# Description of a New Spotted-Thorax *Drosophila* (Diptera: Drosophilidae) Species and Its Evolutionary Relationships Inferred by a Cladistic Analysis of Morphological Traits

ANDREA ACURIO,[1,2] VIOLETA RAFAEL,[3] DIEGO CÉSPEDES[3] AND ALFREDO RUIZ[1]

Ann. Entomol. Soc. Am. 106(6): 000–000 (2013); DOI: http://dx.doi.org/10.1603/AN13028

**ABSTRACT** A phylogenetic approach based on morphological characters is the only alternative applicable in cases where molecular data are unavailable. During a taxonomic inventory of Drosophilidae in 12 localities of Ecuador (South America), we discovered a new species of cactophilic spotted-thorax *Drosophila* Fallen that here we formally describe as *Drosophila machalilla* Acurio 2013. To classify this new species, we analyzed the terminalia of male and female adults, finding similarities with flies of two neotropical spotted-thorax species groups of *Drosophila*, namely *repleta* and *peruensis*. Flies or DNA sequence data are unavailable for the latter species group, hindering a molecular approach. Thus, to accurately classify the new species, we carried out a maximum parsimony cladistic analysis using 52 morphological characters from nine representative taxa of *virilis*, *willistoni*, *repleta*, and *peruensis* species groups. The results indicate that *D. machalilla* sp. nov. belongs neither to the *repleta* group nor to the *peruensis* group and suggest that a new species group should be erected to house *D. machalilla* and *Drosophila atalaia* Vilela & Sene (1982, previously considered a member of the *peruensis* species group).

**KEY WORDS** *Drosophila*, cladistic analysis, *repleta* group, *peruensis* group

Given the striking advances in Molecular Systematics (Moritz and Hillis 1996, Felsenstein 2004), it may seem that there is not much point in reconstructing phylogenies using morphological data anymore. However, a phylogenetic approach based on morphological characters is the only possibility if no molecular material is available.

Taxonomic inventories or species censuses, the fundamental data in biogeography, macroecology, and conservation ecology (Mora et al. 2008), are important in the assessment of species richness, diversity patterns, and provide verifiable information when specimens are deposited in appropriate institutions (Wheeler 1995, 2010).

Systematics requires accurate data on distribution patterns of taxa provided by taxonomic inventories to resolve evolutionary relationships among species (Wheeler 2004, Wilson 2004, Agnarsson and Kuntner 2007). When previously unknown species are discovered, classifications may need revision to reflect their placement. This undoubted may have a large impact on existing classification schemes because, at this time, we cannot say how many more species exist on earth awaiting discovery (Lipscomb 1998).

We are engaged in a taxonomic inventory of Drosophilidae in Ecuador (Rafael and Arcos 1989; Vela and Rafael 2004; Acurio and Rafael 2009a,b; Céspedes and Rafael 2012; Figuero et al. 2012). In December 2010, 12 localities of Central Coast, North and South of Ecuador (A. A. et al., unpublished data) led to the discovery of a new cactophilic spotted-thorax *Drosophila* species (Fig. 1A) described below. To classify the new species, we analyzed the external terminalia on male and female adults. We found similarities with two neotropical species groups of spotted-thorax flies: the *Drosophila repleta* species group with >100 described species (Brake and Bächli 2008) and the *Drosophila peruensis* species group with six species described so far (Ratcov and Vilela 2007, Döge et al. 2011). Although we have the new *Drosophila* species in culture and specimens of *repleta* group are available from our collections and *Drosophila* stock centers around the world, specimens of the *peruensis* group species maintained as culture in laboratory or preserved in alcohol are not available. Although several attempts have been made to collect *D. peruensis*, the first species described from the group, at the Urubamba River in Peru, not one specimen was captured (Ratcov and Vilela 2007, p.310). Therefore, a molecular analysis to find *D. machalilla* phylogenetic affinities to the *peruensis* group has not been possible. Nevertheless, we found an important source of reliable data on species descriptions made by specialists on taxonomy of *Drosophila* Fallen (Supp. Table 1

[1] Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra 08193 Barcelona, Spain.
[2] Corresponding author, e-mail: andrea.acurio@uab.cat.
[3] Laboratorio de Genética Evolutiva, Escuela de Ciencias Biológicas, Pontifícia Universidad Católica del Ecuador, Quito 17012184 Pichincha, Ecuador.

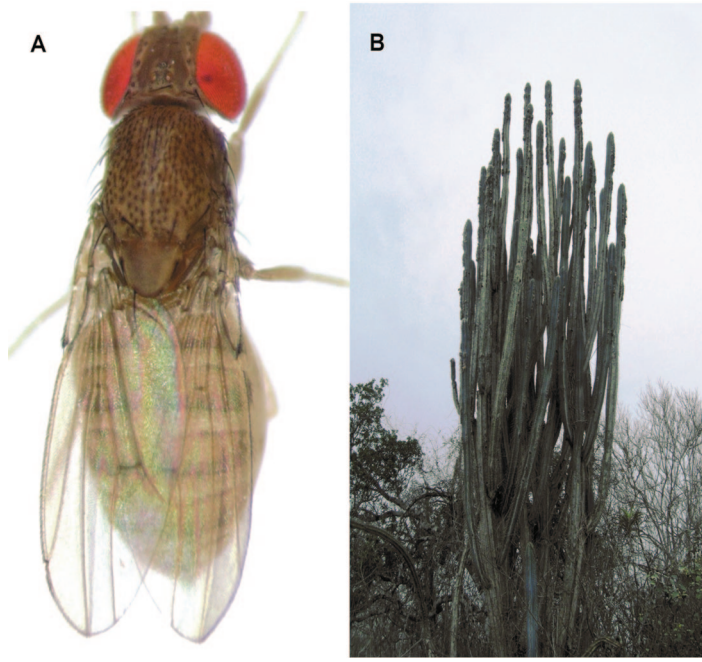


**Fig. 1.** *D. machalilla* sp. nov. and the substrate where it was collected. (A) Female specimen of *D. machalilla* sp. nov. (B) Columnar cactus *A. cartwrightianus.*

[online only]); this information provides not only description, illustration, and data on biological aspects, but also provides the standardized measures and diagnostic characters. This data source contains enough information to create a matrix and perform a cladistic analysis including species with no molecular data available, as those of the *peruensis* group. A cladistic analysis provides us with a solid framework to reconstruct phylogenetic relationships among taxa by looking for shared derived characters (Hennig 1966).

Here, we describe *Drosophila machalilla* Acurio 2013, and place it in the phylogeny of the genus *Drosophila* by performing a cladistic analysis using 52 morphological characters of male and female adults and immature stages with selected representatives of four species groups (*willistoni*, *virilis*, *peruensis*, and *repleta*) of subgenera *Sophophora* and *Drosophila.* The cladograms generated are the basis to propose a new species group (*atalaia*) and formulate a hypothesis of the evolutionary relationships between the spotted-thorax *Drosophila* species groups *repleta*, *peruensis*, and *atalaia.*

## Materials and Methods

**Taxon Sampling. *D. machalilla*** sp. nov. was recorded only at 1 of 12 localities sampled in Ecuador in December 2010. Twenty individuals were collected in San José Beach (01° 13′ 46.4″ S, 80° 49′ 14.6″ W), located on the Central Coast of Ecuador, in Manabí Province. The site of collection is a coastal dry forest with a high density of cacti, particularly the giant columnar cactus ***Armatocereus cartwrightianus*** (Britton & Rose) Backeb. ex A.W. Hill (Fig. 1B). The sampling area is limiting with the northern border of the Machalilla National Park, one of the megadiverse areas of the world (Mast et al. 1997). This park was established in 1979 as World Biosphere Reserve because it harbors high levels of species richness and species endemism.

The method of collection has been described in previous works (Acurio et al. 2010). For terminalia preparation, we followed the method proposed by Bächli et al. (2005) with minor modifications. Once dissected, terminalia were mounted on glass slides using glycerine. The wings were mounted on glass slides using natural Canada balsam to obtain wing indices and measures. Morphological measurements and counts were taken on a Carl Zeiss DiscoveryV8 stereomicroscope equipped with a Zeiss AxioCam MRc (AFX Services, Quito, Ecuador). Genitalia indices were calculated on Zeiss ImagerA2 microscope using Zeiss AxioVision software release 4.8.2. Images of male and female genitalia, pupae, and eggs were processed using Adobe Illustrator CS to produce the figures.

**Analyzed Taxa.** Eight taxa of the *Drosophila* subgenus were selected because: 1) they are representatives of species groups that share morphological characters with ***D. machalilla*** sp. nov.; 2) they are representatives of monophyletic groups; their evolutionary relationships have been inferred by morphological or molecular data; and 3) they have a complete taxonomic description that contains standardized indices and ratios. ***Drosophila willistoni*** Sturtevant 1916 of subgenus ***Sophophora,*** was selected as outgroup. The eight taxa from the ***Drosophila*** subgenus include two represen-

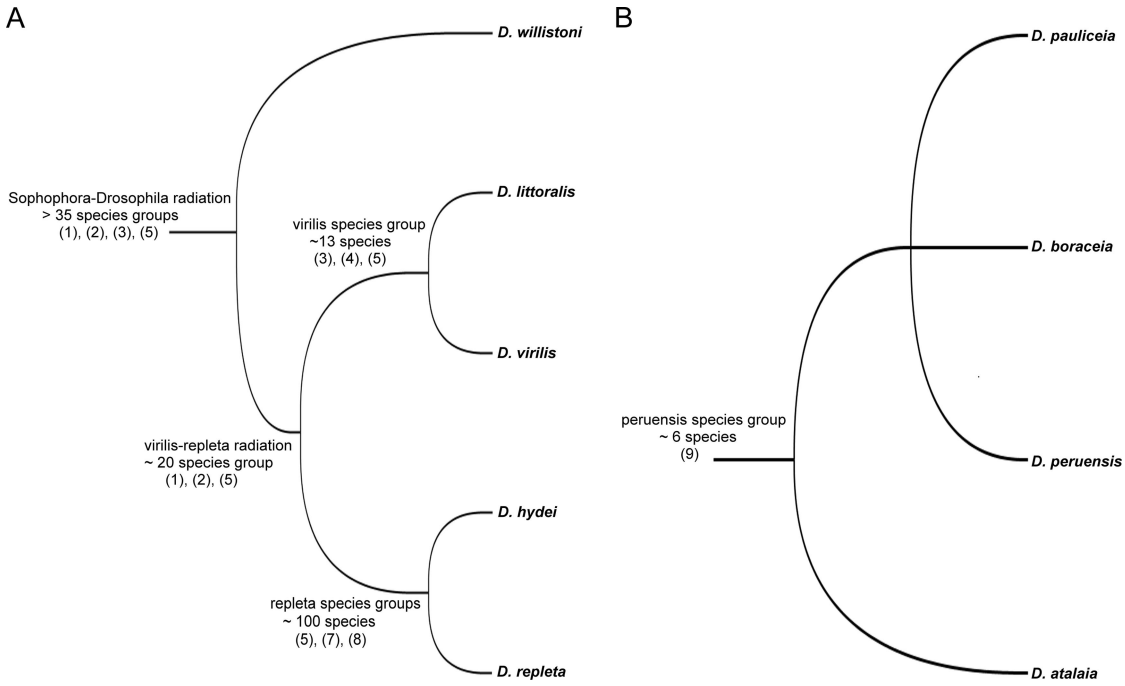


**Fig. 2.** Evolutionary landscape of the species possibly related to *D. machalilla* sp. nov., numbers in parenthesis on each node show phylogenetic studies supporting each evolutionary hypothesis: (A) *Sophophora–Drosophila* radiation hypothesis: (1) (Throckmorton 1975), (2) (Remsen and O'Grady 2002), (3) (Spicer and Bell 2002), (4) (Wang et al. 2006), (5) (Clark et al. 2007), (6) (Wasserman 1992), (7) (Tatarenkov and Ayala 2001), and (8) (Vilela 1983). (B) *peruensis* species group hypothesis: (9) (Ratcov and Vilela 2007).

tatives from the ***virilis group*** (***Drosophila virilis*** Sturtevant 1916, with a worldwide distribution, and ***Drosophila littoralis*** Meigen 1830, with a Holarctic distribution), two representatives from the ***D. repleta*** species group (***D. repleta*** and ***Drosophila hydei*** Sturtevant 1921), and four representatives from the ***peruensis*** species group (***D. peruensis***, ***Drosophila boraceia***, ***Drosophila pauliceia***, and ***Drosophila atalaia*** Vilela & Sene 1982). The selected species span a wide variety of evolutionary distances, from closely related pairs such as ***D. virilis*** and ***D. litoralis*** (8.6 myr) (Morales–Hojas et al. 2011), and ***D. repleta*** and ***D. hydei*** (16.3 myr) (Oliveira et al. 2012) to the distantly related species of the ***Drosophila*** and ***Sophophora*** subgenera (62.9 myr) (Tamura et al. 2004). Figure 2 provides a summary of the known phylogenetic relationships between the nine taxa.

**Selection of Characters.** We made a selection of the most informative characters on *Drosophila* imagines, pupae, and eggs (Throckmorton 1962, 1975; Bock 1976; Vilela and Bächli 1990; Bächli et al. 2005; and the authors' unpublished data). Because we were trying to detect a phylogenetic signal, we were interested only on heritable traits. As previously has been established by Grimaldi (1990) in a morphological systematic approach to Drosophilidae, when we are using morphological characters in a cladistic analysis, we are surveying the expressions of thousands of genes, for instance, quantitative trait locus (QTL) mapping studies (Laurie et al. 1997, Zeng et al. 2000) have identified a minimum of 20 loci underlying the morphological difference between ***Drosophila mauritiana*** Lemeunier and Ashburner 1976 and ***Drosophila simulans*** Sturtevant, 1919, closely related species of the ***Drosophila melanogaster*** species subgroup. Another recent study (Yassin 2013) confirms as well the strong phylogenetic signal that morphological characters provide at different phylogenetic scales.

The following criteria were used to select traits: 1) characters taxonomically informative, they should correlate well with taxonomic grouping; 2) independent characters, the measures should not correlate with specimen size. We used not only discrete characters (traditionally used for phylogenetic analyses) but also continuous characters that contain phylogenetic information and often support or reinforce the results generated by discrete characters (Goloboff et al. 2006). Our dataset contains 52 morphological characters, 27 discrete and 25 continuous (Supp. Table 2 [online only]). Two discrete traits pertain to immature stages, the rest to the imago: head (2), thorax (3), wing (4), leg (1), male genitalia (11), and female genitalia (4). All continuous traits belong to the adult: head (7), thorax (5), and wing (13). An almost complete date set was generated for nine taxa, all except *D. peruensis* (Supp. Table 3 [online only]). Only five of the nine taxa have missing data, usually very few (1, 2, 7, 17, and 1 in *D. littoralis*, *D. virilis*, *D. boraceia*, *D. atalaia*, and *D. hydei*, respectively). However, only 15 characters were recorded from the description of *D.*

*peruensis.* Because specimens of this species have been misidentified frequently (Ratcov and Vilela 2007), the taxon was omitted from analyses.

### Cladistic Analysis

A maximum parsimony cladistic analysis was performed with the program TNT (Goloboff et al. 2008). Continuous characters were analyzed as such to avoid ad hoc methods for discretization. The analysis was carried out using the implied weighting method of Goloboff (1993), with k = 15. Continuous characters were optimized as additive by TNT, and discrete characters were considered as unordered, so an evolutionary change could hypothetically transform freely between any of the described states.

To measure concordance between datasets, two measures of group support—Jackknifing ($P = 0.36$) and Symmetric Resampling ($P = 0.33$)—were calculated under implied weighting, with 500 replications. Measures of raw frequency groups were calculated for both, the strict consensus tree obtained by discrete data set and the optimal tree obtained by the complete data set. Similarity on trees was estimated using subtree pruning and regrafting (SPR) distances implemented in TNT. The most parsimonious tree was obtained by implicit enumeration search using the branch-and-bound algorithm. Polarity on the characters was defined by using *D. willistoni* from *Sophophora* subgenus as outgroup. Character mapping and best tree diagnosis was produced in TNT with the option of common synapomorphies on the optimal tree obtained.

**Taxonomic Description.** We used the traditional morphological terms applied in taxonomic studies of Drosophilidae (Wheeler 1981, Grimaldi 1990, Vilela and Bächli 1990). Abbreviations are as follows: or1 = proclinate orbital seta; or2 = anterior reclined orbital seta; or3 = posterior reclinate orbital seta; vtm = medial vertical seta; vtl = lateral vertical seta; vi = vibrissa; h = postpronotal seta; dc = dorsocentral seta; C = costa; ac = acrostical setae; hb = wing heavy bristles. The indices and measures calculated are based mainly in Bächli et al. (2005).

### *Drosophila machalilla* sp. nov.

**Type Material.** HOLOTYPE: ♂ QCAZ2519. PARATYPE: ♀ QCAZ2534. Remain in the Invertebrate Museum Collection of the Pontificia Universidad Católica del Ecuador (QCAZ). Labeled: "Ecuador: Manabí: San José Beach, 10–XII–2010, (01° 13′46.4″ S, 80° 49′14.6″ W). Acurio A. coll." Both specimens have microvials with terminalia preserved in glycerol. PARATYPES: ♂ QCAZ2520, ♀ QCAZ2535. Same data as holotype. Additional PARATYPES: 2 ♂♂ and 2 ♀♀ have been deposited in the American Museum of Natural History (AMNH).

**Diagnosis.** *D. machalilla* can be differentiated from closely related taxa by having a scutellum light brown, medially darker with brownish spots around scutellar setae, without prescutellar setae. Wing indices 4V = 1.83, 5x = 1.79. Aedeagus apically with one pair of short pointed spurs in the ventral margin, hypandrium with spurious disto-dorsal arms.

*Male.* Head (from live material). Frons yellowish with brownish patches, frontal length 0.43 mm; frontal index = 0.79, top to bottom width ratio 1.44. Frontal triangle narrow, pale brown, as long as frons, ocellar triangle almost completely yellow with dark brown spots around yellow ocellus, ≈45–48% of frontal length. Frontal vittae pale brown. Orbital plates narrow, pale brown with dark brown spots around or1, or2, or3, vtm, and vtl, ≈78–90% length. Orbital setae black, or2 slightly outside of or1, distance of or3 to or1 = 74–80% of or3 to vtm, or1/or3 ratio = 0.8, or2/or1 ratio = 0.5. Postocellar setae 44%, ocellar setae = 70% of frontal length; vibrissal index = 0.55. Face yellowish. Carina yellowish, prominent, nose like, broadened downward, dorsally slightly grooved longitudinally. Gena and postgena light brown. Cheek index ≈6–7. Eyes red bright, eye index 1.2. Occiput dark brown narrowly yellow along eye margins. Pedicel yellowish. Flagellomere one pale brown. Arista with 3–4 dorsal, 2 ventral, and ≈3 small inner branches, plus terminal fork. Proboscis light brown. Clypeus brown, palpus light brown with ≈3 setae and several setulae.

*Thorax.* Length 1.06 mm. Scutum yellowish with a pattern of dark brown spots around bases of most setae and setulae, eight rows of acrostical setulae. H index 1.6. Transverse distance of dorsocentral setae 170–200% of longitudinal distance; dc index = 0.77. No prescutellars. Scutellum light brown medially darker with brown spots around scutellar setae, distance between apical scutellar setae ≈75–80% of that between apical and basal one, basal setae convergent; scut index = 0.83. Pleura predominantly brown with a yellowish central area, subshining, sterno index 0.72, median katepisternal setae ≈36% of anterior one. Haltere brownish-yellow. Legs yellowish brown, preapical setae on all tibiae, apical seta on mesotibia.

*Wings.* Hyaline all veins yellowish with a yellowish shadow in the dorsal part of marginal and submarginal cells, costal section with heavy bristles, R1 + 2 and R3 + 4 slightly darker in older individuals, length 2.16 mm. Length to width radio = 1.92. Indices: C = 2.43, ac = 2.22, hb = 0.38, 4C = 0.98, 4v = 1.64, 5x = 1.72, M = 0.62, prox. x = 0.68.

*Abdomen.* Yellowish, with a narrow brown marginal band, reaching posterior margin of each tergite, subshining.

*Terminalia (Fig. 3).* Epandrium (Fig. 3A) mostly microtrichose, with seven lower setae and no upper setae; ventral lobe roundish at the tip, dorsally broad and ventrally narrow, microtrichose. Cercus anteriorly fused to epandrium, microtrichose and without ventral lobe. Surstylus microtrichose, with a slightly concave row of *ca.* 14 peg-like prensisetae, *ca.* four inner and seven outer setae. Hypandrium (Fig. 3B) slightly shorter than epandrium, anterior margin convex; posterior hypandrial process and hypandrium with spurious disto-dorsal arms; gonopod linked to paraphysis by membranous tissue, with one seta an-

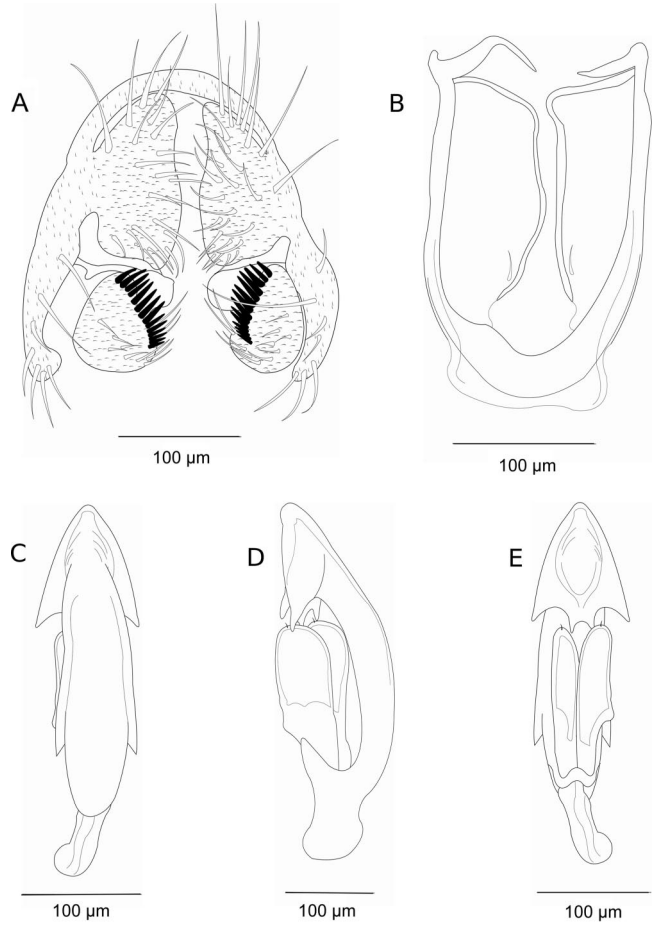


**Fig. 3.** Male terminalia of *D. machalilla* sp. nov.; (A) Epandrium, cerci and surstyli, and decasternum posterior view; (B) Hypandrium; (C–E) aedeagus, aedeagal apodeme, and paraphyses, several views dorsal, ventral, and right lateral, respectively.

teriorly near inner margin. Aedeagus (Fig. 3C–E) apically pointed, ventrally expanded with a pair of subapical pointed spurs and one pair of short pointed spurs in the center of the ventral margin. Aedeagal apodeme shorter than aedeagus anteriorly expanded dorsoventrally, laterally flattened. Ventral rod as long as gonopod, dorsoventrally flattened. Paraphysis linked both to ventrodistal margin of aedeagal apodeme and to gonopod by membranous tissue, medially with one setula near to dorsal margin.

**Female.** *Measurements.* Frontal length 0.44; frontal index = 0.79, top to bottom width radio = 1.43. Ocellar triangle ≈43–44% of frontal length. Orbital plates ≈80–90% of frontal length. Distance of or3 to or1 = 78–80% of or3 to vtm, postocellar setae = 45%, ocellar setae = 64% of frontal lenght; vibrissal index = 0.58. Cheek index ≈6.5. Eye index = 1.27. Thorax length 1.11 mm. H index = 1.4. Transverse distance of dorsocentral setae 180–206% of longitudinal distance; dc index = 0.6. Distance between apical scutelar setae ≈82% of that between apical and basal one; scut index = 0.71, sterno index = 0.69, median katerpisternal setae ≈34% of anterior one. Wing length 2.26 mm, length to width radio = 1.97. Indices: C = 2.37, ac = 2.48, hb = 0.46, 4C = 1.1, 4v = 1.83, 5x = 1.79, M = 0.64, prox. x = 0.77.

*Terminalia (Fig. 4).* Valve of oviscapt (Fig. 4A) brownish, distally rounded, ventrally slightly convex, with *ca.* two distal and *ca.* 11–12 marginal, peg-like outer ovisencilla, first ones roundish and latter ones sharp at tip; trichoid-like outer ovisencilla: three thin, distally positioned and one long curved subterminal.

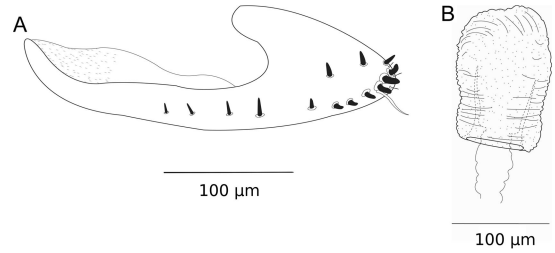


**Fig. 4.** Female terminalia of *D. machalilla* sp. nov.; (A) Left oviscapt valve, lateral view; (B) Spermathecae.

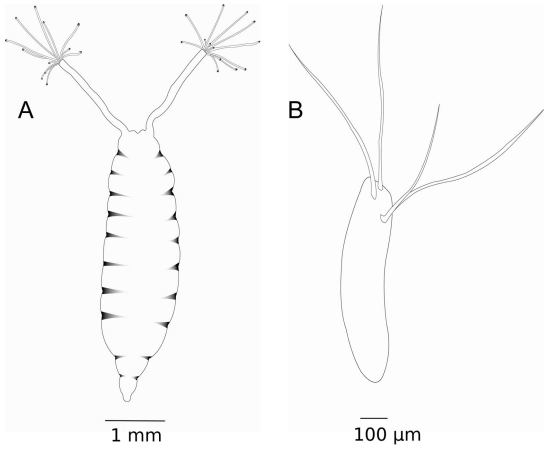


**Fig. 5.** Immature stages of *D. machalilla* sp. nov. (A) Egg; (B) Pupae.

Spermatecae (Fig. 4B) fingertip-shaped slightly invaginated, heavily sclerotized.

*Biology.* Puparia (Fig. 5A) yellowish; horn index ≈1.56; each anterior spiracle with ≈12 branches. Lifespan. At 24°C and 33% humidity: larvae hatches 3 d after the egg is fertilized. First, second, and third instar larvae take ≈ 6 d and pupae ≈ 6.5 d. The imagines reach maturity *ca.* 15.5 d. Eggs with four filaments (Fig. 5B).

*Etymology.* Named to honor the Machalilla culture; one of the most important early societies from Ecuadorian Coast and region where this new species was found. This culture inhabited southern Manabí and Santa Elena Peninsula in a period ranging between: 1400–850 B.C. The Machalilla culture is known by a characteristic pottery style and the practice of skull deformation (Meggers and Evans 1962).

## Results

The implicit enumeration analysis of the 27 discrete characters alone, yielded two most parsimonious trees with six nodes, a total adjusted homoplasy of 0.56 and a length of 51 steps (Fig. 6A and B), the strict consensus cladogram of which is shown in Fig. 6C. The consensus tree has five nodes, a total adjusted homoplasy of 0.61, and 52 steps of length. The phylogenetic signal recovered with the discrete data alone is good enough to recover the evolutionary relationships from taxa of the same species group as the clades *virilis* and *repleta.* The addition of 25 continuous characters to the data matrix and an implicit enumeration search under the same parameters yielded the optimal tree of Fig. 6D; this tree has seven nodes, a length of 129 steps. Autapomorphic features distinguishing *D. machalilla* sp. nov. from other spotted-thorax *Drosophila* species (Table 1) are differences in the sterno index, wing indices 4V and 5X.

The minimum number of SPR moves from strict consensus tree obtained by discrete data set (Fig. 6C) to transforming in the best tree obtained analyzing discrete and continuous data set (Fig. 6D) is 0; no movements are necessary because both trees recover identical relationships. We find no pattern of increase or decrease of group support (Jackknifing or Symmetric Resampling) by addition of continuous characters. However, the additions of continuous characters increase the resolution of the phylogeny, as several synapomorphies belong to the class of continuous traits (Fig. 6C and D).

As is depicted in Fig. 6, *D. machalilla* sp. nov. is a sister taxon of *D. atalaia*, and together conform a separate clade of *peruensis* and *repleta* clades. The *atalaia* clade is recovered using both discrete alone and complete data set; this clade is supported by two synapomorphies (Table 2), character 32, presence of a dark costal lappet on the wing, and character 43, presence of disto-dorsal arms of the hypandrium; this structure and differences between taxa is easily distinguished in a graphical comparison of the male genitalia, the most used morphological structure in *Drosophila* taxonomy (Fig. 7).

One of the synapomorphies found in the *repleta–peruensis–atalaia* clade is the character 27, presence of spots at base of setae on mesonotum. Figure 7 shows this trait, shared by species of the *peruensis* group, *repleta* group, and *D. machalilla* sp. nov., mapped on the optimal tree obtained by implicit enumeration.

## Discussion

The phylogenetic relationships retrieved in our reanalysis of the *peruensis* group mostly corroborated the previous work by Ratcov and Vilela (2007), which was based on a taxonomic analysis. The previous hypothesis and the results obtained in our cladistic analysis of 52 morphological characters are congruent in the respect that *D. pauliceia* is a sister species of *D. boraceia*, and both species conform a monophyletic group separate from *repleta* species group, despite the different taxa analyzed and methods applied on each study. However, our analysis is discordant with Ratcov and Vilela's (2007) in the phylogenetic relationships of *D. atalaia* because, according to our cladistic analysis, this species belongs to a separate clade outside the peruensis group. Ratcov and Vilela (2007) pointed out that *D. atalaia* was the only species from *peruensis* group that: 1) has no prescutellar setae on thorax; 2) has not both main crossveins darker on wing; 3) has a spurious dorsal arch on hypandrium; and 4) has a different disposition of sensilla in the oviscapt. However, they classified *D. atalaia* in the *peruensis* group based on morphological similarities on male and female terminalia, because at the time, those were the closely related species known. Also noteworthy is the difference in habitat and geographical distribution between the other three species that belong to the *peruensis* group and *D. atalaia* as reported by Ratcov and Vilela (2007 p. 310): "The triad of forest-dwelling species, namely *D. boraceia, D. pauliceia*, sp. nov, and *D. peruensis*, are more closely related to each other than they are to the xerophilous and probably cactophilic *D. atalaia.*" It is interesting that both species *D.*

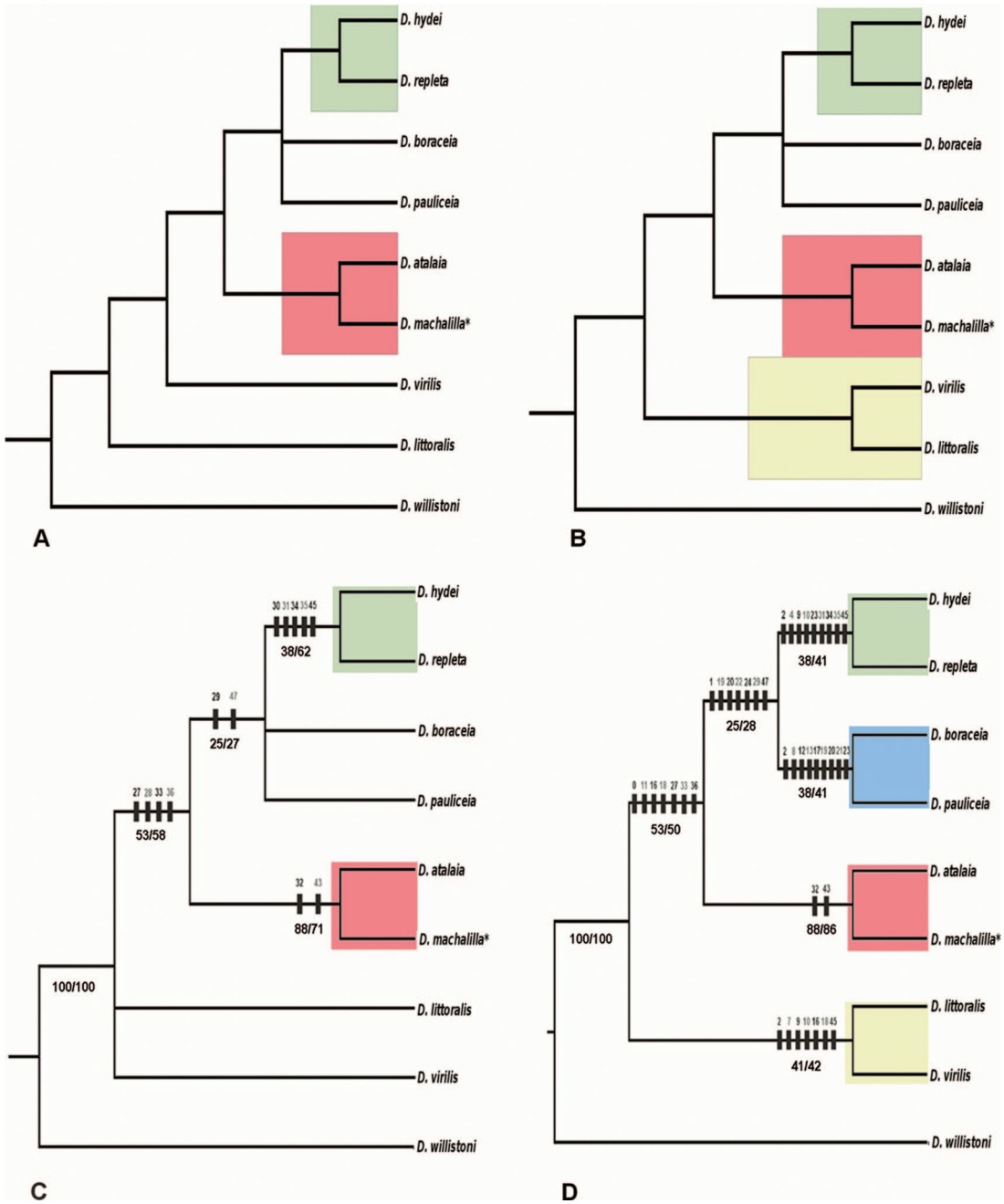


**Fig. 6.** Results of the cladistic analysis of 52 morphological traits in nine *Drosophila* species. (A and B) Two equally parsimonious trees found by implicit enumeration of the discrete data set (27 discrete morphological traits). (C) Strict consensus tree of two most parsimonious trees A and B found in the analysis of 27 discrete morphological traits. (D) Optimal tree obtained by implicit enumeration of the complete data set (27 discrete + 25 continuous morphological traits). In (C) and (D) synapomorphies (black rectangles) are mapped on trees; the numbers above rectangles refer to character numbers (Supp. Table 2 [online only]); the numbers beneath branchings indicate group support Jacknifing ($P = 36$) and Symmetric Resampling ($P = 33$). Colors denote *Drosophila* clades: *repleta* clade (green), *peruensis* clade (blue), *atalaia* clade (red), and *virilis* clade (yellow). Asterisks denote new species here described.

*atalaia* and *D. machalilla* sp. nov. occur on coastal dry forest with predominance of Cactaceae. Although we only can speculate about the area of distribution of both species because more collections are necessary, we know that the type locality of *D. atalaia* was Arraial do Cabo located at Brazilian coast of South Atlantic

**Table 1. Autapomorphies of each taxa obtained in the cladistic analysis of discrete and continuous characters**

| *littoralis* | *virilis* | *boraceia* | *pauliceia* | *atalaia* | *hydei* | *repleta* | *machalilla* |
|---|---|---|---|---|---|---|---|
| **1:** 0.84→0.78 | **5:** 0.85→0.92 | **0:** 1.34→1.5 | **2:** [7.40–10]→5 | **14:** 2.16→1.8 | **0:** 1.3→1.23 | **1:** [0.86–0.92]→0.99 | **10:** 0.70→0.69 |
| **2:** 4.0→3.5 | **6:** 0.54→0.58 | **1:** 0.92→1.0 | **3:** 0.6→0.75 | **15:** 2.26→1.9 | **2:** 6.0→4.5 | **3:** 0.56→0.52 | **21:** 1.69→1.89 |
| **3:** [0.56–0.85]→0.55 | **10:** 0.83→0.87 | **4:** 1.19→[1.2–1.4] | **5:** 0.85→1.0 | **19:** 2.43→2.2 | **6:** 0.54→0.63 | **4:** 1.17→1.16 | **24:** 1.6→1.72 |
| **4:** 1.20→1.14 | **23:** [0.61–0.68]→0.72 | **5:** 0.85→0.80 | **8:** 1.44→1.55 | **20:** 0.98→1.2 | **9:** 1.32→1.41 | **5:** 0.85→0.93 | **48:** 3→2 |
| **5:** 0.85→0.71 | **24:** 1.30→1.0 | **6:** [0.53–0.54]→0.70 | **14:** 2.6→3.0 | **21:** [1.69–1.89]→2.0 | **10:** 0.81→0.82 | **7:** 0.8→0.72 | |
| **7:** 0.96→1.08 | | **7:** [0.80–0.83]→0.90 | **16:** 2.1→2.13 | **22:** 0.62→0.70 | **11:** [33.0–34.0]→29.5 | **11:** [33.0–34.0]→37.5 | |
| **9:** 1.22→1.29 | | **11:** 33.00→27.00 | **19:** 3.4→3.79 | **47:** 3→2 | **18:** [0.40–0.41]→0.48 | **14:** 2.6→2.81 | |
| **12:** 0.67→0.68 | | **12:** 0.63→0.60 | **20:** 0.80→0.65 | | **22:** [0.50–0.51]→0.46 | **17:** [2.04–2.22]→2.24 | |
| **13:** 1.15→1.16 | | **13:** 0.88→0.80 | **21:** 1.6→1.47 | | **23:** 0.79→0.80 | **36:** 1→0 | |
| **15:** 2.64→2.83 | | **16:** 2.1→2.0 | **22:** 0.50→0.43 | | **24:** [1.18–1.27]→1.11 | **41:** 2→4 | |
| **16:** 2.22→2.25 | | **17:** 1.94→1.90 | **32:** 0→1 | | **33:** 1→0 | | |
| **17:** 2.13→2.08 | | **23:** 0.60→0.40 | | | | | |
| **18:** 0.59→0.61 | | **24:** 1.18→1.10 | | | | | |
| **19:** 2.88→2.96 | | **41:** 2→9 | | | | | |
| **20:** 0.94→0.77 | | **42:** 4→5 | | | | | |
| **21:** [1.69–1.89]→1.57 | | **44:** 1→0 | | | | | |
| **22:** 0.56→0.46 | | | | | | | |
| **23:** [0.61–0.68]→0.51 | | | | | | | |
| **38:** 2→15 | | | | | | | |

Numbers in bold denote the characters listed in (Supp. Table 2 [online only]), in brackets ranges of character variation.

Ocean and type locality of *D. machalilla* sp. nov. is San Jose beach located at Ecuadorian Pacific Coast. An analysis of male and female terminalia of both species also confirmed the evolutionary relationship recovered in our cladistic analysis (see above). Besides, the results found here are congruent with a molecular phylogenetic analysis of *D. machalilla* and representatives of six subgroups (*mulleri*, *fasciola*, *hydei*, *mercatorum*, *repleta*, and *inca*) of the *repleta* group and *nannoptera* group using sequences of five molecular markers: three mitochondrial and two nuclear genes (Acurio, Oliveira, Rafael, and Ruiz, unpublished data).

## Classification

***Drosophila peruensis* Species Group.** As lineage of the subgenus *Drosophila* Patterson and Mainland, 1944 (or *Siphlodora* in Yassin 2013, classification scheme proposed). In the absence of a male specimen of *D. peruensis*, the phylogenetic position of this group is based on the female specimen.

*Diagnosis.* sensu lato Ratcov and Vilela (2007) Small flies, with most setae and setulae of the thorax and head arising from dark brown spots, which may be somewhat fused; wings with both main crossveins darker, hypandrium somewhat square-shaped, mostly fused to gonopods and devoid of dorsal arch.

*Discussion.* Previously, both the *peruensis* and *repleta* species groups were included in the *Drosophila* subgenus (Ratcov and Vilela 2007, O'Grady and Markow 2009). In the classification scheme proposed recently by Yassin (2013 p. 11), the *peruensis* group was placed in the reorganized *Drosophila* subgenus along with *Phloridosa*, *Chusqueophila*, and *Palmophila*, whereas the *repleta* group was transferred to the new Subgenus *Siphlodora.* However, this seems to be incorrect because there are no available molecular sequences for *peruensis* group and male genitalia of this group should place it in the subgenus *Siphlodora* (A. Yassin, personal communication). In addition, the bibliographic reference cited in the study of Yassin (2013) to classify the *peruensis* group is Vilela and Pereira (1985), which has been reported as a misidentification (Ratcov and Vilela 2007 p. 310). Our cladistic analysis corroborates that the *peruensis* species group is closely related to the *repleta* species group and therefore both should belong to the same subgenus.
*Taxon content.* Five extant species—*D. peruensis*, *D. boraceia*, *D. pauliceia*, *D. itacorubi*, and *D. paraitacorubi.*

***Drosophila atalaia* new Species Group.** As lineage of the subgenus ***Drosophila*** Patterson and Mainland (or ***Siphlodora*** in Yassin 2013 scheme classification). Inside the ***virilis–repleta*** radiation, one of the three major radiations inside the subgenus ***Drosophila*** according to Throckmorton hypothesis (O'Grady and Markow, 2009).

*Taxon content.* Two extant species: ***D. atalaia*** and ***D. machalilla*** sp. nov.

*Diagnosis.* Small yellowish flies with dark brown spots on mesonotum, hypandrium with disto-dorsal

**Table 2. Common synapomorphies found in each node of the most parsimonious tree obtained in the cladistic analysis of 27 discrete and 25 continuous characters**

| *virilis* node | *peruensis* node | *repleta* node | *atalaia* node |
|---|---|---|---|
| **2:** 6.50→4.00 | **2:** 6.50→7.40 | **2:** 6.50→6.00 | **32:** 0→1 |
| **7:** 0.80→0.83 | **8:** [1.27–1.37]→1.44 | **4:** 1.1–1.2→1.17 | **43:** 0→1 |
| **9:** 1.11→1.22 | **12:** [0.66–0.67]→0.63 | **9:** 1.11→1.32 | |
| **10:** 0.70→0.80 | **13:** 1.06→0.88 | **10:** [0.79–0.80]→0.81 | |
| **16:** 2.18→2.22 | **17:** [2.04–2.22]→1.94 | **23:** [0.61–0.68]→0.79 | |
| **18:** 0.53→0.59 | **19:** [3.12–3.25]→3.40 | **31:** 1→0 | |
| **45:** 1→2 | **20:** [0.81–0.82]→0.8 | **34:** 0→1 | |
| | **21:** [1.69–1.72]→1.60 | **35:** 1→0 | |
| | **23:** [0.61–0.68]→0.60 | **45:** 1→2 | |
| *peruensis–repleta* clade | | *atalaia–peruensis–repleta* clade | |
| **15:** [2.26–2.64]→[2.84–2.93] | | **0:** [1.20–1.27] 0→1 [1.30–1.34] | |
| **19:** [2.43–2.88]→[3.12–3.25] | | **11:** 35.5 0→140.00 | |
| **20:** [0.94–0.98]→[0.81–0.82] | | **16:** 2.18 0→12.10 | |
| **22:** [0.56–0.62]→[0.50–0.51] | | **18:** [0.53–0.40]→0.41 | |
| **24:** [1.30–1.60]→[1.18–1.27] | | **27:** 0→1 | |
| **29:** 0→1 | | **33:** 0→1 | |
| **47:** 3→4 | | **36:** 0→1 | |

Numbers in bold denote the characters listed in (Supp. Table 2 [online only]), in brackets ranges of character variation.

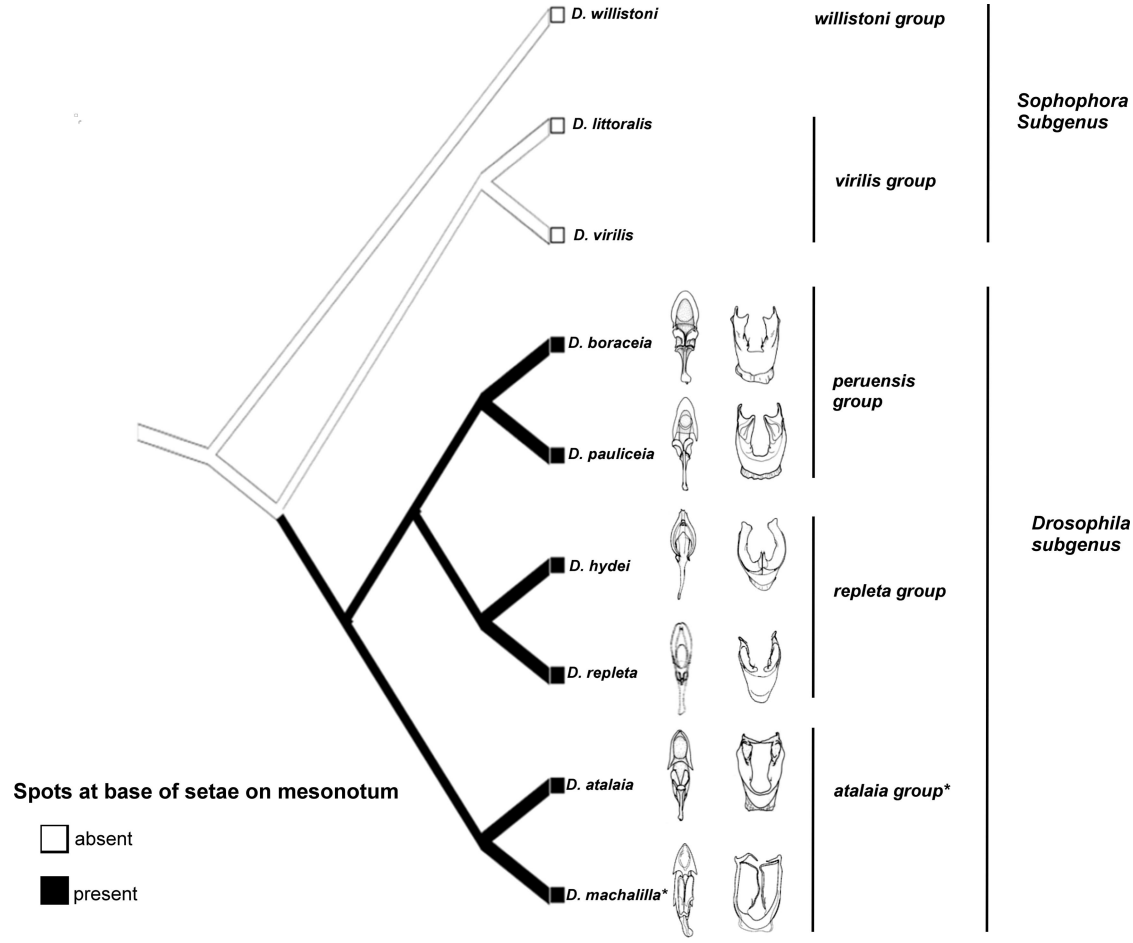


**Fig. 7.** Phylogenetic tree of *Drosophila* relationships based in the cladistic analysis of 52 morphological traits with spotted-thorax character mapped onto it. Draws show the aedeagus and hypandrium structures of male genitalia (taken and modified from [Vilela and Sene 1982, Vilela and Bächli 1990, Vilela and Val 2004, Bächli et al. 2005, Ratcov and Vilela 2007]). Asterisks denote new species and group species here proposed.

arms, females with a lower most-distal ovisensilla on oviscapt, and habitat preference for coastal dry forest with predominance of Cactaceae.

*Discussion. D. atalaia*, previously belonging to the *peruensis* species group (Ratcov and Vilela 2007), and *D. machalilla* sp. nov. are now grouped in the new species group *atalaia* on the basis of male and female genitalia, monophyly on a cladistic analysis, preference of substrate, and habitat ecology.

### The Spotted-Thorax Character

Neotropical species of *Drosophila* with each hair and bristle arising from black or dark brown spot on mesonotum and a substrate preference for Cactaceae plants were, until few years ago, identified as belonging to the *repleta* species group. Species in this group have been studied in morphological and cytological detail (Wasserman 1982, Vilela 1983) and have served as a model system for evolutionary (Ewing and Miyan 1986; Wasserman 1992; Ruiz et al. 1997; Oliveira et al., 2008, 2012) and ecological studies (Markow 1981, Ruiz and Heed 1988, Krebs 1991, Etges 1993). In the light of our results, we recommend caution in the use of this morphological trait for identification at lower taxonomical levels such as species groups.

Currently it is unclear whether the *virilis–repleta* radiation can be defined as monophyletic (Grimaldi 1990, Tatarenkov and Ayala 2001, Remsen and O'Grady 2002, O'Grady and Markow 2009, Yassin 2013) in this context; high quality systematic research including both alpha-taxonomy and phylogenetically supported hypotheses becomes critical to better resolve the evolutionary relationships of a prime model system as *Drosophila.*

### References Cited

**Acurio, A., and V. Rafael. 2009a.** Diversity and geographical distribution of *Drosophila* (Diptera, Drosophilidae) in Ecuador. Dros. Inf. Serv. 92: 20–25.

**Acurio, A., and V. Rafael. 2009b.** Taxonomic survey of Drosophilidae (Diptera) in the Yasuni National Park, Ecuadorian amazon. Acta Amazonica 39: 713–718.

**Acurio, A., V. Rafael, and O. Dangles. 2010.** Biological invasions in the amazonian tropical rain forest: the case of Drosophilidae (Insecta, Diptera) in Ecuador, South America. Biotropica 42: 717–723.

**Agnarsson, I., and M. Kuntner. 2007.** Taxonomy in a changing world: seeking solutions for a science in crisis. Syst. Biol. 56: 531–539.

**Bächli, G., C. R. Vilela, S. S. Escher, and A. Saura. 2005.** The Drosophilidae (Diptera) of Fennoscandia and Denmark. Brill Academic Publishers, Leiden, The Netherlands.

**Bock, I. R. 1976.** Drosophilidae of Australia. I. *Drosophila* (Insecta: Diptera). Aust. J. Zool. Suppl. Ser. 24: 1–105.

**Brake, I., and G. Bächli. 2008.** Drosophilidae (Diptera) world catalogue of insects. Apollo Books, Stenstrup, Denmark.

**Céspedes, D., and V. Rafael. 2012.** Cuatro especies nuevas del grupo de especies *Drosophila mesophragmatica* (Diptera, Drosophilidae) de los andes ecuatorianos. Iheringia Sér. Zool. 102: 71–79.

**Clark, A. G., M. B. Eisen, N. Smith, R. B. Douglas, M.O.B. Casey, T. A. Markow, and T. C. Kaufman. 2007.** Evolution of genes and genomes on the *Drosophila* phylogeny. Nature 450: 203–218.

**Döge, J. S., M. S. Gottschalk, and V.L.S. Valente. 2011.** Two new neotropical species of *Drosophila peruensis* species group (Diptera, Drosophilidae). Iheringia Sér. Zool. 101: 310–316.

**Etges, W. 1993.** Genetics of host-cactus response and life-history evolution among ancestral and derived populations of cactophilic *Drosophila-mojavensis.* Evolution 47: 750–767.

**Ewing, A. W., and J. A. Miyan. 1986.** Sexual selection, sexual isolation and the evolution of song in the *Drosophila-repleta* group of species. Anim. Behav. 34: 421–429.

**Felsenstein, J. 2004.** Inferring phylogenies. Sinauer Associates, Sunderland, MA.

**Figuero, M. L., V. Rafael, and D. Céspedes. 2012.** Grupo *Drosophila asiri* (Diptera, Drosophilidae), un nuevo grupo de especies andinas con la descripción de dos nuevas especies y la redescripción de *Drosophila asiri.* Iheringia Sér. Zool. 102: 33–42.

**Goloboff, P. A. 1993.** Estimating character weights during tree search. Cladistics 9: 83–91.

**Goloboff, P. A., C. I. Mattoni, and A. S. Quinteros. 2006.** Continuous characters analyzed as such. Cladistics 22: 589–601.

**Goloboff, P. A., J. S. Farris, and K. C. Nixon. 2008.** TNT, a free program for phylogenetic analysis. Cladistics 24: 774–786.

**Grimaldi, D. A. 1990.** A phylogenetic, revised classification of genera in the Drosophilidae (Diptera). Bull. Am. Mus. Nat. Hist. 197: 1–39.

**Hennig, W. 1966.** Phylogenetic systematics. University Illinois Press. Urbana-Champaign, IL.

**Krebs, R. 1991.** The mating behavior of *Drosophila mojavensis* on organ pipe and agria cactus. Psyche 98: 101–109.

**Laurie, C. C., J. R. True, J. Liu, and J. M. Mercer. 1997.** An introgression analysis of quantitative trait loci that contribute to a morphological difference between *Drosophila simulans* and *D. mauritiana.* Genetics 145: 339–348.

**Lipscomb, D. L. 1998.** Basics of cladistic analysis. George Washington University Washington, DC.

**Markow, T. 1981.** Courtship behavior and control of reproductive isolation between *Drosophila mojavensis* and *Drosophila arizonensis.* Evolution 35: 1022–1026.

**Mast, R. B., C. G. Mittermeier, R. A. Mittermeier, J. V. Rodríguez-Mahecha, and A. H. Hemphill. 1997.** Ecuador, pp. 314–324. *In* R. A. Mittermeier, P. Robles Gil, and C. G. Mittermeier (eds.), Megadiversity: Earth's Biologically Wealthiest Nations. Cemex, Monterrey, Mexico.

**Meggers, B. J., and C. Evans. 1962.** The machalilla culture: an early formative complex on the ecuadorian coast. Am. Antiq. 28: 186–192.

**Mora, C., D. P. Tittensor, and R. A. Myers. 2008.** The completeness of taxonomic inventories for describing the global diversity and distribution of marine fishes. Proc. R. Soc. B 275. 149–155.

**Morales–Hojas, R., M. Reis, C. P. Vieira, and J. Vieira. 2011.** Resolving the phylogenetic relationships and evolutionary history of the *Drosophila virilis* group using multilocus data. Mol. Phy. Evol. 60: 249–258.

**Moritz, C., and D. M. Hillis. 1996.** Context and controversies, pp. 1–10. *In* D. W. Hillis and C. Moritz (eds.), Molecular Systematics, Sinauer, Sunderland, MA.

**O'Grady, P., and T. Markow. 2009.** Phylogenetic taxonomy in *Drosophila.* Fly (Austin) 3: 10–14.

**Oliveira, D.C.S.G., M. Leonidas, W. J. Etges, P. M. O'Grady, and R. De Salle. 2008.** Species delimitation in the *Drosophila aldrichi* subcluster (Diptera: Drosophilidae) using DNA sequences. Zootaxa 1725: 37–47.

**Oliveira, D.C.S.G., F. C. Almeida, P. M. O'Grady, M. A. Armella, R. De Salle, and W. J. Etges. 2012.** Monophyly, divergence times, and evolution of host plant use inferred from a revised phylogeny of the *Drosophila* repleta species group. Mol. Phy. Evol. 64: 533–544.

**Rafael, V., and G. Arcos. 1989.** Subgrupo *inca,* un nuevo subgrupo del grupo *repleta,* con desacripción de *Drosophila huancavilcae* n. sp (Diptera, Drosophilidae). Evol. Biol. 3: 233–243.

**Ratcov, V., and C. R. Vilela. 2007.** A new neotropical species of spot-thoraxed *Drosophila* (Diptera, Drosophilidae). Rev. Bras. Entomol. 51: 305–311.

**Remsen, J. and P. O'Grady. 2002.** Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support. Mol. Phy. Evol. 24: 248–263.

**Ruiz, A., J. Ranz, M. Caceres, C. Segarra, and A. Navarro. 1997.** Chromosomal evolution and comparative gene mapping in the *Drosophila repleta* species group. Rev. Bras. Gen. 20: 553–565.

**Ruiz, A., and W. B. Heed. 1988.** Host-plant specificity in the cactophilic *Drosophila mulleri* species complex. J. Anim. Ecol. 57: 237–249.

**Spicer, G. S., and C. D. Bell. 2002.** Molecular phylogeny of the *Drosophila virilis* species group (Diptera: Drosophilidae) inferred from mitochondrial 12S and 16S ribosomal RNA genes. Syst. Biol. 95: 156–161.

**Tamura, K., S. Subramanian, and S. Kumar. 2004.** Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. Mol. Biol. Evol. 21: 36–44.

**Tatarenkov, A., and F. J. Ayala. 2001.** Phylogenetic relationships among species groups of the *virilis–repleta* radiation of *Drosophila.* Mol. Phyl. Evol. 21: 327–331.

**Throckmorton, L. H. 1962.** The problem of phylogeny in the genus *Drosophila.* Univ. Tex. Publ. 6205: 207–343.

**Throckmorton, L. H. 1975.** The phylogeny, ecology and geography of *Drosophila,* pp. 421–469. *In* R. C. King (ed.), Handbook of Genetics, Plenum, New York, NY.

**Vela, D., and V. Rafael. 2004.** Three new andean species of *Drosophila* (Diptera, Drosophilidae) of the *mesophragmatica* group. Iheringia Sér. Zool. 94: 295–299.

**Vilela, C. R. 1983.** A revision of the *Drosophila repleta* species group (Diptera, Drosophilidae). Rev. Bras. Entomol. 27: 1–114.

**Vilela, C. R. and G. Bächli. 1990.** Taxonomic studies on neotropical species of seven genera of Drosophilidae (Diptera). Mitt. Schweiz. Entomol. Ges. 63: 1–332.

**Vilela, C. R., and F. M. Sene. 1982.** A new spotted thorax species of the genus *Drosophila* (Diptera, Drosophilidae). Rev. Bras. Entomol. 26: 343–347.

**Vilela, C. R., and F. C. Val. 2004.** A new spot-thoraxed species of *Drosophila* from the Atlantic forest of southeastern Brazil (Diptera, Drosophilidae). Rev. Bras. Entomol. 48: 45–48.

**Wang, B., J. Park, H. Watabe, J. Gao, J. Xiangyu, T. Aotsuka, H. Chen, and Y. Zhang. 2006.** Molecular phylogeny of the *Drosophila virilis* section (Diptera: Drosophilidae) based on mitochondrial and nuclear sequences. Mol. Phyl. Evol. 40: 484–500.

**Wasserman, M. 1982.** The repleta species group, pp. 61–140. *In* M. Ashburner, J. N. Thompson, and H. L. Carson (eds.), The Genetics and Biology of Drosophila. Academic Press, New York, NY.

**Wasserman, M. 1992.** Cytological evolution of the *Drosophila repleta* species group, pp. 455–541. *In* J. R. Powell and C. B. Krimbas (eds.), Inversion Polymorphism in Drosophila. CRC, Inc., Boca Raton, FL.

**Wheeler, M. R. 1981.** The Drosophilidae: a taxonomic overview, pp. 1–97. *In* M. Ashburner, H. L. Carson, and J. N. Thompson (eds.), The Genetics and Biology of *Drosophila.* Academic Press, London, United Kingdom.

**Wheeler, Q. 1995.** Systematics, the scientific basis for inventories of biodiversity. Biodivers. Conserv. 4. 476–489.

**Wheeler, Q. 2004.** Taxonomic triage and the poverty of phylogeny. Phil. Trans. R. Soc. Lond. B 359: 571–583.

**Wheeler, Q. 2010.** What would NASA do? Mission-critical infrastructure for species exploration. Syst. Biodiovers. 8: 11–15.

**Wilson, E. O. 2004.** Taxonomy as a fundamental discipline. Phil. Trans. R. Soc. Lond. B 359: 739–739.

**Yassin, A. 2013.** Phylogenetic classification of the Drosophilidae rondani (Diptera): the role of morphology in the postgenomic era. Syst. Entomol. 32: 349–364.

**Zeng, Z., J. Liu, L. F. Stam, C. Kao, J. M. Mercer, and C. C. Laurie. 2000.** Genetic architecture of a morphological shape difference between two *Drosophila* species. Genetics 154: 299–310.

*Received 8 March 2013; accepted 8 July 2013.*