

Andrachne cuneifolia* (Phyllanthaceae; Euphorbiaceae s.l.) is a *Phyllanthus

MARIA S. VORONTSOVA^{1*}, PETRA HOFFMANN¹, HASHENDRA KATHRIARACHCHI², DUANE A. KOLTERMAN³ and MARK W. CHASE¹

¹Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK

²Department of Systematics and Evolutionary Botany, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria

³Departamento de Biología, Universidad de Puerto Rico, Mayagüez, 00681-9012, Puerto Rico

Received February 2007; accepted for publication August 2007

The phylogenetic relationships, morphology, and distribution of the enigmatic species *Andrachne cuneifolia* of problematic generic affiliation were examined. Nuclear internal transcribed spacer (ITS) and plastid *matK* sequences were analysed with a matrix of *matK* and ITS DNA sequences for *Phyllanthus* and *Andrachne* species. According to this analysis, this taxon belongs in the genus *Phyllanthus*, subgenus *Xylophylla*. Comparison of morphological characters also supports the placement of this species in *Phyllanthus* subgenus *Xylophylla*. The correct name is *Phyllanthus cuneifolius* (Britton) Croizat. A full description, illustration, conservation rating, and distribution map are provided. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 155, 519–525.

ADDITIONAL KEYWORDS: Cuba – internal transcribed spacer (ITS) – Malpighiales – *matK* – molecular phylogenetics – morphology – Puerto Rico – systematics.

INTRODUCTION

Andrachne cuneifolia Britton is a rare and enigmatic endemic of the serpentine and limestone floras of the Caribbean. It was first mentioned in an article on new plants from Cuba (Britton, 1920) as occurring in Punta Maisi, Oriente Province. The only specimen cited in this publication, however, was a collection from Puerto Rico by Underwood and Griggs. Britton, unsure of its generic affiliation, published it with a brief description as *Andrachne* (?) *cuneifolia* Britton (1920). Urban (1926) reported the species from Haiti, mentioning its occurrence in Cuba without a specimen citation. He provided a more extensive description, but did not see the staminate flowers and failed to note the biseriate calyx. He questioned the association of this species with *Andrachne*, suggesting that it belonged either to *Phyllanthus* or a new genus. The new combination *Phyllanthus cuneifolius* (Britton) Croizat was finally published by Croizat

(1943: 12), who argued its similarity to *Phyllanthus orbicularis* Kunth based on the presence of a stipitate ovary and the ‘peculiar male disc’. He unfortunately failed to provide either a detailed staminate flower description or specimen citations. Leon & Alain (1953) followed Croizat (1943), listing Maisi and Jauco in Oriente, but again not citing specimens. The controversy did not end there; in 1954, Webster redetermined the holotype at New York (on a herbarium label, see The New York Botanical Garden Virtual Herbarium: <http://sciweb.nybg.org/VirtualHerbarium.asp>) as *Securinea cuneifolia* (Britton) Webster [comb. ined.]. In his monograph of West Indian *Phyllanthus*, Webster (1958: 208) excluded *P. cuneifolius* but did not offer an alternative generic placement. He changed the determination on the New York holotype again in 1981 to *P. cuneifolius*.

Recently, Phyllanthaceae and Phyllantheae have been the subject of molecular phylogenetic studies (Wurdack *et al.*, 2004; Kathriarachchi *et al.*, 2005, 2006; Samuel *et al.*, 2005). The recent investigation of *Andrachne* s.l. (tribe Poranthereae; Vorontsova *et al.*, in press) has made it possible to address the question

*Corresponding author. E-mail: m.vorontsova@kew.org

of the generic affiliation of *A. cuneifolia* using DNA sequence data. We clarify the affinity of *A. cuneifolia* with a morphological study and a phylogenetic analysis of internal transcribed spacer (ITS) nuclear rDNA and plastid *matK* sequences.

MATERIAL AND METHODS

Silica-dried material (Chase & Hills, 1991) of *A. cuneifolia* was obtained by D. A. Kolterman in 2004 in Puerto Rico. Herbarium material of *Andrachne microphylla* (Lam.) Baill., *A. ciliatoglandulosa* (Millsp.) Croizat, and *A. telephioides* L. was sampled at the Royal Botanic Gardens, Kew. DNA was extracted with the 2 × cetyltrimethylammonium bromide (CTAB) method described by Doyle & Doyle (1987), but with a 2-week precipitation with isopropanol (herbarium specimens) or ethanol (silica-dried samples) and purification on a caesium chloride/ethidium bromide gradient (1.55 g mL⁻¹). DNA of *A. arida* (Warnock & M.C. Johnst.) G.L. Webster was provided by K. J. Wurdack (Smithsonian Institution, Washington, DC, USA). Aliquots of all DNA samples used in this study are available from the Royal Botanic Gardens Kew DNA Bank (<http://www.kew.org/data/dnaBank/homepage.html>).

The ITS region of the above taxa was amplified and sequenced following Kathriarachchi *et al.* (2006); the *matK* gene and its partial flanking *trnK* intron were amplified and sequenced following Samuel *et al.* (2005). These sequences were analysed in conjunction with the combined Phyllanthaceae data set used in Kathriarachchi *et al.* (2006). Sequence alignment was carried out by eye. In this study, the two complete matrices were combined, and phylogenetic analyses using maximum parsimony were performed for the combined data set as implemented in PAUP* version 4.0b10 (Swofford, 2003), according to the method described in Kathriarachchi *et al.* (2006). Analyses were conducted with nucleotide substitutions equally weighted (Fitch parsimony; Fitch, 1971) and gaps treated as 'missing' data. Heuristic searches were performed initially using 1000 random taxon addition replicates, tree bisection–reconnection (TBR) branch-swapping, and 'keeping multiple trees' (MulTrees) in effect, but holding ten trees per replicate to minimize swapping on large numbers of suboptimal trees. All trees thus obtained were used as starting trees for a

further search with the MulTrees option in effect and a limit of 15 000 trees. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 1000 bootstrap replicates, TBR branch-swapping, and simple sequence additions.

As an additional measure of support, phylogenetic relationships were also reconstructed for the combined data set using Bayesian inference, as implemented in the program MrBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001). MrModeltest 2.2 (Nylander, 2004) was used to find the best-fitting substitution model. The GTR + I + G model was selected for the ITS data set, and the SYM + I + G model was selected for the *matK* data set; all other parameters were determined by MrBAYES. ITS and *matK* were treated as separate partitions in the combined analysis. Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 2 000 000 generations and sampled every 100 generations. MCMC reached a plateau in less than 165 000 generations, and the first 1650 trees ('burn in') were excluded. The rest of these trees were compiled as a majority-rule consensus in PAUP*. The frequencies of each clade in this consensus tree are the Bayesian posterior probabilities (Fig. 1).

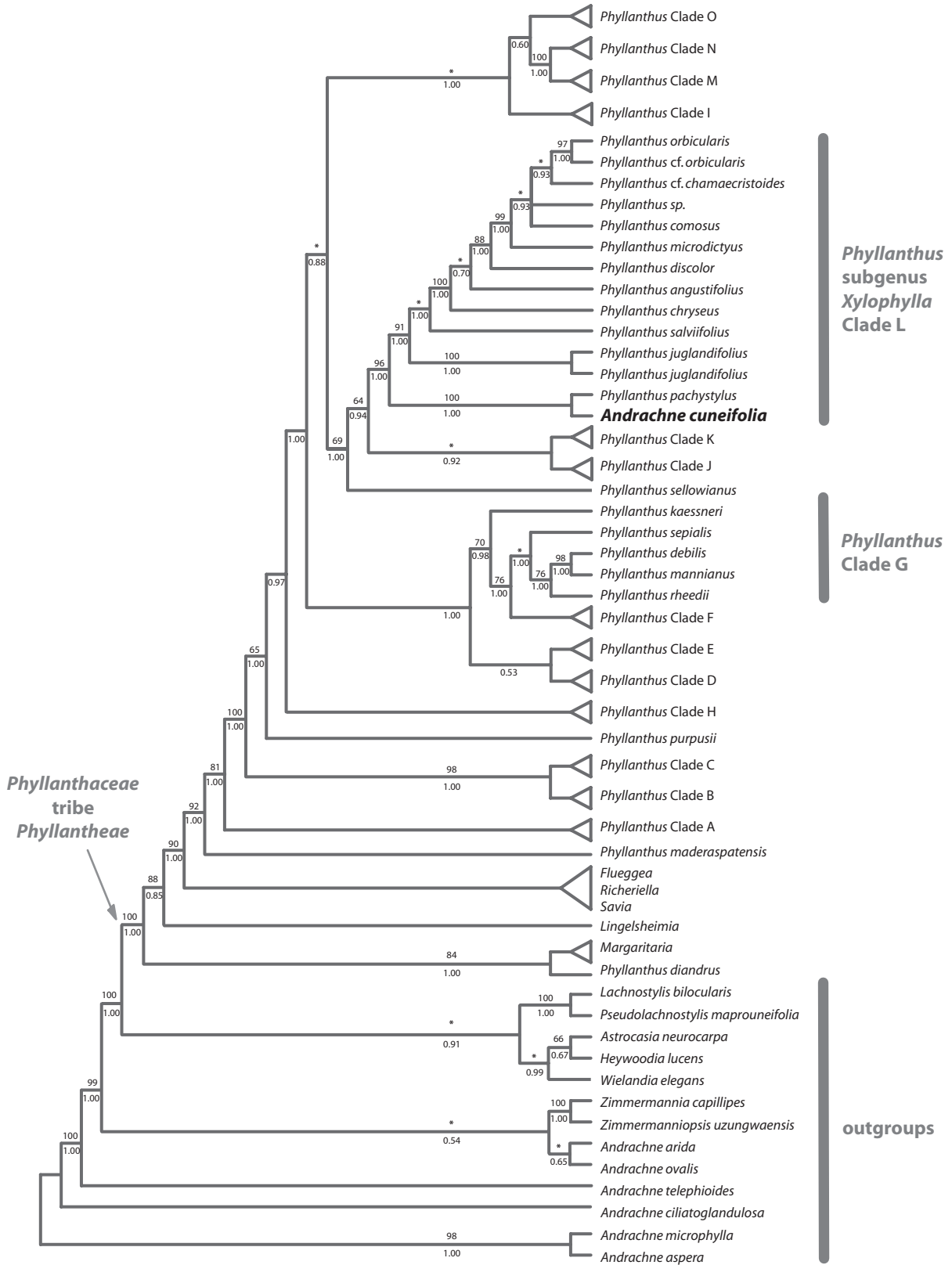
The outgroups of the original data set by Kathriarachchi *et al.* (2006), containing representatives of all other tribes of subfamily Phyllanthoideae (*sensu* Hoffmann, Kathriarachchi & Wurdack, 2006), were used in this study in addition to *A. arida*, *A. ciliatoglandulosa*, *A. microphylla*, *A. ovalis* (E. Mey. ex Sond.) Müll. Arg., and *A. telephioides*.

RESULTS AND DISCUSSION

The ITS and *matK* sequences of *A. cuneifolia* were analysed with the Phyllanthaceae matrix used by Kathriarachchi *et al.* (2006). *Andrachne cuneifolia* was embedded in *Phyllanthus s.s.* as sister to *Phyllanthus pachystylus* Urb. (*Phyllanthus* subgenus *Xylophylla*), with bootstrap support of 100% and a posterior probability of 1.00 (Fig. 1). We conclude that *A. cuneifolia* is in fact a species of *Phyllanthus* and should be considered a member of *Phyllanthus* subgenus *Xylophylla*.

Subgenus *Xylophylla* is centred in the Caribbean, with a few additional sections confined to South America (Webster, 1958). It originally only included taxa with phyllanthoid branching, a character of

Figure 1. Bayesian analysis consensus tree and the strict consensus of 1800 most parsimonious trees [6851 steps; consistency index (CI), 0.39 (including uninformative characters); retention index (RI), 0.67] of tribe Phyllanthaceae inferred from combined nuclear internal transcribed spacer (ITS) and plastid *matK* data. Bootstrap percentages above 50% are shown above the branches and posterior probabilities of 0.50 or above are shown below the branches. Clades not present in the maximum parsimony consensus tree are marked with an asterisk. *Phyllanthus* clades follow Kathriarachchi *et al.* (2006).



great significance in *Phyllanthus* (Webster, 1956). Croizat's section *Elutanthos* (Croizat, 1943) was included by Webster (1958) in the new subgenus *Botryanthus* because of its lack of phyllanthoid branching. Because of the shared distinctive areolate pollen grains, section *Elutanthos* was later included in subgenus *Xylophylla* (Webster & Carpenter, 2002). This was later supported by molecular results (Kathriarachchi *et al.*, 2006).

Caribbean taxa of the subgenus *Xylophylla* have been comprehensively revised by Webster (1958). The lack of phyllanthoid branching in *P. cuneifolius* and its strongly supported sister relationship with *P. pachystylus* allow us to have confidence in placing this taxon in *Phyllanthus* subgenus *Xylophylla* section *Elutanthos*.

The exact placement of *P. cuneifolius* within section *Elutanthos* will require further clarification, because sampling for our analyses included only *P. pachystylus* out of the seven or more species (Webster, 1958) of the section. A comparison between *P. cuneifolius* and *P. pachystylus* is provided below.

COMPARISON BETWEEN *P. CUNEIFOLIUS* AND *P. PACHYSTYLUS*

The sister species of *P. cuneifolius* in our analysis is *P. pachystylus*, a Cuban endemic confined to serpentine soils of eastern Cuba (Fig. 3). The two taxa bear some striking similarities. Both species are sparsely branched shrubs with grey stems giving rise to steeply ascending reddish-brown branches. The leaves of both species are rigidly coriaceous and erect with a grey-bluish tinge. The flowers of both display two series of three sepals and three stamens, with the filaments fused for their entire length. In *P. cuneifolius*, anthers remain discrete with oblique dehiscence, whereas those of *P. pachystylus* are fused to a greater extent with horizontal dehiscence. Further differences include the inflorescence structure and leaf shape. *Phyllanthus pachystylus* inflorescences are nodding pseudo-terminal thyrses, whereas, in *P. cuneifolius*, flowers are borne in fascicles on brachyblasts. Leaf blades are obovate to cuneate in *P. cuneifolius* and elliptic-oblong in *P. pachystylus*. *Phyllanthus pachystylus* is also a bigger shrub with larger leaves, fruits, and seeds, as well as longer petioles and pistillate pedicels.

Phyllanthus cuneifolius (Britton) Croizat (1943)
(Fig. 2)

Andrachne (?) *cuneifolia* Britton

Type: Puerto Rico, Coamo, Coamo Springs, 14.vi.1901–22.vii.1901, Underwood & Griggs 545 (holotype NY).

Description: Monoecious shrub 1–3 m tall. Branching relatively sparse; numerous branches originating from the main stem but with little further division. Main stems 2–3 mm in diameter, terete, slightly zigzag with a small brachyblast at each node bearing branches, leaves, and flowers. Branches more pronouncedly zigzag, slender, glabrous, silver grey to red–brown. Internodes 4–8 mm long. Bark smooth and grey–brown with white spongy lenticels. Leaves fascicled, one to three per fascicle, each fascicle a short brachyblast; brachyblasts strictly distichous on all branches with numerous bracts. Stipules persistent, chartaceous, elongate deltoid, apically acuminate, translucent when young becoming red–brown, 0.6–1.5 × 0.2–0.5 mm, glabrous, entire with a hyaline margin, eglandular. Axillary bud scales deltoid, obtuse apically, dark red to black; basal scales of each brachyblast becoming increasingly thick and woody with age. Petioles terete, adaxially flattened, 0.4–1.5 × 0.2–0.3 mm, < 0.1 times the length of the leaf blade, glabrous, grey–green to pale orange–brown, almost invisible on young leaves. Leaf blade obovate to cuneate (4–)6–11(–13) × (3–)4–6(–7.5) mm, 1.5–2.5 times longer than wide, apically rounded, mucronate (mucro, if present, distinct from the rest of the leaf blade, dark red, with ciliate margin) or emarginate, basally acute, eglandular, coriaceous, smooth, shiny above and dull beneath, glabrous, pale blue–green adaxially, pale green abaxially; margin entire, revolute on drying; midvein adaxially depressed to slightly raised, abaxially raised, pale green to brown–green; a single pair of secondary veins arising from the base of the midvein; six to eight pairs diverging from midvein at *c.* 25°, adaxially almost invisible, abaxially raised, pale brown; tertiary venation present in apical half only, visible on abaxial surface only (×10 magnification), flat, pale brown.

Staminate flowers solitary, geminate or in groups of three, in fascicles together with leaves and sometimes a pistillate flower, 1–1.5 × 1–1.5 mm. Bracts deltoid, apically acuminate, 0.6–2 × 0.2–0.5 mm wide, glabrous, chartaceous, red to ferruginous becoming black with age; margin entire to fimbriate. Buds not seen. Pedicel 4–8 mm long, unarticulated, terete, filiform, glabrous, orange–brown to brown. Sepals six, biseriate, obovate, apically rounded to acute, *c.* 2 × 0.5 mm, eglandular, glabrous, chartaceous, green, margin hyaline, slightly undulate, venation not visible; outer sepals apiculate, acumen bright red, fimbriate (glandular?), inner sepals not apiculate. Petals absent. Disc glands six, alternisepalous, discrete, fleshy, circular, with central depression, 0.1 mm high, 0.3 mm in diameter, entire, glabrous, smooth, grey–green. Stamens three, 1.1 mm long, two-thirds as long as sepals; filaments fused for their entire length, 0.9 mm long; staminal column hexagonal in cross-



Figure 2. *Phyllanthus cuneifolius* (Britton) Croizat: A, habit of fruiting branch; B, pistillate flower, two sepals removed; C, staminate flower, two sepals removed; D, E, cross-sections of the ovary; F, habit at an early stage of flowering; G, columella with persistent sepals and disc; H, mericarp after fruit dehiscence; I, cross-section of the fruit with seed. A, F, G, H, I, drawn from *Ekman 3838*; B, C, D, E, drawn from *Axelrod 10183*. Scale bars: A, F, 1 cm; B, C, 1 mm; D, E, 0.5 mm; G, H, I, 1 mm. Drawn by M. S. Vorontsova.

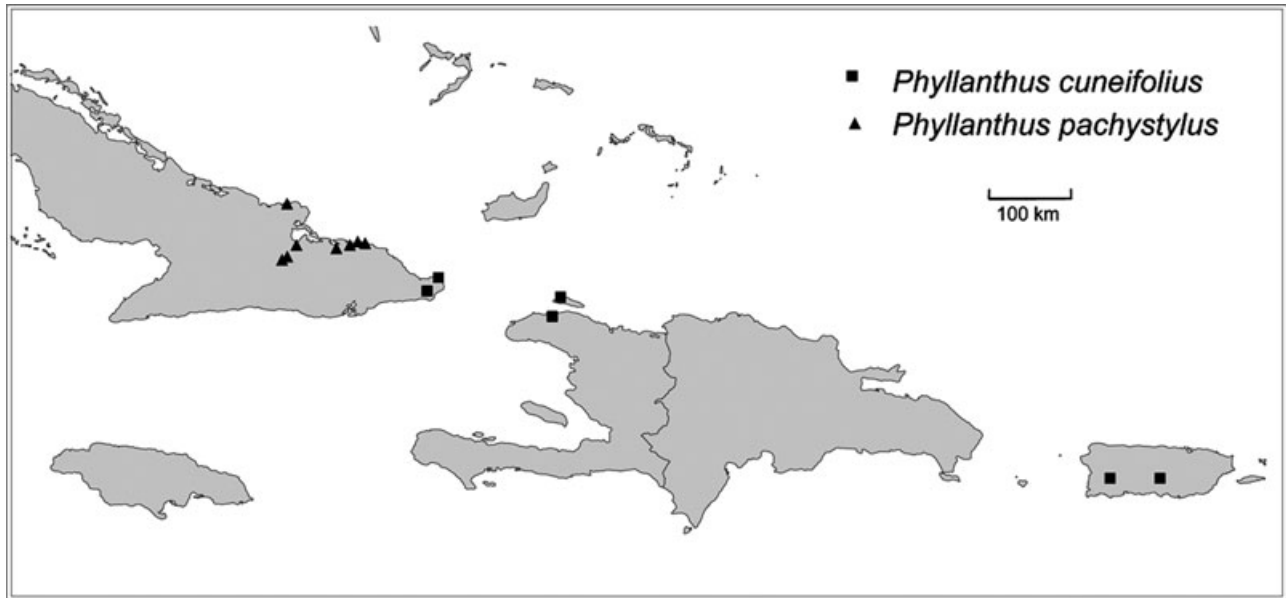


Figure 3. Distribution of *Phyllanthus cuneifolius* (Britton) Croizat (Hispaniola, Puerto Rico, and Cuba) and related *Phyllanthus pachystylus* Urb. (Cuba). *P. cuneifolius* locations in Cuba have been taken from the literature; all other data are based on herbarium collections.

section, narrower in the middle than at base and apex; anthers discrete, 0.3×0.2 mm, two-celled, pale yellow, tilted relative to the filaments *c.* 120° resulting in apparently oblique dehiscence.

Pistillate flowers one per brachyblast, $1.5\text{--}2 \times 1.5\text{--}2$ mm, fascicled with one or two staminate flowers, subtended by one to three leaves. Bracts as in staminate flowers. Buds ellipsoid, mostly covered by the thicker red fimbriate tips of the three apiculate sepals, not accrescent. Pedicel 5–13 mm long, unarticulated, green to red–brown, filiform, terete, glabrous. Sepals six, imbricate, obovate, apically rounded to acute, basally saccate, $1\text{--}1.5 \times 0.7$ mm, glabrous, chartaceous, green; margin hyaline, slightly undulate; basal cavity up to 0.3 mm deep, becoming more pronounced as flower matures; venation not visible; outer sepals apiculate, acumen bright red, fimbriate (glandular?), inner sepals not apiculate. Petals absent. Disc glands six, alternisepalous, adjacent, *c.* 0.4×0.45 mm, fleshy, apically uneven, entire, green to translucent pale brown, glabrous. Ovary globose, trilocular, glabrous, becoming stipitate as fruit matures; ovules two per locule, hemitropous, straight, angle between central column and longitudinal axis of ovule *c.* 40° , with nucellar beak protruding 0.05–0.1 mm from the micropyle; obturator one per ovule, arising from central column, spongy. Styles three, *c.* 0.7 mm long, terete, bifid for all to half their length, red–black, straight, spread horizontally; stigmas rounded, not or hardly dilated.

Fruiting pedicel 10–15 mm long, glabrous; mature disc glands discrete, 0.5×0.7 mm, fleshy dark red;

mature sepals $2\text{--}2.8 \times 0.7\text{--}1$ mm (slightly accrescent longitudinally). Fruits solitary, depressed globose, slightly trilobed, $2.5\text{--}4 \times 3\text{--}6$ mm, glabrous, dark red–brown, smooth except for prominent veins, dehiscing loculicidally, septicidally, septifragally; septifragal dehiscence line irregular. Exo- and mesocarp united, chartaceous, sometimes separating from thin, woody endocarp on dehiscence. Columella usually broken at the stipe, sometimes broken in the middle, rarely preserved intact, but then *c.* $1.6 \times 0.8\text{--}1.2$ mm, clavate, widest in the middle (*c.* 1.2 mm), triangular in cross-section, apically abruptly narrowed (*c.* 0.8 mm long), basally tapering (stipe, *c.* 0.5×0.4 mm), pale yellow to brown.

Seeds two per locule, triquetrous, acute at micropyle, rounded at chalaza, $3 \times 1.5 \times 1.5$ mm, pale yellow around hilum, yellow–brown dorsally, smooth, moderately shiny; micropyle 0.1 mm in diameter; hilum 0.7×0.3 mm, white. Embryo not seen; fide Urban (1926): nearly as long as seed, cotyledons ovate, a little plicate longitudinally, fleshy; radicle tapering with three points, short; endosperm fleshy.

Distribution: Puerto Rico, Haiti (Fig. 3). No specimens of *P. cuneifolius* from Cuba (Britton, 1920; Urban, 1926; Leon & Alain, 1953) could be located in B, F, GH, H, JE, K, LE, MAPR, MO, S, W, WU, or WVA.

Habitat: Limestone or serpentine substrate.

Ecology: Dry scrub. The label of Axelrod 10183 cites 'moist lowland forest'.

Conservation status: Vulnerable (VU) under criteria VU, B1a,b(iii) (IUCN, 2001). Extent of occurrence is below 20 000 km² and only four localities are known. This species is under threat from serpentine mining.

Specimens examined: Puerto Rico: Coamo: Coamo Springs, 14.vi.1901–22.vii.1901, *Underwood & Griggs 545* (holotype NY); El Moreno, 13.v.1922, *Britton et al. 6507* (F!); Prope Coamo, 23.xii.1885, *Sintenis 3212* (B!, MO!); Sabana Grande: Santa Ana, near Sabana Grande, 9.ii.1915, *Britton & Cowell 4025* (MO!); Yauco: Susua Forest Reserve, 27.iii.1997, *Axelrod et al. 10183* (K!); Barrio Susua Alta, Susua Forest Reserve, along trail above (E of) Río Loco, 29.i.2004, *Kolterman et al. 1008* (K!); Susua, 28.vi.1962, *H. Alain 9249* (MAPR!); Susúa Forest Reserve, trail from Reserve Office N along Río Loco, 17.ii.1992, *Axelrod & Chavez 4015* (MAPR!); Susúa Forest Reserve; Drainage of Río Loco, 19.iv.1997, *Breckon & Gonzáles 5078* (MAPR!); Bosque Estatal de Susúa, 13.v.1990, *García 2983* (MAPR!); Bosque Estatal de Susúa, aproximadamente 300 m de la entrada principal, 29.iii.1990, *García & Caminero 2867* (MAPR!); Bosque Estatal de Susua, along dirt road on E side of Quebrada Peces, 4.xii.1990, *Breckon et al. 3661* (MAPR!). Haiti: Ile la Tortue at Boucan-Guepe, 22.v.1925, *Ekman 4091* (S!); Pres qu'île du Nord-Ouest, Port-de-Paix, W of Paline Michel, 19.iv.1925, *Ekman 3838* (F!, K!, MO!, S!).

ACKNOWLEDGEMENTS

Special thanks are due to Olivier Maurin and Laszlo Csiba of the Jodrell Laboratory and Gill Challen of the herbarium K for technical support, and to Ken Wurdack (US) for DNA of *Andrachne arida*. We are grateful to the directors and curators of the herbaria B, F, MAPR, MO, and S for the loan of herbarium specimens.

REFERENCES

- Britton NL. 1920.** Cuban plants new to science. *Memoirs of the Torrey Botanical Club* **16**: 57–118.
- Chase MW, Hills HH. 1991.** Silica gel – an ideal material for field preservation of leaf samples for DNA studies. *Taxon* **40**: 215–220.
- Croizat L. 1943.** Notes on American Euphorbiaceae, with descriptions of 11 new species. *Journal of the Washington Academy of Sciences* **33**: 11–20.
- Doyle JJ, Doyle JL. 1987.** A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.
- Felsenstein J. 1985.** Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Fitch WM. 1971.** Towards defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**: 406–416.
- Hoffmann P, Kathriarachchi H, Wurdack KJ. 2006.** A revised phylogenetic classification of Phyllanthaceae (Malpighiales; Euphorbiaceae sensu lato). *Kew Bulletin* **61**: 37–53.
- Huelsenbeck JP, Ronquist FR. 2001.** MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.
- IUCN. 2001.** *IUCN red list categories*. Gland: IUCN Species Survival Commission.
- Kathriarachchi H, Hoffmann P, Samuel R, Wurdack KJ, Chase MW. 2005.** Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid *atpB*, *matK*, *3'ndhF*, *rbcL*, nuclear *PHYC*). *Molecular Phylogenetics and Evolution* **36**: 112–134.
- Kathriarachchi H, Samuel R, Hoffmann P, Mlinarec J, Wurdack KJ, Ralimanana H, Stuessy TF, Chase MW. 2006.** Phylogeny of the tribe Phyllanthaceae (Phyllanthaceae) based on nrITS and plastid *matK* sequence data. *American Journal of Botany* **93**: 637–655.
- Leon H, Alain H. 1953.** *Phyllanthus*. In: *Flora de Cuba III. Contribuciones Ocasionales del Museo de Historia Natural de la Salle*, No. 13. Havana, Cuba: Imp. P. Fernandez y Cia, S. en C., 44–59.
- Nylander JAA. 2004.** *MrModeltest*, version 2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Samuel R, Kathriarachchi H, Hoffmann P, Barfuss HJ, Wurdack KJ, Davis CC, Chase MW. 2005.** Molecular phylogenetics of Phyllanthaceae: evidence from plastid *matK* and nuclear *PHYC* sequences. *American Journal of Botany* **92**: 132–141.
- Swofford DL. 2003.** *PAUP* phylogenetic analysis using parsimony (*and other methods)*, version 4. Sunderland, MA: Sinauer Associates.
- Urban. 1926.** *Plantae Haitiensis novae vel rariores III*. *Arkiv foer Botanik* **20A**: 1–94.
- Vorontsova MS, Hoffmann P, Maurin O, Chase MW. in press.** Molecular phylogenetics of tribe Poranthereae (Phyllanthaceae; Euphorbiaceae sensu lato). *American Journal of Botany*, in press.
- Webster GL. 1956.** A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* **37**: 91–122.
- Webster GL. 1958.** A monographic study of the West Indian species of *Phyllanthus*. *Journal of the Arnold Arboretum* **39**: 49–100, 111–212.
- Webster GL, Carpenter KJ. 2002.** Pollen morphology and phylogenetic relationships in Neotropical *Phyllanthus* (Euphorbiaceae). *Botanical Journal of the Linnean Society* **138**: 325–338.
- Wurdack KJ, Hoffmann P, Samuel R, Bruijn A, Van der Bank M, Chase MW. 2004.** Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *rbcL* DNA sequences. *American Journal of Botany* **91**: 1882–1990.