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1 **The presence of free D-aspartate in marine macroalgae is restricted to the**
2 **Sargassaceae family**

3

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13

14 *Abbreviations:* Asp, aspartate; OPA, *o*-phthalaldehyde ; NAC, *N*-acetyl-L-cysteine; HPLC,
15 high-performance liquid chromatography.

16

17 **The presence of D-aspartate (D-Asp), a biologically rare amino acid, was evaluated in**
18 **38 species of marine macroalgae (seaweeds). Despite the ubiquitous presence of free**
19 **L-Asp, free D-Asp was detected in only 5 species belonging to the Sargassaceae family of**
20 **class Phaeophyceae (brown algae) but not in any species of the phyla Chlorophyta**
21 **(green algae) and Rhodophyta (red algae). All other members of Phaeophyceae,**
22 **including 3 species classified into the section *Teretia* of Sargassaceae did not contain**
23 **D-Asp. These results indicate that the presence of free D-Asp in marine macroalgae is**
24 **restricted only to the Sargassaceae family, excluding the species in the section *Teretia*.**

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26 **Key words:** D-amino acid; D-aspartate; macroalga (seaweed); Sargassaceae; *Sargassum*

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45 In the past four decades, various D-amino acids have been found in animals and plants.^{1, 2)}
46 Within aquatic animals, D-aspartate (D-Asp) and D-alanine have been identified in
47 cephalopods,^{3, 4)} crustaceans,⁵⁾ and mollusks.⁶⁾ Recently, it was reported that D-Asp and
48 aspartate racemase [EC 5.1.1.13] are involved in anaerobic energy metabolism in the bivalve
49 mollusk *Anadara broughtonii* (formerly *Scapharca broughtonii*).^{7, 8)} Although little attention
50 has been paid to the natural occurrence and physiological role of D-Asp in macroalgae, study
51 on the presence of D-amino acids in macroalgae commenced following the observation of
52 high levels of free D-Asp in the marine macroalga *Sargassum fusiforme*.⁹⁾ *S. fusiforme*
53 germinates in late summer and grows from autumn to spring in the sea near Iwate Prefecture,
54 northern part of the largest island of Japan, showing rapid growth particularly in early spring.
55 The D-Asp content also increases during this early growth period. These findings suggest that
56 D-Asp plays an important role in the early growth stages of *S. fusiforme*,⁹⁾ but the exact
57 biological role of D-Asp in this alga is yet to be clarified. To gain deeper insight into the
58 biological role of D-Asp in *S. fusiforme*, we prepared a polyclonal antibody against D-Asp and
59 confirmed that D-Asp is present in the intracellular structure of *S. fusiforme*.¹⁰⁾ These results
60 exclude the possibility that D-Asp is derived from attached or symbiotic organisms, such as
61 marine bacteria.

62 Nagahisa et al.¹¹⁾ reported the presence of free D-Asp in some marine macroalgae in Japan.
63 They found that D-Asp was present at high levels, with concentrations proportional to those of
64 L-Asp, in *Costaria costata*, *S. fusiforme*, and *S. yezoense* that belong to Phaeophyceae (brown
65 algae). However, D-Asp was not found in many other species of macroalgae, and even if it
66 was detected, it was present only in trace amounts. *Sargassum fulvellum*, *S. fusiforme*, and *S.*
67 *yezoense* thrive in the same intertidal zones in the sea. Although *S. fusiforme* and *S. yezoense*
68 are reported to have high levels of D-Asp, its content in *S. fulvellum* is very low. Thus, the
69 question arises as to why the amount of D-Asp largely differs in marine macroalgae belonging
70 to the same genus, *Sargassum*. In the present study, the distribution of D-Asp in many marine
71 macroalgae, particularly those belonging to the family Sargassaceae, was examined to clarify
72 the seaweeds that contain D-Asp.

73 This is the first report presenting a taxonomical view of the presence of free D-Asp by
74 relating it to the classification of marine macroalgae.

75

76 **Materials and Methods**

77 *Materials.* Marine macroalgae were collected from the coast of Iwate and Kanagawa
78 Prefecture, Japan, in 2013 and 2014 as shown in Table 1. Algal extracts were prepared from 5
79 species of Chlorophyta (green algae), 15 species of Rhodophyta (red algae), and 18 species of
80 Phaeophyceae. All chemicals, including D- and L-Asp, *o*-phthalaldehyde (OPA), and
81 *N*-acetyl-L-cysteine (NAC), were purchased from Wako Pure Chemicals (Osaka, Japan), and
82 were of analytical-grade purity. Milli-Q water for the experiments was obtained by purifying
83 distilled water using a Simplicity UV (Merck Millipore, Billerica, MA, USA) system.

84

85 *Preparation of algal extract.* A part of the thalli of each sampled alga (about 10 g wet
86 weight) was rinsed with distilled water to remove attached foreign materials, such as small
87 organisms. The algae were dried by wrapping the thalli tightly with paper towel and a portion
88 of the thallus was homogenized with 10 volumes of 80% (v/v) ethanol using a polytron
89 homogenizer (PT-K, Kinematica, Lucerne, Switzerland) followed by centrifugation at 50,000
90 $\times g$ at 4 °C for 15 min. The resultant precipitate was extracted with the same volume of
91 ethanol solution for maximal extraction. The supernatants (ethanolic extracts) were combined
92 and evaporated to dryness under reduced pressure at 40 °C. The residue was dissolved in a
93 small amount of Milli-Q water and then rinsed with a sufficient amount of diethyl ether to
94 remove pigments and fatty materials. The aqueous solution layer was transferred to an
95 eggplant-shaped flask and evaporated to dryness. The obtained residue was dissolved in 50
96 mL of Milli-Q water and stored at -20 °C until further analysis with high-performance liquid
97 chromatography (HPLC).

98

99 *Derivatization of amino acids with OPA/NAC.* Each sample extract was passed through a
100 0.20 μm filter. For derivatization of amino acids, 10 μL of the sample solution was mixed with

101 70 μ L of a saturated sodium borate solution and 20 μ L of a mixture of 10 mg OPA and 10 mg
102 NAC in 1 mL of methanol. After allowing the mixture to react for 1 min at room temperature,
103 the reaction mixture was injected directly into the HPLC system.

104

105 *Determination of D- and L-aspartate.* Chromatography was performed as described by
106 Nimura and Kinoshita¹²⁾ with some alterations.¹³⁾ Chromatographic analysis was performed
107 with a JASCO (Tokyo, Japan) HPLC system consisting of a PU-2089 quaternary gradient
108 pump with a degasser, a CO-2065 column oven, an AS-2057 autosampler with a cooling
109 system, an FP-2020 fluorescence detector, and a ChromNAV data processor. The analytical
110 column was a reversed-phase ODS-80T_S (4.6 \times 250 mm) (Tosoh, Tokyo, Japan) with a guard
111 column (3.2 \times 15 mm) packed with the same resin. Elution was performed with a mixture of
112 solvent A (50 mM sodium acetate buffer at pH 5.6) and solvent B (methanol:solvent A =
113 80:20) at 40 °C; the flow rate was set at 1.0 mL min⁻¹. For fluorometric detection of eluted
114 OPA/NAC derivatives, the excitation and emission wavelengths were set to 350 and 450 nm,
115 respectively. The elution gradient was set as follows: 0–20 min, 0–20% solvent B in solvent
116 A; 20–35 min, 20% solvent B in solvent A.

117

118 **Results**

119 *Distribution of free D- and L-aspartate in marine macroalgae*

120 Typical HPLC chromatograms of the converted D- and L-enantiomers of standard amino
121 acids, an extract of *S. fusiforme* with the presence of D-Asp, and an extract of *S. thunbergii*
122 with no D-Asp are shown in Fig. 1. Retention times of both D- and L-Asp derivatives fully
123 corresponded with each peak obtained for extracts of *S. fusiforme* (Fig. 1; peak number 1 and
124 2).

125 Although L-Asp was detected in all species examined, D-Asp was not detected in species
126 belonging to Chlorophyta, Rhodophyta, and most species of Phaeophyceae. However, D-Asp
127 was present in some species of Phaeophyceae belonging to the genus *Sargassum* and
128 *Stephanocystis* of Sargassaceae (Table 1).

129 Figure 2 shows a concentration of D- and L-Asp in Sargassaceae. No traces of D-Asp were
130 detected in *Sargassum confusum*, *S. hemiphyllum*, and *S. thunbergii*. However, *S. fusiforme*, *S.*
131 *horneri*, *S. siliquastrum*, and *S. patens* contained D-Asp in significant concentrations (0.41,
132 0.41, 5.13, and 7.70 $\mu\text{mol} \cdot \text{g}^{-1}$ wet weight, respectively). *Stephanocystis hakodatensis* also
133 contained 0.83 $\mu\text{mol} \cdot \text{g}^{-1}$ wet weight of D-Asp. In addition, the percent content of $[\text{D}/(\text{D} + \text{L})]$
134 $\times 100$ (%) was generally high in *Sargassum fusiforme*, *S. siliquastrum*, and *S. patens* (45.6,
135 45.8, and 51.4 %, respectively), whereas that in *S. horneri* and *S. hakodatensis* was 25.2 and
136 28.4%, respectively.

137

138 **Discussion**

139 The presence of free D-Asp in marine macroalgae was confirmed using pre-column HPLC
140 amino acid analysis. D-Asp was detected only in 5 distinguishable species out of the total of
141 38 species examined (13% of the species). The macroalgae containing D-Asp all belonged to
142 the Sargassaceae family, indicating that free D-Asp is specifically presents in the family
143 Sargassaceae of the class Phaeophyceae, although *S. confusum*, *S. hemiphyllum*, and *S.*
144 *thunbergii*, which also belong to Sargassaceae, contained no D-Asp (Table 1).

145 Generally, Sargassaceae exist at similar locations i.e., on rocks in the lower intertidal zone
146 to the infralittoral zone; Their growth season is also recognized as the duration from
147 autumn to spring in Japan. Therefore, we presumed that the presence of D-Asp is related to
148 algae's taxonomic ranks rather than their habitat. In biological classification, taxonomic ranks
149 comprise species, genus, family, order, class, phylum (division), and kingdom. However,
150 taxonomic ranks in botany include the secondary ranks, such as "section" between genus and
151 species. Thus, the knowledge about detailed classification of Phaeophyceae should be
152 considered to gain deeper insight into the diverse distribution of D-Asp in Sargassaceae.
153 According to detailed taxonomic ranks, it was found that D-Asp was detected in the entire
154 family Sargassaceae, except the section *Teretia* (Fig. 3). Therefore, the presence of free D-Asp
155 in macroalgae is taxonomically restricted to certain sections in Sargassaceae.

156 The taxonomical position of *S. fusiforme* has been discussed for a long time.¹⁴⁾ At present, *S.*

157 *fusiforme* belongs to the section *Hizikia* in the genus *Sargassum*.¹⁴⁾ The genus of *Sargassum* in
158 to subgenus *Bactrophycus* is mainly based on the morphology of the basal part supplemented
159 by the morphology of receptacles. In the present study, we reported high accumulation of
160 D-Asp in *S. fusiforme* from the section *Hizikia*, but its absence in species belonging to the
161 section *Teretia*. Although the section *Hizikia* is positioned near the section *Teretia*
162 morphologically, the presence of D-Asp is unique.

163 Nagahisa et al.¹¹⁾ reported a very low content of D-Asp in *S. fulvellum* from the section
164 *Teretia* (0.0012 $\mu\text{mol}\cdot\text{g}^{-1}$ wet weight) compared to that in *S. yezoense* (0.0485 $\mu\text{mol}\cdot\text{g}^{-1}$ wet
165 weight) from the section *Halochloa*, whereas *C. costata* contained 0.066 $\mu\text{mol}\cdot\text{g}^{-1}$ wet weight
166 of D-Asp; these results were not corroborated in the present study (Table 1). This difference in
167 D-Asp content may have resulted from different protocols implemented during the preparation
168 of the samples before analyses. Namely, in the present study, thalli were carefully cleaned
169 from any attached organisms and the identification of macroalgae species was verified.

170 It is known that the content of amino acids in marine macroalgae changes with seasons
171 and/or locations. Thus, D-Asp is present in *S. fusiforme* throughout the year although its level
172 vary with time.⁹⁾ Similarly, the presence of D-Asp in *S. fusiforme* was confirmed in thalli of
173 samples collected at both Iwate and Kanagawa (data not shown), suggesting that the presence
174 of D-Asp in *S. fusiforme* is independent of season and/or location. It is speculated that the
175 D-Asp is present in other marine macroalgae of the family Sargassaceae, except the section
176 *Teretia*, regardless of the season and/or location, although more detailed studies are necessary.

177 The available information on the biological role and metabolic pathway of D-Asp in marine
178 organisms is limited. It was reported that D-Asp and aspartate racemase are involved in
179 anaerobic energy metabolism in the bivalve mollusk, *A. broughtonii*.^{7,8)} In contrast, the
180 biological role and metabolic pathway of D-Asp in marine macroalgae remain unclear.

181 Funakoshi et al.¹⁵⁾ reported the presence of D-amino acid transaminase [EC 2.6.1.21], which
182 catalyzes transamination between D-Asp and other D-amino acids, in the terrestrial plant,
183 *Arabidopsis thaliana*. Recently, Ito et al.¹⁶⁾ reported that mammalian enzyme serine racemase,
184 the primary enzyme responsible for brain D-serine production also catalyzes Asp racemization.

185 Unfortunately, aspartate racemase, serine racemase, and/or D-amino acid transaminase activity
186 remain to be detected in any macroalgae through biochemical analyses.²⁾ Moreover, there is
187 no genome information about species belonging to the Sargassaceae. Thus, it is necessary to
188 identify and characterize enzymes involved in the metabolism of D-Asp in Sargassaceae
189 through molecular and biochemical testing, which provides novel insights into
190 lineage-specific presence, biosynthetic pathways, and physiological functions of D-Asp in
191 marine brown macroalgae.

192

193 **Author Contribution**

194 T. Yokoyama preformed the experimental design and experiments. Experimental
195 interpretation of data was conducted by all authors. T. Yokoyama wrote the paper, and the
196 other authors commented on the manuscript.

197

198 **Disclosure statement**

199 No potential conflict of interest was reported by the authors.

200

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204

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212 **References**

- 213 [1] Soda K. An essay on D-Amino acids: Retrospection and perspective. In: Konno R,
214 Brückner H, D'Aniello A, et al., editors. D-Amino acids: a new frontier in amino acid and
215 protein research—practical methods and protocols. New York (NY): Nova Science; 2007. p.
216 3–14.
- 217 [2] Yokoyama T, Mikami K. D-Amino acids and amino acid racemases in seaweeds. In:
218 Pomin VH, editor. Seaweeds: Agricultural Uses, Biological and Antioxidant Agents. New
219 York (NY): Nova Science; 2014. p. 135–155.
- 220 [3] D'Aniello A, Giuditta A. Identification of D-aspartic acid in the brain of *Octopus vulgaris*
221 Lam. J Neurochem. 1977; 29:1053–1057.
- 222 [4] D'Aniello A, Giuditta A. Presence of D-aspartate in squid axoplasm and in other regions of
223 the cephalopod nervous system. J Neurochem. 1978;31:1107–1108.
- 224 [5] D'Aniello A, Giuditta A. Presence of D-alanine in crustacean muscle and hepatopancreas.
225 Comp Biochem Physiol. 1980;60:319–322.
- 226 [6] Felbeck H, Wiley S. Free D-amino acids in the tissues of marine bivalves. Biol Bull.
227 1987;173:252–259.
- 228 [7] Shibata K, Watanabe T, Yoshikawa H, et al. Nucleotides modulate the activity of aspartate
229 racemase of *Scapharca broughtonii*. Comp Biochem Physiol. 2003;134:713–719.
- 230 [8] Watanabe T, Shibata K, Kera Y, et al. Effect of hypoxic and osmotic stress on the free
231 D-aspartate level in the muscle of blood shell *Scapharca broughtonii*. Amino Acids.
232 2005;28:291–296.
- 233 [9] Nagahisa E, Kanno N, Sato M, et al. Variations in D-aspartate content with season and part
234 of *Hizikia fusiformis*. Fish Sci. 1994;60:777–779.
- 235 [10] Yokoyama T, Amano M, Sekine M, et al., Immunohistochemical localization of
236 endogenous D-aspartate in the marine brown alga *Sargassum fusiforme*. Biosci Biotechnol
237 Biochem. 2011;75:1481–1484.
- 238 [11] Nagahisa E, Kanno N, Sato M, et al. Occurrence of free D-aspartic acid in marine
239 macroalgae. Biochem Int. 1992;28:11–19.

240 [12] Nimura N, Kinoshita T. o-Phthalaldehyde–N-acetyl-L-cysteine as a chiral derivatization
241 reagent for liquid chromatographic optical resolution of amino acid enantiomers and its
242 application to conventional amino acid analysis. *J Chromatogr.* 1986;52:169–177.

243 [13] Yokoyama T, Kan-no N, Ogata T, et al. Presence of free D-amino acids in microalgae.
244 *Biosci Biotechnol Biochem.* 2003;67:388–392.

245 [14] Stiger V, Horiguchi T, Yoshida T, et al. Phylogenetic relationships within the genus
246 *Sargassum* (Fucales, Phaeophyceae), inferred from its ITS nrDNA, with an emphasis on the
247 taxonomic revision of the genus. *Phycol Res.* 2003;51:1–10.

248 [15] Funakoshi M, Sekine M, Katane M, et al. Cloning and functional characterization of
249 *Arabidopsis thaliana* D-amino acid aminotransferase – D-aspartate behavior during
250 germination. *FEBS J.* 2008;275:1188–1200.

251 [16] Ito T, Hayashida M, Kobayashi S, et al. Serine racemase is involved in D-aspartate
252 biosynthesis. *J. Biochem.* 2016;160:345–353.

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268 **Figure Legends**

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270 **Fig. 1.** Typical HPLC chromatograms of (A) the OPA/NAC derivatives of D- and
271 L-enantiomers of standard amino acids, (B) an extract of the macroalgae *Sargassum fusiforme*
272 and (C) *Sargassum thunbergii*.

273 A 10 µL aliquot of derivatized sample solution was injected into HPLC. Each peak of (A)
274 standard amino acids represents 10 pmol.

275 1. D-Asp, 2. L-Asp, 3. L-Asn, 4. D-Asn, 5. D-Ser, 6. L-Ser, 7. L-Glu, 8. D-Glu, 9. L-Gln, 10.
276 D-Gln, 11. D, L-His, 12. D-Thr, 13. Gly, 14. L-Thr.

277

278 **Fig. 2.** Concentrations of aspartate in macroalgae belonging to the family Sargassaceae.

279 N.D.: not detected.

280

281 **Fig. 3.** Convincing interpretation for the presence of free D-aspartate in Sargassaceae.

282 Infrageneric classification is based on the algae database. The data on *Sargassum fulvellum*
283 (quite low) and *S. yezoense* (*) are based on the report by Nagahisa et al.¹¹⁾

284

285 **Table 1.** Distribution of free D- and L-aspartate in marine macroalgae.

286 N.D.: not detected.

287

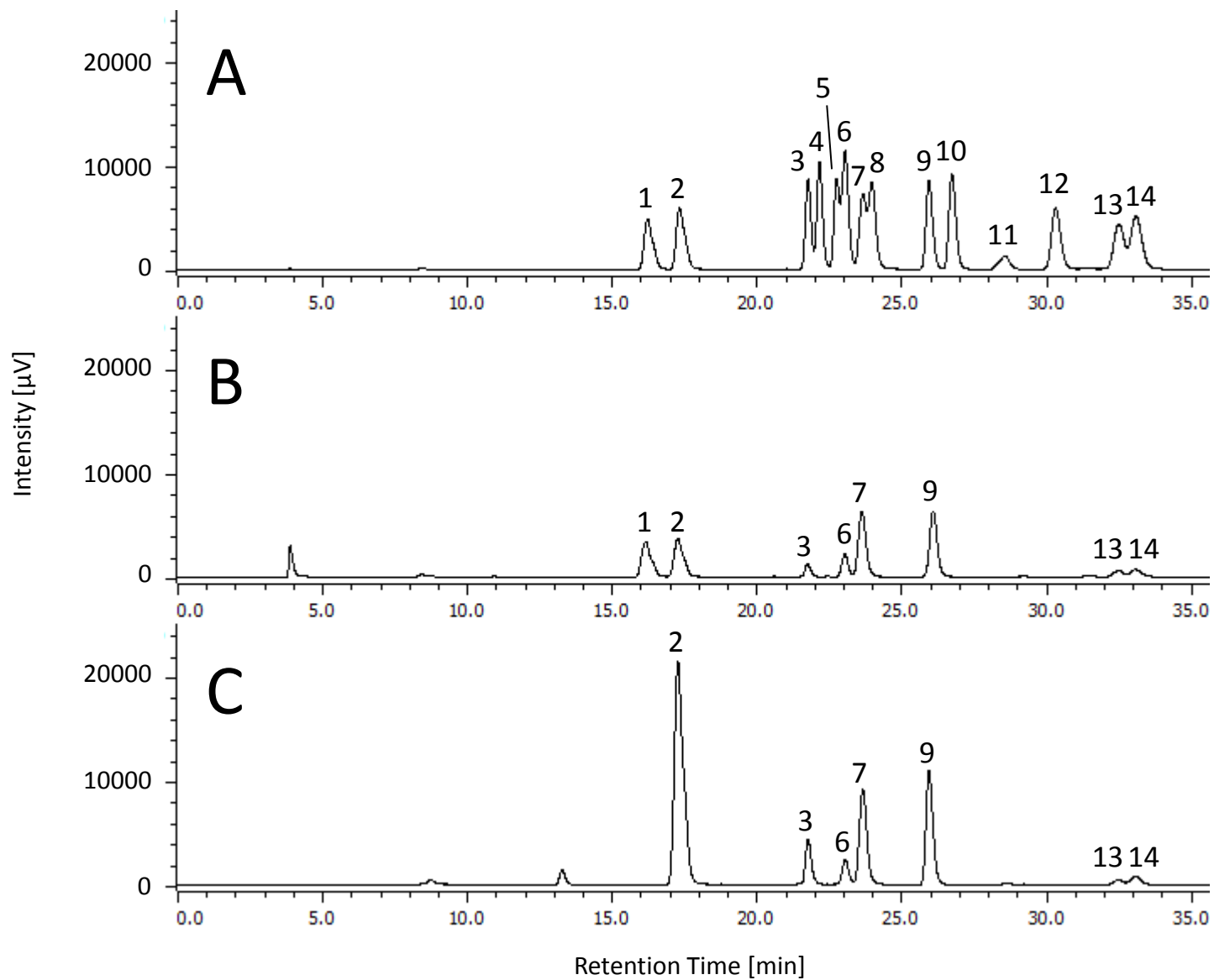


Fig.1. Yokoyama *et al.*

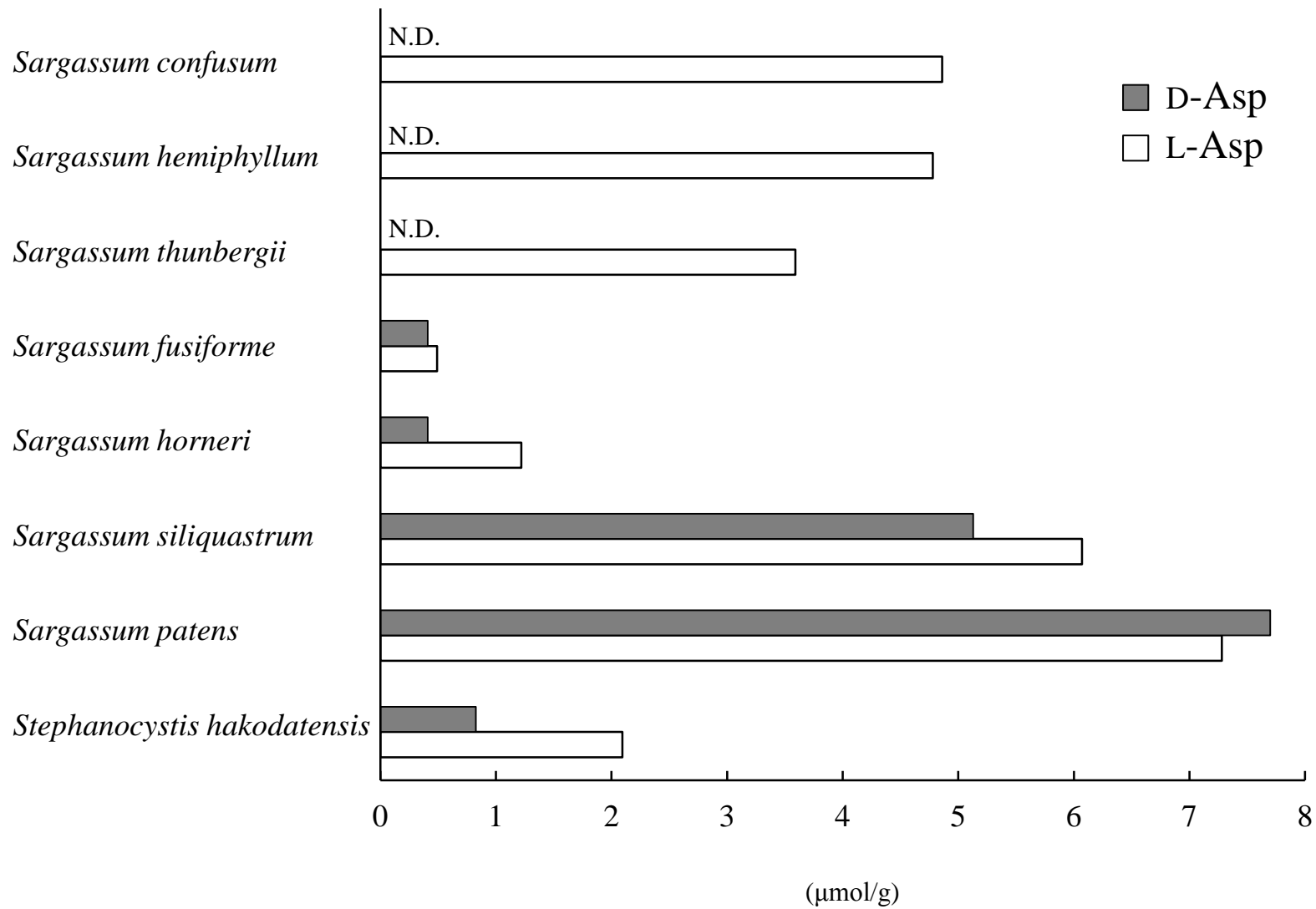


Fig.2. Yokoyama *et al.*

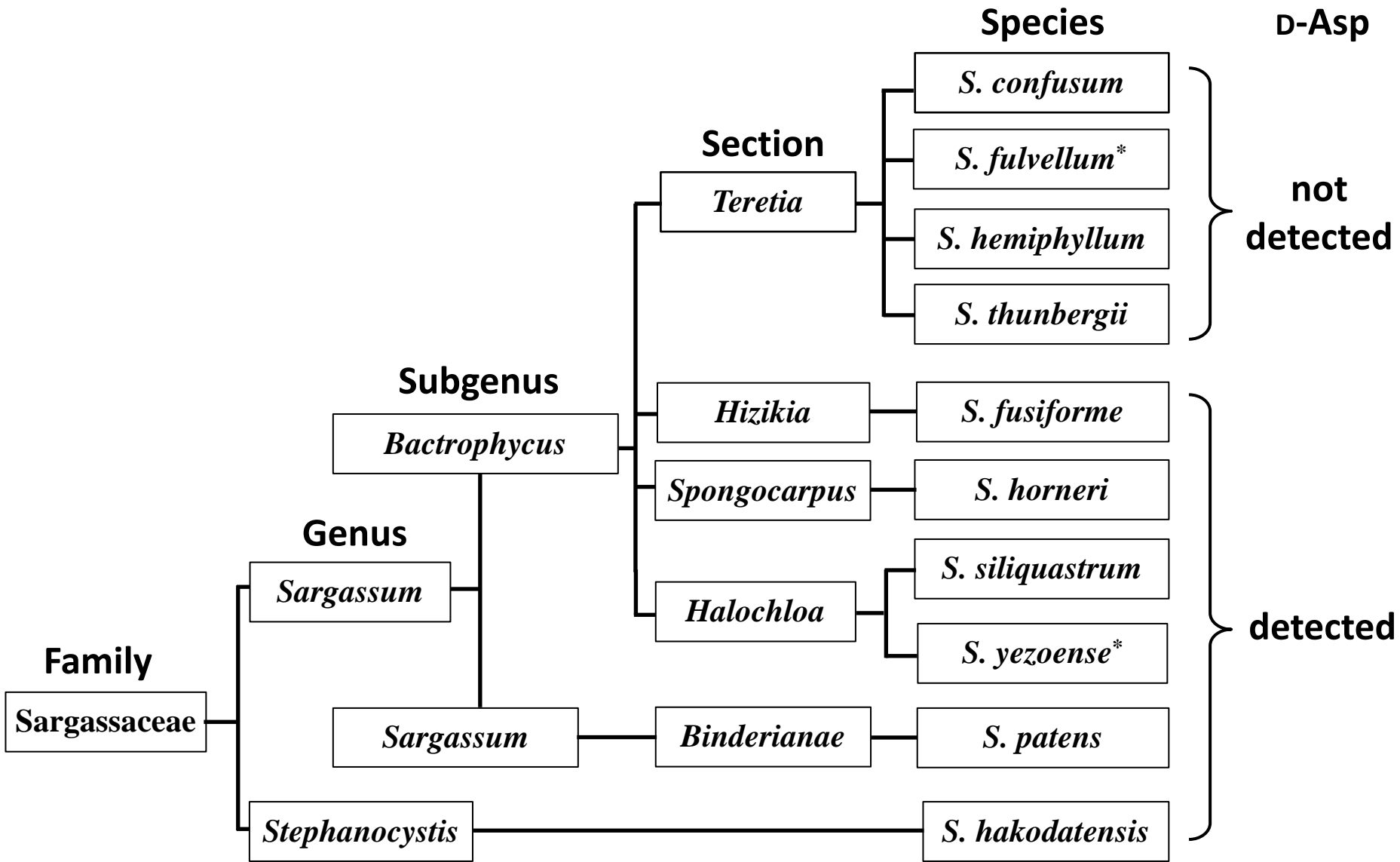


Fig.3. Yokoyama *et al.*

Species	D-Asp ($\mu\text{mol/g}$)	L-Asp ($\mu\text{mol/g}$)	D/(D+L) \times 100 (%)	Sampling date	Sampling area
CHLOROPHYTA					
<i>Cladophora opaca</i>	N.D.	5.69	—	20130610	Iwate
<i>Codium fragile</i>	N.D.	2.40	—	20131217	Iwate
<i>Ulva australis</i>	N.D.	0.18	—	20130610	Iwate
<i>Ulva intestinalis</i>	N.D.	0.28	—	20131104	Kanagawa
<i>Ulva linza</i>	N.D.	1.81	—	20131202	Kanagawa
RHODOPHYTA					
<i>Ahnfeltiopsis paradoxa</i>	N.D.	1.13	—	20130610	Iwate
<i>Chondria crassicaulis</i>	N.D.	2.19	—	20130610	Iwate
<i>Chondrophyucus undulatus</i>	N.D.	17.11	—	20131202	Kanagawa
<i>Chondrus ocellatus</i>	N.D.	1.00	—	20130610	Iwate
<i>Chondrus yendoii</i>	N.D.	0.26	—	20130610	Iwate
<i>Gloiopeltis furcata</i>	N.D.	0.25	—	20130610	Iwate
<i>Grateloupia lanceolata</i>	N.D.	0.89	—	20140614	Iwate
<i>Grateloupia sparsa</i>	N.D.	0.83	—	20131217	Iwate
<i>Hypnea asiatica</i>	N.D.	22.35	—	20131217	Iwate
<i>Hypnea japonica</i>	N.D.	0.25	—	20131202	Kanagawa
<i>Lomentaria hakodatensis</i>	N.D.	0.56	—	20130610	Iwate
<i>Neodilsea yendoana</i>	N.D.	0.69	—	20140614	Iwate
<i>Polysiphonia senticulosa</i>	N.D.	1.83	—	20130610	Iwate
<i>Pyropia yezoensis</i>	N.D.	1.39	—	20140614	Iwate
<i>Schizymenia dubyi</i>	N.D.	1.67	—	20131217	Iwate
PHAEOPHYCEAE					
<i>Analipus japonicus</i>	N.D.	1.73	—	20130610	Iwate
<i>Alaria crassifolia</i>	N.D.	1.48	—	20140614	Iwate
<i>Costaria costata</i>	N.D.	0.89	—	20140614	Iwate
<i>Eisenia bicyclis</i>	N.D.	1.16	—	20131104	Kanagawa
<i>Ishige okamurae</i>	N.D.	1.79	—	20131104	Kanagawa
<i>Petalonia binghamiae</i>	N.D.	4.28	—	20131202	Kanagawa
<i>Saccharina japonica</i>	N.D.	69.70	—	20131111	Iwate
<i>Saccharina japonica var. religiosa</i>	N.D.	1.08	—	20140614	Iwate
<i>Sargassum confusum</i>	N.D.	4.86	—	20130610	Iwate
<i>Sargassum fusiforme</i>	0.41	0.49	45.6	20130610	Iwate
<i>Sargassum hemiphyllum</i>	N.D.	4.78	—	20131104	Kanagawa
<i>Sargassum horneri</i>	0.41	1.22	25.2	20130610	Iwate
<i>Sargassum patens</i>	7.70	7.28	51.4	20131104	Kanagawa
<i>Sargassum siliquastrum</i>	5.13	6.07	45.8	20131219	Iwate
<i>Sargassum thunbergii</i>	N.D.	3.59	—	20140614	Iwate
<i>Scytosiphon lomentaria</i>	N.D.	0.55	—	20130610	Iwate
<i>Stephanocystis hakodatensis</i>	0.83	2.09	28.4	20140614	Iwate
<i>Undaria pinnatifida</i>	N.D.	2.25	—	20140614	Iwate

Table 1. Yokoyama *et al.*