

Two New Species of *Drosophila* (Diptera: Drosophilidae) in the *repleta* Group from Mexico

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ABSTRACT Two new species, *Drosophila huckinsi* Etges & Heed and *D. huichole* Etges & Heed from Mexico are described and compared with close relatives in the *D. longicornis* cluster of the large *D. repleta* group in the subgenus *Drosophila*.

KEY WORDS *Drosophila*, Mexico, cactus, systematics

THE *Drosophila repleta* group comprises 91 species endemic to the New World. Approximately half the members of this group are cactophilic, carrying out their life cycles in and around the fermenting tissues and fleshy fruits of the many species of cacti in North and South America (Heed 1982, Wasserman 1992). Although *repleta* group species have long been the subject of numerous evolutionary, systematic, chromosomal, ecological, and behavioral studies (Patterson and Stone 1952, Throckmorton 1975, Heed and Mangan 1986, Ruiz et al. 1990), new species are still being uncovered and scrutiny of the systematic relationships within this group are being revised (Durando et al. 2000).

Within the monophyletic *mulleri* complex is the *longicornis* species cluster currently comprising six species including *D. longicornis* Patterson & Wheeler, *D. pachuca* Wasserman, *D. propachuca* Wasserman, *D. mainlandi* Patterson, and the more distantly related *D. spenceri* Patterson, and *D. hexastigma* Patterson & Mainland (Wasserman 1992). These species share a common hypothetical ancestor most closely related to ancestors of the *mulleri* and *mojavensis* species clusters (Wasserman 1992). Male genital morphology, principally the shape of the aedeagus, of *D. longicornis*, *D. pachuca*, and *D. propachuca* is indistinguishable, prompting Vilela (1983) to suggest they were conspecific even though Wasserman and Koepfer (1977) had shown interspecific differences in sharing of gene arrangements and strong behavioral isolation between species. Identification of these three species is only possible by field collection of isofemale lines and crossing individuals from these lines to known laboratory stocks. There are two other undescribed mem-

bers of this group, *D. sp.* "S" (from Sonora), homokaryotypic for gene arrangement 2k⁶ (Wasserman 1992), clearly distinguishable based on the shape of its aedeagus, which is most similar to that of *D. spenceri* (W.B.H., unpublished data) and *D. "mull. comp. b."*, an undescribed form known from Puebla and Michoacan, Mexico, that is fixed for gene arrangement 2o⁵ (Wasserman 1992).

We report here two new species from Guanajuato, Nayarit, and San Luis Potosi, Mexico most closely related to the members of the *longicornis* cluster.

Materials and Methods

Wild flies were collected over banana baits in January 2000 at two sites near San Francisco del Rincon, Guanajuato, a single site west of Ixtlan del Rio, Nayarit, and in July 2000 at a site located 54 km northwest of San Luis Potosi, Mexico. Male genitalia were dissected and prepared using a modification of Grimaldi's (1987) microscope slide technique with Berlese's medium (Womersly 1939) substituted for glycerine. Female ovipositors were prepared from specimens taken from laboratory cultures stored in 70% ETOH. These specimens were completely dehydrated via sequential washes with 80, 90, 95, and 100% ETOH and then critical point dried using standard protocols (Grimaldi 1987). The ovipositors of 5–10 females of both new species were dissected from the abdomen and adhered to a specimen mount (Ted Pella, Redding, CA) using double coated, carbon conductive tabs (Ted Pella). The material was sputter coated and visualized using a Hitachi S4700 Field Emission Scanning Electron Microscope (Nissei Sangyo America, Rolling Meadows, IL). All image files were saved in TIF file format. Male genitalia were digitized under a light microscope fitted with a video camera and the resulting images were adjusted for contrast and print size using Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA). Aedeagus sizes were measured with an ocular micrometer. All image files are available upon request.

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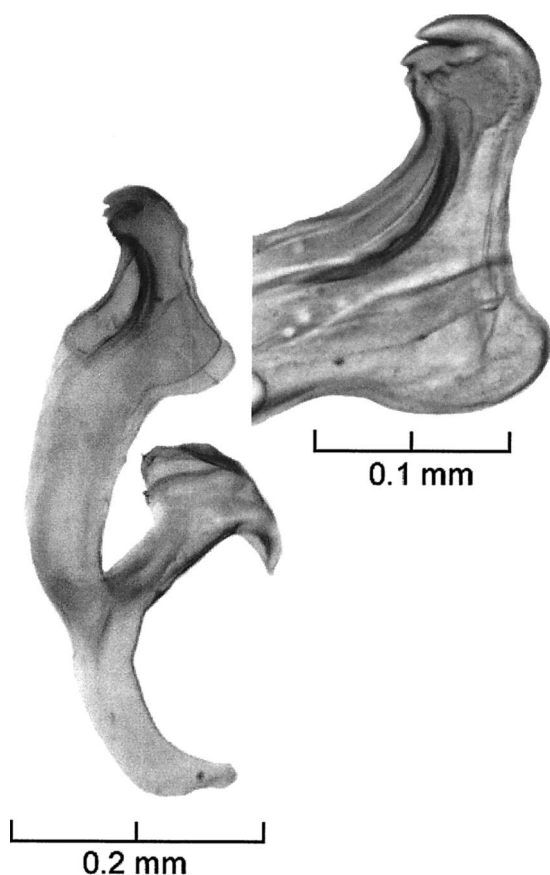


Fig. 1. Aedeagus of *D. huckinsi* in lateral view. Inset shows the enlargement of the tip of the aedeagus with the large central tooth and the lateral margins.

***Drosophila huckinsi* Etges & Heed, sp. nov.**
(Fig. 1)

Head (from live material). *Male, female.* Aristae with four dorsal and two ventral branches in addition to the terminal fork. Third antennal joint blackish at the base. Gena pale, $\approx 1/4$ diameter of eye at widest point. Ground color of the frons is pollinose tan. Anterior proclinate orbital bristle is over half the length of the posterior reclinate and midorbital is over half the length of the anterior proclinate. The front of the head is tan with a dark brown spot on the anterior part of the orbits, at the base of the posterior reclinate, and at the base of the posterior verticals. The frons is brown and V-shaped, with a light spot in front of and on both sides of the ocellus. Carina sulcate broader below with medium brown stripe. Eye color is dark garnet. Pile is light in the lower portion of the eye. One strong oral bristle is present.

Thorax. Ground color is a mixture of light brown in some areas and light gray in others. There are six rows of acrostichial hairs inside the row of dorsocentrals, each hair arising from a darker brown spot. Many of the spots are fused into an irregular pattern. There are three faint stripes, a central and two lateral ones. The

narrow central stripe is grayish and the lateral stripes are tan in color located just outside the anterior and posterior dorsocentral bristles. There are no prescutellar bristles. Anterior scutellar bristles are convergent. Sterno index ≈ 0.8 . Mid-sternopleural bristles are very short. Ground color of the sternopleurae is blackish brown, lighter in males than females. Legs are light tan with dark banding at the base of each tibia. Fore femur with four long bristles on the posterior margin and three shorter bristles on the anterior margin besides the apical bristle.

Abdomen. *Female.* Lightly colored with contrasting heavy posterior dark brown banding interrupted dorsally on each tergite. Lateral margin of each tergite almost solid brown with indistinct central lighter areas more noticeable on first and second visible segments. Other females have all tergites with indistinct "windows" laterally. *Male.* Narrower abdominal banding is usually interrupted on all tergites laterally leaving a dark segment along the margin of each, very similar in appearance to *D. longicornis*. This pattern is less distinct than in other males.

Wings. Wings clear. Costal index ≈ 3.2 , fourth vein index 1.5, $5\times$ index ≈ 1.0 , and $4c$ index is ≈ 0.8 . Third costal section has heavy bristles on its basal one quarter.

External Genitalia. Surstylus with 10–11 teeth and seven to nine marginal bristles, the two innermost being the longest. The inner margin of the ventral lobe of the epandrium is slightly expanded. Tip of the anal plate with ≈ 12 semiclustered bristles. Tip of the aedeagus with one main and two smaller dorsally curved teeth (Fig. 1). The margins of the dorsal cleft near the tip is heavily chitinized. Each gonopod is strongly expanded with one small bristle. The phallosomal index is 2.4.

Internal Characteristics of Imagines. Testes with four inner and three outer gyres. Testes color is light yellow changing to darker yellow–orange with age. Ventral receptacles elongated with ≈ 25 tight coils. Spermathecae are similar to those described for *D. longicornis* (Patterson 1943): they are small and spherical with a chitinized inner structure surrounded by an outer membrane. The ovipositor is pointed with a number of short bristles around the ventral margin (Fig. 2A). Several lateral bristles point posteriorly.

Immature Stages. Eggs with four very long filaments about twice the length of the egg. Puparia amber-colored with about six long and six much shorter terminal branches per horn. Length of horns plus spiracles is \approx two-thirds length of the puparium, similar to *D. longicornis* which Patterson (1943) noted were the longest pupal horns in any species of *Drosophila*.

Chromosomes. Polytene chromosomes show no inversion polymorphism and are homosequential with those of *D. longicornis*, *D. propachuca*, and *D. mainlandi*.

Measurements. Female body length ≈ 3.5 mm and 3.0 for males. Female wing length ≈ 3.3 mm and 3.0 for males.

Type Material. HOLOTYPE ♂, 5 PARATYPE ♂, and 5 ALLATYPE ♀ from laboratory cultures from

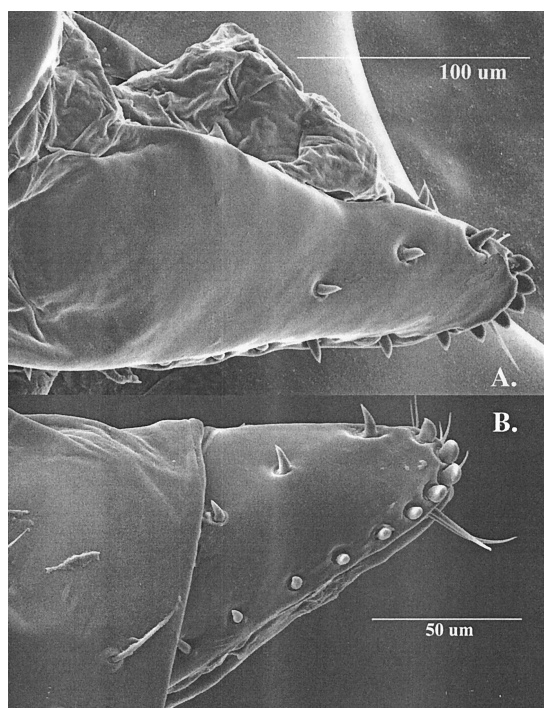


Fig. 2. (A) SEM of the ovipositor of *D. huckinsi*. The lateral bristles point in a posterior direction distinguishing it from that of *D. huichole*. (B) SEM of the ovipositor of *D. huichole*.

San Francisco del Rincon, Guanajuato, Mexico collected by W. J. Etges and M. A. Armella are deposited in the American Museum of Natural History, NY. Live cultures are available from the first author at the University of Arkansas, Fayetteville.

Distribution and Ecology. *D. huckinsi* has been collected in two disparate regions of Mexico spanning a 24-yr period. In October 1976, W.B.H. and M. Wasserman collected a number of individuals in thornscrub near Navojoa, Sonora. In January 2000, more than a thousand individuals were collected at two sites near San Francisco del Rincon, Guanajuato in *Opuntia-Stenocereus* thorn forests and in a similar habitat three km west of Ixtlan del Rio, Nayarit. Near San Francisco del Rincon, this species was abundant, co-occurring mainly with *D. longicornis*, *D. eremophila* Wasserman, and the second new species to be described below. In Nayarit, *D. huckinsi* was collected along with large numbers of *D. longicornis*, *D. aldrichi* Patterson, and *D. eremophila*.

No individuals were reared from numerous *Opuntia* pads returned to the laboratory yet this was the most abundant host plant at the collection sites. Generation times appear to be especially long: male age at first reproduction is roughly 3 wk and some wild-caught adults survived for 10 wk in laboratory conditions.

Diagnosis. Larger than close relative *D. longicornis* in wild-caught material, *D. huckinsi* males have yellow testes, a broader abdomen, longer legs, and a number

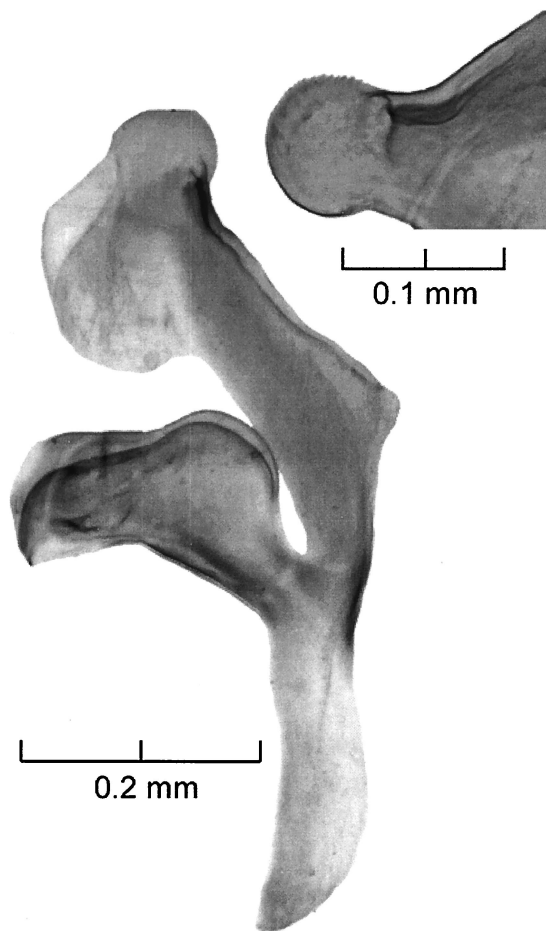


Fig. 3. Aedeagus of *D. huichole* showing the large and laterally flattened gonopods and extended lateral margins of the tip. Sensillae on the dorsal margin of gonopods are not particularly distinguishable in this view. Inset shows details of the ball-shaped tip covered in small teeth along the dorsal margin.

of unique morphological characteristics seen in the aedeagus (Fig. 1).

Etymology. This species is named in honor of Greg Huckins, graduate student at the University of Arizona, durable and trustworthy *Drosophila* field worker, and good friend who passed away in 1999.

Drosophila huichole, Etges & Heed, sp. nov.
(Fig. 3)

Head (from live material). *Males, females.* Aristae with three long and three short dorsal and two ventral branches in addition to the terminal fork. Third antennal joint is brownish-black at the base. Gena pale, approximately one-third diameter of eye at widest point. Ground color of the frons is pollinose tan. Anterior proclinate orbital bristle is about the same length as the posterior reclinate and midorbital is less than half the length of the anterior proclinate. The front of the head is tan with a dark brown stripe on the

anterior part of the orbits, a dark brown spot at the base of the posterior reclinate, and at the base of the posterior verticals. The frons is brown and V-shaped, with a light spot in front of and on both sides of the ocellus. Carina convex broader below with a brown medial stripe. Eye color is brownish-red. Pile is light in the ventral and posterior portions of the eye.

Thorax. Ground color is grayish brown. There are six rows of acrostichial hairs inside the row of dorso-centrals, each hair arising from a darker brown spot. Many of the spots are fused into irregular patterns. There is a faint grayish medial stripe. There are no prescutellar bristles. Anterior scutellar bristles are convergent. Female scutellum with a thick X-shaped pattern of brown pigment, not as obvious on males. Sterno index ≈ 0.9 . Mid-sternopleural bristles are very short. Ground color of the sternopleurae is brown, lighter in males than females. Legs are light tan with dark banding at the base of each tibia except for the foreleg where this band is missing. Fore femur has five long bristles on the posterior margin and three shorter bristles on the anterior margin besides the apical bristle.

Abdomen. *Female.* Light colored with contrasting heavy posterior dark brown banding interrupted dorsally on each tergite. Lateral margin of each tergite almost solid brown with indistinct central lighter areas more noticeable on first and second visible segments. Other females have all tergites with indistinct "windows" laterally that are somewhat darker than the light areas near the dorsal line. *Male.* Thicker and darker abdominal banding is interrupted on first two tergites laterally; the rest appear almost continuously dark brown to the lateral. Abdomen is noticeably thinner and more pointed posteriorly than *D. huckinsi*.

Wings. Wings with a slightly darkened posterior crossvein, especially in mature adults. Costal index ≈ 3.4 , fourth vein index 1.4, 5 \times index ≈ 0.8 , and 4c index is 0.3. Third costal section has heavy bristles on its basal one eighth.

External Genitalia. Surstylus with 12–13 teeth and 10 marginal bristles. The inner margin of the ventral lobe of the epandrium is transparent and broadly overlaps the surstylus. Tip of the anal plate with ≈ 15 –18 bristles. Tip of the aedeagus is ball-shaped in lateral view with minute teeth on the dorsal margin (Fig. 3). The small area adjacent to the margins of the dorsal cleft near the tip is heavily chitinized. The expanded ventral margin is partially chitinized and becomes clearer anteriorly. The dorsal margin of the base of each large gonopod almost contacts the ventral surface of the aedeagus for half its length. Each gonopod is laterally flattened branching from the apodeme with an obvious bristle near the branch point. A ventral rod is visible. The phallosomal index is 1.5. The ball-shaped aedeagus tip and the very broad gonopod closely adjacent to the axis of the aedeagus clearly differentiates *D. huichole* from all other members of the repleta group.

Internal Characteristics of Imagines. Testes with three to four inner and two outer gyres. Testes color is yellow-orange changing to darker orange with age.

Ventral receptacles elongated with ≈ 30 coils. Spermathecae are highly chitinized. The ovipositor is pointed with 16 short bristles (Fig. 2B) with the lateral bristles pointing dorsally, distinguishing the ovipositor from that of *D. huckinsi*.

Immature Stages. Eggs with four filaments approximately two and a half times the length of the egg. Puparia amber-colored with about seven to eight long and eight much shorter terminal branches per horn. Length of horns plus spiracles is about two-thirds the length of the puparium.

Chromosomes. Polytene chromosomes show no inversion polymorphism and are homosequential with those of *D. huckinsi*, *D. longicornis*, *D. propachuca*, and *D. mainlandi*.

Measurements. Female body length ≈ 3.5 mm and for 3.1 males. Female wing length ≈ 3.0 mm and 2.6 for males.

Type Material. HOLOTYPE ♂, 5 PARATYPE ♂, and 5 ALLOTYPE ♀ from laboratory cultures San Francisco del Rincon, Guanajuato, Mexico are deposited in the American Museum of Natural History, NY. Live cultures are available from the first author at the University of Arkansas, Fayetteville.

Distribution. *D. huichole* is known only from the two sites west of San Francisco del Rincon, Guanajuato, and a site near San Luis Potosi described above. Several hundred individuals were collected in Guanajuato in *Opuntia-Stenocereus* thorn forests, co-occurring mainly with *D. longicornis*, *D. huckinsi*, and *D. eremophila*. Fifteen adults were collected near San Luis Potosi along with numerous *D. bifurca* Patterson & Wheeler, *D. ritae* Patterson & Wheeler, *D. racemosa* Patterson & Mainland, *D. eremophila*, *D. longicornis* complex, and *D. hamatofila* Patterson & Wheeler in dense stands of *Opuntia robusta*, *O. phaeacantha*, and *O. ficus-indica*.

Etymology. The species is named for the Huichol people indigenous to the western states of Jalisco and Nayarit, Mexico, who are well-known for their exceptional sacred art and their deer-peyote rituals (Berrin 1978).

Diagnostic Characters. Adult males may easily be differentiated from *D. huckinsi* by the shape of the abdomen and testes color. The shape of the aedeagus is unique (Fig. 3). The ovipositor is blackish-brown, whereas that of *D. huckinsi* is honey brown. Adults are generally larger than *D. longicornis* in wild-caught material, but the aedeagus morphology is the most reliable character for species identification.

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References Cited

- Berrin, K. 1978. Art of the Huichol Indians. Harry N. Abrams, New York.
- Durando, C. M., R. H. Baker, W. J. Etges, W. B. Heed, M. Wasserman, and R. DeSalle. 2000. Phylogenetic analysis of the *repleta* species group of the genus *Drosophila* using multiple sources of characters. *Mol. Phylogenet. Evol.* 16: 296–307.
- Grimaldi, D. A. 1987. Phylogenetics and taxonomy of *Zygothrica* (Diptera: Drosophilidae). *Bull. Am. Mus. Nat. Hist.* 186: 103–268.
- Heed, W. B. 1982. The origin of *Drosophila* in the Sonoran Desert, pp. 65–80. In J.S.F. Barker and W. T. Starmer [eds.], *Ecological genetics and evolution: the cactus-yeast-Drosophila model system*. Academic, Sydney.
- Heed, W. B., and R. L. Mangan. 1986. Community ecology of the Sonoran Desert *Drosophila*, pp. 311–345. In M. Ashburner, H. L. Carson, and J.J.N. Thompson [eds.], *The genetics and biology of Drosophila*. Academic, New York.
- Patterson, J. T. 1943. The Drosophilidae of the southwest. *Univ. Tex. Publ.* 4313: 1–216.
- Patterson, J. T., and W. S. Stone. 1952. *Evolution in the genus Drosophila*. MacMillan, New York.
- Ruiz, A., W. B. Heed, and M. Wasserman. 1990. Evolution of the *mojavensis* cluster of cactophilic *Drosophila* with descriptions of two new species. *J. Hered.* 81: 30–42.
- Throckmorton, L. 1975. The phylogeny, ecology, and geography of *Drosophila*, pp. 421–469. In R. C. King [ed.], *Invertebrates of genetics interest. Handbook of genetics*. Plenum, New York.
- Vilela, C. R. 1983. A revision of the *Drosophila repleta* species group (Diptera: Drosophilidae). *Rev. Bras. Entomol.* 27: 1–114.
- Wasserman, M. 1992. Cytological evolution of the *Drosophila repleta* species group, pp. 455–552. In C. B. Krimbas and J. R. Powell [eds.], *Drosophila inversion polymorphism*. CRC, Boca Raton, FL.
- Wasserman, M., and H. R. Koepfer. 1977. Phylogenetic relationships among *Drosophila longicornis*, *Drosophila propachuca* and *Drosophila pachuca*, a triad of sibling species. *Genetics* 87: 557–568.
- Womersly, H. 1939. *Primitive insects of South Australia*. Government Printer, North Terrace.

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