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A new nightfrog, *Nyctibatrachus minimus* sp. nov. (Anura: Nyctibatrachidae): The smallest frog from India

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A new nightfrog, *Nyctibatrachus minimus* sp. nov. (Anura: Nyctibatrachidae) is described from Kurichiyarmala in the Western Ghats, India. Its most distinctive feature is the small adult snout-vent length, averaging only 12.3 mm in adult males (*N* = 15), making it the smallest known frog from India. Analyses of a fragment of the mitochondrial NADH dehydrogenase 1 gene indicate a minimum divergence of 22% with known small-sized congeners. Miniaturization in *Nyctibatrachus* sp. seems to be associated with absence of

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webbing on toes and fingers, which may have resulted from evolutionary specialization to life in terrestrial habitats.

Keywords: NADH dehydrogenase 1, *Nyctibatrachus minimus* sp. nov., smallest Indian frog, Western Ghats.

THE Western Ghats of Peninsular India is considered an important amphibian hotspot, both in terms of species richness and higher-taxonomic-level endemism[1–5]. The genus *Nyctibatrachus* Boulenger, 1882 is an ancient, endemic lineage[5] in the family Nyctibatrachidae[6] that currently holds 12 recognized species[7,8]. We herein describe a miniature species from Kurichiyarmala, *Nyctibatrachus minimus* sp. nov. and compare it with the descriptions and available material of all species currently recognized in the genus.

N. minimus sp. nov. Holotype: Bombay Natural History Society (BNHS), Mumbai, Maharashtra, India, BNHS 4527, an adult male collected by S.D.B. on 28 July 2001 at an altitude of 1200 m asl, from Kurichiyarmala, 11°35′N, 75°58′E, Wayanad District, Kerala, India; Paratypes: BNHS 4528–4534 (seven adult males), collected along with the holotype, and BNHS 4535–4540 (six adult males) and BNHS 4541 (an adult female), collected by S.D.B. on 24 February 2006 from the type locality. The species epithet *minimus* (Latin: smallest) refers to the diminutive adult size. Suggested vernacular name: miniature nightfrog.

N. minimus sp. nov. can be distinguished from all other members of the genus by the combination of small adult size (males: range 10.0–14.0 mm, $N = 15$; female: 14.9 mm, $N = 1$), presence of granular femoral glands in breeding males, presence of two rows of weakly developed vomerine teeth on each side, lack of webbing on hands and feet, and relatively smooth dorsal skin with faint and interrupted dorsolateral folds and glandular projections (Figures 1–4).

Comparisons were made with the available type specimens of each name bearing taxon in *Nyctibatrachus*, and with other recent collections from the type localities made by the authors. Sexual maturity was determined by examining gonads through a small lateral incision, and by the presence of an advertisement call and femoral glands in breeding males. Nine *Nyctibatrachus* species (*N. aliciae*, *N. deccanensis*, *N. humayuni*, *N. karnatakaensis*, *N. major*, *N. petraeus*, *N. sanctipalustris*, *N. sylvaticus*, and *N. vasanthi*) are clearly distinct from *N. minimus* sp. nov. by their larger adult snout-vent length and relatively robust body. *Nyctibatrachus minimus* sp. nov. can be distinguished from the small-sized *N. kempholeyensis*, a species that has not been observed since its original description[9], by its smaller snout-vent size (maximum 14.9 mm in female *N. minimus* vs 18.0 mm in original description of *N. kempholeyensis*), head about as long as broad (vs head wider than long), and loreal region acute to slightly vertical (vs loreal region vertical to oblique). To support the distinction of *N. minimus* sp. nov. from the remaining smaller *Nyctibatrachus* species, we sequenced a fragment of ~625 base pairs of the mitochondrial NADH dehydrogenase 1 (*ND1*) gene. DNA sequences were obtained by whole-genome extraction[10], PCR amplification, and cycle-sequencing along both strands (GenBank accession numbers EF136395–EF136398 for BNHS 4540, *N. minimus* sp. nov.; BNHS 4520, *N. anamallaiensis*; SDB 1149, *N. beddomii*; BNHS 4522, *N. minor* respectively). Uncorrected pairwise comparison of the sequence of *N. minimus* sp. nov. with those of *N. beddomii*, *N. anamallaiensis*[11] (currently considered a synonym of *N. beddomii*[12–14]), and *N. minor* shows genetic divergences of 25%, 27% and 22% respectively. A 22% sequence divergence between *N. beddomii* and *N. anamallaiensis*, together with a dissimilar morphology (the most conspicuous difference being the presence of dorsolateral folds in *N. anamallaiensis*), allow us to remove the latter from synonymy of *N. beddomii*. Morphologically, *N. minimus* sp. nov. differs from *N. anamallaiensis*, *N. beddomii* and *N. minor* by its still smaller adult size, whose range shows

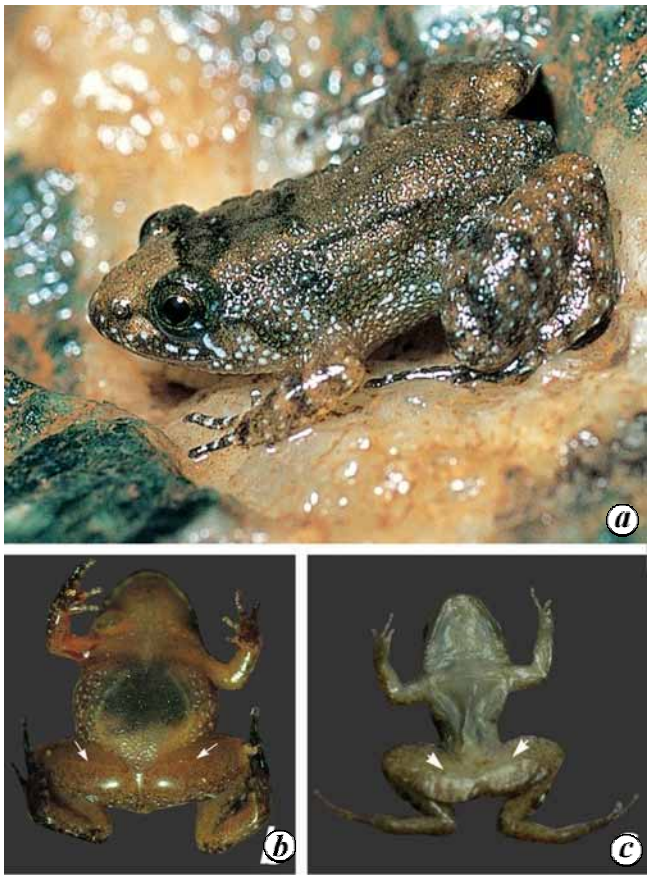

Figure 1. *Nyctibatrachus minimus* sp. nov. ***a***, Holotype (BNHS 4527) in life. ***b***, Holotype in life (ventral side), showing femoral glands. ***c***, Paratype (BNHS 4528) in preservation (ventral side), showing femoral glands.

little or no overlap with those of the other species (Figure 4, Table 1), and by its dorsal skin having faint and interrupted dorsolateral folds and glandular projections (vs distinct dorsolateral folds separated by a row of glandular projections in *N. anamallaiensis*, distinct dorsolateral glandular folds separated by an 'X' pattern on anterior half of back in *N. minor*, and a smooth dorsal skin in *N. beddomii*). In addition, *N. minimus* sp. nov. differs from *N. anamallaiensis* by its weakly developed vomerine teeth (vs well-developed vomerine teeth) and from *N. minor* by the presence of femoral glands (vs absence of femoral glands)[12].

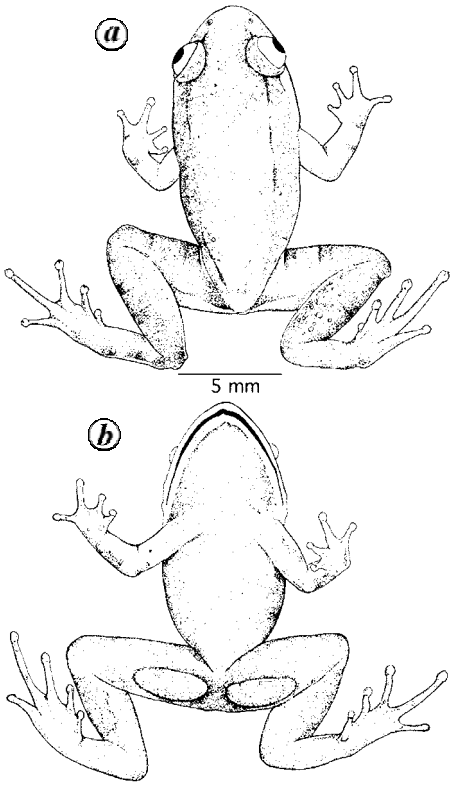

Figure 2. *Nyctibatrachus minimus* sp. nov. holotype. ***a***, Dorsal view; ***b***, Ventral view.

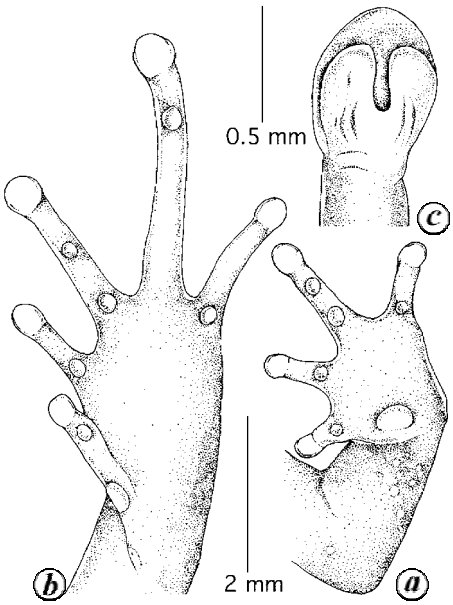

Figure 3. *Nyctibatrachus minimus* sp. nov.. ***a***, Ventral view of hand; ***b***, Ventral view of foot; ***c***. Magnified dorsal view of toe showing groove.

The description (all measurements in mm) of the holotype follows terminology used elsewhere[15]. Small-sized frog (SVL 13.2); head (Figure 2) about as long as broad (HW 5.6; HL 5.5); outline of snout in dorsal view oval, its length (SL 1.8) larger than the horizontal diameter of the eye (EL 1.2); canthus rostralis indistinct, loreal region acute to slightly vertical; interorbital area (IUE 2.3) larger than upper eyelid (UEW 0.7); tympanum indistinct; vomerine teeth weakly developed, pointed, present in two rows on each side, oblique between choanae; tongue nearly cordate, emarginated, without a papilla; button-like projection at the base of the tongue on lower jaw; supratympanic fold absent. Forelimb length (FLL 2.3) slightly shorter than hand (HAL 2.9); tips of fingers with weakly developed disks, with marginal grooves; webbing on fingers absent; subarticular tubercle rather prominent, rounded, single, IV1 absent (Figure 3 *a*), longitudinal groove on the dorsal surface of finger disks; prepollex oval and rather indistinct; supernumerary tubercles absent. Hindlimbs moderately long; shank 3.3 times longer (ShL 6.6) than wide (ShW 2.0), almost equal to femur length (FL 6.5), longer than distance from base of inner metatarsal tubercle to tip of toe IV (FOL 5.9), 1.4 times

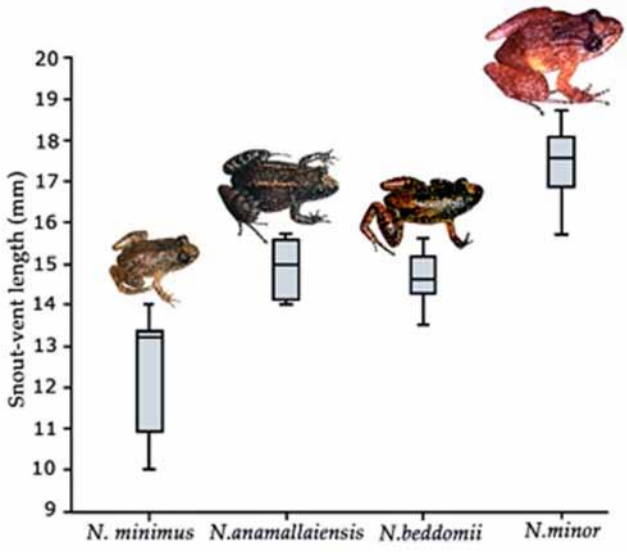

Figure 4. Comparison of snout-vent length of males of the four smallest species of *Nyctibatrachus*. Measurements are summarized in box and whisker plots, with the box spanning the interquartile range, the median indicated by a line inside the box, and the whiskers extending to the highest and lowest observations. From left to right: *N. minimus* sp. nov. ($N = 15$), *N. anamallaiensis* ($N = 10$), *N. beddomii* ($N = 10$) and *N. minor* ($N = 10$).

Table 1. Morphometric measurements (all in mm; range, mean and standard deviation) of *Nyctibatrachus minimus* sp. nov., *N. anamallaiensis*, *N. beddomii* and *N. minor*. Abbreviations are defined in the text. Specimens used in analyses (BNHS; NHM, Natural History Museum, London; SDB, field number: housed at CEMDE, University of Delhi): *N. minimus* sp. nov. – males: BNHS 4527–4540, SDB 421 (all from Kurichiyarmala); female BNHS 4541 (Kurichiyarmala); *N. anamallaiensis* – males: BNHS 4256 and BNHS 4520 (Andiparai Shola), NHM 74.4.29.1372–1373 ('Annamallys'), SDB 40319, SDB 5611–5615 (Puthuthottam); female BNHS 4521 (Andiparai Shola); *N. beddomii* – males: NHM 74.4.29.519, NHM 74.4.29.457 (Malabar), NHM 82.2.10.31 ('Tinivelly'), BNHS 4252 (Ponmudi), BNHS 4253–4254 (Agasthyamala–Athirimala), BNHS 4255 (Ponmudi), SDB 4623 (Sengaltheri), SDB 1149–1150 (Ponmudi); female: NHM 74.4.29.456; *N. minor* – males: BNHS 4522–4526 (Ponmudi), SDB 401, SDB 1154–1155, SDB 6322, SDB 009 (all from Ponmudi); female: SDB 010 (Ponmudi)

	N. minimus sp. nov.				*N. anamallaiensis*				*N. beddomii*				*N. minor*			
	Male (N = 15)			Female	Male (N = 10)			Female	Male (N = 10)			Female	Male (N = 10)			Female
	Range	Mean	SD	N = 1	Range	Mean	SD	N = 1	Range	Mean	SD	N = 1	Range	Mean	SD	N = 1
SVL	10.0–14.0	12.3	1.4	14.9	14.0–15.7	14.9	0.7	17.0	13.5–15.6	14.6	0.6	18.6	15.7–18.7	17.4	0.8	21.8
HW	4.2–5.8	5.2	0.5	5.9	5.1–5.7	5.5	0.2	6.4	5.1–6.5	5.6	0.5	7.1	6.7–8.1	7.3	0.4	10.0
HL	4.0–5.5	5.0	0.5	5.4	4.1–4.9	4.5	0.3	5.6	4.4–6.1	5.1	0.6	0.7	6.2–7.8	6.6	0.7	8.9
SL	1.7–2.5	2.0	0.2	2.4	2.0–2.1	2.0	0.1	2.3	2.1–2.5	2.3	0.1	2.6	2.8–3.5	3.2	0.2	4.3
EL	1.2–1.7	1.5	0.1	1.7	2.4–2.6	2.5	0.1	2.7	1.3–2.2	1.7	0.3	2.3	1.9–2.3	2.0	0.1	3.8
IUE	1.4–2.3	1.8	0.3	2.0	1.5–2.2	1.9	0.2	2.1	1.9–2.3	2.0	0.2	2.4	2.0–2.5	2.3	0.2	3.4
UEW	0.5–0.9	0.7	0.1	0.9	0.8–1.8	1.3	0.4	1.9	0.8–1.1	0.9	0.1	1.3	0.8–1.1	0.9	0.1	1.7
FLL	2.0–2.9	2.5	0.3	2.6	2.4–2.8	2.7	0.2	3.9	2.3–3.4	2.8	0.3	3.7	2.7–3.5	3.0	0.3	4.8
HAL	2.5–3.8	3.1	0.4	3.5	2.9–3.5	3.2	0.2	4.1	2.4–3.1	2.9	0.4	3.9	3.9–4.5	4.3	0.2	5.2
ShL	4.9–6.8	6.1	0.7	6.8	6.3–7.4	6.8	0.4	7.8	5.9–7.4	6.7	0.5	7.8	8.1–9.2	8.6	0.3	10.3
FL	4.2–6.6	5.8	0.7	5.9	5.9–7.6	6.8	0.5	7.8	5.4–7.4	6.5	0.6	7.6	8.1–9.1	8.7	0.3	10.5
TFOL	7.0–11.6	9.8	1.3	11.2	10–10.6	10.3	0.2	11.7	9.1–12.8	10.9	1.2	12.9	12.0–14.2	12.8	0.7	16.5
FOL	4.7–7.6	6.0	0.8	6.5	6.5–7.5	6.9	0.3	8.4	5.3–8.1	6.5	0.8	8.3	7.7–9.2	8.4	0.4	11.0

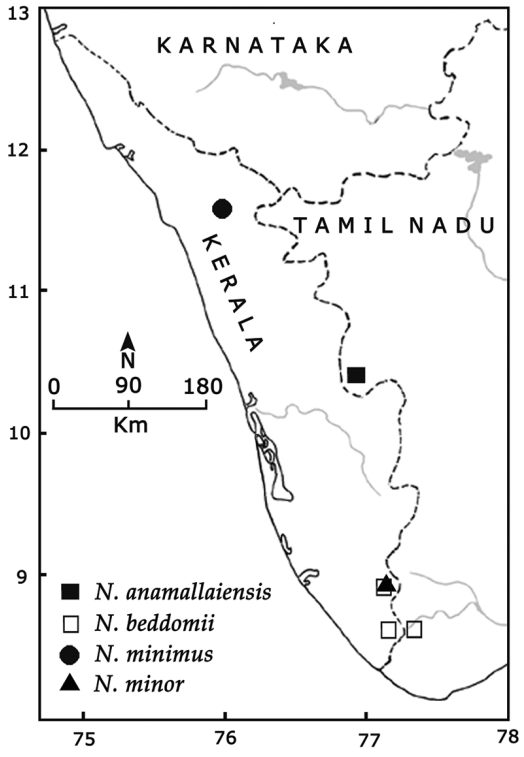

Figure 5. Distribution map for the four smallest species of *Nyctibatrachus* from the Western Ghats.

in distance from heel to tip of toe IV (TFOL 9.4); tips of toes with weakly developed disks, with marginal grooves; webbing absent (Figure 3 *b*); subarticular tubercles rather distinct, rounded, simple, IV2, 3 and V1 absent (Figure 3 *b*); inner metatarsal tubercle indistinct, oval; supernumerary tubercles absent; longitudinal groove on the dorsal surface of toe disks (Figure 3 *c*). Skin of snout shagreened to granular, upper eyelids with a few prominent tubercles; side of head, anterior and posterior parts of dorsum and upper and lower parts of flank granular, with a chain of granular projections forming a faint and interrupted dorsolateral fold from behind the eye to the vent on either side (Figures 1 *a* and 2 *a*); ventral side smooth. A pair of prominent oval-shaped femoral glands (length 3.1 mm × width 1.2 mm) is present on the hind legs (Figures 1 *b* and 2 *b*).

Colour of holotype in alcohol: A dark gray dorsum; dorsal surface of forelimbs and hindlimbs light grey with dark brown cross bands; ventrally uniform greyish with irregular spots, forearm and shank with brownish black markings.

Colour of holotype in life: A light tan dorsum without prominent patterns, except for a few dark brown markings between the eyes; iris light greyish-brown; forelimbs and hindlimbs light greyish-brown, with faint cross-bands; side of the head and lateral side with scattered minute white spots (Figure 1 *a*); ventrally light reddish-grey, forelimbs and hindlimbs with grey spots forming a vermiculated pattern; femoral glands orange (Figure 1 *b*).

Variation: Measurements (range, mean and standard deviation) of the type series are given in Table 1. Femoral glands of male specimens collected during the non-breeding season (February) were weakly developed compared to those of breeding males (July).

Distribution and natural history: *N. minimus* sp. nov. is currently known only from the type locality Kurichiyarmala, north of the Palghat Gap (Figure 5). This region is characterized by Shola forests (patches of high-altitude forest separated from one another by undulating grassland). Males start calling from marshes inside the forest during or immediately after sunset, with two subgular, external vocal sacs laterally inflated.

Size differentiation among nyctibatrachid species has been invoked to warrant recognition of the genus *Nanobatrachus* (*Nano* = small, *Batrachus* = frog)[16]. However, after the description of *N. minor*[12], a small species with typical *Nyctibatrachus* features, subsequent authors considered the two genera to be synonyms[13,14]. With the present description of *N. minimus* sp. nov. and removal of *N. anamallaiensis* from the synonymy of *N. beddomii*, the genus *Nyctibatrachus* now contains 14 species. *N. minimus* sp. nov., *N. anamallaiensis* and *N. beddomii* are among the smallest of approximately 220 currently known Indian species of frogs[6]. The lack of webbing on their feet contrasts with the extensively webbed toes of larger species. Furthermore, while most species of *Nyctibatrachus* are aquatic, the miniature species mainly inhabit the forest floor, where they are mostly found in leaf litter or under rocks. Miniaturization in Nyctibatrachidae may thus have been associated with a morphological specialization to life in terrestrial habitats.

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