

Taxonomy of Symbiotic Dinoflagellates Associated with Korean Anthozoans

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Three species of endosymbiotic dinoflagellates, zooxanthellae, are investigated from six host species of anthozoans from Korea. Three unrecorded endosymbionts species are *Symbiodinium kawagutii*, *Symbiodinium microadriaticum*, and *Symbiodinium* sp. *Symbiodinium kawagutii* is associated with *Alveopora japonica*, *Anthopleura japonica* and *Parasicyonis actinostoloides*. *Symbiodinium microadriaticum* is found in *Anthopleura kurogane* and *Parasicyonis* sp. Unlike the former two symbionts, *Symbiodinium* sp. is associated with *Anthopleura midori*.

Since the symbiotic dinoflagellates, generally called as zooxanthellae, were recorded first by Brandt (cited from Taylor, 1983), more than 25 zooxanthellae species have been reported, including *Symbiodinium microadriaticum* (Freudenthal, 1962; Kevin et al., 1969; Taylor, 1969, 1971a; Thin et al., 1986), *Amphidinium chattonii* (Taylor, 1971a), *Amphidinium klebsii* (Taylor, 1971b), *Gymnodinium beii* (Spero, 1987), *Gloeodinium viscum*, *Scrippsiella velellae* (Banaszak et al., 1993), *Symbiodinium goreauii*, *Symbiodinium kawagutii* and *Symbiodinium pilosum* (Trench and Blank, 1987). Recently, several investigators have used molecular techniques to assess the genetic diversity of the symbiotic dinoflagellates: DNA sequencing (Rowan and Powers, 1992; McNally et al., 1994; Carlos, 1999), RFLP of rRNA genes (Rowan and Powers, 1991a,b; Rowan and Knowlton, 1995; Baker and Rowan, 1997; Carlos et al., 2000), DNA fingerprinting (Goulet and Coffroth, 1997), and RAPD (Baillie et al., 2000).

These symbiotic dinoflagellates and their hosts are known to benefit each other, and the symbionts are directly related to the bleaching phenomenon of hermatypic corals (Hoegh-Guldberg and Salvat, 1995; Brown, 1997). However, biological studies about the symbiotic dinoflagellates have never been performed in Korea, in spite of their importance to host survival. Therefore, this study aimed to investigate the zooxanthellae of anthozoans' host in Korean waters, and to reexamine the biogeography of zooxanthellae based on our former molecular approach (Lanetty et al., 2001).

Materials and Methods

For this study, six anthozoans hosts, *Alveopora japo-*

nica, *Anthopleura japonica*, *Anthopleura kurogane*, *Anthopleura midori*, *Parasicyonis actinostoloides*, *Parasicyonis* sp., were selected. During the period from 1999 to 2001, they were collected at depth between intertidal and sublittoral zone of Korean waters by skin and SCUBA diving and fishing nets. Symbiotic dinoflagellates were extracted by the homogenization of hosts, and cultured in F2 media at 21 °C on a 16:8 h LD cycle. Their size and color were examined under light microscope (LM; Olympus BH2, Olympus CH, Zeiss Axioscop 2 microscope system), and external surface was observed with scanning electron microscope (SEM; JSM 5200). Internal features of symbionts were observed under transmission electron microscope (TEM; HITACHI H-600 ELECTRON MICROSCOPE), and the arrangements of chloroplasts and DAPI stained nucleus were examined under confocal laser scanning microscope (CLSM; Zeiss Axiovert 100M and Zeiss LSM Image Browser). In addition, the volumes of chloroplasts and nucleus were calculated by OPTIMA 6.5 and Excel program.

Results

Symbiotic dinoflagellates from six anthozoans' host in Korea turned out to be three *Symbiodinium* species within family Gymnodiniaceae. They are identified using LM, CLSM, SEM and TEM (Table 1), and compared each other based on the shape of organelles and features.

Class Dinophyceae

Order Gymnodinales

Family Gymnodiniaceae Lemmermann, 1990

Genus *Symbiodinium* Freudenthal, 1962

Symbiodinium kawagutii Trench and Blank, 1987
(Fig. 1, A-I; Fig. 2, A-E)

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Table 1. Character comparison of three symbiotic *Symbiodinium* dinoflagellate species sampled from Korea

Characters		Species		
		<i>S. microadriaticum</i>	<i>S. kawagutii</i>	<i>S. sp.</i>
Position in host		endoderm	endoderm	—
Shape		spheroid	spheroid	spheroid
Color		light green - brown	light green - brown	light green
Size (m)		9.94 ± 0.95	11.47 ± 1.9	9.13 ± 1.52
Amphiesma	No. of outer envelopes	4	4	4
	Pellicle (µm)	0.5	0.045	0.048
	Relationship of plasmalemma and inner membrane	associated	associated	isolated
	Shape of inner envelopes	apposed	apposed	apposed
Cell surface	Shape	smooth	smooth & grooved	sleek
Chloroplast	Arrangement of chloroplast thylakoid	parallel	parallel	parallel
	Vol. of chloroplasts in cell (%)	45.46 ± 10.62	55.71 ± 14.9	28.48 ± 19.55
	No. of envelopes	3	3	3
	No. of thylakoid lamellae	3	3	3
	Peripheral lamellae	presence	presence	presence
	Position of chloroplasts	peripheral	peripheral	peripheral
Mitochondria	No. of envelope	2	2	2
	Shape of cristae	tubular	tubular	tubular
Nucleus	No. of envelopes	2	2	2
	Vol. of nucleus in cell (%)	12.51 ± 4.4	13.51 ± 3.76	16.51 ± 7.28
	Nuclear pore	presence	presence	presence
	Position in cell	eccentric	eccentric	eccentric
	Shape of chromosomes	dinokaryon	dinokaryon	dinokaryon
Pyrenoid	Invasion of thylakoid	absence	absence	absence
	No. of pyrenoid	1	1	1
	No. of stalks	1	2	2
	Shape of pyrenoid	circular	oval	oval
	Starch sheath	presence	presence	presence
Other organelles	Accumulation body	presence	presence	presence
	Calcium oxalate crystal	presence	—	presence
	Dictyosome	presence	presence	presence
	Lipid granule	presence	presence	presence
	Storage product	starch	starch	starch
	Position of storage product	Cy & aP ¹	Cy & aP	Cy & aP
	Trichocyst	absence	absence	absence

¹ Cy & aP: cytoplasm and around pyrenoid as a sheath.

Symbiodinium kawagutii Trench and Blank, 1987, p. 479; Banaszak et al., 1993, p. 526.

Material examined: *Alveopora japonica*: Chagwido, 6 Nov. 2000, 2 inds. (JI Song); Munseom, 13 Feb. 2000, 1 ind. (HS Lim); Munseom, 22. Feb. 2001, 2 ind. (JI Song). *Anthopleura japonica*: Daegae, 27 Jul. 2000, 1 ind. (HS Lim); Yeosu, 13. Jan. 2001, 1 ind. (HS Lim). *Parasicyonis actinostoloides*: Munseom, 7 Nov. 2000, 1 ind. (JI Song); Munseom, 22 Feb. 2001, 1 ind. (JI Song); Munseom, 24 May 2001, 1 ind. (BI Kim).

Description: Unicellular, located within hosts' endodermal cells as coccoid cells, spherical or oval in shape, greenish brown at high irradiance, brown at low irradiance (5-20 m deep) and divided *in hospite* and in culture to produce two daughter cells. Mean diameter of algae 11.47 µm (±SD 1.9 µm, n = 60).

Cell surface smooth and somewhat grooved. Cell wall composed of four outer membranes, pellicle and pressed series of inner membranes associated with plasmalemma; pellicle with membrane bound vesicles near inner envelope and approximately 0.045 µm thick; plasmalemma located under cell wall and associated with inner envelope.

Chloroplasts peripheral, multi-lobed, 38.96% (±SD 9.04%, n = 11) of volume of cell at high irradiance, 64.92% (±SD 7.46%, n = 20) of volume of cell at low

irradiance, enclosed by one envelope comprised of three closely apposed membranes, no girdle lamellae and peripheral lamellae present; thylakoid oriented parallel to long axis of chloroplast; chloroplast stacked in groups of three lamellae and no grana; pyrenoid single, oval in shape, attached to chloroplast by two stalks, no invasive chloroplast thylakoids and surrounded by starch sheath.

Nucleus limited by one envelope composed of two membranes with nuclear pores, off-centered, spherical in shape, 12.1% (±SD 2.79%, n = 11) of volume of cell at high irradiance, 14.37% (±SD 4.09%, n = 20) of volume of cell at low irradiance, peripheral nucleolus and include chromosomes; chromosomes dinokaryon.

Mitochondria limited by double membranes, tubular type cristae, and elongated or circular in shape. Storage products starch, located in cytoplasm and around pyrenoid as a sheath. Trichocysts absent. Accumulation bodies and dictyosomes present. Lipid granules circular, located in cytoplasm and chloroplasts.

Remark: Motile cells of *S. kawagutii* described by Trench & Blank (1987) have not been observed in this study.

Distribution: Korea (Korea Strait, Cheju Island area), Hawaii.

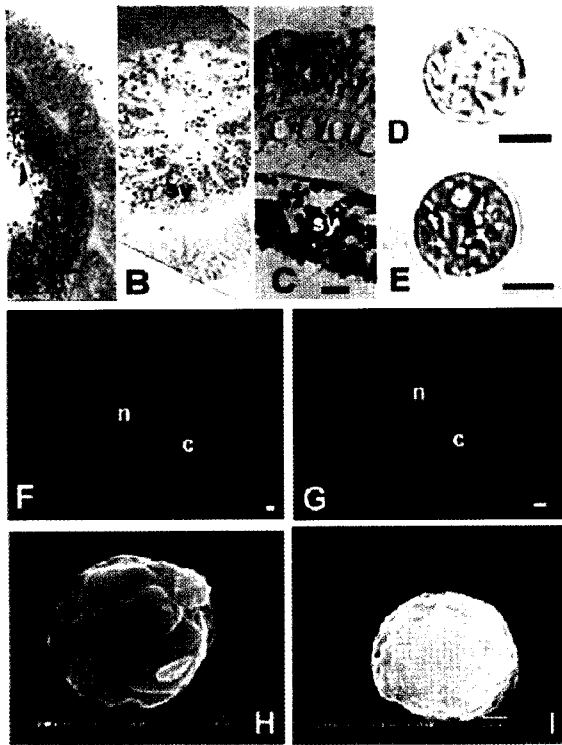


Fig. 1. Location and features of *Symbiodinium kawagutii*. A, From *Anthopleura japonica*; B, From *Alveopora japonica*; C, From *Parasicyonis actinostoloides*; D-E, Microscopic image *in hospite*. D, At high irradiance; E, At low irradiance; F-G, Fluorescent image of chloroplasts and nucleus *in hospite*. F, At high irradiance; G, At low irradiance; H-I, Surface feature *in hospite*. H, At high irradiance. I, Low irradiance. c, Chloroplast; n, Nucleus; sy, Symbiotic dinoflagellates. Scale bars = 100 μm (A-C), 10 μm (D,E), and 1 μm (F-I).

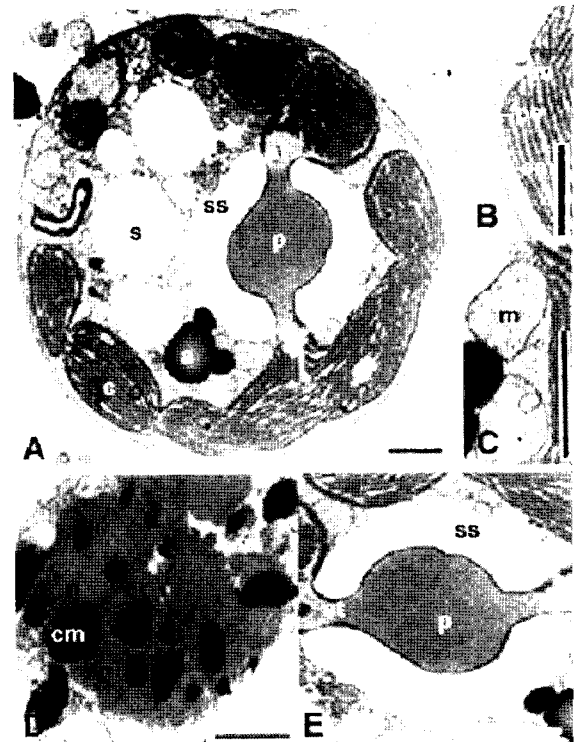


Fig. 2. Ultrastructure of *Symbiodinium kawagutii*. A, *In hospite*. B, Cell wall. C, Mitochondria. D, Nucleus. E, Pyrenoid. a, accumulation body; c, chloroplast; cm, chromosome; im, inner membrane; m, mitochondria; n, nucleus; om, outer membrane; p, pyrenoid; pe, pellicle; s, storage product; ss, starch sheath; st, stalk. Scale bars = 1 μm (A-E).

Symbiodinium microadriaticum Freudenthal, 1962
(Fig. 3, A-H; Fig. 4, A-D)

Symbiodinium microadriaticum Freudenthal, 1962, p. 45; Kevin et al., 1969, p. 341; Taylor, 1969, p. 336; 1971a, p. 232; 1974, p. 248, fig. 2; 1983, p. 19; Loeblich and Sherley, 1979, p. 195; Schoenberg and Trench, 1980a, p. 405; 1980b, p. 429; 1980c, p. 445; Colley and Trench, 1983, p. 61; Blank and Trench, 1985, p. 656; 1986, p. 286; Thin *et al.*, 1986, p. 178; Rowan and Powers, 1991a, p. 1349, fig. 3; 1992, p. 3639; Carlos, 1999, p. 1054, Table 1, Figs. 1-3.

Gymnodinium microadriaticum: Taylor, 1971b, p. 232.

Zooxanthella microadriatica: Loeblich and Sherley, 1979, p. 195.

Material examined: *Anthopleura kurogane*: Anmyeondo, 10 Feb. 2001, 1 ind. (HS Lim); Seogwipo, 19 Jan. 2000, 2 inds. (HR Cha); Yeosu, 13 Jan. 2000, 1 ind. (HS Lim); Yeosu, 31 Oct. 2000, 1 ind. (HS Lim); Yeosu, 13 Jan. 2001, 1 ind. (HS Lim). *Parasicyonis* sp.: Munseom, 22 Feb. 2001, 1 ind. (JI Song).

Description: Unicellular, located within endoderm of

host as coccoid cells, circular or spherical in shape, greenish brown at high irradiance, brown at low irradiance (10-20 m deep) and divided *in hospite* and in culture to produce two daughter cells. Mean diameter of algae 9.94 μm (\pm SD 0.95 μm , n = 40).

Cell surface smooth. Cell wall composed of four outer membranes, approximately 0.5 μm thick pellicle and pressed series of inner membranes associated with plasmalemma; pellicle with membrane bound vesicles near inner envelope.

Chloroplasts peripheral, multi-lobed, 36.43% (\pm SD 7.69%, n = 11) of volume of cell at high irradiance, 51.49% (\pm SD 7.64%, n = 12) of volume of cell at low irradiance, enclosed by one envelope comprised of three closely apposed membranes, no girdle lamellae and peripheral lamellae present; thylakoid oriented parallel to long axis of chloroplast; chloroplast stacked in groups of three lamellae and no grana; pyrenoid single, circular in shape, attached to chloroplast by one stalk, no invasive chloroplast thylakoids and surrounded by starch sheath.

Nucleus limited by one envelope composed of two membranes with nuclear pores, off-centered, spheroid, 16.40% (\pm SD 4.58%, n = 11) of volume of cell at high irradiance, 10.24% (\pm SD 2.24%, n = 12) of volume of cell at low irradiance, peripheral nucleolus and include

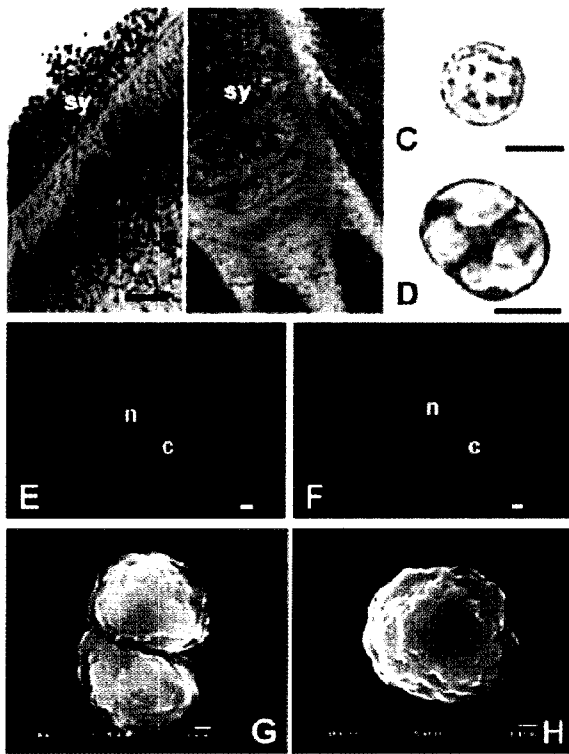


Fig. 3. Location and features of *Symbiodinium microadriaticum*. A, From *Anthopleura kurogane*; B, From *Parascyphis* sp.; C-D, Microscopic image *in hospite*. C, At high irradiance; D, At low irradiance; E-F, Fluorescent image of chloroplasts and nucleus *in hospite*. E, At high irradiance; F, At low irradiance; G-H, Surface feature *in hospite*. G, At high irradiance; H, At low irradiance. c, Chloroplast; n, nucleus; sy, symbiotic dinoflagellates. Scale bars = 100 μ m (A-B), 10 μ m (C-D), and 1 μ m (E-H).

chromosomes; chromosomes dinokaryon.

Mitochondria limited by double membranes, tubular type cristae, and elongated or spherical. Storage products starch, located in cytoplasm and around pyrenoid as a sheath. Trichocysts absent. Accumulation bodies, calcium oxalate crystals and dictyosomes present in cytoplasm. Lipid granules circular, located in cytoplasm and chloroplasts.

Remark: Freudenthal (1962), Loeblich & Sherley (1979), and Trench & Blank (1987) described motile cells of *Symbiodinium microadriaticum*, but motile cells have not been observed in this study.

Distribution: Korea (Yellow Sea, Korea Strait, Cheju Island area), the British Isles, Mediterranean, Caribbean, Great Barrier Reef.

Symbiodinium sp.
(Fig. 5, A-H)

Material examined: *Anthopleura midori*: Sinjindo, 2 July 2000, 1 ind. (HS Lim); Anmyeondo, 5 May 2001, 1 ind. (HS Lim).

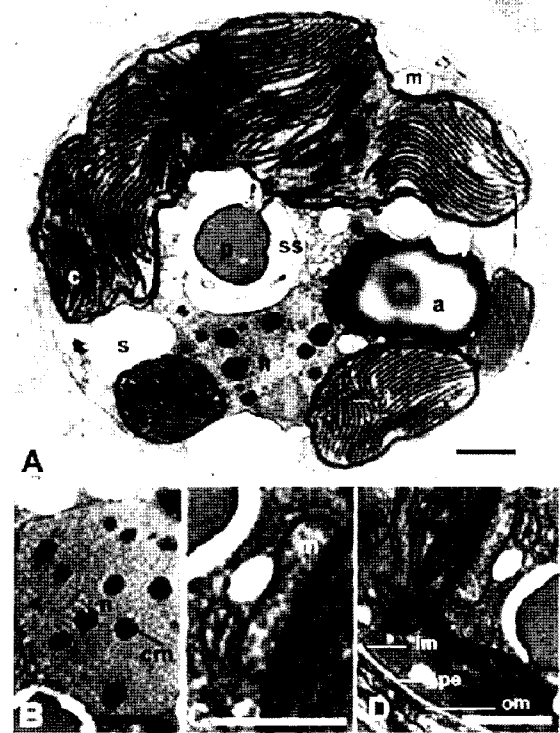


Fig. 4. Ultrastructure of *Symbiodinium microadriaticum*. A, *In hospite*. B, Nucleus and chromosome. C, Mitochondria. D, Cell wall. a, accumulation body; c, chloroplast; cm, chromosome; im, inner membrane; l, lipid droplet; m, mitochondria; n, nucleus; om, outer membrane; p, pyrenoid; pe, pellicle; s, storage product; ss, starch sheath; st, stalk; t, thylakoid. Scale bars = 1 μ m (A-D).

Description: Unicellular, coccoid cells, circular or spherical in shape and light green in color. Mean diameter of algae 9.13 μ m (\pm SD 1.52 μ m, n = 20).

Cell surface smooth and sleek. Cell wall composed of four outer membranes, approximately 0.048 μ m thick pellicle and pressed series of inner membranes; plasmalemma located under cell wall and big empty area located between inner membranes and plasmalemma.

Chloroplasts peripheral, lobed, 55.71% (\pm SD 14.9%, n = 10) of volume of cell, enclosed by one envelope comprised of three closely apposed membranes; thylakoids oriented parallel to long axis of chloroplast; chloroplast stacked in groups of three lamellae and no grana; pyrenoid single, oval in shape, attached to chloroplast by two stalks, no invasive chloroplast thylakoids and surrounded by starch sheath.

Nucleus limited by one envelope composed of two membranes with nuclear pores, off-centered, spheroid in shape, 16.51% (\pm SD 7.28%, n = 10) of volume of cell and include chromosomes; chromosomes dinokaryon.

Storage products starch, located in cytoplasm and around pyrenoid as a sheath. Trichocysts absent. Accumulation bodies, calcium oxalate crystals and lipid granules present; Lipid granules circular, located in cytoplasm and chloroplasts.

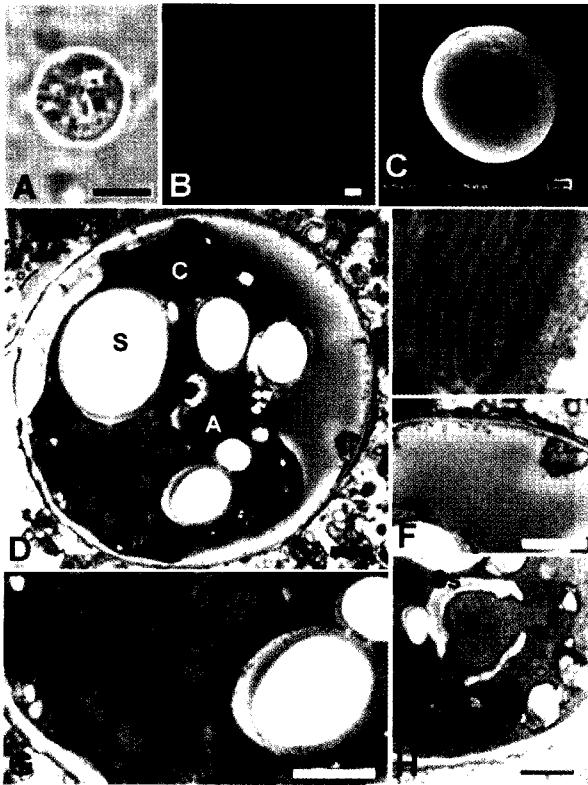


Fig. 5. Features of *Symbiodinium* sp. A, Light microscopic image in hospite. B, Fluorescent image of chloroplasts and nucleus in hospite. C, Surface feature in hospite. D, Ultrastructure in hospite. E, Chloroplast. F, Cell wall. G, Nucleus and chromosome. H, Pyrenoid. a, accumulation body; c, chloroplast; cm, chromosome; im, inner membrane; n, nucleus; om, outer membrane; p, pyrenoid; pe, pellicle; s, storage product; ss, starch sheath; st, stalk; sy, symbiotic dinoflagellates; t, thylakoid. Scale bars = 10 μm (A) and 1 μm (B-D, F-H).

Distribution: Korea (Yellow Sea).

Discussion

The three kinds of symbiotic dinoflagellates recorded in this study, *Symbiodinium kawagutii*, *S. microadriaticum* and *S. sp.* are spheroid in shape, and contains similar chloroplasts, mitochondria, nucleus arrangements and dinokaryon chromosomes. In addition, *S. kawagutii* and

S. microadriaticum change their color and volumes of chloroplasts depending on the intensity of irradiance (Table 2). However, these symbiotic dinoflagellates are different each other in size, *S. kawagutii* being the biggest and *Symbiodinium* sp. the smallest. Also, *S. microadriaticum* has just one stalk of pyrenoid. Moreover, *S. sp.* is the lightest in color, and the most sleek in surface. Furthermore, plasmalemma of *S. sp.* is separated from inner envelope.

These three species show characteristic differences in the volumes of chloroplast and nucleus. In the volume of chloroplast, *S. kawagutii* and *S. sp.* are larger than *S. microadriaticum*, and in the volume of nucleus, *S. sp.* is the largest and *S. microadriaticum* the smallest.

Even though Trench and Blank (1987), Loeblich and Sherley (1979), and Freudenthal (1962) described motile forms of *S. kawagutii* and *S. microadriaticum*, they were not observed in this study because of the difference in cultivating condition.

As a result of examination of marine invertebrates for the symbiotic dinoflagellates, *S. kawagutii* inhabited in *Anthopleura japonica*, *Alveopora japonica*, *Montipora verrucosa* and *Parasicyonis actinostoloides* (Table 3). *S. microadriaticum* was associated with *Aglaophenia helleri*, *Aiptasia* sp., *Cassiopeia* sp., *Anemonia sulcata*, *Anthopleura kurogane*, *Cereus pedunculatus*, *Condylactis aurantiaca*, *Condylactis* sp., *Parasicyonis* sp. and *Zoanthus* sp. *S. sp.* was found in host *Anthopleura midori*.

According to our former molecular study by Lanetty et al. (2001), symbiotic dinoflagellates of a part of *Alveopora japonica* and most of *Parasicyonis actinostoloides* were included in 'clade C' which was identified from *S. kawagutii* by Rowan and Powers (1991a) and Carlos (1999). Even though symbiotic dinoflagellates of *Anthopleura japonica* were considered as 'clade A' by Lanetty et al. (2001), they were expected as 'clade C' in the present study. This suggests that *Anthopleura japonica* could be associated with at least two different symbiotic dinoflagellates. Furthermore, symbiotic dinoflagellates of Korean *Anthopleura kurogane* included in 'clade A' based on the molecular work by Lanetty et al. (2001),

Table 2. Major characters in terms of intensity of irradiance of three symbiotic *Symbiodinium* dinoflagellates identified in the present study.

Characters	<i>S. microadriaticum</i>	<i>S. kawagutii</i>	<i>S. sp.</i>
Size (μm)	9.94 (n = 40)	11.47 (n = 60)	9.13 (n = 20)
Color	light green-brown	light green-brown	light green
At high irradiance	light green	light green	—
At low irradiance	brown	brown	—
Cell surface	smooth	smooth & grooved	sleek
Relationship of plasmalemma and inner membrane	associated	associated	isolated
Vol. of chloroplasts in cell (%)	45.46 (n = 23)	55.71 (n = 31)	55.71 (n = 10)
At high irradiance	36.43 (n = 11)	38.96 (n = 11)	—
At low irradiance	51.49 (n = 12)	64.92 (n = 20)	—
Vol. of nucleus in cell (%)	12.51 (n = 23)	13.51 (n = 31)	16.51 (n = 10)
At high irradiance	16.40 (n = 11)	12.1 (n = 11)	—
At low irradiance	10.24 (n = 12)	14.37 (n = 20)	—
No. of stalks	1	2	2

Table 3. The hosts of Korean anthozoans' and other invertebrates associated with three symbiotic dinoflagellates

Symbiotic dinoflagellates	Host species		
	Korean anthozoans	Other invertebrates	References
<i>Symbiodinium microadriaticum</i>	<i>Anthopleura kurogane</i> <i>Parasicyonis</i> sp.	<i>Aglaophenia helleri</i> <i>Aiptasia</i> sp. <i>Cassiopeia</i> sp. <i>Anemonia sulcata</i> <i>Cereus pedunculatus</i> <i>Condylactis aurantiaca</i> <i>Condylactis</i> sp. <i>Zoanthus</i> sp.	Taylor, 1969 Taylor, 1969 Freudenthal, 1962 Taylor, 1969 Taylor, 1969 Taylor, 1969 Taylor, 1969 Thinh, 1986
<i>S. kawagutii</i>	<i>Anthopleura japonica</i> <i>Parasicyonis actinostoloides</i> <i>Alveopora japonica</i> <i>Anthopleura midori</i>	<i>Montipora verrucosa</i>	Trench and Blank, 1987
<i>S. sp.</i>			

and *S. microadriaticum* was defined as 'clade A' by Rowan and Powers (1991a) and Carlos (1999). As a result, the identification of these symbiotic dinoflagellates is suggested to be better performed through both morphological and molecular studies.

Acknowledgement

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