



Karyological analysis for endangered fish, *Hucho taimen* (Pallas)

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Hucho taimen is also named taimen, belonging to Salmoniformes, Salmonidae, *Hucho* spp. With their ecological environment being damaged and resources being declined, resulting in the number of stocks decreased, their state was endangered now (Dong 1998). *Hucho taimen* is a kind of indigenous cold-water fish with large body size and restrictedly distributed in Heilongjiang River and Eerqisi River of China (Holcik *et al.* 1988). The endangered status of taimen was re-evaluated in 2006. Based on the continue declines the taimen in Heilongjiang River Basin were moved from category III in 1998 to V in 2006 (Pronin 2006). Of the 20000 or so living fish species, the karyotypes of only 10% have been described (Gold 1979). In this paper, we present data on the metaphase chromosomes of *Hucho taimen*, a species in which the karyotype has not previously been published.

Specimens (20) of wild *Hucho taimen* with body weight from 0.5 to 2 kg were collected and reared in Heilongjiang Bohai Cold-Water Fish Station, China. During the

experiment, animals were kept in 200 litre aquaria without feeding. The average water temperature during the period was adjusted to 10°–14 °C.

To stimulate and increase cell mitotic divisions, the fish received 2 identical i/p injections of phytohaemagglutinin, as a T-cell mitogen with a 24 h interval, at a final dose of 20 µg/g body weight in 0.2 mL distilled water solution; 12 h after the second injection, the fish were injected with colchicine, as a mitotic inhibitor, at a final dose of 10 µg/g body weight. The single injection was administered i/p, and the total injected volume was 0.1–0.2 mL/fish. The fish samples were kept in 200 L aquaria at 14°. The head kidney tissue was extracted 4–5 h after colchicine injection and sliced completely for hypotonic treatment. The tissues were immersed in a cold 0.1 M KCl hypotonic solution for 4–5 min.

The hypotonized tissues were centrifuged at 1500 g for 5 min to extract the supernatant and then cold fresh Carnoy's fluid (3: 1v/v methanol and glacial acetic acid) was added.

Table 1. Karyotype indices of *Hucho taimen* (x±s)

No.	Relative length (%)	Arm ratio	No.	Relative length (%)	Arm ratio	No.	Relative length (%)	Arm ratio
m1	6.03±0.32	1.33±0.01	Sm6	4.94±0.11	1.85±0.06	St12	2.83±0.14	3.74±0.15
m2	5.45±0.28	1.26±0.24	Sm7	4.94±0.11	1.81±0.15	St13	2.69±0.01	3.73±0.13
m3	4.56±0.23	1.25±0.21	Sm8	4.94±0.11	1.75±0.21	St14	2.55±0.19	3.67±0.11
m4	4.45±0.12	1.25±0.05	St1	4.67±0.08	6.45±0.11	St15	2.43±0.04	3.61±0.12
m5	4.45±0.18	1.42±0.14	St2	4.49±0.26	6.25±0.12	St16	2.42±0.13	3.53±0.11
m6	4.45±0.09	1.24±0.23	St3	4.24±0.12	5.45±0.29	St17	2.32±0.12	3.21±0.32
m7	4.40±0.38	1.41±0.05	St4	4.18±0.17	5.31±0.11	t1	2.25±0.21	∞
m8	4.04±0.13	1.19±0.15	St5	3.92±0.29	5.04±0.11	t2	4.61±0.31	∞
m9	3.41±0.21	1.03±0.17	St6	3.85±0.11	4.41±0.17	t3	4.19±0.11	∞
Sm1	5.14±0.42	2.51±0.11	St7	3.69±0.16	4.24±0.21	t4	4.07±0.34	∞
Sm2	4.94±0.11	2.18±0.16	St8	3.54±0.16	4.04±0.21	t5	3.97±0.12	∞
Sm3	4.94±0.11	2.11±0.22	St9	3.38±0.12	3.94±0.11	t6	3.81±0.03	∞
Sm4	4.94±0.11	2.05±0.13	St10	3.12±0.21	3.83±0.12	t7	3.81±0.15	∞
Sm5	4.94±0.11	1.96±0.12	St11	3.05±0.11	3.77±0.11	t8	3.75±0.40	∞

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After 45–60 min, the old fixative was replaced with a fresh one following 5 min of centrifugation as above, and the samples were placed in a refrigerator (4°C) for 10 min. Ten

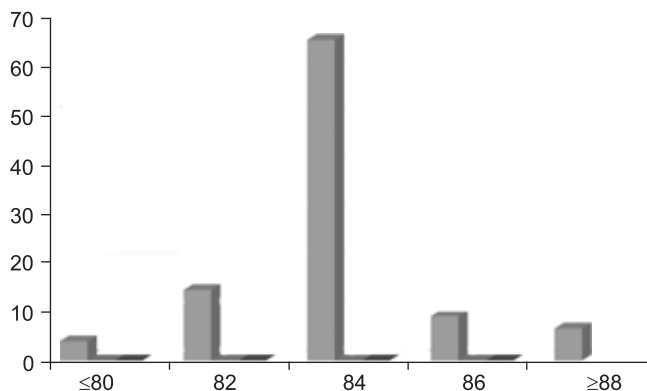


Fig. 1. Statistics of the diploid chromosome number of *Hucho taimen*.

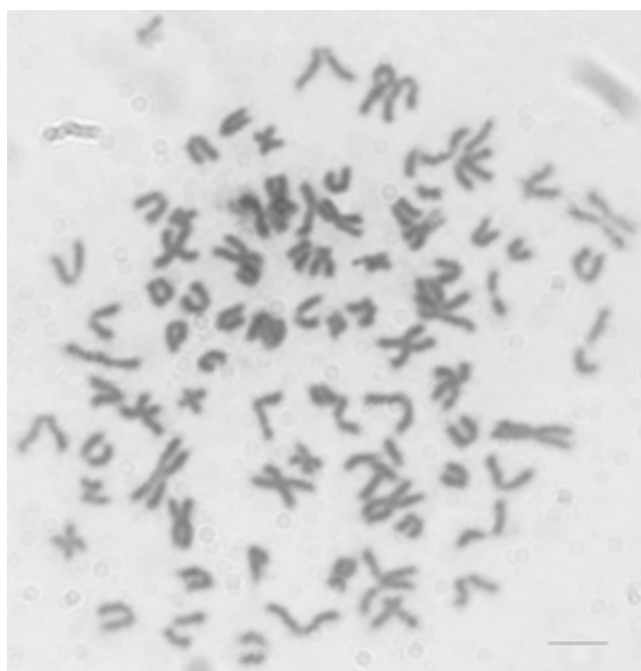


Fig. 2. Metaphase spreads of *Hucho taimen* (5 μ m).

minutes immersion in a fresh fixative was repeated 3 times and after each time, the old fixative was replaced by a fresh one after 5 min of centrifugation as described previously. Finally, the tissues were fixed completely by adding 3–4 mL new fresh fixative and kept at 4°C for 24 h.

The fixed tissues were transferred from Carnoy's fluid to a cold glass slide by dropping 2 to 3 drops of solution at a distance of 60–100 cm. The glass slides were air-dried at room temperature for 24 h, and stained for 10 min by 10% Giemsa solution in Sorenson buffer (40 mol Na_2HPO_4 and 26.6mol KH_2PO_4) at pH 6.8. Slides were dipped into distilled water to wash out extra Giemsa solution and then allowed to air dry at room temperature for 2–3 h (Harvey *et al.* 2002). Chromosomes were designated as having one or two arms following the recommendations of Levan *et al.* (1964).

Thus those chromosomes called metacentric and



Fig. 3. Karyotype of *Hucho taimen*.

submetacentric in this paper have 2 arms and those called subtelocentric and acrocentric have one arm. In some instances metacentric is used to designate all 2-armed and acrocentric to designate all one-armed chromosomes (Harvey 2002).

A total of 76 counts were made of chromosome spreads at metaphase from 11 females and 9 males. Comparison of the karyotypes from males and females did not reveal the presence of any chromosomal differences. The data were therefore combined and the frequency of these counts in relation to the observed chromosome number is shown in Fig. 1. The results indicated clearly that the modal count was 84 ($2n$) with 65.79% of the counts having this number.

An example of one of the metaphase spreads is shown in Fig. 2 and the karyogram produced from this metaphase is shown in Fig. 3. For each chromosome, the arm ratio (r =long arm/short arm) and the relative length were calculated, the karyotype formula proposed for *Hucho taimen* was $2n=84=18m+16sm+34st+16t$, and the arm number was $NF=118$, which was calculated by assigning a value of 2 arm for M/SM chromosomes and 1 arm for the ST/T chromosomes. The karyotype indices of *Hucho taimen* is shown in Table 1.

The study of fish and other aquatic animal chromosomes has become an active area of research in recent decades (Thorgaard and Disney 1990). Karyological studies have provided basic information on the number, size and morphology of chromosomes (Tan *et al.* 2004). It may be useful for addressing a variety of evolutionary and genetic questions about animals (Macgregor 1993) and may permit detection of changes that modified an ancestral karyotype

as it evolved into new lines (Winkler *et al.* 2004). The study of karyotype is also important in aquaculture in connection with the use of chromosome manipulation techniques, including induction of polyploidy, gynogenesis, androgenesis and inter- or intra-species hybridization (Wu and Chen 1986, Diter *et al.* 1993).

This study may provide the first knowledge on chromosome number in *Hucho taimen* and may add basic information useful for chromosomal manipulations to facilitate the culture of this valuable subspecies. The karyotype of *Hucho taimen* is shown to be $2n=84$, like the majority of other Salmonidae, have similar diploid chromosome number, no satellites and sexual dimorphism of the chromosomes were detected in metaphase spreads either in the male or the female karyotypes of these species.

SUMMARY

Metaphase chromosome specimens of *Hucho taimen* injected with PHA and colchicin, were prepared from short-term culture of kidney cells with air-drying technique. Its diploid chromosome number is $2n = 84$. The karyotype formula proposed for *Hucho taimen* was $2n = 18m + 16sm + 34st + 16t$, and the arm number was $NF=118$.

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REFERENCES

- Bethesda M D, Winkler F M, Garcia-Melys D and Palma-Rojas C. 2004. Karyotype of three South East Pacific flounder species of the family Paralichthyidae. *Aquaculture Research* **35**: 1295–98.
- Diter A, Quillet E and Chourrout D. 1993. Suppression of first egg mitosis induced by heat shocks in the rainbow trout. *Journal of Fish Biology* **42**: 777–86.
- Dong C Z, Li H M and Zhao C G. 1998. Properties of *Hucho taimen* and its ecology data. *Chinese Journal of Fisheries* **11** (2): 34–39.
- Gold J R. 1979. Cytogenetics. *Fish Physiology*. Vol. VIII, pp.353–405. (Eds) Hoar W S, Randall D J and Brett J R. Academic Press, London.
- Harvey S C, Powell S F, Kennedy D D, McAndrew B J and Penman D J. 2002. Karyotype analysis of *Oreochromis mortimeri* (Trewavas) and *Sarotherodon*.
- Holcik J K and Hensel J. 1988. *Nieslanik*, The Eurasian Hucho Hucho hucho: Largest melanotheron (Rüppell) [J]. *Aquaculture Research* **33**: 339–42.
- Levan A, Fredya K and Sandberd A. 1964. Nomenclature for centromeric position on chromosomes [J]. *Hereditas Band* **52** (2): 201–20.
- Macgregor U C. 1993. *An Introduction to Animal Cytogenetics*. Chapter 1 and 2. pp.1–30. Chapman & Hall Press, London.
- Pronin N M. 2006. 'About changes in the status of rare and disappearing salmoniformes fish species in the red book of Buryatia republic.' *Second International Symposium on Ecology and Fishery Biodiversity in Large Rivers of Northeast Asia and Western North America*. Harbin, China.
- Tan X, Qin J G, Chen B, Chen L and Li X. 2004. Karyological analysis on redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture* **234**: 65–76.
- Thorgaard G H and Disney J E. 1990. Chromosome preparation and analysis. *Methods for Fish Biology*. (Eds) Schreck C B and Moyle P B. pp. 171–87. American Fisheries Society.
- Wu C, Ye Y and Chen R. 1986. Genome manipulation in carp (*Cyprinus carpio* L.). *Aquaculture* **54**: 57–61.
- Yue P Q and Chen Y Y. 1998. *China Red Data Book of Endangered Animals*. Pisces.