequencing that would be needed to substantiate this identification, consequently we refer to *M.* cf. *keralensis* throughout the study.

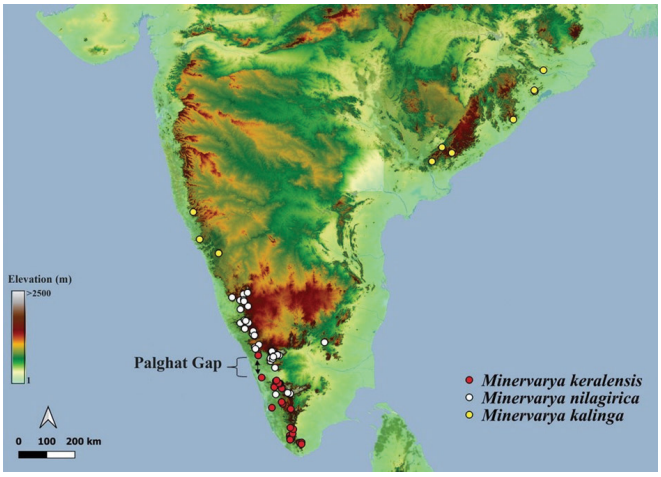

Figure 1. Distribution of the *Minervarya nilagirica* group from peninsular India - *Minervarya kalinga*, *Minervarya nilagirica*, *Minervarya keralensis* (range extension to the north of the Palghat gap)

MATERIALS & METHODS

During this field study, *M.* cf. *keralensis* was observed between January and March at the southern tip of central Western Ghats in Kakandanmpoyii, Nilambur, Kerala 11° 20'36.24" N, 76° 6'16.56" E at an altitude of 700–800 m a.s.l. (Fig. 1). To assess the anuran diversity in different

regions of the central Western Ghats, we have undertaken regular visual encounter and acoustics surveys (Rödel & Ernst, 2004) throughout the year. Frogs were identified to species level by observing morphological characters based on the available taxonomic descriptions and literature (Garg & Biju, 2021; Hegde et al., 2020; Raj et al., 2018). The breeding ecology and the natural history of the species encountered were observed and noted. No specimens were collected during the study.

Call recording and analysis

Calling was observed from January to March, 2019 and calls were recorded on 14 February 2019 in the winter season (post-monsoon) when the air temperature ranged 22.0–22.2 °C and the humidity ranged 60–61 %.

Calls were recorded with a Zoom H1 portable digital audio recorder connected to a unidirectional Rode microphone attached to a Rycote Lyre shock mount (sampling rate of 44100 Hz/16 bit). After locating a calling adult male, the unidirectional microphone was directed at its vocal sac, at a distance of about 30–50 cm. Gain settings of the audio recorder were kept constant throughout the call recording. Calls were recorded choosing randomly selected males without disturbing the frogs. Temperature was measured using 310 CIE (Chung Instrument Electronics) digital thermometer and relative humidity was measured using a Barigo hygrometer (Germany) near the calling individual. Calls with high background noise and overlapping calls were excluded from the analysis. Recorded calls were visualised and randomly analysed in Raven Pro 1.6.2 (K. Lisa Yang, 2022). Temporal measurements were quantified using the waveform/oscillogram window and included - call per minute, call type, call series duration, call duration, call series interval, call interval and the number of calls in a call series. Using the spectrogram, we measured the peak frequency and average entropy. We used a call centred approach and call series parameters were based on previous studies (Köhler et al. 2017; K. Lisa Yang Center for Conservation Bioacoustics, 2022). Calls of six adult males and 20 call series (159 calls) were recorded. These can be played back in BHS video (2023).

Statistical analysis

Call measurements were graphically represented using the R studio (ver. 0.7, R Core Team, 2022) and boxplots were obtained using “ggplot2” (Wickham, 2016).

RESULTS

Morphological diagnosis and range extension

The snout vent length (SVL) of large adult males ranged from 32–44 mm (n=9), the body was robust, head length greater than the head width, the first finger was longer than the second finger, a third subarticular tubercle was present on the fourth toe, with long cylindrical inner metatarsal tubercles, prominent discontinuous folds on the dorsal skin and prominent glandular warts along a dorsal chevron, and prominent reticulations on groin and thigh. The upper lip had horizontal bands, minervaryan lines and rictal glands

Figure 2. Adult male *Minervarya* cf. *keralensis* - **A.** Dorsolateral view, **B.** On calling ground, **C.** Dorsal view

were visible. The digits on the forelimbs were shorter than those on the hind limbs, fingers lacked interdigital webbing, and toes were moderately webbed. Based on the above morphological characteristics, individuals in the population were identified as *M.* cf. *keralensis* (Garg & Biju, 2021; Hegde et al., 2020)(Fig. 2).

The current study is the first report of *M.* cf. *keralensis* from north of the Palghat gap. This species was commonly observed close to the Nilgiris range of the Western Ghats, near the Chaliyar river basin, which is situated on the south-western side of the central Western Ghats (Fig. 1). In this region, human modified landscapes are common within a mosaic of other land-use types including teak plantations and agriculture landscapes (tea, cocoa beans and other crops).

Breeding biology

Breeding season: Calling and breeding activity was observed from January to March; this frog species is a post-monsoon breeder.

Breeding ground/spawning ground: The breeding areas were typical wetlands, paddy fields and marshes, moist microhabitat beside slow-flowing streams and temporary streams which were about to dry out. The adult males called from the banks of the ditches and wetlands, whilst perched on stones, grass patches, gravel and wet soil surfaces that usually had an open or partially open canopy. Spawning grounds were close to the calling areas (Fig. 2).

Secondary sexual characters: Adult males possessed a single subgular vocal sac that was highly distensible, the skin near the vocal sac was lightly pigmented with black, and slightly transparent. While calling, occasional irregular inflation of the vocal sac was observed. Calling adult males had an oval shaped nuptial pad on the first finger. Calling males exhibited yellow colouration near and below the tympanum and below the eye. Adult females were larger than adult males.

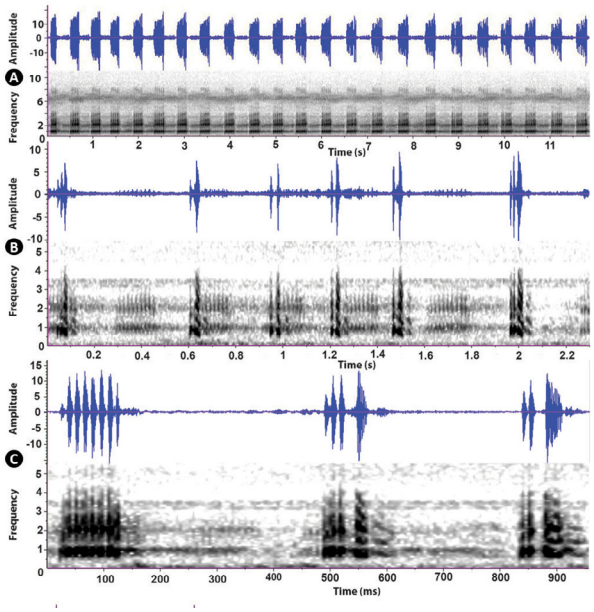

Figure 3. The three advertisement call series of male *Minervarya* cf. *keralensis*, oscillogram showing amplitude (kU) and spectrogram showing frequency (kHz) - **A.** Call series A, **B.** Call series B, and **C.** Call series C (composite call series)

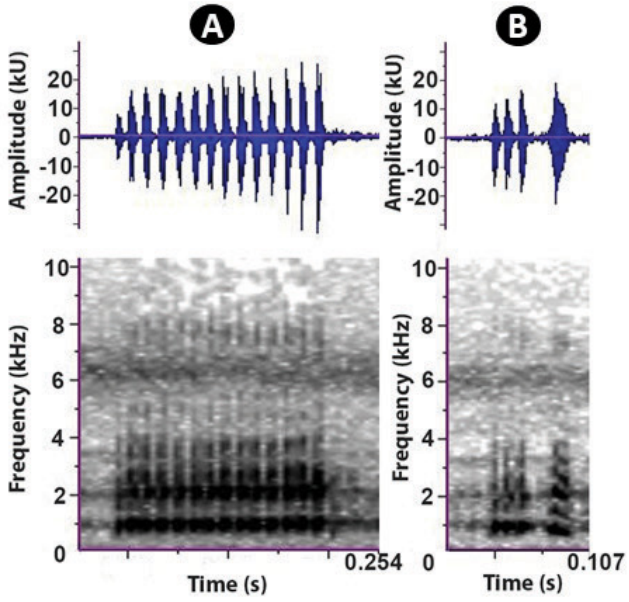

Figure 4. The two types of male advertisement calls (Type A & Type B) of *Minervarya* cf. *keralensis*, oscillogram (blue trace) and spectrogram (grey trace) - **A.** Call type A and **B.** Call type B

Amplexus and egg characteristics: Axillary amplexus was observed (n=8). Multiple small egg clutches were laid in a single spawning. Amplexed pairs moved slowly within the spawning ground and the female laid 3–8 eggs at a time in a clutch (n=8 clutches) and the clutches were laid in more than one place at different distances apart. The eggs were brown in colour and surrounded by a transparent jelly layer. After the eggs were laid, this jelly layer slowly absorbed water from the spawning habitat and the jelly layer swelled gradually.

Bioacoustics

The calls of males were heard from 19:00 h and peak calling time was between 20:00 h and 00:30 h. The majority of amplectant pairs were seen during and after midnight (n=4) and later. After 02:00 h calls slowly subsided.

Call series characters. Calls series contained acoustically multi-call groups that were different and each call comprised several pulsatile groups (Fig. 3). Call series duration/call series length was 3.34 ± 0.32 s and ranged 2.62–3.80 s; Call series interval was 10.71 ± 26.22 s and it ranged from 0.81–117.73 s. The number of calls per call series (n=20) was 8.6 ± 5.91, ranging from 2–25; the average number of call series per minute (n=20) was 16 (range 11–21). The call series contained structurally distinct and different calls, hence we have established three call series categories - A, B, and C.

Call series A: TarrahTarrahTarrah - this was the most frequent call type (n=16), and was most commonly emitted with repetitive and multiple calls, and was relatively long when compared to the call series B and C (Fig. 3A, B & C, Table 1).

Call series B: Kruhrkruhrkruhrrkruhrr - this was emitted before call type A and was more commonly heard in the early hours of the night usually from 18:00 h to 19:30 h and from 04:00 h to 06:00 h on the following day (Fig. 3B; Table 1).

Call series C: TarrahTarrahTarrahkruhr or kruhrkruhrTarrahTarrah - this was a composite series that consists of both type A and type B calls. These were not emitted as frequently as series A calls. They were commonly emitted after the series B calls or at the end of series A calls.

Table 1. The parameters of call series A, B, and C of male *Minervarya* cf. *keralensis*

Call series	Call parameters	Mean ± SD	Range
Series A (n=16)	Call series duration (s)	3.45±3.57	0.51–12.82
	Call series interval (s)	12.88±3.45	0.81–117.73
	No. of calls in a call series	9±6.59	2–25
Series B (n=2)	Call series duration (s)	2.83±1.16	2.01–3.66
	Call series interval (s)	1.39±0.11	1.31–1.74
	No. of calls in a call series	9±3.53	6–11
Series C (composite call) (n=2)	Call series duration (s)	2.52±2.28	0.91–4.14
	Call series interval (s)	4.81±2.33	3.16–6.46
	No. of calls in a call series	6±4.243	3–9

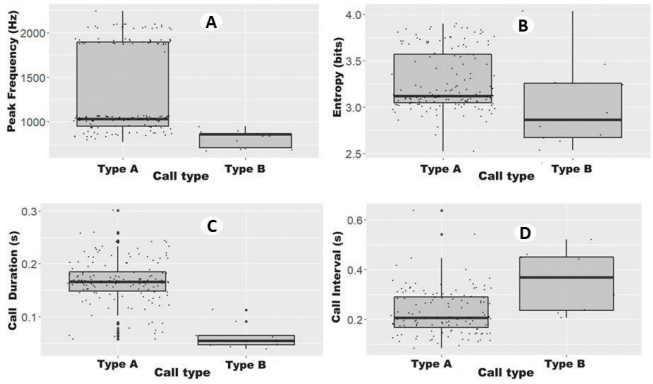

Figure 5. Characteristics of the two types of male advertisement calls of *Minervarya* cf. *keralensis* - **A.** Peak frequency, **B.** Entropy in bits, **C.** Call duration, and **D.** Call intervals

They were frequently not repetitive. In this series, type B calls were emitted at the beginning or at the end of the call series. (Fig. 3C, Table 1).

Call description: Based on structural and audible complexity we have classified calls into two types:-

1) Type A call

In this type, there were more pulsatile groups that were more fused relative to call type B. As there were more pulsatile groups, call duration was greater than in call type B (Figs. 4 & 5, Table 2) and these pulsatile groups were generally more uniformly distanced relative to type B. As the length of call increased, a greater number of pulsatile groups were emitted and pulsatile groups within the call also increased. This call type was the most frequently emitted (n=149). However, type A calls also showed within call type diversity where the number of pulsatile groups were fewer at the beginning of call and there were more pulsatile groups at the end of the call (Fig. 4, Table 2). These contribute to the series A call and were also components of series C.

2) Type B call

These constitute non uniformly placed pulsatile groups which were fused, and with a shorter call duration and with shorter intercall intervals. Type B calls (n=10) were not as frequently emitted as type A (n=149) (Figs. 4 & 5, Table 2). Type B calls were emitted in series B calls and were also components of series C calls.

According to the classification of anuran call guilds (Emmrich et al., 2020), both Call type A and B belong to Call Guild H, which are frequency-modulated, complex calls with multi-unit and multi note or pulsatile groups.

DISCUSSION

During the present study, calling and breeding activities were observed between January and March which is during the dry season. *Minervarya* cf. *kerlaensis* is a post-monsoon breeder, much like *M. kalinga* from the *M. nilagirica* group (Raj et al., 2019; Hegde et al., 2020) (Fig. 6). Among the minervaryans, the *M. nilagirica* group consists of large bodied frogs and large body size may enable them to be

Table 2. Parameters of call types A and B of male *Minervarya* cf. *keralensis*

Call series	Call parameters	Mean ± SD	Range
Type A (n=149)	Peak frequency (Hz)	1334.19±484.62	775.20–2239.45
	Call duration (s)	0.16±0.04	0.05–0.31
	Call interval (s)	6.73±0.08	0.08–0.63
	Average entropy (bits)	3.27±0.31	2.52–3.89
Type B (n=10)	Peak frequency (Hz)	809.64±92.59	689.06–947.46
	Call duration (s)	0.06±0.02	0.03–0.11
	Call interval (s)	0.35±0.12	0.21–0.52
	Average entropy (bits)	3.025±0.47	2.53–4.03

active and reproduce after the monsoon (dry periods) in peninsular India, as they are likely to be less prone to desiccation than the smaller minervaryan frogs (Raj et al., 2018; Hegde et al., 2020; unpublished data). Most minevaryan frogs breed in the pre-monsoon period or during the monsoon (Fig. 6) but as many of them are sympatric (Garg & Biju, 2017; 2021; Phuge et al., 2019; 2020; Kadadevaru et al., 2002; Dubois et al., 2001) there may be micro separation in breeding habitat, calling pattern, and spawning pattern (Garg & Biju, 2017).

The secondary sexual characters observed in adult male *M.* cf. *keralensis* include nuptial pad on the first finger of the forelimbs and black colouration or glandular or transparent skin in the vocal sac region and are typical of minervaryans (Garg & Biju, 2017; 2021; Kadadevaru et al., 2002; Phuge et al., 2019; 2020). In addition to these secondary sexual characteristics, adult males also show different colouration near the tympanum, close to mouth commissural region and in forelimbs which have also been described in *M. mysorensis* (Kuramoto & Joshy, 2001). All the minervaryan species of peninsular India employ axillary amplexus (Garg & Biju, 2017; 2021; Kadadevaru et al., 2002; Phuge et al., 2019; Dubois et al., 2001) and lay their eggs in a variety of sites including aquatic vegetation, wet soil, puddles, and other moist places that are used as temporary spawning grounds (Dubois et al., 2001; present study; unpublished data), which may reduce the risk of predation and desiccation of both the eggs and tadpoles.

The frog *M.* cf. *keralensis* exhibits call complexity. Call type A is more frequent and may be related to male-female interaction. Call type B was heard in the beginning or in between multiple Call A types and these were typically short (Fig. 4, Table 1). Playback experiment studies are needed to ascertain the functionality of all the calls. There is a further complication in our understanding of the calls

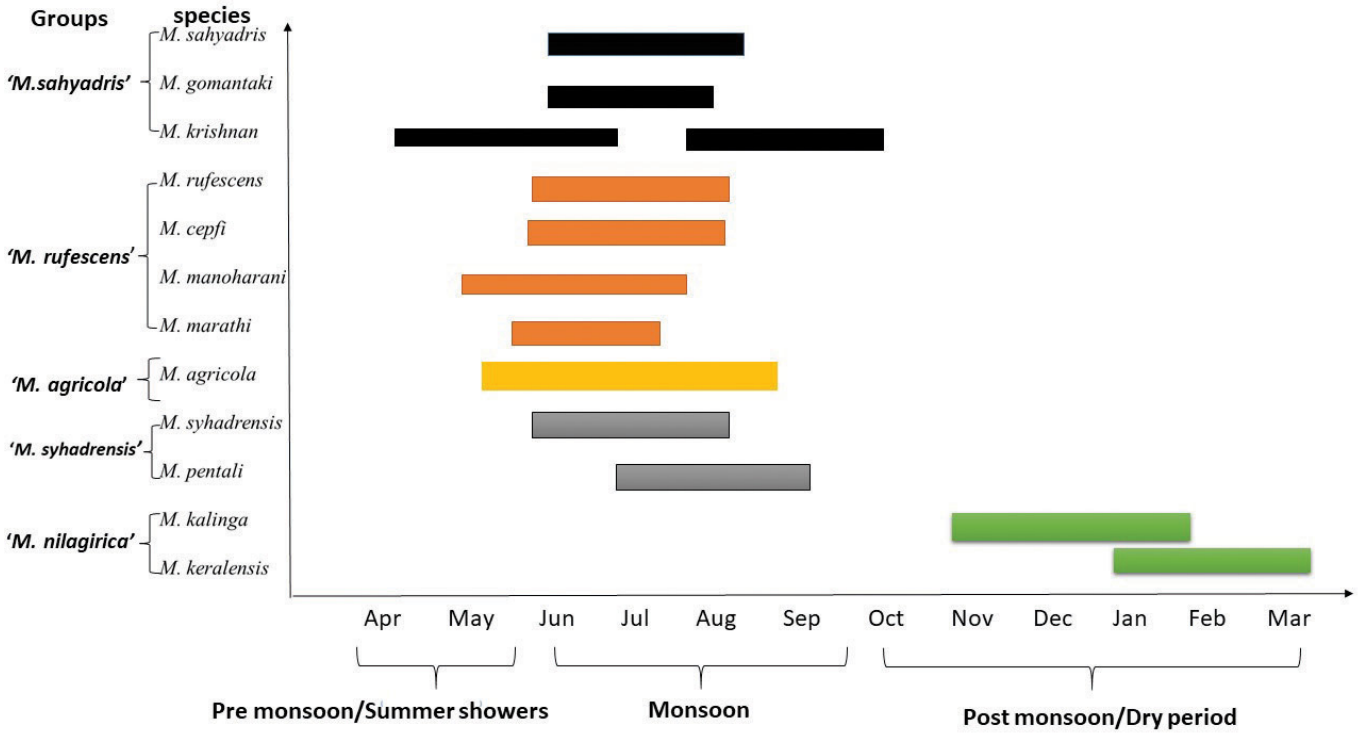

Figure 6. Call phenology of Minervaryan frogs from Western Ghats (after Garg & Biju, 2017; 2021; Phuge et al., 2019; 2020; Kadadevaru et al., 2002; Dubois et al., 2001; present study). Different coloured bars indicate different species groups.

of *M.* cf. *keralensis* as in this study occasionally irregular symmetry was observed when the vocal sac was enlarged during calling. This might have functional significance in producing call variation or different calls, further detailed study of this is needed.

The *M. nilagarica* group of frogs presents an interesting distribution within peninsular India (Fig. 1). *Minervarya nilagirica* is predominantly from the central Western Ghats but is also reported from the southern Western Ghats and the southern part of the Eastern Ghats (Biju & Garg, 2021). *M. kalinga* is known from northern part of the Eastern Ghats (Raj et al., 2018), central Western Ghats (Hegde et al., 2020) and northern Western Ghats (Biju & Garg, 2021) while *M. keralensis* is predominantly from the southern Western Ghats (Biju & Garg, 2021) but this study reports it from north of Palghat gap, in the central Western Ghats with a range extension of about 85 kms. Within the Peninsular India this group is distributed in highly elevated forest areas. Further phylogeographic studies are needed to explain the distribution patterns of the *M. nilagirica* group.

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