

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<sup>8</sup>. Cross-species studies on squirrels, not inclusive of *R. indica* show that karyotypes of squirrels are highly conserved<sup>9,10</sup>.

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.

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

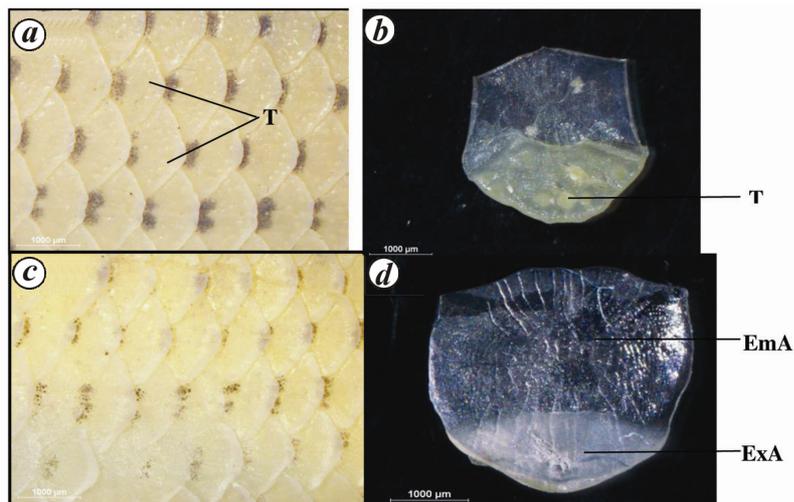
Sexual dimorphism in scales has been reported in some cyprinids like *Chondrostoma nasus*<sup>1</sup>, where breeding tubercles are present on the scales of both males and females, but are smaller and inconspicuous in females. The presence of breeding tubercles in fishes was first reported by Vladykov et al.<sup>2</sup> in species of genus *Gadus*<sup>3</sup> 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<sup>1</sup>. The role and evolution of such tubercles are however not known, they are believed to be used for conspecific recognition<sup>2,4</sup>. 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*<sup>5,6</sup> 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.

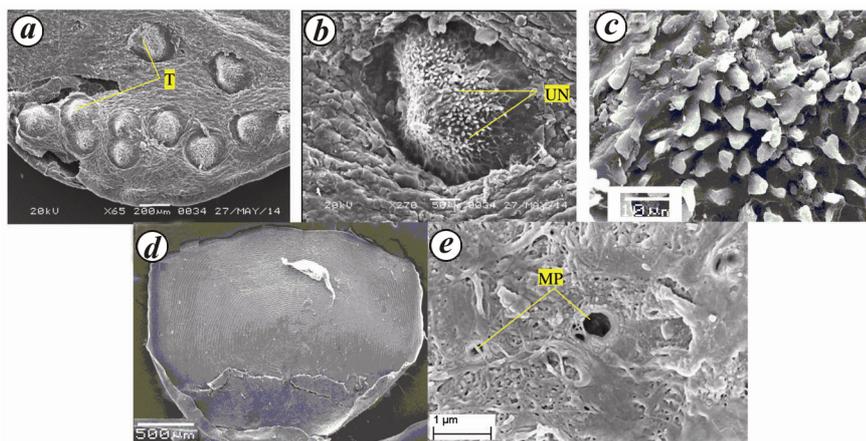
Hill stream fish *B. bendelisis* were captured from Basistha (26°05'38.54"N, 91°46'57.27"E), the only hill stream in the heart of Guwahati city, Assam, India during February 2014 to January 2015. Adult fishes were in the range 107–124 mm total length and 13–26 g body weight. Juvenile as well as adult 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)<sup>7</sup>. 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).



**Figure 1.** Light microscopic images of scales of *Barilius bendelisis*. *a*, Scales in male (M) with distinct tubercles at the ExA. *b*, Dorsal view of a single scale (M) showing tubercles. *c*, Scales in female (F) with no appearance of tubercular outgrowths at the ExA. *d*, Dorsal view of a single scale (F). (Scale bar used: 1000 µm.) T, Tubercles; EmA, Embedded area; ExA, Exposed area.



**Figure 2.** Scanning electron photomicrographs of scales of *B. bendelisis*. *a*, ExA of scale in M displaying T (magnification 65×, scale 200 µm). *b*, Magnified view of a single tubercle (M) (magnification 270×, scale 50 µm). *c*, Clusters of UN from a single tubercle (M) (magnification 1100×, scale 10 µm). *d*, Whole structure of scale of F (magnification 30×, scale bar 500 µm). *e*, MP observed in the ExA of F (magnification 30×, scale bar 1 µm). UN, Unculi; MP, Mucous pores.

There have been several studies on scales of fishes regarding their structure, composition<sup>8,9</sup> and comparative morphology<sup>10</sup>. A detailed study on fish scales is useful in analysing sexual dimorphism, identification of major groups, age and growth determination and pathology of fish scale due to water pollution<sup>8</sup>.

Breeding tubercles in some cyprinids have been reported from different countries<sup>1</sup>. Although the functional significance of breeding tubercles is not clear in most species, the occurrence of different types of breeding tubercles in scales of fishes was observed by many workers who suggested conspecific recognition<sup>2</sup>, protection against mechanical injuries

and maintaining close contact during the breeding period<sup>1,2</sup>.

In the present study, examination of scales in juvenile fish did not reveal the presence of tubercles. Tubercles-bearing uncili were observed in adult fishes only; therefore they are considered as breeding tubercles<sup>1,2</sup>. Since this character was observed only in the adults of males, hence the presence of tubercles is considered to be specific to the males of this species. Uncili are related morphologically to the relatively well-known multicellular horny tubercles, including breeding tubercles of fishes<sup>11</sup>. These structures observed in males are believed to be involved in mechanical protection,

rasping and adhesion<sup>11,12</sup>, while presence of mucous pores in females might aid the adhesion process and help prevent drying and desiccation in fishes.

In conclusion, it is interesting to note the presence of breeding tubercles in scales of male *B. bendelisis* as a sexual dimorphic character present among the Indian cyprinids.

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