Generic affiliations of *Canthium* species placed under *Pyrostria* group B sensu Bridson (Vanguerieae, Rubiaceae) inferred from morphology and molecular data

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**Abstract**

**Background:** *Pyrostria sensu lato* (s.l.) is regarded as one of the polyphyletic group within Vanguerieae formerly comprising of *Pyrostria sensu stricto* (s.s.), *Pyrostria* group A and *Pyrostria* group B delineated by the number of locules and geographical occurrence. Recent molecular phylogenetic studies within the genus have narrowed its circumscription that resulted in the merging of *Pyrostria* group A and *Pyrostria* s.s. Although some species of *Pyrostria* group B were already transferred to *Pyrostria* s.s. and *Psydrax* based on morphology, other representatives of the group remain unsettled.

**Results:** Bayesian and parsimony analysis of the combined ITS (nrDNA) and trnL-F (cpDNA) datasets showed a well-supported clade of the whole Vanguerieae containing four Philippine endemic representatives of *Pyrostria* group B. The placement of *Canthium oligophlebium*, *Canthium obovatifolium* and *Canthium ramosii* within *Pyrostria* s.s. (PP = 0.99; BS = 85%) is robustly supported likewise the affiliation of *C. gynochthodes* with *Psydrax* (PP = 0.94; BS = 85%). Morphological features shared by our species with *Pyrostria* s.s. and *Psydrax* further supports our molecular data.

**Conclusion:** Our study supports the earlier hypothesis that *Canthium oligophlebium*, *C. obovatifolium* and *C. ramosii* should be placed under *Pyrostria* s.s. except for *C. gynochthodes* that grouped with *Psydrax*. Four new combinations are proposed in this study. The generic affiliations of other species of *Pyrostria* group B should be reinvestigated towards a more natural classification in Vanguerieae.

**Keywords:** Bayesian analysis; ITS; *Psydrax*; *Pyrostria*; trnL-F; Vanguerieae
morphology and molecular data, there are still understudied species. When Bridson (1987) reinstated the genus *Pyrostria* Comm. ex A. Juss., she lumped all *Pyrostria* species and representatives of *Canthium* with pair of persistent connate bracts (bracteate species) under *Pyrostria* s.l. and suggested informal groups (*Pyrostria* s.s., *Pyrostria* group A and *Pyrostria* group B) based on the number of locules and geographical occurrence. Both *Pyrostria* s.s. (pluri-locular ovary) and *Pyrostria* group A (bilocular ovary) have unisexual flowers and well represented in Madagascar, the latter extends from Africa to Arabia. Meanwhile, *Pyrostria* group B shares characters with *Pyrostria* group A in having unisexual flowers, bilocular ovaries, broad corolla tube, and 4–5 corolla lobes but the former radiates as far as SE Asia. The polyphyly of *Pyrostria* within the dioecious Vanguerieae by lumping species was initially addressed by Lantz and Bremer (2004) in recovering a clade composed mainly of dioecious species but failed to discussed further this group due to the poor internal support. Razafimandimbison et al. (2009) established a new generic delimitations using molecular data and suggested informal groups (*Pyrostria* group A to *Pyrostria* s.s). Their study, however, failed to include any species under *Pyrostria* group B and made an assumption that this group belongs to *Pyrostria* s.s. due to the presence of paired bracts which is a typical character for this genus. In an earlier study based on morphology, Utteridge and Davis (2009) transferred two SE Asian *Canthium* species belonging to *Pyrostria* group B (*C. brunneens* Craib and *C. cochinchinense* Pierre ex Pit. in H.Lecomte) to *Pyrostria* s.s. Recently, Alejandro et al. (2013) transferred the Philippine endemic *C. subsessifolium* (Merr.) Merr. to *Pyrostria*. In contrast, *Electronia amplifolia* Elmer informally placed under *Pyrostria* group B was transferred to *Psydax* (Ruhsam et al. 2008).

There are still left unresolved endemic Philippine species placed under *Pyrostria* group B probably associated with *Pyrostria* such as *C. brunnenum* (Merr.) Merr., *C. ellipticum* (Merr.) Merr., *C. gynochthodes* Baill., *C. megacarpum* (Merr.) Merr., *C. obovatifolium* (Merr.) Merr., *C. oligophlebium* (Merr.) Merr., *C. ramosii* (Merr.) Merr., and *C. subcapitatum* (Merr.) Merr. Available herbarium materials of these species are scarce and lack reproductive parts for confirmation. In this study, four species of *Canthium* informally placed under *Pyrostria* group B: *C. gynochthodes*, *C. obovatifolium*, *C. oligophlebium*, and *C. ramosii* were collected and challenged their phylogenetic positions within Vanguerieae utilizing molecular sequence data. Furthermore, type specimens were meticulously examined to confirm our molecular results. The present study is a good contribution in understanding a more robust phylogenetic evolutionary trends and lineages within the tribe.

**Methods**

**Taxon sampling**

This study is based on the examination of herbarium sheets from various herbaria as well as field observation. *Canthium gynochthodes*, *C. obovatifolium*, *C. oligophlebium* and *C. ramosii* were collected based on their type protologues. Collected samples (herbarium specimens and preserved reproductive structures in 70% ethanol) were deposited at the USTH for accessioning. Leaf samples were dried in silica-gel for DNA extraction (Chase and Hills 1991).

**Molecular methods**

Genomic DNA was extracted from silica gel-dried leaf samples using the DNeasy Plant Minikit (Qiagen, Germany). The entire ITS region (including the 5.8S gene) was amplified and sequenced using the primer pair P17F/26-82R and P16F/P25R (Popp and Oxelman 2001). Meanwhile, primer pair c/f were used for both amplification and sequencing of the trnL-F region (Taberlet et al. 1991). DNA amplification was carried out following the work of Alejandro et al. (2005, 2011). Amplified DNA was purified using the QIA-quick Purification Kit (Qiagen, Germany). Purified DNA was sent to MACROGEN Inc., Seoul, South Korea for sequencing.

**Phylogenetic analysis**

The ITS and trnL-F sequences were assembled and edited using the Codon Code Aligner version 3.0.1. Novel sequences of the four Philippine *Canthium* from each of the markers used were incorporated with several related sequences from the work of Lantz and Bremer (2004) taken from the GenBank (Table 1). *Ixora coccinea* L. and *Mussaenda erythrophylla* Schumach. & Thonn., considered as closely related to Vanguerieae were used as the outgroups. Sequences were aligned manually using Se-Al v.1.0al (Rambaut 1996).

Bayesian inference (BI) was used to estimate phylogenetic positions of the Philippine endemic *Canthium* species. The analysis was carried out using the MrBayes v.3.1.2p software (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). Model selection for the best-performing evolutionary models were determined under three model selection criteria: a) Akaike Information Criterion (AIC) (Akaike 1974), b) AICc (second order criterion of AIC, necessary for smaller samples) and c) the Bayesian Information Criterion (BIC) (Schwartz 1978). The selected models were HKY and GTR + G for the ITS and trnL-F, respectively. In analyzing single marker, the best performing model was selected and one million generation was considered with a sample frequency of 1000 and four parallel chains. For combined analyses, model selection as well as the settings is similar with that of the single-marker analysis, however there
| Taxon                                                        | GenBank/EMBL Accession Number |
|--------------------------------------------------------------|-------------------------------|
| **ITS**                                                     |                               |
| Afrocanthium burttii (Bullock) Lantz                        | AJ617749 AJ620120             |
| Afrocanthium gillillani (N.E. Br.) Lantz                    | AJ617751 AJ620123             |
| Afrocanthium keniense (Bullock) Lantz                       | AJ617753 AJ620126             |
| Afrocanthium lactescens (Hiern) Lantz                       | AJ617754 AJ620127             |
| Afrocanthium mundianum (Cham. & Schrtl.) Lantz              | AJ315107 AJ620128             |
| Afrocanthium parasiebenlistii (Bridson) Lantz               | AJ617756 AJ620130             |
| Afrocanthium pseudovercillatum (S. Moore) Lantz             | AJ617758 AJ620132             |
| Afrocanthium siebenlistii (K. Krause) Lantz                 | AJ617759 AJ620133             |
| Canthium ciliatum (D. Dietr.) Kuntze                       | AJ617750 AJ620121             |
| Canthium coromandelicum (Burm. f) Alston                    | AJ315081 AJ620122             |
| Canthium glaucum Hiern ssp. glaucum                        | AJ617752 AJ620124             |
| Canthium gynochthodes                                      | HG937666 HG937663             |
| Canthium inerme (L.f.) Kuntze                               | AJ315120 AJ620125             |
| Canthium mirmaense (Verd.) Lantz                            | AJ617775 AJ620174             |
| Canthium obovatifolium (Merr.) Merr.                        | HG937664 HG937661             |
| Canthium oligocarpum Hiern ssp. Captum (Bullock) Bridson    | AJ617755 AJ620129             |
| Canthium oligophlebiurn (Merr.) Merr.                       | HG937665 HG937660             |
| Canthium ramosii (Merr.) Merr.                              | HG937667 HG937662             |
| Fadogia ancylantha Schweinf.                                | AJ315103 AJ620136             |
| Fadogia arenicola K.Schum. & K.Krause                       | AJ874981 AJ874943             |
| Fadogia tetraquetra K. Schum. & K. Krause                   | AJ315099 AJ620139             |
| Fadogia triphylla Baker                                     | AJ874982 AJ874944             |
| Keetia gueinzii (Sond.) Bridson                             | AJ315117 AJ620143             |
| Keetia lukei Bridson                                        | AJ617761 AJ620144             |
| Keetia venosa (Oliv.) Bridson                               | AJ617762 AJ620145             |
| Keetia zanzibarica (Klotzsch) Bridson ssp. zanzibarica      | AJ315105 AJ620138             |
| Psydrax kraussioides (Hiern) Bridson                        | AJ617786 AJ620157             |
| Psydrax livida (Hiern) Bridson                              | AJ617769 AJ620158             |
| Psydrax locuples (K. Schum.) Bridson                        | AJ617770 AJ620159             |
| Psydrax parviflora (Afzel.) Bridson                         | AJ315110 AJ620162             |
| Pynostria ampiporoense (Arènes) Razafim., Lantz & B. Bremer  | AJ617766 AJ719194             |
| Pynostria hystrix (Breneeck.) Bridson                        | AJ315114 AJ620168             |
| Pynostria major (A. Rich. ex DC.) Cavaco                    | EU 584304 FN386344            |
| Pynostria orbiculans A. Rich. ex DC.                        | EU584285 FN386347             |
| Pynostria phylantoidea (Baillon) Bridson                     | AJ315115 AJ620169             |
| Pynostria revoluta (Balf. f.) Razafim., Lantz & B. Bremer    | AJ617776 AJ620176             |
| Pynostria saradanensis Cavaco                               | EU584280 FN386366             |
| Pynostria serpentina Lantz, Klack. & Razafim.                | EU584283 FN386350             |
| Vangueria infausta Burchell                                 | AJ617777 AJ620180             |
| Vangueria prochil Briq.                                     | AJ875009 AJ874975             |
were a total of three million running generations. Clades with posterior probability (PP) exceeding 0.95 were regarded as strongly supported.

Parsimony analysis was conducted using PAUP version 4.0b (Swofford 2000). Heuristic search was carried out to determine the most parsimonious trees utilizing a tree-bisection reconnection (TBR) branch swapping using 10,000 random addition sequences, with MULTREES option on. Consistency index (Kluge and Farris 1969) and retention index (Farris 1989) were calculated to determine if the data is far from being homoplasious. Bootstrapping was determined using 10,000 replicates, MULTREES option off, TBR branch swapping, and five random addition sequences. Clades receiving greater than 90% were considered strongly supported.

Results
Sequence characteristics
Table 2 shows the matrix characteristics of the separate and combined ITS and trnL-F data sets. The aligned matrix of the 43 taxa of the ITS region includes a total of 691 positions, 190 base pairs (bp) of which are phylogenetically informative. The 43 sequences of trnL-F have a total of 1,002 positions, 43 bp of which are informative. The combined ITS/trnL-F of the 43 taxa with 1,693 characters generated a total of 233 bp informative characters.

Phylogenetic analysis
The tree topologies of the separate ITS (PP = 1.00; BS = 100%) and trnL-F (PP = 0.89; BS = 90%) analyses (trees not shown) revealed a monophyletic Vanguerieae. Both trees resolved the phylogenetic positions of C. obovatifolium, C. oligophlebium and C. ramosii in Pyrostria clade with high support in ITS (PP = 0.96; BS = 89%) and trnL-F (PP = 1.00; BS = 85%). However, both separate analyses failed to resolve the placement of C. gynochthodes within the tribe and polytomies were observed for members of Canthium s.s and Psydrax.

Bayesian and parsimony analyses of the combined ITS/trnL-F data sets (Figure 1) shows a robustly supported Vanguerieae (PP = 1.00; BS = 99%) (Figure 1). The majority rule consensus tree of the combined ITS/trnL-F data sets (Figure 1) supports the monophyly of the included genera and recovered tree topologies similar with Lantz and Bremer (2004). For instance, the monophyly of Canthium s.s. is supported (PP = 0.96; BS = 67%) and is closely related to the large flowered group (PP = 1.00; BS = 85%); Keetia (PP = 1.00; BS = 64%) and Afrocanthium (PP = 1.00; BS = 89%) as sister taxa is likewise supported (PP = 0.98; BS = 72%); and the monophyly of Psydrax (PP = 0.94; BS = 85%) and Pyrostria s.s. (PP = 0.99; BS = 85%) were also sustained. The combined data analysis agrees with single marker analyses on the close relatedness of C. obovatifolium, C. oligophlebium and C. ramosii with Pyrostria (PP = 0.99; BS = 85%). Meanwhile, C. gynochthodes is finally resolved within Psydrax (PP = 0.94; BS = 85%).

Discussion
Generic affiliations of species under Pyrostria group
The results presented above clearly shows the polyphyly of Canthium as earlier observed by Lantz et al. (2002), Lantz and Bremer (2004, 2005) and Razafimandimbison et al. (2009). The four Canthium (C. gynochthodes, C. obovatifolium, C. oligophlebium and C. ramosii) should be excluded from Canthium s.s. since these species are spineless. The phylogenetic position of C. obovatifolium, C. oligophlebium and C. ramosii within the Pyrostria clade was already anticipated due to the presence of a persistent, basally paired, connate to acuminate bracts as observed in our recent collections and available herbarium sheets. The synapomorphic characters of Pyrostria such as dioecious sexuality and fleshy corolla with trichomes in the throat (Lantz and Bremer 2004) were also observed in our sampled Canthium species. The placement of these three Canthium species in Pyrostria s.l. was already suggested by Bridson (1987) but she was unsure of the placement in

| Table 1 Nucleotide sequence database accession numbers of taxa used in this study (Continued) |

| Vangueria parvifolia Sond. | AJ315092 | AJ620181 |
|---------------------------|----------|---------|
| ixora cocinea L.          | AJ224826 | AJ620117 |
| Mussaenda erythrophylla Schumach. & Thonn. | AJ224823 | AJ620116 |

Since vouchers of most taxa included in the study were published only the voucher information of the Philippine Canthium included in the study are provided as footnotes.

Table 1 Nucleotide sequence database accession numbers of taxa used in this study

| Table 2 Matrix characteristics of separate and combined datasets |

|                  | ITS     | trnL-F   | Combined |
|------------------|---------|----------|----------|
| Number of taxa   | 43      | 43       | 43       |
| Number of included characters | 691 | 1,002 | 1,693 |
| Number of informative characters | 190 | 43 | 233 |
| Consistency index | 0.56 | 0.93 | 0.64 |
| Retention index  | 0.74    | 0.93     | 0.76     |
the genus due to their geographical occurrence falling outside the known range, i.e. at that time Pyrostria was considered to be a predominately Afro-Madagascan genus.

Although Pyrostria is mostly represented in Africa, Ruhsam et al. (2008) mentioned that the presence of SE Asian Pyrostria could probably be a disjunct part of their African relatives. There is a possibility that species under this genus may have undergone long range dispersal from Africa to Asia as in the case of Mussaenda L. (Alejandro et al. 2005).

Meanwhile, the phylogenetic placement of C. gynochthodes within Psydrax does not support the earlier suggestion of Bridson (1985) in placing the species under Pyrostria s.l. Bridson may have assigned this SE Asian species under Pyrostria group B due to the presence of bracts which resembles that of Pyrostria although Baillon (1879) did not mention the occurrence of this character. However, we examined herbarium sheets of C. gynochthodes [Gaerlan, F.J.M 0542753 (L, PNH); Romero, E.M. 0542751 (L, PNH); Soejarto, D.D. 0219674 (L, PNH); Arriola and Alejandro, 12442 (PNH, USTH); Arriola and Alejandro, 11057 (PNH, USTH)] and revealed that bracts exist in younger inflorescences but totally absent in older ones. The presence of bracts on young inflorescences of C. gynochthodes will not affect its close relatedness with Psydrax. According to Bridson (1987) bracts may be present in some representatives of Psydrax, however, it is distinctive from the paired connate bracts of Pyrostria

Figure 1 Majority-rule consensus tree inferred from the Bayesian analysis of the combined ITS/trnL-F datasets of the 43 included taxa.

The results are congruent with the results of parsimony analysis except for the nodes marked with asterisks. Numbers above branches indicates Bayesian posterior probabilities and those below branches are parsimony bootstrap values. Species under study are highlighted in grey.
which are rare in Vanguerieae. The presence of bracts is not a cardinal character to delimit Psydrax. For instance, Bridson (1985) mentioned of the occurrence of bracts in the Indian _Psydrax umbellata_ (Whit.) Bridson and unnamed Malayan species. Furthermore, examination of _C. gynochthodes_ revealed that it possesses other diagnostic features of _Psydrax_ such as coriaceous leaf blades, keeled stipules with truncate to triangular stipular base and falcate stipular apex, reflexed anthers, long style always exceeding the corolla tube, longer than wide stigmatic knob, cartilaginous seed and a very shallow to nearly inconspicuous apical crest without a lid-like area in the pyrene (Bridson 1985; Cheek and Sonke 2004). Additionally, the occurrence of a unique insertion of 40 bp in the _trnL-F_ region of _Psydrax_ that is non-alignable with other species of Vanguerieae (Lantz and Bremer 2004) exists in _C. gynochthodes_.

The close relatedness of species placed under _Pyrostria_ group B with _Pyrostria_ s.s. and _Psydrax_ are supported by morphology and molecular data. Therefore, it is necessary to recollect the remaining species of _Pyrostria_ group B to determine their correct generic affiliations within the tribe.

**Taxonomic treatment**

We present here novel combinations of four species that were included in our study (Figure 2).

**Pyrostria obovatifolia** (Merr.) Wong, Magdaleno & Alejandro, comb. nov. Basionym: _Canthium obovatifolium_ (Merr.) Merr., Philipp. J. Sci. 35 (1928) 8. _Electronia obovatifolia_ Merr., Philipp. J. Sci. C12 (1917) 167. Philippines, Luzon, Tayabas Prov. Mount Dalindingan, Sept. 1916, _Ramos_ and _Edano_ 26526 (holotype: PNH destroyed; lectotype: designated here K!; isolectotypes: US, HUH) (Figure 2A).

Shrub to small tree, less than 3 m high; branches terete to a more or less quadrangular and glabrous. Leaves obovate, 3.5–7.5 × 1.0–3.0 cm, glabrous on both sides; apex rounded; base acute to acuminate; visible lateral nerves 3 to 4 on each side of the midrib; petiole 2.5–8.0 mm, glabrous. Stipules triangular to broadly ovate, 5.0–6.0 × 1.0 mm, glabrous on both sides. Female inflorescences axillary on 3.0–5.5 mm long peduncles, 6-flowered; peduncular bracts present, 3.0–5.5 mm long, triangular to broadly triangular, glabrous on both sides, enclosing the young inflorescence; pedicels erect, 3.0–4.0 mm long at flowering. Female flowers: calyx limb glabrous; tube 1.2–2.5 mm long; lobes acuminate, 0.2 × 0.4 mm. Corolla 5-merous, white, glabrous outside; tube tubular, 0.8–1.2 mm long, hairs present at the throat; lobes broadly triangular, 2.0–2.5 × 1.0–1.2 mm, recurved. Stamens exerted, attached to corolla tube; anthers narrowly ovate to ovate, 0.3 mm long, exerted. Style including stigmatic knob 3.0–3.9 mm long; stigmatic knob 1 mm long, with a shallow cleft above, style not recessed into the stigmatic head; disk glabrous. Ovary 2-locular. Male flower unknown. Fruits ovoid 8.5–10.5 mm, glabrous.

**Distribution:**—Luzon Island: Ilocos Norte, Quezon

**Habitat:**—In secondary forest; 200–350 m altitude.

**Phenology:**—Flowering from March to June; Fruiting May to December

**Taxonomic notes:** This species approaches _P. subsessifolia_ by its elliptic to ovate leaf shape but differs by its less conspicuous lateral nerves, shorter bracts and non-keeled fruits.

**Pyrostria oligophlebia** (Merr.) Pacia, Quiogue & Alejandro, comb. nov. Basionym: _Canthium oligophlebium_ (Merr.) Merr., Philipp. J. Sci. 35 (1928) 8. _Electronia oligophlebia_ Merr., Philipp. J. Sci. 17 (1921) 442. Philippines, Luzon, Rizal Prov. Mount Susong Dalaga, Aug. 1917, _Ramos_ and _Edano_ 29342 (holotype, PNH destroyed; lectotype: designated here US; isolectotype: HUH, K!) (Figure 2B).

Shrub to small tree, less than 5.0 m high; branches quadrangular to more or less terete and glabrous. Leaves obovate, 3.0–5.5 × 1.0–2.0 cm, glabrous on both sides; apex acute; base acute; visible lateral nerves 2 to 4 on each side of the midrib; petiole 0.7–1.0 mm, glabrous. Stipules broadly triangular to ovate, 1.0–2.0 × 1.0 mm, glabrous on both sides. Female inflorescences axillary on a glabrous peduncle less than 2 mm long; 7–12 flowered; peduncular bracts present, 2.5–3.0 mm long, triangular to broadly triangular, glabrous on both sides, enclosing the young inflorescence; pedicels erect, 4.0–5.0 mm long at flowering, persistent.

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**Figure 2 Images of the four plant species included in the study.**
A. _Pyrostria obovatifolia_ fruiting branch; B. _Pyrostria oligophlebia_ infructescences; C. _Pyrostria ramosii_ fruiting branch; D. _Psydrax gynochthodes_ flowering branch.
Female flowers: calyx limb glabrous 2–2.7 mm long; lobes shortly toothed, 0.1 × 0.3 mm. Corolla 4-merous, white, glabrous outside; tube tubular, 0.8–1.2 mm long, hairs present at the throat; lobes broadly ovate, 2.0–2.5 × 1.0–1.2 mm. Stamens attached to corolla tube adjacent to the throat; anthers ovate, 0.3 mm long, exserted. Style including stigmatic knob 1.0–2.5 mm long; stigmatic knob 1 mm long, with a shallow cleft above; disk glabrous. Ovary 2-locular. Male flower unknown. Fruits ovoid 6.0–6.5 mm, glabrous with distinct indentation when dry.

**Distribution:** Luzon Island: Rizal; Mindanao Island: Davao

**Habitat:** In secondary forest; 500–900 m altitude.

**Phenology:** Flowering from March to December; Fruiting from September to February

**Taxonomic notes:** The smaller and fewer nerved leaves of *P. oligophlebia* approaches *P. gynochthodes*. However, *P. oligophlebia* differs from the latter by having persistent pair of bracts, many-flowered inflorescences and a longer petioles, peduncles and pedicel.

*Pyrostria ramosii* (Merr.) Arriola, Paraguion & Alejandro, comb. nov.

Basionym: *Canthium ramosii* (Merr.) Merr., Philipp. J. Sci. 35 (1928) 9. *Electronia ramosii* Merr., Philipp. J. Sci. 7 (1921) 443. Philippines, Luzon, Tayabas Prov., Mount Umiray, June 1917, Ramos and Edano 28973 (holotype, PNH destroyed; lectotype: designated here NY!; isolecotype: K!) (Figure 2C)

Tree less than 9 m high; branches terete, glabrous. Leaves broadly lanceolate to oblong, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides; apex triangular to ovate, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides; apex triangular to ovate, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides; apex triangular to ovate, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides; apex triangular to ovate, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides; apex triangular to ovate, 2.5–9.5 × 1.5–3.9 cm, glabrous on both sides. Leaf with acuminate apex. However, the latter have longer peduncles and few (2–4) flowered umbel late inflorescences as compared to the numerous (7–12) flowered inflorescence of the former.

*Psydrax gynochthodes* (Baill.) Arriola, Yayen & Alejandro, comb. nov.

Basionym: *Electronia gynochthodes* (Baill.) Merr., Enum. Philipp. Fl. Pl. 3 (1923) 536. *Canthium gynochthodes* Baill., Adansonia 12 (1879) 199. Philippines, Luzon, Batangas Prov., 1917, Cum ing 1848 (holotype, K!) (Figure 2D)

Conclusion

The generic affiliations of four species previously hypothesized under *Pyrostria* group B have been resolved based on morphology and molecular sequence data. We formally proposed three novel combinations in *Pyrostria* and a *Psydrax*. Other species of *Pyrostria* group B should be reinvestigated towards a more natural classification in Vanguerieae. Furthermore, large number of *Pyrostria* and bracteate species temporarily placed under *Canthium* s.l should be sampled to fully understand the evolutionary dispersal of the genus.

Competing interests

The authors declare that they have no competing interests.
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Contributions

GJD and AHA drafted the manuscript; all authors participated in sample collection, molecular work and taxonomic treatment. All authors read and approved the final manuscript.

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References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Alejandro GJD, Razafimandimbison SG, Liede-Schumann S (2005) Polyphyly of Mussaendo inferred from ITS and tmTF data and its implication for generic limits in Mussaendeae (Rubiaceae). Ann J Bot 92:544–557

Alejandro GJD, Meve U, Moulé A, Thiv M, Liede-Schumann S (2011) Molecular phylogeny and taxonomic revision of the Philippine endemic Villarzia Rolfe (Rubiaceae). Plant Syst Evol 296:1–20

Alejandro GJD, Arenas EH, Cremen CM, Arrola AH (2013) A new record of Pyrostria (Vanguerieae:Rubiaceae) from the Philippines inferred from molecular and morphological data. Phil J Syst Bio 8:1–12

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20:407–415

Baillon HE (1879) Canthium gynochthodes, Adansonia 12:199

Bridson DM (1983) The reinstatement of Psydrax (Rubiaceae, subfam. Cinchonidae tribe Vanguerieae) and a revision of the African species. Kew Bull 40:687–725

Bridson DM (1986) The reinstatement of the African genus Keetia (Rubiaceae subfam. Cinchonidae, tribe Vanguerieae). Kew Bull 41:965–994

Bridson DM (1987) Studies in African Rubiaceae: Vanguerieae: a new circumscription of Pyrostria and a new subgenus, Canthium subgen. Bullokia. Kew Bull 42:611–639

Bridson DM (1992) The genus Canthium (Rubiaceae: Vanguerieae) in tropical Africa. Kew Bull 47:353–401

Chase MW, Hils HH (1991) Silica gel: an ideal material for preservation of leaf samples for DNA studies. Taxon 40:215–220

Cheek M, Sonke B (2004) Psydrax brijoniana (Rubiaceae), a new species of tree from western Cameroon. Kew Bulletin 59:605–608

Farris JS (1989) The retention index and the rescaled consistency index. Cladistics 5:417–419

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 8:754–755

Kluge AG, Farris JS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Lantz H, Bremer B (2004) Phylogeny inferred from morphology and DNA data: characterizing well-supported groups in Vanguerieae (Rubiaceae). Bot J Linn Soc 146:257–283

Lantz H, Bremer B (2005) Phylogeny of the complex Vanguerieae (Rubiaceae) genera Fodogia, Pyrignia, and Vangueria with close relatives and a new circumscription of Vangueria. Plant Syst Evol 253:159–183

Lantz H, Andreaeen K, Bremer B (2002) Nuclear rDNA ITS used to construct the first phylogeny of Vanguerieae (Rubiaceae). Plant Syst Evol 230:173–187

Popp M, Oxelman B (2001) Inferring the history of the polyploid Silene angaea (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Mol Phylogenet Evol 20:474–481

Rambaut A (1996) Se-Al v1.0a1. http://tree.bio.ed.ac.uk/software/seal/. Accessed 15 May 2011

Razafimandimbison SG, Lantz H, Moulé A, Bremer B (2009) Evolutionary trends, major lineages and new generic limits in the dioecious group of the tribe Vanguerieae (Rubiaceae): insights into the evolution of functional dioecy. Ann Mo Bot Gard 96:161–181

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rutham M, Vavaerts R, Davis AP (2008) Nomenclatural changes in preparation for a World Rubiaceae Checklist. Bot J Linn Soc 157:115–124

Schwartz G (1978) Estimating the dimensions of a model. Ann Stat 6:461–464

Swofford DL (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:444–448

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl Mol Biol 17:1105–1109

Utteridge TMA, Davis AP (2009) Two new combinations in Pyrostria (Rubiaceae:Vanguerieae) from Thailand. Kew Bull 64:751–752

Verdcourt B (1987) Notes on African Rubiaceae: Vanguerieae. Kew Bull 42:123–199

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