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Comparative Studies of the Genus *Echinometra* from Okinawa and Mauritius

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ABSTRACT—Okinawan sea urchins, the genus *Echinometra*, are four independent species. But which species are the same species as *E. mathaei* and *E. oblonga* as described by Blainville 1825 is still open to question. To answer this question, a field survey of genus *Echinometra* was made in Mauritius (the type locality of *E. mathaei*) according to the characteristics used to classify Okinawan *Echinometra*: appearance, pore pairs, spicules in gonads and tubefeet, sperm shape, and distribution on a reef. The results of cross-fertilization between *Echinometra* from Mauritius and Okinawa are also reported. Mauritian *Echinometra* are classified into three groups which resemble Okinawan *Echinometra* sp. B, D, and violet spine color *Echinometra*. The latter has almost the same characteristics as Okinawan *Echinometra* sp. B but with violet spines, a spine color not found in Okinawa. In cross-fertilization experiments, the sperm of Mauritian *Echinometra* sp. B-like and violet *Echinometra* fertilized Okinawan *Echinometra* sp. B with almost 100% success. However, fertilization was unsuccessful with other Okinawan *Echinometra* species. Therefore, it could be said that Okinawan *Echinometra* sp. B is the same as Mauritian *Echinometra* sp. B-like, and the counterparts of Okinawan *Echinometra* sp. A and C are not distributed in Mauritius. The descriptions of *E. mathaei* most match Mauritian *Echinometra* sp. B-like and the type locality of *E. mathaei* is Mauritius. Thus, it is probable that Mauritian *Echinometra* sp. B-like is *E. mathaei*. Therefore, Okinawan *Echinometra* sp. B, which most resembles Mauritian *Echinometra* sp. B-like, would be also *E. mathaei*. *Echinometra* sp. A and C would be considered to be new species. *Echinometra* sp. D is thought to be the same species as Mauritian black *Echinometra*. Whether Okinawan *Echinometra* sp. D is the same as *E. oblonga* remains as a problem for future research. Because the type locality of *E. oblonga* is not known and, it is suggested that the *E. oblonga* described in the Indo-West Pacific is a complex species.

INTRODUCTION

The sea urchin *Echinometra mathaei* was described by Blainville (1825) on the basis of a single rather small sea urchin from Mauritius. *Echinometra oblonga* was also described in the same paper on the basis of two rather small individuals. The locality of the latter species was unknown. Döderlein (1906) elevated *E. oblonga* to a separate genus, *Mortensia oblonga*, because of the morphology of the spicules in the gonads, but Mortensen (1943) claimed these as morphs of *E. mathaei*, and gave it a trinomial name, *E. mathaei oblonga*, as if it were a subspecies. Clark and Rowe (1971) followed Mortensen's nomenclature. Kelso (1970) did an extensive study on the ecological distribution, test morphology, spicules in gonads, and gamete compatibility of these two morphs, *E. mathaei* and *E. mathaei oblonga*, using Hawaiian individuals. In this study he strongly argued that these two morphs were separate species, *E. mathaei* and *E. oblonga*.

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E. mathaei occurs in abundance on shallow reefs throughout the tropical to warm Indo-Pacific region (Mortensen, 1943). *E. mathaei* is well known as a species showing extensive morphological variation in test shape and spine color (Mortensen, 1943). The Okinawan *Echinometra* species are no exception. Okinawan *E. mathaei* was divided into two types, A and B, based on spine color. These types showed different distribution patterns, habitat preference, and agonistic behavior (Tsuchiya and Nishihira, 1984, 1985). Uehara and Shingaki reported that these two types were impossible to cross-fertilize and that there were differences in larval morphology and karyotype (Uehara and Shingaki, 1984). Uehara and co-workers (Uehara and Shingaki, 1985; Uehara *et al*., 1986, 1990; Uehara and Taira, 1987) discovered two additional types, C and D, based on chromosome and spicule characteristics and gamete incompatibility. These studies showed that the four Okinawan types are different species. Recent biochemical studies on enzyme electrophoresis (Matsuoka and Hatanaka, 1991) and on mitochondrial DNA (Palumbi and Metz, 1991) have also suggested that these types are distinct, but very closely related, species. However, the scientific names of these

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four complex species found in Okinawa are still unclear. This was the reason why the individuals of *Echinometra* from Mauritius, the type locality of *E. mathaei*, and the individuals of *Echinometra* from Okinawa were compared. Okinawan *Echinometra* are tentatively described in this paper as *Echinometra* sp. A, B, C, and D.

The distinguishing criteria of Blainville's classification of *E. mathaei* and *E. oblonga* were based on the number of pore-pairs per ambulacral plate, the number of interambulacral tubercle rows, and the color of the specimen (Blainville, 1825). On the other hand, Mortensen included many criteria for his classification: spicules in gonads, tubefeet and the intestinal wall, the apical system, pedicellaria, shape and color of spines, shape and size of test and larval form (Mortensen, 1943). Overlapping criteria included the number of pore-pairs per ambulacral plate. These characteristics were not enough for describing the differences among the four Okinawan species of *Echinometra*. To distinguish the Okinawan *Echinometra* species, the characteristics observed on the live specimens, such as chromosome, sperm shape (Arakaki, 1989; Arakaki and Uehara, 1989), and cross-fertilization, were needed. The characteristics comparisons and cross-fertilization experiments between Okinawan *Echinometra* and Mauritian *Echinometra* were conducted. Based on these studies, an attempt was

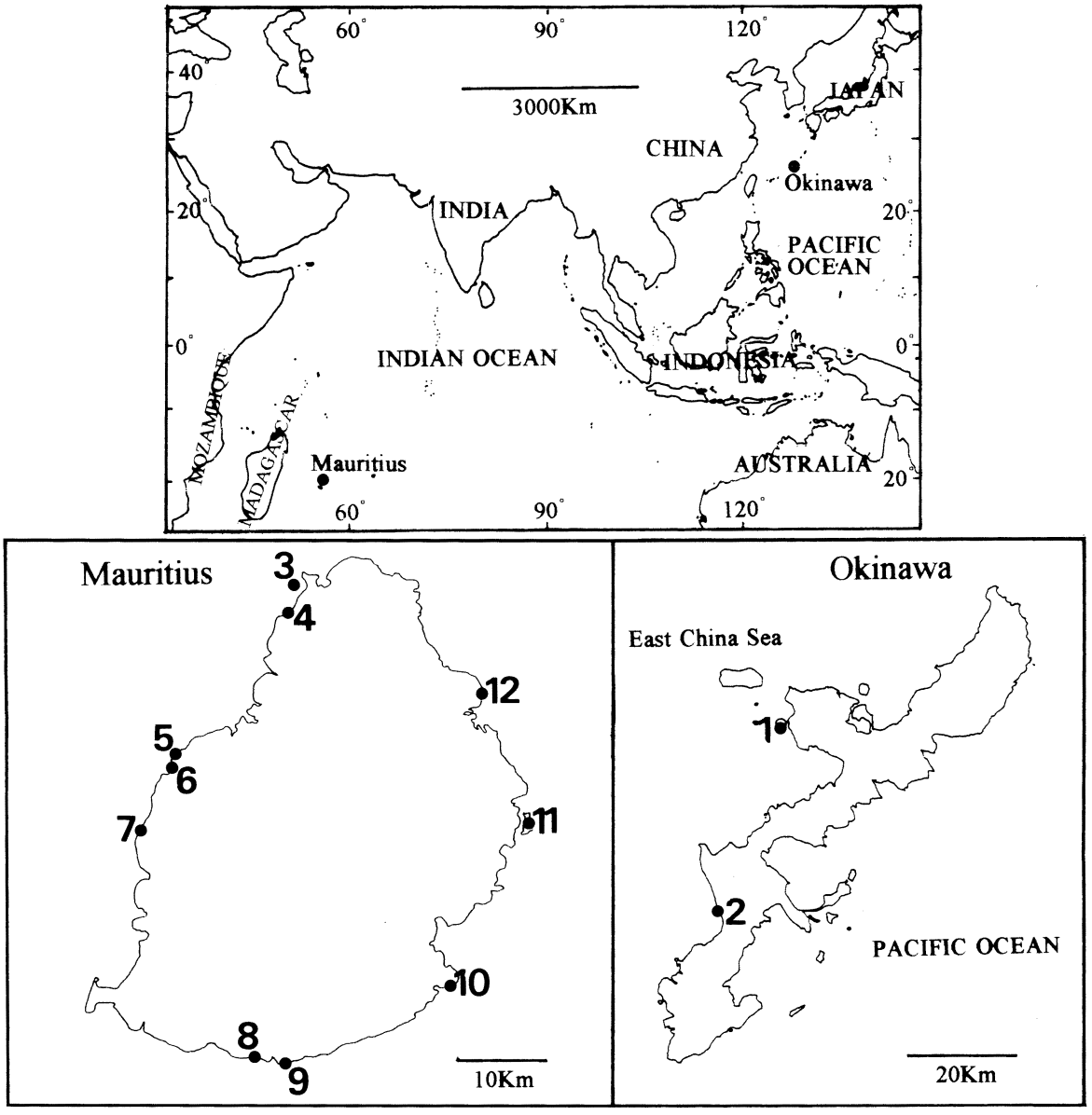

Fig. 1. A map of the Indian Ocean and the Pacific Ocean (above) with enlarged maps of Mauritius (left) and Okinawa (right) below. The figures on the maps indicate the sites where the research was conducted in Mauritius, and the sampling was conducted in Okinawa, respectively. 1, Sesoko Island; 2, Sunabe coast; 3, Off Pointe aux Cannoniers; 4, Trou aux Biches; 5, Pte aux Caves; 6, Albion; 7, Flic en Flac; 8, Riambel; 9, Gris Gris; 10, Blue Bay; 11, Ile aux Cerfs; 12, Poste Lafayette.

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made to find counterparts to Okinawan *Echinometra* among the Mauritian *Echinometra* species. Since the type locality of *E. mathaei* is Mauritius, the *E. mathaei* found in Mauritius must be the same as the original *E. mathaei*. A comparison of the *Echinometra* found in Mauritius with those found in Okinawa shows which species of Okinawan *Echinometra* is *E. mathaei*.

MATERIALS AND METHODS

Individual *Echinometra* specimens were collected at Sesoko Island and at Sunabe along Okinawa's coast in 1995 and 1996. In Mauritius, ten sites were investigated in 1995 (Arakaki and Fagoonee, 1996) and *Echinometra* specimens were collected from Trou aux Biches, Pte aux Caves, Albion, and Post Lafayette. These ten sites were selected to cover almost all possible environments where *Echinometra* were expected to live (Fig. 1). These were categorized in three groups: moats protected by coral reefs, rocky shores exposed to strong wave action and a slope beyond the outer-reef. The moats were shallow, 1 m deep, but wide, ranging from 100 m at their narrowest to 1000 m at their broadest. The beach sand consisted of white biogenic calcareous grains. The substratum of the moat was covered with such sand, mid-distant from the coastline to the reef. The outer-half of the moat had coral growth. Reefs sites were as follows: Trou aux Biches, Albion, Flic en Flac, Riambel, Blue Bay, and Ile aux Cerfs. The rocky shores were subjected to wave action and were unprotected by reefs at Pte aux Caves, Gris Gris, and Poste Lafayette. Although Gris Gris had a reef, it was narrow (about 20 m wide). Within the reef no sand subtratum was formed, and wave action was strong both inside the reef and at the reef edge. The outer-reef slope, off Pointe aux Cannoniers, was studied using SCUBA at a depth of about 20 m.

The characteristics for describing the differences among Okinawan *Echinometra* spp. were: color of spines, brightness of milled ring and skin around the peristome, spicules in the tubefeet and gonads, the number of pore pairs per ambulacral plate and sperm shape. The Brightness of the milled ring and the skin around the peristome were two important factors, besides the color of the spines, for discriminating the four *Echinometra* species in the field in Okinawa. Discrimination for Mauritian *Echinometra* was also done based on these visible characteristics.

The spicules in the gonads were investigated under a microscope (10×10). A small portion of gonad was clipped off using a forceps and put on a slide glass and covered by a cover glass and pressed. Several tubefeet were clipped off with a forceps, and the morphology of the spicules was investigated by the same method as above.

The number of pore-pairs per ambulacral plate was counted under the dissecting microscope in every ambulacral plate in a denuded test.

The observation of sperm was carried out with a scanning electron microscope. Sperm samples were obtained as undiluted semen, i.e., "dry sperm" by removing the testes. The samples were prefixed for 30 min at low temperature (about 4°C) with a 1% osmium tetroxide fixative in filtered (0.2 μm) seawater and then postfixed with the same concentration of the osmium tetroxide fixative for one hour at low temperature (about 4°C). After postfixation, the samples were dehydrated in a graded series of ethanol (50% to 80%) for preservation. For the observation, the preserved sperm samples were rehydrated, terminating at 0% ethanol concentration. Several drops of the sample were dropped on a poly-l-lysine coated round cover glass (15 mm in diameter) to spread and adhere the sperm to the cover glass. The glass was put in a balance bottle. The process of dehydration in a graded series of ethanol (50% to 100%) was carried out in the bottle. After dehydration, these samples were treated with isoamyl acetate for 60 min and dried at the critical point of CO_2, and coated with palladium gold using an Ion Coater (Eiko, IB-1). Observations and photography of sperm morphology were carried out with a Hitachi S-530 scanning electron microscope.

Preservation of gametes, which were used for cross-fertilization, was done as follows: Some part of the gonads was removed from live samples and rinsed with filtered (0.2 μm)seawater and wiped with filter paper and put into a stock tube (1.5 cc) and then kept at low temperature (4-5°C) just before fertilization. Since it was possible to preserve sperm longer than eggs, only sperm was preserved for the purpose of cross-fertilization. The sampling of sperm from Mauritian *Echinometra* spp. for cross-fertilization experiments was carried out with the Albion individuals on the 31st of March, 1996, and the eggs of Okinawan individuals (from Sesoko Island) were fertilized on the 3rd of April, 1996. The sperm from the Mauritian *Echinometra* spp. were preserved for three days before cross-fertilization. The shedding of eggs was induced by injection of 0.5 *M* KCl into the coelomic cavity. Eggs were collected by inverting female urchins on a beaker of filtered (0.2 μm) seawater.

RESULTS

Comparative characteristics

Echinometra from Okinawa and Mauritius are shown in Fig. 2. *Echinometra* sp. A is characterized by white-tipped spines, a definite bright milled ring and dark skin on the peristome. *Echinometra* sp. B is characterized by spines with no white tip, with very faded milled rings and dark skin on the peristome. *Echinometra* sp. C is characterized by spines without a white tip, but with a definite bright milled ring and bright skin on the peristome. In the field, these two *Echinometra* spp. have exhibit a richly colored variation in spines. *Echinometra* sp. D is characterized by deep black spines with a faded milled ring and dark skin around the peristome. Only black-spined individuals have been found so far. Mauritian *Echinometra* individuals could be divided into three groups by appearance. One of the *Echinometra* which has spines without a white tip, but with a definite bright milled ring and dark skin on the peristome resembles Okinawan *Echinometra* sp. B described as *Echinometra* sp. B-like. The spine color of the latter is as abundant as Okinawan *Echinometra* sp. B and C. The individual in Fig. 2 has a bright milled ring, but individuals which do not have a bright milled ring were also found in the field. The second group of individuals resembles the first group in appearence but has violet spines, described as Violet *Echinometra*. Violet spine color is very unusual, as it is found neither in Okinawa nor in the other parts of the western Pacific, such as Indonesia, Guam, or Hawaii. The last one has deep black spines with a faded milled ring and dark skin on the peristome, and bears a resemblance to Okinawan *Echinometra* sp. D described as Black *Echinometra*.

It is reported that Okinawan *Echinometra* have three types of spicules, i.e., bihamate spicules (*Echinometra* sp. A and B) and triradite spicules (*Echinometra* sp. C and D) in the tubefeet and needle spicules (*Echinometra* sp. A and B) and triradite spicules (*Echinometra* sp. C and D) in the gonads (Uehara, 1990). However, in these studies, spicules which are found in the tubefeet and the gonads are divided into four types: bihamete, needle, triradite, and multiple (Fig. 3). "Multiple" means various types of spines. These spicules occur alone or in combination, such as bihamate and triradite, or bihamate-

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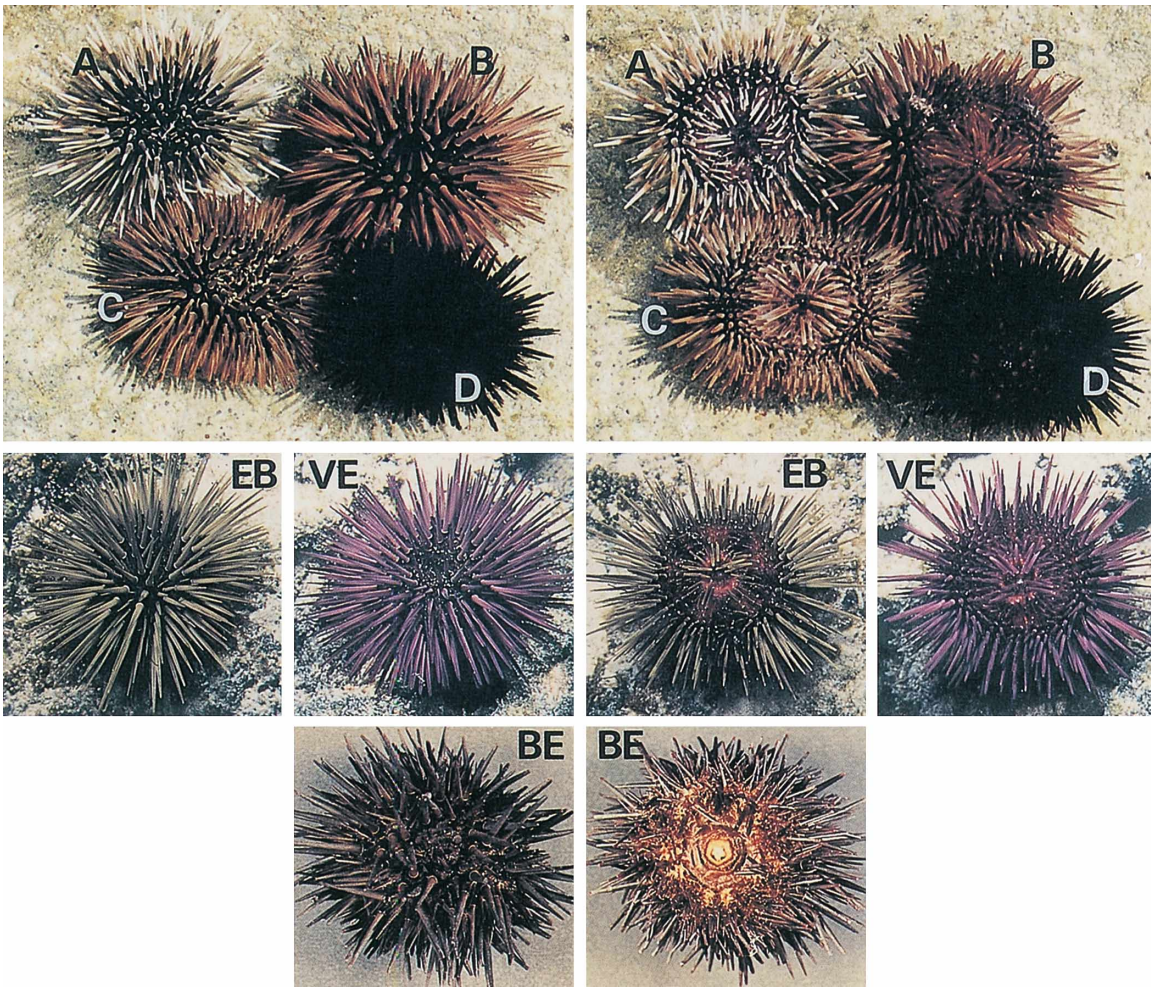

Fig. 2. The species of *Echinometra* from Okinawa and Mauritius. The left and the right half are aboral and oral side views, respectively. The letters, A, B, C, and D indicate *Echinometra* sp. A, B, C, and D from Okinawa, respectively, and VE, EB and BE indicate Violet *Echinometra*, *Echinometra* sp. B-like, Black *Echinometra* from Mauritius, respectively. * The Black *Echinometra* individual photographed as a specimen preserved in alcohol, but the others were live specimens. The color of the Black *Echinometra* was faded due to the effects of alcohol preservation.

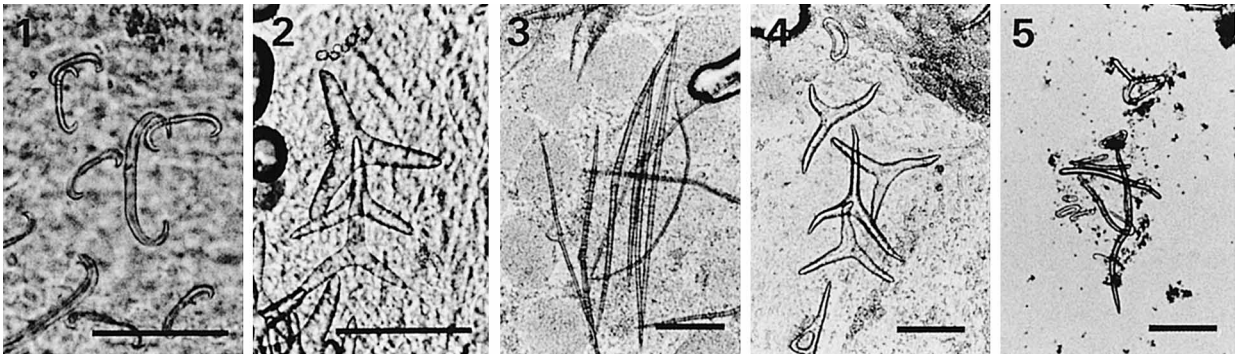

Fig. 3. The shape of spicules from *Echinometra*. 1: Bihamate spicules in tubefeet from Okinawan *Echinometra*. sp. A. 2: Triradiate spicules in tubefeet from Mauritian Black *Echinometra*. 3: Needle spicules in gonads from Okinawan *Echinometra* sp. B. 4: Triradiate spicules in gonads from Okinawan *Echinometra* sp. C. 5: Multiple spicules in gonads from Mauritian Black *Echinometra*. The longer (1 and 2) and shorter bars (3, 4, and 5) indicate 50 μm and 100 μm, respectively.

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Table 1. Appearance and spicules in tubefeet and gonads of *Echinometra* from Okinawa and Mauritius. A, B, C, D, VE, EB, and BE denote *Echinometra* sp. A, B, C, D, Violet *Echinometra*, *Echinometra* sp. B-like, and Black *Echinometra*, respectively. The figure in parentheses indicates the number of individuals.

Region	Species	Color of spines	Milled ring	Skin of peristome	Spicule		Ratio (%)
					Tubefeet	Gonads	
Okinawa	A (n=11)	Various colors with white tip	Bright	Dark	Bihamate	Needle	100
	B (n=11)	Various colors	Dark	Dark	Bihamate	Needle	54.55
			Dark	Dark	Bihamate & Triradiate	Needle	45.45
	C (n=10)	Various colors	Bright	Bright	Bihamate & Triradiate	Triradiate	70
			Bright	Bright	Triradiate	Triradiate	10
			Bright	Bright	None	Triradiate	10
			Bright	Bright	Bihamate & Triradiate	None	10
	D (n=16)	Black	Dark	Dark	Triradiate	Triradiate	43.75
			Dark	Dark	Bihamate	Triradiate	18.75
			Dark	Dark	Bihamate & Triradiate	Triradiate	6.25
			Dark	Dark	None	Triradiate	6.25
			Dark	Dark	Triradiate	Multiple	6.25
			Dark	Dark	Bihamate & Triradiate	Multiple	6.25
			Dark	Dark	Bihamate	Multiple	6.25
			Dark	Dark	None	None	6.25
Mauritius	VE (n=16)	Violet	Dark	Dark	Bihamate & Triradiate	Needle	37.5
			Dark	Dark	Bihamate, Triradiate, Needle	Needle	37.5
			Dark	Dark	Bihamate	Needle	18.75
			Dark	Dark	Triradiate	Needle	6.25
	EB (n=34)	Various colors	Bright	Dark	Bihamate	Needle	26.47
			Dark	Dark	Bihamate & Triradiate	Needle	17.65
			Dark	Dark	Bihamate, Triradiate, Needle	Needle	14.71
			Dark	Dark	Triradiate	Needle	11.76
			Bright	Dark	Bihamate & Triradiate	Needle	11.76
			Dark	Dark	Bihamate	Needle	5.88
			Bright	Dark	Triradiate	Needle	5.88
			Bright	Dark	Bihamate	Multiple	2.94
			Bright	Dark	Bihamate	None	2.94
	BE (n=13)	Black	Dark	Dark	Triradiate	Multiple	46.15
			Dark	Dark	Triradiate	Triradiate & Needle	15.38
			Dark	Dark	None	Needle	15.38
			Dark	Dark	None	Triradiate & Needle	7.69
			Dark	Dark	None	Multiple	7.69
			Dark	Dark	Triradiate	Needle	7.69

triradiate-needle in the tubefeet, and triradiate and needle in the gonads (Table 1). Spicules in the tubefeet are variable, but it is possible to consider the bihamate spicule as a characteristic of *Echinometra* sp. A, B, E. Violet *Echinometra* and *Echinometra* sp. B-like; and triradiate spicules as a characteristic of *Echinometra* sp. C, D, and Black *Echinometra*. Each of these spicule types was found among many individuals of *Echinometra*.

Spicules in the gonads are comparatively stable. The needle spicule is a characteristic of *Echinometra* sp. A, B, Violet *Echinometra*, and *Echinometra* sp. B-like. The triradiate spicule is a characteristc of *Echinometra* sp. C, D, and Black *Echinometra*. The multiple spicule is found only in *Echinometra* sp. D and Black *Echinometra*. Accordingly, *Echinometra* from Okinawa and Mauritius can be gathered into two groups by the spicule shape in the tubefeet and the gonads: *Echihometra* sp. A, B, Violet *Echinometra*, and *Echinometra* sp. B-like; and *Echinometra* sp. C, D, and Black *Echinometra*.

The number of pore-pairs is also a characteristic for discriminating *Echinometra*. The ratio of 4 pore pairs is dominant in all of the *Echinometra* specimens from Okinawa and Mauritius (Fig. 4). These *Echinometra* can be grouped into three according to the ratio of the number of pore-pairs. The first group includes *Echinometra* sp. A, B, Violet *Echinometra*, and *Echinometra* sp. B-like; they have the highest ratio of 4 pore-pairs. Within the group, *Echinometra* sp. B and *Echinometra* sp. B-like differ significantly in pore-pair ratio (Table 2). The second group has only one member, *Echinometra* sp. C. The second group is similar to the first group; however, the number of 4 pore-pairs is less than the first group's while the number of 3 pore-pairs and 5 pore-pairs is more than that of the first group. The ratio of 4 pore-pairs is

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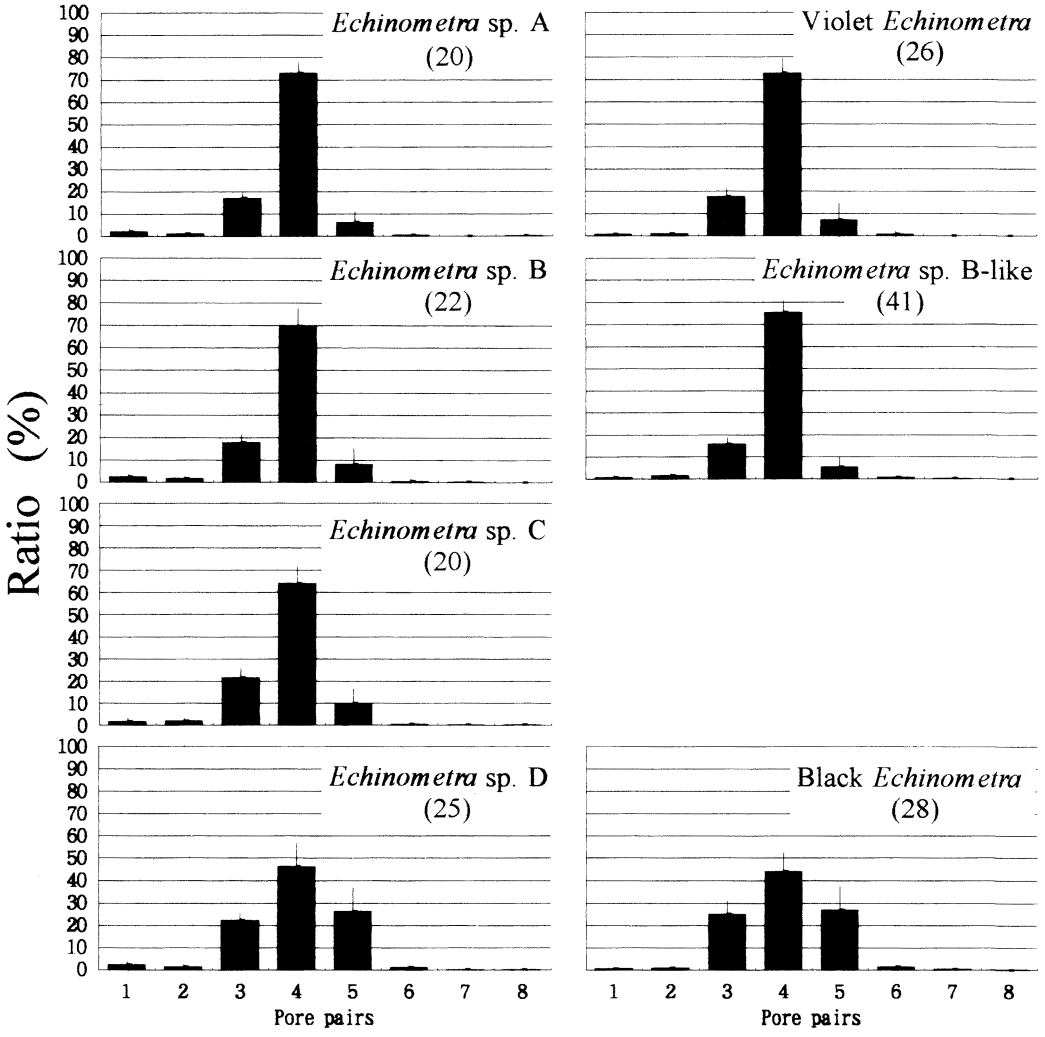

Fig. 4. The ratio of pore pairs of *Echinometra* from Okinawa (left) and Mauritius (right). The figure in parentheses indicates the number of individuals. The values indicate the mean ± S.D.(%).

Table 2. Comparison of the ratio of four pore pairs of *Echinometra* from Okinawa and Mauritius. The values indicate a P value which was calculated by ANOVA. The symbols, ***, **, *, and –, indicate significant levels, $P < 0.001$, $P < 0.01$, $P < 0.05$, and not significant levels, respectively. A, B, C, D, VE, EB, and BE denote *Echinometra* sp. A, B, C, D, Violet *Echinometra*, *Echinometra* sp. B-like, and Black *Echinometra*, respectively. The figure in parentheses indicates the number of individuals.

	Okinawan			
Mauritian	A (n = 20)	B (n = 22)	C (n = 20)	D (n = 25)
VE (n = 26)	0.931 –	0.171 –	2.25×10^{-4} ***	5.84×10^{-14} ***
EB (n = 41)	0.148 –	0.00295 **	3.21×10^{-8} ***	2.35×10^{-21} ***
BE (n = 28)	2.61×10^{-17} ***	2.23×10^{-14} ***	1.18×10^{-10} ***	0.423 –

significantly different from the first group (Table 2). The third group, including *Echinometra* sp. D and Black *Echinometra*, has the lowest ratio of 4 pore-pairs and the highest ratio of 5-pore pairs (Fig. 4). There is no significant difference in the ratio of 4 pore-pairs between the members of the group (Table 2).

Sperm size and shape vary among Okinawan *Echinometra* spp. These differences are also found in Mauritian *Echinometra* (Fig. 5, Table 3). The sperm head size and shape of Okinawan *Echinometra* change gradually to longer and slender in accordance with the order of *Echinometra* sp. A, B, and C. The size changes from 2.91 to 3.45 μm, and the shape (length/width) changes from 2.34 to 3.07. The sperm size and shape of *Echinometra* sp. D are very different from the others, being very long (5.89 ± 0.13 μm) and slender (6.11 ± 0.48). The sperm size and shape of Mauritian Violet *Echinometra* and *Echinometra* sp. B-like are almost the same, resembling those of *Echinometra* sp. B. The sperm of Mauritian Black

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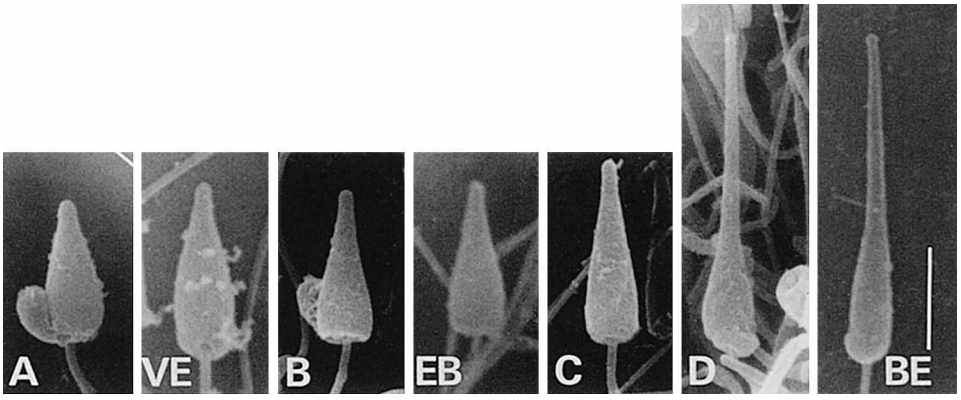

Fig. 5. Sperm of Okinawan *Echinometra* (A, B, C, D) and Mauritian *Echinometra* (VE, EB, BE). A, B, C, D, VE, EB, and BE denote *Echinometra* sp. A, B, C, D, Violet *Echinometra*, *Echinometra* sp. B-like, and, Black *Echinometra*, respectively. The bar indicates 2 μm.

Table 3. Sperm head size (length and width) and shape (length/width) of *Echinometra* from Okinawa and Mauritius. The values indicate the mean ± S.D.. A, B, C, D, VE, EB, and BE denote *Echinometra* sp. A, B, C, D, Violet *Echinometra*, *Echinometra* sp. B-like, and Black *Echinometra*, respectively. The figure in parentheses indicates the number of sperm. Sperm was obtained from one individual of each species. The data for Okinawan *Echinometra* are from Arakaki (1989).

	Okinawan				Mauritian		
	A (n = 50)	B (n = 50)	C (n = 50)	D (n = 50)	VE (n = 20)	EB (n = 20)	BE (n = 20)
Length (μm)	2.91 ± 0.19	3.22 ± 0.16	3.45 ± 0.16	5.89 ± 0.31	3.25 ± 0.13	3.16 ± 0.16	6.28 ± 0.40
Width (μm)	1.24 ± 0.05	1.2 ± 0.05	1.12 ± 0.05	0.97 ± 0.05	1.34 ± 0.04	1.27 ± 0.05	1.06 ± 0.06
Shape (L/W)	2.34 ± 0.18	2.67 ± 0.18	3.07 ± 0.18	6.11 ± 0.48	2.43 ± 0.12	2.49 ± 0.15	5.97 ± 0.55

Table 4. Cross-fertilization among *Echinometra* from Okinawa and Mauritius. The values indicate the mean fertilization ratio ± S.D.(%). These values were calculated by three replicates of 100 inseminated eggs. A, B, C, VE, EB, and BE denote *Echinometra* sp. A, B, C, Violet *Echinometra*, *Echinometra* sp. B-like, and Black *Echinometra*, respectively. The sperm used for the insemination of Okinawan *Echinometra* was collected from different individuals which had inseminated Mauritian *Echinometra*.

	Mauritian (♀)		Okinawan (♀)		
Mauritian (♂)	VE	EB	A	B	C
VE	95 ± 2.83	98 ± 1.41	0.33 ± 0.47	99.67 ± 0.47	12 ± 2.94
EB	98.33 ± 1.25	100	0.33 ± 0.47	100	11.67 ± 1.25
BE	0	2 ± 0.82	–	–	–

Echinometra is a little bit longer and wider than that of Okinawan *Echinometra* sp. D, but the shape is similar (Fig. 5, Table 3).

Cross-fertilization

The ratio of cross-fertilization between Mauritian Violet *Echinometra* and *Echinometra* sp. B-like is very high, with almost 100% success (Table 4). The successful combinations between Black *Echinometra* sperm and Violet *Echinometra*, and *Echinometra* sp. B-like eggs, however, are very low, 0 and 2 ± 0.82%, respectively (Table 4). Sperm from both Mauritian Violet *Echinometra* and *Echinometra* sp. B-like easily fertilize the eggs of Okinawan *Echinometra* sp. B, with 99.67 ± 0.47 and 100% success. Fertilization of eggs from Okinawan *Echinometra* sp. A and C by the sperm of Mauritian Violet *Echinometra* is very low, especially the eggs of *Echinometra* sp. A. The cross-fertilization using Okinawan *Echinometra* sp. A and B is almost impossible (Uehara *et al.*, 1990). *Echinometra* sp. B, Mauritian Violet *Echinometra* and *Echinometra* sp. B-like are not possible to discriminate in terms of cross-fertilization; they are all easily fertilized. However, Okinawan *Echinometra* sp. A and C are apparently different from the Mauritian *Echinometra*, Violet *Echinometra* and *Echinometra* sp. B-like.

Distribution of the *Echinometra* spp. in Mauritius

The ten sites surveyed in Mauritius cover habitats ranging from calm water (eg. off Pointe aux Cannoniers) to shallow (Trou aux Biches, Albion, Flic en Flac, Riambel, Blue Bay, Ile aux Cerfs) and very rough water (Pte aux Caves, Gris Gris,

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Table 5. Abundance and distribution of *Echinometra* in Mauritius. VE, EB, and BE denote Violet *Echinometra*, *Echinometra* sp. B-like, and Black *Echinometra*, respectively. Symbols, ++, +, and –, denote abundance of species: abundant, not many, and not found, respectively.

Site	Environmental condition	Species		
		VE	EB	BE
Off Pointe aux Cannoniers	Beyond reef, calm water	–	–	–
Trou aux Biches	Shallow moats, calm water	++	++	–
Pte aux Caves	Rocky shore, strong waves	–	–	++
Albion	Shallow moats, calm water	++	++	+
Flic en Flac	Shallow moats, calm water	++	++	–
Riambel	Shallow moats, calm water	++	++	–
Gris Gris	Rocky shore, strong waves	+	+	+
Bule Bay	Shallow moats, calm water	++	++	–
Ile aux Cerfs	Shallow moats, calm water	++	++	–
Poste Lafayette	Rocky shore, strong waves	+	+	+

Poste Lafayette) conditions (Table 5). The substratum, varies from sand, sandy bottom with seaweeds, shingle, coral rubble, coral, to rock. These sites cover almost all the possible environments where *Echinometra* live.

Violet *Echinometra* and *Echinometra* sp. B-like tended to be distributed in calm water, and Black *Echinometra* in strong wave areas, such as Pte aux Caves, Gris Gris, and Poste Lafayette (Table 5). These distribution patterns on the reef are similar to those of Okinawan *Echinometra* sp. B and D, respectively (Arakaki, 1989; Nishihira *et al*., 1991; Tsuchiya and Nishihira, 1984).

DISCUSSION

The white-tipped spines of *Echinometra* sp. A are its most prominent characteristc. Therefore discrimination of this species can be done by this characteristic in the field. No Mauritian *Echinometra* individuals have this type of spine. In regard to spicules in the tubefeet and the gonads, pore-pair number, sperm shape, and distribution on a reef, it is rather difficult to discriminate *Echinometra* sp. A from *Echinometra* sp. B-like and Violet *Echinometra*, but cross-fertilization between them is almost impossible. From the results of cross-fertilization it should be understood that *Echinometra* sp. A and those of Mauritian *Echinometra*, Violet *Echinometra* and *Echinometra* sp. B-like are different species. Cross-fertilization between *Echinometra* sp. A and Black *Echinometra* was not carried out because viable sperm of the species was not sampled. However, certain differences (the length and the shape of sperm head and 4 pore-pairs ratio) between them are apparent. It is understood that if the sperm is different, the species should be different (Koike, 1985). Okinawan *Echinometra* sp. C is characterized by a definite milled ring, bright skin on the peristome, triradiate spicule in the tubefeet and the gonads, and a somewhat longer and slender sperm head than *Echinometra* sp. A and B. Among these characteristics, the brightness of the milled ring and the skin on the peristome, and the shape of spicules in the tubefeet are not so reliable because these characteristics vary. However, the shape of spicules in the gonads is stable, with *Echinometra* sp. C having triradiate spicules only. In Mauritian *Echinometra*, Black *Echinometra* have triradiate spicules, but Violet *Echinometra* and *Echinometra* sp. B-like do not have this type of spicule. Cross-fertilization between *Echinometra* sp. C and Violet *Echinometra*/*Echinometra* sp. B-like has a very low success rate. The sperm head size and shape, and the 4 pore-pairs ratio of *Echinometra* sp. C are very different from those of Black *Echinometra*. Thus, it could be said that the counterparts of Okinawan *Echinometra* sp. A and C are not distributed in Mauritius.

Almost all of the characteristics of Violet *Echinometra* and *Echinometra* sp. B-like are similar, namely, the brightness of the milled ring and the skin on the peristome, spicules in tubefeet/gonads, ratio of pore-pairs, and the sperm shape. In addition, they easily fertilize each other. It is not possible to distinguish Violet *Echinometra* from *Echinometra* sp. B-like by these characteristics. However, it is possible to recognize Violet *Echinometra*, and distinguish them from *Echinometra* sp. B-like, solely on the basis of their violet-colored spines as those are only found in the Violet *Echinometra*.

Echinometra sp. B share common characteristics with Violet *Echinometra* and *Echinometra* sp. B-like, and fertilization between them is very successful. However, individuals with violet spines, the spine color of Violet *Echinometra*, were never found in Okinawa. Some *Echinometra* sp. B-like individuals have bright milled rings, but this characteristic is variable. Many individuals, presumed to be the same species as Okinawan *Echinometra* sp. B, with a bright milled ring, are found in Indonesia (Arakaki and Uehara, 1995). Among these three *Echinometra*, Mauritian Violet *Echinometra* are distinguished by violet-colored spines, but it is not possible to distinguish *Echinometra* sp. B from Mauritian *Echinometra* sp. B-like. Thus, it is appropriate to infer that Okinawan *Echinometra* sp. B is the same as Mauritian *Echinometra* sp. B-like.

Black *Echinometra* are distinguished by uniform black spines, 4 pore-pairs ratio, sperm head shape, cross-fertilization, and distribution on a reef from Violet *Echinometra* and

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Echinometra sp. B-like. Black *Echinometra* are also distinguished from Okinawan *Echinometra* sp. A, B, C by these characteristics. Cross-fertilization between Mauritian Black *Echinometra* and Okinawan *Echinometra* was not performed because viable sperm of Black *Echinometra* was not brought back to Okinawa. But the data are sufficient to show Black *Echinometra* are a different species from Okinawan *Echinometra* sp. A, B, C. Cross-fertilization between Okinawan *Echinometra* sp. D and Mauritian *Echinometra* was not conducted, but *Echinometra* sp. D can be distinguished from Violet *Echinometra* and *Echinometra* sp. B-like by the characteristics mentioned above. Resemblances between Okinawan *Echinometra* sp. D and Mauritian Black *Echinometra* are noted in the uniform back spines, dark milled ring and skin on the peristome, 4 pore-pairs ratio, sperm head shape, and distribution on a reef. Therefore, it could be said that Okinawan *Echinometra* sp. D is the same as Mauritian Black *Echinometra*.

The above mentioned Mauritian *Echinometra* were described as *E. mathaei* (*Echinometra* sp. B-like), and *E. mathaei* violacea (Violet *Echinometra*) by Mortensen (1943). *E. mathaei violacea* was described as follows: the test of *E. mathaei violacea* agrees with a typical *E. mathaei*. But it is not possible to apply the naked tests to the *E. mathaei violacea* because when these are naked their distinguishing characteristic is no longer visible. In addition to their highly distinct purplish violet spines, *E. mathaei violacea* abundantly developed tridentate pedicellariae and two dense series of triradiate spicules in the aboral tubefeet. The distribution of this variety is restricted to the western Indian Ocean. Research was carried out by Arakaki and Fagoonee (1996) on this variety which was collected in Mauritius and Mahé (Seychelles). This variety is not found in Okinawa, Indonesia and other parts of the Pacific Ocean. It is found in the west Indian Ocean only. It is meaningful to distinguish this variety from *E. mathaei*.

Mortensen (1943) described *Echinometra*, which has black spines, as *E. mathaei oblonga*. While Kelso (1970) described this sea urchin as *E. oblonga* based on his studies done in Hawaii. Among Hawaiian *Echinometra*, it was shown that *E. mathaei* and *E. oblonga* were impossible to cross-fertilize (Kelso, 1970; Metz *et al*., 1994). Therefore, it is approprite to describe *Echinometra*, which has black spines, as *E. oblonga* or as a species separate from *E. mathaei*.

Among the four species of Okinawan *Echinometra*, it could be said that *Echinometra* sp. B is the same as *E. mathaei* while the other species are different. Because the type locality of *E. mathaei* is Mauritius (Blainville, 1825), *Echinometra* sp. B, which has the most similar characteristics with Mauritian *E. mathaei*, would be *E. mathaei*. However, it is indispensable to consult the holotype of *E. mathaei* in the future. For Okinawan *Echinometra* sp. A and C, a new species name would be needed. Okinawan *Echinometra* sp. D is thought to be the same species as Mauritian *E. oblonga*, but whether Okinawan *Echinometra* sp. D is *E. oblonga* remains as a ploblem for future research. Because the type locality of *E. oblonga* is not known, it is suggested that the *E. oblonga* describe in the Indo-West Pacific is a complex species (Uehara, personal communication). To decide on a species name for Okinawan *Echinometra* sp. D, thorough research is needed in the future.

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