Korean J Biol Sci 4: 319-324, 2000

Phylogenetic Analysis of the Genus *Dendronephthya* (Nephtheidae, Alcyonacea) Based on Internal Transcribed Spacer Sequences of Nuclear rDNA

Young-Ja Lee and Jun-Im Song*

Department of Biological Science, College of Natural Sciences, Ewha Womans University, Seoul 120-750, Korea

Key Words:
Dendronephthya
Soft coral
ITS sequences
Phylogenetic relationship
Interspecies.

Species boundaries among the Alcyonacean soft coral, the genus *Dendronephthya*, are often obscured by inter- and intraspecific morphological variations. In the present study, we attempted to infer the genetic relationships of eight dendronephthians based on their molecular characters, the internal transcribed spacer (ITS) regions of ribosomal DNA, and then compared this result together with the random amplified polymorphic DNA (RAPD) data from our previous investigation. *Dendronephthya. pütteri* and *D. suensoni* formed a divaricate form - VI grade specific clade, whereas *D. castanea, D. gigantea, D. aurea* and *D. spinifera,* formed a umbellate and glomerate form - IV and III grade specific clade. Therefore, we confirmed that the main characters, the growth form and the anthocodial grade and formula, are important in identification of the species in dendronephthians despite some problems. Also, the relationships of the growth form are clarified as the glomerate form is much closer to the umbellate form than to the divaricate form based on two sets of independent molecular data. However, we cannot determine the molecular markers which limit the species boundaries among this genus with ITS sequences.

The recognition of taxonomic boundaries is the first essential step to understand the biogeographical and ecological distributions of organisms. But the species level phylogeny of many marine invertebrate groups is notoriously difficult to confirm due to the paucity of distinguishable their morphological characteristics (Chen and Miller, 1996). In particular, the morphological species boundaries do not often allow determination of the cnidarians. Since the environment has influenced the morphological characters including growth form of some species, morphological variants associated with distinct microhabitats are often assumed to represent ecotypes of a single, environmental plastic species (Knowlton, 1993; Palumbi, 1994; Bruno and Edmunds, 1997; McFadden, 1999).

To date, 121 anthozoan species are known in Korea. Of these, the soft coral genus *Dendronephthya* makes up great association in the southern waters of Cheju Island, the southernmost island of Korea (Je, 1994; Song and Lee, 2000). They are usually defined to include all Nephtheidea (Alcyonacea; Octocorallia) with bushlike or branched appearance. Their polyps are always composed of small groups or bundles, and supporting bundles (Kükenthal, 1906; Roxas, 1933). Their identification is focused on two main characters, the growth form and the anthocodial grade and formula (Kükenthal, 1905; Sherriffs, 1922) (Table 1). However, there is a difficulty due to variability at the intra- and interspecific levels (Utinomi, 1952; Song and Lee, 2000). Thomson and Dean (1931) suggested that these variations in a narrow range are caused by cross-fertilization between sibling species and mutations apart from any hybridizing influences. Since these cryptic or sibling species make it impossible and difficult to identify them based on their morphological characters only (McFadden, 1999), their systematic relationships remain unclear despite their ecological importance.

To resolve these problems, we tried to elucidate their genetic relationships using RAPD analysis (Song and Lee, 2000). As a result, we could confirm that their growth form and anthocodial grade and formula were very important morphological characters in the identification of dendronephthians and compatible with the genetic variation. Also, the relationships based on the growth form clarified that the glomerate was much closer to the umbellate than to the divaricate. Moreover, the RAPD method was found to be useful in confirmation of the barrier between dendronephthians.

In this study, we attempted to revaluate the results from morphological and RAPD data (Song and Lee, 2000) and to search another molecular marker to decide the boundaries between the dendronephthian

* To whom correspondence should be addressed.
Tel: 82-2-3277-2364, Fax: 82-2-3277-2385
E-mail: jisong@mm.ewha.ac.kr

Table 1. Taxonomic information and two main characters of each species in the genus *Dendronephthya* used in this study

Classification	Species	Growth form (Kükenthal, 1905)	Anthocodial grade and formula (Sherriffs, 1922)	Authors
Phylum Cnidaria				
Class Anthozoa				
Subclass Octocorallia				
Order Alcyonacea				
Family Nephtheidae	**Dendronephthya aurea*	Glomerate	III = 1P+(4-5)p+0Cr+very strong S. B.+(0-1)M	Utinomi (1952)
		〃	IV = 1P+(2-4)p+0Cr+strong S. B. +(0-1)M	Song (unpublished data)
	D. gigantea	Glomerate	III = 1P+(4-5)p+0Cr+very strong S. B.	Roxas (1933)
		〃	III = (1-6)P+0Cr+very strong S. B.	Thomson and Dean (1931)
		〃	III = 1P+(4-5)p+0Cr+very strong S. B.+(0 or 1)M	Utinomi (1952)
		〃	III = 1P+(4-5)p+0Cr+very strong S. B.+(1-1½)M	Song (1977)
	**D. spinifera*	Glomerate	III = 1P+(3-4)p+0Cr+strong S. B.	Roxas (1933)
		〃	IV = 1P+(3-4)p+0Cr+very strong S. B.+0M	Utinomi (1952)
		〃	IV = 1P+(2-3)p+0Cr+strong S. B.+(0-1)M	Song (unpublished data)
	D. castanea	Umbellate	IV = 1P+(1-4)p+0Cr+very strong S. B.	Utinomi (1952)
		〃	IV = 1P+(1-3)p+0Cr+strong S. B.+(1-1½)M	Song (1977)
	D. pütteri	Divaricate	VI + 2P+(2-3)Cr+strong S. B.	Roxas (1933)
		〃	VI = 1P+(3-4)Cr+strong S. B.+1M	Song (1977)
	D. suensoni	Divaricate	VI = I+1P+(4-6)Cr+medium S. B.	Thomson & Dean (1931)
		Glomerate	IV = 1P+(4-5)p+0Cr+very strong S. B.+(0 or 1)M	Utinomi (1952)
		〃	IV = 1P+(5-6)p+0Cr+medium S. B.+(1-2)M	Utinomi (1954)
		Divaricate	VI = 1P+(4-6)p+0Cr+medium S. B.+(1-2)M	Song (1977)
	**D.* sp. 1	Divaricate	IV = 1P+(3-4)p+0Cr+strong S. B.+(1-2)M	Song (unpublished data)
	**D.* sp. 2	Divaricate	III = 1P+(1-2)p+(3-4)Cr+medium S. B.+2M	Song (unpublished data)
	***D.* sp. 3	--		GenBank (U64582)
Family Alcyoniidae	***Alcyonium* sp.			GenBank (AF262353)

*species identified in the present study.
**species from GenBank

species. Therefore, we selected ITS and 5.8 S ribosomal DNA containing highly variable sequence areas. Because ITS has been well known to be less subject to selection pressure and more rapidly changing than rDNA regions, it has been widely used to resolve the phylogenetic relationships within genera and both inter- and intraspecies in many taxa (Coffroath, 1997; Leignel et al., 1997; Cerbah et al., 1998; Raahauge and Kristenssen, 2000; Reed et al., 2000). In particular, it has been applied in revealing the relationships of cnidarians at inter- and intraspecific level (Chen and Miller, 1996; Chen et al., 1996; Hunter et al., 1997; Odorico and Miller, 1997a; McFadden et al., 2001, in press).

Materials and Methods

Specimens of *Dendronephthya* were collected from the southern part of Cheju Island between 1997 and 1998 and were identified as 8 species; *D. suensoni, D. pütteri, D. castanea, D. gigantea, D. spinifera, D. aurea, D.* sp. 1, *D.* sp. 2 (Table 1). They were collected from 5-25 m deep by SCUBA diving. Moreover, some specimens of *D. castanea, D. gigantea* and *D. spinifera* were used for this study because of their variable features. The sequences of one dendronephthian, *D.* sp. 3, was obtained from GenBank for analysis (Odorico and Miller, 1997b).

Genomic DNA was extracted from ethanol-preserved polyps using a modification of the standard procedure (Sambrook et al., 1989). Prior to the extraction, the samples were submerged for two or three days in 0.5 M EDTA for decalcification (Song and Won, 1997).

A region of approximately 600 bp of the nuclear ribosomal gene complex spanning the 3'-end of 18 S subunit, ITS 1, 5.8 S subunit, ITS 2 region and the 5'-end of 28 S rDNA was amplified by PCR (5'-G TAACAAGGTTTCCGTAGGT-3', 5'-AT ATGCTTAAATC AGCGGGT-3') (Odorico and Miller, 1997b; Song and Won, 1997). We set PCR solution to 50 μl volume [100 mM Tric-Cl (pH 8.3 at 25℃), 500 mM KCl, 0.01% (w/v) Gelatin, 1.5 mM $MgCl_2$, 0.1% Triton X-100] with 1 unit of *Taq* polymerase and 20 pmol primer. PCR was carried out using the following protocol: 40 cycles at 95℃ (1 min), 52℃ (2 min), and 72℃ (2 min). Each of the amplified products was purified by the PEG purification method. Double stranded amplification products of the ITS and 5.8S subunit regions were sequenced directly. DNA sequencing was conducted on both strands using T7 sequences v2.0 (Amersham).

The nucleotide sequences for all specimens were aligned with Clustal W multiple alignment program (Tompson et al., 1994) and then refined by eye. The phylogenetic trees were constructed by the neighbor joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) methods. We used NEIGHBOR and DNAML programs in PHYLIP 3.54 (Felsenstein, 1994) for NJ and ML analyses. The heuristic search option in PAUP 3.1.1 (Swafford, 1993) was adopted for MP analysis. In NJ analysis, we calculated the distance matrix using the DNADIST program with Kimura's model.

A sequence of a related Alcyonacean genus, *Alcyonium* sp., was obtained from GenBank as an outgroup (McFadden et al., 2001, in press).

Table 2. GenBank database accession numbers, lengths and G+C contents of ITS 1, 5.8 S and ITS 2 sequences of dendronephthian species and *Alcyonium* sp. as an outgroup

Species	Total		ITS 1		5.8 S		ITS 2	
	Accession number	Length (bp)	Length(bp)	G+C content (%)	Length (bp)	G+C content (%)	Length (bp)	G+C content (%)
Dendronephthya suensoni	AF320096	590	212	44.3	156	47.4	187	60.4
D. putteri	AF320095	585	209	44.0	155	48.1	188	60.1
D. castanea-1	AF320097	598	212	44.8	155	47.7	195	58.5
D. castanea-2	AF320098	604	213	46.9	157	47.7	196	58.6
D. castanea-3	AF320099	610	213	47.4	157	47.8	199	59.8
D. castanea-4	AF320100	602	214	45.8	157	47.8	193	59.1
D. castanea-5	AF320101	604	212	46.2	157	47.8	196	59.2
D. gigantea-1	AF320102	603	213	46.5	157	47.8	196	59.7
D. gigantea-2	AF320103	603	213	46.5	157	47.8	196	59.7
D. gigantea-3	AF320104	603	213	47.4	157	47.8	196	58.7
D. spinifera-1	AF320104	603	214	48.1	158	47.5	197	60.4
D. spinifera-2	AF320105	603	214	48.1	158	47.5	197	59.9
D. aurea	AF320106	611	221	47.5	158	46.8	198	60.6
D. sp. 1	AF320107	614	211	46.0	159	46.5	207	58.9
D. sp. 2	AF320108	599	209	46.4	154	47.4	199	59.3
D. sp. 3	U64582	605	216	46.3	157	47.8	197	58.9
Alcyonium sp.	AF262353	633	244	44.7	157	46.5	193	52.3

Results

ITS sequence comparison

After PCR amplification of the entire ITS regions (ITS 1, 5.8S, ITS 2), all of the analysed species displayed a single band of PCR product of about 600 bp. The length of ITS 1 varied from 209 bp in *D.* sp. 2 and *D. pütteri* to 221 bp in *D. aurea*, and that of an outgroup is 244 bp. For 5.8 S, the length varied from 154 bp in *D.* sp. 2 to 159 bp in *D.* sp. 1. Also the length of ITS 2 varied from 187 bp in *D. suensoni* to 207 bp in *D.* sp. 1 (Table 2). The G+C content of ITS 1 varied from 44.0% in *D. pütteri* to 48.1% in two specimens of *D. spinifera*, that of ITS 2 did from 52.3% in the outgroup to 60.6% in *D. aurea*. For 5.8 S, it varied from 46.5% in *D.* sp. 1 and the outgroup to 48.1% in *D. pütteri* (Table 2). Accession numbers of the sequences in the GenBank database were given in Table 2.

Pairwise sequence divergences (Table 3) ranged from 0.7% between specimens of *D. gigantea* to 13.7% between *D. pütteri* and *D. aurea*. The divergence value between the dendronephthians and outgroup varied from 24.1% (*D.* sp. and *A.* sp.) to 30.9% (*D.* sp. 1 and *A.* sp.)

Phylogenetic analysis of ITS region

The total number of alignment of ITS regions was 691 bp with gaps treated as missing data. The alignment had 191 variable sites, 68 of which were informative under the parsimony criterion.

Figure 1 shows the NJ tree based on Kimura's distance (Table 3) with bootstrap values. Two clades can be distinguished. The first one consists of two species: *D. pütteri* and *D. suensoni*, and this node is strongly supported by high bootstrap value (100%). The other clade consists of seven species, *D.* sp. 1, *D.* sp. 2, *D.* sp. 3, *D. castanea, D. gigantea, D. aurea* and *D. spinifera*. 4 specimens of *D. castanea* and 1 specimen of *D. gigantea* form one group, and 1 specimen of *D. castanea*, 2 specimens of *D. gigantea, D. aurea* and 2 specimens of *D. spinifera* form the other. In addition, *D.* sp. 2 and *D.* sp. 3 make up the sister relationship with the other two groups in the second clade. Then, *D.* sp. 1 clusters the second clade. The node clustering the second clade excluding *D.* sp. 1 is supported by 89% bootstrap value, but the division into two groups of the second clade cannot be supported.

Table 3. Pairwise comparison of the taxa used in this study. Lower left triangle is the estimates of sequence divergence computed by DNADIST (PHYLIP 3.45) with Kimura's correction, and upper right triangle shows percent divergence calculated by PAUP 3.1.1

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *Dendronephthya* sp.3	-	4.5	4.7	1.0	2.2	2.0	1.0	1.3	1.2	1.2	2.4	2.9	2.2	9.5	2.3	1.0	24.7
2. *D. suensoni*	0.0466	-	1.0	5.2	5.9	5.6	5.2	5.5	5.4	5.4	6.2	6.3	5.9	13.6	4.1	5.2	25.7
3. *D. putteri*	0.0490	0.0105	-	4.9	5.6	5.6	5.1	5.2	4.9	5.6	6.4	6.1	5.7	13.7	4.2	4.9	25.7
4. *D. castanea*-1	0.0102	0.0550	0.0517	-	1.7	1.9	1.0	0.8	1.5	1.5	1.9	2.7	2.6	9.7	3.0	1.2	24.8
5. *D. castanea*-2	0.0222	0.0625	0.0593	0.0172	-	1.7	2.0	1.3	2.2	2.3	2.7	3.2	3.2	10.3	3.7	1.7	25.3
6. *D. castanea*-3	0.0205	0.0585	0.0587	0.0190	0.0170	-	1.8	1.7	1.8	2.0	2.0	3.0	2.7	10.0	3.0	1.5	24.9
7. *D. castanea*-4	0.0102	0.0550	0.0534	0.0103	0.0206	0.0187	-	1.2	1.2	1.2	2.2	2.5	1.9	9.6	2.7	1.2	25.1
8. *D. castanea*-5	0.0135	0.0583	0.0550	0.0085	0.0135	0.0169	0.0119	-	1.3	1.3	1.8	2.5	2.3	9.4	2.8	1.0	25.1
9. *D. gigantea*-1	0.0118	0.0563	0.0511	0.0154	0.0223	0.0187	0.0119	0.0135	-	0.7	2.2	2.7	1.7	9.2	2.5	1.4	25.3
10. *D. gigantea*-2	0.0118	0.0564	0.0589	0.0154	0.0240	0.0205	0.0119	0.0135	0.0067	-	1.8	2.4	1.3	8.7	2.2	1.4	25.0
11. *D. gigantea*-3	0.0241	0.0665	0.0684	0.0191	0.0278	0.0204	0.0224	0.0189	0.0223	0.0188	-	2.8	2.7	10.2	2.9	2.2	25.6
12. *D. spinifera*-1	0.0295	0.0666	0.0644	0.0282	0.0333	0.0311	0.0261	0.0260	0.0278	0.0243	0.0294	-	1.5	9.8	2.9	2.6	25.4
13. *D. spinifera*-2	0.0222	0.0624	0.0606	0.0262	0.0331	0.0275	0.0189	0.0240	0.0171	0.0136	0.0277	0.0153	-	8.8	2.3	1.9	25.1
14. *D. aurea*	0.1045	0.1565	0.1571	0.1064	0.1135	0.1110	0.1059	0.1027	0.1010	0.0951	0.1134	0.1089	0.0963	-	9.7	9.2	30.9
15. *D.* sp. 1	0.0240	0.0428	0.0432	0.0314	0.0382	0.0310	0.0278	0.0291	0.0258	0.0223	0.0295	0.0294	0.0241	0.1069	-	2.0	24.1
16. *D.* sp. 2	0.0103	0.0549	0.0512	0.0121	0.0172	0.0155	0.0121	0.0102	0.0137	0.0137	0.0226	0.0263	0.0190	0.1003	0.0204	-	24.9
17. *Alcyonium* sp.	0.3143	0.3335	0.3339	0.3173	0.3237	0.3167	0.3215	0.3212	0.3246	0.3195	0.3303	0.3266	0.3225	0.4297	0.3046	0.3183	-

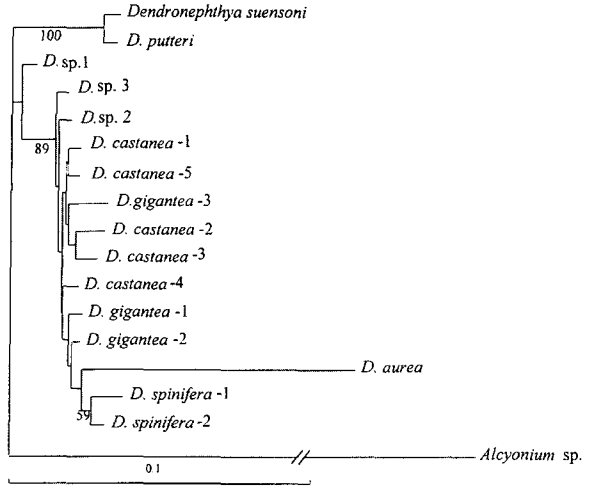

Fig. 1. Phylogenetic relationships of dendronephthians deduced from the neighbor-joining method. This is an unrooted tree, although rooted by the *Alcyonium* sp. as an outgroup. Boostrap values are indicated below corresponing nodes.

ML tree (Fig. 2) also shows two clades that can be distinguished. However, the tree shows similar topology to the NJ tree (Fig. 1) except for the position of *D.* sp. 1 and one specimen of *D. castanea*. Three species, *D. pütteri, D. suensoni* and *D.* sp. 1, form one clade, and the other six species the other. In the first clade, the node of cluster *D. pütteri* and *D. suensoni* is supported by high bootstrap value(100%), which is congruent with NJ tree. The second clade is divided into two groups. One consists of all specimens of *D. castanea, D.* sp. 2 and 1 specimen of *D. gigantea*, and the other two specimens of *D. gigantea, D. aurea* and all specimens of *D. spinifera*. The division into two groups of the

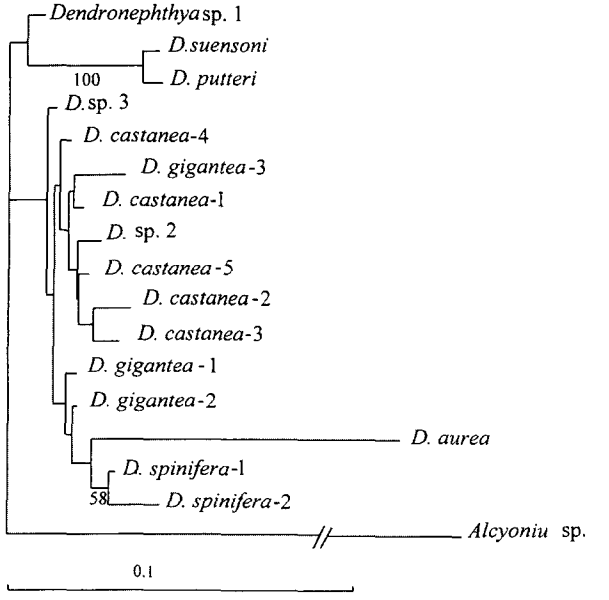

Fig. 2. Relationships within dendronephthians inferred from the maximum likelihood method. All branch length are drawn to scale. Boostrap values are indicated below corresponing nodes. *Alcyonium sp.* served as an outgroup.

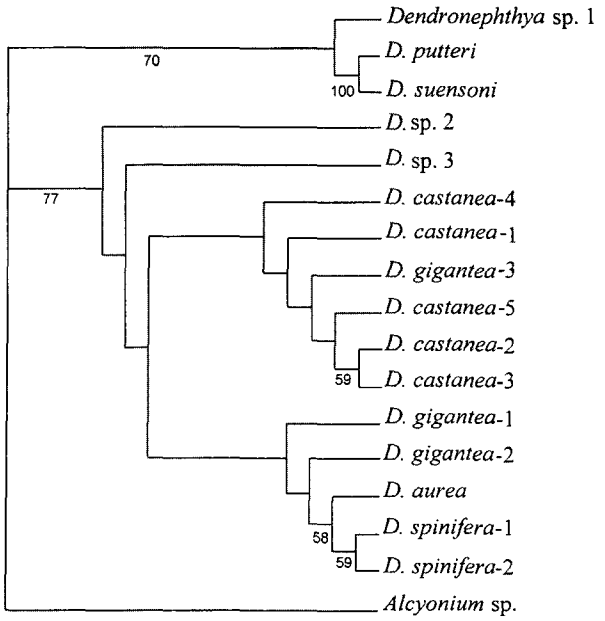

Fig. 3. Realtionships within denderonephthians inferred from the most parsimonious method using the heurestic search option with 1000 bootstrap in PAUP (CI= 0.910). Boostrap values are indicated below corresponing nodes. *Alcyonium sp.* served as an outgroup.

second clade, however, cannot be supported like NJ tree (Fig. 1).

MP tree performed with all informative sites results in a single most parsimony tree (Fig. 3) with 286 steps and the consistency index of 0.717. The tree is almost congruent with the ML tree in topology excluding the position of *D.* sp. 2. It clusters together with the specimens of *D. castanea* into the second group.

Comparison of ITS sequences and RAPD analysis

Figure 4 shows two trees from inferred consensus analysis using ITS sequences and RAPD data (Song and Lee, 2000). They have similar topology despite some differences. First, they are divided into two clades, one consists of *D. pütteri* and *D. suensoni* and the other does of *D.* sp. 1, *D.* sp. 2, *D.* sp. 3, *D. castanea, D. gigantea, D. aurea* and *D. spinifera*. The second clade is then divided again into two groups, one composed of all specimens of *D. castanea*, and the other of most specimens of *D. gigantea, D. aurea* and *D. spinifera*. However, there are some differences between two trees: one is the relation between *D.* sp. 1 and *D.* sp. 2, and the other is the position of *D. gigantea*-3, *D.* sp. 1 and *D.* sp. 2 which make up the sister relationship with each other, then the second clade in the ITS tree. However, they are clustered into one group, and then the group clusters the second clade in the RAPD tree. Also, *D. gigantea*-3 combines with all specimens of *D. castanea* into the first group in the ITS tree, though it is clustered with other specimens of the same species into the second group in the RAPD tree.

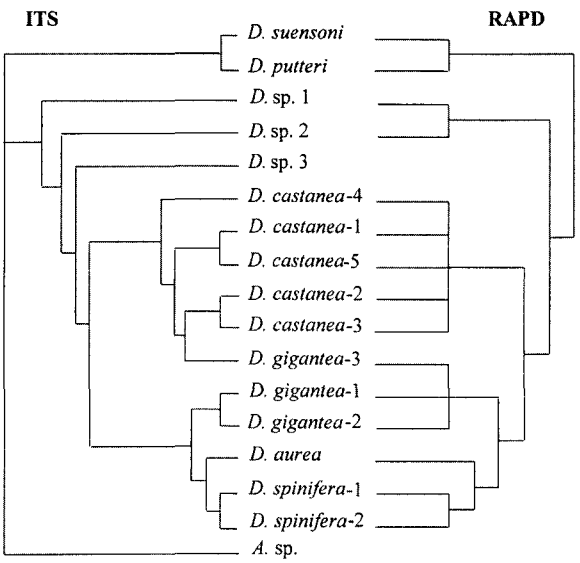

Fig. 4. Combined trees of semi-strict consensus tree and strict tree of ITS sequences and RAPD data (Song and Lee, 2000).

Discussion

Because morphological characters of dendronephthians exhibit variability between intra- and interspecies, some taxonomists described different morphological characters on each species, namely *D. suensoni, D. aurea* and *D. spinifera* (Thomson and Dean, 1931; Roxas, 1933; Utinomi, 1952, 1954; Song, 1977; Song and Lee, 2000) (Table 1).

Thomson and Dean (1931) and Song (1977) recorded that *D. suensoni* had the divaricate form and VI grade in main characters, but Utinomi (1952, 1954) reported glomerate form and IV grade. However, in *D. pütteri,* the divaricate form and IV grade (Raxas, 1933; Song, 1977) are clustered with *D. suensoni.* Moreover, they are strongly clustered into one clade by high value bootstrap (100%) in all phylogenetic trees, and this result coincides with that of RAPD analysis by Song and Lee (2000). *Dendronephthya* sp. 1 has the divaricate form and IV grade, so it clusters with the group of *D. pütteri* and *D. suensoni* which have very similar morphological characters. Therefore, we can conclude that the first clade indicates the dendronephthians with the divaricate form and IV grade.

The relationships of other five species, *D. castanea, D. gigantea, D. aurea* and *D. spinifera*, were inferred as following. All specimens of *D. castanea* clustered into one group in two trees, ML and MP analyses, and all specimens of *D. gigantea, D. aurea* and *D. spinifera* clustered with the other group in all trees excluding one specimen of *D. gigantea.* As the previous studies with morphological characters, *D. castanea* has the umbellate form and IV grade (Utinomi, 1952; Song, 1977), but other species have the glomerate form and III grade (Roxas, 1933; Song, 1977) except two different opinions; that *D. aurea* has IV grade (Song, unpublished) and that *D. spinifera* has IV grade (Utinomi, 1952; Song, unpublished).

However, there are some problems. One is that the division into two groups cannot be supported by bootstrap value, and the divergences within them are very low. Therefore, same species cannot come from the monophyletic group, for example *D. gigantea* and *D. castanea.* So, we can conclude that the ITS method is not sufficient as a molecular marker determining the species boundaries. However, we can infer that the relationships of their growth form, glomerate and umbellate are very similar. According to Thomson and Dean (1931), the relationships among the growth forms have two different opinions. The umbellate form differs from the glomerate and agrees with the divaricate in showing minor branching, but the glomerate and the umbellate are different from the divaricate in the feature of polyp heads. Therefore, we consider that the feature of polyp head is a more important character. Furthermore, this result also suggested that the growth form is not enough to identify dendronephthians. This result is supported by the opinions of Bruno and Edmunds (1977), Knowlton (1993) and McFadden (1999). According to them, difficulties in the identification of marine invertebrates exist, especially in cnidarians with morphological characters, including the growth form.

Another problem is the morphological characters of *D. aurea* and *D. spinifera* described by Song (unpublished). The results of Song (unpublished) did not coincide with those of other taxonomists and both molecular data, ITS and RAPD analysis. Thomson and Dean (1931) reported that the cross-fertilization between sibling species, and the mutations of species apart from any hybridizing influence occurred in the dendronephthians. Therefore, we can guess that our discrepancy in both species is due to these phenomena. So further studies on cross-fertilization between sibling species and hybridization should be done.

Another problem is the outgroup, *Alcyonium* sp. It was very different from dendronephthians and therefore could not be aligned on its regions. Though there is another genus, *Paraspongodes,* that belongs to the family Nephtheidea, we cannot obtain its data. That we have no choice for outgroup is one cause of these problems commented above.

The last problem is the position of *D.* sp. 2 and *D.* sp. 3. First of all, we obtained *D.* sp. 3 from GenBank, and cannot comment about it because of no information is available about its morphological data. In case of *D.* sp. 2, it cannot cluster the first clade, even though it has the divaricate form and IV grade. However, their identification is not concluded yet, and further study on their morphological characters is needed. Also, if many samples with more divergent growth form and anthocodial grade and formula are used, their relationships among dendronephthians can be more clearly delineated.

In spite of some problems, we can confirm once more that the main characters, the growth form and the anthocodial grade and formula, are important characters in the identification of dendronephthians. Also, the relationships of the growth form are clarified in that the glomerate form is much closer to the umbellate form than the divaricate form based on both, RAPD and ITS analyses. Furthermore, the ITS analysis is found to be a useful method to resolve the relationships of morphological characters of the genus *Dendronephthya*, but not useful as a molecular marker to determine the species boundaries, unlike RAPD analysis.

Acknowledgements

This work was supported by grant No. 971-0510-051-2 from the Basic Research Program of the KOSEF.

References

Bruno JF and Edmunds PJ (1997) Clonal variation for phenotypic plasticity in the coral *Madracis mirabilis*. *Ecology* 78: 2177-2190.

Cerbah M, Souza-Chies T, Jubier MF, Lejeune B, and Siljak-Yakovlev S (1998) Molecular phylogeny of the genus *Hypochaeris* using internal transcribed spacers of nuclear rDNA: inference for chromosomal evolution. *Mol Biol Evol* 15: 345-354.

Chen CA and Miller DJ (1996) Analysis of ribosomal ITS1 sequences indicates a deep divergence between *Rhodactis* (Cnidaria: Anthozoa: Corallimorpharia) species from the Caribbean and the Indo-Pacific/Red sea. *Mar Biol* 126: 423-432.

Chen CA, Odorico DM, Ten Lohuis M, Veron JEN and Miller DJ (1995) Systematic relationships within the Anthozoa (Cnidaria: Anthozoa) using the 5'-end of the 28S rDNA. *Mol Phy Evol* 4: 175-183.

Chen CA, Willis BL, and Miller DJ (1996) Systematic relationships between tropical Corallinorpharians (Cnidaria: Anthozoa: Corallimorpharia): utility of the 5.8S and internal transcribed spacer (ITS) regions of the rRNA transcription unit. *Bull Mar Sci* 59: 196-208.

Coffroth MA (1997) Molecular approaches to the study of clonal organisms: deciphering the alphabet soup. *Proc 8th Coral Reef Sym* 2: 1603-1608.

Felsenstein J (1994) PHYLIP (Phylogeny Inference Package) Version 3. 54, Manual. The University of Washington, Seattle.

Hunter CL, Morden CW, and Smith CM (1997) The utility of ITS sequences in assessing relationships among zooxanthellae and corals. *Proc 8th Int Coral Reef Sym* 2: 1599-1602.

Je JG (1994) The distribution of benthic animals on the rocky shore of Munsom area, Chejudo. Report on 93' Natural Ecosystem of Coral Area, pp. 241-259.

Knowlton N (1993) Sibling species in the sea. *Annu Rew Ecol Syst* 24: 189-216.

Kükenthal W (1905) Versuch einer Revision der Alcyonarian. II, Die Familie der Nephthyiden. 2, Teil. Die Gattungen *Dendronephthya n.* g. und *Stereonephthya n.* g. *Zool J Harb Aby Syst* 15: 635-662.

Kükenthal W (1906) Japanische Alcyonacean. Abh. d. II. Kl. d. K. Ak. d. Wiss. Suppl. Bd. Abt. Munchen. pp. 1-86.

Leignel V, Humbert JF, and Elard L (1997) Study by ribosomal DNA ITS2 sequencing and RAPD analysis on the systematics of four *Metastrongylus* species (Nematoda: Metastrongyloidea). *J Parasitol* 83: 606-611.

McFadden CS (1999) Genetic and taxonomic relationships among northeastern Atlantic and Mediterranean populations of the soft coral *Alcyonium coralloides*. *Mar Biol* 133: 171-184.

McFadden CS, Donahue R, Hadland BK, and Weston R (2001) A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus *Alcyonium*. *Evolution*: in press.

Odorico DM and Miller DJ (1997a) Variation in the ribosomal internal transcribed spacer and 5.8S rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. *Mol Biol Evol* 14: 465-473.

Odorico DN and Miller DJ (1997b) Internal and external relationships of the Cnidaria: implications of primary and predicted secondary structure of 5'-end of the 23S-like rDNA. *Proc R Soc Lond B* 264: 77-82.

Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. *Annu Rew Ecol Syst* 25: 547-572.

Raahauge P and Kristensen TK (2000) A comparison of *Bulinus africanus* group species (Planorbidae; Gastropoda) by use of the internal transcribed spacer 1 region combined by morphological and anatomical characters. *Acta Tropica* 75: 85-94.

Reed KM, Hackett JD, and Phillips RB (2000) Comparative analysis of intra-individual and inter-species DNA sequence variation in salmonid ribosomal DNA cistrons. *Gene* 249: 115-125.

Roxas HA (1933) Philippine Alcyonacea. II. The families Alcyoniidae and Nephthydae. *Philippine J Sci* 50: 345-470.

Sambrook J, Fritsch EF, and Maniatis T (1989) Molecular Cloning: a Laboratory manual, 2nd Ed. Cold Spring Harbor Laboratory Press, New York, 1.1-18.85.

Sherriffs WR (1922) Evolution within the genus *Dendronephthya* (Spongodes) (Alcyonaria), with descriptions of a number of species. *Proc Zool Soc* 3: 33-77.

Song JI (1977) A study on the classification of Korea Anthozoa. 2. Alcyonacea. *Korean J Zool* 19: 51-60.

Song JI and Lee YJ (2000) Systematic relationships within the genus *Dendronephthya* (Alcyonacea; Octocorallia; Anthozoa) based on RAPD Analysis. *Korean J Biol Sci* 4: 1-7.

Song JI and Won JH (1997) Systematic relationship of the anthozoan orders based on the partial nuclear 18S rDNA sequences. *Korean J Biol Sci* 1: 43-52.

Swofford DL (1993) PAUP: Phylogenetic Analysis Using Parsimony. Version 3.1.1, Illinois Natural History Survey, Chicago.

Thomson JA and Dean LMI (1931) The Alcyonacea of the Siboga Expedition. Siboga Expeditie, Monaco., pp. 1-227.

Tompson JD, Higgins DG, and Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.

Utinomi H (1952) *Dendronephthya* of Japan. I. *Dendronephthya* collected chiefly along the coast of Kii Peninsula. *Publ Seto Mar Biol Lab* 2: 161-212.

Utinomi H (1954) *Dendronephthya* of Japan. II. New species and new records of *Dendronephthya* and the allied *Steronephthya* from Kii region. *Publ Seto Mar Bio Lab* 3: 319-329.

[Received October 14, 2000; accepted November 13, 2000]