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DNA barcoding for identification of water-onion (*Crinum thaianum*), an endangered species in Thailand

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ABSTRACT: *Crinum thaianum*, also known as water-onion, has been widely used as an ornamental plant in fish tanks and aquariums, an indicator of water quality in natural water sources, and a component in some skincare products. Unfortunately, it is now listed as an endangered species because of the ongoing reduction in its natural area due to habitat destruction, a result of flood protection measures and illegal harvesting for exportation and domestic consumption. This study reported for the first time the use of DNA barcoding, a molecular technique for species-specific identification of this endangered species. Four DNA barcoding regions in six water-onion samples were studied, i.e., the chloroplast-encoded maturase K (*mat*K), ribulose-bisphosphate carboxylase large subunit (*rbc*L) and transfer RNA-histidine (*trn*H)-photosystem II protein D1 (*psb*A) intergenic spacer, and the nuclear-encoded the internal transcribed spacer of ribosomal DNA (ITS of *rDNA*). The six water-onion samples were collected from different locations and 13 other *Crinum* species. The results showed a high genetic similarity among the six water-onion samples. Four nucleotide substitutions were identified in two different regions (*trn*H-*psb*A and ITS) in the 6 water-onion samples. Although all 4 regions could be used to distinguish the species from each other, the ITS of *rDNA* region gave the best result for *Crinum* species discrimination, based on the bootstrap value of the phylogenetic tree and the optimum genetic distance length. This technique could be an efficient tool for species-specific identification and for supporting conservation practices in the future.

KEYWORDS: *Crinum thaianum*, water-onion, *mat*K, *rbc*L, *trn*H-*psb*A, ITS

INTRODUCTION

Crinum (C.) thaianum (water-onion), an aquatic species found only in Thailand, is a native plant of the Southern Provinces of Ranong and Phang Nga [1]. Water-onion is a flowering plant in the family Amaryllidaceae. Its leaves are extremely tough, making it suitable for aquariums with large fish and thus an economic plant that is popular as an ornamental plant in fish tanks and aquariums. In addition, water-onion can be used as an indicator of the quality of stream water because it can only grow in clear, clean water. Furthermore, *C. thaianum* extract has been used in cosmetics and skincare products [2].

Many years ago, during its blooming season, water-onion was promoted by the local tourism industry. At present, it is classified as an endangered species on the International Union for Conservation of Nature (IUCN) Red List due to upstream destruction for flood protection resulting in habitat degradation and reduction [3]. Furthermore, another important issue is an illegal digging of the plant for exportation. These practices have resulted in a substantial reduction of the plant. Although there is a ban on digging up water-onion, it has not been strictly enforced.

DNA barcoding is a concept that applies gene or universal DNA sequence differences to support the identification and classification of organisms [4]. The qualities of the DNA barcoding region must be high interspecific with low intraspecific variation and must have a conservative region. There are many encoded genes or regions (both nuclear and chloroplast) that can be used as DNA barcodes for plants [5]. The chloroplast gene regions, the ribulose-bisphosphate carboxylase large subunit (*rbc*L) gene and the maturase K (*mat*K) gene have been widely used for plant barcoding analysis. In addition, the plastid transfer RNA-histidine-photosystem II protein D1 (*trn*H-*psb*A) intergenic spacer (non-coding) region has been successfully amplified in many plant species and shows a high power of discrimination with its high substitution rate [6]. Moreover, the chloroplast genomes of bamboo species are being studied to provide data beneficial for future research on systematics, genetic diversity, and evolutionary history [7]. The internal transcribed spacer (ITS) in the nuclear genome is located between the large and small subunits of *ribosomal RNA* (*rRNA*) genes. Due to the high copy number of *rRNA* genes, it is easy to amplify even from small quantities of DNA [8]. In addition, the ITS region has a high degree of variation even between closely related species, making this region popular for phylogenetic analysis and species identification [9–11].

Although there is a ban on digging up water-onion, it has not been strictly enforced. In addition, genetic information at the molecular level of water-onion has not yet been reported. Thus, it would be useful to have the genetic information water-onion for species-specific identification and its management planning. Species-specific identification in *Crinum* plants is important to distinguish more abundant species from the endangered one. Therefore, the aim of the current study was to analyze the *mat*K, *rbc*L, *trn*H-*psb*A intergenic spacer, and ITS of *rDNA* regions for information on water-onion genetic diversity and to use the results for the differentiation of water-onion from other *Crinum* plants. The findings would be beneficial for the management, conservation practices, and sustainable uses of water-onion.

MATERIALS AND METHODS

Samples and DNA extraction

Six water-onion (*C. thaianum*) samples were collected from Ranong and Phang Nga Provinces, Southern Thailand and from the White Cane Company, Bangkok, Thailand; including 13 other *Crinum* plants (Fig. 1). Genomic DNAs were extracted from the petal of the *Crinum* plants following the modified CTAB extraction method [12]. The extracted DNA samples were analyzed for quality using electrophoresis in 0.8% agarose gel stained with ethidium bromide to visualize the DNA bands under UV light, and their concentrations were measured using spectrophotometry. Total genomic DNA samples were stored at 4 °C.

PCR amplification

The primers used for the chloroplast-encoded *mat*K, *rbc*L, *trn*H-*psb*A regions and for the nuclear-encoded ITS of *rDNA* region were obtained from other reports [13–16] shown in Table S1. The PCR amplification was conducted using the specific primers of *mat*K, *rbc*L, *trn*H-*psb*A, and ITS. PCR reactions were performed in 25 µl of a mixture containing 100 ng DNA, 5 pmol of each primer, 2.0 mM $MgCl_2$, 1.25 µM of dNTP, and 2 U of phusion high-fidelity DNA polymerase (Thermo Scientific; USA) in 1X PCR buffer. The amplification conditions were 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature (T_a) shown in Table S1, and 1 min at 72 °C; and the extension was completed for 7 min at 72 °C.

DNA sequencing and analysis

PCR products of *mat*K, *rbc*L, *trn*H-*psb*A, and ITS regions, containing the nucleotide sequences of about 900, 600, 700, and 900 bp, respectively, were purified using a FavorPrep gel/PCR purification Kit (Favorgen; Ping-Tung, Taiwan); then, they were electrophoresed in 1% agarose gel, followed by ethidium bromide staining and visualization under UV light. The purified DNA samples were sent for sequencing, except for PCR products of the ITS region which had to be cloned and selected before sequencing to avoid the problem of obtaining multiple peaks of chromatogram. Purified ITS of *rDNA* fragments were cloned into pGEM-T Easy vectors (Promega; WI, USA), and the vectors were transformed into the *Escherichia coli* strain JM 109. For each sample, about 5 colonies with recombinant vectors were selected and cultured. Plasmids from these cultures were purified and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems; CA, USA). The obtained sequences were aligned using MUSCLE (Multiple Sequence Comparison by Log-Expectation) alignments. The aligned DNA sequence data were analyzed using the maximum likelihood (ML) method, and a phylogenetic tree was constructed using the MEGA 5.0 software [17] with 1,000 bootstrap replications. The genetic distance values were also calculated using pair-wise Tamura-3 parameters by MEGA 5.0 software. These analyses facilitated the examination of how *C. thaianum* diverged from other *Crinum* species, thereby supporting the identification of the species.

RESULTS

In total, 19 DNA sequences for each region of the chloroplast-encoded *mat*K, *rbc*L, *trn*H-*psb*A, and 19 sequences at the nuclear-encoded ITS of six water-onion samples collected from different locations and 13 other *Crinum* species were generated and analyzed. The sequence alignments at each locus were shown in the supplementary data (Figs. S1-A and S1-B). The amplicon lengths of *mat*K, *rbc*L, *trn*H-*psb*A, and ITS were in the expected ranges of approximately 900 bp, 600 bp, 700 bp, and 900 bp, respectively (Table S1). All DNA sequences for each region were aligned using MUSCLE alignments. Variable sites and gaps were found in all 4 regions (Table 1). Then, the model testing of the final sequence data was performed to find a suitable model for phylogenetic tree construction. The ML trees were constructed with 1,000 bootstrap replications based on *mat*K, *rbc*L, *trn*H-*psb*A intergenic spacer and ITS of *rDNA* sequence data, using a Tamura 3-parameter (T92) model. The phylogenetic tree of each region indicated that the water-onion (*C. thaianum*) samples were grouped together and separated from the other *Crinum* plants. The constructed ML phylogenetic tree, based on three chloroplast regions of *mat*K, *rbc*L and *trn*H-*psb*A intergenic spacer indicated that all *Crinum* plants could be separated into two major groups: Group I, *C. natans*; and Group II, the rest of the *Crinum* plants, including *C. thaianum*, which was one of many subgroups in the major Group II (Fig. 2A). The ML tree based on nuclear ITS region revealed that the *Crinum* species were clustered into four groups consisting of: Group I, *C. latifolium*, *C. xanthophyllum*, *C. amoenum*, *C. jagus*, and *Crinum* sp. from Indonesi; Group II, *C. natans*; Group III, *C. asiaticum*, *Crinum* sp., *C. erubescens*, *Crinum* × *amablie*, and *C. japonicum*; and Group IV, six samples of *C. thaianum* (Fig. 2B). The ITS region gave the best result for discrimination of *Crinum* species.

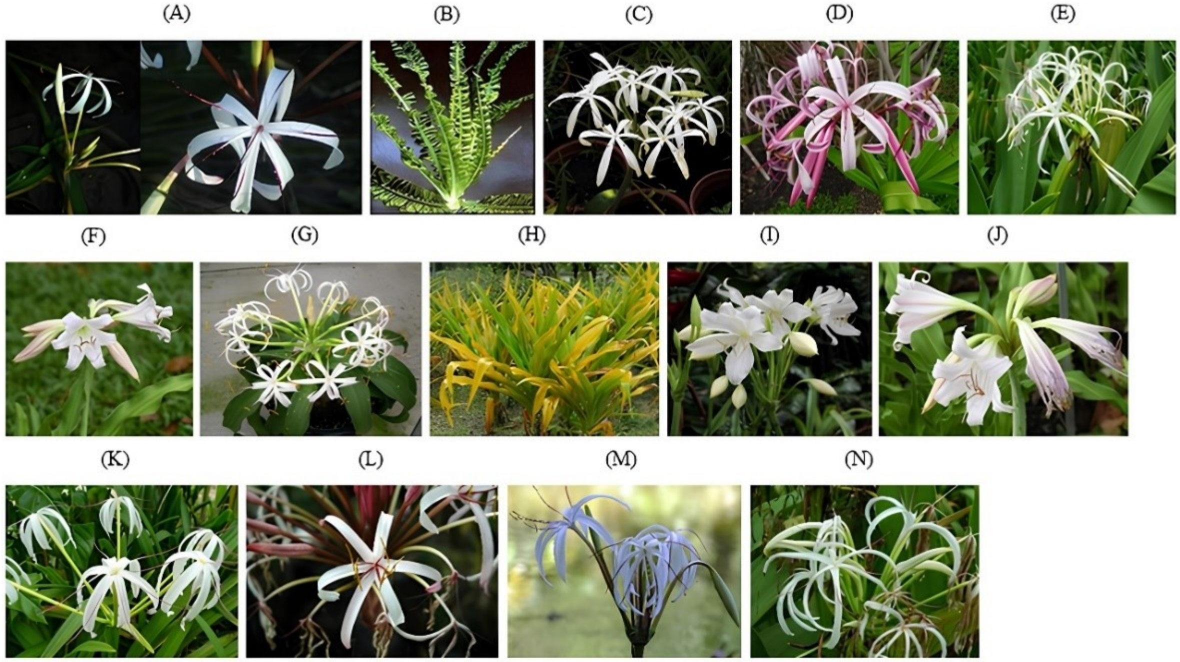

Fig. 1 *Crinum* species used in the present study: (A), *C. thaianum* (SL.382/2558); (B), *C. natans*; (C), *C. amoenum* Ker Gawl. Ex Roxb. (SL.404/2558); (D), *Crinum* × *amabile* Donn ex Ker Gawl. (SL.410/2558); (E), *C. asiaticum* L. (SL.411/2558); (F), *C. latifolium* L. (SL.412/2558); (G), *C. japonicum* (SL.413/2558); (H), *C. xanthophyllum* (SL.414/2558); (I), *C. jagus* (J.Thomps.) Dandy (SL.415/2558); (J), *C. latifolium* L. (SL.416/2558); (K), *C. erubescens* L.f. ex Aiton (SL.417/2558); (L), *Crinum* sp. (SL.434/2558); (M), *C. asiaticum* L. var. anomalum Baker (SL.003/2559); and (N), *Crinum* sp. (Indonesia). SL is the Suan Luang Rama IX Park accession number.

Table 1 Number of nucleotides and nucleotide substitutions of four barcoding regions in *Crinum* species.

Sample	Number of nucleotides (bp)				Number of nucleotide substitutions*			
	*mat*K	*rbc*L	*trn*H-*psb*A	ITS	*mat*K	*rbc*L	*trn*H-*psb*A	ITS
C. thaianum (SL.382/2558)	765	507	628	823	–	–	–	–
*C. thaianum*_1 (Triam canal, Phangnga province)	765	507	628	823	–	–	1	–
*C. thaianum*_2 (White Cane Co.)	765	507	628	823	–	–	–	–
*C. thaianum*_3 (Ta-put canal, Phangnga province)	765	507	628	823	–	–	–	–
*C. thaianum*_4 (Kura canal, Phangnga province)	765	507	628	823	–	–	–	–
*C. thaianum*_5 (Naka canal, Ranong province)	765	507	626	823	–	–	2	1
C. natans (White Cane Co.)	765	507	615	824	50	18	39	9
C. amoenum Ker Gawl. Ex Roxb. (SL.404/2558)	765	507	628	827	3	2	4	25
Crinum × *amabile* Donn ex Ker Gawl. (SL.410/2558)	765	507	627	830	3	1	5	12
C. asiaticum L. (SL.411/2558)	765	507	627	826	4	1	4	31
C. latifolium L. (SL.412/2558)	765	507	628	830	3	1	4	12
C. japonicum (SL.413/2558)	793	507	627	830	66	1	3	13
C. xanthophyllum (SL.414/2558)	765	507	628	826	4	1	3	35
C. jagus (J.Thomps.) Dandy (SL.415/2558)	759	507	628	826	6	2	2	31
C. latifolium L. (SL.416/2558)	765	507	628	826	4	1	2	35
C. erubescens L.f. ex Aiton (SL.417/2558)	765	507	628	830	8	1	12	12
Crinum sp. (SL.434/2558)	765	507	628	830	3	1	4	12
C. asiaticum L. var. anomalum Baker (SL.003/2559)	765	505	628	830	3	7	5	12
Crinum sp. (Indonesia)	765	505	627	826	5	6	5	36

* Number of nucleotide substitutions compared with nucleotide sequence of *C. thaianum* (SL.382/2558).

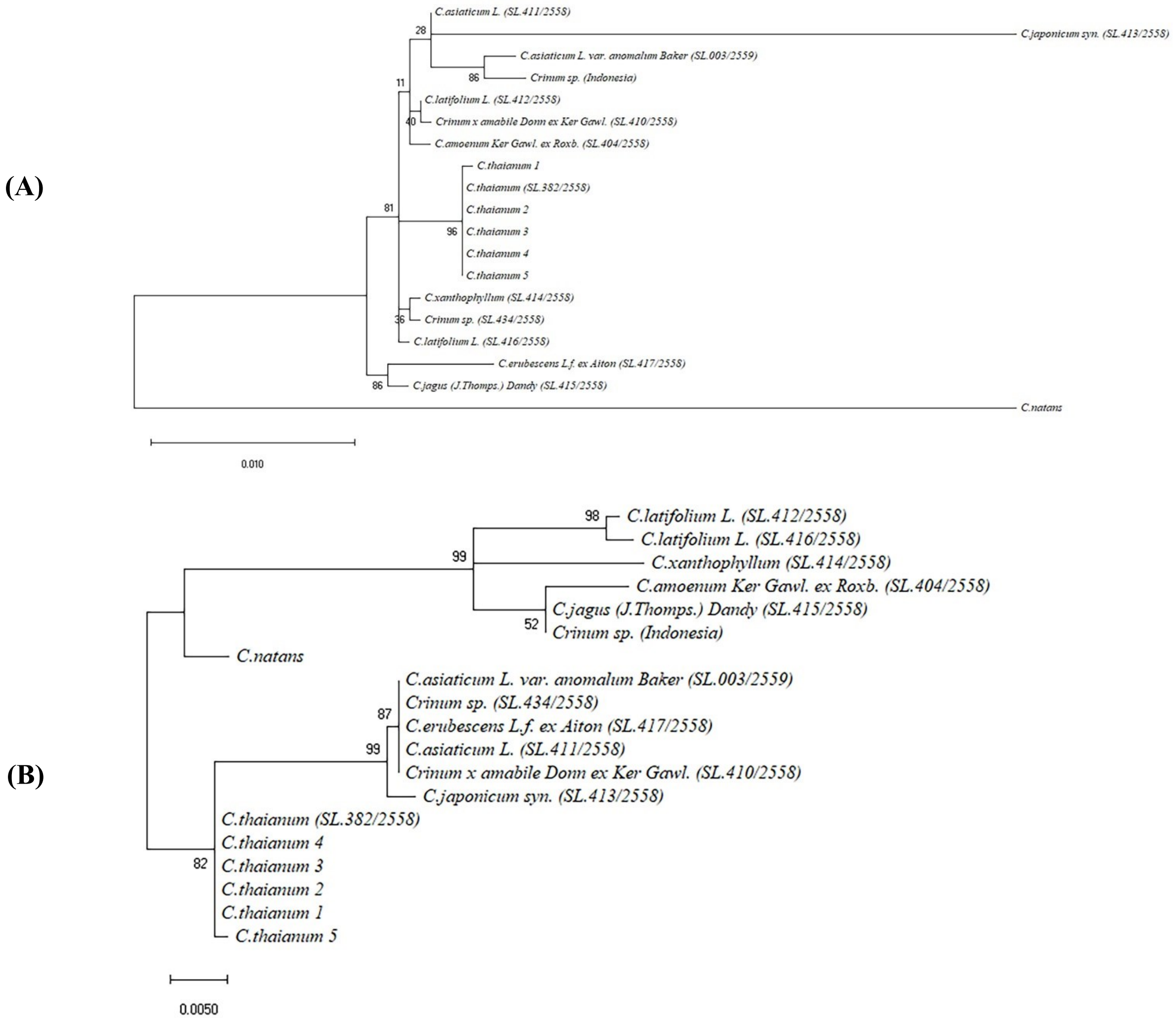

Fig. 2 Phylogenetic tree of 19 samples of *Crinum* species based on ML analysis of specific gene sequences: (A), three chloroplast-encoded gene regions, *mat*K, *rbc*L, and *trn*H-*psb*A intergenic spacer; (B), nuclear-encoded ITS of *rDNA* region.

Sequence analysis of three chloroplast-encoded gene regions

The assembled chloroplast sequences obtained for each specimen were aligned (Fig. S1-A). Then, the phylogenetic tree was generated, and the values of genetic distance (GD) were calculated. The results indicated that the *C. thaianum* samples were grouped together and separated from the other *Crinum* plants, which were clustered in a complex pattern (Fig. 2A). GD values among *Crinum* plants were in the range of 0.0005–0.0883 with a mean distance of 0.0163, while the distances between *C. thaianum* and the other *Crinum* plants were in the range of 0.0037–0.0596 with a mean value of 0.0120. The highest GD value was between *C. thaianum* and *C. natans* (Table 2).

Sequence analysis of *mat*K

The *mat*K sequences of 759–793 bp were amplified from the *Crinum* species. The obtained sequences were submitted to the GenBank database (OL598039–OL598057). The sequences of *C. thaianum* were identical but differed from the sequences of the other *Crinum* species by 3–66 nucleotide substitutions, as shown in Table 1. All 19 sequences of the *Crinum* species were aligned, and a phylogenetic tree was constructed. The constructed ML tree, based on the *mat*K region, revealed that the water-onion (*C. thaianum*) samples were grouped together and separated from the other *Crinum* plants (Fig. S2). However, this dendrogram did not provide clear differentiation among the other *Crinum* plants. The GD values among

Table 2 Genetic distances of pair-wise Tamura-3 parameters determined between studied samples based on three chloroplast-encoded gene regions (*mat*K, *rbc*L, and *trn*H-*psb*A intergenic spacer).

Crinum plant	1	2	3	4	5	6	7	8	9	10	11	12	13
C. thaianum													
C. natans	0.0596												
C. amoenum (SL.404/2558)	0.0046	0.0578											
Crinum × *amabile* (SL.410/2558)	0.0046	0.0584	0.0021										
C. asiaticum (SL.411/2558)	0.0046	0.0578	0.0021	0.0010									
C. latifolium (SL.412/2558)	0.0041	0.0578	0.0016	0.0005	0.0016								
C. japonicum (SL.413/2558)	0.0326	0.0883	0.0309	0.0298	0.0287	0.0303							
C. xanthophyllum (SL.414/2558)	0.0043	0.0572	0.0026	0.0016	0.0016	0.0021	0.0293						
C. jagus (SL.415/2558)	0.0068	0.0575	0.0052	0.0052	0.0052	0.0047	0.0332	0.0047					
C. latifolium (SL.416/2558)	0.0037	0.0572	0.0021	0.0021	0.0021	0.0016	0.0293	0.0016	0.0042				
C. erubescens (SL.417/2558)	0.0109	0.0607	0.0073	0.0084	0.0079	0.0079	0.0369	0.0089	0.0063	0.0084			
Crinum sp. (SL.434/2558)	0.0043	0.0578	0.0026	0.0016	0.0016	0.0021	0.0293	0.0010	0.0047	0.0016	0.0089		
C. asiaticum (SL.003/2559)	0.0078	0.0613	0.0052	0.0031	0.0042	0.0037	0.0331	0.0047	0.0084	0.0052	0.0116	0.0047	
Crinum sp. (Indonesia)	0.0085	0.0613	0.0068	0.0058	0.0047	0.0063	0.0326	0.0052	0.0090	0.0058	0.0132	0.0052	0.0037
Average	0.0163												
Max	0.0883												
Min	0.0005												

the *Crinum* plants were in the range of 0.0000–0.1625 with a mean value of 0.0249. The GD values between *C. thaianum* and the other *Crinum* plants were in the range of 0.0039–0.0937 with a mean GD of 0.0172. The highest GD value was between *C. thaianum* and *C. japonicum* (SL.413/2558). The ML tree after adding the outgroups, including *Allium cepa* (NC 024813.1), *Zea mays* (NC 001666.2), *Arabidopsis thaliana* (NC 000932.1) and *Vauquelinia californica* (AF288129.1) indicated that *Crinum* plants were closely related to the monocots *A. cepa* and *Z. mays* by sharing a recent common ancestor with *A. cepa* (Fig. S2).

Sequence analysis of *rbc*L

The *rbc*L barcoding sequences of 505–507 bp were amplified from the *Crinum* species and submitted to the GenBank database (OL598058–OL598076). The sequences of all *C. thaianum* samples were identical; however, 1–18 nucleotide substitutions were found among the *Crinum* species, as shown in Table 1. The phylogenetic tree was generated based on these sequences. and the GD values were calculated. The results indicated that the *C. thaianum* samples were grouped together and separated from the other *Crinum* plants, which were clustered in a complex pattern (Fig. S3). The GD values among the *Crinum* plants were in the range of 0.0000–0.0450 with a mean distance of 0.0087, while the distances between *C. thaianum* and the other *Crinum* plants were in the range of 0.0020–0.0366 with a mean value of 0.0066. The highest GD value was between *C. thaianum* and *C. natans*. The ML tree after adding *A. cepa* (D38294.1), *Z. mays* (NC 001666.2), *A. thaliana* (NC 000932.1), and *Rubus sieboldii* (MZ128458.1) as the outgroups showed that *Crinum* plants were closely related to the monocots *A. cepa* and *Z. mays* by sharing a recent common ancestor with *A. cepa* (Fig. S3).

Sequence analysis of *trn*H-*psb*A intergenic spacer

The *trn*H-*psb*A intergenic spacer sequences of the *Crinum* species were submitted to the GenBank database (OL598020–OL598038), and all sequences of 615–628 bp were aligned. Among the water-onion samples, the *trn*H-*psb*A sequence of *C. thaianum* collected from the Triam Canal differed from those collected from the other locations by one base transversion. Moreover, two bases of the *trn*H-*psb*A sequence of *C. thaianum* collected from the Naka Canal differed from the others. The sequence of *C. thaianum* differed from the other *Crinum* species by 2–39 substitutions, as shown in Table 1. Then, the ML phylogenetic tree was constructed based on the *trn*H-*psb*A sequences. Although the *C. thaianum* samples were clustered together, they were in a complex group with the other *Crinum* species (Fig. S4). The GD values among the *Crinum* plants were in the range of 0.0000–0.0746 with a mean value of 0.0157, while the values between *C. thaianum* and the other *Crinum* plants were in the range of 0.0035–0.0692 with a mean value of 0.0122. The highest GD value was between *C. thaianum* and *C. natans*. The ML tree, after adding *Lycoris squamigera* (HM748829.1), *Bulbophyllum cylindraceum* (PQ058145.1), *Anamirta cocculus* (LC506309.1), and *Tinospora sagittata* (JF708221.1) as the outgroups showed that *Crinum* plants were clustered with the monocots *L. squamigera* and *B. cylindraceum*. The most distinct *Crinum* species from the *Crinum* plants studied was *C. natans* (Fig. S4).

Sequence analysis of nuclear-encoded ITS of *rDNA* region

The obtained ITS of *rDNA* sequences were submitted to the GenBank database (OL584022–OL584040). All sequences of 823–830 bp were aligned (Fig. S1-B), and the results showed that the ITS of *rDNA* sequences of

Table 3 Genetic distances of pair-wise Tamura-3 parameters determined between studied samples based on nuclear-encoded gene region (ITS of *rDNA*).

Crinum plant	1	2	3	4	5	6	7	8	9	10	11	12	13
C. thaianum													
C. natans	0.0125												
C. amoenum (SL.404/2558)	0.0317	0.0340											
Crinum × amabile (SL.410/2558)	0.0150	0.0249	0.0392										
C. asiaticum (SL.411/2558)	0.0150	0.0249	0.0392	0.0000									
C. latifolium (SL.412/2558)	0.0461	0.0392	0.0261	0.0511	0.0511								
C. japonicum (SL.413/2558)	0.0163	0.0287	0.0432	0.0036	0.0036	0.0524							
C. xanthophyllum (SL.414/2558)	0.0449	0.0405	0.0287	0.0538	0.0538	0.0248	0.0580						
C. jagus (SL.415/2558)	0.0396	0.0340	0.0073	0.0472	0.0472	0.0185	0.0513	0.0210					
C. latifolium (SL.416/2558)	0.0447	0.0378	0.0273	0.0496	0.0496	0.0036	0.0510	0.0286	0.0197				
C. erubescens (SL.417/2558)	0.0150	0.0249	0.0392	0.0000	0.0000	0.0511	0.0036	0.0538	0.0472	0.0496			
Crinum sp. (SL.434/2558)	0.0150	0.0249	0.0392	0.0000	0.0000	0.0511	0.0036	0.0538	0.0472	0.0496	0.0000		
C. asiaticum (SL.003/2559)	0.0150	0.0249	0.0392	0.0000	0.0000	0.0511	0.0036	0.0538	0.0472	0.0496	0.0000	0.0000	
Crinum sp. (Indonesia)	0.0396	0.0340	0.0073	0.0472	0.0472	0.0185	0.0513	0.0210	0.0000	0.0197	0.0472	0.0472	0.0472
Average	0.0303												
Max	0.0580												
Min	0.0000												

C. thaianum were identical, except for the sequence of *C. thaianum* from the Naka Canal, which differed from the other *C. thaianum* by one base transition. Table 1 shows the number of polymorphisms in the ITS of *rDNA* region among the *Crinum* species. The phylogenetic tree was generated from all ITS of *rDNA* sequences (Fig. 2B). The result revealed that the *Crinum* species were clustered into four groups as follows: Group I of six *Crinum* plants, Group II of one *Crinum* plant of *C. natans*, Group III of six *Crinum* plants, and Group IV of six samples of *C. thaianum*. As shown in Table 3, the GD values among the *Crinum* plants were in the range of 0.0000–0.0580 with a mean value of 0.0303, while the values between *C. thaianum* and the other *Crinum* plants were in the range of 0.0125–0.0461 with a mean value of 0.0270. The lowest GD value was between *C. thaianum* and *C. natans*, whereas the highest GD value was between *C. thaianum* and *C. latifolium* (SL.412/2558). The genetic relationship patterns among the *Crinum* plants were different based on the chloroplast and the nuclear database. The ML phylogenetic tree after adding the outgroup species, including *A. cepa* (OP617675.1), *Oryza glumipatula* (KR364805.1), *A. thaliana* (X52320.1), and *Clematis viticella* (PQ888957.1), revealed that *Crinum* plants were closely related to the monocots *A. cepa* (OP617675.1) and *O. glumipatula* by sharing the most recent common ancestor with *A. cepa* (Fig. S5).

DISCUSSION

Plant species currently listed as abundant are being threatened by various factors, such as environmental pollution, exportation for commercial operations, and habitat destruction. In Thailand, plant smuggling is another threatening factor which, if not strenuously addressed, could lead to the risk of species extinction. Species identification is one of several options to protect an endangered plant, and there are various molecular markers that have been developed specifically for plant species identification. One of the interesting molecular techniques for identifying species is DNA barcoding, which has been widely reported in the literature, such as identifying mixed stock of *Ziziphora* species, a medicinally important plant in Iran [18]; and molecular characterization of entomopathogenic fungi [19], using ITS sequences. The *COI* gene has been a useful tool for trade detection of threatened and endangered species in commercial shark products [20] and in marine fish mislabeling investigations in South Korea [21].

The current study used barcoding regions, which included nuclear and chloroplast-encoded genes, to identify *C. thaianum*, an endangered species of Thailand. The Consortium for the Barcode of Life (CBOL) Plant Working Group has recommended the *mat*K and *rbc*L genes as a plant barcode for plant identification at the species level [5]. The results from the constructed phylogenetic tree of the *mat*K and *rbc*L regions among the *Crinum* species showed that the bootstrap value and GD value of the *C. thaianum* clade from the *mat*K ML tree were higher than those from the *rbc*L ML tree. In addition, the GD value between *C. thaianum* and the other *Crinum* plants of the *rbc*L ML tree was lower than that of the *mat*K ML tree. However, the results between these two ML trees were not different. *C. thaianum* samples were clustered in a complex pattern to the other *Crinum* plants, and they were not clearly separated. Thus, these two regions were unsuitable for use as water-onion (*C. thaianum*) species identification. In addition, the *rbc*L sequence was also reported to be not variable enough in some plants for separating some species in the same genus [22].

Generally, the *trn*H-*psb*A intergenic spacer is the best plastid option for sequencing. It has good priming sites with length and interspecific variations [23]. The *trn*H-*psb*A intergenic spacer region is located between the *trn*H and *psb*A genes and has high polymorphism that is more suitable for species identification than *rbc*L [24]. Furthermore, the non-coding *trn*H-*psb*A spacer region has been endorsed as a global land plant barcode that could be utilized for discrimination at the species level [15]. The results from the *trn*H-*psb*A ML tree in the current study were the same as those from the *mat*K and *rbc*L ML trees. However, as the bootstrap value of the *C. thaianum* clade was less than 50, this barcoding region was not effective to identify *C. thaianum* since its lower bootstrap value representing uncertainty for that node.

Analysis of the combined chloroplast regions can provide an accurate result. A combination of the non-coding *trn*H-*psb*A spacer region and a portion of *rbc*L gene analysis [15] and the *trn*H-*psb*A region and portions of two coding regions (*mat*K and *rpo*C1), or three coding regions combined (*mat*K, *rpo*B and *rpo*C1) analysis [25] were previously suggested. In the present study, the three chloroplast regions of *mat*K, *rbc*L and *trn*H-*psb*A intergenic spacer were used to construct a phylogenetic tree. The results indicated that the *C. thaianum* samples were clustered with a higher bootstrap value. Moreover, the complex pattern of some *Crinum* species could be classified. Although the dendrogram obtained from the combined three chloroplast regions was clearer than the dendrogram based on single region analysis, most of the *Crinum* plants could not be distinguished from one another. All *Crinum* plants could be separated into two major groups, Group I consisting of only one sample of *C. natans* and Group II containing the rest of the *Crinum* plants, including *C. thaianum*.

In several researches, the nuclear ribosomal ITS region is recommended as a probable barcoding in plants, fungi and bacteria. The ITS of *rDNA* region is used extensively in phylogenetic analysis and can successfully discriminate species because it provides the highest resolving power for discriminating closely related species of fungi [26]. In addition, it exists in multi-copies and exhibits high levels of within-species sequence differentiation. The ITS1 was earlier suggested as a region for the identification of flowering plants [23]. In the current work, the results based on the ITS of *rDNA* ML tree showed that the *C. thaianum* samples were separated from other *Crinum* species, and they were clustered together, despite being collected from different locations. The other *Crinum* species were clustered into three groups, namely, Group I, consisting of *C. latifolium* (SL.412/2558 and SL.416/2558), *C. xanthophyllum*, *C. amoenum*, *C. jagus* and *Crinum* sp. from Indonesia; Group II, containing only one species, *C. natans*; and Group III, containing *C. asiaticum* (SL.411/2558 and SL.003/2559), *Crinum* sp., *C. erubescens*, *Crinum* × *amablie*, and *C. japonicum*. All six samples of *C. thaianum* were clustered together in Group IV. Although it was not possible to distinguish all *Crinum* plants, it was clear that the *C. thaianum* samples were significantly separated from the other *Crinum* species. Even though the ITS of *rDNA* region was unsuitable for identifying some *Crinum* species, it could be used as an optimum tool for the water-onion (*C. thaianum*) identification.

Different sources of gene sequences used for barcoding provide different patterns of genetic relationship. In this study, the genetic relationship patterns among the *Crinum* plants were different based on the chloroplast-encoded *mat*K, *rbc*L, *trn*H-*psb*A intergenic spacer and the nuclear-encoded ITS of *rDNA* data. The chloroplast-encoded genes are maternal inheritance, whereas the nuclear-encoded genes are inherited from both parents.

CONCLUSION

The four DNA barcoding loci, the chloroplast-encoded *matK*, *rbcL*, and *trn*H-*psb*A intergenic spacer and the nuclear-encoded ITS of *rDNA*, could be used successfully for *C. thaianum* discrimination from other *Crinum* spp. However, the genetic diversity among *Crinum* plants studied, based on nuclear and chloroplast sequence data, resulted in different patterns. The ITS of *rDNA* sequence seemed to give reasonable results. More DNA barcoding loci or DNA markers, or both, should be used to better discern among the *Crinum* plants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.047.

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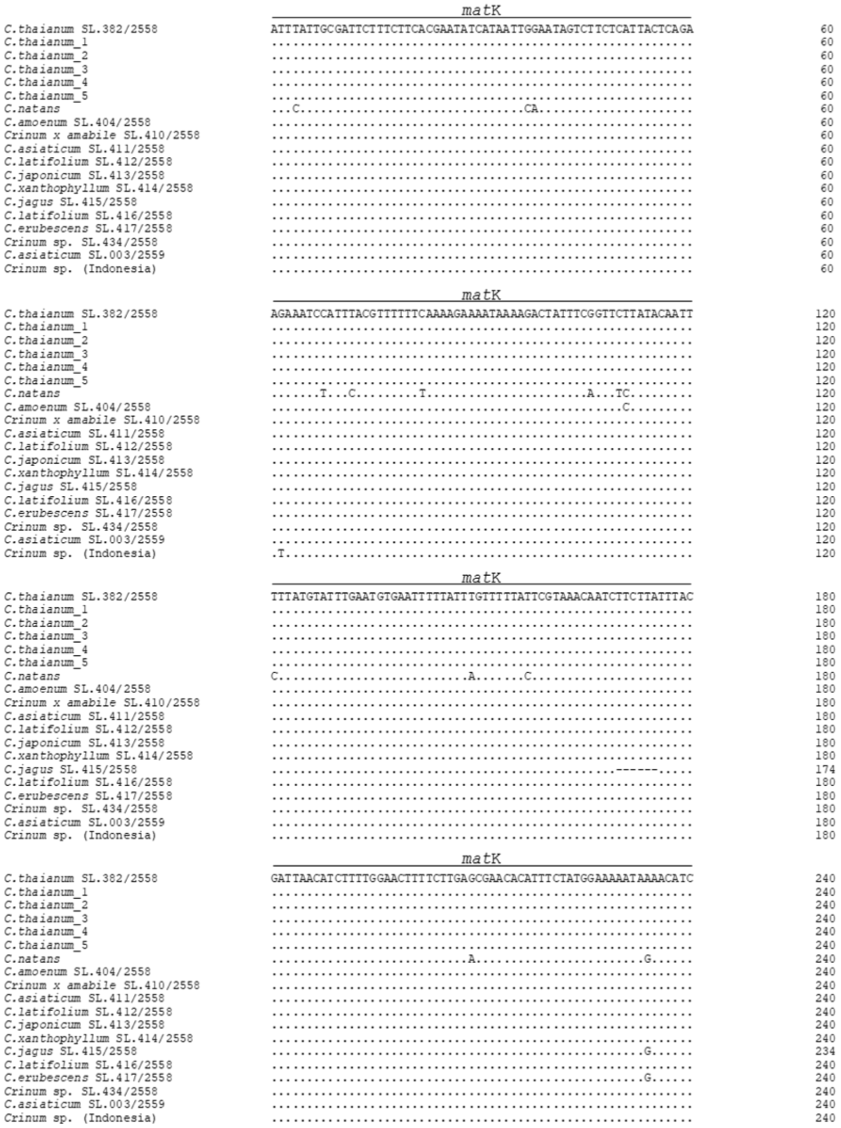
Appendix A. Supplementary data

Table S1 Information on primers used in the present study.

Primer name	Primer sequence (5′–3′)	Annealing temp. (T_a)	Expected amplicon	Reference
*mat*K_F *mat*K_R	ACC CAG TCC ATC TGG AAA TCT TGG TTC CGT ACA GTA CTT TTG TGT TTA CGA AG	52 °C	900 bp	[13]
*rbc*L_F *rbc*L_R	ATG TCA CCA CAA ACA GAG ACT AAA GC GTA AAA TCA AGT CCA CCR CG	52 °C	600 bp	[14]
*trn*H_F *psb*A_R	CGC GCA TGG TGG ATT CAC AAT CC GTT ATG CAT GAA CGT AAT GCT C	56 °C	700 bp	[15]
ITS-18S_F ITS-26S_R	TCG CTC CTA CCG ATT GAA TG TCC TCC GCT TAT TGA TAT GC	56 °C	900 bp	[16]



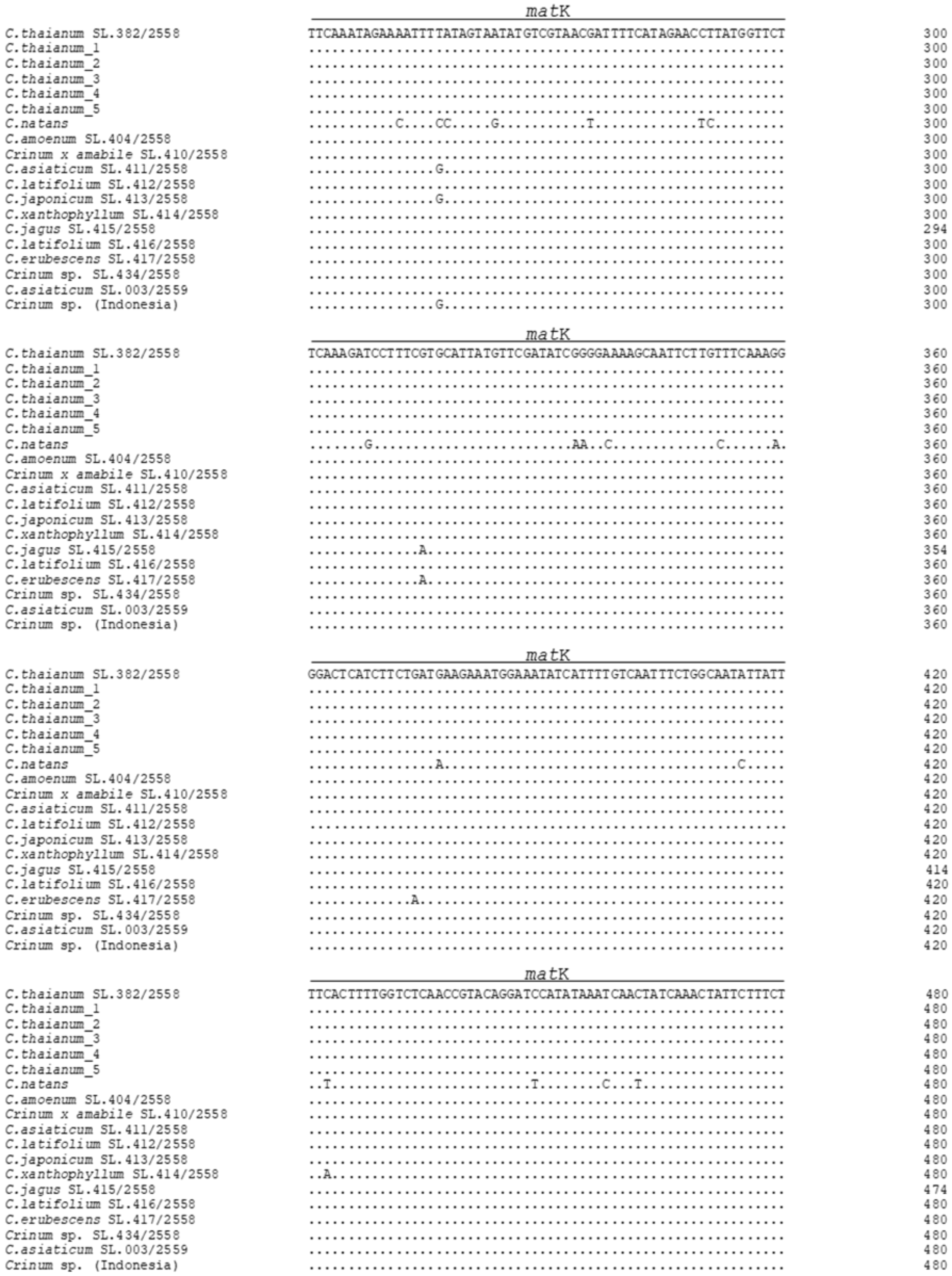
```
                                                        matK
C.thaianum SL.382/2558          ATTTATTGCGATTCTTTCTTCACGAATATCATAATTGGAATAGTCTTCTCATTACTCAGA    60
C.thaianum_1                    ............................................................    60
C.thaianum_2                    ............................................................    60
C.thaianum_3                    ............................................................    60
C.thaianum_4                    ............................................................    60
C.thaianum_5                    ............................................................    60
C.natans                        ...C................................CA......................    60
C.amoenum SL.404/2558           ............................................................    60
Crinum x amabile SL.410/2558    ............................................................    60
C.asiaticum SL.411/2558         ............................................................    60
C.latifolium SL.412/2558        ............................................................    60
C.japonicum SL.413/2558         ............................................................    60
C.xanthophyllum SL.414/2558     ............................................................    60
C.jagus SL.415/2558             ............................................................    60
C.latifolium SL.416/2558        ............................................................    60
C.erubescens SL.417/2558        ............................................................    60
Crinum sp. SL.434/2558          ............................................................    60
C.asiaticum SL.003/2559         ............................................................    60
Crinum sp. (Indonesia)          ............................................................    60

                                                        matK
C.thaianum SL.382/2558          AGAAATCCATTTACGTTTTTTCAAAAGAAAATAAAAGACTATTTCGGTTCTTATACAATT   120
C.thaianum_1                    ............................................................   120
C.thaianum_2                    ............................................................   120
C.thaianum_3                    ............................................................   120
C.thaianum_4                    ............................................................   120
C.thaianum_5                    ............................................................   120
C.natans                        .......T...C.........T.......................A...TC.........   120
C.amoenum SL.404/2558           ..................................................C.........   120
Crinum x amabile SL.410/2558    ............................................................   120
C.asiaticum SL.411/2558         ............................................................   120
C.latifolium SL.412/2558        ............................................................   120
C.japonicum SL.413/2558         ............................................................   120
C.xanthophyllum SL.414/2558     ............................................................   120
C.jagus SL.415/2558             ............................................................   120
C.latifolium SL.416/2558        ............................................................   120
C.erubescens SL.417/2558        ............................................................   120
Crinum sp. SL.434/2558          ............................................................   120
C.asiaticum SL.003/2559         ............................................................   120
Crinum sp. (Indonesia)          .T..........................................................   120

                                                        matK
C.thaianum SL.382/2558          TTTATGTATTTGAATGTGAATTTTTATTTGTTTTTATTCGTAAACAATCTTCTTATTTAC   180
C.thaianum_1                    ............................................................   180
C.thaianum_2                    ............................................................   180
C.thaianum_3                    ............................................................   180
C.thaianum_4                    ............................................................   180
C.thaianum_5                    ............................................................   180
C.natans                        C...........................A.......C.......................   180
C.amoenum SL.404/2558           ............................................................   180
Crinum x amabile SL.410/2558    ............................................................   180
C.asiaticum SL.411/2558         ............................................................   180
C.latifolium SL.412/2558        ............................................................   180
C.japonicum SL.413/2558         ............................................................   180
C.xanthophyllum SL.414/2558     ............................................................   180
C.jagus SL.415/2558             .................................................------.....   174
C.latifolium SL.416/2558        ............................................................   180
C.erubescens SL.417/2558        ............................................................   180
Crinum sp. SL.434/2558          ............................................................   180
C.asiaticum SL.003/2559         ............................................................   180
Crinum sp. (Indonesia)          ............................................................   180

                                                        matK
C.thaianum SL.382/2558          GATTAACATCTTTTGGAACTTTTCTTGAGCGAACACATTTCTATGGAAAAATAAAACATC   240
C.thaianum_1                    ............................................................   240
C.thaianum_2                    ............................................................   240
C.thaianum_3                    ............................................................   240
C.thaianum_4                    ............................................................   240
C.thaianum_5                    ............................................................   240
C.natans                        ............................A........................G......   240
C.amoenum SL.404/2558           ............................................................   240
Crinum x amabile SL.410/2558    ............................................................   240
C.asiaticum SL.411/2558         ............................................................   240
C.latifolium SL.412/2558        ............................................................   240
C.japonicum SL.413/2558         ............................................................   240
C.xanthophyllum SL.414/2558     ............................................................   240
C.jagus SL.415/2558             .....................................................G......   234
C.latifolium SL.416/2558        ............................................................   240
C.erubescens SL.417/2558        .....................................................G......   240
Crinum sp. SL.434/2558          ............................................................   240
C.asiaticum SL.003/2559         ............................................................   240
Crinum sp. (Indonesia)          ............................................................   240
```

Fig. S1-A Multiple DNA sequence alignment of three chloroplast specific gene regions of *Crinum* plants for phylogenetic analysis.



```
                                                        matK
                                  ____________________________________________________________
C.thaianum SL.382/2558            TTCAAATAGAAAATTTTATAGTAATATGTCGTAACGATTTTCATAGAACCTTATGGTTCT               300
C.thaianum_1                      ............................................................               300
C.thaianum_2                      ............................................................               300
C.thaianum_3                      ............................................................               300
C.thaianum_4                      ............................................................               300
C.thaianum_5                      ............................................................               300
C.natans                          ...........C....CC.....G...........T.............TC.........               300
C.amoenum SL.404/2558             ............................................................               300
Crinum x amabile SL.410/2558      ............................................................               300
C.asiaticum SL.411/2558           ................G...........................................               300
C.latifolium SL.412/2558          ............................................................               300
C.japonicum SL.413/2558           ................G...........................................               300
C.xanthophyllum SL.414/2558       ............................................................               300
C.jagus SL.415/2558               ............................................................               294
C.latifolium SL.416/2558          ............................................................               300
C.erubescens SL.417/2558          ............................................................               300
Crinum sp. SL.434/2558            ............................................................               300
C.asiaticum SL.003/2559           ............................................................               300
Crinum sp. (Indonesia)            ................G...........................................               300

                                                        matK
                                  ____________________________________________________________
C.thaianum SL.382/2558            TCAAAGATCCTTTCGTGCATTATGTTCGATATCGGGGAAAAGCAATTCTTGTTTCAAAGG               360
C.thaianum_1                      ............................................................               360
C.thaianum_2                      ............................................................               360
C.thaianum_3                      ............................................................               360
C.thaianum_4                      ............................................................               360
C.thaianum_5                      ............................................................               360
C.natans                          .......G.........................AA..C.............C......A.               360
C.amoenum SL.404/2558             ............................................................               360
Crinum x amabile SL.410/2558      ............................................................               360
C.asiaticum SL.411/2558           ............................................................               360
C.latifolium SL.412/2558          ............................................................               360
C.japonicum SL.413/2558           ............................................................               360
C.xanthophyllum SL.414/2558       ............................................................               360
C.jagus SL.415/2558               ..............A.............................................               354
C.latifolium SL.416/2558          ............................................................               360
C.erubescens SL.417/2558          ..............A.............................................               360
Crinum sp. SL.434/2558            ............................................................               360
C.asiaticum SL.003/2559           ............................................................               360
Crinum sp. (Indonesia)            ............................................................               360

                                                        matK
                                  ____________________________________________________________
C.thaianum SL.382/2558            GGACTCATCTTCTGATGAAGAAATGGAAATATCATTTTGTCAATTTCTGGCAATATTATT               420
C.thaianum_1                      ............................................................               420
C.thaianum_2                      ............................................................               420
C.thaianum_3                      ............................................................               420
C.thaianum_4                      ............................................................               420
C.thaianum_5                      ............................................................               420
C.natans                          ................A.....................................C.....               420
C.amoenum SL.404/2558             ............................................................               420
Crinum x amabile SL.410/2558      ............................................................               420
C.asiaticum SL.411/2558           ............................................................               420
C.latifolium SL.412/2558          ............................................................               420
C.japonicum SL.413/2558           ............................................................               420
C.xanthophyllum SL.414/2558       ............................................................               420
C.jagus SL.415/2558               ............................................................               414
C.latifolium SL.416/2558          ............................................................               420
C.erubescens SL.417/2558          .............A..............................................               420
Crinum sp. SL.434/2558            ............................................................               420
C.asiaticum SL.003/2559           ............................................................               420
Crinum sp. (Indonesia)            ............................................................               420

                                                        matK
                                  ____________________________________________________________
C.thaianum SL.382/2558            TTCACTTTTGGTCTCAACCGTACAGGATCCATATAAATCAACTATCAAACTATTCTTTCT               480
C.thaianum_1                      ............................................................               480
C.thaianum_2                      ............................................................               480
C.thaianum_3                      ............................................................               480
C.thaianum_4                      ............................................................               480
C.thaianum_5                      ............................................................               480
C.natans                          ..T.........................T........C...T..................               480
C.amoenum SL.404/2558             ............................................................               480
Crinum x amabile SL.410/2558      ............................................................               480
C.asiaticum SL.411/2558           ............................................................               480
C.latifolium SL.412/2558          ............................................................               480
C.japonicum SL.413/2558           ............................................................               480
C.xanthophyllum SL.414/2558       ..A.........................................................               480
C.jagus SL.415/2558               ............................................................               474
C.latifolium SL.416/2558          ............................................................               480
C.erubescens SL.417/2558          ............................................................               480
Crinum sp. SL.434/2558            ............................................................               480
C.asiaticum SL.003/2559           ............................................................               480
Crinum sp. (Indonesia)            ............................................................               480
```

Fig. S1-A Continued . . .

```
                                                          matK
C.thaianum SL.382/2558          ATTTTCTGGGTTATCTTTCAAGCCTACTAATAAATTCTTCGGCAGTAAGGAATCAAATGT     540
C.thaianum_1                    ............................................................     540
C.thaianum_2                    ............................................................     540
C.thaianum_3                    ............................................................     540
C.thaianum_4                    ............................................................     540
C.thaianum_5                    ............................................................     540
C.natans                        .....A........T.......T.......G............G................     540
C.amoenum SL.404/2558           ......................T.....................................     540
Crinum x amabile SL.410/2558    ......................T.....................................     540
C.asiaticum SL.411/2558         ......................T.....................................     540
C.latifolium SL.412/2558        ......................T.....................................     540
C.japonicum SL.413/2558         ......................T....A................................     540
C.xanthophyllum SL.414/2558     ......................T.....................................     540
C.jagus SL.415/2558             ......................T.....................................     534
C.latifolium SL.416/2558        ......................T.....................................     540
C.erubescens SL.417/2558        ......................T................T....................     540
Crinum sp. SL.434/2558          ......................T.....................................     540
C.asiaticum SL.003/2559         ......................T.....................................     540
Crinum sp. (Indonesia)          ......................T.....................................     540

                                                          matK
C.thaianum SL.382/2558          TAGAGAATTCATTTCTAATAGATACCGTTACTAAGAAATTTGATACCATAGTCCCGATTA     600
C.thaianum_1                    ............................................................     600
C.thaianum_2                    ............................................................     600
C.thaianum_3                    ............................................................     600
C.thaianum_4                    ............................................................     600
C.thaianum_5                    ............................................................     600
C.natans                        .........TC..............T......................A......AG...     600
C.amoenum SL.404/2558           ........................................................G...     600
Crinum x amabile SL.410/2558    ........................................................G...     600
C.asiaticum SL.411/2558         ........................................................G...     600
C.latifolium SL.412/2558        ........................................................G...     600
C.japonicum SL.413/2558         G..........................A...............A..T.........G...     600
C.xanthophyllum SL.414/2558     ........................................................G...     600
C.jagus SL.415/2558             ........................................................G...     594
C.latifolium SL.416/2558        ........................................................G...     600
C.erubescens SL.417/2558        ........................................................G...     600
Crinum sp. SL.434/2558          ........................................................G...     600
C.asiaticum SL.003/2559         ........................................................G...     600
Crinum sp. (Indonesia)          ........................................................G...     600

                                                          matK
C.thaianum SL.382/2558          TTCTTCTTATTGGATCCCTGTCTAAAGCTAAATTTTGTACCGTATCGGGCCATCCTATTA     660
C.thaianum_1                    ............................................................     660
C.thaianum_2                    ............................................................     660
C.thaianum_3                    ............................................................     660
C.thaianum_4                    ............................................................     660
C.thaianum_5                    ............................................................     660
C.natans                        ................TT..................................C..C....     660
C.amoenum SL.404/2558           ............................................................     660
Crinum x amabile SL.410/2558    ............................................................     660
C.asiaticum SL.411/2558         ............................................................     660
C.latifolium SL.412/2558        ............................................................     660
C.japonicum SL.413/2558         .G...................T.....A.....G..TGT....T.ATCCTTTC.A.CC..     660
C.xanthophyllum SL.414/2558     ............................................................     660
C.jagus SL.415/2558             ............................................................     654
C.latifolium SL.416/2558        ............................................................     660
C.erubescens SL.417/2558        ............................................................     660
Crinum sp. SL.434/2558          ............................................................     660
C.asiaticum SL.003/2559         ............................................................     660
Crinum sp. (Indonesia)          ............................................................     660

                                                          matK
C.thaianum SL.382/2558          GTAAGCCGATCT------GGGCCGATTTATCAGATT------CTGATATTCTTGATCGAT     708
C.thaianum_1                    ............------..................------..................     708
C.thaianum_2                    ............------..................------..................     708
C.thaianum_3                    ............------..................------..................     708
C.thaianum_4                    ............------..................------..................     708
C.thaianum_5                    ............------..................------..................     708
C.natans                        ............GGGCCGATTTATCAGAT.------------................--     706
C.amoenum SL.404/2558           ............------..................------..................     708
Crinum x amabile SL.410/2558    ............------..................------..................     708
C.latifolium SL.412/2558        ............------..................------..................     708
C.japonicum SL.413/2558         T..GTTACTATGGACCCT..T....AA...TCAC.AATCTTGA.T...C...GA...A.A     720
C.xanthophyllum SL.414/2558     ............------..................------..................     708
C.jagus SL.415/2558             ............------..................------..................     702
C.latifolium SL.416/2558        ............------..................------..................     708
C.erubescens SL.417/2558        ............------..................------..................     708
Crinum sp. SL.434/2558          ............------..................------..................     708
C.asiaticum SL.003/2559         ............------..................------..................     708
Crinum sp. (Indonesia)          ............------..................------..................     708
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Fig. S1-A Continued …

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                                                   matK
C.thaianum SL.382/2558        TCG---------GTCGGATATGTAGAAATCTTTCTCATTATCACAGCGGATCCTCAAAAA   759
C.thaianum_1                  ...---------................................................   759
C.thaianum_2                  ...---------................................................   759
C.thaianum_3                  ...---------................................................   759
C.thaianum_4                  ...---------................................................   759
C.thaianum_5                  ...---------................................................   759
C.natans                      -------ATTCG........................................T.......   759
C.amoenum SL.404/2558         ...---------................................................   759
Crinum x amabile SL.410/2558  ...---------................................................   759
C.asiaticum SL.411/2558       ...---------................................................   759
C.latifolium SL.412/2558      ...---------................................................   759
C.japonicum SL.413/2558       .T.GTTTGCGATT...T.G.AA.CATTC.....TAT..C.C.GACT..ATC...T..T..   780
C.xanthophyllum SL.414/2558   ...---------................................................   759
C.jagus SL.415/2558           ...---------..................................T.............   753
C.latifolium SL.416/2558      ...---------................................................   759
C.erubescens SL.417/2558      ...---------..................................T.............   759
Crinum sp. SL.434/2558        ...---------................................................   759
C.asiaticum SL.003/2559       ...---------................................................   759
Crinum sp. (Indonesia)        ...---------................................................   759

                              matK                       rbcL
C.thaianum SL.382/2558        AACAGGCTTATTATACTCCTGATTACGAAACCAAAGATACTGATATCTTGGCAGCATTCC   819
C.thaianum_1                  ............................................................   819
C.thaianum_2                  ............................................................   819
C.thaianum_3                  ............................................................   819
C.thaianum_4                  ............................................................   819
C.thaianum_5                  ............................................................   819
C.natans                      ......A.......................................T.............   819
C.amoenum SL.404/2558         ............................................................   819
Crinum x amabile SL.410/2558  ............................................................   819
C.asiaticum SL.411/2558       ............................................................   819
C.latifolium SL.412/2558      ............................................................   819
C.japonicum SL.413/2558       .TT.TA......................................................   840
C.xanthophyllum SL.414/2558   ............................................................   819
C.jagus SL.415/2558           ............................................................   813
C.latifolium SL.416/2558      ............................................................   819
C.erubescens SL.417/2558      ............................................................   819
Crinum sp. SL.434/2558        ............................................................   819
C.asiaticum SL.003/2559       ............................................................   819
Crinum sp. (Indonesia)        ............................................................   819

                                                   rbcL
C.thaianum SL.382/2558        GAGTAACTCCTCAACCCGGAGTTCCCGCTGAAGAGGCAGGGGCTGCGGTAGCTGCCGAAT   879
C.thaianum_1                  ............................................................   879
C.thaianum_2                  ............................................................   879
C.thaianum_3                  ............................................................   879
C.thaianum_4                  ............................................................   879
C.thaianum_5                  ............................................................   879
C.natans                      ................T.................A..............G..........   879
C.amoenum SL.404/2558         ............................................................   879
Crinum x amabile SL.410/2558  ............................................................   879
C.asiaticum SL.411/2558       ............................................................   879
C.latifolium SL.412/2558      ............................................................   879
C.japonicum SL.413/2558       ............................................................   900
C.xanthophyllum SL.414/2558   ............................................................   879
C.jagus SL.415/2558           ............................................................   873
C.latifolium SL.416/2558      ............................................................   879
C.erubescens SL.417/2558      ............................................................   879
Crinum sp. SL.434/2558        ............................................................   879
C.asiaticum SL.003/2559       ............................................................   879
Crinum sp. (Indonesia)        ............................................................   879

                                                   rbcL
C.thaianum SL.382/2558        CTTCTACCGGTACATGGACAACTGTGTGGACTGATGGACTTACCAGCCTTGATCGTTACA   939
C.thaianum_1                  ............................................................   939
C.thaianum_2                  ............................................................   939
C.thaianum_3                  ............................................................   939
C.thaianum_4                  ............................................................   939
C.thaianum_5                  ............................................................   939
C.natans                      .......T.......................C..C...........T.............   939
C.amoenum SL.404/2558         ............................................................   939
Crinum x amabile SL.410/2558  ............................................................   939
C.asiaticum SL.411/2558       ............................................................   939
C.latifolium SL.412/2558      ............................................................   939
C.japonicum SL.413/2558       ............................................................   960
C.xanthophyllum SL.414/2558   ............................................................   939
C.jagus SL.415/2558           ............................................................   933
C.latifolium SL.416/2558      ............................................................   939
C.erubescens SL.417/2558      ............................................................   939
Crinum sp. SL.434/2558        ............................................................   939
C.asiaticum SL.003/2559       ............................................................   939
Crinum sp. (Indonesia)        ............................................................   939
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Fig. S1-A Continued …

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                                                             rbcL
C.thaianum SL.382/2558          AAGGACGATGCTACCACATTGAGGCCGTTATTGGGGAAGAAAATCAATATATTGCTTATG     999
C.thaianum_1                    ............................................................     999
C.thaianum_2                    ............................................................     999
C.thaianum_3                    ............................................................     999
C.thaianum_4                    ............................................................     999
C.thaianum_5                    ............................................................     999
C.natans                        .............................G..............................     999
C.amoenum SL.404/2558           .............................G..............................     999
Crinum x amabile SL.410/2558    .............................G..............................     999
C.asiaticum SL.411/2558         .............................G..............................     999
C.latifolium SL.412/2558        .............................G..............................     999
C.japonicum SL.413/2558         .............................G..............................    1020
C.xanthophyllum SL.414/2558     .............................G..............................     999
C.jagus SL.415/2558             ..........T..................G..............................     993
C.latifolium SL.416/2558        .............................G..............................     999
C.erubescens SL.417/2558        .............................G..............................     999
Crinum sp. SL.434/2558          .............................G..............................     999
C.asiaticum SL.003/2559         .............................G..............................     999
Crinum sp. (Indonesia)          .............................G..............................     999
                                                             rbcL
C.thaianum SL.382/2558          TAGCTTATCCTTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTG    1059
C.thaianum_1                    ............................................................    1059
C.thaianum_2                    ............................................................    1059
C.thaianum_3                    ............................................................    1059
C.thaianum_4                    ............................................................    1059
C.thaianum_5                    ............................................................    1059
C.natans                        ............................................................    1059
C.amoenum SL.404/2558           ............................................................    1059
Crinum x amabile SL.410/2558    ............................................................    1059
C.asiaticum SL.411/2558         ............................................................    1059
C.latifolium SL.412/2558        ............................................................    1059
C.japonicum SL.413/2558         ............................................................    1080
C.xanthophyllum SL.414/2558     ............................................................    1059
C.jagus SL.415/2558             ............................................................    1053
C.latifolium SL.416/2558        ............................................................    1059
C.erubescens SL.417/2558        ............................................................    1059
Crinum sp. SL.434/2558          ............................................................    1059
C.asiaticum SL.003/2559         ............................................................    1059
Crinum sp. (Indonesia)          ............................................................    1059
                                                             rbcL
C.thaianum SL.382/2558          TGGGTAATGTATTTGGTTTCAAAGCCCTACGAGCTCTACGTCTGGAGGATCTGCGAATTC    1119
C.thaianum_1                    ............................................................    1119
C.thaianum_2                    ............................................................    1119
C.thaianum_3                    ............................................................    1119
C.thaianum_4                    ............................................................    1119
C.thaianum_5                    ............................................................    1119
C.natans                        ............................................................    1119
C.amoenum SL.404/2558           ............................................................    1119
Crinum x amabile SL.410/2558    ............................................................    1119
C.asiaticum SL.411/2558         ............................................................    1119
C.latifolium SL.412/2558        ............................................................    1119
C.japonicum SL.413/2558         ............................................................    1140
C.xanthophyllum SL.414/2558     ............................................................    1119
C.jagus SL.415/2558             ............................................................    1113
C.latifolium SL.416/2558        ............................................................    1119
C.erubescens SL.417/2558        ............................................................    1119
Crinum sp. SL.434/2558          ............................................................    1119
C.asiaticum SL.003/2559         ............................................................    1119
Crinum sp. (Indonesia)          ............................................................    1119
                                                             rbcL
C.thaianum SL.382/2558          CCCCTGCTTATTCCAAAACTTTCCAAGGCCCGCCCCATGGCATCCAAGTTGAAAGAGATA    1179
C.thaianum_1                    ............................................................    1179
C.thaianum_2                    ............................................................    1179
C.thaianum_3                    ............................................................    1179
C.thaianum_4                    ............................................................    1179
C.thaianum_5                    ............................................................    1179
C.natans                        ..A..T......................................................    1179
C.amoenum SL.404/2558           ....C.......................................................    1179
Crinum x amabile SL.410/2558    ............................................................    1179
C.asiaticum SL.411/2558         ............................................................    1179
C.latifolium SL.412/2558        ............................................................    1179
C.japonicum SL.413/2558         ............................................................    1200
C.xanthophyllum SL.414/2558     ............................................................    1179
C.jagus SL.415/2558             ............................................................    1173
C.latifolium SL.416/2558        ............................................................    1179
C.erubescens SL.417/2558        ............................................................    1179
Crinum sp. SL.434/2558          ............................................................    1179
C.asiaticum SL.003/2559         ............................................................    1179
Crinum sp. (Indonesia)          ............................................................    1179
```
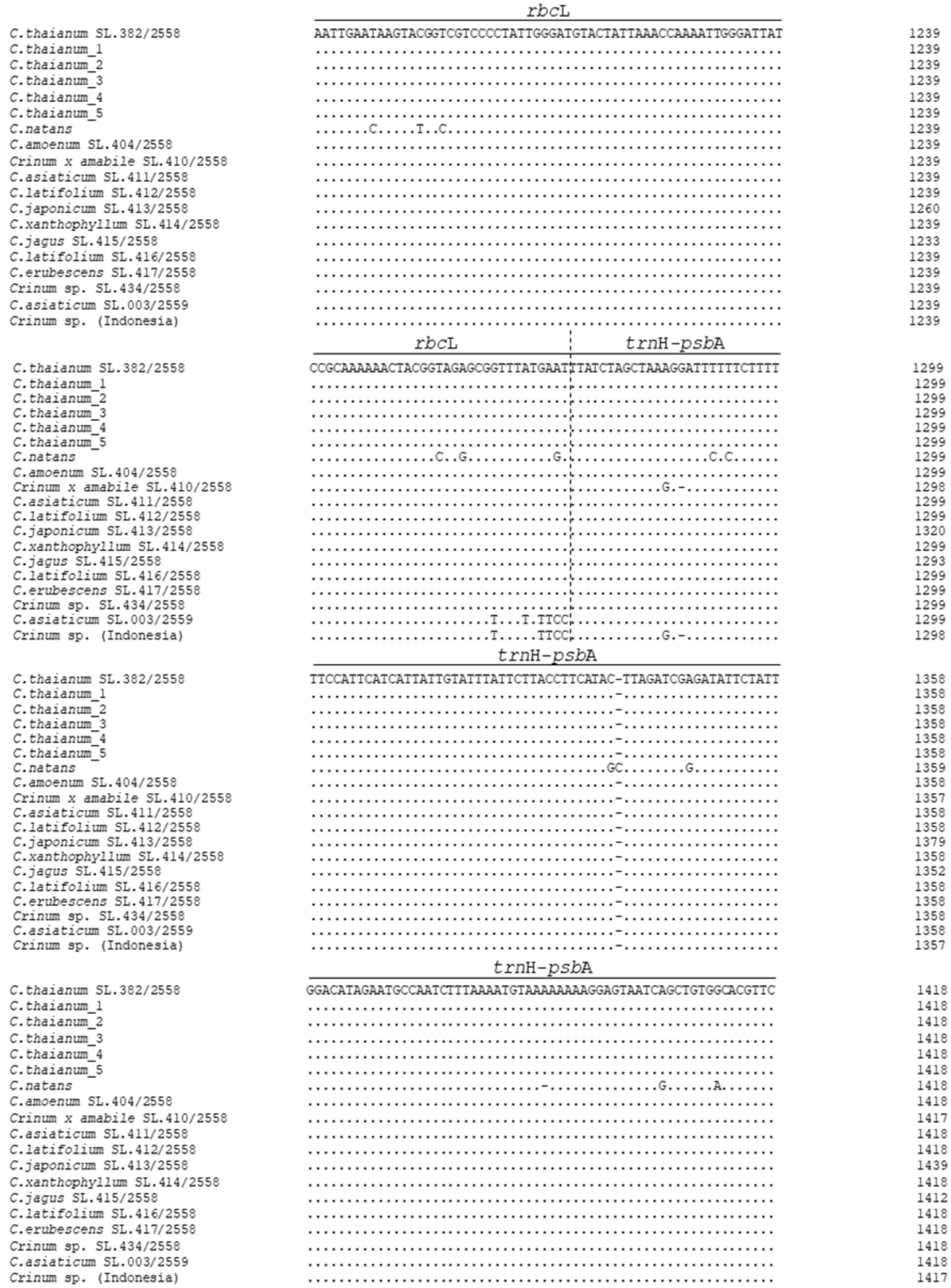

Fig. S1-A Continued …



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                                                                 rbcL
                                ____________________________________________________________
C.thaianum SL.382/2558          AATTGAATAAGTACGGTCGTCCCCTATTGGGATGTACTATTAAACCAAAATTGGGATTAT          1239
C.thaianum_1                    ............................................................          1239
C.thaianum_2                    ............................................................          1239
C.thaianum_3                    ............................................................          1239
C.thaianum_4                    ............................................................          1239
C.thaianum_5                    ............................................................          1239
C.natans                        .......C.....T..C...........................................          1239
C.amoenum SL.404/2558           ............................................................          1239
Crinum x amabile SL.410/2558    ............................................................          1239
C.asiaticum SL.411/2558         ............................................................          1239
C.latifolium SL.412/2558        ............................................................          1239
C.japonicum SL.413/2558         ............................................................          1260
C.xanthophyllum SL.414/2558     ............................................................          1239
C.jagus SL.415/2558             ............................................................          1233
C.latifolium SL.416/2558        ............................................................          1239
C.erubescens SL.417/2558        ............................................................          1239
Crinum sp. SL.434/2558          ............................................................          1239
C.asiaticum SL.003/2559         ............................................................          1239
Crinum sp. (Indonesia)          ............................................................          1239

                                             rbcL                    trnH-psbA
                                ____________________________________________________________
C.thaianum SL.382/2558          CCGCAAAAAACTACGGTAGAGCGGTTTATGAATTTATCTAGCTAAAGGATTTTTTCTTTT          1299
C.thaianum_1                    ............................................................          1299
C.thaianum_2                    ............................................................          1299
C.thaianum_3                    ............................................................          1299
C.thaianum_4                    ............................................................          1299
C.thaianum_5                    ............................................................          1299
C.natans                        ................C..G...........G...................C.C......          1299
C.amoenum SL.404/2558           ............................................................          1299
Crinum x amabile SL.410/2558    .............................................G.-............          1298
C.asiaticum SL.411/2558         ............................................................          1299
C.latifolium SL.412/2558        ............................................................          1299
C.japonicum SL.413/2558         ............................................................          1320
C.xanthophyllum SL.414/2558     ............................................................          1299
C.jagus SL.415/2558             ............................................................          1293
C.latifolium SL.416/2558        ............................................................          1299
C.erubescens SL.417/2558        ............................................................          1299
Crinum sp. SL.434/2558          ............................................................          1299
C.asiaticum SL.003/2559         .......................T...T.TTCC...........................          1299
Crinum sp. (Indonesia)          .......................T.....TTCC............G.-............          1298

                                                        trnH-psbA
                                ____________________________________________________________
C.thaianum SL.382/2558          TTCCATTCATCATTATTGTATTTATTCTTACCTTCATAC-TTAGATCGAGATATTCTATT          1358
C.thaianum_1                    .......................................-....................          1358
C.thaianum_2                    .......................................-....................          1358
C.thaianum_3                    .......................................-....................          1358
C.thaianum_4                    .......................................-....................          1358
C.thaianum_5                    .......................................-....................          1358
C.natans                        ......................................GC........G...........          1359
C.amoenum SL.404/2558           .......................................-....................          1358
Crinum x amabile SL.410/2558    .......................................-....................          1357
C.asiaticum SL.411/2558         .......................................-....................          1358
C.latifolium SL.412/2558        .......................................-....................          1358
C.japonicum SL.413/2558         .......................................-....................          1379
C.xanthophyllum SL.414/2558     .......................................-....................          1358
C.jagus SL.415/2558             .......................................-....................          1352
C.latifolium SL.416/2558        .......................................-....................          1358
C.erubescens SL.417/2558        .......................................-....................          1358
Crinum sp. SL.434/2558          .......................................-....................          1358
C.asiaticum SL.003/2559         .......................................-....................          1358
Crinum sp. (Indonesia)          .......................................-....................          1357

                                                        trnH-psbA
                                ____________________________________________________________
C.thaianum SL.382/2558          GGACATAGAATGCCAATCTTTAAAATGTAAAAAAAAGGAGTAATCAGCTGTGGCACGTTC          1418
C.thaianum_1                    ............................................................          1418
C.thaianum_2                    ............................................................          1418
C.thaianum_3                    ............................................................          1418
C.thaianum_4                    ............................................................          1418
C.thaianum_5                    ............................................................          1418
C.natans                        ..............................-..............G......A.......          1418
C.amoenum SL.404/2558           ............................................................          1418
Crinum x amabile SL.410/2558    ............................................................          1417
C.asiaticum SL.411/2558         ............................................................          1418
C.latifolium SL.412/2558        ............................................................          1418
C.japonicum SL.413/2558         ............................................................          1439
C.xanthophyllum SL.414/2558     ............................................................          1418
C.jagus SL.415/2558             ............................................................          1412
C.latifolium SL.416/2558        ............................................................          1418
C.erubescens SL.417/2558        ............................................................          1418
Crinum sp. SL.434/2558          ............................................................          1418
C.asiaticum SL.003/2559         ............................................................          1418
Crinum sp. (Indonesia)          ............................................................          1417
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Fig. S1-A Continued . . .

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                                                         trnH-psbA
C.thaianum SL.382/2558        ACTAAAAAAAAATCCTTTTGTAGCTAATCATTTATCGAGAAAAATTGAAAAACTCAACAT    1478
C.thaianum_1                  ............................................................    1478
C.thaianum_2                  ............................................................    1478
C.thaianum_3                  ............................................................    1478
C.thaianum_4                  ............................................................    1478
C.thaianum_5                  ............................................................    1478
C.natans                      ....................................T.......................    1478
C.amoenum SL.404/2558         ............................................................    1478
Crinum x amabile SL.410/2558  ............................................................    1477
C.asiaticum SL.411/2558       ............................................................    1478
C.latifolium SL.412/2558      ............................................................    1478
C.japonicum SL.413/2558       ............................................................    1499
C.xanthophyllum SL.414/2558   ............................................................    1478
C.jagus SL.415/2558           ............................................................    1472
C.latifolium SL.416/2558      ............................................................    1478
C.erubescens SL.417/2558      ............................................................    1478
Crinum sp. SL.434/2558        ............................................................    1478
C.asiaticum SL.003/2559       ............................................................    1478
Crinum sp. (Indonesia)        ............................................................    1477
                                                         trnH-psbA
C.thaianum SL.382/2558        GAGGGAGGAGAAAGAAATAATAGTAACTTGGTCTCGGGCATCTACCATTATACCCAAAAT    1538
C.thaianum_1                  ............................................................    1538
C.thaianum_2                  ............................................................    1538
C.thaianum_3                  ............................................................    1538
C.thaianum_4                  ............................................................    1538
C.thaianum_5                  ............................................................    1538
C.natans                      ............................................................    1538
C.amoenum SL.404/2558         ............................................................    1538
Crinum x amabile SL.410/2558  ............................................................    1537
C.asiaticum SL.411/2558       ............................................................    1538
C.latifolium SL.412/2558      ............................................................    1538
C.japonicum SL.413/2558       ............................................................    1559
C.xanthophyllum SL.414/2558   ............................................................    1538
C.jagus SL.415/2558           ..........................................C.................    1532
C.latifolium SL.416/2558      ............................................................    1538
C.erubescens SL.417/2558      ............................................................    1538
Crinum sp. SL.434/2558        ............................................................    1538
C.asiaticum SL.003/2559       ............................................................    1538
Crinum sp. (Indonesia)        ............................................................    1537
                                                         trnH-psbA
C.thaianum SL.382/2558        GATTGGCCATACAATCGCGATTCATAATGGAAAGGAACATTTACCTATTTATATAACAGA    1598
C.thaianum_1                  ............................................................    1598
C.thaianum_2                  ............................................................    1598
C.thaianum_3                  ............................................................    1598
C.thaianum_4                  ............................................................    1598
C.thaianum_5                  ............................................................    1598
C.natans                      ..................T.........................................    1598
C.amoenum SL.404/2558         ............................................................    1598
Crinum x amabile SL.410/2558  ............................................................    1597
C.asiaticum SL.411/2558       ............................................................    1598
C.latifolium SL.412/2558      ............................................................    1598
C.japonicum SL.413/2558       ............................................................    1619
C.xanthophyllum SL.414/2558   ............................................................    1598
C.jagus SL.415/2558           ............................................................    1592
C.latifolium SL.416/2558      ............................................................    1598
C.erubescens SL.417/2558      ............................................................    1598
Crinum sp. SL.434/2558        ............................................................    1598
C.asiaticum SL.003/2559       ............................................................    1598
Crinum sp. (Indonesia)        ............................................................    1597
                                                         trnH-psbA
C.thaianum SL.382/2558        TCGTATGGTAGGTCACAAATTGGGAGAATTCGCGCCTACTATGACTTTCGCGAGACATGC    1658
C.thaianum_1                  ............................................................    1658
C.thaianum_2                  ............................................................    1658
C.thaianum_3                  ............................................................    1658
C.thaianum_4                  ............................................................    1658
C.thaianum_5                  ............................................................    1658
C.natans                      ........................................C...................    1658
C.amoenum SL.404/2558         ........................................C...................    1658
Crinum x amabile SL.410/2558  ........................................C...................    1657
C.asiaticum SL.411/2558       ........................................C...................    1658
C.latifolium SL.412/2558      ........................................C...................    1658
C.japonicum SL.413/2558       ........................................C...................    1679
C.xanthophyllum SL.414/2558   ........................................C...................    1658
C.jagus SL.415/2558           ........................................C...................    1652
C.latifolium SL.416/2558      ........................................C...................    1658
C.erubescens SL.417/2558      ........................................C...................    1658
Crinum sp. SL.434/2558        ........................................C..G................    1658
C.asiaticum SL.003/2559       ........................................C...................    1658
Crinum sp. (Indonesia)        ........................................C...................    1657
```

Fig. S1-A Continued . . .

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                                                         trnH-psbA
C.thaianum SL.382/2558              GAGAAACGATAATAAATCTCGTCGTTAATTTAGAATAAAAATAAAAAATAGATACTTACA             1718
C.thaianum_1                        ............................................................             1718
C.thaianum_2                        ............................................................             1718
C.thaianum_3                        ............................................................             1718
C.thaianum_4                        ............................................................             1718
C.thaianum_5                        ............................................................             1718
C.natans                            ...............................G.........AC.T.GT.CAT.TA..GA.             1718
C.amoenum SL.404/2558               ..........................................T.................             1718
Crinum x amabile SL.410/2558        ............................................................             1717
C.asiaticum SL.411/2558             ............................................................             1718
C.latifolium SL.412/2558            ............................................................             1718
C.japonicum SL.413/2558             ............................................................             1739
C.xanthophyllum SL.414/2558         ............................................................             1718
C.jagus SL.415/2558                 ............................................................             1712
C.latifolium SL.416/2558            ............................................................             1718
C.erubescens SL.417/2558            ..........................................TT................             1718
Crinum sp. SL.434/2558              ............................................................             1718
C.asiaticum SL.003/2559             ............................................................             1718
Crinum sp. (Indonesia)              ............................................................             1717
                                                         trnH-psbA
C.thaianum SL.382/2558              TTGACGGGGGATAACCTTATGATAAAGGGAGCCATACTCAATATCTTACGAAAACAAGA-             1777
C.thaianum_1                        ...........................................................-             1777
C.thaianum_2                        ...........................................................-             1777
C.thaianum_3                        ...........................................................-             1777
C.thaianum_4                        ...........................................................-             1777
C.thaianum_5                        ...........................................................-             1777
C.natans                            TATTGAAGG--------------------AGCTATATCCAATAGCTTAAAAAAACAAGCG             1758
C.amoenum SL.404/2558               ...G.......................................................-             1777
Crinum x amabile SL.410/2558        ...G.......................................................-             1776
C.asiaticum SL.411/2558             ...G.......................................................-             1777
C.latifolium SL.412/2558            ...G.......................................................-             1777
C.japonicum SL.413/2558             ...G.......................................................-             1798
C.xanthophyllum SL.414/2558         ...G.......................................................-             1777
C.jagus SL.415/2558                 ...G.......................................................-             1771
C.latifolium SL.416/2558            ...G.......................................................-             1777
C.erubescens SL.417/2558            ...G.......................................................-             1777
Crinum sp. SL.434/2558              ...G.......................................................-             1777
C.asiaticum SL.003/2559             ...G.......................................................-             1777
Crinum sp. (Indonesia)              ...G.......................................................-             1776
                                                         trnH-psbA
C.thaianum SL.382/2558              ------TATTGGGTATGGCTCCTTCAACGATTCTTAATACATTAAGTTAAGACTTCTGTC             1831
C.thaianum_1                        ------......................................................             1831
C.thaianum_2                        ------......................................................             1831
C.thaianum_3                        ------......................................................             1831
C.thaianum_4                        ------......................................................             1831
C.thaianum_5                        ------......................................................             1831
C.natans                            ATTGCGGG.ATA.CTCCTTCAA....................A..CA......GAGAA...             1818
C.amoenum SL.404/2558               ------......................................................             1831
Crinum x amabile SL.410/2558        ------.........C............................................             1830
C.asiaticum SL.411/2558             ------.........C............................................             1831
C.latifolium SL.412/2558            ------......................................................             1831
C.japonicum SL.413/2558             ------.........C............................................             1852
C.xanthophyllum SL.414/2558         ------.........C............................................             1831
C.jagus SL.415/2558                 ------......................................................             1825
C.latifolium SL.416/2558            ------......................................................             1831
C.erubescens SL.417/2558            ------......................................................             1831
Crinum sp. SL.434/2558              ------.........C............................................             1831
C.asiaticum SL.003/2559             ------.........C............................................             1831
Crinum sp. (Indonesia)              ------.........C............................................             1830
                                                         trnH-psbA
C.thaianum SL.382/2558              TTATCCATTTGTAGCTGTAACTTCAACAGCAGCTAAGTCTAGAGGGAAGTTGTGAGCATT             1891
C.thaianum_1                        ............................................................             1891
C.thaianum_2                        ............................................................             1891
C.thaianum_3                        ............................................................             1891
C.thaianum_4                        ............................................................             1891
C.thaianum_5                        ......................-...........-.........................             1889
C.natans                            ..........A...A..G.................G........................             1878
C.amoenum SL.404/2558               ............................................................             1891
Crinum x amabile SL.410/2558        ............................................................             1890
C.asiaticum SL.411/2558             ............................................................             1891
C.latifolium SL.412/2558            ............................................................             1891
C.japonicum SL.413/2558             ............................................................             1912
C.xanthophyllum SL.414/2558         ............................................................             1891
C.jagus SL.415/2558                 .................G.................G........................             1885
C.latifolium SL.416/2558            ............................................................             1891
C.erubescens SL.417/2558            ....A............G.................G...............A.....T..             1891
Crinum sp. SL.434/2558              ............................................................             1891
C.asiaticum SL.003/2559             ............................................................             1891
Crinum sp. (Indonesia)              .................................................C..C.......             1890

                                  trnH-psbA
C.thaianum SL.382/2558              ACGTTCATG                                                                1900
C.thaianum_1                        ......T..                                                                1900
C.thaianum_2                        .........                                                                1900
C.thaianum_3                        .........                                                                1900
C.thaianum_4                        .........                                                                1900
C.thaianum_5                        .........                                                                1898
C.natans                            .........                                                                1887
C.amoenum SL.404/2558               ......T..                                                                1900
Crinum x amabile SL.410/2558        .....AT..                                                                1899
C.asiaticum SL.411/2558             ....CTTG-                                                                1899
C.latifolium SL.412/2558            .....AT..                                                                1900
C.japonicum SL.413/2558             ....CATG-                                                                1920
C.xanthophyllum SL.414/2558         .........                                                                1900
C.jagus SL.415/2558                 .........                                                                1894
C.latifolium SL.416/2558            .........                                                                1900
C.erubescens SL.417/2558            T.T...T..                                                                1900
Crinum sp. SL.434/2558              .........                                                                1900
C.asiaticum SL.003/2559             .....AT..                                                                1900
Crinum sp. (Indonesia)              .........                                                                1899
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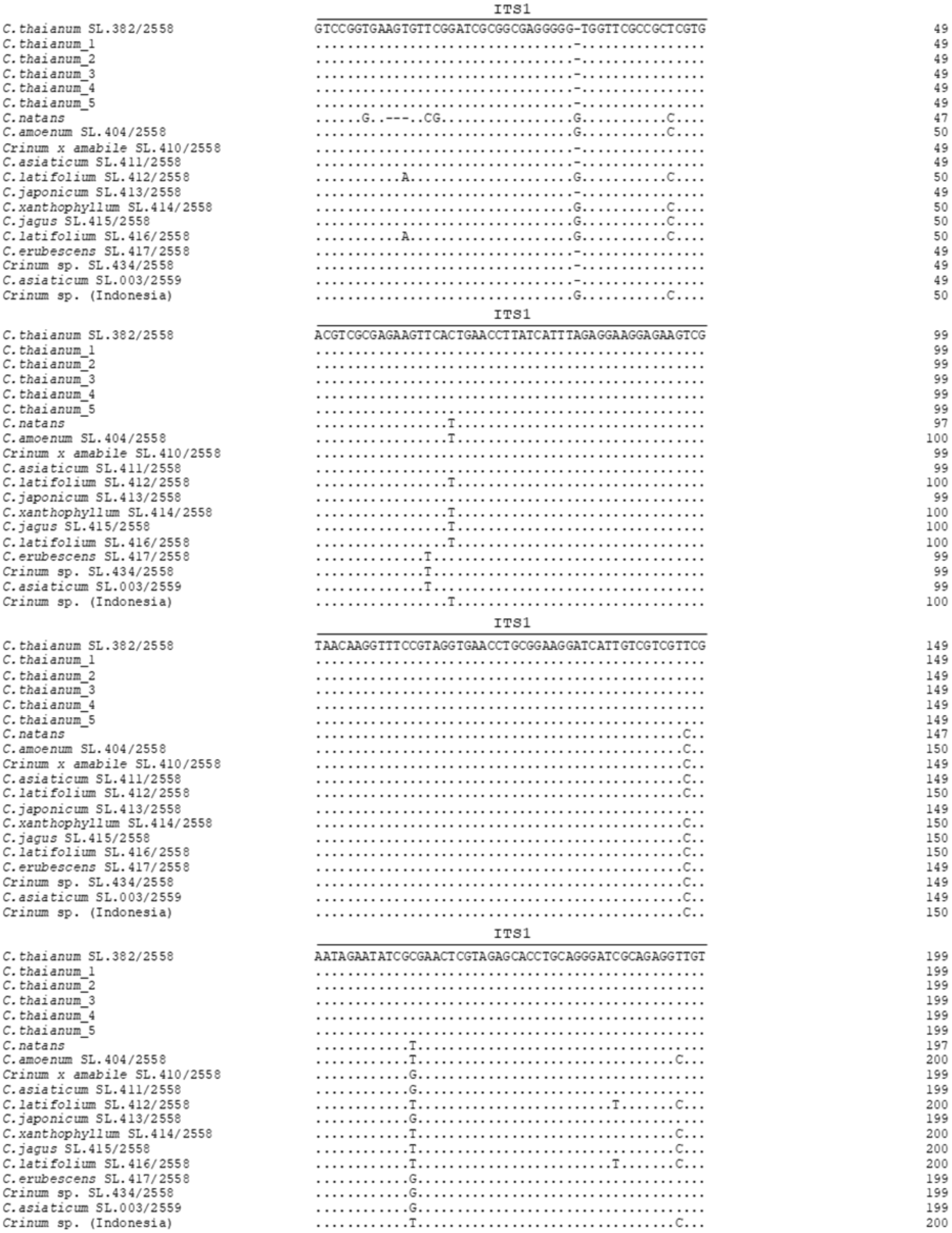
Fig. S1-A Continued . . .



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                                                   ITS1
C.thaianum SL.382/2558          GTCCGGTGAAGTGTTCGGATCGCGGCGAGGGGG-TGGTTCGCCGCTCGTG    49
C.thaianum_1                    .................................-................    49
C.thaianum_2                    .................................-................    49
C.thaianum_3                    .................................-................    49
C.thaianum_4                    .................................-................    49
C.thaianum_5                    .................................-................    49
C.natans                        ......G..---..CG.................G...........C....    47
C.amoenum SL.404/2558           .................................G...........C....    50
Crinum x amabile SL.410/2558    .................................-................    49
C.asiaticum SL.411/2558         .................................-................    49
C.latifolium SL.412/2558        ...........A.....................G...........C....    50
C.japonicum SL.413/2558         .................................-................    49
C.xanthophyllum SL.414/2558     .................................G...........C....    50
C.jagus SL.415/2558             .................................G...........C....    50
C.latifolium SL.416/2558        ...........A.....................G...........C....    50
C.erubescens SL.417/2558        .................................-................    49
Crinum sp. SL.434/2558          .................................-................    49
C.asiaticum SL.003/2559         .................................-................    49
Crinum sp. (Indonesia)          .................................G...........C....    50
                                                   ITS1
C.thaianum SL.382/2558          ACGTCGCGAGAAGTTCACTGAACCTTATCATTTAGAGGAAGGAGAAGTCG    99
C.thaianum_1                    ..................................................    99
C.thaianum_2                    ..................................................    99
C.thaianum_3                    ..................................................    99
C.thaianum_4                    ..................................................    99
C.thaianum_5                    ..................................................    99
C.natans                        .................T................................    97
C.amoenum SL.404/2558           .................T................................   100
Crinum x amabile SL.410/2558    ..................................................    99
C.asiaticum SL.411/2558         ..................................................    99
C.latifolium SL.412/2558        .................T................................   100
C.japonicum SL.413/2558         ..................................................    99
C.xanthophyllum SL.414/2558     .................T................................   100
C.jagus SL.415/2558             .................T................................   100
C.latifolium SL.416/2558        .................T................................   100
C.erubescens SL.417/2558        ..............T...................................    99
Crinum sp. SL.434/2558          ..............T...................................    99
C.asiaticum SL.003/2559         ..............T...................................    99
Crinum sp. (Indonesia)          .................T................................   100
                                                   ITS1
C.thaianum SL.382/2558          TAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGTCGTTCG   149
C.thaianum_1                    ..................................................   149
C.thaianum_2                    ..................................................   149
C.thaianum_3                    ..................................................   149
C.thaianum_4                    ..................................................   149
C.thaianum_5                    ..................................................   149
C.natans                        ...............................................C..   147
C.amoenum SL.404/2558           ...............................................C..   150
Crinum x amabile SL.410/2558    ...............................................C..   149
C.asiaticum SL.411/2558         ...............................................C..   149
C.latifolium SL.412/2558        ...............................................C..   150
C.japonicum SL.413/2558         ..................................................   149
C.xanthophyllum SL.414/2558     ...............................................C..   150
C.jagus SL.415/2558             ...............................................C..   150
C.latifolium SL.416/2558        ...............................................C..   150
C.erubescens SL.417/2558        ...............................................C..   149
Crinum sp. SL.434/2558          ...............................................C..   149
C.asiaticum SL.003/2559         ...............................................C..   149
Crinum sp. (Indonesia)          ...............................................C..   150
                                                   ITS1
C.thaianum SL.382/2558          AATAGAATATCGCGAACTCGTAGAGCACCTGCAGGGATCGCAGAGGTTGT   199
C.thaianum_1                    ..................................................   199
C.thaianum_2                    ..................................................   199
C.thaianum_3                    ..................................................   199
C.thaianum_4                    ..................................................   199
C.thaianum_5                    ..................................................   199
C.natans                        ............T.....................................   197
C.amoenum SL.404/2558           ............T.................................C...   200
Crinum x amabile SL.410/2558    ............G.....................................   199
C.asiaticum SL.411/2558         ............G.....................................   199
C.latifolium SL.412/2558        ............T.........................T.......C...   200
C.japonicum SL.413/2558         ............G.....................................   199
C.xanthophyllum SL.414/2558     ............T.................................C...   200
C.jagus SL.415/2558             ............T.................................C...   200
C.latifolium SL.416/2558        ............T.........................T.......C...   200
C.erubescens SL.417/2558        ............G.....................................   199
Crinum sp. SL.434/2558          ............G.....................................   199
C.asiaticum SL.003/2559         ............G.....................................   199
Crinum sp. (Indonesia)          ............T.................................C...   200
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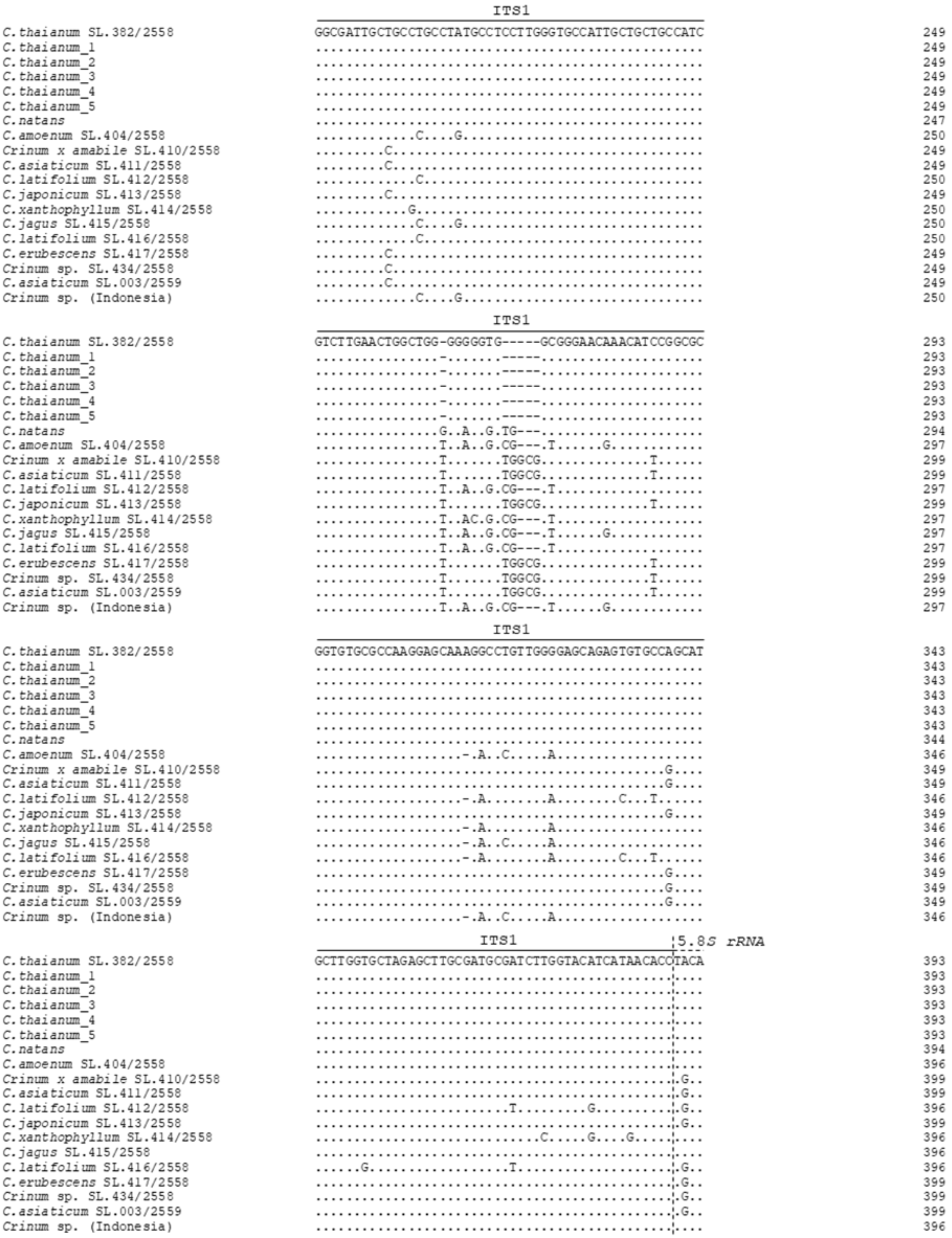
Fig. S1-B Multiple DNA sequence alignment of nuclear ITS of *rRNA* gene region of *Crinum* plants for phylogenetic analysis.



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                                                     ITS1
C.thaianum SL.382/2558        GGCGATTGCTGCCTGCCTATGCCTCCTTGGGTGCCATTGCTGCTGCCATC   249
C.thaianum_1                  ..................................................   249
C.thaianum_2                  ..................................................   249
C.thaianum_3                  ..................................................   249
C.thaianum_4                  ..................................................   249
C.thaianum_5                  ..................................................   249
C.natans                      ..................................................   247
C.amoenum SL.404/2558         .............C....G...............................   250
Crinum x amabile SL.410/2558  .........C........................................   249
C.asiaticum SL.411/2558       .........C........................................   249
C.latifolium SL.412/2558      .............C....................................   250
C.japonicum SL.413/2558       .........C........................................   249
C.xanthophyllum SL.414/2558   ............G.....................................   250
C.jagus SL.415/2558           .............C....G...............................   250
C.latifolium SL.416/2558      .............C....................................   250
C.erubescens SL.417/2558      .........C........................................   249
Crinum sp. SL.434/2558        .........C........................................   249
C.asiaticum SL.003/2559       .........C........................................   249
Crinum sp. (Indonesia)        .............C....G...............................   250
                                                     ITS1
C.thaianum SL.382/2558        GTCTTGAACTGGCTGG-GGGGGTG-----GCGGGAACAAACATCCGGCGC   293
C.thaianum_1                  ................-.......-----.....................   293
C.thaianum_2                  ................-.......-----.....................   293
C.thaianum_3                  ................-.......-----.....................   293
C.thaianum_4                  ................-.......-----.....................   293
C.thaianum_5                  ................-.......-----.....................   293
C.natans                      ................G..A..G.TG---.....................   294
C.amoenum SL.404/2558         ................T..A..G.CG---.T......G............   297
Crinum x amabile SL.410/2558  ................T.......TGGCG..............T......   299
C.asiaticum SL.411/2558       ................T.......TGGCG..............T......   299
C.latifolium SL.412/2558      ................T..A..G.CG---.T...................   297
C.japonicum SL.413/2558       ................T.......TGGCG..............T......   299
C.xanthophyllum SL.414/2558   ................T..AC.G.CG---.T...................   297
C.jagus SL.415/2558           ................T..A..G.CG---.T......G............   297
C.latifolium SL.416/2558      ................T..A..G.CG---.T...................   297
C.erubescens SL.417/2558      ................T.......TGGCG..............T......   299
Crinum sp. SL.434/2558        ................T.......TGGCG..............T......   299
C.asiaticum SL.003/2559       ................T.......TGGCG..............T......   299
Crinum sp. (Indonesia)        ................T..A..G.CG---.T......G............   297
                                                     ITS1
C.thaianum SL.382/2558        GGTGTGCGCCAAGGAGCAAAGGCCTGTTGGGGAGCAGAGTGTGCCAGCAT   343
C.thaianum_1                  ..................................................   343
C.thaianum_2                  ..................................................   343
C.thaianum_3                  ..................................................   343
C.thaianum_4                  ..................................................   343
C.thaianum_5                  ..................................................   343
C.natans                      ..................................................   344
C.amoenum SL.404/2558         ...................-.A..C.....A...................   346
Crinum x amabile SL.410/2558  .............................................G....   349
C.asiaticum SL.411/2558       .............................................G....   349
C.latifolium SL.412/2558      ...................-.A........A........C...T......   346
C.japonicum SL.413/2558       .............................................G....   349
C.xanthophyllum SL.414/2558   ...................-.A........A...................   346
C.jagus SL.415/2558           ...................-.A..C.....A...................   346
C.latifolium SL.416/2558      ...................-.A........A........C...T......   346
C.erubescens SL.417/2558      .............................................G....   349
Crinum sp. SL.434/2558        .............................................G....   349
C.asiaticum SL.003/2559       .............................................G....   349
Crinum sp. (Indonesia)        ...................-.A..C.....A...................   346
                                                   ITS1                 5.8S rRNA
C.thaianum SL.382/2558        GCTTGGTGCTAGAGCTTGCGATGCGATCTTGGTACATCATAACACCTACA   393
C.thaianum_1                  ..................................................   393
C.thaianum_2                  ..................................................   393
C.thaianum_3                  ..................................................   393
C.thaianum_4                  ..................................................   393
C.thaianum_5                  ..................................................   393
C.natans                      ..................................................   394
C.amoenum SL.404/2558         ..................................................   396
Crinum x amabile SL.410/2558  ...............................................G..   399
C.asiaticum SL.411/2558       ...............................................G..   399
C.latifolium SL.412/2558      .........................T.........G...........G..   396
C.japonicum SL.413/2558       ...............................................G..   399
C.xanthophyllum SL.414/2558   .............................C.....G....G.........   396
C.jagus SL.415/2558           ..................................................   396
C.latifolium SL.416/2558      ......G..................T.....................G..   396
C.erubescens SL.417/2558      ...............................................G..   399
Crinum sp. SL.434/2558        ...............................................G..   399
C.asiaticum SL.003/2559       ...............................................G..   399
Crinum sp. (Indonesia)        ..................................................   396
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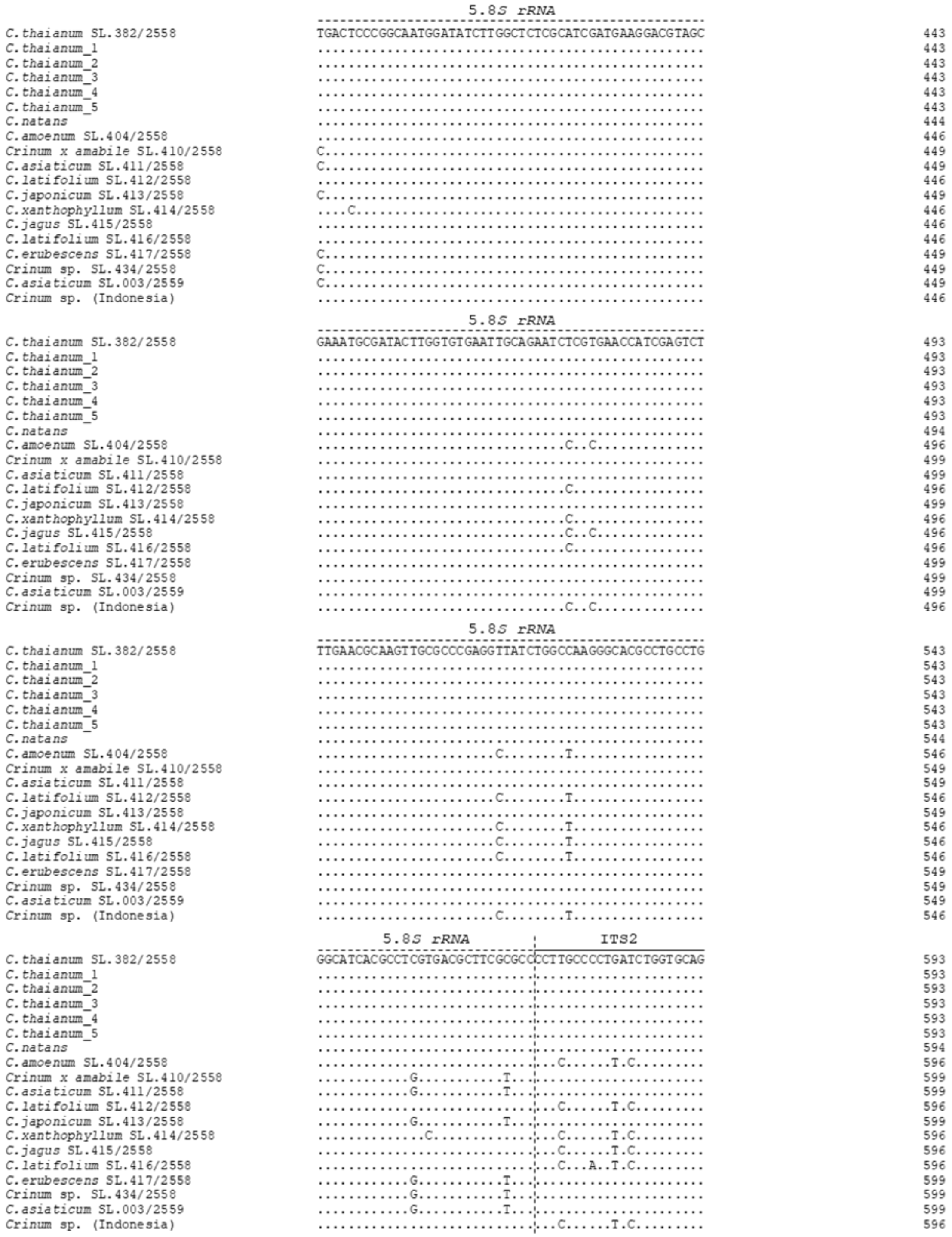
Fig. S1-B Continued …



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                                                  5.8S rRNA
                                 --------------------------------------------------
C.thaianum SL.382/2558           TGACTCCCGGCAATGGATATCTTGGCTCTCGCATCGATGAAGGACGTAGC          443
C.thaianum_1                     ..................................................          443
C.thaianum_2                     ..................................................          443
C.thaianum_3                     ..................................................          443
C.thaianum_4                     ..................................................          443
C.thaianum_5                     ..................................................          443
C.natans                         ..................................................          444
C.amoenum SL.404/2558            ..................................................          446
Crinum x amabile SL.410/2558     C.................................................          449
C.asiaticum SL.411/2558          C.................................................          449
C.latifolium SL.412/2558         ..................................................          446
C.japonicum SL.413/2558          C.................................................          449
C.xanthophyllum SL.414/2558      ....C.............................................          446
C.jagus SL.415/2558              ..................................................          446
C.latifolium SL.416/2558         ..................................................          446
C.erubescens SL.417/2558         C.................................................          449
Crinum sp. SL.434/2558           C.................................................          449
C.asiaticum SL.003/2559          C.................................................          449
Crinum sp. (Indonesia)           ..................................................          446

                                                  5.8S rRNA
                                 --------------------------------------------------
C.thaianum SL.382/2558           GAAATGCGATACTTGGTGTGAATTGCAGAATCTCGTGAACCATCGAGTCT          493
C.thaianum_1                     ..................................................          493
C.thaianum_2                     ..................................................          493
C.thaianum_3                     ..................................................          493
C.thaianum_4                     ..................................................          493
C.thaianum_5                     ..................................................          493
C.natans                         ..................................................          494
C.amoenum SL.404/2558            ................................C..C..............          496
Crinum x amabile SL.410/2558     ..................................................          499
C.asiaticum SL.411/2558          ..................................................          499
C.latifolium SL.412/2558         ................................C.................          496
C.japonicum SL.413/2558          ..................................................          499
C.xanthophyllum SL.414/2558      ................................C.................          496
C.jagus SL.415/2558              ................................C..C..............          496
C.latifolium SL.416/2558         ................................C.................          496
C.erubescens SL.417/2558         ..................................................          499
Crinum sp. SL.434/2558           ..................................................          499
C.asiaticum SL.003/2559          ..................................................          499
Crinum sp. (Indonesia)           ................................C..C..............          496

                                                  5.8S rRNA
                                 --------------------------------------------------
C.thaianum SL.382/2558           TTGAACGCAAGTTGCGCCCGAGGTTATCTGGCCAAGGGCACGCCTGCCTG          543
C.thaianum_1                     ..................................................          543
C.thaianum_2                     ..................................................          543
C.thaianum_3                     ..................................................          543
C.thaianum_4                     ..................................................          543
C.thaianum_5                     ..................................................          543
C.natans                         ..................................................          544
C.amoenum SL.404/2558            .......................C........T.................          546
Crinum x amabile SL.410/2558     ..................................................          549
C.asiaticum SL.411/2558          ..................................................          549
C.latifolium SL.412/2558         .......................C........T.................          546
C.japonicum SL.413/2558          ..................................................          549
C.xanthophyllum SL.414/2558      .......................C........T.................          546
C.jagus SL.415/2558              .......................C........T.................          546
C.latifolium SL.416/2558         .......................C........T.................          546
C.erubescens SL.417/2558         ..................................................          549
Crinum sp. SL.434/2558           ..................................................          549
C.asiaticum SL.003/2559          ..................................................          549
Crinum sp. (Indonesia)           .......................C........T.................          546

                                            5.8S rRNA          |       ITS2
                                 ----------------------------|----------------------
C.thaianum SL.382/2558           GGCATCACGCCTCGTGACGCTTCGCGCC|CCTTGCCCCTGATCTGGTGCAG          593
C.thaianum_1                     ............................|......................          593
C.thaianum_2                     ............................|......................          593
C.thaianum_3                     ............................|......................          593
C.thaianum_4                     ............................|......................          593
C.thaianum_5                     ............................|......................          593
C.natans                         ............................|......................          594
C.amoenum SL.404/2558            ............................|...C......T.C.........          596
Crinum x amabile SL.410/2558     ............G...........T...|......................          599
C.asiaticum SL.411/2558          ............G...........T...|......................          599
C.latifolium SL.412/2558         ............................|...C......T.C.........          596
C.japonicum SL.413/2558          ............G...........T...|......................          599
C.xanthophyllum SL.414/2558      ..............C.............|...C......T.C.........          596
C.jagus SL.415/2558              ............................|...C......T.C.........          596
C.latifolium SL.416/2558         ............................|...C...A..T.C.........          596
C.erubescens SL.417/2558         ............G...........T...|......................          599
Crinum sp. SL.434/2558           ............G...........T...|......................          599
C.asiaticum SL.003/2559          ............G...........T...|......................          599
Crinum sp. (Indonesia)           ............................|...C......T.C.........          596
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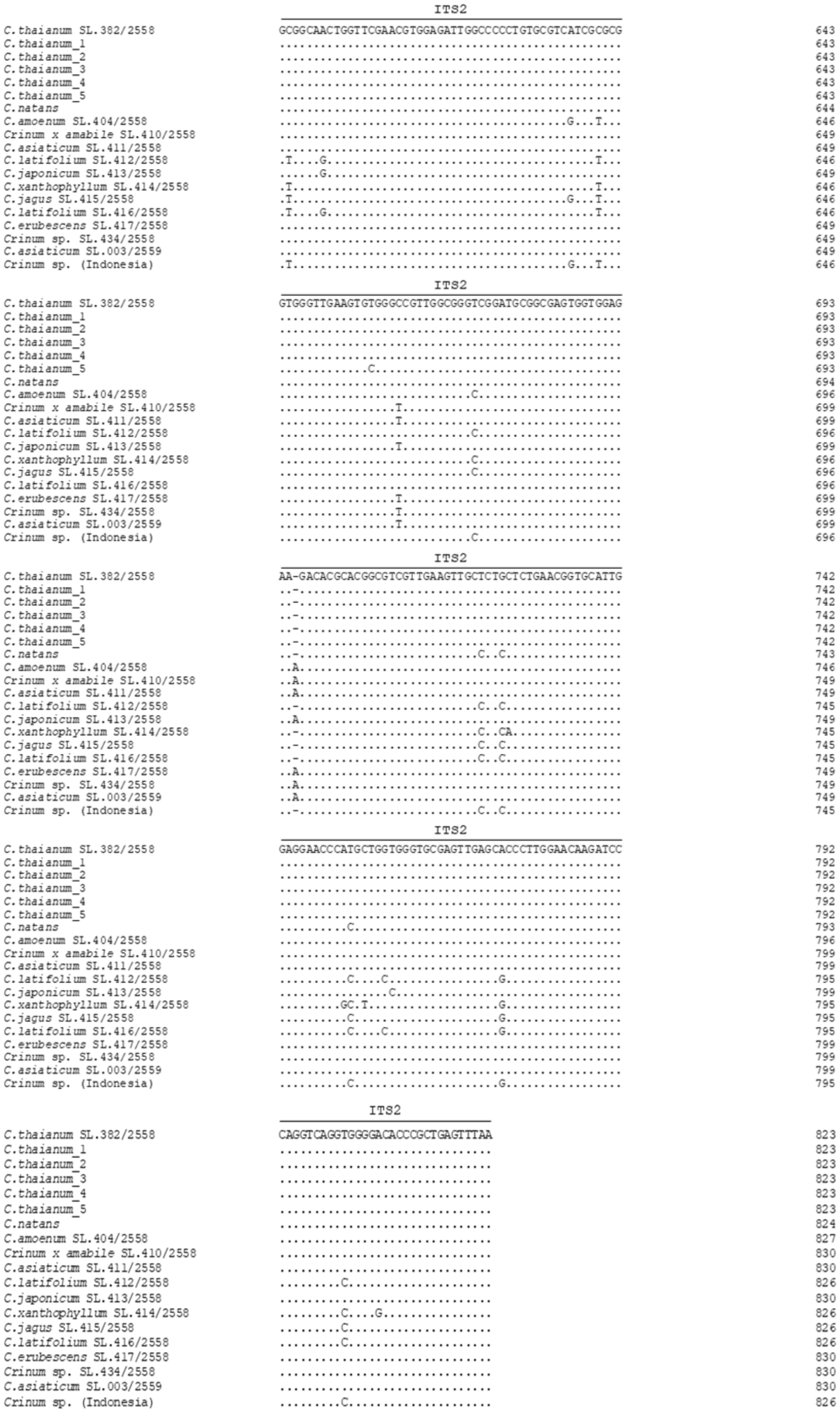
Fig. S1-B Continued . . .



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                                                         ITS2
C.thaianum SL.382/2558          GCGGCAACTGGTTCGAACGTGGAGATTGGCCCCCTGTGCGTCATCGCGCG    643
C.thaianum_1                    ..................................................    643
C.thaianum_2                    ..................................................    643
C.thaianum_3                    ..................................................    643
C.thaianum_4                    ..................................................    643
C.thaianum_5                    ..................................................    643
C.natans                        ..................................................    644
C.amoenum SL.404/2558           ..........................................G...T...    646
Crinum x amabile SL.410/2558    ..................................................    649
C.asiaticum SL.411/2558         ..................................................    649
C.latifolium SL.412/2558        .T....G.......................................T...    646
C.japonicum SL.413/2558         ......G...........................................    649
C.xanthophyllum SL.414/2558     .T............................................T...    646
C.jagus SL.415/2558             .T........................................G...T...    646
C.latifolium SL.416/2558        .T....G.......................................T...    646
C.erubescens SL.417/2558        ..................................................    649
Crinum sp. SL.434/2558          ..................................................    649
C.asiaticum SL.003/2559         ..................................................    649
Crinum sp. (Indonesia)          .T........................................G...T...    646

                                                         ITS2
C.thaianum SL.382/2558          GTGGGTTGAAGTGTGGGCCGTTGGCGGGTCGGATGCGGCGAGTGGTGGAG    693
C.thaianum_1                    ..................................................    693
C.thaianum_2                    ..................................................    693
C.thaianum_3                    ..................................................    693
C.thaianum_4                    ..................................................    693
C.thaianum_5                    .............C....................................    693
C.natans                        ..................................................    694
C.amoenum SL.404/2558           ............................C.....................    696
Crinum x amabile SL.410/2558    .................T................................    699
C.asiaticum SL.411/2558         .................T................................    699
C.latifolium SL.412/2558        ............................C.....................    696
C.japonicum SL.413/2558         .................T................................    699
C.xanthophyllum SL.414/2558     ............................C.....................    696
C.jagus SL.415/2558             ............................C.....................    696
C.latifolium SL.416/2558        ..................................................    696
C.erubescens SL.417/2558        .................T................................    699
Crinum sp. SL.434/2558          .................T................................    699
C.asiaticum SL.003/2559         .................T................................    699
Crinum sp. (Indonesia)          ............................C.....................    696

                                                         ITS2
C.thaianum SL.382/2558          AA-GACACGCACGGCGTCGTTGAAGTTGCTCTGCTCTGAACGGTGCATTG    742
C.thaianum_1                    ..-...............................................    742
C.thaianum_2                    ..-...............................................    742
C.thaianum_3                    ..-...............................................    742
C.thaianum_4                    ..-...............................................    742
C.thaianum_5                    ..-...............................................    742
C.natans                        ..-..........................C..C.................    743
C.amoenum SL.404/2558           ..A...............................................    746
Crinum x amabile SL.410/2558    ..A...............................................    749
C.asiaticum SL.411/2558         ..A...............................................    749
C.latifolium SL.412/2558        ..-..........................C..C.................    745
C.japonicum SL.413/2558         ..A...............................................    749
C.xanthophyllum SL.414/2558     ..-..........................C..CA................    745
C.jagus SL.415/2558             ..-..........................C..C.................    745
C.latifolium SL.416/2558        ..-..........................C..C.................    745
C.erubescens SL.417/2558        ..A...............................................    749
Crinum sp. SL.434/2558          ..A...............................................    749
C.asiaticum SL.003/2559         ..A...............................................    749
Crinum sp. (Indonesia)          ..-..........................C..C.................    745

                                                         ITS2
C.thaianum SL.382/2558          GAGGAACCCATGCTGGTGGGTGCGAGTTGAGCACCCTTGGAACAAGATCC    792
C.thaianum_1                    ..................................................    792
C.thaianum_2                    ..................................................    792
C.thaianum_3                    ..................................................    792
C.thaianum_4                    ..................................................    792
C.thaianum_5                    ..................................................    792
C.natans                        ..........C.......................................    793
C.amoenum SL.404/2558           ..................................................    796
Crinum x amabile SL.410/2558    ..................................................    799
C.asiaticum SL.411/2558         ..................................................    799
C.latifolium SL.412/2558        ..........C....C................G.................    795
C.japonicum SL.413/2558         ................C.................................    799
C.xanthophyllum SL.414/2558     .........GC.T...................G.................    795
C.jagus SL.415/2558             ..........C.....................G.................    795
C.latifolium SL.416/2558        ..........C....C................G.................    795
C.erubescens SL.417/2558        ..................................................    799
Crinum sp. SL.434/2558          ..................................................    799
C.asiaticum SL.003/2559         ..................................................    799
Crinum sp. (Indonesia)          ..........C.....................G.................    795

                                              ITS2
C.thaianum SL.382/2558          CAGGTCAGGTGGGGACACCCGCTGAGTTTAA                       823
C.thaianum_1                    ...............................                       823
C.thaianum_2                    ...............................                       823
C.thaianum_3                    ...............................                       823
C.thaianum_4                    ...............................                       823
C.thaianum_5                    ...............................                       823
C.natans                        ...............................                       824
C.amoenum SL.404/2558           ...............................                       827
Crinum x amabile SL.410/2558    ...............................                       830
C.asiaticum SL.411/2558         ...............................                       830
C.latifolium SL.412/2558        .........C.....................                       826
C.japonicum SL.413/2558         ...............................                       830
C.xanthophyllum SL.414/2558     .........C....G................                       826
C.jagus SL.415/2558             .........C.....................                       826
C.latifolium SL.416/2558        .........C.....................                       826
C.erubescens SL.417/2558        ...............................                       830
Crinum sp. SL.434/2558          ...............................                       830
C.asiaticum SL.003/2559         ...............................                       830
Crinum sp. (Indonesia)          .........C.....................                       826
```

Fig. S1-B Continued . . .

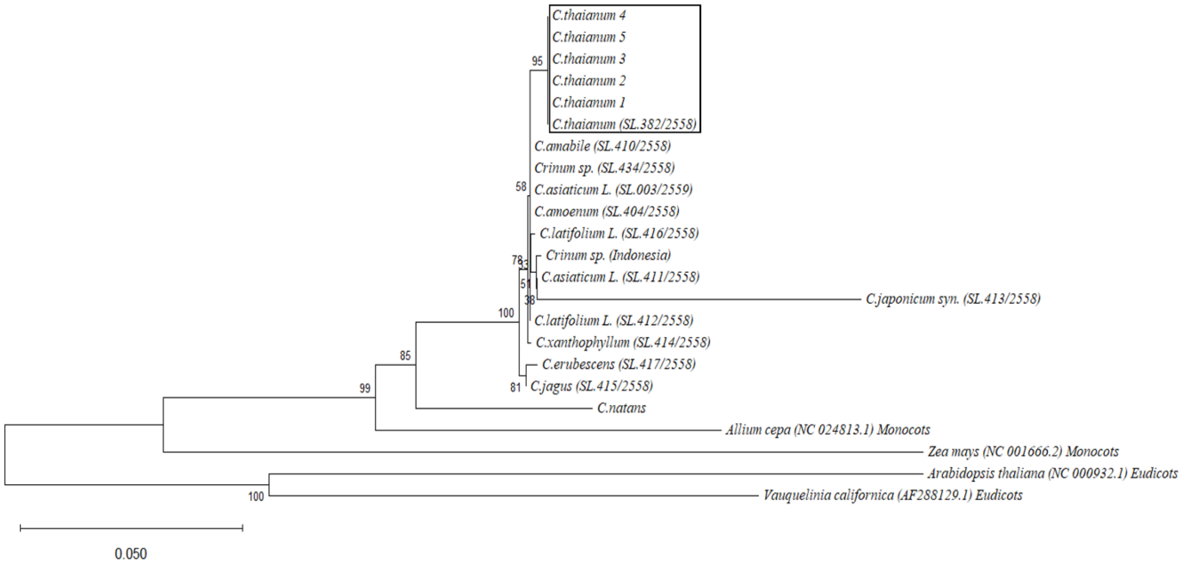

Fig. S2 Phylogenetic tree of 19 samples of *Crinum* species based on maximum-likelihood analysis of *mat*K sequences, including *A. cepa* (NC 024813.1), *Z. mays* (NC 001666.2), *A. thaliana* (NC 000932.1) and *V. californica* (AF288129.1) as outgroup species.

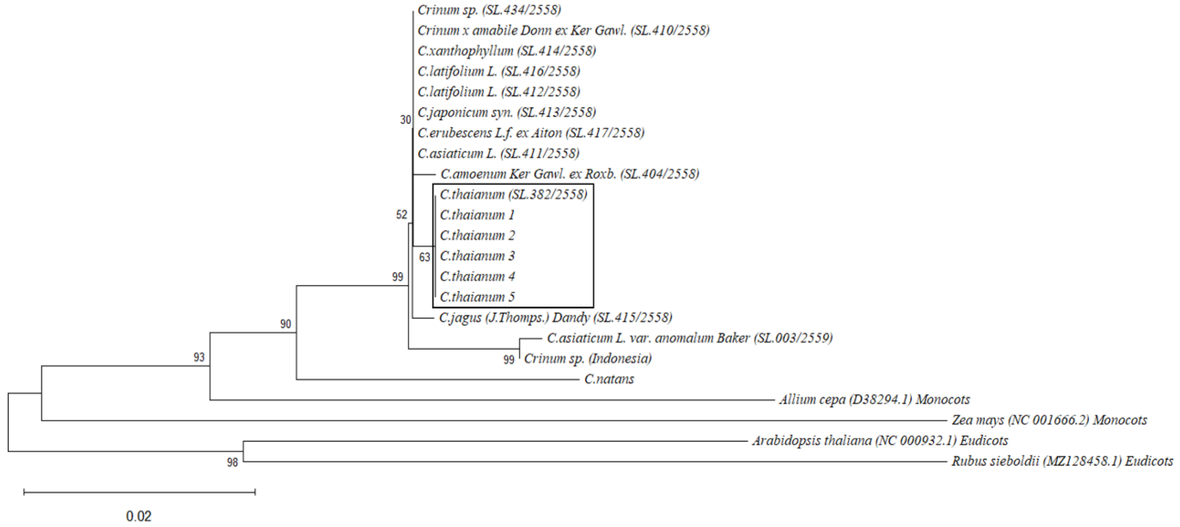

Fig. S3 Phylogenetic tree of 19 samples of *Crinum* species based on maximum-likelihood analysis of *rbc*L sequences, including *A. cepa* (D38294.1), *Z. mays* (NC 001666.2), *A. thaliana* (NC 000932.1), and *R. sieboldii* (MZ128458.1) as outgroup species.

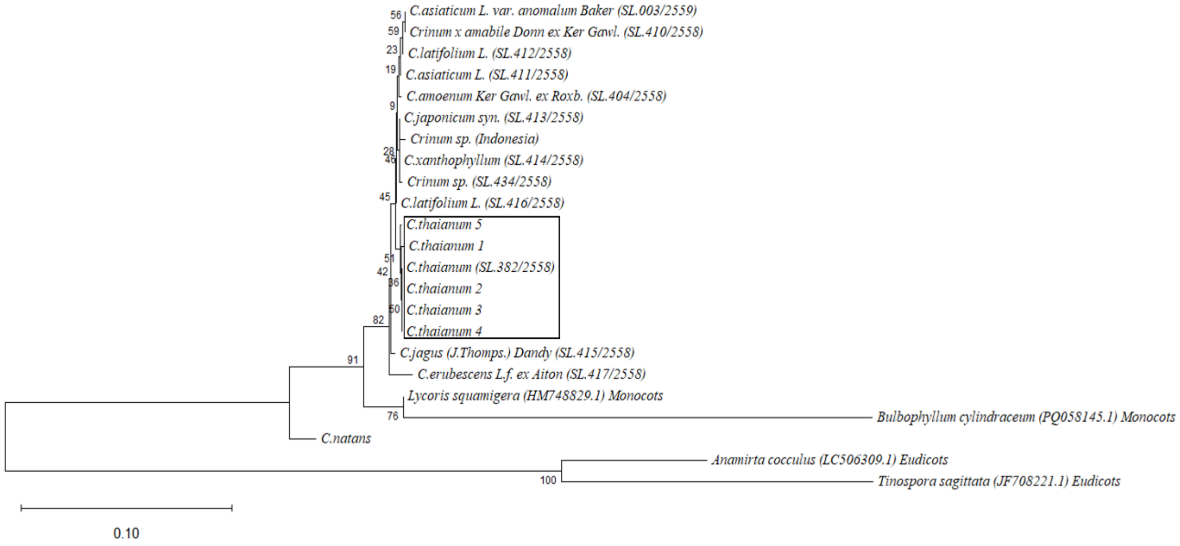

Fig. S4 Phylogenetic tree of 19 samples of *Crinum* species based on maximum-likelihood analysis of *trn*H-*psb*A intergenic spacer sequences, including *L. squamigera* (HM748829.1), *B. cylindraceum* (PQ058145.1), *A. cocculus* (LC506309.1), and *T. sagittata* (JF708221.1) as outgroup species.

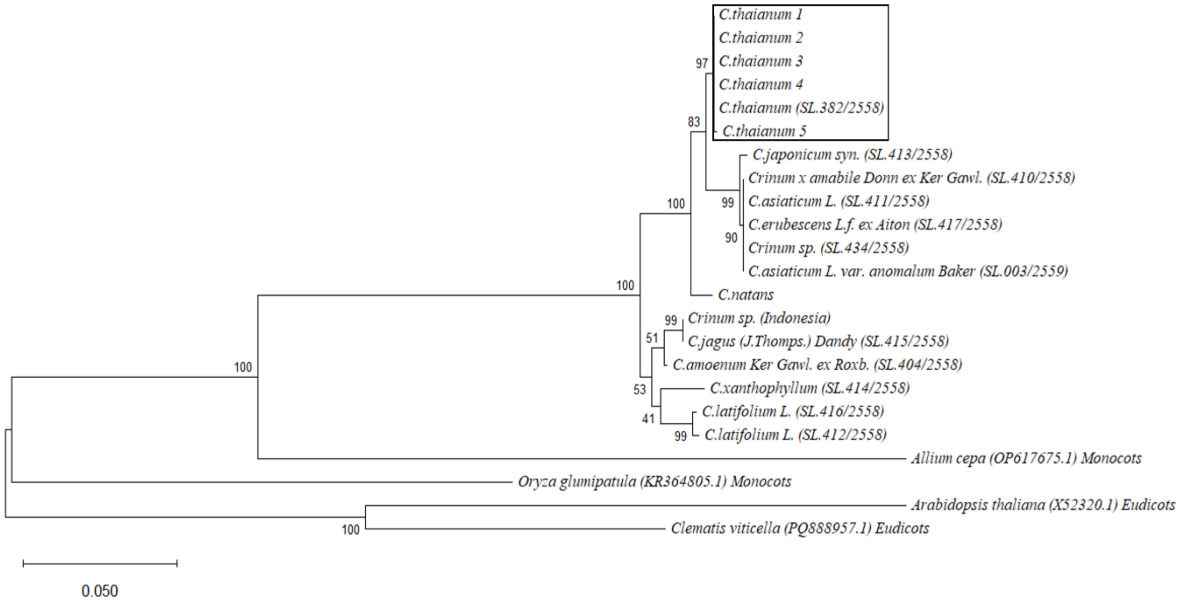

Fig. S5 Phylogenetic tree of 19 samples of *Crinum* species based on maximum-likelihood analysis of Nuclear-encoded ITS of *rDNA* sequences, including *A. cepa* (OP617675.1), *O. glumipatula* (KR364805.1), *A. thaliana* (X52320.1), and *C. viticella* (PQ888957.1) as outgroup species.