CHAPTER 4

RESULTS

4.1 DNA extraction

DNeasy® Plant MiniKit (QIAGEN) was firstly used to extract DNA from dry leaf specimens. Genomic DNA of five *Cassia* specimens (*C. fistula, C. bakeriana, C. javanica* and *C. spectabilis*) were extracted. The extracted DNA were in good quality after checked with 0.8% agarose gel electrophoresis. However, the DNA of *C. spectabilis* was not able to yield any PCR products, although its amplifications had been attempted for three times and the plant was even reextracted with the QIAGEN kit. Nucleospin® DNA miniKit then was subsequently chosen to solve this problem. This was because the Nucleospin kit contains an extraction buffer claimed to be specific to legume plants (i.e. *Pisum sativum*). Fortunately, the extracted genomic DNA of *C. spectabilis* using the Nucleospin kit was successly amplified, given the PCR product visualisable on 0.8% agarose gel electrophoresis. By this mean, all remaining specimens were extracted by the Nucleospin kit. So far, genomic DNA extracted with the Nucleospin® DNA miniKit showed as good quality as that with the DNeasy® Plant MiniKit. Note that although smear DNA bands caused by DNA fragmentation along extraction occurred in most samples, no amplification problem was found. Although, the genomic DNA bands of *C. pumila* and *C. leschenaultiana* could not be seen with gel electrophoresis, these DNA could nicely give PCR amplified products.

4.2 PCR amplification

4.2.1 PCR amplification of *trn*L intron

Amplifications of *trn*L intron regions of all *Cassia* specimens were performed using the *trn*L-C primer as a forward primer and the *trn*L-D primer as a reverse primer. The size of PCR products was around 600 bp compared to 100 bp ladder standard marker. Only *C. siamea* was not able to be amplifed while the remaining specimens were easy to get PCR products. Primer dimers occurred in all of these samples and the control reaction (without DNA added). None of non-specific band was found in any lanes (Fig.15).

Fig. 15 PCR products of *trn*L intron (size around 600 bp) compared with 100 bp ladder marker.

M = 1 kb ladder marker
1 = *C. javanica*
2 = *C. spectabilis*
3 = *C. bakeriana*
4 = *C. fistula*
c = control (blank)
5 = *C. surattensis*
6 = *C. pumila*
7 = *C. leschenaultiana*

4.2.2 PCR amplification of ITS regions

To amplify both ITS1 and ITS2 regions included 5.8S subunit all together, the 5P primer was used as a forward primer and the 8P (or 4P for *C. timoriensis*) primer as a reverse primer. The size of PCR products was around 800 bp compared to standard marker. Non-specific band was not found from all amplification. There were four *Cassia* not successfully amplified: *C. siamea, C. garrettiana, C. pumila* and *C. leschenaultiana*. Other remaining species were amplifiable except *C. timoriensis* which was necessary to use the 4P primer instead of the 8P as a reverse primer and also had to decrese the annealing temperature from 55°C to 50°C in the PCR programme. Primer dimers were not found in all samples and the control reaction (Fig. 16).

Fig. 16 PCR products of ITS regions of nrDNA (~800bp) compared with 100 bp ladder marker

M = 1 kb ladder marker
1 = *C. grandis*
2 = *C. javanica*
3 = *C. spectabilis*
4 = *C. tora*

5 = *C. sophera*
6 = *C. surattensis*
7 = *C. bakeriana*
8 = *C. fistula*
9 = *C. alata*

10 = *C. hirsuta*
11 = *C. occidenatalis*
12 = *C. obtusifolia*
c = control

4.3 PCR product purification and DNA sequencing

QIAquick® PCR Purification Kit (QIAGEN) was used to purify the PCR samples which had no or few primer-dimer. Thirteen samples found having primer-dimers were purified with QIAquick® gel extraction (QIAGEN) with additional gel-slicing procedure. These *Cassia* samples yeilded *trn*L intron PCR products of *C. grandis*, *C. bakeriana*, *C. tora*, *C. sophera*, *C. surattensis*, *C. javanica*, *C. spectabilis*, *C. fistula*, *C. alata*, *C. hirsuta*, *C. occidentalis*, *C. obtusifolia* and *C. pumila.* The purified PCR product was clean and ready to sequence (Fig. 17). After DNA sequencing was performed in both forward and reverse directions, Chromas computer program was used to compare each couple of the complementary sequences (Fig. 18 and Fig. 19). Most electropherogram graphs appeared to be clear without contaminated sequence signals from other plant samples or primer-dimers. Although the *trn*L intron and ITS sequences of almost all samples region were clear and easy to analyse, the sequence results of ITS regions of *C. tora, C. bakeriana, C. obtusifolia* and *C. surattensis* were not clear in both forward and reverse directions (Fig. 20). Sequencing experiments were redone several times for these particularly species but not only in the case of *C. surattensis.* In this case, multiple copies of ITS were strong, but re-sequencing and comparing base by base each time could help to get an accurately data. The DNA sequences were converted to FASTA format (Fig. 21) before further analysed with Clustal X alignment program. Note that band of *C. grandis* was not equally to other members because of the shortest sequences of this species while compared to the others.

Among *trn*L intron sequences of all *Cassia*, the sequence of *C. leschenaultiana* was the longest one with 607 bp long while that of *C. grandis* was the shortest with 548 bp. For ITS sequences, without 16S and 28S regions, the longest total ITS1-5.8S-ITS2 sequence was that of *C. bakeriana* (674 bp) and the shortest one was to *C. grandis* (560bp). Average lengths of both sequence regions were 570 bp and 645 bp (*trn*L intron and ITS regions, respectively). Moreover, these two regions had different characteristics. The *trn*L intron, sequences were easily alignable because they had high similarity to each other in all taxa while alignment of the ITS sequences were much more difficult. GCcontent percentage of each sequence was also observed from nucleotide comparison. The percentages of GC contents of *trn*L intron sequences were range from 35.03% in *C. sophera* and *C. spectabilis* to 36.80% in *C. grandis*. In ITS regions, %GC contents were from 51.61% in *C. grandis* to 62.46% in *C. spectabilis*.

Fig. 17 purified PCR product of *trn*L intron compare with 100 bp ladder marker.

M = 1 kb ladder marker	1 = *C. obtusifolia*	2 = *C. occidentalis*
3 = *C. hirsuta*	4 = *C. alata*	5 = *C. fistula*
6 = *C. bakeriana*	7 = *C. spectabilis*	8 = *C. javanica*
c = control		

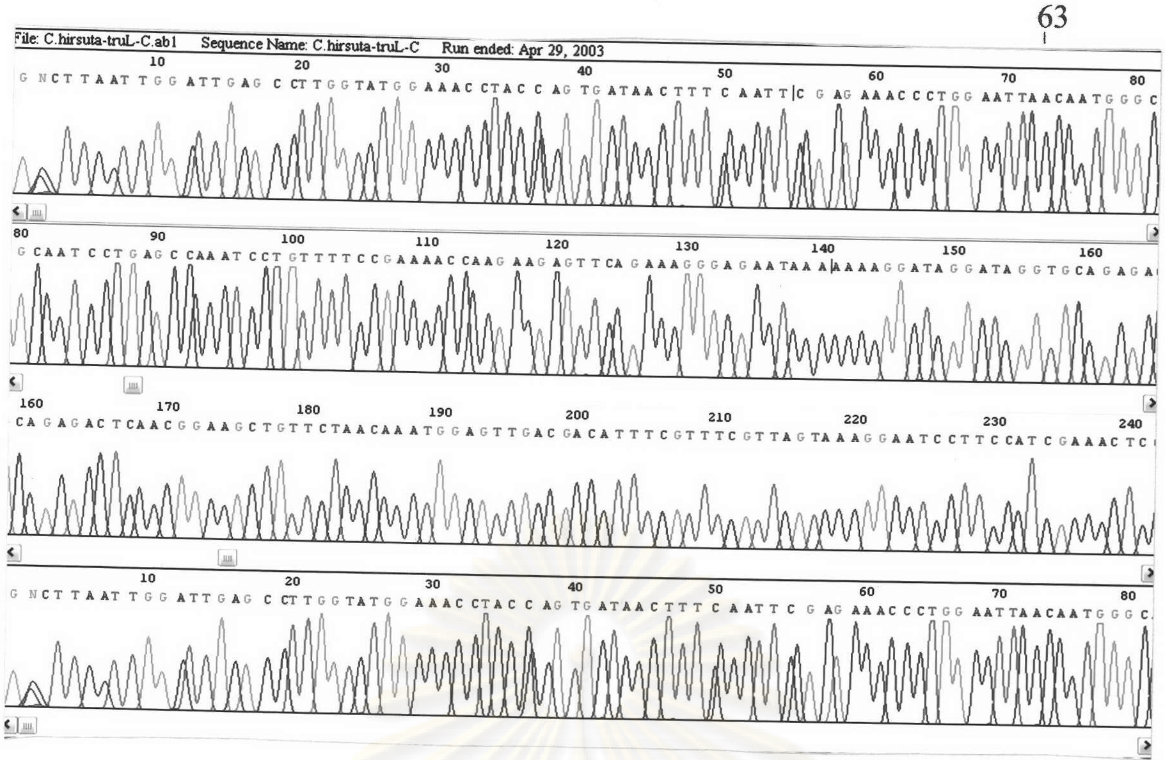

Fig. 18 four-coloured electropherogram of *trn*L intron sequence of *C. hirsuta* with blue peaks represent Cytocine (C), red Thymine (T), green Adenine (A) and cyan Guanine (G) nucleotides.

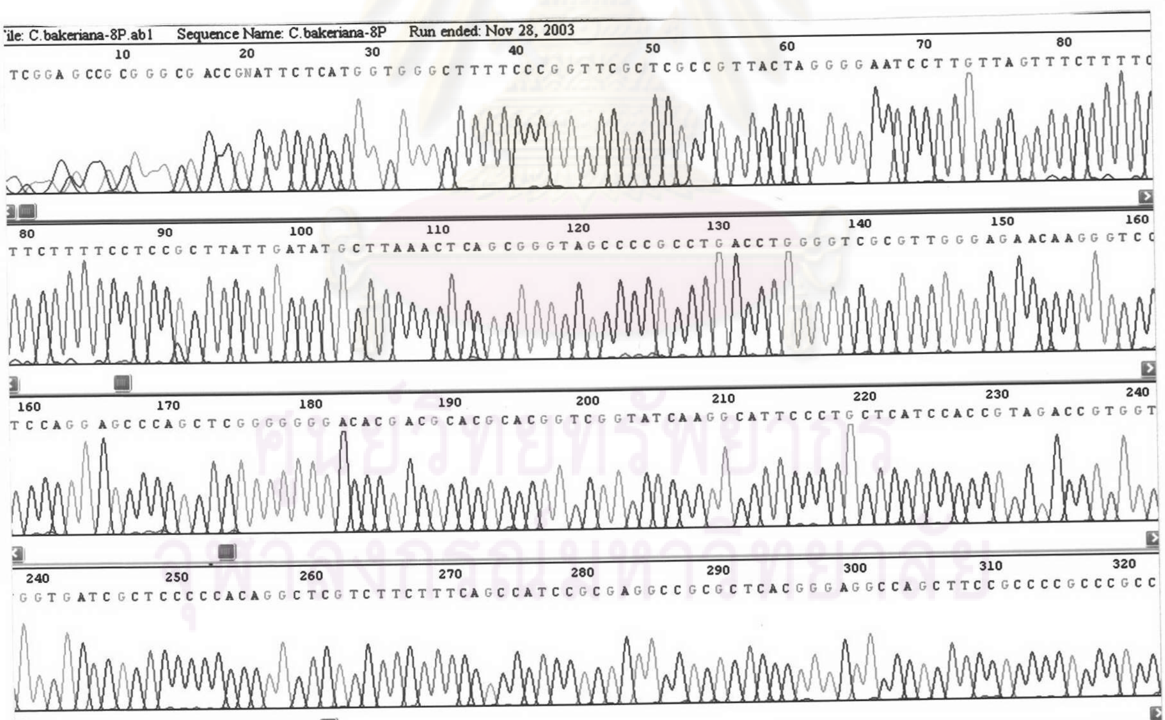

Fig. 19 four-coloured electropherogram of ITS sequence of *C. bakeriana* with blue peaks represent Cytocine (C), red Thymine (T), green Adenine (A) and cyan Guanine (G) nucleotides.

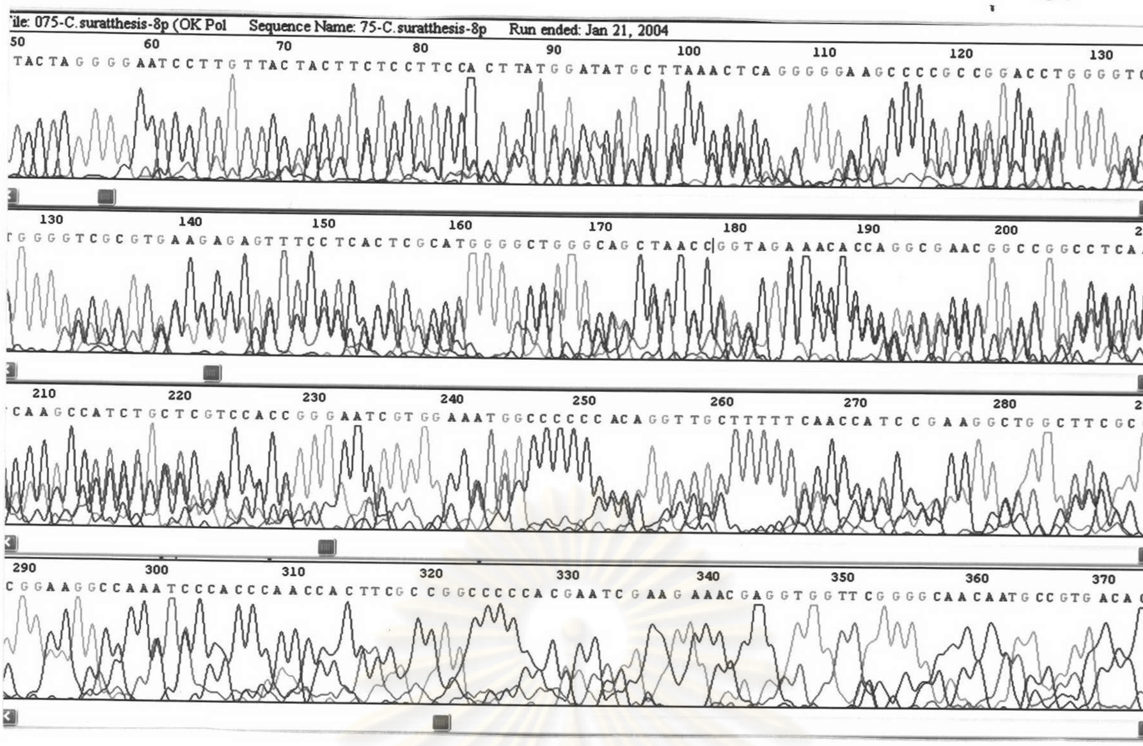

Fig. 20 four-coloured electropherogram of ITS sequence of *C. surattensis* with blue peaks represent Cytocine (C), red Thymine (T), green Adenine (A) and cyan Guanine (G) nucleotides.

Fig. 21 a sample of FASTA sequence format of *trn*L intron sequences

4.4 Alignment of DNA sequences

After converting DNA sequences to FASTA format, the Clustal X computer program was used to align the FASTA file. Three types of sequence alignments were performed: 1.) an alignment of ITS sequences of Thai *Cassia*, 2.) an alignment of *trn*L intron of Thai *Cassia* and 3.) an alignment of *trn*L intron of Thai *Cassia* compared with those of *Cassia* from other countries. These additional *trn*L data for the third alignment were the sequences of *C. grandis* (AF365092), *Senna crassiramea* (AF365090), *S. lindheimeriana* (AF365089), *S. bauhinioides* (AF365087), *S. wislizeni* (AF365028), *S. bacillaris* (AF365031), *Chamaecrista nictitans* (AF365093), *Ch.* sp. Klitgaad (AF365093) and *Ch.* sp. Breteler (AF365094). When completed, the lengths of these character-taxon matrices were 911 bp (excluded 206 bp before doing phylogenetic tree reconstruction which belong to 16S small and 28S large subunit), 646 bp and 657 bp, respectively. These character-taxon matrices (Fig. 22) were then transformed to NEXUS file format (Fig. 23) for further phylogenetic tree reconstruction.

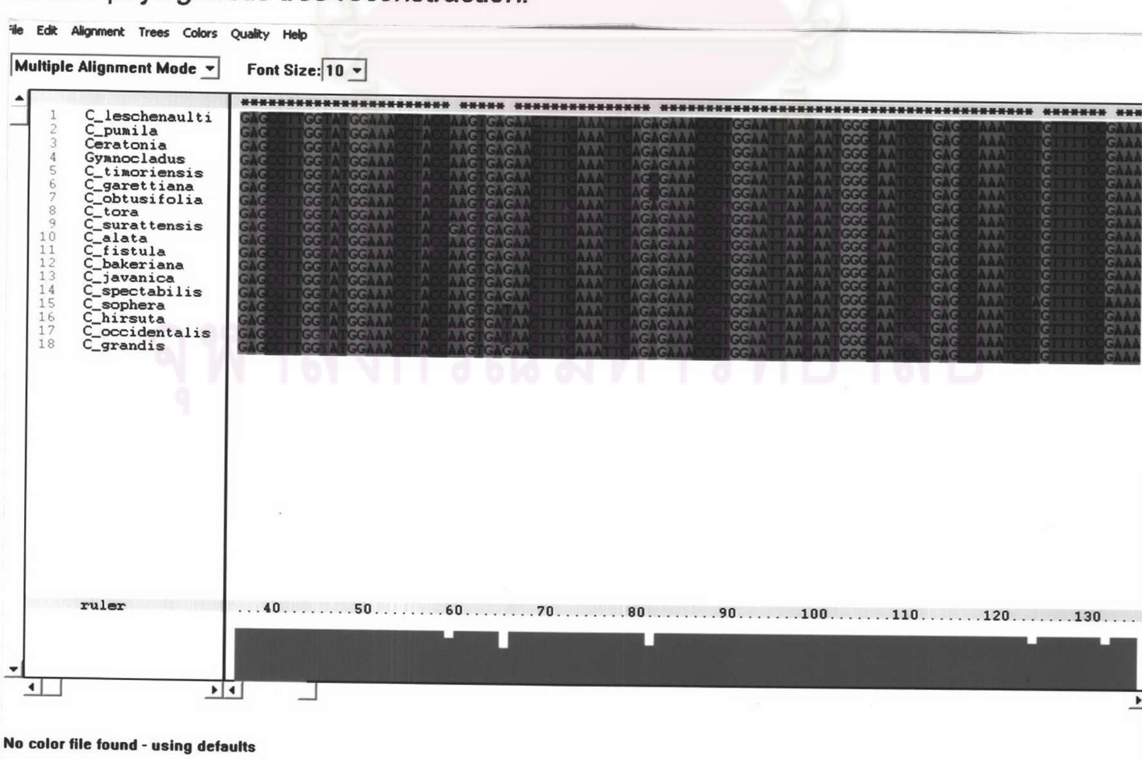

Fig. 22 a sample of an aligned sequence data matrix using ClustalX program

```
#NEXUS

begin data;
       dimensions ntax=18 nchar=597;
       format missing=? gap=- matchchar=. datatype=dna symbols = "01";
       options gapmode=missing;
       matrix

Ceratonia siliqua AF365075      GTCGACTTTA GAAAT-CGTG AG-------- ---------- ------
Gymnocladus dioica AF365095     GTCGACTTTA GAAAT-CGTG AGG------- ---------- ------
C. leschenaultiana              GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCTTCCTAT CCCCAA
C. pumila                       GTCGACTTTA GAAAT-CGTG AGGG------ ---------- ------
C. grandis                      GTCGACTTTA GAAAT-CG-- -GGG------ ---------- ------
C. javanica                     GTCGACTTTA GAAAT-CG-- ---------- ---------- ------
C. bakeriana                    GTCGACTTTA GAAAT-CGTG AG-------- ---------- ------
C. fistula                      GTCGACTTTA GAAAT-CG-G A--------- ---------- ------
C. sophera                      GTCGACTTTA GAAAT-CG-G GG-------- ---------- ------
C. occidentalis                 GTCGACTTTT GAAAT-CGTG A--------- ---------- ------
C. hirsuta                      GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCCTT---- ------
C. obtusifolia                  GTCGACTTTA GAAATGCG-G AG-------- ---------- ------
C. tora                         GTCGACTTTA GAAAT-CGTG AG-------- ---------- ------
C. surattensis                  GTCGACTTTA GAAAT-CG-G AG-------- ---------- ------
C. timoriensis                  GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCC------- ------
C. garettiana                   GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCTCTATCC CCAAC-
C. alata                        GTCGACTTTA GAAAT-CG-G A--------- ---------- ------
C. spectabilis                  GTCGACTTTA GAAAT-CG-- ---------- ---------- ------
;
end;
```

Fig. 23 a sample of NEXUS file format using for phylogenetic analyses in the program PAUP*.

4.5 Phylogenetic analysis

4.5.1 *trn*L intron sequence data set (see Fig. 24)

- *trn*L intron sequence data set of 16 Thai *Cassia* sensu lato

After automatically aligned by ClustalX program and transformed to NEXUS file format, each sequence matrix was adjusted manually to get a highest level of homology before phylogenetically analysed. Some nucleotide positions appeared to be too ambiguous to align and necessarily to be excluded. The beginning and the ending regions of some sequences were also blocked out as incomplete or missing data. Symbols '01' were added to the DNA data matrix to increase informative characters.

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                        10         20         30         40         50
                                ********** ********** **********
Ceratonia siliqua AF365075      ?????????? ?????????? ?GACTTAATT GGATTGAGCC TTGGTATGGA
Gymnocladus dioica AF365095     ?????????? ?????????? ?GACTTAATT GGATTGAGCC TTGGTATGGA
C.(Ch.)leschenaultiana          TCGAAATCGG TAGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(Ch.)pumila                   ?????????? ???????TAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C. grandis                      ?????????? ?????????? ?????????? GGATTGAGCC TTGGTATGGA
C. javanica                     ?????????? ?????????? GACCTTAATT GGATTGAGCC TTGGTATGGA
C. bakeriana                    ?????????? GTGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C. fistula                      ????????CG TAGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)sophera                   ?????????? ?????????? ?????????T GGATTGAGCC TTGGTATGGA
C.(S.)occidentalis              ?????????C GTGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)hirsuta                   ?????????? ?????????? GACCTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)obtusifolia               ?????CGTAG ACGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)tora                      ?????????? ?????????? ?GACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)surattensis               ?????????? ?????????? GACCTTAATT GGCTTGAGCC TTGGTATGGA
C.(S.)timoriensis               ?????????? ???ACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)garettiana                ?????????? TAGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)alata                     ?????????? GTGACGCTAC GGACTTAATT GGATTGAGCC TTGGTATGGA
C.(S.)spectabilis               ?????????? ?????????? GACCTTAATT GGATTGAGCC TTGGTATGGA
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Fig. 24 a 646 bp character-taxon matix of 16 Thai *Cassia* species based on *trn*L intron sequences. Asterisks * and * represent excluded and gap-matrix sites from analyses, respectively. Gap symbol (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data.

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       60         70         80         90        100

Ceratonia siliqua AF365075      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
Gymnocladus dioica AF365095     AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(Ch.)leschenaultiana          AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(Ch.)pumila                   AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. grandis                      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. javanica                     AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. bakeriana                    AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. fistula                      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)sophera                   AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)occidentalis              AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)hirsuta                   AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)obtusifolia               AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)tora                      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)surattensis               AACCTACCGA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)timoriensis               AACCTACCAA GTGAGAACTT TCAAATTCAG CGAAACCCTG GAATTAACAA
C.(S.)garettiana                AACCTACCAA GTGAGAACTT TCAAATTCAG CGAAACCCTG GAATTAACAA
C.(S.)alata                     AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C.(S.)spectabilis               AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                      110        120        130        140        150

Ceratonia siliqua AF365075      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
Gymnocladus dioica AF365095     TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(Ch.)leschenaultiana          TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(Ch.)pumila                   TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. grandis                      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. javanica                     TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. bakeriana                    TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. fistula                      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAA AAGAGTTCAG
C.(S.)sophera                   TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)occidentalis              TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)hirsuta                   TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)obtusifolia               TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)tora                      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)surattensis               TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)timoriensis               TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)garettiana                TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)alata                     TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C.(S.)spectabilis               TGGGCAATCC TGAGCCAAAT CCAGTTTTCC AAAAACCAAG AAGAGTTCAG

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                      160        170        180        190        200
                                                  *** *********
Ceratonia siliqua AF365075      AAAGCGCGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAT
Gymnocladus dioica AF365095     AAAGCGAGAA TAAAACAA-- ---------G GATTGGTGCA GAGACTCAAT
C.(Ch.)leschenaultiana          AAAGGGAGAA TAAAAAAAA- ---------G GATAGGTGCA GAGACTCAAT
C.(Ch.)pumila                   AAAGGGAGAA TAAAAAAAA- ---------G GATAGGTGCA GAGACTCAAT
C. grandis                      AAAGGGAGAA TAAAAAAAA- ---------G GATAGGTGCA GAGACTCAAC
C. javanica                     AAAGGGAGAA TAAAAAAAA- ---------G GATAGGTGCA GAGACTCAAC
C. bakeriana                    AAAGGGAGAA TAAAAAAAA- ---------G GATAGGTGCA GAGACTCAAC
C. fistula                      AAAGGGAGAA TAAAAAAAAA ---------G GATAGGTGCA GAGACTCAAC
C.(S.)sophera                   AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C.(S.)occidentalis              AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C.(S.)hirsuta                   AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C.(S.)obtusifolia               AAAGGGAGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAC
C.(S.)tora                      AAAGGGAGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAC
C.(S.)surattensis               AAAGGGAGAA AAAAAAAAAA AAAA-----G GATAGGTGCA GAGACTCAAC
C.(S.)timoriensis               AAAGGGAGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAC
C.(S.)garettiana                AAAGGGAGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAC
C.(S.)alata                     AAAGGGAGAA TAAAAAAA-- ---------G GATAGGTGCA GAGACTCAAC
C.(S.)spectabilis               AAAGGGAGAA TAAAAAA--- ---------G GATAGGTGCA GAGACTCAAC
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Fig. 24 (continue)

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     210        220        230        240        250
                                                    ********** ********** **********
Ceratonia siliqua AF365075    GGAAGCTGTT CTAACAAATG G--------- ---------- ----------
Gymnocladus dioica AF365095   GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(Ch.)leschenaultiana        GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTTAA
C.(Ch.)pumila                 GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTTAA
C. grandis                    GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C. javanica                   GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C. bakeriana                  GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C. fistula                    GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)sophera                 GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)occidentalis            GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAT
C.(S.)hirsuta                 GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)obtusifolia             GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)tora                    GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)surattensis             GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)timoriensis             GGAAGCTGTT CTAACAAATG GAGTTGACGA TATTTCGTTT ---CG-TTAG
C.(S.)garettiana              GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCCTTT ---CG-TTAG
C.(S.)alata                   GGAAGTTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG
C.(S.)spectabilis             GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CG-TTAG

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     260        270        280        290        300
                              ********** ********** ********** ********** **********
Ceratonia siliqua AF365075    ---------- ---------- ---------- ---------- ----------
Gymnocladus dioica AF365095   TAAAGGAATC CTTCCATCAA AACTCCCGAA AAGAAAGGAT CAAGGATGAA
C.(Ch.)leschenaultiana        TAAAGGAATG CTTCCATCGA AACTCCAGAA AAGAAAGAAT CAAGGATGAA
C.(Ch.)pumila                 TAAAGGAATG CTTCCATCGA AACTCCAGAA AAGAAAGAAT CAAGGATGAA
C. grandis                    TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C. javanica                   TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C. bakeriana                  TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C. fistula                    TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C.(S.)sophera                 TAAAGGAATC CTTCCATCGA AACTCCATAA AAGAAAGGAT CAAGGATGAA
C.(S.)occidentalis            TAAAGGAATC CTTCCATCGA AACTCCATAA AAGAAAGGAT CAAGGATGAA
C.(S.)hirsuta                 TAAAGGAATC CTTCCATCGA AACTCCATAA AAGAAAGGAT CAAGGATGAA
C.(S.)obtusifolia             TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CGAGGATGAA
C.(S.)tora                    TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CGAGGATGAA
C.(S.)surattensis             TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CGAGGATGAA
C.(S.)timoriensis             TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C.(S.)garettiana              TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C.(S.)alata                   TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGGAT CAAGGATGAA
C.(S.)spectabilis             TAAAGGAATA CTTCCATCGA AACTACAGAA AAGAAAGGAT CAAGGATGAA

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     310        320        330        340        350
                              ********** *****                                     *
Ceratonia siliqua AF365075    ---------- -----ATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
Gymnocladus dioica AF365095   CATATAT--- -----ATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(Ch.)leschenaultiana        CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(Ch.)pumila                 CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C. grandis                    CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C. javanica                   CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C. bakeriana                  CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C. fistula                    CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)sophera                 CATATAT--A TACGTATACG TACTGAAATA GTATTTCAAT TGATTAGAC-
C.(S.)occidentalis            CATATAT--A TACGTATACG TACTGAAATA GTATTTCAAT TGATTAGAC-
C.(S.)hirsuta                 CATATAT--A TACGTATACG TACTGAAATA GTATTTCAAT TGATTAGAC-
C.(S.)obtusifolia             CATATATATA TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)tora                    CATATATATA TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)surattensis             CATATAT--A TACGTATACA TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)timoriensis             CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)garettiana              CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
C.(S.)alata                   CATATAT--A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGACC
C.(S.)spectabilis             CATAT----A TACGTATACG TACTGAAATA CTATTTCAAT TGATTAGAC-
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Fig. 24 (continue)

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                             ....|....| ....|....| ....|....| ....|....| ....|....|
                                    360        370        380        390        400
                             ********** **                ****** *****
Ceratonia siliqua AF365075   ---------- --CCCAAATC TCTATTTTTT ----AATATT TATATGACAA
Gymnocladus dioica AF365095  ---------- --CCAAAATC TCTATTTRGT C----ATATT TATATGACAA
C.(Ch.)leschenaultiana       ---CAAACAG ACCCCCAATC TCCATTTTTT ----AATATT TATATGAAAA
C.(Ch.)pumila                ---CAAACAG ACCCCCAATC TCCATTTTTT ----AATATT TATATGAAAA
C. grandis                   ---CAGACAG ACTCCAAATC CCTATTTTTT ----AATATT TATATGACAA
C. javanica                  ---CAGACAG ACCCCAAATC CCTATTTTTT ---GAATATT TATATGACAA
C. bakeriana                 ---CAGACAG ACCCCAAATC CCTATTTTTT ---GAATATT TATATGACAA
C. fistula                   ---CAGACAG ACCCCAAATC CCTATTTTTT TTT-AATATT TATATGACAA
C.(S.)sophera                ---CAGACAG ACCCCAAATC CCTAGTTTTT ----AATATT TATATGACAA
C.(S.)occidentalis           ---CAGACAG ACCCCAAATC CCTAGTTTTT ----AATATT TATATGACAA
C.(S.)hirsuta                ---CAGACAG ACCTCAAATC CCTAGTTTTT ----AATATT TATATGACAA
C.(S.)obtusifolia            ---CAGACAG ACCGCGAATC TCTA------ -----ATATT TATATGACAA
C.(S.)tora                   ---CAGACAG ACCGCGAATC TCTA------ -----ATATT TATATGACAA
C.(S.)surattensis            ---CAGACAG ACCCCGAATC TCTA------ -----ATATT TATATGACAA
C.(S.)timoriensis            ---------G ACCCCAAATC TCTATCTTTT ----AATATT TATATGACAA
C.(S.)garettiana             ---------G ACCCCAAATC TCTATCTTTT ----AATATT TATATGACAA
C.(S.)alata                  AGACAGACAG ACCCCAAATC TCTATTTTTT ----AATATT TATATGACAA
C.(S.)spectabilis            ---CAGACAG ACCCCAAATC CCTATTTTT- ---AAATATT TAGATGACAA

                             ....|....| ....|....| ....|....| ....|....| ....|....|
                                    410        420        430        440        450
                               ******** ****
Ceratonia siliqua AF365075   AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
Gymnocladus dioica AF365095  AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(Ch.)leschenaultiana       AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(Ch.)pumila                AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C. grandis                   AT-------- ----GAAAGA TGTGAATCCA TTCCAAGTTG AAGAAAGAAT
C. javanica                  AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C. bakeriana                 AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C. fistula                   AT-------- ----GAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)sophera                ATTATATGAC AAATAAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)occidentalis           ATTATATGAC AAATAAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)hirsuta                ATTCTATGAC AAATAAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)obtusifolia            AT-------- ----AAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)tora                   AT-------- ----AAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)surattensis            AT-------- ----AAAAGA TGTGAATCGA TTTCAAGTTG AAGAAAGAAT
C.(S.)timoriensis            AT-------- ----AATAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)garettiana             AT-------- ----AAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)alata                  AT-------- ----AAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT
C.(S.)spectabilis            AT-------- ----AAAAGA TGTGAATCGA TTCCAAGTTG AAGAAAGAAT

                             ....|....| ....|....| ....|....| ....|....| ....|....|
                                    460        470        480        490        500
                                  *
Ceratonia siliqua AF365075   CGAAT-ATTC ATTGATCAAA TCATTCACTC CATCATAGTC TGATAGATCT
Gymnocladus dioica AF365095  CGAAT-ATTC ATTGATCAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(Ch.)leschenaultiana       CGAAT-ATTC ATTGATCAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(Ch.)pumila                CGAAT-ATTC ATTGATCAAA TCATTCACTC CATCATAGTC TGATAGATCT
C. grandis                   CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C. javanica                  CAAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C. bakeriana                 CAAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C. fistula                   CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(S.)sophera                CTAATGATTT TTTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(S.)occidentalis           CTAAT-ATTC TTTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(S.)hirsuta                CGAAT-ATTC TTTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(S.)obtusifolia            CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTT TGATAGATCT
C.(S.)tora                   CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTT TGATAGATCT
C.(S.)surattensis            CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTT TGATAGATCT
C.(S.)timoriensis            CGAAT-ATTC ATTGATAAAA TCATTCACTA CATCATAGTC GGATGGATCT
C.(S.)garettiana             CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATGGATCT
C.(S.)alata                  CGAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
C.(S.)spectabilis            CAAAT-ATTC ATTGATAAAA TCATTCACTC CATCATAGTC TGATAGATCT
```

Fig. 24 (continue)

```
                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       510        520        530        540        550
                                  *****
Ceratonia siliqua AF365075      TTT-----GA AAAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
Gymnocladus dioica AF365095     TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(Ch.)leschenaultiana          TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(Ch.)pumila                   TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C. grandis                      TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C. javanica                     TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C. bakeriana                    TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C. fistula                      TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)sophera                   TTTCTTTTGA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)occidentalis              TTTCTTTTGA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)hirsuta                   TTTCTTTTGA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)obtusifolia               TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)tora                      TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)surattensis               TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)timoriensis               TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)garettiana                TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)alata                     TTT-----GA AGAACTGATT AATCGGACGA GAATAAAGAT AGAGTCCCAT
C.(S.)spectabilis               TTT-----GA AGAACTTATT AATCGGACGA GAATAAAGAT AGAGTCCCAT

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       560        570        580        590        600

Ceratonia siliqua AF365075      TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
Gymnocladus dioica AF365095     TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(Ch.)leschenaultiana          TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(Ch.)pumila                   TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C. grandis                      TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C. javanica                     TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C. bakeriana                    TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C. fistula                      TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)sophera                   TCTACATGTC AATACCGACA ACAATGAAAT TTTTAGTAAG AGGAAAATCC
C.(S.)occidentalis              TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)hirsuta                   TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)obtusifolia               TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)tora                      TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)surattensis               TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)timoriensis               TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)garettiana                TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)alata                     TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC
C.(S.)spectabilis               TCTACATGTC AATACCGACA ACAATGAAAT TTATAGTAAG AGGAAAATCC

                                ....|....| ....|....| ....|....| ....|....| ....|.
                                       610        620        630        640
                                                *  ** ********** ********** ******
Ceratonia siliqua AF365075      GTCGACTTTA GAAAT-CGTG AG???????? ?????????? ??????
Gymnocladus dioica AF365095     GTCGACTTTA GAAAT-CGTG AGG??????? ?????????? ??????
C.(Ch.)leschenaultiana          GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCTTCCTAT CCCCAA
C.(Ch.)pumila                   GTCGACTTTA GAAAT-CGTG AGGG?????? ?????????? ??????
C. grandis                      GTCGACTTTA GAAAT-CG?? ?GGG?????? ?????????? ??????
C. javanica                     GTCGACTTTA GAAAT-CG?? ?????????? ?????????? ??????
C. bakeriana                    GTCGACTTTA GAAAT-CGTG AG???????? ?????????? ??????
C. fistula                      GTCGACTTTA GAAAT-CG?G A????????? ?????????? ??????
C.(S.)sophera                   GTCGACTTTA GAAAT-CG?G GG???????? ?????????? ??????
C.(S.)occidentalis              GTCGACTTTT GAAAT-CGTG A????????? ?????????? ??????
C.(S.)hirsuta                   GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCCTT???? ??????
C.(S.)obtusifolia               GTCGACTTTA GAAATGCG?G AG???????? ?????????? ??????
C.(S.)tora                      GTCGACTTTA GAAAT-CGTG AG???????? ?????????? ??????
C.(S.)surattensis               GTCGACTTTA GAAAT-CG?G AG???????? ?????????? ??????
C.(S.)timoriensis               GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCC??????? ??????
C.(S.)garettiana                GTCGACTTTA GAAAT-CGTG AGGGTTCAAG TCCTCTATCC CCAAC?
C.(S.)alata                     GTCGACTTTA GAAAT-CG?G A????????? ?????????? ??????
C.(S.)spectabilis               GTCGACTTTA GAAAT-CG?? ?????????? ?????????? ??????
```


Fig. 24 (continue)

Branch-and-bound searching strategy was used to analyse a data matrix of 597 bp trnL intron sequences of Thai *Cassia* 16 species without *C. siamea* (not amplified both *trn*L intron and ITS regions) included with a 20-position gap matrix (0, 1 symbols). The fully unexcluded matrix had 646 characters, but 62 nucleotides (the first position to the 30th, the 375th to the 385th and 619th to the last one) needed to be excluded. Gap were treated as missing data. *Gymnocladus dioica* (AF365095) and *Ceratonia siliqua* (AF365075) were included into the analysis as outgroup taxa (sequences retrieved from GenBank). All characters used in phylogenetic tree reconstruction were unordered and equally weighted with 37 characters (6.2%) were parsimony-informative as synapomorphy (shared-derived character) and 37 characters were parsimony-uninformative as autapomorphy specifically for each taxon. There were 14 most parsimonious trees found from this data set, with 87 steps in lenth (Fig. 25). Consistency index (CI) was 0.9195 and Homoplasy index (HI) was 0.0805. Retention index (RI) was 0.9041 and rescale consistency index (RC) was 0.8314.

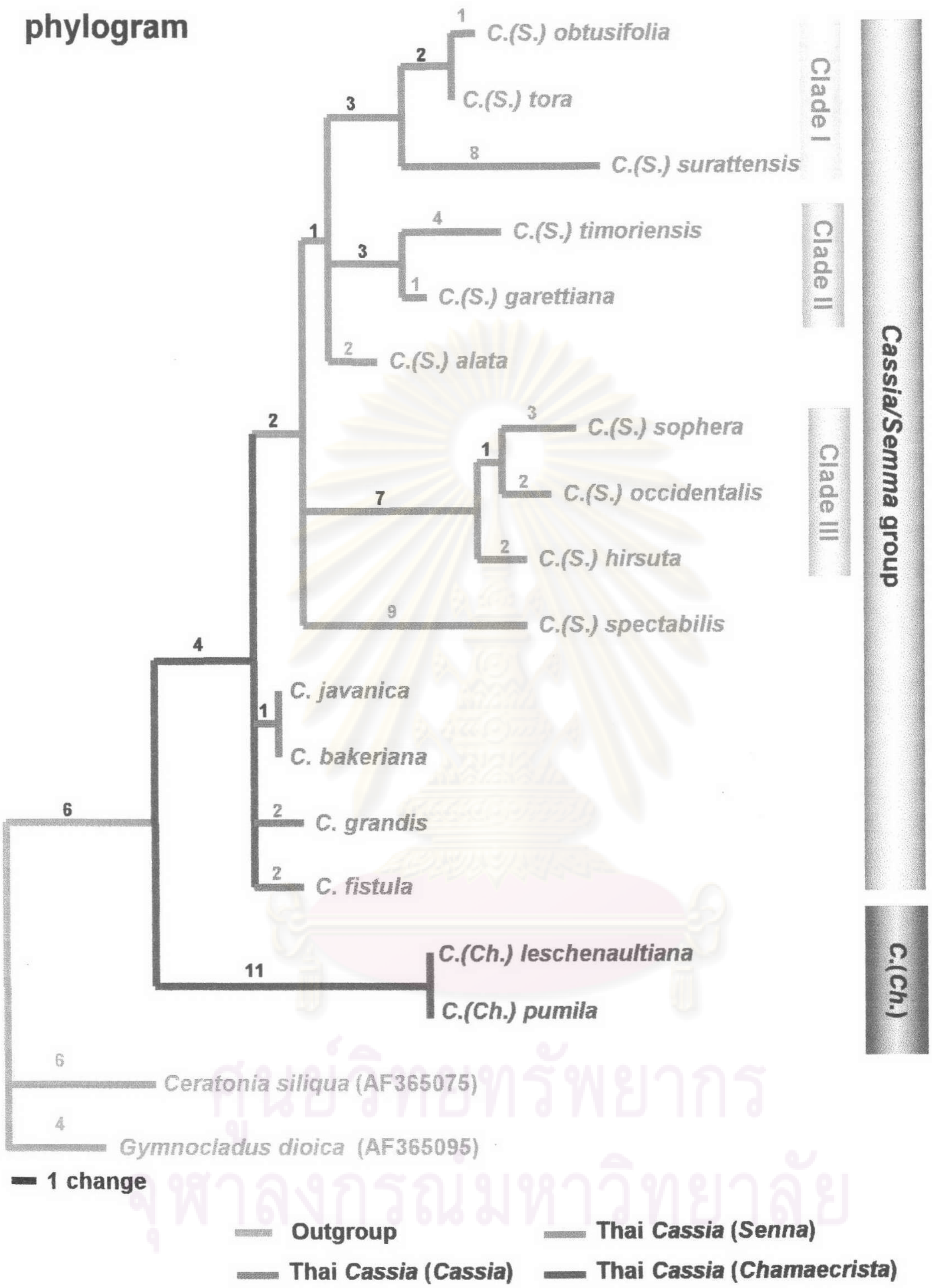

Fig. 25 one phylogram of 14 equally most parsimonious trees (87 steps in length) for 16 *Cassia* in Thailand based on *trn*L intron sequence data with *Ceratonia siliqua* (AF365075) and *Gymnocladus dioica* (AF365095) as outgroups (retrieved from GenBank). Black numbers along branches are amounts of synapomorphy and colouring numbers are amounts of autapomorphy. [CI = 0.9195, RI = 0.9041, RC = 0.8314]

Bootstrap (BS) and jackknife (JK) supporting values were calculated to confirm a reliability of each clade. Some branches were collapsed when these supporting-values were less than 50%. Strict (Fig. 26), semistrict (Fig. 27) and 50% majority-rule (Fig. 28) consensus trees were made from the 14 most parsimonious trees (MPTs). All consensus trees were similar to each other that *Gymnocladus dioica* and *Ceratonia siliqua* the two outgroups were clustered while all Thai *Cassia* species were grouped together with very high bootstrap and jackknife supporting-values (97%, 93%), respectively. Within the Thai *Cassia* group, there were two major clades separated distinctively. One clade contained Thai *Cassia* species that moved to genus *Senna* (blue branches) based on Flora Malesiana and some other species remaining to be in the genus *Cassia* (dark-red branches). The other group had two Thai *Cassia* species which moved to the genus *Chamaecrista*: *Cassia*(*Chamaecrista*) *leschenaultiana* and *C.*(*Ch.*) *pumila* (green branches).

The first *Cassia/Senna* group, with 66% BS and 61% JK, composes of *Cassia* (*Senna*) *obtusifolia*, *C.*(*S.*) *tora*, *C.*(*S.*) *surattensis*, *C.*(*S.*) *timoriensis*, *C.*(*S.*) *garrettiana*, *C.*(*S.*) *alata*, *C.*(*S.*) *sophera*, *C.*(*S.*) *occidentalis*, *C.*(*S.*) *hirsuta*, *C.*(*S.*) *spectabilis*, *C. javanica*, *C. bakeriana*, *C. grandis* and *C. fistula*. The other major group had only two species: *C.*(*Ch.*) *pumila* and *C.*(*Ch.*) *leschenaultiana*, with very high robustness of bootstrap (100%). Considering subgroupings in the first *Cassia/Senna* group, the cluster could be subdivided into three minor clades. The first clade contained *C.*(*S.*) *obtusifolia*, *C.*(*S.*) *tora* and *C.*(*S.*) *surattensis* with strong supporting-values (80% BS and 78% JK). The clade II composed of *C.*(*S.*) *timoriensis* and *C.*(*S.*) *garrettiana* with even higher bootstrap (91%) and jackknife (81%) supporting-values. The last clade III of *C.*(*S.*) *sophera*, *C.*(*S.*) *occidentalis*, and *C.*(*S.*) *hirsuta* had highest supporting-values (100% BS and 99% JK).

Some differences were also found between the strict, semistrict and 50% majority-rule consensus trees. The strict consensus tree showed that there were some remained taxa ungrouped as a clade. These taxa were *C.*(*S.*) *alata*, *C.*(*S.*)

spectabilis, *C. javanica*, *C. bakeriana*, *C. grandis* and *C. fistula*. Grouping between any of these species may have been collapsed after strict consensus analysis to form an unresolved polytomic backbone for the three previously recognised clades. In contrast, the semistrict consensus tree suggested two more low-supported clades. The fourth grouping contained *C. grandis* and *C. fistula* whereas the fifth minor clade composed of *C. javanica* and *C. bakeriana*. Moreover, these four Thai *Cassia* (*Cassia*) formed a distinctive group with each other in the 50% majority-rule consensus tree, though with low supporting-values. Note that *C. javanica* and *C. bakeriana* were clustered in this group. This newly recognised clade therefore had all members belonging to *Cassia* species not moved to the genera *Senna* and *Chamaecrista*. *Cassia* (*Senna*) *spectabilis* and *C.*(*S.*) *alata* were left as unresolved polytomic taxa to other species in the *Cassia/Senna* group in both semistrict and 50% majority-rule consensus trees.

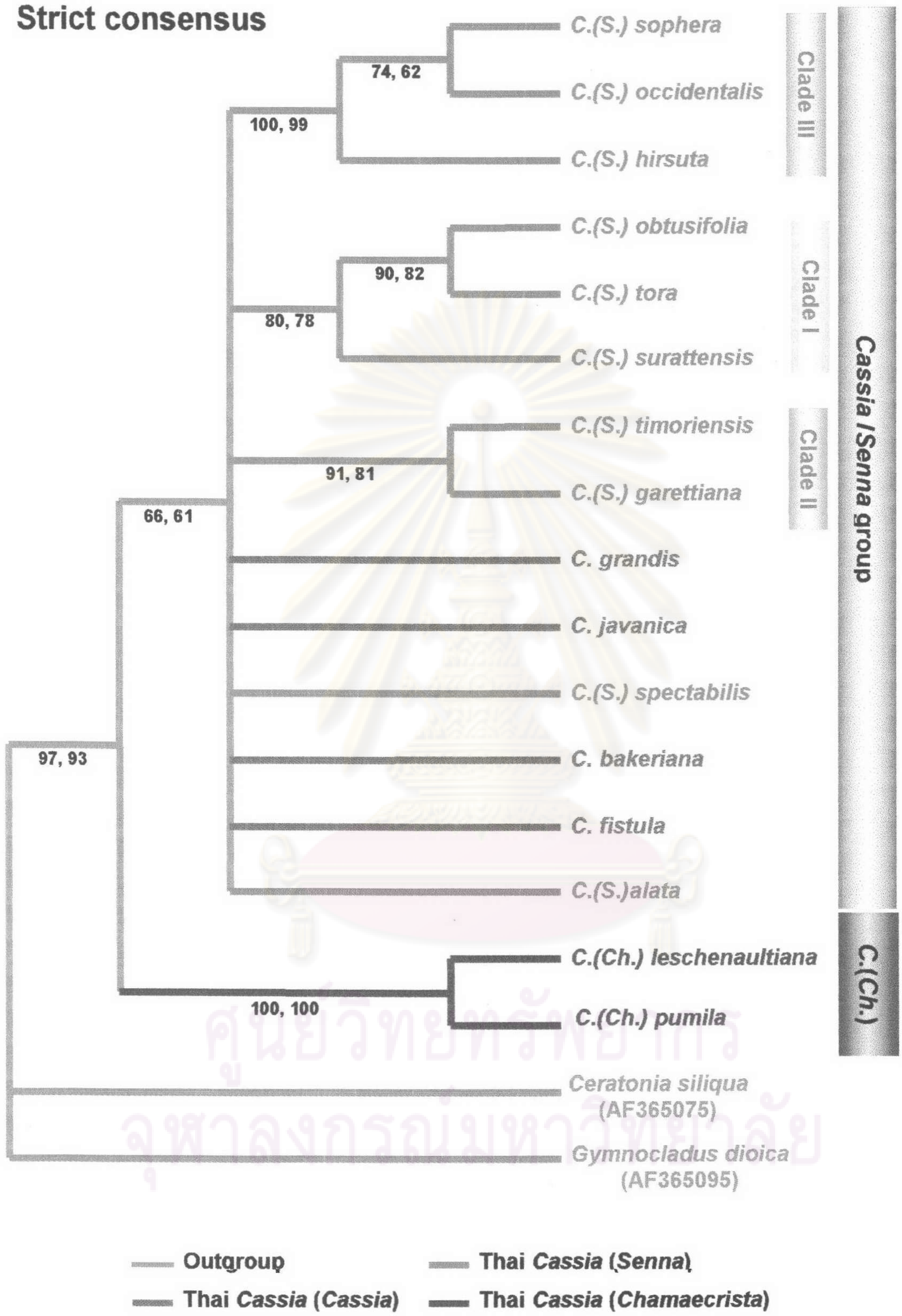

Fig. 26 strict consensus tree from 14 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The two numbers along branches are 1000-replicate bootstrap and jackknife supporting-values, respectively.

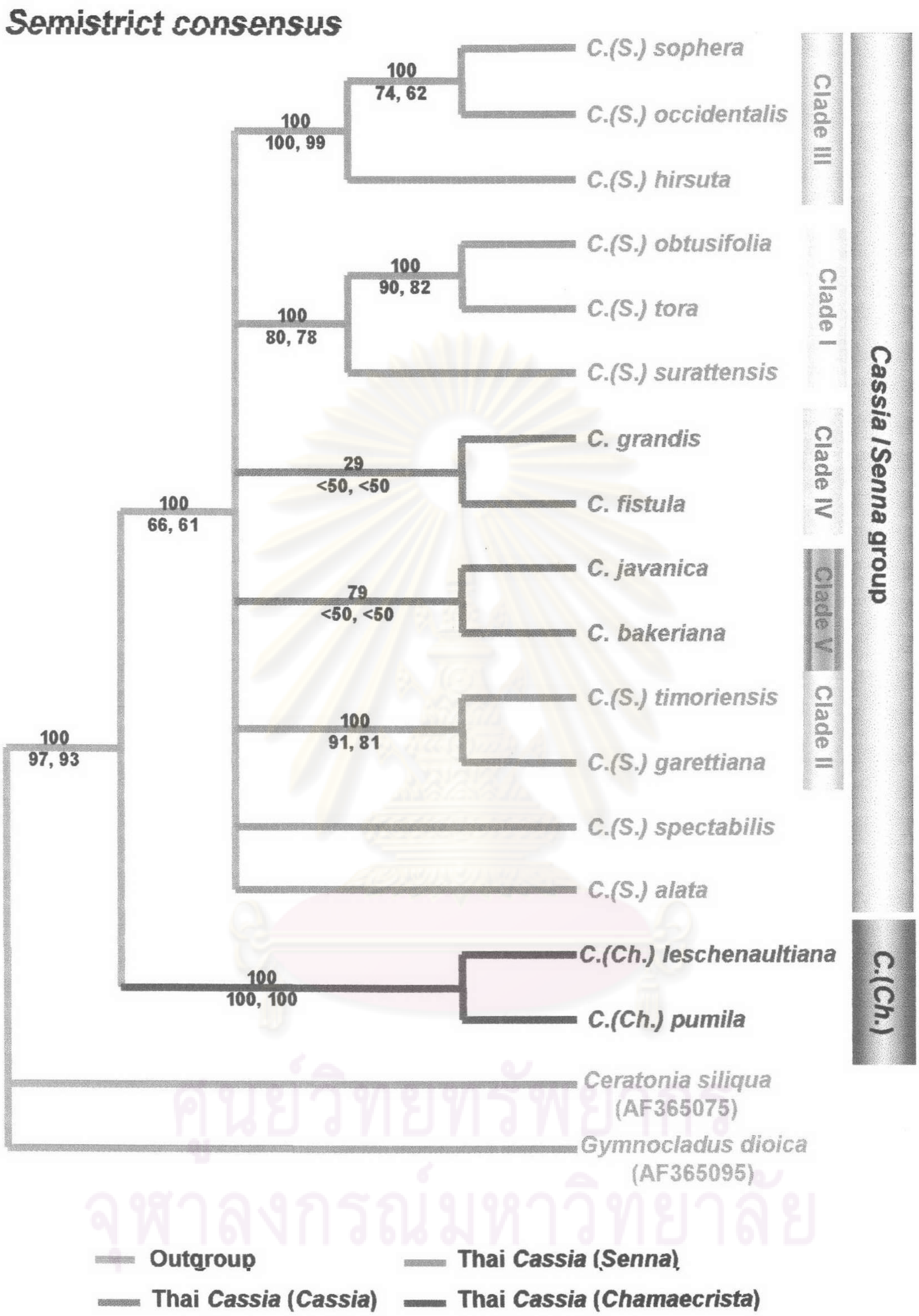

Fig. 27 semistrict consensus tree from 14 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The upper numbers are percentages of congruent MPTs on the consensus trees and the lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively.

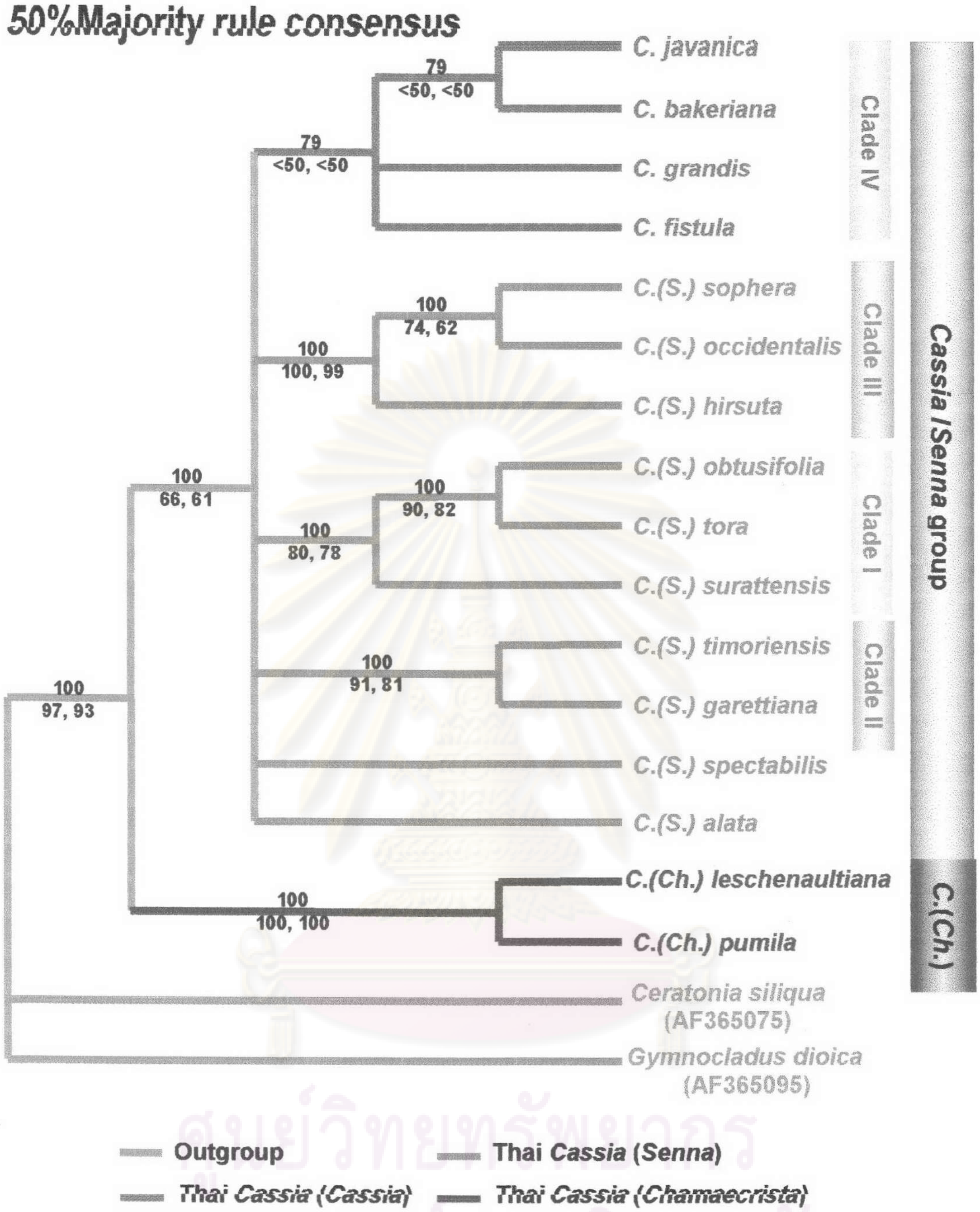

Fig. 28 50% majority-rule consensus tree from 14 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The upper numbers are percentages of similar MPTs tree on the consensus. The lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively.

For distance method analysis, neighbour-joining (NJ) tree was drawn to compare with results from maximum parsimony analyses. The NJ tree showed that *Gymnocladus dioica* was separated from Thai *Cassia* but *Ceratonia siliqua* formed a low supported group with Thai *Cassia* (*Chamaecrsita*). *Cassia* (*Chamaecriata*) *leschenaultiana* and *C.*(*Ch.*) *pumila* were paired with each other like in the parsimony analysis with very high supporting values (100% bootstrap and 100% jackknife). *Cassia* (*Senna*) *sophera*, *C.*(*S.*) *occidentalis* and *C.*(*S.*) *hirsuta* formed their own group with 100% BS and 100% JK supporting-values while *C.*(*S.*) *sophera* were specifically sistered to *C.*(*S.*) *occidentalis* in the group. *Cassia* (*Senna*) *obtusifolia* was paired with *C.*(*S.*) *tora* and both were grouped to *C.*(*S.*) *surattensis* with 80% BS and 76% JK. The next grouping was that of *C.*(*S.*) *alata* forming a cluster with *C.*(*S.*) *garrettiana* and *C.*(*S.*) *timoriensis*. Unexpectedly, *C.*(*S.*) *spectabilis* was transgressed into a group of four still-unmoved *Cassia* species (*C. javanica*, *C. bakeriana*, *C. grandis* and *C. fistula*).

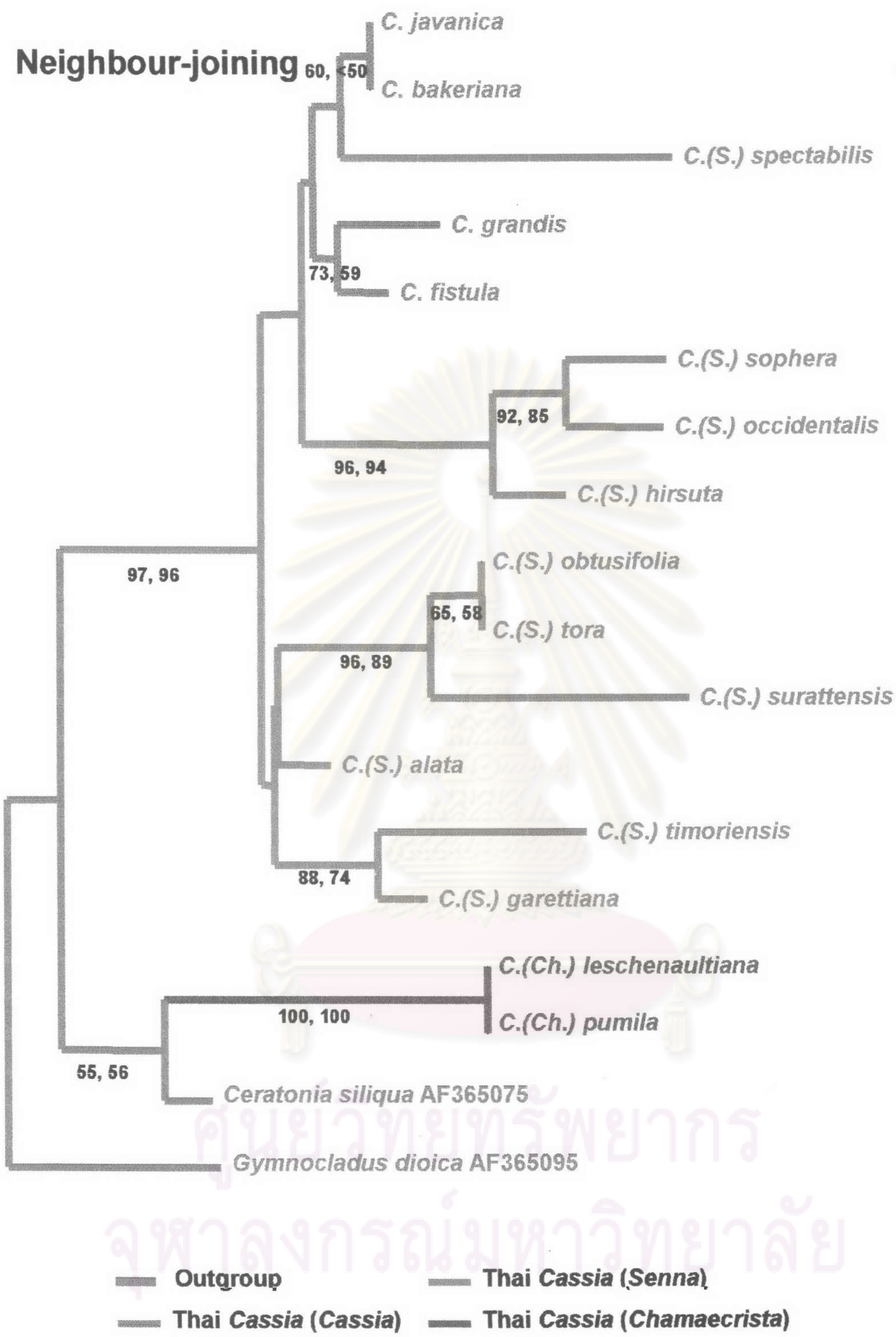

Fig. 29 neighbour-joining tree of 16 *Cassia* in Thailand based on *trn*L intron sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieve from GenBank). The numbers along branches are 1000-replicate bootstraps and jackknife supporting-values, respectively. Note that supporting-values less than 50% were not shown in the tree.

- *trn*L sequence data set of *Cassia* sensu lato 16 species in Thailand compared with those of other members in the subtribe Cassiinae (see Fig. 30)

After the *trn*L character-taxon data matrix of 16 cassia in Thailand was analysed and there were two distinctive major groups were found the next stepwas to ass nine more sequences of other Cassiinae species which were not found in Thailand but their sequences retrieved from GenBank to the analysis. These were *Chamaecrista nictitans* (AF365093), *Ch.* sp. Klitgaad (AF365093), *Ch.* sp. Breteler (AF365094), *Senna crassiramea* (AF365093), *S. lindheimeriana* (AF365089), *S. bauhinioides* (AF365087), *S. wislizeni* (AF365028), *S. bacillaris* (AF365031) and *Cassia grandis* (AF365092). Their trnL sequences were used to align together with 16 *Cassia* species from the previous data set. The new character-taxon matrix was shown in Fig. 30.

	....|....|	....|....|	....|....|	....|....|	....|....|
	10	20	30	40	50
	**********	**********	**********	**********	**********
Ceratonia siliqua AF365075	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
Gymnocladus dioica AF365095	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
Ch_nictitans AF365093	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
Ch. sp. Klitgaad AF365093	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
Ch. sp. Breteler AF365094	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
S. crassiramea AF365090	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
S. lindheimeriana AF365089	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
S. bauhinioides AF365087	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
S. wislizeni AF365028	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
S. bacillaris AF365031	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
C. grandis AF365092	??????????	??????????	??????????	??????????	??????????
C. leschenaultiana	**TCGAAATCGG**	**TAGACGCTAC**	**GGACTTAATT**	**GGATTGAGCC**	**TTGGTATGGA**
C. pumila	**??????????**	**???????TAC**	**GGACTTAATT**	**GGATTGAGCC**	**TTGGTATGGA**
C. grandis	??????????	??????????	??????????	GGATTGAGCC	TTGGTATGGA
C. javanica	??????????	??????????	GACCTTAATT	GGATTGAGCC	TTGGTATGGA
C. bakeriana	??????????	GTGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. fistula	????????CG	TAGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) sophera	??????????	??????????	?????????T	GGATTGAGCC	TTGGTATGGA
C. (S.) occidentalis	?????????C	GTGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) hirsuta	??????????	??????????	GACCTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) obtusifolia	?????CGTAG	ACGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) tora	??????????	??????????	?GACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) surattensis	??????????	??????????	GACCTTAATT	GGCTTGAGCC	TTGGTATGGA
C. (S.) timoriensis	??????????	???ACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) garettiana	??????????	TAGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) alata	??????????	GTGACGCTAC	GGACTTAATT	GGATTGAGCC	TTGGTATGGA
C. (S.) spectabilis	??????????	??????????	GACCTTAATT	GGATTGAGCC	TTGGTATGGA

Fig. 30 a 657 bp character-taxon matrix of 16 Thai *Cassia* species based on *trn*L intron sequences compared with some additional new-world Cassiinae species and two other outgroups. Asterisks * and * represent excluded and gap-matrix sites from analyses, respectively. Gap symbol (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data.

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                          60         70         80         90        100
                                  ********** *******
Ceratonia siliqua AF365075        AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
Gymnocladus dioica AF365095       AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
Ch_nictitans AF365093             AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
Ch. sp. Klitgaad AF365093         AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
Ch. sp. Breteler AF365094         AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
S. crassiramea AF365090           AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
S. lindheimeriana AF365089        AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
S. bauhinioides AF365087          AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
S. wislizeni AF365028             AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
S. bacillaris AF365031            AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. grandis AF365092               ?????????? ?T?????CTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. leschenaultiana                AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. pumila                         AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. grandis                        AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. javanica                       AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. bakeriana                      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. fistula                        AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)sophera                    AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)occidentalis               AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)hirsuta                    AACCTACCAA GTGATAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)obtusifolia                AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)tora                       AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)surattensis                AACCTACCGA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)timoriensis                AACCTACCAA GTGAGAACTT TCAAATTCAG CGAAACCCTG GAATTAACAA
C. (S.)garettiana                 AACCTACCAA GTGAGAACTT TCAAATTCAG CGAAACCCTG GAATTAACAA
C. (S.)alata                      AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA
C. (S.)spectabilis                AACCTACCAA GTGAGAACTT TCAAATTCAG AGAAACCCTG GAATTAACAA

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         110        120        130        140        150

Ceratonia siliqua AF365075        TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
Gymnocladus dioica AF365095       TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
Ch_nictitans AF365093             TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
Ch. sp. Klitgaad AF365093         TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
Ch. sp. Breteler AF365094         TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
S. crassiramea AF365090           TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
S. lindheimeriana AF365089        TGGGCAATCC TGAGCCAAAT CCTATTTTCC GAAAACCAAG AAGAGTTCAG
S. bauhinioides AF365087          TGGGCAATCC TGAGCCAAAT CCTATTTTCC GAAAACCAAG AAGAGTTCAG
S. wislizeni AF365028             TGGGCAATCC TGAGCCAAAT CCAGTTTTCC GAAAACCAAG AAGAGTTCAG
S. bacillaris AF365031            TGGGCAATCC TGAGCCAAAT CCTGTTTTCC AAAAACCAAG AAGAGTTCAA
C. grandis AF365092               TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. leschenaultiana                TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. pumila                         TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. grandis                        TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. javanica                       TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. bakeriana                      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. fistula                        TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAA AAGAGTTCAG
C. (S.)sophera                    TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)occidentalis               TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)hirsuta                    TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)obtusifolia                TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)tora                       TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)surattensis                TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)timoriensis                TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)garettiana                 TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)alata                      TGGGCAATCC TGAGCCAAAT CCTGTTTTCC GAAAACCAAG AAGAGTTCAG
C. (S.)spectabilis                TGGGCAATCC TGAGCCAAAT CCAGTTTTCC AAAAACCAAG AAGAGTTCAG
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Fig. 30 (continue)

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                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                            160        170        180        190        200
                                                          *** *****       *****
Ceratonia siliqua AF365075          AAAGCGCGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAT
Gymnocladus dioica AF365095         AAAGCGAGAA TAAAACAA-- ----GGATTG G-----TGCA GAGACTCAAT
Ch_nictitans AF365093               AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAT
Ch. sp. Klitgaad AF365093           AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAT
Ch. sp. Breteler AF365094           AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAT
S. crassiramea AF365090             AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
S. lindheimeriana AF365089          AAAGGGAGAA TAAAAAA--- ----GGATAG GATAGGTGCA GAGACTCAAC
S. bauhinioides AF365087            AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
S. wislizeni AF365028               AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
S. bacillaris AF365031              AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. grandis AF365092                 AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAC
C.(Ch.)leschenaultiana              AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAT
C.(Ch.)pumila                       AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAT
C. grandis                          AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAC
C. javanica                         AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAC
C. bakeriana                        AAAGGGAGAA TAAAAAAAA- ----GGATAG G-----TGCA GAGACTCAAC
C. fistula                          AAAGGGAGAA TAAAAAAAAA ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)sophera                      AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C. (S.)occidentalis                 AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C. (S.)hirsuta                      AAAGGGAGAA TAAAAAAA-- ----GGATAG GATAGGTGCA GAGACTCAAC
C. (S.)obtusifolia                  AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)tora                         AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)surattensis                  AAAGGGAGAA AAAAAAAAAA AAAAGGATAG G-----TGCA GAGACTCAAC
C. (S.)timoriensis                  AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)garettiana                   AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)alata                        AAAGGGAGAA TAAAAAAA-- ----GGATAG G-----TGCA GAGACTCAAC
C. (S.)spectabilis                  AAAGGGAGAA TAAAAAA--- ----GGATAG G-----TGCA GAGACTCAAC

                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                            210        220        230        240        250
                                                            ********* ********** **********
Ceratonia siliqua AF365075          GGAAGCTGTT CTAACAAATG G--------- ---------- ----------
Gymnocladus dioica AF365095         GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT ---CGTT-AG
Ch_nictitans AF365093               GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTGAA
Ch. sp. Klitgaad AF365093           GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTGAA
Ch. sp. Breteler AF365094           GGAAGCTGTT CTAACAAATG GGGTTGACGA CATTTCATTT TTTTA----G
S. crassiramea AF365090             GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
S. lindheimeriana AF365089          GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
S. bauhinioides AF365087            GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
S. wislizeni AF365028               GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
S. bacillaris AF365031              GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. grandis AF365092                 GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C.(Ch.)leschenaultiana              GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTTAA
C.(Ch.)pumila                       GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCATTT TTTTATTTAA
C. grandis                          GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. javanica                         GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. bakeriana                        GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. fistula                          GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)sophera                      GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)occidentalis                 GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTATT---
C. (S.)hirsuta                      GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)obtusifolia                  GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)tora                         GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)surattensis                  GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)timoriensis                  GGAAGCTGTT CTAACAAATG GAGTTGACGA TATTTCGTTT CGTTA----G
C. (S.)garettiana                   GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCCTTT CGTTA----G
C. (S.)alata                        GGAAGTTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
C. (S.)spectabilis                  GGAAGCTGTT CTAACAAATG GAGTTGACGA CATTTCGTTT CGTTA----G
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Fig. 30 (continue)

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                               ....|....| ....|....| ....|....| ....|....| ....|....|
                                      260        270        280        290        300
                               ********** ********** ********** ********** **********
Ceratonia siliqua AF365075     ---------- ---------- ---------- ---------- ----------
Gymnocladus dioica AF365095    TAAAGGAATC CTTCCATCAA AACTCCCGAA AA-------- ---GAAAGGA
Ch_nictitans AF365093          TAAAGGAATG CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGAA
Ch. sp. Klitgaad AF365093      TAAAGGAATG CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGAA
Ch. sp. Breteler AF365094      TAAAGGAATC CCCCCATCGA AACTCCAGAA AA-----AGA AAAGAAAGGA
S. crassiramea AF365090        TAAAGGAATC CTTCCATCGA AACTCCAGAA AAGAAAGAGA AAAGAAAGGA
S. lindheimeriana AF365089     TAAAGGAATC CTTCCATCGA AACTCCATAA AA-------- -------GGA
S. bauhinioides AF365087       TAAAGGAATC CTTCCATCGA AACTCCATAA AA-------- ---GAAAGGA
S. wislizeni AF365028          TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
S. bacillaris AF365031         TAAAGGAATC CTTCCATCAA AACTACAGAG AA-------- ---GAAAGGA
C. grandis AF365092            TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C.(Ch.)leschenaultiana         TAAAGGAATG CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGAA
C.(Ch.)pumila                  TAAAGGAATG CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGAA
C. grandis                     TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. javanica                    TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. bakeriana                   TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. fistula                     TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)sophera                 TAAAGGAATC CTTCCATCGA AACTCCATAA AA-------- ---GAAAGGA
C. (S.)occidentalis            -AAAGGAATC CTTCCATCGA AACTCCATAA AA-------- ---GAAAGGA
C. (S.)hirsuta                 TAAAGGAATC CTTCCATCGA AACTCCATAA AA-------- ---GAAAGGA
C. (S.)obtusifolia             TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)tora                    TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)surattensis             TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)timoriensis             TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)garettiana              TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)alata                   TAAAGGAATC CTTCCATCGA AACTCCAGAA AA-------- ---GAAAGGA
C. (S.)spectabilis             TAAAGGAATA CTTCCATCGA AACTACAGAA AA-------- ---GAAAGGA

                               ....|....| ....|....| ....|....| ....|....| ....|....|
                                      310        320        330        340        350
                               ********** ********** ******
Ceratonia siliqua AF365075     ---------- ---------- ------ATAC GTACTGAAAT ACTATTTCAA
Gymnocladus dioica AF365095    TCAAGGATGA ACATATAT-- ------ATAC GTACTGAAAT ACTATTTCAA
Ch_nictitans AF365093          TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
Ch. sp. Klitgaad AF365093      TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
Ch. sp. Breteler AF365094      TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
S. crassiramea AF365090        TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
S. lindheimeriana AF365089     TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
S. bauhinioides AF365087       TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
S. wislizeni AF365028          TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
S. bacillaris AF365031         TCGAGGATGA ACATATATA- ---CGTATAC GTACTGAAAT ACTATCTCAA
C. grandis AF365092            TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C.(Ch.)leschenaultiana         TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C.(Ch.)pumila                  TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. grandis                     TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. javanica                    TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. bakeriana                   TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. fistula                     TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)sophera                 TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT AGTATTTCAA
C. (S.)occidentalis            TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT AGTATTTCAA
C. (S.)hirsuta                 TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT AGTATTTCAA
C. (S.)obtusifolia             TCGAGGATGA ACATATATAT ATACGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)tora                    TCGAGGATGA ACATATATAT ATACGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)surattensis             TCGAGGATGA ACATATATAT A--CGTATAC ATACTGAAAT ACTATTTCAA
C. (S.)timoriensis             TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)garettiana              TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)alata                   TCAAGGATGA ACATATATAT A--CGTATAC GTACTGAAAT ACTATTTCAA
C. (S.)spectabilis             TCAAGGATGA ACATATATA- ---CGTATAC GTACTGAAAT ACTATTTCAA
```

Fig. 30 (continue)

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     360        370        380        390        400
                                         ********** *******      ***** ******
Ceratonia siliqua AF365075    TTGATTAGAC C--------- ----CCAAAT CTCTATTTTT T----AATAT
Gymnocladus dioica AF365095   TTGATTAGAC C--------- ----CAAAAT CTCTATTTRG TC----ATAT
Ch_nictitans AF365093         TTGATTAGAC CAAACAGAC- --CCCC-AAT CTCCATTTTT T----AATAT
Ch. sp. Klitgaad AF365093     TTGATTAGAC CAAACAGAC- --CCCC-AAT CTCCATTTTT T----AATAT
Ch. sp. Breteler AF365094     TTGATTAGAC CAGACAGAC- --CCCC-AAT CTCCATTTTT ----GAATAT
S. crassiramea AF365090       TTGATTAGAC CAGACAGAC- --CCC-AAAT CTCTACTTTT T----AATAT
S. lindheimeriana AF365089    TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTAGTTTT T----AATAT
S. bauhinioides AF365087      TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTAGTTTT T----AATAT
S. wislizeni AF365028         TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTATTTTT T----AATAT
S. bacillaris AF365031        TTGATTAGAC CAGACAGAC- --CGC-GAAT CTCTA----- ------ATAT
C. grandis AF365092           TTGATTAGAC CAGACAGAC- --TCC-AAAT CCCTATTTTT T----AATAT
C.(Ch.)leschenaultiana        TTGATTAGAC CAAACAGAC- --CCCC-AAT CTCCATTTTT T----AATAT
C.(Ch.)pumila                 TTGATTAGAC CAAACAGAC- --CCCC-AAT CTCCATTTTT T----AATAT
C. grandis                    TTGATTAGAC CAGACAGAC- --TCC-AAAT CCCTATTTTT T----AATAT
C. javanica                   TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTATTTTT T---GAATAT
C. bakeriana                  TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTATTTTT T---GAATAT
C. fistula                    TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTATTTTT TTTT-AATAT
C. (S.)sophera                TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTAGTTTT T----AATAT
C. (S.)occidentalis           TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTAGTTTT T----AATAT
C. (S.)hirsuta                TTGATTAGAC CAGACAGAC- --CTC-AAAT CCCTAGTTTT T----AATAT
C. (S.)obtusifolia            TTGATTAGAC CAGACAGAC- --CGC-GAAT CTCTA----- ------ATAT
C. (S.)tora                   TTGATTAGAC CAGACAGAC- --CGC-GAAT CTCTA----- ------ATAT
C. (S.)surattensis            TTGATTAGAC CAGACAGAC- --CCC-GAAT CTCTA----- ------ATAT
C. (S.)timoriensis            TTGATTAGAC ------GAC- --CCC-AAAT CTCTATCTTT T----AATAT
C. (S.)garettiana             TTGATTAGAC ------GAC- --CCC-AAAT CTCTATCTTT T----AATAT
C. (S.)alata                  TTGATTAGAC CAGACAGACA GACCCCAAAT CTCTATTTTT T----AATAT
C. (S.)spectabilis            TTGATTAGAC CAGACAGAC- --CCC-AAAT CCCTATTTTT ----AAATAT

                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     410        420        430        440        450
                                             ******* *****
Ceratonia siliqua AF365075    TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
Gymnocladus dioica AF365095   TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
Ch_nictitans AF365093         TTATATGAAA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
Ch. sp. Klitgaad AF365093     TTATATGAAA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
Ch. sp. Breteler AF365094     TTATATGAAA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
S. crassiramea AF365090       TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTTCAAGTT
S. lindheimeriana AF365089    TTATATGACA AATTATATGA CAAATAAAAG ATGTGAATCG ATTCCAAGTT
S. bauhinioides AF365087      TTATATGACA AATTATATGA CAAATAAAAG ATGTGAATCG ATTCCAAGTT
S. wislizeni AF365028         TTAGATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
S. bacillaris AF365031        TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
C. grandis AF365092           TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C.(Ch.)leschenaultiana        TTATATGAAA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C.(Ch.)pumila                 TTATATGAAA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C. grandis                    TTATATGACA AAT------- -----GAAAG ATGTGAATCC ATTCCAAGTT
C. javanica                   TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C. bakeriana                  TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C. fistula                    TTATATGACA AAT------- -----GAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)sophera                TTATATGACA AATTATATGA CAAATAAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)occidentalis           TTATATGACA AATTATATGA CAAATAAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)hirsuta                TTATATGACA AATTCTATGA CAAATAAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)obtusifolia            TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)tora                   TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)surattensis            TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTTCAAGTT
C. (S.)timoriensis            TTATATGACA AAT------- -----AATAG ATGTGAATCG ATTCCAAGTT
C. (S.)garettiana             TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)alata                  TTATATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
C. (S.)spectabilis            TTAGATGACA AAT------- -----AAAAG ATGTGAATCG ATTCCAAGTT
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Fig. 30 (continue)

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     460        470        480        490        500
                                                 *
Ceratonia siliqua AF365075    GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
Gymnocladus dioica AF365095   GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
Ch_nictitans AF365093         GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
Ch. sp. Klitgaad AF365093     GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
Ch. sp. Breteler AF365094     GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
S. crassiramea AF365090       GAAGAAAGAA TCGAAT-ATT CATTGAGAAA ATCATTCACT CCATCATAGT
S. lindheimeriana AF365089    GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATAATTCACT CCATCATAGT
S. bauhinioides AF365087      GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATAATTCACT CCATCATAGT
S. wislizeni AF365028         GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
S. bacillaris AF365031        GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCGTAGT
C. grandis AF365092           GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C.(Ch.)leschenaultiana        GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
C.(Ch.)pumila                 GAAGAAAGAA TCGAAT-ATT CATTGATCAA ATCATTCACT CCATCATAGT
C. grandis                    GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. javanica                   GAAGAAAGAA TCAAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. bakeriana                  GAAGAAAGAA TCAAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. fistula                    GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)sophera                GAAGAAAGAA TCTAATGATT TTTTGATAAA ATCATTCACT CCATCATAGT
C. (S.)occidentalis           GAAGAAAGAA TCTAAT-ATT CTTTGATAAA ATCATTCACT CCATCATAGT
C. (S.)hirsuta                GAAGAAAGAA TCGAAT-ATT CTTTGATAAA ATCATTCACT CCATCATAGT
C. (S.)obtusifolia            GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)tora                   GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)surattensis            GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)timoriensis            GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT ACATCATAGT
C. (S.)garettiana             GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)alata                  GAAGAAAGAA TCGAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
C. (S.)spectabilis            GAAGAAAGAA TCAAAT-ATT CATTGATAAA ATCATTCACT CCATCATAGT
```

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     510        520        530        540        550
                                            *****
Ceratonia siliqua AF365075    CTGATAGATC TTTT-----G AAAAACTGAT TAATCGGACG AGAATAAAGA
Gymnocladus dioica AF365095   CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
Ch_nictitans AF365093         CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
Ch. sp. Klitgaad AF365093     CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
Ch. sp. Breteler AF365094     CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGTCG AGAATAAAGA
S. crassiramea AF365090       CTGATAGATC TTTT-----G AAGAACTTAT TAATCGGACG AGAATAAAGA
S. lindheimeriana AF365089    CTGATAGATC TTTTCTTTTG AAGAACTGAT TAATCGGACG AGAATAAAGA
S. bauhinioides AF365087      CTGATAGATC TTTTCTTTTG AAGAACTGAT TAATCGGACG AGAATAAAGA
S. wislizeni AF365028         CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
S. bacillaris AF365031        TTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. grandis AF365092           CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C.(Ch.)leschenaultiana        CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C.(Ch.)pumila                 CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. grandis                    CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. javanica                   CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. bakeriana                  CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. fistula                    CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)sophera                CTGATAGATC TTTTCTTTTG AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)occidentalis           CTGATAGATC TTTTCTTTTG AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)hirsuta                CTGATAGATC TTTTCTTTTG AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)obtusifolia            TTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)tora                   TTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)surattensis            TTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)timoriensis            CGGATGGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)garettiana             CTGATGGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)alata                  CTGATAGATC TTTT-----G AAGAACTGAT TAATCGGACG AGAATAAAGA
C. (S.)spectabilis            CTGATAGATC TTTT-----G AAGAACTTAT TAATCGGACG AGAATAAAGA
```

Fig. 30 (continue)

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         560        570        580        590        600
                                                                                 *******
Ceratonia siliqua AF365075        TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
Gymnocladus dioica AF365095       TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
Ch_nictitans AF365093             TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
Ch. sp. Klitgaad AF365093         TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
Ch. sp. Breteler AF365094         TAGAGTCCCA TTTTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
S. crassiramea AF365090           TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
S. lindheimeriana AF365089        TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
S. bauhinioides AF365087          TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
S. wislizeni AF365028             TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATTGTAA
S. bacillaris AF365031            TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C. grandis AF365092               TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGGGA TTT-------
C.(Ch.)leschenaultiana            TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(Ch.)pumila                     TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C. grandis                        TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C. javanica                       TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C. bakeriana                      TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C. fistula                        TAGAGTCCCA TTCTACATGT CAATACCGAC AACAA-GAAA TTTATAGTAA
C.(S.)sophera                     TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTTTAGTAA
C.(S.)occidentalis                TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)hirsuta                     TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)obtusifolia                 TAGAGTCCCA TTCTACATGT CAATACCGAC AACAA-GAAA TTTATAGTAA
C.(S.)tora                        TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)surattensis                 TAGAGTCCCA TTCTACATGT CAATACCGAC AACAA-GAAA TTTATAGTAA
C.(S.)timoriensis                 TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)garettiana                  TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)alata                       TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA
C.(S.)spectabilis                 TAGAGTCCCA TTCTACATGT CAATACCGAC AACAATGAAA TTTATAGTAA

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         610        620        630        640        650
                                  ********** ********** ********** ********** **********
Ceratonia siliqua AF365075        GAGGAAAATC CGTCGACTTT AGAAAT-CGT GA???????? ??????????
Gymnocladus dioica AF365095       GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
Ch_nictitans AF365093             GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAG??????? ??????????
Ch. sp. Klitgaad AF365093         GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAG??????? ??????????
Ch. sp. Breteler AF365094         GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAG??????? ??????????
S. crassiramea AF365090           GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
S. lindheimeriana AF365089        GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
S. bauhinioides AF365087          GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
S. wislizeni AF365028             GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
S. bacillaris AF365031            GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGG?????? ??????????
C. grandis AF365092               ---------- ---------- ---------? ?????????? ??????????
C.(Ch.)leschenaultiana            GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGGGTTCAA GTCCTTCCTA
C.(Ch.)pumila                     GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGGG????? ??????????
C. grandis                        GAGGAAAATC CGTCGACTT- AGAAAT-CG? ??GGG????? ??????????
C. javanica                       GAGGAAAATC CGTCGACTTT AGAAAT-CG? ?????????? ??????????
C. bakeriana                      GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAG??????? ??????????
C. fistula                        GAGGAAAATC CGTCGACTTT AGAAAT-CG? GA???????? ??????????
C.(S.)sophera                     GAGGAAAATC CGTCGACTTT AGAAAT-CG? ??GGG????? ??????????
C.(S.)occidentalis                GAGGAAAATC CGTCGACTTT TGAAAT-CGT GA???????? ??????????
C.(S.)hirsuta                     GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGGGTTCAA GTCCCTT???
C.(S.)obtusifolia                 GAGGAAAATC CGTCGACTTT AGAAATGCG? GAG??????? ??????????
C.(S.)tora                        GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAG??????? ??????????
C.(S.)surattensis                 GAGGAAAATC CGTCGACTTT AGAAAT-CG? GAG??????? ??????????
C.(S.)timoriensis                 GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGGGTTCAA GTCC??????
C.(S.)garettiana                  GAGGAAAATC CGTCGACTTT AGAAAT-CGT GAGGGTTCAA GTCCTCTATC
C.(S.)alata                       GAGGAAAATC CGTCGACTTT AGAAAT-CG? GA???????? ??????????
C.(S.)spectabilis                 GAGGAAAATC CGTCGACTTT AGAAAT-CG? ?????????? ??????????
```

Fig. 30 (continue)

	....|..
	*******
Ceratonia siliqua AF365075	???????
Gymnocladus dioica AF365095	???????
Ch_nictitans AF365093	???????
Ch. sp. Klitgaad AF365093	???????
Ch. sp. Breteler AF365094	???????
S. crassiramea AF365090	???????
S. lindheimeriana AF365089	???????
S. bauhinioides AF365087	???????
S. wislizeni AF365028	???????
S. bacillaris AF365031	???????
C. grandis AF365092	???????
C.(Ch.)leschenaultiana	**TCCCCAA**
C.(Ch.)pumila	**???????**
C. grandis	???????
C. javanica	???????
C. bakeriana	???????
C. fistula	???????
C.(S.)sophera	???????
C.(S.)occidentalis	???????
C.(S.)hirsuta	???????
C.(S.)obtusifolia	???????
C.(S.)tora	???????
C.(S.)surattensis	???????
C.(S.)timoriensis	???????
C.(S.)garettiana	CCCAAC?
C.(S.)alata	???????
C.(S.)spectabilis	???????

Fig. 30 (continue)

A 586 bp *trn*L intron sequences of Thai *Cassia* 16 species (no *C. siamea*) and other New-World Cassiinae was analysed using branch-and-bound searching strategy. A 35 position gap matrix (0, 1 symbols) was included. The full data matrix had 657 characters but 107 nucleotides (the first position to the 67th, the 385th to the 395th and the 629th to the 657th) were necessary to be excluded. Gaps were treated as missing data. *Gymnocladus dioica* (GenBank AF365095) and *Ceratonia siliqua* (AF365075) were included into the analysis as outgroup taxa. All characters used in phylogenetic tree reconstruction were unordered and equally weighted with 57 characters (9.7%) were parsimony-informative as synapomorphy and 46 characters were parsimony-uninformative as autapomorphy. There were 6 most parsimonious trees found from this data set, with 127 steps in length (Fig. 31). Consistency index (CI) was 0.8425 and homoplasy index (HI) was 0.1575. Retention index (RI) was 0.8788 and rescaled consistency index (RC) was 0.7404.

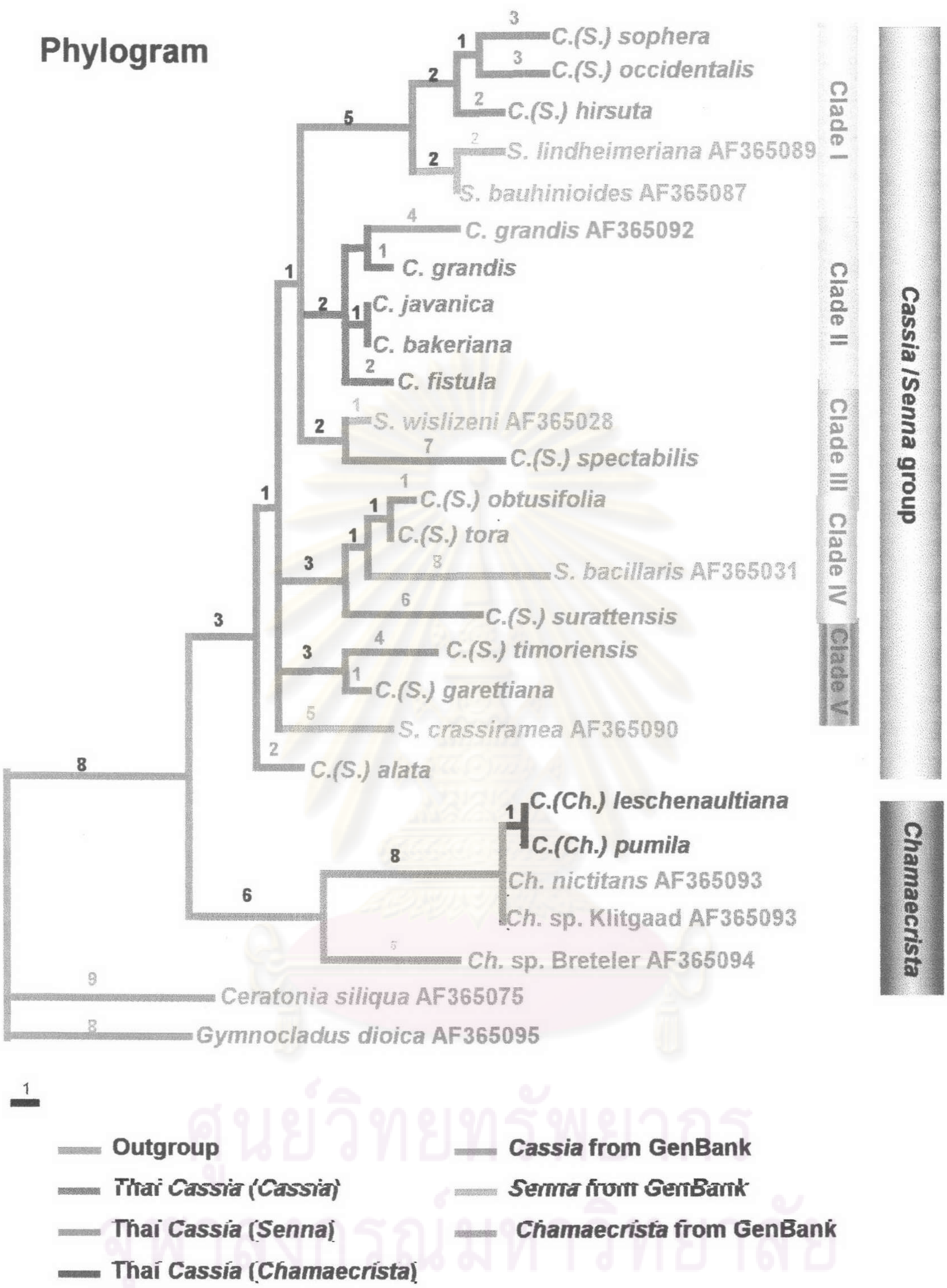

Fig. 31 one phylogram of 6 equally most parsimonious trees (127 steps in length) for 16 *Cassia* in Thailand based on *trn*L intron sequence data compared with nine additional Cassiinae New-World species and two outgroups, *Ceratonia siliqua* and *Gymnocladus dioica*. Numbers in black are amounts of synapomorphy and colouring numbers are amounts of autapomorphy. [CI = 0.8425, RI = 0.8788, RC = 0.7404]

All nine most equally most parsimonious trees (MPTs) were further analysed to produce strict (Fig. 32), semistrict (Fig. 33) and 50% majority-rule (Fig. 34) consensus trees. All three consensus trees were similar to each other that all members of Cassiinae were grouped together with high bootstrap and jackknife supporting-values (99%, 98%), respectively, while the two outgroups *Gymnocladus dioica* (AF365095) and *Ceratonia siliqua* (AF365075) were clustered. Within the Cassiinae group, there were two major clades separated distinctively. The fist clade had *C.*(*Ch.*) *leschenaultiana* and *C.*(*Ch.*) *pumila* which moved to the genus *Chamaecrista* based on Flora Malesiana, grouped with other New-World *Chamaecrista* species: *Chamaecrista nictitans*, *Ch.* sp. Klitgaad and *Ch.* sp. Breteler, with very high robustness of bootstrap (97%) and jackknife (95%) supporting-values. The other group contained all Thai *Cassia* species not moved to the genus *Chamaecrista*, clustered with other New-World *Senna* species and *C. grandis* (AF365092) from GenBank.

The *Cassia/Senna* group (85% BS and 82% JK) composed of *Cassia* (*Senna*) *sophera*, *C.*(*S.*) *occidentalis*, *C.*(*S.*) *hirsuta*, *Senna lindheimeriana*, *S. bauhinioides*, *S. wislizeni*, *C. grandis* (AF365092), *C. grandis*, *C. javanica*, *C. bakeriana*, *C. fistula*, *C.*(*S.*) *spectabilis*, *C.*(*S.*) *obtusifolia*, *C.*(*S.*) *tora*, *S. bacillaris*, *C.*(*S.*) *surattensis*, *C.*(*S.*) *timoriensis*, *C.*(*S.*) *garrettiana*, *S. crassiramea* and *C.*(*S.*) *alata*. Considering subgroupings in this *Cassia/Senna* group, the clade could be subdivided into five minor clades. The first clade contained three Thai *Cassia* (*Senna*), (*C.*(*S.*) *sophera*, *C.*(*S.*) *occidentalis* and *C.*(*S.*) *hirsuta*) and New-World *Senna* (*S. lindheimeriana* and *S. bauhinioides*) with very high supporting-values (99% BS and 97% JK). The clade II composed of New-World *C. grandis* and all Thai *Cassia* (*Cassia*) species with 65% BS and 53% JK supporting-values. The clade III contained *S. wislizeni* and *C.*(*S.*) *spectabilis* with 68% BS and 64% JK supporting-values. The fouth clade composed other three Thai *Cassia* (*Senna*) (*C.*(*S.*) *obtusifolia*, *C*(*S.*) *tora* and *C.*(*S.*) *surattensis*) and *S. bacillaris* (69% both BS and JK). The last clade of *C.*(*S.*) *timoriensis* and *C.*(*S.*) *garrettiana* had high supporting-values (94% BS and 86% JK).

Apart from the similarity between the strict, semistrict and 50% majority-rule consensus trees as above, the strict onsensus tree however indicated that there were two remaining taxa unable to be grouped as a clade. These taxa were *S. crassiramea* and *C.*(*S.*) *alata,* though *S. crassiramea* was being a less significant member of the clade IV of the semitrict consensus tree. Moreover, the semistrict and 50% majority-rule revealed that *Ch. nictitans* paired with *Ch.* sp. Klitgaad and *C.*(*Ch.*) *leschenaultiana* coupled with *C.*(*Ch.*) *pumila* within the *Cassia* (*Chamaecrista*) group, though with low supporting-values. Note that *C.*(*S.*) *alata* was always not able to form a resolved grouping with other species in any kind of consensus trees.

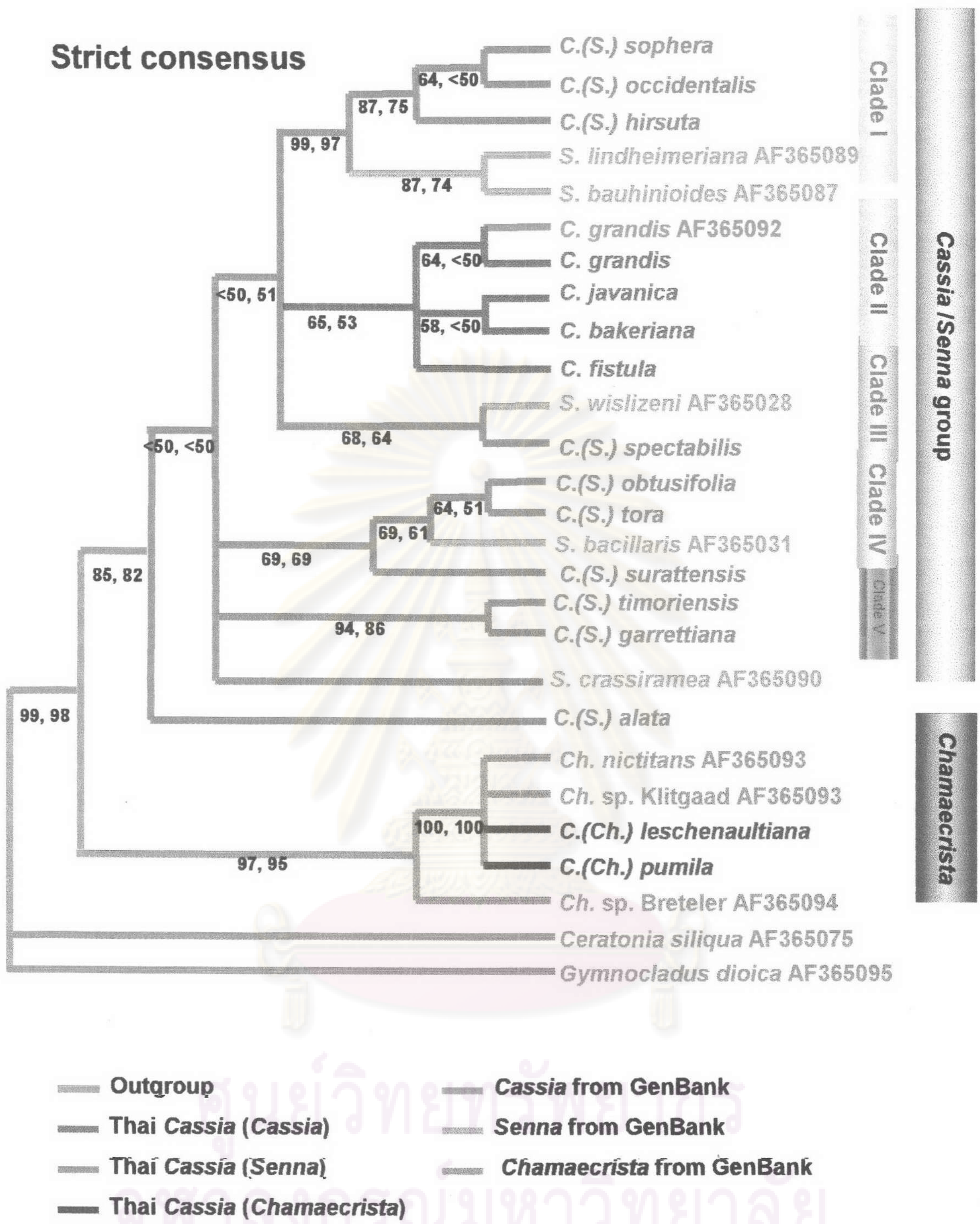

Fig. 32 strict consensus tree from 6 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data compared with nine additional Cassiinae new-world species, *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The numbers along branches are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively.

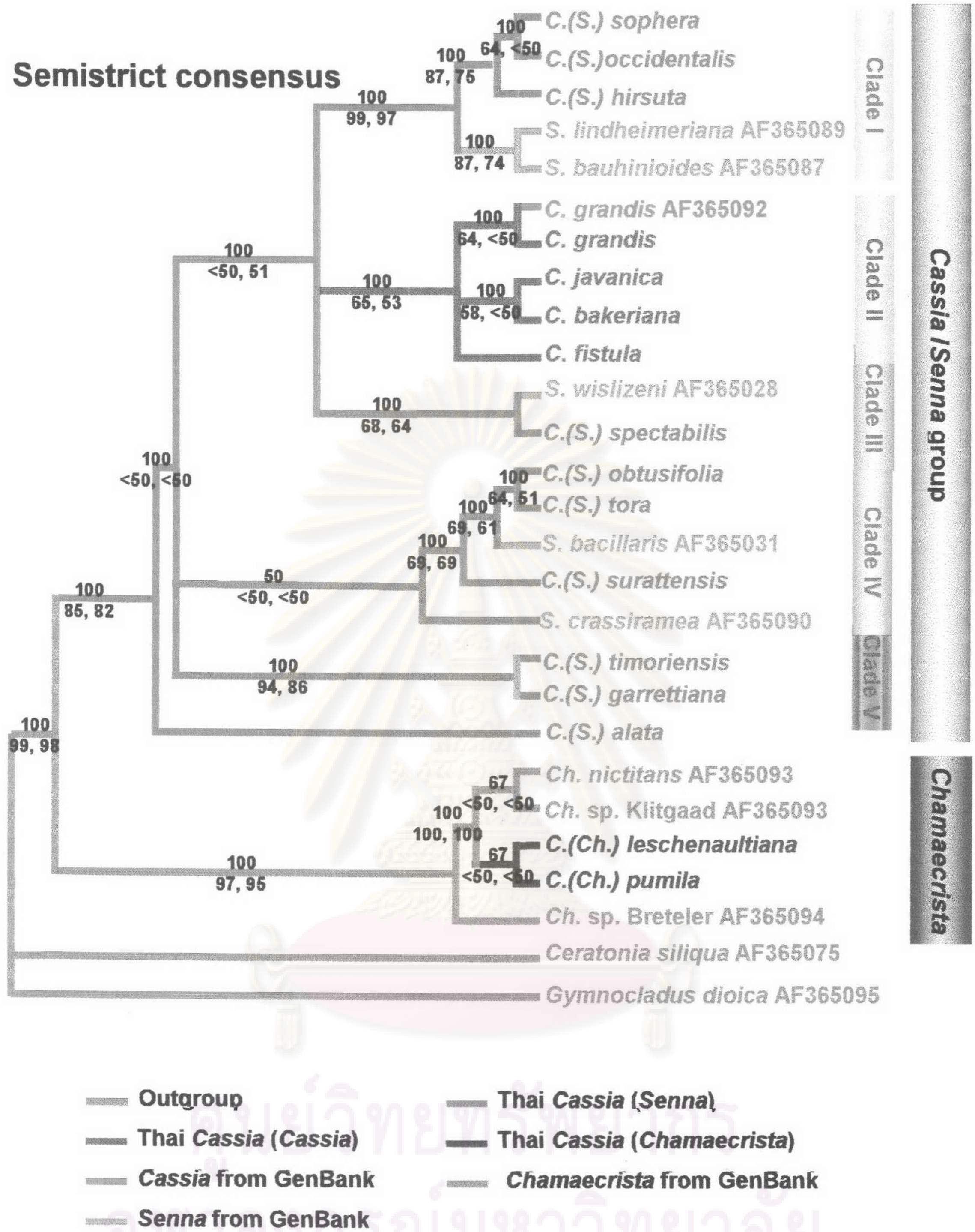

Fig. 33 semistrict consensus tree from 6 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data compared with nine additional Cassiinae new-world species, *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The upper numbers are percentages of congruent MPTs on the consensus tree and the lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively.

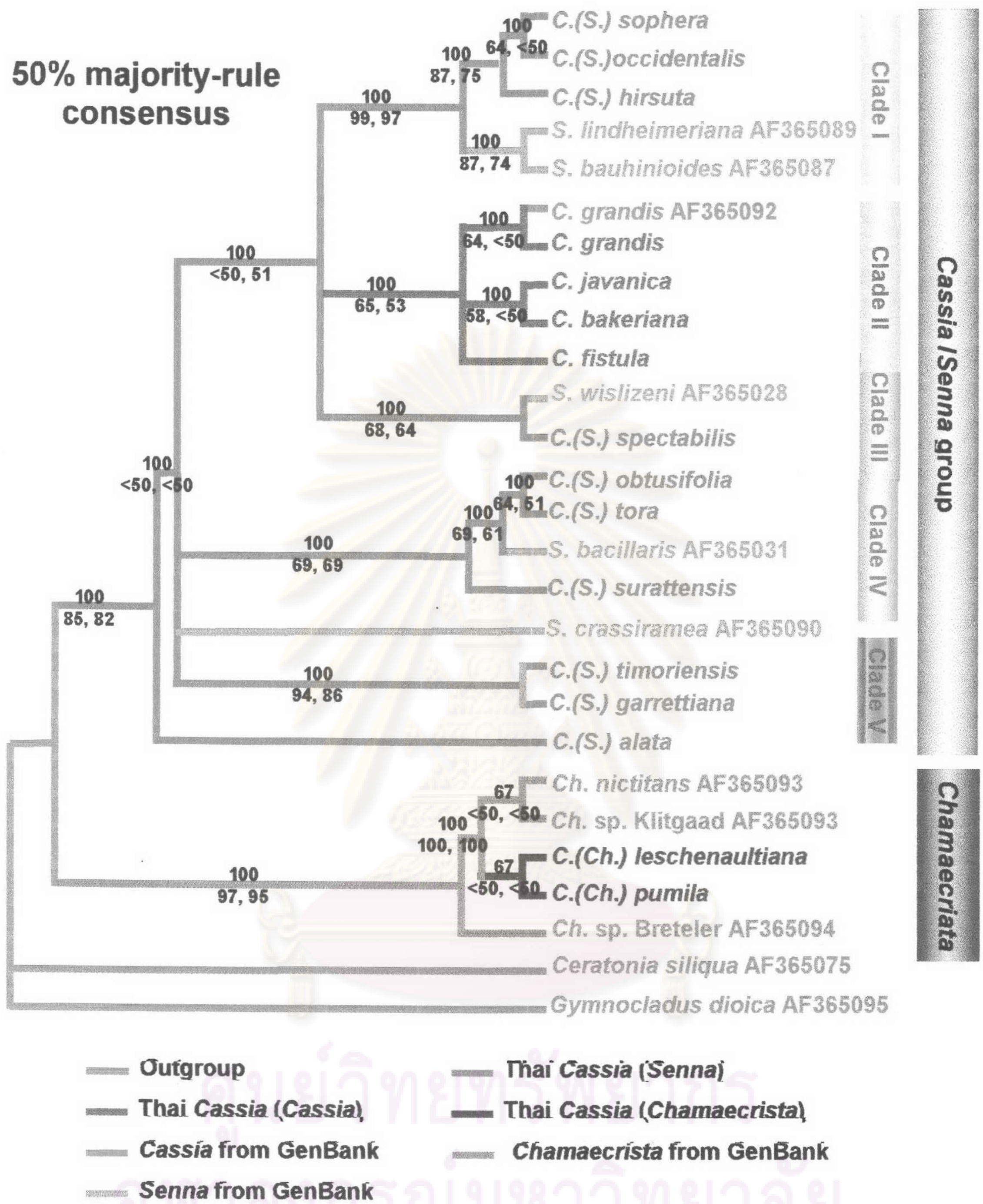

Fig. 34 50% majority-rule consensus tree from 6 most parsimonious trees of 16 *Cassia* in Thailand based on *trn*L intron sequence data compared with nine additional Cassiinae new-world species, *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieved from GenBank). The upper numbers are amounts of similarity MPTs on the consensus trees and the lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively.

Neighbour-joining (NJ) tree was also made for this new data matrix to compare with the results from maximum parsimony analyses. The NJ tree showed that all Thai *Cassia* species suggested to be *Chamaecrista* (*C.*(*Ch.*) *leschenaultiana* and *C.*(*Ch.*) *pumila*) were grouped as sister with each other to other New-World *Chamaecrista* as in result of parsimony analysis. Four true Thai *Cassia* (*Cassia*) species were also clustered as a unique group with 65% BS and 53% JK. Several more resolved groups were found from NJ tree but two taxa *C.*(*S.*) *alata* and *S. crassiramea* were still unable to group with any other Cassiinae.

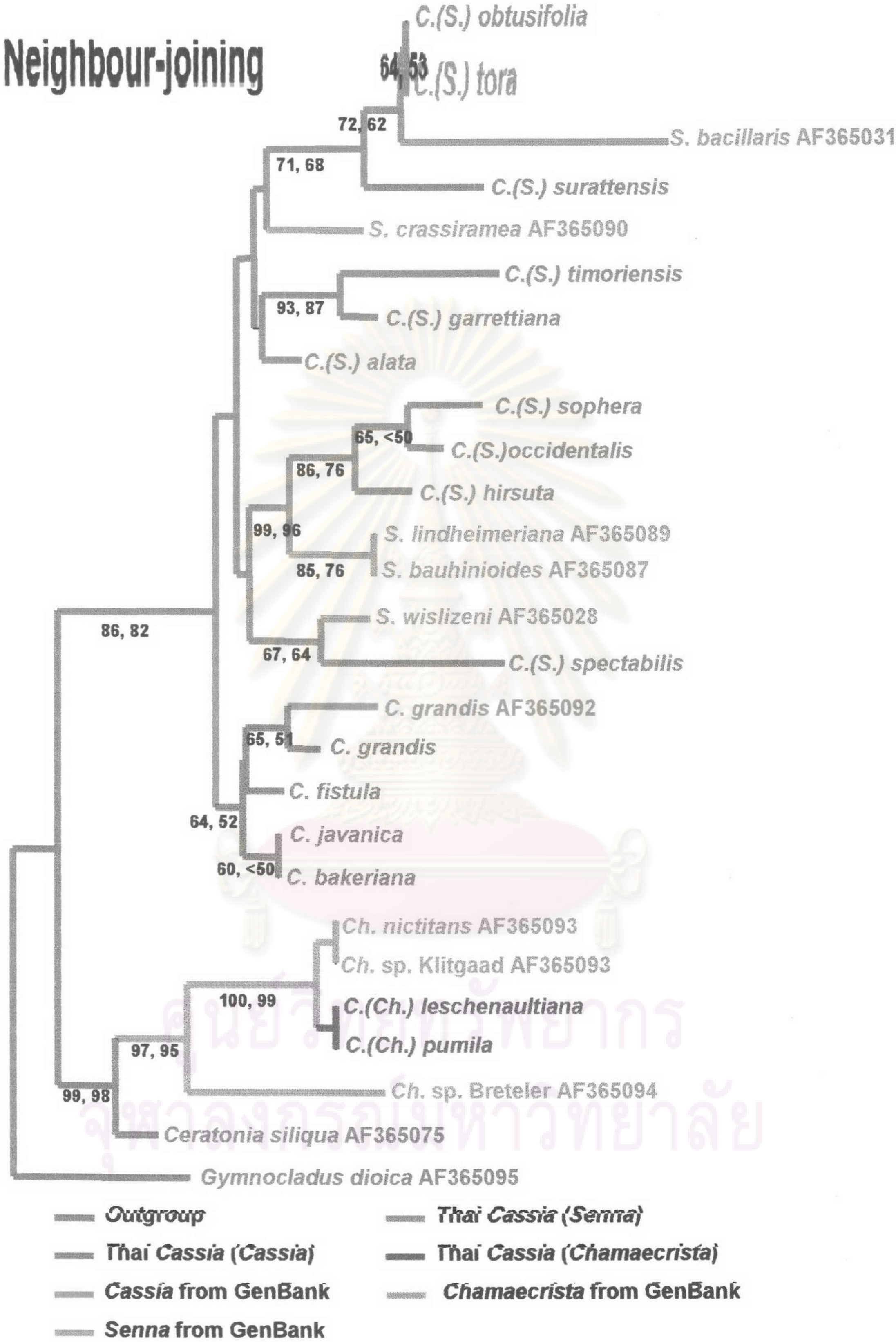

Fig. 35 neighbour-joining tree of 16 *Cassia* in Thailand based on *trn*L intron sequence data compared with nine additional Cassiinae New-World species, *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieve from GenBank). The numbers along branches are 1000-replicate bootstraps and jackknife supporting-values, respectively. Note that supporting-values less than 50% were not shown in the tree.

4.5.2 ITS1-5.8S-ITS2 sequences data set (see Fig. 36)

A total ITS1-5.8S-ITS2 sequences data matrix of 13 Thai *Cassia* was also prepared and phylogenetically analysed. Partial sequences of 16S small and 28S large subunits of nuclear ribosomal DNA were prior excluded. The sequence matrix was automatically aligned by Clustal X program and transformed to NEXUS file format, and adjusted manually to get a highest level of homology before phylogenetically analyses (Fig. 40). Gaps were treated as missing data. The data set composed of 913 bp sequence data which the 290 bp belong to the ITS1 regions, 160 bp to the 5.8S subunit and 255 bp to the ITS2 region. The DNA data matrix had total ITS sequences of 13 Thai *Cassia* species. Those of *C.(S.) siamea*, *C.(S.) garrettiana*, *C.(Ch.) pumila* and *C.(Ch.) leschenaultiana* could not be obtained because of difficulty in PCR amplification. The 206 bp regions of 16S (the first position to the 53rd position) and 28S subunits (the 759 position to the last one) were excluded before analysed and were not shown in the data matrix. Sequences of *Gymnocladus dioica* (AF510030, 33) and *Ceratonia siliqua* (AJ245575) retrieved from GenBank were included into the analysis as outgroup taxa.

```
                               ....|....| ....|....| ....|....| ....|....| ....|....|
                                      10         20         30         40         50
                               ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 ?????????? ?????????? ???TTTCTGT AGGTGAACCT GCGG??AAGG
Ceratonia siliqua AJ245575     TGGAAGGAGA AGTCGTAACA AGGTTTTCGT AGGTGAACCT GTGG??AAGG
C. grandis                     ?????????? ?????????? ??AGTTACGT AGGTGAACTT GTGA??AAGG
C. javanica                    ?????????? ?????????? ?????TTCGT AGGTGA?CCT GCGG??AAGG
C. bakeriana                   ?????????? ?????????? ?????TTCGT AGGTGA?CCT GCGG??AAGG
C. fistula                     ?????????? ?????????? ????TTCCGT AGGTGAACCT GCGG??AAGG
C.(S.) sophera                 ?????????? ?????????? ??CACGTCGT AGGTGA?CCT GCGG??AAGG
C.(S.) occidentalis            ?????????? ?????????? ?CAGTTCCGT AGGTGAACCT GCGG??AAGG
C.(S.) hirsuta                 ?????????? ?????????? ?CAGTTCCGT AGGTGAACCT GCGG??AAGG
C.(S.) obtusifolia             ?????????? ?????????? ?????TTCGT AGGTGAACCT GCGG??AAGG
C.(S.) tora                    ?????????? ?????????? ??AGTTCCGT AGGTGAACCT GCGG??AAGG
C.(S.) surattensis             ?????????? ?????????? ????TT?CGT AGGTGAACCT GCGG???AGG
C.(S.) timoriensis             ?????????? ?????????? ?????????? ????GGGCC? ??GG??AAGG
C.(S.) alata                   ?????????? ?????????? ????TT?CGT AGGTGAGC?T ??GGAGAAGG
C.(S.) spectabilis             ?????????? ?????????? ?ACAGTCCGT AGGTGAACCT GCGG??AAGG
```

Fig. 36 a 913 bp character taxa matrix of Thai *Cassia* based on ITS regions sequences for doing tree reconstruction. Asterisks *, *, *, * and * represent 18S, ITS1, 5.8S, ITS2 and 26S regions, respectively. Gaps symbols (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data.

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       60         70         80         90        100
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030, 33 ATCATTGTCG ATGCCTATCA AACTGGACGG CCCGCGAATC GGTTAAG--A
Ceratonia siliqua AJ245575      ATCATTGTCG ATGCCTCACA AAACGAACGA CCTGCGAATT GGTTAA---A
C. grandis                      ATCCTTGTTG ATGCCT--CA ACCAGAATGA CACATGAGCC AG--------
C. javanica                     ?TCATTGTCG ATGCCT--CA ACCAGAACGA CACGCGAACC GGTTCAAGAG
C. bakeriana                    ATCATTGTCG ATGCCT--CA ACCAGAACGA CACGCGAACC GGTTAAAGAG
C. fistula                      ATCATTGTCG ATGCCT--CA ACCAGAACGA CACGCGAACC AGTTAAGTGT
C.(S.) sophera                  ATCATTGTCG ATGCCTCGCA AACTGGACGA CTCGTGAATC GGTTGAAACA
C.(S.) occidentalis             ATCATTGTCG ATGCCTCGCA AACTGGACGA CTCGTGAACC GGTTGAAACA
C.(S.) hirsuta                  ATCATTGTCG ATGCCTCGCA AACTGGACGA CTCGTGAACT GGTTGAAACA
C.(S.) obtusifolia              ATCATTGTCG TTGCCTCACA AACGGGACCA CTCGCGAATT GGTTGAAACA
C.(S.) tora                     ATCATTGTCG TTGCCTCACA AACGGGACCA CTCGCGAATT GGTTGAAACA
C.(S.) surattensis              ATCATTGTCG ATGCCTCGCA AAYGGTACCA CTCGCGAACA GGTTGAAATG
C.(S.) timoriensis              ATCATTGTTG ATGCCTCACA AACAGGACGA TTTGCGAATC GGTTGAACTA
C.(S.) alata                    ATCATTGTCG ATGCCTCGCA AACAGGACGA CCCGCGAACC GGTTGAACCA
C.(S.) spectabilis              ATCATTGTCG TTGC-TCACA AACAGCACGA CGCGCGAATC GGTTGAACCA

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       110        120        130        140        150
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33  CCACCGGGG- ----AGGCGG AGGGCGTGC- ---------- --GTCGCCCC
Ceratonia siliqua AJ245575      CTATCGGG-- -----GGCGG GGGGCGTGC- ---------- --GTCCTCCC
C. grandis                      ---------- --G-AGG--A ---------- ---------- ----------
C. javanica                     TACCA-ACCG AGGGGGAGGA GGGATGGGGC GTGCTGTGCT CTGCCTGCCT
C. bakeriana                    TACCGTACCG TGGGGGAGGA GGGATGGGGG ----CGTGCC CTGCCTGCCT
C. fistula                      ACCATGCCAG GGGAGGAGGA GGGATGGGGG CGTGCTATGC CTGCCTCCAG
C.(S.) sophera                  ATCTCGGGT- TGGGAGACGA GTGGTGTGC- ---------- --GTCCCACT
C.(S.) occidentalis             ATCTCGGGG- TGGGAGACGA GTGGTGTGC- ---------- --GTCCCACT
C.(S.) hirsute                  ATCTCGGGG- TGGGAGACGA GTGGTGTGC- ---------- --GTCCCACT
C.(S.) obtusifolia              CTCCCGAGG- TGGTAGACGA GCGGCGTGC- ---------- --GTCGCCCT
C.(S.) tora                     CTCCCGAGG- TGGTAGACGA GCGGCGTGC- ---------- --GTCGCCCT
C.(S.) surattensis              CTCCCGAGGC GGG-AGGCGA GGGGCGTGC- ---------- --GTCGCCCT
C.(S.) timoriensis              ATCTCGGGCT AGGCAAATGA GGGGTCCATG ---------- --ATTGCCCC
C.(S.) alata                    ATCCCGGGG- AGGGAGGCGA GGGGCATGC- ---------- --ACTGCCTC
C.(S.) spectabilis              ATACGGGGGA GGGAGGCCGA AGGGGTGCCC ---------- --GTCGCGCC

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       160        170        180        190        200
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33  G-AGCCCCCC ---------- ---CGCCCGG GGACGCTT-- GCAGTCT-TG
Ceratonia siliqua AJ245575      A-AGCCTCCA ---------- ---TGTCGGG AGGCGCCT-- GTGGCCC-CC
C. grandis                      -CACCCACCA TAT------- -AATGTTGCA CAGGGAAGCT GGTGCCGGTA
C. javanica                     CCAGCCACCC GACCCCCTCG GGTAGTTGCA CGGGGAAGCC AGCGCCTTCG
C. bakeriana                    CCAGCCACCT TACCCCCTCG GGTAGTTGCA CGGGGAAGCC AGCGCCTTTG
C. fistula                      CCACCTGCCC TAC------- -GTCGTTGCA CGGGGAAGCC AGCGCCACTG
C.(S.) sophera                  T-AGTTGCCC GCCC------ -CGTGTCGGG GGTGTGAC-G GTGGCCTAGT
C.(S.) occidentalis             T-AGTTGCCC GCCT------ -CGTGTCGGG GGTGTGACCG GTGGCCTAGT
C.(S.) hirsuta                  T-AGTTGCTC GCCC------ -CGTGTCGGG GGTGTGAC-G GTGGCCTACT
C.(S.) obtusifolia              G-AGTCCCCC GCTC------ -GGTGCCCGG G--------- ----------
C.(S.) tora                     G-AGTCCCCC GCTC------ -GGTGCCCGG G--------- ----------
C.(S.) surattensis              TCAGTCCCCC GCTG------ -GGTGTCCGG GGCGTGAT-A GTGGCCTACG
C.(S.) timoriensis              GTAGTTGCTA GCCC------ -CTTGGTCGG GGGTGCAAGA GTGGCCT-CG
C.(S.) alata                    G-AGTCGCCA GCCC------ -CATG-CCAG GGGTGCGAGT GCGGCCT-CG
C.(S.) spectabilis              G-AGTCGCCC TCCC------ -CGAGCCCCA GGGTACGAGA GTGGCCT-CG
```


Fig. 36 (continue)

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                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       210        220        230        240        250
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33  CGCTGCTTGT GCTCGCCTGG A--AA----- -AATAACAAA CCTCGGCGCC
Ceratonia siliqua AJ245575      CGCCACTCGT GCTACCTCGA C--CA----- -AAAAACTAA CCCTGGCGTT
C. grandis                      AGGCCCAAGA GAAACTGTGG C--AATAAAA TAATCACAA- CTCTGGCACT
C. javanica                     AGGCACGGGC GGAACCGTGG C--AACGAAA TAACCACAA- CCCCGGCGCT
C. bakeriana                    AGGCACGGGC GGAATCGTGG C--AACGAAA TAACCACAA- CCCCGGCGCT
C. fistula                      AGGCACTAGC GGAACCGTGG CGCAACGAAA CAACCACAA- TTTCGGCGCT
C.(S.) sophera                  TGCTGCATC- GCACCCCTGG C--AACCCAA CAACTATAA- CCCCGGCGCC
C.(S.) occidentalis             TGCTGCCTC- GCACCCCCGG C--AACCCAA CAACTATAA- CCCCGGCGCC
C.(S.) hirsuta                  TGCTGCCCC- GCTCCCTCGG C--AACCCAA CAACTATAA- CCCCGGCGCC
C.(S.) obtusifolia              TGCTAACTA- TAGCCTCGGG C--AACCCC- -AATAAAAA- CCCCGGCGCT
C.(S.) tora                     TGCTAACTA- TAGCCTCGGG C--AACCCC- -AATAAAAA- CCCCGGCGCT
C.(S.) surattensis              TGCAGCCTT- GCGCCTCGGG C--AACCCCT CAACAATAA- CCCCGGCGCT
C.(S.) timoriensis              TGCTGCTTGT GTGCCCCGGG C--AACAAAA CAACCATAA- CCCCGGCGCC
C.(S.) alata                    TGCTGCAAA- GCGCCCCGGG C--AACACAA CAACTGCAAA CCCCGGCGCG
C.(S.) spectabilis              TGCCGCT-GC GCTACCCAGG C--AACCCAA CAACTAAAA- CCCCGGCGCG

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       260        270        280        290        300
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33  -GGACGCGTC AAGGAACTCG AACGAACTAG CGTGCTCCCG GCGGCCCGGA
Ceratonia siliqua AJ245575      T-AACGCGCC AAGGAACTAC AACCAGTGAG CGTGCTCCCG ATGACCTGGT
C. grandis                      -AGATGCACC AAGGAACCTT AAGAGATAGG CACGGCCTTG GCAGCCTAGA
C. javanica                     -AGATGCGCC AAGGACCCAT AAGAAACAGG CACGACCCTG GCTGCCCGGA
C. bakeriana                    -AGATGCGCC AAGGACCCAT AAGAAATAGG CACGACCCTG GCTGCCCGGA
C. fistula                      -AGATGCGCC AAGGACCCAT AAGAAACAGG CACGGCCTTG GCTGCCCGGA
C.(S.) sophera                  -GGATGCGCC AAGGAACTCA AACCAACGTG CGTGGCCTCG GCGAACCGGA
C.(S.) occidentalis             -GGATGCGCC AAGGAACCAA AACCAACGTG CGTGGCCTCG GCGAACCGGA
C.(S.) hirsuta                  -GGATGCGCC AAGGAACTCA AACCAACGTG CGTGGCCTCG GCGAACCGGA
C.(S.) obtusifolia              -GGTT-CGCC AAGGAAATGA AACTTACGTG TGTGGCCTCG GCGAACCGGA
C.(S.) tora                     -GGTTGCGCC AAGGAAATGA AACTTACGTG TGTGGCCTCG GCGAACCGGA
C.(S.) surattensis              TGGACGCGCC AAGGAACTCA AACGAATGTG TGTGGCCCCG ACGAATCGAA
C.(S.) timoriensis              -GAACGCGCC AAGGAACTCA AACAAACGTG CGTAGCCTCG GCGATCCGGA
C.(S.) alata                    -AGAAGCGTC AAGGAACTCG AACAAAAGCG CGTGGCCTCG GCGACCCGGA
C.(S.) spectabilis              -AGAAGCGCC AAGGAACTCA AACAAACGTG CGTGGCCTCG GCGAGCCGGA

                                ....|....| ....|....| ....|....| ....|....| ....|....|
                                       310        320        330        340        350
                                ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33  GACGGG-CCC CGCCCGGAGA GC-GTCCCGA CGATTGTATC CA-AAACGAC
Ceratonia siliqua AJ245575      AACGGC-GAT CGATCGATGA GC-GTCGTGA CATTCTTATC CA-AAATGAC
C. grandis                      GACGGAGCCT TGCTAAGGAT GC-GCCAAGG AACTAACAAC AA-AAATGAC
C. javanica                     GACGGAGACC CGCCAGGGAA GC-GCCGCTG AACTTGTAAC CA-AAACGAC
C. bakeriana                    GACGGAGCCT CGCCAGGGAA GC-GCCACTG AACTCGTAAC GA-AAACGAC
C. fistula                      GACGGAGCCA TGCCAGGGAA GCTGCCGCGG AACTAGTAAC AA-AAACGAC
C.(S.) sophera                  GACGGA-TCT CGCCATGGCC C--GCCGCGA AAATGATGTC TA-AAACGAC
C.(S.) occidentalis             GACGGA-TCT CGCCATGGCC C--GTCGCGA AAACGATGTC TA-AAACGAC
C.(S.) hirsuta                  GACGGA-TCT CGCCATGGCC C--GCCGCGA AAACGATGTC AA-AAACGAC
C.(S.) obtusifolia              GACGGT-TCT TGTCCGGGGC C--GTGACGA AAATGAAATT AA-AAATGAC
C.(S.) tora                     GACGGT-TCT TGTCCGGGGC C--GTGACGA AAATGAAATT AA-AAATGAC
C.(S.) surattensis              GACGCT-TCT AGCCCGGGGT C--CCTACGT AAACGATGTC CA-AAATGAC
C.(S.) timoriensis              TACGGA-TGT CGCTCGGGGC AA-GTCGTGA AAATGATGTC TA-AAATGAC
C.(S.) alata                    GACGGA-TCT CGTTCGGGGC CA-GCAGCGA AAATGATGTC TA-GAATGAC
C.(S.) spectabilis              GACGGT-TGT GGC-CGGGGC TG-ATCGCGA AAAGGATGTC TAGAAACGAC
```

Fig. 36 (continue)

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                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                          360        370        380        390        400
                                    ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33      TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
Ceratonia siliqua AJ245575          TCTCGGTAAC GGATATCTCG GCTCTCG--- ---------- -------AAA
C. grandis                          TCTCGGCAAT GGATATCTAG GCTCTCACAT TGATGAAGAA CGTAGTGAAG
C. javanica                         TCTCGGCAAC GGATATCTAG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C. bakeriana                        TCTCGGCAAC GGATATCTAG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C. fistula                          TCTCGGCAAC GGATATCTAG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) sophera                      TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) occidentalis                 TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) hirsuta                      TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) obtusifolia                  TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) tora                         TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) surattensis                  TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) timoriensis                  TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) alata                        TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA
C.(S.) spectabilis                  TCTCG-CAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA

                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                          410        420        430        440        450
                                    ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33      TGCGATACTT GGTGTGAATT GCAGAATCCC GCGAACCATC GAGTCTTTGA
Ceratonia siliqua AJ245575          TGCAATACTT GGTGTGAATT GCAGAATCTT GTGAACCATC AAGTCTTTGA
C. grandis                          TGCGATACTT AGTGTGAGTT GCAGAATCCC ATGAACAATC GAGTCTTTGA
C. javanica                         TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C. bakeriana                        TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C. fistula                          TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) sophera                      TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) occidentalis                 TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) hirsuta                      TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) obtusifolia                  TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) tora                         TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) surattensis                  TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) timoriensis                  TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) alata                        TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA
C.(S.) spectabilis                  TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC GAGTCTTTGA

                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                          460        470        480        490        500
                                    ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33      ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
Ceratonia siliqua AJ245575          ACACAAGTTG TGCCCGAAGC CATCAAGCCG AAGGCACGTC TGCCTGGGTG
C. grandis                          ATGCAAGTTA CGCCCGAAGC CATTAGGCCG AGGGCACGTC TGCTTAGGCG
C. javanica                         ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGCG
C. bakeriana                        ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGCG
C. fistula                          ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGCG
C.(S.) sophera                      ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) occidentalis                 ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) hirsuta                      ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) obtusifolia                  ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) tora                         ACGCAAGTTG CGCCCGAAGC CACTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) surattensis                  ACGCAAGTTG CGCCCGAAGC CACTAGGCCA AGGGCACGTC TGCCTGGGTG
C.(S.) timoriensis                  ACGCAAGTTG CGCCCGAAGC CATTAGGCTG AGGGCACGTC TGCCTGGGTG
C.(S.) alata                        ACGCAAGTTG CGCCCGAAGC CATTAGGCCG AGGGCACGTC TGCCTGGGTG
C.(S.) spectabilis                  ACGCAAGTTG CGCCCGAAGC CATTAGGCCG AGGGCACGTC TGCCTGGGTG
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Fig. 36 (continue)

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                    510        520        530        540        550
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 TCACACAACG TTGCCCCCA- -CTC------ -CACCGCCTC TCAGGGGGCG
Ceratonia siliqua AJ245575      TCACACACTG TCGCCCCCA- -CCC------ -CGTGGCCTC TCGCGTGGCT
C. grandis                      TTACCCAACG TTGCTCCCAA TCCCATCTTT GCTTACTCCC TCCCATTGGA
C. javanica                     TCACACAACG TTGCCCCCAA TCCCACTCTC ACTCCCTCCC C---ATCGGA
C. bakeriana                    TCACACAACG TTGCCCCCAA TCCCATTCCC ACTCCCCCCC CCTCATCGGA
C. fistula                      TCACACAACG TTGTTCCCGA CCCCATT--- -CTCGCTCCC TCCCACCGGA
C.(S.) sophera                  TCACGCATCG TTGCCCCAAA -CCA------ -CGTCGTCCC TCCGGTATGT
C.(S.) occidentalis             TCACGCATCG TTGCCCCAAA -CCA------ -CGTCGTCCC TCCGGTATGT
C.(S.) hirsuta                  TCACGCATCG TTGCCCCAAA -CCA------ -CGTCGTCCC TCCGGTATGT
C.(S.) obtusifolia              TCACGCATCG TAGCCCCAAG -CCA------ -CGTCCACCC CCCGATTGAT
C.(S.) tora                     TCACGCATCG TAGCCCCAAG -CCA------ -CGTCCACCC CCCGATTGAT
C.(S.) surattensis              TCACGCATTG TTGCCCCAAA -CCA------ -CTTTGTCCC TCCGATCGGT
C.(S.) timoriensis              TCACGCACTG TTGCCCCAAA -CCC------ -TAATGCCTC TCAAATTGAT
C.(S.) alata                    TCACGCATCG TTGCCCCAAA ACCC------ -CGTCGTCCC TCCGGTCAAT
C.(S.) spectabilis              TCACGCATCG TTGCCCCAAG -CCC------ -CGCCGTGCC TCCTGTTGAT
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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                    560        570        580        590        600
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 TGTCG----- -------GGC GGGGCGGATG CTGGCCTCCC GTGAGCTCCT
Ceratonia siliqua AJ245575      TCGAG----- -------GAA TGGGCAGATT ATGGCCTTCC GTGAGCT--T
C. grandis                      GGGGTGAGTA CCGGGCGAGC ATGGCAGAAG CTGACCTCCC ATGAGCG--T
C. javanica                     GGGGCGAG-- --CGGCGGGC GGGGCGGAAG CTGGCCTCCC GTGAGCG--C
C. bakeriana                    GGGGCGAG-- --CGGCGGGC GGGGCGGAAG CTGGCCTCCC GTGAGCG--C
C. fistula                      GCGGCGGG-- -ATGGTGGTT GGGGAGGAAG TTGGCCTCCC GTGAGCG--T
C.(S.) sophera                  CGGAGCGGG- -GGAGGTGCT TGGGCGGAAG TTGGCCTCCC GTGAGCA--A
C.(S.) occidentalis             CGGAGCGGG- -CGAGGTGCT TGGGCGGAAG TTGGCCTCCC GTGAGCA--A
C.(S.) hirsuta                  CGGAGCGGT- -CGAGGTGCT TGGGCGGAAG TTGGCCTCCC GTGAGCA--A
C.(S.) obtusifolia              CCGGGGCGA- -CGAGGTGCT TGGGGGGAAT TTGGCCTCCC GTGATCC--G
C.(S.) tora                     CCGGGGCGA- -CGAGGTGCT TGGGGGGAAT TTGGCCTCCC GTGATCC--G
C.(S.) surattensis              GGGGGCCGG- -CGAGGTGCT CGGGTGGAAT TTGGCCTCCC GTGAGCC--A
C.(S.) timoriensis              CAGAGTGGG- -CGAGGTGCT TGGGCAAAAT TTGGCCTCCC GTGAGCA--T
C.(S.) alata                    CGGAGGCGG- -CGAGGTGCT TGGGCGGAAG CTGGCCTCCC GTGAGCA--T
C.(S.) spectabilis              CGGGGCAGG- -CGGGTTGCT TGGGCGGAAT CTGGCCTCCC GTGAGAA--C
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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                    610        620        630        640        650
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 TGCCTCGCGG ATGGCCGAAA ---GACGAGT CTGCGGTGT- -TAGCCGCCG
Ceratonia siliqua AJ245575      CGCCTTATGG ATGGCCCAAA ---AGAGAGT TCGCGGTGG- -CGACTGCCA
C. grandis                      GGCCTCGCGG ATGGCTGAAA ---GATGAGC CTGTGGGGGA GCGATCACCA
C. javanica                     GGCCTCGCGG ATGGCCGAAA ---GATGAGC CTGTGGGGGA GCGATCACCA
C. bakeriana                    GGCCTCGCGG ATGGCTGAAA GAAGACGAGC CTGTGGGGGA GCGATCACCA
C. fistula                      GGCCTCGCGG ATGGCTGAAA ---GACGAGC CTGTGGGGGA GCGATCACCA
C.(S.) sophera                  TGCCTCGTGG ATGGTTGAAA ---AAGGATC CTGTGGGGGG GCGACCGCCA
C.(S.) occidentalis             TGCCTCGTGG ATGGTTGAAA ---AAGGAGC CTGTGGGGGG GCGACCGCCA
C.(S.) hirsuta                  TGCCTCGTGG ATGGTTGAAA ---AAGGAGC CTGTGGGGGG GCGACCGCCA
C.(S.) obtusifolia              TGCATTGCGG ATGGCCGAAA ---AAGGAGC CTGTGCGGGG -CAATCGCCA
C.(S.) tora                     TGCATTGCGG ATGGCCGAAA ---AAGGAGC CTGTGCGGGG -CAATCGCCA
C.(S.) surattensis              TGCCTCGTGG ATGGTCAAAA ---AAGTAGC CTGTGGGGGG -CAATTGCCA
C.(S.) timoriensis              TGCCTTGTGG ATGGTTGAAA ---AATGAGC CTATGAGTAA -TGATTGCCA
C.(S.) alata                    TGCCTTGCGG ATGGCCGAAA ---TTAGAGC CTGTGAGGGG -CAATCGCCA
C.(S.) spectabilis              TGGCTCGTGG ATGGCCGAAA ---GTAGAGC CTGCGGGTGG -GCATTGCCA
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Fig. 36 (continue)

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                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     660        670        680        690        700
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 CGACGGACGG TGGATGAGTG ATC-----CT CGAGACCGGT CGCGCGCGCG
Ceratonia siliqua AJ245575     CGACGCACGG TGGATGAGCA AAGA----CT CAAGACCAGT CGTGCAAGTG
C. grandis                     CGTTCTGCGG TGGATGTGTA GAAAATGCCT TGATACCA-- ----------
C. javanica                    CGGTCCACGG TGGACGAGCA GGGAATGCCT TGATACCGAC CGTGCGTGCG
C. bakeriana                   CGGTCTACGG TGGATGAGCA GGGAATGCCT TGATACCGAC CGTGCGTGCG
C. fistula                     CGTTCCACGG TGGATGAGCA GAAAATGGCT TGATACCGAT CGTGCGTGAG
C.(S.) sophera                 CGTTCCACGG TGGATGAGCG CTAG---CCT CGAGACCGAA CGTGCGCGAG
C.(S.) occidentalis            CGTTCCACGG TGGATGAGCG CTAG---CCT CGAGACCGAA CGTGCGCGAG
C.(S.) hirsuta                 CGTTCCACGG TGGATGAGCA CTAG---CCT CGAGACCGAA CGTGCGCGAG
C.(S.) obtusifolia             CGTTCCACGG TGGATGAGCA GATG---CCT CGAGACCGAC CTTGTGTTGG
C.(S.) tora                    CGTTCCACGG TGGATGAGCA GATG---CCT CGAGACCGAC CTTGTGTTGG
C.(S.) surattensis             CGTTCCACGG TGGA--AGCA AGAT---GGT CGAGACCGAC CGTGCGGGGG
C.(S.) timoriensis             CGTTC-ACGG TGGATGAGCG AATGA--CCT TGAGACCGAC CGTGTGCATG
C.(S.) alata                   CGTTCCACGG TGGTTGAGCA GATG---CCT CGAGGCCGAC CGTGCGCGAG
C.(S.) spectabilis             CGCTCCACGG TGGATGAGCT GATG---CCT CGAGATCGAT CGTGCGCGAG


                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     710        720        730        740        750
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 TCTTCCCGTC AAGCAGGCTG CGAGACCCTG CCGCGTCGCT CGTTGCGAGC
Ceratonia siliqua AJ245575     TCATACCCGG GATTGCGCTC GGAGACCCTT CAGCATCGCG AGGTGCATAT
C. grandis                     ---------- ---------- ---------- ---------- ----------
C. javanica                    TCGTGTCCCC CC-CGAGCCG GGCTCCTGGA CCCTCGTT-- ----------
C. bakeriana                   TCGTGTCCCC CC-CGAGCTG GGCTCCTGGA CCCTTGTT-- ----------
C. fistula                     TCGTGCCCCC CTGTTGGGCT CTTGGACCCT CGTTCGTT-- ----------
C.(S.) sophera                 CTGTCCCTCC GACTAGGCTG CGAGACCCT- TGCGAGTG-- ---AGGAATC
C.(S.) occidentalis            CTGTCCCTCC GACTAGGCTG CGAGACCCT- TGCGAGCG-- ---AGGAATC
C.(S.) hirsuta                 CTGTCCCTAC GACTAGGCTG CGAGACCCC- TGCGAGCG-- ---AGGAATC
C.(S.) obtusifolia             TTGTCCCTAC GGATGGGCTG TCAGACCCTT TGGGAGCG-- ---ACGAAGC
C.(S.) tora                    TTGTCCCTAC GGATGGGCTG TCAGACCCTT TGGGAGCG-- ---ACGAAGC
C.(S.) surattensis             TTGTTCTTAC GGTTAGGCTG CCAAACCCTA TGAGGGAG-- ---AGGAAGC
C.(S.) timoriensis             TTGTTCCTTT GTTTAGGCTG CGAGACCCT- TGCAAGCA-- ---AGAAAGC
C.(S.) alata                   TTGTCCCCAC GACAAGGCTG CGAGACCCT- TGCGAGCA-- ---AGTAAGT
C.(S.) spectabilis             CTGTCCCCCT GTCCAGGCTG CAAGGCCCC- CGGTGGTG-- ---TGAATGC


                              ....|....| ....|....| ....|....| ....|....| ....|....|
                                     760        770        780        790        800
                              ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33 GCTTCCGACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAA?
Ceratonia siliqua AJ245575     GCCTCGAACG G?ACCCTAAG ?TCAGGCGGG GCTACTCG?C TGAGTTTAAG
C. grandis                     ---------? ?????????? ?????????? ?????????C TAAGTTTAAG
C. javanica                    -CTCCTAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C. bakeriana                   -CTCCCAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C. fistula                     -CTCCTAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) sophera                 GCTCCCAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) occidentalis            GCTCCCAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) hirsuta                 GCTCCCAACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) obtusifolia             TTTCCCGAAC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) tora                    TTTCCCGAAC G?ACCCCAGG ?TCAGGCGGG GCTACCCG?C TGAGTTTAAG
C.(S.) surattensis             TCTCTCAACC CCACCCC?GG GTCGGGGGGG GCTACCCGTC TGAGTT?AAC
C.(S.) timoriensis             ACTCACAACC G?ACCCCAGG GTCAG????? ?????????? ????TT????
C.(S.) alata                   GCTCCCAACC G?ACCCCAGG ?TCAGGCGGG GCCACCCG-C TGAGTTTAAG
C.(S.) spectabilis             GCTCCCGACC G?ACCCCAGG ?TCAGGCGGG GCTACCCG-C TGAGTTTAAG
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Fig. 36 (continue)

```
                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                          810        820        830        840        850
                                    ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33      ?????????? ?????????? ?????????? ?????????? ??????????
Ceratonia siliqua AJ245575          CATATCAATA AG?????CGG AGGA?????? ?????????? ??????????
C. grandis                          CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C. javanica                         CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C. bakeriana                        CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C. fistula                          CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) sophera                      CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) occidentalis                 CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) hirsuta                      CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) obtusifolia                  CATATCAATA AG?????TGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) tora                         CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) surattensis                  CCTATCA-TG AG?????GCG AGGAAAAAAA ACTAACAAGG GTTTCCTTTT
C.(S.) timoriensis                  ?????????? ?????????? ?????????? ?????????? ??????????
C.(S.) alata                        CATATCAATA AG?????CGG AGGAAAAGAA ACTAACAAGG ATTCCCCTAG
C.(S.) spectabilis                  CATATCAATA AGTGGAACGG AGGAAAAGAA -CTAACAAGG ATTCCCCTAG

                                    ....|....| ....|....| ....|....| ....|....| ....|....|
                                          860        870        880        890        900
                                    ********** ********** ********** ********** **********
Gymnocladus dioica AF510030,33      ?????????? ?????????? ?????????? ?????????? ??????????
Ceratonia siliqua AJ245575          ?????????? ?????????? ?????????? ?????????? ??????????
C. grandis                          TAACGGTGAG TGAGTCGGGA AAAGCCCACC ATGA?GAATC GGT?CGCCCC
C. javanica                         TAACGGCGAG CGAACCGGGA AAAGCCCACC ATGA?GAATC G?T?CGCCCG
C. bakeriana                        TAACGGCGAG CGAACCGGGA AAAGCCCACC ATGA?GAATC GGT?CGCCCG
C. fistula                          TAACGGCGAG CGAACCGGGA AAAGCCCACC ATGA?GAATC GGTTCGCCCC
C.(S.) sophera                      TAACGGCGAG CGAACCGGGA AGAGCCCACC ATGA?GAATC GGT?CG????
C.(S.) occidentalis                 TAACGGCGAG CGAACCGGGA AGAGCCCACC ATGAAGAATC GGT?CGCCCT
C.(S.) hirsuta                      TAACGGCGAG CGAACCGGGA AGAGCCCACC ATGA?GAATC GGT?CGCCCT
C.(S.) obtusifolia                  TAACGGCGAG CGAACCGGGA ATAGCCCACC ATGA?GAATC GG?CCGCCCT
C.(S.) tora                         TAACGGCGAG CGAACCGGGA ATAGCCCACC ATGA?GAATC GG?CCGCCCT
C.(S.) surattensis                  TGGCGGAG?G ?GAACCGGGA A?AGCCC?CC ?????????? ??????????
C.(S.) timoriensis                  ?????????? ?????????? ?????????? ?????????? ??????????
C.(S.) alata                        TAACGGCGAG CGAACCGGGA AAAGCCCACC ATGA?GAATC GGT?CGCCC?
C.(S.) spectabilis                  TAACGGCGAG CGAACCGGGA AGAGCCCACC ATG??????C G?TCTGCCCC

                                    ....|....| ...
                                          910
                                    ********** ***
Gymnocladus dioica AF510030,33      ?????????? ???
Ceratonia siliqua AJ245575          ?????????? ???
C. grandis                          C?AGCGTCTA ATG
C. javanica                         C?G?C?TC?G A??
C. bakeriana                        C?GGC?TCCG A??
C. fistula                          C?GGC?TCCG AT?
C.(S.) sophera                      ?????????? ???
C.(S.) occidentalis                 CCGGCGTCGA TG?
C.(S.) hirsuta                      C?GGCGTCGA TG?
C.(S.) obtusifolia                  CGGCGTCT?A TG?
C.(S.) tora                         CGGCGTCTGA TG?
C.(S.) surattensis                  ?????????? ???
C.(S.) timoriensis                  ?????????? ???
C.(S.) alata                        TCGGCGTCAG ???
C.(S.) spectabilis                  TCGGCGTGGA T??
```

Fig. 36 (continue)

Branch-and-bound searching strategy was used to analyse this ITS data matrix. All characters used in phylogenetic tree reconstruction were unordered and equally weighted and unordered with 295 parsimony-informative characters (41.7%) and parsimony-uninformative 136 characters.

There was only one most parsimonious tree found from this data set, with 956 steps in length (Fig. 37). Consistency index (CI) was 0.7103 and homoplasy index (HI) was 0.2897. Retention index (RI)was 0.6884 and rescaled consistency index (RC) was 0.4889. Bootstrap and jackknife supporting values were calculated. Strict, semistrict and 50% majority-rules consensus trees were not necessary to perform. The phylogenetic tree was fully-resolved; the two outgroups, *Gymnocladus dioica* and *Ceratonia siliqua*, were clustered while all *Cassia* were grouped together with high bootstrap and jackknife supporting-values (98% and 98%, respectively). Within the Thai *Cassia* group, there were two major clades separated distinctively; the group of Thai *Cassia* species that moved to genus *Senna* (blue branches) based on Flora Malesiana, and the other group composed of species remaining to be in the genus *Cassia* (dark-red branches).

The first *Cassia* (*Senna*) clade, with 70% BS and 68% JK, composed of *C.* (*S.*) *occidentalis*, *C.*(*S.*) *sophera*, *C.*(*S.*) *hirsuta*, *C.*(*S.*) *obtusifolia*, *C.*(*S.*) *tora*, *C.*(*S.*) *surattensis*, *C.*(*S.*) *alata*, *C.*(*S.*) *timoriensis* and *C.*(*S.*) *spectabilis*. The other clade had four *Cassia* (*Cassia*) species: *C. javanica*, *C. bakeriana*, *C. grandis* and *C. fistula* with very high robustness of bootstrap (100%) and jackknife (100%) supporting-values. Considering subgroupings in the first *Cassia* (*Senna*) group, two subclade could recognized from the phylogeny. The first minor clade contained *C.*(*S.*) *occidentalis*, *C.*(*S.*) *sophera* and *C.*(*S.*) *hirsuta* with strongest supporting-values (100% BS and 100% JK). The other clade composed of *C.*(*S.*) *obtusifolia*, *C.*(*S.*) *tora* and *C.*(*S.*) *surattensis* with slightly lower bootstrap (98%) and jackknife (97%) supporting-values than the previous one. Moreover, another weak subclade (<50% of both BS and JK), *C.*(*S.*) *alata* and *C.*(*S.*) *timoriensis* was also drawn. For the *Cassia*

(*Cassia*) major clade, there were two recognizable minor clades: *C. bakeriana* paired with *C. javanica* with highest supporting-values (100% of both BS and JK) and *C. grandis* sistered to *C. fistula* with very weak supporting-values (55% BS and < 50% JK).

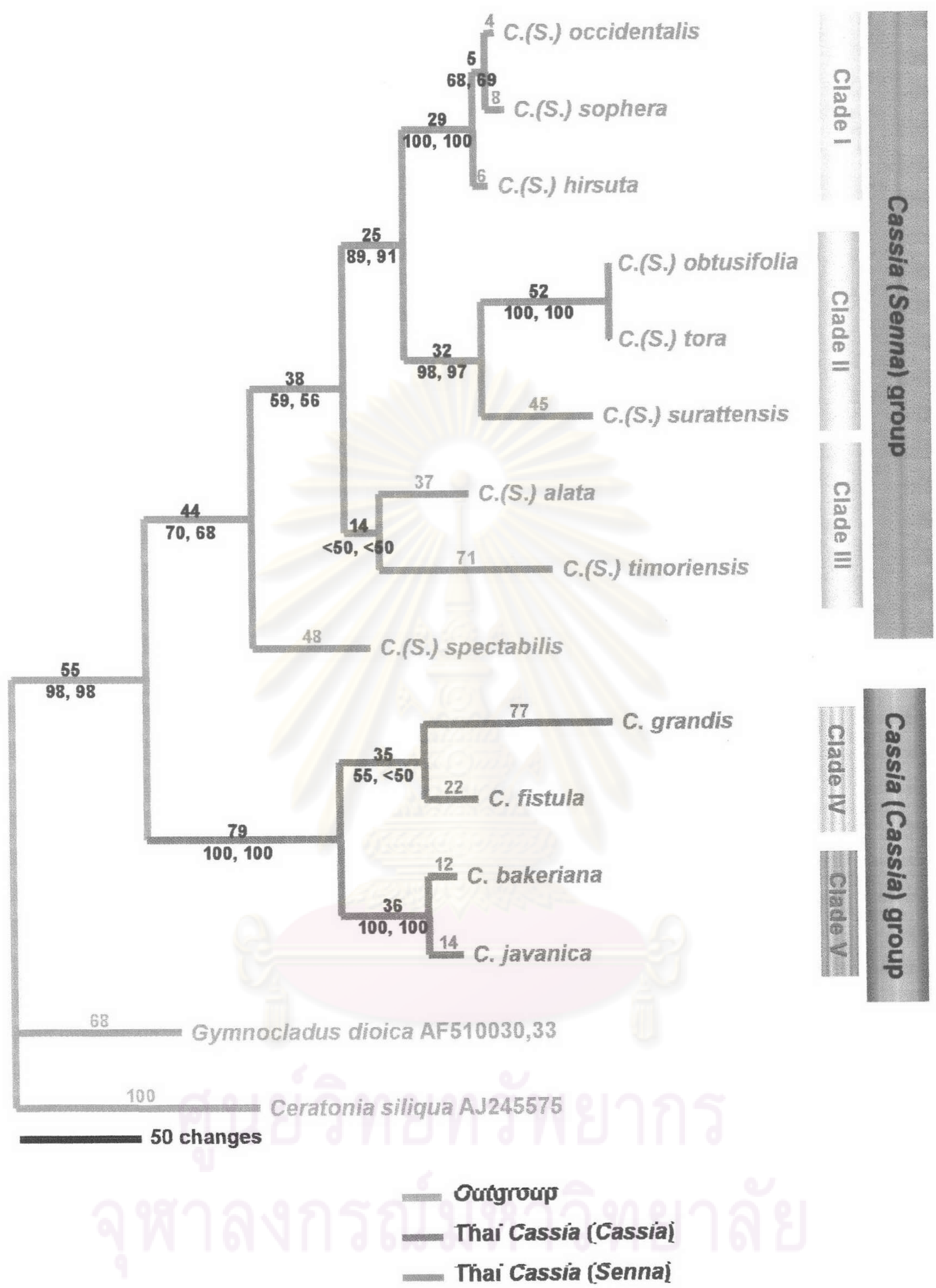

Fig. 37 only one most parsimonious trees (956 steps in length) found from ITS data matrix of 13 *Cassia* species in Thailand based on ITS region sequence data with two outgroups, *Ceratonia siliqua* and *Gymnocladus dioica* the upper numbers are amounts of synapomorphy and the coloured numbers are amounts of autapomorphy. The lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively. [CI = 0.7103, RI = 0.6884, RC = 0.4889]

ITS phylogenetic tree from parsimony analysis was compared with the neibour-joining (NJ) tree of the same data set. The NJ tree also showed that both *Gymnocladus dioica and* ITS sequences were very much different from those of Thai *Cassia* as seen previously in the results of *trn*L intron analyses. Neighbour-joining distance analysis of the ITS sequence data fully supported taxon-arrangement topology on the tree from the parsimony analysis in *Cassia* (*Senna*) group. The second *Cassia* (*Cassia*) group revealed a slightly difference within this group; that is *C. bakeriana* was paired with *C. javanica* before grouped firstly to *C. fistula* and then to *C. grandis*.

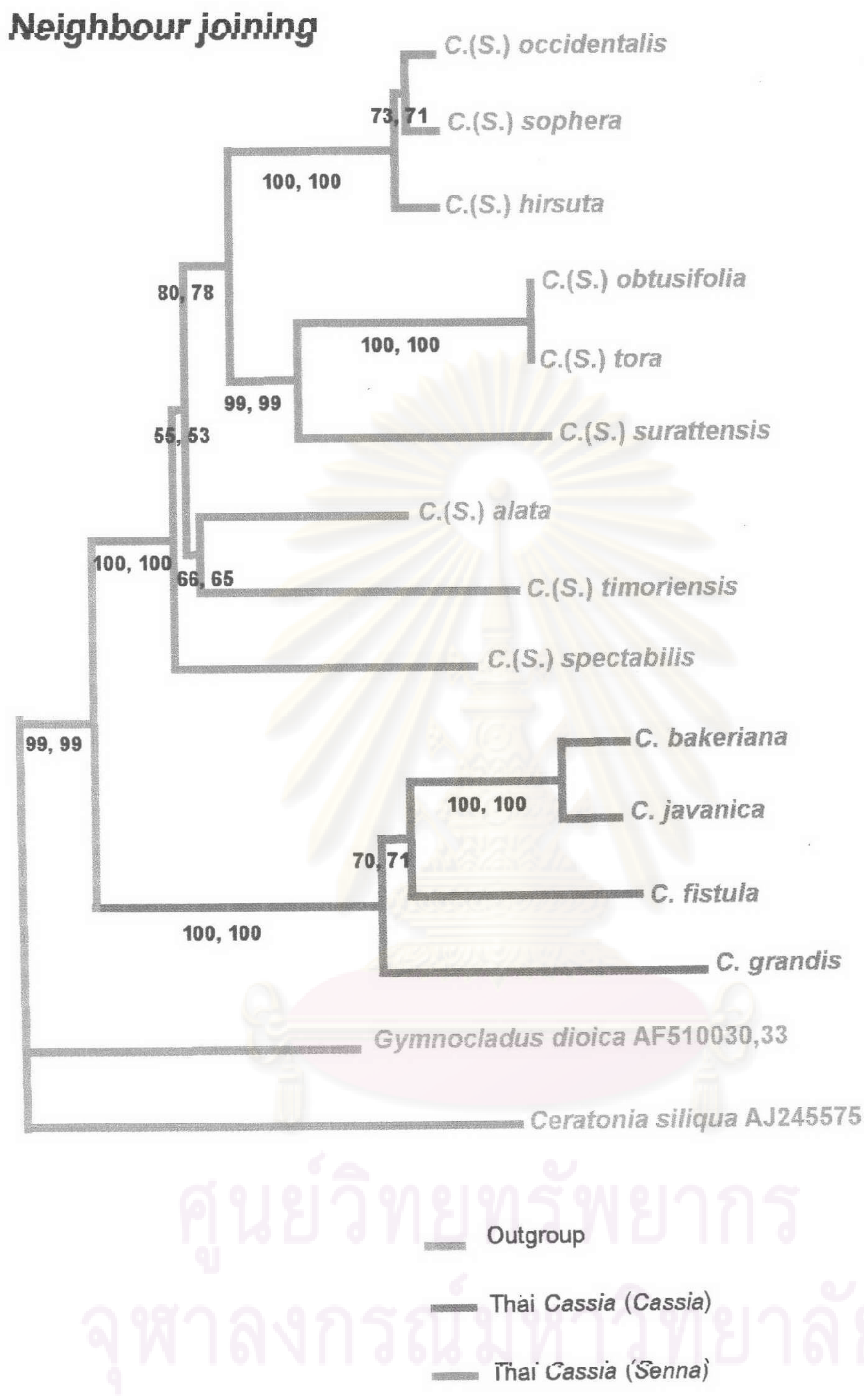

Fig. 38 Neighbour-joining tree of 13 *Cassia* in Thailand based on ITS regions sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieve from GenBank).

4.5.3 Combined sequence data of *trn*L intron and ITS regions

One best way to make a phylogenetic conclusion from two different DNA data sets is to combine both sequence matrices and rerun an analysis. A combined data matrix between sequences data sets of *trn*L intron and ITS regions of Thai *Cassia* species was prepared. Again, branch-and-bound searching strategy was used to analyse the combined data matrix which had 1303 bp long (597 bp belong to *trn*L intron sequence and 706 bp to the ITS regions). Only 13 Thai *Cassia* species were suitable to be in the analysis as the others (*C. siamea*, *C. garrettinana*, *C. pumila* and *C. leschenaultiana*) could not be PCR amplified either form *trn*L intron or ITS regions. *Gymnocladus dioica* and *Ceratonia siliqua* were still the only two most suitable outgroups from this matrix. Three hundred and nineteen characters (24.5%) were parsimony-informative as synapomorphy (shared-derived characters) and 177 characters were parsimony-uninformative as autapomorphy specifically for each taxon.

As expected, there was only one most parsimonious tree found from this newly combined data set, with 1030 steps in length (Fig. 39). Consistency index (CI) was 0.7243 and homoplasy index (HI) was 0.2757. Retention index (RI)was 0.6072 and rescaled consistency index (RC) was 0.5050. Reliability of each internal branch was estimated using bootstrap and jackknife analyses. The new phylogeny had the same topology as the tree resulted from ITS data alone. The fully-resolved phylogram from combined data set show that *Gymnocladus dioica* and *Ceratonia siliqua* were separated as an outgroup clade while all Thai *Cassia* species were grouped together with full bootstrap and jackknife supporting-values (both 100%). Two major clades were found within the Thai *Cassia* group. As same as from ITS only, both Thai *Cassia* species that moved to the genus *Senna* (blue branches) based on Flora Malesiana are *Cassia* species remaining to be in the genus *Cassia* (dark-red branches) had their own distinctive clades. The *Cassia* (*Senna*) clade (67%

BS and 66% JK) composed of C.(*S.*) *occidentalis*, C.(*S.*) *sophera*, C.(*S.*) *hirsuta*, C.(*S.*) *obtusifolia*, C.(*S.*) *tora*, C.(*S.*) *surattensis*, C.(*S.*) *alata*, C.(*S.*) *timoriensis* and C.(*S.*) *spectabilis*. C. *javanica*, C. *bakeriana*, C. *grandis* and C. *fistula* were grouped together with 100% bootstrap and jackknife branch supporting-values. Within *Cassia* (*Senna*) group, three minor clades could be pointed out: the first clade 100% BS and JK contained C.(*S.*) *occidentalis*, C.(*S.*) *sophera* and C.(*S.*) *hirsuta*; the second clade composed of C.(*S.*) *obtusifolia*, C.(*S.*) *tora* and C.(*S.*) *surattensis* (99% BS and JK); and the last clade of C.(*S.*) *alata* and C.(*S.*) *timoriensis* with weak supporting-values (<50% of both BS and JK). Considering subgrouping in the second major clade of Cassia (Cassia) species, there were two minor clades found that C. *bakeriana* paired with C. *javanica* (100% of both BS and JK) and C. *grandis* paired with C. *fistula* with weak supporting-values (54% of BS and 52% of JK).

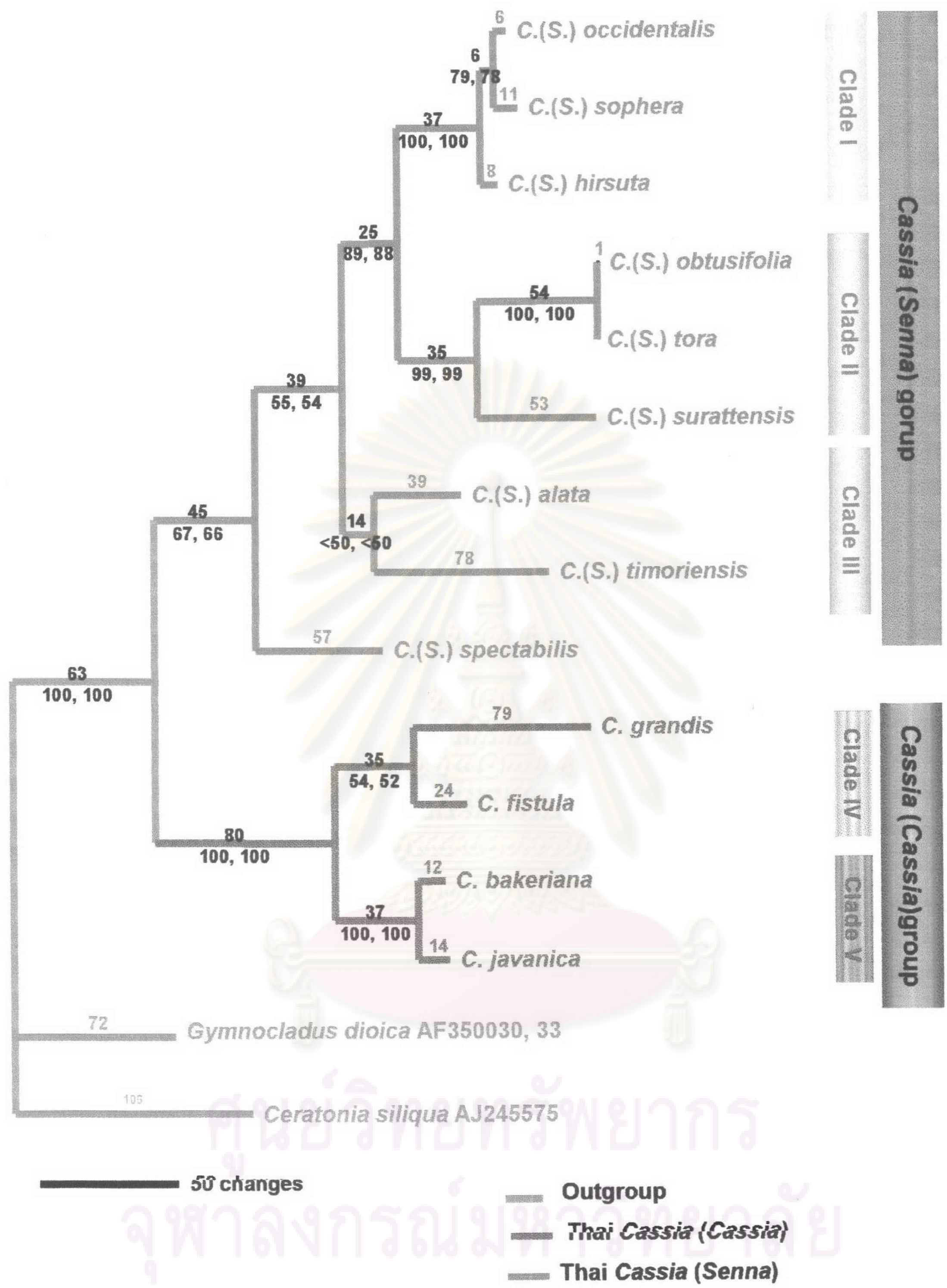

Fig. 39 only one most parsimonious trees (1030 steps in length) found from ITS data matrix of 13 *Cassia* species in Thailand based on combined *trn*L intron and ITS region sequence data with two outgroups, *Ceratonia siliqua* and *Gymnocladus dioica*. The upper in black numbers are amounts of synapomorphy and the coloured numbers are amounts of autapomorphy.The lower numbers are 1000-replicate bootstrap and jackknife supporting-values of each clade, respectively. [CI = 0.7243, RI = 0.6972, RC = 0.5050]

To complete the phylogenetic analysis procedure, NJ method was also performed with the combined DNA matrix. The NJ tree showed the same result as the analysis of ITS data alone that *Gymnocladus dioica* and *Ceratonia siliqua* were separated from all 13 Thai *Cassia* taxa. Neighbour-joining result from ITS sequence data fully supported the parsimony analysis in the first major clade (*Cassia* (*Senna*) clade). While the second major clade (*Cassia* (*Cassia*) clade) were slightly different from parsimony method that this clade had *C. bakeriana* paired with *C. javanica* before grouping to *C. fistula* and *C. grandis*, respectively.

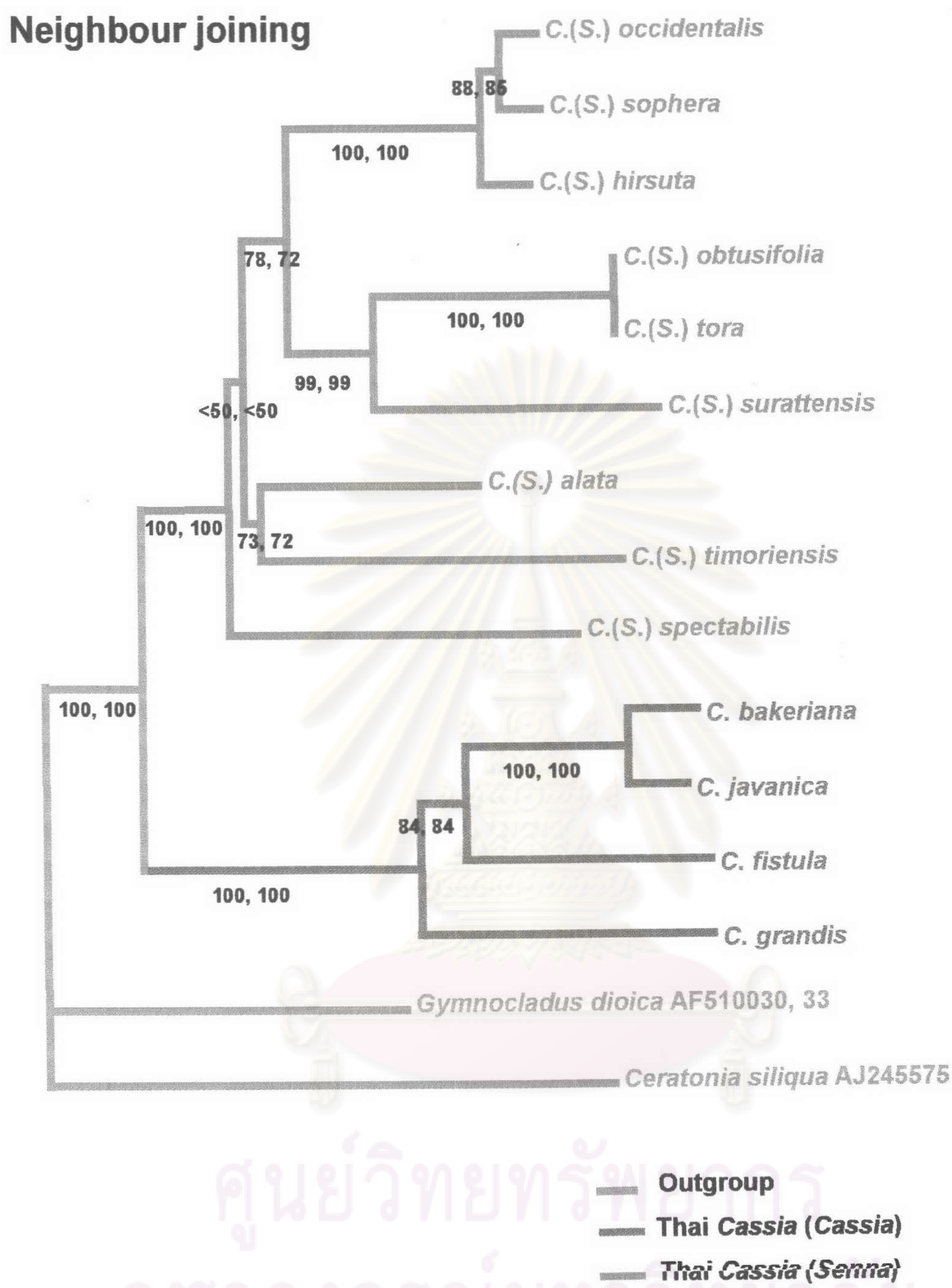

Fig. 40 Neighbour-joining tree of 13 *Cassia* in Thailand based on ITS regions sequence data with *Ceratonia siliqua* and *Gymnocladus dioica* as outgroups (retrieve from GenBank).

4.6 Cytogenetic study

Cytogenetic study of some *Cassia* in Thailand was performed. Two species (*C. timoriensis* and *C. tora*) were studied on somatic cells from their root tips. Other ten species (*C. occidentalis*, *C. surattensis*, *C. siamea, C. garrettiana*, *C. fistula*, *C. sophera*, *C. javanica*, *C. spectabilis*, *C. timoriensis* and *C. tora*) were investigated cytogenetically on their germline cells (Fig. 41 to Fig. 50). The chromosome number of most species could be counted more or less from their bivalent numbers in the late prophase to late metaphase stage. So far, the cytogenetic results were not good enough for karyotyping analysis because the chromosomes were too small and the chromosome from root tips were too difficult to be counted because of contaminated bacteria. The chromosome numbers reporting from meiotic study were not exact as a single number (Fig. 42 to Fig. 50). Only the chromosome numbers *C. occidentalis* (Fig. 41) could be counted ambiguously as 2n=26 while those of the other nine species (Fig. 42 to Fig. 50) were recorded from estimates. For instance, both chromosome numbers of *C. surattensis* (Fig. 42) and *C. tora* (Fig. 43) were revealed to be 2n=56 since 28 bivalent pairs per cell were found in the metaphase I stage. Another four species *C. siamea*, *C. fistula*, *C. sophera* and *C. timoriensis* could be estimated to be 2n=28 as in the Fig. 44, 46, 47 and 50, respectively. The estimated chromosome numbers from the other three remaining species, however, were unclear as around 12-14 bivalent pairs in *C. garrettiana* (Fig. 45), 13-14 in *C. javanica* (Fig. 48) and 11-12 in *C. spectabilis* (Fig. 49).

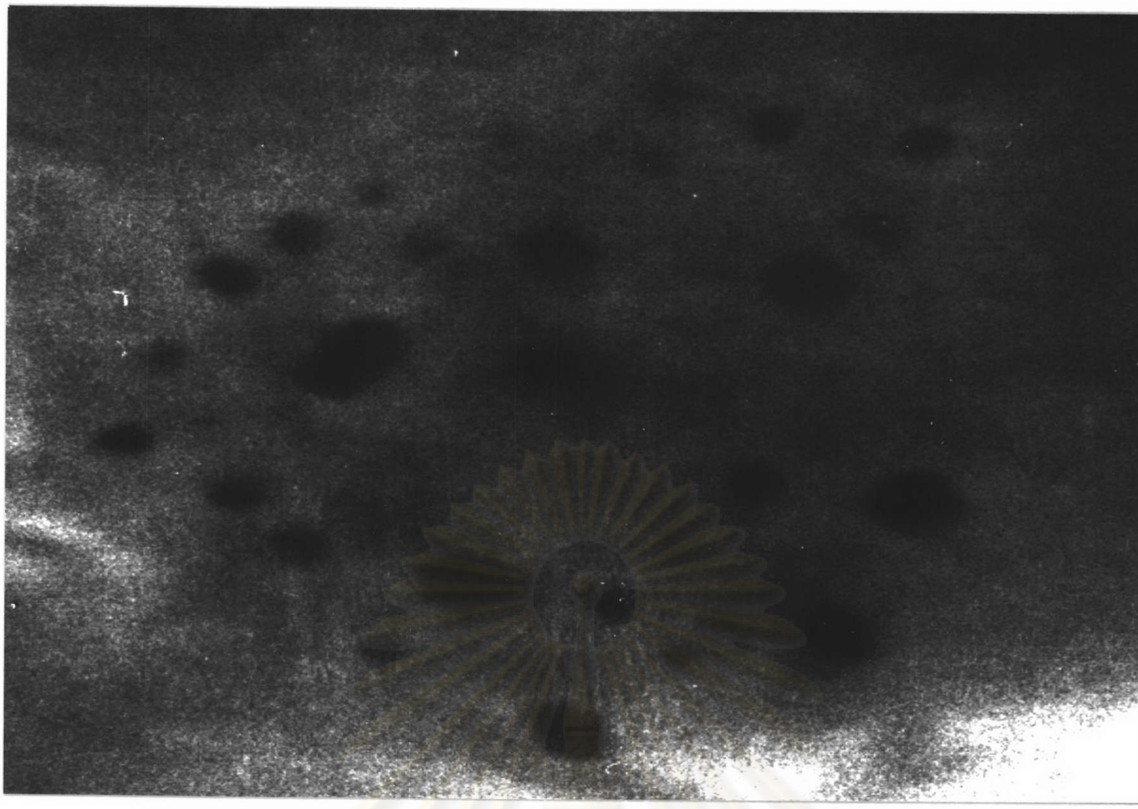

Fig. 41 late metaphase in microsporocyte of *C. occidentalis* (estimated 2n = 26)

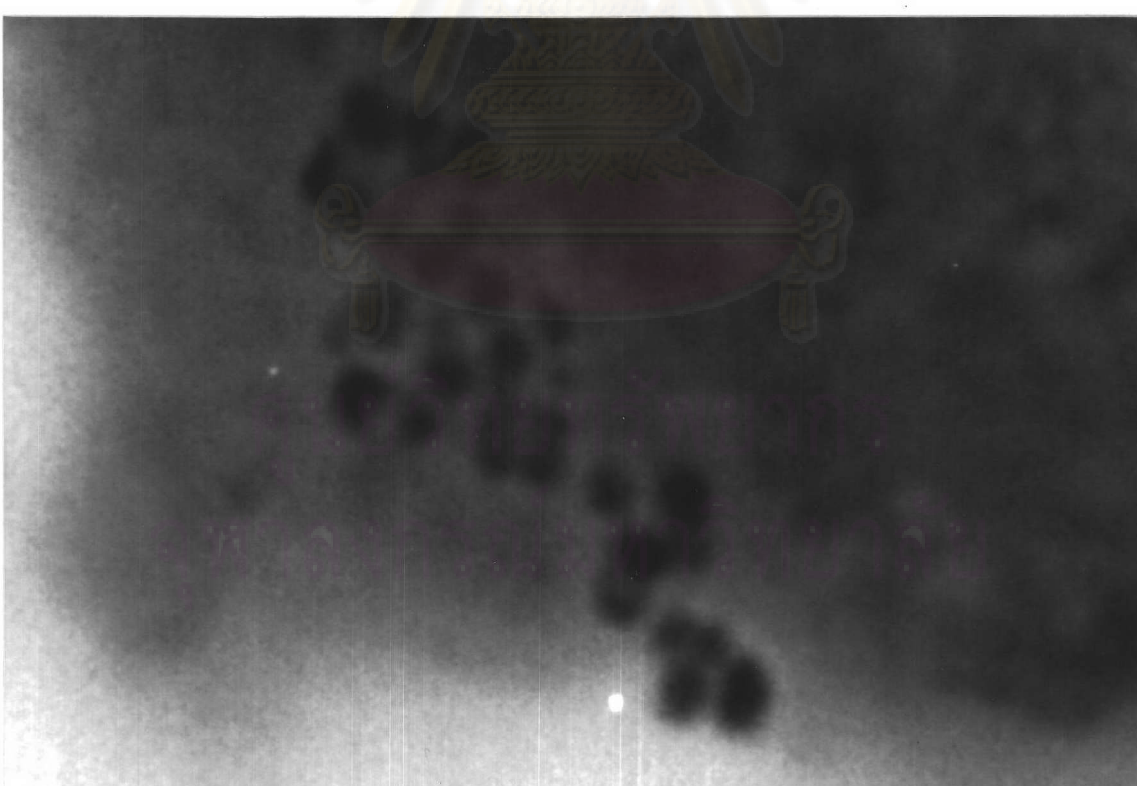

Fig. 42 metaphase I in microsporocyte of *C. surattensis* (estimated 28 bivalent, 2n=56)

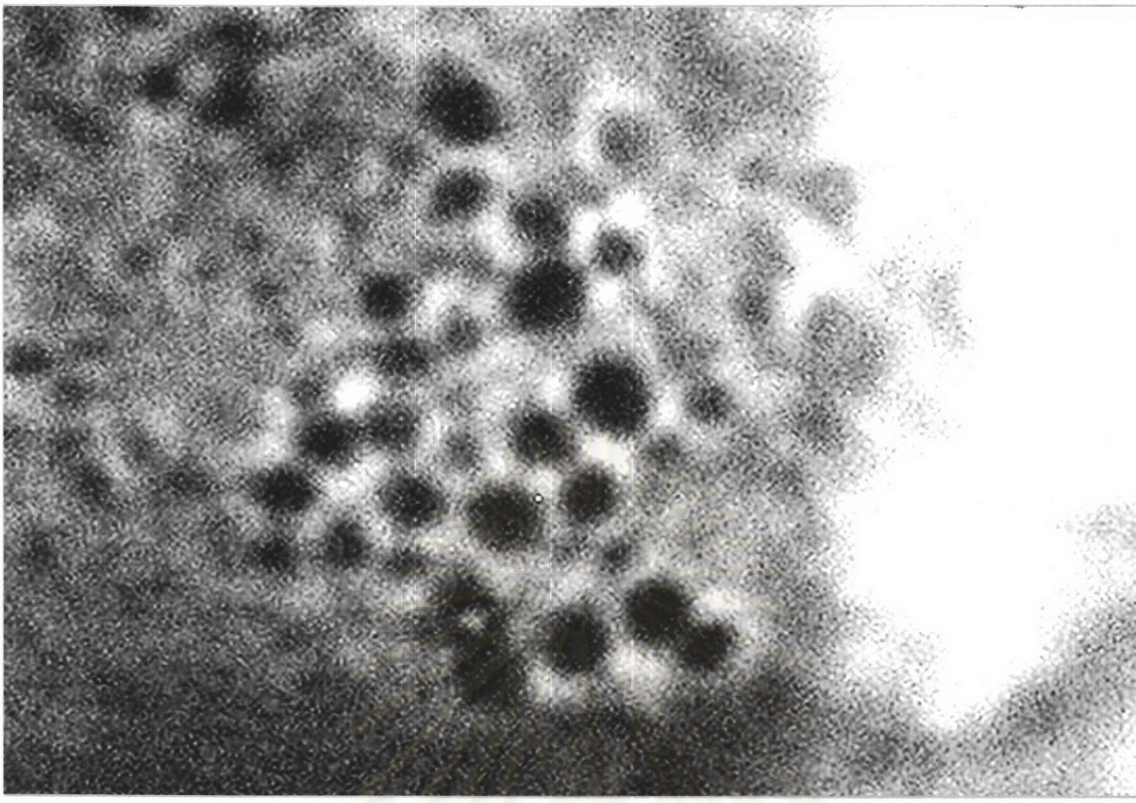

Fig. 43 metaphase I in microsporocyte of *C. tora* (estimated 28 bivalent, 2n=56)

Fig. 44 early metaphase I in microsporocyte of *C. siamea* (estimated 14 bivalent, 2n=28)

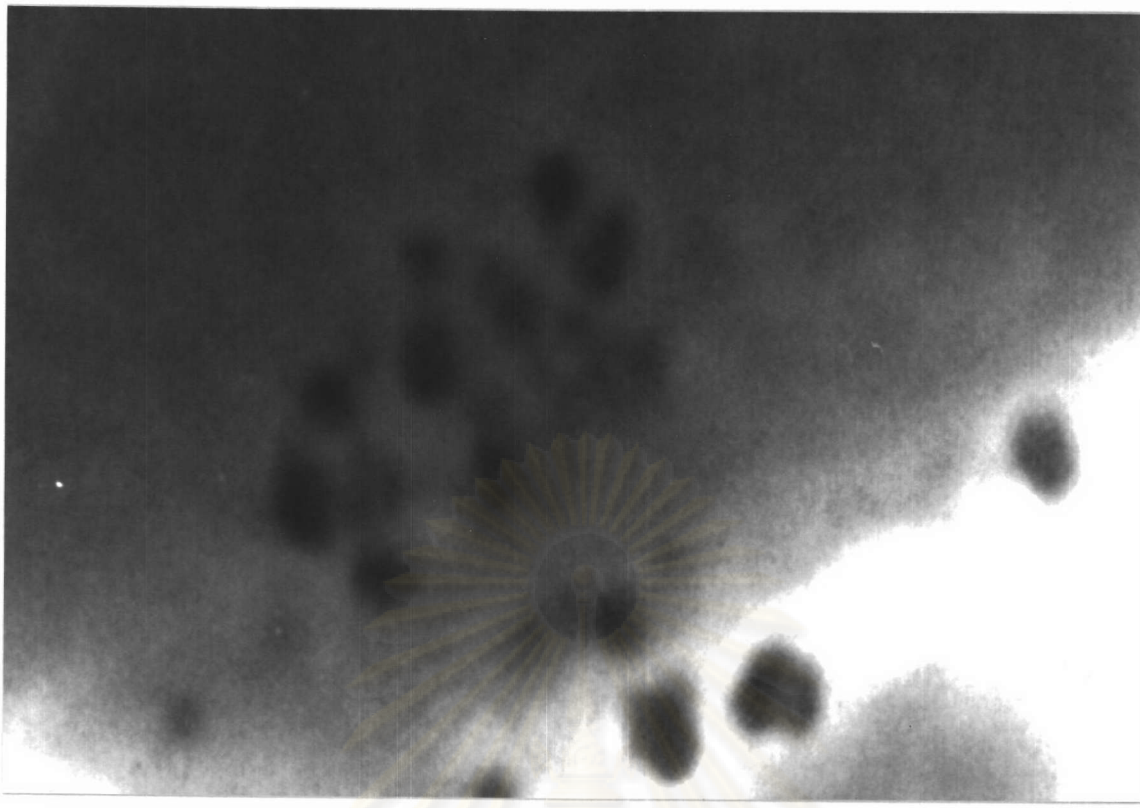

Fig. 45 metaphase I in microsporocyte of *C. garrettiana* (estimated 12-14 bivalent, 2n=24-28)

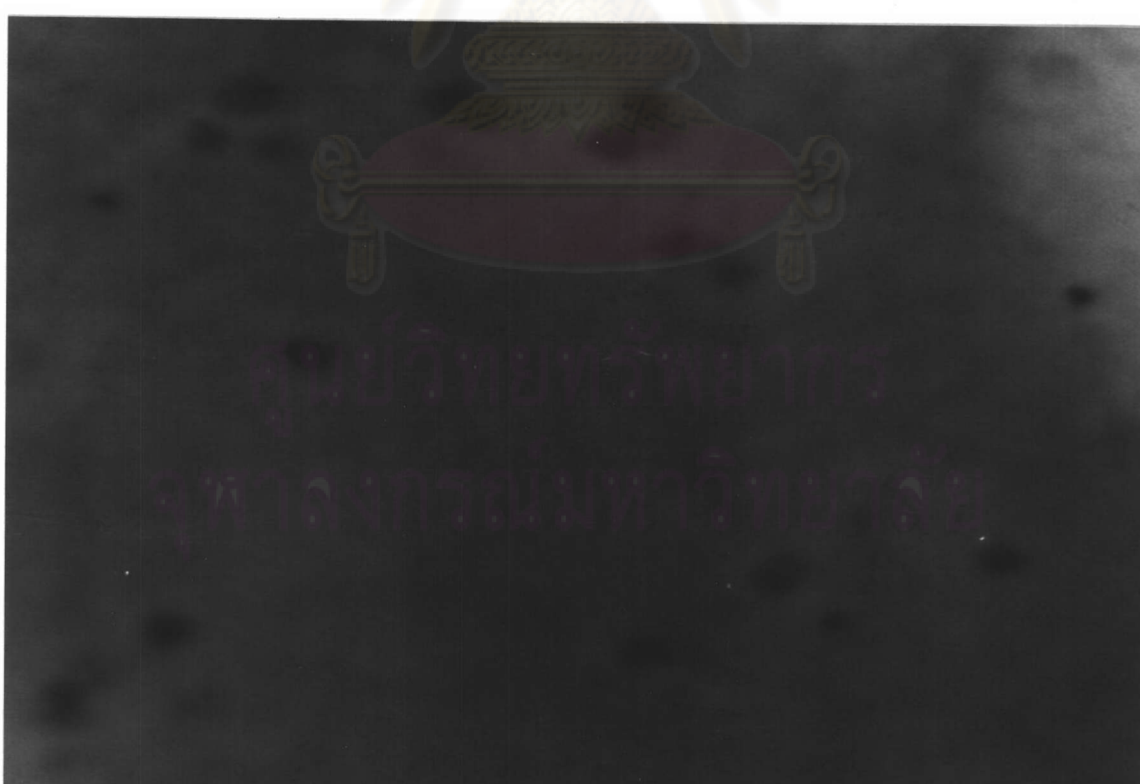

Fig. 46 late prophase (diakinesis) in microsporocyte of *C. fistula* (estimated 14 bivalent, 2n=28)

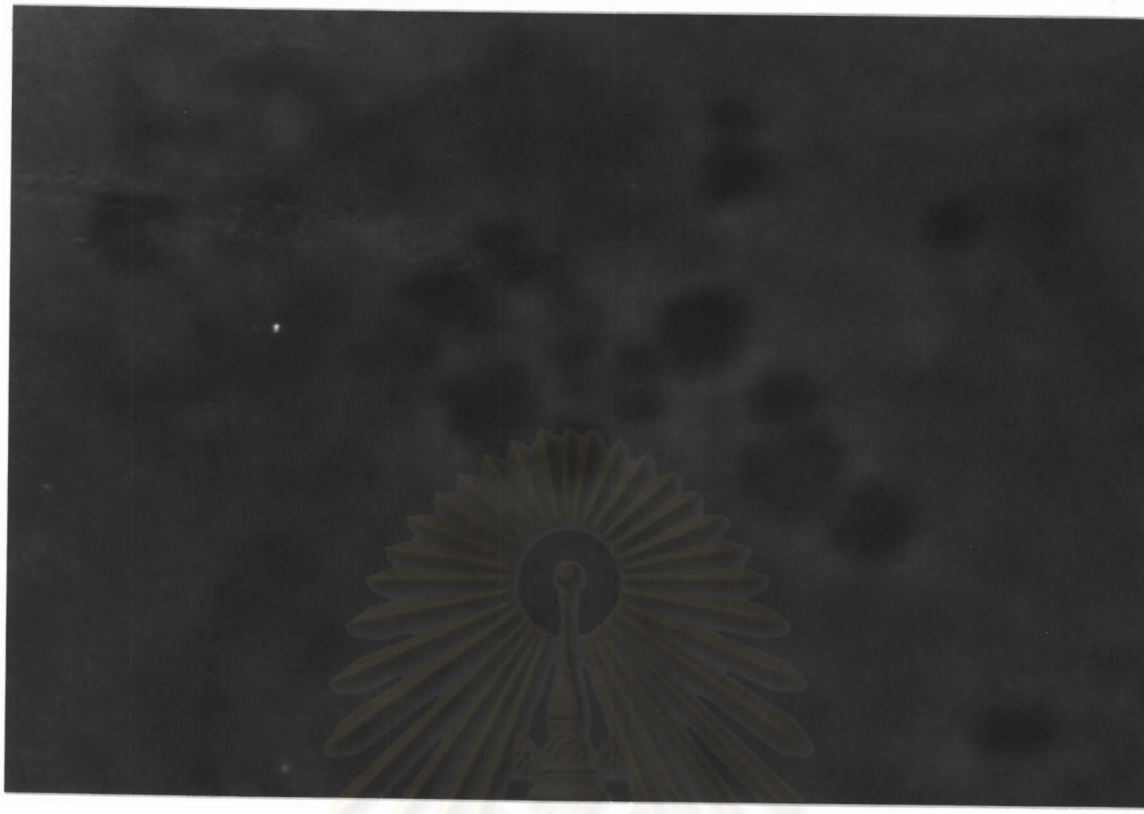

Fig. 47 early metaphase I in microsporocyte of *C. sophera* (estimated 14 bivalent, 2n=28)

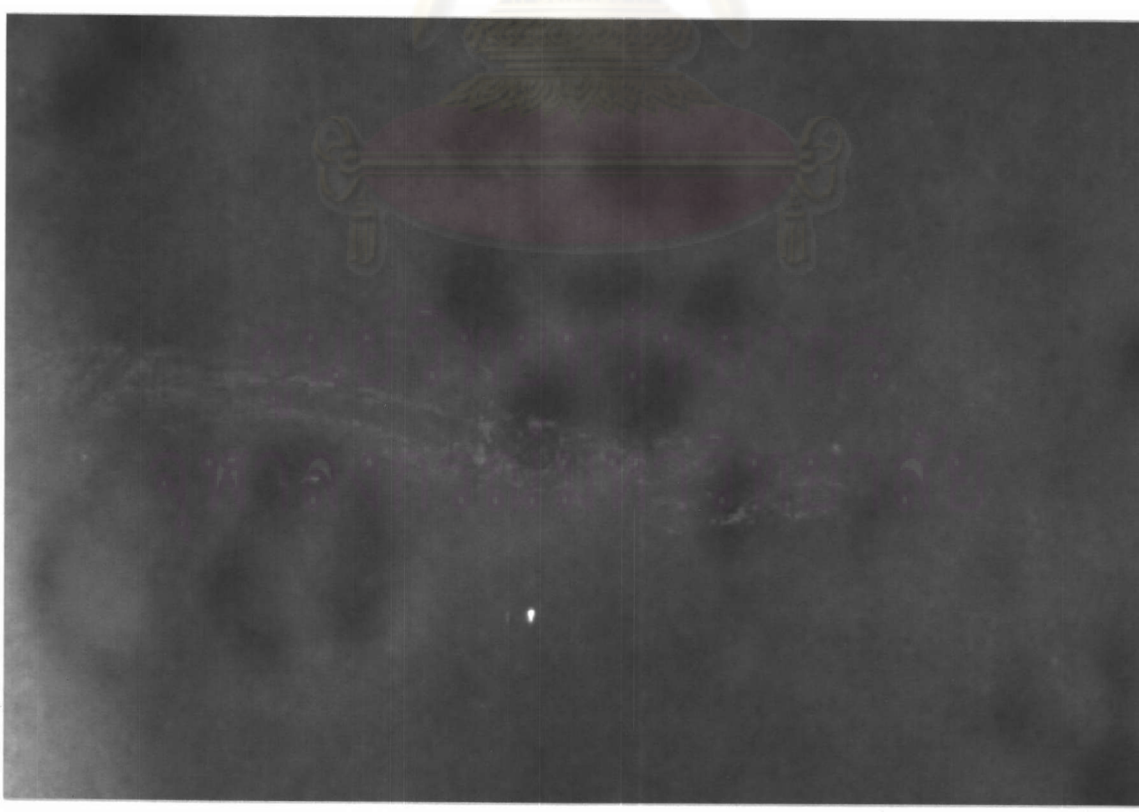

Fig. 48 late prophase (diakinesis) in microsporocyte of *C. javanica* (estimated 13-14 bivalent, 2n=26-28)

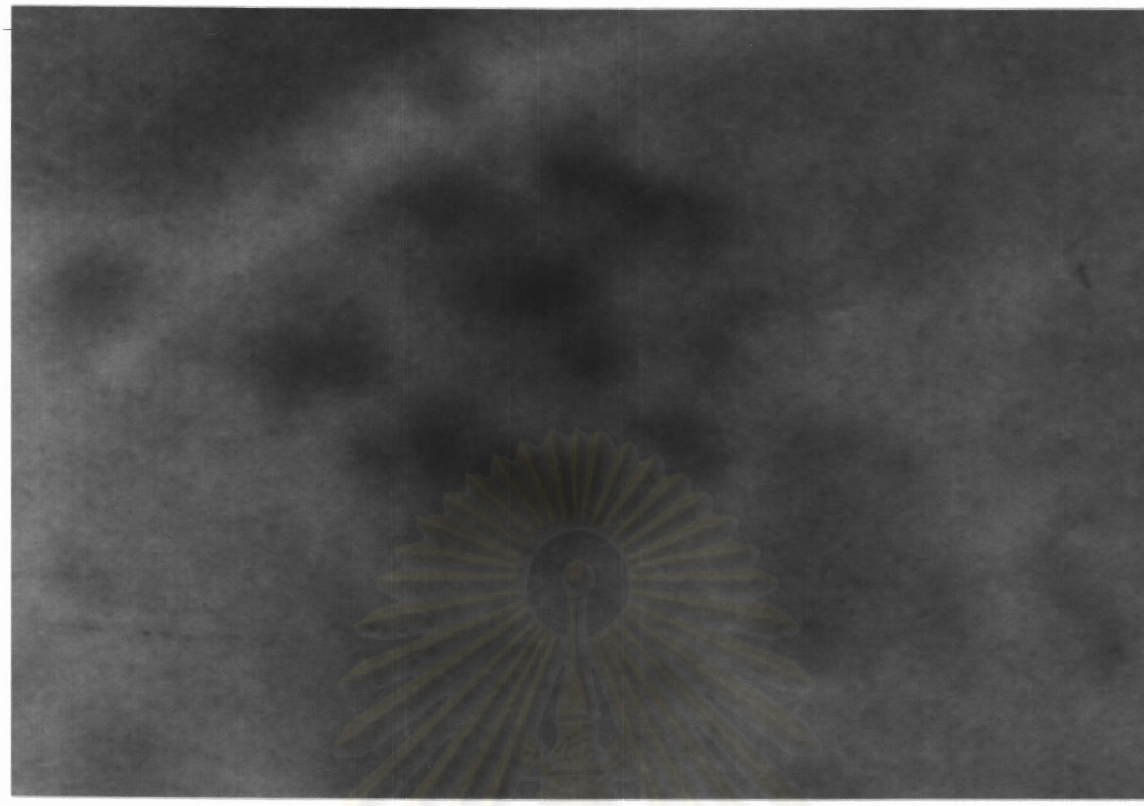

Fig. 49 late prophase (diakinesis) of microsporocyte of *C. spectabilis* (estimated 11-12 bivalent, 2n=22-24)

Fig. 50 prophase I (diakinesis) of microsporocyte of *C. timoriensis* (estimated 14 bivalent, 2n=28)