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Stipules and colleters of the mangrove Rhizophoraceae: morphology, structure and comparative significance

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ABSTRACT. One characteristic possessed by the entire mangrove Rhizophoraceae is the strong enclosure of its young shoots by conspicuous rounded or flattened stipules. Young leaves are conspicuously immersed in resinous to milky exudates from colleters (multicellular external secretory emergences) located at the adaxial stipule bases. We systematically studied and compared morphological and structural features of stipules and their colleters from 18 taxa of the mangrove Rhizophoraceae. Three types of sclereid idioblasts (*Ceriops* and *Rhizophora*), collenchymas, and thick cuticles, were found to provide a structural basis for the mechanical support of stipules. Several to hundreds (35-580) of finger-like colleters aggregate into genus-specific shapes: rectangular to trapezoidal (*Bruguiera*), triangular (*Ceriops* and *Kandelia*), or as a band (*Rhizophora*). Number of rows, total number of colleters per stipule, and their individual size, also vary by taxon, and have taxonomic value. All colleters of this subfamily are considered anatomically 'standard', regardless of whether they appear as short-stalked rods (*Bruguiera*), long-stalked rods (*Ceriops*, *Kandelia*) or acuminate rods (*Rhizophora, no stalk*). Colleters in these taxa are comprised of a central axis of slender, elongated cells and an outer palisade-like epidermis, with a secretory function, perpendicular to the axis. Based on stipule and colleter characteristics, *Ceriops* and *Kandelia* are closely related, but *Rhizophora* shows more derived features. The structural and mechanical protection provided by stipules and colleter exudates may help shield the young shoots of these mangrove plants from their harsh environments.

Keywords: Anatomy; *Bruguiera*; *Ceriops*; Colleter; *Kandelia*; Mangroves; *Rhizophora*; Sclereid; Standard type; Stipule.

INTRODUCTION

Rhizophoraceae is well known as the richest mangrove family, having four exclusively mangrove genera (*Bruguiera*, *Ceriops*, *Kandelia* and *Rhizophora*) (Tomlinson, 1986). About 21 mangrove species of this family are currently recognized, including new taxa from *Kandelia* (Sheue et al., 2003b) and *Ceriops* (Sheue et al., 2009a, b; Sheue et al., 2010). The family is diversified both ecologically (habitat) and morphologically (trait). In addition to the mangroves mentioned above, the family has 15 inland genera with 135 species occurring in inland forests (Juncosa and Tomlinson, 1988a). All members of the Rhizophoraceae *s. s.* (excluding *Anisophyllea*) have large and conspicuous interpetiolar, glabrous and caducous stipules on both vegetative and reproductive shoots, strongly ensheathing the young leaves and inflorescences (Hou, 1958). Several to hundreds of aggregated finger-like colleters mixed with milky mucilage can be observed with the naked eye at the adaxial base of stipules in the species of mangrove Rhizophoraceae (Tomlinson, 1986; Sheue et al., 2003a, b; Sheue et al., 2005). Except for the genus *Bruguiera,* colleters, although few in number, are also present within the bracteoles of mangrove Rhizophoraceae inflorescences (Sheue, 2003; Sheue et al., 2003a).

Colleters are external secretory structures (Fahn, 1990), characterized by the production of a viscous substance that covers, protects from desiccation, and lubricates the bud and young leaves of plants (Kronestedt-Robards and Robards, 1991; Evert, 2007). Hou (1958) mentioned that colleters in Rhizophoraceae were first observed by Alston in African *Cassipourea*, and later by Metcalfe and Chalk in *Carallia* (both inland genera). These glandular emergences inside the adaxial regions of stipules appear to occur in all Malaysian genera, both mangrove and inland (Hou, 1858). Colleter exudates from this family are assumed to be composed of phenolic compounds (Roshchina and Roshchina, 1993).

Colleters have long been associated with a number of flowering plants, notably Apocynaceae and Rubiaceae. The color and consistency of colleter exudates are variable in

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a somewhat diagnostic manner from species to species of the mangrove Rhizophoraceae (Tomlinson, 1986). However, the knowledge of colleters of Rhizophoraceae is still under-reported and remains obscure. The presence of colleters in *R. mangle* was noticed by Warming in 1883 and briefly described (Gill and Tomlinson, 1969): "a feature of some physiological significance in the bud of *Rhizophora* is a double row of yellow, glandular scales on the side of each stipule. The primodia enclosed by the stipules are always bathed by a viscous fluid which seems to originate from these glands." It is the only species in this family to have the colleters anatomically studied (Lersten and Curtis, 1974). The main objectives of the present work are to provide a systematic study of stipules and colleters of the mangrove species of this family in order to understand their morphology, structure, taxonomic value, and comparative significance.

MATERIALS AND METHODS

Plant materials containing stipules with colleters were collected from 18 mangrove Rhizophoraceae taxa (including two hybrids of *Rhizophora*) from Asia and Australia from 1999 to 2009 (Table 1). The voucher specimens were deposited in the Herbarium of National Chung Hsing University (TCB), Taichung, Taiwan.

Three individual plants were sampled from a population, and from each individual, three stipules were carefully detached from the shoot apices when the young leaves were about to emerge from the stipules. Stipules were stored in 70% ethanol in the field, observed and photographed with a Zeiss dissecting microscope to determine stipule morphology, number of colleters, and their forms of aggregation within the stipule. A portion of the materials was observed with a S-2400 Scanning Electron Micro-

Table 1. Taxa and collection information of the mangrove species of Rhizophoraceae in this study.

Species	Collection locality	Habitat
Bruguiera cylindrica (L.) Blume	Pichavarum, Tamil Nadu, India Sungei Buloh Wetland Reservoir, Singapore	Back or middle mangroves
Bruguiera exaristata Ding Hou	Cairns, Queensland, Australia	Back or middle mangroves
Bruguiera hainesii C. G. Rogers	Pulau Ubin, Singapore	Within mangroves
Bruguiera gymnorhiza (L.) Savigny	Sungei Buloh Wetland Reservoir, Singapore Cardwell, Queensland, Australia	Middle mangroves
Bruguiera parviflora (Roxb.) W. & A. *ex* Griff.	Pasir Ris Nature Park, Singapore Cardwell, Queensland, Australia	Back or middle mangroves
Bruguiera sexangula (Lour.) Poir.	Benut, Johor, Malaysia	Middle mangroves
Ceriops australis (C. T. White) Ballment, T. J. Sm. & J. A. Stoddart	Cardwell, Queensland, Australia Moreton Bay, Queensland, Australia	Back mangroves, landward
Ceriops decandra (Griff.) Ding Hou	Pichavarum, Tamil Nadu, India	Back mangroves
Ceriops pseudodecandra Sheue, H. Y. Liu, C. C. Tsai and Yuen P. Yang	Darwin, Northern Territory, Australia Cairns, Queesland, Australia	Middle mangroves, along river bank Back mangroves
Ceriops tagal (Perr.) C. B. Rob.	Sungei Buloh Wetland Reservoir, Singapore Cairns/ Cardwell, Queensland, Australia	Within mangroves Back mangroves
Ceriops zippeliana Blume	Pasir Ris Nature Park, Singapore	Back mangroves
Kandelia candel (L.) Druce	Benut, Johor, Malaysia Malaysia	Along river bank
Kandelia obovata Sheue, H. Y. Liu & J. W. H. Yong	Tanshui, Taipei, Taiwan Tongshi, Chaiyi, Taiwan	Along river bank, pure stand River bank
Rhizophora apiculata Blume	Sungei Buloh Wetland Reservoir, Singapore Pichavarum, Tamil Nadu, India Cairns/ Cardwell, Queensland, Australia	Seaward mangroves
Rhizophora × *annamalayana* Kathiresan	Pichavarum, Tamil Nadu, India	Back mangroves
Rhizophora × *lamarckii* Montr.	Cairns/ cardwell, Queensland, Australia	Back mangroves
Rhizophora mucronata Lam.	Pichavarum, Tamil Nadu, India Sungei Buloh Wetland Reservoir, Singapore	Seaward mangroves
Rhizophora stylosa Griff.	Tainan, Taiwan Cairns/ Moreton Bay, Queensland, Australia	Along salty canal Seaward mangroves

scope (Hitachi, Tokyo, Japan), after treating with ethanol series dehydration, critical point drying and gold coating, allowing shape and size determination in particular. These stipules were also treated with the clearing method (Chiang, 1990) to investigate features such as sclereid idiobalsts and crystals.

For field fixation for anatomical study, the base of each stipule with colleters was cut into several small pieces and put into 1.25-1.5% glutaraldehyde in 0.1 M phosphate buffer with 5% sucrose. Materials were stored in this fixative for 5-7 days during collection abroad. For the second fixation, materials were subsequently transferred to 1% OsO_4 in 0.1 M phosphate buffer for about 4 hours. After dehydration through an ethanol series, materials were infiltrated for 3 days and embedded within Spurr's-resin. The embedded materials were polymerized in an oven at 70°C for 12 hours. Semi-thin sections (1 μm) were made by an Ultracut E (Leica, Wetzlar, Germany) or a MTX Microtome (RMC, Tucson, USA), stained with 0.1% Toluidine blue for 1 minute or so, and then observed and photographed with a BH-2 Light Microscope (Olympus, Tokyo, Japan).

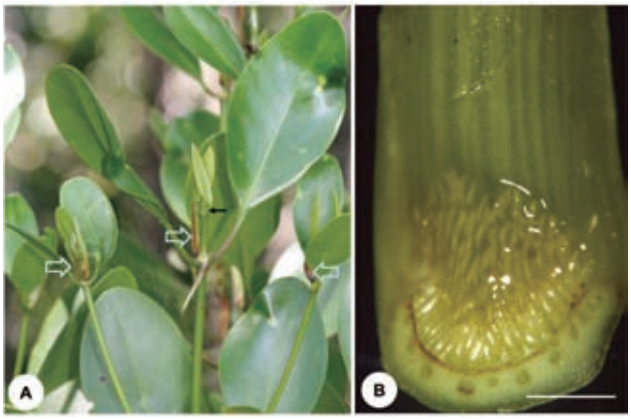

Figure 1. The mangrove Rhizophoraceae with shoots and stipules. (A) The shoot apices and top nodes of *Ceriops australis* with stipules (broad arrows), which are interpetiolar and caducous. Primodia enclosed by the stipules are always bathed by a viscous fluid. Young expanded leaves coated with resin-like mucilage (thin arrow); (B) A fresh stipule of *Bruguiera exaristata* showing yellowish aggregated finger-like colleters with viscous exudation at the adaxial base. Scale bar = 1 mm.

Table 2. Morphological and structural characters of stipules and colleters of the mangrove Rhizophoraceae, including aggregated form, number of rows and total number of colleters in each stipule, and individual shape and color of colleters.

Taxon	Stipule	Colleter	
	Morphology/ length (cm)/ Sc	Aggregated form/ row no./ total no.	Shape/ color
Bruguiera cylindrica	Round/ 3.0-4.0/ no Sc	▮▲ / 4-7/ 70-90	SRs/ MW
Bruguiera exaristata	Round/ 2.0-3.0/ no Sc	▲ / 9-14/ 120-135	SRs/ MW-MY
Bruguiera hainesii	Round/ 3.5-4.2/ no Sc	▲ / 12-15/ 100-146	SRs/ MW
Bruguiera gymnorhiza	Round/ 4.0-5.0/ no Sc	▮▲ / 9-13/ 200-220	SRs/ MW
Bruguiera parviflora	Round/ 4.5-6.5/ no Sc	▮▲ / 6-8/ 160-170	SRs/ MW
Bruguiera sexangula	Round/ 4.5-5.5/ no Sc	▲◠/ 3-4 / 35-40	SRs/ MW
Ceriops australis	Flattened/ 1.0-1.2/ Sc 2 types	▲/ 9-11/ 90-140	SRl/ MW
Ceriops decandra	Flattened/ 1.2-2.4/ Sc 2 types	▲/ 7-8/ 50-70	SRl/ MW
Ceriops pseudodecandra	Flattened/ 2.0-3.0/ Sc 2 types	▲/ 8-12/ 80-100	SRl/ MW
Ceriops tagal	Flattened/ 1.5-2.8/ Sc 2 types	▲/ 24-26/ 165-205	SRl/ MW
Ceriops zippeliana	Flattened/ 2.5-3.6/ Sc 2 types	▲/ 18-20/ 280-310	SRl/ MW
Kandelia candel	Flattened/ 3.0-4.0/ no Sc	▲/ 6-7/ 75-96	SRl/ MY
Kandelia obovata	Flattened/ 2.5-3.2/ no Sc	▲/ 8-9/ 95-110	SRl/ MY
Rhizophora apiculata	Round/ 5.0-8.5/ Sc 3 types	▬/ 2-4/ 160-200	ARse/ Y
Rhizophora mucronata	Round/ 4.5-6.5/ Sc 3 types	▬/ 6-9/ 460-580	ARse/ MY
Rhizophora stylosa	Round/ 4.5-5.5/ Sc 3 types	▬/ 4- 6/ 180-300	ARse/ MY
Rhizophora × annamalayana	Round/ 4.5-6.0/ Sc 3 types	—/ 6-7/ 130-160	ARse/ MY
Rhizophora × lamarckii	Round/ 4.5-5.5/ Sc 3 types	—/ 2-3/ 75-90	ARse/ MY

Symbols: aggregated form: broad band ▬; narrow band —; rectangle ▮; trapezoid ▲; semicircle ◠; triangle ▲; Abbreviations: ARse-Sessile acuminate rod; MW-milky white; MY-milky yellow; Y-yellow; Sc: sclereid idioblast; SRl-long stalked rod; SRs-short stalked rod.

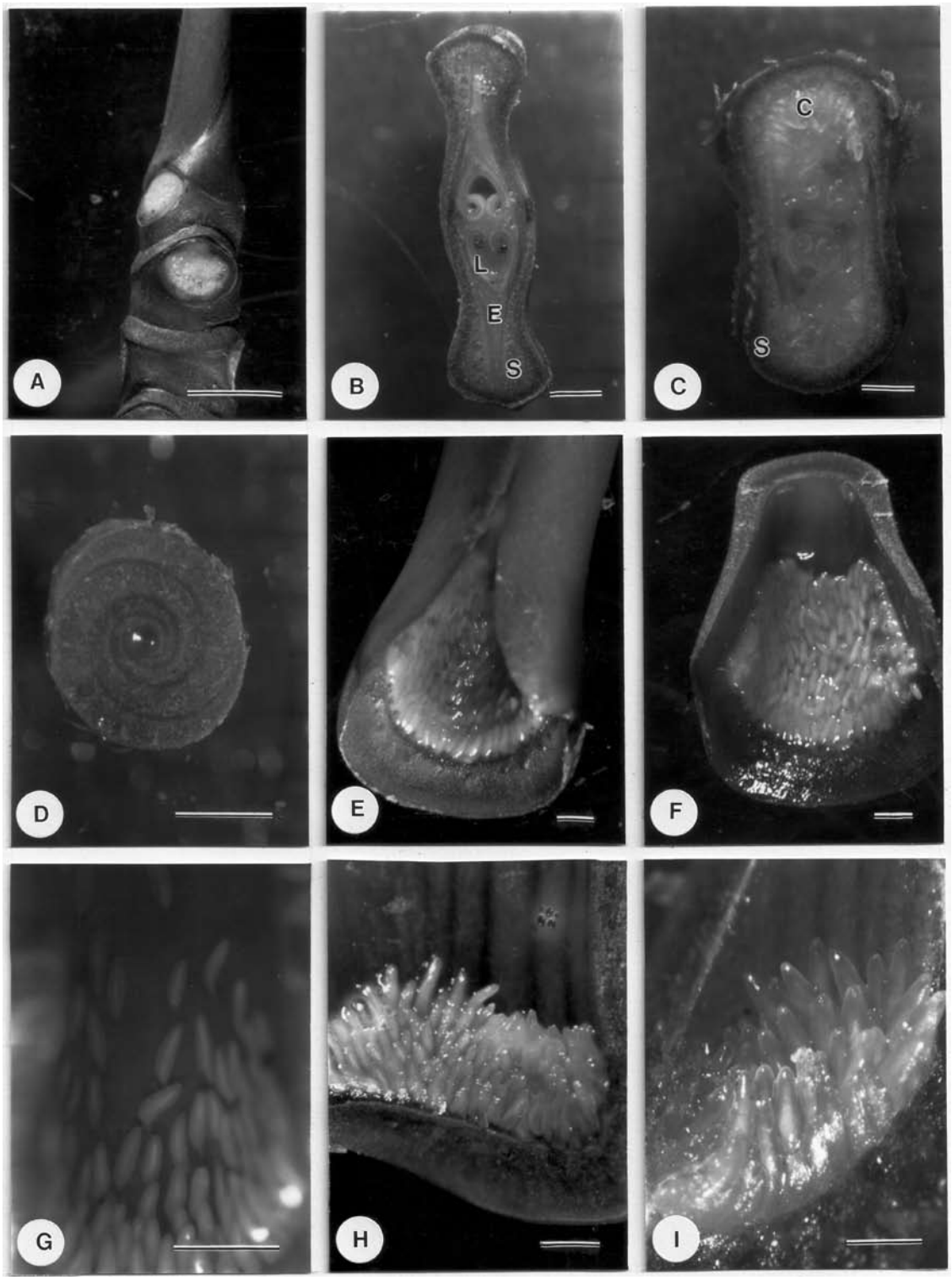

Figure 2. Morphological features of stipules and colleters in mangrove Rhizophoraceae. Abbreviations: C: colleter, E: exudates, L: leaf, S: stipule. (A) A shoot of *Rhizophora apiculata* with stipules, annular stipule scars and leaf scars; (B-C) Cross-section of a *Ceriops zippeliana* shoot showing a pair of clasping stipules appearing as flattened type, with thickest part in the folding region and its thickness gradually thinning out toward its margin and apex; (C) A position closer to the base of the stipule with colleters. The convolute young leaves, colleters and their exudates appear between the space of stipules; (D) Cross-section of a *R. apiculata* shoot with a pair of clasping stipules appearing as rounded type; (E-F) A *Bruguiera gymnorhiza* stipule with aggregated colleters forming a rectangle at the adaxial base. Removing some stipule tissues reveals colleters in (F); (G) Aggregated colleters in *B. cylindrica*. Note that colleters are less compact at the distal end; (H) A broad band of aggregated colleters in *R. mucronata*; (I) Part of a colleter aggregation in *R. apiculata*. Scale bars: (A) and (D) = 5 mm; (B), (C), (E), (F), (G) and (H) = 1 mm; (I) = 500 μm.

RESULTS

Stipule morphology

All the members of mangrove Rhizophoraceae have distinct stipules paired outside young leaves (Figures 1A and 2A). Most of the stipules are green to yellow- green; however, some species (e.g. *R. apiculata, R. stylosa, B. gymnorhiza*) may have both red-purple and green stipules. The morphology of stipules is consistent within a genus, although size may vary interspecifically (Table 2). Two types of stipule morphology can be recognized: rounded (*Bruguiera* and *Rhizophora*) (Figure 2A, 2D) and flattened (*Ceriops* and *Kandelia*, Figure 2B-C). The adaxial base of each stipule contains a space filled with aggregated colleters and a slightly sticky resin-like substance, which can be observed from detached stipules (Figures 1B, 2E-I) or stipule cross-sections (Figure 2B-C). Despite the morphological difference, clasping stipule vernation is present in all species of the mangrove Rhizophoraceae, whereby one margin is free and is thickest in the middle region and gradually thins out towards its edge (Figure 2B-D).

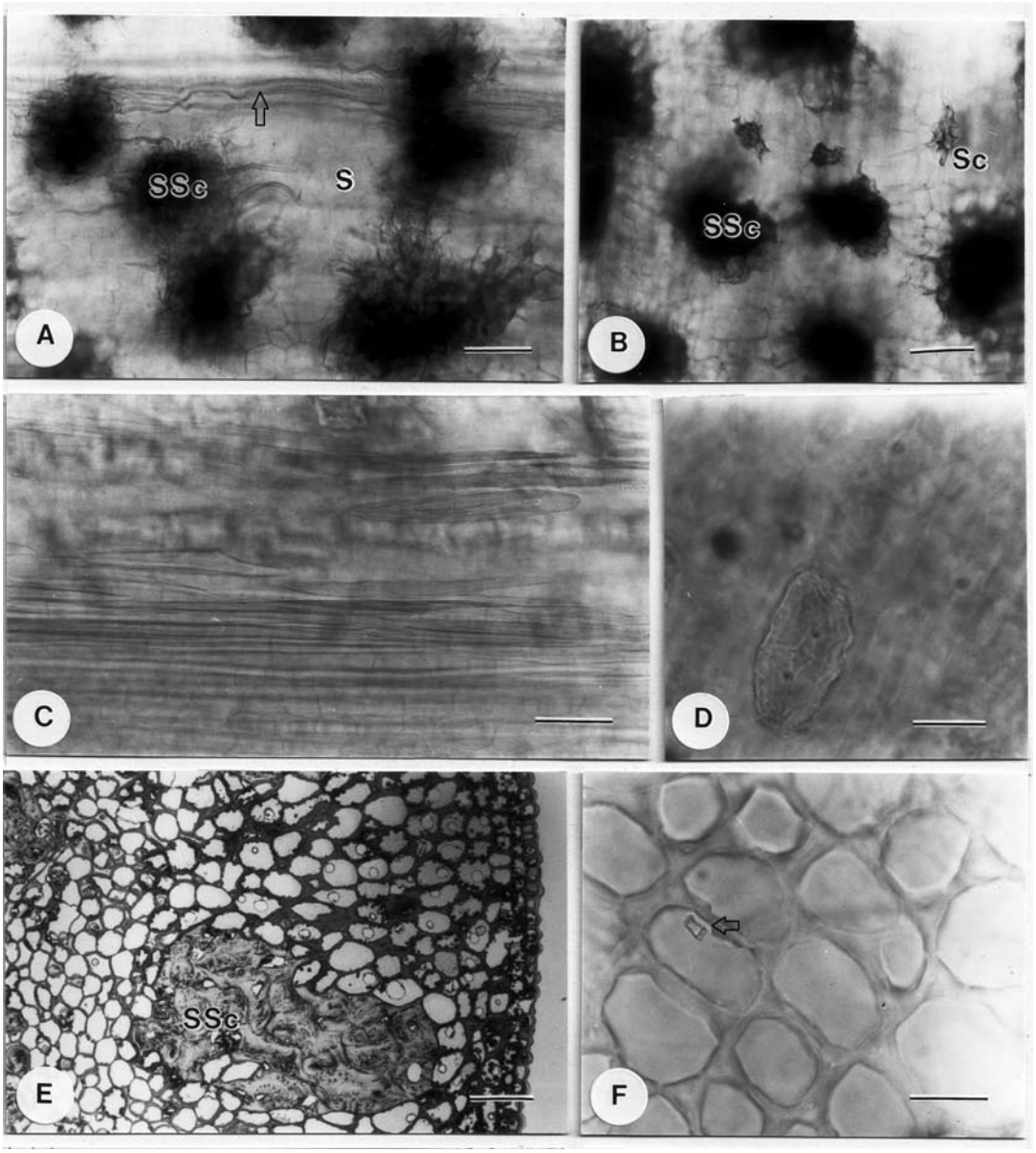

Figure 3. Anatomical features of mangrove Rhizophoraceae stipules. Abbreviations: S: stipule, Sc: small sclereid idioblast, SSc: strands of aggregated sclereid idioblast. (A-B) Sclereid idioblasts in *Rhizophora apiculata,* several strands of aggregated sclereid idioblasts (multibranched) and elongated rod idioblasts (arrow) parallel to the long axis of the stipules in (A); three isolated small sclereid idioblasts and aggregated sclereid idioblasts in (B); (C-D) Sclereid idioblasts in *Ceriops australis*, elongated rod idioblasts in (C) and an isolated small sclereid idioblast in (D); (E) Cross-section of *R. stylosa* stipule showing a strand of aggregated sclereid idioblasts; (F) The collenchyma in the base part of a stipule in *Bruguiera parviflora*, with a prismatic crystal inside a cell (arrow). Scale bars: (A), (B) and (E) = 100 μm; (C), (D) and (F) = 30 μm.

Stipule structure

The clearing method and cross-sections reveal that two genera of the mangrove Rhizophoraceae, *Ceriops* and *Rhizophora*, have sclereid idioblasts inside the stipules. *Rhizophora* has three types of sclereid idioblasts: strands of aggregated sclereid idioblasts (Figure 3A-B), isolated small idioblasts (Figure 3B) and elongated rod idioblasts, which are mostly parallel to the long axis of stipules (Figure 3A). In *Ceriops*, two kinds of sclereid idioblasts are observed: elongated rod idioblasts along the thickest mid region (Figure 3C) and isolated small idioblasts (Figure 3D). In contrast, *Bruguiera* and *Kandelia* have no sclereid idioblast in

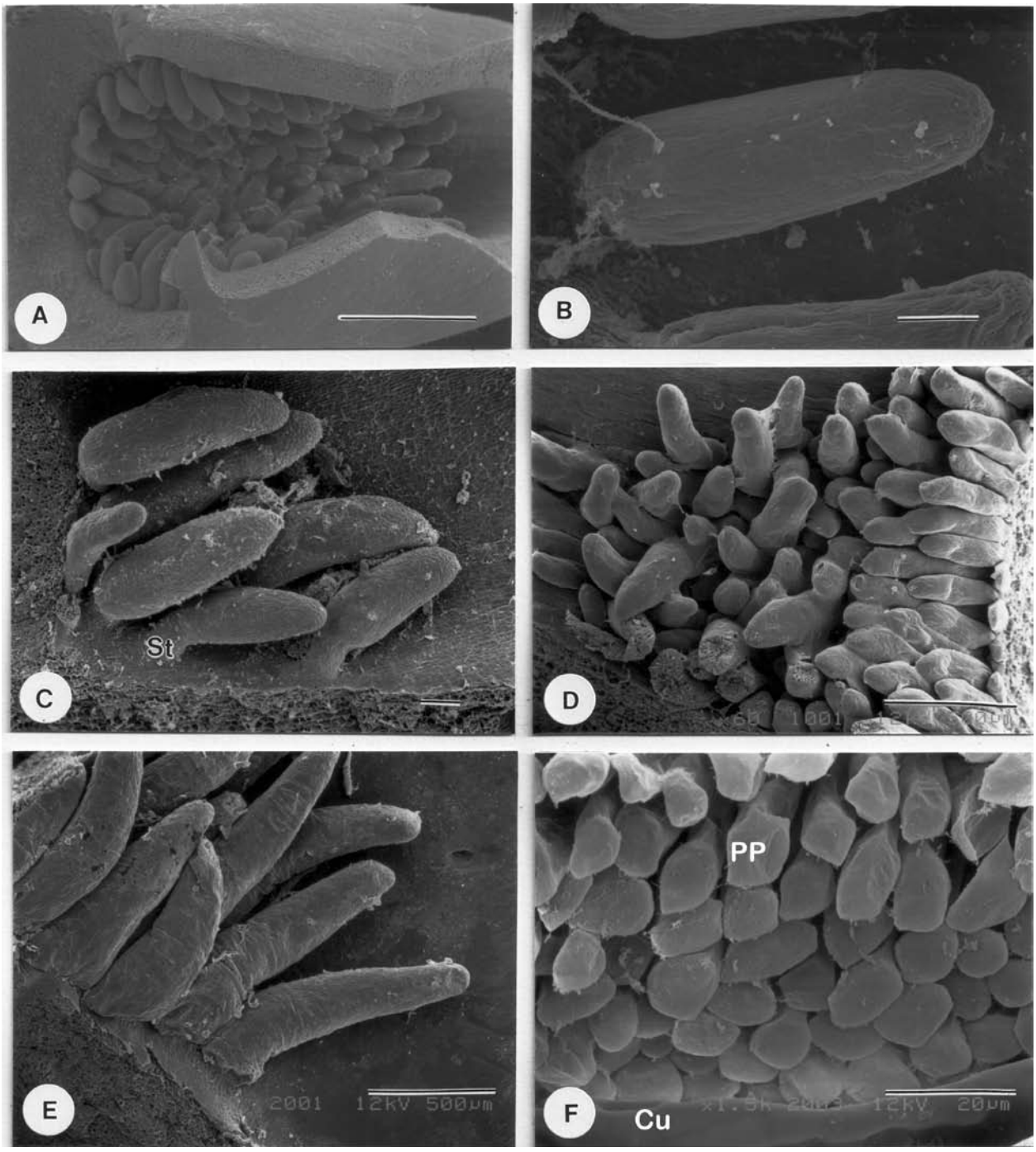

Figure 4. SEM view of mangrove Rhizophoraceae colleters. Abbreviations: Cu: cuticle, PP: peripheral parenchyma, St: stalked rod colleter. (A- B) *Bruguiera exaristata*, colleters aggregate as a trapezoid form in (A) and an individual colleter appears as a short stalked rod in (B); (C) *Ceriops decandra*, with long stalked rod colleters; (D) *Kandelia obovata*, a part of the aggregated colleters in a triangular form; (E-F) *Rhizophora apiculata*; (E) Colleters appear as sessile acuminate rods; (F) A surface view of a colleter showing the detached cuticle and inner peripheral parenchyma cells. Scale bars: (A) = 1 mm; (B) and (C) = 100 μm; (D) and (E) = 500 μm; (F) = 20 μm.

their stipules, both instead possess collenchyma tissue near the abaxial side of their stipules (Figure 3F).

Stipule structure among mangrove Rhizophoraceae species is similar, except for the presence of sclereid idioblasts (Table 2, Figure 3E). Unlike in normal leaves, there is no differentiated palisade and spongy parenchyma in these stipules. A thick cuticle covers the epidermis of both sides. The ground tissue consists mostly of collenchyma, but parenchyma is found near the adaxial side. If present, sclereid idioblasts mostly occur within collenchyma. Stipule tissues of mangrove Rhizophoraceae are also abundant in tannins (Figure 6A) and form druse (Figure 6A), or occasionally prismatic (Figure 3F), calcium oxalate crystals.

Colleter

Several to numerous milky-white to yellowish colleters of various shapes aggregated in multiple rows were found attached to the base of the adaxial side of the stipule (Figure 1B, Table 2). These colleters remain attached to the stipule, but shrink and gradually lose their fresh color as the stipule drops from the parent tree. However, colleters may attach to the corresponding side of the stipule scar along with the basal residue of the stipule if the stipule is removed while it is still strongly enclosing a shoot. Obvious secretions often glue the colleters together in all mangrove taxa observed in this study.

Aggregated form, rows and numbers of colleters

A mass of colleters occurring at the adaxial base of each stipule aggregates as a band, rectangle, trapezoid or triangle. It is clear that these aggregated forms are generically consistent (Table 2).

In *Bruguiera*, multirow colleters aggregate as a rectangle or trapezoid (Figure 4A), and colleters located at the distal end of a stipule may appear more or less loosely organized (Figure 2G). There are from 3-14 rows, and c. 40-210 colleters in each stipule of the various *Bruguiera* species (Table 2). It is apparent that row number and total colleter number are species specific, and this could be a diagnostic character for species differentiation. In the genus *Bruguiera*, *B. gymnorhiza* has the greatest number of colleters (9-12 rows, 200-210 colleters) (Figure 2E-F), while *B. sexangula* has the least (3-4 rows, 35-40 colleters) (Table 2), although their stipules are nearly the same size.

The stipules of *Kandelia* and *Ceriops* are flattened (Figure 2B-C) and both colleters are aggregated into a triangular shape (Figure 4D). Due to the tightly overlapping margins of the stipule, the colleters of these two genera are difficult to observe unless the stipule is cut into two halves. It is noteworthy that *K. candel*, with a larger stipule (3-4 cm long), has fewer colleters (75-96) than *K. obovata*, with a smaller stipule (2.5-3.2 cm long, 95-110 colleters) (Table 2).

In the genus *Rhizophora*, colleters are aggregated either as narrow or broad bands, comprising 2-9 rows (Table 2; Figure 2H-I). *Rhizophora apiculata* colleters are bright yellow, but those of other taxa of this genus are milky white. This feature could distinguish *R. apiculata* from other species of *Rhizophora* in the field. In addition, row numbers of colleters differ between species of this genus. *Rhizophora apiculata* has just 2-3 rows of colleters, while *R. stylosa* has 4-6, and *R. mucronata* has 6-9 (460-580 colleters).

Individual shape and size of colleter

Colleter attachment comes in both sessile and stalked forms in the mangrove Rhizophoraceae (Table 2; Figures 4-5). Only the genus *Rhizophora* has sessile colleters (Figures 4E, 5D), while the colleters of *Bruguiera*, *Ceriops* and *Kandelia* have stalks near the base. Colleters of the species of *Bruguiera* appear as short stalked rods (stalk less than 30 μm long) with similar width from the base to the tip (Figures 4B, 5A). Colleters of *Ceriops* and *Kandelia* have long stalked rods, with stalks about or longer than 100 μm in length (Table 3; Figures 4C, 5B-C). Colleters of *Rhizophora* are best described as sessile acuminate rods due to the uneven width (Figures 4E, 5D).

The size (most conveniently measured by length) of individual colleters may vary within the same aggregated group within a stipule. For example, smaller colleters are often observed near the base, but these smaller colleters are relatively few in number. There are larger numbers of larger similarly-sized colleters within a stipule. These colleters are the basis of the comparative measurements in this study. It is clear that the genus *Rhizophora* has the largest colleters in these four genera of mangrove Rhizophoraceae. The colleter size of six selected species of mangrove Rhizophoraceae is compared in Table 3 (stalks included if stalks present). The colleters of *R. stylosa* reach 1008.9 ± 270 μm in length with a broad base (up to

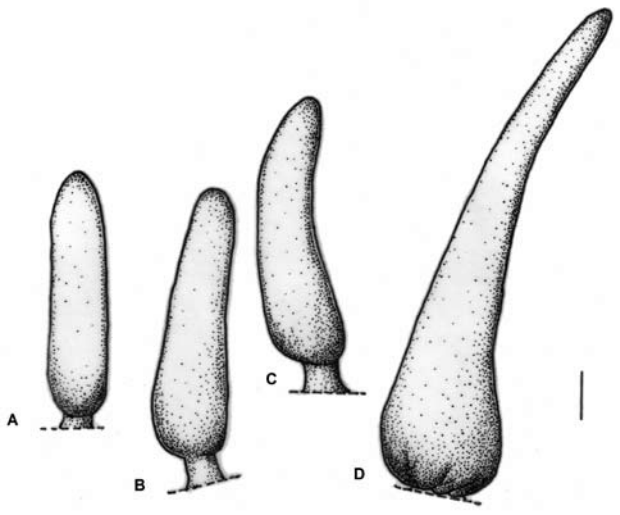

Figure 5. Diagram of the individual shapes of colleters in mangrove Rhizophoraceae stipules. (A) *Bruguiera*, short stalked rod; (B) *Ceriops*, long stalked rod; (C) *Kandelia*, long stalked rod; (D) *Rhizophora*, sessile acuminate rod. Scale bar = 100 μm.

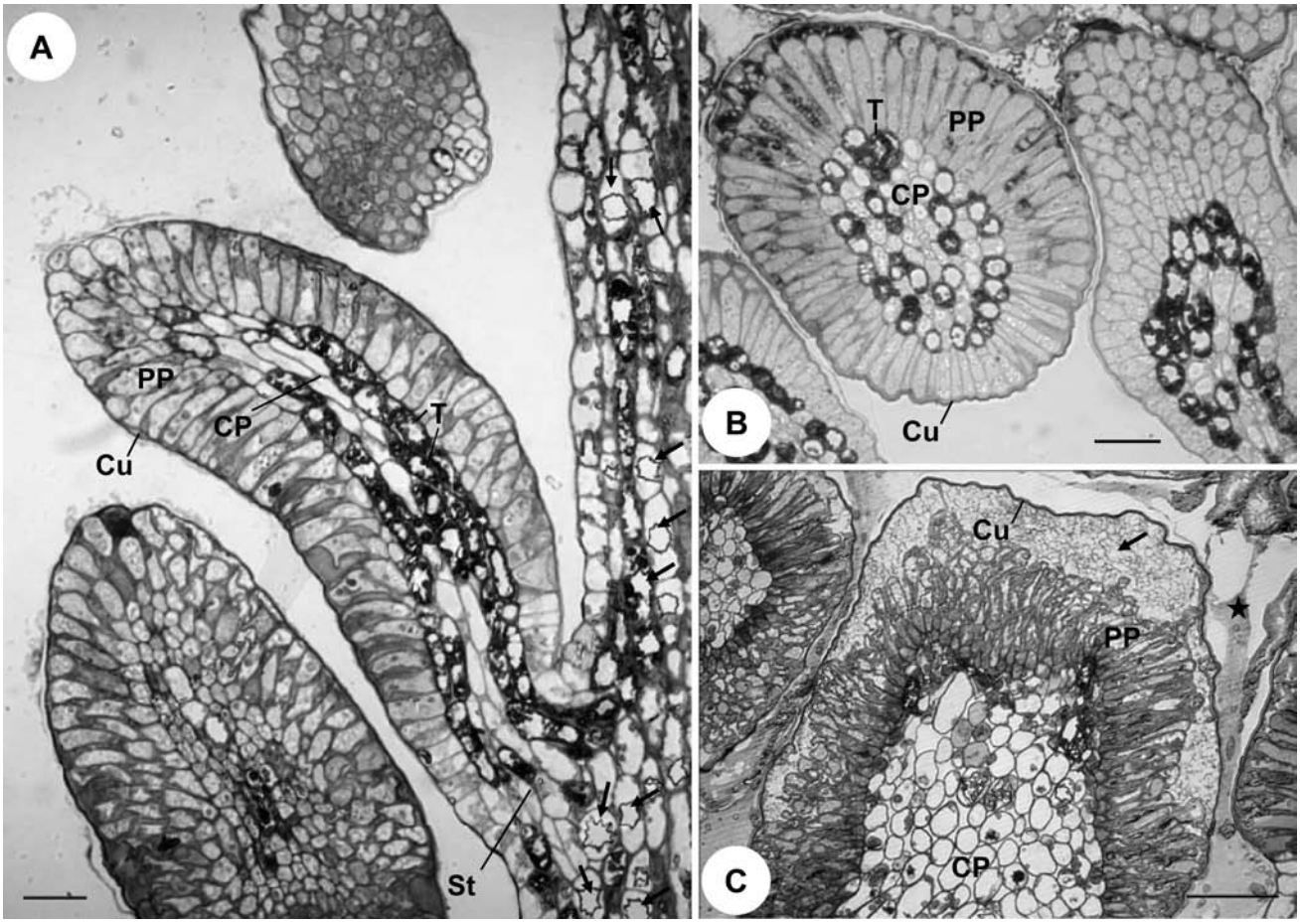

Figure 6. Anatomical structure of colleters in mangrove Rhizophoraceae stipules. Abbreviations: Cu: cuticle, CP: central core of parenchyma, PP: peripheral parenchyma, St: stalked rod colleter; T: Tanninferous cells. (A-B) *Kandelia obovata*; (C) *Rhizophora stylosa*. (A) A longitudinal section of a colleter showing the central core of elongated parenchyma, peripheral parenchyma and a stalk attached to a stipule. Abundant drused crystals (arrows) appear in the stipule tissue, but are absent from colleter cells. Tanninferous cells commonly occur in colleter and stipule core parenchyma, but not in the colleter epidermis; (B) Cross-section of a colleter showing central parenchyma surrounded by radically elongated peripheral epidermis; (C) A terminal part of a colleter showing an apparent space filled with exudates (arrow) between peripheral epidermis and the cuticle. Some exudates (starred) that drench young shoots are released after the rupture of the cuticle. All scale bars = 50 μm.

Table 3. Morphometric characters of colleters of six selected species of the mangrove Rhizophoraceae.

Species	N	Length[1] (μm) (min) avg (max) ± std	Width (μm) (min) avg (max) ± std	Stalk length (μm)
Bruguiera exaristata	12	(238) 406.6 (540) ± 106.6	(119) 153.3 (200) ± 27.7	Less than 30
Ceriops australis	10	(204) 371.2 (530) ± 100.8	(82) 97.9 (102) ± 9.1	160 ± 24
Ceriops decandra	10	(269) 516.0 (672) ± 119.9	(77) 157.2 (211) ± 46.1	92.7 ± 5.8
Ceriops tagal	10	(510) 674.8 (856) ± 115.2	(102) 130.6 (142) ± 18.2	Not measured
Kandelia obovata	10	(275) 500.5 (680) ± 129.2	(100) 155.8 (220) ± 39.2	119 ± 23.9
Rhizophora stylosa	10	(713) 1008.9 (1490) ± 270.0	Base[2]: 300.0 ± 28.2 Middle: 222.5 ± 11.6 Tip: 100.1 ± 18.1	No stalk

[1]Stalks included if stalks present.
[2]Because colleter is an acuminate rod, widths were measured from the base, middle and tip.

300 μm) tapering to one-third of its basal width at the tip. *Ceriops australis* has the smallest colleters judged both by length (371.2 ± 100.8 μm) and by width (97.9 ± 9.1 μm). In the genus *Ceriops*, colleter size varies in a gradation (e.g. *C. tagal*: 674.8 ± 115.2 μm, *C. decandra*: 516.0 ± 119.9 μm, and *C. australis*: 371.2 ± 100.8 μm).

Structure of colleter

Despite the variation in stalk presence and the size of colleters, the anatomical structure of colleters of all taxa studied is remarkably similar. Viewed in transverse section, colleters consist of a rather massive core of elongated parenchymatous cells (c. 8-10 rows) with unlignified walls in the center (Figure 6), sheathed by many peripheral parenchyma (palisade-like epidermal cells) arranged in one layer perpendicular to the axis (Figures 4F; 6A-B). Usually, the cell walls of the core parenchyma are thicker than those of the palisade-like epidermis. Many of these core parenchyma cells are tanniferous. Drused crystals rarely occur in the parenchyma of the core region, and not at all in the outer epidermal cells (Figure 6A). The outer thin-walled palisade-like epidermial cells are the secretory cells, with dense cytoplasm. The mucilage accumulates in the intercellular spaces of epidermal cells as well as between the cuticle and the secretory epidermal cells (Figure 6C).

DISCUSSION

All members of the mangrove Rhizophoraceae have conspicuous stipules enclosing young shoots. However, compared to leaf structure and function, stipules are much less studied and under-reported. In this study, we recognized three types of sclereid idioblasts in stipules of *Ceriops* and *Rhizophora* genera. The strands of aggregated sclereid idioblasts and the isolated small idioblasts in *Rhizophora* are astrosclereids, as reported for the stipules of *R. mucronata* (Shah and Sundarraj, 1965). This is the first report of idioblasts in the *Ceriops* stipules. These isolated small idioblasts appear as short rods without branch arms. The elongated rod idioblasts are parallel to the long axis of *Ceriops* stipules, similar to the arrangement of elongated idioblasts along the leaf midrib in *Rhizophora* (Sheue, 2003). These idioblasts and collenchyma, along with vascular tissues and the thick cuticle of stipules, provide strong mechanical support that presumably protects the shoot inside.

Both rounded and flattened stipules were recognized in the mangrove Rhizophoraceae. Stipule morphology correlates with the shape of their contained colleter aggregations. Flattened stipules appear in the two mangrove genera, *Ceriops* and *Kandelia*. These stipules are thickest in the folding regions, and their thickness gradually thins out toward the margin and apex. As a consequence, a triangular space filled with colleters is formed in its adaxial base.

The work reported here suggests that the forms of colleter aggregations, and the shapes of the individual colleters in the stipules, could have taxonomic value at the generic level in the mangrove Rhizophoraceae. The number of colleter rows in an aggregation could have value at the specific level for most of these species. For example, the five taxa of *Ceriops* appear very similar in morphology (Sheue et al., 2009a, 2010). The characteristics of colleters can serve as valuable vegetative diagnostic traits for *Ceriops* materials in the absence of reproductive parts. This taxonomically valuable character has also been applied to differentiate between species within *Bruguiera* (Sheue et al., 2005) and *Kandelia* (Sheue et al., 2003a, b). A hand lens (c. 10X) is recommended to observe this feature in the field.

The similarity in stipule and colleter morphology demonstrates a close relationship between *Ceriops* and *Kandelia* that is in agreement with the molecular phylogeny (Setoguchi et al., 1999; Schwarzbach and Ricklefs, 2000). However, two types of sclereid idioblasts are present in *Ceriops* stipules, but are absent from those of *Kandelia*. No sclereid was found in *Bruguiera* or *Kandelia* stipules, but two and three sclereid types were found in *Ceriops* and *Rhizophora* stipules, respectively. This may imply that *Ceriops* and *Rhizophora* are more derived than the former two genera (*Bruguiera* and *Kandelia*), with *Rhizophora* being the most derived genus in this subfamily. This result is consistent with the phylogeny within this subfamily based on molecular (Setoguchi et al., 1999; Schwarzbach and Ricklefs, 2000) and morphological evidence (Juncosa and Tomlinson, 1988b).

In this study, colleters of two reported *Rhizophora* hybrids were compared with colleters of their putative mother plants. As is usual, the morphological features of hybrids were consistently intermediate to their putative parents. Interestingly, only one hybrid showed an intermediate number of rows. *Rhizophora* × *annamalayana* Kathiresan (Kathiresan, 1995), was reported from southern India and was established as a hybrid of *R. apiculata* and *R. mucronata* (Parani et al., 1997). The hybrid from India, *R.*× *annamalayana*, has 5-6 rows of colleters, which appear as an intermediate between its parents (2-4 rows and 6-9 rows). In contrast, the hybrid, *R.* × *lamarckii,* reported as a hybrid of *R. apiculata* (2-4 rows) and *R. stylosa* (4-6 rows) (Duke and Bunt, 1979; Tomlinson, 1986), has 2-3 rows of colleters, which is closer to those of *R. apiculata*.

The 'standard' type colleter was first described by Lersten (1974a), which is one of the widespread colleter types in Rubiaceae. In addition, 'dendroid' and 'brush-like' types were described as Rubiaceous colleters (Lersten, 1974a, b). The members of Apocynaceae also have standard type colleters intermingled with petiolar hairs on the adaxial side of the petiole (Thomas and Dave, 1991). In this study, the colleters of all mangrove species of this family are anatomically standard, as Lersten and Curtis (1974) observed in *R. mangle,* and are remarkably similar to those found in several other families. However, various shapes of colleters were recognized in this study: short-stalked rods (*Bruguiera*), long-stalked rods (*Ceriops*, *Kandelia*), or sessile acuminate rods (*Rhizophora*). The stalk structure

of a colleter has seldom been reported, but *Mandevilla* is one example (Appezzato-da-Glória and Estelita, 2000). The foliar and intrapetiolar colleters of *Mandevilla* can exhibit vascularization, but its interpetiolar colleters lack vascularization (Appezzato-da-Glória and Estelita, 2000). Althought no vascularization similar to that observed for *R. mangle* (Lersten and Curtis, 1974) was found in the present study, we recently observed subtle vascularization in *K.obovata* by more sensitive methods (Sheue et al., in preparation).

Lersten (1974a) found crystals in the colleters of the Rubiaceae. However, in the present study, an abundance of drused crystals was observed within the stipule tissues beneath the colleters, but rarely inside colleters. This difference may imply differentiated cellular functions and metabolic activities between colleters and stipules. Significant spaces between the peripheral parenchyma and the cuticle containing a secretory product were commonly found in colleters of the mangrove Rhizophoraceae as observed in those of *Gardenia* (Mangalan et al., 1990). The secretory product is released after the rupture of the cuticle and drenches the shoot apex inside the stipule (Mangalan et al., 1990).

Rhizophora, a genus usually occurring at the front of mangroves facing the sea, has the largest colleters with an average length over 1 mm. The colleters of *Ceriops*, a back mangrove closer to land, are relatively smaller (c. 370 μm in *C. australis*). The prominent size and numerous colleters of *Rhizophora* (460-580 in *R. mucronata*) represent the most developed colleters of this study. In addition, the sessile acuminate rod of colleters in *Rhizophora* is considered to be more derived in this family, since the colleters of the other three genera are stalked.

Erythroxylaceae was recently confirmed as a sister family of Rhizophoraceae (Setoguchi et al., 1999; Schwarzbach and Ricklefs, 2000). Thiebaut and Hoffmann (2005) described colleters in four genera of Erythroxylaceae, and compared to two genera of Rhizophoraceae. Colleters of Erythroxylaceae are of the same type as those of Rhizophoraceae, but position, arrangement, shape and size differ between all examined genera (Thiebaut and Hoffmann, 2005). In addition, the authors commented that the obvious secretion gluing the colleters together occurs in the mangrove *R. mucronata*, but not in the inland member of the Rhizophoraceae, *Cassipourea malosana* (Thiebaut and Hoffmann, 2005). In terms of colleter number and size, it is evident that the mangrove Rhizophoraceae has more and larger colleters, as reported here, than those observed in the inland Rhizophoraceae, *Cassipourea malosana* (Thiebaut and Hoffmann, 2005), *Carallia* and *Gynotroches* (Sheue, 2003).

The combined evidence from morphological and anatomical features of these mangroves and observations in the field, suggest that the stipules provide a physical barrier, and the colleter secretions provide a viscous fluid barrier, that protect the young shoots from the strong wind, salt spray, tidal immersion and high temperatures that characterize their harsh environment. Since the colleters of this family are still under-reported, it would be interesting to compare the differences between the inland and mangrove species with special reference to the ecological adaptations represented by stipule and colleter characters.

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紅樹科紅樹林植物的托葉與指狀腺體的形態與構造之比較研究

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紅樹科紅樹林植物的枝稍具有圓形或扁平之托葉，緊密包圍著幼葉與頂芽。此托葉向軸面基部的指狀腺體分泌出明顯可見之樹脂狀至乳狀物質浸潤幼芽。本文首次系統性地觀察及比較本科紅樹林植物的 18 個分類群之托葉與指狀腺體的形態與構造。托葉內三種形態的厚壁異形細胞 (細蕊紅樹與紅樹屬)、發達的厚角組織或與表皮細胞外之厚角質層，提供了機械性支持的結構基礎。數十個至數百個 (35-580) 似手指狀之指狀腺體聚集呈特定的形狀，具屬的專一性：紅茄苳屬呈長方形至梯形、細蕊紅樹屬和水筆仔屬為三角形 、紅樹屬則呈帶狀。各托葉內指狀腺體的排數、總數與指狀腺體的個別大小，在各分類群並不相同，具分類價值。依據托葉和指狀腺體的特徵顯示細蕊紅樹屬和水筆仔屬的關係最接近，而紅樹屬則可能呈現較後起的特徵。本群植物的指狀腺體儘管在形態上呈現短柄桿狀 (紅茄苳屬)，長柄桿狀 (細蕊紅樹屬和水筆仔屬) 或無柄漸尖桿狀 (紅樹屬)，但其解剖構造均為標準型。此腺體的中心軸由細長的薄壁細胞構成，其外圍似柵狀的薄壁表皮細胞具分泌功能，與中心薄壁細胞呈垂直排列。由托葉所具的結構與機械性的保護功能，和其內指狀腺體的生理浸潤作用，可能對此紅樹林植物的頂芽提供了重要的保護，以助其應付嚴酷的環境。

關鍵詞：解剖；紅茄苳屬；細蕊紅樹屬；指狀腺體；水筆仔屬；紅樹林；紅樹屬；標準型；厚壁細胞；托葉。