Karyological and Osteological Approach to Phylogenetic Systematics of Tetraodontiform Fishes

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Introduction

Karyotypes of 25 species of tetraodontiform fishes have been reported in the world, i.e., 2 species of Triacanthidae (RISHI, 1973; CHOUDHURY *et al.*, 1982), 4 species of Balistidae (ARAI & NAGAIWA, 1976), 6 species of Monacanthidae (ARAI & NAGAIWA, 1976; MUROFUSHI & YOSIDA, 1979; MUROFUSHI *et al.*, 1980), a species of Ostraciidae (ARAI & NAGAIWA, 1976), 11 species of Tetraodontidae (HINEGARDNER & ROSEN, 1972; ARAI & KATSUYAMA, 1973; NATARAJAN & SUBRAHMANYAM, 1974; ARAI & NAGAIWA, 1976; CHOUDHURY *et al.*, 1982), and a species of Diodontidae (ARAI & NAGAIWA, 1976). Among 10 families of living tetraodontiform fishes, karyotypes of 4 families such as Triacanthodidae, Triodontidae, Molidae and Aracanidae, have not been reported.

Recently, I observed chromosomes of five tetraodontiform fishes, *Lactoria diaphana* (BLOCH et SCHNEIDER), *Ostracion immaculatus* TEMMINCK et SCHLEGEL of the family Ostraciidae and *Canthigaster coronata* (VAILLANT et SAUVAGE), *Fugu pardalis* (TEMMINCK et SCHLEGEL), *Fugu poecilonotus* (TEMMINCK et SCHLEGEL) of the family Tetraodontidae, and the results are here reported.

Some monographic studies on tetraodontiform osteology were recently published (MATSUURA, 1979; TYLER, 1980; TYLER & MATSUURA, 1981). Among them, MATSUURA (1979) examined 32 osteological characters of Balistoidei and discussed on the polarities. As additional character to MATSUURA's, structural morphology of the relation between the axial skeleton and pterygiophores is treated in this paper.

The present study aims at phylogenetic systematics of tetraodontiform fishes on the basis of karyotypes and morphological characters by a newly revised method which reflects evolution as much as possible. A phylogenetic classification follows the reconstruction of phylogenetic interrelationships.

The classification of material fishes follows that by TYLER (1980).

Methods

Karyology. Method of chromosome preparation is the same as that of ARAI (1973). Classification of chromosomes is adopted from LEVAN *et al.* (1964). Metacentrics and submetacentrics are described as two-arm chromosomes, and subtelo-

centrics and acrocentrics as one-arm chromosomes. The definition of the new arm number (NAN) is referred to Arai & Nagaiwa (1976). However, the definition of NAN in the centric fission should be revised, because NAN changes by whether minute chromosomes (MC) are derived from the same chromosome or not. When the number of MC is two and 2n is 50 in acanthopterygian fish karyotypes, NAN is defined as follows, NAN=2n−number of MC.

Osteology. For the osteological observations, some specimens were cleared and stained with Alizarin Red "S". Meristic characters were counted mainly on radiographs.

Phylogenetic systematics. Polarity of the structural morphology of the relation between the axial skeleton and pterygiophores was determined by the method of Maslin (1952), especially by his "principle of chrono- and morpho-clines" and "principle of divergence".

The systematic methodology proposed in this study is a revised method of "Wagner's groundplan/divergence method" (Wagner, 1961, 1969, 1980), and different from both "Hennigian cladistics" and "evolutionary taxonomy". The present method agrees with Hennigian cladistics in cladistic analysis, but differs from Hennigian cladistics in the following points: (1) both plesiomorphic and apomorphic character states are used for analysis of interrelationships, and (2) the number of sister groups is not always two, i.e., branching patterns are not always dichotomous (Hennig, 1966; Wiley, 1981). The present method also differs from evolutionary taxonomy in that the morphological gaps cannot be adopted for analysis of interrelationships, and that weighting of characters is abandoned (Mayr, 1969, 1974). The present method is similar to "character compatibility analysis" in the use of cladistics as an objective means of estimating evolutionary history, but differs from character compatibility analysis in the following points: (1) branching patterns are not always restricted to dichotomy, (2) weighting of characters is basically abandoned, and (3) characters need not be tested for compatibility (Le Quesne, 1969, 1972; Esta-brook, 1972; Meacham, 1981). Therefore, a systematic methodology from the viewpoint of evolutionary phylogenetics will be described in detail in the following lines.

Method of Reconstruction of Phylogenetic Interrelationships

In this paper, phylogenetic interrelationships are reconstructed by the following method.

1) Only characters whose polarities are determined can be adopted for the reconstruction of phylogenetic interrelationships.

2) Interrelationships in taxa under study are analyzed by the combinations of characters as many as possible. In Hennigian cladistic analysis, as shown in Fig. 1 (Felsenstein, 1981), the kinship between taxa can be changed by the order in which apomorphic characters are added to the cladogram. In order to avoid the possibility of above variation of interrelationships, all character states that form character combinations corresponding to taxa under study cannot be separated to be compared.
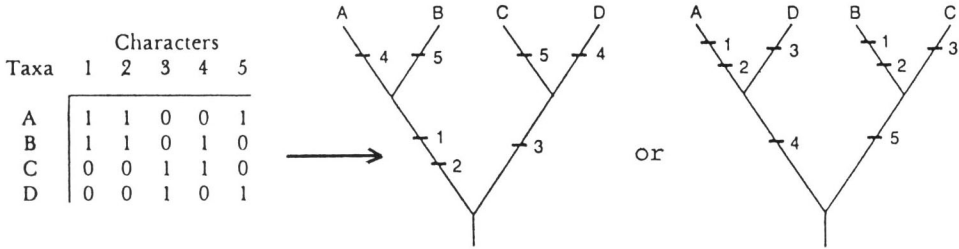
Fig. 1. Different phylogenies obtained from the data table, under different weightings of the characters. (After FELSENSTEIN, 1981.)

Ancestor-descendant relationship : Taxa I and II
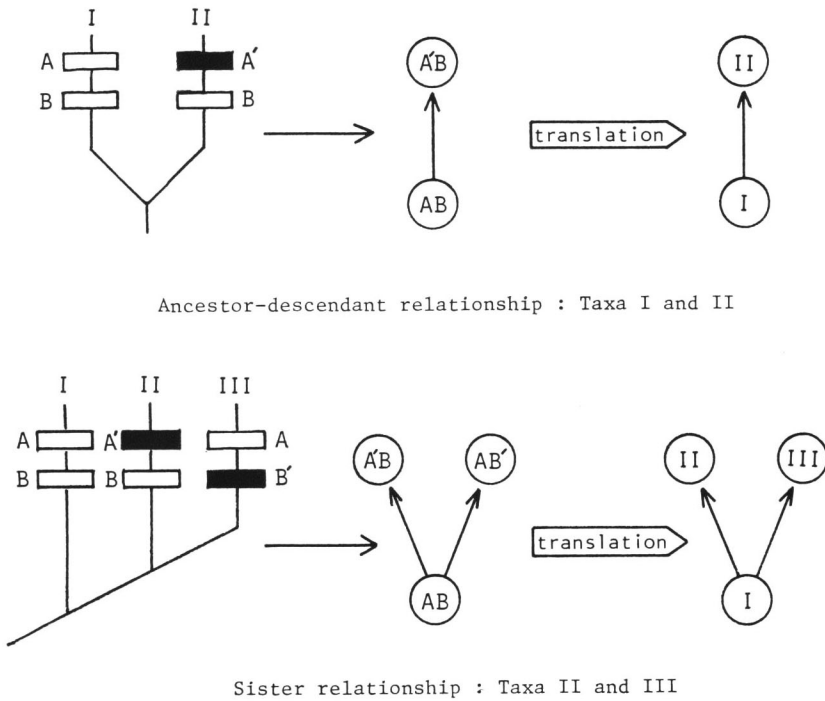

Sister relationship : Taxa II and III

Fig. 2. Ancestor-descendant relationships and sister group relationships.

3) All taxa under study are interpreted as derivatives of the character combinations (Fig. 2). If it is hypothesized that the concept of synapomorphy is applied to character combinations corresponding to taxa under study, synapomorphy defines both sister group relationships and ancestor-descendant relationships (ENGELMANN & WILEY, 1977; SZALAY, 1977; WILEY, 1981, pp. 105–107). That is, ancestor-descendant relationships cannot be excluded by only synapomorphy. Sister group relationships are discriminated from ancestor-descendant relationships by whether or not taxa under
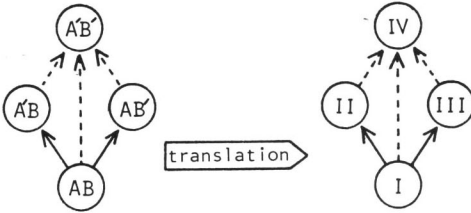
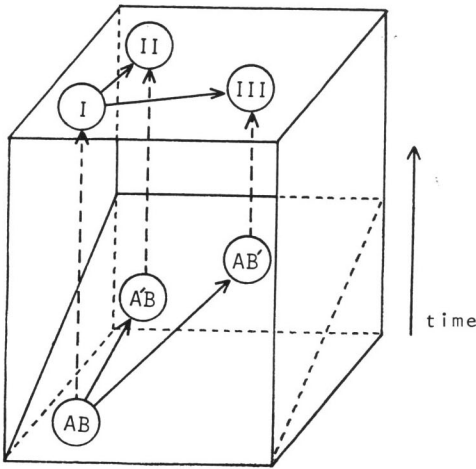Fig. 3. Interrelationships in 4 character combinations and 4 taxa.

Fig. 4. Anagenetic interrelationships in 3 character combinations and 3 taxa.

study have character combinations which include character states contradicted to each other (HENNIG, 1966, fig. 22).

4) Pathways in character combinations should be parsimonious. For example, we assume that there are 4 taxa (I, II, III and IV) comprising two characters whose polarity is determined. Each character is assumed to have two character states (A, A'; B, B'). Accordingly, each character combination belonging to Taxa I, II, III and IV is AB, A'B, AB' and A'B', respectively. As regards polarity of two characters, it is assumed that character state A' is derived from character state A and character state B' from character state B. Then, interrelationships of 4 character combinations can be schemed like in Fig. 3 in which arrows show the direction of differentiation.

In Fig. 3, ancestral character combination of A'B or AB' is AB, and that of A'B' is one of three character combinations, AB, A'B and AB'. Considering the principle of parsimony (KLUGE & FARRIS, 1969; WILEY, 1981; SOBER, 1983), the ancestral character combination of A'B' is A'B or AB'. However, it cannot be determined which of A'B and AB' could be ancestral to A'B'. In such case, systematic position of Taxon IV is left for a future study, until more useful characters will be found.

5) Anagenetic interrelationships in 3 taxa and 3 character combinations are shown in Fig. 4.

Method of Construction of Phylogenetic Classification

Many taxonomists have discussed on the relationship between phylogeny and classification. It is difficult to compare the relation between phylogeny and classification in cladistics with that in its opponents such as evolutionary taxonomy, because the definition of phylogeny or classification in cladistics is different from that in its opponents (Sokal & Camin, 1965; Mayr, 1974; Hennig, 1975; Farris, 1982).

In Hennigian cladists[1], kinships in taxa but not evolution are shown in the cladogram, and classification corresponds to phylogenetics, i.e., phylogenetic interrelationships can be translated automatically to classification (Hennig, 1966; Wiley, 1975).

In evolutionary taxonomy, classification is partly different from phylogeny. The raw data permit (1) the reconstruction of phylogeny and (2) the establishment of classification. Yet, neither is "phylogeny based on classification" nor "classification based on phylogeny". Classification and phylogeny are based on a study of "natural groups" found in nature, group having character combinations one would expect in the descendants of a common ancestor. Both phylogeny and classification are based on the same comparisons of organisms and their characteristics and on a careful evaluation of the established similarities and differences (Hull, 1967; Mayr, 1969).

At any rate, a classification reflects basically a hierarchy, which is arranged by gaps and needs limited kinds of categorical ranks, while polarity based on transformation series of character states is very important factor in phylogenetic interrelationships in both cladistics and evolutionary taxonomy. In other words, discontinuity is a philosophy for classification, while continuity for phylogenetic interrelationships (Mayr, 1969, pp. 229–230). In this sense, a classification based on phylogenetic interrelationships is contradictory in logic.

However, a classification is in need for practical purpose. Then, for the sake of convenience, a method of construction of the classification which reflects phylogenetic interrelationships as much as possible will be proposed here.

From the viewpoint of evolutionary phylogenetics, Wagner (1969, 1980) proposed the patristic distance which indicates the estimated amount of evolution, i.e., the grade or level, and suggested determination of categorical rank of taxa under study by patristic distance. However, his patristic distance as the barometre for ranking taxa cannot be adopted here, because no one knows whether the evolution rate of each character is the same or not. It seems to me that the most important key in ranking of taxa is the fact "when a monophyletic group under study has not its sister group, its categorical rank cannot be determined."

A newly revised method for ranking taxa above the species level is described below.

At first, "the stem character combination", which may correspond to ground-

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1) Excepting Hennigian cladists, there are many cladists who have studied the classification which reflects evolutionary history, e.g., Wagner, W. H. Jr. (1961, 1969), Wagner, W. (1962), Camin & Sokal (1965), Kluge & Farris (1969), Cichocki (1976), Estabrook *et al.* (1977), Duncan (1980), Meacham (1980) and others.
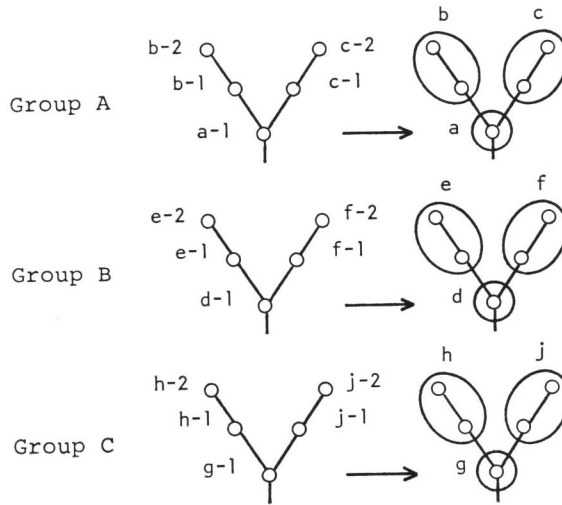
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Fig. 5.   Species and genera in Group A, Group B and Group C.

plan *sensu* WAGNER (1961, 1969), is here proposed.   The stem character combination is defined as the character combination corresponding to the stem taxon (HENNIG, 1966).

In the ancestor-descendant relationships (the straight-line evolution[2]), a categorical rank of each taxon forming a monophyletic group is the same.

In the sister group relationships (the branching evolution[2]), each of sister groups can be grouped into a higher rank of category than that of any taxon in a sister group. In this case, all taxa corresponding to character combinations derived directly from the stem character combination are considered to belong to the same rank of category. A taxon or taxa corresponding to the stem character combination can be classified into the same categorical rank as that of sister groups, annotated with "stem".   Accordingly, the number of taxa being ranked to one step higher category in a phylogenetic classification of a monophyletic group is the number of branches *plus* one, which corresponds to a group comprising stem taxa or a stem taxon.

For explanation, we assume that there are three monophyletic groups (Groups A, B and C), each of which consists of 5 species and 3 genera (Fig. 5).

Group A   Stem genus a (Species: a-1)
                    Genus b (Species: b-1, b-2)
                    Genus c (Species: c-1, c-2)
Group B   Stem genus d (Species: d-1)
                    Genus e (Species: e-1, e-2)
                    Genus f (Species: f-1, f-2)
Group C   Stem genus g (Species: g-1)

2) Concepts of the straight-line evolution and branching evolution based on interrelationships of character combinations are referred to WAGNER, W. (1962) and WAGNER, W. H., JR. (1969).

Genus h (Species: h-1, h-2)
Genus j (Species: j-1, j-2)

1) When Group A, Group B and Group C are ancestor-descendant relationships (Fig. 6), a categorical rank of each group cannot be determined. As a result, a phylogenetic classification is as follows.

Group A   Stem genus: a
            Genera: b, c
Group B   Stem genus: d
            Genera: e, f
Group C   Stem genus: g
            Genera: h, j

Fig. 6. Ancestor-descendant relationships.

2) When Group A, Group B and Group C are sister group relationships (Fig. 7), each of three groups can be classified into one step higher categorical rank than generic level. In this case, a phylogenetic classification is as follows.

Tribe A   Stem genus: a
            Genera: b, c
Tribe B   Stem genus: d
            Genera: e, f
Tribe C   Stem genus: g
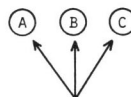   Genera: h, j

Fig. 7. Sister group relationships.

3) When Group A and Group B are sister group relationships, and Group B and Group C are ancestor-descendant relationships (Fig. 8), each of three groups can be classified into one step higher categorical rank than generic level. Moreover, a monophyletic group comprising Group B and Group C can be classified into one step higher categorical rank than that of Group B or Group C, i.e., subfamily level. Accordingly, Group A can be also classified into one step higher categorical rank than that of Group B or Group C.

As a result, a phylogenetic classification is as follows.

Subfamily X Tribe A Stem genus: a
                      Genera: b, c
Subfamily Y Tribe B Stem genus: d
                      Genera: e, f
             Tribe C Stem genus: g
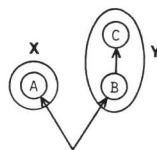   Genera: h, j

Fig. 8. Complex of ancestor-descendant relationships and sister group relationships.

Results and Discussion

Karyology

For the further discussion, chromosomes of 5 tetraodontiform fishes will be
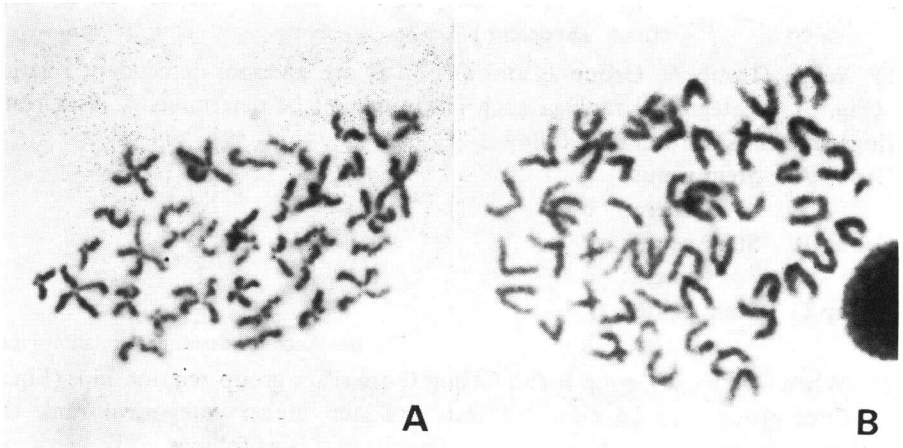
Fig. 9. Photomicrographs of mitotic metaphase chromosomes from gill epithelial cells of two ostraciid fishes. —— A, *Lactoria diaphana* (No. E·98·93), 2n=36, ×1,470; B, *Ostracion immaculatus* (No. E·98·75), 2n=50, ×1,770.

described here.   Chromosomes of 5 species, *Lactoria diaphana*, *Ostracion immaculatus*, *Canthigaster coronata*, *Fugu pardalis* and *Fugu poecilonotus*, have never been reported.

Lactoria diaphana (BLOCH et SCHNEIDER) "Umi-suzume" (Figs. 9A, 10A).   A specimen (No. E·98·93), 108.6 mm in total length, was caught at Shirahama, Waka-yama Prefecture.   Characters of material fish are shown in Table 1.

As shown in Table 2, the diploid chromosome number of this species is 36.   The karyotype comprises 4 pairs of larger metacentric, one pair of smaller metacentric, one pair of submetacentric and 12 pairs of subtelocentric-acrocentric chromosomes. All the larger metacentrics are approximately two times longer in size than any other chromosome, and hence they seem to have been produced by centric fusion.   The arm number is 48.   The new arm number is at least 44.

Ostracion immaculatus TEMMINCK et SCHLEGEL "Hako-fugu" (Figs. 9B, 10B).   A specimen (No. E·98·75), 108.5 mm in total length, was collected from the same locality as that of *Lactoria diaphana* (Table 1).

The diploid chromosome number is 50 (Table 2).   The karyotype comprises 2 pairs of submetacentric and 23 pairs of subtelocentric-acrocentric chromosomes. The arm number is 54.   Among acrocentric chromosomes, there are two minute chromosomes, which are smaller than half the size of any other chromosome and hence may have been produced by centric fission.   The new arm number is 48.

The karyotype of this species agrees well with that of *Ostracion cubicus* LINNAEUS from Ishigaki Island, Ryukyus and from Yakushima Island, off southern Kyushu (ARAI & NAGAIWA, 1976), but differs from that of *Lactoria diaphana* in both 2n and NF.

Canthigaster coronata (VAILLANT et SAUVAGE) "Hana-kinchaku-fugu" (Figs. 11). Two specimens (Nos. E·98·60 and E·98·71), 64.0 and 59.6 mm in total length, were

Table 1.   Characters of 5 species of material fishes.

Species	No. of fish	S.L. (mm)	Dorsal	Anal	Vertebrae
Ostraciidae					
Lactoria diaphana	1	90.5	9	9	18
Ostracion immaculatus	1	86.8	9	9	18
Tetraodontidae					
Canthigaster coronata	2	47.4–50.4	9–11	9	17
Fugu pardalis	2	61.6–95.8	12	10–11	23
F. poecilonotus	2	50.7–62.7	13	11	21

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

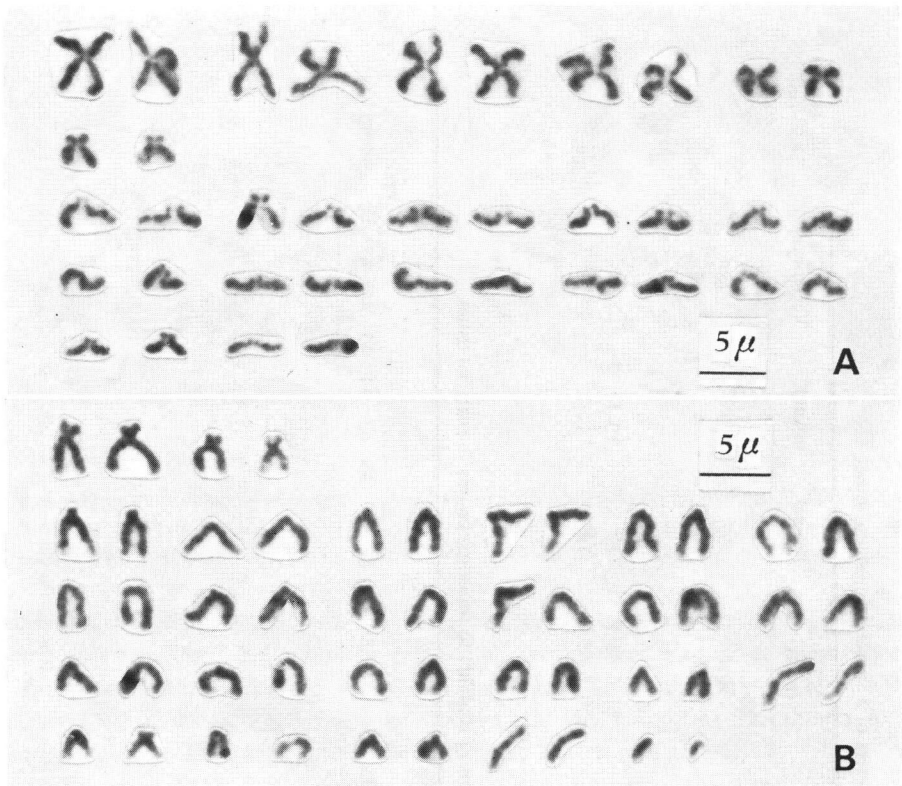| Species	2n																									Total
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	
Ostraciidae																									
Lactoria diaphana							1		7	1															9
Ostracion immaculatus																					1	4	6	1	12
Tetraodontidae																									
Canthigaster coronata	5	1	1																						7
Fugu pardalis														2	1	3	4								10
F. poecilonotus													1	2	4	4	9	1							21

Fig. 10.   Karyotypes of two species of trunkfishes. —— A, *Lactoria diaphana*, from Fig. 9A,
NF=48, ×1,780; B, *Ostracion immaculatus*, from Fig. 9B, NF=54, ×1,930.

caught at Shirahama, Wakayama Prefecture (Table 1).

As shown in Table 2, the diploid chromosome number is 28.   The karyotype of
this species comprises 3 pairs of metacentric, one pair of submetacentric, 10 pairs of
subtelocentric-acrocentric chromosomes.   The arm number is 36.   This diploid
chromosome number is the smallest in tetraodontiform fishes whose karyotypes have
been reported.   The karyotype of this species is very different from that of *Canthi-
gaster rivulata*, i.e., 2n and NF of *C. rivulata* is 34 and 44, while 28 and 36 in *C. coronata*
(ARAI & NAGAIWA, 1976).

Fugu pardalis (TEMMINCK et SCHLEGEL) "Higan-fugu" and *Fugu poecilonotus*
(TEMMINCK et SCHLEGEL) "Komon-fugu".   Two specimens of *Fugu pardalis* (Nos.
E·98·67 and A·13·45), 78.8 and 122.2 mm in total length, and two specimens of *F.
poecilonotus* (Nos. E·98·28 and E·98·29), 78.0 and 60.4 mm in total length, were
used for chromosome observation.   Three specimens, Nos. E·98·28, E·98·29 and
E·98·67, were caught at Shirahama, Wakayama Prefecture, and a specimen, No.
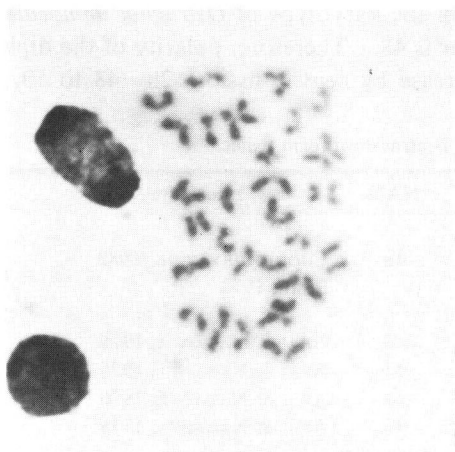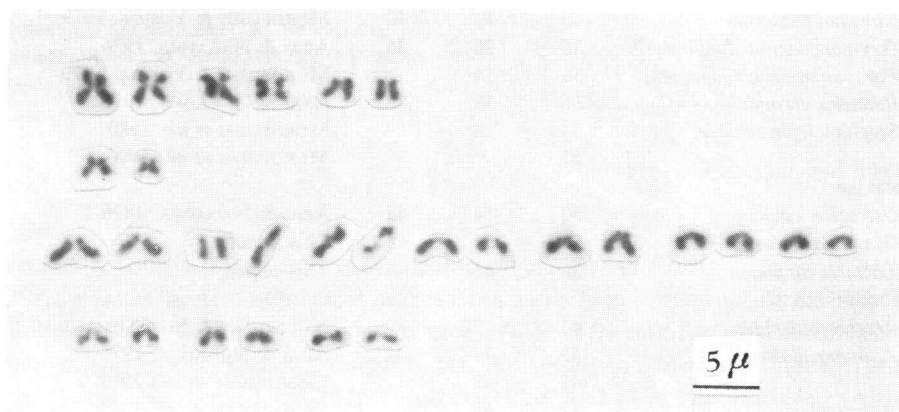A·13·45, at Nabeta Bay, Shimoda, Shizuoka Prefecture (Table 1).

Fig. 11. A photomicrograph of mitotic meta-phase chromosomes (above) and the karyo-type (below) from a gill epithelial cell of *Canthigaster coronata* (No. E·98·71), 2n= 28, NF=36, × 1,970.

The diploid chromosome number of *Fugu pardalis* and *F. poecilonotus* is 44 (Table 2). As clear chromosome figures of two species could not be obtained, their arm number and new arm number could not be described here.

The diploid chromosome number of the present two species is the same as that of *Fugu chrysops* and *F. niphobles* (ARAI & KATSUYAMA, 1973; ARAI & NAGAIWA, 1976).

Chromosomes of Tetraodontiform Fishes

As shown in Table 3, the diploid chromosome number ranges from 28 to 50 in tetraodontiform fishes. In the family level, it is 48 in Triacanthidae and 46 in Diodontidae, and ranges from 44 to 46 in Balistidae, from 33 to 40 in Monacanthidae, from 36 to 50 in Ostraciidae, and from 28 to 44 in Tetraodontidae. The diploid chro-mosome number of two species of *Ostracion* is 50, which is the largest number in tetrao-

dontiform fishes. However, as described on the karyotype of *Ostracion immaculatus* in the foregoing section, its new arm number is 48. Therefore, polarity of the diploid chromosome number is as follows, (1) increase by centric fission, $2n=48$ to 50, (2)

Table 3.　Chromosomes of tetraodontiform fishes.

Species	2n	NF	NAN	Literature
Triacanthidae				
Triacanthus brevirostris	48	49	48	CHOUDHURY *et al.*, 1982
T. strigilifer	48			RISHI, 1973
Balistidae				
Pseudobalistes flavimarginatus	44	46	46	ARAI & NAGAIWA, 1976
Rhinecanthus aculeatus	44	44	44	ARAI & NAGAIWA, 1976
R. verrucosus	44	44	44	ARAI & NAGAIWA, 1976
*Sufflamen chrysopterus	46	46	46	ARAI & NAGAIWA, 1976
Monacanthidae				
**Cantherhines pardalis	40	40	40	ARAI & NAGAIWA, 1976
Navodon modestus	40	40	40	MUROFUSHI & YOSIDA, 1979
Oxymonacanthus longirostris	36	36	36	ARAI & NAGAIWA, 1976
Paramonacanthus japonicus	34	34		MUROFUSHI & YOSIDA, 1979
Rudarius ercodes	36	36	36	ARAI & NAGAIWA, 1976
Stephanolepis cirrhifer ♀	34	34		MUROFUSHI *et al.*, 1980
♂	33			MUROFUSHI *et al.*, 1980
Ostraciidae				
**Ostracion cubicus	50	54	48	ARAI & NAGAIWA, 1976
O. immaculatus	50	54	48	This paper
Lactoria diaphana	36	48	44?	This paper
Tetraodontidae				
Arothron hispidus	42	*ca.* 78		NATARAJAN & SUBRAHMANJAM, 1974
*A. immaculatus	42	72	42	ARAI & NAGAIWA, 1976
	42	68		CHOUDHURY *et al.*, 1982
A. reticularis	42	68		CHOUDHURY *et al.*, 1982
A. leopardus	40	68		CHOUDHURY *et al.*, 1982
*A. nigropunctatus	38	72	40	ARAI & NAGAIWA, 1976
Canthigaster coronata	28	36		This paper
C. rivulata	34	44	34?	ARAI & NAGAIWA, 1976
Chelonodon patoca	40	70	42	ARAI & NAGAIWA, 1976
*Fugu chrysops	44	64	46	ARAI & NAGAIWA, 1976
F. niphobles	44	64	46	ARAI & KATSUYAMA, 1973
F. pardalis	44			This paper
F. poecilonotus	44			This paper
*Lagocephalus lunaris	44	68	46	CHOUDHURY *et al.*, 1982
*Monotreta palembangensis	42			HINEGARDNER & ROSEN, 1972
Diodontidae				
Diodon bleekeri	46	58	46	ARAI & NAGAIWA, 1976

　* Genera *Sufflamen, Arothron, Fugu, Lagocephalus* and *Monotreta* were reported as *Hemibalistes, Tetraodon, Sphoeroides, Gastrophysus* and *Tetraodon*, respectively.

** *Cantherhines pardalis* and *Ostracion cubicus* were reported under the names of *Amanses sandwichiensis* and *Ostracion tuberculatus*, respectively.
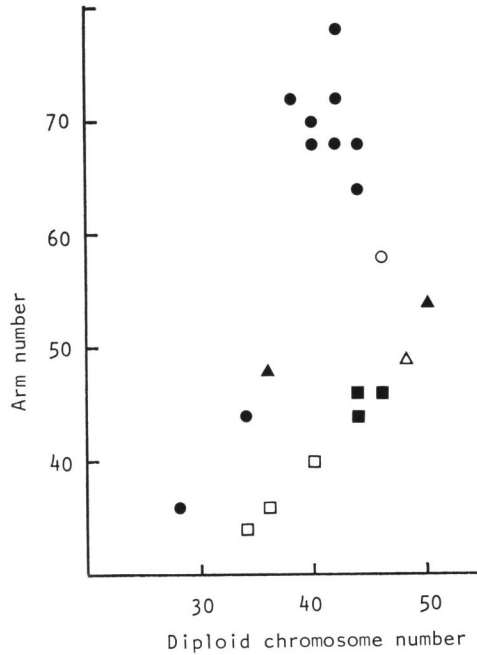
Fig. 12. Chromosomes in tetraodontiform fishes. Data from Table 3. ●, Tetraodontidae; ○, Diodontidae; ▲, Ostraciidae; △, Triacanthidae; ■, Balistidae; □, Monacanthidae.

(Figure 12: scatter plot with y-axis "Arm number" marked 40, 50, 60, 70 and x-axis "Diploid chromosome number" marked 30, 40, 50.)

decrease by centric fusion or loss of chromosomes, $2n = 48$ to 28.

As regards the arm number, it ranges from 34 to 78 in tetraodontiform fishes. The relation between $2n$ and NF is shown in Fig. 12. The number of two-arm chromosomes ranges from 0 to 2 in Triacanthidae, Balistidae and Monacanthidae, from 4 to 12 in Ostraciidae, from 10 to 36 in Tetraodontidae, and 12 in Diodontidae. Although karyological data are not enough to analyze interrelationships of tetraodontiform fishes, Fig. 12 seems to show that the relation between $2n$ and NF is characteristic to each family.

Notes. As regards chromosomes of porcupine fishes, $2n$ of *Diodon bleekeri* is more numerous than that of any species of puffers whose chromosomes have hitherto been reported, although NAN of both the porcupine fish and puffers with $2n = 44$ is the same, i.e., NAN $= 46$. DNA content of a porcupine fish, *Chilomycterus schoepfii*, is about two times of those of 4 species of puffers, *Sphoeroides maculatus*, *S. nephelus*, *Tetraodon palembangensis* and *T. fluviatilis* (HINEGARDNER & ROSEN, 1972).

On the other hand, osteological characters suggest that porcupine fishes are derived from puffers (see p. 188 in the text). Similar cases seem to be found in the relation between wrasses and parrotfishes (HINEGARDNER & ROSEN, 1972; ARAI & KOIKE, 1980). These facts suggest that $2n = 46$ of the porcupine fish has been produced from chromosomes of puffers by the duplication of DNA content without polyploidization of chromosome number.

Structural Morphology of the Relation between the Axial
Skeleton and Pterygiophores

MATSUURA (1979) and TYLER (1980) studied tetraodontiform osteology and determined the polarity of character states of many bones. As regards the relation between the vertebral column and pterygiophores, they did not mention on its phylogenetic significance. As shown in Table 4, the number of vertebrae anterior to the first pterygiophore of the soft dorsal (VN-pre-soft-dorsal) is very conservative, i.e., VN-pre-soft-dorsal is 8 in Triacanthodidae, 5 and 4 in Triacanthidae, 5 in Balistidae, 5 and 4 (exceptionally 6 or 8) in Monacanthidae, 7 (exceptionally 8) in Aracanidae, Ostraciidae, Triodontidae and Tetraodontidae, 12 in Diodontidae, and 4 in Molidae. As regards fossil fishes, it is 6 in *Protoacanthodes ombonii* and *Cryptobalistes brevis*. Eight, seven, five and four in VN-pre-soft-dorsal compose transformation series.

Above data suggest that VN-pre-soft-dorsal has phylogenetic significance. Therefore, its polarity was checked by the method of MASLIN (1952). Both TYLER (1980) and MATSUURA (1979) considered that each of Balistidae and Triacanthidae is derived from Triacanthodidae or its ancestor. Fossil species such as *Protoacanthodes ombonii* and *Cryptobalistes brevis* are considered to be the links between Triacanthodidae and Triacanthidae or Balistidae (TYLER, 1980). When two hypotheses described above are accepted, the polarity of character states of VN-pre-soft-dorsal is from 8 to 4, i.e., 8 is the most primitive and 4 is the most advanced character state.

On the other hand, VN-pre-soft-dorsal of Diodontidae is more numerous than that of both Triacanthodidae and Tetraodontidae.

However, the character state in Diodontidae cannot be considered to be more primitive than that of Triacanthodidae or Tetraodontidae by the following reasons. Diodontidae is more specialized than Triacanthodidae in such characters as (1) absence of the spinous dorsal and the pelvis, (2) jaw teeth fused into two plates, (3) posession of 3 pairs of pharyngobranchials, and (4) absence of the posttemporal, the basihyal, the urohyal and the interhyal. Moreover, the diploid chromosome number of Triacanthidae is 48, while that of Diodontidae is 46. From comparative karyology, this means that Diodontidae is not so primitive as Triacanthidae, which is considered as derivatives from Triacanthodidae.

Diodontidae is also more specialized than Tetraodontidae in the following characters, (1) jaw teeth fused into two plates, (2) absence of the interhyal, (3) number of the postcleithrum being one, (4) 9 caudal fin rays, (5) the fused parhypural, and (6) number of the hypurals being one. The diploid chromosome number of Diodontidae is larger than that of Tetraodontidae whose karyotypes have been reported. However, 2n of Tetraodontidae cannot be compared with 2n of Diodontidae because chromosomes of the porcupine fish may have been produced by duplication of DNA content.

Such facts as discussed above are found in two species of Monacanthidae and *Chonerhinos*, *Fugu* and *Monotreta* in Tetraodontidae. Each of VN-pre-soft-dorsal of two monacanthids, *Pseudalutarius nasicornis* and *Psilocephalus barbatus*, is 6 and

Table 4. Numbers of vertebrae anterior to the first pterygiophore of
the soft dorsal fin (VN-pre-soft-dorsal), total vertebrae (VN),
abdominal vertebrae (AV) and caudal vertebrae (CV) in tetraodontiform fishes.

Species	VN-pre-soft-dorsal	VN=AV+CV
Triacanthodidae		
†*Protobalistum imperiale*	8?	?
†*Spinacanthus cuneiformis*	8?	20= ?+?
†*Eoplectus bloti*	8	20= 9+11
Atrophacanthus japonicus	8	20= 8+12
Halimochirurgus centricoides	8	20= 8+12
Johnsonina eriomma	8	20= 8+12
Macrorhamphosodes uradoi	8	20= 8+12
Parahollardia lineata	8	20= 8+12
Paratriacanthodes retrospinis	8	20= 8+12
Triacanthodes anomalus	8	20= 8+12
Tydemania navigatoris	8	20= 8+12
Triacanthidae		
†*Cryptobalistes brevis*	6	20= 8+12?
†*Protoacanthodes ombonii*	6	20= 8+12?
Pseudotriacanthus strigilifer	4	20= 8+12
Triacanthus biaculeatus	5	20= 8+12
Tripodichthys augustifrons	5	20= 8+12
Trixiphichthys weberi	4	20= 8+12
Balistidae		
Balistapus undulatus	5	18= 7+11
Balistes polylepis	5	18= 7+11
Canthidermis maculatus	5	18= 7+11
Rhinecanthus rectangulus	5	18= 7+11
Sufflamen frenatus	5	18= 7+11
Xanthichthys lineopunctatus	5	18= 7+11
Monacanthidae		
Acanthaluteres spilomelanurus	4	20= 6+14
Alutera heudelotti	5	20= 7+13
A. monoceros	4	20= 7+13
A. scripta	5	20–23
Amanses scopas	5	19= 7+12
Brachaluteres trossulus	5	20= 7+13
B. ulvarum	5	20= 7+13
Cantherhines pardalis	5	19= 7+12
C. sandwichiensis	5	19= 7+12
Chaetoderma penicilligera	5	20= 7+13
C. spinosissimus	5	20= 7+13
Meuschenia trachylepis	4	20= 7+13
Monacanthus chinensis	5	19= 7+12
M. ciliatus	4	19= 6+13
Navodon modestus	5	19= 7+12
Oxymonacanthus longirostris	5	26= 8+18
Paraluterus prionurus	5	20= 7+13
Paramonacanthus cryptodon	5	19= 7+12

Table 4   (continued).

Species	VN-pre-soft-dorsal	VN=AV+CV
P. japonicus	5	19= 7+12
Pervagor spilosomus	5	19= 7+12
Pseudaluteres nasicornis	6	27= 8+19
Pseudomonacanthus peroni	5	19= 7+12
Psilocephalus barbatus	8	29= 7+22
Rudarius ercodes	5	20= 7+13
R. minutus	4	19= 7+12
Stephanolepis hispidus	5	19= 7+12
S. cirrhifer	5	19= 7+12
Aracanidae		
Aracana aurita	7	18*
A. ornata	7 or 6	18*
Kentrocapros rosapinto	7	18*
K. aculeatus	7	18*
Strophiurichthys robustus	7	18*
Ostraciidae		
Acanthostracion quadricornis	7	19*
Lactoria cornuta	7	18*
Ostracion cubicus	7	18*
Rhinesomus triqueter	7	18*
Tetrosomus gibbosus	7	18*
Triodontidae		
Triodon macropterus	7	20= 9+11
Molidae		
Mastrus lanceolatus	4	16**
Mola mola	4	17**
Ranzania laevis	4	18**
Tetraodontidae		
Amblyrhynchotes honckenii	7	19= 8+11
A. piosae	7	19= 8+11
Arothron armilla	7	19= 8+11
Arothron immaculatus	7	18= 8+10
A. nigropunctatus	7	18= 9+ 9
A. stellatus	7	18= 8+10
Carinotetraodon lorteti	7	17= 7+10
Chelonodon fluviatilis	7	18= 8+10
C. patoca	7	19= 8+11
Canthigaster amboinensis	7	17= 8+ 9
C. coronata	7	17= 8+ 9
C. rivulata	7	17= 8+ 9
C. rostrata	7	17= 8+ 9
C. valentini	7	17= 8+ 9
Chonerhinos modestus	8	26=10+16
Colomesus psittacus	7	19= 8+11
Contusus richei	7	18= 8+10
Ephippion guttifer	7	20= 8+12
Fugu chrysops	8	21= 9+12

Table 4 (continued).

Species	VN-pre-soft-dorsal	VN=AV+CV
F. niphobles	7	21= 8+13
F. pardalis	9	23=10+13
F. poecilonotus	7	21= 8+13
F. vermicularis	7	22= 8+14
Guentheridia formosa	7	17= 8+ 9
Lagocephalus laevigatus	7	19= 8+11
L. lagocephalus oceanicus	7	18= 8+10
L. spadiceus	7	19= 8+11
L. scleratus	7	17= 8+ 9
Monotreta leiurus	8	21=10+11
Pelagocephalus coheni	7	17= 8+ 9
Sphoeroides maculatus	7	19= 8+11
S. pachygaster	7	18= 8+10
Tetraodon mbu	7	18= 8+10
Torquigener hamiltoni	7	19= 8+11
T. pleurostictus	7	19= 8+11
Xenopterus naritus	7	29=10+19
Diodontidae		
Chilomycterus schoepfi	12	20=12+ 8
Diodon holocanthus	12	21=12+ 9

† Fossil species.

* Total vertebrae cannot be divided into abdominal vertebrae and caudal vertebrae.

** As the genuine caudal fin is absent in Molidae, the numbers of caudal vertebrae and total vertebrae cannot be compared with those of species in other families.

8, respectively. Their VN-pre-soft-dorsal are more numerous than those in most species of Monacanthidae. However, morphological characters of *P. nasicornis* and *P. barbatus* are not so primitive as those of most monacanthid fishes.

As regards *Fugu*-species, VN-pre-soft-dorsal correlates strongly with abdominal vertebral number, i.e., VN-pre-soft-dorsal of species with 8 abdominal vertebrae is 7, while that of species with more than 8 abdominal vertebrae is 8 or more. Since species with VN-pre-soft-dorsal exceeding 7 are not so primitive as species belonging to *Sphoeroides* whose VN-pre-soft-dorsal is 7, VN-pre-soft-dorsal exceeding 7 is considered to be derived character state.

From above discussion, it seems to be reasonable that character states of VN-pre-soft-dorsal are bipolar, i.e., (1) decrease from 8 to 4, and (2) increase from 7 to 12 in Tetraodontidae and Diodontidae, and from 5 to 8 in Monacanthidae. Polarity in Tetraodontidae, Diodontidae and Monacanthidae is caused by the increase of VN-pre-soft-dorsal accompanied with the increase of the number of abdominal vertebrae.

Phylogenetic Systematics

TYLER (1980) studied phylogeny of tetraodontiform fishes on the basis of osteolo-

gical characters. He grouped each family by conventional method. MATSUURA (1979) tried to reconstruct the phylogenetic interrelationships of Balistoidea by cladistic analysis. However, in his classification of Balistidae and Monacanthidae, he did not use HENNIG's method analyzing sister group relationships (HENNIG, 1966, p. 91, fig. 22). Sister groups need to have character combinations which include character states contradicted to each other. If MATSUURA followed HENNIG's method, Balistidae and Monacanthidae should be sister groups. Actually, Balistidae is ancestral to Monacanthidae in all characters that MATSUURA observed.

In the following lines, phylogenetic interrelationships of tetraodontiform fishes will be reconstructed and then, their phylogenetic classification will be constructed.

Tetraodontiformes. TYLER (1980) reported that living 10 groups classified in family level are monophyletic. However, as their phylogenetic interrelationships are obscure, interrelationships among these 10 groups are required to be studied here.

Tetraodontiform osteological data except those examined by me are adopted from MATSUURA (1979), TYLER (1980) and TYLER & MATSUURA (1981).

Polarities of karyotypes and the structural morphology of the relation between the axial skeleton and pterygiophores were determined in this work. Excepting bipolar characters whose polarities were estimated here, polarities of other characters follow those by previous works (MATSUURA, 1979; TYLER, 1980).

For analysis of phylogenetic interrelationships, following 17 characters are used. Polarity of each character is from character state a to character state e, i.e., $a \rightarrow b \rightarrow c \rightarrow d \rightarrow e$. There are two polarities in Character 17, i.e., $a \rightarrow b \rightarrow c$ and $b \rightarrow x$.

Skull

(1) Mesopterygoid. a: present. b: far more reduced than state a or none.

Jaws

(2) Teeth. a: separated. b: fused into 4 plates. c: fused into 3. d: fused into 2.

Hyoid apparatus

(3) Urohyal. a: present. b: absent. (4) Basihyal. a: present. b: absent.
(5) Interhyal. a: present. b: absent.

Branchial arches

(6) Number of pharyngobranchials. a: 4. b: 3 or 2.

Pectoral girdle

(7) Posttemporal. a: present. b: absent. (8) Postcleithrum. a: two bones. b: one bone. (9) Number of actinosts. a: 4. b: 3.

Pelvic fin and girdle

(10) Pelvis. a: paired. b: unpaired. c: none. (11) Fin rays. a: present. b: absent.

Dorsal fin

(12) Number of spinous dorsal fin rays. a: 6. b: 1–3. c: none.

Caudal fin and skeletons

(13) Number of fin rays. a: 12. b: 11. c: 10. d: 9. e: none.

(14) Parhypural. a: autogenous. b: fused. c: none. (15) Epural. a: free.

b: fused. *c*: none. (16) Number of hypurals. *a*: 5. *b*: 4. *c*: 2 (a free hypural and a fused hypural plate or the upper and the lower hypural plates). *d*: 1 (a fused hypural plate). *e*: none.

Vertebrae

(17) Number of vertebrae anterior to the first pterygiophore of the soft dorsal fin. *a*: 8. *b*: 7. *c*: 5–4. *x*: 12.

Character states of 17 characters in 10 families in the sense of TYLER (1980) are shown in Table 5. Interrelationships in 10 character combinations (Types A to J) are shown in Fig. 13. Phylogenetic interrelationships of 10 groups are translated from interrelationships in 10 character combinations corresponding to 10 groups (Fig. 14). Among 17 characters under study, the number of derived characters is none in Type A, 5 in Type B, 7 in Types E and C, 8 in Type G, 9 in Type D, 10 in Type H, 11 in Type F, 14 in Type J, and 15 in Type I.

Figures 13 and 14 suggest following points, (1) Type A is the most primitive, (2) from Type A, each of Types B, C, D, E and F is differentiated independently, i.e., Type B has sister relationships with Types C, D, E and F in Characters 1 and 8, Type C with Types D, E and F in Characters 2, 6, 10 and 17, Type D with Types E and F in Characters 2, 14 and 15, Type E with Type F in Characters 2, 3 and 13, (3) Type C is ancestral to Type G in Character 8, (4) it is unknown whether or not Type C and Type G are classified into the same categorical rank, because Type C and Type G are ancestor-descendant relationships, (5) Type D is ancestral to Type H in Characters 6 and 13, (6) it is unknown whether or not Type D and Type H are classified into the same categorical rank, because Type D and Type H are ancestor-descendant relationships, (7) Type F is ancestral to Type J in Characters 2, 5, 8, 13, 14, 16 and 17, and (8) it is unknown whether or not Type F and Type J are classified into the same categorical rank, because Type F and Type J are ancestor-descendant relationships.

The relation among Types E, I and F is difficult to be analyzed. Type I can be derived from both Type E and Type F. By 17 characters under study, it cannot be determined which of Type E and Type F could be ancestral to Type I.

Table 5. Character states of 17 characters in tetraodontiform fishes.

Group No.	Family (TYLER, 1980)	Character No.																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Triacanthodidae	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
2	Triacanthidae	b	a	a	b	a	a	a	b	a	a	a	a	a	a	a	c	c
3	Balistidae	a	a	a	b	a	b	a	a	a	b	b	b	a	a	a	c	c
4	Monacanthidae	a	a	a	b	a	b	a	b	a	b	b	b	a	a	a	c	c
5	Aracanidae	a	a	a	b	a	a	a	a	a	c	b	c	b	b	b	d	b
6	Ostraciidae	a	a	a	b	a	b	a	a	a	c	b	c	c	b	b	d	b
7	Triodontidae	a	c	a	b	a	a	b	a	a	a	b	b	a	a	a	b	b
8	Molidae	a	d	b	b	a	b	b	b	b	c	b	c	e	c	c	e	c
9	Tetraodontidae	a	b	b	b	a	b	b	a	a	c	b	c	b	a	a	c	b
10	Diodontidae	a	d	b	b	b	b	b	b	a	c	b	c	d	b	a	d	x

Accordingly, the systematic position of Group 8 (Molidae in the sense of TYLER, 1980) is left for a future study, until more useful characters will be found.

Analysis of karyotypes does not conflict with the present phylogenetic interrela-
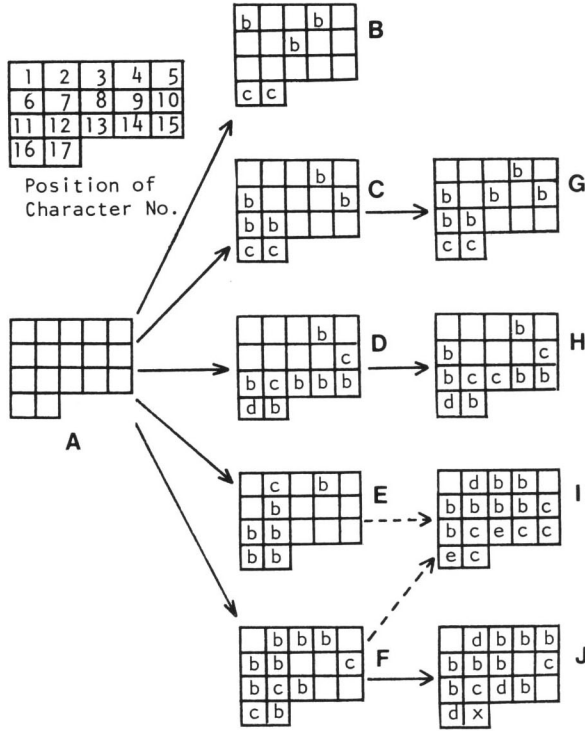

Fig. 13. Ten types of character combinations based on 17 characters of 10 groups of tetraodonti-form fishes (Types A to J). Character No. corresponds to that of Table 5. Blank squares mean character state *a*.
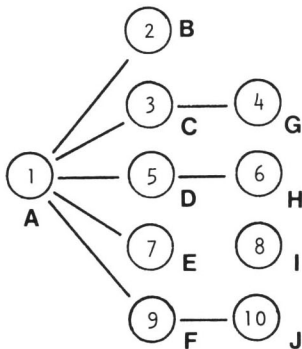

Fig. 14. Interrelationships of 10 types of character combinations (A to J) and 10 groups in tetraodontiform fishes. Figures in circles correspond to Group No. in Table 5.

tionships excepting those between Group 9 and Group 10. As regards this case, chromosomes of the porcupine fish may have been produced from those of puffers by the duplication of DNA content (see p. 187 in the text).

A phylogenetic classification of living tetraodontiform fishes based on phylogenetic interrelationships is as follows.

Order Tetraodontiformes
    Stem family Triacanthodidae
        Family Triacanthidae
        Family Balistidae (including Monacanthidae)
        Family Ostraciidae (including Aracanidae)
        Family Triodontidae
        Family Tetraodontidae (including Diodontidae)
        Taxon *sedis incertae*: a monophyletic group corresponding to Molidae in the sense of TYLER (1980)

Notes. It is not checked whether or not tetraodontiform fishes are ranked as order level. However, following TYLER (1980), they are ranked *a priori* as order level in the present paper. Family Triacanthidae is more closely related to the stem family Triacanthodidae than any of the other 4 families. This may suggest that Triacanthidae is the satellite branch of the stem family. When this hypothesis is adopted, the present classification is more similar to that of WINTERBOTTOM (1974) than that of TYLER (1980).

Interrelationships between Groups 3 and 4, Groups 5 and 6 or Groups 9 and 10 will be more closely examined in the forthcoming lines.

Balistidae (Group 3 and Group 4). Following 12 characters are used for inter-relationship analysis. The source of osteological data and polarities of characters are the same as those in tetraodontiform fishes, although one more character state in Characters 9 and 11 is added to that in MATSUURA (1979), i.e., 3rd character state of Character 9 is divided into two states, and 1st character state of Character 11 into two states. Direction of differentiation in Characters 1–8, 10 and 12 is from character state a to character state c. There are two polarities in Characters 9 and 11, i.e., $a{\rightarrow}b{\rightarrow}c{\rightarrow}e{\rightarrow}f$ and $b{\rightarrow}d{\rightarrow}e{\rightarrow}f$ in Character 9, and $a{\rightarrow}c$ and $b{\rightarrow}c$ in Character 11.

Cranium

(1) Ethmoid. a: not articulated with pterygiophore. b: articulated with pterygiophore.

Jaws

(2) Number of outer teeth on premaxillary. a: 8. b: 6. (3) Number of inner teeth on premaxillary. a: 6. b: 4. (4) Number of dentary teeth. a: 8. b: 6. c: 4.

Hyoid apparatus

(5) Number of branchiostegals. a: 6. b: 5. c: 4.

Branchial arches

(6) Number of basibranchials. a: 3. b: 2. (7) Number of pharyngobranchials. a: 3. b: 2.

Table 6. Character states of 12 characters in Balistidae
(Balistidae and Monacanthidae in the sense of TYLER, 1980).

Species		Character No.											
No.	Scientific name	1	2	3	4	5	6	7	8	9	10	11	12
	Balistidae of TYLER												
1	*Abalistes stellatus*	a	a	a	a	a	a	a	a	a	a	a	a
2	*Balistapus undulatus*	a	a	a	a	a	a	a	a	a	a	a	a
3	*Balistes capriscus*	a	a	a	a	a	a	a	a	a	a	a	a
4	*B. forcipatus*	a	a	a	a	a	a	a	a	a	a	a	a
5	*B. polylepis*	a	a	a	a	a	a	a	a	a	a	a	a
6	*B. vetula*	a	a	a	a	a	a	a	a	a	a	a	a
7	*Balistoides viridescens*	a	a	a	a	a	a	a	a	a	a	a	a
8	*Canthidermis maculatus*	a	a	a	a	a	a	a	b	a	a	a	a
9	*Melichthys niger*	a	a	a	a	a	a	a	a	a	a	a	a
10	*M. vidua*	a	a	a	a	a	a	a	a	a	a	a	a
11	*Odonus niger*	a	a	a	a	a	a	a	a	a	a	a	a
12	*Pseudobalistes flavimarginatus*	a	a	a	a	a	a	a	a	a	a	a	a
13	*P. fuscus*	a	a	a	a	a	a	a	a	a	a	a	a
14	*Rhinecanthus aculeatus*	a	a	a	a	a	a	a		a	a	a	a
15	*R. echarpe*	a	a	a	a	a	a	a	b	a	a	a	a
16	*R. verrucosus*	a	a	a	a	a	a	a	a	a	a	a	a
17	*Sufflamen bursa*	a	a	a	a	a	a	a	a	a	a	a	a
18	*S. chrysopterus*	a	a	a	a	a	a	a	a	a	a	a	a
19	*S. fraenatus*	a	a	a	a	a	a	a	b	a	a	a	a
20	*Xanthichthys mento*	a	a	a	a	a	a	a	a	a	a	a	a
	Monacanthidae of TYLER												
21	*Acreichthys hajam*	b	b	b	b	b	a	b	b	b	a	a	b
22	*Alutera heudelotii*	a	b	b	b	a	a	b	b	b	e	b	c	b
23	*A. monoceros*	a	b	b	b	a	a	b	b	b	e	b	c	b
24	*A. schoepfi*	a	b	b	b	a	a	b	b	b	e	b	c	b
25	*A. scripta*	a	b	b	b	a	a	b	b	b	e	b	c	b
26	*Amanses scopas*	b	b	b	b	a	a	b	b	b	b	b	a	b
27	*Arotrolepis filicaudus*	a	b	b	b	b	a	b	b	b	a	a	b
28	*Brachaluteres ulvarum*	a	b	b	b	b	a	b	b	f	b	a	c
29	*Cantherhines dumerili*	b	b	b	b	a	a	b	b	b	b	a	b
30	*C. pardalis*	b	b	b	b	a	a	b	b	b	b	a	b
31	*C. pullus*	b	b	b	b	a	a	b	b	b	b	a	b
32	*C. sandwichiensis*	b	b	b	b	a	a	b	b	b	b	a	b
33	*Chaetoderma penicilligera*	a	b	b	b	b	a	b	b	b	a	a	b
34	*Eubalichthys mosaicus*	a	b	b	b	a	a	b	b	d	b	b	b
35	*Meuschenia freycineti*	b	b	b	b	a	a	b	b	c	b	a	b
36	*M. hippocrepis*	b	b	b	b	a	a	b	b	c	b	a	b
37	*M. trachylepis*	b	b	b	b	a	a	b	b	c	b	a	b
38	*Monacanthus chinensis*	a	b	b	b	b	a	b	b	b	a	a	b
39	*M. ciliatus*	a	b	b	b	b	a	b	b	b	a	a	b
40	*M. mylii*	a	b	b	b	b	a	b	b	b	a	a	b
41	*M. tuckeri*	a	b	b	b	b	a	b	b	b	a	a	b
42	*Navodon modestus*	b	b	b	b	a	a	b	b	c	b	a	b

Table 6 (continued).

No.	Scientific name	1	2	3	4	5	6	7	8	9	10	11	12
43	*N. setosus*	b	b	b	b	a	a	b	b	b	c	b	a	b
44	*N. tesselatus*	b	b	b	b	a	a	b	b	b	c	b	a	b
45	*Nelusetta ayraudi*	a	b	b	b	a	a	b	b	b	d	b	b	b
46	*Oxymonacanthus longirostris*	b	b	b	c	b	a	b	b	b	f	b	a	b
47	*Paraluteres prionurus*	a	b	b	c	b	a	b	b	b	f	b	c	b
48	*Paramonacanthus barnardi*	a	b	b	b	b	a	b	b	b	b	a	a	b
49	*P. cryptodon*	a	b	b	b	b	a	b	b	b	b	a	a	b
50	*P. curtorhynchus*	a	b	b	b	b	a	b	b	b	b	a	a	b
51	*P. japonicus*	a	b	b	b	b	a	b	b	b	b	a	a	b
52	*Pervagor melanocephalus*	b	b	b	b	b	a	b	b	b	b	a	a	b
53	*P. spilosomus*	b	b	b	b	b	a	b	b	b	b	a	a	b
54	*Pseudalutarius nasicornis*	b	b	b	b	b	a	b	b	b	f	b	a	b
55	*Psilocephalus barbatus*	a	b	b	c	c	b	b	b	b	f	b	b	c
56	*Pseudomonacanthus peroni*	b	b	b	b	a	a	b	b	b	c	b	a	b
57	*Rudarius ercodes*	b	b	b	c	b	a	b	b	b	c	b	a	b
58	*R. minutus*	b	b	b	c	b	a	b	b	b	c	b	a	b
59	*Scobinichthys granulatus*	a	b	b	b	a	a	b	b	b	c	b	a	b
60	*Stephanolepis auratus*	a	b	b	b	b	a	b	b	b	b	a	a	b
61	*S. cirrhifer*	a	b	b	b	b	a	b	b	b	b	a	a	b
62	*S. hispidus*	a	b	b	b	b	a	b	b	b	b	a	a	b
63	*S. setifer*	a	b	b	b	b	a	b	b	b	b	a	a	b

Pectoral girdle

(8) Postcleithrum. *a*: two bones. *b*: one bone.

Pelvic complex

(9) Encasing scales. *a*: IV-11. *b*: III-6, 7. *c*: II-4 at posterior end of pelvis. *d*: II-4 at ventral part of pelvis. *e*: I-1. *f*: 0-0. (10) Fin ray element. *a*: present. *b*: absent. (11) Cartilage plug. *a*: present at posterior end of pelvis. *b*: present at ventral part of pelvis. *c*: absent.

Dorsal fin

(12) Number of dorsal spines. *a*: 3. *b*: 2. *c*: 1.

Interrelationships in 15 character combinations (Types A to O) are shown in Table 6 and Fig. 15. Monacanthidae in the sense of TYLER (1980) is considered to be a monophyletic group, but there is not found the most primitive character combination. Therefore, Type C was hypothesized as the stem character combination of Types, D, E and F.

Phylogenetics of 63 species are translated from interrelationships in 14 character combinations corresponding to 63 species (Figs. 16 and 17).

From Figs. 15–17, the following facts become clear, i.e., (1) Type C is hypothetical and the most primitive in the monophyletic group comprising 13 types (C to O) of character combinations, (2) from Type C, each of Types D, E and F is independently
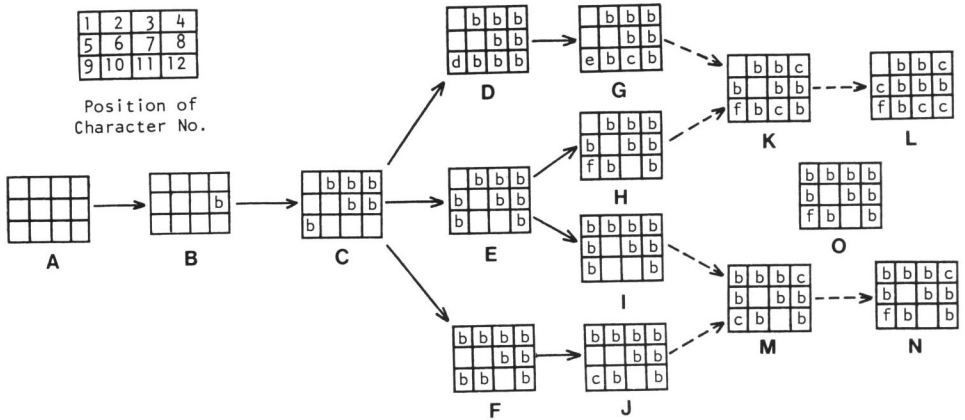
Fig. 15. Fifteen types of character combinations based on 12 characters of 63 species of [Balistidae (Types A to O). Type C is hypothetical. Character No. corresponds to that [of Table 6. Blank squares mean character state *a*.
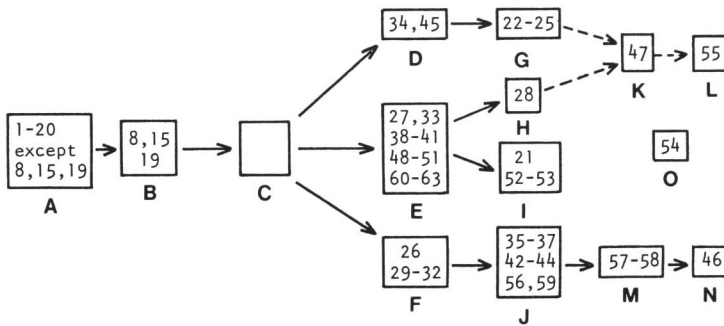

Fig. 16. Interrelationships of 15 types of character combinations (A to O) and 63 species in Balistidae. Type C is hypothetical. Figures in squares correspond to Species No. in Table 6. Pathways from Type J to Type M and from Type M to Type N were determined by karyology.

differentiated, i.e., Type E has sister relationships with Types D and F in Characters 1, 5 and 9, Type D with Type F in Characters 1 and 9, (3) Type E is ancestral to Types H and I in Characters 1, 9 and 10, (4) Type H has sister relationships with Type I in Characters 1, 9 and 10, (5) Type D is ancestral to Type G in Characters 9 and 11, and (6) Type F is ancestral to Type J in Character 9.

Types D to J are classified into 3 groups. However, it cannot be determined that to which group each of Types K, L, M, N and O belongs, i.e., Types K and L can be derived from Types G and H, Types M and N from Types I and J.

As regards chromosomes, 2n of *Paramonacanthus* and *Stephanolepis* belonging to Type E is 34, and 2n of *Cantherhines* and *Navodon* belonging to Types F and J is 40. Diploid chromosome number of *Rudarius* (Type M) and *Oxymonacanthus* (Type
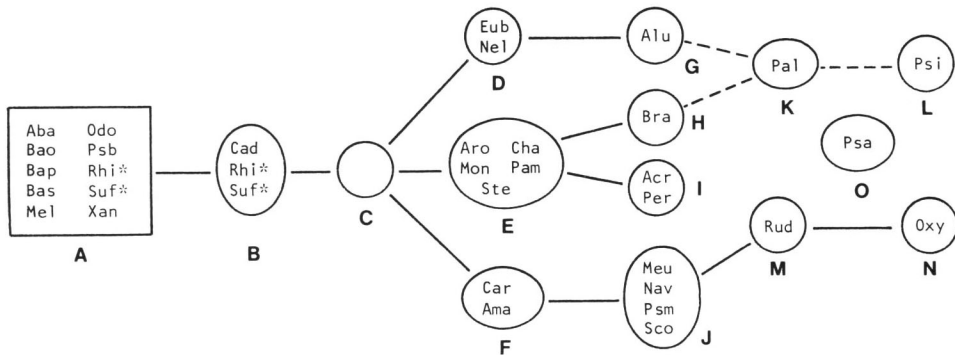
Fig. 17. Interrelationships in 33 genera in Balistidae. Aba, *Abalistes*; Acr, *Acreichthys*; Alu, *Alutera*; Ama, *Amanses*; Aro, *Arotrolepis*; Bao, *Balistoides*; Bap, *Balistapus*; Bas, *Balistes*; Bra, *Brachaluteres*; Cad, *Canthidermis*; Car, *Cantherhines*; Cha, *Chaetoderma*; Eub, *Eubalichthys*; Mel, *Melichthys*; Meu, *Meuschenia*; Mon, *Monacanthus*; Nav, *Navodon*; Nel, *Nelusetta*; Odo, *Odonus*; Oxy, *Oxymonacanthus*; Pal, *Paraluteres*; Pam, *Paramonacanthus*; Per, *Pervagor*; Psa, *Pseudalutarius*; Psb, *Pseudobalistes*; Psi, *Psilocephalus*; Psm, *Pseudomonacanthus*; Rhi, *Rhinecanthus*; Rud, *Rudarius*; Sco, *Scobinichthys*; Ste, *Stephanolepis*; Suf, *Sufflamen*; Xan, *Xanthichthys*. Genera with an asterisk are distributed in two types.

N) is 36. Karyologically, 2n=36 is plesiomorphic to 2n=34, but apomorphic to 2n=40. Therefore, *Rudarius* and *Oxymonacanthus* can be derived from *Cantherhines* (Type F) and *Navodon* (Type J), but not from *Paramonacanthus* and *Stephanolepis* (Type E). Chromosomes of *Paraluteres*, *Psilocephalus*, *Pseudalutarius* have not been reported up to this date, and their systematic positions cannot be determined, until more useful characters will be found.

A phylogenetic classification of Balistidae based on above interrelationships is shown below.

Family Balistidae

Subfamily Balistinae

          Genera: *Abalistes, Balistapus, Balistes, Balistoides, Canthidermis, Melichthys, Odonus, Pseudobalistes, Rhinecanthus, Sufflamen, Xanthichthys*

Subfamily Monacanthinae

    Stem tribe is hypothetical.

        Tribe Monacanthini

            Stem genera: *Arotrolepis, Chaetoderma, Monacanthus, Paramonacanthus, Stephanolepis*

                Genera: *Acreichthys, Pervagor, Brachaluteres*

        Tribe Aluterini

                Genera: *Eubalichthys, Nelusetta, Alutera*

        Tribe Cantherhinini, new tribe

                Genera: *Cantherhines, Amanses, Navodon, Meuschenia,*

Pseudomonacanthus, Scobinichthys, Rudarius,
Oxymonacanthus
Taxa *sedis incertae*: *Paraluteres, Psilocephalus, Pseudalutarius*

Notes. In the present study, each of Group 3 (Balistidae *sensu* TYLER) and Group 4 (Monacanthidae *sensu* TYLER) is monophyletic, and comparable to each other, although Group 3 has ancestor-descendant relationships with Group 4.

In TYLER (1980), *Stephanolepis* is considered as the most primitive genus in Monacanthidae. However, in the present systematics, *Stephanolepis* is not the stem stock of Monacanthidae, but only one of stem genera in a tribe in Monacanthinae (Monacanthidae *sensu* TYLER). Other many differences between TYLER's and the present phylogenetic systematics are clearly shown by comparing Fig. 17 with TYLER's figure (1980, p. 178, fig. 128).

Ostraciidae (Group 5 and Group 6). Following 11 characters are used. Polarity of each character follows that of TYLER (1980). Direction of differentiation in each character is from character state *a* to character state *d*.

Cranium

(1) Myodome. *a*: present. *b*: absent.

Vertebrae

(2) Degree of fusion in the first 5 vertebrae. *a*: 1st fused with 2nd, remainders being free. *b*: 1st fused with 2nd, and 3rd with 4th. *c*: all of the first 4 vertebrae fused. *d*: all of the first 5 vertebrae fused. (3) Trifid neural spines. *a*: absent. *b*: present. (4) Neural spine of the 7th vertebra. *a*: developed. *b*: reduced. (5) Haemal spine of penultimate vertebra. *a*: autogenous. *b*: fused. (6) Number of post anal vertebrae. *a*: 5. *b*: 4. *c*: 3. (7) Foramen of caudal plate. *a*: present. *b*: absent.

Pterygiophores

(8) Expansion of the last anal fin pterygiophore for carapace support. *a*: no. *b*: yes. (9) Expansion of the last dorsal fin basal pterygiophore for carapace support. *a*: no. *b*: yes. (10) Relation in the last anal pterygiophore and haemal spines of the 14th and 15th vertebrae. *a*: not connected. *b*: connected partly. *c*: tightly connected without any space in between.

Hyoid apparatus

(11) Number of pharyngobranchials. *a*: 4. *b*: 3 or 2.

Interrelationships in 8 character combinations (Types A to H) are shown in Table 7 and Fig. 18.

Ostraciidae *sensu* TYLER is considered to be a monophyletic group, but there is not found the most primitive character combination. Therefore, Type B was hypothesized as the stem character combination of Types C, D and E.

Phylogenetics of 15 species are translated from interrelationships in 7 character combinations corresponding to 15 species (Figs. 19 and 20).

From Figs. 18–20, following points seem to be shown, i.e., (1) character combination corresponding to all species that belong to Aracanidae *sensu* TYLER is included

Table 7. Character states of 11 characters in Ostraciidae (Aracanidae and Ostraciidae in the sense of TYLER, 1980).

| No.	Scientific name	1	2	3	4	5	6	7	8	9	10	11
	Aracanidae of TYLER											
1	*Aracana aurita*	a	a	a	a	a	a	a	a	a	a	a
2	*A. ornata*	a	a	a	a	a	a	a	a	a	a	a
3	*Kentrocapros aculeatus*	a	a	a	a	a	a	a	a	a	a	a
4	*Strophiurichthys robustus*	a	a	a	a	a	a	a	a	a	a	a
	Ostraciidae of TYLER											
5	*Acanthostracion guineensis*	a	d	a		b	b	a	a	a	b	b
6	*A. notacanthus*	a	d	a		b	b	a	a	a	b	b
7	*A. polygonius*	a	d	a		b	b	a	a	a	b	b
8	*A. quadricornis*	a	d	a	a	b	b	a	a	a	b	b
9	*Lactophrys trigonus*	a	d	a		a	c	a	a	a		b
10	*Lactoria cornuta*	b	c	b	a	b	c	a	b	b	b	b
11	*L. diaphana*	b	c	b	b	b	c	b	b	b	c	b
12	*L. fornasinii*	b		b		b	c	b	b	b		b
13	*Ostracion lentiginosum*	b	b	b		b	b	b	b	b	b	b
14	*O. cubicus*	b	b	b	a	b	b	b	b	b	b	b
15	*Rhinesomus triqueter*	a	d	a	a	a	c	a	a	a	b	b
16	*Rhynchostracion rhinorhynchus*	b	c	b		b	b	b	b	b	b	b
17	*Tetrosomus concatenatus*	b	c	b	b	b	c	b	b	b	c	b
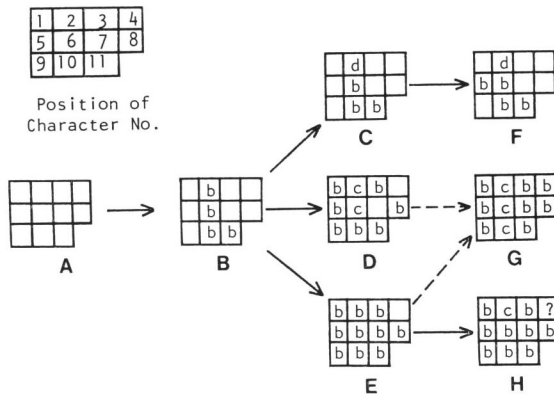
Fig. 18. Eight types of character combinations based on 11 characters of 17 species of Ostraciidae (Types A to H). Type B is hypothetical. Blank squares mean character state *a*.

in Type A, (2) Type B is hypothetical and the most primitive in the monophyletic group comprising 7 types of character combinations (Types B to H), (3) from Type B, each of Types C, D and E is independently differentiated, i.e., Type C has sister relationships with Types D and E in Characters 1, 2, 3, 5 and 9, Type D with Type E in Characters 2, 6 and 7, (4) Type C is ancestral to Type F in Character 5, and (5) Type
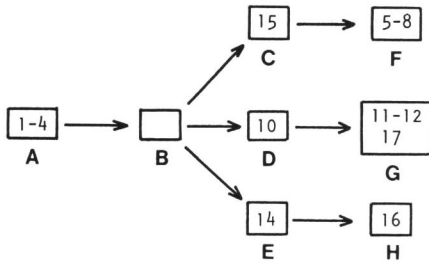
Fig. 19. Interrelationships of 8 types of character combinations (A to H) and 15 species of Ostraciidae. Type B is hypothetical. Figures in squares correspond to Species No. in Table 7. Pathway from Type D to Type G was determined by karyology.
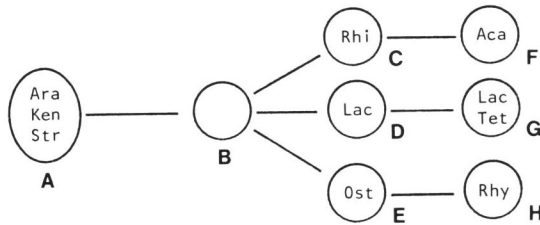

Fig. 20.   Interrelationships in 9 genera in Ostraciidae. Aca, *Acanthostracion*; Ara, *Aracana*; Ken, *Kentrocapros*; Lac, *Lactoria*; Ost, *Ostracion*; Rhi, *Rhinesomus*; Rhy, *Rhynchostracion*; Str, *Strophiurichthys*; Tet, *Tetrosomus*.

E is ancestral to Type H in Character 2.

The relation among Types D, G and E is difficult to be analyzed.   That is, Type G can be derived from Type D in Characters 4, 7 and 10, and also from Type E in Characters 2, 4, 6 and 10.

As regards chromosomes, 2n of *Ostracion cubicus* belonging to Type E is 50, and 2n of *Lactoria diaphana* belonging to Type G is 36.   Karyologically, 2n=50 cannot be plesiomorphic to 2n=36, because 2n=50 is apomorphic to 2n=48 and 2n=36 also apomorphic to 2n=48, i.e., the cline of character states including 2n=50 is different from that including 2n=36.   Therefore, *Lactoria diaphana* (Type G) can be derived from *Lactoria cornuta* (Type D), but not from *Ostracion cubicus* (Type E).

Although character states in Characters 4 and 10 of *Lactophrys trigonus* are unknown, plesiomorphic state in Characters 1, 3, 5, 8 and 9 of this species seems to show that *Lactophrys trigonus* is classified into a monophyletic group including Type C and Type F.

A phylogenetic classification of Aracanidae and Ostraciidae in the sense of TYLER (1980) based on above interrelationships is shown as follows.

        Family Ostraciidae

        Subfamily Aracaninae

                Genera: *Aracana, Kentrocapros, Strophiurichthys*

        Subfamily Ostraciinae

           Stem tribe is hypothetical.

Tribe Lactophrysini
    Genera: *Lactophrys, Rhinesomus, Acanthostracion*
Tribe Lactoriini, new tribe
    Genera: *Lactoria, Tetrosomus*
Tribe Ostraciini
    Genera: *Ostracion, Rhynchostracion*

Notes. Present phylogenetics agree well with those of TYLER (1980, p. 239, fig. 178). Each of Group 5 and Group 6 is considered to be monophyletic and comparable to each other, although Group 5 is ancestral to Group 6.

It may be noteworthy that the distribution of Ostraciinae differs in tribe level, i.e., species belonging to Tribe Lactophrysini are distributed in the Atlantic Ocean, while species belonging to Tribe Lactoriini and Tribe Ostraciini in the Indo-Pacific Oceans (TYLER, 1980, p. 238).

Tetraodontidae (Group 9 and Group 10). Characters which have intrageneric variations cannot be, as a rule, adopted for analysis of phylogenetic interrelationships above species level. Following 10 characters are used. Direction of differentiation in each character is from character state *a* to character state *b*. Character 10 is bipolar and its polarities are from *a* to *b* and from *a* to *x*.

Cranium

(1) Parasphenoid dorsal lobe reaching frontal. *a*: absent. *b*: present. (2) Pterosphenoid. *a*: present. *b*: absent. (3) Prefrontal. *a*: present. *b*: absent.

Hyoid apparatus

(4) Interhyal. *a*: present. *b*: absent.

Suspensorium

(5) Mesopterygoid. *a*: present. *b*: absent.

Supraneural element and pterygiophores

(6) Supraneural element anterior to dorsal pterygiophores. *a*: present. *b*: absent. (7) A posteriorly directed spine of the last dorsal pterygiophore. *a*: absent. *b*: present.

Caudal fin and skeleton

(8) Number of fin rays. *a*: 11. *b*: 9 or 10. (9) Parhypural. *a*: autogenous. *b*: fused.

Nasal apparatus

(10) *a*: upright sac with 2 nostrils. *b*: a tube with a single nostril or a bifid tentacle or a simple flap. *x*: an open cup.

Character states of 10 characters in Group 9 and Group 10 are shown in Table 8. Interrelationships in 14 character combinations (Types A to N) are shown in Fig. 21. Phylogenetic interrelationships of 48 species are translated from interrelationships in 15 character combinations corresponding to 48 species (Figs. 22 and 23).

Figures 21–23 show following points, (1) Type A is the most primitive, (2) from Type A, each of Types B and C is independently differentiated, i.e., Type B has sister relationships with Type C in Characters 4 and 7, (3) Type B is ancestral to Type D in

Table 8.   Character states of 10 characters in Tetraodontidae
(Tetraodontidae and Diodontidae in the sense of TYLER, 1980).

Species		Character No.									
No.	Scientific name	1	2	3	4	5	6	7	8	9	10
	Tetraodontidae of TYLER										
1	*Amblyrhynchotes honckenii*	b	a	a	b	a	a	a	a	a	a
2	*A. hypselogenion*	b	a	a	b	a	a	a	a	a	a
3	*A. piosae*	b	a	a	b	a	a	a	a	a	a
4	*Arothron hispidus*	a	a	a	b	a	a	a	a	a	b
5	*A. immaculatus*	a	a	a	b	a	a	a	a	a	b
6	*A. meleagris*	a	a	a	b	a	a	a	a	a	b
7	*A. nigropunctatus*	a	a	a	b	a	a	a	a	a	b
8	*A. stellatus*	a	a	a	b	a	a	a	a	a	b
9	*Canthigaster amboinensis*	a	a	a	b	a	a	a	a	a	b
10	*C. rostrata*	a	a	a	b	a	a	a	a	a	b
11	*Carinotetraodon lorteti*	a	a	a	b	a	b	a	a	a	b
12	*Chelonodon fluviatilis*	b	a	a	b	a	b	a	a	a	b
13	*C. patoca*	b	a	a	b	a	a	a	a	a	b
14	*Chonerhinos modestus*	a	a	b	b	a	a	a	a	a	x
15	*Colomesus asellus*	a	a	a	b	a	a	a	a	a	a
16	*C. psittacus*	b	a	a	b	a	a	a	a	a	a
17	*Contusus richei*	b	a	a	b	b	a	a	a	a	a
18	*Ephippion guttifer*	b	a	a	b	a	a	a	a	a	b
19	*Fugu chrysops*	b	a	a	b	a	a	a	a	a	a
20	*F. oblongus*	b	a	a	b	a	a	a	a	a	a
21	*Guentheridia formosa*	b	a	a	b	a	a	a	a	a	a
22	*Lagocephalus inermis*	a	a	a	a	a	a	b	a	a	a
23	*L. laevigatus*	b	a	a	a	a	a	b	a	a	a
24	*L. lagocephalus*	b	a	a	a	a	a	b	a	a	a
25	*L. lunaris*	b	a	a	a	a	a	b	a	a	a
26	*L. sceleratus*	a	a	a	a	a	a	b	a	a	a
27	*L. spadiceus*	b	a	a	a	a	a	b	a	a	a
28	*Monotreta leiurus*	b	a	a	b	a	b	a	a	a	b
29	*Omegophora armilla*	b	a	a	b	a	a	a	a	a	b
30	*Pelagocephalus coheni*	a	a	a	b	a	a	a	a	a	x
31	*Sphoeroides annulatus*	b	a	a	b	a	a	a	a	a	a
32	*S. angusticeps*	a	a	a	b	a	a	a	a	a	a
33	*S. dorsalis*	a	a	a	b	a	a	a	a	a	a
34	*S. greeleyi*	a	a	a	a	a	a	a	a	a	a
35	*S. maculata*	a	a	a	a	a	a	a	a	a	a
36	*S. marmoratus*	a	a	a	b	a	a	a	a	a	a
37	*S. nepheus*	a	a	a	b	a	a	a	a	a	a
38	*S. pachygaster*	a	a	a	a	a	a	a	a	a	a
39	*S. sechurae*	a	a	a	b	a	a	a	a	a	a
40	*S. testudineus*	a	a	a	b	a	a	a	a	a	a
41	*S. trichocephalus*	a	a	a	b	a	a	a	a	a	a
42	*Tetraodon mbu*	b	b	a	b	a	b	a	a	a	b
43	*T. lineatus*	b	a	a	b	a	a	a	a	a	b

Table 8   (continued).

No.	Scientific name	Character No.									
		1	2	3	4	5	6	7	8	9	10
44	*Torquigener pleurogramma*	b	a	a	b	a	a	a	a	a	a
45	*T. pleurostictus*	b	a	a	b	a	a	a	a	a	a
46	*Xenopterus naritus*	a	a	b	b	a	a	a	a	a	x
	Diodontidae of TYLER										
47	*Chilomycterus schoepfi*	a	a	a	b	a	b	a	b	b	a
48	*Diodon holocanthus*	a	a	a	b	a	b	a	b	b	a
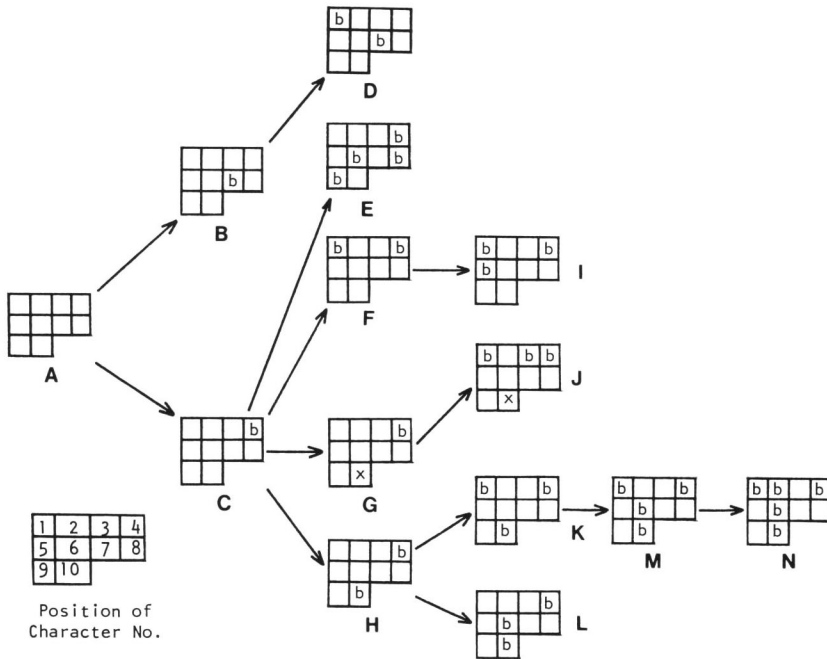
Fig. 21.   Fourteen types of character combinations based on 10 characters of 48 species in Tetraodontidae (Types A to N).   Blank squares mean character state *a*.

Character 1, (4) each of Types E, F, G and H is independently differentiated from Type C, i.e., Type E has sister relationships with Types F, G and H in Characters 1, 9 and 10, Type F with Types G and H in Characters 1 and 10, Type G with Type H in Character 10, (5) Type F is ancestral to Type I in Character 5, (6) Type G is ancestral to Type J in Characters 1 and 3, (7) from Type H, each of Types K and L is independently differentiated, i.e., Type K has sister relationships with Type L in Characters 1 and 6, (8) Type K is ancestral to Type M in Character 6, and (9) Type M is ancestral to Type N in Character 2.

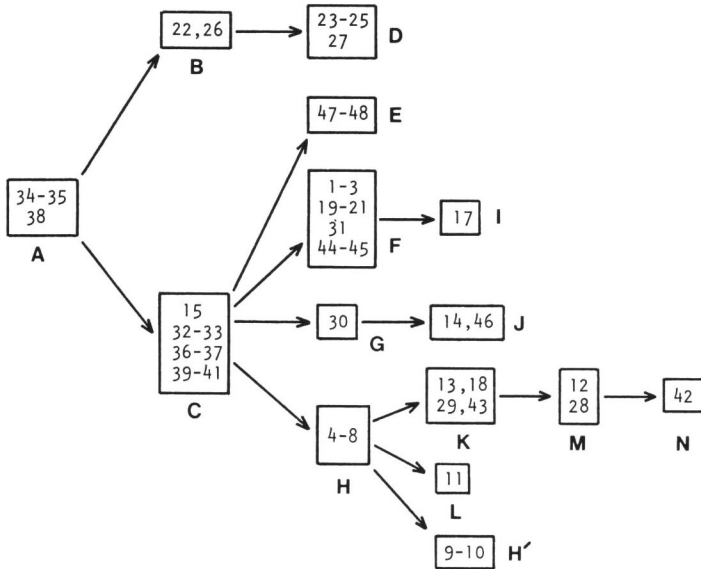Type E represents the character combination of porcupine fishes, which were

Fig. 22. Interrelationships of 15 types of character combinations (A to N and H′) and 48 species in Tetraodontidae. Figures in squares correspond to Species No. in Table 8.

separated from puffers in family level by TYLER (1980). However, Type E is derived from Type C. Such interrelationships are translated as it that *Diodon* and *Chilomycterus* are derived from *Sphoeroides* or its ancestor. Accordingly, the categorical rank of porcupine fishes becomes to be lower than that of puffers. *Pelagocephalus* (Type G) has sister relationships with *Arothron* (Type H) in Character 10. However, as described by TYLER & PAXTON (1979), *Pelagocephalus* is much more closely related to *Sphoeroides* than to *Arothron*. This may suggest that *Pelagocephalus* is the satellite branch of *Sphoeroides* belonging to Type C.

Analysis of karyotypes does not conflict with the present phylogenetic interrelationships. Type H′ is not separated from Type H in 10 characters under study, but is separated by karyological character, i.e., 2n of Type H ranges from 38 to 42, while 2n of Type H′ ranges from 28 to 34.

From phylogenetic interrelationships of Tetraodontidae, a phylogenetic classification of living tetraodontid fishes is constructed as follows.

Family Tetraodontidae
Stem subfamily Liosaccinae, new subfamily
Genus: *Liosaccus* (including *Sphoeroides greeleyi, S. maculata, S. pachygaster*)
Subfamily Lagocephalinae
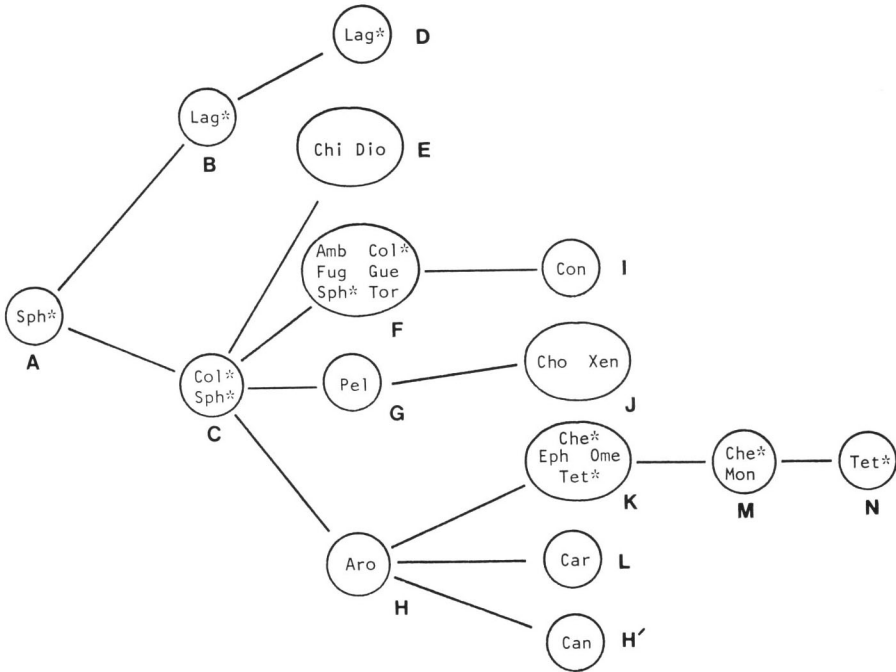Genus: *Lagocephalus*
Subfamily Tetraodontinae

Fig. 23.   Interrelationships in 20 genera in Tetraodontidae. Amb, *Amblyrhynchotes*; Aro, *Arothron*; Can, *Canthigaster*; Car, *Carinotetraodon*; Che, *Chelonodon*; Chi, *Chilomycterus*; Cho, *Chonerhinos*; Col, *Colomesus*; Con, *Contusus*; Dio, *Diodon*; Eph, *Ephippion*; Fug, *Fugu*; Gue, *Guentheridia*; Lag, *Lagocephalus*; Mon, *Monotreta*; Ome, *Omegophora*; Pel, *Pelagocephalus*; Sph, *Sphoeroides*; Tet, *Tetraodon*; Xen, *Xenopterus*.   Genera with an asterisk are distributed in two or more types.

Stem tribe Sphoeroidini
          Genus: *Sphoeroides* (including *Colomesus asellus*)
     Tribe Colomesini
          Stem genera: *Colomesus* (including *Sphoeroides annu-latus*), *Guentheridia*, *Amblyrhynchotes*, *Fugu*, *Torquigener*
          Genus: *Contusus*
     Tribe Diodontini
          Genera: *Chilomycterus*, *Diodon*
     Tribe Tetraodontini
          Stem genus: *Arothron*
          Genera: *Canthigaster*, *Carinotetraodon*, *Chelonodon*, *Ephippion*, *Omegophora*, *Tetraodon* (including *Monotreta*)
     Tribe Chonerhinini

Genera: *Pelagocephalus, Chonerhinos, Xenopterus*
Notes. Phylogenetic interrelationships of Tribe Tetraodontini agree basically with those of TYLER (1980, p. 341, fig. 278).   However, the present phylogenetic classification of Tetraodontidae is different from that of TYLER in the following points, (1) categorical rank of Diodontidae *sensu* TYLER changed from family level to tribe level, (2) species belonging to *Sphoeroides* were separated into different subfamilies, Liosaccinae (*nov.*) and Tetraodontinae, (3) a monophyletic group including *Chonerhinos* and *Xenopterus* was ranked to tribe level, (4) categorical rank of Canthigasterinae *sensu* TYLER changed from subfamily level to generic level in Tetraodontinae, although systematic position of *Canthigaster* in TYLER (1980) is similar to that in the present study, and (5) *Monotreta* was synonymized with *Tetraodon* but not with *Chelonodon*, *Ephippion* and *Omegophora*, because no trituration teeth in both jaws are shared by *Tetraodon* and *Monotreta*.

Summary

1.   Chromosomes of five tetraodontiform fishes (*Lactoria diaphana, Ostracion immaculatus, Canthigaster coronata, Fugu pardalis, Fugu poecilonotus*) were observed, and the structural morphology of the relation between the axial skeleton and pterygiophores of 102 species in Tetraodontiformes was reported.

2.   Phylogenetic systematics of tetraodontiform fishes were studied on the basis of karyotypes and osteological characters by a newly revised method of WAGNER's (1969) groundplan/divergence method.

By analysis of 17 osteological characters, living tetraodontiform fishes were classified into 6 families (Triacanthodidae, Triacanthidae, Balistidae, Ostraciidae, Triodontidae and Tetraodontidae), although the systematic position of a monophyletic group corresponding to Molidae *sensu* TYLER (1980) was left for a future study.

Among above 6 families, phylogenetic classification of Balistidae, Ostraciidae and Tetraodontidae was constructed after phylogenetic interrelationships in above 3 families were reconstructed.

On the basis of 12 osteological characters and karyotypes, 63 species of Balistidae were classified into 2 subfamilies, Balistinae and Monacanthinae.   Balistinae was considered to be ancestral to Monacanthinae which consists of 3 tribes.

On the basis of 11 osteological characters and karyotypes, 17 species of Ostraciidae were grouped into 2 subfamilies, Aracaninae and Ostraciinae.   Aracaninae was considered to be ancestral to Ostraciinae which was separated to 3 tribes.

On the basis of 9 osteological characters, the nasal apparatus and karyotypes, 48 species of Tetraodontidae were classified into 3 subfamilies, Liosaccinae (*nov.*), Lagocephalinae and Tetraodontinae.   Tetraodontinae was classified into 5 tribes, one of which corresponds to Diodontidae *sensu* TYLER (1980).

Acknowledgments

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