RESEARCH COMMUNICATIONS


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A comparative analysis of the molars of Mus booduga, Mus dunnii and fossil Mus of the Indian subcontinent: Phylogenetic and palaeobiogeographic implications

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Upper molars of Mus booduga collected from Varanasi and Mus dunnii (chromosome type-I) show slightly derived features compared to those of Mus booduga collected from Mysore and Mus dunnii (chromosome type-II). Mus auctor, Mus elegans, Mus flynnii, Mus sp. indet., Mus jacobi, Mus sp., Mus booduga and Mus dunnii are closely related to each other in having moderately elongated and rather strongly distorted M1's and moderately reduced M2's (except for Mus elegans, Mus sp. and Mus jacobi). Representation of Mus, both in terms of taxonomic diversity and considerable numbers, in the Plio—Pleistocene deposits of the Indian subcontinent is indicative of an early diversification event in the history of Mus.

In all murids, M1 (first upper molar) is relatively longer than M2 (second upper molar), which in turn is longer than M3 (third upper molar). According to Misonne1 lengthening of M1 in a forward direction accompanied by reduction of M2 brings the whole series forward and occupies the place left by P4 (fourth upper premolar). The length of M1 and M2 is given in relation to M2, which has a standard relative length of 100% (ref. 1). Generally ancient murids have broad molars, thus Misonne1 suggested that broad molars correspond to the more generalized types. Jacobs proposed that in primitive murids, labial cingulum on lower molars are poorly developed and anterostyle on M1 relative to lingual antercone, but not on the posterior extremity.

During the preliminary studies on Late Pliocene Mus, it was observed that it resembles Mus booduga, the Indian Pigmy Field mice3, most closely. In the light of evolutionary trends of murids suggested by Misonne1 and generalized dental characters of murids proposed by Jacobs, an attempt has been made here to study Mus booduga (extant taxon, collected from Varanasi and Mysore), Mus dunnii (extant taxon, chromosome types I, II and III), Mus auctor and Mus sp. reported from Late Miocene and Early Pleistocene Siwalik deposits, respectively2, Mus flynnii and Mus sp. indet. recovered from Late Pliocene (around 2.5 m.y.) Siwalik sediments.

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of India, *Mus jacobisi* discovered from Late Pliocene (around 2.4 m.y.) deposits of Karewas (Kashmir, India) and *Mus elegans* reported from Pliocene deposits of Paul-I-Charkhi, Afghanistan.

Eleven skulls (with 132 teeth) each of adult *Mus booduga* and *Mus dumni* housed at Cytogenetics Laboratory, Centre of Advanced Study in Zoology, Banaras Hindu University, were examined. Forty isolated teeth of fossil *Mus* housed at Vertebrate Palaeontology Laboratory, Centre of Advanced Study in Geology, Panjab University have been used here for comparison.

Measurements were made using a microscope fixed with a reticle. Dental terminology of murid molars proposed by Jacobs is followed here.

Matthey and Petter were first to distinguish between *Mus booduga* and *Mus dumni* on the basis of divergent karyotypes but they also observed a slight difference in shape of molar and colour of underparts. The colour of underparts however is at times misleading. The pygmy field mice *Mus booduga* and *Mus dumni*, which are endemic to the Indian subcontinent, are morphologically very similar and they inhabit the same ecological fields.

The diploid number of chromosomes is 40 in them but while the karyotype of *Mus booduga* with all acrocentric chromosomes is identical to *Mus musculus*, that of *Mus dumni* is distinct due to presence of composite submetacentric X and large acrocentric Y chromosomes. The works carried out in the Cytogenetics Laboratory of BHU over the last several years have revealed that *Mus dumni* is in active phase of speciation. In sharp contrast to the extreme conservation observed in the karyotype of *Mus booduga* throughout India, *Mus dumni* populations of different places have divergent karyotypes. Three chromosome types (I, II and III), which are apparently parapatric in distribution, have been found so far, and on conducting mate-preference behaviour in them have shown some degree of ethological isolation. Recently, on hybridization between chromosome types I and III, it has been observed that they are also to some extent reproductively isolated since varying conditions of hybrid fertility of males and inviability of hybrid females have been observed. The stable karyotypic difference among the three chromosomes types of *Mus dumni* has been achieved by establishing homozygosity for heterochromatin variation at the centromeric regions and as prominent short arms of the autosome pairs 1, 3 and 6. The chromosome types are incipient biological species.

Marshall examined specimens of *Mus booduga* collected from Utter Pradesh, Madhya Pradesh, Karnataka and Tamil Nadu states, and of *Mus dumni* collected from Haryana, Uttar Pradesh, Madhya Pradesh, Maharashtra and Tamil Nadu states of India. According to him, *Mus booduga* has a long and slender first upper molar with a long anterior cusp (lingual anterocone) surrounded by an accessory cusp (prestyle) and an inconspicuous antero-external cusp (labial anterocone). During the present investigation, the above-mentioned features were clearly observed on *Mus booduga* collected from Varanasi but specimens of *Mus booduga* collected from Mysore have relatively shorter lingual anterocones on M1 and lacked a prestyle (Figure 1a, b).

Incisive foramina of *Mus booduga* collected from Varanasi penetrate deeper (reaching between antero- and ento-style of the M1) compared to those of *Mus booduga* collected from Mysore and *Mus dumni* (chromosome types I, II and III), where it reaches the level of antero-style of M1 (Figure 1f–g). Upper incisors of *Mus booduga* collected from Mysore are more curved compared to those of *Mus booduga* (Varanasi) and *Mus dumni* (types I, II and III) (Figure 1k–o).

*Mus* is characterized by having a reduced M3 and M1, antero-style on M1 anteroposteriorly compressed and

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**Figure 1.** Occlusal views of upper and lower molars of *a. Mus booduga* (Varanasi specimen no. Mbw-3); *b. Mus booduga* (Mysore sp. no. M3); *c. Mus dumni* (type I, sp. no. Md-4); *d. Mus dumni* (type II, sp. no. M2); *e. Mus dumni* (type III, sp. no. Mdh-1); *f* and *k–o. skulls showing position of incisive lamina and curvature of upper incisors (sp. nos. Nbv 3, M3, Md-4, M2 and Mdh-1 respectively). Bar = 1 mm.
posterior relative to the lingual antercone and posterior cingulum reduced or absent. Further, M₃ has an ‘X’ pattern formed by the joining of labial and lingual anterconids, proconid and metaconid. *Mus auctor* is considered to be the most primitive form of *Mus* recovered so far. It differs from *Mus flynni*, *Mus sp. indet.*, *Mus jacobi*, *Mus elegans*, *Mus sp.*, *Mus booduga* and *Mus dumi* in having a reduced M₁ with distinct precingulum, M₂ with a conspicuous labial antercone and a reduced posterior cingulum and M₃ with a labial antercone (Figure 2a). Possible phylogenetic relationships between *Mus flynni* and *Mus auctor* and *Mus jacobi* and *Mus auctor* have been suggested.

Molars of *Mus dumi* (type-II), *Mus booduga* (Mysore) and *Mus flynni* resemble each other in having moderately elongated lingual antercones and M₃ lacking labial cingulum (Figure 1b, d and 2b). On the other hand, molars of *Mus dumi* (type I and III), *Mus booduga* (Varanasi), *Mus sp. indet.* and *Mus jacobi* are quite similar to each other in having considerably elongated lingual antercones, prestyles (variably present) M₃ with distinct labial cingulum and medial anterconids (Figure 1a, c, e, 2c and d).

Missoni suggested that in advanced murids, M₁ is extremely elongated relative to M₂ and M₃ and some forms of *Mus* even have M₁ with length over 240% of M₂ and M₃ around 40%; this gives a total of
240 + 100 + 40 = 380% which implies that in spite of having three teeth, such specimens would have a surface equal to that of four teeth of dental series of primitive rodents. Such calculations were carried out on fossil and extant taxon compared herein. The results show that Mus auctor should be most generalized of all in this character followed by Mus flynni and Mus elegans (Table I). Mus sp. indet. and Mus jacobsi are considered here to be relatively derived in this character. Among the extant forms, Mus booduga (Mysore) and Mus dunnii (type II) are generalized in this character compared to Mus booduga (Varanasi) and Mus dunnii (type I), whereas Mus dunnii (type III) falls somewhere in between these two types.

Three specimens of Mus sp. have been reported from the Early Pleistocene Siwalik deposits, near Pabbi Hills, Pakistan. Forty-three specimens of Mus jacobsi come from Late Pliocene deposits of Kacwa, Kashmir. Twenty-nine specimens of Mus flynni and 11 specimens of Mus sp. indet. have been collected from Late Pliocene, Siwalik sediments, near Saketi Village, Himachal Pradesh, India. Such a representation of Mus species may suggest an early diversification in the history of Mus. On the basis of occurrences of Mus in Pliocene and Pleistocene deposits, a palaeobiogeographic province has been constructed (Figure 3).

Apart from the Indian subcontinent, Plio-Pleistocene Mus is known from Hadar Formation, Ethiopia (represented by four molars) and Lake Turkana, Kenya (represented by six molars). As this collection from Africa lacks M1’s, it is at the moment difficult to compare it with that of the Indian subcontinent from the phylogenetic point of view.

In Figure 4, values (length and width) of M1’s and M2’s of all the Mus specimens fall in one domain. This observation encourages us to propose that on the whole Mus booduga, Mus dunnii, Mus flynni, Mus auctor, Mus elegans, Mus sp. indet., Mus jacobsi and Mus sp. are very close to each other and further recovery of fossil material, particularly from Pleistocene deposits may provide a better picture on the evolutionary lineages of Mus.

Prostatic inhibin has a predominantly anti-parallel β-sheet structure

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Prostatic inhibin (94 amino acid residues, $M_r = 10,540$) is a protein isolated from the human and animal prostate glands. Three-dimensional structure of this cysteine-rich (10/94) protein has been studied by NMR spectroscopy. Preliminary investigations provide valuable information on the secondary structure of this protein. It is found to acquire a predominantly anti-parallel β-sheet structure and possibly the molecule is locked into several such sheets through disulphide linkages.

Prostatic inhibin is a protein with 94 amino acids and molecular weight of 10 kDa. It has been isolated from the human and animal prostate glands. More than a decade of research has established a wide range of its biological activities, ranging from preventing pregnancy to curing prostate cancer. Inhibin prevents pregnancy by modulating the level of circulating follicle-stimulating hormone (FSH) in mammals. It suppresses prolactin, a hormone that promotes lactation. Therefore, neutralizing inhibin through active immunization has been found to increase milk production. Although the primary structure of this molecule has been determined, no information is available so far about the three-dimensional structure. Our preliminary NMR investigations throw light on the three-dimensional structure of prostatic inhibin - an information of great value, in view of the useful biological activities of this molecule.

About 20 mg of HPLC pure protein was dissolved in 0.5 ml of an appropriate solvent (approximately 3 mM) and buffered with 100 mM acetate buffer. The pH was adjusted to 4.2. NMR measurements were carried out in 99.9% 2H$_2$O and in a mixed solvent consisting of 90% H$_2$O and 10% 2H$_2$O. Temperature was optimized for the best possible resolution and all spectra were recorded at 310 K. NMR experiments were carried out on a Bruker AMX 500 spectrometer with a 1H frequency of 500 MHz and involve (i) two-dimensional (2D) two-quantum-filtered correlation spectroscopy (2QF COSY), (ii) 2D clean total correlation spectroscopy (clean TOCSY) with a mixing time of 100 ms, and (iii) 2D nuclear Overhauser enhancement spectroscopy (NOESY).

Prostatic inhibin has about 600 observable protons. The 500 MHz NMR spectrum shows reasonably well resolved features. Figure 1 shows the 1D 1H spectrum of the protein in a mixed solvent of 90% H$_2$O and 10% 2H$_2$O. Figure 2A and 2B show 2QF COSY and NOESY spectra, respectively, in 99.9% 2H$_2$O. A detailed analysis of these and other spectra has enabled us to identify several spin systems. These include eight threonines, five valines, two glycines, one leucine, one isoleucine and twenty-one AMX spin systems (belonging to Cys, Ser, Asp, Asn, Tyr, Trp, His and Phe). The subspectral features are well dispersed. For example, the C$_a$H–C$_b$H correlations for all the eight threonine residues present in the prostatic inhibin are shown in Figure 3. Thus, at this stage, we have been able to identify almost half of the spin systems. Even in the absence of sequential resonance assignments, the NMR data provide valuable information on the secondary structure of this protein.

In the first instance, the 1D spectrum (Figure 1) indicates that the protein has a well-defined and ordered structure. There are several downfield-shifted C$_a$H protons as well as several upfield-shifted methyl resonances. The downfield shift of the C$_a$H protons is a