peated SSRs are ubiquitous in plants. The SSRs flanked by DNA sequences that are present only once in a genome, i.e. 'microsatellites', can be used as valuable molecular markers, and may be ideal for assessment of genetic diversity and facilitating core collections. (iv) The retroelements comprising repetitive DNA elements with dispersed organization constitute the major component of plant nuclear genome, and exhibit regions of depletion or amplification scattered throughout. Identification of changes associated with retroelements, particularly retrotransposons that account for most of the variation in genome size[13] would be of great value to understand means and ways to genome evolution.

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Increased bilirubin binding to erythrocytes of tobacco chewers than non-chewers

The binding of unconjugated bilirubin, a catabolic product of haemoglobin, to various types of cells including erythrocytes and its toxic effects are well known[1]. Under physiological conditions, human erythrocytes bind a fixed amount of bilirubin from a given bilirubin load[2,3]. This bilirubin binding property of erythrocytes, however, changes with the variation in the physico-chemical properties of erythrocyte membranes[4]. Recently, we have demonstrated that the erythrocytes from healthy smokers bind more bilirubin than erythrocytes from healthy non-smokers[5]. This increase in bilirubin binding to the smokers' erythrocytes can be correlated to the smoke-induced changes in the physico-chemical properties of erythrocyte membrane[6,7]. The nicotine in tobacco can also be absorbed simply by contact with the mucus membranes of the mouth[8]. But, unlike the effects of cigarette smoke, incubation of nicotine with erythrocytes is reported to show decreased deformability of the cells[9]. Whether the effect of tobacco leaf extract on the bilirubin uptake by erythrocytes is same as that observed with cigarette smoke is not known. Therefore, it is interesting to study the binding of bilirubin to erythrocytes from healthy tobacco chewers. Here, we present our data on the binding of bilirubin to erythrocytes from healthy tobacco chewers and healthy non-chewers. Our data suggest that erythrocytes from healthy tobacco chewers bind more bilirubin than that from healthy non-chewers.

Blood samples of both healthy tobacco chewers consuming more or less similar amount of nicotine per day and non-chewers (females of the age group between 25 and 40 years) were collected in 4% sodium citrate after interviewing the persons concerned thoroughly. Erythrocytes were isolated from the blood samples by centrifugation at 1000 *g* for 20 min. Then, 1.0 ml of albumin (2.0%) in 0.07 M sodium phosphate buffer, pH 7.4, containing 0.08 M sodium chloride was added and the mixture was incubated for 20 min in

order to remove traces of bilirubin previously bound to erythrocytes. The cells were then washed three times with the same buffer (without albumin). Finally, to the final packed cell volume, the same volume of buffer was added to get the 50% haematocrit value. No difference in the total cell count was found in the prepared 50% haematocrit value from both tobacco chewers and non-chewers. This observation was fairly in agreement with the earlier observation[10].

Fresh bilirubin solution was prepared by dissolving a few crystals of bilirubin in a known volume of 38 mM sodium carbonate solution containing 5 mM EDTA, pH 11.0. Its concentration was determined by the method of Fog[11]. The solution was protected from light and used within 1 h and experiments with bilirubin were performed in yellow light.

Binding of bilirubin to erythrocytes was studied by incubating 1.0 ml of erythrocyte suspension of 50% haematocrit value (containing 3.5×10^8 cells) with 1.0 ml of bilirubin solution containing varying amounts of bilirubin in the range of 0–600 nmoles. The final volume of the incubation mixture was made up to 6.0 ml with 0.07 M sodium phosphate buffer, pH 7.4, containing 0.08 M sodium chloride. After incubation at 37°C for 30 min, it was centrifuged at 1000 *g* for 20 min and the supernatant discarded. The cells were washed 3 times with 5.0 ml of the same buffer. The last supernatant was always found devoid of bilirubin as determined by the method of Fog[11]. Erythrocyte-bound bilirubin was eluted with 2.5 ml of 2.5% albumin solution after incubation at 37°C for 20 min. The supernatant containing bilirubin was collected after centrifugation for 20 min at 1000 *g* and the amount of bilirubin in the eluate was determined by the method of Fog[11].

Figure 1 shows the binding of bilirubin to erythrocytes obtained from different individuals (healthy tobacco chewers and healthy non-chewers) at a given amount of bilirubin in the incubate. The percentage coefficient of variation in the amount of bound bilirubin to erythrocytes obtained from different samples of each group (chewers and non-chewers) at all the bilirubin concentrations used in this study was found to be within 5%. In case of chewers' samples, it seems reasonable as consumption of tobacco was more or less the same in these individuals. The amount of bilirubin in the incubate was varied from 10 to 520 nmoles. A comparison of the amount of bilirubin bound to erythrocytes of healthy non-chewers and healthy tobacco chewers at a given amount of bilirubin in the incubate suggests that erythrocytes from healthy tobacco chewers remained indistinguishable ($P > 0.05$) from those of non-chewers as both of them bound the same amount of bilirubin when 110 nmoles of bilirubin were present in the incubate. On the other hand, any increase in the amount of bilirubin in the incubate beyond 110 nmoles led to a distinction in them. Erythrocytes from healthy tobacco chewers bound more bilirubin as compared to those from non-chewers. This difference in the amount of bilirubin bound to these erythrocytes became more pronounced on increasing the bilirubin concentration in the incubate. The increase in the amount of bilirubin bound to erythrocytes from healthy tobacco chewers compared to those from healthy non-chewers when 152, 180, 360 and 520 nmoles of bilirubin were present in the incubate was 17.8% ($P = 1.69 \times 10^{-7}$), 16.4% ($P = 9.24 \times 10^{-7}$), 28.9% ($P = 1.54 \times 10^{-5}$) and 43.9% ($P = 2.12 \times 10^{-9}$), respectively. From these results, it appears that bilirubin binding sites on the erythrocytes from healthy non-chewers and healthy tobacco chewers have more or less the same affinity for bilirubin. Interaction of tobacco extract with erythrocyte membranes resulted in the formation of a few more low affinity binding sites which could only be detected when higher amount of bilirubin was present in the incubate. This explanation seems to be quite reasonable as a similar pattern of bilirubin binding to erythrocytes has been reported when erythrocytes were incubated with bilirubin along with lipid extract of soybean[12]. However, the increase in the amount of erythrocyte-bound bilirubin observed with erythrocytes from healthy tobacco-chewers was relatively less compared to smoke-induced increase in bound bilirubin to erythrocytes from healthy smokers. Since the composition of cigarette smoke and tobacco is entirely different, this might be one of the reasons for the difference in bilirubin binding behaviour of erythrocytes from smokers as reported earlier[5]. Thus, it can be concluded that the erythrocytes from healthy tobacco chewers are capable of binding more bilirubin compared to erythrocytes from healthy non-chewers. It has been reported that the frequency of neonates suffering from bilirubin encephalopathy is more if mothers are addicted to tobacco[13]. However, from our results, it is difficult to predict the effect of tobacco chewing on the health of individuals suffering from jaundice, but in view of the low survival of bilirubin-bound erythrocytes[14], it is expected that erythrocytes of tobacco chewers may be more prone to premature senescence which will result in higher production of bilirubin.

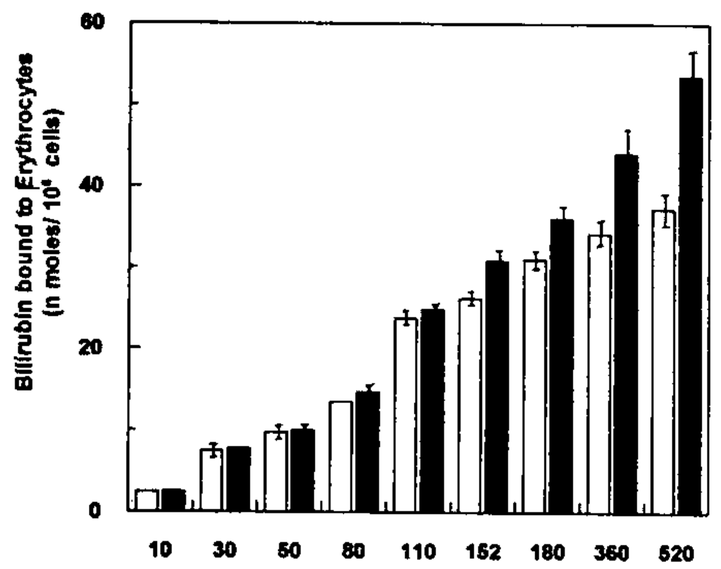

Figure 1. Binding of bilirubin to erythrocytes from healthy non-chewers (□) and healthy tobacco chewers (■). The values represent a mean of eight observations from eight independent samples. The standard deviation is shown by the symbol ⊥.

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The new sponge resources of Orissa coast

From the 215 records of sponges in Indian museums[1], only 20 specimens were reported from Orissa coast before 1920. The collections were made (1908–1910) by trawl in *Golden Crown* and *Investigator* expeditions mostly from depths of 36–54 m. Thereafter, there has been no information regarding the coral-based sedentary organisms off Orissa coast.

During the last two decades, active research is being carried out by scientists throughout the world on 'drugs from the sea'. In India too, during the last six years, considerable amount of work has been done in several universities and institutes. Regional Research Laboratory (RRL), Bhubaneswar has taken up investigations on the marine organisms off Orissa coast (Bay of Bengal). While collecting non-edible/poisonous/venomous benthic fauna mostly by trawling, several indications of the existence of sedentary fauna like gorgonids, sponges, hard corals and soft corals had been noticed by the authors and it was decided to collect these organisms.

Under this project, the geophysical survey of the sea bed was carried out by NIO, RC, Visakhapatnam, utilizing dual frequency echosounder, side scan sonar, sub-bottom profiler and portable global positioning system. The area of study is off Gopalpur coast between

Figure 2. *Azorica pfeifferae* Carter, species no. 46.

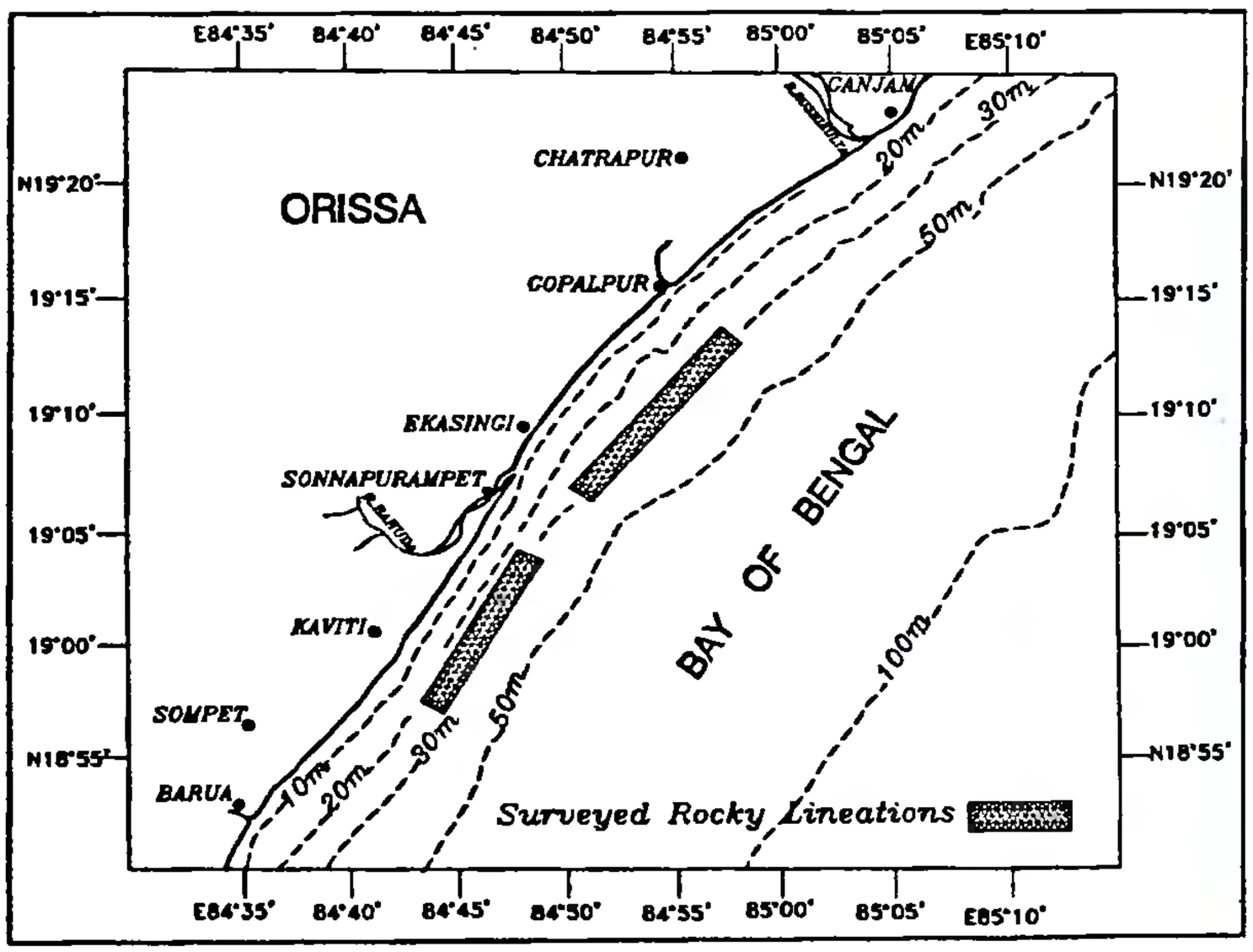

Figure 1. Location map of Reefal sedentary resources.

Figure 3. *Aurora globostellata* Carter, species no. 47.