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中国地胆草属和假地胆草属(菊科)分合的分子依据

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摘要　为探讨中国菊科地胆草属和假地胆草属在DNA分子水平上的亲缘关系以及这两个属的分合问题，以香港野生的地胆草属植物地胆草(*Elephantopus scaber* L.)、白花地胆草(*E. mollis* H. K. B.)和香港、台湾归化的假地胆草属植物假地胆草(*Pseudelephantopus spicatus* (B. Juss. ex Aublet) C. F. Baker)为材料，选取6种18－24 mer和5种10 mer的随机单引物进行任意引物PCR(AP-PCR)和随机扩增多态DNA(RAPD)分析，以相似度指数值作亲缘关系分析。初步结果显示：2种地胆草与1种假地胆草之间的DNA指纹图谱差异较大，提示地胆草属与假地胆草属植物的亲缘关系较远，属于属间差异。这与形态学、组织学和细胞学结果呈相关性，认为这两个属合并成一个广义的地胆草属是不合适的。

关键词　地胆草属；假地胆草属；分子系统学；聚合酶链反应；DNA指纹技术

中图分类号　Q949.783.5

MOLECULAR EVIDENCES ON SEPARATION AND COMBINATION OF THE GENERA *ELEPHANTOPUS* L. AND *PSEUDELEPHANTOPUS* ROHR (COMPOSITAE) IN CHINA

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Abstract Two *Elephantopus* species and one *Pseudelephantopus* species have been placed in one single genus *Elephantopus* L. *sensu lato* by some earlier systematists. The genomic profile obtained by amplifying the DNA with different single primers were distinctive to these three species and two genera. The Similarity Indexes indicated that *Elephantopus mollis* is more closely related to *E. scaber* than to *Pseudelephantopus spicatus*. The present study indicated that the *Elephantopus scaber* L., and its closed species *E. mollis* as well as *Pseudelephantopus spicatus* were genetically to be distinguished. The estimates of genomic DNA fingerprints based on Similarity Index values calculated from amplified DNA band profiles show a close association with known levels of morphological and histological, cytological relatedness.

Key words *Elephantopus; Pseudelephantopus;* Molecular systematics; Polymerase chain reaction; DNA fingerprinting

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The genus *Elephantopus* was established by Linnaeus in 1753, the lectotype species is *Elephantopus scaber* L., A genus of about 30 species centered in the Neotropics but also found in the Old World, with 2 species (*Elephantopus scaber* L., *E. mollis* H. B. K.) occurring in southern, southwestern mainland China and Taiwan[1]. The genus *Pseudelephantopus* was established by Rohr in 1792, the type species is *Elephantopus spicatus* (Aublet) C. F. Baker. Two closely related but quite distinct species, *Pseudelephantopus spiralis* (Less.) Cronq. and *P. spicatus* (Aubl.) C. F. Baker widespread in the American tropics, the latter also is naturalized in Africa, eastern Asia, and Guam, and was introduced only in Guangdong, Hong Kong and southern Taiwan[2,3].

Although the genus *Elephantopus* is easily recognized, the species, which are based on characters of pappus, leaves, bracts, branching patterns, pubescence, and glomerules, are not always sharply defined and require some experience to identify[4]. The genera *Elephantopus* L. *sensu stricto* and *Pseudelephantopus* Rohr have been placed in one single genus *Elephantopus* L. *sensu lato* by some earlier systematists[2,5-7], but it differs from the latter mainly in the sessile instead of long-pedunculate heads and in the unequal instead of equal pappus-bristles. Chromosome numbers of 2n=22 have been reported for the five taxa— four species and two forms— of *Elephantopus* by Baldwin and Speese[8], the genus *Pseudelephantopus* differs also from *Elephantopus* in chromosome number (2n=26 vs. 2n=22)[4].

Earlier Chinese botanical literature recorded *E. mollis* H. B. K., as the synonym of *E. tomentosus* L. (=*E. bodinieri* Gegnep., *E. elatus* Bertol.)[1,9,10], but according to B. Seenmann's description in *Flora of the Isthmus of Panama*[7] and the authentic specimen of Humboldt in Kunth's Herbarium, the genuine *E. tomentosus* L. has a much taller habit, violet-purple flowers. *E. mollis* species is distinguished by a slender habit, the white flowers and particularly the pappus, of which the paleaceous portion at the base is much shorter, and more abruptly attenuated in the bristle, than in any other species of *Elephantopus*. Therefore this white flower species, which was considered as *E. tomentosus sesus* H. B. K. in Nov. Gen. Sp. Pl. 4:26. 1820; Gleason in Bull. N. Y. Bot. Gard. 4:241. 1906 *non* L. would be *E. mollis* H. B. K. now.

For organisms with practical value or those used in academic studies, molecular biological characterization using specific markers is necessary. That is, both biochemical and molecular markers that discriminate among individual strains are essential for the molecular biological characterization of an organism. Actually, the usefulness of biochemical marker in various kinds of molecular biological research is obvious. Nowadays, several different kinds of biochemical markers have been used in the identification and phylogeny analysis of organisms and their population, the most frequently used biochemical

markers are those concerning the isoenzyme and protein analysis.

Karyological analysis can very often reveal significant chromosomal changes such as structural rearrangements. But chromosomal changes can not reveal alternations in individual genes. A precise determination of changes in a particular gene sequence can be obtained by restriction fragment length polymorphism (RFLP) analysis[12]. The foundation for this concept was established with the hallmark observation by Wyman and White[13] of a polymorphic DNA locus characterized by a number of variable-length restriction fragments. However, the method is limited in two main ways. Firstly, the length of time required to undertake an RFLP analysis is usually 5–6 days and secondly, the result of such an analysis is limited only to the gene sequence which is used as a probe. On the other hand, the development of the polymerase chain reaction (PCR) has led to the major technical advances in molecular biology in recent years[14, 15]. Unfortunately, the application of PCR usually requires information to be present on the sequence of the target DNA, thereby often limiting its usefulness to characterizing genes. The development of a PCR process using only a single arbitrary oligonucleotide primer, known as arbitrarily primed PCR (AP-PCR)[16] and random amplified polymorphic DNA (RAPD)[17], makes the amplification of random sequences in genomic DNA possible. These sequences can in many cases be used as molecular markers. Actually, AP-PCR and RAPD are able to generate polymorphisms and such polymorphisms are strain-specific[18,19].

The purpose of this study is at molecular level to investigate the phylogenetic relationship between genus *Elephantopus* and genus *Pseudelephantopus* in China.

1 Materials and methods

Plant materials Fresh leaves from three species collected in different localities of Hong Kong and Taiwan were used, all the voucher specimens are deposited in the Herbarium, Department of Biology, The Chinese University of Hong Kong (Table 1).

Total genomic DNA preparation This DNA microextraction procedure was based on a method developed for lyophilized leaf tissue and the protocol was modified by Rogers and Bendich[20]. 200 mg of ground sample powder were weighed out and added into a 1.5 ml microfuge tube with 6 vol of 1×CTAB extraction buffer preheated to 56℃, respectively, and incubated for 30 min with occasional shaking, then cooled to room temperature and extracted by an equal volume of chloroform/isoamyl alcohol (24:1). After spinning at 14 000×g for 10 min, the top phase was transferred to a fresh microfuge tube and 0.1 vol of preheated at 56 ℃ 10% CTAB solution was added. After 10 min at room temperature, the solution was extracted with equal vol of chloroform/isoamyl alcohol (24:1) again. The top phase was collected and an equal vol of 1×CTAB precipitation buffer was

Table 1 Plant species used in DNA fingerprinting

Origin	Locality*	Date	Voucher No.
Elephantopus mollis			
Em1	CU Campus, Sha Tin	Jan. 10, 1994	H Cao 1001
Em2	CU Campus, Sha Tin	Sep. 8, 1994	H Cao 1005
Em3	Ng Tung Chai	Sep. 30, 1994	H Cao 1015
Em4	Yung Shue Au	Oct. 10, 1994	H Cao 1019
Em5	Pat Sin Range	Oct. 17, 1994	H Cao 1023
Em6	Fengkang, Pingtung, Taiwan	Nov. 12, 1994	H Cao 1030
E. scaber			
Es1	Ng Tung Chai	Sep. 5, 1994	H Cao 1003
Es2	CU Campus, Sha Tin	Sep. 30, 1994	H Cao 1016
Es3	Yung Shue Au	Oct. 10, 1994	H Cao 1020
Es4	Ng Tung Chai	Oct. 17, 1994	H Cao 1024
Es5	Yung Shue Au	Oct. 24, 1994	H Cao 1027
Pseudelephantopus spicatus			
Ps1	Green House/Biol. Dept./CU, Sha Tin	Sep. 5, 1994	H Cao 1004
Ps2	Anping, Tainan, Taiwan	Nov. 16, 1994	H Cao 1031

*Except indicated, all are in Hong Kong. CU: The Chinese University of Hong Kong

added, mixed and stood for 30 min. The solution was centrifuged at 13 000×g for 15 min at 20 ℃; then the pellet was suspended in 500 μl 1.0 mol/L NaCl and 2 vol of cooled 100% alcohol were added and stored at -20℃ overnight. The solution was spun at 13 000×g for 5 min and the pellet was washed with 65% and 85% EtOH twice, respectively. The pellet was dried in oven (<60 ℃) for 30 min and resuspended in 50 μl TE buffer.

RNAase treatments were repeated once, particularly those in which RNA were present in the DNA solution. The RNA can be digested by restriction enzyme, 10 μl of RNAase stock (heat-treated) was added to the TE buffer containing sample DNA and incubated at 37 ℃ for 1 h. The chloroform/isoamyl alcohol extraction was repeated and the purified DNA was finally dissolved in TE buffer.

Genomic DNA amplification

AP-PCR Fifteen plants (three species) of two genera collected from the wild-growing in Hong Kong have been involved in the AP-PCR study. For fingerprinting a plant species, AP-PCR was used for generating specific DNA profiles for all the samples in Table 1. The following method was a simple modification of the procedure of Welsh and McClelland[16]. Amplification reactions were performed in volumes of 25 μl containing 1×*Taq* buffer (Promega), 1.5 mmol/L $MgCl_2$ (Sigma), 0.2 mmol/L of each dNTPs (Promega), 2 μmol/L primer, 2.5 U *AmpliTaq* polymerase (Perkin-Elmer Cetus) and

100−200 ng template DNA. The reaction mixture was pipetted into a 0.5 ml microcentrifuge tube. Six different primers (18−24 mers) were used in the present study (Table 2).

Light mineral oil (Sigma) was overlaid onto the mixture and the tube was placed into the Thermolyne Thermocycler (Barnstead/Thermolyne Corp., USA). The mixture was then subjected to the following thermal cycles after using a "hot start" step to minimize nonspecific amplification, in which the mixture was heated at 94 ℃ for 10 min before adding polymerase. Two cycles of low stringency amplification: 94 ℃, 5 min; 35 ℃, 5 min; 72 ℃, 5 min, 40 cycles of high stringency amplification: 94 ℃, 1 min; 55 ℃, 1 min, 72 ℃, 1 min, 1 cycle of high stringency amplification with longer primer extension time: 94 ℃, 1 min; 55 ℃, 1 min; 72℃, 10 min.

Table 2 The six arbitrarily chose primers used for DNA fingerprinting by AP-PCR

Name of primer	DNA sequence of the corresponding primer
M13 For	5' d-CGCCAGGGTTTTCCCAGTCACGAC-3' (24 mer)
M13 Rev	5' d-AGCGGATAACAATTTCACACAGGA-3' (24 mer)
Gal K	5' d-TACGGTGGCGGAGCGCAGCA-3' (20 mer)
Seq 2	5' d-CTGGTCAAGGCACAAGAGAT-3' (20 mer)
P4	5' d-GCTTCTCAAGCAGGACCT-3' (18 mer)
SH 1133	5' d-CATCGGATCCACCACGTC-3' (18 mer)

RAPD Three representive species were also involved in the RAPD study. The method was a modification of the procedure of Williams et al.[17]. For the RAPD reactions, 25 ng template DNA were used in 1×*Taq* buffer, 2 mmol/L $MgCl_2$, 0.1 mmol/L of each dNTP with 0.2 μmol/L primer and 0.5 U *AmpliTaq* polymerase in a final volume of 25 μl. The mixture was not covered with light mineral oil and the tube was placed in a FTS-960 Microplate Fast Thermal Sequencer (Corbett Research Co., USA). The reaction was programmed as follows: 1 min at 94℃, 1 min at 36℃ and 2 min at 72℃ for 45 cycles. A "hot start" step (addition of the enzyme at 94℃) was also used. RAPD primers were random 10-mers from Operon Kit AM and Kit C (Operon Technologies, USA). The specific primers and their sequences are listed in Table 3.

Each unit was tested in two replicated experiments where a single large PCR reaction was separated in several tubes and loaded in each wells. The amplified products were kept at 4 ℃ for further analysis. Agarose gel electrophoresis was carried out for the amplified product. 10 μl of the AP-PCR product was analyzed by 2.5% agarose gel electrophoresis in the 1×TBE buffer system and stained with EtBr prior to UV photography. 10 μl of solution was loaded on the gel; the 100 base-pair DNA ladder (Pharmacia, USA) was used as a size marker in all gels.

Table 3 List of the primers used in RAPD amplification

Name of primer	DNA sequence of corresponding primer	%G+C content
OPAM-02	5' d-ACTTGACGGG-3'	60
OPAM-11	5' d-AGATGCGCGG-3'	70
OPC-06	5' d-GAACGGACTC-3'	60
OPC-08	5' d-TGGACCGGTG-3'	70
OPC-20	5' d-ACTTCGCCAC-3'	60

Data analysis DNA band patterns of the species were compared in a pairwise manner using the Similarity Indexes (S.I.) in the following equation[21]: $S_{xy}=2n_{xy}/n_x+n_y$ where S_{xy}=the similarity index between samples in lanes x and y, n_{xy}=the number of bands common to both lane x and lane y, n_x=the number of bands in lane x, n_y=the number of bands in lane y.

Gel image was stored as TIF file and sizes of AP-PCR or RAPD product were analyzed by ImageQuant^{TM} program (Molecular Dynamics, USA). The S.I. value of fingerprints calculated for each possible pairwise comparison between samples by the program DNA SIMDEX.

2 Results and discussion

Genomic DNA fingerprints of all leaf specimens were successfully generated. The representative DNA profiles of the genomic fingerprints by AP-PCR using all six random long primers were presented in Fig. 1 to Fig. 2. After the species *Pseudo-elephantopus spicatus* (Ps1) was screened with 40 random short primers, the following 17 were found to give scorable bands of RAPD markers: OPAM: 01–02, 09, 11 and 20; OPC: 02–04, 06–08, 14–16 and 18–20. The genomic DNA fingerprints by RAPD among the three representive species showed that distinctive DNA bands were generated for five primers listed in Table 3 (Fig. 3).

In order to study the systematics of *Elephantopus* L. and *Pseudelephantopus* Rohr, the phylogenetic relationship of *Elephantopus scaber* and its two closely related species *Elephantopus mollis* and *Pseudelephantopus spicatus* was investigated according to the genomic DNA fingerprints.

DNA fingerprinting of suitably amplified genomic DNA to a random primer in AP-PCR and RAPD usually results in the detection of a large number of fragments. These fragments tend to become progressively more crowded with diminishing size. Thus, the larger ones, 0.8–1.2 kb, are relatively easy to score, whereas fragments smaller than 0.2–0.3 kb are almost impossible to evaluate unambiguously. The number of useful fragments detected usually ranges between 5 and 15 in the present study.

DNA patterns are commonly evaluated by scoring the individual bands as either present or absent for each individual samples. Computer-aided scanning of gel electrophoresis photographs may become a valuable tool[22]. Assuming that the fragments are in linkage equilibrium, the probability that two different samples will exhibit identical fragment profiles can then be calculated as the mean S.I. values raise to the mean number of fragments scored per individual. Assuming that all bands are monomorphic, have equal frequencies within the samples studied, and occur independently of one another, for closely

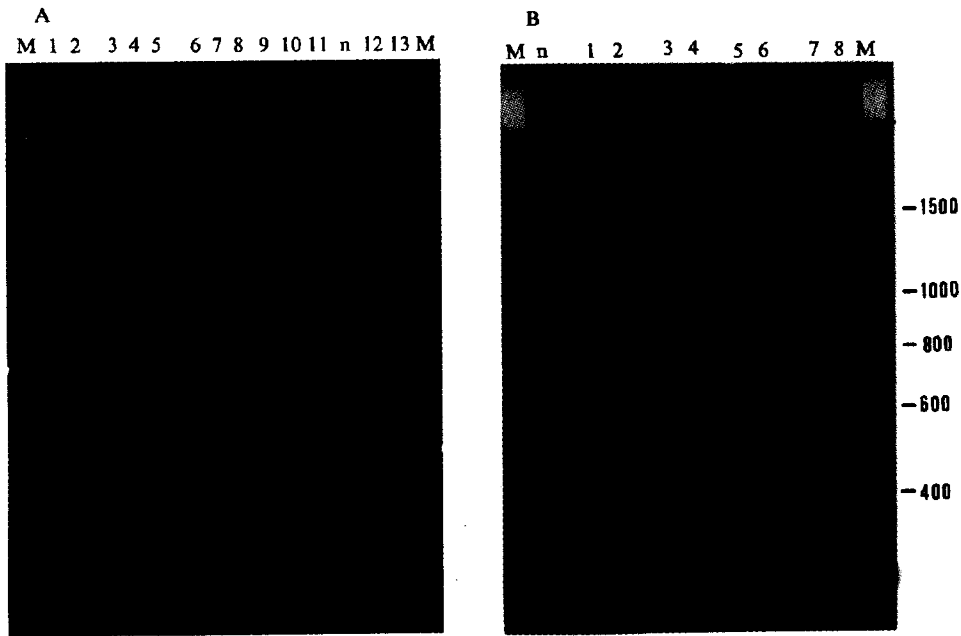

Fig. 1 Genomic fingerprints generated by AP-PCR using M13 For primer (A) and Gal K primer (B). A: 1−5: Es1−Es5; 6−11: Em1−Em6; 12−13: Ps1−Ps2. B: 1−6: Em1−Em6; 7: Es1; 8: Ps1. M: 100 bp DNA ladder, migration positions of molecular size markers are indicated in bp. The sample codes were listed in Table 1.

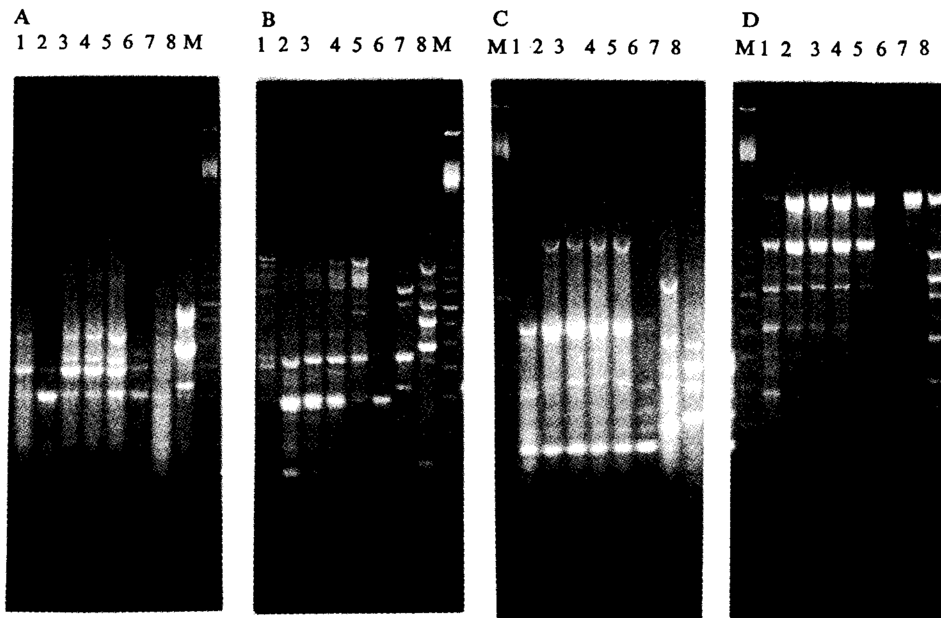

Fig. 2 Genomic fingerprints generated by AP-PCR using SH 1133 (A), P4 (B), Seq 2 (C) and M13 Rev (D) primers. The AP-PCR patterns were different for same species when the primer used for DNA amplification was different. 1−6: Em1−Em6; 7: Es1; 8: Ps1; M: 100 bp DNA ladder. The sample and primer codes were listed in Tables 1 and 2.

related species the value of S.I. value should approach 100%, and for unrelated species the S.I. value should approach 0. This assumption was validated by calculating the S.I. values for each of the 11 primers used for comparing 3 species tested.

The two *Elephantopus* species and one *Pseudelephantopus* species have been placed in one single genus by some authors. In fact, *E. scaber* and *E. mollis* as well as *Pseudelephantopus spicatus* belong to two genera in the family Compositae. The genomic profile obtained by amplifying the DNA with different primers were distinctive to these species (Figs. 1−3). The Similarity Indexes indicated that *Elephantopus mollis* is more closely related to *E. scaber* than to *Pseudelephantopus spicatus* (Tables 4 and 5).

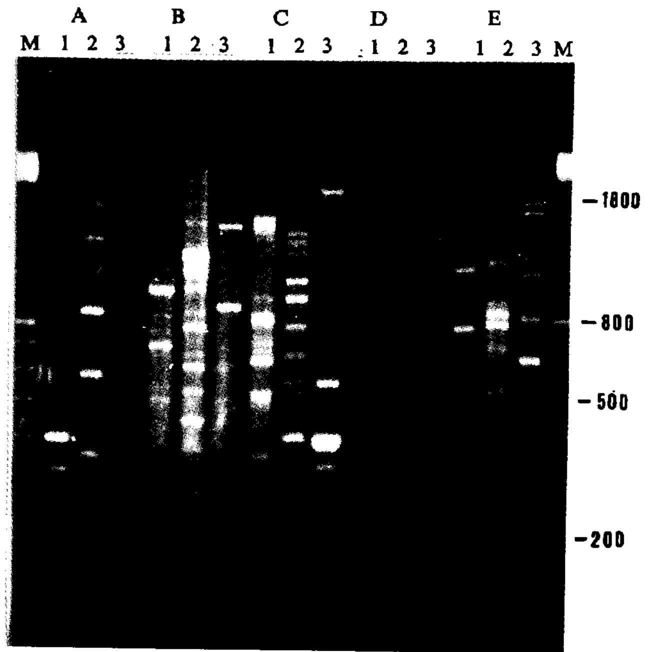

Fig. 3 Genomic fingerprints generated by RAPD using OPAM-02 (A), OPAM-11 (B), OPC-06 (C), OPC-08 (D) and OPC-20 (E) primers.
The RAPD patterns were different for same species when the primer used for DNA amplification was different. 1: Es1; 2: Em1; 3: Ps1; M: 100-bp DNA ladder, migration positions of molecular size are indicated in bp. The sample (one representive plant) and primer codes were listed in Tables 1 and 3.

The present study indicated that the *Elephantopus scaber* L., and its closed species *E. mollis* as well as *Pseudelephantopus spicatus* were genetically to be distinguished. The estimates of genomic DNA fingerprinting based on Similarity Indexes calculated from amplified DNA band profiles show a close association with known levels of morphological and histological, cytological relatedness[4,8,22].

The DNA fingerprinting techniques were showed to be useful for differentiating among

Table 4 Similarity Indexes of two *Elephantopus* species and one *Pseudelephantopus* species deduced by AP-PCR

Sample*	Similarity Indexes (S.I.)						Average S.I.**
	M13 For	SH1133	P4	Seq 2	M13 Rev	Gal K	
Es vs. Em	0.42	0.63	0.38	0.38	0.40	0.37	0.43
Es vs. Ps	0.20	0.27	0.27	0.23	0.23	0.26	0.24
Em vs. Ps	0.17	0.11	0.11	0.22	0.13	0.13	0.15

*Letters refer to Table 1; **Percentage S.I. was calculated from pairwise comparsion done between a representive species (a sample) using six long primers from Figs. 1 and 2.

Table 5 Similarity Indexes of two *Elephantopus* species and one *Pseudelephantopus* species deduced by RAPD

Sample*	Similarity Indexes (S.I.)					Average S.I.**
	OPAM-02	OPAM-11	OPC-06	OPC-08	OPC-20	
Es vs. Em	0.22	0.30	0.40	0.40	0.52	0.37
Es vs. Ps	0.20	0.18	0.12	0.00	0.19	0.14
Em vs. Ps	0.21	0.21	0.17	0.00	0.17	0.15

*Letters refer to Table 1; **Percentage S.I. was calculated from pairwise comparsion done between a representive species (a sample) using five short primers from Fig. 3.

species within genera as well as between the genera themselves[23]. We conclude, therefore, that random-primed PCR-based AP-PCR and RAPD markers are well suited for use as molecular markers for the assessment of the systematics of plants.

Otherwise, the molecular phylogeny between genus *Elephantopus* and genus *Pseudelephantopus* and their evolution relationship in family Compositae will be futher studied by better molecular markers such as DNA sequencing using both nuclear genes and chloroplast genes[24].

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References

1 Chen Y L. *Elephantopus* L.; *Pseudelephantopus* Rohr. In: Ling Y, Chen Y L, Shih C eds. Flora Reipublicae Popularis Sinicae. Tomus 74. Beijing: Science Press, 1985, 43－47

2 Dunn S T, Tutcher W J. Flora of Kwangtung and Hongkong (China). In: Bulletin of Miscellaneous Information (Royal Botanical Gardens, Kew). Add series X. London: His Majesty's Stationary Office, 1912, 139

3 You Y D, Chen Y F, Wu Y. Originally Growing Plants of Taiwan. Vol. 1. Taipei: Shu Qing Publishing Co., Ltd., 1990, 104

4 Jones Jr. S B. The genera of Vernonieae (Compositae) in the Southeastern United States. J Arn Arbor, 1982, 63: 503－507

5 Anonymous. Iconographia Cormophytorum Sinicorum. Tomus 4. Beijing: Science Press, 1980, 803

6 Merrill E D. An Enumeration of Philippine Flowering Plants. Vol. 3. Manila: Bureau of Printing, 1923, 596

7 Seemann B. Flora of the Isthmus of Panama. In: The Botany of the Voyage of H. M. S. Herald, under the Command of Captain Henry Kellett, R. N., C. B., during the years 1845－51. London: Covent Garden, 1852－1857, 141

8 Baldwin Jr. J T, Speese B M. Chromosomes of *Elephantopus* and of *Pluchea* in the range of Gray's Manual of Botany. Am J Bot, 1955, 42:123－125

9 How F C. Flora Cantonensis. Beijing: Science Press, 1956, 530－531

10 Hu S Y. The Compositae of China. Quart J Taiwan Museum, 1966, 19:378

11 Baker C F. A revision of Elephantopeae— I. Trans Acad Sci St. Louis, 1902, 12:43－56

12 Kesseli R, Ochoa O, Michelmore R. Variation at RFLP loci in *Lactuca* spp. and origin of cultivated Lettuce (*L. sativa)*. Genome, 1991, 34:430－436

13 Wyman A R, White R. A Highly polymorphic locus in human DNA. Proc Natl Acad Sci USA, 1980, 77:6754－6758

14 Saiki R K, Scharf S J, Faloona F et al. Enzymatic amplification of *β*-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science, 1985, 230:1350－1354

15 Mullis K B, Faloona F, Scharf S J et al. Specific enzymatic amplification of DNA *in vitro:* The polymerase chain reaction. Cold Spring Harbor Sym Quan Biol, 1986, 51:263－273

16 Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucl Acids Res, 1990, 18: 7213－7218

17 Williams J G K, Kubelik A R, Livak K J et al. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res, 1990, 18:6531－6535

18 Nybom H. Applications of fingerprinting in plants population studies. In: Pena S D J, Chakraborty R, Epplen J T et al. eds. DNA Fingerprinting: State of the Science. Basel: Birkhauser Verlag, 1993, 293－309

19 Weising K, Ramser J, Kaemmer K et al. Oligonucleotide fingerprinting in plants and fungi. In: Burke T, Dolf G, Jeffreys A J et al. eds. DNA Fingerprinting: Approaches and Applications. Basel: Birkhauser Verlag, 1991, 313－329

20 Rogers S O, Bendich A J. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol, 1985, 5:69－76

21 Nei M, Li W H. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA, 1979, 76:5269－5273

22 Lin C C, Yen M H. Development of natural crude drug resources from Taiwan (ix)— pharmacognostical studies on the crude drug "Ding-Kia-U". J Chin Med, 1992, 2:33－49

23 Sytsma K J. Hahn W J. Molecular systematics: 1991－1993. Pro Bot, 1994, 55:307－333

24 Wang X Q, Hong D Y. Progress in molecular systematics of plants in recent five years. Acta Phytotax Sin, 1997, 35:464－480