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SYSTEMATICS

Comparison of *Ceriops pseudodecanda* sp. nov. (Rhizophoraceae), a new mangrove species in Australasia, with related species

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ABSTRACT. The notion that the widespread mangrove genus *Ceriops* consists of three species, *C. australis* (White) Ballment, Smith & Stoddart, *C. decandra* (Griff.) Ding Hou and *C. tagal* (Perr.) C. B. Rob., is still widely accepted. However, our recent studies have shown that the previously recognized species *Ceriops decandra* can be separated into three species, *C. decandra*, *C. zippeliana* Blume and *Ceriops pseudodecandra* sp. nov. Sheue, Liu, Tsai & Yang based on morphological and molecular evidence. The last entity is newly described in this treatment, which has been misapplied with the name *C. decandra* for several decades in Australia, Papua New Guinea and Seram, New Guinea (Irian Jaya) of Indonesia. Morphologically, the new species is more similar to *C. decandra* and *C. zippeliana* than to *C. australis* and *C. tagal*. Furthermore, molecular phylogenetic analysis also supports the notion that *C. pseudodecandra* is distinctly a new species. Here, the botanical descriptions with an illustration and the distribution ranges of the related species are provided. A key to differentiate species of *Ceriops* and a comparison with an emphasis on these three morphologically similar species are given as well.

Keywords: Australia; *Ceriops decandra*; *Ceriops pseudodecandra*; *Ceriops zippeliana*; Indonesia; Mangroves; Papua New Guinea.

INTRODUCTION

Mangroves are the characteristic intertidal plant formations found along the sheltered tropical and subtropical coastlines. The mangrove flora consists of around 36 genera in 26 families (Saenger, 2002). Among the members, the pan-tropical family Rhizophoraceae, which comprises 16 genera and about 120 species of evergreen trees and shrubs (Hou, 1958), is the richest mangrove family with four exclusively mangrove genera. However, a detailed morphological and anatomical study of the mangrove Rhizophoraceae showed that two species belonged to *Kandelia* and *Ceriops*, separately, and should be added or described (Sheue, 2003). The species *Kandelia obovata* Sheue, Liu and Yong was later reported as a new species from East Asia (Sheue et al., 2003) and received a strong support by the study using microsatellite markers (Giang et al., 2006).

The last revision of the genus *Ceriops* was put forward by Hou (1958), with two species recognized: *C. tagal* (Perr.) C. B. Rob. and *C. decandra* (Griff.) Ding Hou. *Ceriops tagal* is a common and familiar constituent of mangroves with a wide distribution range, from East Africa throughout Malaysia to Micronesia, Melanesia and northern Australia (Hou, 1958; Duke, 2006). *Ceriops decandra* is thought to range from India through Thailand, Vietnam and the Malay Peninsula, Java, Borneo, Philippines to New Guinea (Hou, 1958; Tomlinson, 1986; Field, 1995; Naskar and Mandal, 1999; Duke, 2006).

Ceriops australis (White) Ballment, Smith & Stoddart (1988) was added as the third member of the genus which was originally recognized as a variety of *C. tagal* by White (1926). The evidence for its elevation to species level was based on isozyme characters as well as the morphological feature of hypocotyl (Ballment et al., 1988). A recent evaluation concerning the taxonomic status of the species based on morphological and molecular evidence (Sheue et al., 2009a) supports this treatment.

As previously mentioned, another uncertain species was noticed in the genus *Ceriops* Arn. (Sheue, 2003). This uncertain species of *Ceriops* has been reverted to its original name *C. zippeliana* from its current applied name

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C. decandra (Sheue et al., 2009b). *Ceriops zippeliana* is morphologically and genetically discernible from *C. decandra* which occurs from India, Burma and the west coast of Thailand; and *C. zippeliana* should be the correct name for the populations in southeastern Asia to which the name *C. decandra* is misapplied (Sheue et al., 2009b). Moreover, a further analysis on the populations of the current '*C. decandra*' in New Guinea and Australia reveals that they are morphologically different from *C. zippelianan* and *C. decandra*. This raises the question about the taxonomic status of the populations and attracts our interest to clarify their species identity.

After a detailed study using herbarium specimens, fresh materials, and visits to the habitats in Australia and Indonesia over four years, the authors have come to the conclusion that the so-called '*C. decandra*' of Australia, New Guinea and Seram actually is yet an undescribed species based on morphological characters, palynological evidence and molecular evidence obtained in this study. Here, the botanical descriptions and the distribution ranges of this new species and related species are presented. A key to distinguish the five taxa of *Ceriops* is provided and the interspecific morphological differences are discussed, with an emphasis on the three morphologically similar species of *C. decandra*, *C. pseudodecandra* and *C. zippeliana*.

MATERIALS AND METHODS

Morphological and pollen characteristics

Fresh samples and herbarium materials were examined and photographed. Three to ten branches with flowers and viviparous seedlings were collected from each of five individuals of *C. pseudodecandra* along the riverbank of Blackmore River at Darwin of Northern Territory, Cairns of Queensland (October, 2005) and Cardwell of Queensland in Australia (May, 2007). In addition, a field survey for distribution range of *Ceriops* was especially carried out in Ambon, Seram, Sulawesi, Bali and Lombok of Indonesia (July- August, 2009). Voucher specimens were deposited in the Herbarium of National Chiayi University (CHIA). The specimens examined were from herbaria BM, BO, CAL, DNA, GH, HAST, IBSC, K, L, MO, PPI, SING, SINU and TAI (Holmgren and Holmgren, 1998).

Pollen grains were smeared on the surface of a microscope slide and examined with an Olympus BH-2 Light Microscope first to measure the size (N=30). Also, pollen grains were air dried and scattered on the surface of the stub covered with double-side tape and coated with gold directly. These samples were examined and photographed with a Hitach S-2400 Scanning Electron Microscope.

Molecular evidence

Materials. Samples of *Ceriops decandra*, *C. zippeliana* and *C. pseudodecandra* were collected at different localities of India, Singapore and Australia. Both *C. tagal* and *C. australis* were included as outgroups for comparison (Table 1). 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, PCR amplification and electrophoresis, DNA recovery and sequencing. The detailed procedures of DNA extraction, conditions of PCR and sequencing for this study were similar to those described in the previous report (Sheue et al., 2009a) and the universal primers for amplifying the *trn*L intron of chloroplast DNA were the same as described by Taberlet et al. (1991).

Data analyses. DNA sequence alignment was conducted using the program ClustalW multiple alignment in BioEdit (Hall, 1999). Genetic relationships were then determined using the program MEGA version 4 (Tamura et al., 2007). 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 4 (Tamura et al., 2007). 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 4 (Tamura et al., 2007).

TAXONOMIC TREATMENT

Ceriops pseudodecandra Sheue, Liu, Tsai, and Yang, sp. nov.—TYPE: Australia: Darwin Harbour: on banks of tidal Blackmore River (12°3′54.4″ S, 130°57′56.9″ E), *C.-R. Sheue M234*, 26th October 2005 (holotype: DNA; isotypes: BM, BO, DNA, GH, HAST, K, L, MO and SING). Figures 1-3 and Table 2

Ceriops decandra auct. non (Griff.) Ding Hou: Hou, Flora Malesiana, ser. 1, vol. 5: 471, 1958. *pro part*.

Folia pseudodecandra vel obovato-elliptica, 6-12 cm longa, 2.5-5 cm lata, venis lateralibus primariis 8-12, petiolis 1.2-3.5 cm longis. Pedunculis brevissimis. Petala 5, multi-fida, lobis apice 12-18 laciniatis, cilia in dimidio inferiore.

Small trees up to 6 m tall; bark grayish brown with horizontal fissures of main stem, flaky and flanged buttresses at base. Leaves oblong to elliptic-obovate, 6-12(-13) cm × 2.5-5(-6) cm, apex obtuse, rounded to emarginate, base attenuate, lateral veins 8-12 pairs; petiole 1.2-3.5 cm long. Stipules 2-3 cm long at extension stage before dropping, with 8-12 layered, 80-100 colleters inside at adaxial base. Inflorescence compact bifurcate cyme-like, usually borne at the upper nodes of a branch, axillary, (2-)3-20 flowered. Bracteole 2-lobed, disc-like, sessile, 2 mm long, apex rounded with small fissures. Calyx lobes 5, erect while flowering and fruiting, ovate, acute, 3 mm

Table 1. A list of molecular study for the 20 specimens of *Ceriops decandra* (Griff.) Ding Hou, *C. pseudodecandra* Sheue, Liu, Tsai and Yang, *C. zippeliana* Blume and two outgroup species of this genus, and their different geographical distributions and accession numbers.

No.	Taxon	Locations	Accession no.
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
Rh-30	*C. decandra*	West Sundarbans, India (IN)	EF118955
Rh-34	*C. decandra*	West Sundarbans, India (IN)	EF118956
Rh-35	*C. decandra*	West Sundarbans, India (IN)	EF118957
Rh-36	*C. decandra*	West Sundarbans, India (IN)	EF118958
Rh-14	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118977
Rh-43	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118973
Rh-44	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118974
Rh-45	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118975
Rh-46	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118976
Rh-56	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118982
Rh-57	*C. zippeliana*	Pasir Ris Nature Park, Singapore (SING)	EF118983
Rh-74	*C. pseudodecandra*	Cairns, Australia (AU)	EF118959
Rh-84	*C. pseudodecandra*	Cairns, Australia (AU)	EF118963
Rh-75	*C. pseudodecandra*	Darwin, Australia (AU)	EF118960
Rh-76	*C. pseudodecandra*	Darwin, Australia (AU)	EF118961
Rh-77	*C. pseudodecandra*	Darwin, Australia (AU)	EF118962
Rh-13	*C. australis*	Moreton Bay, Australia (AU)	EF118948
Rh-31	*C. tagal*	West Sundarbans, India (IN)	EF118987

× 1.5 mm, calyx tube *c.* 2 mm in height. Petals 5, white, turning brownish, linear-rectangular, 2-2.5 mm × 1.5 mm (including terminal cilia), apex fingers-like, fringed with 12-18 sinuate cilia, 0.5 mm long, base broad, upper half margins glabrous, lower half margins hairy (< 0.1 mm),. Stamens 10, almost equal in length; filament 1.3 mm long; anther 0.9 mm long, ovoid, dorsifixed, with one short connective protrusion. Ovary inferior, 3-locular, 2 ovules in each locule, style 1.5 mm long, stigma 1, very shortly trifid. Persistent calyx tube hemi-globular, 4-5 mm in height, 6-9 mm in width, persistent lobes 5, 2.5-3.0 mm × 1.0 mm, slightly obliquely erect or ascending. Fruit ovoid-conical, 1.0-1.3 cm × 0.5-0.8 cm (calyx tube not included). Hypocotyl clavate, 10-16 cm × 0.5-0.8 cm, sharply ridged and sulcate, with an elongated acuminate apex (root tip), erect to pendant; epicotyl 10-13 mm long.

Distribution. Australia: northern coastal areas of Northern Territory and northern and eastern Queensland; Indonesia: Provinsi Papua, Provinsi Irian Jayat Barat (Irian Jaya), the Daru Islands and Seram (east, Hoti and Bula); Papua New Guinea: entire coastal mangrove areas (Figure 4).

Specimens examined. **AUSTRALIA.** Northern Territory: *Brennan 4563* (DNA), *Craven 4611*, *4613, 6430* (DNA), *Dunlop 8644* (DNA), van *Kerkhof 50* (DNA), *Mckean 1026*, *1027* (DNA), *Rankin 1721* (DNA), *Risler and Kerrigan 186* (DNA), *Risler and Mangion 346* (DNA), *Russel-Smith and Lucas 5801* (DNA), *Sheue M232-M235* (CHIA), *Smith D3126* (DNA), *Wells s. n.* [1975], [1978], [1979] (DNA), *Wells 26* (DNA), *Wightman 388, 476*, *535*, *542, 1480, 2104*, *2471, 2289, 7328* (DNA). Queensland: *Macnae s. n.* [1962] (GH, L), *Neldner 3733* (DNA), *Sheue M241, M315-M317* (CHIA), *Smith 3998*, *11593*, *11617*, *12443* (GH, L). **PAPUA NEW GUINEA.** Boroi (Prov. Madang, Dist. Bogia): *Iserentant 9052*, *9423* (BM); Central District: *Darbyshire 777* (BO, GH), *Wiakabu* et al. *LAE70426* (BO); East Papua: *Pullen 8155* (GH, MO); Gulf District: Kerema Bay: *Schodde 4204* (K). **INDONESIA.** ARU ISLANDS: *Turner and Mamesah 22* (BO, GH, K); Irian Jaya: *Fanani and Wiharja 405* (BO), *Johns 9648* (BO), *Salverda 150* (BO), *Utteridge 37* (BO), *van Royen 4921* (BO, GH); Moluccas: Seram (east): *Buwalda 5981* (BO), *Mirmanto and Rukandi ERI46* (BO), *Komassi 927* (BO), *Sheue M481-482* (CHIA); Western Division: Daru Island: *Foreman et al. LAE60490* (GH), *Brass 6213* (BM), *6214* (K), *Pringgo 42* (BO), *Streimann and Lelean NGF18467* (GH), *Simaga 786* (GH), *Versteegh BW4928* (GH); West of Hisiu: *Leach 3803* (GH); Yapen Island: *Aet and Idjan 682* (GH, K, L), *971* (BO, K).

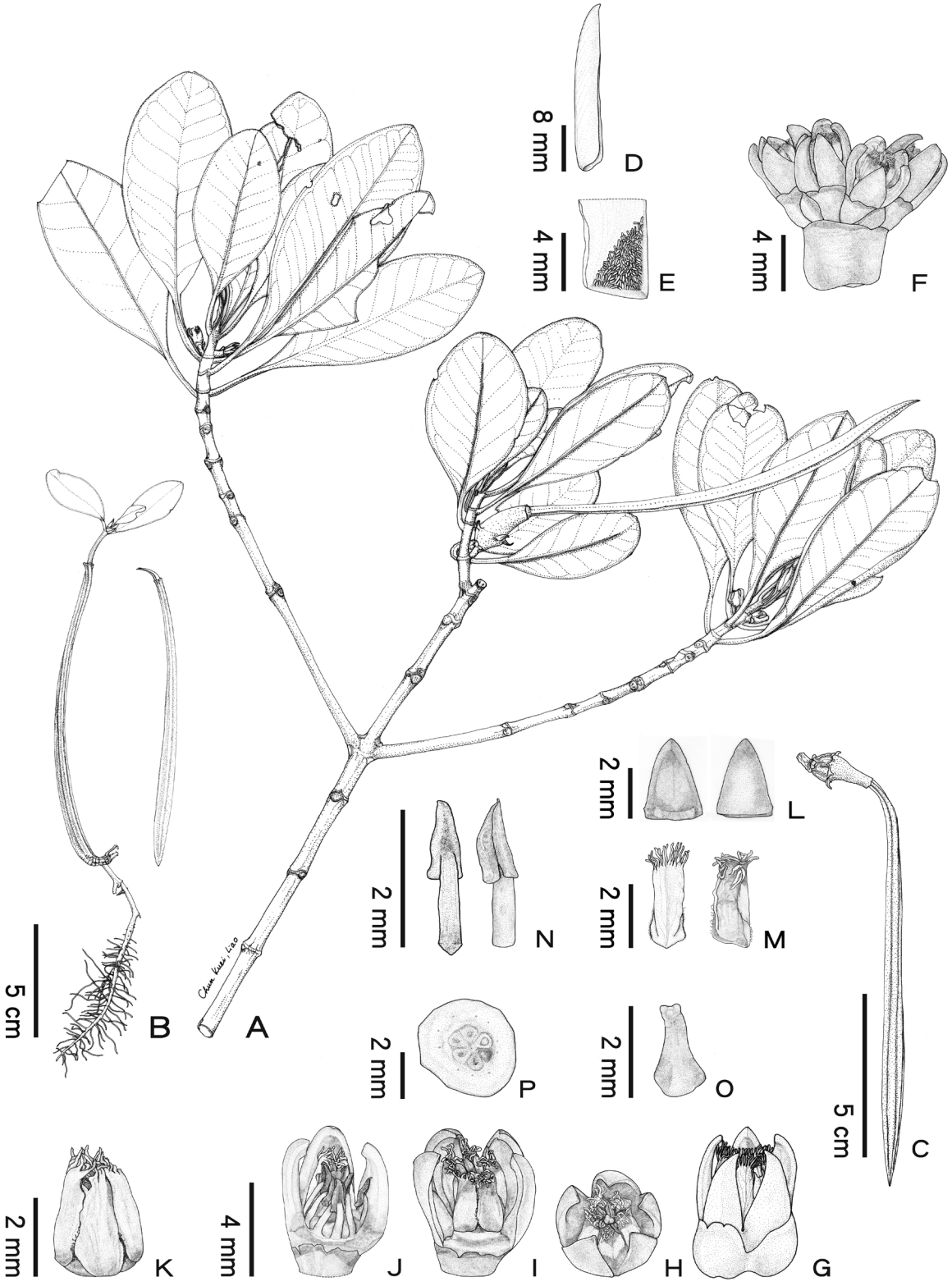

Figure 1. Illustration of *Ceriops pseudodecandra* Sheue, Liu, Tsai, and Yang. A, A shoot with flowers, fruit and hypocotyl; B, Seedlings with a prominent epicotyl up to 1.3 cm and elongated oblong young leaves; C, Fruit with a persistent hemi-globular calyx tube, erect calyx lobes, and a clavate hypocotyl with an acuminate sharp apex; D, Stipule; E, Half part of the adaxial base of stipule with colleters; F, A dense bifurcate cyme-like inflorescence; G, Lateral view of a flower; H, Top view of a flower; I-J, Lateral views of flowers, part of calyx lobes and petals removed; K, Lateral view of petals from a flower; L, Sepals of adaxial (left) and abaxial (right) sides; M, Petals of adaxial (right) and abaxial (left) sides; N, Stamens; O, Style; P, Cross section of ovary. (Material from Holotype: DNA, *C.-R. Sheue M234*, 26th October 2005, collected from banks of tidal Blackmore River of Darwin Harbour, Australia)

Ceriops decandra (Griff.) Ding Hou, Flora Males. 1, 5: 471. 1958. *pro part*. & *descr. emend.* & *excl. syn*. *C. zippeliana* Blume (Table 2) (Holotype, CAL: Ejusdem Icones, vol. 8, t. 116 !)

Bruguiera decandra Griff. (1836) 10.

Ceriops roxburgiana Arn. (1838) 364. *pro part.*

Rhizophora decandra Roxb. (1814) 36. *nomen.*

Shrub or small tree, 2-5 m tall; bark light-gray, peeling off into thin flakes, stilt roots developed at base. Leaves oval to obovate, 4-9 cm × 2.5-6 cm, apex obtuse, rounded to emarginate, base obtuse to cuneate, lateral veins 8-10 (-11) pairs; petiole 1.2-1.8 cm long. Stipules 1.2-2.4

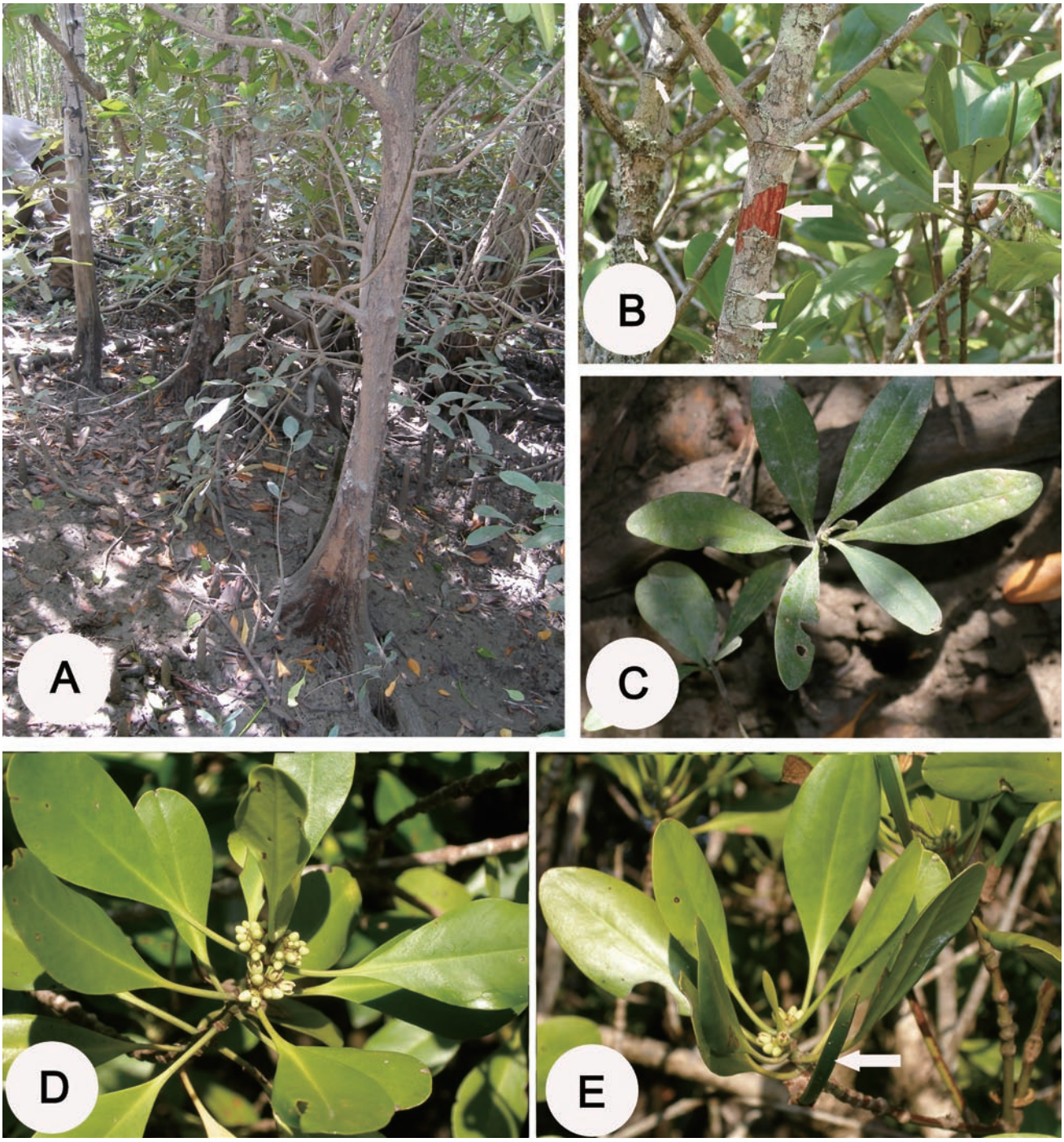

Figure 2. Habitat and morphological characters of *Ceriops pseudodecandra* Sheue, Liu, Tsai, and Yang. A-B, Plants in the mangroves of Darwin, Northern Territory of Australia; C-E, Plants in the mangroves of Cairns, Queensland of Australia. A, An individual tree with patch-like detached bark and buttress near trunk base; B, Stems with horizontal fissures (narrow arrow) on bark and reddish brown wood (broad arrow). Note a fruit with a lifted hypocotyl (H); C, Two young seedlings with typically oblong leaves; D, Dense bifurcate cyme-like inflorescences, each with 6-10 flowers; E, Fruit (brown color) and the elongated hypocotyls toward different directions (arrows).

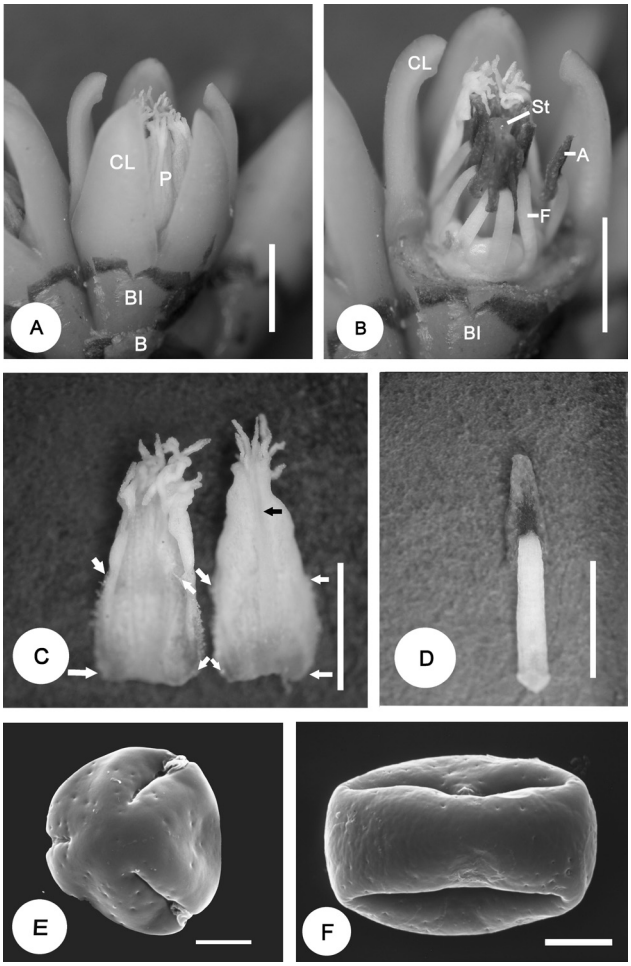

Figure 3. Floral characters of *Ceriops pseudodecandra* Sheue, Liu, Tsai, and Yang. Abbreviations: A: anther; B: secondary bract; Bl: bracteole; CL: calyx lobe; F: filament; P: petal; St: style. A-B, Floral lateral views, two calyx lobes and petals removed in B; C, Petals of adaxial (left) and abaxial (right) sides. Note the lower half margins with loose and short hairs (< 0.1 mm) (between the two white arrows) and a longitudinal ridge of the abaxial side (black arrow); D, Stamen; E-F, Polar view (E) and equatorial view (F) of pollen grains, exine smooth with sparsely distributed punctae. Scale bars: 1.5 mm in A-B; 1 mm in C-D; 5 μm in E-F.

cm long, with 7-8 layered colleters inside adaxial base. Inflorescence compact bifurcate cyme-like, axillary, generally 16 buds, (4-)6-10(-12) matured. Bracteole 2-lobed, disc-like, sessile, 1-2 mm in length, apex rounded, with 0-3 colleters inside. Calyx lobes 5, erect while in flowering and in fruiting, ovate, acute, 4 mm × 2 mm. Petals 5, white, turning brownish, linear-rectangular, 4 mm × 1.8-2.0 mm (including terminal cilia), apex fingers-like, fringed with 20-25 sinuate cilia, 0.8-1.25 mm long, margins entire with long and dense hairs (0.5 mm), base broad, folded longitudinally with a distinctive ridge. Stamens 10, equal in size; filament 1.6-2.0 mm long; anther 1-1.2 mm long, ovoid, dorsifixed, with one long connective protrusion. Ovary inferior, 3-locular, 2 ovules in each locule, style 2.5-3 mm long, stigma 1, very shortly trifid. Persistent calyx tube hemi-globular, 5-9 mm in height, persistent lobes 5, 4 × 1.6-2.0 mm, ascending. Fruit ovoid, 0.6-1.0 cm × 0.5-0.6 cm. Hypocotyl clavate, 8-13 × 0.5-0.7 cm, ridged and sulcate, width approximately the same, slightly tappering towards a blunt apex (root tip), erect to pendant; epicotyl 2-3 mm long.

Notes. The type of this species (Ejusdem Icones, vol. 8, t. 116) and the drawing of Roxburgh's collection 1140 in Kew Library were examined. Basically, the latter was a copy from the former but omitting the dissected flower with pistil and a stamen. See Sheue et al. (2009b) for the illustration and specimens examined.

Distribution. India, Bangladesh through Burma to southeastern Thailand (Satun) (Figure 4).

Ceriops zippeliana Blume, Mus. Bot. 1: 143. 1849.

Ceriops decandra auct. non (Griff.) Ding Hou (1958) 471. *pro part*.

Main diagnostic characters were listed in Table 2. See Sheue et al. (2009b) for detailed description, illustration and specimens examined.

Distribution. The southwestern coast of southern Malay Peninsula through Singapore, Bintan Island, the east coast of the Malay Peninsula to the Gulf of Thailand to Vietnam, the Philippines, Borneo, Sulawesi (Celebes), Moluccas (Seram), Lesser Sunda Islands and Java (Figure 4).

Key to species of *Ceriops* of Rhizophoraceae

1a. Petal apex with 3(-5) clavate appendages; inflorescence axis relatively long and slender (10-30 mm × 2 mm), bending downwards.

2a. Hypocotyl terete (without ridges) usually less than 10 cm long at maturity; base of calyx lobe in flowering 12-15 mm in width; stipule usually shorter than 1.2 cm long at extension stage ..... *C. australis*

2b. Hypocotyl angular (with ridges) 9- 25 cm long at maturity; base of calyx lobe in flowering 18-25 mm in width; stipule usually longer than 1.2 cm long at extension stage ..... *C. tagal*

1b. Petal apex fringed with 12-25 sinuate cilia; inflorescence axis relatively short and stout (3-10 mm × 3-4 mm), erect.

3a. Inflorescence simple and head-like with 3-5 flowers; petals hairless along margins; persistent calyx tube short (2-3 mm) and disc-like ..... *C. zippeliana*

3b. Inflorescence dense bifurcate cyme-like with 6-20 flowers; petal hairy at least along lower margins; persistent calyx tube long (4-9 mm) and hemi-globular (dome-like).

4a. Entire margins of petals with dense and long hairs (0.5 mm); style 2.5- 2.8 mm long, epicotyl 2-3 mm long; length ratio of fruit to calyx tube 1~1.5 at maturity ..... *C. decandra*

Table 2. The comparison of morphological characters among *Ceriops decandra* (Griff.) Ding Hou, *C. pseudodecandra* Sheue, Liu, Tsai and Yang and *C. zippeliana* Blume.

Characters	*Ceriops decandra*	*Ceriops pseudodecandra*	*Ceriops zippeliana*
Stipule	1.2-2.4 cm before dropping, with 50-70 colletors at adaxial base (7-8 layered)	2.0-3.0 cm before dropping, with 80-100 colletors at adaxial base (8-12 layered)	2.5-3.6 cm before dropping, with 154-190 colletors at adaxial base (18-20 layered)
Leaf	Oval to obovate, 4.0-9.0 cm × 2.5-6.0 cm, lateral veins 8-10(-11), petiole 1.2-1.8 cm in length	Oblong to elliptic-obovate, 6.0-12.0(-13.0) cm × 2.5-5.0(-6.0) cm, lateral veins 8-12, petiole 1.2-3.5 cm in length	Obovate-elliptic, 5.5-11.0 cm × 3.0-7.5 cm, lateral veins (9-)11-12(-13), petiole 1.5-2.6 cm in length
Inflore-scence	16 buds, (4-)6-10(-12) matured, dense bifurcate cyme-like, with primary and additional bracts	(2-)3-20 flowered, dense bifurcate cyme-like, with primary and additional bracts	3-5(-7) flowered, simple head-like, with primary bract only
Bracteole	1-2 mm in length	2 mm in length	2.6 mm in length
Calyx	5 lobed, 4.0 mm × 2.0 mm	5 lobed, 3.0 mm × 1.5 mm	5 lobed, 2.0-3.0 mm × 2 mm
Corolla	5, 4.0 mm × 1.8-2.0 mm (including terminal cilia), entire margins with dense and long hairs (0.5 mm), apex finger-like fringed with 20-25 sinuate cilia, 0.8-1.25 mm long	5, 2.0-2.5 mm ×1.5 mm (including terminal cilia), upper half margins glabrous, lower half margins with loose and short hairs (< 0.1 mm), apex finger-like fringed with 12-18 sinuate cilia, 0.5-0.6 mm long	5, 3.0-3.5 mm ×1.8 mm (including terminal cilia), margins hairless, apex finger-like fringed with sinuate 13-17 cilia, 0.5-0.8 mm long
Stamens	10, filament 1.6-2.0 mm long, anther 1.0-1.2 mm long, with one long connective protrusion	10, filament 1.3 mm long, anther 0.9 mm long, with one short connective protrusion	10, filament 1.0 mm long, anther 1.0 mm long, with one short connective protrusion
Style	Style 2.5-2.8 mm long	Style 1.4-1.5 mm long	Style 2.0-2.2 mm long
Pollen	L = 21.0 ± 1.49 μm in equator view, exine scabrate with punctae	L = 19.7 ± 1.2 μm in equator view, exine smooth with sparsely distributed punctae	L = 15.43 ±1.16 μm in equator view, exine irregularly regulo-reticulate
Fruit	Calyx hemi-globular (dome-like), 5-9 mm high, persistent lobes 5, 4.0 ×1.6-2.0 mm; fruit ovoid, 0.6-1.0 cm × 0.5-0.6 cm, no special decoration	Calyx tube hemi-globular, 4-5 mm high, persistent lobes 5, 2.5-3.0 mm × 1.0 mm; fruit ovoid-conical, 1.0-1.3 cm × 0.5-0.8 cm, no special decoration	Calyx tube shallow disc-like, 2-3 mm high, persistent lobes 5, 2-2.5 × 1-1.5 mm; fruit ovoid-conical, 1.2-1.5 cm × 1.0 cm, with netted decoration
Hypocotyl	8-13 cm × 0.5-0.7 cm, gradually thickening with a blunt apex (root tip)	10-16 cm × 0.5-0.8 cm, gradually thickening with an acuminate sharp apex	9-17 cm × 0.7-0.8 cm, gradually thickening with a acute sharp apex
Epicotyl	2-3 mm long	10-13 mm long	2-3 mm long

4b. Lower half margins of petals with loose and short hairs (0.1 mm); style 1.4-1.5 mm long, epicotyl 10-12 mm long; length ratio of fruit to calyx tube 2~3 at maturity ..... *C. pseudodecandra*

Molecular evidence

Sequence alignment and characteristics. PCR products from all samples studied were directly sequenced. The accession numbers of those plastid DNA sequences from the seven accessions of *C. decandra*, seven accessions of *C. zippeliana*, and five accessions of *C. pseudodecandra* plus two outgroups are shown in Table 1. Those sequences were aligned and resulted in 662 characters, from which 17 and 13 were variable and informative parsimony sites, respectively. The aligned data matrix and tree files are available from the author (tsaicc@mail.kdais.gov.tw). The genetic distance estimated from the 2-parameter method of Kimura (1980) is 0.0034 between *C. decandra* and *C. pseudodecandra* and 0.0051 between *C. zippeliana* and *C. pseudodecandra*. Two stable transversions are found within this DNA region between *C. pseudodecandra* and *C. decandra*/*C. zippeliana* (data not shown).

Phylogeny reconstruction. The phylogenetic tree for the intron of *trn*L used characters that are equally weighted. Based on the MP method, the analysis yields 300 equally parsimonious trees with a length of 18 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 are highly congruent (Figure 5, MP tree presented only).

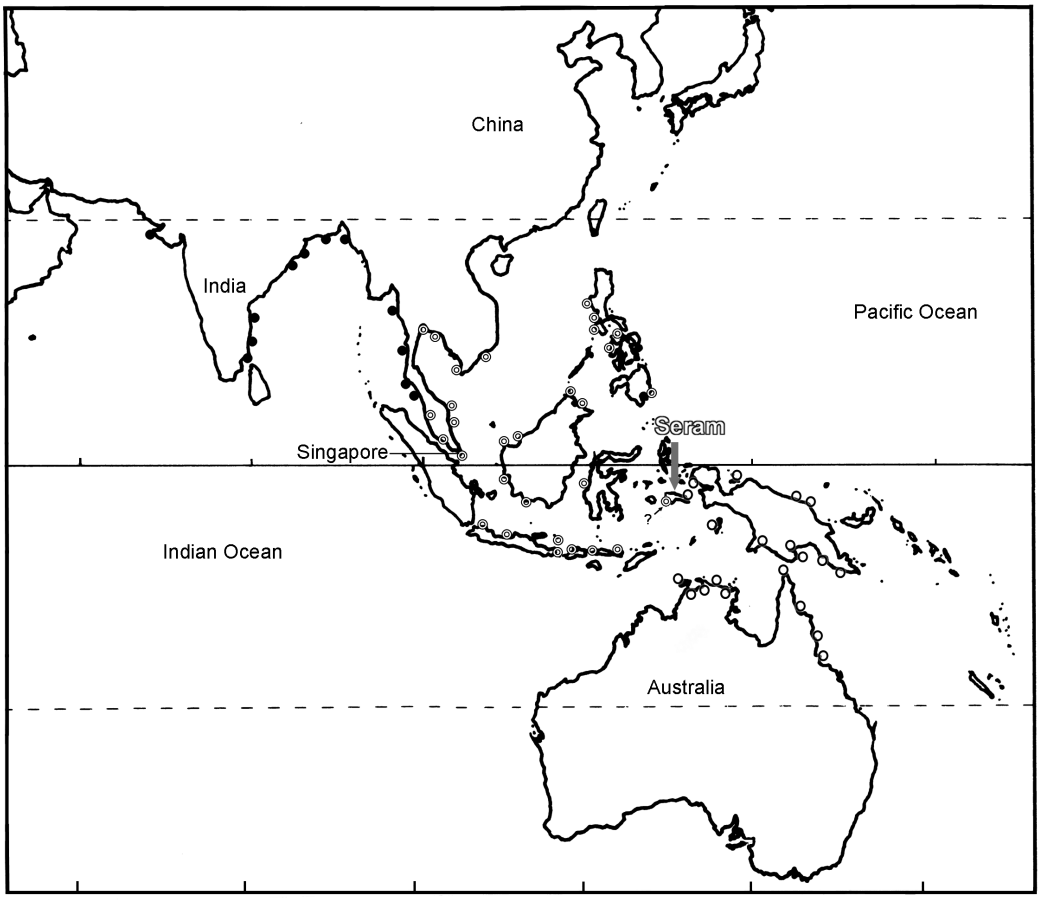

Figure 4. The distribution ranges of *Ceriops decandra* (Griff.) Ding Hou (solid circle), *C. pseudodecandra* Sheue, Liu, Tsai, and Yang (open circle) and *C. zippeliana* Blume (double circle). The question mark refers to the doubtful locality in area of Ambon and western Seram where no population of *C. zippeliana* has been found currently.

Based on the MP tree, accessions of *C. pseudodecandra* form a clade supported by a 82% bootstrap value and are separated from either accessions of *C. decandra* or accessions of *C. zippeliana*. Therefore, the molecular data also support the distinctness of accessions of *C. pseudodecandra*.

Ceriops pseudodecandra often appears as a small tree less than 5 m in height, with grayish brown bark, flaky and flanged buttresses at base (Figure 2A) with horizontal fissures of main stem (Figure 2B). However, a sheet of specimen *Simaga 786* (GH) recorded it a tree 12 m in height at Daru of Papua New Guinea. Compared to the brown stem and flaky base of *C. pseudodecandra*, *C. australis* and *C. tagal* usually have a gray-white to orange-brown stems with protruded and rounded lenticels.

The leaf shape of the new species is mostly oblong to elliptic-obovate with 8-12 pairs of lateral veins (Table 2), and interestingly the populations from New Guinea have especially typically oblong leaves up to 13 cm in length. The elongated oblong leaves of the young seedlings in shade environment could easily be observed from the habitat (Figure 2C). The features of somewhat oblong leaves and distinctly acuminate leaf bases of the

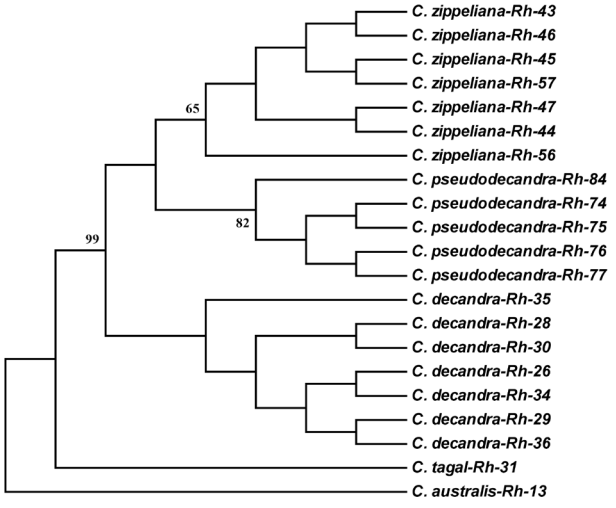

Figure 5. The strict consensus parsimonious tree of seven accessions of *Ceriops decandra* (Griff.) Ding Hou, five accessions of *C. pseudodecandra* Sheue, Liu, Tsai, and Yang, seven accessions of *C. zippeliana* Blume and plus two outgroups derived from the *trn*L intron sequence. Bootstrap values > 50% are shown on each branch.

new species are helpful to discern it from other species of *Ceriops*. Wightman (2006) accurately described *C. decandra* (i.e. *C. pseudodecandra*) in Northern Territory of Australia as having elliptic-oblong leaves. In addition, Tomlinson (1986) noticed the larger and darker leaves of *C. decandra* (i.e. *C. pseudodecandra*) in Queensland and assumed that it might be a result of habitat differences. The sun leaves of *C. pseudodecandra* with slightly reflex margins are often orientated straight up in the air to avoid strong sunlight (Figure 2D-E). This phenomenon of sun leaves is also quite common for *C. australis* and *C. tagal* in Australia and they have even more strongly reflexed margins (Sheue, per. observ.).

The feature of inflorescence has an important diagnostic value for differentiating the two groups of *Ceriops*, namely *C. australis* and *C. tagal*, and *C. decandra*, *C. pseudodecandra* and *C. zippeliana.* The former group has relatively long and slender peduncles and the latter group has short and stout ones. Moreover, the inflorescence of *C. zippeliana* is simple head-like (Sheue et al., 2009b); however, those of *C. decandra* and *C. pseudodecandra* are dense bifurcate cyme-like (Table 2). This difference can be observed from the series of bracts below each bracteole of a flower and has been noted by Sheue (2003). *Ceriops zippeliana* only has a primary bract (Sheue et al., 2009b) while *C. decandra* (Sheue et al., 2009b) and *C. pseudodecandra* have additional secondary bracts or even more multi-ranked bracts of an inflorescence with more flowers.

Features of petal morphology also correlated with the inflorescence features used to divide the genus into two groups, petals of *C. australis* and *C. tagal* with 3 (-5 of *C. australis*) clavate appendages (Sheue et al., 2009a); petals of *C. decandra* (Sheue et al., 2009b), *C. pseudodecandra* and *C. zippeliana* (Sheue et al., 2009b) with finger-like fringes containing 12-25 sinuate cilia (Table 2; Figure 3A-C). Furthermore, the most conspicuous difference of the petal for the latter three taxa is the feature of petal margins. The margins of the petals of *C. zippeliana* are hairless (Sheue et al., 2009b) whereas those of *C. decandra* are densely covered with long hairs *c.* 0.5 mm in length (Sheue et al., 2009b) and only the margins of the lower half petals of *C. pseudodecandra* are covered with loose and short hairs less than 0.1 mm (Figure 3C).

The difference of the floral parts and pollen grains of these three species of *Ceriops* is distinctive (Table 2). *Ceriops decandra* has relatively larger flower size (4 mm long calyx lobes) and floral parts (including sepal, petal, stamen and style) than those of *C. pseudodecandra* and *C. zippeliana* (2-3 mm long of calyx lobes). All the species of *Ceriops* have tri-colporate pollens (Das and Ghose, 1990; Sheue, 2003). In terms of the size of pollen grains, those of *C. decandra* are the largest (21 μm in length) and those of *C. zippeliana* are the smallest (15.4 μm in length). The pollen grains of the new species (19.7 μm in length) are slightly smaller than those of *C. decandra*. The most evident pollen character served for differentiating these taxa is the micro-surface features. The pollen grains of new species have smooth with sparsely distributed punctae exine (Figure 3D-E) while those of *C. decandra* have scabrate with punctae exine (Das and Ghose, 1990; Sheue et al., 2009b) and those of *C. zippeliana* have irregularly regulo-reticulate exine (Sheue et al., 2009b).

The ovoid fruit of *Ceriops* has persistent calyx tubes and calyx lobes and their detailed morphology is useful for interspecific differentiation (Table 2). The new species (Figure 1C) and *C. decandra* (Sheue et al., 2009b) have hemi-globular (dome-like) calyx tubes, 5- 9 mm in height while *C. zippeliana* (Sheue et al., 2009b) has shallow disc-like calyx tubes about 2-3 mm in height. However, the fruit of the new species is more similar to *C. zippeliana* appears as ovoid-canical. This feature leads to a higher ratio of fruit length to calyx tube length of *C. pseudodecandra* (2-3) than that of *C. decandra* (1-1.5). The hypocotyl of the viviparous seedling of *C. decandra* is gradually thickened with a blunt apex (root tip) and that of *C. zippeliana* is also gradually thickened but with a sharp apex (Sheue et al., 2009b). The hypocotyl of the new species slightly resembles that of *C. zippeliana*, but has a more distinctly elongated acuminate sharp apex (Figure 1C). In addition, an easily recognizable feature of the seedling is the length of the epicotyls. The epicotyls of the new species are prominent and much longer (usually 10-13 mm in length) than those of the other species of *Ceriops* (2-3 mm in length) (Figure 1B). It is noteworthy that the length of epicotyl of the new species is constant and could serve as a good character to differentiate *C. pseudodecandra* from other taxa of this genus although this feature has not been mentioned before.

The major flowering peak of *C. pseudodecandra* is during September to November, and fruiting occurs from October to February in both the Northern Territory (Wightman, 2006) and northern Queensland (Duke, 2006) of Australia. Based on the observation of herbarium specimens and a field trip (Sheue, personal observation), the populations of New Guinea (including Papua New Guinea and Indonesia) seem to flower earlier, from July to October, and some may start from January to June. It is quite common to have the mature viviparous seedlings with cotyledon collars on the same tree while flowering. It is highly possible that the fruit and propagule maturation takes about 12 months in *C. pseudodecandra*, as for *C. australis* in the Darwin area of northern Australia (Coupland et al., 2005).

Based on our current field survey and examination of herbarium specimens, the distribution range of *C. pseudodecandra* includes Seram, the Daru Islands, Irian Jaya of Indonesia, Papua New Guinea and Northern Territory and Queensland of northern Australia (Figure 4). The southern boundary in Queensland is down to Hinchinbrook Channel in the east of Queensland (Duke, 2006; Sheue, personal obser.). In Australia, it occurs scattered through tidal forest, but more commonly toward landward margins of tidal waterways (Wightman, 2006).

Generally, this new species does not form a monotypic stands, but prefers mixed forests (Duke, 2006), occurring with *Ceriops*, *Bruguiera*, *Xylocarpus* and *Rhizophora* (Sheue, personal obser.).

It is interesting to examine the distributional relationship of *C. pseudodecandra* and *C. zippeliana* in South Asia. According to herbarium specimen information, *C. zippeliana* ranges from West Malaysia through Singapore, eastern Malay Peninsula, to the Philippines, Borneo and most of Indonesia, but not including Irian Jaya (Sheue et al., 2009b). Although two herbarium specimens of *C. zippeliana* had been collected from Maluku area in 19th century, there is no such population found in Seram and Ambon (the major islands of Maluku) according to the current field trip in 2009 by the first author and the surveys of Indonesian scientists (Suhardjono, personal communication). However, the new species, *C. pseudodecandra* was found by the first author in eastern Seram (Hoti, Bula) which was accordant with the early historical collection. Due to the misapplication of the name *C. decandra* for these two species prevailing in these areas over several decades, a detailed survey for the distributional ranges of these two taxa around New Guinea and its surrounding islands is still necessary.

Besides the previous morphological and palynological evidence, the molecular evidence of the *trn*L intron of plastid DNA supports the taxonomic status of *C. pseudodecandra* as a new taxon as well. In this study, the genetic distance is 0.0034 between *C. decandra* and *C. pseudodecandra* and 0.0051 between *C. zippeliana* and *C. pseudodecandra*. Based on the MP tree, accessions of *C. pseudodecandra* form a clade supported by a 82% bootstrap value and are separated from either accessions of *C. decandra* or accessions of *C. zippeliana* (Figure 5). Using the inter-simple sequence repeat (ISSR), Tan et al. (2005) attempted to understand the population genetic structure of the previously recognized *C. decandra* populations (including three taxa), in the Malay Peninsula and North Australia. At the species level, high genetic variation (P=72%, H_E=0.253, and I=0.379) and high estimate of G_{ST} (up to 0.882) were detected and the populations were grouped into different major geographic regions from a UPGMA dendrogram (Tan et al., 2005). After the attentive taxonomic studies on *Ceriops*, the UPGMA dendrogram reported by Tan et al. (2005) could be harmonized with the geographic range of the three taxa of *Ceriops* reported in this study (Figure 4).

Over all, the new species exhibits a high morphological similarity to *C. decandra* and *C. zippeliana*, and shows several intermediate features between these two taxa. The occurrence of this new species appears to restrict in Australasia geographic region of Indo West Pacific, according to world distribution of mangroves (Duke, 1992). A further intensive survey of this taxon in this region still needed. In addition, as Juncosa and Tomlinson's (1988) suggested 'If we can devote at least as much time to observation as we are doing speculation about phylogenies, then our understanding is likely to improve', a comprehensive observation for the mangrove species with their distributional ranges may be still needed.

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LITERATURE CITED

Ballment, E.R., T.J. Smith III, and J.A. Stoddart. 1988. Sibling species in the mangrove genus *Ceriops* (Rhizophoraceae), detected using biochemical genetics. Aust. Syst. Bot. **1:** 391-397.

Coupland, G.T., E.I. Paling, and K.A. McGuinness. 2005. Vegetative and reproductive phenologies of four mangrove species from northern Australia. Aust. J. Bot. **53:** 109-117.

Das, S. and M. Ghose. 1990. Pollen morphology of some mangrove plants of Sunderbans, West Bengal. J. Natl. Bot. Soc. **44:** 59-75.

Duke, N.C. 1992. Mangrove floristics and biogeography. *In* A.I. Robertson and D.M. Alongi (eds.), Tropical Mangrove Ecosystems (Volume 41), Coastal and Estuarine Studies Series, American Geophysical Union, Washington, D.C., pp. 63-100.

Duke, N.C. 2006. Australia's Mangroves: the Authoritative Guide to Australia's Mangrove Plants. University of Queensland, Queensland, 200 pp.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:** 783-791.

Field, C.D. 1995. Journey amongst Mangroves. International Society for Mangrove Ecosystems, Okinawa, Japan, 140 pp.

Fitch, W.M. 1971. Towards defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. **20:** 406-416.

Giang, L.H., G.L. Geada, P.N. Hong, M.S. Tuan, N.T.H. Lien, S. Ikeda, and K. Harada. 2006. Genetic variation of two mangrove species in *Kandelia* (Rhizophoraceae) in Vietnam and surrounding area revealed by microsatellite markers. Int. J. Plant Sci. **167:** 291-298.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. **41:** 95-98.

Hillis, D.M. and J.J. Bull. 1993. An empirical test of

bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42:** 182-192.

Holmgren, P.K. and N.H. Holmgren. 1998. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Hou, D. 1958. Rhizophoraceae. *In* C.G.G.J. van Steenis (ed.), Flora Malesiana, series 1, vol. 5. Noordhoff-Kolff NV, Djakarta, pp. 429-473.

Juncosa, A.M. and P.B. Tomlonson. 1988. A historical and taxonomic synopsis of Rhizophoraceae and Anisophylleaceae. Ann. Missouri Bot. Gard. **75:** 1278-1295.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. Mol. Evol. **16:** 111-120.

Naskar, K., and R. Mandal. 1999. Ecology and Biodiversity of Indian Mangroves. Daya Publishing House, Delhi, pp. 754.

Rzhetsky, A. and M. Nei. 1992. Statistical properties of the ordinary least-squares, generalized least-squares, and minimum-evolution methods of phylogenetic inference. J. Mol. Evol. **35:** 367-375.

Saenger, P. 2002. Mangrove Ecology, Silviculture and Conservation. Kluwer Academic Publishers, The Netherlands, pp. 360.

Saitou, N. and M. Nei. 1987. The Neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406-425.

Sheue, C.R., F.S. Chou, Y.P. Yang, J.W.H. Yong, H.Y. Liu, S.C. Chang, P. Saenger, G. Wightman, C. Mangion, J.W.H. Yong, and C.C. Tsai. 2009a. Reevaluating the taxonomic status of *Ceriops australis* (Rhizophoraceae) based on morphological and molecular evidence. Bot. Stud. **50:** 89-100.

Sheue, C.R., H.Y. Liu, C.C. Tsai, S.M.A. Rashid, J.W.H. Yong, and Y.P. Yang. 2009b. On the morphology and molecular basis of segregation of two species *Ceriops zippeliana* Blume and *C. decandra* (Griff.) Ding Hou (Rhizophoraceae) from southeastern Asia. Blumea **54:** (in press).

Sheue, C.R. 2003. Comparative Morphology and Anatomy of the Eastern Mangrove Rhizophoraceae. Ph. D. Dissertation, National Sun Yat-sen University, Kaohsiung, Taiwan, pp. 228.

Sheue, C.R., H.Y. Liu, and J.W.H. Yong. 2003. *Kandelia obovata* (Rhizophoraceae), a new mangrove species from Eastern Asia. Taxon **52:** 287-294.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. **17:** 1105-1109.

Tamura, K., J. Dudley , M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. **24:** 1596-1599.

Tan, F., Y. Huang, X. Ge, G. Su, X. Ni, and S. Shi. 2005. Population genetic structure and conservation implications of *Ceriops decandra* in Malay Peninsula and North Australia. Aquat. Bot. **81:** 175-188.

Tomlinson, P.B. 1986. The Botany of Mangroves. Cambridge University Press, Cambridge, pp. 419.

White, C.T. 1926. A variety of *Ceriops tagal* C. B. Rob. (= *C. candolleana* W. and A.). J. Bot. Lond. **64:** 220-221.

Wightman, G. 2006. Mangroves of the Northern Territory, Australia: Identification and traditional use. Department of Natural Resources, Environment and the Arts, and Greening Australia, Northern Territory, Australia, pp. 168.

擬印度細蕊紅樹 (紅樹科)── 產自大洋洲的新種紅樹林植物及其相近種的比較

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分布廣泛的細蕊紅樹屬 (*Ceriops*) 包含三種紅樹林植物：澳洲細蕊紅樹 (*C. australis*)、印度細蕊紅樹 (*C. decandra*) 與細蕊紅樹 (*C. tagal*) 之看法至今仍廣爲接受。然而，近年的研究發現原來所認知的 *C. decandra* 實則包含三個分類群，*C. decandra*、齊氏細蕊紅樹 (*C. zippeliana*) 和一新種 ─ 擬印度細蕊紅樹 (*C. pseudodecandra*)。本文即報導此一產於澳洲、巴布亞新幾內亞及印尼的新種紅樹林植物，數十年來本物種一直被錯誤地認定爲 *C. decandra*。本新種較接近於 *C. decandra* 與 *C. zippeliana*，且表現出若干介於此兩者之間之形態特性；除此，分子生物學的證據亦支持 *C. pseudodecandra* 成爲一新種的地位。本文提供了新種植物與其相近種的描述、繪圖和分布、本屬植物的檢索表及此三種相近物種間的詳盡比較與討論。

關鍵詞：澳洲；印度細蕊紅樹；擬印度細蕊紅樹；齊氏細蕊紅樹；印尼；紅樹林；巴布亞新幾內亞。