CHAPTER IV

RESULTS

4.1 Plant sample collection

4.1.1 Collection of *Aeschynanthus* cultivars from Jatujak market.

Some cultivated samples of *Aeschynanthus* were obtained from a plant-selling area of Jatujak flee market, Bangkok, on March 13th, 2003. Only one from about 30 shops was selling *Aeschynanthus* at that time. This shop had six *Aeschynanthus* cultivars which were different in morphology from each other, such as in their leaf characters and stem colour. The plants were named as *Aeschynanthus* sp. JJ_001 to *A.* sp. JJ_006 and then were photographed digitally, except *A.* sp.JJ_006 which died very soon after brought to the glass house. Moreover, as *A.* sp. JJ_001 had strange and interesting pinkish petal colour (discuss more in details in Chapter 5), a herbarium specimen of this plant was prepared and sent to Mary Mendum of Royal Botanic Garden Edinburgh, UK, to check whether *A.* sp. JJ_001 was actually a new species. Photos of the five cultivars *Aeschynanthus* from Jatujak market (*A.* sp. JJ_001-JJ_005) were shown respectively in Fig. 8, 9, 10, 11 and 12.

Fig. 8 Morphological characteristics of *Aeschynanthus* sp. JJ _001.

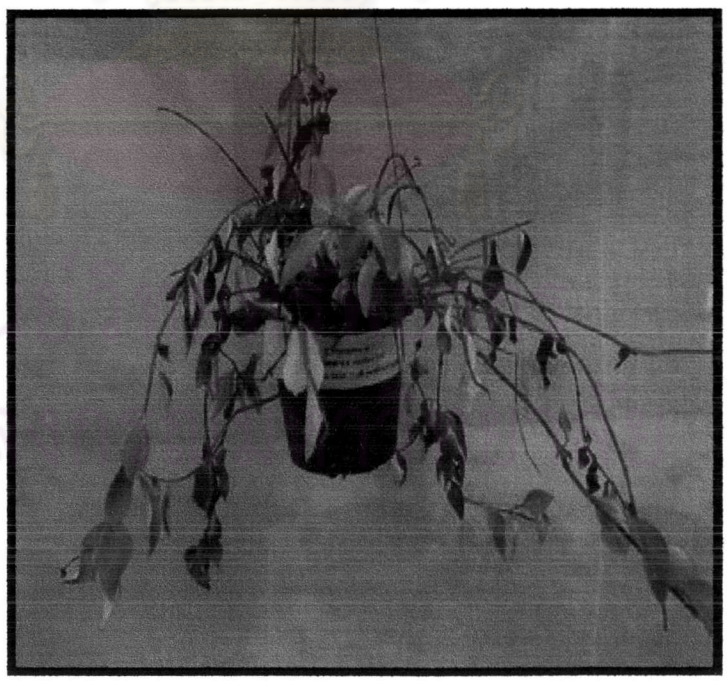


Fig. 9 Morphological characteristics of *Aeschynanthus* sp. JJ_002.

Fig. 10 Morphological characteristics of *Aeschynanthus* sp. JJ_003.

Fig. 11 Morphological characteristics of *Aeschynanthus* sp. JJ_004.

Fig. 12 Morphological characteristics of *Aeschynanthus.* sp. JJ_ 005.

4.1.2 Expedition in Chiangmai to collect wild *Aeschynanthus* samples.

The first expedition set off on December 19th-20th, 2003 to visit San-ku and Doi Pui areas of Sutep Mountains. These two areas were 600 and 800 metres above sea level, respectively. At San-ku area, many flowering *Aeschynanthus* were found along side the trail. Although this location was difficult to survey with limited walk-ways, the plants were easily recognised with their red colour flowers hanging from the tree trunks (Fig. 13). Majority of the large trees in San-Ku were in the family Palionaceae and most *Aeschynanthus* were found on these tree as high as 8-10 metres above the ground.

These *Aeschynanthus* specimens collected in San-Ku were identified as of *A. hildebrandii*.

Another field-work location of the first expedition was Doi Pui, this location was easier to survey than San-Ku as most plants were growing along the road. Various kinds and sizes of trees were recognised here. *Aeschynanthus* was common in this place (Fig.14). Many *Aeschynanthus* flowers were found falling on the ground and then were collected to keep in 70% ethanol. Although majority of large trees in this areas were both pines and Palionaceae, no *Aeschynanthus* was seen growing on pines

The second expedition to San-Ku and Doi Pui areas in Chiangmai province took place on June 5th – 12th, 2004. The survey date was not in the flowering season of *Aeschynanthus* and then gave difficulty on distinguishing the plant out of other vegetatives in the forest. In additionally, Doi Inthanon National Park in Sutep Mountains was also visited in this second expedition. Doi Inthanon is the highest mountain in Thailand and much higher than San-Ku and Doi Pui with an attitude over 2,500 metres above sea level. Most of *Aeschynanthus* were found on trunks of large trees along the road going up to the top of the mountain (Fig. 15). The weather on the top of Doi Inthanon was very cold, humid, and lightly raining. An expedition in this forest followed a man-made walking trail of the national park. Many *Aeschynanthus* were found on various kinds of trees along the trail (Fig. 16). These plants were supposed to be *A. macranthus* with their unique large-leaf characteristics. Several other plants which looked like

Aeschynanthus in both habit and morphology were also found in this area. Sample collection was prohibited by regulations of the national park. None of *Aeschynanthus* found in either San-Ku and Doi Pui or Doi Inthanon was flowering and only their vegetative parts were photographed in the second expedition.

Fig. 13 The habitat of wild *Aeschynanthus* at San-Ku area on winter season. The plants were flowering and some of them had already been pollinated, turning to capsule-like fruits. The orange arrow indicates an *Aeschynanthus* flower.

Fig. 14 The habitat of wild *Aeschynanthus* at Doi-Pui on winter season. Capsule-like fruits of *Aeschynanthus* were also observed in this area. The orange arrow shows an *Aeschynanthus* flower.

Fig. 15 The habitat of wild *Aeschynanthus* at Doi-Pui on rainy season. The plants were not flowering on that time. The orange arrow indicates one *Aeschynanthus* plant growing on a tree trunk.

Fig. 16 The habitat of wild *Aeschynanthus* at Doi Inthanon National Park on rainy season. These plants did not have flowers. The orange arrow shows an *Aeschynanthus* which is supposed to be *A. macranthus* settling on the tree trunk.

4.2 DNA extraction

Total genomic DNA of 45 *Aeschynanthus* smples was extracted from either fresh or dry leaf specimens of individual. The extracted genomic DNA was dissolved in 100 ul of AE buffer of QIAGEN DNA minikit. An amount of each extracted DNA was estimated by agarose gel electrophoresis compared with the M23 1.5 kb + 100 bp DNA ladder marker and the estimated DNA concentration was mostly suitable for subsequent PCR experiments. All of *Aeschynanthus* samples gave high enough quality and quantity of yielded genomic DNA as shown in Fig. 18, 19, and 20. However, the DNA of *A. superbus* was not able to yield any genomic band product, though reextracted with either the QIAGEN DNA minikit or any other DNA extraction kit. Note that, although smear DNA bands caused by DNA fragmentation along extraction occurred in some samples, no PCR amplification problem was later found. Moreover, the genomic DNA band of *A.* sp. CM_034 could not be observed with gel electrophoresis, the DNA gave nicely PCR amplified products.

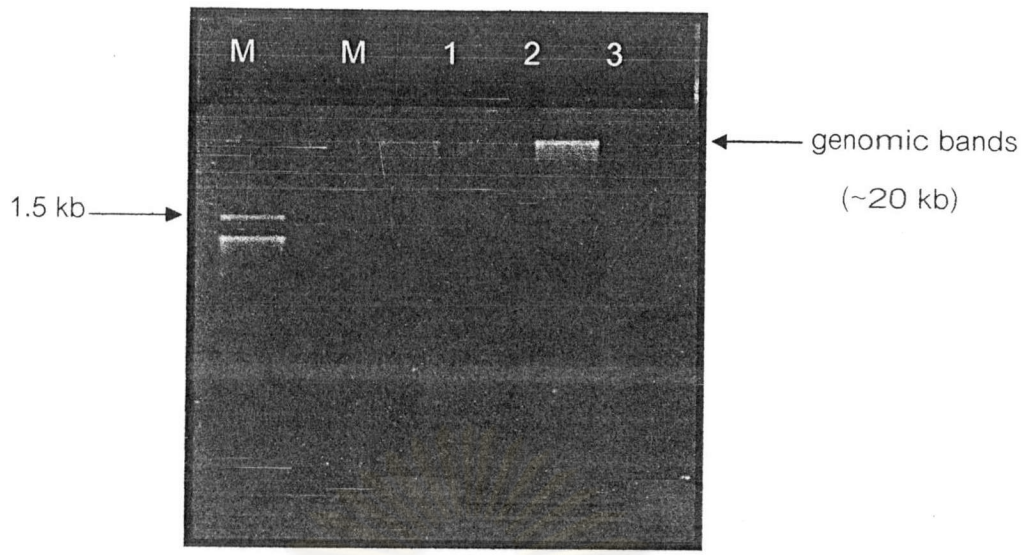


Fig. 17 Genomic DNA bands of some *Aeschynanthus* samples provided by RBGE compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1 = *A. fecandus*
2 = *A. fulgen*
3 =*A. garrettii*
4 = *A. hookeri*

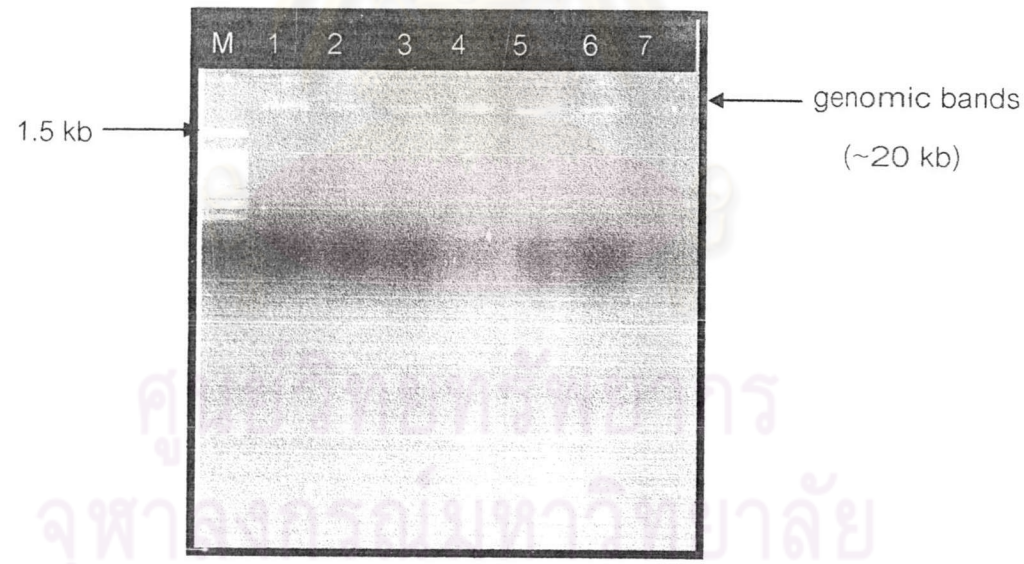


Fig. 18 Genomic DNA bands of *Aeschynanthus* samples collected from Chiangmai province compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1= *A.* sp. CM_007
2 = *A.* sp. CM_009
4 = *A.* sp. CM_022
3 = *A.* sp. CM_013
5 = *A.* sp. CM_026
6 = *A.* sp. CM_030
7 = *A.* sp. CM_034

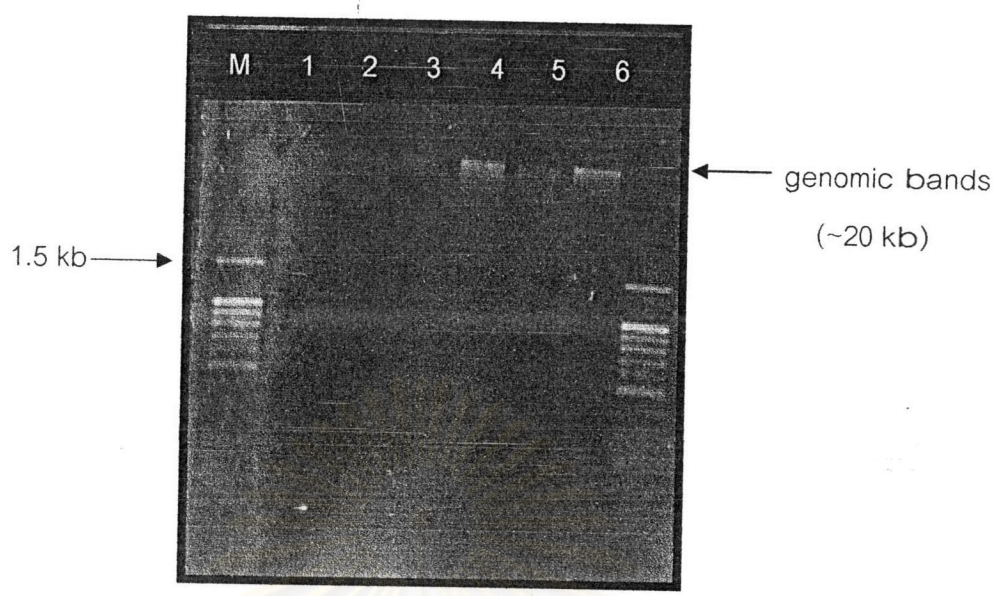


Fig. 19 Genomic DNA bands of *Aeschynanthus* samples collected from Jatujak market compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1= *A.* sp. JJ_001
2 =*A.* sp. JJ_002
3 = *A.* sp.JJ_003
4 = *A.* sp.JJ_ 004
5 = *A.* sp. JJ_005
6 = *A.* sp. JJ_006

4.3 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 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 was only one *Aeschynanthus* not successfully amplified *A. superbus*. Other remaining species were amplifiable and did not have to adjust the annealing temperature. Primer dimers were not found from all samples and the control reactions except *Aeschynanthus* bought from Jatujak market.

The approximated sizes of PCR products of ITS regions are about 800 bp as shown in Fig. 21, 22, and 23.

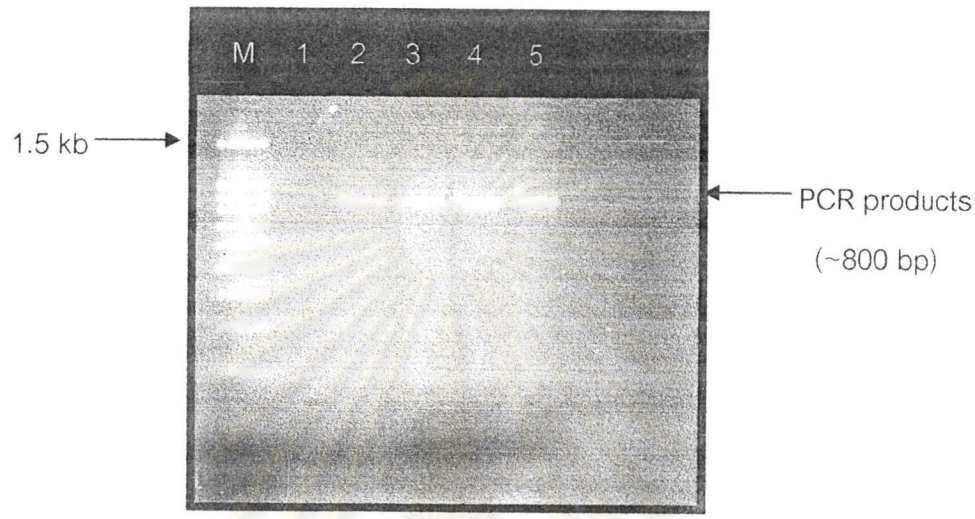


Fig. 20 PCR products of ITS regions of some *Aeschynanthus* samples (provided by RBGE) compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1 = negative control
2 = *A. fecandus*
3 = *A. flugen*
4 = *A. garrettii*
5. = *A. hookeri*

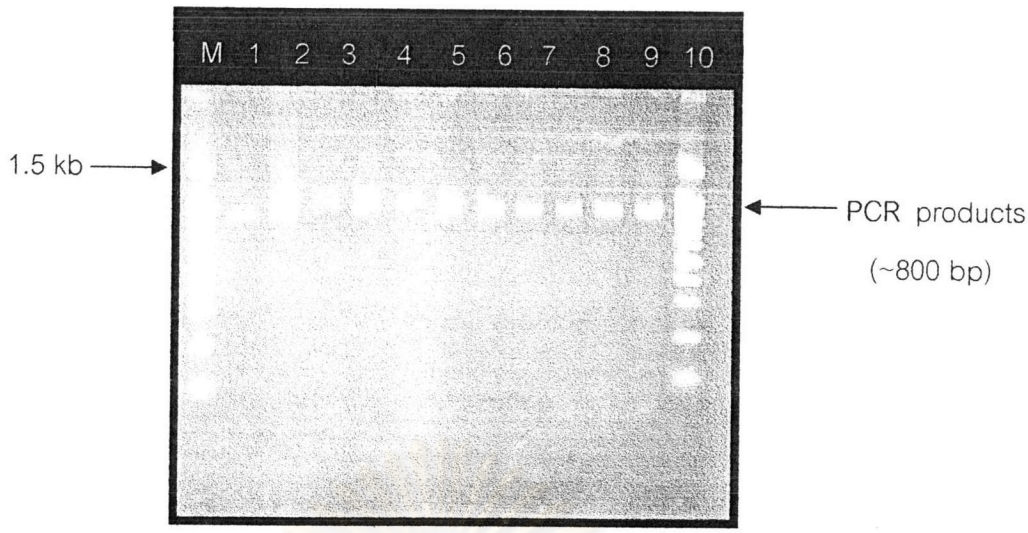


Fig. 21 PCR products of ITS regions of *Aeschynanthus* samples collected from Chiangmai province compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1 = *A.* sp. CM_007
2 = *A.* sp. CM_007
3 = *A.* sp. CM_ 013
4 = *A.* sp. CM_013
5 = *A.* sp. CM_022
6 = *A.* sp. CM_022
7 = *A.* sp. CM_026
8 = *A.* sp. CM_026
9 = *A.* sp. CM_030
10= *A.* sp. CM_034
11= *A.* sp. CM_034

Fig. 22 PCR products of ITS regions of *Aeschynanthus* samples collected from Jatujak market compared with 1.5 kb + 100 bp DNA marker.

M = 1.5 kb + 100 bp DNA marker
1 = negative control
2 = *A.* sp. JJ_001
3 = *A.* sp. JJ_002
4 = *A.* sp. JJ_003
5 = *A.* sp. JJ_004
6 = *A.* sp. JJ_005
7 = *A.* sp. JJ_006

4.4 PCR product purification and DNA sequencing

After the ITS regions of each *Aeschynanthus* species was amplified with suitable primers, the PCR products were cleaned up before DNA sequencing. In this study QIAquick PCR purification kit was used to purify the DNA products. Although, the DNA concentration might have been decreased a little bit after purifed, the DNA concentration was enough for sequencing. Each PCR product was sequenced 4 times with primer 5P, and 1P in the forward direction and with primers 4P and 8P in the reverse direction. After DNA sequencing was performed, Chromas computer program was used to compare each couple of the complementary sequences. Sequencing experiments were redone several times for some species and base-by-base comparison could help getting an accurate data. All of Thai *Aeschynanthus* gave clear ITS sequences without contaminated sequence signals from other plant samples or primer-dimers (Fig. 24) except *A. parviflorus* which had some intraindividual sequence polymorphism (Fig. 25). All DNA sequences were converted to FASTA format before further analysed with Clustal X alignment program. Not that, sequence retrived from leaf material which same species either identical accession number or not same accession number with the previous study (Denduangboripant *et al.*, 2001) were used to compared in this study.

Complete sequences of the whole ITS regions were successfully generated from leaf materials of almost all 32 *Aeschynanthus* samples supplied by RBGE. The boundaries of ITS1 and ITS2 and adjacent-coding regions were determined by

comparing to published *Aeschynanthus* ITS sequences retrieved from GenBank. The lengths of ITS sequences from these 32 taxa varied from 634 to 665 bp. The lengths of ITS1 regions of these *Aeschynanthus* ranged from 222 bp to 233 bp and the 5.8s rDNA of all samples was 156 bp in length while the lengths of ITS2 was 240 to 259 bp. For *Aeschynanthus* specimens bought from Jatujak market and those collecting from Chiangmai province, the sequences lengths and the boundaries of ITS regions were similar to other *Aeschynanthus* from the previous study (Denduangboripant *et al.*, 2001). Almost all ITS sequences were clear except those of *Aeschynanthus* sp.JJ_005 which had intra-individual sequence polymorphism and then gave only a short readable sequence. All of the sequences were compared to Genbank nucleotide database to identify the species name.

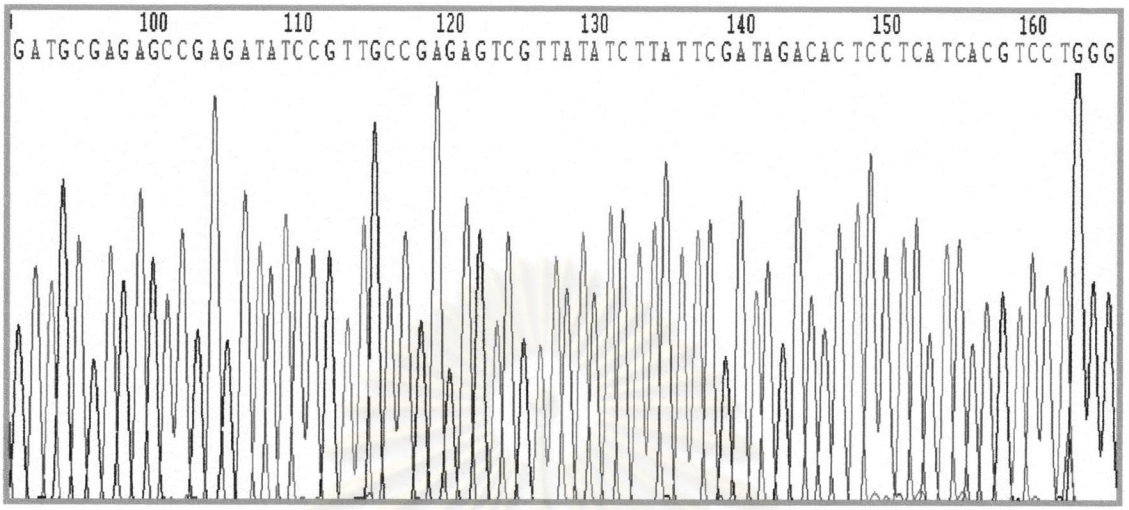


Fig. 23 Four-coloured electropherogram of ITS sequence of *A. javanicus* (clear sequence) with blue peaks represent Cytocine (C), red Thymine (T), green Adenine (A) and cyan Guanine (G) nucleotides.

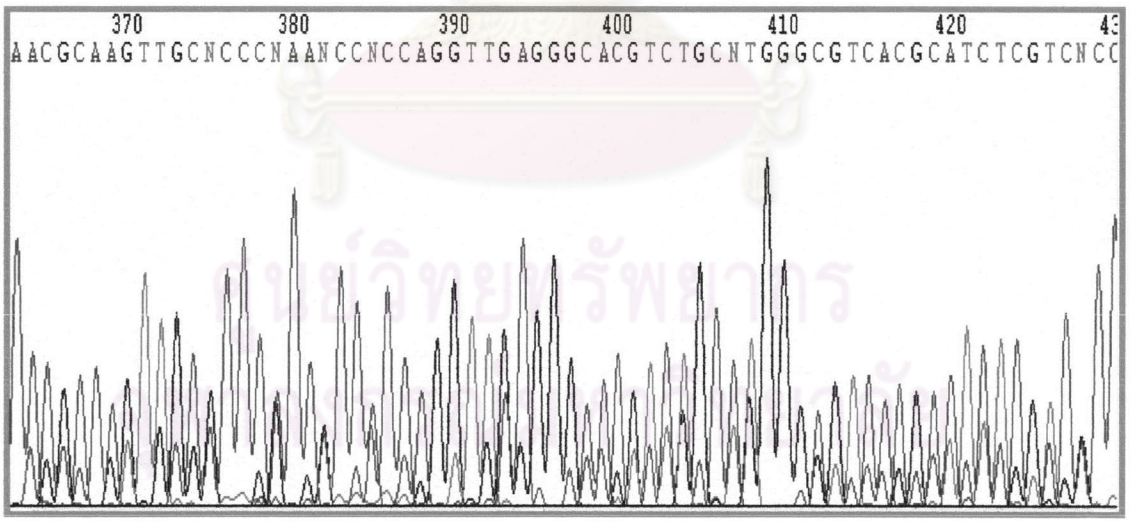


Fig. 24 Four-coloured electropherogram of ITS sequence of *A. parviflorus* (some intra individual sequence polymorphism) with blue peaks represent Cytocine (C), red Thymine (T), green Adenine (A) and cyan Guanine (G) nucleotides.

4.5 DNA data matrix preparation

After converting DNA sequences to FASTA format, Clustal X computer program was used to align the FASTA file. Three types of sequence data matrices were prepared (1) an ITS data matrix of all *Aeschynanthus* samples supplied from RBGE, aligned with all previously studied *Aeschynanthus* ITS sequence (Fig. 26) (2) an ITS data matrix of all *Aeschynanthus* samples brought from Jatujuk market, aligned with all previously studied *Aeschynanthus* ITS sequences (Fig. 27) (3) an ITS data matrix of some *Aeschynanthus* collected in Chiangmai, aligned with all Thai *Aeschynanthus* ITS sequences (Fig. 28). The lengths of these character-taxon matrices were 665 bp, 571 bp and 531 bp, respectively. The three ITS data matrices were then transformed to NEXUS file format for further phylogenetic tree reconstruction.

Fig. 25 A 572 bp character-taxon matrix of 80 *Aeschynanthus* taxa based on ITS sequences. Gap symbol (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data. Asterisks * represent excluded sites from analyses.

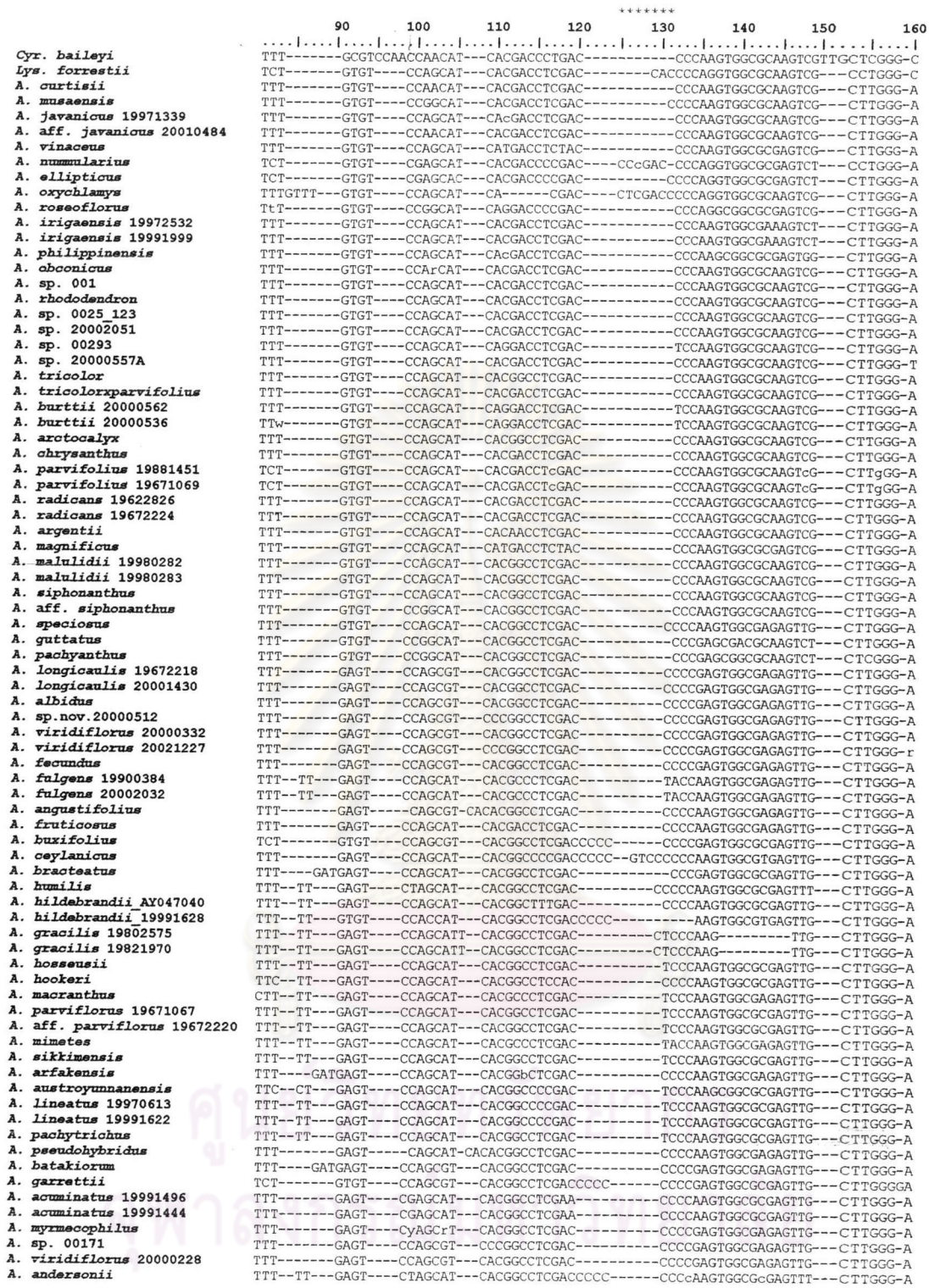
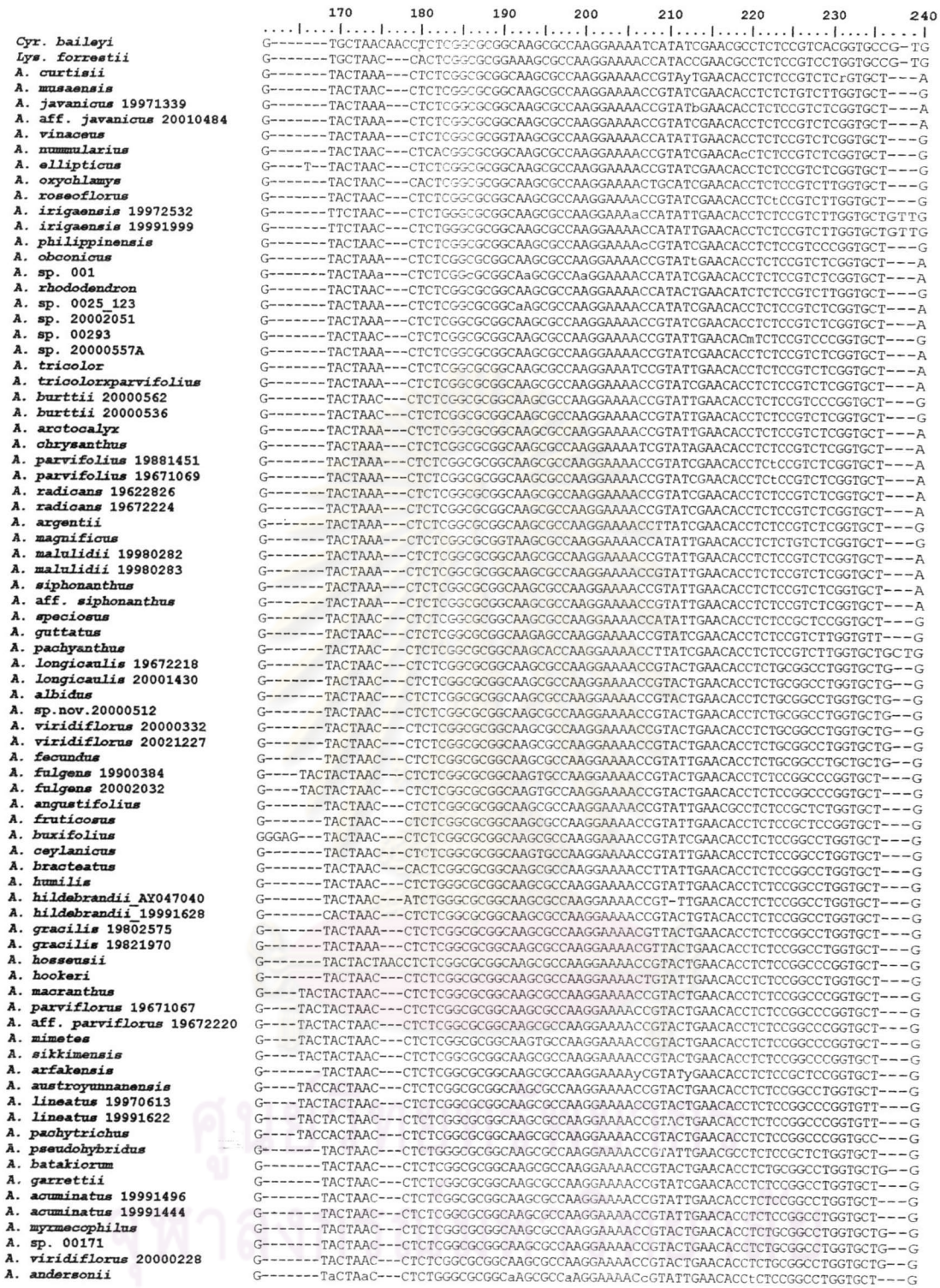


Fig. 25 (continued)



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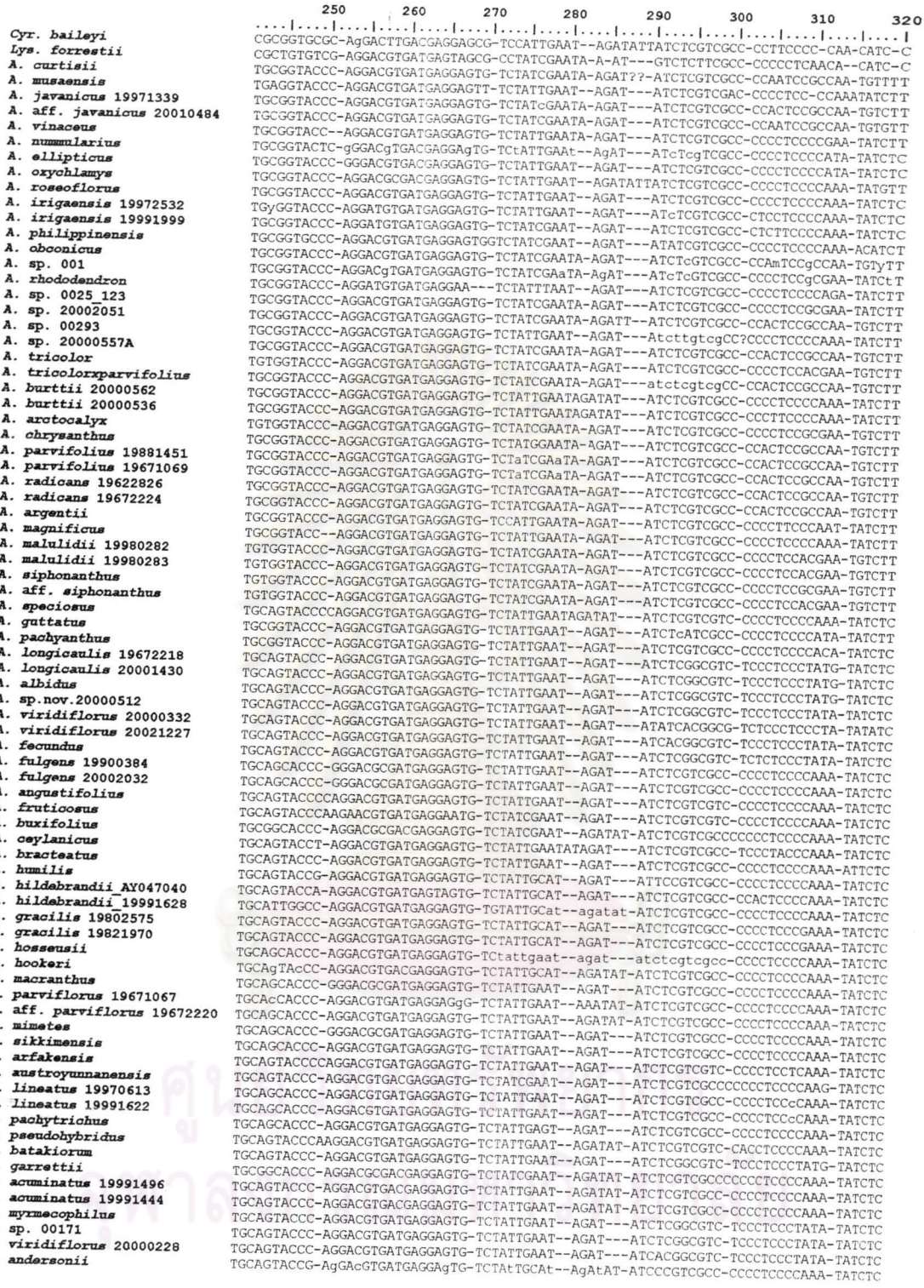

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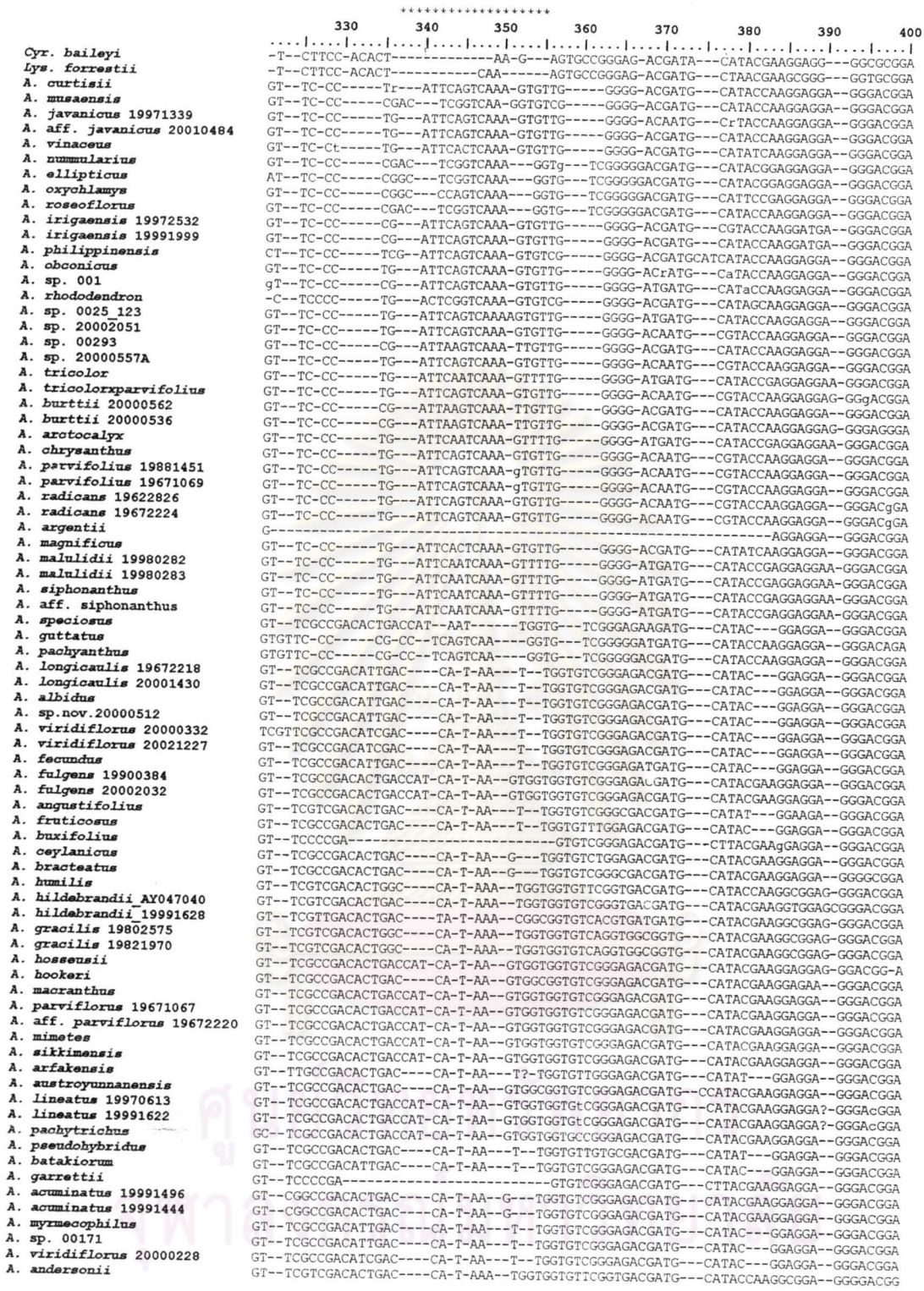

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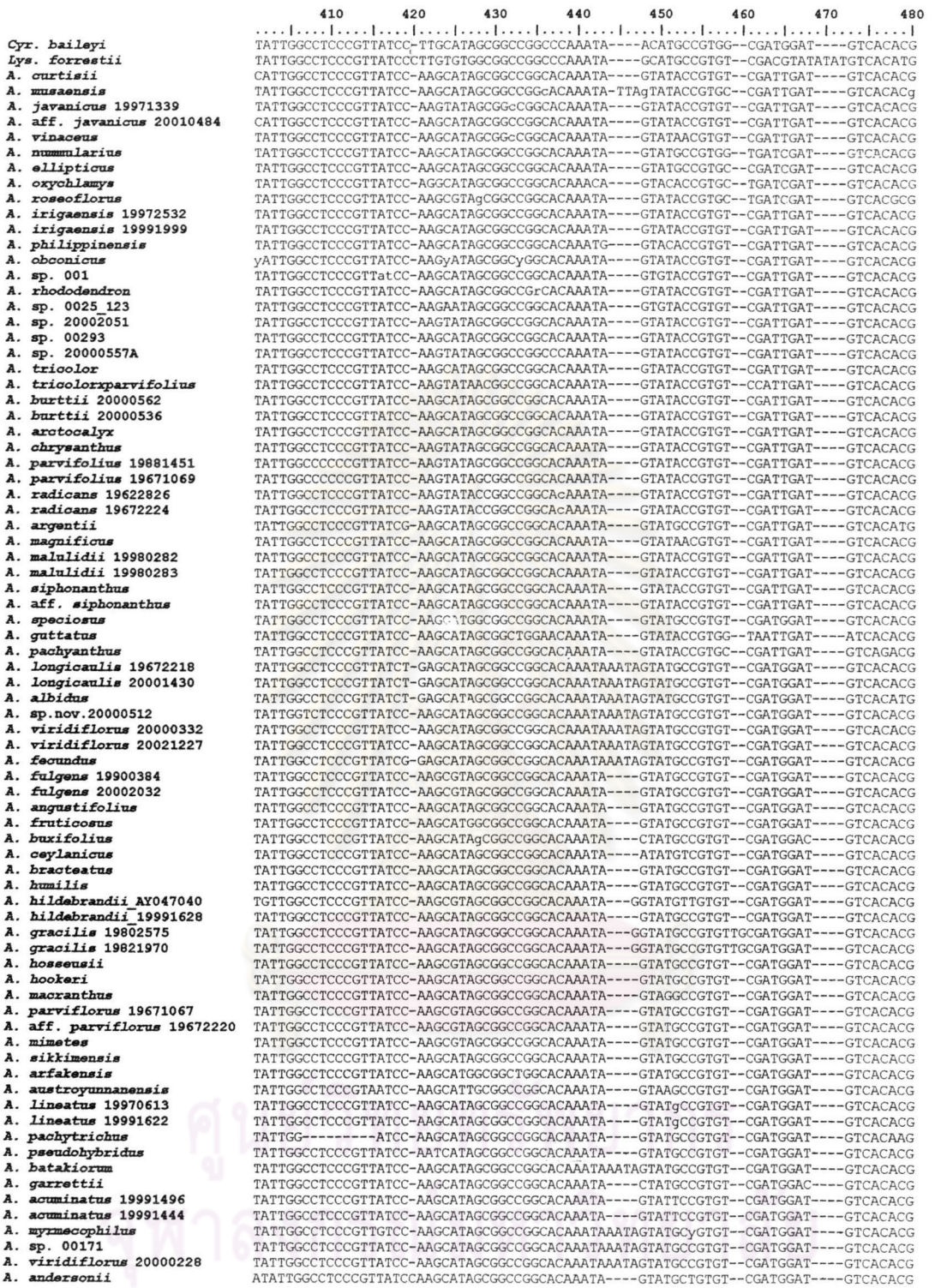

Fig. 25 (continued)

Fig. 25 (continued)

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                                570        580        590        600
                                ....|....|....|....|....|....|....|....|.
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Lys. forrestii                  C-GACCCAA-C-----GGCAC----GAGAT-TG------CCCTCGA
A. curtisii                     T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. musaensis                    C-GACCCAA-T-CAATGGCGC----AAGAT-CG------CcCTCGA
A. javanicus 19971339           T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. aff. javanicus 20010484      T-GACCCAA-T-----GGCAC----aagat-tg------CCCTCGA
A. vinaceus                     C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. nummularius                  C-GACCCAAA------GGCAC----AAGAT-GG------CCcTCGA
A. ellipticus                   A-GACCCAAA------GGCAC----AAGAT-CG------CCCTCGA
A. oxychlamys                   C-GACCCAA-C-----GGCAC----AAGAT-GG------CCCTCGA
A. roseoflorus                  C-GACCCAA-C-----GGCAC----AAGAT-GG------CCCTCGA
A. irigaensis 19972532          C-GACCCAA-T-----GGCAC----AAGAT-TG------CTCTCGA
A. irigaensis 19991999          C-GACCCAA-T-----GGCAC----AAGAT-TG------CTCTCGA
A. philippinensis               C-GACCCAA-T-----GGCAC----GAGAT-TG------CTCTCGG
A. obconicus                    T-GACCCAA-T-----GGCAC----mAGAT-TG------CCCTCGA
A. sp. 001                      T-GACCCAA-T-----GgCAC----AAGAT-TG------CCCTCGa
A. rhododendron                 C-GACCCAAAT-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 0025_123                 T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 20002051                 T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 00293                    C-GACCCAA-T-----GGCAC----TagAt-tG------CCCTCGA
A. sp. 20000557A                T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. tricolor                     C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. tricolorxparvifolius         T-GACCCAA-T-----GGCAC----AAGAT-TG------CCTTCGA
A. burttii 20000562             C-GACCCAA-T-----GGCAC----TAGAT-TG------CCCTCGA
A. burttii 20000536             C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. arctocalyx                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. chrysanthus                  T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. parvifolius 19881451         T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. parvifolius 19671069         T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. radicans 19622826            T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. radicans 19672224            T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. argentii                     C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. magnificus                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. malulidii 19980282           C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. malulidii 19980283           C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. siphonanthus                 C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. aff. siphonanthus            C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. speciosus                    C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. guttatus                     C-GACCCAA-T-----GGCAC----AAGAT-CG------CCCTCGA
A. pachyanthus                  C-GACCCAA-C-----GGCAC----AAGAT-CG------CCCTCGA
A. longicaulis 19672218         C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. longicaulis 20001430         C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. albidus                      C-GACCCAA-T-----GGCAC----AAGTT-TG------CCCTCGA
A. sp.nov.20000512              C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
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A. fecundus                     C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. fulgens 19900384             C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. fulgens 20002032             C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. angustifolius                C-AACCCAA-T-----GGCAC----AAGAT-TG---CCGCCCTCGA
A. fruticosus                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. buxifolius                   G-GACCCAA-TT----GGCATAAATAAGATCTG------CCCtCGA
A. ceylanicus                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. bracteatus                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. humilis                      C-GACCCAAG------------AAGAT-TG------CCCACGA
A. hildebrandii_AY047040        C-GACCCAA-C-------------AAGAT-TG------CCCTCGA
A. hildebrandii_19991628        C-GACCCAA-C-------------AAGAT-TG------CCCTCGA
A. gracilis 19802575            G-GACCCAG-C-------------AAGTT-CG------CCCTCGA
A. gracilis 19821970            G-GACCCAG-C-------------AAGTT-CG------CCCTCGA
A. hosseusii                    C-GACCCAA-T-----GGCAC----AAgat-tg------CCCTCGA
A. hookeri                      C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. macranthus                   T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. parviflorus 19671067         C-GACCCAA-T---  -GGCAC----AAGat-tg------CCCTCGA
A. aff. parviflorus 19672220    C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. mimetes                      C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sikkimensis                  C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. arfakensis                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. austroyunnanensis            C-GACCCAA-T-----GGCAC----GAGAG-TG------CCCTCGA
A. lineatus 19970613            C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. lineatus 19991622            C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. pachytrichus                 T-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. pseudohybridus               C-AACCCAA-T-----GGCAC----AAGAT-TG---CCGCCCTCGA
A. batakiorum                   C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. garrettii                    G-GACCCAA-C-----GGCATAA-TAA-AT-TG------CCCTCAA
A. acuminatus 19991496          C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. acuminatus 19991444          C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. myrmecophilus                C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 00171                    C-GACCCAA-T-----GGCAC----AagAt-tG------CCCTCGA
A. viridiflorus 20000228        C-GACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. andersonii                   C-GACCCAA-C-------------AAGAT-TG------CCCACGA
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Fig. 25 (continued)

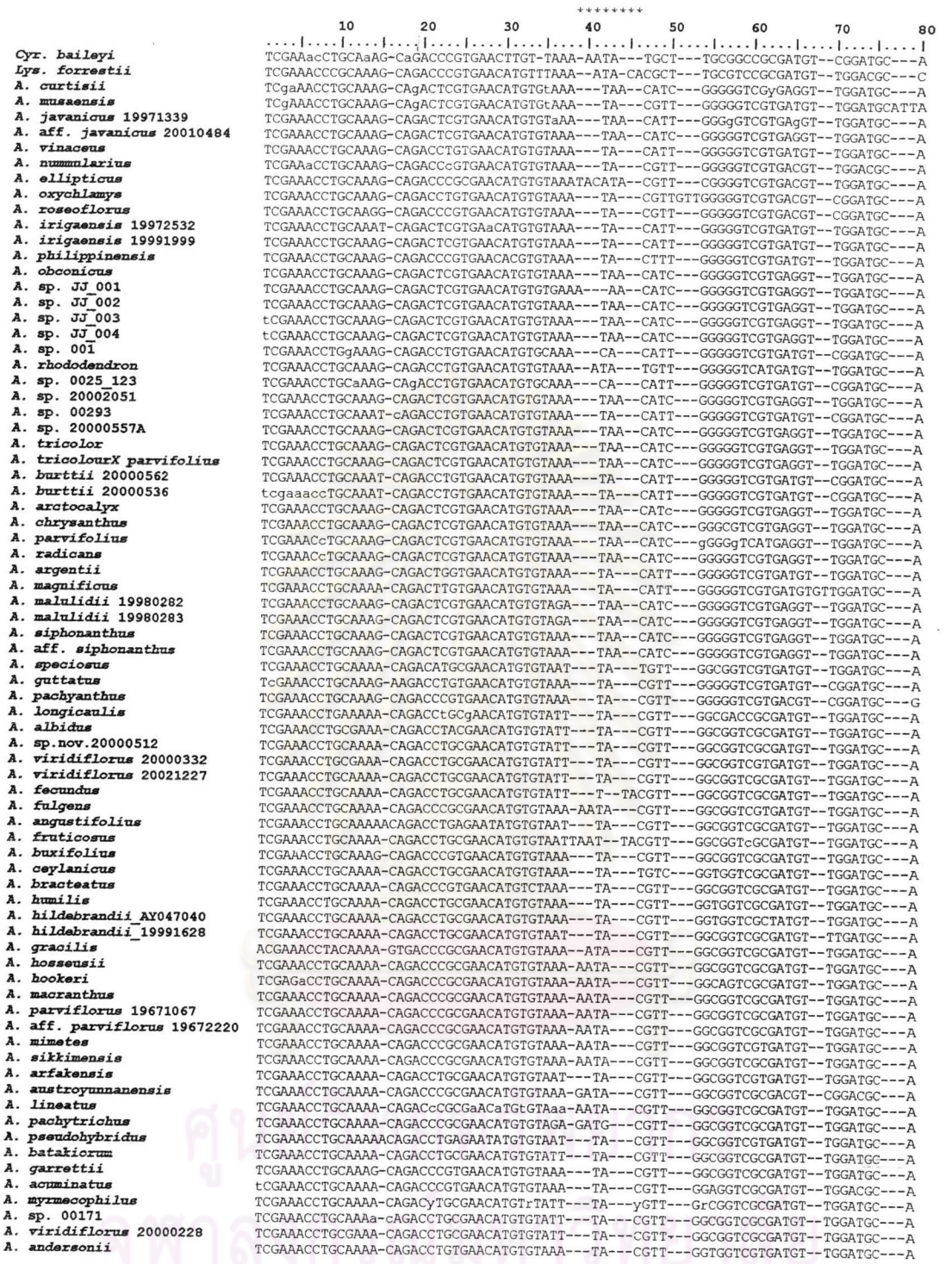
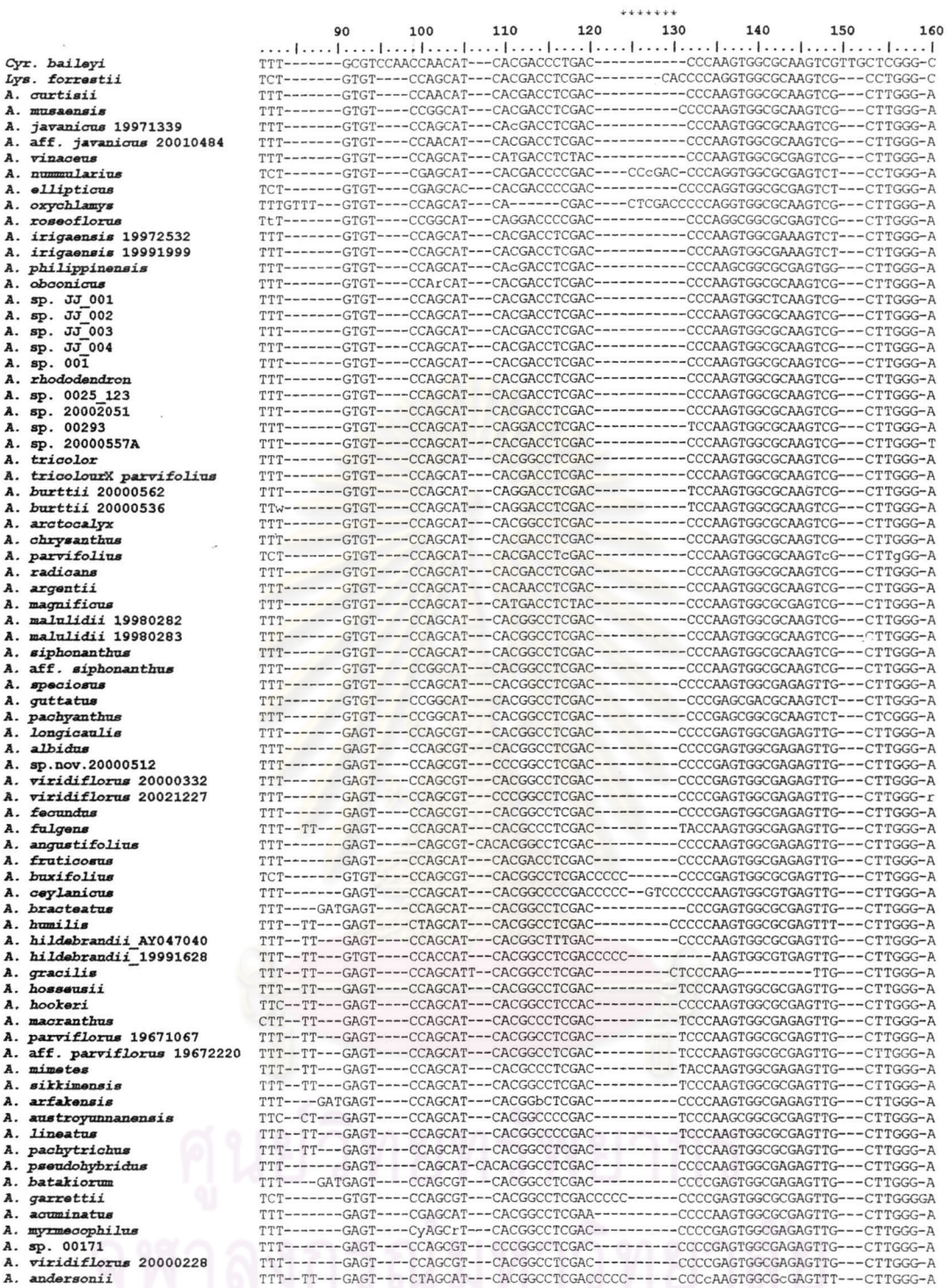


Fig. 26 A 571 bp character-taxon matrix of 77 *Aeschynanthus* taxa based on ITS sequences. Gap symbol (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data. Asterisks * represent excluded sites from analyses.



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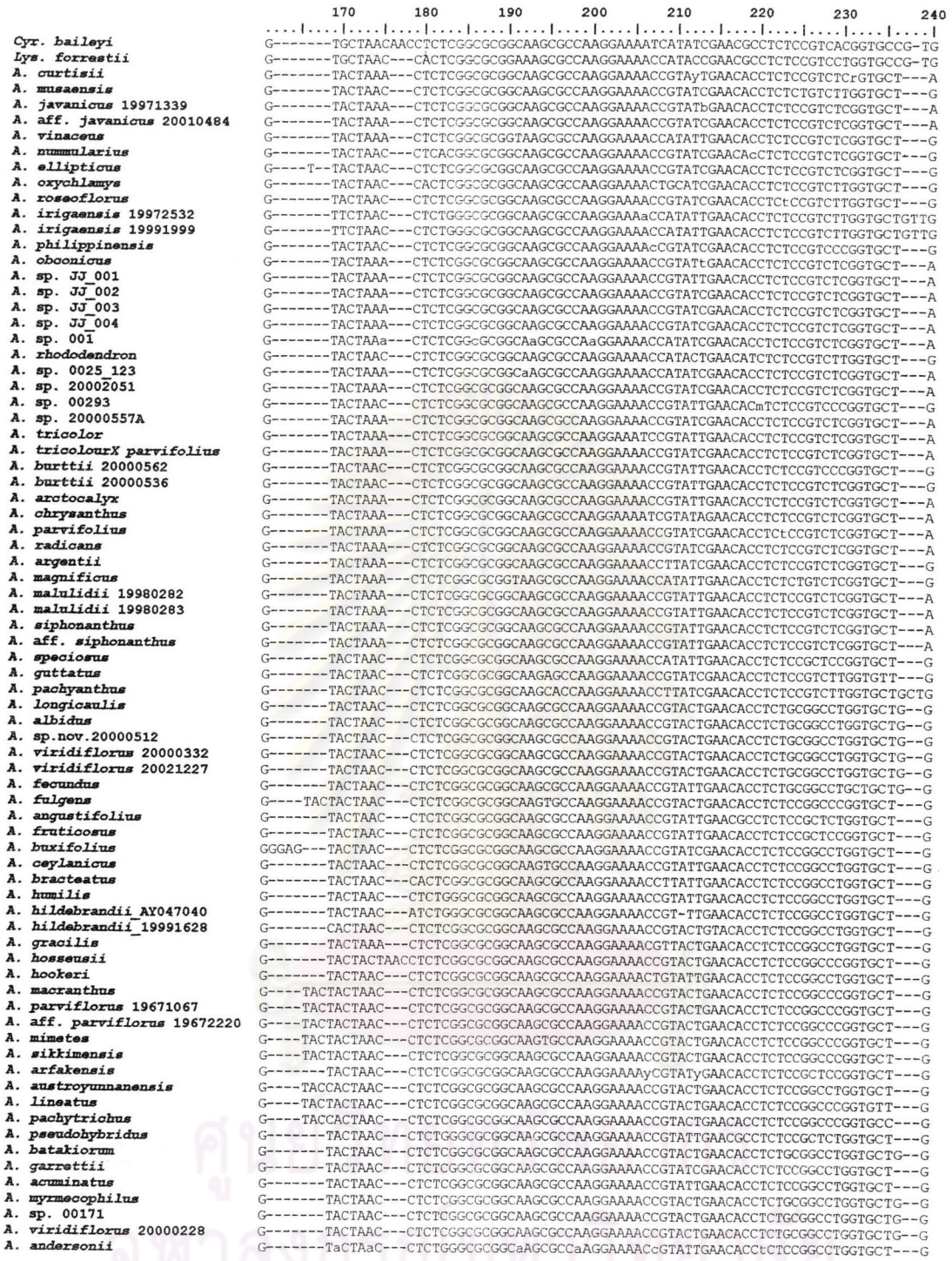

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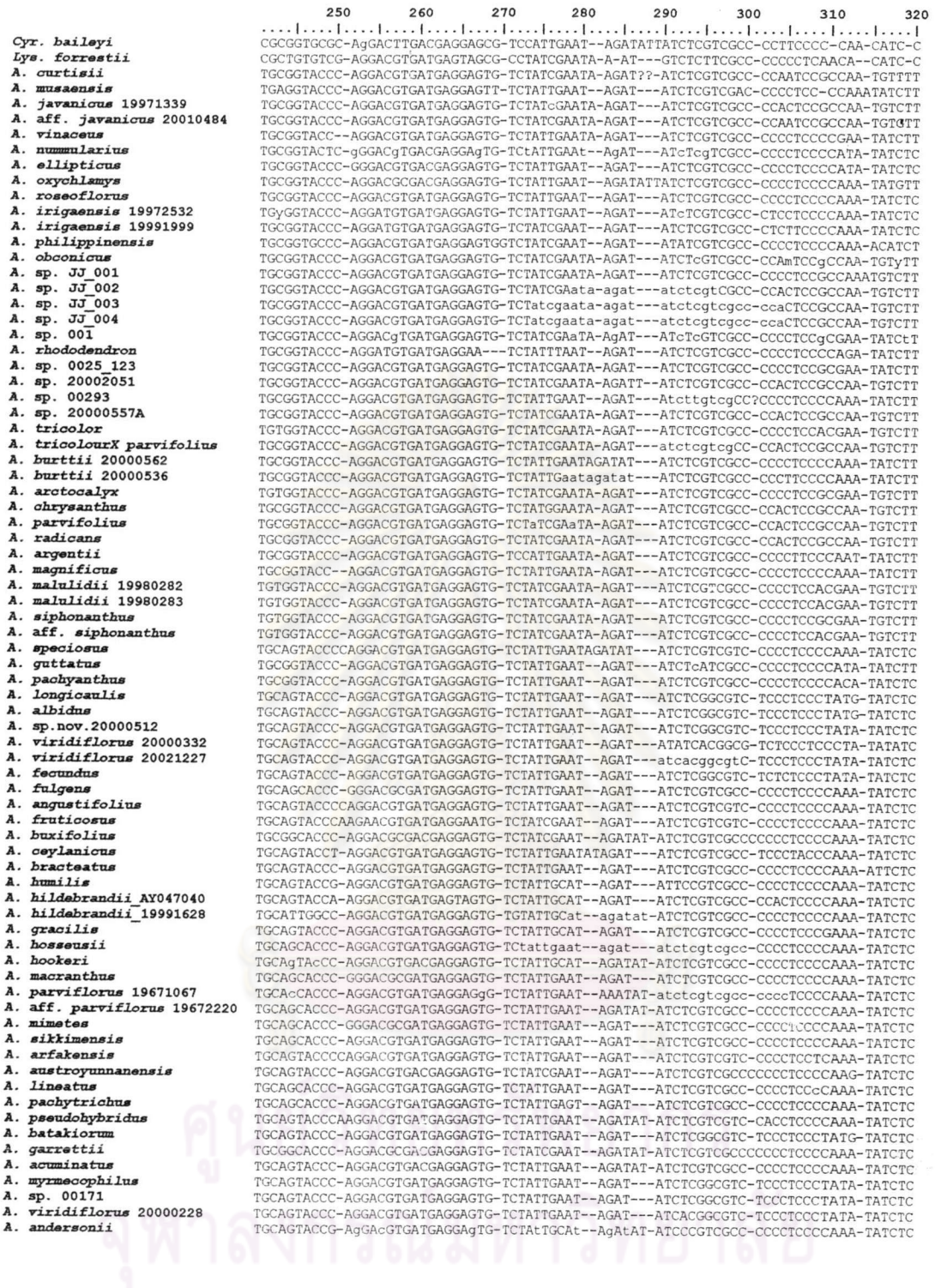

Fig. 26 (continued)

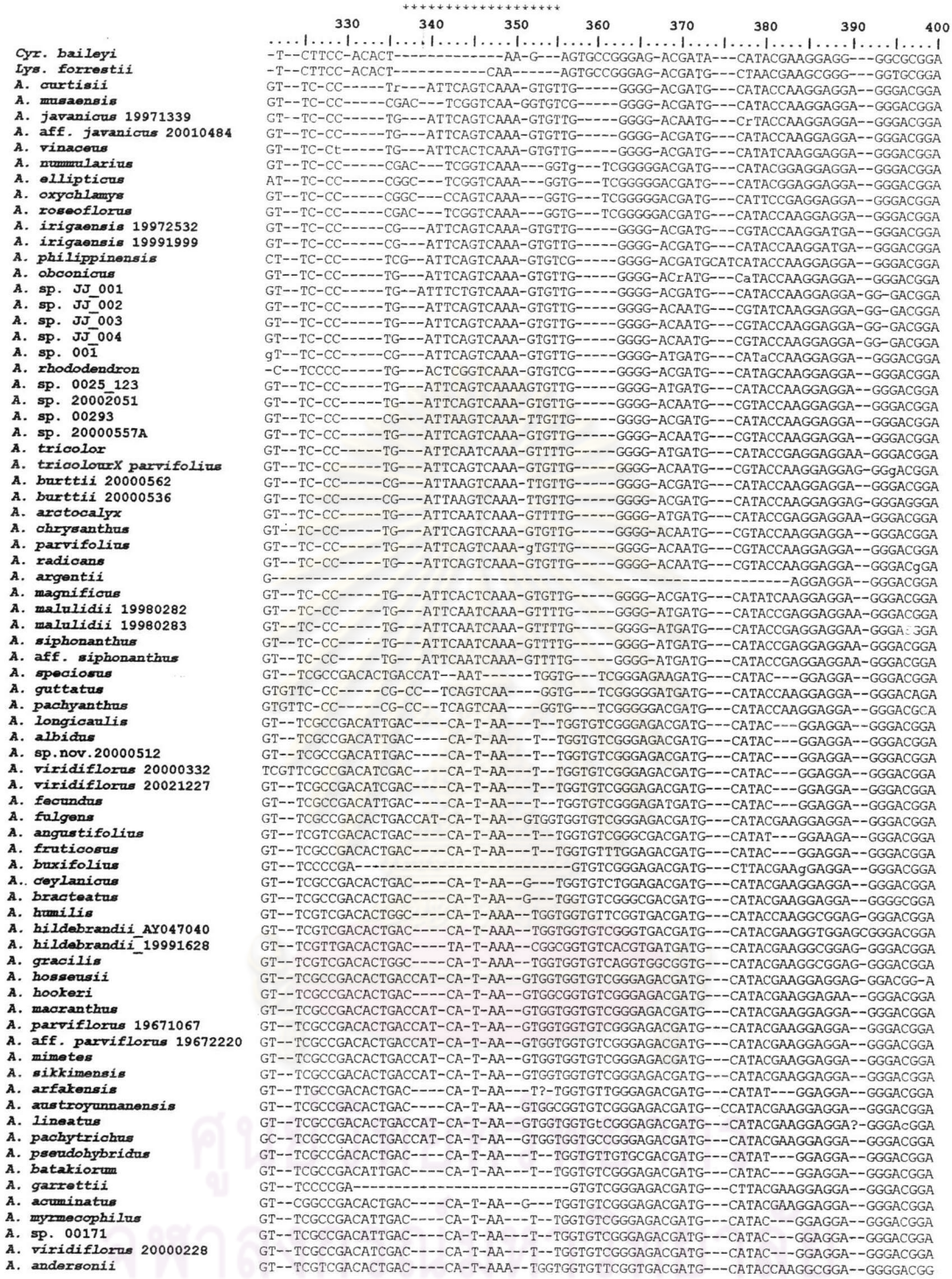

Fig. 26 (continued)

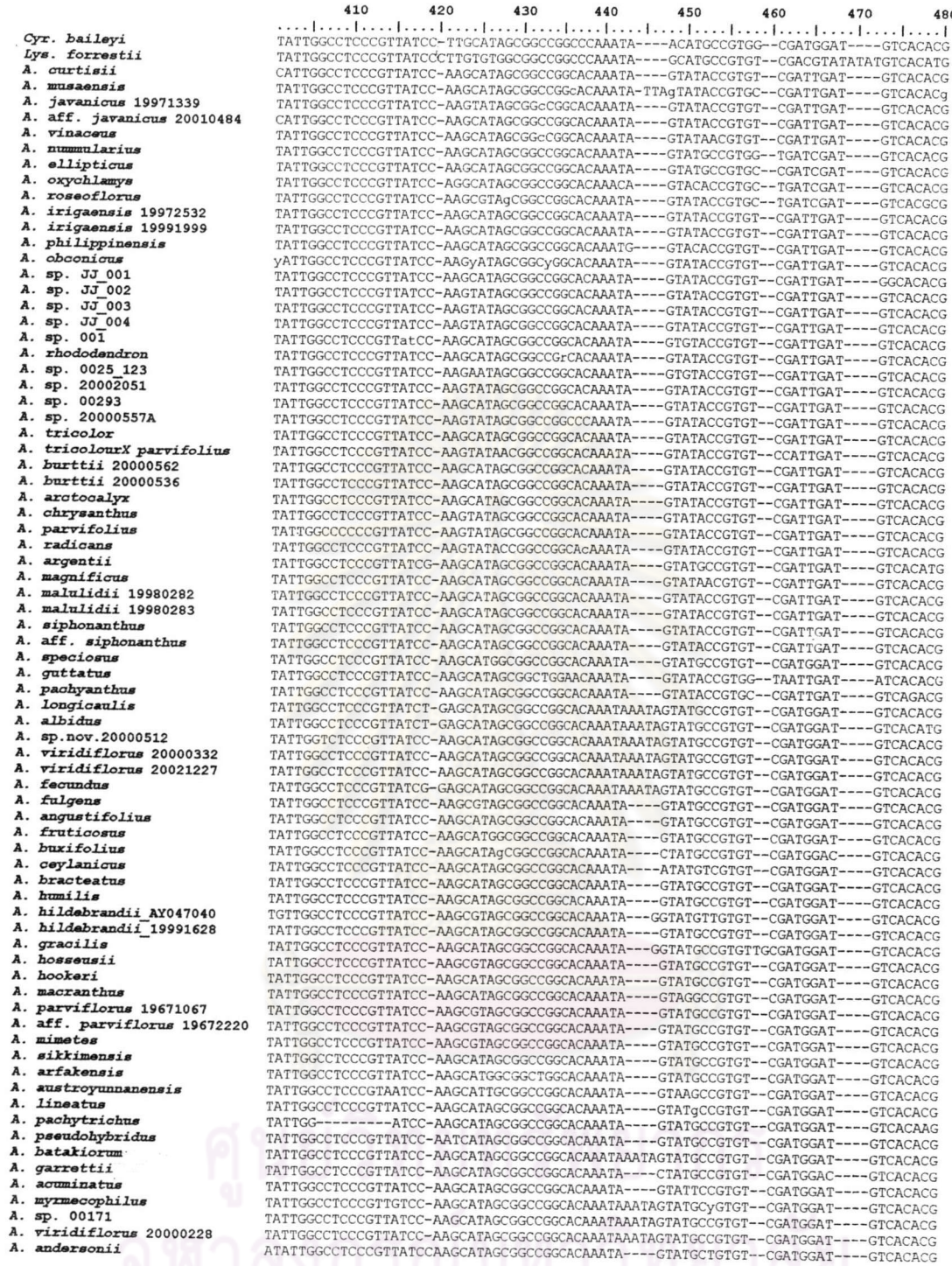

Fig. 26 (continued)

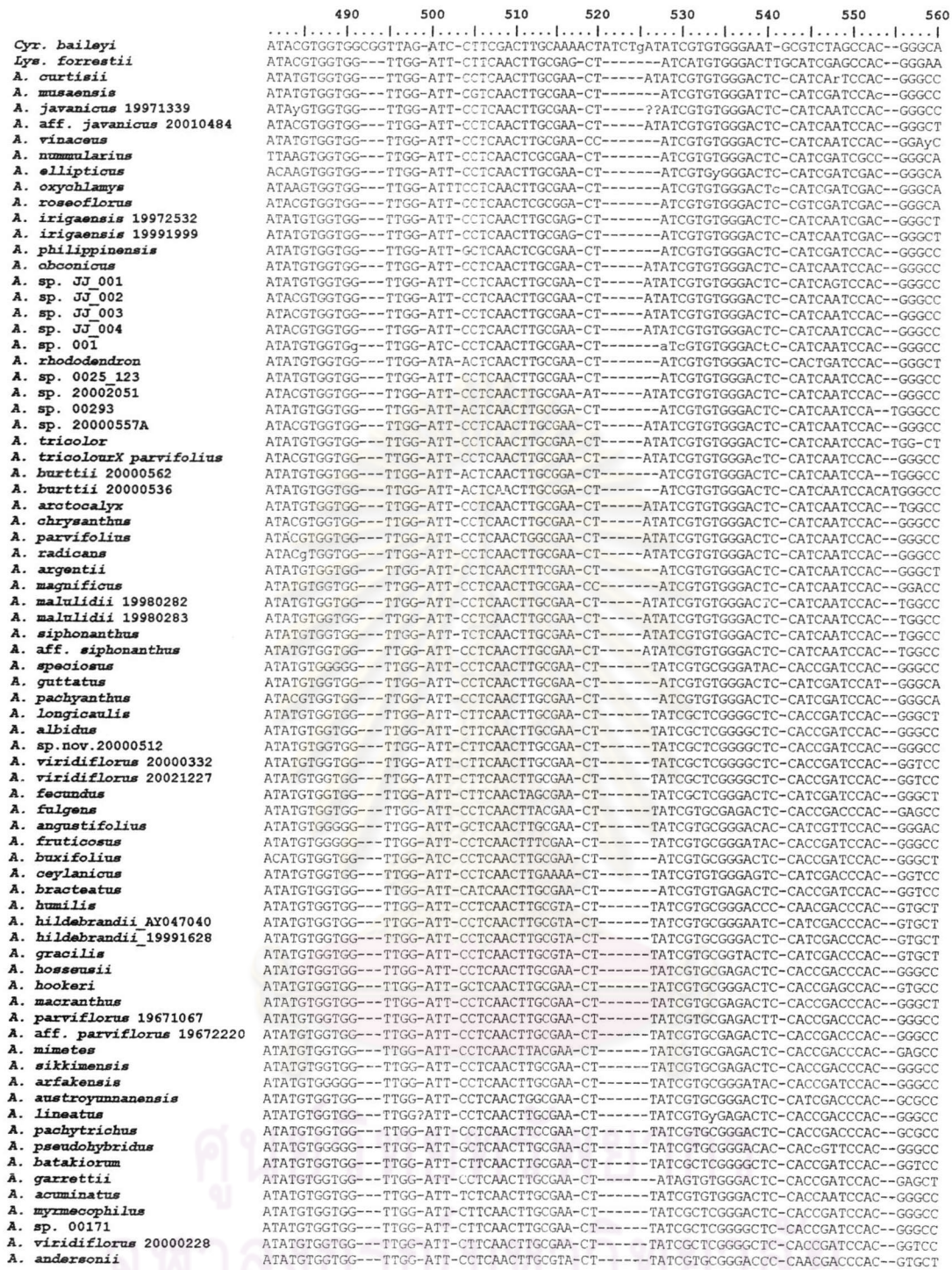

Fig. 26 (continued)

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                                570        580        590        600
                                ....|....|....|....|....|....|....|....|....
Cyr. baileyi                    CGACCCTG-T-----GGCAG----CAGAT-TGGTGCTGCCTTCCA
Lys. forrestii                  CGACCCAA-C-----GGCAC----GAGAT-TG------CCCTCGA
A. curtisii                     TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. musaensis                    CGACCCAA-T-CAATGGCGC----AAGAT-CG------CcCTCGA
A. javanicus 19971339           TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. aff. javanicus 20010484      TGACCCAA-T-----GGCAC----aagat-tg------CCCTCGA
A. vinaceus                     CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. nummularius                  CGACCCAAA------GGCAC----AAGAT-GG------CCCTCGA
A. ellipticus                   AGACCCAAA------GGCAC----AAGAT-CG------CCCTCGA
A. oxychlamys                   CGACCCAA-C-----GGCAC----AAGAT-GG------CCCTCGA
A. rosseoflorus                 CGACCCAA-C-----GGCAC----AAGAT-GG------CCCTCGA
A. irigaensis 19972532          CGACCCAA-T-----GGCAC----AAGAT-TG------CTCTCGA
A. irigaensis 19991999          CGACCCAA-T-----GGCAC----AAGAT-TG------CTCTCGA
A. philippinensis               CGACCCAA-T-----GGCAC----GAGAT-TG------CTCTCGG
A. obconicus                    TGACCCAA-T-----GGCAC----mAGAT-TG------CCCTCGA
A. sp. JJ_001                   TGACCCAA-C-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. JJ_002                   TGACCCAA-T-----GGCAC----AAGAT-tg------CCCTCGA
A. sp. JJ_003                   TGACCCAA-T-----GGCAC----AAGat-tg------CCCTCGA
A. sp. JJ_004                   TGACCCAA-T-----GGCAC----AAGat-tg------CCCTCGA
A. sp. 001                      TGACCCAA-T-----GgCAC----AAGAT-TG------CCCTCGa
A. rhododendron                 CGACCCAAAT-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 0025_123                 TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 20002051                 TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 00293                    CGACCCAA-T-----GGCAC----TagAt-tG------CCCTCGA
A. sp. 20000557A                TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. tricolor                     CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. tricolourX parvifolius       TGACCCAA-T-----GGCAC----AAGAT-TG------CCTTCGA
A. burttii 20000562             CGACCCAA-T-----GGCAC----TAGAT-TG------CCCTCGA
A. burttii 20000536             CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. arctocalyx                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. chrysanthus                  TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. parvifolius                  TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. radicans                     TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. argentii                     CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. magnificus                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. malulidii 19980282           CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. malulidii 19980283           CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. siphonanthus                 CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. aff. siphonanthus            CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. speciosus                    CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. guttatus                     CGACCCAA-T-----GGCAC----AAGAT-CG------CCCTCGA
A. pachyanthus                  CGACCCAA-C-----GGCAC----AAGAT-CG------CCCTCGA
A. longicaulis                  CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. albidus                      CGACCCAA-T-----GGCAC----AAGTT-TG------CCCTCGA
A. sp.nov.20000512              CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. viridiflorus 20000332        CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. viridiflorus 20021227        CGACCCAA-T-----GGCAC----aagat-tg------CCCTCGA
A. fecundus                     CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. fulgens                      CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. angustifolius                CAACCCAA-T-----GGCAC----AAGAT-TG---CCGCCCTCGA
A. fruticosus                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. buxifolius                   GGACCCAA-TT----GGCATAAATAAGATCTG------CCCtCGA
A. ceylanicus                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. bracteatus                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. humilis                      CGACCCAAG---------------AAGAT-TG------CCCACGA
A. hildebrandii_AY047040        CGACCCAA-C---------------AAGAT-TG------CCCTCGA
A. hildebrandii_19991628        CGACCCAA-C---------------AAGAT-TG------CCCTCGA
A. gracilis                     GGACCCAG-C---------------AAGTT-CG------CCCTCGA
A. hosseusii                    CGACCCAA-T-----GGCAC----AAgat-tg------CCCTCGA
A. hookeri                      CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. macranthus                   TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. parviflorus 19671067         CGACCCAA-T-----GGCAC----AAGat-tg------CCCTCGA
A. aff. parviflorus 19672220    CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. mimetes                      CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sikkimensis                  CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. arfakensis                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. austroyunnanensis            CGACCCAA-T-----GGCAC----GAGAG-TG------CCCTCGA
A. lineatus                     CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. pachytrichus                 TGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. pseudohybridus               CAACCCAA-T-----GGCAC----AAGAT-TG---CCGCCCTCGA
A. batakiorum                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. garrettii                    GGACCCAA-C-----GGCATAA-TAA-AT-TG------CCCTCAA
A. acuminatus                   CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. myrmecophilus                CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. sp. 00171                    CGACCCAA-T-----GGCAC----AagAt-tG------CCCTCGA
A. viridiflorus 20000228        CGACCCAA-T-----GGCAC----AAGAT-TG------CCCTCGA
A. andersonii                   CGACCCAA-C---------------AAGAT-TG------CCCACGA
```

Fig. 26 (continued)

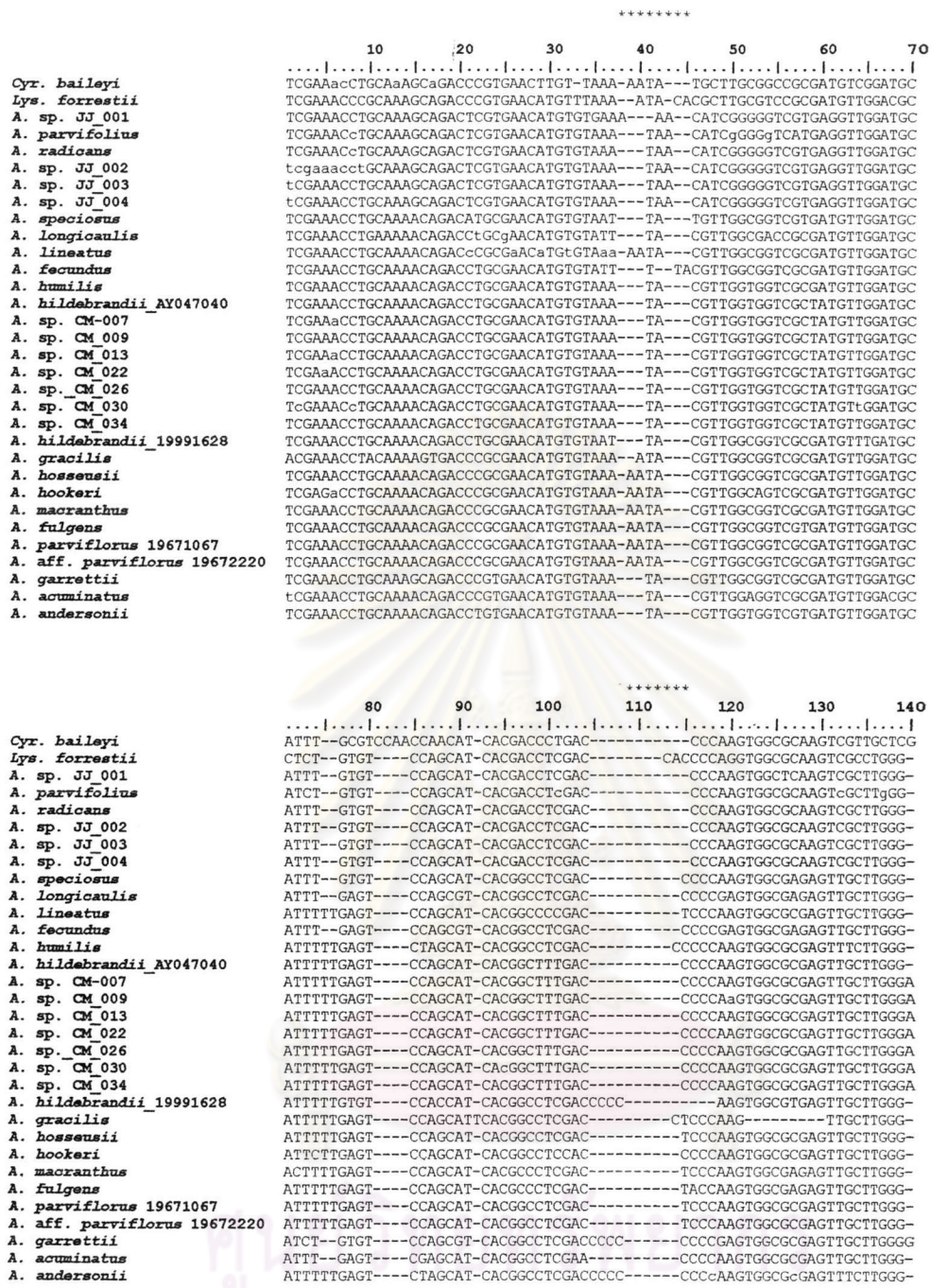

Fig. 27 A 531 bp character-taxon matrix of 30 *Aeschynanthus* taxa based on ITS sequences. Gap symbol (-) indicates insertion or deletion at the site. ? symbol shows missing nucleotide data. Asterisks * represent excluded sites from analyses.

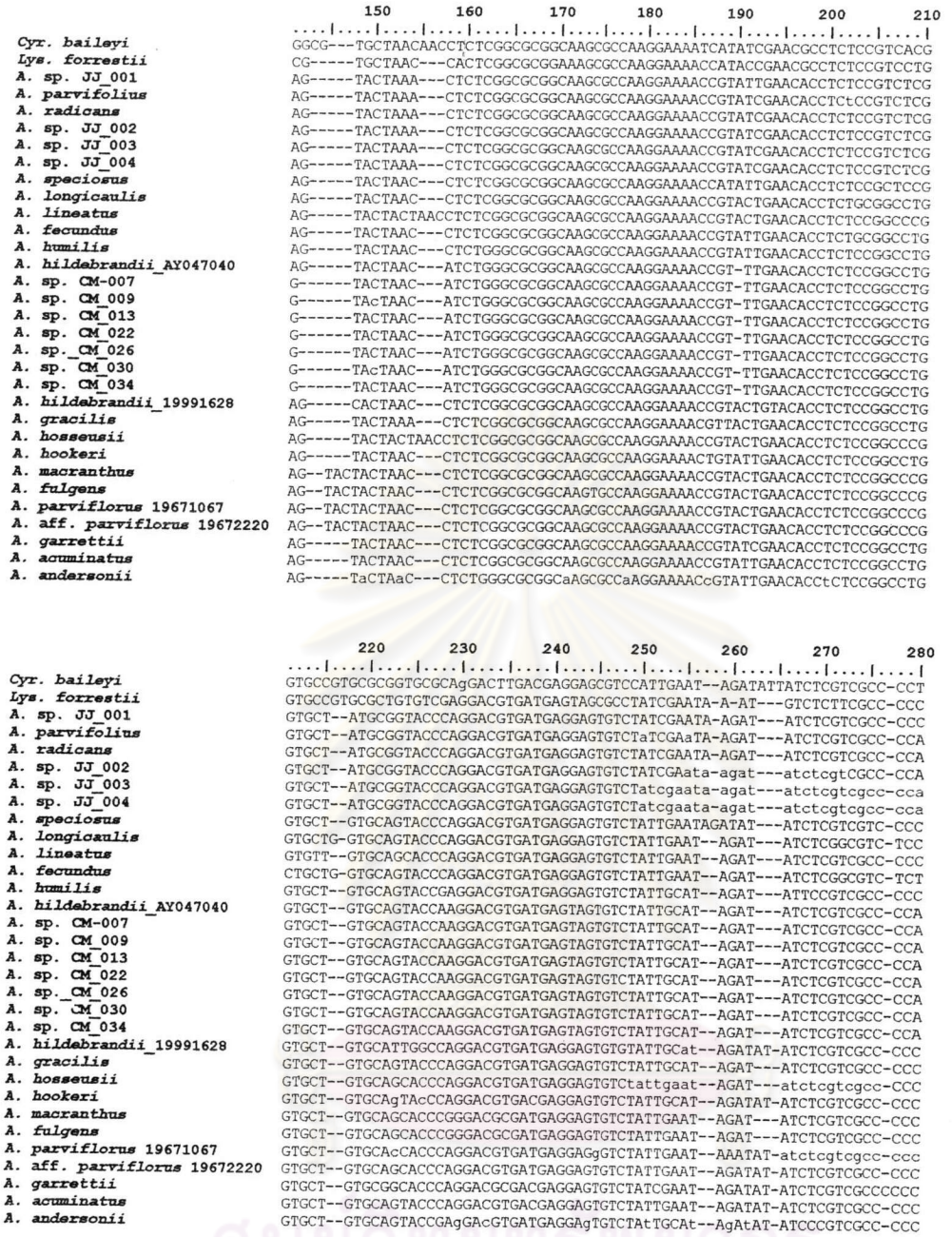

Fig. 27 (continued)

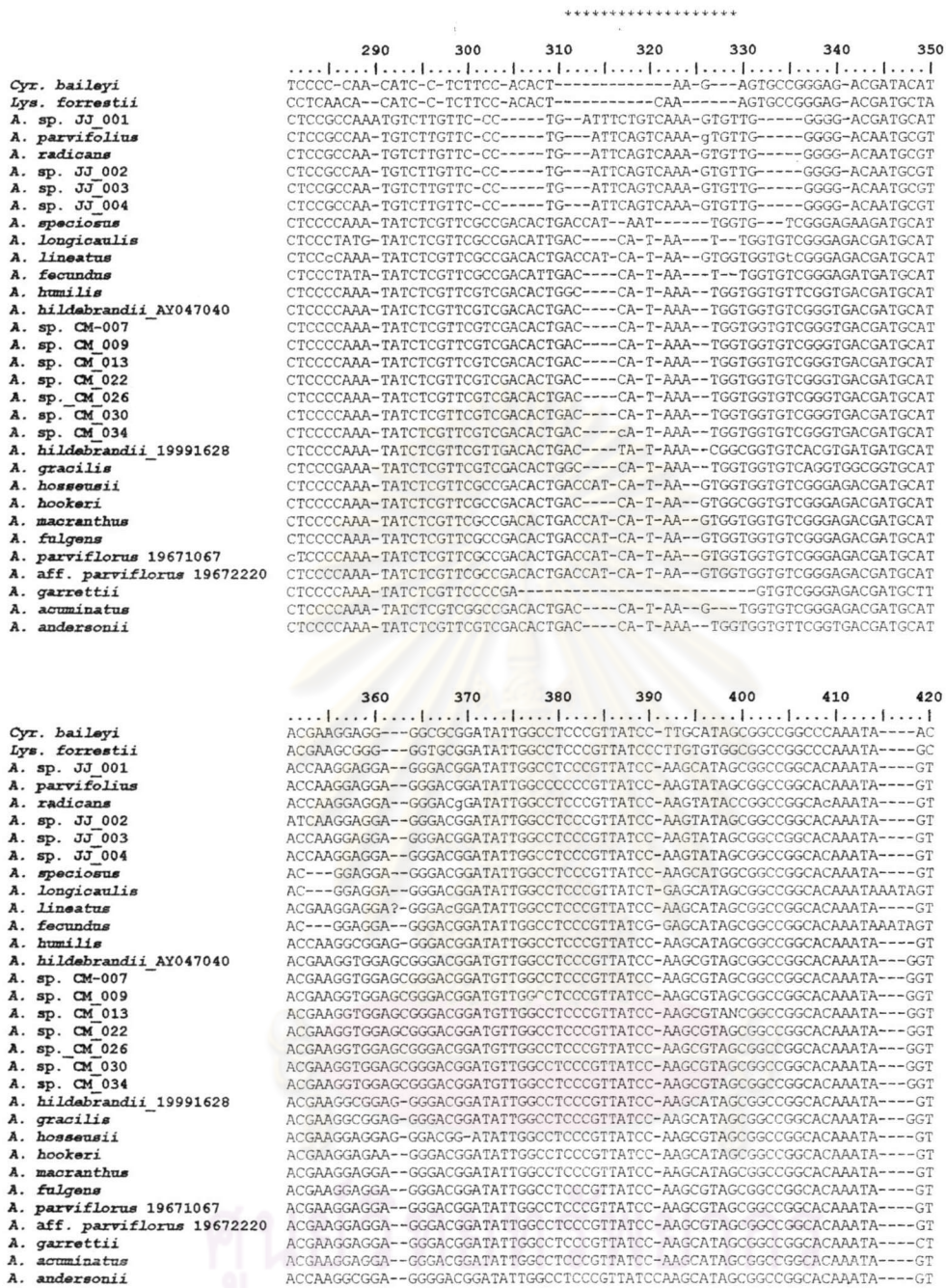

Fig. 27 (continued)

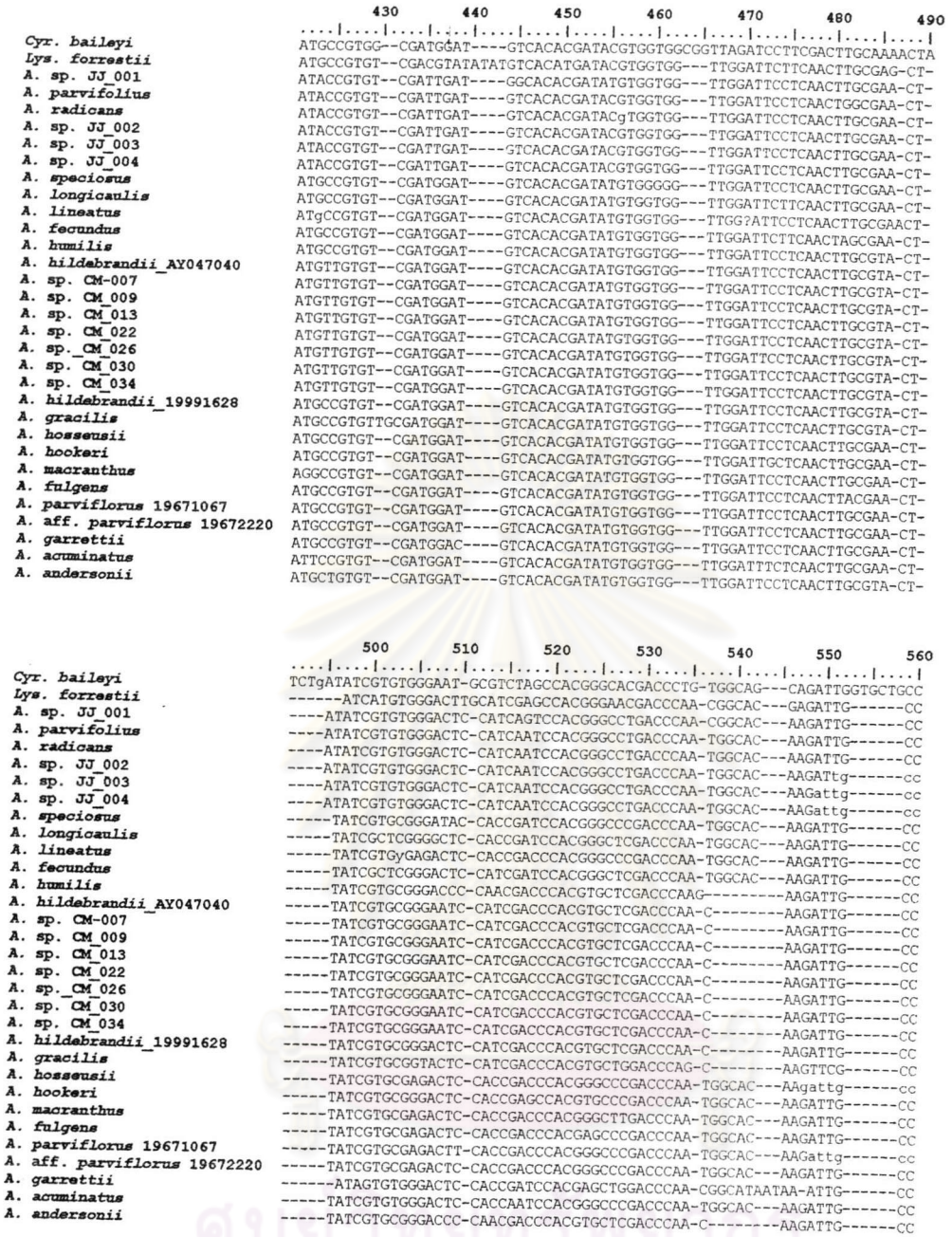

Fig. 27 (continued)

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                                ....|
Cyr. baileyi                    TTCCA
Lys. forrestii                  CTCGA
A. sp. JJ_001                   CTCGA
A. parvifolius                  CTCGA
A. radicans                     CTCGA
A. sp. JJ_002                   CTCGA
A. sp. JJ_003                   CTCGA
A. sp. JJ_004                   CTCGA
A. speciosus                    CTCGA
A. longicaulis                  CTCGA
A. lineatus                     CTCGA
A. fecundus                     CTCGA
A. humilis                      CACGA
A. hildebrandii_AY047040        CTCGA
A. sp. CM-007                   CTCGA
A. sp. CM_009                   CTCGA
A. sp. CM_013                   CTCGA
A. sp. CM_022                   CTCGA
A. sp._CM_026                   CTCGA
A. sp. CM_030                   CTCGA
A. sp. CM_034                   CTCGA
A. hildebrandii_19991628        CTCGA
A. gracilis                     CTCGA
A. hosseusii                    CTCGA
A. hookeri                      CTCGA
A. macranthus                   CTCGA
A. fulgens                      CTCGA
A. parviflorus 19671067         CTCGA
A. aff. parviflorus 19672220    CTCGA
A. garrettii                    CTCAA
A. acuminatus                   CTCGA
A. andersonii                   CACGA
```

Fig. 27 (continued)

4.6 Phylogenetic analyses

4.6.1 Analyses of the ITS data matrix of *Aeschynanthus* samples supplied from RBGE

The ITS sequences of all *Aeschynanthus* specimens supplied from RBGE and those of all previously studied *Aeschynanthus* were first automatically aligned by ClustalX program and then 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.

Phylogenetic analyses by maximum parsimony approach using heuristic searching strategy were performed to analyse this data matrix of 571 bp. *Cytandra baleyi* (GenBank acession number AF349151 and AF34923) and *Lysionotus forrestii* (AF349152 and AF 349233) were included into the analysis as outgroup taxa. Two hundreads and nine characters were parsimony-informative as synapomorphy (shared-derived character) and 97 characters were parsimony-uninformative as autapomorphy specifically for each taxon. There were more than 202,700 most parsimonious trees (MPTs) (trees exceed a computer memory used by PAUP*) found from this data set, with 736 steps in length (Fig. 29) without a gap matrix included (0,1 symbol). Consistency index (CI) was 0.600 and homoplasy index (HI) was 0.400. Retention index (RI) was 0.832 and rescaled consistency index (RC) was 0.500. When a 94-positions gap matrix was included to the analysis to increase informative characters, more than 202,700 maximally parsimonious trees (trees exceed a computer memory used by

PAUP*) were obtained with a tree length of 969 steps and higher tree-resolution. Semistrict (Fig. 30) and 50% majority-rule (Fig. 31) consensus trees were made from all of 202,700 MPTs. Both consensus trees were primarily similar to each othe in that all *Aeschynanthus* species were devided into two major clades: Clade I and Clade II. Bootstrap (BS) and jackknife (JK) supporting-values were calculated to confirm a reliability of each branch (Fig. 30). For distance method analysis, a neighbour-joining (NJ) tree with statistic supporting-values (Fig. 32) was drawn to compare with results from maximum parsimony analyses.

Regarding to a topology of the semistrict consensus tree (Fig. 30), almost all of *Aeschynanthus* species which had been studied on different RBGE accession number (for example, *A. fulgens* 20002032 VS *A. fulgens* 19900384) were paired or clustered together as sister sequences. The exceptional taxa were *A. javanicus* 19971339 VS *A.* aff. *javanicus* 20010484 and also *A. hildebrandii* 19991628 of RBGE VS *A. hildebrandii* AY047040 from GenBank. All phylogenetic trees produced by all methods used in this study confirmed the division of *Aeschynanthus* into two major clades. Both major clades had high bootstrap and jackknife supporting-values: 89% BS and 88% JK for clade I, and 78% BS and 78% JK for clade II (Fig. 30). In the major clade I, *Aeschynanthus parviflorus* 19671067 paired to *A.* aff. *parviflorus* 19672220 with 85% BS and 73% JK high supporting-values. *Aeschynanthus linaetus* 19991622 which was newly added to the tree was paired to *A. lineatus* 19970613 of the previous work with high supporting-values (98% BS and 93% JK) before weakly joining with *A. pachytrichus*.

Aeschynanthus hookeri was sistered to *A. austroyunnanensis* with low supporting-values. This couple was then joined to the group of *A. humilis*, *A. andersonii*, *A. hildebrandii* AY047040, *A. gracilis* 19802570 and 198219970, *A. hildebrandii* 19991628 with less than 50% supporting-values. The position of *A.* sp. nov. 20000512 on the phylogenetic tree was unresolved because it formed a polytomic node with other species such as *A. viridiforus* 20000332 and 20021277, the polytomic grouping of *A. batakiorum*, *A. myrmecophilus*, *A.* sp. 0017, and *A. viridiflorus* 20000228, and the subclade of *A. longicaulis* 19672218 and 20001430 and *A. albidus*. Interestingly, the two newly-added *A. viridiflorus* 20000332 and 20021227 were not clustered with *A. viridiflorus* 20000228 from previous study. In addition, the three synonymously problematic species, *Aeschynanthus andersonii*, *A. humilis* and *A. hildebrandii* (GenBank accession number AY047040) were clustered together in this major clade I. *Aeschynanthus humilis* was firstly paired with *A. andersonii* with very high supporting-values (98% BS and 99% JK) before joined to *A. hildebrandii* with 69% BS and 64% JK. However, *A. hildebrandii* 19991628 which were given by RBGE was not grouped with these three taxa in the semistrict consensus tree but equally close to *A. gracilis* 19802570 and *A. gracilis* 19821970.

In the major clade II, *Aeschynanthus burttii* 20000562 was paired with *A.* sp. 00293 with 59% BS and 57% JK low supporting-values before joined to *A. burttii* 20000536 with very high BS and JK supporting-values (99% and 98%, respectively). Likewise, the additional *A. irigaensis* 19991999 was sistered to *A. irigaensis* 19972532

with hundred-percentages BS and JK supporting values. The molecular phylogenetic tree also showed several subgroupings of taxa in this clade. One interesting subgroup IIA (88% BS and 81% JK) is the cluster of all taxa in the section *Aeschynanthus* and some members of section *Microtricium*. This subgroup could be divided into two minor groups. *Aeschynanthus pavifolius* 19881451 and 19671069, *A. radicans* 19622826 and 19672224, *A. javanicus* 19971339, *A.* sp. 20002051, *A.* sp. 20000557A, *A. tricolor* x *parvifolius*, *A. chrysanthus*, *A. curtisii*, *A. obconicus* and *A.* aff *javanicus* 20010484 were first clustered together as a distinctively strong group with 75% and 68% bootstrap and jackknife supporting-values. Noted that the supposingly *Aeschynanthus* hybrid, *A. tricolor* X *parvifolius*, was placed in this minor group close to *A. parvifolius*, its paternal side. The other minor group in the subgroup IIA of the clade II included with *A. malulidii* 19980282 and 19980283, *A.* aff. *siphonanthus*, *A. siphonanthus*, *A. arctocalyx*, and *A. tricolor* with very high bootstrap and jackknife supporting-values (95% and 97%, respectively). Apparently, lack of knowledge of sectional classification of many taxa in the major clade II gave dificulty in recoginition of mono or non-mono phylogenetic groupings in this clade.

Considering specifically on Thai *Aeschynanthus*, the positions of most of them on the ITS phylogenetic tree were in the major clade I. These clade I members were *A. flugens* (both 20002032 and 19900384), *A. macranthus*, *A. parviflorus* 19671067 and *A.* aff. *parviflorus* 19672220, *A. lineatus* (both 19970613 and 19991622), *A. hosseusii*, *A. humilis*, *A. andersonii*, *A. hildebrandii* 19991628, *A. acuminatus* (both 19981469 and

19981444), *A. longicaulis* (19872218 and 20001430), *A. fecundus*, *A. speciosus* and *A. garrettii*. The two other Thai *Aeschynanthus*, *A. parvifolius* (both 19881451 and 19671069) and *A. radicans* (19622826 and 19672224) were placed in the clade II and their positioning supported the previous study of Denduangboripant *et al.* (2001). Since a majority of *Aeschynanthus* species in the clade I occur in mainland, Indochina (including Thailand) while those of the clade II mostly occur in both oceanic areas and Malay Peninsular, the phylogenetic positions of these 16 *Aeschynanthus* reported in Thailand reasonably follow their biogeographical trend.

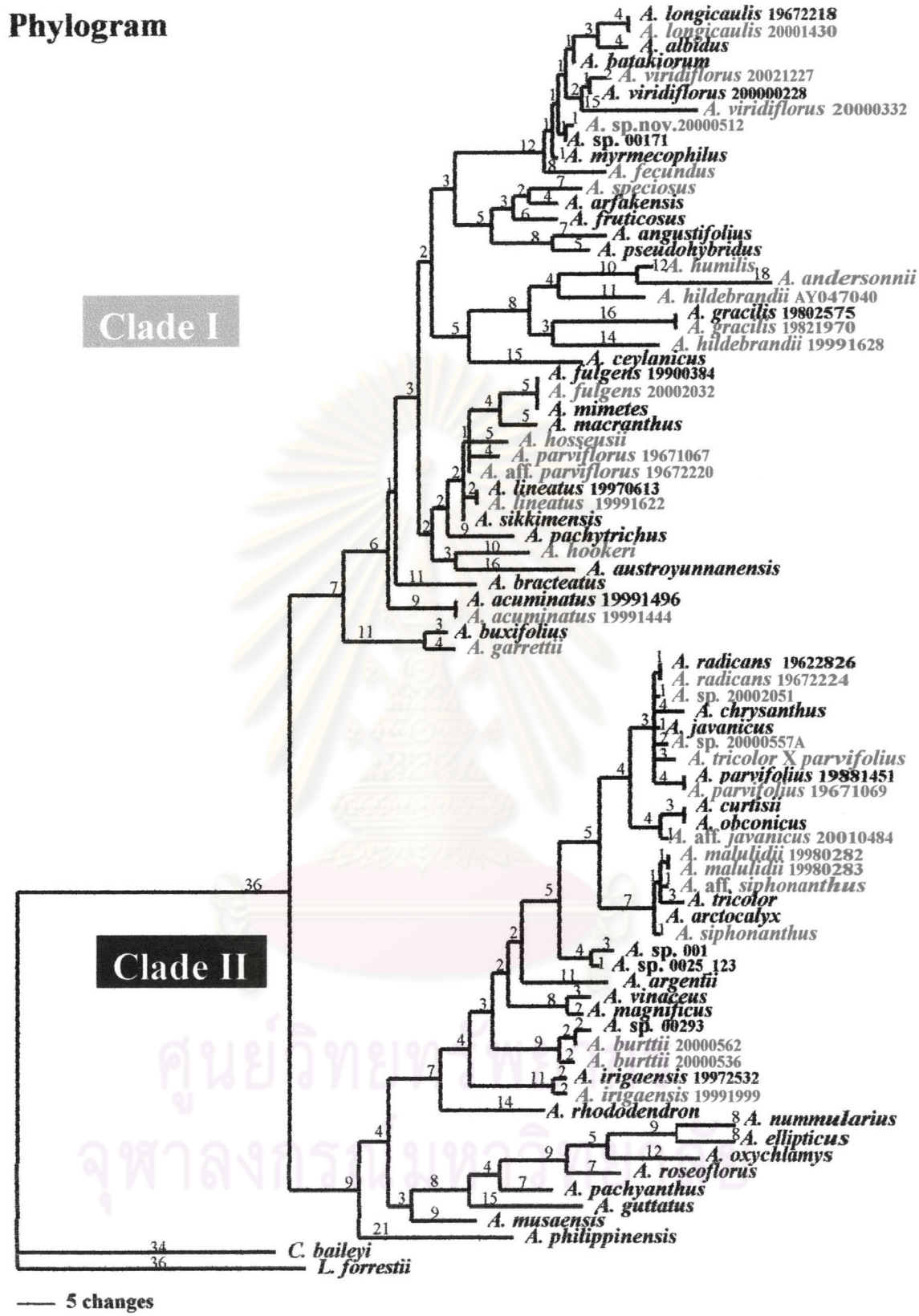

Fig. 28 One phylogram of 202,700 equally most parsimonious trees (736 steps in length) for 80 *Aeschynanthus* taxa and two outgroup Gesneriaceae taxa (*Cytandra baileyi* and *Lysionotus forrestii*) based on parsimony analysis of the combined ITS1 and ITS2 sequence data without gap matrix [CI =0.601, RI =0.832, RC =0.500]. Taxon names in colour letters are *Aeschynanthus* samples used in this study. The number along branches indicates the amount of character change (branch length).

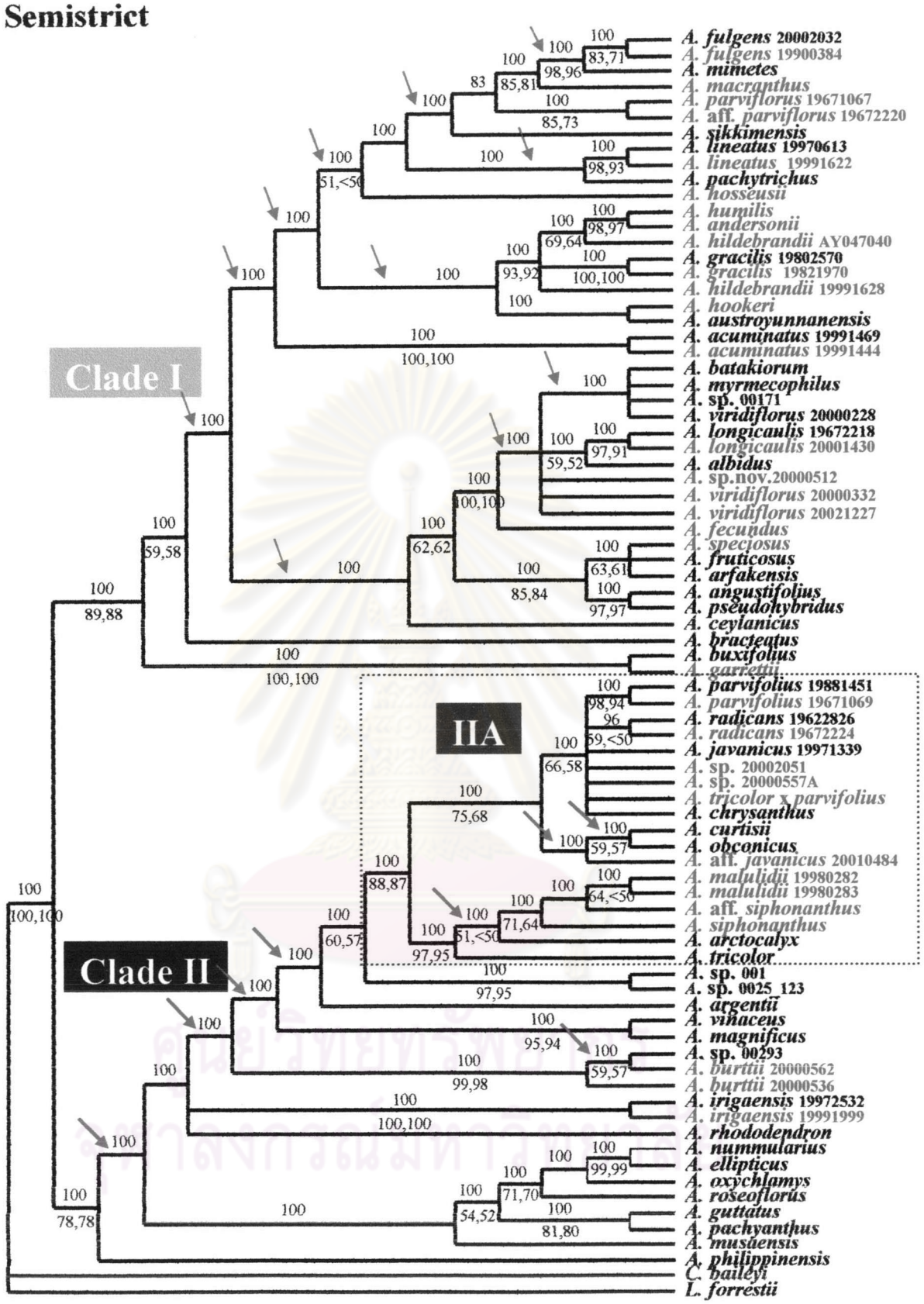

Fig. 29 A semistrict consensus tree of 202,700 most parsimonious trees (969 steps in length) for 80 *Aeschynanthus* taxa and two outgroup Gesneriaceae taxa (*Cytandra baileyi* and *Lysionotus forrestii*) based on parsimony analysis of the combined ITS1 and ITS2 sequence data without gap matrix. The upper numbers are percentages of clade-congruity between MPTs. The lower numbers are "fast" stepwise-addition Bootstrap and Jacknife supporting-values of 10,000 replicates. The arrows indicate branches that collapse when gap matrix is excluded. Taxon names in colour letters are *Aeschynanthus* samples used in this study.

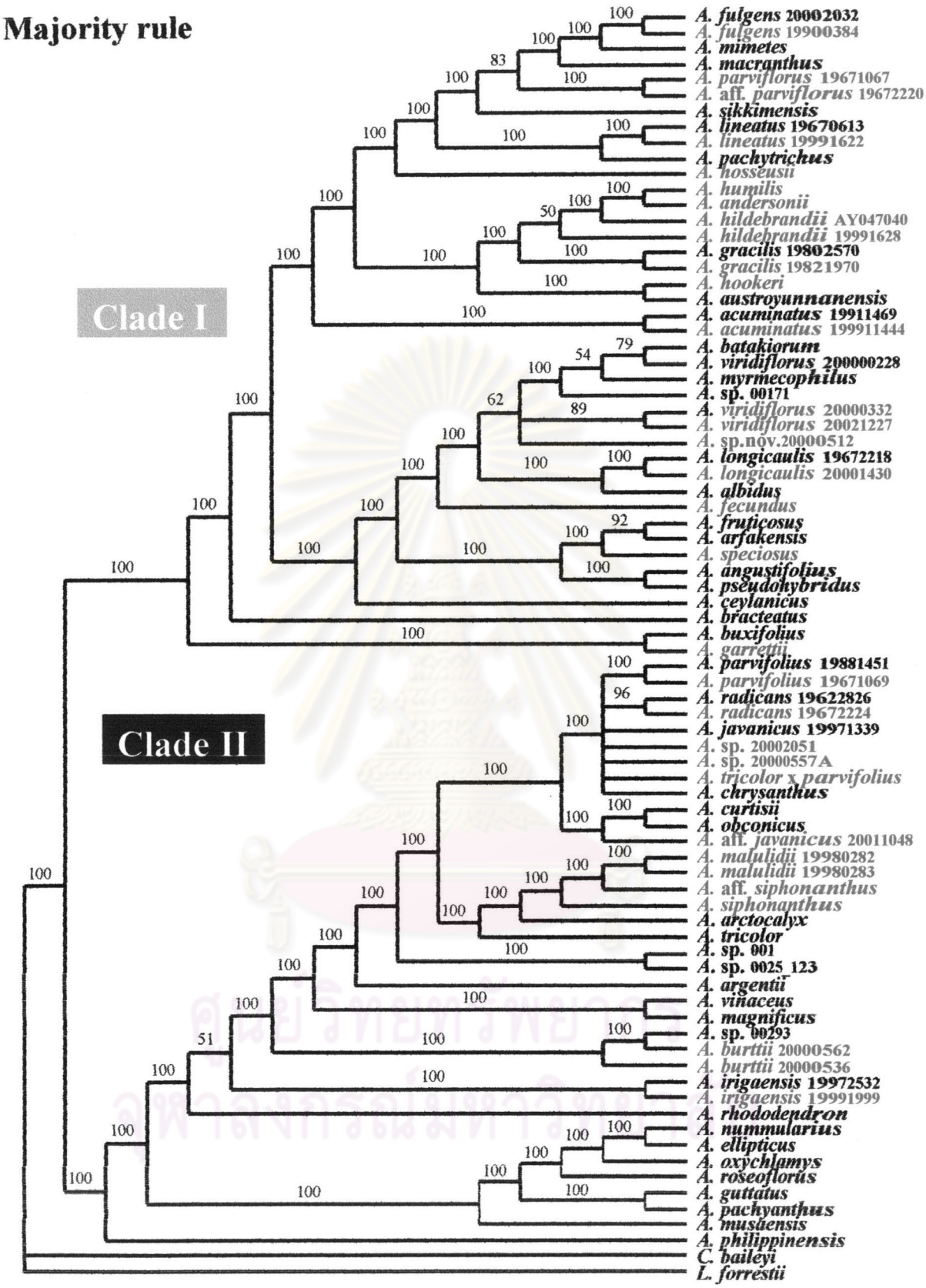

Fig. 30 A 50% majority-rule consensus tree of 202,700 most parsimonious trees of 80 *Aeschynanthus* based on ITS sequence data with gap matrix and using *C. Baileyi* and *L. forrestii* as outgroups. The upper numbers are percentages of identical clade between MPTs on the consensus tree. Taxon names in colour letters are *Aeschynanthus* samples used in this study.

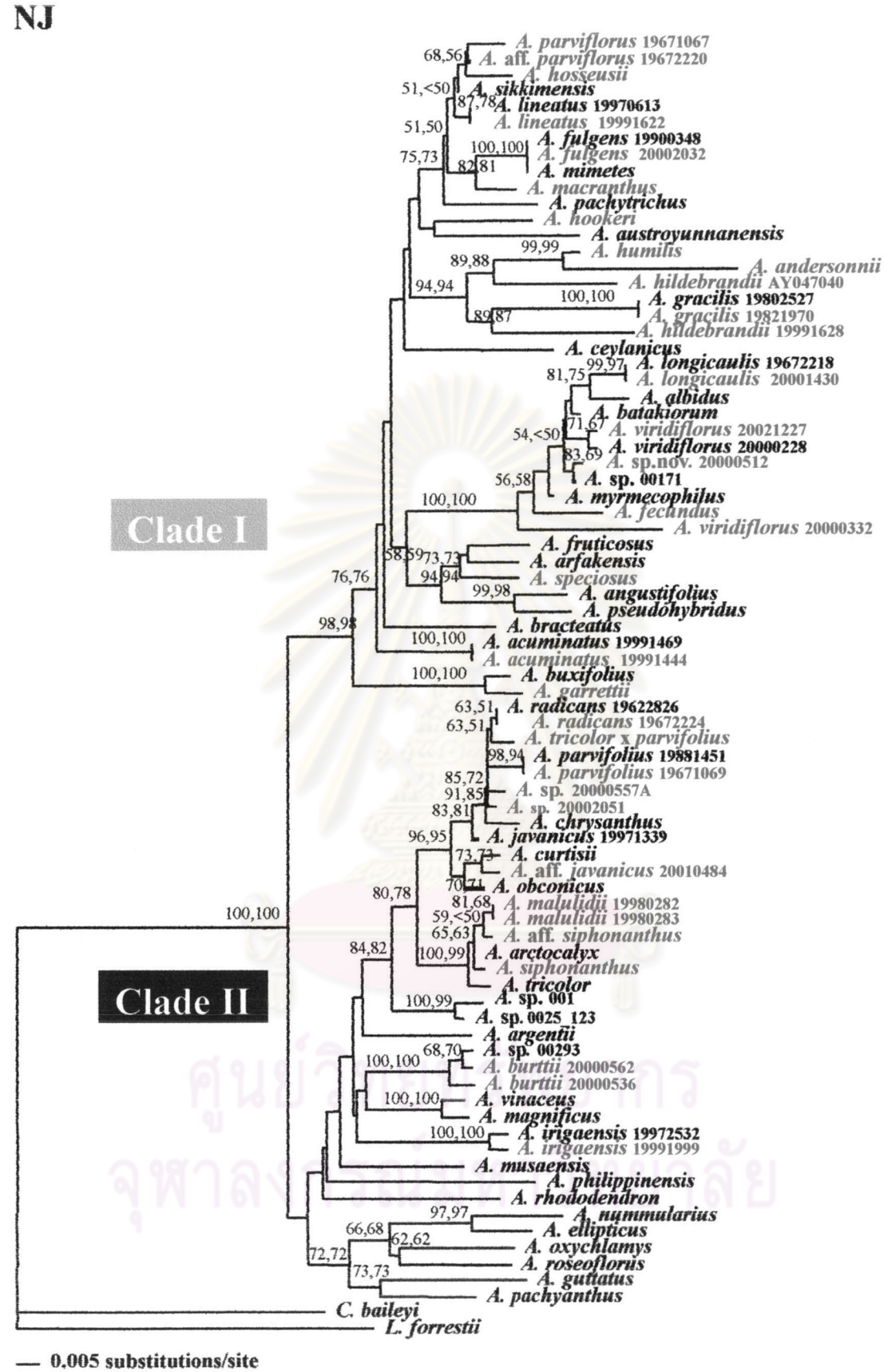

Fig. 31 A neighbour-joining tree of 80 *Aeschynanthus* taxa based on ITS sequence data without gap matrix and using *C. baileyi* and *L. forrestii* as outgroups. The numbers along branches are 10,000-replicate bootstrap and jackknife supporting-values, respectively. Note that supporting-values less than 50% were not shown in the tree. Taxon names in colour letters are *Aeschynanthus* samples used in this study.

4.6.2 Analyses of the ITS data matrix of *Aeschynanthus* samples bought from Jatujak flee market

ITS regions of six additional *Aeschynanthus* specimens bought from Jatujak market were sequenced and Blast-searched with the GenBank nucleotide database. *Aeschynanthus* sp. JJ_001, *A.* sp. JJ_002, *A.* sp. JJ_003, and *A.* sp. JJ_004 except *A.* sp. JJ_006 were found having ITS sequences similar to those of members of the genus *Aeschynanthus*. Even *A.* sp. JJ_005 which showed ITS sequence–length polymorphism problem also gave a high similarity to *Aeschynanthus* ITS sequences in GenBank. *Aeschynanthus* sp. JJ_002 showed ITS sequence similar to those of *A. radicans* clone A (AF34178, AF349259) and gave 100% identities. *Aeschynanthus* sp. JJ_003 and *A.* sp. JJ_004 gave the same ITS sequences and also had high similarity to *A. radicans* clone A with 99% identities. However, *Aeschynanthus* sp. JJ_001 gave a unique sequence which is different from any other *Aeschynanthus* reported in GenBank. *Aeschynanthus* sp. JJ_006 did not give ITS sequences similar to *Aeschynanthus* and its sequences were found closing to *Columnia linaris* with 96 % identities.

Maximum parsimony analyses using heuristic searching strategy were performed to study a molecular phylogenetic relationship between these four Jatujak *Aeschynanthus* and other sequenced taxa. One hundred and eighty-nine characters of the 571 bp ITS sequence data matrix were parsimony-informative and 112 characters were phylogenetically uninformative as autapomorphy specifically for each taxon. There were 203,700 most parsimonious trees (MPTs) found from this data set without using

gapmatrix, with 743 steps in length (for example, Fig. 33). Consistency index (CI) was 0.602 and Homoplasy index (HI) was 0.395. Retention index (RI) was 0.822 and rescaled consistency index (RC) was 0.494. A semistrict consensus tree (Fig. 34) was made from all of 203,700 most parsimonious trees after analysed the data matrix without gap matrix. Moreover, a neighbour joining (NJ) tree with bootstrap and jackknife (JK) supporting-values (Fig. 35) was also made. Both *Aeschynanthus* molecular phylogenies either from maximum parsimony or distance method showed that the cultivars *A.* sp. JJ_002, *A.* sp. JJ_003, and *A.* sp. JJ_004 were grouped with *A. chrysanthus*, *A. parvifolius*, and *A. radicans* in the section *Aeschynanthus*. For the unique pinkish *Aeschynanthus*, *A.* sp. JJ_001, this plant was placed separately from other Jatujak *Aeschynanthus*, located distinguishly as a unique branch among other members of the section *Aeschynanthus*.

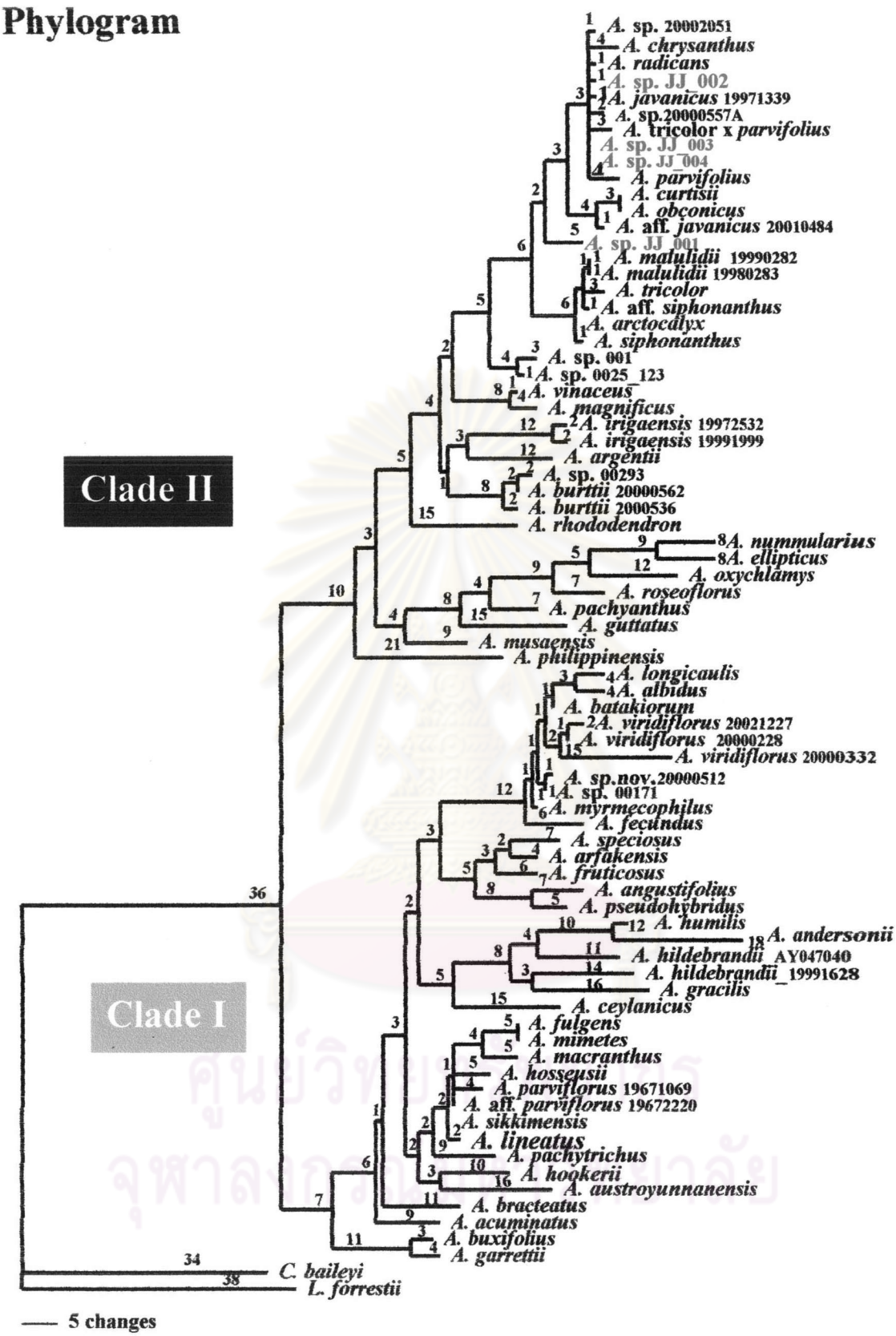

Fig. 32 One phylogram of 203,700 equally most parsimonious trees (743 steps in length) for 77 *Aeschynanthus* taxa and two outgroup Gesneriaceae taxa (*Cytandra baileyi* and *Lysionotus forrestii*) based on parsimony analysis of the combined ITS1 and ITS2 sequence data without gap matrix [CI =0.602, RI =0.821, RC =0.494]. Taxon names in colour letters are *Aeschynanthus* samples bought from Jatujak market. The number along branches indicates the amount of character changes (branch length).

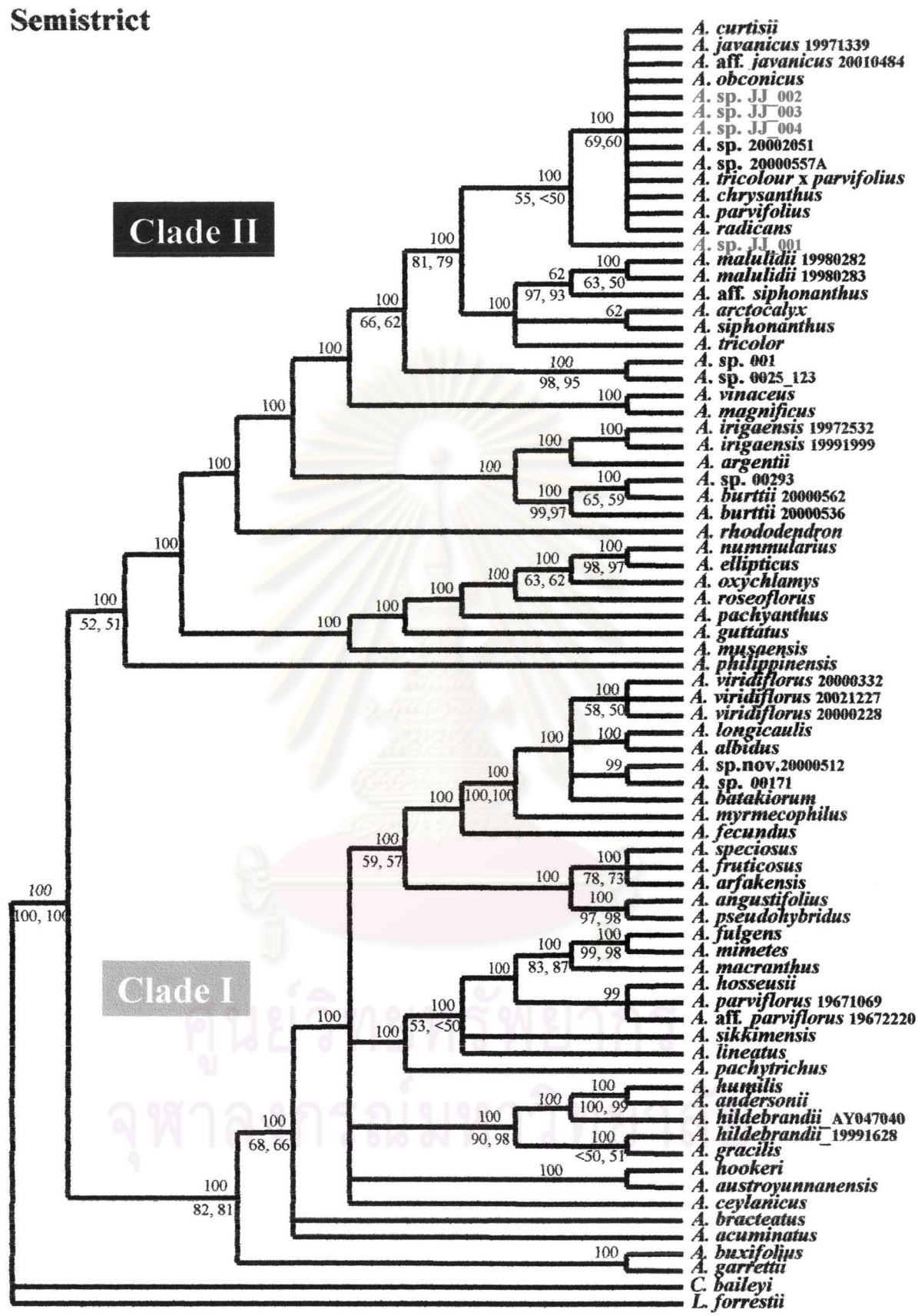

Fig. 33 A semistrict consensus tree of 203,700 most parsimonious trees (743 steps in length) for 77 *Aeschynanthus* taxa and two outgroup Gesneriaceae taxa (*Cytandra baileyi* and *Lysionotus forrestii*) based on parsimony analysis of the combined ITS1 and ITS2 sequence data without gap matrix. The upper numbers are percentages of clade-congruity between MPTs. The lower numbers are "fast" stepwise-addition Bootstrap and Jacknife supporting-values of 10,000 replicates. Taxon names in colour letters are *Aeschynanthus* samples bought from Jatujak market.

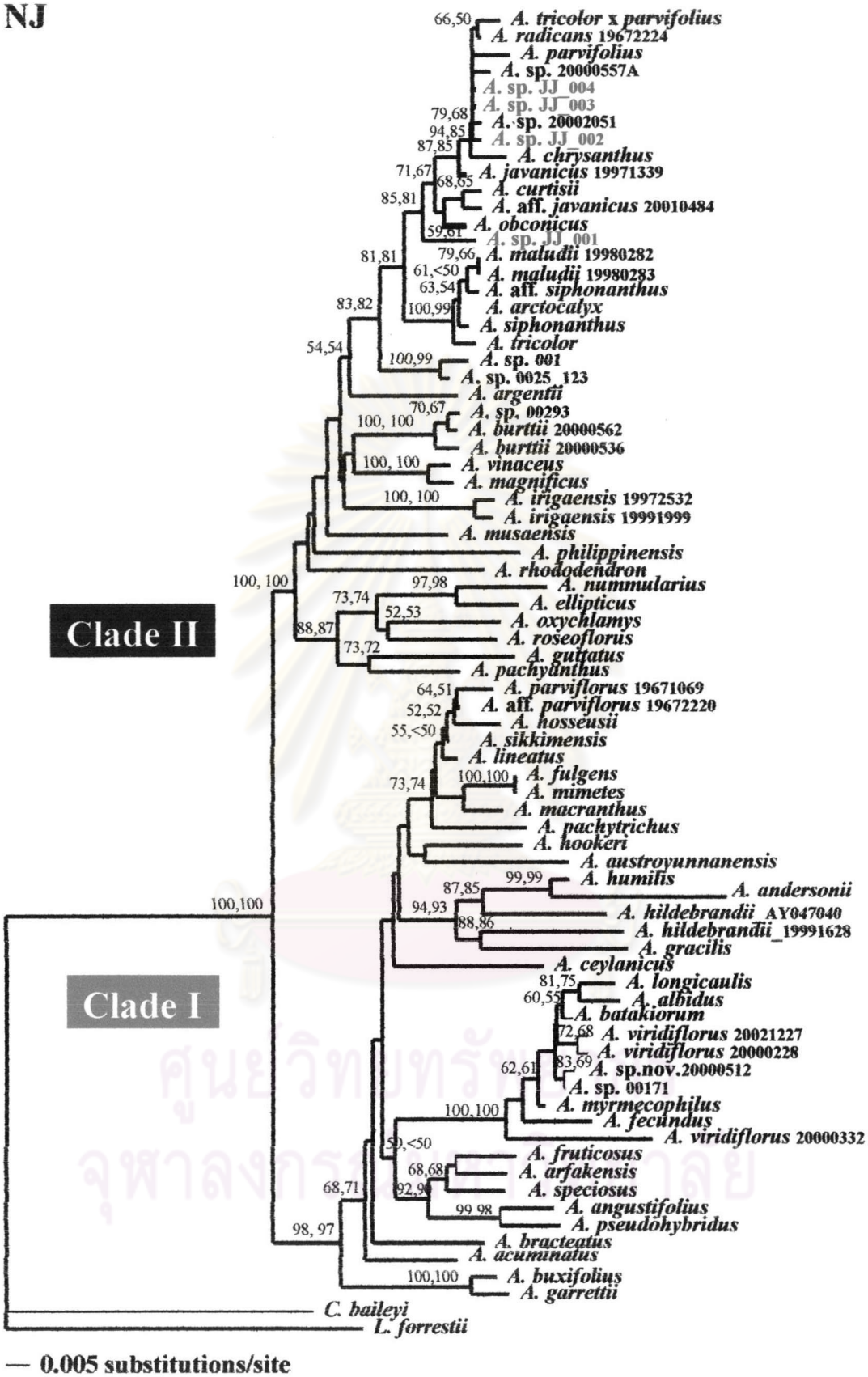

Fig. 34 A neighbour-joining tree of 77 *Aeschynanthus* taxa based on ITS sequence data without gap matrix and using *C. baileyi* and *L. forrestii* as outgroups. The numbers along branches are 10,000-replicate bootstrap and jackknife supporting-values, respectively. Note that supporting-values less than 50% were not shown in the tree. Taxon names in colour letters are *Aeschynanthus* samples bought from Jatujak market.

4.6.3 Analyses of the ITS data matrix of *Aeschynanthus* collected in Chiangmai

The ITS sequences of seven of *Aeschynanthus* samples collected in San-ku and Doi Pui, two nearby areas in Chiangmai province, were Blast-searched with Genbank nucleotide database. All *Aeschynanthus* sp. CM_007, *A.* sp. CM_009, *A.* sp. CM_013, *A.* sp. CM_022, *A.* sp. CM_030, *A.* sp. CM_034 had the ITS sequences which were similar to members of the genus *Aeschynanthus*. In fact, all of these ITS sequences were identical to that of *A. hildebrandii* (Genbank accession number AY047040) with 100% identities. Phylogenetic analysis using distance method to make a neighbour-joining (NJ) tree (Fig. 36) supported the Blast-search result. After phylogenetic analysed with other 21 Thai *Aeschynanthus* taxa, all seven *Aeschynanthus* collected from Chiangmai were clustered together with high bootstrap and jackknife supporting-values (94% and 86%, respectively). Moreover, these seven Chiangmai *Aeschynanthus* were also grouped with *A. hildebrandii* AY047040 with 100% bootstrap and jackife supporting values, but separated distinctively from *A. hildebrandii* 19991628 given from RBGE.

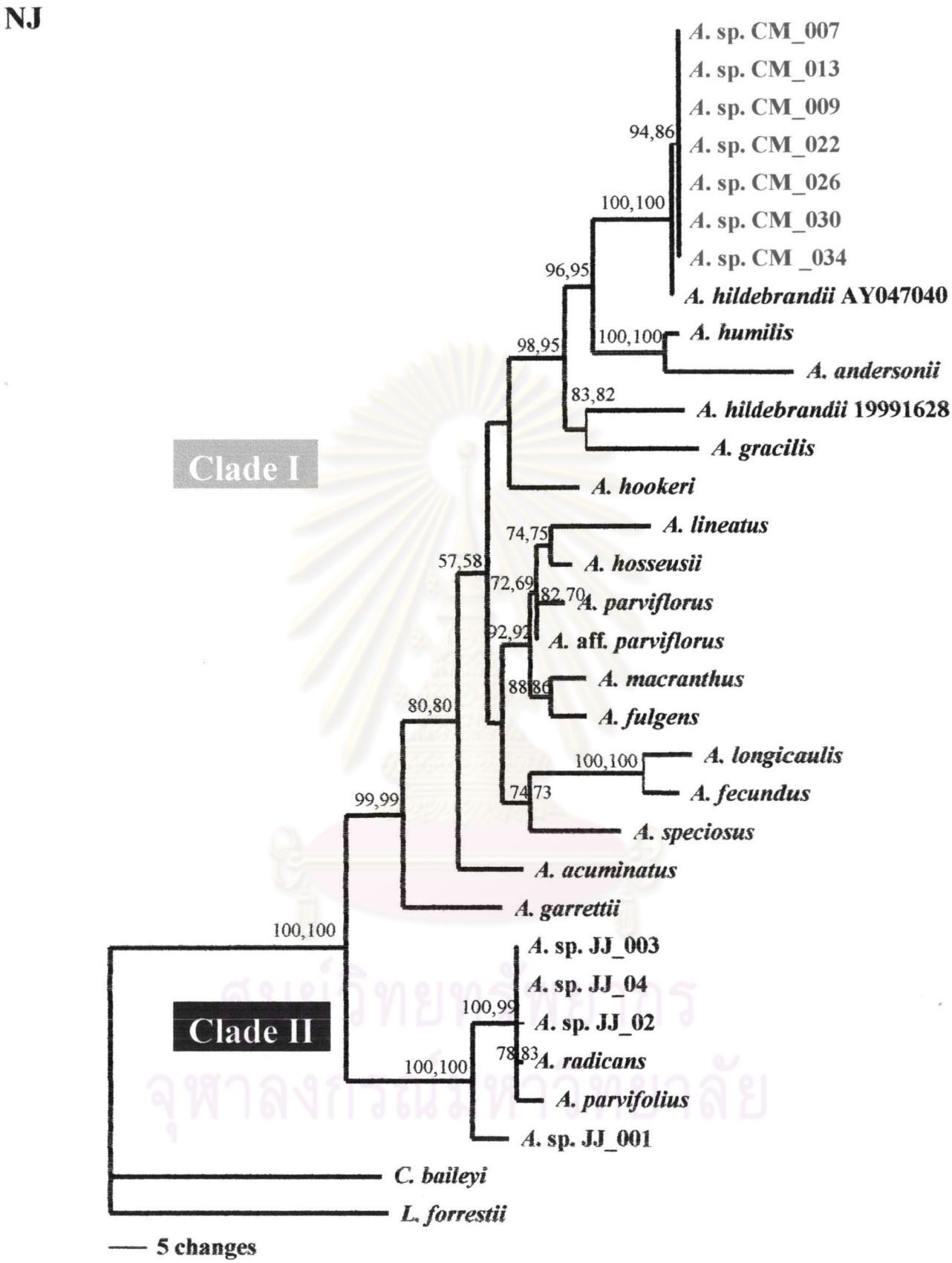

Fig. 35 A neighbour-joining tree of 30 *Aeschynanthus* taxa based on ITS sequence data without gap matrix and using *C. baileyi* and *L. forrestii* as outgroups. The numbers along branches are 10,000-replicate bootstrap and jackknife supporting-values, respectively. Note that supporting-values less than 50% were not shown in the tree. Taxon names in colour letters are *Aeschynanthus* samples collected in Chiangmai province.

4.7 PCR RAPD experiments

From PCR-RAPD amplification of *A. andersonii* 19970465, *A. humilis* 19850473, and *A. hildebrandii* 19991628 for solving synnonymous problem of these *Aeschynanthus*, PCR RAPD products of these plants were checked with agarose gel electrophoresis for presence or absence of major bands which showed a reproducible pattern among genotypes. Each RAPD band represents different alleles of a locus, do not co-migrate. Only two from three RAPD primers yielded informative and reproducible PCR products. Most of the observed RAPD products were in the range of 500 bp – 1.5 kb while the products belows 500 bp or above 1 kb were faint or non-reproducible bands. The RAPD primer UBC 001 amplified 5 bands from genomic DNA of *A. andersonii* and *A. humilis* with molecular size approximately 1.6 kb, 1.3 kb, 900 bp, 800 bp, and 700 bp. This primer gave only 3 bands for *A. hildebrandii* DNA: 1.3 kb, 900 bp and 700 bp. The other RAPD primer, UBC 002, amplified 3 bands from every samples with molecular size ca. 1.25 kb, 800 bp and 500 bp. Figure 37 shows an example of RAPD patterns using all three RAPD primers.

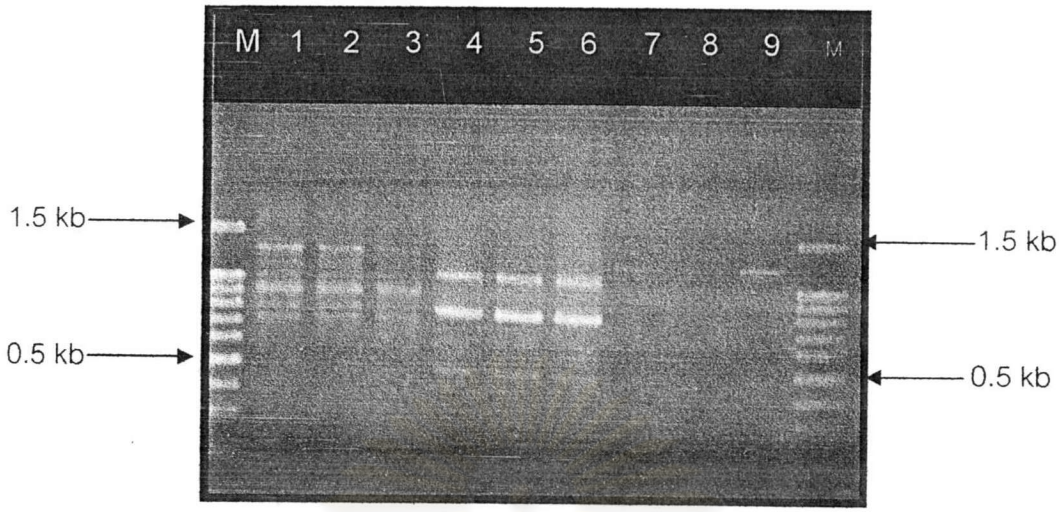

Fig. 36 PCR-RAPD products of three synonymous *Aeschynanthus* species compared with 1.5 kb + 100 bp DNA marker. Amplified products generated from genomic DNA with RAPD primers UBC 001, UBC 002, and UBC 003.

M = 1.5 kb + 100 bp DNA marker

1 = *A. andersonii* + UBC 001

2 = *A. humilis* + UBC 001

3 = *A. hildebrandii* + UBC 001

4 = *A. andersonii* + UBC 002

5 = *A. humilis* + UBC 002

6 = *A. hildebrandii* + UBC 002

7 = *A. andersonii*+ UBC 003

8 = *A. humilis* +UBC 003

9 = *A. hildebrandii* + UBC 003

4.8 Cytogenetic experiments

All chromosome numbers of six *Aeschynanthus* counted in this study are listed in Table 9. After investigating the root tip cells in mitotic cell division of each plant under light microscope, *A. andersonii* showed distinguishly separated cells which had best dye absorption and showed dark reddish colour chromosomes (2n=28) (Fig. 38). *Aeschynanthus humilis* gave a chromosome number of 2n= 28 with less dye-absorbing, light reddish colour chromosomes (Fig. 39). The chromosome number of the *A. hildebrandii* was also found being 28 (Fig. 40). The root tip cells of this species were separated distinguishly while having least dye absorption, made their chromosome having light grayish colour. *Aeschynanthus obconicus,* however, showed ambiguous chromosome number per cell since most of its cells were still not separated from each other. Its chromosomes were not dispersed enough to count an exactly chromosome number. The estimated chromosome numbers of *A. obconicus* were 28, 30 and 32 (Fig. 41). In the case of *A. radicans* (Fig. 42), the chromosome number was found to be 32 while its root cells were still attached together and its chromosomes could absorb the acetocarmine dye enough to darken the chromosomes. The last cytogentic experiment was done on *Aeschynanthus* sp. JJ_001 (Fig. 43 and 44). This plant gave a chromosome number of 2n=32, and the root cells were separated distinguishly.

The chromosomes of all *Aeschynanthus* used in these experiments were very small, less than 1.5 micron in size. Even under the highest magnification (100X) of the

light microscope with blueish-colour hematoxylin staining method (for example, Fig. 44), these chromosomes appeared to be as small as dots. Because of this difficulty, type of each chromosome could not be indicated.

Table 9 A list of chromosome numbers of some Thai *Aeschynanthus*

Name	chromosome number counted in this study
Aeschynanthus andersonii	28
Aeschynanthus hildebrandiii	28
Aeschynanthus humilis	28
Aeschynanthus obconicus	32
Aeschynanthus radicans	32
Aeschynanthus sp. JJ_001	32

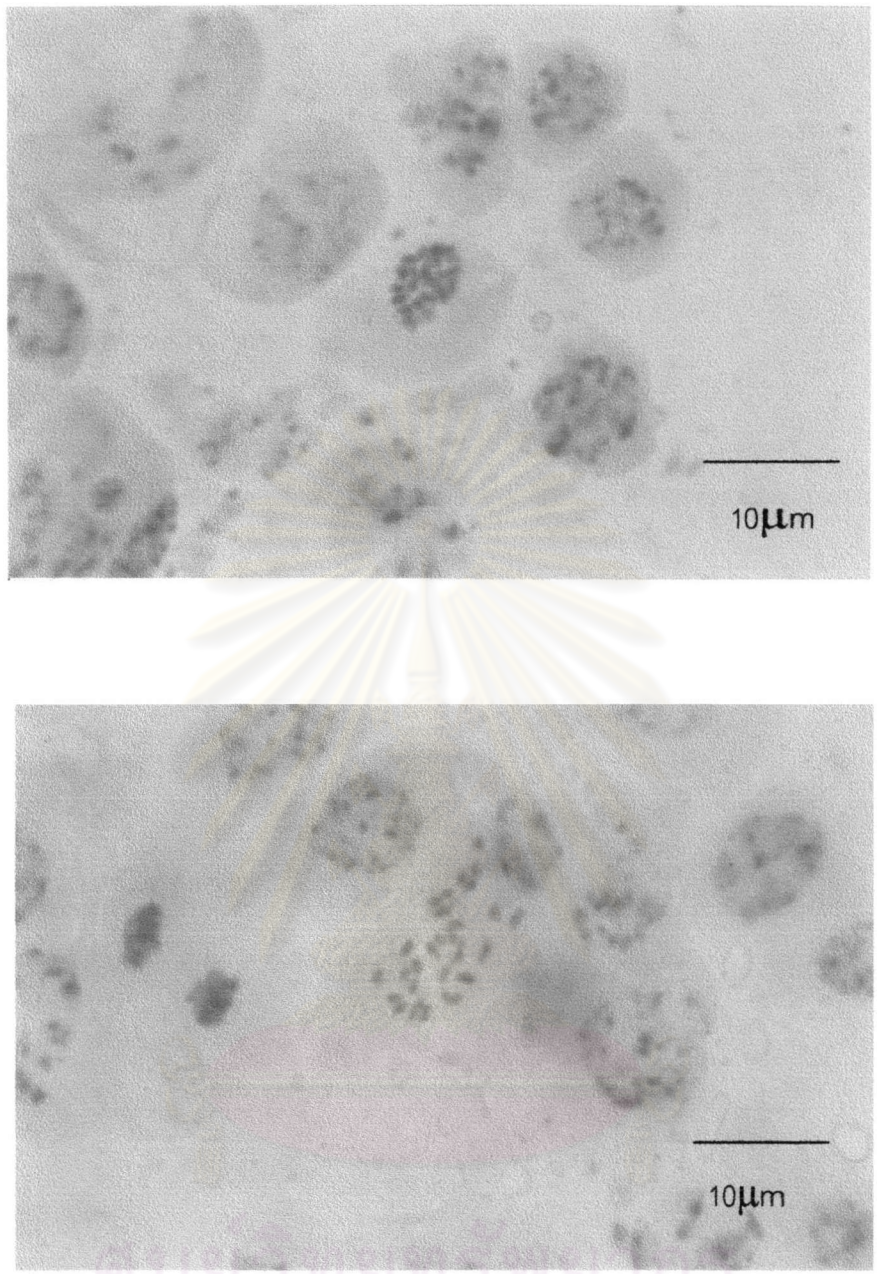

Fig. 37 Root tip cells of *A. andersonii* in metaphase showing an estimated chromosome number of 2n=28.

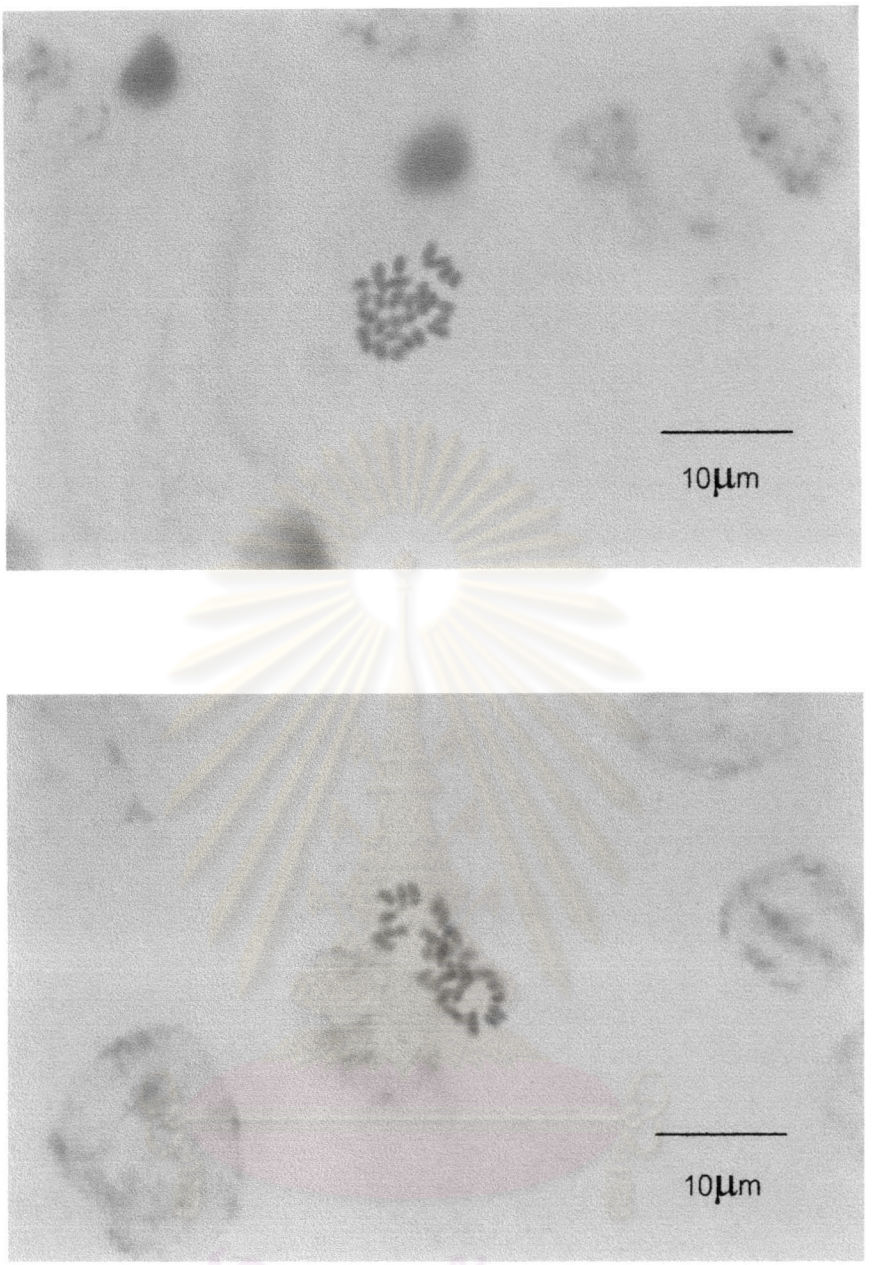

Fig. 38 Root tip cells of *A. humilis* in metaphase showing an estimated chromosome number of 2n=28.

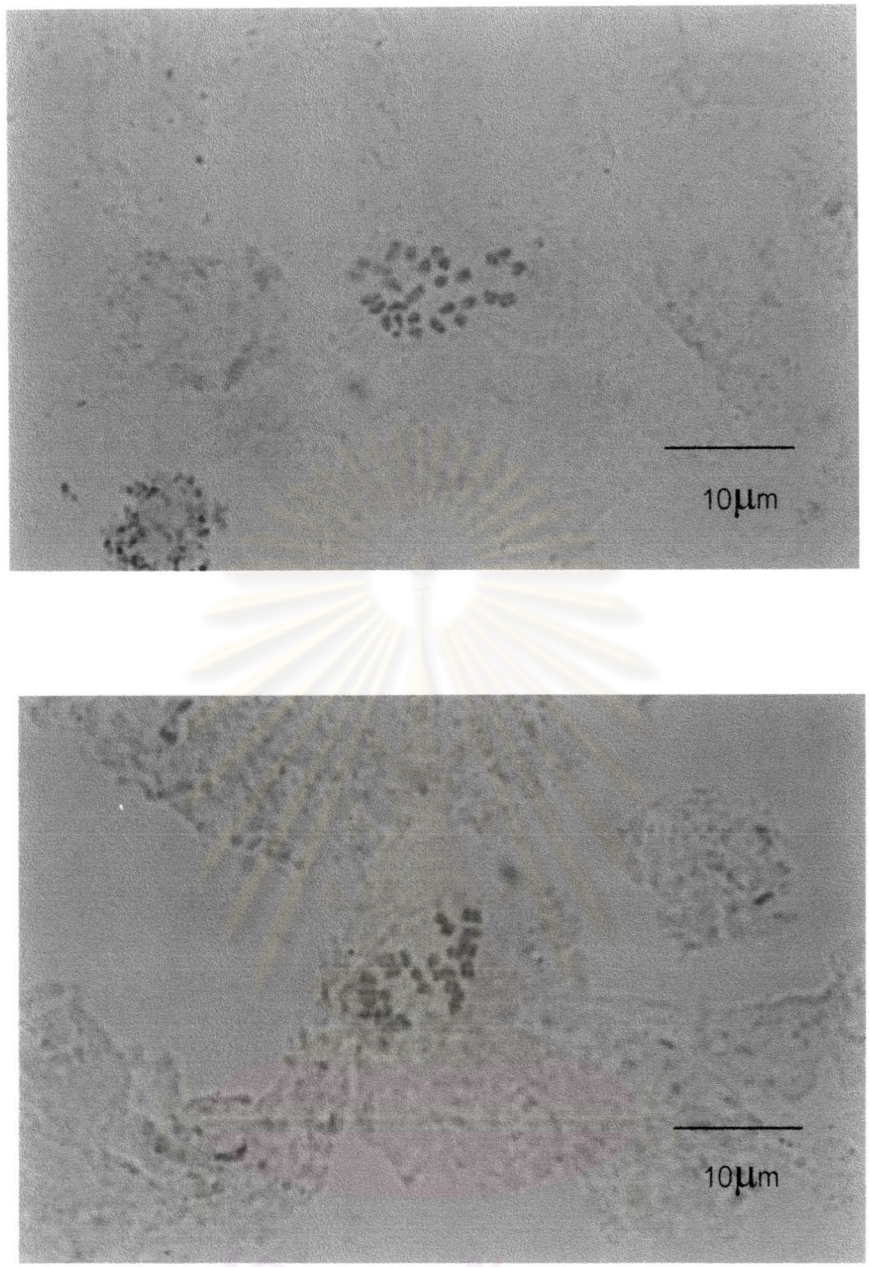

Fig. 39 Root tip cells of *A. hildebrandii* in metaphase showing an estimated chromosome number of 2n=28.

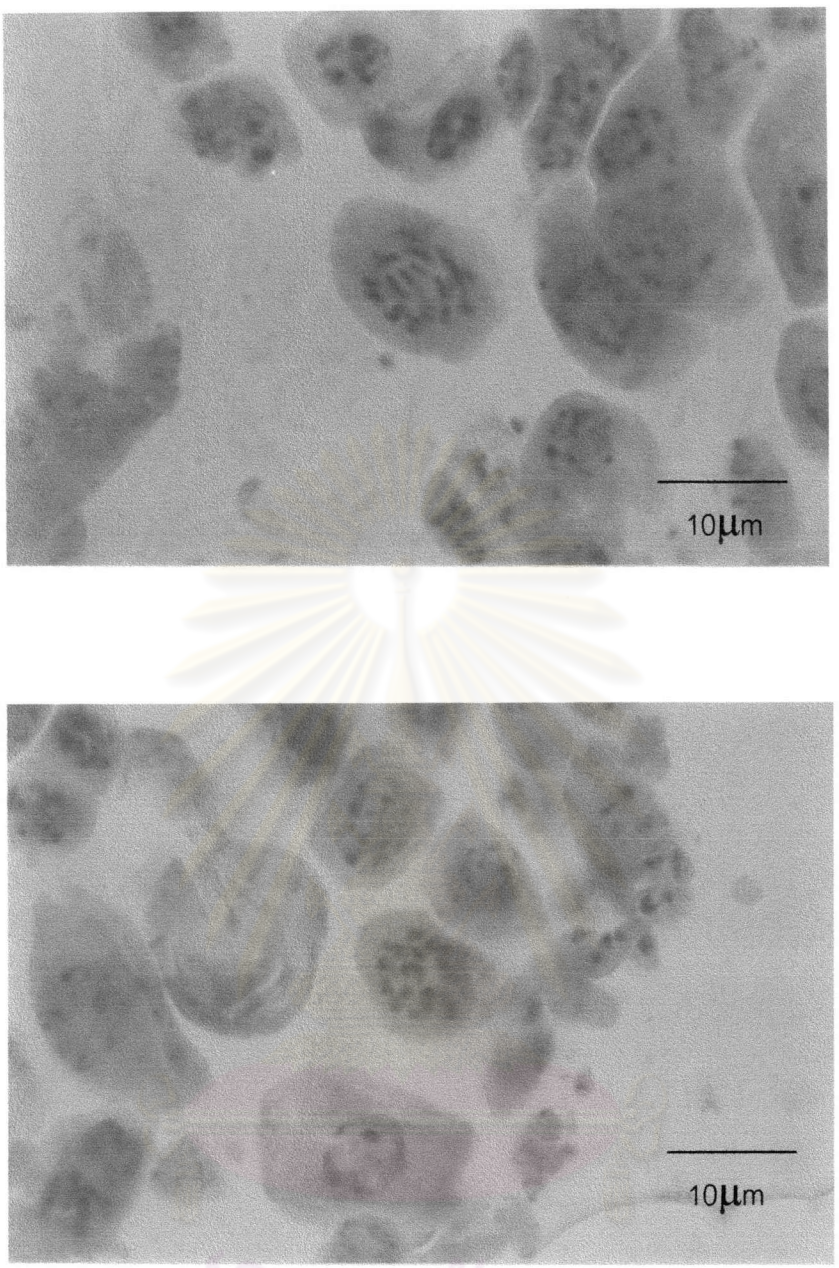

Fig. 40 Root tip cells of *A. obconicus* in metaphase showing an estimated chromosome number of 2n =28, 2n = 32 from the top to the bottom.

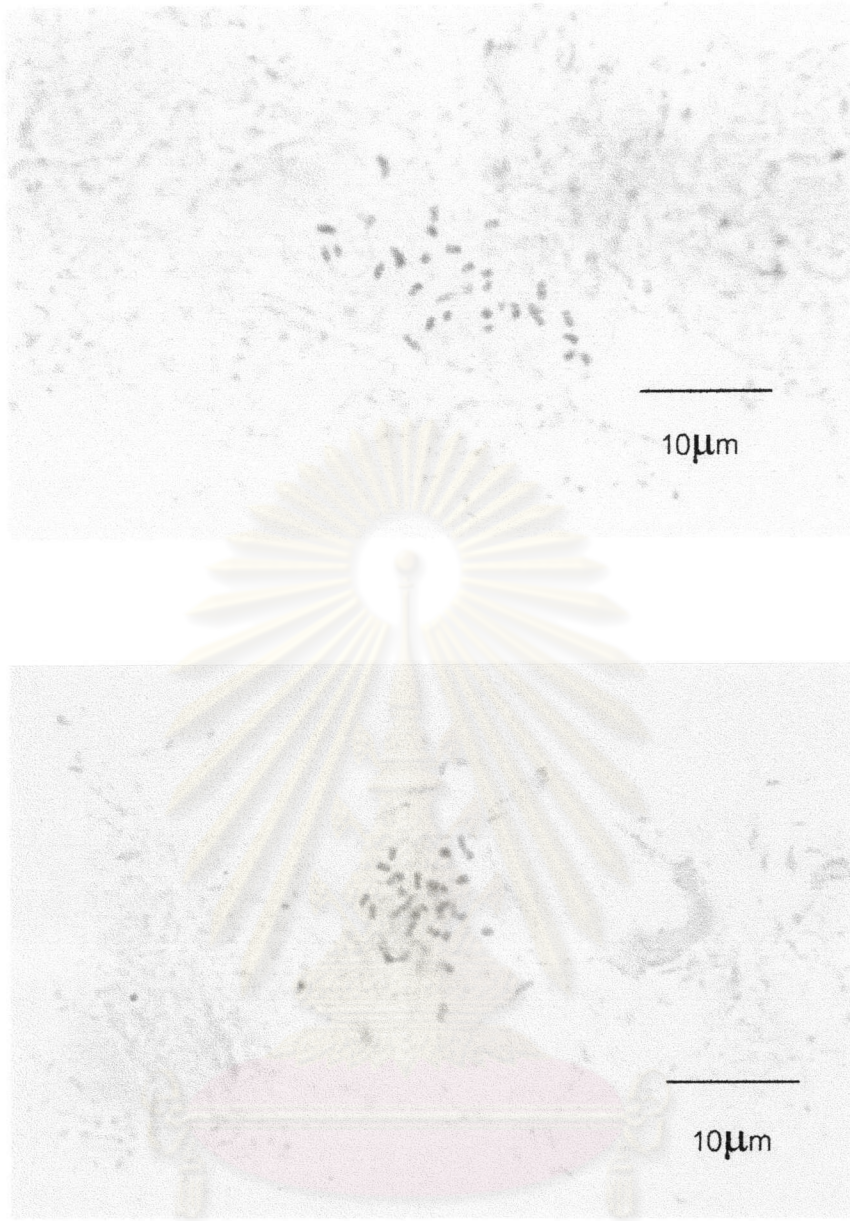

Fig. 41 Root tip cells of *A. radicans* in metaphase showing an estimated chromosome number of 2n=32.

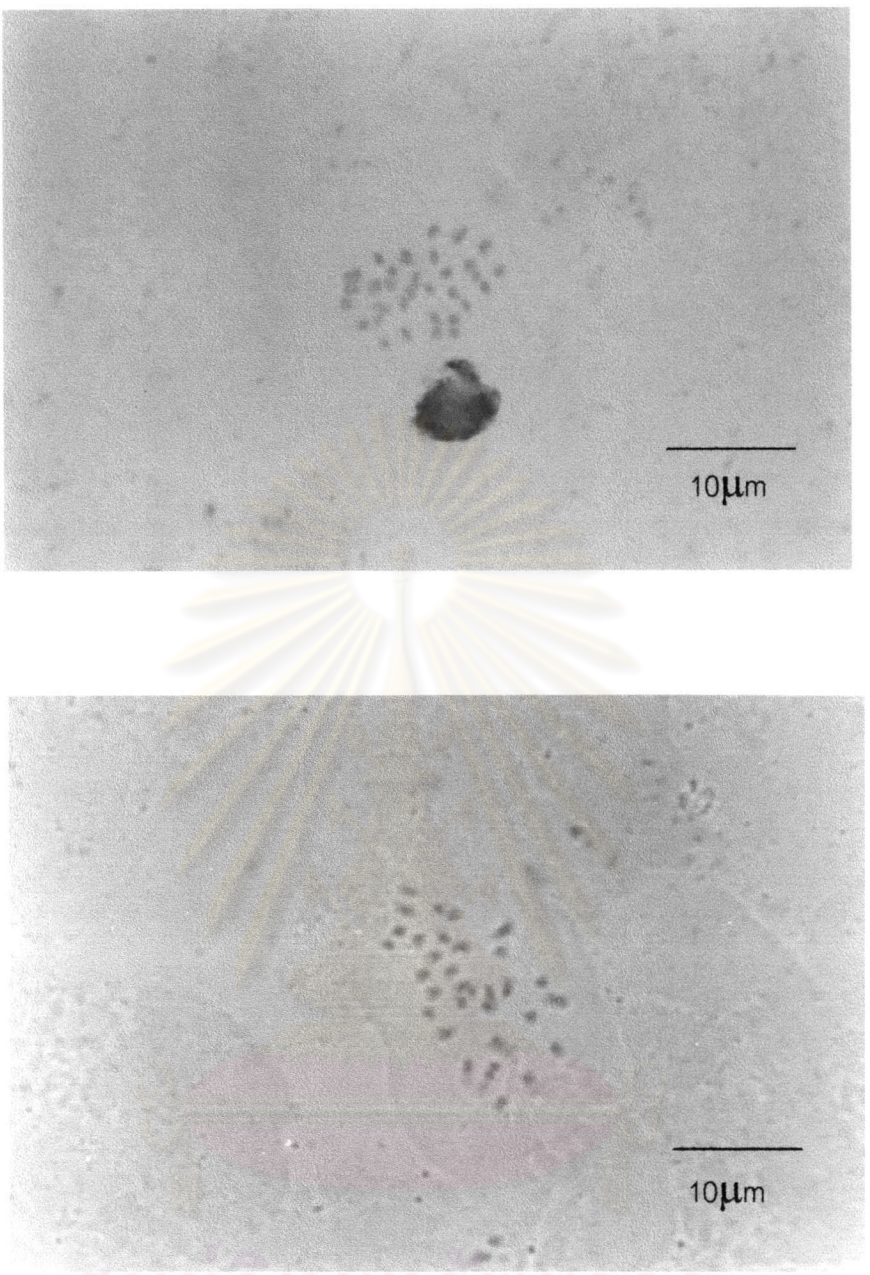

Fig. 42 Root tip cells of *A.* sp. JJ_001 in metaphase showing an estimated chromosome number of 2n=32.

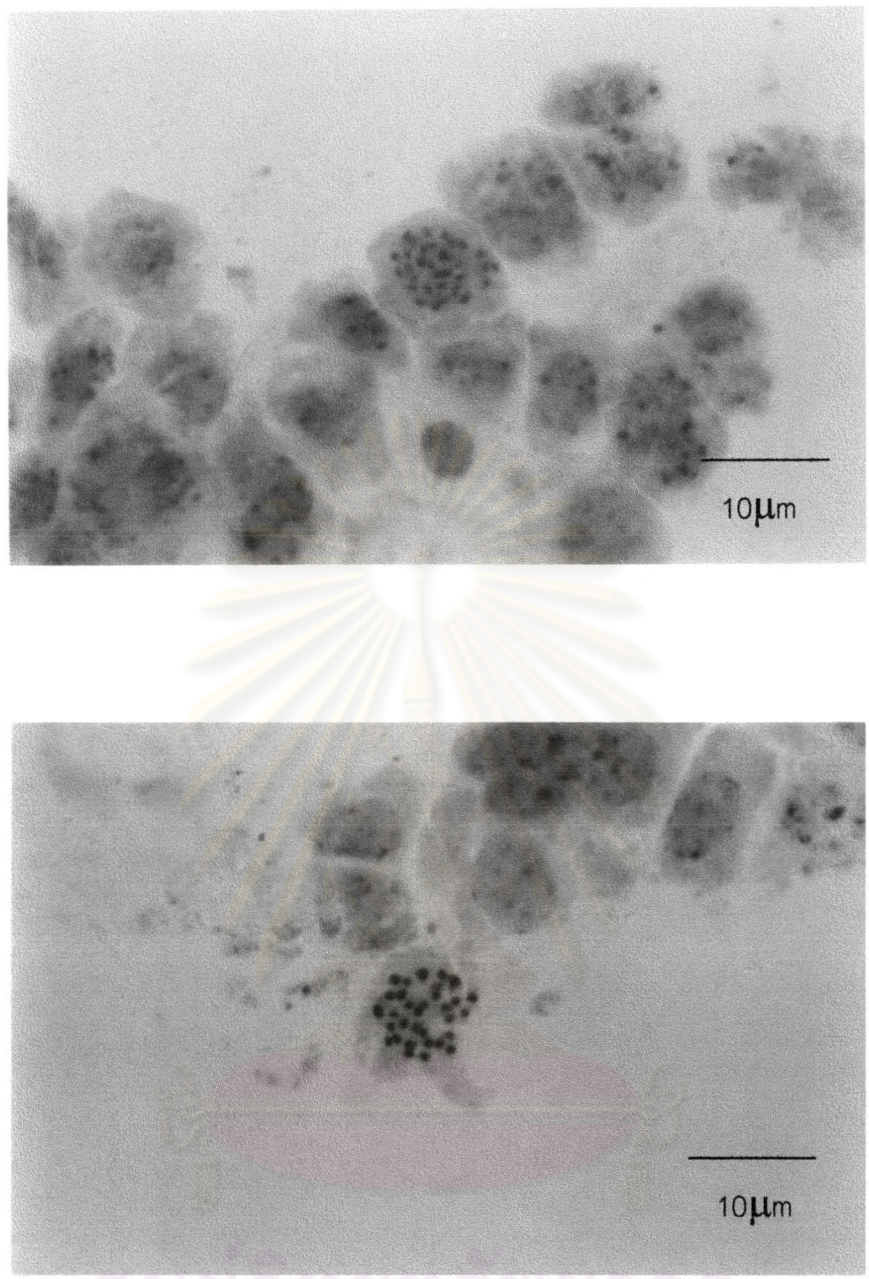

Fig. 43 Root tip cells of *A.* sp. JJ_001 in metaphase (stained with hematoxoxylin dye) showing an estimated chromosome number of 2n =32.