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Botany and physiology

Rubus alutaceus (Rosaceae), a new species for Colombia with agronomic potential

Brigitte Liliana Moreno–Medina[1] , Fánor Casierra–Posada[2] , Sofía Albesiano[3]

Abstract – In Gachantivá, Colombia, *Rubus alutaceus* has been used for commercial purposes for more than one decade but so far with unknown taxonomic identity. For its identification, the anatomical and morphological characteristics of its flowers, fruits, leaves, stems, and seeds, as well as the physicochemical characteristics of its fruits such as color, equatorial and polar diameter, fresh weight, pH and total soluble solids (TSS), were studied. Qualitative and quantitative morphological parameters were evaluated, and longitudinal and transverse cuts were made for histological studies. The plants were found to have trifoliate and pentafoliate leaves; whitish–yellow subglobose polydrupes, fully mature fruits with 8.8°Brix TSS; elliptical, subglobose seeds with ruminated endocarps; anatomical calcium oxalate crystals in the form of druses and raphides in the leaves and branches; and abundant hairs, simple and glandular (colleters) addition to vascular tissue with a more or less continuous appearance. High value in TSS, positively influences fruit agribusiness and the presence of simple hair and colleters can reduce the attack of pests, these variables identify the new species and give it agronomic potential to strengthen its cultivation and marketing.
Index terms: Calcium oxalate crystals, colleters, histology, taxonomic novelty, postharvest parameters.

Rubus alutaceus (Rosaceae), uma nova espécie para a Colômbia com potencial agrícola

Resumo – No município de Gachantivá, Colômbia, *Rubus alutaceus* é usada com fins comerciais, ha mais de uma década, sem que até o momento se conheça a sua identidade taxonômica. Com o propósito de identifica-la, estudaram–se as características anatômicas e morfológicas das suas flores, frutos, folhas, caules, sementes; características físico-químicas dos frutos, sua cor, segundo a escala CIELAB, o diâmetro equatorial e polar, peso fresco, pH e sólidos solúveis totais (SST). Avaliaram-se as variáveis morfológicas qualitativas e quantitativas, e realizaram-se cortes longitudinais e transversais com o objetivo de fazer estudos histológicos. Encontraram-se plantas que apresentam folhas trifoliadas e pentafoliadas; polidrupas subglobosas de cor amarela–esbranquiçada, SST de 8,8 °Brix em frutos completamente maduros; sementes elípticas, subglobosas com endocarpo ruminado; anatomicamente apresentam-se cristais de oxalato cálcico em forma de drusas e ráfides nas folhas e ramos e abundantes pelos simples e coléteres, além do tecido vascular de aspecto mais ou menos contínuo. O alto teor em TSS influencia positivamente o agronegócio dos frutos e a presença de estruturas secretoras incluindo tricomas e coléteres simples podem reduzir o ataque de pragas; essas variáveis caracterizam a nova espécie e conferem potencial agronômico para fortalecer seu cultivo e sua comercialização.
Termos para indexação: cristais de oxalato cálcico, coléteres, histologia, novidade taxonômica, parâmetros pós-colheita.

Corresponding author:
brigitte.moreno@uptc.edu.co

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[1]PhD Student in Chemical Sciences of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, Colombia. E–mail: brigitte.moreno@uptc.edu.co. (ORCID 0000-0003-0920-2744). Beneficiary call 733 of Boyacá.

[2]Agronomist, Ph.D., Professor, Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, Colombia. E–mail: fanor.casierra@uptc.edu.co (ORCID 0000-0001-7508-5174)

[3]Biologist, M. Sc., Ph.D. Professor, Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja, Colombia. E–mail: adriana.albesiano@uptc.edu.co (ORCID 0000-0002-8672-6018)

Introduction

Rosaceae is represented by 3000–3200 species, 90–100 genera, 20 tribes and four subfamilies (WALTERS, 1961; ROMOLEROUX, 1996; POTTER et al., 2007; ZHANG et al., 2017). They have a worldwide range, but are most diverse in the Northen Hemisphere and in some subtropical regions (ZHANG et al., 2017). The species include edible fruits, ornamental trees and shrubs deciduous or evergreen, with alternate leaves that are sometimes stipulated, simple or compound, imparipinnate or webbed, with three to five leaflets, with axillary or terminal inflorescences that is racemose, in panicles or solitary, and persistent, complete, actinomorphic, pentameric flowers, sometimes with numerous stamens and white to purple petals. The receptacle expands into a flat or convex disc, forming a cup on whose edges insert the sepals, petals and stamens. The fruits can be achenes, capsules, drupes, or knobs, which sometimes take part of the receptacle during formation, in addition to the ovary, (HEYWOOD et al., 2007). Owing to the fact that enormous morphological diversity, the species of this family present difficulties for taxonomic identification (GONZALEZ-ROMANO and CANDAU, 1989; ROMOLEROUX, 1996; KALKMAN, 2004; HUMMER and JANICK, 2009; XIANG et al., 2017). Additionally, some taxa are important from the economic viewpoint because they are fruit and ornamental species such as *Malus domestica* or *Rosa* sp. respectively.

Rubus (Rubeae) is the genus with the highest number of species, 750 (ALICE and CAMPBELL, 1999), distributed in 12 subgenera (ROMOLEROUX, 1996). Morphologically, they are shrubs or sub–shrubs, often stoloniferous, with needles from 1 to 2 mm in length, racemose inflorescences, simple or compound panicles, flowers that are complete, perfect, hypogynous, actinomorphic, dichlamydeous, heterochlamydeous, dialisepal and dialipetal, with free stamens, convex receptacle, apocarpous gynoecium, more than 15 carpels, and fruits that are grouped, solitary or polydrupes (ROMOLEROUX, 1996; BUSHWAY, et al., 2008; AYALA et al., 2013; DRENCKHAHN, 2019). In Colombia, as in other countries of the Andes region, representatives of this genus are found at altitudes between 0 and 4500 m a.s.l. (MARULANDA et al., 2012; BERNAL et al., 2015). *Rubus glaucus,* called mora de Castilla, stands out as the most cultivated and commercialized; in addition, there are other taxa with important productive and commercial characteristics, such as *R. adenotrichos, R. alpinus, R. bogotensis, R. floribundus, R. giganteus, R. megalococus, R. nubigenus* and *R. rosifolius*, which enrich production systems in Colombia (CANCINO et al., 2011; MORALES and VILLEGAS, 2012). Along with the numerous species and varieties of the *Rubus* genus that are established as crops, there are great morphological diversity and different physicochemical and medicinal properties that are attributed to biological factors such as apomixis, hybridization, polyploidy, and the environments of cultivated areas (ROMOLEROUX, 1996; BALLESTERO et al., 2004; MARULANDA et al., 2012; CORREA et al., 2014; MORENO–MEDINA et al., 2018). On the other hand, in Colombia, agronomic and post–harvest management is inadequate, which is aggravated by the fact that the fruits have a limited shelf–life and are characterized as being highly perishable, resulting in low profitability of the crops (MORENO–MEDINA and DEAQUIZ, 2016).

This paper deals, the anatomy and morphology of the flower, leaf and stem were described in addition to the physicochemical parameters of the fruits of the cultivated species *Rubus alutaceus*, which is reported as a taxonomic novelty, along with the agricultural potential of this specie.

Materials and methods

Field phase: Three field trips were made to the El Carmen farm in Gachantivá, Colombia. This municipality is located at 5°45'03''N and 73°32'58''W and 2504 m a.s.l. The site has moderate, scattered slopes and forms narrow valleys. The climatic conditions include a temperature range of 12–18 °C, annual average pluvial precipitation above 1000 mm, altitudes between 2,100 and 2,600 m a.s.l. and native forests, including oak stands (EOT–GACHANTIVÁ, 2013). Were collected 5–10 samples, in the flowering and fruiting stadium. The samples were processed in the Laboratory of Genetics and Molecular Biology–GEBIMOL of the Pedagogical and Technological University of Colombia (UPTC).

Laboratory phase: The morphometric data, both reproductive and vegetative, were recorded as follows: 1.) *Vegetative morphology*: *Growth habit*; *branches*: consistency, shape, thickness, length, type of branching, color of the epidermis; *leaf*: composition, duration, limb leaf shape consistency, length and width, type of margin, apex shape, length and shape of the leaf base, length of petioles and petiolules, arrangement on the stem, type of venation, type of stipulations, presence or absence of prickles and presence colleters. 2) *Reproductive morphology*: *flower*: type, color, phytography, position of the gynoecium, symmetry, type of calyx and corolla, type of inflorescence; *fruit*: external color, shape; Seeds: number, shape, size and type of surface. The minimum and maximum values registered in the individuals collected were taken into account (STEARN, 1996; MORENO, 1984; FONT–QUER, 2001; BECERRA et al., 2002; RAMÍREZ and GOYES, 2005; JUDKEVICH et al., 2017).

Anatomical description: stems, leaves and flowers were prepared to establish the anatomical characteristics using cross sections, which were made with a manual rotary microtome (AO Rotary Microtome model 820, American Optical Corporation, Buffalo, NY, USA). In addition, the plant tissue was treated with xylol and stained with safranin and fast–green. They were photographed and analyzed with an OMAX trinocular microscope and AMSCOPE trinocular stereoscope (MONTERO, 2001; SANDOVAL, 2005; RAMOS et al., 2013).

Description of post–harvest parameters: 30 fruits were harvested randomly, in two stages of maturity (green and fully mature), for which the physical parameters were determined, such as: equatorial and polar diameters (with a vernier), fresh weight (with an analytical balance: OHAUS® 0.001 precision), number of seeds, fruit color according to the CIELAB scale (Cartesian coordinate system determined by three rectangular coordinates L*: lightness; a*: corresponds to red if a* > 0 or to green if a* <0; and b*: corresponds to yellow if b* > 0, and to blue if b* < 0, with a MINOLTA® PCE–RGB 147436 digital colorimeter). In addition, the chemical parameters total soluble solids (TSS) in the fully mature fruits (with a HANNA® digital refractometer, HI968033, Hanna Instruments, Eibar, Spain, with a 0 to 85% range and 0.1°Brix precision) and pH in the fully mature fruits (METROHM® potentiometer, model E–744, Series 18415) were determined. The quantitative data were represented by mean and standard error of mean, this analysis was done with the R program, version 3.1.1.

Results

The new, studied species was identified as:

Rubus alutaceus B. Moreno, Casierra, Albesiano, **sp. nov.** TYPE: – COLOMBIA, Boyacá, Municipality of Gachantivá, El Carmen Farm, 2504 m a.s.l. March 31, 2017, *B. Moreno* 2. (Holotype: COL) (Fig. 1).

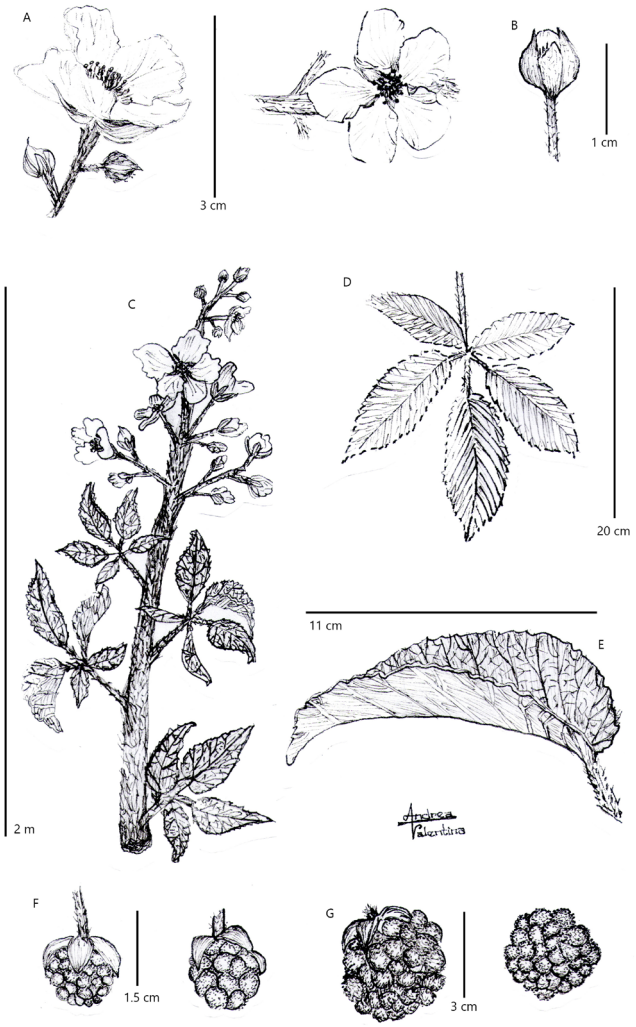

Figure 1 – Morphological characteristics: **A**. Dialisepal and dialipetal flower with numerous stamens; **B**. Floral button with glandular hairs; **C**. Branch with alternate leaves and dichasium–compound inflorescence; **D**. Composite–pentafoliar leaf, craspedodromous venation; **E**. Leaflet with serrated margin and acuminate apex; **F**. Subglobose polydrupe; **G**. Drupes from a polycarpal ovary.

Etymology: Species name due to the whitish–yellow color of ripe fruits.

Diagnosis: –sub–shrub, branches and leaves with simple and glandular hairs; medium branches with five ribs, trifoliate and pentafoliar leaves; subglobose, whitish–yellow polydrupes.

Morphological description:

Growth habit: sub–shrub, up to 6 m high, simpodial. *Branches*: green with abundant hairs simple and colleters, 3–6 m long, 0.1–1.0 cm in diameter. *Apical branches*: terete. *Middle branches*: angular, with five ribs. *Needles*: light green with some purple tones at the base, up to 4 mm in length. *Leaves*: persistent; alternate membranous compound, palmate, trifoliate and pentafoliar; elliptical; blades, 1.2–5.0 cm wide, 3.0–11 cm long, abundant glandular hairs (colleters) and simple, opaque; serrated margin; acuminate apex, 0.1–1.1 mm in length; rounded base; craspedodromous venation, 8–17 pairs of veins; petiole, 4–7 cm in length; petiolules 0.02–1.7 cm in length; needles on the main vein of the abaxial side, 1 mm long, light brown; trifoliate bract; adnate stipules, 4–9 cm in length; green pulvinus, tomentose, 1–4 mm in diameter at the base. *Inflorescence*: cyme, dichasium–compound, with more than 50 flowers; complete; perfect, hermaphrodites; actinomorphic, hypogynous (Fig. 1 and 5); dialisepal, light green, with glandular trichomes; dialipetal, white; numerous stamens, ca. 70, dorsifixed anthers; apocarpic, polycarpic, ca. 120; $X\varnothing K_5$, C_5, A_{70}, $\underline{G}_{120}$. *Polydrupe*: subglobose, 1–2 cm in equatorial diameter, 1.3–3.0 cm in polar diameter, ca. 102 drupes, whitish–yellow at complete maturity and green in the immature state (Fig. 2). *Seeds*: reniform, ovate, 2.0–2.5 mm long, 1–2 mm wide, covered by a ruminated, yellowish–matt surface endocarp.

PARATYPE: COLOMBIA. Boyacá: Municipality of Gachantivá, El Carmen Farm, 2504 m a.s.l., August 31 2017, *B. Moreno, S. Albesiano* and *L. Arrieta* 1, 3 & 4 (COL).

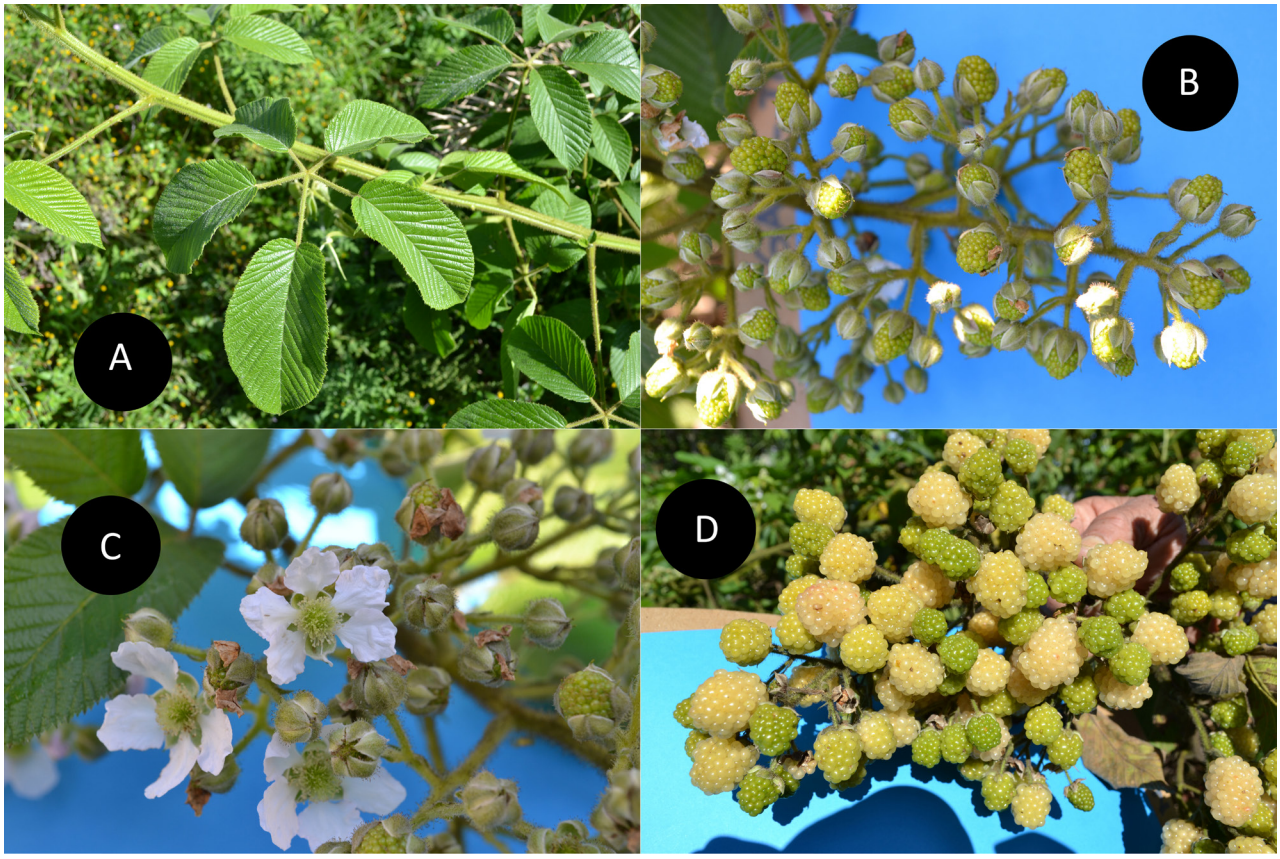

Figure 2. Characteristics of the leaves, flowers and fruits: **A.** Terete branches with alternating leaves and pentafoliates; **B.** Cyme inflorescence with more than 50 flowers; **C.** Complete, perfect flowers and white petals; **D.** Whitish–yellow polydrupes.

Anatomical description:

STEM:

Table 1. Anatomical characteristics of the stem cross section of *R. alutaceus*

TISSUE			CHARACTERISTICS
Dermal		Cuticle	More or less thick (less than 0.5 mm), which covers the epidermis
		Epidermis	Unstratified, with anticlinal cells, simple hair type trichomes and full–length colleters
Fundamental	Cortex	Collenchyma	Subepidermal of angular type
		Sclerenchyma	In the form of fibers, which form a ring around the vascular bundles, very noticeable towards the ribs
	Medulla	Parenchyma	Druses crystals of calcium oxalate
Vascular		Vascular bundles	The number of cells increases continuously and towards the ribs of the xylem and phloem

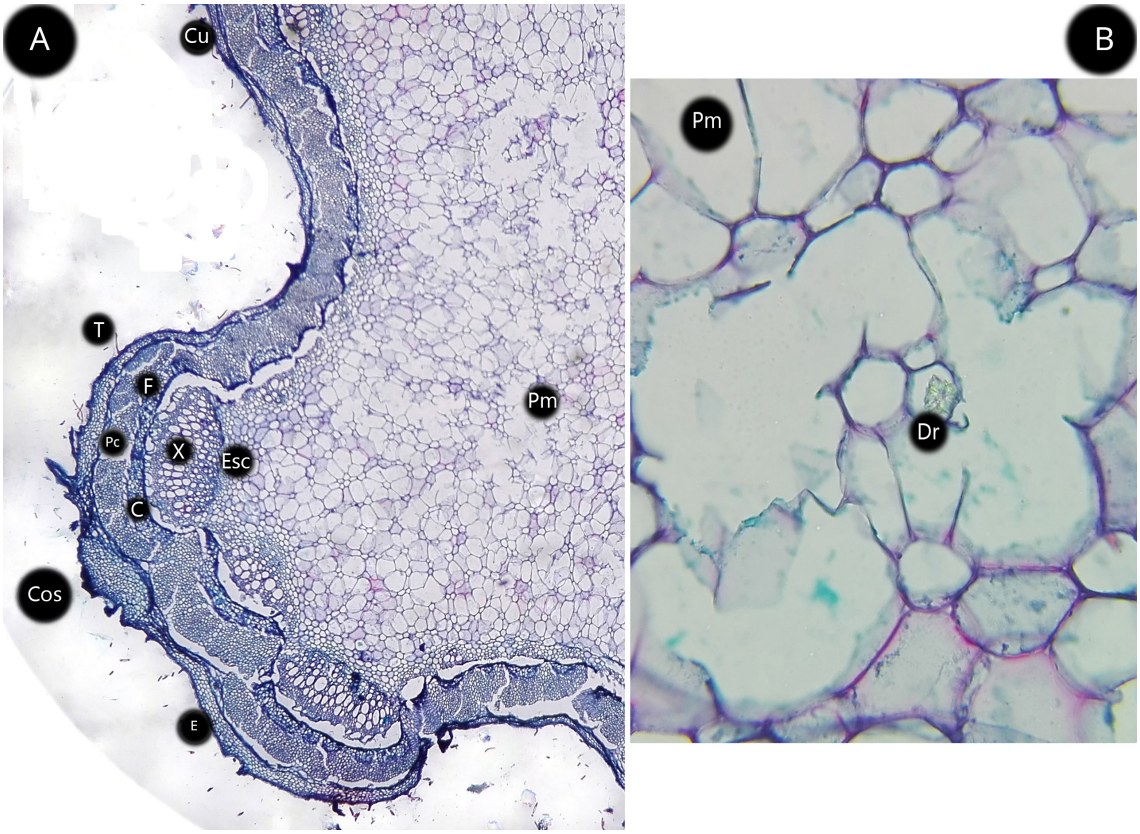

Figure 3. A. Cross section of the stem with one of the five ribs (4X); **B.** Medullary parenchyma and presence of druses (40X). **Cu**–Cuticle; **E**–Epidermis; **Pm**–medullary parenchyma; **Esc**–Sclerenchyma; **F**–Phloem; **X**–Xylem; **T**–Trichome; **Dr**–Druse; **Cos**–Ribs; **C**–Cambium; **Pc**–cortical parenchyma.

LEAF:

Table 2. Anatomical characteristics of leaf cross section in *R. alutaceus*

TISSUE		CHARACTERISTICS
Dermal	Cuticle	Thick (less than 0.5 mm) and covers the epidermis
	Epidermis	Unstratified, with quadrangular cells, simple hair type tricomes, and secondary and main nerve colleters
Mesophyll	Palisade parenchyma	The mesophyll of the leaf is bifacial, well differentiated and relatively compact. The Palisade parenchyma is located next to the adaxial epidermis, multi–layered, two to three strata, with druses
	Spongy parenchyma	Condensed with very few inter–cellular spaces
Vascular	Vascular bundles	Xylem oriented to the stalk and the phloem towards the underside. The primary nerve has druses and raphides around the vascular bundle

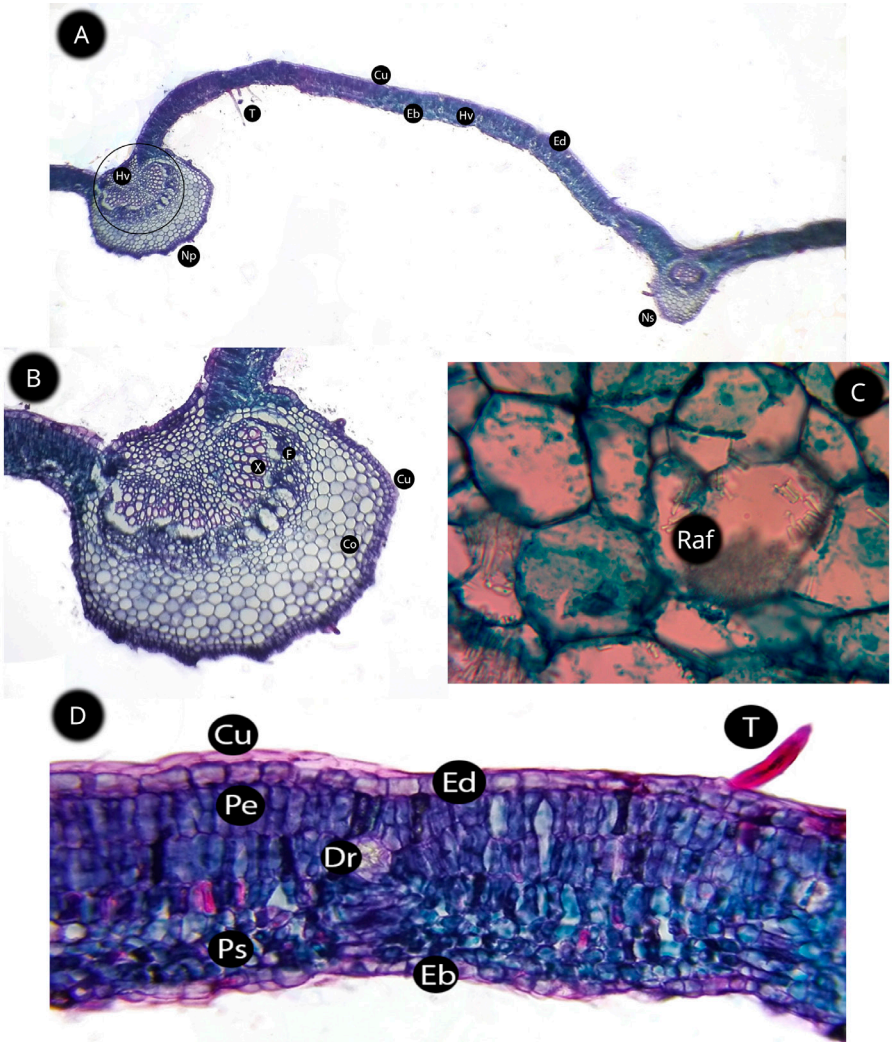

Figure 4. Cross section of *R. alutaceus* leaf: **A**. General aspect (4X); **B**. Central nerve (10X); **C**. Raphides in primary nerve (40X); **D**. Mesophilic (40X). **Np**–Primary nerve; **Ns**–Secondary Nerve; **Cu**–Cuticle; **Pe**–Palisade Parenchyma; **Ps**–Spongy parenchyma; **Hv**–Vascular bundles; Adaxial epidermis; **Eb**–abaxial epidermis; **Co**–Collenchyma; **X**–Xylem; **F**–Phloem; **T**–Trichomes; **Dr**–Druses; **Raf**– Raphides.

FLOWER:

Table 3. Anatomical characteristics of the cross section of the floral button in *R. alutaceus*

FLOWER PARTS		CHARACTERISTICS
Floral peduncle	Receptacle	With unstratified epidermis, widened at the apex and convex
Floral wraps	Sepal	Persistent, covered by simple hairs and colleters of different sizes
Essential organs	Gynoecium	Carpel structure on which the ovules develop

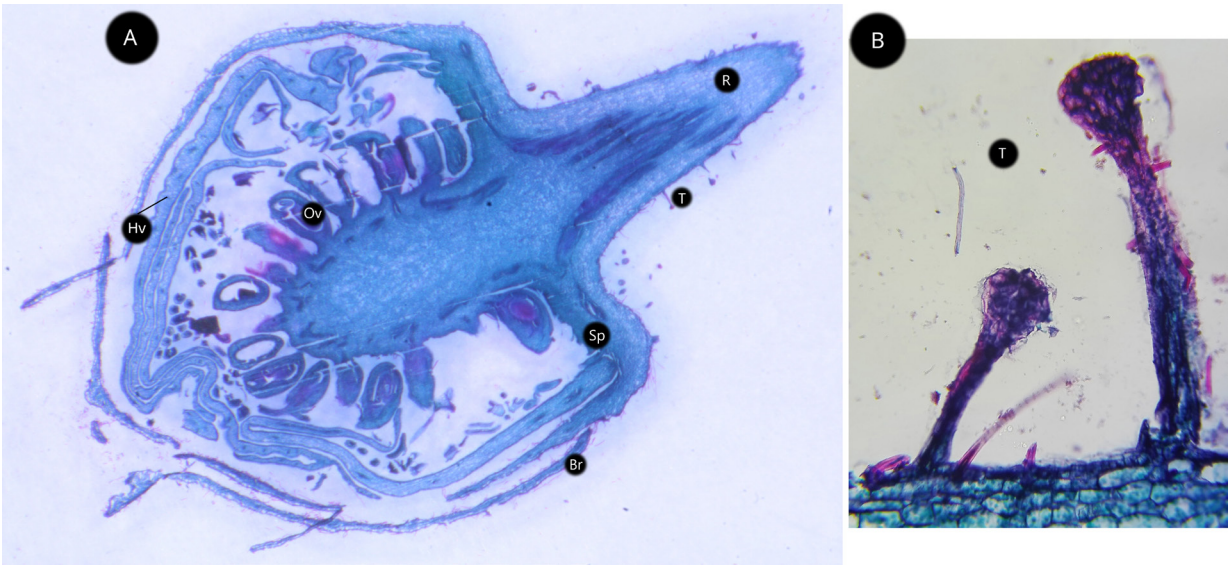

Figure 5. A. Floral button features (4X); B. Colleters and simple hairs in the receptacle. Unstratified epidermis (40X). R–Receptacle; T–Trichomes; Ov–ovule; Hv–Vascular bundles; Br–Bracts; Sp–Sepals.

Agronomic description:

Table 4. Postharvest agronomic parameters evaluated in *R. alutaceus* fruits.

Parameters	Unit	Maturity stage	
		Green	Completely mature
Polar diameter	cm	1.4 ± 0.03	1.9 ± 0.03
Equatorial diameter	cm	1.2 ± 0.02	1.5 ± 0.02
Total soluble solids	(°Brix)	–	8.8 ± 0.26
Ph	–	–	2.98 ± 0.03
Mass	g	1.1 ± 0.03	3.5 ± 0.18
Color (CIELAB)	Lightness (L)	46.46 ± 0.31	48.306 ± 0.35
	a*	–8.43 ± 0.18	3.427 ± 0.08
	b*	33.492 ± 0.34	14.743 ± 0.24
Seeds per fruit	Number	102 ± 9.98	102 ± 9.98

The quantitative data were represented by mean and standard error of mean.

Discussion

Morphological description:

This study characterizes the species *R. alutaceus* for the first time, collected in the municipality of Gachantivá–Colombia and identified as a sub–shrub, up to 6 m high with needles, and simple and colleters on the branches and leaves (KELLOGG et al., 2011). The latter are established as characteristics that can distinguish taxa, as in the case of *R. canescens, R. hirtus* and *R. ulmifolius* (ABBATE et al., 2006). It presents middle branches with five ribs, trifoliate leaves and pentafoliates, and inflorescence, ca. 60 flowers, that ends in a solitary flower. Green, subglobose polydrupes are seen in the first stages of maturity, which are whitish–yellow at full maturity (ROMOLEROUX, 1996; MORENO et al., 2011).

In this species, the morphological and genetic variability recorded for *Rubus* (STEARN, 1996; MARULANDA et al., 2007; CANCINO et al., 2011) is shown, as well as interesting agronomic possibilities due to the morphological (stems, leaves and fruits) and physicochemical characteristics (fruits), making it an important resource for agricultural development in Boyacá, Colombia.

Anatomical description

The presence of trichomes in *R. alutaceus,* Fig. 4 and 5, matches what was reported for other *Rubus* species (FELL and ROWSON, 1956, 1957, 1960; ÁLVAREZ and ESTÉBANEZ, 2015). Apparently, these excrescences of epidermal tissue of glandular hairs are involved in the evolutionary relationships of this type of plant and the development of spines and needles (KELLOGG et al., 2011).

R. alutaceus has colleters (Fig. 5) in the leaves, stem (Table 1 and 2) and floral structures (Table 3); what coincides with Fuks and Guimarães (1984); Thomas (1991) and Vitarelli (2009), who reported colleters in different dicotyledonous organs. Fahn (1988) stated that this type of trichomes secretes sticky substances that are generally mixtures of terpenes, which are essential oils and some resins (ESAU and RAY, 2008). Although there is no consensus regarding the function of trichomes, in the case of *Rubus* species, it can be inferred that this type of structures can protect reproductive and vegetative organs against desiccation, UV radiation and attack from microorganisms (DEMARCO, 2005).

This study and the research of Fell and Rowson (1956, 1957, 1960), Lersten and Horner (2000), show inclusions of calcium oxalate (Fig. 4) in the form of crystals in species of the family Rosaceae and the genus *Rubus*. On the other hand, Wada et al. (2011) observed, in the seeds of *R. caesius, R. hoffmeisterianus and R. occidentalis*, druses in the macroesclereids of the exotesta; likewise, Álvarez and Estébanez (2015) found this type of salts in the form of crystals in the mesophile of *Rubus ulmifolius*. These deposits of inorganic substances, which, in *R. alutaceus*, are found in the mesophyll, stem and leaf nerves (Table 2), can be useful for the taxonomic classification of the species (LERSTEN and HORNER, 2000; ESAU and RAY, 2008). An interesting agronomic value resulting from the formation of this type of crystals indicates that this plant has a mechanism of regulation of calcium levels in different organs, which can intervene in the protection of the plant against herbivores (SALTZ and WARD, 2000) and act as sites to store heavy metals and contribute to their detoxification (JÁUREGUI–ZUÑIGA and MORENO, 2004).

Other important compounds that develop in the vacuoles and appear in the cell walls are phenolic substances, which are found in *Rubus* (ÁLVAREZ and ESTÉBANEZ, 2015; MORENO–MEDINA et al., 2018). These compounds are synthesized mainly in the leaves, epidermis, and palisade parenchyma and usually perform several functions in plants, including waterproofing for the reduction of transpiration (ESAU and RAY, 2008) and protection against pests (NAVA–PÉREZ et al., 2012) and some phytopathogens (SILVA–ADAME et al., 2013). However, in *Rubus* seeds, high concentrations of protoanthocyanidins in combination with strong and coarse exotics are directly involved in the regulation of germination by the dormancy mechanism (ISAZA, 2007; WADA et al., 2011). Therefore, these characteristics of *Rubus* allow the formation of seed banks under wild conditions over time as a strategy for survival (DÍAZ, 2011).

In the transverse section of the stem (Table 1), the structural characteristics showed parenchymal cells, which can possibly assume specific characteristics when storing starches, anthocyanins, oleifera bodies, proteins or tannins; in this particular case, deposits of calcium oxalate crystals (Fig. 3). At the same time, it is important to highlight that there is an important reserve of water in active parenchyma cells and vacuoles (ESAU and RAY, 2008), which would give an advantage to this species for the productive activity, in relation to an eventual water deficit and possible stress events that may affect its agronomic potential.

Agronomic description

Table 4 shows the highest values for the polar and equatorial diameters and fresh weight of the *R. alutaceus* fruits in the fully mature state, coinciding with Ayala et al. (2013), who found the highest values for physical parameters in the maximum state of maturity for the species *R. glaucus*.

In addition, the values can be compared with those reported by Moreno and Deaquiz (2016), who found fruits with smaller equatorial diameters for the cultivar *R.*

alpinus and similar values in the polar diameters in Alto Ricaurte (Arcabuco), Colombia, possibly because of the agronomic management in this region.

The TSS results (8.78°Brix) for the fully mature fruits show the commercial potential of this species, taking into account the values reported by NTC 4106, developed for Colombia, which establishes the requirements that must be met by mora de Castilla (*Rubus glaucus*) intended for fresh consumption or as raw material for processing. In this standard, maximum values of 8.5°Brix were established for the fully mature state of fruits. The fresh weight the fruits had a mass of 3.5 g in the maximum state of maturity, which differs from that reported by Ayala et al. (2013), who found values of 7.7 g in the maximum state of maturity for *R. glaucus*.

The unit weight of the seeds in the present cultivar coincides with those reported by Hummer and Peacock (1994), who found *Rubus* taxa seeds weighing less than 2 mg.

The lightness parameter (CIELAB) showed that the fully mature fruits had higher values, possibly because, at full maturity, the size is larger and, therefore, there is higher water content, as reported by Rincon et al. (2015) for the species *R. alpinus*. Parameter a* values of –8.43 were evident in the first stages of maturation, showing a hue oriented toward the color green; in the fully mature fruits, the value was 3.42, and the hue was slightly red. The b* parameter showed positive values in the two evaluated maturity stages such as 33.49 green, 14.74 mature, placing this parameter in the yellow zone. This coincides with the report by Ballestero et al. (2004), who described *R. ellipticus*, with globular–ovoid and yellow berries, with pleasant organoleptic characteristics (sweet) and a high potential for market.

Conclusions

R. alutaceus is considered a new species and is named after the whitish–yellow color of the fully ripe fruits.

R. alutaceus presents calcium oxalate crystals in the form of druses and raphides in stems and leaves, showing that there is a mechanism for regulating calcium levels in the plant.

The simple trichomes and colleters found in the anatomy of *R. alutaceus* generate positive expectations for its agronomic potential and possible resistance to pests and diseases once this plant is established as a crop in different production units.

Because of its whitish–yellow color and the high value of the TSS, this fruit can be considered as having enormous potential for production and agroindustry in Colombia.

The botanical details evidence the potential that exists in the Alto Ricaurte region in Boyacá, Colombia for the diversity of *Rubus,* and the implementation of strategies that improve the management of these species as a crop could strengthen the productive chain of berries.

Conflict of interest: The authors declare that they have no competing interests.

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