GRAMMISTIN, THE SKIN TOXIN OF SOAPFISHES, AND ITS SIGNIFICANCE IN THE CLASSIFICATION OF THE GRAMMISTIDAE

John E. RANDALL[1], Katsumi AIDA[2], Takashi HIBIYA[2],
Nobuhiro MITSUURA[3], Hisao KAMIYA[3],
and Yoshiro HASHIMOTO[3]

With 23 Text-figures

Introduction

The soapfishes are named for their ability to produce large amounts of mucus, particularly if they are handled or otherwise disturbed; when confined in a small volume, the surface of the water may become frothy. In the older literature these fishes have usually been classified as the subfamily Grammistinae of the Serranidae (seabasses, groupers, etc.). GOSLINE (1960) suggested that they be regarded as a family, the Grammistidae, in which he included *Grammistes, Rypticus, Pogonoperca, Grammistops,* and *Pseudogramma* and its allies. He characterized the family as follows: Whole upper border of opercle attached to the body by a flap of skin. Nasal organ dorso-ventrally elongate, made up of a number of nearly parallel, longitudinally oriented lamellae (instead of the usual rosette). Mouth large; supramaxillary present. Teeth mostly villiform, many of them depressible. No enlarged scales on operculum. Upper portion of preopercular border usually with one to four projecting spines. Two (or one) pyloric caeca. Dorsal spines II to VIII. Anal with no or with III spines, in the latter instance with the last spine shorter than the penultimate. Pelvics I, 5, the inner ray attached to the abdomen by a membrane.

GOSLINE noted that *Diploprion* of the Serranidae seemed to be the closest relative of the Grammistidae as he defined the group.

SCHULTZ (1966) followed GOSLINE in regarding the grammistids as a family. He divided it into two subfamilies, the Grammistinae and the Pseudogramminae. He distinguished the latter by its having an incomplete primary lateral line, ending beneath the soft dorsal fin (the Grammistinae have a complete lateral line), some

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1 Bernice P. Bishop Museum, Honolulu, Hawaii 96818.
2 Laboratory of Fish Physiology, Faculty of Agriculture, University of Tokyo.
3 Laboratory of Marine Biochemistry, Faculty of Agriculture, University of Tokyo.

species with a secondary lateral line on the caudal peduncle, scales of moderate size and not embedded (small and often embedded in the Grammistinae), and 26 to 28 vertebrae (24 or 25 in the Grammistinae). He added three more genera to the Grammistinae: *Rainfordia, Diploprion,* and *Aulacocephalus.* He did not examine a specimen of *Rainfordia* (as is discussed in the section on classification below, it is not a grammistid). He included *Diploprion* and *Aulacocephalus* because he noted that these genera have the anterior dorsal pterygiophores associated with the first four neural spines "fundamentally the same as in *Grammistes.*" He then contradicted himself, pointing out that the arrangement of the dorsal pterygiophores in these two genera is more serranid-like than in *Grammistes* and *Rypticus* and "cast doubt on the appropriateness of continuing to recognize the Grammistidae as a family separate from the Serranidae".

GREENWOOD *et al.* (1966) on the other hand, recognized both the Grammistidae and Pseudogrammidae as distinct families; however these authors presented no discussion for such a classification.

While diving in the Florida Keys in 1958 the senior author became aware, in an unusual way, that something exuded from the soapfish *Rypticus saponaceus* is very noxious. About a 9–inch (228.6 mm) adult was speared in 90 feet. Rather than carry the fish all the way to the boat at the surface, it was temporarily stored inside his bathing trunks. Very soon it became apparent that a secretion from this fish was a powerful urethral irritant, and it was promptly removed from the bathing suit. Later in the Virgin Islands and Puerto Rico, conversation with fishermen revealed that keeping a soapfish in a restricted volume of seawater with other fishes often resulted in the death of the other fishes.

MARETZKI and del CASTILLO (1967) conducted a preliminary study of the toxic property of *Rypticus saponaceus* in Puerto Rico. By placing a soapfish in a small volume of seawater and agitating the container, they noted that the water became foamy and opalescent. Intraperitoneal injection of crude toxin solution into mice resulted in death, often preceded by "motor unrest". Freshwater guppies, which these authors stated can survive 24 hours in seawater without obvious ill effects, were used to test the toxin in varying concentrations. In a concentrated solution the guppies almost immediately lose their sense of equilibrium and die in 3 to 5 minutes. Small reef fishes exposed to the same concentration show similar effects. The substance they found responsible for the effects was non-dialysable and insoluble in solutions of low ionic strength. Seawater solutions of the crude toxin at neutral pH was rapidly inactivated, but this can be delayed by shifting the pH in either direction. The toxin was particularly stable at pH 3–4. It could not be extracted with diethyl ether, chloroform or methyl acetate but was soluble in water-saturated *n*-butanol. When the butanolic extract was evaporated in a porcelain dish, it was possible to achieve a partial physical separation of the yellowish gummy toxic residue from the crystalline inorganic salt deposits which collected in the bottom of the dish. The toxic residue was completely dissolved by acidification with dilute HCl. Crude toxin solutions

warmed to 65°C for 2 hours retained a high level of activity. Nevertheless, MARETZKI and del CASTILLO suggested that a protein or polypeptide may be responsible for or associated with the toxic effects. The results after acid hydrolysis of *n*-butanol purified toxin, followed by chromatography in a mixture of *n*-butanol, acetic acid and water (4 : 1 : 1) revealed a number of amino acids (but no cysteine and a paucity of sulfur-containing amino acids, in general), although the concentrate gave a negative reaction with ninhydrin prior to hydrolysis. The same preparation contained only 4.4 per cent nitrogen as determined by micro-Kjeldahl assay.

RANDALL (1967), reporting on the food habits of West Indian reef fishes, noted that no soapfishes were found in the stomachs of predatory fishes and suggested that the toxic slime of *Rypticus* might be repelling to predators.

LIGUORI et al. (1963) demonstrated that the golden-striped soapfish (*Grammistes sexlineatus*) of the tropical Indo-Pacific region secreted copious amounts of viscid mucus by tactile stimulation which had both antibiotic and toxic activity. Both aqueous and 95% ethanolic extracts of lyophilized mucus inhibited the growth of *Escherichia coli* when assayed by the paper disc method on plate cultures at 37°C. The fish *Fundulus heteroclitus* was killed in one hour or less when immersed in a solution of 70 or more parts per million of the ethanol extractable material. The symptoms were primarily neurological. Striking effects were produced on sea urchin egg development. Various concentrations were capable of immobilizing sperm and causing cytolysis of unfertilized eggs.

HASHIMOTO and KAMIYA (1969) investigated the toxic substance in the skin of another Indo-Pacific soapfish, *Pogonoperca punctata*. Skin extracts force-fed in capsules to cats or injected subcutaneously induced ciguatera-like effects. The cats displayed diarrhea, followed by a loss of motor activity. Vomiting and increased salivation were frequently observed. Paralysis of the limbs and coma occurred within a few days. Extracts of the muscle tissue of the fish were nontoxic at a level of 10% body weight. Intraperitoneal injections in mice resulted in paralysis of the hind limbs, labored breathing and death. The minimum lethal dose was 320μg per gram, equivalent to 70 mg of the raw skin; death time was 24 to 48 hours. When immersed in a solution of the toxin, the medaka or Japanese ricefish (*Oryzias latipes*) showed motor excitation, followed by a loss of equilibrium and death. The minimum lethal dose was 0.78 mg per 50 ml (or 15.6 ppm). Hemolytic activity was demonstrated using a 2% rabbit blood cell suspension. No hemolytic activity was detected from extracts of flesh, liver, and viscera other than liver. The toxin is only partially extractable with water, physiological saline, 10% acetic acid or hot ethanol, but effectively with hot 70% ethanol. It is soluble in *n*-butanol and *n*-amyl alcohol but insoluble in diethyl ether, petroleum ether, or chloroform. In an alkaline medium (pH 12) it is heat labile. About 80% of the hemolytic activity is lost on heating in a bath of boiling water for 15 minutes. No marked loss of activity, however, may be observed when heating on the acidic side (pH 2).

On thin-layer chromatography, the preparation partially purified by precipitation with NaCl and subsequent extraction with n-butanol consisted of four or five toxic components, each positive to the Dragendorff reagent and negative to both the biuret and ninhydrin reagents. Dialysis carried out against 50 volume of distilled water at 5°C for 5 hours resulted in 35% of the original activity being found in the outer solution and 60% in the tube.

A comparison was made between ostracitoxin[1], the external toxin of trunkfishes (Ostraciontidae) and the toxin of *Pogonoperca*. Although there is some resemblance in pharmacological properties, the soapfish toxin displays differences in other respects. The toxin from *Pogonoperca* is extractable from the skin of a frozen fish whereas that from ostraciontid fishes may be obtained only from live fishes. Also there are distinct dissimilarities in thin-layer chromatography and in solubilities. For example, ostracitoxin is soluble in chloroform. It was noted that the toxin from *Pogonoperca* behaves similarly to that of *Rypticus* as reported by MARETZKI and del CASTILLO.

HASHIMOTO and KAMIYA also obtained an ichthyotoxin from the skin of *Grammistes sexlineatus* and found by thin-layer chromatography that it consisted of three components corresponding to those of the toxin from *Pogonoperca punctata*. They suggested that the same or similar toxins might be widespread within the soapfish family. This concept has provided the stimulus for the present paper.

Investigation of the Toxin

Taste Test: We found that placing a small amount of the mucus of soapfishes on one's tongue results in an immediate unpleasant bitter taste and slight stinging sensation which is presumably due to the skin toxin of these fishes. If these fishes are confined to a small volume of water and agitated, the taste may be detected in the water. This water easily forms a long-lasting foam like a soap solution when shaken vigorously.

The direct tasting of mucus provides a rapid method of initial screening for toxin. With this taste test we have determined that all of the grammistids which occur in the tropical Pacific, plus the four species of *Rypticus* that were examined alive or freshly dead, were positive for the toxin.

The specimens of *Grammistes sexlineatus* and *Pogonoperca punctata* for testing of toxicity were obtained from the Ryukyu Islands, and those of *Aulacocephalus temmincki* and *Diploprion bifasciatum* from the Seto Marine Biological Laboratory in southern Honshu. *Grammistops ocellatus* was taste tested in Tahiti, *Rypticus saponaceus*, *R. subbifrenatus*, and *R. randalli* were initially examined in Puerto Rico and later Japan.

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1 Ostracitoxin was named by THOMSON (1964). BOYLAN and SCHEUER (1967) named it pahutoxin. The earlier name of THOMSON is preferred, however, for it refers to *Ostracion* the well-known type genus of the trunkfish family, whereas the term proposed by BOYLAN and SCHEUER draws on a Hawaiian fish name which is rarely used.

Rypticus bicolor was collected and tested in the Gulf of California. Subsequent tests were made on a live individual shipped to Japan.

The following 16 serranid fishes were also taste-tested from freshly dead or live material obtained from the Central Wholesale Market of Tokyo, Ueno Zoo Aquarium, Enoshima Aquarium, Keikyu-Aburatsubo Marine Park Aquarium, and the Nagasaki Aquarium: *Doderleinia berycoides, Lateolabrax japonicus, Coreoperca kawamebari, Cephalopholis argus, C. urodelus, Epinephelus akaara, E. awoara, E. caeruleopunctatus, E. fario, E. fasciatus, E. hoedtii, E. merra, E. moara, E. septemfasciatus, Stereolepis ischinagi,* and *Variola louti.* Drs. Tokiharu ABE and Fujio YASUDA assisted in the identification of these fishes. All were obviously negative for the characteristic bitter taste of grammistids except *Epinephelus merra* and *E. ischinagi* for which the first tester reported slight reactions. Subsequent taste tests for these species, plus tests for hemolytic activity, were all negative.

Similarly, taste tests were performed on the following pseudogrammid fishes, after being collected at Central Pacific localities: *Pseudogramma polyacantha, Aporops bilinearis,* and *Suttonia lineata.* All were negative for the toxin.

In addition to the bitter taste, the senior author noted that the mucus of *Grammistes* and *Pogonoperca* has a distinctive pungent and unpleasant odor, and after handling these fishes, one's hands smell of it. The purified skin toxin of these fishes has no detectable odor, however.

With something so subjective as tasting, it is difficult to make a precise assessment of the relative strength of the skin toxin of a fish. Also the amount of toxin produced by an individual fish varies with the degree of stress to which it is exposed, and probably other factors as well. Nevertheless it seemed evident that the strongest taste could be obtained from *Grammistes sexlineatus.* The mucus of *Pogonoperca punctata* also produced a strong taste. That of *Grammistops ocellatus* and *Rypticus,* by comparison, was relatively weak. The four species of *Rypticus* tested could be rated by the strength of the bitter taste as follows: *bicolor* > *saponaceus* > *subbifrenatus* > *randalli.* Aulacocephalus temmincki* and *Diploprion bifasciatum* were about equal in their taste reaction and intermediate to *Grammistes* and *Pogonoperca* on one hand and *Grammistops* and *Rypticus* on the other.

Ichthyotoxic Activity: Tests were conducted on the effect of the secretions of *Grammistes sexlineatus, Aulacocephalus temmincki,* and *Diploprion bifasciatum* on nongrammistid fishes similar to that carried out by HASHIMOTO and KAMIYA (1969) on *Pogonoperca punctata.*

When a live *Grammistes sexlineatus* 93 mm in standard length (SL) was kept in 150 ml of distilled water for 1.5 minutes, the water became turbid and foamy. Five individuals of *Oryzias latipes* placed in this water died in 11 minutes on the average.

One *Diploprion bifasciatum* 158 mm in standard length was agitated in 800 ml of seawater for 5 minutes. Five adults of *O. latipes* were then placed in this water. They showed immediate motor excitation and died within 15 minutes. Although a fresh-

water species, *O. latipes* can ordinarily survive in seawater for at least a day.

An individual of *Aulacocephalus temmincki* about 200 mm in standard length was kept under agitation in 400 ml of seawater for 3 minutes. One *O. latipes* was killed in 81 minutes when placed in this water, and a girellid fish (*Girella punctata*) succumbed in 63 minutes in the same water.

Tests were also made of the effect of partially purified toxin from the mucus and skin of the following soapfishes on live *O. latipes*: *P. punctata, G. sexlineatus, D. bifasciatum, A. temmincki, G. ocellatus,* and *R. saponaceus.* The tests were run with the same *n*-butanolic extracts as used for the investigation of hemolytic activity (see below). One saponin unit of toxin (determined from the hemolytic activity equivalent to 1 mg of standard saponin) from each of the six species was dissolved in separate containers containing 50 ml of distilled water at 20°C. Five live medaka were placed in each container and the death time of the fish recorded. The results are shown in Table 1. They indicate that the relationship between hemolytic and ichthyotoxic activity is similar regardless of the species of soapfish.

Table 1. The death time of medaka (*Oryzias latipes*) in solutions of skin toxin from grammistid fishes.

Species	*Pogonoperca punctata*	*Grammistes sexlineatus*	*Diploprion bifasciatum*	*Aulacocephalus temmincki*	*Grammistops ocellatus*	*Rypticus saponaceus*
Death time (min.)	68, 80, 84 84, 100	61, 64, 68 78, 84	84, 88, 90 91, 95	68, 71, 71 72, 111	47, 95, 102 116, 120	72, 95, 103 125, 130
Average	83	69	89	79	96	105

Effect of a Soapfish on a Predator: On December 4, 1968 it was noted that a *Grammistes sexlineatus* 21 mm in standard length was eaten during the night by another fish of the same species 38 mm in standard length in an aquarium at the Eniwetok Marine Biological Laboratory, Eniwetok Atoll, Marshall Islands. Since grammistids do not die in water in which they have been provoked into liberating skin toxin which kills other fishes in the same container, it is presumed that they are tolerant of their own toxin. They would not, therefore, be expected to be deterred by the toxin in feeding. As mentioned previously, it has been hypothesized that the skin toxin of grammistids is repelling to predators. To test this, the 38-mm *G. sexlineatus* was placed in a large aquarium at Eniwetok which contained a lionfish (*Pterois volitans*) about 150 mm in standard length which was routinely fed small live fishes as food. Before the little grammistid had reached the bottom of the aquarium, the lionfish was already in slow pursuit. It soon seized the soapfish in its jaws, but immediately expelled it. It made two expelling movements of its mouth after the fish was released, suggesting that a lingering bad taste was being experienced. No further attempt was made by the *Pterois* to eat the *Grammistes*.

Hemolytic Activity: The skin toxin of grammistids causes hemolysis of rabbit blood

cells. Hemolytic activity was tested for six species of these fishes. For *Pogonoperca punctata, Grammistes sexlineatus,* and *Grammistops ocellatus* the test solutions were prepared by first grinding up the skin without removing the mucus and washing with distilled water. The residue was then extracted three times with hot 70% ethanol for 30 minutes. The three extracts were combined and concentrated *in vacuo,* acidified with acetic acid, and defatted with diethyl ether. The residue was then dissolved in distilled water. Some of this solution was used for testing, and some was extracted three times with *n*-butanol; the three *n*-butanol layers were combined, the residue dissolved in distilled water, and this also used for testing.

The test solutions for *Diploprion bifasciatum, Aulacocephalus temmincki,* and *Rypticus saponaceus* were made from the mucus and then the skin with the external mucus removed. The mucus was carefully scraped off the skin, suspended in 50 ml of distilled water and shaken three times with an equal volume of *n*-butanol. The *n*-butanol layers were combined and evaporated to dryness under reduced pressure. The residue was dissolved in distilled water and acidified with acetic acid. The skin freed from slime was treated in the same manner as the first three species mentioned.

Hemolytic activity was determined with 2% rabbit blood cell suspension by the method given by YASUMOTO, WATANABE, and HASHIMOTO (1964) for asterosaponin. The results (Table 2) are expressed in terms of saponin units (S.U.). One saponin

Table 2. The hemolytic activity of skin and mucus toxins of grammistid fishes

Species	No.	Skin			Mucus	
		Hot 70% EtOH Ext.		n-BuOH Ext.	n-BuOH Ext.	
		S.U./g skin	S.U./mg	S.U./mg	S.U./g mucus	S.U./mg
Pogonoperca punctata	3	17.4	0.75	0.78		
	4	51.8	0.99	1.70		
	5	3.5	0.10	0.21		
	6	8.0	0.26	0.53		
	7	19.2	0.41	0.58		
	8	15.6	0.27	0.50		
	9	19.5	0.22	0.91		
	10	20.5	0.23	0.94		
Grammistes sexlineatus	6	31	0.36	2.3		
	7	25	0.31	0.86		
Diploprion bifasciatum	1	0.22	0.004		1.2	0.15
	2	0.18	0.003	0.02	0.8	0.05
	3	0	0.003		1.8	0.09
Aulacocephalus temmincki	1	0.14	0.002		1.0	0.03
	2	0.30	0.004		1.9	0.08
	3	0.21	0.003	0.16	3.2	0.08
Grammistops ocellatus	1	1.67	0.15	0.60	—	—
Rypticus saponaceus	1	0.21	0.04	0.15	1.6	0.04

unit is the hemolytic activity equivalent to 1 mg of standard saponin (Merck, B6, Darmstadt).

For the three species in which a separate test was made on the mucus, it is evident that much more toxin is found in the mucus than the skin alone. Considerable individual variation is evident within a species, undoubtedly reflecting in part the treatment of the individual fishes prior to their death. Nevertheless it seems evident that more toxin may be found per unit of skin in *Grammistes sexlineatus* and *Pogonoperca punctata* than the other species.

The same procedure for testing hemolytic activity of *G. sexlineatus* and *P. punctata* was carried out for a specimen of the pseudogrammid fish *Suttonia lineata* from Hawaii 75 mm in standard length. No hemolysis of the rabbit blood cells was detectable for this species.

Thin-layer Chromatography: The preparations for determination of hemolytic activity obtained by extraction with *n*-butanol were examined by thin-layer chromatography. Each resulted in several components which were positive to the Dragendorff reagent. The toxic components were located on the cellulose plate by spraying with 2% rabbit blood cell suspension. The spots with hemolytic activity were almost the same as those positive to the Dragendorff reagent. The Rf values obtained are summarized in Table 3; those of the main spots are underlined. The patterns of the chromato-

Table 3.　Thin-layer chromatogram of the skin toxin of grammistid fishes.

Species	I	II
Pogonoperca punctata (skin)	0.50, 0.45, 0.43, 0.37	0.52, 0.39, 0.31, 0.26, 0.08
Grammistes sexlineatus (skin)	0.75, 0.58, 0.51, 0.44, 0.36	0.57, 0.51, 0.46, 0.41, 0.32
Diploprion bifasciatum (mucus)	0.90, 0.75, 0.58 0.51, ~0.35 (tailing)	0.71, 0.51, 0.63, 0.39, 0.27
Aulacocephalus temmincki (mucus)	0.90, 0.75, 0.58, 0.50~0.30 (tailing)	0.79, 0.53, 0.42, 0.34
Grammistops ocellatus (skin)	0.60, 0.50, 0.40 (weak)	0.70~0.40 (tailing, weak)
Rypticus saponaceus (mucus)	0.91, 0.52, 0.35	0.75, 0.63, 0.53, 0.34

System:　I.　Cellulose .... *n*-BuOH : EtOH : H$_2$O (4 : 1 : 2)
　　　　　II.　Kieselgel G .... *n*-BuOH : *n*-PrOH : 0.1N HCl (1 : 1 : 1)

grams for the different species were similar to each other. The spots for *Grammistops ocellatus* were not as clear, however. This was probably due to initial preservation of the specimen in 10% formalin, followed by soaking in water, and storage in 40% isopropanol.

For comparison, the Rf values for ostracitoxin or pahutoxin are 0.81 (System I) and 0.50 (System II).

Properties of Purified Skin Toxin: The preparations of skin toxin of three of the grammistid fishes, *Grammistes sexlineatus*, *Pogonoperca punctata*, and *Rypticus saponaceus*, were further purified by silicic acid column chromatography. The hemolytic activity and certain chemical and physical properties are summarized in Table 4. We also attempted to purify the skin toxin from *Diploprion bifasciatum* but could only obtain a preparation of very low purity.

Although there were some differences in the relative amounts of the various amino acids, the four dominant amino acids were the same for all three species. We do not know to what extent the differences in composition of amino acids, ultraviolet and infrared spectra may be attributed to the different purity of the preparations from the different species or how much may be due to possible differences in the chemical structure of the toxin of the different species (see Table 4). More detailed biochemical study of the skin toxin in grammistids is now in progress.

Table 4. Properties of the skin toxin of grammistid fishes.
(Each preparation was purified by silicic acid column chromatography.)

Species	*Grammistes sexlineatus*	*Pogonoperca punctata*	*Rypticus saponaceus*
Hemolytic activity (S.U./mg dried residue of extract)	4.55	3.08	0.11
Dragendorff reagent	+	+	+
Dominant Amino Acid	Leucine, Isoleucine, Phenylalanine, Glycine	Leucine, Isoleucine, Phenylalanine, Glycine	Leucine, Isoleucine, Phenylalanine, Glycine
Total nitrogen (%)	12.7	10.1	8.8
Amino acid nitrogen* (% of total nitrogen)	72	91	54
Infrared - IR (cm^{-1})	1660, 1530 (peptide)	1655, 1540 (peptide)	1660, 1540 (peptide)
Ultraviolet - UV (mμ)	258	245	240

* Nitrogen of ammonia detected in amino acid analysis is included.

Histological Investigation: Histological preparations were made of the skin of nine species of soapfishes. Those of *Grammistes sexlineatus*, *Rypticus bicolor*, *Diploprion bifasciatum*, and *Aulacocephalus temmincki* were the most satisfactory, for these fishes were available alive. The other preparations were made from frozen or preserved specimens; the epidermis was sufficiently altered on these to preclude adequate study of surface mucous cells.

Two types of mucous cells in the epidermis and well-developed mucous glands in the dermis under the scales were found in the skin of both *Grammistes sexlineatus* (Figs. 1–3) and *Rypticus bicolor* (Figs. 4–6). The first type of mucous cell (here designated Type I) is the usual type found in the epidermis of fishes in general. On both *G. sexlineatus* and *R. bicolor* these are small and arranged along the upper layer of the epidermis. They are positive to PAS reaction and negative to Sudan Black B staining. The second kind of mucous cell (Type II) is negative to the PAS reaction and positive to Sudan Black B staining. To our knowledge, this cell type is not found in non-grammistid fishes. In *Grammistes sexlineatus,* the smaller cells of this type are found at the upper layer of the epidermis; the larger cells, or possibly mucous depots which contain many small granules stainable with Sudan Black B, are found in the lower layer. In *R. bicolor* mucous cells of Type II are found only in the lower layer. In both species, the dermal mucous glands have a duct leading to the epidermal surface and are composed of small compartments. Material in these glands, being PAS-negative, is strongly stained with Sudan Black B, and presumably is discharged through the duct to the exterior of the fish.

The skin toxin of *Grammistes* and other soapfishes also stains positive (black) with Sudan Black B.

Pogonoperca punctata, Grammistops ocellatus, Rypticus randalli, R. saponaceus, and *R. subbifrenatus* have the same type of dermal mucous glands as *Grammistes sexlineatus* and *Rypticus bicolor.* As stated, the epidermis of these five were not examined due to alteration from preservation and freezing.

Diploprion bifasciatum and *Aulacocephalus temmincki,* on the other hand, lack the dermal mucous glands. They have both types of mucous cells in the epidermis, however, as may be seen in the histological sections of Figs. 7 to 9 for *D. bifasciatum* and Figs. 10 to 12 for *A. temmincki.*

Discussion: The skin toxin of the different grammistid fishes seems to be the same or very similar in all of the species investigated. All gave the same distinctive bitter taste. Each preparation could be extracted and purified by the same procedures, including the extraction with hot 70% ethanol, *n*-butanol extractions, and silicic acid column chromatography. All displayed similar thin-layer chromatograms, and they showed essentially the same ratio between ichthyotoxic and hemolytic activity. Each preparation consisted of a peculiar peptide with a moiety of tertiary or quarternary amine. Special mucous cells stainable with Sudan Black B were found in all the genera. The purified skin toxin is also stained by Sudan Black B.

We propose at this time to name the toxin grammistin. If differences are ultimately demonstrated in its chemical structure among the species, we believe they will be minor and may be given subsidiary designations such as A, B, C, etc.

Grammistin is distinct from ostracitoxin, the skin toxin of trunkfishes (Ostraciontidae). As mentioned in the Introduction, there are noteworthy differences in the thin-layer chromatograms and in solubilities. There are also histological dif-
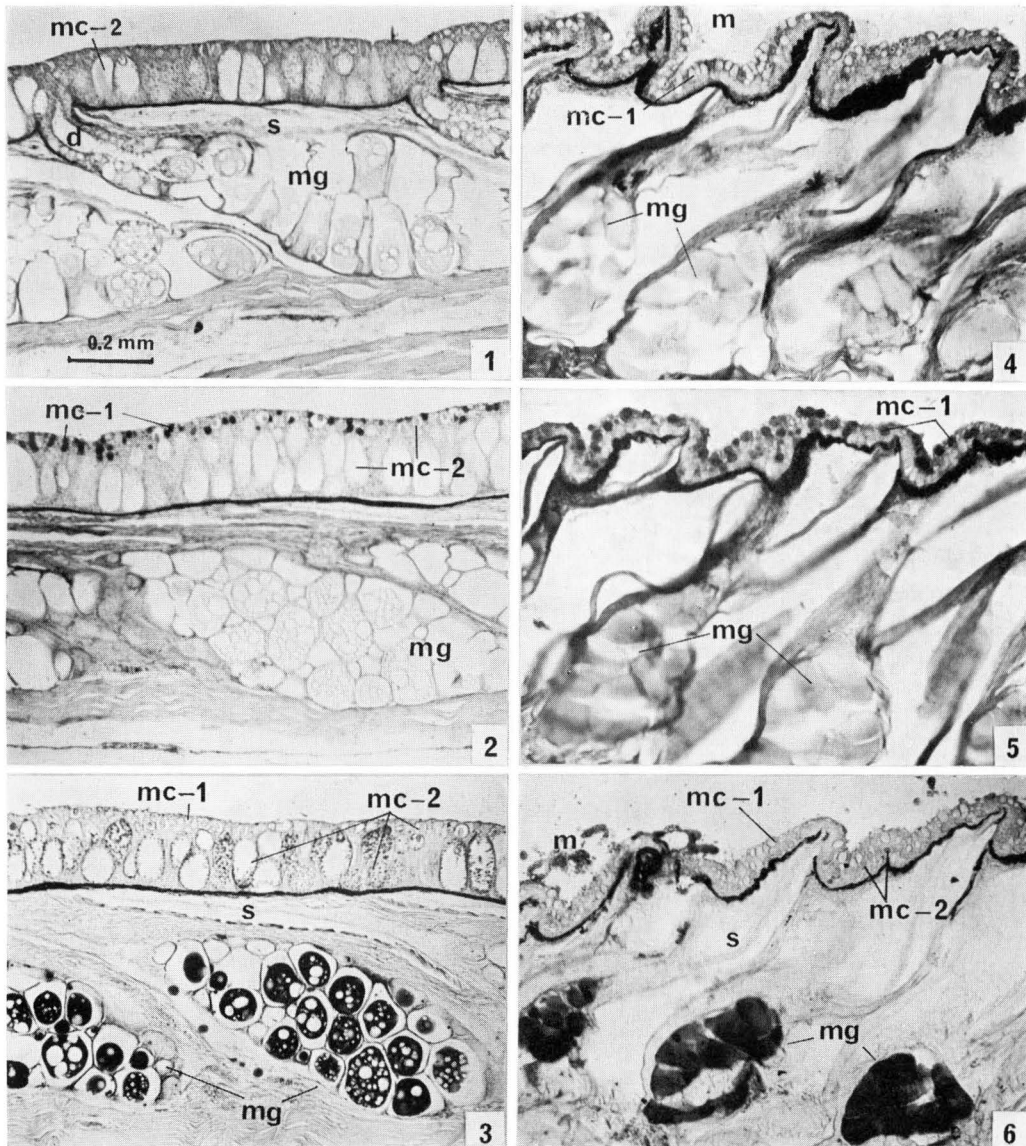
Figure 1. *Grammistes sexlineatus*, Hematoxylin-eosin preparation.
Figure 2. *Grammistes sexlineatus*, PAS-hematoxylin preparation.
Figure 3. *Grammistes sexlineatus*, Sudan Black B preparation.
Figure 4. *Rypticus bicolor*, Hematoxylin-eosin preparation.
Figure 5. *Rypticus bicolor*, PAS-hematoxylin preparation.
Figure 6. *Rypticus bicolor*, Sudan Black B preparation.

(mc-1 = mucous cell, Type I; mc-2 = mucous cell, Type II; mg = mucous gland; s = scale; m = mucus; d = duct. Same magnification for all figures)
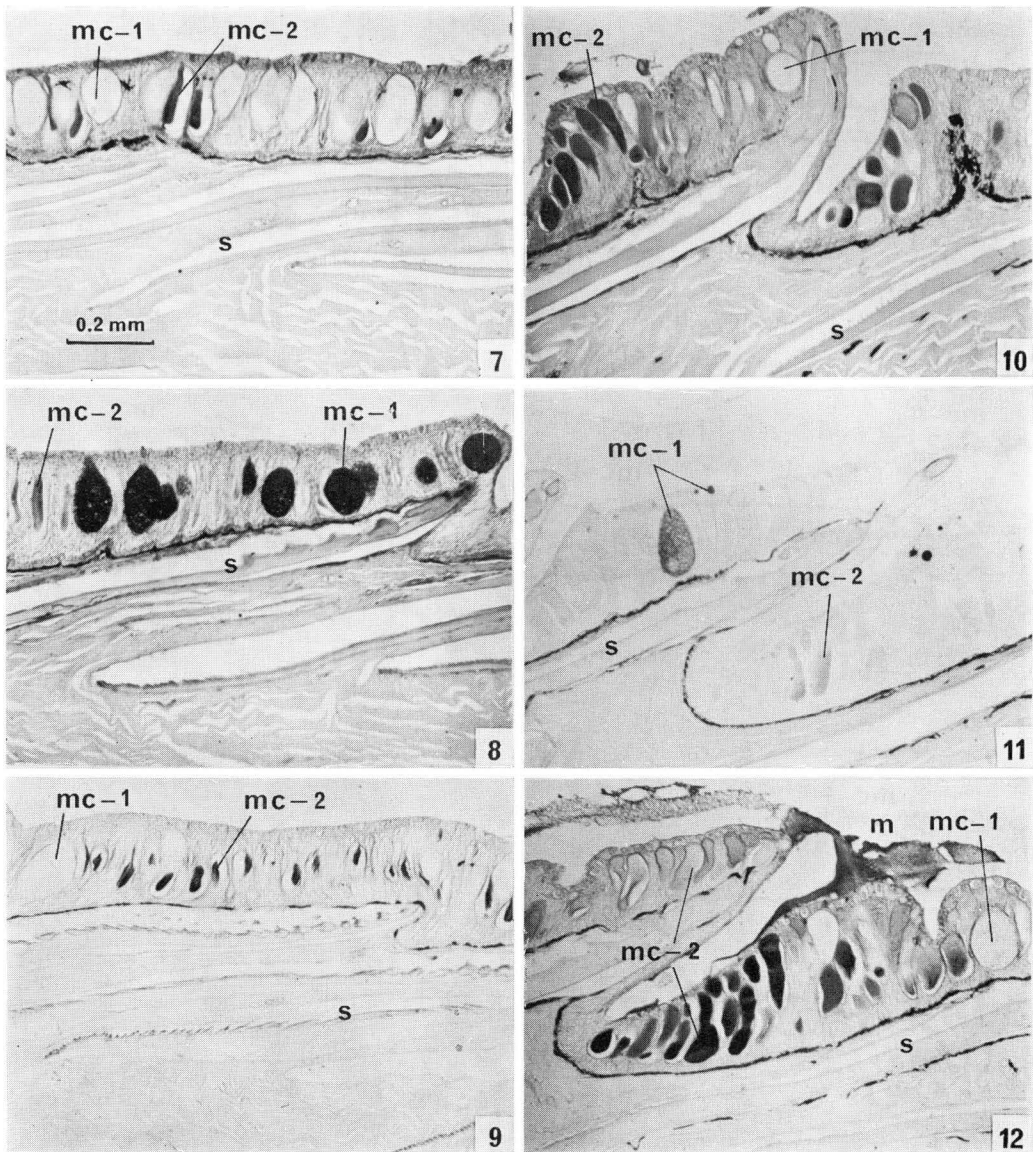
Figure 7.   *Diploprion bifasciatum*, Hematoxylin-eosin preparation.
Figure 8.   *Diploprion bifasciatum*, PAS-hematoxylin preparation.
Figure 9.   *Diploprion bifasciatum*, Sudan Black B preparation.
Figure 10.   *Aulacocephalus temmincki*, Hematoxylin-eosin preparation.
Figure 11.   *Aulacocephalus temmnicki*, PAS preparation.
Figure 12.   *Aulacocephalus temmincki*, Sudan Black B preparation.

(See foot-note of the preceeding page)

ferences in the glands believed responsible for secreting the toxins. Those in *Ostracion* are in the form of club-shaped single cells or simple multicellular glands derived from them. These cells lie in mid-epidermis (THOMSON, 1968). With the exception of *Diploprion* and *Aulacocephalus,* the grammistids have simple tubular or alveolar glands in the dermis, in addition to unicellular glands in the epidermis.

As will be seen from the section on Classification below, *Diploprion* and *Aulaco-cephalus* are morphologically intermediate to the Serranidae and the remaining fishes of the Grammistidae in a number of respects. The lack of multicellular toxin-secreting glands in the dermis of these fishes is an additional feature suggesting that they are primitive members of the family.

The unique color patterns of *Grammistes* and *Aulacocephalus,* and to a lesser extent *Diploprion* and *Pogonoperca* are more distinctive than those of most serranid fishes. One might assume that these patterns serve as warning coloration to potential predators. *Rypticus* and *Grammistops,* on the other hand, are very drab. The species of *Rypticus* appear to be mainly nocturnal (see below), and the individuals of *Grammistops* we have collected have all been taken with rotenone from deep recesses in caves (the fish were not seen prior to the liberation of the poison). Possibly the species of these two genera are more retiring by day than other soapfishes and thus would have less need for colors that would serve to advertise their unpleasant skin exudation.

Classification

As discussed in the previous section, the presence of grammistin in the genera *Grammistes, Pogonoperca, Grammistops, Rypticus, Aulacocephalus,* and *Diploprion* is an additional unifying principle for the group, thus making a stronger case for family status. Conversely, the absence of the toxin from *Pseudogramma* and related genera, along with the difference in structure of the lateral line, more vertebrae and fewer scales, provides a more convincing separation of this group from the Grammistidae. Also the small size of the pseudogrammids might be mentioned. The maximum SL for the family is about 90 mm.

The family Grammistidae may be defined collectively by the following characters: upper border of opercle attached to body by a flap of skin; mouth large, the lower jaw projecting, the maxillary extending to or posterior to a vertical through center of eye; supramaxillary present; teeth in villiform bands in jaws and on vomer and palatines; 3 opercular spines (except one species of *Rypticus* with 2); preopercular margin strongly serrate or with 1 to 4 spines; head and body moderately to strongly compressed, the width of body behind gill opening contained about 2 to 3.5 times in maximum depth; interorbital slightly to strongly convex; ventral profile of head usually jutting below that of thorax; a single complete lateral line; scales small, often embedded, about 75 to 140 rows between origin of lateral line and caudal base; no enlarged scales on operculum; one or two dorsal fins (when two, not separated at

base); borders of caudal, pectoral, and soft portions of dorsal and anal fins bluntly rounded; branched caudal rays 15 (except *Rypticus* with 16 or 17); dorsal spines II to IX; dorsal soft rays 12 to 27; anal spines 2 or 3 (except *Rypticus* which has none); anal spines, when present, very short, the longest half or less than half the length of longest anal soft ray; anal soft rays 8 to 17; pectoral rays 13 to 18; pelvic rays I, 5, the last ray broadly joined to abdomen by a membrane; origin of pelvic fins below or anterior to base of pectoral fins; gill membranes free from isthmus, those of the two sides barely connected anteriorly; branchiostegal rays 7; vertebrae 24 or 25; one interneural spine (pterygiophore) between second and third neural spines and two interneurals between third and fourth neural spines; a free interneural (predorsal) near tip of first interneural spine and a second near tip of second interneural spine; two nasal openings on each side, the anterior in a short tube with posterior edge longer; nasal organ either a rosette or vertically elongate; skin with a thick coat of viscid mucus containing the toxin grammistin, believed secreted by special mucous cells which stain dark with Sudan Black B.

In his excellent publications on serranid fishes, Katayama (1959, 1960a, 1960b) placed *Diploprion* and *Aulacocephalus* in a separate subfamily, Diploprioninae, from the Grammistinae. The first character used in his key to subfamilies to distinguish the two groups is the greater convexity of the interorbital region of the Diplorioninae. If one were comparing just *Diploprion* with *Grammistes*, such a difference is evident, but it breaks down when other genera are considered. The interorbital of *Aulacocephalus* seems no more convex than that of *Pogonoperca*. Another distinction is the length of the "posterior process" of the premaxillary. This is reported as very long in the Diploprioninae but only moderate in length in the Grammistinae. If the posterior process of the premaxillary of Katayama is the median ascending process, then again we fail to note any marked difference when all genera are compared. *Diploprion* has the longest process, but that of *Aulacocephalus* is only of moderate length; the distal end of the process of this genus does not approach the interorbital space whereas it enters the interorbital on all other grammistid genera (admittedly, this is due in part to the longer snout of *Aulacocephalus*).

As indicated in the Key to Genera below, there are some significant differences between the two genera *Diploprion* and *Aulacocephalus* and the other grammistids. These may well be regarded as of sufficient magnitude to warrant the recognition of a subfamily Diploprioninae. The genus *Rypticus*, on the other hand, is also very distinctive. If subfamilies are established, then this genus might also qualify as a separate subfamily.

The Indo-Pacific genera of Grammistidae are all monotypic, except for *Diploprion* with two species. By contrast, the genus *Rypticus* is represented by eight species in the Atlantic and three in the eastern Pacific (see further discussion under *Rypticus*).

There was no opportunity to test the possible toxic quality of the skin of the rare Australian fish *Rainfordia opercularis* McCulloch which Schultz (1966) placed in

the Grammistidae. A preserved specimen 96 mm in standard length (SL) from Heron Island, Great Barrier Reef was examined at the U. S. National Museum (USNM 203247). There is no suggestion of the thick whitish mucus so characteristic of gram-mistids on the specimen. Other features clearly separate this fish from the Gram-mistidae. The head is strongly depressed; there is no membraneous connection between the inner pelvic ray and the abdomen; there are two widely spaced dorsal fins; the preopercular margin is weakly serrate.

Rainfordia has also been regarded in recent classifications as a member of the Serranidae (GREENWOOD *et al.*, 1966) and as a monotypic family (NORMAN, 1957; WOODLAND and SLACK-SMITH, 1963).

KEY TO THE GENERA OF GRAMMISTIDAE

1a. Scales mainly ctenoid, not embedded; preopercular margin strongly serrate; subopercle and interopercle partially serrate; nasal organ with lamellae arranged in a nearly complete circle; mucous cells stainable with Sudan Black B present only in epidermis 2

1b. Scales cycloid, often embedded; preopercular margin smooth except for 1 to 4 spines on upper limb; subopercle and interopercle smooth; nasal organ vertically elongate, the lamellae horizontal; mucous cells stainable with Sudan Black B present in both the epidermis and in glands in the dermis 3

2a. Dorsal rays VIII, 13 to 16; anal rays II, 12 or 13; pelvic fins long, reach-ing posterior to anus; body very compressed, the width behind gill opening 3.3 to 4 in depth ....*Diploprion*

2b. Dorsal rays IX, 12; anal rays III, 9; pelvic fins not long, not reaching anus; body not very compressed, the width about 2.8 in depth ..*Aulacocephalus*

3a. No anal spines; anal soft rays 13 to 17; dorsal spines II to IV; dorsal soft rays 20 to 27; no deep notch between spinous and soft portions of dorsal fin; branched caudal rays 16 or 17 (usually 17); fins very fleshy; small embedded scales on interorbital space, snout below nostrils, max-illary, and more than half way out on fins ....*Rypticus*

3b. Anal spines II or III (embedded in *Grammistes*); anal soft rays 8 or 9; dorsal spines VII; dorsal soft rays 12 to 14; a deep notch between spinous and soft portions of dorsal fin; branched caudal rays 15; fins not very fleshy (except basally); no scales on interorbital, maxillary or more than half way out on fins 4

4a. No fleshy flap on chin; pectoral rays 14 or 15; dorsal soft rays 12 (rarely 13); first dorsal spine 1.2 to 1.5 in length of second dorsal spine; scales extending onto proximal portion of posterior dorsal and anal rays beyond basal scaly sheath ....*Grammistops*

4b.   A fleshy flap on tip of chin; pectoral rays 16 to 18; dorsal soft rays 13
      or 14; first dorsal spine about 1.7 in length of second dorsal spine; no
      scales extending beyond basal scaly sheath of dorsal and anal fins 5

5a.   Fleshy flap on chin large, about as long as eye diameter; anal rays III, 8
      . . . .*Pogonoperca*
5b.   Fleshy flap on chin very small; anal rays II, 9 . . . .*Grammistes*

Diploprion bifasciatum

Figure 13

Diploprion bifasciatum (KUHL and VAN HASSELT) CUVIER and VALENCIENNES, 1828. Hist. Nat. Poissons,
     vol. 2, p. 137, pl. 21 (type locality, Java).

Diagnosis: Dorsal rays VIII,13 to 16; anal rays II,12 or 13; pectoral rays 17 or 18;
lateral-line scales 80 to 88; vertical scale rows above lateral line to caudal base 100
to 110; scales on body ctenoid except those on nape, thorax, abdomen, and bases of
dorsal and anal rays; no scales on interorbital, snout, or maxillary; gill rakers long,
9 to 10 + 20 to 22 on first arch; body very deep, the depth 2 to 2.4 in standard length,
and very compressed, the width behind gill opening 3.3 to 4 in greatest depth; head
length 2.65 to 2.9 in standard length; mouth very oblique; preopercular margin
coarsely serrate; subopercle and interopercle serrate; interorbital space strongly
convex; pelvic fins very long, the tips extending posterior to spinous portion of anal
fin; a deep notch between spinous and soft portions of dorsal fin; third dorsal spine
the longest, its length 1.6 to 1.8 in head length; pale (light yellow in life) with two
black bars, one on head through eye and a broader one on side of body.

Remarks: *Diploprion bifasciatum* is known from India and Ceylon through the Indo-
Malayan region and China to southern Japan. It is also recorded in Australia from
Queensland to western Australia. It has not been taken from the islands of Oceania
or from the western Indian Ocean. KAMOHARA (1961) illustrated a specimen which
is 250 mm in total length. WEBER and DE BEAUFORT (1931) have recorded the total
length as about 240 mm. ROFEN (1963) stated that the species attains a maximum
length of about 8 inches (203 mm) in the Gulf of Thailand.

     Phillip C. HEEMSTRA called our attention to an all-black specimen of *D. bifasciatum*
180 mm in standard length which was collected by Victor G. SPRINGER at One Tree
Island, Great Barrier Reef. This specimen (USNM, uncatalogued) resembles
D. bifasciatum in all respects except color. Two other specimens collected at the same
island include a normal-colored individual of 153 mm standard length and one of
130 mm on which the black body bar encompasses nearly all of the posterior three-
fourths of the body. The latter specimen is thus somewhat intermediate in color to
the black specimen and normal-colored individuals, therefore suggesting that the
black is merely a melanistic form of *bifasciatum*.

HUBBS and CHU (1934) noted that the second and third dorsal spines of the late postlarval stage of this species are prolonged into filaments.

Diploprion drachi ESTÈVE

Figure 14

Diploprion drachi ESTÈVE in ROUX-ESTÈVE and FOURMANOIR, 1955. Ann. Inst. Océanogr. Paris, N. S. 30: 197 (type locality, Abulat, Red Sea).

Diagnosis: Dorsal rays VIII,15 or 16; anal rays II,12; pectoral rays 15 or 16; lateral-line scales 87; vertical scale rows above lateral line to caudal base 113; scales ctenoid; no scales on interorbital, snout, maxillary or fins except basally on caudal fin; gill rakers 8 + 19 or 20; body not very deep, the depth about 2.4 to 2.6 in standard length, and very compressed, the width behind gill opening 3 to 3.5 in depth; head length 2.6 to 2.8 in standard length; mouth very oblique; maxillary reaching posterior to center of eye; preopercular margin coarsely serrate; subopercle and interopercle serrate; interorbital space moderately convex; pelvic fins long, the tips reaching to or well beyond origin of anal fin; a deep notch between spinous and soft portions of dorsal fin; third dorsal spine the longest, its length 2.25 to 2.45 in head length (fin spines slightly abnormal in holotype); brown, the fins pale except spinous portion of dorsal fin and base of soft portion of dorsal fin which are black.

Remarks: *Diploprion drachi* was described from a single specimen 84 mm in standard length from the island of Abulat in the Red Sea. This fish was kindly sent on loan by M. L. BAUCHOT of the Muséum National d'Histoire Naturelle. The most obvious differences between it and *Diploprion bifasciatum* are less body depth for *drachi*, fewer pectoral rays (15 or 16 for *drachi*, 17 or 18 for *bifasciatum*), and color. *D. drachi* lacks the blackish bars of *bifasciatum*, but it has a nearly black spinous dorsal fin, and the base of the soft portion of the fin is also blackish.

Victor G. SPRINGER cf the U. S. National Museum recently collected a specimen of *D. drachi* (USNM 205489, 108 mm SL) in the Strait of Jubal, Red Sea (southern end of Sinai Peninsula at Ras Muhammad) at a depth of 30 feet. The specimen was sent on loan, and its descriptive data are incorporated into the diagnosis above.

Aulacocephalus temmincki

Figure 15

Aulacocephalus temmincki BLEEKER, 1857. Verh. Bat. Gen., vol. 26, p. 15, pl. 5, fig. 2 (type locality, Nagasaki, Japan).

Diagnosis: Dorsal rays IX,12; anal rays III,9; pectoral rays 14 to 16; lateral line scales 76 to 82; vertical scale rows above lateral line to caudal base 83 to 87; scales ctenoid; no scales on interorbital, snout, maxillary, or fins except basally on caudal;

gill rakers 7 to 8 + 18 to 20; depth of body about 2.9 to 3 in standard length and width of body behind gill opening about 2.8 in depth; head length 2.4 to 3 in standard length; snout relatively long, its length about 2.7 in head length; mouth slightly oblique; preopercle coarsely serrate; subopercle and interopercle serrate; interorbital space moderately convex; pelvic fins inserted beneath pectoral fins, not very long (about 2 in head), the tips not reaching anus; third and fourth dorsal spines the longest, their length 3.1 to 3.5 in head; first and last dorsal spines subequal, about half the length of longest spines; color in life deep blue with a bright yellow band running from near front of upper jaw through eye, along upper part of back and basally in dorsal fin to upper base of caudal fin; a spot of yellow at lower base of caudal fin and a streak of yellow along upper part of maxillary; soft portions of median fins with black rays and translucent membranes.

Remarks: In the *Proceedings of the Zoological Society of London* (1833, pt. 1, p. 117) there is an anonymous report of ichthyological contributions from Mauritius which includes the statement, "M. LIÉNARD, sen., has described a new species of *Grammistes,* Cuv., distinguishable from the two previously known by its compressed form, on which account it has been designated *Gramm. compressus*: its first dorsal fin has nine rays." BOULENGER (1895) placed this name with a question mark at the head of his list of synonymy for *Pogonoperca ocellata.* We have been unable to find any publication by LIÉNARD in which *Grammistes compressus* is formally described. If nine rays can be construed as meaning nine dorsal spines, there is a possibility that *compressus* could be *A. temmincki,* since it is the only known grammistid with nine dorsal spines. However, *G. compressus* cannot be positively identified from the sparse descriptive data given, and we propose that this name be suppressed.

Aulacocephalus temmincki is one of the most strikingly colored of all the serranoid fishes. It has an unusual disjunct distribution: Japan, northeastern China and Korea (REEVES, 1927), Natal coast of South Africa (GILCHRIST and THOMPSON, 1917), Mauritius (GÜNTHER, 1859), Thailand (BOULENGER, 1895), Kermadec Islands (WAITE, 1911), and New Zealand (WHITLEY, 1968). In his *The Sea Fishes of Southern Africa,* SMITH (1949) referred to this species as unmistakable and reported it as occurring as far south as Durban. He also stated that it has excellent flesh and attains 14 inches (356 mm) in length. The senior author has examined a specimen 178 mm SL from Taiwan at the University of Tokyo (No. 22615). With the exception of Thailand, these localities lie within the range in latitude of about 20° to 36°.

Suspecting that there might be some error in BOULENGER's Thailand record, which was based on a skeleton of a half-grown fish, we wrote A. C. WHEELER of the British Museum (Natural History). He confirmed the identification and noted that the label with the skeleton reads, "Siam Purchd. of Mr. JAMRACH". He added, "JAMRACH was a general dealer in natural history items, and as such his localities may be open to more suspicion than those of a collector from an area." This skeleton represents the only record of the species from a truly tropical locality. If *A. temmincki* could exist in

a region where the seas remain warm throughout the year, it is difficult to understand why it has not been recorded from areas such as the East Indies or Philippines. It would seem more reasonable to assume that JAMRACH's specimen came from a cooler locality than Thailand than to explain the apparent absence of this fish from tropical localities intermediate within its present range. Perhaps this species lived in the lower latitudes at some time in the past when seas were cooler, as during an ice age. It is also possible that it will be found in tropical regions in deeper water.

Genus *Rypticus*

Diagnosis: Dorsal spines II to IV; dorsal soft rays 20 to 27; no anal spines; anal soft rays 13 to 17; pectoral rays 13 to 17; branched caudal rays 16 or 17 (usually 17; other genera with 15); vertical scale rows above lateral line to caudal base 75 to 130 (from SCHULTZ and REID, 1939); scales cycloid, embedded, present on interorbital, side of snout below nostrils, maxillary, and more than half way out on fins; fully-formed gill rakers of adults not numerous, the total count 5 to 11 on first arch; body moderately elongate and compressed; head moderately pointed, the length 2.4 to 3.4 in standard length; mouth large and oblique, the maxillary usually reaching to or beyond a vertical at posterior edge of eye; opercular spines 3 (except *R. randalli* with 2); preopercular margin smooth except 1 to 3 spines on upper portion; subopercle and interopercle smooth; interorbital space narrow, nearly flat to moderately convex; pelvic fins small, their length contained more than 3 times in head length; fins fleshy; no deep notch between spinous and soft portions of dorsal fin; dorsal spines short; color drab - gray, brown, or olivaceous, some species marked with dark or light spots.

Remarks: COURTENAY (1967) has revised the Atlantic species of *Rypticus*. He recognized the following eight species: *saponaceus* (BLOCH and SCHNEIDER), *subbifrenatus* GILL, *macrostigmus* COURTENAY, *bistrispinus* (MITCHILL), *bornoi* BEEBE and TEE VAN, *maculatus* HOLBROOK, *randalli* COURTENAY, and *brachyrhinus* COURTENAY. All occur in the western Atlantic and one of them, *saponaceus*, is found also at St. Helena, Ascension, the Cape Verde Islands, and the coast of Africa from Senegal to the Congo. *R. maculatus* occurs from southern Florida to Cape Hatteras and in the northern Gulf of Mexico. The other species are more tropical in their distribution.

Boyd W. WALKER and George C. WILLIAMS have worked on the eastern Pacific species of *Rypticus* but have not yet published their findings. WALKER (personal communication) informed us that he and WILLIAMS will recognize *bicolor* (VALENCIENNES) and *nigripinnis* GILL. In addition they have an undescribed species from the Revillagigedo Islands.

The species of *Rypticus* range in size from the small *bornoi* (still known only from the type from Haiti which measures 49.4 mm standard length) and *macrostigmus* (the twelve recorded specimens from the Bahamas and Panama are 40 to 57 mm in standard

length) to *saponaceus* as large as 258 mm standard length and *bicolor* to about 250 mm standard length.

RANDALL (1967) reported that 12 of 27 West Indian specimens of *saponaceus* with food in their stomachs fed about equally on fishes and crustaceans, and HOBSON (1965, 1968) found that 13 individuals of *bicolor* from the Gulf of California fed mainly on benthic crustaceans. Both of these species are primarily nocturnal. It is expected from observations of the secretive behavior of other species of grammistids during daylight hours that they will prove to be principally nocturnal also.

For the analysis of the skin toxin of *Rypticus* we obtained fresh material of the following species: *saponaceus, subbifrenatus, randalli,* and *bicolor.* Photographic illustrations are presented of these (Figs. 16 to 19).

Grammistops ocellatus

Figure 20

Grammistops ocellatus SCHULTZ in SCHULTZ *et al.*, 1958. Bull. U.S. Natl. Mus. 202, vol. 1, p. 386, fig. 62 (type locality, Bikini Atoll, Marshall Islands).

Diagnosis: Dorsal rays VII,12 (one with VI,13); anal rays III,9 (one with 8); pectoral rays 14 or 15; lateral-line scales 58 to 67; vertical scale rows above lateral line to caudal base 82 to 100; scales cycloid, embedded, none on interorbital, snout, or maxillary; fins scaled basally, those on caudal reaching about half way to edge of fin; scales extending onto proximal portion of posterior 7 or 8 dorsal rays and 3 or 4 anal rays beyond the scaled basal sheath; gill rakers 1 or 2 + 6 to 9 on first arch (plus 5 to 7 low tubercles on upper limb and 4 to 8 on lower); body moderately elongate, the depth 3.1 to 3.7 in standard length, and moderately compressed, the width behind gill opening about 2 in depth; head length 2.55 to 2.65 in standard length; mouth large and oblique, the maxillary reaching to or beyond hind margin of eye; ventral profile of head jutting below that of thorax; preopercular margin smooth except for 1 or 2 short broad-based spines on upper margin (absent from one side of two specimens); subopercle and interopercle smooth; pelvic fins 2.1 to 2.2 in head length; fins not fleshy except basally; dorsal fin nearly notched or just notched to base between spinous and soft portions; dorsal spines short, the second or third the longest, 3.4 to 4.4 in head length; first dorsal spine 1.2 to 1.5 in second dorsal spine; color gray-brown, the head yellowish in life, with a prominent dark ocellus nearly as large as eye on opercle (one Tahitian example with a group of three small ocelli at this location); a dark brown spot near tip of lower jaw on each side; front edge of eye narrowly blackish; membranes of fins pale except basally, the rays dark.

Remarks: *Grammistops ocellatus* is known in the literature from the type specimen, 85.5 mm in standard length taken in 20 to 40 feet in the Marshall Islands, and two specimens, 63 and 88 mm in length, from 12 to 60 feet off East Africa (5° S and 14°

10′ S). The African specimens were described as *Tulelepis canis* by SMITH (1954); this name was correctly referred to the synonymy of *G. ocellatus* by GOSLINE (1960).

We here record the species from Tahiti (BPBM 8330: 2 specimens, 88 and 107 mm SL), Guam (BPBM 9021: 1 specimen, 94 mm SL), and Palau (BPBM 9556: 1 specimen, 75 mm SL). The Tahitian specimens were collected by the senior author from a cave in a coral reef at a depth of 50 feet in Popote Bay, district of Papara on March 6, 1969. The Guam specimen was taken in 10 to 26 feet by Harry T. KAMI on December 6, 1969. The Palauan specimen was collected by the senior author, A. R. EMERY, and E. S. HELFMAN in a cave in 60 feet near the entrance to Malakal Pass on April 17, 1970. Further collecting in the tropical Indo-Pacific will no doubt further reduce the present discontinuities in the distribution of this rare species.

Pogonoperca punctata

Figure 21

Grammistes punctatus CUVIER and VALENCIENNES, 1830, Hist. Nat. Poiss., vol. 6, p. 504 (type locality, South Seas, near Vanikoro Islands).

Diagnosis: Dorsal rays VIII,12 or 13 (reports of VII are probably due to overlooking the small eighth spine); anal rays III,8; pectoral rays 17 or 18; lateral-line scales 59 to 70; vertical scale rows above lateral line to caudal base about 88; scales cycloid, embedded, none on interorbital, snout, maxillary, or fins except basal fleshy portion of dorsal and anal fins, basal two-fifths of caudal fin, and basal part of paired fins; gill rakers shorter than longest gill filaments, with 1 or 2 + 7 or 8 fully formed on first arch (plus about 7 low tubercles on both upper and lower limb of arch); a prominent flat fleshy protuberance about as large in area as eye oriented transversely at tip of chin; body oblong, the depth 2.2 to 2.7 in standard length, and compressed, the width behind gill opening 2.2 to 2.7 in depth; head length 2.5 to 2.75 in standard length; mouth moderately large and very oblique, the maxillary nearly reaching or extending slightly posterior to a vertical through center of eye; preopercle with 3 to 5 short spines on upper margin; subopercle and interopercle smooth; interorbital moderately convex; length of pelvic fins 1.7 to 2 in head length; fins not fleshy except basally; a deep notch nearly separating spinous and soft portions of dorsal fin; second or third dorsal spines the longest, 2 to 3 in head length; first dorsal spine 1.6 to 2.1 in length of second dorsal spine; color brown, a little blotchy, with numerous small dark-edged white spots (smaller dorsally than ventrally) on head, body, chin flap, basally on fins and extending part way out on soft rays (some areas on side of body with fewer or almost no white spots); four triangular black saddles on back, the largest on nape, the next largest beneath spinous portion of dorsal and extending as a broad blackish area into fin, covering most of the second and third interspinous membranes (rest of spinous portion of fin with white spots as on body); third black saddle beneath middle-anterior part of soft portion of dorsal fin and the last, about

as large as eye, on front of caudal peduncle; in addition there is a round black spot about the size of the pupil on upper middle base of caudal fin, another at midbase of anal fin, and a third at origin of anal fin; a fourth black spot about twice the diameter of the previous spots lying midventrally in front of anus; a blackish zone across upper interorbital and extending as a diffuse bar below eye (unlike other black areas, this contains faint white dots); soft portions of fins with membranes pale and rays dark except for the previously mentioned white basal spots.

Remarks: CUVIER and VALENCIENNES based their description of *Grammistes punctatus* on a drawing by MERTENS of a 7-inch (177.8 mm) fish from "la mer du Sud, prés de Vanicolo" (Vanikoro Islands). There is no type specimen.

GÜNTHER (1859) described *Pogonoperca ocellata* from a dried specimen 12 inches (304.8 mm) long from a locality unknown to him. In the same work he recognized *Grammistes punctatus* CUVIER and VALENCIENNES. BOULENGER (1895) placed *G. punctatus* in *Pogonoperca* and gave its distribution as the Malay Archipelago and Polynesia. He ascertained that the type locality of *ocellata* is Mauritius and listed other specimens from the Seychelles and Madras, India. He stated that *ocellata* is "very closely allied" to *punctata*, differing in the more elongate body (depth 2.5 to 2.75 in total length, as opposed to 2.25 to 2.33 for *punctata*), a shorter head (length 3.0 to 3.5 times in total length, as opposed to 2.8 to 3.0 for *punctata*), and white spots more numerous and entirely covering the fins.

BLISS (1883) described *Pogonoperca reticulatus* (attributing the name to STEINDACHNER, MS) from a specimen 190 mm in standard length from Mauritius. This specimen was examined at the Museum of Comparative Zoology at Harvard University (MCZ 5933). The depth of the body is 2.7 in the standard length, and the head length 2.5 in the standard length. The fins are covered with relatively large close-set pale spots. In addition, the dark areas on the back are not as well developed, and no obvious black pigment extends into the spinous dorsal fin. There is no black spot at the base of the caudal fin, nor are any visible at the origin or mid-base of the anal fin. All of the meristic data of this fish match that of Pacific specimens. A figure of a 9-inch (228.6 mm) specimen from the Seychelles in SMITH and SMITH (1963) also reveals white spots running to the periphery of the fins. From the illustration the depth of the body appears to be about 2.3 in the standard length and the head about 2.7 in the standard length.

More specimens of *Pogonoperca* are needed to more fully document the differences between Indian Ocean and Pacific Ocean fishes of this genus. We do not believe, however, that these differences are specific in magnitude. Therefore we provisionally refer *ocellata* and *reticulatus* to the synonymy of *punctata*.

Judging from the few records in the literature and paucity of specimens in museums, *Pogonoperca punctata* is not a common species. In addition to the localities mentioned above, it is reported from Ambon (BLEEKER, 1857), Ulea (Caroline Islands) (KITTLITZ, 1858), Society Islands (GÜNTHER, 1873), Réunion and Madagascar
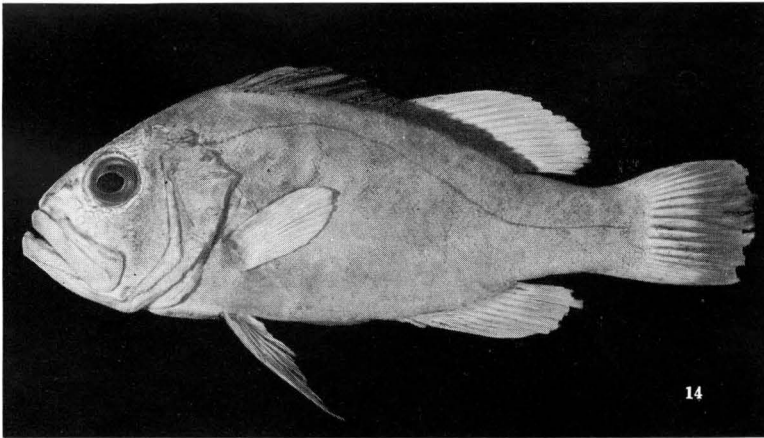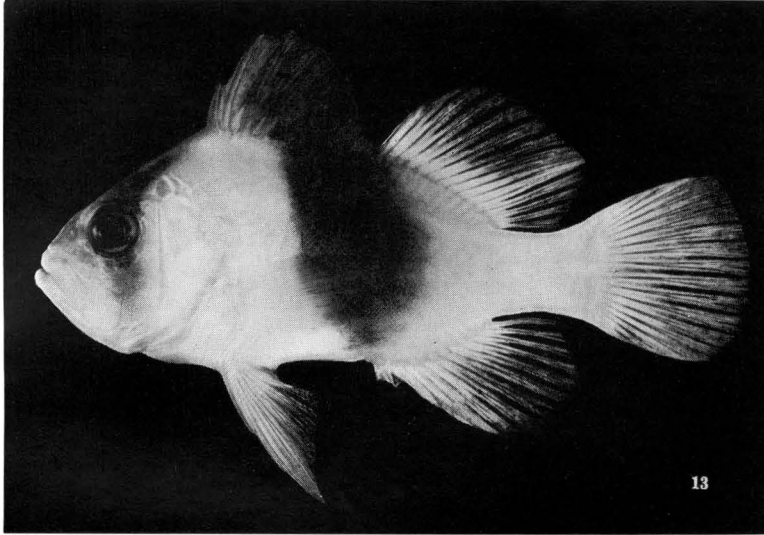
Figure 13. *Diploprion bifasciatum*, 67 mm SL, Philippine Islands.
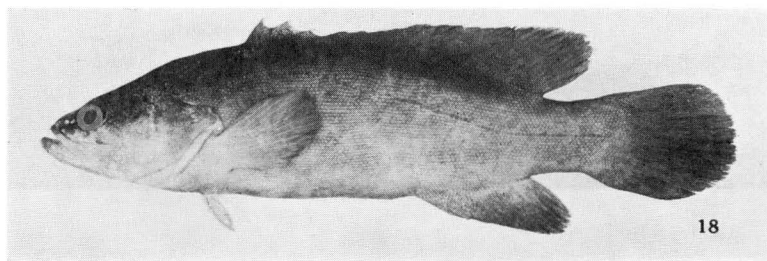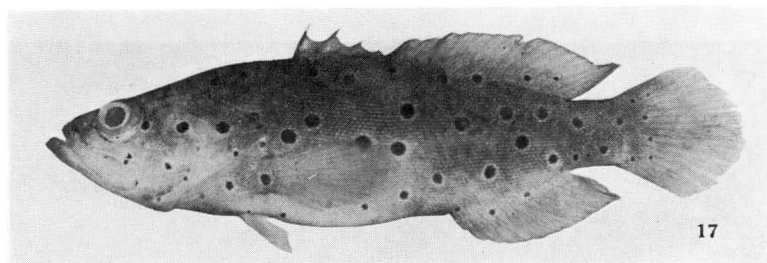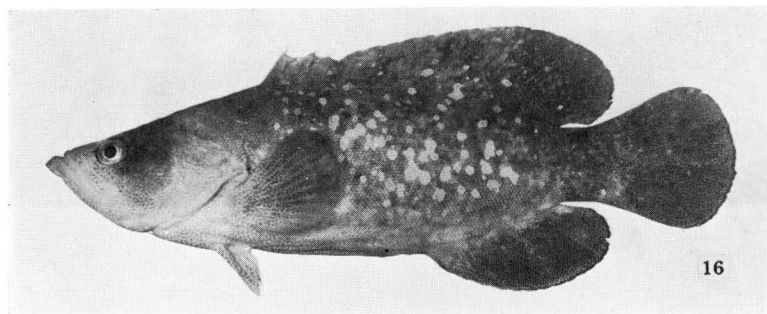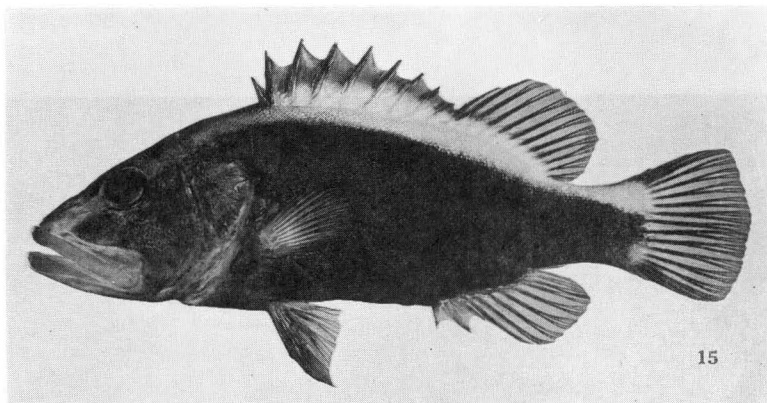Figure 14. *Diploprion drachi*, holotype, 84 mm SL, Abulat, Red Sea.

Figure 15.   *Aulacocephalus temmincki*, 147 mm SL, Shirahama, Japan.
Figure 16.   *Rypticus saponaceus*, 127 mm SL, St. John, U. S. Virgin Islands.
Figure 17.   *Rypticus subbifrenatus*, 63 mm SL, Puerto Rico.
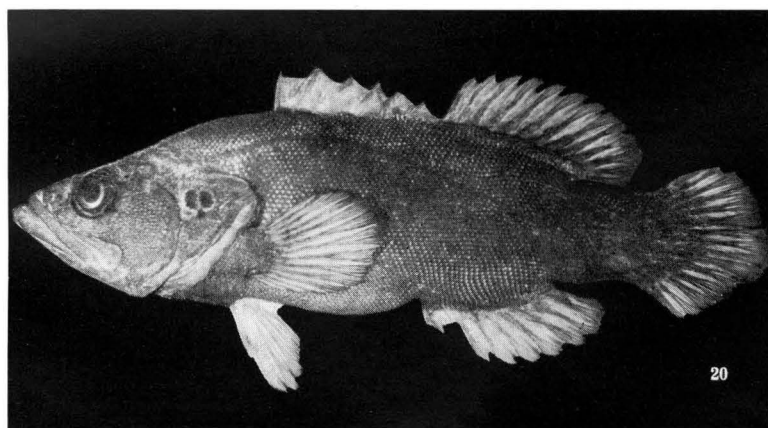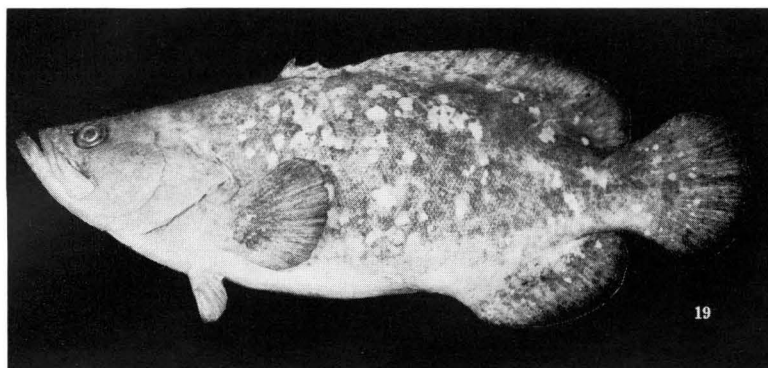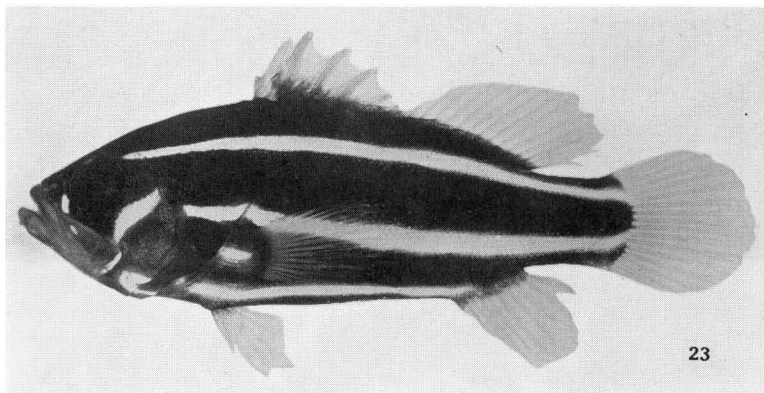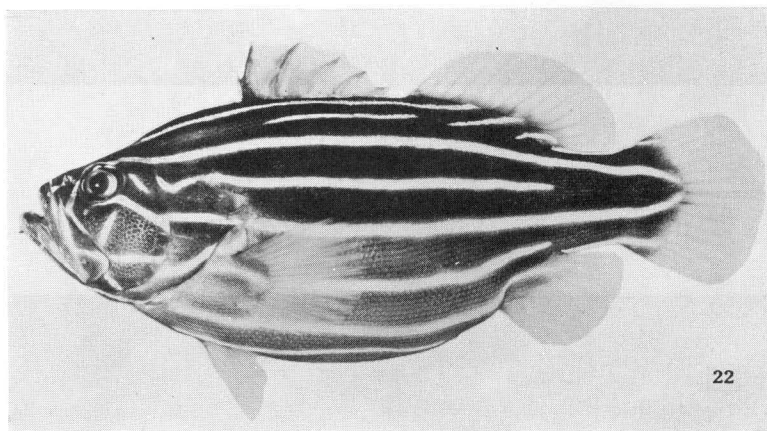Figure 18.   *Rypticus randalli*, 127 mm SL, Puerto Rico.

Figure 19. *Rypticus bicolor,* 199 mm SL, Isla Las Animas, Gulf of California.

Figure 20 *Grammistops ocellatus,* 112 mm SL, Tahiti.
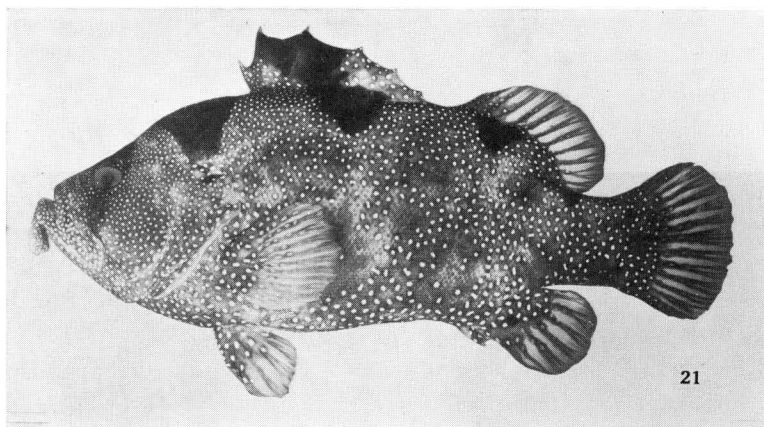
Figure 21.   *Pogonoperca punctata*, 173 mm SL, Washington Island, Line Islands.
Figure 22.   *Grammistes sexlineatus*, 75 mm SL, Guam. Abdomen distended from a half-digested fish (about 45 mm in SL and 10 mm in depth) in stomach.
Figure 23.   *Grammistes sexlineatus*, 34 mm SL, Eniwetok Atoll, Marshall Islands.

(BLEEKER, 1874), Misol and Seychelles (BOULENGER, 1895), Nauru (WHITLEY and COLEFAX, 1938), Japan (KATAYAMA, 1960b and KAMOHARA, 1962), and the Ryukyu and Amami Islands (HASHIMOTO and KAMIYA, 1969).

We record the species herein from Jarvis Island (1° 55′ N; 157° 20′ W) and Washington Island (4° 43′ N; 160° 26′ W) in the Line Islands. The Jarvis record is based on a specimen (USNM 205492, 148 mm SL) collected by F. SIBLEY in a channel on the SW side of the island at a depth of 10 feet on November 18, 1964. The Washington specimen (BPBM 7746, 173 mm SL) was collected off the west side of the island from beneath a small ledge in 55 feet by the senior author and J. McVEY on November 3, 1968.

A second specimen of *Pogonoperca punctata* at the Museum of Comparative Zoology (MCZ 25771, 158 mm standard length) collected by Andrew GARRETT bears the label Sandwich Islands (=Hawaiian Islands). There is no date of collection. In spite of intensive fish collecting in the Hawaiian Islands since GARRETT's time, no additional specimens of *Pogonoperca* have been obtained. GARRETT collected numerous fishes elsewhere in the Pacific, especially in French Polynesia, and there is a strong possibility of locality error for the Hawaiian record.

Our largest specimen (from Okinawa) measured 300 mm in total length (about 250 mm SL) and weighed 510 g. BOULENGER reported the total length of his largest specimen (under the name *P. ocellata*) as 330 mm.

Grammistes sexlineatus

Figures 22 and 23

Perca sexlineata THUNBERG, 1792. Vet. Akad. Nya Handl., vol. 13, p. 142, pl. 5 (type locality, East Indies).

Diagnosis: Dorsal rays VII,13 or 14; anal rays II,9; pectoral rays 16 to 18; lateral-line scales 60 to 72; vertical scale rows above lateral line to caudal base 82 to 88; scales cycloid, embedded, none on interorbital, snout, maxillary, or fins except basal fleshy portion of dorsal and anal fins, basal half of caudal fin, and basal part of paired fins; gill rakers shorter than longest gill filaments, with 1 to 3 + 7 to 9 fully formed on first arch (plus 4 to 6 low tubercles on upper limb and 4 to 7 on lower); a small fleshy flap (smaller than pupil of eye) on tip of chin; body oblong, the depth 2.3 to 2.8 in standard length, and compressed, the width 2 to 2.7 in depth; head length 2.3 to 2.7 in standard length; mouth large and very oblique, the maxillary reaching from posterior to center of eye to slightly beyond eye; upper preopercular margin with 2 to 4 short broad-based spines; subopercle and interopercle smooth; interorbital slightly to moderately convex; pelvic fins 1.8 to 2.5 in head length; fins not fleshy except basally; a deep notch nearly separating spinous and soft portions of dorsal fin; third (rarely the second) dorsal spines the longest, 2.9 to 3.4 in head length; first dorsal spine 1.7 to 2 in second; color dark brown with light yellow stripes; fin

rays of median fins pink to reddish brown, the membranes pale. Juveniles up to about 50 mm SL, such as the one of Figure 23, have three yellow stripes on the side, one from behind upper eye to upper base of caudal fin, one from opercle running above pectoral base to lower base of caudal fin, and the third along lower chest and abdomen; in addition there is a median yellow band from snout to origin of dorsal fin and prominent yellow markings on head; there is a small black spot at front of upper lip. With increasing size, secondary yellow stripes appear in the dark brown zones between the yellow stripes, the first on the side of nape, running to beneath the soft portion of the dorsal fin; these stripes develop from the anterior part of fish and are narrower than the initial three stripes. By a length of about 75 mm the stripes are complete, resulting in six on a side (not including median dorsal band on head and nape), but with the original stripes still wider. With increasing size, the stripes become equal in width and two more may be added, as shown in Figure 22. In large adults the yellow stripes break up into series of dashes.

Remarks: This species has often been recorded under the name *Grammistes orientalis* Bloch and Schneider (1801). Three other synonyms have long been recognized, *vittata, triacantha* and *pentacantha,* all described by Lacépède (1802).

Arnoult in Arnoult, Bauchot-Boutin, and Roux-Estève (1958) named *Grammistes lineatus* as new from the island of Aldabra in the Indian Ocean. The description, which was based on a single specimen 46 mm in total length, and the figure strongly suggest that *G. lineatus* is a juvenile of *G. sexlineatus*. The specimen has three pale stripes on the body as may be seen on Fig. 23 herein of *G. sexlineatus,* a specimen of about the same size (the stripes are broader on *lineatus,* but we have other specimens of *sexlineatus* which have stripes this broad). The only significant difference between *lineatus* and *sexlineatus* appeared to be the dorsal ray count of VI,7 for the former. Oddly, this was not mentioned specifically by Arnoult as a distinction between the species. M. L. Bauchot examined the holotype for us at the Muséum National d'Histoire Naturelle in Paris. She reported that the dorsal rays are VII,13. Therefore we refer *G. lineatus* to the synonymy of *G. sexlineatus*.

Fowler (1946) described a new subspecies, *Grammistes sexlineatus proerythraeus,* from a single specimen 33 mm in total length from the Ryukyu Islands. He distinguished it by the scarlet front of the spinous dorsal fin. Kamohara (1964) recognized the subspecies, giving its range as Kochi Prefecture, Japan, and Ryukyu Islands. He recorded the range of *Grammistes sexlineatus sexlineatus* as Mie Prefecture, Japan to the Red Sea; Africa; Australia and Polynesia. Such a distribution for two subspecies is illogical. More important, the distinction given by Fowler is probably normal juvenile coloration for the species. The specimen of Figure 23, 34 mm in standard length and 43 mm in total length, from Eniwetok, Marshall Islands, had a dorsal fin which was bright red in life anterior to the second dorsal spine; this color extended onto the basal portion of the second interspinous membrane; therefore, there seems to be no justification for the subspecific differentiation as proposed by Fowler.

Grammistes sexlineatus has the broadest distribution of any species of soapfish. It is recorded from east Africa as far south as Durban (by SMITH, 1949, who noted that the flesh is bitter) to the islands of eastern Oceania except Hawaii and Easter Island. It is known from western and northern Australia, and ranges north to southern Japan. The Bishop Museum has specimens from the Philippines, Palau Islands, Solomon Islands, Guam, Marshall Islands, Marcus Island, Fiji, Samoa, Society Islands, and Tuamotus. SMITH (1949) stated that the species attains 10 inches (254 mm). Our largest specimen measured 144 mm in total length (about 5.7 inches); it weighed 64 grams. Mr. S. MIKAMI of the aquarium at Yomiuriland, Japan informed us that he has a preserved specimen which is 270 mm (10.6 inches) in total length.

Summary

A toxin in the mucus of soapfishes (Grammistidae) has been detected by a number of methods, including its distinctive bitter taste. It is present to a varying degree in all the genera. By the taste test *Grammistes* and *Pogonoperca* gave the strongest reaction, *Aulacocephalus* and *Diploprion* an intermediate reaction, and *Grammistops* and *Rypticus* the weakest. The skin toxin appears to be completely lacking in the related families Serranidae and Pseudogrammidae.

The amount of toxin secreted increases when a fish is frightened. Other fishes confined in a small volume of water with a soapfish under stress soon die. Previous investigations have revealed that soapfish toxin is lethal to mammals when force-fed in capsules or injected. The symptoms in cats show some similarity to ciguatera.

The toxin causes hemolysis of mammalian blood cells. Through tests of hemolytic activity on rabbit blood cells it was shown that the soapfish mucus alone contains much more toxin than ground skin tissue. Judging from hemolysis, the toxin of *Grammistes sexlineatus* and *Pogonoperca punctata* is more concentrated than that of other soapfishes. Taste tests also indicate that these two species have a strong toxin.

When equivalent amounts of toxin (as determined by hemolytic activity) from a species of each of the six grammistid genera were dissolved in water for the determination of ichthyotoxic activity, it was noted that the death time of the test fish, *Oryzias latipes*, was nearly the same for all preparations.

The principal function of the toxin would seem to be defensive. When a lionfish (*Pterois volitans*) was fed a small live *Grammistes sexlineatus* in an aquarium, it quickly expelled the soapfish and made no further attempt to feed upon it.

Chemical properties of the toxin, drawing in part on HASHIMOTO and KAMIYA (1969), are as follows: insoluble in diethyl ether, petroleum ether, and chloroform; partially soluble in water, physiological saline, 10% acetic acid and hot ethanol; effectively soluble in hot 70% ethanol, *n*-butanol, and *n*-amyl alcohol. In an alkaline medium (pH 12) the toxin is not heat stable, but no loss of activity takes place when heating in an acid medium (pH 2). Dialysis carried out at 5°C for 5 hours resulted

in 35% of the activity in the outer solution.

Thin-layer chromatograms from the preparations of toxin for hemolytic activity were similar for the six species tested. Spraying the cellulose plate with 2% rabbit blood cell suspension revealed 4 or 5 toxic components. These were almost the same as those positive to the Dragendorff reagent.

Further purification was carried out for the toxin of *Grammistes sexlineatus*, *Pogonoperca punctata* and *Rypticus saponaceus* by silicic acid column chromatography. Each preparation consisted of a peculiar peptide with a moiety of tertiary or quarternary amine. The total nitrogen varied from 8.8 to 12.7%, and amino acid nitrogen from 54 to 91%. The four dominant amino acids of the toxin of all three species are leucine, isoleucine, phenylalanine, and glycine.

The differences in the composition of amino acids and the ultraviolet and infrared spectra among the species may be due to differences in purity of the preparations. In view of the many similarities, we believe the toxin, which we name grammistin, to be the same or very similar throughout the family Grammistidae.

Histological preparations of the skin of soapfishes revealed two types of mucous cells in the epidermis, one like that found in fishes in general, and the other which was negative to PAS reaction but positive to Sudan Black B staining. In addition, for all the species tested except *Diploprion bifasciatum* and *Aulacocephalus temmincki*, there are multicellular glands in the dermis between the scales which stain with Sudan Black B and have ducts that lead to the surface. Grammistin also stains black with the same reagent.

The soapfishes have generally been regarded as a subfamily, at best, of the Serranidae in the older literature. GOSLINE (1960) proposed that they be classified as a separate family, the Grammistidae, but he included the pseudogrammids, excluded *Diploprion,* and did not consider *Aulacocephalus.* SCHULTZ (1966) recognized the family Grammistidae, included the genera *Diploprion* and *Aulacocephalus,* added *Rainfordia* and retained the pseudogrammids. Nevertheless, he expressed doubt of the validity of the family, stating that the only character common to all grammistid genera and not the Serranidae is the membraneous attachment of the inner pelvic ray to the body.

We can exclude *Rainfordia* on morphological grounds from the Grammistidae. The pseudogrammids, with an incomplete lateral line (and for some a secondary lateral line), 26 to 28 vertebrae, and scales of moderate size, might also be separated on morphology alone. One additional character, a heavy coat of mucus containing the skin toxin grammistin serves to distinguish the Grammistidae from both the Serranidae and the Pseudogrammidae.

We recognize six genera in the Grammistidae. Of these, *Diploprion* and *Aulacocephalus* appear to be more closely related to the Serranidae than the other grammistid genera. They stand apart morphologically from the rest of the grammistids in their possession of ctenoid scales, a nasal organ with the lamellae arranged as radii of a

circle, a strongly serrate preopercle, and some serrations on the interopercle and subopercle.

Diploprion is characterized by a dorsal fin formula of VIII,13 to 16 and pelvic fins which reach posterior to the anus. It is represented by two species, the deep-bodied *D. bifasciatum* which is light yellow with two broad black bars, and *D. drachi* with less body depth and no black bars. The latter is known from only two specimens from the Red Sea; additional descriptive data are presented for it, and it is illustrated for the first time. *D. bifasciatum* ranges from India through the Indo-Malayan region to China and Japan.

Aulacocephalus is represented by one species, *temmincki*, which is bright blue with a yellow stripe running from the snout through the eye and along the upper back; its dorsal rays are IX,12. With the exception of a dubious record from Thailand, the distribution of this fish consists of the western Pacific and Indian Oceans between latitudes 20° to 36° N and S.

Rypticus is a distinctive genus which lacks anal spines, has II to IV dorsal spines and 20 to 27 rays, and 16 or 17 (instead of 15) caudal rays. COURTENAY (1967) revised the eight Atlantic species; in addition, three are known from the eastern Pacific (one of which is undescribed); none are colorful.

Also drab is *Grammistops ocellatus,* named for the dark ocellus on the opercle. It has a dorsal formula of VII,12 (rarely VI,13). It was described from one specimen from the Marshall Islands and recorded since from two specimens from East Africa. The range is here extended to include Palau, Guam, and Tahiti.

Pogonoperca appears to be monotypic. We regard *P. ocellata* GÜNTHER and *P. reticulatus* STEINDACHNER (in BLISS) as probable synonyms of *P. punctata* (CUVIER and VALENCIENNES). This fish is characterized by a large fleshy flap on the tip of the chin, dorsal rays VIII,12 or 13; anal rays III,8, and a brown color with numerous white spots and black saddle-like areas along the back. It is recorded from several islands of the Indian Ocean, the western Pacific, and in Oceania from Tahiti, Nauru, and the Caroline Islands. We collected the species at Washington Island in the Line Islands and the Ryukyus.

Grammistes sexlineatus also has a flap on the chin but it is very small. It is otherwise distinguished by dorsal rays VII,13 or 14; anal rays II,9 and its dark brown to black color with bright yellow stripes. Juveniles have 3 such stripes on the side; these increase in number to 8 in adults, and they break up into series of dashes on large adults. This fish has the broadest distribution of all the soapfishes: east Africa to French Polynesia, and it is the most common of the Indo-Pacific species.

Grammistes lineatus ARNOULT (1958) is a synonym of *Grammistes sexlineatus*.

FOWLER's separation of a subspecies, *Grammistes sexlineatus proerythraeus,* based on a single 33 mm specimen from the Ryukyus, is not tenable.

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