CHAPTER 4

RESULTS

The results could be divided into 2 sections. First section was result in orchid morphology that based on information from plant representatives in each species. Second section was result in genetic relationship between two genera. Genetic relationship results were referred to orchid morphology and classified taxonomy.

4.1 Orchid morphology

There were 4 species of *Habenaria* used in this study. In each species, 4 plants were recorded and average value was presented.

4.1.1. There are 3 different flower colors of *H. rhodocheila* Hance.

- *H. rhodocheila* Hance. (orange flower) (Figure 11)

Leaves: green to brown–green, undulate leaves, 4 to 10 leaves per plant, spiral leaf arrangement

Flowers: bright to deep orange, 2 to 7 flowers per plant

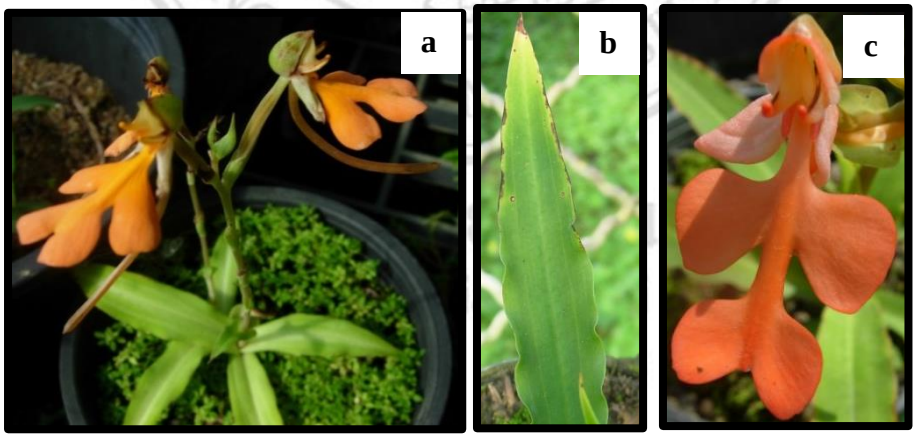

Figure 11 Morphology of *H. rhodocheila*, orange flower

a) plant; b) leaf; c) flower

- *H. rhodocheila* Hance. (pink flower) (Figure 12)

Leaves: green to brown-green and spot, 4 to 10 leaves per plant, spiral leaf arrangement

Flowers; bright pink, 3 to 7 flowers per plant

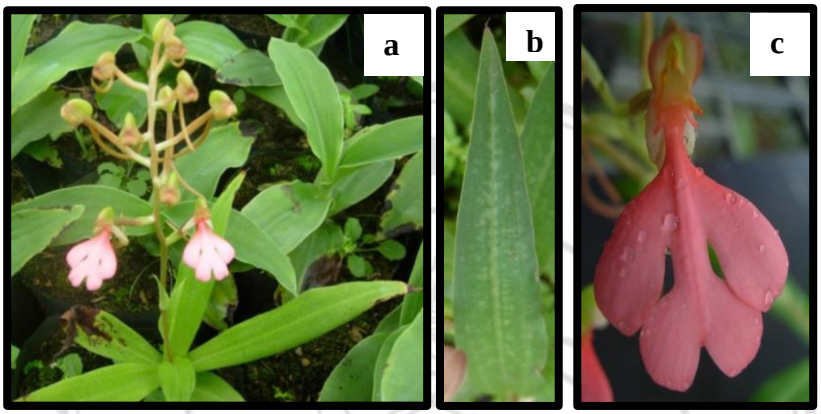

Figure 12 Morphology of *H. rhodocheila*, pink flower

a) plant; b) leaf; c) flower

- *H. rhodocheila* Hance. (red flower) (Figure 13)

Leaves: green some undulate leaves, 2 to 8 leaves per plant, spiral leaf arrangement

Flowers: bright to deep red, 2 to 7 flowers per plant

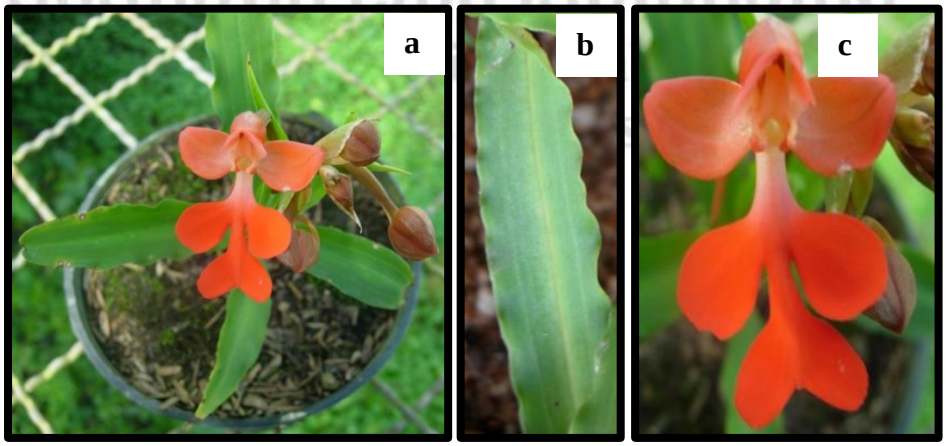

Figure 13 Morphology of *H. rhodocheila* (red flower)

a) plant; b) leaf; c) flower

4.1.2. *H. lindleyana* Steud. (Figure 14)

Leaves: green and undulate leaves, 2 to 4 leaves per plant, spiral leaf cover ground arrangement

Flowers: white color, 7 to 10 flowers per plant

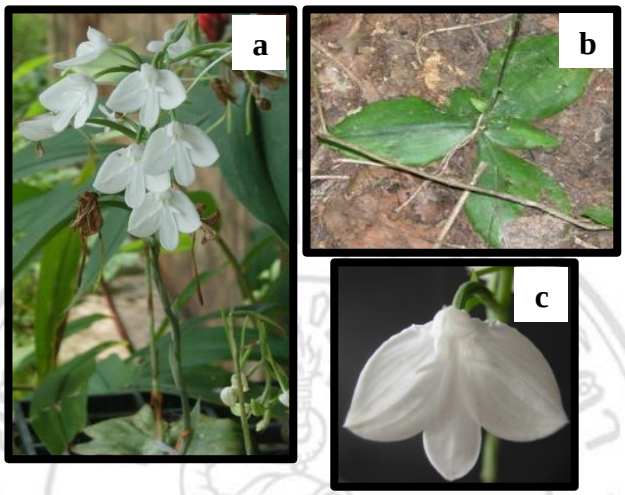

Figure 14 Morphology of *H. lindleyana*

a) plant; b) leaf; c) flower

4.1.3. *H. myriotricha* Gagnep. (Figure 15)

Leaves: bright green, 4 to 8 leaves per plant, spiral leaf arrangement.

Flowers: white color, orange spit at the center, 9 to 12 flowers per plant

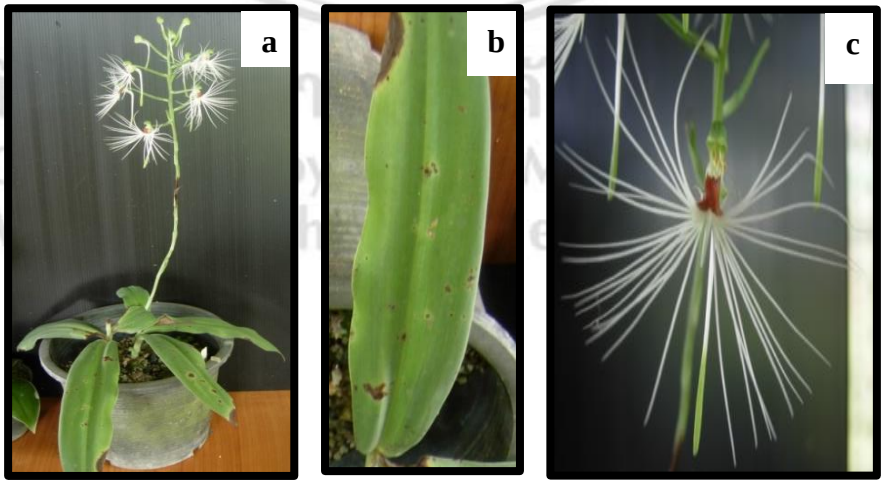

Figure 15 Morphology of *H. myriotricha*

a) plant; b) leaf; c) flower

4.1.4. *H. xanthocheila* Ridl. (Figure 16)

Leaves: green to some deep green and brown to black margin, 2 to 6 leaves per plant, spiral leaf arrangement

Flowers: yellow to orange–yellow, 2 to 4 flowers per plant

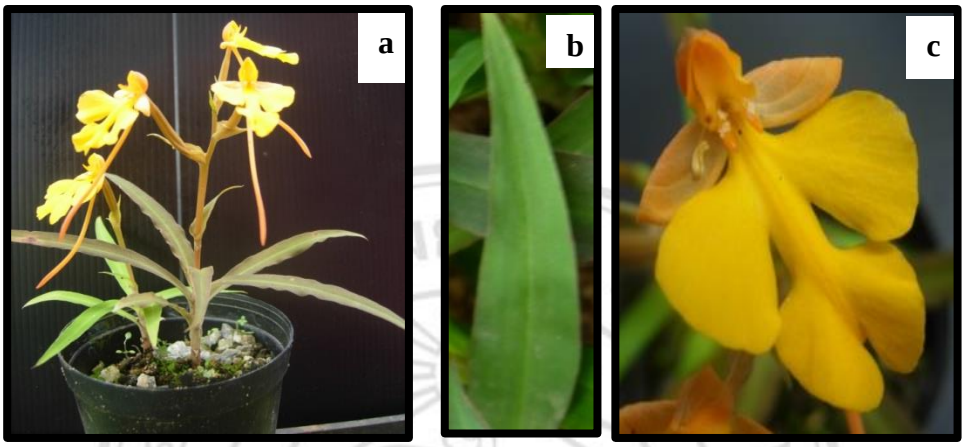

Figure 16 Morphology of *H. xanthocheila*

a) plant; b) leaf; c) flower

There are 2 species of *Pecteilis* used in this experiment.

4.1.5. There are two varieties of this species

- *P. hawkesiana* King & Pantl. (white lip) (Figure 17)

Leaves: green and white margin, 2 to 4 leaves per plant, spiral leaf cover ground

Flowers: all white, 7 to 10 flowers per plant

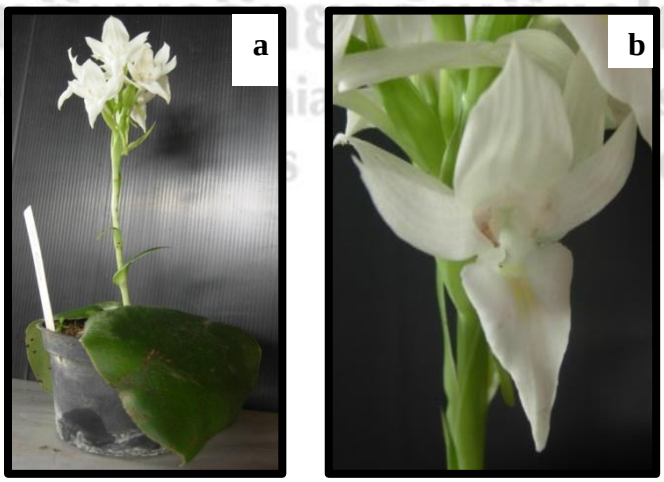

Figure 17 Morphology of *P. hawkesiana*, white lip

a) plant; b) flower

- *P. hawkesiana* King & Pantl. (yellow lip) (Figure 18)

Leaves; green and white margin, 2 to 4 leaves per plant, spiral leaf cover ground

Flowers; white flower with yellow lip, 7 to 10 flowers per plant

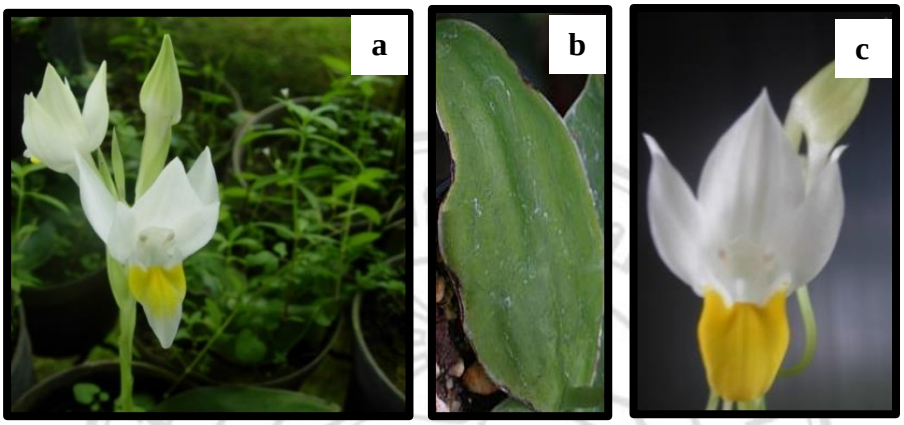

Figure 18 Morphology of *P. hawkesiana*, yellow lip

a) plant; b) leaf; c) flower

4.1.6. *P. susannae* (L.) Rafin (Figure 19)

Leaves; green, 6 to 8 leaves per plant, spiral leaf arrangement

Flowers; white and cream, 7 to 8 flowers per plant

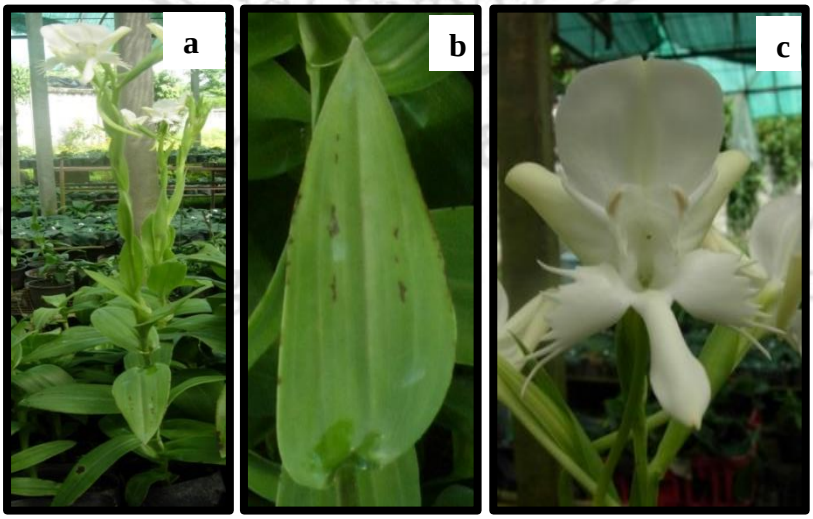

Figure 19 Morphology of *P. susanae*

a) plant; b) leaf; c) flower

Table 1 Flower size of different *Habenaria* and *Pecteilis*

Name	Leaves		Inflorescences	Flowers		Lip	
	Width (cm)	Length (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)
H. rhodocheila (orange flower)	1.7±0.2	14.1±0.9	17.5±1.5	1.8±0.1	2.7±0.0	1.9±0.1	1.9±0.2
H. rhodocheila (pink flower)	0.9±0.5	7.4±2.5	13.0±3.5	1.1±0.1	1.4±0.1	0.9±0.2	1.1±0.1
H. rhodocheila (red flower)	1.0±0.5	6.7±2.6	10.6±0.4	1.8±0.2	2.2±0.2	1.7±0.3	2.0±0.2
H. xanthocheila	1.3±0.3	8.7±3.9	8.2±2.0	2.0±0.3	2.3±0.1	2.0±0.2	1.7±0.1
H. lindleyana	4.5±1.3	16.2±4.0	28.0±6.9	3.4±0.3	4.9±0.7	0.8±0.5	0.6±0.1
H. myriotricha	5.5±2.7	8.9±4.0	28.0±7.4	4.8±0.2	5.0±0.7	1.8±0.2	2.1±0.1
P. hawkesiana (white lip)	10.0±3.6	11.3±2.8	25.7±3.9	3.8±0.6	4.2±0.6	1.7±0.5	1.8±0.5
P. hawkesiana (yellow lip)	8.2±4.0	12.7±3.4	25.4±6.2	5.5±0.2	5.1±0.5	2.8±0.1	2.1±0.1
P. susannae	5.7±0.9	14.8±2.0	43.3±1.9	8.1±0.2	6.1±0.1	3.3±0.2	4.2±0.2

4.2 Analysis of genetic relationship of genus *Habenaria* and *Pecteilis* by RAPD technique

4.2.1 DNA extraction

Leaf DNAs were extracted and compared using four kinds of buffers, CTAB (Doyle and Doyle, 1990), CTAB + PVPP (modification of Doyle and Doyle, 1990), SDS (Ichiro *et al.*, 2013) and SDS + PVPP (modification of Ichiro *et al.*, 2013) buffer. After the chloroform: isopropanol step, a dirty oily layer was found on top of supernatant when extracted by CTAB buffer. The supernatant provided by SDS buffer was clear and green (Figure 20) but did not contain oily contaminant. Additional PVPP did not confer any differences. DNA qualities were checked by electrophoresis in 1.5% agarose gel. The electrophoresis result showed single DNA bands from each CTAB-extracted sample (Figure 21). Extraction by SDS + PVPP buffer also gave DNA bands similar to those by CTAB buffer (Figure 22). DNA contents were checked by Nanodrop spectrophotometer 2000 (Thermo Scientific, USA) at 260 and 280 nanometer. $A_{260/280}$ ratios of nine independently isolated samples extracted by CTAB and SDS buffers were 1.55 to 1.92 and 1.61 to 1.77, respectively (Table 2).

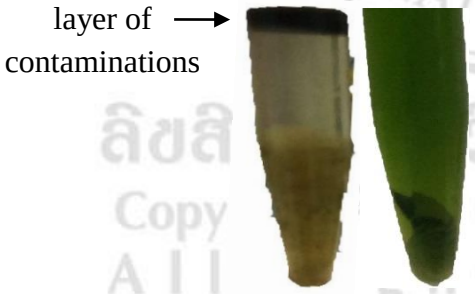

Figure 20 *Habenaria* and *Pecteilis* DNAs extracted by CTAB buffer (Left) and SDS + PVPP buffer (Right).

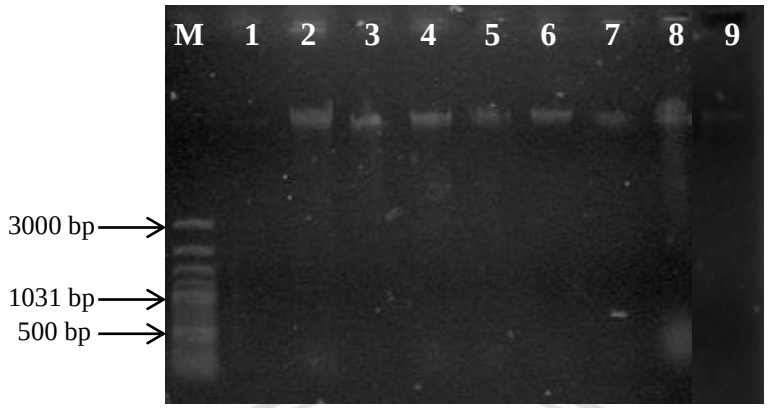

Figure 21 Gel electrophoresis of *Habenaria* and *Pecteilis* DNA extracted by CTAB buffer. [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (orange flower); 2: *H. rhodocheila* (pink flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *H. myriotricha*; 7: *P. hawkesiana* (white lip); 8: *P. hawkesiana* (yellow lip); 9: *P. susannae*]

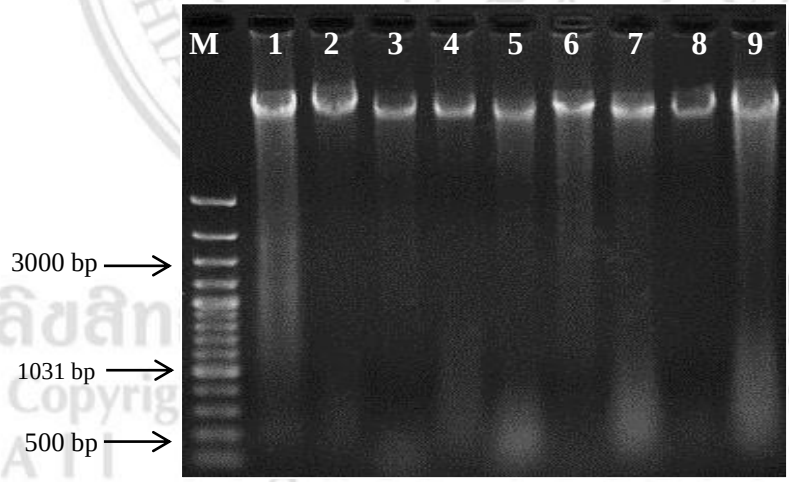

Figure 22 Gel electrophoresis of *Habenaria* and *Pecteilis* DNA extracted by SDS + PVPP buffer. [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (orange flower); 2: *H. rhodocheila* (pink flower); 3: *H. rhodocheila* red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *H. myriotricha*; 7: *P. hawkesiana* (white lip); 8: *P. hawkesiana* (yellow lip); 9: *P. susannae*]

Table 2 DNA concentration and absorbance ratios ($A_{260/280}$) of DNAs extracted by CTAB and SDS buffers.

Name	CTAB buffer		SDS buffer	
	DNA concentrations (ng/µl)	$A_{260/280}$	DNA concentrations (ng/µl)	$A_{260/280}$
H. rhodocheila (orange flower)	158.2	1.92	1369.6	1.65
H. rhodocheila (pink flower)	113.8	1.55	2134.3	1.71
H. rhodocheila (red flower)	273.1	1.82	476.3	1.77
H. xanthocheila	105.4	1.37	427.4	1.76
H. lindleyana	575.0	1.75	627.1	1.76
H. myriotricha	605.7	1.70	2390.8	1.73
P. hawkesiana (white lip)	120.5	1.67	2192.0	1.66
P. hawkesiana (yellow lip)	256.7	1.81	1533.9	1.71
P. susannae	753.5	1.74	3993.9	1.61

4.2.2 RAPD analysis

Genetic relationships of nine orchid samples as representatives of 2 genera were investigated. One-hundred and forty decamer primers, OPA01-20, OPC01-20, OPD01-20, OPF01-20, OPG01-20, OPN01-20 and OPU01-20 (Appendix B, Table 1) were screened. The results showed 77 primers, OPA01, OPA02, OPA03, OPA07, OPA09, OPA10, OPA13, OPA15, OPA16, OPA18, OPA20, OPC02, OPC04, OPC05, OPC06, OPC07, OPC08, OPC09, OPC10, OPC11, OPC14, OPC16, OPC17, OPC19, OPC20, OPD01, OPD02, OPD03, OPD05, OPD06, OPD08, OPD11, OPD12, OPD13, OPD15, OPD18, OPD20, OPF01, OPF02, OPF05, OPF08, OPF09, OPF10, OPF13, OPF16, OPF17, OPG01, OPG04, OPG05, OPG07, OPG09, OPG10, OPG13, OPG15, OPG17, OPN04, OPN05, OPN07, OPN10, OPN13, OPN14, OPN16, OPN17, OPN18, OPN19, OPN20, OPU01, OPU02, OPU03, OPU06, OPU10, OPU11, OPU12, OPU15, OPU16, OPU17 and OPU19, by which a total of 3,046 bands were generated (Figure 23 to 75).

Out of 77 primers, 53 primers could reveal polymorphism between *Habenaria* and *Pecteilis*. There were 1,628 polymorphic bands. Some of these primers yielded polymorphic bands specific to each subgroup. Banding patterns from these 53 primers were also recorded and analyzed by UPGMA and PCoA for identifying genetic relationship.

OPA02 primer produced 100% polymorphic bands but there was no common band within a group of *Habenaria* or *Pecteilis*. (Figure 23, Table 3).

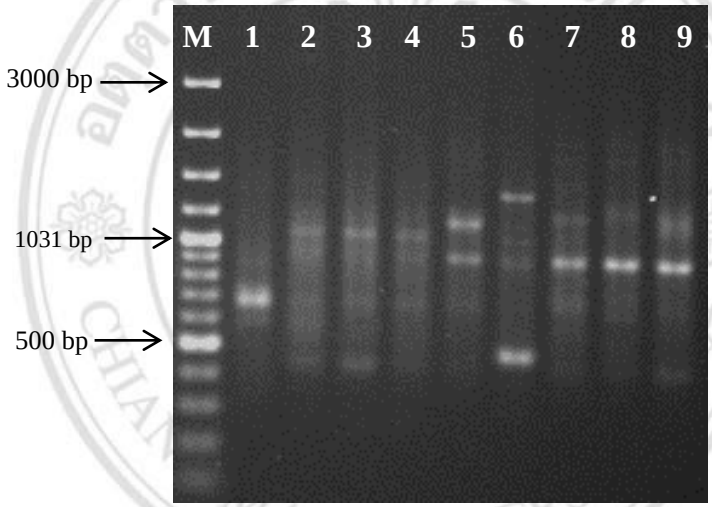

Figure 23 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA02 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPA07 primer generated $OPA07_{1,153}$ marker within *H. rhodocheila* (pink, orange and red flower) group (Figure 24).

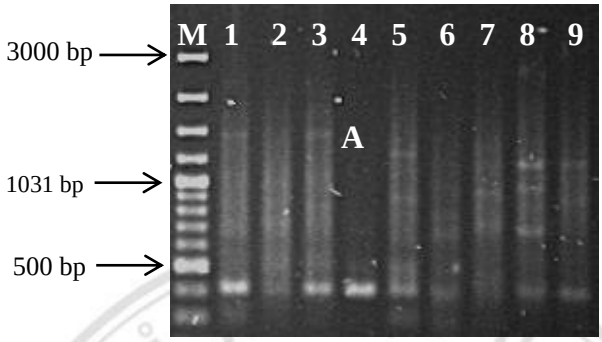

Figure 24 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA07 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,153 bp]

OPA09 primer amplified polymorphic bands in all samples but did not produce any specific bands (Figure 25, Table 3).

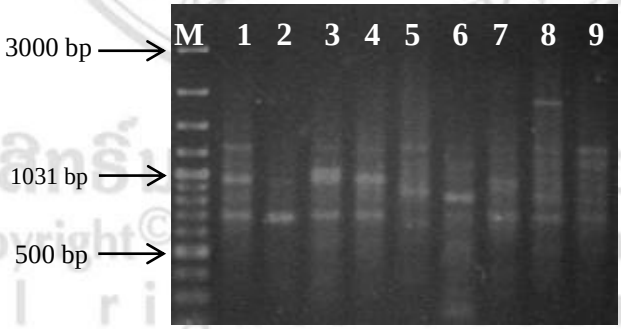

Figure 25 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA09 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPA10 produced 100% polymorphic bands (Figure 26, Table 3). $OPA10_{680}$ marker was found in *H. rhodocheila* and *H. xanthocheila* and $OPA10_{362}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 26).

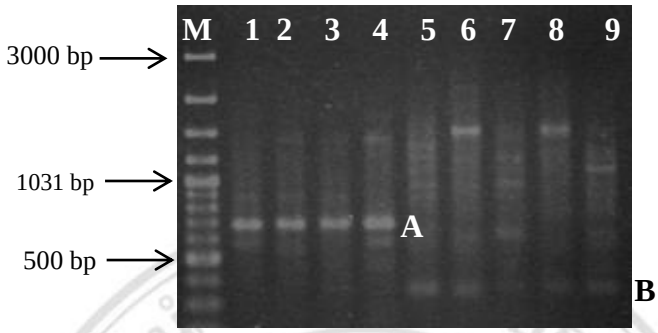

Figure 26 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 680 bp; B: 362 bp]

OPA15 produced 100% polymorphic bands (Figure 27, Table 3).

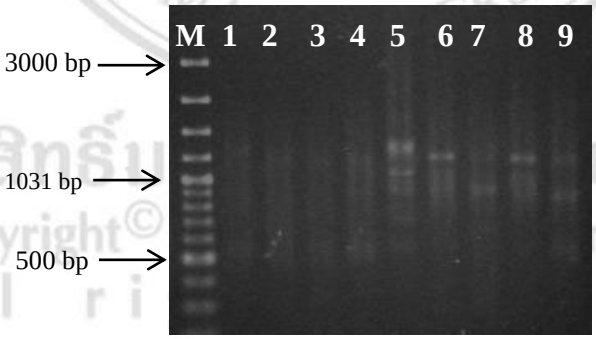

Figure 27 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA15 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPA16 generated $OPA16_{1,255}$ marker in *H. rhodocheila* and *H. xanthocheila* and $OPA16_{858}$ marker within *H. rhodocheila* (pink, orange and red flower) group (Figure 28).

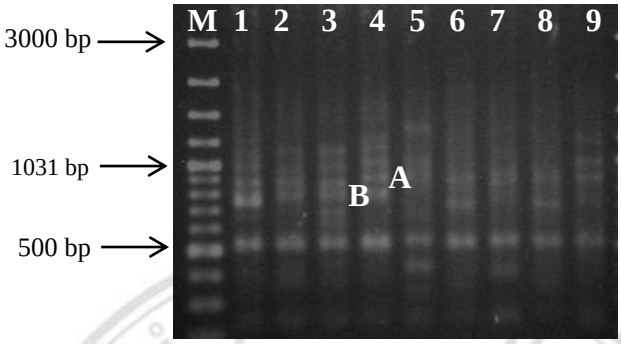

Figure 28 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA16 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,255 bp; B: 858 bp]

OPA18 produced 100% polymorphic bands (Figure 29, Table 3). It generated $OPA18_{888}$ marker within *Pecteilis* genus (Figure 29).

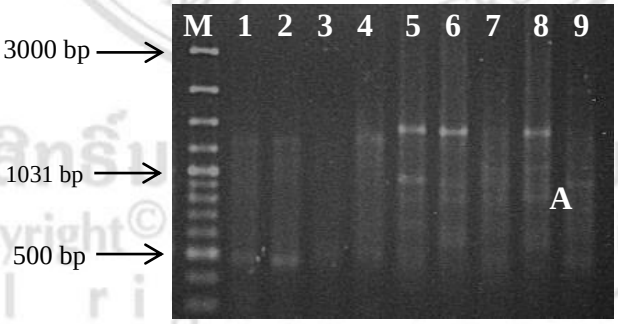

Figure 29 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA18 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 888 bp]

OPA20 produced 100% polymorphic bands (Figure 30, Table 3). It generated $OPA20_{1,842}$ and $OPA20_{259}$ markers in *H. rhodocheila* and *H. xanthocheila* and $OPA20_{864}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 30).

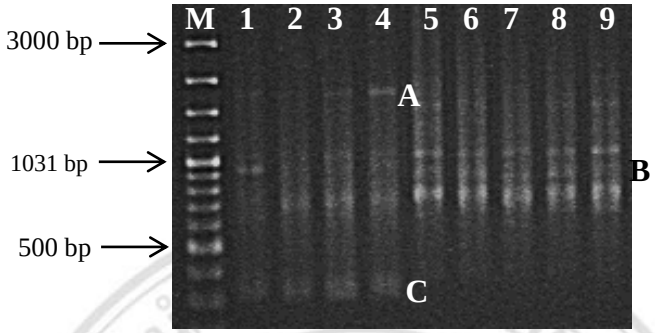

Figure 30 RAPD profile of *Habenaria* and *Pecteilis* generated by OPA20 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,842 bp; B: 864 bp; C: 259 bp]

Table 3 PCR amplifications by 8 OPA primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPA02	428-1,537	35	35	100
OPA07	202-1,599	41	32	78
OPA09	552-1,906	41	32	78
OPA10	162-1,716	39	39	100
OPA15	569-1,448	28	28	100
OPA16	288-1,661	50	41	82
OPA18	485-1,483	29	29	100
OPA20	259-1,842	33	33	100
Total		263	236	
Average		37.57	33.71	91.14

OPC04 primer generated $OPC04_{786}$ and OPC_{641} markers among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus and $OPC04_{498}$ marker *H. rhodocheila* and *H. xanthocheila* (Figure 31).

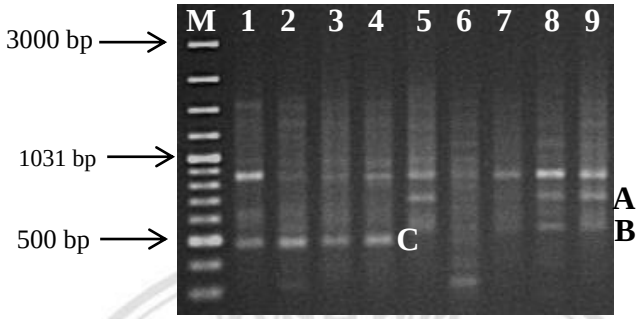

Figure 31 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC04 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 786 bp; B: 641 bp; C: 498 bp]

OPC05 primer generated $OPC05_{1,322}$ marker in *H. rhodocheila* and *H. xanthocheila* and $OPC05_{1,280}$ marker among *H. lindleyana, H. myriotricha* and *Pecteilis* genus (Figure 32).

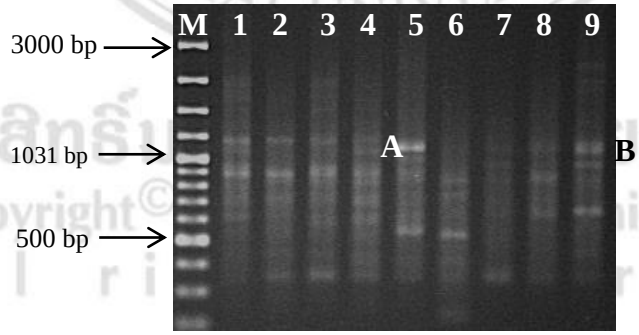

Figure 32 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC05 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,322 bp; B: 1,280 bp]

OPC06 primer generated polymorphic bands in all samples (Figure 33).

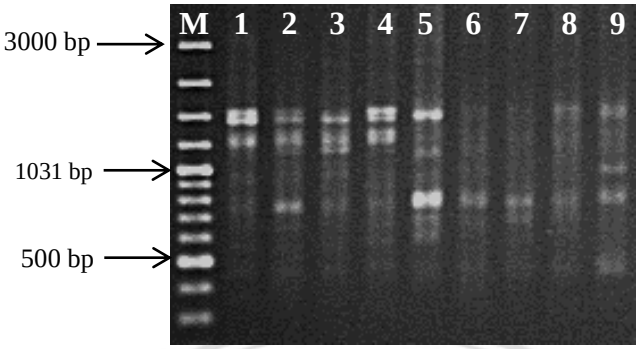

Figure 33 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC06 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC07 primer produced $OPC07_{655}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 34).

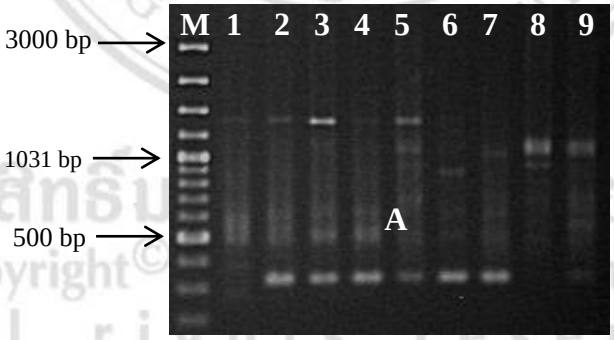

Figure 34 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC07 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 655 bp]

OPC08 primer produced 100% polymorphic bands (Figure 35, Table 4). It generated $OPC08_{1,886}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 35).

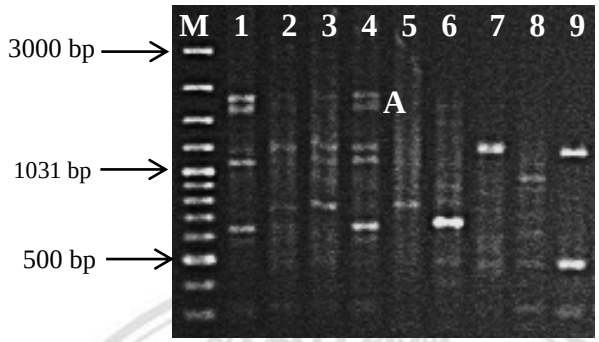

Figure 35 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC08 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,886 bp]

OPC09 primer amplified the highest number of 85 bands and produced 100% polymorphic bands (Figure 36, Table 4).

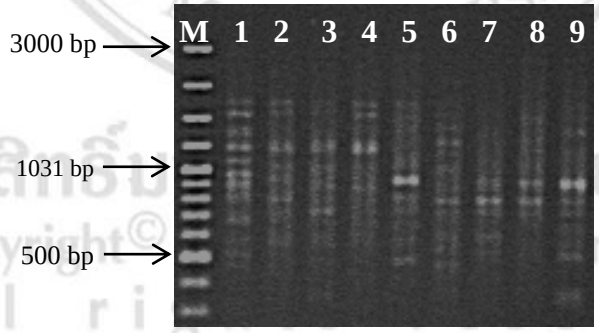

Figure 36 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC09 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC10 primer amplified polymorphic bands in all samples (Figure 37).

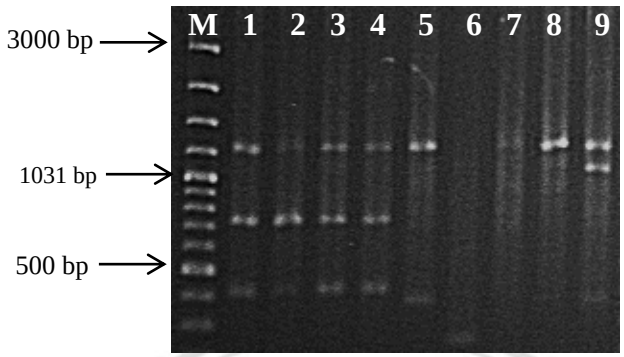

Figure 37 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC11 primer produced 100% polymorphic bands (Figure 38, Table 4).

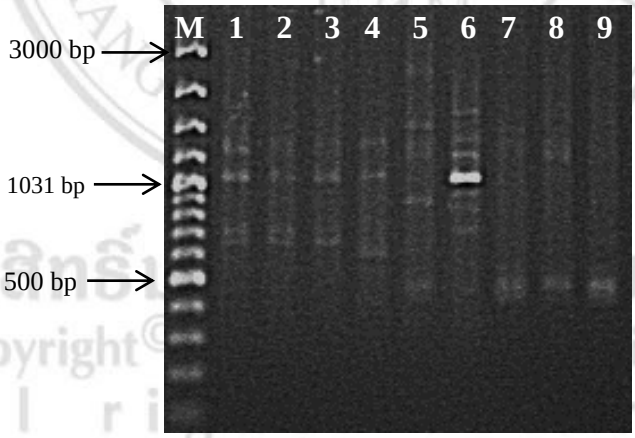

Figure 38 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC11 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC14 primer generated polymorphc bands in all samples (Figure 39).

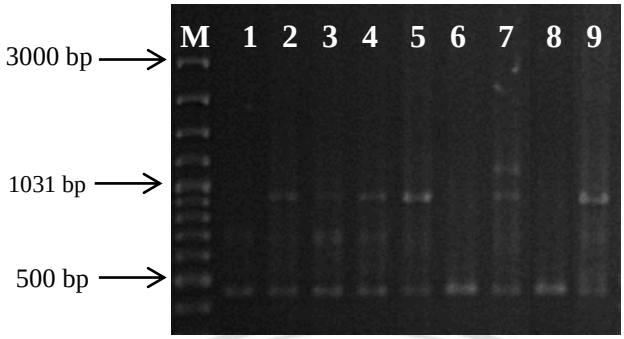

Figure 39 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC14 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC16 primer produced $OPC16_{1,268}$ marker among *H. xanthocheila*, *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 40).

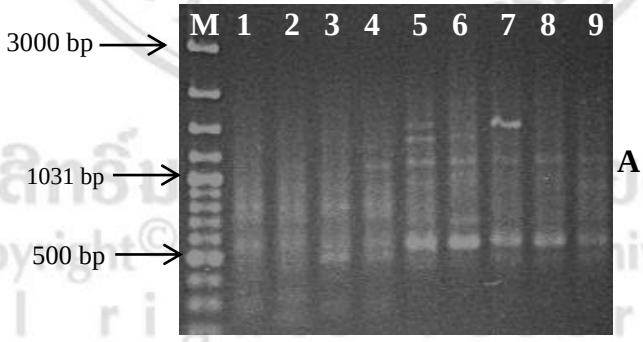

Figure 40 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC16 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,268 bp]

OPC17 primer generated polymorphic bands in all samples (Figure 41).

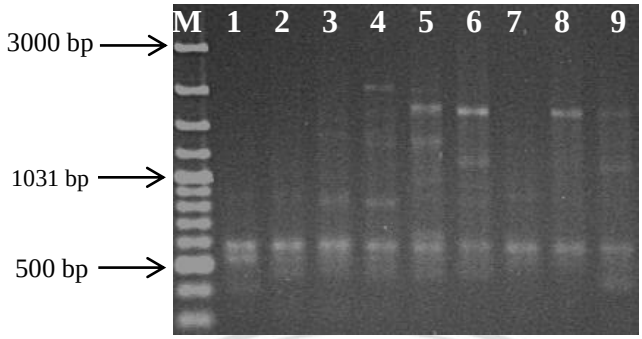

Figure 41 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC17 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPC19 primer generated polymorphic bands in all samples (Figure 42).

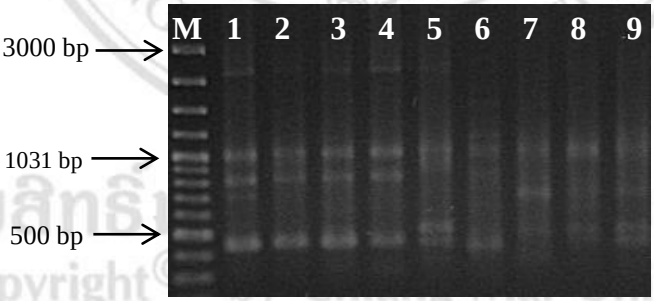

Figure 42 RAPD profile of *Habenaria* and *Pecteilis* generated by OPC19 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

Table 4 PCR amplifications by 11 OPC primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPC04	266-1,658	53	44	83
OPC05	139-1,981	63	54	86
OPC06	441-1,709	61	16	26
OPC07	252-1,476	39	30	77
OPC08	149-1,886	73	73	100
OPC09	299-1,850	85	85	100
OPC11	474-2,380	45	45	100
OPC14	452-1,285	25	7	28
OPC16	487-1,607	44	17	39
OPC17	616-1,954	44	26	59
OPC19	420-2,122	36	18	50
Total		568	415	
Average		51.64	37.73	68

OPD01 primer amplified $OPD01_{492}$ marker within *P. hawkesiana* (white and yellow lip) (Figure 43).

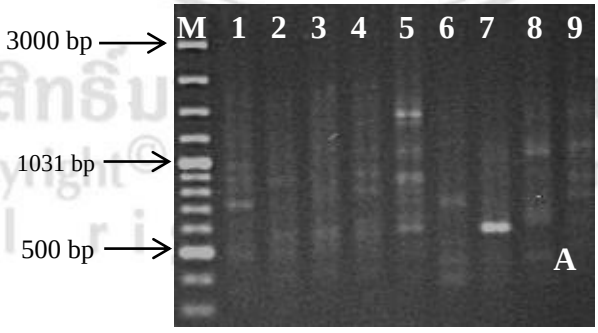

Figure 43 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD01 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 492 bp]

OPD05 primer amplified $OPD05_{2,240}$ marker in *H. lindleyana* and *H. myriotricha* (Figure 44).

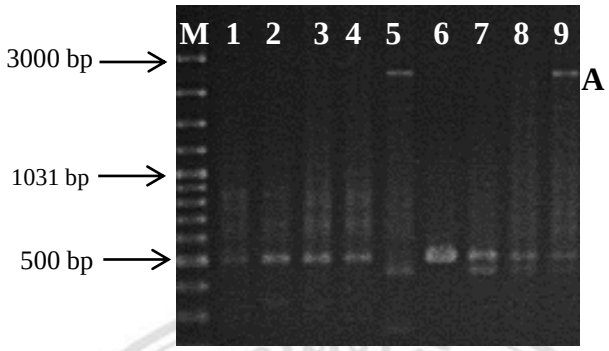

Figure 44 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD05 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 2,240 bp]

OPD06 primer generated $OPD06_{1,297}$ marker in *H. rhodocheila* and *H. xanthocheila* and $OPD06_{295}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 45).

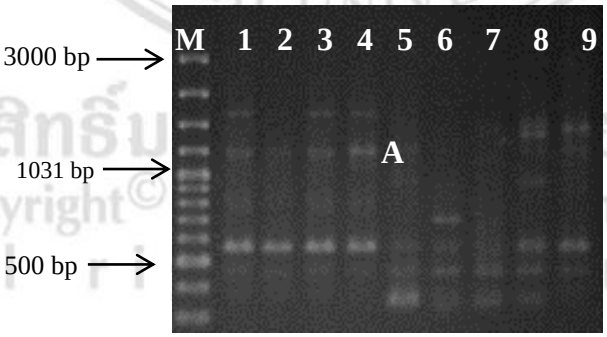

Figure 45 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD06 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,297 bp; B: 295 bp]

OPD08 primer generated $OPD08_{1,194}$ and $OPD08_{790}$ markers among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus and $OPD08_{534}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 46).

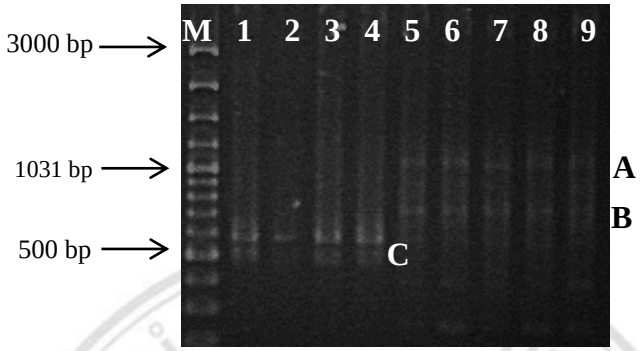

Figure 46 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD08 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,194 bp; B: 790 bp; C: 534 bp]

OPD12 primer amplified polymorphic bands in all samples (Figure 47)

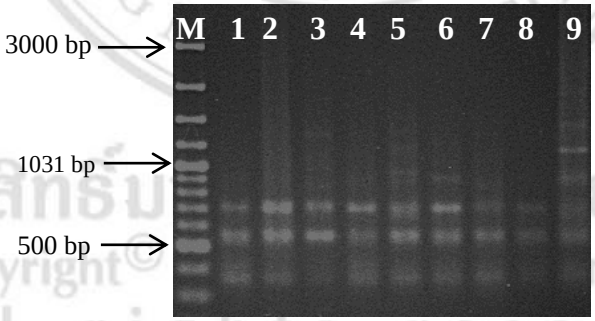

Figure 47 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD12 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPD13 primer produced $OPD13_{1,543}$ marker in *H. lindleyana* and *H. myriotricha* (Figure 48).

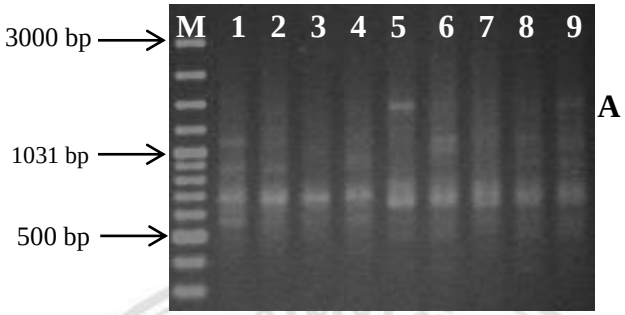

Figure 48 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD13 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,543 bp]

OPD15 produced $OPD15_{1,474}$ marker within *Pecteilis* genus (Figure 49).

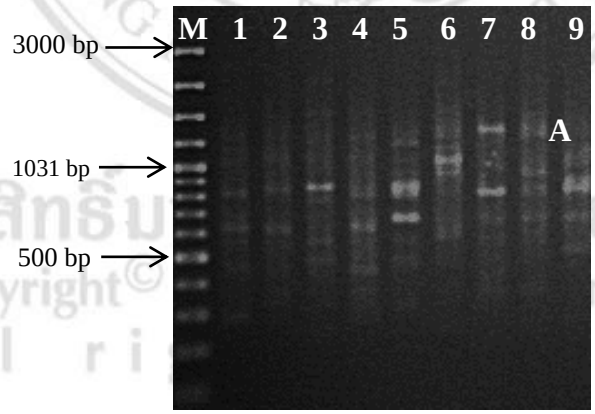

Figure 49 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD15 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,474 bp]

OPD18 primer produced $OPD18_{553}$ marker within *Pecteilis* genus (Figure 50).

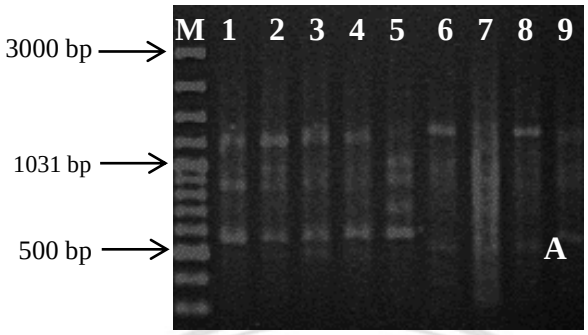

Figure 50 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD18 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 533 bp]

OPD20 primer generated $OPD20_{899}$ and $OPD20_{573}$ markers within *P. hawkesiana* (white and yellow lip) (Figure 51).

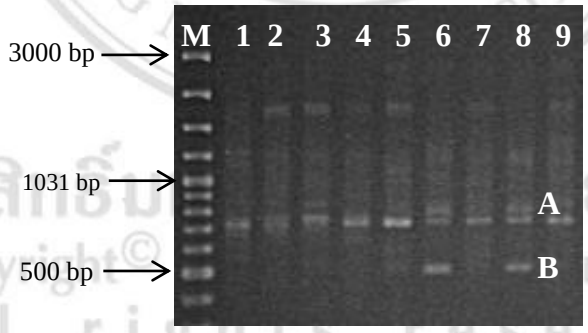

Figure 51 RAPD profile of *Habenaria* and *Pecteilis* generated by OPD20 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 899 bp; B: 573 bp]

Table 5 PCR amplifications by 9 OPD primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPD01	390-1,592	46	37	80
OPD05	137-2,240	32	23	72
OPD06	295-1,769	40	22	55
OPD08	107-1,194	40	31	78
OPD12	327-1,919	40	13	33
OPD13	592-1,543	37	10	27
OPD15	459-1,474	30	12	40
OPD18	553-1,496	39	30	77
OPD20	573-1,888	42	15	36
Total		346	193	
Average		38.44	21.44	55.33

OPF01 primer produced $OPF01_{959}$ maker within *H. rhodocheila* (pink, orange and red flower) (Figure 52).

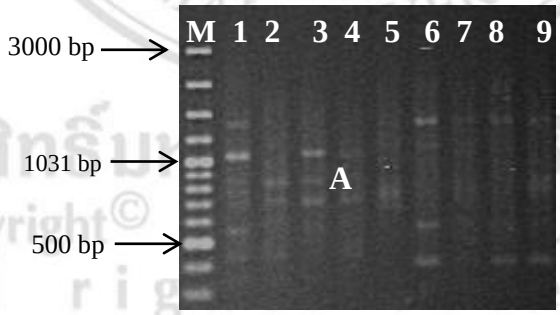

Figure 52 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF01 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 959 bp]

OPF05 primer amplified polymorphic bands in all samples (Figure 53).

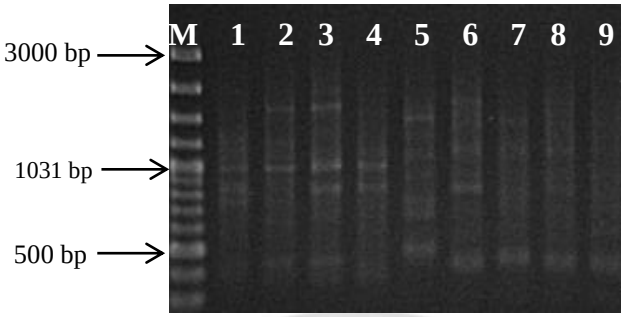

Figure 53 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF05 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPF09 primer amplified polymorphic bands in all samples (Figure 54).

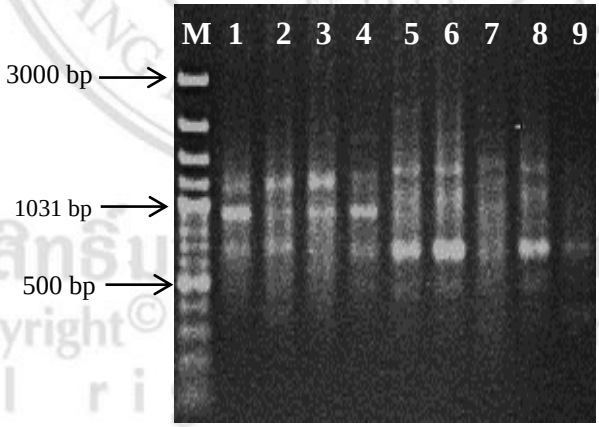

Figure 54 RAPD profile of *Habenaria* and *Pecteilis* generated by OPF09 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

Table 6 PCR amplifications by 3 OPF primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPF01	374-1,476	42	33	79
OPF05	383-1,744	48	21	44
OPF09	522-2,275	42	24	57
Total		132	78	
Average		44.00	26.00	60.00

OPG04 primer amplified $OPG04_{1,209}$ and $OPG04_{903}$ markers in *H. rhodocheila* and *H. xanthocheila* (Figure 55).

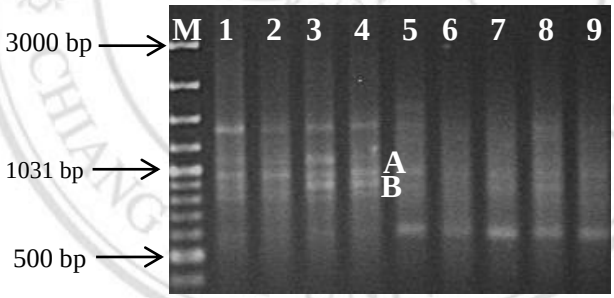

Figure 55 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG04 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,209 bp; B: 903 bp]

OPG07 amplified $OPG07_{580}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 56).

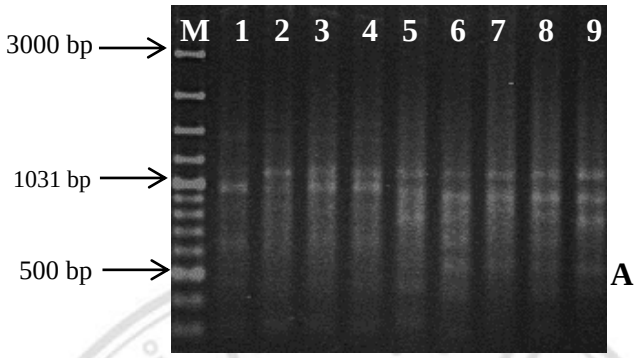

Figure 56 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG07 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 580 bp]

OPG09 primer produced $OPG09_{635}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 57).

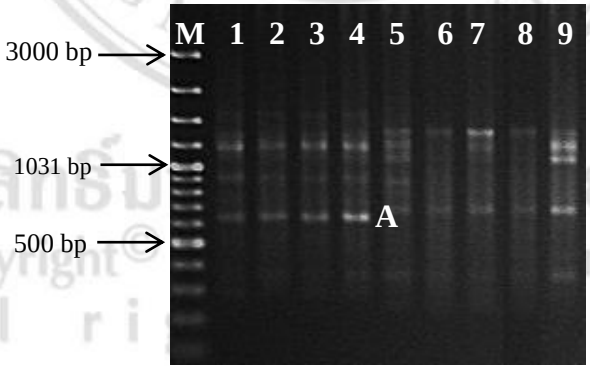

Figure 57 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG09 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 735 bp]

OPG10 primer generated $OPG10_{600}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 58).

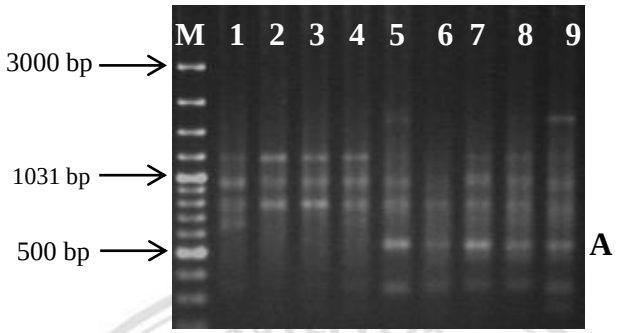

Figure 58 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 600 bp]

OPG13 primer amplified 100% polymorphic bands (Figure 59, Table 7). It generated $OPG13_{972}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 59).

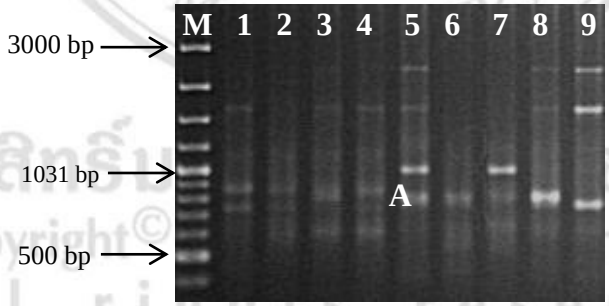

Figure 59 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG13 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 972 bp]

OPG15 amplified $OPG15_{1,352}$ and OPG_{507} markers in *H. rhodocheila* and *H. xanthocheila* and $OPG15_{817}$ marker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 60).

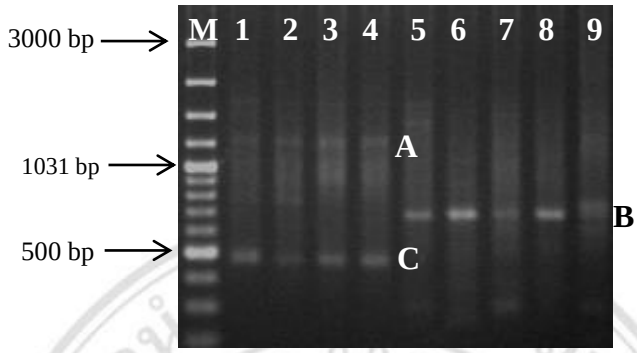

Figure 60 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG15 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,352 bp; B: 817 bp; C: 507 bp]

OPG17 primer generated $OPG17_{1,194}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 61).

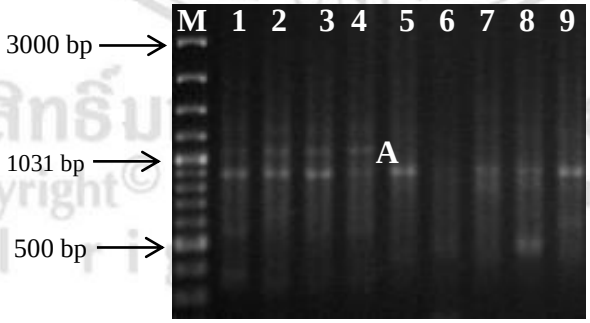

Figure 61 RAPD profile of *Habenaria* and *Pecteilis* generated by OPG17 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,194 bp]

Table 7 PCR amplifications by 7 OPG primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPG04	639-1,755	41	32	78
OPG07	470-1,696	54	18	33
OPG09	325-1,737	64	37	58
OPG10	297-1,781	41	14	34
OPG13	682-2,257	47	47	100
OPG15	240-1,793	45	36	80
OPG17	123-1,270	38	20	53
Total		330	204	
Average		47.14	29.14	62.29

OPN04 primer produced $OPN04_{759}$ maker among *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 62).

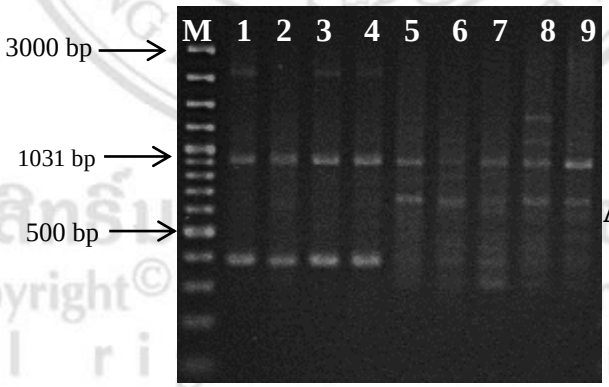

Figure 62 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN04 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 759 bp]

OPN05 primer amplified 100% polymorphic bands (Figure, Table 8). It generated $OPN05_{605}$ markers in a group of *H. rhodocheila* (pink, orange and red flower) and 2 species of *Pecteilis*, and $OPN05_{268}$ marker within *H. rhodocheila* (pink, orange and red flower) group (Figure 63).

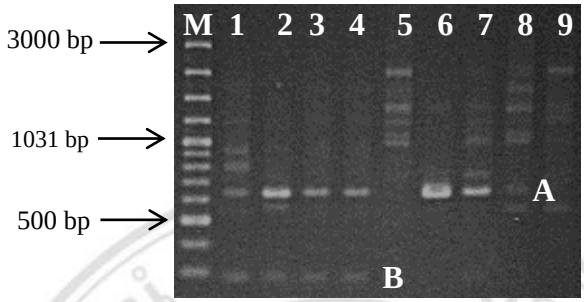

Figure 63 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN05 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 605 bp; B: 268 bp]

OPN10 amplified $OPN10_{1,591}$ marker in *H. lindleyana* and *H. myriotricha* and $OPN10_{1,151}$ marker between *H. rhodocheila* and *H. xanthocheila* (Figure 64).

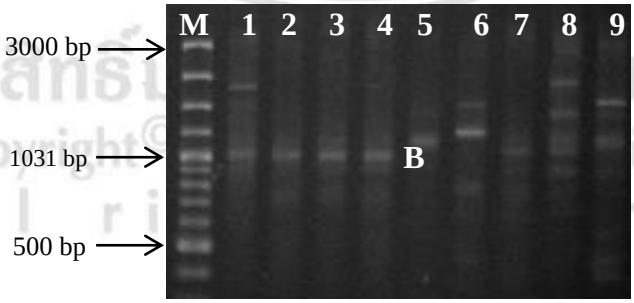

Figure 64 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,591 bp; B: 1,151 bp]

OPN16 primer generated $OPN16_{910}$ marker in *H. lindleyana*, *H. myriotricha* and *Pecteilis* genus (Figure 65).

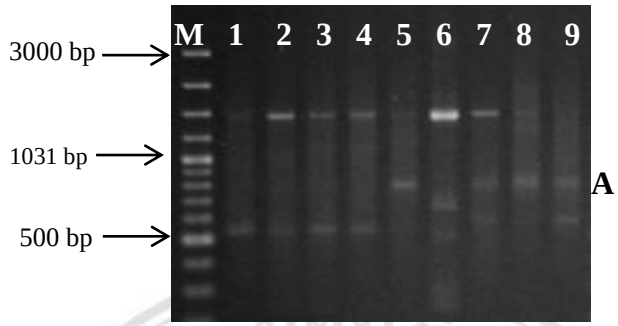

Figure 65 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN16 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 910 bp]

OPN20 primer amplified $OPN20_{646}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 66).

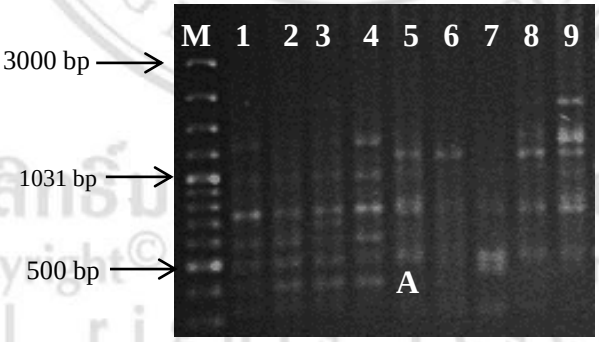

Figure 66 RAPD profile of *Habenaria* and *Pecteilis* generated by OPN20 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 646 bp]

Table 8 PCR amplifications by 5 OPN primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPN04	227-2,121	44	26	59
OPN05	268-2,055	35	35	100
OPN10	446-1,893	38	29	76
OPN16	203-1,606	29	20	69
OPN20	231-1,832	62	26	42
Total		208	136	
Average		41.6	27.2	69.2

OPU01 amplified polymorphic bands in all samples (Figure 67).

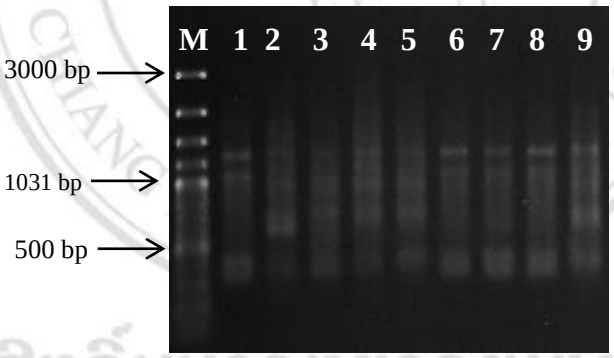

Figure 67 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU01 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU06 amplified polymorphic bands in all samples (Figure 68).

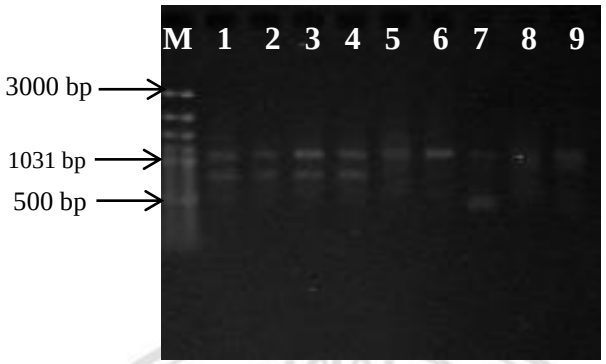

Figure 68 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU06 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU10 amplified polymorphic bands in all samples (Figure 69).

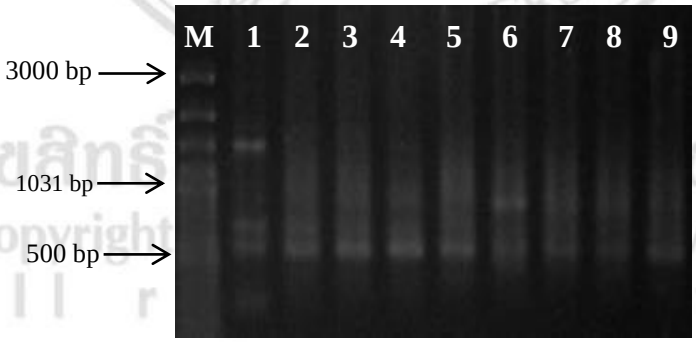

Figure 69 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU10 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU11 amplified polymorphic bands in all samples (Figure 70).

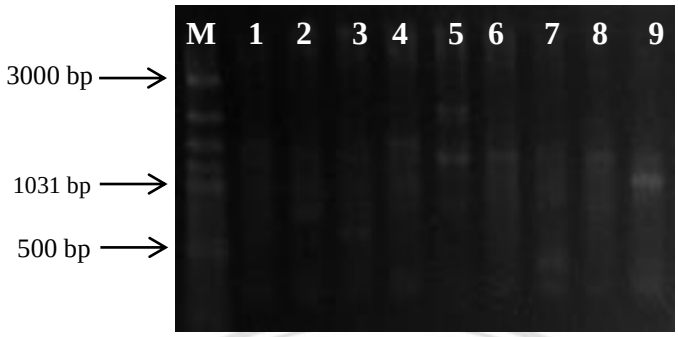

Figure 70 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU11 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU12 amplified polymorphic bands in all samples (Figure 71).

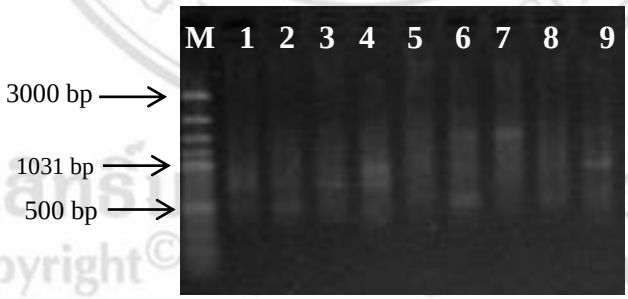

Figure 71 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU12 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU15 primer generated $OPU15_{1,315}$ marker in *H. rhodocheila* and *H. xanthocheila* (Figure 72).

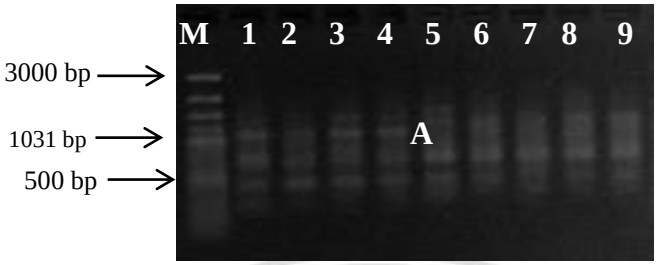

Figure 72 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU15 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 1,315 bp]

OPU16 generated 100% polymorphic bands (Figure 73, Table 9).

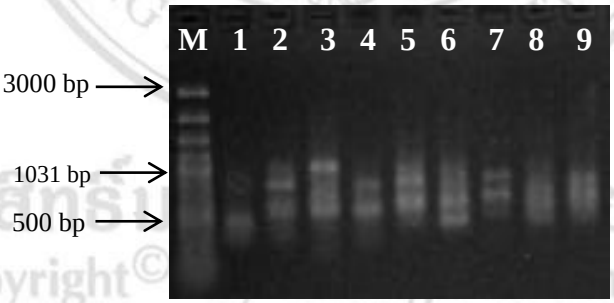

Figure 73 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU16 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU17 generated 100% polymorphic bands (Figure 74, Table 9).

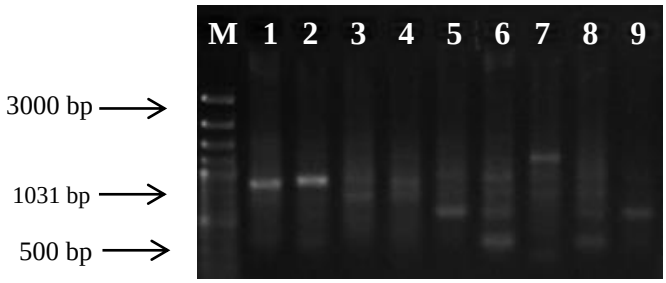

Figure 74 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU17 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*]

OPU19 primer amplified 100% polymorphic bands (Figure 75, Table 9). It generated $OPU19_{2,090}$ marker within *P. hawkesiana* (white and yellow lip) (Figure 75).

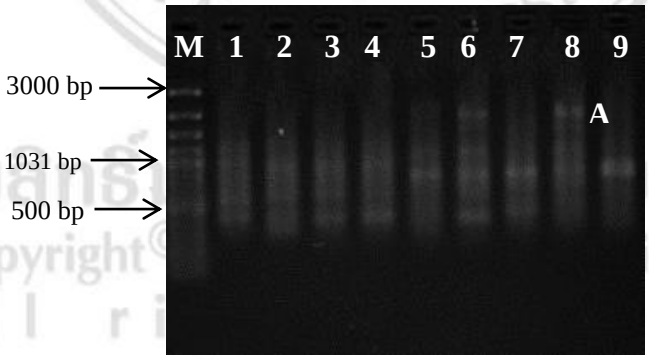

Figure 75 RAPD profile of *Habenaria* and *Pecteilis* generated by OPU19 primer [M: 100 bps plus DNA ladder; 1: *H. rhodocheila* (pink flower); 2: *H. rhodocheila* (orange flower); 3: *H. rhodocheila* (red flower); 4: *H. xanthocheila*; 5: *H. lindleyana*; 6: *P. hawkesiana* (yellow lip); 7: *P. susannae*; 8: *P. hawkesiana* (white lip); 9: *H. myriotricha*; A: 2,090 bp]

Table 9 PCR amplifications by 9 OPU primers

Primers	Size of DNA bands (bps)	Total no. of bands	No. of polymorphic bands	% polymorphism
OPU01	392-1,603	39	30	77
OPU06	606-1,377	26	8	31
OPU10	144-1,756	44	44	100
OPU11	406-2,204	42	42	100
OPU12	407-1,902	43	34	79
OPU15	270-1,626	46	28	61
OPU16	334-1,222	32	32	100
OPU17	450-1,765	45	45	100
OPU19	404-2,090	49	49	100
Total		366	312	
Average		40.67	34.67	83.11

The other 24 primers including OPA01, OPA03, OPA13, OPC02, OPC20, OPD02, OPD03, OPD11, OPF02, OPF08, OPF10, OPF13, OPF16, OPF17, OPG01, OPG05, OPG07, OPN13, OPN14, OPN17, OPN18, OPN19, OPU02 and OPU03 could not generate polymorphic bands (Appendix C, Figure 1 to 24). All of DNA patterns from 24 primers were not used to calculate genetic relationship of *Habenaria* and *Pecteilis* in this study.

4.3 Analysis of genetic relationships

Genetic relationships among 9 samples of 6 species from *Habenaria* and *Pecteilis* genera were estimated by NTSYSpc version 2.01c program and mode dendrogram by using Unweighted Pair Group with Arithmetic Mean (UPGMA). PCoA dendrograms were made by GenAIEx 6.5 program (Peakall and Smouse, 2012).

OPA10 primer could separate plant samples into 3 groups at 0% similarity. The primer could group *H. rhodocheila* (pink, orange and red flower) and *H. xanthocheila*. Cluster analysis did not correspond with PCoA (Figure 76 to 77).

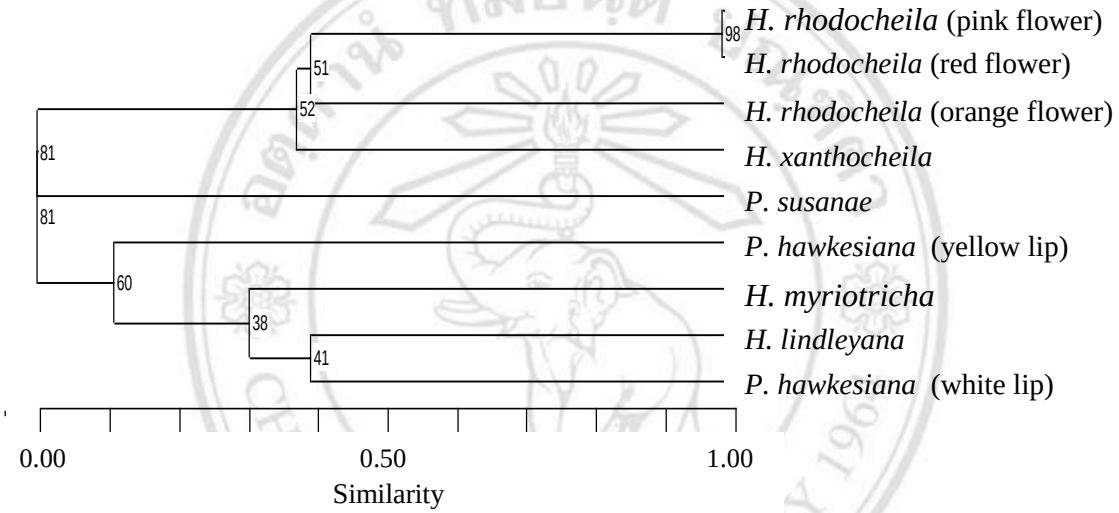

Figure 76 Dendrogram of *Habenaria* and *Pecteilis* based on OPA10 primer

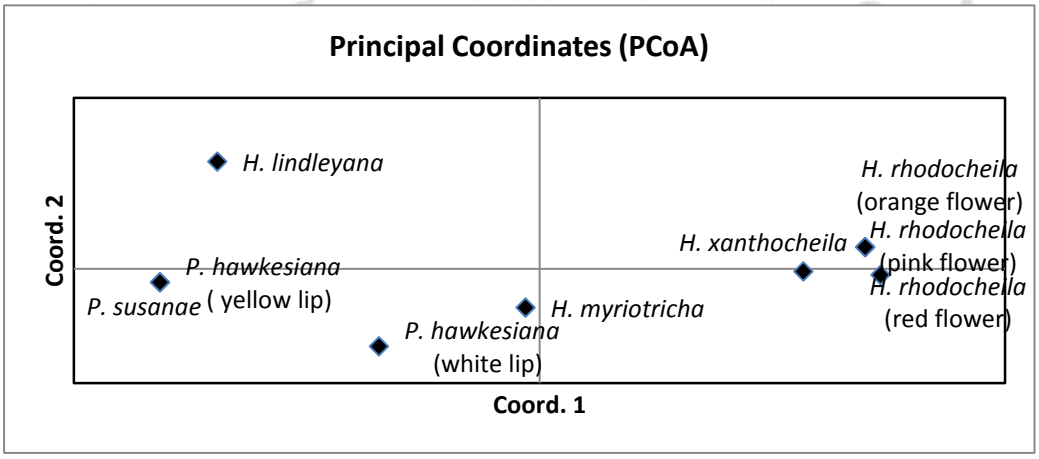

Figure 77 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPA10 primer

OPC05 primer could divide plant samples into 2 groups at 3% similarity. The primer could separate subgroup *H. rhodocheila* (pink, orange and red flower) and *H. xanthocheila* from another group. Cluster analysis corresponded with PCoA (Figure 78 to 79).

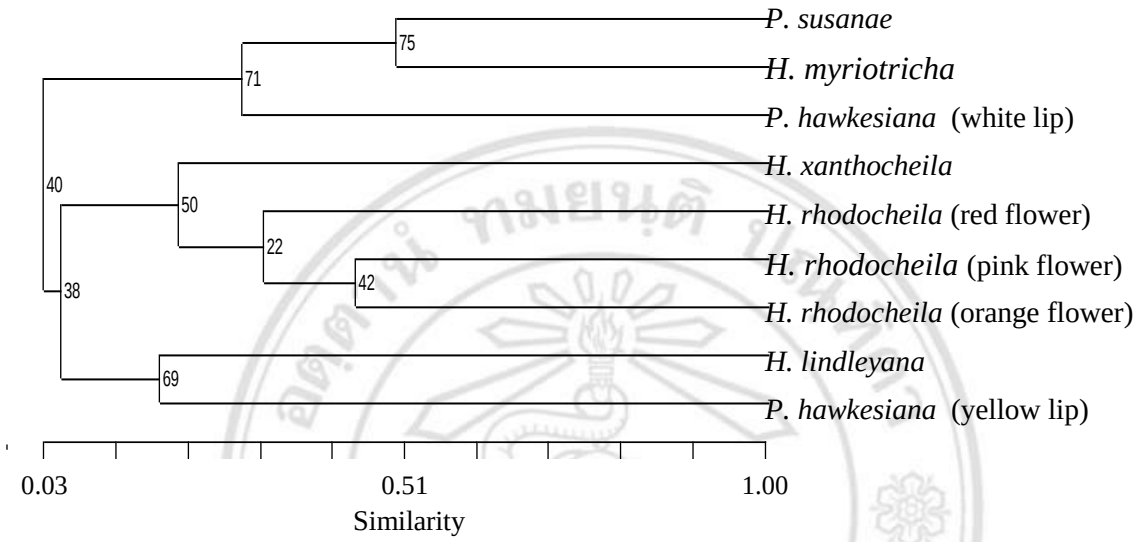

Figure 78 Dendrogram of *Habenaria* and *Pecteilis* based on OPC05 primer

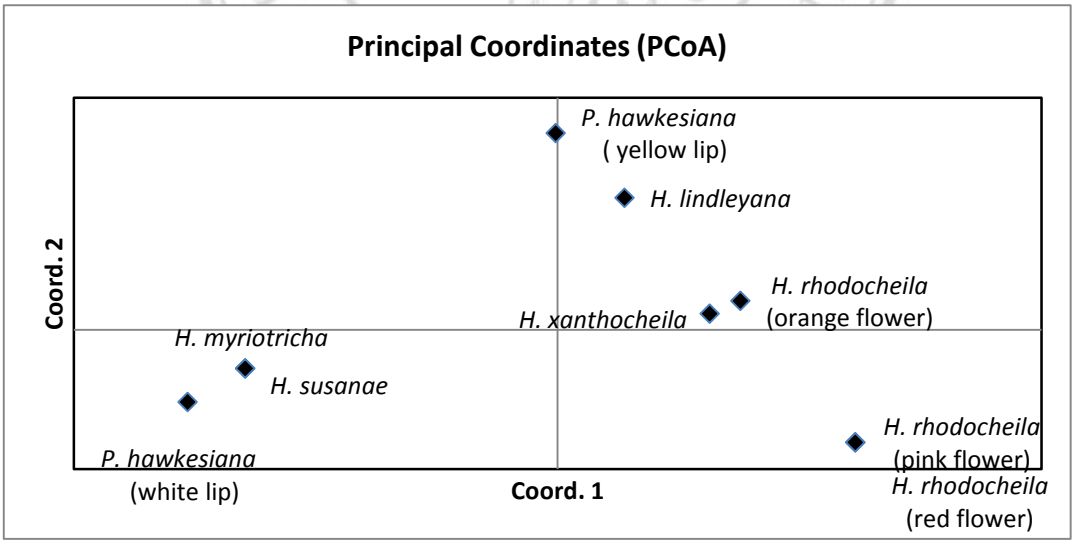

Figure 79 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC05 primer

OPC06 primer could divide plant samples into 2 groups at 0% similarity. OPC06 primer could cluster a subgroup of *H. rhodocheila* (pink, orange and red flower). Cluster analysis did not correspond with PCoA (Figure 80 to 81).

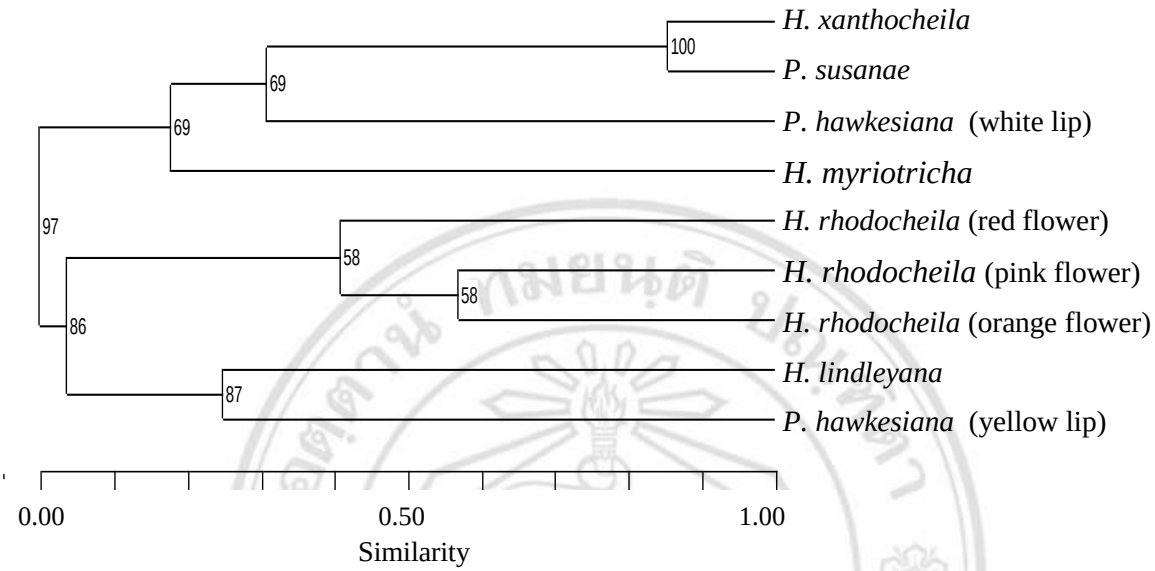

Figure 80 Dendrogram of *Habenaria* and *Pecteilis* based on OPC06 primer

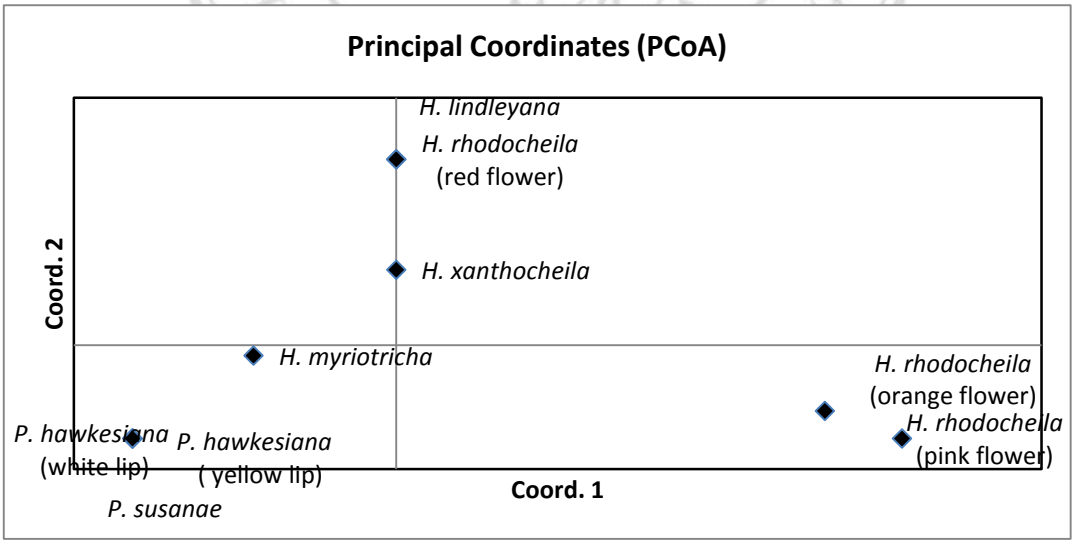

Figure 81 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC06 primer

OPC07 primer could divide plant samples into 2 groups at 3% similarity. The primer further separated a subgroup of *H. rhodocheila* (pink and orange) from another sub group of *H. rhodocheila* (red flower) and *H. xanthocheila*. Cluster analysis corresponded with PCoA (Figure 82 to 83).

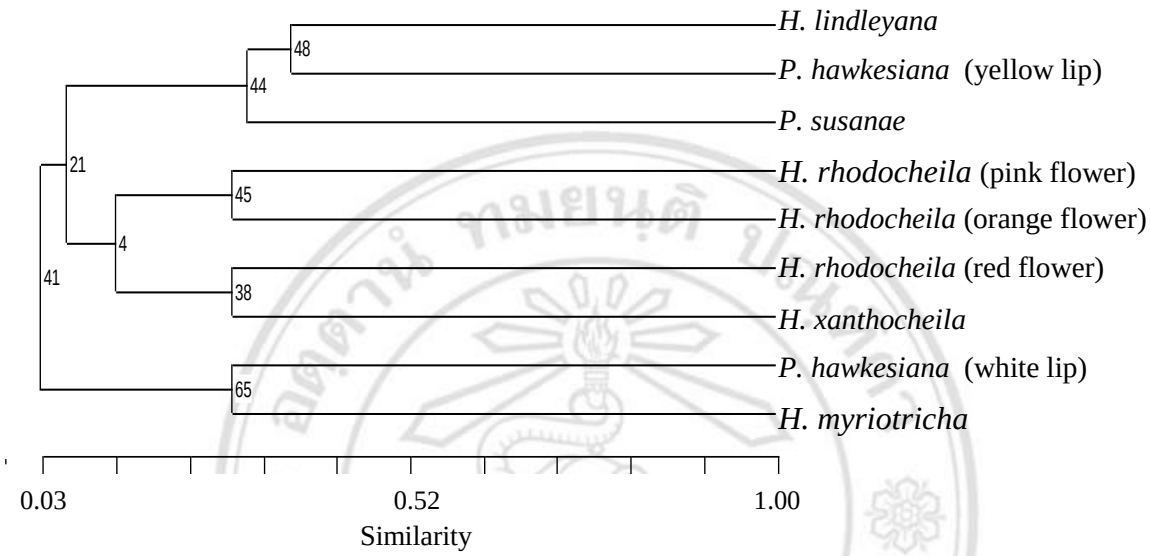

Figure 82 Dendrogram of *Habenaria* and *Pecteilis* based on OPC07 primer

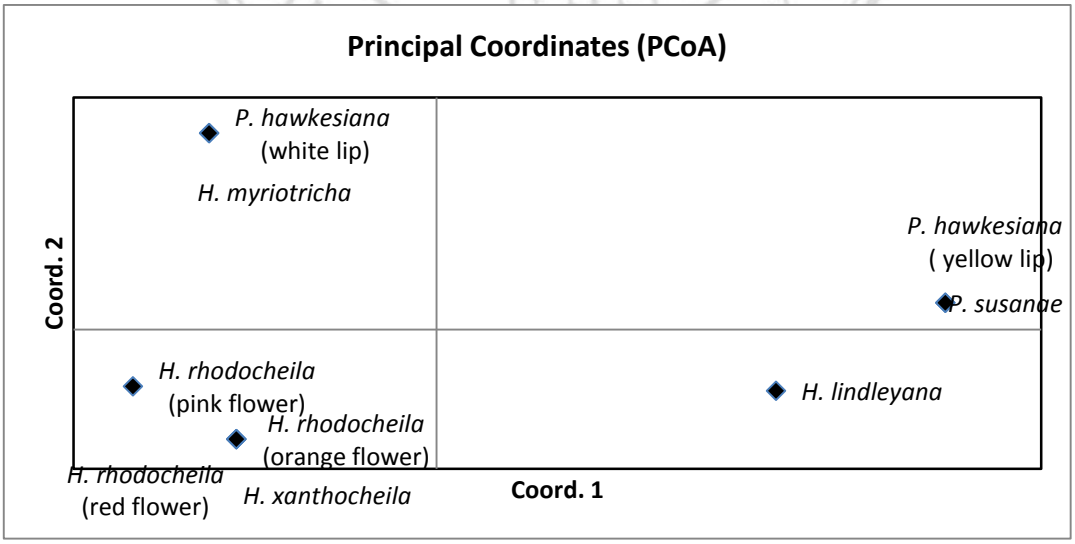

Figure 83 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC07 primer

OPC11 primer could divide plant samples into 2 groups at 2% similarity. The primer could separate a subgroup of *H. rhodocheila* (pink, orange and red flower) and *H. xanthocheila* from *P. hawkesiana* (white lip). Cluster analysis did not correspond with PCoA (Figure 84 to 85).

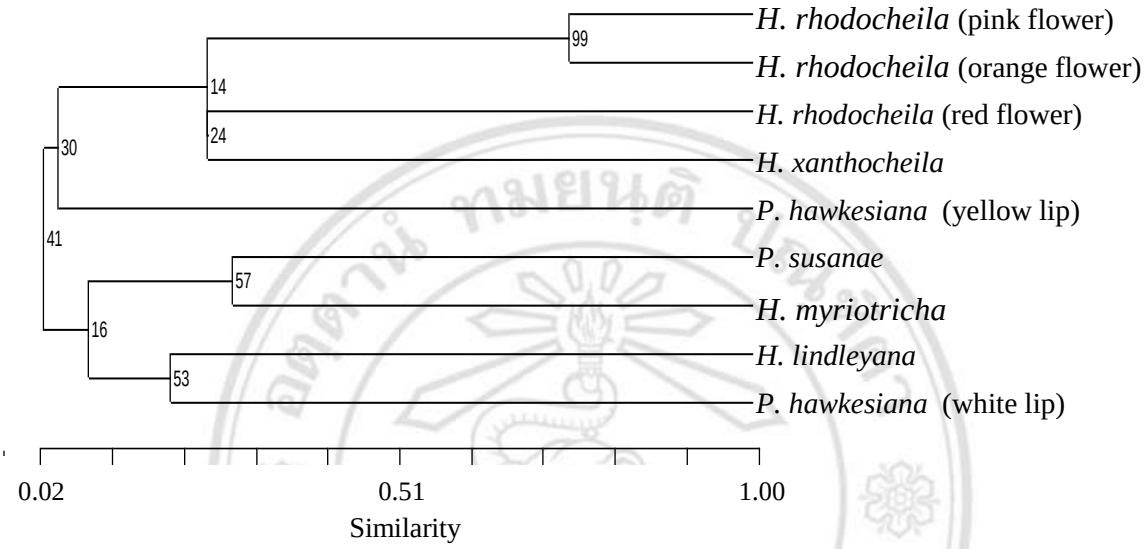

Figure 84 Dendrogram of *Habenaria* and *Pecteilis* based on OPC11 primer

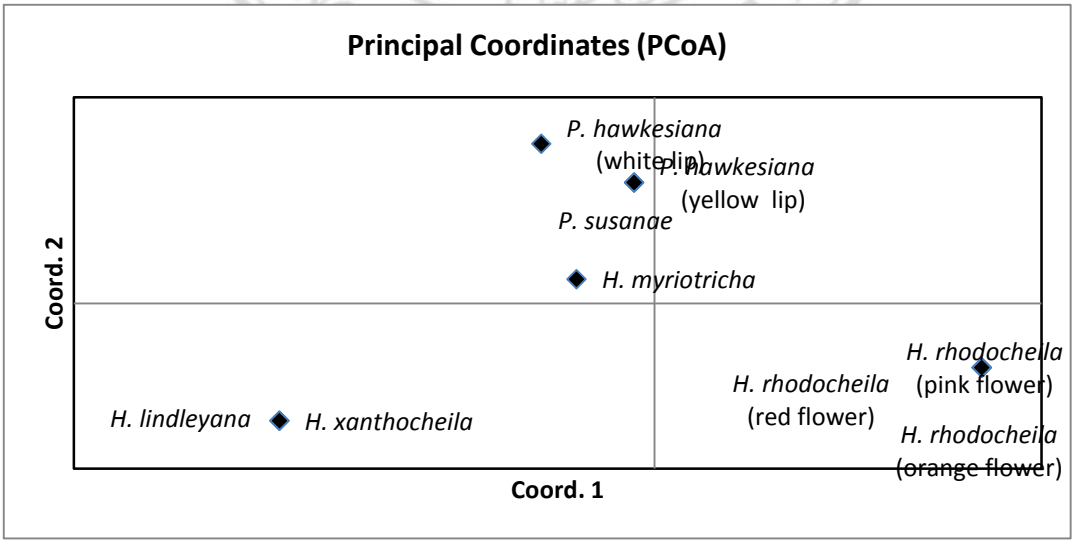

Figure 85 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC11 primer

OPC14 primer could divide plant samples into 2 groups at 2% similarity. The primer could separate subgroup of *P. hawkesiana* (yellow and white lip) from *H. lindleyana*. Another group was separated as subgroup of *H. rhodocheila* (pink flower) and *H. xanthocheila* and a subgroup of *H. rhodocheila* (orange and red flower). Cluster analysis corresponded with PCoA (Figure 86 to 87).

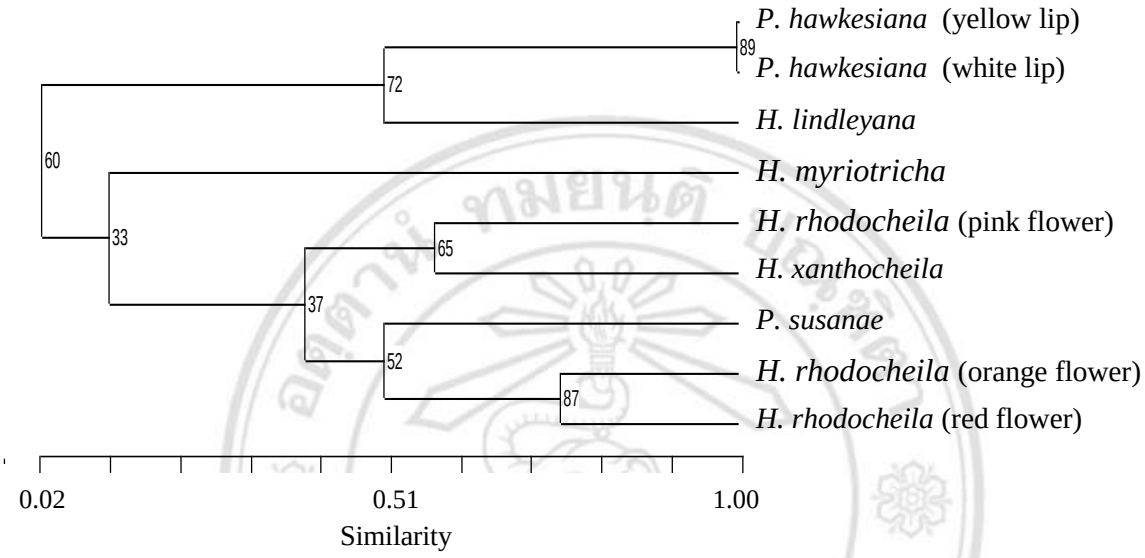

Figure 86 Dendrogram of *Habenaria* and *Pecteilis* based on OPC14 primer

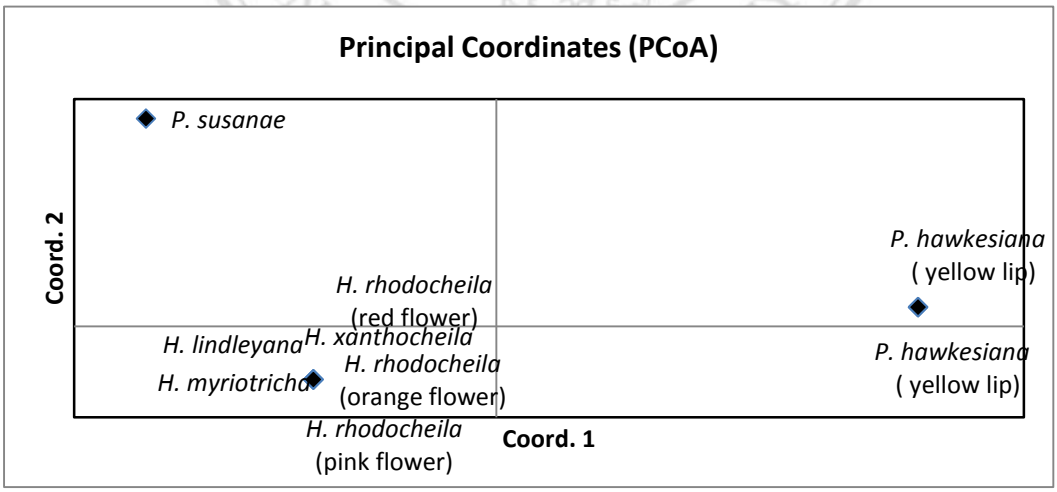

Figure 87 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC14 primer

OPC16 primer could divide plant samples into 2 groups at 5% similarity. The primer could separate a group of *H. rhodocheila* (red and pink flower) and *H. xanthocheila* from the others. Cluster analysis did not correspond with PCoA (Figure 88 to 89).

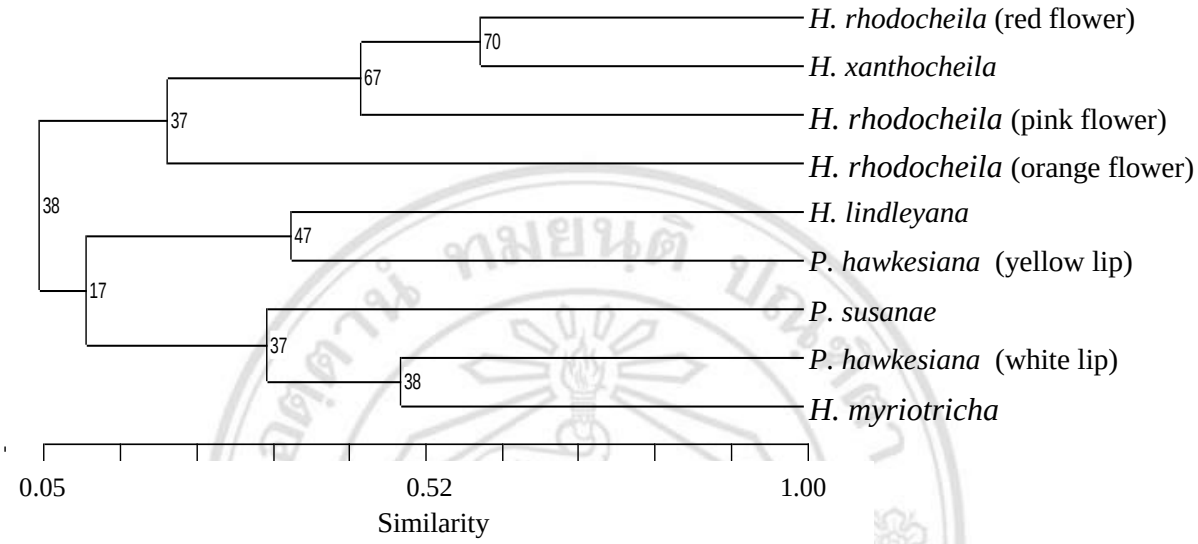

Figure 88 Dendrogram of *Habenaria* and *Pecteilis* based on OPC16 primer

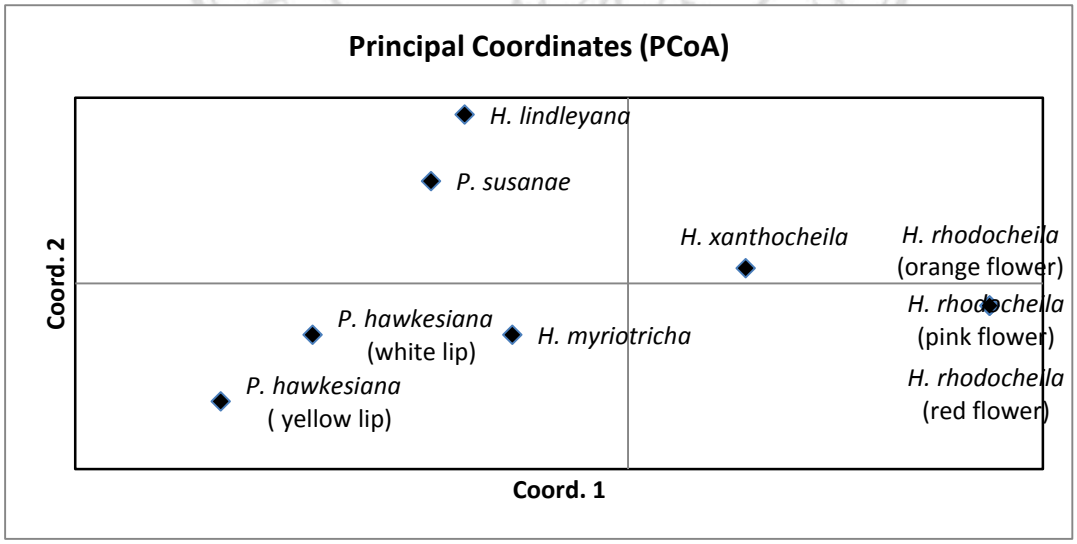

Figure 89 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC16 primer

OPD05 primer could divide plant samples into 2 groups at 3% similarity. The primer could group of *H. rhodocheila* (pink, orange and red flower). *P. hawkesiana* (yellow and white lip) were also clustered at 69% similarity. Cluster analysis did not correspond with PCoA (Figure 90 to 91).

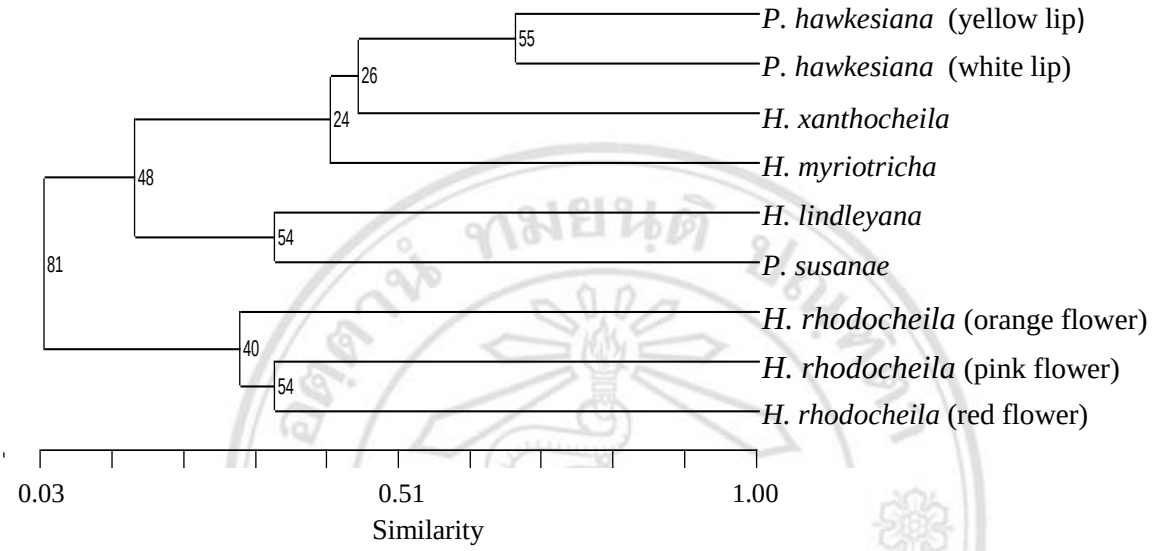

Figure 90 Dendrogram of *Habenaria* and *Pecteilis* based on OPD05 primer

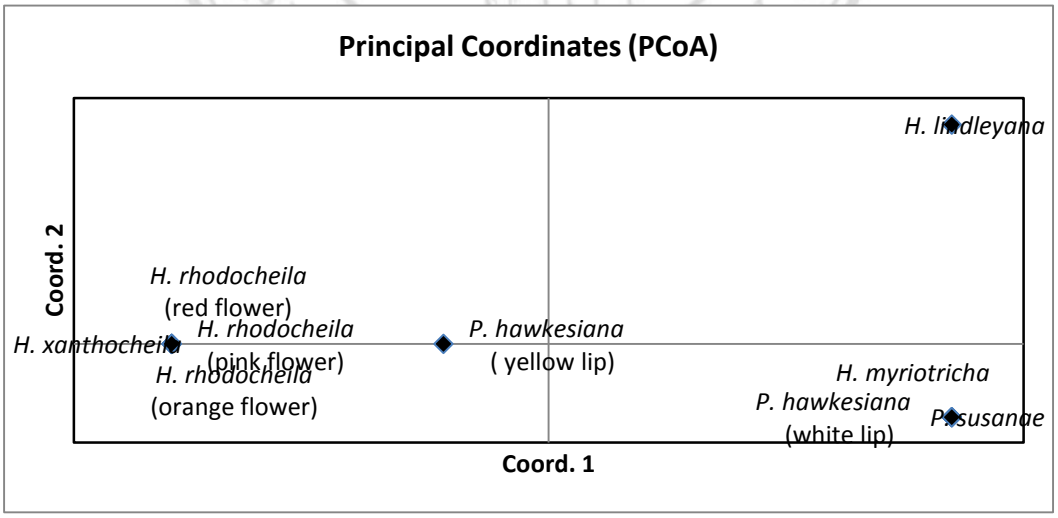

Figure 91 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPD05 primer

OPD08 primer could separate *H. xanthocheila* from the others at 0% similarity. The primer could group *H. rhodocheila* (pink, orange and red flower). Cluster analysis did not correspond with PCoA (Figure 92 to 93).

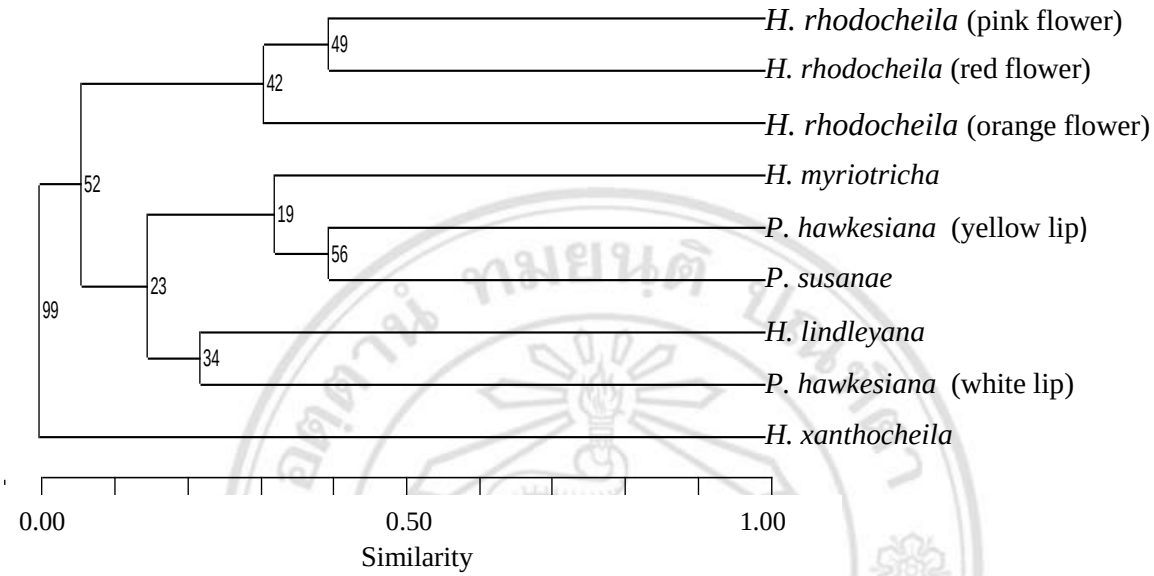

Figure 92 Dendrogram of *Habenaria* and *Pecteilis* based on OPD08 primer

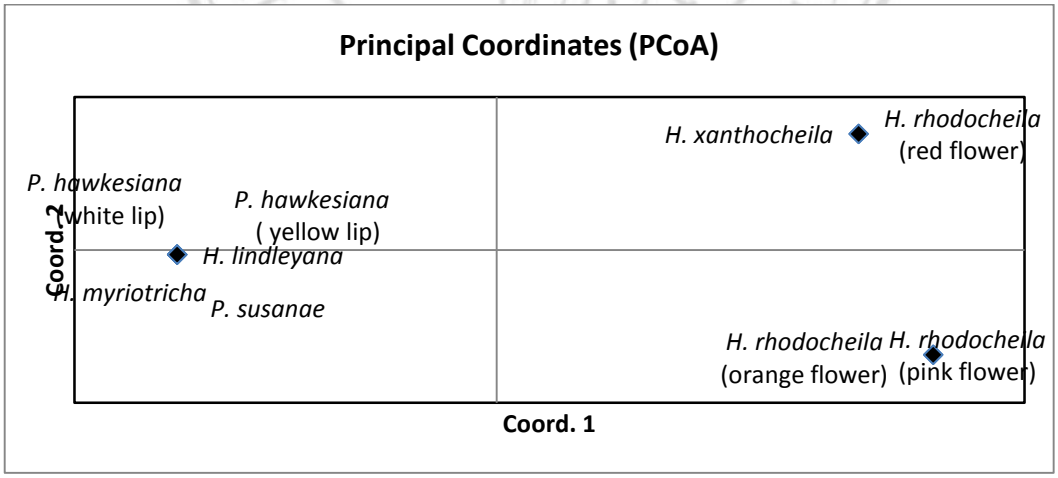

Figure 93 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPD08 primer

OPD12 primer could divide plant samples into 2 groups at 14% similarity. The primer could separate a subgroup of *H. rhodocheila* (pink, orange and red flower) from a subgroup of *H. lindleyana*, *H. xanthocheila* and *P. susanae*. Cluster analysis did not correspond with PCoA (Figure 94 to 95).

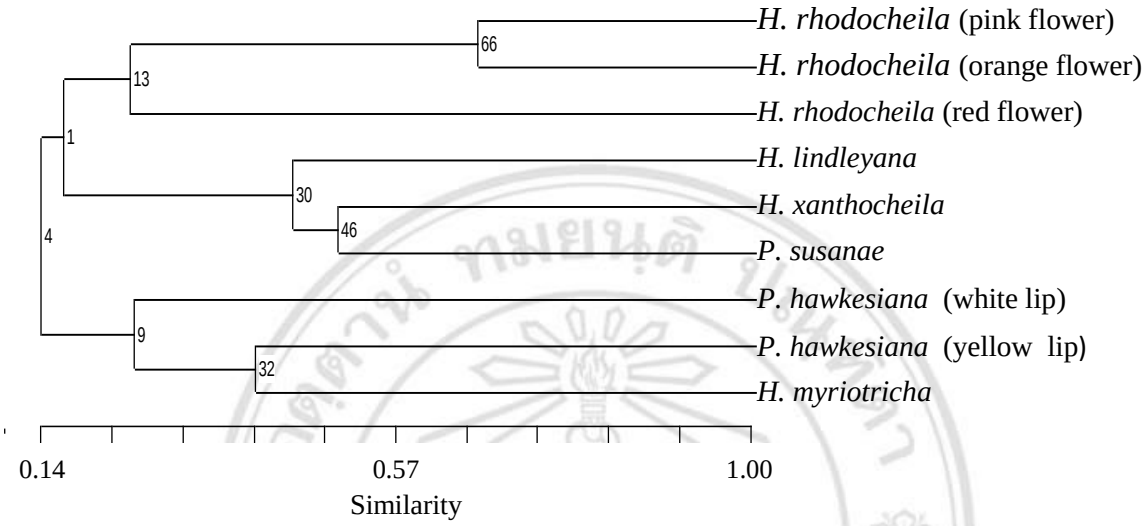

Figure 94 Dendrogram of *Habenaria* and *Pecteilis* based on OPC16 primer

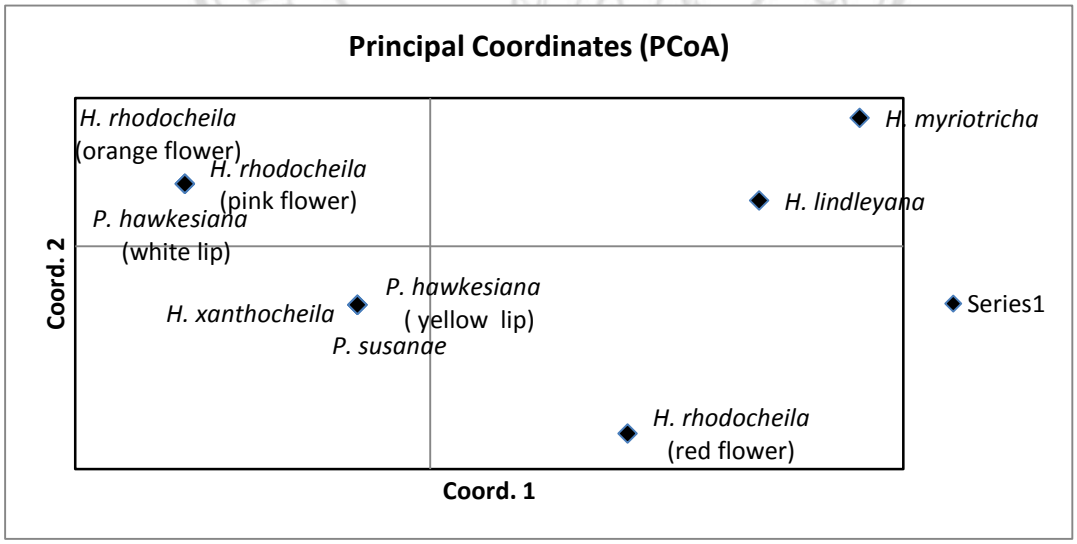

Figure 95 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPC16 primer

OPG09 primer could separate *P. kawkesiana* from the others at 2% similarity. The primer could separate a subgroup of *H. rhodocheila* (pink, orange and red flower) and *H. xanthocheila* from another subgroup. Cluster analysis did not correspond with PCoA (Figure 96 to 97).

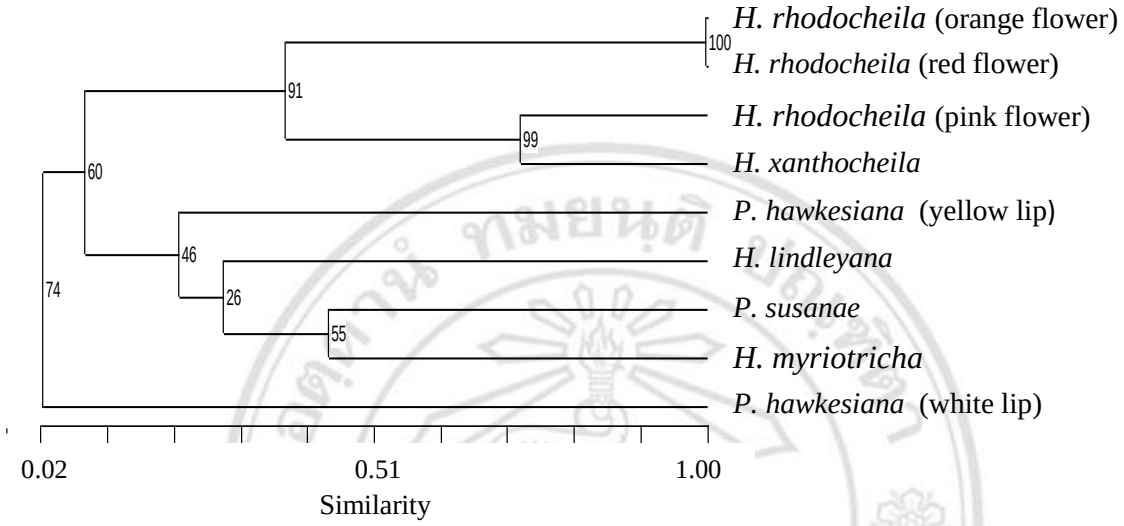

Figure 96 Dendrogram of *Habenaria* and *Pecteilis* based on OPG09 primer

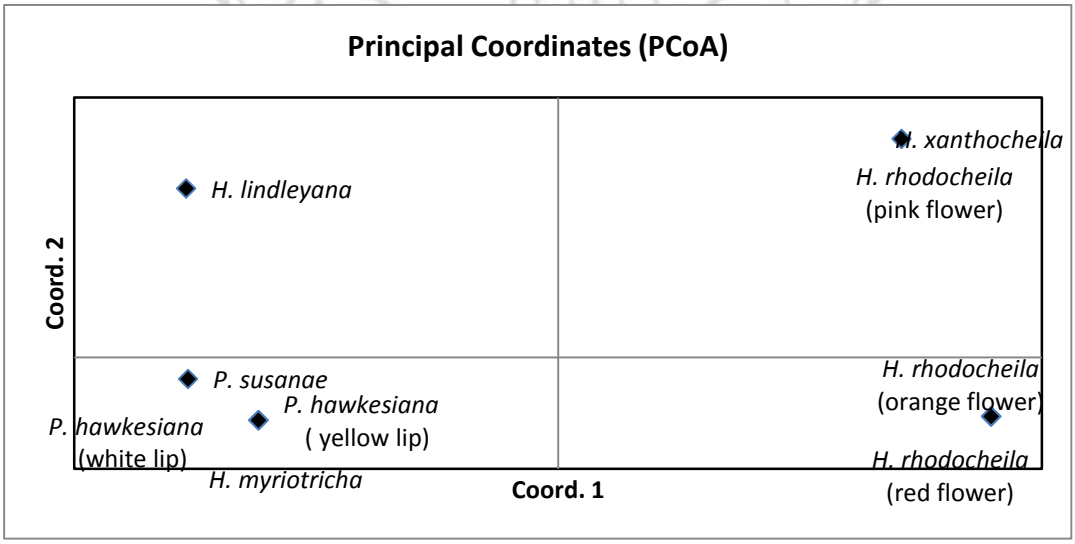

Figure 97 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPG09 primer

OPG15 primer could divide plant samples into 3 groups at 0% similarity. The primer could separate a subgroup of *H. rhodocheila* (orange and red flower) and *H. lindleyana* from a subgroup of *H. rhodocheila* (pink flower) and *H. xanthocheila*. It could divide a group of *Pecteilis* from the others. Cluster analysis did not correspond with PCoA (Figure 98 to 99).

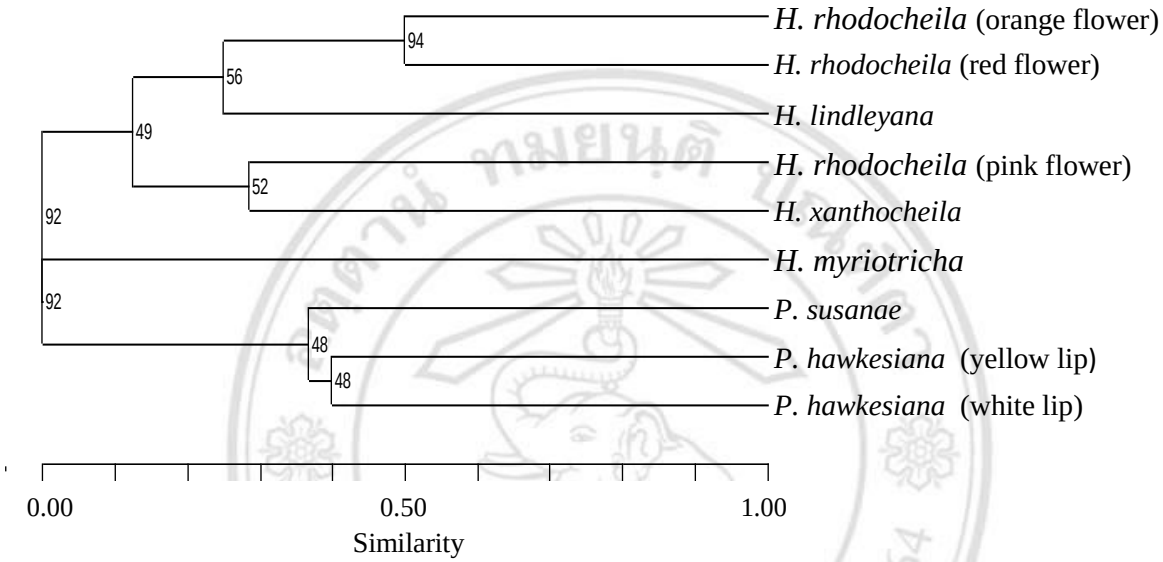

Figure 98 Dendrogram of *Habenaria* and *Pecteilis* based on OPG15 primer

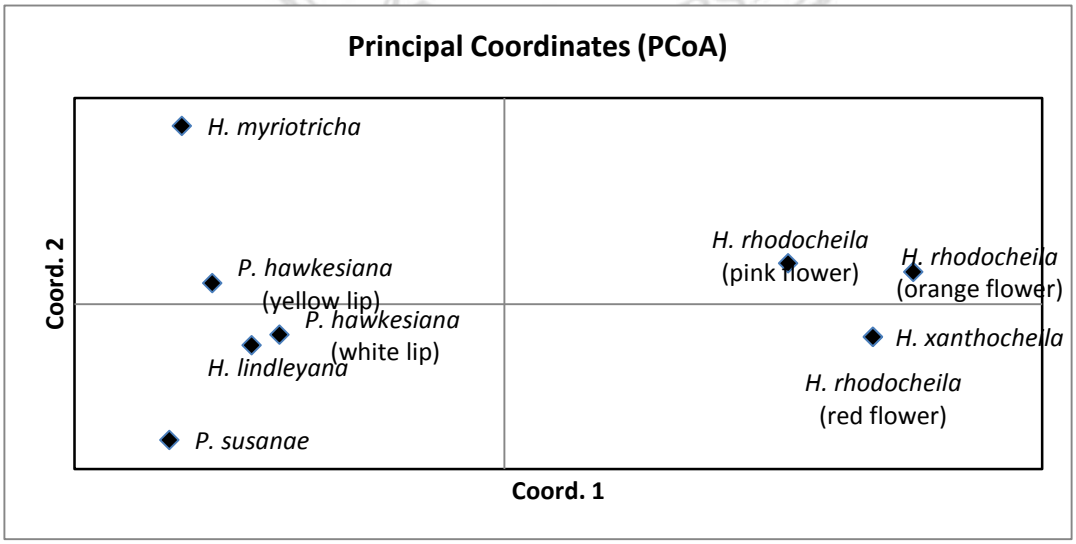

Figure 99 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPG15 primer

OPN04 primer could divide plant samples into 2 groups at 16% similarity. The primer could separate a group of *H. rhodocheila* (pink, red and orange flower) and *H. xanthocheila* from another group. Cluster analysis did not correspond with PCoA (Figure 100 to 101).

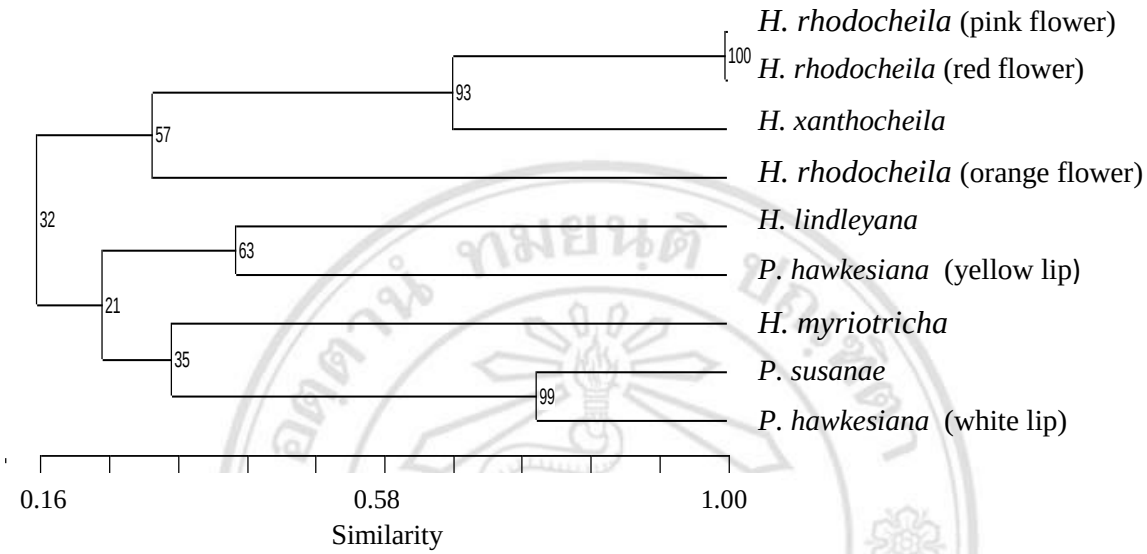

Figure 100 Dendrogram of *Habenaria* and *Pecteilis* based on OPN04 primer

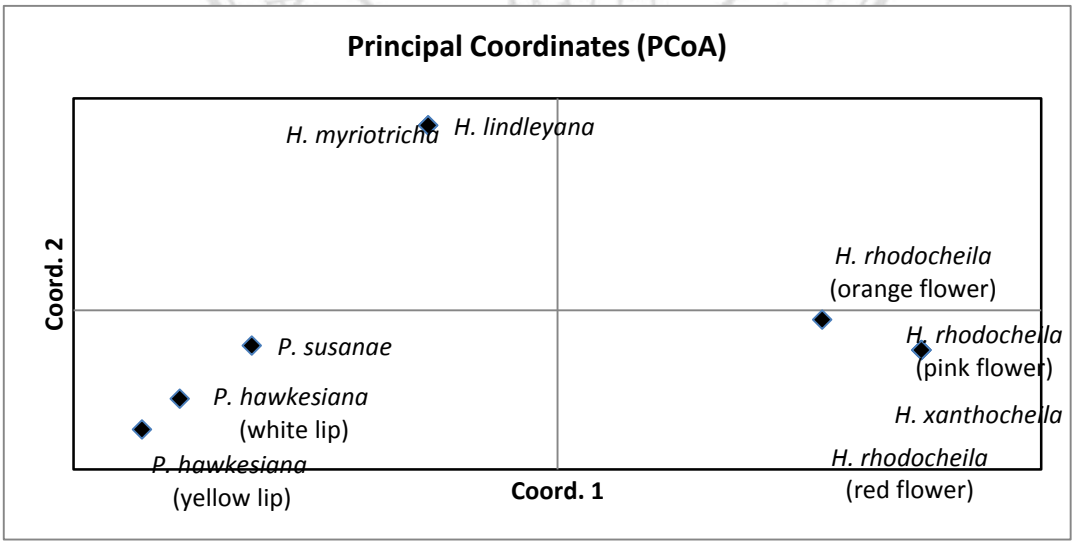

Figure 101 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPN04 primer

OPN05 primer could divide plant samples into 3 groups at 0% similarity. The primer could separate a group of *H. rhodocheila* (red and pink flower) and *H. xanthocheila* from the other groups. Cluster analysis corresponded with PCoA (Figure 102 to 103).

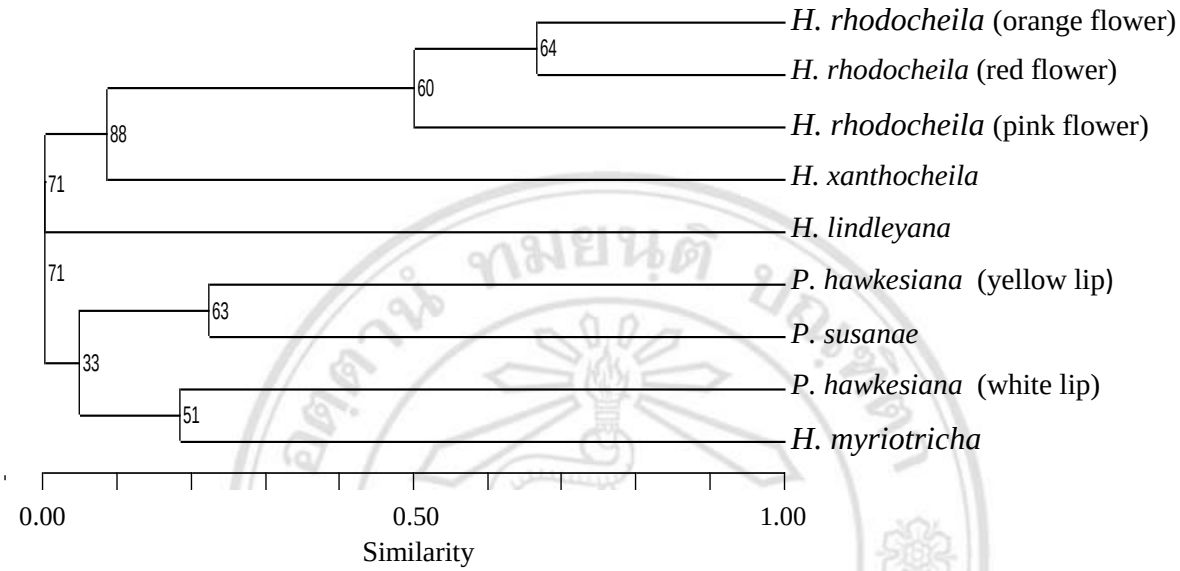

Figure 102 Dendrogram of *Habenaria* and *Pecteilis* based on OPN05 primer

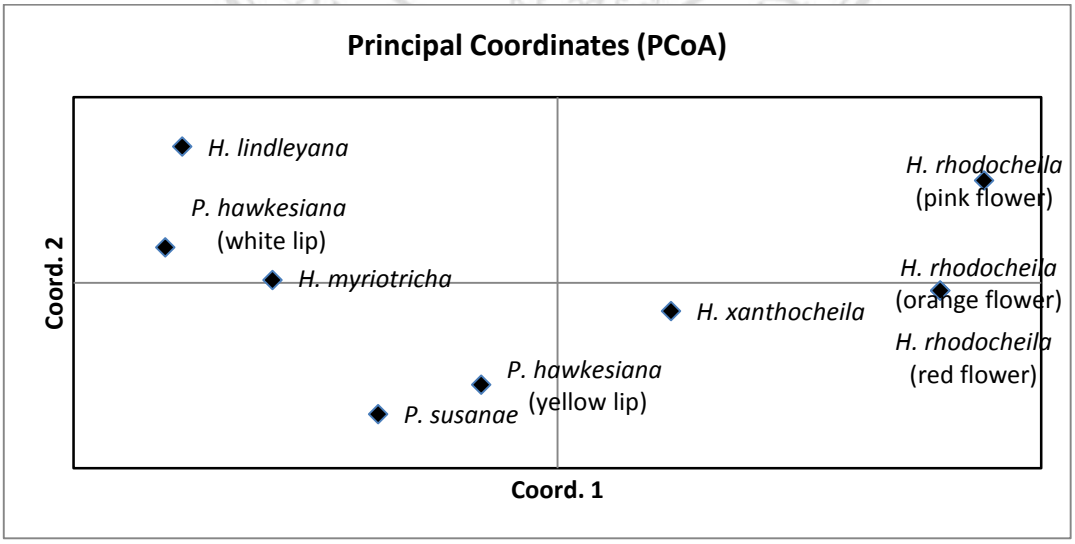

Figure 103 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPN05 primer

OPU12 primer could divide plant samples into 5 groups at 0% similarity. The primer could separate a group of *H. rhodocheila* (red and pink flower) and *H. xanthocheila* from the other groups. Cluster analysis did not correspond with PCoA (Figure 104 to 105).

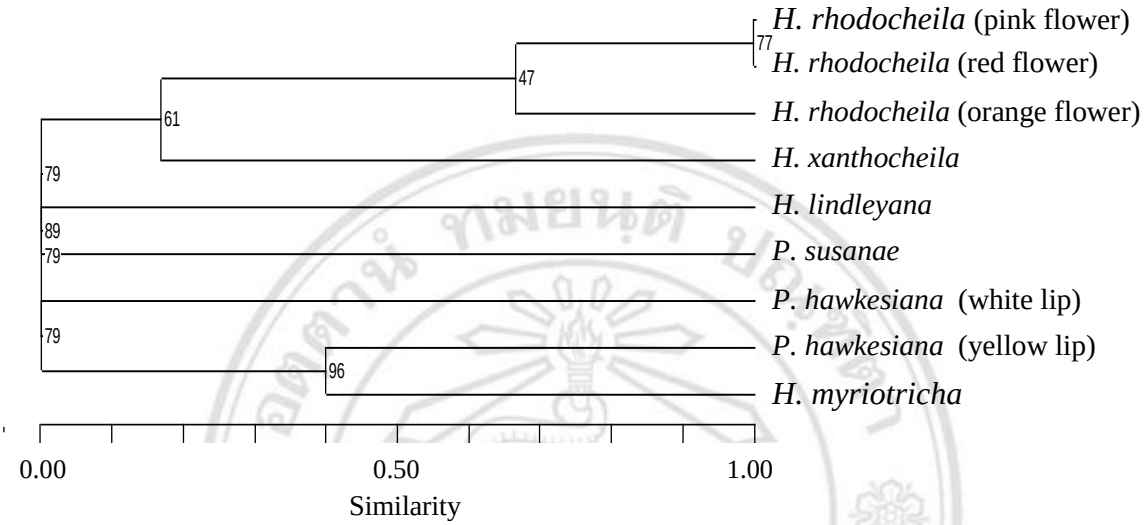

Figure 104 Dendrogram of *Habenaria* and *Pecteilis* based on OPU12 primer

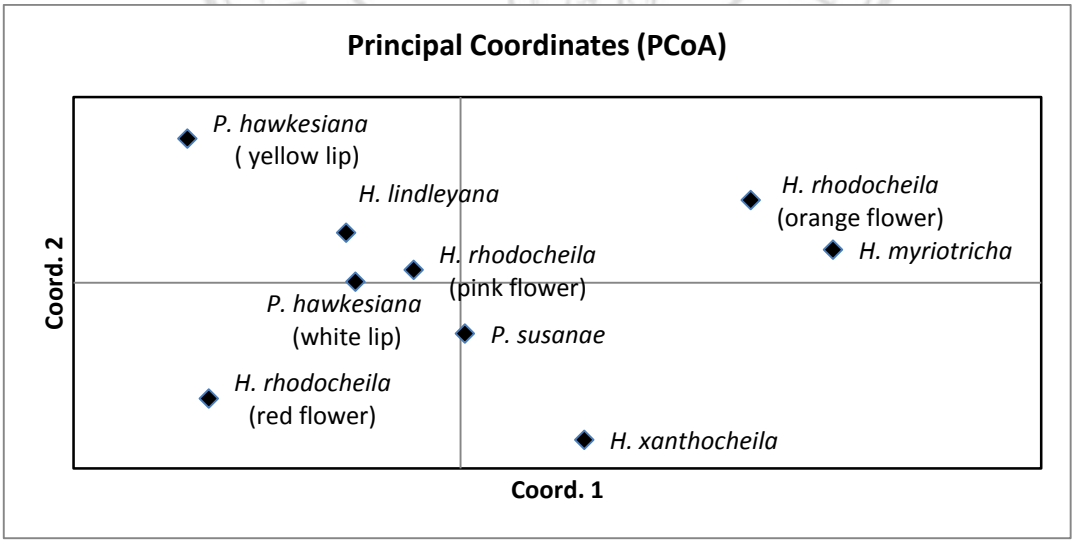

Figure 105 PCoA dendrogram of *Habenaria* and *Pecteilis* based on OPU12 primer

Combinations of 15 primers, OPA10, OPC05, OPC06, OPC07, OPC11, OPC14, OPC16, OPD05, OPD08, OPD12, OPG09, OPG15, OPN04, OPN05 and OPU12, could divide plant samples into 2 groups at 6% similarity. These primers could cluster a group of *H. rhodocheila* (red and pink flower) with *H. xanthocheila* while *H. myriotricha* and *H. lindleyana* were grouped with *Pecteilis* genus (figure 106).

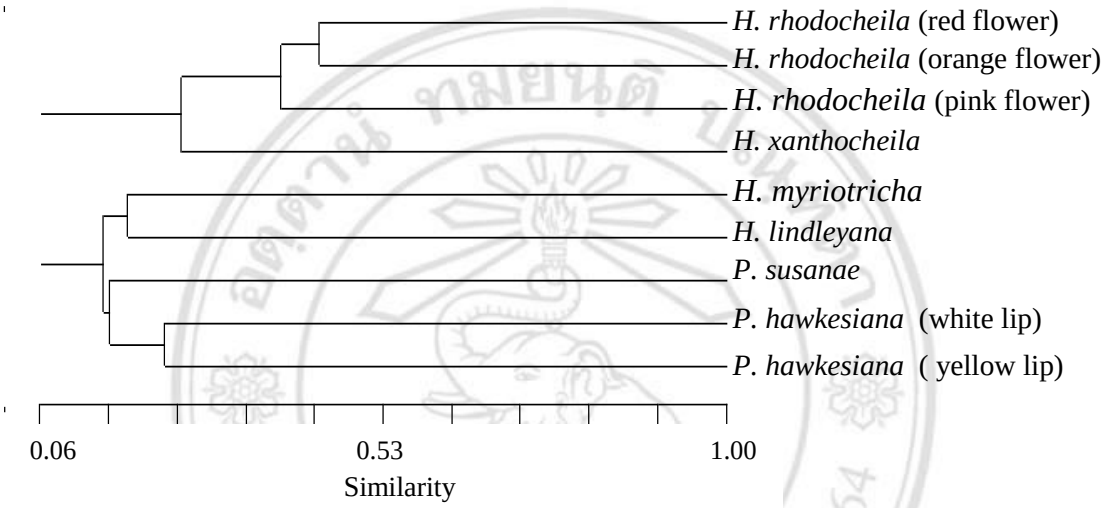

Figure 106 Dendrogram of *Habenaria* and *Pecteilis* based on fifteen primers