RESEARCH ARTICLE

Extent and structure of genetic diversity in a collection of the tropical multipurpose shrub legume *Cratylia argentea* (Desv.) O. Kuntze as revealed by RAPD markers

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Abbreviations: cv.: cultivated variety
MCA: multiple correspondence analysis
RAPD: random amplified polymorphic DNA

The tropical multipurpose shrub legume *Cratylia argentea* is well adapted to acid soils of low to medium fertility and has excellent drought-tolerance. Due to its high nutritive value it is particularly suited as forage for dry-season supplementation. A collection of 47 *C. argentea* accessions in a collection, derived from seed

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replicating of original accessions with differing geographic origin and morphological and agronomic characteristics was investigated using molecular markers (RAPD (random amplified polymorphic DNA)). Genetic diversity (H_T = 0.145) in the collection was low, with 30% of differentiation among groups and high genetic similarity among accessions (*GS* = 0.805). Within-accession variability was high. One taxonomic mismatch and five possible duplicate accessions were identified. Our results suggest that the genetic diversity in the *C. argentea* accessions studied is relatively homogeneously distributed, indicating the likelihood of extensive outcrossing. The genetic diversity of original accessions should be assessed to determine if outcrossing has occurred during or before *ex situ* storage. This might also support any decision on whether accessions should be bulked rather than maintaining them individually.

Cratylia argentea (Desv.) O. Kuntze (syn. *C. floribunda* Benth., *Dioclea argentea* Desv.) is a drought-tolerant tropical multipurpose shrub legume with high potential for animal nutrition, particularly for dry season supplementation and silage, due to its high nutritive value (Argel and Lascano, 1998). The leafy shrub usually reaches a height of 1.5 to 3 m and has the potential to remain green and productive during dry seasons up to seven months. It is well adapted to acid soils of low to medium fertility and to elevations up to 1200 m.a.s.l.

Cratylia argentea contains only traces of tannins and its nutritive value is higher than that of most other shrub legumes adapted to acid soils (Schultze-Kraft, 1996). The species shows excellent regrowth after cutting and can be used as forage, soil cover, mulch and green manure (Argel and Lascano, 1998; Lascano et al. 2002). It is a valuable protein source in livestock production systems, particularly during the dry season, both in cut-and-carry (fresh fodder and silage) and in grazing systems (Argel and Lascano, 1998; Lascano et al. 2002). *C. argentea* can be easily propagated by seed, whereas vegetative propagation was unsuccessful (Pizarro et al. 1996).

The genus *Cratylia* is native to South America where it occurs south of the Amazon river, from western Peru to the state of Ceará in Brazil. The taxonomic delimitations within the genus are not yet defined. According to Queiroz and Coradin (1996) there are five species distinguished based on morphological characteristics and geographical distribution. Four of them, *C. mollis* Mart. ex Benth., *C. hypargyraea* Mart. ex Benth., *C. bahiensis* L.P. Queiroz and *C. intermedia* (Hassl.) L.P. Queiroz and R. Monteiro, are restricted to Brazil. The distribution range of *C. argentea* extends from western Peru to Bolivia and Brazil. The species occurs in a broad range of biogeographical regions such as the Cerrado, Caatinga, Yungas and Amazon provinces.

Collections of *C. argentea* are held in Brazil by Embrapa Recursos Genéticos e Biotecnologia, Brasília and Embrapa Cerrados, Planaltina (45 and 48 accessions, respectively), and by the International Center for Tropical Agriculture (CIAT), Cali, Colombia (57 accessions). However, a reconciled total collection probably comprises not more than 50 different accessions. The germplasm was mostly collected during general wild-legume collecting missions, *i.e.*, where the target was a wide range of species. Depending on topography and soil variability, not only the distance between general legume sampling sites in these missions was variable but, subsequently, also the distance between individual *C. argentea* accessions. The accessions were collected in a diversity of environments and broadly represent the natural distribution of the species.

Agronomic and morphological evaluations across a range of sites in tropical America concentrated on a collection of 11 accessions (Schultze-Kraft, 1996) and as a result, acommercial variety consisting of a mixture of accessions CIAT 18516 and CIAT 18668 was released in Costa Rica as cv. (cultivated variety) Veraniega and in Colombia as cv. Veranera (Lascano et al. 2002). Recently, the agronomic and morphological diversity in an expanded collection of 38 accessions was described by Andersson et al. (2006a).

The aim of the present study was to complement the latter variability assessment by a molecular marker approach. The specific objectives were to assess and describe the genetic diversity (a) within a collection of 47 accessions, and (b) within accessions, and (c) identify possible duplicates and taxonomic mismatches. When choosing a molecular marker technique, one should not only consider the particular objective(s) of the study, but also the technical requirements and the cost and time investments implied. In the present case, random amplified polymorphic DNA (RAPD) markers were chosen since they are an effective and relatively inexpensive technique and do not require any prior sequence information (Jones et al. 1997; Chenuil, 2006). These dominant neutral markers sample the genome randomly generating multiple numbers of amplifiable polymorphic fragments, and can be applied to a wide range of plant and animal taxa. They are especially useful for distinguishing individuals, cultivars or accessions, and allow the quantification of similarities or differences (Jones et al. 1997; Parker et al. 1998).

MATERIALS AND METHODS

Plant material and DNA extraction

The 47 *Cratylia argentea* accessions used in this study were collected mainly in the Brazilian states of Mato Grosso and Goiás in three collection missions in 1984, 1993 and 1995 (Figure 1, Table 1). Accessions are bulk samples of three plants per accession, representing populations at their respective collection sites. Two accessions (CIAT 18672 and CIAT 18957) were collected at the northern limit of the natural distribution range of the

species in the Brazilian states of Pará and Tocantins, south of the Amazon river (03º 45' S and 06º 30' S, respectively). The only non-erect but prostrate and strongly climbing accession (CIAT 22397 'Yapacaní') was collected in Santa Cruz, Bolivia. *Cratylia mollis* CIAT 7940 was included as additional reference to validate the genetic assessment of *C. argentea*, expecting the genetic similarity among *C. argentea* accessions to be greater than between accessions of *C. argentea* and *C. mollis*, another drought tolerant legume shrub native to the dry Caatinga of north-eastern Brazil (Queiroz and Coradin, 1996).

At CIAT, the entire *C. argentea* collection is replicated every 7 to 10 years. Accordingly, the plant material used in this study has undergone one (accessions collected between 1993 and 1998) or two (accessions collected in 1984) multiplication cycles, respectively (A. Ciprián, CIAT GRU, personal communication 2006). Seedlings were raised in the greenhouse and transplanted to the field at the CIAT-Quilichao Experimental Station (Cauca department, Colombia; 03º 06' N, 76º 31' W; 990 m.a.s.l). Young leaves were harvested in the field just before reaching fully developed size. For the determination of genetic variability among accessions, bulked samples were taken from three individual plants per accession. For the assessment of within-accession genetic variability, leaves were harvested separately from ten individual plants of each of five accessions (CIAT 18516, CIAT 18668, CIAT 18674, CIAT 22408 and CIAT 22409). Samples were macerated in liquid nitrogen and total genomic DNA extracted from 50 mg tissue using a small-scale DNA extraction method (Qiagen DNeasy® Plant Mini Extraction Kit, Qiagen Inc., Valencia, CA, USA) with minor modifications: 600 instead of 400 µl Buffer AP1, 2 instead of 4 µl RNAse, and 150 instead of 130 µl Buffer AP2. DNA was quantified by means of a DyNA Quant^{TM} 200 fluorometer (Hoefer Scientific Instruments, San Francisco, CA, USA) and diluted to a final concentration of 5 ng DNA/µl.

RAPD markers

The protocol for RAPD analysis was as described in Andersson et al. (2006b), with the volume of the final reaction (25 µl) being composed of 1X PCR buffer (50 m*M* KCl, 10 m*M* Tris-HCl pH 8.8, 0.1% Triton x-100), 2.5 m*M* MgCl2, 0.2 m*M* of each dNTPs, 0.2 µ*M* primer (Series OPD, OPG, OPI and OPJ from Operon Technologies, Alameda, CA, USA), 1 U Taq DNA polymerase (Promega Corp., Madison, WI, USA) and 25 ng of template DNA. A negative control without template DNA was included in each round of reactions. Amplifications were performed in a thermocycler (PTC-100^{TM}, MJ Research Inc., Watertown, MA, USA) with an initial denaturing step of 5 min at 94ºC followed by 40 cycles of 30 sec at 94ºC, 30 sec at 38ºC and 1 min at 72ºC and a final extension step of 5 min at 72ºC. The PCR products were run on a 1.4% agarose gel at 20 V/cm during 45 min. The amplified DNA fragments were visualised by ethidium bromide staining (0.5 µg/ml bromide in gel and buffer) under UV-light, and

photographed with a Kodak digital camera DC 120 (Software Kodak Digital Science^{TM} 1997).

Data analysis

Amplified DNA fragments were manually scored as present (1) or absent (0) for each primer, and variations in band presence were recorded as polymorphisms.

Data were converted into a similarity matrix using the Nei and Li similarity coefficient based on the proportion of shared fragments. The Nei and Li similarity coefficient (Nei and Li, 1979) calculates the genetic similarity (*GS*) between two samples, *i* and *j*, with the formula $GS(i,j) = 2Nij / (2Nij + Ni + Nj)$, where *Nij* is the number of bands present in both *i* and *j*, *Ni* is the number of bands present in *i* and absent in *j*, and *Nj* is the number of bands present in *j* and absent in *i*.

Then, a similarity tree was produced by clustering the similarity matrix based on the Average Linkage UPGMA (unweighted pair group method with arithmetic averages) algorithm. The dendrogram was cut considering a cophenetic value of 0.97 (R^2), which resulted in 7 groups and a level of 77% of genetic similarity. The calculation of genetic similarity and cluster analysis were performed using the NTSYS package version 2.1. In addition, multiple correspondence analysis (MCA) was performed on the original matrix to provide an additional representation of genetic similarity and to visualize the dispersion of individuals in relation to their molecular profile, based on the first three principal axes of variation. Means of genetic similarity and genetic diversity within and among groups were determined for both methods (cluster analysis and MCA) to analyse the coherence of the resulting classifications. MCA and means of genetic diversity were calculated using SAS version 8.2.

The efficiency of the RAPD primers to differentiate among accessions was evaluated by calculating the discriminatory power $D_L = 1 - \sum p_i^2$, with p_i being the proportion of accessions carrying the *i*th banding pattern at the *j*th primer and calculating p*i* for each pattern generated by the primer (Tessier et al. 1999). D_L is an extension of the polymorphism information content (PIC) and provides an estimate of the probability that two randomly chosen individuals show different banding patterns for the same primer, and thus are distinguishable from one another.

RESULTS

RAPD profiles

Out of the 47 oligonucleotide decamer primers initially screened with three *C. argentea* accessions, 17 gave smeared or faint bands and seven gave no amplification products. Fifteen primers identified high levels of polymorphism and were repeated to test for reproducibility. Out of these, six primers detected distinct, clearly resolved

and consistently reproducible amplification products and were, therefore, selected for the amplification of RAPD sequences. They generated a total of 72 scorable fragments across the 47 *C. argentea* accessions and the *C. mollis* reference accession (Table 2). On average 11 scorable fragments were obtained per primer, ranging from six (primer OPJ 12) to 16 (primers OPG 12 and OPI 07). The band size ranged from 320 to 2900 bp. A RAPD profile generated by primer OPD 15 is shown in Figure 2.

Out of the 72 fragments detected across all analysed accessions, 68 (94%) were polymorphic. Among *C. argentea* accessions, 61 fragments were detected, 56 (92%) being polymorphic. Eleven markers generated by primers OPD 15, OPG 12, OPI 07 and OPJ 12 were unique to *C. mollis*, and five markers (all generated by primer OPI 07) were unique to *C. argentea* accessions. Of these, three were unique to the only non-erect *C. argentea* accession CIAT 22397 ('Yapacaní'), and the other two were found in CIAT 22399 and CIAT 22403, respectively. In addition, three markers (generated by primers OPJ 06 and OPJ 12) were common to *C. mollis* and 'Yapacaní', but were absent in the other *C. argentea* accessions. Although the two accessions CIAT 18672 and CIAT 18957 were collected at the northern limit of the natural distribution range of the species and were geographically as distant from the rest of the collection as was the Bolivian accession 'Yapacaní' (Figure 1), no fragments unique to these accessions were detected.

The discriminatory power (D_L) of the selected primers ranged from 0.615 to 0.942, with four primers having > 85% probability of discriminating between two accessions (Table 2). As reported by Tessier et al. (1999) our data showed that the discriminatory power of a primer does not solely depend on the number of bands and banding patterns it generates. For example, primer OPD 15, which generated nine polymorphic bands and 18 different banding patterns, had a discriminatory power of 90%, whereas primer OPI 07 with more polymorphic bands (16) and a similar number of banding patterns (16) had a much lower discriminatory power (75%).

Cluster and multiple correspondence analyses

The dendrogram based on Nei and Li genetic similarity, provides seven groups at the 77% level of genetic similarity (Figure 3). First, the *C. mollis* reference accession is separated, followed by the only non-erect accession 'Yapacaní' (CIAT 22397). Subsequently, two groups each containing only one accession are split: CIAT 18668 and CIAT 22389. Next, a 4-member group comprising accessions CIAT 18674, CIAT 22384, CIAT 22386 and CIAT 22401, and a single accession (CIAT 22408) are separated. The remaining 39 accessions (81%) fall into one main cluster (Figure 3).

MCA confirms the separation of the *C. mollis* reference and the non-erect *C. argentea* accession 'Yapacaní' from the remaining accessions (first and second dimension, accounting for 27% and 14% of the total variation, respectively). The multiple correspondence coordinates of each of the three groups (*C. mollis*, 'Yapacaní' and the remaining *C. argentea* accessions) form the extreme points of an almost equally-sided triangle, indicating that the non-erect *C. argentea* accession 'Yapacaní' is genetically almost as distant from all other *C. argentea* accessions as is *C. mollis*. The third dimension discriminates the remaining 46 *C. argentea* accessions into two main and three smaller groups (Figure 4). Accession CIAT 18668 is grouped together with CIAT 22389 and CIAT 22403 (group 5 in Figure 4), and CIAT 22386 with CIAT 22387 (group 4). Accession CIAT 18674 makes up a group of its own (group 3). The remaining 40 accessions (83%) fall into two main clusters, containing 28 and 12 accessions, respectively.

Geographic origin (*i.e.* distance) had only limited influence on the composition of the groups. In several instances, genetically similar accessions were detected from geographically very distant areas (*e.g.* CIAT 22373 and CIAT 22409), whereas geographically close accessions often appeared to be genetically very distant in both cluster analysis and MCA (*e.g.* CIAT 18675 and CIAT 22408). Here again, it was not possible to distinguish the two accessions CIAT 18672 and CIAT 18957, which were the most distant from the rest of the collection in terms of geographic distance (Figure 1). Both accessions clustered tightly together with other accessions distributed widely throughout Central Brazil, in cluster as well as in MCA.

Genetic diversity and genetic similarity among and within groups

Total genetic diversity in the sample studied here was low (H_T = 0.169), with only 30% of differentiation among groups (G_{ST} = 0.302) (Table 3). The mean genetic similarity was high (*GS* = 0.776) and ranged from 0.388 to 0.814 among groups (Table 4) and from 0.316 to 0.947 among accessions (data not shown). When excluding the two most genetically distant accessions, 'Yapacaní' and *C. mollis* (groups 6 and 7, respectively), total diversity decreased to H_T = 0.145, and the coefficient of gene differentiation was reduced by half to 15.2% (Table 3). Mean *GS* increased to 0.805, ranging from 0.720 to 0.814 among groups (Table 4) and from 0.604 to 0.947 among accessions (data not shown). The genetic similarity between groups (Table 4, above diagonal) was as high as or even higher than within groups (Table 4, diagonal).

Genetic diversity and genetic similarity within accessions

To compare mean genetic diversity within and among *C. argentea* accessions, ten individual plants of each of five accessions were chosen. Total genetic diversity H_T among these accessions was 0.180, while mean genetic diversity within accessions (H_S) was 0.152, representing 84% of total genetic variation. Mean genetic similarity *GS* was 0.720,

ranging from 0.730 to 0.800 within the five accessions studied (Table 5).

Possible duplicates in the collection

If considering accessions as genetically identical when their genetic similarity *GS* was equal to or greater than 0.95, then all accessions were uniquely identified, indicating that the collection does not contain genetic duplicates. However, a group of possible duplicate candidates was identified (CIAT 22373, CIAT 22378, CIAT 22380, CIAT 22381 and CIAT 22411) with pairwise genetic similarities very close to this value (*GS* > 0.94).

DISCUSSION AND CONCLUDING REMARKS

In this first study of genetic diversity in *Cratylia* with RAPD molecular technique, the level of DNA polymorphism detected with six decamer random primers was very high (> 90%) and allowed the distinction of all accessions analysed. The high discriminatory power of the primers used (83% on average) indicates that the RAPD technique provides an effective tool for germplasm analysis in *Cratylia*.

Genetic structure within the collection

Overall genetic diversity in the collection was low, with high genetic similarity among accessions. In other words, the frequencies/abundances of polymorphic RAPD fragments in the analysed collection were relatively low, and the distribution pattern of these few polymorphic fragments was very similar for the majority of the accessions, leading to high genetic similarity values. Cluster and multiple correspondence analyses showed the clear separation of *C. mollis* and the non-erect accession 'Yapacaní'. The grouping of the remaining 46 *C. argentea* accessions differed slightly when based on UPGMA clustering or MCA, indicating that there was no clear pattern within this bulk of accessions. The low genetic differentiation and high genetic similarity among groups show that the low genetic diversity is fairly homogeneously distributed in the collection, without any particular pattern. Also, geographic origin (*i.e.* distance) apparently did not affect the composition of these groups, since in several instances genetically similar accessions were detected from geographically very distant areas, whereas geographically close accessions often appeared to be genetically very distant in both cluster analysis and MCA. In addition, the genetic variability within accessions was high, with genetic similarities within accessions being only slightly greater than mean similarity among accessions. These findings suggest the existence of a single, widely shared genepool with limited genetic separation among accessions, and indicate the likelihood of extensive outcrossing. These results agree with studies of tropical tree population structures. Tropical tree species appear to have large genepools, with high diversity and little genetic structure,

due to extensive outcrossing and geneflow occurring over distances of several kilometres (Schierenbeck et al. 1997).

Mating system

Among various factors that determine the genetic composition of plant populations, mating system is the most influential. High polymorphism levels of the magnitude observed in the present study for *C. argentea* (92%) are reported for outcrossing species, whereas predominantly selfing and/or clonal species generally show much higher proportions (45-80%) of monomorphic loci (Zhivotovsky, 1999). Similarly, the relatively low differentiation among groups and the high genetic diversity within groups, are characteristic for predominantly outcrossing species, whereas for inbreeding species this relationship tends to be inverted (Nybom, 2004).

Generally, tropical legumes have been considered as predominantly self-pollinated, but there is increasing evidence of cross-pollination in many species, suggesting that some outcrossing may occur in most legume species (Maass and Torres, 1998). Very little is known about the genetics of *C. argentea* and it is unclear whether *C. argentea* is allogamous or autogamous. While Queiroz et al. (1997) reported from Planaltina, Brazil, the occurrence of outcrossing and suggested a mixed mating system, Bystricky et al. (2006) found *C. argentea* to be self-incompatible under the conditions of CIAT-Quilichao. Our findings provide further evidence for this. Furthermore, field observations suggest that tripping is involved in fertilization (Andersson et al. 2006a). Pollinators such as the exotic honeybee *Apis mellifera*, and other bees and bumblebees belonging to the genera *Bombus*, *Centris* and *Xylocopa* (Hymenoptera: Apoidea) have been observed to visit *C. argentea* flowers loaded with pollen (Queiroz, 1996). Some of these pollinators have been reported to be capable of flight distances of several kilometers thus effecting pollen dispersal between plants at great distances, e.g. the honeybee *A. mellifera* (Ricketts, 2004) and the carpenter bee *Xylocopa fimbriata* Fabr. (Gonzalez and Engel, 2004). At CIAT, *C. argentea* accessions are multiplied in plots that are at least 300 m distant and hence, isolated from each other to a certain extent (A. Ciprián, CIAT GRU, personal communication 2006). The accessions studied here have not undergone more than two multiplication cycles since seed increase has started. It thus appears that cross-pollination may have occurred also among and within original populations in the wild.

However, we suggest precautions to be taken by genebanks during the regeneration of *C. argentea* accessions to avoid cross-pollination, and the re-consideration of multiplication protocols in order to maintain the genetic integrity of accessions. More detailed studies (*e.g.* with co-dominant molecular markers) are required to identify the prevailing reproductive strategy of *C. argentea* and to assess the rate and impact of outcrossing in this species.

Possible duplicates and taxonomic mismatches in the collection

Although genetically all accessions were significantly different ($P < 0.05$) from each other, a group of five possible duplicate accessions (CIAT 22373, CIAT 22378, CIAT 22380, CIAT 22381, CIAT 22411) was identified, which shared more than 94% of their banding pattern, differing in three bands only. Re-analysing these accessions with an additional primer or with additional marker types such as AFLP (amplified fragment length polymorphisms) would likely lead to a clearer molecular differentiation between pairs.

Ordination was particularly useful in visualising that the non-erect accession 'Yapacaní', the only germplasm from Bolivia, was genetically almost as distinct from all other *C. argentea* accessions as was *C. mollis*. This, together with its unusual growth habit (climbing vine), suggests that there may be a misclassification of the 'Yapacaní' accession - in spite of the fact that according to the botanical literature (Queiroz and Coradin, 1996), the only *Cratylia* speciesthat occurs in Bolivia is *C. argentea*. Botanical specimens from Bolivia and Peru should be re-examined for an eventual taxonomic revision of the genus *Cratylia*. Also, further germplasm from longitudes higher than 58º W (Figure 1) should be collected and its genetic diversity analyzed in support of taxonomic re-considerations.

With respect to accessions CIAT 18516 and CIAT 18668 which form the commercial cultivar, it was striking that CIAT 18668 was genetically very different from CIAT 18516 as well as from most other *C. argentea* accessions (Figure 3). In agronomic evaluations, however, these two accessions were not only very similar regarding morphological characteristics and forage quality (Andersson et al. 2006a), but also regarding consistently high DM production in different environments. The fact that in terms of RAPD variation these accessions appear to be very distinct might be an indicator of the neutral (*i.e.* non-functional) nature of the polymorphisms detected by this molecular technique. Furthermore, it is unknown to which extent the agronomic traits above are influenced by the environment. RAPD markers therefore are not an adequate tool for predicting or explaining agronomic characteristics. These contradictions probably could be solved either using morphological characters that are less sensitive to environmental variation, or performing more thorough examinations with more sophisticated techniques (*e.g.* ISSR-PCR).

Acknowledgments

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APPENDIX
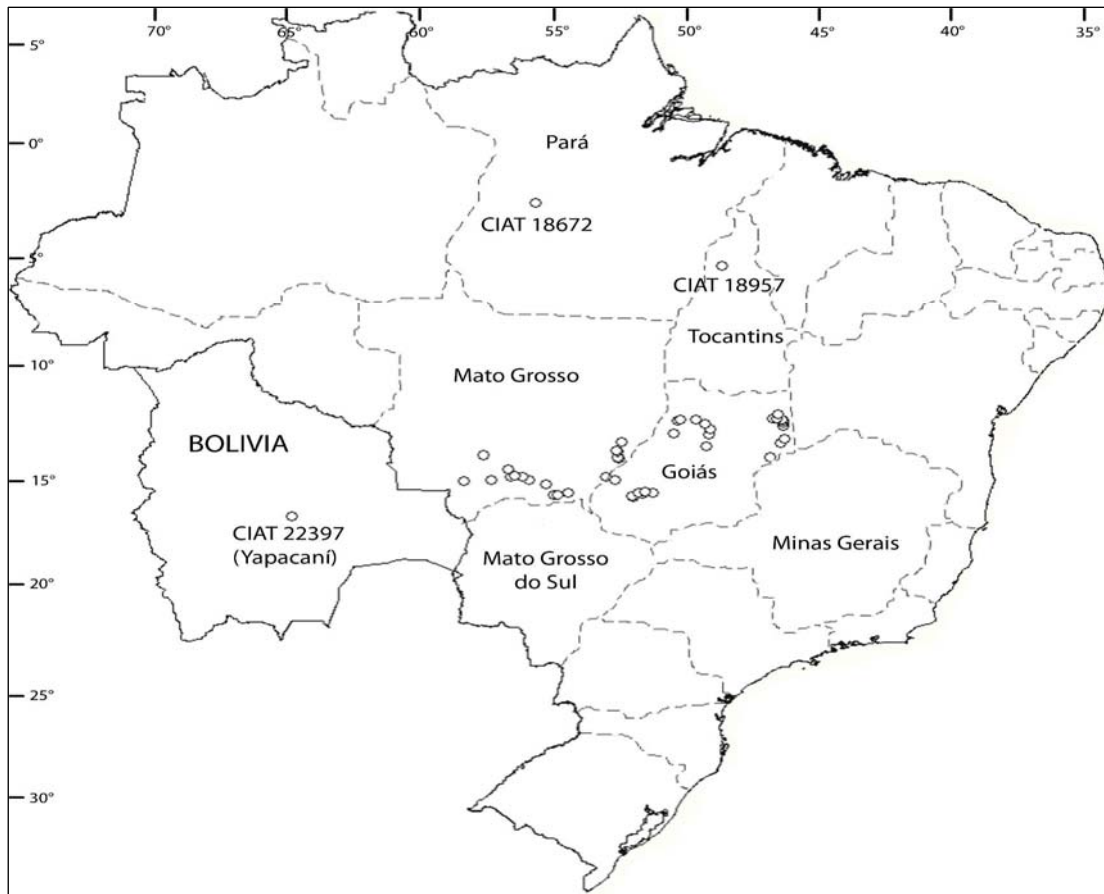
Figure 1. Geographical distribution of the 47 *Cratylia argentea* accessions used in this study. The accessions were originally collected in the states Mato Grosso, Goiás, Tocantins and Pará in Brazil, and in Bolivia.
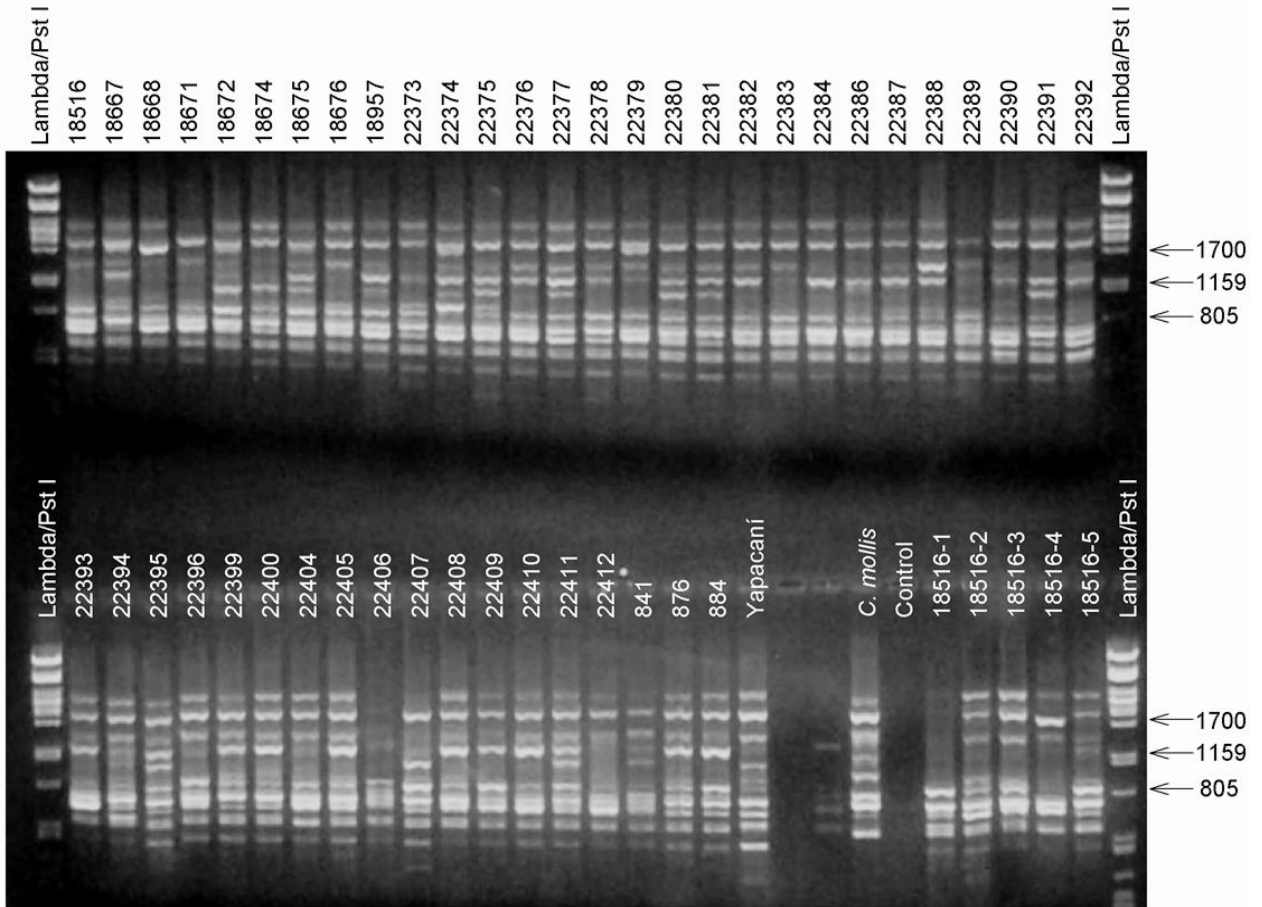
Figure 2. RAPD profile obtained using primer OPD15 for 47 *Cratylia argentea* accessions, *C. mollis*, one DNA-free control, and five samples of different individuals of accession CIAT 18516 for the assessment of within-accession variability. Size markers (λ-DNA/PstI, Invitrogen, USA) for assessing base pair lengths are shown in the first and last lanes.
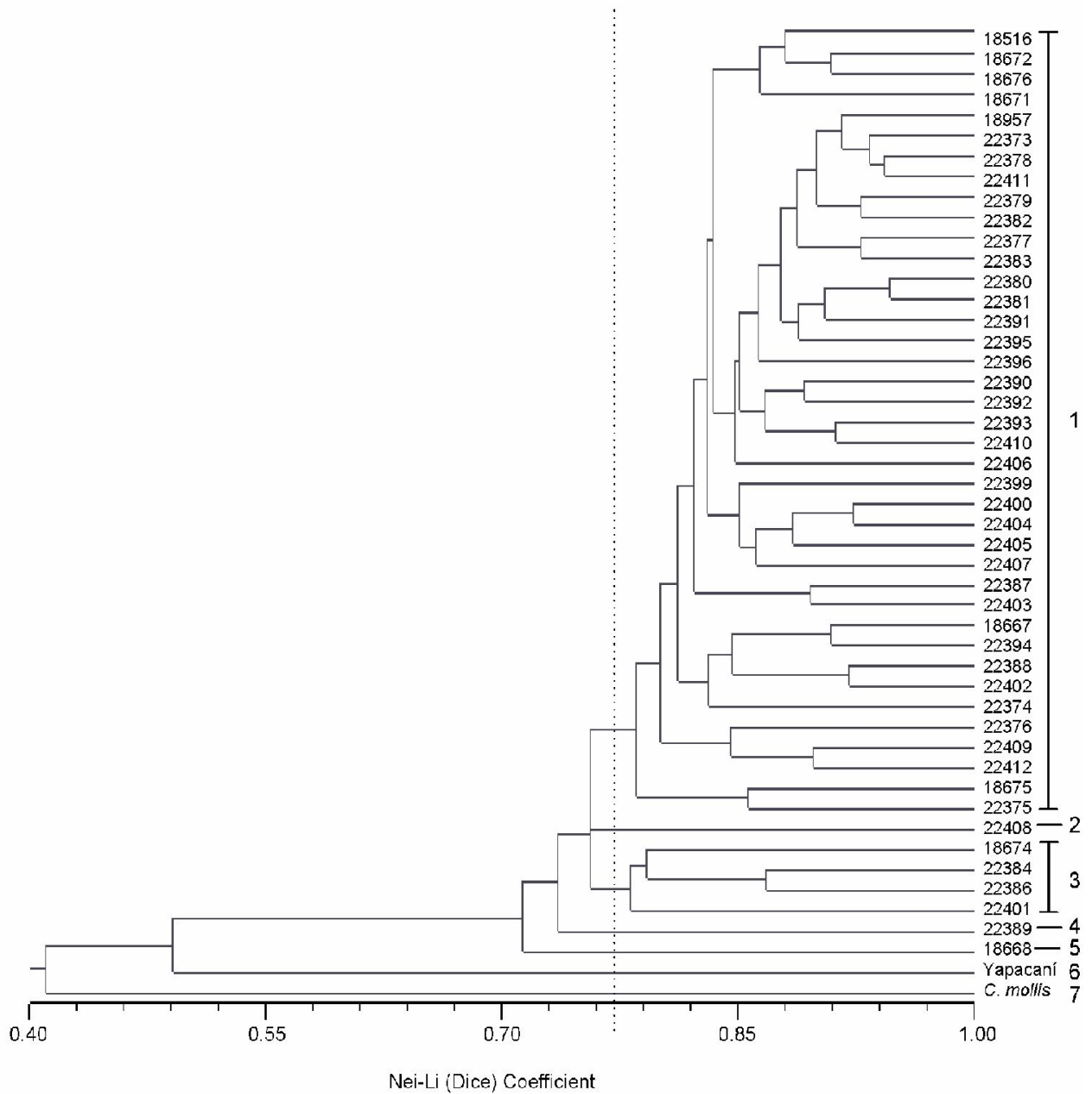
Figure 3. Grouping of 47 *Cratylia argentea* accessions and a *C. mollis* reference accession, using UPGMA. Genetic distances are according to Nei and Li based on RAPD markers. Clusters were analysed at the 0.77 similarity level (dotted line).

Figure 4. Three-dimensional representation of five groups derived from MCA of RAPDs among 46 *Cratylia argentea* accessions (without 'Yapacaní').

Table 1. Origin of the *Cratylia argentea* accessions used in the present study (Queiroz and Coradin, 1996; CIAT Tropical Forages Database and G.P. da Silva, Embrapa Recursos Genéticos e Biotecnologia [personal communication]).
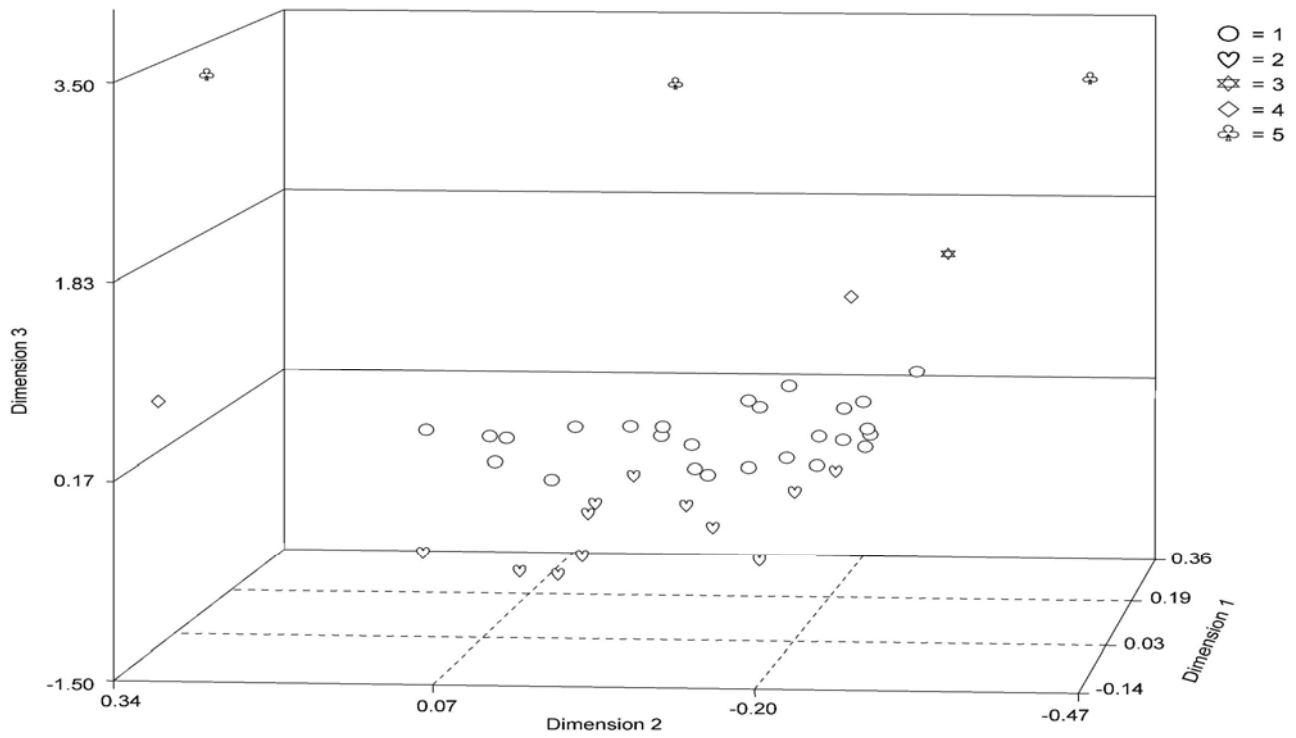
CIAT No.	EMBRAPA No.	State	Latitude	Longitude	Altitude (masl)	Year of collection
18516	000167	Goiás	13º 22' S	46º 25' W	800	pre-1980
18667	000027	Mato Grosso	15º 43' S	55º 43' W	455	1984
18668	000035	Mato Grosso	15º 22' S	56º 13' W	175	1984
18671	000060	Mato Grosso	14º 46' S	57º 05' W	230	1984
18672	000086	Pará	03º 45' S	55º 14' W	140	1984
18674	000116	Mato Grosso	14º 38' S	52º 22' W	320	1984
18675	000124	Mato Grosso	14º 54' S	52º 17' W	380	1984
18676	000132	Goiás	16º 21' S	51º 20' W	450	1984
18957	000175	Tocantins	06º 30' S	48º 37' W	350	pre-1980
22373	000591	Goiás	14º 05' S	46º 23' W	780	1995
22374	000612	Goiás	13º 16' S	46º 25' W	660	1995
22375	000639	Goiás	13º 01' S	46º 36' W	620	1995
22376	000655	Goiás	14º 23' S	49º 09' W	550	1995
22377	000663	Goiás	13º 54' S	49º 03' W	510	1995
22378	000671	Goiás	13º 37' S	49º 02' W	390	1995
22379	000680	Goiás	13º 21' S	47º 07' W	380	1995
22380	000698	Goiás	13º 14' S	49º 28' W	340	1995
22381	000701	Goiás	13º 14' S	50º 40' W	360	1995
22382	000710	Goiás	13º 17' S	50º 12' W	330	1995
22383	000728	Goiás	13º 51' S	50º 20' W	300	1998
22384	000736	Mato Grosso	14º 14' S	52º 10' W	360	1995
22386	000752	Mato Grosso	14º 34' S	52º 21' W	320	1995
22387	000761	Mato Grosso	15º 50' S	52º 25' W	370	1995
22388	000787	Mato Grosso	16º 23' S	54º 01' W	330	1995
22389	000795	Mato Grosso	16º 26' S	54º 19' W	400	1995
22390	000809	Mato Grosso	16º 01' S	54º 55' W	300	1995
22391	000817	Mato Grosso	15º 49' S	55º 30' W	400	1995
22392	000825	Mato Grosso	15º 58' S	55º 00' W	240	1995
22393	000833	Mato Grosso	15º 42' S	56º 42' W	210	1995
22394	000868	Mato Grosso	15º 52' S	57º 49' W	200	1995
22395	000892	Goiás	16º 25' S	51º 35' W	460	1995
22396	000906	Goiás	16º 32' S	51º 03' W	510	1995
22397	'Yapacaní'	Bolivia, Santa Cruz	17º 25' S	63º 56' W	350	1998
22399	000604	Goiás	14º 30' S	46º 24' W	650	1995
22400	000621	Goiás	13º 10' S	46º 40' W	660	1995
22401	000841	Mato Grosso	15º 51' S	56º 49' W	360	1995
22402	000876	Mato Grosso	16º 30' S	54º 36' W	270	1995
22403	000884	Mato Grosso	16º 34' S	51º 45' W	600	1995
22404	000191	Goiás	13º 01' S	56º 37' W	650	1993
22405	000213	Goiás	13º 15' S	46º 28' W	700	1993
22406	000221	Goiás	14º 15' S	46º 30' W	780	1993
22407	000514	Goiás	14º 54' S	46º 56' W	500	1995
22408	000540	Mato Grosso	14º 06' S	46º 25' W	810	1995
22409	000566	Goiás	13º 27' S	46º 22' W	540	1995
22410	000574	Goiás	13º 17' S	46º 25' W	660	1995
22411	000647	Goiás	15º 12' S	46º 47' W	550	1995
22412	000779	Mato Grosso	15º 42' S	52º 43' W	400	1995

Table 2. Oligonucleotide primers employed in RAPD analysis of *Cratylia argentea*, their sequence, number of polymorphic (P) and monomorphic (M) bands obtained, percentage of polymorphic bands (% PB), and discriminatory power (D_L).

Primer code	Sequence (5' to 3')	Number of bands				D_L
		(including *C. mollis*)		(only *C. argentea*)		
		P	M	P	M	
OPD 15	CATCCGTGCT	9	1	7	1	0.903
OPG 12	CAGCTCACGA	16	1	11	1	0.942
OPI 07	CAGCGACAAG	16	1	12	2	0.755
OPJ 06	TCGTTCCGCA	13	0	13	0	0.898
OPJ 07	CCTCTCGACA	8	0	8	0	0.862
OPJ 12	GTCCCGTGGT	6	1	5	1	0.615
Sum		68	4	56	5	
Total		72		61		
PB (%)		94.4		91.8		

Table 3. Nei estimates of genetic diversity (heterogeneity) among groups of *Cratylia argentea*.

Group	n	$H_{i(1-7)}$	$H_{i(1-5)}$
1	28	0.128	0.128
2	12	0.126	0.126
3	1	0.0	0.0
4	2	0.101	0.101
5	3	0.120	0.120
6 ('Yapacaní')	1	0.0	-
7 (*C. mollis*)	1	0.0	-
HS		0.118	0.123
HT		0.169	0.145
GST		0.302	0.152

n: number of accessions.
H_i: genetic diversity within each group.
H_S: average genetic diversity within groups.
H_T: total genetic diversity.
G_{ST}: coefficient of genetic differentiation (proportion of total genetic diversity found among groups).

Table 4. Average genetic similarity (*GS*) values between (above diagonal) and within (diagonal) groups of *Cratylia argentea* germplasm, the non-erect accession 'Yapacaní' and the *C. mollis* reference accession, based on all pairwise similarities between accessions according to molecular marker information (RAPDs).

Group	*n*	1	2	3	4	5	Total	'Yapacaní'	*C. mollis*
1	28	0.825	0.814	0.769	0.774	0.759		0.487	0.413
2	12		0.839	0.720	0.764	0.721		0.515	0.404
3	1			1.000	0.757	0.754		0.426	0.433
4	2				0.717	0.748		0.479	0.400
5	3					0.757		0.457	0.388
C. argentea	46						0.805		
'Yapacaní'	1							1.000	0.444
C. mollis	1								1.000
Total	48								0.776

n: number of accessions.

Table 5. Genetic similarity among (above diagonal) and within (diagonal) five *Cratylia argentea* accessions, based on all pairwise similarities between individuals according to molecular marker information (RAPDs).

CIAT No.	*n*	18516	18668	18674	22408	22409	Total
18516	10	0.730	0.704	0.724	0.669	0.735	
18668	10		0.763	0.727	0.667	0.719	
18674	10			0.782	0.692	0.753	
22408	10				0.737	0.710	
22409	10					0.800	
Total	*50*						0.720

n: number of individuals per accession.