

## Structure of the femoral gland and habitat features of an endemic anuran, *Nyctibatrachus major* (Boulenger)

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### Abstract

*Nyctibatrachus major* (Boulenger) is one of the uncommon ranid anurans endemic to the Western Ghats. This paper describes the habitat features, sexual dimorphism in the male and female and morphometry of the species. The males of this species possess sex-specific, bilaterally symmetrical, ventral femoral glands, which are modified mucous glands and are likely to aid in amplexus. The structure of the femoral glands is described in relation to the seasons.

**Key words:** *Nyctibatrachus major*, habitat, morphometry, femoral gland, histology.

### 1. Introduction

The richness and diversity of amphibian fauna of India in general and of Western Ghats in particular is well documented<sup>1–4</sup>. The Western Ghats with its heavy annual precipitation and concomitant large areas of tropical evergreen forests is known to offer the optimum habitat features for several species of amphibians. Despite the degradation of natural forests, even today, the area is the home of a variety of amphibians including caecilians<sup>5–6</sup>. On account of the availability of stable micro-environments, of the 104 endemic Indian amphibian species, 84 are distributed in the Western Ghats<sup>4</sup>. One such endemic anuran is *Nyctibatrachus major* (Boulenger).

A faunistic survey and morphometry of the species collected from Wynad, Kerala, has been reported by Pillai<sup>7</sup>. The males of this species possess femoral glands, a brief morphological description of which has been reported by Inger *et al*<sup>8</sup> and Pillai<sup>9</sup>. Due to infrequent availability of this hill-stream species, more details about the species have so far not been documented. The paper presents general observations on the habitat and morphology of the species with particular reference to the histology of the femoral glands in the male.



FIG. 1. Photograph of (a) male and (b) female *Nyctibatrachus major* (dorsal view)

## 2. Materials and methods

Individuals of *Nyctibatrachus major* (Fig. 1) were collected after intense search of the forest area around Sringeri (Lat.  $13^{\circ} 25' 05''$  - Long.  $75^{\circ} 15' 14''$ ) and a total of 24 adult frogs were collected. Of these, six were males. After recording the morphometric measurements of the bilateral femoral glands of these, a few of them were processed for histological staining<sup>10</sup> using Haematoxylin-eosin. The stained sections ( $8\mu$ ) were observed under a binocular research microscope and necessary measurements were taken using a calibrated ocular micrometer. Micro-photographs of the histological structure of the femoral glands were taken by using an Olympus OM 1N camera.

## 3. Results

### 3.1. Occurrence and habitat features of the frog

Table I presents the data on the availability of *N. major* from the areas around Sringeri. Unlike many other anurans of this area, *N. major* is a difficult species to locate and capture. Almost all the specimens collected were found near submerged rocks of lotic habitats streaming through the core forest areas at medium elevations (see Table I). Adults of the species are mostly aquatic. Except for their snout and nostrils, which are exposed, the rest of the body is totally submerged in the water. A record of the temperature and pH of water at the site of collection indicated that the frogs inhabited acidic waters (pH range 6.0-6.5) at a temperature of  $18.0$ - $24.5^{\circ}\text{C}$ . An intense search for these frogs in other habitats at lower altitudes in Sringeri was futile. That the species is a forest dwelling and aquatic has also been reported by Pillai<sup>7</sup>. Hitherto, the maximum number of individuals (42) of *N. major* collected from Wynad is by Pillai<sup>7</sup>, and his observations, as well as those of Inger *et al*<sup>6</sup> confirm the present observations regarding the habitat features of this species. Further, it is to

Table 1  
Occurrence of *Nyctibatrachus major* in Sringeri

Date	Place	Nos collected		Habitat (Approx altitude) in m > MSL
		Male	Female	
09.07 1990	Sannanegunda	1	2	Forest streams (760)
09.09 1990	Sannanegunda	1	1	Seepage water – small streams of the forest (760)
24.10.1990	Sirtmane	–	1	Small tributary (= 900)
17.12 1990	Meegnakallu forests	1	2	Seepage water (= 850)
24.12 1990	Sirtmane	–	1	Margin of a small tributary (= 900)
15.01.1991	South Kanara border	1	3	Small litter-covered streams (1000)
01 05.1991	Sannanegunda Meegnakallu forests	2	7	Seepage streams (820)
19.06 1991	Sannanegunda	–	1	Seepage water (760)

be noted that the present collection of *N. major* from the environs of Sringeri is a first record of its occurrence in this area.

### 3.2. Sexual dimorphism

Table II presents the data on the morphometry of males and females. From this table, the following points of interest can be noted:

- i) The range of the snout-vent length (SVL) of six males was 22.0–52.0 mm, while that of the eighteen females was 17.0–48.0 mm.
- ii) Most of the other morphometric parameters are apparently related to the SVL and average values indicate higher ranges in the males than in the females.
- iii) On the basis of external morphometry alone (not observing the femoral glands in the males), it is difficult to distinguish the sexes of the species (see Fig. 1). Therefore, the males which were morphologically segregated based on the presence of femoral glands, were also dissected to confirm the presence of testes. Since even the smallest male of 22 mm SVL had distinct femoral glands, it is evident that these glands are typically sexually dimorphic parameters of the species (see Figs 2a and 2b). Although males were observed to produce sex-specific vocalization, no external vocal sacs could be distinguished, indicating that the vocal sacs were internal.

### 3.3. Morphology and morphometry of the femoral glands

As illustrated in Fig. 2, the femoral glands of male *N. major* are bilaterally symmetrical, elliptical glands situated on the ventral side of the thighs. In the freshly

**Table II**  
**Morphometry of the males and females of *Nyctibatrachus major***  
 (Average values and ranges are denoted in mm)

Parameters	Males <i>n</i> = 6	Females <i>n</i> = 18
Snout-vent length	40-66 (22.0-52.0)	39-50 (17.0-48.0)
Fore limb length	21-66 (09.0-28.0)	20-05 (09.0-27.0)
Hind limb length	58-17 (30.0-70.0)	54-00 (24.0-69.0)
Inter orbital width	05-83 (03.0-08.0)	04-70 (02.5-06.0)
Length of upper eyelid	04-50 (03.0-05.0)	03-80 (02.5-06.0)
Snout length	06-50 (04.0-08.0)	06-28 (03.5-08.0)

collected specimens, the glands appear rather translucent and pinkish (Fig. 2a), the latter indicating its rich vascularity through the branches of the femoral artery as confirmed by dissection. On preservation, the glands became more distinct, opaque and cream coloured (Fig. 2b). On close observations, the gland surface appeared granular. Figure 3 presents the data on the length of femur, tibia and femoral gland, as well as the width of the femoral gland, in relation to the SVL of the males. With increase in SVL, while relative increases in the first three parameters were evident, the femoral gland width was more or less similar for frogs beyond 35 mm SVL.

Since the males were collected during different times of the year(s), the data on the length of femoral gland as a percentage of SVL was plotted against the collection



**FIG. 2a.** Photograph of male and female *Nyctibatrachus major*. (Ventral view reduced to 0.4 times; arrows denote the position of the femoral glands in the male).

**FIG. 2b.** Closer view of the femoral glands in male *N. major* (specimen preserved in formaldehyde).

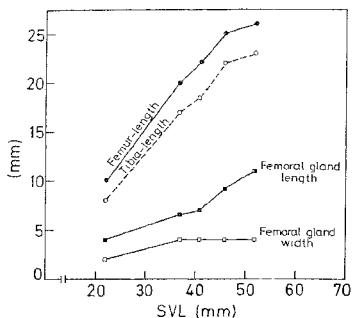


FIG. 3. *Nyctibatrachus major*: Morphometry of males in relation to SVL.

months to ascertain any seasonal bearing, if any, on the size of the femoral gland (Fig. 4). The areas of collection fall in the seasonal climatic rainforest belt<sup>11</sup>, where the seasonality is largely based on monsoons. Each year can be divided into three distinct seasons of premonsoon (February through May), monsoon (June through September) and postmonsoon (October through December and extending to the end of January). Based on this seasonality, not considering the smallest male of 22 mm SVL (which may be an immature frog), it is evident from the figure that during the present studies the males of *N. major* have been collected in all three seasons and

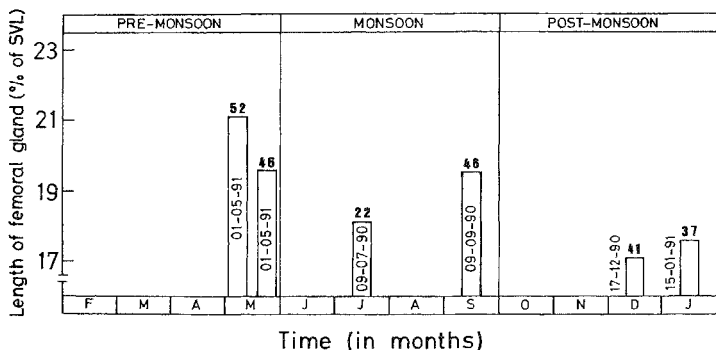


FIG. 4. Variation in the length of femoral glands in relation to seasons. Values indicated above each histogram correspond to the SVL (in mm) of the respective specimen.

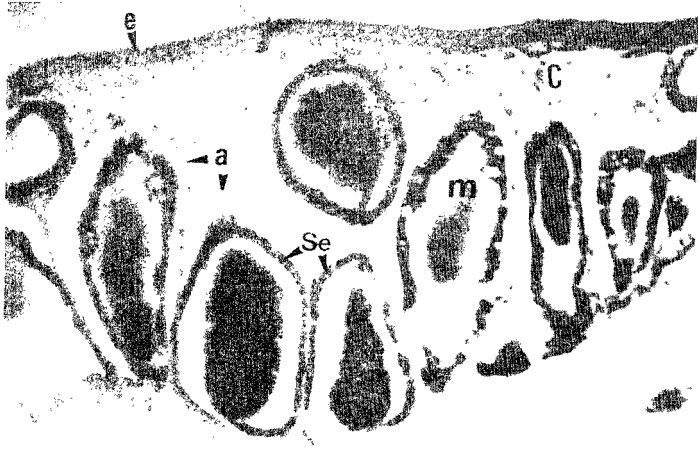


FIG. 5a. T.S. of the femoral gland collected in December 1990 (H&E stained;  $\times 170$  times).

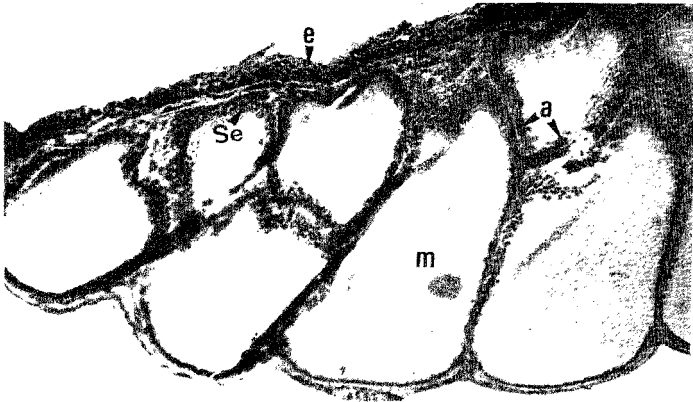


FIG. 5b. T.S. of the femoral gland of the specimen collected during May 1991 (H&E stained;  $\times 175$  times). e: Surface epithelium of the femoral gland, a: Acini, c: Connective tissue matrix, Se: Secretory epithelium and m: Mucoid secretions of the acinus.



FIG. 6. A magnified view of a single acinus of the femoral gland showing histological details (H&E stained;  $\times 1600$  times). e: Surface epithelium of the femoral gland, v: vacuoles, o: Opening of the acinus to the exterior and m: Mucoid secretions of the acinus.

that the relative length of the femoral gland may have a seasonal bearing—longer during premonsoon and monsoons and shorter during postmonsoons. However, further work is needed to confirm this observation.

#### 3.4. Histology of the femoral glands

Figures 5a and b illustrate the cross-sections of a femoral gland of *N. major* collected during December 1990 and May 1991, respectively, corresponding to the post- and premonsoon seasons. In general, the femoral gland is composed of a large number of spherical or elongated acinii, arranged mostly in one layer and each opening by a distinct micropyle-like pore (Fig. 6) to the exterior, through multilayered skin epidermis. It appears that these femoral glands are specialized hypertrophied mucous glands<sup>12</sup> serving some special function. In the postmonsoon-collected specimen, the number of acinii were fewer than in the one collected in summer and in the former,

the inter-acinar space consisted of a connective tissue matrix which was feebly basophilic. Each acinus was filled with mucoid eosinophilic secretions which filled the entire lumen of the acinus in the premonsoon-collected frogs but not so in the postmonsoon-collected specimens. The periphery of the mucoid contents of the postmonsoon-collected frog also showed vacuolization along the secretory epithelium, which was absent in the premonsoon-collected frog. A comparison of acinar epithelium also indicated that the cells were more basophilic during premonssoons than in postmonssoons. On the whole, it appears that there are subtle differences in the structure of the femoral glands of frogs collected during the two seasons.

#### 4. Discussion

The presence of male-specific sex characters in many anurans is well known<sup>13</sup>. The most commonly encountered secondary sex characters include the thumb pad elaboration in the males, the structure of which is believed to vary with sexual cycle thereby indicating its apparent regulation by the male hormone<sup>13-17</sup>. The exclusive presence of femoral glands in the males of *N. major* and its variation in relation to seasons may also reflect the pattern of its activity. Since the complex environmental factors in the Western Ghats<sup>4</sup> also show a strict seasonality relative to the rainfall<sup>11</sup>, the size and function of the femoral gland may also have a seasonality and reproductive function like the thumb pad of Anurans<sup>15,16</sup>. The latter needs to be investigated. The structure and histology of the femoral glands of the male *N. major* indicate that they may aid in amplexus of this hill-stream anuran. However, this needs to be confirmed further through observations on amplexant pairs. Earlier reports mention that the femoral glands are present in the adult males of this species<sup>8</sup> and during the present studies, even the smallest male of an SVL of 22 mm had the femoral glands. Therefore, it would also be interesting to study the ontogeny of the femoral glands in the species.

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