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Feeding specificity and photosynthetic activity of Korean sacoglossan mollusks

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During feeding on algal cytoplasm, some sacoglossans are known to keep the chloroplasts photosynthetically active for days to months in their digestive cells. Korean sacoglossan mollusks containing functional chloroplasts were screened using an *in vivo* chlorophyll fluorescence measuring system (pulse amplitude modulation, PAM). We collected six sacoglossans feeding on siphonous and siphonocladous green algae (*Elysia atroviridis, E. nigrocapitata*, *E. ornata, Ercolania boodleae, Placida dendritica, Stiliger* sp.) and one feeding on ceramiaceaen algae (*Stiliger berghi*) and performed feeding experiments using 37 algal species. Three species of *Elysia* showed strong photosynthetic activity for months. However, *P. dendritica* maintained functional chloroplasts only for several hours after feeding. *E. boodleae, S. berghi*, and *Stiliger* sp. showed no photosynthetic activity in any circumstances. Among all species, *E. nigrocapitata* was capable to tolerate the longest period of starvation for over 4 months. Four 'solar powered' sacoglossans bonded avidly to their specific algal food. Each species attached to and consumed only one algal species when several algae were given together. While they occasionally consumed other algae after prolonged starvation, they always reverted to their specific algae when available.

Key Words: *Elysia*; feeding specificity; green algae; kleptoplast; *Placida*

INTRODUCTION

Sacoglossan mollusks are suctorial herbivores that live on the cell sap of coenocytic macroalgae (Jensen 1993). As an adaptation for suctorial feeding, these sea slugs have uniserial radula and show narrow specificity for their algal diet (Händeler and Wägele 2007). With few exceptions, algae that have been identified as food for sacoglossan sea slugs belong to the class Ulvophyceae sensu Floyd and O'Kelly (1990). However, food organisms are not known for many sacoglossans yet.

Some sacoglossan mollusks incorporate algal chloroplasts inside their cells, and the incorporated chloroplasts are functional for days to months, depending on the species. Trench et al. (1972) provided conclusive evidence that photosynthetic assimilates are released from the chloroplasts in the sea slug cells. This incorporation and maintenance of foreign chloroplasts is known as kleptoplasty (e.g., Clark et al. 1990). It has long been suspected that genes have been transferred from algae to the sea slugs, because many components of photosystems in active algal plastids are very short-lived, with turnover

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times especially under bright light condition (Schuster et al. 1988, Vass et al. 1992, Warner et al. 1999). Thus, if the plastids are maintained in an active state for weeks and months, it is logical to assume that the mollusks could produce necessary proteins to keep the photosynthetic activity because the photosynthetic proteins in chloroplasts are turned over quickly (often in less than a day). Recently, accumulating evidences show that algal nuclear genes have been laterally transferred to these sacoglossan species (Rumpho et al. 2001, 2008, Pierce et al. 2007, Schwartz et al. 2010).

Sacoglossan mollusks bind avidly to their food algae. In most cases the sacoglossan mollusks eat less than ten species of algae throughout their life cycle, and the chloroplasts from only one or two species could be sequestered and maintained in the sea slug cells. This feeding specificity is also necessary for the development of successful lateral gene transfer which might render the acquired chloroplast photosynthetically active for long time.

Here, we screened for Korean sacoglossans containing functional chloroplasts using an *in vivo* chlorophyll fluorescence measuring system (pulse amplitude modulation, PAM). The feeding specificity of the sea slugs was tested using 37 species of cultured algae.

MATERIALS AND METHODS

Sea slug collection, identification and culture

The sacoglossans (Table 1, Fig. 1) were collected monthly from Wando and Gangneung, Korea from July 2009 until October 2010. They were identified following Koh (2006) and Internet resources on the subject (Sea slug forum 2010). The animals were kept in IMR medium (Klochkova et al. 2006) in 9 × 5 cm Petri dishes at 15-20°C in 12 : 12 h L : D cycles with 15 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent bulbs.

Feeding experiment

The algae used in the feeding experiment, their collection site or source and conditions for laboratory culture are given in Table 2. All algae were grown in IMR medium as described above. While performing feeding experi-

Table 1. Sacoglossans identified in this study

Species	Algal diet in the field[a]	Number of individuals per sampling site	F_v/F_m [b]	Peak of population
Elysia atroviridis Baba[c]	*Bryopsis* spp.	100 (Wando)	0.31-0.76	January-March
Elysia nigrocapitata Baba[d]	*Cladophora sakaii* *Bryopsis* spp.	272 (Wando)	0.53-0.67	October
Elysia ornata Swainson[c]	*Bryopsis* spp.	1 (Wando)	0.54	-
Ercolania boodleae Baba[e]	*Chaetomorpha moniligera*, *Bryopsis* spp.	4 (on *B. plumosa*, Wando) 10 (on *C. moniligera*, Gangneung)	0	April-June
Placida dendritica	*Bryopsis* spp., *Codium hubsii*, *Codium fragile*	305 (on *Bryopsis* spp., Wando) 1 (on *C. hubsii*, Gangneung) 9 (on *C. fragile*, Wando)	Fed on *B. plumosa* before assay: 0.12 Fed on *Codium* spp. or *D. tenuissima* before assays: 0 Fed on *B. plumosa* and then starved for 6-12 h before assay: 0	July-mid October
Stiliger berghi Baba[e]	*Polysiphonia japonica* var. *forfex*	5 (Wando)	0	August
Stiliger sp.[e]	*Cladophora sakaii*	3 (Wando)	0	August

[a]Algal species from which the sea slugs were collected were considered as primarily eaten in the field.
[b]F_v/F_m in intact plants of *Bryopsis plumosa*, *Codium minus*, and *Derbesia tenuissima* (sporophytes) were over 0.8 (control).
[c]Animals starved for 1 week before the assay.
[d]Animals starved for 1.5 months before the assay.
[e]Animals fed until the assay.

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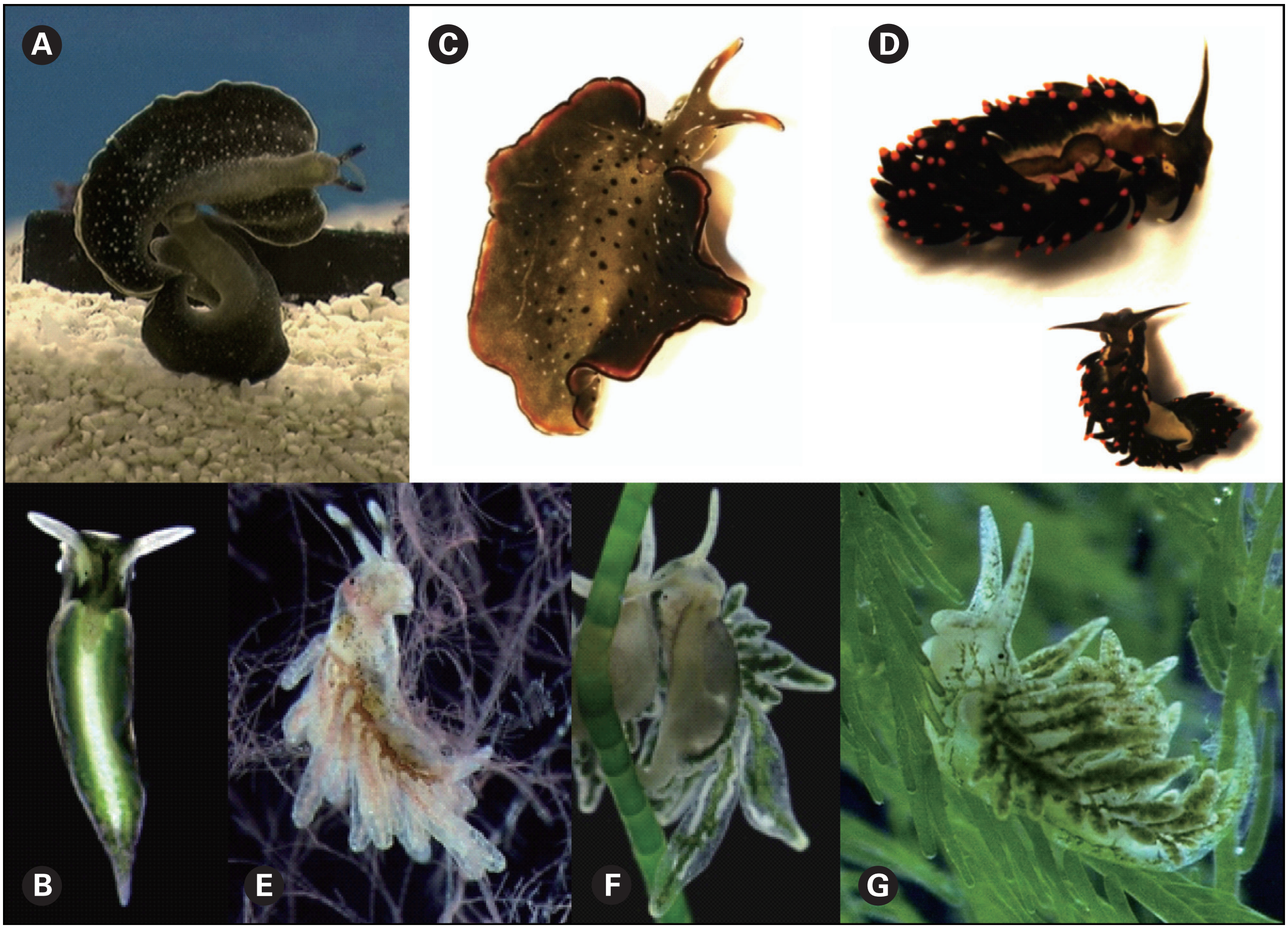

Fig. 1. Sacoglossans identified in this study. (A) *Elysia atroviridis*. (B) *Elysia nigrocapitata*. (C) *Elysia ornata*. (D) *Ercolania boodleae*. (E) *Stiliger berghi*. (F) *Stiliger* sp. (G) *Placida dendritica*.

ments for *Elysia atroviridis* and *E. ornata*, each potential algal food was given to sea slugs for a period of 10-14 days. If the algae were completely or partially consumed during that time, other tested algae were added. However, if a particular algal species was not consumed, it was considered non-eatable and was replaced with another algal species. In case of *E. nigrocapitata*, the longest starvation period exceeded 4 months. In case of *Ercolania boodleae*, *Placida dendritica*, *Stiliger berghi*, and *Stiliger* sp. the starvation time was 2-7 days, since they do not cope well with longer starvation times. Chlorophyll fluorescence measurements were performed *in vivo* in the fed and starved sea slugs using Diving-PAM Chlorophyll *a* Fluorometer (Walz, Effeltrich, Germany).

Phylogenetic analyses in *Placida dendritica*

Genomic DNA was isolated using a genomic DNA isolation kit following the manufacturer's instructions (Intron Biotech, Seoul, Korea). Polymerase chain reaction (PCR) was performed using primers displayed in Table 3, with an initial denaturation at 95°C for 3 min, followed by 35 cycles of amplification (denaturation at 95°C for 20 s, annealing at 55°C for 30 s and extension at 72°C for 1 min and 1.5 min for 16S and 28S rDNA, respectively) with a final extension at 72°C for 10 min. Amplified PCR products were purified using a gel extraction kit (Qiagen, Valencia, CA, USA) and cloned into a T-easy vector (Promega, Madison, WI, USA) to determine DNA sequences. The phylogenetic analyses were performed using sequences for *Placida* spp. (Fig. 2) obtained from GenBank (NCBI 2010). *Alderia* spp. (*A. willowi* and *A. modesta*) and *Ercolania fuscata* were used as outgroups. The DNA sequences were aligned using ClustalW version 1.6 matrix (European Bioinformatics Institute, Cambridge, UK) at 50% of delay divergent cut off and the obtained alignments were manually refined. Maximum parsimony (MP) analyses were performed using Windows based program, MEGA

Table 2. List of algae used in the slug feeding experiment, their collection site and conditions for laboratory culture, and results of sacoglossan feeding experiment

No	Species	Temperature for algal laboratory culture	Collection site/Source and date (mo, y)	Sacoglossan						
				Elysia atroviridis	*Elysia nigrocapitata*	*Elysia ornata*	*Ercolania boodleae*	*Placida dendritica*	*Stiliger berghi*	*Stiliger* sp.
1	*Aglaothamnion byssoides* (Arnott *ex* Harvey) Boudouresque *et* Perret-Boudouresque (♂)	15	UTEX culture collection	-	-	-	-	-	+	-
2	*Aglaothamnion* sp. (♂) (hybrid of *A. oosumiense* + *A. callophyllidicola*)	15	Daecheon (Korea)/09.2003. (*A. oosumiense*, *A. callophyllidicola*)	-	-	-	-	-	-	-
3	*Antithamnion densum* (Suhr) Howe (♀)	15	Gangneung (Korea)/07.1986.	-	-	-	-	-	+	-
4	*A. glanduliferum* Kylin (♂)	15	UTEX culture collection	-	-	-	-	-	+	-
5	*A. nipponicum* Yamada *et* Inagaki (♂)	15	Gacheon (Korea)/05.1995. Daecheon (Korea)/09.2003.	-	-	-	-	-	-	-
6	*Audouinella* sp.	20	JWc (4243)	-	-	-	-	-	-	-
7	*Bostrychia tenuissima* King *et* Puttock	20	JWc (3663)	-	-	-	-	-	-	-
8	*Dasya sinicola* (Setchell *et* Gardner) Dawson	15	JWc (646)	-	-	-	-	-	-	-
9	*Dasysiphonia chejuensis* Lee *et* West	15	Jeju Island (Korea)/05.2003.	-	-	-	-	-	-	-
10	*Griffithsia monilis* Harvey	15	JWc (3435)	-	-	-[b]	-	-	-	-
11	*Gracilaria chilensis* Bird, McLachlan *et* Oliveira	20	JWc (3948)	-	-	-	-	-	-	-
12	*Heterosiphonia japonica* Yendo	15	Wando (Korea)/09.2004.	-	-	-	-	-	-	-
13	*Polysiphonia japonica* var. *forfex* (Harvey) Yoon[a]	15	Wando (Korea)/08.2009.	-	-	-	-	-	+	-
14	*Pterocladia capillacea* (Gmelin) Bornet[a]	15	Wando (Korea)/10.2009.	-	-	-	-	-	-	-
15	*Rhodosorus marinus* Geitler	20	JWc (4026)	-	-	-	-	-	-	-
16	*Anadyomene stellata* (Wulfen) Agardh	20	JWc (1573)	-	-	-	-	-	-	-
17	*Bryopsis corticulans* Setchell	15-20	Wando (Korea)/05.2006.	+	+	+	-	+	-	-

18	*B. corymbosa Agardh*	15-20	Peter the Great Bay, Japan Sea (Russia) / 09.2003.	+	+	+	-	+	-	-
19	*B. indica* Gepp *et* Gepp[a]	15-20	Wando (Korea) / 07.2009.	+	-	+	-	+	-	-
20	*B. maxima* Okamura *ex* Segawa	15-20	Wando (Korea) / 02.2008.	+	+	+	-	+	-	-
21	*B. muscosa* Lamouroux	15-20	Gangneung (Korea) / 10.2007.	+	+	+	-	+	-	-
22	*B. plumosa* (Hudson) Agardh	15-20	Gacheon (Korea) / 04.2003.	+	+	+	+[c]	+	-	-
23	*Bryopsis* sp.	15-20	Jeju Island (Korea) / 10.2007.	+	-	+	-	+	-	-
24	*Derbesia tenuissima* (Moris *et* De Notaris) Crouan *et* Crouan (vesicular gametophytes)	20	JWc (4303)	-	-	+	-	-	-	-
25	*D. tenuissima* (sporophyte)	20	JWc (4303)	+	+	+	-	+	-	-
26	*Derbesia* sp. (sporophyte)	20	JWc (4419)	+	-	+	-	+	-	-
27	*Chaetomorpha moniligera* Kjellman	15		-	+	-	+	-	-	+
28	*Cladophora japonica* Yamada[a]	15	Wando (Korea) / 10.2009.	-	-	-	-	-	-	-
29	*C. sakaii* Abbott[a]	15	Wando (Korea) / 10.2009.	-	+	-	-	-	-	+
30	*Codium fragile* (Suringar) Hariot[a]	15	Wando (Korea) / 07.2009, 10.2009.	-	-	-	-	+	-	-
31	*C. minus* (Schmidt) Silva	20	Gampo (Korea) / 07.2007.	-	-	+	-	+	-	-
32	*Microdictyon umbilicatum* (Velley) Zanardini	15-20	JWc (3674)	-	-	-	-	-	-	-
33	*Phyllodictyon orientale* (Gepp *et* Gepp) Kraft *et* Wynne	20	1631	-	+	-	-	-	-	-
34	*Ulva fenestrata* Postels *et* Ruprecht[a]	15	Wando (Korea) / 07.2009, 10.2009.	-	-	-	-	-	-	-
35	*U. linza* Linnaeus[a]	15	Wando (Korea) / 07.2009, 10.2009.	-	-	-	-	-	-	-
36	*Valonia* sp.	20	JWc (1528)	-	-	-	-	-	-	-
37	Planktonic pennate diatom	20	Wando (Korea) / 07.2009, 10.2009.	-	-	-	-	-	-	-

JWc: isolates obtained from culture collection of Dr. John A. West (West 2010).
[a] Field-collected plants.
[b] The animal punctured several cells in the filament, but obviously did not eat.
[c] Only species of *Bryopsis* consumed by *E. boodleae.*

version 4.1 (MEGA 2007, Tamura et al. 2007, Kumar et al. 2008). The MP analyses were tested for robustness by bootstrapping with 1,000 replications.

RESULTS

Elysia atroviridis (Fig. 1A)

This species was reported from Korea by Koh (2006) under the name *E. flavomacula* Jensen. The latter name, however, was regarded as a synonym of *E. atroviridis* (Sea slug forum 2010).

Diet. The animals ate little but continuously when food was available. In the field, they were always found on *Bryopsis* spp. In the laboratory, they fed specifically on *Bryopsis* spp. They also fed on the sporophytes of *Derbesia tenuissima,* but went back to *Bryopsis* spp. when the algae were available (Table 2). Prolonged periods of starvation of up to 2 months could be tolerated.

Seasonality. This species appeared in Wando in December and had peak of population from January to mid

Table 3. Oligonucleotide primers used for amplification of 16S and 28S rDNA

Gene	Primer	Sequence	Reference
16S rDNA	16S1	CGCCTGTTTATCAAAAACAT	Händeler et al. 2009
	16S2	CCGGTCTGAACTCAGATCACGT	Maeda et al. 2010
28S rDNA	28SD1-D3F1	ACCCGCTGAATTTAAGCA	Händeler et al. 2009
	28SD1-D3R1	GACGATCGATTTGCACGTCA	Maeda et al. 2010

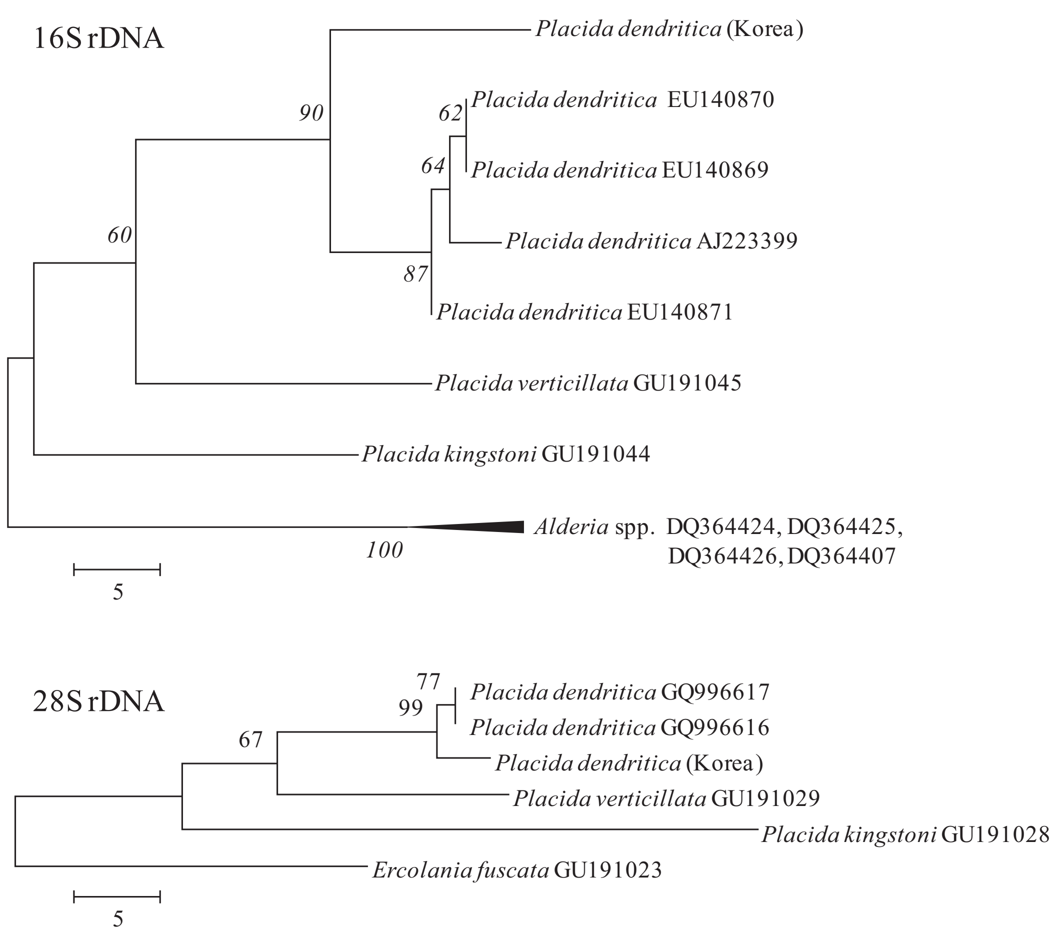

Fig. 2. Phylogenetic analyses in *Placida dendritica*.

March. During this period, it was possible to collect over 40 animals during one survey. In other months of the year, this species was not detected at this site.

***In vivo* chlorophyll fluorescence assay.** In animals starved for 1 week, F_v/F_m was 0.31-0.76, which represented 38.7-95% of the chlorophyll fluorescence values in intact plants of *B. plumosa*.

Elysia nigrocapitata (Fig. 1B)

This species was reported from Japan and Hong Kong (Sea slug forum 2010). This is the first report of this species in Korea. Our specimens ranged from several mm to 1.7 cm in length, and ranged in color from light to very dark green when fed and pale grayish-yellow when starved. Parapodia were leaf-like when flattened, with a wide black band along the reverse side edges and thin white band along the upper side edges. The head was black, while the rhinophores and areas around eyespots were white.

Diet. In the field, the animals fed on *Cladophora sakaii*. In laboratory culture, they switched to feed on *Chaetomorpha moniligera* and *Phyllodictyon orientale*. Some animals (< 30%) switched to feed on *Bryopsis* spp. and sporophytes of *Derbesia* (Table 2) after 4 month-long starvation. Species *C. moniligera* and *P. orientale* did not grow in the sampling site of *E. nigrocapitata*.

Seasonality. In October, 58-214 animals were easily collected from a sampling site in Wando. However, from late November to mid March, only 2-6 animals were collected from the same site during each survey. In other months of the year, this species was absent from this site.

***In vivo* chlorophyll fluorescence assay.** In animals starved for 1.5 months, F_v/F_m was 0.53-0.67, which represented 66.2-83.7% of the chlorophyll fluorescence values in intact coenocytic green algal thalli.

Elysia ornata (Fig. 1C)

This species was reported from Jeju Island by Koh (2006). We herein extend knowledge on its distribution on the southern coast of Korea.

Diet. The animal ate continuously when food was available. In the field it was found on *B. plumosa*. In laboratory, it fed specifically on *Bryopsis* spp. and vesicular gametophytes of *D. tenuissima* (Table 2).

Seasonality. During the course of this study, we found 1 animal from Wando site on October 4, 2009 and were unable to find more specimens from the same sampling site at other times during that year.

***In vivo* chlorophyll fluorescence assay.** In animal starved for 1 week, F_v/F_m was 0.54, which represented 67.5% of the chlorophyll fluorescence values in intact plants of *B. plumosa*.

Ercolania boodleae (Fig. 1D)

Ten animals were found on *C. moniligera* from Gangneung and 4 animals were found in-between rhizoids of *B. plumosa* attached epiphytically to the red alga *Plocamium* sp. from Wando. It is noteworthy that *Chaetomorpha* spp. do not grow in that locality in Wando. *E. boodleae* is a common sacoglossan species in the Peter the Great Bay (Russia, Sea of Japan) (Minichev 1976). Martynov (1997) listed this species for the intertidal zone of South Sakhalin (Sea of Okhotsk). This species was reported from Jeju Island and eastern coast of Korea (Koh 2006). We herein extend knowledge on its distribution on the southern coast of Korea.

Diet. The animals were fed on *C. moniligera* in the laboratory and ate continuously when food was available. Two 4-5 mm long animals consumed a 20 cm-long *Chaetomorpha* filament within 6 h. The animals collected from Wando consumed *B. plumosa* occasionally, however those collected from Gangneung fed only on *C. moniligera* (Table 2). Short periods of starvation (4-7 days) could be tolerated.

Seasonality. In Gangneung, this species was more abundant in spring and the early summer months, correlating with the abundance of *C. moniligera*. In Wando, we found 4 animals on October 4, 2009 and were unable to find any more specimens from this site in other months of the year.

***In vivo* chlorophyll fluorescence assay.** In animals fed until the analysis, F_v/F_m was 0, implying that algal protoplasm and chloroplasts were digested/damaged after consumption.

Placida dendritica (Fig. 1G)

Since a number of very similar-looking taxa around the world have been identified as *P. dendritica* (Sea slug forum 2010), we subjected our specimens to 16S and 28S rDNA analyses. The animals from Wando showed affinity with an isolate from Spain identified as *P. dendritica*. However, it differed from it in both 16S and 28S rDNA (Fig. 2). The taxonomic re-appraisal of Korean species will be necessary when more sequences are available in the GenBank, however at this time we called it *P. dendritica* based on morphology. Our specimens were aeolid-like in shape; the anterior surface was covered with cerata down each side of the body. The body was dirty white, fairly transparent, and the color of food in the digestive system showed through the body.

This species is a new record for Korean sacoglossan fauna, although very common and distributed all around the Korean peninsula. In north Atlantic waters, *P. dendritica* is commonly found associated with *Codium fragile* (Evertsen and Johnsen 2009). During the course of this study, we collected 305 animals from various *Bryopsis* species. Thus, this species is more associated with *Bryopsis* spp. in Korea. It is noteworthy that *C. fragile* and *C. tomentosum* were very abundant in Wando sampling site, but *P. dendritica* was found mainly on *Bryopsis* plants. We found only 10 animals feeding on *Codium* spp. in the field (Table 1).

Diet. The animals ate continuously when *Bryopsis* spp. were available. In the laboratory, approximately 10% of animals switched to *C. minus* or *Derbesia* sporophytes when no *Bryopsis* spp. were available (Table 2), but went back to *Bryopsis* spp. as soon as they were provided. In the absence of food, the green coloration turned brownish within 2-3 days and then faded. The animals usually died after 10-14 days of starvation.

Seasonality. This species had peak of population in summer and early autumn. From July to mid October, it was possible to collect 50-100 animals from several plants of *Bryopsis* spp. during one survey. From December to March, the population declined, so that only 10-12 animals were collected from over 100 large plants of *Bryopsis* spp. But the host algae *Bryopsis* spp. were very abundant in winter months when sea slugs were reduced in number and were less abundant in summer and early autumn.

***In vivo* chlorophyll fluorescence assay.** A difference in F_v/F_m values was observed depending on an algal diet. In animals continuously fed on *B. plumosa* until the assays, F_v/F_m was 0.12, but it abruptly reduced to 0 within 6-12 h after feeding. In animals continuously fed on *C. minus* and *D. tenuissima*, F_v/F_m was always 0.

Stiliger berghi (Fig. 1E)

This species is a new record for Korean sacoglossan fauna. It is known from Japan and Peter the Great Bay, Russia (Sea slug forum 2010). We herein extend knowledge on its distribution on the southern coast of Korea. Our specimens were aeolid-like in shape; the anterior surface was covered with irregularly arranged and irregularly sized cerata down each side of the body. The body was slightly yellowish to dirty white, fairly transparent, and the color of food in the digestive system showed through the body wall. The rhinophores were tapering and cylindrical, with a dark band about one-third of the way down from the tip.

Diet. This species has been reported to live and feed on the red algae, especially *Neorhodomela larix*. In the field, our specimens fed on *Polysiphonia japonica* var. *forfex*. In laboratory culture, they switched to feed on *Antithamnion densum, A. glanduliferum* and *Aglaothamnion byssoides* (Table 2). Short periods of starvation (4-7 days) could be tolerated.

Seasonality. During the course of this study, we found 5 animals from Wando on August 10, 2009 and were unable to find more specimens from the same sampling site in other months of the year.

***In vivo* chlorophyll fluorescence assay.** In animals continuously fed on the red ceramiaceaen algae until the assays, F_v/F_m was 0, implying that algal protoplasm and chloroplasts were digested/damaged after consumption.

Stiliger sp. (Fig. 1F)

This species is a new record for Korean sacoglossan fauna. Three animals were found on *C. sakaii* from Wando. They were aeolid-like in shape and 6-7 mm long. The anterior surface was covered with irregularly arranged and irregularly sized cerata down each side of the body. The body was dirty white, fairly transparent, and the color of food in the digestive system showed through the body wall.

Diet. In the field, the animals fed on *C. sakaii*. In laboratory culture, they switched to feed on *C. moniligera* (Table 2), but did not eat it continuously and did not consume all the protoplasm from the damaged *Chaetomorpha* cells. It allowed *C. moniligera* cells to regenerate or make intracellular protoplasts.

Seasonality. During the course of this study, we found 3 animals from Wando on October 4, 2009. No other specimens were recovered from the same sampling site in other months of the year.

***In vivo* chlorophyll fluorescence assay.** In animals continuously fed on *C. moniligera* until the assays, F_v/F_m was 0, implying that algal protoplasm and chloroplasts were digested/damaged after consumption.

DISCUSSION

During the course of this study, we collected 7 sacoglossan species from the same site in Wando where *B. plumosa* grows as a dominant species all year round, except in July and August. *E. atroviridis*, *E. nigrocapitata*, and *P. dendritica* were the most abundant sacoglossans in that area. *In vivo* chlorophyll fluorescence analysis indicated that all three species of *Elysia* possessed active chlorophylls and, thus, photosynthetically functional chloroplasts even after prolonged starvation. Considering that these species belong to sacoglossans, the results suggest that they have kleptoplasts (Yamamoto et al. 2009). Thus, our results add two more potential species, *E. atroviridis* and *E. nigrocapitata*, to the list of sacoglossans that are known to possess functional chloroplasts (e.g., Yamamoto et al. 2009). Moreover, in *E. atroviridis* and *E. nigrocapitata* the kleptoplasty seems to be long-term (for over 1 month to 4 months). To date, only four species are known to perform long-term maintenance of acquired plastids, including *E. chlorotica, E. timida, E. crispata,* and *Plakobranchus ocellatus* (Wägele et al. 2010).

Kawaguti and Yamasu (1965) described functional chloroplasts of *C. fragile* in the 'hepatic diverticula' of *E. atroviridis* by detailed investigation through electron microscopy, but did not present data to support the possibility of an endosymbiotic relationship. More detailed studies using transmission electron microscopy analysis and PAM measurements will be necessary to fully understand kleptoplasty by *E. atroviridis* and *E. nigrocapitata*.

In the case of *P. dendritica* fed on *Bryopsis*, a decrease of F_v/F_m was observed within 6-12 h after feeding, while in animals fed on *Derbesia* and *Codium*, F_v/F_m was always 0. Yamamoto et al. (2009) reported that *Placida* sp. fed on *Codium* showed a F_v/F_m value of 0.48, although it survived for less than a week during starvation. On the contrary, Evertsen and Johnsen (2009) reported that *P. dendritica* fed on *C. fragile* until assays did not have any photosynthetic responses. More studies will be necessary to confirm *in vivo* chloroplast functionality according to algal diet in *Placida*.

Also, Yamamoto et al. (2009) reported a high F_v/F_m value of 0.6 in *Stiliger ornatus*; however in our study, *S. berghi* and *Stiliger* sp. were negative species, together with *E. boodleae*.

In sea slugs feeding on green algae, the diet was restricted to either siphonous or siphonocladous species, especially *Bryopsis* spp. *Elysia nigrocapitata* could switch from its primary diet (siphonocladous green algae) to *Bryopsis* after being starved for 4 months, and *E. boodleae* occasionally fed on *B. plumosa* instead of *Chaetomorpha*. Considering that in the field three sacoglossans were constantly feeding on *B. plumosa* and two species could also feed on it occasionally, one might expect a rapid shortage of food resources from a small sampling site. On the contrary, *B. plumosa* was very abundant in that sampling site, despite the seasonal abundance of particular sea slug species. *Bryopsis* spp. are known for their ability to regenerate viable plants from protoplasm extruded in seawater upon wounding (Tatewaki and Nagata 1970, Pak et al. 1991, Kim et al. 2001). Protoplast formation in *Bryopsis* was interpreted as a method of propagation because one branch of a plant could generate hundreds of viable new cells spontaneously in seawater (Kim et al. 2001). Thus, grazing by sacoglossans might have been beneficial for *Bryopsis* abundance in that area. It is noteworthy that all the green algal species specifically consumed by these mollusks have ability of protoplast regeneration (Klotchkova et al. 2003, Kim and Klotchkova 2004). Wounding in *Chaetomorpha* can also trigger the development of aplanospores or swarmers (Klotchkova et al. 2003). Thus, the appearance of sea slugs feeding on *Chaetomorpha* could also contribute to its propagation to some extent. More ecological studies will be necessary to elucidate the symbiotic nature among the sea slugs and algae involved in this specific feeding and kleptoplasty.

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