5.0 RESULTS AND DISCUSSIONS

5.1 DNA extraction

5.1.1 Quality comparisons

DNA from *Sargassum oligocystum* and *S. binderi* collected from the intertidal coral reef flat bed at Cape Rachado and Teluk Kemang, Port Dickson, Negeri Sembilan was isolated by using two protocols, both involving the use of CTAB. Quality of DNA isolated from protocol 1 and protocol 2 is shown in Table 6. Protocol 2 was chosen for extraction of intact DNA as this protocol is simple to handle, safe, inexpensive and less time consuming as compared to protocol 1. A higher purity of DNA and no pigmentation was obtained using protocol 2. The $A_{260/280}$ ratios of the DNA extracted using protocol 2 are shown in Table 7. The value of a pure DNA is shown by the $A_{260/280}$ ratio in the range 1.800 to 2.000. However, when working with the DNA of plants, the presence of the secondary compounds such as phenolic compounds and polysaccharides will somehow give a lower value of $A_{260/280}$ ratios. As in this study, the value of $A_{260/280}$ ratios is in between 1.642 and 1.956. Although the $A_{260/280}$ ratios is slightly lower than expected, the amplifications of PCR using random primers were still giving a positive result.

A total of 60 samples were extracted by using protocol 2. Clear, non-pigmented intact DNA was spooled out by using a glass rod after precipitation by isopropanol. The $A_{260/280}$ ratio of the first extraction ranged from 1.114 to 1.625 (Table 7). In order to obtain a successful amplification of RAPD-PCR, the extraction was carried out once again to get purer DNA with a higher $A_{260/280}$ ratio (Table 8). The genomic DNA bands isolated from protocol 1 and 2 (first and second extraction) is shown in Figure 2. When tested with RAPD-PCR, 95% of the samples gave positive results compared with 55% for the DNA without re-extraction.

S. oligocystum and *S. binderi* need extra attention while cleaning as they are normally dirty. The plant may be full of sand, salt and epiphytised with diatoms, fungi and bacteria. The stem of the plants was too difficult to grind into powder form due to the stiffness of the cell wall thickening and polysaccharide deposition. The receptacles of the plants were always full of epiphytes and the probality to get them clean is very low and the structure of the receptacles themselves were very small and the surface were not even. Therefore, only leaves and young shoots were used as these parts are easy to clean. More DNA can be obtained from young shoots as these parts are actively involved in mitosis.

Thoroughly clean tissues were ground using liquid nitrogen to break the tough cell walls in order to release the cellular constituents. The cell membrane was then disrupted by using a detergent, usually PVP (polyvinylpropaline), SDS (sodium

dodecyl sulfate) or CTAB so that the DNA is released into the extraction buffer. The DNA was protected from endogenous nucleases by adding EDTA as a chelating agent that binds magnesium ions, generally considered a necessary cofactor for most nucleases. To denature and separate the proteins from the DNA, emulsified homogenate of chloroform : isoamylalcohol (24:1) was added (Rogers and Bendich, 1994).

In protocol 1, according to Doyle and Doyle (1990), 1% (w/v) of PVP-40 added into DNA isolation buffer to isolate DNA from plants was found to be effective as an absorbent for tannin and other secondary plant compounds, including high concentration of phenolic compounds. However, this was not good enough to get rid of the polysaccharides and phenolic compounds in *S. oligocystum* and *S. binderi*. A "jelly-like" and insoluble DNA preparation was obtained and this is most probably due to the carbohydrates as only 1% CTAB was used initially, which was insufficient. 10 % of CTAB used after the first chloroform-isoamyalcohol extraction generated significant amounts of hazardous waste that will endanger the environment as the disposal of hazardous waste will add to the actual extraction costs.

The described procedure in protocol 2 is reliable, relatively quick and simple to perform. This method works very well with small quantities of fresh algal tissues as compared to protocol 1 which required a large amount of dried tissues. Also, in protocol 1 DNA is lost at each step , thus reducing the overall efficiency of the

isolation as too many steps were involved. Thus, a rather simple and quick procedure was developed in protocol 2. By incubating tissue powder with 4% CTAB in the extraction buffer, nucleic acids are precipitated selectively despite the presence of phenolic compounds, chlorophyll and cell proteins. Insoluble polysaccharides are precipitated by hot CTAB (Murray and Thompson, 1980 and Moller, 1992). As a replacement for 1% PVP, 0.2% (w/v) of 2-β-mercaptoethanol was used in order to absorb secondary plant compounds including the phenolic compounds. The polysaccharides can be removed from isolated DNA by adjusting the salt concentration from 0.7 M to 1.4 M (Michaels *et al.*, 1994).

DNA extraction method	Numer of samples	$A_{260/280}$ ratio	Pigmentation
Protocol 1	10	0.830-1.125	30%
Protocol 2	60	1.114-1.625	0%

Table 6: Comparison of DNA extraction methods

DNA samples	$A_{260/280}$ ratio	Amount of DNA (μg/ml)	DNA samples	$A_{260/280}$ ratio	Amount of DNA (μg/ml)
SO 01	1.556	28.0	SB 01	1.262	26.5
SO 02	1.192	28.0	SB 02	1.268	26.0
SO 03	1.225	24.5	SB 03	1.281	32.0
SO 04	1.625	47.0	SB 04	1.176	40.0
SO 05	1.197	42.5	SB 05	1.229	43.0
SO 06	1.217	36.5	SB 06	1.233	45.0
SO 07	1.128	48.5	SB 07	1.282	32.0
SO 08	1.154	45.0	SB 08	1.254	44.5
SO 09	1.158	44.0	SB 09	1.241	54.0
SO 10	1.151	46.0	SB 10	1.265	43.0
SO 11	1.158	44.0	SB 11	1.329	54.5
SO 12	1.152	45.5	SB 12	1.267	38.0
SO 13	1.156	44.5	SB 13	1.243	43.5
SO 14	1.114	49.0	SB 14	1.214	62.5
SO 15	1.161	44.0	SB 15	1.202	59.5
SO 16	1.188	67.0	SB 16	1.296	35.0
SO 17	1.356	30.5	SB 17	1.308	51.0
SO 18	1.464	50.5	SB 18	1.277	60.0
SO 19	1.476	46.5	SB 19	1.297	41.5
SO 20	1.288	71.5	SB 20	1.247	48.0
SO 21	1.409	58.5	SB 21	1.264	57.5
SO 22	1.444	65.0	SB 22	1.327	36.5
SO 23	1.401	38.5	SB 23	1.204	59.0
SO 24	1.517	69.5	SB 24	1.227	54.0
SO 25	1.367	30.0	SB 25	1.231	91.0
SO 26	1.261	84.5	SB 26	1.187	42.5
SO 27	1.337	69.5	SB 27	1.269	87.0
SO 28	1.261	68.0	SB 28	1.344	40.5
SO 29	1.258	63.5	SB 29	1.303	76.0
SO 30	1.185	65.0	SB 30	1.512	65.0

SO : *Sargassum oligocystum*
SB : *Sargassum binderi*

Table 7: DNA quality of protocol 2 (first extraction)

DNA samples	$A_{260/280}$ ratio	Amount of DNA (μg/ml)	DNA samples	$A_{260/280}$ ratio	Amount of DNA (μg/ml)
SO 01	1.722	8.0	SB 01	1.940	8.0
SO 02	1.956	7.5	SB 02	1.866	8.0
SO 03	1.642	6.0	SB 03	1.778	10.0
SO 04	1.855	15.0	SB 04	1.684	12.0
SO 05	1.773	10.0	SB 05	1.750	15.0
SO 06	1.655	9.0	SB 06	1.850	15.0
SO 07	1.922	12.0	SB 07	1.865	12.0
SO 08	1.930	12.0	SB 08	1.920	13.5
SO 09	1.885	12.0	SB 09	1.690	18.0
SO 10	1.722	15.0	SB 10	1.725	10.5
SO 11	1.763	15.0	SB 11	1.750	14.0
SO 12	1.695	15.0	SB 12	1.859	12.5
SO 13	1.880	12.0	SB 13	1.880	15.0
SO 14	1.900	18.0	SB 14	1.900	18.0
SO 15	1.778	12.0	SB 15	1.925	15.0
SO 16	1.748	25.0	SB 16	1.910	10.0
SO 17	1.802	12.0	SB 17	1.880	17.5
SO 18	1.667	20.0	SB 18	1.875	20.0
SO 19	1.890	15.0	SB 19	1.790	15.0
SO 20	1.920	28.0	SB 20	1.800	15.0
SO 21	1.960	25.0	SB 21	1.800	25.0
SO 22	1.918	28.0	SB 22	1.836	12.0
SO 23	1.899	12.0	SB 23	1.720	25.0
SO 24	1.793	25.0	SB 24	1.900	25.0
SO 25	1.699	8.5	SB 25	1.930	33.0
SO 26	1.800	30.0	SB 26	1.800	15.0
SO 27	1.825	25.0	SB 27	1.850	30.0
SO 28	1.927	20.0	SB 28	1.750	15.0
SO 29	1.880	20.0	SB 29	1.875	28.0
SO 30	1.900	20.0	SB 30	1.790	25.0

SO : *Sargassum oligocystum*

SB : *Sargassum binderi*

Table 8: DNA quality of protocol 2 (re-extraction)

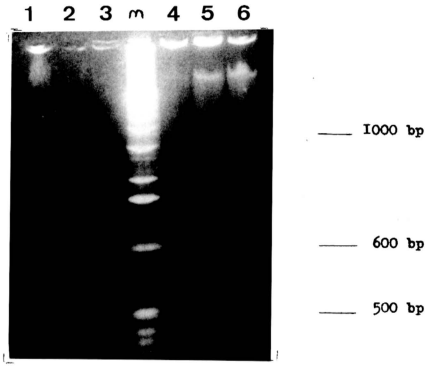


Figure 2. Example of *Sargassum oligocystum* genomic DNA on 0.8% agarose gel.
Lanes 1 and 2, Protocol 1;
Lanes 3 and 4, Protocol 2 (single extraction)
Lanes 5 and 6, Protocol 2 (double extraction).
The lane marked with m is a 100-bp DNA ladder (from 1500 to 100 bp, respectively).

5.2 Optimisation of PCR

5.2.1 PCR parameters

To optimise the PCR conditions with various RAPD primers is a laborious task. Each PCR parameter has to be considered in order to obtain stable and reliable band patterns. The parameters of RAPD-PCR amplification include Mg^{2+} concentration, amount of dNTP, amount of primer, DNA concentration and annealing temperature, number of cycles, and most importantly, the amount of *Taq* polymerase, which plays a very important role in successful amplification.

5.2.2 Optimised conditions

From the study, five primers (OPA13, OPK7 and 9, OPN6 and 16) were examined with the two *Sargassum* species: *S. oligocystum* and *S. binderi*. For the optimisation of the RAPD-PCR, seven parameters were optimised. The DNA with the concentration of 25 ng, 50 ng and 100 ng were tested. The primers with a different concentrations of 5 pmol and 10 pmol were tested. 100 μM and 200 μM of the dNTP concentrations were also tested. For the *Taq* DNA polymerase, one and two units were tested. The concentrations of 1.5 mM, 2.0 mM and 2.5 mM of $MgCl_2$ were tested. The number of cycles for the amplification of 30, 35, 40, 45, 50, 55 and 60 cycles were tested. The annealing temperature of the reactions were tested at 30°C,

33°C, 36°C, 40°C and 43°C. A combination of the optimised conditions for each primer with the PCR parameters are shown in Table 9. With the optimisation of the reaction conditions and parameters, the variability of the replicon was reduced.

For the optimisation of the number of cycles, fewer than 30 thermal cycles provided insufficient amplification, while more than 55 cycles did not significantly increase yield. Magnesium concentrations below 1.5 mM, primer concentrations below 5 pmole , and individual dNTP concentrations below 100 μM failed to yield sufficient amplification.

Among the parameters tested, Mg^{2+} concentration was found to be very important for reproducibility of the method. At lower concentrations (1.0-1.5 mM), only lower molecular weight bands were amplified (Figure 3, a and b, lane 1-5). As the concentration was increased to 2.0 -2.5 mM, the banding patterns were consistent with both high and low molecular weight bands (Figure 3, a and b, lane 6-10). At a concentration of 2.5 mM, the higher molecular weight bands were more intense and sharp but the bands of lower molecular weight were very faint (Figure 3, a and b, lane 6-10). Therefore, 2.5 mM of Mg^{2+} concentration was chosen for the rest of the RAPD-PCR amplification.

In this study, not only the Mg^{2+} concentration (Figure 3) but annealing temperature (Figure 4) should be optimised for each species and primer combinations separately according to the suggestion of Wolff *et al.* (1993). Differences in results were noted

with various amounts of *Taq* polymerase (Figure 5), dNTP used (Figure 6), primer (Figure 7) and DNA (Figure 8) used. Therefore, optimisation of each parameter was carried out to obtain the most suitable conditions to gain the consistent and sharpest bands in the amplification.

Amplification of target DNA loci occurred at all tested *Taq* polymerase concentrations. The yield and complexity of amplified products were relatively low at 0.5 and 1 unit of polymerase per 25 µl reaction. As the quantity of enzyme was increased to 2 units per reaction, many additional bands, both prominent and faint, became evident (Figure 5). This result suggests a competition between loci for a limiting amount of enzyme. Presumably, some loci are bound by one or two sites that are less perfectly complementary to the primer that are bound to other sites. Loci bound by these former sites are at a disadvantage when polymerase is limiting and are efficiently amplified only with an excess of enzyme.

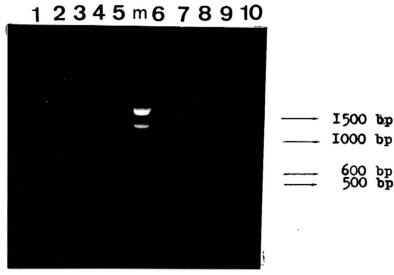
If secondary bands are produced under a condition of excess enzyme, then the addition of sufficient DNA should overcome this condition. When 2.0 unit of polymerase and varying quantities of DNA were used in standard amplifications (Figure 8), the secondary band formation observed with 25 ng of DNA was suppressed with 50 ng. Primer concentrations of 10 ρmole enhanced the production of some bands (Figure 7) but did not improve the discrimination of major bands from the background signal.

Significant changes in the DNA fingerprint generated are not related to the annealing temperature as in the study carried out by Welsh and McClleland, 1990. The above statement did not apply to this study where the annealing temperature affected the amplified RAPD-PCR products (Figure 4), that is, changes in annealing temperature affected the number of bands produced.

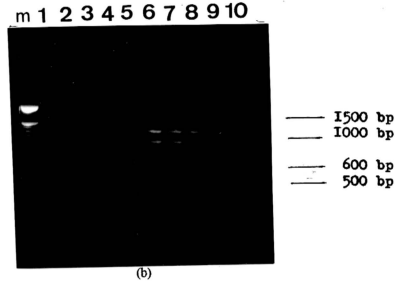
From this study, it is clear that the RAPD technique requires careful attention to detail in execution and cautious interpretation only when sufficient replication has been performed. The plant material should be clean and as free as possible of contaminating organisms. Extreme care must be taken during the preparation of reagents and amplification reaction mixtures to prevent contamination or DNA carryover from one vessel to another so as to reduce the possibility of false positives or false negatives (Kwok, 1989). Variation in efficiency of amplification among heating block wells in the temperature cycler can lead to variable results (Linz, 1990). Therefore, it is necessary to determine the temperature of each well, both in preliminary experiment and from experiment to experiment.

Primer / Optimised PCR parameter	OPA 13	OPK 7	OPK 9	OPN 6	OPN 16
Mg^{2+}	2.5 mM	2.5 mM	2.5 mM	2.5 mM	2.5 mM
Taq polymerase	1 unit	2 unit	2 unit	2 unit	2 unit
dNTP	100 μM	200 μM	200 μM	200 μM	200 μM
primer	5 pmol	5 pmol	10 pmol	10 pmol	10 pmol
annealing temperature	40°C	30°C	30°C	36°C	33°C
DNA concentration	25 ng	50 ng	50 ng	50 ng	50 ng

Table 9: Optimised conditions for various randomised primers



(a)



(b)

Figure 3. The effect of Mg^{2+} concentration on RAPD amplification of *Sargassum oligocystum* SO17 by OPN16 .
a. Lane 1-5, 1.0 mM Mg^{2+} ; Lane 6-10 2.0 mM Mg^{2+}.
b. Lane 1-5, 1.5mM Mg^{2+} ; Lane 6-10, 2.5 mM Mg^{2+}.
The lane marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp, respectively).

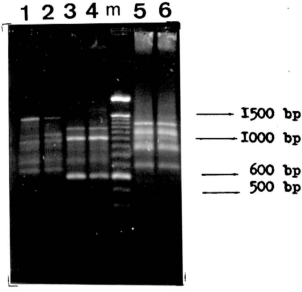


Figure 4. The effect of annealing temperature on RAPD amplification of *Sargassum oligocystum* SO25 by OPA13.
Lane 1 and 2, 30°C
Lane 3 and 4, 36°C
Lane 5 and 6, 40°C
The lane marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp, respectively).

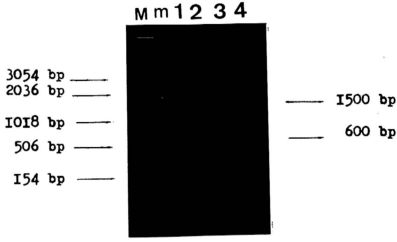


Figure 5. The effect of *Taq* polymerase on RAPD amplification of *Sargassum oligocystum* SO11 by OPK7.
Lane 1 and 3, 1 unit of *Taq* polymerase;
Lane 2 and 4, 2 unit of *Taq* polymerase.
The lanes marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

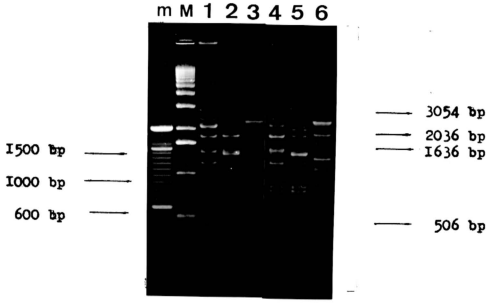


Figure 6. The effect of dNTP concentration on
RAPD amplification of *Sargassum oligocystum* SO9 by OPK7.
Lane 1-3, 100 µmole of dNTP;
Lane 4-6, 200 µmole of dNTP.
The lanes marked with m is a 100 bp DNA ladder
(from 1500 bp to 100 bp) and M is a 1 kb DNA ladder
(from 5095 bp to 154 bp), respectively.

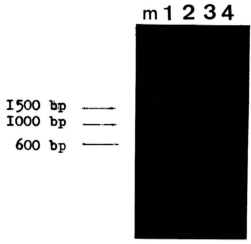


Figure 7. The effect of primer concentration on RAPD amplification of *Sargassum oligocystum* SO20 by OPK7.
Lane 1 and 2, 5 ρmole of primer;
Lane 3 and 4, 10 ρmole of primer.
The lane marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp), respectively.

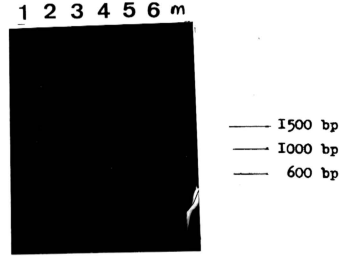


Figure 8. The effect of DNA concentration on RAPD amplification of *Sargassum oligocystum* SO12 by OPK7.
Lane 1-2, 25 ng of genomic DNA;
Lane 3-4, 50 ng of genomic DNA;
Lane 5-6, 100 ng of genomic DNA.
The lane marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp), respectively.

5.3 RAPD analysis

RAPD-PCR has been used in recognition of animal species (Fong *et al.*, 1995; Pfenninger *et al.*, 1995). In higher plants, this technique has been used for the identification, that is, *Apium* (Yang and Quiros, 1993), *Solanum* (Singsit and Ozias-Akins, 1993), *Stylosanthes* (Kazan *et al.*, 1993), *Theobroma* (Wilde *et al.*, 1992) and *Brassica* (Hu and Quiros, 1991). Application in algae such as heterosis study in *Gelidium vagum* (Rhodophyta) (Patwary *et al.*, 1994), gene mapping study in *Chlamydomonas eugametos* (Chlorophyta) (Haring *et al.*, 1996), assessment study of populations of *Gracilaria chilensis* (Rhodophyta) (Meneses, 1996), and comparison of polymorphisms in *Gelidium vagum* (Rhodophyta) (Patwary *et al.*, 1993). In chracterisation of three species of *Porphyra* (Bangiales, Rhodophyta), a 20-mer M 13 primer yields amplification products which can be used to fingerprint specific genotypes (Dutcher and Kapraun, 1994). In Alberto and Santos study (1997), RAPD analysis is appropriate to characterise the genetic variability of three natural populations of *Gelidium sesquipedale* (Rhodophyta). As for *Sargassum* species, Ho *et al.*, 1995 has reported that this technique is suitable to use in characterisation of *Sargassum polycystum* and *S. siliquosum* (Phaeophyta). In 1996, van Oppen *et al.* reported that RAPD data are robust in identifying large-scale biogeographic populations study of *Lophocladia trichoclados* (Ceramiales, Rhodomelaceae).

5.3.1 RAPD size

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Primer	Selected Region
OPA13	400 - 4000 bp
OPK7	300 - 4000bp
OPK9	300 - 4000 bp
OPN6	300 - 2072 bp
OPN16	400 - 3000 bp

Table 10: The sizes of amplified DNA fragments generated by RAPD primers which are selected for the analysis.

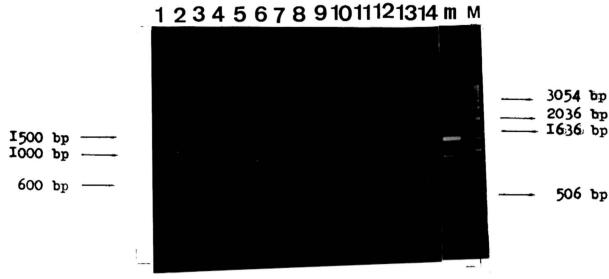


Figure 9. RAPD banding patterns of *Sargassum binderi* and *S. oligocystum*. Results obtained with primer OPA13. Lane 1, SB2; Lane 2, SO15; Lane 3, SB12; Lane 4, SB5; Lane 5, SO27; Lane 6, SO8; Lane 7, SO14; Lane 8, SB11; Lane 9, SO18; Lane 10, SB29; Lane 11, SB17; Lane 12, SO22; Lane 13, SB6; Lane 14, SO19. The lanes marked with m is a 100-bp DNA ladder (from 1500 bp to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp and 154 bp), respectively.

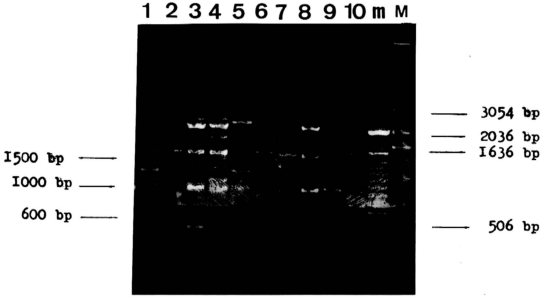


Figure 10. RAPD banding patterns of *Sargassum oligocystum* and *S. binderi*. Results obtained with primer OPK7. Lane1, SB3. Lane 2, SB6. Lane 3, SO8. Lane 4, SO11, Lane 5, SO14, Lane 6, SB12, Lane 7, SO18, Lane 8, SO 21, Lane 9, SO30. Lane 10, SB28. The lanes marked with m is a 100 bp DNA ladder (from 1500 bp to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

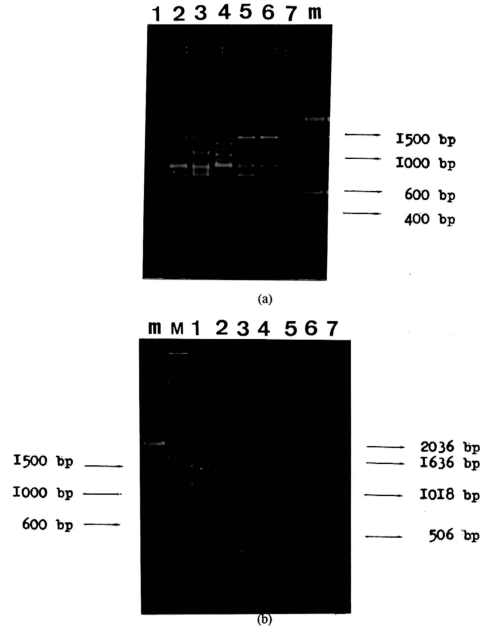


Figure 11. RAPD banding patterns of *Sargassum oligocystum* and *S. binderi*. Results obtained with primer OPN16. a. Lane1, SO1; Lane 2, SO4; Lane 3, SO9; Lane 4, SO14; Lane 5, SO19; Lane 6, SO 25; Lane 7, SO28. b. Lane 1, SB2; Lane 2, SB6; Lane 3, SB10; Lane 4, SB16; Lane 5, SB20; Lane 6, SO23; Lane 7, SO27. The lanes marked with m is a 100-bp DNA ladder (from 1500 to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

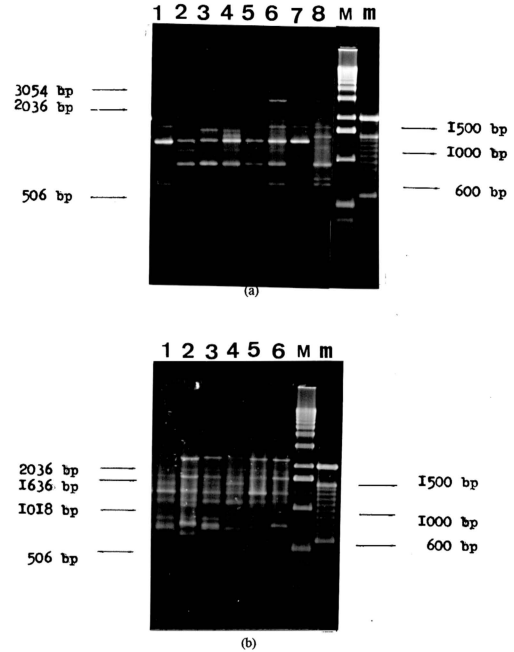


Figure 12. RAPD banding patterns of *Sargassum oligocystum* and *S. binderi*. Results obtained with primer OPK9. a. Lane 1, SO1; Lane 2, SO5; Lane 3, SO10; Lane 4, SO14; Lane 5, SO19; Lane 6, SO20; Lane 7, SO25; Lane 8 SO28. b. Lane 1, SB3; Lane 2, SB9; Lane 3, SB 12; Lane 4, SB17; Lane 5, SB25; Lane 6, SO28. The lanes marked with m is a 100-bp DNA ladder (from 1500 to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

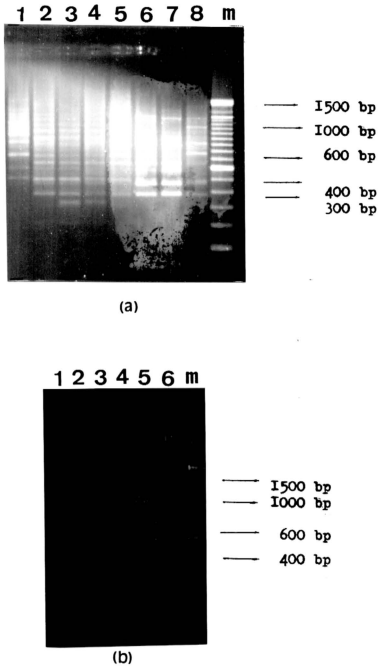


Figure 13. RAPD banding patterns of *Sargassum oligocystum* and *S. binderi*. Results obtained with primer OPN6. a. Lanel, SO1; Lane 2, SO7; Lane 3, SO11; Lane 4, SO18; Lane 5, SO23; Lane 6, SO25; Lane 7, SO29; Lane 8, SO30. b. Lane 1, SB2; Lane 2, SB8; Lane 3, SB 13; Lane 4, SB19; Lane 5, SB24; Lane 6, SB29. The lanes marked with m is a 100-bp DNA ladder (from 1500 to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

5.3.3 RAPD profiles

There are bands that are shared between the individual samples of *S. binderi* and *S. oligocystum* (intraspecifically) by the five primers tested. In addition to that, there are bands which are shared by samples between *S. binderi* and *S. oligocystum* (interspecifically).

For OPA13, four bands were common to *S. binderi* in 27 out of 30 samples tested (Figure 9). They were bands at 500 bp, 700 bp, 900 bp and 1100 bp. For *S. oligocystum*, 28 out of 30 samples tested, there were three bands which were common, that is, a 500 bp band, a 700 bp band and a 1300 bp band (Figure 9). The bands that were common between the two *Sargassum* species were 500 bp and 700 bp. Therefore, 900 bp and 1100 bp were only found in *S. binderi* and band 1300 bp was only found in *S. oligocystum*. These bands may be species specific bands for *S. binderi* and *S. oligocystum*, respectively, when amplifying using OPA13.

For OPK7, two common bands appeared in 26 out of 30 samples of *S. binderi* tested, that is, a 1500 bp band and 1636 bp band (Figure 10). Three common bands sharing with 28 out of 30 samples of *S. oligocystum* were 900 bp, 1500 bp and 2072 bp (Figure 10). The single band that is shared between the two *Sargassum* species was a 1500 bp band. Therefore, a 1636 band is a species specific band for *S. binderi* and

bands 900 bp and 2072 bp are species specific for *S. oligocystum* when OPK7 is the primer for the amplification.

For OPK9, three common bands were found in 27 out of 30 samples of *S. binderi* tested (Figure 12a). They were 1100 bp, 1300 bp and 1636 bp. For *S. oligocystum*, three common bands were also found in 27 out of 30 samples tested (Figure 12b). They were 800 bp, 1000 bp and 1400 bp. This is a very specific primer to the two *Sargassum* species tested as there was not even one common band found between the two *Sargassum* speices.

For OPN6, five common bands were found in 28 out of 30 samples of *S. binderi* tested, that is, 400 bp, 500 bp, 600 bp, 750 bp and 1100 bp (Figure 13a). In 28 out of 30 samples of *S. oligocystum* tested, three common bands were found, that is, 500 bp, 600 bp and 900 bp (Figure 13b). There were two bands which were shared between *S. binderi* and *S. oligocystum*, that is, 500 bp and 600 bp. There were three species specific bands found in *S. binderi*, that is, band 400 bp, 750 bp and 1100 bp. One species specific band, 900 bp, was found in *S. oligocystum* samples. This is not a very good primer to use for *S. binderi* as there are too many bands in common.

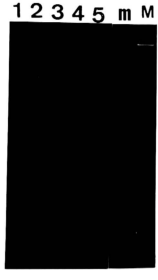
For OPN16, six bands were found to be common in 26 out of 30 samples of *S. binderi* tested (Figure 11a). They were 400 bp, 550 bp, 800 bp, 900 bp, 1100 bp and 1500 bp. For *S. oligocystum*, four common bands appeared within 27 samples out of 30

samples tested (Figure 11b). They were 700 bp, 900bp, 1100 bp and 1500 bp. There were three common bands found between the samples of *S. binderi* and *S. oligocystum*, that is, 900 bp, 1100 bp and 1500 bp. This primer is not a good primer to use for DNA polymorphisms as there were too many sharing bands between intra- and inter- speices.

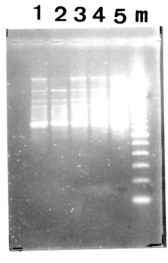
A 500 bp band was found in both *Sargassum* species tested while using OPA13 and OPN6 as the primers. When testing with OPK7 and OPN16, a 1500 bp band was found in both *Sargassum* species. There is a possibility that the two pair of primers of OPA13 and OPN6, OPK7 and OPN16 are amplifying the same region of the genomic DNA of the two *Sargassum* species.

5.3.4 Reproducibility

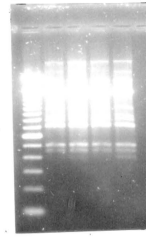
When appropriate precautions were taken in conducting the RAPD procedure and with the optimised conditions for PCR by a particular primer was used, the major banding pattern of a particular RAPD-PCR reaction was remarkably reproducible for independent DNA extractions of any given sample. This was true for replicates both in an experiment and between experiments (Figure 14 a, b, c, d, and e). The patterns were stable regardless of whether total nucleic acid or RNase treated DNA was used as a substrate. This showed that the method was well-standardised since only minor variations among duplicates were detected.



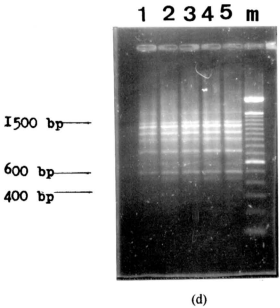
(a)



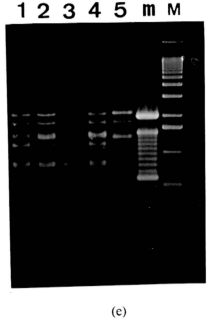
(b)



(c)



(d)



(e)

Figure 14. Reproducibility of RAPD profiles for independent DNA extractions *of Sargassum oligocystum* SO25. a. RAPD patterns generated by OPA 13. b. RAPD patterns generated by OPK 7. c. RAPD patterens generated by OPK 9. d. RAPD patterns generated by OPN 6. e. RAPD patterns generated by OPN 16. The lanes marked with m is a 100-bp DNA ladder (from 1500 to 100 bp) and M is a 1 kb DNA ladder (from 5095 bp to 154 bp), respectively.

5.4 Data analysis

5.4.1 Tables of F values

The F values for the five primers used to compare individuals of *Sargassum binderi* and *S. oligocystum* and between *S. binderi* and *S. oligocystum* are shown in Tables 13-27.

5.4.2 Comparison of F values

The application of similarity coefficient calculations formulated by Nei and Li (1979) gave results showing a high degree of similarity between individuals of *S. binderi* and also between individuals of *S. oligocystum*. This applied to all the primers tested (OPA13, OPK7, OPK9, OPN6 and OPN16) being used to compare for polymorphisms. The summary of the coefficient of similarity values for the 5 primers used with *S. binderi* and *S. oligocystum* are given in Table 11.

For primer OPA13, samples that showed F value > 0.75 for *S. binderi* is 94.67 % and 92.67 % for *S. oligocystum*. 95 % (*S. binderi*) and 74 % (*S. oligocystum*) of the samples gave a F value > 0.75 for primer OPK7. As for OPK9, 88.89% and 85.11% of samples from *S. binderi* and *S. oligocystum* presented a F value > 0.75, respectively. The F value > 0.75 amplified by OPN6 for *S. binderi* was 88.67% and

84.22% for *S. oligocystum* of all the samples examined. For OPN16, it was a 100% and 91% of samples from *S. binderi* and *S. oligocystum* that gave a F value > 0.75. Therefore, all the five primers used in this experiment showed high level (F value > 0.75) of intraspecific similarity for the two *Sargassum* species.

The two samples (species) exhibited different degrees of similarity when tested with different primers (Table 11). This is due to the fact that the primers amplify different regions of the DNA randomly. A primer that amplifies a more conserved region will give a higher degree of similarity whereas a higher dissimilarity will be obtained when a primer amplifies a more variable region of DNA.

The results of a comparison between *S. oligocystum* and *S. binderi*, is shown in Table 12. The results showed that *S. oligocystum* and *S. binderi* are not closely related as the range of the F values varies from 0.222 to 0.625 for OPA13; 0.190 to 0.667 for OPK7; 0.261 to 0.667 for OPK9; 0.250 to 0.720 for OPN6 and 0.281 to 0.632 for OPN16. The majority of the pairwise comparisons of the samples show that the two species of *Sargassum* are dissimilar (F value < 0.50) while less than 25% of the samples showed F value > 0.50. F value > 0.50 was shown in 13.89%, 16.56%, 21.33% and 24.33% using OPA13, OPK7, OPK9, OPN6, and OPN16, respectively.

Nei and Li's (1979) coefficient considers only the common presence of bands and errors may occur when considering the common presence of bands as the sole

evidence for identity. Similarly sized RAPD bands that co-migrate can have different sequences that represent different polymorphism. The problem of co-migration of non homologous bands may be a concern, particularly when testing for similarities between members of higher taxonomical levels (Hardy *et al.*, 1992). However, the possible co-migration of the RAPD-generated bands was not considered in this study.

Primer / Sample	OPA13	OPK7	OPK9	OPN6	OPN16
Sargassum binderi	0.588 - 1.000	0.737 - 1.000	0.609 - 1.000	0.615 - 1.000	0.783 - 1.000
Sargassum oligocystum	0.667 - 1.000	0.636 - 1.000	0.632 - 1.000	0.545 - 1.000	0.667 - 1.000

Table 11: Pairwise similarity coefficient (F value) of *S. binderi* and *S. oligocystum* (Intraspecies relationship studies)

Primer	F value of *Sargassum binderi* and *S. oligocystum*
OPA13	0.222 - 0.625
OPK7	0.190 - 0.667
OPK9	0.261 - 0.667
OPN6	0.250 - 0.720
OPN16	0.281 - 0.632

Table 12: Pairwise similarity coefficient (F value) between *Sargassum binderi* and *S. oligocystum* (Interspecies relationship studies)

Sample SB / SB	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.824																													
3	0.933	0.875																												
4	0.933	0.875	1.000																											
5	0.750	0.706	0.800	0.800																										
6	0.875	0.941	0.933	0.933	0.857																									
7	0.933	0.875	1.000	1.000	0.923	0.933																								
8	0.933	0.875	1.000	1.000	0.923	0.933	1.000																							
9	0.875	0.941	0.933	0.933	0.857	1.000	0.933	0.933																						
10	0.857	0.800	0.923	0.923	1.000	0.857	0.923	0.923	0.857																					
11	0.857	0.800	0.923	0.923	1.000	0.857	0.923	0.923	0.857	1.000																				
12	0.824	0.889	0.875	0.875	0.800	0.941	0.875	0.875	0.941	0.800	0.800																			
13	0.875	0.941	0.933	0.933	0.857	1.000	0.933	0.933	1.000	0.857	0.857	0.941																		
14	0.800	0.875	1.000	0.857	0.923	0.800	0.857	1.000	0.800	0.923	0.769	0.875	0.800																	
15	0.800	0.750	0.857	1.000	0.769	0.800	1.000	0.857	0.800	0.923	0.923	0.875	0.800	0.857																
16	0.875	0.706	0.933	0.800	0.857	0.750	0.800	0.800	0.750	0.857	0.857	0.824	0.750	0.800	0.933															
17	0.800	0.875	1.000	0.857	0.923	0.800	0.857	0.857	0.933	0.923	0.769	0.875	0.800	0.857	1.000	0.800														
18	0.941	0.778	0.875	0.875	0.933	0.588	0.750	0.875	0.706	0.800	0.933	0.889	0.588	0.875	0.875	0.941	0.875													
19	0.875	0.824	0.933	0.933	0.857	0.750	0.800	0.800	0.750	0.857	1.000	0.941	0.750	0.800	0.933	0.875	0.933	0.824												
20	0.941	0.888	0.750	0.875	0.800	0.706	0.750	0.750	0.588	0.800	0.800	0.889	0.706	0.750	0.750	0.824	0.750	0.889	0.706											
21	0.800	0.750	0.857	0.857	0.769	0.933	0.714	0.857	0.800	0.923	0.923	0.875	0.933	0.857	0.857	0.933	0.857	0.750	0.800	0.750										
22	0.800	0.875	1.000	0.857	0.923	0.800	0.857	0.857	0.800	0.923	0.769	0.875	0.800	0.857	1.000	0.800	1.000	0.750	0.667	0.750	0.857									
23	0.875	0.824	0.933	0.933	0.857	0.750	0.800	0.933	0.750	0.857	0.857	0.824	0.750	0.933	0.933	0.750	0.933	0.824	0.750	0.706	0.800	0.933								
24	0.875	0.706	0.800	0.800	0.857	0.625	0.933	0.933	0.750	0.714	0.857	0.824	0.625	0.933	0.800	0.875	0.800	0.824	0.750	0.706	0.800	0.800	0.750							
25	0.750	0.941	0.933	0.800	0.857	0.750	0.800	0.800	0.750	0.857	0.857	0.941	0.750	0.800	0.933	0.875	0.933	0.824	0.750	0.824	0.933	0.933	0.875	0.750						
26	0.941	0.889	0.875	0.875	0.800	0.706	0.750	0.750	0.706	0.800	0.933	0.889	0.706	0.750	0.875	0.824	0.875	0.778	0.824	0.778	0.875	0.875	0.824	0.706	0.824					
27	0.800	0.875	0.857	0.857	0.923	0.800	0.714	0.857	0.800	0.923	0.923	0.875	0.800	0.857	0.857	0.933	0.857	0.875	0.800	0.750	0.857	0.857	0.800	0.933	0.933	0.875				
28	0.800	0.750	0.857	0.714	0.923	0.800	0.857	0.857	0.800	0.923	0.923	0.875	0.800	0.857	0.857	0.933	0.857	0.875	0.800	0.875	0.857	0.857	0.800	0.933	0.800	0.750	0.857			
29	0.875	0.706	0.800	0.800	0.857	0.875	0.800	0.933	0.875	0.857	0.857	0.824	0.875	0.933	0.800	0.750	0.800	0.824	0.750	0.824	0.933	0.800	0.875	0.875	0.750	0.706	0.800	0.933		
30	0.875	0.824	0.933	0.800	1.000	0.875	0.800	0.800	0.750	0.857	0.857	0.824	0.875	0.800	0.933	0.750	0.933	0.824	0.750	0.824	0.800	0.933	0.875	0.750	0.875	0.824	0.800	0.800	0.750	

SB : *Sargassum binderi*

Table 13: F value of *Sargassum binderi* by OPA13 intraspecifically

Sample SB SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.375	0.471	0.267	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.235	0.375	0.400	0.400	0.375	0.400	0.471	0.375	0.353	0.267	0.400	0.375	0.375	0.375	0.471	0.267	0.400	0.375	0.375
2	0.353	0.444	0.250	0.375	0.400	0.353	0.375	0.375	0.353	0.400	0.400	0.222	0.353	0.375	0.250	0.353	0.375	0.222	0.471	0.333	0.375	0.500	0.353	0.235	0.353	0.444	0.250	0.500	0.353	0.471
3	0.353	0.444	0.250	0.375	0.400	0.353	0.375	0.375	0.353	0.400	0.400	0.222	0.353	0.375	0.500	0.471	0.375	0.333	0.353	0.333	0.375	0.375	0.353	0.353	0.353	0.222	0.375	0.250	0.353	0.353
4	0.353	0.444	0.250	0.375	0.400	0.353	0.375	0.375	0.353	0.400	0.400	0.222	0.353	0.250	0.375	0.353	0.500	0.222	0.353	0.333	0.375	0.375	0.353	0.353	0.353	0.333	0.250	0.375	0.233	0.471
5	0.375	0.471	0.267	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.233	0.375	0.400	0.400	0.500	0.533	0.471	0.500	0.471	0.533	0.533	0.500	0.500	0.500	0.471	0.400	0.533	0.500	0.375
6	0.375	0.471	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.233	0.375	0.400	0.533	0.375	0.533	0.353	0.375	0.353	0.400	0.400	0.375	0.375	0.375	0.353	0.267	0.400	0.250	0.375
7	0.375	0.471	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.233	0.375	0.267	0.400	0.500	0.533	0.588	0.375	0.353	0.533	0.400	0.500	0.500	0.375	0.353	0.267	0.267	0.375	0.500
8	0.353	0.444	0.375	0.375	0.400	0.353	0.375	0.375	0.353	0.400	0.400	0.222	0.353	0.250	0.375	0.471	0.500	0.444	0.353	0.333	0.500	0.500	0.471	0.471	0.353	0.222	0.250	0.500	0.353	0.588
9	0.375	0.471	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.235	0.375	0.400	0.400	0.500	0.400	0.471	0.375	0.471	0.533	0.533	0.500	0.500	0.500	0.471	0.400	0.533	0.375	0.375
10	0.471	0.556	0.500	0.500	0.533	0.471	0.500	0.500	0.471	0.400	0.533	0.333	0.471	0.375	0.500	0.471	0.500	0.333	0.353	0.333	0.375	0.375	0.353	0.353	0.353	0.333	0.375	0.375	0.471	0.353
11	0.375	0.471	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.235	0.375	0.400	0.267	0.375	0.533	0.353	0.500	0.471	0.533	0.533	0.500	0.500	0.500	0.471	0.533	0.533	0.500	0.375
12	0.375	0.588	0.533	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.353	0.500	0.533	0.400	0.500	0.400	0.353	0.500	0.471	0.533	0.533	0.500	0.500	0.500	0.471	0.400	0.533	0.500	0.375
13	0.500	0.471	0.533	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.353	0.500	0.533	0.400	0.250	0.400	0.353	0.375	0.353	0.400	0.400	0.375	0.375	0.375	0.353	0.400	0.400	0.375	0.500
14	0.375	0.353	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.353	0.375	0.400	0.267	0.250	0.533	0.353	0.375	0.353	0.400	0.400	0.375	0.375	0.375	0.353	0.400	0.400	0.375	0.375
15	0.500	0.471	0.533	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.353	0.500	0.400	0.533	0.250	0.400	0.353	0.375	0.353	0.400	0.400	0.375	0.375	0.500	0.353	0.400	0.400	0.250	0.375
16	0.500	0.353	0.400	0.400	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.353	0.375	0.400	0.533	0.500	0.400	0.471	0.500	0.471	0.533	0.533	0.500	0.500	0.500	0.471	0.533	0.533	0.375	0.500
17	0.250	0.353	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.353	0.375	0.267	0.400	0.500	0.533	0.353	0.500	0.471	0.533	0.533	0.500	0.375	0.500	0.471	0.400	0.400	0.375	0.500
18	0.471	0.444	0.375	0.500	0.533	0.471	0.500	0.500	0.471	0.533	0.533	0.333	0.471	0.500	0.500	0.471	0.500	0.444	0.471	0.444	0.500	0.500	0.471	0.471	0.471	0.444	0.375	0.500	0.471	0.353
19	0.471	0.556	0.500	0.500	0.533	0.471	0.500	0.500	0.471	0.533	0.400	0.333	0.471	0.375	0.500	0.588	0.375	0.222	0.235	0.444	0.500	0.375	0.471	0.471	0.588	0.444	0.500	0.375	0.235	0.236
20	0.471	0.444	0.375	0.375	0.400	0.353	0.375	0.500	0.353	0.533	0.533	0.444	0.471	0.375	0.375	0.471	0.500	0.333	0.353	0.333	0.375	0.375	0.471	0.363	0.471	0.444	0.500	0.500	0.353	0.353
21	0.500	0.471	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.353	0.375	0.533	0.400	0.375	0.400	0.235	0.375	0.471	0.400	0.400	0.375	0.375	0.375	0.353	0.400	0.400	0.375	0.375
22	0.471	0.444	0.375	0.375	0.400	0.353	0.375	0.375	0.353	0.400	0.400	0.333	0.353	0.375	0.375	0.471	0.500	0.444	0.471	0.444	0.500	0.500	0.471	0.471	0.471	0.444	0.375	0.250	0.233	0.471
23	0.376	0.353	0.400	0.400	0.429	0.375	0.400	0.400	0.375	0.429	0.429	0.235	0.375	0.533	0.400	0.250	0.533	0.471	0.375	0.471	0.533	0.533	0.667	0.500	0.500	0.375	0.235	0.400	0.400	0.375
24	0.471	0.444	0.375	0.250	0.400	0.353	0.375	0.250	0.353	0.400	0.400	0.222	0.353	0.375	0.500	0.353	0.375	0.333	0.353	0.333	0.375	0.375	0.353	0.471	0.353	0.333	0.375	0.250	0.353	0.471
25	0.588	0.556	0.500	0.500	0.533	0.471	0.500	0.500	0.471	0.533	0.533	0.444	0.471	0.375	0.500	0.471	0.500	0.444	0.353	0.333	0.500	0.375	0.471	0.588	0.353	0.222	0.250	0.500	0.471	0.353
26	0.625	0.588	0.533	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.471	0.500	0.533	0.400	0.500	0.533	0.471	0.375	0.471	0.533	0.400	0.500	0.375	0.375	0.471	0.533	0.533	0.500	0.500
27	0.588	0.556	0.500	0.500	0.533	0.471	0.500	0.500	0.471	0.533	0.533	0.444	0.471	0.375	0.500	0.353	0.375	0.333	0.353	0.333	0.375	0.375	0.353	0.471	0.471	0.444	0.500	0.500	0.471	0.471
28	0.588	0.556	0.500	0.500	0.533	0.471	0.500	0.500	0.471	0.533	0.533	0.444	0.471	0.375	0.500	0.471	0.500	0.444	0.471	0.333	0.500	0.375	0.471	0.471	0.471	0.444	0.500	0.500	0.353	0.471
29	0.625	0.471	0.400	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.471	0.500	0.400	0.533	0.500	0.533	0.471	0.500	0.353	0.533	0.533	0.500	0.500	0.375	0.353	0.533	0.400	0.500	0.375
30	0.625	0.588	0.533	0.533	0.571	0.500	0.533	0.533	0.500	0.571	0.571	0.353	0.500	0.400	0.533	0.500	0.533	0.353	0.375	0.471	0.400	0.533	0.625	0.375	0.250	0.235	0.533	0.533	0.375	0.500

SB : *Sargassum binderi*
SO : *S. oligocystum*

Table 15: F value of *Sargassum binderi* and *S. oligocystum* by OPA13 interspecifically

Sample SB SB	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.900																													
3	0.800	0.778																												
4	0.857	0.842	0.842																											
5	0.857	0.947	0.737	0.900																										
6	0.909	0.900	0.900	0.857	0.857																									
7	0.842	0.941	0.824	0.889	0.889	0.842																								
8	0.842	0.941	0.824	0.889	0.889	0.842	1.000																							
9	0.800	0.889	0.778	0.842	0.947	0.800	0.824	0.824																						
10	0.952	0.947	0.737	0.900	1.000	0.857	0.889	0.889	0.947																					
11	0.800	0.889	0.778	0.842	0.947	0.800	0.824	0.824	1.000	0.947																				
12	0.900	1.000	0.778	0.842	0.842	0.900	0.941	0.941	0.889	0.947	0.889																			
13	0.857	0.842	0.842	0.900	0.900	0.952	0.889	0.778	0.842	0.900	0.842	0.947																		
14	0.818	0.800	0.800	0.952	0.857	0.909	0.842	0.737	0.800	0.952	0.800	0.800	0.857																	
15	0.800	0.778	0.889	0.947	0.947	0.800	0.941	0.941	0.889	0.947	0.778	0.889	0.842	0.947																
16	0.800	0.889	0.889	0.842	0.842	0.900	0.824	0.824	0.889	0.947	0.778	0.889	0.842	0.947	0.889															
17	0.842	0.941	0.824	0.889	0.889	0.842	0.875	0.875	0.824	0.778	0.824	0.941	0.778	0.889	0.941	0.778														
18	0.842	0.824	0.824	0.889	0.889	0.842	0.875	0.875	0.824	0.778	0.824	0.824	0.778	0.889	0.941	0.889	0.941													
19	0.900	0.889	0.889	0.842	0.947	0.800	0.824	0.824	0.778	0.842	0.889	0.778	0.947	0.842	0.778	0.947	0.842	0.889												
20	0.857	0.842	0.947	0.900	0.900	0.857	0.889	0.889	0.737	0.900	0.947	0.842	0.900	0.900	0.737	0.800	0.842	0.737	0.889											
21	0.857	0.947	0.947	0.800	0.800	0.857	0.889	0.889	0.842	0.800	0.842	0.737	0.800	0.800	0.842	0.800	0.842	0.842	0.889	0.842										
22	0.800	0.778	0.889	0.842	0.947	0.800	0.941	0.824	0.889	0.737	0.778	0.889	0.842	0.737	0.889	0.842	0.889	1.000	0.824	0.778	0.824									
23	0.800	0.778	0.778	0.842	0.947	0.800	0.824	0.824	0.889	0.842	0.889	0.889	0.842	0.737	0.889	0.842	0.889	1.000	0.941	0.889	0.824	0.824								
24	0.857	0.842	0.947	0.900	0.900	0.857	0.778	0.889	0.737	0.900	0.947	0.842	0.900	0.800	0.737	0.800	0.737	0.737	0.778	0.737	0.889	0.889	0.800							
25	0.952	0.947	0.842	0.800	0.900	0.857	0.778	0.889	0.842	0.900	0.947	0.842	0.800	0.800	0.842	0.900	0.842	0.842	0.889	0.842	0.889	0.889	0.800	0.947						
26	0.950	0.947	0.842	0.900	0.800	0.762	0.889	0.778	0.947	0.800	0.842	0.737	0.800	0.800	0.947	0.900	0.842	0.842	0.889	0.842	0.778	0.778	0.800	0.842	0.900					
27	0.909	0.800	0.900	0.952	0.952	0.909	0.947	0.842	0.900	0.857	0.900	0.800	0.857	0.952	0.900	0.952	0.900	0.900	0.737	0.900	0.842	0.737	0.762	0.800	0.762	0.762				
28	0.800	0.889	0.778	0.947	0.947	0.800	0.824	0.824	0.889	0.737	0.778	0.889	0.947	0.947	0.889	0.842	0.778	0.889	0.824	0.778	0.824	0.941	0.947	0.889	0.842	0.842	0.900			
29	0.900	0.778	0.778	0.947	0.842	0.800	0.824	0.824	0.778	0.842	0.778	0.778	0.842	0.842	0.889	0.737	0.778	0.889	0.941	0.889	0.824	0.824	0.842	0.889	0.737	0.737	0.800	0.778		
30	0.947	0.941	0.824	0.778	0.778	0.842	0.875	0.875	0.824	0.778	0.824	0.941	0.889	0.778	0.941	0.778	0.824	0.824	0.875	0.824	0.875	0.857	0.889	0.941	0.778	0.778	0.842	0.824	0.889	

SB : *Sargassum binderi*

Table 16: *F* value of *Sargassum binderi* by OPI7 intraspecifically

Sample SO / SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	1.000																													
3	1.000	1.000																												
4	0.952	0.952	0.952																											
5	0.952	0.952	0.952	1.000																										
6	0.800	0.800	0.800	0.857	0.857																									
7	0.900	0.900	0.900	0.952	0.952	0.900																								
8	0.842	0.842	0.842	0.800	0.800	0.737	0.842																							
9	0.857	0.857	0.857	0.818	0.818	0.857	0.857	0.800																						
10	0.947	0.947	0.947	0.900	0.900	0.842	0.842	0.778	0.800																					
11	0.857	0.857	0.857	0.818	0.818	0.762	0.762	0.800	0.818	0.800																				
12	0.762	0.762	0.762	0.727	0.727	0.667	0.667	0.700	0.636	0.700	0.727																			
13	0.737	0.737	0.737	0.700	0.700	0.737	0.737	0.667	0.800	0.667	0.800	0.900																		
14	0.762	0.762	0.762	0.727	0.727	0.667	0.667	0.700	0.636	0.700	0.727	1.000	0.900																	
15	0.700	0.700	0.700	0.667	0.667	0.700	0.700	0.737	0.762	0.842	0.762	0.952	0.947	0.952																
16	0.700	0.700	0.700	0.667	0.667	0.700	0.700	0.737	0.762	0.842	0.667	0.952	0.947	0.952	1.000															
17	0.762	0.762	0.762	0.727	0.727	0.667	0.667	0.700	0.636	0.700	0.727	1.000	0.900	1.000	0.952	0.952														
18	0.762	0.762	0.762	0.727	0.727	0.667	0.667	0.700	0.636	0.700	0.727	1.000	0.900	1.000	0.952	0.952	1.000													
19	0.952	0.952	0.952	0.909	0.909	0.857	0.857	0.800	0.818	0.900	0.818	0.727	0.700	0.727	0.762	0.762	0.727	0.727												
20	0.700	0.700	0.700	0.667	0.667	0.700	0.700	0.737	0.762	0.842	0.667	0.857	0.947	0.952	1.000	1.000	0.952	0.952	0.667											
21	0.762	0.762	0.762	0.727	0.727	0.667	0.667	0.700	0.727	0.800	0.727	1.000	0.900	1.000	0.952	0.952	1.000	1.000	0.727	0.952										
22	0.800	0.800	0.800	0.762	0.667	0.700	0.800	0.797	0.762	0.842	0.762	0.952	0.842	0.952	0.900	0.900	0.952	0.952	0.667	0.900	0.952									
23	0.800	0.800	0.800	0.762	0.762	0.800	0.700	0.737	0.762	0.737	0.762	0.952	0.842	0.952	0.900	0.900	0.952	0.952	0.667	0.900	0.952	0.900								
24	0.700	0.700	0.700	0.667	0.667	0.700	0.800	0.842	0.667	0.737	0.762	0.952	0.947	0.952	1.000	1.000	0.952	0.952	0.762	1.000	0.952	0.900	0.900							
25	0.818	0.909	0.818	0.696	0.870	0.818	1.000	0.762	0.696	0.857	0.696	0.870	0.762	0.870	0.909	0.818	0.870	0.870	0.783	0.909	0.783	0.909	0.727	0.727						
26	0.952	0.857	0.762	0.909	0.909	0.762	0.952	0.800	0.727	0.900	0.818	0.909	0.800	0.909	0.857	0.857	0.909	0.909	0.727	0.952	0.818	0.952	0.762	0.762	0.783					
27	0.857	0.857	0.762	0.818	0.909	0.952	0.952	0.800	0.727	0.800	0.818	0.909	0.900	0.909	0.857	0.857	0.818	0.818	0.727	0.952	0.818	0.857	0.667	0.762	0.957	0.857				
28	0.857	0.857	0.857	0.909	0.909	0.857	0.857	0.800	0.818	0.800	0.727	0.818	0.900	0.909	0.952	0.857	0.818	0.909	0.818	0.952	0.818	0.857	0.667	0.857	0.870	0.857	0.909			
29	0.909	0.909	0.818	0.870	0.870	0.818	0.818	0.667	0.783	0.762	0.783	0.783	0.857	0.870	0.818	0.818	0.870	0.957	0.696	0.818	0.696	0.818	0.727	0.909	0.833	0.818	0.870	0.870		
30	0.909	0.727	0.818	0.870	0.870	0.909	0.818	0.667	0.783	0.667	0.696	0.870	0.857	0.870	0.727	0.818	0.870	0.952	0.783	0.909	0.783	0.909	0.727	0.818	0.750	0.909	0.783	0.870	0.833	

SO : *Sargassum oligocystum*

Table 17: F value of *Sargassum oligocystum* by OPKT intraspecifically

Sample SB SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.381	0.526	0.526	0.600	0.600	0.476	0.444	0.444	0.421	0.500	0.421	0.421	0.400	0.286	0.316	0.421	0.444	0.556	0.526	0.400	0.400	0.421	0.421	0.300	0.400	0.400	0.286	0.632	0.421	0.444
2	0.286	0.316	0.316	0.400	0.400	0.381	0.333	0.333	0.421	0.400	0.421	0.316	0.300	0.286	0.316	0.316	0.333	0.333	0.421	0.400	0.400	0.316	0.316	0.400	0.400	0.400	0.400	0.381	0.526	0.556
3	0.476	0.526	0.526	0.600	0.600	0.381	0.444	0.556	0.421	0.500	0.526	0.526	0.300	0.476	0.316	0.316	0.556	0.556	0.632	0.400	0.400	0.421	0.421	0.400	0.500	0.400	0.381	0.421	0.632	0.556
4	0.545	0.600	0.600	0.478	0.478	0.455	0.316	0.632	0.400	0.381	0.400	0.400	0.286	0.364	0.300	0.300	0.316	0.316	0.500	0.286	0.476	0.400	0.400	0.286	0.476	0.476	0.455	0.400	0.400	0.526
5	0.455	0.500	0.500	0.478	0.478	0.364	0.526	0.421	0.400	0.476	0.300	0.300	0.190	0.273	0.200	0.200	0.421	0.421	0.400	0.381	0.381	0.400	0.400	0.381	0.476	0.476	0.455	0.300	0.500	0.421
6	0.476	0.526	0.526	0.500	0.500	0.381	0.444	0.333	0.421	0.400	0.421	0.316	0.400	0.286	0.421	0.421	0.556	0.556	0.421	0.300	0.400	0.421	0.421	0.300	0.500	0.400	0.476	0.421	0.526	0.556
7	0.381	0.421	0.421	0.400	0.400	0.286	0.333	0.556	0.526	0.400	0.421	0.421	0.300	0.381	0.316	0.316	0.444	0.444	0.421	0.300	0.400	0.421	0.421	0.300	0.400	0.400	0.286	0.526	0.421	0.444
8	0.500	0.556	0.556	0.526	0.526	0.300	0.471	0.588	0.444	0.526	0.556	0.444	0.316	0.400	0.333	0.333	0.471	0.471	0.556	0.316	0.421	0.556	0.556	0.316	0.421	0.421	0.400	0.556	0.444	0.471
9	0.364	0.400	0.400	0.381	0.381	0.455	0.421	0.632	0.500	0.381	0.500	0.400	0.381	0.364	0.400	0.400	0.316	0.316	0.500	0.381	0.476	0.500	0.500	0.381	0.381	0.381	0.364	0.400	0.500	0.421
10	0.400	0.444	0.444	0.421	0.421	0.400	0.471	0.688	0.444	0.421	0.556	0.444	0.421	0.400	0.444	0.444	0.353	0.353	0.444	0.316	0.421	0.556	0.556	0.316	0.421	0.526	0.500	0.556	0.556	0.471
11	0.364	0.400	0.500	0.286	0.381	0.364	0.526	0.421	0.400	0.571	0.300	0.300	0.381	0.364	0.500	0.300	0.526	0.316	0.400	0.571	0.476	0.300	0.500	0.381	0.381	0.381	0.364	0.500	0.400	0.526
12	0.455	0.500	0.300	0.478	0.286	0.455	0.632	0.421	0.500	0.381	0.400	0.500	0.381	0.455	0.500	0.400	0.421	0.421	0.300	0.381	0.476	0.400	0.500	0.476	0.381	0.476	0.364	0.400	0.500	0.526
13	0.500	0.444	0.444	0.526	0.421	0.500	0.588	0.353	0.556	0.526	0.444	0.556	0.526	0.500	0.556	0.444	0.471	0.353	0.333	0.421	0.526	0.444	0.444	0.526	0.316	0.526	0.400	0.444	0.667	0.588
14	0.364	0.400	0.400	0.478	0.476	0.364	0.421	0.526	0.500	0.381	0.500	0.400	0.286	0.364	0.300	0.300	0.421	0.421	0.500	0.286	0.381	0.400	0.400	0.286	0.381	0.476	0.273	0.300	0.400	0.526
15	0.381	0.316	0.421	0.400	0.500	0.381	0.444	0.444	0.421	0.500	0.421	0.526	0.400	0.476	0.421	0.421	0.333	0.556	0.421	0.500	0.300	0.421	0.526	0.300	0.300	0.400	0.476	0.421	0.421	0.556
16	0.286	0.421	0.316	0.500	0.400	0.286	0.444	0.556	0.421	0.500	0.526	0.526	0.500	0.381	0.421	0.316	0.333	0.444	0.421	0.400	0.400	0.526	0.526	0.600	0.300	0.300	0.381	0.526	0.632	0.333
17	0.364	0.400	0.500	0.478	0.381	0.545	0.316	0.526	0.500	0.381	0.500	0.400	0.476	0.273	0.500	0.500	0.421	0.421	0.600	0.381	0.286	0.500	0.400	0.381	0.286	0.381	0.273	0.600	0.400	0.421
18	0.455	0.300	0.400	0.286	0.286	0.455	0.421	0.526	0.500	0.571	0.400	0.400	0.381	0.545	0.400	0.400	0.316	0.316	0.400	0.286	0.476	0.400	0.300	0.476	0.381	0.286	0.273	0.400	0.500	0.421
19	0.364	0.400	0.300	0.381	0.476	0.455	0.421	0.421	0.400	0.476	0.400	0.300	0.286	0.455	0.400	0.400	0.421	0.421	0.500	0.381	0.476	0.300	0.300	0.476	0.571	0.476	0.455	0.400	0.500	0.526
20	0.381	0.421	0.421	0.400	0.500	0.381	0.556	0.444	0.526	0.500	0.316	0.421	0.500	0.381	0.421	0.421	0.333	0.556	0.526	0.300	0.400	0.316	0.421	0.400	0.500	0.500	0.476	0.316	0.526	0.444
21	0.455	0.500	0.500	0.381	0.381	0.455	0.526	0.526	0.600	0.381	0.600	0.500	0.286	0.455	0.300	0.300	0.526	0.526	0.500	0.381	0.476	0.400	0.400	0.381	0.476	0.381	0.364	0.300	0.500	0.526
22	0.381	0.316	0.421	0.500	0.400	0.571	0.444	0.556	0.421	0.400	0.632	0.632	0.500	0.381	0.526	0.421	0.444	0.556	0.316	0.500	0.500	0.316	0.526	0.500	0.600	0.400	0.476	0.526	0.526	0.556
23	0.381	0.421	0.421	0.400	0.500	0.476	0.556	0.556	0.526	0.421	0.400	0.526	0.421	0.500	0.381	0.421	0.421	0.444	0.632	0.500	0.400	0.316	0.421	0.500	0.300	0.500	0.381	0.526	0.421	0.444
24	0.286	0.316	0.526	0.500	0.500	0.476	0.556	0.444	0.632	0.500	0.526	0.421	0.400	0.286	0.316	0.316	0.333	0.444	0.526	0.400	0.400	0.421	0.632	0.400	0.400	0.400	0.571	0.421	0.316	0.444
25	0.348	0.476	0.286	0.455	0.455	0.348	0.400	0.400	0.381	0.364	0.381	0.286	0.364	0.348	0.286	0.381	0.400	0.400	0.476	0.545	0.273	0.381	0.476	0.455	0.364	0.455	0.435	0.381	0.381	0.300
26	0.455	0.400	0.400	0.381	0.286	0.364	0.632	0.316	0.500	0.286	0.500	0.300	0.476	0.455	0.500	0.400	0.316	0.316	0.600	0.571	0.571	0.500	0.500	0.381	0.381	0.476	0.455	0.400	0.300	0.632
27	0.364	0.300	0.400	0.381	0.476	0.273	0.526	0.316	0.500	0.381	0.300	0.500	0.381	0.455	0.500	0.600	0.316	0.316	0.300	0.476	0.571	0.400	0.300	0.286	0.476	0.381	0.455	0.600	0.600	0.632
28	0.455	0.400	0.500	0.286	0.381	0.364	0.526	0.526	0.500	0.381	0.300	0.400	0.381	0.364	0.600	0.600	0.421	0.526	0.400	0.571	0.381	0.500	0.400	0.381	0.571	0.476	0.455	0.400	0.500	0.526
29	0.348	0.381	0.381	0.364	0.364	0.364	0.348	0.400	0.400	0.286	0.455	0.381	0.381	0.273	0.522	0.381	0.476	0.300	0.400	0.381	0.364	0.455	0.476	0.381	0.364	0.545	0.455	0.348	0.571	0.381
30	0.348	0.381	0.286	0.364	0.455	0.435	0.500	0.400	0.381	0.455	0.571	0.476	0.455	0.522	0.476	0.381	0.300	0.500	0.381	0.455	0.455	0.476	0.476	0.545	0.455	0.364	0.348	0.476	0.571	0.400

SB : *Sargassum binderi*
SO : *S. oligocystum*

Table 18: F value of *Sargassum binderi* and *S. oligocystum* by OPK1 interspecifically

Sample SB / SB	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.952																													
3	0.957	0.909																												
4	1.000	0.952	0.957																											
5	0.952	1.000	0.909	0.952																										
6	0.957	0.909	1.000	0.957	0.909																									
7	0.952	1.000	0.909	0.952	1.000	0.909																								
8	1.000	0.952	0.957	1.000	0.952	0.957	0.952																							
9	1.000	0.952	0.957	1.000	0.952	0.957	0.952	1.000																						
10	0.952	1.000	0.909	0.952	1.000	0.909	1.000	0.952	0.952																					
11	0.952	1.000	0.909	0.952	1.000	0.909	1.000	0.952	0.952	1.000																				
12	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909																			
13	0.952	1.000	0.909	0.952	1.000	0.909	1.000	0.952	0.952	1.000	1.000	0.909																		
14	1.000	0.952	0.957	1.000	0.952	0.957	0.952	1.000	1.000	0.952	0.952	0.957	0.952																	
15	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909	1.000	0.909	0.957																
16	0.952	1.000	0.909	0.952	1.000	0.909	1.000	0.952	0.952	1.000	1.000	0.909	1.000	0.952	0.909															
17	0.952	1.000	0.909	0.952	1.000	0.909	1.000	0.952	0.952	1.000	1.000	0.909	1.000	0.952	0.909	1.000														
18	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909	1.000	0.909	0.957	1.000	0.909	0.909													
19	0.909	0.952	0.957	0.909	0.952	0.957	0.952	0.909	0.909	0.952	0.952	0.957	0.952	0.909	0.957	0.952	0.952	0.957												
20	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909	1.000	0.909	0.957	1.000	0.909	0.909	1.000	0.957											
21	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909	1.000	0.909	0.957	1.000	0.909	0.909	1.000	0.957	1.000										
22	1.000	0.952	0.957	1.000	0.952	0.957	0.952	1.000	1.000	0.952	0.952	0.957	0.952	1.000	0.957	0.952	0.952	0.957	0.909	0.957	0.957									
23	0.957	0.909	1.000	0.957	0.909	1.000	0.909	0.957	0.957	0.909	0.909	1.000	0.909	0.957	1.000	0.909	0.909	1.000	0.957	1.000	1.000	0.957								
24	0.909	0.952	0.870	0.909	0.952	0.870	0.952	0.909	0.909	0.952	0.952	0.870	0.952	0.909	0.864	0.952	0.952	0.870	0.909	0.870	0.870	0.909	0.870							
25	0.957	0.909	0.917	0.957	0.909	0.917	0.909	0.957	0.957	0.909	0.909	0.917	0.909	0.957	0.917	0.909	0.909	0.917	0.870	0.917	0.917	0.957	0.917	0.870						
26	0.857	1.000	1.000	0.857	1.000	1.000	1.000	0.857	0.857	1.000	1.000	1.000	1.000	0.857	1.000	1.000	1.000	1.000	0.857	1.000	1.000	0.857	1.000	0.857	0.818					
27	0.909	0.952	0.870	0.909	0.952	0.870	0.952	0.909	0.909	0.952	0.952	0.870	0.952	0.909	0.870	0.952	0.952	0.870	0.909	0.870	0.870	0.909	0.870	0.909	0.870	0.952				
28	0.818	0.952	0.870	0.818	0.952	0.870	0.952	0.818	0.818	0.952	0.952	0.870	0.952	0.818	0.870	0.952	0.952	0.870	0.909	0.870	0.870	0.808	0.870	0.909	0.783	0.952	0.909			
29	0.870	0.909	0.833	0.870	0.909	0.833	0.909	0.870	0.870	0.909	0.909	0.833	0.909	0.870	0.833	0.909	0.909	0.833	0.870	0.833	0.833	0.870	0.833	0.870	0.833	0.909	0.870	0.870		
30	0.857	1.000	0.909	0.857	1.000	0.909	1.000	0.857	0.857	1.000	1.000	0.909	1.000	0.857	0.909	1.000	1.000	0.909	0.857	0.909	0.909	0.857	0.909	0.857	0.818	1.000	0.952	0.857	0.909	

SB : *Sargassum binderi*

Table 19: F value of *Sargassum binderi* by OPN16 intraspecifically

Sample SO / SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.889																													
3	0.889	0.889																												
4	0.778	0.778	0.778																											
5	0.842	0.842	0.842	0.842																										
6	0.889	0.778	0.778	0.778	0.842																									
7	0.778	0.778	0.778	0.778	0.737	0.778																								
8	0.889	0.889	0.889	0.889	0.842	0.889	0.778																							
9	0.778	0.778	0.778	0.778	0.947	0.778	0.889	0.667																						
10	0.889	0.778	0.889	0.667	0.842	0.889	0.778	0.778	0.778																					
11	0.824	0.941	0.824	0.706	0.778	0.826	0.706	0.706	0.706	0.706																				
12	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.824																			
13	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.824	1.000																		
14	0.889	0.889	0.889	0.889	0.947	0.778	0.889	0.889	0.889	0.778	0.824	0.889	0.889																	
15	0.889	1.000	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.941	0.889	0.889	0.889																
16	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.824	1.000	1.000	0.889	0.889															
17	0.889	0.778	0.778	0.778	0.737	0.667	0.667	0.778	0.889	0.667	0.824	0.889	0.889	0.889	0.778	0.889														
18	0.778	0.889	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.941	0.889	0.889	0.889	0.889	0.889	0.889													
19	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.778	0.778	0.778	0.941	0.889	0.889	0.889	0.889	0.889	0.889	1.000												
20	0.889	0.889	0.889	0.667	0.842	0.778	0.778	0.889	0.778	0.778	0.941	0.889	0.889	0.889	0.889	0.889	0.889	1.000	1.000											
21	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.778	0.778	0.778	0.941	0.889	0.889	0.889	0.889	0.889	0.889	1.000	1.000	1.000										
22	1.000	0.889	0.889	0.778	0.842	0.889	0.778	0.889	0.778	0.889	0.824	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889									
23	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.778	0.778	0.778	0.941	0.889	0.889	0.889	0.889	0.889	0.889	1.000	1.000	1.000	1.000	0.889								
24	0.889	1.000	0.889	0.778	0.842	0.778	0.778	0.778	0.778	0.778	0.941	0.889	0.889	0.889	1.000	0.889	0.889	0.889	0.889	0.889	0.889	0.889	0.889							
25	0.778	0.778	0.778	0.889	0.842	0.778	0.778	0.889	0.778	0.667	0.706	0.778	0.778	0.889	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778	0.778						
26	0.824	0.824	0.824	0.706	0.778	0.706	0.706	0.824	0.706	0.706	0.750	0.824	0.824	0.824	0.824	0.824	0.706	0.706	0.706	0.706	0.706	0.824	0.706	0.824	0.706					
27	0.941	0.824	0.706	0.706	0.778	0.824	0.824	0.706	0.706	0.706	0.750	0.824	0.824	0.824	0.824	0.824	0.824	0.706	0.706	0.706	0.706	0.941	0.706	0.824	0.706	0.750				
28	0.889	1.000	0.889	0.778	0.842	0.778	0.778	0.889	0.778	0.778	0.824	0.889	0.889	0.889	1.000	0.889	0.889	0.778	0.778	0.778	0.778	0.889	0.778	1.000	0.778	0.824	0.824			
29	0.778	0.889	0.778	0.667	0.737	0.889	0.667	0.778	0.778	0.778	0.824	0.889	0.889	0.889	0.889	0.889	0.889	0.778	0.778	0.778	0.778	0.778	0.889	0.667	0.824	0.941	0.889			
30	0.889	0.889	0.889	0.778	0.842	0.778	0.778	0.778	0.667	0.778	0.941	0.778	0.778	0.778	0.889	0.778	0.778	0.778	0.778	0.778	0.778	0.889	0.889	0.889	0.778	0.941	0.824	0.889	0.889	

SO : *Sargassum oligocystum*

Table 20: F value of *Sargassum oligocystum* by OPN16 intraspecifically

Sample SB SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.400	0.421	0.476	0.500	0.476	0.476	0.421	0.500	0.500	0.444	0.526	0.476	0.500	0.500	0.571	0.421	0.421	0.476	0.500	0.476	0.381	0.400	0.381	0.421	0.500	0.500	0.476	0.400	0.421	0.500
2	0.500	0.526	0.476	0.500	0.400	0.571	0.526	0.600	0.500	0.500	0.500	0.476	0.476	0.600	0.571	0.632	0.526	0.381	0.500	0.571	0.287	0.300	0.381	0.526	0.400	0.500	0.476	0.500	0.526	0.400
3	0.400	0.421	0.571	0.500	0.526	0.476	0.526	0.500	0.400	0.526	0.444	0.472	0.472	0.500	0.476	0.526	0.421	0.381	0.500	0.381	0.038	0.300	0.476	0.526	0.500	0.500	0.571	0.500	0.526	0.500
4	0.500	0.632	0.571	0.500	0.455	0.571	0.632	0.500	0.400	0.421	0.500	0.571	0.500	0.500	0.476	0.632	0.526	0.571	0.400	0.381	0.286	0.600	0.476	0.421	0.400	0.500	0.381	0.500	0.526	0.500
5	0.571	0.500	0.455	0.476	0.400	0.455	0.500	0.476	0.381	0.500	0.500	0.455	0.500	0.571	0.455	0.500	0.400	0.455	0.476	0.545	0.364	0.381	0.455	0.500	0.381	0.476	0.455	0.476	0.500	0.381
6	0.400	0.421	0.381	0.500	0.444	0.476	0.526	0.500	0.500	0.526	0.421	0.476	0.526	0.400	0.381	0.421	0.421	0.476	0.400	0.476	0.476	0.476	0.500	0.571	0.421	0.400	0.400	0.476	0.400	0.421	0.500
7	0.500	0.526	0.476	0.500	0.526	0.476	0.526	0.500	0.400	0.400	0.526	0.476	0.444	0.400	0.571	0.526	0.526	0.476	0.500	0.571	0.476	0.500	0.381	0.526	0.500	0.500	0.500	0.476	0.500	0.526	0.500
8	0.500	0.421	0.476	0.500	0.476	0.571	0.500	0.476	0.600	0.632	0.500	0.571	0.500	0.500	0.476	0.632	0.526	0.476	0.500	0.500	0.476	0.600	0.476	0.500	0.500	0.500	0.476	0.476	0.500	0.421	0.500
9	0.400	0.444	0.381	0.400	0.421	0.381	0.421	0.400	0.400	0.421	0.421	0.381	0.421	0.400	0.476	0.421	0.421	0.476	0.300	0.381	0.476	0.500	0.476	0.421	0.300	0.400	0.476	0.500	0.421	0.500
10	0.300	0.421	0.381	0.400	0.421	0.381	0.421	0.400	0.400	0.421	0.421	0.381	0.421	0.300	0.476	0.421	0.421	0.476	0.400	0.476	0.571	0.400	0.571	0.421	0.500	0.500	0.571	0.400	0.421	0.400
11	0.526	0.444	0.500	0.381	0.556	0.476	0.556	0.500	0.526	0.526	0.400	0.600	0.556	0.632	0.500	0.476	0.556	0.500	0.500	0.600	0.300	0.316	0.400	0.556	0.526	0.421	0.400	0.421	0.444	0.421
12	0.500	0.632	0.455	0.600	0.476	0.500	0.500	0.600	0.600	0.556	0.632	0.571	0.526	0.500	0.476	0.500	0.526	0.476	0.500	0.476	0.381	0.400	0.381	0.476	0.500	0.500	0.476	0.476	0.500	0.476
13	0.500	0.500	0.571	0.500	0.500	0.571	0.632	0.600	0.600	0.556	0.500	0.571	0.526	0.500	0.476	0.476	0.526	0.476	0.500	0.476	0.381	0.400	0.381	0.444	0.500	0.400	0.476	0.500	0.421	0.500
14	0.600	0.526	0.400	0.500	0.444	0.476	0.500	0.500	0.400	0.526	0.421	0.476	0.545	0.400	0.421	0.500	0.526	0.571	0.500	0.476	0.381	0.500	0.571	0.500	0.500	0.400	0.476	0.300	0.421	0.500
15	0.500	0.632	0.571	0.600	0.632	0.476	0.526	0.500	0.500	0.556	0.526	0.476	0.526	0.476	0.526	0.400	0.476	0.500	0.526	0.381	0.500	0.476	0.500	0.476	0.421	0.400	0.300	0.476	0.400	0.421	0.400
16	0.500	0.444	0.476	0.500	0.500	0.571	0.526	0.500	0.500	0.526	0.500	0.476	0.526	0.400	0.571	0.500	0.526	0.472	0.500	0.476	0.526	0.400	0.476	0.526	0.500	0.400	0.476	0.476	0.500	0.526	0.600
17	0.400	0.381	0.476	0.300	0.526	0.476	0.526	0.600	0.500	0.600	0.632	0.476	0.526	0.400	0.381	0.526	0.500	0.476	0.600	0.476	0.526	0.500	0.476	0.421	0.600	0.500	0.476	0.526	0.500	0.526	0.400
18	0.400	0.421	0.476	0.500	0.421	0.476	0.526	0.400	0.500	0.421	0.421	0.381	0.526	0.300	0.281	0.421	0.556	0.381	0.500	0.476	0.526	0.500	0.476	0.526	0.400	0.500	0.476	0.500	0.526	0.500
19	0.400	0.421	0.476	0.500	0.421	0.476	0.526	0.400	0.500	0.421	0.421	0.381	0.526	0.300	0.281	0.421	0.556	0.381	0.500	0.476	0.526	0.500	0.476	0.526	0.400	0.500	0.476	0.500	0.526	0.500
20	0.400	0.421	0.476	0.500	0.421	0.476	0.526	0.400	0.500	0.421	0.421	0.381	0.526	0.300	0.281	0.421	0.556	0.381	0.500	0.476	0.526	0.500	0.476	0.526	0.400	0.500	0.476	0.500	0.526	0.500
21	0.400	0.421	0.476	0.500	0.421	0.476	0.526	0.400	0.500	0.421	0.421	0.381	0.526	0.300	0.281	0.421	0.556	0.381	0.500	0.476	0.526	0.500	0.476	0.526	0.400	0.500	0.381	0.500	0.316	0.500
22	0.400	0.421	0.476	0.500	0.421	0.381	0.421	0.500	0.400	0.421	0.421	0.476	0.421	0.400	0.381	0.421	0.526	0.286	0.500	0.381	0.381	0.500	0.571	0.526	0.500	0.400	0.381	0.500	0.526	0.500
23	0.500	0.526	0.476	0.500	0.526	0.381	0.421	0.500	0.400	0.526	0.526	0.476	0.526	0.400	0.571	0.500	0.421	0.476	0.500	0.476	0.476	0.600	0.476	0.421	0.500	0.400	0.476	0.500	0.421	0.400
24	0.600	0.632	0.571	0.600	0.632	0.381	0.526	0.500	0.500	0.526	0.632	0.476	0.500	0.500	0.500	0.526	0.632	0.571	0.400	0.571	0.476	0.500	0.476	0.421	0.500	0.500	0.381	0.500	0.421	0.400
25	0.526	0.556	0.400	0.526	0.556	0.400	0.444	0.421	0.421	0.444	0.444	0.400	0.444	0.421	0.500	0.556	0.444	0.400	0.421	0.500	0.500	0.500	0.400	0.444	0.476	0.526	0.500	0.632	0.556	0.500
26	0.421	0.444	0.400	0.421	0.444	0.300	0.444	0.526	0.421	0.444	0.556	0.500	0.476	0.526	0.500	0.556	0.556	0.500	0.526	0.500	0.500	0.526	0.500	0.556	0.421	0.556	0.600	0.632	0.526	0.526
27	0.316	0.333	0.400	0.316	0.333	0.400	0.444	0.421	0.421	0.333	0.333	0.500	0.444	0.421	0.500	0.333	0.444	0.400	0.421	0.300	0.500	0.421	0.400	0.500	0.556	0.526	0.400	0.421	0.444	0.500
28	0.500	0.421	0.571	0.400	0.526	0.286	0.421	0.600	0.500	0.526	0.632	0.476	0.526	0.600	0.571	0.526	0.526	0.476	0.600	0.571	0.500	0.600	0.476	0.500	0.556	0.600	0.571	0.500	0.421	0.500
29	0.300	0.316	0.381	0.300	0.421	0.286	0.421	0.500	0.500	0.421	0.526	0.381	0.500	0.400	0.476	0.316	0.526	0.381	0.500	0.476	0.476	0.500	0.381	0.421	0.500	0.500	0.381	0.500	0.421	0.500
30	0.400	0.421	0.381	0.300	0.526	0.286	0.632	0.600	0.600	0.526	0.632	0.476	0.500	0.400	0.571	0.632	0.632	0.476	0.600	0.286	0.571	0.500	0.381	0.632	0.400	0.600	0.476	0.500	0.526	0.400

SB : *Sargassum binderi*
SO : *S. oligocystum*

Table 21: F value of *Sargassum binderi* and *S. oligocystum* by OPN16 interspecifically

Sample SB SB	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.960																													
3	1.000	0.960																												
4	0.923	0.960	0.923																											
5	0.960	0.917	0.960	0.880																										
6	0.833	0.870	0.833	0.833	0.783																									
7	0.783	0.818	0.783	0.783	0.818	0.857																								
8	0.783	0.818	0.783	0.870	0.818	0.762	0.800																							
9	0.833	0.783	0.833	0.833	0.870	0.818	0.952	0.762																						
10	0.846	0.800	0.846	0.846	0.880	0.833	0.783	0.783	0.917																					
11	0.800	0.750	0.800	0.720	0.833	0.609	0.636	0.636	0.696	0.600																				
12	0.960	0.917	0.960	0.880	1.000	0.783	0.818	0.818	0.870	0.880	0.833																			
13	0.880	0.917	0.880	0.800	0.917	0.696	0.727	0.818	0.783	0.800	0.833	0.917																		
14	0.833	0.783	0.833	0.750	0.870	0.636	0.762	0.762	0.818	0.833	0.783	0.870	0.870																	
15	0.880	0.750	0.880	0.800	0.917	0.696	0.727	0.818	0.783	0.880	0.833	0.917	0.833	0.870																
16	0.960	0.833	0.800	0.800	0.833	0.870	0.909	0.727	0.870	0.800	0.833	0.833	0.750	0.783	0.833															
17	0.917	0.870	0.833	0.750	0.870	0.727	0.857	0.762	0.727	0.833	0.783	0.783	0.783	0.818	0.696	0.783														
18	0.783	0.818	0.783	0.783	0.818	0.762	0.900	0.900	0.762	0.696	0.818	0.727	0.818	0.762	0.727	0.818	0.857													
19	0.870	0.727	0.783	0.870	0.727	0.857	0.900	0.800	0.857	0.783	0.818	0.818	0.727	0.857	0.818	0.727	0.857	0.800												
20	0.833	0.783	0.833	0.833	0.870	0.818	0.762	0.857	0.818	0.750	0.870	0.783	0.870	0.727	0.783	0.870	0.727	0.952	0.857											
21	0.880	0.833	0.880	0.880	0.917	0.870	0.727	0.818	0.783	0.720	0.750	0.833	0.833	0.870	0.750	0.833	0.696	0.909	0.727	0.783										
22	0.846	0.800	0.846	0.923	0.880	0.833	0.696	0.783	0.750	0.846	0.720	0.720	0.880	0.833	0.720	0.880	0.833	0.870	0.783	0.833	0.720									
23	0.923	0.880	0.769	0.923	0.960	0.917	0.783	0.696	0.750	0.769	0.800	0.800	0.880	0.917	0.720	0.880	0.750	0.783	0.783	0.750	0.720	0.923								
24	0.917	0.783	0.833	0.917	0.957	0.909	0.857	0.857	0.818	0.833	0.870	0.783	0.870	0.909	0.783	0.870	0.818	0.762	0.952	0.909	0.783	0.917	0.917							
25	0.846	0.800	0.846	0.846	0.880	0.833	0.870	0.783	0.833	0.846	0.880	0.880	0.800	0.833	0.800	0.800	0.833	0.783	0.783	0.750	0.720	0.846	0.769	0.917						
26	0.800	0.833	0.960	0.800	0.833	0.783	0.909	0.727	0.783	0.880	0.917	0.833	0.750	0.870	0.750	0.750	0.870	0.818	0.909	0.957	0.833	0.880	0.800	0.870	0.720					
27	0.750	0.870	0.917	0.833	0.783	0.818	0.762	0.762	0.818	0.917	0.870	0.870	0.783	0.818	0.783	0.783	0.727	0.952	0.857	0.909	0.783	0.917	0.917	0.909	0.750	0.783				
28	0.833	0.783	0.917	0.750	0.783	0.727	0.762	0.857	0.727	0.917	0.870	0.763	0.696	0.818	0.696	0.696	0.727	0.857	0.952	0.909	0.783	0.750	0.833	0.909	0.833	0.870	0.727			
29	0.783	0.727	0.870	0.783	0.909	0.762	0.800	0.900	0.762	0.870	0.818	0.818	0.818	0.762	0.727	0.818	0.769	0.800	1.000	0.857	0.727	0.870	0.696	0.952	0.870	0.909	0.762	0.952		
30	0.800	0.750	0.800	0.720	0.833	0.870	0.727	0.818	0.783	0.800	0.750	0.833	0.833	0.870	0.750	0.833	0.696	0.818	0.909	0.783	0.750	0.800	0.800	0.870	0.880	0.917	0.783	0.870	0.909	

SB : *Sargassum binderi*

Table 22: F value of *Sargassum binderi* by OPK9 intraspecifically

Sample SO / SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.778																													
3	0.706	0.706																												
4	0.824	0.824	0.750																											
5	0.824	0.889	0.824	0.824																										
6	0.824	0.824	0.750	0.750	0.824																									
7	0.889	0.778	0.750	0.706	0.778	0.941																								
8	0.889	0.778	0.750	0.706	0.778	0.941	1.000																							
9	0.889	0.778	0.750	0.706	0.778	0.941	1.000	1.000																						
10	0.824	0.824	0.750	0.750	0.824	1.000	0.941	0.941	0.941																					
11	0.778	0.778	0.706	0.824	0.778	0.941	0.889	0.889	0.889	0.941																				
12	0.750	0.778	0.778	0.750	0.778	0.750	0.778	0.778	0.778	0.706	0.667																			
13	0.750	0.750	0.824	0.824	0.778	0.706	0.750	0.750	0.750	0.706	0.750	0.778																		
14	0.778	0.750	0.778	0.800	0.889	0.824	0.778	0.778	0.778	0.824	0.750	0.778	0.778																	
15	0.750	0.750	0.778	0.750	0.824	0.842	0.800	0.800	0.750	0.800	0.824	0.842	0.842	0.737																
16	0.900	0.900	0.842	0.824	0.800	0.842	0.900	0.900	0.900	0.842	0.800	0.700	0.700	0.800	0.750															
17	0.706	0.941	0.750	0.875	0.941	0.875	0.824	0.824	0.824	0.875	0.824	0.706	0.706	0.824	0.824	0.842														
18	0.706	0.706	0.750	0.875	0.824	0.875	0.824	0.824	0.824	0.875	0.824	0.824	0.824	0.706	0.778	0.737	0.875													
19	0.706	0.941	0.750	0.875	0.941	0.875	0.824	0.824	0.824	0.875	0.824	0.824	0.706	0.706	0.667	0.842	1.000	0.875												
20	0.667	0.778	0.889	0.941	0.889	0.824	0.778	0.778	0.778	0.824	0.778	0.778	0.778	0.778	0.737	0.800	0.941	0.941	0.941											
21	0.842	0.737	0.750	0.778	0.778	0.778	0.842	0.842	0.842	0.778	0.600	0.800	0.800	0.800	0.842	0.857	0.778	0.778	0.667	0.737										
22	0.706	0.824	0.750	0.750	0.778	0.875	0.706	0.706	0.706	0.875	0.824	0.706	0.824	0.706	0.667	0.737	0.750	0.875	0.750	0.824	0.889									
23	0.842	0.947	0.667	0.778	0.750	0.778	0.842	0.842	0.842	0.778	0.842	0.737	0.737	0.842	0.700	0.857	0.667	0.778	0.667	0.737	1.000	0.889								
24	0.824	0.824	0.750	0.750	0.842	0.875	0.824	0.824	0.824	0.875	0.824	0.824	0.706	0.706	0.667	0.842	0.750	0.750	0.750	0.706	0.889	0.875	0.889							
25	0.778	0.778	0.706	0.706	0.750	0.750	0.800	0.778	0.800	0.842	0.889	0.857	0.857	0.842	0.824	0.824	0.778	0.800	0.800	0.824	0.857	0.842	0.842	0.706						
26	0.778	0.824	0.706	0.824	0.824	0.800	0.824	0.824	0.750	0.842	0.889	0.857	0.857	0.842	0.824	0.824	0.778	0.800	0.800	0.824	0.857	0.842	0.737	0.706	0.889					
27	0.842	0.800	0.778	0.750	0.750	0.778	0.750	0.778	0.778	0.778	0.842	0.857	0.842	0.824	0.800	0.762	0.824	0.889	0.857	0.842	0.800	0.778	0.800	0.778	0.947	0.842				
28	0.842	0.824	0.778	0.750	0.750	0.778	0.800	0.778	0.800	0.778	0.737	0.778	0.800	0.824	0.800	0.762	0.778	0.889	0.857	0.842	0.800	0.778	0.700	0.778	0.947	0.842	1.000			
29	0.706	0.800	0.750	0.750	0.750	0.750	0.778	0.778	0.750	0.750	0.706	0.750	0.750	0.750	0.667	0.737	0.750	0.750	0.750	0.778	0.778	0.750	0.778	0.750	0.941	0.824	0.889	0.889		
30	0.737	0.750	0.667	0.778	0.750	0.778	0.800	0.824	0.800	0.778	0.737	0.778	0.778	0.800	0.800	0.762	0.667	0.889	0.778	0.842	0.900	0.778	0.900	0.778	0.947	0.842	1.000	1.000	0.889	

SO : *Sargassum oligocystum*

Table 23: F value of *Sargassum oligocystum* by OPKI intraspecifically

Sample SB SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.400	0.316	0.545	0.381	0.476	0.500	0.421	0.526	0.400	0.364	0.476	0.476	0.476	0.500	0.381	0.476	0.400	0.421	0.526	0.500	0.381	0.364	0.364	0.500	0.455	0.381	0.600	0.400	0.526	0.476
2	0.636	0.667	0.455	0.476	0.571	0.600	0.526	0.400	0.500	0.455	0.476	0.571	0.476	0.600	0.676	0.571	0.500	0.421	0.526	0.600	0.571	0.545	0.455	0.500	0.455	0.381	0.400	0.500	0.526	0.476
3	0.381	0.400	0.381	0.381	0.400	0.421	0.444	0.444	0.316	0.381	0.300	0.400	0.500	0.421	0.400	0.400	0.421	0.333	0.444	0.421	0.500	0.381	0.476	0.526	0.381	0.500	0.316	0.316	0.444	0.400
4	0.476	0.500	0.476	0.476	0.400	0.421	0.444	0.444	0.421	0.381	0.400	0.500	0.500	0.526	0.400	0.500	0.526	0.444	0.556	0.526	0.500	0.571	0.571	0.526	0.476	0.400	0.421	0.526	0.444	0.300
5	0.636	0.421	0.636	0.364	0.571	0.600	0.526	0.500	0.400	0.455	0.381	0.476	0.571	0.500	0.381	0.476	0.600	0.421	0.526	0.500	0.381	0.364	0.545	0.500	0.545	0.571	0.600	0.400	0.526	0.381
6	0.476	0.600	0.571	0.571	0.500	0.526	0.444	0.444	0.421	0.381	0.500	0.500	0.500	0.421	0.500	0.500	0.526	0.556	0.364	0.526	0.400	0.381	0.381	0.421	0.381	0.500	0.421	0.421	0.566	0.500
7	0.545	0.571	0.545	0.455	0.381	0.500	0.421	0.421	0.400	0.364	0.381	0.476	0.381	0.300	0.381	0.476	0.400	0.444	0.526	0.500	0.381	0.364	0.364	0.400	0.364	0.476	0.400	0.300	0.316	0.381
8	0.545	0.500	0.545	0.421	0.476	0.500	0.421	0.421	0.400	0.364	0.381	0.476	0.381	0.400	0.476	0.476	0.400	0.421	0.526	0.500	0.571	0.364	0.364	0.400	0.455	0.476	0.400	0.400	0.421	0.381
9	0.381	0.571	0.545	0.545	0.476	0.500	0.421	0.421	0.400	0.364	0.381	0.476	0.381	0.400	0.476	0.476	0.500	0.421	0.421	0.400	0.381	0.364	0.364	0.400	0.455	0.476	0.400	0.400	0.421	0.444
10	0.476	0.600	0.476	0.476	0.500	0.526	0.444	0.444	0.421	0.381	0.400	0.500	0.500	0.421	0.500	0.500	0.421	0.444	0.556	0.526	0.400	0.381	0.476	0.526	0.381	0.500	0.526	0.421	0.444	0.400
11	0.455	0.571	0.545	0.545	0.476	0.500	0.421	0.421	0.400	0.364	0.381	0.476	0.381	0.400	0.476	0.476	0.400	0.526	0.421	0.400	0.476	0.364	0.455	0.400	0.364	0.381	0.400	0.400	0.526	0.476
12	0.455	0.381	0.455	0.364	0.476	0.300	0.316	0.316	0.400	0.364	0.476	0.476	0.476	0.500	0.476	0.476	0.400	0.421	0.421	0.400	0.381	0.455	0.455	0.400	0.364	0.381	0.300	0.400	0.421	0.381
13	0.455	0.286	0.364	0.364	0.381	0.400	0.316	0.316	0.300	0.364	0.381	0.381	0.476	0.400	0.381	0.381	0.300	0.526	0.316	0.300	0.381	0.364	0.455	0.400	0.455	0.476	0.500	0.400	0.526	0.476
14	0.455	0.381	0.545	0.364	0.476	0.500	0.316	0.421	0.400	0.364	0.381	0.476	0.476	0.500	0.381	0.476	0.400	0.526	0.421	0.400	0.476	0.455	0.455	0.500	0.455	0.381	0.400	0.500	0.526	0.476
15	0.435	0.545	0.522	0.261	0.364	0.478	0.300	0.300	0.478	0.435	0.364	0.364	0.455	0.435	0.381	0.364	0.364	0.381	0.500	0.400	0.478	0.545	0.522	0.435	0.478	0.348	0.364	0.478	0.400	0.455
16	0.417	0.348	0.333	0.417	0.348	0.636	0.476	0.381	0.455	0.333	0.522	0.435	0.435	0.273	0.348	0.435	0.545	0.381	0.381	0.545	0.522	0.417	0.417	0.364	0.333	0.522	0.455	0.455	0.381	0.348
17	0.381	0.300	0.571	0.476	0.500	0.526	0.556	0.556	0.526	0.476	0.400	0.500	0.600	0.526	0.500	0.500	0.526	0.556	0.556	0.526	0.400	0.381	0.476	0.526	0.571	0.400	0.526	0.381	0.444	0.400
18	0.476	0.500	0.476	0.476	0.400	0.421	0.556	0.556	0.526	0.381	0.400	0.400	0.526	0.400	0.526	0.400	0.526	0.444	0.556	0.526	0.400	0.381	0.476	0.421	0.476	0.400	0.444	0.526	0.444	0.500
19	0.571	0.600	0.571	0.571	0.500	0.526	0.556	0.667	0.526	0.476	0.500	0.500	0.600	0.526	0.400	0.500	0.421	0.444	0.444	0.421	0.500	0.476	0.381	0.526	0.476	0.400	0.526	0.526	0.444	0.400
20	0.364	0.381	0.364	0.455	0.286	0.500	0.526	0.421	0.500	0.455	0.381	0.381	0.381	0.300	0.381	0.381	0.500	0.316	0.316	0.400	0.381	0.455	0.364	0.400	0.364	0.476	0.500	0.300	0.421	0.381
21	0.348	0.545	0.435	0.522	0.455	0.478	0.500	0.400	0.478	0.348	0.455	0.545	0.455	0.381	0.545	0.545	0.381	0.300	0.400	0.476	0.364	0.435	0.522	0.571	0.348	0.364	0.571	0.476	0.500	0.545
22	0.476	0.500	0.476	0.476	0.500	0.421	0.444	0.333	0.421	0.381	0.400	0.500	0.600	0.421	0.500	0.600	0.421	0.333	0.333	0.316	0.400	0.381	0.476	0.526	0.476	0.400	0.421	0.526	0.444	0.500
23	0.435	0.545	0.522	0.435	0.545	0.478	0.500	0.400	0.478	0.348	0.455	0.545	0.545	0.476	0.545	0.545	0.364	0.476	0.600	0.400	0.381	0.455	0.522	0.522	0.381	0.348	0.455	0.571	0.286	0.400
24	0.381	0.400	0.381	0.381	0.400	0.526	0.444	0.333	0.526	0.476	0.500	0.500	0.400	0.381	0.400	0.500	0.526	0.444	0.333	0.316	0.400	0.381	0.476	0.421	0.476	0.500	0.421	0.526	0.444	0.400
25	0.636	0.667	0.636	0.636	0.571	0.600	0.421	0.526	0.400	0.364	0.476	0.381	0.381	0.400	0.571	0.381	0.400	0.421	0.526	0.500	0.381	0.364	0.545	0.500	0.364	0.381	0.300	0.400	0.421	0.476
26	0.545	0.571	0.545	0.545	0.476	0.500	0.316	0.421	0.400	0.364	0.381	0.476	0.381	0.300	0.381	0.381	0.500	0.526	0.526	0.500	0.571	0.545	0.364	0.400	0.455	0.476	0.400	0.400	0.316	0.381
27	0.609	0.455	0.609	0.478	0.500	0.571	0.500	0.500	0.478	0.522	0.545	0.636	0.455	0.478	0.455	0.545	0.478	0.400	0.500	0.478	0.500	0.522	0.348	0.478	0.435	0.455	0.381	0.381	0.500	0.364
28	0.522	0.455	0.609	0.522	0.400	0.478	0.500	0.600	0.478	0.522	0.545	0.455	0.455	0.381	0.455	0.455	0.571	0.400	0.500	0.571	0.545	0.435	0.435	0.478	0.348	0.364	0.476	0.478	0.400	0.364
29	0.571	0.600	0.476	0.571	0.600	0.421	0.444	0.556	0.421	0.476	0.500	0.600	0.500	0.421	0.400	0.500	0.632	0.556	0.556	0.421	0.500	0.476	0.571	0.526	0.476	0.400	0.421	0.526	0.556	0.500
30	0.609	0.500	0.609	0.478	0.636	0.571	0.500	0.600	0.476	0.522	0.636	0.636	0.455	0.476	0.455	0.545	0.476	0.400	0.400	0.381	0.455	0.348	0.348	0.381	0.522	0.455	0.476	0.381	0.500	0.364

SB : *Sargassum binderi*
SO : *S. oligocystum*

Table 24: F value of *Sargassum binderi* and *S. oligocystum* by OPK3 interspecifically

Sample SB SB	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2	0.846																													
3	0.880	0.880																												
4	0.769	0.769	0.800																											
5	0.846	0.769	0.720	0.846																										
6	0.769	0.692	0.720	0.769	0.923																									
7	0.800	0.800	0.833	0.720	0.800	0.880																								
8	0.769	0.846	0.880	0.769	0.846	0.923	0.880																							
9	0.615	0.769	0.800	0.769	0.769	0.769	0.640	0.692																						
10	0.692	0.846	0.880	0.846	0.846	0.769	0.640	0.846	0.692																					
11	0.769	0.769	0.800	0.769	0.846	0.846	0.800	0.769	0.692	0.846																				
12	0.800	0.800	0.917	0.880	0.720	0.800	0.750	0.800	0.720	0.880	0.800																			
13	0.769	0.769	0.800	0.923	0.846	0.769	0.720	0.769	0.692	0.846	0.923	0.880																		
14	0.769	0.692	0.800	0.846	0.769	0.769	0.720	0.769	0.769	0.923	0.846	0.880	0.923																	
15	0.769	0.692	0.800	0.769	0.769	0.769	0.720	0.769	0.769	0.923	0.846	0.880	0.923	1.000																
16	0.769	0.692	0.800	0.769	0.769	0.846	0.800	0.769	0.769	0.923	0.923	0.800	0.846	0.923	0.923															
17	0.769	0.846	0.720	0.769	0.769	0.769	0.720	0.692	0.769	0.846	0.923	0.720	0.846	0.846	0.846	0.923														
18	0.769	0.692	0.800	0.769	0.769	0.769	0.800	0.769	0.769	0.923	0.923	0.800	0.846	0.923	0.923	1.000	0.923													
19	0.769	0.846	0.880	0.769	0.769	0.846	0.800	0.769	0.769	0.923	0.923	0.880	0.846	0.846	0.846	0.923	0.846	0.923												
20	0.769	0.846	0.880	0.769	0.769	0.846	0.800	0.769	0.769	0.923	0.923	0.880	0.846	0.846	0.846	0.923	0.846	0.923	1.000											
21	0.846	0.769	0.800	0.769	0.692	0.692	0.800	0.769	0.769	0.846	0.923	0.800	0.846	0.846	0.846	0.923	1.000	0.923	0.846	0.846										
22	0.846	0.846	0.880	0.692	0.692	0.769	0.800	0.692	0.846	0.923	0.846	0.800	0.769	0.846	0.846	0.923	0.923	0.923	0.923	0.923										
23	0.923	0.769	0.800	0.846	0.692	0.769	0.800	0.769	0.769	0.923	0.846	0.880	0.923	1.000	1.000	0.923	0.846	0.923	0.846	0.846	0.846	0.846								
24	0.923	0.769	0.800	0.846	0.692	0.769	0.800	0.769	0.769	0.923	0.846	0.880	0.923	1.000	1.000	0.923	0.846	0.923	0.846	0.846	0.846	0.846	1.000							
25	0.769	0.846	0.800	0.769	0.846	0.846	0.880	0.846	0.692	0.846	0.846	0.880	0.923	0.846	0.846	0.769	0.769	0.769	0.692	0.692	0.769	0.692	0.769	0.769						
26	0.769	0.769	0.800	0.923	0.923	0.923	0.800	0.846	0.615	0.769	0.846	0.880	0.923	0.846	0.846	0.846	0.769	0.769	0.769	0.769	0.769	0.692	0.846	0.769	1.000					
27	0.846	0.769	0.800	0.769	0.769	0.692	0.800	0.692	0.769	0.769	0.769	0.800	0.846	0.846	0.846	0.692	0.769	0.769	0.615	0.692	0.769	0.692	0.769	0.769	0.769	0.769				
28	0.846	0.769	0.800	0.769	0.692	0.769	0.800	0.769	0.769	0.846	0.769	0.880	0.846	0.846	0.846	0.769	0.692	0.769	0.769	0.769	0.692	0.769	0.846	0.846	0.846	0.846	0.846			
29	0.846	0.769	0.800	0.769	0.692	0.769	0.800	0.769	0.769	0.846	0.769	0.880	0.846	0.846	0.846	0.769	0.692	0.769	0.769	0.769	0.692	0.769	0.846	0.846	0.846	0.846	0.846	1.000		
30	0.846	0.769	0.800	0.769	0.692	0.769	0.800	0.769	0.769	0.846	0.769	0.880	0.846	0.846	0.846	0.769	0.692	0.769	0.769	0.769	0.692	0.769	0.846	0.846	0.769	0.769	0.846	0.846	0.846	

SB : *Sargassum binderi*

Table 25: F value of *Sargassum binderi* by OPN5 intraspecifically

Sample SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
SO																														
1																														
2	0.818																													
3	0.818	1.000																												
4	0.818	1.000	1.000																											
5	0.870	0.957	0.957	0.957																										
6	0.917	0.833	0.833	0.833	0.880																									
7	0.783	0.696	0.696	0.696	0.667	0.800																								
8	0.783	0.957	0.957	0.957	0.917	0.800	0.750																							
9	0.818	0.727	0.727	0.727	0.696	0.667	0.696	0.696																						
10	0.818	1.000	1.000	1.000	0.957	0.833	0.696	0.957	0.545																					
11	0.818	0.909	0.909	0.909	0.870	0.833	0.609	0.957	0.545	0.909																				
12	0.917	0.833	0.833	0.833	0.880	1.000	0.880	0.880	0.667	0.833	0.833																			
13	0.833	0.833	0.833	0.833	0.880	0.923	0.800	0.880	0.583	0.833	0.833	0.923																		
14	0.870	0.696	0.696	0.696	0.750	0.800	0.833	0.750	0.696	0.696	0.696	0.800	0.720																	
15	0.833	0.833	0.833	0.833	0.800	0.769	0.800	0.800	0.667	0.750	0.667	0.769	0.769	0.960																
16	0.750	0.750	0.750	0.750	0.800	0.769	0.800	0.800	0.667	0.750	0.667	0.769	0.769	0.960	1.000															
17	0.750	0.750	0.750	0.750	0.880	0.769	0.800	0.800	0.667	0.833	0.750	0.769	0.769	0.960	1.000	1.000														
18	0.750	0.750	0.750	0.750	0.720	0.769	0.720	0.720	0.750	0.667	0.667	0.846	0.846	0.800	0.769	0.769	0.769													
19	0.750	0.750	0.750	0.750	0.720	0.769	0.720	0.720	0.750	0.667	0.667	0.846	0.846	0.800	0.769	0.769	0.769	1.000												
20	0.667	0.750	0.750	0.750	0.880	0.846	0.720	0.600	0.750	0.833	0.750	0.846	0.923	0.800	0.846	0.846	0.846	0.846	0.846											
21	0.727	0.818	0.818	0.818	0.870	0.833	0.783	0.870	0.727	0.818	0.818	0.833	0.917	0.696	0.750	0.750	0.750	0.833	0.833	0.917										
22	0.697	0.783	0.783	0.783	0.917	0.800	0.750	0.833	0.696	0.870	0.783	0.720	0.800	0.750	0.800	0.800	0.800	0.720	0.720	0.800	0.783									
23	0.783	0.783	0.783	0.783	0.833	0.800	0.750	0.833	0.696	0.783	0.783	0.720	0.880	0.750	0.800	0.800	0.800	0.800	0.800	0.880	0.870	0.833								
24	0.783	0.783	0.783	0.783	0.833	0.800	0.750	0.833	0.696	0.783	0.783	0.720	0.880	0.750	0.800	0.800	0.800	0.800	0.800	0.880	0.870	0.833	1.000							
25	0.667	0.750	0.750	0.750	0.800	0.769	0.800	0.640	0.750	0.833	0.833	0.769	0.615	0.720	0.880	0.917	0.846	0.846	0.833	0.833	0.750	0.720	0.720	0.640						
26	0.783	0.783	0.783	0.783	0.833	0.800	0.833	0.833	0.696	0.783	0.870	0.800	0.800	0.750	0.800	0.800	0.800	0.800	0.800	0.800	0.783	0.750	0.750	0.667	0.960					
27	0.800	0.800	0.800	0.800	0.783	0.750	0.696	0.696	0.727	0.727	0.818	0.750	0.833	0.769	0.750	0.750	0.750	0.750	0.750	0.750	0.727	0.696	0.696	0.783	0.917	0.957				
28	0.800	0.800	0.800	0.800	0.783	0.750	0.696	0.696	0.727	0.727	0.818	0.750	0.833	0.769	0.750	0.750	0.750	0.750	0.750	0.750	0.727	0.696	0.696	0.783	0.917	0.957	1.000			
29	0.800	0.800	0.800	0.800	0.783	0.750	0.783	0.783	0.727	0.727	0.818	0.750	0.833	0.769	0.750	0.750	0.750	0.750	0.750	0.750	0.727	0.696	0.696	0.783	0.917	0.957	1.000	1.000		
30	0.818	0.833	0.750	0.750	0.800	0.846	0.769	0.720	0.833	0.818	0.870	0.727	0.917	0.769	0.917	0.800	0.960	0.960	1.000	0.909	0.957	0.833	0.783	0.870	0.917	0.783	0.667	0.800	0.909	

SO : *Sargassum oligocystum*

Table 26: F value of *Sargassum oligocystum* by OPNS intraspecifically

Sample SB SO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	0.417	0.538	0.522	0.500	0.462	0.462	0.522	0.583	0.500	0.500	0.417	0.435	0.417	0.417	0.417	0.462	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.500	0.500	0.583
2	0.333	0.500	0.435	0.500	0.333	0.250	0.348	0.417	0.333	0.333	0.333	0.435	0.417	0.333	0.333	0.293	0.250	0.250	0.333	0.333	0.333	0.333	0.333	0.333	0.417	0.417	0.417	0.417	0.417	0.417
3	0.333	0.500	0.435	0.500	0.333	0.250	0.348	0.417	0.333	0.333	0.333	0.435	0.417	0.333	0.333	0.250	0.250	0.250	0.333	0.333	0.333	0.333	0.333	0.417	0.417	0.333	0.333	0.417	0.417	0.417
4	0.333	0.500	0.435	0.500	0.333	0.250	0.348	0.417	0.333	0.333	0.333	0.435	0.417	0.333	0.333	0.250	0.250	0.250	0.333	0.333	0.333	0.333	0.417	0.417	0.333	0.333	0.417	0.417	0.417	0.417
5	0.480	0.640	0.583	0.560	0.400	0.400	0.500	0.560	0.480	0.480	0.480	0.500	0.480	0.400	0.400	0.400	0.400	0.400	0.480	0.480	0.480	0.480	0.480	0.480	0.400	0.400	0.400	0.480	0.480	0.480
6	0.538	0.615	0.560	0.615	0.538	0.462	0.480	0.538	0.462	0.462	0.538	0.480	0.538	0.462	0.462	0.462	0.462	0.462	0.462	0.462	0.462	0.462	0.538	0.538	0.462	0.462	0.538	0.538	0.538	0.615
7	0.480	0.400	0.417	0.480	0.640	0.560	0.417	0.560	0.400	0.480	0.320	0.417	0.400	0.480	0.480	0.320	0.320	0.400	0.320	0.320	0.320	0.320	0.480	0.480	0.400	0.400	0.400	0.400	0.400	0.560
8	0.480	0.560	0.500	0.560	0.480	0.480	0.500	0.480	0.400	0.400	0.480	0.417	0.400	0.320	0.320	0.400	0.400	0.400	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.560
9	0.417	0.417	0.522	0.333	0.250	0.333	0.435	0.417	0.462	0.417	0.417	0.435	0.333	0.333	0.333	0.417	0.333	0.417	0.500	0.500	0.417	0.500	0.417	0.417	0.417	0.500	0.500	0.500	0.417	0.417
10	0.417	0.583	0.522	0.500	0.462	0.500	0.435	0.417	0.462	0.417	0.500	0.435	0.417	0.333	0.333	0.417	0.417	0.417	0.500	0.500	0.500	0.500	0.417	0.417	0.417	0.417	0.333	0.417	0.417	0.500
11	0.417	0.583	0.522	0.500	0.500	0.500	0.522	0.583	0.500	0.500	0.583	0.435	0.500	0.500	0.500	0.583	0.583	0.583	0.500	0.500	0.583	0.500	0.500	0.500	0.500	0.500	0.417	0.500	0.500	0.417
12	0.462	0.538	0.480	0.538	0.538	0.538	0.560	0.538	0.462	0.462	0.538	0.400	0.462	0.462	0.462	0.462	0.462	0.462	0.385	0.385	0.462	0.385	0.462	0.462	0.462	0.538	0.538	0.538	0.462	0.538
13	0.462	0.538	0.480	0.538	0.538	0.538	0.560	0.538	0.462	0.462	0.538	0.400	0.462	0.462	0.462	0.462	0.462	0.462	0.385	0.385	0.462	0.385	0.462	0.462	0.480	0.480	0.538	0.462	0.462	0.500
14	0.400	0.400	0.417	0.400	0.480	0.480	0.500	0.400	0.400	0.480	0.480	0.417	0.480	0.480	0.480	0.480	0.560	0.500	0.560	0.480	0.480	0.560	0.480	0.400	0.400	0.417	0.462	0.480	0.480	0.500
15	0.462	0.385	0.480	0.385	0.462	0.462	0.480	0.385	0.385	0.462	0.538	0.480	0.538	0.462	0.462	0.500	0.538	0.538	0.538	0.538	0.500	0.538	0.462	0.462	0.417	0.462	0.538	0.538	0.538	0.480
16	0.385	0.462	0.400	0.462	0.538	0.480	0.480	0.385	0.462	0.462	0.385	0.400	0.462	0.462	0.538	0.500	0.500	0.462	0.462	0.385	0.417	0.462	0.462	0.462	0.480	0.538	0.462	0.462	0.462	0.480
17	0.385	0.462	0.400	0.462	0.480	0.480	0.480	0.385	0.462	0.462	0.385	0.400	0.462	0.462	0.538	0.500	0.500	0.462	0.462	0.385	0.417	0.462	0.462	0.462	0.480	0.500	0.462	0.462	0.462	0.480
18	0.462	0.538	0.560	0.462	0.500	0.480	0.480	0.538	0.462	0.462	0.615	0.540	0.692	0.615	0.615	0.500	0.500	0.538	0.538	0.538	0.462	0.615	0.615	0.500	0.480	0.615	0.615	0.615	0.815	0.500
19	0.462	0.462	0.560	0.538	0.462	0.462	0.400	0.462	0.538	0.462	0.462	0.400	0.462	0.615	0.462	0.500	0.500	0.462	0.538	0.462	0.500	0.538	0.462	0.538	0.500	0.500	0.462	0.538	0.462	0.480
20	0.538	0.462	0.560	0.538	0.462	0.538	0.400	0.462	0.462	0.615	0.462	0.480	0.538	0.615	0.462	0.462	0.538	0.462	0.538	0.615	0.462	0.538	0.462	0.462	0.615	0.615	0.538	0.462	0.538	0.538
21	0.417	0.583	0.522	0.500	0.500	0.583	0.609	0.462	0.583	0.583	0.500	0.522	0.583	0.500	0.500	0.583	0.462	0.417	0.500	0.667	0.500	0.583	0.583	0.583	0.500	0.500	0.417	0.500	0.500	0.583
22	0.400	0.560	0.500	0.480	0.480	0.400	0.500	0.500	0.560	0.480	0.480	0.538	0.480	0.560	0.480	0.400	0.462	0.400	0.480	0.560	0.480	0.560	0.480	0.400	0.500	0.560	0.560	0.480	0.400	0.480
23	0.640	0.720	0.667	0.640	0.560	0.480	0.462	0.500	0.640	0.640	0.500	0.667	0.640	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.640	0.560	0.560	0.560	0.560	0.400	0.400	0.560
24	0.400	0.560	0.500	0.480	0.480	0.480	0.500	0.500	0.480	0.480	0.480	0.417	0.400	0.480	0.400	0.560	0.500	0.480	0.480	0.480	0.560	0.480	0.480	0.480	0.480	0.480	0.480	0.480	0.400	0.480
25	0.462	0.462	0.400	0.500	0.462	0.538	0.500	0.500	0.462	0.385	0.538	0.480	0.538	0.462	0.615	0.462	0.500	0.462	0.462	0.615	0.538	0.462	0.462	0.538	0.538	0.462	0.615	0.615	0.462	0.480
26	0.480	0.480	0.417	0.417	0.480	0.400	0.462	0.400	0.480	0.400	0.480	0.500	0.480	0.560	0.450	0.480	0.500	0.480	0.400	0.560	0.480	0.560	0.640	0.640	0.480	0.480	0.560	0.480	0.480	0.500
27	0.538	0.615	0.522	0.500	0.500	0.462	0.462	0.500	0.583	0.417	0.583	0.480	0.583	0.500	0.500	0.480	0.500	0.480	0.417	0.583	0.500	0.583	0.417	0.417	0.500	0.583	0.500	0.583	0.500	0.500
28	0.500	0.417	0.522	0.500	0.583	0.500	0.500	0.583	0.500	0.417	0.583	0.522	0.500	0.667	0.500	0.500	0.500	0.480	0.500	0.500	0.583	0.583	0.500	0.500	0.667	0.583	0.583	0.500	0.500	0.480
29	0.417	0.500	0.435	0.500	0.500	0.500	0.436	0.500	0.583	0.417	0.500	0.500	0.500	0.583	0.500	0.500	0.500	0.480	0.583	0.500	0.417	0.500	0.500	0.417	0.417	0.417	0.583	0.500	0.583	0.500
30	0.333	0.417	0.462	0.538	0.522	0.250	0.333	0.400	0.385	0.400	0.480	0.500	0.435	0.480	0.560	0.333	0.640	0.538	0.417	0.417	0.462	0.480	0.333	0.417	0.400	0.500	0.480	0.400	0.400	0.500

SB : *Sargassum binderi*
SO : *S. oligocystum*

Table 27: *F* value of *Sargassum binderi* and *S. oligocystum* by OPN6 interspecifically

5.4.3 Phylogenetic analysis

The phylogenetic analysis has been widely used in studying the relationships of algae. Saunders *et al.* (1995) reported that this technique is useful to study the relationships of species of uncertain taxonomic postition within the Achrochaetales-Palmariales complex (Rhodophyta) using 18S rDNA sequence. In that particular study, the phylogenetic trees were inferred by neighbour-joining (Saitou and Nei, 1987) using the DNADIST program of the PHYLIP package version 3.5 (Felsenstein, 1985). The cryptic diversity study of *Porphyra* (Rhodophyta) by mapping the restriction sites of several restriction enzymes to the small sub-unit (SSU) rRNA genes and the phylogenetic analysis was done by using the mix parsimony and maximum likelihood programs in the PHYLIP computer package (version 3.41) (Felsenstein, 1989) by Stiller and Waaland (1993). In 1996, Lee and King had reported the usefulness of the phylogenetic analysis to characterise five genera of Dictyotaceae (Phaeophyta) based on the DNA sequences of nuclear rDNA internal transcribed spacer (ITS) and 5.8S. The tree distance analyses were conducted using DNADIST in the PHYLIP 3.57 package (Felsenstein, 1993). In the close evolutionary relationships study among the Sporochnales, Desmarestiales and Laminariales (Phaeophyceae) (Tan and Druehl, 1996), parsimony analysis of the 18S rDNA sequences was done with the PAUP computer package (PAUP 3.1.1, Swofford, 1993) and the phylogenetic trees were conducted by neighbour-joining analysis (neighbour -joining MEGA; Kumar *et al.*, 1993).

The following is a discussion on the results of the cluster analysis conducted for the phylogenetic relationships between the two *Sargassum* species using the different primers.

5.4.3.1 OPA13

Two main clusters (*S. binderi* and *S. oligocystum*) (Figure 15) which consist of two and three subclusters, respectively. The two subclusters from *S. binderi*, that is, first subcluster: samples 1, 18, 20 and second subcluster: samples 2, 25, 15, 17, 22, 30, 26, 3, 4, 7, 8, 6, 9, 13, 12, 5, 10, 11, 27, 19, 16, 21, 14, 24, 23, 28, 29. Samples 31, 59, 32, 33, 34, 38, 35, 41, 36, 37, 45, 56, 55, 58, and 57 are under the first subcluster of *S. oligocystum*. The second subcluster consists of samples 40, 60, 48, 42, 46, 44, and 47. As for the last subcluster of *S. oligocystum*, the members are samples 39, 50, 53, 43, 49, 51, 52, and 54.

Samples 1 to 30 belonging to *S. binderi* are 81.4% similar. From the second subcluster, four groups of samples, that is, samples 3, 4, 7, 8, samples 6, 9, 13, samples 5, 10, 11, and samples 14, 24 give a 100% of similarity. There are two individual lineages, that is, samples 26 and 19 which show 85.7 % and 87.9% similarity to the main cluster of *S. binderi*, respectively.

Samples 31 to 60 belonging to *S. oligocystum* are 80% similar. There are eight groups of samples sharing a 100% similarity. Five out of eight are from the first subcluster, that is, samples 31, 59, samples 32, 33, 34, 38, samples 36, 37, samples 45, 56 and samples 55, 58, 57. Samples 42, 46 and samples 44, 47 from the second subcluster and samples 52, 54 from the last subcluster have a similarity level of 100%.

By comparing between the two main clusters, *S. binderi* and *S. oligocystum*, only 42.1 % similarity is shown. Therefore, by using OPA 13 as a primer, *S. binderi* is distantly related to *S. oligocystum* at the species level.

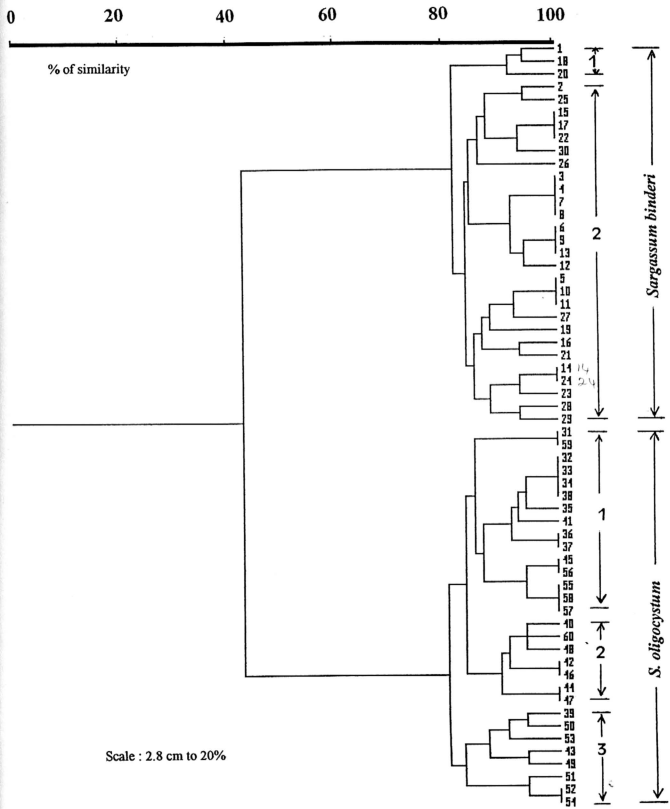


Figure 15. Phylogenetic tree of *Sargassum binderi* and *S. oligocystum* using OPA13.

similarity. For the individual lineages, sample 49 showed 93.57% similarity, sample 40 gave 92.14% similarity, sample 59 and 38 is 83.57% and 78.57% similar to the main cluster of *S. oligocystum*, respectively.

The two main clusters, that is, *S. binderi* and *S. oligocystum* are only 42.14% similar. Therefore, OPK7 as a primer indicates that *S. binderi* is also distantly related at the species level.

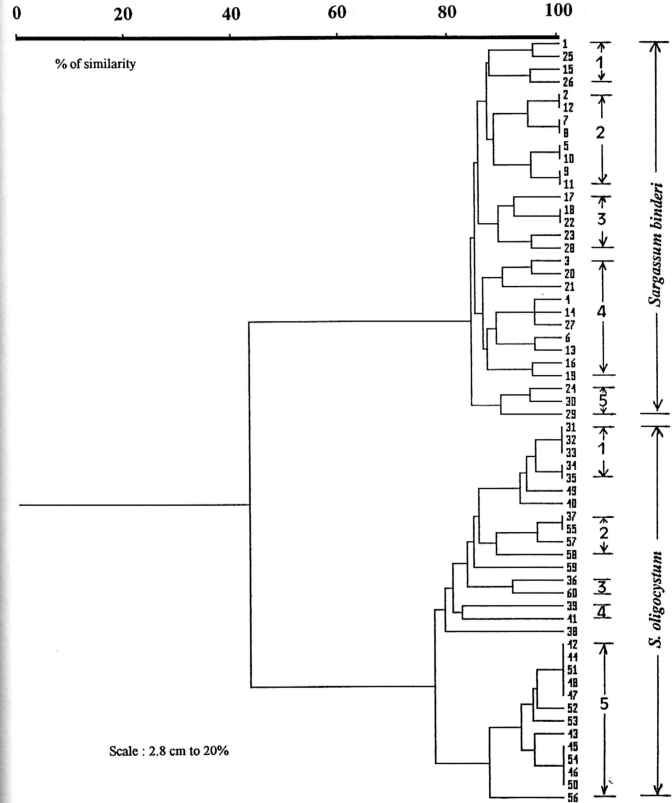


Figure 16. Phylogenetic tree of *Sargassum binderi* and *S. oligocystum* using OPK7.

5.4.3.3 OPK9

The two main clusters, representing *S. binderi* and *S. oligocystum* (Figure 17), has six subclusters each. Samples 1, 3, 2 are grouped under the first subcluster of *S. binderi*. In the second subcluster, there are only three members, which include samples 5, 12, 13. Third subcluster contains samples 4, 22, 23. Fourth subcluster is made up by samples 10, 15, 14. Samples 8, 18, 27, 11, 20, 19, 29, 24, 30 are in the fifth subcluster and samples 6, 21, 7, 9, 16 are grouped under the last subcluster. Samples 13, 25, and 17 are individual lineages under the main cluster of *S. binderi*. As for *S. oligocystum*, the first subcluster consists of samples 31, 46. The second subcluster has six members, samples 36, 40, 37, 38, 39, 41. Samples 51, 53, 52,54 are grouped under the third subcluster. In the fourth subcluster, are samples 32, 47, 49, 35, 34, 50, 48. The fifth subcluster consists of samples 55, 57, 60, 58, 59, 56 and samples 42, 43, 45, 44 are separated into the sixth subcluster. There is only one individual lineage, that is, sample 33.

S. binderi has 80% similarity intraspecifically. There are two pairs of samples sharing a 100% similarity in the second subcluster, that is, samples 5 and 12 and the fifth subcluster, that is, samples 19 and 29. The individual lineages are sample 13, 17 and 25 which are 90%, 80.7% 82.9% similar to the main cluster of *S. binderi*, respectively.

For *S. oligocystum*, the intraspecific similarity level is 72.9%. Two pairs of samples under the second subcluster, that is, samples 36, 40, and samples 37, 38, 39, one pair under the third subcluster, samples 51, 53, one pair in the fourth subcluster, samples 47, 49 and one pair of sample grouped under the fifth subcluster, samples 57, 60, 58 indicated a 100% similarity intraspecifically. The individual lineage, sample 33 only showed 72.9% similarity to the main cluster of *S. oligocystum*..

By using OPK9 as a primer, the similarity level interspecifically is only 45.4%. Therefore, *S. binderi* is only distantly related to *S. oligocystum* genetically.

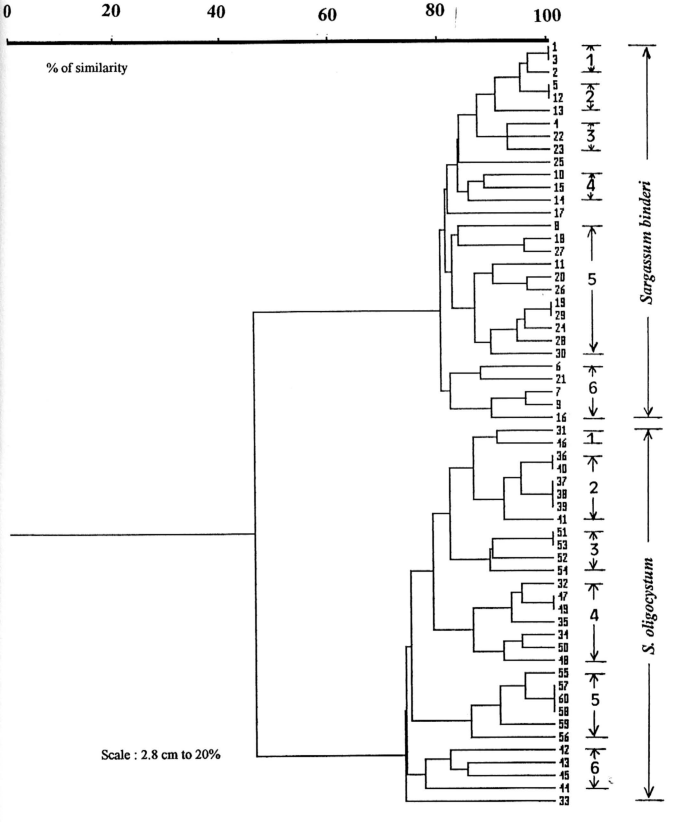


Figure 17. Phylogenetic tree of *Sargassum binderi* and *S. oligocystum* using OPK9.

5.4.3.4 OPN6

Two clusters are formed (Figure 18), that is, one from *S. binderi* and another from *S. oligocystum*. The cluster of *S. binderi* contained three subclusters, that is, samples 1, 2, 3, 12, 27, 28, 29, 30 (first subcluster), samples 10, 14, 15, 23, 24, 11, 16, 18, 17, 21, 20, 19, 22 (second subcluster), samples 4, 13, 25, 26, 5, 6, 8, 7 (third subcluster) and one individual lineage, that is, sample 9. Four subclusters can be found from the cluster of *S. oligocystum*, that is, samples 31, 36, 42, 43, 32, 33, 34, 40, 35, 38, 41 (first subcluster), samples 48, 49, 60, 50, 51, 53, 54 and samples 37, 44, 45, 46, 47 (second subcluster) samples 55, 56, 57, 58, 59 (third subcluster) and two individual lineages, that is, samples 52 and 39.

There are 77.2% similarity showed from samples 1 to 30 belonging to *S. binderi*. Four groups of samples showed the highest similarity level at 100%. Samples 28, 29 are grouped under the first subcluster, samples 14, 15, 23, 24, samples 16, 18, and samples 17, 21 from the second subcluster, and samples 25, 26 from the third subcluster. As for the individual lineage, that is, sample 9, the similarity level is 72.8% to the mian cluster of *S. binderi*.

Samples 31 to 60 belonging to *S.* oligocystum are 74.2% similar. From the dendrogram, there are four groups of samples showing 100% similarity, that is, samples 36, 42, and samples 32, 33, 34 from the first subcluster, samples 49, 60 and

samples 53, 54 from the second subcluster, samples 45, 46, 47 from the third subcluster and samples 57, 58, 59 from the last subcluster. The two individual lineages, samples 52 and 39 are 79.3% and 70% similar to the main cluster of *S. oligocystum*, respectively.

By comparing the clusters of *S. binderi* and *S. oligocystum* using OPN6 as a primer, these two species are distantly related at the similarity level of 47.9%.

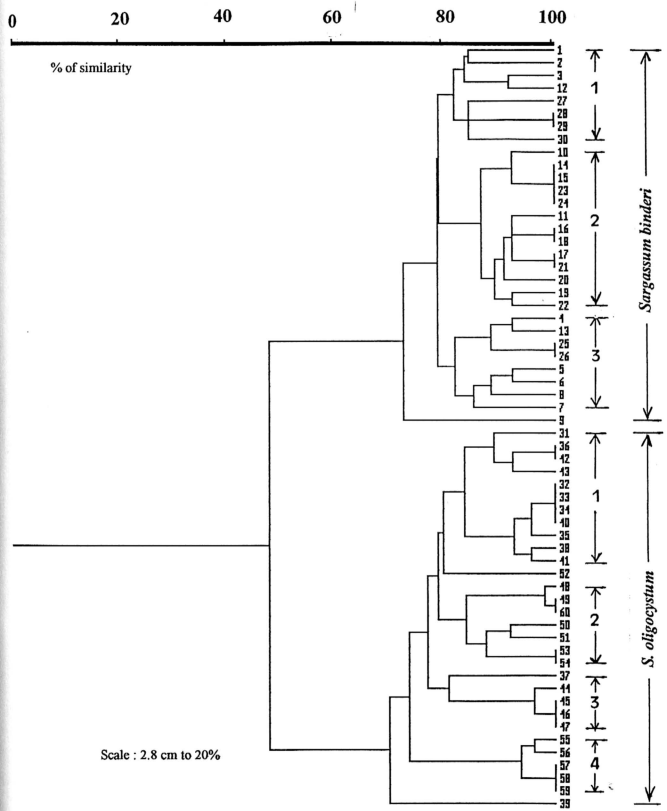


Figure 18. Phylogenetic tree of *Sargassum binderi* and *S. oligocystum* using OPN6.

5.4.3.5 OPN16

There are generally two main clusters (Figure 19) involved here, that is, one cluster from *Sargassum binderi* which contains two subclusters, that is, samples 1, 4, 8, 9, 14, 22, 3, 6, 12, 15, 18, 20, 21, 23, 19 25 (first subcluster), samples 2, 5, 7, 10, 11, 13, 16, 17, 26, 30, 27, 28, 24 (second subcluster) and one individual lineage, that is, sample 29. The other cluster, that is, *S. oligocystum*, has five subclusters, that is, samples 31, 52, 38, 33, 42, 46, 43, 47, 48, 50, 53, 51, 49 (first subcluster), samples 32, 58, 54, 45, 41, 60 (second subcluster), samples 57, 59 (third subcluster), samples 34, 55, 35, 39, 44, 37 (fourth subcluster), and samples 36, 40 (fifth subcluster) and one individual lineage, that is, sample 56.

Samples 1 to 30 belonging to *S. binderi* showed 91% similarity. From the first subcluster, there are two groups of samples which have 100% similarity, that is, samples 1, 4, 8, 9, 14, 22 and samples 3, 6, 12, 15, 18, 20, 21, 23. In the second subcluster, samples 2, 5, 7, 10, 11, 13, 16, 17, 26, and 30 appear to be 100% similar. For the individual lineage (sample 29), it showed 87.9% similarity to the cluster of *S. binderi*.

Samples 31 to 60 belonging to *S. oligocystum* showed 78.6% similarity. From the first subcluster of this species, there are three groups of samples sharing 100% similarity, that is, samples 31, 52, samples 42, 46, 43 and samples 48, 50, 53, 51, 49. In the

second subcluster, the 100% similarity are contributed by samples 32, 58, 54, and 45. Sample 56 was an individual lineage with 77.1% of similarity to the main cluster of *S. oligocystum*.

Between the two main clusters, there is only 52% similarity and this shows that *S. binderi* is only distantly related to *S. oligocystum* when primer OPN16 is used to obtain polymorphisms, for comparison.

As a result of too many pairs of samples having 100% similarity, OPN16 is not a good primer to separate the samples among the species. Therefore, this primer is not very suitable to use as a marker to generate DNA fingerprints to screen *S. binderi* and *S. oligocystum*.

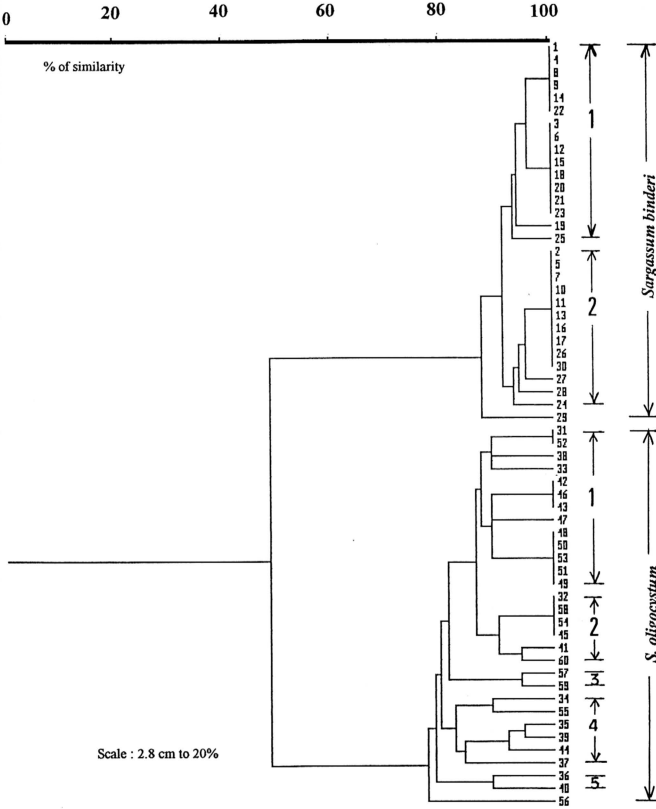


Figure 19. Phylogenetic tree of *Sargassum binderi* and *S. oligocystum* using OPN16.

5.4.4 Probe for DNA fingerprinting

All the primers, OPA13, OPK7, OPK9, OPN6 and OPN16 showed that *S. binderi* is only distantly related (similarity level in between 42 % to 52 %) to *S. oligocystum* based on the similarity level. The five primers can easily separate out the two *Sargassum* species into two main clusters. In between the individuals of *S. binderi*, all the samples showed a very high similarity level (71 % to 91 %). For all the individual samples of *S. oligocystum*, the similarity level obtained also gave high values (72.0 % to 80 %).

Among all the primers, OPK9 is the best primer to characterise the two *Sargassum* species. This primer can separate out very clearly even up to the individual level of all the samples tested (figure 17). Therefore, OPK9 may be suitable to use as a probe for DNA fingerprinting.

For OPK7, it is only to good use to separate out the individuals of *S. binderi* but not *S. oligocystum* although the separation between the two *Sargassum* species is clear (figure 16). This is because 16 out of 30 samples of *S. oligocystum* tested showed a similarity level of 100 %.

15 out of 30 samples of *S. binderi* and 19 out of 30 samples showed a similarity level of 100 % indicating that OPA13 is not a good primer to use to separate out the

individuals between *S. binderi* and *S. oligocystum* although the separation between the two *Sargassum* species is clearly obtained (figure 15).

When OPN6 was used, 12 out of 30 samples of *S. binderi* and 16 out of 30 samples showed a similarity level of 100 % (figure 18). Although the relationship of the two *Sargassum* species is clearly showed, it is not a good primer to separate the samples at the individual level.

OPN16 is the most unsuitable primer if samples were to sort out by individuals as 24 out of 30 samples of *S. binderi* tested showed 100 % similar to the main cluster of *S. binderi* (figure 19). 14 out of 30 samples belonging to *S. oligocystum* also showed 100 % similar to the main cluster of *S. oligocystum* (figure 19). Therefore, it is not suitable to use as a probe for DNA fingerprinting.

5.5 Molecular data versus morphology

The results obtained from this study using molecular technique, that is, RAPDs clearly showed that *S. binderi* and *S. oligocystum* are two different species. They are not synonyms as reported by Womersley and Bailey (1970). This study supported the hypothesis proposed by Ajisaka *et al.* (1998) that *S. binderi* is different from *S. oligocystum* by two characters, that is, the vesicles and the receptacles of the plant. The vesicles of *S. binderi* are winged and *S. oligocystum* are not. The receptacles of *S. binderi* are twisted and with sharply dentate margins but *S. oligocystum* usually have spines only at the tips of the receptacles.