H. binata is a biologically significant system because it retains embryogenic potential even after 4 years of repeated subcultures. In this tree species, we obtained direct somatic embryogenesis without an intervening callus phase. Somatic embryos developed in clusters and multiplied rapidly. Several generations of somatic embryos have been initiated with no decrease in embryogenic potential. Bipolarity of the somatic embryos was confirmed by histological studies. The results showed that in *H. binata*, it is possible to obtain direct somatic embryogenesis from semi-mature zygotic embryos. Such studies are potentially useful in obtaining artificial seeds and experiments on genetic transformation.

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ACKNOWLEDGEMENT. We thank the Department of Biotechnology, Ministry of Science and Technology, Govt of India, New Delhi for providing financial assistance.

Received 31 August 2000; revised accepted 13 November 2000

A new species of frog in the genus *Nyctibatrachus* (Anura: Ranidae) from Western Ghats, India

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A new species of *Nyctibatrachus* is described from the Western Ghats part of Kudremukh National Park, Karnataka, South India. This torrent species is differentiated from all known species in the genus by its very large size (SVL: snout vent length range 52–84 mm), stout body, rough and highly wrinkled dorsum, presence of two prominent folds in the tympanic region, prominent vomerine teeth located horizontally on highly-elevated strong ridges situated far behind the choanae. Hindlimb is short, tibio-tarsal articulation reaching the tympanic region, tips of fingers and toes dilated into prominent discs with circum-marginal grooves. Prominent and highly developed tubercles equal to the size of their respective terminal phalangial discs are present. The new species is compared with closely related congeneric species (*N. humayuni*, *N. major* and *N. vasanthi*). It markedly differs in colouration, size of tubercles, length of the forelimb, hindlimb, foot and tibia (calculated as a per cent of respective SVL) with a high degree of squared Euclidean dissimilarity.

HILLY terrain and forests of the Western Ghats of India are an abode for various amphibian species. This region harbours as many as 123 species[1–3]. Kudremukh National Park is located in the central Western Ghats (13°10′–13°26′N and 75°05′–75°10′E) and comprises highly complex vegetation mosaic of tropical evergreen forest, shola-grassland and mixed semi-evergreen forest, richly nourished by several hill streams and torrents. Biotic

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assemblage of this biodiversity-rich province is little known, especially for plants and other animals, while natural history information of the region is available for amphibians[4]. However, repeated and in-depth surveys have resulted in either addition of new species or extension of range of distribution of others. It has been felt that intensive observations will certainly result in the discovery of new species and improve the understanding of the distribution of amphibian fauna[5].

During the fieldwork of a research project sponsored by the Department of Science and Technology (DST) on amphibian diversity and habitat ecology, on 21 April 2000, some large and robust species of *Nyctibatrachus* were recorded in a torrential stream 'Kadambi Hole', flowing amidst the Kudremukh National Park (altitude: 850–1110 m msl). These frogs inhabited caves beneath the boulders in the riffle zone of water. On careful observation following the literature[6–13] describing various species of the genus *Nyctibatrachus* and comparison with closely-related congeneric species, it has been found that it is a hitherto undescribed species.

*Nyctibatrachus hussaini** sp. nov: Species description is based on four adult females and one adult male (Field No. KUES 176–180). Since collecting the specimen is prohibited in this national park, only one specimen (Field No. KUES 177), which was dead in the process of sampling was deposited in the office of the Range Forest Officer (Wildlife Division), State Forest Department, Kudremukh, Chickmagalore district. Photograph of the adult male (Field No. KUES 176) was submitted to the Natural History Museum, UK.

A large torrent species of *Nyctibatrachus* is different from other congeneric species by the presence of stout body, short limbs and indistinct tympanum (Figure 1). Two prominent skin folds are present in the region of tympanum, a supra-tympanic fold commencing at the base of the posterior border of upper eyelid and ending at the base of shoulder and another fold commencing at the base of the lower eyelid and ending on the angle of lower jaw are diagnostic. A prominent Y-shaped ridge commences from the tip of snout, the arms of which end at the anterior edge of the eye. The inter-orbital space bears a prominent black-coloured ridge connecting the two eyelids. Body is black, mottled with yellow. Limbs are not cross-barred; however speckled with body colouration, which extends up to the disc of the fourth toe on the dorsal side. Discs are prominent at the tip of fingers and toes with circum-marginal grooves and prominent sub-articular tubercles in limbs and metatarsal tubercle.

Vomerine teeth are very strong, located horizontally on an elevated ridge at a distance behind the choanae. The number of teeth is 9 to 10. Head is depressed and broader than long and snout blunt less than the diameter of eye. Canthus rostralis is indistinct and loreal region slightly concave. Nostril openings are dorsally oriented and slightly elevated from the skin surface. Pupil is rhomboidal and dorsally slightly elevated. Distance between eye and nostril is smaller than the diameter of eye. Inter-nasal distance is smaller than the inter-orbital distance and diameter of eye and is equal to distance between the eye and nostril. Inter-orbital space is equal to upper eyelid. Tympanum is indistinct. Forelimb is stout, fingers moderately long, webbing absent and the length is half of the snout vent length. The first finger is shorter than the second. Fingertips are dilated into discs with circum-marginal grooves. The fingers bear prominent sub-articular tubercles.

Hindlimbs are stout, nearly 1.3 to 1.5 times the length of SVL. Tibio-tarsal articulation reaches the posterior border of the eye. The length of femur and tibia is 2/5 the length of SVL. Length of the foot is nearly 80% that of the forelimb. The morphometry of the new species is detailed in Table 1. Outer metatarsals are separated by the web nearly to the base. Toe tips are with prominent discs, with circum-marginal grooves separating the upper and

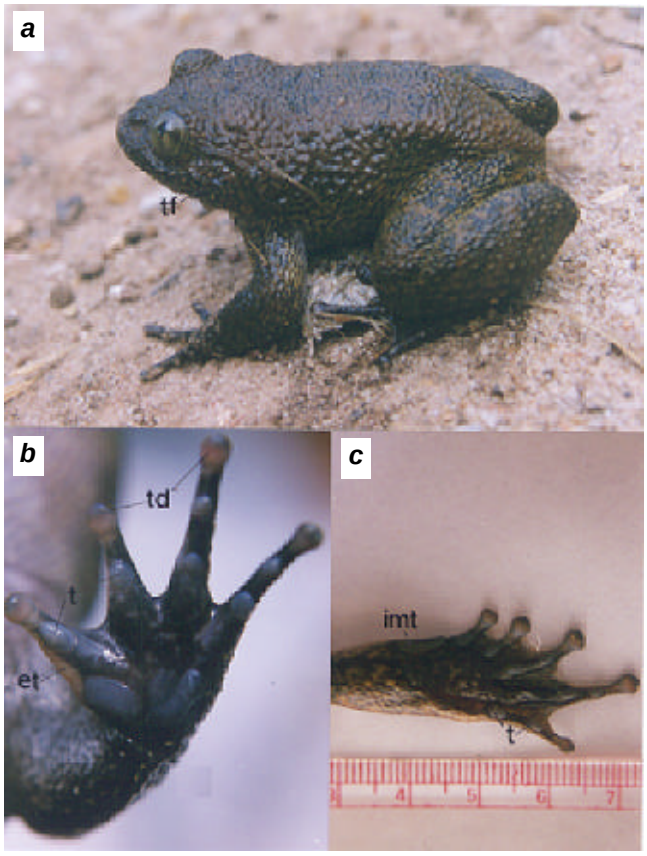

Figure 1. The new species of large wrinkled frog *Nyctibatrachus hussaini*. ***a***, Adult male (SVL 84 mm); ***b***, Details of under parts of palm (length, 21.5 mm); ***c***, Foot ventral side (length, 38 mm). (tf, Tympanic folds; td, Terminal discs with circum-marginal groove; t, Tubercles; et, Elongated thumb pad; imt, Inner metatarsal tubercle.)

*We take pleasure in naming this species after Shri S. A. Hussain, as a modest token of appreciation for his valuable suggestions, guidance and help in the diversity and ecological studies on amphibians of Kudremukh National Park, Western Ghats.

lower surfaces. Webbing is full and extends to the terminal discs of the toes. Sub-articular tubercles are prominent and are nearly equal to the diameter of the terminal discs. A prominent inner metatarsal tubercle is more than half the length of the first toe. Males possess a prominent cream-coloured elongated thumb pad on the lateral side of the first finger (thumb) and prominent femoral glands.

Skin on the dorsal side possesses small vermiculated foldings extending up to the toes. A prominent inverted Y-shaped ridge is seen from the anterior tip of the snout to the anterior corner of the eye. A black prominent horizontal ridge connects the two eyelids. The ventral surface of the lower jaw possesses longitudinal folds. The colour is black with yellow mottling. Ventral side is cream-coloured with black and yellow mottling on thigh and the space between the hands.

The adult body size, skin folds on the dorsum, webbing in the toes, circum-marginal groove on the finger tip dilation and canthus rostralis are the important characters used for the segregation of the species of *Nyctibatrachus*[6,8,10,12,13]. Characters of the present species show remarkable differences from congeneric species, with large adult body size, prominent corrugation of skin on the body surface and limbs, presence of circum-marginal grooves in terminal dilation of fingers and toes, complete webbing in foot and by absence of canthus rostralis with rounded snout.

However, this species possesses certain common characters with *N. humayuni*. The presence of rounded snout, absence of canthus rostralis, presence of circum-marginal grooves, shape of pupil and narrow eyelid and webbing patterns do exhibit similarity to that of *N. humayuni*. But it differs in having (a) Shorter snout, less than the diameter of the eye (which is equal in *N. humayuni*); (b) Narrow inter-obital space (equal to upper eyelid) compared to twice the width of upper eyelid in *N. humayuni*; (c) Prominent, highly elevated, strong and horizontally located vomerine teeth (9–10 in each ridge) located far beyond posterior border of choanae against the oblique series located close to choanae in *N. humayuni*; (d) Short and stout hindlimb, tibio-tarsal articulation reaching posterior border of eye (tympanic region), against the eye in case of *N. humayuni*; (e) Very prominent sub-articular tubercles with diameter equal to the terminal discs, compared to moderately developed ones in *N. humayuni*.

On the other hand, the depressed head, elevated nostril, narrow upper eyelid, indistinct tympanum, presence of a papilla on the hind corner of the lower eyelid and elongated metatarsal tubercles are similar to *N. major*. It however differs from *N. major* by (a) Presence of circum-marginal grooves, both in fingers and toe-tips, which are restricted in *N. major* only to toes; (b) Diameter of the eye shorter than inter-orbital space, against the slightly larger eye diameter in *N. major*; (c) Strong vomerine teeth located on an elevated ridge, with 9–10 teeth and a prominent gap between the two ridges, against the oval, oblique and closely-situated vomerine teeth in *N. major*; (d) Fully webbed toes.

The present specimen differs from *N. vasanthi* by its larger adult size, presence of prominent skin folds on the dorsum and prominent supratympanic folds. It differs from *N. deccanensis* by the full webbing in the foot and larger adult body size. All other congeneric species are smaller.

Table 1. Body measurements of *N. hussaini* (values are in mm)

	Field no. (Specimen no.)				
Parameter	176	177	178	179	180
Snout vent length	84	66	68	52	67
Length of head	26	18	19	16	19
Width of head	39	29	28	22	29
Width of body behind shoulder	46	28	33	25	33
Inter-nasal distance	6	6	4	4.5	5
Inter-orbital distance	11	8	9	6.5	8
Diameter of eye	10	9	7.5	7	8
Distance between eye and nostril	6	5	5.5	5	5
Diameter of tympanum	Indistinct				
Length of forelimb	43	38	38	29	37
Length of first finger	10	8	7	6	7
Length of second finger	12	10	8	7.5	9
Webbing in the hand	0	0	0	0	0
Length of hindlimb	109	88	94	72	90
Length of femur	32	24	24	28	27
Width of femur	21.25	16	16.6	14.5	17
Length of tibia	30	26	22	20	24
Width of tibia	14.6	10.5	11.6	8	11
Length of tarsus	12	13	12	9	12
Width of tarsus	7	5	5.6	3.5	5.4
Extent of webbing	Full				
Length of foot	38	29	32	22	32

Table 2. Mean SVL and tibial length of studied specimens (Values are in mm)

	N. hussaini (*n* = 5)	*N. humayuni* (*n* = 6)	*N. vasanthi* (*n* = 3)	*N. major* (*n* = 8)
Mean SVL	67.5 ± 11.347	42.0 ± 5.97	28.4 ± 6.67	41.77 ± 13.596
Length of tibia	24.5 ± 3.8410	20.33 ± 1.862	12.17 ± 0.306	16.08 ± 3.685

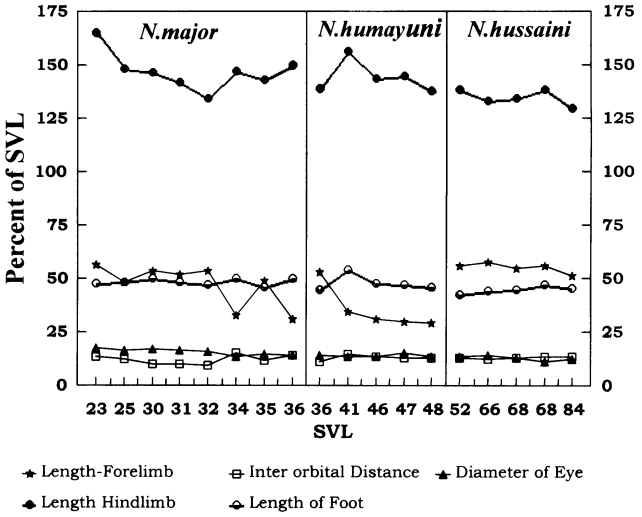

Figure 2. Comparison of some morphometric parameters of closely-related species of *Nyctibatrachus*.

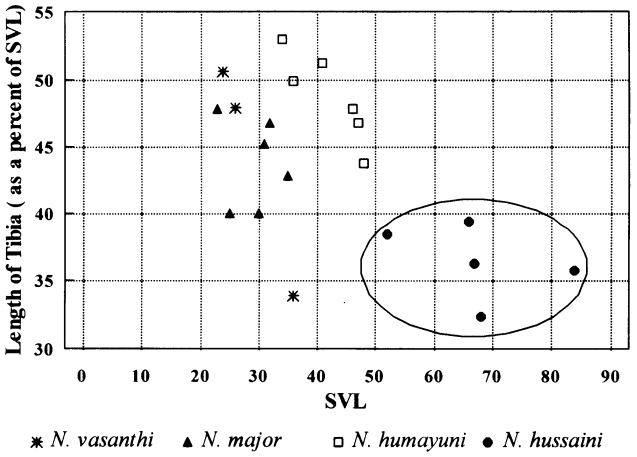

Figure 3. Comparison of tibial length (as per cent of SVL) of closely-related species of *Nyctibatrachus*, indicating clear differences in the ratio of tibial length to SVL in the new species.

Figures 2 and 3 give a comparison of the present species with other congeneric species, viz. *N. major*, *N. humayuni* and *N. vasanthi*. Compared with *N. major* and *N. humayuni*, the adult body size of the new species is the largest. The ratio of length of forelimb (calculated as per cent of SVL) in *N. humayuni* is less than the length of the foot (< 35%), while in *N. hussaini*, it is more than the length of the foot (> 50%). Further, the length of the hindlimb (calculated as per cent of SVL) is much greater in *N. major* and *N. humayuni* compared to *N. hussaini*. The length of tibia has further indicated this as it forms a separate group when plotted against corresponding SVL (Figure 3). The mean length of the tibia (as a per cent of SVL) is the shortest in *N. hussaini* (36.3%) followed by *N. major* (38.5%), *N. vasanthi* (42.8%) and *N. humayuni* (48.4%), respectively (Table 2).

Table 3. Absolute squared Euclidean dissimilarity coefficient calculated for the length of tibia (as a per cent of SVL) for the close congeneric species of *N. hussaini*

	Squared Euclidean dissimilarity coefficient		
Variable	*N. humayuni*	*N. hussaini*	*N. major*
N. hussaini	622.3325	–	–
N. major	87.08	289.2525	–
N. vasanthi	259.5781	296.4926	235.8781

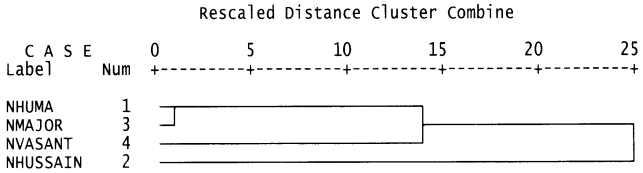

Figure 4. Dendrogram plotted for the absolute squared Euclidean dissimilarity coefficient for the length of tibia among the closely related species of *Nyctibatrachus*. NHUMA, *N. humayuni*; NMAJOR, *N. major*; NVASANT, *N. vasanthi*; NHUSSAIN, *N. hussaini*.

The squared Euclidean dissimilarity (Table 3) calculated for the data on the length of tibia as per cent of SVL, clearly depicts very high dissimilarity between *N. hussaini* and *N. major* (289.25), *N. humayuni* (622.33) and *N. vasanthi* (296.49), respectively. However, the dissimilarity is less between *N. major* and *N. humayuni* (87.08). The hierarchical cluster analysis using the centroid method (Figure 4) clearly depicts that *N. major* and *N. humayuni* are closer congeneric species compared to *N. hussaini* and the latter is totally separated from the other three congeneric species.

Thus *N. hussaini* is clearly distinguishable by its large size, very prominent body fold on the dorsum and limbs, presence of two folds at the tympanic regions, shorter hindlimbs and presence of very prominent circum-marginal grooves in both fingers and toes.

Table 4. Key for the identification of species of the genus *Nyctibatrachus*

1. Skin of the dorsum smooth – 2
   Skin of the dorsum with closely-set fold – 5
2. Webbing on the toe is less than one-forth or rudimentary – 3
   Toes half webbed – 4
3. Tibio-tarsal articulation reaches the tip of the snout; limbs are cross-barred – *beddomei*
   Tibio-tarsal articulation not reaching the eye; limbs without cross-bars (pupil red, body stout and toad-like appearance) – *kempholeyensis*
4. Webbing half to three-forth; tips of the fingers and toes without discs – *pygmaeus*
   Webbing full, tips of the fingers and toes with discs – *vasanthi*
5. Toes less than one-forth webbed – *minor*
   Toes half webbed – 6
   Toes more than half webbed – 7
6. Canthus rostralis present – *sylvaticus*
   Canthus rostralis absent – *sanctipalustris*
7. Adult body size small, toes three-fourth webbed; light dorsolateral band and presence of circum-marginal groove on the terminal discs of toes and fingers – *aliceae*
   Adult body size medium to large – 8
8. Toes two-third to three forth webbed – *major*
   Toes fully webbed – 9
9. Inter-orbital space equal to length of upper eyelid; tibio-tarsal articulation reaching the posterior border of eye; sub-articular tubercles equal to the diameter of terminal discs – *hussaini*
   Inter-orbital space twice the length of upper eyelid; tibio-tarsal articulation reaching eye; sub-articular tubercles moderate and smaller than the diameter of terminal discs – *humayuni*

Based on the extensive study on the specimens in the collection at the Kuvempu University, the new species and the literature[6–8,10,12,13], a key for the identification of the species of the genus of *Nyctibatrachus* has been proposed (Table 4).

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ACKNOWLEDGEMENTS. This work has been carried out as a part of DST (SERC Division, Govt of India) sponsored research project (No. SP/SO/C-39/97) awarded to S.V.K. Shri S. A. Hussain, Biodiversity Initiative Trust, Mangalore has helped us with his valuable suggestions during the study. We thank suggestions and constructive comments on the manuscript made by Dr R. J. Ranjit Daniels, CARE EARTH, Chennai. The help rendered by Mr Shankar, Range Forest Officer (Wildlife Division), Kudremukh National Park and Dr J. Keshavaiah, Department of Chemistry, Kuvempu University are acknowledged. K.V.G and A.H.M.R. thank DST for fellowship.

Received 5 December 2000; revised accepted 16 January 2001