



Mud-packing frog: A novel breeding behaviour and parental care in a stream dwelling new species of *Nyctibatrachus* (Amphibia, Anura, Nyctibatrachidae)

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Abstract

Reproductive modes are diverse and unique in anurans. Selective pressures of evolution, ecology and environment are attributed to such diverse reproductive modes. Globally forty different reproductive modes in anurans have been described to date. The genus *Nyctibatrachus* has been recently revised and belongs to an ancient lineage of frog families in the Western Ghats of India. Species of this genus are known to exhibit mountain associated clade endemism and novel breeding behaviours. The purpose of this study is to present unique reproductive behaviour, oviposition and parental care in a new species *Nyctibatrachus kumbara* sp. nov. which is described in the paper. *Nyctibatrachus kumbara* sp. nov. is a medium sized stream dwelling frog. It is distinct from the congeners based on a suite of morphological characters and substantially divergent in DNA sequences of the mitochondrial 16S rRNA gene. Males exhibit parental care by mud packing the egg clutch. Such parental care has so far not been described from any other frog species worldwide. Besides this, we emphasize that three co-occurring congeneric species of *Nyctibatrachus*, namely *N. jog*, *N. kempholeyensis* and *Nyctibatrachus kumbara* sp. nov. from the study site differ in breeding behaviour, which could represent a case of reproductive character displacement. These three species are distinct in their size, call pattern, reproductive behaviour, maximum number of eggs in a clutch, oviposition and parental care, which was evident from the statistical analysis. The study throws light on the reproductive behaviour of *Nyctibatrachus kumbara* sp. nov. and associated species to understand the evolution and adaptation of reproductive modes of anurans in general, and *Nyctibatrachus* in particular from the Western Ghats.

Key words: Axillary amplexus, oviposition, Kathalekan, mud pack, *Myristica* swamps, reproductive character displacement, Western Ghats

Introduction

Reproductive modes including oviposition sites, egg development, larval development sites, presence or absence of parental care and details of parental care are diverse among anurans (Jameson 1955; Toledo *et al.* 2012). Up until now, 40 anuran reproductive modes are known, which vary both inter and intra specifically (Morrison & Hero 2003; Gururaja 2010; Toledo *et al.* 2012). Intraspecific variation in reproductive mode is known in very few species (Haddad & Prado 2005), but interspecific variation is widespread. Such variations are usually attributed to evolutionary and ecological factors (Morrison & Hero 2003; Rice 2008). Reproductive character displacement (RCD) is one such evolutionary determinant that arises with the interactions between co-occurring congeneric (COCG) species and imposes directional selection on each species' reproductive characteristics (including mating signals or mating preferences), leading to divergence between COCG species in these characteristics and a related reduction in reproductive interfaces (Howard 1993; Rice 2008).

The family *Nyctibatrachidae* forms one of the three oldest frog families in the Indian subcontinent (Roelants *et al.* 2007), with genus *Nyctibatrachus* endemic to the Western Ghats and genus *Lankanectes* endemic to Sri Lanka.

Nyctibatrachus consists of 27 species (Biju *et al.* 2011) and have exhibited clade endemism within the Western Ghats Mountains (Boclaer *et al.* 2012). Natural history notes and description of tadpoles, adult feeding habits, adult size and scaling, ecology, growth and population estimation of a few species of *Nyctibatrachus* have been documented ever since the discovery of the first species of *Nyctibatrachus* in 1882 (Boulenger 1882; Annandale 1918a, 1918b, 1919; Rao 1920; Rao 1937; Bhaduri & Kripalani 1955; Shaffer 1988; Krishnamurthy *et al.* 2001; Gururaja *et al.* 2003; Krishnamurthy & Reddy 2008; Girish & Krishnamurthy 2009; Biju *et al.* 2011). Among the studies on breeding behaviour in the genus *Nyctibatrachus*, three kinds of amplexus are known, namely; loose amplexus in *N. jog*, *N. humayuni* and *N. minor* (Biju *et al.* 2011); pseudo amplexus or no amplexus in *N. petraeus* (Kunte 2004) and lack or abbreviated amplexus in *N. humayuni* (Narahari *et al.* 2011). Call pattern, oviposition, reproduction and natural history notes were observed for *N. major* (now *N. sanctipalustris*) by Kuramoto & Joshy (2001), *N. petraeus* by Kunte (2004) and *N. humayuni* by Narahari *et al.* (2011) and Biju *et al.* (2011). The purpose of this study is to describe a new species, *Nyctibatrachus kumbara* sp. nov. and its associated unique reproductive behaviour, oviposition and parental care. In addition, the paper presents a possible case of RCD amongst three COCG species, suggesting that this process might have influenced the evolution of the diverse breeding behavior in *Nyctibatrachus*. Phylogenetic relationships of the new species are determined based on DNA sequences of the mitochondrial 16S rRNA gene.

Material and methods

Study area and species. As a part of ongoing studies on anuran diversity and distribution in the central Western Ghats (12°N–14°N latitude) of India, observations of the *Nyctibatrachus kumbara* sp. nov. and other congeneric *Nyctibatrachus* species were made in several localities in the Western Ghats (Figure 1) since 2004 (details will be published elsewhere). On site behavioural studies and specimen collection were made from Kathalekan, Uttara Kannada district, Karnataka state, India (14.27414°N, 74.74704°E, 572 m above sea level). Kathalekan represents *Myristica* swamp forests which are relict forests of ancient origin, characterized by first and second order perennial streams and evergreen forest vegetation (Chandran *et al.* 2010; Gururaja 2010). Streams flowing through the swamps are characterised by slow flow rate, network of modified knee roots of *Gymnacranthera canarica* across the stream, stilt roots of *Myristica fatua*, presence of rocks and boulders and intermittent puddles with sandy bottom. Three co-occurring congeneric species of *Nyctibatrachus* (*N. jog*, *N. kempholeyensis* and *Nyctibatrachus kumbara* sp. nov.) inhabit these streams and swamps.

Behavioural observations. Observations on individuals of the new species described herein, *Nyctibatrachus kumbara* sp. nov. were made at a stream in Kathalekan from 17:00–22:00 hr on 10 occasions (26-06-2006; 18-08-2006; 18-02-2008; 25-03-2008; 24-06-2008; 15-07-2008; 16-07-2008; 29-06-2009, 19-08-2012; 16-09-2012). Among these, on a few occasions, observations and searches were also carried out on *N. jog* and *N. kempholeyensis* which co-occurs with *Nyctibatrachus kumbara* sp. nov. Subsequent sections on behavioural observations focus mainly on *Nyctibatrachus kumbara* sp. nov. unless otherwise mentioned. Individuals of *Nyctibatrachus kumbara* sp. nov. were searched using torch lights and by tracing calling males along the edges of stream, in cavities and under small rocks/boulders. On finding an individual of *Nyctibatrachus kumbara* sp. nov., one of us (KVG/KPD) would sit at a safe distance (1-2m) from the individual and record the activities using torch lights intermittently. Observations were also made during day time between 06:00–17:00 hr. Individuals were not seen, but a few advertisement calls were heard. Measuring tapes were used to measure depth of water, distance between individuals and oviposition height. Snout-vent length (SVL) and diameter of eggs were measured in the field to the nearest 0.1 mm with a digital caliper. Air temperature and relative humidity were recorded using TFA digital Thermo-Hygrometer. Calls were recorded using an Olympus stereo digital voice recorder (LS-11). Calls with less background noise were manually selected from different individual call records and were analysed using Audacity Ver.1.3 (Beta) and Song Scope Ver. 4.1.3A. Eighteen calls from *N. kempholeyensis*, ten from *N. jog* and three from *Nyctibatrachus kumbara* sp. nov. were selected for further analysis. Duration and peak frequency of each call was recorded. Call terminology was based on Kok and Kalamandeen (2008). To study the reproductive character displacement, both personal observations as well as information available in literature (Bhaduri & Kripalani 1955; Kunte 2004; Biju *et al.* 2011; Narahari *et al.* 2011) were compiled. PAST ver 2.17c was used for statistical analysis. We assumed normal distribution for sample data and used unpaired one tailed T test with Bonferroni corrections

for repeated measures for comparison. For size-corrected analysis of call characteristic features between species, we used ANCOVA. We used distribution free multivariate analysis, non-metric multidimensional analysis (NMDS) for behavioural data. Video clippings were made using Sony DSC H2 and Canon Powershot SX 30IS digital still cameras. Supplementary video clips on advertisement calls, amplexus, oviposition and male parental care are available at <http://www.gururajakv.net>.

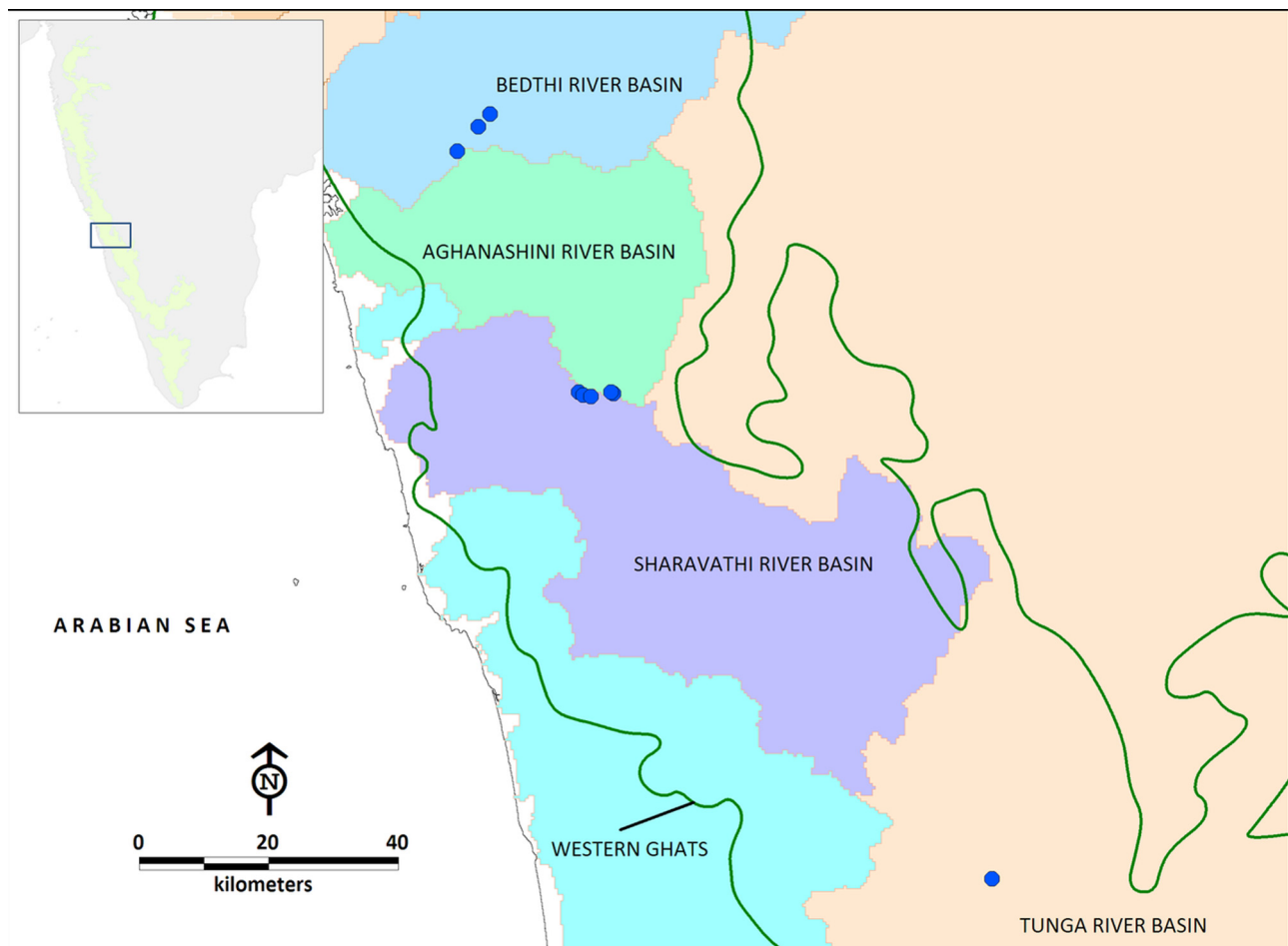


FIGURE 1. Study localities (blue circles) of *Nyctibatrachus kumbara* sp. nov. in the Western Ghats of India.

Specimen collection. Adult calling males were collected during their active breeding period. Specimens collected were euthanized with dilute alcohol, fixed in 4% formalin and preserved in 70% alcohol. Tissue samples for genetic analysis were preserved in molecular grade alcohol. Morphological measurements were taken with Mitutoyo vernier calliper (to the nearest 0.1 mm). Microscopic observations were made using Leica MZ75 (6.3 magnification under 1.0x objective) binocular microscope. Color of live specimens and natural history notes were recorded at the type locality during multiple visits.

DNA extraction, amplification and sequencing. For genetic studies, genomic DNA extraction was carried out from ethanol preserved tissues (2 individuals). The extraction was carried out using salt extraction protocol (Vences *et al.* 2012). The extracted DNA was quantified and amplified using 16s mitochondrial DNA (Palumbi *et al.* 2002). The reaction was performed in 15 μ l volume reaction containing 2 μ l of 10X PCR buffer (Sigma-Aldrich), 2 μ l of deoxyribonucleotide triphosphates (1 mM), 2 μ l of each primer (5 pmol/ μ l) and 0.2 μ l of Taq DNA polymerase (5 units/ μ l) (Sigma-Aldrich), 5.8 μ l of dH₂O and 1 μ l of template DNA (>80 ng). The PCR profile consisted of a denaturing step at 95°C for 5 min, followed by 40 cycles (95°C for 30 sec, 55°C for 40 sec, 72°C for 90 sec,) and a final extension of 72°C for 5 min. The amplified product was sent for purification and sequencing to Shrimpex Biotech services Pvt Ltd. The sequences were checked for quality and deposited in GenBank (Accession numbers KF935242 and KF935244).

Genetic analysis. The chromatogram files were checked manually using the program Chromas lite 2.01 (<http://>

/www.technelysium.com.au/chromas_lite.html). For alignment, sequences of other *Nyctibatrachus* species were retrieved from GenBank (For Accession numbers see Appendix 1, gene sequence to corresponding species were referred to Biju *et al.* 2011 and Bocxlaer *et al.* 2012). The sequences were first aligned using MAFFT algorithm (Katoh *et al.* 2002) and manually corrected in MacClade v4.08 (Maddison and Maddison 2005). Genetic distances between the species were estimated using MEGA 5.10 software (Tamura *et al.* 2011) by calculating uncorrected pairwise distance. For constructing the phylogenetic relationship, Maximum Likelihood (ML) analysis was performed in raxmlGUI v1.3 (Silvestro & Michalak 2012). Using Akaike Information Criterion (-lnL=3079.97; AIC=6295.95) in jModel test (Posada 2008), GTR+I+G model of nucleotide substitution was selected. ML was executed with 10,000 bootstrap replications. For levels of genetic divergence, pair wise distance up to 2% was considered as low divergent, 2–4% as medium, 4–6% as high and >6% as very high.

Abbreviations. AG—axilla to groin distance; BW—body width behind shoulders, from right axilla to left axilla; BWG—body width in front of groin; EL—eye length, i.e. the horizontal distance between the bony orbital borders of the eye; EN—eye to nostril distance, i.e., distance between anterior orbital border to —most point of eye and middle of nostril; fd I, II, III and IV—maximum disc width on fingers I, II, III and IV respectively; FFL—first finger length (tip of finger disc to proximal palmar tubercle); FFTF—distance from maximum incurvature of web between fourth and fifth toe to tip of fourth toe; FGB—femoral gland breadth; FGL—femoral gland length; FL—thigh length; FLL—forelimb length, measured from the elbow to the base of the outer palmar tubercle; FOL—foot length, measured from the base of the inner metatarsal tubercle to the tip of the fourth toe; FrFL—fourth finger length, measured from base of proximal sub-articular tubercle to finger tip; FW—femur width; fw I, II, III and IV—width of finger I, II, III and IV respectively, measured at the base of the disc; HAL—hand length, measured from the base of the outer palmar tubercle to the tip of the third finger; HD—head depth, measured as a vertical profile at region behind eyes to chest; HL—head length, from the rear of the mandible to the tip of the snout; HW—head width, at the angle of the jaws; IBE—distance between posterior corner of eyes, i.e., the shortest distance between the posterior—most orbital borders of the eyes; IFE—distance between anterior corner of eyes, i.e., the shortest distance between the anterior orbital borders of the eyes; IMT—length of inner metatarsal tubercle; IN—internarial distance, i.e., least distance between the inner margins of nares; IUE—inter upper eyelid width, i.e., the shortest distance between the upper eyelids; MBE—distance from the rear of the mandible to the posterior—most orbital border; MFE—distance from the rear of the mandible to the anterior—most orbital border; MN—distance from the rear of the mandible to the centre of the nostril; MTFF—distance from distal edge of metatarsal tubercle to maximum incurvature of web between fourth and fifth toe; MTTF—distance from distal edge of metatarsal tubercle to maximum incurvature of web between third and fourth toe; NS—nostril to snout distance, i.e., distance between middle of nostril and tip of snout; SFL—second finger length; SL—snout length, measured from the tip of the snout to the anterior—most orbital border; SVL—snout to vent length; T1L—toe one length (tip of disc to proximal sub articular tubercle); T2L—toe two length; T3L—toe three length; T4L—toe four length; T5L—toe five length; td I, II, III, IV and V—maximum disc width on toes I, II, III, IV and V respectively; TFL—third finger length; TFOL—distance from the heel to the tip of the fourth toe; TFTF—distance from maximum incurvature of web between third and fourth toe to tip of fourth toe; TL—tibia length; tw I, II, III, IV and V—width of toes I, II, III, IV and V respectively, measured at the base of disc; UEW—maximum upper eyelid width. Webbing formula used follows Dubois *et al.* (2001).

Other abbreviations used. amsl (above mean sea level), ANCOVA (Analysis of Covariance), COCG (co-occurring congeneric), KVG (K.V. Gururaja), KPD (K.P. Dinesh), SA (Sameer Ali), NMDS (Non-metric multidimensional Scaling), ZSIC (Zoological Survey of India, Kolkata), ZSI/WGRC/V/A (Zoological Survey of India/ Western Ghats Regional Centre, Calicut/Vertebrata/Amphibia).

Results

Field setting. The first observation of breeding behaviour in *Nyctibatrachus kumbara* **sp. nov.** was made on 26-6-2006 along a stream edge amidst tree roots and small rocks. We observed six complete sequences of breeding. Field observations were made from 17:00–22:00 hr in the streams of *Myristica* swamps. Individuals were calling and exhibiting breeding behaviour at very shallow depth (0–2 cm) along the edges of the stream. *Nyctibatrachus kumbara* **sp. nov.** is a medium sized nocturnal frog that feeds, breeds and inhabits these streams predominantly

along the edges. Congeneric co-occurring species in the streams and habitats with *Nyctibatrachus kumbara* sp. nov. are *N. jog* Biju *et al.* (2011), a medium sized nocturnal frog and *N. kempholeyensis* (Rao 1937), a small sized nocturnal frog.

Male advertisement calls. No external vocal sac dilations were observed during the call. Male produced advertisement calls during night throughout the year; however, call prevalence was more during the monsoon season (June–October) compared to other seasons of the year. Calls were occasionally heard during the day time from the crevices along the sides of the stream. Two types of calls were noticed, calls with single ‘tok’ note and calls with double ‘tok tok’ notes. Double note calls were predominant when a female was close-by. Advertisement call pattern of male individuals were recorded on 16-09-2012, and was used for call analysis (individual not collected, identified on the basis of call pattern, size and behaviour). Calls generally started at 18:00 hr and peaked between 19:00 and 21:00 hrs. Simultaneously in the same area calls were also recorded for *N. jog* and *N. kempholeyensis*. Air temperature was 25.3 ± 0.3 °C (N=3), relative humidity was $80.33 \pm 0.33\%$ (N=3). Call characteristics of *Nyctibatrachus kumbara* sp. nov. are given in Table 1 and Figure 2. In single calls, peak frequency was 1342.17 ± 197.1 Hz and in double calls it was 1524.78 ± 130.84 Hz. Call duration for single and double call was 0.11 ± 0.03 sec and 0.44 ± 0.05 sec respectively. Call characteristic features of *N. jog* and *N. kempholeyensis* are given in Table 2 and spectrograms are given in Figure 3 and 4 (a&b) respectively. *Nyctibatrachus jog* has a paired vocal sac that opens laterally during a call. It has a soft bird like ‘hooors’ call and gets intensified when a female is very close. In *N. kempholeyensis*, paired vocal sac opens along the sides of the head and has two types of calls. Type I call is ‘chirr...chi..chi..chi..chi..chi..chi’ having a chirp in the beginning followed by 5–9 pulses. Type II call is ‘few..few..few..few..few..few..chirr..chi..chi..chi..chi..chi’ having soft call pulses (7–10 pulses) in the beginning and ending with Type I call pulses.

TABLE 1. Single (*) and double call (**) characteristics of *Nyctibatrachus kumbara* sp. nov.

Call characters	Type I call			Type II call		
	Duration* (sec) (n=12)	Fundamental Frequency* (Hz) (n=12)	Peak frequency* (Hz) (n=12)	Duration** (sec) (n=9)	Fundamental Frequency** (Hz) (n=9)	Peak frequency** (Hz) (n=9)
Mean±SD	0.11±0.03	601.75±12.71	1342.17±197.07	0.44±0.05	584.22±42.17	1524.78±130.84
Range	0.07–0.20	581–620	956–1581	0.39–0.53	538–671	1232–1724

TABLE 2. Call characteristics of *Nyctibatrachus jog* and *N. kempholeyensis* (# indicates Type I call and @ indicates Type II call).

Call characters	<i>N. jog</i>		<i>N. kempholeyensis</i>			
	Duration (sec) (n=10)	Peak frequency (Hz) (n=10)	Duration# (sec) (n=18)	Peak frequency# (Hz) (n=18)	Duration@ (sec) (n=3)	Peak frequency@ (Hz) (n=3)
Mean±SD	0.59±0.09	1512.9±101.34	5.17±1.45	3895.76±85.86	11.69±1.36	1633.67±106.52
Range	0.48–0.75	1359–1679	3.47–9.22	3717.84–4113.52	10.17–12.78	1539–1749

Male and female interaction. On 16-08-2012, first call from the male (SVL 45 mm) was heard at 18:40 hr with single ‘tok’ note. At 18:57 hr, a female (SVL 50 mm) came out of a crevice (crevice formed by eroded soil amidst tree roots along a stream edge) and moved very close to calling male from behind (~5 cm). On sighting this female, the male started calling more frequently with single notes interspersed with double (tok-tok) notes. At 19:10 hr, the male stood upright on its hind limbs and with its forelimbs touched a few naturally fallen twigs. The twigs were 4 cm above the flowing stream. The female moved to the place where male was standing and it also stood on its hind limbs and touched same places where male had previously touched. On two occasions, the female and the male were seen standing on their hind limbs and touching each other with their forelimbs (Figure 5a).

Amplexus. By 19:22 hr, the female was back in her squatting position and the male started calling with double notes and continued until the amplexus. The female crawled very close to the calling male, facing the male. At 19:41hr, the male mounted the female and clasped her by the arm pits with its fore limbs (Figure 5b) forming axillary amplexus. Both male and female remained in axillary amplexed position for at least 5 minutes. At 19:46

hr, the amplexed pair stood upright on their hind limbs and the female touched the twigs again (Figure 5c). Immediately, the amplexed pair rotated in clock wise direction (initiated by the female) to do a handstand and positioned their cloacae on the place where they had touched earlier (19:47 hr). The amplexed pair remained in this position for 14 seconds.

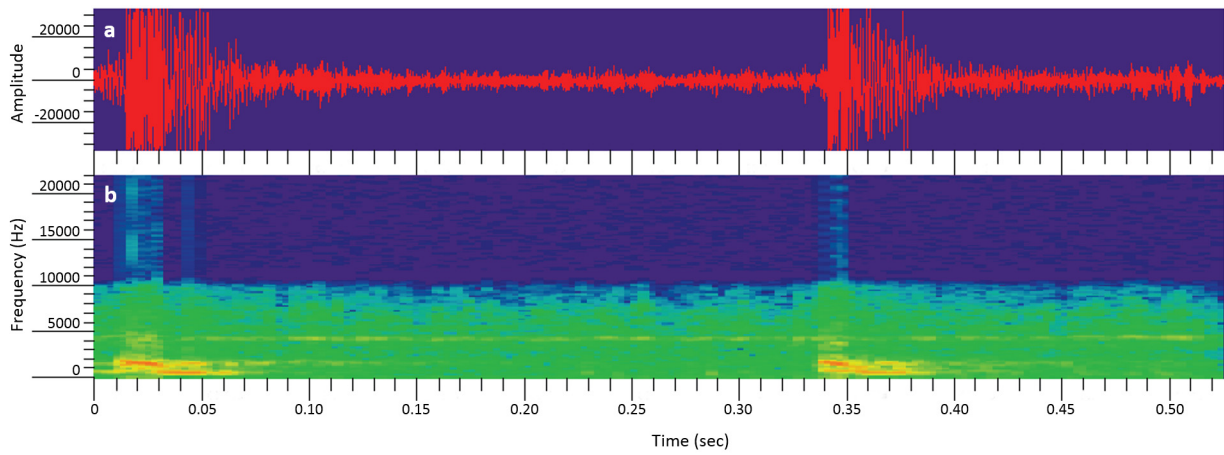


FIGURE 2. Advertisement call spectrogram of *Nyctibatrachus kumbara* sp. nov. a. Amplitude and b. Spectrogram.

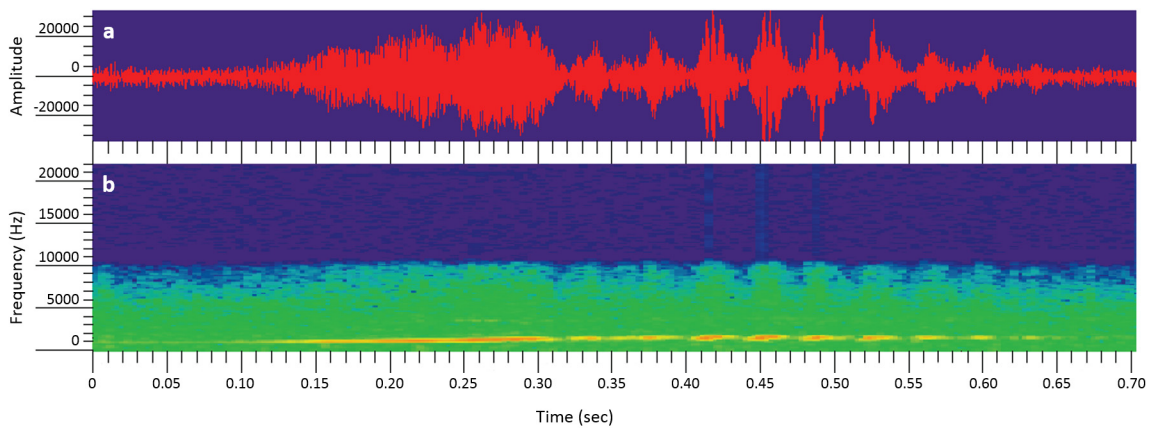


FIGURE 3. Advertisement call spectrogram of *Nyctibatrachus jog*. a. Amplitude and b. Spectrogram.

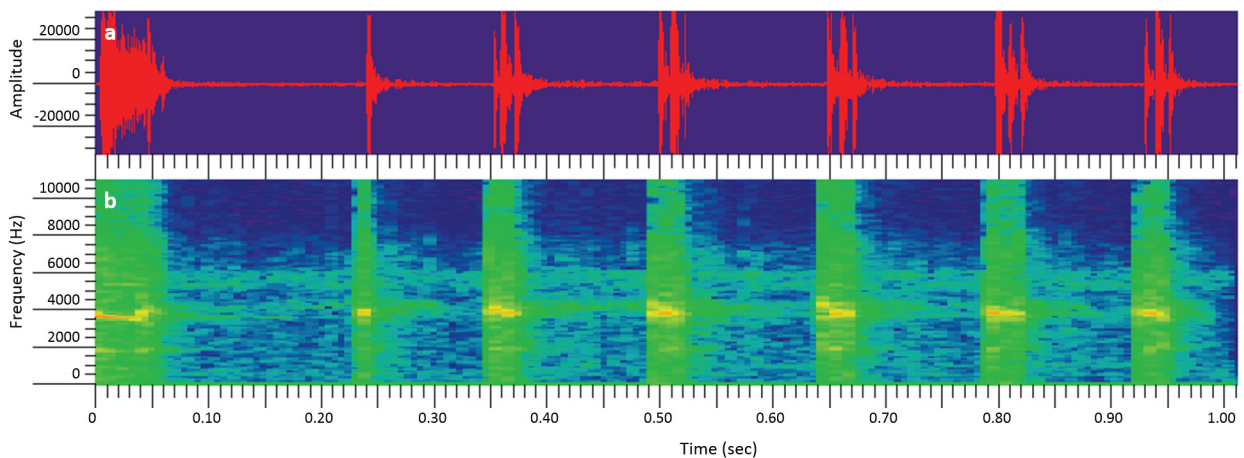


FIGURE 4a. Type I advertisement call spectrogram of *Nyctibatrachus kempholeyensis*. a. Amplitude and b. Spectrogram.

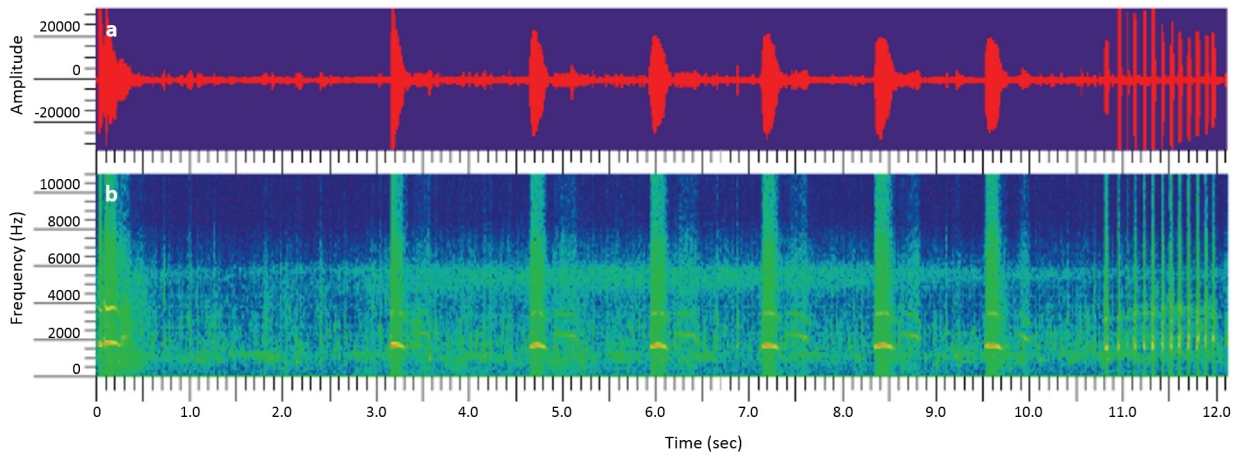


FIGURE 4b. Type II advertisement call spectrogram of *Nyctibatrachus kempholeyensis*. a. Amplitude and b. Spectrogram.



FIGURE 5. a. Male and female in courtship (male and female are marked with symbols); b. Axillary amplexus in the pair; c. Amplexed pair standing upright on their hindlimb before oviposition; d. Female doing a handstand at the time of releasing eggs.

Oviposition. After vigorously shaking its hind limbs, the male got separated from the female and sat in a squatting position looking at the female. The female slowly released 5 pigmented eggs (in a span of 6 seconds) in the handstand position and stuck them to the twig which it had touched earlier (Figure 5d). After releasing the eggs, the female very slowly moved away from the spot, but remained within 30 cm of the area. We observed six different egg clutches with average clutch size of five eggs (range 4–6 eggs) on various substrates overhanging

slow flowing streams (oblique rock faces, knee root bases, twigs and so on) placed at an average height of 6.33 ± 0.52 cm ($n=6$) from the ground. Mean egg diameter was 3.98 ± 0.1 mm ($n=4$).

Parental care. At 19:57 hr, the male called twice and moved towards egg clutch (Figure 6a). At 19:58 hr, the male stood on its hind limbs and touched the twigs to identify the clutch position on twigs (Figure 6b). After repeating this two times, the male collected mud from the stream bed on its hands and spread it on the egg clutch while standing on its hind limbs. This was repeated 14-15 times (in one instance it was 38 times) to cover all the eggs with mud (Figure 6c). The duration for covering the egg clutch with mud was about 12 minutes. By 18:10 hr, the male moved away from the spot (Figure 6d). In other instances ($n=3$), we observed that male individuals were calling next to mud covered egg clutches and females oviposited next to these clutches. However, as of now, we do not know the reason for such multiple clutches. Eggs hatched into tadpoles by day eight. Although, we have observed various stages of tadpoles throughout the year, we have not been able to determine the exact time taken for metamorphosis.

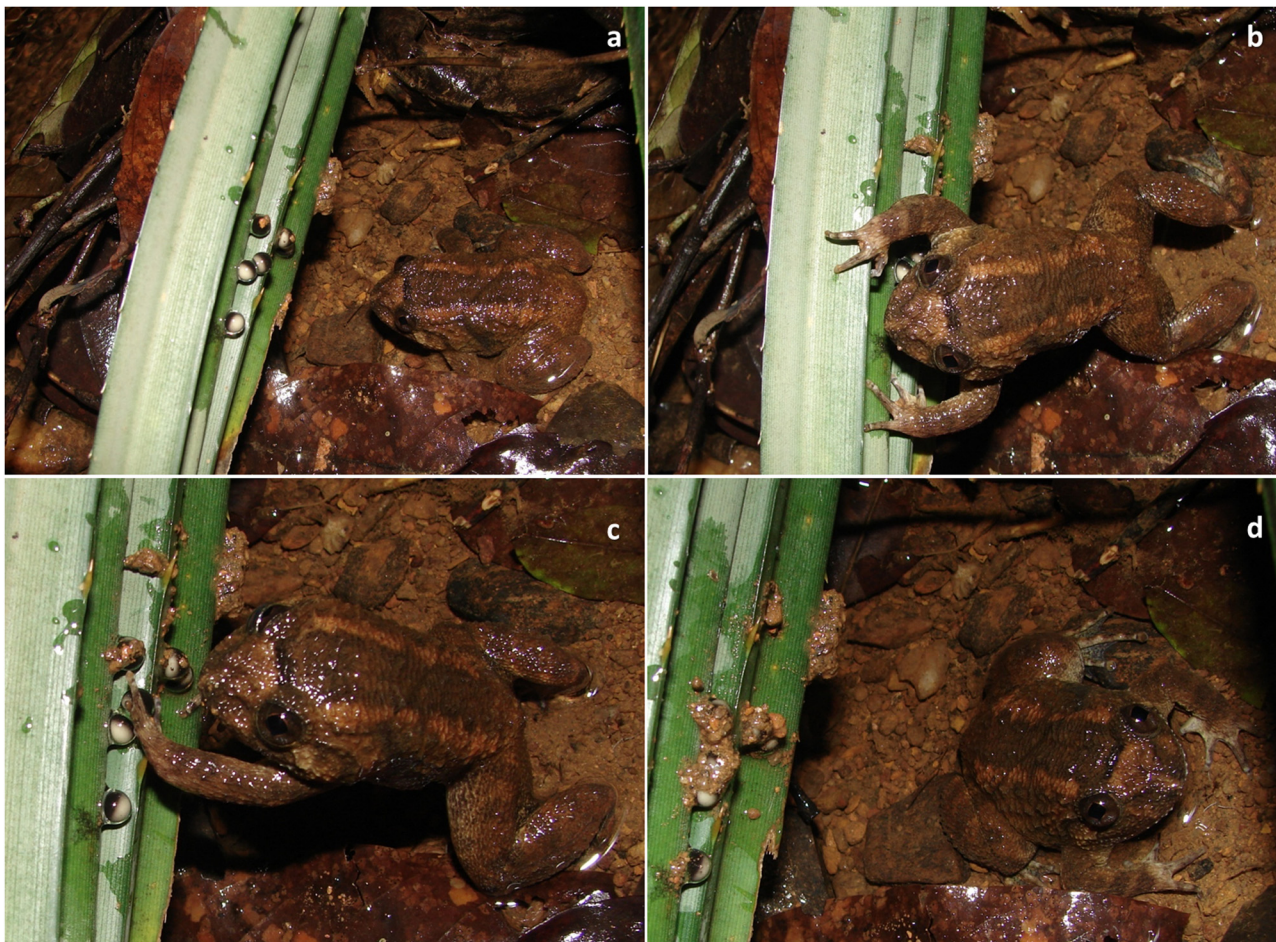


FIGURE 6. a. Male approaching the eggs; b. Male standing upright locating the egg clutch; c. Male covering the egg clutch with sand gravels and mud from the stream bed; d. Male leaving the place after covering the egg mass with sand and mud.

Genetic analysis. The pairwise uncorrected genetic distances for mitochondrial 16S rRNA for all 28 nominal species of *Nyctibatrachus* including *Nyctibatrachus kumbara* **sp. nov.** were in the range 1.2% to 12.25% (Table 1S). Minimum genetic divergence for *Nyctibatrachus kumbara* **sp. nov.** was 3.41% with *N. dattatreyaensis* and maximum divergence of 10.84% with *N. jog*, a syntopic congener. *Nyctibatrachus kumbara* **sp. nov.** is a member of the *N. sanctipalustris* clade in the gene tree generated from the 16S sequences, comprising *N. dattatreyaensis*, *N. shiradi*, *N. vrijeuni*, *N. karnatakaensis* and *N. sanctipalustris* (Figure 7).

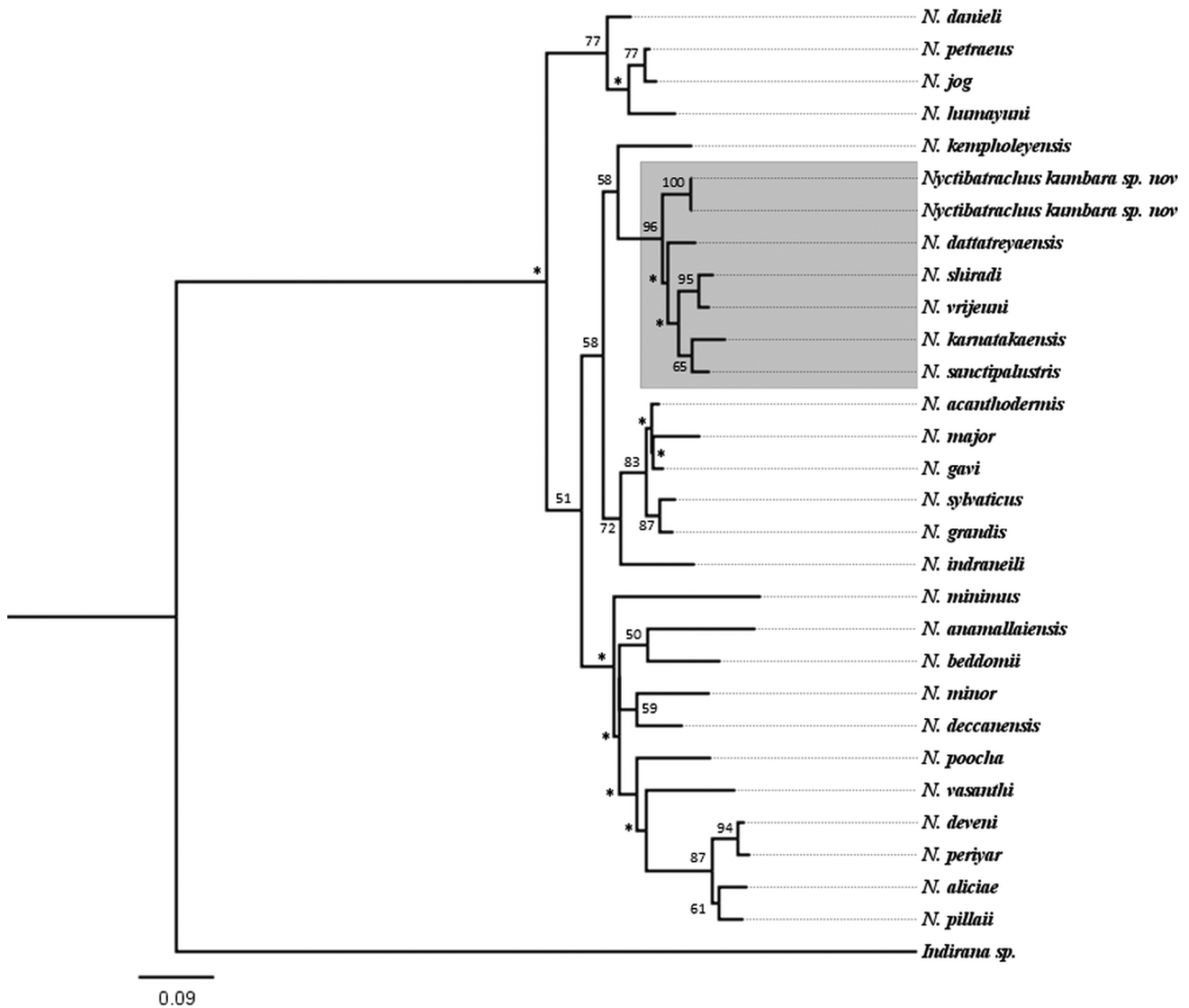


FIGURE 7. Maximum likelihood tree for all 28 nominal species of *Nyctibatrachus* and an outgroup (*Indirana sp.*) based on mitochondrial 16S rRNA. Number at the branches indicate bootstrap values. Bootstrap values less than 50 are indicated with an asterisk. Area marked with grey belong to *N. sanctipalustris* clade.

Species description

Nyctibatrachus kumbara sp. nov.

(Table 2S, Figures 5, 6, 8–9, 10c.)

Holotype. ZSI/WGFRS/V/A/860, adult male collected on 09-06-2006 by KVG and SA along a flowing rivulet of Sharavathi river basin (14.27323°N; 74.74756°E, 590m amsl) at Kathalekan, Uttara Kannada District, Karnataka, India.

Paratypes. ZSI/WGFRS/V/A/861 and ZSI/WGFRS/V/A/863, adult females collected on 03-10-2006 by KVG and SA along a flowing rivulet of Sharavathi river basin (14.27499°N; 74.73695°E, 583m amsl) at Malemane, Uttara Kannada District, Karnataka, India; ZSI/WGFRS/V/A/862 and ZSI/WGFRS/V/A/864, adult males collected on 09-06-2011 by KVG and KPD along a flowing rivulet of Sharavathi river basin (14.27323°N; 74.74756°E, 590m amsl) at Kathalekan, Uttara Kannada District, Karnataka, India.

Diagnosis. This species is assignable to the genus *Nyctibatrachus* because of its semi aquatic to aquatic habitat, medium to large size, pupil rhomboidal, glandular wrinkled skin, presence of vomerine teeth, notched

tongue, finger and toe discs with disc, absence of webbing on fingers and presence of webbing on toes and presence of sub ocular gland (Biju *et al.* 2011).

Nyctibatrachus kumbara **sp. nov.** can be easily distinguished from all other species in the genus by the following combination of characters: (1) adult male size medium to large (SVL 46.5±0.74 mm); (2) head wider than long (HW 18.7–20.9 mm; HL 14.8–15.7 mm); (3) dorsum glandular without any spiny projections in the anterior half, glandular corrugations irregular without specific pattern; in males, throat and chest finely dotted with glandular folds rest of the region smooth, belly white; (4) webbing on toes medium (I ½-1 II ½-1 III 0-2 IV 2-0 V); (5) nuptial pad and femoral glands prominent in adult males; (6) dorsal body color dark brown, ventrally buff colored except belly; (7) finger disc weakly developed (fd3 0.9±0.24 mm; fw3 0.8±0.19 mm); (8) toe disc moderately developed (td4 1.7±0.32 mm; tw4 0.8±0.05 mm); (9) third finger disc with dorso-terminal groove, cover notched distally, fourth toe disc with dorso-terminal groove cover bifurcate distally; (10) hand-stand oviposition, fertilized egg clutch covered with mud pack; (11) genetically belongs to *N. sanctipalustris* clade including *N. dattatreyaensis* (3.41% divergent on mitochondrial 16S rRNA), *N. vrijeuni* (4.02%), *N. shiradi* (4.02%), *N. karnatakaensis* (4.62%) and *N. sanctipalustris* (4.62%).



FIGURE 8. *Nyctibatrachus kumbara* **sp. nov.** in axillary amplexus. Adult male (Holotype ZSI/WGFRS/V/A/860) on top of adult female in swampy evergreen forest of Kathalekan, Uttara Kannada district, Karnataka, India. Female not collected.

Description of the holotype. ZSI/WGRC/V/A/860, adult male (Figure 9), terminology follows Biju *et al.* (2011) and Dinesh *et al.* (2008a). Morphometric data are given in Table 2S.

A medium-sized species of *Nyctibatrachus* (SVL 46.2 mm); habitus compact and squat; head wider (HW 18.7 mm) than long (HL 15 mm; MN 11.9 mm; MFE 9.6 mm; MBE 3.4 mm), rounded in anterior view; snout rounded (SL 7.1 mm), marginally protruding, its length longer than horizontal diameter of eye (EL 5.7 mm); canthus rostralis rounded; loreal region concave; interorbital space flat (IUE 6.3 mm) and double the upper eye lid width (UEW 3.1 mm); internarial distance (IN 4.3 mm) less than interorbital distance (IUE 6.3 mm); distance between back of eyes (IBE 14.5 mm) is greater than double the distance between front of eyes (IFE 6.9 mm); nostrils oval, without a flap of skin, and is closer to eye (EN 2.9 mm) than snout tip (NS 3.5 mm); eyes large (EL/HL = 0.38%)

protruding on the sides of head, its diameter more than eye to nostril distance (EL/EN=1.96); pupil rhomboidal; tympanum indistinct; subocular gland distinct (Figure 9C); pineal ocellus absent; vomerine ridge present posterior to choanae, oval bearing three on left and six sharp spinose teeth on right ridge; symphyseal knob 'W' shaped, moderately developed; tongue moderate, oval slightly emarginated, median lingual process absent; parotoid glands, cephalic ridges and co-ossified skin absent.

Forelimbs (FLL 10.9 mm) strong and sub-equal to hand length (HAL 10.1 mm); third finger thin, long, rounded (TFL 5.3mm); thin dermal fringe on finger III; webbing absent (Figure 9D); relative length of fingers, shortest to longest: I<II<IV<III; tips of fingers enlarged with weakly developed disc and third finger disc with dorso-terminal groove, cover notched distally (Figure 9F); subarticular tubercles moderate, round one each on finger I and II and two each on finger III and IV; prepollex and palmar tubercle distinct (Figure 9D).

Hind limbs moderately long; shank length (TL 21.3 mm) more than two times longer than wide (TW 8.1 mm), shorter than thigh length (FL 22.6 mm) and sub-equal to the distance from the base of the internal metatarsal tubercle to the tip of toe IV (FOL 20.1 mm); heels touch when the tibia are folded at right angles to the body; toes thin and long; toe IV long (T4L 9.9 mm) and less than the distance from the base of the tarsus to the tip of toe IV (TFOL 26.7 mm); relative length of toes, shortest to longest: I<II<V<III<IV (Figure 9E); tips of toes with moderately developed discs and fourth toe disc with dorsoterminal groove, cover bifurcate distally (Figure 9G), webbing medium (3/4th) (I ½-1 II ½-1 III 0-2 IV 2-0 V) (MTTF 13.5 mm; MTF 12.8 mm; TFTF 6.6 mm; FFTF 8.4 mm) (Figure 9H); dermal fringe along toe V distinct; subarticular tubercles present, moderate, oval, one each on toe I and II, two each on toe III and V, and three on toe IV; inner metatarsal tubercle short prominent, spade shaped; its length (IMT 3 mm) shorter than toe I (T1L 3.5 mm) length; outer metatarsal tubercle, supernumerary tubercles and tarsal tubercles absent (Figure 9E).

Skin is corrugated on the dorsal and lateral surfaces of the body with glandular folds; forelimbs and hind limbs with short longitudinal glandular folds; snout with a 'Y' shaped fold from middle of upper lip, its arms bifurcating between nares and running towards orbits; three small dotted horizontal folds between posterior corner of eyes to region above shoulder; supratympanic fold and subocular fold distinct. Throat with dense glandular longitudinal folds and rest of the venter soft glandular without any folds; belly smooth; lower lip dotted with a single row of small discontinuous glandular folds; thigh ventrally smooth (Figure 9B); dorsally tarsus with fine spinules (Figure 9A). Distinct raised femoral gland present (FGL 9.2 mm; FGB 3.8 mm).

Color of holotype. *In life*, iris golden yellow in its upper part, pupil rhomboidal, black in color (Figure 8). Sides of head light colored, region between subocular gland and tympanic region cream colored. A black horizontal band connecting upper eye lids. Dorsum brown with two light brick red dorsolateral bands laterally bordered by dark brown from orbits to vent. Region between dorsolateral bands light brown with three black horizontal bands, one at between orbits, one at anterior region of shoulders and other at posterior region of shoulders. Forearm barred with small black and larger dark brown bands; hind limbs barred with small light brick red and larger dark brown bands. Sides of the body brownish. Nuptial pads on base of first fingers cream colored. Throat translucent with fine black spots on the glandular skin. Chest, belly and anterior part of thighs translucent, where internal organs can be clearly seen. Posterior part of thigh with raised glossy light yellow femoral gland in males.

In preservative (Figure 9A), dorsal and lateral parts of body mottled with brown and cream color, with a pair of faint dorsolateral lines from posterior orbit to above vent; faint horizontal band between eyes; femoral glands cream white; dorsal parts of limbs, forelimbs, thigh, shank and foot up to tip of fingers and toes barred; throat and chest pale brown; belly and thighs white; femoral glands granular, white in color (Figure 9B).

Secondary sexual characters. Males have a nuptial pad covering the base of the dorsal surface of the first finger and a pair of moderately raised femoral glands which are more than two times longer (FGL 9.2 mm) than wide (FGB 3.8 mm), and is conspicuous both in life and preservative (Figure 9B). Females have pigmented eggs.

Additional information from paratypes and variations. Morphological data are given in Table 2S. Paratypes range from 42.8 mm to 47.4 mm in SVL; in all the external morphological characters they are similar to holotype.

Etymology. The specific epithet 'Kumbara' is derived from Kannada language, a name given to the community of people involved in making pottery. The species name *Kumbara* is a noun in apposition to generic name.

Suggested common name. Kumbara night frog.

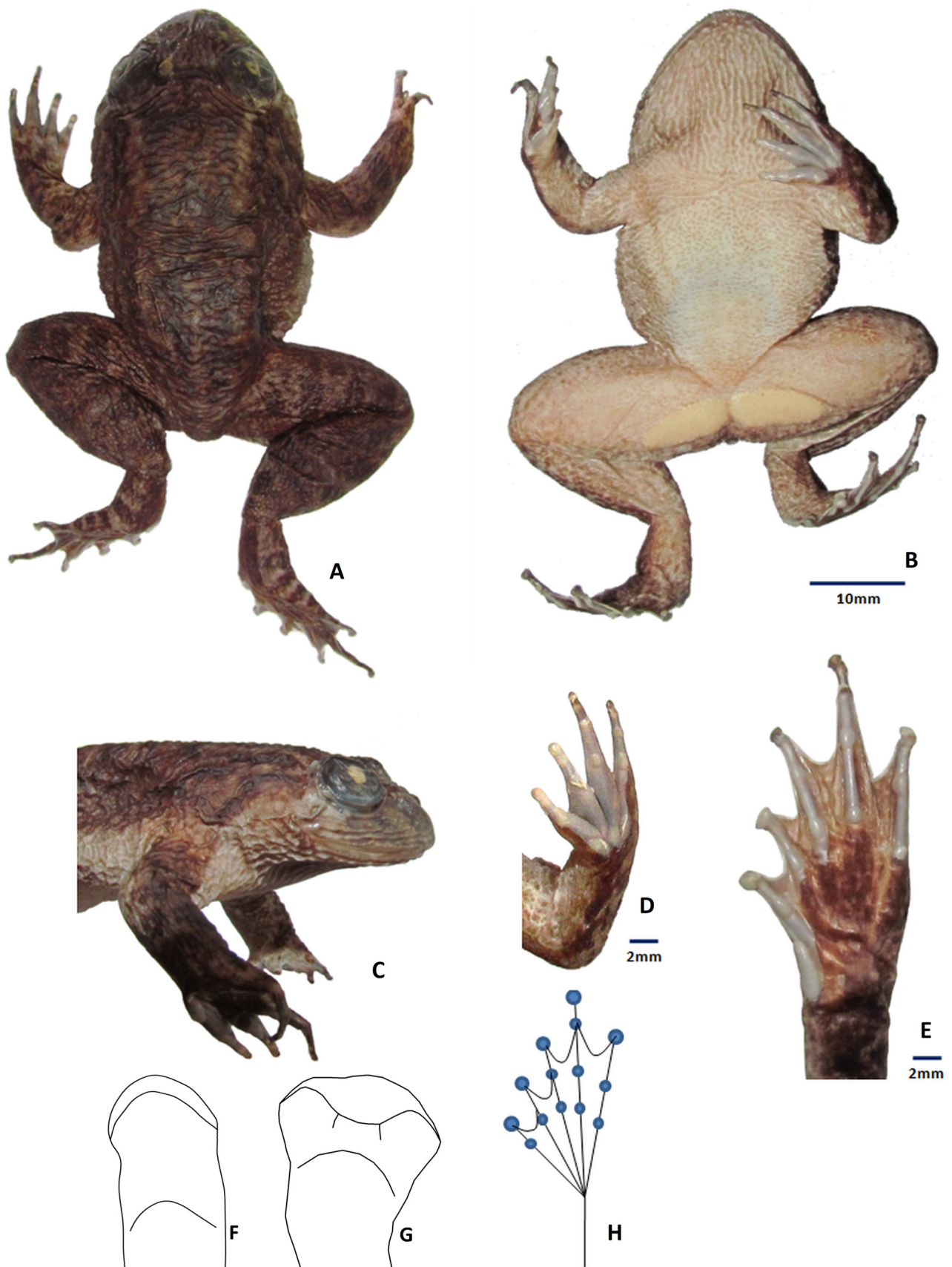


FIGURE 9. Holotype (ZSI/WGFRS/V/A/860) of *Nyctibatrachus kumbara* sp. nov. A—Dorsal view; B—Ventral View; C—Lateral profile of head; D—Ventral view of Forelimb; E—Ventral view of Hindlimb; F—Dorsal view of third finger; G—Dorsal view of fourth toe and H—Schematic view of webbing in hindlimb.



FIGURE 10. Co-occurring congeneric (COCG) species of *Nyctibatrachus* in Kathalekan. a. *Nyctibatrachus kempholeyensis*, b. *N. jog* and c. *Nyctibatrachus kumbara* **sp. nov.**

Distribution and Natural History. Distribution of *Nyctibatrachus kumbara* **sp. nov.** is here confirmed from the streams and rivulets of the Tunga, Sharavathi, Aghanashini and Bedthi rivers (between 13.60515°N–14.61582°N and 74.75576°E–75.320638°E) in Shimoga and Uttara Kannada districts of South India (Figure 1). Based on our field observations, this species is locally abundant in the surroundings of the type locality and is very much restricted to perennial streams of the evergreen and semi-evergreen forests.

Comparison. We compared *Nyctibatrachus kumbara* **sp. nov.** with all 27 valid species of *Nyctibatrachus* based on either the examination of holotypes or non-type vouchers collected from their respective type localities (listed in Appendix 2).

Possession of a combination of characters like; medium to large size; irregular dorsal glandular skin corrugations without specific pattern and spiny projections on the anterior half; third finger disc with dorso-terminal groove, cover notched distally; fourth toe disc with dorso-terminal groove cover bifurcate distally; webbing medium; nuptial pad and femoral glands present in adult males, and egg laying with handstand oviposition and covering of eggs with mud, are unique to *Nyctibatrachus kumbara* **sp. nov.**

Detailed morphological comparisons for *Nyctibatrachus kumbara* **sp. nov.** is made only for sympatric species. But other allopatric congeners are compared with a suite of characters, namely, SVL, third finger disc, fourth toe disc, femoral gland in male and webbing in foot (Table 3).

Genetically *Nyctibatrachus kumbara* **sp. nov.** belongs to *N. sanctipalustris* clade (Figure 7). The other species included in the *N. sanctipalustris* clade differ from the *Nyctibatrachus kumbara* **sp. nov.** as follows: *N. dattatreyaensis* is smaller (SVL 36.2 to 42.3 mm), third finger disc without dorso-terminal groove, fourth toe disc with dorso-terminal groove cover notched distally; *N. vrijeuni* is smaller (SVL 37.4 to 43.1 mm), third finger disc without dorso-terminal groove, fourth toe disc with dorso-terminal groove cover notched distally; *N. shiradi* is smaller (SVL 18.8 to 27.5 mm), with small webbing, fourth toe disc with dorso-terminal groove cover notched distally; *N. sanctipalustris* is smaller (SVL 25.7 to 37.6 mm), third finger disc without dorso-terminal groove, fourth

toe disc with dorso-terminal groove cover notched distally; *N. karnatakaensis* is larger (SVL 56.0 to 63.8 mm), with extensive webbing, third finger disc with dorso-terminal groove cover rounded distally, fourth toe disc with dorso-terminal groove cover rounded distally. *Nyctibatrachus kumbara* **sp. nov.** exhibited 3.41–4.62% genetic divergence to these species (Table 1S).

Nyctibatrachus kumbara **sp. nov.** is syntopic with *N. kempholeyensis* (Figure 10a) and *N. jog* (Figure 10b) but can be distinguished in the field from *N. kempholeyensis* in having medium to large adult size SVL 42.8 to 47.4 mm (vs small adult size SVL 15.5 to 24.4mm in *N. kempholeyensis*), finger and toe disc weak to moderately developed (vs finger and toe disc well developed in *N. kempholeyensis*), glandular dorsum without any spiny projections in the anterior half, glandular corrugations irregular without specific pattern (vs less wrinkled dorsal skin with prominent granular projections in *N. kempholeyensis*), webbing medium (vs webbing small in *N. kempholeyensis*), egg clutches covered with mud (vs eggs without mud packing in *N. kempholeyensis*), eggs are laid at the edges of the slow flowing streams (vs eggs are laid at the slushy edges of streams in *N. kempholeyensis*).

Nyctibatrachus kumbara **sp. nov.** can be distinguished in the field from *N. jog* in having medium to large adult size SVL 42.8 to 47.4 mm (vs medium adult size SVL 33.1 to 39.0 mm in *N. jog*), finger and toe disc weak to moderately developed (vs finger and toe discs well developed in *N. jog*), glandular dorsum without any spiny projections in the anterior half, glandular corrugations irregular without specific pattern (vs relatively weakly wrinkled dorsal skin with prominent glandular projections in *N. jog*), egg clutches with 8 eggs and are covered with mud (vs egg clutches with 52 eggs without mud packing in *N. jog*), eggs are laid at the edges of the slow flowing streams (vs eggs are laid at the edges and above the flowing streams in *N. jog*).

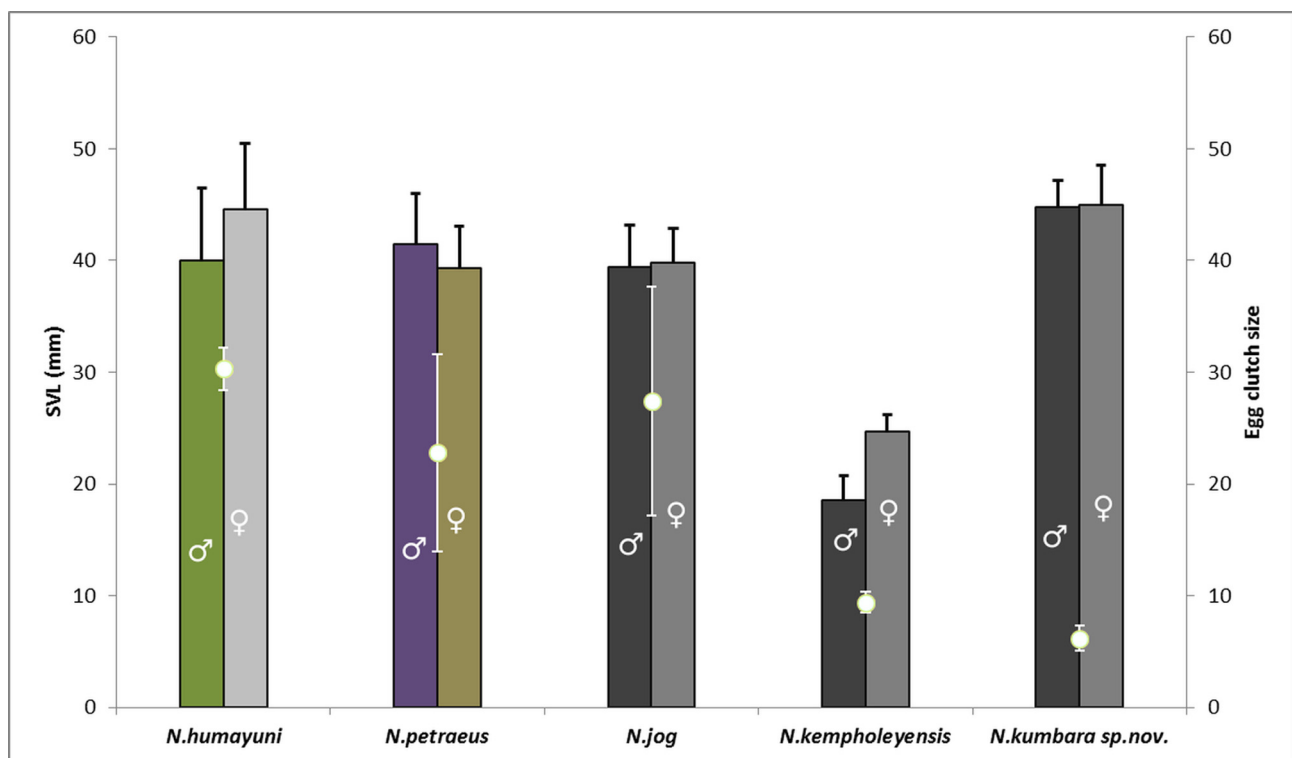


FIGURE 11. Female and Male SVL (Mean±Sd, in mm) and clutch size (Mean±Sd) in three allopatric (*N. humayuni*, *N. petraeus* and *N. jog*) and three sympatric (*N. jog*, *N. kempholeyensis* and *Nyctibatrachus kumbara* **sp. nov.**) species of *Nyctibatrachus*.

Reproductive character displacement. Reproductive characteristics of 13 species of *Nyctibatrachus* are presented in Table 4. Detailed reproductive characteristics were available for *N. humayuni*, *N. petraeus*, *N. jog* (*N. humayuni* clade, spatially non-overlapping and allopatric, as evidenced from the genetic tree in Figure 7), *N. kempholeyensis* and *Nyctibatrachus kumbara* (spatially overlapping and sympatric). On comparing reproductive characters between these two groups, we found less difference among the allopatric species and significant difference between the sympatric species with respect to SVL (Figure 11). Table 5 details unpaired one tailed T test to compare allopatric and sympatric species. The remaining reproductive characters were subjected to non-metric

TABLE 3. Morphological variations among the species of *Nyctibatrachus* (*third finger and fourth toe disc as seen under the microscope Leica MZ75 (6.3 magnification under 1.0x objective)).

Species	SVL (mm, maximum size of ♂ and ♀)	*Third finger disc	*Fourth toe disc	Femoral gland in adult male	Webbing
<i>N. acanthoderms</i>	49.7 to 66.2	without groove	with dorso-terminal groove, cover rounded distally	Present	Medium
<i>N. aliciae</i>	20.5 to 32.0	with dorso-terminal groove, cover notched distally	with dorso-terminal groove cover bifurcate distally	Present	Medium
<i>N. anamallaiensis</i>	13.1 to 17.4	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Present	Absent
<i>N. beddomii</i>	13.3 to 18.0	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Present	Absent
<i>N. danieli</i>	24.9 to 35.1	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Absent	Medium
<i>N. dattatreyaensis</i>	36.2 to 42.3	without dorso-terminal groove	with dorso-terminal groove, cover notched distally	Present	Medium
<i>N. deccanensis</i>	17.4 to 26.2	without dorso-terminal groove	with dorso-terminal groove cover bifurcate distally	Present	Rudiment
<i>N. deveni</i>	22.6 to 33.6	with dorso-terminal groove, cover notched distally	with dorso-terminal groove, cover bifurcate distally	Absent	Medium
<i>N. gavi</i>	49.5 to 60.1	without dorso-terminal groove	with dorso-terminal groove, cover rounded distally	Present	Medium
<i>N. grandis</i>	56.8 to 76.9	without dorso-terminal groove	with dorso-terminal groove, cover rounded distally	Present	Medium
<i>N. humayuni</i>	32.8 to 50.6	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Present	Medium
<i>N. indraneili</i>	42.5 to 50.8	without groove	without groove	Present	Medium
<i>N. jog</i>	33.1 to 39.0	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Present	Medium
<i>N. karnatakensis</i>	56.0 to 63.8	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Present	Extensive
<i>N. kempholeyensis</i>	15.5 to 24.4	with dorso-terminal groove, cover rounded distally	with dorso-terminal groove, cover rounded distally	Present	Small

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TABLE 3. (Continued)

Species	SVL (mm, maximum size of ♂ and ♀)	*Third finger disc	*Fourth toe disc	Femoral gland in adult male	Webbing
<i>Nyctibatrachus kumbara</i> sp. nov.	42.8 to 47.4	with dorso-terminal groove cover notched distally	with dorso-terminal groove, cover bifurcate distally	Present	Medium
<i>N. major</i>	31.5 to 54.2	without dorso-terminal groove	with dorso-terminal groove, covers rounded distally	Present	Medium
<i>N. minimus</i>	10.0 to 14.8	with dorso-terminal groove, cover bifurcate distally	with dorso-terminal groove, cover bifurcate distally	Present	Absent
<i>N. minor</i>	15.4 to 20.5	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Absent	Absent
<i>N. periyar</i>	24.2 to 29.9	with dorso-terminal groove, cover notched distally	with dorso-terminal groove, cover bifurcate distally	Present	Medium
<i>N. petraeus</i>	35.1 to 47.6	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Present	Medium
<i>N. pillaii</i>	20.0 to 25.4	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Absent	Medium
<i>N. poocha</i>	25.3 to 37.5	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Present	Medium
<i>N. sanctipalustris</i>	25.7 to 37.6	without dorso-terminal groove	with dorso-terminal groove, cover notched distally	Present	Medium
<i>N. shiradi</i>	18.8 to 27.5	with dorso-terminal groove cover notched distally	with dorso-terminal groove cover notched distally	Present	Small
<i>N. sylvaticus</i>	27.5 to 36.2	with dorso-terminal groove cover rounded distally	with dorso-terminal groove cover rounded distally	Present	Medium
<i>N. vasanthi</i>	21.9 to 37.0	with dorso-terminal groove cover bifurcate distally	with dorso-terminal groove cover bifurcate distally	Present	Medium
<i>N. vrijeuni</i>	37.4 to 43.1	without dorso-terminal groove	with dorso-terminal groove, cover notched distally	Present	Medium

TABLE 4. Egg clutch size, amplexus, egg attendance and oviposition site in the genus *Nyctibatrachus*. (* personal observations)

Species	Clutch size	Amplexus	Mud covering Behavior	Egg attendance by parent	Oviposition site
<i>N. aliciae</i>	17	Loose amplexus (Biju <i>et al.</i> 2011)	Absent*	Present (both male and female (Biju <i>et al.</i> 2011))	Over hanging leaf and wet rock ~60cm above the stagnant and flowing water (Biju <i>et al.</i> 2011)
<i>N. danieli</i>	40*	-	Absent*	Present (only male)*	Over hanging leaf and wet rocks up to ~150cm above the running water*
<i>N. grandis</i>	-	-	-	Present (both male and female (Biju <i>et al.</i> 2011))	Wet rocks (Biju <i>et al.</i> 2011)
<i>N. humayuni</i>	38*	Loose abbreviated amplexus (Biju <i>et al.</i> 2011; Narahari <i>et al.</i> 2011)	Absent	Present (male only (Narahari <i>et al.</i> 2011))	Over hanging leaf and wet rocks up to ~150cm above the running water (Narahari <i>et al.</i> 2011)
<i>N. jog</i>	52*	Loose amplexus (Biju <i>et al.</i> 2011)	Absent*	Present (both male and female, (Biju <i>et al.</i> 2011); only male*)	Over hanging leaf, wet rocks and tree bark up to ~150cm above the running water*
<i>N. karnatakaensis</i>	25*	-	Absent*	Present (only male)*	On the rock next to flowing water up to ~30cm*
<i>N. kempholeyensis</i>	10*	Axillary*	Absent*	Present (only male)*	Over hanging leaf and dry leaf litter (<30cm above running/slushy water)*
<i>Nyctibatrachus kumbara</i> sp. nov.	8*	Axillary*	Present*	Present (only male)*	On to the rocks and roots at the bank of slow flowing streams (4 to 10 cm above water surface)*
<i>N. major</i>	17*	-	Absent*	Present (male)*	On to the rocks next to flowing stream (<30cm)*
<i>N. minor</i>	-	Loose amplexus (Biju <i>et al.</i> 2011)	-	-	Below moist dead leaves in water slush (Biju <i>et al.</i> 2011)
<i>N. periyar</i>	27*	-	Absent*	Present (only male)*	On to the rock next to water spills in the flowing water*
<i>N. petraeus</i>	35	Lack of amplexus (Kunte 2004)	absent	Present (only male)	Over hanging leaf, tree bark and wet rocks up to ~150cm above the running water
<i>N. vrijeuni</i>	-	-	-	Present (only male)	On the wet rocks next to slow flowing streams (Biju <i>et al.</i> 2011)

multidimensional analysis. Figure 12 illustrates the NMDS plot based on SVL (male and female), maximum number of eggs in a clutch, type of amplexus (absence=0, loose abbreviated=1, axillary=2), parental care (absent=0, by male=1, by both male and female=2), mud covering behavior (absence=0, presence=1), oviposition site (<10cm from water=1, >10 and <30 cm from water=2, >30cm from water=3) and position of male and female at the time of oviposition (upright=1, handstand=2) among the overlapping and non-overlapping groups (stress value = 0.11). There is a clear clustering of allopatric species of *Nyctibatrachus* to sympatric species (Figure 12).

For the co-occurring congeneric (COCG) species call characteristic features were subjected to statistical analysis. Overall call characteristic features (duration and peak frequency) between all COCG species were significantly different (ANCOVA, $F=669.1$, $P<0.00001$). On pairwise comparison of call characteristics of COCG species, except for *N. jog* and *N. kumbara* (ANCOVA, $F<1$, $p>0.5$), remaining COCG species exhibited significant difference (Table 6). Amplexus in *N. jog* is loose amplexus, while in *N. kempholeyensis* (KVG personal observation) and in *N. kumbara* it is axillary. Oviposition site for *N. jog* is wet rock surfaces, overhanging leaves and tree bark above 15 cm to ~200 cm from the bottom of stream, but it was only 4–10 cm for *N. kumbara* and within 30 cm for *N. kempholeyensis*. Egg clutch size significantly differed among COCG species (Table 5).

Most significant reproductive character displacement is in terms of parental care. *Nyctibatrachus kumbara* covers eggs with mud, while *N. jog* and *N. kempholeyensis* do not cover the eggs, but attend the egg clutches. In terms of breeding period, again there is an overlap between *N. kumbara* and *N. kempholeyensis*, but they differ from *N. jog* which breeds only during monsoon (KVG personal observation).

TABLE 5. Comparison of SVL (in mm, male and female) of allopatric and sympatric species of *Nyctibatrachus* using unpaired one tailed T-test with Bonferroni corrections for repeated measures. Numbers in parenthesis are p-values. Area in grey indicate allopatric species. NH_M—*Nyctibatrachus humayuni* male, NH_F—*N. humayuni* female, NP_M—*N. petraeus* male, NP_F—*N. petraeus* female, NJ_M—*N. jog* male, NJ_F—*N. jog* female, NK_M—*N. kempholeyensis* male NK_F—*N. kempholeyensis* female, NKU_M—*Nyctibatrachus kumbara* **sp. nov.** male, NKU_F—*Nyctibatrachus kumbara* **sp. nov.** female.

	NH_F n=8	NP_M n=11	NP_F n=8	NJ_M n=13	NJ_F n=9	NK_M n=11	NK_F n=9	NKU_M n=6	NKU_F n=5
NH_M n=9	1.53 (0.15)	0.57 (0.58)	0.27 (0.79)	0.28 (0.78)	0.1 (0.92)	9.64 (<0.001)	7.00 (<0.001)	2.01 (0.07)	1.86 (0.09)
NH_F		1.25 (0.23)	2.13 (0.06)	2.25 (<0.05)	2.07 (0.06)	11.97 (<0.001)	9.33 (<0.001)	0.07 (0.95)	0.14 (0.90)
NP_M			1.12 (0.28)	1.23 (0.23)	0.99 (0.34)	15.11 (<0.001)	11.54 (<0.001)	1.95 (0.07)	1.67 (0.13)
NP_F				0.01 (0.99)	0.27 (0.79)	14.02 (<0.001)	10.32 (<0.001)	3.28 (<0.01)	2.73 (0.02)
NJ_M					0.3 (0.77)	16.81 (<0.001)	12.66 (<0.001)	3.77 (0.002)	2.97 (0.02)
NJ_F						17.3 (<0.001)	13.15 (<0.001)	3.49 (0.004)	2.75 (0.03)
NK_M							7.44 (<0.001)	22.19 (<0.001)	15.49 (<0.001)
NK_F								18.29 (<0.001)	12.28 (<0.001)
NKU_M									0.11 (0.91)

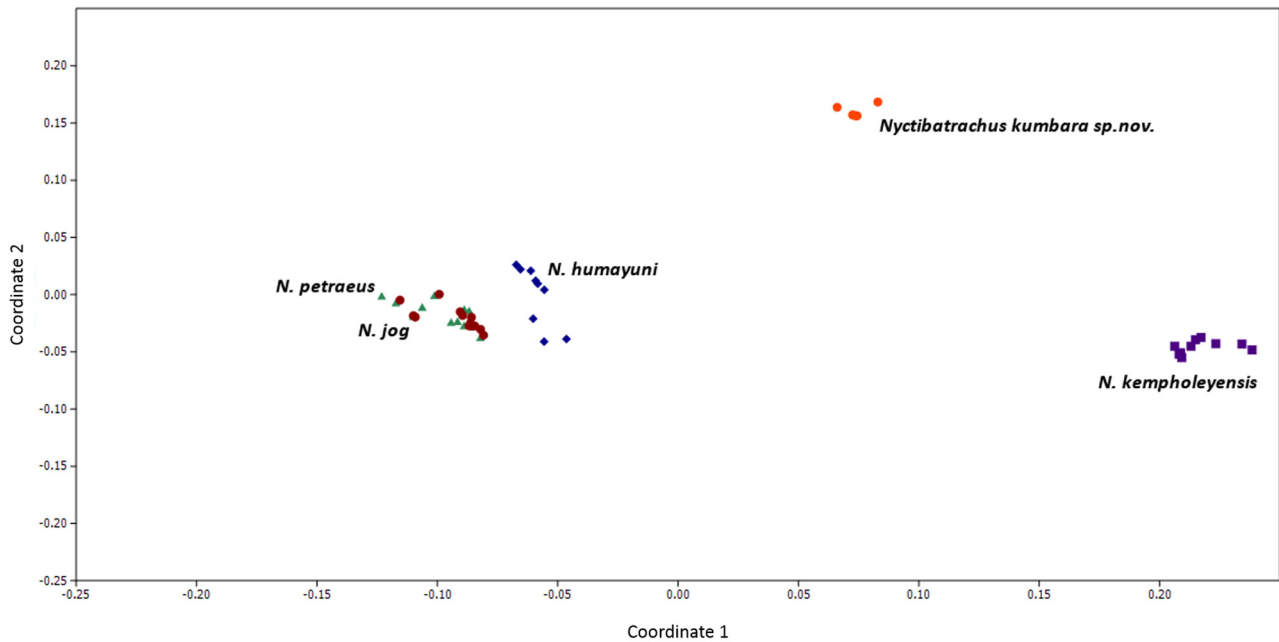


FIGURE 12. Non-metric multidimensional scale (NMDS) analysis of allopatric and sympatric species of *Nyctibatrachus* based on SVL (both male and female), maximum number of eggs in a clutch, type of amplexus, parental care, mud covering behavior, oviposition site and position of male and female at oviposition. Allopatric species namely *N. humayuni*, *N. petraeus* and *N. jog* are clustered in one group, while *N. kempholeyensis* and *Nyctibatrachus kumbara sp. nov.* forms two distinct clusters.

TABLE 6. Size corrected pairwise comparison of call characteristic features of COCG species using ANCOVA. p-values in parenthesis. NJ_pf/du—*N. jog* peak frequency and duration; NK_pf1/du1—*N. kempholeyensis* peak frequency and duration for call Type I; NK_pf2/du2—*N. kempholeyensis* peak frequency and duration for call Type II; NKU_pf1/du1—*Nyctibatrachus kumbara sp. nov.* peak frequency and duration for single call; NKU_pf2/du2—*Nyctibatrachus kumbara sp. nov.* peak frequency and duration for double call.

	NK_pf1/du1	NK_pf2/du2	NKU_pf1/du1	NKU_pf2/du2
NJ_pf/du	863.8 (<0.01)	0.4 (0.54)	0.2 (0.65)	0.01 (0.97)
NK_pf1/du1		529.7 (<0.01)	354.7 (<0.01)	630.2 (<0.01)
NK_pf2/du2			0.06 (0.82)	0.28 (0.61)
NKU_pf1/du1				0.09 (0.77)

Discussion

Anurans exhibit diverse reproductive strategies to overcome the limitation posed by external or internal fertilisation (Beck 1998), selective pressure of predators on aquatic eggs and tadpoles (Prado *et al.* 2005), prevailing environmental conditions both in space and time; and as a part of evolutionary process (Jameson 1955; Duellman & Trueb 1994; Wells 2007; Pfenning & Stewart 2010). Also r- and K-selection (MacArthur & Wilson 1967) influence the reproductive strategies in anurans (Nichols *et al.* 1976). However, according to Stearns (1992) the concept of r- and K-selection is less meaningful due to varied range in life-history strategies seen in nature and does not make precise predictions. In the present study, we compared three co-occurring species of *Nyctibatrachus*, namely *N. jog*, *N. kempholeyensis* and *N. kumbara* and three allopatric species, namely *N. humayuni*, *N. petraeus* and *N. jog* (Figure 10). *Nyctibatrachus jog* is allopatric with the *N. humayuni* clade, however it is sympatric with *N. kempholeyensis* and *N. kumbara*.

Significance of courtship. Mate identification through tactile cues is an important non-vocal signal in anurans (Duellman & Trueb 1994). Male individuals of *N. kumbara* called from a fixed site and amplexed with an approaching female, which is similar to the species of *Bombina*, *Discoglossus*, *Pelobates*, *Scaphiopus*, *Bufo* and *Rana* (Wells 2007). In *N. kumbara* the courtship behaviour exhibited by males and females by touching each other is perhaps to recognise the potential mate and to gauge the size of both individuals. However, all this remain very speculative and calls for further studies to ascertain the behaviour.

Significance of mud pack on egg clutches. Parental care in amphibians is the energy investment into offspring after fertilisation (for details refer to Wells 2007). Egg attendance by male individuals has been attributed to protection of eggs from being damaged by intruding males, and deterring egg predation and preventing desiccation (in *Hyalinobatrachium valerioi* (Vockenhuber *et al.* 2009)). Parental care involving both male and female, only females and only males are well known (Wells 2007). So far, there is no report of any other anuran species covering egg clutch with mud. Mud packing on egg clutches by *N. kumbara* might be attributed to, 1. minimising clutch dehydration as the outer capsule of terrestrial amphibian eggs are susceptible to dehydration by losing moisture to drier air (Duellman & Trueb 1994), 2. camouflage eggs as the eggs are very prominently pigmented to avoid predators (Crump 1995; Wells 2007).

Reproductive Character Displacement. Observed reproductive characters in *N. jog*, *N. humayuni* and *N. petraeus* are similar with loose to abbreviated amplexus (Kunte 2004; Narahari *et al.* 2011; Biju *et al.* 2011, detailed in Table 4). They belong to the *N. humayuni* clade from the Northern Western Ghats (Bocxlaer *et al.* 2012 and Figure 7). The minimal aerial distance between the known populations of this clade are *N. jog*-*N. petraeus* is 97 km; *N. jog*-*N. humayuni* is 331 km and *N. petraeus*-*N. humayuni* is 164 km (distance calculated from the nearest verified population of the respective species using Google Earth). However, information on reproductive characters from other clades of *Nyctibatrachus* from the Western Ghats is lacking. Despite such limitation, on comparing reproductive characters between spatially non-overlapping allopatric to overlapping sympatric clades, we found differences in reproductive characters among the spatially overlapping taxa and reproductive character similarities in spatially non-overlapping taxa in a suite of character, which could be an indication for reproductive character displacement.

Significance of new species. Taxonomy for the genus *Nyctibatrachus* has been stabilised in the recent past (Das & Kunte 2005; Dinesh *et al.* 2007; Dinesh *et al.* 2008b) and a complete revision with the description of twelve new species has been recently made by Biju *et al.* (2011) providing baseline for comparisons across taxa. The discovery of *N. kumbara* reiterates that there is need for systematic sampling across the Western Ghats for providing a comprehensive listing of amphibian species in the region. For delimiting species ranges and to appreciate mountain associated clade endemism in *Nyctibatrachus*, sampling has to be very systematic covering altitudinal, latitudinal and longitudinal gradients. Although *N. kumbara* **sp. nov.** is distinct from known species of *Nyctibatrachus* in morphology, acoustics, breeding behavior and single gene genetic study, we still warrant multiple gene phylogenetic studies on *N. kumbara* to substantiate its specific phylogenetic relationship with the other members of *Nyctibatrachus*. Apart from taxonomy and phylogeny, we stress the importance of studies on breeding behaviour, ecology and parental care in amphibians of the Western Ghats, which would provide a holistic and inclusive approach to understand the evolutionary significance of anurans in the Western Ghats.

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APPENDIX 1. GenBank accession numbers for the 16s rDNA of congeneric species of *Nyctibatrachus* in the Western Ghats. Species identity to the respective gene is referred to Bossuyt and Milinkovitch (2000), Biju et al. (2011) and Bocxlaer et al. (2012).

Species	GenBank Accession #	Reference
<i>N. acanthodermis</i>	JN644915	Bocxlaer et al. 2012
<i>N. aliciae</i>	JN644893	Bocxlaer et al. 2012
<i>N. anamallaiensis</i>	JN644899	Bocxlaer et al. 2012
<i>N. beddomii</i>	JN644913	Bocxlaer et al. 2012
<i>N. danieli</i>	JN644902	Bocxlaer et al. 2012
<i>N. dattatreyaensis</i>	JN644891	Bocxlaer et al. 2012
<i>N. deccanensis</i>	JN644911	Bocxlaer et al. 2012
<i>N. deveni</i>	JN644895	Bocxlaer et al. 2012
<i>N. gavi</i>	JN644918	Bocxlaer et al. 2012
<i>N. grandis</i>	JN644904	Bocxlaer et al. 2012
<i>N. humayuni</i>	JN644901	Bocxlaer et al. 2012
<i>N. indraneili</i>	JN644909	Bocxlaer et al. 2012
<i>N. jog</i>	JN644900	Bocxlaer et al. 2012
<i>N. karnatakaensis</i>	JN644910	Bocxlaer et al. 2012
<i>N. kempholeyensis</i>	JN644903	Bocxlaer et al. 2012
<i>N. major</i>	AF249052	Bossuyt and Milinkovitch 2000
<i>N. minimus</i>	JN644896	Bocxlaer et al. 2012
<i>N. minor</i>	JN644908	Bocxlaer et al. 2012
<i>N. periyar</i>	JN644897	Bocxlaer et al. 2012
<i>N. petraeus</i>	JN644912	Bocxlaer et al. 2012
<i>N. pillaii</i>	JN644892	Bocxlaer et al. 2012
<i>N. poocha</i>	JN644907	Bocxlaer et al. 2012
<i>N. sanctipalustris</i>	JN644917	Bocxlaer et al. 2012
<i>N. shiradi</i>	JN644898	Bocxlaer et al. 2012
<i>N. sylvaticus</i>	JN644916	Bocxlaer et al. 2012
<i>N. vasanthi</i>	JN644894	Bocxlaer et al. 2012
<i>N. vrijeuni</i>	JN644905	Bocxlaer et al. 2012

APPENDIX 2. Museum specimens examined.

N. acanthodermis; ZSI/WGRC/V/A/775; holotype; Kaikatti, Nelliampathy, Palakkad, Kerala.
N. aliciae; ZSI/WGRC/V/A/777; Ponmudi, Thiruvananthapuram, Kerala.
N. beddomei; ZSI/WGRC/V/A/480; Meenmootty, Idduki (dist), Kerala.
N. danieli; ZSI/WGRC/V/A/778; holotype; Humbarli, Koyna, Satara, Maharashtra.
N. dattatreyaensis; ZSI/WGRC/V/A/646; holotype; Datta Peeta, Chikkamagaluru, Karnataka.
N. deccanensis; ZSI/WGRC/V/A/611; holotype; Kolukkumalai, Idukki, Kerala.
N. deveni; ZSI/WGRC/V/A/786; holotype; Kaikatti, Nelliampathy, Palakkad, Kerala.
N. gavi; ZSI/WGRC/V/A/789; holotype; Gavi, Idukki, Kerala.
N. grandis; ZSI/WGRC/V/A/791; holotype; Thirunelly, Wayanad, Kerala.
N. humayuni; ZSIC. Cat. No. 20628; holotype; Mahableshwar, Satara (dist), Maharashtra.
N. indraneili; ZSI/WGRC/V/A/795; holotype; Longwood shola, Kotagiri, Nilagiri, Tamil Nadu.
N. jog; ZSI/WGRC/V/A/796; holotype; Jog Falls, Shimoga, Karnataka.

N. karnatakaensis; ZSI/WGRC/V/A/579; holotype; Manikyadhara Betta, KNP, Chikkamagaluru, Karnataka.
N. kempholeyensis; ZSI/WGRC/V/A/798; neotype; Kempholay, Hassan, Karnataka.
N. major; ZSI/WGRC/V/A/802; Athirimala, Thiruvananthapuram, Kerala.
N. minimus; ZSI/WGRC/V/A/ 671; Kurichiyarmala, Wayanad (dist), Kerala.
N. minor; ZSI/WGRC/V/A/ 596; Mahendra giri, Tamil Nadu.
N. periyar; ZSI/WGRC/V/A/806; holotype; Vallakadavu, Periyar TR, Idukki, Kerala.
N. petraeus; ZSI/WGRC/V/A /610; Kathlaekhan, Uttara Kannada (dist), Karnataka.
N. pillaii; ZSI/WGRC/V/A/808; holotype; Kakachi, Tirunelveli, Tamil Nadu.
N. poocha; ZSI/WGRC/V/A/812; holotype; Munnar, Idukki, Kerala.
N. sanctipalustris; ZSI/WGRC/V/A/198; holotype; Nemankolly, Chikkamagaluru, Karnataka.
N. shiradi; ZSI/WGRC/V/A/815; holotype; Kottigehar, Chikkamagaluru, Karnataka.
N. sylvaticus; ZSI/WGRC/V/A/818; neotype; Kempholay, Hassan, Karnataka.
N. vasanthi; ZSI/WGRC/V/A/ 822; Kakachi, Tirunelveli, Tamil Nadu.
N. vrijeuni; ZSI/WGRC/V/A/824; male; holotype; Suganthagiri, Wayanad, Kerala.

Supplementary information

TABLE 1S. Uncorrected pairwise genetic distance (%) between congeners of Nyctibatrachus described from the Western Ghats based on 570bp of mitochondrial 16S rRNA gene.

	<i>N. kumbara</i> sp. nov.	<i>N. aliciae</i>	<i>N. dattatreyaensis</i>	<i>N. minimus</i>	<i>N. periyar</i>	<i>N. vasanthi</i>	<i>N. pillaii</i>	<i>N. major</i>	<i>N. acanthodermis</i>
<i>N. aliciae</i>	8.84								
<i>N. dattatreyaensis</i>	3.41	3.41							
<i>N. minimus</i>	9.44	9.44	9.04						
<i>N. periyar</i>	7.83	7.83	3.61	9.84					
<i>N. vasanthi</i>	8.43	8.43	7.63	8.63	10.24				
<i>N. pillaii</i>	8.03	8.03	3.21	7.83	9.44	8.03	7.83		
<i>N. major</i>	7.43	7.43	10.24	7.43	10.44	10.64	9.84	10.24	
<i>N. acanthodermis</i>	7.03	7.03	10.04	7.03	9.64	9.84	8.84	9.44	3.61
<i>N. anamallaiensis</i>	8.63	8.63	8.43	9.44	10.84	9.44	8.84	8.63	9.24
<i>N. beddomii</i>	8.23	8.23	7.43	8.63	9.44	7.23	8.63	7.03	10.04
<i>N. danieli</i>	9.24	9.24	10.84	9.24	10.84	10.84	9.24	10.04	9.44
<i>N. decanensis</i>	8.23	8.23	8.03	8.63	9.44	8.43	8.23	8.23	9.44
<i>N. gavi</i>	7.23	7.23	10.24	7.63	9.04	9.44	8.63	9.04	3.82
<i>N. grandis</i>	7.03	7.03	10.04	7.23	9.24	9.44	9.24	9.24	4.22
<i>N. humayuni</i>	9.84	9.84	10.84	10.24	10.44	10.24	9.84	9.24	10.04
<i>N. indranelli</i>	6.43	6.43	9.24	6.63	8.84	8.84	9.24	9.24	6.22
<i>N. jog</i>	10.84	10.84	10.84	10.84	10.64	11.85	9.84	10.24	9.84
<i>N. karnatakaensis</i>	4.62	4.62	10.04	4.42	9.64	8.63	9.24	9.04	8.23
<i>N. kempholeyensis</i>	7.43	7.43	9.84	6.83	8.63	8.84	9.84	8.23	7.83
<i>N. minor</i>	9.44	9.44	8.43	9.64	8.43	8.43	7.63	7.63	9.84
<i>N. petraeus</i>	10.64	10.64	11.24	11.04	10.24	11.85	9.04	10.64	10.04
<i>N. poocha</i>	8.43	8.43	7.43	8.03	8.84	8.23	6.83	6.83	9.24
<i>N. sanctipalustris</i>	4.62	4.62	9.04	4.42	10.44	9.64	9.64	8.03	8.03
<i>N. shiradi</i>	4.02	4.02	9.64	4.42	9.84	9.44	8.03	8.23	9.04
<i>N. sylvaticus</i>	6.83	6.83	9.84	7.23	9.04	9.04	8.84	8.84	4.42
<i>N. vrijeuni</i>	4.02	4.02	9.84	4.42	9.64	9.24	8.63	8.03	8.43
<i>N. deveni</i>	7.83	7.83	4.02	8.43	10.04	1.61	8.03	3.61	10.44

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TABLE 1S. (continued)

	<i>N. anamallaiensis</i>	<i>N. beddomii</i>	<i>N. danieli</i>	<i>N. deccanensis</i>	<i>N. gavi</i>	<i>N. grandis</i>	<i>N. humayuni</i>	<i>N. indranelli</i>	<i>N. jog</i>
<i>N. anamallaiensis</i>	8.43								
<i>N. beddomii</i>	8.84	6.83							
<i>N. danieli</i>	8.43	9.04	8.43						
<i>N. deccanensis</i>	9.04	7.43	7.23	9.44					
<i>N. gavi</i>	1.41	8.84	9.04	7.83	8.63				
<i>N. grandis</i>	2.01	8.63	9.04	8.63	8.63	2.41			
<i>N. humayuni</i>	8.43	7.83	8.63	4.82	8.23	7.63	9.04		
<i>N. indranelli</i>	5.02	9.24	9.24	9.44	8.23	5.62	5.62	9.44	
<i>N. jog</i>	8.63	8.63	9.84	3.61	9.24	7.83	9.24	3.82	9.84
<i>N. karnatakaensis</i>	7.43	8.84	8.63	9.44	9.44	7.23	7.63	9.84	7.23
<i>N. kenpholeyensis</i>	8.03	10.04	8.84	8.84	9.64	7.83	8.23	10.04	7.83
<i>N. minor</i>	9.04	8.63	8.23	8.43	5.82	8.43	9.24	8.23	8.63
<i>N. petraeus</i>	8.84	8.84	9.44	3.41	9.44	8.03	9.44	3.82	10.44
<i>N. poocha</i>	8.03	8.23	7.43	8.03	7.03	7.63	7.83	8.43	8.43
<i>N. sanctipalustris</i>	8.03	8.84	8.43	9.44	7.83	7.83	8.43	9.84	7.83
<i>N. shiradi</i>	8.63	9.84	9.64	10.24	8.84	8.63	9.04	9.84	8.23
<i>N. sylvaticus</i>	2.61	8.63	8.23	8.84	9.24	3.01	1.81	8.84	5.82
<i>N. vrijeuni</i>	8.43	10.04	9.44	10.04	9.04	8.23	8.43	10.04	8.03
<i>N. deveni</i>	10.04	9.24	7.63	11.24	8.23	9.64	9.84	9.84	9.44

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TABLE 1S. (continued)

	<i>N. karnatakaensis</i>	<i>N. kempholeyensis</i>	<i>N. minor</i>	<i>N. petraeus</i>	<i>N. poocha</i>	<i>N. sancipalustris</i>	<i>N. shiradi</i>	<i>N. sylvaticus</i>	<i>N. vrijeuni</i>	<i>N. deventi</i>
<i>N. karnatakaensis</i>	10.84									
<i>N. kempholeyensis</i>	10.44	7.23								
<i>N. minor</i>	8.63	8.84	8.43							
<i>N. petraeus</i>	1.20	10.64	10.44	8.23						
<i>N. poocha</i>	8.84	8.03	7.83	6.43	8.43					
<i>N. sancipalustris</i>	10.04	3.82	7.63	8.23	10.24	8.43				
<i>N. shiradi</i>	11.04	4.42	7.23	8.43	10.84	8.23	4.02			
<i>N. sylvaticus</i>	9.44	6.83	8.03	8.03	9.24	7.63	7.43	8.03		
<i>N. vrijeuni</i>	11.04	4.02	6.63	8.63	10.84	8.43	4.02	1.41	7.43	
<i>N. deventi</i>	11.85	9.04	8.84	8.63	12.25	8.63	9.24	8.84	9.24	8.63

TABLE 2S. Some morphometric data (in mm) for the type series of *Nyctibatrachus kumbara* sp. nov. Prefix ZSI/WGFRS/V/A for holotype and paratypes (* Holotype, # Paratype).

Reg. No.	860*	862#	864#	Mean ± SD (range)	861#	863#	Mean ± SD
Sex	Male	Male	Male		Female	Female	
SVL	46.2	47.4	46	46.5±0.7 (46.0–47.4)	43.3	42.8	43.0±0.3
EL	5.7	6	5.7	5.8±0.2 (5.7–6.0)	5.1	5.4	5.3±0.2
EN	2.9	3.5	3.7	3.4±0.4 (2.9–3.7)	3.5	3.7	3.6±0.1
HL	15	14.8	15.7	15.1±0.5 (14.8–15.7)	14.6	15.3	15.0±0.6
HW	18.7	20.3	20.9	20.0±1.1 (18.7–20.9)	19.7	19.1	19.5±0.4
HD	11.2	12.3	13.7	12.4±1.3 (11.2–13.7)	11.8	11.1	11.5±0.5
IBE	14.5	15.5	14.8	14.9±0.5 (14.5–15.5)	13.6	13.4	13.5±0.1
IFE	6.9	7.9	7.7	7.5±0.5 (6.9–7.9)	7.2	7.1	7.1±0.1
IN	4.3	4.3	4.2	4.2±0.1 (4.2–4.3)	4.4	3.8	4.1±0.4
IUE	6.3	5.1	5.4	5.6±0.6 (5.1–6.3)	5.1	5	5.1±0.1
MBE	3.4	4.6	5.2	4.4±1.0 (3.4–5.2)	5.3	4.4	4.8±0.6
MFE	9.6	8.8	8.8	9.1±0.5 (8.8–9.6)	9.1	8.6	8.8±0.3
MN	11.9	12.6	12.4	12.3±0.4 (11.9–12.6)	12.6	10.7	11.6±1.3
NS	3.5	4.5	4.9	4.3±0.7 (3.5–4.9)	3.8	4	3.9±0.2
SL	7.1	7.9	8.2	7.7±0.6 (7.1–8.2)	6.9	6.9	6.9±0.0
UEW	3.1	3.1	2.9	3.0±0.1 (2.9–3.1)	2.7	2.5	2.6±0.2
fd1	0.6	0.9	1.2	0.9±0.3 (0.6–1.2)	0.9	0.9	0.9±0.0
fd2	0.6	0.8	1.1	0.8±0.2 (0.6–1.1)	1.1	0.9	1.0±0.2
fd3	0.6	0.8	1.1	0.9±0.2 (0.6–1.1)	1.1	0.9	1.0±0.1
fd4	0.7	0.8	1	0.8±0.1 (0.7–1.0)	1	1	1.0±0.0
fw1	0.6	0.8	0.9	0.8±0.2 (0.6–0.9)	0.7	0.7	0.7±0.0
fw2	0.6	0.8	1	0.8±0.2 (0.6–1.0)	0.8	0.8	0.8±0.0
fw3	0.6	0.7	1	0.8±0.2 (0.6–1.0)	0.6	0.8	0.7±0.1
fw4	0.6	0.7	0.8	0.7±0.1 (0.6–0.8)	0.6	0.7	0.7±0.1
FFL	3	3.2	3.9	3.4±0.5 (3.0–3.9)	3.3	2.8	3.1±0.3
SFL	3	3.5	3.9	3.5±0.5 (3.0–3.9)	3.8	2.4	3.1±1.0
TFL	5.3	5.6	6.4	5.8±0.6 (5.3–6.4)	6	4.9	5.4±0.8
FrFL	4.6	4.7	5.5	5.0±0.5 (4.6–5.5)	5.1	4.5	4.8±0.4
HAL	10.1	11.6	12.2	11.3±1.1 (10.1–12.2)	11	10.4	10.7±0.4
FLL	10.9	10	9.6	10.2±0.7 (9.6–10.9)	8.1	9.1	8.6±0.7
AG	16.7	15.3	13.7	15.3±1.5 (13.7–16.7)	14.5	14.1	14.3±0.3
BW	19.1	20.3	19.1	19.5±0.7 (19.1–20.3)	17.8	19.2	18.5±1.0
BWG	12.1	13.9	13.5	13.2±0.9 (12.1–13.9)	12.2	14.9	13.5±1.9
FGL	9.2	9.5	9.7	9.5±0.3 (9.2–9.7)	-----	-----	-----

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TABLE 2S. (Continued)

Reg. No.	860*	862#	864#	Mean ± SD (range)	861#	863#	Mean ± SD
Sex	Male	Male	Male		Female	Female	
FGB	3.8	4	5	4.2±0.7 (3.8–5.0)	-----	-----	-----
FL	22.6	22.1	25.6	23.5±1.9 (22.1–25.6)	22.5	21.6	22.1±0.6
FW	8.4	12.1	10.5	10.4±1.9 (8.4–12.1)	10.8	10	10.4±0.5
TL	21.3	19.6	22.1	21.0±1.3 (19.6–22.1)	20.6	19.1	19.8±1.1
TW	8.1	8.9	8.1	8.4±0.5 (8.1–8.9)	8.3	7.8	8.1±0.4
FOL	20.1	20.1	21.9	20.7±1.1 (20.1–21.9)	21.3	19.9	20.6±1.0
TFOL	26.7	27.6	30.6	28.3±2.1 (26.7–30.6)	29.7	27.9	28.8±1.2
T1L	3.5	3.8	4.7	4.0±0.6 (3.5–4.7)	4.1	4.2	4.1±0.1
T2L	3.9	4.1	5.3	4.4±0.7 (3.9–5.3)	4.7	4.2	4.5±0.4
T3L	6.8	6.6	8.7	7.4±1.1 (6.6–8.7)	7.6	7	7.3±0.5
T4L	9.9	9.9	12.3	10.7±1.4 (9.9–12.3)	11.4	10.5	11.0±0.6
T5L	7.2	7.2	8.6	7.7±0.8 (7.2–8.6)	8	7.6	7.8±0.3
td1	1.1	2.1	1.4	1.5±0.5 (1.1–2.1)	1.5	1.2	1.4±0.2
td2	1.1	1.4	1.5	1.4±0.2 (1.1–1.5)	1.4	1.3	1.4±0.1
td3	1.1	1.4	1.6	1.4±0.3 (1.1–1.6)	1.6	1.4	1.5±0.1
td4	2.1	1.4	1.7	1.7±0.3 (1.4–2.1)	1.8	1.3	1.6±0.4
td5	1.1	1.2	1.4	1.3±0.2 (1.1–1.4)	1.1	1.2	1.2±0.1
tw1	0.8	0.8	1	0.8±0.1 (0.8–1.0)	0.8	0.9	0.9±0.1
tw2	0.8	1	1	0.9±0.1 (0.8–1.0)	1	0.8	0.9±0.1
tw3	0.7	1	0.9	0.9±0.1 (0.7–1.0)	0.9	0.9	0.9±0.0
tw4	0.7	0.8	0.8	0.8±0.1 (0.7–0.8)	0.7	0.8	0.8±0.1
tw5	0.7	0.9	1	0.9±0.1 (0.7–1.0)	0.9	0.9	0.9±0.0
IMT	3	3.3	3.9	3.4±0.5 (3.0–3.9)	3.4	3.7	3.5±0.2
FFTF	8.4	7.1	9.4	8.3±1.2 (7.1–9.4)	8.8	8.4	8.6±0.3
MTFF	12.8	14.1	14.7	13.9±1.0 (12.8–14.7)	11.1	12.9	12.0±1.3
MTTF	13.5	13.5	15.1	14.0±0.9 (13.5–15.1)	14.2	13.3	13.7±0.7
TFTF	6.6	6.6	7.8	7.0±0.7 (6.6–7.8)	7.8	6.7	7.3±0.7

Call pattern, breeding behaviour of *Nyctibatrachus kumbara* available in <http://www.gururajakv.net>