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COMMUNICATION

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NYCTIBATRACHUS MEWASINGHI, A NEW SPECIES OF NIGHT FROG (AMPHIBIA: NYCTIBATRACHIDAE) FROM WESTERN GHATS OF KERALA, INDIA

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Abstract: A new species *Nyctibatrachus mewasinghi* is described from the Malabar Wildlife Sanctuary of Western Ghats of Kozhikode District, Kerala. The new species can be distinguished from known congeners based on small adult size, head equal to or slightly wider than long, less wrinkled dorsal skin with prominent granular projections, absence of dorso-lateral glandular folds, a ridge extending from the lip over the tip of the snout to between the nostrils bifurcate posteriorly producing an inverted 'Y', finger and toe discs well developed with dorso-terminal groove, cover rounded distally, third finger disc slightly wider than finger width and fourth toe disc almost equal to or slightly wider than toe width, presence of two palmar tubercles, moderate webbing and thigh, shank and foot almost of equal length. Molecular phylogeny based on two mitochondrial genes (ND1 and 16S rRNA) reveals that the species is genetically distinct from others within the genus, and is a sister taxon to *N. athirappillyensis* and *N. kempholeyensis*. Multivariate morphometric analysis clearly distinguishes the new night frog species *Nyctibatrachus mewasinghi* from *N. athirappillyensis* and *N. kempholeyensis*.

Keywords: 16S rRNA, additional records, discriminant analysis, Malabar Wildlife Sanctuary, molecular phylogeny, ND1 gene, new record, new species, *Nyctibatrachus athirappillyensis*, *N. kempholeyensis*.

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Author Contribution: KK collected the specimens, field data, studied comparative material and generated morphometric data. ND and KK generated molecular data. ND performed the molecular, statistical analysis and diagnosed the species. ND and SM wrote the manuscript with inputs from KK.

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INTRODUCTION

Genus *Nyctibatrachus* is diagnosed based on a combination of characters including small to large body sizes (10.0–77.0 mm snout to vent length); oval or diamond shaped pupil; shagreened, glandular or wrinkled dorsal skin; tympanum not visible externally, or with borders obscured by rugose skin; vomerine teeth present; tongue notched or emarginated posteriorly; tips of fingers with or without discs, discs with or without distinct dorso-terminal grooves; webbing absent on fingers; tips of toes with or without discs, discs with or without dorso-terminal grooves; and subocular gland present on all species (Biju et al. 2011). Although originally considered as a member of the family Ranidae by Boulenger (1882), based on molecular phylogenetic studies by Roelants et al. (2004), Bossuyt et al. (2006) and Frost et al. (2006), this genus is now placed under the family Nyctibatrachidae. The Western Ghats endemic genus *Nyctibatrachus* has 35 known species (Garg et al. 2017).

While studying the distribution of species of *Nyctibatrachus* along the Western Ghats mountain ranges using molecular analysis of mitochondrial NADH dehydrogenase subunit 1 (ND1) and 16S rRNA genes, we discovered a population that is sister taxa to, but genetically divergent from *N. kempholeyensis* and *N. athirappillyensis*. We describe this population as a new species, *Nyctibatrachus mewasinghi*, based on morphology, multivariate morphometric analysis and molecular analysis.

MATERIALS AND METHODS

Specimen collection and museum deposition

Specimens of *Nyctibatrachus* were studied from the Western Ghats of Kerala, Tamil Nadu, Karnataka and Goa, India. Specimen collection was kept to a minimum with collection of mainly road kills, injured or potentially infected individuals. Specimens from protected areas were collected with permissions from the Kerala Forest Department (permit no. WL WL10-3548/2013) and Karnataka Forest Department (permit no. PS/PCCF/WL/CR/22/2013-14). Specimens were preserved in either absolute ethanol or 4% formaldehyde. Studied specimens are in the museum collections of the Bombay Natural History Society (BNHS), Mumbai, and the Wildlife Information Liaison Development (WILD), Coimbatore (Appendix 1).

Morphometry and morphological description

Measurements were taken to the nearest 0.1mm using a digital caliper (Mitutoyo) as defined in Biju et al. (2011), and include: SVL (snout-vent length), HW (head width), HL (head length), MN (distance from rear of mandible to nostril), MFE (distance from rear of mandible to anterior orbital border), MBE (distance from rear of mandible to posterior orbital border), SL (snout length), EL (eye length), IUE (inter upper eyelid width), UEW (maximum upper eyelid width), IFE (internal front of eyes), IBE (internal back of eyes), FAL (forearm length), HAL (hand length), FDIII (disc width of finger III), FWIII (width of finger III), ShL (shank length), TL (thigh length), FOL (foot length), TFOL (distance from heel to tip of fourth toe), TD (disc width of toe IV), TW (width of toe IV). Methods for morphological descriptions follow Biju et al. (2011) for ease of comparison. Webbing formula was determined following the method provided by Savage & Heyer (1967) with modifications by Myers & Duellman (1982).

Statistical analysis

All the measurements showed a positive linear relationship with SVL. Thus to remove the effect of size, morphometric data were normalized by expressing measurements as a ratio of SVL. Apart from the *N. kempholeyensis* and *N. athirappillyensis* comparative material (see Appendix 1) we also used data from Biju et al. (2011) and Garg et al. (2017). Discriminant analysis (DA) was performed to understand whether related species of *Nyctibatrachus* form significantly different clusters (Huberty & Olejnik 2006). Statistical analysis was performed with PAST 3.09 (Hammer et al. 2001).

Molecular analysis

Thigh muscle tissue was harvested from 10 specimens (WILD-13-AMP-144, 341, WILD-14-AMP-400, 403, 431, WILD-15-AMP-561, 623, 624, 625 and BNHS 5983). DNA was extracted using QIAamp DNA Mini Kit (QIAGEN, USA) following manufacturer's protocol. Mitochondrial ND1 gene sequence was amplified using the primer pair NDH-L (5'-AAA CTA TTT AYY AAA GAR CC-3') and NDH-M (5'-GGG TAT GAN GCT CGN ACT CA-3') (Roelants & Bossuyt 2005), and 16S rRNA gene was amplified using primer pair 16SF (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SR (5'-CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi et al. 1991). PCR reaction was performed in a 25µl reaction volume containing 5µl of template DNA (~200ng), 2.5µl of 10X reaction buffer (100 mM Tris pH 9.0, 500 mM KCl, 15 mM MgCl₂, 0.1% Gelatin), 2µl of 25 mM MgCl₂, 1µl of 10 mM dNTPs, 1µl of each primer, 1µl Taq polymerase

and 16.5µl nuclease free water. The thermal profile for both the genes was 10 minutes at 95°C, and 35 cycles of 1 minute at 94°C, 1 minute at 50°C and 2 minutes at 72°C, followed by extension of 10 minutes at 72°C. Amplified DNA fragments were purified using the Promega Wizard Gel and PCR clean up system and sequenced. The purified PCR products were sequenced using ABI prism 3730 sequencer (Applied Biosystems, USA) and Big dye terminator sequencing kit (ABI Prism, USA). These sequences have been deposited in GenBank (accession numbers are provided in Appendix 2).

Sequences were analyzed for similar sequences in NCBI GenBank database (<http://www.ncbi.nlm.nih.gov/>) using BLAST (Altschul et al. 1990). Additional ND1 and 16S gene sequences for the genus *Nyctibatrachus* and the outgroup *Lankanectes corrugatus* were retrieved from the NCBI GenBank database. GenBank accession numbers for the sequences used in the study are provided in Appendix 2. Gene sequences were aligned separately using MUSCLE (Edgar 2004) and genes were concatenated. Data was partitioned in four parts, one for 16S rRNA gene and three for respective codon positions of ND1 gene to create a full partition, and then the greedy strategy (Lanfear et al. 2012) implemented in IQ Tree (Nguyen et al. 2015) was used to find the right partitioning scheme based on minimum Bayesian Information Criterion (BIC) (Schwarz 1978; Nei & Kumar 2000). Maximum likelihood analysis was performed in IQ Tree (Nguyen et al. 2015) with ultrafast bootstrap support (Minh et al. 2013) for 1000 iterations. The phylogenetic tree was edited in FigTree v1.4.2 (Rambaut 2009). Kimura two parameter (K2P) genetic distances between sequences were determined in MEGA 7 (Kumar et al. 2016).

RESULTS

Molecular analysis

Best partition scheme suggested nucleotide substitution model TPM2+I+G4 for 16S rRNA gene and first two codon positions of ND1 gene (BIC = 23697.804) and TIM2+I+G4 for the third codon position of ND1 gene (BIC = 10121.790). Maximum likelihood analysis (Fig. 1) indicates that *Nyctibatrachus mewasinghi* is genetically distinct from known congeners. *Nyctibatrachus mewasinghi* formed a monophyletic group with its sister taxa *N. athirappillyensis* and *N. kempholeyensis*.

For 16S rRNA gene, K2P distance between *N. mewasinghi* and *N. athirappillyensis* was 2.1–2.5 %, while between *N. mewasinghi* and *N. kempholeyensis*

was 4.0–4.7 %. These distances were more than for other known species, including 2.1% distance between *N. shiradi* and *N. vrijeuni*, 1.5% between *N. jog* and *N. petraeus*, 1.4% between *N. gavi* and *N. acanthodermis*, and 1.2% between *N. periya* and *N. deveni*.

For ND1 gene, K2P distance between *N. mewasinghi* and *N. athirappillyensis* was 6.8%, while between *N. mewasinghi* and *N. kempholeyensis* was 12.2–12.9 %. These distances were more than for other known species, including 4.6% distance between *N. shiradi* and *N. vrijeuni*, 4.8% between *N. jog* and *N. petraeus*, 8.1% between *N. gavi* and *N. acanthodermis*, and 4.0% between *N. periya* and *N. deveni*.

The phylogeographic map of the subtree containing only *N. mewasinghi*, *N. athirappillyensis* and *N. kempholeyensis* (Image 1) shows that *N. mewasinghi* and its sister taxon *N. athirappillyensis* are separated by the Palghat gap. Although, the latitudinal distribution of *N. mewasinghi* and *N. kempholeyensis* overlap, we did not record *N. kempholeyensis* in the type locality of *N. mewasinghi*.

Morphometric analysis

Nyctibatrachus mewasinghi, *N. athirappillyensis* and *N. kempholeyensis* formed distinct clusters in multivariate morphometric space (Fig. 2). *Nyctibatrachus mewasinghi* differs from *N. athirappillyensis* and *N. kempholeyensis* in having relatively larger EL and smaller HL, HW, ShL, FOL, TFOL and SL (Table 1).

Table 1. Factor loadings for variables in discriminant analysis.

Character	DA axis 1	DA axis 2
HW	0.3484	0.1898
HL	0.5931	0.1065
MN	0.0844	-0.0438
MFE	0.1482	-0.0770
MBE	0.0765	0.1551
SL	-0.0142	-0.2263
EL	-0.1422	0.0912
IUE	-0.0782	-0.0244
UEW	-0.0045	-0.1213
IFE	0.0529	-0.0778
IBE	-0.0279	-0.0368
FAL	0.0078	0.0325
HAL	0.1091	-0.1466
ShL	0.2537	0.0129
TL	0.0846	0.2299
FOL	0.1915	-0.2369
TFOL	0.1564	-0.2187
FDIII	0.0788	0.0413
FWIII	0.0036	0.0172
TD	0.1188	0.0846
TW	0.0302	0.0392

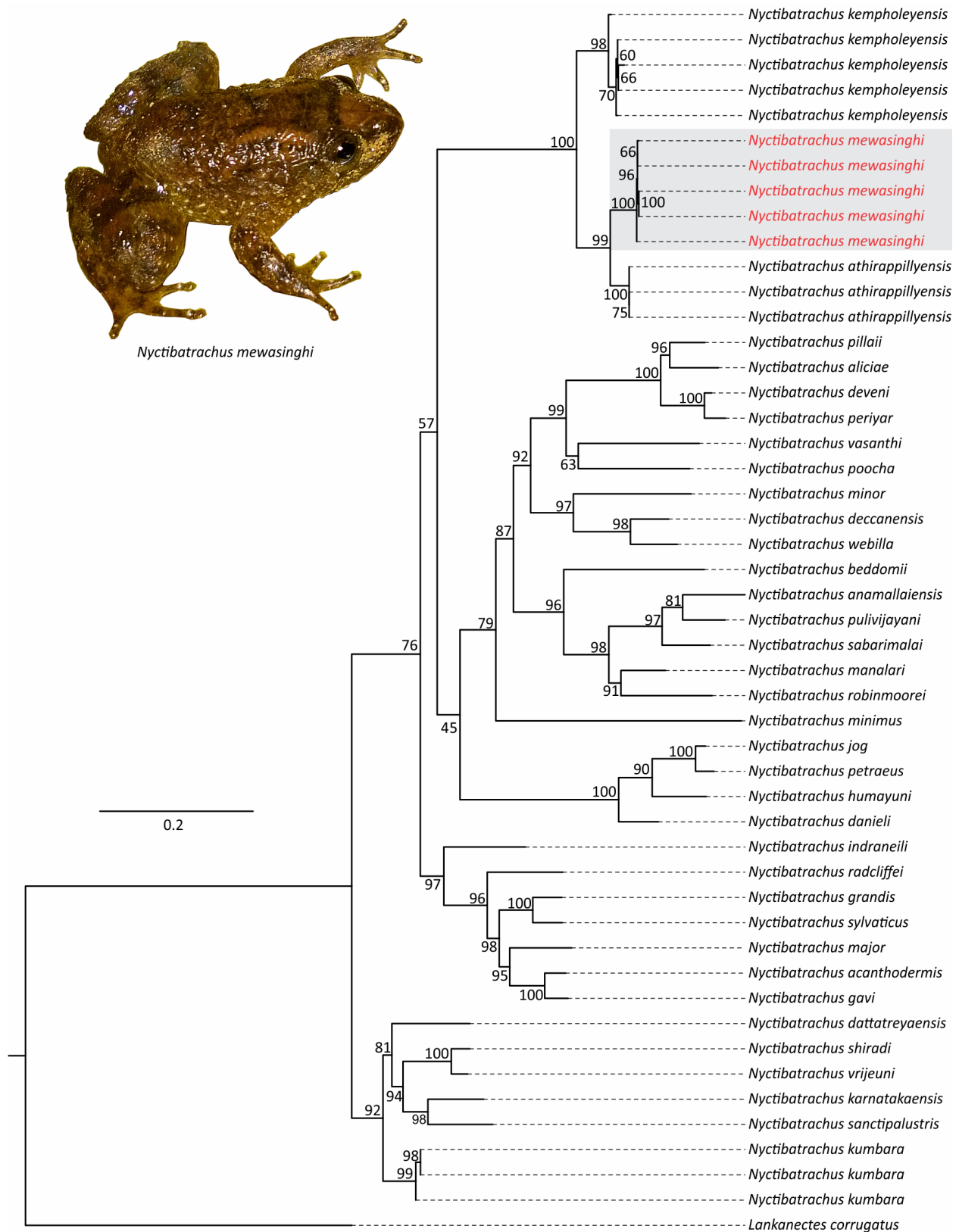


Figure 1. Maximum likelihood tree based on concatenated 16S rRNA and ND1 gene sequences and best partition scheme. *Lankanectes corrugatus* is used as an outgroup. Values along the nodes are percent bootstraps out of 1000 iterations.

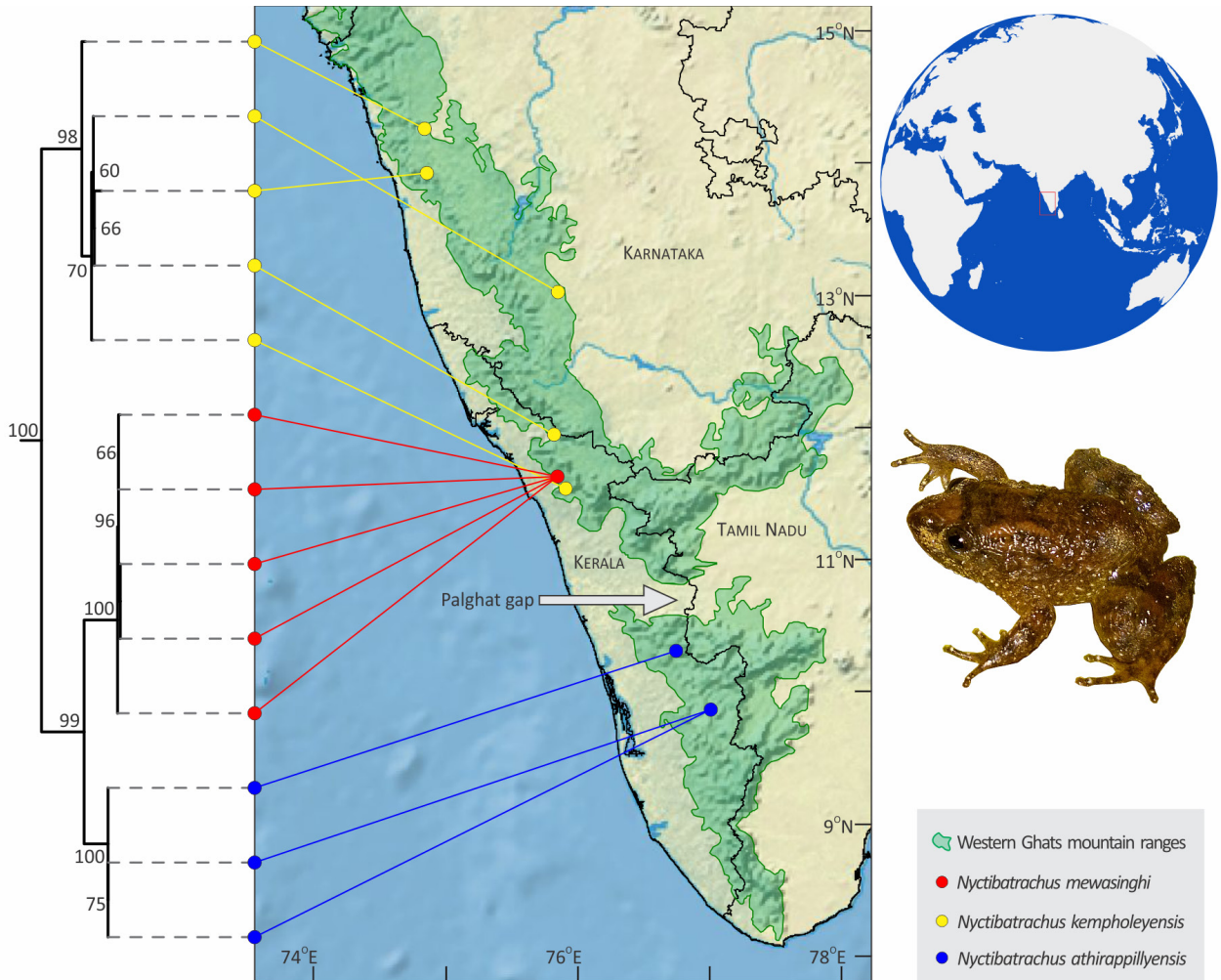


Image 1. Phylogeographical map of *Nyctibatrachus mewasinghi* sp. nov. along with its sister taxa *N. kempholeyensis* and *N. athirappillyensis*. Subtree from Figure 1 is plotted along the geographical distribution of specimens.

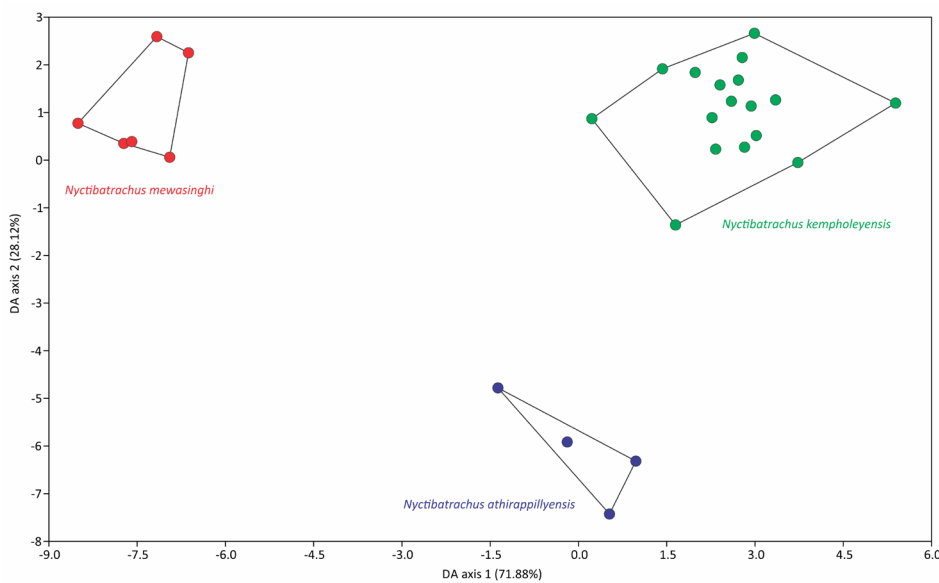


Figure 2. Discriminant analysis (DA) of morphometric data of *Nyctibatrachus mewasinghi* sp. nov. along with its sister taxa *N. kempholeyensis* and *N. athirappillyensis*. Values in parenthesis are percent of total variation explained by each DA axis.

TAXONOMY

***Nyctibatrachus mewasinghi* sp. nov.**
(Images 2, 3a,b)

urn:lsid:zoobank.org:act:CF14F2CB-2107-4DC8-86BD-6C6866D8C334

Holotype: BNHS 5984, female, 22.1mm SVL, 26.xii.2013, Peruvannamuzhi (11.599 N & 75.819 E, 38m elevation), Malabar Wildlife Sanctuary, Kozhikode District, Kerala, India, coll. K. Krutha & B. Kumar.

Paratypes: 3 ex., WILD-15-AMP-623 (female, 21.4mm SVL), WILD-15-AMP-624 (male, 22.3mm SVL) and WILD-15-AMP-625 (male, 22.1 SVL), 26.xii.2013, collected along with the holotype; 2 ex., WILD-13-AMP-341 (female, 23.0mm SVL) and WILD-14-AMP-403 (male, 21.9mm SVL), 26.xii.2013, same location as holotype,

coll. K. Krutha & B. Kumar.

Diagnosis: *Nyctibatrachus mewasinghi* sp. nov. can be distinguished from all its known congeners by a combination of characters including: small adult size (21.4–23.0 mm SVL); head equal to or slightly wider than long (HW/HL ratio 1.0–1.3); internal back of eyes to eye length ratio 2.0–2.4; less wrinkled dorsal skin with prominent granular projections; absence of dorso-lateral glandular folds separated by an 'X' pattern on anterior half of back; a ridge extending from the lip over the tip of the snout to between the nostrils bifurcate posteriorly producing an inverted 'Y'; finger and toe discs well developed; third finger disc slightly wider than finger width (FDIII/FWIII ratio 1.3–2.5); fourth toe disc almost equal to or slightly wider than toe width (TD/TW ratio 1.0–1.8); third finger and fourth toe discs with

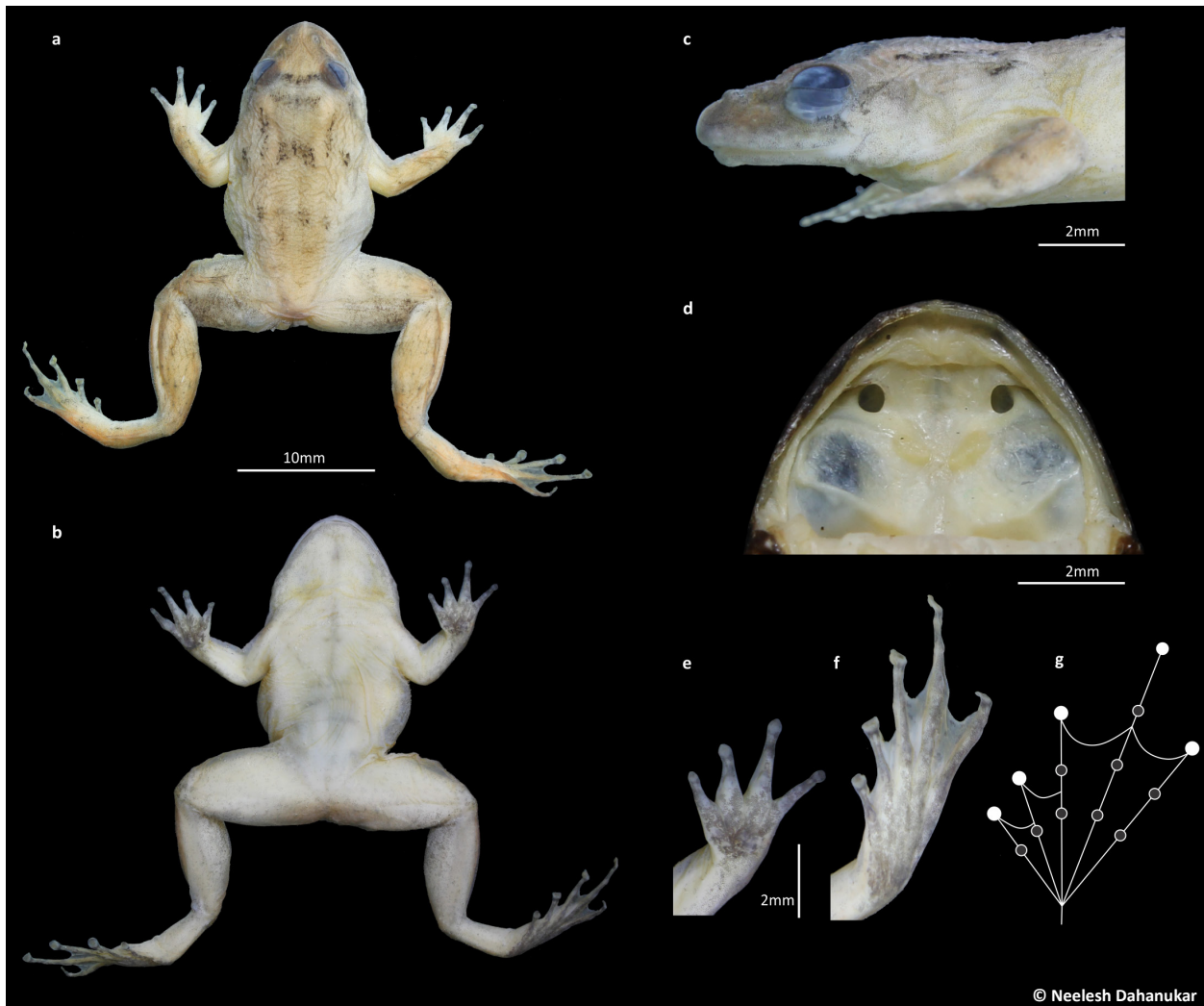


Image 2. Holotype of *Nyctibatrachus mewasinghi* sp. nov. (BNHS 5984, 22.1mm SVL). (a) Dorsal view, (b) ventral view, (c) lateral view of head, (d) buccal cavity showing placement, size and shape of choanae and vomerine teeth, (e) left hand, (f) left foot and (g) illustration of webbing extent.

Table 2. Morphometry (in mm) of *Nyctibatrachus mewasinghi* sp. nov.

Characters	BNHS 5984	WILD-15-AMP-623	WILD-13-AMP-341	WILD-15-AMP-624	WILD-15-AMP-625	WILD-14-AMP-403
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype
	Female	Female	Female	Male	Male	Male
SVL	22.1	21.4	23.0	22.3	22.1	21.9
HW	7.2	7.6	8.8	7.7	8.0	8.3
HL	6.4	6.1	7.1	6.3	7.8	7.3
MN	4.7	4.7	6.1	4.9	6.2	6.0
MFE	3.2	3.1	3.9	3.0	4.8	3.5
MBE	1.3	1.6	1.7	1.2	3.1	2.5
SL	3.2	3.1	3.8	3.3	3.4	3.7
EL	2.6	2.8	3.5	2.7	3.0	2.8
IUE	3.1	2.7	3.5	2.8	2.6	2.7
UEW	1.0	1.0	0.9	1.4	1.0	1.1
IFE	2.9	2.8	3.6	2.9	3.3	3.7
IBE	6.2	6.2	7.2	5.9	6.4	6.8
FAL	4.2	4.5	5.4	4.7	3.5	4.2
HAL	4.8	4.8	6.5	4.9	4.9	5.5
ShL	9.7	10.4	11.3	9.4	9.6	10.8
TL	9.9	11.1	11.8	8.8	10.3	11.4
FOL	9.3	10.6	12.1	9.5	10.0	10.4
TFOL	8.6	10.1	10.7	9.9	10.1	12.3
FDIII	0.4	0.4	0.5	0.4	0.5	0.3
FWIII	0.2	0.2	0.3	0.3	0.2	0.2
TD	0.5	0.5	0.7	0.6	0.5	0.4
TW	0.4	0.5	0.4	0.4	0.3	0.3

dorso-terminal groove, cover rounded distally; presence of two palmar tubercles; webbing small, reaching above the second subarticular tubercle on either side of toe IV; thigh nearly equal to shank (TL/SHL ratio 0.9–1.1); and shank nearly equal to foot length (SHL/FOL ratio 0.9–1.0).

Comparisons: *Nyctibatrachus mewasinghi* sp. nov. differs from *N. anamallaiensis*, *N. beddomii*, *N. manalari*, *N. minimus*, *N. minor*, *N. pulivijayani*, *N. robinmoorei* and *N. sabarimalai* in presence of webbing between toes (vs. absent). *Nyctibatrachus mewasinghi* differs from congeners *N. acanthodermis*, *N. dattatreyaensis*, *N. gavi*, *N. humayuni*, *N. major*, *N. petraeus*, *N. pillaii*, *N. poocha*, *N. radcliffei*, *N. sylvaticus* and *N. vrijeuni* in having dorsal skin less prominently wrinkled (vs. prominently wrinkled). Further, *Nyctibatrachus mewasinghi* differs in having the third finger with a dorso-terminal groove (vs. without groove in *N. acanthodermis*, *N. dattatreyaensis*, *N. deccanensis*, *N. gavi*, *N. grandis*, *N. indraneili*, *N.*

major, *N. radcliffei*, *N. sanctipalustris*, *N. vrijeuni* and *N. webilla*), fourth toe disc with a dorso-terminal groove, cover rounded distally (vs. bifurcate distally in *N. aliciae*, *N. anamallaiensis*, *N. beddomii*, *N. deveni*, *N. kumbara*, *N. manalari*, *N. minimus*, *N. minor*, *N. periyar*, *N. pillaii*, *N. poocha*, *N. pulivijayani*, *N. robinmoorei*, *N. sabarimalai* and *N. vasanthi*; or notched distally in *N. athirappillyensis* and *N. shiradi*). From species that have finger and toe discs, covered rounded distally, *Nyctibatrachus mewasinghi* differs in having reduced webbing with webbing formula I1–2II1–2½III1–2¼IV2¼–1V (vs. extensive webbing with webbing formula I1–1II1–2½III1–2¼IV2¼–1V in *N. danieli*; I1–1II1–1¼III1–2¼IV2¼–1V in *N. humayuni*; I1–1¼II1–1½III1–2¼IV2¼–1V in *N. jog*; I1–1II1–1III1–1½IV1½–1V in *N. karnatakaensis*; I1–1½II1–2III1–2¼IV2¼–1V in *N. petraeus*; I1–1¾II1–2¼III1–2¼IV2¼–1V in *N. sylvaticus*). *Nyctibatrachus mewasinghi* also has flesh or off-white ventral coloration in life (vs. red, reddish-orange or reddish-brown in *N. deccanensis*



Image 3. *Nyctibatrachus mewasinghi* sp. nov. in life. (a-b) Holotype (BNHS 5984, 22.1mm SVL), (c-e) male paratype (WILD-15-AMP-624, 22.3mm SVL).

and *N. webilla*). *Nyctibatrachus mewasinghi* differs with a genetic distance between 7.8–13.7 % in 16S rRNA gene and 25.0–32.4 % in ND1 gene from all its congeners except *N. athirappillyensis* and *N. kempholeyensis*.

Compared to its sister taxa, *Nyctibatrachus mewasinghi* sp. nov. differs from *N. athirappillyensis* in having larger eye length to snout length ratio (EL/SL ratio 0.8–0.9 vs. 0.6–0.7), smaller internal back of eyes to eye length ratio (IBE/EL ratio 2.0–2.4 vs. 2.5–2.7), fourth toe with a dorso-terminal groove and cover rounded distally (vs. fourth toe with a dorso-terminal groove and cover notched distally) and webbing formula I1–2⁻I11–2¹/₂III1–2¹/₄IV2¹/₄–1V (vs. extensive webbing with webbing formula I1–1³/₄I11–2⁺III1–2⁺IV2⁺–1V). The genetic distance between *N. mewasinghi* and *N. athirappillyensis* is 2.1–2.5% for 16S rRNA gene and 6.8% for ND1 gene.

Nyctibatrachus mewasinghi sp. nov. differs from *N. kempholeyensis* in having two palmar tubercles (vs. single), a significantly shorter head (HL/SVL = 31.0 ± 3.2 vs. 37.0 ± 4.5, $t = 2.999$, $df = 21$, $P = 0.007$), significantly narrower head (HW/SVL = 36.0 ± 2.1 vs. 39.5 ± 2.9, $t = 2.669$, $df = 21$, $P = 0.014$) and webbing formula I1–2⁻I11–2¹/₂III1–2¹/₄IV2¹/₄–1V (vs. I1–2⁻I11–2¹/₄III1–2¹/₂IV2¹/₂–

1V). The genetic distance between *N. mewasinghi* and *N. kempholeyensis* is 4.0–4.7% for 16S rRNA gene and 12.2–12.9% for ND1 gene.

Description of the female Holotype BNHS 5984 (all measurements are in mm): Small sized frog (SVL 22.1); head slightly wider than long (HL 7.2; HW 6.4); outline of snout in dorsal and ventral view oval (Image 2a,b), snout (SL 3.2) longer than horizontal diameter of eye (EL 2.6); loreal region obtuse, canthus rostralis indistinct; interorbital area (IUE 3.1) wider than upper eyelid (UEW 1.0); distance between posterior margins of eyes (IBE 6.2) about 2.1 times the distance between anterior margins of eyes (IFE 2.9); supratympanic fold distinct, from posterior corner of upper eyelid to near the shoulder (Image 2c); choanae small, its largest diameter less than half of the largest diameter of vomerine teeth patch (Image 2d).

Forearm (FAL 4.2), smaller than hand (HAL 4.8); fingers with dermal fringes, third finger discs with a dorso-terminal groove, cover rounded distally, third finger disc two times wider than finger (FDIII 0.4, FWIII 0.2); all subarticular tubercles present, distinct, oval; pre-pollex distinct, oval; two palmar tubercles, oval, distinct.



Image 4. Distribution of *Nyctibatrachus mewasinghi* sp. nov. along with its sister taxa *N. kempholeyensis* and *N. athirappillyensis*.

Hind limbs moderately long, shank (ShL 9.7) slightly smaller than thigh (TL 9.9) and larger than foot length (FOL 9.3); distance from heel to tip of toe IV (TFOL 8.6); webbing small, webbing formula I1–2II1–2½III1–2¼IV2¼–1V; reaching beyond the second subarticular tubercle on either side of toe IV (Image 2f, g); fourth toe disc with dorso-terminal groove, cover rounded distally, fourth toe disc 1.3 times wider than toe (TD 0.5, TW 0.4); subarticular tubercles prominent, oval, single, all present.

Skin of snout and between eyes shagreened, upper eyelids sparsely tuberculated; anterior and posterior parts of back wrinkled (Images 2a, 3a); two large longitudinal folds on the back extending from just behind upper eyelid towards dorsum; a ridge extending from the lip over the tip of the snout to between the nostrils bifurcate posteriorly producing an inverted ‘Y’; a glandular fold between the eyes; dorsal parts of forelimb, thigh, and shank have longitudinal folds; ventral side smooth; glandular projections on margin of



Image 5. Habitat at the type locality of *Nyctibatrachus mewasinghi* sp. nov.

lower jaws and anterior margin of thighs.

Coloration of holotype: In life (Image 3a,b), dorsum light grayish-brown, lateral side lighter than dorsum; a dark stripe between the eyes connecting upper eyelids mid-dorsally, demarcating a triangular light brown patch on dorsal side of the snout; a pair of light reddish-brown longitudinal dorsal bands extend from the back of the eyelids to the middle of dorsum; glandular folds brown; limbs dorsally light brown; fore limbs, hind limbs, fingers and toes with dark brown cross-bands; ventral side off-white with very few and scattered grayish-brown spots on throat and ventral side of fore limb and hind limb; belly plain; upper and lower jaw margin grayish with white glandular projections on lower jaw margin; palm and feet grayish-brown; fingers, toes and webbing dark gray. In preservation (Image 2), coloration as per life just more faded, longitudinal dorsal bands cream to light brown color.

Variation: Morphometric variation in five paratypes is provided in Table 2. Males have a prominent femoral gland (Image 3e).

Distribution and habitat: *Nyctibatrachus mewasinghi* sp. nov. is known only from its type locality Peruvannamuzhi in Malabar Wildlife Sanctuary, Western Ghats of Kerala, north of Palghat Gap (Image 4). The

species was seen in a stream with riparian cover (Image 5) running alongside a wall after the toe drain of the Peruvannamuzhi Dam. Males were observed calling on top of rocks and leaves around 19:00hr.

Etymology: The species is named after Dr. Mewa Singh, Distinguished Professor (for Life), University of Mysore; J.C. Bose Fellow, Institution of Excellence, and Honorary Professor of Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru. This species is in honour of his extraordinary contributions in behavioral ecology and primatology, and his immense contribution to the conservation of Indian primates.

Suggested common name: Mewa Singh's Night Frog.

DISCUSSION

Nyctibatrachus mewasinghi sp. nov. and *N. athirappillyensis* are sister taxa within a monophyletic clade that includes *N. kempholeyensis*. Genetic divergence of *N. mewasinghi* from *N. athirappillyensis* can be attributed to their separation across the Palghat gap, as observed for *Nyctibatrachus* species earlier (see Van Bocxlaer et al. 2012) and other related taxa such as *Indirana* (see Dahanukar et al. 2016). The genetic divergence of *N. mewasinghi* from *N. kempholeyensis*, however, raises new questions on the biogeography. While *N. kempholeyensis* is widely distributed from 11.5–14.3°N (Image 4) with little genetic divergence (Fig. 1; Image 1), the type locality of *N. mewasinghi*, which falls within this latitudinal range has only a single species with high genetic divergence from *N. kempholeyensis*. The genetic divergence between *N. mewasinghi* and *N. athirappillyensis* and between *N. mewasinghi* and *N. kempholeyensis* is much above the expected barcode gap as evidenced from the fact that for both the genes there are several other species pairs with much lower genetic divergence (see molecular analysis above).

Nyctibatrachus athirappillyensis has been recorded from a new location in Idukki District, while *N. kempholeyensis* is recorded from four additional locations (see Image 2 and Appendix 1).

Family Nyctibatrachidae with its two extant genera (Frost et al. 2006), namely the monotypic genus *Lankanectes* endemic to Sri Lanka (Dubois & Ohler 2001) and the genus *Nyctibatrachus* endemic to the Western Ghats of India (Biju et al. 2011), is an ancient frog lineage that emerged about 85–61mya (Roelants et al. 2004). In an important contribution, Biju et al. (2011) revised the genus, clarified taxonomy of several known species, described 12 new species based on new genetic

and morphological information and provided novel behavioral observations. This was followed by discovery of a new mud packing behavior and description of a new species by Gururaja et al. (2014) and seven new species of small sized night frogs by Garg et al. (2017). All these papers elucidated our limitations in the knowledge of amphibian diversity in Western Ghats biodiversity hotspot. Description of a new species in the current communication further bolsters this assertion and raises possibilities of discovering more species from this region.

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Appendix 1. Comparative material studied.

Nyctibatrachus acanthodermais: Paratypes, 6 ex., BNHS 5430–5431 adult males, BNHS 5432–5435 adult females, 46.7–62.4 mm SVL, 8.vi.2010, Kaikatti, Nelliampathy, Palakkad District, Kerala State, coll. S.D. Biju.

Nyctibatrachus aliciae: 2 ex., BNHS 5436–5437, 11.vii.2004, adult males, 20.3–22.2mm SVL, Ponmudi, coll. S.D. Biju.

Nyctibatrachus anamallaiensis: 1 ex., WILD-13-AMP-320, 25.x.2009, Shendurney (8.909 N & 77.119 E, 222m), coll. K. Krutha, Nisha, Sivakumar; 1 ex., WILD-15-AMP-599, 14.xii.2013, Kurinjimala (10.208 N & 77.264 E), coll. K. Krutha.

Nyctibatrachus athirappillyensis: BNHS 5983, female, 24.4mm SVL, 17.xii.2013, Idukki (9.874 N & 77.076 E, 797m elevation), Kerala, India, coll. K. Krutha and B. Kumar; 1 ex., WILD-14-AMP-431, female, 19.2mm SVL, 16.xii.2013, Idukki (9.843 N & 76.979 E, 682m elevation), Kerala, India, coll. K. Krutha and B. Kumar.

Nyctibatrachus danieli: Paratype, 1 ex., BNHS 5438, adult male, 30.5mm SVL, 10.vii.2002, Humbarli Village, Koyna, Satara District, Maharashtra State, coll. S.D. Biju; Paratype, 2 ex., BNHS 5439–5440, two adult males, 27.9–29.7mm SVL, 18.viii.2004, Amboli, coll. S.D. Biju; Paratype, 2 ex., 20.viii.2004, BNHS 5441, adult male, 23.5mm SVL, and BNHS 5443, adult female, 31.3mm SVL, Amboli, coll. S.D. Biju; Paratype, 1 ex., BNHS 5442, adult male, 28.1mm SVL, 12.vii.2005, Amboli, coll. V. Giri.

Nyctibatrachus deccanensis: 1 ex., BNHS 5445, adult female, 20.5mm SVL, 6.vi.2004, Eravikulam (Hut shola), coll. S.D. Biju; 1 ex., BNHS 5444, adult female, 24.2mm SVL, 6.viii.2009, Anamudi summit, Eravikulam, coll. S.D. Biju.

Nyctibatrachus deveni: Paratypes, 2 ex., BNHS 5448–5449, adult females, 30.8–33.2mm SVL, 8.vi.2010, Kaikatti, Nelliampathy, Palakkad District, Kerala State, coll. S.D. Biju; Paratypes, 2 ex., BNHS 5446–5447, adult males, 22.9–11.6mm SVL, 11.viii.2008, Kaikatti, Nelliampathy, Palakkad District, Kerala State, coll. S.D. Biju.

Nyctibatrachus gavi: Paratype, 1 ex., BNHS 5450, adult female, 10.vi.2006, Gavi, Pathanamthitta District, Kerala State, coll. S.D. Biju.

Nyctibatrachus grandis: Paratype, 1 ex., BNHS 5452, adult female, 55.4mm SVL, 24.vi.2007, Thirunelly, Wayanad District, Kerala State, coll. S.D. Biju; Paratype, 1 ex., BNHS 5451, adult male, 62.5mm SVL, Chembra, coll. S.D. Biju.

Nyctibatrachus humayuni: Paratypes, 2 ex., BNHS 1403, adult female, 45.7mm SVL, and BNHS 1404, subadult female, 34.0mm SVL, Khandala; Paratype, 1 ex., BNHS 1413, adult female, 45.8mm SVL, Mahabaleshwar.

Nyctibatrachus indraneili: Paratype, 1 ex., BNHS 5456, 50.4mm SVL, adult female, 12.vii.2004, Long-wood Shola, Kotagiri, Nilgiris District, Tamil Nadu State, coll. S.D. Biju.

Nyctibatrachus jog: Paratype, 1 ex., BNHS 5457, adult male, 37.9 mm SVL, 4.vi.2005, Jog Falls, Mavingundi, Shimoga District, Karnataka State, coll. S.D. Biju.

Nyctibatrachus karnatakaensis: 1 ex., BNHS 5458, adult male, 59.9mm SVL, Manikyadhara Betta, Kudremukh National Park (13°21.6 N & 75°07.9 E, 900m), Sringeri Taluk, Chikmagalur District, Karnataka, coll. S.D. Biju.

Nyctibatrachus kempholeyensis: 1 ex., BNHS 5459, adult male, 18.2mm SVL, 30.vii.2005, Kempholey, Hassan District, Karnataka State, coll. S.D. Biju; 2 ex., 20.viii.2006, BNHS 5460, adult male, 20.9mm SVL, and BNHS 5461, adult female, 23.3mm SVL, Banasura, Wayanad, coll. S.D. Biju; 1 ex., WILD-13-AMP-022, 30.xi.2012, Rainforest Retreat Coorg (12.424 N & 75.738 E), coll. K. Krutha and H. Tripathi; 1 ex., WILD-13-AMP-144, 15.vii.2013, Aralam (11.922 N & 75.789 E, 78m), K. Krutha, S. Kudalkar and A. Raj; 1 ex., WILD-13-AMP-147, 15.vii.2013, Aralam (11.933 N & 75.801 E, 124m), K. Krutha, S. Kudalkar and A. Raj; 1 ex., WILD-14-AMP-400, 27.xii.2013, Kakkayam (11.548 N & 75.889 E, 60m), coll. K. Krutha and B. Kumar; 1 ex., WILD-15-AMP-561, 8.ix.2014, Nagodi, coll. K. Krutha and G.P. Dwivedi.

Nyctibatrachus major: 1 ex., BNHS 5463, adult female, 26.9mm SVL, Bonakkad, coll. S.D. Biju; 1 ex., BNHS 5464, adult female, Kallar, coll. S.D. Biju; 1 ex., BNHS 5462, adult male, Sengaltheri, coll. S.D. Biju.

Nyctibatrachus minimus: 5 ex., BNHS 4470–4774, 8.6–16.4 mm SVL, coll. S.D. Biju.

Nyctibatrachus minor: 1 ex., BNHS 5465, adult male, 15.6mm SVL, Ponmudi, coll. S.D. Biju.

Nyctibatrachus periyar: Paratype, 1 ex., BNHS 5466, adult female, 13.vii.2008, Vallakadavu, Periyar Tiger Reserve, Idukki District, Kerala State, coll. Anil.

Nyctibatrachus petraeus: 2 ex., BNHS 5467–5468, adult males, 40.4–47.6mm SVL, Amboli, coll. S.D. Biju.

Nyctibatrachus pillaii: Paratypes, 2 ex., BNHS 5469–5470, adult males, 4.vi.2005, 19.2–21.0mm SVL, Ooth, coll. S.D. Biju; Paratype, 2 ex., BNHS 5471–5472, adult males, 19.4–21.7mm SVL, 23.vii.2005, Thenmala, coll. S.D. Biju.

Nyctibatrachus poocha: Paratypes, 2 ex., BNHS 5473–5474, adult males, 27.0–30.5mm SVL, 12.viii.2005, Mattupetti, coll. S.D. Biju; Paratype, 1 ex., BNHS 5475, adult female, 12.viii.2005, 32.7mm SVL, Munnar, coll. S.D. Biju.

Nyctibatrachus shiradi: Paratype, 1 ex., BNHS 5476, adult male, 22.0mm SVL, 10.viii.2004, Kudremukh-Malleshwaram, coll. S.D. Biju; Paratype, 1 ex., BNHS 5480, an adult male, 17.0mm SVL, Kottigehara, Chikmagalur District, Karnataka State, coll. S.D. Biju, 07.viii.2004.

Nyctibatrachus vasanthi: 2 ex., BNHS 5477–5478, adult males, 20.3–26.3 mm SVL, 20.viii.2007, Kakachi, coll. S.D. Biju.

Nyctibatrachus vrijeuni: Paratype, 1 ex., BNHS 5479, adult female, 42.9mm SVL, 23.vii.2004, Suganthagiri, Wayanad District, Kerala State, coll. S.D. Biju.

Data for *Nyctibatrachus beddomii*, *N. dattatreyaensis*, *N. sanctipalustris* and *N. sylvaticus* was obtained from Biju et al. (2011); for *N. kumbara* from Gururaja et al. (2014); and for *Nyctibatrachus manalari*, *Nyctibatrachus pulvijayani*, *Nyctibatrachus radcliffei*, *Nyctibatrachus robinmoorei*, *Nyctibatrachus sabarimalai* and *Nyctibatrachus webilla* from Garg et al. (2017).

Appendix 2. GenBank accession numbers for sequences used in molecular analysis.

Species	Voucher	16S	ND1
<i>Nyctibatrachus acanthoderms</i>	SDB 23	JN644915	JF274066
<i>Nyctibatrachus aliciae</i>	SDB 1142	JN644893	JF274075
<i>Nyctibatrachus anamallaiensis</i>	SDB 40321	JN644899	JF274079
<i>Nyctibatrachus athirappillyensis</i>	ZSI/WGRC/ V/A/891	KY447300	---
<i>Nyctibatrachus athirappillyensis</i>	BNHS5983	MG719959	MG717878
<i>Nyctibatrachus athirappillyensis</i>	WILD-14- AMP-431	MG719960	MG717879
<i>Nyctibatrachus beddomii</i>	SDB (DEL) 1479	JN644913	JF274080
<i>Nyctibatrachus danieli</i>	SDB (Varad) E2	JN644902	JF274057
<i>Nyctibatrachus dattatreyaensis</i>	SDB (DEL) 0267	JN644891	JF274062
<i>Nyctibatrachus deccanensis</i>	SDB 1056	JN644911	JF274077
<i>Nyctibatrachus deveni</i>	SDB 25	JN644895	JF274072
<i>Nyctibatrachus gavi</i>	SDB 4836	JN644918	JF274067
<i>Nyctibatrachus grandis</i>	SDB 40360	JN644904	JF274064
<i>Nyctibatrachus humayuni</i>	SDB (Varad) 8	JN644901	JF274056
<i>Nyctibatrachus indraneili</i>	SDB 4017	JN644909	JF274063
<i>Nyctibatrachus jog</i>	SDB 40144	JN644900	JF274054
<i>Nyctibatrachus karnatakaensis</i>	SDB 1098	JN644910	JF274060
<i>Nyctibatrachus kempholeyensis</i>	SDB 40150	JN644903	JF274069
<i>Nyctibatrachus kempholeyensis</i>	SDBDU 2012.53	KY447307	---
<i>Nyctibatrachus kempholeyensis</i>	WILD-14- AMP-400	MG719961	MG717880
<i>Nyctibatrachus kempholeyensis</i>	WILD-13- AMP-144	MG719962	MG717881
<i>Nyctibatrachus kempholeyensis</i>	WILD-15- AMP-561	MG719963	MG717882
<i>Nyctibatrachus kumbara</i>	Isolate A1	KF935242	---
<i>Nyctibatrachus kumbara</i>	Isolate A2	KF935243	---
<i>Nyctibatrachus kumbara</i>	Isolate A3	KF935244	---

Species	Voucher	16S	ND1
<i>Nyctibatrachus major</i>	SDB0006	AF249052*	JF274068
<i>Nyctibatrachus manalari</i>	SDBDU 2015.2994	KY447301	---
<i>Nyctibatrachus mewasinghi</i> sp. nov.	WILD-15- AMP-623	MG719964	MG717883
<i>Nyctibatrachus mewasinghi</i> sp. nov.	WILD-15- AMP-624	MG719965	MG717884
<i>Nyctibatrachus mewasinghi</i> sp. nov.	WILD-15- AMP-625	MG719966	MG717885
<i>Nyctibatrachus mewasinghi</i> sp. nov.	WILD-13- AMP-341	MG719967	MG717886
<i>Nyctibatrachus mewasinghi</i> sp. nov.	WILD-14- AMP-403	MG719968	MG717887
<i>Nyctibatrachus minimus</i>	SDB33	JN644896	JF274070
<i>Nyctibatrachus minor</i>	SDB1146	JN644908	JF274078
<i>Nyctibatrachus periyar</i>	SDB4813	JN644897	JF274073
<i>Nyctibatrachus petraeus</i>	SDB023/SDB 2004-023	JN644912	JF274055
<i>Nyctibatrachus pillai</i>	SDB40286	JN644892	JF274074
<i>Nyctibatrachus poocha</i>	SDB1175	JN644907	JF274071
<i>Nyctibatrachus pulivijayani</i>	ZSI/WGRC/ V/A/906	KY447302	---
<i>Nyctibatrachus radcliffei</i>	SDBDU 2014.2771	KY447303	---
<i>Nyctibatrachus robinmoorei</i>	ZSI/WGRC/ V/A/925	KY447304	---
<i>Nyctibatrachus sabarimalai</i>	ZSI/WGRC/ V/A/932	KY447305	---
<i>Nyctibatrachus sanctipalustris</i>	SDB6012	JN644917	JF274061
<i>Nyctibatrachus shiradi</i>	SDB0233	JN644898	JF274059
<i>Nyctibatrachus sylvaticus</i>	SDB4672	JN644916	JF274065
<i>Nyctibatrachus vasanthi</i>	SDB2044	JN644894	JF274076
<i>Nyctibatrachus vrijeuni</i>	SDB3028	JN644905	JF274058
<i>Nyctibatrachus webilla</i>	ZSI/WGRC/ V/A/935	KY447306	---
<i>Lankanectes corrugatus</i>	VUB 0106	AY948730	AY948730

* Voucher number is not available for sequence AF249052, provided voucher number is for JF274068.





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