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# *Clusia hirsuta*, a New Species from *Clusia* sect. *Retinostemon* and the First Description of Trichomes in the Genus

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**ABSTRACT.** *Clusia hirsuta* Hammel (Clusiaceae), a new species from the Pacific wet forest in Colombia and Ecuador, is described. Trichomes are reported here for the first time in the genus and described using micro-morphological techniques. Morphological and molecular data are used to establish the phylogenetic position of this newly described taxon within the genus *Clusia* L.

**Key words:** *Clusia*, Clusiaceae, section *Retinostemon*, trichomes.

The Neotropical genus *Clusia* L. (Clusiaceae) includes approximately 350 species of woody hemiepiphytes, shrubs, and trees. The group is most species rich in northern South America, including the northern Andes, the Amazonian lowlands east of the Andes, the Pacific lowlands from northwest Ecuador to Panama, and the Guayana Highlands (Gustafsson et al., 2007). Nearly all species are dioecious, though a few are hermaphroditic and some are apomictic (Maguire, 1976; Pipoly & Graff, 1995; Hammel, 2010). Subgeneric classification has been based mainly on morphological characteristics of the androecium. Modern phylogenetic reconstructions based on molecular data have shown that the genus, as well as some of the previously recognized sections, are monophyletic, but evolutionary relationships among closely related taxa remain largely unresolved (Gehrig et al., 2003; Gustafsson et al., 2007).

Since early taxonomic work on the genus (e.g., Planchon & Triana, 1860), it has been established that *Clusia* includes species with glabrous leaves. Here, we describe a new species of *Clusia* and report for the first time the presence of trichomes in the genus. Using morphological and molecular data we estimate the phylogenetic position of the species within the genus.

## METHODS

Fresh plant material and herbarium specimens housed at MO were studied. Leaf tissue samples were

dried in silica gel for molecular work and preserved in FPA fixative (formalin, propionic acid, ethanol 50%) for anatomical work. Leaf transversal sections were obtained following Ocampo and Columbus's (2010) protocol for fresh material. Leaf tissue samples were critical point dried, coated with gold in a Cressington 108 Auto Sputter Coater (Cressington Scientific Instruments UK, Watford, U.K.), and imaged in a Hitachi SU3500S Variable Pressure Scanning Electron Microscope (Hitachi, Tokyo, Japan).

DNA extraction was performed using a modified version of the CTAB protocol (Doyle & Doyle, 1987). The entire internal transcribed spacer (ITS) of the nuclear ribosomal repeat was amplified via polymerase chain reaction (PCR) using primers N-nc18S10 and C26A (Wen & Zimmer, 1996). PCR products were sequenced on an ABIxl sequencer facility at Rancho Santa Ana Botanic Garden following the BigDye terminator protocol (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). ITS sequences generated for the newly described taxon were deposited in GenBank at the National Center for Biotechnology Information public repository (accession numbers MF188241.1 and MF188242.1). Additional ITS sequences from previous studies (Gehrig et al., 2003; Gustafsson et al., 2007), available in GenBank, were used for phylogenetic reconstructions, which were performed following Luján (2016).

***Clusia hirsuta* Hammel, sp. nov.** TYPE: Colombia.

Valle del Cauca: orilla de carretera entre El Queremal y La Elsa—El Engaño, 19.2 km W de El Queremal, selva húmeda primaria arriba del Río San Juan, 3°53.028'N, 76°47.064'W, 702 m, 19 ene. 2015, M. Luján, E. A. Tripp, G. T. Godden, T. Lemieux, N. Medina & A. Manjarrés 496 (♂) (holotype, HUA!; isotype, RSA!).

**Diagnosis.** *Clusia hirsuta* Hammel differs from all other species of *Clusia* L. by having simple trichomes on young

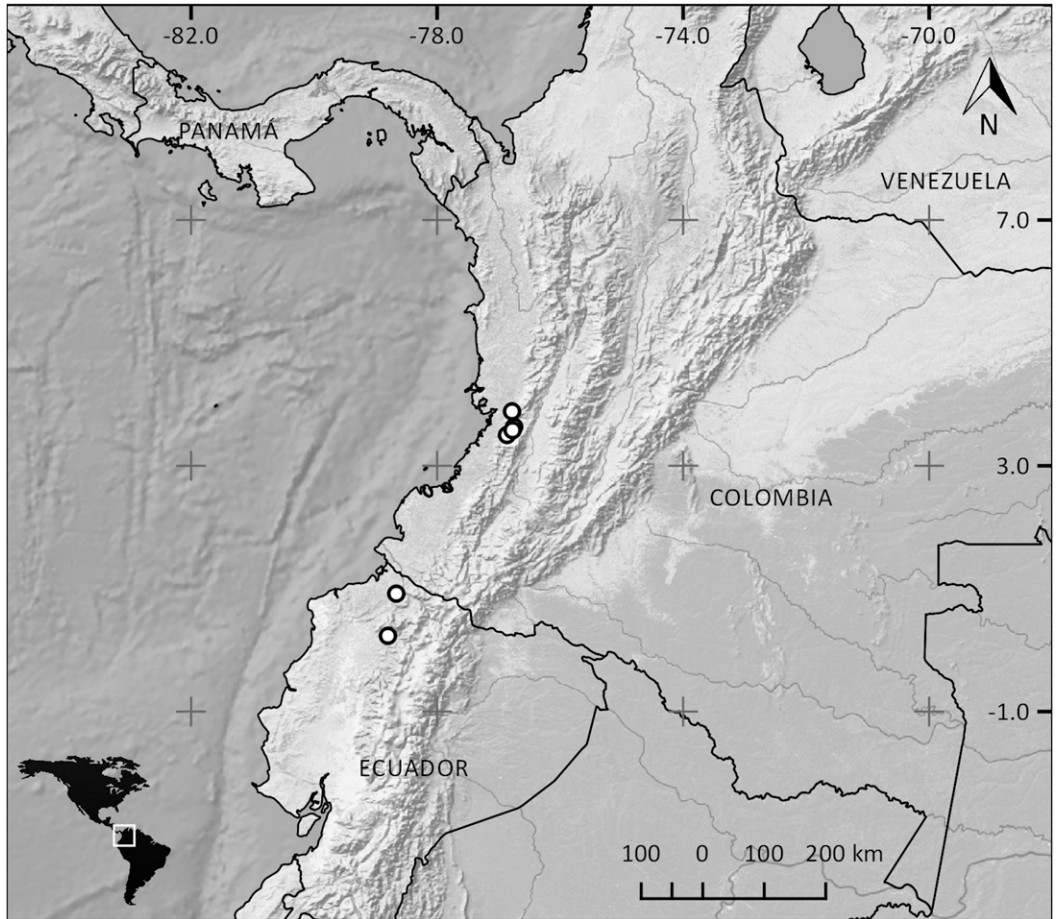


Figure 1. Distribution map of known collections of *Clusia hirsuta* Hammel.

vegetative parts, peduncles, pedicels, and on the abaxial leaf surface; and subsessile, elliptical leaves with pinnate brochidromous venation with strong intersecondaries and infra-marginal veins.

Decumbent, often hemiepiphytic, shrub or liana up to 6 m, with scarce, clear to cream-colored latex; dioecious. Young branches, inflorescence axis, and abaxial surface of leaves hirsutulous with simple, multicellular trichomes, green when fresh turning brown when dry, 300–400  $\mu\text{m}$ . Leaves subsessile with the petiole 1–3 mm, unwinged; blade membranaceous, discolorous (abaxial surface lighter green than adaxial surface), 20–25  $\times$  10–18 cm, broadly elliptic to oblong, base cordate, apex broadly acuminate, margin revolute; venation pinnate brochidromous, the primary and secondary veins prominent abaxially and impressed adaxially, with 22 to 28 pairs of secondary veins ending apically in an intramarginal vein, the angle to primary vein smoothly increasing toward the base, with strong intersecondary veins. Inflorescences terminal, erect, paniculate,

20–22  $\times$  10–15 cm, the staminate with 36 to 40 flowers, the pistillate with 18 to 24 flowers; bracts and bracteoles triangular, 2–4  $\times$  2–3 mm, apex acute. Flowers with pedicel 3–5 mm, cylindrical; sepals 4 or 5, white to light green, 5–8  $\times$  3–5 mm, broadly ovate to triangular; perianth 15–20 mm diam. Staminate flowers with petals 4 or 5, translucent white, 5–8  $\times$  3–5 mm, obovate with narrowed base, margin hyaline, sinuous; androecium formed by a dome-shaped, prominent synandrium, 2–3 mm diam., of 28 to 30 sessile stamens, central staminodia producing abundant bright yellow resin during anthesis, only distal stamens showing white, presumably viable pollen, proximal stamens immersed in resin. Pistillate flowers with petals 4 or 5, dull white to pink, 5–8  $\times$  4–5 mm, broadly ovate, margin entire; staminodial ring surrounding base of ovary; ovary white to light yellow, 8–9 mm high  $\times$  3–4 mm diam., ovoid; stigmas 8 to 10, sessile, white, obtusely triangular, located 1–2 mm below apex of ovary. Fruits dark pink to red when submature, 2–3 cm in diam., semispherical to

oblate, smooth; stigmas turning black, persistent on fruits; seeds 6 to 8 per locule, covered with orange-red aril.

**Phenology.** *Clusia hirsuta* has been collected with flowers and fruits in January, February, and July.

**Distribution and habitat.** *Clusia hirsuta* has been collected on the Pacific slopes of the Cordillera Occidental in Colombia and the Cordillera Real in Ecuador (Fig. 1), between 500 and 1800 m elevation in primary montane pluvial forest.

**Etymology.** The specific epithet refers to the unique characteristic of this species of having simple, erect trichomes.

**Discussion.** *Clusia hirsuta* is the first known species described in the genus that has trichomes, further expanding the morphological variability seen in the genus. A number of other *Clusia* species have been reported to have exfoliating epidermis on young branches (e.g., *C. minor* L., *C. pratensis* Seem. [Hammel, 1986, 2010; pers. obs.]), but this may reflect a developmental phase in secondary growth of young shoots and is merely tearing of the epidermis rather than real indumentum. Trichomes in *C. hirsuta* are formed of 12 to 15 elongated epidermal cells. Leaf transverse sections showed that a druse is often present at the base of the trichomes (Fig. 2).

Morphological characteristics of the androecium in staminate flowers of *Clusia hirsuta* suggest the species is included in *Clusia* sect. *Retinostemon* Planch. & Triana, which is characterized by staminate flowers having a resin-secreting synandrium of fused stamens and staminodes (Fig. 3A). Moreover, phylogenetic analysis of molecular data (ITS) also indicates that *C. hirsuta* is part of section *Retinostemon*, and that it is closely related to *C. petiolaris* Planch. & Triana (bootstrap value 96%, Fig. 4), a species known from wet mountain forests on the eastern Andes in Colombia and Ecuador. Section *Retinostemon* includes ca. 32 species distributed in northern South America and Central America and is the most species-rich section of resin-producing flowers in the genus.

Petals in staminate flowers of *Clusia hirsuta* have a different consistency compared to petals in pistillate flowers (translucent vs. dull; Fig. 3A, B). Differences in corolla traits between pistillate and staminate flowers have also been reported in *C. heterocolorata* L. Marinho & Bittrich (Marinho et al., 2015). *Clusia heterocolorata* is classified in section *Phloianthera* Planch. & Triana, a section not phylogenetically proximate to *C. hirsuta*. It is probable that corolla traits that differ between pistillate and staminate conspecifics have evolved independently in different lineages, although further investigation is needed to fully understand this character.

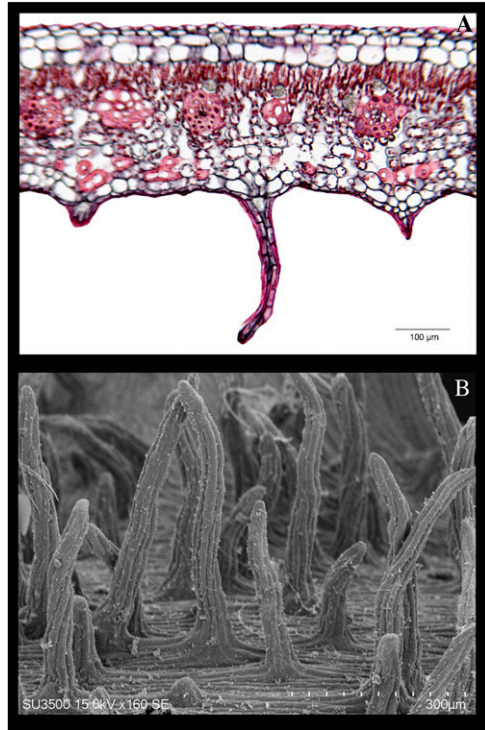


Figure 2. Detail of trichomes on abaxial leaf surface of *Clusia hirsuta* Hammel. —A. Leaf transversal section. —B. SEM photograph of trichomes on lower surface of the leaf base. A and B from *M. Luján et al.* 496.

**Paratypes.** COLOMBIA. **Valle del Cauca:** orilla de carretera entre El Queremal y La Elsa—El Engaño, 19.2 km W de El Queremal, selva húmeda primaria arriba del Río San Juan, 3°34.654'N, 76°46.548'W, 829 m, 19 ene. 2015, *M. Luján, E. A. Tripp, G. T. Godden, T. Lemieux, N. Medina & A. Manjarres* 495 (♀) (HUA, RSA); Yatacué, Alto Anchicaya, near CVC hydroelectric plant headquarters, wet/pluvial forest transition, valley of Río Dagua (tributary of Anchicaya), 3°38'N, 76°45'W, 710–880 m, 17 July 1984, *A. Gentry & M. Monsalve* 48261 (♀) (MO); short rd. above CVC camp Yatacué, Alto Anchicaya, 3°30'N, 76°52'W, 600 m, 7 Feb. 1984, *A. Juncosa* 2067 (♀) (MO). ECUADOR. **Esmeraldas:** San Lorenzo Canton Carretera Lita—San Lorenzo, 38 km N de Lita, bosque pluvial tropical, 78°40'W, 0°55'N, 500 m, 9 July 1990, *D. Rubio, C. H. Dodson, E. Hagsater & N. Revelo* 455 (♂) (MO, QCNE [not seen]). **Pichincha:** Reserva Natural Río Guajalito, Km. 59 on Quito—Chiriboga—Santo Domingo Rd., 3.5 km NE of main rd., 78°48'10"W, 0°13'53"S, 1800 m, 11 Feb. 1992, *T. Croat* 72121 (♀) (MO).

We were unable to find and reconfirm *Zak & Jaramillo* 2523 (MO), determined by one of us (BH), to be this entity in 1989. By its label data “corola morada, latex amarillento” it is somewhat aberrant and may represent a different entity.

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Figure 3. *Clusia hirsuta* Hammel. —A. Staminate flower; note pubescence and prominent veins in leaf above flower (from M. Luján et al. 496). B–D. Pistillate material (from M. Luján et al. 495). —B. Pistillate flower. —C. Branch with pistillate inflorescence; note leaves with depressed secondary and inframarginal veins. —D. Immature fruits; note apical crown of brown stigmata. Scale bars: A, B, D = 1 cm; C = 5 cm.

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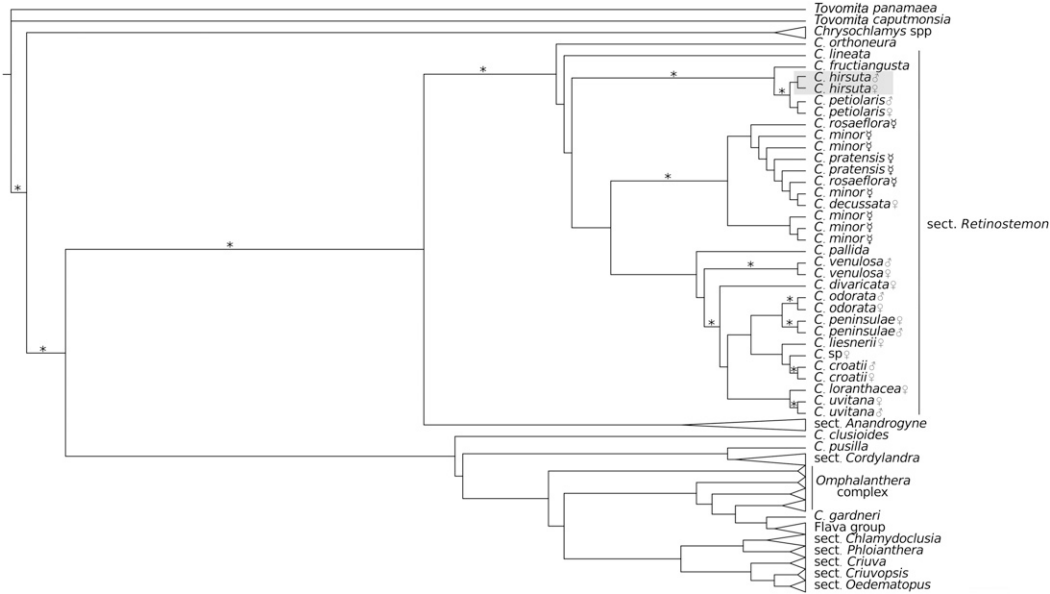


Figure 4. Phylogenetic relationships among *Clusia* L. species based on maximum likelihood analysis of nuclear ITS data. *Clusia hirsuta* Hammel is highlighted in gray box; star symbol (\*) above branches denotes bootstrap values greater than 90%. Pistillate (♀), staminate (♂), or bisexual (♂) form is indicated next to species names.

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