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Systematic Relationships among Species of the Genus *Dendronephthya* (Alcyonacea; Octocorallia; Anthozoa) Based on RAPD Analysis

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The genus *Dendronephthya*, generally known as soft corals, is reported as an abundant and variable taxon. They mostly distribute in warmer waters of the Indo-Pacific Ocean region including Korea. In spite of their abundance and ecological importance as habitats of marine organisms, there are difficulties in the study of their identification and systematics because they have morphological variabilities and limited taxonomic characters. To resolve the problems, we attempted to elucidate the genetic relationships in the genus *Dendronephthya* by using random amplified polymorphic DNA (RAPD) analysis. This study was based on eight dendronephthian species and one Alcyoniidae species, *Alcyonium gracillimum*, as an outgroup. The results from all analysis suggest that they could be classified into four groups by the growth form and the anthocodial grades as follows: the first one, *D. pütteri* and *D. suensoni* with the divaricate form and VI grade; the second one, *D.* sp. 1 and *D.* sp. 2 with the divaricate form and III or IV grade; the third one, *D. gigantea* and *D. aurea* being closer than *D. spinifera* with the glomerate form and III grade; the last one, *D. castanea* related to *D. gigantea* rather than *D. pütteri* with the umbellate form and IV grade. Moreover, the divaricate form was separated from the group of the glomerate and umbellate form. At the intraspecies level, the types of the *D. castanea, D. gigantea* and *D. spinifera* were separated depending on the feature of spicules in the polyp head, and the coloration could not influence genetic variation. From this study, we can confirm that their morphological characters are compatible with the genetic variation, also RAPD analysis is a very useful method for resolving the systematic relationships of dendronephthians.

The Anthozoa is very variable and important taxon for the habitats of marine organisms. The order Alcyonacea belongs to the subclass Octocorallia that is under the Anthozoa, generally known as soft corals and mainly distributed in the southern sea of Korea including Cheju Island area.

In the alcyonarians, three families (Alcyoniidae, Nephtheidae and Siphonogorgiidae) have been recorded from the Korean waters (Rho and Song, 1976; Song, 1977). Especially, the Nephtheidae that contains nine dendronephthian species is reported as an abundant taxon in the Cheju Island area (Je, 1994).

The genus *Dendronephthya* shows bushlike or branching feature and their polyps are always provided with supporting bundles (Roxas, 1933). The genus contains an enormous number of species, not fewer than 150 species all over the world (Thomson and Dead, 1931; Roxas, 1933; Utinomi, 1952) and is mostly distributed in the warmer waters of the Indo-Pacific region from the Red Sea to Japan except one species, *D. mexicana* (Utinomi, 1952).

To identify them, observations are focused on the growth form (Kükenthal, 1905), and the anthocodial grade and formula (Sherriffs, 1922). The former divided the genus into three main groups - Glomerate, Divaricate and Umbellate, and the latter classified them into six grades by the number and arrangement of point and crown sclerites in anthocodial armature (Thomson and Dean, 1931; Roxas, 1933). However, their identification retains difficult problems because they have minute variability in the intraspecies (Utinomi, 1952). The variation in a narrow range is caused by the cross-fertilization between sibling-species, and the mutations of species apart from any hybridizing influence. For example, *D. gigantea, D. ehrenbergi* and *D. brebirama* having much variability brought about this influence (Thomson and Dean, 1931). Therefore, the systematic

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Table 1. Two taxonomic characters of the genus *Dendronephthya* used in this study

Species	Growth form by Kükenthal (1905)	Anthocodial grade by Sheriffs (1922)	Authors
Dendronephthya gigantea	Glomerate	III	Thomson and Dean (1931) Roxas (1933) Utinomi (1952) Song (1977)
*D. aurea**	Glomerate	III	Utinomi (1952)
*D. spinifera**	Glomerate	III IV	Roxas (1933) Utinomi (1952)
D. suensoni	Divaricate	VI	Thomson and Dean (1931) Song (1977)
	Glomerate	IV	Utinomi (1952, 1954)
D. pütteri	Divaricate	VI	Roxas (1933) Song (1977)
D. castanea	Umbellate	IV	Utinomi (1952) Song (1977)
D. sp. 1**	Divaricate	IV	-
D. sp. 2**	Divaricate	III	-

*Species identified in the present study, **Species added for analyzing

relationships within the genus *Dendronephthya* remain uncertain in spite of their abundance and ecological importance, and some species are described by different opinions according to scientists (e. g. *D. suensoni* and *D. spinifera*) (Table 1).

Recently, to resolve many problems occurred from the intraspecies and interspecies of many taxon, the method of random amplified polymorphic DNAs (RAPDs) (Williams et al. 1990, Welsh et al. 1990) has been used (Borowsky et al., 1995; Clossland et al., 1993; Espinasa and Borowsky, 1998; Foo et al., 1995; Heun et al., 1997; Krane et al., 1999; Sültman et al., 1995; Tamate et al., 1995; Tibayrence et al., 1993). The RAPD markers are made of relatively short DNA fragments (a few Kb, or less in length) and amplified via PCR (Polymerase Chain Reaction, with low annealing temperature, 35-45℃) by small arbitrary primers (usually 10 bases in length, with G+C contents > 50%) (Grosberg et al., 1996).

In the present study, we analyzed RAPD markers in an attempt to determine the extent of genetic variations within intraspecies and interspecies of dendronephthians. Also another aim of this study was to examine the usefulness of the RAPD marker for genetic analysis of soft coral populations.

Materials and Methods

We collected 39 specimens in total from the southern part of Cheju Island by scuba diving between 1997 and 1998. This study was based on eight species of the genus *Dendronephthya* - *D. suensoni, D. pütteri, D. castanea, D. gigantea, D. spinifera, D. aurea, D.* sp. 1, *D.* sp. 2, and one Alcyoniidae species, *Alcyonium gracillimum,* as an outgroup (Table 1). *A. gracillimum* is a common and abundant species in Cheju Island and has same habitat with dendronephthians. So we selected it as an outgroup for this study. However, three species, *D. castanea, D. gigantea and D. spinifera,* among them showed variation of anthocodial armature and coloration. Therefore, we divided *D. castanea* into five groups (Aw, Ay, Bw, By, C), *D. gigantea* into three groups (D, E, F) and *D. spinifera* into two groups (G, H) (Table 2).

Table 2. Taxonomic information and types of each species collected from the Cheju Island area

Classification	Species	Types (N)	Main characters
Phylum Cnidaria Class Anthozoa Subclass Octocorallia Order Alcyonacea			
Family Nephtheidae	*Dendronephthya suensoni*	(3)	
	D. pütteri	(3)	
	D. castanea	Aw (2), Ay (2)	no M
		Bw (3), By (2)	with M
		C (2)	with many M
	D. gigantea	D (3)	equal P polyp neck with spicules
		E (4)	subequal P polyp neck without spicules
		F (6)	subequal P polyp neck with spicules
	D. aurea	(2)	
	D. spinifera	G (1)	number of S.B.=3 long and thin S.B.
		H (3)	No. of S.B.=more than 4 short and thick S.B.
	D. sp.1	(1)	
	D. sp.2	(1)	
Family Alcyoniidae	*Alcyonium gracillimum*	(1)	

A - H, types of each species; M, intermediate sclerities; P, large point sclerities,
S. B., supporting bundles; Bw, white supporting bundles; By, yellow supporting bundles.

The genomic DNA were extracted from polyps by using the phenol extraction method described by Sambrook et al. (1989). Before the extraction, they had been submerged for two or three days in 0.5M EDTA for decalcification (Song and Won, 1997). The concentration of DNA was adjusted to 10 μg/μl for the subsequent PCR reaction. Total 40 primers were used from QUIAGEN 10-mer Kits series A and B, and then 17 primers with polymorphic results were selected. We set PCR solution to 20 μl [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 10 pmol primer. The PCR-reaction was

Table 3. Matrix of similarity coefficient among the genus *Dendronephthya*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	-																			
2	0.264	-																		
3	0.387	0.360	-																	
4	0.529	0.538	0.583	-																
5	0.435	0.412	0.282	0.509	-															
6	0.424	0.387	0.424	0.489	0.346	-														
7	0.678	0.678	0.700	0.574	0.700	0.678	-													
8	0.651	0.651	0.667	0.604	0.674	0.644	0.324	-												
9	0.648	0.655	0.663	0.608	0.692	0.670	0.346	0.291	-											
10	0.604	0.620	0.644	0.587	0.667	0.644	0.367	0.300	0.254	-										
11	0.659	0.674	0.674	0.578	0.674	0.651	0.393	0.387	0.418	0.447	-									
12	0.604	0.620	0.628	0.587	0.644	0.604	0.380	0.360	0.406	0.374	0.331	-								
13	0.612	0.628	0.636	0.587	0.644	0.612	0.367	0.387	0.393	0.387	0.331	0.141	-							
14	0.651	0.667	0.674	0.578	0.674	0.651	0.380	0.424	0.380	0.412	0.346	0.400	0.374	-						
15	0.624	0.648	0.655	0.574	0.655	0.624	0.435	0.380	0.360	0.406	0.339	0.367	0.353	0.254	-					
16	0.640	0.648	0.678	0.574	0.655	0.624	0.447	0.406	0.435	0.452	0.406	0.380	0.367	0.406	0.400	-				
17	0.651	0.674	0.689	0.561	0.674	0.644	0.452	0.424	0.430	0.458	0.360	0.412	0.400	0.360	0.353	0.234	-			
18	0.624	0.624	0.616	0.547	0.624	0.616	0.479	0.463	0.458	0.463	0.474	0.484	0.474	0.452	0.458	0.447	0.463	-		
19	0.636	0.636	0.620	0.570	0.644	0.612	0.514	0.458	0.441	0.479	0.479	0.479	0.479	0.479	0.452	0.484	0.469	0.308	-	
20	0.632	0.616	0.616	0.538	0.632	0.624	0.509	0.504	0.500	0.494	0.543	0.494	0.504	0.474	0.479	0.500	0.494	0.346	0.308	-
21	0.636	0.636	0.644	0.587	0.659	0.636	0.494	0.500	0.463	0.479	0.509	0.469	0.469	0.469	0.494	0.474	0.500	0.418	0.435	0.441
22	0.636	0.651	0.636	0.587	0.659	0.651	0.494	0.500	0.484	0.489	0.489	0.489	0.489	0.479	0.474	0.524	0.509	0.430	0.435	0.452
23	0.632	0.648	0.640	0.583	0.670	0.640	0.529	0.514	0.500	0.474	0.504	0.494	0.504	0.494	0.500	0.519	0.504	0.424	0.418	0.458
24	0.624	0.616	0.608	0.547	0.632	0.624	0.489	0.514	0.479	0.494	0.524	0.494	0.504	0.524	0.519	0.547	0.552	0.447	0.430	0.435
25	0.636	0.628	0.636	0.561	0.651	0.636	0.524	0.509	0.494	0.489	0.500	0.500	0.509	0.529	0.524	0.514	0.509	0.463	0.458	0.484
26	0.620	0.644	0.644	0.543	0.659	0.644	0.463	0.489	0.452	0.458	0.469	0.479	0.469	0.469	0.504	0.514	0.489	0.441	0.435	0.484
27	0.624	0.632	0.640	0.547	0.648	0.640	0.479	0.504	0.458	0.484	0.494	0.494	0.484	0.474	0.509	0.479	0.484	0.435	0.463	0.500
28	0.624	0.632	0.624	0.547	0.640	0.648	0.519	0.533	0.479	0.494	0.494	0.494	0.484	0.474	0.500	0.500	0.484	0.412	0.418	0.447
29	0.612	0.628	0.628	0.561	0.636	0.612	0.504	0.479	0.463	0.458	0.489	0.469	0.469	0.458	0.463	0.484	0.479	0.406	0.412	0.452
30	0.612	0.636	0.644	0.561	0.651	0.636	0.494	0.500	0.463	0.458	0.489	0.458	0.458	0.469	0.484	0.484	0.489	0.430	0.424	0.463
31	0.648	0.648	0.624	0.556	0.640	0.640	0.509	0.533	0.519	0.524	0.463	0.494	0.484	0.452	0.489	0.519	0.514	0.458	0.494	0.509
32	0.612	0.612	0.612	0.533	0.628	0.620	0.514	0.519	0.504	0.500	0.500	0.500	0.489	0.500	0.494	0.514	0.519	0.474	0.479	0.494
33	0.636	0.644	0.628	0.595	0.651	0.628	0.533	0.519	0.504	0.500	0.500	0.489	0.489	0.469	0.474	0.514	0.509	0.474	0.458	0.504
34	0.648	0.648	0.640	0.565	0.663	0.640	0.529	0.543	0.529	0.524	0.514	0.514	0.524	0.494	0.489	0.556	0.524	0.500	0.474	0.489
35	0.595	0.604	0.604	0.543	0.628	0.604	0.463	0.447	0.430	0.400	0.509	0.469	0.479	0.489	0.484	0.474	0.469	0.430	0.435	0.452
36	0.632	0.600	0.616	0.565	0.632	0.608	0.458	0.474	0.447	0.452	0.514	0.494	0.494	0.484	0.479	0.519	0.494	0.447	0.484	0.479
37	0.616	0.600	0.616	0.608	0.624	0.632	0.583	0.561	0.547	0.524	0.628	0.561	0.570	0.604	0.600	0.600	0.628	0.583	0.578	0.574
38	0.578	0.570	0.595	0.552	0.595	0.587	0.561	0.565	0.543	0.529	0.600	0.556	0.556	0.583	0.587	0.552	0.583	0.514	0.556	0.561
39	0.632	0.624	0.685	0.640	0.685	0.663	0.692	0.689	0.692	0.644	0.717	0.681	0.689	0.696	0.692	0.692	0.710	0.685	0.703	0.678

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
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19																			
20																			
21	-																		
22	0.360	-																	
23	0.353	0.308	-																
24	0.393	0.406	0.387	-															
25	0.412	0.400	0.406	0.339	-														
26	0.387	0.346	0.367	0.406	0.360	-													
27	0.339	0.380	0.387	0.400	0.367	0.308	-												
28	0.406	0.406	0.400	0.374	0.393	0.324	0.316	-											
29	0.374	0.360	0.353	0.353	0.360	0.331	0.291	0.324	-										
30	0.360	0.374	0.353	0.353	0.360	0.300	0.308	0.308	0.200	-									
31	0.463	0.452	0.469	0.489	0.504	0.452	0.435	0.435	0.463	0.463	-								
32	0.479	0.435	0.452	0.463	0.489	0.435	0.430	0.406	0.424	0.435	0.339	-							
33	0.447	0.424	0.418	0.484	0.489	0.447	0.452	0.430	0.387	0.424	0.418	0.346	-						
34	0.484	0.418	0.447	0.469	0.504	0.484	0.469	0.458	0.441	0.452	0.400	0.308	0.324	-					
35	0.469	0.458	0.418	0.452	0.447	0.447	0.452	0.463	0.400	0.447	0.514	0.458	0.424	0.452	-				
36	0.474	0.452	0.469	0.447	0.452	0.441	0.447	0.447	0.393	0.441	0.489	0.463	0.463	0.447	0.308	-			
37	0.587	0.587	0.616	0.574	0.570	0.552	0.591	0.583	0.570	0.570	0.583	0.570	0.570	0.591	0.514	0.538	-		
38	0.538	0.565	0.570	0.514	0.509	0.489	0.514	0.514	0.509	0.500	0.533	0.519	0.556	0.570	0.519	0.514	0.441	-	
39	0.689	0.689	0.670	0.678	0.689	0.681	0.707	0.678	0.689	0.674	0.632	0.636	0.651	0.655	0.667	0.663	0.574	0.604	-

※ 1-3, *D. suensoni;* 4-6, *D. pütteri;* 7-8, *D. castanea* Aw; 9-10, *D. castanea* Ay; 11-13, *D. castanea* Bw; 14-15, *D. castanea* By; 16-17, *D. castancea* C; 18-20, *D. gigantea* D; 21-24, *D. gigantea* E; 25-30, *D. gigantea* F; 31, *D. spinifera* G; 32-34, *D. spinifera* H; 35-36, *D. aurea*; 37, *D.* sp. 1; 38, *D.* sp. 2; 39, *A. gracillimum* carried out using the following protocol: 1 cycle at 95℃ (1 min), 35℃ (2 min), and 72℃ (2 min); 35 cycles at 95℃ (1 min), 35℃ (2 min), and 72℃ (2 min); and I cycle at 95℃ (1 min), 35℃ (2 min), 72℃ (5 min) (Williams et al., 1993; Grosberg et al., 1996). Each of the amplified products were run on a 1.5% agarose gel. The gels were stained with 0.5 µg/µl ethidium bromide solution, and then photographed. RAPDs markers were coding as "0" (absence of band) or "1" (presence of band), and made up of the data matrix. The genetic similarity coefficients and the dendrogram were estimated by the UPGMA method in the NTSYS program (Rohlf, 1992).

Results

A total of 200 polymorphic RAPD markers amplified by 17 different primers was used to analyze the genetic relationships among the dendronephthians (Appendix 1). The genetic similarity coefficient among 39 specimens (9 species, 15 types) was estimated by the UPGMA method (Table 3). The average of similarity coefficient (ASC) crosses all specimens ranged from 0.141 (between the specimens of *Dendronephthya castanea* Bw) to 0.717 (*D. castanea* Bw and *A. gracillimum*). In the same species, the specimens of *D. gigantea* were considerately divergent (ASC=0.312). The ASC across all specimens ranged from 0.446 (*D. suensoni* and *D. pütteri*) to 0.692 (*D. castanea* and *A. gracillimum*), and the ASC between outgroup, *A. gracillimum* and the other specimens was 0.646 (Table 3).

As shown in Fig. 1, dendronephthians are divided into two clades; *D. suensoni* and *D. pütteri* consist of the first one, and the remaining species the second one. From the first clade, six specimens were used from *D. pütteri* and *D. suensoni*, they are clustered together without branching each other species. And in the second clade, a total of 32 specimens (8 species) were used; 11 specimens of *D. castanea*, 13 specimens of *D. gigantea*, two specimens of *D. aurea*, four specimens of *D. spinifera* and each one specimens of two species, *D.* sp. 1 and *D.* sp. 2. Especially, many specimens of *D. gigantea* and *D. castanea* were used to examine the variability in the anthocodial armature. As a result, they are divided again into two clades, one has *D. gigantea, D. aurea, D. spinifera* and *D. castanea*, the other does *D.* sp. 1 and *D.* sp. 2. In the first one, *D. gigantea* and *D. aurea* bind together, and then *D. spinifera* binds again with them, and the last, *D. castanea* binds. Of course, there are no mixing with different species.

Furthermore, the specimens of *D. castanea, D. gigantea* and *D. spinifera* are composed of each of two branching groups (Fig. 1). In the *D. castanea*, four specimens of the type A separate from the other types and seven specimens of the types B and C divide into groups according to the types despite of color variation. *D. gigantea* are divided into two parts, three

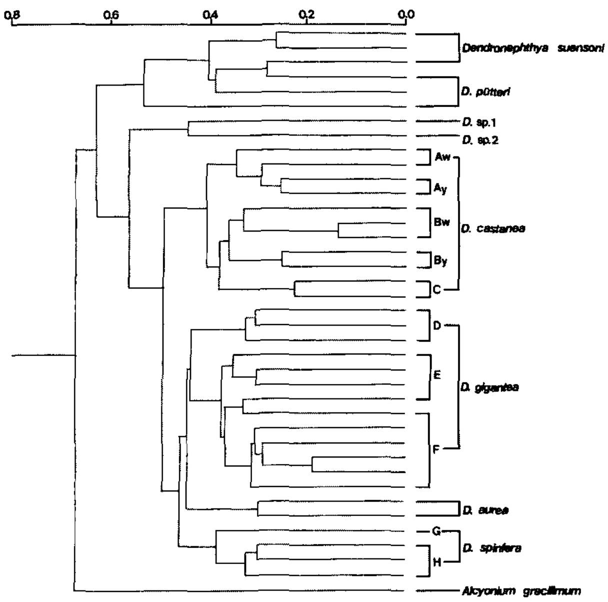

Fig. 1. Dendrogram of the 39 dendronephthian specimens derived from genetic similarity coefficient estimated from RAPD analysis.

specimens of the type D and 10 specimens of the types E and F, and the 10 specimens of the second part are compound together. Finally, the two types of the *D. spinifera*, one specimen of the type G and three of the type H of *D. spinifera*, are also divided following their types.

Discussion

The genus *Dendronephthya* was named by Kükenthal (1905) and it's systematic studies were continued by Sherriffs (1922), Thomson and Dean (1931), Roxas (1933), Utinomi (1952, 1954) and Song (1977) for a long time. However, in spite of all their studies, we find difficulties in identifying the relationships within dendronephthians because of their variabilities and limited taxonomic characters.

As a result of the dendrogram (Fig. 1), a total of eight dendronephthians species is divided into two clades; *D. suensoni* and *D. pütteri* are in the first clade and the remaining species are in the second one. The first clade, six specimens of *D. suensoni* and *D. pütteri*, is clustered together. And it is supported by their common characters which are described as divaricate from and VI grade (Roxas, 1933; Song, 1977; Thomson and Dean, 1931). Therefore, we consider that they are more closely related than other species.

Within the second clade, 32 specimens of six species, *D. castanea, D. gigantea, D. aurea, D. spinifera, D.* sp. 1 and *D.* sp. 2, are divided again into two clades. One has *D. gigantea, D. aurea, D. spinifera* and *D. castanea* and the other has *D.* sp. 1 and *D.* sp. 2. In the first clade, all specimens of *D. gigantea* and *D. aurea* appeared as a cluster without any confusion, and then

D. spinifera binds into them. Those three species are reported as organisms having the glomerate form and III grade (Roxas, 1933; Song, 1977; Thomson and Dean, 1931). These results mostly coincide with one another. However, the growth form and anthocodial grade of *D. suensoni* and *D. spinifera* by Utinomi (1952) are not supported in terms of our present results. In the second one, *D.* sp. 1 and *D.* sp. 2 were clustered together. Their growth form is inferred as the divaricate form, but they bind to the group of the glomerate and the umbellate, and separate from the group of the divaricate. We got that result, because their feature of the divaricate forms were different from *D. suensoni* and *D. pütteri*. And their grade is inferred as IV for *D.* sp. 1 and III for *D.* sp. 2. However, their identification isn't concluded yet, so more study about their growth form is needed.

D. castanea reported as the umbellate form and IV grade was clustered with the group of the glomerate form and III grade (*D. gigantea, D. aurea* and *D. spinifera*), but separated from which the divaricate form and VI grade (*D. pütteri* and *D. suensoni*). That is the glomerate form has a closer relationship with the umbellate than the divaricate form. According to Thomson and Dean (1931), the relationships among the growth forms have two different opinions which the umbellate form differs from the glomerate and agrees with the divaricate in showing much minor branching, but the glomerate and the umbellate different from the divaricate in the feature of polyp heads. Therefore, we consider that the feature of polyp head is a more important character because of the agreement on the latter by results.

Especially, the results divided *D. castanea, D. gigantea* and *D. spinifera* into six groups. *D. castanea* is divided into two groups by their absence or presence of intermediate sclerities, but coloration does nto effect our results. *D. gigantea* is seperated into two groups by the feature of large point sclerities and *D. spinifera* is divided into two groups by the number and shape of support bundles. With these data, we suggest that some kinds of characters on anthocodial armature are more important than others. These results are accordant to the report of Thomson and Dean (1931). They report that the variation of the *D. gigantea* is caused by the mutation, and our result agree with that. Also, the variation of *D. castanea* and *D. spinifera* are caused by the same reason of *D. gigantea*.

From the result of this study, we confirm that three characters of growth form, anthocodial grade and formula are very important characters in identifiying dendronephthians and compatible with the genetic variation. Also, the relationships of the growth form identified that the glomerate is much closer to the umbellate than the divaricate. The RAPD analysis is defined as very useful method to resolve the systematic relationships within the genus *Dendronephthya*.

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Appendix 1. Data matrix of RAPD markers in the genus *Dendronephthya*

	P1 ABCDEFGH	P2 ABCDEFGHIJKLMNOPQ	P3 ABCDEFGHI	P8 ABCDEFGH	P9 ABCDEFGHIJ	P10 ABCDEFGHIJK	P12 ABCDEFGH	P14 ABCDEFGHIJ	P15 ABCDEFGHIJKL
1	00111000	00100001001000100	100100010	00010101	0000100110	00111011001	00001000	0010000101	001001001100
2	00011000	00100001100000100	100100010	00010101	0000100110	00011011000	00001010	0010000001	001001001100
3	10011100	00100001001000100	100000010	11110101	0000100110	00011011001	10001010	0010000001	001011001100
4	01100100	00001000010100000	111100111	00000010	0000100100	00111011000	11001010	1110000000	000100010000
5	10011100	00100001001000100	101000010	11110001	0000100100	00011011001	11001010	1110000000	000011001100
6	00001100	11001001100000110	100100010	00010101	0000100100	00011011001	01001010	1110000000	000111001000
7	01000110	00001000010101100	101101111	00000011	0010011100	01100000010	00110010	0101000100	000101010000
8	00100101	00001000010101100	100100000	00000011	0010011100	01000000000	00110010	0101000000	000111010000
9	00100101	00001000010100100	100100001	00000011	0010010000	01000000000	00010000	0001010001	000001010000
10	00100100	00001000010100100	100100010	00000011	0010010000	01000000000	00010000	0001000001	000001010000
11	01100101	00001000010101100	101100111	00000011	0010001100	01111010011	11100110	0000000001	000111010000
12	00000100	00001000010101100	100100010	00000011	0010001100	01110010011	00100010	0000000001	000101010000
13	00000100	00001000010101100	101100010	00000011	0010001100	01110010011	00100010	0000010001	000101010000
14	00100100	00001000010100110	101001101	00000011	0010000100	01011010010	01000110	0000010101	000111010000
15	00100101	00001000010101100	101100001	00000011	0010000100	01011010001	00000110	0000010101	000111010000
16	00100100	00001000010101000	101100000	00000011	0110001100	01000010010	01000110	1100110000	000101010001
17	00100101	00001000010100000	101100101	00000011	0010001100	01000010011	01000110	1100110001	000111010001
18	01100100	00001000010100000	101100100	00000011	0110000101	00001000100	10000110	0010000000	000111010000
19	00100101	00001001110100000	100100001	00000011	0010000101	00000010100	10000110	0010000000	000111010000
20	10000100	00001000110100000	110000000	00000011	0010000101	00000010100	10000110	0010000100	000111010000
21	00000100	00001000010100000	110100000	00000001	0010010101	11010010010	01000100	0010101001	100100010000
22	00000111	00001000010101100	101000111	00000001	0010010101	11110010000	00000100	0010000001	100111010000
23	00100100	00001000010100000	100100011	00000001	0010010101	11111000010	00000100	0010100001	100111010000
24	01000100	00001000010100000	110101011	00000001	0010110101	01110010000	10000000	0000001001	000011000001
25	01100101	00001000010101100	110100011	00000001	1010110101	01010010010	01000000	0000000001	100011000000
26	00100101	00001000010101100	101100111	00000001	0110110101	01110010010	01000000	0000000001	100100010000
27	00100100	00001000010100000	101100111	00000001	1010110101	01010010010	01000000	0000100001	100101011101
28	00100100	00001000010100000	101100111	00000001	0010110101	01010010110	11000000	0000000001	000010011100
29	00100100	00001000010100011	101100011	00000001	1010110101	01010010000	01000000	0000000001	000111010000
30	00100100	00001000010100011	101100011	00000001	1010110101	01110010010	01000000	0000000001	000000010000
31	01100100	00001000010100010	101000110	00000010	0110001101	11111010010	11000110	0000000001	000111011100
32	01100110	00001000010100001	101100010	00000001	0100000100	01110010000	01000110	0000000001	000111011101
33	00100110	00001000010100110	000100011	00000001	0100000100	01110010100	01000110	0000100001	000111010000
34	01000111	00001000010100011	000100111	00000001	0100000100	01110010100	01000110	0000000001	000111011100
35	00100100	00001000010100100	100100011	00000001	0010010100	00000000000	00000000	0001100000	000111010000
36	00000101	00001000010100111	001100111	00000001	0010010101	00000000000	00000000	0001000001	000111010000
37	00000100	00010010000001100	000010000	00000000	0001010000	00000111100	00000000	0000000000	000000010000
38	01000000	00001000001000000	001100000	00000001	0000100101	00000011010	01000000	0000000000	000000000010
39	01000000	00000100010100010	000010010	00010010	0001000110	10101101000	00010101	0000000010	000000000101

Appendix 1. Continued

	P17 ABCDEFGHIJKL	P18 ABCDEFGHIJKLM	P19 ABCDEFGHIJKLMNOPQ	P20 ABCDEFGHIJKLMNOP	P24 ABCDEFGHIJKLMN	P26 ABCDEFGHIJKLMNO	P27 ABCDEFGHIJ	P28 ABCDEFGHIJ
1	000000000110	1010100110010	10000101001010010	0001000001100000	00100100110000	010000001000000	0011010100	0000000001
2	100000000110	1010100110010	10000100001010010	1001000001000000	00100100110000	010000001000000	0011011100	0000000010
3	100000000110	1010100110010	10000100001001000	1001000011000000	00100100001100	111000001000000	0010011100	1010000001
4	010000000110	1000010100010	00000001001000000	0001000001100010	01001101110000	111000001000000	0110100000	0000000110
5	100000000110	1010100110010	10000100001001000	1001000011000000	01001101110000	111000001000000	0010011100	1010000001
6	000000000110	1010101110010	10000100001010000	1001000001000000	01001101110000	111000001000000	0011011100	1010000001
7	011000001001	0100010000010	01000001000100000	0100000100000010	00000100001001	001010110000001	1100111000	0100000000
8	011000001001	0100010000010	01000001000100000	0110000000001000	00000100001001	000000110000001	1100111100	0100000001
9	011000001001	0100010000010	01000001000100000	0110000100000010	00000100001101	001010110000001	1100111100	0100000001
10	011000001001	0100000000010	01000001000100000	0100000000000000	00000100000101	000000110000001	1100110000	0100000001
11	011010001001	0110010000010	01000011000110000	1110000100001000	00000101001001	001010110000001	1100110100	0100000101
12	011010001001	0110101100010	01000001000100000	0100000000001000	00000101001001	001010110000001	1100110100	0000000001
13	011010001001	0110101100010	01000001000100000	0100000100000000	00000101001001	001010110000001	1100110100	0000000001
14	011010001001	0110010001010	01000011000100000	0100000100000010	00000100101001	001010110000001	1100110000	0100000001
15	011010001001	0110010001010	01000011000100000	0110000000000010	00000101101001	001000110000001	1100110100	0000000001
16	011010001010	0110100100010	01000011000110000	0110000000100000	00000100101001	001010110000001	1100111110	0000000101
17	011010001010	0110010100010	01000011000110000	0110000000100000	00000100101001	001010110000001	1100110010	0000000101
18	011000001010	1110000000110	00000001000100000	0000001110000000	00000110101101	011010100000101	0100111110	0000000001
19	011100001010	1110011000110	00000001000100000	0010000110000000	00000110001101	011010100000101	0100100100	0010000001
20	011100001010	1110010110110	00000001000100000	0000000010000010	00000110101101	011010100000101	0100100000	0000000010
21	011000001010	1010110001110	00000001000100000	0000100110000010	00000110001001	001010100000101	0100111110	0100000001
22	011000001010	1110110001110	00000001000100000	0000101010000010	00000110001101	001000100000100	0100000110	0100000101
23	010100001010	1110111001110	00000001000100000	1000101010000000	00000110001101	001000100000100	0100100010	0100000101
24	010100001010	1100110000110	00000001000100000	1000101010000010	00000110001001	001010100000100	0100101100	0010000000
25	011000001010	1000000000110	00000001000100000	1000101010000000	00000110001001	001010100000100	0100100110	0010000110
26	010100001010	1000010000110	00000001000100000	0000001110000000	00000100001101	001010100000100	0100000010	0100000001
27	011000001010	1000110000110	00000001000100000	0000001110010010	00000100001001	001010100000100	0100101110	0100000101
28	010110001010	1110110000110	00000001000100000	0000001110000010	00000100001101	001010100000100	0100100110	0000000011
29	010000001010	1110110000110	00000001000100000	0000001010010000	00000110001001	001010100000100	0100100110	0100000001
30	011100001010	1110110000110	00000001000100000	0000001010000000	00000110001001	001010100000100	0100100110	0100000001
31	011000001000	0000110001110	00100000010101000	1000000100000010	00000100001100	001010100000000	0100000110	0000000001
32	010100001000	1100110000110	00100000010101000	0000000100000010	00000100001100	001000100000000	0100000110	0000000111
33	010010001000	1110110001110	00100000010101000	0000000110010000	00000110001100	001000100000000	0100100110	0100000001
34	011100001000	1110110001110	00100000010101000	0000100000010010	00000110001100	001000100000000	0100100000	0000000101
35	010000001010	1110000000110	01000000001101000	0000000000110000	00000110001100	001010100000000	0100110000	0000000100
36	010000001010	1110000000110	01000000001101000	1000000000010010	00000100001000	001010100000000	0100111010	0000000010
37	100000001000	0000000000001	00010000100001000	0000010000000000	00000100000010	001010000000000	0100000100	0001000000
38	000000000000	0000000000010	00010000100000100	0000001000000000	10000100000100	001010000000000	0100001110	0000000000
39	000111000000	0001100011001	10101000000010001	0000010000000101	01001100000000	000101001010000	0011000011	0001101010

1-3, *D. suensoni;* 4-6, *D. pütteri;* 7-8, *D. castanea* Aw; 9-10, *D. castanea* Ay; 11-13, *D. castanea* Bw; 14-15, *D. castanea* By; 16-17, *D. castancea* C; 18-20, *D. gigantea* D; 21-24, *D. gigantea* E; 25-30, *D. gigantea* F; 31, *D. spinifera* G; 32-34, *D. spinifera* H; 35-36, *D. aurea*; 37, *D.* sp. 1; 38, *D.* sp. 2; 39, *A. gracillimum*