Phytotaxa 284 (3): 203–210
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.284.3.5

Effectiveness of DNA barcoding markers in the description of a new and unusual calyptrate species of *Myrcianthes* (Myrtaceae)

CARLOS PARRA-O.[1, 2] & A. F. BOHÓRQUEZ-OSORIO[3]
[1]*Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Carrera 30 No. 45-03, Bogotá, D.C., Colombia.*
[2]*Corresponding author: caparrao@unal.edu.co*
[3]*Herbario Universidad de Caldas – FAUC, Calle 65 No. 26-10, Manizales, Colombia (andresfelipebo@gmail.com)*

Abstract

A new species, *Myrcianthes roncesvallensis* is described and illustrated from Andean forests of Tolima, Colombia. Due to the unusual combination of morphological characters in this new species never been found previously in a Colombian Myrtaceae, such as having a closed calyx, dichasial inflorescence and an eugenioid embryo, three DNA barcoding markers (*rbcL*, *matK* and ITS) were used to confirm the genus in which this species should be described. Taxonomic affinities of the new species within the genus are discussed.

Key words: Calyx morphology, Colombia, *Eugenia* group, Neotropics

Resumen

Se describe e ilustra a *Myrcianthes roncesvallensis*, una nueva especie que crece en bosques andinos del departamento del Tolima, Colombia. Debido a la inusual combinación de caracteres de esta nueva especie (cáliz cerrado, inflorescencia en dicasio y embrión eugenioide), combinación que nunca ha sido encontrada en ninguna Myrtaceae que crece en Colombia, se usaron tres marcadores de Código de Barras de ADN (*rbcL*, *matK* y ITS) para confirmar el género en que esta especie debería ser adecuadamente descrita. Se discuten las afinidades taxonómicas de esta nueva especie dentro de su género.

Palabras clave: Colombia, grupo *Eugenia*, morfología del cáliz, Neotrópico

Introduction

During the study of Myrtaceae specimens collected in the Andean forest of Roncesvalles, Tolima, a new species of this family was identified. Interestingly, this new species has an unusual combination of morphological characters that have never been found in a Colombian Myrtaceae, such as having a closed calyx in the flower bud, dichasial inflorescence and seeds with two separate cotyledons (*i.e.*, eugenioid embryo, see Landrum & Kawasaki 1997). Such a combination of characters, at generic level, was not mentioned in the study of genera of Myrtaceae in Brazil (Landrum & Kawasaki 1997). Among Colombian Myrtaceae, the genera that have species with dichasial inflorescences and seeds with eugeniod embryos are *Myrcianthes* O. Berg (1855–1856: 315) and *Pseudanamonis* Kausel (1956: 511), but the latter is a monospecific genus where the dichasium is often branched resembling an umbel, and the cotyledons are partially fused (Grifo 1992, 2003).

Myrcianthes is a genus of 30–35 species growing from the south of Florida, the Caribbean and Mexico to Chile (Grifo 1992, Landrum & Kawasaki 1997, Proença *et al*. 2011, Parra-O. 2012). This genus is characterized by usually having coriaceous leaves, uniflorous inflorescences or more frequently simple to compound dichasia, calyx open with four (rarely five) well separated calyx lobes, ovary usually 2-locular (sometimes 3-locular), 5–30 ovules per locule, fruits with 1–2 seeds (sometimes up to 4) and embryo with two free plano-convex cotyledons (Landrum & Kawasaki 1997, Proença *et al*. 2011). *Myrcianthes* belongs to a clade named by Lucas *et al*. (2007) as '*Eugenia* group', where the genus seems to be monophyletic. In Colombia there are 12–13 species of this genus growing commonly in Andean forests between 1800–3500 m (Parra-O. 2014).

Accepted by Marcos Sobral: 2 Nov. 2016; published: 16 Nov. 2016

Although some of the characters, such as having simple or compound dichasia and an embryo with two separate cotyledons would lead us to describe this new species within *Myrcianthes*, the presence of a clearly closed calyx in the flower buds of this new species raised doubts if it could belongs to this genus; *Myrcianthes* has been characterized by always having an open calyx (Grifo 1992, Landrum & Kawasaki 1997, Proença *et al*. 2011). In order to confirm the proper genus where this new species should be described we also used, along with morphological characters, three DNA barcoding markers (*rbcL*, *matK* and ITS) as a source of additional characters.

Materials & Methods

Field collections were made in natural areas around the 'Reserva Natural de las aves Loros Andinos' of Proaves, located in the Yerbahuena Páramo (municipality of Roncesvalles, department of Tolima) at elevations between 3100–3300 m. Comparisons between this new species and related species of *Myrcianthes* were made after the study of herbarium specimens at CAS, CAUP, COAH, COL, CUVC, FMB, GH, HUA, JAUM, LLANOS, MEDEL, MICH, MO, NY, PSO, SURCO, TOLI, US, and UTMC (acronyms follow Thiers 2016). Flowers and fruits were dissected and analysed under a stereomicroscope. Morphological descriptions were made following the terminology used by McVaugh (1956, 1968), Grifo (1992), Landrum & Kawasaki (1997), and Beentje (2010).

For molecular analysis, twenty milligrams of dried (silica gel) tissue of specimen *C. Parra-O. & A. F. Bohórquez 849* (COL) was ground with a mortar and pestle. Total genomic DNA was extracted using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions, and including additional centrifugation between steps three and four. The chloroplast *rbcL* and *matK* regions, suggested as standard plant barcode markers (CBOL Plant Working Group 2009), and the nuclear ITS region, also proposed as a barcode marker (Chen *et al.*, 2010; China Plant BOL Group 2011) were used. For *matK*, amplifications were obtained using the primer combination 3F_KIM and 1R_KIM (Dunning & Savolainen 2010) as suggested by the CBOL Plant Working Group (CBOL Plant Working Group 2009). For *rbcL* the primer combination *rcbL*a_F-*rbcL*a_R was used as recommended by CBOL Plant Working Group (CBOL Plant Working Group 2009), but sequencing of the PCR products of this primer combination was not always successful; as an alternative, primer combination *rbcL*1f and *rbcL*1724r (Fay *et al.* 1997) were employed. The ITS region was amplified using the ITS4 and ITS5 primers of White *et al*. (1990). All PCR reactions included 0.6–10 µM of each primer, 200 µM of each dNTP, 5 µl of DNA extract, and 2.5 U Taq DNA polymerase and its accompanying 10X PCR buffer (Invitrogen, Brazil; including a final Mg^{2+} concentration of 1.5 mM) or 1.25 U HotStarTaq DNA polymerase and its accompanying 10X PCR buffer (QIAGEN, Germany; including a final Mg^{2+} concentration of 1.5 mM), for a final volume of 25 µl. PCR amplification was carried out with a Maxygene Gradient thermal cycler (Axygene); after initial denaturation (94° C for 3 min or 95° C for 15 min) the following cycling conditions were used for the PCR: 35–40 cycles of denaturation (97° C for 1 min [ITS], 94° C for 1 min [*mat*K], 94° C for 30–45 sec [*rbc*L]), primer annealing (48° C for 1 min [ITS], 48° C for 30 sec [*mat*K], 55° C or 58° C for 45–60 sec [*rbc*L]), primer extension (72° C for 1 min), and an additional elongation of 10 min at 72° C after the end of all cycles. Amplified products were visualised after gel electrophoresis on a 0.8% agarose gel. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Germany). Sequencing reactions were analysed at the Genetic Institute of the Universidad Nacional de Colombia (Bogotá), on an ABI 3500 8–capillary Genetic Analyzer. Sequences in both directions for each marker were assembled and edited in Sequencher v. 4.1.4 (Genecodes). Individual sequences are available from Genbank (accession numbers KX462191- KX462193). Edited sequences were compared with sequences stored in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), using the BLAST (Standard Nucleotide BLAST) tool.

Taxonomic treatment

Myrcianthes roncesvallensis C. Parra-O. & Bohórquez-Osorio, *sp. nov.* (Figures 1, 2)

Type:—COLOMBIA. Tolima: Roncesvalles, "vereda Yerbahuena, páramo Yerbahuena, Reserva Natural de las aves Loros Andinos (Proaves)", 3221 m, 04°05'04.4''N, 75°42'19.8''W, 12 September 2015 (buds, fl., fr.), *Carlos Parra-O. & A. F. Bohórquez 853* (holotype COL!, isotypes COL!, CUVC!, FAUC!, FMB!, HUA!, JAUM!, TOLI!).

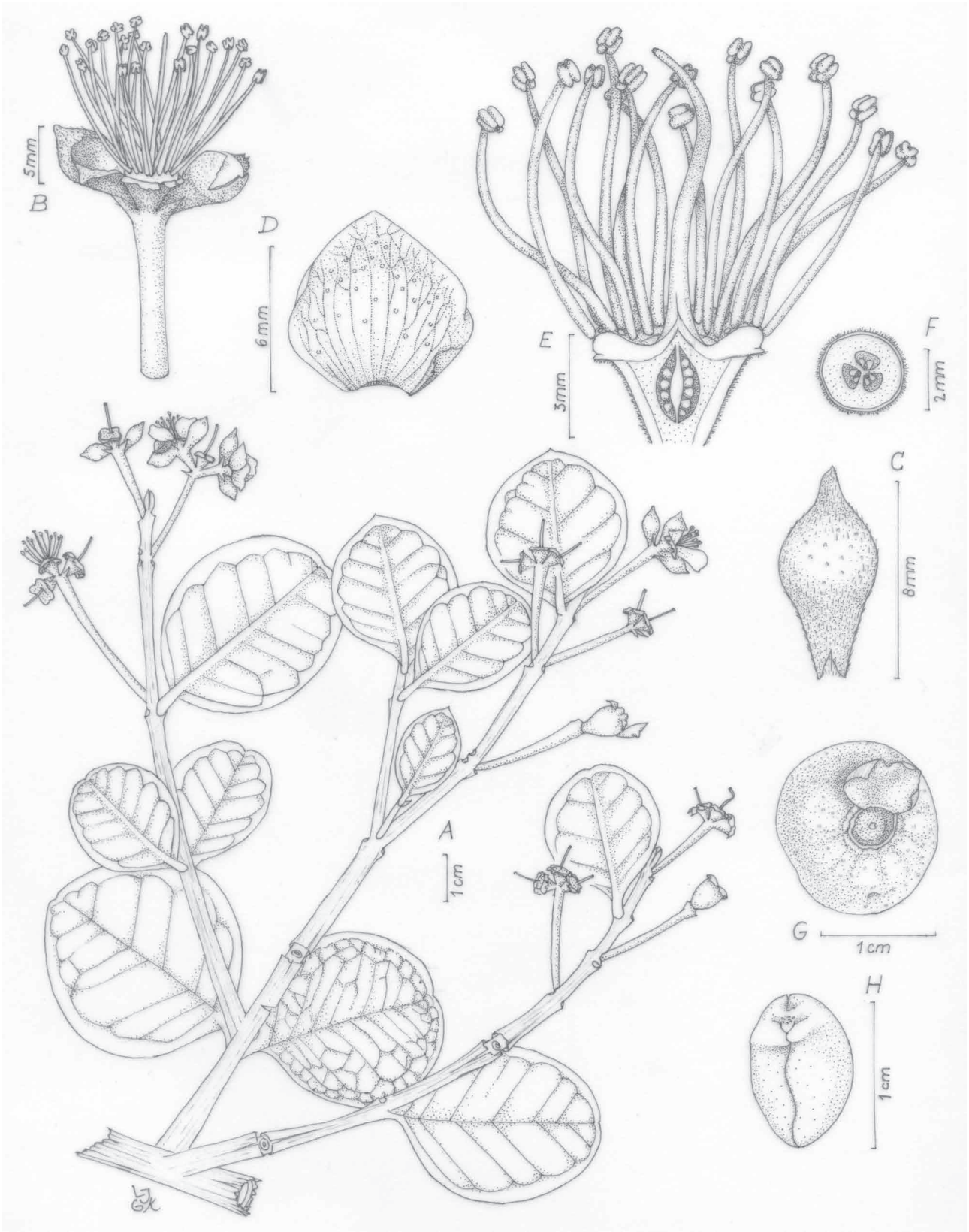

FIGURE 1. *Myrcianthes roncesvallensis*. (A) flowering branch, (B) inflorescence, (C) flower bud, (D) petal, (E) longitudinal section of hypanthium and ovary, (F) cross section of ovary, (G) fruit, (H) embryo. Illustration by Laura Giraldo Kalil; A, C, D, E, and F drawn from the holotype, B drawn from *C. Parra-O. & A. F. Bohórquez 857*, G and H drawn from *C. Parra-O. & A. F. Bohórquez 852*.

This species is most similar to *Myrcianthes rhopaloides* (Kunth 1823: 137) McVaugh (1958: 771), from which it is distinguished by having a closed calyx (versus open calyx in *M. rhopaloides*), moderately pubescent inflorescence and flower buds (versus glabrous inflorescence and flower buds in *M. rhopaloides*), 75–100 stamens (versus 150–175 in *M. rhopaloides*), and anthers 0.4 mm long (versus anthers 1 mm long in *M. rhopaloides*).

FIGURE 2. *Myrcianthes roncesvallensis*. (A) Closed calyx detaching as a unit (*C. Parra-O. & A. F. Bohórquez 853*), (B) closed calyx tearing in two or three more or less regular lobes (*C. Parra-O. & A. F. Bohórquez 849*), (C) both types of calyx opening in flowers of the same inflorescence (*C. Parra-O. & A. F. Bohórquez 857*), (D) tissue remnants from the calyx opening that persist attached to the hypanthium (*C. Parra-O. & A. F. Bohórquez 852*), (E) remnants of the calyx in the fruit (*C. Parra-O. & A. F. Bohórquez 852*), (F) remnants of the calyx in the fruit (*C. Parra-O. & A. F. Bohórquez 856*). Photos: A. F. Bohórquez (A); C. Parra-O. (B, C, D, E, and F). Scale bar = 5 mm.

Tree, 2–15 m tall; bark light brown or grey peeling in irregular plates; hairs when present 0.4–4 mm, simple, light reddish drying brownish yellow; young branches quadrangular in cross-section, vinaceous, glabrous; old branches subcompressed to terete, grey, glabrous; branches with young buds densely puberulous, light reddish. Leaf blades wide

elliptic, elliptic or suborbicular, occasionally almost orbicular, (1.5) 2.2–6 (7.5) × (1.2) 1.8–4.5 (6.7) cm, coriaceous, discolorous, the upper surface glabrescent with hairs becoming hyaline in older leaves, with impressed blackish to dark yellow glandular dots, the lower surface glabrescent or sparsely to moderately puberulous, with raised dark vinaceous glands; apex rounded, sometimes obtuse or retuse, frequently mucronate; base obtuse, sometimes almost rounded; margin entire to slightly revolute; midvein sulcate and glabrous to slightly puberulous above, convex and glabrous to glabrescent below; lateral veins 7–10 pairs, slightly sulcate to sulcate and slightly to moderately puberulous above, convex and glabrous to slightly puberulous below; petiole 2.3–4.4 mm long, vinaceous or blackish, slightly rugose, glabrous to moderately puberulous, canaliculate. Inflorescence axillary, simple or sometimes compound dichasium, (1.6) 2–4.6 (5.6) cm long, usually solitary or sometimes paired and opposite, with 3–7 flowers, the axes compressed, glabrous to densely puberulous, light reddish when young, vinaceous to dark brown when mature; peduncles (10) 13–37 (41) × 1.7–2.1 mm; bracts lanceolate, 1.3–3.2 × 0.5–0.6 mm, glabrescent to moderately pubescent, truncate in the base, persistent; bracteoles 2, opposite at base of hypanthium, lanceolate or ovate, 1.4–1.8 × 0.3–0.7 mm, glabrescent to moderately pubescent, persistent after anthesis; flower buds globose, green to dark brown, sometimes with violet spots at the apex, 4–7.5 mm long, 2.7–4.4 mm in diameter, moderately pubescent, sessile or a subcompressed pedicel 1.1–1.5 (2) × 0.5–0.8 mm, moderately puberulous; calyx closed, in anthesis detaching as a regular or irregular calyptra, or tearing in 2–4 more or less regular lobes, calyptra (when present) discoid, 4.9–6.5 mm in diameter, pubescent, prominently apiculate, the apiculum 0.8–1.5 mm; petals 4, white, sometimes with pink or red strips or spots, broadly to very broadly ovate, 5.5–6.7 × 7–7.6 mm, glabrous or sometimes with tiny sparse hairs at margin and apex, apex obtuse to rounded, base truncate; hypanthium 2.4–4.2 mm in diameter, not prolonged above the ovary, moderately to densely pubescent outside, glabrous inside, sometimes with irregular tissue remnants of the dehiscence of the calyx; disk semi-quadrangular or almost quadrangular, white to whitish, 4.2–5.6 mm, slightly pubescent; style white, becoming pink to dark red with age, generally straight, sometimes geniculate, 8.6–11 mm long, glabrous; stamens 75–100; filaments white, 5.2–9.5 mm, anthers ellipsoid, cream, 0.4 mm long, without glands; ovary 1–1.7 mm in diameter, 2(–3)-locular, 20–25 ovules per locule. Fruits globose, dark violet when mature, 1.3–1.7 cm in diameter, glabrous; seeds 2–4, slightly reniform, 13 × 7.6 mm, seed coat papyraceous, brownish to light brownish, smooth; embryo with two separate plano-convex cotyledons, with minute light brownish glands.

Etymology:—The specific epithet of the new species refers to the municipality of Roncesvalles, which is located in the west of the department of Tolima in Colombia.

Phenology:—Collected with buds and flowers in January and September; collected with fruits in September.

Distribution, habitat and ecology:—*Myrcianthes roncesvallensis* seems to be endemic to Colombia and is only known from Andean forest in Roncesvalles between 3127–3286 m elev. This species is growing together with species of *Baccharis* Linnaeus (Asteraceae; Linnaeus 1753b: 860), *Ilex* Linnaeus (Aquifoliaceae; Linnaeus 1753a: 125), *Vaccinium* Linnaeus (Ericaceae; Linnaeus 1753a: 349), *Viburnum* Linnaeus (Adoxaceae; Linnaeus 1753a: 267), *Hesperomeles* Lindley (Rosaceae; Lindley 1837: tabula 1956) and *Rubus* Linnaeus (Rosaceae; Linnaeus 1753a: 492), in secondary forests or in open areas associated with human disturbance.

Fruits of *M. roncesvallensis* are eaten by certain species of birds, such as the 'loro coroniazul' (*Hapalopsittaca fuertesi*) and the 'mirla ojiamarilla' (*Turdus fuscater*) (G. Cardona & A. López, personal communication). In fact, *M. roncesvallensis* is being planted in some areas of the Natural birds Reserve 'Loros Andinos', in order to provide food for *Hapalopsittaca fuertesi*; this parrot is endemic to the area and is classified as a Critically Endangered species (BirdLife International 2016).

Common name:—"guayabo" (*Parra-O. & Bohórquez 853*).

Conservation status:—Because this species is only known from one location, and no additional collections have been found, the conservation status of *M. roncesvallensis* can only be assessed as Data Deficient, or DD, following IUCN Red List criteria (IUCN 2012).

Molecular affinities:—Sequences of all three selected barcode markers were obtained. For ITS, a 647 base pairs length fragment was recovered; a BLAST search of it found the top match (KJ187656.1) to be a specimen of *Myrcianthes pungens* (Berg 1857-1859: 224) Legrand (1968: 52), and the following two matches were a specimen (AM234100.1) of *Myrcianthes pseudomato* (Legrand 1944: 477) McVaugh (1963: 493) and an additional accession (AM234099.1) of *M. pungens*. The three top matches had an identity value of 98% compared to the sequence of *M. roncesvallensis*. For *mat*K, a 783 bp length fragment was recovered and a BLAST search of it found the top match (KR867678.1) to be a specimen of *Eugenia uniflora* Linnaeus (1753a: 470) with an identity value of 99%; for *E. uniflora* the complete plastid genome has been sequenced. The next top matches were four *mat*K sequences (DQ088554.1, AY521545.1, KM065289.1, KM065298.1) of specimens of *Pimenta* Lindley (1821: 19), also with identity values of 99%. In GenBank there are only two *mat*K sequences of *Myrcianthes* [*i.e.*, *M. fragrans* (Swartz 1788: 79) McVaugh

(1963: 485); KJ772955.1) and *M. cisplatensis* (Cambessèdes 1832: 342) Berg (1856: 315); JN661013.1)], but neither of them were found as matches in the BLAST search. Differences between *mat*K sequences of *M. cisplantensis*, *M. fragrans*, and *M. roncesvallensis* are explained by the use of different primer pairs to obtain the sequences, where each primer combination recovers different portions of the *mat*K region and not the entire region. Between *M. fragrans* and *M. roncensvallensis mat*K sequences there is a difference of 129 bp, where the *M. fragrans* sequence is shorter (654 bp) than that of *M. roncesvallensis*. Although *M. roncesvallensis* and *M. cisplatensis* (757 bp) are quite similar in length, they only match by 276 bp; the primers used for obtaining the sequence of *M. cisplatensis* recover a portion of the *mat*K gene and the flanking *trn*K intron (Murillo-A. *et al*. 2012), whereas the 3F_KIM and 1R_KIM primers falls into the *mat*K gene.

For *rbc*L, a 551 bp length fragment was recovered; a BLAST search of it found the top match (KF981267.1) to be a specimen of *Pimenta pseudocaryophyllus* (Gomes 1812: 92) Landrum (1984a: 242), and the second match was a specimen of *Myrcianthes fragrans* (U26328.2), both with an identity value of 99%.

Discussion and affinities:—*Myrcianthes roncesvallensis* is the first species of the genus found to have a closed calyx; all the other species of *Myrcianthes* described to date have flowers with an open calyx that has four or five well defined calyx lobes. In this species, when anthesis occurs, the closed calyx detaches as a unit (*i.e.*, regular calyptra; equivalent to the 'closed calyptra' or 'well defined calyptra' of Lucas *et al*. 2011) in some of the flowers (Figure 2A), whereas in other flowers the calyx tears in two, three or four more or less regular lobes (Figure 2B); sometimes both types of anthesis occur in flowers of the same inflorescence (Figures 1B, 2C). Interestingly in the flowers where the calyx starts to open as a unit, the calyptra does not always remain as a unit; sometimes it breaks up into irregular portions, leaving an irregular calyptra and some tissue remnants that persist attached to the hypanthium, resembling calyx lobes (Figure 2D); in this case it seems to be that the calyptra is not detached from the rim of the hypanthium at anthesis, but somewhere above it. The calyptra and/or the tissue remnants sometimes remain attached to the fruits during their development, growing bigger in size. In Neotropical Myrtaceae the remnants of the calyx in the fruit are useful for generic identification, along with other characters, because most of the times it allows the observer to infer the type of flowering calyx of the specimen (Parra-O. 2014). The remnants of the calyx in the fruit of *M. roncesvallensis* are not always useful for identifying the type of calyx, because they could be derived from regular or irregular calyptras (that easily fall), calyx lobes, or tissue remnants that were attached to the hypanthium when the breaking up of the irregular calyptra occurred (Figures 2E, 2F).

There have been reports of species having a closed calyx appearing in at least one other genus (*i.e.*, *Myrceugenia* O. Berg, 1855–1856: 5) that was traditionally accepted as having an exclusively open calyx (Landrum 1984b). The presence of a closed calyx in the flower has evolved independently in different genera of Myrtaceae, and genera such as *Campomanesia* Ruiz & Pavón (1794: 72) and *Psidium* Linnaeus (1753a: 470) have a variable degree of calyx fusion (from closed calyx to open calyx) among their species (Landrum 1984b). McVaugh (1968) was reluctant to use calyx characters as the main feature for delimiting genera in American Myrtaceae.

Myrcianthes roncesvallensis is apparently related to *M. rhopaloides*, and both species are vegetatively similar; they can be differentiated by the floral characters mentioned in the diagnosis. Although both species show wide variation of shape of the leaf blade, in *M. roncesvallensis* it is common to find suborbicular to occasionally almost orbicular leaf blades in adult as well as young branches; in *M. rhopaloides* such leaf blade shapes are more common to be found in sprouts or, sometimes, in some of the old leaves.

Because the three DNA barcode markers used here were useful to confirm the proper genus where this new species had to be described, we recommend that this approach could be used in situations where generic placement of a Myrtaceae species is unclear.

Paratypes:—COLOMBIA. Tolima: Roncesvalles, "vereda Yerbahuena, Reserva Natural de la aves Loros Andinos (Proaves), 'borde del río Cucuana'", 3127 m, 21 January 2014 (buds, fl.), *A. F. Bohórquez-O. 862* (COL!); Roncesvalles, "vereda Yerbahuena, páramo Yerbahuena, Reserva Natural de las aves Loros Andinos (Proaves)", 3286 m, 04°05'14.8''N, 75°42'24.1''W, 11 September 2015 (buds, fl.), *C. Parra-O. & A. F. Bohórquez 849* (COL!, CUVC!, FAUC!, FMB!, HUA!, TOLI!); *ibidem*, 11 September 2015, *C. Parra-O. & A. F. Bohórquez 850* (COL!); *ibidem*, 11 September 2015, *C. Parra-O. & A. F. Bohórquez 851* (COL!, FAUC!); Roncesvalles, "vereda Yerbahuena, páramo Yerbahuena, Reserva Natural de las aves Loros Andinos (Proaves)", 3221 m, 04°05'04.4''N, 75°42'19.8''W, 12 September 2015 (buds, fl., fr.), *C. Parra-O. & A. F. Bohórquez 852* (COL!, FAUC!, FMB!, HUA!); *ibidem*, 3272 m, 04°04'57''N, 75°42'18.2''W, 12 September 2015 (buds, fl.), *C. Parra-O. & A. F. Bohórquez 854* (COL!, FAUC!); Roncesvalles, "vereda Yerbahuena, finca La Riviera", 3280 m, 04°05'04.2''N, 75°42'09.6''W, 12 September 2015 (fr.), *C. Parra-O. & A. F. Bohórquez 856* (COL!, HUA!); *ibidem*, 12 September 2015 (buds, fl.), *C. Parra-O. & A. F. Bohórquez 857* (COL!, FAUC!, TOLI!).

Acknowledgements

We thank Gonzalo Cardona and Albeiro López for their assistance during field work at the 'Reserva Natural de las aves Loros Andinos' (Proaves); we also thank Laura Giraldo Kalil, who prepared the illustration. The first author acknowledges the División de Investigación (DIB) – sede Bogotá of the Universidad Nacional de Colombia for research funds awarded to the project "Código de Barras de ADN: aplicabilidad en especies andinas de *Myrcianthes* (Myrtaceae), código 201010016581". The first author also thanks the California Academy of Sciences and the fellowships of the Lakeside Foundation, for allowing him to do molecular work at the Center for Comparative Genomics in April 2013; at the Academy he is grateful to Darin Penneys, Gilberto Ocampo, Anna Sellas, Marcela Alvear, and Frank Almeda for their support. The first author is also grateful to CAS, CAUP, COAH, COL, CUVC, FMB, GH, HUA, JAUM, LLANOS, MEDEL, MICH, MO, NY, PSO, SURCO, TOLI, US, and UTMC for providing him access to their collections. The first author expresses his special gratitude to the Herbario Nacional Colombiano (COL), and particularly the Instituto de Ciencias Naturales, at the Universidad Nacional de Colombia for their support.

References

Beentje, H. (2010) *The Kew Plant Glossary: an illustrated dictionary of plant terms*. Royal Botanic Gardens, Richmond, Surrey, UK, 160 pp.

Berg, O.C. (1855–1856) Revisio Myrtacearum Americae. *Linnaea* 27: 1–472.

Berg, O.C. (1857–1859) Myrtaceae. *Flora Brasiliensis* 14 (1): 1–656.

BirdLife International. (2016) Species factsheet: *Hapalopsittaca fuertesi*. Available from: http://www.birdlife.org/datazone/speciesfactsheet.php?id=1650 (accesed 17 June, 2016).

Cambessèdes, J. (1832) *Flora Brasiliae Meridionalis* 2: 1–381.

CBOL Plant Working Group. (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences* 106 (31): 12794–12797.
http://dx.doi.org/10.1073/pnas.0905845106

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. & Leon, C. (2010) Validation of the ITS2 region as a novel DNA Barcode for identifying medicinal plant species. *PLOS One* 5 (1): e8613.
http://dx.doi.org/10.1371/journal.pone.0008613

China Plant BOL Group. (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences* 108 (49): 19641–19646.
http://dx.doi.org/10.1073/pnas.1104551108

Dunning, L.T. & Savolainen, V. (2010) Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. *Botanical Journal of the Linnean Society* 164: 1–9.
http://dx.doi.org/10.1111/j.1095-8339.2010.01071.x

Fay, M.F., Swensen, S.M. & Chase, M.W. (1997) Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin* 52 (1): 111–120.
http://dx.doi.org/10.2307/4117844

Gomes, B.A. (1812) Observações Botanico-Medicas sobre algumas Plantas do Brazil. *Memórias de Mathematica e Phisica da Academia Real das Sciencias de Lisboa* 3 (1): 1–104.

Grifo, F. (1992) *A revision of Myrcianthes Berg (Myrtaceae)*. Unpublished Ph.D. Thesis, Cornell University, Ithaca, New York.

Grifo, F. (2003) *Pseudanamomis*. *In*: Steyermark, J.A., Berry, P.E., Yatskievych, K. & Holst, B.K. (Eds.) *Flora of the Venezuelan Guayana. Volume 7. Myrtaceae-Plumbaginaceae*. Missouri Botanical Garden Press, St. Louis, pp. 87–88.

IUCN (2012) *Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0*. Gland, Switzerland and Cambridge, UK: IUCN. Available from: https://portals.iucn.org/library/efiles/edocs/RL-2012-002.pdf (accesed 27 April 2016)

Kausel, E. (1956) Beitrag zur systematic der Myrtaceen. *Arkiv för Botanik, serie 2* 3: 491–516.

Kunth, C.S. (1823) Myrtaceae. *In*: Humboldt, A. & Bonpland, A. (Eds.) *Nova Genera et Species Plantarum* 6: 1–542.

Landrum, L.R. (1984a) A new name and new combinations in *Campomanesia* and *Pimenta* (Myrtaceae). *Brittonia* 36 (3): 241–243. Available from: http://www.jstor.org/stable/2806515 (accessed 1 November 2016)

Landrum, L.R. (1984b) Taxonomic implications of the discovery of calyptrate species of *Myrceugenia* (Myrtaceae). *Brittonia* 36 (2): 161–166.
http://dx.doi.org/10.2307/2806624

Landrum, L.R. & Kawasaki, M.L. (1997) The genera of Myrtaceae in Brazil: an illustrated synoptic treatment and identification keys. *Brittonia* 49 (4): 508–536.
http://dx.doi.org/10.2307/2807742

Legrand, C.D. (1944) Contribución al conocimiento de tres árboles argentinos de la familia de las Mirtáceas. *Lilloa* 10: 471–482.

Legrand, C.D. (1968) Las Mirtáceas del Uruguay III. *Boletín de la Facultad de Agronomía de la Universidad de la República, Montevideo* 101: 1–80.

Lindley, J. (1821) *Collectanea Botanica* 4: 16–20.

Lindley, J. (1837) *Edward's Botanical Register* 23: pls. 1920–2014.

Linnaeus, C. (1753a) *Species Plantarum*. Vol. I. Laurentius Salvius, Stockholm, 560 pp.

Linnaeus, C. (1753b) *Species Plantarum*. Vol. II. Laurentius Salvius, Stockholm, 640 pp.

Lucas, E.J., Harris, S.A., Mazine, F.F., Belsham, S.R., Nic Lughadha, E.M., Telford, A., Gasson, P.E. & Chase, M.W. (2007) Suprageneric phylogenetics of Myrteae, the generically richest tribe in Myrtaceae (Myrtales). *Taxon* 56: 1105–1128.
http://dx.doi.org/10.2307/25065906

Lucas, E.J., Matsumoto, K., Harris, S.A., Nic Lughadha, E.M., Benardini, B. & Chase, M.W. (2011) Phylogenetics, morphology, and evolution of the large genus *Myrcia* s.l. (Myrtaceae). *International Journal of Plant Sciences* 172 (7): 915–934.
http://dx.doi.org/10.1086/660913

McVaugh, R. (1956) Tropical American Myrtaceae, I - Notes on generic concepts and description of previously unrecognized species. *Fieldiana, Botany* 29 (3): 145–228.
http://dx.doi.org/10.5962/bhl.title.2444

McVaugh, R. (1958) Flora of Peru—Myrtaceae. *Field Museum of Natural History, Botanical Series* 13 (2): 569–818.

McVaugh, R. (1963) Tropical American Myrtaceae, II - Notes on generic concepts and description of previously unrecognized species. *Fieldiana, Botany* 29 (8): 395–532.
http://dx.doi.org/10.5962/bhl.title.3851

McVaugh, R. (1968) The genera of American Myrtaceae - An interim report. *Taxon* 17: 354–418.
http://dx.doi.org/10.2307/1217393

Murillo-A., J., Ruiz-P., E., Landrum, L.R., Stuessy, T.F. & Barfuss, M.H.J. (2012) Phylogenetic relationships in *Myrceugenia* (Myrtaceae) based on plastid and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 62: 764–776.
http://dx.doi.org/10.1016/j.ympev.2011.11.021

Parra-O., C. (2012) Una nueva especie de *Myrcianthes* (Myrtaceae) de Colombia. *Caldasia* 34 (2): 277–282.

Parra-O., C. (2014) Sinopsis de la familia Myrtaceae y clave para la identificación de los géneros nativos e introducidos en Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 38 (148): 261–277.
http://dx.doi.org/10.18257/raccefyn.128

Proença, C.E.B., Jennings, L.V.S. & Lucas, E.J. (2011) Two new species of Myrtaceae (Myrteae) from northern South America. *Brittonia* 63 (1): 46–50.
http://dx.doi.org/10.1007/s12228-010-9125-5

Ruiz, H. & Pavón, J.A. (1794) *Florae Peruvianae, et Chilensis Prodromus*. Sancha, Madrid, 154 pp.

Swartz, O. (1788) *Nova Genera et Species Plantarum seu Prodromus*. Stockholm, Uppsala and Turku, 158 pp.

Thiers, B. (2016) *Index Herbariorum*: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/science/ih/ (accesed 27 April 2016)

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and directing sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.