in our case the order of enhancement of SERS intensity due to pyridine is comparable to that of the best silver aggregates reported earlier.

It has been observed that Kr and He–Ne lasers enhance the SERS spectra of pyridine adsorbed onto the green sol, to the maximum extent and hence best suited for the excitation purpose unlike Ar laser. This is because the wavelength for green absorption due to fractal aggregates of silver resonates with the Kr and He–Ne lasers. The He–Ne laser which was available could generate only 20 mW power. Hence we have used Kr laser for its high power efficiency.

The SERS results were obtained in Prof. A. J. Creighton's laboratory at the University of Kent at Canterbury, England.

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Species identification of snake-head fishes by nuclear DNA RFLP: Its taxonomic implications

The snake-heads (genus *Channa*, family Channidae) are commercially important air-breathing fishes. They occur in freshwater marshes, swamps and ditches of the Indian sub-continent[1]. Three species of snake-head fishes namely, *Channa punctatus*, *C. striatus* and *C. gachua*, which occur sympatrically in this locality were studied. The natural populations of these species show a declining trend owing to (i) the modification and encroachment of their habitat by developmental activities, and (ii) their over-exploitation. Inter-specific hybridization has been attributed to be yet another cause of decline of populations in some species of fishes like apache trout[2,3]. Environmental changes and habitat modification have led to increased frequency of hybridization in various groups of fishes including Cyprinidae, Salmonidae, Cyprinodontidae, Cichlidae, Catostomidae, Escocidae, Poeciliidae, Antherinidae, Centrarchidae and Percidae[4].

Although these species are sympatric, there is no report on hybrids. Unlike cyprinids, salmonids and other groups of fishes, are these species not susceptible to natural hybridization (due to the stringent reproductive isolation mechanism!) or has the natural hybridization between these species escaped the notice in the absence of suitable markers that would have helped in identifying the hybrids? This study was undertaken to find a species-specific molecular marker, that might allow detection of natural hybridization between these species.

Restriction fragment length polymorphism (RFLP) of ribosomal rRNA gene has been useful for systematic analysis in fishes at the species level[5,6], species and hybrid identification in carps and catfishes[7,8]. In the hybrids, the restriction fragments of ribosomal rRNA gene were found to inherit biparentally[7,8]. Therefore,

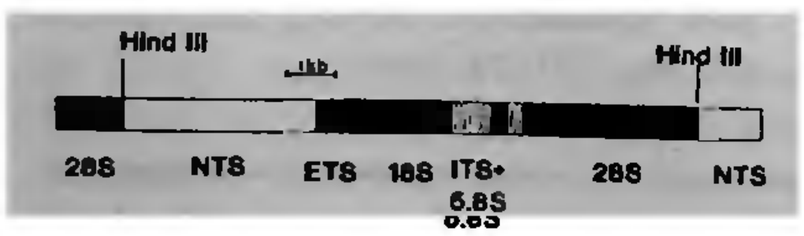

Figure 1. A diagrammatic representation of ribosomal RNA gene structure. The 5.8S, 18S and 28S are the regions coding for 5.8S, 18S and 28S rRNAs. ETS, External transcribed spacers; ITS, Internal transcribing spacer; NTS, Non-transcribing spacer.

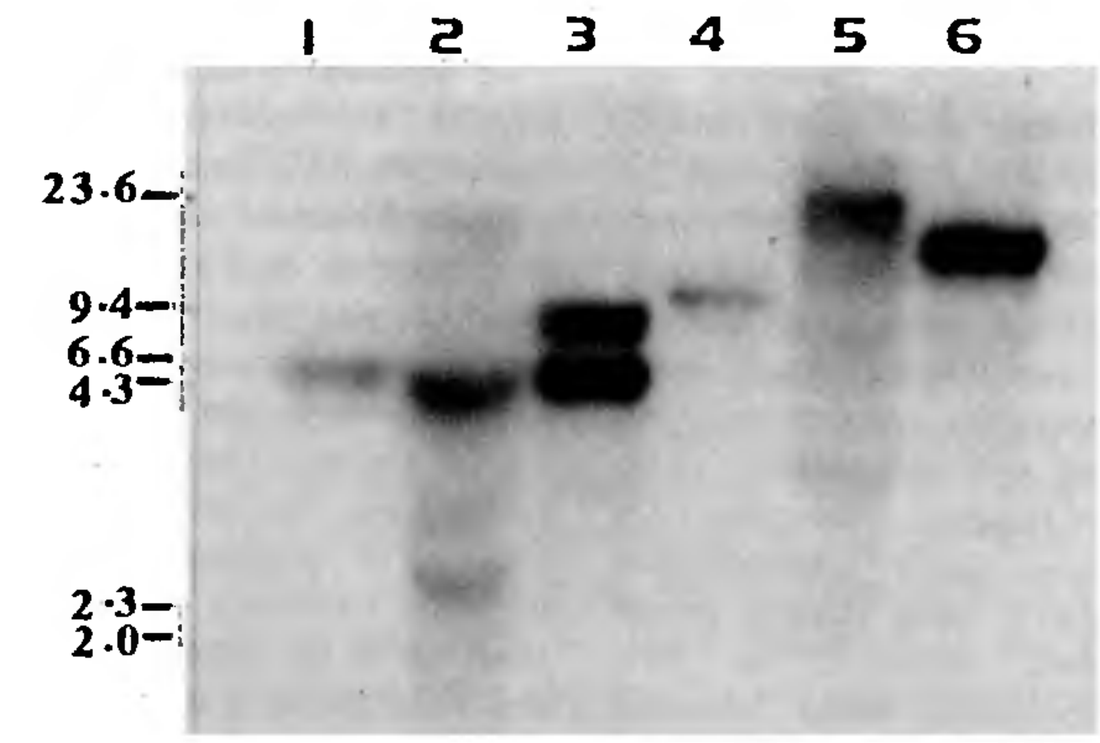

Figure 2. Ribosomal RNA gene RFLP in *Channa*. Restriction enzyme digested nuclear DNA was subjected to electrophoresis and Southern hybridized using labelled pXlr 101 probe. *Eco*RI digests (lanes 1–3): 1, *Channa punctatus* (Cp); 2, *C. striatus* (Cs); 3, *C. gachua* (Cg). *Hin*dIII digests (lanes 4–6): 4, Cp; 5, Cs; 6, Cg DNA. The approximate positions of the lambda *Hin*dIII size markers are indicated.

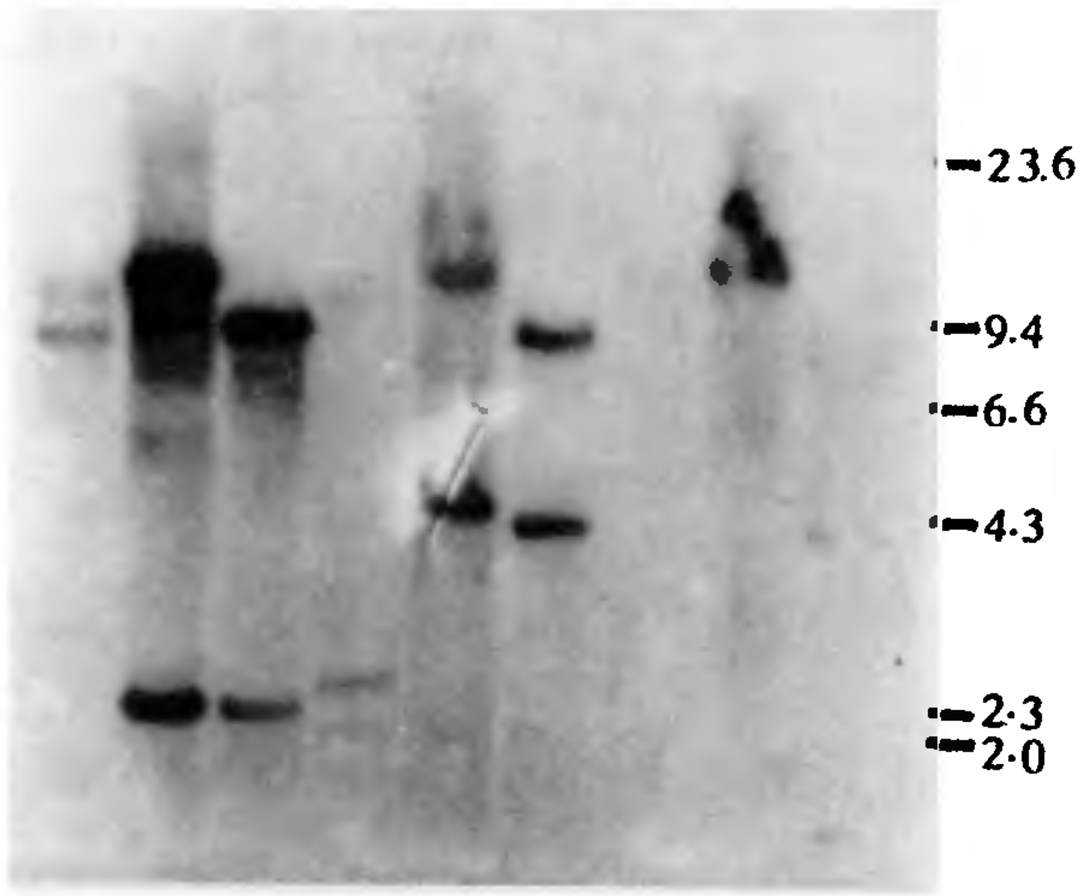

Figure 3. Ribosomal RNA gene RFLP in *Channa*. Nuclear DNA digested with *Pst*I (lanes 1–3), *Bam*HI (4–6) and *Bgl*II (7–9). Lanes 1, 4 & 7 for Cp; 2, 5 & 8 for Cs 3, 6 & 9 for Cg.

nuclear DNA RFLP using ribosomal RNA gene probe was used in the present study to find out species-specific marker, which can later be used in the hybrid identification programme.

Live specimens of *C. punctatus*, *C. striatus* and *C. gachua* were bought from the local market. About one ml of blood collected by cutting the caudal peduncle of anaesthetized live fish was used for nuclear DNA isolation following the method of Marmur[9] with some modifications as described by Ghosh *et al.*[8].

Nuclear DNA was digested with appropriate restriction enzymes and fractionated on 0.8% agarose gel by electrophoresis, Southern blotted onto Hybond N^+ nylon membrane (Amersham, UK) and hybridized with the isolated 12 kb *Hin*dIII rDNA fragment from clone pXlr 101 of *Xenopus laevis*[10], radiolabelled with 32αP dATP using random primer kit (Bangalore Genei)[11], in 50% formamide containing buffer following Maniatis *et al.*[11]. The 12 kb ribosomal RNA gene clone of *Xenopus laevis*, pXlr 101, carries 5′ETS-18S-ITS-5.8S-28S-ETS-NTS-3′ (Figure 1) and was cloned at *Hin*dIII site in pBR322. After hybridization and washing[11], the filters were autoradiographed using ORWO or Konika X-ray film. The molecular sizes of the hybridized fragments were determined with reference to *Hin*dIII digested lambda phage DNA.

By digestion with *Eco*RI, a common band of 5 kb was obtained in these three species (Figure 2, lanes 1–3). In *C. punctatus* one 5 kb band was obtained, whereas in *C. gachua* an 8 kb band along with the 5 kb was observed. In *C. striatus* besides the common 5 kb band a high- and a low-molecular weight band (14.8 and 2.5 kb) were found. The low-molecular band was often not detectable possibly because of polymorphism. By *Eco*RI digestion a common 5 kb band was also observed in the fishes examined earlier[8] and it might be a conserved feature in fishes.

*Hin*dIII did cut once in the rDNA repeat (Figure 2, lanes 4–6) of these species producing about 9, 18.8 and 14 kb fragments in *C. punctatus*, *C. striatus* and *C. gachua*, respectively. *Pst*I and *Bam*HI (Figure 3) had two sites each in these fishes. *Bgl*II (Figure 3) had one site each in *C. punctatus* and *C. striatus* but *C. gachua* showed polymorphism either having a single or three-banded phenotype (Figure 3, lane 9). Thus, barring a limited polymorphism, these species showed species-specific RFLP pattern with respect to *Hin*dIII, *Bam*HI and *Pst*I, which could be useful as marker in hybrid identification programme.

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