(*Dinophysis* spp.), which form a component of harmful algal blooms.

1. Mohanty, A. K., Satpathy, K. K., Sahu, G., Sasmal, S. K., Sahu, B. K. and Panigrahy, R. C., *Curr. Sci.*, 2007, **93**(5), 616–618.
2. Sasamal, S. K., Panigrahy, R. C. and Misra, S., *Int. J. Remote Sensing*, 2005, **26**(17), 3853–3858.
3. Taylor, F. J. R., Blackbourn, D. J. and Blackbourn, J., *J. Fish. Res. Board Can.*, 1971, **28**, 391–407.
4. Montagnes, D. J. S. and Lynn, D. H., *J. Plankton. Res.*, 1989, **11**, 193–201.
5. Lindholm, T., *Adv. Aquat. Microbiol.*, 1985, **3**, 1–48.
6. Crawford, D. W., *Mar. Ecol. Prog. Ser.*, 1989, **58**, 161–174.
7. Crawford, D. W., Purdie, D. A., Lockwood, A. P. M. and Weissman, P., *Estuarine Coastal Shelf Sci.*, 1997, **45**, 799–812.
8. Hansen, P. J., Nielsen, L. T., Johnson, M., Berge, T. and Flynn, K. J., *Harmful Algae*, 2013, **28**, 126–139.
9. Jankowski, A. W., In *Materials II. All-Union Conference on Protozoology. Part 1, General Protozoology* (eds Markevich, A. P. and Yu, I.), Naukova Dumka, Kiev, 1976, pp. 167–168.
10. Garcia-Cuetos, L., Moestrup, O. and Hansen, P. J., *J. Eukaryot. Microbiol.*, 2012, **59**(4), 374–400.
11. Lindholm, T., Lindroos, P. and Mork, A. C., *Biosystems*, 1988, **21**, 141–149.
12. Peterson, T. D. *et al.*, *Aquat. Microb. Ecol.*, 2013, **68**, 117–130.
13. Park, M. G. *et al.*, *Aquat. Microb. Ecol.*, 2006, **45**, 101–106.

ACKNOWLEDGEMENTS. We thank the Head, Department of Marine Sciences, Berhampur University for providing the necessary facilities and INCOIS, Hyderabad for sponsoring the SATCORE project. B.K.S. thanks DST, New Delhi for providing INSPIRE fellowship.

Received 25 February 2015; revised accepted 20 October 2015

BIRAJA KUMAR SAHU[1,*]
R. C. PANIGRAHY[1]
S. K. BALIARSINGH[2]
C. PARIDA[1]
K. C. SAHU[1]
ANEESH A. LOTLIKER[2]

[1]*Department of Marine Sciences, Berhampur University, Berhampur 760 007, India*
[2]*Indian National Centre for Ocean Information Services, Hyderabad 500 090, India*
**For correspondence.*
e-mail: birajkumar@gmail.com

Karyotype of the Indian giant squirrel (*Ratufa indica*)

The Indian Giant squirrel syn. Malabar giant squirrel (*Ratufa indica*) is an upper-canopy dwelling mammal, endemic to southwestern, central and eastern peninsular India, found specifically in the Western Ghats, Satpuras and Eastern Ghats. It is seen at elevations of 180–2300 m and is widely distributed[1]. The species has been classified under the Class, Mammalia; Order, Rodentia; Suborder, Sciuromorpha; family, Sciuridae; subfamily, Ratufinae; Genus, Ratufa and species *Ratufa indica*[2]. According to the IUCN, ver 3.1, *R. indica* is classified under the 'Least Concern' category, but its population shows a decreasing trend due to habitat loss[3]. It is also listed under the Schedule II of the Indian Wildlife (Protection) act, 1972.

Karyotyping is an important source of genomic information from a species. Karyotype studies in wild species are confronted with difficulties. First, the habitats of wild animals are remote and away from lab facilities. Bone marrow cells and tissue samples for *in vitro* culture can be used only if available from fresh carcasses. Most often, the carcasses in the wild are noticed late after death and putrefied. Even if fresh, the cells extracted from bone marrow have to be processed immediately or transported within a very short duration. Other tissue samples have to be transported without exposure to extremes of temperature and under sterile conditions[4]. Whole blood in heparin is suitable in terms of handling, transport and maintenance of sterility, but collecting fresh blood from wild species is impractical. The procedures are

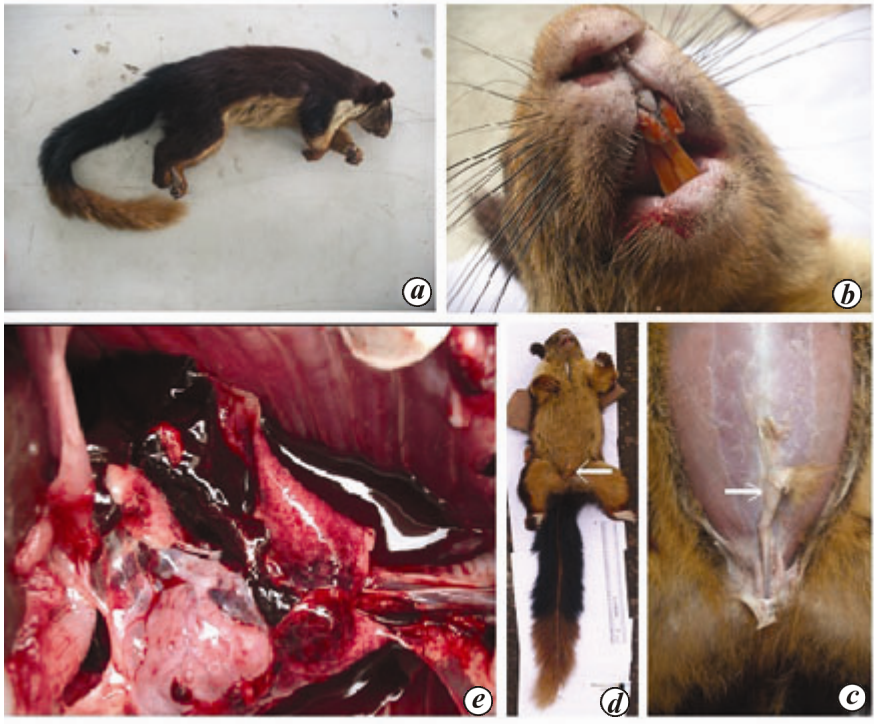

Figure 1. ***a***, Carcass of *Ratufa indica*; ***b***, Bleeding from mouth and nostrils; ***c***, ***d***, Arrows indicating male genitalia; ***e***, Unclotted blood in thoracic cavity.

not only difficult, but also bound by legal implications. A thorough search of the literature indicated absence of information on karyotype of *R. indica*. The karyotype of the Indian Giant squirrel (*R. indica*) was studied by chromosome preparation of leukocyte culture from unclotted blood collected during necropsy.

A male Indian Giant squirrel (Figure 1 *c*, *d*) was found dead under a cyprus tree in Cairn hill Reserve Forest, Udhagamandalam, in the Nilgiri Biosphere Reserve, and its necropsy was conducted. The carcass was fresh and death might have occurred a short while ago, as its body was warm, rigor mortis was absent and fresh bleeding noticed from the nostrils and mouth (Figure 1 *b*). A large blood clot was found adhering to the lung. About 20 ml of unclotted blood was found in the thoracic cavity (Figure 1 *e*). The animal had probably died of shock due to pulmonary rupture. Ten ml of unclotted blood from the thoracic cavity was collected in heparinized vacutainer and transported in ice to Veterinary College and Research Institute, Namakkal, the nearest facility available for culture and karyotyping.

The chromosome spreads were prepared from leucocyte cultures by standard procedure[5]. Cultures were set up in RPMI 1640 culture medium (8 ml) with Pokeweed (0.1 ml) as mitogen. Autologous plasma (1.5 ml) and buffy coat (0.2 ml) from the centrifuged sample were added to cultures. The cultures were incubated at 37°C for three cell cycles (72 h). Ninety minutes prior to harvest 0.01% colchicine was added to the cultures and the incubation was continued. The cultures were subjected to hypotonic treatment (0.075 M KCl) and fixed in Cornoy's fixative (methanol – 3 parts: acetic acid – 1 part). The slides were prepared and stained with 4% Giemsa. Non-overlapping metaphase spreads with full chromosome ($2n$) complement were chosen and examined under Leica model DM 2500 microscope at 1000× magnification.

Photomicrographs were obtained for preparation of karyotypes and assessing morphometric characteristics. Each chromosome was cut out separately and matching pairs were arranged according to decreasing size with small arm upwards. The sex chromosomes were placed in the end. The length of each chromosome was measured from tip to tip using a vernier caliper with an accuracy of 0.05 mm.

Relative length was calculated as the ratio of individual chromosome length to that of total haploid genome length (length of autosomes + X + Y chromosome). The centromeric index was calculated as the ratio of short arm (p) length to total length ($p + q$) of the chromosome.

The carcass of the squirrel was fresh with unclotted blood available in the thoracic cavity, which was successfully used for chromosome preparation. Figure 2 shows the karyotype of the male Indian giant squirrel obtained in the present study. From the spreads analysed, it was found that the diploid number of chromosomes in *R. indica* was 42 ($2n$), comprising 20 pairs of autosomes and a pair of sex chromosomes. As there was no reference karyotype available for the species, chromosomes were arranged following standard procedure based on morphology and decreasing size. Relative length of autosomes varied from 2.09% to 6.96% (Figure 3). All the 20 pairs of autosomes were biarmed with metacentric and submetacentric positions of centromere. The Centromeric Index (CI) varied from 0.53 to 0.75. The sex chromosomes showed marked difference in size and the relative length of X and Y chromosomes was 5.04 and 2.42 respectively. The sex chromosomes were metacentric with CI values of 0.56 and 0.62 respectively.

Literature survey indicated absence of information on the karyotype of *R. indica*. Family Sciuridae includes 273 species[6], in which 10 species studied cytogenetically were characterized by a stable diploid number of $2n = 40$, and little variation in fundamental number (FN) = 72 or 76. The X chromosome was always metacentric or submetacentric. Among other arboreal squirrels in the family Sciuridae reviewed for chromosome studies, chromosome number ranged from 38 to 62 with majority of species having 40 chromosomes[7]. The

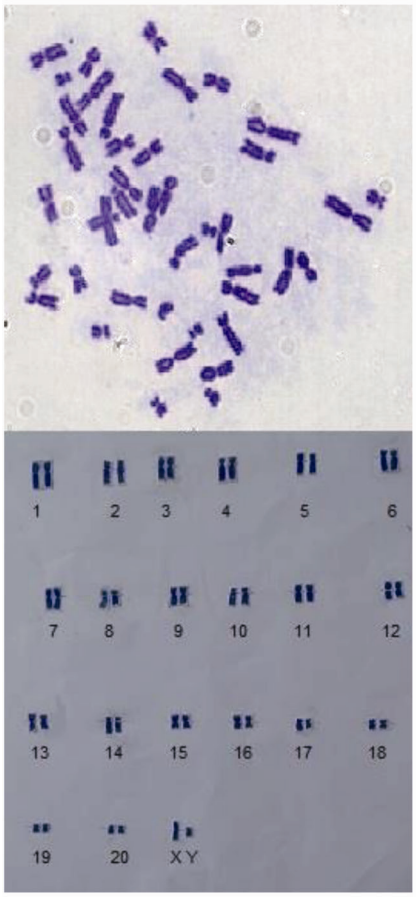

Figure 2. Karyotype of *Ratufa indica*.

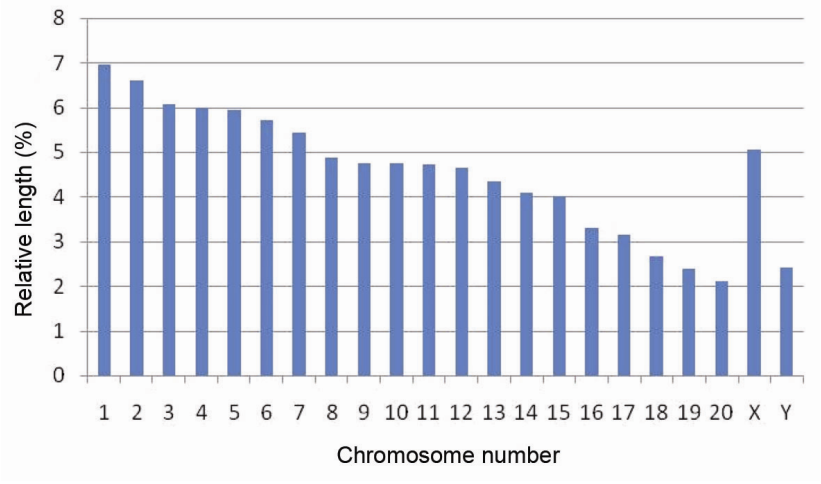

Figure 3. Relative length of 20 autosomes, X and Y chromosome in *R. indica*.

Venezuelan species *Sciurus granetensis* had a similar number to *R. indica* with 42 chromosomes. However, the FN in *S. granetensis* was 78 with 19 pairs of biarmed and one pair of acrocentric autosome. Karyotype obtained for *R. indica* in the present study indicates FN of 80 as all autosomes were biarmed. The Iranian arboreal species *S. anomalus* had 40 chromosomes with FN of 76, as the 19th acrocentric autosome was absent[8]. Cross-species studies on squirrels, not inclusive of *R. indica* show that karyotypes of squirrels are highly conserved[9,10].

The diploid number of wild arboreal squirrel, *R. indica* endemic to South, Central and Eastern Peninsular India is confirmed through karyotype obtained from lymphocyte culture using unclotted blood available from fresh carcass.

1. Molur, S., Srinivasulu, C., Srinivasulu, B., Walker, S., Nameer, P. O. and Ravikumar, L., Status of South Asian non-volant small mammals: Conservation assessment and management plan, Workshop report, Zoo Outreach Organisation/CBSG-South Asia, Coimbatore, India, 2005, p. 618.
2. Erxleben, J. C. P. (in Latin), *Systema regni animalis per classes, ordines, genera, species, varietates cum synonymia et historia animalium. Classis I. Mammalia*, Leipzig, Germany: Impensis Weygandianis, 1777, 42; http://www.worldcat.org/oclc/14843832
3. Rajamani, N., Molur, S. and Nameer, P. O., *IUCN Red List of Threatened Species*, Version 2011.2, 2010; www.iucnredlist.org
4. Bulatova, N. S., Searle, J. B., Nadjafova, R. S., Pavlova, S. V. and Bystrakova, N. V., *Comp. Cytogenet.*, 2009, **3**(1), 57–62.
5. Halnan, C. R. E., *Res. Vet. Sci.*, 1977, **22**, 40–43.
6. Fernando de, J., Lima, S. and Langguth, A., *Folia Zool.*, 2002, **51**, 201–204.
7. Fagundes, V., Christoff, A. U., Amaro-Ghilard, R. C., Scheibler, D. R. and Yonenaga-Yassuda, Y., *Gen. Mol. Biol.*, 2003, **26**(3), 253–257.
8. Nadler, C. F. and Hoffmann, R. S., *Experientia*, 1970, **26**, 1383–1386.
9. Li, T. *et al.*, *Chromosome Res.*, 2004, **12**, 317–335.
10. Li, T., Wang, J., Su, W., Nie, W. and Yang, F., *Cytogenet. Genome Res.*, 2006, **112**, 270–276.

ACKNOWLEDGEMENTS. We acknowledge the assistance rendered by staff of office of the Ranger, Nilgiris South Division. We also thank the Dean, Veterinary College and Research Institute, Namakkal, for laboratory facilities.

Received 11 November 2015; revised accepted 26 December 2015

R. VENKATARAMANAN[1,*]
N. MURALI[2]
C. SREEKUMAR[1]
K. V. GOWRIMANOKARI[1]

[1]*Postgraduate Research Institute in Animal Sciences, Tanuvas, Kancheepuram 603 203, India*
[2]*Veterinary College and Research Institute, Namakkal 637 001, India*
**For correspondence.*
e-mail: venkyvet@gmail.com

Breeding tubercles in scales of male *Barilius bendelisis* (Hamilton, 1807) identified as sexual dimorphic character

Sexual dimorphism in scales has been reported in some cyprinids like *Chondrostoma nasus*[1], where breeding tubercles are present on the scales of both males and females, but are smaller and inconspicuous in females. The presence of breeding tubercles in fishes was first reported by Vladykov *et al.*[2] in species of genus *Gadus*[3] which were used in taxonomic identification, since both male and female fish possess this character. There have been studies on breeding tubercles in scales suggesting a correlation to the pre-spawning behaviour in males[1]. The role and evolution of such tubercles are however not known, they are believed to be used for conspecific recognition[2,4]. It was also suggested that the morphology of such tubercles may vary among different species. Although there are reports on the use of scales as a key character for analysing age and growth in *Barilius bendelisis*[5,6] there is no report on sexual dimorphism in this fish using scales as key structures. Thus in the present study, the surface topography of scales from adult male and female fishes was analysed by both light microscopy and scanning electron microscopy (SEM) to confirm the presence of breeding tubercles and study the structures in detail.

Hill stream fish *B. bendelisis* were captured from Basistha (26°05′38.54″N, 91°46′57.27″E), the only hill stream in the heart of Guwahati city, Assam, India during February 2014 to January 2015. Adult fishes were in the range 107–124 mm total length and 13–26 g body weight. Juvenile as well as adult fishes were studied throughout the seasons. Sexes of the fishes were confirmed through dissection and scales were carefully taken out from near the head region above the lateral line and also from other areas for microscopic observations. Scales were superficially cleaned with 70% alcohol using a fine brush to remove any attached debris and immersed in 3% glutaraldehyde solution for fixation. Buffer treatment consisted of washing the scales three times in 0.1 M solution of sodium cacodylate buffer at an interval of 15 min and finally storing in the same buffer until processed further. Scales were gradually dehydrated in acetone grades (30–100%) and then treated with tetramethylsilane (TMS)[7]. Clean and dried samples were gold-coated for 10 min before viewing in SEM (model JSM-6360 JEOL) at 20 kV.

The study revealed that surface topography of scales in both sexes differed in *B. bendelisis*. Light microscopic studies revealed that in the male fish the exposed area (ExA) of scales is studded with tubercles (Figure 1 *a* and *b*). SEM images revealed that tubercles contained numerous elongated spine-like structures called unculi (Figure 2 *b* and *c*). These were observed to be smooth surfaces with pointed distal ends and broad proximal edges. When observed under the light microscope (Figure 1 *c* and *d*) as well as in SEM (Figure 2 *d*), scales of adult female fish were devoid of tubercles. However, the exposed area of scales revealed numerous mucous pores with depressions (Figure 2 *e*).