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Morphological diversity of larval skeletons in the sea urchin family Echinometridae (Echinoidea: Echinodermata)

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To clarify the morphological variety of larval skeletons, a detailed morphological comparison among the species of the family Echinometridae was performed. Through conspecific comparison of larval skeletons among different ages, we found five skeletal characters of the body skeleton that are stable in the four-armed pluteus and thus useful in homologous comparison among the species. The morphological variation was summarized as the difference in the number of spines and posteroventral transverse rods, and differences in the shape of the body skeleton. Significant correlations were found between some skeletal characters, such as between upper body length and bottom width of body skeleton and between lower body length and the number of spines. We found that the larval skeletons of tropical species tend to have fewer spines and rods than those of temperate species, which is consistent with the hypothesis that a reduction in skeletal elements decreases the specific gravity of larvae as an adaptation to tropical waters.

INTRODUCTION

Sea urchin larvae, echinoplutei, have a unique form with long projecting arms and a calcite skeleton in the body. The larval skeleton not only keeps the shape of the larval body and protects the digestive organs but also helps orient the arms upward in the water column (Pennington & Hadfield, 1989; Pennington & Strathmann, 1990). This orientation is balanced by the arrangement of the skeleton and allows for more effective swimming and feeding (Pennington & Strathmann, 1990). The larval skeleton is greatly reduced or even lost in non-feeding larvae (Amemiya & Emlet, 1992; Emlet, 1995; Parks et al., 1989; Wray & Raff, 1991), suggesting the importance of the larval skeleton for feeding activity.

Larval calcite skeletons display considerable morphological diversity. The number and length of arm skeletons, the fenestrated structure of arm skeletons and the basket-like structure of the body skeleton vary considerably among orders and families (Mortensen, 1921; Onoda, 1936, 1938; Wray, 1992; Emlet et al., 2002). The abundance of spines on the skeletal rods and the shape of the basket-like structure of the body skeleton also vary among the species within the families (Ishikawa & Noguchi, 1988; Wray, 1992).

These morphological differences in larval skeletons influence larval life and vice versa. Larvae with longer arms (hence longer arm skeletons) can feed more effectively, because larvae feed by using ciliated bands running along the arms (Strathmann, 1971; Hart, 1991). Emlet (1982, 1983) indicted that a fenestrated arm rod is stronger against drag than a non-fenestrated one and thus may be adaptive in protection against predators. The morphological diversity of larval skeletons is likely to be generated by continuous adaptation to its environment.

Sea urchins have long been one of the model organisms for early embryogenesis. The morphogenesis of the larval skeleton in particular, has been extensively investigated. Recent results based on EST analyses revealed a detailed genetic cascade for early skeletogenesis (Zhu et al., 2001; Davidson et al., 2002; Ettensohn et al., 2003). However, compared with the differentiation process of the spiculogenic mesenchyme cells, less is known about the morphogenesis of the larval skeletal structure. The interaction between the skeletogenic mesenchyme and the surrounding epidermis has been shown to be essential for skeletal morphogenesis (Ettensohn & Malinda, 1993; Cavalieri et al., 2003). In addition, Armstrong & McClay (1994) performed an interspecific swap of skeletogenic mesenchyme cells, and proved that the primary information for the skeletal structure is carried by skeletogenic mesenchyme cells. Therefore, the variation of larval skeletal morphology is an excellent system to understand its evolutionary history both from an ecological standpoint (to understand WHY the variation has been generated), and from a developmental standpoint (to understand HOW the variation is produced).

Some characteristics of the larval skeleton, such as the number and length of arm skeletons, the fenestrated structure of the arm skeleton and the basket-like structure of the body skeleton are conserved in higher taxonomic groups (Mortensen, 1921; Wray, 1992). For example, the species in the family Diadematidae have two long arms, while Echinoida have eight short arms. Species in the family Arbacidae have 12 long arms. These skeletal characters are well conserved at the order- and

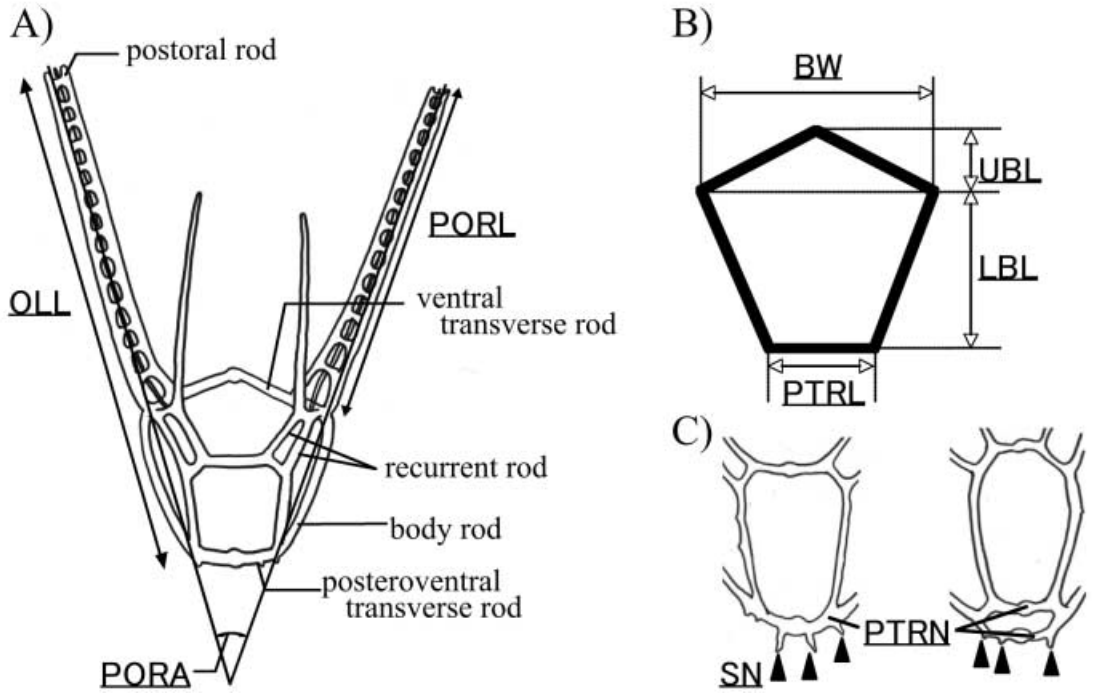

Figure 1. Anatomy and measured characters of the larval skeleton. (A) Drawing of a whole skeleton in ventral view; (B) schematic of the body skeleton in ventral view; and (C) magnification of the posterior portion of the larval skeleton. BW, body width; LBL, lower body length; OLL, overall length; PORA, angle of bilateral postoral rods; PORL, length of postoral rod; PTRL, length of posteroventral transverse rod; PTRN, number of posteroventral transverse rods; UBL, upper body length; SN, number of spines. Larval skeleton with single posteroventral transverse rod (C, left), and with double rods (C, right). Arrowheads indicate small spines projecting from skeletal rod(s).

family-level, and thus evolutionarily stable for about 200 million years (Wray, 1992).

On the other hand, there are some skeletal traits that vary even among related species within a family, such as abundance of spines on skeletal rods and the shape of the body skeleton. Some species of the family Toxopneustidae lack spines on their skeletal elements, and the others have small spines (Komatsu & Noguchi, 1997); two species of the Strongylocentrotidae have recurrent rods of different lengths (Mortensen, 1921; Kryuchkova, 1976; Strathmann, 1979; Hata & Osanai, 1994). The morphological variations among closely related species have evolved in a relatively short period of time, and thus it may be feasible to seek relationships between morphological characteristics and environmental factors. Therefore, comparative studies focusing on related species could provide clues to understand the evolution of the morphological diversity of larval skeletons in sea urchins.

Numerous studies have been conducted on the morphology of larval skeletons in sea urchins (Mortensen, 1921; Onoda, 1936, 1938; Kryuchkova, 1976; Strathmann, 1979). However, precise interspecific comparisons of larval morphology with attention to compare homologous developmental stages have seldom been made. In addition, larval morphology displays phenotypic plasticity depending on the availability of food (Boidron-Metairon,

Table 1. *Morphometric characters of sea urchin larval skeletons and their abbreviations. The left table shows morphometric characters for comparison between two different ages within a species. The right table shows morphometric characters that we used for morphological comparison among nine species.*

Comparison between two ages		
Number	Character	Abbreviations
1	Overall length	OLL
2	Length of postoral rod	PORL
3	Angle of bilateral postoral rod	PORA
4	Upper body length	UBL
5	Lower body length	LBL
6	Body width	BW
7	Length of posteroventral transverse rod	PTRL
8	Number of spines	SN

Comparison among nine species		
Number	Character	Abbreviations
1	Upper body length	UBL
2	Lower body length	LBL
3	Body width	BW
4	Length of posteroventral transverse rod	PTRL
5	Number of spines	SN
6	Number of posteroventral transverse rod	POTRN

*, PTRL is excluded from principal component analysis because it is a categorical value.

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Table 2. *Comparison of morphological values of larval skeletons between two different ontogenetic stages. Mean, standard deviation and range (in parentheses) of skeletal characters for two- and five-day old larvae in* Colobocentrotus mertensii *and two- and four-day old larvae in* Echinometra oblonga.

	C. mertensii			*E. oblonga*		
Character	2-days-old	5-days-old	*P*	2-days-old	4-days-old	*P*
OLL (μm)	147.7 ±15.7 (118.3–174.6)	475.1 ±24.3 (438.2–523.4)	<0.001	340.8 ±13.9 (303.0–361.6)	510.0 ±21.7 (468.1–539.5)	<0.001
PORL (μm)	82.2 ±16.7 (48.8–107.1)	403.7 ±24.5 (368.9–447.5)	<0.001	279.1 ±17.7 (235.1–301.0)	422.4 ±22.3 (390.0–461.6)	<0.001
PORA (°)	52.7 ±4.1 (51.8–64.1)	42.2 ±4.0 (35.3–51.2)	<0.001	42.5 ±5.0 (34.7–53.4)	42.9 ±4.7 (34.3–52.4)	n.s.
UBL (μm)	20.8 ±1.6 (17.9–23.8)	20.4 ±2.4 (16.6–25.8)	n.s.	23.0 ±2.3 (19.1–28.0)	23.1 ±3.7 (16.9–29.0)	n.s.
LBL (μm)	61.0 ±3.2 (55.8–66.2)	63.9 ±4.9 (50.4–79.3)	n.s.	76.6 ±3.4 (70.4–82.4)	74.9 ±5.4 (61.1–86.2)	n.s.
BW (μm)	84.2 ±2.1 (81.3–87.9)	82.9 ±3.4 (75.7–89.9)	n.s.	96.3 ±3.3 (90.6–102.1)	94.6 ±3.7 (88.0–103.8)	n.s.
PTRL (μm)	33.2 ±2.7 (28.9–38.8)	31.3 ±3.6 (25.7–37.8)	n.s.	41.1 ±2.7 (33.5–44.8)	41.7 ±4.1 (35.4–51.6)	n.s.
SN	1.9 ±1.4 (0–4)	1.5 ±1.2 (0–3)	n.s.	7.7 ±1.3 (6–10)	8.9 ±2.4 (6–13)	n.s.

n.s., not significant.

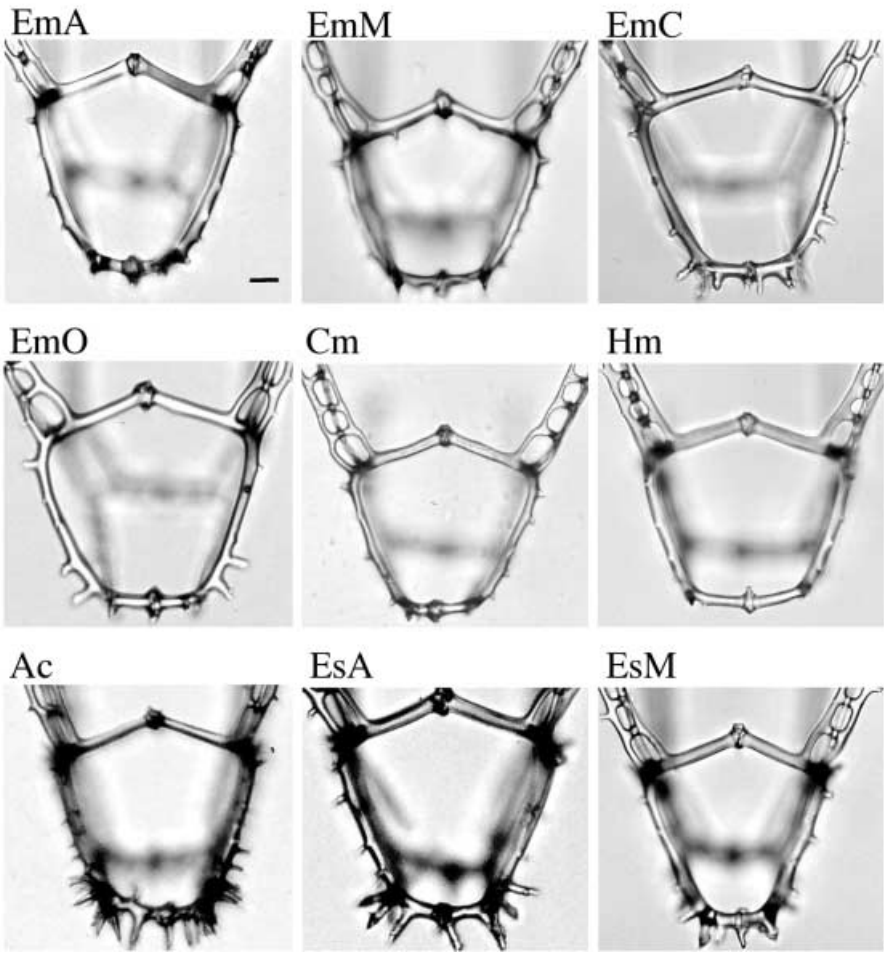

Figure 2. Ventral view of body skeletons in echinometrid sea urchin larvae. EmA, *Echinometra* sp. A; EmM, *Echinometra mathaei*; EmC, *Echinometra* sp. C; EmO, *Echinometra oblonga*; Cm, *Colobocentrotus mertensii*; Hm, *Heterocentrotus mammillatus*; Ac, *Anthocidaris crassispina*; EsA, *Echinostrephus aciculatus*; EsM, *Echinostrephus molaris*. Scale bar: 10 μm.

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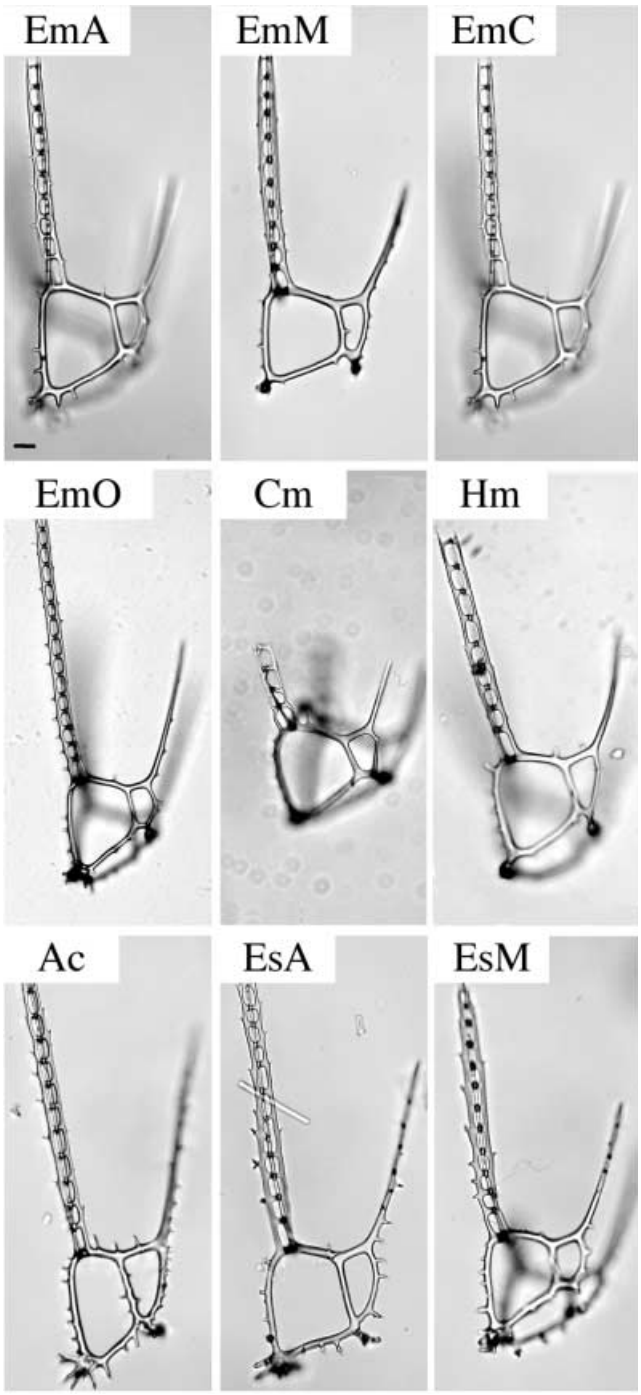

Figure 3. Lateral view of larval skeletons in the Echinometridae. Species abbreviations are same as Figure 2. Scale bar: 10 μm.

1988; Fenaux et al., 1988; Hart & Scheibling, 1988; Strathmann et al., 1992; Hart & Strathmann, 1994; Orr 1999). Because less investment into feeding structures is required when food is abundant, larvae cultured with abundant food develop shorter arms than do those cultured with less food. For precise interspecific comparisons, the plasticity must be considered. Furthermore, most of the morphological differences in larval skeletons are quantifiable, and thus morphometric analyses can be used to describe the variations in larval skeletons.

In the present study, we describe the morphological diversity of larval skeletons in the family Echinometridae with detailed, quantitative comparisons of characters. The sea urchin family Echinometridae includes about 20 species in the world, and nine of them are found in Japan. Most of the echinometrid species are widely distributed in tropical to subtropical waters but some species inhabit the temperate zone. Some echinometrid species are distributed sympatrically where they have different habitats (Ebert, 1982; Uehara, 1990; Nishihira et al., 1991) and the spawning period is slightly different among the species (Ishikawa & Noguchi, 1988; Arakaki & Uehara, 1991) suggesting that their larvae may utilize different ecological niches. First, in order to identify the skeletal characters that change during development, we compare the morphology of larval skeletons between different ages for two species whereby to decide the skeletal characters used for the comparison among the species. Secondly, we compare the morphology of larval skeletons among nine species in the Echinometridae. To exclude external effects on larval growth, measurements of the larval skeletons were made prior to feeding. Thirdly, we conduct a correlation analysis and a canonical discriminant analysis on the morphometric values of skeletal characters to clarify pattern in morphological variation of larval skeleton in Echinometridae.

MATERIALS AND METHODS

Sea urchins

Nine echinometrid species were used in this study. The genus *Echinometra* (Gray, 1825) in the Indo-Pacific area is now recognized to consist of four different species (Uehara, 1990; Matsuoka & Hatanaka, 1991; Palumbi, 1996; Palumbi et al., 1997, McCartney et al., 2000), but proper scientific names have not yet been given to the species. In this paper, we follow the nomenclature in McCartney et al. (2000): the genus *Echinometra* sp. A, *E. mathaei*, *Echinometra* sp. C, and *E. oblonga*, which correspond to types A, B, C, and D, respectively, in Okinawa (Uehara, 1990).

Adults of *Echinometra* sp. A, *Echinometra* sp. C, and *Colobocentrotus mertensii* (Brandt, 1835) were collected off Okinawa Island. *Echinometra mathaei* (Blainville, 1825), *E. oblonga* (Blainville, 1825), *Echinostrephus molaris* (Blainville, 1815), and *Heterocentrotus mammillatus* (Linneus, 1758) were collected off the Ogasawara Islands. *Anthocidaris crassispina* (A. Agassiz, 1863) and *Echinostrephus aciculatus* (A. Agassiz, 1863) were collected in Tanabe Bay.

Fertilization and culture of larvae

Gametes were obtained by injecting 1 ml of 1 mM acetylcholine chloride suspended in seawater into the coelom. Eggs were washed at least twice with seawater and then inseminated. Fertilized eggs were cultured in a watch glass at room temperature until hatching. Hatched larvae were transferred into 500 ml of filtered seawater in glass beakers and cultured at 27°C for two days without food. In each species, one parental pair was used for the comparison. In order to measure growth change in larval skeleton after feeding, larvae of *Echinometra oblonga* and

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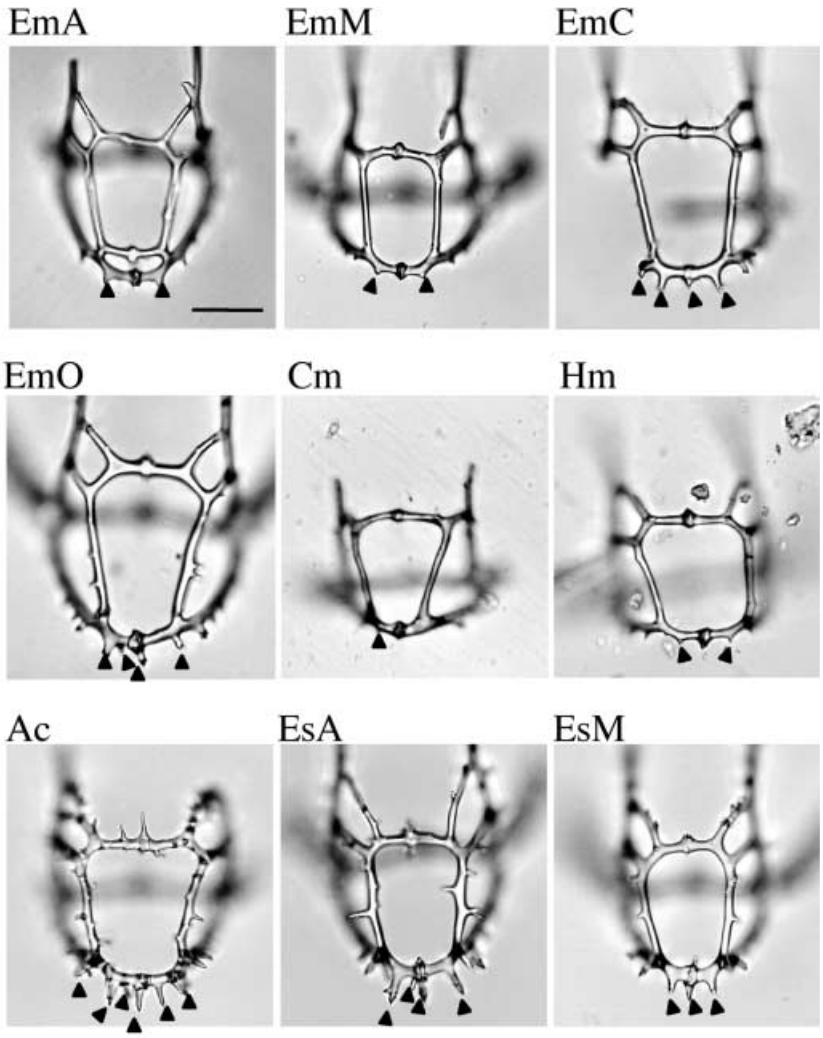

Figure 4. Posterior view of larval skeletons in echinometrid sea urchins. Arrowheads indicate small spines that run on posteroventral transverse rods. Species abbreviations are same as Figure 2. Scale bar: 50 μm.

Table 3. *Mean, standard deviation and range of morphological values of larval skeletons (N=15). The abbreviations of the characters are shown in Figure 1.*

Species	*E.* sp A	*E. mathaei*	*E.* sp.C	*E. oblonga*	*C. mertensii*	*H. mammillatus*	*A. crassispina*	*E. aciculatus*	*E. molaris*
OLL (μm)	245.7 ±7.1	217.6 ±18.8	216.0 ±18.8	216.0 ±16.5	340.8 ±13.9	228.8 ±16.7	286.8 ±20.5	289.5 ±29.5	241.1 ±28.7
	(231.9–256.6)	(183.7–256.6)	(181.0–235.9)	(303.0–361.6)	(98.1–150.3)	(201.3–254.8)	(234.3–322.6)	(238.0–341.6)	(200.5–287.5)
PORL (μm)	177.7 ±6.4	154.7 ±19.2	148.1 ±19.6	279.1 ±17.7	69.1 ±17.5	173.4 ±15.6	222.2 ±21.8	220.5 ±30.5	174.8 ±30.0
	(170.8–195.1)	(130.8–208.0)	(105.6–167.6)	(235.1–301.0)	(36.7–93.3)	(137.9–196.4)	(158.9–243.9)	(149.7–265.3)	(128.0–220.3)
PORA (μm)	45.5 ±1.8	41.4 ±4.5	45.6 ±4.2	42.7 ±5.2	52.7 ±4.1	44.6 ±3.0	42.9 ±4.0	45.9 ±4.3	47.2 ±3.0
	(41.2–48.1)	(35.7–51.3)	(38.2–53.7)	(34.7–53.4)	(51.8–64.1)	(39.1–49.4)	(35.7–49.7)	(37.6–56.8)	(39.3–51.3)
UBL (μm)	25.6 ±3.0	19.7 ±2.3	18.9 ±2.0	23.0 ±2.3	20.9 ±1.9	18.6 ±1.5	17.9 ±1.7	21.9 ±2.3	19.5 ±1.6
	(16.5–29.8)	(15.8–23.2)	(14.8–22.6)	(19.1–28.0)	(18.5–23.8)	(15.8–21.3)	(14.8–21.0)	(16.5–24.8)	(16.4–22.4)
LBL (μm)	74.7 ±2.8	66.4 ±3.3	76.7 ±3.7	76.6 ±3.4	62.4 ±2.5	64.2 ±4.9	73.6 ±4.2	82.5 ±4.9	73.4 ±2.6
	(69.6–79.3)	(60.8–73.0)	(70.1–85.6)	(70.4–82.4)	(58.5–66.8)	(56.7–72.9)	(64.4–80.5)	(70.5–89.3)	(68.3–76.8)
BW(μm)	88.3 ±2.5	86.5 ±2.7	93.4 ±3.5	96.6 ±3.3	84.5 ±2.2	86.7 ±3.8	86.0 ±3.2	92.8 ±4.4	80.8 ±2.5
	(83.9–92.9)	(81.8–90.4)	(87.1–98.8)	(90.0–100.6)	(79.7–87.9)	(78.7–92.6)	(81.8–94.8)	(82.1–100.6)	(76.9–83.6)
PTRL (μm)	37.7 ±3.8	38.9 ±5.3	43.1 ±4.1	41.1 ±2.7	37.2 ±3.3	51.4 ±4.4	49.9 ±6.2	43.3 ±4.4	33.2 ±4.0
	(32.1–44.9)	(30.8–51.8)	(35.3–50.6)	(33.5–44.8)	(32.3–44.1)	(43.8–57.7)	(40.3–62.1)	(36.9–51.1)	(25.5–38.5)
SN	4.7 ±2.19	3.5 ±2.07	9.5 ±2.07	7.7 ±1.35	2.3 ±1.45	2 ±0.76	15.1 ±2.63	12 ±2.75	10 ±1.46
	(1–8)	(0–7)	(7–13)	(6–10)	(0–5)	(1–3)	(10–20)	(7–18)	(7–13)
PTRN (S:D)	2:13	15:0	15:0	10:5	15:0	15:0	13:2	15:0	15:0
BL/BW	1.13 ±0.03	1.00 ±0.04	1.03 ±0.04	1.03 ±0.05	0.99 ±0.03	0.96 ±0.04	1.06 ±0.04	1.13 ±0.03	1.15 ±0.04
	(1.06–1.18)	(0.92–1.07)	(0.96–1.09)	(0.97–1.15)	(0.92–1.02)	(0.89–1.04)	(1.00–1.10)	(1.08–1.17)	(1.10–1.23)
BW/PTRL	2.37 ±0.25	2.26 ±0.26	2.18 ±0.20	2.09 ±0.12	2.29 ±0.20	1.70 ±0.15	1.75 ±0.22	2.16 ±0.17	2.47 ±0.34
	(1.98–2.78)	(1.74–2.77)	(1.84–2.57)	(1.93–2.35)	(1.97–2.64)	(1.48–1.96)	(1.48–2.15)	(1.92–2.44)	(2.03–3.20)

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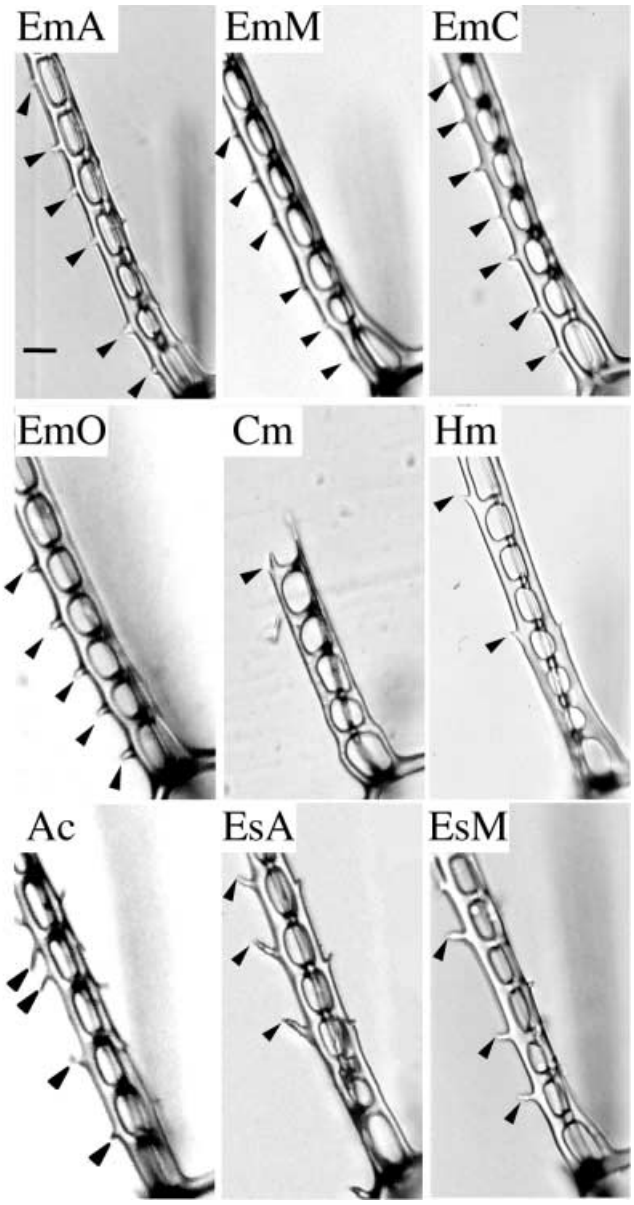

Figure 5. Close-up photographs of fenestrated postoral rods in sea urchin larvae of the family Echinometridae. Arrowheads indicate small spines that run on postoral rod. Species abbreviations are same as Figure 2. Scale bar: 10 μm.

C. mertensii were cultured for two or three additional days, respectively, with food (*Chaetoceros gracilis*).

Preparation of larvae

To examine interspecific differences, skeletons of the two-day-old larvae of nine species were measured and compared. Fifteen larvae were picked randomly from a culture beaker. After the body tissue had been removed by soaking in a bleach solution, each larva was placed in a drop of glycerol on a glass slide, and positioned with a tungsten needle with the anterior-posterior axis parallel to the plane of the glass slide and with the ventral side down. Specimens were mounted using glass beads of 100–125 μm diameter as spacers to prevent the larvae from being squashed.

Table 4. *Number of larvae with double or single posteroventral transverse rod(s)*

Species	Single	Double
Anthocidaris crassispina	13	2
Colobocentrotus mertensii	15	0
Echinometra sp. A	2	13
Echinometra sp. C	15	0
Echinometra mathaei	15	0
Echinometra oblonga	10	5
Echinostrephus aciculatus	15	0
Echinostrephus molaris	15	0
Heterocentrotus mammillatus	15	0

Morphometry of the larval skeleton

Nine characters of the larval skeleton were measured (Figure l; Table l). Overall length (OLL), length of postoral rods (PORL), angle of bilateral postoral rods (PORA), upper and lower body length (UBL and LBL), body width (BW), length of posteroventral transverse rods (PTRL), number of posteroventral transverse rods (PTRN) and number of spines on the body rods and posteroventral transverse rods (SN). The OLL is from the posterior end of the body rod to the anterior end of the

Table 5. *Correlation coefficients between morphological characters of the body skeleton of echinometrid sea urchin larvae. A significant correlation is marked with an asterisk ($P < 0.05$)*

	UBL	LBL	BW	PTRL
UBL	1			
	0.159	1		
	0.301*	0.497*	1	
	−0.255*	0.184*	0.383*	1
	−0.160	0.647*	0.124	0.126

UBL, upper body length; LBL, lower body length; BW, body width; PTRL, posteroventral transverse rods length; SN, number of spines on the body rods and posteroventral transverse rods.

Table 6. *Standardized canonical coefficients, eigenvalues, and proportions of variation explained for the first three canonical variants.*

Character	CAN1	CAn2	CAN3
UBL	0.183	−0.840	0.490
LBL	1.234	−1.309	0.269
BW	−0.348	0.928	1.311
PTRL	0.354	0.946	0.158
SN	1.926	0.644	−0.612
Eigenvalue	7.282	2.721	2.273
Proportion (%)	55.6	20.8	17.4

UBL, upper body length; LBL, lower body length; BW, body width; PTRL, posteroventral transverse rods length; SN, number of spines on the body rods and posteroventral transverse rods.

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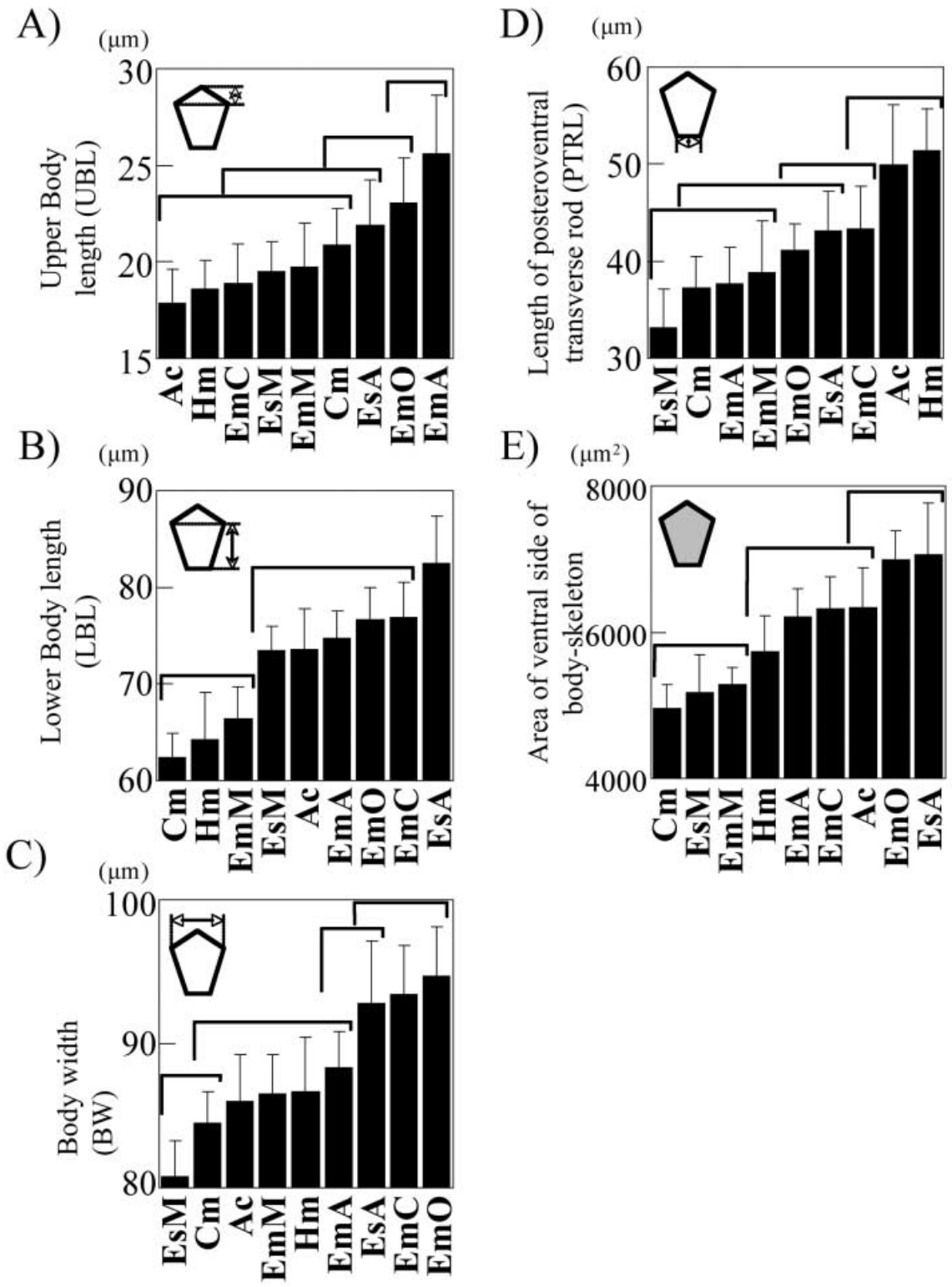

Figure 6. Mean and standard deviations of morphological measurements of two-day old larvae in nine echinometrid species. (A) Upper body length (UBL); (B) lower body length (LBL); (C) body width (BW); (D) length of posteroventral transverse rod (PTRL); and (E) area of ventral side of body skeleton. Insets indicate measured parts. Species under the same horizontal line show no significant differences.

postoral rod. The PORL is the distance from the base of the postoral rod to the tip. Upper body length (UBL) is the height of the upper part of the body skeleton along the anterior-posterior axis as measured from the apex of the ventral transverse rod to the midpoint of the widest part of body skeleton; lower body length (LBL) is the height of the lower part of the body skeleton along the anterior-posterior axis as measured from the midpoint of the widest part of body skeleton to the midpoint of posteroventral transverse rod (Figure 1A). Body width (BW) is defined as the length of the widest part of the body skeleton along the right-left axis. Posteroventral transverse

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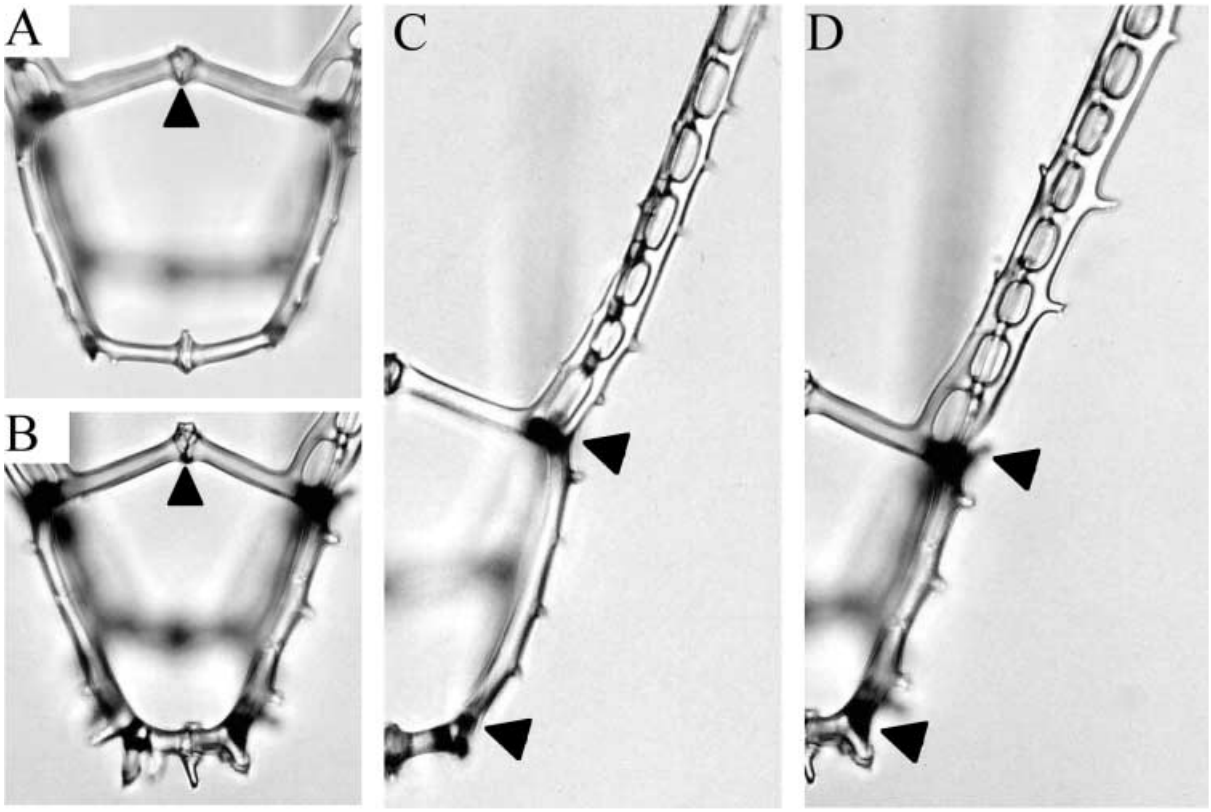

Figure 7. Representative photographs of variation in the shape of the body skeleton (A&B) and joint between body and postoral rods (C&D). (A) *Heterocentrotus mammillatus*; (B&D) *Echinostrephus molaris*; and (C) *Echinometra* sp. A. The shape of the body skeleton characterized as vertically short (BL is short), wide (PTRL is long), and plane apex (UBL is long); (A) vs vertically longer bottom-narrow, and pointed apex; (B) open arrowheads indicate the apex of ventral transverse rod. The joint of body rod and postoral rod incurved; (C) vs relatively straight in the other species; (D) closed arrowheads indicate the ends of the body rod; incurved (C) and relatively straight (D).

rod (PTRL) is measured from the posterior end of the right body rod to the left body rod. Angle of bilateral postoral rods (PORA) is the interior angle created by the left and right postoral rods. The posteroventral transverse rods, which connect the bilateral body rods at their posterior positions, vary in number within and between species and were therefore counted (PTRN). Number of spines on the body rods and posteroventral transverse rods (SN) refers to the number of small spines running along the body rods and posteroventral transverse rods.

Statistical analysis

The Statview 5.0 software package (SAS Institute Inc., Cary, NC) was used for *t*-tests, analysis of variance (ANOVA), multiple comparison tests and correlation analysis. To test growth changes, the morphological values for larval skeletons of *C. mertensii* and *E. oblonga* at different ages were compared using a *t*-test. To test the interspecies differences of larval skeletons, the morphological values of larval skeletons were compared using ANOVA followed by pairwise multiple comparison tests using Scheffe's method. Correlation analysis was conducted for five morphological characters to detect the relationships among the skeletal characters (Table 1, character nos. 1–5 on the right). A canonical discriminant analysis was performed using the CANDISC procedure of SAS to distinguish the species with five morphological characters that compose the body skeleton. The measured values were log-transformed before canonical discriminant analysis except for SN, which is not a continuous variable.

RESULTS

Comparison between larval skeletal morphologies at two different ages

Larval skeletons of *Colobocentrotus mertensii* and *Echinometra oblonga* were compared at two different ages with eight skeletal characters (Table 1) in order to distinguish the growth changes during development. In both species, OLL and PORL were significantly different between larvae at the two ages (Table 2). Angle of bilateral postoral rod (PORA) also differed significantly between larvae of the two different ages in *C. mertensii*, but not in *E. oblonga* (Table 2). In contrast, no significant differences were observed in UBL, LBL, BW, PTRL, or SN, which comprise the body skeleton (Figure 1). Growth changes during development were found in the skeletal characters of the arms but not in those of the body. Thus, the characters of the body skeleton can be regarded as typical for each species.

Comparison among morphological values of larval skeletons of nine species

Larval skeletons of nine species were compared at the two-day-old stage prior to feeding, in order to show

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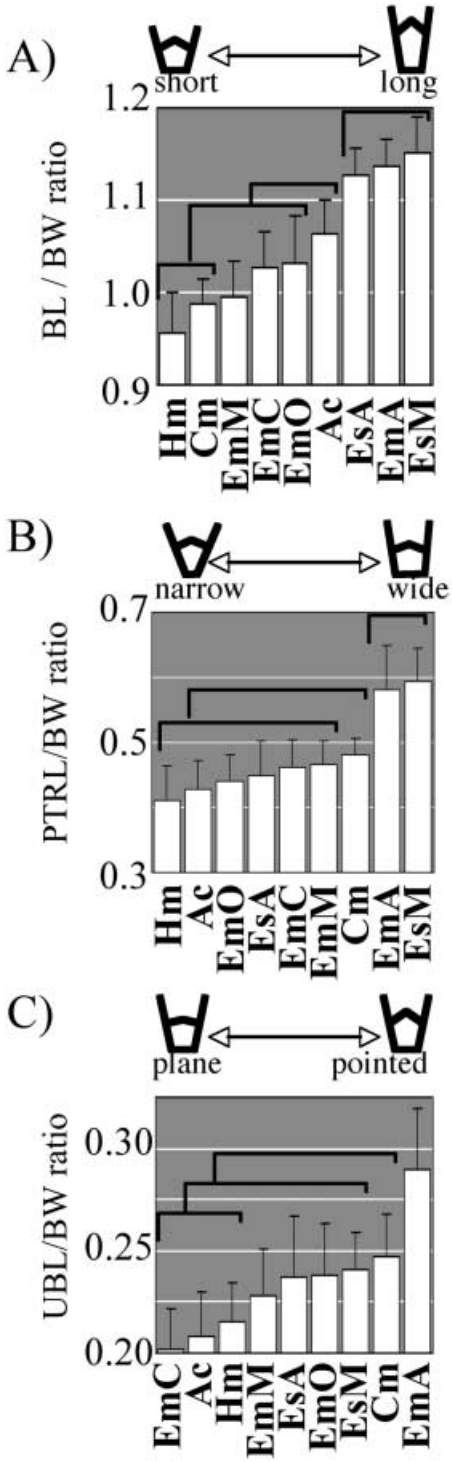

Figure 8. The shape of the body skeleton in larvae of nine species in the Echinometridae. (A) The ratio of BL to BW; (B) PTRL to BW; and (C) UBL to BW. Insets indicate the schematic morphology of the body skeleton: 'long' means body length was longer than body width, while 'short' means body width was longer than body length. These character states are also called 'vertically long' and 'vertically short', respectively, in the text. 'Wide' means posteroventral transverse rod length was greater, resulting in a wider posterior end of the body skeleton, while 'narrow' means posteroventral transverse rod length was smaller resulting in a narrower posterior end of the body skeleton. 'Pointed' means upper body length was longer resulting in a more pointed apex of the ventral transverse rod, while 'plane' results in a relatively plane apex of the ventral transverse rod.

morphological differences in larval skeleton among the echinometrid species. Because of their ontogenetic variability, we do not use the characters OLL, PORL and PORA for the comparison among nine Echinometrid species. Six morphological values, upper body length (UBL), lower body length (LBL), body width (BW), length of posteroventral transverse rod (PTRL), number of spines (SN) and number of posteroventral transverse rod (PTRN) were compared as follows.

Upper body skeleton (UBL)

Upper body skeleton (UBL) varied from 17.9 to 26.6 μm (Figure 6A) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests showed four non-discrete groups (see the inserted lines of Figure 6A). Larvae of *Echinometra* sp. A have the longest UBL averaging 25.6 μm. Larvae of *E. oblonga* and *Echinostrephus aciculatus* and *C. mertensii* have medium UBLs averaging between 20.9 and 23.0 μm. In the other five species, UBLs of larvae are rather short, between 17.9 and 19.7. The larvae with the shortest UBL are those of *Anthocidaris crassispina*.

Lower body skeleton (LBL)

Lower body skeleton (LBL) varied from 62.4 to 82.5 μm (Figure 6B) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests showed three discrete groups (see the inserted lines of Figure 6B). Lower body skeletons (LBLs) of *E. aciculatus* larvae are significantly longest of all, with an average of 82.5 μm. Larvae of *Echinometra* sp. C, *E. oblonga*, *Echinometra* sp. *A*, *A. crassispina* and *Echinostrephus molaris* have mid-sized LBLs averaging between 73.4 and 76.8. The larvae with the shortest LBL, those of *Echinometra mathaei*, *Heterocentrotus mammillatus* and *C. mertensii* had average LBLs of 66.4, 64.2 and 62.4 μm, respectively.

Body width (BW)

Body width (BW) varied from 80.8 to 96.6 μm (Figure 6C) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests showed four non-discrete groups (see the lines of Figure 6C). The larvae of *E. oblonga*, *Echinometra* sp. *C* and *Echinostrephus aciculatus* had average BWs of more than 90 μm. *Echinometra oblonga* had the widest larvae with an average BW of 96.6 μm followed by *Echinometra* sp. C and *Echinostrephus aciculatus* larvae that had BW averages of 93.4 and 92.8, respectively. The larvae of *E. molaris* were narrowest averaging 80.8 μm.

Length of posteroventral transverse rod (PTRL)

Length of posterovental transverse rod (PTRL) varied from 33.2 to 51.4 μm on average (Figure 6D) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests revealed four non-discrete groups (see the lines of Figure 6D). The shorter PRTL values were found in larvae of *E. molaris*, *C. mertensii*, *Echinometra* sp. A and *E. mathaei* and averaged 33.2, 37.2, 37.7, and 38.9 μm, respectively. Larvae of *Echinometra* sp.

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Table 7. *Summary of characteristics of the larval skeleton in nine species of echinometrid sea urchins.*

	Body-skeleton										
					Spine		Number of TCR[e]	Joint of body rod and POR[f]	Spine on postoral rod		
	Size[a]	BL/BW	Shape[b] PTRL/BW	UBL/BW	length[c]	abundance[d]			length[c]	density[g]	direction[g]
Anthocidaris											
A. crassispina	medium	M	W	PL	long	abundant	S-main	Less In.	Long	medium	Oblique
Echinostrephus											
E. aciculatus	large	L	N	MP	long	medium	S	Less In.	Long	medium	Oblique
E. molaris	small	L	N	MP	long	medium	S	Less In.	Long	medium	Oblique
Colobocentrotus											
C. mertensii	small	S	N	MP	short	few	S	Incurved	Short	low	Oblique
Hemicentrotus											
H. mammillatus	medium	S	W	PL	short	few	S	Incurved	Short	low	Oblique
Echinometra											
E. sp. nov. A	medium	L	N	P	medium	few	D-main	Incurved	Short	high	Perpendicular
E. mathaei	small	S	N	MP	medium	few	S	Incurved	Short	high	Perpendicular
E. sp. nov. C	medium	M	N	PL	medium	medium	S	Incurved	Short	high	Perpendicular
E. oblonga	large	M	N	MP	medium	medium	S-main	Incurved	Short	high	Perpendicular

[a], The size of the body skeleton is the area of the ventral side of body skeleton calculated using UBL, LBL, BW and PTRL (Figure 6). [b], The shape of the body skeleton is calculated by the ratios BL/BW, PTRL/BW and UBL/BW (Figure 8). The vertical shape of the body skeleton is classified by the BL/BW ratio: S, vertically short (average BL/BW <1.0); M, middle lengthwise ($1.0 \leqslant$ average BL/BW <1.1); L, vertically long (average BL/BW $\geqslant 1.1$). Width at posterior end is classified by the PTRL/BW ratio: N, narrow at posterior end (average PTRL/BW $\leqslant 0.5$); W, wide at posterior end (average PTRL/BW >0.5). The pointedness of the apex of the ventral transverse rod is classified by the UBL/BW ratio: PL, plane (average UBL/BW $\leqslant 0.255$); MP, moderately pointed ($0.255 <$ average UBL/BW $\leqslant 0.25$); P, pointed (average UBL/BW >0.25). [c], Length of spines in the body skeleton. 'long' spines are more than 10 μm long, 'medium' spines are 5–10 μm long and 'short' spines are less than 5μm long. On the postoral rod, 'long' spines are 3–5 μm long and 'short' spines are 1–3 μm long. [d], Abundant, average SN $\geqslant 15$; middle, $5 <$ average SN $\leqslant 15$; few, average SN $\leqslant 5$. [e], D-main, larvae with a double rod were dominant; S-main, larvae with a single rod were dominant; S, only larvae with a single rod were observed (Table 5). [f], See Figure 4 and text. [g], See Figure 8 and text.

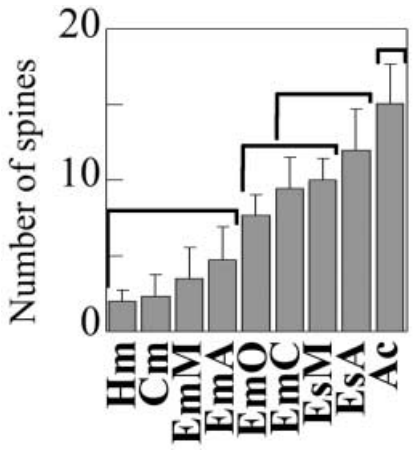

Figure 9. Means and standard deviations of SN. Species abbreviations are same as Figure 2. Letters indicate the result of multiple comparisons among pairs of means. Species under the same line show no significant differences.

C, *Echinostrephus aciculatus* and *Echinometra oblonga* had mid-sized PTRLs, averaging 43.1, 43.3 and 46.3μm, respectively. Larvae of *A. crassispina* and *H. mammillatus* had the longest PTRLs with averages of 49.9 and 51.4 μm, respectively.

Total size of the body skeleton

As an index of the total size of the body skeleton, the area of the ventral side of the body skeleton was calculated using UBL, LBL, BW and PTRL (Figure 6E). Larvae of *Echinostrephus aciculatus* and *Echinometra oblonga* had the largest areas averaging 7062.6 and 6989.5 μm^2, respectively. Larvae of *A. crassispina, Echinometra* sp. C, *Echinometra* sp. A and *H. mammillatus* had intermediate sizes averaging between 5731.1 and 6338.7 μm^2. In the other three species, the total size of the body skeleton was small, averaging from 4962.3 to 5283.4 μm^2

Shape of the body skeleton (ratios of BL to BW, PTRL to BW and UBL to BW)

The ventral view of the body skeleton revealed a pentagonal shape (Figure 7A,B). The pentagonal shape was vertically short and wide at the posterior end in some species, such as *H. mammillatus* (Figure 7A), and vertically longer and narrow at the posterior end in other species, such as *Echinostrephus molaris* (Figure 7B). To compare the shapes of body skeletons, the ratios of body length (BL, i.e. UBL+LBL) to body width (BW), of the length of posteroventral transverse rod (PTRL) to BW and of upper body length (UBL) to BW were calculated (Figure 8). A body skeleton with a higher BL to BW ratio appeared vertically longer, as seen in Figure 7B; a body skeleton with a lower ratio appeared vertically shorter, as seen in Figure 7A. A body skeleton with a lower PTRL to BW ratio looked narrow at the posterior end, as seen in Figure 7B; a body skeleton with a higher ratio looked wide at the posterior end, as seen in Figure 7A. In the species with a higher UBL to BW ratio, the apex of ventral transverse rod (Figure 7, open arrowheads) was more pointed as seen in Figure 7B and a body skeleton with lower ratio looked more obtuse at the apex of the ventral rod as seen in Figure 7A.

Ratios of BL to BW. The average BL to BW ratio varied between 0.96 and 1.15 (Figure 8A) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests indicated two discrete, significantly different groups (see the lines in Figure 8A). *E. molaris* had the highest BL to BW ratio (1.15 on average). *Echinostrephus aciculatus* and *Echinometra* sp. A had ratios that averaged 1.13. Body length (BL) to BW ratios in these three species were significantly higher than in the other six species (Figure 8A). The average BL to BW ratios in the remaining species ranged from 0.96 to 1.06. *Heterocentrotus mammillatus* and *C. mertensii* had the lowest average BL to BW ratios, 0.96 and 0.99, respectively.

Ratios of PTRL to BW. The average PTRL to BW ratio varied between 0.41 and 0.59 (Figure 8B) and differed significantly among the nine species ($P<0.0001$). Pairwise multiple comparison tests produced two discrete groups, which differed significantly (Figure 8B). Larvae of *H. mammillatus* and *A. crassispina* had the highest average PTRL to BW ratios, 0.59 and 0.58, respectively. The PTRL to BW ratios in these two species were significantly higher than those in the other seven species (Figure 8B). Average ratios of PTRL to BW ranged between 0.41 and 0.48 in the remaining species, and pairwise multiple comparison tests showed two non-discrete groups (Figure 8B).

Ratios of UBL to BW. The average UBL to BW ratio varied between 0.20 and 0.29 (Figure 8C) and differed significantly among the nine species ($P<0.0001$). *Echinometra* sp. A had the highest average UBL to BW ratio, 0.29, and was significantly higher than the other species. In the other eight species, UBL to BW ratios varied between 0.20 and 0.25. Pairwise multiple comparison tests produced three non-discrete groups. Among them, the average UBL to BW ratios were rather low in *Echinometra* sp. C, *A. crassispina* and *H. mammillatus* (Figure 8C).

Number of spines on the body skeleton (SN)

In all species, small spines projected from each rod of the body skeleton (Figures 2–5, arrowheads in Figure 4). The spines were relatively longer in *A. crassispina* and in two species of *Echinostrephus* (Ac, EsA, and EsM in Figure 4). In all the species, the spines on the posteroventral transverse rods and on the ends of the body rods were usually more abundant and longer than the spines on other rods (Figures 2 & 4).

The average SN varied from 2.0 to 15.1 (Figure 9) and differed significantly among the species ($P<0.0001$). Pairwise multiple comparison tests revealed three discrete groups (Figure 9). Spines were significantly less abundant in the larvae of *H. mammillatus, C. mertensii, E. mathaei*, and *Echinometra* sp. A, which averaged 2 to 4.7 spines. Spines were moderately abundant, averaging between 7.7 and 12.0, in *Echinostrephus aciculatus, E. molaris, Echinometra* sp. C, and *E. oblonga. A. crassispina* larvae had significantly more spines than any other species, with an average of 15.1.

To compare the density of spines, SN was divided by the sum of double the body rod length and the PTRL. Spine

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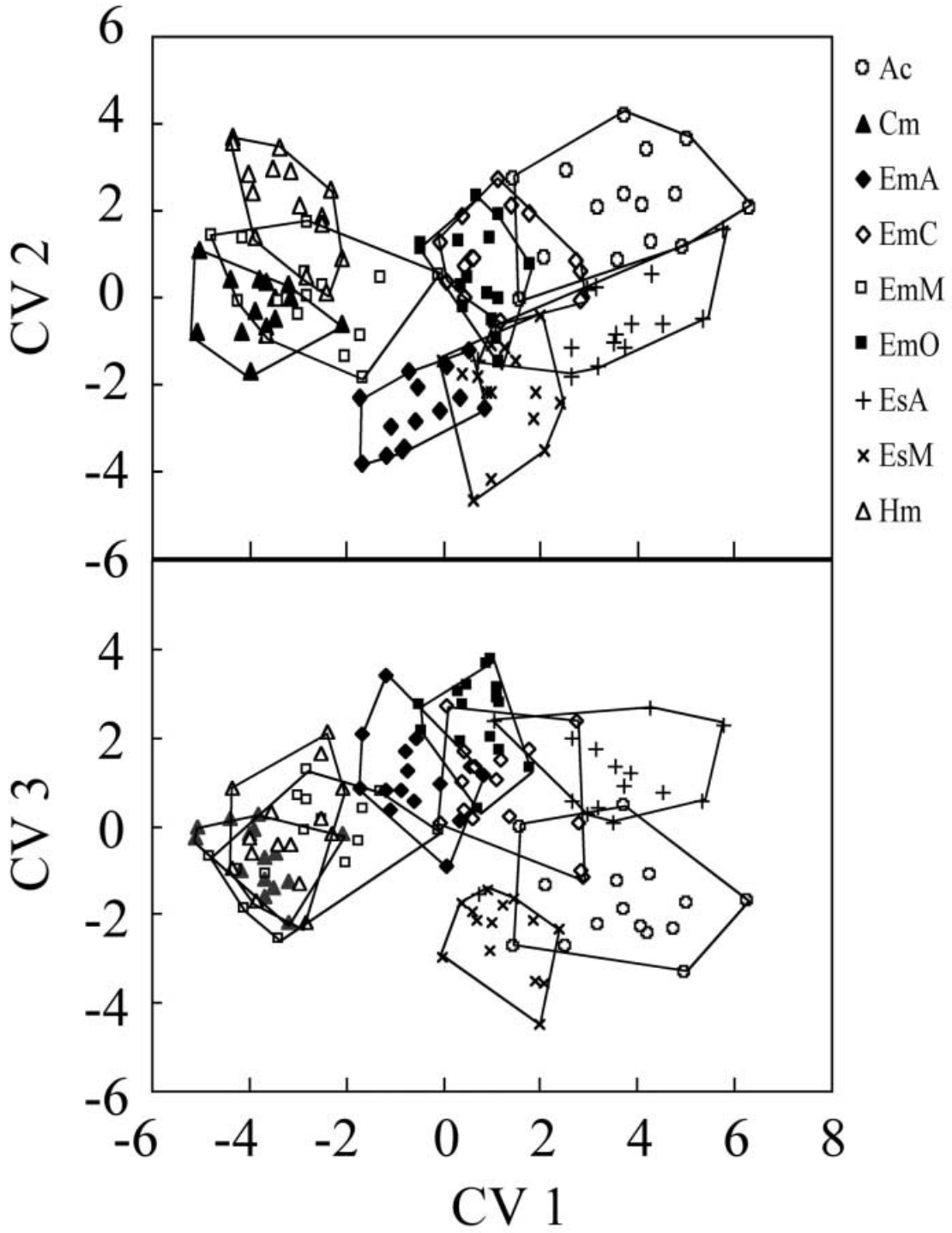

Figure 10. Results of canonical discriminant analysis with plots of the first three scores. Abbreviations of the species are shown in Figure 2.

density differed significantly among the nine species ($P < 0.0001$). An analysis of multiple comparisons of spine density gave the same results as those obtained for SN (data not shown).

Number of posteroventral transverse Rods (PTRN)

Number of posteroventral transverse rods (PTRN) differed among and within species, being either double or single (Figure 4). In *Echinometra* sp. A, *E. oblonga*, and *A. crassispina*, PTRN varied within the species (Table 4). In *Echinometra* sp. A, the majority of larvae had double posteroventral transverse rods. On the other hand, in *Echinometra* sp. C and *A. crassispina*, larvae with single posteroventral transverse rods were abundant. In the other six species, each larva had a single posteroventral transverse rod.

Correlation among the morphological characters

To show the correlation between the morphological characters, correlation coefficients are shown in Table 5.

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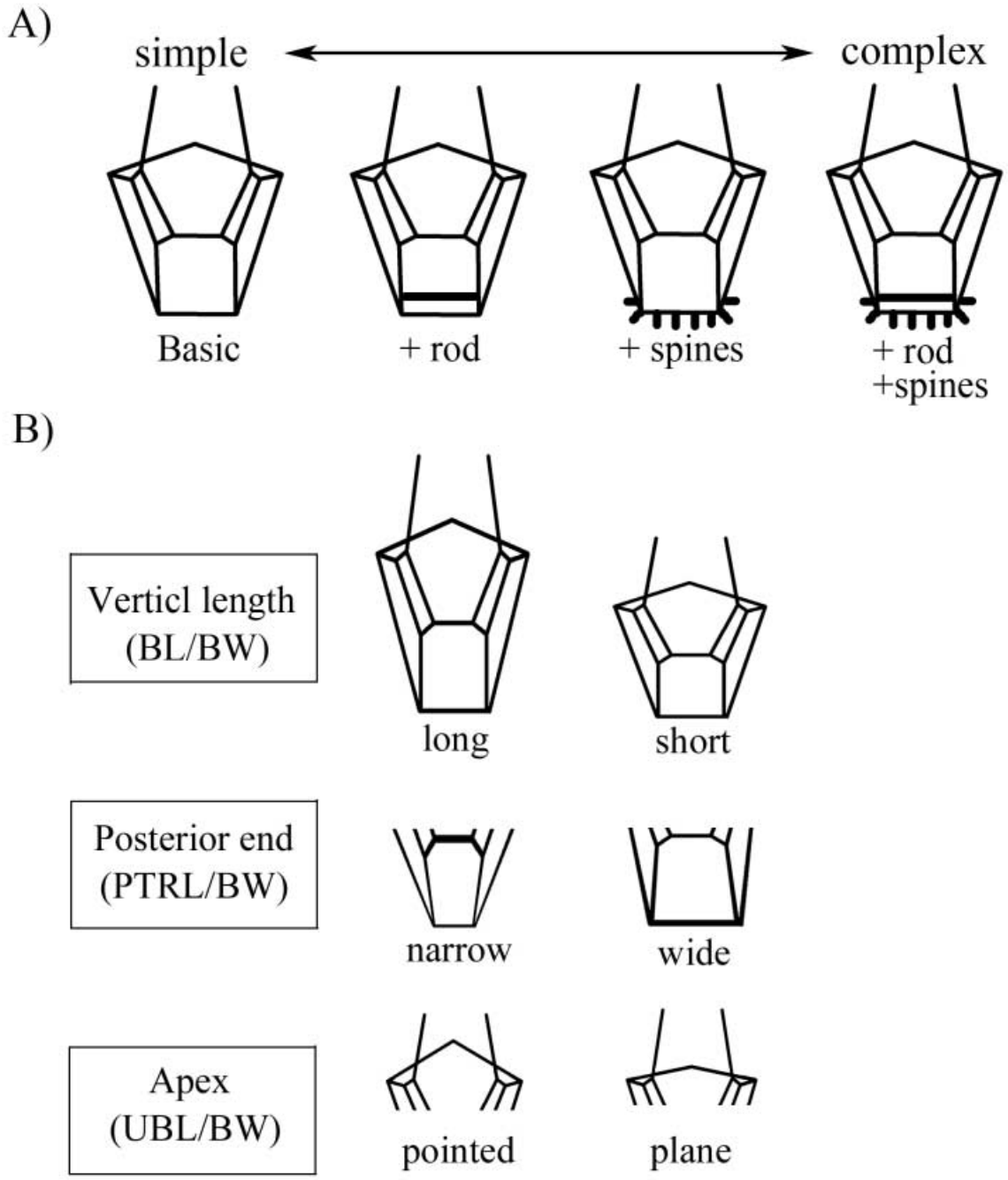

Figure 11. Summary of morphological variation in the body skeleton of sea urchin larvae. (A) Variation in skeletal accessories, spines and posteroventral transverse rod; and (B) variation in the shape of the body skeleton.

Lower body length (LBL), body width (BW) and length of posteroventral transverse rod (PTRL) were positively and significantly correlated, meaning that larval skeletons with a longer body skeleton tend to have a wider body and a longer posteroventral transverse rod. In addition, LBL is positively and highly correlated with the number of spines (SN), namely, larvae with a long body skeleton tend to have many spines. Conversely, upper body length (UBL) was negatively correlated with PTRL (Table 5), indicating that larval skeletons with a pointed apex of the ventral transverse rod tend to have a long posteroventral transverse rod. The correlation coefficients are not significant for other pairs of characters (Table 5).

Canonical discriminant analysis

Canonical discriminant analysis (CDA) was conducted on five skeletal characters in nine species (Table 6 and Figure 10). The CDA was highly significant (Wilks' lambda=0.00515, $P<0.0001$). The first three canonical variables accounted for 93.8% of the variance, explaining 55.6, 20.8, and 17.4% of the variation, respectively.

The first canonical variable (CV1) is most weighted for SN and LBL in which eigenvalues were 1.234 and 1.926, respectively (Table 6). The CV1 separated *C. mertensii*, *E. mathaei* and *H. mammillatus* as a group with shorter LBL and fewer spines, from the rest of the species (Figure 10). *Anthocidaris crassispina* and *Echinostrephus aciculatus* with

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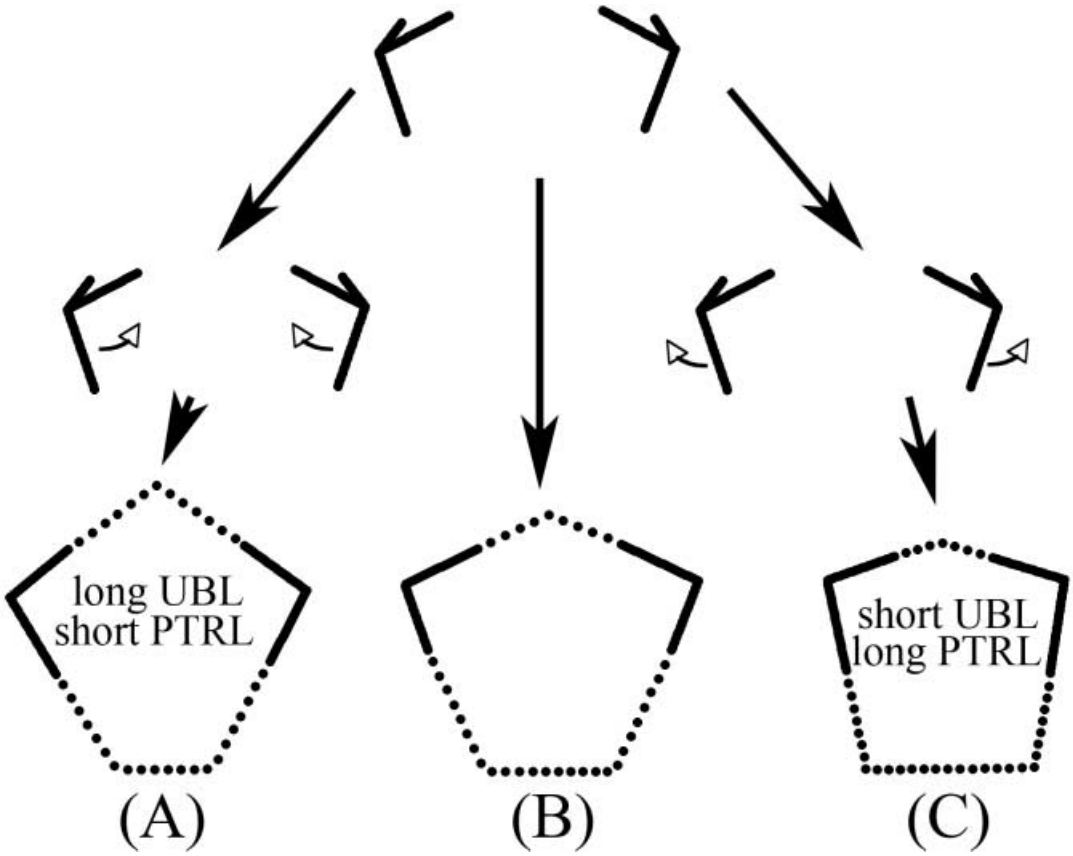

Figure 12. Schematic representation of a hypothetical way of generating variation in the shape of the body skeleton. In the Echinometridae, the larval skeleton is initiated from triradiate spicules with a specific angle, 95°. If the spicules rotate toward each other in the body, they could generate larval skeletons with a pointed apex of the ventral transverse rod and a narrow at posterior end (A). If the triradiate spicules rotate exteriorly when they are generated, they could generate a larval skeleton with a relatively plane apex in the ventral transverse rod and that is wide at the posterior end (C).

longer LBL and more spines were also well separated from the others. The second canonical variable (CV2) is negatively correlated with UBL and LBL in which coefficients were −0.840 and −1.309, respectively, and positively correlated with BW and PTRL in which coefficients were 0.928 and 0.946, respectively. The CV2 separated *Echinometra* sp. A and *Echinostrephus molaris* in which larval skeletons had longer UBL and LBL, and narrower BW and PTRL than the other species. *Anthocidaris crassispina* and *H. mammillatus* had similar CV2 values since their larval skeleton had greater BW and PTRL. The third canonical variable (CV3) is most weighted for BW and negatively weighted for SN, with the coefficients 1.311 and −0.612 respectively. The CV3 values mostly overlapped among the species although *Echinometra oblonga* clearly separated from both *A. crassispina* and *Echinostrephus molaris*.

Other characteristics

Small spines usually projected from the postoral rods in each species (arrowheads in Figure 5). Spines on the postoral rod were relatively abundant in the four species of *Echinometra* and less abundant in the other five species (compare the arrowheads in Figure 5). In *C. mertensii*, *H. mammillatus*, and the four species of *Echinometra*, the spines on the postoral rods were relatively short (Figure 5: Cm, EmA, EmC, EmM, EmO, and Hm) as compared with those of the other three species (Figure 5: Ac, EsA, and EsM). Furthermore, in the four *Echinometra* species, the spines usually projected outward and perpendicular to the postoral rod; in the other five species, the spines were oblique to the postoral rod.

Although it is not easy to quantify, the length of the spines on the body skeleton also varies among the species. In *C. mertensii* and *H. mammillatus*, the spines were less than 5 μm long or even bulge-like as in Figures 4 and 5. The spines of the four species of *Echinometra* were longer; in most specimens, the longest spines reached 5–10 μm. *Anthocidaris crassispina* and the two species of *Echinostrephus* had longer spines; their longest spines were usually longer than 10 μm. The species with more spines on body skeleton clearly tend to have longer spines on the postoral rods.

The ends of the body rod are also characteristic of some species. In *C. mertensii*, *H. mammillatus*, and the four *Echinometra* species, both ends of the body rod were curved inwards, and hence the joint of the body rod and the postoral rod curved inward (Figure 7C and Figure 2: Cm, EmA, EmC, EmM, EmO, and Hm). In contrast, in *A. crassispina*, *Echinostrephus aciculatus*, and *E. molaris*, both ends of the body rod were less curved, and hence the joint between the body and the postoral rod was relatively straight (Figure 7D and Figure 2: Ac, EsA, and EsM).

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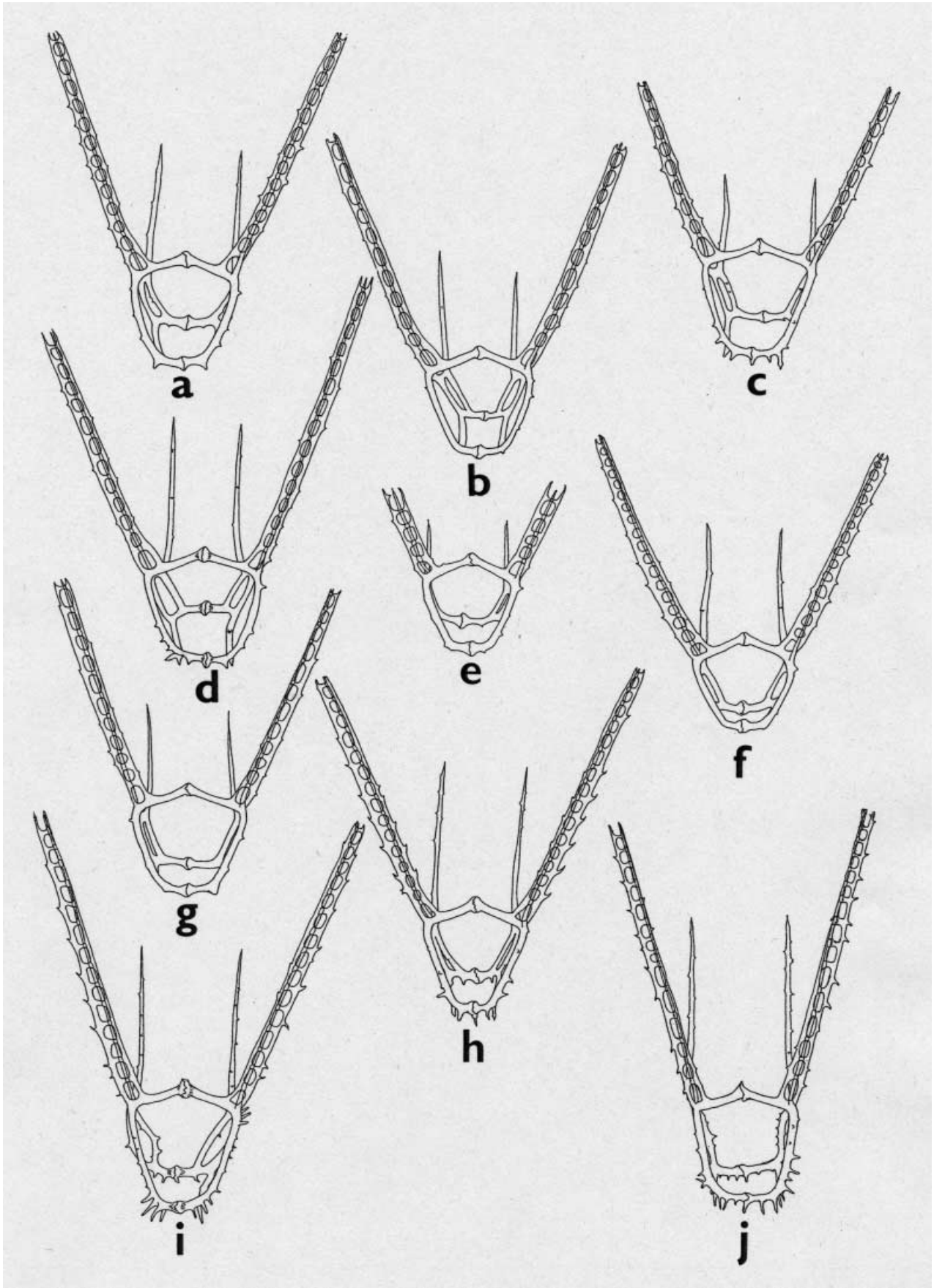

Figure 13. Skeletons of two-day old and five-day old larvae of nine species in the sea urchin family *Echinometridae*. (A–E, G–I) Two-day old larvae; (F) five-days old larva. (A) *Echinometra* sp. A; (B) *Echinometra mathaei*; (C) *Echinometra* sp. C; (D) *Echinometra oblonga*; (E&F) *Colobocentrotus mertensii*; (G) *Heterocentrotus mammillatus*; (H) *Echinostrephus molaris*; (I) *Echinostrephus aciculatus*; (J) *Anthocidaris crassispina*.

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Summary of morphological variation in larval skeletons

Morphological variations of the body skeleton in Echinometridae are summarized in Figure 11. The number of spines and posteroventral transverse rods can be regarded as accessories of the larval skeleton. The most basic and simple type of larval skeleton has no spines and no second posteroventral transverse rod (Figure 11A, left). By adding of skeletal accessories to the basic larval skeleton, morphological variations of larval skeleton are generated. In addition, the shape of the body skeleton varies considerably in many ways (Figure 11B). The characteristics of the larval skeletons of the nine species are summarized in Table 7. Drawings of the skeletons of two-day-old larvae of each of the nine species and of five-day-old larvae of *C. mertensii* are presented in the Figure 13.

DISCUSSION

Variation in skeletal traits

The comparison between larval skeletons of different ages within the same species demonstrated that the length of the postoral rod (PORL) increased during development at early stages; BL, BW, PTRL, and SN did not (Table 2). This indicates that the elements of the body skeleton do not grow after they form the basket-like structure (i.e. once all skeletal elements of the body skeleton have connected to each other). Most studies showed that plasticity is observed only in the arms but not in the larval body skeleton. McEdward & Herrera (1999) showed that the elements of the body skeleton are not affected by food supply, but those of the arm skeleton are affected considerably. Sinervo & McEdward (1988) cultured the halves of two-cell stage embryos and found that the larval skeleton that developed from a half of a two-cell stage embryo can develop body rods close to normal size. Yanagisawa (2001) also observed that the size of the body skeleton of *Colobocentratus mertensii* is kept unchanged during the four-arm stage. Therefore, elements of the body skeleton can be regarded as characteristic of each species. In addition, although PTRN varied even within a species (Table 4), there were apparent and stable differences in the frequency of larvae with single or double posteroventral transverse rods (S.K., personal observation). Thus this frequency can also be regarded as a species-specific characteristic.

Angle of bilateral postoral rod (PORA) differed significantly between two ages in *C. mertensii* (Table 2). Although two-day-old *C. mertensii* larvae had the widest PORA of all species (Table 3), five-day-old larvae had nearly the same PORA as those of the other species. The PORA might be wide when the postoral rod is short and becomes narrower during the early phase of postoral rod elongation. Variation in the angle of the bilateral postoral rods among orders and families is one of the notable characteristics of morphological diversity in sea urchin larvae (Wray, 1992), but it is not species-specific in the echinometrid species we examined.

Morphological differences of larval skeletons among nine species

The morphology of the larval skeletons could be characterized for each species by a combination of several of the characteristics presented here (Table 7).

The larval skeletons of *Anthocidaris crassispina* had many spines and were wide at the posterior end, and thus distinguishable from the other eight species. The two *Echinostrephus* species shared most character states (Table 7) meaning that their larval skeletons are similar. However, the size of the body skeleton was significantly different between them (Figure 6), and CDA also separated them clearly (Figure 10). The larval skeletons of *A. crassispina* shared more character states with *Echinostrephus aciculatus* and *E. molaris* than the other species (Table 7). These three species had characteristically straight body rod ends, more spines, and a vertically longer body skeleton.

The larval skeletons of *C. mertensii* and *Heterocentrotus mammillatus* shared eight of 11 character states (Table 7). These two species had a vertically shorter body skeleton, and fewer and shorter spines and could thus be distinguished from the other species. The shape of the body skeleton differed between them; *C. mertensii* was characterized by a body skeleton with a narrow posterior end; *H. mammillatus* had a body skeleton with a wider posterior end.

The four species of *Echinometra* we examined shared the character states of the arm skeleton, and were thus distinguishable from the other species. Within *Echinometra*, *Echinometra* sp. A had a significantly longer body skeleton (Figure 8A) and higher frequency of double posteroventral transverse rods, which distinguished it from the other three species. *Echinometra mathaei* differed from *Echinometra* sp. C and *E. oblonga* by having fewer spines (Figure 9). On the other hand, no significant differences were noted between *Echinometra* sp. C and *E. oblonga*. Although the size of the body skeleton differed between them, CDA could not separate them (Figure 10). *Echinometra* species shared more characters with, and thus were more similar to, *C. mertensii* and *H. mammillatus* than to the other three species (Table 7).

Larvae of four of the species collected in the field, *A. crassispina*, *Echinometra* sp. A, *E. mathaei*, and *Echinostrephus aciculatus*, can be classified using the keys established in this study (S.K., unpublished observations).

Functional implications of the morphological variation in larval skeletons in the Echinometridae

Lower body length (LBL), body width (BW), and length of the posteroventral transverse rod (PTRL) were positively and significantly correlated to each other (Table 5). These significant correlations indicate that the larval body skeleton tends to increase in size in all dimensions. Increasing rates of UBL, BW and PTRL, however, are not uniform among the species, thus generating the various shapes of body skeletons (Figure 11B).

Another interesting correlation among the skeletal characters is that between lower body length (LBL) and number of spines (SN), namely, larvae with a long body skeleton tend to have many spines (Table 5). The body skeleton, especially the posterior part, is considered a counterweight that lowers the centre of gravity of larvae and thereby orients larvae with the arms upward (Pennington & Emlet, 1986; Pennington & Strathmann, 1990). If a body skeleton is vertically long, the centre of gravity is located more anteriorly, and thus more counterweight might be required for larval orientation. This

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might generate the correlation between LBL and SN as well as the correlation between lower body length (LBL) and PTRN (compare Table 4 with Figure 6B). In *Echinometra* sp. A, which has a greater LBL but fewer spines, the double PTR may contribute as a counterbalance.

The larval skeletal variation in the Echinometridae could be explained another way. The viscosity of water is important to small floating plankton (Tait, 1980; Thurman & Burton, 2001). Small plankton sinks rapidly in warmer, less viscous water, for example in the surface water at lower latitudes. In some copepod species, morphological adaptations to decrease sinking rate are observed in the tropics (Thurman & Burton, 2001). Reduction of high-density material such as the mineral skeleton is generally accepted to be an adaptation to warmer water (Nishimura, 1981). In sea urchin larvae, reduction of the skeletal elements, such as size, spines, and rods of the larval skeleton also appear to be morphological adaptations to decrease sinking rate. Because the density of calcium carbonate, the main component of the larval skeleton, is greater than two-fold that of seawater (approximately 2.71 g/cm^3 vs 1.023 g/cm^3), variations in the length and abundance of spines and the number of posteroventral transverse rods should influence the density of larvae. Indeed, Pennington & Strathmann (1990) found that larvae with more complex skeletons are heavier than those with simpler skeletons. Interestingly we found that, in the Echinometridae, tropical species tend to have fewer and shorter spines. The reduction in skeletal accessories is most prominent in *C. mertensii* and *H. mammillatus*: they are close to the basic larval skeleton type in Figure 1l, and they occur primarily in subtropical and tropical zones (Shigei, 1974). In contrast, spiny larval skeletons are seen in *A. crassispina* and *Echinostrephus aciculatus*, which are distributed mainly in temperate zones (Shigei, 1974). Although it is not clear whether the number of spines and posterior transverse rods affects the specific gravity or sinking rate, the reduction of skeletal elements may be, at least partly, explained as an adaptation to tropical waters. Thus, the variation in size, and the abundances of spines and rods of the larval skeleton may correspond to the temperature, viscosity and density of seawater that each species inhabits.

Upper body length (UBL) was negatively correlated with PTRL (Table 5), indicating that larval skeletons with a pointed apex of ventral transverse rod tend to have a narrow posterior end. We know of no functional reasons for this correlation; rather we prefer to interpret this correlation as being a developmental constraint. The larval skeleton is initiated from triradiate spicules, and two processes of the spicules grow up to form the ventral transverse rod and body rod, and finally make up the ventral side of the body skeleton (Figure 1l) (Okazaki, 1960). In the Echinometridae, the angle of the ventral transverse rod to the body rod is relatively constant among the species (approximately 95° ±3.5, data not shown), suggesting that the angle is under developmental constraint in the way these rods are produced. Therefore, the correlation between UBL and PTRL may be due to a constraint on the initial angle of the processes of the triradiate spicules (Figure 1l).

We have described the morphological diversity of larval skeletons in nine echinometrid sea urchins. We found that, although most of the skeletal characters can evolve independently, some characters are correlated. The correlation between spine number and lower body length can be explained from a functional point of view. We also found that developmental constraint may account for the correlation of skeletal characters, such as that between UBL and PTRL. Since variation of the larval skeleton of sea urchins is one of the few subjects accessible from both ecological and developmental standpoints, the detailed descriptions presented here are the first step in linking ecology, developmental biology and evolution.

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