Silicoflagellates and ebridians from the Seto Inland Sea and Kuroshio, including the description of *Octactis pulchra* var. *takahashii* var. nov.

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Abstract Three silicoflagellate species, *Octactis pulchra*, *Dictyocha stapedia* and *Stephanocha speculum*, and the ebridian *Ebria tripartita*, were observed in water samples from the Seto Inland Sea and the adjacent Kuroshio. Both *Octactis* and *Dictyocha* exhibit two distinct morphologies, with skeletons made of thicker components predominating in the Seto Inland Sea, and of thinner components in the Kuroshio. The skeletons of *O. pulchra* var. *takahashii* var. nov. have a more fragile appearance, with thinner components, longer spines and a smaller apical ring, compared to basal ring diameters, than the better known type variety *O. pulchra* var. *pulchra*. Both thick and thin forms of *Octactis* and *Dictyocha* were often found as double skeletons, with skeletons of *Octactis* apparently attached at the abbasal surfaces of the basal corners by organic matter, while those of *Dictyocha* were held in place by pikes. Only one specimen of *S. speculum* and a small number of ebridians were observed in the Seto Inland Sea, with both being absent in the Kuroshio samples.

Keywords Japan, Kuroshio, Seto Inland Sea, ebridians, silicoflagellates, living assemblages, oceanography

1. Introduction

The Seto Inland Sea (also known as simply the Inland Sea) is 450km long from east to west, and is relatively shallow, with an average depth of 37.3m and a greatest depth of 105m (Imai *et al*., 2006). It is almost entirely surrounded by Honshu, Shikoku and Kyushu Islands, but has two open connections to the North Pacific *via* the Kii and Bungo Channels, and a narrow connection to the Sea of Japan *via* the Kanmon Strait (Figures 1a, b). In comparison to the Kuroshio, the waters of the Seto Inland Sea have a lower salinity and higher nutrient load associated with dense human populations and fluvial input, notably from the Yodo, Takahashi, Kako and Ohta Rivers (Figure 1b). The Seto Inland Sea is characterised by red tides (Okaichi, 2003), which have increased in frequency and scale since the 1960s, due to intense eutrophication along the coastline (Goto, 1975).

The Kuroshio is about 100km wide, 1km deep and some 3000km in length (Barkley, 1970), and is characterised by straight and meandering paths (see Figure 1a; Kawabe, 1980). Fluctuations in these paths bring warmwater intrusions to Enshu Nada and Kumano Nada for different lengths of time, about 20 and 50 days, respectively (Kasai *et al*., 1993). Cold water rises to the surface between the Kuroshio meander and the Japanese coast, causing a temperature decrease of about 10°C as the meander develops (Barkley, 1970). The changing position of the meander results in fluctuating oceanographic conditions, which have a great effect on the distribution of the marine biota, notably the position of the fishing grounds. The Kuroshio core-waters are generally warm (>23°C), salty (>34.7‰) and low in nutrients (Lie *et al*., 1998; Du *et al*., 2013).

Only one significant report on silicoflagellates and ebridians in the Seto Inland Sea exists in the literature. Yanagisawa (1943) recorded *Dictyocha stapedia* Haeckel (as *Dictyocha fibula* var. *stapedia* (Haeckel) Lemmermann, and maybe some of the other subtaxa) in large num-
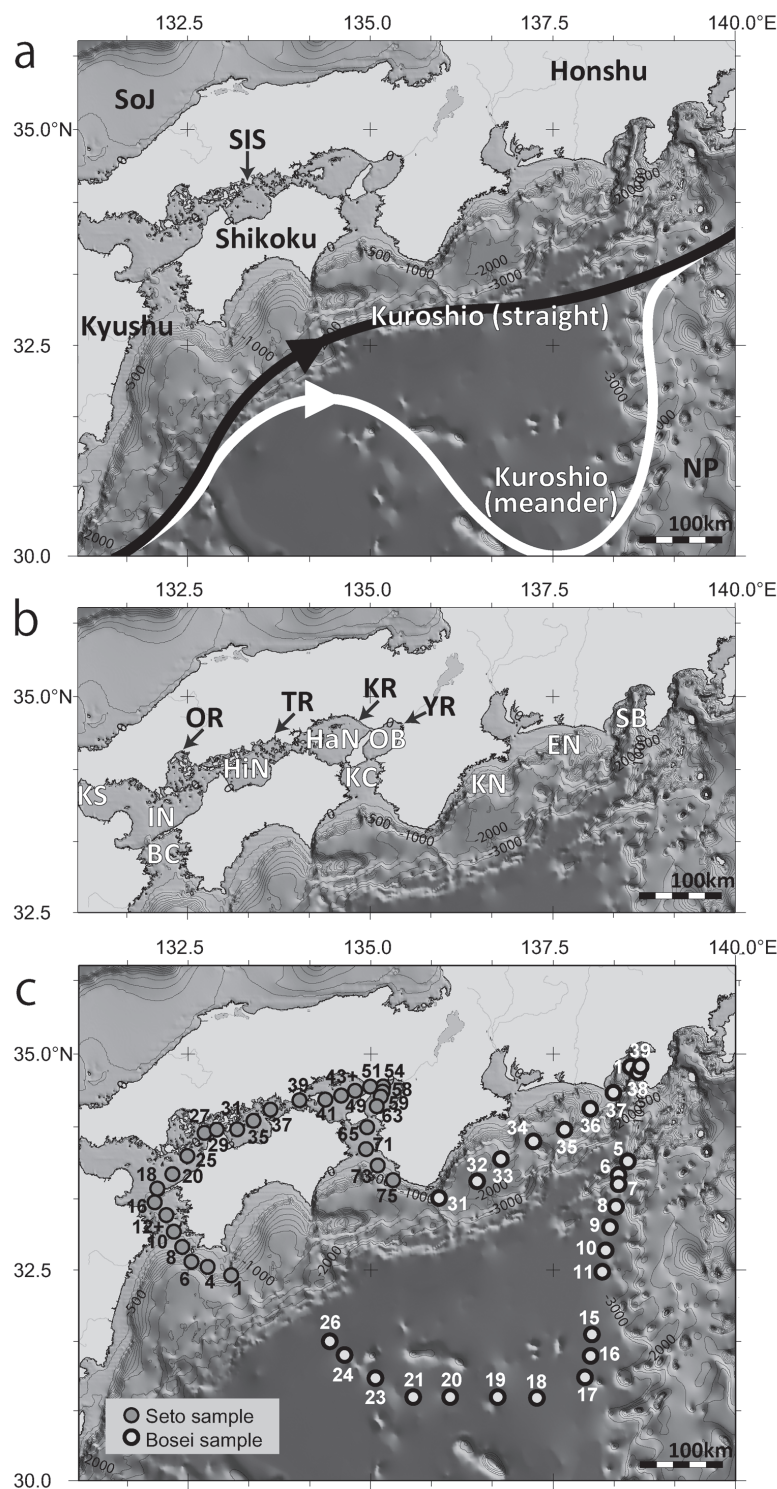
Figure 1: Seto Inland Sea and adjacent Kuroshio: (**a**) Map of sampling area. Isobaths shown in metres. Approximate straight (black line) and meandering (white line) positions of the Kuroshio modified from Kawabe (1980). SIS - Seto Inland Sea, SoJ - Sea of Japan, NP - North Pacific. (**b**) Map showing locations of rivers and oceanographic areas mentioned in the text. KS - Kanmon Strait, BC - Bungo Channel, IN - Iyo Nada, OR - Ohta River, HiN - Hiuchi Nada, TR - Takahashi River, HaN - Harima Nada, KR - Kako River, OB - Osaka Bay, YR - Yodo River, KC - Kii Channel, KN - Kumano Nada, EN - Enshu Nada, SB - Suruga Bay. (**c**) Map showing location of Seto and Bosei samples

bers in Osaka Bay, with *Stephanocha speculum* (Ehrenberg) McCartney & Jordan (as *Distephanus speculum* (Ehrenberg) Haeckel), *Octactis pulchra* Schiller (as *Mesocena polymorpha* var. *bioctonaria* (Ehrenberg) Lemmermann and *Distephanus speculum* var. *octonarius* (Ehrenberg) Jørgensen), and the ebridian *Ebria tripartita* (Schumann) Lemmermann was also present. It should be noted that the illegitimate name *Distephanus* Stöhr was recently replaced by *Stephanocha* McCartney & Jordan (Jordan & McCartney, 2015).

During a search for silicoflagellate double skeletons (also called doublets) in a separate study (McCartney *et al*., 2014a), several illustrations of double skeletons of *O. pulchra* were found in an unpublished thesis on the Seto Inland Sea, written by Yuko Fukunaga. These samples, and others from the adjacent Kuroshio, have been re-examined in the scanning electron microscope (SEM) to determine the silicoflagellate and ebridian assemblage composition. Three silicoflagellate species were observed, including double skeletons of *Dictyocha* and *Octactis*. Examination of the *Octactis* double skeletons revealed new information on their fine structure and skeletal variability, and may give clues to their evolution. In addition, specimens of the rarely-studied ebridian *E. tripartita* were observed and incorporated into this study.

2. Materials and methods

Surface-water samples were collected from, and south-east of, the Seto Inland Sea during the *Bosei-maru* Cruise (26th April to 2nd May, 2008) of the Department of Ocean Science, Tokai University. The samples were obtained using the shipboard sea-water supply (for research use), with temperature and salinity data recorded using a continuous salinity temperature recorder (Horiba U-22XD water quality monitor). Two sets of samples were collected, Seto samples from

the Seto Inland Sea and Bosei samples from the Kuroshio and nearby Enshu Nada (Figure 1b). In total, 55 filter samples were examined for this study (Figure 1c): 28 samples from the Seto Inland Sea, including three from southern Shikoku (Seto-1 to Seto-6), four from the Bungo Channel (Seto-8 to Seto-16), four from Iyo Nada (Seto-18 to Seto-27), four from Hiuchi Nada (Seto-29 to Seto-37), five from Harima Nada (Seto-39 to Seto-51), four from Osaka Bay (Seto-54 to Seto-63) and four from the Kii Channel (Seto-65 to Seto-75), as well as 27 samples in, or near to, the Kuroshio, south-east of Shikoku Island and Kii Channel, including three from Kumano Nada (Bosei-31 to Bosei-33), three from Enshu Nada (Bosei-34+ to Bosei-36), four from Suruga Bay (Bosei-1, Bosei-37 to Bosei-39) and 17 from the open ocean within the Kuroshio (Bosei-5 to Bosei-26). A list of the sample data, water temperature, salinity, nutrients (silicate, nitrate, phosphate) and chlorophyll-*a* is provided in Table 1.

On board the ship, about 0.7-2.0 litres of surface seawater were filtered through a 47mm-diameter Millipore HA-type nitrocellulose filter (0.45μm porosity). The filters were dried at room temperature and stored in plastic Petri dishes. Later, in a shore-based laboratory at Yamagata University, the filter samples were rinsed with distilled water to remove salt crystals, and then re-dried and stored again in plastic Petri dishes.

For the SEM study, samples were prepared by cutting off a small piece of filter (3x3mm), which was mounted on an aluminium SEM stub, then coated with Pt/Pd in an Eiko IB-3 ion sputter-coater. The filter samples were then observed in a JEOL JSM-6510LV SEM. All silicoflagellates and ebridians on the entire filter piece were counted (Table 2), and many were photographed using the digital camera built into the SEM (Plates 1-4). Most specimens lay flat on their basal or apical rings, so that single skeletons were generally observed in apical or abapical views and double skeletons in apical axis view. Some specimens were oriented, or could be tilted, to provide lateral or oblique views (see Figure 2 for the terminology used in this paper).

Specimens were generally in excellent condition, although those of Seto-29 were abraded. Fragmented specimens were unusual, but those with more than half the basal ring were included in the counts. The absolute abundances (number of skeletons/litre) of each taxon were calculated using the equation:

$$A = NS/V$$

, where A = the absolute abundance, N = the number of individual skeletons counted on the stub, S = the scaling factor and V = the volume of sea-water (in litres) filtered. Double skeletons were counted as two individuals.

It should be noted that some specimens were photographed on either an extra stub of Seto-35 (Pl.3, fig.2), or an extra filter of Seto-59 (Pl.2, fig.4; Pl.4, fig.6), but these were not included in Table 2.

3. Results

A total of 256 specimens of *Octactis* were observed (Plates 1-3) and counted (Table 2), of which 229 (89%) were *O. pulchra* var. *pulchra* and 70 (~27%) were double skeletons, with all but five of the latter being from the Seto Inland Sea. Of the *Octactis* specimens, 220 (86%) were eight-sided, with nine seven-sided and 22 nine-sided examples (Table 2). There were also five doublet specimens, composed of skeletons with a contrasting number of basal and apical sides (herein labeled 'mix'). *Octactis* can be separated into two varieties, based on the thickness of the components (elements or spines) that make up the skeletons.

A total of 57 specimens (representing 53 single and four double skeletons) of *Dictyocha stapedia* were also observed (Pl.4, figs 1-7) and counted (Table 2), of which 44 (77%) were found in the Seto Inland Sea. One specimen of *Stephanocha speculum* var. *bispicatus* (Bukry) McCartney & Jordan and 15 specimens of *E. tripartita* were found only in the Seto Inland Sea (Pl.4, fig.8 and figs 9-12, respectively). Observations on the doublets, as well as on some interesting aberrant skeletons, constitute the main focus of this study. Some basic information on single skeletons can be found in the Systematics section, below.

3.1 Observations on *Octactis pulchra* double skeletons

In the study area, skeletons composed of both thick (Plates 1, 2) and thin (Plate 3) components of this species are present, but with different distributions. Skeletons made of thicker components were mostly observed in the Seto Inland Sea samples, while those with thinner components occur more commonly in the Kuroshio samples. These differences have prompted the proposal of a new variety, *O. pulchra* var. *takahashii* (see description in the System-

Sample code	Date/Time	Latitude N	Longitude E	Temp. [°C]	Salinity	NO$_3$ [µmol/l]	PO$_4$ [µmol/l]	Si [µmol/l]	Chl-a [µg/l]	Water filtered [L]
Bosei-01	2004/4/25 11:21	34.88	138.44	20.23	34.38	0.39	0.14	2.82	0.91	1
Bosei-05	2004/4/25 20:05	33.73	138.44	19.09	34.50				0.51	2
Bosei-06	2004/4/25 20:49	33.58	138.41	19.32	34.50	0.77	0.04	2.54	0.68	2
Bosei-07	2004/4/25 21:20	33.49	138.39	19.45	34.51	0.47	0.06	2.67	0.44	2
Bosei-08	2004/4/25 22:36	33.25	138.34	19.64	34.48	0.30	0.05	2.01	0.53	2
Bosei-09	2004/4/25 23:58	33.00	138.29	21.48	34.45	1.40	0.06	1.42	0.76	2
Bosei-10	2004/4/26 01:31	32.75	138.24	22.53	34.50	0.35	0.04	2.12	0.43	2
Bosei-11	2004/4/26 02:53	32.50	138.19	22.47	34.52	1.40	0.03	1.91	0.28	2
Bosei-15	2004/4/26 11:06	31.75	138.03	22.64	34.69	0.15	0.03	2.38	0.20	2
Bosei-16	2004/4/26 12:10	31.50	137.98	22.88	34.65	0.18	0.04	1.40	0.19	2
Bosei-17	2004/4/26 13:13	31.24	137.93	23.21	34.70	0.10	0.02	1.49	0.19	2
Bosei-18	2004/4/26 18:35	31.00	137.28	19.96	34.82	0.08	0.01	1.63	0.14	2
Bosei-19	2004/4/26 20:48	31.00	136.69	20.06	34.79	0.12	0.02	1.50	0.16	2
Bosei-20	2004/4/26 22:57	31.00	136.09	22.30	34.51	0.39	0.02	1.03	0.27	2
Bosei-21	2004/4/27 01:08	31.00	135.50	21.45	34.63	0.58	0.06	1.66	0.63	2
Bosei-23	2004/4/27 05:36	31.54	134.66	18.89	34.80					2
Bosei-24	2004/4/27 05:04	31.54	134.67	18.88	34.81	0.14	0.02	1.27	0.43	2
Bosei-26	2004/4/27 14:35	31.67	134.39	19.88	34.79	0.40	0.02	1.35	0.20	2
Seto-1	2004/4/27 21:17	32.45	133.09	22.96	34.47	0.80	0.05	2.04	0.25	2
Seto-4	2004/4/27 23:00	32.58	132.77	22.74	34.49	0.14	0.03	0.99	0.33	2
Seto-6	2004/4/28 00:00	32.64	132.56	21.05	34.45	0.11	0.06	0.89	0.46	2
Seto-8	2004/4/28 01:00	32.77	132.40	19.97	34.48	0.23	0.04	0.76	0.32	2
Seto-10	2004/4/28 02:00	32.94	132.31	20.10	34.48	0.52	0.07	0.76	0.41	2
Seto-12+	2004/4/28 03:14	33.13	132.19	18.04	34.50					2
Seto-16	2004/4/28 05:00	33.29	132.03	15.29	34.20	1.78	0.19	4.46	0.16	1
Seto-18	2004/4/28 06:00	33.46	132.08	14.52	34.07	0.86	0.18	5.19	0.81	2
Seto-20	2004/4/28 07:02	33.61	132.26	14.00	33.94	0.07	0.14	4.55	0.44	2
Seto-25	2004/4/28 09:04	33.81	132.52	14.11	33.89	0.28	0.15	4.81	0.66	2
Seto-27	2004/4/28 10:01	33.96	132.72	14.07	33.84	0.28	0.16	5.15	1.86	2
Seto-29	2004/4/28 11:00	34.14	132.90	13.58	33.72	0.44	0.20	4.01	1.41	2
Seto-31	2004/4/28 12:01	34.13	133.11	14.90	33.51	0.08	0.17	1.37	0.58	2
Seto-35	2004/4/28 14:00	34.22	133.47	16.12	33.07					1
Seto-37	2004/4/28 15:01	34.33	133.74	15.02	33.08	0.14	0.18	0.95	2.02	1
Seto-39	2004/4/28 15:59	34.42	133.99	15.34	32.74	0.12	0.17	0.76	5.00	1
Seto-41	2004/4/28 17:01	34.39	134.29	15.50	32.39	0.06	0.18	0.87	1.40	1
Seto-43	2004/4/28 17:59	34.46	134.57	14.98	32.49	0.10	0.76	3.91	0.93	1
Seto-49	2004/4/29 10:02	34.54	134.76	13.64	32.37	0.62	0.18	4.86	2.41	1
Seto-51	2004/4/29 11:01	34.62	135.02	13.68	32.17	0.31	0.15	4.18	3.43	1
Seto-54	2004/4/29 12:01	34.63	135.18	17.90	27.34	0.09	0.03	3.39	24.10	0.7
Seto-58	2004/4/30 09:50	34.54	135.17	14.81	29.85	0.09	0.00	0.76	10.82	1
Seto-59	2004/4/30 10:28	34.47	135.13	14.42	31.69	0.28	0.05	14.90	0.83	1
Seto-63	2004/4/30 11:01	34.41	135.06	15.20	32.10	0.35	0.17	4.07	0.63	1
Seto-65	2004/4/30 12:02	34.17	134.95	14.20	32.60	0.35	0.19	5.28	1.76	1
Seto-71	2004/4/30 14:03	33.83	134.94	15.78	33.00	0.20	0.45	5.34	0.38	1
Seto-73	2004/4/30 15:03	33.66	135.14	18.94	34.46	0.16	0.09	1.73	0.57	1
Seto-75	2004/4/30 16:03	33.50	135.35	21.11	34.46	0.42	0.09	1.59	0.75	1
Bosei-31	2004/4/30 18:01	33.36	135.93	22.33	34.47	0.05	0.04	1.11	0.41	2
Bosei-32	2004/4/30 20:01	33.58	136.41	19.45	34.33	0.07	0.03	2.01	0.47	1.5
Bosei-33	2004/4/30 22:02	33.80	136.82	19.81	34.38	0.36	0.07	3.05	0.28	2
Bosei-34+	2004/5/1 00:13	34.00	137.27	20.00	34.60					2
Bosei-35	2004/5/1 02:02	34.17	137.62	20.03	34.51	10.28	0.33	14.56	0.22	2
Bosei-36	2004/5/1 04:03	34.36	138.02	19.66	34.48	0.83	0.03	0.83	0.27	2
Bosei-37	2004/5/1 06:01	34.58	138.37	19.68	34.23	0.08	0.02	0.97	0.18	2
Bosei-38	2004/5/1 08:02	34.66	138.66	20.27	34.28	0.09	0.01	1.21	0.69	2
Bosei-39	2004/5/1 10:03	34.89	138.58	19.65	34.31	0.15	0.03	1.24	0.27	1

Table 1: List of Seto and Bosei samples examined in this study and oceanographic data

atics section), for the skeletal morphology that has thinner components and often longer basal spines. Although single skeletons were numerous, the samples also yielded a large number of double skeletons, particularly of *O. pulchra* var. *pulchra* (Table 2; double skeletons:single skeletons = 65:159 in the Seto Inland Sea for var. *pulchra*, 3:20 in the Kuroshio for var. *takahashii*). In some cases, the components of the daughter and mother skeletons are of similar thickness (Pl.1, fig.3), whilst in others, the daughter skeleton has visibly thinner components (Pl.1, fig.5a; Pl.2, fig.2), particularly in the case of double-skeleton specimens of *O. pulchra* var. *pulchra*. This difference in thickness is not restricted to the basal sides and spines, but also to the elements that compose the apical ring (as seen in Pl.2, fig.1).

The double skeletons of *Octactis* are always in the corner-to-corner configuration (see McCartney *et al.*, 2015 for a discussion on configurations), but since they lack pikes, it is assumed that the abbasal surfaces of the two skeletons are held together by organic matter (cf. *D. stapedia*). Most spines of the paired skeletons touch only near the basal ring and separate distally, and thus are not generally in contact over their entire lengths, as is the case for *Stephanocha* (McCartney *et al.*, 2014a). There is also

Taxon	O. pulchra var. pulchra									O. pulchra var. takahashii						D. stapedia				S. speculum var. bispicata			E. tripartita			
	single skeletons			double skeletons						sing.	sing.	doub.	sing.			sing.	doub.			single						
# sides	7	8	9	7	8	9	mix	N	A	7	8	8	9	N	A	4	4	N	A	6	N	A	N	A	total N	total A
Seto-1	0	0	0	0	0	0	0	0	0	0	2	1	0	4	239	2	1	4	239	0	0	0	0	0	8	478
Seto-4	0	1	0	0	0	0	0	1	60	0	0	0	0	0	0	1	0	1	60	0	0	0	0	0	2	120
Seto-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-8	0	0	0	0	0	0	0	0	0	0	0	1	0	2	120	0	0	0	0	0	0	0	0	0	2	120
Seto-10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-12+	0	2	0	0	0	0	0	2	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	120
Seto-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	120	0	0	0	0	0	1	120
Seto-18	1	0	0	0	0	0	0	1	60	0	0	0	0	0	0	0	0	0	0	1	1	60	0	0	2	120
Seto-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-25	0	3	1	0	1	0	0	6	359	0	0	0	0	0	0	0	0	0	0	0	0	0	1	60	7	418
Seto-27	0	25	2	2	14	0	3	65	3886	0	0	0	0	0	0	0	0	0	0	0	0	0	2	120	67	4005
Seto-29	0	16	1	0	0	0	0	17	1016	0	0	0	0	0	0	0	0	0	0	0	0	0	2	120	19	1136
Seto-31	0	3	0	0	0	0	0	3	179	0	0	0	0	0	0	3	0	3	179	0	0	0	6	359	12	717
Seto-35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-37	0	1	0	0	0	0	0	1	120	0	0	0	0	0	0	0	0	0	0	0	0	0	2	239	3	359
Seto-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-41	0	1	0	0	0	0	0	1	120	0	0	0	0	0	0	0	0	0	0	0	0	0	1	120	2	239
Seto-43	0	17	1	0	0	1	0	20	2391	0	0	0	0	0	0	2	0	2	239	0	0	0	0	0	22	2630
Seto-49	1	14	3	0	13	2	0	48	5739	0	0	0	0	0	0	14	0	14	1674	0	0	0	0	0	62	7412
Seto-51	1	19	3	0	18	1	2	65	7771	0	0	0	0	0	0	12	1	14	1674	0	0	0	0	0	79	9445
Seto-54	0	0	0	0	0	1	0	2	342	0	0	0	0	0	0	1	0	1	171	0	0	0	0	0	3	512
Seto-58	0	4	1	0	0	0	0	5	598	0	0	0	0	0	0	1	0	1	120	0	0	0	1	120	7	837
Seto-63	0	8	0	0	2	0	0	12	1435	0	0	0	0	0	0	1	0	1	120	0	0	0	0	0	13	1554
Seto-65	2	13	3	0	5	0	0	28	3348	0	0	0	0	0	0	4	0	4	478	0	0	0	0	0	32	3826
Seto-71	0	11	1	0	0	0	0	12	1435	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	1435
Seto-73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seto-75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-09	0	0	0	0	0	0	0	0	0	0	3	0	1	4	239	0	0	0	0	0	0	0	0	0	4	239
Bosei-10	0	0	0	0	0	0	0	0	0	0	5	1	0	7	418	2	0	2	120	0	0	0	0	0	9	538
Bosei-11	0	0	0	0	0	0	0	0	0	1	3	1	0	6	359	1	0	1	60	0	0	0	0	0	7	418
Bosei-15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	60	0	0	0	0	0	1	60
Bosei-17	0	0	0	0	0	0	0	0	0	0	1	1	0	3	179	1	0	1	60	0	0	0	0	0	4	239
Bosei-18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-20	0	0	0	0	0	0	0	0	0	0	4	0	0	4	239	1	0	1	60	0	0	0	0	0	5	299
Bosei-21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	60	0	0	0	0	0	1	60
Bosei-26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	120	0	0	0	0	0	2	120
Bosei-31	0	0	0	0	0	0	0	0	0	0	1	0	0	1	60	2	0	2	120	0	0	0	0	0	3	179
Bosei-32	0	1	0	0	0	0	0	1	80	0	1	0	0	1	80	2	1	2	159	0	0	0	0	0	4	319
Bosei-33	0	1	0	0	0	0	0	1	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	60
Bosei-34+	0	1	0	0	0	0	0	1	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	60
Bosei-35	1	1	0	0	0	0	0	2	120	0	0	0	0	0	0	1	0	1	60	0	0	0	0	0	3	179
Bosei-36	0	1	0	0	0	0	0	1	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	60
Bosei-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosei-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2: Absolute abundances (in skeletons/l) and raw counts of the five silicoflagellate and ebridian taxa recorded in this study. *Octactis* and *Dictyocha* specimens further separated into single and double skeletons, and number of basal sides. In column of total skeletons (N), a double skeleton = 2 and a single skeleton = 1. V = volume of sea-water filtered
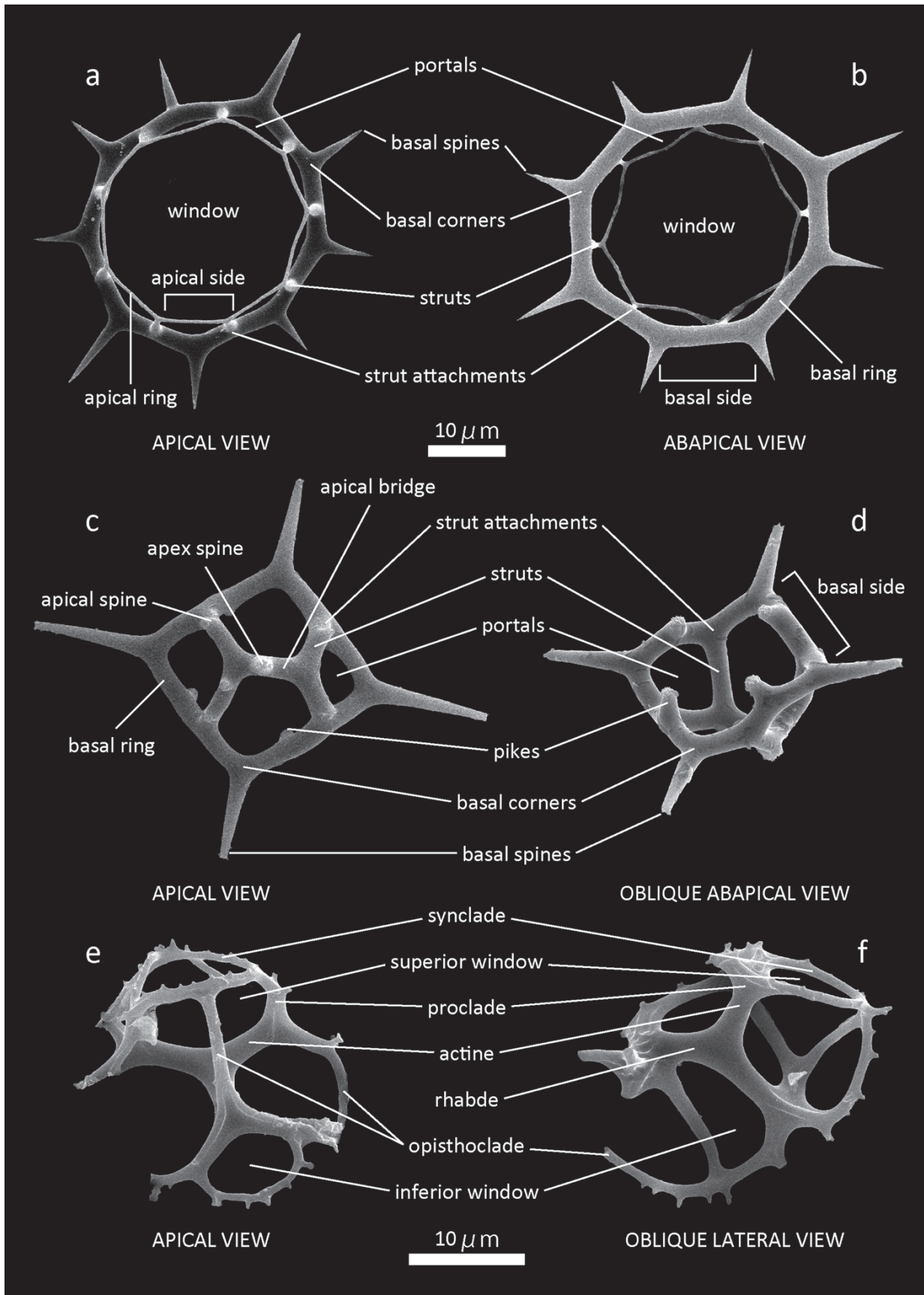
Figure 2: Terminology of silicoflagellate and ebridian skeletons, based on McCartney *et al*. (2014a) and Deflandre (1934), respectively. *Octactis pulchra* var. *pulchra* in (**a**) apical and (**b**) abapical view. *Dictyocha stapedia* in (**c**) apical and (**d**) oblique abapical view. *Ebria tripartita* in (**e**) apical and (**f**) oblique lateral view

a gap between the basal rings along the length of the basal sides, with the gap being most pronounced opposite the strut attachments (Pl.1, fig.4b; see also McCartney *et al.*, 2014a). The cementing agent appears to be quite strong, as shown by one single skeleton in abapical view (Pl.2, figs 6a, b) that had a fragment of the basal corner of the other skeleton still attached, after the rest had broken away. This specimen was from sample Seto-29, in which there were no intact double skeletons, presumably due to abrasion or partial dissolution, yet this specimen retained the connected fragment.

One interesting double skeleton (Pl.1, figs 5a-c) has a mother skeleton in apical view nearest the observer and in front of an incompletely formed daughter skeleton. The mother skeleton has two apical ring elements, positioned on opposite apical sides, which are incompletely formed, with small gaps in the middle (see inset Pl.1, figs 5b, c). The ends are closed, showing that these are not broken. This provides further evidence that the apical ring elements form initially at the triple junctions and develop towards the middle. This specimen also shows that the mother skeleton may not be complete before the daughter forms. Two other specimens were observed with this feature on one apical side; in one case the gap is near the triple junction, rather than in the middle.

3.2 Aberrants of *Octactis pulchra*

While truly distorted or unusual (see McCartney *et al.*, 2014b) teratoid skeletons were rare, and are not presented in this study, some minor aberrants are discussed here. A few specimens possessed a slightly longer side with an additional, small spine, providing a very short, vestigial ninth basal side that was not associated with a strut (Pl.2, fig.9). Another specimen (Pl.2, fig.4) appears to have grown two extra basal spines on the inner surface of the basal ring; their bases seem to be continuous with the basal ring, and so do not appear to be remnants of a previously attached skeleton of a doublet. Five doublets possessed skeletons with different numbers of sides ('mix' in Table 2). Four of these specimens (three in Seto-27 and one in Seto-51) had eight- and nine-sided basal rings (*e.g.* Pl.2, figs 11a, b), while another specimen from Seto-51 had seven- and eight-sided basal rings, where one of the seven corners was bypassed by a long side (Pl.2, fig.10). Two single skeletons with bulbs at various locations on the basal ring (Pl.2, figs 4, 7), and a specimen that had a divided apical ring (Pl.2, fig.8), were also present in the Seto-51 sample. Furthermore, single skeletons of the daughter lacking an apical ring were also observed and are the subject of a separate study (McCartney *et al.*, submitted). A similar example has been illustrated already in a previous study (McCartney *et al.*, 2014a, fig.6c).

3.3 Observations on *Dictyocha stapedia* double skeletons

Specimens of *D. stapedia* were less abundant than *Octactis* in most of the samples we observed. *D. stapedia* skeletons made of relatively thinner (Pl.4, figs 1-3) and thicker (Pl.4, figs 4-6) components were present in the study area, with distributions similar to those of the *O. pulchra* thin and thick forms: *i.e.* the thin forms were mostly found in the Kuroshio samples, while the thick forms were observed in the Seto Inland Sea. However, apart from distribution and degree of skeletal thickness, the morphology of the two forms was identical. Of particular interest, though, are the double skeletons, which are always in the corner-to-corner configuration and use pikes to hold the two skeletons together, in contrast to *O. pulchra*, which lacks pikes and is presumably held together by organic material between the basal corners (McCartney *et al.*, 2014a). In some cases, the daughter and mother skeletons are of similar thickness (Pl.4, fig.3), whilst in others, the thinner daughter skeleton is easily visible (Pl.4, figs 6, 7). It is clear from Table 2 that double skeletons are quite rare, compared to single skeletons (two *versus* 42 in the Seto Inland Sea, two *versus* 11 in the Kuroshio).

3.4 Absolute abundances of silicoflagellates and ebridians

Although silicoflagellate absolute abundances were quite low, they were much higher in the Seto Inland Sea than in the Kuroshio (up to 9.4×10^3 *versus* 0.5×10^3 skeletons/l, respectively), particularly in the Osaka Bay area. With regard to the silicoflagellate and ebridian assemblage, *Octactis* was predominant and *Dictyocha* common in the Seto Inland Sea (up to 7.8×10^3 *versus* up to 1.7×10^3 skeletons/l, respectively), while both were uncommon in the Kuroshio (up to 0.4×10^3 *versus* up to 0.2×10^3 skeletons/l, respectively). On the other hand, ebridians and *Stephanocha* were uncommon in the Seto Inland Sea, and not observed in the Kuroshio samples.

Plate 1

Octactis pulchra var. *pulchra*

single skeletons - 1, 2; **double skeletons** - 3-6

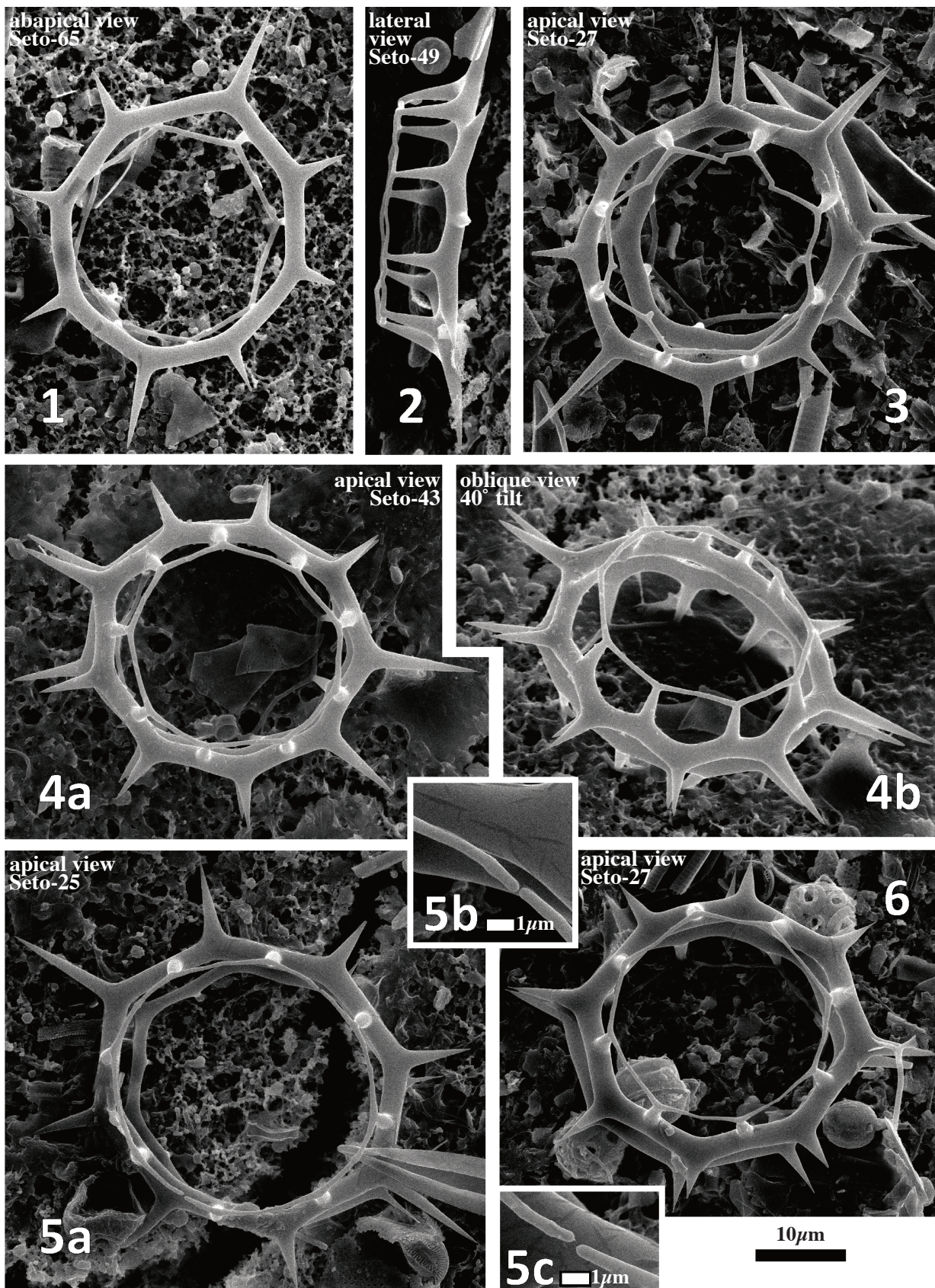(5b - magnification of upper right region of apical ring; 5c - magnification of lower left region of apical ring)

Plate 2

Octactis pulchra var. *pulchra*

single skeletons - 3 (b - broken strut attachment), 4 (aberrant form with spines on bottom of basal ring, swollen basal sides), 5 (a - with broken struts, lacking apical ring; b - close-up of broken hollow struts), 6 (b - detail of corner of daughter(?) still attached), 7 (aberrant form with basal ring expansion at corners of strut attachments), 9;
double skeletons - 1, 2, 8, 10 (near skeleton 7-sided, far skeleton 8-sided), 11
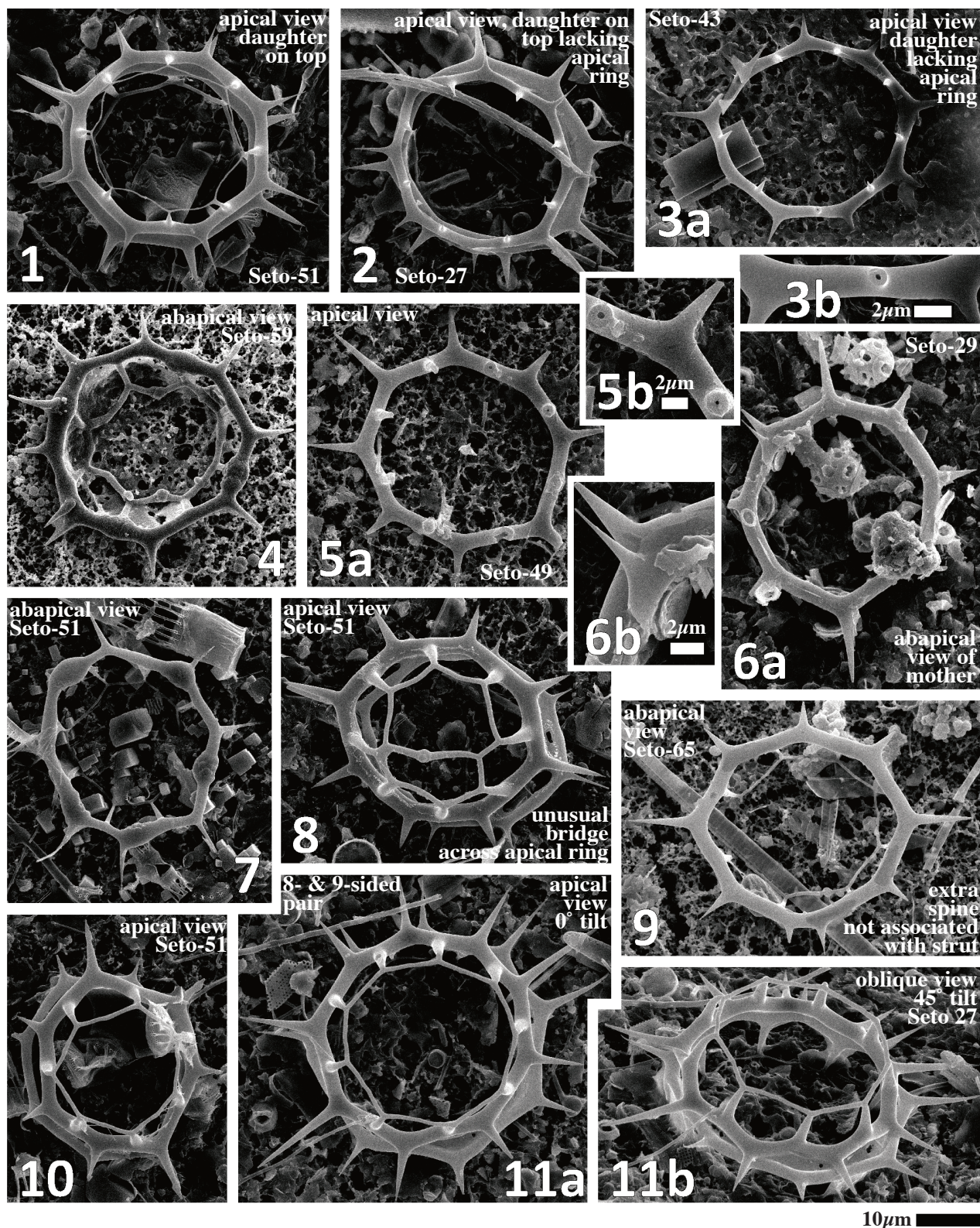
Plate 3

Octactis pulchra var. *takahashii* var. nov.

single skeletons - 1, 3, 5, 6; **double skeletons** - 2, 4, 7 (type specimen)
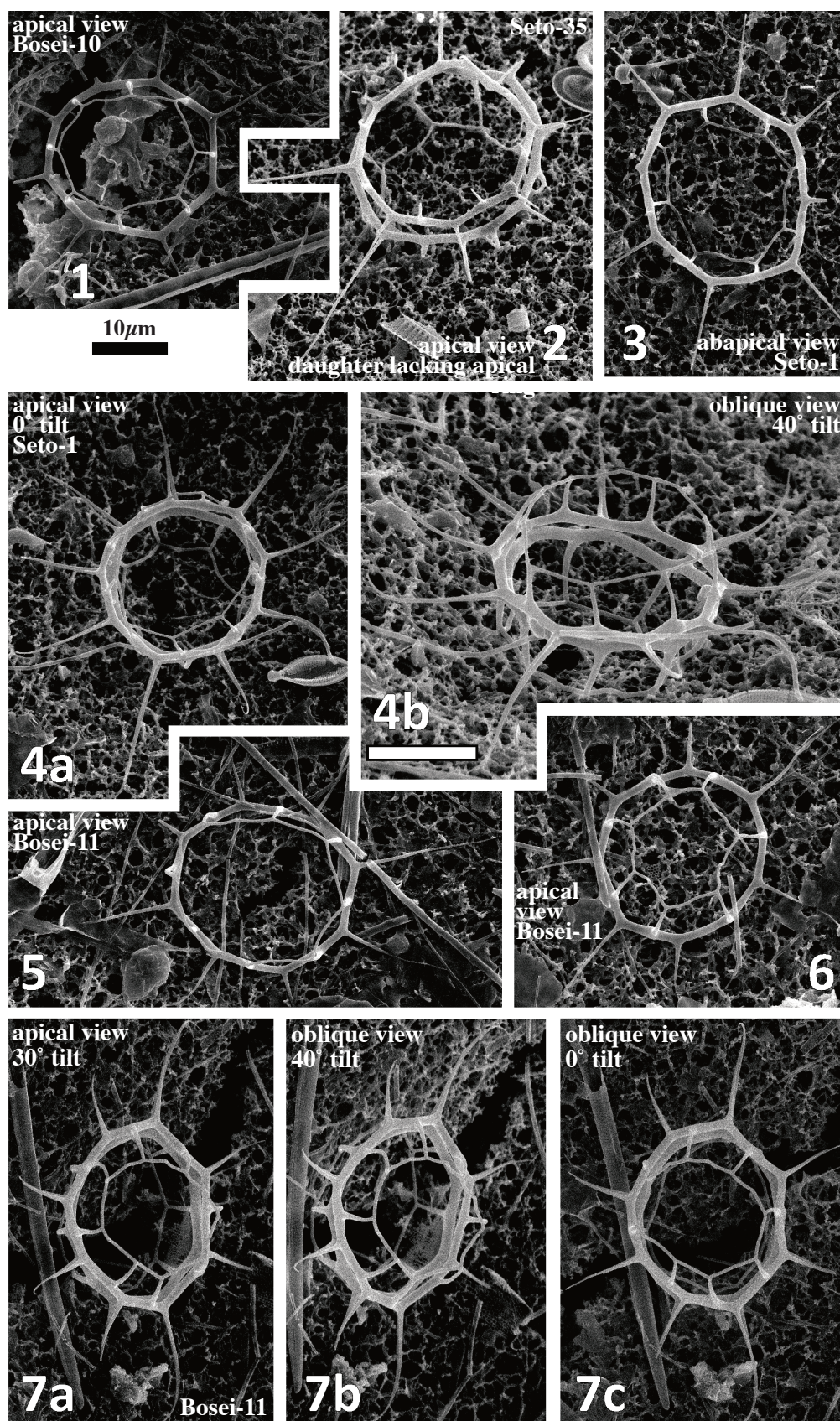
Plate 4

Dictyocha stapedia, Stephanocha speculum, Ebria tripartita

single skeletons - 1, 2, 4, 5; **double skeletons** - 3, 6, 7 (thinner daughter skeleton in foreground)

D. stapedia apical view Bosei-26

D. stapedia oblique abapical view Bosei-26

D. stapedia lateral view, 30° tilt Bosei-31

5μm (1-3)

10μm (4-8)

D. stapedia apical view Seto-49

D. stapedia oblique abapical view Seto-49

D. stapedia lateral view 30° tilt Seto-59

D. stapedia oblique lateral view Seto-51

Seto-18

S. speculum apical view

E. tripartita Seto-37

5μm (9-12)

E. tripartita, Seto-27

E. tripartita, Seto-25

E. tripartita, Seto-31

4. Discussion
4.1 Double skeletons

Although many illustrations and photographs of *O. pulchra* exist in the literature, only a few of these show double skeletons (*e.g.* Hovasse, 1932, pl.6, figs 15-17 as *Distephanus octonarius* (Ehrenberg) Haeckel; Ling & Takahashi, 1985, pl.1, fig.5 and pl.2, figs 1, 2, 4 as *Distephanus pulchra* (Schiller) Ling & Takahashi; Takahashi, 1991, pl.2, fig.7 as *D. pulchra*). McCartney *et al.* (2014a) compared the double skeletons of *O. pulchra* and *S. speculum* (as *D. speculum*). *Stephanocha*, as shown in the specimen illustrated in this study (Pl.4, fig.8), has the strut attachment positions rotated sinistrally, when observed in apical view, with the pikes being closer to the dextral corner of each basal side. When observed in lateral view, the basal side has a zig-zag design, because the elements trend apically towards the strut attachment and abapically towards the pike attachment, which breaks the basal side into three elements, two that connect to the basal corners and a middle element between the strut and pike attachments (McCartney *et al.*, 2014a).

O. pulchra does not have these features. The basal sides are composed of two elements, both connecting the basal corners to the strut attachments, as there are no pikes. Between the basal corners, the basal elements trend slightly in the apical direction, to produce a gap between the basal sides of the skeleton pair when observed in oblique or lateral view (Pl.1, fig.4b), but the basal ring is more flat and lacks the zig-zag design of *Stephanocha*. *Octactis* also does not have the sinistral rotation of the apical structure observed on most species of *Dictyocha* and all *Stephanocha* since the Late Miocene. There are other differences as well. In general, the basal elements of *O. pulchra* var. *pulchra* are more massively built than in *Stephanocha*. The basal spines also appear to narrow more quickly (compare Pl.1, fig.2 with Pl.4, fig.8), and the spines of the paired skeletons separate distally, where the abbasal surfaces of *Stephanocha* spines lie against each other. These distinctions are cited by McCartney *et al.* (2014a) as evidence that *Octactis* and *Stephanocha* should be retained as separate genera.

4.2 Taxonomic considerations

Ling & Takahashi (1985) observed *Octactis* from several locations, and concluded that *O. pulchra* should be included in *Distephanus*, since *D. speculum* subsp. *tenuis*

Bukry (now *Stephanocha speculum* var. *tenuis* (Bukry) McCartney & Jordan) possessed a six-sided morphology that has an apical ring made of thinner elements, similar to those observed in *O. pulchra*. However, there are pronounced differences in the basal ring structures and strut attachment locations of *S. speculum* and *O. pulchra* that strongly suggest that these are distinct genera.

In the present study, a few single skeletons and one double skeleton of *O. pulchra* with an incomplete or broken apical ring were observed (Pl.2, figs 2, 3a, 5a), as well as one specimen completely lacking struts (not shown here). In other specimens, we have observed apical rings with swollen parts near the middle of the apical side (see Pl.2, fig.4), while another specimen forming a bridge across the ring has a similar swelling in the middle of the bridge (see Pl.2, fig.8). This suggests to us that the apical ring elements form from both ends and subsequently meet in the middle. However, no double skeletons of *Stephanocha* with the daughter skeleton lacking an apical ring have been observed in this or our previous studies, and single skeletons that have basal rings with incomplete struts are rare in both the modern and fossil records. This may suggest that *Octactis* and *Stephanocha* form apical structures in a somewhat different manner.

In addition, an eight-sided skeleton of *D. speculum* var. *octonarius* with an extra basal spine was illustrated by Gemeinhardt (1930, fig.59b), which appears similar to one shown here as *O. pulchra* (Pl.2, fig.9).

4.3 Biogeography and ecology

In general, the Kuroshio was warmer, slightly more saline and with fewer nutrients than the Seto Inland Sea (Table 1, Figure 3), with a particularly significant drop in salinity in Osaka Bay due to inflowing rivers. These rivers were probably responsible for transporting nutrients to Osaka Bay, where eutrophication is known to be a problem (Hoshika *et al.*, 1998). The nutrient data in Table 1 and Figure 3 show higher silicate concentrations in the Seto Inland Sea than in the Kuroshio (up to 15 *versus* 1-3μmol/l, respectively), particularly in Osaka Bay, whereas those of nitrate and phosphate remained low throughout the cruise (except at Bosei-35, where silicate reached 15μmol/l and nitrate reached 10μmol/l). The absolute abundance data for *O. pulchra* var. *pulchra* and *D. stapedia* show higher values in and around Osaka Bay, where there is also a peak in chlorophyll-*a* (Figure 3), seemingly caused by
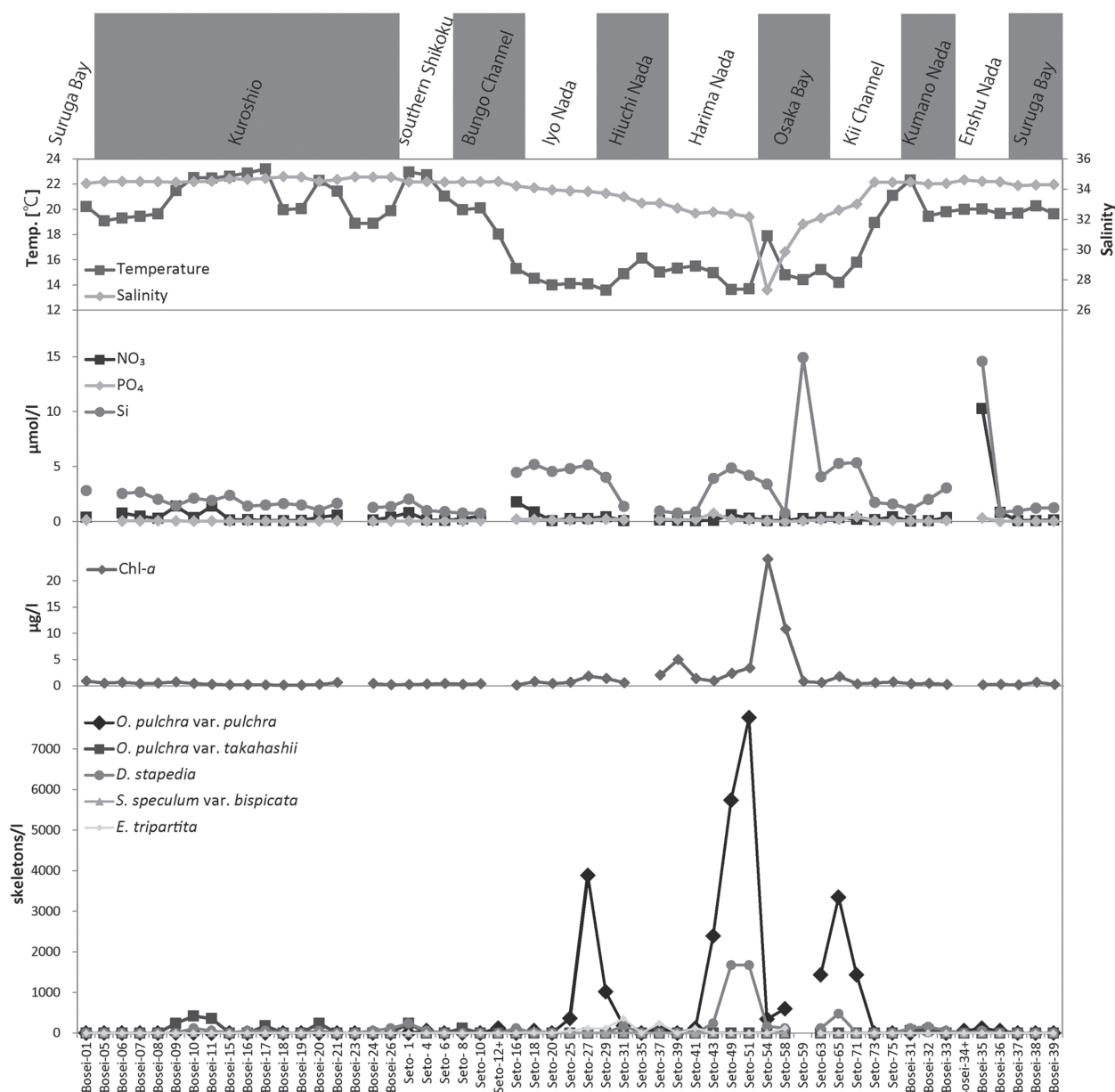
Figure 3: Temperature, salinity, nutrient concentration, chlorophyll-*a* and absolute abundances of the five taxa recorded in this study (in skeletons/l), with stations arranged in chronological order. Physico-chemical data downloadable from http://kutty.og.u-tokai.ac.jp/~kunio/2008kaijitsu/2008og_kaijitsu3.htm

dinoflagellates and the centric diatom *Skeletonema* (Fukunaga, unpubl. obs., 2009), with the latter species often responsible for red tides in the bay (Oshima *et al*., 2009). Relatively higher silicoflagellate abundances were also recorded by Yanagisawa (1943) from Osaka Bay, but skeletons of *Octactis pulchra* were probably misidentified as *Mesocena* and *Distephanus* in his counts, making direct comparisons difficult.

The ecological conditions that might be responsible for the *O*. *pulchra* skeletal variations that have relatively thick or thin components is not easy to identify, but peaks

in temperature tend to equate with higher numbers of *O*. *pulchra* var. *takahashii* and the thin form of *D*. *stapedia*, suggesting a change in oceanographic conditions (increased temperature combined with low silicate values) is associated with the morphological change. In addition, many of the aberrants were found in Samples Seto-51 and Seto-59 in Osaka Bay, which is characterised by low salinities caused by river inflow, but also high silicates, high diatom and dinoflagellate abundances, as well as the highest silicoflagellate abundances. Interestingly, Tsujita (1949) reported a red tide of *Dictyocha fibula* (presumed

herein to be *D. stapedia*) in Omura Bay in Kyushu during August, caused by lowered salinities due to the mixing of river- and sea-waters. In the red tide, some of the skeletons were aberrants, echoing our observations. Clearly, further studies of this type need to be carried out in order to draw some conclusions on whether skeletal plasticity is related to environmental stress.

5. Systematics

Class DICTYOCHOPHYCEAE **Silva, 1980**
Order DICTYOCHALES **Haeckel, 1894**
Family **DICTYOCHACEAE** Lemmermann, 1901a

Octactis pulchra Schiller, 1925 var. *pulchra*
Pl.1, figs 1-6; Pl.2, figs 1-11

Description: Skeletons robust, with circular (Pl.1, figs 4-6), or slightly elongate (Pl.1, figs. 1, 3) basal rings of 7-9 basal sides and with length:width ratios of up to 1.15:1. One basal spine at junction of two basal sides. Usually, one pair of opposing basal spines longer than the others (*e.g.* Pl.1, fig.1). Pikes absent. Generally, strut attachments at midpoint of each side, but not rotated sinistrally in apical view, as with modern *Dictyocha* and *Stephanocha* (McCartney *et al*., 2014a). Struts unusual, as thickness diminishes markedly from strut attachment towards apical ring, with apical ring elements being thinner (Pl.1, fig.2). Sometimes, apical ring elements offset (Pl.1, fig.3), or with expanded region or knob (Pl.2, fig.8) near midpoint of element. These features are not apical spines, but apical spines may occur in Southern Ocean (Hallegraeff, pers. comm., 2013) and Mediterranean Sea (Malinverno, pers. comm., 2013). **Dimensions**: Major axis outside diameters of basal ring ~35μm; major axis spines ~10μm long, ~2.5μm thick at basal ring; minor axis spines ~80% of major axis thickness and length. Strut ~5μm long, ~2μm thick near basal side, about a quarter that thickness near apical ring. Apical ring elements up to 1μm thick. **Occurrence**: Abundant to uncommon in the Seto Inland Sea (Seto-4, 12, 18, 25, 27, 29, 31, 37, 41, 43, 49, 51, 54, 58, 63, 65, 71), although occasionally absent. Largely absent in the Kuroshio, sometimes uncommon (Bosei-32, 34, 35, 36). **Previous records**: The type variety, var. *pulchra,* has been generally associated with coastal enclosed areas, such as the Gulf of California (Murray & Schrader, 1983; Martínez-López *et al*., 2012), Seto Inland Sea (Yanagisawa, 1943) and the Adriatic Sea (Rigual-Hernández *et*

al., 2010), and has been observed off the coast of Panama (Takahashi, 1991). However, this variety has also been recorded in sediment traps in open-ocean equatorial locations in the Pacific and Atlantic Oceans (Takahashi, 1991), as well as in the subarctic Pacific (Onodera & Takahashi, 2007) and in an Atlantic Ocean warm-core ring (Takahashi & Blackwelder, 1992). In addition, it has been found in Holocene sediments in the Mediterranean (Dumitrică, 1973), off California (Barron & Bukry, 2007a), in the Gulf of California (Barron *et al*., 2004, 2005; Barron & Bukry, 2007b), and in deep ocean sediments of the equatorial Pacific Ocean by Bukry & Foster (1973), Bukry (1976, 1978, 1980, 1982a, 1983) and McCartney *et al*. (1995).

Octactis pulchra var. *takahashii* McCartney, Abe & Jordan var. nov.

Pl.3, figs 1-7. **Derivation of name**: After Professor Kozo Takahashi (Hokusei Gakuen University, Japan), micropalaeontologist, in recognition of his earlier observations on this taxon. **Diagnosis**: Skeletons, fragile-looking, with circular to slightly elongate basal ring, composed of 7-9 basal sides of varying length. Strut attachments near middle of each side. Basal spines long, narrow and curved in various directions. Basal spine length may be equal to basal ring diameter. Apical ring often with different symmetry to basal ring; apical ring may be same size as basal ring, or appreciably smaller. Apical ring elements appreciably thinner than basal elements. **Differentiation**: Compared to var. *pulchra*, the skeleton of the new variety is smaller (outside basal ring diameters of 21.0-25.5μm *versus* 28.3-33.3μm in var. *pulchra* in this study), more fragile-looking (*i.e.* with relatively thinner basal spines and sides) and with much longer basal spines (lengths of 8.3-18.9μm *versus* 6.7-12.8μm in var. *pulchra* in this study). In some specimens, the basal spines are sometimes strongly curved (Pl.3, figs 4a, b), while in others they are straight (Pl.3, figs 2, 3). **Dimensions**: Type specimen with basal ring major-axis outside diameter of 24.3μm, minor-axis diameter of 21.5μm, length:width ratio of 1.13. Longest basal spine = 14μm. Strut length ~4μm. **Holotype**: Pl.3, figs 7a-c (double skeleton in apical view). **Paratype**: Pl.3, fig.6 (single skeleton in apical view). **Type locality**: Bosei-11 (32.498°N, 138.191°E), in the Kuroshio, south of Japan. **Repository**: Since it is difficult to preserve stubs and filters, the holotype is an illustration, in accordance with Article 40.5 of the *International Code of Nomenclature*

(McNeill *et al*., 2012). A digital image file will be deposited in the National Science Museum, Tokyo. **Occurrence**: Uncommon in the Kuroshio (Bosei-9, 10, 11, 17, 20, 31, 32), but sometimes absent. Largely absent in the Seto Inland Sea, although sometimes rare (Seto-1, 8). **Previous records**: Takahashi (1991) illustrated five specimens of *O. pulchra* (as *Distephanus pulchra* (Schiller) Ling & Takahashi), one of which was a double skeleton (his pl.2, fig.7), while another had 10 basal sides, with apical sides bearing small spines (his pl.2, fig.10). In addition, a specimen from the Indian Ocean was illustrated by McCartney *et al*. (2014a, fig.6B). A further two specimens from a Gulf Stream warm-core ring, observed by Takahashi & Blackwelder (1992) using the light microscope, may also belong to this new taxon, although the basal spines cannot be seen clearly enough to be certain.

Dictyocha stapedia Haeckel, 1887
Pl.4, figs 1-7

Description: The bridge is relatively long and canted sinistrally. Looking down the length of the bridge in apical view, the sinistral strut is longer and attaches further from the major axis basal corner than the dextral strut. There is a pronounced apex spine, and there may be small apical spines on the flanks of the struts, which may occur inconsistently and vary in location on all struts. Prominent pikes occur on all basal sides. Four double skeletons, including a mother-daughter doublet (Pl.4, fig.7), were counted, whilst another double skeleton, also as a mother-daughter doublet (Pl.4, fig.6), was found on a stub made from another piece of filter, but not counted. The double skeleton featured in Pl.4, fig.3 is almost identical to that illustrated by Haeckel (1887, pl.101, fig.12). **Differentiation**: *Dictyocha mandrai* Ling possesses accessory spines on the outside of the middle part of the basal ring, while *D. messanensis* apparently lacks pikes ("Stützstacheln bisher nicht beobachtet" in Lemmermann, 1901b, p.261), thus distinguishing them from both the thick and thin forms of *D. stapedia*. Haeckel (1887, p.1561) also mentions that *D. stapedia* is "similar to the hats of *Dictyocha fibula* and *Dictyocha messanensis*, but distinguished by four small centripetal teeth, which start from the inside of the basal ring at the side of the four ascending beams", implying that *D. messanensis* lacks pikes. **Occurrence**: Common in the Seto Inland Sea (Seto-1, 4, 16, 31, 43, 49, 51, 54, 58, 63, 65), although occasionally absent. Uncommon in

the Kuroshio (Bosei-10, 11, 16, 17, 20, 24, 26, 31, 32, 33, 35), but sometimes absent. **Previous records**: This species is often recorded in deep-sea drilling cores, but rarely in water samples, despite being described from water samples from the Atlantic, Pacific and Indian Oceans by Haeckel (1887). The specimens observed in this study are consistent with those identified as *D. stapedia* by Loeblich *et al*. (1968), while those by Poelchau (1976), Murray & Schrader (1983), Onodera & Takahashi (2007) and McCartney *et al*. (2014a) identified as *D. messanensis*, and many others identified as *D. fibula* in the biological literature, are actually *D. stapedia*.

Stephanocha speculum var. *bispicata* (Bukry, 1982b)
McCartney & Jordan *in* Jordan & McCartney, 2015
Pl.4, fig.8

Only one specimen was observed in this study. **Occurrence**: Rare in the Seto Inland Sea (Seto-18). **Previous records**: Although described from Upper Miocene to Upper Pliocene sediments of the eastern equatorial Pacific (Bukry, 1982b), it has been recorded in Holocene sediments from the North Pacific (Poelchau, 1976) and as extant in Southern Ocean surface waters (Malinverno, 2010). This taxon is therefore in need of further investigation.

Class THECOFILOSA **Cavalier-Smith** *in* **Cavalier-Smith & Chao, 2003**
Order EBRIIDA **Deflandre, 1936**
Family **EBRIACEAE** Lemmermann, 1901a

Ebria tripartita (Schumann, 1867) Lemmermann, 1899
Pl.4, figs 9-12

Description: Internal siliceous skeleton triradiate, with Y-shaped triode, composed of three thick elements (a rhabde and two actines). Each actine bifurcates into slightly thinner elements, the proclade and opisthoclade, which diverge in opposite directions. The two proclades bifurcate again into synclades, which connect to an apical ring. The openings between the actines, proclades, synclades and apical ring are called superior windows. The two opisthoclades of the actines bifurcate, with one branch joining the base of the rhabde and the other forming an abapical ring. Another opisthoclade arises from the base of the rhabde and joins the apical ring. The openings between the rhabde, opisthoclades and abapical ring are called inferior windows. Short spines often adorn some of the thinner elements. All elements are solid. **Dimensions**: According to Hargraves

(2002), skeleton is 24-34μm in length, 22-31μm in width, with a length:width ratio of 1.1. Specimens in the present study are 28.2-32.0μm in length, 23.0-24.5μm in width. **Occurrence**: Only low abundances (<400 skeletons/l) were recorded in the present study. Uncommon in the Seto Inland Sea (Seto-25, 27, 29, 31, 37, 41, 58), or absent. Absent in the Kuroshio. **Previous records**: Absolute abundance rarely more than 10^4 cells/l (Hargraves, 2002), but higher abundances have been recorded during the summer in Long Island Sound (4.9x10^4 cells/l: Conover, 1956) and off Rhode Island (Ernissee & McCartney, 1993). Usually, *E. tripartita* is found in cold to warm, temperate coastal localities (*e.g.* Vancouver, Canada: Hoppenrath & Leander, 2006; coastal Brazil: Gomes *et al*., 2007). However, Vørs (1992) found this taxon in the Gulf of Finland, while Onodera & Takahashi (2007) reported this taxon as rare in their sediment-trap (50°N) study in the subarctic Pacific.

6. Conclusions

The specimens of *O. pulchra* provided an opportunity to study its fine structure and morphological variation, particularly for double skeletons, but also for aberrants. The species is unusual, compared to modern *Dictyocha* and *Stephanocha*, in that the basal rings are made of sides that are of more linear shape and lack pikes. More fragile-looking specimens of *O. pulchra* with longer spines were found in the Bungo Channel entrance and outside the Seto Inland Sea. These specimens are described as *O. pulchra* var. *takahashii* var. nov. Thinner skeletons of *D. stapedia* were also observed in the Kuroshio, but their morphology was identical to that of the Seto Inland Sea specimens, so a new variety was not warranted. In this study, double skeletons of *Octactis* were quite numerous, while some *D. stapedia* doublets were also present. Further observations on mother-daughter skeletal differences in doublets of both genera corroborated previous findings, and provided further examples of what is still essentially a poorly-documented phenomenon. Many of the aberrants of *Octactis* documented herein by SEM images were only known from drawings in the old literature. Comparison of the absolute abundances and distributions of the thick and thin types of *O. pulchra* and *D. stapedia*, as well as those of the aberrants, suggests that changes in the skeletal morphology may be related to increased temperature (thin-type skeletons) and decreased salinity, high silicate concentrations and increased productivity (aberrants). These environmental studies warrant further investigation, both in the Seto Inland Sea and in other areas (*e.g.* the Southern Ocean), where changes in skeletal thickness and aberrants are known to occur.

Acknowledgments

References

Barkley, R.A. 1970. The Kuroshio current. *Science Journal*, **6**: 54-60.

Barron, J.A. & Bukry, D. 2007a. Development of the California Current during the past 12,000 yr based on diatoms and silicoflagellates. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **248**: 313-338.

Barron, J.A. & Bukry, D. 2007b. Solar forcing of Gulf of California climate during the past 2000 yr suggested by diatoms and silicoflagellates. *Marine Micropaleontology*, **62**: 115-139.

Barron, J.A., Bukry, D. & Bischoff, J.L. 2004. High resolution paleoceanography of the Guaymas Basin, Gulf of California, during the past 15, 000 years. *Marine Micropaleontology*, **50**: 185-207.

Barron, J.A., Bukry, D. & Dean, W.E. 2005. Paleoceanographic history of the Guaymas Basin, Gulf of California, during the past 15,000 years based on diatoms, silicoflagellates, and biogenic sediments. *Marine Micropaleontology*, **56**: 81-102.

Bukry, D. 1976. Silicoflagellate and coccolith stratigraphy, southeastern Pacific Ocean, Deep Sea Drilling Project Leg 34. *Initial Reports of the DSDP*, **34**: 715-735.

Bukry, D. 1978. Cenozoic silicoflagellates and coccolith stratigraphy, southeastern Atlantic Ocean, Deep Sea Drilling Project Leg 40. *Initial Reports of the DSDP*, **40**: 635-649.

Bukry, D. 1980. Silicoflagellate biostratigraphy and paleoecology in the eastern equatorial Pacific, Deep Sea Drilling Project Leg 54. *Initial Reports of the DSDP*, **54**: 545-573.

Bukry, D. 1982a. Cenozoic silicoflagellates from offshore Guatemala, Deep Sea Drilling Project Site 495. *Initial Reports of the DSDP*, **67**: 425-445.

Bukry, D. 1982b. Neogene silicoflagellates from the eastern equatorial Pacific, Deep Sea Drilling Project Hole 503A. *Initial Reports of the DSDP*, **68**: 311-323.

Bukry, D. 1983. Upper Cenozoic silicoflagellates from offshore Ecuador, Deep Sea Drilling Project Site 504. *Initial Reports of the DSDP*, **69**: 321-342.

Bukry, D. & Foster, J.H. 1973. Silicoflagellate and diatom stratigraphy, Leg 16, Deep Sea Drilling Project. *Initial Reports of the DSDP*, **16**: 815-871.

Cavalier-Smith, T. & Chao, E.E.-Y. 2003. Phylogeny and classification of phylum Cercozoa (Protozoa). *Protist*, **154**(3-4): 341-358.

Conover, S.A.M. 1956. Oceanography of Long Island Sound, 1952-1954. IV. Phytoplankton. *Bulletin of the Bingham Oceanographic Collection*, **15**: 62-112.

Deflandre, G. 1934. Nomenclature du squelette des Ebriacées et description de quelques formes nouvelles. *Annales de Protistologie*, **4**: 75-96.

Deflandre, G. 1936. Les flagellés fossiles. Aperçu biologique et paléontologique; Role géologique. *Actualités scientifiques et industrielles*, **No.335**: 1-98. Paris, Hermann & Cie.

Du, C., Liu, Z., Dai, M., Kao, S.-J., Cao, Z., Zhang, Y., Huang, T., Wang, L. & Li, Y. 2013. Impact of the Kuroshio intrusion on the nutrient inventory in the upper northern South China Sea: insights from an isopycnal mixing model. *Biogeosciences*, **10**: 6419-6432.

Dumitrică, P. 1973. Miocene and Quaternary silicoflagellates in sediments from the Mediterranean Sea. *Initial Reports of the DSDP*, **13**: 902-933.

Ernissee, J.J. & McCartney, K. 1993. Ebridians. *In*: J.H. Lipps (Ed.). *Fossil Prokaryotes and Protists*: 131-140. Blackwell Scientific Publications, UK.

Gemeinhardt, K. 1930. Silicoflagellatae. *Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*, **10**: 1-87. Akademische Verlagsgeselleschaft, Leipzig.

Gomes, E.A.T., Santos, V.S., Tenenbaum, D.R. & Villac, M.C. 2007. Protozooplankton characterization of two contrasting sites in a tropical coastal ecosystem (Guanabara Bay, RJ). *Brazilian Journal of Oceanography*, **55**(1): 29-38.

Goto, M. 1975. Pollution problems of the Seto Inland Sea. *Pure and Applied Chemistry*, **42**: 155-166.

Haeckel, E. 1887. Report on the radiolaria collected by *H.M.S.* *Challenger* during the years 1873-1876. *Report on the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873-1876*, **18**: 1-1803.

Haeckel, E. 1894. Systematische Phylogenie. Entwurf eines Natürlichen Systems der Organismen auf Grund ihrer Stammesgeschichte. Erster Theil. *Systematische Phylogenie der Protisten und Pflanzen*. Georg Reimer, Berlin: 400pp.

Hargraves, P.E. 2002. The ebridian flagellates *Ebria* and *Hermesinum*. *Plankton Biology & Ecology*, **49**: 9-16.

Hoppenrath, M. & Leander, B.S. 2006. Ebriid Phylogeny and the Expansion of the Cercozoa. *Protist*, **157**: 279-290.

Hoshika, A., Tanimoto, T. & Mishima, Y. 1998. Eutrophication and organic pollution in the Osaka Bay, Seto Inland Sea. *Journal of Water and Environment Technology*, **21**(11): 765-771 [in Japanese].

Hovasse, R. 1932. Contribution a l'étude des Silicoflagellés. Multiplication, variabilité, hérédité, affinités. *Bulletin Biologique de la France et de la Belgique*, **66**: 447-501.

Imai, I, Yamaguchi, M. & Hori, Y. 2006. Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankton Benthos Research*, **1**(2): 71-84.

Jordan, R.W. & McCartney, K. 2015. *Stephanocha* nom. nov., a replacement name for the illegitimate silicoflagellate genus *Distephanus* (Dictyochophyceae). *Phytotaxa*, **201**(3): 177-187.

Kasai, A., Kimura, S. & Sugimoto, T. 1993. Warm water intrusion from the Kuroshio into the coastal areas south of Japan. *Journal of Oceanography*, **49**: 607-624.

Kawabe, M. 1980. Sea level variations along the south coast of Japan and the large meander in the Kuroshio. *Journal of the Oceanographical Society of Japan*, **36**: 97-104.

Lemmermann, E. 1899. Ergebnisse einer Reise nach dem Pacific. H. Schauinsland 1896-97. Zoologische Jahrbücher. *Abhandlungen herausgegeben vom Naturwissenschaftlichen Verein im Auftrag der Wittheit zu Bremen*, **16**: 313-398.

Lemmermann, E. 1901a. Beitrage zur Kenntniss der Planktonalgen. *Deutsche Botanische Gesellschaft, Berichte*, **19**: 85-95.

Lemmermann, E. 1901b. Silicoflagellatae. *Deutsche Botanische Gesellschaft, Berichte*, **19**: 247-271.

Lie, H.-J., Cho, C.-H., Lee, J.-H., Niiler, P. & Hu, J.-H. 1998. Separation of the Kuroshio water and its penetration onto the continental shelf west of Kyushu. *Journal of Geophysical Research*, **103**(C2): 2963-2976.

Ling, H.-Y. & Takahashi, K. 1985. The silicoflagellate genus *Octactis* Schiller, 1925: A synonym of the genus *Distephanus*. *Micropaleontology*, **31**: 76-81.

Loeblich, A.R., III, Loeblich, L.A., Tappan, H. & Loeblich, A.R., Jr. 1968. Annotated Index of Fossil and Recent Silicoflagellates and Ebridians with descriptions and illustrations of validly proposed taxa. *The Geological Society of America, Memoir*, **106**: 1-319.

Malinverno, E. 2010. Extant morphotypes of *Distephanus speculum* (Silicoflagellata) from the Australian sector of the Southern Ocean: Morphology, morphometry and biogeography. *Marine Micropaleontology*, **77**: 154-174.

Martinez-López, A., Álvarez-Gómez, I.G. & Durazo, R. 2012. Climate variability and silicoflagellate fluxes in Alfonso Basin (southern Gulf of California). *Botanica Marina*, **55**: 177-185.

McCartney, K., Abe, K., Harrison, M.A., Witkowski, J., Harwood, D.M., Jordan, R.W. & Kano, H. 2015. Silicoflagellate double skeletons in the geologic record. *Marine Micropaleontology*, **117**: 65-79.

McCartney, K., Churchill, S. & Woestendiek, L. 1995. Silicoflagellates and ebridians from ODP Leg 138, Eastern Equatorial Pacific. *Proceedings of the ODP, Scientific Results*, **138**: 129-162.

McCartney, K., Jordan, R.W. & Abe, K. Submitted. Did the silicoflagellate genus *Octactis* evolve from *Bachmannocena*? *Journal of Micropalaeontology*.

McCartney, K., Malinverno, E., Schweizer, M., Witkowski, J. & Young, J.R. 2014b. Two unusual silicoflagellate double skeletons. *Journal of Nannoplankton Research,* **33**: 19-22.

McCartney, K., Witkowski, J., Jordan, R.W., Daugbjerg, N., Malinverno, E., van Wezel, R., Kano, H., Abe, K., Scott, F., Schweizer, M., Young, J.R., Hallegraeff, G.M. & Shiozawa, A. 2014a. Fine structure of silicoflagellate double skeletons. *Marine Micropaleontology,* **113**: 10-19.

McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J., Prud'homme van Reine, W.F., Smith, G.F., Wiersema, J.H. & Turland, N.J. 2012. International Code of Nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. *Regnum Vegetabile* **154**: 1-240. Koeltz Scientific Books.

Murray, D. & Schrader, H. 1983. Distribution of silicoflagellates in plankton and core top samples from the Gulf of California. *Marine Micropaleontology,* **7**: 517-539.

Okaichi, T. 2003. Red-tide phenomena. *In*: T. Okaichi (Ed.). *Red Tides*. Terra Scientific Publishing Company, Tokyo: 7-60.

Onodera, J. & Takahashi, K. 2007. Diatoms and siliceous flagellates (silicoflagellates, ebridians, and endoskeletal dinoflagellate *Actiniscus*) from the subarctic Pacific. *Memoirs of the Faculty of Science, Kyushu University. Series D, Earth and Planetary Sciences*, **31**: 105-136.

Oshima, A., Nishio, T., Fujiwara, Y., Shinya, M. & Kitano, M. 2009. Dynamics of a red tide of *Skeletonema costatum* and *Thalassiosira* spp. (Bacillariophyceae) around the Yume-Shima landfill site in Osaka Bay. *Osaka Municipal Environmental Report*, **71**: 49-55 [in Japanese].

Poelchau, H.S. 1976. Distribution of Holocene silicoflagellates in North Pacific sediments. *Micropaleontology*, **22**: 164-193.

Rigual-Hernández, A.S., Bárcena, M.A., Sierro, F.J., Flores, J.-A., Hernández-Almeida, I., Sanchez-Vidal, A., Palanquez, A. & Heussner, S. 2010. Seasonal to interannual variability and geographic distribution of the silicoflagellate fluxes in the Western Mediterranean. *Marine Micropaleontology,* **77**: 46-57.

Schiller, J. 1925. Die planktonischen Vegetationen des Adriatischen Meeres. B. Chrysomonadina, Heterokontae, Cryptomonadina, Eugleninae, Volvocales. 1. Systematischer Teil. *Archiv für Protistenkunde*, **53**: 59-123.

Schumann, J. 1867. Preussische Diatomeen. *Schriften der Physik-Oek*. *Gesellschaft zu Königsberg*, **8**: 37-68.

Silva, P.C. 1980. Names of families and classes of living algae with special reference to their use in the Index Nominum Genericorum (Plantarum). *Regnum Vegetabile*, **103**: 1-156.

Takahashi, K. 1991. Silicoflagellates and *Actiniscus*: Vertical fluxes at Pacific and Atlantic sediment trap stations. *Woods Hole Oceanographic Institution, Ocean Biocoenosis Series,* **No. 2**: 35pp.

Takahashi, K. & Blackwelder, P.L. 1992. The spatial distribution of silicoflagellates in the region of the Gulf Stream warm-core ring 82B: Application to water mass tracer studies. *Deep-Sea Research I*, **39**: 327-346.

Tsujita, T. 1949. Red water caused by Silicoflagellata in the Ohmura-wan. *The Report of the Nagasaki Marine Laboratory*, **No.2**: 17-29 [in Japanese with English abstract].

Vørs, N. 1992. Heterotrophic amoebae, flagellates, and heliozoans from the Trärminne area, Gulf of Finland, in 1988-1990. *Ophelia*, **41**: 197-301.

Yanagisawa, T. 1943. On Silicoflagellates. *Umi to Sora*, **23**: 11-29 (451-469) [in Japanese].