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Molecular phylogeny of conifers using RFLP analysis of PCR-amplified specific chloroplast genes

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Abstract We investigated the molecular phylogeny of conifers using restriction endonuclease fragment length polymorphism of six polymerase chain reaction-amplified chloroplast genes – *frx*C, *rbc*L, *psb*A, *psb*D, *trn*K, and 16S. We detected 227 total site changes among species, representing 23, 26, 38, 48, 67, and 25 site changes in *frx*C, *psb*A, *psb*D, *rbc*L, *trn*K and 16S, respectively. The mean nucleotide substitution was 10.75% (SD 0.573) among species in five families. Forty maximally parsimonious trees were obtained using the Wagner parsimony method, and a 50% majority-rule consensus tree was obtained from them. Data analysis produced similar basic patterns when both the Wagner parsimony and the neighbor-joining methods were applied, and the main lineages were clearly separated. Taxaceae and Cephalotaxaceae species were used as the out-groups when applying Wagner parsimony methods. With the Wagner method, the consistency index was 0.510, the retention index was 0.879, and tree length was 435 steps. Our results indicated that Cupressaceae and Taxodiaceae are closely related families and that *Sciadopitys verticillata* is the basal lineage of Cupressaceae and Taxodiaceae. The neighbor-joining tree is similar to the 50% majority-rule consensus of the 40 Wagner parsimony trees except for the position of *Keteleeria daversifolia,* the *Picea* and *Cedrus* group, and the divergence within Cupressaceae.

Key words Molecular phylogeny · Chloroplast genes · Conifers · RFLP (restriction fragment length polymorphism) · PCR (polymerase chain reaction)

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Introduction

Conifers are extensively distributed worldwide and important for forestry and natural conservation. On the basis of fossil records, conifers dominated the forest vegetation in the Mesozoic Era and although they are the largest and most diverse group of living gymnosperms, the phylogenetic relationship between families and genera is not clear. Based on morphology and some aspects of secondary product chemistry, Eckenwalder (1976) concluded that Taxodiaceae and Cupressaceae have a great degree of overall similarity. Immunological data also supported this conclusion (Price and Lowenstein 1989). In Taxodiaceae, a number of characters distinguish the position of *Sciadopitys* as unusual, these include its chromosome number and karyotype (Khoshoo 1961; Schlarbaum and Tsuchiya 1984, 1985), embryology (Doyle and Brennan 1971), wood anatomy (Phillips 1941), and immunological data (Price and Lowenstein 1989). In the Taxodiaceae family, the chloroplast DNA (cpDNA) structure of *Cryptomeria japonica* (Tsumura et al. 1993) is quite different from species in the Pinaceae family (Strauss et al. 1988; Lidholm and Gustafsson 1991; Tsudzuki et al. 1992). Recently, as more sophisticated molecular biology techniques have been developed, molecular phylogenetic trees based on *rbc*L sequence data have been reported (Soltis et al. 1992; Chase et al. 1993; Brunsfeld et al. 1994). The phylogeny based on the *rbc*L sequence also showed that Taxodiaceae and Cupressaceae form a monotypic group (Brunsfeld et al. 1994). The molecular phylogeny of other conifer species has also been studied: namely, *Pinus* species, using restriction endonuclease fragment length polymorphism (RFLP) of cpDNA, mitochondrial DNA (mtDNA), and several other nuclear genes (Strauss and Doerksen 1990); *Pinus* species, using ribosomal DNA RFLP (Govindaraju et al. 1992); Asian pine species, using RFLP of cpDNA (Wang and Szmidt 1993); *Pinus* species using RFLP of 5S RNA spacer region (Moran et al. 1992); Pseudotsuga species, using RFLP of cpDNA, mtDNA, and other nuclear genes (Strauss et al. 1990); the phylogenetic position of Taxaceae, based on 18S

rRNA sequences (Chaw et al. 1993); a Miocene Taxodium species, using the *rbc*L sequence (Soltis et al. 1992); and seed plants including conifers, using the *rbc*L sequence (Chase et al. 1993; Bousquet et al. 1992). However, most studies have used only a few genus or *rbc*L sequence data. Actually, the literature is replete with *rbc*L sequence data giving us abundant information on the phylogenetic studies among genera. However, to clarify the species phylogeny, we investigated many other genes.

In this study, we focused on conifer phylogeny, in particular, the evolutionary position of species in Taxodiaceae. We used the RFLP analysis of six polymerase chain reaction (PCR)-amplified chloroplast genes and investigated site changes between species to construct a phylogenetic tree.

Materials and methods

Plant materials

Needle samples were collected from 45 conifer species in the arboretums listed in Table 1. These included all of the Taxodiaceae species, 6 species of the Cupressaceae, 19 Pinaceae species, 2 Taxaceae species, and 1 Cephalotaxaceae specie.

Manipulation of DNA

Total DNA was extracted from the needles of each species by a modified method of Greenwood et al. (1989). Five grams of each needle was frozen in liquid nitrogen and ground in an Iwatani grinder (IFM-150). The ground tissue was added to 50 ml of ice-cold buffer I (50 m*M* TRIS-HCl, pH 8.0, 5 m*M* EDTA, 350 m*M* sorbitol, 0.1% BSA, 0.1% β-mercaptoethanol, and 10% polyethylene glycol 6000) and then homogenized in the Iwatani grinder for 30 s. The homogenate was filtered through two layers of cheesecloth and one layer of miracloth and pelletized by centrifugation at 3 000 rpm for 10 min at 4°C. The pellet was then resuspended in 10 ml of ice-cold buffer II (50 m*M* TRIS-HCl, pH 8.0, 5 m*M* EDTA, 350 m*M* sorbitol, 0.1% BSA, 0.1% β-mercaptoethanol, and 1% sodium sarcosyl) and incubated for 30 min at room temperature. An aliquot of 10 ml of 2×CTAB buffer (100 m*M* TRIS-HCl, pH 8.0, 20 m*M* EDTA, 1.4m*M* NaCl, 2% Cetytrimethylammoniumbromide, 0.4% β-mercaptoethanol; Murray and Thompson 1980) was added, followed by incubation at 60°C for 10 min It was then extracted with 20 ml of chloroform-isoamyl alcohol (24:1) and the layers separated by centrifugation at 4 000 rpm for 10 min at room temperature. The aqueous layer was transferred to a new tube and the DNA precipitated by adding a two-thirds volume of cold isopropanol. The DNA was recovered with a disposable pipette, washed with 70% cold ethanol, and subsequently dissolved in TE (10 m*M* TRIS-HCl, pH 8.0, 0.1 m*M* EDTA). To remove RNA contamination, 20 µl/ml of RNase was added to each DNA sample and incubated at 37°C for 60 min, followed by phenol extraction and precipitation with ethanol.

Six genes found in cpDNA (*frx*C, *rbc*L, *psb*A, *psb*D, *trn*K and 16S) were amplified by PCR (Table 2). Primers were designed using the DNASIS program (Takara Co., Kyoto) based on nucleotide sequence data from the EMBL database and synthesized by Nippon Gene Co. PCR amplification of these cpDNA regions was performed as follows: reaction mixtures (100 µl) contained 10 m*M* TRIS-HCl, pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 9.1 m*M* of each dNTP, 100 pmol of each primer, 200 ng of template DNA, and 2.5 units of *Taq* polymerase. Amplification was carried out for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, with a final 5-min incubation at 72°C with a PC700 model of Astech Co. The amplified DNAs were purified by ethanol precipitation and dissolved in 100 µl of TE. In order to detect changes in specific genes among species the DNAs were digested separately into the following groups: *Sau*3AI, *Taq*I, *Xba*I, *Sty*I, *Sca*I, *Rsa*I, *Msp*I, *Hinf*I, *Eco*RV, *Hae*III, and *Bgl*II for *frx*C; *Pst*I, *Msp*I, *Rsa*I, *Sau*3AI, *Taq*I, *Sty*I, *Hae*III, *Hin*fI and *Hin*dIII for *psb*A; *Hae*III, *Hin*fI, *Msp*I, *Sau*3AI, *Sty*I, *Alu*I, *Rsa*I, *Bsm*I, *Hha*I, and *Taq*I for *psb*D; *Hae*III, *Bgl*I, *Bam*HI, *Bsm*I, *Msp*I, *Pst*I, *Rsa*I, *Sau*3AI, *Sty*I, *Xba*I, *Hha*I, and *Taq*I for *rbc*L; *Eco*RV, *Bam*HI, *Sty*I, *Sca*I, *Msp*I, *Dra*I, and *Bsm*I for *trn*K; and *Hha*I, *Hin*fI, *Eco*RI, *Sty*I, *Sau*3AI, *Sma*I, *Taq*I, and *Rsa*I for 16S. Electrophoresis was performed on 2% agarose gel in TAE buffer (40 m*M* TRIS-HCl, 20 m*M* sodium acetate and 2 m*M* EDTA, pH 8.0).

Scoring of site changes and construction of a phylogenetic tree

After PCR amplification 5 µl of the reaction mixture was used for estimating the sizes of fragment-amplified genes by gel electrophoresis on 2% agarose gel in TAE buffer before digestion with restriction endonucleases. The digested DNAs were also subjected to electrophoresis on 2% agarose gel and the molecular size of each fragment was estimated by comparison with the size marker, *Hinc*II-digested Ø X174, using the DNASIS program. Each fragment's estimated molecular size included ±5% error. Site changes between specific genes in the various species were determined from these data.

Phylogenetic analysis was conducted using the heuristic search condition of the PAUP 3.0 program (Phylogenetic Analysis Using Parsimony; Swofford 1991) with the Wagner parsimony option and also by using the neighbor-joining (NJ) method (Saitou and Nei 1987) in the MEGA (Molecular Evolutionary Genetics Analysis) program (Kumar et al. 1993). Using the NJ method, we calculated a matrix of pairwise distances between species with the PAUP program. If phylogenetic trees constructed by several different methods agree, confidence in the phylogenetic tree and the data used for its construction will increase.

Estimation of nucleotide substitutions

To estimate nucleotide substitutions, we used the equation,

$$p = p_1\langle \Sigma_i r_i (m_i - m_{XYi})/[\{1-(1-p_i)^{ri}\}\{2-(1-p_i)^{ri}\}]\rangle / [\Sigma_i r_i m_i/\{2-(p=p_i)^{ri}\}]$$

where *pi* is a trial value of *p* (Nei and Tajima 1983). When *p*=*p1*, *p* is the maximum-likelihood estimate of *p*. In this equation m_X and m_Y indicate the number of restriction sites in DNA sequences *X* and *Y*, and m_{XY} is the number of restriction sites shared by the two sequences. The value of *mi* can also be calculated from the formula $mi=(m_X+m_Y)/2$. In the first equation, *i* refers to the *i*th type of enzyme with *ri* recognition nucleotides, and *k* is the number of different kinds of restriction enzymes. The proportion of nucleotide differences is *p*. We also calculated the rate of nucleotide substitution (*d*) from the formula, $d=-3/4\log e\,(1-4p/3)$. The variance of *d* thus obtained is given by $V(d)=1/\{1/V(di)\}$.

Results

Variation among species

We amplified six chloroplast genes. The predominant fragment sizes of the different genes showed no variation among species. After digesting these fragments by restriction endonucleases, we investigated site changes between species (Fig. 1). In total, 227 site changes were detected by RFLP analysis of PCR-amplified specific genes in cpDNA (Appendix 3). The length of the amplified genes

Table 1 Species subjected to an analysis of molecular phylogeny, and their origin

No.	Species	Family	Origin[a]
1	*Athrotaxis cupressoides*	Taxodiaceae	Tasmania, Australia
2	*Athrotaxis laxifolia*	Taxodiaceae	Tasmania, Australia
3	*Athrotaxis selaginoides*	Taxodiaceae	Tasmania, Australia
4	*Cryptomeria japonica*	Taxodiaceae	For. and For. Prod. Res. Inst.
5	*Cryptomeria fortunei*	Taxodiaceae	For. and For. Prod. Res. Inst.
6	*Cunninghamia konishii*	Taxodiaceae	Univ. Forest of Chiba, Univ. Tokyo
7	*Cunninghamia lanceolata*	Taxodiaceae	Univ. Forest, Univ. Tsukuba
8	*Glyptostrobus pensilis*	Taxodiaceae	Botanical Barden, Univ. Tokyo
9	*Metasequoia glyptostroboides*	Taxodiaceae	For. and For. Prod. Res. Inst.
10	*Sequoia sempervirens*	Taxodiaceae	For. and For. Prod. Res. Inst.
11	*Sequoiadendron giganteum*	Taxodiaceae	Univ. Forest, Kyoto Univ.
12	*Taiwania cryptomerioides*	Taxodiaceae	Univ. Forest of Chiba, Univ. Tokyo
13	*Taiwania flusiana*	Taxodiaceae	Univ. Forest, Kyoto Univ.
14	*Taxodium mucronatum*	Taxodiaceae	Botanical Barden, Univ. Tokyo
15	*Taxodium ascendens*	Taxodiaceae	For. Tree Breed. Inst.
16	*Taxodium distichum*	Taxodiaceae	For. Tree Breed. Inst.
17	*Sciadopitys verticillata*	Taxodiaceae	For. and For. Prod. Res. Inst.
18	*Chamaecyparis obtusa*	Cupressaceae	For. and For. Prod. Res. Inst.
19	*Chamaecyparis pisifera*	Cupressaceae	For. and For. Prod. Res. Inst.
20	*Juniperus chinensis*	Cupressaceae	For. and For. Prod. Res. Inst.
21	*Juniperus rigida*	Cupressaceae	For. and For. Prod. Res. Inst.
22	*Thuja standishii*	Cupressaceae	For. and For. Prod. Res. Inst.
23	*Thujopsis dolabrata*	Cupressaceae	For. and For. Prod. Res. Inst.
24	*Abies homolepsis*	Pinaceae	For. and For. Prod. Res. Inst.
25	*Abies mariesii*	Pinaceae	For. and For. Prod. Res. Inst.
26	*Abies sachalinensis*	Pinaceae	For. and For. Prod. Res. Inst.
27	*Abies veitchii*	Pinaceae	For. and For. Prod. Res. Inst.
28	*Cedrus deodara*	Pinaceae	For. and For. Prod. Res. Inst.
29	*Keteleeria davidiana*	Pinaceae	For. and For. Prod. Res. Inst.
30	*Larix kaempferi*	Pinaceae	For. and For. Prod. Res. Inst.
31	*Picea abies*	Pinaceae	For. and For. Prod. Res. Inst.
32	*Picea jezoensis*	Pinaceae	For. and For. Prod. Res. Inst.
33	*Pinus densiflora*	Pinaceae	For. and For. Prod. Res. Inst.
34	*Pinus koraiensis*	Pinaceae	For. and For. Prod. Res. Inst.
35	*Pinus pentaphylla*	Pinaceae	For. and For. Prod. Res. Inst.
36	*Pinus strobus*	Pinaceae	For. and For. Prod. Res. Inst.
37	*Pinus thunbergii*	Pinaceae	For. and For. Prod. Res. Inst.
38	*Pseudolarix amabilis*	Pinaceae	For. and For. Prod. Res. Inst.
39	*Pseudotsuga japonica*	Pinaceae	For. and For. Prod. Res. Inst.
40	*Pseudotsuga wilsoniana*	Pinaceae	For. and For. Prod. Res. Inst.
41	*Tsuga diversifolia*	Pinaceae	For. and For. Prod. Res. Inst.
42	*Tsuga sieboldii*	Pinaceae	For. and For. Prod. Res. Inst.
43	*Taxus cuspidata*	Taxaceae	For. and For. Prod. Res. Inst.
44	*Torreya nucifera*	Taxaceae	For. and For. Prod. Res. Inst.
45	*Cephalotaxus harringtonia*	Cephalotaxaceae	For. and For. Prod. Res. Inst.

[a] Plant materials were sampled in the arboretums of the universities and institutes listed, except for *Athrotaxis*, which was from natural populations found in Tasmania, Australia

Table 2 Primers, fragment sizes, and sources of information for amplification by PCR of six specific genes in chloroplast DNA

Gene	Primer (forward and reverse)	Size	Source
*frx*C	5′-ATAGCAGTTTACGGGAAAGG-3′ 5′-TGAATAATTCCCGATCTGGA-3′	779 bp	*Pinus contorta* (Lidholm and Gustafsson 1991)
*rbc*L	5′-TGTCACCAAAAACAGAGACT-3′ 5′-TTCCATACTTCACAAGCAGC-3′	1387 bp	*Pseudotsuga menziesii* (Hipkins et al. 1990)
*psb*A	5′-TACGTTCGTGCCATAACTTCC-3′ 5′-CTAGCACTGAAAACCGTCTT-3′	939 bp	*Pinus contorta* (Lidholm and Gustafsson 1991)
*psb*D	5′-TATGACTATAGCCCTTGGTA-3′ 5′-TAGAACCTCCTCAGGGAATA-3′	1042 bp	*Nicotiana tabacum* (Shinozaki et al. 1986)
*trn*K	5′-AACCCGGAACTAGTCGGATG-3′ 5′-TCAATGGTAGAGTACTCGGC-3′	2569 bp	*Oryza sativa* (Hiratsuka et al. 1989)
16S	5′-ACGGGTGAGTAACGCGTAAG-3′ 5′-CTTCCAGTACGGCTACCTTG-3′	1375 bp	*Nicotiana tabacum* (Shinozaki et al. 1986)

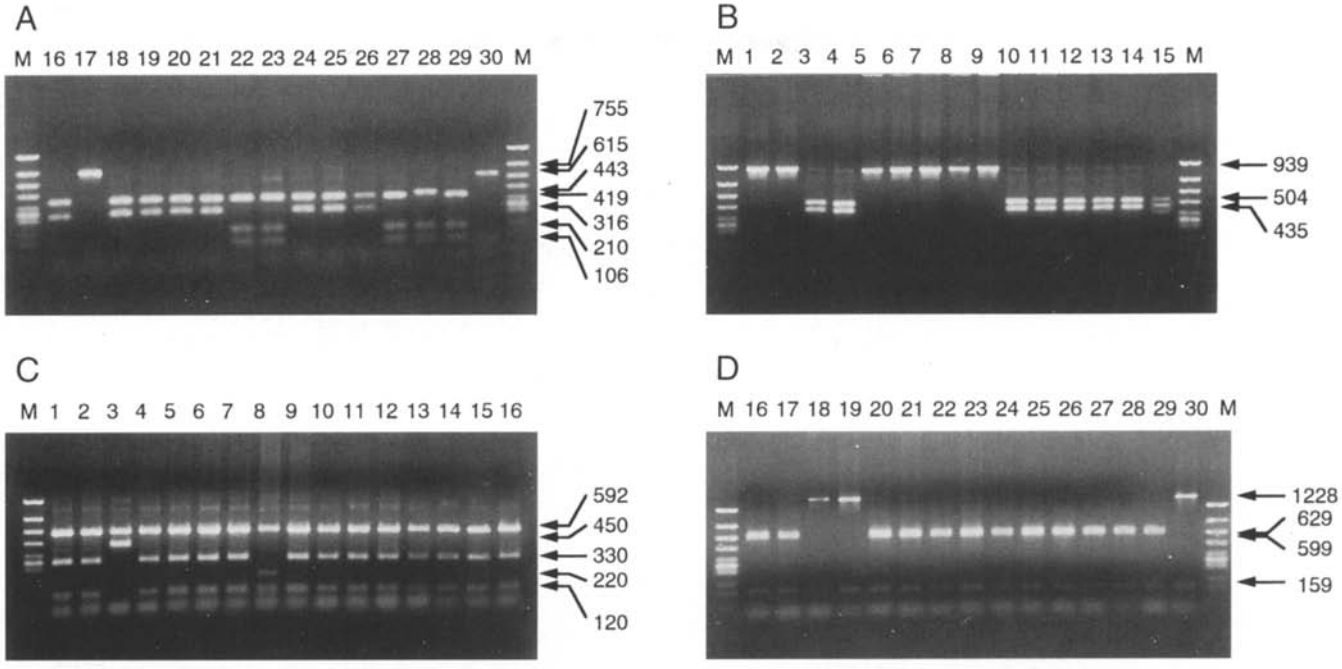

Fig. 1A–D RFLP patterns of specific PCR-amplified genes of chloroplast DNA. **A** *Hin*fI-digested *frx*C region, **B** *Hin*sIII-digested *psb*A region, **C** *Hin*fI-digested *psb*D region, and **D** *Sty*I-digested *rbc*L. *Numbers* above each photograph refer to numbering in Table 1. Number 8 of **C** is *Pinus strobus* and no. 30 of **A** and **D** is *Ginkgo bibora*. *M* indicates molecular size markers, namely, a *Hin*cII digest of Ø X174

ranged from 779 bp for *frx*C to 2569 bp for *trn*K. The PCR-amplified DNA fragments were mostly coding regions except for *trn*K. The *trn*K genes usually have a large intron, for example, 2504 bp long in rice, 2526 bp long in tobacco, and 2111 bp long in liverwort. We detected the following site changes among the species in each gene –23, 26, 38, 48, 67, and 25 in *frx*C, *psb*A, *psb*D, *rbc*L, *trn*K, and 16S, respectively. Nucleotide substitution also ranged from 8.30% in *psb*D to 27.05% in *trn*K (Tables 3 and 4. The mean nucleotide substitution was 10.75% (S.D. 0.00573) among species in the five families.

Phylogenetic tree

Forty maximally parsimonious trees were obtained when the Wagner parsimony method was used, and a 50% majority-rule consensus tree was obtained from these (Fig. 2). The NJ tree is shown in Fig. 3. Data analysis produced similar patterns when both the Wagner parsimony and the NJ methods were applied, and the main lineages were clearly separated (Figs. 2 and 3). Pinaceae species were used as the out-groups when Wagner parsimony methods were applied. The Wagner method produced a consistency index of 0.510, a retention index of 0.879, and a tree length of 435 steps. Our results indicate that Cupressaceae and Taxodiaceae are closely related families and that *Sciadopitys verticillata* is the basal lineage of Cupressaceae and Taxodiaceae (Figs. 2 and 3). Cupressaceae species are derived from Taxodiaceae. *Cunninghamia* is a basal lineage of Taxodiaceae and Cupressaceae. *Cunninghamia* is the sister to Taiwania. *Sequoia* and *Sequoiadendron* are closely related species and the sister to *Metasequoia*. *Cryptomeria fortunei* and *Cryptomeria japonica* share exactly the same data (Appendix), and *Cryptomeria* is the sister to *Glyptostrobus* and *Taxodium*. *Athrotaxis selaginoides* and *A. laxifolia* have exactly the same data, and *A. cupressoides* forms one group with two *Athrotaxis*, and that group becomes the sister to *Cryptomeria*, *Glyptostrobus*, and *Taxodium*. Genera in Pinaceae are clearly separated, with *Pinus* species diverging first, and the *Picea*, *Cedrus*, *Pseudotsuga*, and *Larix* group and the *Abies*, *Tsuga*, *Ketelleria*, *Pseudolarix* group, second.

The NJ tree is similar to the 50% majority-rule consensus of the 40 Wagner parsimony trees except for the position of *Keteleeria daversifolia* and the divergence within Cupressaceae. In the NJ tree, *K. daversifolia* lies between *Abies* and *Tsuga*, and the positions of *Thuja* and *Thjopsis* do not match the Wagner tree where the *Picea* and *Cedrus* group lies between the *Abies*, *Ketelleria,* and *Tsuga* group and *Pseudolarix* (Fig 3).

Discussion

Relationship between Taxodiaceae and Cupressaceae

Our results show Taxodiaceae and Cupressaceae to be a monophyletic group that does not include *Sciadopitys*. Hart (1987) suggested that, based on morphological and chemical traits, Taxodiaceae and Cupressaceae form a single monophyletic family. Results from an investigation on the immunological similarity of seed proteins (Price and Lowenstein 1989) indicate no major discontinuity between Tax-

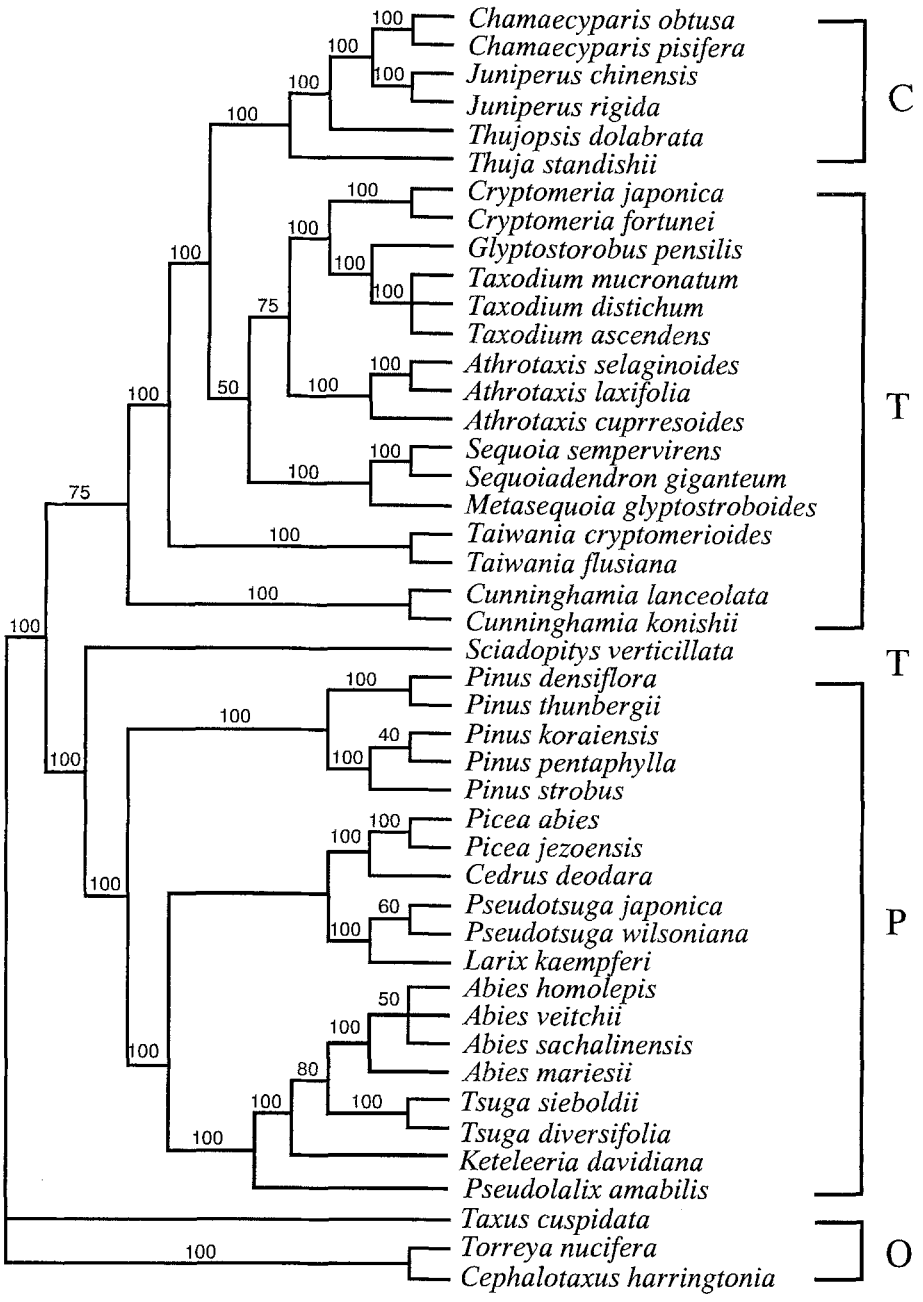

Fig. 2 The 50% major consensus tree obtained from 40 maximally parsimonious Wagner trees using RFLP analysis of six PCR-amplified chloroplast conifer genes. The *numbers* on a branch indicate the probability supporting that branch out of 40 maximally parsimonious trees "T" denotes traditional members of Taxodiaceae, "C" and "P" denote members of Cupressaceae and Pinaceae, respectively, and "O" are outgroups

odiaceae and Cupressaceae. Based on *rbc*L sequencing data, Chase et al. (1993) and Brunsfeld et al. (1994) also suggested that Taxodiaceae and Cupressaceae form a single monophyletic family. Our results suggest that *Sciadopitys* is a basal lineage of Taxodiaceae and Cupressaceae and that Cupressaceae diverged from Taxodiaceae. The molecular phylogeny using *rbc*L sequence data also suggests similar results (Chase et al. 1993; Brunsfeld et al. 1994).

Species positions within Taxodiaceae and Cupressaceae

Brunsfeld et al. (1994) stated that (1) *Cryptomeria* and *Glyptostrobus* form a group; (2) *Metasequoia*, *Sequoia* and *Sequoiadendron* also form a group; (3) *Cunninghamia* is the basal lineage of Taxodiaceae; and (4) although the positions of *Athrotaxis*, *Taxodium* and *Taiwania* are not fully resolved, at least *Athrotaxis* is basal to *Taxodium* and *Taiwania*. Our phylogenetic tree is basically similar to this tree, however, we determined that (1) the positions of *Taiwania* and *Taxodium* are different; (2) *Taiwania* lies between *Cunninghamia* and the other genera of Taxodiaceae, and (3) *Taxodium* forms a group with *Cryptomeria* and *Glyptostrobus*. In the Wagner tree, the *Cryptomeria*, *Glyptostrobus*, and *Taxodium* group, the *Athrotaxis* group, and the *Sequoia*, *Sequoiadendron*, and *Metasequoia* group are each considered to be well-defined groups. However, the divergence between them is not fully resolved by the

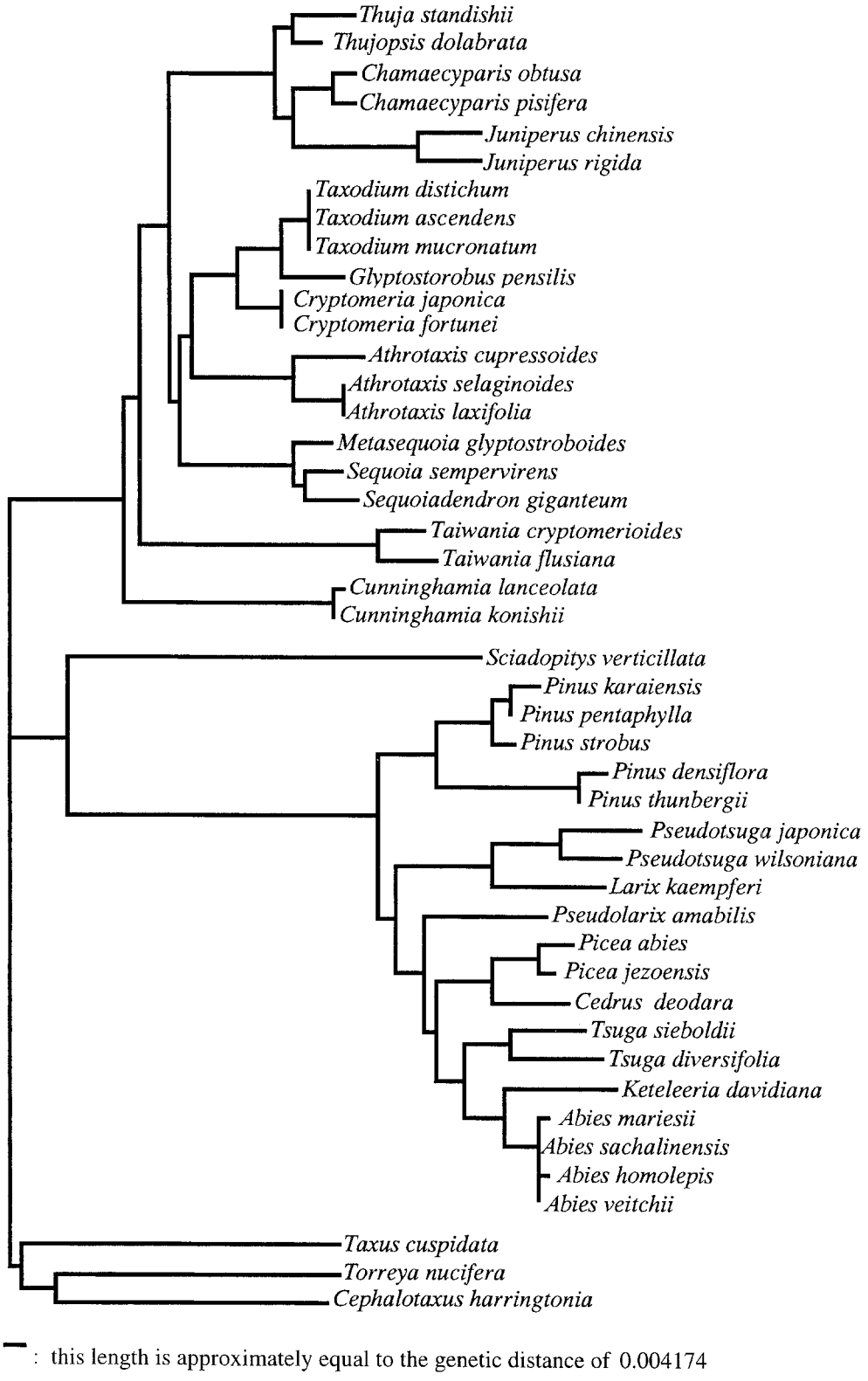

Fig. 3 The neighbor-joining tree constructed from RFLP analysis of six PCR-amplified genes in conifer chloroplast DNA

Wagner method because the divergence between the *Sequoia*, *Athrotaxis*, and *Cryptomeria* groups exhibit low reliability with probability values of 50 and 75, respectively (Fig. 2).

We consider the 3 *Taxodium* species to be closely related because they have exactly the same data sets in this analysis. There has been considerable disagreement with respect to the number of species recognized within *Taxodium* (Hart and Price 1990). The 3 varieties recognized are indistinguishable in reproductive characteristics and are continuously intergrading in morphological and phenological characteristics, although extremely pure populations

Table 3 Estimated percentage of nucleotide substitutions (below diagonal) and the number of changes of restriction sites (above diagonal) for 45 conifer species (as identified in Table 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	–	6	6	18	18	27	26	24	25	25	27	37	40	22	22	22	49	31	31	43	41	29	28
2	0.96	–	0	18	18	27	26	24	25	27	29	35	38	20	20	20	49	31	31	43	41	31	30
3	0.96	0.00	–	18	18	27	26	24	25	27	29	35	38	20	20	20	49	31	31	43	41	31	30
4	3.28	3.45	3.45	–	0	29	30	10	17	19	21	35	36	8	8	8	51	23	23	35	35	23	22
5	3.28	3.45	3.45	0.00	–	29	30	10	17	19	21	35	36	8	8	8	51	23	23	35	35	23	22
6	4.92	4.95	4.95	4.95	4.95	–	1	31	34	34	34	36	39	31	31	31	52	36	38	50	48	36	35
7	4.66	4.69	4.69	5.17	5.17	0.20	–	32	35	35	35	37	40	32	32	32	51	37	39	51	49	37	36
8	4.36	4.55	4.55	1.82	1.82	5.43	5.66	–	23	25	27	37	38	6	6	6	57	27	27	35	39	29	26
9	4.08	4.41	4.41	3.03	3.03	5.95	6.17	3.95	–	6	6	32	33	21	21	21	56	28	26	34	34	26	25
10	4.27	4.77	4.77	3.36	3.36	6.00	6.23	4.30	0.95	–	4	34	35	23	23	23	56	28	28	36	36	26	25
11	4.51	5.02	5.02	3.44	3.44	5.92	6.15	4.54	1.29	1.02	–	34	35	25	25	25	56	26	26	34	34	34	26
12	6.52	6.57	6.57	6.39	6.39	6.64	6.87	6.21	5.88	6.27	6.20	–	7	37	37	37	62	40	38	48	46	38	35
13	6.98	6.85	6.85	6.32	6.32	7.10	7.34	6.31	5.98	6.20	5.96	1.65	–	38	38	38	63	37	35	45	47	35	32
14	4.20	3.91	3.91	1.68	1.68	5.60	5.83	1.11	3.79	4.14	4.38	6.21	6.31	–	0	0	55	25	25	35	35	35	27
15	4.20	3.91	3.91	1.68	1.68	5.60	5.83	1.11	3.79	4.14	4.38	6.21	6.31	0.00	–	0	55	25	25	35	35	27	24
16	4.20	3.91	3.91	1.68	1.68	5.60	5.83	1.11	3.79	4.14	4.38	6.21	6.31	0.00	0.00	–	55	25	25	35	35	27	24
17	9.56	9.62	9.62	10.64	10.64	11.11	10.75	11.90	11.19	11.29	11.21	13.14	13.30	11.68	11.68	11.68	–	56	58	70	70	54	55
18	6.13	6.34	6.34	4.46	4.46	6.95	7.19	5.13	5.31	5.36	5.11	7.83	6.81	4.79	4.79	4.79	11.82	–	2	22	20	12	9
19	5.90	6.11	6.11	4.59	4.59	7.08	7.32	5.09	4.78	5.15	5.24	7.39	6.58	4.75	4.75	4.75	11.94	0.43	–	20	18	10	7
20	7.77	8.01	8.01	6.75	6.75	9.20	9.25	6.74	6.05	6.45	6.37	8.98	8.15	6.74	6.74	6.74	13.06	4.30	3.79	–	8	22	19
21	7.48	7.71	7.71	6.82	6.82	8.70	8.76	7.34	6.12	6.51	6.44	8.48	8.79	6.63	6.63	6.63	13.13	4.21	3.70	1.04	–	22	19
22	5.50	5.87	5.87	4.38	4.38	6.64	6.87	5.03	4.72	4.76	4.69	7.30	6.68	4.70	4.70	4.70	10.90	2.20	1.88	4.06	4.46	–	5
23	5.25	5.62	5.62	4.14	4.14	6.56	6.79	4.62	4.48	4.52	4.45	6.85	6.06	4.29	4.29	4.29	11.25	1.67	1.36	3.51	3.90	0.92	–
24	15.65	15.53	15.53	15.04	15.04	13.71	13.76	15.74	16.08	15.73	15.67	16.41	16.09	15.98	15.98	15.98	17.30	15.50	15.61	15.06	15.84	14.22	15.07
25	15.93	15.81	15.81	15.32	15.32	14.21	14.25	16.02	16.36	16.27	15.95	16.71	16.38	16.27	16.27	16.27	17.60	15.79	15.90	15.33	16.12	14.49	15.35
26	15.74	15.62	15.62	15.13	15.13	13.80	13.85	15.83	16.16	15.82	15.76	16.50	16.18	16.07	16.07	16.07	17.38	15.60	15.70	15.15	15.93	14.31	15.17
27	15.59	15.47	15.47	14.98	14.98	13.65	13.70	15.68	16.02	15.67	15.61	16.35	16.03	15.92	15.92	15.92	17.24	15.44	15.55	15.00	15.78	14.16	15.01
28	16.32	16.20	16.20	15.70	15.70	14.14	14.18	16.65	15.56	16.16	16.10	17.10	16.78	16.65	16.65	16.65	17.22	16.43	16.54	16.19	16.75	15.35	15.99
29	17.01	16.89	16.89	16.38	16.38	14.98	15.03	17.11	17.20	16.60	16.54	17.83	17.50	17.36	17.36	17.36	18.50	16.90	17.00	15.67	16.47	15.78	16.43
30	16.20	16.08	16.08	15.58	15.58	14.45	14.50	16.54	16.63	16.79	16.46	16.99	16.66	16.54	16.54	16.54	17.63	16.31	16.42	16.32	16.88	15.45	16.12
31	16.55	16.43	16.43	15.93	15.93	14.58	14.63	16.89	15.78	16.64	16.32	17.34	17.01	16.89	16.89	16.89	17.19	16.67	16.78	16.42	16.98	15.57	16.22
32	16.29	16.17	16.17	15.68	15.68	14.34	14.39	16.63	15.53	16.39	16.07	17.07	16.75	16.63	16.63	16.63	16.93	16.41	16.51	16.17	16.72	15.32	15.96
33	17.08	16.70	16.70	16.45	16.45	15.52	15.57	17.44	16.53	17.18	16.85	17.91	17.85	17.44	17.44	17.44	18.84	17.24	17.34	15.98	16.54	15.85	16.76
34	16.39	16.27	16.27	15.01	15.01	14.37	14.42	15.95	16.58	16.47	16.41	17.19	16.86	15.95	15.95	15.95	18.10	16.51	16.62	15.53	16.09	15.15	16.05
35	16.28	16.16	16.16	14.90	14.90	14.04	14.08	15.84	16.47	16.37	16.30	17.09	16.75	15.84	15.84	15.84	18.00	16.40	16.51	15.42	15.98	15.04	15.94
36	15.67	15.54	15.54	14.31	14.31	13.46	13.51	15.24	15.86	15.75	15.69	16.45	16.12	15.24	15.24	15.24	17.60	15.77	15.88	15.54	16.11	14.44	15.32
37	16.96	16.58	16.58	16.33	16.33	15.40	15.45	17.32	16.41	17.06	16.73	17.79	17.46	17.32	17.32	17.32	18.72	17.12	17.22	15.86	16.42	15.73	16.64
38	16.11	15.99	15.99	15.50	15.50	14.38	14.43	16.20	16.29	16.21	15.89	16.89	16.56	16.45	16.45	16.45	18.05	15.97	16.08	15.51	16.30	14.90	15.53
39	16.40	16.28	16.28	15.79	15.79	14.45	14.50	16.74	16.59	16.74	16.42	17.19	16.68	16.74	16.74	16.74	17.31	16.52	16.63	17.26	17.83	15.44	16.07
40	16.14	16.02	16.02	15.53	15.53	14.19	14.23	16.48	16.33	16.48	16.16	16.92	16.60	16.48	16.48	16.48	16.78	16.26	16.36	17.00	17.57	15.17	15.81
41	17.06	16.94	16.94	16.43	16.43	15.27	15.31	17.16	17.49	17.41	17.08	17.62	17.29	17.41	17.41	17.41	18.82	16.94	17.05	16.44	17.25	15.82	16.48
42	17.03	16.91	16.91	16.39	16.39	15.22	15.27	17.13	16.72	17.38	17.05	17.60	17.27	17.39	17.39	17.39	18.52	16.92	17.02	16.40	17.22	15.78	16.44
43	7.17	7.75	7.75	7.39	7.39	6.92	6.64	7.92	7.72	7.79	7.71	8.51	8.81	7.74	7.74	7.74	10.13	8.68	8.80	9.82	9.70	8.32	7.87
44	7.72	6.89	6.89	7.95	7.95	8.20	7.89	7.94	8.10	8.17	8.09	9.11	9.04	7.22	7.22	7.22	10.98	7.57	7.70	9.47	9.35	7.60	7.16
45	7.51	7.19	7.19	6.47	6.47	7.25	7.31	6.82	7.35	7.60	7.52	6.72	8.45	6.11	6.11	6.11	11.05	7.54	7.67	9.89	9.56	7.39	6.94

Table 4 The amplified fragment size, the surveyed sequence, the mean estimates of nucleotide substitutions (%), and the corresponding standard deviations in six chloroplast genes

Gene	Fragment size (bp)	Surveyed sequence[a]	Site change	Nucleotide substitution (%)	Standard deviation
*frx*C	779	127 bp (16.30%)	23	8.92	0.0031
*rbc*L	1387	241 bp (17.36%)	48	8.35	0.0021
*psb*A	939	123 bp (13.10%)	26	8.30	0.0028
*psb*D	1042	167 bp (16.03%)	38	11.0	0.0032
*trn*K	2569	310 bp (12.07%)	67	28.47	0.0261
16S	1375	141 bp (10.25%)	25	11.17	0.0057
Total	8091	1109 bp (13.71%)	227	10.81	0.0033

[a] Surveyed sequence means the surveyed number of nucleotide sequence and the percentage against the amplified fragment sizes, which was inferred from restriction endonuclease analysis

appear to be morphologically and ecologically distinct (Watson 1985). We used 3 species but didn't detect any differences between the species; therefore, based on cpDNA, the 3 *Taxodium* varieties can be considered to be genetically very similar. *Taxodium* has often been thought to be closely related to *Glyptostrobus*, which is similar in its habitat, possession of knees, pattern of cone-scale development, relatively large number of cotyledons, winter-deciduous branchlets, and immunological characteristics (Hart and Price 1990; Price and Lowenstein 1989). Our results

Table 3 Continued

24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
87	88	86	86	87	94	90	90	89	92	88	86	84	90	85	92	89	93	90	42	44	45
87	88	86	86	87	94	90	90	89	92	88	86	84	90	85	92	89	93	90	44	38	41
87	88	86	86	87	94	90	90	89	92	88	86	84	90	85	92	89	93	90	44	38	41
87	88	86	86	87	94	90	88	87	92	84	82	80	90	85	92	87	93	90	42	44	41
87	88	86	86	87	94	90	88	87	92	84	82	80	90	85	92	87	93	90	42	44	41
82	83	81	81	82	89	85	85	84	87	83	81	79	85	80	87	84	88	85	39	43	42
83	84	82	82	83	90	86	86	85	88	84	82	80	86	81	88	85	89	86	38	42	43
93	94	92	92	93	100	96	94	93	98	90	88	86	96	91	98	83	99	96	44	44	43
92	93	91	91	88	99	95	91	90	95	93	91	89	93	90	97	94	98	93	45	47	46
90	91	89	89	90	95	95	93	92	95	93	91	89	93	88	97	94	96	93	45	47	48
90	91	89	89	90	95	95	93	92	95	93	91	89	93	88	97	94	96	93	45	47	48
88	89	87	87	88	95	91	91	90	93	89	87	85	91	86	93	90	94	91	47	51	48
87	88	86	86	87	94	90	90	89	92	88	86	84	90	85	92	89	93	90	50	52	49
93	94	92	92	93	100	96	94	93	98	90	88	86	96	91	98	93	99	96	42	40	39
93	94	92	92	93	100	96	94	93	98	90	88	86	96	91	98	93	99	96	42	40	39
93	94	92	92	93	100	96	94	93	98	90	88	86	96	91	98	93	99	96	42	40	39
90	91	89	89	92	97	91	89	88	91	89	87	85	89	90	93	88	96	91	55	57	58
84	85	83	83	86	91	89	89	88	89	87	85	83	87	82	91	88	90	87	47	43	44
86	87	85	85	88	93	91	91	90	91	89	87	85	89	84	93	90	92	89	49	45	46
92	93	91	91	94	97	97	97	96	95	93	91	91	93	90	101	98	98	95	57	53	56
90	91	89	89	92	95	95	95	94	93	91	89	89	91	88	99	96	96	93	57	53	54
82	83	81	81	84	89	87	87	86	87	85	83	81	85	80	89	86	88	85	47	45	46
85	86	84	84	87	92	90	90	89	90	88	86	84	88	83	92	89	91	88	44	42	43
–	3	1	1	24	15	33	27	28	31	23	25	27	31	22	29	32	20	19	91	99	86
0.57	–	2	2	23	16	32	26	25	32	22	24	26	30	19	28	31	19	20	92	100	87
0.76	0.94	–	0	23	14	32	26	27	32	22	24	26	30	21	28	31	19	18	90	98	85
0.19	0.38	0.57	–	23	14	32	26	27	32	22	24	26	30	21	28	31	19	18	90	98	85
2.94	2.86	3.47	2.88	–	21	23	3	4	25	19	17	19	23	16	17	14	22	21	87	97	81
2.55	2.87	1.86	2.49	2.97	–	28	24	23	28	20	22	22	26	19	22	27	19	20	94	98	91
5.33	5.07	5.29	5.27	4.56	5.04	–	27	27	34	26	24	24	32	29	18	19	29	32	94	94	87
4.64	4.40	4.46	4.58	1.49	3.49	4.70	–	3	28	20	18	20	26	19	20	13	25	24	90	100	87
4.49	4.11	4.31	4.42	1.36	3.80	4.69	0.37	–	29	21	19	27	16	19	12	26	25	25	89	99	86
5.60	5.66	5.41	5.85	4.83	5.32	6.14	4.97	4.96	–	20	18	20	2	27	30	33	27	32	92	100	93
4.31	4.21	3.98	4.25	4.01	3.75	4.97	3.72	3.57	3.61	–	2	4	18	21	26	23	23	24	86	98	87
4.79	4.69	4.45	4.73	3.60	4.22	4.55	3.32	3.17	3.35	0.39	–	2	16	21	24	21	25	24	84	96	85
5.28	5.18	4.93	5.22	4.06	4.24	4.57	3.77	3.62	3.81	0.78	0.39	–	18	21	22	19	27	26	82	94	83
5.79	5.38	5.13	5.57	4.70	5.04	6.01	4.85	4.84	0.38	3.34	3.08	3.53	–	25	28	31	25	30	90	98	91
3.44	2.80	3.00	3.24	3.44	3.20	5.10	3.85	3.30	4.78	3.53	3.98	4.45	4.51	–	23	22	20	21	87	95	82
5.34	5.10	4.57	5.28	3.33	3.63	3.11	3.60	3.59	5.53	4.69	4.29	3.87	5.41	4.40	–	7	21	28	92	104	89
5.81	5.56	5.62	5.75	3.73	4.62	3.37	2.50	2.49	6.16	4.26	3.85	3.43	6.04	4.54	1.05	–	28	29	89	101	86
3.86	3.49	2.99	3.65	4.14	3.33	5.40	4.28	4.42	4.77	4.24	4.56	5.04	4.35	3.11	3.84	5.13	–	11	95	105	92
3.71	3.75	3.39	3.50	3.56	3.74	5.72	4.13	4.12	5.53	4.68	4.56	5.05	5.10	3.94	5.13	5.44	1.85	–	90	100	87
16.05	16.33	16.14	15.99	15.05	16.17	16.11	15.27	15.03	16.72	15.56	15.21	14.62	16.60	15.77	15.14	14.88	16.94	16.65	–	46	37
17.64	17.69	17.47	17.33	17.28	16.75	16.19	17.52	17.25	17.81	17.64	17.54	16.90	17.44	16.83	17.62	17.36	18.84	18.55	7.36	–	35
15.87	16.17	15.97	15.81	15.09	16.49	14.95	15.31	15.06	17.85	16.11	15.75	15.13	17.46	15.35	15.18	14.90	16.79	16.50	6.42	5.92	–

also support the relationship that *Taxodium* is most similar to *Glyptostrobus* and *Cryptomeria*.

On the basis of morphological traits, *Athrotaxis laxifolia* is said to be most certainly a hybrid between *A. selaginoides* and *A. cupressoides* (Cullen and Kirkpatrick 1988). In our study, data on *A. laxifolia* coincided with *A. selaginoides* completely. The difference between *A. cupressoides* and the other 2 species was 6 site changes. Chloroplast DNA in coniferous species has been reported to be paternally inherited (Neale et al. 1986, 1989, 1991; Szmidt et al. 1987; Neale and Sederoff 1989; Stine et al. 1989; Sutton et al. 1991). Thus, as shown by our data, if *A. laxifolia* is the hybrid between *A. selaginoides* and *A. cupressoides* and if cpDNA is paternally inherited, then *A. selaginoides* must be a pollen donor for *A. laxifolia*.

The difference between the 2 *Cunninghamia* species is only 1 site change, the *Bgl*II site in the *rbc*L gene. Within the *Taiwania* species, we detected 7 site differences, 1 in *frx*C, 2 in *rbc*L, 3 in *trn*K, and 1 in 16S.

Sequoia and *Sequoiadendron* have 4 site differences, the *Hha*I site in *psb*D, 2 *Dra*I sites in *trn*K, and the *Rsa*I site in 16S. The tree based on *rbc*L sequence data also indicated that these are closely related species (Brunsfeld et al. 1994) and immunological data showed similar results (Price and Lowenstein 1989).

We investigated only 6 species of four genera of Cupressaceae, these were found to be closely related to the Taxodiaceae species, an observation supported by morphological traits (Hart 1987) and *rbc*L sequence data (Brunsfeld et al. 1994).

The position of Sciadopitys

Sciadopitys has often been classified as a morphologically isolated species of Taxodiaceae or as the separate family Sciadopityaceae (Hayata 1931; Hart 1987; Schlarbaum and Tsuchiya 1985). However, *Sciadopitys* is closely related to

Cupressaceae and Taxodiaceae in some morphological traits (Hart and Price 1990). Its chromosome number is n=10, which suggests that it apparently branched from its ancestors Cupressaceae and Taxodiaceae (n=11) by aneuploid reduction (Schlarbaum and Tsuchiya 1985). Using *rbc*L sequence data, Chase et al. (1993) and Brunsfeld et al. (1994) concluded that *Sciadopitys* is not closely related to Taxodiaceae and Cupressaceae and should be excluded from the family on the basis of molecular phylogeny. An immunological comparison between Taxodiaceae and Cupressaceae species indicated that *Sciadopitys* is quite distant immunologically from all of the other groups examined and is as distant from the cupressaceous-taxodiaceous group as the other families are from one another (Price and Lowenstein 1989). Our results also show that *Sciadopitys* is quite different from Taxodiaceae and the other families. In our tree, based on cpDNA, *Sciadopitys* is not included in Taxodiaceae but is considered to be a basal lineage of Taxodiaceae and Cupressaceae.

Phylogenetic relationship within Pinaceae

Pinaceae is also considered to be a monophyletic group and is separated into two major sub-groups, *Pinus* and the other genera. Genus *Pinus* is also clearly separated into two groups, diploxylon and haploxylon. Hart (1987) suggested that the monophyly of the Pinaceae is well-established. However, our data do not completely support his results in the position of each genus. Hart (1987) concluded that the grouping of Pinaceae into two lineages is based on only a few characteristics: the presence of resin ducts in the seeds and of cleavage polyembryony supports the monophyly of *Abies*, *Pseudolarix*, *Keteleeria*, *Cedrus*, and *Tsuga*, while resin ducts in the secondary wood and leaves with an endodermis having a thickened Casparian strip support the monophyly of *Cathaya*, *Pinus*, *Larix*, *Pseudotsuga*, and *Picea*. Immunological comparison data showed that the Abietoid group (*Keteleeria*, *Abies*, *Cedrus*, *Tsuga*, and *Pseudolarix*) is clearly separate from the Pinoid group (*Picea*, *Pinus*, *Larix*, and *Pseudotsuga*) (Price et al. 1987). The tree based on *rbc*L sequence data also supports this, except for *Cedrus*. In our tree, *Abies*, *Tsuga*, *Keteleeria*, and *Pseudotsuga* are of the same group, but the other 2 groups (*Pinus* species and *Picea*, *Cedrus*, *Pseudotsuga*, and *Larix* species) are not supported by our results. Thus, in our phylogenetic tree the placement of these latter 2 groups were not completely supported by the results of Hart (1987), Price et al. (1987), and Brunsfeld et al. (1994). When the Wagner and NJ trees are compared, the divergence between the Picea-Cedrus group and the *Abies*, *Tsuga*, *Keteleeria*, and *Pseudolarix* group is not fully resolved, but the *Abies*, *Tsuga*, *Keteleeria* and *Pseudolarix* group is considered to be well-defined.

PCR-RFLP method for molecular phylogeny

The PCR-RFLP method is very effective and convenient for the determination of molecular phylogeny (Liston 1992) for if we have some primers to amplify the specific regions, we can survey many species simultaneously and investigate many regions conveniently. Currently, many sequencing data are available from the database; for example, whole chloroplast (Ohyama et al. 1986; Shinozaki et al. 1986; Hiratsuka et al. 1989; Wakasugi et al. 1994) and mitochondrial sequence data (Oda et al. 1992) and much nuclear gene sequence data. When sequencing methods are used for molecular phylogeny, it is not so easy to survey many genes simultaneously. When using the PCR-RFLP method, we can also choose the target region depending on the level of classification. Meanwhile, we can choose a highly stable region like the coding region of mitochondrial genes or chloroplast genes when we survey the relationship between a genus or family, and when we survey within a genus (as with closely related species), we can choose the spacer regions between coding regions.

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Appendix Change at restriction sites of six chloroplast genes in 45 coniferous species

Gene	Enzyme		Site change (bp ± 5%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
frxC	*Sau*3AI		165 – 115 + 50[a]	1[b]	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1	1	0	1	1	1	1	0	1	1	1
	*Taq*I	1	779 – 422 + 357	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	422 – 335 + 87[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	779 – 619 + 160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0	1	1	1	1	1
		4	619 – 589 + 30[a]	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	619 – 379 + 240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Xba*I	1	779 – 629 + 150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	779 – 542 + 237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0
		3	237 – 160 + 77[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0
	*Sty*I		779 – 605 + 174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Sca*I		130*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Rsa*I	1	280 – 241 + 39[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	241 – 210 + 31[a]	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	*Msp*I		779 – 506 + 273	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Hin*fI	1	779 – 755 + 24[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
		2	755 – 419 + 336	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	755 – 615 + 140	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
		4	316 – 210 + 106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0
		5	336 – 316 + 20[a]	1	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		6	615 – 570 + 45[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	*Eco*RV		779 – 619 + 160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Hae*III	1	779 – 520 + 259	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		2	779 – 577 + 202	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
*psb*A	*Msp*I	1	939 – 766 + 173	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	173 – 140 + 33[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
		3	173 – 106 + 67[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	1
	*Rsa*I		468 – 400 + 68[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Sau*3AI	1	939 – 605 + 334	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	605 – 485 + 120	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		3	334 – 229 + 105	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		4	605 – 413 + 192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		5	413 – 366 + 47[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		6	385 – 265 + 120	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	485 – 385 + 100	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		8	120*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Taq*I	1	939 – 697 + 242	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	939 – 599 + 340	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	*Sty*I		554 – 324 + 230	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
			230 – 120 + 110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Hae*III	1	235 – 168 + 67[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	0	0	1	0
		2	168 – 98 + 70[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
		3	939 – 704 + 235	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		4	704 – 424 + 280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	235 – 120 + 115	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	0	1
	*Hin*fI	1	315 – 209 + 106	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
		2	624 – 461 + 163	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	461 – 421 + 40[a]	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		4	939 – 624 + 315	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	1

Appendix Continued 1

Identification number (see Table 1), columns 1–23:

Gene	Enzyme		Site change (bp ± 5%)			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	*Hind*III		939 –	504 +	435	1	1	1	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1
*psb*D	*Hae*III	1	845 –	545 +	300	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0
		2	545 –	360 +	185	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
		3	1042 –	845 +	197	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1
		4	845 –	708 +	137	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Hin*fI	1	450 –	330 +	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
		2	330 –	220 +	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
		3	592 –	310 +	282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	1042 –	592 +	450	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Msp*I	1	492 –	392 +	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	712 –	492 +	220	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
		3	110*			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	330 –	220 +	110	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Sau*3AI	1	542 –	482 +	60[a]	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1
		2	482 –	440 +	42[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	*Sty*I	1	1042 –	932 +	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		2	1042 –	562 +	480	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	1042 –	742 +	300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Alu*I	1	872 –	532 +	340	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
		2	340 –	180 +	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	532 –	332 +	200	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
		4	532 –	382 +	150	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Rsa*I	1	1042 –	622 +	420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
		2	420 –	260 +	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
		3	622 –	392 +	230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Bsm*I	1	1042 –	612 +	430	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	430 –	250 +	180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Hha*I	1	1042 –	621 +	421	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		2	1042 –	892 +	150	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
		3	892 –	532 +	360	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1
		4	360 –	200 +	160	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	1042 –	782 +	260	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	1042 –	712 +	330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	260 –	130 +	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Taq*I	1	1042 –	772 +	270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		2	1042 –	632 +	410	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
		3	632 –	492 +	140	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
		4	410 –	370 +	40[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	632 –	382 +	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
*rbc*I	*Hae*III	1	1387 –	863 +	524	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	863 –	449 +	414	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1
		3	863 –	618 +	245	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
		4	863 –	536 +	327	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		5	863 –	478 +	385	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	478 –	300 +	178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	478 –	366 +	112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8	366 –	319 +	47[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		9	385 –	265 +	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Identification number (see Table 1), columns 24–45:

Gene	Enzyme		Site change (bp ± 5%)			24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
	*Hind*III		939 –	504 +	435	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1
*psb*D	*Hae*III	1	845 –	545 +	300	0	0	0	0	1	0	1	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1
		2	545 –	360 +	185	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	1042 –	845 +	197	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		4	845 –	708 +	137	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	*Hin*fI	1	450 –	330 +	120	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	330 –	220 +	110	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	1	1	0	0	0
		3	592 –	310 +	282	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
		4	1042 –	592 +	450	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
	*Msp*I	1	492 –	392 +	100	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0
		2	712 –	492 +	220	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
		3	110*			0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	330 –	220 +	110	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
	*Sau*3AI	1	542 –	482 +	60[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
		2	482 –	440 +	42[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Sty*I	1	1042 –	932 +	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
		2	1042 –	562 +	480	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
		3	1042 –	742 +	300	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Alu*I	1	872 –	532 +	340	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
		2	340 –	180 +	160	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	1
		3	532 –	332 +	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	532 –	382 +	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Rsa*I	1	1042 –	622 +	420	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	420 –	260 +	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	622 –	392 +	230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1
	*Bsm*I	1	1042 –	612 +	430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	430 –	250 +	180	1	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	*Hha*I	1	1042 –	621 +	421	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
		2	1042 –	892 +	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	892 –	532 +	360	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	360 –	200 +	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	1042 –	782 +	260	1	1	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0
		6	1042 –	712 +	330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		7	260 –	130 +	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	*Taq*I	1	1042 –	772 +	270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	1042 –	632 +	410	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	632 –	492 +	140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		4	410 –	370 +	40[a]	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0
		5	632 –	382 +	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
*rbc*I	*Hae*III	1	1387 –	863 +	524	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	863 –	449 +	414	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		3	863 –	618 +	245	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	863 –	536 +	327	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		5	863 –	478 +	385	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		6	478 –	300 +	178	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		7	478 –	366 +	112	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0
		8	366 –	319 +	47[a]	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0
		9	385 –	265 +	120	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0

Appendix Continued 2

Gene	Enzyme		Site change (bp ± 5%)	Identification number (see Table 1)																																								
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
	*Bgl*III	1	1175 – 860 + 315	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		2	860 – 767 + 93[a]	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
		3	1387 – 1175 + 212	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	*Bam*HI		1387 – 971 + 416	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	*Msp*I	1	1387 – 1199 + 188	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	1199 – 774 + 425	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	188 – 137 + 51[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0
		5	1114 – 1035 + 79[a]	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		6	1387 – 1114 + 273	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
		7	425 – 280 + 145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	*Pst*I		1387 – 732 + 655	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Rsa*I	1	296 – 209 + 87[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	0	1	1	1	0	1	0	0	0	1	1	0	0	0
		2	422 – 219 + 203	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	252 – 203 + 49[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
		4	192 – 143 + 49[a]	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	*Sau*3AI	1	414 – 330 + 84[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		2	738 – 414 + 324	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	0	1	1
		3	414 – 229 + 185	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
		4	137[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	267 – 137 + 130	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	*Sty*I	1	599 – 444 + 155	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		2	1228 – 629 + 599	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1
		3	1387 – 1228 + 159	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Xba*I		1387 – 1269 + 118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	*Hha*I	1	501 – 428 + 73[a]	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		2	501 – 356 + 145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	676 – 528 + 148	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	749 – 501 + 248	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
		5	1387 – 749 + 638	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	*Taq*I	1	316 – 189 + 127	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	1387 – 1071 + 316	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	1260 – 1076 + 184	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	1061 – 471 + 590	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0
		5	471 – 312 + 159	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	590 – 267 + 323	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
		7	260 – 230 + 30[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
		8	323 – 260 + 63[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0
		9	267 – 147 + 120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	1064 – 540 + 524	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
*trn*K	*Eco*RV	1	2569 – 2019 + 550	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	2019 – 1709 + 310	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0
		3	2019 – 1529 + 490	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1	0	0	0
		4	2019 – 1319 + 700	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0
		5	2569 – 2309 + 260	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	2309 – 1589 + 720	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	2039 – 1739 + 300	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		8	2569 – 2039 + 530	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		9	550 – 330 + 220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Appendix Continued 3

Gene	Enzyme		Site change (bp ± 5%)	Identification number (see Table 1)																																										
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
	*Bam*HI	1	2569 – 1329 + 1240	1	1	1	0	0	1	1	0	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0
		2	1240 – 940 + 300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
		3	940 – 500 + 440	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		4	440 – 280 + 160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0
		5	500 – 470 + 30[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	1329 – 809 + 520	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	1329 – 1069 + 260	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		8	520 – 420 + 100	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		9	1329 – 1029 + 300	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	1029 – 649 + 380	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Sty*I	1	2569 – 1889 + 680	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	680 – 420 + 260	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
		3	2569 – 1989 + 580	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	2569 – 2169 + 400	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	2569 – 2289 + 280	0	0	0	0	0	1	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	2569 – 2329 + 240	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	2569 – 2429 + 140	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8	1989 – 1799 + 190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Sca*I	1	2569 – 2049 + 520	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0
		2	2569 – 2079 + 490	1	0	0	1	1	0	0	1	1	1	1	0	0	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
		3	2569 – 2119 + 450	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	2569 – 2169 + 400	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	2569 – 1329 + 1240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	2079 – 1749 + 330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	2169 – 2029 + 140	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	*Msp*I	1	2569 – 1294 + 1275	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	0
		2	1294 – 1063 + 231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	1063 – 577 + 486	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0
		4	577 – 486 + 91*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		5	1275 – 670 + 605	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
		6	1275 – 925 + 350	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		7	2569 – 1469 + 1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
		8	1469 – 750 + 719	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		9	719 – 490 + 229	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
		11	490 – 380 + 110	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		12	1100 – 760 + 340	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		13	2340 – 1360 + 980	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		14	229 – 180 + 49*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	*Dra*I	1	2569 – 2199 + 370	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	1	0	1	1	1	0	0	0	0
		2	2199 – 1379 + 820	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
		3	2569 – 1889 + 680	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		4	2569 – 1569 + 1000	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	1000 – 730 + 270	0	1	1	1	1	0	0	1	1	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		6	2569 – 1689 + 880	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		7	2569 – 2179 + 390	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8	1529 – 1379 + 150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix Continued 4

Gene	Enzyme		Site change (bp ± 5%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
		9	1729 – 880 + 849	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		11	849 – 740 + 100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		12	2569 – 1409 + 1160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	*Bsm*I	1	2569 – 1389 + 1180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		2	1389 – 1059 + 330	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		3	1059 – 829 + 230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		4	2569 – 1480 + 1089	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
		5	1480 – 1040 + 440	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	440 – 270 + 170	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	2569 – 1569 + 1000	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		8	1089 – 740 + 349	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		9	1040 – 550 + 490	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16S	*Hha*I	1	425 – 250 + 175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
		2	410 – 260 + 150	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		3	965 – 710 + 255	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		4	965 – 880 + 85[a]	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	880 – 540 + 340	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	*Hin*fI	1	644 – 514 + 130	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		2	217 – 123 + 94[a]	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	185 – 125 + 60[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Eco*RI	1	1375 – 1215 + 160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
		2	1225 – 765 + 460	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	150 – 120 + 30[a]	0	0	0	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	150 – 80 + 70[a]	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		5	1375 – 1315 + 60[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
		6	1375 – 1225 + 150	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	*Sau*3AI		180 – 160 + 20[a]	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Sma*I		1375 – 1225 + 150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
	*Taq*I		555 – 470 + 85[a]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	*Rsa*I	1	1375 – 1255 + 120	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
		2	520 – 440 + 80[a]	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		3	755 – 520 + 235	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
		4	520 – 500 + 20[a]	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
		6	235 – 200 + 35[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		7	235 – 180 + 55[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		8	500 – 380 + 120	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Identification number: see Table 1.

[a] At least one change was confirmed, but the resultant fragments were not recognized on a 2% agarose gel because the sizes of fragment were too small

[b] 0 or 1 indicates that a restriction site was absent or present, respectively