Zootaxa 1069: 1–32 (2005)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)
1069

Molecular systematics and geographical distribution of the *Drosophila longicornis* species complex (Diptera: Drosophilidae)

DEODORO C. S. G. OLIVEIRA[1, 2], PATRICK M. O'GRADY[1, 3], WILLIAM J. ETGES[4], WILLIAM B. HEED[5] & ROB DeSALLE[1]

[1]*Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA; email: desalle@amnh.org*

[2]*Department of Biology, University of Rochester, Rochester, NY, USA; email: dolivei2@mail.rochester.edu*

[3]*Department of Biology, University of Vermont, VT, USA; email: pogrady@uvm.edu*

[4]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA; email: wetges@uark.edu*

[5]*Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, AZ, USA; email: WHeed@email.arizona.edu*

Abstract

Here we examine the phylogenetic relationships of eleven species previously hypothesized to be members of the *Drosophila longicornis* complex (*repleta* group, *mulleri* subgroup) using combined analyses of four mitochondrial genes. This complex, as currently redefined, is composed of the *longicornis* cluster (*D. longicornis*, *D. pachuca*, *D. propachuca*, and *D. mainlandi*), the *ritae* cluster (*D. desertorum*, *D. mathisi*, and *D. ritae*), and several miscellaneous species (*D. hamatofila*, *D. hexastigma*, *D. spenceri*, and an undescribed species "from Sonora"). A maximum likelihood inference also includes the *huckinsi* cluster (*D. huckinsi* and *D. huichole*) as the most distant members in the *longicornis* complex, a condition not recovered using maximum parsimony. We were unable to diagnose species in the triad of sibling species *D. longicornis*, *D. pachuca*, and *D. propachuca* using rapidly evolving mitochondrial DNA data, and we discuss possible species concept conflict for this triad. Comprehensive distribution information for these species, gathered over the past 60 years, is synthesized and displayed in range maps. Available information about the ecology and host plants of each species is also included.

Key words: *Drosophila longicornis* complex; molecular systematics; species concept conflict; host cactus

Introduction

The *Drosophila repleta* species group is composed of the *fasciola, hydei*, *mercatorum*,

Accepted by D. Bickel: 19 Sept. 2005; published: 25 Oct. 2005

mulleri, and *repleta* subgroups (Vilela 1983; Wasserman 1982, 1992). The *mulleri* subgroup includes approximately 50 species, almost half the species-level diversity in the *repleta* group. Cytological investigations of salivary gland chromosome banding patterns have been used to infer the phylogenetic relationships among the flies in the *mulleri* subgroup and have served as the basis for several complexes and clusters of species (Wasserman 1982, 1992).

The *longicornis* cluster was proposed to include five members of the *mulleri* subgroup, *D. longicornis* Patterson & Wheeler, *D. mainlandi* Patterson, *D. pachuca* Wasserman, *D. propachuca* Wasserman, and the undescribed "from Sonora" (Wasserman 1982). Other species have, at one time or another, been suggested as either members or relatives of the *longicornis* lineage based on a number of different analyses. For example, two species, *D. hexastigma* Patterson & Mainland and *D. spenceri* Patterson, were considered to be relatives of the *longicornis* cluster based on cytological data (Wasserman 1982). Another species, *D. hamatofila* Patterson & Wheeler, has been difficult to place using cytological methods because of a large number of unique chromosome inversions (Wasserman 1962, 1992). Some molecular work has indicated that *D. hamatofila* is related to the *longicornis* cluster (Oliveira *et al.* unpublished results). Two new species, *D. huckinsi* Etges & Heed and *D. huichole* Etges & Heed, were recently described from Mexico. These two species were considered to be most closely related to the members of the *longicornis* cluster based on morphological and cytological data (Etges *et al.* 2001). Finally, Durando *et al*. (2000), using both DNA characters and chromosomal inversion data, found the *longicornis* cluster to be polyphyletic with respect to two members of the *ritae* cluster, *D. ritae* Patterson & Wheeler and *D. desertorum* Wasserman. This is in agreement with allozyme electrophoresis studies that placed *D. desertorum* in the *longicornis* cluster (Richardson *et al.* 1975; Richardson and Smouse 1976). The *ritae* cluster also contains a third member, *D. mathisi* Vilela (for a recent review of the *ritae* cluster see Etges and Heed 2004).

The geographical distribution of the species in the *mulleri* subgroup, along with that of their host cacti is significant from an evolutionary point of view because it is through this strong association that these flies have been able to invade the arid lands, deserts and semi-deserts, of the New World. The last efforts to plot the distribution range of many *repleta* group species were in the 1940s (Patterson and Wagner 1943; Patterson and Mainland 1944). These surveys, while comprehensive at the time, cover only a subset of the present-day species diversity (*D. longicornis*, *D. hamatofila*, *D. hexastigma*, *D. mainlandi*, *D. ritae*, and *D. spenceri*). Species described since, as well as subsequent collections, range extensions and taxonomic revisions have not been incorporated since the 1940s.

Here we present a phylogenetic analysis of eleven species supposed to belong to the *longicornis* lineage. Four mitochondrial loci were employed to reconstruct a maximum parsimony and a maximum likelihood trees. Updated distribution maps along with data on cactus host usage are also presented.

Materials and Methods

Distribution Records and Taxon Sampling

Collecting information was gathered from several sources: the authors' personal records, Tucson Stock Center, Ambrose Monell Cryo Collection (American Museum of Natural History) and available data in the literature (Appendix 1). When available, multiple populations of members of the *longicornis* lineage were sampled for molecular work. Outgroup taxa were chosen from all major *repleta* group lineages as well as those species considered close to the *longicornis* complex based on the published phylogeny (Durando *et al.* 2000). Two members of the *nannoptera* group, *D. acanthoptera* Wheeler and *D. pachea* Patterson & Wheeler, were included as more distant outgroups and served to root our phylogenetic tree. In total, sixteen outgroup species were included in this study. Collecting data for the ingroup taxa is presented in Appendix 1. Further information for both the ingroup and outgroup species is available at the Ambrose Monell Cryo Collection website (http://research.amnh.org/amcc/).

Maps were plotted using the ArcView 8.1 software (ESRI). The geographical coordinates for the localities were obtained on the websites GeoNet Names Server (http://earth-info.nga.mil/gns/html/) and Geographic Names Information System (http://geonames.usgs.gov/index.html).

DNA Isolation and PCR Amplification

Three to five flies were macerated using a micropestle in a 1.5 ml PCR tube with buffer provided by the DNeasy Tissue Kit and DNA was isolated using the manufacturer's instructions (Qiagen). Loci of interest were PCR-amplified and sequenced using standard protocols (as in Remsen and O'Grady 2002). Four mitochondrial fragments were selected for this study: NADH2 (F192 – AGCTATTGGGTTCATACCCC, R732 – GAAGTTTGGTTTAAACCTCC), COI (F2640 – GCWGTMTTTGCTATTATAGCAGG, R3037 – TYCATTGCACTAATCTGCCATATTAG), COII (COIIa – ATGGCAGATTAGTGCAATGG, COIIb – GTTTAAGAGACCAGTACTTG), 16S/12S (F14029 – ATTTAATAAACSCTGATACAC, R14735 – AWAAACTAGGATTAGATACCC). Primer sequences are named based on their direction (forward or reverse) and position in the aligned mitochondrial genomes of *Drosophila yakuba* Burla (Clary and Wolstenholme 1985) and *Anopheles quadrimaculatus* Say (Mitchell *et al.* 1993); they are part of a larger set of primer pairs designed to work in a broad taxonomic range (unpublished data). The COII primers that were used are from Beckenbach *et al.* (1993). We were able to generate sequences for the four mitochondrial regions to all taxa sampled. Sequences have been submitted to GenBank under accession numbers DQ201970 to DQ202009 (16S/12S), DQ202010 to DQ202049 (COII), DQ202050 to DQ202089 (COI), and DQ202090 to DQ202129 (NADH2).

Sequence Editing and Phylogenetic Analysis

Sequences were edited in Sequencher 4.0 (Gene Codes Corp.) and exported into NEXUS formatted files (Maddison *et al.* 1997). Alignment for the 16S/12S fragment was trivial and was done manually using MacClade (Maddison and Maddison 2000). The three protein coding fragments did not require alignment.

Phylogenetic analyses, using maximum parsimony (MP) and maximum likelyhood (ML) were done in PAUP* 4.0b10 (Swofford 2002). All data was combined into a single analysis with a total of 2247 included characters, 534 of which were parsimony-informative. Settings for MP analysis were as follows: search type = heuristic, addition sequences = random, number of replicates = 500, branch swapping = TBR. Support at each node was assessed using bootstrap proportions (Felsenstein 1985, 1988) and Jackknife (Farris *et al.* 1996) with 1000 bootstrap or jackknife replicates (10 heuristic searches, other settings as above). Jackknife was done using a 37% deletion (Farris *et al.* 1996). Decay indices (Bremer 1988), a measure of the number of extra steps in the tree length required before a node collapses, were calculated using TreeRot.v2b (Sorenson 1999). The Modeltest program was used to select the model of evolutionary change the best fits the data (Posada and Crandall 1988). The ML tree was found using 10 searches (random addition). Support for the ML tree was estimated by 100 bootstrap replications.

Results

Phylogenetic Hypothesis

A total of twenty-four terminals, representing taxa previously hypothesized as being members or relatives to the *longicornis* cluster were included in our ingroup. Several outgroup taxa from other closely related lineages were also chosen to examine monophyly and placement of the *longicornis* lineage. The strict consensus of the four most parsimonious trees obtained in the combined analysis is shown in Fig. 1A and the ML tree constructed with the GTR + G + I model is shown in Fig. 1B. The monophyly of all eleven taxa hypothesized to belong to the *longicornis* complex is recovered by the ML tree. Nine of eleven species in our ingroup form a monophyletic group in the MP tree. The overall topologies corroborate the conclusions of some cytological and allozyme electrophoretic analyses (Richardson *et al.* 1975; Richardson and Smouse 1976; Wasserman 1982, 1992). Our results also agree with most relationships depicted in a recently published molecular phylogeny of the *repleta* group (Durando *et al.* 2000). The present analysis includes the enigmatic species *D. hamatofila* within the *longicornis* lineage, while previous molecular data suggested that *D. hamatofila* was outside this clade (Durando *et al.* 2000). MP and ML trees show conflicting topologies for several poorly supported nodes. The most relevant one is that *D. huichole* and *D. huckinsi* are placed outside the *longicornis* complex in the MP analyses, in apparent inconsistency with chromosomal inversion similarities and morphological resemblance (Etges *et al.* 2001). Moreover, the MP analyses groups them with *D. mulleri,* which is quite distinct morphologically. A possible explanation is that this might be a spurious result due to a high number of parallel changes accumulated by the rapidly evolving mitochondrial genes.

Our analysis fails to recover reciprocal monophyly within the *longicornis* triad of sibling species (*D. longicornis*, *D. pachuca*, and *D. propachuca*). Multiple representatives of species within this triad are intermixed. Similarly, we are unable to identify diagnostic molecular characters for each of these taxa under a population aggregation analysis (data not shown; Davis and Nixon 1992). In comparison *D. mainlandi* has nine diagnostic nucleotides spread in the combined data that differentiates it from *longicornis–pachuca–propachuca* (data not shown). Support for the sister group relationship between *D. mainlandi* and the species of the *longicornis* triad is consequently quite strong (Fig. 1).

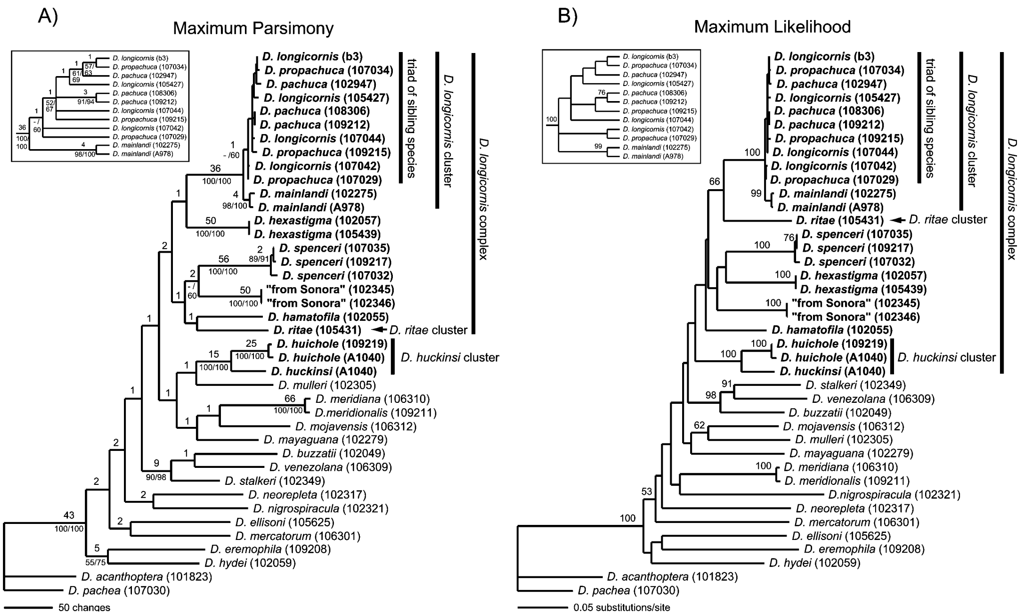

FIGURE 1. A—Strict consensus of the four most parsimonious trees (2262 steps, CI = 0.43, RI = 0.585). The Decay index is provided above the nodes. Bootstrap / Jackknife support values are provided below the nodes. The ingroup is shown in bold. The inset highlights the branching within *longicornis* cluster. B—Maximum likelihood tree obtained using the GTR + G + *I* model (-ln *L* = 13228.243; substitution rate matrix—AC = 1.625, AG = 9.369, AT = 3.825, CG = 2.092, CT = 36.885, GT = 1.000; estimated nucleotide frequencies—A = 0.358, C = 0.099, G = 0.090, T = 0.453; α = 0.652; *I* = 0.567). Bootstrap values are provided above the nodes. The inset highlights the branching within the *longicornis* cluster.

Geographical distribution

Our aim is to synthesize disparate distributional information and host cactus usage accumulated for a number of the species related to the *longicornis* lineage (Figs. 2–5). It should be noted, however, that some historical distribution data might be inaccurate in light of current taxonomic definitions. For example, it is possible that some records for *D. longicornis* might actually be either *D. pachuca* or *D. propachuca* (Wasserman and Koepfer 1977), depending on when they were obtained. In the same way, some records for *D. ritae* could be due to misidentification of *D. mathisi* (Vilela 1983). Details of collecting information are presented in Appendix 1.

D. desertorum—This species was traditionally known only from Hidalgo, Mexico (Wasserman 1962, Vilela 1983). Recent collection suggests that *D. desertorum* has spread northward into the Chisos Basin of Big Bend National Park in southwest Texas (Etges and Heed 2004). This species has been associated with the flat-leaf *Opuntia* (Wasserman 1982, 1992).

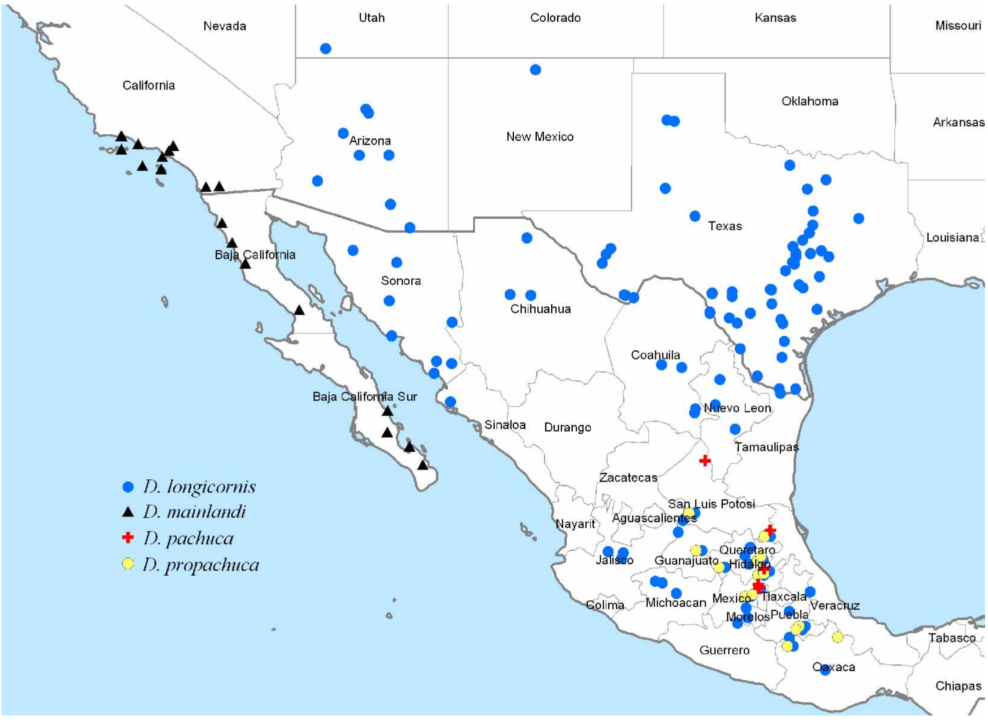

FIGURE 2. Distribution map of the known records of *D. longicornis*, *D. mainlandi*, *D. pachuca*, and *D. propachuca*.

D. hamatofila—The distribution of *D. hamatofila* extends from south California to central Texas and runs down through Mexico (Patterson and Wagner 1943). This species occurs in the highlands of the Mexican Plateau and passes onto the dryer lowlands in the northern of its range, areas of occurrence of *Opuntia* species (Patterson and Mainland 1944). It has been recorded on the fruit of the common prickly pear *Opuntia lindheimeri* Engelmann (Patterson 1943), associated to *O. phaeacantha* Engelmann (Heed new record), *O. ficus-indica* (Linnaeus) Miller, *O. oricola* Philbrick (Wasserman 1992), *O.*

demissa Griffiths (Heed new record), and also to *Ferocactus wislizeni* (Engelmann) Britton and Rose (Heed new record) and *Ferocactus sp* (Wasserman 1992). It has been found in the Mexican States of Baja California, Chihuahua, Coahuila, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico, Michoacan, Morelos, Nuevo Leon, Puebla, Queretaro, San Luis Potosi, Sonora, and Tamaulipas. It has also been collected at Arizona, California, New Mexico, Texas, and Utah in the United States.

FIGURE 3. Distribution map of the known records of *D. hexastigma* and *D. spenceri*.

D. hexastigma—This species is mainly an inhabitant of the cactus forests of the Hidalgan Deserts which extend intermittently from southern San Luis Potosi to northern Oaxaca at higher elevations (Axelrod 1979 and references therein). It has been reported from Guanajuato, Hidalgo, Morelos, Oaxaca, Puebla, Queretaro, and San Luis Potosi in Mexico. *Drosophila hexastigma* has been associated with six distinct species of columnar cacti, they are *Cephalocereus columna-trajani* (Karwinsky ex Pfeiffer) Schumann (syn. *C. hoppenstedtii*; Heed new record), *Escontria chiotilla* (F.A.C. Weber) Rose (Etges new record), *Myrtillocactus geometrizans* (Martin ex Pfeiffer) Console (Etges new record, Heed new record), *Neobuxbaumia tetetzo* (J. M. Coulter) Backeberg (Heed new record), *Pachycereus marginatus* (De Candolle) Britton & Rose (Cornejo new record, Heed new record), and *Stenocereus stellatus* (Pfeiffer) Riccobono (Etges new record, Heed new record).

D. huckinsi—This newly described taxon has been collected in *Opuntia*-*Stenocereus* thorn forests, but no individuals were reared from *Opuntia* pads returned to the laboratory (Etges *et al.* 2001). It has been collected in areas where the only host plants are *Opuntia* species, so we assume it is an *Opuntia* breeder. It has been found in Guanajuato, Nayarit, Puebla, and Sonora, Mexico.

D. huichole—Like *D. huckinsi*, this newly described species has been collected in *Opuntia–Stenocereus* thorn forests (Etges *et al.* 2001). It is known from the states of Guanajuato, San Luis Potosi, and Puebla, Mexico.

FIGURE 4. Distribution map of the known records of *D. hamatofila*, *D. huckinsi*, *D. huichole*, and “from Sonora”.

D. longicornis—This species is quite widespread from central Texas to central Arizona and ranges from southern Utah in the north to southern Mexico (Patterson and Wagner 1943). In Mexico it is present in the eastern part of the Sonoran desert and the northern part of the Sinaloan thornscrub, and in the central Mexican tableland. It follows the major distributional areas of several *Opuntia* species (Patterson and Mainland 1944). *Drosophila longicornis* has been reared from *O. ficus-indica*, *O. lindheimeri*, *O. phaeacantha*, *O. violacea* Engelmann Ex B. D. Jackson (Ruiz and Heed 1988), *O. wilcoxii* Britton and Rose (Ellen Heed new record), and “O. velutina complex”. In Tucson, Arizona, it has also been reared from *F. wislizeni* (Heed new record, C. May new record). It has been reported from Chihuahua, Coahuila, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico, Michoácan, Morelos, Nuevo Leon, Oaxaca, Puebla, Queretaro, San Luis Potosi, Sinaloa, Sonora, Tamaulipas, Veracruz, and the Federal District in Mexico, and at Arizona, New Mexico, Texas, and Utah in Unites States.

D. mainlandi—This species has been collected in the Mediterranean climatic zone of the coastal sage scrub and chaparral of California, including three of the Channel Islands, and the succulent sage scrub of adjacent Baja California. The remaining localities are in or near the short tree forest of the Cape region, and a single locality, San Borja, is mid-peninsular. *Drosophila mainlandi* has been collected chiefly in the non-desert regions where species the genus *Opuntia* are abundant (Heed 1982). It breeds in rotting cladodes of prickly pear cacti and has been reared from the following species: *O. oricola* (Heed new record), *O. demissa* (Heed new record), *O. ficus-indica* (Heed new record), and *Opuntia sp* (A. Beckenbach personal communication). We find no evidence for the existence of *D. mainlandi* in Colombia (Ruiz and Fontdevila 1981 as cited by Vilela 1983).

D. mathisi—This species has been found in Arizona and New Mexico, United States. It was described by Vilela (1983) based on a series of pinned material previously identified as *D. ritae*.

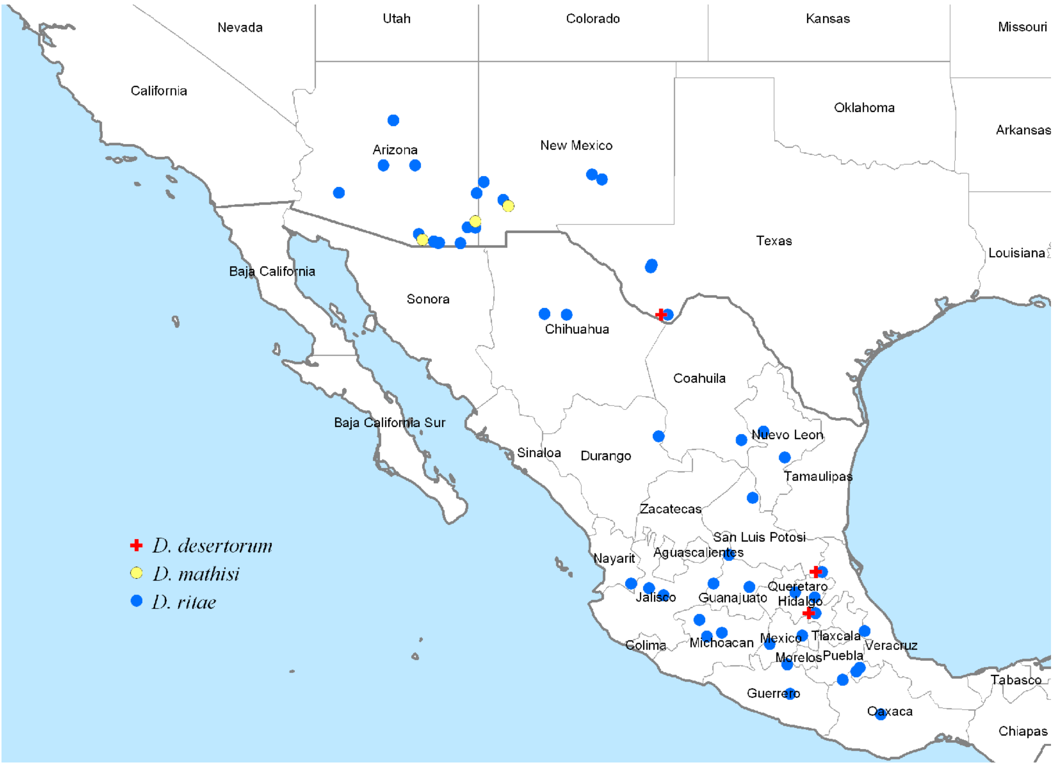

FIGURE 5. Distribution map of the known records of *D. desertorum*, *D. mathisi*, and *D. ritae*.

D. pachuca—This species is known from the northeast of Mexico City (Wasserman and Koepfer 1977), where it has been associated with the flat-leaf *Opuntia* (Wasserman 1982, 1992). It has been collected in the Mexican States of Hidalgo, Mexico and San Luis Potosi.

D. propachuca—The center of distribution of *D. propachuca* appears to be in the high elevations of the state of Hidalgo and it radiates outward to San Luis Potosi in the north end to Oaxaca in the south, again at high elevations. *Drosophila propachuca* has been identified by Wasserman from the states of Aguascalientes, Guanajuato, Hidalgo, Mexico, Oaxaca, Puebla, Queretaro, and San Luis Potosi (Wasserman and Koepfer 1977; Wasserman 1982, 1992) and later. This species has been associated with and reared from the flat-leaf *Opuntia* in Hidalgo, Oaxaca, and Puebla.

D. ritae—This taxon is a mountain species, common in the desert region around the mountains and the mountains themselves (Patterson and Wagner 1943). Its range encompasses the highlands in the Mexican Central Plateau and extends northwest into the northern Arizona (Patterson and Wagner 1943). It has been collected in Chihuahua, Coahuila, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico, Michoacan, Nayarit, Nuevo Leon, Oaxaca, Puebla, San Luis Potosi, Vera Cruz, and the Federal District in Mexico, and in Arizona, New Mexico, and Texas in United States.

"from Sonora"—This undescribed species is known only from Sonora and Sinaloa, Mexico, where it has been reared from *O. wilcoxii* (Ruiz and Heed 1988) and several other species of *Opuntia* (Heed unpublished data).

D. spenceri—This species is most common in the frost-free environment of the thorn forest of southern Sonora and northern Sinaloa. *Drosophila spenceri* is also found in the tropical deciduous forests in the cape region of Baja California Sur and the Pacific coastal plain (including the Balsas Basin) of Jalisco, Michoacan, Guerrero, Morelos and Oaxaca. Its distribution range includes the additional Mexican states of Nuevo Leon, Puebla, and San Luis Potosi. It is a lowland species found especially in the driest parts of these areas. *Drosophila spenceri* has been reared from six species of columnar cacti and two species of barrel cactus: *Carnegiea gigantea* (Engelmann) Britton and Rose, *P. pecten-aboriginum* (Engelmann ex S. Watson) Britton and Rose (Heed new record), *P. pringlei (*S. Watson) Britton and Rose (Heed new record), *P. weberi* (J. M. Coulter) Backeberg (Etges new record), *S. quevedonis* (J. G. Ortega) Buxbaum (Etges new record), *S. thurberi* (Engelmann) Buxbaum, *F. cylindraceus* (Engelmann) Orcutt (Etges unpublished data), *F. emoryi* (Engelmann) Orcutt (Heed new record). In summary, *D. spenceri* is most assuredly a species of the Neotropics and is largely dependent upon a variety of cacti in the subtribe Pachycereinae.

In summary the species listed are restricted to the arid lands of southwestern United States and Mexico. Seven species have been recorded only from Mexico, *D. hexastigma*, *D. huckinsi*, *D. huichole*, *D. pachuca*, *D. propachuca*, "from Sonora", and *D. spenceri*.

Discussion

The longicornis *complex*

We are erecting a more inclusive group, the *longicornis* complex (*mulleri* subgroup: *repleta* group), based on our phylogenetic analysis as summarized in Table 1. This group includes the *longicornis* (*D. longicornis*, *D. pachuca*, *D. propachuca*, *D. mainlandi*), *ritae* (*D. ritae, D. mathisi, D. desertorum*), and *huckinsi* (*D. huckinsi* and *D. huichole*) clusters plus four other species (*D. hamatofila*, *D. hexastigma*, *D. spenceri*, "from Sonora") and is sister to the *mulleri* complex.

The composition of *ritae* cluster presented here is based on polytene chromosome similarities as originally proposed by Wasserman (1982, 1992). This can be considered a conservative definition, since allozyme and phylogenetic results have suggested that *D. desertorum* could actually be closer related to the *longicornis* cluster (Richardson *et al.* 1975; Richardson and Smouse 1976; Durando *et al.* 2000; Etges and Heed 2004). *Drosophila mathisi* is very similar to *D. ritae* on both morphological and cytological grounds (Wasserman 1992, Vilela 1983), but it has never been investigated at the molecular level. Unfortunately, at the time of this study *D. desertorum* and *D. mathisi* were not available for molecular work and the monophyly of the *ritae* cluster could not be tested. Anyhow, the placement of the three species of the *ritae* cluster as members of an expanded *longicornis* complex is supported by previous molecular (Durando *et al.* 2000), cytological studies (Wasserman 1982, 1992), and the current investigation. The grouping of *D. hamatofila* in the *longicornis* complex is somewhat surprising, as it has been considered an enigmatic species not clearly related to any other taxa in the *mulleri* subgroup. Chromosome banding patterns suggest that this species is quite variable cytologically (Wasserman 1992), and this high degree of variability may have masked the key inversions, synapomorphies, linking this species to the *longicornis* complex. Another surprising result is the exclusion of *D. huckinsi* and *D. huichole* of the *longicornis* complex in the MP tree (Fig. 1A). *Drosophila huckinsi* and *D. huichole* were initially placed in the *longicornis* complex because their polytene chromosomes are homosequential with those of *D. longicornis*, *D. propachuca*, and *D. mainlandi*, and also due to morphological similarities (Etges et. al. 2001). We believe that the best interpretation of the current knowledge is as depicted in the ML tree (Fig. 1B), the *huckinsi* cluster is the most basal lineage within the *longicornis* complex.

The species concept conflict in the longicornis *triad of sibling species*

Within the *longicornis* cluster, there is a triad of morphologically indistinguishable sympatric forms *D. longicornis*, *D. pachuca*, and *D. propachuca.* Laboratory crosses between these three sibling species failed to produce fully fertile offspring, prompting Wasserman and Koepfer (1977) to conclude that they are different species under a biological species concept. This view is further supported by the fact that each species has its own unique chromosomal polymorphisms (Wasserman and Koepfer 1977). However, the similarity in male genitalic structure has led to the suggestion that they could be conspecific (Vilela 1983).

This triad is not possible to differentiate morphologically and all three species are sympatric in central Mexico (Fig. 2). As pointed out by Wasserman and Koepfer (1977), the method to correctly identify these species involves crossing isofemale lines from field-collected specimens with reference laboratory strains. Therefore, the prospect of finding molecular markers for species identification would be advantageous for future research in this clade. However, mitochondrial sequences generated for this study failed to produce diagnostic characters and to recover reciprocal monophyly for these taxa (Fig. 1 and Results). A possible explanation for this pattern is that these species are of recent origin and are still undergoing differentiation at the molecular level. Albeit, applying a phylogenetic species concept to our data (Cracraft 1989; Davis and Nixon 1992), it can be considered evidence that only a single species exists. Therefore, under a biological species concept *D. longicornis*, *D. pachuca*, and *D. propachuca* maintain their status as valid species, but not by a phylogenetic concept of species. Further understanding of a possible ecological isolation and the genetic basis of infertility are needed to solve this dilemma, whether *D. longicornis*, *D. pachuca*, and *D. propachuca* are indeed good species or not.

TABLE 1. Revised taxonomic relationships for the species analyzed in this study.

Complex	Cluster	Species
longicornis	*longicornis*	*D. longicornis* Patterson & Wheeler, 1942
		D. pachuca Wasserman, 1962
		D. propachuca Wasserman, 1962
		D. mainlandi Patterson, 1943
	ritae	*D. desertorum** Wasserman, 1962
		*D. mathisi** Vilela, 1983
		D. ritae Patterson & Wheeler, 1942
	miscellaneous	*D. hamatofila* Patterson & Wheeler, 1942
		D. hexastigma, Patterson & Mainland, 1944
		D. spenceri Patterson, 1943
		“from Sonora”, undescribed
	huckinsi	*D. huckinsi* Etges & Heed, 2001
		D. huichole Etges & Heed, 2001

*Not included in the phylogenetic analysis.

Host cactus usage

Most species in the *longicornis* lineage are *Opuntia* breeders, as this is the probable plesiomorphic conditions for the *mulleri* subgroup. Only two species, *D. hexastigma* and *D. spenceri* utilize the more chemically complex columnar cacti in the subfamily Pachycereeae. Interestingly, the ML phylogeny shows them as sister taxa, not supported by the MP analysis, an indication that this trait evolved only once in the *longicornis* complex. These two species overlap little in their distribution (Fig 3). *Drosophila hexastigma* is found in higher elevations along the Mexican plateau and *D. spenceri* is a lowland species (Patterson and Mainland 1944). *Drosophila spenceri* has also been reared from the barrel cactus *Ferocactus sp.*, subfamily Cactoideae. The use of the barrel cactus is shared with the typically *Opuntia* breeders *D. longicornis* and *D. hamatofila*. The shift of host cactus usage by *D. spenceri* and *D. hexastigma* and the ecological role of the barrel cactus deserve further attention.

It has been suggested that the driest part of Sonoran Desert acts as an ecological barrier for *Opuntia* breeding flies (Heed 1982). In more pluvial times the ancestral species to *D. longicornis* and *D. mainlandi* had its distribution across the Sonoran Desert. As the condition became drier in the interior, a population was isolated in the region of southern California and northern Baja California giving rise to *D. mainlandi* (Heed 1982). This allopatric speciation model is well corroborated by our data (Figs. 1 and 2).

Acknowledgments

Financial assistance was provided by grant DEB 01-C29105 to R. DeSalle and P. M. O'Grady and by grant NSF INT-9724790 to W. J. Etges and W. B. Heed. Deodoro Oliveira was an Ambrose Monell Research Fellow at the AMNH.

References

Axelrod, D.I. (1979) Age and Origin of the Sonoran Desert vegetation. *Occasional Papers of the California Academy of Sciences*, 132, 1–74.

Beckenbach, A.T., Wei, W. & Liu, H. (1993) Relationships in the *Drosophila obscura* species group, inferred from mitochondrial cytochrome oxidase II sequences. *Molecular Biology and Evolution*, 10, 619–634.

Bremer, K. (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, 42, 795–803.

Cracraft, J. (1989) Speciation and its ontogeny: the empirical consequences of alternative species concepts and processes of differentiation. *In*: Otte, D. & Endler, J. A. (Eds.), *Speciation and its consequences*. Sinauer, Sunderland, pp. 28–59.

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecular of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution*, 22, 252–257.

Davis, J.I. & Nixon, K.C. (1992) Populations, genetic variation, and the delineation of phylogenetic species. *Systematic Biology*, 41, 421–435.

Durando, C.M., Baker, R.H., Etges, W.J., Heed, W.B., Wasserman, M. & DeSalle, R. (2000) Phylogenetic analysis of the *repleta* species group of the genus *Drosophila* using multiple sources of characters. *Molecular Phylogenetics and Evolution,* 16, 296–307.

Etges, W.J., Armella, M.A., O'Grady, P.M. & Heed W.B. (2001) Two new species of *Drosophila* (Diptera: Drosophilidae) in the *repleta* group from Mexico. *Annals of the Entomological Society of America,* 94, 16–20.

Etges, W.J. & Heed W.B. (2004) A new geographical record for *Drosophila desertorum* and a review of the *D. ritae* cluster in the *repleta* group of Drosophila. *Drosophila Information Service,* 87, 30–32.

Farris, J.S., Albert, V., Kallersjo, M., Lipscomb, D. & Kluge, A.G. (1996) Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, 12, 99–124.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.

Felsenstein, J. (1988) Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics,* 22, 521–565.

Heed, W.B. (1982) The origin of *Drosophila* in the Sonoran Desert. *In*: Barker, J. S. F. & Starmer, W. T. (Eds.), *Ecological Genetics and Evolution: The Cactus–Yeast–Drosophila Model System*. Academic Press, Sydney, pp. 65–80.

Maddison, D.R. & Maddison, W.P. (2000) *MacClade: Analysis of phylogeny and character evolution, version 4.0*, Sinauer Associates, Sunderland, Mass.

Maddison, D.R., Swofford, D.L. & Maddison, W.P. (1997) Nexus: An extensible file format for systematic information. *Systematic Biology,* 46, 590–621.

Mitchell, S.E., Cockburn, A.F. & Seawright, J.A. (1993) The mitochondrial genome of *Anopheles quadrimaculatus* species A: complete nucleotide sequence and gene organization. *Genome,* 36, 1058–1073.

Patterson, J.T. (1943) The Drosophilidae of the Southwest. *The University of Texas Publications,* 4313, 7–203.

Patterson, J.T. & Mainland, G. B. (1944) The Drosophilidae of Mexico. *The University of Texas Publications*, 4445, 9–101.

Patterson, J.T. & Wagner, R.P. (1943) Geographical distribution of species of the genus *Drosophila* in the United States and Mexico. *The University of Texas Publications*, 4313, 217–281.

Patterson, J.T. & Wheeler M.R. (1942) Description of new species of the sub-genus *Hirtodrosophila* and *Drosophila*. *The University of Texas Publications,* 4213, 67-109.

Posada, D & Crandall, K.A. (1988) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.

Remsen, J. & O'Grady, P.M. (2002) Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support. *Molecular Phylogenetics and Evolution,* 24, 249–264.

Richardson, R.H., Richardson, M.E. & Smouse, P.E. (1975) Evolution of electrophoretic mobility in the *Drosophila mulleri* complex. *In*: Markert, C. L. (Ed.). *Isozymes IV: Genetics and Evolution*. Academic Press, New York, pp. 533–545.

Richardson, R.H. & Smouse, P.E. (1976) Patterns of molecular variation. I. Interspecific comparison of electromorphs in the *Drosophila mulleri* complex. *Biochemical Genetics*, 14, 447–465.

Ruiz, A. & Fontdevila, A. (1981) Ecologia y evolucion del subgroupo *mulleri* en *Drosophila* de Venezuala y Colombia. *Acta Cientifica Venezolana* 32, 338–345.

Ruiz, A. & Heed, W. B. (1988) Host–Plant specificity in the cactophilic *Drosophila mulleri* species complex. *Journal of Animal Ecology,* 57: 237–249.

Sorenson, M.D. (1999) *TreeRot, version 2.* Boston University, Boston, MA.

Swofford, D.L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (and other methods), version 4.0 beta.* Smithsonian Institution, Washington DC.

Vilela, C.R. (1983) A revision of the *Drosophila repleta* species group (Diptera, Drosophilidae). *Revista Brasileira de Entomologia*, 27, 1–114.

Wasserman, M. (1962) Cytological studies of the *repleta* group of the genus *Drosophila*. V. The *mulleri* subgroup. *The University of Texas Publications*, 6205, 85–117.

Wasserman, M. (1982) Evolution of the *repleta* group. *In*: Ashburner, M., Carson, H.L. & Thompson, J.N. (Eds.), *The genetics and biology of* Drosophila*, vol 3b*. Academic Press, London, pp. 61–139.

Wasserman, M. (1992) Cytological evolution of the Drosophila repleta species group. In: Krimbas, C. B. & Powell, J. R. (Eds.), *Drosophila Inversion Polymorphism*. CRC Press, Boca Raton, pp. 455–552.

Wasserman, M. & Koepfer, H.R. (1977) Phylogenetic relationships among *Drosophila longicornis*, *Drosophila propachuca*, *Drosophila pachuca*, a triad of sibling species. *Genetics*, 87, 557–568.

APPENDIX 1 – Collection records.

Locality[1]	References; collecting information; and identification numbers[2]
D. desertorum	
Pachuca, Hidalgo, Mexico – 20.07N / 98.44W	J, I; 2519.22
San Pedro Mines, Hidalgo, Mexico – 21.23N / 98.31W	A, I; 102946, E26.3K, E42.1–2
Chisos Basin (Big Bend NP), Texas, USA – 29.15N / 103.15W	L; WE, iii / 2005 – 2116 ind.
D. hamatofila	
Ejido Uruapan, Baja California, Mexico – 31.37N / 116.26W	C; A754 WH, JR, WS, xii / 1979 – 2 ind. ex: *O. ficus-indica*
Ensenada, Baja California, Mexico – 31.52N / 116.37W	A, I; A756 JR, xii / 1979 – 4 ind. ex: *O. ficus-indica*; 102885, E10.7
San Matias Pass., Baja California, Mexico – 30.45N / 115.16W	C; A857 WH, PB, iii / 1984 – 331 ind. ex: *O. oricola*
San Telmo, Baja California, Mexico – 30.58N / 116.06W	C; A757 WH, JR, xii / 1979 – 707 ind. ex: *O. oricola*
Valle San Quintin, Baja California, Mexico – 30.35N / 115.58W	A1049 WE, JMu, HW, i / 2003 – 6 ind.
Candelaria*, Chihuahua, Mexico – 31.07N / 106.28W	H
Charco*, Chihuahua, Mexico – 28.25N / 106.10W	H
Chihuahua, Chihuahua, Mexico – 28.38N / 106.05W	H
Encinillas*, Chihuahua, Mexico – 29.15N / 106.21W	H
Meoqui, Chihuahua, Mexico – 28.17N / 105.29W	H
Santa Clara*, Chihuahua, Mexico – 29.17N / 107.01W	H
Muzquiz, Coahuila, Mexico – 28.40N / 100.30W	G, H
Piedra Blanca, Coahuila, Mexico – 29.05N / 102.19W	H
Ramos Arizpe, Coahuila, Mexico – 25.33N / 100.58W	H
Canelas, Durango, Mexico – 25.06N / 106.34W	C; 201.5
Lerdo, Durango, Mexico – 25.32N / 103.32W	H
San Miguel de Allende, Guanajuato, Mexico – 20.55N / 100.45W	C, N; W110
Zumpango, Guerrero, Mexico – 17.39N / 99.30W	H
Ixmiquilpan, Hidalgo, Mexico – 20.29N / 99.14W	H
Zimapan, Hidalgo, Mexico – 20.14N / 99.21W	N
Guadalupe, Jalisco, Mexico – 20.51N / 103.20W	H
Tequila, Jalisco, Mexico – 20.53N / 103.50W	N
Pelillos (Valle de Bravo), Mexico, Mexico – 19.11N / 100.08W	H
Teotihuacan, Mexico, Mexico – 19.41N / 98.51W	H
Patzcuaro, Michoacan, Mexico – 19.31N / 101.36W	H

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Alpuyeca, Morelos, Mexico – 18.44N / 99.16W	H
Monterrey, Nuevo Leon, Mexico – 25.40N / 100.19W	H
Sabinas Hidalgo, Nuevo Leon, Mexico – 26.30N / 100.10W	C; 642
Petlalcingo, Puebla, Mexico – 18.05N / 97.54W	H
San Juan del Rio, Queretaro, Mexico – 20.23N / 100.00W	C, N; W111
Arriaga, San Luis Potosi, Mexico – 21.54N / 101.23W	H
Matehuela, San Luis Potosi, Mexico – 23.39N / 100.39W	C, N; W106
Magdalena (El Alamo Ranch), Sonora, Mexico – 30.19N / 110.44W	C, G, H; 201.4
Guemez, Tamaulipas, Mexico – 23.56N / 99.00W	H
Hidalgo, Tamaulipas, Mexico – 26.03N / 98.12W	H
Tampico, Tamaulipas, Mexico – 22.13N / 97.51W	H
Aravaipa Valley, Arizona, USA – 32.51N / 110.38W	C, J; A8 WH iv / 1959
Ashton Draw, Arizona, USA – NF	F
Cave Creek, Arizona, USA – 33.50N / 111.57W	F
Coolidge Dam, Arizona, USA – 33.11N / 110.32W	F
Flagstaff, Arizona, USA – 35.12N / 111.39W	F
Hart Prairie, Arizona, USA – 35.20N / 111.44W	F
Kofa Mountains, Arizona, USA – 33.25N / 113.57W	A999 WE, GH, PO, xi / 1996 – 1 ind.
Patagonia, Arizona, USA – 31.32N / 110.46W	C, J; A6 (.2)
Portal, Arizona, USA – 31.55N / 109.08W	B, I; 15081-1301.1, E32.2
Prescott, Arizona, USA – 34.32N / 112.28W	F
Roosevelt Lake, Arizona, USA – 33.44N / 111.11W	F
Rose Creek, Arizona, USA – 33.50N / 110.59W	F
Rustler's Park, Arizona, USA – 31.54N / 109.16W	F, G
Sabino Canyon, Arizona, USA – 31.52N / 111.28W	F
San Bernardino, Arizona, USA – 32.59N / 113.19W	F, G
Tucson, Arizona, USA – 32.13N / 110.59W	A, B, C, F, I; A52 WH, i / 1962 – 29 ind. ex: *F. wislizeni*; A59 WH, ii / 1962 – 22 ind. ex: *O. ficus-indica*; A67 WH, ix / 1962 – 68 ind. ex: *O. ficus-indica*; A102 WH, ix / 1963 – 20 ind. ex: *O. ficus-indica*; A243 SJ, ii / 1969 – 56 ind. ex: *O. phaeacantha*; A247 WH, iii / 1969 – 121 ind. ex: *O. phaeacantha*; A666 WH, ii / 1977 – 50 ind. ex: *O. phaeacantha*;

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
	A841 WS, HP, iii / 1983 – 289 ind. ex: *O. phaeacantha*; A862 CM, iii / 1984 – 13 ind. ex: *F. wislizeni*; A864 AR, iii / 1984 – 1167 ind. ex: *O. phaeacantha*; A885 WE, iii / 1985 – 73 ind. ex: *O. phaeacantha*; A912 WH, iii / 1986 – 20 ind. ex: *O. ficus-indica*; A914 WH, iv / 1986 – 174 ind. ex: *O. phaeacantha*; A1030 WH, iii / 2001 – 43 ind. ex: *O. ficus-indica*; 102054, 15081-1301.0 (.4), A764, E2.6
Anza-Borego Desert, California, USA – 33.05N / 116.36W	C; 553, 554
Arcadia, California, USA – 34.08N / 118.02W	C; 567
Bell, California, USA – 33.59N / 118.10W	F
Del Mar, California, USA – 32.58N / 117.16W	C; A863 PB, iii / 1984 – 17 ind. ex: *O. ficus indica*
Escondido, California, USA – 33.07N / 117.05W	C; 212
Highway 101, California, USA – NF	F
Lauro Canyon, California, USA – 34.27N / 119.44W	F
Malibu Canyon, California, USA – 34.06N / 118.44W	C; 211
Mission Canyon*, California, USA – 34.27N / 119.43W	F
Pomona, California, USA – 34.04N / 117.45W	C; 215
San Gabriel Canyon, California, USA – 34.10N / 117.55W	F
Santa Barbara, California, USA – 34.25N / 119.42W	F
Santa Barbara Island, California, USA – 33.28N / 119.02W	C; A853 RG, i / 1984
Santa Catalina Island, California, USA – 33.23N / 118.25W	A; C; A826 WH, MH, DS, x / 1981 – 17 ind. ex: “O. demissa”; A956 WH, HP, ED, xi / 1991 – 22 ind. ex: “O. demissa”; 102055
Santa Cruz Island, California, USA – 34.01N / 119.43W	C; A825 JM, x / 1981
Topanga Canyon, California, USA – 34.02N / 118.31W	C; 210
Verdemont, California, USA – 34.11N / 117.22W	I
Wohlford, California, USA – 33.10N / 117.00W	C; 213
Bonita Canyon, New Mexico, USA – 36.37N / 106.12W	F
Las Cruces, New Mexico, USA – 32.19N / 106.47W	F, G
National Monument, New Mexico, USA – NF	F
Radium Springs, New Mexico, USA – 32.30N / 106.56W	F, G

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Silver City, New Mexico, USA – 32.46N / 108.17W	F
Abernathy, Texas, USA – 33.50N / 111.50W	F
Alice, Texas, USA – 27.45N / 98.04W	F
Arlington, Texas, USA – 32.44N / 97.07W	F
Austin, Texas, USA – 30.16N / 97.44W	F
Austin (Aldrich Farm), Texas, USA – 30.16N / 97.44W	F
Bell County, Texas, USA – 31.03N / 97.28W	F
Belton, Texas, USA – 31.04N / 97.28W	F
Bexar County, Texas, USA – 29.27N / 98.32W	F
Blanco, Texas, USA – 30.06N / 98.25W	F
Brackettville, Texas, USA – 29.19N / 100.25W	F
Brownsville, Texas, USA – 25.54N / 97.30W	F
Brownwood*, Texas, USA – 31.43N / 98.59W	F
Carrizo Springs, Texas, USA – 28.31N / 99.52W	F
Catarina, Texas, USA – 28.21N / 99.37W	F
Childress, Texas, USA – 34.29N / 100.12W	F
Chisos Basin (Big Bend NP), Texas, USA – 29.15N / 103.15W	WE, iii / 2005 – 1262 ind.
Chisos Mountains, Texas, USA – 29.16N / 103.18W	F
Comal County, Texas, USA – 29.49N / 98.18W	F
Del Rio, Texas, USA – 26.37N / 98.56W	F
Dilley, Texas, USA – 28.40N / 99.10W	F
Eagle Pass, Texas, USA – 28.43N / 100.30W	F
Eddy, Texas, USA – 31.17N / 97.15W	F
Fayette County, Texas, USA – 29.52N / 96.55W	F
Florence, Texas, USA – 30.50N / 97.47W	F
Fort Davis, Texas, USA – 30.35N / 103.53W	C, J, I; 1981.1
Fort Stockton, Texas, USA – 30.53N / 102.53W	F
Fort Worth, Texas, USA – 32.43N / 97.19W	F
Fredericksburg, Texas, USA – 30.16N / 98.52W	F
Georgetown, Texas, USA – 30.38N / 97.41W	F
Giddings, Texas, USA – 30.11N / 96.56W	F
Grand Canyon, Texas, USA – NF	F
Guadalupe Mountains, Texas, USA – 31.53N / 104.52W	F
Harlingen, Texas, USA – 26.11N / 97.42W	F

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Henley (Hays County), Texas, USA – 30.03N / 98.02W	F
Johnson City, Texas, USA – 30.17N / 98.25W	F
Lamesa, Texas, USA – 32.44N / 101.57W	F
Laredo, Texas, USA – 27.31N / 99.31W	F
Limpia Canyon, Texas,USA – 30.23N / 103.44W	F
Marfa, Texas, USA – 30.18N / 104.01W	F
McAllen, Texas, USA – 26.12N / 98.14W	F
Mineral Wells, Texas, USA – 38.48N / 98.07W	F
Ozona, Texas, USA – 30.43N / 101.12W	F
Palo Duro Canyon, Texas, USA – 34.38N / 100.56W	F
Plainview*, Texas, USA – 34.11N / 101.43W	F
Plano, Texas, USA – 33.01N / 96.42W	F
Round Rock, Texas, USA – 30.31N / 97.41W	F, G, I
San Angelo, Texas, USA – 31.28N / 100.26W	F
San Antonio, Texas, USA – 29.26N / 98.29W	F, I
Selma*, Texas, USA – 33.29N / 97.53W	F
Sierra Blanca, Texas, USA – 31.10N / 105.22W	F
Sonora, Texas, USA – 30.34N / 100.38W	F
Sterling, Texas, USA – 31.50N / 100.59W	F
Tahoka, Texas, USA – 33.10N / 101.47W	F
Throckmorton, Texas, USA – 33.11N / 99.11W	F
Toyahvale, Texas, USA – 30.56N / 103.47W	F
Travis County, Texas, USA – 30.20N / 97.48W	F
Uvalde, Texas, USA – 29.13N / 99.47W	F
Uvalde County, Texas, USA – 29.22N / 99.46W	F
Victoria, Texas, USA – 28.48N / 97.00W	F
Waco*, Texas, USA – 31.33N / 97.08W	F
Wichita Falls, Texas, USA – 33.55N / 98.29W	F
Wild Rose Pass, Texas, USA – 30.43N / 103.47W	F
Zion National Park, Utah, USA – 37.18N / 113.03W	F, G
D. hexastigma	
San Miguel de Allende, Guanajuato, Mexico – 20.55N / 100.45W	N

......*continued on the next page*

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Metztitlan (Gan. Las Vaquerias), Hidalgo, Mexico – 20.36N / 98.59W	A960 WH, HP, ED ix / 1992 – 19 ind.; A1015 WE, MA, GH, viii / 1997 – 8 ind.; A1016 WE, MA, GH, viii / 1997 – 32 ind.
Venados, Hidalgo, Mexico – 20.10N / 98.44W	A; 102929; E27.5
Zimapan; Hidalgo, Mexico – 20.14N / 99.21W	N
Cuernavaca, Morelos, Mexico – 18.55N / 99.15W	N
Joluxtla, Oaxaca, Mexico – 18.09N / 97.45W	A1043 WE, MA, CL, AA, viii / 2000 – 523 ind. asp: *S. stellatus* & *Escontria sp.*
Oaxaca, Oaxaca, Mexico – 17.02N / 96.44W	I
Acatlan, Puebla, Mexico – 18.12N / 98.27W	N
Petlalcingo, Puebla, Mexico – 18.05N / 97.54W	H, I
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A, B, D; A739, MJ, ix / 1978; A842 DC, iii / 1983 – 6 ind. ex: *P. marginatus*; A955 WH, HP, ED ix / 1991 – 874 ind + 4 ind. ex: *S. stellatus* + 43 ind. ex: *M. geometrizans* + 197 ind. asp: *C. columna-trajani* + 113 ind. asp: *S. stellatus* + 87 ind. asp: *P. marginatus* + 55 ind. asp: *N. tetetzo* + 43 ind. asp: *M. geometrizans*; A1013 WE, MA, GH viii / 1997 – 32 ind.; A1048 WE, MA, PO, DO, i / 2002 – 288 ind.; 102057, 102056, 105439, 107033, 107069, 107073, 107074, 15081-1302.0, 199, 250, 266, 267
San Juan del Rio, Queretaro, Mexico – 20.23N / 100.00W	N
La Parada, San Luis Potosi, Mexico – 22.21N / 101.13W	A1045 WE, MA, CL, AA, viii / 2000 – 9 ind.
Matehuala, San Luis Potosi, Mexico – 23.39N / 100.39W	N
San Luis Potosi, San Luis Potosi, Mexico – 22.09N / 100.59W	I; E30.1
No collecting information is available	A; 100093, 124
D. huckinsi	
San Francisco del Rincon, Guanajuato, Mexico – 21.01N / 101.51W	E; A1040 WE, MA, RGr, i / 2000 – 325 ind.; A1041 WE, MA, RGr, i / 2000 – 356 ind.; B9
Ixtlan del Rio, Nayarit, Mexico – 21.02N / 104.22W	E; A1042 WE, MA, RGr, i / 2000 – 632 ind.

......*continued on the next page*

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A1048 WE, MA, PO, DO, HW, i / 2002 – 38 ind.
Navojoa, Sonora, Mexico – 27.06N / 109.28W	E; A657.17A WH, MW, x / 1976 – 15 ind.
No collecting information is available	A; 108296, 212
D. huichole	
San Francisco del Rincon, Guanajuato, Mexico – 21.01N / 101.51W	E; A1040 WE, MA, RGr, i / 2000 – 29 ind.; A1041 WE, MA, RGr, i / 2000 – 20 ind.; B10
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A; A1048 WE, MA, PO, DO, HW, i / 2002 – 4 ind.; 109219
San Luis Potosi, San Luis Potosi, Mexico – 22.09N / 100.59W	E; A1044WE, MA, CL, AA, vii / 2000 – 15 ind.
D. longicornis	
Candelaria*, Chihuahua, Mexico – 31.07N / 106.28W	H
Encinillas*, Chihuahua, Mexico – 29.15N / 106.21W	H
Santa Clara*, Chihuahua, Mexico – 29.17N / 107.01W	H
Cuatro Cienegas, Coahuila, Mexico – 26.59N / 102.05W	B, I; 15081-1311.4 (.5, .6), E67.6 (.6D, .6G, .6K)
Monclova*, Coahuila, Mexico – 26.54N / 101.25W	H
Muzquiz, Coahuila, Mexico – 28.40N / 100.30W	H
Ramos Arizpe, Coahuila, Mexico – 25.33N / 100.58W	H
Saltillo, Coahuila, Mexico – 25.25N / 101.00W	H
Mexico City, D. F., Mexico – 19.23N / 99.08W	H
San Miguel de Allende, Guanajuato, Mexico – 20.55N / 100.45W	N
Taxco, Guerrero, Mexico – 18.33N / 99.36W	H
Ixmiquilpan, Hidalgo, Mexico – 20.29N / 99.14W	H
Jacala, Hidalgo, Mexico – 21.01N / 99.11W	H
Metztitlan (Gan. Las Vaquerias), Hidalgo, Mexico – 20.36N / 98.45W	A, A1015 WE, MA, GH, viii / 1997 (stock ident. MW); A1016 WE, MA, GH, viii / 1997 (stock ident. MW); 107044, 107047, 216, 219
Pachuca, Hidalgo, Mexico – 20.07N / 98.44W	H
San Pedro Mines, Hidalgo, Mexico – 21.23N / 98.31W	I; E26.12, E47.36E

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Santa Maria de Regla, Hidalgo, Mexico – 20.15N / 98.33W	B; A961A WH, EP, ED, v / 1992 – 3 ind. asp: *Opuntia sp.* (stock ident. MW); 15081-1311.11
Zimapan, Hidalgo, Mexico – 20.14N/ 99.21W	N
Guadalajara, Jalisco, Mexico – 20.40N / 103.23W	N
Guadalupe, Jalisco, Mexico – 20.51N / 103.20W	H
Tequila, Jalisco, Mexico – 20.53N / 103.50W	H, N
State-line, Jalisco – Guanajuato, Mexico – 21.31N / 101.32W	A; B3
Teotihuacan, Mexico, Mexico – 19.41N / 98.51W	H
Zempoala, Mexico, Mexico – 19.03N / 99.19W	H
Camecuaro, Michoacan, Mexico – 19.55N / 102.17W	H
Carapan, Michoacan, Mexico – 19.52N / 102.03W	H
Patzcuaro, Michoacan, Mexico – 19.31N / 101.36W	H, K
Alpuyeca, Morelos, Mexico – 18.44N / 99.16W	H
Hualahuises, Nuevo Leon, Mexico – 24.53N / 99.40W	H
Monterrey, Nuevo Leon, Mexico – 25.40N / 100.19W	H
Sabinas Hidalgo, Nuevo Leon, Mexico – 26.30N / 100.10W	B; 15081-1311.8, SB21.1W
Huajuapan de Leon, Oaxaca, Mexico – 17.48N / 97.46W	K
Oaxaca, Oaxaca, Mexico – 17.02N / 96.44W	H
Astacingo, Puebla, Mexico – 18.56N / 97.54W	H
Petlalcingo, Puebla, Mexico – 18.05N / 97.54W	H
Tehuacan, Puebla, Mexico – 18.27N / 97.23W	N
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A, D; A955 WH, HP, ED, ix / 1991; A1048 WE, MA, PO, DO, HW, i / 2002 – 245 ind.; 105427, 108281
San Juan del Rio, Queretaro, Mexico – 20.23N / 100.00W	N
San Luis Potosi, San Luis Potosi, Mexico – 22.09 / 100.59W	B, I; 15081-1311.2, E30.6
Arriaga, San Luis Potosi, Mexico – 21.54N / 101.23W	H
Los Mochis, Sinaloa, Mexico – 25.46N / 108.58W	A236 DF, WH, i / 1969 – 2 ind. ex: *O. wilcoxii*
Alamos, Sonora, Mexico – 27.01N / 108.58W	I, M; A81 JR, xi / 1962 – 35 ind. ex: *Opuntia sp.*; A659 WH, MW, x / 1976 – 1 ind. ex: *Opuntia sp.*; A892 WE, AR, iii / 1985 – 161 ind. ex: “O. velutina complex”, E37.2
Caborca, Sonora, Mexico – 30.43N / 112.09W	A854 AR, RT, ii / 1984 – 194 ind.

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Guaymas (Punta Gorda), Sonora, Mexico – 27.56N / 110.54W	A, A909, MH, DS, xii / 1985 – 152 ind. ex: *O. wilcoxii*; 102274,
Hermosillo, Sonora, Mexico – 29.04N / 110.58W	G, H
Magdalena, Sonora, Mexico – 30.19N / 110.44W	G; H; A94 JR iii / 1963 – 44 ind. ex: *Opuntia sp.*
Navojoa, Sonora, Mexico – 27.06N / 109.28W	M; A80 JR, ix / 1962 – 1 ind. ex: *Opuntia sp.*; A233 WH, DF, SJ, i / 1968 – 6 ind. ex: *O. wilcoxii*; A298 WH, BF, i / 1971 – 28 ind. ex: *Opuntia sp.*; A893 WE, AR, iii / 1985 – 25 ind. ex: *O. wilcoxii*
Playa Cochorit, Sonora, Mexico – 26.42N / 109.30W	M; A881 WH, MH, DS, xii / 1984 – 74 ind. ex: *O. violacea*; A890 WE, AR, iii / 1985 – 10 ind. ex: *O. violacea*
Yecora, Sonora, Mexico – 28.22N / 108.55W	A966 GH, ix / 1992 – 15 ind.
Hidalgo, Tamaulipas, Mexico – 26.03N / 98.12W	H
Perote, Veracruz, Mexico – 19.34N / 97.14W	H
Ashton Draw, Arizona, USA – NF	F
Cave Creek, Arizona, USA – 33.50N / 111.57W	F
Flagstaff, Arizona, USA – 35.12N / 111.39W	F
Hart Prairie, Arizona, USA – 35.20N / 111.44W	F
Prescott, Arizona, USA – 34.32N / 112.28W	F
Ramsey Canyon, Arizona, USA – 31.25N / 110.17W	F
Rose Creek, Arizona, USA – 33.50N / 110.59W	F
San Bernardino, Arizona, USA – 32.59N / 113.19W	F
Tucson (Saguaro NME), Arizona, USA – 32.13N / 110.59W	A, B, I; 105612 PO, ii / 1999; A52 WH, i / 1962 – 176 ind. ex: *F. wislizeni*; A59 WH, ii / 1962 – 590 ind. ex: *O. ficus-indica*; A67 WH, iv / 1962 – 5539 ind. ex: *O. ficus-indica*; A102 WH iv / 1963 – 58 ind. ex: *O. ficus-indica*; A413 WS, i / 1973 – 75 ind. ex: *O. ficus-indica*; A666 WH, ii / 1977 – 18 ind. ex: *O. phaeacantha*; A765 WH, i / 1980 – 364 ind. ex: *O. ficus-indica*; A862 CM iii / 1984 – 10 ind. ex: *F. wislizeni*; A864 AR, iii / 1984 – 53 ind. ex: *O. phaeacantha*; A884 WH, iii / 1985 – 39 ind. ex: *O. lindheimeri*; A885 WE, iii / 1985 – 15 ind. ex: *O. phaeacantha*; A908

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
	WH, JR, xi / 1985 – 19 ind. ex: *O. phaeacantha*; A912 WH iii / 1986 – 694 ind. ex: *O. ficus-indica*; A914 WH, iv / 1986 – 14 ind. ex: *O. phaeacantha*; A915 WH, iv / 1986 – 727 ind. ex: *O. ficus-indica*; A1030 WH, iii / 2001 – 139 ind ex: *O. ficus-indica*; 105612, 107042, 107046, 15081-1311.9, A286, A1005, 214, 218
Bonita Canyon, New Mexico, USA – 36.37N / 106.12W	F
Alice, Texas, USA – 27.45N / 98.04W	F
Austin (Aldrich Farm), Texas, USA – 30.16N / 97.44W	A, B, F, G J, K, I; 102944, 15081-1311.7, E25.1F, 2513.1
Bell County, Texas, USA – 31.03N / 97.28W	F
Bexar County, Texas, USA – 29.27N / 98.32W	F
Brackettville, Texas, USA – 29.19N / 100.25W	F
Burleson County, Texas, USA – 30.31N / 96.37W	F
Carrizo Springs, Texas, USA – 28.31N / 99.52W	F
Catarina, Texas, USA – 28.21N / 99.37W	F
Chisos Basin (Big Bend NP), Texas, USA – 29.15N / 103.15W	L; WE, iii / 2005 – 2116 ind.
Chisos Mountains, Texas, USA – 29.16N / 103.18W	F
Del Rio, Texas, USA – 26.37N / 98.56W	F
Dilley, Texas, USA – 28.40N / 99.10W	F
Eagle Pass, Texas, USA – 28.43N / 100.30W	F
Eddy, Texas, United State – 31.17N / 97.15W	F
Falfurrias, Texas, USA – 27.14N / 98.08W	F
Fayette County, Texas, USA – 29.52N / 96.55W	F
Florence, Texas, USA – 30.50N / 97.47W	F
Fort Davis, Texas, USA – 30.35N / 103.53W	F
Fort Worth, Texas, USA – 32.43N / 97.19W	F
Geoge West, Texas, USA – 28.20N / 98.07W	F
Georgetown, Texas, USA – 30.38N / 97.41W	F
Gonzales, Texas, USA – 29.30N / 97.27W	F
Grand Canyon, Texas, USA – NF	F
Harlingen, Texas, USA – 26.11N / 97.42W	F
Hays County, Texas, USA – 30.03N / 98.02W	F

......*continued on the next page*

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Hillsboro, Texas, USA – 32.01N / 97.08W	F
Hot Springs, Texas, USA – 29.11N / 102.59W	F
Kinney County, Texas, USA – 29.20N / 100.25W	F
Lamesa, Texas, USA – 32.44N / 101.57W	F
Laredo, Texas, USA – 27.31N / 99.31W	F
Limpia Canyon, Texas, USA – 30.23N / 103.44W	F
Marfa, Texas, USA – 30.18N / 104.01W	F
McAllen, Texas, USA – 26.12N / 98.14W	F
Milano, Texas, USA – 30.43N / 98.52W	F
Ottine Park, Texas, USA – 29.36N / 97.35W	F
Palestine*, Texas, USA – 31.46N / 95.38W	F
Palo Duro, Texas, USA – 34.56N / 101.40W	F
Plano, Texas, USA – 33.01N / 96.42W	F
Pleasanton, Texas, USA – 28.58N / 98.35W	F
Randall County, Texas, USA – 34.58N / 101.54W	F
Round Rock, Texas, USA – 30.31N / 97.41W	F
San Antonio, Texas, USA – 29.26N / 98.29W	F, I
Selma*, Texas, USA – 33.29N / 97.53W	F
Sterling, Texas, USA – 31.50N / 100.59W	F
Thorndale, Texas, USA – 30.37N / 97.13W	F
Three Rivers, Texas, USA – 28.28N / 98.11W	F
Travis County, Texas, USA – 30.20N / 97.48W	F
Uvalde, Texas, USA – 29.13N / 99.47W	F
Uvalde County, Texas, USA – 29.22N / 99.46W	F
Victoria*, Texas, USA – 28.48N / 97.00W	F
Waco, Texas, USA – 31.33N / 97.08W	F
Zion National Park, Utah, USA – 37.18N / 113.03W	G
No collecting information is available.	A; 107043, 107045, 215, 217
D. mainlandi	
Arroyo Socorro, Baja California, Mexico – 30.18N / 115.40W	A, I; A519 WH, WS, DV, MJ, vii / 1974 – 138 ind.; 102957
Ejido Uruapan, Baja California, Mexico – 31.37N / 116.26W	A754 WH, JR, JF, WS, xii / 1979 – 91 ind. ex: *O. ficus-indica*

......*continued on the next page*

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
San Borja, Baja California, Mexico – 28.47N / 113.55W	A972 PO, iv / 1994 – 6 ind.
San Telmo, Baja California, Mexico – 30.58N / 116.06W	A762 WH, JR, JF, WS, xii / 1979 – 60 ind. ex: *O. oricola*
Puerto Balandra, Baja California Sur, Mexico – 24.19N / 110.18W	A789 WH, WS, i / 1981 – 24 ind.
Punta Agua Verde, Baja California Sur, Mexico – 25.03N / 111.12W	A978 WE, CB, CI, iii / 1996 – 6 ind.
San Bartolo*, Baja California Sur, Mexico – 23.44N / 109.52W	A793 WH, WS, i / 1981 – 11 ind.
Santiago, Baja California Sur, Mexico – 24.48N / 110.02W	A976 WE, CB, CI, iii / 1996 – 4 ind.
Arcadia, California, USA – 34.08N / 118.02W	M. Wasserman notes (1975); A751 WH, MH, x / 1979 – 60 ind.
Bell (Los Angeles), California, USA – 33.59N / 118.10W	F, G, I
El Cajon, California, USA – 32.47N / 116.58W	M. Wasserman notes (1975)
Lauro Canyon, California, USA – 34.27N / 119.44W	F, G
Mission Canyon*, California, USA – 34.27N / 119.43W	F, G
Oxnard, California, USA – 34.12N / 119.10W	A951 AB, iv / 1991 – 4 ind. ex: *Opuntia sp*
Palos Verdes Peninsula, California, USA – 33.46N / 118.22W	A852 WH, MH, DS, i / 1984 – 163 ind. ex: *O. oricola*
Pine Valley, California, USA – 32.49N / 116.31W	A520 WH, WS, DV, MJ, vii / 1974 – 2 ind.
Santa Barbara Island, California, USA – 33.28N / 119.02W	A853 RG, i / 1984 – 15 ind.
Santa Catalina Island, California, USA – 33.23N / 118.25W	A, B, D; A826 WH, MH, DS, x / 1981 – 70 ind.; A956 WH, HP, ED, xi / 1991 – 1163 ind. + 59 ind. ex: *O. demissa*; 102275, 15081–1315.0
Santa Cruz Island, California, USA – 34.01N / 119.43W	A825 JM, x / 1981 – 190 ind.; BC, xii / 2004 – 252 ind.
D. mathisi	
Patagonia, Arizona, USA – 31.32N / 110.46W	I; A6.4
Portal, Arizona, USA – 31.55N / 109.08W	I
Whitewater, New Mexico, USA – 32.35N / 108.08W	I; 2360.2
D. pachuca	
Pachuca, Hidalgo, Mexico – 20.07N / 98.44W	A, B, I, J, K, N; 106315, 15081-1391.5 (.14), E16.1 (.1A), 2519.1 (.20, .21), E29.17, 401.3A2 (.3N2, .4D2, .4G, .4T, .4U, .4Y)

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
San Pedro Mines, Hidalgo, Mexico – 21.23N / 98.31W	B, I; 15081-1391.7 (.8), E26.1 (.3-1, .15)
Acolman, Mexico, Mexico – 19.37N / 98.55W	K
Chapingo, Mexico, Mexico – 19.29N / 98.54W	A, B, I, K; 102947, 107027, 107037, 108306, 109212, 15081-1391.0 (.2, .3), E14.3 (.10A, .11A, .12A), E143, 189, 192, 205
Matehuala, San Luis Potosi, Mexico – 23.39N / 100.39W	N
D. propachuca	
Mexico City, D. F., Mexico – 19.23N / 99.08W	I; E7.7A
San Miguel de Allende, Guanajuato, Mexico – 20.55N / 100.45W	K
Metztitlan, Hidalgo, Mexico – 20.36N / 98.59W	A, B; A960 HP, ED, WH, v / 1992 (stock ident. MW); 107034, 107039, 15081-1411.3, 200, 208
Pachuca, Hidalgo, Mexico – 20.07N / 98.44W	A, B, I, J, K, N; 102915, 106318, 15081-1411.1, E13.1A, E16.2A, E29.1, 2519.18
San Pedro Mines, Hidalgo, Mexico – 21.23N / 98.31W	I; E26.3-2
Santa Maria de Regla, Hidalgo, Mexico – 20.15N / 98.33W	A, B; A961B WH, HP, ED, v / 1992 – 27 ind. asp: *Opuntia sp.* (stock ident. MW); 107029, 107036, 15081-1411.4, 195, 204
Tianguistengo, Hidalgo, Mexico – 20.44N / 98.38W	A, B, K; 109215, 15081-1411.0; E15.1
Venados, Hidalgo, Mexico – 20.10N / 98.44W	I; E28.2D
Chapingo, Mexico, Mexico – 19.29N / 98.54W	I; E14.1, E18.3
Huajuapan de Leon, Oaxaca, Mexico – 17.48N / 97.46W	K
Tuxtepec, Oaxaca, Mexico – 18.06N / 96.07W	A1014 / A1021 WE, MA, GH, iii / 1997 – 6 ind. ex: *Opuntia sp.* (stock ident. MW)
Tehuacan, Puebla, Mexico – 18.27N / 97.23W	K, N
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A955 WH, ED, HP, x / 1991 – 143 ind. ex: *Opuntia sp.* (stock ident. MW)
San Juan del Rio, Queretaro, Mexico – 20.23N / 100.00W	K
San Luis Potosi, San Luis Potosi, Mexico – 22.09N / 100.59W	I, B; 15081-1411.3, E30.7
D. ritae	
Encinillas*, Chihuahua, Mexico – 29.15N / 106.21W	H

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Santa Clara*, Chihuahua, Mexico – 29.17N / 107.01W	H
Saltillo, Coahuila, Mexico – 25.25N / 101.00W	H
Mexico City, D. F., Mexico – 19.23N / 99.08W	J; 2521d2
Lerdo, Durango, Mexico – 25.32N / 103.32W	H
San Francisco Del Rincon, Guanajuato, Mexico – 21.01N / 101.51W	A1040/A1042 WE, MA, i/2000 – 2 ind.
San Miguel de Allende, Guanajuato, Mexico – 20.55N / 100.25W	N
Taxco, Guerrero, Mexico – 18.33N / 99.36W	H
Zumpango, Guerrero, Mexico – 17.39N / 99.30W	H
Metztitlan, Hidalgo, Mexico – 20.36N / 98.59W	A960 WH, HP, ED, v / 1992 – 1 ind.
Pachuca, Hidalgo, Mexico – 20.07N / 98.44W	A, H, I; 102948, 401.4c
San Pedro Mines, Hidalgo, Mexico – 21.23N / 98.31W	I; E26.3-3
Zimapan, Hidalgo, Mexico – 20.14N / 99.21W	N
Guadalajara, Jalisco, Mexico – 20.40N / 103.23W	N; A821 WH, EH, MH, viii / 1981 – 12 ind.
Tequila, Jalisco, Mexico – 20.53N / 103.50W	H, N
Pelillos (Valle de Bravo), Mexico, Mexico – 19.11N / 100.08W	H
Camecuaro, Michoacan, Mexico – 19.55N / 102.17W	H
Patzcuaro, Michoacan, Mexico – 19.31N / 101.36W	H, N
Uruapan, Michoacan, Mexico – 19.24N / 102.03W	H
Ixtlan del Rio, Nayarit, Mexico – 21.02N / 104.22W	A1042 WE, MA, i / 2000 – 7 ind.
Hualahuises, Nuevo Leon, Mexico – 24.53N / 99.40W	H
Monterrey, Nuevo Leon, Mexico – 25.40N / 100.19W	H
Oaxaca, Oaxaca, Mexico – 17.02N / 96.44W	H
Petlalcingo, Puebla, Mexico – 18.05N / 97.54W	H
Tehuacan, Puebla, Mexico – 18.27N / 97.23W	I, B, D; 15081-1471.2, 402.2
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A, B; A955 WH, HP, ED, ix / 1991 – 7 ind.; A1048 WE, MA, PO, DO, HW, i / 2002 – 15 ind.; 102344, 105431, 15081-1471.3 (.4)
Arriaga, San Luis Potosi, Mexico – 21.54N / 101.23W	H
Matehuala, San Luis Potosi, Mexico – 23.39N / 100.39W	N
Perote, Veracruz, Mexico – 19.34N / 97.14W	H
Carr Canyon, Arizona, USA – 31.28N / 110.14W	F
Cave Creek, Arizona, USA – 33.50N / 111.57W	F, I; A386

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Chiricahua Mountains, Arizona, USA – 31.56N / 109.23W	F
Flagstaff, Arizona, USA – 35.12N / 111.39W	F
Huachuca Mountains, Arizona, USA – 31.29N / 110.25W	F
Mule Mountain, Arizona, USA – 32.58N / 109.06W	G
Portal, Arizona, USA – 31.55N / 109.08W	I; E32.3
Ramsey Canyon, Arizona, USA – 31.25N / 110.17W	F, G
Rose Creek, Arizona, USA – 33.50N / 110.59W	F
San Bernardino, Arizona, USA – 32.59N / 113.19W	F
Santa Rita, Arizona, USA – 31.43N / 110.52W	G
Tombstone Canyon*, Arizona, USA – 31.26N / 109.36W	F, G
Capitan, New Mexico, USA – 33.33N / 105.34W	F
Glenwood, New Mexico, USA – 33.29N / 108.53W	F, G
Hondo, New Mexico, USA – 33.23N / 105.16W	F
Silver City, New Mexico, USA – 32.46N / 108.17W	F, G
Chisos Basin (Big Bend NP), Texas, USA – 29.15N / 103.15W	WE, iii / 2005 – 9 ind.
Chisos Mountains, Texas, USA – 29.16N / 103.18W	F, G
Grand Canyon, Texas, USA – NF	F
Limpia Canyon, Texas, USA – 30.23N / 103.44W	F, G
Wild Rose Pass, Texas, USA – 30.43N / 103.47W	F, G, I
“from Sonora”	
El Dorado, Sinaloa, Mexico – 24.19N / 107.22W	M; A878 WH, WE, AR, xi/1984 – 10 ind. ex: *O. wilcoxii*
Los Hornos, Sinaloa, Mexico – 25.10N / 107.30W	A, M; A877 WH, WE, AR, xi/1984 – 116 ind. ex: *O. wilcoxii*; 102345, 107028, 107038, 194, 206
Alamos, Sonora, Mexico – 27.01N / 108.58W	A, M; A657 WH, MW, x/1976 – 60 ind. ex: *Opuntia sp*; A892 WE, AR, iii/1985 – 40 ind. ex. “O. velutina complex”; 102345, 102346, 190
Navojoa, Sonora, Mexico – 27.06N / 109.28W	M; A657 WH, MW, x/1976 – 3 ind. ex: O. ficus-indica; A893 WE, AR, iii/1985 – 62 ind. ex: *O. wilcoxii*
D. spenceri	
Cuñaño, Baja California Sur, Mexico – 23.54N / 100.47W	A, B, I; A202 WH, JR, iv / 1968 – 501 ind. ex: *P. pecten-aboriginum*; 109217, 15081-1441.0, E310.1

......*continued on the next page*

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Los Planes, Baja California Sur, Mexico – 23.58N / 109.56W	A270 WH, iii / 1970 – 88 ind. asp: *P. pecten-aboriginum*
Santiago; Baja California Sur; Mexico – 24.48N / 110.02W	A976 WE, CB, CI, iii / 1996 – 1 ind.
Taxco, Guerrero, Mexico – 18.33N / 99.36W	G, H
Zapilote Canyon (Mezcala), Guerrero, Mexico – 17.57N / 99.37W	A1018 WE, MA, PO, GH, i / 1998 – 1041 ind. + 3 ind. ex: *P. weberi*
Chamela, Jalisco, Mexico – 19.32N / 105.05W	A1009 WE, MA, PO, iii / 1997 – 9 ind.
Tequila (El Medinena), Jalisco, Mexico – 20.53N / 103.50W	G, H
Playa Azul, Michoácan, Mexico – 18.40N / 102.05W	A; A1010 WE, MA, SP, GH, iii / 1997 – 3 ind.; 107032, 198
Playa Azul (25 km North), Michoácan, Mexico – 18.45N / 102.05W	A814 WH, EH, viii / 1981 – 21 ind.
Presa del Infiernillo, Michoácan, Mexico – 18.36N / 101.51W	A1017 WE, MA, PO, GH, i / 1998 – 98 ind. + 1 ex: *S. quevedonis*
Uruapan, Michoácan, Mexico – 19.24N / 102.03W	G, H
Alpuyeca (Rio Chalma), Morelos, Mexico – 18.44N / 99.16W	G, H, I
Hualahuises, Nuevo Leon, Mexico – 24.53N / 99.40W	G, H
Monterrey (Huasteca Canon), Nuevo Leon, Mexico – 25.40N / 100.19W	G, H
Huatulco, Oaxaca, Mexico – 15.45N / 96.08W	A1047 WE, MA, PO, DO, HW, i / 2002 – 2 ind.
Zapotitlan de Salinas, Puebla, Mexico – 18.20N / 97.29W	A1048 WE, MA, PO, DO, HW, i / 2002 – 7 ind.; 105430
Tamazunchale, San Luis Potosí, Mexico – 21.16N / 98.47W	G, H
El Dorado, Sinaloa, Mexico – 24.19N / 107.22W	M; A878 WH, WE, AR, xi / 1984 – 5 ind.
Los Hornos, Sinaloa, Mexico – 25.10N / 107.30W	M; A877 WH, WE, AR, xi / 1984 – 1 ind.
Mazatlan, Sinaloa, Mexico – 23.13N / 106.25W	A717 GD, i / 1978 – 250 ind. ex: *P. pecten- aboriginum*
Presa Huites (El Fuerte), Sinaloa, Mexico – 26.54N / 108.20W	A; A1019 JS, CB, viii / 1998 – 6 ind.; 107035, 107041, 201, 211
Empalme, Sonora, Mexico – 27.58N / 110.49W	A239 WH, i / 1969 – 58 ind. ex: *P. pringlei*
Navojoa, Sonora, Mexico – 27.06N / 109.28W	A657 WH, MW, x / 1976 – 10 ind.
Peon (Guaymas), Sonora, Mexico – 27.54N / 110.32W	A, D, M; A875 WH, WE, AR, x / 1984 – 19 ind. ex: *C. gigantea* + 5 ind. ex: *S. thurberi*; 102347

......continued on the next page

APPENDIX 1 (continued)

Locality[1]	References; collecting information; and identification numbers[2]
Punta Onah, Sonora, Mexico – 29.05N / 112.11W	A906 WH, SB, AB, viii / 1985 – 1 ind.
San Carlos, Sonora, Mexico – 27.58N / 111.04W	A, B; A882 MH, DS, xii / 1984 – 20 ind. ex: *F. emoryi*; 102348, 15081-1441.1

1 – Each locality may represent several distinct collections. * There are more than one place in this State with the same name, therefore the localization is tentative; NF – Localities not found.

2 – References: A – Ambrose Monell Cryo Collection, AMNH; B – Tucson *Drosophila* Stock Center; C – Wasserman 1992; D – Durando et al. 2000; E – Etges et al. 2001; F – Patterson and Wagner 1943; G – Patterson 1943; H – Patterson and Mainland 1944; I – Vilela 1983; J – Wasserman 1962; K – Wasserman and Koepfer 1977; L – Etges and Heed 2004; M – Ruiz and Heed 1988; N – Wasserman 1967.

Collectors: AA – Adolpho Alvarado, AB – Andrew Beckenbach, AR – Alfredo Ruiz, BF – Ben Foote, BC – Brian Counterman, CB – Christina Babcock, CD – Celeste Durando, CI – Conrad Istock, CL – Carolyn Lewis, CM – C. May, DC – Dennis Cornejo, DF – David Fellows, DO – Deodoro Oliveira, DS – Daniel Siebert, DV – Don Vacek, ED – Eric Dyreson, EH – Emily Heed, GD – Garry Duncan, GH – Greg Huckins (deceased), HP – Henar Pimentel, HW – Harry Wistrand, JF – Jim Fogleman, JM – John Moore, JMu – Josh Mutic, JR – Jean Russell, JS – Joana Silva, MA – Miguel Armella, MH – Margaret Heed, MJ – Margo Jefferson, MW – Marvin Wasserman, PB – Peter Brussard, PO – Patrick O'Grady, RG – Ronald Garthwaite, RGr – Robin Gray; RR – Richard Richardson, RT – Richard Thomas, SB – Stuart Barker, SJ – Spencer Johnston; SP – Sandra Perez, WE – William Etges, WH – William Heed, WS – William Starmer

Association with cacti species were done by aspirating from rots, sweeping over fruits or other cacti tissue in the field, and by flies emerging from rotting cactus brought to the laboratory. For details contact one of the authors.

