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Reevaluating the taxonomic status of *Ceriops australis* (Rhizophoraceae) based on morphological and molecular evidence

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ABSTRACT. *Ceriops australis* (White) Ballment, Smith & Stoddart, a member of the mangrove family Rhizophoraceae, was originally recognized as *C. tagal* var. *australis* White but was raised to species rank based solely on isozyme features and the only distinctive morphological feature of the hypocotyl. Therefore, it was considered a sibling species of *C. tagal* (Perr.) C. B. Rob. The goal of this study was to test the previous assessment that *C. australis* and *C. tagal* differ consistently only in hypocotyl morphology, in order to reevaluate the taxonomic status and to establish its geographic range. Principal components analysis was employed to analyze 29 morphological characters of herbarium specimens from Australia, Madagascar, and Sumatra tentatively identified as *C. australis* and *C. tagal*, and two well differentiated distinct taxa were recognized. In addition, both of the detailed morphological features based on fresh and herbarium materials and the intron sequences of *trn*L gene from plastid DNA support this conclusion. This finding disagrees with previous assessment and supports the current taxonomic status of *C. australis*. Here, a key to these two species is provided, and a revised distribution range of *C. australis* is established. This is the initial report of *C. australis'* occurrence in a part of Indonesia, in addition to areas of Australia and Papua New Guinea.

Keywords: Australia; *Ceriops tagal*; *Ceriops*; Distribution; Indonesia; Mangroves; Papua New Guinea; Plastid DNA; Principal components analysis.

INTRODUCTION

Ceriops Arn. is one of the mangrove genera in the family Rhizophoraceae, with a widespread geographical range from eastern Africa throughout tropical Asia, and northern Australia to Melanesia, and through Micronesia north to southern China (Tomlinson, 1986). It typically grows in the inner mangroves, often forming pure stands on better drained sites or becoming stunted in exposed and highly saline sites, within the reach of occasional tides (Hou, 1958).

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The last revision of the genus *Ceriops* was done by Hou (1958), with two species recognized: *C. tagal* (Perr.) C. B. Rob. and *C. decandra* (Griff.) Ding Hou. Some 20 additional names, including several infraspecific names were synonymized for them, but a variety name *C. tagal* (Perr.) C. B. Rob. var. *australis* C. T. White named by White (1926) was not listed.

White (1926) noticed a form of *C. tagal* in which the propagules had smooth, terete hypocotyls rather than the angled or ribbed hypocotyls typical of *C. tagal* from Australia and Papua New Guinea. He initially intended to describe this form as a new species distinct from *C. tagal*, based on the "less distinctly veined, and more inclined to recurved" leaves (White, 1926). After examining additional specimens, however, he found those differences between the new form and *C. tagal* were not constant

except for the hypocotyl morphology. Thus, White described the form as a variety, *C. tagal* var. *australis*.

Based on the analysis of starch gel electrophoresis of isozymes from *C. tagal* var. *tagal*, *C. tagal* var. *australis* and *C. decandra* in northern Australia, Ballment et al. (1988) found a uniform genetic structure within each taxon and a high level of genetic divergence among taxa. For each taxon having a distinct isozyme profile and the evidence of reproductive isolation, the authors proposed three distinctive species and hence raised White's variety to specific rank as *C. australis*, despite the fact that the extent of divergence in morphological characters other than propagule morphology remained unclear (Ballment et al., 1988). *Ceriops australis* was then claimed as a sibling species of *C. tagal* (Ballment et al., 1988).

Due to the confusion regarding diagnostic characters, it is still unknown how far north of Australia *C. australis* extends (Duke, 2006). Misidentification of these two morphologically similar taxa has been quite common in herbaria (Sheue, personal observation). Making a field identification of *C. australis* is very difficult at any time other than the fruiting stage with a hypocotyl. Thus, a detailed study of these two morphologically similar species is vital.

The goals of this study, therefore, are to detect the differences between *C. australis* and *C. tagal*, based on a broad and detailed morphological assessment aided by molecular data, and to establish the geographic distribution range of *C. australis*. Here we apply principal components analysis to morphometric data obtained from herbarium specimens and investigate the DNA features of the *trn*L intron of cpDNA. We also use detailed characters from fresh and herbarium materials to reevaluate the taxonomic status of these species. The results will be useful for field work identification and herbarium examination, conservation, and for clarification of the relationship between these species.

MATERIALS AND METHODS

Herbarium specimens and morphometric analysis

As it has been reported that only viviparous seedlings could be used to differentiate *Ceriops australis* from *C. tagal*, the former having terete (smooth) hypocotyls and the latter having ridged hypocotyls (White, 1926; Ballment et al., 1988), specimens of branches with both vegetative and reproductive features, including viviparous seedlings, were examined for this morphometric study. Fifteen specimens from the Northern Territory (OTUs 1-8) and Queensland (OTUs 9-15) of Australia, tentatively identified as *C. australis*, and 15 herbarium specimens tentatively identified as *C. tagal* representing populations of Northern Territory from Australia (OTUs 16-22), Madagascar (OTUs 23-26), and Sumatra (OTUs 27-30) were used in the morphometric study (Appendix). Principal components analysis (PCA) was conducted to analyze 29 morphological characters (25 quantitative and 4 binary characters, Table 1), and the PC-ORD package (McCune and Mefford, 1999) was used to analyze character variable matrices. The possible differentiated characters identified in this analysis will be used to detect diagnostic features in the following analysis.

Fresh plant materials for morphological characterization

Fresh plant materials of *C. australis* and *C. tagal* were sampled from Cape York, Cairns, and Cardwell of northeastern Queensland and from the Darwin area of the Northern Territory of Australia during 2005 to 2007 for morphological characters investigation and molecular study. Three branches from each of five individuals in a population were collected. Characters of fresh materials were investigated by a Leica MZ75 stereoscope and photographed with an Olympus C7070 digital camera. Voucher specimens were deposited at the Herbarium of the Department of Biological Resources, National Chiayi University (CHIA).

Herbarium specimens for determining distribution range

The loaned specimens (Appendix) from herbaria BM, DNA, GH, K, L and MO were identified as *C. australis* or *C. tagal* through the following two steps. In the first step, specimens with viviparous seedlings attached on the shoots were determined and used for getting diagnostic features for identification. In the second step, specimens lacking hypocotyls were identified according to the diagnostic features obtained from the previous first step. Each specimen was carefully examined at least thrice. In addition, a few specimens of *C. australis* examined from Herbaria BO and CAL were incorporated in the results.

Molecular evidence

Materials. Populations of *C. australis* and *C. tagal* were mainly sampled at five sites on the northeast Queensland coast and north Northern Territory coast in Australia during the period from 2003 to 2007. In addition, *C. tagal* collected from Singapore and India and *C. decandra* collected from India were analyzed together (Table 2). Three to five leaves were taken from each individual and stored with silica gel in zip-lock plastic bags until DNA isolation. Voucher specimens were deposited at the Herbarium of National Chiayi University (CHIA).

DNA extraction. Using the cetyltrimethylammonium bromide (CTAB) method described previously (Doyle and Doyle, 1987), total DNA was extracted from fresh etiolated leaves. Ethanol-precipitated DNA was dissolved in TE (Tris-EDTA) buffer and stored at -20ºC. Qiagen (Valencia, CA, USA) columns were used to clean the DNA samples, which were difficult to amplify by PCR. The approximate DNA yields were then determined using a spectrophotometer (model U-2001, Hitachi).

PCR amplification and electrophoresis. The protocols for PCR were as follows. A 50-µl mixture contained 40 mM Tricine-KOH (pH 8.7), 15 mM KOAc, 3.5 mM Mg $(OAc)_2$, 3.75 µg/ml BSA, 0.005% Tween 20, 0.005% Nonidet-P40, four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of Advantage 2 DNA polymerase (Clontech), 10 ng genomic DNA, plus a 50-µl of mineral oil. Amplification reactions were carried out in a dry-block with two-step thermal cycles (Biometra). The universal primers for amplifying the *trn*L intron of chloroplast DNA were the same as described by Taberlet et al. (1991). The first step of PCR reaction conditions for the *trn*L intron were: incubation at 94ºC for 3 min, 10 cycles of denaturation at 94ºC for 30 s, annealing at 68ºC for 10 s, and extension at 72ºC for 45 s. The second step was carried out with 30 cycles of denaturation at 94ºC for 30 s, annealing at 66ºC for 10 s, extension at 72ºC for 45 s, and a final extension for 5 min at 72ºC. The PCR products were analyzed by agarose gel electrophoresis (1.0%, w/v in TBE), stained with 0.5 µg/ml ethidium bromide, and photographed under UV light exposure.

DNA recovery and sequencing. The PCR products in this study were recovered using glassmilk (BIO 101, California) and directly sequenced following the method of dideoxy chain-termination using an ABI377 automated sequencer with the Ready Reaction Kit (PE Biosystems, California) of the BigDye™ Terminator Cycle Sequencing. Primers for sequencing were the same as those used for PCR. Each sample was sequenced two or three times to ensure the accuracy of the sequences. The reactions were performed following the recommendation of the manufacturers. These reactions were performed based on the recommendations of the manufacturer.

Data analyses. DNA sequence alignment was conducted using the program Clustal W multiple alignment in BioEdit (Hall, 1999). Genetic relationships were then determined using the program MEGA version 2.1 (Kumar et al., 2001). The genetic distance matrix was calculated by the two-parameter method of Kimura (1980) and then used to construct the phylogenetic trees using the Neighbor-joining (NJ) method (Saitou and Nei, 1987). Maximum parsimony (MP) analyses (Fitch, 1971) were done using code modified from the Close-Neighbor-Interchange (CNI) algorithm (Rzhetsky and Nei, 1992) in MEGA version 2.1 (Kumar et al., 2001). Bootstrapping (1000 replicates) was carried out to estimate the support for both NJ and MP topologies (Felsenstein, 1985; Hillis and Bull, 1993). The strict consensus parsimonious tree was then constructed using the program MEGA version 2.1 (Kumar et al., 2001).

RESULTS

Morphometric analysis

We performed a principal coordinate analysis, and the result is shown in Figure 1. These two species are well separated, and 51.8% of the variation can be explained by the first two principal coordinates. Only the first ordination axis was considered (Table 1). For components, the ten highest eigenvector values belonged to reproductive characters, except leaf length; accordingly, these include surface of hypocotyl (HS), length of fruit (FL), length of hypocotyl (HL), length of calyx lobe/ width of calyx lobe (CLL/CLW), width of calyx lobe (CLW), thickness of the middle part of calyx lobe (CLT), width of hypocotyl (HW), width of fruit (FW), length of style (STL) and leaf length (LL). The highest three eigenvector values of vegetative characters were leaf length (LL), stipule length at the naturally expanded stage (SL) and leaf width (LW). These characteristic variables represented the relative contribution of the first component in explaining the total variation within the dataset. Each two selected diagnostic characters of organs belonging to leaf (LL, SL), flower (CLW, STL), and fruit (HS, FL) are suggested for use in identification and are shown in Figure 1.

Morphological features

Ceriops australis and *C. tagal* have very similar morphological characteristics, including a grey-white trunk and stem with buttressed base, elliptic-obovate leaves with reflexed margins, and small flowers with white petals (Figure 2). The most distinctive basis upon which to differentiate the two species is the viviparous seedling (hypocotyl), as reported before. Based on field experience, *C. tagal* usually has dark green and elliptic to obovate leaves and longer stipules (the expanded stipules usually longer than 1.5 cm) than *C. australis*, which has more yellow-green and obovate leaves and shorter stipules (the expanded stipules usually less than 1.2 cm) (Figure 3; Table 1).

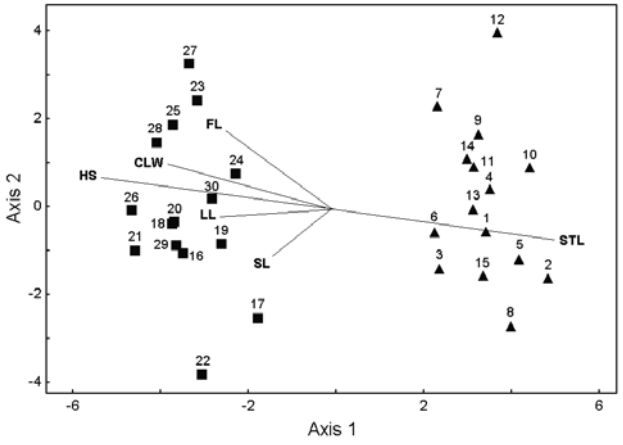

Figure 1. PCA ordination diagram of OTUs and prominent variables. OUTs 1-15: *Ceriops australis*; OUTs 16-30: *C. tagal*. Each two selected diagnostic characters belonged to organs of leaf (LL, SL), flower (CLW, STL) and fruit (FL, HS) for differentiating the two species are suggested. Abbreviations: CLW: Width of the base of calyx lobe; FL: length of fruit; HS: surface of hypocotyl; LL: leaf length; SL: stipule length at the naturally expanded stage; STL: length of style.

Table 1. A List of the selected 29 morphological characters, examined from each 15 herbarium specimens of *Ceriops australis* and *C. tagal* from Madagascar, Sumatra and Australia for principal components analysis. Characters 1-11 are leaf characters, 12-22 are flower characters, 23-29 are fruit and hypocotyl characters.

No.	Character (unit) or (character state)	Character abbreviation	*C. australis* Mean±std or character state	*C. tagal* Mean±std or character state	Eigenvector value of axis 1
1	Leaf blade length (mm)	LL	55.7±5.3	68.5±8.2	-0.2169
2	Leaf width (mm)	LW	27.5±3.4	32.3±5.1	-0.1792
3	The length between the maximum width of leaf to leaf apex (mm)	LWmax	22.9±3.6	27.7±5.1	-0.1680
4	Petiole length (mm)	PL	18.4±4.4	19.1±4.5	-0.0326
5	Leaf length/ leaf width	LL/LW	2.05±0.20	2.13±0.23	-0.0278
6	The ratio of leaf length to the length between the maximum width of leaf to leaf apex	LL/LWmax	2.5±0.3	2.5±0.3	-0.0058
7	Leaf length/ petiole length	LL/PL	3.2±0.8	3.7±0.6	-0.1105
8	Leaf apex	LAE	0 = 6	0 = 12	-0.1260
	with (0) or without emarginated (1)		1= 9	1 = 3	
9	The degree of acute of leaf apex	LAA	61.7±5.0	63.1±7.8	-0.0471
10	Number of lateral vein	V	7.3±0.8	8.0±0.7	-0.1334
11	Stipule length of naturally expanded (mm)	SL	11.8±1.4	16.1±2.7	-0.2060
12	Length of calyx lobe (mm)	CLL	4.3±0.3	4.1±0.4	0.0784
13	Thickness of the middle part of calyx lobe (mm)	CLT	0.24±0.04	0.35±0.05	-0.2402
14	Width of the base of calyx lobe (mm)	CLW	1.4±0.2	2.0±0.1	-0.2530
15	Length of calyx lobe/width of calyx lobe	CLL/CLW	3.0±0.4	2.1±0.2	0.2538
16	Length of petal (not included bristle) (mm)	PL	2.8±0.3	3.04±0.19	-0.1519
17	Number of bristles of each petal apex	B	3.5±0.7	3.0±0.0	0.1228
18	Length of bristle (mm)	BL	0.77±0.15	0.61±0.11	0.1554
19	Length of enlarged part of bristle (mm)	BHL	0.23±0.06	0.31±0.05	-0.1902
20	Width of enlarged part of bristle (mm)	BHW	0.08±0.01	0.13±0.04	-0.2180
21	Trichome on abaxial surface of petal	PT	0 = 15	0 = 6	0.1991
	with (0) or without (1)			1 = 9	
22	Length of style (mm)	STL	3.07±0.39	2.08±0.49	0.2259
23	Length of fruit (mm)	FL	11.6±1.39	19.3±1.62	-0.2645
24	Width of fruit (mm)	FW	6.2±0.7	9.2±1.28	-0.2258
25	Length of fruit/ width of fruit	FL/FW	1.9±0.3	2.1±0.2	-0.1377
26	Persistent calyx lobe	CLR	0 = 7	0 = 15	-0.1651
	reflex (0) or patent (1)		1 = 8		
27	Length of hypocotyl (mm)	HL	106.6±23.0	241.3±38.6	-0.2596
28	Width of hypocotyl (mm)	HW	3.2±0.6	5.9±1.11	-0.2393
29	Surface of hypocotyl	HS			-0.2818
	smooth (0) or ridged (1)		0 = 15	1 = 15	

It was evident that features like calyx lobe, petal morphology, style (Figure 4), fruit length, and hypocotyl surface (Figure 3C) could aid the differentiation of these sibling species. *Ceriops australis* has longer flowers (Figure 4A), narrower and longer calyx lobes (Figure 4C; Table 1), longer petals (Figure 4D-E) and longer styles (Figure 4F) than *C. tagal* (Figure 4B, C, E-F). Three to five more slender clavate appendages were commonly found on the petal apex of *C. australis*, but only three such appendages (more short) were observed on *C. tagal* (Figure 4D-E; Table 1).

DNA evidence

Sequence alignment and characteristics. PCR products from each sample studied were directly sequenced. The accession numbers of those plastid DNA sequences

Table 2. A list of molecular study for 14 accessions of the *Ceriops australis* and 15 accessions of *C. tagal,* as well as three outgroup accessions of *C. decandra*, and their different geographical distributions.

Abb.	Taxon	Collection location	Accessions No.
Rh-13	*C. australis*	Moreton Bay, QLD, Australia (AU)	EF118948
Rh-70	*C. australis*	Cairns, QLD, Australia (AU)	EF118971
Rh-71	*C. australis*	Darwin, NT, Australia (AU)	EF118949
Rh-72	*C. australis*	Darwin, NT, Australia (AU)	EF118950
Rh-73	*C. australis*	Darwin, NT, Australia (AU)	EF118951
Rh-113	*C. australis*	Cardwell, QLD, Australia (AU)	EF673713
Rh-114	*C. australis*	Cardwell, QLD, Australia (AU)	EF673714
Rh-115	*C. australis*	Cardwell, QLD, Australia (AU)	EF673715
Rh-120	*C. australis*	Cardwell, QLD, Australia (AU)	EF673717
Rh-132	*C. australis*	Cardwell, QLD, Australia (AU)	EF673721
Rh-133	*C. australis*	Cardwell, QLD, Australia (AU)	EF673722
Rh-134	*C. australis*	Cardwell, QLD, Australia (AU)	EF673723
Rh-135	*C. australis*	Cardwell, QLD, Australia (AU)	EF673724
Rh-136	*C. australis*	Cardwell, QLD, Australia (AU)	EF673725
Rh-31	*C. tagal*	West Sundarbans, India (IN)	EF118987
Rh-32	*C. tagal*	West Sundarbans, India (IN)	EF118964
Rh-33	*C. tagal*	West Sundarbans, India (IN)	EF118965
Rh-65	*C. tagal*	Cairns, QLD, Australia (AU)	EF118966
Rh-85	*C. tagal*	Cairns, QLD, Australia (AU)	EF118986
Rh-86	*C. tagal*	Cairns, QLD, Australia (AU)	EF118988
Rh-66	*C. tagal*	Darwin, NT, Australia (AU)	EF118967
Rh-67	*C. tagal*	Darwin, NT, Australia (AU)	EF118968
Rh-68	*C. tagal*	Cape York, QLD, Australia (AU)	EF118969
Rh-69	*C. tagal*	Cape York, QLD, Australia (AU)	EF118970
Rh-82	*C. tagal*	Pulau Ubin, Singapore (SING)	EF118972
Rh-116	*C. tagal*	Cardwell, QLD, Australia (AU)	EF673716
Rh-121	*C. tagal*	Cardwell, QLD, Australia (AU)	EF673718
Rh-127	*C. tagal*	Cardwell, QLD, Australia (AU)	EF673719
Rh-131	*C. tagal*	Cardwell, QLD, Australia (AU)	EF673720
Rh-26	*C. decandra*	Pichavarum, India (IN)	EF118952
Rh-28	*C. decandra*	West Sundarbans, India (IN)	EF118953
Rh-29	*C. decandra*	West Sundarbans, India (IN)	EF118954

Abbreviations: AU: Australia, IN: India; QLD: Queensland; NT: Northern Territory; SING: Singapore.

from the 14 accessions of *C. australis* and 15 accessions of *C. tagal* plus three outgroup accessions are shown in Table 2. Those sequences were aligned and resulted in 606 characters, from which 13 were variable sites. The sequence alignment was submitted to TreeBase (Submission ID: SN4033). Each variable site was a potentially informative parsimony site. Neither *C. australis* nor *C. tagal* showed any sequence variation at the species level. The genetic distance between *C. australis* and *C. tagal* was 0.003 using the 2-parameter method of Kimura (1980). Two stable transitions were found within this DNA region between *C. tagal* and *C. australis* (data not shown).

Phylogeny reconstruction. The phylogenetic tree for the intron of *trn*L used characters that were equally weighted. Based on the MP method, the analysis yielded 270 equally parsimonious trees with a length of 13 steps, a consistency index (CI) of 1.0, and a retention index (RI) of 1.0. The strict consensus tree is shown in Figure 5. More than 50% of the bootstrap values are shown below/above the supported branches for MP tree. The NJ tree and the MP strict consensus tree constructed from plastid DNA data were highly congruent (Figure 5, MP tree presented only). Based on the phylogenetic tree, accessions of *C. australis* formed a clade supported by a 69% bootstrap value, and accessions of *C. tagal* formed a clade supported by a 72% bootstrap value. Molecular data also supported the distinctness of *C. australis* and *C. tagal*, even in the sympatric populations of Queensland and Darwin, Australia.

Distribution range of *C. australis*

The whole distributional range of *C. australis* includes eastern (Moreton Bay) and northern Queensland (Cape York, Nassau River), the coast of the Northern Territory, through northern and northwestern Western Australia (to the Ashburton River), the southern part of Papua New Guinea (Port Moresby, Daru Island), through Timor, Flores, Sumbawa, Java and Pulau Bilinton, close to Sumatra, Indonesia (Figure 6). This is the first report that *C. australis* occurs in parts of Indonesia.

According to the examination of herbarium specimens, *C. australis* has a much wider distribution range than *C. tagal* in Australia, although *C. tagal* is widely distributed from East Africa through India and Asia to New Caledonia. However, *C. tagal* is only found in northeastern and northern Queensland, through Cape York, Arnhem Land, and Melville Island in Australia. There are only about five colonies with a few individuals of *C. tagal* growing closely with *C. australis* found in the Darwin area (Sandy Creek) in the Northern Territory according to our field survey.

DISCUSSION

In this study, both of the morphological features revealed by PCA and molecular evidence demonstrate that *C. australis* should be recognized as distinct from *C. tagal*, rather than as a sibling species only slightly different

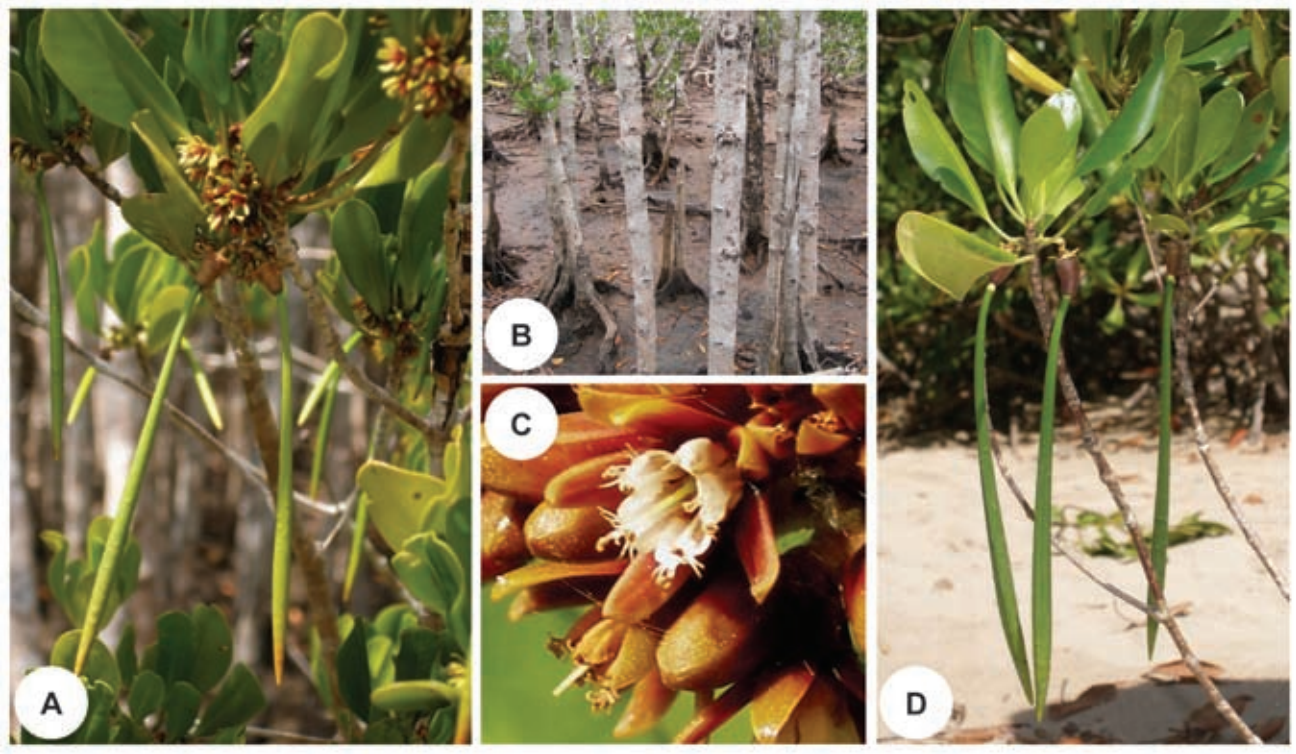

Figure 2. Habitats of *Ceriops australis* (A-C) and *C. tagal* (D). A, Close-up of *C. australis* with flowers and viviparous seedlings with smooth surface; B, The grey-white bark with buttress base of *C. australis* at Cairns, Queensland; C, Flowers of *C. australis*. Note the evident long style; D, *C. tagal* with viviparous seedlings with ridges.

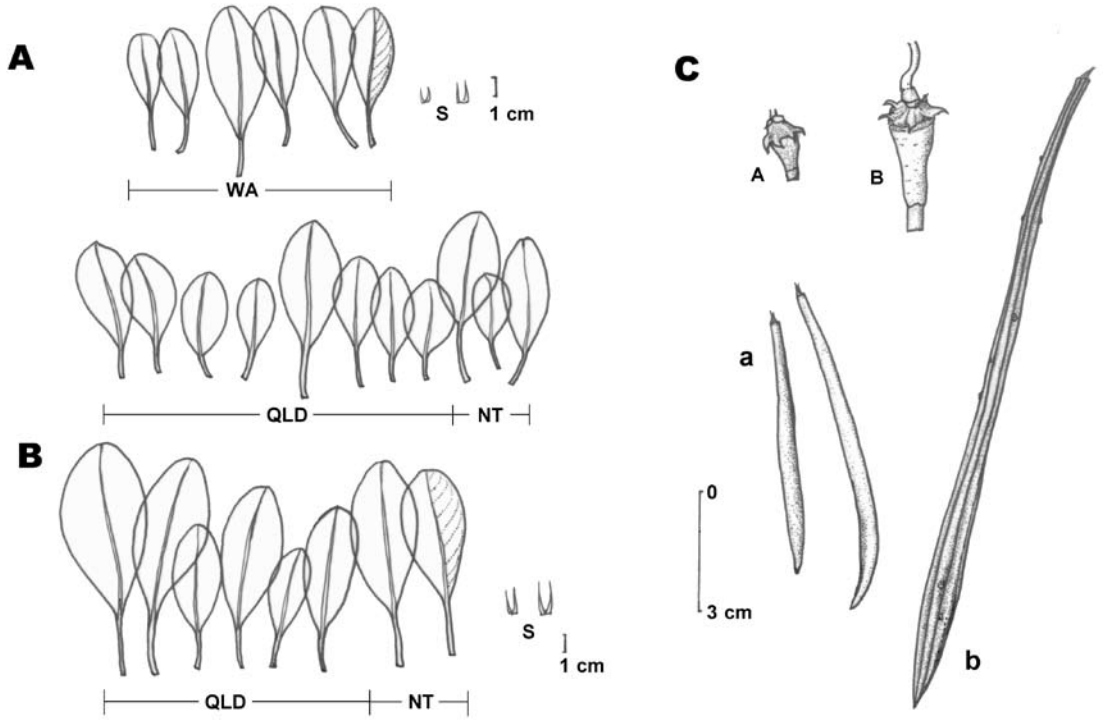

Figure 3. Characters of leaves, fruits and hypocotyls of *Ceriops australis* and *C. tagal*. A, Leaves of *C. australis* tend to be more obovate in shape and stipules at naturally expanded stage are usually less than 1.2 cm; B, Leaves of *C. tagal* are more oblong in shape, and stipules at naturally expanded stage are usually longer than 1.4 cm; C, The fruit is smaller and the hypocotyl is shorter and ridge-free of *C. australis* (A & a); while the fruit is larger and the hypocotyl is longer and ridged of *C. tagal* (B & b). Abbreviations: NT: Northern Territory, QLD: Queensland, WA: Western Australia.

in hypocotyl feature and genetic structure as proposed by White (1926) and Ballment et al. (1988). However, for a practical application of the concept of species, it is necessary to provide some diagnostic characters. According to the morphometric results obtained in this study, the most distinctive characters differentiating the two species are reproductive features. The diagnostic characters of style length (STL) and width of calyx lobe (CLW) are recommended for the plants with flowers; those of hypocotyl surface (HS) and fruit length (FL) are recommended for plants with fruits. Nevertheless, we suggest that the features of leaf length (LL) and stipule length of the naturally expanded stage (SL) could also aid the identification of plants in the field without flower or fruit.

Based on the results of morphological features and PCA, a key to differentiating the populations of *C. australis* and *C. tagal* is here provided:

Key to *C. australis* and *C. tagal*

1a. Leaf blade usually shorter than 6.5 cm in length; stipule less than 1.2 cm long at the naturally expanded stage; base of calyx lobe 12-15 mm in width; style 2.7-4 mm in length; fruit 9-14 mm long; hypocotyl terete (without longitudinal ridges), 5-12 cm in length ..... *C. australis*

1b. Leaf blade usually longer than 6.5 cm in length; stipule longer than 1.4 cm long at naturally expanded stage; base of calyx lobe 18-25 mm in width; style 1.5 mm in length (-3.5 of populations from Darwin area, Northern Territory of Australia); fruit 18-25 mm long; hypocotyl angular (with longitudinal ridges), 15-35 cm in length ..... *C. tagal*

According to Wightman (2006), populations of *C. tagal* in Northern Territory generally have elliptic leaves and relative shorter petiole length (usually less than 1/4 of the blade length) than the mostly obovate leaves and relative longer petiole length (generally reaching 1/3 or more of the blade length) of *C. australis*. Based on the observation of this study, we agreed with Wightman's statement and found that the populations of *C. australis* in Western Australia have the most typical obovate and smaller leaves than other populations. It is likely that *C. australis* is the only one species of this genus occurring in Western Australia, which results in much less opportunity to have gene flow with other species of *Ceriops*, if compared to the other sympatric populations of *Ceriops*.

In addition, we noted some of the detailed differences between these two taxa, including the number of colleters inside the adaxial base of the stipule (Sheue, 2003), the thickness of the middle of the calyx lobe, and the number and shape of the clavate appendages on the petal apex.

However, to observe these delicate features a hand lens (10X) or a stereoscope may be needed.

It is notable that the observation of herbarium specimens in this study revealed no evident morphological variations of *C. tagal* between the populations of Madagascar and Sumatra and those from northern Australia. The low levels of morphological variation across a big geographic range of *C. tagal* noted by this study were consistent with the inter simple sequence repeat (ISSR) markers of *C. tagal* studied in Asia (Ge and Sun, 2001) and the *trn*L intron sequences of plastid DNA from different locations of *C. tagal* in this study.

Correct information for identification is essential to getting an accurate biogeographic description. Since the confusion in diagnostic characters applies to these two taxa in Australia, obtaining accurate information on their distribution ranges is not easy. This is perhaps why Australia's Virtual Herbarium (AVH) could not supply the correct information for these two taxa (http://www.anbg.gov.au/cgi-bin/avhxml.cgi). In terms of AVH Mapper, *C. australis* only occurs in the Nothern Territory and northeastern Western Australia while *C. tagal* has a much wider distribution range from Queensland, through the Northern Territory to Western Australia. Based on a detailed examination of herbarium specimens in this study, we have reconstructed the geographic range of *C. australis* and *C. tagal* in Australia. The dominant species of *Ceriops* in Australia is *C. australis*. It ranges from Western Australia and the Northern Territory to Queensland. This result is consistent with the report of Duke (2006). In Papua New Guinea, *C. australis* has only been observed from Port Moresby and Idlers Bay. This was consistent with the observations of White (1926), McCusker (1984), and Wells (1983).

It is quite interesting that *C. australis* occurs in Timor, Flores, Sumbawa, Java and Pulau Bilinton of Indonesia. The first collector of *C. australis* from Indonesia may have been Teijsmann in 1875 (specimens found at BO herbarium, Sheue, personal observation). Due to the limited herbarium specimens available from Indonesia, an extensive field survey for *C. australis* from the nearby islands of Indonesia would be useful. This information would be valuable for mangrove conservation and the study of phytogeography and dispersal ecology.

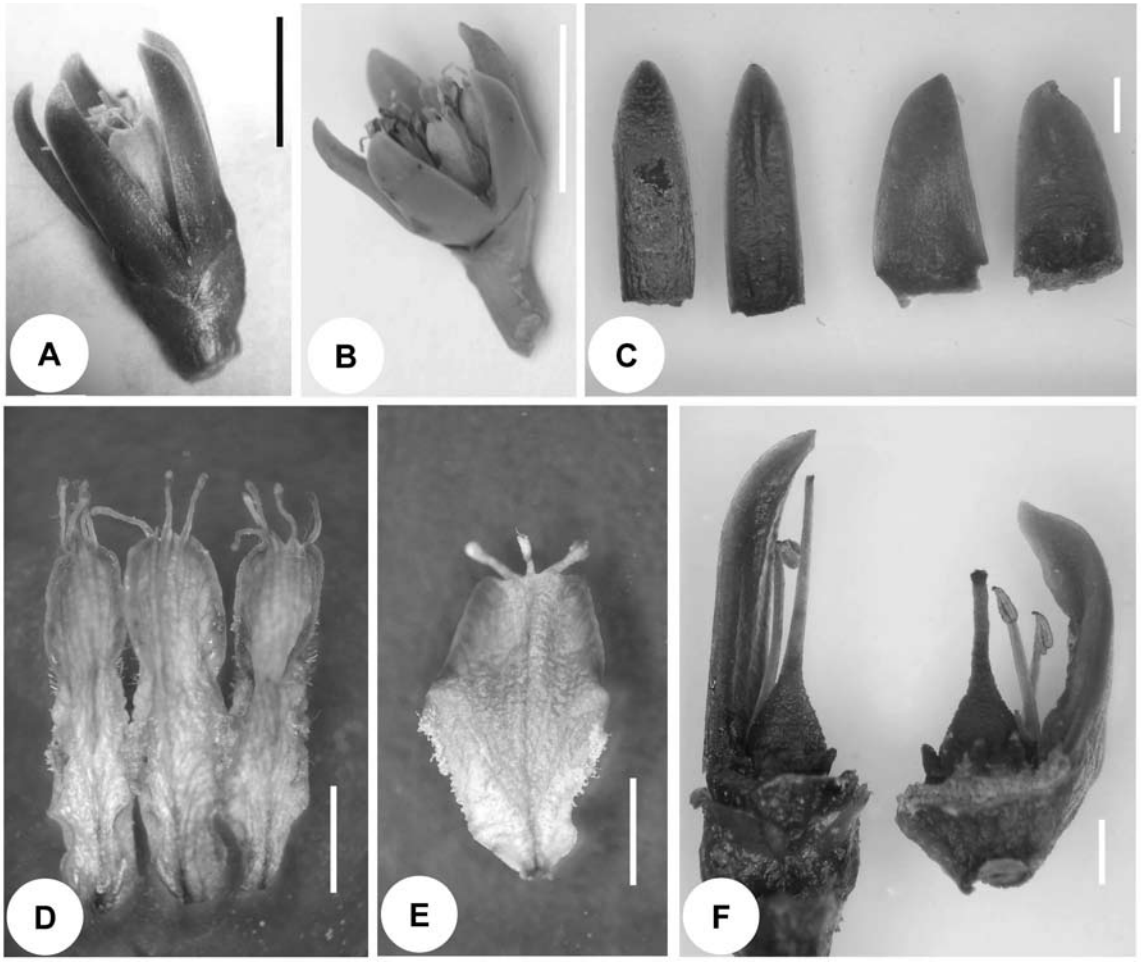

Figure 4. Flower morphology of of *Ceriops australis* and *C. tagal*. A, Lateral view of a flower of *C. australis*; B, Lateral view of a flower of *C. tagal*; C, Calyx lobes with abaxial and adaxial sides of *C. australis* (left) and *C. tagal* (right); D, Petals of *C. australis*, with 3-5 more slender clavate appendages; E, Petal of *C. tagal*, usually with 3 short clavate appendages; F, Lateral view of detached flowers of *C. australis* (left) and *C. tagal* (right) showing calyx lobe, anther and style. Scale bars: A-B = 5 mm, C-F = 1 mm.

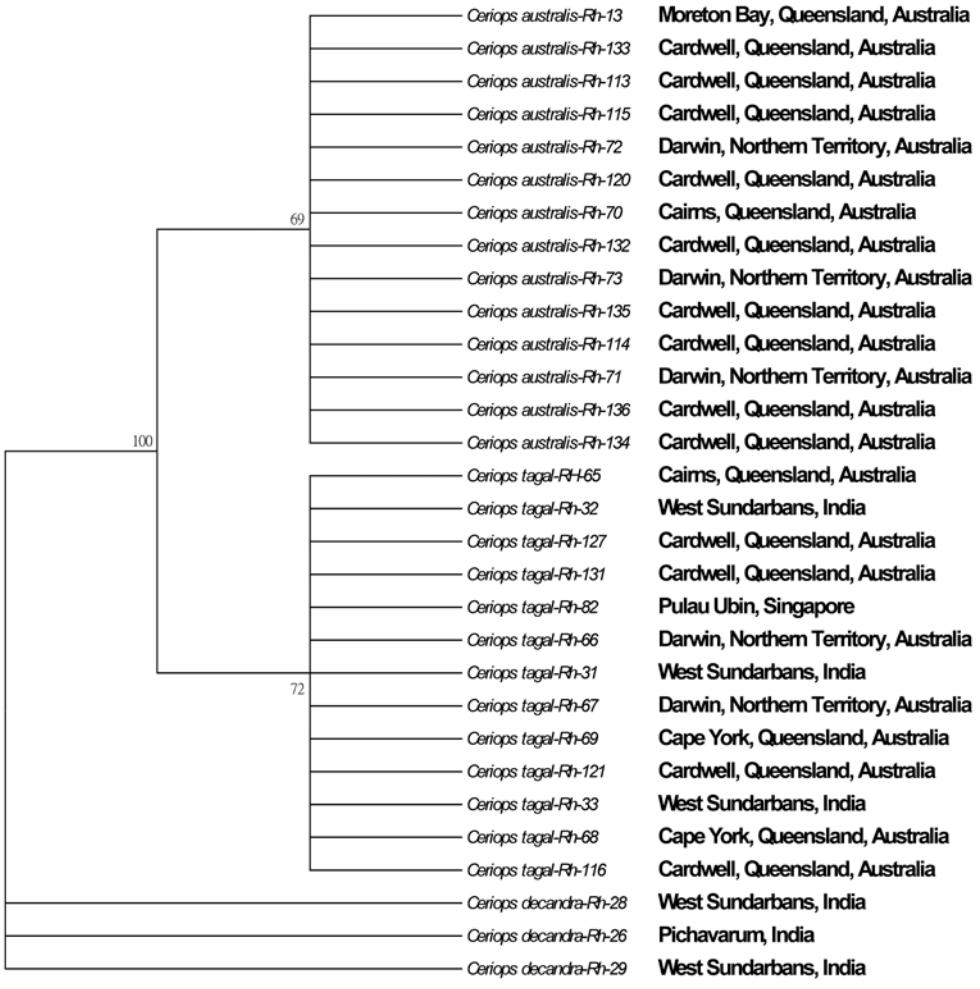

Figure 5. The strict consensus parsimonious tree of 14 accessions of *Ceriops australis* and 15 accessions of *C. tagal* plus three outgroup accessions of *C. decandra* derived from the *trn*L intron sequence. Bootstrap values > 50% are shown on each branch.

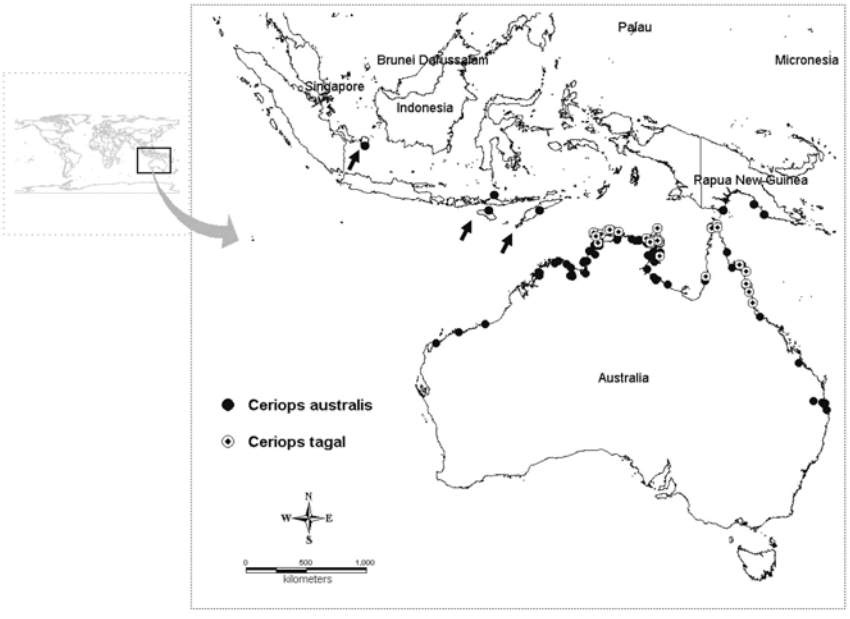

Figure 6. The distribution range of *Ceriops australis* and the sympatric localities of *C. tagal* in Australia. The arrows indicate the new localities of *C. australis* in Indonesia first reported in this study.

These two species are sympatric in Papua New Guinea and northern Queensland (White, 1926; McMillan, 1986), and both occur on the northern coast of Northern Territory (Wells, 1983). No intermediate forms between them has been recorded, as reported by McCusker (1984). After the examination of numerous herbarium specimens and limited fresh materials collected from Darwin area, we found that several characters of flowers of *C. tagal* collected from Northern Territory and Papua New Guinea are closer to those of *C. australis*. Namely, the populations of *C. tagal* from the Northern Territory and Papua New Guinea have narrower and oblong calyx lobes, longer clavate appendages on the petal apex and longer styles than *C. tagal* from other populations in the world. However, the characters of fruit and propagule of *C. tagal* from this area resemble those of other global populations of *C. tagal*. We assume that a possible hybridization between these two taxa may have occurred. According to Duke et al. (1984), the major flowering season of the populations from northeastern Australia are November and January to March for *C. australis* and *C. tagal*, respectively. Based on the observation of herbarium specimens, a broader period of flowering season for both species could be inferred. The possible overlap of flowering season may increase the opportunity of hybridization between these two species. An anecdotal report notes that hybridization has occurred, and some trees with both types of propagules from the Murray River, Admiralty Island, and Pigeon Island in northeastern Queensland have been observed (Ballment et al., 1988). However, except for the flower variation in the Northern Territory and Papua New Guinea previously mentioned, we did not find such intermediate forms in this study.

A further study to compare the morphological and genetic variations of populations of *C. tagal* in the Northern Territory, Australia, and Papua New Guinea and the other populations from the world should be useful and interesting. Moreover, the factors influencing the sympatric populations of *C. australis* and *C. tagal* in the Northern Territory and Queensland may be worth exploring, in order to reveal why a possible hybridization only occurs in the Northern Territory, but not in northern Queensland.

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以形態及分子特徵再評估南方細蕊紅樹（紅樹科）的分類位階

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南方細蕊紅樹為紅樹科的紅樹林植物，White 原發表此為細蕊紅樹的一變種，Ballment 等人隨後僅以同功酵素特徵的差異，將其提昇至種的位階，由於兩者極為相似，形態上僅胎生苗下胚軸的外表略有差別，故被指稱為細蕊紅樹的親緣種。本研究欲驗證此兩分類群的形態特徵是否僅具下胚軸的差異，並輔以分子特徵，再評估其分類位階及確立其地理分布範圍。以來自澳洲、馬達加斯加與蘇門答臘的標本材料做形態測量分析，選出 29 項形態特徵，以主成分分析（PCA），可將原先試驗性地分成此兩個分類群的材料明顯地區分為二大群。另外，比較此兩分類群的新鮮植物與臘葉標本之多項細微形態特徵和葉綠體 DNA 的 *trn*L intron 基因序列也支持此結論。本研究的結果不同意先前所認為之此兩分類群僅具下胚軸的形態差異，支持南方細蕊紅樹之種的分類位階。本文並提供此兩種植物的檢索表及重新建立南方細蕊紅樹的分布範圍，除了澳洲和巴布亞新幾內亞外，本研究並首次報導印尼地區亦有本種的分布。

關鍵詞：澳洲；細蕊紅樹屬；細蕊紅樹；分布；印尼；紅樹林；巴布亞新幾內亞；葉綠體 DNA；主成分分析。

Appendix. Specimens list (herbarium acronyms follow Index Herbariorum, available at http://sweetgum.nybg.org/ih/).

Specimens list for morphometric analysis for principal components analysis. *Ceriops australis*: **AUSTRALIA: Northern Territory:** *Bowman & Wilson 263* (DNA), *Dunlop 3629* (DNA), *Dunlop & Munns 7512* (L), *Egan 2821* (DNA), *Forster & Russell-Smith PIF5920* (DNA), *Henshall 857* (DNA), *Latz 3192* (DNA, L, MO), *Must 1649* (DNA), *Russell-Smith & Lucas 5884* (DNA). **Queensland:** *Smith 4825* (L), *12441* (GH, L) *Stoddart 4536*, *4699*, *4761*, *4903* (L, MO), *4992* (L). *Ceriops tagal*: **AUSTRALIA: Northern Territory:** *Brock 116* (DNA), *Dunlop 3899* (DNA), *Dunlop & Wightman 9709* (DNA), *J. & Eurell 78/20* (DNA, MO), *Scarlett 164* (DNA), *Wightman 458*, *786* (DNA). **MADAGASCAR:** *Birkinshaw & Jules 13* (MO), *Darcy & Rakotozafy 15470* (MO), *Dorr & Koenders 3063* (GH, MO), *Rahajasoa 356* (MO). **SUMATRA:** *Iwatsuki* et al. *S1319* (MO), *S1321* (L), *Schmad 146* (L), *Teijsmamn & Miquee s. n.* [no date] (K).

Specimens examined for revising the distribution range of *C. australis* and the sympatric localities of *C. tagal* in Australia. *Ceriops australis*: **AUSTRALIA:** New Holland: *Banks & Solander s. n.* [1770] (BM), **Queensland:** *Blake 14127* (MO), *Clarkson 2016* (MO), *3875* (DNA, MO), *Cribb & Newton s. n.* [1950] (BM), *Dietrich s. n*. [1863-65], *657* (MO), *Durrington* (L), *Everist 7881A* (L), *Fosberg 61833* (MO), *Macnae s. n.* [1962], Mrs. *Stephenson 569* (BM), *Neldner & Clarkson 2993* (DNA), *Smith 4825*, *11435* (L), *12441* (GH, L), *Stoddart AQ14784* (K), *4510* (MO), *4527*, *4536*, *4699*, *4761*, *4786*, *4903* (L, MO), *4992* (L), *Webster & Hildreth 15005* (GH), *White s. n.* [1915] (BM), *3372A* (K, type), *3373A* (GH); **Northern Territory:** *Bardsley s .n.* [1985] (DNA), *Barlow 506* (DNA), *Blake 17050* (K, GH), *Bowman & Wilson 263* (DNA), *Brennan 2619* (DNA), *Brooker 3258* (DNA), *Byrnes NB275* (DNA), *Byrnes & Maconochie 1077* (DNA), *Calliss 63* (DNA), *Chippendale s. n.* [1961], *8180* (DNA), *Clark 948* (DNA), *Cowie 5183* (DNA), *Cowie & Dunlop 4131*, *7926* (DNA), *Dunlop 1869* (DNA), *2782* (DNA, MO), *3984* (DNA), *Dunlop & Leach 8062* (DNA), *Dunlop & Munns 7512* (L), *Dunlop & Wightman 9203* (DNA), *Egan 2391*, *2821* (DNA), *Forster & Russell-Smith PIF5920* (DNA), *Gill s. n.* [1970] (GH), *Henry 88* (DNA), *Henshall 857* (DNA), *Hodder s. n.* [1971] (K), *D4044* (DNA), *Latz 3192* (DNA, L, MO), *3390* (DNA), *Leach 3993*, *4231* (DNA), *Leach & Cowie 3641* (DNA), *Martensz & Schodde AE737* (DNA), *McKean B142*, *974* (DNA), *Michell & Ingraham 27* (DNA), *Must 884*, *1310* (DNA), *1348*, *1649* (DNA, MO), *Nelson 1078* (DNA), *Rankin 1171*, *1248*, *1380*, *2220* (DNA), *Ridpath Mck B7* (DNA), *Russell-Smith 8918* (DNA), *Russell-Smith & Lucas 4375*, *5642*, *5884*, *8368* (DNA), *Scarlett 163* (DNA), *Shaw & Dunlop 3629* (DNA, MO), *Smith 1030*, *Specht 591* (GH), *Story 8337* (DNA), *Thomson 661*, *1878*, *2621*, *2661* (DNA), *van Kerckhof 29*, *33*, *39* (DNA), *Waddy 560* (DNA), *Wells s. n.* [1975, 1978] (DNA), *Wheelwright DW8* (DNA), *24* (DNA), *Wightman 475*, *488*, *504*, *506*, *520*, *543*, *619*, *673*, *701*, *814*, *1070*, *1544*, *1663*, *2290*, *2389*, *2453*, *2472* (DNA), *4603* (DNA, MO), *6162*, *6652* (DNA), *Wightman & Dunlop 551*, *563* (DNA), *Wightman & Giulian 2926* (DNA), *Wightman & Smith 3531*, *4523* (DNA), *Williams 350* (DNA), *Williams & Wightman 135* (DNA), *Wilson 790* (DNA); **Western Australia:** *Croat 52316A* (MO), *Cunningham 235* (K), *Fstyguold s. n.* [1906] (BM), *George 12724* (DNA), *14829* (K), *Hartley 14587* (DNA), *Mitchell 5949* (DNA), *Morrison s. n.* [1950], *Paijmans 2469* (DNA), *Perry 2548* (DNA), *Wightman 7111* (DNA); **PAPUA NEW GUINEA:** Central District: near Barune: *Frodin UPNG4444* (K); Fairfax Harbour: *Gillison NGF22159* (GH); Kairuku subdistriction: *Darbyshire 773* (K); near Lae Lae: *Schodde 2681* (GH); Kappa Kappa Papua: *Brass 786* (BM); Port Moresby: *Frodin & Millar UPNG562* (L). **INDONESIA**: Timor: Anonymous *s. n.* [1923] (BO), *L. v. d. Pijl 820* (BO); Flores: *M. Kew s. n.* [1905] (BO); Subawa: *Kostermans & Wirawan 348* (BO); Java: *Teijsmann s. n.* [no date] (CAL); Bilinton Island: *Teijsmann s. n.* [1875] (BO).

Ceriops tagal: **AUSTRALIA:** no data: *Leschenault s. n.* [1802] (BM), **Queensland:** *Stoddart 4086, 4113, 4154* (MO), *4317* (MO, L), *4385* (MO), *4634* (MO, L), *4637* (MO), *Smith 11409* (GH), *11618* (K), *11619* (L), *12445* (GH), *12521* (L), *Smith & Webb 3243* (L), Mrs. *Stephenson 486*, *545*, *570*, *606* (BM), *Thom 4168*, *4170*, *4171*, *4172* (MO), *Clarkson 3387* (MO). **Northern Territory:** *Bardsley 15* (DNA), *Brennan 4563, 2627, 2877* (DNA), *Brock 116* (DNA), *Byrnes & Maconochie NB1078* (DNA), *Cowie 3397, 5140, 6924* (DNA), *Dunlop 3899* (DNA), *Dunlop & Wightman 6541, 9709, 9739* (DNA), *Egan 2713* (DNA), *Eurell s.n.* (MO), *s. n.* [1978] (GH), *J. & Eurell 78/ 20* (DNA, MO), *Kerrigan & Risler 57* (DNA), *Scarlett 164* (DNA), *Stocker GS79* (DNA), *Wells s. n.* [1975] (DNA), *Wightman 458, 786, 1970, 4113, 4185, 4457, 6506* (DNA), *Wightman & Giuliana 2993* (DNA), *Wightman & Smith 3531* (DNA).