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# Chromosomes of Tetraodontiform Fishes from Japan

By

**Ryoichi ARAI**

Department of Zoology, National Science Museum, Tokyo

**and**

**Kazuo NAGAIWA**

College of Agriculture and Veterinary Medicine, Nihon University, Tokyo

Various attempts have been made in the past to arrange the tetraodontiform fishes in a phylogenetic sequence, and life history (BREDER and CLARK, 1947; FRASER-BRUNNER, 1951) and comparative anatomy have been studied by many investigators, e. g., skull (GREGORY, 1933; FRASER-BRUNNER, 1943; KURONUMA, 1943; ABE, 1952; TYLER, 1968), vertebral characters (ABE, 1942; TYLER, 1963, 1968), caudal skeletons (FRASER-BRUNNER, 1951; MONOD, 1968; TYLER, 1970), digestive tract and inflation (BREDER and CLARK, 1947), and comparative myology (WINTERBOTTOM, 1974).

Recently, karyological approach to fish systematics has become more important. As far as we know, however, chromosome studies of tetraodontiform fishes are very few, chromosomes of only four species having been reported in the world (NOGUSA, 1960; HINEGARDNER and ROSEN, 1972; ARAI and KATSUYAMA, 1973).

In this paper, karyotypes of fourteen species of four families, Balistidae, Tetraodontidae, Diodontidae, and Ostraciontidae, are described, and the relationships of the diploid chromosome number (2n), the arm number (NF), and the new arm number (NAN) are discussed for analysis of comparative karyology of fishes.

Systematics of families of tetraodontiform fishes are those by GREENWOOD *et al.* (1966), and the generic classification except for Tetraodontidae, that of SMITH (1965).

Method of chromosome preparation is the same as was given in ARAI and KATSUYAMA (1973).

Classification of chromosomes is adopted from LEVAN *et al.* (1964). Metacentrics and submetacentrics are described as two-arm chromosomes, and subtelocentrics and acrocentrics as one-arm chromosomes.

All the specimens used for the experiments are deposited in the fish collection of the Department of Zoology, National Science Museum, Tokyo.

### ***Hemibalistes chrysopterus*** (BLOCH et SCHNEIDER) "Tsumajiro-mongara"

(Plate 1, figs. 1 and 3)

Two specimens, 59.5 and 78.5 mm in total length, were collected from Kusugô

(30°25′N, 130°36′E), Yakushima Island, off southern Kyushu (Table 1).

As shown in Table 2, the diploid chromosome number is 46. The karyotype of this species comprises 23 pairs of acrocentric chromosomes. The chromosomes are comparable in appearance and show a gradation in size that makes it impossible to arrange them in size groups. The arm number is 46.

### ***Pseudobalistes flavimarginatus*** (RÜPPELL) "Kiheri-mongara"

(Plate 1, figs. 2 and 4)

A specimen, 49.0 mm in total length, was caught at Kusugô, Yakushima Island, and another specimen of 52.5 mm in total length, at Sunosaki (30°44′N, 130°60′E), Nishino-omote City, Tanegashima Island, off southern Kyushu (Table 1).

The diploid chromosome number is 44 (Table 2). The karyotype comprises a pair of large metacentrics, and 21 pairs of subtelocentric-acrocentric chromosomes. Large chromosomes are nearly twice longer in size than any other chromosome or more than that, and hence seem to be formed by centric fusion. The arm number is 46. The karyotype of this species differs from that of *H. chrysopterus* in the diploid chromosome number, but agrees well with the latter in the arm number.

### ***Rhinecanthus aculeatus*** (LINNAEUS) "Murasame-mongara"

(Plate 2, figs. 1 and 3)

A specimen, 70.0 mm in total length, was collected at Yonehara (24°27′N, 124° 11′E), Ishigaki Island, southern Ryukyus (Table 1).

As shown in Table 2, the diploid chromosome number is 44. The karyotype comprises 22 pairs of acrocentric chromosomes. The karyotype of this species agrees with that of *Pseudobalistes flavimarginatus* in the diploid chromosome number, but differs from the latter in the arm number.

### ***Rhinecanthus verrucosus*** (LINNAEUS) "Kurakake-mongara"

(Plate 2, figs. 2 and 4)

Two specimens, 53.0 and 59.0 mm in total length, were caught at Sunosaki, Nishino-omote City, Tanegashima Island (Table 1).

The diploid chromosome number is 44 (Table 2). The karyotype comprises 22 pairs of acrocentric chromosomes. In size, one-arm chromosomes show a gradation from largest to smallest, hence cannot be easily divided into size groups. The arm number is 44. The karyotype of this species is similar to that of *R. aculeatus*.

### ***Amanses sandwichiensis*** (QUOY et GAIMARD) "Goma-umazura"

(Plate 3, figs. 1 and 4)

A specimen, 94.0 mm in total length, was collected at Shimama (30°28′N, 130°

52′E), Tanegashima Island (Table 1).

As shown in Table 2, we could not obtain clear chromosome figures sufficient for determining the karyotype of this species. However, we tentatively report it as follows. The diploid chromosome number is 40, and the karyotype comprises 20 pairs of subtelocentric-acrocentric chromosomes. The arm number is 40. The diploid chromosome number of this species is smaller than that of *Rhinecanthus*.

Table 1. Characters of fourteen species of material fishes.

| Species | No. of fish | S.L. (mm) | D | A | VN |
|---|---|---|---|---|---|
| Balistidae | | | | | |
| *Hemibalistes chrysopterus* | 2 | 49.5– 68.0 | III, 26 | 24 | 7+11 |
| *Pseudobalistes flavimarginatus* | 2 | 42.0– 43.5 | III, 25 | 23–24 | 7+11 |
| *Rhinecanthus aculeatus* | 1 | 60.0 | III, 24 | 22 | 7+11 |
| *R. verrucosus* | 2 | 47.0– 51.0 | III, 25–26 | 22–23 | 7+11 |
| *Amanses sandwichiensis* | 1 | 80.0 | II, 35 | 30 | 7+12 |
| *Rudarius ercodes* | 3 | 33.5– 47.0 | II, 26 | 25 | 7+13–14 |
| *Oxymonacanthus longirostris* | 4 | 55.5– 76.0 | II, 31–33 | 28–30 | 8+17 |
| Tetraodontidae | | | | | |
| *Sphoeroides chrysops* | 2 | 86.0– 92.5 | 12–13 | 11–12 | 22–23 |
| *Tetraodon immaculatus* | 1 | 43.5 | 9 | 9 | 18 |
| *T. nigropunctatus* | 2 | 82.0–114.0 | 10–11 | 10–11 | 18 |
| *Chelonodon patoca* | 2 | 40.5– 56.0 | 10 | 8 | 20 |
| *Canthigaster rivulata* | 5 | 71.5– 84.0 | 10 | 10 | 17 |
| Diodontidae | | | | | |
| *Diodon bleekeri* | 2 | 124.0–129.0 | 15 | 15 | |
| Ostraciontidae | | | | | |
| *Ostracion tuberculatus* | 2 | 27.0– 34.5 | 9 | 9 | |

## ***Rudarius ercodes*** JORDAN et FOWLER "Amime-hagi"

(Plate 3, figs. 2 and 5)

Three specimens, 42.5 to 60.0 mm in total length, were caught at Tsumekizaki, Shimoda, Izu Peninsula, Shizuoka Prefecture (Table 1).

The diploid chromosome number is 36 (Table 2). The karyotype comprises 18 pairs of acrocentric chromosomes. In size, one-arm chromosomes show a gradation from largest to smallest, hence cannot be easily divided into size groups. The arm number is 36. The diploid chromosome number of this species is smaller than those in *Amanses sandwichiensis* and *Navodon modestus* (see NOGUSA, 1960).

## ***Oxymonacanthus longirostris*** (BLOCH et SCHNEIDER) "Tengu-kawahagi"

(Plate 3, figs. 3 and 6)

Four specimens, 62.0 to 86.0 mm in total length, were collected at Yonehara, Ishigaki Island, southern Ryukyus (Table 1).

As shown in Table 2, the diploid chromosome number is 36. The karyotype comprises 18 pairs of acrocentric chromosomes. The arm number is 36. The karyotype of this species is similar to that of *Rudarius ercodes*.

### ***Sphoeroides chrysops*** (HILGENDORF) "Akame-fugu"

(Plate 4, figs. 1 and 3)

Two specimens, 104.0 and 115.5 mm in total length, were caught at Tsumekizaki, Shimoda, Izu Peninsula (Table 1).

The diploid chromosome number is 44 (Table 2). The karyotype of this species comprises 3 pairs of metacentric, 7 pairs of submetacentric, and 12 pairs of subtelocentric-acrocentric chromosomes. Among two-arm chromosomes there were found a pair of large submetacentrics which seem to be formed by centric fusion. The arm number is 64. The karyotype of this species resembles that of *S. niphobles* which was reported as *Fugu niphobles* in ARAI and KATSUYAMA (1973).

### ***Chelonodon patoca*** (HAMILTON-BUCHANAN) "Okinawa-fugu"

(Plate 4, figs. 2 and 4)

Two specimens, 52.5 and 69.5 mm in total length, were collected at the river mouth near Nagura (24°25′N, 124°08′E), Ishigaki Island (Table 1).

As shown in Table 2, the diploid chromosome number is 40. The karyotype comprises 7 pairs of metacentric, 8 pairs of submetacentric, and 5 pairs of subtelocentric-acrocentric chromosomes. There were found a pair of large metacentrics among two-arm chromosomes. The arm number is 70. The karyotype of this species differs from that of *Sphoeroides chrysops* in the diploid chromosome number.

### ***Tetraodon immaculatus*** (BLOCH et SCHNEIDER) "Sujimoyô-fugu"

(Plate 5, figs. 1 and 4)

A specimen, 58.0 mm in total length, was caught at the river mouth near Nagura, Ishigaki Island (Table 1).

The diploid chromosome number is 42 (Table 2). The karyotype comprises 7 pairs of metacentric, 8 pairs of submetacentric, and 6 pairs of subtelocentric chromosomes. The arm number is 72. The diploid chromosome number of this species is larger than that of *C. patoca*, but smaller than that of *S. chrysops*.

### ***Tetraodon nigropunctatus*** (BLOCH et SCHNEIDER) "Yogore-fugu"

(Plate 5, figs. 2 and 5)

Two specimens, 104.0 and 135.0 mm in total length, were collected at Yonehara, Ishigaki Island (Table 1).

Table 2. Frequency distributions of diploid chromosome counts in tetraodontiform fishes.

| Species | 2n | | | | | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | |
| Balistidae | | | | | | | | | | | | | | | | | | | | | | |
| *Hemibalistes chrysopterus* | | | | | | | | | | | 1 | 3 | 3 | 2 | 6 | 20 | 2 | | | | | 37 |
| *Pseudobalistes flavimarginatus* | | | | | | | | | | 1 | 2 | 1 | 4 | 24 | 1 | 1 | | | | | | 34 |
| *Rhinecanthus aculeatus* | | | | | | | | 3 | 1 | | 2 | 7 | 7 | 17 | 2 | 1 | | | | | | 40 |
| *R. verrucosus* | | | | | | | | | | | 3 | 4 | 6 | 9 | 2 | 1 | | | | | | 25 |
| *Amanses sandwichiensis* | | | | | | 1 | | | 1 | 2 | | | | | | | | | | | | 4 |
| *Rudarius ercodes* | | | 1 | 4 | 3 | 26 | 1 | | | | | | | | | | | | | | | 35 |
| *Oxymonacanthus longirostris* | | 1 | 5 | 3 | 4 | 18 | 1 | | | | | | | | | | | | | | | 32 |
| Tetraodontidae | | | | | | | | | | | | | | | | | | | | | | |
| *Sphoeroides chrysops* | | | | | | | | | 1 | 1 | 2 | 5 | 8 | 40 | 2 | 1 | | | | | | 60 |
| *Tetraodon immaculatus* | | | | | | | 1 | | | | 1 | 5 | 1 | | | | | | | | | 8 |
| *T. nigropunctatus* | | | | | 4 | 8 | 9 | 28 | 2 | 1 | | | | | | | | | | | | 52 |
| *Chelonodon patoca* | | | | | | | 2 | 6 | 7 | 21 | | | | | | | | | | | | 36 |
| *Canthigaster rivulata* | | 1 | 5 | 20 | 2 | | | | | | | | | | | | | | | | | 28 |
| Diodontidae | | | | | | | | | | | | | | | | | | | | | | |
| *Diodon bleekeri* | | | | | | | | | | | | 1 | 2 | 3 | 1 | 11 | | 1 | | | | 19 |
| Ostraciontidae | | | | | | | | | | | | | | | | | | | | | | |
| *Ostracion tuberculatus* | | | | | | | | | | | | | | | | | | | | 3? | | 3 |

As shown in Table 2, the diploid chromosome number is 38. The karyotype comprises 7 pairs of metacentric, 10 pairs of submetacentric, and 2 pairs of subtelocentric chromosomes. The arm number is 72. As regards marine acanthopterygian fishes, congeneric species very rarely have different karyotypes. In the case of *Tetraodon*, *immaculatus* and *nigropunctatus* have 42 and 38 chromosomes in the diploid number, respectively. Nostrils of *T. nigropunctatus* are morphologically different from those of *Chelonodon* and *T. immaculatus*.

### ***Canthigaster rivulata*** TEMMINCK et SCHLEGEL "Kitamakura"

(Plate 5, figs. 3 and 6)

Five specimens, 88.0 to 102.5 mm in total length, were caught at Amatsu-kominato, Awa, Chiba Prefecture (Table 1).

The diploid chromosome number is 34 (Table 2). The karyotype comprises 2 pairs of metacentric, 3 pairs of submetacentric, 5 pairs of subtelocentric, and 7 pairs of acrocentric chromosomes. The arm number is 44. The diploid chromosome number of this species is the smallest among those of tetraodontiform fishes. SMITH (1965) adopted the family Canthigasteridae. From karyological point of view, however, *C. rivulata* may be considered as a specialized fish.

### ***Diodon bleekeri*** GÜNTHER "Hitozura-harisenbon"

(Plate 6, figs. 1 and 3)

Two specimens, 145.0 and 155.0 mm in total length, were collected from Yonehara, Ishigaki Island, southern Ryukyus (Table 1).

As shown in Table 2, the diploid chromosome number is 46. The karyotype comprises 3 pairs of metacentric, 3 pairs of submetacentric, and 17 pairs of subtelocentric-acrocentric chromosomes. The arm number is 58. The diploid chromosome number of this species is larger than those of tetraodontid fishes.

### ***Ostracion tuberculatus*** LINNAEUS "Hako-fugu"

(Plate 6, figs. 2 and 4)

A specimen, 48.0 mm in total length, was caught at Yonehara, Ishigaki Island, and another specimen of 32.5 mm in total length at Kusugô, Yakushima Island (Table 1).

We could not get clear chromosome figures sufficient for determining the karyotype of this species. However, we tentatively report it as follows. The diploid chromosome number is 50, and the karyotype comprises 2 pairs of submetacentric, and 23 pairs of subtelocentric-acrocentric chromosomes. The arm number is 54. There were a pair of minute chromosomes, which are smaller than half the size of any other chromosome, hence may be produced by centric fission. As far as we know, the diploid

Table 3. Comparison of characters within tetraodontiform fishes whose karyotypes have been reported.

| Genus | Abdominal vertebrae | Nostrils (pairs) | Teeth | Dorsal spines | Pelvis | Movable pelvic spine | Cover | Inflation | Branchio-stegals | 4th gill |
|---|---|---|---|---|---|---|---|---|---|---|
| Balistidae | | | | | | | | | | |
| *Hemibalistes* | 7 | 2 | not fused | 3 | + | + | large scales | rudimentary | 6 | + |
| *Pseudobalistes* | 7 | 2 | not fused | 3 | + | + | large scales | rudimentary | 6 | + |
| *Rhinecanthus* | 7 | 2 | not fused | 3 | + | + | large scales | rudimentary | 6 | + |
| *Amanses* | 7 | 2 | not fused | 2 | + | − | small scales | rudimentary | 6 | + |
| *Rudarius* | 7 | 2 | not fused | 2 | + | − | small scales | rudimentary | 6 | + |
| *Oxymonacanthus* | 8 | 2 | not fused | 2 | + | − | small scales | rudimentary | 6 | + |
| Tetraodontidae | | | | | | | | | | |
| *Sphoeroides* | 8–9 | 2 | fused, 4 | 0 | − | − | spines | developed | 5 | − |
| *Tetraodon* | 8 | 1 | fused, 4 | 0 | − | − | spines | developed | 5–6 | − |
| *Chelonodon* | 8 | (1)* | fused, 4 | 0 | − | − | spines | developed | | − |
| *Canthigaster* | 8 | 1 | fused, 4 | 0 | − | − | spines | developed | 5–6 | − |
| Diodontidae | | | | | | | | | | |
| *Diodon* | 12 | 1 | fused, 2 | 0 | − | − | strong spines | developed | 6 | − |
| Ostraciontidae | | | | | | | | | | |
| *Ostracion* | 9 | 2 | not fused | 0 | (+)** | − | carapace | absent | 6 | + |

* Nostril is a depression surrounded by a low rim.

** Pelvic ridges are considered as a remnant of pelvis (MATSUBARA, 1955).

chromosome number of this species is the largest among those of tetraodontiform fishes whose chromosomes have been known (NOGUSA, 1960; HINEGARDNER and ROSEN, 1972; ARAI and KATSUYAMA, 1973).

## Direction of Differentiation of Characters in the Family Balistidae

Although it is unknown whether or not diagnostic characters within the family Balistidae are directly related with different karyotypes, from the karyological point of view, we tried to find out the relationship between the comparative anatomy and karyotypes in taking up such characters as vertebral characters, teeth number, dorsal spines, and scales. Data given in Table 3 were derived from reports of ABE (1942), FRASER-BRUNNER (1943), BREDER and CLARK (1947), MATSUBARA (1955), BEAUFORT and BRIGGS (1962), TYLER (1963), SMITH (1965) and others.

*Vertebrae.* As shown in Tables 1, 3 and 4, the diploid chromosome number ranges from 36 to 46, and the total vertebrae, from 18 to 25. The abdominal vertebrae of 5 genera and 6 species excepting *Oxymonacanthus longirostris* are 7. When only the caudal vertebral number is considered, the diploid chromosome number decreases as the caudal vertebral number becomes larger.

*Teeth.* *Hemibalistes, Pseudobalistes* and *Rhinecanthus* have an outer series of 8 teeth, and *Amanses, Rudarius* and *Oxymonacanthus* have an outer series of 6 teeth on the upper jaw. *Hemibalistes, Pseudobalistes* and *Rhinecanthus* have an inner series of 6 teeth, and *Amanses, Rudarius* and *Oxymonacanthus* have an inner series of 4 teeth on the upper jaw. In this case, the diploid chromosome number becomes smaller as the number of teeth decreases.

*Dorsal spines.* *Hemibalistes, Pseudobalistes* and *Rhinecanthus* are 3, and *Amanses, Rudarius* and *Oxymonacanthus* are 2 in the number of dorsal spines. Decrease of dorsal spine number agrees well with that of the diploid chromosome number. This fact may suggest that the dorsal with two spines is more specialized than that with three spines.

*Scales.* The body of *Hemibalistes, Pseudobalistes* and *Rhinecanthus* is covered with large stout scales. However, scales of *Amanses, Rudarius* and *Oxymonacanthus* are transformed into small scales or minute rough scales which generally do not form regular series and have spinules. The fact seems to show that transformation of scales from large to small or variously formed scales reflects strongly the direction of differentiation in the comparative morphology of scales.

On the other hand, the systematic position of filefishes has not been fixed. Many ichthyologists have classified them as the family Aluteridae or Monacanthidae, but recent investigators, GREENWOOD *et al.* (1966) and TYLER (MS.) included them in the family Balistidae. Our karyological data suggest that Aluteridae or Monacanthidae is not an independent family, but is a specialized group in Balistidae.

Table 4. Karyotypes of tetraodontiform fishes.

| Species | 2n | Chromosome forms | | NF | Large chromosome* | NAN* | Literature |
|---|---|---|---|---|---|---|---|
| | | two-arm | one-arm | | | | |
| Family Balistidae | | | | | | | |
| *Hemibalistes chrysopterus* | 46 | 0 | 46 | 46 | 0 | 46 | This paper |
| *Pseudobalistes flavimarginatus* | 44 | 2 | 42 | 46 | 2 | 46 | This paper |
| *Rhinecanthus aculeatus* | 44 | 0 | 44 | 44 | 0 | 44 | This paper |
| *R. verrucosus* | 44 | 0 | 44 | 44 | 0 | 44 | This paper |
| *Amanses sandwichiensis* | 40 | 0 | 40 | 40 | 0 | 40 | This paper |
| *Navodon modestus*** | 40 | — | — | — | — | — | Nogusa (1960) |
| *Rudarius ercodes* | 36 | 0 | 36 | 36 | 0 | 36 | This paper |
| *Stephanolepis cirrhifer*** | 34 | — | — | — | — | — | Nogusa (1960) |
| *Oxymonacanthus longirostris* | 36 | 0 | 36 | 36 | 0 | 36 | This paper |
| Family Tetraodontidae | | | | | | | |
| *Sphoeroides niphobles**** | 44 | 20 | 24 | 64 | 2 | 46 | Arai and Katsuyama (1973) |
| *S. chrysops* | 44 | 20 | 24 | 64 | 2 | 46 | This paper |
| *Tetraodon immaculatus* | 42 | 30 | 12 | 72 | 0 | 42 | This paper |
| *T. palembangensis* | 42 | — | — | — | — | — | Hinegardner and Rosen (1972) |
| *T. nigropunctatus* | 38 | 34 | 4 | 72 | 2 | 40 | This paper |
| *Chelonodon patoca* | 40 | 30 | 10 | 70 | 2 | 42 | This paper |
| *Canthigaster rivulata* | 34 | 10 | 24 | 44 | 0 | 34 | This paper |
| Family Diodontidae | | | | | | | |
| *Diodon bleekeri* | 46 | 12 | 34 | 58 | 0 | 46 | This paper |
| Family Ostraciontidae | | | | | | | |
| *Ostracion tuberculatus* | 50? | 4 | 46? | 54? | 0 | 49? | This paper |

* As for definition, see text.
** The diploid chromosome number by classical gonad section method.
*** Reported as *Fugu niphobles* in Arai and Katsuyama (1973).

## Significance of NAN in Comparative Karyology

Before advancing the discussion, we propose a new term, "new arm number" (NAN) in this paper. For this concept is very important and necessary for comparative karyology. NAN is a total of the diploid chromosome number and the large chromosome number. The large chromosome (LC) is defined as follows; 1) LC can be divided into size groups and separated from the other size group or groups in the karyotype, 2) LC belongs to a larger size group, and 3) LC is nearly twice or more than twice longer in size than any chromosome of the other size group or groups. Hence chromosomes which are produced by centric fusion are LC, and if three conditions described above are all filled, not only metacentrics or submetacentrics but also subtelocentrics or acrocentrics are defined as LC. NAN is the same or more numerous than the diploid chromosome number, i. e., NAN$\geqq$2n, and NAN is the same or smaller than the arm number, i. e., NAN$\leqq$NF. For example, LC corresponds to "large (L) chromosome" in CHEN (1971). In his report, the diploid chromosome number in 20 species of *Fundulus* ranges from 32 to 48, but their NAN is always 48. NAN may be an indicator which has more important significance than the diploid chromosome number in the fish phylogeny.

On the other hand, in acanthopterygian fish karyology, as far as we know, the cases of centric fission are vague, hence we have not considered NAN in the case of centric fission. However, in the case where centric fission occurs, two minute chromosomes (MC) by fission should be counted as one in NAN, i. e., NAN=2n−half of number of MC.

When a karyotype comprises chromosomes by fusion as well as chromosomes by fission, it is very difficult to differentiate LC and MC from the other chromosomes.

At any rate, the degree of importance of 2n, NF, and NAN in fish phylogeny within a family may be shown as follows, NAN$\geqq$2n$\geqq$NF. The direction of differentiation of NAN, 2n and NF may be large to small in NAN, large to small in 2n, and small to large in NF having the same number of 2n and NAN.

## Karyological Interrelationships of Four Families in Tetraodontiform Fishes

As shown in Table 4, when we consider NAN of tetraodontiform fishes, 46 in NAN is found in three families, Balistidae, Tetraodontidae and Diodontidae; a pair of large submetacentrics are LC in NAN of *Sphoeroides*. Karyological data are not so many in tetraodontiform fishes, hence it is unknown whether or not species with 48 chromosomes are present in these three families. However, it is possible that Balistidae, Tetraodontidae and Diodontidae are specialized fish groups and their basic diploid chromosome number is 46, and that species with 46 chromosomes will be found in Tetraodontidae in near future. Ostraciontidae differs from the other three families by the diploid chromosome number. As shown in Table 3, Ostraciontidae has many basic characters of tetraodontiform fishes. When we divide these four families into two groups, Ostraciontidae may be separated from the other three families. Concerning with families with 46 in NAN, Balistidae differs from Tetraodontidae and Diodontidae by the absence of two-arm chromosomes by pericentric inversion.

Karyological results in the present paper associate the phylogenetic tree by BREDER and CLARK (1947, p. 315, fig. 8) with interrelationships of tetraodontiform fishes.

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## Explanation of Plates 1–6

### Plate 1

Figs. 1–2. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of triggerfishes. —— 1. *Hemibalistes chrysopterus*, 2n=46. ×1,900. —— 2. *Pseudobalistes flavimarginatus*, 2n=44. ×1,900.

Figs. 3–4. Karyotypes of triggerfishes. —— 3. *Hemibalistes chrysopterus*, from Fig. 1, NF=46. ×1,840. —— 4. *Pseudobalistes flavimarginatus*, from Fig. 2, NF=46. ×1,940.

### Plate 2

Figs. 1–2. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of triggerfishes. —— 1. *Rhinecanthus aculeatus*, 2n=44. ×1,900. —— 2. *R. verrucosus*, 2n=44. ×1,900.

Figs. 3–4. Karyotypes of *Rhinecanthus*. —— 3. *R. aculeatus*, from Fig. 1, NF=44. ×2,130. —— 4. *R. verrucosus*, from Fig. 2, NF=44. ×1,520.

### Plate 3

Figs. 1–3. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of filefishes. —— 1. *Amanses sandwichiensis*, 2n=40. ×1,900. —— 2. *Rudarius ercodes*, 2n=36. ×1,900. —— 3. *Oxymonacanthus longirostris*, 2n=36. ×1,900.

Figs. 4–6. Karyotypes of filefishes. —— 4. *Amanses sandwichiensis*, from Fig. 1, NF=40. ×2,250. —— 5. *Rudarius ercodes*, from Fig. 2, NF=36. ×2,330. —— 6. *Oxymonacanthus longirostris*, from Fig. 3, NF=36. ×1,590.

### Plate 4

Figs. 1–2. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of puffers. —— 1. *Sphoeroides chrysops*, 2n=44. ×1,900. —— 2. *Chelonodon patoca*, 2n=40. ×1,900.

Figs. 3–4. Karyotypes of puffers. —— 3. *Sphoeroides chrysops*, from Fig. 1, NF=64. ×2,450. —— 4. *Chelonodon patoca*, from Fig. 2, NF=70. ×2,600.

### Plate 5

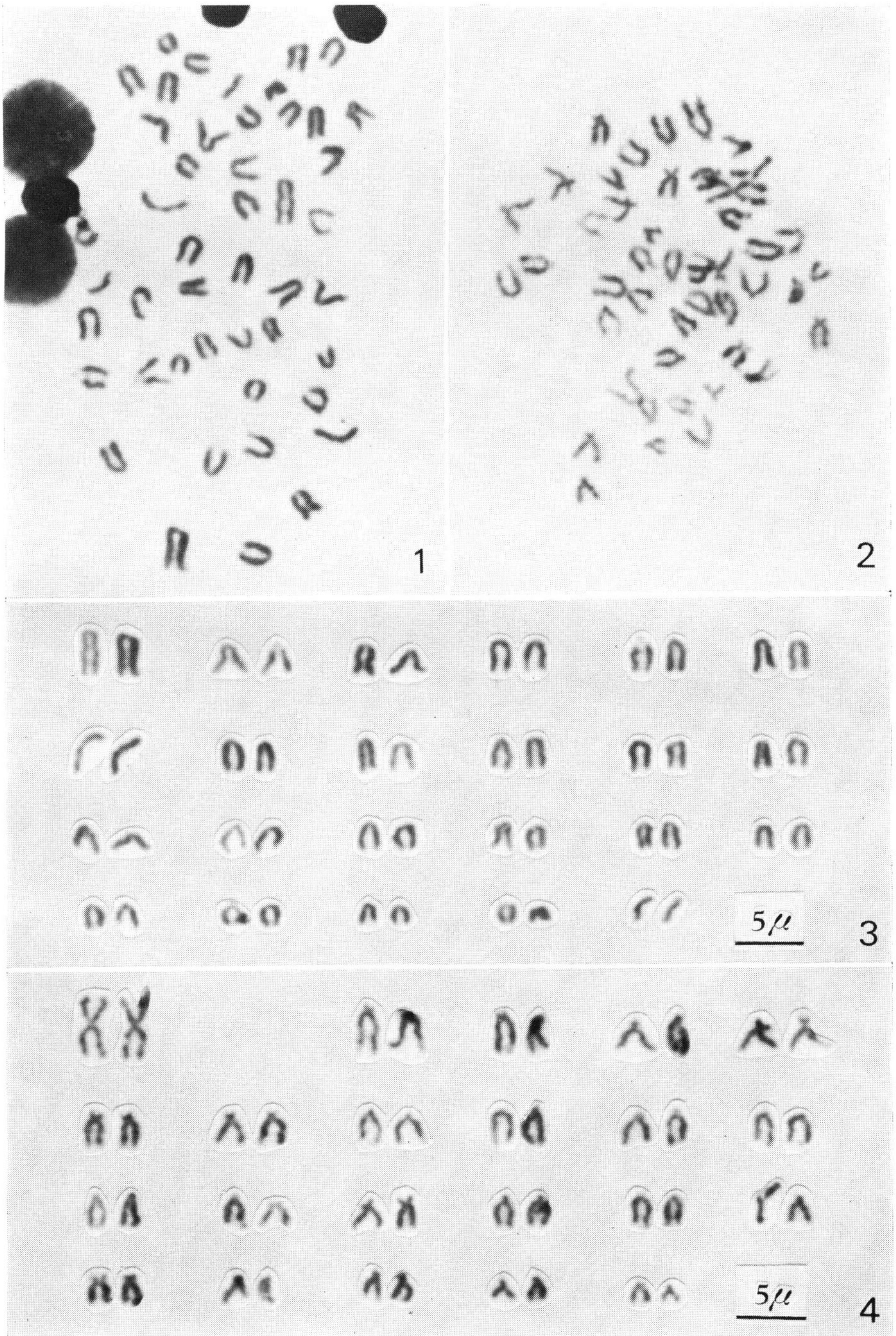
Figs. 1–3. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of puffers. —— 1. *Tetraodon immaculatus*, 2n=42. ×1,900. —— 2. *T. nigropunctatus*, 2n=38, ×1,900. —— 3. *Canthigaster rivulata*, 2n=34. ×1,900.

Figs. 4–6. Karyotypes of puffers. —— 4. *Tetraodon immaculatus*, from Fig. 1, NF=72. ×2,010. —— 5. *T. nigropunctatus*, from Fig. 2, NF=72. ×2,110. —— 6. *Canthigaster rivulata*, from Fig. 3, NF=44. ×2,930.

### Plate 6

Figs. 1–2. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of a porcupine fish and a trunkfish. —— 1. *Diodon bleekeri*, 2n=46. ×1,900. —— 2. *Ostracion tuberculatus*, 2n=50? ×1,900.

Figs. 3–4. Karyotypes of a porcupine fish and a trunkfish. —— 3. *Diodon bleekeri*, from Fig. 1, NF=58. ×1,900. —— 4. *Ostracion tuberculatus*, from Fig. 2, NF=54? ×1,510.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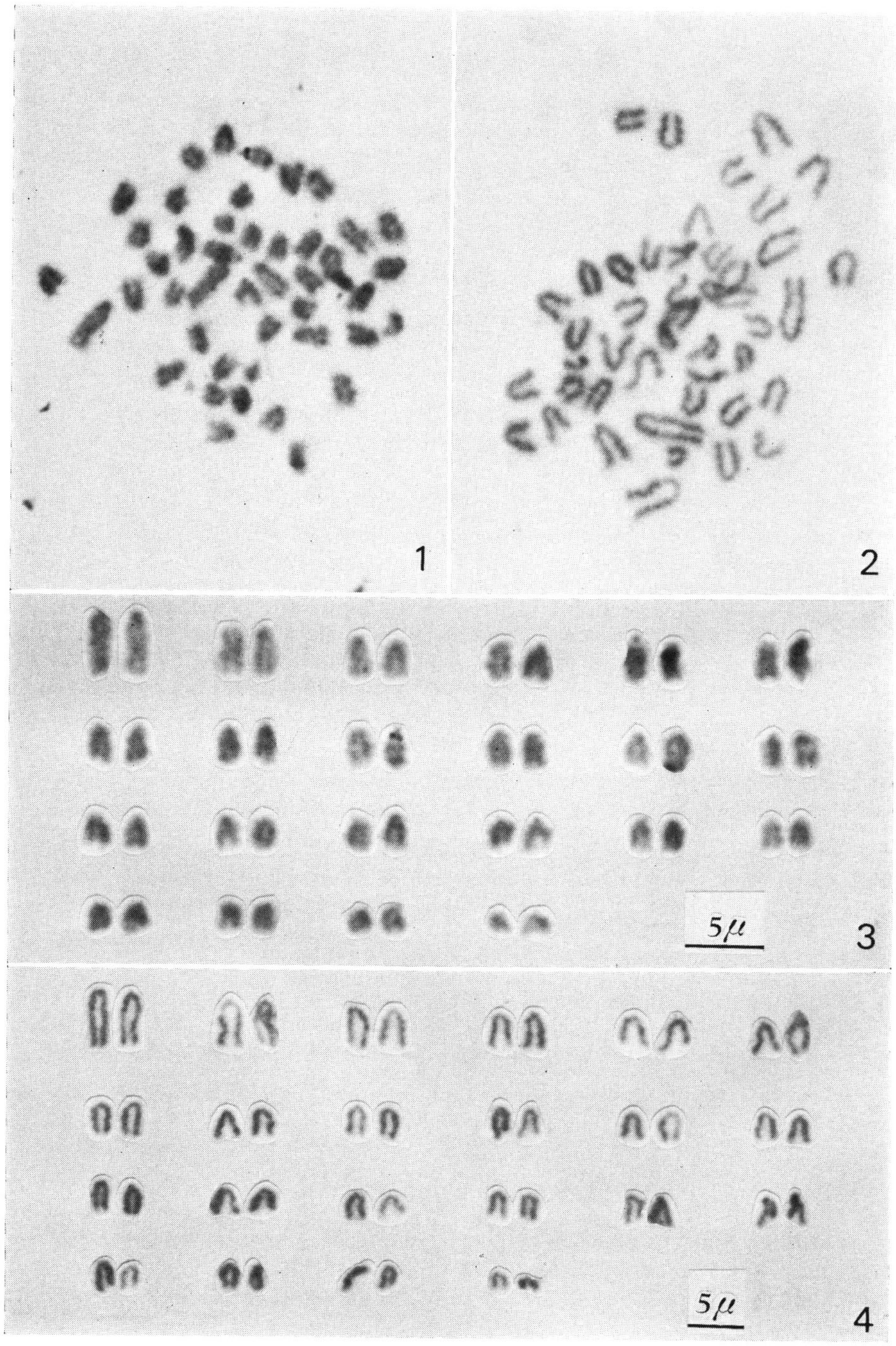
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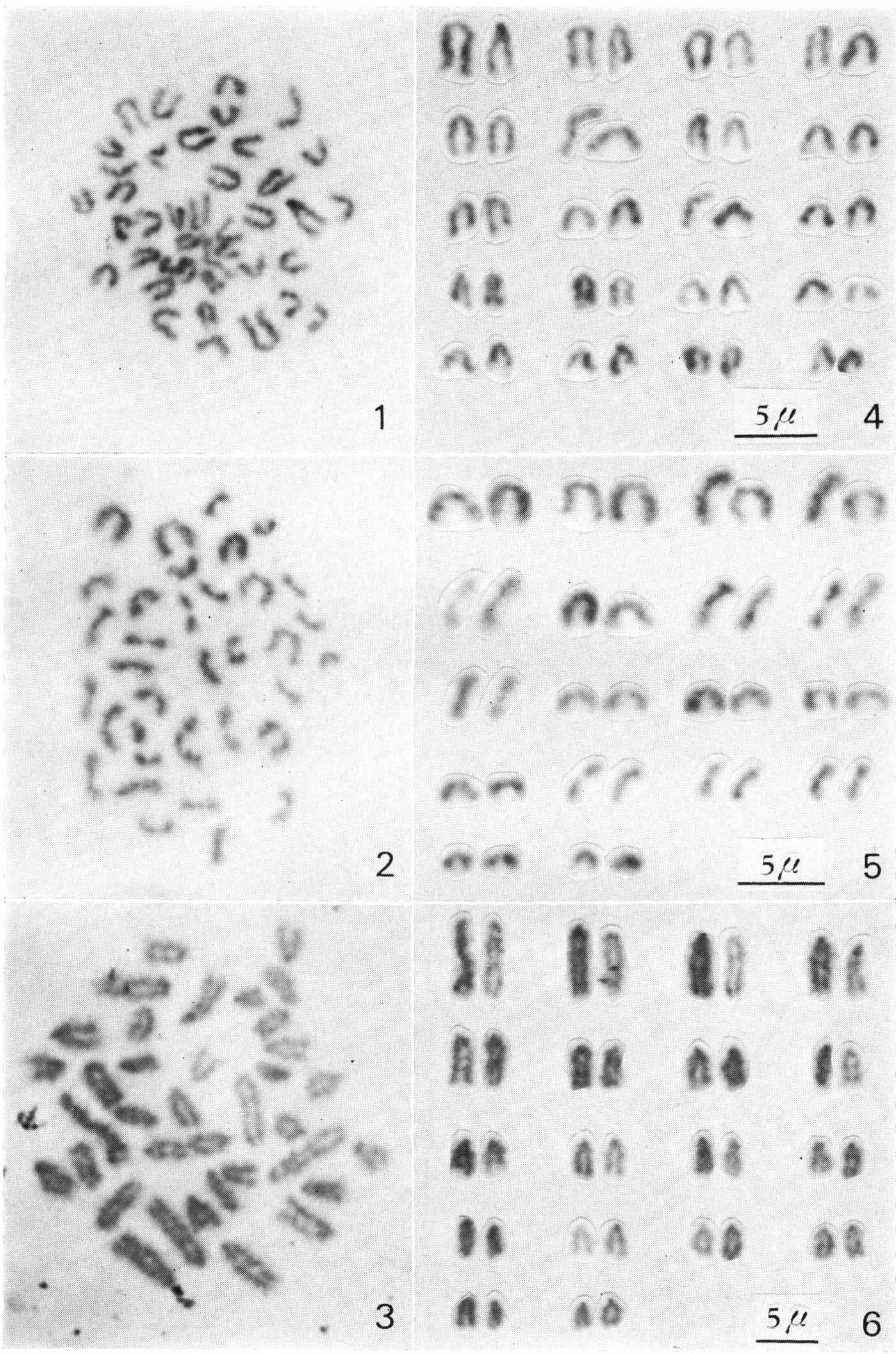
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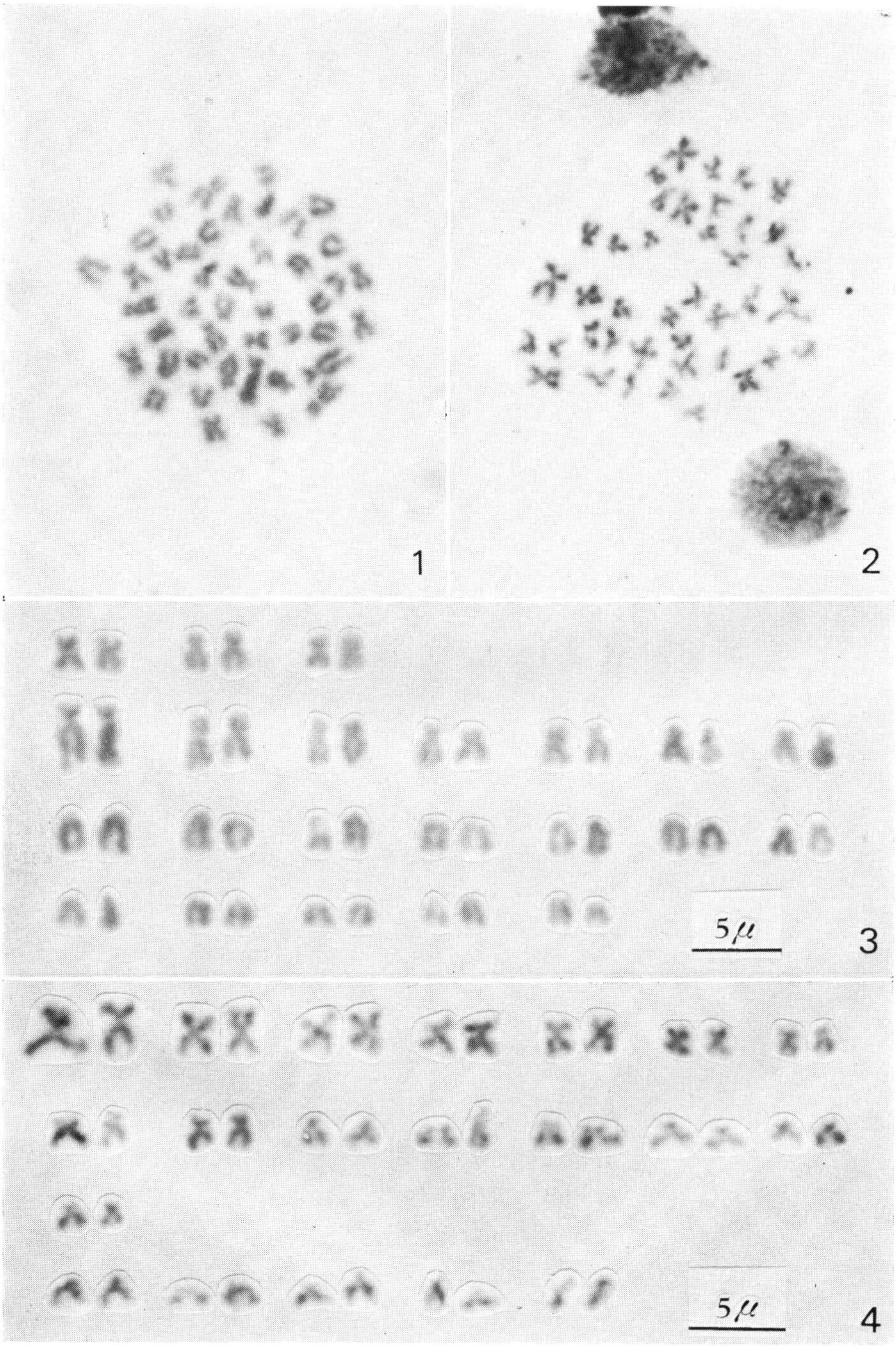
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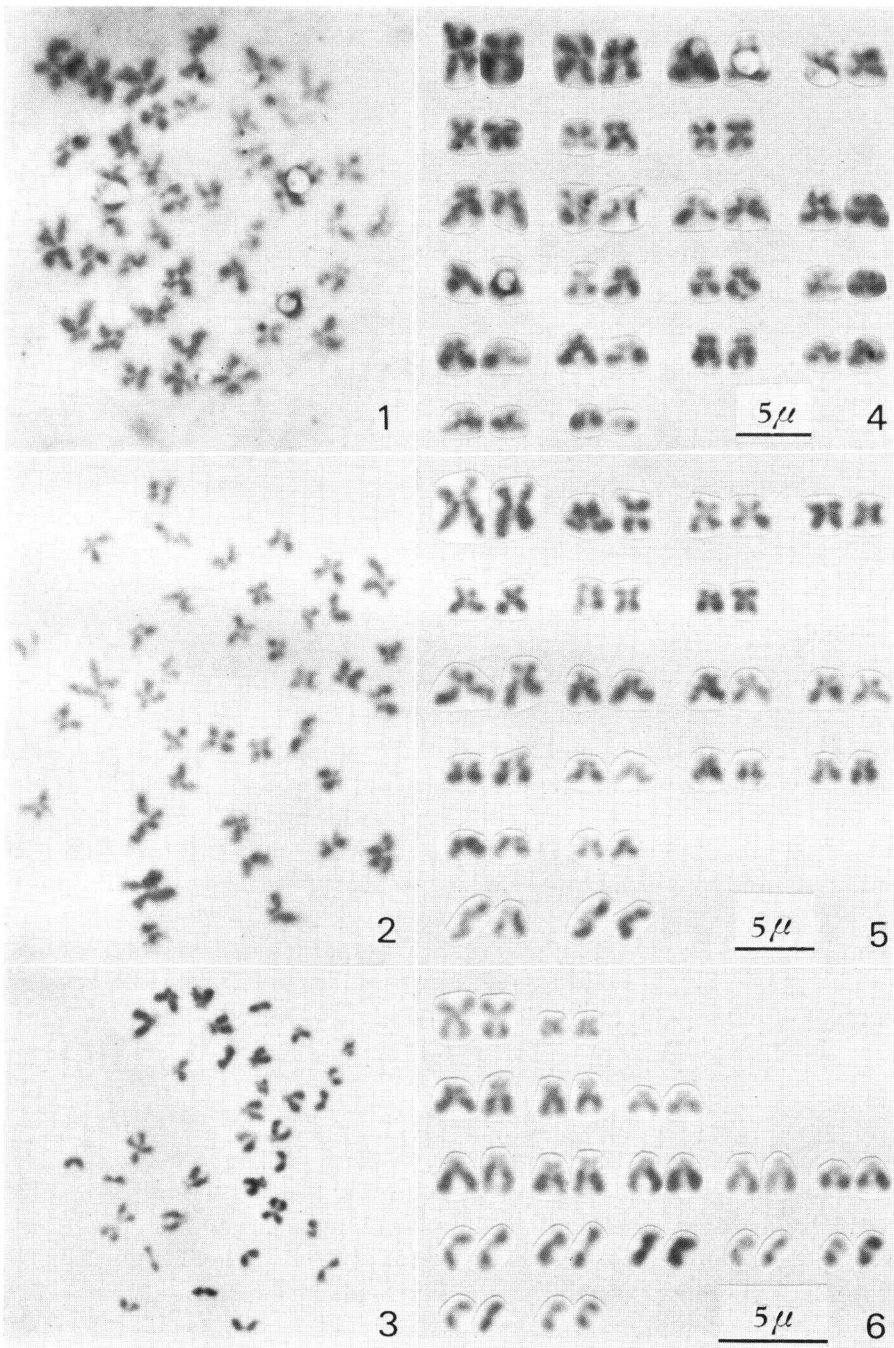
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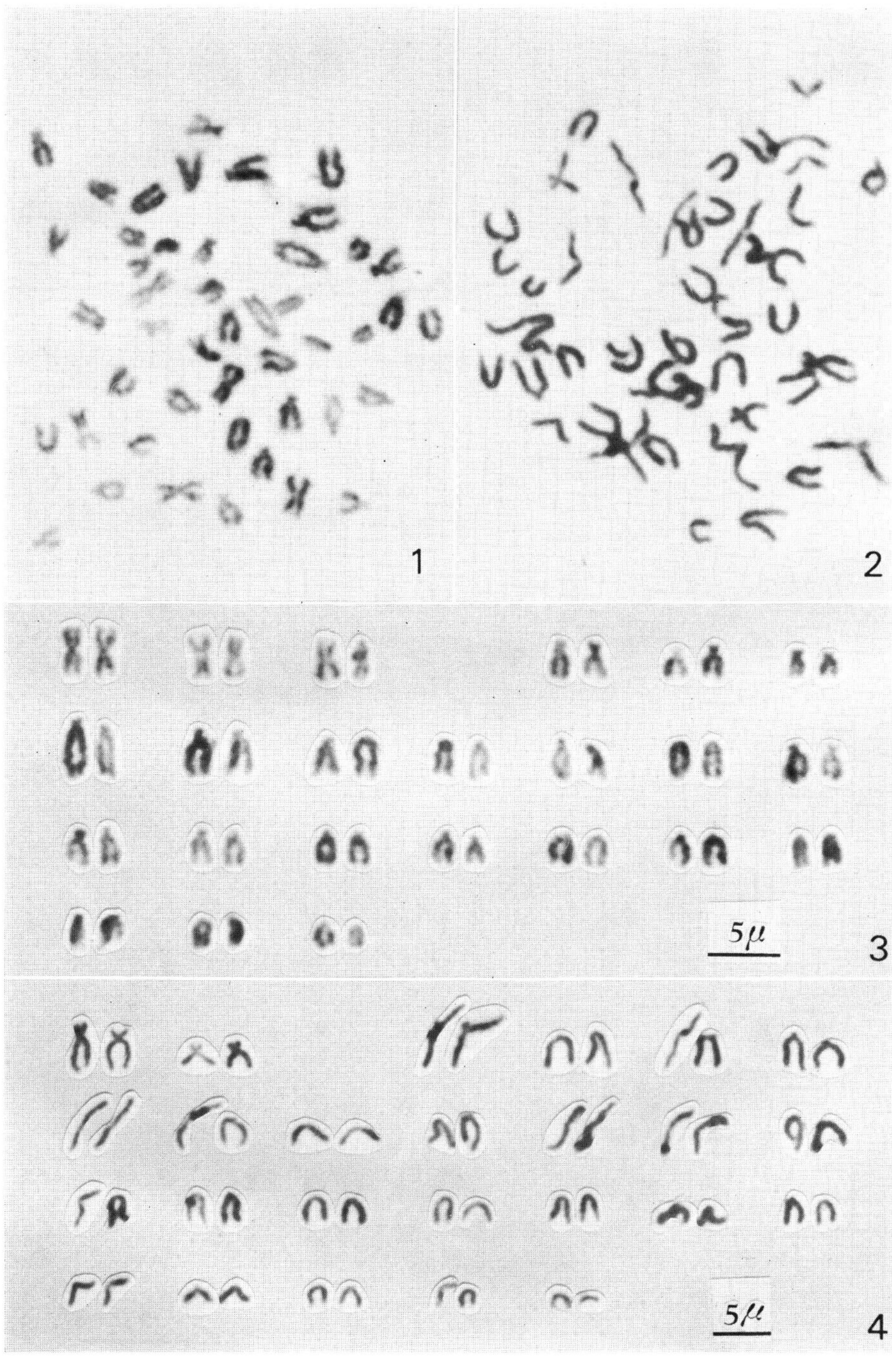
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