Molecular and Morphological Characterization of a New Monotypic Genus of Annonaceae, Mwasumbia, from Tanzania

Thomas L. P. Couvreur,¹,5 Raymond W. J. M. van der Ham,² Youssoufa M. Mbele,¹ Frank M. Mbago,³ and David M. Johnson⁴

¹Nationaal Herbarium Nederland - Wageningen branch, Biosystematics Group, Wageningen University, Generaal Foulkesweg 37, 6703 BL Wageningen, The Netherlands
²Nationaal Herbarium Nederland – Leiden branch, Leiden University, 2300 RA Leiden, The Netherlands
³The Herbarium, Botany Department, Box 35060, University of Dar es Salaam, Dar es Salaam, Tanzania
⁴Department of Botany-Microbiology, Ohio Wesleyan University, Delaware, Ohio 43015 U. S. A.
⁵Author for correspondence (tlpcouvreur@gmail.com)

Abstract—The coastal lowland rain forests of eastern Africa are well known for their high levels of plant endemism. A new genus of Annonaceae, Mwasumbia, is described from Tanzania, underscoring this high biodiversity and represented by a single species, Mwasumbia alba. The new genus presents several morphological characters suggesting a close relationship to two other African genera, Greenwayodendron and Polyceratocarpus. Maximum parsimony and Bayesian molecular phylogenetic analyses based on two plastid markers, rbcL and the trnL-trnF region, strongly support the close relationship of these three genera to one another as well as to two other African genera, Annickia and Piptostigma. Together these five genera form a moderately supported clade within the so-called short-branch clade of Annonaceae. A detailed morphological and palynological comparison between Mwasumbia and the four other genera shows that this new genus exhibits a combination of features unique within this group: intermediate tertiary leaf venation, exclusively bisexual flowers, slightly imbricate sepals, valvate petals, outer and inner petals equal in length, numerous stamens, four carpels, few and uniseriate ovules, few and sessile monocarps, and verrucate sulcate pollen grains. Phylogenetic analyses also suggest the genus Piptostigma, as currently circumscribed, to be paraphyletic. An IUCN conservation status of VU D2 is proposed, reflecting the narrow distribution of the single species.

Keywords—Annonaceae, biodiversity hotspot, coastal forest, East Africa, Kimboza Forest Reserve, pollen, Tanzania, taxonomy, Uluguru Mountains.

Annonaceae are pan-tropical trees, shrubs, and lianas belonging to the order Magnoliales (APG II 2003), representing the most diverse family within this comparatively early diverging order, comprising some 130 genera and approximately 2,500 species (Chatrou et al. 2004). They form an easily recognizable and monophyletic group with alternate extipulate distichous leaves, a trimerous perianth usually with two whorls of petals, numerous stamens, mostly free carpels, and large seeds with ruminate endosperm (Kessler 1993). Classification within the family and the delimitation of genera, however, has been much debated (Walker 1971; Koek-Noorman et al. 1990), but more recent studies based on analyses of morphology (Doyle and Le Thomas 1994, 1996) and molecular characters (Doyle et al. 2000; Mols et al. 2004; Richardson et al. 2004; Pirie et al. 2006; Couvreur et al. 2008) have begun to clarify phylogenetic relationships within the family. The genus Anaxagorea has been recovered as sister to the rest of the family (Richardson et al. 2004; Scharaschkin and Doyle 2005), followed by a small clade of genera called the “ambavoids” by Doyle and Le Thomas (1996). The rest of the family is split into two equally large groups: the “long-branch clade” (LBC, Richardson et al. 2004), which contains mostly tropical American and African genera, and the so-called “short-branch clade” (SBC, Richardson et al. 2004), with mostly Asian and tropical American genera. Within the latter, a small group of African genera, called the “Piptostigmoids” (Doyle and Le Thomas 1994, 1996) has emerged, initially defined on the basis of pollen characters, but then supported and expanded by molecular characters (Doyle et al. 2000). This grouping includes the core genera Greenwayodendron, Polyceratocarpus, and Piptostigma, with the genus Annickia more loosely allied to it (Doyle et al. 2000).

In Africa, knowledge of specific and generic diversity is still incomplete, despite the ecological importance of Annonaceae in lowland rain forests (Gentry 1993; Tchouto et al. 2006a, b). This ecological importance is especially apparent in the coastal forests of eastern Africa, one of the 34 biodiversity hotspot regions (Mittermeier et al. 2004), where numerous new taxa of Annonaceae have been described in the two last decades (Verdcourt 1986, 1996; Verdcourt and Mwasumbi 1988; Johnson et al. 1999; Luke and Deroin 2005; Couvreur et al. 2006). These coastal forests consist of numerous small patches of fragmented lowland forests not bigger than 370 km² occurring from southern Somalia to southern Mozambique (Burgess et al. 1998; Burgess and Clarke 2000). They also include the lowland rain forests at the base of the Eastern Arc Mountain range (e.g. Usambara or Uluguru mountains, Burgess et al. 1998). The coastal and montane rain forests of eastern Africa harbour an exceptional density of endemic plants, which is one of the highest on the planet (Myers et al. 2000). Concentrated in a total area of only approximately 7,000 km² they support approximately 3,500 plant species, of which approximately 1,100 (31%) are endemic (Burgess et al. 1998; Burgess and Clarke 2000; Burgess et al. 2007). It has been shown that Annonaceae present a high diversity of species and genera within the Eastern Arc Mountains as well as in the Coastal Forests (Couvreur et al. 2006).

Here we propose a new genus of Annonaceae, Mwasumbia, endemic to a single coastal rain forest reserve at the base of the Uluguru Mountains in Tanzania. Verdcourt (1971), in his account of the Annonaceae for the Flora of Tropical East Africa, drew initial attention to this plant as a “species of uncertain generic position”, and suggested on the basis of the single fruiting collection then known that it could belong to the small African genus Greenwayodendron because of the structure of the seeds. However, during the past decade additional flowering and fruiting collections have been made, enabling us to clarify the position of this species. The flowers show a great resemblance to those of Polyceratocarpus, another African genus, with two subequal whorls of coriaceous petals and a
conical torus with a flattened apex. They differ, however, in their leaf morphology, _Polyceratocarpus_ has a characteristic parallel tertiary venation, whereas in the new genus the tertiary venation is intermediate between parallel and reticulate. Moreover, _Polyceratocarpus_ is androdioecious whereas the new species appears to have strictly bisexual flowers. The fact that this new taxon appears to have strong morphological similarities to several other genera as well as certain dissimilarities excludes a straightforward conclusion as to its correct position. We therefore adopted an integrated approach using a combination of morphological and molecular data to highlight differences and resemblances between this new genus and the four genera closely related to this plant.

**Materials and Methods**

**Morphology**—For the new genus _Mwasumbia_, original specimens from K, MO and WAG were examined. One field trip to Tanzania was also undertaken in 2006 to the unique known locality for collection of fresh material such as DNA and spirit samples and to gather ecological data. For observations on _Annickia_, _Greenswayodon_, _Piptostigma_, and _Polyceratocarpus_, spirit and herbarium specimens available at M, MO, and WAG were used.

**Pollen Preparation**—Pollen samples of _Mwasumbia_, _Piptostigma_ and _Polyceratocarpus_ were studied (see Appendix 1). Further, published pollen data of _Annickia_ and _Greenswayodon_ was available for comparison (Walker 1971; Le Thomas 1980). Pollen of _Mwasumbia_ was prepared for scanning electron microscopy (SEM) and transmission electron microscopy (TEM), while that of _Piptostigma_ and _Polyceratocarpus_ only for TEM. _Annickia_ pollen is fragile and the acetolysis preparation method (Erdtman 1990) is often too drastic and damages the pollen grains, making observations difficult. Therefore, we used an alternative method based on three consecutive baths of mature stamens in n-hexane, an organic solvent, following Couvreur et al. (2006). M was prepared in 70% alcohol which was given an extra bath in 100% alcohol prior to the n-hexane baths. The pollen was then gold-coated and examined with SEM. The samples for TEM were prepared according to the techniques described by van der Ham (1990). The subdivision of the exine into tectum, infratectum, and basal layer with foliations (all ectexinous) follows Le Thomas (1980). Further terminology follows Punt et al. (2007).

**Molecular Phylogenetic Analyses**—Preliminary phylogenetic analyses indicated that _Mwasumbia_ clustered in the so-called short-branched clade (SBC). Thus, we focused our sampling within the SBC. Based on Richardson et al. (2004), 36 out of the approximately 40 genera of the SBC were sampled, representing all major lineages. The other major clades of _Annonaceae_ (long-branched clade (LBC), ambavioids, and _Anaxagorea_) were also sampled. Within the LBC, 17 out of approximately 30 genera were included, representing all major well-supported lineages identified to date (Richardson et al. 2004; Couvreur et al. 2008). Two from the approximately eight genera in the ambavioid clade and two species of _Anaxagorea_ were also included. _Eupomatia bennettii_ (_Eupomatia_ et al. has been recovered as sister to _Annonaceae_, Qiu et al. 2000; Sautu et al. 2003) was chosen as outgroup. Sampling within genera was always restricted to one species within the LBC and ambavioids, and varied from one to two species within the SBC. Two genera within the SBC, namely _Oxandra_ and _Polyalthia_, have been shown to be polyphyletic based on molecular data (Mols et al. 2004; Pirie et al. 2006) and thus more than two species were included representing the two different clades. Finally, the two genera thought to be most closely related to _Mwasumbia_ based on morphological observations, _Piptostigma_ and _Polyceratocarpus_, were more thoroughly sampled at the species level. They were represented by four out of approximately 12 and ten species, respectively. Voucher specimens of all 78 taxa included in the analyses are listed in Appendix 1.

DNA extractions were performed using a modified CTAB (Doyne and Doyle 1987) method following Bakker et al. (1998). The universal primers C/D and E/F (Taberlet et al. 1991) were used to amplify and sequence the _trnL_ intron and _trnL-trnF_ spacer. The _rbcL_ marker was amplified using two primer combinations, 1F/724R and 636F/1460R (Fay et al. 1997). PCR reactions were performed with 35-50 ng of genomic DNA, 0.8% of BSA, 0.2 μM of each primer, 0.2 mM dNTP PCR mix (Promega, Madison, Wisconsin), 3 μM MgCl₂, 1× PCR buffer (Promega), and 0.5 U of Tag DNA polymerase (Promega) in a total volume of 50 μL. The PCR program was as follows: 35 thermal cycles at 94°C for 1 min, 50-55°C for 50 s, 72°C for 50s and a final extension at 72°C for 3 min. Sequences were edited using Staden (http://staden.sourceforge.net/) and manually aligned using the software PAUP* (version 4.10b; Swofford 2002). Gaps were coded following the simple coding model of Simmons and Ochoterena (2000). Microsatellites were excluded from the analysis, as these structures probably originate through slipped-strand mispairing (Levinson and Gutman 1987) and are highly homoplastic.

Maximum Parsimony (MP) analyses were performed using PAUP* (version 4.10b; Swofford 2002). Heuristic searches were performed with 100 random sequence addition iterations, saving 100 trees at each iteration, with tree bisection-reconnection branch swapping. Relative support for each node was assessed by performing 1,000 bootstrap (BS) replications (Felsenstein 1985; Salamin et al. 2003) with TBR branch swapping (20 random addition sequences, saving 20 trees per replicate).

**Bayesian Analyses**—All analyses were run using the Metropolis-coupled Monte Carlo Markov chain algorithm as implemented in MrBayes (ver. 3.1.2; Ronquist and Huelsenbeck 2003) with the program’s default parameters for the priors. For each model, two separate runs were started from random trees. Each run was composed of one cold and three heated chains with the temperature parameter T set to 0.05 to ensure good mixing. Gap characters were always included in the analysis and were set to follow the model implemented in MrBayes for binary data, using the “iset coding = variable” command. Four alternative partition strategies varying from simple to complex were considered for this study (Table 1). The best performing evolutionary model for each partition was identified under two different model selection criteria, the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC; Akaike 1973) as implemented in MrModeltest (Nylander 2004). The parameters for each partition were allowed to evolve independently using the “unlink” command. An initial analysis for each of the four partition strategies was run for two million generations sampling every 100th generation. To decide which partition strategy best agreed with the data, the Bayes Factor (Kass and Raftery 1995; Sinheimer et al. 1996; Nylander et al. 2004; Brandley et al. 2005) was computed using the harmonic means after each MCMC run provided by the sump function in MrBayes. The best partition strategy was then rerun with the same parameters, but with three separate runs and for four million generations. In order to assess that the MCMC reached stationarity we examined the loglikelihood (lnL) plots using Tracer v. 1.3 (Rambaut and Drummond 2003). In particular, we searched for evidence that model likelihoods and parameter estimates reached stationarity after a burn-in period. Additionally, we checked that independent runs converged using the online software AWTY (Nylander et al. 2008). Trees are posted at TreeBASE (study number S2275).

**Results**

**Pollen Morphology**—SEM (Fig. 1A, B): Pollen grains solitary, disulculate, bilateral, heteropolar: consisting of an exinous ‘hemisphere’ (assumed to be the proximal side) and an innitious ‘hemisphere’ (assumed to be the distal side). Polar axis 59 μm. Exinous part ± conical, slightly elliptic in cross-section, verrucate-(rugulate); verrucae irregularly shaped, up to 5 μm diameter at the pole, decreasing to approximately 1 μm at the margin of the exine, surrounded by narrow fos-

<table>
<thead>
<tr>
<th>Table 1. Partitioning strategies explored for the Bayesian analyses of this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partition strategy</strong></td>
</tr>
<tr>
<td>T0</td>
</tr>
<tr>
<td>T1</td>
</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
</tbody>
</table>
sulae and/or small (up to 0.5 μm) perforations. Intinous part ± kidney-shaped, 44 × 1 μm thick. Exinous part, irregularly scabrate. Exinous part and intinous part largely separated by two crescent-shaped more or less depressed areas (concave sides towards exinous part), which are considered to be the apertures (sulculi). TEM (Fig. 1C, D): Exine 0.6–1.8 μm thick, thinning out near the sulci. Tectum 0.4–1.3 μm thick. Infratectum inconspicuous, approximately 0.1 μm thick, indistinctly columellate and/or granular. Basal layer 0.1–0.4 μm thick, irregularly granular, subtended by 1–2 indistinct, discontinuous foliations. Intine consisting of two layers. Exintine tubular, up to 1.5 μm under the exine, almost absent at the two crescent-shaped depressed areas, thickening up to 3.5 μm towards the assumed distal pole. Endintine homogeneous, approximately 1 μm thick throughout, except under the two crescent-shaped depressed areas (up to 3 μm).

**Molecular Phylogeny**—Both markers combined represent 2,669 characters, 187 of which were excluded because of ambiguity in the alignment. Of the remaining 2,482 characters included in the analyses 405 (16.3%) were parsimony-informative. After the maximum parsimony heuristic search, 8,400 most parsimonious trees of 1,422 steps long were found (consistency index (CI) of 0.622 and retention index (RI) of 0.747).

In the Bayesian analyses, stationarity was reached after 150,000 generations (as visualized with Tracer) for all four partition strategies (Table 1) and the lnL values were the same between independent runs. All strategies generated majority-rule consensus trees with identical topologies. Small fluctuations in PP occurred for moderately supported clades, but the strongly supported ones (PP ≥ 0.99) remained constant. The Bayes Factor always indicated strong support (BF > 200) in favour of partition strategy T3 over the other three strategies considered (results not shown). This partition strategy was then rerun for four million generations with three independent runs. All three runs reached stationarity after 250,000 generations with all of the parameters converging to the same values as seen with Tracer. For all parameters the effective sample size (ESS) values were above 100, indicating appropriate sampling of the posterior distributions. Finally, the posterior probabilities of all splits were indistinguishable between independent runs as visualized with AWTY, indicating convergence (results not shown).

MP and Bayesian analyses generated similar topologies. Three major clades were recovered with strong support (MP: BS > 95%; Bayesian analyses: PP > 0.95): the SBC, LBC and the ambavioids (Fig. 2). In all analyses *Anaxagorea* appeared as sister to the rest of the family. The new genus *Mwasumbia* clusters within the SBC in an early diverging clade together with four other African genera: *Anniccia, Greenwayodendron, Piptostigma*, and *Polyceratocarpus*. *Mwasumbia* is strongly supported as sister to *Piptostigma* and *Polyceratocarpus*. *Greenwayodendron* was recovered as sister to the latter three genera. The position of *Anniccia* is unresolved in the MP or assigned a low PP in the Bayesian (0.66) analyses with respect to the latter clade and the rest of the SBC. *Polyceratocarpus* was strongly supported as monophyletic in both analyses, while *Piptostigma* appeared to be a paraphyletic genus (with respect to *Polyceratocarpus*).

**Taxonomic Treatment**

*Mwasumbia* Couvreur & D. M. Johnson, gen. nov.—**TYPE**: *Mwasumbia alba* Couvreur & D. M. Johnson.

Genus novum affinis *Polyceratocarpus* floribus axillaribus solitariis, petalis coriaceis subaequalibus, sub anthesis petalis exterioribus erectis et petalis exterioribus patentibus, et monocarpis sessilibus pluriseminalibus valde similis, sed a quo nervis tertiariorum foliorum irregularibus, sepalis imbricatis, ovulis uniseriatis, pollinis granis verrucatis, et seminibus rotundatis differt. Species unicum: *Mwasumbia alba* Couvreur & D. M. Johnson.

Small trees or shrubs with irregular tertiary venation of the leaves. Inflorescences one-flowered, axillary, with a short sympodial rachis and a longer pedicel bearing a single bract...
Fig. 2. Majority rule consensus tree of 30,000 trees sampled from the last 3 million generations; trees from the Bayesian analysis using the partition strategy T3 with four million generations. Thick branches indicate branches with both > 95% bootstrap and > 0.99 posterior probability support. Posterior probability (left) / bootstrap support (right). The arrow indicates the position of *Mwasumbia alba*.
proximal to the midpoint. Flowers bisexual; sepals free, slightly imbricate at base, shorter than petals; petal whorls subequal, valvate in bud, the petals coriaceous; outer petals spreading at anthesis, inner petals erect. Stamens numerous, clavate, with a short (< 0.2 mm) filament and flat glabrous apex of the anther connective. Carpels 3–4, oblong; ovaries densely hairy; stigmas bilobed; ovaries 5–8, attached laterally in a single row. Torus short-cylindrical. Monocarps sessile, ellipsoid, densely hairy. Seeds approximately 2 per monocarp, rounded and compressed laterally; seed coat rugose, brown; raphe/antiraphe sunken in a circumferential groove; endosperm ruminations spiniform.

A single species, known from one locality in east-central Tanzania.

**Etymology**—The generic name is in honor of the Tanzanian botanist Leonard Mwasumbi, a leading authority of the Tanzanian flora and former head of the herbarium at the University of Dar es Salaam.

*Mwasumbia alba* Couvreur & D. M. Johnson, sp. nov.—TYPE: TANZANIA. Morogoro region: Kimboza Forest Reserve, approximately 2 km after Kimboza village; 7°01.32'S 37°48.20'E; 26 Nov 2006 [Fl. & Fr.]; T.L.P Couvreur 85 (holotype: WAG; isotypes: MO, NHTI, DSMI, OWU!).

Tree 2–3 m tall, DBH 3–5 cm. Twigs slender, longitudinally striate, densely covered with erect hairs 1.0–1.3 mm long, soon glabrate, brown. Leaf blades 7–14 cm long, 3–6 cm wide, length:width ratio 1.9–2.6, obovate or rarely elliptic, obliquely broadly cuneate to subcordate at base, acuminate to cuspidate at the apex, the acumen 5–22 mm long; midrib slightly impressed and glabrous above, raised and sparsely covered with appressed hairs to glabrous below; secondary veins 8–10 per side, diverging at 45–60° from midrib, slightly impressed to slightly raised above, slightly raised below, higher-order veins irregular, plane to slightly raised above, slightly raised below; petiole 1–2.5 mm long, 0.8–1 mm in diameter, covered with short erect hairs to glabrous, leaf lamina inserted on top. Inflorescences axillary, sometimes cauliflorous, a 1-flowered rhipidium. Buds not enclosed in sepals, ovoid to conic, densely covered with short appressed hairs, white to white-cream. Sympodial rachis to 7 mm long, densely covered with short appressed hairs, brown, with 0–6 minute lower bracts which are densely covered with short appressed hairs and brown. Flowering pedicels 10–22 mm long, densely covered with short appressed hairs, green, upper bract inserted at 1/3 from the base, minute, appressed against pedicel, covered with short appressed hairs, green. Flowers bisexual, with two whorls of 3 free petals. Sepals 3, free, slightly imbricate at base, 4–3 mm long, 4–5 mm wide, length:width ratio 0.75–0.8, broadly ovate, base subcordate, apex acute, densely covered with short appressed hairs outside, glabrous inside, appressed against petals, persistent in fruit. Petals white to white-cream in vivo, drying dark black; outer petals 12–18 mm long, 10–7.5 mm wide, length:width ratio 1.8–2.1, elliptic, base truncate, apex obtuse; sericeous outside, glabrous inside except for fine puberulence towards apex and along margins, flat, spreading horizontally at anthesis; inner petals 13–18 mm long, 5–6 mm wide, length:width ratio 2.6–3, narrowly oblong, base truncate, apex acute, glabrous on both sides, the margins with short appressed hairs, concave and erect at anthesis. Stamens in 6–8 whorls, 2–2.7 mm long, filament < 0.2 mm long, narrowed, apical prolongation of connective flat, approximately 2 mm long, glabrous, white cream. Torus approximately 1.8–2.2 mm long, 2–2.8 mm in diameter at apex, short-cylindric. Carpels 4(3?), 2–3 mm long, approximately 1 mm wide, oblong, densely covered with appressed hairs; ovaries uniseriate, 5–8 in number, placentalation lateral; stigmas bilobed, glabrous, bright yellow to white cream. Fruiting pedicels approximately 10–15 mm long, covered with short appressed hairs. Monocarps 18–25 mm long, 10–15 mm in diameter, ellipsoid, sessile, densely covered with short dark brown erect hairs in combination with additional sparsely spaced longer light brown hairs. Seeds approximately 2 per monocarp, approximately 11 mm long, approximately 10 mm wide, 4–5 mm thick, spherical and flattened; testa rugose, brown; raphe depressed; hilum approximately 4 mm long, 2 mm wide, elliptic. Figure 3.

**Phenology**—Mature flowers collected in March, June, and September to November, and fruits collected in June, July and November.

**Distribution and Habitat**—The species occurs in a single known locality, the Kimboza Forest Reserve located on both sides of the Ruvu River in the eastern foothills of the Uluguru Mountains in the Morogoro Region. It lies at approximately 7°00'S and 37°48'E with an altitudinal range of 200–500 m above sea level and covers a total area of 386 ha (approximately 4 km²). The soil is characterized by Tropical Rendzina on Precambrian dolomitic marble base rocks, which creates a distinctive tropical karstic landscape with large isolated blocks and pinnacles of marble scattered throughout the forest (Rodgers et al. 1983). The mean annual rainfall is approximately 1,700 mm with the main rainy season extending from November to April. Only 3 months (June-August) present less than 50 mm of rain. Humidity is high all year round. The forest has two main vegetation types: swamp forest dominated by dense stands of *Pandanus engleri* and lowland rain forest with a closed canopy at approximately 20 m and a few emergents at 40 m (e.g. *Serculicia appendiculata*) (Rodgers et al. 1983). *Mwasumbia alba* occurs frequently in the latter habitat, where collectors have noted *Bromadia salicina* (Rubiaceae), *Ricinodendron heudelotii* (Euphorbiaceae), and *Zenkerella* sp. (Fabaceae) as other dominants. The lowland rain forests adjacent to the Uluguru Mountains (which includes the Kimboza forest) have been identified as one of the subcenters of endemism within the coastal forests (Burgess et al. 1998). The Kimboza forest now counts three endemic genera and approximately 17 endemic species of angiosperms (Burgess et al. 1998; Clarke et al. 2000). The restricted distribution of *Mwasumbia* is a common pattern in the coastal East African belt vegetation, with about 40% of all coastal forest plant endemics known from a single forest (Clarke et al. 2000). Within Annonaceae, another example can be found in the monotypic genus *Sanrafaelia*, restricted to the lowland rain forests adjacent to the East Usambara Mountains (Verdcourt 1996; Couvreur et al. 2006). Compared to other families found in the East African rain forests, Annonaceae appear particularly rich in endemic genera, now totaling six (approximately 21% of total number of endemic genera, Clarke et al. 2000). Other highly diverse families such as Rubiaceae have, for example, just four endemic genera (approximately 14% of total number of endemic genera, Clarke et al. 2000).

**Additional Specimens Examined**—TANZANIA. Morogoro Region: Kimboza Forest Reserve, 2 km after Kimboza village, 45 km from Morogoro, 7°01.32'S, 37°48.28'E; 250 m, 25 November 2006, Couvreur & Mbogo 73 (DSM, OWU, WAG); Makuyuni Ward, Kimboza Community Forest Reserve, N of River Ruvu, SE end of Kimboza Kibangire commun- ity forest, 07°01′29″S, 35°14′32″E; 250–260 m, 20 March 2006, Kayombo et al. 5323 (MO, OWU); Kimboza Forest Reserve, 225 m, 19 September
Fig. 3. *Mwasumbia alba*. A. Flowering branch. B. Detail of young branch with erect hairs. C. Detail of flower bud. D. Detail of full grown flower. E. Detail of sepals. F. Flower with petals removed showing receptacle with stamens and carpels. G. Carpel with missing stigma longitudinally sectioned showing uniseriate row of ovules. H. Detail of inner petal, outer side. I. Detail of outer petal, outer side. J. Detail of outer petal surface. K. Detail of stamen, front view. L. Detail of broad stamen. M. Fruiting pedicel bearing one monocarp. N. Detail of seed, above view. O. Detail of seed with hilum and raphe, side view. All drawings from Couvreur 85 (WAG), except M-O which are from Semsei 810 (K).
IUCN Conservation Status—VU D2. *Mwasumbia* has been collected seven times, always from the same locality in the Kimboza Forest Reserve. During the 1980s the reserve was severely logged, depriving the forest of its tall canopy trees, even though human activities had been prohibited since 1982 (Madoffe et al. 2006). There appears to be no decline in collections during the past half century. Although no precise population studies have been conducted, the first author encountered it a few times near the road. In addition, it has been shown that even though there has been a significant loss of forest in lowland and submontane areas in the Ulugurus, most endemic plant species still survive, having been collected again recently (Burgess et al. 2002). However, there is no doubt that the distribution area of *Mwasumbia* is very restricted in size being concentrated in less that 20 km², and we therefore apply the category of “Vulnerable” under criterion D2. This is regarded as a special criterion. Because this species is restricted to a small area and occurs in just one locality it is “prone to the effects of human activities or stochastic events within a very short time period in an uncertain future, and is thus capable of becoming Critically Endangered or even Extinct in a very short time period” (IUCN 2001).

**Discussion**

The African Short-Branch Clade—The infrafamilial classification of Annonaceae has always been problematic mainly due to the absence of unambiguous floral, fruit, and seed characters (Walker 1971; van Heusden 1992; Doyle and Le Thomas 1996). However, recent morphological cladistic analyses (Doyle and Le Thomas 1994; Johnson and Murray 1995; Doyle and Le Thomas 1996; Chatrou et al. 2000) as well as molecular phylogenetic studies (Doyle et al. 2000; Mols et al. 2004; Richardson et al. 2004; Pirie et al. 2006; Couvreur et al. 2008) have elucidated many intergeneric and higher-level relationships. The grouping of Greenwayodendron with *Piptostigma* in an early diverging position within the SBC was already inferred in previous molecular phylogenetic analyses (Richardson et al. 2004; Pirie et al. 2006). Although the position of *Annickia* is unresolved with our dataset we shall nevertheless include it in the discussion. Indeed, based on a seven plastid DNA marker dataset (approximately 7,200 characters), *Annickia* was recovered as sister to both Greenwayodendron and *Piptostigma* with moderate support (BS = 76%, Lars Chatrou, pers. comm.). Well supported clades based on molecular data within Annonaceae have yet to receive formal infrafamilial names. We shall thus refer to this clade as the African short-branch clade (ASBC). The close relationship of the ASBC genera was, however, not apparent from morphology or palynology alone. Informal family level classifications (i.e. without any analysis of the data) based on different morphological characters such as pollen (Walker 1971; Walker 1972), flower (Fries 1959; van Heusden 1992) or fruit (van Setten and Koek-Noorman 1992) did not provide support for their grouping. When using floral morphology, three of the four genera were not assigned to any group (van Heusden 1992). Additionally, a morphological cladistic analysis of the Annonaceae resolved *Piptostigma* as sister to both *Polyceratocarpus* and the Neotropical genus *Sapranthus*, while Greenwayodendron was linked with the early diverging ambavioid clade and *Annickia* clustered within the malmeoid clade (Doyle and Le Thomas 1996). Thus, this group is hard to characterize from morphology alone, a common situation for African Annonaceae systematics (Couvreur et al. 2008). Even the recently published revision of *Annickia* (Versteegh and Sosef 2007) did not shed any more light on this situation.

**Morphological Affinities of Mwasumbia**—*Mwasumbia* presents several floral affinities with Greenwayodendron, *Piptostigma*, and *Polyceratocarpus* (Table 2). This similarity is also found at the molecular phylogenetic level, where *Mwasumbia* appears in an intermediate position between the Greenwayodendron and *Piptostigma*/Polyceratocarpus clades (Fig. 2). Without conducting any formal comparative analyses, we

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Annickia</em></th>
<th><em>Greenwayodendron</em></th>
<th><em>Mwasumbia</em></th>
<th><em>Piptostigma</em></th>
<th><em>Polyceratocarpus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiary venation</td>
<td>intermediate</td>
<td>reticulate</td>
<td>intermediate</td>
<td>parallel</td>
<td>parallel</td>
</tr>
<tr>
<td>Inflorescence position</td>
<td>terminal</td>
<td>terminal</td>
<td>lateral</td>
<td>parallel</td>
<td>parallel</td>
</tr>
<tr>
<td>Sex distribution</td>
<td>bisexual</td>
<td>androdioecious</td>
<td>biseual (?)</td>
<td>biseual</td>
<td>androdioecious</td>
</tr>
<tr>
<td>Sepal asteivation</td>
<td>valvate</td>
<td>imbricate</td>
<td>imbricate</td>
<td>valvate</td>
<td>valvate</td>
</tr>
<tr>
<td>Petal number</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Petal relative length</td>
<td>outer absent</td>
<td>outer = inner</td>
<td>outer = inner</td>
<td>outer &lt; inner</td>
<td>outer = inner</td>
</tr>
<tr>
<td>Torus (stamen portion)</td>
<td>flat/conical</td>
<td>flat/conical</td>
<td>short cylindrical</td>
<td>short cylindrical</td>
<td>short cylindrical</td>
</tr>
<tr>
<td>Torus (carpel portion)</td>
<td>flat/convex</td>
<td>flat/convex</td>
<td>concave</td>
<td>concave</td>
<td>concave</td>
</tr>
<tr>
<td>Apex of connective</td>
<td>discoid/tongue-shaped</td>
<td>discoid/tongue-shaped</td>
<td>discoid</td>
<td>discoid</td>
<td>discoid</td>
</tr>
<tr>
<td>Pollen: ornamentation</td>
<td>psilate</td>
<td>weakly verrucate</td>
<td>verrucate</td>
<td>psilate</td>
<td>psilate</td>
</tr>
<tr>
<td>Pollen: infractum</td>
<td>colcemelle</td>
<td>granular</td>
<td>granule</td>
<td>granule</td>
<td>granule</td>
</tr>
<tr>
<td># of carpels</td>
<td>15-20</td>
<td>4</td>
<td>3-14</td>
<td>3-14</td>
<td>2-20</td>
</tr>
<tr>
<td>Number of ovules per carpel</td>
<td>1</td>
<td>2</td>
<td>5-8</td>
<td>6-10</td>
<td>20-30</td>
</tr>
<tr>
<td>Ovule arrangement</td>
<td>basal</td>
<td>1-seriate lateral</td>
<td>1-seriate</td>
<td>2-seriate</td>
<td>2-seriate</td>
</tr>
<tr>
<td>Monocarps</td>
<td>stipitate</td>
<td>stipitate</td>
<td>sessile</td>
<td>sessile</td>
<td>sessile</td>
</tr>
<tr>
<td>Seeds per monocarp</td>
<td>1</td>
<td>1-2</td>
<td>2-3</td>
<td>numerous</td>
<td>numerous</td>
</tr>
<tr>
<td>Raphe</td>
<td>raised</td>
<td>grooved</td>
<td>grooved</td>
<td>flat</td>
<td>grooved/flats</td>
</tr>
</tbody>
</table>
briefly discuss a few of the important characters that either unite or distinguish *Mwasumbia* from these genera.

Three characters have been found to be potential synapomorphies of the *Mwasumbia*, *Piptostigma* and *Polyceratocarpus* clade.

1. **Position of inflorescences.** Within Annonaceae the position of the inflorescences can be either terminal (including leaf opposed) or axillary (Fries 1959). In both cases the inflorescence can appear supra- or extra-axillary, but these positions are achieved via metatopic displacement (Weberling and Hoppe 1996) and have variable importance for classification. In *Mwasumbia* the inflorescences are axillary as in *Piptostigma* and *Polyceratocarpus*, whereas *Annickia* and *Greenwayodendron* have terminal ones (Table 2).

2. **Shape of the floral torus.** This character is fairly constant within genera (van Heusden 1992) but has been shown to be highly homoplasious at the family level (Doyle and Le Thomas 1996). *Mwasumbia*, *Piptostigma*, and *Polyceratocarpus* have a short cylindrical shape for the stamen bearing part and a concave shape for the carpel bearing one (Table 2).

3. **Monocarp base.** Within Annonaceae individual monocars can be either stipitate or sessile, a feature thought to contain little taxonomic information (van Setten and Koek-Noorman 1992). However, within African Annonaceae, a sessile monocarp did provide one of the few morphological characters allowing the African long-branch clade to be distinguished from the uvarioid clade (Couvreur et al. 2007). Here, sessile monocars are also a synapomorphy for the *Mwasumbia*, *Piptostigma* and *Polyceratocarpus* clade (Table 2).

A few characters, however, can be used to distinguish *Mwasumbia* from *Piptostigma* and *Polyceratocarpus*.

1. **Leaf architecture.** This character has been shown to be phylogenetically useful within Annonaceae (Scharaschkin and Doyle 2006). Tertiary venation appears particularly interesting. Three types are found in Annonaceae, all represented within the ASBC: reticulate, parallel (or percurrent), and intermediate between the two (with mixed parallel and reticulate tertiary veins, see Fig. 6 of Doyle and Le Thomas 1996). Parallel venation occurs independently in a few genera across the family (e.g. *Monanthotaxis*, *Sapranthus*) and is a synapomorphy for *Piptostigma* and *Polyceratocarpus*. In contrast, the intermediate venation, found in *Mwasumbia* and *Greenwayodendron*, is the most common type for the family. The reticulate state (found in *Annickia*) appears less common but was inferred to be ancestral for the family (Doyle and Le Thomas 1996; Scharaschkin and Doyle 2006).

2. **Sepal aestivation.** The general flower pattern in Annonaceae is one whorl of three sepals alternating with two whorls of three free petals. *Annickia* is unique within the family in that the outer petal whorl is absent (Table 2, van Heusden 1992; Versteegh and Sosef 2007). *Mwasumbia* most strongly resembles *Polyceratocarpus* in petal morphology (Table 2), both having petal whors equal in length and thickness, and a valvate petal aestivation, with the outer ones spreading horizontally at anthesis. In *Greenwayodendron* the petals are also equal in length but are much narrower and are imbricate. However, in contrast to *Polyceratocarpus*, *Mwasumbia* possesses slightly imbricate sepals, a character shared with *Greenwayodendron*. Sepal and petal aestivation were used by Fries (1959) to separate the family into two main groups. However, this character has been shown repeatedly to be highly homoplasious providing little useful taxonomic information for Annonaceae classification (Doyle and Le Thomas 1996; Couvreur et al. 2008).

3. **Ovule rows.** In Annonaceae, when more than two ovules are present they are either positioned in one (uni-seriate) or in two (biseriate) rows. *Polyceratocarpus* was indicated as having numerous ovules in a single row (van Heusden 1992). However, this observation is not consistent with a previous description provided by Le Thomas (1969) who indicated *Polyceratocarpus* as having two rows of ovules. Our personal observations also confirm this latter view. Thus, both *Piptostigma* and *Polyceratocarpus* have a biseriate placentation, while *Mwasumbia* has a uni-seriate one as in *Greenwayodendron*.

**Pollen Morphology**—The most remarkable feature of *Mwasumbia* pollen (Fig. 1), the conspicuous intine bulge, also occurs in *Polyceratocarpus*. The intine bulge is small in *Greenwayodendron* (Le Thomas 1980, plate 19.1, as *Polyalthea suaveolens*) and in *Piptostigma*. The state of this character is unknown in *Annickia*, although, judging from the extensive acetylated exine shown by Walker (1971, plate 5.4, as *Enantia*), it might be relatively small. Strongly bulging intines are found also outside the African short-branch clade in *Metocarpidium* (Le Thomas 1980, plate 5.2) and *Polyalthea sumatrana* (Waha and Hesse 1988, Fig. 1F). This character might not be so rare within Annonaceae as it will only be apparent if mature pollen is preserved and prepared in the right way (see Material and Methods).

Two features distinguish the pollen of *Mwasumbia* from that of the other genera in the ASBC: exine ornamentation and infratectal structure. *Annickia*, *Piptostigma*, and *Polyceratocarpus* have a psilate exine, while ornamentation is weakly verrucate in *Greenwayodendron* (Le Thomas 1980) and verrucate (-rugulate) in *Mwasumbia*. The infratectum is columnellate in *Annickia* (Le Thomas 1980) and granular in *Greenwayodendron* (Le Thomas 1980), *Piptostigma*, and *Polyceratocarpus*, but indistinctly columnellate/granular in *Mwasumbia*. The pollen of *Mwasumbia* thus underlines the distinctiveness of the genus. While it is concordant with the pollen morphology in the other four genera, it does not show a particular connection with the pollen of any one, but seems to be intermediate between the pollen of *Annickia* and that of the others.

**Paraphyly of Piptostigma**—Our analyses showed that *Piptostigma* is paraphyletic, but the taxonomy of this genus is poorly known. It is, however, interesting to note that *Piptostigma fisculatum* (De Wild.) Boutique, which renders the genus paraphyletic (see Fig. 2), was initially placed in a distinct genus, *Brieya* De Wild. (De Wildeman 1914), to which was later added *B. latipetala* Exell (Exell 1950). Both *Brieya* and *Piptostigma* are characterized by much longer inner petals with sepaloid outer petals, a unique character for African Annonaceae genera. This situation is also encountered in several other South-East Asian SBC genera such as *Miliusa*, *Phaeanthus* (van Heusden 1992; Mols and Kessler 2000, 2003) and *Crabella* (Saunders et al. 2004), and in the South American genus *Heteropetalum*.

*Brieya*, however, was distinguished from *Piptostigma* by having fasciculate flowers with basally free monocars (De Wildeman 1914). Additionally, *Brieya* species have a slightly different leaf morphology with significantly fewer secondary veins (Exell 1950), thus presenting some morphological similarity to those of *Polyceratocarpus*. We shall not make any formal taxonomic decisions here as this will require an in-depth comparison of the two genera.
Acknowledgments. The curators of K and MO are thanked for the loan of material. Marc Sosef (WAG) is deeply thanked for critically reading through an earlier version of the manuscript. Roy Gereau (MO) is thanked for his comments on pollen morphology within Annonaceae. We are grateful to Wim Star and Ben Kiet (NNH-Leiden) for preparing the TEM photographs and pollen plate, respectively. Hans de Vries prepared the fine botanical illustration. The governmental authorities of Tanzania (COSTECH) as well as national park directors are thanked for granting collecting permits. Funding for fieldwork in Tanzania in 2006 came from the Netherlands Organization for Scientific Research (N.W.O.), the Albertina Memmenga Stichting, the Hugo de Vries Fonds and Air France-KLM. Finally, two anonymous reviewers are thanked for their useful comments.

Literature Cited


