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# PHYLOGENETIC RELATIONSHIPS OF SAMYDACEAE AND TAXONOMIC REVISION OF THE SPECIES OF *CASEARIA*

# IN SOUTH-CENTRAL ASIA

by

Tharangamala Samarakoon

Abstract of a Dissertation Submitted to the Graduate School of The University of Southern Mississippi in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

# ABSTRACT

# PHYLOGENETIC RELATIONSHIPS OF SAMYDACEAE AND TAXONOMIC REVISION OF THE SPECIES OF *CASEARIA* IN SOUTH-CENTRAL ASIA

# by Tharangamala Samarakoon

# May 2015

The flowering plant family Samydaceae was recently reinstated to include 14 genera and about 256 species of tropical trees and shrubs. Preliminary analyses of relationships in the family, however, indicated that the largest genus in the family, *Casearia*, is not monophyletic and that several smaller groups are probably misplaced. In order to affirm or refute those hypotheses, additional DNA sequence data with broader taxon sampling from the Old World were gathered for phylogenetic analysis. Phylogenetic inferences were drawn from parsimony, likelihood, and Bayesian analyses of plastid and nuclear DNA data. The results affirm the monophyly of Samydaceae and consistently recover four major clades, which are here circumscribed as four tribes. The results also affirm that *Casearia* is not monophyletic. Rather, several small genera (Samyda, Laetia sensu stricto, Hecatostemon, and Zuelania) are nested within it. One previously recognized genus, *Piparea*, which is more closely related to *Ryania* and Trichostephanus, should be reinstated. Nomenclatural changes are presented here to bring generic circumscriptions in line with phylogenetic relationships within Samydaceae and with the proposed conservation of the name *Casearia* over *Samyda* and *Laetia*. A taxonomic revision of *Casearia* in south-central Asia, including the area from Pakistan to Burma and south from the Himalayas to Sri Lanka, is also presented. Thirteen species are

recognized, nine of which are endemic to south-central Asia and four of which are more widespread, also occurring eastward into China, southeast Asia, and Malesia.

Descriptions, taxonomic keys, and distributional maps are provided for the species.

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Dr. Carl A. Reese

Dr. Karen S. Coats Dean of the Graduate School

May 2015

# DEDICATION

To my parents and family, who believe in me and deserve much credit for the success in my life. My caring husband Methil Karunanayake and my sweet little angels, Omandee, Kalana, and Selini, without whom this would not have been completed.

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# CHAPTER I

# PHYLOGENETIC RELATIONSHIPS OF SAMYDACEAE (MALPIGHIALES) INFERRED FROM PLASTID AND NUCLEAR DNA SEQUENCE DATA

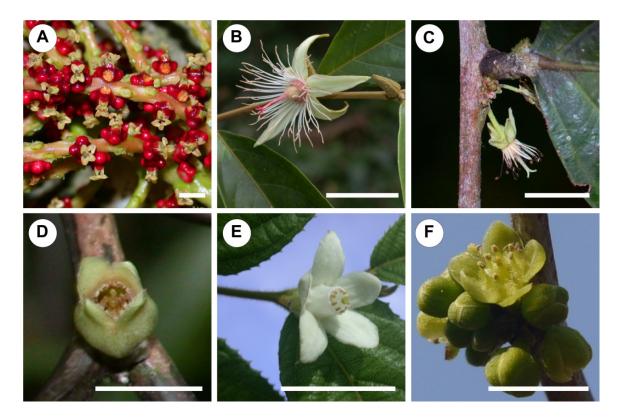
# Abstract

The flowering plant family Samydaceae was recently reinstated to include 14 genera and about 256 species of tropical trees and shrubs. Preliminary analyses of relationships in the family, however, indicated that the largest genus in the family, *Casearia*, is not monophyletic and that several smaller groups are probably misplaced. In order to affirm or refute those hypotheses, additional DNA sequence data with broader taxon sampling from the Old World were gathered for phylogenetic analysis. In particular, rapidly evolving plastid (matK, ndhF, psbA-trnH, trnL, and trnL-F) and nuclear (EMB2765 and GBSSI) DNA regions were sampled for characters. Four of these regions (matK, ndhF, EMB2765, GBSSI) could be confidently aligned across the family, and phylogenetic inferences were drawn from parsimony, likelihood, and Bayesian analyses primarily of these data. The results affirm the monophyly of Samydaceae and consistently recover four major clades, which are here circumscribed as four tribes, namely Lunanieae, Ryanieae, Samydeae, and Tetrathylacieae. The results also affirm that *Casearia* is not monophyletic. Rather, several small genera (*Samyda, Laetia* sensu stricto, *Hecatostemon*, and *Zuelania*) are nested within it. One previously recognized genus, Piparea, which is more closely related to Ryania, and Trichostephanus, should be reinstated. A key to the newly circumscribed tribes and genera is provided.

## Introduction

Samydaceae are a family of tropical, woody flowering plants recently resurrected to include about 256 species of the former Flacourtiaceae (Alford, 2003, 2005, in prep.). Flacourtiaceae as traditionally circumscribed (e.g., Warburg, 1894; Gilg, 1925; Lemke, 1988) were a polyphyletic assemblage principally placed together because they have ovaries with parietal placentation and lack features of the other few families with parietal placentation. As a "garbage bin" family, Flacourtiaceae were widely known for intractability. Even the experienced field botanist Alwyn Gentry wrote, "If you don't have any idea what family it is, try Flacourtiaceae or Euphorbiaceae" (Gentry, 1993). Furthermore, because identification is often based on technical characteristics of the highly diverse, yet minute, flowers (Figure 1.1), many Samydaceae (and other former Flacourtiaceae) are filed in herbaria among the indets. Although many Samydaceae have medicinal, cytotoxic, insecticidal, fungicidal, or other interesting chemical constituents (e.g., Anaya et al., 2004; Ashik et al., 2006; Beutler et al., 2000; Borges et al., 2000; Itokawa et al., 1990; Jullian et al., 2005; Monachino, 1949; Taylor, 2002), can accumulate metals at some of the highest recorded levels (Jaffré et al., 1979), are hosts to the weird angiosperm parasites of the Apodanthaceae, and are common elements of tropical forests worldwide (Phillips and Miller, 2002; Guilherme et al., 1998; Silva Jr. et al., 1998), the family has received little attention because there are almost no economically important members of the family, save a few species sporadically used for lumber (Chudnoff, 1984; Richter and Dallwitz, 2000). Furthermore, even though many species in the family are common and widespread, more than 30% are threatened (Walter and Gillett, 1997). Despite lack of attention until now, the morphological diversity of the

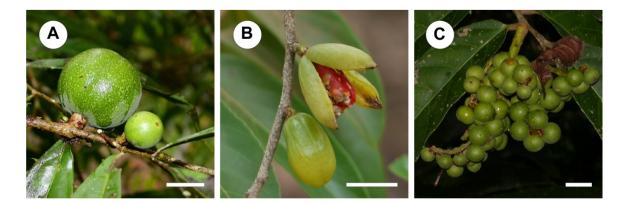
family holds many opportunities for developmental, physiological, and evolutionary study (Cronk, 2005) because of its close relationship to Salicaceae, for which an entire genome sequence is now available (Tuskan et al., 2006).



*Figure 1.1.* Flower diversity in Samydaceae. A. *Tetrathylacium macrophyllum*, with fleshy, 4-merous flowers arranged in spicate inflorescences. B. *Ryania speciosa*, with many stamens, intrastaminal staminodes (pink), and style divided at the apex. C. *Casearia javitensis*, with one whorl of stamens, intrastaminal staminodes, and style divided at the apex. D. *Neoptychocarpus killipii*, dioecious, with a fleshy, urceolate calyx. E. *Samyda dodecandra*, with fusion of androecium and lacking staminodes. F. *Casearia tomentosa*, with a single whorl of stamens and interstaminal staminodes. Scale bars = 1 cm. Photos A–D courtesy of Mac H. Alford; E courtesy of Jacqueline Salazar; and F courtesy of Dinesh Valke.

The natural history of Samydaceae is also poorly known. Pollination studies of a few species (Weber, 2008; Machado and Oliveira, 2000; Barbosa, 1997; Kress and Beach, 1994) and fruit dispersal studies of even fewer species (Janzen, 1983; Howe and

Vander Kerckhove, 1979; Howe, 1977; Howe and Primack, 1975) have been conducted. One species, *Tetrathylacium macrophyllum*, may even have the first recognized case of katydid ('locust') pollination (Weber, 2008), and at least two genera have fruit dispersal by fish (*Laetia suaveolens, Casearia aculeata*, possibly *Ryania*; Correa, 2015; *Laetia corymbulosa*, M. H. Alford, pers. obs.). Several species have ant associations (Schmidt, 2001; M. H. Alford, pers. obs.). The karyology of only three genera has been studied: *Casearia, Ryania*, and *Trichostephanus*. Counts of 2*n*=22, 24, and 44 were found for *Casearia* and *Ryania* (Grill, 1990), and a count of 2*n*=126 was found for *Trichostephanus* (Hallé and de Wilde, 1978). Clearly, variation exists, but the sampling has been too poor at this point to make broader generalizations.



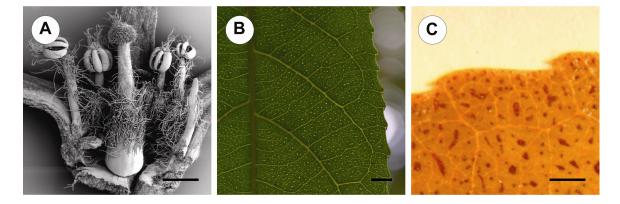
*Figure 1.2.* Fruit diversity in Samydaceae. A. *Casearia obovalis*, thick-walled, tardily dehiscent fruit. B. *Casearia tomentosa*, capsule with red arillate seed. C. *Tetrathylacium macrophyllum*, dry berry. Scale bars = 1 cm. Photos A, C courtesy of Mac H. Alford; photo B courtesy of Dinesh Valke.

This lack of study on many fronts is surprising, given the abundance of many species, the ease of observation (many are 'treelets'), and the diversity of habitats, flower morphology (although most very small), and fruit size, color, and ornamentation (Figure

1.2). Most importantly, this lack of data hinders our understanding of the biogeography and evolution of the family. In a genus (*Aglaia*, Meliaceae) with similar 'functional syndromes' as the genus *Casearia*, a greater understanding of the natural history has permitted correlations to distributions of dispersers (birds versus mammals, in particular) and the proposal of interesting, testable hypotheses about speciation and character evolution (Pannell, 1998; Muellner et al., 2008).

Analyses of DNA sequence data have been critical in clarifying the taxonomic mess of the former Flacourtiaceae. These analyses place Flacourtiaceae in the Malpighiales, a highly diverse order of flowering plants which includes families such as Salicaceae (willows and cottonwoods), Euphorbiaceae (rubber, cassava, poinsettias), Violaceae (violets), Passifloraceae (passion fruits), Hypericaceae (St. John's worts), and Erythroxylaceae (coca) (Davis and Wurdack, 2004; Davis et al., 2005; Tokuoka and Tobe, 2006; Korontkova et al., 2009; Wurdack and Davis, 2009; Xi et al., 2012). Chase et al. (2002) demonstrated that Flacourtiaceae as commonly circumscribed belong to two major lineages, one that is cyanogenic and associated with Achariaceae and another that is non-cyanogenic and associated with Salicaceae. Later, Alford (2003, 2005, in prep.) followed with a study using morphological and more variable DNA sequence data on a broader sampling of taxa and revealed that the non-cyanogenic clade were best divided into three families: Samydaceae, Scyphostegiaceae, and Salicaceae sensu medio. Each of these families is highlighted by suites of synapomorphies and is well-supported by both morphological and molecular data (cf. Wurdack and Davis, 2009; Xi et al., 2012). Like related families such as Passifloraceae, Samydaceae have the plesiomorphic character states of arillate seeds, hypanthia, and introrse anther dehiscence (Figure 1.3A).

Synapomorphies of the Samydaceae include loss of petals, presence of deciduous, nonglandular ("theoid") leaf teeth (Figure 1.3B), and pellucid-punctate and/or -lineate leaves (Figure 1.3B, C).



*Figure 1.3.* A. Scanning electron micrograph of the flower of *Casearia pitumba*, showing hypanthium, introse anther dehiscence, and expanded glandular connective on the anther. B. *Casearia corymbosa*, showing pellucid dots and lines and deciduous teeth (reddish tips). C. *Casearia graveolens*, showing pellucid dots and lines and deciduous teeth. Scale bars = 2 mm. Photos A–B courtesy of Mac H. Alford.

Previous results indicated that two genera, *Casearia* and *Laetia*, are probably not monophyletic (Alford, 2005, in prep.). This did not come as a complete surprise, as keys to identify the genera of Samydaceae (or Flacourtiaceae) are often based on single floral characters which require careful dissections to use (Sleumer, 1980) or are long and unsatisfying due to the lack of clear, correlated generic characteristics (Alford, 2003). Individuals in fruit can be almost impossible to identify—even to genus—without direct comparison to well-curated herbarium material. Those analyses indicated that there are two groups of *Casearia*: (1) *Casearia* sect. *Piparea*, which is closely related to *Ryania* and *Trichostephanus*, and (2) all of the other sections of *Casearia*, in which *Hecatostemon*, *Laetia* sensu stricto, *Samyda*, and *Zuelania* are nested. That phylogeny,

however, was based on a small sample of the species (and was biased to the Neotropics), lacked three small genera (*Osmelia*, *Pseudosmelia*, and *Ophiobotrys*), and only included a matrix of 32 morphological characters variable among members of Samydaceae. Before significant changes in classification are proposed, a greater sampling of characters and species, especially in *Casearia* and Old World taxa, is needed. Although the greatest diversity of *Casearia* in terms of named groups (approximately corresponding to morphological diversity) occurs in the New World (Sleumer, 1980), the Old World dominates in numbers of species (Sleumer, 1954). The thoroughness and robustness of the phylogeny is important because some disruption of the nomenclature is inevitable. The name *Laetia* predates the *Casearia* and *Samyda*, and any updates to the classification will involve either a significant number of name changes or the need for a conservation proposal. *Casearia* and *Samyda* already have a complex nomenclatural history (Sleumer, 1956; Kiger, 1972, 1984), both having been published simultaneously by Jacquin and used by other authors in several different ways.

This study has several goals: (1) to infer robust phylogenetic relationships within Samydaceae, using a broader character set drawn from both plastid and nuclear DNA data and a broader taxon set, with better sampling from the Old World, (2) to provide a modern classification of the family, based on phylogenetic relationships and morphological distinctiveness, (3) to provide a phylogenetic framework for effectively and efficiently studying natural groups in future work, and (4) to use phylogenetic relationships to better understand character evolution and biogeography within the family.

# Materials and Methods

# Taxon sampling

Taxon sampling and outgroups are based on previous molecular phylogenetic studies of Samydaceae (Alford, 2005), which included sampling for three plastid regions (*ndhF*, *trnL*, and *trnL-F*) for 11 of the 14 recognized genera and 26 of the 256 recognized species. Sampling was augmented by adding 74 species, including 23 Old World species. Data were generated for the three genera missing in Alford's (2005) study, *Ophiobotrys*, Osmelia, and Pseudosmelia. Sampling includes all major morphological and biogeographical representatives of Casearia and Laetia, two heterogeneous genera. Outgroups include Lacistema aggregatum (Lacistemataceae), Lacistema robustum (Lacistemataceae), Lozania pittieri (Lacistemataceae), Hasseltia floribunda (Salicaceae), Prockia crucis (Salicaceae), Carpotroche longifolia (Achariaceae), Kiggelaria africana (Achariaceae), and Scyphostegia borneensis (Scyphostegiaceae), all from closely related families of the Malpighiales and some of which were formerly placed in Flacourtiaceae together with Samydaceae. Outgroup taxa and close relatives were determined based on Chase et al. (2002), Alford (2005), and Wurdack and Davis (2009). Carpotroche longifolia and Kiggelaria africana (Achariaceae) were considered the global outgroups for the analysis.

### DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from 0.2–1.0 g of herbarium material or of fresh or silica-dried leaf material using a Qiagen DNeasy Plant Mini Kit (Cat. No. 69104, Qiagen, Hilden, Germany) following the manufacturer's protocol, except that tissue was crushed at ambient temperature in extraction buffer with a mortar and pestle. For some herbarium specimens, modifications were made to the Qiagen DNeasy kit to enhance DNA yield, following Costa and Roberts (2014). Samydaceae are known to have phenolic compounds (e.g., Ashik et al., 2006; Rayanil, 2012), and some samples had a dark-colored final elute. Those samples, as well as some other DNA extractions, were never successful in DNA amplification, even though quality and quantity of the DNA was good as determined by spectophotometer. An additional washing step was done for those samples using wash buffer W2 to remove phenolic compounds which tend to persist in the elute, inhibiting subsequent DNA amplification. At the amplification stage, a PCR additive composed of trehalose, bovine serum albumin (BSA), and polysorbate 20 (Tween20) (Samarakoon et al., 2013; CHAPTER 6) was added to the PCR mixture to overcome the effects of PCR inhibitors. Sometimes dilution series (1:10, 1:100, DNA:water) were also used to overcome PCR inhibition. However, the above methods were incapable of completely overcoming the effect of PCR inhibitors in all cases.

Previous analyses based on plastid, nuclear, and mitochondrial regions have shown enough variation to confidently determine relationships among families within Malpighiales (Alford, 2005; Chase et al., 2002; Wurdack and Davis, 2009; Davis et al., 2002, 2005). For analyses here, though, in order to obtain better resolution within the family and within genera, highly variable regions were selected. Data were collected for five plastid loci and two nuclear loci. Plastid regions include the non-coding *trnL* intron, *trnL-F* intragenic spacer, intergenic spacer for photosystem II protein D1 gene and flanking *tRNA-His* gene (*psbA-trnH*); and the coding gene for a subunit of nicotinamide dehydrogenase (3' end of *ndhF*) and *maturase K* gene and flanking 3' *trnK* intron region (*matK-trnK*). Nuclear regions include part of the granule bound starch synthase (*GBSSI*) gene, which is commonly called waxy in grasses, and embryo defective 2765

(EMB2765). Primers used in this study are listed in Table 1.1.

Table 1.1

Primers used for PCR amplification and sequencing. References are given for previously published primer sequences. Primers for GBSSI were developed herein using the tools in Phytozome (Goodstein et al., 2012) across the Populus, Manihot, and Ricinus genomes.

Region	Primer Name	Primer Sequence $(5'-3')$ , Reference
EMB2765	EMB2765ex F2 EMB2765ex99R	TATCCAAATGAGCAGATTATGTGGGA TTGGTCCAYTGTGCWGCAGAAGGRT Wurdack and Davis, 2009
GBSSI	GBSSI_R GBSSI_F	GTTCCATATCCCGCATAGCATGC ACTGTRAGCCCTTACTATGC
matK	matK_1176F trnK_2_R	CAATTCATTCAATATTTCCTT AACTAGTCGGATGGAGTAG Johnson and Soltis, 1994; Kelly Steele in Xiang et al., 1998
ndhF	ndhF_5.5F ndhF_10.2R	TTAGTRAYAGTYGGTTGTATTCACC CCATCRATNACTCGYCYATCAAAAA Nyffeler in Davis et al., 2001
trnH-psbA	trnH_F psbA_R	CGCGCATGGTGGATTCACAATCC GTTATGCATGAACGTAATGCTC Tate and Simpson, 2003; Sang et al., 1997
trnL-trnF	trnLF_e trnLF_f	GGTTCAAGTCCCTCTATCCC ATTTGAACTGGTGACACGAG Taberlet et al., 1991
trnL	trnL_c trnL_d	CGAAATCGGTAGACGCTACG GGGGATAGAGGGACTTGAAC Taberlet et al., 1991

DNA was amplified by using a Thermo PCR Sprint Thermal Cycler (Thermo Electron Corporation, Milford, MA). Reactions were performed in a final volume of 50  $\mu$ L, with the sample containing 8  $\mu$ L sterile H<sub>2</sub>O, 10  $\mu$ L of TBT-PAR enhancer (Samarakoon et al., 2013), 25  $\mu$ L Taq polymerase (Takara Bio Inc. Otsu, Shiga, Japan), and 2.5  $\mu$ L of each primer at a 50 nmol concentration. For shorter regions like *trnL-F*, *trnH-psbA*, and *EMB2765*, PCR amplification was performed with an initial denaturation at 95°C for 3 minutes followed by 35 cycles of denaturation at 98°C for 10 s, primer annealing at 55°C for 30 s, and primer extension at 72°C for 40 s. For longer regions, amplification was performed with an initial denaturation at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for 30 s, and primer extension at 72°C for 30 s, and primer extension at

PCR amplification products were separated in 1% agarose gel and were subsequently stained with ethidium bromide to visualize. Amplified products were purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and DNA sequencing was performed at Eurofins MWG Operon, Huntsville, Alabama. *Sequence Editing Alignment and Phylogenetic Analyses* 

DNA sequence chromatograms were proofed, edited, and assembled into contigs using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were initially aligned using ClustalX 2.0 (Thompson et al., 1997) and then corrected manually using WinClada (Nixon, 2002). Sequence alignment introduced gaps that were later coded as binary presence-absence or multiple character states (Simmons and Ochoterena, 2000). Indels associated with homopolymer regions were not coded. Due to the extensive homopolymer regions, homology assessment in the non-coding regions (*trnL*, *trnL-F*, and *trnH-psbA*) was difficult, and alignment was sometimes ambiguous. Autapomorphic and parsimony uninformative characters were not scored, and were excluded along with gaps prior to analysis.

Parsimony analyses were performed using NONA (Goloboff, 1993), as implemented in WinClada (Nixon, 2002). Analyses were conducted for individual DNA datasets, combined plastid DNA datasets, combined nuclear DNA datasets, and combined datasets of both plastid and nuclear data (Nixon and Carpenter, 1996). Searches began with 10 sequential rachets (Nixon, 1999), with 200 iterations holding one tree per iteration at a 10% random constraint level, followed by subsequent heuristic searches based on the ratchet tree files, holding a maximum of 1000 trees. Clade stability for the parsimony analyses was estimated with 1000 jackknife replicates (Farris et al., 1996), where each replicate consisted of 10 random addition TBR searches, holding 2 trees per replicate.

The combined matrix of *ndhF*, *matK*, *EMB2765*, and *GBSSI* was also analyzed using the methods of Bayesian inference (BI) and Maximum Likelihood (ML). Prior to analysis, the best fit substitution models for the data matrices were determined using the AIC rankings, as implemented in jModelTest 2.1.4 (Guindon and Gascuel, 2003; Posada, 2008; Darriba et al., 2012). The best fit model selected for the combined data set was HKY + I + G. For the ML inference, four separate tree searches were performed using GARLI v. 2.0 (Zwickl, 2006), applying the HKY+ I + G model. Additionally, to assess clade support, 1000 ML bootstrap replicates (Felsenstein, 1985) were run in GARLI v. 2.0 (Zwickl, 2006). All four replicates resulted in the same tree topology. Consensus trees were calculated with Sumtrees from DendroPy 3.12.0 (Sukumaran and Holder, 2010).

Bayesian inference was performed using MrBayes v.3.2.2 (Ronquist et al., 2012). Two independent Bayesian analyses were run from different random starting trees for 100,000,000 generations, sampling every 1000 generations, and 25% of the sampled trees were discarded as burn-in. The majority-rule consensus tree containing the posterior probabilities of the phylogeny was determined from 10,000 trees. The average standard deviation values were less than 0.01. Both ML and BI trees were displayed using FigTree v1.4.0 (Drummond and Rambaut, 2007).

### Divergence Times Estimation

Because *Casearia* is a genus which has high species richness and is broadly distributed in both the Neotropics and Paleotropics, the phylogeny and inferred divergence times were estimated in order to infer timing of major radiations and geographical migrations. Divergence times were estimated by analyzing the sequences of three DNA regions (*ndhF*, *matK*, and *GBSSI*) from 24 ingroup taxa and two outgroup taxa. A Bayesian Markov chain Monte Carlo (MCMC) approach was taken simultaneously to estimate the phylogenic history and divergence times by using Bayesian molecular dating analysis, BEAST v1.7.5 (Drummond and Rambaut, 2007). Instead of fossil calibration points, which cannot be placed confidently in the phylogeny within the family, age constraints for the family divergence time were based on those inferred in a broader analysis of the order Malphigiales, which was based on broader taxonomic sampling and several fossil calibrations (Wurdack and Davis, 2009). Accordingly, the Salicaceae branch was calibrated to 80.1–91.9 Mya (95% confidence interval). Here divergence times were estimated using relaxed molecular clock (uncorrelated lognormal; Drummond et al., 2006), and a Yule branching process with a

uniform prior was adopted, which is appropriate for divergent rather than reticulate relationships. The GTR+I+G substitution model from jModelTest was used as the best fitting model. The final topology was estimated by combining four independent iterations of Markov chain Monte Carlo (MCMC), each with 50,000,000 generations sampling every 1000 generations. Convergence was checked in Tracer v1.5 and the files were combined using Log Combiner v1.7.5. All post-burn-in trees yielded an effective sample size (ESS) of >200 for all model parameters. Summary trees were generated using TreeAnnotator v1.7.5. (Tracer v1.5 and TreeAnnotator v1.7.5 are part of the BEAST software package). Final trees were displayed using FigTree v1.4.0 (Drummond and Rambaut, 2007).

## Results

Good amplification and sequencing were obtained for plastid *trnL*, *trnL-F*, *trnH-psbA*, *matK*, and *ndhF* and for nuclear *GBSSI* and *EMB2765*. In addition, good amplification and sequences were obtained for mitochondrial *matR* and *nad6*, although limited sampling was performed due to low variation. Phylogenetic utility of the nuclear internal transcribed spacer (*ITS*) and plastid *ncpGS* and *trnS-trnG* intergenic spacer were also tested. *ITS* was commonly confounded by contamination, *ncpGS* suffered from poor amplification, and *trnS-trnG* had two homopolymer regions, resulting in short, good reads, where the middle third was often of poor quality and subsequently missing for contiguous sequences. Of the well-sampled regions, plastid *trnH-psbA* was difficult to align even within the family, plastid *trnL* and *trnL-F* were alignable but yielded little parsimony informative variation within the family, and at least two copies (as well as within-copy, presumably allelic, polymorphism) of nuclear *EMB2765* were recovered.

For *EMB2765*, the most commonly amplified copy was used for broader analyses, and polymorphic sites were retained and coded as polymorphisms. For these reasons, the DISCUSSION (below) will be primarily based upon analyses of plastid *matK* and *ndhF* and nuclear *EMB2765* and *GBSSI* DNA data. These regions were confidently aligned (=confident homology assessment) and variable enough to resolve many key relationships. *Ophiobotrys, Osmelia,* and *Pseudosmelia,* genera not previously sampled, were successfully amplified and sequenced for only one region each, lamentably only *Pseudosmelia* for one of the cleanly aligned regions. Characteristics of the final datasets and statistics from the parsimony analyses are given in Table 1.2.

# Table 1.2

Summary of parsimony statistics for individual plastid, nuclear, and combined matrices. Combined matrix contained plastid matK and ndhF and nuclear EMB2765 and GBSSI.

	EMB276	5 GBSSI	matK	ndhF	trnH-psbA trnL-F		trnL	cpDNA	nrDNA Combined	
Number of taxa	31	38	46	53	41	65	55	47	44	39
Number of characters in aligned matrix	2824	771	1286	894	673	578	813	3727	1283	3706
Parsimony- informative characters	126	219	222	182	110	95	110	494	349	622
% missing characters		7	32	13	44	34	23	25	66	22
Number of MP trees	277	5	500	581	102	1041	1025	1024	39	61
Length of MP tree	234	496	410	447	533	196	234	1480	824	1311
CI	63	60	67	54	47	65	61	60	56	61
RI	78	75	85	73	58	78	70	75	70	74

The phylogenies yielded several consistent patterns (Figures 1.4 and 1.5,

Appendices A–J), across both sampled DNA regions and analytical techniques

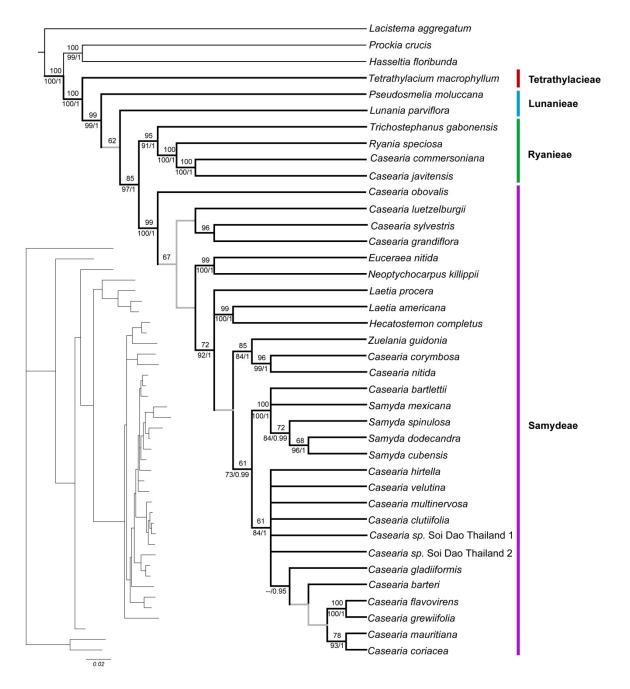
(parsimony, ML, BI). The Bayesian inference tree and Maximum Likelihood tree for combined *matK*, *ndhF*, *EMB2765*, and *GBSSI* is highly congruent with the most parsimonious tree (Figure 1.4, Appendices I and J). Posterior probabilities indicate high support for most of the clades. In the Bayesian tree, the large clade with *Casearia* and other nested genera is better resolved than in the most parsimonious tree.

The family itself was recovered as monophyletic. *Tetrathylacium* is sister to the other genera, *Lunania* is then sister to the remaining genera, and then there was a clade of Trichostephanus + Ryania + Casearia sect. Piparea (C. javitensis and C. commersoniana sampled here) and a clade of other *Casearia* with *Euceraea*, *Neoptychocarpus*, *Samyda*, Laetia, Hecatostemon, and Zuelania nested within, often with poor internal resolution. *Neoptychocarpus* + *Euceraea* were often recovered together; *Laetia americana* (sect. Laetia) and Hecatostemon were often recovered together; Casearia nitida and C. corymbosa (sect. Casearia informal group 'Nitidae') and Zuelania were often recovered together; Casearia bartlettii (sect. Casearia informal group 'Guidonia') and C. oblongifolia (sect. Casearia informal group 'Aculeatae') were often recovered with Samyda; and all Old World taxa were often recovered in a single clade. Unfortunately, within the broader *Casearia* clade, jackknife, bootstrap, and posterior probability values were often low. However, among Old World taxa, C. grewiifolia and C. flavovirens were often recovered together, and C. coriacea and C. mauritiana were recovered together, both pairs sharing similar geographical distributions, southeast Asia and the Mascarene Islands, respectively.

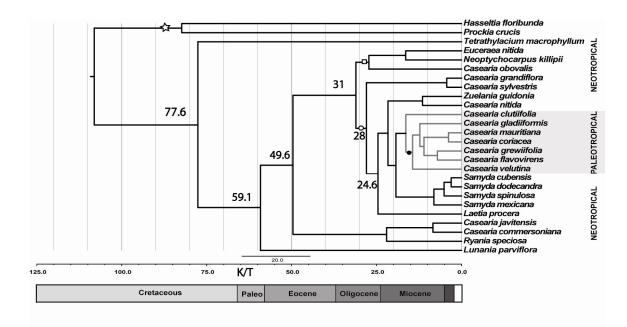
No significant incongruence between individual consensus trees was found except with the *trnH-psbA* region and with some taxa in results from the *EMB2765* region. The

plastid region *trnH-psbA* showed extensive variability and multiple homopolymer regions, and were thus not aligned well. Therefore, the resulting tree probably does not reflect a realistic phylogeny. Three Old World *Casearia* spp. (*C. flavovirens*, *C. velutina*, and *Casearia* sp. from Madagascar) appear as sister to *Prockia crucis* (Salicaceae) in the full *EMB2765* phylogeny, and this highly incongruent position is likely due to a duplication event in which a different paralog has been amplified. A taxonomic study across a range of Malpighiales and distant relatives suggested non-functional pseudogene copies of *EMB2765* (Wurdack and Davis 2009) which can be amplified by same primer. Later Andrea et al. (2013) confirmed the presence of at least two copies of *EMB2765* in a study based on *Hypericum* (Hypericaceae), and they used a modified EMB primer to recover orthologous copies of this marker.

The phylogeny from the divergence times estimation is congruent with the broader parsimony, ML, and BI analyses (Figure 1.5). The divergence times estimation indicates that *Tetrathylacium* diverged from other Samydaceae during late Cretaceous (mean 77.6 Mya, 95% HDP: 33–133 Mya). *Lunania* diverged from the remaining clades during late Paleocene (mean 59.2 Mya, 95% HDP: 25.9–102.9 Mya), and the clade of *Trichostephanus*, *Ryania*, and *Casearia* sect. *Piparea* diverged from the other *Casearia* (and nested genera) during the mid-Eocene (mean 49.6 Mya, 95% HDP: 21.5–86.4 Mya). Within the broader *Casearia* clade, the first divergence occurred during the mid-Oligocene (mean 31.1 Mya, 95% HDP: 14–52.7 Mya), followed by a close succession of other cladogenetic events. The Old World *Casearia* diverged from other *Casearia* during the mid-Miocene (mean 16.4 Mya, 95% HDP: 6.9–28.9 Mya).



*Figure 1.4.* Strict consensus tree of 61 most parsimonious trees and Bayesian phylogram with branch lengths, constructed from combined plastid (*ndhF* and *matK*) and nuclear (*EMB2765* and *GBSSI*) data. L=1311, CI=61, RI=74. Jackknife values are above the branches; ML bootstrap values and Bayesian posterior probability values shown below branches. Branches with no numbers are not supported by > 50% jackknife value, ML bootstrap >70%, or Bayesian posterior probability values > 0.95.



*Figure 1.5.* Divergence times estimates chronogram from BEAST for Samydaceae. All posterior probabilities were above 50%. A square indicates posterior probability of 0.51, the filled circle 0.68, and the open circle 0.81. No mark indicates posterior probabilities > 99%. The scale bar indicates time in million years. Bars correspond to 95% HPD intervals. Star shows calibration point. Gray branches show the paleotropical divergence.

# Discussion

Analyses of individual markers as well as of combined data strongly support the monophyly of the family, affirming earlier phylogenetic studies (Alford, 2005, in prep.). *Tetrathylacium* is sister of the rest of the family with high resolution (jackknife support=100%). *Tetrathylacium*, a genus of two species endemic to the Neotropics, differs from other genera in the family in lacking arils and pellucid-punctations. Within the other clade, *Lunania* is sister to the other genera. Morphologically, *Ophiobotrys*, *Osmelia*, and *Pseudosmelia* are similar to *Lunania* in that they have 3(–5)-veined leaves, although much more weakly so, and inflorescences of long spike-like racemes. Only one region for each of these genera, though, was amplified, and their placements are still ambiguous. In the *matK* phylogeny, a divergence to *Pseudosmelia* occurs before *Lunania*,

but the clade of *Lunania* plus other genera is poorly supported (52% jackknife). *Ophiobotrys* is placed with Old World *Casearia* in *trnL* and *trnL-F* phylogenies, but informative characters were few. Morphologically, *Osmelia* bears great resemblance to *Antidesma* (Euphorbiaceae; Hoffmann, 2005), and even though the current analysis could not place *Osmelia*, *Pseudosmelia*, and *Ophiobotrys* with great confidence, their placement within Samydaceae is affirmed.

All analyses indicate that *Casearia* Jacq. as commonly circumscribed is not monophyletic. One section of *Casearia*, *C*. sect. *Piparea* (3 spp.), is more closely related to *Ryania*, and the other sections of *Casearia* are in a clade among which are nested several small genera, including *Hecatostemon* (1 sp.), *Samyda* (9 spp.), *Zuelania* (1 sp.), and most of the putatively polyphyletic *Laetia* (7 spp., including the type species, *L*. *americana* L.).

Data strongly support the placement of *Casearia* sect. *Piparea* (*C. javitensis*, *C. commersoniana*, and *C. spruceana*) with *Ryania*. *Trichostephanus*, a rare African genus whose family-level placement was uncertain until recently (Alford, 2005, in prep.), is sister to *Ryania* plus *Casearia* sect. *Piparea*. This group is well-supported morphologically and anatomically. Morphologically, these taxa have intrastaminal staminodes, a divided style, and often a stipitate ovary/fruit. Anatomically, these taxa are characterized by having very long and wide rays in the wood, readily visible even to the naked eye (Miller, 1968). This group is also characterized by elongate (non-spherical) pollen grains with equatorially elongate endoapertures (*C. javitensis, Ryania*: Keating, 1973; *Trichostephanus*: Hallé and de Wilde, 1978) Given the strong and consistent molecular and morphological evidence, the genus *Piparea* should be reinstated for

species placed in *Casearia* sect. *Piparea*. One other group, *Laetia* sect. *Scypholaetia* (3 spp.), also likely belongs to this clade. They lack staminodes, but share the features of wood anatomy, style, and ovary/fruit. Unfortunately, DNA data were not obtained for a representative of this group.

*Euceraea* and *Neoptychocarpus* always resolve as sister groups. Both have 4merous flowers with alternating stamens of two different lengths. *Neoptychocarpus* is dioecious, and the sexuality of *Euceraea* is poorly known and perhaps mixed. In some trees, *Euceraea* and *Neoptychocarpus* are nested within a broader clade of *Casearia*, but with that internal position having low jackknife/ML bootstrap/PP support. In other trees, *Euceraea* and *Neoptychocarpus* are part of a polytomy. Because of the distinctive morphology of these genera relative to the other *Casearia* (and nested genera), these genera are conservatively held separate here.

The most speciose clade consists of *Casearia* (minus section *Piparea*), *Samyda*, *Laetia* (sects. *Laetia* and *Casinga*), *Hecatostemon*, and *Zuelania*. Within this clade there is little resolution, even in the highly variable DNA regions sampled, hinting at a rapid radiation. This clade includes both New World and Old World species. Within this clade, though, there are several consistent, but small, pairs or groups of taxa. *Casearia grewiifolia* and *C. flavovirens* are recovered together; morphologically they are united by large fruits, and geographically they are both found in south-central and southeastern Asia. *Casearia coriacea* and *C. mauritiana* are recovered together; geographically they are both found in the Mascarene Islands of the Indian Ocean. *Laetia americana* and *Hecatostemon completus* are recovered together, and they share pellucid-punctate sepals (as well as leaves). *Casearia* sect. *Casearia* informal group 'Nitidae,' represented by *C.* 

*nitida* and *C. corymbosa* in these trees, informal group 'Ilicifoliae,' represented by *C. aquifolia*, and *Zuelania* are recovered together; these share a Central American and West Indies geography and many representatives have corymbiform inflorescences. Finally, *Samyda* is recovered with *Casearia* sect. *Guidonia* (*C. bartlettii*) and *C. sect. Casearia* informal groups 'Aculeatae' and 'Decandrae,' represented by *C. oblongifolia* and *C. emarginata*, respectively. *Casearia* sect. *Guidonia* exhibits much fusion of the androecium, like *Samyda*, and its placement was expected (e.g., Kiger, 1972). The relationship with groups 'Aculeatae' and 'Decandrae' need further sampling for making broader generalizations. Group 'Decandrae' is the largest in the genus, is found throughout both the New World and Old World tropics, and, as far as determined, has no unique features in combination with *Samyda*. *Samyda* may or may not be monophyletic, with one of the Mexican species possibly related to other *Casearia*.

# Divergence Times Estimates

Tropical rainforest has been inferred to be the ancestral habitat for most of Malpighiales, and fossil evidence indicates that tropical rainforest appeared after the K/T event (Wolfe and Upchurch, 1987). The BEAST analysis indicates that the first divergence in Samydaceae, between *Tetrathylacium* and other genera, occurred approximately 77.6 Mya in the Campanian (Late Cretaceous), before the K/T boundary. Thereafter, divergence of *Lunania* from other ingroups occurred in the mid-Paleocene approximately 59.1 Mya, which coincides with the most pronounced global warming trend from the mid-Paleocene (59 Mya) to early Eocene (52 Mya) (Zachos et al., 2000). This global warming trend peaked in the early Eocene, which was followed by a 17 My long trend towards cooler conditions (Zachos et al., 2000). The origin of the *Ryania* + *Casearia* sect. *Piparea* clade (49.6 Mya) is associated with this peak warmth era. The origin of the broader *Casearia* clade occurred during last warm period, in the Oligocene (31 Mya), when thereafter global temperature is much colder and not as favorable for tropical plants. However, higher speciation around 24.6–28 Mya suggests that diversification in the *Casearia* clade occurred during this time period when global temperatures were lower due to the Antarctic ice sheets and when broadleaf forests declined (Zachos et al., 2001). A similar, although less pronounced, climatic optimum occurred during the mid-Miocene (~15 Mya; Zachos et al., 2001), resulting in expansion and diversification of rainforests (Moreley, 2000). Diversification in the Old World *Casearia* clade occurred during this time. The earliest divergence appears during the mid-Miocene (~16.4 Mya), followed by later divergence into the Afro-Malagasy land masses.

Clearly, the divergence of Samydaceae and the wide distribution of *Casearia* post-dates the break-up of Gondwana, as low latitude connection between these continents broke up around 105 Mya (McLoughlin, 2001); diversification of Samydaceae begins at ~77.6 Mya. The divergence of the Old World species of *Casearia* begins at about 15–20 Mya in the Miocene (Figure 1.5). Two competing hypotheses then are that (1) there was a "Laurasian migration" (Davis et al., 2001) or (2) there was a long-distance dispersal event (Renner, 2004). Both of those mechanisms have been proposed for taxa closely related to Samydaceae in the Malpighiales. By whatever mechanism, the most likely interpretation of the relationships is that species diversified in the Americas and then moved into Asia and finally to Africa and Madagascar. Diversification from Asia to Madagascar may have been along "Lemurian Stepping Stones" via India (Schatz, 1996) or from recent long-distance dispersal event(s). Regardless, more species from Africa and

Indo-Malaya and more robust phylogenetic relationships are needed to choose among these alternative hypotheses.

## Taxonomic Treatment

# Tribal Classification

Tetrathylacieae T.Samarakoon & M.H.Alford, trib. nov., TYPE: *Tetrathylacium* Poepp. Diagnosed by having four stamens, exarillate seeds, and leaves lacking pellucidpunctations or -lineations. One genus: *Tetrathylacium* Poepp.

Lunanieae T.Samarakoon & M.H.Alford, trib. nov., TYPE: *Lunania* Hook. Diagnosed by having leaves 3(–5)-veined from base, sometimes weakly so, and long spike-like racemes. Genera: *Lunania* Hook., *Ophiobotrys* Gilg, *Osmelia* Thwaites, *Pseudosmelia* Sleumer.

Ryanieae T.Samarakoon & M.H.Alford, trib. nov., TYPE: *Ryania* Vahl. Diagnosed by having staminodes located inside the whorl(s) of stamens, a 3-parted style, and dark-colored heartwood with rays that are visible to the naked eye (3000–7000 µm long). Genera: *Laetia* L. sect. *Scypholaetia* Warb., *Piparea* Aubl. (*=Casearia* Aubl. sect. *Piparea* (Aubl.) Benth.), *Ryania* Vahl, *Trichostephanus* Gilg.

Samydeae (Vent.) Dumort., TYPE: *Samyda* (=*Casearia*, nom. cons. prop.). Diagnosed by having usually having staminodes alternating with the stamens (inside in a few *Casearia*) or staminodes reduced or absent, usually a single style (3-parted in *Casearia* sects. *Gossypiosperma* and *Crateria*), and having short rays in the wood (300– 2000 µm). Genera: *Euceraea* Mart., *Neoptychocarpus* Buchheim, *Casearia* Jacq. (including *Hecatostemon* S.F.Blake, *Laetia* Loefl. ex L. sect. *Laetia*, *Samyda* Jacq., and *Zuelania* A.Rich.).

# Key to Tribes and Genera of Samydaceae

1. Stamens 4; leaves lacking pellucid-punctations or -lineations; seeds exarillate

1. Stamens (5–)6–numerous (or plants dioecious); leaves usually pellucid-punctate or lineate; seeds arillate

2. Leaves 3(–5)-veined, sometimes weakly so..... [Lunanieae]

3. Leaves 3(-5)-veined from base to near apex, exstipulate, calyx irregularly split at anthesis into 2–3(-5) lobes, anther dehiscence latrorse to extrorse; plants bisexual ...... *Lunania*3. Leaves weakly 3-veined at the base, pinnately veined above, stipulate, calyx with 4(-5) imbricate lobes, anther dehiscence introrse; plants dioecious

4. Sepals 5; stamens 5(-6); style basally 1, apically divided into 3
stigmas Ophiobotrys
4. Sepals $4(-5)$ ; stamens $8(-10)$ ; styles 3, separate, each with a
bilobed or flattened stigma Osmelia

2. Leaves pinnately-veined (or weakly 3-veined at base)

.....Trichostephanus

6. Plants bisexual; style divided at apex into 3–9 branches, each with a capitate stigma

7. Flowers subtended by bracts fused into an obvious cup; staminodes lacking ...... Laetia sect. Scypholaetia 7. Flowers subtended by free bracts; staminodes present 8. Stamens 10–25; pubescence, if present, simple .....Piparea 8. Stamens 30–70; pubescence simple or stellate ......Ryania 5. Disk lobes (if present) usually interstaminal, heartwood light-colored and wood rays small (mostly <2 mm high, no wider than 7 cells), leaves uncommonly drying black, style one (rarely lacking), usually with 1 capitate stigma or a 3-lobed stigma (except Casearia sylvestris and 9. Plants dioecious; calyx urceolate, fleshy ...... Neoptychocarpus 9. Plants bisexual or rarely/possibly polygamous or dioecious; calyx reflexed to cup-like, not fleshy 10. Inflorescences composite spikes ..... Euceraea 10. Inflorescences fascicles, glomerules, or cymes .....Casearia

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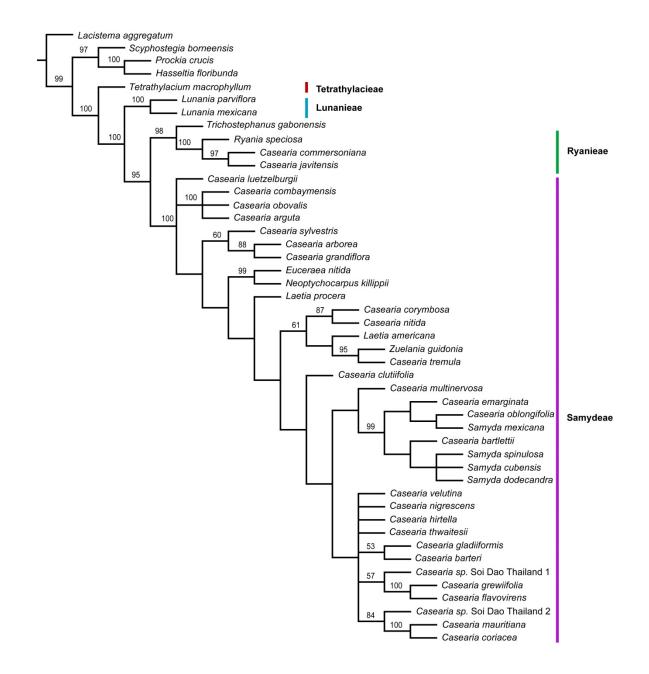
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## APPENDIX A

Strict consensus tree of 1024 most parsimonious trees of combined plastid (*ndhF*, *trnL*-*F*, *trnL*, and *matK*) data. L=1480, CI=60, RI=75. > 50% Jackknife values are above the branches.



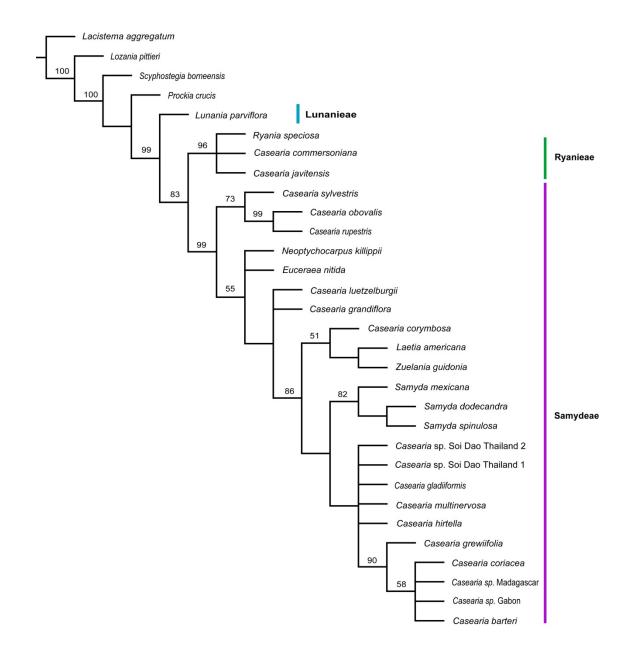
### APPENDIX B

Strict consensus tree of 39 most parsimonious trees of combined nuclear (*EMB2765* and *GBSSI*) data matrices. L=824, CI=56, RI=70. >50% jackknife values are above the branches.



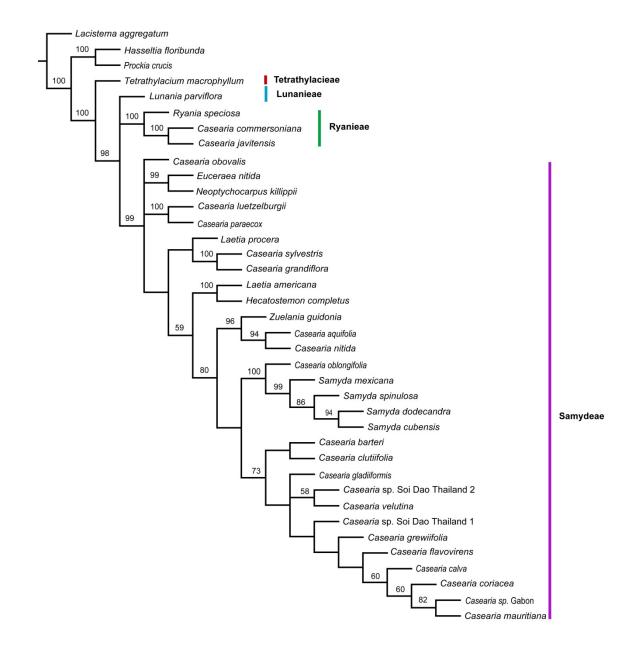
## APPENDIX C

Strict consensus of 277 most parsimonious trees of the *EMB2765* data set. >50% jackknife values are indicated above the branches. L=234, CI=63, RI=78.



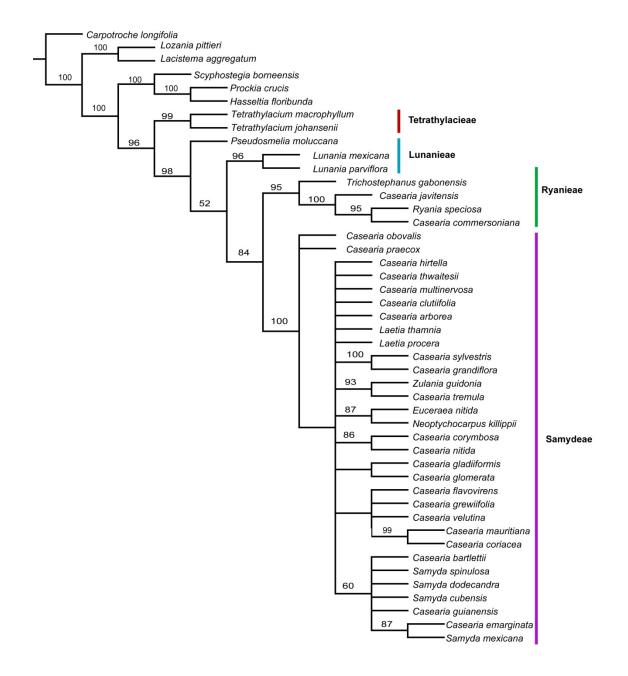
# APPENDIX D

Strict consensus of 5 most parsimonious trees of the *GBSSI* data set. >50% jackknife values are indicated above the branches. L=496, CI=60, RI=75.



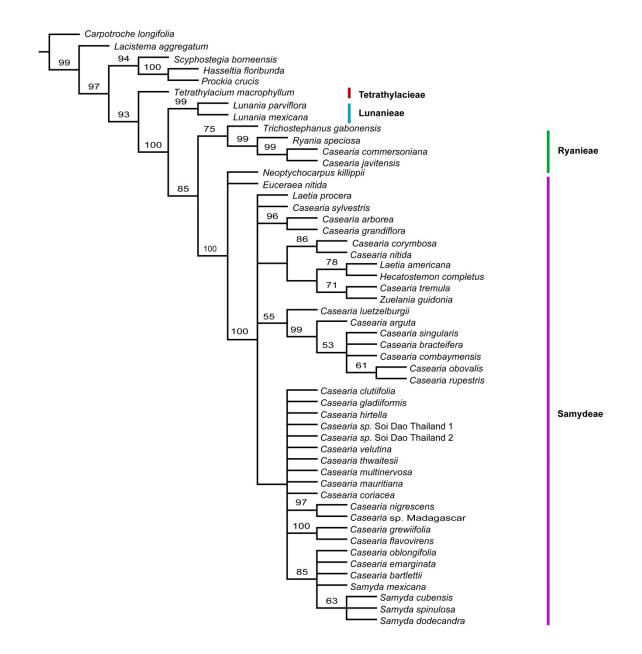
## APPENDIX E

Strict consensus of 500 most parsimonious trees of the *matK* data set. >50% jackknife values are indicated above the branches. L=410, CI=67, RI=85.



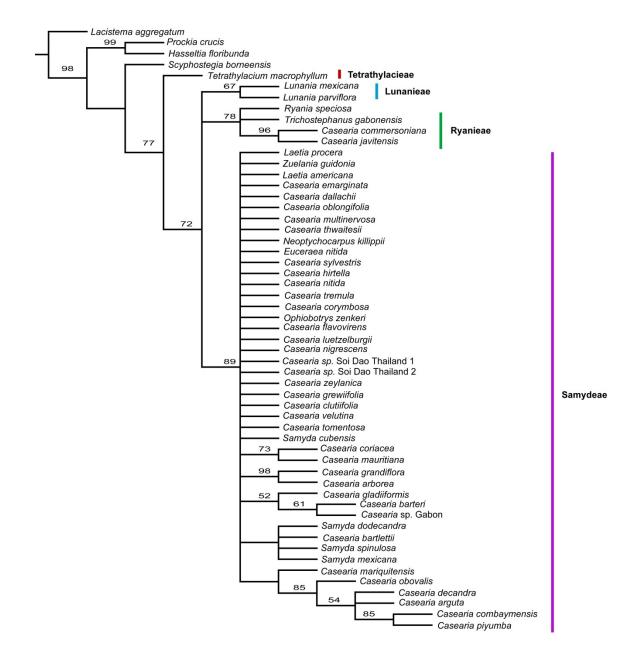
#### APPENDIX F

Strict consensus of 581 most parsimonious trees of the *ndhF* data set. >50% jackknife values are indicated above the branches. L=447, CI=54, RI=73.



## APPENDIX G

Strict consensus of 1025 most parsimonious trees of the *trnL* data set. >50% jackknife values are indicated above the branches. L=234 CI=61, RI=70.



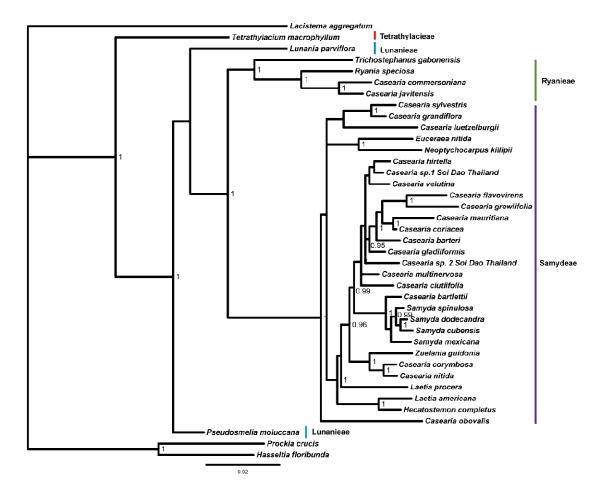
#### APPENDIX H

Strict consensus of 1041 most parsimonious trees of the *trnL-F* data set. >50% jackknife values are indicated above the branches. L=196, CI=65, RI=78.



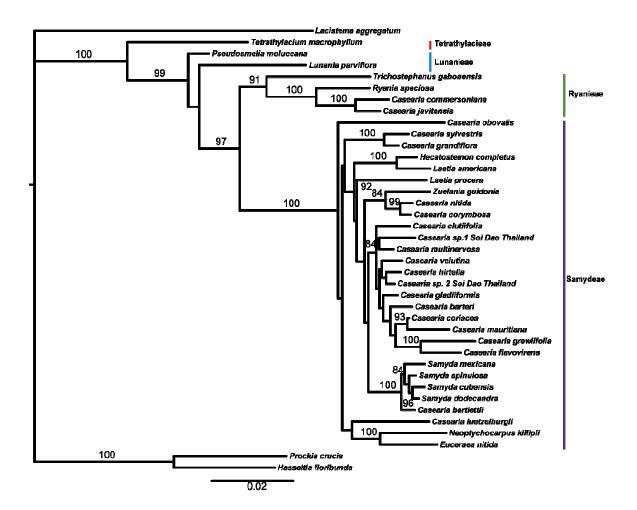
# APPENDIX I

Phylogram produced by Bayesian analysis of combined plastid (*matK*, *ndhF*) and nuclear (*EMB2765* and *GBSSI*) DNA data in MrBayes. Bayesian posterior probability (PP) values are above the branches.



## APPENDIX J

Phylogram produced by Maximum Likelihood analysis of combined plastid (*matK*, *ndhF*) and nuclear (*EMB2765* and *GBSSI*) DNA data in GARLI. Bootstrap values are above the branches.



#### CHAPTER II

# PROPOSAL TO CONSERVE THE NAME CASEARIA AGAINST LAETIA AND SAMYDA (SAMYDACEAE)

## Proposal

*Casearia* Jacq., Enum. Syst. Pl. 4: 21. Aug–Sep 1760. [Dicot.: Samyd.], nom. cons. prop. Lectotypus: *Casearia nitida* Jacq. (Bahama Fl. [Britton & Millspaugh] 25. 1920.)

(=) *Laetia* Loefl. ex L., Syst. Nat., ed. 10. 2: 1068, 1074, 1373. 7 Jun 1759, nom. cons., nom. rej. prop. Typus: *Laetia americana* L.

(=) Samyda Jacq., Enum. Syst. Pl.: 4, 21. Aug–Sep 1760, nom. cons., nom. rej. prop.
 Lectotypus: Samyda dodecandra Jacq. (Taxon 5: 194. 1956.)

Recent phylogenetic studies (CHAPTER 1) indicate that *Casearia* Jacq. as commonly circumscribed is not monophyletic. One section of *Casearia*, *C*. sect. *Piparea* (3 spp.), is more closely related to *Ryania* Vahl, and the other sections have nested among them several small genera, including *Hecatostemon* S.F.Blake (1 sp.), *Samyda* Jacq. (9 spp.), *Zuelania* A.Rich (1 sp.), and most of the polyphyletic *Laetia* Loefl. ex L. (7 spp., including the type species, *L. americana* L.). If one wishes to recognize the large group as a single genus, which we propose to do, the oldest available name is *Laetia*.

Unfortunately, *Laetia* as currently circumscribed consists of only 10 species, all Neotropical, and less than 30 names or combinations are currently available in *Laetia* for the approximately 215 species that we propose to circumscribe within this single genus. *Casearia*, on the other hand, is currently circumscribed to include about 200 species, is distributed in tropical and subtropical areas worldwide, and is a name employed in many large-scale revisionary and floristic works (e.g., Sleumer, 1954, 1980; Breteler, 2008; Samarakoon, CHAPTER 4).

The name *Casearia* also competes with *Samyda*. *Casearia* was published at the same time as the name *Samyda* (as conserved), in Jacquin's *Enumeratio Systematica Plantarum* (1760). Because early authors (e.g., Linnaeus, 1762; Lamarck, 1805) united *Casearia* Jacq. under *Samyda* Jacq., *Samyda* should then take priority (Art. 11.5, ICN). Furthermore, the name *Samyda* has been used as the source for the tribal name Samydeae and family name Samydaceae. However, *Samyda* as currently circumscribed consists of only nine species, all of which are neotropical, and already has a complex nomenclatural history (Sleumer, 1956; Kiger, 1972). Although more names and combinations are available in *Samyda* than in *Laetia*, over 100 combinations or new names would still be needed in order to provide names for all of the currently recognized species. If *Casearia* were conserved over both *Laetia* and *Samyda*, only 18 (12 combinations and 6 new names) are needed.

Thus, we propose that the name *Casearia* be conserved over *Laetia* and *Samyda*. Such conservation would result in greater stability of names, resulting in fewer needed combinations or new names and no name changes for the approximately 120 species occurring in the Old World.

#### Literature Cited

Breteler, F. J. 2008. A synopsis of *Casearia* Jacq. (Samydeae–Salicaceae) in West- and
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#### CHAPTER III

## NOMENCLATURAL NOVELTIES IN SAMYDACEAE

#### Abstract

Nomenclatural changes are presented here to bring generic circumscriptions in line with recent advances in the understanding of phylogenetic relationships within Samydaceae and with the proposed conservation of the name *Casearia* over *Samyda* and *Laetia*. Names of species formerly recognized in *Hecatostemon*, *Laetia* sect. *Laetia*, *Laetia* sect. *Casinga*, *Samyda*, and *Zuelania* are provided combinations or new names in *Casearia*, and the appropriate names for species of *Casearia* sect. *Piparea* when recognized as the separate genus *Piparea* are clarified. One new combination is also provided for *Piparea*.

## Introduction

Recent phylogenetic studies of Samydaceae (Alford, 2005; Samarakoon et al., in prep., CHAPTER 1) have indicated that the large pantropical genus *Casearia* Jacq. is not monophyletic. A genus of about 200 species, as commonly circumscribed (e.g., Sleumer, 1980), *Casearia* consists of trees, treelets, and shrubs that are common elements of tropical forests worldwide (e.g., Phillips and Miller, 2002). Infrageneric classification and species delimitation within the genus are difficult, as they are often based on microscopic characters of the flowers, most of which seldom exceed a few millimeters in length or width (e.g., Warburg, 1894; Gilg, 1925; Kiger, 1972; Sleumer, 1980; Alford, 2003, 2005). Related genera within Samydaceae, too, are distinguished from *Casearia* based on elements of floral morphology, often making their identification even to genus difficult, especially when vegetative or in fruit. These genera, which are phylogenetically nested

within Casearia, include Hecatostemon S.F.Blake (1 sp.), Samyda Jacq. (9 spp.),

*Zuelania* A.Rich. (1 sp.), and two sections of *Laetia* Loefl. ex L. (7 of the 10 spp.). These genera have been recognized apart from *Casearia* due to greater fusion of calyx and filaments (*Samyda*), greater number of stamens (*Hecatostemon, Zuelania*), lack or reduction of staminodes (*Laetia, Samyda*), and presence of a thick, peltate stigma with no style (*Zuelania*). Combinations and new names are provided here so that species formerly recognized in these genera nested within *Casearia* may now have names in and be recognized as *Casearia*.

Phylogenetic analyses (Alford, 2005; Samarakoon et al., in prep, CHAPTER 1) also indicate that one section of *Casearia*, *C*. sect. *Piparea*, is distantly related to other sections of *Casearia*, being more closely related to *Ryania*, *Trichostephanus*, and *Laetia* sect. *Scypholaetia*. This group was formerly recognized as the genus *Piparea*, and the appropriate names are summarized here so that its use may be easily reinstated. One combination was needed and is provided herein.

### Taxonomy

## Samyda

*Samyda* has been recognized as distinct from *Casearia* based on its greater fusion of sepals and filaments and its reduction of staminodes (Kiger, 1972). Kiger (1972: 57) foresaw the current problem when he wrote: "Save tradition and practicality, there are no really compelling reasons why *Samyda* could not be submerged in *Casearia* rather than held separate. On morphological, anatomical, palynological and, presumably, phylogenetic grounds, either disposition would be equally justifiable." Revealing their remarkable similarity, Kiger's key to the two genera takes up about  $1\frac{1}{3}$  pages of text and highlights combinations of characteristics (1972: 57–59).

- *Casearia macrantha* (Wilson) T.Samarakoon & M.H.Alford, comb. nov. Basionym:
   *Samyda macrantha* Wilson, Torreya 30: 72. 1930. TYPE: CUBA. Matanzas, in
   savannis Guamacaro (Limonar) and La Palma Sola, *Wright 1897* (lectotype:
   GOET; isolectotypes: BM, BREM, G, GH, K, MO, NY, P, S, US, W).
- *Casearia littoralis* T.Samarakoon & M.H.Alford, nom. nov. Basionym: Samyda microphylla Urb., Repert. Spec. Nov. Regni Veg. 20: 307. 1924, non Casearia microphylla Eichler, Fl. Bras. (Martius) 13(1): 474, t. 96. 1871. TYPE: CUBA. Oriente, Nipe Bay, near Antilla, Ekman 7323 (holotype: S; isotype: NY [fragment]). Etymology: This species is found in littoral forest in Cuba.
- Casearia yucatanensis (Standl.) T.Samarakoon & M.H.Alford, comb. nov. Basionym:
   Samyda yucatanensis Standl., Contr. U.S. Natl. Herb. 23: 842. 1923. TYPE:
   MEXICO. Yucatán, Mérida, Schott 603 (holotype: US; isotypes: BM, F, MO).
- *Casearia mexicana* (Rose) T.Samarakoon & M.H.Alford, comb. nov. Basionym: Samyda mexicana Rose, Contr. U.S. Natl. Herb. 5: 199. 1899. TYPE: MEXICO. Guerrero, Acapulco, Palmer 81 (holotype: US; isotypes: A, BM, F, GH, K, MICH, MO, NY).
- Casearia villosa (Sw.) T.Samarakoon & M.H. Alford, comb. nov. Basionym: Samyda
   villosa Sw., Prodr. [O. P. Swartz] 68. 1788. TYPE: JAMAICA. In the mountains,
   Swartz s.n. (lectotype: S; isolectotypes: C, G-DC, GOET [fragment], S, SBT).

*Casearia dodecandra* (Jacq.) T.Samarakoon & M.H.Alford, comb. nov. Basionym: *Samyda dodecandra* Jacq., Enum. Syst. Pl. 21. 1760. TYPE: ILLUSTRATION.
Pl. Amer. t. 146, f. 2. 1757. (See Kiger, 1972, for additional information.)

*Casearia kigeri* T.Samarakoon & M.H.Alford, nom. nov. Basionym: *Samyda glabrata*Sw., Prodr. [O. P. Swartz] 68. 1788, non *Casearia glabrata* Blume, Mus. Bot.
1(16): 253. 1851. TYPE. JAMAICA. Mountains of the southern part, *Swartz s.n.*(holotype: S [photos at A, NY]; isotypes: BM, G-DC, SBT). This name honors
Robert William Kiger (1940–), whose dissertation (1972) on *Samyda* provided the foundation for this and much other work in the family.

*Casearia lemkeana* T.Samarakoon & M.H.Alford, nom. nov. Basionym: *Samyda cubensis* P.Wilson, Torreya 30: 72. 1930, non *Casearia cubensis* Urb., Repert.
Spec. Nov. Regni Veg. 22: 91. 1925. TYPE: CUBA. *Wright 1896* (holotype: NY; isotypes: NY, S, US). Etymology: This name honors David E. Lemke (1953–), who synthesized pre-molecular research in Flacourtiaceae (now divided mostly among Achariaceae, Samydaceae, and Salicaceae) and provided a revised tribal classification of the family.

Casearia spinulosa (Vent.) T.Samarakoon & M.H.Alford, comb. nov. Basionym: Samyda spinulosa Vent., Choix Pl. 43, t. 43. 1808. TYPE: U.S.A. U.S. Virgin Islands. St. Thomas, Riedlé anno 1797 (holotype G-Vent; isotype(?): P).

Laetia

*Laetia* has been distinguished from *Casearia* based on its lacking staminodes and its having very little (or no) fusion of the calyx and stamens at the base (i.e., clearly hypogynous, rather than perigynous). Some *Laetia* have cymose inflorescences, much like *Casearia* sect. *Casearia* informal group 'Nitidae,' and phylogenetic analyses based on DNA place those groups together. One section of *Laetia*, *L*. sect. *Scypholaetia*, is not closely related to *Casearia* and is being moved to another genus (Alford and Dement, in prep.); these three species have bracts fused into an obvious cup and have a 3-lobed style, the latter a rare feature in *Casearia* (e.g., *Casearia* sects. *Crateria* and *Gossypiospermum*, ca. 6 spp.).

*Casearia ternstroemioides* (Griseb.) T.Samarakoon & M.H.Alford, comb. nov.
Basionym: *Laetia ternstroemioides* Griseb., Pl. Wright. (Grisebach) 156. 1860.
TYPE: CUBA. Oriente, Monteverde, *Wright 1107* (holotype: GOET; isotypes: BR, F, G, GH, K, MO, NY, P, W).

*Casearia americana* (L.) T.Samarakoon & M.H.Alford, comb. nov. Basionym: *Laetia americana* L., Syst. Nat., ed. 10: 1074. 1759. TYPE: COLOMBIA. Bolívar:
Cartagena, *Jacquin s.n.* (neotype [as "holotype," Sleumer, 1980: 239]: BM). (See Dorr and Wiersema, 2010, for additional information.)

*Casearia corymbulosa* (Spruce ex Benth.) T.Samarakoon & M.H.Alford, comb. nov.
Basionym: *Laetia corymbulosa* Spruce ex Benth., J. Proc. Linn. Soc., Bot. Suppl. 5(Suppl. 2): 83. 1861. TYPE: BRAZIL. Amazonas, ad oram meridionalem flum.
Amazonum adostium flum. Solimões, *Spruce 1599* (holotype: K; isotypes: BM, FI-Webb, K, M, P, W).

Casearia thamnia (L.) T.Samarakoon & M.H.Alford, comb. nov. Basionym: Laetia thamnia L., Pl. Jamaic. Pug. 31. 1759. TYPE: JAMAICA. Red hills above the Angels, P. Browne s.n. (holotype: LINN Cat. 680.1).

Casearia panamensis T.Samarakoon & M.H.Alford, nom. nov. Basionym: Laetia micrantha A. Robyns, Ann. Missouri Bot. Gard. 54: 190. 1967, non Casearia micrantha G.Don, Gen. Hist. 2: 52. 1832. TYPE: PANAMA. Darién, vicinity of Caná, Stern et al. 513 (holotype: MO; isotypes: G, GH, L, US). Etymology: The name recognizes the country of Panama, where this species is endemic.

*Casearia supra-axillaris* T.Samarakoon & M.H.Alford, nom. nov. Basionym: *Samyda procera* Poepp., Nov. Gen. Sp. Pl. (Poeppig & Endlicher) 3: 67. 1845, non *Casearia procera* A.C.Sm., J. Arnold Arbor. 31: 318. 1950. TYPE: BRAZIL.
Amazonas, Ega, *Poeppig 2853* (holotype: W; isotypes: F, G, GH, P). Etymology: The name highlights a characteristic of this common species: the distinct supra-axillary inflorescences.

Casearia suaveolens (Poepp.) T.Samarakoon & M.H.Alford, comb. nov. Basionym:
Samyda suaveolens Poepp., Nov. Gen. Sp. Pl. (Poeppig & Endlicher) 3: 66, t. 274.
1845. TYPE: BRAZIL. Amazonas, Ega, Poeppig 2912 (holotype: W; isotypes: F, P).

#### Hecatostemon

Hecatostemon has been distinguished from Casearia based on its having a large number of stamens (80–100), which are arranged in three series, and by having its single whorl of staminodes (ca. 15) located within the stamens, instead of alternating with them.
Casearia completa (Jacq.) T.Samarakoon & M.H.Alford, comb. nov. Basionym: Laetia completa Jacq., Enum. Syst. Pl. 24. 1760. TYPE: COLOMBIA. Bolívar, Cartagena, La Quinta, Jacquin s.n. (holotype: BM).

*Zuelania* has been distinguished from *Casearia* based on its greater number of stamens (20–40) and subsessile, large ("peltate") stigma. Sleumer (1980: 278) noted that it was "close to *Casearia*."

*Casearia peltata* T.Samarakoon & M.H.Alford, nom. nov. Replaced name: *Laetia guidonia* Sw., Prodr. [O. P. Swartz] 83. 1788. TYPE: JAMAICA. *Swartz s.n.* (lectotype: S; isolectotypes: BM, SBT). Etymology: The new name reflects the characteristic feature of the flower: its large, peltate stigma.

# Casearia

CASEARIA Jacq., Enum. Syst. Pl. 4, 21. 1760, nom. cons. prop. TYPE: *Casearia nitida* Jacq. (as designated in Bahama Fl. [Britton & Millspaugh] 25. 1920.)

Guidonia Mill., Gard. Dict. Abr., ed. 4. 1754, nom. rejic. (see Sleumer, 1956).

- Laetia Loefl. ex L., Syst. Nat., ed. 10. 2: 1068, 1074, 1373. 1759, nom. cons., nom. rej. prop. TYPE: Laetia americana L. (≡Casearia americana (L.) T.Samarakoon & M.H.Alford).
- Samyda Jacq., Enum. Syst. Pl. 4, 21. 1760, nom. cons., nom. rej. prop. TYPE: Samyda dodecandra Jacq. (as designated by Sleumer, H.O. 1956. Taxon 5: 194)
   (≡Casearia dodecandra (Jacq.) T.Samarakoon & M.H.Alford)
- *Anavinga* Adans., Fam. Pl. (Adanson) 2: 448. 1763. TYPE: *Anavinga lanceolata* Lam. (≡*Casearia tomentosa* Roxb.).
- *Iroucana* Aubl., Hist. Pl. Guiane 1: 328, t. 127. 1775. TYPE: *Iroucana guianensis* Aubl. (=*Casearia guianensis* (Aubl.) Urban).

- *Pitumba* Aubl., Hist. Pl. Guiane 2(Suppl.): 29, t. 385. 1775. TYPE: *Pitumba guianensis* Aubl. (*≡Casearia pitumba* Sleumer).
- Melistaurum J.R.Forst. & G.Forst. Char. Gen. Pl., ed. 2. 143. 1776. TYPE: Melistaurum distichum J.R.Forst. & G.Forst. (=Casearia melistaurum Spreng.).
- *Valentinia* Sw., Prodr. [O. P. Swartz] 63. 1788, non Heist. ex Fabr. 1763. TYPE: *Valentinia ilicifolia* Sw. (=*Casearia comocladifolia* Vent.).
- Vareca Gaertn., Fruct. Sem. Pl. i. 290. t. 60. 1788. TYPE: Vareca zeylanica Gaertn. (≡Casearia zeylanica (Gaertn.) Thwaites).
- Athenaea Schreb., Gen. Pl., ed. 8[a]. 1: 259. 1789. TYPE: Athenaea guianensis (Aubl.) J.F.Gmel. (≡Casearia guianensis (Aubl.) Urb.).
- Chaetocrater Ruiz & Pav., Fl. Peruv. Prodr. 61, t. 35. 1794. LECTOTYPE: Chaetocrater capitatum Ruiz & Pav. (=Casearia arborea (Rich.) Urban).
- *Clasta* Comm. ex Vent., Choix Pl. sub t. 47. 1803. TYPE: *Clasta fragilis* Comm. ex Vent. (=*Casearia fragilis* Vent.).
- Crateria Pers., Syn. Pl. [Persoon] 1: 485. 1805. SYNTYPES: Crateria capitata Pers. (=Casearia arborea (Rich.) Urb.) and Crateria fasciculata Pers. (=Casearia fasciculata (Ruiz & Pav.) Sleumer), nom. illeg., based on Chaetocrater Ruiz & Pav.
- Bigelovia Spreng., Neue Entdeck. Pflanzenk. 2: 150. 1821, nom. rejic., non Bigelovia
  Spreng., Syst. Veg. (ed. 16) [Sprengel] 1: 366, 404. 1824. TYPE: Bigelovia
  brasiliensis Spreng. (=Casearia obliqua Spreng.).

- *Lindleya* Kunth, Nov. Gen. Sp. [H.B.K.] v. t. 480 .1821, nom. nud., non *Lindleya* Kunth, Nov. Gen. Sp. [H.B.K.] 6: 239 (ed. qto.), 188 (ed. fol.). 1824 [5 Jan], nom. cons., nec Lindleya Nees, Flora 4: 299. 1821 [21 May], nom. rej.
- Antigona Vell., Fl Flumin. 186. 1829. TYPE: Antigona serrata Vell. (=Casearia lasiophylla Eichler).
- *Bedusia* Raf., Sylva Tellur. 11. 1838. TYPE: *Bedusia aromatica* Raf. (*=Casearia esculenta* Roxb.).
- Chetocrater Raf., Sylva Tellur. 149. 1838. SYNTYPES: Chetocrater hirta Raf. (=Casearia aculeata Jacq.), Chetocrater javitensis (H.B.K.) Raf. (=Casearia javitensis H.B.K.), and Chetocrater tinifolia (Vent.) Raf. (=Casearia tinifolia Vent.).
- *Zuelania* A.Rich. in Sagra, Hist. Phys. Cuba, Pl. Vasc. 10: 88. t. 12. 1845. TYPE: *Zuelania laetiodes* A.Rich (*=Casearia peltata* T.Samarakoon & M.H.Alford).
- *Corizospermum* Zipp. ex Blume, Mus. Bot. 1(16): 255. 1851, nom. inval. (not accepted by author). TYPE: *Corizospermum clutiifolium* Zipp. ex Blume (≡*Casearia clutiifolia* Blume).
- Sadymia Griseb., Fl. Brit. W.I. [Grisebach] 25. 1859. TYPE: Sadymia villosa (Sw.) Griseb. (≡Casearia villosa (Sw.) T.Samarakoon & M.H.Alford).
- Casinga Griseb., Abh. Königl. Ges. Wiss. Göttingen 9: 27, 29. 1861. TYPE: Casinga suaveolens (Poepp.) Griseb. ex Benth. (≡Casearia suaveolens (Poepp.)
   T.Samarakoon & M.H.Alford).
- *Hecatostemon* S.F.Blake, Contr. Gray Herb. (ser. 2) 53: 42. 1918. TYPE: *Hecatostemon dasygynus* S.F.Blake (=*Casearia completa* (Jacq.) T.Samarakoon & M.H.Alford)

Gossypiospermum (Griseb.) Urb., Repert. Spec. Nov. Regni Veg. 19: 6. 1923. TYPE: Gossypiospermum praecox (Griseb.) P.Wilson (≡Casearia praecox Griseb.).

Synandrina Standl. & L.O.Williams, Ceiba 3: 75. 1952. TYPE: Synandrina riparia Standl. & L.O.Williams (≡Casearia williamsiana Sleumer).

Tardiella Gagnep., Notul. Syst. (Paris) 15: 32. 1955. TYPE: Tardiella annamensis Gagnep. (≡Casearia annamensis (Gagnep.) Lescot & Sleumer).

## Piparea

- PIPAREA Aubl., Hist. Pl. Guiane 2: App. 30, t. 386. 1775. TYPE: Piparea dentata Aubl.
- Piparea dentata Aubl., Hist. Pl. Guiane 2(Suppl.): 31, t. 386. 1775. TYPE: FRENCH
  GUIANA. Montagne du Serpent près de Cayenne, Aublet s.n. (holotype: BM; isotype: W). =Casearia commersoniana Cambess., Fl. Bras. Merid. (A. St.-Hil.).
  2: 235. 1830.
- Piparea multiflora C.F.Gaertn., Suppl. Carp. 231, t. 224. 1807. TYPE: FRENCH GUIANA. Cayenne, Patris s.n. (holotype: TUB [?]; isotype: G-DC). =Casearia javitensis H.B.K., Nov. Gen. Sp. [H.B.K.] 5: 366, t. 479 (labeled as "Lindleya glabra"). 1823.
- Piparea spruceana (Benth. ex Eichler) T.Samarakoon & M.H.Alford, comb. nov.
  Basionym: Casearia spruceana Benth. ex Eichler, Fl. Bras. (Martius) 13(1): 486.
  1871. TYPE: BRAZIL. Amazonas. Prope São Gabriel de Cachoeira, Rio Negro,
  Spruce 2024 (lectotype: W; isolectotypes: B, GOET, K, P).

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#### CHAPTER IV

# A TAXONOMIC REVISION OF *CASEARIA* (SAMYDACEAE) IN SOUTH-CENTRAL ASIA

## Abstract

A taxonomic revision of *Casearia* (Samydaceae) in south-central Asia, including the area from Pakistan to Burma and south from the Himalayas to Sri Lanka, is presented. Thirteen species are recognized, nine of which are endemic to south-central Asia and four of which are more widespread, also occurring eastward into China, southeast Asia, and Malesia. Descriptions, taxonomic keys, distributional maps, and illustrations are provided for the species.

## Introduction

*Casearia* Jacq. (1760) is the most widely distributed and speciose genus of Samydaceae, a family which was recently resurrected from the former Flacourtiaceae (Alford 2003, 2005; Wurdack and Davis 2009) and now occasionally included in a broadly circumscribed Salicaceae (Chase et al. 2002; APG III 2009). Samydaceae are characterized by introse anther dehiscence, pellucid punctuations in the leaves, theoidtype deciduous leaf teeth, arillate seeds, lack of petals, and often presence of a hypanthium (Alford 2003, 2005). The genus *Casearia*, as most commonly circumscribed (e.g., Sleumer 1954, 1956, 1971, 1980, 1985), can be distinguished from other genera of Samydaceae by the presence of hermaphroditic flowers with a single series of staminodes (= "disk glands" sensu Sleumer) and a well-developed style, although phylogenetic data indicate that several small genera are nested within it (Samarakoon et al., in prep.; CHAPTER 1). The genus consists of shrubs to medium-sized trees and typically has fascicles or glomerules (rarely cymes) of non-showy, small flowers in the axils of the leaves. Infrageneric groups have been described based on inflorescence morphology, position of staminodes, number of stamens, and branching of the style (Sleumer 1980), the latter three characters often difficult to observe without careful dissection, given the small size of the flowers. Despite the non-showy flowers, however, *Casearia* often has showy fruits and/or brightly colored arillate seeds. The genus consists of about 200 species (about 75% of the family) and is found throughout the tropics, including Asia and the Pacific Islands (about 98 spp.), North and South America (about 75 spp.), Africa, Madagascar, and the Indian Ocean islands (16 spp.), and Australia (5 spp.). They are common elements in ecological or biodiversity plots in the tropics (Gentry 1992).

Due to the importance of small morphological features in species delimitation, classification, and keying, the number of species, and the wide distribution of the genus, no worldwide treatment of *Casearia* has been completed. Hermann Sleumer completed treatments of Neotropical, African, Malagasy, Australian, and Malesian species (Sleumer 1954, 1956, 1971, 1980, 1985), but he did not study species present in south-central Asia, including the area from Pakistan east to Burma and south to Sri Lanka, and no other comprehensive modern treatment is available. When specimens have been studied or regional checklists compiled, names have been drawn from old floras and out-of-date treatments which continue to perpetuate historical mistakes in the nomenclature and species boundaries based on little data. Attempts to better circumscribe species of *Casearia* by several taxonomists were far from complete due to reliance upon highly variable morphological characters, such as leaf morphology and pubescence, as well as lack of ample sampling and available herbarium material.

The region included in this study contains one of the world's 34 biodiversity hotspots, Sri Lanka and the Western Ghats of India. Biodiversity hotspots are defined as regions with >1500 endemic species of plants and <70% habitat remaining (Mittermeier et al. 2004). This hotspot consists of 189,611 km<sup>2</sup> of tropical and subtropical moist broadleaf forest, of which only 23% remains. Of the 5916 known plant species there, 3049 (52%) are endemic (Kumar et al. 2004), and thus the region is rightly targeted as a key area for conservation. One of the species included in this treatment, *Casearia wynadensis*, is listed as Vulnerable B1+2c ver 2.3 (IUCN 1998, 2014).

#### Taxonomic History

Western scientific study and publication about plants in Asia started in the 16th century with Dutch colonization, and thus the first record of *Casearia* is in *Horti Indici Malabarici* (1683) by Hendrik Adrian van Rheede, a Dutch surgeon who offered his services as a botanist to explore the Malabar coast (southwestern India) and Ceylon (Sri Lanka) to search for medicinal plants and herbs (Manilal et al. 1977). In his list of medicinal plants, *Casearia* was introduced as the genus *Anavinga*. There Rheede used that name, accompanied by a good illustration, to describe what is probably *C*. *graveolens* Dalz. (see below and CHAPTER 5). In a following volume of his book (1685), Rheede recognized another *Casearia* species as *Tsjerou kanneli*, which is here recognized as Roxburgh's *Casearia esculenta*.

Jean-Baptiste Lamarck (1783) first introduced the genus to modern (i.e., post-1753) systematics. He received a specimen of a plant from Pierre Sonnerat which he thought belonged to the same genus that Rheede described, and he gave it the name *Anavinga lanceolata*, now known as *Casearia tomentosa* Roxb. He included Rheede's original description under the name *Anavinga ovata*. Slightly before Lamarck's 1783 work, though, Nicolai Joseph Jacquin (1760) had used the name *Casearia* for New World *Casearia* species in his *Enumeratio Systematica Plantarum*, honoring Johannes Casearius (1642–1678), a missionary and church minister of the Dutch East India Company who helped to prepare the Latin version of the two volumes of Rheede's *Hortus Malabaricus*. Willdenow (1797) recognized the Indian *Anavinga* species as *Casearia*, noted that Jacquin's name preceded Lamarck's, and made the needed combinations.

Thereafter, with the rise of British East India Company, William Roxburgh (1751–1815), Nathaniel Wallich (1786–1854), Francis Buchanan-Hamilton (1762–1829), John Forbes Royle (1798–1858), Robert Wight (1796–1872), J. D. Hooker (1817–1911), and George Henry Kendrick Thwaites (1812–1882), who served as botanists for the Company, worked to identify and collect not only other species of *Casearia* but also other Indian plants in that region. Among them, Roxburgh (1751–1815), the Superintendent of the Royal Botanic Garden, Calcutta, and known as the "founding father of Indian botany" (Robinson, 2008), was the key botanist who worked for the East India Company. He prepared descriptions of 2579 Indian plants and had life-size paintings and dissections of each of the plants made by Indian artists for his *Flora Indica*. In *Flora Indica* (1832) Roxburgh recognized six species of *Casearia* in India, which is nearly half of the *Casearia* species currently known in the region.

Wallich also contributed to the collection of *Casearia* after Roxburgh. Wallich worked as Roxburgh's assistant from 1809, became Superintendent of the Royal Botanic Garden, Calcutta, in 1815 following Roxburgh's death, and served in this post until 1846. Wallich made a name for himself through his numerical list of dried specimens of plants in the East India Company's Museum (1828–1849), which had over 8500 specimens. He collected and described new plants, had them drawn and painted, and distributed specimens to the chief gardens and herbaria in Europe and North America. Many of those names are *nomina nuda*. All new names given by him for *Casearia* are synonyms of *Casearia tomentosa* or *C. graveolens* (or misidentified as *Casearia*). Nonetheless, Wallich made a large collection of *Casearia*.

Competition of collecting plants and naming new species during that time also led to future confusion in the taxonomy of this genus. A good collection of Sri Lankan *Casearia* was made by a British botanist and entomologist, Thwaites, who worked as a superintendent of the Royal Botanical Gardens at Peradeniya, Ceylon (Sri Lanka) (1849– 1880, from 1857 as Director). He documented new taxa in his Enumeratio Plantarum Zeylaniae (1864), but his new species and varieties are synonyms of *Casearia esculenta* Roxb. A more recent and well-curated collection of *Casearia* species from southeast Asia is located at the herbarium of the University of Michigan (MICH), which was supplemented by collections of Dr. Walter Koelz and Rup Chand. Walter Koelz was an American zoologist and anthropologist who was fascinated by natural history, ornithology, and botany, as well as Tibetan culture and artifacts. He collected plant and bird specimens from India, Tibet, Assam, Nepal, and Iran for the Himalayan Research Institute of the Roerich Museum, New York City. He collected plants in association with Rup Chand, an Indian Thakur who was introduced to the University of Michigan by him and later worked there for 40 years until he died in 1994. Chand and Koelz worked and traveled together for nearly 30 years, and each collected over 10,000 plant specimens,

including more *Casearia* from a wider geographical area in the Western Himalayas and borderlands of Western Tibet than any other botanists since.

Despite those contributions, collections of *Casearia* in south-central Asia are still scarce, precluding a good assessment of variation and detailed morphological study of all putative taxa. This revision presents an update of the genus *Casearia* in south-central Asia to clarify taxonomic problems by including nomenclatural justifications, updated species circumscriptions, taxonomic descriptions, illustrations, and known geographical distributions of each species and a robust taxonomic key to identify them.

#### Materials and Methods

The revision presented here is based on examination of 719 herbarium specimens, including type specimens, which were borrowed from BM, CAL, G, K, L, MICH, PDA, and US (abbreviations follow *Index Herbariorum*, Thiers 2014). Online or e-mailed images of several type specimens and historical collections were examined from CAL, K (http://apps.kew.org/herbcat/navigator.do), KUN, LIV (Royle collection), MH, and P-LAM (Lamarck collection, http://www.lamarck.cnrs.fr/herbier.php?lang=en). Digital images of drawings at Kew made for Roxburgh's *Flora Indica* were also viewed online (http://apps.kew.org/floraindica/home.do). Newly collected specimens were examined from Thailand and Sri Lanka, and 2 species were observed in the field in Sri Lanka to augment the morphological observations and to check the accuracy of the key. The geographic scope of the study covers the countries of Bangladesh, Bhutan, Burma, India, Nepal, Pakistan, and Sri Lanka. However, collections examined from Burma are few. Additional species may be expected there. According to *The Checklist of the Trees, Shrubs, Herbs and Climbers of Myanumar* (Kress et al., 2003), four species of *Casearia* 

are recorded in Burma, including *Casearia canziala* Wall., *C. lobbiana* Turcz., *C. esculenta* Roxb., and *C. andamanica* King. Here *Casearia canziala* Wall. is considered a synonym of *Casearia tomentosa* Roxb.; *C. lobbiana* is known to be distributed in the Malay Peninsula (Singapore, Malacca, Selangor, Negri Sembilan, Perak Penang), Riouw (P.Durian), and east coast of Sumatra (Sleumer, 1954), but no material was found from the study area in this study. *Casearia andamanica* is endemic to Andaman Islands, but according to Kress et al. (2003), it is also recorded from Taninthayi (a southern coastal region of Myanmar, adjacent to the Andaman Islands); again, no specimens were seen. For Burma, the treatment here may be used together with recent treatments for China (Yang and Zmarzty 2007) and Thailand (Sleumer 1985, Harwood and Tathana 2011).

Preliminary species circumscription was done by examining herbarium specimens borrowed from major herbaria, by thoroughly examining and recording vegetative and reproductive characters, and by sorting specimens into groups based on character discontinuity (cf. Phylogenetic Species Concept, Nixon and Wheeler 1990). Ideally, species differ in several putatively unrelated characters (e.g., vegetative and reproductive) that are not known in closely related groups to be strongly affected by environmental conditions (e.g., pubescence, Ehleringer 1984), which serve as evidence of their evolutionary independence (i.e., lack of gene flow with other such groups). Morphological measurements on herbarium specimens were carried out using a Zeiss Stemi 2000-C stereo-microscope and IP54 iGaging EZ Cal Digital Calipers or rulers. All measurements were based on dried material. Color data of fresh material, bark details, height of the plant, and other data were included in species descriptions when noted on collection labels. Geographical distribution maps were built by using the data present on herbarium specimen labels with SimpleMappr (http://www.simplemappr.net) (Shorthouse, 2010).

## Results

Morphological discontinuities provide evidence for the recognition of 13 species of *Casearia* in south-central Asia. *Casearia rubescens* and *C. tomentosa* are rather variable, and two historically used varieties are recognized here, too. *Casearia esculenta* and *C. zeylanica*, which are occasionally lumped together as a single species, are recognized as two distinct species which are distributed mainly in southern India and Sri Lanka.

#### Taxonomy

- CASEARIA Jacq., Enum. Syst. Pl. 4, 21. 1760. TYPE: *Casearia nitida* (L.) Jacq. (as designated by Sleumer, 1980).
- *Anavinga* Adans., Fam. Pl. (Adanson) 2: 448. 1763; Lam. Illustr. t. 355. 1823. TYPE: *Anavinga lanceolata* Lam. (*=Casearia tomentosa* Roxb.).
- Melistaurum J.R.Forst. & G.Forst., Char. Gen. Pl., ed. 2. 143. 1776. TYPE: Melistaurum distichum J.R.Forst. & G.Forst. (=Casearia disticha (J.R.Forst. & G.Forst.) A.Gray).
- *Vareca* Gaertn., Fruct. Sem. Pl. i. 290. t. 60. 1788. TYPE: *Vareca zeylanica* Gaertn. (=*Casearia zeylanica* (Gaertn.) Thwaites).
- Tardiella Gagnep., Notul. Syst. (Paris) 15: 32. 1955. TYPE: Tardiella annamensis Gagnep. (=Casearia annamensis (Gagnep.) Lescot & Sleumer).
- Additional generic synonyms used in other parts of the world may be found in Samarakoon and Alford (in prep., CHAPTER 3).

Shrubs, treelets, or trees, commonly 5–15 m tall and up to 30 cm dbh,

uncommonly up to 30 m tall and 70 cm dbh with buttresses up to 1 m high; vestiture of simple trichomes, sparse to dense, variable in length, density, and location, mostly white to light yellow, rarely rusty brown, commonly found on sepals and young parts, a few species completely glabrous; mature stems mostly rough with small bumps or fissures; young branches usually terete and lenticulate, sometimes compressed and quadrangular; branches infrequently thorny [none in study area]. Leaves simple, alternate, usually distichous, sometimes subdistichous, uncommonly spiraled, petiolate, stipulate, evergreen or sometimes deciduous; lamina chartaceous to membranaceous to coriaceous, pinnately veined, with veins broadly arching and anastomosing near margins, rarely obscurely 3-4 veined from the base, almost always with pellucid dots and/or lines that are visible against the light or at 10×; margin entire, crenate, serrate, denticulate, or rarely spiny [none in study area] and most young leaf margins with obvious deciduous, conical teeth, soon shed, leaving an almost entire or wavy margin; stipules small, scale-like, and often early caducous, rarely large and/or persistent. Inflorescences of few to many flowers in axillary sessile or pedunculed fascicles or glomerules, rarely with solitary flowers or in cymes [none in study area]; pedicels short, the flowers sometimes practically sessile, articulate, most articulations not visible due to the basal bracts; basal bracts of the inflorescence free, papery, scale-like, often ovate, often numerous and congested forming a persistent raised cushion or coral-like pad. Flowers bisexual and small, almost always less than 1 cm in length, usually perigynous, uncommonly hypogynous [none in study area]; sepals deeply (4–)5-lobed, greenish yellow or white, rarely pink, free and imbricate above, connate at the base, sometimes up to halfway, forming a shallow or deep floral

cup (hypanthium), never adnate to the ovary, persistent in fruit; petals absent; and roccium a single whorl of free or perigynous stamens, rarely several whorls (those formerly recognized as *Hecatostemon* and *Zuelania*, not in study area); stamens (6-)8-10(-12, -24)[-numerous in species formerly recognized in *Hecatostemon* and *Zuelania*]); anthers globose to ovoid, sometimes apiculate by a glabrous or barbate gland-like connective; staminodes (= "disk glands" sensu Sleumer) present or rarely absent (those formerly recognized as the New World genus *Laetia*), generally equaling the stamens in number, found in the same whorl with the stamens and alternating with them, very rarely fused to form an almost extra-staminal corona (those formerly recognized as the New World genus Samyda), otherwise very short and filaments appearing nearly free, staminode lobes triangular, clavate, or oblong, mostly hairy at the apex; pollen singly-shed, grains spheroidal to prolate, 3–5-colporate (see Keating 1973); gynoecium a single pistil with a 1-locular, superior ovary, usually with 2-4 parietal placentae, multi-ovulate with several (up to 12) or numerous ovules; ovules anatropous; style 1, or rarely 0 (formerly recognized as the New World genus Zuelania) or divided into 3 at the apex (subgenera Crateria and Gossypiospermum of the New World); stigma capitate, sometimes obscurely 3-lobed, rarely peltate (Zuelania). Fruits fleshy, but firm ("dry berry"), subglobose or ellipsoid, often with 3-6 ridges when fresh, dehiscing to (2-)3(-4) valves, sometimes tardily so, dehisced valves naviculate (boat-shaped); flower parts persistent at the base of the fruit; style remnants often persisting at the apex. Seeds generally ovoid or obovoid, few to numerous, glabrous or pubescent, completely to partially enveloped by a soft, often brightly colored (white, yellow, orange, red) and sometimes fimbriate aril;

testa crustaceous, often striated, albumen fleshy, cotyledons flat, embryo straight. x = 11 or 12, 2n = 22, 24, 44 (Grill, 1990).

A pantropical genus of about 195 species, or about 213 species when circumscribed to include *Hecatostemon*, *Laetia* (sect. *Laetia* and *Casinga*), *Samyda*, and *Zuelania* (see Samarakoon et al., in prep.; CHAPTER 1).

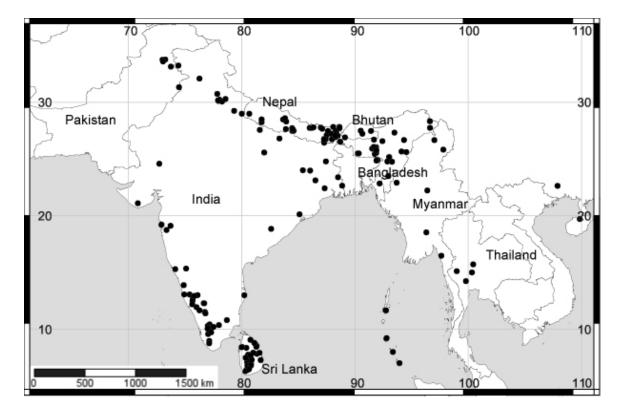


Figure 4.1. Distribution of Casearia in the study area.

Key to the Species of Casearia of South-Central Asia

- Mature leaves conspicuously publication to the naked eye or at 10× magnification, at least along the midvein and veins abaxially
  - 2. Mature leaves, petioles, and shoots densely pubescent on both sides, velvet-like
    - 3. Leaf lanceolate with an acuminate apex, acumen 1–1.5 cm; hairs rusty

golden-brown in color ..... C. wynadensis

- 3. Leaf elliptic with an acute apex; hairs white or not a rusty color

  - 4. Leaf base obtuse, infrequently acute; lower secondary veins diverging separately and clearly pinnate; leaf margin serrate, infrequently crenate; fruits small, 1–2.5 × 1–2 cm...... *C. tomentosa*
- 2. Mature leaves densely pubescent abaxially, at least along midvein and secondary veins, but glabrous or sparsely pubescent on upper side
  - Margin finely serrulate (about 8 teeth per cm); fruits small, 5–6 × 5–7 mm
     *C. vareca*
  - 5. Margin shallowly crenate, serrate, or almost entire; fruits large, 10–15 ×
    6–8 mm
- 1. Mature leaves apparently glabrous or glabrescent to the naked eye on both sides

- 7. Young stem quite pubescent; terminal bud pubescent
  - Terminal bud sparsely pubescent; young leaves sparsely puberulous or completely glabrous; pedicel and calyx glabrous or minutely puberulous; deciduous leaf teeth not seen or shed very early
    - Several stipules can be seen at the same time, stipules subpersistent, sometimes down to fifth mature leaf; leaf base symmetric (rarely oblique), lamina lanceolate to elliptic, midvein glabrous, thickly papery, more coriaceous when dry .....C. zeylanica
    - 9. Stipules early caducous; leaf base oblique, lamina oblanceolate, minutely pubescent along midvein, leaves membranous and drying brittle; pedicel densely pubescence, not articulated or articulation not prominent......C. sikkimensis
  - 8. Terminal bud densely pubescent; young shoots, petiole, leaves, pedicels, and calyx pubescent; leaf teeth clearly visible in very young leaves
    - 10. Leaf margin finely crenate or shallowly crenate, lamina oblong,
      lower 3–5 secondary veins diverging from mid-rib close together
      or from one point ......C. grewiifolia var. gelanoides
    - Leaf margin mostly entire, infrequently serrate, lamina variable in shape, mostly elliptic, ovate, or lanceolate, less often oblong, secondary veins diverging separately and clearly pinnate

..... C. glomerata

7. Young stem and terminal bud completely glabrous

- 11. Deciduous; leaf margins conspicuously crenate, serrate, or shallowly crenate or serrate (infrequently entire); stipules linear, or lanceolate with an acuminate tip, subpersistent, clustered at the flushing stem; flowers with offensive smell; young leaves very chartaceous, turning blackish green when dry, pellucid streaks and dots clearly visible in brown color at 10×; tertiary leaf veins finely reticulate; stem purplish grey with conspicuous white lenticels ...... *C. graveolens*
- 11. Evergreen; leaf margins entire; stipules linear, ovate, or triangular with an acute tip, early caducous, normally only one stipule remaining at the tip; flowers lacking smell; young and older leaves coriaceous or thickly papery, not turning blackish green when dry; pellucid streaks and dots not visible in brown color at 10×; tertiary veins broadly reticulate; stem brown

  - 12. Leaves <9 cm long; young leaf petiole midvein purplish red; not in the Andaman Islands
    - 13. Leaves obovate or orbicular, congested at the apex of a branch; flowers subterminal, few, 1–3, coriaceous
      - 14. Leaf length almost as long as the width; margin revolute; apex obtuse or emarginated; pellucid glands very conspicuous, appearing as punctures on dried leaves; abaxial tertiary veins finely reticulate, raised;

- 14. Leaf length at least 2 times as long as the width; margin not revolute; apex acute; pellucid glands faint; abaxial tertiary veins broadly reticulate, not raised; young leaves, petioles, and midvein green ......C. esculenta
- 13. Leaves elliptic to ovate, not congested at the apex of a branch; flowers not strictly subterminal, many, crowded glomerules >10, thickly papery

15. Pedicels pubescent, fruits  $2.5 \times 1$  cm ......*C. rubescens* 

15. Pedicels glabrous, fruit 1 × 1 cm .....C. esculenta

 CASEARIA ANDAMANICA King, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 67: 16. 1898.— TYPE: INDIA, Andaman Islands, *King's Collectors s.n.* (holotype: CAL [photo!]; isotypes: CAL [photo!], BM!).

Evergreen trees, 6-15 m; bark yellowish brown (Chakrabarty and Gangopadhyay, 1992; Hajra et al., 1999), branches spreading, dark brown to pale brown, smooth, glossy, terete, young branches more or less quadrangular, glabrous, purplish black when dry, lenticulate, lenticels few, approximately round; terminal bud completely glabrous. Leaves 9-25 (-35) × 4.5–10 (-14) cm, oblong to oblong elliptic, grayish green, glossy, greenish brown to pale green above when dry, paler beneath, subcoriaceous, pellucid punctations or striations not visible even at 10×, glabrous on both sides, even on the midvein; margin subentire, undulate, thick; apex acute to shortly acuminate, acumen

ca. 1 cm with a blunt tip; base broadly obtuse to subcuneate, noticeably oblique; midvein flat or slightly grooved above, prominent and raised beneath; lateral veins 8–13 pairs, ascending, broadly arched, 2–4 pairs near the base usually are closely pinnate from the midvein, anastomosing near margins (each secondary vein bends slightly and joins the vein above it), flat and obscure above, markedly raised and slightly prominent beneath, tertiary veins broadly reticulate; petiole 2–3 cm long, slender, ca. 3 mm in diameter, glabrous, terete, canaliculate; stipules  $3 \times 2.5$  mm, triangular, robust, glabrous, apex acuminate, margin ciliate, persistent down to six leaves. Inflorescence axillary, subterminal, glomerules on short tubercles, usually many flowered, up to 20 (Hajra et al., 1999); bracts numerous, minute, ca. 1 mm, ovate, scale like, sparsely pubescent to quite glabrous, completely glabrous within, margin ciliate; pedicels 3–5 mm, rather slender, articulated at the base, glabrous both above and below the articulation; buds ca. 3 mm, elliptic, glabrous. Flowers 5–7 mm in diameter, greenish white, calyx deeply 5-lobed, lobes ovate ca. 1.5 mm long, apex acuminate; stamens 10, 1–1.5 mm long, glabrous or sparsely pubescent towards the base, straight and slender; anthers 0.8 mm, staminodes 1 mm, oblong, flat, tufted hairs at the apex, glabrous towards the base; ovary  $2 \times 1$  mm, trigonous- ovoid (pyramidal); style short, ca. 0.2 mm, obscure, stigma capitate, ovules numerous. Fruits  $3-5 \times 1.5-2$  cm at maturity, ellipsoid or ovoid, yellow when ripe, smooth, glabrous, glossy, thickly 3–6-ridged when dry, apiculate, 3-valved with thick pericarp. Seeds not seen; aril white, turning pink on drying (Hajra et al., 1999).

*Representative Specimens*—INDIA. Andaman and Nicobar Islands: Andamans, (11.68°N 92.77°E), *King's collectors*, BM000948323 (BM).

*Distribution and Habitat*—Endemic to the Andaman Islands of India, recorded between Diglipur and Kalight near Yatrik camp in North Andaman. Extremely rare. Evergreen forests from sea level up to about 150 m altitude (Figure 4.2).

*Phenology*—Collected in flower January to April. Fruits seen in August to September.

*Etymology*—The specific epithet refers to the Andaman Islands, where the species is endemic.

*Taxonomic Comments—Casearia grewiifolia* var. *gelonoides* is commonly mistaken with this species. When the two species are side by side, they are clearly distinguishable. *Casearia grewiifolia* var. *gelonoides* has smaller leaves which are not glossy and are dark brown or blackish brown when dry, and there are usually 2–4 pairs of secondary veins in *C. grewiifolia* var. *gelonoides* starting from the base or at one point of the midvein, but in *C. andamanica* the basal veins are separate and clearly pinnate. In addition, *C. andamanica* has glabrous terminal buds, stipules, and flowers while *Casearia grewiifolia* var. *gelonoides* has quite pubescent young parts and indeed most of the whole plant.

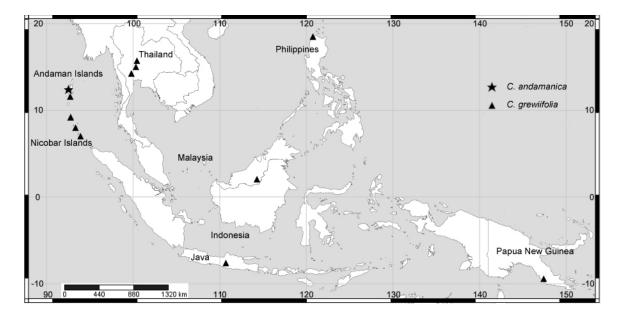


Figure 4.2. Distribution of Casearia and amanica and C. grewiifolia.

 CASEARIA ESCULENTA Roxb., Hort. Bengal. 90. 1814, nom. nud. Fl. Ind. ii. 420, 1832.—TYPE: INDIA. Circar Mts., Roxburgh drawing 148 (holotype: CAL, n.v.).

*Casearia laevigata* Dalz., Hooker's J. Bot. Kew Gard. Misc. 4: 108. 1852.—TYPE: INDIA. Southern Concarn, *Dalzell s.n.* (n.v.).

Casearia championii Thwaites, Enum. Pl. Zeyl. 19. 1858.—TYPE: SRI LANKA (CEYLON). Hewaheta, *Thwaites C.P. 2608* (holotype: PDA!, isotypes: BM!, G!, K!, L!).

*Casearia varians* Thwaites, Enum. Pl. Zeyl. 19. 1858.—TYPE: SRI LANKA
(CEYLON). Ratnapura District, Palabaddala; Kandy District, Hunnasgiriya and
Palagalla, *Thwaites C.P. 2657* (lectotype: PDA! here designated, isolectotypes:
BM!, G, K!, L!).

*Casearia varians* var. β *minor* Thwaites, Enum. Pl. Zeyl. 19. 1858.—TYPE: SRI

LANKA (CEYLON). Central Province, Kandy District, Raxawa, *Thwaites C.P.* 3365 (lectotype: PDA!, here designated; isolectotypes: BM!, G!, K!, L!).

*Casearia varians* Thwaites var. α *ovata*, Enum. Pl. Zeyl. 19. 1858.—TYPE: SRI LANKA (CEYLON). Rathnapura district, Palabaddala; Kandy district, Hunnasgiriya and Palagalla *Thwaites C.P. 2604* (lectotype: PDA!, here designated; isolectotypes: BM!, G!, K!, L!).

Evergreen, small to medium-sized shrubs 2–3 m or small trees 3–15 m; trunk 5– 40 cm dbh, slender; bark rough and fluted, grey, brown, or yellowish grey, sometimes with small raised dark patches, live bark 5 mm thick, honey color, hard, inner bark pale brown; sparsely branched, branches slender, glabrous, lenticulate, lenticels linear or round, size variable; terminal bud glabrous. Leaves  $4-15.5 \times 2.5-7.5$  cm, broadly elliptic to oblong-elliptic or ovate, thickly subcoriaceous; young and old leaves glabrous both abaxially and adaxially, pellucid dots and streaks very small and faint, hard to see in dried material even at 10× magnification, but clearly seen in fresh material; lamina dull, dark green, dying pale brown or copper brown; margin entire; apex acute, frequently with a short acumen (ca. 1 cm), rarely acumen curved; base acute or cuneate, midvein narrow, flat above, glabrous on both sides, lateral veins 6–10 pairs, slightly arching upwards, approximately straight and parallel to each other, tertiary veins broadly reticulate, not clearly visible on upper side; petiole 0.5–1 cm long, stout, terete, sometimes canaliculate, glabrous; stipules  $2.5 \times 1.5$  mm, scale-like, glabrous, early caducous. Inflorescences subterminal on raised axillary knobs, numerous per branch, glomerules sparse or moderately dense with 5-10 (-20) flowers; flower bracts numerous, minute,

membranous, pubescent; pedicels articulated near the base, glabrous both above and below articulation. Flowers ca. 2.5 mm across; calyx lobes ca. 1.5 mm, broadly ellipticoblong, green, pale yellow or pale white, round, completely glabrous; stamens 6–8; filaments 1 mm, pubescent; staminodes 6–8, oblong, truncate and villous at the apex, about as long as the stamens; ovary ca. 1.5 cm, ellipsoid, glabrous, the style indistinguishable and minute, stigma capitate with a thick margin, obscurely 2-fid. Fruit a capsule, mostly globular,  $1.5-2 \times 1$  cm wide, orange-yellow when ripe, distinctly 3ribbed, glabrous, dehiscing to 2–3 valves; fruiting pedicel (3–)7–8 mm long. Seeds up to 12, ca.  $3 \times 2$  mm, mostly globose but flattened on one side, brick brown when dry with few pale white ornamental markings; aril dark scarlet.

Representative Specimens—India. KARNATAKA: Mysore, Hassan, Bisle ghat, [12°42'N, 75°40'E], Saldanha 13615 (MICH).—KERALA: near Munar, Mankulam forest, [10° 03'N, 76°59'E], Kostermans 26186 (G, L, US).— MIZORAM: Lushai hills, Hmuntha, [23°29'N, 92°56'E], Koeltz 32624 (MICH).—TAMILNADU: Anamalais, Paralai, [10°25'N, 77°10'E], Barber 4054 (K); Anaimalai Hills, Udumanparai, 10°10'16"N, 77°03'48"E, collector unknown 5797 (K); Pulney (Palni Hills), Dindigul, Kodaikana, Shembaganur, [10°11'N, 77°29'E], Fieldmen 49916 (K); Madras, Nilgiri northern slope, [11°29'N, 76°44'E], Lawson s.n. (1885) (K); Mount Nilgiri, [11°32'N, 76°43'E], BM000948411 (BM). Sri Lanka. CENTRAL: Galewela, [7°46'N, 80°34'E], Worthington 4249 (BM, K); Gampola, Dolosbage Kellie estate, [7°06'N, 80°28'E], Worthington 1882 (BM); Knuckles, Corbats Gap, [7°22'N, 80°50'E], Kostermans 23486 (G[2], L); Knuckles, Kalupahana, [7°26'N, 80°47'E], Jayasuriya 2539 (PDA); Knuckles, Madulkelle, [7°24'N, 80°44'E], Kostermans 25035 (G, L(2)); Knuckles, Madulkelle, Lebanon Estate, [7°24'N, 80°44'E], *Kostermans* 27188 (G, L); Knuckles, Madulkelle, Lebanon Estate, [7°24'N, 80°44'E], *Kostermans* 27561 (G, L); Knuckles, North of Madulkelle, near summit of Gombiya ridge, [7°24'N, 80°44'E], *Kostermans* 28528 (K, L); Knuckles, Rangala to Corbats Gap, [7°21'N, 80°46'E], *Kostermans* 23486 (L); Knuckles, [7°24'N, 80°44'E], *Kostermans* s.n. [May-71] (K).—NORTH WESTERN: Kurunegala, Beligama estate, [7°41'N, 80°31'E], *Worthington* 493 (K).— SABARAGAMUWA: Sinharaja forest, Weddagala entrance, [6°26'N, 80°25'E], *Kostermans* 29160 (L); Sinharaja forest, Sinha gala, [6°23'N, 80°28'E], *Kostermans* 26798 (L, PDA).— SOUTHERN: Hiniduma, Kanneliya forest, [6°18'N,80°19'E], *Kostermans* 28632 (L).—Locality unknown: *Walker* 245 (K); *Thomson* s.n. (L); *Thwaites* 2604 (G[3], L); *Thwaites* 2608 (BM,G, L); *Thwaites* 2657 (BM, G[2]); *Thwaites* 3365 (G[5], L); *Kostermans* 23486 (L); *Kostermans* 28632 (L); *Thwaites* 2604 (BM).—Place M.... [text undecipherable], *Lob...* [text undecipherable] (K 42).

*Distribution and Habitat*—Mainly distributed in southern India (Malabar-Bombay to Kurg) and in Sri Lanka (Figure 4.3). Also reported from Nepal (Banerji, 1966)), Bhutan (Clement, 1991), Burma (Kress et al., 2003), Singapore (King, 1898), although no specimens from those areas were examined in this study. Sea level up to 1500 m.

*Phenology*—Flowers seen May to October; fruits seen June to November. *Etymology*—The specific epithet refers to the ovate shape of the leaves.

Common Names—Commonly known as the esculent (edible)-leaved false Kamela/Kamala (the "true" Kamela being *Mallotus philippensis* (Lam.) Mull.Arg., Euphorbiaceae), wild cowrie fruits (English), ससरंगी saptrangi (Hindi), ചാറുക്കുന്നം cherukkunnam, മലമ്പാവട്ടെ malampaavetta, വള്ളക്കുന്നം vellakkunnam (Malayalam), किरमीरा kirmira, कुळकुळटा kulkulta (Marathi), सप्तचक्र saptachakra, स्वर्णमूल svarnamulah (Sanskrit), മമ്പ്രമാനം (Sinhala), ട്രാംപ്രം പ്രസ്ഥം konda gangudu (Telugu).

*Uses*—The wood is yellow, honey, or biscuit color, fine grained with fine rays, moderately hard, heavy, and durable, but is exploited for medicinal uses over timber use. Fruits are sweet and said to be eaten in Sri Lanka (Verdcourt, 1996), but there is no mention of whether it is eaten by humans or birds. Roxburgh (1832) mentioned that leaves are eaten in stews by the natives in Circar Mountains (Malabar Mountains); Roxburgh coined the name *C. esculenta* for this reason ("esculenta" means edible). Roots are purgative and said to be used by natives in Circar Mountains (Roxburgh, 1832). They are commonly used in Indian Ayurveda, as roots are hypoglycemic, astringent, cathartic, antiperoxidative, and antioxidant. Therefore, root decoctions are used for liver troubles, diabetes, and piles indigestion (Umberto, 2012).

*Taxonomic Comments*—Roxburgh claimed that his *Casearia esculenta* seemed to be Rheede's *Tserou kanneli* (Hort. Mal. 5: 99, t. 50. 1685). Both Rheede's illustration and the description do not include anything that disagrees with *C. esculenta*. However, neither the illustration nor the description is very detailed. Rheede mentioned that the root is red in color, and Roxburgh also tried to extract red color from roots. Based on those details, Rheede's *Tserou kanneli* is likely *C. esculenta*.

Thwaites identified different varieties of *Casearia varians* based on leaf size and shapes. *Casearia varians* var. *ovata* and *Casearia championii* have larger, ovate leaves

and occur in wet areas. These resemble more the Indian *C. esculenta*, which has broadly ovate leaves, than the Sri Lankan individuals with more narrowly ovate leaves. Thwaite's *Casearia varians* var  $\beta$  *minor*, which has narrower and smaller leaves than typical *C. esculenta* leaves, is most common on the leeward side of the mountains, which are also known to have prevalent wind. Obovate leaf shape, congested leaves at the end of the branch, fewer flowers in the inflorescence, and smaller flowers make it resemble more *C. thwaitesii*, but the leaves are not that small and lack other unique characters of *C. thwaitesii*. Although *Casearia varians* var.  $\beta$  *minor* Thwaites is considered here a synonym, further study is needed.

Dried Indian *C. esculenta* is hard to distinguish from *Casearia rubescens*. See the description under *C. rubescens* for differences between these taxa.

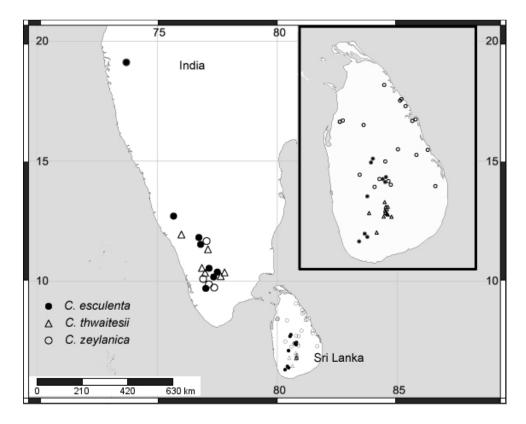


Figure 4.3. Distribution of Casearia esculenta, C. thwaitesii, and C. zeylanica.

 CASEARIA GLOMERATA Roxb. ex DC., Prodr. [A. P. de Candolle] 2: 49. 1825.— TYPE: INDIA. Roxb. Drawing 2250 *Roxburgh s.n.* (lectotype: G!, here designated; isolectotypes: K [photo!], CAL).

Casearia ovata Wall., Numer. List (Wallich) n. 7192 E. 1832. Nomen nudum.

Guidonia glomerata (Roxb.) Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46, 92. 1877.

*Guidonia glomerata* Kurz var. *α glabriuscula*. J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46, 92. 1877.

*Casearia glomerata* var. α *glabriuscula* Kurz. J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46,

92. 1877.

*Casearia glabra* Kurz ex C.B.Clarke. Fl. Brit. India (J. D. Hooker) ii. 591. 1879. Deciduous shrub or medium sized tree, 2–15 m; trunk 17– 45 cm dbh; bark cream colored, rough; branches grey when older, often leafless, stem purplish black, spreading, sometimes 3–4 sided, twig tips densely pubescent, hairs appressed and puberulous down to about the third leaf, then hairs short and spreading thereafter, older branches completely glabrous; lenticels linear, prominent in leafless stems; terminal buds densely pubescent, hairs appressed. Leaf blade  $4-12(-15) \times 3-6.5(-8)$  cm, variable in shape, mostly elliptic, ovate, or lanceolate, less often oblong, thickly papery, sparsely puberulous when young, soon glabrescent on both surfaces; translucent glands elongate and round; young leaf margin often serrulate (7–9 teeth per cm), teeth quickly shed, older leaf margin entire, obscurely denticulate, or crenate (3–5 teeth per cm), less often serrate, serrulate, or crenulate (about 5 teeth per cm); apex acuminate, acute, less often obtuse, often forming a short acumen to 1 cm gradually or more abruptly; base mostly cuneate or acute, less often obtuse, rarely asymmetric; lateral veins mostly 4–6, rarely up to 8 pairs,

broadly arching upward, conspicuous on both surfaces, tertiary veins broadly reticulate; petiole 8–12 mm long, young leaf petiole pubescent, sparsely puberulose down to third leaf, glabrescent when older; stipules  $0.1 \times 0.1$  mm, ovate, small, scale-like, early caducous, deciduous by the second leaf, adaxially sparsely appressed hairy, more often glabrous, margin ciliate, apex acute, stipule scar linear, often lined with few tufted hairs (collaters). Inflorescence of crowded glomerules, 8-15(-20) flowers in the axils of leaves or leafless nodes; bracts scaly, many, broadly ovate or triangular, ca. 1 mm, abaxially semiappressed densely hairy; pedicels 4–6 mm in flower, 7–10 mm in mature fruits, articulate near base, both above and below articulation pubescent, hairs semiappressed. Flowers with offensive smell (Brandis, 1971), buds small, ca. 2 mm; calyx 5, yellowish green, broadly elliptic, obovate, or oblong, 2–3 mm, sometimes veined, outside sparsely pubescent, hairs semiappressed, glabrous towards margins, glabrous within or rarely sparsely pubescent, margin minutely ciliate to nearly glabrous; stamens 8, alternating with hairy oblong staminodes, staminodes more than half the length of the stamens, adaxially glabrous, apex densely hairy, hairs white and rather long; filaments 1–2 mm, pubescent, rarely glabrous; anthers suborbicular, ca. 0.3 mm. Ovary ca. 1.6 mm, ovoid, glabrous or sparsely hairy near the style; style about 0.5 mm, glabrous or sparsely hairy close to ovary; stigma capitate, obscurely three lobed. Fruits  $1-1.4 \times 0.8-1$  cm, ellipsoid, oblong ellipsoid, or obovoid, bright yellow when ripe, glabrous, leathery, usually not or scarcely rigid, dark reddish or blackish brown when dry, in dried material pericarp conspicuously veined, 2–3-valved, split half way upon dehiscence. Seeds several (ca. 5), ca. 3–5 mm, ovoid to round, pale brown when dry; aril orange scarlet (*Thakur Rup Chand* 3160), pale yellow in dried material.

*Representative Specimens*—**Bangladesh**. East Bengal, *Griffith* 73 (G). **Bhutan**. Mishichen-Khosa, Hara et al. 13546 (BM); TRONGSA (TONGSA): Tashiling, Tongsa bridge, [27°29'N, 90°30'E], Hara et al. 5204 (BM).—ZHEMGANG: Zhemgang hillside, 3 km NE of Shamgong, [27°14'N, 90°39'E], Grierson & Long 1613 (K).—Mishichen Khosa, Harra et al. 13546 (BM). China. GUANGDONG (Kwangtung), [23°07'N, 113°15'E], Levine 2331 (BM).—GUANGXI. Sui luk, southwest of Nanning, Pa Lau, [22°38'N, 107°53'E], Tsang 21920 (BM).—HAINAN: Ngai, Yeung Ling Shan, [20°01'N, 110°20'E?], Lau 180 (BM, G).— Hung tung, Huem, Tseng uen, [22°17'N, 113°57'E?], To Kang Peng 2331 (BM). India. ARUNACHAL PRADESH: Delei valley, 28°20'N, 96°37'E, Ward 8160 (K).—ASSAM: Dima Hasao, Haflong, [25°10'N, 93°01'E], Koeltz 27726 (MICH).— MEGHALAYA: East Khasi hills, Cherrapunji, [25.3°N, 91.7°E], Koeltz 29554 (L, MICH), Koeltz 29594 (MICH), Koeltz 29885 (MICH), Koeltz 30302 (MICH), Koeltz 30537 (L, MICH), Koelts & Rup Chand 29649 (L, MICH), Rup Chand 5495 (L. MICH), Rup Chand 5621 (L, MICH), Rup Chand 5670 (MICH), Rup Chand 5681 (L, MICH), Rup Chand 5939 (L, MICH), Rup Chand 5992 (MICH), Rup Chand 6209 (MICH), Rup Chand 6219 (L, MICH), Rup Chand 6241 (L, MICH), Rup Chand 6266A (L, MICH), Rup Chand 7557 (MICH); East Khasi, Shillong, Dirangdong [text undecipherable] hill slope exit of political colony, [25°34'N, 91°53'E], Rao 7528 (L); East Khasi hills, Umran, [26°03'N, 91°51'E], Rup Chand 8368 (MICH); Garo Hills, Tura Mountain, [25°35'N, 90°17'E], Rup Chand 2870 (MICH); Khasia, hill slope, collector unknown L0793820 (L); Khasia, Nyrman [text undecipherable], Clarke 44002c (G); Khasia, Hooker f. 1100 (K), Hooker f. 1588 (K), Hooker f. s.n. (G), Hooker f. & Thomson s.n. (L), Hooker f. & Thomson s.n. (BM); Locality unknown, Cave s.n. (G).—

MIZORAM. Lushai hills, Hmuntha, [23°31'N, 92°56'E], Rup Chand 4463 (L, MICH).— NAGALAND: Naga hills, Kohima, [25°40'N, 94°06'E], Rup Chand s.n. (L), Rup Chand 3160 (MICH); Naga Hills, Takubama, [25°37'N, 94°32'E], Koeltz 26062 (L, MICH), Rup Chand 3607 (MICH).—SIKKIM: between Pemiongchi and Sangachelling, [27°29'N, 88°18'E], collector unknown s.n. 04/05/1913 (BM); Teling Kung [text undecipherable] 04/05/1913 (BM); Rasang, 27°18'N, 88°22'E, Stainton 5382 (BM); unknown locality, Clarke 27950 (BM); Hooker f. s.n. (BM, L, G), Thomson s.n. (L).—WEST BENGAL: Darjeeling, Lebong, [27°03'N, 88°16'E], Clarke 35223d (G), Gamble 9695 (K), Meebold 416 (G[3]), Clarke 26741 (BM), Clarke 27505 (BM), Clarke 35182 (BM, G[2]), Clarke 27505D (K), Clarke 35224A (G), Clarke 35224C (G[3]). Nepal. KOSHI: Sankhuwasabha, Arun Valley, Iswa Khola, 27°33'N, 87°15'E, *Stainton 5917* (BM); Sankhuwasawa, Hongu Khola, 27°30'N, 86°50'E, Stainton 5943 (BM); Sunsari, Dharan, 10 min East of Dharan, 26°50'N, 87°25'E, Stainton 6538 (BM), Stainton 6540 (BM); Terhathum, Saguri, Sangur Bhanjyang, 26° 52'N, 87°18'E, Stainton 5698 (BM).— MECHI: Jhapa, Hans po khari, 26°50'N, 88°05'E, west of Mechi River, Stainton 6449 (BM); Jhapa, west of Nehlajam, 26°51'N, 87°34'E, Williams 157 (BM); Panchthar, Memeng, NE of Ilam, 27°12'N, 87°58'E, Stainton 6831 (BM). East Himalaya, Rongbe, Cave s.n. (G). East Himalaya, Tang Ta-Tho-Dha, Biswas 9294 (G). East Himalaya, Wight 198 (G[2]). Locality unknown: *Punkosany* [text undecipherable] 612 (BM).

*Distribution and Habitat—Casearia glomerata* occurs in deciduous, mixed evergreen and warm broad leaved forests in the sub-Himalayan tract and outer ranges in India, Bhutan, Burma, and Nepal at an altitude of 1000–3000 m (Figure 4.4). In addition, it is recorded as a very common species in forest patches in the mountains of China and Taiwan. Also recorded in Vietnam, Malaya, and Sumatra, but no specimens from those areas examined for this study.

*Phenology*—Collected in flower in April to July. Flowering occurs December to June (Brandis, 1971). Fruits seen in April to July. Trees leaf out in May.

*Etymology*—The specific epithet refers to the closely collected flowers in glomerules. *Casearia glomerata* has very dense inflorescences with up to 20 flowers, in contrast to the more-or-less loose glomerules of the other species.

Common Names—Since Casearia glomerata is very common in Madya Pradesh, Andra Pradesh, and neighboring states, there are many vernacular names. Loor joor (Bengali) is the vernacular name in Sylheti (Roxburgh, 1832, 1814). 球花脚骨脆 qiu hua jiao gu cui is the Chinese common name (Yang and Zmarzty, 2007). Other common names in India include: Kunnan (Malayalam), Giridi (Odia (Oriya/Uriya), Gilchi (Gondi), Bokhada (Marathi) (Brandis, 1971), Tillingkung and Barkaunle (Nepalese).

*Taxonomic Comments*—Morphologically, *Casearia glomerata* is a highly variable species (e.g., in leaf size, shape, margin, base) compared to all of the other south-central Asian *Casearia*, but that does not make it hard to distinguish from other species since it always has quite glabrous leaves along with glabrous stipules and densely pubescent terminal buds. Sometimes *Casearia esculenta* can be confused with *C*. *glomerata* in having ovate and glabrous leaves with quite entire leaves, but *Casearia esculenta* does not grow together with *C. glomerata* nor has the compact glomerules nor densely pubescent terminal buds of *C. glomerata*.

*Casearia sikkimensis* is also commonly misidentified as *C. glomerata*. However, *C. sikkimensis* has oblanceolate leaves, is minutely pubescent along the midvein, and has

slender flowers. *Casearia sikkimensis* also has denser glomerules than *C. glomerata*. (Mukherjee, 1972).

*Excluded Names*—In the *Flora of China*, *Casearia merrillii* Hayata, Icon.Pl. Formosan. 3: 30. 1913 (Type—TAIWAN, Pintung (TAI) [photo!]), described from Taiwan, is mentioned as a synonym for *C. glomerata*. However, the type specimen is very different than *C. glomerata*. *Casearia glomerata* is never seen with deeply dentate to crenate margins, asymmetric cordate leaf bases, such oblong leaves, and less arched 8– 12 pairs of veins as in that type specimen. Therefore *Casearia merrillii* Hayata should not be considered a synonym of *C. glomerata*.

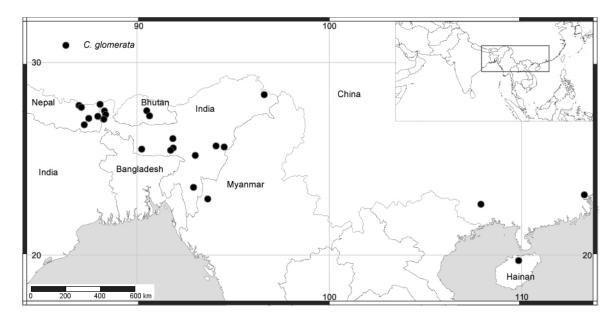


Figure 4.4. Distribution of Casearia glomerata.

4. CASEARIA GRAVEOLENS Dalzell, Hooker's J. Bot. Kew Gard. Misc. 4: 107. 1852.— TYPE: INDIA. Southern Concan, *Dalzell s.n.* (lectotype, here designated: L [photo!]-K000591432; isolectotypes: K-K000591431 [photo!], K000591433 [photo!].

- *Casearia hamiltonii* Wall., Numer. List (Wallich) Cat. n. 7195 B. 1832, nom. nud.— TYPE: INDIA. *Wallich 7195 B*. (lectotype, here designated: G!-G00161557; isolectotypes: BM!-BM000948377).
- Samyda glabra Buch.-Ham., Trans. Linn. Soc. London 17(2): 227. 1835. Buch.-Ham. ex
  Wall., Numer. List [Wallich], 7195 A, 1832, nom. nud., non *Casearia glabra*Roxb., 1814, nom. nud., non 1832.—TYPE: INDIA, Mangga, 4 January 1811, *Hamilton s.n.* (holotype: K, *Wallich 7195a*).
- *Casearia macrogyna* Turcz., Bull. Soc. Nat. Mosc. 1: 463. 1858.—TYPE: INDIA. *Metz. Coll. n. 105* (n.v.).
- Casearia alnifolia Royle, Ill. Bot. Himal. Mts. (Royle) 1, 170. 1839.—TYPE: INDIA. Royle s.n. (lectotype, here designated: LIV [photo!]- LIV.1952.121.1581ii, isolectotypes: LIV [photo!]-LIV.465308).
- *Casearia graveolens* var. *lintsangensis* S.Y.Bao, Acta Bot. Yunnan. 5(4): 378. 1983.— TYPE: CHINA: Yunnan, *T. T. Yu 18120* (holotype: KUN [photo!]).
- *Casearia bourdillonii* N.Mukh. Bull. Bot. Soc. Bengal 19(2): 109. 1967—TYPE: INDIA. Colatoorpolay, *Bourdillon 104* (holotype: K!).

Deciduous shrubs or small trees, 3–6 m, trunk 20 cm dbh; bark dark grey with white specks, rough and fissured; branches green, smooth, with grey-white patches, young branches drying purplish black, terete, sometimes 3-sided, spreading, sometimes drooping, older branches grayish brown with green patches; twig tips, young branches, and older branches completely glabrous, lenticulate; lenticels mostly linear, sometimes appearing as concentrated dots; terminal bud glabrous. Leaf blade  $6-19 \times 4-9$  cm, surface dull, pale green or obviously olivaceous green, dry leaves pale brown or greenish

brown, pellucid dots and streaks prominent abaxially, closely arranged, irregularlyshaped, reddish brown, clearly visible in dried material at 10× magnification without holding leaf to light, broadly elliptic to elliptic oblong, thin papery, young leaves membranous, drying to greenish brown or blackish green, both abaxial and adaxial surfaces glabrous; margin shallowly crenate, sometimes serrate, young leaf margin serrate and becoming crenate, crenation even, sometimes deciduous teeth may remain on the older leaves, otherwise often entire; apex variable, broadly acute, obtuse, or rounded, often contracting gradually or abruptly to a short acumen, ca. 1 cm; base rounded or broadly obtuse, rarely cuneate, mostly symmetrical, rarely oblique; midvein narrow, canaliculate, lateral veins 6–10 pairs, arching upward, nearly almost parallel to each other, tertiary veins finely reticulate and clearly visible on both sides, in dry leaves raised and very conspicuous in pale brown or yellowish brown color; petiole 8–15 mm, glabrous, canaliculate; stipules  $5-10 \times 2$  mm, conspicuous, narrowly lanceolate, papery, glabrous, early caducous, but observed down to seven leaves in flushing branches, margin smooth, rarely ciliate near apex, apex mostly acuminate, sometimes acute, base evidently auriculate, stipule scar large, pale brown, ovate to irregular in shape, scar conspicuous on young stems. Inflorescences axillary glomerules, 1-5(-8) per axil, mostly seen on leafless stems; pedicels 3–6 mm, articulate near base, glabrous above articulation or pubescent with short semi-spreading hairs above articulation near to the base, more densely pubescent below articulation; bracts ovate, ca. 2 mm, densely appressed hairy, longitudinally striate, glabrous within (adaxially), margin smooth, not ciliate. Flower buds globose, sometimes club shaped, densely appressed hairy, sometimes short sparsely spreading hairy mixed with long white spreading hairs, margin ciliate, especially towards

the apex; flowers pale green to green with foul smell; sepals 5, ovate to ovate-oblong,  $3.5-4 \times 1.3-2$  mm, outside pubescent, more densely so towards base, hairy or glabrescent above with semi-spreading short hairs, sometimes completely glabrous, inside sparsely hairy, margin practically glabrous, not ciliate, veined; stamens 6–8, filaments glabrous, 1–1.5 mm, anthers oblong, ca. 0.5 mm; staminodes oblong, ca. 0.6 mm, half as long as stamen filaments, densely pubescent throughout, hairs white when dry, long; ovary ovoid, ca. 1.5 mm, mostly glabrous, rarely pubescent with spreading short hairs close to style, smooth; style distinct, ca. 0.8 mm, glabrous or hairy in lower part; stigma distinctly capitate. Fruit a capsule, orange yellow, glabrous, shiny when ripe, distinctly longitudinally 6-ribbed, dark reddish or blackish brown when dry, ellipsoid oblong or nearly globose,  $2-5 \times 1-1.5$  cm, pericarp fleshy and thick, densely but shallowly warty, veined when dry, 3-valved, dehisced completely, valves navicular (boat-shaped) when dehisced. Seeds several, ca. 12, pale yellowish brown with dark brown striations when dry, ovoid to trigonous, 4–5 mm, surface smooth, enclosed in thin fleshy red aril, partly fimbriate, pale yellowish brown when dry.

*Representative Specimens*—**Bhutan**. SAMTSE: Deo Pani Khola above Samtse, 26°54'N, 89°07'E, *Grierson & Long 3508* (K).—TRASHIGANG: Trashigang, Telsta, 27°19N, 91°34'E, *Clarke 27956* (BM); Trashigang, *Grierson & Long 2046* (K).—BAGO: Pegu Range, Eastern slope, pegu yomah, [18°27'N, 96°19'E], *Kurz 1937* (K).—KACHIN: Upper Burma, [26°40'N, 97°00'E], *Mokim s.n.* (G).—MANDALAY: Pyin Oo Lwin (Maymyo), [22°14'N, 96°23'E], *Lace 5190* (K). Locality unknown: *Dr. King 584* (K). **India**. ASSAM: Haflong, [25°10'N, 93°00'E], *Koeltz 27725* (MICH).—JHARKHAND: Hazaribagh, Chota Nagapur, North base of Gilbralta (Canary) hill, [24°00'N, 85°23'E], *Kerr 3*9 (BM); Hazaribagh, Chota Nagapur, beyond Gilbralta (Canary) Hill, [24°00'N, 85°23'E], *Kerr 2069A* (BM), Hazaribagh, *Haines CN 311a* (K).—KARNATAKA: Madras Presidency, Terr Canara (South Canara [text undecipherable]), Manglore, [13°03'N, 75°23'E], *Hohenacker 105* (BM); Hassan, Kadamana, [12°53'N, 75°41'E], *Ramamoorthy 1893* (MICH); Kurg (Coorg), [12°30'N, 75°38'E], *Madrad* [text undecipherable] *53* (K); Mysore, Hassan, Vanagur, [12°17'N, 76°38'E], *Saldanha 16865* (MICH); Mysore, Hassan, Bisle ghat, [12°42'N, 75°40'E], *Saldanha 13615* (MICH).— KERALA: Idukki, Cardamom hills, Munar Periyar road, [9°52'N, 77°08'E], *Ridsdale 207* (K, L); Kollam, Ariyankavu, Thenmalai, [8°57'N, 77°08'E], *Ridsdale 534* (K, L); Kerala road to Poonmudi, [8°45'N, 77°07'E], *Kostermans 26080* (L, G[2], K); near Munar,

Mankulam forest, [10° 03'N, 76°59'E], Kostermans 26186 (G, L, US).--

MAHARASHTRA: Konkan, [19°13'N, 72°54'E], *Shocks vc s.n.* (K[2]); Pune, Khandala, Behrauis plat. [18°44'N, 73°21'E], *HS 9118* (K); Pune, Ambegaon, [19° 07'N, 73°42'E], *Dr. Ritchie 919/3* (K).—MEGHALAYA: Khasi Hills, E. Khasia hills, Umran, [25°46'N, 91°52'E], *Rup Chand 8368* (L); Khasi Hills, Nongphoh, Shillong, [25°55'N, 91°48'E], *Rup Chand 1484* (L, MICH), Nongphoh, *Clarke 43283* (G), *Clarke 38118D* (BM).— MIZORAM: Lushai hills, Hmuntha, [23°31'N, 92°56'E], *Rup Chand 4432* (MICH).— ORISSA: Matlili, Kalyan forest, *Raju 1690* (CAL). —TAMIL NADU: Anaimalai Hills, [11°30'N, 76°44'E], *Beddome 8203* (BM); Nilgiri, [11°30'N, 76°44'E], *Beddome 3152* (BM), Mount Nilghim, *Hook s.n.* (G).—UTTAR PRADESH. Gorakhpur, upper Gangetic plain, [26°48'N, 83°19'E], *Har*... [text undecipherable] *21619a* (K); Kheri, upper gangetic plain, [30° 7'N, 77°56'E], *Inayat* [text undecipherable] *s.n.* (G[2]); *Doswals*[text undecipherable] *3090* (K).—UTTARAKHAND: Garhwal, Tehri Garhwal, Dewat,

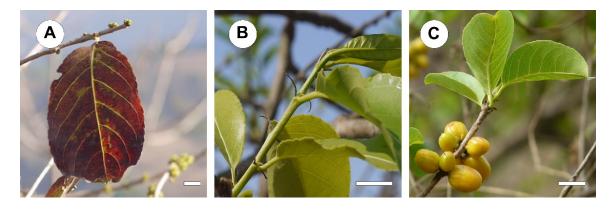
[30°18'N, 78°33'E], Naithani 48252 (G); Kumaon, [29°15'N, 79°19'E], Duku 806 (K); Locality unknown: Falconer 363 (K).— JHARKHAND: Parasnath, [23°59'N, 86° 2'E], collector unknown (BM); India. Locality unknown: Ridsdale 207 (L), Kostermans 26080 (L). Nepal. DHAWALAGIRI: Myagdi, Ghorepani, 28°11'N, 83°44'E, Dobremez & Ramanathen 3235 (BM).— [LUMBINI], [Gulmi], Nahapani [text undecipherable], Dobremez 1879 (BM).—CHITWAN: Narayani, Churia Hills (Sivalik Hills), [27°28'N, 84°17'E], Troth 772 (BM, MICH).-DHAWALAGIRI: Myagdi, Tatopani, north of Beni, [28°29'N, 83°39'E], Stainton et. al. 615 (BM).—GANDAKI: Kaski, Mardi Khola, Sisaghat, [28°18'N, 83°54'E], *Stainton 6064* (BM).—KOSHI: Biratnagar, [26°27'N, 87°16'E], Stainton 46 (BM); Sunsari, Chyan, Teloh (Telok[text undecipherable]), 27°17'N, 87°53'E, Williams 570 (BM).-LUMBINI: Nawal Parasi, Girwari Forest, *Makin 254* (BM). MECHI: Taplejung, Tamur valley, Mewa Khola, [27°28'N, 87°36'E], Stainton 5865 (BM).—NARAYANI: Chitwan, Chiteu, 25°83'N, 83°42'E, Dobremez & Ramanathen 3187 (BM); Chitwan, Ramnagar, Chilwan, [27°43'N, 84°26'E], Wesche 1137 (BM). RAPTI: Rukum, Ganbari, 26°45'N, 87°58'E, Stainton 5732 (BM). SAGARMATHA: Khmbu, Bhota Kosi, 27°45'N, 86°00'E, McCosh 99 (BM); Khmbu, Bhota Kosi, 27°45'N, 86°12'E, Stainton 4467 (BM); Khmbu, Bhota Kosi, Khari Khola, 27°48'E, 86°20'E, Stainton 4650 (BM); Udayapur, Rani haura, Yali Valley, Stainton et al. 5451 (BM). Nepal, Jarda Klada [text undecipherable], *Polunin et al.* 3725 (BM). Nepal, Tohlari (Jhalari [text undecipherable]), Stainton 440 (BM). Pakistan. KHYBER: Pakhtunkhwa, Charsadda, Kangra, Bhadwar, Koeltz 4477 (MICH). Thailand. Doi sulth, Kerr 5259 (BM). KANCHANABURI: Lai yori (Khwae yai [text undecipherable]), Kanbusri, [15°7'N, 98°59'E], Marcan 2418 (BM).

*Distribution and Habitat—Casearia graveolens* has a wide distribution in Bangladesh, Bhutan, Burma, Cambodia, India, Laos, Nepal, Pakistan, Thailand, Vietnam, and China (Yunnan) (Figure 4.6). It is common in deciduous forest at higher elevations in the Himalayas and sub-Himalayas, at altitudes of 250–2000 m. Also recorded in rocky deciduous hardwood forest at low elevations to 600 m. *Casearia graveolens* is very common in subtropical forest of valleys and ravines in southern India and the Western Ghats.

*Phenology*—Loses leaves in February, flushes new leaves in June–July. Collected in flower in February–March. Flowers mostly set on leafless branches. Fruits seen in July–November.

*Etymology*—The specific epithet refers to the strong, offensive smell of the flowers.

Common Names—Due to its wide distribution, *Casearia graveolens* has many common names. Chilla छिल्ला (Hindi) is the most common name used in India. Other common names used in India include: ईरि१४०ी२१ Kirambira (Gujarati), गिलची Gilchi, Safed-Karai, Phempri (Hindi); Haniche, Konje, Bokara, Hanise, Killangi (Kannada [Karnataka state]); Anavananni, Anavinga, Cherukannan, Chirakonna (Malayalam [state of Kerala]); पिंपरी Pimpari (Konkani), बोखाडा Bokhada, Mori, Pimpari (Marathi [Maharashtra state]), Mando, Beniman, Jamurdo, Kokra (Oriya); Girivudi, Vasanga, Veska, Vaasanga (Telegu); 香味脚骨脆 Xiang wei jiao gu cui (Chinese); Sano dedri (Nepalese). *Uses*—Edible oil is extracted from the seeds (Umberto, 2012). Crushed stem bark, especially unripe fruit and leaves, are used locally to kill fish. Also used in Ayurveda for anticancer and antiviral properties. Root paste used to treat piles, and juice given for jaundice (Umberto, 2012).



*Figure 4.5.* Morphology of *Casearia graveolens.* A. Copper-colored deciduous leaves. Flowers borne on mostly leafless branches. B. Linear subpersistent stipules. C. Globular fruits which turn bright yellow when ripe; note crenate leaf margin. Scale bars = 1 cm. Photos courtesy of Dinesh Valke.

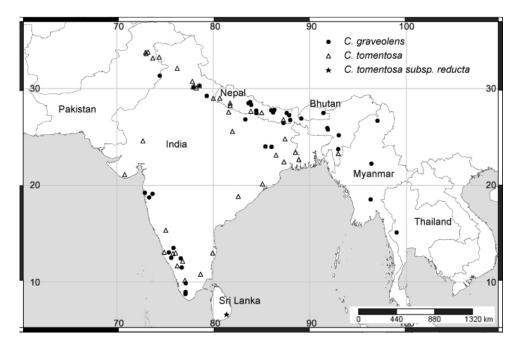
*Taxonomic Comments*—*Casearia graveolens* has a wide distribution throughout India and neighboring countries, yet it is very easy to identify even from herbarium material by its linear, clustered, subpersistent stipules, especially seen in flushing stems, and from its very chartaceous leaves which turn olivaceous green when drying, particularly young leaves (Figure 4.5). Further if young leaves are examined, prominent, evenly distributed, brown colored pellucid lines can be seen. Older leaves show abaxial raised, finely reticulate venation in dried leaves. In addition, the leaves are usually broadly elliptic with an obtuse base, which does not occur in any other species coexisting with this species.

Rheede introduced the genus Casearia in his Hortus Malabaricus, under the name Anavinga along with an illustration. He indicated that the plant had crenate margins and glabrous leaves. Among the *Casearia* that grow in the Malabar region, only *C. tomentosa* and C. graveolens have crenate margins. Casearia tomentosa, though, has quite obvious tomentose vestiture. The illustration is also more similar to C. graveolens than to C. esculenta, as interpreted by others. Dalzell (1852) mentioned that the flowers of Casearia graveolens have an unpleasant odor, whereas Rheede stated that flowers do not have an odor, which is the only character that disagrees with C. graveolens. Confusingly, though, he mentioned that its *leaves* have an unpleasant odor. Clarke (1879) thought Rheede's Anavinga is C. tomentosa, which has more glabrous southern individuals. But it is erroneous since at least young parts and the abaxial surface of the leaves of C. tomentosa are quite obviously tomentose, even in southern individuals. Consequently, C. graveolens is the most likely species to agree with Rheede's Anavinga, even though that would suggest that C. ovata is its correct name according to the rule of priority. Based on usage, I argue (Samarakoon, in prep.; CHAPTER 5) that the name *C. ovata* should be rejected.

Mukherjee (1965) described a new species *Casearia bourdillonii* to provide a name for Beddome's *Casearia varians* non Thw. However, he chose a wrong type specimen, *Bourdillon 104*, which does not represent Beddome's *Casearia varians*. Collectively considering the glabrous stipules with auriculate bases clustered at the apex of the branches, the glabrous terminal buds, the densely reticulated, abaxial, raised veins of the leaves, the cuneate leaf bases, the glabrous pedicels, and the glabrous, evidently veined calyx on that type specimen, *Casearia bourdillonii* N.Mukh. is here considered a synonym of *Casearia graveolens* Dalzell. However, it lacks the linear stipules, prominent

brown pellucid-punctations, and chartaceous leaves as typical *C. graveolens*, making its placement tentative.

The specimen selected as lectotype here (L!-L0010648) was previously annotated as an "isotype." However, no specimen annotated "lectotype" or "holotype" was found, nor did I find a lectotypification in the literature. The L specimen is a high quality specimen.



*Figure 4.6.* Distribution of *Casearia graveolens, C. tomentosa* and *C. tomentosa* subsp. *reducta*.

5. CASEARIA GREWIIFOLIA var. GELONIOIDES (Blume) Sleumer, Fl. Males., Ser. 1,

Spermat. 6(6): 944. 1972. Casearia hexagona Decne. var. gelonioides Blume,

Mus. Bot. Lugd.-Bat. 1: 255. 1850.—TYPE: TIMOR. "Obsolete remote crenulatis coriaceis."

Samyda greviaefolia Poir. Encycl. (J. Lamarck & al.) Suppl. 5:32, 1817.—TYPE:

INDONESIA (Java). 1791–1797, La Haye s.n. (holotype: G).

Casearia leucolepis Turcz., Bull. Soc. Imp. Naturalistes Moscou 31(1): 463. 1858.—

TYPE: SINGAPORE. 1858, *T. Lobb. no. 468* (lectotype: K! here designated; isolectotype: BM [Photo!], G [Photo!], P [Photo!]).

Casearia grewiaefolia Vent. var. deglabrata Koord. & Valeton, Bijdr. Boomsoort. Java

1: 174. 1894.—TYPE: INDONESIA: Celebes, 1920, S. H. Koordens no. 18796.

*Casearia insularis* Vasudeva Rao & T. Chakrab., J. Econ. Taxon. Bot. Addit. Ser., 5(4):
991. 1984; *Casearia grewiaefolia* Vent. var. *insularis* (Vasudeva Rao & T. Chakrab.) T. Chakrab. & M. G. Gangop., J. Econ. Taxon. Bot. 16(3): 717.
1992.—TYPE: INDIA. Andaman-Nicobar Islands, North Andaman Island, Saddle Peak Range, Lamia Bay Slope, 31 Mar 1977, *Balakrishnan 5389 A* (holotype: CAL; isotype: PBL).

Evergreen (or sometimes deciduous? cf. Umberto, 2012), small shrubs, 3–6 m, or trees up to 20(-35) m; trunk dbh 30 cm, greyish pale brown or whitish, rough, with small bumps, fluted at the base when old, buttresses up to 1 m, branches spreading, purplish grey with paler, prominent, raised lenticel markings, young branches compressed, more or less quadrangular, olivaceous, densely woolly tomentose, eventually glabrescent, lenticulate, lenticels more or less round; terminal bud densely tomentose. Leaves 8–10(– 16) × 3.5–6(–8) cm, more or less oblong, sometimes ovate-oblong, olivaceous green, paler beneath, dark brown or blackish brown when dry, thinly coriaceous, densely pellucid-punctate and striate, striations comet shaped, visible to the naked eye in young leaves, glabrous above even on the midvein; margin mostly regularly minutely to rather coarsely crenate, sometimes nearly entire and undulate; apex shortly acuminate, acumen ca. 1–2 cm, or obtuse; base broadly cuneate to round, sometimes subtruncate or

subcordate, always noticeably oblique; midvein flat or slightly grooved above, prominent and raised beneath, densely rusty to yellowish tomentose above, more greyish pilose to tomentose beneath, often glabrescent with age; lateral veins (8-)10-14 pairs, slightly arched, ascending and more or less transverse, 2-4 pairs near the base, usually starting from the leaf base or at one point at the midvein, flat or little raised above, markedly raised beneath, but not prominent on both faces, tertiary veins broadly reticulate, visible but not prominent; petiole 5-10(-15) mm, stout, ca. 2 mm in diameter, pubescent, glabrescent in older leaves; stipules  $1.5-2 \times 0.8$  mm, triangular, robust, glabrous to sparsely hairy near the center, densely appressed hairy towards the margin, margin ciliate, early caducous. Inflorescences fascicles, usually many flowered with up to10 flowers, but sometimes reduced to few flowers or even single flower, often in the axil of fallen leaves; bracts numerous, ca. 1 mm, membranous, pubescent; pedicels 5-6(-8) mm, rather stout, pubescent, articulate, velvety both above and below the articulation; buds ca. 2 mm, velvety with dense, short appressed hairs outside and within. Flowers ca. 7 mm diameter, white, green, or yellowish; calyx deeply 5-lobed, lobes ca. 2–3 mm long, 1.5–2 mm broad, ovate, apex acuminate, reflexed at full anthesis, appressed hairy outside, puberulose within; stamens usually 8, rarely up to 10, slightly different in length; filaments1–2 mm long, glabrous or sparsely pubescent towards the base, straight and slender; staminodes 1 mm, oblong, thick, densely hairy, with tufted hairs at the apex; ovary  $2 \times 1$  mm, ovoid, glabrous or sparsely pubescent towards the style; style short, ca. 0.2 mm, but distinct; stigma capitate, ovules numerous. Fruits  $2.5-4 \times 1-2$  cm at maturity, globose to ellipsoid, smooth, glabrous, glossy, thickly 3-ridged when dry, apiculate, bright yellowish orange at maturity, 3-valved with thick pericarp, with bitter

burning taste (*Kostermans*, 1296). Seeds numerous, angular, ca. 5 mm, black, with bitter taste (*Kostermans* 1188); aril red orange, laciniate.

*Representative Specimens*—India. ANDAMAN AND NICOBAR ISLANDS: Andaman Islands, South Andamans, South Sentinel Island, [11°39'N, 92°44'E], Balakrishnan 7618 (CAL); Nicobar Islands, Car Nicobar, Arong, [9°10'N, 92°45'E], *Nair 3717* (CAL); Nicobar Islands, Car Nicobar, Lapathy, Scarce, [9°13'N, 92°47'E], *Nair 3544* (CAL, L); Nicobar Island, Great Nicobar, Campbell Bay, [6°59'N, 93°55'E], *Hore* 7283 (CAL); Nicobar Islands, Katchal Island, west bay, [8°00'N, 93°20'E], Chakraborty 5206 (CAL). Indonesia. BORNEO: Locality unknown, collector unknown, (K000591383).—JAVA: Locality unknown, Lobb 468 (G[2]), collector unknown 238 (K). New Guinea. CENTRAL PROVINCE: Sogeri, [9°25'S, 147°24'E], Forbes 377 (K). **Philippines.** Ilocos Norte: Bangui, [18°29'N, 120°45'E], *Ramos s.n.* (K); Philippines, locality unknown, collector unknown 13650. Thailand. KANCHANABURI: Ku-jae, about 150 km northwest of Kanburi, [14°14'N, 99°47'E], Kostermans 1296 (US); near Kannyu, about 100 km northwest of Kanburi, [15°00'N, 100°19'E], Kostermans 1188 (US).—NAKHON SAWAN: Nakhon Sawan city, 39 km northwest of nakhon sawan, [15°43'N, 100°26'E], King's collector 5429 (US), King's collector 5430 (US); Thailand, southeastern Thailand, Collins 885 (US), Collins 917 (US), Collins 1503 (US); Locality unknown, collector unknown, K000591464.

*Distribution and Habitat*—The full distribution of the species and varieties of *Casearia grewiifolia* is unknown. It is recorded in Australia, Burma, Indochina (Cambodia, Laos, Vietnam, Thailand), Malaysia, Indonesia (Java, Lesser Sunda Islands [Bali], Borneo, Celebes, Moluccas [Sula]), the Philippines, and New Guinea (Sogeri). In the study area, *C. grewiifolia* var. *grewiifolia* is not recorded. *Casearia grewiifolia* var. *gelonioides* is recorded in the Andaman and Nicobar Islands (India) (Figure 4.2). In Great Nicobar Island and Andaman Islands it is distributed in an altitudinal range from near sea level to 200 m. It is not a common tree. It grows in well-developed lowland rain forest and gallery forest. It is critically endangered on these islands.

*Phenology*—Collected in flower in September to April. Fruits seen in November to May.

*Etymology*—The specific epithet refers to the *Grewia*-like leaves (Tiliaceae; Malvaceae s.l.). The variety name *gelonioides* refers to the three ribbed fruits which look like the fruits of *Acronychia pedunculata* Miq. (Rutaceae), formerly known as *Gela lanceolata* Lour.

Common Names—Kill toung (India), Salokdan (Sabah), Kruai paa (Tahiland).

*Uses*—Indigenous people use the leaves as a tobacco substitute. The leaves also have medicinal applications, and it is occasionally used for timber for posts and beams. The arillate seeds are eaten by fruit pigeons (Hajra et al., 1999).

*Taxonomic Comments*—*Casearia grewiifolia* var. *gelonioides* differs from var. *grewiifolia* by a pronounced glabrescence in all parts. Var. *grewiifolia* has a densely hairy plant body or at least densely hairy young parts and abaxial midvein. Almost always var. *grewiifolia* has a minutely crenate margin while var. *gelonioides* has a shallowly crenate or slightly undulate margin. The leaf texture also differs considerably between the two varieties, as var. *gelonioides* has somewhat thinly coriaceous leaves while var. *grewiifolia* has very papery, soft leaves. Var. *grewiifolia* has larger fruits, more than 3–4 cm, but fruits of var. *gelonioides* are smaller and rarely become larger than 3 cm. On the other hand, the fruits of var. *grewiifolia* are said to have a bitter, burning taste and are used for fish poison while var. *gelonioides* has a mild, sweet taste and are eaten by fruit pigeons (Hajra et al., 1999). However, which part(s) of the fruit is eaten and which part is used for as fish poison are unknown.

*Casearia grewiifolia* differs little from *Casearia tomentosa*, but one can distinguish them by considering the leaf characters collectively. *Casearia grewiifolia* has very *Grewia*-like leaves, with rounder, oblique leaf bases, which appear almost cordate, with 2–3 secondary veins that starts very close to the base. The lamina is oblong with a finely crenate margin.

- 6. CASEARIA KURZII C.B.Clarke, Fl. Brit. India (J. D. Hooker) 2(6): 594. 1879. *Casearia glomerata* var. *puberula* Kurz, Forest Fl. Burma i. 530. 1877; *Guidonia glomerata* (Roxburgh) Kurz var. (β) *puberula* Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46(2): 92. 1877—TYPE: INDIA. Chittagong.
- *Casearia kurzii* var. *gracilis* S.Y.Bao, Acta Bot. Yunnan. 5(4): 376. 1983.—TYPE: CHINA. *Yunnan exped.* 9507 (holotype: KUN [photo!]).

Small tree or shrub, 1–15 m; branches spreading, purplish grey when dying, sometimes with 3 sides, twig tips and branchlets pubescent, hairs spreading in older branches; lenticels not prominent; terminal bud densely pubescent, hairs appressed. Leaf blade  $9-15(-20) \times 4-5(-8)$  cm, oblong or oblong-lanceolate, sometimes lanceolate, rarely oblong-elliptic, thin and papery, upper surface glabrous above, rarely with few hairs towards base, lower surface pubescent throughout or sparsely pubescent only along midvein and lateral veins, rarely subglabrous, hairs yellowish and pale brown; translucent

glands very dense and tiny, not visible to the naked eye, hard to see even under a microscope at 10×, appearing as evenly distributed, tiny, black or brown dots; margin shallowly serrate (3 teeth per cm) or sometimes subentire; base rounded to cordate, often oblique; lateral veins 6–11 pairs, broadly arching upward, clearly visible in pale color, apex acute or gradually tapering to form a short acumen of ca. 1.5 cm; petiole 8–15 mm, public p broadly triangular to ovate, minute, papery, glabrous, sparsely to densely appressed pubescent in the middle towards apex, margin ciliate, very early caducous, not seen even subtending the first mature leaf; stipule scar more or less round, sometimes increasing in size with age and becoming conspicuous. Inflorescence of few to many flowers (up to 15) in axillary sessile glomerules; bracts papery, many, broadly ovate, 0.5–0.7 mm, sparsely pubescent with appressed hairs, margins densely ciliate, glabrous within; pedicels 5–8 mm in flower (ca. 1 cm in fruits), articulate, articulation ca. 2 mm from the base, completely glabrous or sparsely hairy below the articulation, hairy above the articulation, hairs spreading, yellowish. Flowers whitish, small; calyx 5-lobed, lobes broadly ovate, 2– 3 mm, outside pubescent, glabrous towards margin, hairs appressed to spreading and yellowish, glabrous within or with few hairs; stamens 7–8, filaments pubescent, 0.7 mm long, anthers ovoid, 1–2 mm, glabrous; staminodes oblong, mostly slightly shorter than filaments (1/2-3/4 shorter than filaments), hairy, hairs tufted at the apex, long and becoming whitish when drying; ovary ovoid, ca. 2 mm, glabrous; style short, ca. 0.5 mm, glabrous; stigma capitate, enlarged, easily disintegrating. Fruits capsules, 1-1.5 cm  $\times 6-8$ mm, obovoid to oblong, orange when ripe, drying black, pericarp varicose/wrinkled, with many ellipsoid inclusions, prominent in dried material. Seeds several (ca. 5), drying pale

brown, ovoid, ca. 5 mm, surface smooth, enveloped by a thin, fleshy, partly fimbriate, pale yellowish aril.

Representative Specimens—Bangladesh. CHITTAGONG: Chittagong, Hill Tracts, [22°51'N, 92°10'E], *King's collectors 296* (CAL); Chittagong, Kaptai, [22°51'N, 92°10'E], *Gamble 7754* (K).—NORTH BENGAL: *Biswas 1592* (US). Burma. MON: Mawlamyine, Kowpok, [16°29'N, 97°37'E], *Meebold 16860* (L), *Meebold 17294* (CAL). India. ASSAM: collector unknown 97K.—MEGHALAYA: Garo Hills, Dambu Reserve near Rongmigiri, [25°30'N, 90°19'E], *R. N. De. F. 19968* (CAL).—SIKKIM: Himalaya, [27°43'N, 88°38' E], *Burman* [text undecipherable] *187* (CAL); Sikkim, *D. s.n.* (BM); Sikkim, *King's collector s.n.* (BM).—WEST BENGAL: Darjeeling, Cheuga valley, [27°02'N, 88°15'E], *N...* [text undecipherable] *2432A* (K); Darjeeling, Kalimpong, [27°03'N, 88°28'E], *Haines 999* (K).

*Distribution and Habitat—Casearia kurzii* grows in subtropical forest in the southeastern Himalayas; 500–1500 m (Figure 4.7). This region includes a few of the northern states of India, including Assam, Arunachal Pradesh, Meghalaya, Sikkim, Tripura, and West Bengal, and also the surrounding countries such as Bangladesh, Bhutan, China, and Nepal.

*Phenology*—Collected in flower in July to August. Fruits seen from October to March.

*Etymology*—The specific epithet honors Wilhelm Sulpiz Kurz (1834–1878), a famous German botanist who worked for the Dutch East Indies Company. He worked as a garden director at Bogor (Indonesia) and as curator of the herbarium in the Royal Botanic Garden, Calcutta. The outcome of his expeditions to the Andaman Islands,

Burma, India, and Singapore resulted in the two-volume *Forest Flora of British Burma* (1877). He identified *Casearia kurzii* as a hairy variety of *C. glomerata*, giving the name *Casearia glomerata* var. *puberula*.

*Taxonomic Comments*—Pedicels of this species are much longer than any other species. Leaf shape and size of *Caseaia kurzii* resemble *Casearia vareca*, but the regularly close serrate margin of *C. vareca* differs from the shallowly serrate margin of *C. kurzii*.

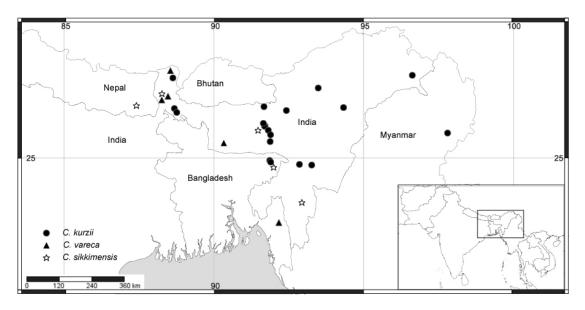


Figure 4.7. Distribution of Casearia kurzii, C. vareca, and C. sikkimensis.

7. CASEARIA RUBESCENS Dalzell, Hooker's J. Bot. Kew Gard. Misc. 4: 108. 1852.— TYPE: INDIA. Karnataka, Western Ghats, Syhadree Mountain, Lat. 15°, *Dalzell s.n.*(lectotype, here designated: K!-K000591430; isolectotype: K!-K65).

Evergreen trees, medium sized, 8–20 m; trunk 10–30 cm dbh, often branched from the base; bark 1 mm thick, cream-colored when young, turning brown with age, phloem smooth or slightly cracked; crown dense; branches conspicuously horizontal,

glabrous; branchlets terete, glabrous, with dense, large, irregular lenticels, young branchlets purplish red (Brandis, 1971), drying to purplish black; terminal bud glabrous. Leaf blade dark green glossy above, blackish green when dry,  $5-19 \times 3-7.5$  cm, ellipticoblong or elliptic-lanceolate; apex broadly acute or with short acumen (1-2 cm), acumen frequently curved; base cuneate or subacute, infrequently asymmetric, margin entire; coriaceous; both surfaces of both young and old leaves glabrous, pellucid transparent dots and lines closely arranged, midvein raised above, purplish (Brandis, 1971), glabrous on both sides, secondary veins 10–12 pairs, sometimes purplish red, tertiary veins broadly reticulate, but most short and perpendicular to the midvein and secondary veins, giving a half railroad track (or curved comb) appearance, clearly seen when dry or if specimen was collected in alcohol; petiole 0.7-1.6 cm, canaliculate, glabrous, noted as purplish red in color; stipules  $2.5 \times 1.5$  mm wide, scale-like, mostly glabrous or rarely appressed puberulose in the adaxial center, early caducous, leaving conspicuous triangular scars, sometimes persistent to the second leaf. Inflorescence subterminal, axillary clusters, glomerules sparse or dense with 5-10(-20) flowers; pedicels short, less than 5 mm, articulate near the base, sparsely spread minute hairs present, lower articulation densely pubescent. Flowers ca. 2.5–4 mm across, greenish white, with wine red tinge when young, calyx lobes 5, broadly elliptic-oblong,  $2 \times 2.5$  mm, glabrous, sometimes minutely pubescent, margin sometimes ciliate; stamens 8, filaments pubescent, staminodes oblong, truncate and villous at the apex, half as long as the stamens; ovary ellipsoid, glabrous, sometimes hairy, the style indistinguishable and minutely pubescent between stigma and upper end of the ovary, stigma globose with a thick margin. Fruit a capsule, mostly ellipsoid, sometime globular,  $1-2.5 \times 1-1.2$  cm wide, orange-yellow when ripe, glabrous

and varicose (having vein-like folding) when dry, 3-valved. Seeds up to 12, ca. 3 mm, mostly globose but flattened on one side, brick brown when dry with few pale white ornamental markings; aril not seen.

*Representative Specimens*—India. KARNATAKA: Shimogo, Kodachadri range,
13°N, 77°07'E [*sic*, 13°53'N, 74°52'E], *Saldanha et al.* 6836 (CAL).—KERALA:
Cardamom hills, 30 km from Munnar on Munnar-Periyar Road, [9°52'N, 77°08'E], *Ridsdale 170* (L[2]); Cardamom hills, Devicolam-Periyar road, mostly beyond the gap,
[10°03'N, 77°06'E], *Ridsdale* 673 (L[2]), *Ridsdale* 713 (L[2]); Idukki, Peermade,
Travancore, [9°34'N, 77°01'E], *Bourdillon s.n.* (K).— MAHARASHTRA: Bombay,
Thane, [19°13'N, 72°55'E], *Dalzell s.n.* (K); Konkan, [19°12'N, 72°54'E], *Hooker s.n.*(K); Konkan, *Shocks vc s.n.* (L[2]); Konkan, Malabar hill, [12°11'N, 75°37'E] *Shocks v.c. s.n.* (BM, G). TAMILNADU: Anaimalai Hills, 10°10'N, 77°03'E, *Barber 5994* (K);
Mount Nilghiri, [11°30'N, 76°45'E], V Kurg G00161563 (G); Nilgiris, Coonoor,
[11°23'N, 76°47'E], *Bourne s.n.* (K); Nilgiris, BM000948360 (BM). Place unknown, *Griffiths s.n.* (K), *Ridsdale s.n.* (L), *collector unknown*; 62K, 102K, K000591430.

*Phenology*—Collected in flower February to April. Fruits seen in June to September.

*Etymology*—The specific epithet refers to the veins and petiole which are red when they are young. Even the young fruit has a reddish tinge, a feature unique to this species in south-central Asia.

*Distribution and Habitat*—Endemic to the Western Ghats of India (Figure 4.9). A common subcanopy tree in higher elevation evergreen forests of the Western Ghats; 200–2500 m.

Casearia rubescens var. gamblei N.Mukh., J. Bombay Nat. Hist. Soc. 69(2): 393.

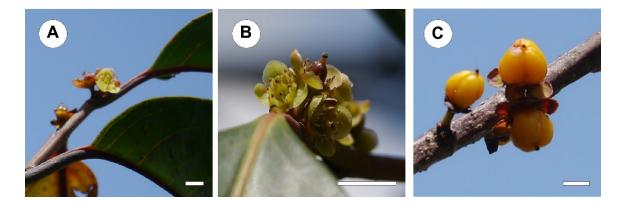
1972.—TYPE: INDIA. Peermerd, South India, *Bourdillon 181* (holotype: CAL; isotype: K!-K66).

*Casearia rubescens* var. *gamblei* was described by Mukherjee (1972) as it differs from the type of *C. rubescens* in having broadly lanceolate leaves and 9–11 pairs of lateral veins while *Casearia rubescens* sensu stricto has ovate-oblong leaves with obtusely acuminate apices and 6–8 pairs of lateral veins.

## KEY TO THE VARIETIES OF CASEARIA RUBESCENS

Leaves broadly lanceolate, $5.5-10.5 \times 3-6$ cm, apex broadly acute, infrequently curved,
base rounded to broadly acute, 9–11 pairs of lateral veins; fruits ellipsoid, large, ca. 2.5
cm, densely varicosevar. gamblei
Leaves broadly ovate to ovate-oblong, $7-19 \times 4-7.5$ cm, apex obtusely acuminate (1 cm),
commonly curved, base acute to cuneate, 6-8 pairs of lateral veins; fruits globose, small,
< 1.3 cm, glabrous var. <i>rubescens</i>

The stipule of the type specimen of *Casearia rubescens* var. *rubescens* is minutely puberulose in the middle, glabrous towards the margin, and the pedicels are puberulose. However, almost all specimens that are identified as var. *rubescens* have completely glabrous stipules, and some have completely glabrous pedicels. On the other hand, *C. rubescens* var. *gamblei* has completely glabrous stipules and pedicels. Consistency of those characters among var. *gamblei* is not known due to lack of specimens other than the type. (The type of var. *gamblei* does not have fruits. Fruit characters included from the specimens that are recognized as var. *gamblei*).



*Figure 4.8.* Morphology of *Casearia rubescens.* A. Inflorescence with few flowers; note reddish colored petioles and purplish color of young branches. B. Greenish yellow flowers with red tinge of ovary. C. Illipsoid to trigonous fruits which turn bright yellow when ripe. Scale bars = 1 cm. Photos courtesy of Dinesh Valke.

*Taxonomic Comments*—The morphology of *Casearia esculenta* Roxb. and *Casearia rubescens* Dalzell is very similar. *Casearia rubescens* is easy to identify from fresh material due to its glossy, slightly coriaceous leaves, mostly with acuminate, curved apices, purplish-red veins, petioles, and young stems, reddish tinge in the sepals and young fruits, and ellipsoid young fruits (Figure 4.8). *Casearia esculenta*, on the other hand, has dull, papery leaves, acute leaf apices, and globose fruits and lacks any prominent purplish red coloration. However, identifying dried material is very difficult. In the *Flora of British India* (1879), C. B. Clarke mentioned that the only difference between those species is pedicel pubescence, *C. esculenta* having glabrous pedicels and *C. rubescens* having pubescent pedicels. Most Indian *C. esculenta* have glabrous pedicels, stipules, and buds, but there are *C. rubescens* with glabrous pedicels. Therefore, I could not find any consistent character in dried herbarium specimens to delimit these two species. However, shorter tertiary veins starting from the midvein like railroad tracks are noted in *C. rubescens* which are not evident in *C. esculenta*. Therefore, we have to consider all characters collectively, including the geographic occurrence, to distinguish the two species.

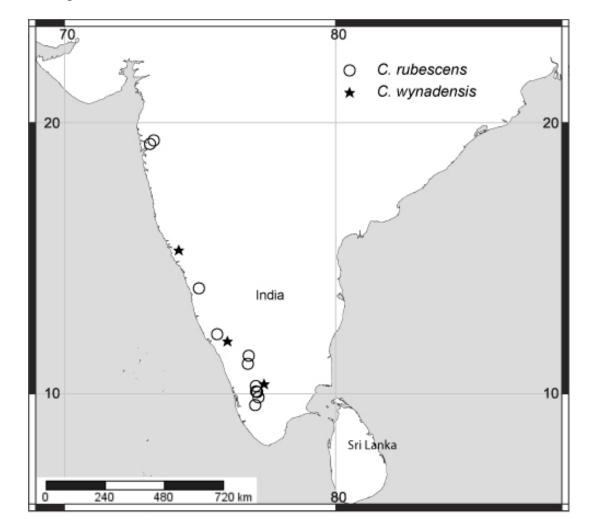


Figure 4.9. Distribution of Casearia rubescens and C. wynadensis.

8. CASEARIA SIKKIMENSIS N.Mukh., J. Bombay Nat. Hist. Soc. 69(2): 392. 1972;

Casearia glomerata Roxb. & DC. var. sikkimensis (N.Mukh.) R.C.Srivast.-

TYPE: J. S. Gamble in Dhobijhua ad Kurseong mense April anni 1882, *J. S. Gambel 10316* (CAL).

Large to medium sized trees, 4-15 m; branches grey, purplish black when dry, stem quadrangular, young branches puberulose, hairs short and spreading, soon becoming glabrous, completely glabrous after fourth leaf, leaves more or less perpendicular to the branch; bark lenticulate, lenticels prominent, more or less elongate; terminal bud densely pubescent, hairs spreading. Leaf blade  $6-9 \times 2.5-3.5$  cm, lanceolate or oblong-lanceolate, membranous, sparsely puberulous when young, soon becoming glabrous on both surfaces except the midvein of the lower surface, midvein mostly sparsely pubescent; translucent glands elongate and round; young leaf margin often serrulate (~7 teeth per cm), teeth quickly shed, older leaf margin distantly serrate or entire, apex shortly acuminate, acumen to 0.5 cm, gradually or more abruptly; base acute, less often obtuse, oblique; midvein canaliculate above; lateral veins 5–7 pairs, broadly arching upward, conspicuous on both surfaces, reticulations not so prominent; petioles 0.6–1 mm, slender, glabrous, canaliculate, inside the groove puberulose in very young leaves but soon becoming glabrous; stipules  $1 \times 0.75$  mm, triangular to ovate, small, scale-like, early caducous, sometimes seen as 3–5 tufted stipules together at the apex, glabrous, margin ciliate, apex acute, stipuler scar prominent. Inflorescence axillary glomerules, 1–1.5 mm diameter, much crowded, 8–15(–25) flowers; bracts scaly, many, broadly ovate, ca. 0.5–1 mm, rusty brown, glabrous or sparsely hairy, margin ciliate; pedicels 3-5(-8) mm long, non articulated or articulation not clear, slightly pubescent, hairs semiappressed; buds small, ca. 2 mm. Flowers with calyx 5-lobed, yellowish green, broadly ovate, 2.5 mm, outside tomentose, glabrous within or rarely sparsely pubescent, margin minutely ciliate to nearly

glabrous; stamens 7–8, filaments 2 mm long, hairy; anthers ca. 0.3 mm, ovate, basified; staminodes, filiform, equal in length to filaments, slightly narrowed to the apex; ovary ca. 1 mm, conical, glabrous, 1–2 ovules in each placenta (Mukherjee, 1972); style about 0.5 mm, glabrous; stigma discoid. Fruits 8–10 mm, globose (Mukherjee, 1972). Seeds 3–4, ovate and slightly compressed (Mukherjee, 1972).

Representative Specimens—Bangladesh. SYLHET: [24°54'N, 91°54'E], collector unknown 7987 (G). India. MEGHALAYA: Khasia, [25°55'N, 91°49'E], Hooker f. & Thomson 1100 (K).—MIZORAM: Lushai hills, [23°31'N, 92°56'E], Parney 223 (K).— WEST BENGAL: Darjeeling, [27°03'N, 88°16'E], Clarke 35224 (G), Gamble 6881A (K); India, locality unknown, Wallich, 7192D (K), Wallich 8003 (G). Nepal. KOSHI: Sunsari, Dharan, Dangi, 26°45'N, 87°25'E, Stainton 5708 (BM). Locality unknown, Wallich 8003 (G), collector unknown K000591428.

*Distribution and Habitat—Casearia sikkimensis* occurs in deciduous, mixed evergreen and warm broad leaved forests in the sub-Himalayan tract around Sikkim; 1000–1500 m (Figure 4.7).

Phenology-Collected in flower in April. Fruits seen in October.

*Etymology*—The specific epithet refers to the Sikkim area in India where it was first reported.

*Taxonomic Comments—Casearia sikkimensis* is sometimes considered a variety of *C. glomerata*. However, *C. sikkimensis* has smaller, lanceolate to oblong-lanceolate leaves with minute pubescence along the midvein and slender flowers in denser glomerules. Moreover, *Casearia glomerata* has densely pubescent twig tips, whereas *C. sikkimensis* has puberulose or almost glabrous tips. The leaf texture also differs considerably between the two species as *C. sikkimensis* has more membranous leaves and dries to have brittle leaves, while *C. glomerata* has thick, papery leaves. *Casearia glomerata* is a deciduous tree; the deciduousness of *C. sikkimensis* is not known.

In the original description, Mukherjee writes that it is related to *C. graveolens* but has membranous leaves, distantly serrate margin, pedicels not articulated, a hairy petiole, staminodes and filaments alike and equal in length, and globular fruits (Mukherjee, 1972). Although *C. sikkimensis* grows together with *C. graveolens*, the resemblance of both species is weak. However, *C. sikkimensis* may be easily mistaken as *Casearia tomentosa*, unless the vestiture is noted, and may be easily mistaken as *Casearia kurzii*, unless leaf size is noted.

- 9. CASEARIA THWAITESII Briq., Annuaire Conserv. Jard. Bot. Genève 2: 62. 1898. *Casearia coriacea* Thwaites, Enum. Pl. Zeyl. (Thwaites). 20. 1858—TYPE: SRI LANKA. Adam's Peak. *Thwaites C. P. 465* (holotype: PDA!; isotypes: BM!, G!, L!).
- *Casearia varians* var. *y obovata* Thwaites, Enum. Pl. Zeyl. (Thwaites). 20. 1858—TYPE: SRI LANKA. Nuwaraeliya, 7000–8000 ft (2000–2500 m), *Thwaites C.P. 1217*, *1247* (syntypes: BM!, G! [2], L!, PDA!). *Thwaites C. P. 1217* completely agrees with the protologue and is here designated as lectotype (lectotype: BM!; isolectotypes: G!, L!, PDA!).

Evergreen, medium-sized tree, 4–18 m or a shrub 2–3 m with a dense canopy; trunk up to 60 cm dbh, stout, gnarled, very rough with uneven bumps and cracks (*Worthington 6774*); bark 1 mm thick, pale yellowish; branches confined to the upper

part of the stem, grey with pale grey and black patches, completely glabrous, gnarled, lenticulate, lenticels prominent, raised, irregular in shape; young branches dark green or grey, terete but with uneven knots and peels, with conspicuous mold-like black speckles; terminal bud glabrous. Leaves congested mostly at the tips, red purple when young, blade 1.5-6 cm long (Indian individuals up to 11.5 cm), 1-4 cm wide (Indian individuals up to 7.5 cm), obovate or sometimes elliptic, thickly coriaceous or subcoriaceous, dark green (blackish green), dull, sometimes glossy, glabrous on both surfaces, midvein raised, purplish red tinge on the lower side, pale yellow above, lateral veins 5–6 (Indian individuals up to 7), sometimes young leaf veins have a slight red tinge, more or less straight and parallel to each other, vein parallel to margin is prominent, tertiary veins broadly reticulate, indistinct, translucent punctations or lines mostly round, very prominent, becoming rusty colored in dried material, sometimes seen as even small punctures on the leaf; apex mostly emarginate, obtuse to rounded; margin entire, often revolute, sometimes thick membranous; base cuneate or attenuate; petiole 4–8 mm long, flat on one side and convex on the other in cross section, young petiole purplish red, glabrous, leaf teeth present down to second young leaf, deciduous thereafter; stipules  $2 \times$ 1.5 mm, ovate or broadly triangulate, scale-like, glabrous, margin entire, early caducous, stipule scar raised, not prominent in the older leaves. Inflorescences on raised knobs, axillary fascicles, subterminal, not common in leafless axils, few flowered (up to 6 per knob or axil), but mostly flowers solitary; bracts ovate to triangular, small, ca. 1 mm, completely glabrous, longitudinally striate within, margin smooth, not ciliate; buds small, ca. 1 mm, globose to club-shaped, glabrous, sometimes with ciliate margin, pinkish white to greenish white, mostly with a red tinge; pedicels 2–4 mm long, articulated 0.5 mm

above the base (1 mm in fruits) but not clear in the flowers, below and above articulation glabrous. Flowers with 5-lobed calyx, lobes  $1.5 \times 2.8$  mm, rounded or broadly ovate, green with purplish red tinge, sometimes pale white, glabrous outside and within, margin slightly ciliate in the distal end; stamens 6–8, filaments 0.7 mm long, glabrous or puberulose, anthers 0.5 mm long; staminodes 6–8, 0.8 mm long, shaped like a square flap or club shape, glabrous, margin smooth or slightly ciliate; ovary ca. 2 mm, ovoid, glabrous; stigma broadly discoid; style indistinguishable. Fruits  $1.5-2 \times 1$  cm, orange or yellow, ellipsoid to oblong, mostly asymmetrical, apiculate, pericarp thick, smooth, mostly have three conspicuous ribs, glabrous, glossy when ripe, varicose / wrinkled when dry, 2-valved and split half way upon dehiscence. Seeds 6–9,  $5.5 \times 3$  mm, ovate to spindle shaped, pale brown with dark brown longitudinal streaks; aril orange-red, becoming pale brown, a thin film covering the seeds when dry.

*Representative Specimens*—India. KERALA: Wayanad, [11°56'N, 76°00'E], *Beddome 3146* (BM), *Beddome 3147* (BM).—TAMIL NADU: Anaimalai Hills, Coimbatore, Konolar, [10°18'N, 77°00'E], *Chandrabose 69008* (CAL); Anaimalai Hills, 10°10'N, 77°03'E, *collector unknown* (BM); Dindigul, Kodaikanal, Mahilkundram shola 15 km from Kodi to Berijam, [10°22'N, 77°58'E], *Matthew 53193* (K); Nilgiris, [11°23'N, 76°47'E], *Gamble 17320* (BM); Pulni hills, Gunudattu shoal, [10°12'N, 77°29'E], *Dalzell s.n* (K). India, place unknown, Jurwelly hills [text undecipherable], *Beddome 3149* (BM); place unknown, *Beddome 3148* (BM). Sri Lanka. CENTRAL: Nuwara Eliya, Adams Peak, slopes of Adams Peak along the SE trail from the Moray GroupTea Estates, [6°48'N, 80°29'E], *Davidse 8648* (US); Nuwara Eliya, Adam's Peak, [6°48'N, 80°29'E], *Bernardi 15783* (G[2]); Nuwara Eliya, Elk plains near Ambawella, [6°52'N, 80°48'E],

Kostermans s.n (G[2], L); Nuwara Eliya, Ambewela pattipola road near to Kande ela reserve, [6°55'N, 80°47'E], Samarakoon 100 (PDA, USMS[2]); Nuwara Eliya, Bambara Kellie, Worthington 6774 (L); Nuwara Eliya, Hakgalle jungle, [6°55'N, 80°49'E], Kosterman 24190 (US,L); Nuwara Eliya, Hortain plains between Farr Inn and Small World's End, [6°48'N 80°47'E], Waas 845 (L); Nuwara Eliya, Hortain plains Ohiya road, [6°47'N, 80°48'E], Nowicke et al. 272 (MICH); Nuwara Eliya, Hortain plains road to World's End, [6°46'N, 80°47'E], Cramer & Tirvengadum CP275 (L), Balakrishnan 1045 (MICH); Nuwara Eliya, Horton Plains along road from Ohiya to Farr Inn, [6°48'N, 80°48'E], Huber 662 (US); Nuwara Eliya, Horton plains along trail from Big World's End, near Galagama falls, [6°46'N, 80°46'E], *Theobald 2830* (US); Nuwara Eliya, Horton Plains, [6°48'N, 80°47'E], Farr Inn-Big World's End jungle patch, Cramer 4343 (US), Sohmer 10047 (US), road to World's End, Cramer & Tirvengadum 4025 (US), Cramer & Tirvengadum CP275 (G, US), Balakrishnan NBK1045 (K, US), Balakrishnan NBK1205 (US), Cramer 4343 (K), Bernardi 15881 (G[2]); Nuwara Eliya, Mount Piduruthalagala, [7°00'N, 80°46'E], Nooteboom 3262 (L, US); Nuwara Eliya, Mount Piduruthalagala slopes directly north of Nuwara Eliya, [7°00'N, 80°46'E], Davidse & Sumithraarachchi 8073 (L, US); Nuwara Eliya, Pattipola, mid way between horton plains and pattipola, [6°49'N, 80°48'E], Cramer & Tirvengadum CP113 (G, K, L, PDA, US); Nuwara Eliya, Sita-Eliya, Kandapola, [6°55'N, 80°48'E], Jayasuriya 8254 (PDA); Nuwara Eliya, Sita-Eliya, [6°55'N, 80°48'E], Jayasuriya & Sohmer 8459 (US); Nuwara Eliya, Jhon Miers 192 (BM), Worthington 6774 (K); Ratnapura, Adam's Peak, on the top cone at the north side, 6°48'N, 80°30'E, Bremer 990 (US).—SABARAGAMUWA,

Handapan Ella plains, above Bulutota pass, [6°28'N, 80°37'E], *Kostermans s.n.* (L). Sri Lanka, place unknown, *Thwaites 1217* (BM, G[2], L).

*Distribution and Habitat*—India and Sri Lanka (Figure 4.3), found in primary and secondary montane or cloud forests; 1500–2300 m. It is a canopy tree also found in forest edges and roadsides. In India it is recorded in high altitude evergreen montane forest patches (shola) of Agasthyamalai, Anaimalai, Palani hills, and Nilgiri (Western Ghats). In Sri Lanka it is found only in montane forests in the central hill range–Hortain planes, Piduruthalagala, and Knuckels forest reserves.

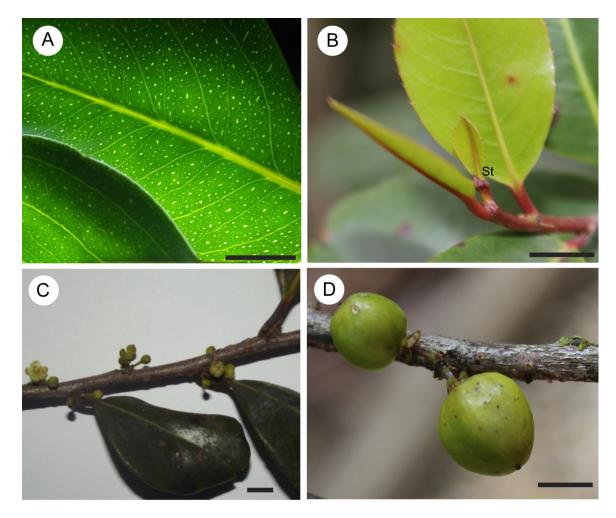
*Phenology*—Collected in flower November to June. Fruits seen in May to October.

*Etymology*—The specific epithet honors the British botanist and entomologist, George Henry Kendrick Thwaites (1812–1882), who worked as a superintendent of the Royal Botanical Gardens at Peradeniya, Ceylon (Sri Lanka) (1849–1880, from 1857 as Director). He is the one who first documented this plant in his *Enumeratio Plantarum Zeylaniae* (1864), but he misidentified it as Ventenat's *Casearia coriacea* (native to Java).

*Excluded Names*—Non *Casearia coriacea* Vent., Choix t. 45. 1803. Non *Casearia coriacea* Wall. Numer. List, 1828 (Wallich) *n.* 7196.

Taxonomic Comments—Indian Casearia thwaitesii has larger leaves  $(4.5-11.5 \times 2-7.5 \text{ cm})$  compared to Sri Lankan specimens with smaller leaves which never exceed 6  $\times$  4 cm. Verdcourt, in the *Revised Handbook to the Flora of Ceylon* (1996) considered *C*. *thwaitesii* from India as the same species, mentioning the extremely large leaves. The other features are more-or-less similar to each other. Other than larger leaves, few

subsequent differences are noted in the Indian material including orbicular leaf shape, deciduous teeth that are not evident or early caducous even before unfolding (Figure 4.10), and pellucid dots and lines that are not prominent, as in Sri Lankan *C. thwaitesii* material. However, not enough Indian material is available to check for more characters. Therefore, Indian *C. thwaitesii* is here considered as same as Sri Lankan *C. thwaitesii*.



*Figure 4.10.* Morphology of *Casearia thwaitesii*. A. Pellucid dots and lines. B. Glabrous, early caducous stipules with red tinge on young petioles and stem. C. Few-flowered axillary inflorescence. D. Globular fruits which turn bright yellow when ripe. Scale bars = 1 cm.

- CASEARIA TOMENTOSA Roxb., Hort. Bengal. 90. 1814, nom. nud.; Fl. Ind. Ed. Carey,
   2: 421. 1832; *Guidonia tomentosa* (Buch.-Ham.) Kurz, J. Asiat. Soc. Bengal, Pt.
   2, Nat. Hist. 46, 92. 1877.—TYPE: INDIA. Circar Mts., *Roxb. s.n.*; Illustration no
   147 of Icones Roxburghianae (lectotype; K! K000591434 here designated;
   isolectotype, BM!, CAL).
- Anavinga lanceolata Lam., Encycl. (Lamarck) 1(1): 148. 1783, non Casearia lanceolata
  Miq. Linnaea 18: 753. 1845 (=Casearia arborea (Rich.) Urb.). Casearia elliptica
  Willd., Sp. Pl. 2(1): 628. 1799, nom. illegit., non Casearia elliptica Klotzsch.
  Reis. Br.-Guiana (Ri. Schomburgk). 3: 1167, 1848, nom. nud.—TYPE: INDIA.
  'India orientali', Sonnerat s.n. (holotype: P-LAM [photo!]).
- *Casearia ovata* non (Lam.) Willd., sensu Roxb. Hort. Bengal. 90. 1814; Fl. Ind. 2. 420. 1832. nom. nud.—TYPE: INDIA. Roxburgh drawing 1431 (lectotype, here designated: CAL; isolectotype, K [photo!]).
- *Casearia anavinga* Dalz, Dalz and Gibs. Bomb. Fl. 11. 1861.—TYPE: INDIA. Caranjah hill and Rajahpoor.
- Samyda bazanica Buch.-Ham. ex Willd. 7192C Wallich cat. 1832, nom. nud.—TYPE: INDIA. Goyalpara, July 1808, Hamilton s.n. (Wallich cat. no. 7192C) (syntype, K [photo!]).

Samyda canziala Buch.-Ham., Trans. Linn. Soc. London 17(2): 225. 1837 (1837 publ. 25 May 1835); Numer. List (Wallich) 7192B, 1832; Casearia canziala (Buch.-Ham.) Wallich, Hort. Suburb. Calcutt. 78. 1840; Guidonia canziala (Buch.-Ham.) Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46, 92. 1877.—TYPE: INDIA. Habora, 21 March 1803, Hamilton s.n. (Wallich cat. no. 7192B) (holotype, K [photo!]). Samyda piscida Ham., Trans. Linn. Soc. London 17(2): 225. 1837; 7193D Numer. List (Wallich), 1832 ("piscidia"), nom. nud., orth. mut.—TYPE: Karik Kal, INDIA. 10 April 1811, *Hamilton s.n. Wallich cat. no. 7193D* (lectotype: K(7193D infra)!) Sukanazar, INDIA, 24 March 1810, Hamilton s.n. (syntype: K 7193D supra!).

*Casearia cheela* Royle, Ill. Bot. Himal. Mts. (Royle), 1, 170. 1839.—TYPE: INDIA. *Royle s.n.* (holotype: LIV [photo!]).

*Casearia piscidia* Buch.-Ham. ex Royle Ill. Bot. Himal. Mts. (Royle), 1, 170. 1839.— TYPE: INDIA. *Royle s.n.* (holotype: LIV [photo!]).

Casearia villosa Wight, 7191B Numer. List (Wallich), nom. nud. 1832.—TYPE: INDIA. Wallich 7191B (syntype: BM!, G!, K [photo!]).

Shrubs or small trees (2-)8-12(-16) m; trunk to 12-25(-35) cm dbh; bark brownish grey, rough, irregularly cracked, peeling with rounded or square shape flakes, inner bark red brown; living bark beefy red outside, dirty white or red brown inside (Verdcourt, 1996); branches spreading, horizontal, bare, leaves restricted to the tips of the branches, grey with paler prominent lenticel markings, fissured, sparsely pubescent or glabresent; young shoots densely tomentose; terminal bud densely pubescent. Leaves variable in size, 6-13 (northern Indian specimens 18-23) cm × 2.5-5 (northern Indian specimens 5-8) cm, narrowly elliptic to narrowly oblong elliptic, rarely oblong to lanceolate, both surfaces densely pubescent, sometimes upper surface becoming glabrescent when old, lower surface densely tomentose, papery, mostly glaucous above, pellucid dots and lines not visible to naked eye, clear at  $10\times$ , closely arranged; young leaf margin crenate or serrate, 6-9 teeth per cm, older leaves obscurely crenate or serrate, 1-3teeth per cm, mostly deep enough to count, infrequently entire to erose with a translucent

edge, frequently hairy; apex acute, obtuse, or slightly acuminate with an acumen up to 1-1.5 cm; base cuneate to rounded, frequently oblique; adaxial midvein pubescent to sparsely publication between the second and the second area of the second and the second area of the second ascending, broadly arched, mostly anastomosing near margins, sparsely pubescent above, densely pubescent below; tertiary veins broadly reticulate, pubescent below; petiole stout, 8-10 mm long, ca. 1.5 mm in diameter, densely tomentose; stipules  $15 \times 10 \text{ mm}$ , lanceolate to ovate, leafy, early caducous, rarely seen in the second young unfolded leaf, woolly tomentose, margin serrate, apex acute. Inflorescences of dense clusters of flowers or infrequently in few-flowered clusters or flowers solitary; bracts numerous, densely pubescent; pedicels articulated, pubescent above articulation, densely pubescent below articulation, pedicel above articulation 2–3 mm long, to ca. 4 mm in fruits. Flowers with calyx lobes 4-5,  $2-4 \times 2$  mm, ovate to obovate, or lanceolate, whitish or pale green, pubescent on both surfaces, upper surface frequently glabrous, margin ciliate; stamens 6– 10, filaments pubescent; staminodes 1–2 mm long, clavate, densely pubescent, ca. 1.5 mm long, about half long as stamens; ovary ovoid to globose, 1.5–2.2 mm, sparsely pubescent; style short, 1.5–2 mm long, obscure; stigma capitate. Fruits ellipsoid, oblong to globose,  $1-2.5 \times 1-2$  cm, fleshy, shining, glabrous, yellow when ripe, with six ridges, splitting into 3 (-4) valves, varicose when dry. Seeds up to 5, embedded in soft red pulp, angular or obovoid; aril is red colored (Figure 4.11).

*Representative Specimens*—**India.** BIHAR: *Hooker f. s.n.* (BM, G), *Hooker f. & Thomson* (K). GUJARAT: Gir Forest National Park, Pilipat 1 mile north of Saoam gir forest, [21° 6'N, 70°49'E], *Hodd 140* (K).—HIMAL: *Thomson s.n.* (BM, G, L).— JHARKHAND: Bengal, Rajmehal hiles, [24°47'N, 87°25'E], *Kurz 5767* (BM); Shikharji, Parasnath Hill, Behar, [23°59'N, 86°02'E], *Clarke 14867A* (BM); Hazaribagh, Chota Nagapur, [24°00'N, 85°23'E], Kerr 2069 (BM[2]), Kerr 2069c (BM), Parasnath, Behar, [23°59'N, 86°02'E], *Clarke 14062B* (BM).— KARNATAKA: Bombay presidency, North Canara, [13°03'N,75°23'E], Young s.n. (BM[2]); Dharwad, Kappatagudda hills, [15°21'N, 75°04'E], Sedgwick & Bell 5248 (K); Hassan, Mysore, Balupet-Saklespur road, 13°N, 76°E, Saldanha 12976 (US); Hassan, Mysore, 6 miles before Saklespur, 13°N, 76°E, Saldanha 13246 (MICH, US); Hassan, Mysore, Bagi, 13°N, 76°E, Saldanha 16331 (MICH, US); Hassan, Mysore, Konnaur road, [12°56'N, 75°48'E], Saldanha 16722 (MICH); Sultan Batlary, [11°39'N, 76°15'E], Suresh & Manilal 32870 (US).-KASHMEER: Rajauri, Nowshehra, [33°15'N, 74°23'E], *Clarke* 28187E (BM).— MAHARASHTRA: Konkan, [13°04'N, 74°54'E], *Slocks* (L).—MIZORAM: Lushai hills, [23°29'N, 92°56'E], *collector unknown* (K).—ODISHA: Jeypore hils, [18°51'N, 82°35'E], Beddome 3158 (BM); Odisha, Nayagarh district (Khandpara state), [20°07'N, 85°05'E], Mooney 931 (K).—RAJASTHAN: Ahmedabad, Mount Abu, [24°35'N, 72°42'E], Saxton 699 (K); Jodhpur, Bharatpur, between Sikandra & Talcher, [25°34'N, 81°58'E], Perumal 8750 (CAL).— TAMILNADU: Anaimalai Hills, [10°10'N, 77°03'E], Beddome 8207 (BM); Annamalai Nagar, Chidambaram, [10°10'N, 77°03'E], *Perumal 17879* (CAL); Coimbatore, Thanjavur, [10°18'N, 77°00'E], Nair 57162 (CAL); Madras, Iriguis [text undecipherable], [12°59'N, 80°13'E], Gamble 18370 (BM); Nochiyam, Tiruchirappalli, [10°48'N, 78°41'E], Xavier 23666 (K).—UTTAR PRADESH: Bahraich, [27°34'N, 81°35'E], Inayat 23654 (G), Bindal Nala, [30°14'N, 78°01'E], Bhardwaja 17 or 12 (K); Chowki Dhani, [30°04'N, 78°15'E], Koul s.n. (US); Dehradun, Jaunsar, Daukpattes, [30°44'N, 77°49'E], Bhatuagan 105 (US); Garhwal, [30°18'N, 78°33'E], collector

unknown 364 (L); Lachiwala, Mohand, [30°10'N, 77°54'E], Sriwastava (US); Nainital, Kumaon, Kaladhungi, [29°15'N, 79°19'E], Strachey & Winterbottom, 7191 (BM, K); Siwalik and Jaunsar, Ihans[text undecipherable], [29°00'N, 80°00'E], Sharma 222 (G[2]).—WEST BENGAL: Basirhat, 24 Pergumal, [22°39'N, 88°52'E], *Clarke 34828* (G-2,BM); Krishnanagar, Nadia, [23°24'N, 88°30'E], Sinclair 3555 (US); Midnapore, Bodadihi, [22°25'N, 87°19'E], *Maji 1134* (K); Murshidabad, *Clarke 26187C* (BM); Turang forest, [23°07'N, 86°30'E], Haines 999a (K); West Bengal, place unknown, *Clarke 11751* (BM). India, Deakiracrtah hills[text undecipherable], *Beddome 3156* (BM); Del. [text undecipherable], G.K... [text undecipherable] 1869 (BM); Monghpr jungle, Lockwood 7 77 (K), Nazaria [text undecipherable], A.V. M... [text undecipherable] s.n. (K); Ramandrooy [text undecipherable], Beddome 3155 (BM); Sandore hills [text undecipherable], *Beddome 3154* (BM); Zeyhur hills [text undecipherable], Beddome 3157 (BM); Culd Hills [text undecipherable], Beddome 3153 (BM). India, locality unknown, Wight 517 (G); Wight 517 (G); Hole 137 (US); Wallich 7192D (BM, G); Campbell s.n. 1836 (BM, G[2]); Hooker f. s.n. (US); Hooker f. & Thomson s.n. (L); Royle s.n. (K); Thomson s.n. (BM, L); Wight s.n. (BM); collector unknown 1019 (L), collector unknown 1021 (L), 1022 (L); collector unknown (K4); collector unknown (K89). Nepal. BAGMATI: Lalitpur, Godawari, 29°00'N, 80°40'E, Dobremez 1878 (BM), Dobremez 1818 (G).—BHERI: Bardiya, Bardia, 28°15'N, 81°45'E, Dobremez & Ramanathan 2393 (BM), Dobremez 2593 (G); Surkhet, Ramghat, 28°30'N, 81°45'E, Stainton 6216 (BM).—KOSHI: Sunsari, Dharan, 26°43'N, 87°16'E, Williams 56 (BM).—LUMBINI: Nawal Parasi, Tribenighat, [27°38'N, 83°53'E], Makin 129 (BM); Nawalparasi, Bishnanagar forrest, [27°38'N, 83°53'E], Makin 245 (BM);

Rupandehi, Khasyauli forest, *Makin 234* (BM).—NARAYANI: Chitwan, Churia hills, 27°30'N, 84°35'E, Troth 773 (BM, MICH); Chitwan, Tikoli, 27°38'N, 84°30'E, Stainton 5269 (BM); Chitwan, Wesche 1186 (BM); Nepal, Kuchkudu jungle [text undecipherable], Shrestha 3931 (BM); Nepal, Terai, Inayat 23654 (G). Pakistan. AZAD KASHMIR: Mirpur, [33°09'N, 73°44'E], Stuward 27250 (BM).—PUNJAB. Kangra, Bhadwar, [32°06'N, 76°15'E], Koeltz 4232 (MICH, US), Koeltz 4378 (MICH), Koeltz 4468 (MICH, US[2]), Rup Chand & Koelz 4419 (MICH, US); Rawalpindi, Chattar, [33°46'N, 73°14'E], Anwar 310 (US); Rawalpindi, Daman-e-Koh, Margalla hills, [33°45'N, 72°59'E], Hussain 70 (G); Rawalpindi Nurour, [33°36'N, 73°02'E], Nasir & Siddiqe 300 (US), Nasir 257 (MICH); Rawalpinidi, Saidpun, Stewart 13756 (US). Locality unknown: Bouton s.n. (G), Dr. Ritchie 1011 (K); Falconer 364 (G), Hb. Dc 1898 (G); Wallich 797 (BM); Wallich 7191B (BM); Wallich 7192E (K); Wallichi 7195 (BM); Wallich s.n. (BM); Wight 1019 (G); Wight 7191B (G); Young s.n. (BM-3). Locality unknown, collector unknown: 383 (G), BM000624264, BM000624272, BM000948378, G00161583, 325469US.

*Distribution and Habitat*—Burma, India, Nepal, Pakistan, and Sri Lanka. Widespread throughout India and in sub Himalayan countries including western Pakistan, Nepal, and Burma (Figure 4.6). Very common in moist, hilly subtropical forests up to 900 m elevation from the foothills of the Himalayas southwards. In southern India commonly distributed in open grasslands at lower elevations. Uncommon in the Western Ghats (Parrotta, 2001). In Sri Lanka, mostly distributed in lowland scrub and gallery forest in dry and intermediate zones where rain is sparse. Former indications of its distribution (e.g., Flora of British India) in Malaysia and Northern Australia are erroneous and may refer to another species.

Phenology—Flowers seen in February to March. Fruits seen in May and June. *Etymology*—The specific epithet refers to the thickly tomentose plant parts. *Common Names*—Commonly known as the downy-leaved false Kamela/Kamala ("true" kamala = Mallotus philippensis (Lam.) Mull.Arg., Euphorbiaceae),  $\dot{H}$ 3US maun

(Bengali) dholo-con, gholoum, munjhaad, หู่ชุด munjaal, munzal, sunjhal, (Gujarati),

छिल्ला chilla, bairi, beri, bheri, chilara, kirchi (Hindi), biliyubina, hanise, hlingi, konje (Kannada) bhedsi (Konkani), anakkarana, anavananni, vapunnakannan (Malayalam), chillara, karei, lainja, massei, modi, मोदगी modgi (Marathi), सानो बेथे sano bethe (Nepalese), girari, giridi, kakoli, khonji, kirtti, kokra (Oriya), chilhaka, kampillaka (Sanskrit), கடிச்சை katiccai, kaludukkutti, kottal, kutti (Tamil), charcho, చిలుకదుదుడి

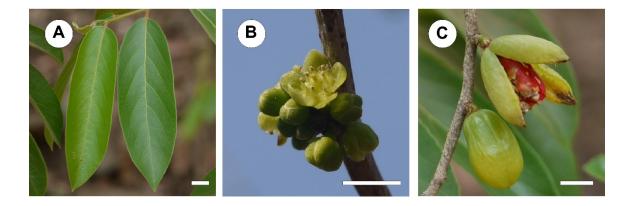
chilaka-dududi, gamudu, girugudu, kalamisvari, pisiki, vasanga (Telugu), tulo dedri (Nepali).

*Uses*—The juice from the fruit is used as a fish poison. The wood is good for making combs (Nasir and Ali, 1970). In India extractions obtained from various parts of this tree are used as an adulterant, anodyne by indigenous doctors also used to treat dog bite, snake bite, colic, diarrhea, diuretic, dropsy, fever, neuralgia, pleurisy, pneumonia, rabies, ringworm, scabies, sore, splenitis, etc. (Parrotta, 2001). The root bark is used to treat anemia and diabetes and to stimulate liver function. The leaf paste is used as an anthelmintic. The edible fruit pulp is a useful diuretic and has hypoglycemic properties. Oil extraction from seed is used extremely for sprains (Parrotta, 2001).

*Taxonomic Comments*—In some books *Casearia glabra* Roxb. is considered a synonym of *C. tomentosa*, but it is erroneous because Roxburgh mentioned that *Casearia glabra* is from the Molucca Islands and its leaves are glabrous.

Since *Casearia grewiifolia* var. *grewiifolia* does not occur in this area, it is easy to identify all tomentose material as *C. tomentosa* (*C. wynadensis* is also tomentose, but has a rusty colored tomentum). Morphologically, however, this species shows wide variation. Normally the leaf size is  $6-13 \times 2.5-5$  cm, but among northern Indian material I found material with extra large leaves about 18–24 cm and distinctly cuneate leaf bases (*Koelz 4419, Makin 129*). Leaf shape can also vary a lot, from ovate to elliptic to lanceolate to oblong.

Sri Lankan material is somewhat different from Indian material. Sri Lankan material has narrowly lanceolate to elliptic leaves, and the whole plant body is more glabrescent; only buds and young leaves are tomentose, and the inflorescence is few-flowered. Verdcourt, in the *Revised Handbook to the Flora of Ceylon* (1996), mentioned that it differs little from *C. zeylanica*, which is not entirely glabrous all the time, and he hypothesized that a once widespread population of *C. tomentosa* may have been virtually eliminated by introgression with *C. zeylanica*. Here, this revision applies Verdcourt's subspecies level for Sri Lankan C. *tomentosa*. It is important to mention that among the studied specimens none of the Sri Lankan *C. tomentosa* are as tomentose as Indian material and none of the Indian material is as glabrescent as the Sri Lankan *C. tomentosa*. Pollen from Sri Lankan *C. tomentosa* shows foldings in the ektexine (Figure 4.12).



*Figure 4.11.* Morphology of *Casearia tomentosa.* A. Oblong leaves with densely tomentose vesture. B. Crowded inflorescence with greenish yellow sepals. C. Oblong fruits which have bright red arils. Scale bars = 1 cm. Photos courtesy of Dinesh Valke.

Casearia tomentosa subsp. reducta Verdc., Revis. Handb. Fl. Ceylon (Dassanayake), 10:

234 (1996).—TYPE: CEYLON. Jaffna, Gardner in CP 1248 (K, holotype

[photo!]; PDA, isotype!). Casearia tomentosa Thwaites, Enum. Pl. Zeyl.

(Thwaites). 20. 1858. non Roxb. sensu stricto. Casearia elliptica Alson in Handb.

Fl. Ceylon 6:131.1931, non Willd. sensu stricto.

## KEY TO THE SUBSPECIES OF CASEARIA TOMENTOSA

Leaves lanceolate, leaf base cuneate; young stem, lower surfaces of leaves and

inflorescences much less tomentose, inflorescences few-flowered

...... Casearia tomentosa subsp. reducta

Leaves narrowly elliptic to oblong elliptic, oblong lanceolate, leaf base rounded; young

stem, lower surfaces of leaves and inflorescence densely tomentose, inflorescent densely

flowered ...... Casearia tomentosa subsp. tomentosa

Representative Specimens—Sri Lanka. SOUTHERN: Hambantota, Yala National park, [6°30'N, 81°27'E], Menik ganga 1 mile above yalu bungalow, Fosberg et al. 51054 (G, L, US), about 1 mile above mouth of Manik ganga, Fosberg 50220 (US), along Menik ganga, Cooray 69073132R (US), Bernardi 15551 (G[2], K, PDA), Davidse 7789 (L), Fosberg & Mueller-Dombois 50160 (US), Meijer 220 (K, PDA, US); Place unknown, Thwaites CP1248 (BM, G, K), collector unknown, 2664 (L).

11. CASEARIA VARECA Roxb., Fl. Ind. (Roxburgh) 2: 418. 1832; Hort. Bengal. 33,

1814—TYPE: INDIA. Silhet, Roxb drawing 2249 (lectotype: K [photo!], here designated; isolectotype: CAL). *Guidonia vareca* (Roxb.)Baill. ex Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46, 92. 1877.

Deciduous shrub or small tree, 2–6 m; bark pale brown to cream color, smooth, sometimes furrowed; young stems and branchlets terete, mostly drying black or greenish black, densely villose, hairs yellowish; older branches grey, puberulose, never quite glabrous; lenticels not prominent, like round dots, if present; terminal buds densely pubescent, golden brown. Leaf blade 8–11 × 2.5–4 cm, lamina usually oblong, rarely somewhat obovate, papery and brittle when dry, young leaf blade glabrous above, puberulose towards the margin and petiole, with yellowish, dense, soft, short hairs present along midvein and secondary veins, completely pubescent beneath, blackish green when dry, older leaves glabrous above, pubescent along midvein and secondary veins, softly long hairs beneath both lamina and veins, dense along the margin, older leaves drying to copper color, translucent glands

mostly round, a few as lines, very prominent, dense, evenly spaced; margin serrulate, 8 teeth per cm, evenly spaced, adaxial side of tooth concave, abaxial side straight, often ending in a very sharp point; apex abruptly narrowed to acute point 1.5–3 mm, base rounded to cuneate, slightly asymmetric; midvein sunken; lateral veins 6–10, almost equally spaced, arching at about a 50° angle, clearly visible in greenish brown or in blackish brown in the copper color background, raised underneath; tertiary veins broadly reticulate not prominent; petiole 5–8 mm long, ca. 1.3 mm diameter, densely hairy, sometimes with densely appressed short hairs on very old leaves, cylindrical, not grooved; stipules  $1.6 \times 1$  mm, quite glabrous, rarely found with few hairs, margin densely ciliate, stipule base densely hairy, often appearing as a hairy ball. Inflorescences of sessile crowded glomerules of 8-13(-18) flowers, rarely 1-5 in leaf axils, found in almost all leaf axils when in flowering; bracts membranous, brown, and very brittle in dried material, triangular to ovate, ca. 1.4 mm, semi-appressed hairs in the middle, sparsely hairy to glabrous towards the margin, margin ciliate; pedicel articulation prominent, pedicel approximately equal below and above articulation, up to 2 mm above articulation, very hairy below articulation, hairy and cup-like at the articulation, glabrous above the articulation, flower buds ca. 2 mm, globose, small, greenish yellow to greenish white, buds drying to copper color, glabrous, only top portion of the sepals ciliate. Flowers with calyx 5-lobed, lobes  $1.7 \times 1.4$  mm, obovate to ovate, completely glabrous, margin ciliate; staminal tube more elongated than any other species, brown, translucent in between stamens and staminodes, stamens often 8 rarely up to 10, filament broad, spasely hairy; staminodes almost long as stamens, margin densely hairy with long hairs, middle sparsely hairy to glabrous, top hairs drying white; style 0.4 mm, glabrous; stigma capitate

to conical, obscurely 3-lobed; ovary ca. 0.8 mm, conical, glabrous. Fruits 3–5 per inflorescence,  $5-6 \times 5-7$  mm, subglobose or broadly ellipsoid, hexagonal with three sharp lines, glabrous, rough but not varicose, orange or orange yellow when ripe, splitting into 3 valves to base, pericarp thin, 0.4 mm, aromatic. Seeds up to 6, ca.  $2.3 \times 3.8$  mm, arranged in two layers, three top and three bottom, in a bright red pulp, off white when dry, with brown longitudinal ornamental marks, asymmetrically ellipsoid; aril color not known.

*Representative Specimens*—Bangladesh. EAST BENGAL : locality unknown, Griffith 195 (G, L).—SYLHET: Bhola, [24°54'N, 91°54'E], Clarke 17418A (BM), Clarke 17418B (BM); Sylhet, 5 miles South of Sylhet, [24°52'N, 91°53'E], Gill 19 (CAL); Sylhet, Hooker f. & Thomson s.n. (BM); Sylhet, Hooker f. & Thomson s.n. (L); SYLHET: locality unknown, Abu Hussain 108 (CAL); Clarke 44789A (BM); Hooker f.s.n. (G[2]); Hooker f. & Thomson s.n. (BM, K, L); collector unknown (L0793760). Burma. KACHIN: N Jang Yang, 25°50'N, 97°48'E, Kaulback 183 (BM). India. ARUNACHAL PRADESH: Akha Hills, [26°42'N, 91°40'E], Badul Khan 18 (L); Daphla Hills, [27°19'N, 93°29'E], Badui Khan 45 (US); Tirap, Khonsa, Menoka, [27°45'N, 96°37'E], Koeltz 29262 (MICH, L); Triap, Tirap-Namchik divide 16 mile mark on Ledo road, Menoka, [27°45'N, 96°37'E], Juan 97 (G).—ASSAM: Cachar, [24°47'N, 92°51'E], Prazer s.n. (L); Darrang, Chari Duar, [26°35'N, 92°25'E], Parry 1269 (K); Stainton 6688 (K); Jorhat, Hoollongapar Gibbon Sanctuary, Sibsagar, [26°40'N, 94°19'E], JOR 247 (K); Kamrup, Bamanigaon, Koeltz 24271 (MICH, L), Rup Chand 2531 (MICH, L); Kamrup, Guwahati, along the road side 16/4 miles from gauhati, [26°04'N, 91°42'E], Panigrah 5274 (L); Kamrup, Guwahati, [26°03'N, 91°41'E], Clarke 37122 (G), Clarke 37122A (BM);

Kamrup, Palasbari, [26°07'N, 91°32'E], Koeltz 29193 (MICH, L).—ASSAM: Colonel Jenkins s.n. (L); Dr. King's collector s.n. (US); Dr. Prain's Collector 273 (BM, G); Jenkins s.n. (L).—MANIPUR: Kala Naga hills, [24°46'N, 93°15'E], Prazer 8 (US); Manipur, place unknown, Meebold 6101 (K).—MEGHALAYA: East Khasi hills, Cherrapunji, collector unknown 28216 (L); East Khasi hills, East Nurting, Clarke 44789 (G[2]); East Khasi hills, Shillong, Nongphoh, [25°55'N, 91°49'E], Koeltz 23045 (L, MICH); East Khasi hills, Shillong, [25°32'N, 91°52'E], Rao 10755 (L), Rao 10821 (L[2]); East Khasi hills, Umran, [25°46'N, 91°53'E], Koeltz 22466 (MICH, L), Rup Chand 8277 (MICH), Rup Chand 19989 (L); Garo hills, Parry 1269 (K); Khasi hills, Mawryngkeneng, Rup Chand 5104 (MICH).—NAGALAND: Naga Hills, Baligam, Dr. Prain's collector 245 (L, US).—SIKKIM: [27°43'N, 88°38'E], Clarke 17973C (BM), Clarke 26541A (BM), Hooker f. s.n. (K[2], L, G, US); collector unknown 198 (L).— UTTAR PRADESH: Lakhimpur Kheri, Between Tinsukia and Rangagora, [27°55'N, 80°47'E], Rock 872 (US), Rock 877 (US).—WEST BENGAL: Jalpaiguri, [26°31'N, 88°43'E], Chandra 1022 (CAL), Mukerjee 1341 (CAL). India, locality unknown, Clarke 40731 (US), Clarke 40716 (G), Griffith 195 (G), Toppin 4483 (K), Wallich 7192D (G), collector uknown G00161639; Place unknown, Wallich 7191 (G), Wallich 7194a (G[3]), Wallich 7197a (G); collector unknown, BM000624261, G00161597, G00161600, G00161603, G00161605, G00161638, G00161643.

*Distribution and Habitat—Casearia vareca* occurs in subtropical deciduous forests, in sub-Himalayan tracts in India, Bangladesh, Bhutan, and Nepal (Figure 4.7). Especially common in "Sal" (*Shorea robusta*) forests in Assam, Khassia, and Sylhet at altitudes of 0–1000 m. *Phenology*—Flowers seen in February to October. Fruits seen in September to February.

*Etymology*—The specific epithet refers to the *Vareca* of Gaertner. Roxburgh thought this species is the same one that Gaertner described as *Vareca zeylanica*.

Common Names—Sylhet (India): "Tetahehera" (Roxburgh, 1832).

 CASEARIA WYNADENSIS Bedd., Icon. Pl. Ind. Or. 34. 1874.—Type: India. Kerala, Wynaad, alt. 2000–3000 ft. (500–1000m), *Beddome s.n.* (holotype, here designated: K!; isotypes: MH!).

Trees or treelets, up to 5–7 m; trunk 6 cm; bark smooth; branches plagiotropous, horizontal, terete, softly pubescent, older branches puberulose; branchlets and young parts densely golden to dull brownish yellow pubescent; terminal buds densely pubescent, hairs semiappressed. Leaf blades 5–15 × 2.5–5.5 cm, lanceolate, narrowly elliptic, elliptic oblong, or oblanceolate, thickly papery, translucent glands very clear in brown color in dried material under 10×, glabrous above except veins; apex acute to acuminate, acumen 1–1.5 cm, mostly curved; base acute; margin serrate, membranous and translucent, 4–5 teeth per cm, teeth concave both sides, regular spacing with a rounded sinus, tooth apex having an early deciduous nipple-shaped thickened cap, mature leaves obscurely serrate with membranous margin, midrib glabrous at the distal end, becoming densely pubescent and grooved toward the base on the upper surface and densely pubescent throughout beneath, lateral nerves/secondary veins 8–12 pairs, pinnate, arching upwards, glabrous upper side, sparsely hairy beneath, tertiary veins reticulate; petiole 6–12 mm, slender, fulvous tomentose, deeply canaliculate; stipules small, linear,

early caducous, not seen even in the first leaf,  $2.5 \times 0.1$  mm, densely pubescent with appressed hairs. Inflorescences of loose fascicles, in axils of older leaves, 1–4 flowers; pedicel 4 mm long, not articulated or articulation not clear, pubescent with appressed hairs. Flowers greenish white, calyx lobes 4, 3 mm long, ovate, hairy within, glabrous outside; stamens 8, filaments hairy, 2 mm long, alternating with staminodes, tufted at their apex; ovary oblong, 1-locular, ovules many; style to 3 mm, simple, hairy; stigma conspicuously 3–4 lobed (trifid [Gamble, 1997]). Fruit a capsule, 2–2.5 × 1 cm, oblong, 3 ridged, orange (Gamble, 1997), 3-valved. Seeds not seen; aril color not known.

*Representative Specimens*—**India.** GOA: Bombay Presidency, Mulgaon, Pro parte salsotte, [15°17'N, 74°07'E], *Santapau 5315/214* (L).—KERALA: Munar (*sic*. Palghat), Silent valley, [10°05'N, 77°03'E], *Kostermans 26201* (L, K); Wayanad, [11°56'N, 76°00'E], *Beddome s.n.* (K).

*Distribution and Habitat—Casearia wynadensis* is endemic to the Western Ghats of India in Kerala and Tamil Nadu Districts (Figure 4.9). It is an understory tree in wet evergreen forests at higher elevations, 600–2000 m.

Phenology—Collected in flower in October–February.

*Etymology*—The specific epithet refers to the Wayanad District (*Wynaad*), a district in northeastern Kerala state, India.

*Taxonomic Comments*—Differs considerably from *Casearia tomentosa* by the narrower leaves with acuminate tip and having rusty color hairs. *Casearia wynadensis*, is listed as Vulnerable B1+2c ver 2.3 (IUCN 1998, 2014).

13. CASEARIA ZEYLANICA (Gaertn.) Thwaites, Enum. Pl. Zeyl. 19. 1858. Vareca zeylanica Gaertn., Fruct. Sem. Pl. 1. 290. t. 60, fig 6. 1788.—TYPE: SRI LANKA. Ceylon E collect. sem. hort. Lugdb. ("Seeds from Leiden Botanic Garden") (holotype: L, n.v.); C.P. 415 (lectotype: here designated, L!)

Casearia esculenta Roxb. var. angusta Fl. Brit. India (J. D. Hooker) 2: 592. 1879.-

TYPE: SRI LANKA (CEYLON). *Thwaites C. P. 2603* (holotype; PDA!, isotopes: L!, G!).

Evergreen tree 8–30 m; crown dense; trunk dbh 12–70 cm; bark smooth, sometime cracked, grey to whitish, live bark 10 mm, pale brown to cream color; crown ca. 5 m, sometimes branched from the base, branches conspicuously horizontal, sagging and straggling, young branches terete, conspicuously angular with ridges and furrows, glabrous, lenticels present but not many, blackish purple in color when dry, older glabrous; terminal buds densely pubescent. Leaf blades  $10-17 \times 4-7.5$  cm, ovate, ovatelanceolate to broadly elliptic-oblong; both young and older leaves completely glabrous, coriaceous, older leaves drying to dark brown color, translucent glands prominent and dense, varying in size and shape, appearing as small punctures under 10x; margin entire, undulate when dry; apex broadly acute or shortly acuminate with an acumen of ca. 0.5 cm, apex infrequently curved; base rounded and shortly cuneate, usually slightly oblique; midvein glabrous, prominent, grooved; lateral veins 7–9 pairs, faint, raised underneath; tertiary veins broadly reticulate, not prominent; petiole long, 1-1.5 cm long, ca. 1.5 mm diameter, slender, puberulose, grooved, puberulose, especially inside the groove; stipules  $2 \times 1$  mm, scale-like, glabrous throughout, rarely puberulose in the adaxial centre, margin smooth, several buds releasing at the same time having a cluster of stipules at the same

time, subpersistent, mostly can see up to 5–6 mature leaves. Inflorescences subterminal, mostly found at leafless nodes, crowded glomerules, 1.5 cm of diameter, with 10–20 flowers; bracts membranous, brown, minute, ovate, ca. 0.5 mm, hairy, glabrous within, margin smooth; pedicel, articulation not prominent, minute as 0.01 mm, ca. 3–5 mm above articulation, comparatively long, slender (ca.15 mm in fruits), minutely densely to sparsely pubescent below articulation, densely to sparsely appressed hairy above the articulation; flower buds ca. 2 mm, oval to globose, small, ca. 2 mm, semi-appressed hairy, margin not ciliate. Flowers with calyx lobes 5, lobes  $2 \times 2$  mm, obovate to ovate, densely hairy outside and within, margin ciliate, persistent; stamens often 6–8, filaments 1.5 mm, gradually tapering, sparsely hairy; staminodes half as long as stamens, truncate, margin densely hairy, apex with densely tufted long hairs which turn white when dry; anthers 0.7 mm, basifixed; style 0.5 mm, glabrous; stigma capitate; ovary ca. 1 mm, glabrous, one locular, with 3 parietal placentae. Fruits ellipsoid, ca.  $1-1.5 \times 0.6$  cm, mature fruits apparently glabrous but minutely, densely hairy with yellowish hairs, appearing as dust on fruits, dehisced fruits not seen and dehiscing sutures not prominent, yellow when ripe, has an unpleasant odor (*Worthington*, 1651). Seeds 5,  $4 \times 2.5$  mm, ovate, angular, black when dry; arils fleshy and scarlet (*Hladik 1057*).

*Distribution and Habitat*—Southern India and Sri Lanka (Figure 4.3). In India, the species is found in Central Kerala, in wet evergreen forest at 1200 m. It is recorded in the Cardamom Hills, on the road from Thekadi to Devicolam, in the Anamallays, and is said to be common in the Western Ghats in dense moist forests at 500–1000 m (Beddome, 1873). In Sri Lanka the species is very common along the entire coast and has a wide elevation range, from sea level up to 1500 m, and a wide habitat range, found in dry zone scrubs, disturbed secondary forests, mangroves and swamp margins, sand dunes and in sand spits with jungle, and very common in gallery forest along sandy river banks. It is also recorded in primary and secondary submontane forest as well as tropical forests.

*Phenology*—Collected in flower from March to May. Fruits seen in June to October.

*Etymology*—Joseph Gaertner (1732–1791) named the species *Vareca zeyalnica* based on the vernacular name of the seeds, "Wal-wareca," which he recieved from Sri Lanka.

Common Names—Walwaraka (Sinhala), Kakkapalai, Kakapelar, காக்கைப்பலா Kakkaipalai, குட்டி kutti, Tey pala (Tamil)

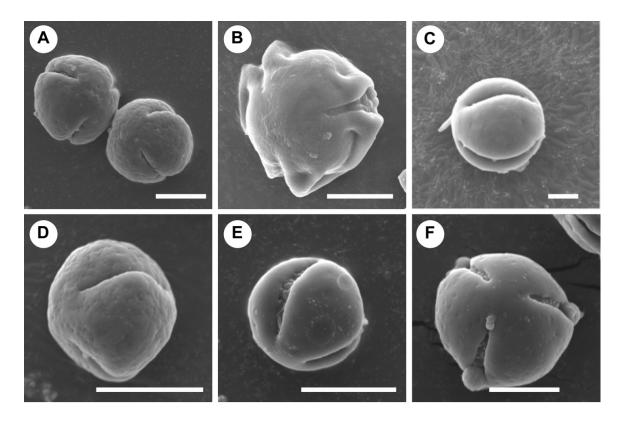
*Notes*—Beddome (1873) included one species of *Casearia* in his *Flora Sylvatica*, which covered the trees of southern India and Sri Lanka. He referred to this species as *Casearia varians* Thw., but unfortunately his circumscription included both *C. esculenta* Roxb. and *C. thwaitesii* Briq. as circumscribed here. His description was accompanied by a good illustration based on living material. Gamble (1920) and Mukherjee (1965) noted that the illustration did not match typical *C. varians* as described by Thwaites, and it also did not easily match *C. esculenta* Roxb. or *C. rubescens* Dalzell. Therefore Gambel used *C. varians* Bedd. not of Thw. for Beddome's species. But Beddome's species cannot have the binomial "varians" as it was already used to describe another species. For that reason Mukherjee introduced *Casearia bourdillonii* to describe Beddome's figure in *Flora Sylvatica* t. 208. Unfortunately, he picked *Bourdillon 104* from evergreen forest near Colatoorpolay to describe that species, which is not as same as Beddome's figure as well as most of the diagnostic features he used to differentiate two species are not present in the type specimen he used, which is here considered a synonym of *C. graveolens* (see

note under *C. graveolens*). Few specimens collected by Kostermans (26138, 710) around Codamom hills, which has hairy terminal buds and subpersistent stipules, beyond a doubt match Beddome's figure t. 208.

*Representative Specimens Examined*—India. KERALA: Cardamom hills Devicolam-Periyar road, [9°52'N, 77°08'E], Ridsdale 710 (L[2]), road Thekkady to Devi colam, [10°03'N, 76°58'E], Kostermans 26138 (G, K, L).— THAMILNADU: Cumbum, [9°43'N, 77°17'E], Jacob 77247 (K), Jacob 17790 (K); Madras (Chennai), Sriharikota, [11°29'N, 76°44'E], *Gambel 12832* (K); India, Jurvvelly hills [text undecipherable], Beddome 3151 (BM). Sri Lanka. CENTRAL: Kandy, Peradeniya Botanical garden, [7°16'N, 80°35'E], Kostermans 24504 (L[2]); Kanneliya forest near Hiniduma, [6°18'N, 80°19'E], Kostermans 28632 (G); Knuckles, [7°22'N, 80°50'E], Kostermans s.n. (G, L[2]); Madugoda, Kaluwella road, Hunasgiriya, [7°24'N, 80°42'E], Kostermans 26731 (G[2], L); Peradeniya, Botanic Gardens, [7°16'N, 80°35'E], Kostermans 24584 (G).— EASTERN: Ampara, Gallodai near Padiyatawala, [7°16'N, 81°40'E], Huber 473 (PDA, US).—EASTERN: Batticaloa, Trincomalee road 4 miles northwest of Batticaloa, [7°49'N, 81°20'E], Davidse & Sumithraarachchi 8989 (L); Batticaloa, Keeli-Kudah, [7°55'N, 81°34'E], Waas 2127 (US); Batticaloa-Trincomalee road, mile marker 30/1, Balakrishnan NBK 364 (US); Man. Keni, East Coast, Worthington 6704 (L); Trincomalee, behind the Pandanus zone in dense coastal scrub, *Kostermans* 27720 (L-3); Trincomalee, Foul point, 8°30'N, 81°19'E], collector unknown 9677 (BM); Trincomalee, Kuchchaveli road from Nilla, [8°41'N, 81°11'E], Nowicke & Jayasuriya 292 (US); Trincomalee, Kuchchavelli, [8°48'N, 81° 5'E], Kosterman 24807 (BM, G[2], K, L, US); Trincomalee, Muttur, [8°27'N, 81°16'E], Worthington 1651 (BM-3); Trincomalee,

Bernardi 15290 (G[2]).—NORTH CENTRAL: Anuradapura, Wilpattu national park, [8°21'N, 80°24'E], *Tirvengadum & Waas 782* (L, US); Polonnaruwa, 1 mile north eastern of Elahera along Aban ganga, [7°43'N, 80°47'E], *Davidse 7366* (K, L, PDA, US); Polonnaruwa, Sacred area along N-S P270, Hladik 1057 (US); Polonnaruwa Sacred area inside the forest, *Hladik 921* (US).—NORTH WESTERN: Belligama, Kurunegala, [7°29'N, 80°20'E], Worthington 955 (BM); Kurunegala, Weerambugedara, Samarakoon 99 (PDA, USMS[2]); Puttalam, Wilpattu National park, Kokkare villu, [7°35'N, 79°52'E], Wirawan 1066 (L, MICH, US); Puttalam, Wilpattu National park, Kuda Patessa, [8°26'N, 80°02'E], Wirawan 1147 (K, MICH); Wilpattu national park, 3 miles west of office, [8°26'N, 80°02'E], Meijer 352 (L); Wilpattu national park, close to plot W39, north of Marai villu, on Puttalam-Mannar road side, *Wirawan et al.* 890 (L); Wilpattu national park, West of WNP between Perya & Periya Naga villus, [8°26'N, 80°02'E], Mueller-Dombois & Wirawan s.n. (US); Polonnaruwa, Polonnaruwa jungle, Kosterman 24312 (US); Polonnaruwa, Sacred area, [7°55'N, 81°00'E], Ripley 75 (US).— Sri Lanka. place unknown, Thwaites C.P. 260 (BM); Thwaites 413 (BM); Thwaites 415 (BM, G[4], L), *Thwaites C.P. 2603* (G[2], L).

Palaiottu, [9° 3'N, 80°45'E], Cramer 4375 (US); Trincomalee, [8°47'N, 81°07'E],



*Figure 4.12.* Pollen diversity of *Casearia* spp. in study area. A. *Casearia graveolens*, oblique polar view with ektexine proliferations B. *Casearia tomentosa* subsp. *reducta*, oblique polar view with folding of ektexine. C. *Casearia sikkimensis*, equatorial view with smooth ektexine. D. *Casearia thwaitesii*, oblique polar view with ektexine proliferations. E. *Casearia kurzii*, smooth ektexine. F. *Casearia glomerata*, with few ektexine proliferations. Scale bars =  $10 \mu m$ . SEM images produced from FEI Quanta 200 scanning electron microscope; samples sputter coated with silver.

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#### CHAPTER V

# PROPOSAL TO REJECT THE NAME ANAVINGA OVATA (SAMYDACEAE) Proposal

Anavinga ovata Lam., Encycl. (J. Lamarck & al.) Suppl. 1(1): 148. 1783. Casearia ovata
(Lam.) Willd., Sp. Pl. 2(1): 629. 1799.—TYPE: India, Malabar, Cochin, Rheede, Hort.
Mal. 4: t. 49, 1683. [Angiosp.: Samyd.], nom. utique rej. prop.

Rheede (1683) introduced the genus *Anavinga* in his *Hortus Malabaricus*. He described one species from sandy places near Kochi (=Cochin, Kerala state, India) and provided an illustration (Tab. 49). The illustration is somewhat stylized but includes significant details of the leaf shape, leaf margins, and fruits, and the accompanying description (101–102) is rather thorough, even including habitat and phenology. Adanson (1763) was the first to recognize *Anavinga* in the post-1753 systematics literature. Lamarck (1783) then recognized the first two species in the genus, and one of those species was based on Rheede's Tab. 49 and named *A. ovata*. Because there are no original specimens of this *Anavinga* from Rheede (or collaborators) and no specimens are cited by Lamarck, the type of *Anavinga ovata* is by default the illustration in Rheede.

In 1760 Jacquin introduced the genus *Casearia* for New World species in his *Enumeratio Systematica Plantarum*. Willdenow (1799) later recognized the close affinities of *Anavinga* and Jacquin's *Casearia* and transferred *Anavinga ovata* to *Casearia*, the name with priority. The species and related species have been recognized in *Casearia* ever since.

# Rheede's illustration

(http://www.biodiversitylibrary.org/item/14376#page/305/mode/1up) shows a branch

with ovate leaves with crenate/serrate margins and globose fruits, somewhat 3-angled. The description augments the illustration by indicating that the leaves are glabrous, evergreen, and have an unpleasant odor and that the flowers do not have an odor. Unfortunately, the illustration and description do not permit unambiguous assignment of this name to a particular species. There are two currently recognized species in the Malabar region which mostly fit this description: *Casearia tomentosa* Roxb. and *C*. graveolens Dalzell. Casearia tomentosa has tomentose leaves that would surely have been noted by Rheede, one of few botanists whom Linnaeus referred to as a trustworthy author (Linnaeus, 1737; Müller-Wille and Reeds, 2007). Casearia graveolens has deciduous leaves and flowers with an unpleasant odor, which, too, disagrees with the description. So, the application of the name *Casearia ovata* is ambiguous. The illustration best matches C. graveolens, and the mistakes in the description (i.e., evergreen leaves, inodorous flowers) could easily have been misinterpretations or a wrong attribution to the source of the odor, as an odor was reported from the leaves. Stipule morphology is an important character in *Casearia*, and could perhaps resolve this dispute, although this character is rarely mentioned in the older works.

The name *Casearia ovata* is also historically confusing. Roxburgh (1832) provided a thorough description of *Casearia ovata*, even citing Rheede's illustration, but he described the species as having deciduous, downy, and serrulate leaves, contradicting the description in Rheede (and Willdenow). Roxburgh's own illustration (Icon. Roxb. unpublished, http://apps.kew.org/floraindica/img/illustration/large/4590.jpg) indicates that he was actually describing *C. tomentosa*. Roxburgh also introduced *C. esculenta* in his *Flora Indica* (2[1]: 422), which he described as glabrous with entire leaves. Since

then, the names *C. ovata* and *C. esculenta* have been used interchangeably for the same entity, even though Rheede's illustration clearly shows toothed leaves. Furthermore, Roxburgh indicated that his *C. esculenta* was probably the same as *another* species described by Rheede called "Tsjerou Kanneli" (1685).

C. B. Clarke (1879) completely ignored or failed to cite both Lamarck's *Anavinga ovata* and Willdenow's *Casearia ovata*, but he did note that Wallich (1832) misapplied the name *C. ovata* (in the sense of Roxburgh, i.e., *C. tomentosa*) to a specimen of *C. glomerata*. Mukherjee (1972) later tried to solve these nomenclatural and taxonomic problems. He argued that *C. ovata* sensu Rheede and Willdenow is the same entity as *C. esculenta* and Gaertner's *Vareca zeylanica* ( $\equiv C.$  *zeylanica* (Gaertn.) Thwaites). However, *Casearia esculenta* and *C. zeylanica* differ in having entire leaves and in lacking odor . *Casearia zeylanica* also has densely pubescent buds.

Thus, the name *C. ovata* has been used inconsistently in the literature, while *C. esculenta*, *C. graveolens*, and *C. tomentosa* have been used consistently. Displacing any of these would introduce instability.

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#### CHAPTER VI

# ENHANCING PCR AMPLIFICATION OF DNA FROM RECALCITRANT PLANT SPECIMENS USING A TREHALOSE-BASED ADDITIVE

### Abstract

PCR amplification of DNA extracted from plants is sometimes difficult due to the presence of inhibitory compounds. An effective method to overcome the inhibitory effect of compounds that contaminate DNA from difficult plant specimens is needed. The effectiveness of a PCR additive reagent containing trehalose, bovine serum albumin (BSA), and polysorbate-20 (Tween-20) (TBT-PAR) was tested. PCR of DNA extracted from fresh, silica-dried, and herbarium leaf material of species of Achariaceae, Asteraceae, Lacistemataceae, and Samydaceae that failed using standard techniques were successful with the addition of TBT-PAR. The addition of TBT-PAR during routine PCR is an effective method to improve amplification of DNA extracted from herbarium specimens or plants that are known to contain PCR inhibitors.

# Introduction

Despite wide application of polymerase chain reaction (PCR) over two decades, PCR inhibitors are still a major impediment to successful DNA amplification of plant DNA. Various compounds, including polysaccharides and phenolic compounds in plant tissues, can inhibit PCR amplification (Jobes et al., 1995; Wilson, 1997). Although commercial DNA extraction kits claim to remove most inhibitory compounds from plant tissues more efficiently than "home brew" DNA isolation methods (e.g., Peist et al., 2001), many plant molecular biologists still encounter negative PCR results due to the presence of inhibitors derived from the plant tissue itself. In our case, failure to amplify DNA from particular plant species was a persistent problem even when the template DNA was extracted from fresh tissue and spectrophotometric analysis indicated high DNA yield and quality.

To overcome the effects of PCR inhibitors, several studies indicate that diluting DNA extracts is a successful solution (e.g., Kontanis, 2006; Ma, 2007; pers. obs.). With the dilution of template DNA, though, one has to compensate for the sensitivity of the PCR reaction (Kontanis, 2006), especially when the DNA extractions are from old or poorly preserved plant materials, which normally give a low yield of DNA, or when fungal (or other) contaminants are likely present. In our experience, dilution produces inconsistent results. DNA from some recalcitrant taxa still fails to amplify even when diluted 1:1000. To overcome this PCR inhibition problem, the efficacy of a PCR additive reagent was tested. The reagent, containing trehalose, bovine serum albumin and Tween-20<sup>®</sup> (TBT-PAR), has been routinely used for the detection of a virus in shrimp samples (Cao et al., 2010). Trehalose stabilizes proteins and backbone fluctuations of DNA (cf. Butler and Falke, 1996). BSA, a common additive to PCR solutions, is known to have high lysine content, and phenolic compounds may bind with lysine and thereby prevent their binding to and inactivation of Taq polymerase (Kreader, 1996). Addition of nonionic detergents like polysorbate-20 (Tween-20) or Triton X-100 can neutralize negative effects of sodium dodecyl sulfate (SDS) (Wilson, 1997). As little as 0.01% SDS contamination of the template DNA left-over from the extraction procedure can inhibit PCR by reducing *Taq* polymerase activity to as low as 10% (Wilson, 1997; Peist, 2001). Polysorbate-20 may also suspend other plant inhibitors.

#### Methods

TBT-PAR was prepared as a 5X solution. The 5X solution contains 750 mM trehalose (Cat # T9531, Sigma-Aldrich, St. Louis, MO, USA), 1 mg/mL non-acetylated BSA (Cat # B4287, Sigma-Aldrich), 1% Tween-20<sup>®</sup> (Cat # 23336, Acros, Geel, Belgium), and 8.5 mM Tris hydrochloride (Cat # BP1758, Fisher Scientific, Pittsburg, PA, USA), pH 8.0. See Appendix for step-by-step protocol for preparing the 5X solution. It is important to note that the effectiveness of different TBT-PAR preparations in enhancing PCR can vary. The most critical factor appeared to be the source (manufacturer) of trehalose. We found trehalose from Sigma-Aldrich (Cat # T9531) to work well whereas several lots of trehalose from another chemical company did not work.

A 50 mL screw-cap polypropylene centrifuge tube was used to prepare the reagent because 10 mL can be mixed thoroughly by swirling without excessive foaming. The reagent was aliquoted into smaller tubes depending on the estimated amount needed for each experiment and frequency of use. The reagent was stored at 4°C when frequent use was anticipated and discarded after one week or frozen at -20°C in a non-frost-free freezer for long-term storage. Multiple freeze-thaw cycles of the reagent was avoided. During PCR, TBT-PAR was used at 1X concentration (e.g., 5  $\mu$ L of 5X TBT-PAR in a total PCR volume of 25  $\mu$ L).

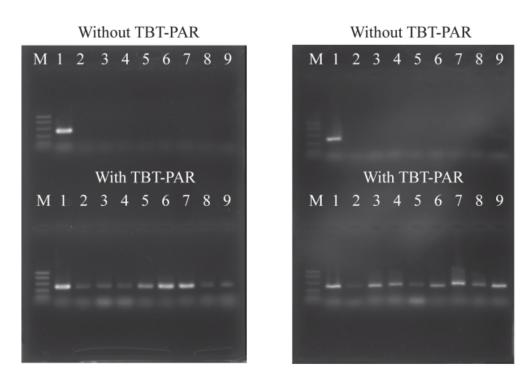
The efficacy of TBT-PAR was tested with template DNA from species of Achariaceae, Asteraceae, Lacistemataceae, and Samydaceae extracted from fresh samples, field samples collected in silica gel, and old herbarium specimens that had previously never produced positive PCR results using standard techniques (see Figure legends for species and vouchers). PCR amplification with and without the enhancer was compared. Plant genomic DNA was isolated using a Qiagen DNeasy plant kit (Qiagen, Inc., Valencia, CA). The plastid intragenic spacer trnL-F was used to test TBT-PAR activity, due to the relative abundance of plastid DNA and the small size of the trnL-F fragment (ca. 350 bp). DNA was amplified using Takara Premix Ex Taq (ver. 2.0) (Takara Bio, Otsu, Japan) in 50  $\mu$ L reactions consisting of 25  $\mu$ L of Premix solution, 2.5  $\mu$ L of each primer at 100  $\mu$ M concentration, 2  $\mu$ L of template DNA at a concentration of approximately 2-27 ng/ $\mu$ L, and either 18  $\mu$ L of water or 8  $\mu$ L of water plus 10  $\mu$ L of TBT-PAR. The amplification reaction consisted of 3 minutes initial denaturation at 94°C followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 minute followed by 2 minutes of final extension at 72°C. PCR products were separated by size in 1% agarose gels, stained with ethidium bromide, and photographed with 312 nm UV transillumination.

#### Results

Results with the TBT-PAR enhancer reveal consistent and widespread efficacy in improving PCR amplification (Figure 6.1). Among DNA samples extracted from fresh or silica-dried leaves from eight recalcitrant species of Samydaceae, one (the control) was PCR amplified successfully in the absence of TBT-PAR (Figure A, upper panel), but all amplified with the addition of TBT-PAR (Figure A, lower panel). Similarly, among DNA extracted from herbarium specimens of eight species, one (a control known to amplify under standard conditions) amplified successfully without TBT-PAR (Figure B, upper panel), but all amplified with the addition of TBT-PAR (Figure B, lower panel). Slight differences in size of the PCR product are likely due to variable-length repeats within the trnL-F spacer, which often different in length even in closely related species. For example, among *Casearia sylvestris, C. bartlettii*, and *C. commersoniana* (AY757012-AY757014), the trnL-F sequences range from 340 to 372bp. Although TBT-PAR enhanced amplification of recalcitrant DNA, PCR inhibition was probably not eliminated completely as evidenced by the varying yields. Other possible causes for the difference in PCR yield among samples include low template DNA concentration (2-27ng/µL) and/or possible partial DNA degradation. All trials with fresh or silica-dried material to date have yielded positive results when TBT-PAR was used, including amplification of other regions (e.g., plastid trnL intron, ndhF; nuclear ITS, embryo defective 2765 (EMB2765), nuclear granule-bound starch synthase (GBSSI)). Similarly, about 80% of trials with herbarium material dating to 1989 have yielded positive results. Sequences obtained from the amplified DNA have been acceptable to excellent.

# Conclusions

TBT-PAR was an effective additive in improving PCR amplification of DNA from plant specimens or from old and potentially degraded museum specimens. The additive is prepared using relatively inexpensive components and thus can be included in all routine amplifications to maximize the probability of PCR success. Demonstrated here to be effective in enhancing PCR of DNA from two dicot lineages (Malpighiales and Asterales), TBT-PAR has potential for widespread use in amplifying recalcitrant samples.



*Figure 6.1.* A. Gel electrophoresis of PCR products obtained from amplification of template DNA extracted from fresh or silica-dried leaves of nine Samydaceae species with and without the TBT-PAR additive. The species name is followed by voucher specimen in the herbarium of USMS, unless otherwise noted, and year of collection. Upper lanes are without TBT-PAR and lower lanes are the same samples with TBT-PAR. 1-Casearia grewiifolia Vent. (Harwood 4, 2010), positive control, 2-Casearia sp. (Harwood 1, 2010), 3-C. calva Craib. (Harwood 3, 2010), 4-C. flavovirens Blume (Harwood 5, 2010), 5-C. velutina Blume (Harwood 8, 2010), 6-C. coriacea Vent. (Baider & Florens 25058, 2011), 7-C. corymbosa Kunth (Alford s.n., 2011), 8-C. tacanensis Lundell (Davidse 36910 [MO], 2000), 9-C. mauritiana Bosser (Baider & Ploseus 2309, 2011). B.Gel electrophoresis of PCR products obtained from amplification of template DNA extracted from herbarium specimens of eight species with and without the TBT-PAR additive. Upper lanes are without TBT-PAR and lower lanes are the same samples with TBT-PAR. 1-Casearia grewiifolia Vent. (Harwood 4, 2010), positive control, 2-Ophiobotrys zenkeri (Chatelain 1446 [WAG], 2004), 3-Casearia tremula (Griseb.) Griseb. ex C.Wright (Machuca 7051 [NY], 1993), 4-C. hirtella Hosok. (Victor LR26709 [US], 1996), 5-Osmelia maingayi King. (Church et al. 2777 [A], 1995), 6-Gamochaeta simplicicaulis (Willd. ex Spreng.) Cabrera (Ahles & Liesner 32138, 2007), 7-Pseudognaphalium obtusifolium (L.) Hilliard & B.L.Burtt. (Alford 4197, 2011), 8-Lacistema aggregatum (P.J.Bergius) Rusby. (Alford s.n., 2010), 9-Mocquerysia multiflora Hua (Dechamp 13215 [WAG], 1989).

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