

buffer and with viscosity between 10,000 and 30,000 centipoise in pre-filled syringes was produced under license from the Drugs Control authorities and injected into knees of people suffering from osteoarthritis. The results are given in Table 6. It can be seen from Table 6 that the formulation had excellent rejuvenating effect on all the recipients. Moreover, none of the patients had any noteworthy inflammatory reaction. After the completion of the efficacy studies, more than 4500 sterile, pre-filled syringes were made available to more than 1500 patients suffering from osteoarthritis of the knee. The results of application have been excellent.

Sodium hyaluronate of molecular weight between 1.0 and 1.3 MDa in the form of 1% solution in phosphate buffer, with a viscosity between 10,000 and 30,000 centipoises behaves like a viscoelastic fluid. It shows excellent therapeutic effects on all recipients suffering from osteoarthritis of the knee. More than 4500 pre-filled syringes were made available to about 1500 patients from all over the country.

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Extension of range of distribution of *Nasikabatrachus sahyadrensis* Biju & Bossuyt (Amphibia: Anura: Nasikabatrachidae) along Western Ghats, with some insights into its bionomics

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***Nasikabatrachus sahyadrensis* Biju & Bossuyt is observed to occur in the forested habitats of the Nilgiri ranges north of Palakkad Gap, indicating its extension of range of distribution from the hitherto known limit south of the Palakkad Gap between 8 and 11°N lat, i.e. Anamalai and Cardamom Hills in the Western Ghats. The adult individual of *N. sahyadrensis* freshly unearthed from a scooping pit in the habitat was tested for its behavioural tendencies for burrowing in the field and feeding in the laboratory under captive conditions in a glass tank filled with damp soil and prey. Some aspects of bionomics of this fossorial frog focusing on its burrowing and underground foraging behaviours with reference to morphological and ethological characteristics of the taxon are presented here.**

Keywords: Bionomics, burrowing, foraging behaviour, *Nasikabatrachus sahyadrensis*, Palakkad Gap.

BIJU and Bossuyt¹ described the burrowing frog *Nasikabatrachus sahyadrensis*, a new amphibian species from India, veritably acknowledged by animal taxonomists and biogeographers all over the world as one of the rarest kinds of 'once in a century find'². Molecular dating estimates and phylogenetic DNA analyses of the frog, recognized under a new family, indicate the new taxon's relationship with frogs of the family Sooglossidae endemic to Sey-

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chelles, thereby suggesting its evolutionary origin 130 million years ago, even before the break-up of the ancient Gondwanaland mass. The discovery of *N. sahyadrensis* from India and its relationship with sooglossids in Seychelles have revived the concept of the prehistoric land bridge between Africa and India that might have been a dispersal corridor for animals between the two countries.

Biju and Bossuyt¹ collected the holotype of *N. sahyadrensis* from a degraded forest near a cardamom plantation at Kattappana (type locality, 09°45'N, 77°05'E, altitude approximately 900 m asl), Idukki District, Kerala, Western Ghats, India. Dutta *et al.*³ considered three specimens of 'pignose frog' as similar to *N. sahyadrensis*, and therefore placed it under the family Nasikabatrachidae. Two specimens (one male and one female: SVL 52.8 and 89.9 mm respectively) were collected from a rainforest fragment, at Sankaran Kudi (10°14'46"N, 76°55'55"E) in the Anamalais, Tamil Nadu, and the third one (a gravid female, SVL 78.3 mm) from Murikkassery (near Kothamangalam), Ernakulam District. They have also examined additional specimens: one male (SVL 67.0 mm) collected from the Manimala River at Erumely, Kottayam District, Kerala, one immature male (45.0 mm) from Najayappilli village, near Thattekkad Bird Sanctuary, Ernakulam District, and one mature male (SVL not known) from Indira Gandhi Wildlife Sanctuary, Pollachi, Tamil Nadu. Dutta *et al.*³ have also studied four tadpoles, supposedly of the fossorial frog taxon, collected from a fast-flowing stream in a coffee estate at Manamboli (10°22'59"N, 76°55'23"E) in the Anamalais. Based on the above-mentioned collection data, Dutta *et al.*³ presumed the distribution limits of the frog taxon as being the forested habitats in the southern Western Ghats, falling within the range between 8° and 11°N lat. The presumed range evidently falls just south of the Palakkad Gap of the Western Ghats.

On 3 August 2004, a specimen collector brought to us one plump specimen of a frog (SVL 57.2 mm), reportedly obtained while digging out pits in a cleared site of a rubber plantation at Karuvarakundu, Malappuram District, Kerala. The frog, which was in its burrowed habitude, about 3 ft beneath the ground surface, got exposed while scooping the pit.

The next year, in a faunal exploration trip to this area, we could make a fortuitous collection of a second specimen (SVL 87.5 mm) of the species on 6 July 2005 from the same locality, again during soil-digging. The exact collection site falls within a plantation land, viz. Kundode Estate (lat. 11°06.4'N, long. 76°64'E, elevation approximately 500 m asl). Notably, it was during the monsoon period that both the specimens were collected.

Both the specimens (ZSI/CLT-V/A: 575 and 576) were identified as *N. sahyadrensis* based on the following salient characteristics. Body plump and relatively large in size; skin smooth but thick, and colouration dorsally black and ventrally dark grey. Head small, relatively short to body length, and sub-conical; snout tapered to a knob-like white

protuberance; a pair of nostrils, one each on either side of the snout-protuberance at its base; eyes small, upper eyelid prominent, lower eyelid merely an integumentary fold, iris black with a rounded pupil (horizontal pupil as mentioned in the original description¹), eye-diameter less than the distance between anterior eye-margin and the nostril, or inter-orbital distance; tympanum absent; mouth ventral, vomerine teeth absent, tongue small and rounded in front. Fore limbs short, palm hard, with fingers hardly/rudimentarily webbed, and tips rounded without discs; hind limbs small, tibia short, toes three-fourth webbed and rounded at the tips without discs, and with a large, elongated, shovel-like inner metatarsal tubercle.

The characteristics of the specimens available with us resembled the described features of *N. sahyadrensis* Biju & Bossuyt¹ and the fossorial frog taxon of Dutta *et al.*³ thereby indicating the identity of the latter with none other than the species *N. sahyadrensis* itself.

Following the collection of the specimen, we made some key observations on the bionomics of this amphibian species evidenced from its habitat site and the surrounding environs.

The collection site formed an area of disturbed/altered forest habitat on the gentle slope of a hill. The altered habitat comprised a mixed vegetation of plantation crops of cocoa and coffee on the hilltops, and rubber plantation on the slopes. A stream almost in a depleted phase was also observed in the area. The soil was mostly red, with a texture of top-layered forest loam on the surface and red soil underneath. The eco-habitat area veritably forms an integral part of the peripheral hilly-habitat environs that adjoin the Silent Valley National Park.

The plantation workers who enabled us to collect the specimen are knowledgeable about the existence of the burrowing frog *N. sahyadrensis* in the habitat mentioned above. They find this uncommon frog while cutting trenches in the plot during the monsoon period (June to October) and are aware of some behavioural peculiarities. According to them, live individuals are sparingly visible in the vicinity of the water paths/pools, at times found in pairs clasping each other, or buoying up in the side pools of the swelling streams, especially during the beginning of the monsoon season.

We made an attempt to observe the burrowing habit of the frog in the field by leaving it on a heap of loose soil. The lone robust frog freshly collected from the habitat was released at four different spots of varying surface hardness on the ground to observe some possible clues on its bionomics and behaviour. When left in the damp red-loam soil, the frog instinctively started to dig down and burrow itself beneath the loose soil and disappeared in the heap within 3–5 min. On hard-ground surface it made a vigorous attempt to burrow down, but not gaining enough progress, moved away from the spot. It looked uncomfortable to burrow down a ground with a thick mat of cluster weeds. When kept on pebbled-gravel-strewn

ground in an open and dry stream-bed, instead of exhibiting burrowing tendency, it frantically tried to escape from the spot with long stretches, characteristically without any leaping movement aided by the hind limbs.

The observation made by us in the laboratory revealed more information on the bionomics of this species. The frog left exposed on hard or dry soil of the ground did not show any leaping movement aided by its hind limbs. This feature, owing to its short hind limbs, is in stark contrast to the characteristic feature of other anurans which have markedly long and powerful hind limbs modified for leaping. As regards the visual acumen, its smaller eyes, unlike the large and well-developed ones found in other anurans, apparently seemed less advanced. The pointed snout, on the other hand, was found to be sensitive to the touch-stimuli from its surrounding environment.

We closely observed the burrowing behaviour of the species by keeping the lone robust frog collected inside a glass tank (90 cm length, 45 cm width and 60 cm height), filled with damp soil admixed with soil termites, ants and small worms, up to three-fourth depth, i.e. 45 cm in the tank. The mode of burrowing (Figure 1 *a*) was observed to be characteristic. The hind limbs with strong, tubercled feet were primarily used for digging. The frog assumed a squatting posture, with thigh and shank of each hind limb closely tucked postero-lateral to the body. The frog's broad, outwardly directed feet laterally worked like spades, making use of the enlarged, crescentic metatarsal tubercles (Figure 1 *b*), scrubbing-off the under-soil outwardly and over to the back of the body. The forelimbs with hard palms (Figure 1 *c*) pushed the body downward, besides stabilizing it in the process. To the space voided by the digging feet, the thigh and shank were in-pushed, dragging the body along, thereby gradually being covered and concealed by the displaced soil. When the frog in its just burrowed condition was gently uncovered to observe its digging process, its dorsal trunk (epaxial) musculature appeared compactly contracted to become a pair of bulbous blocks on the back (vide Figure 1 *a*), apparently working in unison with the musculature of the pelvic girdle and hind limbs, enabling the digging process. The whole body assumed a compact mass. The frog at rest, in its burrowed condition, was found to have a horizontal posture, with the limbs closely tucked to the body.

The fossorial frog in its captive condition in the laboratory resumed its burrowed living in the damp soil and never came out of its concealment, even for feeding during night. It did not remain idle underneath the soil. It gave an indirect hint about its 'under the soil' movement by shifting positions from the initially burrowed and settled spot to others within the damp soil. This was realized when the frog was gently exposed, occasionally, in order to replenish the soil with food such as soil-termites and ants, and also to check whether it was alive during longer periods of captivity. We maintained the frog in its subterranean living conditions for five months from July to November 2005. Underneath the soil, it thrived on termites. The frog presumably depended more on its olfactory and tactile cues rather than on the visual acumen, to detect/locate prey. Its strong head having a hard-knobbed snout and a small ventral mouth with a narrow gape indicated that this species was more likely an underground feeder that ingests only small prey such as termites, ants and small worms.

The feeding mechanism of most anurans, which have wider gape of mouth and considerable diversity in tongue structure, normally involves lingual flip. The limitation of this fossorial anuran, *N. sahyadrensis* to its underground foraging by tongue-flipping mode was very evident by the fact that the frog possessed a small ventral mouth with narrow gape and a small, basally attached and fluted tongue. These characters apparently enable it to capture and ingest only small prey.

N. sahyadrensis mainly feeds on soil termites³, which is evident from our observation also. In the laboratory, initially earthworms and ants were fed to the frog to know its feeding preference. Commonly available earthworms (10 nos, 5–10 cm long) released in the damp soil of the glass tank remained unaffected without being preyed upon by the frog. As it was difficult to get small ants to feed the frog, taking cue from the literature³, the frog was tested with the termite prey. Two to three specimen tubes (20 ml capacity) of termites collected from wild and homestead terrestrial habitats were mixed with the soil in the glass tank thrice a week. The feeding behaviour of the frog became evident with clear indications of its subsoil movements, whenever usual supply of termite-prey was ensured, in the soil of the tank. The gut



Figure 1. *Nasikabatrachus sahyadrensis*. *a*, Mode of burrowing; *b*, Foot showing enlarged inner metatarsal tubercle; *c*, Palms showing hardened areas; *d*, Frontal view showing the calloused snout tip and oral groove.

analysis carried out later showed the presence of semi-digested traces of termite-prey.

Intake of small prey, such as termites, can be reasonably linked with the functional adaptations of the frog's head and mouth. The sub-conical head is short and stout, and the pointed snout is calloused at the tip (Figure 1 d), as a protuberance, overhanging the mouth, facilitating the fossorial frog to penetrate the subterranean termite niches while foraging underground. The lower jaw, unlike the more rigid upper jaw, is flap-like and flexible. Bordering with the upper jaw it could form a grooved aperture (Figure 1 d) through which the basally attached fluted tongue can be protruded out to stick or suck up the termite-prey from subsoil fissures, or even from underground termite galleries/tunnels.

The feeding ecology of *N. sahyadrensis* appears to be well integrated with the ecology of soil-termites to the extent that the latter could even become the preferred prey of the frog. Most moist rainforests have relatively large number of genera of termites⁴, and many termite species are subterranean insects colonizing in their abundant population/biomass, thriving on dead wood and humus in the predominantly monsoon type climate of the region. The burrowing and mound-building activities of termites increase the rate of percolation of rainwater and aeration of both the top and subsoil keeping the underground soil temperature low and the moisture content high^{5,6}. This might become indirectly beneficial for the fossorial frog taxon.

The feeding mechanism enabling the underground subsistence of this fossorial frog is apparently evolved in synergic relationship with the ecology of the subterranean termites, which resolves many puzzles on the frog's tackling of the crucial physiological requirements in the underground environment. In India, *N. sahyadrensis* from the southern Western Ghats may be the only known amphibian species that is a fully underground forager. All other burrowing frogs are either open burrow feeders or diurnal burrow dwellers that are open ground feeders in the night.

Other fossorial frogs, which assume a similar foraging strategy are the species of *Rhinophrynus* (Anura: Rhinophrynidae) inhabiting the sub-humid lowland areas from southern USA, Mexico to Costa Rica of North and Central America, and *Hemisus* (Ranidae: Hemisinae) found in tropical and subtropical sub-Saharan Africa⁷. Although both have no phylogenetic relationship with *Nasikabatrachus*, their member species (e.g. *Rhinophrynus dorsalis* and *Hemisus guttatum*) have striking similarity with *N. sahyadrensis* in some of their structural and physiological adaptive features concerned with the feeding mechanism. Both have robust bodies, short limbs, smooth skin and small head. The enlarged, spade-like inner metatarsal tubercles are used to dig into soil rapidly by lateral movements of the feet. They spend most of their life underground, and feed on termites/ants by the unique method of tongue protrusion through a buccal groove. The snout

pointed with calloused tip can penetrate into termitarium or tunnel. They emerge from underground only after heavy rains. *R. dorsalis* exhibits inguinal amplexus, floating on surface of temporary pools⁷.

The morphological and ethological characteristics of the taxon have enabled it to adapt to the present mode of life. In spite of its structural/physiological limitations related to locomotion, feeding, respiration and reproduction, this species considered as a phylogenetic relict one, still shackles itself to its environment, which accounts for its success. While most of the modern amphibians have exhibited amazing evolutionary diversity, this species has taken less progressive changes in the mode of life that is well answered by its phylogenetic senility.

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Vegetative and reproductive phenophases in *Aesculus indica* Colebr. at two different altitudes in Himalayan forests

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Phenological features of *Aesculus indica* Colebr. were studied in Kumaun Central Himalaya in relation to leaf sprouting, anthesis, pollen production, fruit setting, development and retention, and leaf and fruit drop at two different altitudes. Leaf initiation in this species starts in the middle of February and leaf formation occurs in March. Initiation of flowering was observed during the first fortnight of April at both the sites,

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