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Phylogenetic relationships among genera *Eucalyptus* and *Corymbia* species based on rnDNA internal transcribed spacers sequences

Relações filogenéticas entre espécies dos gêneros Eucalyptus e Corymbia inferidas com base na análise das seqüências dos espaçadores internos não transcritos (ITS) do DNA ribossômico nuclear (rnDNA)

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RESUMO: Relações filogenéticas entre espécies introduzidas no Brasil, pertencentes ao gênero *Eucalyptus*, subgênero *Symphyomyrtus* (secções *Adnataria, Exsertaria*, *Maidenaria* e *Transversaria*) e espécies do gênero *Corymbia* (secções *Politaria* e *Ocharia*) foram inferidas por meio da análise das seqüências do gene 5.8S do DNA ribossômico nuclear e das regiões ITS1 e ITS2 que o flanqueiam. As seqüências das espécies *E. globulus* ssp*. globulus* e *A. bakeri* (gênero *Angophora*) foram extraídas do banco de dados "Genbank" e incluídas na análise. A espécie *Psidium guajava* foi utilizada como "outgroup". O programa computacional Clustal X foi utilizado para o alinhamento das seqüências e a árvore filogenética foi obtida a partir do Método de Agrupamento do vizinho mais próximo (Saitou and Nei, 1987). A seqüência região 5.8S mostrou ser bastante conservada em todas as espécies investigadas, embora algumas variações tenham sido detectadas nas seqüências desse gene, principalmente quando foram comparadas às seqüências das espécies dos gêneros *Angophora, Corymbia* e *Eucalyptus*. As regiões ITS1 e ITS2 apresentaram variações em suas seqüências, para todas as espécies investigadas. A análise da árvore filogenética permitiu constatar nítida separação entre os gêneros *Corymbia* e *Eucalyptus*. A espécie *A. bakeri*, agrupou-se com as espécies do gênero *Corymbia.* Dentro do gênero *Symphyomyrtus*, gênero *Eucalyptus*, as espécies da secção *Maidenaria* formaram um agrupamento que incluiu a espécie *E. globulus* ssp *globulus*, cuja seqüência foi extraída do banco de dados e incluída na análise. As espécies das secções *Adnataria, Exsertaria* e *Transversaria* não formaram grupos distintos, característicos de cada secção. As divergências observadas entre os dados morfológicos (classificação de Pryor and Johnson, 1971) e os moleculares obtidos no presente estudo são, provavelmente, devido aos sucessivos cruzamentos interespecíficos e às possíveis introgressões que ocorreram no material em estudo.

PALAVRAS-CHAVE: Corymbia, *Eucalyptus*, Filogenia molecular, ITS, rnDNA

ABSTRACT: Phylogenetic relationships between *Eucalyptus* species, subgenus *Symphyomyrtus* (sections *Adnataria*, *Exsertaria*, *Maidenaria*, and *Transversaria*), and *Corymbia* species (sections *Politaria* and *Ocharia*) were established based on the sequence of internal transcribed rDNA spacers (ITS1 and ITS2). The species analyzed were obtained from a collection kept in Brazil. Fragments obtained using primers ITS1 and ITS2 were sequenced and part of the sequence of ITS1 and ITS2 and the complete sequence of 5.8S rDNA were used in the analysis. ITSs and 5.8S rDNA sequences from *E. globulus* ssp. *globulus* and *A. bakeri* (Genus *Angophora*) were downloaded from the Genbank database and included in the analysis. *Psidium guajava* was the selected outgroup used. The sequence alignment and a Neighbor-joining tree were obtained using Clustal X. Few variations were detected in the 5.8S rDNA sequences obtained, occurring mainly between *Eucalyptus* and *Corymbia*, thus defining these genera. Variations in ITS sequences occurred in all investigated species. Phylogenetic analysis showed a clear separation between the genera *Corymbia* and *Eucalyptus*. *A. bakeri* was more closely related to species belonging to genus *Corymbia*. Regarding the subgenus *Symphyomyrtus* (Genus *Eucalyptus*), only species from section *Maidenaria* grouped together according to their common section. This could have been caused by the removal of natural reproductive barriers when these species were introduced in Brazil, with a consequent increase in the rate of interspecific crossings and introgression events.

KEYWORDS: *Corymbia, Eucalyptus*, Molecular Phylogeny, ITS, rnDNA

INTRODUCTION

Eucalyptus species occur naturally in Australia and on the Indonesian islands and comprises about 700 species (Chippendale, 1988). *Eucalyptus* was introduced in Brazil by Edmundo Navarro de Andrade in 1904. Nowadays, about 60% of the reforested areas in Brazil are occupied by *Eucalyptus* species (Santos, 1990).

The generic term Eucalypts includes the genus *Eucalyptus* and the small genus *Angophora* Cav., which is characterized by the presence of flowers with free sepals and petals, as opposed to the general opercular flower condition observed in the genus *Eucalyptus*.

Pryor and Johnson (1971) proposed the division of the genus *Eucalyptus* into seven subgenera: *Blakella*, *Corymbia*, *Eudesmia*, *Gaubaea*, *Idiogenes*, *Monocalyptus*, and *Symphyomyrtus*. Johnson (1976) later proposed an eighth subgenus named *Telocalyptus*. The classification proposed by Pryor and Johnson (1971) was based on morphological and ecological characters, and on the null crossability among the subgenera. Based on morphological and molecular studies, Hill and Johnson (1995) described the genus *Corymbia*, which is comprises the subgenera *Corymbia* and *Blakella*. According to Pryor and Johnson (1981), these subgenera are closely related and both are more closely related to the genus *Angophora* than to the other *Eucalyptus* subgenera.

The taxonomic relationships in each subgenus of Eucalypts are very complex. The main reasons for complexity are the existence of a high number of species in each of them, the notorious existence of clinal variation, a phenomenon in which the species present gradual phenotypic differences according to the place where it is settled, and the natural occurrence of interspecific hybrids, which leads to the appearance of new variants, often well adapted and of difficult identification (Goes, 1985). Nowadays, the introduction of Eucalypts in other countries, including Brazil, has allowed the occurrence of many interspecific crossings of unlikely occurrence in the natural habitat because of the absence of previously existing geographical barriers (Jacobs, 1981).

Eucalypts is very important in Brazil. The main species (*E. urophylla*, *E. grandis*, *E. saligna*, and their interspecific hybrids) are

included in section *Transversaria.* This section was divided by Pryor and Johnson (1971) into series and subseries according to morphological similarities shared by the species. However, interspecific crossings among and inside series and subseries are very common, making this classification quite subjective.

Molecular studies of the 5S rDNA gene are gradually solving phylogenetic relationships inside the genus *Eucalyptus* (Udovicic et al., 1995; Ladiges et al., 1995). However, the gene sequences obtained thus far were not sufficient to infer the phylogenetic relationships between genera with good statistical reliability.

Molecular biology techniques using rDNA regions have been applied with success to the solution of taxonomic problems related to genus and species relationships in several plant species (Bayer et al., 1996). These regions include the ribosomal subunit coding genes (18S, 5.8S and 26S rDNA), whose sequences are highly conserved and repeated in tandem along the eukaryotic genome. Spacer sequences occur between rDNA genes inside and outside the coding units. The two spacers between the ribosomal subunit genes are ITS1 (between 18S and 5.8S rDNA) and ITS2 (between 5.8s and 26S rDNA). These spacers are part of the primary transcript but have no known function and are removed before the final transcripts leave the nucleus. They also diverge a lot in sequence as well as length between different species (Long and David, 1980). ITS sequence analysis produced positive results in phylogenetic studies with good resolution up to the genus and species level (Lanoue et al., 1996; Quijada et al., 1998). Steane et al. (1999) determined and analyzed ITS sequences of 35 *Eucalyptus* and five outgroup species, clearly distinguishing the subgenera as well as the sections evaluated.

The aim of study was to analysis the relationships among introduced *Eucalyptus* species based on the sequences of ITS1, ITS2 and 5.8S rDNA.

MATERIAL AND METHODS

Material

Plants belonging to three *Corymbia* species (sections *Politaria* and *Ocharia*) and fifteen *Eucalyptus* species, subgenus *Symphyomyrtus* (sections *Adnataria*, *Exsertaria*, *Maidenaria* and *Transversaria*), were analyzed. The species *Psidium guajava* was used as an outgroup in this analysis because it is phylogenetic and taxonomically distant from the studied species, and also belongs to the same Family Myrtaceae. The classification of the species analyzed, shown in Table 1 was according to Hill and Johnson (1995).

The plant material was collected at the Experimental Station of Itatinga and Anhembi (São Paulo, Brasil).

DNA extraction

Total DNA was extracted from 150 mg of fresh or frozen leaf tissue using liquid nitrogen and a solution of 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 8.0, 2% CTAB, 1.0% PVC, and 2% mercaptoethanol as extraction buffer, as described by Ferreira and Grattapaglia (1994). Only one individual of each species studied species was used in the present investigation.

PCR

Primers ITS1 and ITS4 were used for amplification of the DNA fragment containing both ITSs and the 5.8S rRNA coding sequences. Primer ITS4 (5 ' TCCTCCGCTTATTGATATGC 3 ') anneals close to the beginning of the 26S rDNA, while primer ITS1 (5 ' TCCGTAGGTGAACCTGCGG 3') anneals at the end of the 18S rDNA, thus encompassing the region of interest during the amplification reaction. The primer sequences were reported by White et al. (1990). The amplification reactions followed the protocol proposed by Yuan et al. (1996). Each reaction comprised: 2.5 μl of each primer (10 mM), 4.0μl of a solution containing

all dNTPs (2.5 μM of each nucleotide), 5.0 μl of 10x reaction buffer (Pharmacia Biotech Inc.), 0.25 μl of *Taq* DNA polymerase (5U/μl - Pharmacia Biotech Inc.), 3.0 μl of total DNA solution (5.0 ng/μl), and 32.75 μl of autoclaved milli-Q water. Two drops of mineral oil were added to each reaction to avoid evaporation. The amplification was performed in PTC 100 (M. J. Research) according to the following cycle: an initial denaturation step of 95°C for 2 minutes, and a 35 cycles of 1 min. at 95° C, 1 min. at 55° C, 1.5 min at 72° C, and a final extension of 10 min. at 72°C. The amplification reaction qualities were verified by electrophoresis on 0.8% agarose gels with 1X TBE buffer (Tris-borate-EDTA) for 30 min. at 80V. The amplified fragments were stained with ethidium bromide, visualized using a UV light source and photographed with a Polaroid 667 camera (black and white film). A molecular length marker of 1 kb (GIBCO-BRL) was used to estimate the size of the amplification products. The reaction products were then purified using the Consert™ Rapid PCR Purification System (GIBCO-BRL) according to manufacturer instructions.

Table 1
Classification and origin (Brazil) of the *Eucalyptus* and *Corymbia* species analyzed (Hill and Johnson, 1995).
(Classificação e local de coleta (no Brasil) das espécies dos gêneros *Eucalyptus* and *Corymbia* estudados, de acordo com Hill and Johnson,1995)

Species	Collection site	Genus	Subgenus	Section
E. paniculata Sm.	E. E. Itatinga[5]	*Eucalyptus*	*Symphyomyrtus*	*Adnataria*
E. camaldulensis Dehn.	*E. E. Itatinga*	*Eucalyptus*	*Symphyomyrtus*	*Exsertaria*
E. tereticornis Sm.	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Exsertaria*
E. globulus Labil	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Maidenaria*
E. benthamii Maiden & Cambage	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Maidenaria*
E. dunnii *Maiden*	E. E. Anhembi[6]	*Eucalyptus*	*Symphyomyrtus*	*Maidenaria*
E. urophylla S.T. Blake	E. E. Anhembi –Ilha de Flores (Indonésia)[2]	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. urophylla S.T. Blake	E. E. Anhembi- ex Timor Português[2]	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. grandis Hill ex Maiden	E. E. Anhembi - Atherton[2] QLD[3]	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. grandis Hill ex Maiden	E. E. Anhembi - Coff's Harbour[2] NSW[4]	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. resinifera Sm.	E. E. Anhembi	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. propinqua Deane & Maiden	E. E. Anhembi	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. pellita F. Muell	E. E. Anhembi	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. saligna Sm.	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. botryoides Sm.	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. robusta Sm	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
E. deanei *Maiden*	E. E. Itatinga	*Eucalyptus*	*Symphyomyrtus*	*Transversaria*
C. maculata (Hook.) [1]	E. E. Itatinga	*Corymbia*		*Politaria*
C. citriodora (Hook.)[1]	E. E. Anhembi	*Corymbia*		*Politaria*
C. torelliana (F. Muell)[1].	E. E. Itatinga	*Corymbia*		*Ocharia*

[1]K.O. Hill & L. A. S. Johnson;
[2] Origin;
[3] Queesland - Australia;
[4] New South Wales - Australia;
[5] E. E. Itatinga - Latitude: 23° 10' S Longitude: 48° 40' W Altitude: 857 m
[6]E. E. Anhembi - Latitude: 22° 47' S Longitude: 48° 09' W Altitude: 500 m

DNA sequencing

The PCR products were sequenced by the method proposed by Sanger et al. (1977). Each sequence reaction contained: 1.0 μl of Big Dye™ Terminator kit, 3.0 μl of 2.5X reaction buffer (200 mM Tris-HCl, pH 9.0, and 5.0 mM $MgCl_2$), 5.0 μl of PCR product (250 ng/μl), and 3.0 μl of primer solution (0.25 mM). The primers used in the sequencing reactions were the same as used in the amplification of the target fragments. The sequencing reactions were processed with an automated PCR machine (PT 100 - M.J. Research) using the following cycles: initial denaturation step of 1 minute at 96°C, 40 cycles of 20s at 96° C, 20s at 52° C, 4 min. at 60° C. Sequencing was performed with an ABI PRISM 377 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems). All samples had both strands sequenced.

Analysis of the sequences

The DNA sequences were determined using the Sequencher™ software, v3.0 (Gene Codes Corporation, Inc. 1995). The exact ITS1, 5.8S rDNA, and ITS2 regions were determined using *Eucalyptus globulus* ssp. *globulus* sequences downloaded from GenBank (identification no. AF058463, Steane et al., 1999). The sequence of the species *Angophora bakeri* was also downloaded from GenBank (identification no. AF058456, Sale et al., 1996). Both downloaded sequences were used in the analysis. The sequences were aligned and edited using the softwares Clustal X (Thompson et al., 1997) and SeqApp (version 1.8a154 for Apple/ Macintosh written by Don Gilbert, Indiana University, and available by anonymous ftp from ftp.bio.indiana.edu/molbio/seqapp.hqx) , respectively. A phylogenetic tree was obtained by Neighbor Joining (Saitou and Ney, 1987). The statistical reliability of each tree branch was determined by the Bootstrap Method (Felsenstein, 1985) using 500 replicates.

RESULTS

The alignment of the sequences of each species analyzed is shown in Figure 1. The length of the entire ITS1, 5.8S rDNA and ITS2 region of *Eucalyptus* was around 634 bp (Steane et al., 1999). In the present study, only part of this region (505 bp) was used in the alignment. The beginning of the ITS1 sequence was not analyzed because of nucleotide ambiguities in this region. This happened because the annealing site of primer ITS1 was too close to the beginning of the ITS1 region and unincorporated marked ddNTPs caused reading interferences. However, this primer was used due to its good performance in the amplification reaction.

The 5.8S rDNA region started at position 119 and ended at position 282 in the alignment (Figure 1). Almost no differences were detected among the sequences of the species studied. According to Steane et al. (1999), the size of the 5.8S rDNA region for *E. globulus* ssp. *globulus* was 161 bp.

Variations in the 5.8S rDNA sequence were detected in *C. citriodora*, *C. torelliana*, *C. maculata*, and *Psidium guajava*. These species had adenine and guanine at positions 147 and 250, respectively, while the remaining species presented an adenine and a gap at these positions. The species *E. robusta* and *E. tereticornis*, both belonging to the subgenus *Symphyomyrtus*, presented an adenine at position 251, as opposed to a thymine observed at this position for the remaining species. At position 256, the species *C. torelliana*, *C. maculata* and *Psidium guajava* presented a cytosine, while the base observed in the other species was thymine. The species *C. citriodora* had a thymine at position 257, as opposed to a cytosine detected in all other species studied (Figure 1).

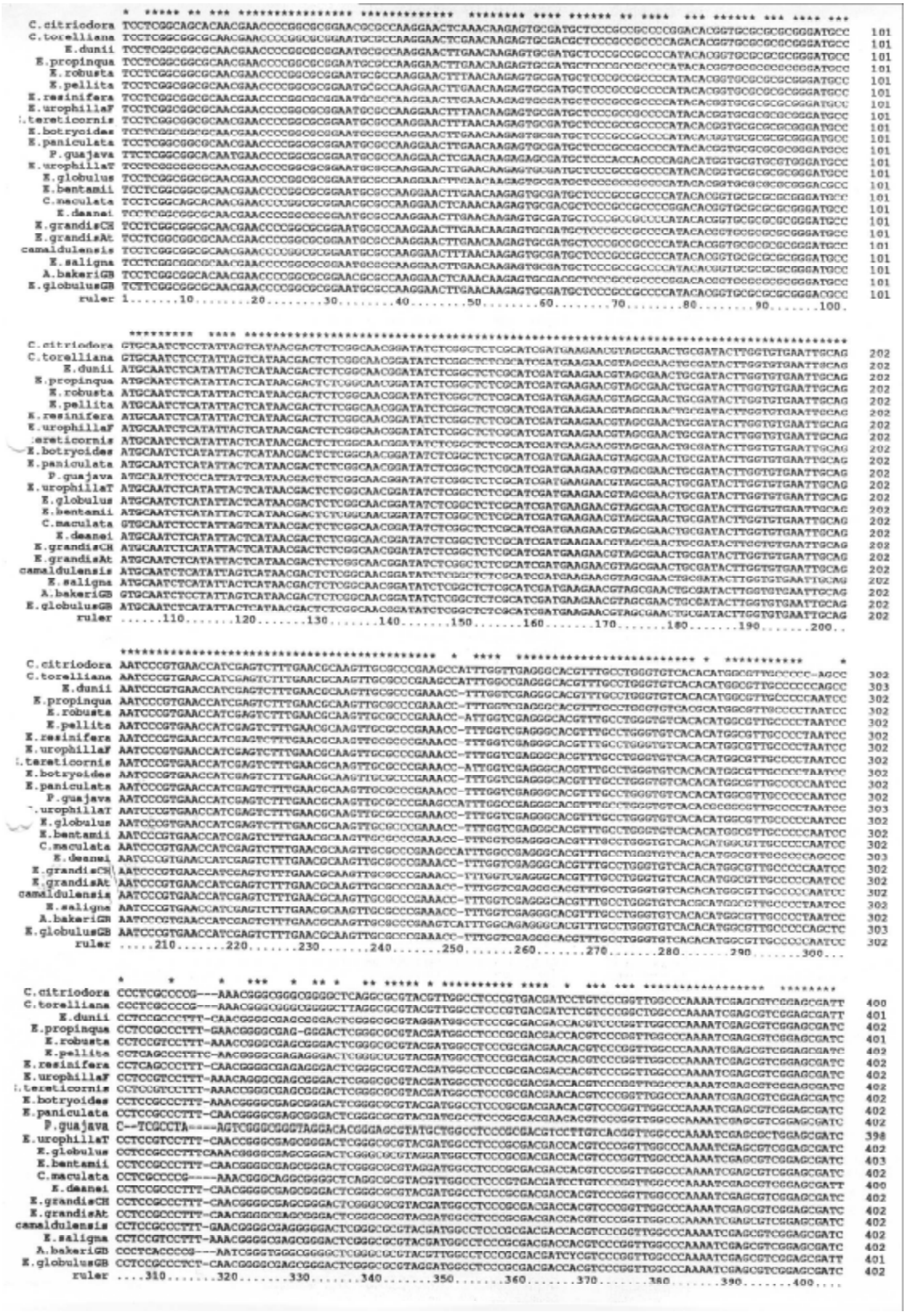

Figure 1
Alignment of the obtained ITSs and 5.8S nrDNA sequences from the *Eucalyptus* and *Corymbia* species studied in this work and outgroup. The alignment was obtained using the sofware Clustal X. Positions where bases are equal for species are marked (*). Number indicate the consecutive positions of 1 to 505 from the beginning of the ITS1 region to the ITS 2 region.
(Seqüências alinhadas correspondentes às regiões ITSs e ao gene 5.8S do nrDNA, das espécies dos gêneros *Eucalyptus* e *Corymbia* analisadas e do "outgroup". O alinhamento das seqüências foi obtido a partir do programa computacional Clustal X. As posições marcadas com (*) indicam similaridade de bases em todas as seqüências apresentadas. Os hífens (-) indicam "lacunas" nas seqüências. A seqüência da região ITS 1 corresponde às posições 1 a 118; a seqüência do gene 5.8S corresponde às posições 119 a 282; e a seqüência da região ITS 2 corresponde às posições 283 a 501. As posições 502 a 505 referem-se ao início da seqüência da região codificadora da subunidade ribossomal 26S)

The phylogenetic tree showing the genetic relationships between the studied species, and *E. globulus* ssp. *globulus* and *A. bakeri* is shown in Figure 2. The percent values given above the tree branches were obtained using the Bootstrap method.

The fifteen species belonging to the genus *Eucalyptus*, subgenus *Symphyomyrtus* were divided into four distinct groups. The first group showed no differences between its sequences and included two species belonging to section *Transversaria*: *E. deanei* and two *E. grandis* varieties from Atherton and Coff's Harbour. The second group was close to the first and included the species *E. paniculata* (section *Adanataria*), *E. botryoides* (section *Transversaria*), *E. globulus*, *E. globulus ssp. globulus* (GenBank access no. AF058463, Steane et al., 1999), *E. bentami*, and *E. dunnii* (Section *Maidenaria*). The third group was formed by the species *E. urophylla* (originally from the Timor Island), *E. saligna*, *E. urophylla* (originally from the Flowers Island), *E. tereticornis*, and *E. robusta*. All species in this group belong to section *Transversaria*, except *E. tereticornis* (section *Exsertaria*). The fourth group included section *Transversaria* species *E. resinífera*, *E. pellita*, and *E. propinqua*, and *E. camaldulensis* from section *Exsertaria*. *E. propinqua* was closer to *E. camaldulensis* than to the two species from its own section.

Another large distinct group was formed by the species belonging to the genera *Corymbia* (*C. torelliana*, *C. maculata*, and *C. citriodora*) and *Angophora* (*A. bakeri*) with a bootstrap value of 100%.

DISCUSSION

The divergence between the genus *Corymbia* and the genus *Eucalyptus* (subgenus *Symphyomyrtus*) was evident. This was mainly due to variations in the 5.8S rDNA sequences. Differences between these two genera have already been detected by ITS sequence analysis (Steane et al., 1999). These authors included in their analysis the genus *Angophora*, which was closely related to the genera *Corymbia* and *Eucalyptus*. The segregation of the genera *Corymbia* and *Eucalyptus* has, however, been opposed by Brooker (2000).

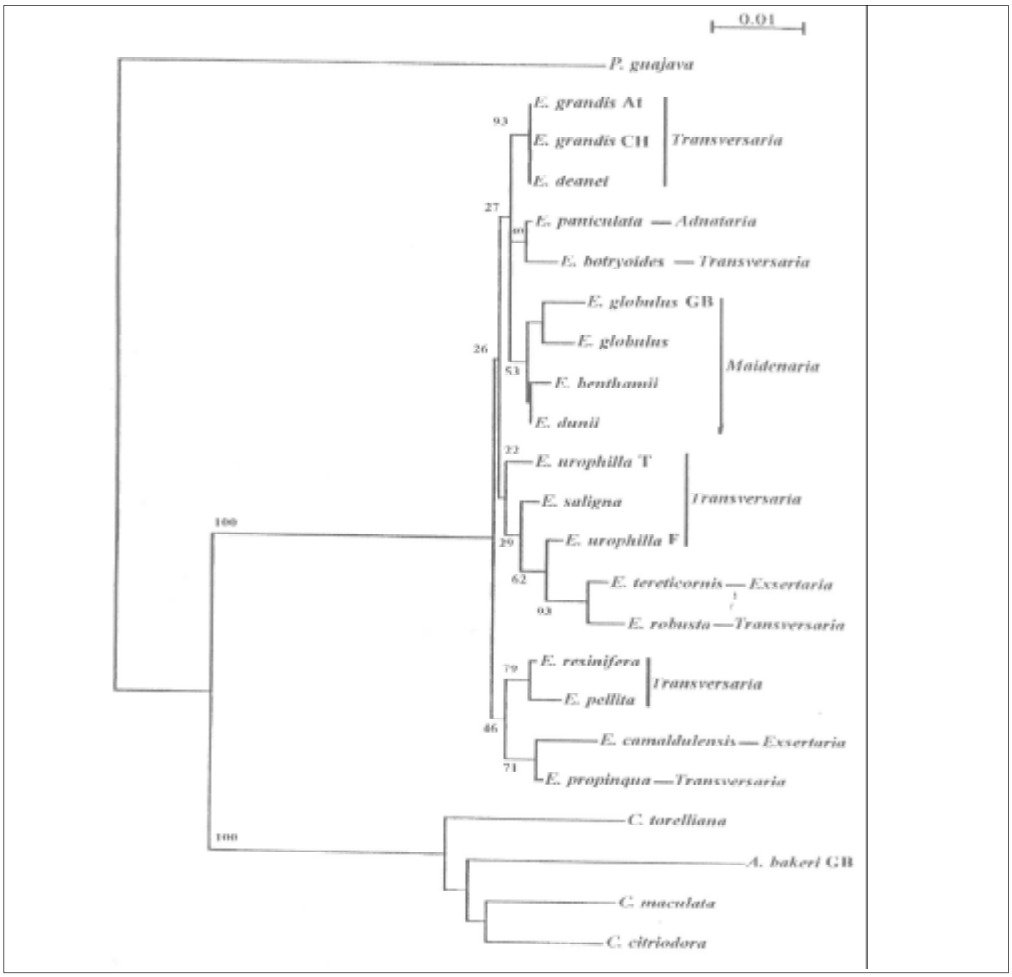

Figure 2
Neighbor-joining (Saitou and Ney, 1987) phylogenetic tree of the ITSs and 5.8S rDNA sequences of the species studied in this work. Numbers on the branches are Bootstrap values (%) obtained from 500 replicates analyses. The initials AT, CH, T and F mean respectively Atherton, Coff's Harbour, Timor and Flores.
(Árvore filogenética obtida a partir da análise das seqüências da regiões ITSs e do gene 5.8S do nrDNA das espécies do gênero *Corymbia* e do gênero *Eucalyptus* (subgênero *Symphyomytus*) avaliadas no presente estudo, utilizando o Método de agrupamento Neighbor-joining (Saitou and Ney, 1987). Os valores localizados nos ramos da árvore correspondem aos valores (%) de Bootstrap. As iniciais At, CH, T e F, referem-se às localidades de Atherton. Coff's Harbour, Timor e Flores, respectivamente)

The phylogenetic analysis showed that *A. bakeri* was closely related to the genus *Corymbia* (Figure 2). This also agreed with the results obtained by Steane et al. (1999). According to morphologic and molecular data, the genera *Angophora* and *Corymbia* were included in a monophyletic group (Hill and Johnson, 1995; Ladiges et al., 1995; Udovicic et al., 1995; Steane et al., 1999). In the present study, these genera also formed a monophyletic group where *Angophora* originated from the genus *Corymbia*, as also observed by Steane et al. (1999).

The genus *Angophora* has flowers with free petals and sepals instead of the opercular flower structure observed in the genera *Eucalyptus* and *Corymbia*. According to Brooker (1986), the *Eucalyptus* ancestral genus had flowers with free sepals and petals, and the opercular structure

is a derived character. If the genus *Angophora* really originated from the genus *Corymbia*, as shown by Steane et al. (1999) and by the present study, this would have important implications in the concept of flower evolution for these groups, since it would mean a reversal from the opercular condition to the free sepals and petals condition.

The species of the subgenus *Symphyomyrtus* (genus *Eucalyptus*) were divided into four different groups. The species positions in the phylogenetic tree did not entirely follow the classification proposed by Pryor and Johnson (1971). Steane et al. (1999) successfully discriminated the different sections of the subgenus *Symphyomyrtus* using ITS sequence analysis. The divergences found between our results and those obtained by Steane et al. (1999) may be due to the fact that our analysis included more than one species per section and some species could be hybrids since the occurrence of interspecific crossings within and between sections of the subgenus *Symphyomyrtus* is common (Pryor and Johnson, 1971; Jacobs, 1981).

Eucalyptus bentamii, *E. dunnii*, *E. globulus* and *E. globulus* ssp *globulus* grouped together. All these species are from section *Maidenaria*. The agreement between the morphological and molecular data could be explained by a lower frequency of interspecific crossings between section *Maidenaria* species and the species of the other sections, thus maintaining the identity of the group.

Section *Transversaria* species were scattered in the tree and were clustered into four different groups. *Eucalyptus grandis* and *E. deanei*, two species of very similar morphology (Boland et al., 1984), grouped together and away from *E. saligna*, which is morphologically very similar to *E. deanei* and *E. grandis* (Boland et al., 1992). The probable cause of this divergence is the fact that hybrids may have been analyzed since the rate of interspecific crossings between *E. saligna* and other species of the same genus is known to be high, and Brazilian material was used.

Eucalyptus saligna did not group with *E. botryoides* (section *Transversaria*) even though both were collected from the same population in Itatinga (Brazil), that was considered to be a hybrid generation, with *E. saligna* as the predominant species, followed by *E. botryoides* (Baez, 1994). *Eucalyptus botryoides* grouped with *E. paniculata* (Section *Adnataria*), suggesting that the used accession of *E. botryoides* could be a hybrid originating from a crossing with some species other than *E. saligna*.

Eucalyptus resinifera and *E. pellita* grouped together. These two species share many morphological similarities (Goes, 1985). Both species and *E. urophylla* belong to the subseries *Resiniferinae*, section *Transversaria* (Pryor and Johnson, 1981). According to Pryor et al. (1995), *E. pellita*, *E. resinifera*, and *E. urophylla* are closely related. However, *E. urophylla* grouped closer to the species of the subseries *Saligninae* (*E. saligna*, *E. robusta*, *E. botryoides*, *E. grandis*, *E. deanei*). Martin and Cossalter, (1975), who suggested that *E. urophylla may* really belong to the subseries *Saligninae*, obtained similar results. Both origins of *E. urophylla* evaluated (Flowers and Timor Islands) were included in the same group, but their sequences were not 100% identical, probably due to natural interspecific crossings. According to Pryor et al. (1995), the different origins of *E. urophylla* were really, origins of two different species. Based on morphometric analysis of *E. urophylla* of different origins, these authors described two species, *E. orophilla* from Timor Island, and *E. wetariensis* from Wetar Island, besides the typical *E. urophylla*, from Flowers Island.

Eucalyptus tereticornis and *E. camaldulensis*, section *Exsertaria*, did not group together. *Eucalyptus tereticornis* grouped with *E. robusta* (Section *Transversaria*). According to Goes (1985), it is not rare to find inside *E. robusta* populations natural hybrids between *E. robusta* and *E. tereticornis*, denoted *E. kirtoneana*. *Eucalyptus camaldulensis* grouped closer to *E. propinqua* and to a lesser extent with *E. resinífera*

and *E. pellita*, all belonging to section *Transversaria*. This was another situation that could be explained by a high rate of interspecific crossings inside and between the sections evaluated.

The taxonomic relationships were successfully determined by ITS sequence analysis between the genera *Corymbia* and *Eucalyptus* (subgenus *Symphyomyrtus*), for example. However, at the species level, where interspecific crossings are common, the phylogenetic and taxonomic relationships could not be determined precisely, requiring application of techniques with higher resolution power. The frequent interspecific crossings in the genus *Eucalyptus* could compromise the genetic integrity of the species studied, representing an interfering factor in future phylogenetic studies (Jackson et al.,1999).

Nowadays, the integrity of *Eucalyptus* species is only maintained in their natural habitat (Australia and surrounding islands) due to the existence of geographic and ecological barriers such as altitude differences, different flowering seasons, and pollen incompatibility. However, the removal of these barriers, in a way or another, may greatly increase and is increasing, the possibilities of interspecific hybridization events in the genus *Eucalyptus* (Brooker, 1986).

For further study, more conserved sequences, such as the cloroplast DNA (cp cloroplast) maybe more suitable for analyses of *Eucalyptus* species. Since cp -DNA maintains its integrity thorough hybridization.

AUTHORS AND ACKNOWLEDGMENTS

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