

Also, inclusion of the neurotoxin P2 (Amm P2)⁶ among leurotoxin I, charybdotoxin and noxiustoxin³ is not correct. Alignment of the relevant cysteine residues in register keeping in view the structural motif that exists in CsEV3 structure and also in the neurotoxin P2 structure, the structure should actually be classified under 'I5A' type tertiary structural category⁴. Classification of these disulphide-containing proteins/peptides under correct structural categories is relevant and important, because the predicted structures can be used as initial models to interpret the three-dimensional structure data by X-ray diffraction and NMR techniques.

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pH in the digestive system of some gastropod molluscs

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Here we show that the gut and digestive gland of *Pila globosa*, *Bellamyia bengalensis* and *Achatina fulica* is slightly acidic.

ENORMOUS work has been done on the digestive physiology of gastropod molluscs. Optimum conditions for the activity of a number of digestive enzymes have been determined^{1,2} but there is no information regarding pH milieu in the digestive tract itself. Since efficiency of digestion depends upon pH, efforts were made to measure the pH of different parts of the gut of some gastropods *Pila globosa*, *Bellamyia bengalensis* (Prosobranchia) and *Achatina fulica* (Pulmonata).

Freshly collected animals were refrigerated for 30 min at 5°C for immobilization. The entire alimentary canal along with the digestive glands was dissected out and kept on a clean slide. Digestive glands were separated and teased. Different parts of the gut were cut and slit open and the pH was determined using pH paper³. One snail of each species was dissected for one experiment and each experiment repeated six times.

Results are summarized in Table 1. Careful analysis of the data indicates that buccal mass is very slightly acidic (pH ranges 6.0 to 6.5) in *Pila* but almost neutral (pH 6.5 to 7.0) in *Bellamyia* and *Achatina*. Oesophagus is slightly alkaline (pH 7.0 to 7.5) in *Bellamyia* but acidic (pH 6.0 to 6.5) in *Pila* and *Achatina*. Stomach is the most acidic part in all the three species. In *Pila* and *Achatina*, pH is almost similar to digestive gland (5.5 to 6.0). In *Bellamyia*, pH of stomach is higher (6.0 to 6.5) compared to digestive glands (5.5 to 6.0). Intestine is slightly acidic in *Pila* (pH 6.0 to 6.5) but almost neutral in *Bellamyia* and *Achatina* (pH 6.5 to 7.0). The pH of hind gut is identical (6.0 to 6.5) and of digestive

Table 1. pH in the digestive system of some gastropod molluscs

Gastropod	pH range					Digestive gland
	Buccal mass	Oesophagus	Stomach	Intestine	Hind gut	
<i>Pila globosa</i>	6.0-6.5	6.0-6.5	5.5-5.8	6.0-6.5	6.0-6.5	5.8-6.0
<i>Bellamyia bengalensis</i>	6.7-7.0	7.0-7.5	6.0-6.5	6.5-7.0	6.0-6.5	5.5-6.0
<i>Achatina fulica</i>	6.5-7.0	6.0-6.5	5.5-6.0	6.5-7.0	6.0-6.5	5.5-6.0

glands is more or less the same (5.5 to 6.0) in all the three species.

However, there is no marked difference in the pH milieu of the gut of *Pila*, *Bellamya* and *Achatina*. All are slightly acidic. Gut of *Pila* is more acidic and oesophagus of *Bellamya* shows slight alkalinity.

Since *Pila*, *Bellamya* and *Achatina* are mainly phytophagous, similar pH environment in their gut is not unexpected. Amongst insects, pH of the gut appears to be characteristic of taxonomic group rather than feeding habit³⁻⁵. The same may be true for molluscs as well. Slightly acidic gut may be characteristic of gastropod molluscs.

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Courtship, amplexus and advertisement call of the frog, *Microhyla rubra*

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Microhyla rubra has a single reproductive season (April–October) in a year. Sexually mature males possess a single subgular vocal sac during breeding season. Males produce advertisement calls which are species-specific and consists of a number of calls produced in series at variable intervals forming a call group. Each call group comprises 8–104 calls. Each call is formed of a pulse group of 15–21 pulses. The frequency spectrum is continuous and broad. The sound energy is concentrated between 300 Hz and 3300 Hz. Gravid females are attracted by the advertisement call, and this results in axillary amplexus.

MICROHYLA RUBRA is a small microhylid frog found in the vicinity of Dharwad (Karnataka, India). The information on its reproductive biology is scarce. Many recent studies demonstrate that acoustic communication has reached a remarkably high level of development of differentiation even though there are surprisingly few different types of calls among various anuran species¹. Bioacoustics in Indian microhylids are limited to *Ramanella variegata*². The present study concerns some aspects of breeding behaviour and advertisement calls of *M. ornata*.

Field observations to study the courtship, amplexus and vocalization of *M. rubra* were carried out for three years (1988–1991). During frog calling, the calls were identified and recorded on a tape recorder (at 4–8 cm/s speed). Microphones were held within a distance of 15–20 cm of a calling frog. Recordings were done at 24–26°C air temperature and 85–90% relative humidity. The sound pressure level was measured by MEONIX sound level meter. Calls of ten frogs ($N=10$) were analysed at the Zoologisches Institut, Bonn Universität by examining oscillogram (Textronix oscilloscope 502 A; Tonnie's Recordine Camera; film speed 25 cm/s) and by sonagram analysis (with computer program MOSIP (R) spectro analyses V6 41/89, MEDAV GmbH). Statistical analysis was carried out with program STSC, Inc. (Knoxville, USA).

M. rubra are burrowing and nocturnal frogs and have a single reproductive period. Males possess a single subgular (Figure 1) vocal sac which assumes black colour during the breeding season. Adult males give advertisement call. The calling is in chorus, and the shortest distance between nearest calling males measured is 10 cm. Calling period begins with the first heavy showers of premonsoon (April/May)/monsoon (June) rains and ends in October. Between April and June calling is maximum, and from July onwards the number of callers becomes less and less, calls becoming rare between September and October. The advertisement call attracts the gravid female. Females come out of their burrows and quickly jump towards calling males. Sometimes females come jumping from a distance of 15–20 m and move nearer the calling males. With the next call the female moves quickly to reach the calling male and pushes itself under it. Then it is suddenly clasped by the male. After proper adjustment between both, it results into axillary amplexus (Figure 2). After the amplexus the male does not produce advertisement call.

Frogs call during night by sitting in open field and also hiding under grass within a distance of about 10 m from the pond. Advertisement call is audible to human beings from a distance of 20–25 m. The sound pressure level of the call varies from 83 to 96 dB. Table 1 shows analysis of the call. The advertisement call of *M. rubra* consists of a number of calls produced in series at variable intervals forming a call group³. Each call group comprises 8–104 calls. Duration of call group varies from 7 to 76 seconds. The call duration varies between 138 ms and 228 ms. Each call is formed of a pulse group of 15–21 pulses (Figure 3) and the pulses overlap in any part of the call. The amplitude of the first pulse is always low and rises quickly in second pulse. By 4th or 5th pulse it reaches a maximum and is followed by a gradual decrease. The frequency spectrum is continuous and broad. The sound energy is concentrated between 300 Hz and 4150 Hz. The fun-