The tropical African legume Scorodophloeus clade includes two undescribe Hymenostegia segregate genera and *Micklethwaitia*, a rare, monospecific genus from Mozambique

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ARTICLE INFO

ABSTRACT

Legume subfamily Caesalpinioideae accommodates approximately 2250 species in 171 genera which traditionally are placed in four tribes: Caesalpinieae, Cassieae, Cercideae and Detarieae. The monophyletic tribe Detarieae includes the Amherstieae subclade which contains about 55 genera. Our knowledge of the relationships among those genera is good in some cases but for many other genera phylogenetic relationships have been unclear. The non-monophyletic nature of at least two amherstioid genera, *Cynometra* and *Hymenostegia* has also complicated the picture. During the course of a multi-disciplinary study of *Hymenostegia* sensu lato, which includes phylogenetic analyses based on *mat*K and *trn*L data, we have recovered the "Scorodophloeus clade", an exclusively tropical African clade of four genera which includes the eponymous genus *Scorodophloeus*, two undescribed generic segregates of *Hymenostegia* sensu lato, and the previously unsampled rare monospecific genus *Micklethwaitia* from Mozambique. *Zenkerella* is suggested as a possible sister genus to the Scorodophloeus clade. A distribution map is presented of the seven species that belong to the Scorodophloeus clade.

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1. Introduction

Leguminosae is the third largest angiosperm family estimated at 19, 500 described species (LPWG, 2013) divided among three subfamilies of which Papilionoideae and Mimosoideae are monophyletic. The third subfamily, Caesalpinioideae is a paraphyletic basal grade from within which the other two subfamilies arose. In terms of species, the caesalpinioid legumes are the smallest subfamily at around 2250 species in c. 170 genera (Lewis et al., 2005). Traditionally, Caesalpinioideae has been recognised in four tribes; Caesalpinieae, Cassieae, Cercideae and Detarieae sensu lato. The latter is a strongly supported monophyletic pantropical group which accommodates approximately a third of all caesalpinioid legume species and is most diverse in tropical Africa. Members of Detarieae sensu lato (Detarieae from here on) are very diverse morphologically. Several classifications have been proposed for the approximately 750 species (Mackinder, 2005) but Polhill (1994) was first

to recognise Detarieae as a single broadly circumscribed taxon. Earlier classifications instead recognised the diversity in two smaller tribes but with differing delimitations (Breteler, 1995; Cowan and Polhill, 1981; Léonard, 1957). A series of higher level reconstructions based on plastid data have produced a broad phylogenetic framework for Detarieae including establishing the Amherstieae clade (AMC) (Bruneau et al., 2008). The pantropical AMC comprises some 55 genera containing an estimated 565 species of trees or occasionally shrubs, but no lianas or herbs. Clade biodiversity is highest in tropical Africa from where about 290 species are known; approximately 155 species are found in the Neotropics and some 120 species are recorded from the SE Asian tropics.

Within the AMC, four well supported subclades are consistently recovered in phylogenetic analyses (Bruneau et al., 2000, 2001, 2008): the Saraca clade, (tropical Asian distribution; 4 genera); the Afzelia clade (pantropical distribution; 3 genera); the Brownea clade (neotropical distribution; 7 genera) and the Berlinia clade (tropical African distribution; 17 genera). Together these four clades account for 31/55 described genera. An ongoing combined taxonomic and phylogenetic study of *Hymenostegia* sensu lato has led to an improved understanding of relationships among several other AMC taxa which we report here.

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1.1. Taxonomic focus of the study: Hymenostegia sensu lato

Hymenostegia sensu lato is a genus of small to large tropical African forest trees. The c. 17 species currently assigned to the genus have (1) 2 to many-jugate paripinnate leaves, bear large, often showy, persistent paired petaloid bracteoles on the pedicel and have compressed, single (rarely 2-) seeded dehiscent pods.

1.2. Generic delimitation of Hymenostegia sensu stricto

1.2.1. Insight from morphology

Despite the shared combination of characters detailed above, there is evidence from a suite of vegetative morphological characters that the genus as currently delimited is an artificial assemblage (Mackinder et al., 2010). Several characters such as stipule fusion, presence of an auricle at the stipule base, presence of crater-shaped glands on the lower surface of the leaflets, type of lower leaflet surface indumentum and leaf rachis morphology together suggested that only six species of Hymenostegia sensu lato were congeneric with the type Hymenostegia floribunda Harms, (Mackinder et al., 2010). Since then, three additional Hymenostegia sensu stricto species, H. elegans Wieringa & Mackinder, H. robusta Wieringa & Mackinder (Wieringa and Mackinder, 2012) and H. viridiflora (Mackinder and Wieringa, 2013) have been published. Of the species considered not congeneric, it was hypothesised that Hymenostegia afzelii (Oliv.) Harms and H. laxiflora (Benth.) Harms may represent an undescribed genus and also that H. ngouniensis Pellegr. may be best recognised as a new monospecific genus (Mackinder et al., 2010).

1.2.2. Insight from phylogenetic analyses

Phylogenetic analysis of caesalpinioid legumes based on trnL intron data sampled six and eight species of Hymenostegia sensu lato respectively (Bruneau et al., 2000, 2001) including Hymenostegia afzelii and H. ngouniensis. Both studies recovered a clade of "core" Hymenostegia species (Hymenostegia sensu stricto from here on) which comprised the type species and four (Bruneau et al., 2000) or five (Bruneau et al., 2001) other Hymenostegia species. H. afzelii and H. ngouniensis were neither resolved within Hymenostegia sensu stricto, nor placed together, findings that are congruent with the predictions based on the suite of vegetative morphological characters discussed under Section 1.2.1. H. afzelii was recovered with Scorodophloeus zenkeri Harms as a weakly supported species pair whilst the position of H. ngouniensis was either unresolved (Bruneau et al., 2000) or in an unsupported sister relationship to a clade containing three Afzelia species and Intsia bijuga (Colebr.) Kuntze (Bruneau et al., 2001).

More recently, Bayesian analyses based on combined trnL intron and matK data recovered a strongly supported Hymenostegia sensu stricto clade, and confirmed, again with strong support, a close relationship between H. afzelii and Scorodophloeus zenkeri (Bruneau et al., 2008). The combined cpDNA analyses placed Hymenostegia ngouniensis as sister to the H. afzeli–S. zenkeri pairing (Bruneau et al., 2008) with strong support. Hymenostegia laxiflora, the putative sister species of H. afzelii, was not sampled, nor were the two other species of Scorodophloeus, S. fischeri (Taub.) J.Léonard and S. torrei Lock.

1.3. Relevance of the genus Cynometra to this study

Cynometra sensu lato is a pantropical genus of approximately 90 species of caesalpinioid legumes with species diversity divided fairly evenly among the three main tropical regions (Mackinder, 2005). The monophyly of Cynometra sensu lato (Cynometra from here on) has long been doubted (Léonard, 1951). South East Asian species may be congeneric with the genus Maniltoa (Bruneau et al., 2001, 2008; Mackinder, 2005; LPWG, 2013). The c. 30 species endemic to the Neotropics are the subject of an ongoing PhD study (Radosavljevic, in prep.). The considerable morphological diversity of the c.30 African species of Cynometra led the Belgian Botanist, Jean Léonard, one of the most knowledgeable African Caesalpinioideae taxonomists of all time, to declare that Cynometra in Africa is "un veritable depotoir dans lequel ont et glissees d'innombrables especes qu'on ne savait ou classer" that is "a veritable dump where countless species have been slipped that no one knew where to classify" (Léonard, 1951). He divided African Cynometra into three morphologically defined groups. There exists the possibility that part (or parts) of the species diversity presently accommodated within Cynometra may be more closely related to some of those species currently misplaced in Hymenostegia sensu lato.

The monospecific genus Micklethwaitia was originally described in Cynometra by Harms (1899) but was given generic status by Léonard (as Brenaniodendron) (Léonard, 1999). For nomenclatural reasons it was subsequently renamed as Micklethwaitia (Lewis and Schrire, 2004). Micklethwaitia carvalhoi (Harms) G.P.Lewis & Schrire, sampled for DNA analysis for the first time here, cannot be accommodated readily in one of Léonard's proposed three groups of Cynometra and was considered as closely resembling the genus Scorodophloeus (Lewis, 1996).

1.4. Research questions addressed in this study

Using phylogenetic analyses the following morphology based hypotheses are tested.

1. H. laxiflora and H. afzelii are sister species which neither belong in Hymenostegia sensu stricto nor in any described genus.
2. Micklethwaitia does not belong in Cynometra sensu lato but is closely related to Scorodophloeus.

2. Materials and methods

2.1. Taxon sampling

The sampling for the present study included taxa sampled in previous studies Bruneau et al., 2001, 2008) as well as 12 newly sampled taxa. Voucher details of newly sequenced accessions are given in Table 1. New additions include Hymenostegia laxiflora, Scorodophloeus fischeri and a further accession of the genus not yet confirmed to species, as well as Micklethwaitia carvalhoi. Due to the suspected affinities between Hymenostegia sensu lato and Cynometra sensu lato, six species of Cynometra (C. abrahamii Du Puy & R.Rabev., C. brachyrrhachis Harms, C. crassiflora Benth., C. filifera Harms, C. mannii Oliv., and C. sp.) and two species of its close relative Maniltoa (M. gemmipara Scheff. ex Backer and M. lenticellata C.T.White) were sampled. Two accessions of the genus Plagiosiphon additional to those used in Bruneau et al. (2008) were sequenced here, as it was resolved, in part, to be affiliated to H. afzelii, H. ngouniensis and S. zenkeri in that earlier study. We also included several accessions from the Afzelia, Berlinia and Brownea clades of the AMC (Bruneau et al., 2008), and the genera Leonardoxa, Loesenera, and Talbotiella that were resolved in a clade with Hymenostegia sensu stricto in that study, as well as several taxa whose position within the AMC is currently unresolved (Amherstia, Crudia, Cryptosepalum, Dicymbe, Humboldtia, Neochevaliodendron, Normandiodendron, Paramacrolobium, Polystemonanthus, Tamarindus and Zenkerella). Finally, several accessions (Saraca declinata Miq., Lysidice rhodostegia Hance and Endertia spectabilis Steenis & de Wit) from the Saraca clade were included as outgroups because the Saraca clade is the sister lineage to the remaining members of the AMC (Bruneau et al., 2008). The complete taxon sampling and GenBank accession numbers for sequences included in this study is presented in Appendix 1. To minimise the amount of missing data in the analyses, we selected accessions for which both DNA markers (see below) were available.

Table 1
Voucher details of new accessions sampled for this study. Taxa included for the first time in a published study are denoted with a double asterisk.

Taxon	Collector and collector number	Country collected	Herbarium code where voucher is deposited
Brownea jaramilloi 1**	Pérez 34/2	Ecuador	K
Brownea jaramilloi 2**	Villa 1606	Ecuador	K
*Cynometra abrahamii**	Nusbaumer LN 1220	Madagascar	K
*Cynometra brachyrrhachis**	Clark 3449	Tanzania	K
*Cynometra crassiflora**	Klitgaard 424	Ecuador	K
*Cynometra filifera**	Luke 10205	Tanzania	K
Hymenostegia afzelii 2	Van Andel 4244	Cameroon	WAG
*Hymenostegia laxiflora**	Mbou 398	Congo Brazzaville	K
Hymenostegia ngouniensis	Wieringa 4493	Gabon	WAG
Hymenostegia neoaubrevillei	Breteler 14793	Gabon	WAG
Lebruniodendron leptanthum	Wieringa 1659	Gabon	WAG
Maniltoa lenticellata	Bray 08937	Australia (CULT)	K
*Micklethwaitia carvalhoi**	Muller 4168	Mozambique	K
*Plagiosiphon decipher**	Wieringa 2316	Gabon	WAG
Plagiosiphon sp. nov. 2**	Wieringa 4039	Gabon	WAG
*Scorodophloeus fischeri**	Faulkner 4025	Tanzania	K
Scorodophloeus cf. *torrei**	Luke 8788	Tanzania	K
Scorodophloeus zenkeri 2	Breteler 14073	Gabon	WAG
Scorodophloeus zenkeri 3	Wieringa 5067	Gabon	WAG

2.2. DNA sequencing

Total DNA was extracted from 0.2 to 0.3 g of leaf and/or flower tissue from herbarium or silica gel dried material using a modification of the Doyle and Doyle's (1987) method (Csiba and Powell, 2006). We sampled the plastid *trn*L intron and the *mat*K gene and flanking 3′ intron DNA regions, due to their proven utility in elucidating phylogenetic relationships of Caesalpinioideae in previous studies (Bruneau et al., 2001, 2008). DNA was purified using QIAquick columns (Qiagen, Crawley, West Sussex, UK) and following manufacturer's protocol. Amplifications were performed in 25 μL volume reactions. The *trn*L intron was amplified with primers c and d of Taberlet et al. (1991). The amplification of *mat*K was performed in two fragments, using primer pairs *mat*KLa and *mat*K1932R (Wojciechowski et al., 2004), and *mat*K1100F (Wojciechowski et al., 2004) and *trn*KRdet (Bruneau et al., 2008), as outlined in Bruneau et al. (2008). For problematic specimens, the first fragment was sequenced in three fragments using the additional primer trnK685F (Hu et al., 2000; Lavin et al., 2000). The PCR protocol for *trn*L included 4 min initial denaturation (94 °C) and 30 cycles of 1 min denaturation (94 °C), 1 min annealing (50 °C), and 1 min elongation (72 °C). The PCR protocol for *mat*K included 1 min initial denaturation (94 °C) and 38 cycles of 30 s denaturation (94 °C), 1 min annealing (48 °C), and 1 min elongation (72 °C). Both protocols included an initial denaturation phase (94 °C) of 2 min and a final elongation phase (72 °C) of 7 min.

Sequences from both strands were acquired from an Applied Biosystems 3730 capillary DNA automated sequencer (ABI, Warrington, Cheshire, UK) using Big Dye terminator v3.1 chemistry, following manufacturer's protocols (ABI). Both contigs were assembled and edited with Sequencher version 4.5 (Gene Codes, Ann Arbor, Michigan, USA). All sequences have been submitted to GenBank (Appendix 1). Sequences were aligned in BioEdit v. 7.0 (Hall, 1999), using ClustalW (Thompson et al., 1994) and by making manual adjustments.

2.3. Phylogenetic analyses

Maximum parsimony analyses were performed with PAUP* 4.0b10 (Swofford, 2002). Heuristic searches were conducted with 1000 random taxon addition replicates, and tree-bisection-reconnection (TBR)

branch swapping, holding 10 trees at each step with MULtrees on. Clade support was estimated with the bootstrap (Felsenstein, 1985): 1000 bootstrap replicates, simple taxon addition, TBR branch swapping, and holding 10 trees at each step. Bayesian analyses were conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). The programme MrModeltest version 2.2 (Nylander, 2004) was used to find the most appropriate model for the data set for each DNA region separately. The model selected was GTR + I + G for both *trn*L and *mat*K, with four substitution rate categories. Two analyses were performed simultaneously, with 10,000,000 generations of four Markov chain Monte Carlo searches (MCMCs) sampling every 1000th generation. Bayesian analyses were run on the cluster of CIPRES Science Gateway (http://www.phylo.org/) and the parameters were checked for convergence with the software Tracer v.1.5 (Drummond and Rambaut, 2007). The first 2500 (25%) trees of low posterior probability were deleted from each run and all remaining trees were imported into PAUP* 4.0b10 to compute a majority rule consensus tree, which is the maximum clade credibility Bayesian tree, referred to hereafter as the Bayesian tree (Fig. 1). Percent frequencies of each clade in the post burn-in trees are the Bayesian posterior probabilities (PP).

3. Results

3.1. Sequence characteristics

The lengths of the DNA regions and ranges among taxa included are similar to those reported in Bruneau et al. (2008). The *mat*K region was 1911p long in the aligned matrix, and the initial aligned length of the *trn*L intron was 901 bp. However, due to ambiguous alignment, 285 characters (323–358 and 399–648) were excluded from the trnl data. Of the 2526 characters included, 275 (10.9%) were potentially parsimony informative; 61 (9.8%) for *trn*L and 214 (11.2%) for *mat*K.

3.2. Phylogenetic analyses

The maximum parsimony analysis yielded 4440 equally most-parsimonious trees of 985 steps with consistency index (CI) = 0.72 and retention index (RI) = 0.78. The maximum clade credibility of Bayesian analysis shows similar overall topology to the strict consensus of the parsimony analysis, but it is generally more resolved, and support in posterior probabilities (PP) for individual clades is relatively higher than the respective BPs. As the strict consensus and the Bayesian tree are congruent, here we present only the Bayesian tree (Fig. 1).

3.3. Phylogenetic relationships

The Saraca, Brownea and Berlinia clades were all recovered in the strict consensus parsimony tree (not shown) and Bayesian analyses (Fig. 1). The Afzelia clade was recovered in the Bayesian analyses but accessions of its constituent genera (*Afzelia*, *Intsia* and *Brodriguesia*) were placed in unresolved positions in the parsimony strict consensus tree (not shown). In addition to those clades, several other groups were recovered in both the parsimony strict consensus and Bayesian trees.

The three species of *Hymenostegia* sensu stricto sampled are placed together with accessions of *Leonardoxa*, *Talbotiella* and *Loesnera*. *Cynometra* species are recovered in two separate clades, one exclusively *Cynometra* and the other with species of *Maniltoa*. *Plagiosiphon* is resolved as a monophyletic genus. *Cryptosepalum staudtii* Harms and *Paramacrolobium coeruleum* (Taub.) J.Léonard are resolved as a species pair, as are *Dicymbe alstonii* Sandwith and *Polystemonanthus dinklagei* Harms.

A clade of 11 accessions, representing seven species, three of *Scorodophloeus*, three of *Hymenostegia* sensu lato and *M. carvalhoi* is recovered (Fig. 1). *Hymenostegia laxiflora* and *H. afzelii* are resolved as sister species with strong support. *Mi. carvalhoi* is placed as a weakly supported sister of *H. ngouniensis*. The "Scorodophloeus clade" is recovered in all equally most parsimonious trees, but with low bootstrap
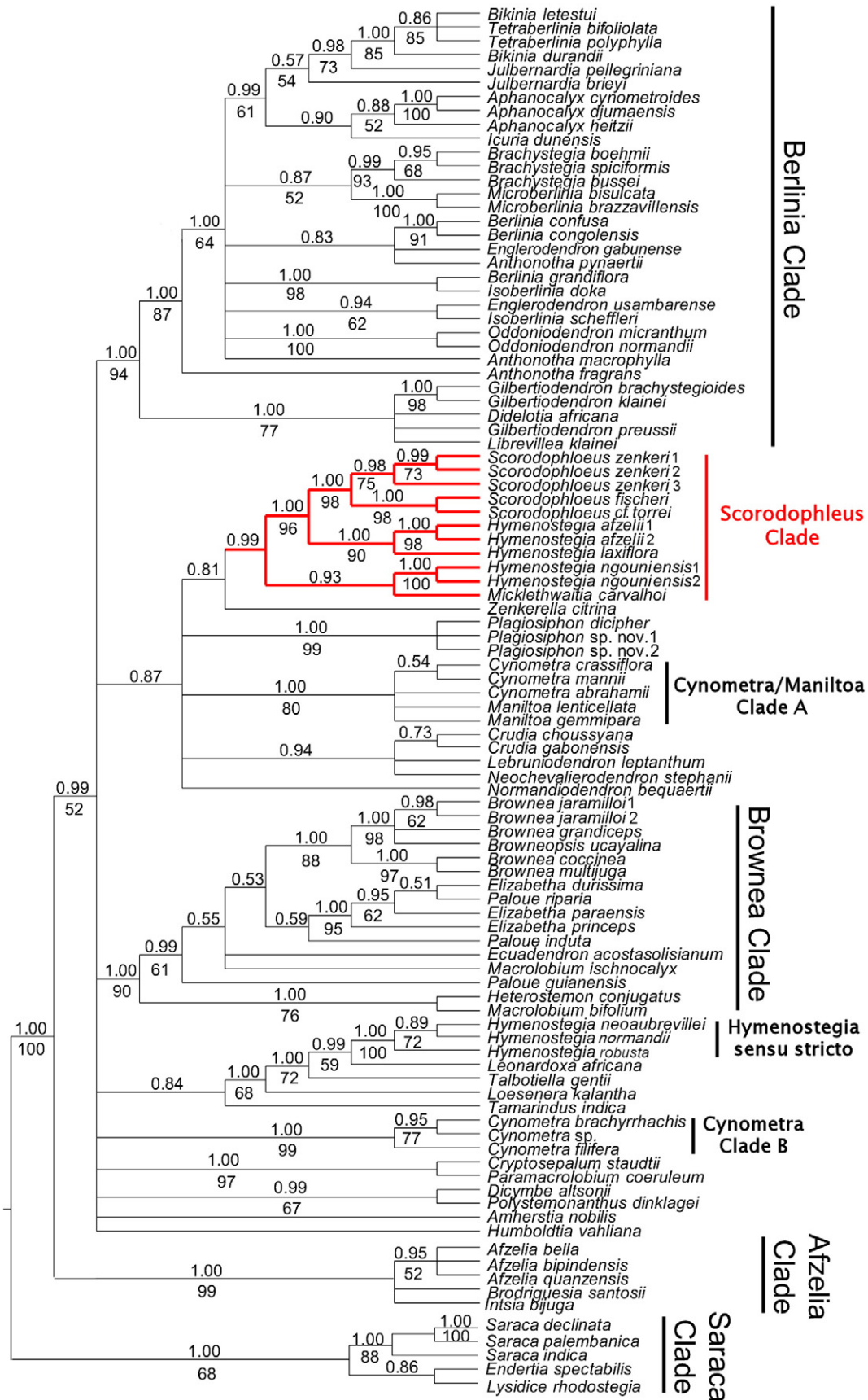
Fig. 1. Phylogenetic analyses of the *mat*/3′-*trn*K and *trn*L intron regions of the Amherstieae clade (sensu Bruneau et al., 2008). Bayesian tree showing posterior probabilities above the line and bootstrap support from the parsimony analyses below the line. The Scorodophloeus clade is highlighted in red. Clades referred to in the text are labelled. Members of the Saraca clade were used as outgroups.

support. *Scorodophloeus* as currently circumscribed is strongly supported as a monophyletic genus.

4. Discussion

The broad phylogenetic framework of the AMC including the recovery of the Brownea, Berlinia and Afzelia clades (Fig. 1) is congruent with the findings of Bruneau et al. (2008), unsurprisingly, since much of the data analysed here was drawn from that study.

4.1. Hymenostegia segregate genera

The Scorodophloeus clade accommodates three species of *Hymenostegia* sensu lato which, as predicted from morphology (Mackinder et al., 2010) do not belong in *Hymenostegia* sensu stricto. Also as predicted from morphology *H. afzelii* and *H. laxiflora* are sister species. This morphologically homogenous species pair is readily recognisable as distinct from *Scorodophloeus* which is resolved with strong support as sister.

The findings of this phylogenetic study are consistent with those of previous morphology based studies (Mackinder et al., 2010) indicating that *H. afzelii* and *H. laxiflora* constitute an undescribed segregate genus. We propose therefore to transfer them to *Annea* gen. nov. ined. (Mackinder and Wieringa, accepted for publication).

H. ngouniensis is placed within the Scorodophloeus clade as sister to *Micklethwaitia* with weak support (Fig. 1). However, in some of the most equally parsimonious trees based on phylogenetic analyses of *mat*K alone (unpublished results) *H. ngouniensis* was placed as sister to the *Scorodophloeus–Annea* gen. nov. pairing (weakly supported) with *Micklethwaitia* in turn placed as sister to the *Scorodophloeus–Annea–H.ngouniensis* group (weakly supported), hence the phylogenetic relationship between *H. ngouniensis* and *Micklethwaitia* remains unclear. Whilst we do not rule out the possibility that they may represent sister lineages, we rule out transferring *H. ngouniensis* to *Micklethwaitia* as a second species of the genus because numerous morphological characters distinguish *H. ngouniensis* from *Micklethwaitia*. We therefore propose the transfer of *H. ngouniensis* to *Gabonius* gen. nov. (Wieringa et al., accepted with minor revision), a newly erected monospecific genus.

4.2. Micklethwaitia

Micklethwaitia carvalhoi is a rare monospecific genus endemic to Mozambique (Mackinder, 2005). The species was originally described as *Cynometra carvalhoi* by Harms (1899) from fragmentary material. When better material was later collected, Lewis (1996) wrote a more detailed description, reiterated Brenan's view (unpublished results) that *Cynometra carvalhoi* was closely related to *Scorodophloeus* and presented a table comparing differences between the flowers of *C. carvalhoi* and *S. fischeri*. Soon after, Léonard (1999) raised *Cynometra carvalhoi* to generic rank as *Brenaniodendron* although that generic name (deemed a homonym of *Brenandendron* in the Asteraceae) was subsequently replaced by *Micklethwaitia* (Lewis and Schrire, 2004).

The phylogenetic analyses presented in this study are congruent with Léonard's decision to recognise *Micklethwaitia* (as *Brenaniodendron*) as a monotypic genus and confirm the close relationship of *Micklethwaitia* to *Scorodophloeus* as posited by Lewis (1996). As discussed under Section 4.1, we do not consider *Gabonius* (*Hymenostegia*) *ngouniensis* to be congeneric with *Micklethwaitia* given the many morphological differences between these two taxa. Differences are largely in (but not confine to) floral characters such as bracteole aestivation, persistence, shape and their position on the pedicels, the number of well-developed petals, the size and shape of anthers and their relative length to the filaments, as well as the depth of the hypanthium and the different attachment points of the stipe to the hypanthium wall.

4.3. Cynometra

Our findings are consistent with the hypothesis that *Cynometra* is not monophyletic (Léonard, 1951, 1996; Bruneau et al., 2008). In our analyses, accessions of *Cynometra* are resolved in two well-supported clades. Three *Cynometra* species are placed together with two *Maniltoa* species (Cynometra/Maniltoa Clade A). Based on morphology, a close relationship between SE Asian *Cynometra* species with the SE Asian genus *Maniltoa* has long been hypothesised (Knaap van Meeuwen, 1970). However, in this study, the three *Cynometra* accessions resolved in a clade with *Maniltoa* are in turn from Africa (*C. mannii*), Madagascar (*C. abrahamii*) or Ecuador (*C. crassiflora*) suggesting, albeit based on low sampling, that a more complicated geographical pattern of relationships may exist.

Cynometra clade B is composed of three other accessions, *Cynometra* sp. (Herendeen 97-X11-1), *C. brachyrrhachis* and *C. filifera*, all from Tanzania in East Africa; the latter two are country endemics. That *C. filifera* could be accommodated, even within the diffuse bounds of *Cynometra* sensu lato was questioned by Brenan (1967) who wrote "the racemose inflorescences, strobiliform when young and the definitely alternate arrangement of at least most of the leaflets combine to make *C. filifera* very anomalous among the other East African species of *Cynometra*". Despite the unusual morphology, our study indicates a strong phylogenetic relationship with at least some other East African *Cynometra* species.

4.4. Properties of the Scorodophloeus clade

4.4.1. Geography

The Scorodophloeus clade is exclusively tropical African and restricted to two regional centres of endemism. In the Guineo–Congolian Centre of endemism, diversity is widespread throughout the upper and lower Guinea regions including through the Dahomey interval (Map 1), extending into the Congolian region and represented there by *S. zenkeri*. A single species *Annea* (*Hymenostegia*) *afzelii* spans the Dahomey interval, the arid corridor which separates the West African rainforest block from the rainforests of Central African. Three species, *S. fischeri*, *S. torrei* and *M. carvalhoi* are East African endemics falling within the Swahilian regional centre of endemism (Clarke, 1998). Compared to the four Guineo–Congolian species, the three East African species have relatively narrow distributional ranges, a common pattern among species endemic to the coastal forest of eastern Africa which is interpreted as a relict of a once pan-African tropical forest (Burgess et al., 1998).

4.4.2. Morphology

Numerous morphological characters were drawn from several sources (Lewis, 1996; Herendeen et al., 2003; Mackinder et al., 2011) and their presence/absence in the Scorodophloeus clade was compared with their presence/absence among species of the wider AMC clade. We were not able to identify any unambiguous synapomorphies for the Scorodophloeus clade. We note, however, that persistent imbricate distichous bud scales which are found in many AMC species, for example, *Brownea* spp., *Cynometra* spp., *Hymenostegia* sensu stricto spp., *Maniltoa* spp., *Plagiosiphon* spp. and *Talbotiella* spp. do not occur in any members of the Scorodophloeus clade. Such bud scales are also absent from *Zenkerella* spp. and so we speculate that the weakly supported placement of *Zenkerella* as sister to the Scorodophloeus clade, represented by *Zenkerella citrina* Taub. (Fig. 1), may prove correct when more data are available.

Within the Scorodophloeus clade, the strongly supported sister relationship of the Annea and Scorodophloeus lineages is consistent with seedling morphology. Seedlings (when available) have been shown to be rich in characters that have taxonomic utility at genus level in Detarieae (Léonard, 1957; Léonard, 1994; Léonard, 1996; Léonard and Doucet, 1997). Seedling morphology is known for both *Annea* species (Mackinder and Wieringa, accepted for publication) as well as for *S. zenkeri* (Léonard, 1957), *S. fischeri* (Temu, 1990) and

S. torrei (Lock, 2006). In all cases, the first two seedling leaves are alternate, not the more common arrangement among the AMC genera where the first two seedling leaves are opposite. Typical AMC seedlings produce an obvious epicotyl followed by the a first pair of leaves. The growth of the shoot is temporarily halted whilst those leaves fully mature. Subsequently, the mature shoot develops distally, and from there on, alternate leaves are produced. We interpret the production of only alternate leaves as a loss of the epicotyl. We cannot make a statement concerning this character of epicotyl loss with respect to the entire Scorodophloeus clade as seedling morphology of *Micklethwaitia* is not recorded. Nevertheless, a single seedling of *Gabonius* gen. nov. ined. (Wieringa et al., accepted with minor revision) presents a previously unrecorded intermediate form between the two established opposite or alternate first leaf pair morphologies. In *Gabonius* gen. nov. ined., based on J.J.F.E. de Wilde 9329, epicotyl scars suggest the presence of a first pair of rudimentary opposite caducous leaves, beyond which the epicotyl continues growing until a pair of fully developed alternate leaves appear. Despite the indicated presence of a rudimentary opposite first leaf pair, we consider *Gabonius* seedling morphology to be functionally akin to seedlings of *Annea* gen. nov. ined. and *Scorodophloeus*. It seems improbable that the reduced leaf pair is functional if it does not persist as suggested by the growth of the unchanged epicotyl beyond them.

Of the estimated five species of *Zenkerella* (Mackinder, 2005), *Zenkerella grotei* (Harms) J.Léonard [(as *Z. capparidacea* (Taub.) J.Léonard subsp. *grotei* (Harms) Temu)], *Z. egregia* J.Léonard and *Z. perplexa* Temu are also known to produce alternate leaves from the outset (Léonard, 1994). Seedling morphology of *Zenkerella citrina*, the only species sampled in this study, is not recorded but Léonard's postulate (Léonard, 1957) of morphological homogeneity among seedlings of the same genus has been supported through numerous new seedling records across many genera (Léonard, 1994; Léonard, 1996; Léonard and Doucet, 1997; Lock, 2006; Temu, 1990) so it is reasonable to suggest that *Z. citrina* will share the first alternate seedling leaves character too. This would again be consistent with the idea that *Zenkerella* may be sister to the Scorodophloeus clade.

4.4.3. Biodiversity

Using data extrapolated from Lewis et al. (2005), the average legume genus includes 27 species. Caesalpinioideae genera are however, generally smaller accommodating an average of 13 species (data extrapolated from Lewis et al., 2005). Average genus size among AMC genera is lower still at around 11 species and 30% of AMC genera are monospecific compared to 26% across the family (data extrapolated from Lewis et al., 2005). The four genera of the Scorodophloeus clade together comprise only seven described species and may thus be considered a more extreme example of the species poor wider pattern of generic diversity that characterises the pan tropical AMC. Whilst alpha (species) diversity is often lower in African forests than in the forests of the New World (Parmentier et al., 2007), and generic diversity is often greater (Prance, 1994) as exemplified by the AMC. Of the c. 55 AMC genera, 32 are tropical African endemics, 11 are neotropical endemics and nine genera are restricted to the Asiatic tropics.

4.4.4. Conservation and Threat

Five of the seven species of the Scorodophloeus clade, *Annea* (*Hymenostegia*) *afzelii*, *Annea* (*Hymenostegia*) *laxiflora*, *Scorodophloeus zenkeri*, *S. fischeri* and *Gabonius* (*Hymenostegia*) *ngouniensis* have wide geographical ranges such that they do not qualify for a category of threat according to the criteria of IUCN (2001).

Scorodophloeus torrei is known from a handful of collections, collected 40–50 years ago in Mozambique coastal forest. Lock (2006) noted that *S. torrei* is "probably threatened by exploitation for timber and firewood and by clearance of the forest for agriculture" and suggested that an IUCN category of Vulnerable was appropriate. In our phylogenetic analyses,

we include *Scorodophloeus* cf. *torrei* (Luke 8788), a voucher that comprises seedlings, mature foliage and a separate pod, gathered in Tanzania from the Udzungwa Mountains (Map 1). Whilst the collection locality of Luke 8788 is geographically closer to some collections of *S. fischeri* than those of *S. torrei*, the morphology of Luke 8788 is quite distinct from that of *S. fischeri*. We cannot yet confirm the species the identification of the Luke gathering, which was collected in submontane habitat, >600 km from the nearest known confirmed *S. torrei* locality in coastal forest (the typical habitat of *S. torrei*). One plausible explanation is that Luke 8788 represents an undescribed species of *Scorodophloeus*, morphologically similar to *S. torrei* but endemic to the Udzungwa Mountains, an area where numerous species have been recognised as endemic in the last 20 years (Luke personal communication).

No forest remains of *Micklethwaitia* at the type locality in Mozambique but following recent exploration it is known to be extant in four sites in northern coastal Mozambique (Clarke, 2010), in part of the same coastal forest system where *Scorodophloeus torrei* grows. Of the four known *Micklethwaitia* localities, two are actively threatened by timber extraction and fire, respectively (Clarke, 2010), so that after a full IUCN assessment is carried out, it is probable that *Micklethwaitia* will also qualify for a category of threat.

5. Conclusion

The genus *Scorodophloeus*, two *Hymenostegia* segregate genera and *Micklethwaitia* sampled here for the first time are a natural group which we call the Scorodophloeus clade. Molecular phylogenetic analyses presented here support morphology based hypotheses that the genus *Zenkerella* may be sister to the Scorodophloeus clade and this putative relationship will be explored further.

Acknowledgements

Appendix 1. Complete taxon sampling and GenBank accession numbers for sequences used in this study

Species	GenBank accessions	
	trnL	matK
Afzelia bella	AF365128	EU361846
Afzelia bipindensis	AF365131	EU361847
Afzelia quanzensis	AF365130	EU361848
Amherstia nobilis	AF365210	EU361849
Anthonotha fragrans	AF365233	EU361850
Anthonotha gabunensis	AF365236	EU361851
Anthonotha macrophylla	AF365234	EU361853
Anthonotha pynaertii	AF365235	EU361854
Aphanocalyx cynometroides	AF365244	EU361855
Aphanocalyx djumaensis	AF365249	EU361856
Aphanocalyx heitzii	AF365247	EU361857
Berlinia confusa	EU361747	EU361879
Berlinia congolensis	AF365216	EU361881
Berlinia grandiflora	EU361748	EU361882
Bikinia durandii	AY116896	EU361883
Bikinia letestui	AF365231	EU361884
Brachystegia boehmii	EU361749	EU361886
Brachystegia bussei	AF549290	EU361887
Brachystegia spiciformis	AF365253	EU361888
Brodriguesia santosii	EU361750	EU361890
Brownea coccinea	EU361752	EU361891
Brownea grandiceps	AF365193	EU361892
Brownea jaramilloi 1	KF294036	KF294051
Brownea jaramilloi 2	KF294037	KF294052

Appendix 1 (*continued*)

Species	GenBank accessions	
	*trn*L	*mat*K
Brownea multijuga	AF365194	EU361893
Browneopsis ucayalina	AF365199	AF365198
Crudia choussyana	EU361788	EU361921
Crudia gabonensis	AF365172	EU361922
Cryptosepalum staudtii	AF365258	EU361923
Cynometra abrahamii	KF294038	KF294053
Cynometra brachyrrhachis	KF294039	KF294054
Cynometra crassiflora	KF294040	KF294055
Cynometra filifera	KF294041	KF294056
Cynometra mannii	AF365114	EU361925
Cynometra sp.	EU361791	EU361924
Dicymbe altsonii	AF365217	EU361932
Didelotia africana	AF365260	EU361933
Ecuadendron acostasolisianum	AF365207	EU361938
Elizabetha durissima	EU361804	EU361940
Elizabetha paraensis	AF365208	EU361941
Elizabetha princeps	EU361805	EU361942
Endertia spectabilis	AF365136	EU361943
Englerodendron usambarense	AF365218	EU361944
Gilbertiodendron brachystegioides	AF365238	EU361954
Gilbertiodendron klainei	EU361811	EU361955
Gilbertiodendron preussii	AF365239	EU361956
Heterostemon conjugatus	EU598689	EU361968
Humboldtia vahliana	AF365212	EU361970
Hymenostegia afzelii 1	KF294042	KF294057
Hymenostegia afzelii 2	AF365146	EU361975
Hymenostegia laxiflora	KF294043	KF294458
Hymenostegia neoaubrevillei	AF365144	KF294058
Hymenostegia ngouniensis 1	AF365142	EU361977
Hymenostegia ngouniensis 2	KF314820	KF294059
Hymenostegia robusta	AF365141	EU361976
Hymenostegia normandii	AF365148	AF365148
Icuria dunensis	AF365232	EU361979
Intsia bijuga	AF365149	EU361981
Isoberlinia scheffleri	AF365221	EU361983
Leonardoxa africana	AF365118	EU361992
Librevillea klainei	AF365262	EU361993
Lebruniodendron leptanthum	EU361817	KF294060
Julbernardia brieyi	AF365264	EU361985
Julbernardia pellegriniana	AF365266	EU361986
Loesenera kalantha	AF365150	EU361994
Lysidice rhodostegia	AF365152	EU361995
Macrolobium bifolium	AF365200	EU361996
Macrolobium ischnocalyx	AF365201	EU361997
Maniltoa gemmipara	AF365120	EU361998
Maniltoa lenticellata	KF294044	KF294061
Micklethwaitia carvalhoi	KF294045	KF294062
Microberlinia bisulcata	AF365223	EU362002
Microberlinia brazzavillensis	AF365222	EU362003
Oddoniodendron micranthum	AF365225	EU362008
Oddoniodendron normandii	AF365224	EU362009
Neochevalierodendron stephanii	AF365151	EU362006
Normandiodendron bequaertii	AF365119	EU362007
Paloue guianensis	EU361825	EU362014
Paloue induta	AF365205	EU362015
Paramacrolobium coeruleum	AF365242	EU362017
Plagiosiphon dicipher	KF294046	KF294068
Plagiosiphon sp. nov.1	EU361789	EU361926
Plagiosiphon sp. nov.2	KF294047	KF294069
Paloue riparia	EU361826	EU362016
Polystemonanthus dinklagei	AF365226	EU362028
Saraca declinata	AF365156	EU362033
Saraca indica	EU361832	EU362034
Saraca palembanica	AF365157	EU362035
Scorodophloeus fischeri	KF294048	KF294063
Scorodophloeus cf. torrei	KF294049	KF294064
Scorodophloeus zenkeri 1	AF365125	EU362041
Scorodophloeus zenkeri 2	–	KF294065
Scorodophloeus zenkeri 3	KF294050	KF294066
Talbotiella gentii	AF365159	EU362055
Tamarindus indica	AF365206	EU362056
Tetraberlinia bifoliolata	AF365227	EU362060
Tetraberlinia polyphylla	AF365230	EU362061
Zenkerella citrina	AF365127	EU362066
Isoberlinia doka	AF365220	EU361982

Appendix 2. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sajb.2013.07.002.

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