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A molecular test of Huxley's line: *Cyrtandra* (Gesneriaceae) in Borneo and the Philippines

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A biogeographic and phylogenetic study of *Cyrtandra* (Gesneriaceae) in the Sundaland region (Borneo and Peninsular Malaysia) and the Philippines using nuclear ribosomal (ITS) DNA sequence data reveals a major division between the *Cyrtandra* floras of Sundaland and the Philippines. Palawan, the most westerly of the Philippine islands, emerges as an area of mixing between these two. The Bornean element in the *Cyrtandra* flora of Palawan (two species in our sample) appears to result from recent (i.e. Pleistocene) dispersal from Borneo. The remaining seven species sampled from Palawan are most closely related to those from elsewhere in the Philippines. However, the Palawan clade is sister to the other Philippine taxa, suggesting an ancient (possibly Pliocene) vicariance event. Huxley's line—a zoogeographic boundary placing Palawan and Borneo together—receives some support from this study as there is evidence of recent dispersal of Bornean flora into Palawan. However, in terms of more ancient biogeographic patterning of the region, Palawan has stronger links with the other Philippine islands.

ADDITIONAL KEYWORDS: biogeography – ITS – Wallace's line – phylogeny – systematics – SE Asia – Palawan.

INTRODUCTION

BIOGEOGRAPHY OF SE ASIA

The islands of SE Asia form one of the most geologically complex and biogeographically interesting areas in the world. This is due to their position at the meeting point of the two supercontinents of Laurasia and Gondwanaland. The islands in the west of this region, including part of Borneo and the whole of Palawan (the most westerly of the Philippine islands), are on the Sunda continental shelf and Laurasian in origin. The islands in the east of the region, including New Guinea, are on the Sahul shelf and are essentially Gondwanan in origin. The islands in the middle, including Sulawesi, the Moluccas and most of the islands in the Philippine archipelago, lie in deep sea between the two shelves (Fig. 1).

The tectonic history of the Philippines is extremely complex. Recent evidence (Hall, 1996) suggests that the main landmass of the Philippines originated more than 50 million years ago as a series of 'island arcs' far out in the Pacific Ocean. As the Australian continent moved northwards towards the Asian continent, undersea volcanoes were formed by the plate tectonic movement. These gradually emerged from the sea and according to the reconstructions of Hall (1996) underwent considerable tectonic movement and rotation. As recently as the Miocene (15 Mya) Mindanao, for example, was widely separated from Luzon and situated east of the Sulawesi landmass and a little distance north of the New Guinea part of the Australian plate. The Philippine archipelago has only taken on its current shape over the last 10 Myr.

Wallace's Line, one of the best known biogeographic boundaries in the world, runs through the south of this region and demarcates the boundary between faunas and, to a lesser extent, floras with predominantly Laurasian affinities and those of Gondwanic origin. Huxley (1868) modified Wallace's Line and extended it north into the Philippines. Based on zoological data Huxley drew his line to the east of Palawan, effectively linking the island biogeographically with Borneo and Asia and separating it from the rest of the Philippine Archipelago (Fig. 1). Both of these lines were originally drawn using zoogeographic evidence, and subsequent detailed study of the biogeography of terrestrial mammals of the

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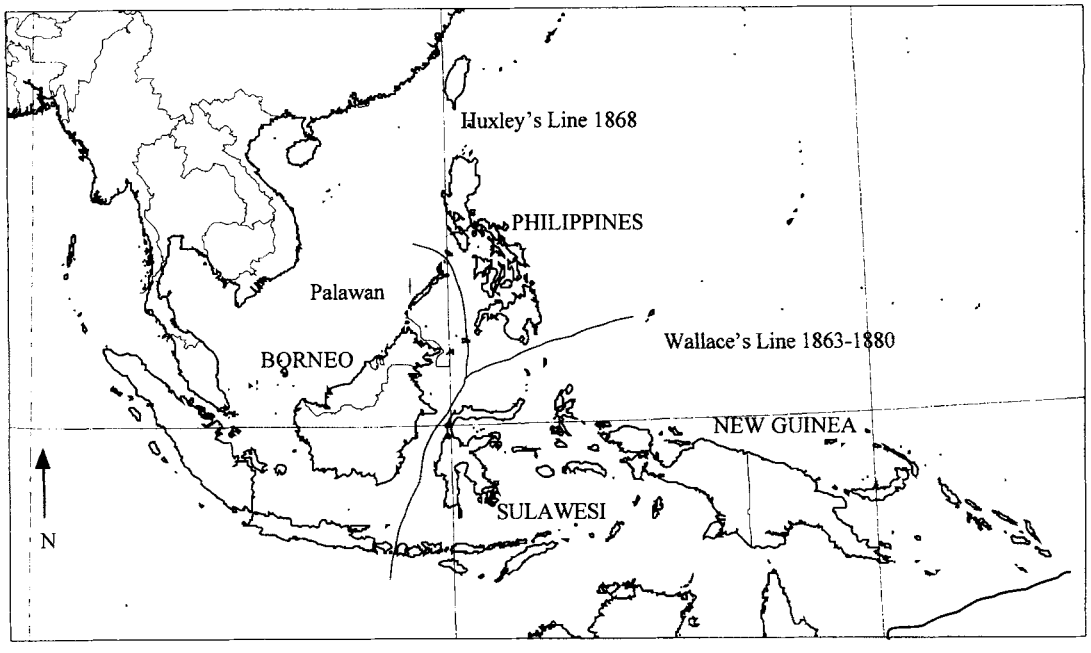

Figure 1. Map of SE Asia showing the positions of Wallace's Line and Huxley's Line and of the islands mentioned in the text.

Sunda Shelf and the Philippines (Heaney, 1986) has supported Huxley's hypothesis. Heaney found that some 96% of the genera of the terrestrial mammal fauna of Palawan is shared with Borneo (Heaney, 1986).

The botanical evidence is less clear-cut. However, Merrill in his *Enumeration of Philippine Plants* (1923) noted that the Balabac-Palawan-Calaman-Cuyo subprovince contained some striking Bornean elements not found elsewhere in the archipelago. Furthermore there are some plant species which are reported as having distinctive Palawan-Borneo distributions, such as the bamboo *Dinochloa robusta* which occurs in northern Sabah and Palawan (Wong, 1998) and others such as *Nepenthes mira*, recently described from Palawan, which belongs to a species group previously known only from Borneo (Cheek & Jebb, 1998). Unfortunately, there are few examples of phytogeographic studies which focus explicitly on the islands of Borneo and the Philippines.

DISTRIBUTION OF *CYRTANDRA*

Cyrtandra (Gesneriaceae) is a large genus of some 500–600 species of herbs, shrubs and lianas. It is found from the Nicobar Islands in the Indian Ocean, throughout Malesia, in Taiwan, southeast to Queensland and east to the high islands of the Pacific and Hawaii. It reaches its most easterly position in the Marquesas Islands in French Polynesia. Its centres of diversity are New Guinea and Borneo (each with over 150 species) and the Philippines (over 80 species) (Fig. 2). The distribution of *Cyrtandra* across this region and its remarkable propensity for evolutionary radiation make this an exceptionally promising genus for considering both biogeographic and evolutionary patterns and for testing the validity of Huxley's Line.

One of the most significant features of this genus throughout its range is the occurrence of high numbers of local endemics. Despite the almost continuous distribution of the genus there are relatively few examples of widespread *Cyrtandra* species and many examples of species unique to one region, one archipelago and even one island. There are no major disjunctions in the distribution of *Cyrtandra*. It crosses not only Wallace's and Huxley's Lines but, unlike some 23% of Fijian genera (Smith, 1955), it also crosses the Andesite Line, another pronounced biogeographic boundary in the east of the region (Mueller-Dombois & Fosberg, 1998). However, although *Cyrtandra* has clearly been able to spread around the Pacific, the very high levels of local endemism in the genus suggest that inter-island dispersal may be a rare event.

METHODS

PLANT MATERIAL

Material of 30 *Cyrtandra* accessions representing 26 species was obtained for this study (Table 1, Fig. 2).

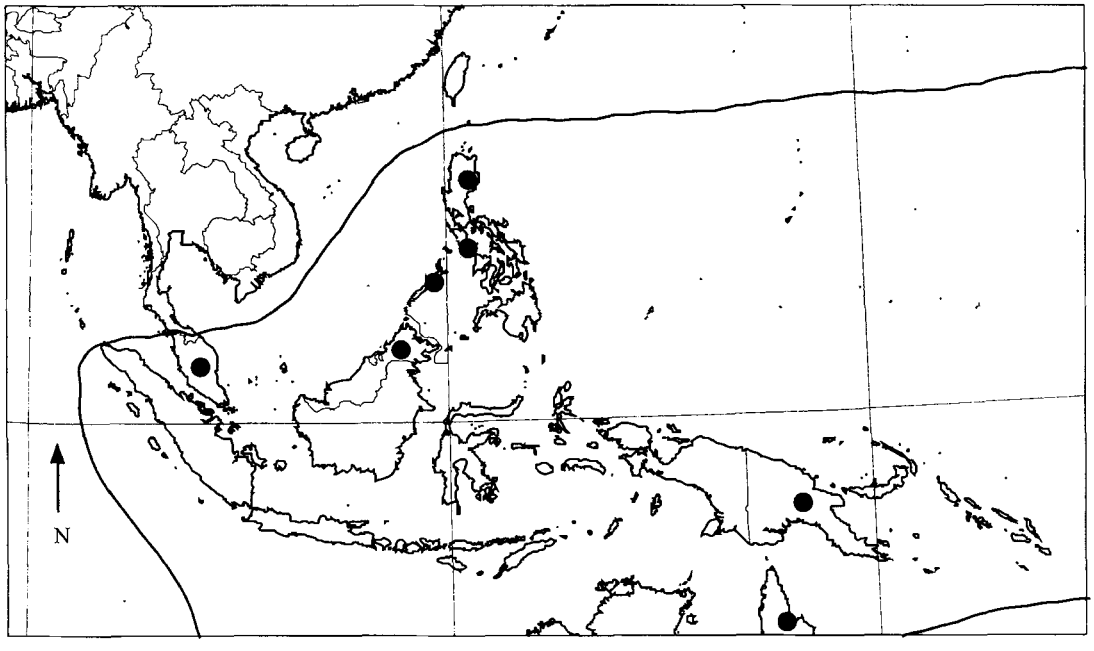

Figure 2. Map of SE Asia showing the limit of distribution of *Cyrtandra* in the region and the approximate localities of specimens included in the phylogenetic analysis.

Twenty-five of these accessions were collected as silica gel dried leaf material collected during Royal Botanic Garden Edinburgh (RBGE) expeditions to Borneo and the Philippines and stored frozen. A further three leaf samples were obtained from Vienna Botanic Garden. One specimen was obtained from the Australian National Botanical Garden, Canberra, and one specimen was sequenced from a herbarium specimen (see Table 1 for full details of the origin of the specimens included in the analysis). Species B and C from Palawan, C. sp (Naga), C. sp (Isabella) from Luzon, C. sp (Lantuyang), C. sp (Halcon 1), and C. sp (Halcon 2) from Mindoro are potential new species. Further taxonomic work on the genus is in progress (Atkins & Cronk, in press).

Aeschynanthus pulcher (Bl.) G.Don was selected as the outgroup. From recent molecular research on relationships within the Cyrtandroideae (Smith *et al.*, 1997) and from sequencing at RBGE (Q. Cronk and M. Möller, unpublished) *Aeschynanthus* appears to be one of the most closely related genera to *Cyrtandra* sequenced so far. This study focuses on the species from Sundaland and the Philippines. *C. monticola* K.Schum. and *C. baileyi* F.Muell. from New Guinea and Australia respectively are included to elucidate the relationships of the Sundaland and Philippine species further.

DNA EXTRACTION AND PCR

DNA was extracted from all specimens using the standard CTAB small scale DNA extraction procedures (Möller & Cronk, 1997). The whole ITS region was amplified using the Polymerase Chain Reaction (PCR) with the '5P' forward and '8P' reverse primers. Details of primers and PCR conditions follow Möller & Cronk (1997).

Following successful amplification, the PCR product was purified using the QIA quick PCR purification kit. The cycle sequencing reactions were performed using ABI PRISM Dye Terminator Sequencing Ready Reaction Kit, with the purified product, AmpliTaq DNA polymerase and fluorescent dye labelled terminators. Each spacer was sequenced individually in both forward and reverse directions for sequence confirmation (Möller & Cronk, 1997). These products were precipitated using 70% ethanol with 0.5 mM magnesium chloride and the resulting DNA pellet was dried and frozen.

In preparation for sequencing, the pellet was resuspended in 6 ml of 'gel loading mixture' containing EDTA, formamide and blue dextron. The mixture was then heated to denature the DNA (94°C for 2 min) and placed on ice. DNA sequencing was carried out on an acrylamide-bisacrylamide gel (final concentration 4%) containing urea, in a Perkin Elmer ABI PRISM 337 DNA sequencer.

SEQUENCE ANALYSIS

Initial alignment of the 31 sequences was done using the programme Clustal. The alignments were subsequently refined manually. Alignment gaps were

Table 1. Names marked *ined.* are unpublished and will be validated in a forthcoming publication (Atkins & Cronk, in press)

Species	Voucher number & collector(s)	Origin	Herbarium
C. cleopatrae H.J.Atkins & Cronk *ined.*	25437 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Cleopatra Needle	E
C. elatostemoides Elmer	25498 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Thumb Peak	E
C. inaequifolia Elmer	25414 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Cleopatra Needle	E
C. inaequifolia Elmer	25499 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Thumb Peak	E
C. hirtigera H.J.Atkins & Cronk var. *hirtigera ined.*	25433 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Cleopatra Needle	E
C. hirtigera H.J.Atkins & Cronk var. *chlorina* H.J. Atkins & Cronk *ined.*	25518 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Thumb Peak	E
C. pulgarensis [Elmer ex] H.J.Atkins & Cronk *ined.*	25522 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Thumb Peak	E
C. sp. B	25389 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Cleopatra Needle	E
C. sp. C	25520 Cronk, Mendum, Argent, Middleton, Wilkie, Fuentes, Chaves	PALAWAN: Thumb Peak	E
C. cumingii C.B.Clarke	29034 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	MINDORO: Oriental Province	E
C. sp (Halcon 1)	29053 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	MINDORO: Oriental Province	E
C. sp (Halcon 2)	29054 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	MINDORO: Oriental Province	E
C. sp (Lantuyang)	29035 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	MINDORO: Oriental Province	E
C. incisa C.B.Clarke	MAK 4 Cronk & Sagun	LUZON: Laguna Province	E
C. cf *lagunae*	MAK 1 Cronk & Sagun	LUZON: Laguna Province	E
C. cf *lagunae*	MAK 3 Cronk & Sagun	LUZON: Laguna Province	E
C. sp (Isabella)	29009 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	LUZON: Isabella Province Barangay San Jose	E
C. sp (Naga)	29130 Mendum, Argent, Pennington, Wilkie, Romero, Fuentes	LUZON: Camarine Sur, Naga Province	E
C. burbidgei C.B.Clarke	CBHM 22 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Crocker Range	E

continued

Table 1. *continued*

Species	Voucher number & collector(s)	Origin	Herbarium
C. chrysea C.B.Clarke	CBHM 20 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mount Kinabalu	E
C. clarkei Stapf	CBHM 19 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mount Kinabalu	E
C. corniculata B.L.Burtt	CBHM 9 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mesilau Park	E
C. gibbsiae S.Moore	CBHM 11 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mount Kinabalu	E
C. kermesina B.L.Burtt	CBHM 8 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mesilau Park	E
C. mesilauensis B.L.Burtt	CBHM 7 Cronk, Burtt, Hilliard, Mendum	BORNEO: (Sabah) Mesilau Park	E
C. splendens C.B.Clarke	45-89-04 [GS-89-04]	BORNEO: Sarawak	W
C. pendula Blume	19860730-1/2 Weber, Antogsamy	MALAYSIA: (cultivated in Vienna Botanic Garden)	W
C. wallichi (C.B.Clarke) B.L.Burtt	Weber s.n.	MALAYSIA: Maxwell's Hill	W
C. baileyi F.v.M.	T118 Cronk & Percy	AUSTRALIA: Queensland (from cultivated material)	E
C. monticola K.Schum.	6002 Takeuchi	NEW GUINEA: Morobe Province, Lae	E
Aeschynanthus pulcher (Bl.) G. Don	19882557 Argent	INDONESIA: Java, Gunung Salak	E

scored following Simmons & Ochoterena (2000) and added to the data matrix as extra characters. A summary of the characteristics of the 31 informative indels is given in Table 2. An alternative method of gap coding in which gaps may be coded as more than one character (Möller & Cronk, 1997) was also used for comparison and gave an identical strict consensus tree (data not shown). The analysis used a Macintosh Performa 6400/200 computer to implement equally weighted parsimony with the program PAUP* version 4.0b2a (Swofford, 1999).

Bootstrap values (Felsenstein, 1982) were calculated from a 1000 replicate analysis using a HEURISTIC search strategy, RANDOM addition sequence of the taxa and TBR branch swapping with a MAXTREE limit of 1000 per replicate. For this study we applied the following scheme of support: bootstrap values of 50–74% represent weak support, 75–84% moderate support and 85–100% strong support (Richardson *et al.*, 2000). Decay indices were determined by running sequential analyses increasing the branch length of trees saved by one until particular clades collapsed (Bremer, 1988). Further analyses were run which excluded ambiguously alignable regions in the matrix and the indel matrix.

RESULTS

Phylogeny of Philippine and Bornean *Cyrtandra*

With the indel matrix included and with 20 characters from two ambiguously alignable regions excluded from the analysis, 24 trees of 564 steps were obtained. A summary of the matrix and tree statistics is given in Table 3. One of the trees arbitrarily chosen from the 24 most parsimonious trees obtained in the analysis

Table 2. Characteristics of the 31 potentially informative indel characters. The gap matrix is shown at the end of the sequence matrix

Indel no.	Position	No. base pairs
1	35–36	2
2	46–51	6
3	61–63	3
4	69–74	6
5	83–86	4
6	104–109	6
7	125–127	3
8	131	1
9	144–146	3
10	151–152	2
11	216–217	2
12	223–224	2
13	224	1
14	244	1
15	253	1
16	334–338	5
17	334–336	3
18	338	1
19	321	1
20	341–343	3
21	341–342	2
22	356–357	2
23	363–369	7
24	441–443	3
25	451–454	4
26	451–462	12
27	468–476	9
28	468–475	8
29	531–533	3
30	533	1
31	546–551	6

Table 3. Results of molecular analysis

Total characters in matrix	589
No. of excluded characters	20
No. of constant characters	284
Variable uninformative characters	129
Parsimony informative characters	156
Number of informative indels	31
Size of informative indels	1–12 bp
Number of MPT*	24
Consistency index (CI)	0.654
Homoplasy index (HI)	0.346
Retention index (RI)	0.692
Rescaled consistency index	0.453

* With gap matrix included and unambiguously alignable regions excluded.

is displayed as a phylogram with branch lengths (Fig. 3).

The strict consensus tree (Fig. 4) has three major clades. These correspond broadly to three geographical areas. Clade I contains the two species from Australia and New Guinea, *C. baileyi* and *C. monticola* respectively. This clade is well supported with a bootstrap value of 100 (DI of at least 3). The second major clade (II) with moderate bootstrap support of 79 is a broadly Sundaic group, containing predominantly species from Borneo (Sabah and Sarawak) and Peninsular Malaysia. It also contains, however, two of the Palawan species and one from Mindoro. The third major clade (III) is a Philippine clade with bootstrap support of 74. Within the Philippine clade the Palawan species form a sister clade (bootstrap = 81, DI = +1) to those from Luzon and Mindoro (bootstrap = 97, DI = > +3). See Figure 5 for a map showing the positions of these three major clades.

In the strict consensus tree the two species from New Guinea and Australia are resolved as sister to the Sundaic and Philippine clades. This relationship, however, has no bootstrap support and it is not possible to state the relative position of the New Guinea/Australia clade with confidence.

Reanalysing the data matrix without the gap matrix yielded 28 most parsimonious trees of 519 steps: the Sundaic clade had a lower bootstrap value (67 as opposed to 74) and the Philippine clade collapsed in the strict consensus into two separate clades; a Palawan one (with a bootstrap value of 78) and a Luzon/Mindoro one (with a bootstrap value of 83). The New Guinea/Australia clade retained its high bootstrap support (100).

The presence or absence and type of foliar sclereids has been shown to vary significantly in *Cyrtandra* and their potential utility as taxonomic characters has been shown (Bokhari & Burtt, 1970; Burtt & Bokhari, 1973). For this reason, the presence or absence of foliar sclereids in each of the terminal taxa has been indicated on the strict consensus tree. Although the type of sclereid present is not indicated on the tree *C. elatostemoides* and *C. gibbsiae* share the same sclereid pattern of vermiform sclereids in the hypodermis and polymorphic sclereids in the mesophyll confirming their placement together by the molecular data. Similarly, *C. clarkei* and *C. kermesina*, also placed together by the molecular analysis share the same sclereid pattern, this time of osteosclereids in the hypodermis and astrosclereids in the mesophyll.

SEQUENCE EVOLUTION WITHIN *CYRTANDRA*

Two significant indel events are shared by all of the Palawan species except *C. elatostemoides* and *C.* sp. *C*, which are the two Palawan species which occur in

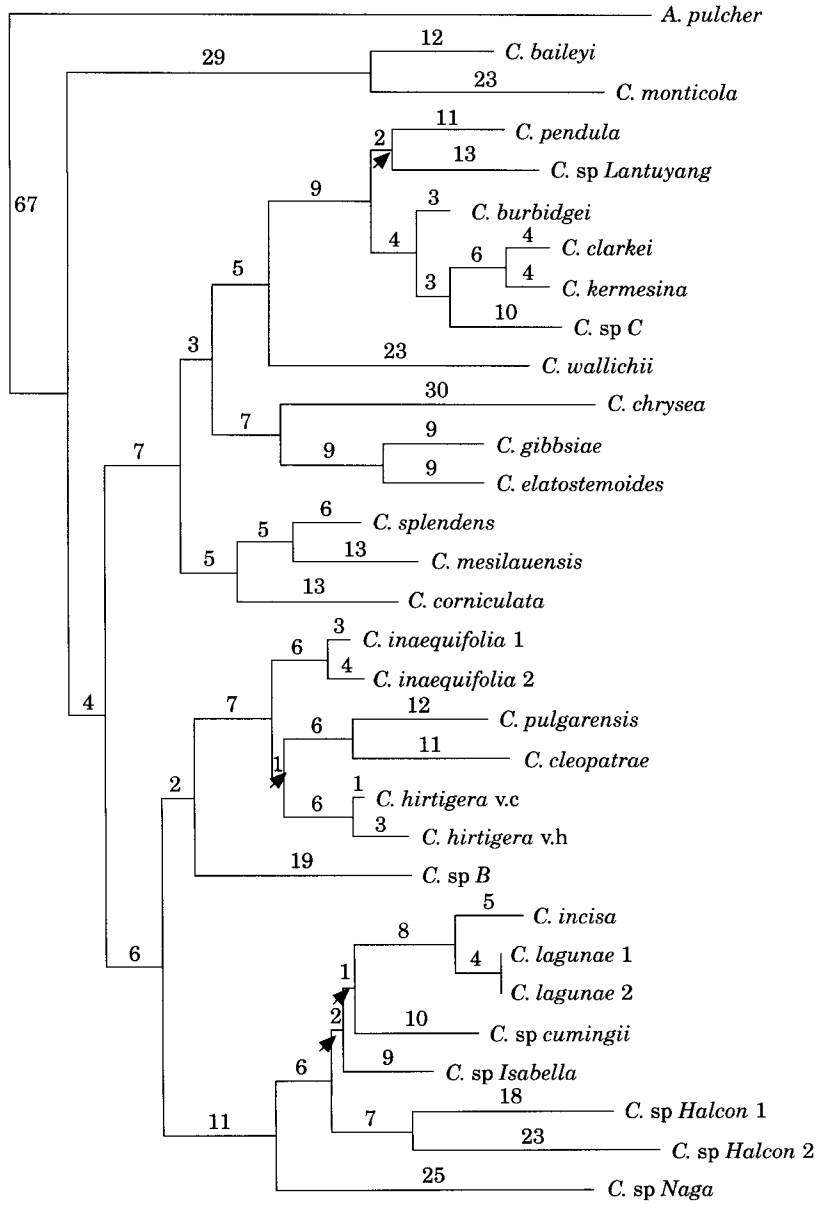

Figure 3. Phylogram of one of the 24 most parsimonious trees of 564 steps based on parsimony analysis of the combined ITS1 and ITS2 sequence data plus the alignment gap matrix. Numbers above the branches are branch lengths. Arrows indicate nodes that collapse in the strict consensus.

the Sundaic clade. One of these indel events (number 25 at position 451) involves a four base pair (GATT) tandem repeat. All of the non-Palawan Philippine species, with the exception of the Sundaic *C.* sp. (Lantuyang), share a 12 bp deletion at position 455 (indel event number 26). *C. baileyi* and *C. monticola*—the Australian and New Guinean species respectively—share a number of indel events, including a synapomorphic 6-base pair insertion (GTGCTG) at position 546 (indel event number 31).

Maximum interspecific divergence rates for *Cyrtandra* in this study was 15.7% for ITS1 and ITS2 combined. These rates compare with, for example, maximum sequence divergence within *Streptocarpus* (Gesneriaceae) of 23.5% for ITS1 and ITS2 combined (Möller & Cronk, 1997).

DISCUSSION

BIOGEOGRAPHIC IMPLICATIONS OF THE PHYLOGENY

With these results in place a number of biogeographic observations can be made. Firstly, a division emerges in the molecular analysis between the Sundaland and

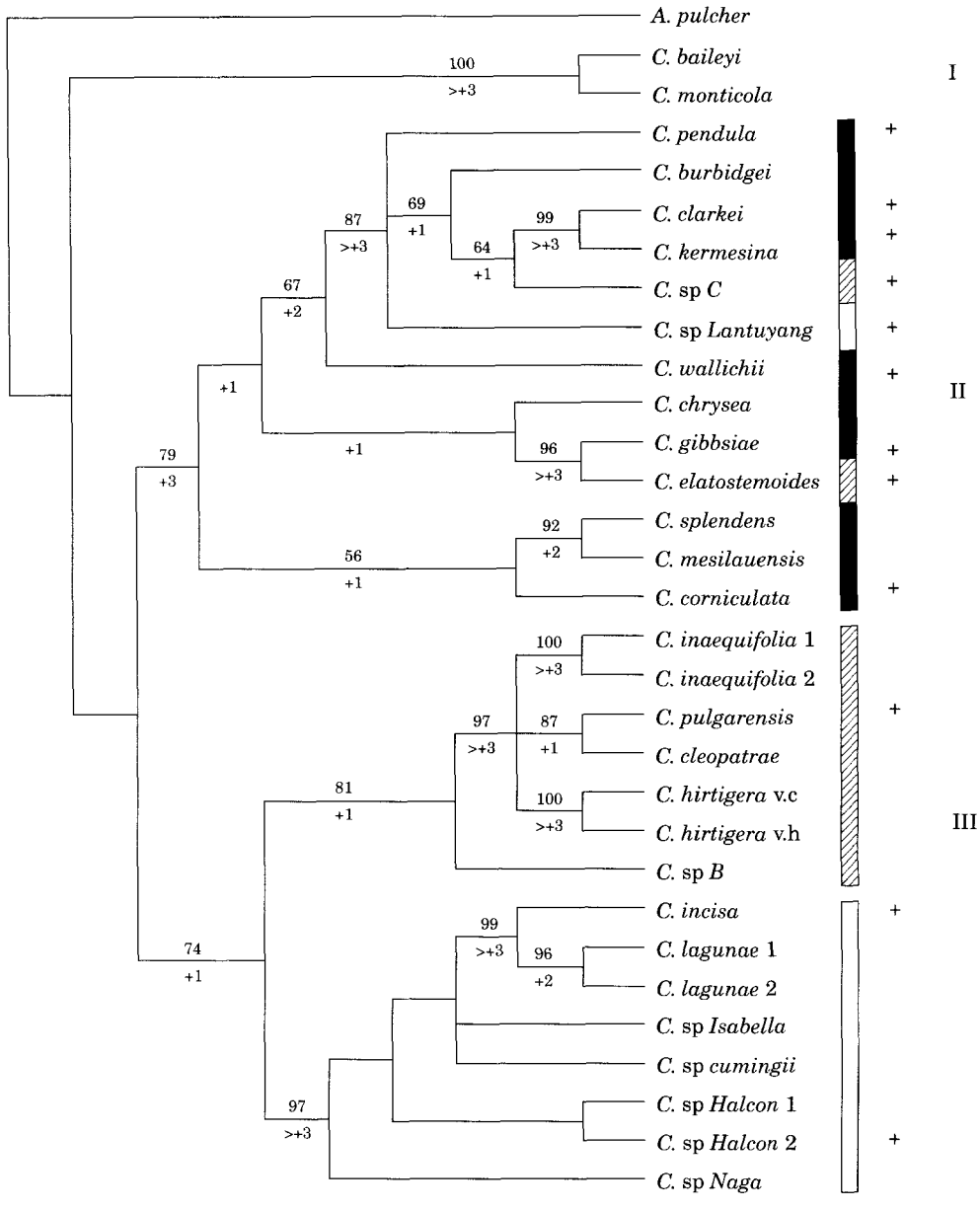

Figure 4. Strict consensus tree of 24 most parsimonious trees based on parsimony analysis of combined ITS1 and ITS2 sequence data plus the alignment gap matrix. Numbers above the branches are bootstrap values of 1000 replicates, numbers below are decay indices. (+) Sclereids present. (■) Sundaland. (□) Philippines (excluding Palawan). (▨) Palawan.

Philippine species. Secondly, Palawan emerges as an area of mixing between these two. Within the Palawan *Cyrtandra* flora there are two distinct groups of taxa. Firstly, there are two species whose closest relatives are in Sundaland and fall within the Sundaland clade. Secondly, the majority of species on the island are a monophyletic group which emerge as sister to those from the rest of the Philippines. These two different groups of Palawan *Cyrtandra* clearly have different evolutionary histories and these patterns require different explanations.

Group 1: C. elatostemoides *and* C. *sp. C.—the Sundaland species*

These two Palawan species fall within the Sundaland clade. Their presence in this clade is given support by evidence from sclereid data. *C. elatostemoides* Elmer

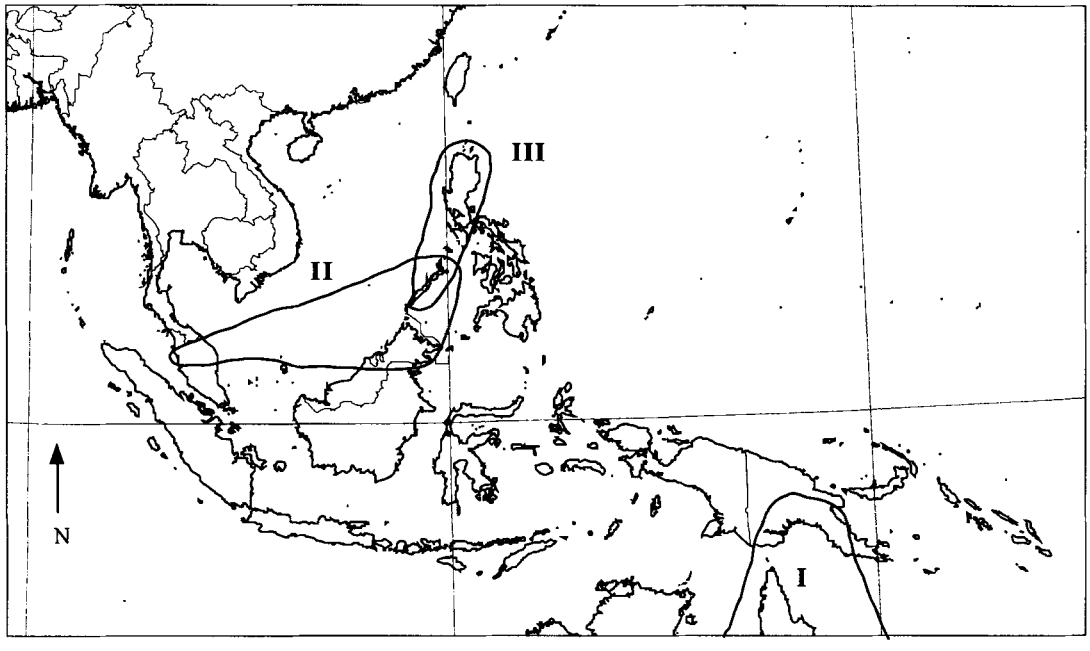

Figure 5. Map of SE Asia showing the position of the three major clades which emerged in the analysis. Roman numerals refer to the three major clades identified in the analysis.

is known also to occur in Borneo. This is the only example, so far known, of a *Cyrtandra* species with a Borneo-Palawan distribution. It might be expected that, unless it has become extinct in this part of its range, *C.* sp. C may also be found in Borneo in the future. A third Philippine species, from Mindoro, also falls in this clade.

The occurrence of Palawan species in this clade is consistent with a dispersal explanation. These two appear to be relatively recent arrivals on Palawan from Borneo. Their closest relatives are on Borneo. They have apparently not had time to radiate on Palawan. It is possible that these species arrived on Palawan during one of the glacial periods of the Pleistocene when these two islands would have been joined due to a lower sea-level. Furthermore, favoured by many *Cyrtandra* species, are likely to have been more widespread during the Pleistocene cool montane conditions. These factors would have reduced significantly the barriers to dispersal of these species between Borneo and Palawan at that time.

All of the dispersal events identified by this analysis are from west to east. The sampling is low but there does not, on the evidence from this analysis, appear to be any movement in the opposite direction.

Group 2: the Palawan clade

The relationship between the seven taxa that fall within this clade and those from the rest of the Philippines is very different from the one described above. The pattern here cannot easily be explained in terms of dispersal. The Palawan taxa emerge in this analysis as sister to those from the rest of the Philippines. In biogeographic terms this pattern is more consistent with a vicariance explanation. This is not a 'nesting' pattern—one group has not evolved from within the other—but rather consistent with both the Philippine and Palawan clades having evolved from a common ancestor and subsequently become isolated from each other.

It is likely that this vicariance event is older than that suggested for the dispersal events between Borneo and Palawan. The Philippine and Palawan groups have radiated significantly. The sequence divergence between the two groups varies from 6 to 13% suggesting separation no later than the Pliocene, as ITS sequence divergence rates of 0.1% per Myr has been estimated by some authors (Richardson *et al.*, submitted; Sang *et al.*, 1994).

It is more difficult to find an explanation for this pattern that is consistent with our current understanding of the geological history of the region. The geological history of this region, however, is extremely complex and theories are constantly changing. There is no evidence, at present (Hall, 1996) that Palawan was formerly joined with other parts of the Philippines and separated subsequently. This would be the most satisfactory explanation for the pattern seen in the data. Alternatively, there could have been simultaneous ancient dispersal events to Palawan and

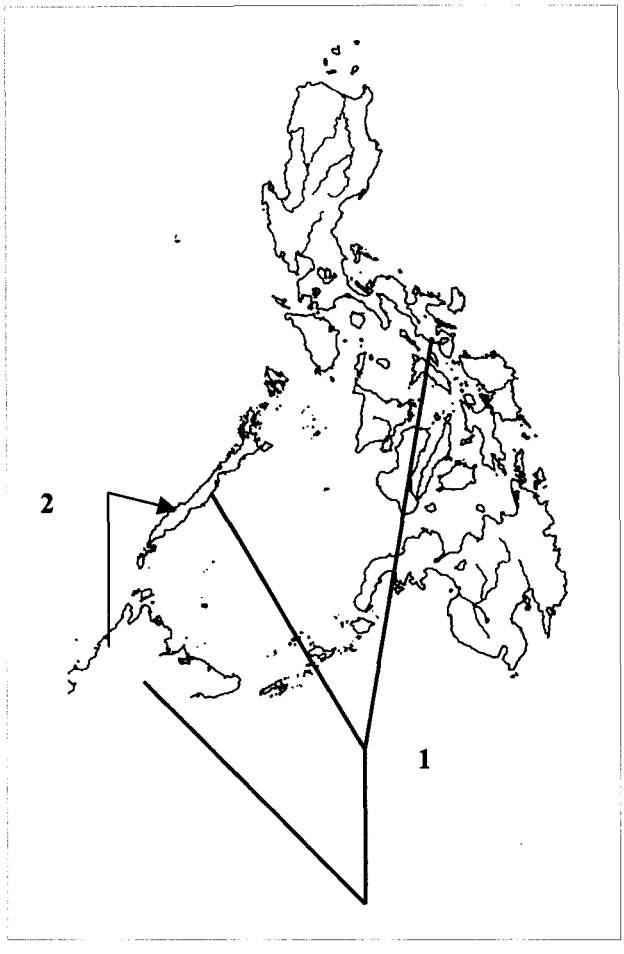

Figure 6. Map illustrating the hypotheses presented here based on *Cyrtandra*. (1) A hypothetical Pliocene vicariance event. (2) A hypothetical Pleistocene dispersal event.

Luzon/Mindoro from which the species in the study evolved (see Fig. 6 for a summary of these hypotheses).

With over 150 species of *Cyrtandra* known in Borneo and at least 80 from the Philippines the analysis of just 30 species is likely to reveal only part of the story of the evolution of *Cyrtandra* in this region. Obtaining more samples of these rainforest herbs is difficult as they are often overlooked by collectors, but this should be a priority for the future. Some species described in the early 20th century from the Philippines may now be extinct. However, despite being scattered at low density within the forest, their ubiquity in the rainforests of Malesia makes them ideal for studies of this sort.

PHYLOGENETIC EVIDENCE FOR AND AGAINST HUXLEY'S LINE

The results of this study provide some evidence in support of Huxley's Line and other evidence which challenges its existence. Huxley found that there were greater similarities between the Alectoromorphae (megapodes and pheasants) of Palawan and Borneo than between these groups on Palawan and the rest of the Philippines. He consequently drew his line to the east of Palawan and linked the island biogeographically with Borneo. A link between Palawan and Borneo is supported in this study by the existence on Palawan of the two *Cyrtandra* species that have clear affinities with Borneo. As mentioned earlier these two species are thought to have arrived by dispersal during the glacial maxima of the Pleistocene. Heaney (1986) in his study of land mammals in this region also found support for Huxley's Line. He found striking similarities between the land mammal fauna of Palawan and Borneo. He showed that the distinct mammalian faunal regions in the area correspond almost exactly to the limits of dry land during the most recent glacial period. The altered conditions of the glacial maxima appeared to allow the movement of effective dispersers, such as birds and land mammals, around the region. This is reflected in their current distribution patterns. The distribution of land mammals and birds and, to a lesser extent, *Cyrtandra*, seem therefore to be excellent indicators of these recent events in the geological history of Borneo and the Philippines.

The *Cyrtandra* species with links to Borneo, however, only represent a fraction of the *Cyrtandra* flora of Palawan. Huxley's Line is challenged by the presence on the island of a monophyletic group of seven species which emerge in the analysis as sister to those from the rest of the Philippines. As has been discussed earlier, these possibly reflect a more ancient (Pliocene) vicariance event between Palawan and the other islands in the archipelago; an event apparently not reflected in the contemporary distributions of Huxley's birds and Heaney's mammals.

The results of this study underline the difficulty in drawing sharp biogeographic boundaries in this region. Contrary to Huxley's findings, Palawan does not fall neatly into the same biogeographic region as Borneo in this study. Simpson (1977) observed that there were "too many lines" in this region (in a paper by that title). The different ages, evolutionary histories and methods of dispersal of different biota seem likely to result in different distribution patterns and consequently the drawing of different conclusions and biogeographic boundaries.

Analysis using ITS data has proved effective at resolving relationships in *Cyrtandra*. A secondary issue of the relationship of the Bornean and Philippine groups to the Australian and New Guinean *Cyrtandra* has not been resolved in this analysis. Higher sampling density is needed and it also may be useful to look at a gene that is evolving at a slower rate than ITS, such as the *trnL* intron from the chloroplast genome, to

resolve more satisfactorily relationships between these different clades.

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APPENDIX

MOLECULAR DATA MATRIX OF ALIGNED SEQUENCES FOR ITS1 AND ITS2 REGIONS AND INDEL MATRIX

```
                                                                                                                  60
A.pulcher                 TCGAAACCTGCAAAGCAGACTCGTGAACATGTGT--AAATAACATCGGGGGTCGTGAGGT
C.baileyi                 TCGAAACCTGCAAAGCAGACCCGTGAACTTGTTA-AAAATATGCTTGCGGCCGCGATGTC
C.monticola               TCGAAACTTGCAAAGCAGACCCGTGAACTTGTTT--AAATA-GCTTGCGGCCGCGATGTC
C.pendula                 TKGAAACCTGCAAAGCAGACC-GTGAACACGTTT--AAATATGCTCGCGGCCGCGGTGGC
C.splendens               TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATACGATTGCGGCCGCGATGTC
C.wallichii               TCGAAACCTACAAAGAAGACCCGCGAACATGTTT--AAATATGCTTGCGGCCGCGATGTC
C.burbigei                TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATATGATTGCGGCCGCGGTGGC
C.chrysea                 TCGTAACCTGCAAAGCAGACCCGTGAACATGTTTAAAAACACGCTTGCGGCCACGGTGTC
C.clarkei                 TCGAAACATGCAAAGCAGACCCGTGAACATGTTT--AAATATGCTTGCGGCCGCGGTGGC
C.corniculata             TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATACGCTTGTGGCCGCGATGTC
C.gibbsiae                TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATACGCTTGCGGACGCGATGTC
C.kermesina               TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATATGCTTGCGGCCGCGGTGGC
C.mesilauensis            TCGAAACCTGCAAAGCAGACCCGTGAAMATGTTT--AAATACGATTGCGGCCGCGAWGTY
C.elatostemoides          TCGAAACATGCAAAGCAGACCCGTGAACATGTTT--AAATACGCTTGCGGACGCGATGTC
C.inaequifolia1           TCGAAACCTGCAAAGCAGACCCGTGAACGTGTTT--AAATACGCTTGCGGCCGCGATGTC
C.inaequifolia2           TCGAAACCTGCAAAGCAGACCCGTGAACGTGTTT--AAATACGCTTGCGGCCGCGATGTC
C.pulgarensis             ??????????????????ACCGTGGGACATGTTT--AAATACGCTTGCGGCCGCRATGTC
C.hirtigerav.c            TCGAAACCTGCAAAGTAGACCCGTGAACATGTTT--AAATACGCTTGCGGCCGTGATGTC
C.hirtigerav.h            TCGAAACCTGCAAAGTAGACCCGTGAACATGTTT--AAATACGCTTGCGGCCGTGATGTC
C.cleopatrae              ?????????????ATATACCGTGAAACATGTTT--AAATACGCTTGCGGCCGCGATGTC
C.spB                     TCGAAACCTGCAAAGCAGACCCGTGAACATGTTT--AAATACGCTTGCGGCCGCGATGTC
C.spC                     TCGAAACCTGCAAAGTAGACCCGTGAACATGTTT--AAATATGCTTGCGGCCGCGGTGGC
C.cumingii                TCGAAACCTGCAAAGCAGACCCGCGAACATGTTT--AAATACGCTTGCGGCCGCGATGTC
C.incisa                  TCGAAACCTGCAAAGCAGACCCGCGAACATGTTT--AAATACGCCT------GCGATGTC
C.lagunae1                TCGAA-CCTGCAAAGCAGACCCGCGAACATGTTT--AAATACGCC------CGCGATGTC
C.lagunae2                TCGAAACCTGCAAAGCAGACC-GCGAACATGTTT--AAATACGCC------CGCGATGTC
C.spHalcon1               TCGAAACCTGCAAAGCAGACCCGCGAACATGTTT--AAATACGCTTGCGGCCGCGATGTT
C.spHalcon2               TCGAAACCTGTAAAGCAGACCCGCGAACACGTTT--AAACACGCTTGCGGTCGCGATGTG
C.spIsabella              TCGAAACCTGCAAAGCAGACCCGCGAACATGTTT--AAATACGCTTGCGGCCGAGGTGTC
C.spLantuyang             TCGAAACCTACAAAGTAGACCCGTGAACATGTTT--GAATATGCTTGCGGTCGTGGTAGC
C.spNaga                  TCGAAACCCGCAAAGCAGACCCGCGAACATGTTT--AAAAACGCC-GCG-----GACGTC
                                                                                      120
A.pulcher                 --TGGATGCATTTGTGTCCAGC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.baileyi                 ---GGATGCATTTGCGTCCAACCAACATCACGACCCTGACCCC------AAGTGGCGCAA
C.monticola               GTCGGATGCATTTGCGTCCAACCGACATCACGGCCCTGACCCC------GAGTGGCGCAA
C.pendula                 ---GGATGTGTTTGCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.splendens               ---GGATGCGTTTGCGTCCAAT----GTCACGACCTCGACCCC------AAGTGGCGCAA
C.wallichii               ---GGATGTGTTTGCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.burbigei                ---GGATGTGTTTGCATCCAAC----ATCACGACTTCGACCCC------AAGTGGCGCAA
C.chrysea                 ---GGATGCGTCTGCGTCCCAC----ATCGCGACTTCGGCCCC------GAGTGGCGCAA
C.clarkei                 ---GGATGCGTTTGCATCCAAC----ATCACGACCCTGACCCC------AAGTGGCGCAA
C.corniculata             ---GGATGCGTTTGCGTCCAAT----ATCACGACTTCGACCCC------AAGTGGCACAA
C.gibbsiae                ---GGATGCGCTTGCGCCCGAT----ATCACGACTTCGACCCC------AAGTGGCGCAA
C.kermesina               ---GGATGCGTTTGCATCCAAC----ATCACGACCCAGACCCC------AAGTGGCGCAA
C.mesilauensis            ---GGAWGCGTTTGCGTCCAAT----ATYACGACCTTGACCCC------AAGTGGSGCAA
C.elatostemoides          ---GGATGCGTTTGCGCCCGAC----ATCACGACATCGACCCC----------------A
C.inaequifolia1           ---GGATGCATTTGCGTCCAAC----ATCACGACCTCGACCCC----CCAAGTGGCGCAA
C.inaequifolia2           ---GGATGCATTTGCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.pulgarensis             ---GGATGCGTTTGCGTCCAAC----ATCACGATCTCGAACCC------AAGTGGCGCAA
C.hirtigerav.c            ---GGATGCATTTGCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.hirtigerav.h            ---GGATGCATTTGCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.cleopatrae              ---GGATGCGTTTGCGTCCAAC----ATCATGACCCTGACCCC------AAGTGGCGCAA
```

APPENDIX – *continued*

```
C.spB              ---GGATGCGTT-GCGTCCAAC----ATCACGACCTCGACCCC------AAGTGGCGCAA
C.spC              ---GGATGCGTTTGCGTCCAAT----ATCAAGACCCCGACCCC------AAGTGGCGCAA
C.cumingii         ---GGATG------CGTCCAAC----ATCAAGGCTTCGACCCC------AAGTGGCGCAA
C.incisa           ---GGATG------CGTCCAAC----ATCAAGGGCTCGACCCC------AAGTGGCGCAA
C.lagunae1         ---GGATG------CGTCCAAC----ATCAAGGGCTCGACCCC------AAGTGGCGCAA
C.lagunae2         ---GGATG------CGTCCAAC----ATCAAGGGCTCGACCCC------AAGTGGCGCAA
C.spHalcon1        ---GGATG------CGTCCAAC----ATCACGGCCTCGACCCC------AAGTGGCGCAA
C.spHalcon2        ---GGATG------CGTCCAAC----ATCACGGCCTCGGCCTCGACCCCAAGTGGCGCGA
C.spIsabella       ---GGATG------CGTCCAAC----ATCAAGGCCTCGACCCC------AAGTGGCGCAA
C.spLantuyang      ---GGACGTGTTTGCGTCCAAC----ATCACGACCTTGACCCC------AAGTGGCGCAA
C.spNaga           ---GGATGTGT--GCGTCCAAC----GTCACGGCTTCGGCCCC------AAGTGGCGCAA
                                                                            180
A.pulcher          GTCG---CTT-GGGNGTACTAAA---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCG
C.baileyi          GTCGTTGCTC-GGGCGTGCTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAATCA
C.monticola        GTCGTTGCTT-GGGCGTGCTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.pendula          GTCG---CTC-GGGCGTACTAAC---CTTT--TGGCGCGGCAA-GCGCCAAGGAAAAAAG
C.splendens        GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACA
C.wallichii        GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAAAGCGCCAAGGAAAAACA
C.burbigei         GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACG
C.chrysea          GTCG---CTC-GGGCGTACCAAC---CTCT--CGGCGCGGGAA-GCGCCAAGGAAAAACA
C.clarkei          GTCK---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACG
C.corniculata      GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGTGGCAA-GCGCCAAGGAAAAACA
C.gibbsiae         GTCG---CTG-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACA
C.kermesina        GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACG
C.mesilauensis     GTYG---CTY-GGGSGTACCAAC--CTTTT---GGCGCGGCAA-GCGCCAAGGRAAAAMG
C.elatostemoides   GTCG---CTG-GGGTGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGARAAACA
C.inaequifolia1    GTCG---CTC-GGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.inaequifolia2    GTCG---CTC-GGGCATACTAACAACCTAT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.pulgarensis      GTCG---CTT-GGGTATACTAACAACCTCT--CGGCGCGGAAA-GCGCCAAGGAAAACCA
C.hirtigerav.c     GTCG---CTC-GGGCAAACTAACAACCTCT--CGGCGCRGCAA-GCGCCAAGGAAAACCA
C.hirtigerav.h     GTCG---CTC-GGGCAAACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.cleopatrae       GTCG---CTC-GGGCATACAAACAACCTCT--GGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spB              GTCG---CTC-GGGCAAACTAACACAACCTCTCGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spC              GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAACG
C.cumingii         GTCG---CTCGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.incisa           GTCG---CTCGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.lagunae1         GTCG---CACGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.lagunae2         GTCG---CACGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spHalcon1        GTCG---CTCGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spHalcon2        GTCG---CTCGGGGCATACTAACAACCTCT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spIsabella       GTCG---CTGGGGGCAAACTAACAACCTTT--CGGCGCGGCAA-GCGCCAAGGAAAACCA
C.spLantuyang      GTCG---CTC-GGGCGTACTAAC---CTCT--CGGCGCGGCAA-GCGCCAAGGAAAAATG
C.spNaga           GTCG---CTCGGGGCATCCTAACAACCTCC--CGGCGCGGCAA-GCGCCAAGGAAAACCA
                                                                            240
A.pulcher          TATCGAACACCTCTCCGTCTCGGTGC--TATGCGG--TACCC--AGGACGTGATGAGGA-
C.baileyi          TATCGAACGCCTCTCCGTCACGGTGCCGTGCGCGG--TGCGC--AGGACTTGACGAGGA-
C.monticola        TATCGAACGCCTCTCCGTCTCGGTGCCGTGCGCGG--TACGC--AGGACGTGACGATGA-
C.pendula          TATCGAAMGCCTTTCCGTCTCGGTGCCGTGCGCGG--TACCCC-AGGACGCGATGAGGA-
C.splendens        TACCGAACACCTCTCCGTCTCGGTGCCGTGCGCGG--TACTC--AGGATGTGACGAGGA-
C.wallichii        TATTGAACGCCTCTTCGTCTCGGTTCCGTGCGCGG--TACCY--GGGACGTGACGAGGA-
C.burbigei         TATCGAACGTCTCTCCGTCTCGGCGCCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
C.chrysea          TACCGAACGCCTCTCCGTCTCGGCGCCGTCCGCGG--CACCCCCGGGACGTGACGAGGAA
C.clarkei          TATCGAACGCCTCTCCGTCTCGGCGCCGTTCGCGG--TACCC--AGGACGTGACGAGGA-
C.corniculata      TATCGAACACCTCTCTGTCTCGGTGCCGTGCGCGG--TACCC--AGAACGTGATGAGGA-
C.gibbsiae         TACCGAACGCCTCTCCGTCTCGGCGCCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
C.kermesina        TATCGAACGCCTCTCCGTCTCGGCACCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
C.mesilauensis     TACCGAACACCTTTCCGTTTGGGTGCCGTGCGCGG--TAACCC-AGGATGTGACGAGGA-
C.elatostemoides   TACCGAACGCTTCTCTGTTTCGGCGCCGTGCGCGG--TACCC--AGGAARTGACGAGGA-
C.inaequifolia1    TACCGAGCACCTCTCCGTCTCGGTGCCGTGCGCGG--TACAC--AGGACGTGACGAGGA-
C.inaequifolia2    TACCGAGCACCTCTCCGTCTCGGTGCCGTGCGCGG--TACAC--AGGACGTGACGAGGA-
```

APPENDIX – *continued*

```
C.pulgarensis      TACCGAGCACCTCTCCGTCTCGGTGCCGTGCGCGG--TACAC--AGGACGTGACGAGGA-
C.hirtigerav.c     TACCGAGCACCTCTCCGTCCCGGTGCCGTGCGCGG--TACAC--AGGACGTGACGAGGA-
C.hirtigerav.h     TACCGAGCACCTCTCCGTCCCGGTGCCG-GCGCGG--TACAC--AGGACGTGACGAGGA-
C.cleopatrae       TACCGAGCACCTCTCCGTCTCG-------GCGCGG--TACAC--AGGACGTGACGAGGA-
C.spB              TACCGAACACCTCTCCGTCTCGGTGCCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
C.spC              TATCGATCACCTCTCCGTCTCGGCGCCGTGCGCGG--TACCC--GGGACGTGACGAGGA-
C.cumingii         TACGGAACACCTCTCCGTCTCGGCGCCGTGCGCGGTATACCC--AGGACGTGACGAGGA-
C.incisa           TACGGAACACCTCTCCGTCTCGGTGCCGTGCGCGG--TGCCC--AGGACGTGACGAGGA-
C.lagunae1         TACGGAACACCTCTCCGTCTCGGTGCCGTGCGCGG--TGCCC--AGGACGTGACGAGGA-
C.lagunae2         TACGGAACACCTCTCCGTCTCGGTGCCGTGCGCGG--TGCCC--AGGACGTGACGAGGA-
C.spHalcon1        TACGGACCACCTCTCCATCTCGGTGCCGTGCGCGG--TACCA--GGGATGTGATGAGGA-
C.spHalcon2        TATCGAACGCCTCTCCTTCCCGGCGCCGTGCGCGG--CACCA--GGGACGTGAGGAGGA-
C.spIsabella       TACGGAACGCCTCTCCGTCTCGGTGCCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
C.spLantuyang      TATCGAACGCCTCTCCGTCTCGGTGCCGTGCGCGG--TACCC--AGGACGCGACGAGGA-
C.spNaga           TACGGACCACCTCTCCGTCTCGGTGCCGTGCGCGG--TACCC--AGGACGTGACGAGGA-
                                                                            300
A.pulcher          GTGTCTATCGAATAAGATAT-ATCTCGTCGCCCCACTCCGCC----AATGTCTT-GTTCC
C.baileyi          GCGTCCATTGAA-TAGATATTATCTCGTCGCCCCTTCCCCC-----AACATCCT-CTTCC
C.monticola        GCGTCCATTGAA-TTGATATCATCTCGTCGCCCCCCCCTCATCCC-AACATCCT-CTTCC
C.pendula          GCGTCTATTGAA-TAGATTCTATCTCGTCGCCTCCTCCCCCG----AGCATCGT-CTTCC
C.splendens        GCGTCTATTGAA-TAGATACTATCTCGTCGCCCCCC----------AACATCCT-CTTCC
C.wallichii        CCATCTATGGAA-TAGATTTTATCTCGTAGCCCCCCAGC-------GGCATTGT-CTTCC
C.burbigei         GCGTMTAYTGAA-TAGATTCTATCTCGTCGCCCCCCC---------AGCATCGT-CTTCC
C.chrysea          GCGTCTATTGAA-T--ATATAATCTCGTCGCCCCCC----------AGCATCCT-CTCCC
C.clarkei          GCGTCTATTGAA-TAGATTATATCTCGTCGCCCCCCC---------AGCATCGT-CTTCC
C.corniculata      GCGTCTATCGAA-TAGATACTATCTCGTCGCCCCCC----------AACATCCT-CTTCC
C.gibbsiae         GCGTCTATTGAA-TATATAGAATCTCGTCGCCCCC-----------AACATCCT-CTTCC
C.kermesina        GCGTCTATTGAA-TAGATTATATCTCGTCGCCCCCCC---------AGCATCGT-CTTCC
C.mesilauensis     GCGTCTATCGAA-TAGATACTATCTCGTCGCCCCCC----------AACATCCT-CTTCC
C.elatostemoides   GCGTCTATTGAA-TATATATAATCTCGTCGCCCCC-----------AACATCCT-CTTCC
C.inaequifolia1    GCA-CTATCGAA-TAGATATTATCTCGTTGCCCCCCCC--------AGCATCCT-CTTCC
C.inaequifolia2    GCA-CTATCGAA-TAGATATTATCTCGTTGCCCCCCC---------AACATCCT-CTTCC
C.pulgarensis      GCA-CTATCGAATTAGATATTATCTCGTTGCCCCTCTTCTCCCC--AACATTCT-CTTCC
C.hirtigerav.c     GCA-CTATCGAA-TAGATATTATCTCGTTGCCCCCCCC--------AACATCCT-CTTCC
C.hirtigerav.h     GCA-CTATCGAA-TAGATATTATCTCGTTGCCCCCCCC--------AACATCTT-CTTCC
C.cleopatrae       GCA-CTATCGAA-TAGATATTATCTCGTTGCCCCCC----------AACATCCT-TTTCC
C.spB              GAA-CTATCGAA-TAGATATTATCTTGTCGTCCCCCC---------AACATCCT-CTTCC
C.spC              GCATCTATTGAA-TAGATTCTATCTCGTCGCCCCCCCC--------AGCATCGT-CTTCC
C.cumingii         GCA-CCATTGAA-TAGATATAATCTCGTCGCCCCCCCCCCCCCCAA-AACATCTT-CTTCC
C.incisa           GGA-CCATTGAA-TAGATATTATCTCGTCGCCCTGTCCCCCC----AACATCGT-CTCCC
C.lagunae1         GGA-CCATTGAA-TAGATATCATCTCGTCGCCCTCTCCCCCC----AACATCCT-CTCCC
C.lagunae2         GGA-CCATTGAA-TAGATATCATCTCGTCGCCCTCTCCCCCC----AACATCCT-CTCCC
C.spHalcon1        GAA-CGATTGAA-TAGATATTATCTCGTCGCCCCTCCCCCCC----AGCATCCT-CTTCC
C.spHalcon2        GCA-CGATTTAA-TAGATACTGTCTCGTCGCCCCCCC---------AACATCCT-CTTCC
C.spIsabella       GCA-CCATTGAA-TAGATATTATCTCGTCGCCCCCCC---------AACATCCT-CTTCC
C.spLantuyang      GCGTCTATTGAA-TAGATTCTATCTCGTCGCCCCCTCCCCC-----AGCATCGT-CTTCC
C.spNaga           GGA-CTATCGAA-AGGATATTATTTTGTCGCCCCTCCCC-------AGCA-CCTTGTTCC
                                                                            360
A.pulcher          CTGATTCAGTCAAAGTGTTG-GGGGAC-AATGCGTA--CCAAGGAGGA-GGGACG--GAT
C.baileyi          ACA-----CTAAGAGTGCCG-GGAGAC-GATACATA--CG---AAGGA-GGGGCGCGGAT
C.monticola        AAA-----CTAAGAGTGCCG-GGAGAC-GATAGATA--CG---GAGGA-GGGGCGCGGAT
C.pendula          CCA-----CTCAGAGTGCCG-GGAGAA-GATGCATA--CG---AAAGA-GGGGCG--GAT
C.splendens        CCA-----CTCAGAGTGTCG-GGAGAC-GATGCATA--CG---AAAGA-GGGGCG--GAT
C.wallichii        CCA-----CTCAGAGTGCCG-GGAGAT-GATGCATATACG---AAAGA-GGGGTG--GAT
C.burbigei        CCA-----CTCAGAGTGCCG-GGAGAC-GACGCATA-- CG---GAAGA-GGGGCG--GAT
C.chrysea          CCT-----CTCAGAATGCCG-GGAGAC-GACGCATA--CG---AAAGA-AGGGCG--GAT
C.clarkei          CCA-----CTCAGAGTGCCG-GTAGGC-GACGCATA--CG--AAAAGA-GGGGCG--GAT
C.corniculata      CCA-----CTCAGAGTGCCG-GGAGAC-GATGCATA--CG---AAAGAGGGGGCG--GAT
C.gibbsiae         CCA-----CTCCGAGTGCCG-GGAGAC-GATGCATA--CG---AAAGA-GGGGCG--GAT
C.kermesina        CCA-----CTCAGAGTGCGG-GTAGGC-GACGCATA--CG--AAAAGA-GGGGCG--GAT
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APPENDIX – *continued*

```
C.mesilauensis      CCA-----CTCAGAGTGTCG-GGAGAC-GATGCATA--CG---AAAGA-GGGGCG--GAT
C.elatostemoides    CCA-----CTCCGAGTGCCG-GGAGAC-GATGCATA--CG---AAAGA-GGGGCG--GAT
C.inaequifolia1     CCA-----CTCTGAGTGTCG-GGAGAC-GATGCACA--CG---GAGGA-GGGGCG--GAT
C.inaequifolia2     CCA-----CTCTGAGTGTCG-GGAGAT-GATGCACA--CG---GAGGA-GGGGCG--GAT
C.pulgarensis       CCA-----CTCTGAGTGTCG-GGAGAC-TATGCACA--CG---AAGGA-GGGGCG--GAT
C.hirtigerav.c      CCA-----CTCTGAGTGTCG-GGAGAC-GATGCACA--CG---AAGGA-GGGGCG--GAT
C.hirtigerav.h      CCA-----CTCTGAGTGTCG-GGAGAC-GATGCACA--CG---AAGGA-GGGGCG--GAT
C.cleopatrae        CCA-----CTCTGAGTGTCG-GGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
C.spB               CCA-----CTCTGCGTGTCG-GGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
C.spC               CCA-----CTCAGAGTGCCG-GGAGAC-GACGCATA--CG---GAAGA-GGGGCG--GAT
C.cumingii          CCA-----CTCAGAGTGCCG-GGAGAC-GATGCGTA--CG---AAGGA-GGGGCG--GAT
C.incisa            CCA---C-CCCAGAGTGCCG-GGAGACCGATGCATA--CG---AAGGA-GGGGCG--GAT
C.lagunae1          CCA---C-CCCAGAGTGCCG-GGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
C.lagunae2          CCA---C-CCCAGAGTGCCG-GGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
C.spHalcon1         ACA-----CTCAGAGTGCCGAGGAGAA-GATGCACA--CC---GAGGA-GGGGCG--GAT
C.spHalcon2         CCA-----CTCAGAGTGTCGGGGAGAC-GATGCATA--CG---ACGGA-GGGGCG--GAT
C.spIsabella        CCA-----CTCAGAGTGCCG-GGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
C.spLantuyang       CCA-----CTCAAAGTGCCG-GGAGAC-GATACATA--CG---AAAGA-GGGGCG--GAT
C.spNaga            CCA-CTCACTCAGAGTGCCGGGGAGAC-GATGCATA--CG---AAGGA-GGGGCG--GAT
                                                                            420
A.pulcher           ATTGGCCTCCCGTTATCCAA-GTATAGCGGCCGGCACAAATAGTATACCGTGTCGATTGA
C.baileyi           ATTGGCCTCCCGTTATCCTT-GCATAGCGGCCGGCCCAAATAACATGCCGTGGCGATGGA
C.monticola         ATTGGCCTCCCGTAATGCTA-GCATAGCGGCCGGCCCAAATAACATGCTGTGGCKATGCA
C.pendula           ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGCCGGCGGA
C.splendens         ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGAAGGA
C.wallichii         ATTGGCCTCCCGTTATCCTA-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGACGGA
C.burbigei          ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGCCGACGGA
C.chrysea           ATTGGCCTCCCGTTATCCTC-ATGTAGCGGCCGGCCCAAATAACATGCCGTGTCTGCGCA
C.clarkei           ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATTCCGTGCCGACGGA
C.corniculata       ATTGGCCTCCCGTTATCTTC-GTGCAGCGGCCGGCCCAAATAACATACCGTGTCGATGGA
C.gibbsiae          ATTGGCCTCCCGTTATCTTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGACGGA
C.kermesina         ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATGCCGTGCCGACGGA
C.mesilauensis      ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGTGGACGGA
C.elatostemoides    ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATGCCGTGTCGACGGG
C.inaequifolia1     ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAATAACATACTGTGCCGATGGA
C.inaequifolia2     ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAATAACATGCTGTGCCGATGGA
C.pulgarensis       ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAATAACATACCGTGCCGATGGA
C.hirtigerav.c      ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGATGGA
C.hirtigerav.h      AT-------CCGTTATCCTT-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGATGGA
C.cleopatrae        AT-------CCGTTATCCTT-GTGTTGCGGCCGGCCCAAATAACATACCGTGCCGATGGA
C.spB               ATTGGCCTCCCGTTATCCT--------CGGTCGGCCCAAATAATATACCGTGCCGATGCA
C.spC               ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATGCCGTGCCGACGGA
C.cumingii          ATTGGCCTCCCGTTATCTTG-GCGTAGCGGCCGGCCCAAACAAGATACCGTGTCGATGGA
C.incisa            ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAACAACATACCGTGTCKATGGA
C.lagunae1          ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAACAACATACCGTGTCGATGGA
C.lagunae2          ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAACAACATACCGTGTCGATGGA
C.spHalcon1         ATTGGCCTCCCGTTACCCTT-GTGTAGCGGCCGGCCCAAACAACATGCCGTGTCGATGGA
C.spHalcon2         GTTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAACAACATACCGTGTCGATGGA
C.spIsabella        ATTGGCCTCCCGTTATCCTT-GTGTAGCGGCCGGCCCAAACAACATGCCGTGTCGATGGA
C.spLantuyang       ATTGGCCTCCCGTTATCCTC-GTGTAGCGGCCGGCCCAAATAACATACCGTGTCGACGGA
C.spNaga            ATTGGCCTCCCGTTATCACT-GCGTAGCGGCCGGCCCAAACAACATACCGTGCCGATGGA
                                                                            480
A.pulcher           TGTCACACGATACGTGGTGG---TTGGATT----CCTCAACTTGCGA---------ACTA
C.baileyi           TGTCACACGATACGTGGTGGCGGTTAGATC----CTTCGACTTGCAA--------AACTA
C.monticola         TGTCACACGATACGTGGTGGCGGTTAGATT----CCTCGACTTGCAA--------AACTA
C.pendula           TGTCACACGATAAGTGGTGG---TTGGATT----CCTCAACTCGCGG---------ACTA
C.splendens         TGTCACACGATAAGTGGTGG---TCGTGTT----CCTAAACTTGCGG---------ACTA
C.wallichii         TGTCACACGATAAGCGGTGG---TTGGATT----CCTCAACTTGCGT---------AGTA
C.burbigei          TGTCACACGATAAGTGGTGG---TTGGATT----CCTCAACTCGCGG---------ACTA
C.chrysea           TGTCACACGATGAGTGGTGG---TTGGATT----CCTCAACTTGCGG---------ACTA
C.clarkei           TGTCACACGATAAGTGGTGG---TTGGATT----CCTCAACTCGCGG---------ACTA
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APPENDIX – *continued*

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C.corniculata        TGTCACACGATAAGTGGTGG---TCGGATT----CCTCAACTTGCGG---------ACTA
C.gibbsiae           TGTCACACGATAAGTGGTGG---TCGGATT----CCTCAACTTGCGT---------ACTA
C.kermesina          TGTCACACGATAAGTGGTGG---TTGGATT----CTTCAACTCGCGG---------ACTA
C.mesilauensis       TGTCACACGATAAGTGGTGG---TCGTATT----CCTCAACTTGCGG---------ACTA
C.elatostemoides     TGTCACACGATAAGTGGTGG---TCGGATT----CCTCAACTTGCGT---------ACTA
C.inaequifolia1      TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAACTCGCGA---------ACTA
C.inaequifolia2      TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAACTCGCGA---------ACTA
C.pulgarensis        TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAACTCGCAA---------ACTA
C.hirtigerav.c       TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAACTCGTGA---------ACTA
C.hirtigerav.h       TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAAYTCGTGA---------ACTA
C.cleopatrae         TGTCACACGATACGTGGTGG---TTGGATTGATTCCTCAACTCGCGA---------ACTA
C.spB                TGTCACACGATACGTGGTGG---TTGGATTGATTCTTCAACTTGCGA---------ACTA
C.spC                TGTCACACGATAAGTGGTGG---TCGGATT----CCTCAACTCGCGG---------ACTA
C.cumingii           TGTCACACGATACGTGGTGG---TTGGATT------------CGTGA---------ACTA
C.incisa             TGTCACAAGATATGTGGTGG---TTGGATT------------CGTGA---------ACTA
C.lagunae1           TGTCACACGATACGTGGTGG---TTGGATT------------CGTGA---------ACTA
C.lagunae2           TGTCACACGATACGTGGTGG---TTGGATT------------CGTGA---------ACTA
C.spHalcon1          TGTCACACGATACGTGGTGG---TTGGATT------------CGCGA---------ACTA
C.spHalcon2          TGTCGCACGATACGTGGTGG---TTGGATT------------TGTGA---------ACTA
C.spIsabella         TGTCACACGATACGTGGTGG---TTGGATT------------CGTGAACTTTACGAACTA
C.spLantuyang        TGTCACACGATAAGTGGTGG---TTGGATT----CCTCAACT-GCGG---------ACTA
C.spNaga             TGTCACACGATACGTGGTGG---TCGGATT------------CGTGA---------ACTA
                                                                            540
A.pulcher            TAT--------CGTGTGGGACTCCATCAATCCACGGGCCTGACCCAATGG---CACA--A
C.baileyi            TCT---GATATCGTGTGGGAATGCGTCTAGCCACGGGCACGACCCTGTGG---CAGC--A
C.monticola          TCT---AATATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCGGTGGCGGCAGC--A
C.pendula            TAT------ATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CACC--A
C.splendens          TCT-----TATCGTGTGGGAATGCGTCGAGCCACGGCCAAGACCCAATGG---CACC--A
C.wallichii          TCT------ATCGTGTGGGAATGCGTCGAGCCACGAGCACGGCCCAATGG---CACA--A
C.burbigei           TAT------ATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CACC--A
C.chrysea            TTC-----CCTCGTGTGGGAATGCGTCTCGCCACGGGCACGACCCAACGG---CACC--A
C.clarkei            TAT------ATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CACC--A
C.corniculata        TCT-----TATCGTGTGGGAATGCGTCGAGCCACGGCCACGACCCAATGG---CATC--T
C.gibbsiae           TCT-----TATCGNGTGGGAATGTGTCGAGCCACGGCCAAGACCCAATGG---CACG--A
C.kermesina          TAT------ATCGTGTGGGAATGCATCGAGCCAMGGGCACGACCCAACGG---CACC--A
C.mesilauensis       TCT-----TATCGTGTGGGAATGCGTCGAGCCACGGCCAAGACCCAATGG---CACC--A
C.elatostemoides     TCT-----TATCGTGTGGGAATGCGTCGAGCCACGGCCACGACCCAATGG---CAGC--A
C.inaequifolia1      TCAAGTTATTTCGTGTGGGAATGCGTCGAGCCAAGGGCACGACCCAATGGAG-CACT--A
C.inaequifolia2      TCAAGTTATTTCGTGTGGGAATGCGTCGAGCCAAGGGCACGACCCAATGGCG-CACT--A
C.pulgarensis        TCT--ATATATCGTG-GAGAATGCGTCGAGCCACGGGCACGACCCAATGA---CACT--A
C.hirtigerav.c       TCT--ATATATCGTGTGGGAATGCGTCGAGCCACGGGCATGACCCAATGG---CACT--A
C.hirtigerav.h       TCT--ATATATTGTGTGGGAATGCGTCGAGCCACGGGCATGACCCAATGG---CACT--A
C.cleopatrae         TCT--ATATATCGTGTGGGAATGCGTCCAGCCACGGGCACGACCCAATGG---CACT--A
C.spB                T-T---TATATCGCGTGAGAATGCGTCGAGCTACGGGCACGACCCAATGG---CATC--A
C.spC                ------TATATCGTGTGGGAATGCGTCGAGCCACGGGCAYGACCCAAAGG---CACC--A
C.cumingii           TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CAGC--A
C.incisa             TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CAGC--A
C.lagunae1           TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CAGC--A
C.lagunae2           TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGGCACGACCCAACGG---CAGC--A
C.spHalcon1          TCT---TATATCGTGTGGGAATGCGCCGAGCCACGGTCACGACCCAACGG---CAGC--A
C.spHalcon2          TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGCCACGACCCAACGG---CAGCGCA
C.spIsabella         TCT---TATATCGTGTGGGAATGCGTCGAGCCACGGGAACGACCCAACGG---CAGC--A
C.spLantuyang        TAT------ATCGTGTGGGAATGCGTCGAGCCACGGGTACGACCCAACGG---CACC--A
C.spNaga             TCT---TATATCGTGTGGGAACGCGTCGAGCCACGGGCACGACCCAACGG---CAGC--A
                                                 558                        589
A.pulcher                      GATTG------CCCTCGA100011111111?000001011011 01?1?1
C.baileyi                      GATTGGTGCTGCCTTCCA001001010111?011??11?0001001 1?0
C.monticola                    GATCGGTGCTGCCCTCCA100001010111?011??11?0001001000
C.pendula                      GATCG------CCCTCGA101011111110101 1??11?101101?1?1
C.splendens                    GTTTG------CCCTCGA101011111111?011??11?101101?1?1
C.wallichii                    GATTG------CCTTCGA101011111111?011??11?101101?1?1
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APPENDIX – *continued*

C.burbigei	GATCG------CCCTCGA101011111111?011??11?101101?1?1
C.chrysea	GATTG------CCCTCGA001011111110001 1??11?101101?1?1
C.clarkei	GATCG------CCCTCGA101011111111?011??101101101?1?1
C.corniculata	GTTTG------CCCTCGA101011111111?011??11?101101?1?1
C.gibbsiae	GGT-G------CCCTCGA101011111111?011??11?101101?1?1
C.kermesina	GATCG------CCCTCGA101011111111?011??10?101101?1?1
C.mesilauensis	GATTG------CCCTCGA101011110?101011??11?101101?1?1
C.elatostemoides	GATTG------CCCTCGA10101?111111?011??11?101101?1?1
C.inaequifolia1	GATTG------CCTTCGA101010110111?111??11?101001?011
C.inaequifolia2	GATTG------CCTTCGA101011110111?111??11?101001?011
C.pulgarensis	GATTG------CCTTCGA101011110111?101??11?101001?1?1
C.hirtigerav.c	GATGG------CCTTCGA101011110111?111??11?101001?1?1
C.hirtigerav.h	GATTG------CTTTCGA101011110111?111??11?111001?1?1
C.cleopatrae	GA?????????????????101011110 1?1?111??11?111001?1??
C.spB	GATTG------CCCTCGA101011110011?111??11?101001?1?1
C.spC	GATCG------CCCTCGA101011111111?011??11?101101?1?1
C.cumingii	GATTG------CCTTCGA101111100101?111??11?101?11?1?1
C.incisa	GTTTG------CCTTCGA101111100111?1101111?101?11?1?1
C.lagunae1	GATTG------CCCTCGA111111100111?1101111?101?11?1?1
C.lagunae2	GATTG------CCCTCGA111111100111?1101111?101?11?1?1
C.spHalcon1	GATTG------CCCTCGA101111100111?111??01?101?11?1?1
C.spHalcon2	GATTG------CCCTCGA101110100111?111??01?101?11?1?1
C.spIsabella	GATTG------CCCTCGA101111100111?111??11?101?1001?1
C.spLantuyang	GATCG------CCCTCGA101011111111?011??11?101101?1?1
C.spNaga	GATCG------CCCTCGA101011100111?1100001?101?11?1?1