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Genetic diversity and differentiation of *Taxodium* in the south-eastern United States using cleaved amplified polymorphic sequences

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Two taxa of *Taxodium*, bald cypress and pond cypress, occur in the south-eastern United States. The ranges of these taxa overlap in the south-eastern Coastal Plain, with the range of the latter being more restricted. Although these taxa co-occur throughout a portion of the more expansive range of bald cypress (*Taxodium distichum* (L.) L. C. Rich), the habitats of the two taxa appear to differ. Consequently, considerable debate has occurred regarding the taxonomic status of pond cypress. Some authors recognize pond cypress as a distinct species (*Taxodium ascendens* Brongn.), whereas others recognize it as a variety/ecotype (*Taxodium distichum* var. *imbricarium* (Nutt.) Croom). In this study, the genetic diversity of these two taxa was investigated using 10 DNA markers based on sequences from cDNA clones of *Cryptomeria japonica*. *Cryptomeria* is a monospecific genus native to Japan, and is a close relative of *Taxodium*. These markers were codominant in *Cryptomeria* and were presumed to be codominant in *Taxodium*. DNA was extracted from leaf tissue collected from six populations of bald cypress and seven populations of pond cypress throughout Florida and Georgia. The average heterozygosities of bald cypress and pond cypress were 0.386 (SE 0.040) and 0.380 (SE 0.040), respectively. Most of the genetic variation (91.9%) was found within populations, 4.9% was found between populations and 3.2% between taxa. Results of DNA analysis using cleaved amplified polymorphic sequences (CAPS) in this study did not suggest that pond cypress was a species distinct from bald cypress. Our conclusion is that the two taxa of *Taxodium* should be given varietal status.

Keywords: bald cypress, CAPS, *Cryptomeria*, pond cypress, STS, *Taxodium*.

Introduction

Fossil records suggest that *Taxodium* was widely distributed throughout North America, Europe, and East Asia from the Cretaceous to the Pleistocene (Small, 1931; Florin, 1963). As the Pleistocene seas receded, *Taxodium* settled into its present distribution in the south-eastern United States and Mexico. Two taxa, bald cypress and pond cypress, occur in the south-eastern United States. The ranges of these taxa overlap in the south-eastern Coastal Plain, with the range of the latter being more restricted.

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The taxonomic status of *Taxodium* has been debated since the early 19th century (Watson, 1985). Britton (1926), Dallimore & Jackson (1966) and Rehder (1940) recognized three species: *T. distichum* (L.) L. C. Rich. (bald cypress), *T. ascendens* Brongn. (pond cypress) and *T. mucronatum* Ten. (Montezuma bald cypress). Godfrey (1988) concurred with this taxonomic treatment of pond cypress and bald cypress, suggesting temporal differences in phenology.

Watson (1983, 1985) evaluated morphological, anatomical, biochemical and cytological aspects of pond cypress and bald cypress, and concluded that the differences between them for the characteristics he considered were minor. He suggested that pond cypress be considered as a variety of bald cypress (*T. distichum*

var. *imbricatum* (Nutt.) Croom). He supported the hypothesis that pond cypress diverged from bald cypress, via isolated populations of bald cypress throughout the Coastal Plain during the Wisconsin Continental Glaciation. At this time, the genus was displaced southward, in conjunction with directional selection pressure exerted by habitats with reduced nutrient status and increased frequency or intensity of drought (Watson, 1983). Neufeld (1986) reported the ecophysiological implications of tree architecture for the two taxa, concluding that the morphology exhibited by pond cypress includes evolutionary adaptations resulting from climatically induced habitat changes. Miller *et al*. (1993) also found differences in interstitial soil pH between pond cypress (≈4.5) and bald cypress (≈6–7) populations. In the same study, Miller *et al*. repeated bioflavonoid analysis of leafy branchlet tissue from one of the populations Watson (1983) had evaluated, and concluded that these chemical 'fingerprints' can be altered by the condition, or health of the tree. Therefore, biflavonoids appear to have limited use as evidence of taxonomic status.

In molecular phylogenetic studies, no differences were apparent between the two taxa using PCR-RFLP analysis of six genes of chloroplast DNA (Tsumura *et al*., 1995). Lickey (1996) also failed to find significant differences between the two taxa after allozyme analysis of young shoot tissue from trees across their geographical range.

Sequence-Tagged Site (STS) primers have been used to generate PCR-based markers in many plant species, including *Populus* (Bradshaw *et al*., 1994), *Cuphea* (Slabaugh *et al*., 1997), *Pinus taeda* (Harry *et al*., 1998) and *Picea mariana* (Perry & Bousquet, 1998). STS-based cleaved amplified polymorphic sequences (CAPS) (Konieczny & Ausubel, 1993; Drenkard *et al*., 1997), are more reliable and convenient for genomic mapping and population genetics. The cDNA clones represent expressed genes and are extremely useful for establishing anchor points in genomic mapping and other genetic studies. Recently, we have developed STS markers in *Cryptomeria japonica*, a species closely related to *Taxodium* (Brunsfeld *et al*., 1994; Tsumura *et al*., 1995). The STS markers were derived from cDNA sequences of *C. japonica* (Tsumura *et al*., 1997) and half have been mapped in linkage groups (Mukai *et al*., 1995). The sequences of PCR primers for STSs therefore are especially well conserved in closely related genera, such as *Taxodium* and *Cryptomeria* (Brunsfeld *et al*., 1994; Tsumura *et al*., 1995). Tsumura *et al*. (1997) reported that about half of the STS markers from *C. japonica* could be amplified by PCR in *Taxodium* as single fragments.

We have applied the CAPS markers of *C. japonica* to *Taxodium* populations to investigate the genetic variation within and between populations, and between the two taxa in question. Using these results, we have evaluated the efficiency of CAPS markers, and the genetic relationship and taxonomic status of the two cypresses using these conserved markers.

Materials and methods

Plant materials

In early September 1994, fresh leafy branchlets were collected from 122 trees representing seven populations of pond cypress, and 130 trees representing six populations of bald cypress, in Georgia and Florida (Fig. 1). Samples were collected from an average of 19.55 trees in each population, with considerable distance between sampled trees to minimize sampling half-sib individuals. Ages of sampled trees were not determined but sizes of sampled trees tended to be similar within each population. All collection locations contained populations of pond cypress and bald cypress in sufficiently close proximity for potential cross-pollination except possibly Highlands Hammock, Tates Hell and Tocoi. Collected plant tissue was stored at −30°C prior to DNA extraction.

Laboratory analysis

Total DNA was extracted from each sample using the modified CTAB method of Murray & Thompson (1980). CAPS markers based on sequences of cDNA clones of *C. japonica* (Tsumura *et al*., 1997) were used to evaluate the genetic diversity and differentiation of pond cypress and bald cypress. Eighty STS markers of *C. japonica* were evaluated for use in this study. Basic PCR amplification conditions were as follows: reaction mixtures (100 μL) contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 100 pmol of each primer, 50 ng of template DNA, and 2.5 units of *Taq* polymerase. PCR amplification was carried out for 5 min at 94°C, followed by 36–45 cycles of 40 s at 94°C, 40 s at 58 or 60°C, and 80 s at 72°C, with a final 5-min incubation at 72°C using a PC700 model from Astech Co. Ltd, or an MJ Research Programmable Thermal Controller PTC100. In the survey for polymorphic STS markers in *Taxodium*, we used four individuals of each *Taxodium* variety.

Each PCR product was digested with eight four-base-cutter restriction enzymes (*Alu*I, *Hae*III, *Hha*I, *Msp*I, *Nde*II *Hin*fI, *Taq*I and *Rsa*I) and electrophoresed in either 2% agarose gels or 7.5% polyacrylamide gels. Ten suitable polymorphic STS markers were identified out of the total of 80 primer gels tested in *Taxodium* (Table 1).

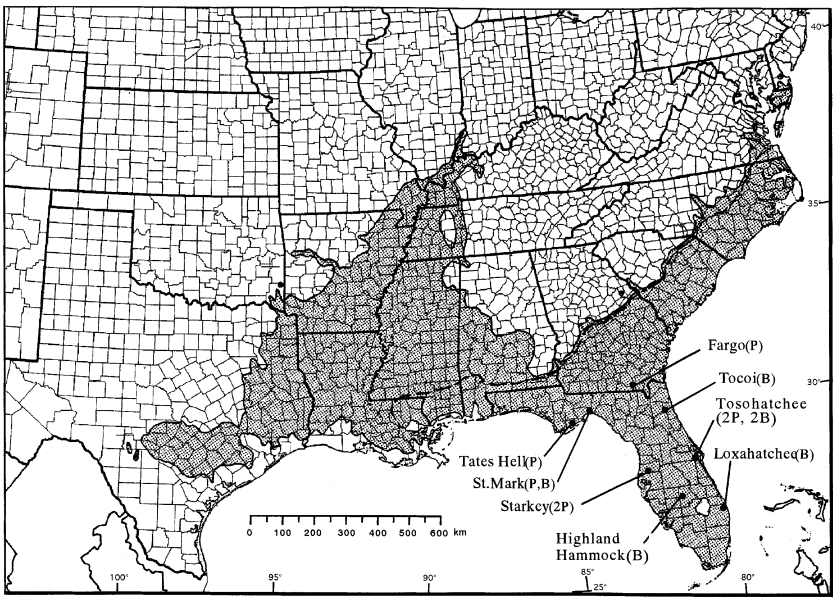

Fig. 1 Natural distribution of *Taxodium* in the U.S.A. (Wilhite & Toliver, 1990) and sampling sites. P and B indicate pond cypress and bald cypress populations, respectively. The dashed line indicates the range of pond cypress. The following abbreviations were used for each population: Fargo, FAR; Tocoi, TCI; pond cypress populations in Tosohatchee, TP1 and TP2; bald cypress in Tosohatchee, TB1 and TB2; Loxahatchee, LXH; pond cypress populations in Starkey, SD1 and SD2; Tates Hell, THL; pond cypress in St. Mark, SMP, bald cypress in St. Mark, SMB; and Highland Hammock, HHM.

Statistical analysis

To estimate within-population variation, we used the following measures calculated from the allele frequencies of all loci analysed: the proportion of polymorphic loci (*Pl*) at the 95% criterion; the average number of alleles per locus (N_a), and the averages of expected heterozygosities (H_e), where unbiased values for H_e were also estimated (Nei, 1978).

The fixation indices, F_{IS} ($= 1 - H_o/H_e$), F_{IT} and F_{ST}, for polymorphic loci and over all loci, were determined (Nei & Chesser, 1983) for comparison of the observed genotype frequencies to the expectations of Hardy–Weinberg equilibrium. Deviations from such expectations were analysed using the chi-squared test (Workman & Niswander, 1970).

Between-population variation was estimated in terms of the following statistics. Nei's gene diversity statistics (Nei, 1973) were estimated as follows: $H_T = H_P + D_{PV} + D_{VT}$, $H_V = H_P + D_{PV}$ and $G_{PV(T)} = D_{PV}/H_T$, $G_{VT} = D_{VT}/H_T$, where H_T is the total population gene diversity, H_P and H_V are the average gene diversities within populations and variety, respectively, and D_{VT} and D_{PV} are the average gene diversity between variety and between populations within variety, respectively, and $G_{PV(T)}$ and G_{VT} are the relative extent of gene differentiation among populations within variety and between taxa, respectively. These analyses were carried out using the GENESTRUT program (Constantine *et al.*, 1994). To estimate the amount of gene flow between populations, the number of migrants exchanged per generation, *Nm*, also was calculated indirectly from values of $G_{PV(T)}$ (or G_{VT}) at each locus and the values over all loci by application of Wright's infinite island formula (Wright, 1931): $Nm = (1 - G_{PV(T)}$ or $(G_{VT})/4G_{PV(T)}$ or G_{VT}, where N is the effective population size and m is the proportion of migrants exchanged per generation. Finally, Nei's unbiased genetic distances were calculated for all population pairs (Nei, 1972, 1978). The dendrogram was constructed based on the genetic distance matrix using UPGMA (Sokal & Michener, 1958) and neighbour-joining (NJ) methods (Saito & Nei, 1987). Bootstrap analysis was carried out using the DISPAN computer program (Ota, 1993).

Results

STS-based codominant CAPS markers

In *Taxodium*, single PCR products were obtained for 40 of the *Cryptomeria* STSs. These were cleaved with eight different restriction endonucleases. Ten of these 40 markers showed polymorphic restriction patterns, and all patterns appeared to be codominant (Fig. 2). Therefore, these 10 markers were used to evaluate the genetic diversity of *Taxodium* (Table 1).

Genetic diversity of Taxodium

Genetic diversity of *Taxodium* was investigated using only polymorphic CAPS markers. Therefore, the average unbiased heterozygosities were relatively high, ranging from 0.325 to 0.432 (Table 3). The average heterozygosities of pond cypress and bald cypress were 0.380 and 0.395, respectively. The average number of alleles per locus was 2.00 for most populations. The

Table 1 PCR-based codominant markers developed from cDNA clones of *Cryptomeria japonica* used to assess genetic diversity in *Taxodium*

Locus	Primer	PCR condition†	Fragment size (bp)	Enzyme‡	Fragment size in *C. japonica* (bp)	Homology§
CD618	5′-CAA GGA CAA CGG GCA AAA AT-3′ 5′-GAA CTG GGT TCC AAG GCT AT-3′	60, 40 cycles	1000	*Taq*I	1000	
CD41	5′-GAA TCC AAA ACC ACT TGC TA-3′ 5′-ACA TTC ACG ACC CTC CGT AT-3′	58, 45 cycles	2000	*Hin*f I	2000	*Arabidopsis thaliana* cDNA clone 113J6T7
CD1514	5′-GGT CGG TCT GAC ATT CCA TT-3′ 5′-CGA GAA GCG TCC AAA CAT TA-3′	60, 40 cycles	500	*Nde*II	500	*Pinus sylvestris CHS* gene for chalcone synthase
CD620	5′-AGG CCA AAC CCT CAG AAG TA-3′ 5′-GCT GGG AAG TCC TCT AAG AA-3′	56, 40 cycles	550	*Rsa*I	550	*Arabidopsis thaliana* cDNA clone 118 A8T7
CD1852	5′-GCA TAG CAT TTT CCC AAT CA-3′ 5′-AAG GGA TCG AAG AGG GTC AT-3′	58, 36 cycles	350	*Rsa*I	350	
CD1894	5′-ACC CTT TCC TCG CCT ACA TT-3′ 5′-GCC GAC TGA GTA AAC AAA CC-3′	60, 40 cycles	750	*Hha*I	750	Oat TUB1 mRNA for beta-tubulin (partial)
CD1237	5′-GGA ATC GGA TGG GTT ATC TG-3′ 5′-AGA ATC CGG GAC CAA ATC TA-3′	60, 40 cycles	900	*Hha*I	900	*Arabidopsis thaliana* cDNA clone 120C7T7
CD1613	5′-GGT GAA CAA GAA AGG GAA AT-3′ 5′-ATG TGT TGT CTG GCT TGG TA-3′	60, 40 cycles	800	*Hae*III	800	
CD1179	5′-TGG GTT TGG GCA TAA GTC TG-3′ 5′-TTG CCC CTG TTG TTT TAT CC-3′	58, 40 cycles	800	*Hae*III	800	
CD402	5′-CCT GCC CAT GGT GAA AGT AA-3′ 5′-TTG AAT CCA GAG GCT TGA AA-3′	58, 40 cycles	350	*Alu*I	350	

†Annealing temperature and PCR cycles. ‡Polymorphic enzyme. §Significant with BLAST homology test.

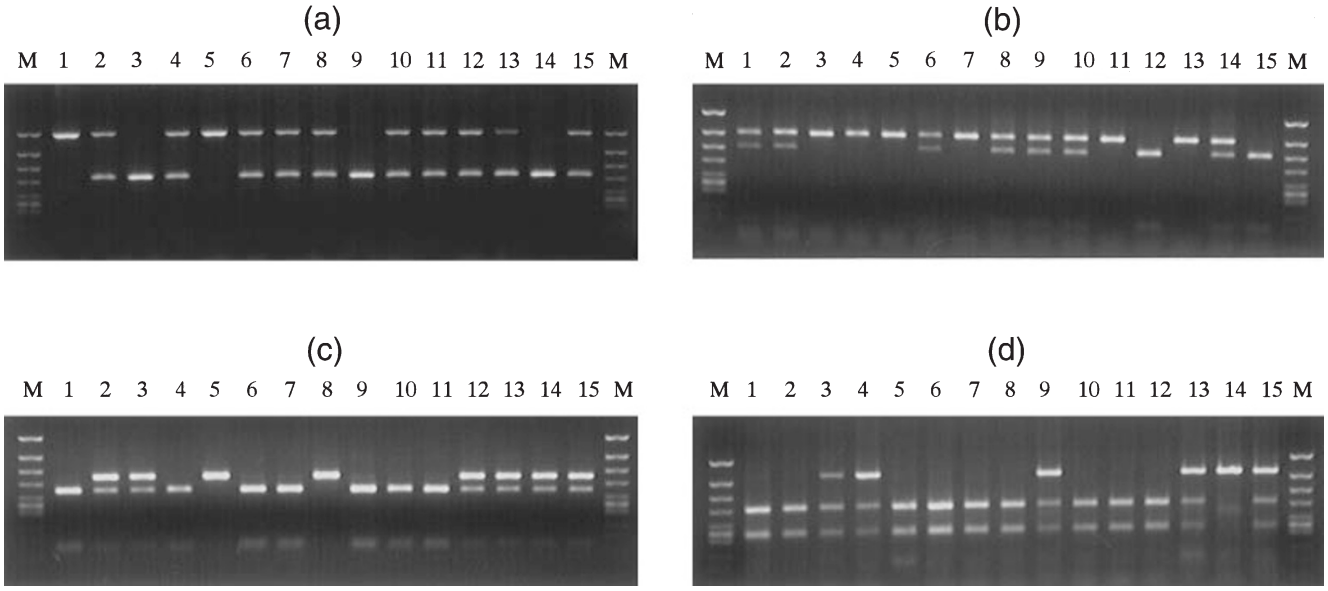

Fig. 2 Restriction patterns of CAPS markers in *Taxodium*. (a) *CD618*/*Taq*I; (b) *CD1894*/*Hha*I; (c) *CD620*/*Rsa*I; (d) *CD1613*/*Hae*III. Both marginal lanes in each panel show DNA size markers of X174/*Hin*cII, and the other lanes indicate the investigated individuals.

CD1237 and *CD1179* loci in the Tosohatchee P2 (TP2) population were monomorphic, as was the *CD1179* locus of the Loxahatchee (LXH) population. Consequently, the average heterozygosities of these two loci were lower than those of the other eight (Table 2). A clear difference for the average allele frequencies of *CD618* between the two cypress taxa was noted. The average frequencies of the *a* allele at the *CD618* locus in pond cypress and bald cypress were 0.875 and 0.390, respectively (Fig. 3). The *b* allele frequency of *CD1179* in the Fargo population (0.940) was greater than for the remaining populations (0.000–0.196).

The gene diversity within populations (H_P) varied for each locus, ranging from 0.129 for *CD1237* to 0.500 for *CD1514*. The gene diversity in the total populations (H_T) also ranged from 0.130 to 0.500 (Table 5). Half of the fixation indices (F_{IS}) showed statistically significant values, at the 5% probability level for *CD618* and *CD1179* and at the 1% level for *CD1514*, *CD620* and *CD402* (Table 4). The chi-squared value of *CD1514* had particularly high significance. The F_{ST} values showed statistical significance at seven loci, and the value of F_{ST} over all loci also was statistically significant.

Genetic variation within populations, between populations within taxa, and between taxa was 0.919, 0.049 and 0.032, respectively (Table 5). The numbers of migrants exchanged per generation among populations within and between taxa (*Nm*) were 4.85 and 7.56, respectively. The unbiased genetic distances between populations ranged from 0.000 to 0.166 and their average was 0.042. There was no clear geographical tendency in the allele frequencies or heterozygosities among populations. The dendrogram using the UPGMA method showed little differentiation between the two taxa of *Taxodium* (Fig. 4), and the NJ tree showed a very similar dendrogram to the UPGMA tree.

Discussion

We have applied CAPS markers from cDNA sequences of *C. japonica* to investigate the genetic diversity of natural populations of *C. japonica* and concluded that there is no large difference in results between allozyme and CAPS studies (Tsumura & Tomaru, 1999). Because *Taxodium* and *Cryptomeria* are closely related genera, we believe that the CAPS markers we have used are as appropriate for investigating the genetic diversity of *Taxodium* as allozyme studies.

The polymorphic DNA markers, derived from STS sequences of cDNA from *C. japonica*, have been used for other coniferous species (Tsumura *et al.*, 1997). When screening for suitable markers in *Taxodium*, dominant markers and those considered to encode multicopy genes were excluded. The STS markers were presumed to correspond to expressed genes because they were derived from sequences of cDNA clones of *C. japonica*. These regions were presumed to correspond to genes in *Taxodium* because cDNA sequences between the closely related taxa (e.g. *Taxodium* and *Cryptomeria*) generally are conserved (Brunsfeld *et al.*, 1994; Tsumura *et al.*, 1995). The inheritance of these markers was not investigated in *Taxodium* because plant material from

Table 2 Allele frequency at 10 loci in 13 populations of *Taxodium*

		Pond cypress								Bald cypress						
Locus	Allele	FAR	SMP	SD2	SD1	THL	TP1	TP2	Mean	TB2	HHM	LXH	TCI	TB1	SMB	Mean
CD618	a	0.620	0.933	0.933	0.900	0.940	0.912	0.889	0.875	0.380	0.360	0.260	0.460	0.611	0.267	0.390
	b	0.380	0.067	0.067	0.100	0.060	0.088	0.111	0.125	0.620	0.640	0.740	0.540	0.389	0.733	0.610
CD41	a	0.227	0.308	0.367	0.400	0.208	0.143	0.438	0.299	0.220	0.313	0.104	0.300	0.382	0.143	0.244
	b	0.773	0.692	0.633	0.600	0.792	0.857	0.562	0.701	0.780	0.687	0.896	0.700	0.618	0.857	0.756
CD1514	a	0.520	0.500	0.433	0.533	0.520	0.500	0.500	0.501	0.500	0.500	0.500	0.500	0.500	0.500	0.500
	b	0.480	0.500	0.567	0.467	0.480	0.500	0.500	0.499	0.500	0.500	0.500	0.500	0.500	0.500	0.500
CD620	a	0.300	0.367	0.500	0.600	0.640	0.563	0.556	0.504	0.600	0.440	0.360	0.400	0.333	0.367	0.417
	b	0.700	0.633	0.500	0.400	0.360	0.437	0.444	0.496	0.400	0.560	0.640	0.600	0.667	0.633	0.583
CD1852	a	0.660	0.633	0.800	0.700	0.740	0.533	0.333	0.628	0.660	0.680	0.760	0.720	0.533	0.500	0.642
	b	0.340	0.367	0.200	0.300	0.260	0.467	0.667	0.372	0.340	0.320	0.240	0.280	0.467	0.500	0.358
CD1894	a	0.780	0.667	0.633	0.700	0.740	0.813	0.750	0.726	0.660	0.760	0.900	0.700	0.700	0.900	0.770
	b	0.220	0.333	0.367	0.300	0.260	0.187	0.250	0.274	0.340	0.240	0.100	0.300	0.300	0.100	0.230
CD1237	a	0.120	0.033	0.133	0.067	0.040	0.031	0.000	0.061	0.120	0.060	0.060	0.140	0.071	0.033	0.081
	b	0.880	0.967	0.867	0.933	0.960	0.969	1.000	0.939	0.880	0.940	0.940	0.860	0.929	0.967	0.919
CD1613	a	0.320	0.067	0.167	0.033	0.160	0.214	0.111	0.153	0.160	0.260	0.220	0.260	0.467	0.100	0.245
	b	0.680	0.933	0.833	0.967	0.840	0.786	0.889	0.847	0.840	0.740	0.780	0.740	0.533	0.900	0.756
CD1179	a	0.060	0.967	0.833	0.933	0.875	0.962	1.000	0.804	0.940	0.680	1.000	0.960	0.833	0.933	0.891
	b	0.940	0.033	0.167	0.067	0.125	0.038	0.000	0.196	0.060	0.320	0.000	0.040	0.167	0.067	0.109
CD402	a	0.480	0.433	0.533	0.367	0.420	0.375	0.444	0.436	0.300	0.420	0.560	0.420	0.633	0.500	0.472
	b	0.520	0.567	0.467	0.633	0.580	0.625	0.556	0.564	0.700	0.580	0.440	0.580	0.367	0.500	0.528

Table 3 The average heterozygosity (H_e), the average number of alleles per locus (N_a), the proportion of polymorphic loci (*Pl*, 95% criterion), and the unbiased heterozygosity (unbiased H_e) in 13 populations of *Taxodium*

Population	*n*	*Pl*	N_a (SE)	H_e (SE)	Unbiased H_e (SE)
Pond cypress					
FAR	25	100	2.00 (0.00)	0.439 (0.214)	0.387 (0.041)
SMP	15	80	2.00 (0.00)	0.365 (0.148)	0.328 (0.063)
SD2	15	100	2.00 (0.00)	0.440 (0.215)	0.377 (0.044)
SD1	15	90	2.00 (0.00)	0.340 (0.128)	0.337 (0.058)
THL	25	90	2.00 (0.00)	0.355 (0.140)	0.329 (0.048)
TP1	17	80	2.00 (0.00)	0.352 (0.171)	0.325 (0.057)
TP2	10	80	1.80 (0.18)	0.435 (0.224)	0.339 (0.069)
Mean	17.43	100	2.000 (0.00)	0.389 (0.168)	0.380 (0.040)
Bald cypress					
TB2	25	100	2.00 (0.00)	0.452 (0.238)	0.378 (0.043)
HHM	25	100	2.00 (0.00)	0.494 (0.273)	0.419 (0.037)
LXH	25	90	1.90 (0.10)	0.369 (0.180)	0.309 (0.056)
TCI	25	90	2.00 (0.00)	0.396 (0.197)	0.399 (0.043)
TB1	15	100	2.00 (0.00)	0.473 (0.255)	0.432 (0.039)
SMB	15	90	2.00 (0.00)	0.341 (0.165)	0.326 (0.057)
Mean	21.67	100	2.000 (0.00)	0.423 (0.216)	0.386 (0.040)
Mean	19.55	100	2.000 (0.00)	0.407 (0.184)	0.395 (0.036)

crosses was not available. However, inheritance of these markers in *C. japonica* provided the basis for use of these genes as putative loci.

The average unbiased heterozygosities of pond cypress and bald cypress ranged from 0.325 to 0.432, whereas the average heterozygosities were 0.380 and 0.395, respectively. Hamrick & Godt (1989) summarized levels of allozyme variation at the species and population levels for species with different attributes. This allows us to compare the data between isozyme and CAPS marker studies because Hamrick & Godt (1989) also used only polymorphic loci when they calculated their values (H_T and H_S). The average heterozygosity found for CAPS loci was approximately 30% greater than that of the isozymes.

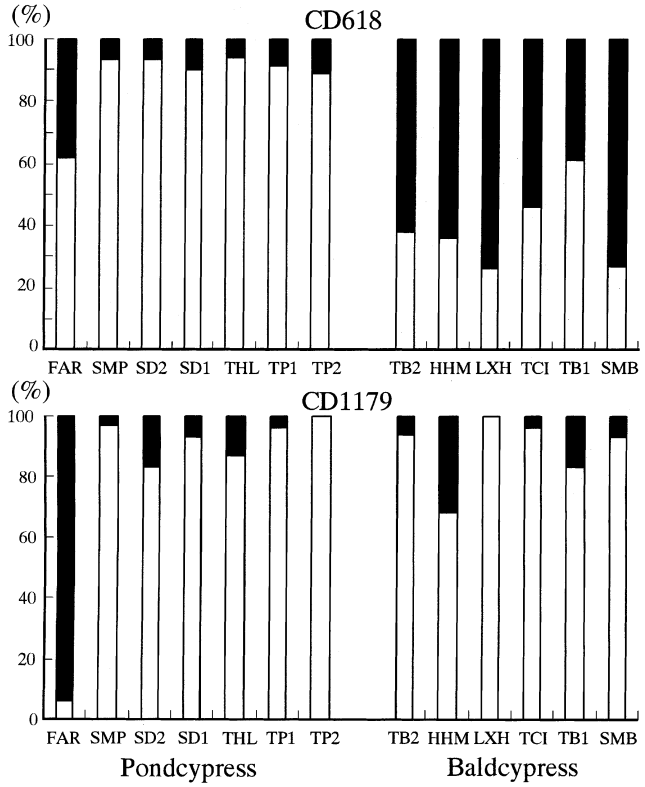

Fig. 3 Allele frequencies of 13 populations of *Taxodium* at the *CD618* and *CD1179* loci. Open and solid portions of bars show *a* and *b* alleles, respectively.

Ten putative CAPS loci were investigated for populations of two *Taxodium* taxa that are sympatric in the south-eastern Coastal Plain. Based on our data, only one of these loci (*CD618*) showed clear differences in allele frequencies between the two taxa (Table 2). All pond cypress populations except the population in Fargo, Georgia (FAR) had high frequencies of the *a* allele. Bald cypress populations tended to have low frequencies of the same allele.

Five loci were not in Hardy–Weinberg equilibrium according to the F_{IS} values. The mean values of F_{IS} indices across populations varied from locus to locus, except for loci *CD1514* and *CD620*. This observation corresponds with the negative mean values of F_{IS} found across these loci in most populations of the two taxa. These small excesses of heterozygotes may be the result of selection but it remains unclear in these data. The genetic distance between the two taxa was only 0.043, except for the Fargo population of pond cypress, which had a value of 0.071 (Fig. 3). However, the genetic differentiation among the remaining pond cypress populations was very small.

At the *CD1179* locus, all populations surveyed had high frequencies of the *a* allele, except the Fargo population, which was almost fixed for the *b* allele. The pond cypress population in Fargo was the most northern population investigated in this study, and was in close proximity to the Suwannee River. Unfortunately, plant material could not be collected from a sufficient number of trees from the co-occurring bald cypress population for reliable analysis. Therefore,

Table 4 F-statistics for 10 loci in *Taxodium*

Locus	F_{IS}	χ^2	d.f.	P	F_{ST}	χ^2	P	F_{IT}
CD618	0.125	4.00	12	<0.05	0.293	149.50	<0.01	0.381
CD41	−0.056	0.80	12	NS	0.030	15.40	<0.01	−0.024
CD1514	−0.935	223.00	12	<0.01	0.000	0.20	NS	−0.935
CD620	−0.382	37.20	12	<0.01	0.036	18.30	<0.01	−0.332
CD1852	0.102	2.60	12	NS	0.039	19.70	<0.01	0.136
CD1894	0.033	0.30	12	NS	0.009	4.40	<0.05	0.041
CD1237	−0.078	1.60	12	NS	0.005	2.50	NS	−0.073
CD1613	0.046	0.50	12	NS	0.053	27.10	<0.01	0.097
CD1179	0.147	5.50	12	<0.05	0.427	217.60	<0.01	0.511
CD402	0.195	9.60	12	<0.01	−0.002	0.80	NS	0.193
Over all loci	−0.126	285.20	120	<0.01	0.080	455.60	<0.01	−0.036

Table 5 Gene diversity within and between populations and varieties in *Taxodium*

Locus	H_P	H_T	D_{PV}	D_{VT}	$G_{PV(T)}$	G_{VT}	H_P/H_T
CD618	0.321	0.455	0.017	0.117	0.038	0.256	0.706
CD41	0.386	0.398	0.011	0.001	0.028	0.002	0.970
CD1514	0.500	0.500	0.000	0.000	0.000	0.000	1.000
CD620	0.480	0.498	0.015	0.003	0.031	0.006	0.963
CD1852	0.447	0.465	0.019	−0.001	0.040	−0.002	0.962
CD1894	0.376	0.379	0.003	0.000	0.008	0.000	0.991
CD1237	0.129	0.130	0.001	0.000	0.008	0.000	0.992
CD1613	0.298	0.315	0.014	0.004	0.044	0.011	0.945
CD1179	0.150	0.263	0.109	0.003	0.416	0.013	0.572
CD402	0.497	0.497	0.000	−0.001	0.001	−0.001	1.001
Mean	0.358	0.390	0.019	0.013	0.049	0.032	0.919

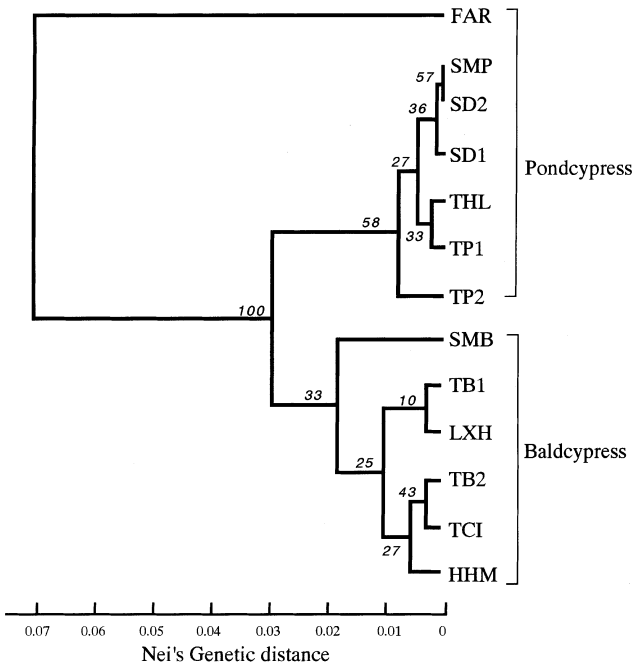

Fig. 4 Dendrogram based on genetic distance using UPGMA of 13 populations of two cypresses in *Taxodium*. Numbers in italics within the phylogenetic tree represent bootstrap values based on 1000 replicates.

direct comparison of these sympatric populations of bald cypress and pond cypress could not be carried out.

The genetic diversities of pond cypress and bald cypress were similar (Table 3), with the greatest genetic variation contained within populations (91.9%), 4.9% between populations, and 3.2% between taxa (Table 4). Liu *et al.* (1990) reported the genetic structure of bald cypress using three isozyme loci. According to their data, the genetic variation within populations was 96.4%, whereas variation between streams was only 0.4%, and between populations within streams was 1.5%. However, their study was confined to a single stream system in South Carolina, and the area had been affected by thermal effluent for many years. Consequently, it is difficult to compare the genetic variation between populations in these two studies. In our study, the genetic differentiation between populations within variety ($G_{PV(T)}$) was 4.9% and between taxa (G_{VT}) it was 3.2%. These values were high compared to those from the isozyme study (Liu *et al.*, 1990). One of the reasons for this might be the large area of our study (Coastal Plain region of Florida and Georgia, ≈500 km long) compared to the single stream system in South Carolina in the allozyme study (about 15 km). Liu *et al.* (1990) also estimated the number of migrants per generation to have an *Nm* value of 8.72. In our study, the *Nm* values within and between taxa were 4.85 and 7.56, respectively. *Nm* values between isozyme and CAPS studies were similar. Lickey (1996) reported the genetic variation of 22 populations of two taxa in *Taxodium*. The heterozygosities of the two taxa were approximately the same, $H_e = 0.125$ in bald cypress and $H_e = 0.122$ in pond cypress, and the F_{ST} over all populations was 0.203. The pond cypress populations made a cluster when the dendrogram based on Nei's genetic distance was constructed but the differentiation between pond cypress and bald cypress populations was very small.

The results of this study do not suggest that pond cypress is a species distinct from bald cypress. This is consistent with the findings of Watson (1983) and of Lickey (1996). Watson's research focused on morphological, anatomical, biochemical and cytological characteristics, whereas Lickey evaluated genetic structure of the two taxa using allozymes. Considering the historical background of classification of this species, we have concluded that the two taxa of *Taxodium* should be classified as varieties, as suggested by Watson (1983) from the analysis of several characters.

The genetic diversity and differentiation in each species is different depending on its evolutionary history and distribution. Therefore, it is not easy to understand the level of genetic diversity and differentiation in each taxonomic level such as family, genus and species, which means there is no criterion to decide on each taxonomy level using genetic data. However, if we could add genetic data to traditional data such as morphological traits, it may be possible to decided on a suitable status in each species for which there is a taxonomic problem.

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