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Phylogenetic relationships in the genus *Arachis* based on seed protein profiles

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Summary

Seed protein profiles of 19 accessions representing seven sections of the genus *Arachis* were studied using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The general profile showed appreciable homology between these taxa, supporting their classification based on morphology and cross-compatibility relationships. The accessions of section *Arachis* expressed a high variation confirming inferences from earlier studies. Variation between accessions of a species is limited. Accessions of the section *Ambinervosae* and *Caulorhizae* formed one cluster and accessions of sect. *Erectoides* and *Procumbensae* formed another. Whereas the representative accessions of sect. *Triseminalae* and *Extranervosae* formed two independent clusters. Using the percentage of dissimilarity in electrophoretic bands as a statistical genetic distance between accessions, sect. *Arachis* (containing the cultivated groundnut, *A. hypogaea*) is phylogenetically closest to sect. *Erectoides* followed by *Procumbensae, Ambinervosae, Caulorhizae, Triseminalae* and *Extranervosae*, respectively.

Introduction

Cross-incompatibility between species belonging to various sections of the genus *Arachis* has restricted the collection of evidence from cytogenetical analysis to draw phylogenetic relationships between them. Studies based on isozymes (Stalker et al., 1990), DNA restriction fragment length polymorphisms (RFLPs) (Kochert et al., 1991; Halward et al., 1991) and random amplified polymorphic DNA (RAPDs) (Halward et al., 1992) have been made, but seed protein profiles are still a powerful tool to ascertain genetic homology at the molecular level, and to resolve taxonomic and phylogenetic problems. They have recently been used successfully in sect. *Arachis* of the genus *Arachis* to confirm phylogenetic relationships based on cytogenetic investigations (Singh et al., 1991). The general profile in sect. *Arachis* showed considerable homology between the taxa despite ploidy differences. However, appreciable genetic differences exist between species which support the genomic subdivision and species limits based on a biosystematic definition of species. This study further indicated that seed protein profiles can be very useful at a macroevolutionary level in differentiating between species, but are of relatively lesser help at a microevolutionary level in differentiating between accessions of the same species. Therefore, the present study was undertaken as an extension of a previous study to explore the degree of genetic difference between species of different sections of genus *Arachis* at protein level, to elucidate phylogenetic relationships, and to identify marker proteins for each section/species, if possible.

Materials and methods

Details of seeds from taxa used in the present investigation have been summarized in Table 1.

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Table 1. Identity and taxonomic affinity of species/accessions used in the investigation

S. No.	ICG No.	Identity	Taxonomy	
			Section	Series
1	8200	GKBPSSc 30067; PI 468202 *A. duranensis*	*Arachis*	*Annuae*
2	8124	K 9484; PI 298639 *A. batizocoi*	*Arachis*	*Annuae*
3	8210	GKBPSSc 30081; PI 468327 *A. batizocoi*	*Arachis*	*Annuae*
4	8144	*A. villosa* (Coimbtore)	*Arachis*	*Perennes*
5	8216	GKP 10017; PI 262141 *A. cardenasii*	*Arachis*	*Perennes*
6	8906	HLK 410; PI 338280 *A. stenosperma*	*Arachis*	*Perennes*
7	8135	K 7264; PI 263393 *A. monticola*	*Arachis*	*Amphiploides*
8	156	M 13; *A. hypogaea* ssp. *hypogaea*	*Arachis*	*Amphiploides*
9	221	TMV 2; *A. hypogaea* ssp. *fastigiata*	*Arachis*	*Amphiploides*
10	13211	6676; *A. pusilla*	*Ambinervosae*	–
11	13219	6110 ORFL; *A. pusilla*	*Ambinervosae*	–
12	13222	VSW 6791 Wt FL; *A. pintoi*	*Caulorhizae*	–
13	8973	GKPSc 30134; PI 468176 *A. paraguariensis*	*Erectoides*	*Tertafoliolatae*
14	8215	GKPSc 30126; PI 468170	*Erectoides*	*Tertafoliolatae*
15	8945	GK 30003; PI 468149	*Procumbensae*	–
16	8904	GKP 10034; *A. rignoi*	*Procumbensae*	–
17	11557	KSSc 36008-2; PI 475986	*Procumbensae*	–
18	8131	GK 12922; PI 338449 *A. triseminalis*	*Triseminalae*	–
19	8142	*A. villosulicarpa* (Coimbtore)	*Extranervosae*	–

Sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis

Protein profiles were resolved using SDS-polyacrylamide gel electrophoresis (Singh et al., 1991). However, since it was observed in the previous study that the general protein profiles have considerable homology in their major bands, and that most genetic differences were confined to minor bands, protein extraction was carried out on a defatted meal (100 mg) in 20 ml of 2% SDS-buffer (pH 6.9) using Kinematica Polytron Homogenizer (Kinematica GmbH Kviens Luzean, Switzerland). The homogenates were centrifuged for 20 minutes at 3000 ×g and the extracts were filtered and electrophorized.

Variation in the position of bands was measured using Resolution factor value (Rf value) as described earlier (Singh et al., 1991). Both minor and major bands were included in counting the number of bands, calculating percentage similarity and identifying unique bands which differentiate species belonging to different sections. Percentage similarity between pairs of accessions was calculated using Ladizinsky & Hymowitz (1979) method. The species were clustered following Tocher's method (Rao, 1952), using percentage dissimilarity as the generalized statistical distance. In this method first species are arranged in order of their relative distance from each other. Then two species/accessions having smallest distance are considered first, to which third species having smallest

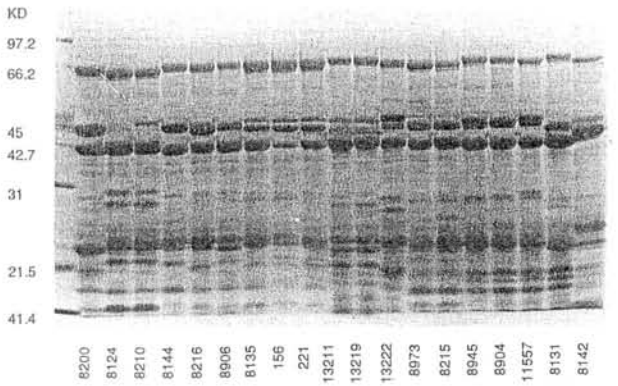

Fig. 1. Seed protein profiles of 19 accessions of *Arachis* species representing seven sections. Track 1 contains molecular weight of markers. Number below each track represents identity of genotypes as per Table 1.

distance from first two species is added. Then fourth and so it goes on. When it is felt that addition of a particular species, results in an abrupt increase of average distance, this species is not included in that cluster and a second cluster is formed. This process is continued till all species are included in one or other cluster.

Results

The electrophoretic seed protein profiles of 19 species/accessions representing seven sections of the genus *Arachis* are presented in Fig. 1. Modification of the earlier electrophoretic technique resolved a greater number of bands (29) in these taxa. The maximum number of bands in any one taxon was 21, recorded in *A. pintoi* belonging to sect. *Caulorhizae*, closely followed by the two accessions of sect. *Ambinervosae* (18) (Table 2). Rf value of these bands ranged from 0.14 to 0.96, and they grouped almost identically across various accessions (Table 2). The accessions belonging to sect. *Ambinervosae, Caulorhizae, Erectoides Procumbensae* and *Triseminalae*, which are wild, have a greater number of fast-moving bands than do the accessions of *Arachis* and *Extranervosae*, which also have some cultivated accessions.

Intra- and intersectional homologies

Nine accessions belonging to sect. *Arachis*, including representatives of both A and or B genomes showed a similar number of bands. However, two accessions of *A. batizocoi* carrying the B genome, expressed a comparatively lower degree of similarity (40.90-63.2%) with other species of sect. *Arachis* (Table 3) and therefore, statistically formed a separate cluster as in previous studies (Singh & Moss, 1982, 1984; Singh et al., 1991). Among the remaining species of sect. *Arachis*, the three tetraploid accessions, wild *A. monticola* and the two subspecies of cultivated *A. hypogaea*, resolved nearly identical number of bands with similar mobility, expressing 78-94% of genetic homology. The four diploid species representing the A genome, also resolved many similar bands between them, with the percentage of similarity ranging from 58 to 88% (Table 3).

Among the other accessions representing the remaining six sections of genus *Arachis*, two accessions of *A. pusilla* (ICG 13211 and 13219) belonging to sect. *Ambinervosae* resolved identical number of bands with similar mobility and thereby 100% genetic homology (Table 3). Their homology with the species of sect. *Arachis* ranged from 32 to 50%, while with the species belonging to five other sections it ranged from 42 to 56% (Table 3). They expressed highest genetic homology (56.0) with the *A. pintoi* (ICG 13222) of sect. *Caulorhizae*, (the three together formed a cluster) followed by *A. triseminalis* of sect. *Triseminalae, A. villosulicarpa* of sect. *Extranervosae* and accessions of sect. *Procumbensae* and *Erectoides*. The lone representative of sect. *Caulorhizae, A. pintoi* expressed a similar degree of homology with two accessions sect. *Erectoides, A.* sp. 30134 and *A.* sp. 30126 followed by the species of sect. *Extranervosae, Triseminalae*, and *Procumbensae*. The two accessions of sect. *Erectoides* and three of sect. *Procumbensae* resolved nearly equal number of bands with identical mobility. Their genetical homology based on the similarity of bands ranged from 65 to 93%, they thereby formed a single cluster. Though the two accessions, GKPSC 30134 and GKPSC 30126 of sect. *Erectoides* have greater similarity between them than to three accessions belonging to sect. *Procumbensae*. The two representative accessions of sect. *Triseminalae* and *Extranervosae* stood distinct from rest of the species belonging to five other sections. They showed genetic homology ranging from 22 to 56% (Table 3). Section *Triseminalae* was closest to three accessions of sect. *Procumbensae, A. rigonii*, and *A.* sp. KSSC 36008-2 and GK 30003 with genetic homology ranging from 53 to 56%, followed by the accessions belonging to sect. *Ambinervosae* and *Caulorhizae* (Table 3). The accession from sect. *Triseminalae* was farthest in genetic homology

Table 2. Distribution of denatured polypeptides of 19 species of genus *Arachis* representing seven sections

RF value	*Arachis* species representing seven sections of genus *Arachis*																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0.14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.22	+	+	+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-
0.25	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-
0.29	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
0.31	+	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-
0.33	+	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	+
0.35	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.37	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+
0.39	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.41	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+
0.45	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+
0.49	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
0.57	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
0.59	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.61	-	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-
0.65	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
0.68	+	-	-	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	+
0.71	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+
0.72	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.74	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
0.76	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.78	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-
0.81	-	+	+	+	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-
0.83	+	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-
0.84	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
0.87	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-
0.90	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
0.93	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-
0.96	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+

Serial number of species as per Table 1.

\+ Denote present, - denote absent.

from the two accessions of *A. batizocoi* from sect. *Arachis* carrying the B genome. *A. villosulicarpa* of sect. *Extranervosae*, besides showing more than 50% homology with the accession of sect. *Caulorhinae*, also showed a similar degree of homology with *A. triseminalis* of sect. *Triseminalae* and to accessions of sect. *Procumbensae*, sect. *Erectoides* and species of sect. *Arachis* representing the A genome, such as *A. villosa* and *A. stenosperma* (Table 3). The variation between the protein profiles of accessions of the same species (*A. pusilla, A. batizocoi, A. hypogaea*) and between accessions of same series or section (*Tetrafoliolatae* and *Procumbensae*) was limited (Fig. 1, Table 3).

The dendrogram (Fig. 2) drawn on percentage dissimilarity in bands and clustering using Tocher's method (using dissimilarity in bands as genetical distance between a pair of accessions) grouped the accessions into 6 clusters. Species of sect. *Arachis*, which contain cultivated *A. hypogaea* grouped into two clusters – one represented by the most diploid species, the wild tetraploid *A. monticola* and the two cultivars from two subspecies of *A. hypogaea* and the other by the two accessions of diploid *A. batizocoi*. The two accessions of *A. pusilla* from sect. *Ambinervosae* together with *A. pintoi* of sect. *Caulorhizae* formed third cluster, while the two accessions belonging to sect. *Erectoides*

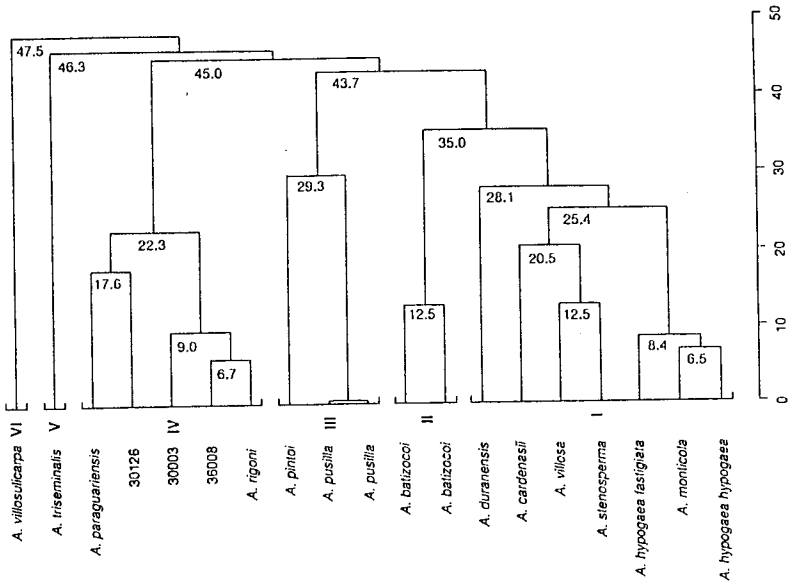

Fig. 2. Dendrogram and clusters constructed on the basis of percentage dissimilarities in the protein profiles.

and three accessions of sect. *Procumbensae* (earlier considered as a series of sect. *Erectoides*) formed the fourth cluster. The sole representatives sect. *Triseminalae* (monotypic) and *Extranervosae* formed fifth and sixth clusters (Fig. 2).

Possible markers

The protein profiles in genus *Arachis* are quite conservative, without substantial variations between the accessions belonging to different sections. This suggests a common ancestry of the accessions studied. The whole profile can be divided into three major regions classified by Cherry (1990): – region 1 of canarachin, region 2 of arachin of acidic subunits, and region 3 of arachin of basic subunits. The major variation representing genetic divergence between accessions is mostly confined to regions 2 and 3 of arachin. In sect. *Arachis*, the B genome species can be characterized by two typical marker bands at the Rf values of 0.57 and 0.61 of proteins of 29 and 29.5 KD. The A genome have bands similar to those of different sections, without any specific band typical to it, except that of *A. duranensis*, which has an additional band at Rf value 0.72. The tetraploid accessions of sect. *Arachis*, both wild and cultivated, also do not have any specific typical marker band. The two accessions of sect. *Ambinervosae* have a typical band at the Rf values 0.37 and 0.41; the former is shared with the only accession of *Extranervosae, A. villosulicarpa*, and the latter with the sole accession of *Caulorhizae, A. pintoi*. The total profile picture of *Ambinervosae* and *Caulorhizae* is strikingly different from that of other sections. The sole member of *Caulorhizae, A. pintoi* has marker bands at Rf values 0.29 and 0.68. It shares the former band with an accession *A.* sp. 30134 of Sect. *Erectoides*, and the latter with *A. triseminalis* of sect. *Triseminalae*. The five accessions belonging to sect. *Erectoides* and *Procumbensae* showed nearly identical profiles, with most bands similar to one or the other accessions of other sections, except for the one at Rf value 0.84, which they shared with all other accessions, except those belonging to sect. *Arachis*. The lone accession representing sect. *Triseminalae* and *Extranervosae* has a different profile from the rest, and have a comparatively smaller number of bands. *A. villosulicarpa* has a typical major band at Rf value 0.37 which it shares with two accessions of *Ambinervosae*, but with a quantitative (intensity) difference. Similarly, *A. triseminalis* of sect. *Triseminalae* has a typical band at an Rf value 0.65 which it shares only with the lone accession of *Caulorhizae*.

Table 3. Percentage of similarity among species belonging to different sections of genus *Arachis*

	Arachis species representing seven sections of genus *Arachis*																	
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	40.9	40.9	57.9	61.1	68.4	68.4	72.2	63.2	36.0	36.0	48.0	55.0	60.0	38.1	42.9	40.9	36.4	45.0
2		87.5	61.1	55.6	55.0	55.0	50.0	50.0	32.0	32.0	38.5	50.0	55.0	47.4	52.6	57.9	31.8	27.3
3			52.6	47.4	55.0	63.2	57.9	57.9	37.5	37.5	38.5	42.9	47.6	40.0	45.0	50.0	31.8	21.7
4				80.0	87.5	66.7	61.1	61.1	33.3	33.3	52.2	45.0	57.9	50.0	55.6	52.6	40.0	50.0
5					70.6	70.6	64.7	64.7	40.9	40.9	47.8	55.6	61.1	52.9	58.8	55.6	42.1	44.4
6						77.8	72.2	72.2	41.7	41.7	60.9	47.6	60.0	52.6	57.9	55.0	42.9	52.6
7							93.8	93.8	47.8	47.8	54.2	55.0	60.0	45.0	50.0	47.6	42.9	38.1
8								87.5	43.5	43.5	50.0	57.9	55.0	40.0	45.0	42.9	38.1	33.3
9									50.0	50.0	50.0	50.0	55.0	47.4	45.0	42.9	38.1	33.3
10										100.0	56.0	37.5	41.7	47.6	45.5	43.5	52.4	47.6
11											56.0	37.5	41.7	47.6	45.5	43.5	52.4	47.6
12												56.5	60.9	47.8	52.2	50.0	52.2	54.5
13													82.4	64.7	70.6	66.7	38.1	40.0
14														70.6	76.5	72.2	42.9	52.6
15															92.9	86.7	50.0	44.4
16																93.3	55.6	50.0
17																	52.6	47.4
18																		50.0

Serial number of species as per Table 1.

Discussion

The protein profiles in the species/accessions representing seven different sections of genus *Arachis* broadly appear similar, corroborating earlier findings of conservative protein profile patterns in the genus *Arachis* (Klozova et al., 1983a, 1983b; Singh et al., 1991). These differences and similarities observed support the broad sub-classification of genus *Arachis* proposed by Gregory et al. (1980) and Krapovickas (1990). Taking the percentage of dissimilarity in bands as a genetical distance between a pair of accessions, the total accessions studied grouped into six clusters (Fig. 2). The section *Arachis*, which contains cultivated tetraploid species, *A. hypogaea* (AABB) and several diploid species with A or B genomes (Table 1), clustered in two groups – one represented by tetraploid species having both A and B genomes and diploid species with the A genome and the other by *A. batizocoi* with the B genome. These support our earlier findings (Singh et al., 1991). Species with the A genome show greater homology with accessions of other sections while accessions with the B genome are farthest from them (Table 3).

The two accessions of *Ambinervosae* and an accession of *Caulorhizae* formed the other cluster, while the two accessions belonging to sect. *Erectoides* and three accessions of sect. *Procumbensae* (earlier considered a series of sect. *Erectoides*) formed another. The later grouping suggests that though the series *Procumbensae* has been raised to the status of section on the basis of morphological variation (Krapovickas, 1990), but at molecular level, it is still close to sect. *Erectoides*. The lone representatives of sect. *Triseminalae* (monotypic) and *Extranervosae*, form two separate clusters.

Phylogenetically, based on relative genetic homology (similarity in bands), the closest to sect. *Arachis* (containing cultivated groundnut) are the accessions of sect. *Erectoides*, and *Procumbensae* followed by representatives of *Caulorhizae* and *Ambinervosae*. The two accessions belonging to sect. *Triseminalae* and *Extranervosae* are the farthest (Table 3). These relationships support the earlier conclusions of Cherry (1975) based on electrophoretic protein and enzyme profile, and of Neucere & Cherry (1975) and Klozova et al. (1983a, 1983b) based on immunochemical similarity. The accessions belonging to sections phylogenetically closer to section *Arachis* have greater prospects in genetic improvement of groundnut than distant ones.

The protein profiles of the accessions of the same species presented a very limited or no variation. This

means that in certain cases protein profile may resolve the differences between accessions of the same species, while in other cases it may not. Probably it depends on degree of genetic divergence. For example, the two accessions of *A. batizocoi* and that of *A. hypogaea* (representing two subspecies, not cultivars of subspecies) differ in major bands which can be used to differentiate accessions of these species, whereas the two accessions of *A. pusilla* did not show such differences even when they have different flower color (Fig. 1, Table 3). Nevertheless, the profiles as a whole, are unique to accessions belonging to different sections, and to species from different sections. Sect. *Arachis* has the maximum variability, as it contains maximum number of species (Krapovickas, 1990). It also appears to be highly evolved as it shows great variability in habitat (both perennial and annual), ploidy level (both diploid and tetraploid) and in chromosome morphology (Singh & Moss, 1982, 1984). Therefore, these results corroborate earlier conclusions of Ladizinsky & Hymowitz (1979) and highlight that protein profile can be used at macroevolutionary to delimit boundaries between sections and species. In addition the present study also indicates that in certain cases protein profiles can also differentiate between accessions of the same species at microevolutionary level, as is being reported for several genera (Crawford, 1990) and also for *Arachis* (Bianchi-Hall et al., 1993).

In conclusion the diversity in protein profiles can be utilized for identification, for classification and for tracing phylogenetic relationships between species, sections and accessions. Further, the protein profile has the advantage of representing full genome of a species unlike RFLPs and RAPDs, which represent a segment of DNA. This limitation sometimes may complicate the inferences, for example in RFLP studies, one probe/enzyme combination may implicate a set of species, while the other combinations may rule it out. Therefore, in broader perspectives phylogenetic relationship drawn on similarity between protein profile should be more direct and simple than molecular techniques such as RFLPs which need all possible probe/enzyme combinations and RAPDs a large number of primers to achieve conclusive phylogenetic inferences.

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