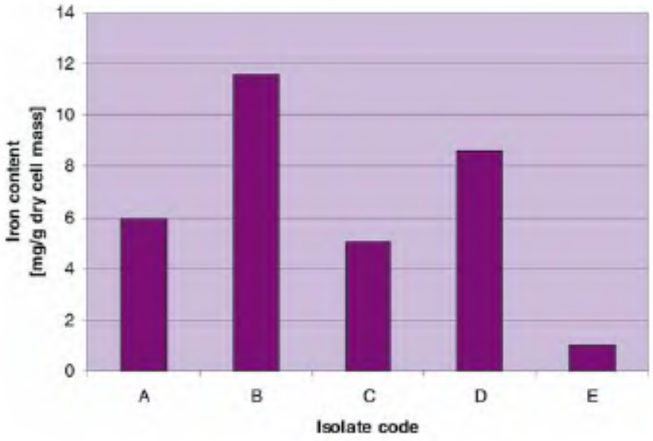

Figure 3. Intracellular iron content of the isolates.

In light of the ecological importance of MTB in biogeochemical cycles, the study of such bacteria in hitherto unexplored environments can be significant. Fossil magnetosomes are considered to contribute significantly to magnetic properties of sediments and soils[7,11]. The magnetic nano-crystals in the 4.5-Ga-old Martian meteorite ALH84001 are found to be similar to the bacterial magnetosomes[12]. This seems to hint at a certain correlation between the MTB and the meteorites that could have tremendous implications on the search for extraterrestrial life.

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Karyotype analysis of Persian stone lapper, *Garra persica* Berg, 1913 (Actinopterygii: Cyprinidae) from Iran

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The karyotypic and cytological characteristics of an endemic cyprinid fish *Garra persica* Berg, 1913 have been investigated by examining metaphase chromosome spreads obtained from gill epithelial and kidney. The diploid chromosome number of this species was $2n = 48$. The karyotype consisted of 15 pairs of metacentric, eight pairs of submetacentric and one pair of subtelocentric chromosomes (15m, 8Sm, 1St). The arm number was 94. No heteromorphic sex chromosomes were cytologically detected. Chromosome number, karyotype formula and arm number of *G. persica* differentiate it from *G. rufa*, a closely related species.

Keywords: Chromosome, *Garra persica*, idiogram, karyotype analysis.

CYPRINIFORMES or carps are a group of freshwater fishes with six families, 321 genera and about 3268 species found throughout the world, except Australia and South America[1]. The Cyprinidae or minnows are found in North America, Africa and Eurasia. The Cyprinidae with 220 genera and about 2420 species is the largest family of freshwater fishes and with possible exception of Gobiidae, the largest family of vertebrates[1]. They are mostly small (<5 cm) although some are very large (3 m). The genus *Garra* is one among this diverse group found throughout Southwest Asia and from Africa to Southeast Asia. There are about 73 species and four are recognized from` Iran, including *Garra rufa* (Heckel, 1843), *G. rossica* (Nikol'skii, 1900), *G. variabilis* and *G. persica* (Heckel, 1843)[2]. *G. persica* (Figure 1) is recognized only as a sub-

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species of *G. rufa* by Bianco and Banarescu[3], while some researchers[4,5] synonymize it with *G. rufa*. This species is found in the Hormuzgan, Makran and Jaz Murian basins and possibly the Sistan and Yazd basins of Iran[3,6].

Although *G. persica* has been described and compared morphologically, its karyotype has not yet been investigated. As study of fish chromosomes has received considerable attention in recent years because of its usefulness in addressing problems of classification, evolution and heredity, in this study, karyological features of this endemic carp from Iran have been reported.

G. persica specimens were collected from Rudan River at Rudan (Hormuzgan Province, southeast Iran; 27°28′24.1″N, 57°15′18.8″E, altitude 211 m) using electrofishing device and dip net. Other fish species, including *Aphanius dispar* (Cyprinodontidae), *Cyprinion watsoni* (Cyprinidae) and *Schistura* cf. *sargadensis* (Balitoridae) were also collected from this river. The fishes were transported live to the laboratory and kept in a well-aerated aquarium at 20–25°C before analysis. For karyological studies the modified method of Uwa[7] was used. Colchicine solution was prepared with 0.005 g in 20 ml physiological serum. The fishes were injected intraperitoneally with 0.02 ml of colchicine per gram of body weight using an insulin syringe, and then were replaced in the aquarium for 4–5 h. The gill filaments and kidneys of these specimens were then removed and placed in hypotonic 0.36% KCl solution for 45 min at room temperature (25°C). Thereafter, the solutions were centrifuged for 10 min at 1000 rpm, adding 2–3 drops of fresh and cold Carnoy fixative (1 : 3 acetic acid : methanol) before centrifugation. The supernatants were then discarded and 5 ml fresh and cold fixative was added to sediments, mixed thoroughly and then left for 1 h. The fixation and centrifugation stages were repeated two times. The suspensions were then trickled to cold slides. These slides were stained with 10% Giemsa for 20 min. Chromosomes were observed, selected and photographed using Olympus light microscope mounted by a camera. Karyotypes were prepared by arranging chromosomes in pairs by size. For each chromosome, the average length of the short and long arms and arm ratio (the ratio of the long arm length to the short arm length of the chromosomes) were calculated. The chromosomes were then classified according to the criteria of Levan *et al.*[8]. Fundamental number (NF) expressed as twice the number of atelocentric plus the number of telocentric chromosomes, was found. The idiogram was prepared in Harvard Graphics 2.0 software.

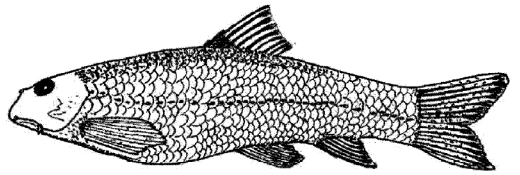

Figure 1. *Garra persica* from Iran.

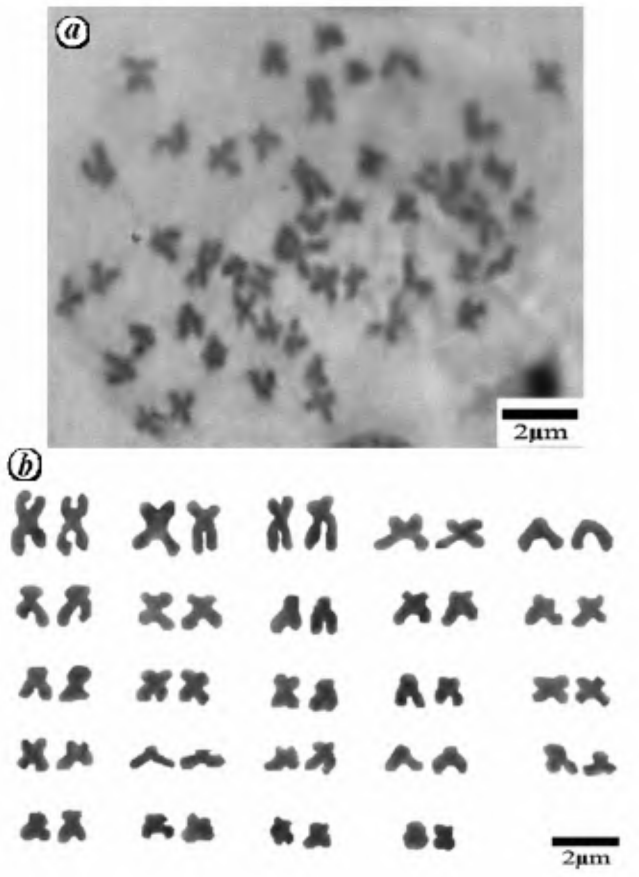

Figure 2. Giemsa-stained chromosome spread (***a***) and karyotype (***b***) of *G. persica* from Iran.

Metaphase spread of this species is given in Figure 2 *a*. The diploid chromosome number was $2n = 48$ (Figure 2 *b*). Quantitative data of the different measurements used to classify chromosomes and idiogram are given in Table 1 and Figure 3. The karyotype consisted of 15 metacentric, eight submetacentric and one subtelocentric chromosomes (15m, 8Sm, 1St), and the arm number was 94. No heteromorphic elements indicating sex chromosomes were detected in this endemic carp.

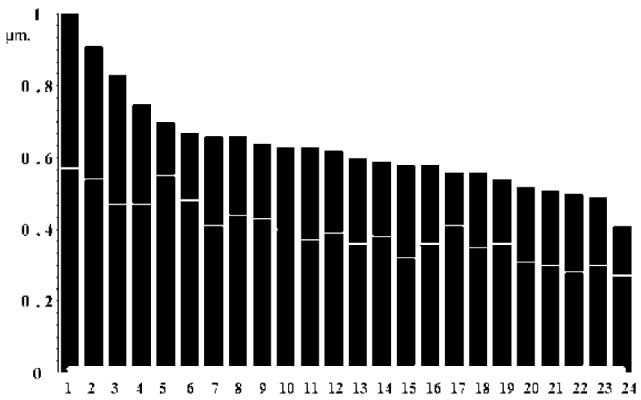

Figure 3. Haploid idiogram of *G. persica* from Iran.

Table 1. Chromosome measurements (in μm) and classification of *Garra persica* chromosomes

Chromosome number	Long arm length (μm)	Short arm length (μm)	Arm value	Chromosome type
1	0.57 ± 0.13	0.43 ± 0.04	1.32	m
2	0.54 ± 0.02	0.37 ± 0.12	1.47	m
3	0.47 ± 0.04	0.36 ± 0.09	1.31	m
4	0.47 ± 0.17	0.28 ± 0.03	1.68	m
5	0.55 ± 0.15	0.15 ± 0.07	3.69	St
6	0.48 ± 0.14	0.19 ± 0.10	2.58	Sm
7	0.41 ± 0.08	0.25 ± 0.04	1.64	m
8	0.44 ± 0.11	0.22 ± 0.05	2.06	Sm
9	0.43 ± 0.08	0.21 ± 0.07	2.10	Sm
10	0.40 ± 0.07	0.23 ± 0.11	1.70	Sm
11	0.37 ± 0.05	0.26 ± 0.06	1.43	m
12	0.39 ± 0.05	0.23 ± 0.03	1.69	m
13	0.36 ± 0.05	0.24 ± 0.04	1.48	m
14	0.38 ± 0.03	0.21 ± 0.06	1.84	Sm
15	0.32 ± 0.02	0.26 ± 0.05	1.21	m
16	0.36 ± 0.09	0.22 ± 0.04	1.61	m
17	0.41 ± 0.05	0.15 ± 0.05	2.72	Sm
18	0.35 ± 0.01	0.21 ± 0.06	1.65	m
19	0.36 ± 0.04	0.18 ± 0.06	1.98	Sm
20	0.31 ± 0.03	0.21 ± 0.04	1.43	m
21	0.30 ± 0.04	0.21 ± 0.05	1.41	m
22	0.28 ± 0.03	0.22 ± 0.02	1.30	m
23	0.30 ± 0.03	0.19 ± 0.02	1.59	m
24	0.27 ± 0.12	0.14 ± 0.03	1.95	Sm

M, Metacentric; Sm, Submetacentric; St, Subtelocentric.

The diploid chromosome number of fishes varies from $2n = 22–26$ in some species of Nototheniidae (an Antarctic fish group) to $2n = 240–260$ in some anadromous Acipenseridae which show several microchromosomes[9].

The diploid chromosome number of *G. persica* was $2n = 48$, and it is in conformation with the chromosome number of its closely related species (*G. rufa*)[10]. However, the diploid chromosome number of *G. rufa* was reported[11–13] to vary from $2n = 44$ to 52. There are also reports on the karyotype of other species of this genus. Krysanov and Golubtsov[14] reported the chromosome number of *G. demebeensis* (Ruppell, 1837), *G. makiensis* (Boulenger, 1903) and *G. quadrimaculata* (Ruppell, 1837) to be $2n = 52$. It was also $2n = 52$ in *G. cambodgiensis* (Tirant, 1883). According to Kilinkhadrt[15] and Khuda-Bukhsh *et al.*[16], the diploid chromosome number of *G. lampta*, *G. pingi pingi* and *G. gotyla gotyla* is $2n = 50$.

Although the diploid number variation was found in the genus *Garra*, the majority of *Garra* species have $2n = 50$; thus $2n = 50$ may be regarded as characteristic of the primitive karyotype in this genus. However, Robertsonian translocation may have played an important role in karyotype evolution of this genus. The majority of the cyprinid species have $2n = 50$ chromosomes[17,18], while *Cyprinus carpio* has $2n = 98–100$ and the polyploid *Barbus* species from southern Africa has $2n = 148$ or 150 chromosomes[17–19].

In the present study, no cytological evidence was found for sex chromosome dimorphism in *G. persica*, which is in agreement with reports on many fish species[20–23]. In marine fishes also, despite the large number of living species, the occurrence of cytologically differentiated sex chromosomes appears to be rare, although it has been described in platyfishes and in some catfishes[24–26].

The arm number of *G. persica* was 94. This is near the limits (78–90) reported for the Asian and African *Garra* species[13,14,16,20]. Chromosome number, karyotype formula and arm number of *G. persica* may differentiate this species from *G. rufa*, another native fish of Iran. This is in agreement with Coad[2], who considers the *Garra* populations of the Hormuzgan Province as distinct species (*G. persica*). As pointed out earlier, this species was recognized only as a subspecies of *G. rufa* by Bianco and Banarescu[3], while others[4,5] synonymize it with *G. rufa*. Further molecular, cytological, anatomical, morphological and biological investigations towards better recognition and understanding of the cyprinid fish are suggested.

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In vitro micropropagation of *Bambusa balcooa* Roxb. through nodal explants from field-grown culms and scope for upscaling

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An efficient and reproducible *in vitro* procedure for large-scale multiplication of *Bambusa balcooa* Roxb. has been described. Multiple shoot formation (8–10) was observed from excised tender node (12–18 mm in length) containing axillary bud isolated from secondary branches of 1½-yr-old culms, when implanted on Murashige and Skoog (MS) medium containing 6-benzylaminopurine (BAP, 1.0 mg l^{-1}). Continuous shoot proliferation, tenfold, every 4 weeks was achieved by sub-culturing shoot clumps (2–3 shoots/cluster) in BAP (1.0 mg l^{-1}) fortified medium. Seventy-five per cent of shoots could be rooted efficiently on excised propagules when transferred to MS medium supplemented with BAP (1.0 mg l^{-1}) and naphthaleneacetic acid (3.0 mg/l). Using this protocol, the first batch of plantlets can be obtained after six months from the initial establishment and then every month new batches of plantlets can be regenerated, depending upon the availability of efficient shoots. After hardening, rooted plantlets were successfully transferred to the soil in polythene sleeves with over 90% survivability and recorded normal growth. Macroproliferation can be done after a period of 5 months from the soil transfer, by which propagules can be increased by more than three times. The tech-economic viability was studied with consideration that the production will be executed in a well-established plant tissue culture laboratory. Factors for characterization at various stages for tissue culture work of *B. balcooa* have also been studied and evaluated.

Keywords: *Bambusa balcooa*, culms, micropropagation, nodal explants.

BAMBUSA BALCOOA Roxb., an indigenous widespread bamboo of North East India, is considered as the best, tallest, strongest, and highly durable, and is utilized mostly for structural use and pulping. It has maximum girth of culms and rind thickness among all species of the genus *Bambusa.* The species is also valued for its edible tender shoots, mainly for food and pickle industry. But, the rapidly increasing bamboo-based industries have resulted in severe loss of forest stocks, which demands adequate re-plantation of elite bamboo species to strengthen the raw material stock.

Seed production in *B. balcooa* is generally not recorded after gregarious flowering[1]. Moreover, its flowering cycle is reported as 40–100 years and only once during its life time[2]. In this regard, large-scale elite bamboo replantation necessitates sufficient ready stock of planting material, for which application of plant tissue culture technique is the only justified methodology. Alternative methods of vegetative propagation using offsets or culms are slow, expensive and also cumbersome to handle and liable to desiccation.

Research on tissue culture of bamboo was reported by Alexander and Rao[3] on embryo culture of *Dendrocalamus strictus*. Since then, starting from Huang and Murashige[4], a good start has been made on bamboo tissue culture and a number of laboratories have begun to make progress. But a majority of the successful achievements are made through somatic embryogenesis and/or regeneration from juvenile seedlings. Consequently, reports on regeneration from mature/field source materials followed by upscaling work for commercial use are limited. To the best of our knowledge, reports on clonal multiplication from six species of *Dendrocalamus* and three species of *Bambusa* are available, among which large-scale field transfer (quantity-wise, total 30,000 nos) was reported for *Dendrocalamus asper*, *D. membranaceus*, *D. strictus*, *D. giganteus*, *Bambusa arundinacea* and *B. vulgaris*[5].

However, reports on *in vitro* regeneration of *B. balcooa* are limited, except those of Das and Pal[6], and Gillis *et al.*[7], where protocols about field transfer and survivability are not available. The present study reports an upscaling procedure for mass multiplication of this commercially important bamboo species.

Tender nodes (12–18 mm in length) obtained from minor branches of 1½-year-old culms of *B. balcooa* regenerated from approximately 80-year-old elite plants were used as explants.

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