

Karyotypes of Two Antarctic Fishes, *Notothenia gibberifrons* and *Notothenia coriiceps neglecta**

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Abstract The chromosomes of two species of Antarctic fishes, *Notothenia (Gobionotothen) gibberifrons* and *Notothenia (Notothenia) coriiceps neglecta*, were prepared by the air-drying method at the Polish Antarctic Station "Henryk Arctowski" during the austral summer 1984–1985. For *N. (G.) gibberifrons* the diploid number is $2n=46$ consisting of 2 metacentric (m) pairs, 1 submetacentric (sm) pair and 20 telocentric (t) or subtelocentric (st) pairs. For *N. (N.) coriiceps neglecta* the diploid number is $2n=22$ consisting of 9 m pairs, 1 sm pair and 1 st pair. Some aspects of karyological evolution of these fishes are discussed.

Members of the family Nototheniidae are the main constituents of the Antarctic ichthyofauna. The systematics of this family, however, is still not well established. Hureau (1970), for instance, believed that the genera *Notothenia* and *Trematomus* were artificial and should be rearranged. Andersen and Hureau (1979) defined three genera, *Patagonotothen*, *Pagothenia* and *Notothenia*, for the subfamily Nototheniinae based on the structure of the caudal skeleton and of the cephalic canals. They considered *Trematomus* at a subgenus level. In a more recent work, Andersen (1984) made a phylogenetical analysis of the family Nototheniidae based on several systematic characters. Besides other redefinitions, the genus *Notothenia* was divided into three subgenera (*Notothenia*, *Lepidonotothen* and *Gobionotothen*) and the species *Trematomus hansonii* and *Trematomus bernacchii* were transferred to genus *Pagothenia*. Other species have also been transferred among subfamilies and subgenera. Cytogenetical methods have been used to study phylogeny, evolution, hybridization, speciation and systematics of many fish groups. Data on cytogenetics of Antarctic fish species are still scanty. As far as we know about 12 species from this region have been examined (Prirodina, 1984; Prirodina and Neelov, 1984; Bazignan and Ozouf-Costaz, 1985; Phan *et al.*, 1986) revealing very useful information. In this paper karyotypes of *Notothenia (Gobionotothen) gibberifrons* and *Notothenia (Notothenia) coriiceps*

neglecta are described and some aspects of their karyological evolution are discussed.

Material and methods

Fish specimens were collected with a gill net at Admiralty Bay, in front of the Polish Antarctic Station "Henryk Arctowski" during the austral summer 1984–1985. Six specimens of *N. (G.) gibberifrons* (2 males and 4 females) ranging from 219 to 305 mm in total length and 5 females of *N. (N.) coriiceps neglecta* ranging from 248 to 380 mm in total length were used in this study. The specimens were kept alive in tanks with running water and were injected intraperitoneally with 0.3 ml/100 g body weight of 0.3% colchicine in saline solution. Eight hours after the injection, the specimens were sacrificed and the kidney was removed for chromosome preparations. Various procedures were tried with good results but the air-drying method described by LeGrande and Fitzsimons (1976), with slight modification, was found to be the best one. The kidney was minced into small pieces and hypotonized in 10 ml of 0.9% sodium citrate solution for 35 min, with frequent mixing by aspiration-expiration using a Pasteur pipette. The suspension was then filtered through cheesecloth, then centrifuged at about 1000 rpm for 6 min. The supernatant was decanted and the cell suspension was fixed in 10 ml of Carnoy (3:1, methanol: acetic acid) for 10 min. After two further rinses in fixative the cells were resuspended in 0.5 ml of the same solution. The

* Cytogenetical studies of Antarctic fishes II.

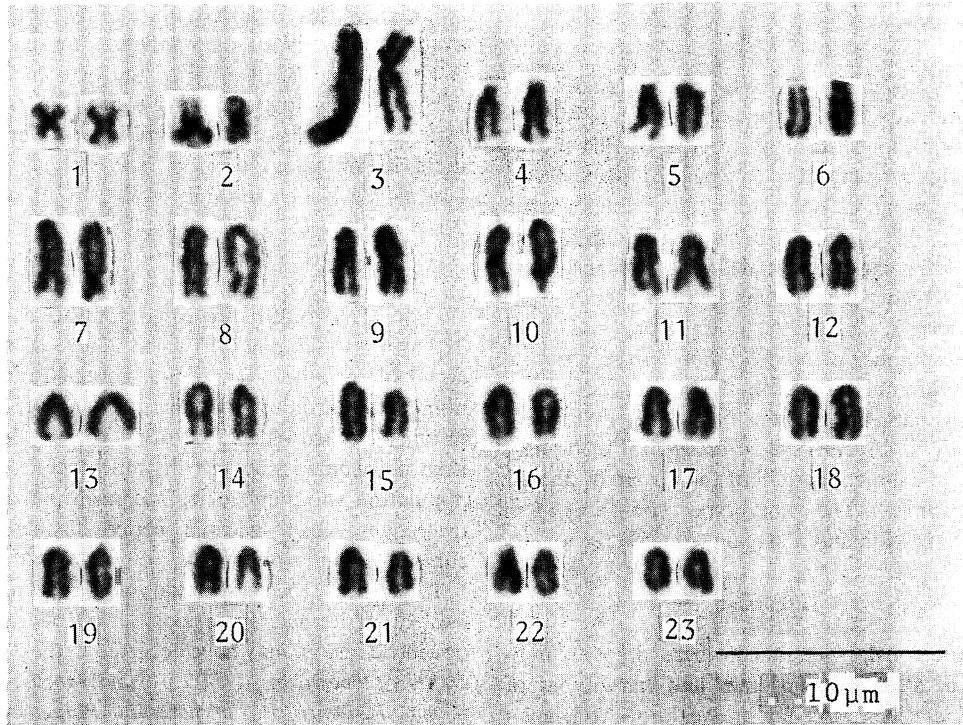


Fig. 1. Karyotype of *Notothenia gibberifrons*.

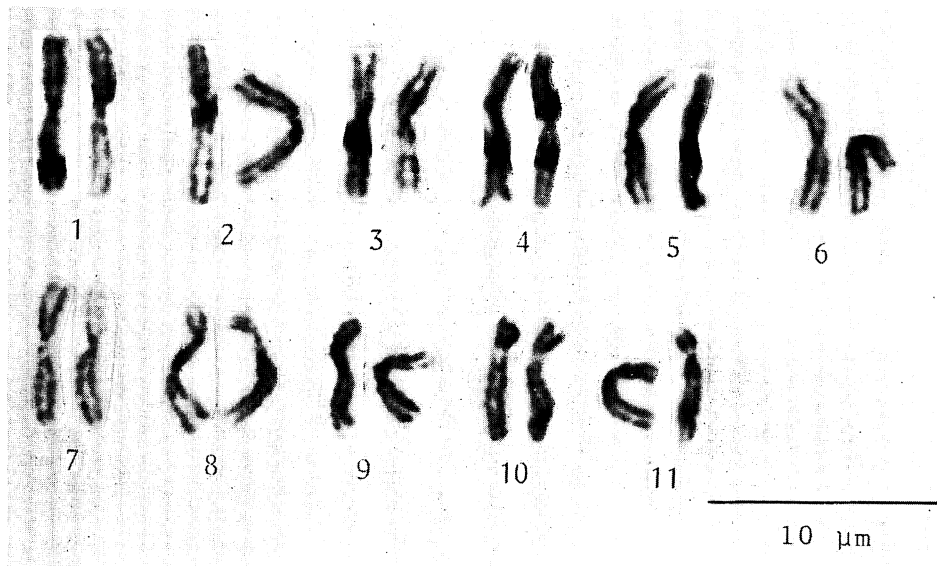


Fig. 2. Karyotype of *Notothenia coriiceps neglecta*.

suspension was then dropped onto cold glass slides and ignited or air dried. Slides were stained with Giemsa diluted to 1:10 by pH 7.0 phosphate buffer, dried and mounted in Permount.

Chromosomes were counted and the karyograms were prepared from photographic prints. Terminology for centromeric position followed the criteria of Levan *et al.* (1964).

Results

For *N. (G.) gibberifrons*, 23 metaphase cells were counted. Twenty one of them were of $2n=46$ chromosomes. The karyotype of this species consists of 2 metacentric (m) pairs, 1 submetacentric (sm) pair and 20 telocentric (t) or subtelocentric (st) pairs (Fig. 1). Remarkable differences between karyotypes of males and females were not observed. The results obtained clarify the uncertainty regarding the morphology and chromosome number of *N. (G.) gibberifrons* reported in a preliminary work on the karyotype of this species (Phan *et al.*, 1986).

For *N. (N.) coriiceps neglecta* 15 metaphase cells were counted, and all of them were of $2n=22$ chromosomes. The karyotype comprises 9 m pairs, 1 sm pair and 1 st pair (Fig. 2).

Discussion

As previously mentioned, there are few published data on chromosomes and karyotypes of Antarctic fishes. As far as we know, Bazignan and Ozouf-Costaz (1985) karyotyped 7 species, of which 4 belong to the family Nototheniidae. Prirodina (1984) (cited by Bazignan and Ozouf-Costaz, 1985) and Prirodina and Neelov (1984) karyotyped 4 species of this family. Phan *et al.* (1986) reported karyotypes of 3 species of Nototheniidae obtained during the II Brazilian Expedition to Antarctica, in the austral summer of 1983–1984. Table 1 summarizes data on karyotypes of

Antarctic fishes reported up to now and made available directly or indirectly to our team.

The karyotypic analysis of the two species described in this paper revealed that *N. (G.) gibberifrons* has 23 chromosomal pairs, most of them (21) of t or st type. *N. (N.) coriiceps neglecta* has only 11 chromosomal pairs whose majority (9) are of the m type. In relation to the karyotype of *N. (N.) coriiceps neglecta*, our results differ slightly from those of Prirodina and Neelov (1984). The authors reported for this fish $2n=22$ chromosomal pairs of which 20 are of m type and 2 of sm type. Our results revealed the same number of chromosomal pairs, of which 18 are of m type, 2 of sm type and 2 of st type. It has not been determined whether this difference is due to population variability or to specimen preparation techniques which allowed better distinction of chromosome morphology.

Two main processes of karyological evolution, namely pericentric inversions and Robertsonian or centric fusion, may have occurred in these fishes. *N. (G.) gibberifrons*, *N. (G.) cyanobranca*, *Pagothenia hansonii*, *P. bernacchii*, *Patagonotothen longipes*, *P. ramsayi* and *Dissostichus eleginoides* have a greater number of chromosomes, most of them occupied by t elements. Ohno *et al.* (1968) and other authors believe that the ancestral karyotype of the perciform fishes consisted of 24 t type pairs of chromosomes and that the presence of t elements in the chromosomal set indicate more primitive character. The differences between the karyotypes of these fishes may be explained mainly

Table 1. Antarctic fish karyotypes of the family Nototheniidae.
Classification follows that by Andersen (1984).

Species	2n	m	sm	st	st or t	Literature
<i>Notothenia (Notothenia) coriiceps neglecta</i>	22	20	2			Prirodina and Neelov, 1984
<i>N. (N.) coriiceps neglecta</i>	22	18	2	2		Phan <i>et al.</i> , 1986; present study
<i>N. (N.) rossii marmorata</i>	24	24				Prirodina and Neelov, 1984
<i>N. (N.) rossii rossii</i>	24	22	2			Bazignan and Ozouf-Costaz, 1985
<i>N. (Gobionotothen) gibberifrons</i>	46	4	2		40	Phan <i>et al.</i> , 1986; present study
<i>N. (G.) cyanobranca</i>	48	4			44	Bazignan and Ozouf-Costaz, 1985
<i>Paranotothenia magellanica</i>	26	24		2		Bazignan and Ozouf-Costaz, 1985
<i>Paranotothenia microlepidota</i>	26	22	2		2	Prirodina and Neelov, 1984
<i>Pagothenia hansonii</i>	48	2	4		42	Phan <i>et al.</i> , 1986
<i>Pagothenia bernacchi</i>	48	2			46	Phan <i>et al.</i> , 1986
<i>Patagonotothen longipes</i>	48	4			44	*Prirodina, 1984
<i>Patagonotothen ramsayi</i>	48		2		46	*Prirodina, 1984
<i>Dissostichus eleginoides</i>	48	2			46	Bazignan and Ozouf-Costaz, 1985

* Cited by Bazignan and Ozouf-Costaz (1985).

by pericentric inversions. *N. (N.) coriiceps neglecta*, *N. (N.) rossii marmorata*, *N. (N.) rossii rossii*, *Paranotothenia magellanica* and *P. microlepidota*, on the other hand, have reduced number of chromosomes mostly of m type. Mechanisms of centric fusion, in which two t type chromosomes fused together to originate one m type chromosome may have occurred. This process transformed a karyotype of $2n=48$ of mainly t elements into a karyotype $2n=24$ with only m elements as found in *N. (N.) rossii marmorata*. Other species, having mainly m elements could have suffered the same process, together with other mechanisms such as pericentric inversions and deletions.

Andersen (1984) proposed 4 models of cladograms to illustrate the phylogeny of the Nototheniidae. Karyological data, although scarce and scattered, are in general agreement with some aspects of his classification. For instance, fishes of subgenus *Notothenia* are of $2n=22$ to 24; subgenus *Gobionotothen* are of $2n=46$ to 48 and genus *Paranotothenia* are of $2n=26$ (Table 1). Prirodina and Neelov (1984) discuss that *N. (G.) cyanobranca* was once included in the same group of *N. (N.) coriiceps* and *N. (N.) rossii*. Its karyotype, however, is more related to that of *N. (G.) gibberifrons* than either of the 2 species mentioned (Table 1).

Fishes belonging to the genus *Pagothenia* have a great number of chromosomes mostly of t elements. Phylogenetic position of this genus is one of the great doubts which remains from Andersen's study. We believe that cytogenetical studies on other nototheniid fishes mainly of the genera *Trematomus*, *Pagothenia*, *Pleuragramma*, *Aethotaxis* and *Cryothernia* would be very helpful in solving this problem. The modifications of karyotypes undergone by nototheniid fishes during evolution seem to be of a magnitude big enough to be used as an instrument for their phylogenetical analysis.

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南極海産魚類 *Notothenia gibberifrons* と *Notothenia coriiceps neglecta* の核型

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南極大陸周辺海域に生息する *Notothenia (Gobionotothen) gibberifrons* と *N. (Notothenia) coriiceps neglecta* の 2 種の染色体を空気乾燥法を用いて調べた。 *N. (G.) gibberifrons* は $2n=46$ で、その染色体構成は $4m \cdot 2sm \cdot 40t$ もしくは st であった。一方、 *N. (N.) coriiceps neglecta* は $2n=22$ でその構成は $18m \cdot 2sm \cdot 2st$ であった。これら 2 種の核型進化の様相についても考慮した。