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Authors: Arakaki, Yuji, and Uehara, Tsuyoshi

Source: Zoological Science, 16(3) : 551-558

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.16.551

ZOOLOGICAL SCIENCE **16**: 551–558 (1999)

Morphological Comparison of Black *Echinometra* Individuals among Those in the Indo-West Pacific

Yuji Arakaki[1]* and Tsuyoshi Uehara[2]

[1]*Department of Tourism, Meio University, Bimata, Nago, Okinawa, Japan and*
[2]*Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa, Japan*

ABSTRACT—It is almost impossible to discriminate each region's black *Echinometra* based on appearance, such as the color of spines, milled ring, and skin around the peristome. However, it is possible to divide them into several groups based on such characteristics as the spicules of the gonads and tubefeet, the pore-pairs, the shape of the sperm, and the pedicellaria. The percentage of spicules of the gonads and tubefeet depends on the region the individuals inhabit. The five pore-pair percentage of Guamanian individuals is very low, and this is significant compared to the individuals of the other regions. The sperm morphology of Guamanian and Hawaiian individuals is significantly more compact than that of Mauritian, Indonesian, Okinawan, and Boninian individuals, while the difference between the Guamanian and Hawaiian individuals is also significant. The tridentate pedicellaria of the Hawaiian individuals are longer than those of the other regions' individuals, and the difference is significant. The length differences of the ophiocephalous pedicellaria and trifiliate pedicellaria are significant depending on the individuals of the region, and it is possible to discriminate individuals of each region into several groups. Guamanian and Hawaiian individuals are separated into different groups based on all these characteristics, and it is also possible to discriminate these two groups from the other regions' individuals by several characteristics. Therefore, black *Echinometra* individuals living in the Indo-West Pacific are divided into three groups, i.e., Mauritian, Indonesian, Okinawan, Boninian individuals; Guamanian individals; and Hawaiian individuals. The number of pore-pairs and morphology of the sperm in the Okinawan *Echinometra* species complex reflect species level differences, and it is suggested that cross-fertilizations between black *Echinometra* from Okinawa and Guam as well as Okinawa and Hawaii were not successful. This indicates that the three groupings based on these characteristics reflect a species level difference. In other words, the black *Echinometra* inhabiting the Indo-West Pacific are a species complex, or a cryptic species composed of at least three species.

INTRODUCTION

The species are too similar to discriminate in appearance, but they are regarded as independent species in the natural population called cryptic species or sibling species (Walker, 1964). The four species of Okinawan *Echinometra* are one such example. In the beginning, these four species of Okinawan *Echinometra* were regarded as a single species, *Echinometra* mathaei. However, later, ecological (Nishihira *et al*., 1991), morphological (Uehara and Shingaki, 1985), and biochemical studies (Matsuoka and Hatanaka, 1991), showed that these species are independent, and they are presently considered to be sibling species (Matsuoka and Hatanaka, 1991). These four species are described as *Echinometra* spp. A, B, C, and D, respectively (Arakaki *et al*., 1998). *Echinometra* spp. B and D are considered *E. mathaei* and *E. oblonga*, respectively, whereas *Echinometra* spp. A and C are considered altogether new species (Arakaki *et al*., 1998).

E. oblonga was first described by Blainville in 1825, but the type locality was not described. The body color was described as "white or perhaps violet" in the original paper (Blainville, 1825). That means the color of the spines or the color of the naked test was not evident. But Kelso (1970) understood that the color was the color of the naked test. Döderlein (1906) described the *E. oblonga* as always having unicolored spines, whereas the spines of *E. mathaei* were often lightly colored at their tip. Clark (1925) suggested *E. oblonga*'s spines were black, particularly the thick ones. Edmondson (1935) also described *E. oblonga*'s spines as black, and those of *E. mathaei* as other than black; that is, green, gray, or reddish-brown. His discrimination of these *Echinometra* based on the color of the spines was criticized by Mortensen (1943) as too simple. However, it is common to regard the black spined *Echinometra* as *E. oblonga* in the Indo-

* Corresponding author: Tel. +81-980-51-1081;
FAX. +81-980-52-4640.
E-mail: arakaki@tor.meio-u.ac.jp

West Pacific (Kelso, 1970; Michel, 1974; Russo, 1977; Paulay, 1989; Metz *et al*., 1991, 1994; Metz and Palumbi, 1996; Palumbi, 1996; Palumbi *et al*., 1997).

The black *Echinometra* comparisons were conducted on Okinawan individuals (identical with *Echinometra* sp. D) and Mauritian individuals (classified as *E. oblonga* by Michel, 1974) and they were considered the same species (Arakaki *et al*., 1998). However, the comparisons on black *Echinometra* individuals other than from these regions in the Indo-West Pacific were not conducted. In this paper, black *Echinometra* comparisons conducted in the six regions in the Indo-West Pacific show that the black *Echinometra* populations inhabiting the Indo-West Pacific are not a single species but rather a species complex, or cryptic species.

MATERIALS AND METHODS

Individuals collected in Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii were used as the materials for the comparison (Fig. 1). Until recently, only two species of *Echinometra*, identical with Okinawan *Echinometra* spp. A and B, respectively, were known from Guam (Uehara, 1990; Uehara *et al*., 1996). However, it was revealed that black *Echinometra* are also distributed in Guam. To date, black *Echinometra* individuals have been found only in the central part of Guam east coast in front of "Guam Aquaculture Development and Training Center" which is washed by strong wave action. The characteristics used for comparisons are the color of the spines, the milled ring, the color of the peristome, the number of pore-pairs, the spicules in the gonads and tubefeet, the pedicellaria morphology, and the sperm morphology. The color of the spines was described from live samples whereas characteristics other than sperm were studied from samples preserved in 70% ethanol. Sperm from the samples was preserved in 80% ethanol fixed with 1% osmium tetroxide-seawater.

Number of pore-pairs

The number of pore-pairs on every ambulacral plate (of the five ambulacra from the apical system to the peristomial system) was counted under a dissecting microscope.

Morphology of the sperm

Sperm was observed with a scanning electron microscope. The sperm was fixed with a 1% osmium tetroxide-seawater dehydrate in a graded series of ethanol, dried at the critical point of CO_2. Photographs were taken of the sperm. The size of the sperm was measured using a pair of calipers on a photographic image of the sperm. For detailed observation methods, see Arakaki *et al*. (1998).

Morphology of the pedicellaria

For easy collection of the pedicellaria, all the spines around the body were removed under a dissecting microscope with a pair of tweezers. The pedicellaria were preserved in 70% ethanol and were prepared for observations/photography. The area on a body where the pedicellaria were collected was from the peristomial to the apical system on both sides of the long and short axis. An attempt was made to collect all pedicellaria in an area on a body, but there were too many, particularly ophiocephalous pedicellaria, which are very small. The size of the pedicellaria was measured with a pair of calipers on a photographic image. The classification of the pedicellaria is based on Campbell's classification (Campbell, 1983).

RESULTS

Color of spines, milled reing, and skin of peristome

The milled ring is dark, and the skin color around the peristome is also dark in all of the individuals from each region (Table 1). There are no differences in these characteristics. However, the color of the spines is not the same in all of the individuals. Four Boninian individuals have dark brown spines, not black spines (Table 1); however, this difference is very minute.

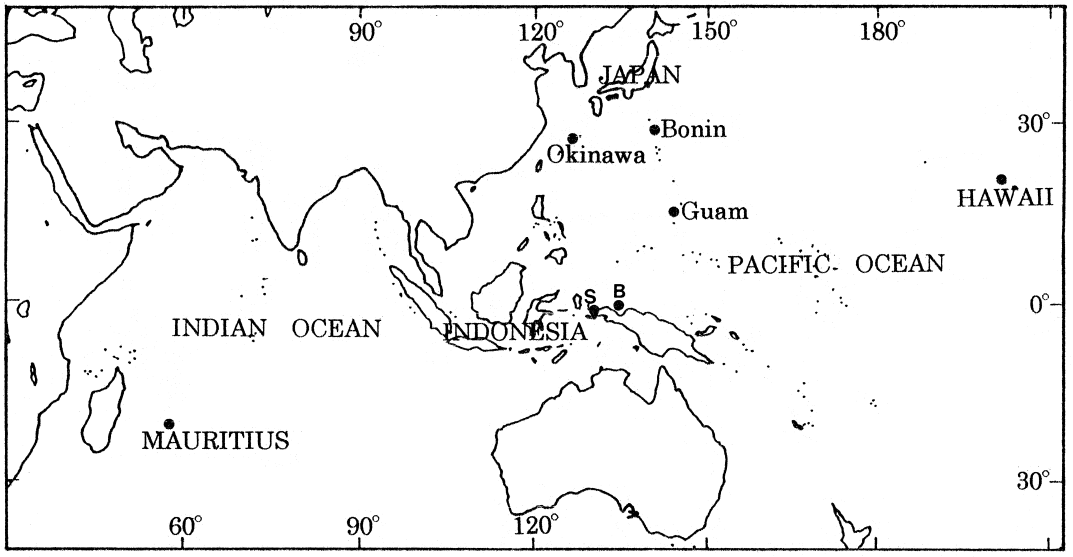

Fig. 1. Sampled regions of black *Echinometra* in the Indo-West Pacific. The sampling was conducted in: Mauritius, Trou aux Biches, Pet aux Cave, Albion, Post Lafayette; Indonesia, Kasuari Cape (Sorong, marked "S" in the figure), Arunamu (Biak I., marked "B" in the figure); Okinawa, Sunabe, Sesoko I.; Bonin Is, Sakaiura (Chichi-jima), Miyanohama (Chichi-jima); Guam, the central part of Guam east coast; Hawaii, Turtle Bay (Oahu I.).

Table 1. Color of spines, milled ring, skin of the peritome, and spicules in gonads and tubefeet of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The figure in parentheses indicates the number of individuals.

Region	Color of spines	Milled ring	Skin of peristome	Spicule		Percentage
				Gonads	Tubefeet	
Mauritius	Black	Dark	Dark	Multiple	Triradiate	46.2
(n=13)	Black	Dark	Dark	Triradiate & Needle	Triradiate	15.4
	Black	Dark	Dark	Needle	None	15.4
	Black	Dark	Dark	Triradiate & Needle	None	7.7
	Black	Dark	Dark	Multiple	None	7.7
	Black	Dark	Dark	Needle	Triradiate	7.7
Indonesia	Black	Dark	Dark	Triradiate	None	44.4
(n=9)	Black	Dark	Dark	Triradiate	Triradiate	22.2
	Black	Dark	Dark	None	None	22.2
	Black	Dark	Dark	None	Triradiate	11.1
Okinawa I.	Black	Dark	Dark	Triradiate	Triradiate	43.8
(n=16)	Black	Dark	Dark	Triradiate	Bihamate	18.8
	Black	Dark	Dark	Triradiate	Triradiate & Bihamate	6.3
	Black	Dark	Dark	Triradiate	None	6.3
	Black	Dark	Dark	Multiple	Triradiate	6.3
	Black	Dark	Dark	Multiple	Triradiate & Bihamate	6.3
	Black	Dark	Dark	Multiple	Bihamate	6.3
	Black	Dark	Dark	None	None	6.3
Bonin I.	Black	Dark	Dark	Multiple	Triradiate	15.4
(n=13)	Black	Dark	Dark	Triradiate	Triradiate	7.7
	Black	Dark	Dark	Triradiate	Bihamate	7.7
	Black	Dark	Dark	Triradiate & Multiple	Bihamate	7.7
	Black	Dark	Dark	Multiple	None	7.7
	Black	Dark	Dark	Multiple	Bihamate	7.7
	Black	Dark	Dark	None	None	7.7
	Black	Dark	Dark	–	Bihamate	7.7
	Dark brown	Dark	Dark	Triradiate	Bihamate	7.7
	Dark brown	Dark	Dark	Needle	Triradiate & Bihamate	7.7
	Dark brown	Dark	Dark	None	Triradiate	7.7
	Dark brown	Dark	Dark	–	Triradiate	7.7
Guam	Black	Dark	Dark	Multiple	Bihamate	45.5
(n=11)	Black	Dark	Dark	Multiple	Triradiate	27.3
	Black	Dark	Dark	Multiple	Triradiate & Needle	9.1
	Black	Dark	Dark	Multiple	Triradiate & Bihamate	9.1
	Black	Dark	Dark	Multiple	None	9.1
Hawaii	Black	Dark	Dark	Multiple	Triradiate	35.3
(n=17)	Black	Dark	Dark	Multiple	None	11.8
	Black	Dark	Dark	Needle	Triradiate	11.8
	Black	Dark	Dark	None	Triradiate	11.8
	Black	Dark	Dark	Triradiate	Triradiate	5.9
	Black	Dark	Dark	Triradiate	Triradiate & Bihamate	5.9
	Black	Dark	Dark	Multiple	Triradiate & Bihamate	5.9
	Black	Dark	Dark	Multiple	Bihamate	5.9
	Black	Dark	Dark	Needle	–	5.9

Spicules

The spicules found in the gonads are triradiate, multiple, needle, and a combination of these (Table 1). Two individuals out of seventy-nine individuals in all appear without spicules. The majority of the spicules is multiple and triradiate, but this depends on the region: the Guamanian individuals have multiple spicules only; Mauritian and Hawaiian individuals have more multiple than triradiate spicules; the Indonesian individuals have triradiate spicules only; and the Boninian individuals have about 50% multiple and about 50% triradiate spicules. Needle spicules are found in Mauritian, Boninian, and Hawaiian individuals, but at a low percentage.

The spicules found in the tubefeet are triradiate, bihamate, needle, and a combination of these. One individual out of seventy-nine individuals in all appears without spicules. The majority of the spicules is triradiate and bihamate while needle spicules are found in just one individual. The individuals which have more triradiate spicules than other types of spicule are from Okinawa and Hawaii, and the individuals which have about 50% triradiate and about 50% bihamate spicules are

from Bonin and Guam. Mauritian and Indonesian individuals have only triradiate spicules.

Number of pore-pairs

The percentage of pore-pairs of each region's individuals is shown in Table 2. The four-pore-pair percentage (40% to 50%) is the highest, while the three- and five- pore-pair percentages (20% to 30%) are the next highest in the individuals from Mauritius, Indonesia, Okinawa, Bonin, and Hawaii. In contrast, the Guamanian individuals have almost the same percentage, both of three- and four-pore-pairs, while the percentage of five-pore-pairs is very low (7%). The difference in five-pore-pairs between Guamanian individuals and the other regions' individuals is significant ($p<0.005$) (Table 3).

Morphology of the sperm

Sperm from Mauritian, Okinawan, and Boninian individuals is slender while sperm from Guamanian and Hawaiian individuals is compact (Table 4). Differences between these two, the slender sperm and the compact sperm, are significant (Table 5). Differences between the sperm from Guamanian and Hawaiian individuals are significant in length and shape, but not in width. Differences among the sperm from Mauritian, Okinawan, and Boninian individuals are small. These differences, however, are statistically significant in the shape at least.

Morphology of the pedicellaria

The pedicellaria found are tridentate, ophiocephalous, trifoliate, and globiferous. Among these, the head-length of

Table 2. The percentage of each pore-pair of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The values indicate the mean±S.D. (%). The figure in parentheses indicates the number of individuals.

Region	Number of pore-pairs (%)							
	1	2	3	4	5	6	7	8
Mauritius (n=28)	0.61±0.69	0.95±0.79	25.27± 5.86	44.11± 8.75	27.17±10.49	1.41±1.03	0.40±0.42	0.09±0.26
Indonesia (n=16)	1.55±1.26	1.65±0.93	24.04± 4.23	49.76±11.71	21.45±11.41	1.24±1.68	0.20±0.43	0.12±0.47
Okinawa (n=25)	2.32±1.40	1.48±1.01	22.33± 3.36	46.30±10.73	26.24±10.92	1.01±1.14	0.25±0.39	0.08±0.24
Bonin (n=16)	0.90±0.62	2.75±1.45	24.53± 3.69	44.46± 7.24	25.19± 7.84	1.99±2.76	0.15±0.26	0.03±0.12
Guam (n=13)	0.89±0.64	2.74±1.45	42.28±11.73	46.37± 7.54	7.08± 8.25	0.56±0.94	0.08±0.26	0.00
Hawaii (n=20)	2.08±1.97	2.10±1.35	28.51± 3.71	41.63±14.14	24.23±14.04	1.22±1.57	0.17±0.26	0.05±0.24

Table 3. Comparison of the percentage of five pore-pairs of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The values indicate a P value calculated by ANOVA. The symbols, ***, **, *, and –, indicate significant levels, $P<0.001$, $P<0.01$, $P<0.05$, and insignificant levels, respectively. The figure in parentheses indicates the number of individuals.

	Indonesia (n=16)	Okinawa (1=25)	Bonin (n=16)	Guam (n=13)	Hawaii (n=20)
Mauritius (n=28)	0.107 –	0.758 –	0.526 –	6.43×10^{-7} ***	0.42 –
Hawaii (n=20)	0.537 –	0.278 –	0.813 –	0.000543 ***	
Guam (n=13)	0.001051 **	4.33×10^{-6} ***	3.31×10^{-6} ***		
Bonin (n=16)	0.303 –	0.748 –			
Okinawa (n=25)	0.197 –				

Table 4. Sperm head-size (length and width) and shape (length/width) of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The values indicate the mean±S.D. The figure in parentheses indicates the number of sperm. The sperm from Guam and Hawaii was collected from four individuals each while the sperm from Okinawa and Bonin was collected from one individual each.

	Mauritius (n=20)	Okinawa (n=50)	Bonin (n=20)	Guam (n=80)	Hawaii (n=42)
Length (μm)	6.46±0.81	5.97±0.61	6.89±0.45	2.70±0.17	3.16±0.21
Width (μm)	1.08±0.12	0.97±0.05	1.01±0.06	1.19±0.06	1.19±0.08
Shape (L/W)	5.97±0.55	6.19±0.76	6.34±0.56	2.27±0.18	2.67±0.26

Table 5. Comparison of sperm head-size (length and width) and shape (length/width) of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The values indicate a P value calculated by ANOVA. The symbols, ***, **, *, and –, indicate significant levels, P<0.001, P<0.01, P<0.05, and insignificant levels, respectively. The figure in parentheses indicates the number of sperm. The sperm from Guam and Hawaii was collected from four individuals each while the sperm from Okinawa and Bonin was collected from one individual each.

	Okinawa (n=50)			Bonin (n=20)			Guam (n=80)		
	Length	Width	Shape	Length	Width	Shape	Length	Width	Shape
Mauritius (n=20)	0.0093 **	4.05×10^{-7} ***	0.25 –	0.046 *	0.812 –	0.05 –	2.83×10^{-60} ***	2.17×10^{-7} ***	3.53×10^{-71} ***
Hawaii (n=42)*	5.98×10^{-66} ***	1.22×10^{-32} ***	3.24×10^{-66} ***	5.65×10^{-69} ***	3.95×10^{-6} ***	2.61×10^{-60} ***	1.35×10^{-31} ***	0.85 –	6.64×10^{-21} ***
Guam (n=80)*	4.61×10^{-80} ***	7.27×10^{-45} ***	8.3×10^{-79} ***	2.76×10^{-82} ***	1.65×10^{-9} ***	1.52×10^{-74} ***			
Bonin (n=20)	7.75×10^{-8} ***	1.91×10^{-8} ***	0.45 –						

	Hawaii (n=42)		
	Length	Width	Shape
Mauritius (n=20)	1.59×10^{-49} ***	2.03×10^{-5} ***	9.16×10^{-57} ***
Hawaii (n=42)*			
Guam (n=80)*			
Bonin (n=20)			

Table 6. Pedicellaria, tridentate, ophiocephalous, and trifoliate head-length of black *Echinometra* from Mauritius, Indonesia, Okinawa, Bonin, Guam, and Hawaii. The values indicate the mean±S.D. The figure in parentheses indicates the number of pedicellaria.

Type of pedicellaria	Size of pedicellaria (μm)					
	Mauritius	Indonesia	Okinawa	Bonin	Guam	Hawaii
Tridentate	347±114 (n=32)	330±110 (n=14)	402±201 (n=14)	427±195 (n=40)	347±62 (n=8)	675±307 (n=42)
Ophiocephalous	249± 26 (n=43)	289± 35 (n=28)	310± 47 (n=60)	351± 25 (n=40)	302±25 (n=59)	373± 60 (n=25)
Trifoliate	111± 13 (n=41)	118± 12 (n=19)	145± 16 (n=38)	138± 13 (n=110)	121±13 (n=32)	146± 14 (n=7)

tridentate, ophiocephalous, and trifoliate pedicellaria was measured (Table 6). The length of the tridentate pedicellaria in Hawaiian individuals is extremely long, and it is significantly different from that of the other regions' individuals (Table 7). The length of the ophiocepalous pedicellaria in Hawaiian individuals is the longest, but it is not significantly different from that of Boninian individuals while it is significantly different from that of the other regions' individuals. The length differences among Indonesian, Okinawan, and Guamanian individuals are not significant although these are significantly different from the other regions' individuals. And finally, the length differences of Mauritian individuals are significantly different from all the other regions' individuals. The length of the trifoliate pedicellaria in Hawaiian individuals is also longest, but it is not significantly different from that of Okinawan and Boninian individuals while it is significantly different from that of the other regions' individuals. While length differences between Indonesian and Guamanian individuals are not significant, these are significantly different from the other regions' individuals. The length differences of Mauritian individuals are significantly different from all the other regions' individuals; this is true for the ophiocephalous pedicellaria as well.

DISCUSSION

It is almost impossible to discriminate black *Echinometra* among the regions based only on appearances. However, it is possible to discriminate them based on the spicules of the

Table 7. Comparison of the pedicellarias, tridentate, ophiocephalous, and trifoliate head-length of black *Echinometra* from Mauritius, Indonetate pedicellaria, ophiocephalous pedicellaria, and trifoliate pedicellaria, respectively. The values indicate a P value calculated by ANOVA. The insignificant levels, respectively. The number of pedicellaria used for this analysis is the same as those shown in Table 6.

	Hawaii			Guam			Bonin		
Region	td	o	tf	td	o	tf	td	o	tf
Mauritius	2.87×10^{-7} ***	1.35×10^{-17} ***	1.48×10^{-7} ***	0.993157 –	2.68×10^{-17} ***	1.84×10^{-22} ***	0.046189 *	2.16×10^{-30} ***	1.84×10^{-22} ***
Indonesia	0.000177 ***	1.05×10^{-7} ***	9.7×10^{-5} ***	0.710468 –	0.058191 –	0.490147 –	0.091053 –	2.95×10^{-12} ***	4.95×10^{-9} ***
Okinawa	0.003527 **	2.07×10^{-6} ***	0.976073 –	0.482692 –	0.228499 –	1.33×10^{-9} ***	0.687901 –	1.74×10^{-6} ***	0.00492 *
Bonin	5.17×10^{-5} ***	0.054158 –	0.136913 –	0.268204 –	5.87×10^{-16} ***	3.85×10^{-10} ***			
Guam	0.005018 **	4.44×10^{-11} ***	6076×10^{-5} ***						

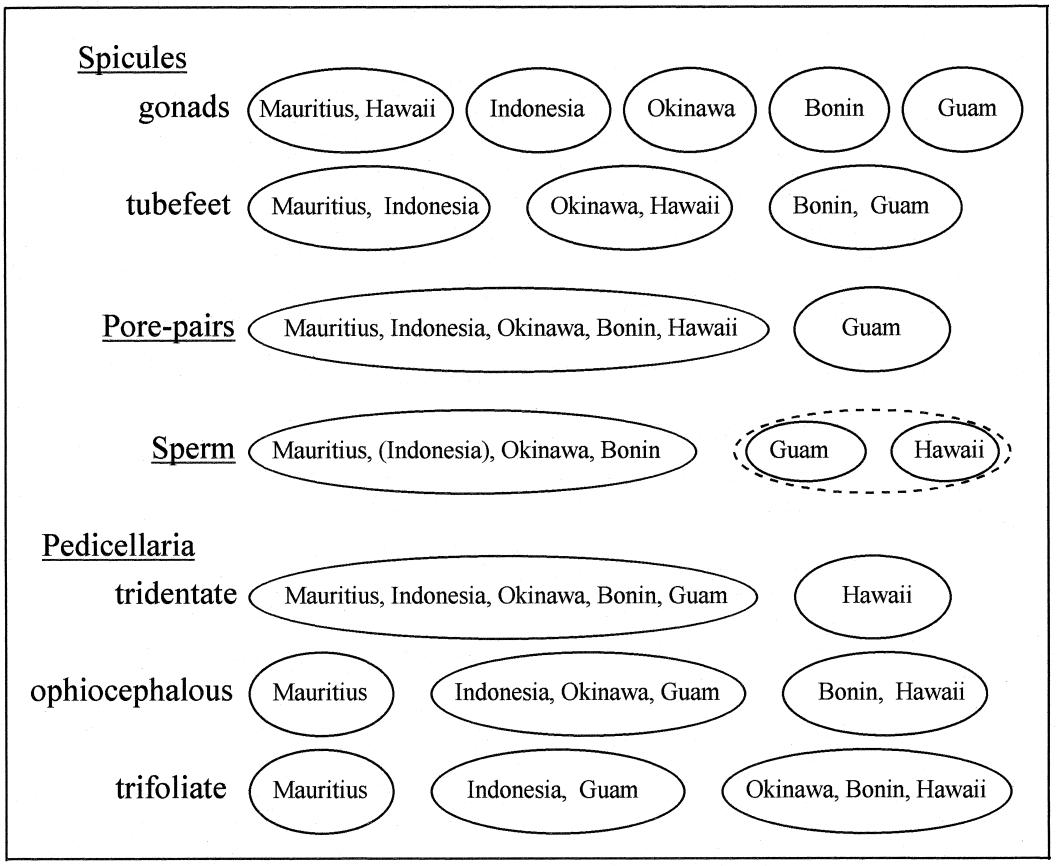

Fig. 2. The diagram shows the groupings of each region's black *Echinometra* based on each of the characteristics. The groupings based on the spicules of the gonads and tubefeet are grounded on each type spicule's percentage (Table 1), but a statistical analysis for comparisons was not conducted. For characteristics other than spicules, the groupings are based on statistical analysis for comparisons. Individduals of the respective regions were allocated to different groups or the same group depending on whether the comparison was at significant level or not. For statistical analysis of the characteristics, see tables: Pore-pair, Table 3; Sperm, Table 5; Pedicellaria, Table 7. Sperm of the Indonesian individuals is based on biological microscope observation alone. The size was not measured.

gonads and tubefeet, the number of pore-pairs, the morphology of the sperm, and the pedicellaria. The grouping of each region's individuals based on these characteristics is shown in Fig. 2. For all characteristics, the black *Echinometra* from Guam and Hawaii are allocated to different groups. The differences of the two regions' (Guamanian and Hawaiian) individuals, from the other regions' (Mauritian, Indonesian, Okinawan, and Boninian) individuals are: Guamanian individu-

sia, Okinawa, Bonin, Guam, and Hawaii. The abbreviations, td, o, and tf, denote triden-
symbols, ***, **, *, and –, indicate significant levels, P<0.001, P<0.01, P<0.05, and

Okinawa			Indonesia		
td	o	tf	td	o	tf
0.256371	1.17×10^{-11}	2×10^{-16}	0.661445	7.47×10^{-7}	0.043944
–	***	***	–	***	*
0.271282	0.038408	2.7×10^{-8}			
–	*	***			

als can be discriminated from Mauritian, Indonesian, Okinawan, and Boninan individuals by the spicules of the gonads and tubefeet, the number of pore-pairs, and the morphology of the sperm; Hawaiian individuals can be discriminated from Mauritian, Indonesian, Okinawan, and Boninian individuals by the morphology of the sperm and the head-length of the tridentate pedicellaria, at least. The Mauritian, Indonesian, Okinawan, and Guamanian individuals are allocated to the same group by virtue of the number of pore-pairs, the morphology of the sperm, and the head-length of the tridentate pedicellaria, but they are allocated to different groups with reference to the spicules of the gonads and tubefeet, the head-length of the ophiocephalous and trifoliate pedicellaria.

Groupings by the number of pore-pairs, the morphology of the sperm, and the head-length of the tridentate pedicellaria capture four to five regions, which is more regions than groupings using other characteristics. Note that four of the regions are common to each of the three groupings based on these three characteristics. Note further the differences within these three characteristics are rather conspicuous, the percentage of five-pore-pairs of the Guamanian individuals being extremely low, the sperm shape of the Guamanian and Hawaiian individuals being compact, and the head-length of the tridentate pedicellaria of the Hawaiian individuals being extremely long. On the other hand, groupings by the spicules of the gonads and tubefeet, and the head-length of the ophiocephalous and trifoliate pedicellaria capture fewer members than groupings based on the number of pore-pairs, the morphology of the sperm, and the head-length of the tridentate pedicellaria. Furthermore, the regions in such groupings do not reappear in a cluster as they do in groupings based on the number of pore-pairs, the morphology of the sperm, and the head-length of the tridentate pedicellaria. Also the differences within the spicules of the gonads and tubefeet, and the head-length of the ophiocephalous and trifoliate pedicellaria are comparatively small. Therefore, it is appropriate to consider the former three characteristics as more reliable than the latter four characteristics for discriminating groups of black *Echinometra* inhabiting the Indo-West Pacific. This consideration is supported by the notion that the number of pore-pairs is one of the important factors for the classification of the genus *Echinometra* (Mortensen, 1943). Based on these three characteristics (i.e., the number of pore-pairs, the morphology of the sperm, and the head-length of tridentate pedicellaria), black *Echinometra* from the six regions in the Indo-West Pacific may be divided into three groups: the Mauritian, Indonesian, Okinawan, Boninian individuals making one group while the Guamanian

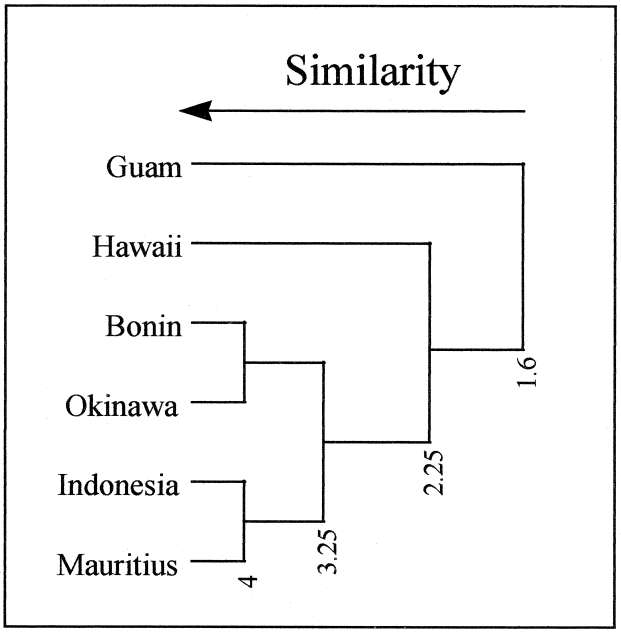

Fig. 3. The phenogram shows the similarity of each region's black *Echinometra*. The phenogram was constructed based on the number of common characteristics calculated by the association coefficient. The number of common characteristics is counted as the number of common groups shown in Fig. 2. For example, Mauritius and Indonesia are allocated to the same group by four common characteristics: tubefeet, pore-pairs, sperm, tridentate pedicellaria. The figures on the left side of the phenogram show the number of common characteristics between each region's individuals or each cluster.

and Hawaiian individuals make up each a different group. These three groupings are supported by an overall similarity calculated by the association coefficient (Fig. 3). Three clusters as the same as the groupings based on the three characteristics above are recognized at 3.25 common characteristics level in the phenogram.

Since the number of pore-pairs and the morphology of the sperm in the Okinawan *Echinometra* species complex reflect species level differences (Uehara, 1990; Arakaki *et al*., 1998), Guamanian black *Echinometra* should be considered a species independent from the other regions' black *Echinometra* by the significant difference in five pore-pairs. And Guamanian, Hawaiian, or the other regions' black *Echinometra* should be regarded as independent species also, respectively, by the significant difference in sperm morphology. Besides, cross-fertilizations between black *Echinometra* from Okinawa and Guam as well as Okinawa and Hawaii are not successful (Arakaki, unpublished). Therefore, these three groupings would reflect a species level difference. In other words, the black *Echinometra* inhabiting the Indo-West Pacific should be regarded as a species complex, or a cryptic species composed of at least three species.

ACKNOWLEDGMENTS

We heartily thank Dr. I. Fagoonee and the staff and students of the Faculty of Science, University of Mauritius, Mauritius; Dr. J. D. Kusen and the staff and students of the Faculty of Fishery, Sam Ratulangi University, Manado, Indonesia; Miss P. Kussoy, Yayasan, KELOA, Manado, Indonesia; Dr. T. Yanagisawa, Professor Emeritus, Saitama Medical School Junior College, Japan; Dr. R. H. Richmond and Mrs. S. C. Wilkins, Marine Laboratory, University of Guam, USA; and Dr. R. Yanagimachi, University of Hawaii, USA, for helping to collect animals and using their facilities. We also extend our appreciation to T. C. Guile, Meio University, Okinawa, Japan, for his editorial assistance. This research was partly supported by a grant from the Uruma Trust Fund for Research into Science and Humanities.

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(Received November 16, 1998 / Accepted February 22, 1999)