

Research Article

Algae 2019, 34(4): 303-313

<https://doi.org/10.4490/algae.2019.34.12.10>

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Epibionts associated with floating *Sargassum horneri* in the Korea Strait

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Floating seaweed rafts are a surface-pelagic habitat that serve as substrates for benthic flora and fauna. Since 2008, *Sargassum horneri* clumps have periodically invaded the Korea Strait. In this study, the polymerase chain reaction-free small-organelles enriched metagenomics method was adopted to identify the species of epibiotic eukaryotes present in floating *S. horneri* fronds. A total of 185 species were identified, of which about 63% were previously undetected or unreported in Korean waters. The rafts harbored a diverse assemblage of eukaryotic species, including 39 Alveolata, 4 Archaeplastida, 95 Opisthokonts, 4 Rhizaria, and 43 Stramenopiles. Of these 185 taxa, 48 species were found at both *Sargassum* rafts collection stations and included 24 Stramenopiles, 17 Alveolata, and 7 Opisthokonts. Among these, the highest proportion (50%) of species was photo-autotrophic in basic trophic modes, while the proportion of phagotrophic, osmo- or saprotrophic, and parasitic modes were 43.8%, 4.2%, and 2.1%, respectively. This study demonstrates the contribution of floating *Sargassum* rafts as dispersal vectors that facilitate the spread of alien species.

Key Words: alien species; floating seaweed; Korea Strait; *Sargassum horneri*; small-organelles enriched metagenomics (SoEM); trophic mode

INTRODUCTION

Sargassum horneri rafts, which were sparsely detected in the open ocean in the past century, have periodically invaded the Korea Strait since 2008 (Kim et al. 2019). They now comprise the vast majority of marine debris in the Korea Strait, Yellow Sea, and East China Sea. These large biotic rafts that can support an extensive pelagic habitat are far from uncommon but few studies have focused on these and there exist no insights on the diversity of their epibiotic organisms. The most common floating brown alga, *S. muticum* (Yendo) Fensholt, has high fecundity

but cannot reproduce vegetatively. Therefore, the presence of conceptacles bearing antheridia and oogonia as well as air-bladders in this monoecious species has proven to be a great advantage for its successful dispersal to appropriate habitats (Strong et al. 2006). In contrast, *S. horneri* (Turner) C. Agardh can propagate through both germlings and vegetative reproduction and branches separated from a shoot will continue to grow into a new fronds (Uchida 1993).

S. horneri (hereafter *Sargassum*) is distinct from other



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Received October 5, 2019, Accepted December 10, 2019

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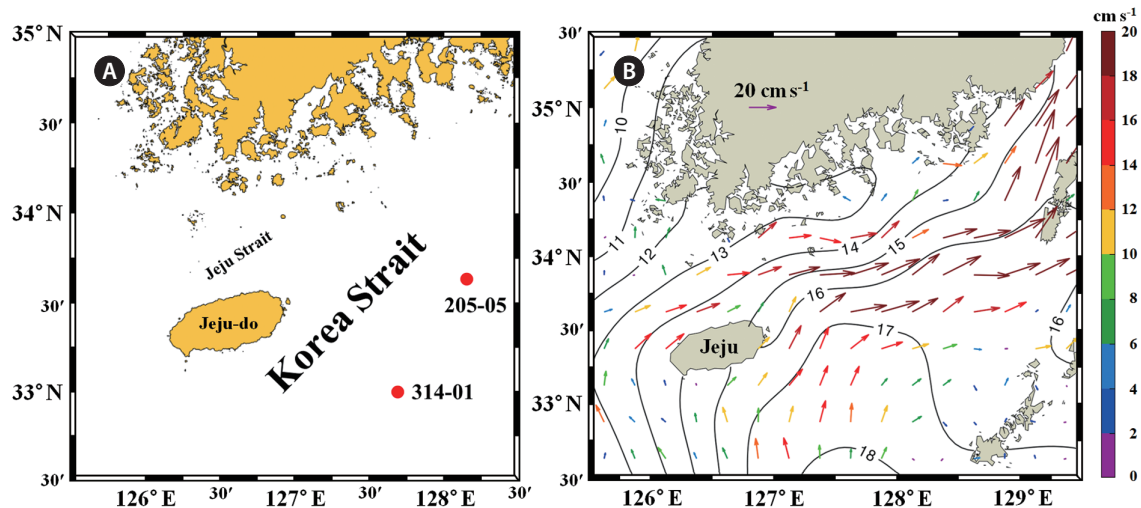


Fig. 1. Sampling stations (205-05 and 314-01) in the Korea Strait (A) and mean surface current from surface drifter observations (B). Contours in (B) are monthly mean sea surface temperature (°C) in April 2017.

congeneric species due to its phylogenetic distinctness (Stiger et al. 2003) and its unique morphology, such as its long main stem, long-elliptical bladders with a dentate coronal leaf, and conspicuous terete receptacles (Yoshida 1983). This species is an important component of benthic assemblages on the rocky shores of intertidal to subtidal zones in temperate eastern Asia (Choi et al. 2003, Pang et al. 2009, Watanabe et al. 2019). It grows very rapidly, reaching height of 2-5 m from winter to summer, and is recognized for variations in its reproductive traits, such as reproductive phenology and sexuality (Watanabe et al. 2019). The rapid growth rates and life history characteristics of *Sargassum* may explain its success as an invasive species, as it has proven to be in Southern California (Kaplanis et al. 2016).

Driven by currents and winds, a buoyant seaweed, such as *Ascophyllum nodosum* (Linnaeus) Le Jolis, may transport associated organisms over distances as long as at 5,500 km (Macaya et al. 2016). Floating seaweeds have played host to a wide range of eukaryotic organisms in the pelagic environment by providing a substratum for adherence, a food source, and a refuge during travel (Thiel and Gutow 2005a, 2005b, Tala et al. 2019). In addition, floating rafts can support the growth of epibionts (cyanobacteria, fungi, macroalgae, protists, and invertebrates) and their transfer to new habitats, thereby introducing alien species and posing a threat to the local biodiversity and economy (Carlton and Geller 1993, Masó et al. 2003, Thiel and Gutow 2005a, 2005b, Zettler et al. 2013, Carlton et al. 2017, Larsson et al. 2018). Such epibiotic organisms can also have a potentially detrimental effect on the growth and fitness of their host species.

The surveys of seaweed-associated assemblages that have been conducted so far have been geographically restricted and did not provide for a wide range of taxonomic groups. A wide range of eukaryotic taxa in the pelagic rafting assemblages, particularly *Sargassum*-associated, remains unexplored, and fungi, macroalgae, protists and invertebrates may be present. A newly established polymerase chain reaction (PCR)-free small-organelles enriched metagenomics (SoEM) method is an effective and accurate tool for identifying species across all taxonomic groups of eukaryotes from the smallest unicellular protists (>0.2 micrometers) to small animals that are a few millimeters long (Jo et al. 2019). This method would be a useful alternative approach in evaluating the biodiversity and community structure of both plankton and benthos in marine ecosystems.

In this study, we aimed to identify the eukaryotic epibiont community found on floating *Sargassum* fronds. We summarized the species diversity by analyzing an innovative SoEM method and discussed the ecological implications of epibionts associated with this specialized habitat in the Korea Strait. Although only epibionts associated with floating rafts are reported, this result will help fill the knowledge gap regarding the ecological roles of floating algae.

MATERIALS AND METHODS

Sample collection and filtration

Floating *Sargassum* rafts were collected from two off-

shore stations in the Korea Strait during cruises of the National Institute of Fisheries Science (NIFS) research vessel TAMGU8 from Apr 16 to 20, 2017 (Fig. 1). The sampling of rafts was performed using a landing net and conducted as near as possible to the stations 205-05 (33°37'18.1" N, 128°09'11.9" E) and 314-01 (33°00'00.0" N, 127°42'00.0" E) of the NIFS monitoring program. At the same time, an approximately 10 L sample of surface seawater was collected at each station.

Floating fronds over 100 cm in length, with holdfast regions, were immediately transferred into a plastic bag with 4 L of the surface seawater. Any organisms that fell off the thallus were quickly collected and added into the bag. All contents of the bag were immediately transported to the shipboard laboratory, and were shaken violently for several minutes to remove all mobile associated taxa and scraped to remove as many sessile invertebrates as possible. After shaking, *Sargassum* fronds were discarded from the plastic bag, and the remaining seawater was filtered using the cellulose acetate (CA) filter with a pore size of 0.45 μm . The surface seawater was also filtered using the same filter to provide control plankton communities, to exclude plankton species that were observed in *Sargassum* fronds. All CA filters were placed separately into sterile screw-cap tubes, and were frozen in liquid nitrogen. Before they were delivered to the laboratory, they were stored at -80°C until needed.

Subsequently, macroscopic specimens on all flora and fauna associated with the *Sargassum* rafts were identified under a dissecting microscope to the lowest possible identification levels using the available identification keys. The subsamples were preserved in 5% formalin for later identification.

DNA extraction, SoEM library preparation, and sequencing

To profile eukaryotic biodiversity of *Sargassum* rafts hitchhikers, we utilized a previously developed metagenomics method, SoEM, with slight modification (Jo et al. 2019). The CA filter contained trapped environmental samples that were subsequently chopped into tiny pieces with sterile scissors on ice, and then resuspended in 10 mL of ice-chilled homogenizing buffer (250 mM sucrose, 30 mM Tris-HCl, 10 mM EDTA, and pH 7.5). Chopped filter papers within the buffer were homogenized using an IKA-T10 tissue homogenizer (IKA, Staufen, Germany) at maximum RPM on ice for five cycles of 15 s and 15 s off ice. The homogenate was firstly centrifuged at 4°C at $1,300 \times g$ for 10 min to remove nuclei, cellular debris, and

filter paper pieces, and then the supernatant was transferred to a fresh tube. It was then centrifuged at 4°C at $17,000 \times g$ for 30 min to enrich small organelles. The supernatant was discarded, and small-organelles enriched DNA was extracted from the pellet using the Qiagen DNeasy Blood/Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The DNA was quantified and qualified using the Qubit 3.0 fluorometer (Thermo Fisher, Waltham, MA, USA) and an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Preparation of SoEM libraries and sequencing were carried out following the same procedures followed in the previous study (Jo et al. 2019).

Bioinformatics analysis

To remove sequencing adaptors and low-quality sequences, all paired-end sequences of SoEM libraries were preprocessed using Trimmomatic (v0.36) (Bolger et al. 2014). To extend the sequence length in further taxonomy-assignment processes, the preprocessed read-pairs were merged to one read by overlapped sequences using PEAR (Paired-End reAd mergeR, v0.9.10) (Zhang et al. 2014) with a minimum length '-n 400,' and minimum overlap '-v-10' options. To assign taxonomic information of the merged reads, a BLAST search was performed against the NCBI NT database (download date: Jun 7, 2017) with queries of the merged reads. After the BLAST search, the results were filtered according to the following criteria, (1) E-value $< 1e-10$, (2) aligned length ≥ 400 bp, and (3) pick the top one result based on the bit-score. And, NCBI GenBank accession numbers were converted to NCBI taxonomy IDs of the results that satisfied above criteria using 'nucl_gb.accession2taxid' file in NCBI taxonomy FTP (<ftp://ftp.ncbi.nih.gov/pub/taxonomy/accession2taxid/>), and an in-house python script. To eliminate false positive results in this process, accession numbers with less than five distinct assigned reads were discarded in further steps.

Taxonomy assignment of eukaryotic plankton and epibionts

To profile hierarchical taxonomic information of taxonomy IDs obtained in the previous step (e.g., Phylum, Class, Order, Genus, and Species levels), we utilized the 'taxize' tool, a package of R program (Chamberlain and Szocs 2013). After this assignment, we first excluded results that assigned taxonomic IDs to superkingdoms of 'Bacteria,' 'Virus,' 'Archaea,' and 'Unclassified' groups

to investigate eukaryotic biodiversity. Results following cases of ambiguous assignments were discarded or combined in further steps: (1) taxonomy IDs with unavailable taxonomic information at the genus level were discarded; (2) environmental entries in the NCBI taxonomy database, such as '283062; eukaryotic picoplankton environmental sample,' '212038; Copepoda environmental sample,' and '693772; invertebrate environmental sample,' were discarded; (3) uncultured entries in the database, such as '175278; uncultured metazoan' and '100272; uncultured eukaryote' were discarded; and (4) to avoid duplicate species assignments, ambiguous results in species level, such as '*Cyclotella* sp. WC03_2,' and '*Cyclotella* sp. L04_2,' were combined into same species as '*Cyclotella* sp.' Finally, to investigate the biodiversity of marine eukaryotes within the data, we manually curated the taxonomic hierarchy of the remaining taxonomic IDs by matching them with the World Register of Marine Species (WoRMS; <http://www.marinespecies.org>, accessed on Nov 19, 2019) database and the Korea National List of Marine Species (KNLMS; <https://www.mbris.kr/pub/marine/natilist/nationalist.do>, accessed on Aug 1, 2019) from Marine Bio Resource Information System (MBRIS) of Republic of Korea. Here, the species that was recorded in at least one database between both databases (i.e., WoRMS or KNLMS) was regarded as a marine eukaryotic species in this study.

For *Sargassum*-specific epibiotic species, we determined the trophic mode of each taxon from our own biological knowledge and from reference to the literature as necessary (e.g., Adl et al. 2019). When the feeding ecology of a specific taxon was unknown, we assigned it to the most probable feeding type.

Chlorophyll-*a* fluorometry

The photosynthetic activity of *Sargassum* was measured via chlorophyll-*a* fluorescence using a Diving-PAM fluorometer (Walz GmbH, Effeltrich, Germany). The maximum efficiency of PSII photochemistry (F_v / F_m) was determined for all fronds after being acclimatized to darkness for 10 min using leaf clips (Diving-LC; Walz), where F_v represents the variable fluorescence calculated as the maximum (F_m) minus the minimum (F_o) fluorescence (Genty et al. 1989). To compare these results with those of the floating fronds, the benthic *Sargassum* was collected on Apr 15, 2003 from the low intertidal to shallow subtidal zone of Wando (34°18' N, 126°41' E) along the southwestern coast of Korea.

RESULTS

Environments in the study area

To understand the mean surface circulation in the Korea Strait, mean surface current vectors were calculated in each lattice with 0.25° grid spacing using the velocity data of surface drifters that passed through the Korea Strait from 1991 to 2017 (Fig. 1B). The Tsushima Warm Current flowing northeast through the Korea Strait is a branch of the Kuroshio Current (Lie and Cho 2016). The Cheju Warm Current flows clockwise around Jeju-do and transports warm and saline seawater into the Jeju Strait. Annual mean current speeds estimated from the surface drifter observations were 18-20 cm s⁻¹ at station 205-05 and 12-13 cm s⁻¹ at station 314-01 (Park et al. 2019). The

Table 1. Sampling date and geographic position of two floating rafts in the Korea Strait

Station	Date	Latitude	Longitude	SST	SSS	F_v / F_m
205-05	Apr 16, 2017	33°37'18.1" N	128°09'11.9" E	16.5	34.5	0.636 ± 0.011
314-01	Apr 20, 2017	33°00'00.0" N	127°42'00.0" E	18.4	34.7	0.644 ± 0.009

Sea surface temperature (SST, °C), sea surface salinity (SSS), and maximum quantum yield (F_v / F_m) for floating fronds of *Sargassum horneri* (mean ± standard error, n = 25).

Table 2. Summary of sequencing statistics for samples used in the SoEM method using Trimmomatic (v0.35) and PEAR (v0.9.10)

Station	Total reads (raw data)	Total read bases pairs (raw data)	Total read (trimmed data)	PEAR-assembled read pairs to single-end	Assembled reads (%)
205-05 Seawater	45,155,648	13,195,601,272	41,839,682	6,458,940	30.9
205-05 <i>Sargassum</i>	34,396,480	10,015,710,427	32,786,872	4,927,012	30.0
314-01 Seawater	41,694,760	12,249,347,061	39,224,952	5,999,949	30.6
314-01 <i>Sargassum</i>	38,910,710	11,337,831,984	36,281,754	6,621,541	36.5

SoEM, small-organelles enriched metagenomics; PEAR, Paired-End reAd mergeR.

current is faster at station 205-05 than at station 314-01 because the flows from the Jeju Strait and the south converge at this juncture. In April, the water column is not yet vertically stratified and there is a surface meridional temperature gradient in the Korea Strait. The sea surface temperature data in Fig. 1 were obtained from the Operational Sea Surface Temperature and the Sea Ice Analysis (OSTIA) dataset was generated by the UK Met Office.

Photosynthetic performance of floating *Sargassum*

Twenty-five fronds from each raft and a benthic habitat were selected at random and the maximum quantum yield (F_v / F_m) which is an indicator of photosynthetic activity, was measured with a Diving-PAM. The F_v / F_m of the benthic fronds was 0.704 ± 0.011 (mean \pm standard error, $n = 25$), which is normal for the natural vegetative fronds, whereas the F_v / F_m values of the floating fronds from stations 205-05 and 314-01 were approximately 0.636 and 0.644, respectively (Table 1). As a result, floating fronds F_v / F_m values exhibited negligible differences between the two stations at which they were sampled and were only about 10% lower than those of benthic vegetation fronds.

Eukaryotic plankton and epibiont diversity

To profile eukaryotic biodiversity of plankton and epibionts at stations 205-05 and 314-01, small-organelles enriched DNAs from the four samples (two seawater and two *Sargassum* samples) were sequenced using an Illumina Miseq (v2, 301-cycle). A total of 34.4-45.1 million raw data reads (10.0-13.2 Giga bases) were obtained from each sample. After trimming and filtering adapter and low-quality reads, a range of 32.8-41.8 million high-quality reads per sample remained. To obtain longer sequences with more than 400 bp, paired-end reads were merged to single-end read, and 4.9-6.6 million read-pairs were merged (i.e., 30.0-36.5% of paired-end reads were merged) (Table 2). A BLAST search was performed on the NCBI nt database in May 2018 with an e-value option of $1e-10$. We first obtained a total of 2,243 NCBI Taxonomy IDs by taxonomic profiling, and 609 eukaryote IDs were extracted by hierarchical taxonomy status using the “taxize” package in R.

The SoEM output showed that a total of 366 eukaryotic operational taxonomic units (OTUs) were identified at the species level. These eukaryotes taxa comprised 7 supergroups, 25 phyla (8 not available [NA]), 62 classes

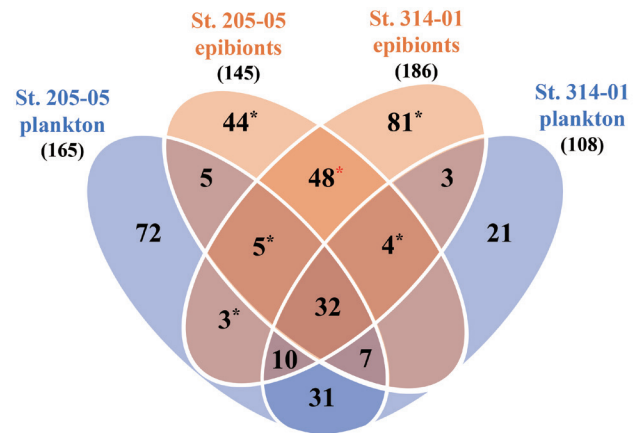


Fig. 2. Eukaryotic operational taxonomic units (OTUs) shared among four samples, including seawater and floating *Sargassum* analyzed by small-organelles enriched metagenomics. The blue circle shows the number of species analyzed in seawater plankton and the brown circle shows the number of the *Sargassum* epibionts at stations 205-05 and 314-01. The *Sargassum* epibionts (*) excluded plankton from both seawater samples. A total of 185 epibionts occurred in at least one *Sargassum* raft. The 48 species (marked with a red asterisk) are *Sargassum*-specific epibionts.

(11 NA), 128 orders (10 NA), 196 families (6 NA), and 280 genera, including the “not available (NA)” taxa. Of these 366 OTUs, 201 (54.9%) taxa that had been previously unreported in Korean waters were detected at the species level and were compared with the integrated database from KNLMS. The information of the taxon-assigned OTUs is presented in Supplementary Table S1.

To determine the *Sargassum* epibiotic species, eukaryotic plankton communities that were not associated with floating rafts were excluded from a complete OTUs list. This clarified whether the epibiotic species specifically inhabited the floating *Sargassum* or had floated on by chance from the surrounding seawater.

There were obvious differences in the diversity of specimens obtained from the four different samples. For the seawater and floating *Sargassum* samples, the highest number of eukaryotic OTUs were observed from the *Sargassum* of station 314-01, followed by those from the seawater and the *Sargassum* of station 205-05. The lowest number of OTUs (108) was obtained from the seawater sample from station 314-01 (Fig. 2).

Sargassum-specific epibionts

We identified 185 taxa associated with floating *Sargassum*, with representatives of nearly all supergroups, including 95 (51.4%) Opisthokonts, 43 (23.2%) Strameno-

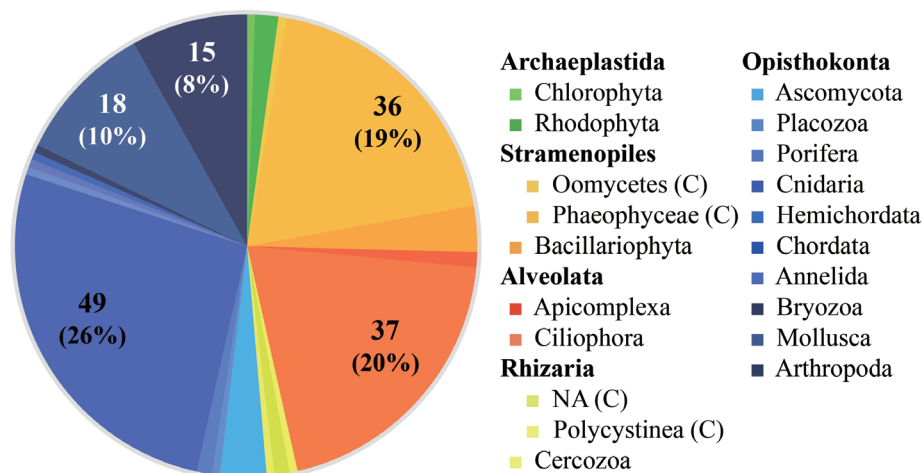


Fig. 3. Species richness of *Sargassum* epibionts. The 185 eukaryotic taxa comprised 18 phyla, including 2 not available (NA) taxa. The NAs are presented by class (C).

phyla, 39 (21.1%) Alveolata, 4 (2.2%) Archaeplastida, and 4 (2.2%) Rhizaria (Fig. 3). These eukaryotes taxa comprised 6 supergroups, 18 phyla (2 NA), 35 classes (2 NA), 67 orders (5 NA), 111 families (1 NA), and 148 genera, including NA taxa. Of all 185 identified species, 117 (63.2%) taxa were previously undetected or unreported in Korean waters. Representatives of 19 phyla / classes were found in the *Sargassum* fronds, with highest richness group being the Cnidaria (26.5%), followed by Ciliophora (20.0%), Phaeophyceae (19.5%), Mollusca (9.7%), Arthropoda (8.1%), Bacillariophyta (3.2%), Ascomycota (3.4%), and Rhodophyta (1.6%) (Fig. 3).

In contrast, the specifically *Sargassum* epibiotic species (hereafter referred to as *Sargassum*-specific epibionts), including 22 Phaeophyceae, 16 Ciliophora, 2 Bacillariophyta, 2 Ascomycota, 2 Cnidaria, 2 Arthropoda, 1 Mollusca, and 1 Apicomplexa were also obtained from both *Sargassum* rafts (Fig. 4). A total of 48 *Sargassum*-specific epibionts were classified as belonging to only 3 supergroups with 8 (1 NA) phyla, 14 classes, 21 orders, 29 families, and 44 genera (Supplementary Table S1). The majority of these taxa were photo-autotrophs (50% of the total *Sargassum*-specific epibionts) and phagotrophs (44%), although two osmo- or saprotrophic and one parasitic species were also found (Fig. 4). Alveolata contains 16 species of Ciliophora that exhibit a phagotrophic mode, and *Toxoplasma gondii* of Apicomplexa exhibits a parasitic mode. Opisthokonta has two fungi with an osmotrophic mode and five metazoan with a phagotrophic mode. A few epiphytic diatoms represented the autotrophic fraction of *Sargassum*-specific epibionts.

DISCUSSION

Although it was difficult to determine the exact origin of the *Sargassum* rafts collected from the South Sea of Korea (the Korea Strait) during the last several years, there have been reports of different origins and pathways from those in the main Kuroshio Current, East China Sea, northwestern East China Sea, and southern Yellow Sea (Chang and Isobe 2003, Abé et al. 2013, Komatsu et al. 2014a, 2014b, Qi et al. 2017). Considering the direction of flow of the Kuroshio Current, however, we do not argue the origin of our samples as most of the floating *Sargassum* rafts possibly originated somewhere in the southern part of East China Sea. The Tsushima Warm Current flowing northeast through the Korea Strait is a branch of the Kuroshio Current (see Fig. 1B).

It has been shown that certain hitchhikers on the seaweed rafts (e.g., stalked barnacles of the genus *Lepas*) can help determine the time period for which the host rafts have been floating as well as their origin and path they traversed during the time spent in the water. Free-floating or detached seaweeds that are just beginning to float in surface waters are immediately colonized by stalked barnacles. Therefore, the sizes of these barnacles can be used as a proxy of floating duration because they only adhere to buoyant objects, and their size provides a good estimate of the duration for which a hitchhiker has been afloat (Thiel and Gutow 2005b, Fraser et al. 2011, Macaya et al. 2016). However, contrary to our expectations, these species were not found in epibiotic communities of *Sargassum* rafts during the present study.

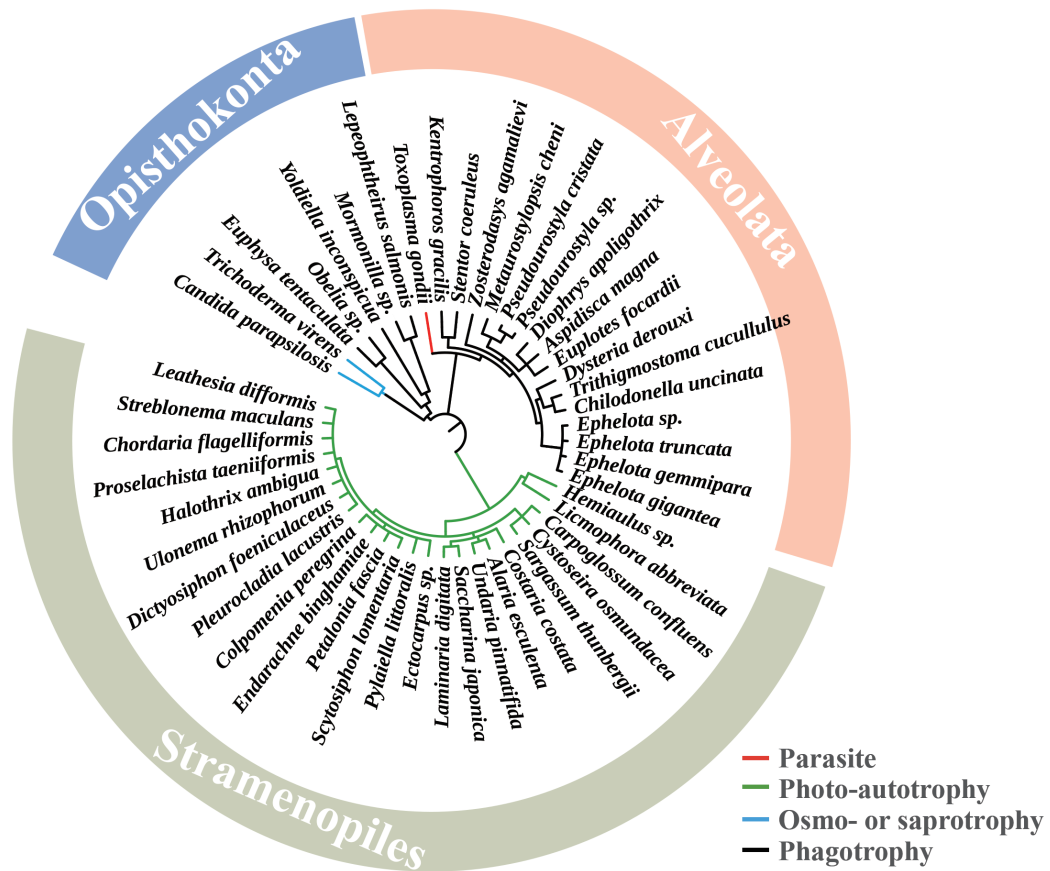


Fig. 4. Schematic phylogeny and trophic mode of *Sargassum*-specific epibionts. The 48 species, *Sargassum*-specific epibionts, belong to 3 supergroups. Each species has four broad ecological traits. The trophic modes were expressed as parasitic (red line), osmo- or saprotrophic (blue line), photo-autotrophic (green line), and phagotrophic mode (black line). The majority of these taxa are photo-autotrophs (50% of the *Sargassum*-specific epibionts) and phagotrophs (44%). The circular phylogenetic tree was created using phyloT (<http://phylot.biobyte.de/>) and the tree was visualized using iTOL (<http://itol.embl.de/>).

Floating *Sargassum* was found to harbor a diverse epibiotic assemblage with 185 species, belonging to 18 different phyla and 35 classes from only two rafts sampled in the Korea Strait (Fig. 3, Supplementary Table S1). It is difficult to determine whether the *Sargassum* epibionts are truly endemic or nonindigenous species to Korean waters due to incomplete knowledge of their taxonomy and as the many geographical areas and habitats where they originate are not well studied.

The taxonomic classification of not only protists but also many other groups of eukaryotes is continuously changing due to small differences in the taxonomic criteria used. Thus, many species described as new for an area, later turn up as synonyms to previously described species. This makes it difficult to judge whether they have existed in a particular region earlier or not. Furthermore, many eukaryotic species have not been detected or prop-

erly identified before now due to the lack of taxonomists in this country. These taxonomical changes are likely to shift even more in the future, when molecular tools will be used more frequently than they are today. In particular, the use of the SoEM method for species identification across the whole taxonomic group of eukaryotes from an environmental sample has greatly contributed towards the better understanding and the extension of marine biodiversity considerations.

Approximately two-thirds of the species found in our studies are newly recorded from Korean waters. Many epibionts may have been originally living on the *Sargassum* fronds or have colonized it before it became a floating raft. This study demonstrates some further evidence that floating *Sargassum* rafts are an important pelagic habitat that provides vital resources for a diverse epibiotic assemblage.

Although many of the large brown algae cannot propagate vegetatively, floating fertile parts can drift away and establish new populations. Whether floating *Sargassum* fronds are capable of releasing viable embryos is unknown, but mature fronds bearing reproductive conceptacles have been observed often in this study (throughout the Korea Strait in the spring of 2017). Moreover, photosynthetic activity determined by chlorophyll-*a* fluorescence measurements revealed normal or slightly decreased (ca. 90%) maximum quantum yields when compared with those collected from nearby natural rocky habitats (Table 1). *Sargassum* rafts sampled in this study may have been exposed to sunlight and air for a long period of time considering that their fronds had the potential to stay afloat. However, the complex interaction of abiotic and biotic stressors (temperature, irradiance, and grazing) on their floating persistence at the sea surface still remains poorly understood, although it is known that the persistence of seaweeds at the sea surface plays an important role in promoting long-distance dispersal and connectivity (Arroyo and Bonsdorff 2016, Rothäusler et al. 2018).

Chlorophyll-*a* fluorescence analysis has become one of the most powerful approaches for measuring photosynthetic activity of algae *in vivo* (Schreiber et al. 1994). It is particularly useful for measuring the impact of environmental stress on the physiological state of photosynthesis (e.g., Kang et al. 2016). Maximum quantum yield (F_v / F_m) can be used to reflect the photosynthetic activity and stress levels of both floating and benthic vegetative fronds. When values decline, this can reflect the development of mechanisms either for photoprotection or photodamage. Benthic fresh fronds from natural *Sargassum* habitats had negligible impact on the photosynthetic performance and had no detectable impact on health. However, the F_v / F_m values were about 0.64 for all floating *Sargassum* fronds. It indicates that in the spring, the floating *Sargassum* fronds in the Korea Strait are under exposed to adverse conditions that are not conducive to their growth.

An important role of the seaweeds in temperate waters is their architectural structure, which provides shelter for the mobile epifauna and an increased surface area for epibionts (Wallentinus 2002). The richness of epibiotic species associated with the floating seaweeds was strongly influenced by the raft size, weight or volume and age, and varied according to seasonal and geographical changes (Stoner and Greening 1984, Thiel and Gutow 2005a, 2005b, Vandendriessche et al. 2007, Wichmann et al. 2012, Abé et al. 2013). The composition of these epibi-

onts was also affected by physical environments such as temperature, salinity, ocean currents, and even weather conditions (Clarkin et al. 2012, Thiel and Fraser 2016). In addition, the health and physiological status of seaweed rafts are likely to play important roles in the development of epibiotic communities. Although we could not analyze the effects of frond age or floating times on the diversity of epibionts, in the floating *Sargassum* fronds, which presumably reached the senescence stage, the F_v / F_m remained relatively high at about 90% of that in control fronds.

The economic impact of these seaweed rafts on fisheries and aquaculture mainly occurs through clogging of fish nets and / or by creating a nuisance in aquaculture through fouling supporting structures and aquaculture target species (e.g., seaweeds, oysters, and mussels), clogging of cages, and, in extreme cases, by lifting and sailing away with free-living oysters and mussels. Massive rafts of floating seaweeds are both an economic and a recreational problem as they can hinder navigation of boats around aquaculture grounds and fishing ports. In Korea, great efforts have been made to clear *Sargassum* strandings to protect the biodiversity of an area or because they hinder shellfish and seaweed harvests, but often with limited success. Furthermore, other introduced species cause problems by fouling aquaculture structures and getting entangled in fishing tools and have to be cleared away.

The other side, *Sargassum* species are well-known ecosystem engineers of shallow hard-bottom ecosystems, forming extended canopies, structuring the algae and invertebrate communities, and attracting predators in the temperate areas of China, Japan, and Korea. Although *S. horneri* appears often as a free-floating object in temperate eastern Asia, it is capable of reproducing nearly year round in these countries, and is known to rapidly colonize new habitats, growing up to 5 m in height in dense forests and forming dense thickets on rocky shores that provide a habitat and spawning ground for a diverse assemblage of organisms (Choi et al. 2003, Sun et al. 2009).

In the eastern YS, large rafts of floating *Sargassum* were observed in March 2017 covering an area of 1,300 m² (Kim et al. 2019). Seaweeds forming dense mats floating on the surface may be detrimental for the benthos habitats as they can drastically decrease the light penetration needed for the benthic plants and by reducing oxygen for aquatic animals by decreasing air exchange through the water surface. The problems with almost total *Sargassum* rafts are well-known, as are those that arise when such huge biomasses descend to the sea bottom and smother

benthic communities and increase hypoxia conditions.

In conclusion, species dispersal and connectivity via seaweed rafts may be highly effective in some areas and limited in others. For this reason, studies of their biodiversity are important. The surveys conducted so far have been geographically restricted and have not account for the full range of taxonomic groups. To assess the diversity of eukaryotic epibionts, *Sargassum* fronds were collected from the Korea Strait, during which massive amounts of floating rafts invaded episodically. The recently established PCR-free SoEM method was adapted to assess eukaryotic diversity, ranging from the smallest unicellular eukaryotes (protists) to small animals a few millimeters long. Beyond taxonomic assignation, we inferred basic trophic modes to 48 *Sargassum*-specific epibionts on basis of their genetic affiliation to trophic group of reference. We explored the potential impacts of floating seaweeds and their role in the dispersal of alien eukaryotes into new habitats.

ACKNOWLEDGEMENTS

We would like to thank Dr. Keunyoung Kim, Juhee Min and Jingyo Lee for supporting the field sampling. This research was financially supported by NRF-2016 R1A6A1A03012647 to KYK.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. A complete list of eukaryotic plankton and *Sargassum* epibionts in this study, with the Korea National List of Marine Species (KNLMS) (<https://www.e-algae.org>).

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