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TRABAJOS ORIGINALES

Three new species of *Drosophila tripunctata* group (Diptera: Drosophilidae) in the eastern Andes of Ecuador

Tres especies nuevas del grupo Drosophila tripunctata (Diptera: Drosophilidae) en los Andes Orientales de Ecuador

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Abstract

Three new species of the *Drosophila tripunctata* group are described and illustrated. These new species were captured using plastic bottles containing pieces of fermented banana with yeast. The collections were from Napo Province, Ecuador at 2 200 m and 3 362 m above sea level. The new species are: *Drosophila napoensis* sp. nov., *Drosophila cuyuja* sp. nov. and *Drosophila quijos* sp. nov. The first two species belong to subgroup I and the latter species belong to subgroup III of the *Drosophila tripunctata* group.

Keywords: Diversity; Drosophila tripunctata group; Neotropical Region; new species; genitalia.

Resumen

Se describen e ilustran tres especies nuevas del grupo *Drosophila tripunctata*. Estas especies fueron capturadas con trampas hechas de botellas de plástico que contenían pedazos de plátano fermentados con levadura. Las colectas fueron realizadas en la provincia de Napo, Ecuador, a 2 200 y 3 362 metros sobre el nivel del mar. Las especies nuevas son: *Drosophila napoensis* sp. nov., *Drosophila cuyuja* sp. nov. y *Drosophila quijos* sp. nov. Las dos primeras especies pertenecerían al subgrupo I y la última especie al subgrupo III del grupo *D. tripunctata*.

Palabras clave: Diversidad; grupo Drosophila tripunctata; Región Neotropical; especies nuevas; genitalia.

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Introduction

We describe three new species of *Drosophila* that belong to the *tripunctata* group of the subgenus *Drosophila*, as originally established by Sturtevant (1942). Based on morphological characters, this group has been divided into four subgroups: Subgroup I is probably transitional to the *guarani* group (Frota-Pessoa, 1954). Subgroup II is the most typical of the group. Subgroups I and II are considered to be more related to each other than to subgroups III and IV. Subgroup III is probably transitional to the *cardini* group (Frota-Pessoa, 1954). Subgroup IV also has some affinities to this group, since three of these species have white faces like some members of the *cardini* group (Frota-Pessoa, 1954).

However, the identification of this group of species is difficult, due to the great intraspecific variation observed in the bands and spots on the distal tergites. Characters of the male terminalia have been the most reliable for differentiating between closely related species (Vilela 1992). According to Frota-Pessoa (1954), the representative characteristics of the *tripunctata* group are: the mesonota are without markings, the carina are never sulcate and the anal plates are separated from the genital arch.

The tripunctata group is endemic to the Neotropical Region, where it represents the second largest formal group, surpassed only by the D. repleta species group (Vilela 1921). In Ecuador, 23 species of the group have been recorded: D. metzii Sturtevant, 1921; D. paraguayensis Duda, 1927; D. bandeirantorum Dobzhansky & Pavan, 1943; D. mediosignata Dobzhansky & Pavan, 1943; D. mediopicta Frota-Pessoa, 1954; D. johnstonae Pipkin & Heed, 1964; D. roehrae Pipkin & Heed, 1964; D. cuaso Bächli, Vilela & Ratcov, 2000; D. loewi Vilela & Bächli, 2000; D. carlosvilelai Vela & Rafael, 2001; D. fontdevilai Vela & Rafael, 2001; D. machachensis Vela & Rafael, 2001; D. pasochoensis Vela & Rafael, 2001; D. pilaresae Vela & Rafael, 2001; D. tomasi Vela & Rafael, 2001; D. valenciai Vela & Rafael, 2001; D. ichubamba Vela & Rafael, 2005; D. ninarumi Vela & Rafael, 2005; D. patacorona Vela & Rafael, 2005; D. quillu Vela & Rafael, 2005; D. surucucho Vela & Rafael, 2005; D. urcu Vela & Rafael, 2005 and *D. condorhuachana* Céspedes & Rafael, 2012.

The *tripunctata* group radiated from the Paleotropics and diversified in the Neotropics. The progentitors of the *tripunctata* radiation probably fed as larvae on living fungi, flowers and fruits in the process of fermentation (Throckmorton 1975). Currently, the members of the *tripunctata* group are generalist feeders as larvae. Ovipositing females typically choose mushrooms over fruits, but mushrooms also constitute a favorable breeding site for larvae; this variation in oviposition site could have a genetic basis potentially leading to speciation by larval host (Jaenike 1989). In the Neotropics, species in the *tripunctata* group have a high diversity of yeast associated with different strategies for utilization of substrates for larval feeding (Morais et al. 1992) The *tripunctata* species group has a Neotropical distribution with Brazil the center of diversity. Only *Drosophila tripunctata* is found in the Neartic region (Markow & O'Grady 2005).

Material and methods

The flies were collected in Napo Province in northeastern Ecuador in the foothills of the Andes descending to the Amazon region. Collections were made at two altitudes, 2200 m $(00^{\circ}37'08.7"S; 77^{\circ}50'21.2"W)$ and 3362 m $(00^{\circ}22'52.6"S, 78^{\circ}09'44.4"W)$. Traps (40) were placed in each location five

meters apart and one meter high from the base of the trees that marked the sampling locations. The traps were made with a 500 ml plastic bottle and baited with banana pieces (2 cm) previously fermented for 24 hours with yeast.

Living flies in the traps were captured with an entomological aspirator and collected in a tube with standard *Drosophila* culture media. Females were individually isolated after mating to produce iso-female lines. The larvae and adults were maintained on gelatin-banana media. Adult specimens were stored in microcentrifuge tubes with ethanol (70%). The external morphology of each fly was examined under a stereomicroscope (Zeiss; Discovery V8) and measured with the AxioVision program. Male terminalia were dissected out of the abdomen and placed in KOH (10%) and boiled for ten minutes. They were then placed in glycerol (60%) for description, photography and preservation before being double-mounted in microvials and as dried, mounted specimens. Indices of the paratypes are presented in parentheses. Descriptive terms and indices follow the system of Bächli et al. (2004).

The holotypes and paratypes of the new species have been deposited in the Museo de Zoología – Invertebrados, Pontificia Universidad Católica del Ecuador, Quito (QCAZ).

Results

These three new species in the *tripunctata* group have been described as members of two subgroups, I and III.

Subgroup I: Drosophila napoensis sp. nov. Drosophila cuyuja sp. nov.

Drosophila napoensis sp. nov.

(Figs. 1 - 6)

Type material. Male holotype (dissected, terminalia in microvial), labelled "*D. napoensis* Holotype 3, E. L. Ramos & V. Rafael det. 2014 Ecuador, Napo, Papallacta (opposite the west side of Papallacta Lake, across the road), 00°22'52.6"S, 78°09'44.4"W, 3362 m). II. 08/09/2012, E.L. Ramos col., (QCAZI 3004). Paratype: 13 (dissected, terminalia in microvial), same data as holotype (QCAZI 3005).

Diagnosis. Aristae generally with 5 dorsal and 2 ventral branches on the right antenna and 4 dorsal and 3 ventral branches on the left, plus terminal fork. Basal scutellar setae divergent. Surstylus with a row of 10 prensisetae in the left and 8 (9) on the right, 14 inner setae on the right and 18 on the left. Aedeagus with two lateral serrate projections (ear shaped) whose distal edge almost reaches the apex, apex invaginated with membranous center and with striae. Hypandrium shield-shaped.

Description. Head. Frons yellowish brown, frontal length 0.40 mm; frontal index = 0.78 (0.83), top to bottom width ratio = 1.31 (1.31). Frontal triangle yellowish brown. Ocellar triangle brown, ocellus yellow. Orbital plate brown, the medial vertical setae was closer to lateral vertical setae and slightly toward the outer edge of the orbital plate, distance of or3 to or1, 12% (10%) of or3 to vtm, 0.15 (0.13), or1/or3 ratio 0.69 (0.70) or2/or1 ratio 0.60 (0.48) postocellar setae 65% (62.50%) and ocellar setae 90% (87.50%) of frontal length; vt index = 0.79 (0.67), vibrissal index = 0.65 (0.68). Frontal vitta brown. Gena and postgena yellow.



Figures 1 – 6. *Drosophila napoensis* sp. nov. (1) abdomen (scale bar = 1mm); (2) epandrium, cerci, surstyli and decasternum; (3) hypandrium and gonopods in ventral view; (4 - 6) aedeagus in dorsal, lateral and ventral view, respectively (scale bar = $100 \mu m$).

Face yellowish brown. Carina yellowish brown, prominent, not sulcate. Cheek index = 11 (9.14). Eyes red; eye index = 1.27 (1.33). First flagellomere brown. Aristae plumose, right aristae with 5 dorsal and 2 ventral branches and 4 dorsal and 3 ventral branches in the left, plus terminal fork. Proboscis beige.

Thorax. Brown; length 1.45 (1.05) mm with 6 rows of acrostical setulae between the two anterior dorsocentral setae, h index = 0.94 (1.01). Tranverse distance of dorsocentral setae 1.73 (1.62) of longitudinal distance, dc index = 0.56 (0.57). Scutellum brown. Distance between apical scutellar setae 62% (78%) of that between apical and basal setae; basal scutellar setae divergent; scut index = 1.11 (1.80, apical scutellar setae broken). Pleura yellow, sterno index = 0.92 (0.88), median katepisternal setae slightly smaller than the anterior. Legs yellow.

Wings beige, length 3.81 mm (3.83), length to width ratio = 2.11 (2.29). Indices: C= 3.42 (3.92); ac= 2.45 (2.22); hb= 0.30 (0.33); 4c= 0.58 (0.53); 4v= 1.25 (1.35); 5x= 1.12 (1.47); M= 0.34 (0.38); prox.x= 0.30 (0.31).

Abdomen (Fig. 1). Yellow; tergite 1 without pigmentation; 2 – 6 with an apical band uninterrupted in the midline.

Length (Body + wings) 4.79 (4.90) mm

Male terminalia (Figs. 2 – 3). Epandrium microtrichose without setae above, with 2 lower short setae weakly sclerotized. Cerci not linked to epandrium. Surstylus almost elliptic and microtrichose; with a row of 10 prensisetae on the left and 8 (9) on the right, 14 inner setae in the right and 18 in the left. Hypandrium shield-shaped. Gonopod microtrichose with one seta in the middle.

Aedeagus (Figs. 4 - 6). Weakly sclerotized with two lateral serrate projections (ear shaped) whose distal edges almost reach at apex. Apex invaginated with a membranous center and with striae. Aedeagal apodeme less sclerotized. Paraphyses broken with fine hairs.

Etymology. Name refers to the Ecuadorian province, Napo, where the specimens were collected.

Relationship to other species. The general shape of the male terminalia, especially the aedeagus, suggests a close relationship to *D. loewi* Vilela & Bächli, 2000

Drosophila cuyuja sp. nov.

(Figs. 7 - 12)

Type material. Male Holotype (dissected, terminalia in microvial), labelled "*D. cuyuja* Holotype 3, E. L. Ramos & V. Rafael det. 2014 Ecuador, Napo, Papallacta (opposite to Papallacta Lake), 00°22'52.6"S, 78°09'44.4"W, 3362 m). II. 11/11/2012, E.L. Ramos col., (QCAZI 3003).

Diagnosis. Aristae plumose, right arista with 4 dorsal and 2 ventral branches and 3 dorsal and 2 ventral branches on the left, plus terminal fork. Abdomen beige; tergite 1 with less pigmentation; tergite 2 with a median pigmented triangular spot, tergites 2 - 4 with a dark pigmented hourglass shape. Aedeagus sclerotized with two crests serrated laterally on the ventral part and two membranous projections with striae on the upper dorsal part.

Description. Head. Frons yellowish brown, frontal length 0.43 mm; frontal index = 0.84, top to bottom width ratio = 1.68. Frontal triangle yellow. Ocellar triangle yellowish brown, ocellus yellow. Orbital plate yellowish brown, the medial vertical seta was closer to lateral vertical seta and slightly towards the outer edge of the orbital plate, distance of or3 to or1 7% of or3 to vtm 16%, or1/or3 ratio 0.52, or2/or1 ratio 0.55, postocellar setae 67% and ocellar setae 88% of frontal length; vt index = 0.89, vibrissal index = 0.57. Frontal vitta yellow. Gena and postgena yellow. Carina yellow, prominent and not sulcate. Cheek index = 8.11. Eyes wine red; eye index = 1.35. First flagellomere yellow. Aristae plumose, right arista with 4 dorsal and 2 ventral branches and 3 dorsal and 2 ventral branches on the left, plus the terminal fork. Proboscis yellow.

Thorax. Yellowish brown; length 1.70 mm with 6 rows of acrostic setulae between the two anterior dorsocentral setae, h index = 0.83. Transverse distance of dorsocentral setae 1.81 of longitudinal distance, dc index= 0.89. Scutellum yellow. Distance between apical scutellar setae 73% of that between apical and basal setae; basal scutellar setae divergent; scut index = 1.62. Sterno index= 0.83. Legs yellow.

Wings beige, length 2.42 mm, length to width ratio= 2.39. Indices: C= 5.0; ac= 1.94; hb= 0.25; 4C= 0.41; 4v= 1.17; 5x= 1.08; M= 0.30; prox. x= 0.33.

Abdomen (Fig. 7). Beige; tergite 1 with less pigmentation; tergite 2 with a middle pigmentation like a triangle, tergites 2 - 4 with dark pigmented hourglass shape.

Length (body + wings) 3.8 mm.

Male terminalia (Figs. 8 – 9). Epandrium microtrichose with 1 upper long seta in the right side and 3 lower long setae in the left side. Cerci not linked to epandrium. Surstylus microtrichose and rectangular with a row of 11 prensisetae in the left and 10 in the right, 11 inner setae in both sites of ventral lobe. Hypandrium sclerotized, shield – shaped. Gonopod oval and microtrichose.

Aedeagus (Figs. 10 – 12). Sclerotized with two crests serrated laterally on the ventral part and two membranous projections with striae on the upper dorsal part. Aedeagal apodeme less sclerotized and strongly bent inventral direction. Ventral rod triangle – shaped. Paraphyses with a small sclerotized microprojections, "C" shaped with a long seta on the upper edge and 2 on the inner edge of middle gonopod.

Etymology. This species is named after the Cuyuja region of Napo Province, Ecuador.

Relationship to other species. The general shape of the male terminalia does not suggest any relationship to other species of *Drosophila tripunctata* group.

SUBGROUP III: DROSOPHILA QUIJOS SP. NOV.

Drosophila quijos sp. nov.

(Figs. 13 - 18)

Type material. Male holotype (dissected, terminalia in microvial), labelled "*D. quijos* Holotype ♂, E. L. Ramos & V. Rafael det. 2014 Ecuador, Napo, Cordillera de los Guacamayos, 00°22'52.6"S, 78°09'44.4"W, 3362 m). II. 16/05/2012, E.L.





Figures 7 – 12. *Drosophila cuyuja* sp. nov. (7), abdomen (scale bar = 1mm); (8) epandrium, cerci, surstyli and decasternum; (9) hypandrium and gonopods in ventral view; (10 – 12) aedeagus and paraphyses in dorsal, lateral and ventral view, respectively (scale bar = 100μ m).



Figures 13 – 18. *Drosophila quijos* sp. nov. (13) abdomen (scale bar = 1mm); (14) epandrium, cerci, surstyli and decasternum; (15) hypandrium and broken gonopods in ventral view; (16 – 18) aedeagus, paraphyses and broken gonopods in dorsal, lateral and ventral view, respectively (scale bar = 100μ m).

Ramos col., (QCAZI 3001). Paratype: 13 (dissected, terminalia in microvial), with same data as holotype (QCAZI 3002).

Diagnosis. Aristae plumose, right arista with 8 dorsal and 3 ventral branches and 7 dorsal and 3 ventral branches on the left, plus the terminal fork. Abdomen yellow with dorsal midline; tergite 1 with little pigmentation on the lower part of the tergite (without dorsal midline); tergites 2 - 5 with dark brown lateral bands which increase in wide apically and slightly narrowed laterally. Apex of aedeagus with a wide invagination and dorsally with a voluminous projection with bright studs. Aedeagal apodeme sclerotized (broken in the paratype). Paraphyses long with two pedunculated setae.

Description. Head. Frons brown, frontal length 0.45 mm (0.47); frontal index= 0.80 (0.86), top to bottom width ratio= 1.48 (1.40). Frontal triangle yellowish brown. Ocellar triangle dark brown, ocellus yellow. Orbital plate brown, the medial vertical seta is closer to lateral vertical seta and slightly towards the outer edge of the orbital plate, distance of or3 to or1 8% (10%) of or3 to vtm 15% (15%), or1/or3 ratio 0.73 (0.70), or2/ or1 ratio 0.60 (0.62), postocellar setae 66% (65%) and ocellar setae 82% (80%) of frontal length; vt index= 1.10 (1.0), vibrissal index= 0.52 (0.50). Frontal vitta brown. Gena and postgena yellowish brown. Carina yellow, prominent, not sulcate. Cheek index = 10.85 (9.21). Eyes wine red; eye index = 1.31 (1.33). First flagellomere yellowish brown. Aristae plumose, right arista with 8 dorsal and 3 ventral branches and 7 dorsal and 3 ventral branches on the left, plus terminal fork. Proboscis yellow.

Thorax. Brown; length 0.87 mm (0.81) with 6 rows of acrostic setulae between the two anterior dorsocentral setae, h index= 0.89 (0.80). Transverse distance of dorsocentral setae 2.0 (1.98) of longitudinal distance, dc index = 0.80 (0.83). Scutellum brown. Distance between apical scutellar setae 92% of that between apical and basal setae; basal scutellar setae divergent; scut index= 1.01 (1.30). Sterno index= 0.63 (0.71), median katepisternal setae slightly smaller than the previous one. Legs yellow.

Wings beige, posterior crossvein slightly infuscate, length 3.77 mm (3.90), length to width ratio= 2.11 (2.0). Indices: C= 4.09 (4.0); ac= 2.16 (2.3); hb= 0.23 (0.30); 4c= 0.50 (0.54); 4v= 1.16 (1.0); 5x= 1.20 (1.02); M= 0.32 (0.45); prox.x= 0.33 (0.43).

Abdomen (Fig. 13). Yellow with dorsal midline; tergite 1 with little pigmentation in the distal part of the tergite and without dorsal midline; tergites 2 – 5 with dark brown lateral bands which increase in width apically and slightly narrowed laterally; tergite 6 pigmented entire width and length.

Length (body + wings), 4.64 mm (4.82).

Male terminalia (Figs. 14 – 15). Epandrium microtrichose with 2 long lower setae. Cerci not linked to epandrium. Cerci microtrichose with long setae. Surstylus sclerotized with a row of 12 prensisetae on the left and 14 on the right, 9 inner setae on the right and 10 on the left. There were two groups of outer setae: 6 inward and 19 outward. Hypandrium shield – shaped with sclerotized edge (broken in the paratype).

Aedeagus (Figs. 16 – 18). Sides of the aedeagus sclerotized with two lateral and serrate projections. Apex with a wide invagination and dorsally with a voluminous projection with

bright studs near to the lateral projections. Aedeagal apodeme sclerotized (broken in the paratype). Paraphyses long with two pedunculated setae.

Etymology. Named after the Quijos region of Napo Province, Ecuador where the specimens were collected.

Relationship to other species. The general shape of the male terminalia, especially the aedeagus, suggests a close relationship to *D. bandeirantorum* in the *tripunctata* group.

Discussion

The two new species, *Drosophila napoensis* and *Drosophila cuyuja* should be placed in subgroup I of the *tripunctata* species group because these species are yellowish, without pollinosity, the mesonotum is without markings, the anal plate is without spikes and the anterior crossvein is not clouded. Another group that could be appropriate for these species is subgroup IV, however the species described in this paper have a clouded crossvein and the species in subgroup IV do not have clouded anterior crossveins. Other characteristics of subgroup IV are similar to these two new species including: distal tergites with posterior bands interrupted in the middle and expanded laterally, convergent anterior scutellar setae and a costal index greater than or equal to 4.0 (Frota-Pessoa 1954).

Drosophila cuyuja is not similar to other species in the tripunctata group therefore it could not be assigned to a subgroup. However, the external characteristics of Drosophila cuyuja clearly indicate that this species should be placed in the D. tripunctata group because species in this group are all yellowish, in general dull or only slightly shining, without markings on the mesonotum; and with red eyes, darker dorsally than ventrally; sterno index about 0.6 and basal scutellar setae are generally divergent (Frota-Pessoa 1954). The genitalia characteristics of D. cuyuja, the general shape of the aedeagus, the hypandrium with a distal bow that surrounds the penis, the sharp terminus of the ventral rod and the anal plates are not fused to the genital arch are characteristic of species in the tripunctata group (Frota-Pessoa 1954).

Drosophila napoensis is very similar to D. loewi but the most important difference is in the aedeagus. First, D. napoensis has the aedeagus tip strongly envaginated without a dorsocentral projection and D. loewi has the aedeagus tip only slightly invaginated with a projection in the dorsocentral part. Second, D. napoensis has semicircular, ear-shaped projections with a small serrated projection on the dorsal part of the aedeagus and D. loewi has completely circular projections without small projections. Third, the aedeagal apodeme in D. napoensis is curved on the dorsal part and D. loewi has a straight aedeagal apodeme. Finally, in D. napoensis the hypandrium is without setula and in D. loewi the hypandrium has one inner setula.

External morphology indicated that *Drosophila quijos* should be placed in subgroup III. These species are dull or sub-shining, have distal tergites with posterior bands of pigment expanded in the middle, the anterior scutellar setae are divergent, the costal index is near 4.0 and the anterior crossvein is slightly clouded (Frota-Pessoa 1954).

In the first publication describing *D. bandeirantorum* (Dobzhansky & Pavan, 1943) the authors did not describe the male terminalia. Eleven years later, Frota-Pessoa (1954) completed the description and emphasized that this species has forceps with a row of 10 - 12 primary teeth, 14 - 20 secondary teeth on upper surface that are longer and weaker than the primary ones and 7 - 12 marginal bristles. Vilela (1992) considered that this species has 8 primary teeth, no secondary teeth and 14 marginal bristles that come up to upper surface. This disagreement is probably because the secondary teeth and marginal bristles are the same dark color and they end in sharp points. In contrast, Drosophila quijos has the primary teeth, secondary teeth and marginal bristles clearly differentiated. In D. quijos the aedeagus tip is wide and bifid. Drosophila bandeirantorum has an aedeagus with a bifid tip but it is not wide. The aedeagus tip in D. quijos has lateral serrate, ear - shaped projections that end in the middle and a dorsal voluminous projection with bright spots. In D. bandeirantorum, the aedeagus tip has small lateral projections and the projections are on the ventral part. In D. quijos the paraphyses have with two pedunculated setae and the aedeagal apodeme is not curved. Drosophila bandeirantorum has only one pedunculated seta and the aedeagal apodeme is curved.

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