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Marine and freshwater palynomorphs preserved in surface sediments of Osaka Bay, Japan

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大阪湾表層堆積物中の海産・淡水産パリノモルフ

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抄録：大阪湾表層堆積物中に観察される花粉，シダ植物胞子，菌類以外の渦鞭毛藻シスト，チンチノモルフ，有孔虫ライニング，甲殻類の一部，アクリタークなどから構成される海産・淡水産パリノモルフを記載した．甲殻類遺骸や淡水産パリノモルフを除くと渦鞭毛藻シストが最も優占（Station 8で21,567 cysts/g）し，有孔虫ライニングがそれに次いだ（St. 5で4,224 linings/g）．麻痺性貝毒原因種*Alexandrium* 属の楕円形シストはSt. 7で最高密度 3,610 cysts/g で出現して湾全域に分布していた．魚毒性ラフィド藻*Chattonella* シストも St. 8で最高密度 455 cysts/gで出現し，湾全域に分布していた．淡水産パリノモルフは主に緑藻類*Staurastrum*で構成され，淀川河口付近のSt. 7 と St.．8（St. 7 で49,875 cells/g）で極めて多産していた．この種の分布状況は東部沿岸残渣流の方向を反映しているようである．この様に海産・淡水産パリノモルフは環境復元にとって有効な遺骸であることが判明した．

Abstract：Various marine and freshwater palynomorphs consisting of dinoflagellate cysts, tintinnomorphs, microforaminiferal linings, crustacean eggs and fragments, and acritarchs, as well as pollen and fern spores were found in surface sediments of Osaka Bay, Japan are briefly described. Except for crustacean remains and freshwater palynomorphs, the most dominant palynomorpha are dinoflagellate cysts（21,567 cysts/g at Station 8）followed by microforaminiferal linings（4,224 linings/g at St. 5）. Ellipsoidal cysts identical to those produced by PSP causative *Alexandrium tamarense/catenella* were distributed throughout the bay（highest abundance of 3,610 cysts/g at St. 7）. Cysts of ichthyo-toxic raphydophycean *Chattonella* were also observed throughout the bay with the highest occurrence of 455 cysts/g at St. 8）. Freshwater palynomorphs mainly consisting of chlorophycean *Staurastrum* were extremely dominated at Sts. 7 and 8, near the mouth of the Yodo River（49,875 cells/g at St. 7）. Its distribution appears to reflect the flowing direction of the East Coast Residual Current. Thus, marine and freshwater palynomorphs are useful tools for reconstructing environments.

Key words: acritarch; *Chattonella* cyst; dinoflagellate cyst; microforaminiferal lining; Osaka Bay; tintinnomorph; palynomorph

Introduction

Sediments contain organic and inorganic remains of various planktonic and benthic organisms. Diatoms, silicoglagellates, foraminifers, coccoliths, and ostracods are typical sources of inorganic remains. Pollen, fern spores, fungal spores, resting cysts of dinoflagellates and ciliates, resting cells of macro- and microalgae are composed of organic compounds and generally called as palynomorphs. Some of these remains are used as good fossil tools for reconstructing terrestrial and oceanic paleoenvironments. Among these palynomorphs, pollen and fern spores have been used to understand climatic changes and reconstructing paleovegetation for long time. On the other hand, along with other marine microfossils dinoflagellate cysts are regarded as indicators for biostratigraphy and a proxy for changing oceanographic environments as well as other marine microfossils. However, in the sediments, there are also additional remains, other than those of not only these palynomorphs. These are called as marine palynomorphs（e.g. Matsuoka, 1994）and composed of palynomorphs but also other remains different from pollen, fern spores, and dinoflagellate cysts. These are called as resting cysts and lorica of tintinnids（tintinnomorphs）, organic linings of smaller benthic foraminifers（microforaminiferal linings）, appendages, body parts and resting eggs of crustaceans（mainly copepods）, and micro-remains of unknown organisms called acritarchs. Recent studies in the Arctic attempted to clarify the Holocene paleo-environmental change based on a combination of information provided by different palynomorphs icluding dinoflagellate cysts, acritarchs, tintinnid

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remains, microforaminiferal linings, and pollen and fern spores (Mudie, 1992; Pieńkowski et al., 2012, 2014). In temperate regions, non-pollen palynomorphs consisting of dinoflagellate cysts, acritarchs, colonial algae, cyanobacteria, zygnemataceaen remains, fungal spores, microforaminiferal linings, thecamoebians, copepod eggs, rotifers, and scolecodonts were employed for the reconstruction of salinity and environmental changes around the Caspian-Black Sea-Mediterranean corridor by Mudie et al. (2011). However, it is very difficult to understand fully the significance of these non-pollen palynomorphs in environmental studies, because the members of non-pollen palynomorphs consist of taxonomically and ecologically diverse organisms and are also used as specific terms in palynology. For example, tintinnomorphs is for the loricae of tintinnids and resting spores of ciliata, (micro) foraminiferal linings for organic linings of smaller benthic foraminifers, stomatocysts for chrysophycaean cysts and acritarchs for micro-remains of unknown organisms (e.g. Traverse, 1988). In addition to them, appendages, body parts and resting eggs of crustaceans (mainly copepods) are also frequently found in modern sediments.

Various and well-preserved marine and freshwater palynomorphs were found in modern sediments of Osaka Bay where many studies on phytoplankton, zooplankton, and benthic foraminifers have been carried out as introduced later. Careful morphological comparison of these parent organisms led to a deeper understanding of these palynomorphs and their environmental shifts over past geologic times in modern coastal areas. In this article, marine and freshwater palynomorphs found in surface sediments of Osaka Bay are preliminary described with their environmental significance, and the present taxonomic issues of each palynomorph are briefly introduced.

Environments of Osaka Bay

Osaka Bay is a typical semi-enclosed sea, ellipsoidal in shape, oriented in a northeast to southwest direction (Fig.1). The bay is connected to Harima-Nada and Kii-Suido through the Akashi and Tomogashima channels respectively. According to Tsujimoto et al. (2006), the bay is approximately 1450 km^2 in surface area, and is about 20 m in mean water depth. The central to eastern parts are rather flat in bottom topography. The bottom sediments are muddy in the northeastern to central parts and sandy in the western and southern parts. Very coarse gravelly sediments are distributed near the two connecting mouths (Nagaoka et al., 2004). Fujiwara et al. (1989) suggested that the following currents are observed in Osaka Bay; the clockwise Okinose Circulation Current in the center of the bay, the Nishinomiya-oki Circulation current and the following East Coast Residual Current along the northern and eastern margins of the bay, and the Suma-oki Reversed Current off Kobe to Suma (Fig.1).

Several rivers flow into Osaka Bay along the northern to northeastern coast of the bay. Among these rivers, the largest one (Yodo River) has an annual mean discharge is 193 m^3/s, which is approximately an order of magnitude greater than that from the other rivers (data on river discharges were provided by the Ministry of Land, Infrastructure, Transport and Tourism, Japan). Oceanic water from the shelf sea flows into the bay at its south mouth (Kii-Suido).

According to Osaka Bay Environmental Conservation Council (https://www.osaka-wan.jp /index.php/189/194), COD, TN, and TP were the highest along the northern to northeastern coast of the bay (COD; 3.1-4.9 mg/L, TN; 0.2-0.89 mg/L, TP; 0.03-0.081 in 2011), while other areas they were generally lower (COD; 1.6-2.4 mg/L, TN; 0.22-0.31 mg/L, TP; 0.026-0.034 in 2011). During the ten years since 2003, the total nitrogen reduced from 0.49 to 0.38 mg/L, while the total phosphate was generally constant around 0.05 mg/L. Approximately 20 cases of harmful algal blooms occurred during 2003 to 2012. According to the Research Institute of Environment, Agriculture and Fisheries of Osaka Prefecture (http://www.kannousuiken-osaka.or.jp/suisan/gijutsu/do/), the bottom of the inner most part of the bay is covered by anoxic sea-water from June to September in most years. These data indicate that the most eutrophicated area in Osaka Bay is the innermost part, in which is off the most populated areas.

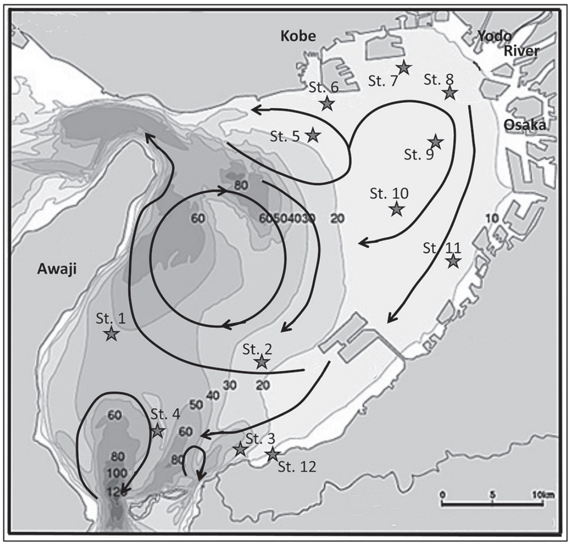

Fig. 1. Map showing sampling sites, water depth and current circulation patterns in Osaka Bay. Topographic map is provided by Department of Environmental Agriculture, Forest, and Fisheries in Osaka Prefectural Government. Current circulation patterns are based on Fujiwara et al. (1989).

Previous studies related to marine and freshwater palynomorphs in Osaka Bay

Many palynological studies in the Osaka Bay area have been carried out on the Plio-Pleistocene Osaka Group by Tai (1963), Nasu (1970), Furutani (1989), and Hongo (2009). Only a few reports included marine and freshwater palynomorphs other than pollen and fern spores (Hongo and Nakajo, 2003), however most of these reports introduced them as additional components in the pollen-spore studies. On the other hand, only *Alexandrium* cysts (Dinophyceae) studied by Yamaguchi et al. (1996) and Yamamoto et al. (2009), and raphidophycean algae (cyst of *Chattonella*) studied by Imai (1990) were included as part of investigations into harmful algal bloom events. Other important components of marine palynomorphs (microforaminiferal linings, benthic foraminifers) were studied by Takayanagi (1953), Nakaseko (1953) and Tsujimoto et al. (2006) from the view points of micropaleontology. Crustaceans (resting eggs and body remains) were reported by Koga (1987) and Jo and Uno (1983) for understanding a mechanism of production of fisheries. Until now, no systematic study on tintinnids has been performed in Osaka Bay.

Materials and Methods

Sampling site

In total, 12 surface samples were collected using a TFO-type II handy corer and an Eckman Berge bottom sampler on October 13 and 14 of 2016 (Fig.1). The top 2cm of sediments were removed for palynological analysis, grain-size analysis and measurements of water contents.

Analytical method

The analytical method followed that of Matsuoka and Fukuyo (2000). The samples were treated with HCl and HF for removing calcium carbonate and silicate particles at room temperature respectively and then sieved with stainless-steel screens of opening mesh size of 125 μm and 20 μm. Materials caught by a 20 μm mesh-size screen were observed under the microscope.

Results and Discussion

The sampled marine palynomorphs found in the sediments of Osaka Bay consisted of several different taxonomic groups including dinoflagellate cysts, tintinnomorphs, microforaminiferal linings, resting eggs and chitin bodies of copepods, acritarchs and others (Table 1). Since some dinoflagellates produce resting cysts that can be preserved as fossils in the sediment for a long time, dinoflagellate cysts are the most investigated marine palynomorphs (e.g. Traverse, 1988). Ciliate remains (tintinomorphs) are frequently observed in modern sediments, since their loricae are made of mostly transparent organic membranes called pseudo-chitin (e.g. Dolan, 2013). In addition, some ciliates can also produce chitinous resting cysts that are preserved in sediments. The classification systems of some of these palynomorphs have been well documented, such as dinoflagellate cysts, while others have not been studied as much.

Dinoflagellate cysts (planktonic, photo-mixotrophic and heterotrophic micro-protoctista) (Fig. 2; Fig. 7A; Tables 1 and 2)

Since some dinoflagellates can produce resting cysts that are preserved in sediment for a long time, dinoflagellate cysts are one of the well-investigated marine palynomorphs. In this article, we followed the classification system of modern dinoflagellate cysts given by Matsuoka and Fukuyo (2000).

In total, dinoflagellate cysts consisting of more than 43 species of 18 genera were identified; belonging to 3 genera and 10 species in the Gonyaulacales, 2 genera and 4 species to the Gymnodiniales, and 16 genera and 39 species to the Peridiniales. Cyst densities of dinoflagellates ranged from 1,836 cysts/g (St. 3) to 20,657 cysts/g (St. 8) with 7,974 cysts/g in average. The cysts in the innermost part of the bay were more abundant than those of the central and southeastern coasts. Cysts of photo-mixotrophic species belonging to the Gonyaulacales represented 526 cysts/g in the southern part and 1,076 cysts/g in the northern part of the bay. On the other hand, heterotrophic peridinioid and gymnodinioid cysts represented 1,289 cysts/g in the northern parts and 1076 cysts/g in the southern parts. Combining the data from all stations, the cysts of heterotrophic species were equal to the over half of the total dinoflagellate cysts. This feature of the innermost part of the bay was clearer than that of the southern to central parts.

Cyst of *Alexandrium catenella/tamarense* (Fig. 2a, b; Fig. 7B; Table 2)

In Osaka Bay, the occurrence of several PSP causative dinoflagellates such as *Gymnodinium catenatum*, *Alexandrium tamarense*, *A.*

Table 1. Summary of ecological and morphological characteristics of major marine and brackish palynomorphs.

Taxa	Habitat	Life mode	Mode of nutrition	Common remaining parts	Morphological types and features	Representative name	Reference
Dinophyceae (Dinoflagellate cyst, Dinocyst)	**abundantly in marine (commonly in blackish, freshwater)**	**mainly planktonic**	**autotrophic, heterotrophic** (bacteria, diatoms, other phototrophic dinoflagellates, ciliates, nannoflagellates, colorless deterial particles) **mixotrophic**	**resting cyst**	**Spherical to subspherical type ellipsoidal type, peridinioid type, ovoidal type with various surface ornamentations, transvers and longitudinal fullows archeopyle**	***Spiniferites Brigantedinium***	**Wall and Dale 1968 Jacobson and Anderson 1986 Hansen 1991**
Acritarch	**mainly in marine (commonly brackish water, freshwater)**	**probably planktonic**	**mainly autotrophic, heterotrophic (?) mixotrophic (?)**	**resting cell?**	**Spherical to subspherical type ellipsoidal type, ovoidal type ornamented with various appendages and openings**	***Bion Halosphaera Michystridium***	**Downie et al. 1963**
Prasinophyceae	**mainly marine**	**planktonic**	**autotrophic**	**resting phycoma**	**Spherical to subspherical type with or without fenestrate walls and membranous surface ornaments**	***Tasmanites Cymatiosphaera Pleurozonaria Pterosperma Pterospermella***	**Wall 1962 Park et al. 1978**
Tintinomorph	**marine**	**planktonic**	**heterotrophic** (bacteria, diatoms, dinoflagellates, microflagellates (chlorophytes, chrysophytes, pelagophytes, prasinophytes, and pyrenomonadaceae) , raphidophytes, other ciliates, and small organic particles)	**lorica**	**Circular cylindrical, inverted frustum**		**Van Wavern 1993 Montagnes 2013**
				resting cyst	**Spherical type, Elliptical type, Egg-shaped, aborally blunt types Egg-shaped, orally blunt types, Hexagonal type Asymmetrically rhombic, orally blunt type, Triangular type**		
Testate Amoebae	**freswater to blackish water**	**benthic**	**heterotrophic (organic detritus?)**	**organic shell**	**discoidal, cup-shaped and elongated ellipsoidal with simple opening,**		**Meisterfeld 2000**
Foraminifera (micro-foraminiferal linings)	**marine**	**benthic heterotrophic**	**heterotrophic** (bacteria, pennate diatoms, micro-algae, other protozoans, and dead organic material)	**organic inner lining**	**Single chamber type, Uniserial type, Biserial type, Coiled type, Compound type**	**micro-foraminiferal linings**	**Stancliffe 1987 Kitazato 1981 Topping et al. 2006 Armstrong and Brasier 2007**
Crustaceae	**mainly marine**	**planktonic heterotrophic**	**heterotrophic** (diatoms, dinoflagellates, ciliates, fecal pellets, and organic detritus)	**exoskelton**	**intercoxal plate, tail sternite, appendage, etc**		**Van Wavern 1993**
				egg envelops	**Discoid type, Folded discoid type, Double saucer shaped type, Fissured sphere type, Hemisphere type, Ellise type, Fusiform type, Double fusiform type**	***Cobricosphaeridium***	**Van Wavern 1993 McMinn et al. 1992 Taniguchi 1975 Sherr and Sherr 1988**
Polychaeta	**mainly marine**	**benthic**	**heterotrophic**	**mouth-lining parts**		**Scolecodont**	**Traverse 1988**

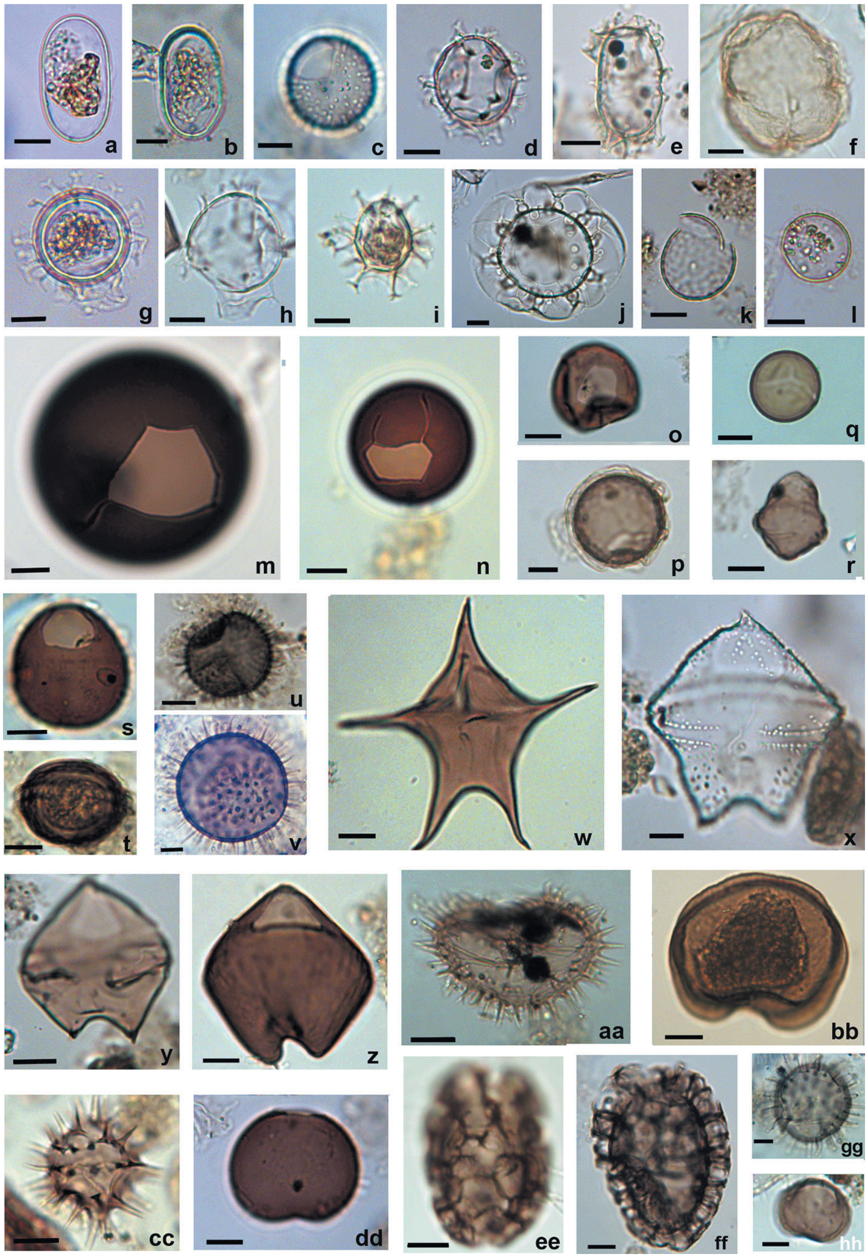

Fig. 2. Dinoflagellate cysts (Dinophyceae). a, b: *Alexandrium tamarense/catenella* cyst, c: *Operculodinium centrocarpum* (Deflandre & Cookson) Wall sensu Wall et Dale, d: *Spiniferites delicatus* Reid (short process form), e: *Spiniferites elongatus* Reid, f: Cyst of *Levanderina fissa* (Levander) Moestrup, Hakanen, Hansen, Daugbjerg et Ellegaard (=*Gyrodinium instriatum*), g: *Spiniferites mirabilis* (Rossignol) Matsuoka, h: *Spiniferites nanus* Matsuoka, i: *Spinifeites bulloideus* (Deflandre et Cookson) Sarjeant, j: *Tuberculodinium vancampoae* (Rossignol) Wall, k: cyst of *Scrippsiella* sp. (calcareous processes removed), l: Colorless spherical cyst without ornament, m: *Brigantedinium majusclum* Reid, n: *Brigantedinium cariacoense* (Wall) Reid, o: *Brigantedinium simplex* (Wall) Reid, p: Cyst of *Protoperidinium parthenopes* Zingone et Montresor, q: *Brigantedinium* sp. (pale brown type), r: Cyst of *Protoperidinium* sp. 1, s: Cyst of *Protoperidinium* sp. 2, t: *Dubridinium caperatum* Reid, u: Cyst of *Niea acanthocysta* (Kawami, Iwataki et Matsuoka) T. Liu, K.N. Mertens et H. Gu (=*Oblea acanthocysta*), v: *Echinidinium* sp. large and accuminate type, w: *Stelladinium robustum* Zonneveld, x: *Trinovantedinium applanatum* Bujak et Davies, y: *Lejeunecysta* sp., z: Cyst of *Protoperidinium paraoblongum* Sarai, Yamaguchi, Kawami et Matsuoka, aa: *Selenopemphix quanta* (Bradford) Matsuoka, bb: *Selenopemphix alticinctum* (Bradford) Matsuoka, cc: *Selenopemphix* sp. (small type), dd: Cyst of *Protoperidinium latidosale* (Dangeard) Sarai, Yamaguchi, Kawami et Matsuoka, ee, ff: Cyst of *Polykrikos kofoidii* Chatton, gg: *Echinidinium* sp. (long type), hh: Cyst of *Protoperidinium* sp. 1 (apical view). Scale bars: 20 μm.

Table 2. Abundance of marine and freshwater palynomorphs observed in surface sediments of Osaka Bay, west Japan. Concentrations are shown for 1g of dry sediment. Colorless ovoidal and spherical cysts without any ornament were tentatively included in dinoflagellate cysts, however, it is possible that these are different from dinoflagellate cysts.

	St. 1		St. 2		St. 3		St. 4		St. 5		St. 6		St. 7		St. 8		St. 9		St. 10		St. 11		St. 12	
	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g
DINOFLAGELLATA																								
GONYAULACALES																								
*Spiniferites bulloideus**	10	350	8	288	7	238	8	224	15	720	15	465	4	380	8	728	10	800	15	795	15	840	6	420
Spinferites dellicatus			1	36			3	84			1	31					1	80			1	56	5	350
Spiniferites elongatus			1	36							1	31									4	224		
Spiniferites hyperacanthus					1	34			2	96	1	31	2	190			2	160	2	106	2	112		
Spiniferites mirabilis			1	36															1	53				
Spiniferites ramosus							3	84			1	31					1	80	1	53			1	70
Spiniferites nanus							3	84	1	48			1	95									2	140
Spiniferites spp.											1	31	2	190			1	80						
Lingulodinium machaerophorum									1	48	2	62	2	190	1	91					2	112		
Tuberculodinium vancampoae			1	36	1	34			3	144	2	62	1	95	1	91	1	80	1	53				
Alexandrium tamarense/catenella	23	805	12	432	4	136	20	560	46	2208	29	899	38	3610	32	2912	8	640	31	1643	58	3248	10	700
PERIDINIALES																								
Brigantedinium simplex			5	180					2	96	3	93	3	285	1	91	1	80	4	212	2	112	1	70
Brigantedinium cariacoense	1	35					1	28	2	96	7	217	3	285	7	637	5	400	4	212	2	112		
Brigantedinium majuscrum	1	35	2	72							1	31	1	95			1	80	2	106	1	56		
Brigantedinium grande			3	108									1	95					1	53				
Brigantedinium irregulare					1	34					2	62	1	95	1	91								
Brigantedinium spp.	11	385	16	576	13	442	7	156	18	864	40	1240	34	3230	50	4550	29	2320	27	1431	38	2128	6	420
Brigantedinium spp. (pale brown)	9	315	7	252	18		17	476	10	480	12	372	22	2090	31	2821	21	1680	19	1007	26	1456	1	70
Lejeunecysta concreta			2	72			4	112					4	380	3	273	2	180	2	106	2	112	1	70
Lejeunecysta sp.											1	31	1	95										
Selenopemphix nephroides					1	34			2	96	1	31	2	190			1	80						
Selenopemphix quanta L					1						3	93					1	80	4	212				
Selenopemphix quanta S									4	192	1	31	4	380	2	182	1	80			1	56		
Stelladinium reidii													3	285	1	91			1	53	1	56	1	70
Trinnovantedinium applanatum							1	28			1	31	2	190			2	160			6	336		
Votatdinium calvum (*cordate*)	1	35	2	72	1	34							3	285					4	212	2	112		
Votatdinium calvum (*rhombic*)					2	68							1	95					2	106	4	224	2	140
Protoperidinium lattisinum	1	35	2	72			2	56	1	48	2	62			1	91	2	160	1	53	3	168		
Protoperidinium americanum									1	48	2	62	2	190										
Protoperidinium ponticum					1	34													1	53	1	56		
Protoperidinium leonis					1	34			1	48	1	31			3	273	1	80						
Protoperidinium acromaticum									1	48														
Protoperidinium sp. (small perid)							2	56							3	273	1	80			1	56		
Protoperidinium sp.									1	48			1	95	2	182			1	53				
Peridinium quinqucorne																							1	70
Scrippsiella spp.	3	105	5	180	7	238	6	168	4	192	3	93	2	190	18	1638	9	720	12	636	6	336	2	140
Dubridinium cavatum	2	70	4	144	4	136	6	168	7	336	6	186	8	760	8	728	19	1520	7	371	15	840	1	70
Oblea acanthocysta							1	28	1	48	3	93	1	95			2	160	4	212	5	260	4	280
Echinidinium acliatum															1	91	1	80			1	56		
Echinidinium sp. (long)	3	105	7	252	1	34	11	308	4	192	4	124	11	1045	10	910	7	560	6	318	12	672	1	70
Echinidinium sp. (short)	3	105	8	288	2	68	4	112	5	240	6	186	13	1325	14	1274	15	1200	9	477	10	560	4	280
Echinidinium sp. (hair)	1	35			2	68			2	96	1	31	6	570	4	364					5	260		
Echinidinium sp. (large & acuminate)									1	48														
Echinidinium sp. (dense)															8	728								
GYMNODINIALES																								
Polykrikos kofoidii			1	36	3	102			4	192			1	95	5	455	2	160	1	53	3	168		
Polykrikos schwartzii					1	34							1	95										
Gyrodinium instriatum													1	95					1	53				
INCERTAE SEDIS																								
Ovoidal cyst			3	108			3	84	2	96	6	186	3	285	4	364	2	160	5	265	4	224	2	140
Spherical cyst	9	315	6	216	10	340	10	280	3*	144	15	465	10	950	18	1638	7	560	14	742	43	2408	9	639

Table 2. *Continued*.

	St. 1		St. 2		St. 3		St. 4		St. 5		St. 6		St. 7		St. 8		St. 9		St. 10		St. 11		St. 12	
	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g	original	cysts/g
ACRITARCHA																								
Baltisphaeridium sp.	5	175	1	36					1	48	2	62	2	190	4	364	2	160	2	106			2	140
Michystridium ariakense			2	72	3	102	2	56	2	96	2	62			3	273	2	160	6	318	4	224	4	280
Michystridium spp.			3	108	6	204	4	108	1	48	1	31	4	380	5	455	6	480	10	530	8	448	6	420
Concentricystes rubina											1	31												
Halodinium minor							2	56			3	93	1	95	4	364	2	160	5	265			1	70
Halodinium sp.							1	28									2	160	2	106				
Ovoidites spp.			1	36			4	108			1	31	4	380	1	91	2	160	4	212	5		3	210
RAPHYDOPHYCEAE																						260		
Chattonella marina	10	350	2	72	5	170	9	252			10	310	3	285	5	455	3	240	14	742	4	224	3	210
PRASINOPHYCEAE																								
Cymatiosphaera spp.	1	35			1	34	4	108															2	140
Pterosperma sp.																					2	112		
CHLOROPHYCEAE																								
Pediastrum simplex			1	36	2	68	1	28			1	31	6	570	12	1092	2	160						
Pediastrum biwae													1	95	2	182	1	80						
Staurastrum spp.**	1	35	6	216			5	140	1	48	24	744	525	49875	212	19292	29	2320	12	636	7	392	2	140
Desmodesmus spp.			1	36									3	285										
TINTINNID																								
Resting spore	14	490	13	468			13	356	22	1056	15	465	35	3325	22	2002	16	1280	15	795	10	560	16	1120
lorica	2	70	3	108			6	168	7	336	2	62	6	570	6	546	1	80	1	53	1	56		
TESTATE AMEBOA			1	36	1	34	1	28							2	182			1	53				
FORAMINIFERA																								
Uniserial type	7	245	14	504	13	442	9	252	14	672	18	558	6	570	5	455	15	1200	19	1007	9	504	3	210
Biserial type	6	210	13	468	8	272	5	140	6	288	24	744	5	475	1	91	2	160	15	795	6	336	5	350
Coiled type	41	1435	21	756	60	2040	45	1260	40	1920	57	1767	5	475	8	728	9	720	34	1802	22	1232	11	770
Compound type	5		8	288	5	170	4	108	28	1344	9	279			4	364	2	160	4	212			3	210
CRASTACEA																								
Resting egg (reticurate)	6	210	7	252	10	340	11	308	21	1008	13	403	12	1140	20	1820	28	2240	14	742	17	954	1	70
Resting egg (spinate)	29	1015	64	2304	42	1428	50	1400	109	5232	97	3007	53	5035	50	4550	50	4000	68	3604	60	3360	13	940
Body***	52	1820	34	1224	28	952	47	1316	43	2064	44	1364	34	3230	30	2730	32	2560	46	2438	36	2016	8	560
TOTAL		8820		10512		8398		9316		21072		15438		85590		57603		28980		24115		26094		10049

*: *Spiniferites bulloideus* and *Operculodinium centrocarpum* involve nodular process forms.
**: *Staurastrum* spp. is mainly consisting of *S. dorsidentiferum* W. et G.S. West var. *ornatum* Grönbland.
***: Crustacean body included parts of appendages.

catenella, and *A. tamiyavanichii* have been known for some time (Yamamoto, 2004). In 2002 and 2007, PSP toxication in *Ruditapes philippinarum*, *Fulvia mutica*, and *Corbicula japonica* were reported (Yamamoto et al., 2011). The elongate cyst with a colorless wall is identical to those of *Alexandrium acathenella*, *A. tamarense*, or *A. catenella*. However, since these cysts are not differentiated based on external morphology, Yamamoto et al. (2011) conducted cyst incubation experiments and concluded that most ellipsoidal cysts are identical to *A. tamarense*. *A. acathenella* occurs extremely rarely, however a small amount of *A. catenella* also occurred in Osaka Bay. Therefore, these ellipsoidal cysts were identified as cysts of *Alexandrium tamarense/catenella* in this paper.

After PSP events, the distribution of *A. tamarense* cysts was investigated in order to detect the seed beds in 2006 and 2007 by Yamamoto et al. (2009). Consequently, *A. tamarense* cysts were observed throughout the bay, but the cyst densities were considerably different in these two years. In 2006 before the bloom, the maximum density of the cysts was recorded as 112±28 cysts/g at the east coast, while in 2007 after the bloom, it reached at 5,683±631 cysts/g (Yamamoto et al., 2009). This means that the cysts preserved in the sediment are closely related to the plankton cell densities (blooms). However, in both years, the maximum cyst density was located at the mouth of the Yodo River along the east coasts of the bay. In spite of the lower cyst densities, as shown in dry sediment weight (maximum of 3,610 cysts/g at St. 7.), the cyst were observed in all samples and recorded more concentrated areas on the east coasts. These horizontal distributions suggest that the cysts of *A. tamarense* seem to be constantly transported by the East Coast Residual Current. Also, the occurrence of the cyst of *A. tamarense/catenella* in the studied samples suggests that there is a high risk of the PSP events caused by *A. tamarense* in the future.

Cyst of *Chattonella* (Raphidophyceae; planktonic and phototrophic micro-protoctista) (Fig. 3; Fig. 7C; Table 2)

This is the first report in which the cyst of *Chattonella* has been observed after palynological processing. These cysts are mostly hemispherical and have a transparent wall. The opening is circular and formed around the top of the hemispherical cyst. This suggests that these cysts may have been originally attached to siliceous sediment particles before palynological processing as shown by Imai and Itoh (1985). For identification and counting of *Chattonella* cysts in sediments the following two methods currently exist; the MPM (Most Probable Method) method (Ishida and Kadota, 1979) improved for *Chattonella* cysts by Itoh and Imai (1987) and the direct count method (CD) is that the cysts in sediments are identified under an inverted fluorescence microscope with blue excitation light (Itoh and Imai, 1987).

In Osaka Bay, the cyst of *Chattonella* was first identified under the MPM method by Imai and Itoh (1987). Cyst density was recorded as 2-155 cysts/cm^3 and the highest density of 155 cysts/cm^3 were observed in the western part of the bay. Thereafter, Itakura et al. (1991) reinvestigated the horizontal distribution of *Chattonella* cyst in surface sediments of Osaka Bay collected in 1989 and 1988 under two different extraction methods after the blooming of *Chattonella*. By the DC the *Chattonella* cysts were recorded in 0-284 (ave. 62) cells/cm^3 in 1988 and 0-378 (av. 79) cells/cm^3 in 1989 samples. While, the *Chattonella* cysts were recorded in 0-110 (ave. 13) cells/cm^3 by the MPM (Itakura et al., 1991). In general, along the east coasts of the bay, the *Chattonella* cysts were more concentrated in both years. The direct count after palynological processing in this study revealed the *Chattonella* cysts are distributed entirely in Osaka Bay with 72-742 cysts/g. There may be two reasons for explaining these differences. One is that since *Chattonella* does not make a big bloom in recent years, cyst production has decreased. The other is caused by different counting methods. The direct count method includes two different processing techniques. In the samples processed palynologically, both living and empty

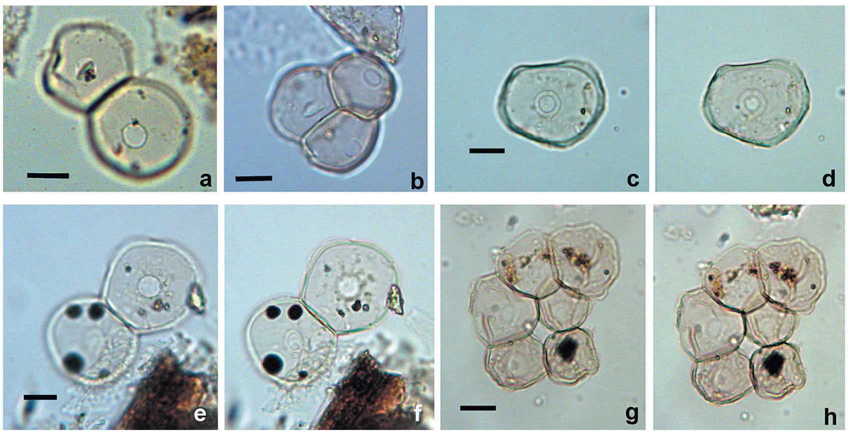

Fig. 3. Cysts of *Chattonella* (Raphidophyceae).a-h: Empty cysts of *Chattonella* showing circular opening at the top. Usually cysts of *Chattonella* were observed as colonies. Scale bars: 20 μm.

cysts are observed, however, under the direct count using a fluorescence microscope with blue excitation light, only living cysts with chloroplasts are detected as well as the MPM. This is important when designing the cyst survey for *Chattonella*, to consider which method is preferable for the purposed study.

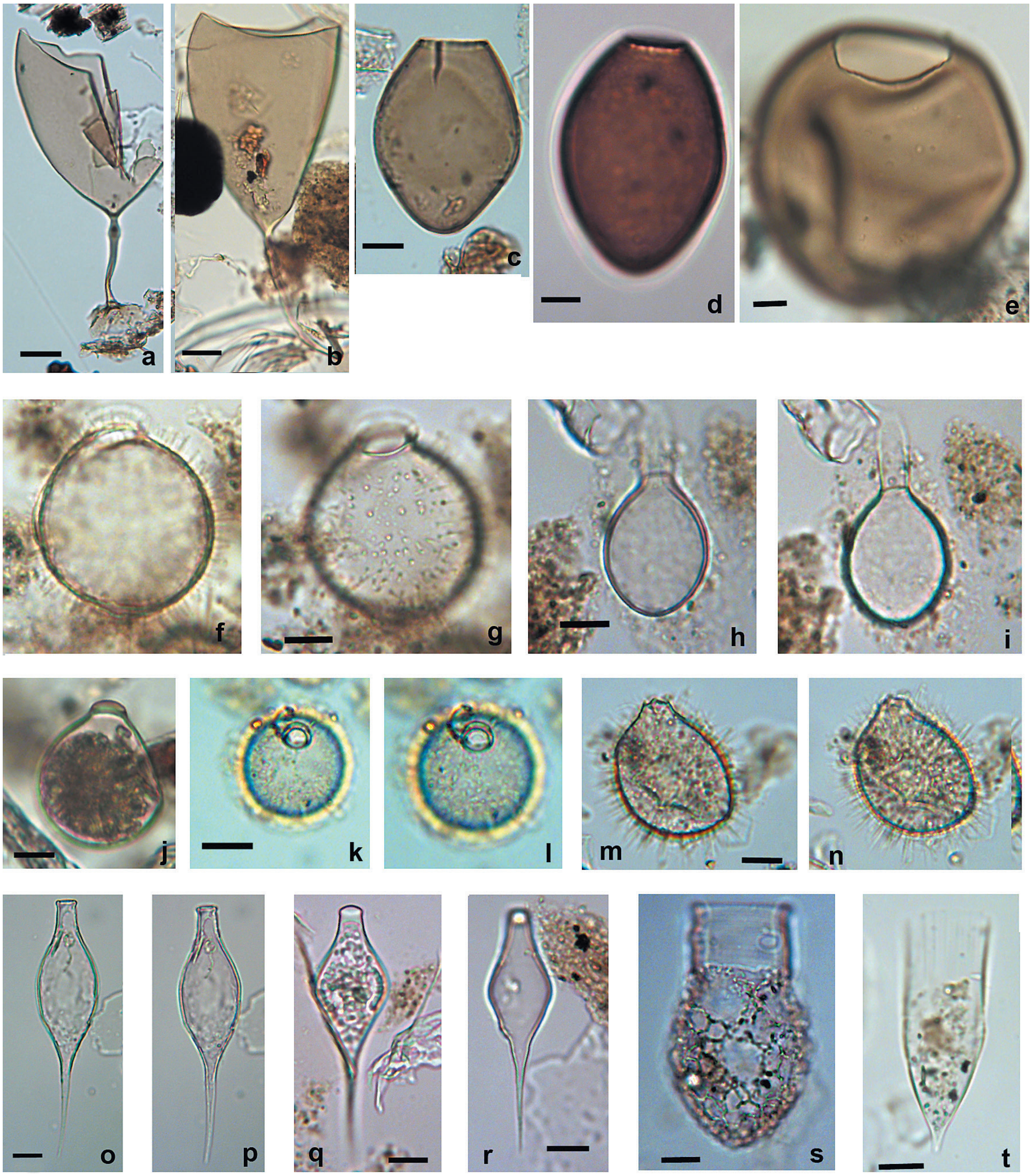

Fig. 4. Resting cysts and loricae of tintinnids (Ciliata). a-b: Hexagonal, not ornamented type (Hexano-5) of Van Wanveren (1993), c-e: Asymmetrically rhombic, orally blunt, not ornamented type of Van Wanveren (1993), f-g: Cyst of *Strombidium* sp. 1, h-i: Cyst of *Strombidium* sp. 2, j: Cyst of *Strombidium* sp. 3, k-l: Cyst of *Strombidium* sp. 4, m-n: Cyst of *Strombidium* sp. 5, o-p: Cyst of *Crytrostrombidium* sp. 1, q-r: Cyst of *Crytrostrombidium* sp. 2, s: lorica of *Codonellopsis* sp., t: lorica of *Protorhabdonella* sp.. Scale bars: 20 μm.

Tintinnomorphs (planktonic ciliated protoctista) (Fig. 4; Tables 1 and 2)

Resting cyst

Hexagonal, not ornamented type (Hexano-5) of Van Wanveren (1993)

Asymmetrically rhombic, orally blunt, not ornamented type of Van Wanveren (1993)

Cyst of *Strombidium* sp. 1

Cyst of *Strombidium* sp. 2

Cyst of *Strombidium* sp. 3

Cyst of *Strombidium* sp. 4

Cyst of *Strombidium* sp. 5

Cyst of *Crytrostrombidium* sp. 1

Cyst of *Crytrostrombidium* sp. 2

Lorica

Codonellopsis sp.

Protorhabdonella sp.

Tintinnid remains are frequently observed in modern sediments, since their loricae are made of mostly transparent organic membranes called pseudo-chitin. In addition, some tintinnid can produce chitinous resting cysts that are also preserved in sediments. Van Waveren (1993) proposed a classification system of tintinnomorphs' remaining parts as loricae and resting cysts based on their external morphological features. Since most tintinnid resting cysts lack the sculptures and ornaments developed on the lorica surface of living forms, Van Waveren (1993) introduced and used several terms for the description of the lorica shape. On the other hand, Kamiyama (2013) introduced several living resting cysts of marine oligotrich ciliates preserved in sediments. Meunier (1910) studied living microplankton of Barents Sea and Kara Sea and described many organisms morphologically similar to present resting cysts of tintinnid as *Fusporis* (eg Fig. 4o, p, q, r). However, related plankton species of these cysts found in sediments are not yet clarified.

Microforaminiferal linings (benthic protoctista) (Fig. 5; Fig. 7D; Tables 1 and 2)

Microforaminiferal linings are small (less than 150 μm in total size) and foraminiferal organic remains found in palynological preparations (Wilson and Hoffmeister, 1952). Several authors have proposed formal or informal classification systems for microforaminifers (Goczan, 1962; Macko, 1963; Deak, 1964; Pantic and Bjraktarevic, 1988). Finally, Stancliffe (1987) classified fossils and remains of microforaminiferal linings into five major morphological types: single chambers, uniserial, biserial, coiled, and compound types. This classification system was also used in the Holocene microforaminiferal study (Stancliffe and Matsuoka 1991). In this article, we used the informal classification system for microforaminiferal linings proposed by Stancliffe (1987); however, we did not adopt the categories beyond the five major types because of difficulties in observation.

Takayanagi (1953) and Nakaseko (1953) first studied benthic foraminifers of Osaka Bay. Since then, several studies of foraminifers have been published. Tsujimoto et al. (2006) recently described 61 species and 37 genera of benthic foraminifers in bottom sediments of Osaka Bay. Their abundance ranged from 3 to 148 individuals/g and they were very abundant in the northwestern part, but rather less in the east coast. On the other hand, microforaminiferal linings in sediments were 1,540-4,224 linings/g. This is more than ten to twenty times that of benthic foraminifers' concentration recorded by Tsujimoto et al. (2006). At the moment, it is difficult to explain this much difference, however one possibility is that two studies had different target sizes. Tsujimoto et al. (2006) observed specimens over 105 μm in size, while in this study we observed microforaminifers of less than 125 μm in size. This suggests that abundant smaller benthic foraminifers have not yet been fully investigated until now.

Meta-zooplankton (mainly crustacean remains) (Tables 1 and 2)

The palynomorphs belonging to this group consists of pseudochitinous body remains and resting eggs. Apart from modern biological terminology, Van Waveren (1993) proposed proper terms for crustacean remains preserved in sediments of Banda Sea as palynomorphs. In this study, crustacean remains were tentatively classified into two categories; resting eggs and body fragments including appendages. The parents of some spinous resting eggs are known from sediments using egg incubation experiments (Kasahara et al., 1974). However, the parents of many resting eggs have been not yet known. Also crustacean bodies have been preserved as fragments in sediments. In this case, the number (or density of fragments) of remains observed in sediments has a different meaning from other palynomorphs that are single-celled. Handling of crustacean fragments is rather difficult and should be confirmed in future.

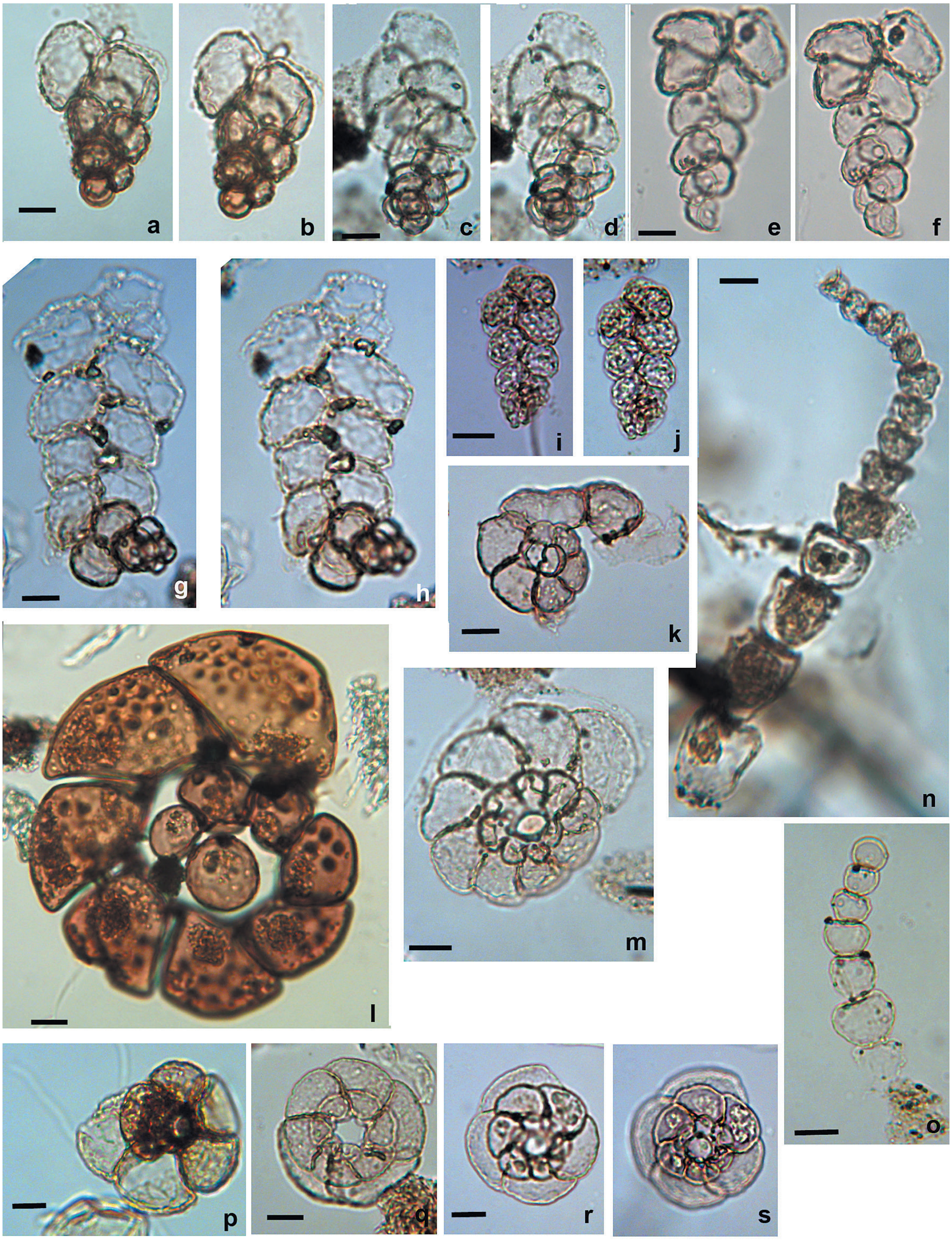

Fig. 5. Microforaminiferal linings (Benthic foraminifera). a-b, c-d, e-f, i-j: Biserial types, g-h: Compound type, k, l, m, p, q, r-s: Coiled types, n, o: Uniserial type. Scale bars: 20 μm

Acritarcha (**resting cells of mainly planktonic and phototrophic micro-protoctista**) (Fig. 6; Tables 1 and 2)

Halodinium minor Bujak

Halodinium sp.

Michystridium ariakense Takahashi

Michystridium sp.

Acritarch is an artificial taxonomical group consisting of resting cells and/or resting eggs of various organisms (Downie et al., 1963). An acritarch is usually spherical to subspherical, ellipsoidal, or ovoidal in shape and ornamented with various appendages, processes, spines, and membrane with slit, circular and polygonal openings. Based on the previous records, acritarchs are mainly considered to be marine (but sometimes common in brackish water and freshwater) and probably planktonic and phototrophic. In this study, *Michystridium ariakense* described from the Pleistocene sediments of the Ariake Sea by Takahashi (1971) occurred at several stations.

Chlorophyceae (**planktonic phototrophic micro-protoctista**) (Fig. 6; Fig. 7E; Table 2)

Pediastrum biwae Negoro

Pediastrum simplex (Meyen) Lemmermann

Staurastrum dorsidentiferum W. et G.S. West var. *ornatum* Grönbland

Botryococcus braunii Kützing

A Chlorophyceaa remain of *Staurastrum pingue* was once described from a middle Albian samples collected from Terrant Country of Texas, USA, however, its occurrence was considered to be contamination in water released from Lake Worth Dam (Srivastava, 1997). In sediments of Osaka Bay, a few freshwater planktonic algal remains occurred in almost all samples. These are identical to *Staurastrum dorsidentiferum* W. et G.S. West var. *ornatum* Grönbland, *Pediastrum biwae* Negoro, *Pediastrum simplex* (Meyen) Lemmermann, and *Botryococcus braunii* Kützing. Among them, *S. dorsidentiferum* var. *ornatum* was extremely abundant with over 10^4 cells/g at St. 7 and St. 8 situated in the innermost part of the bay. Along the east coast of the bay, *S. dorsidentiferum* var. ornatum occurred abundantly while in the western part of the bay, this species was less abundant. The distribution of *S. dorsidentiferum* var. *ornatum* is quite different from those of other marine palynomorphs consisting of dinoflagellate cysts, *Chattonella* cysts, and microforaminiferal linings. According to Wakabayashi and Ichinose (1982) and Kishimoto et al. (2013), this species is the most dominant desmids in Lake Biwa and occurs as an endemic for the Lake Biwa-Yodo River system. Therefore, the abundant occurrence of this species around the mouth of the Yodo River suggests that such algal remains were transported by the Yodo River from Lake Biwa to Osaka Bay.

Hongo and Nakajo (2003) discussed sedimentary characteristics in Osaka Bay based on the abundance of another endemic freshwater green algae *Pediastrum biwae*, and concluded that fine-grained sediments from the Yodo River were transported south-southwestward by residual currents. The horizontal distribution of *S. dorsidentiferum* var. *ornatum* is similar to that of *P. biwae* shown by Hongo and Nakajo (2003).

Concluding summary

Marine and Fresh water palynomorphs have been used for reconstruction of coastal paleoenvironments. Matsuoka (1992) clarified the late Holocene regression of marine environments by increase of freshwater algal remains (spore of Zygnemataceae) and acritarch *Chomoriletes rubinus* (Christopher) (=*Pseudoschzaea rubina* (Christopher)) in Hirado Island, West Kyushu. Mudie et al. (2011) showed the relative abundance of marine, brackish-water, and freshwater palynomorphs which was called as nonpollen palynomorphs by Mudie et al. (2011) indicated change of salinity and environment in the Caspian-Black Sea-Mediterranean corridor in the Holocene. Rubino et al. (2017) investigated plankton resting cells in recent sediments of Haifa port, Israel and discussed the relation between their distribution and environmental consequence. Matsuoka et al. (2018) suggested the role of microfolaminiferal linings (benthic foraminifers) in the tropical shallow water ecosystems in tha case of shouthern Myanmar. In this study, the horizontal distribution of the elongate *Alexandrium* cysts suggests the direction of coastal currents together with the abundance and distribution of freshwater algal remains *Staurastrum*. Thus, marine and freshwater palynomorphs become useful indicators for the reconstruction of environments. To date, however, the significance of these palynomorphs in a paleoenvironmental study has not yet been fully understood except for dinoflagellate cysts due to the lack of basic information on their parental organisms. The most fundamental and essential subject for further development on these palynomorphs in terms of environmental studies is to clarify the relationship to their

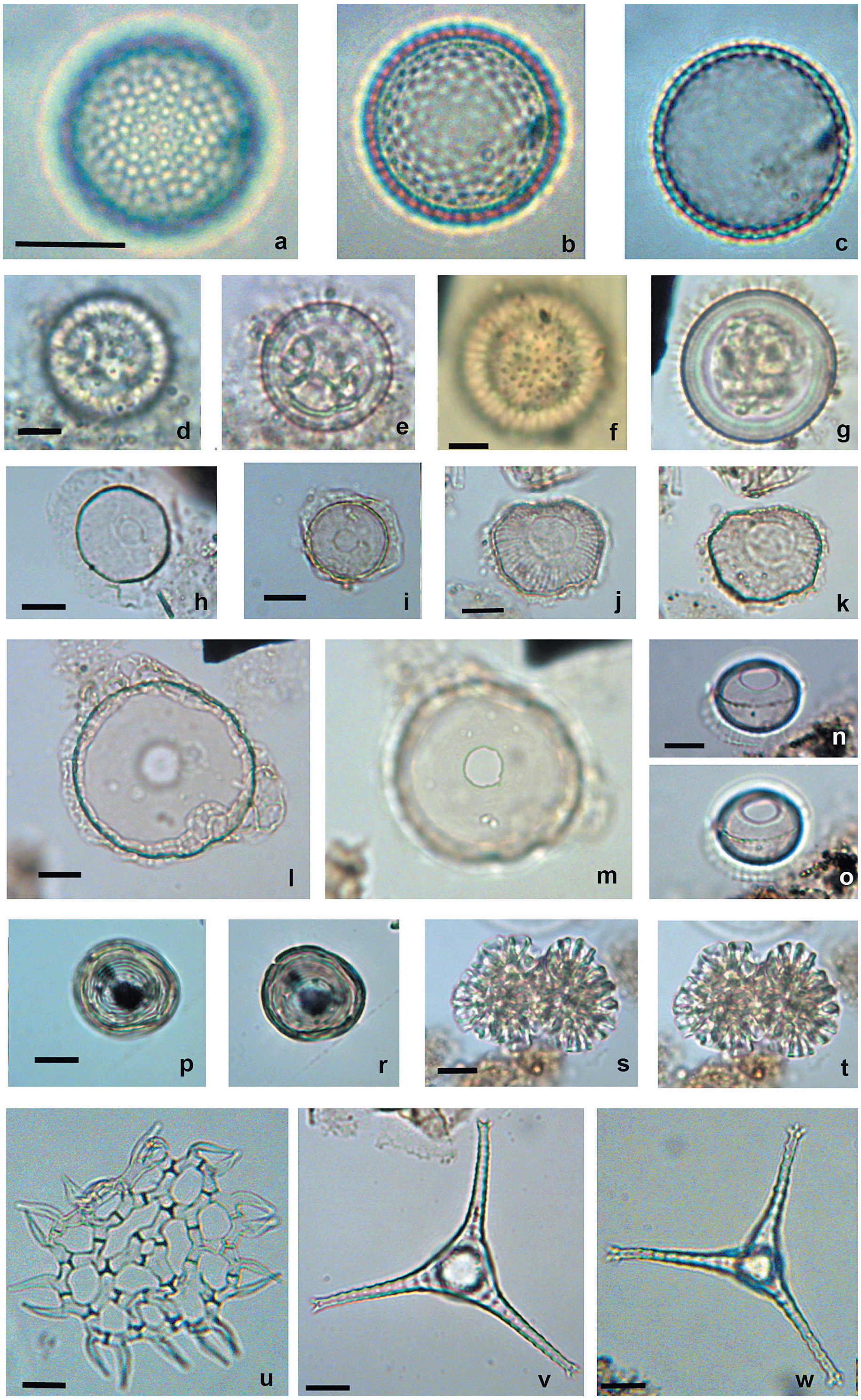

Fig. 6. Acritarch and freshwater microalgal remains (Chlorophyceae). a-c: *Michystridium* sp., d-e, f-g: *Michystridium ariakense* Takahashi, h, i, j-k: *Halodinium* sp., l-m: *Halodinium minor* Bujak, n-o: *Radiosperma* sp., p-r: *Concentricystes rubinus* Rossignol, s-t: *Botryococcus braunii* Kützing, u: *Pediastrum biwae* Negoro, v,w: *Staurastrum dorsidentiferum* W. et G.S. West var. *ornatum* Grönbland. Scale bars: 20 μm

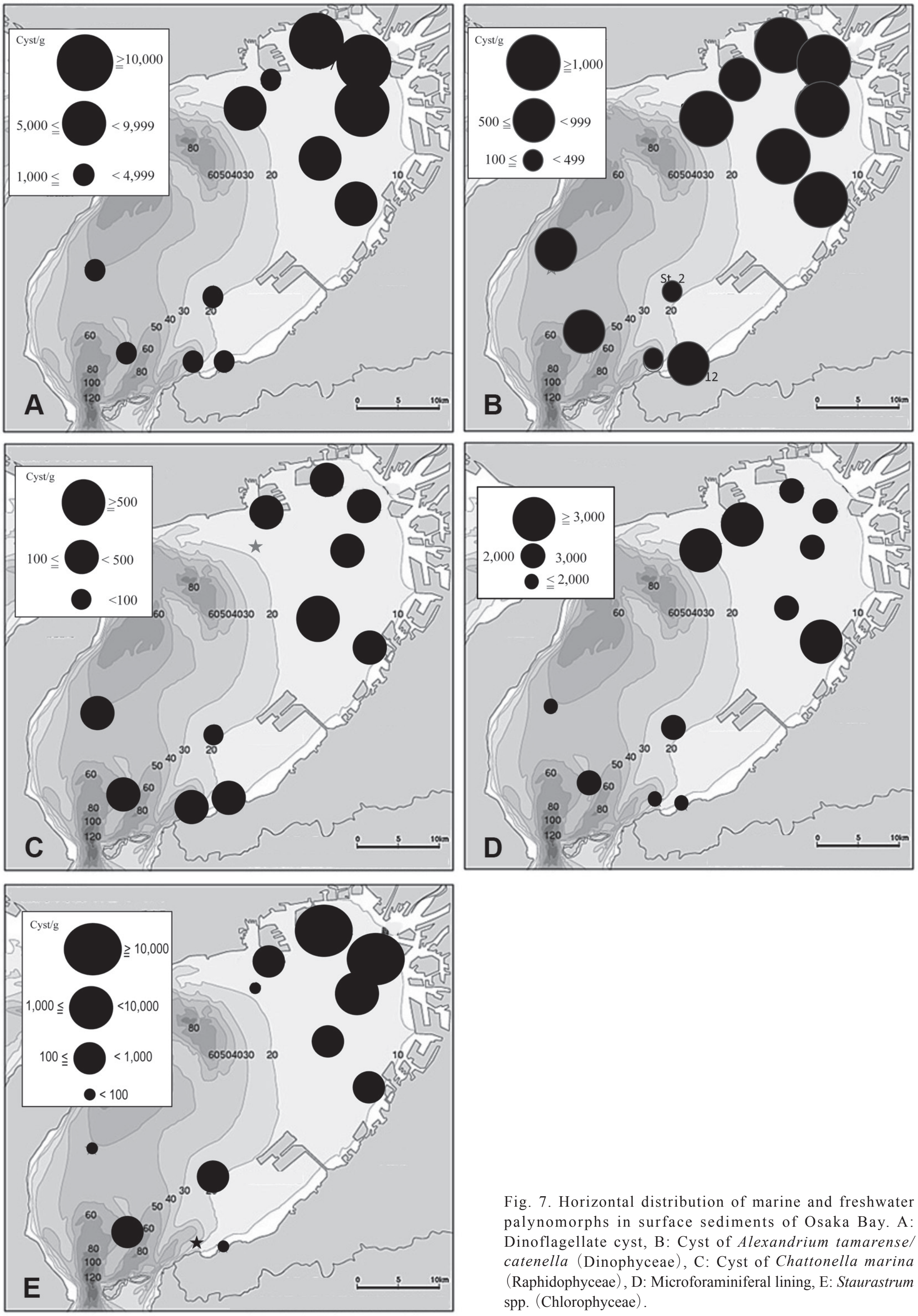

Fig. 7. Horizontal distribution of marine and freshwater palynomorphs in surface sediments of Osaka Bay. A: Dinoflagellate cyst, B: Cyst of *Alexandrium tamarense/catenella* (Dinophyceae), C: Cyst of *Chattonella marina* (Raphidophyceae), D: Microforaminiferal lining, E: *Staurastrum* spp. (Chlorophyceae).

parents. In the case of dinoflagellates, acritarchs and ciliate cysts, cysts-incubation experiments and/or molecular identification may be useful methods to achieve this goal. While in the case of microforaminiferal linings, further taxonomic study of smaller benthic foraminifera is needed with a different way. For crustacean remains, the establishment of a handling method is important.

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