

Morphological Variation in the Forelegs of the Hawaiian Drosophilidae. I. The AMC Clade

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ABSTRACT The Hawaiian Drosophilidae possess spectacular diversity in male foreleg modifications, many of which are unknown in other Diptera. The greatest diversity in foreleg morphology is in the *antopocerus*, *modified tarsus*, and *ciliated tarsus* clade (AMC Clade), a group of 95 species. The *modified tarsus* flies are divided into the *bristle*, *ciliated*, *split*, and *spoon tarsus* subgroups. The *bristle tarsus* species feature one or two rows of thickened setae on the basitarsus. The *split tarsus* species are characterized by only having four tarsal segments, in contrast to five tarsomeres in the remainder of Diptera. Based on comparisons of the apparent ground state of ventral setal rows across the Hawaiian *Drosophila*, we suggest that it is the second tarsal segment which has been lost. The *spoon tarsus* species are characterized by having the second tarsomere modified into a setae-filled, concave-shaped spoon. The *ciliated tarsus* species, all of which possess one or more elongate setae on the tarsus of males, are probably not monophyletic with respect to the *bristle tarsus* subgroup. The *antopocerus* flies are characterized by a long basitarsus, with extensive setation on the tibia and basitarsus of some species. The use of these foreleg modifications in courtship behavior has been previously described and it is suggested that they represent the results of sexual selection. The current work expands on previous morphological analyses, presenting a level of detail not previously possible without SEM images. The new characters revealed will figure prominently in future cladistic studies. *J. Morphol.* 271:86–103, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: Hawaiian Drosophilidae; Drosophilidae; Diptera; foreleg

INTRODUCTION

The Hawaiian Drosophilidae has long been recognized as the premier example of adaptive radiation and rapid speciation in nature (Carson, 1987). The endemic Hawaiian drosophilid fauna is divided into two main lineages, the Hawaiian *Drosophila* and *Scaptomyza* (Throckmorton, 1975; Kaneshiro, 1976), which together comprise a large, diverse radiation, consisting of perhaps 1,000 species (Kaneshiro, 1997). These two genera form a monophyletic group, indicating that all are

descended from a single common ancestor that arrived in the Hawaiian Islands approximately 25 million years ago (Russo et al., 1995). A combination of adaptation to specific host plants and microhabitats (Heed, 1968, 1971; Carson, 1971; Montgomery, 1975; Magnacca et al., 2008), extreme genetic isolation within the geographically dissected Hawaiian Archipelago, and behavioral isolation leading to reproductive barriers between closely related species (Spieth, 1966; Kaneshiro and Boake, 1987), has led to the impressive species diversity in the Hawaiian Drosophilidae.

The Hawaiian Drosophilidae, in addition to having high species diversity, also display an impressive array of morphological variation. Many species have spectacular modifications of their mouthparts, wings, genitalia, and/or forelegs, which are thought to be the result of sexual selection (Kaneshiro and Boake, 1987). The Hawaiian *Drosophila* is divided into eight species groups: *antopocerus*, *modified tarsus*, *picture wing*, *modified mouthpart*, *nudidrosophila*, *ateledrosophila*, *rustica*, and *haleakalae*, all of which possess some male-specific foreleg modifications. Foreleg modification is most extensive in the AMC clade (Magnacca et al., 2008), formerly the Leaf Breeder Clade, a monophyletic group of species including the *antopocerus*, *modified tarsus*, and *ciliated tarsus* species groups (Throckmorton, 1966; Heed, 1968; Baker and DeSalle, 1997; Kambyssellis and Craddock, 1997; Bonacum, 2001; Fig. 1). Spieth (1966) recorded laboratory observations of the courtship behavior of representatives of most groups of Hawaiian *Drosophila* and reported on the widespread use of the leg in mating, particularly within

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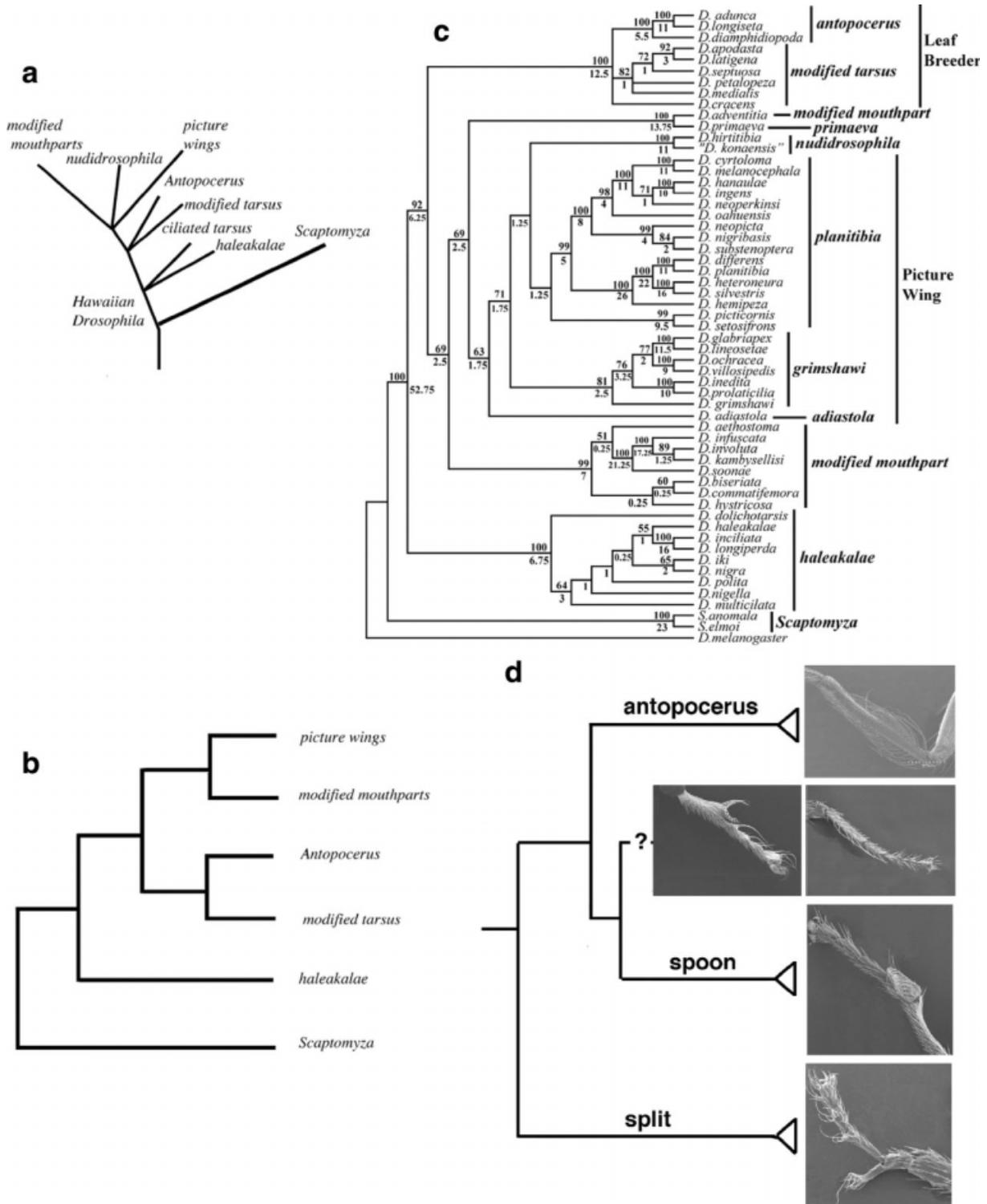


Fig. 1. Proposed phylogenies of Hawaiian *Drosophila* species groups and clades, containing the AMC Clade groups. (a) Throckmorton, 1966. (b) Bonacum, 2001, small dataset. (c) Kambyzellis and Craddock, 1995; Baker and DeSalle, 1997. Numbers above the branches reflect percent bootstrap support, those below the branches are Bremer Support indices. Vertical lines delimit species groups in italics (single horizontal lines show groups with only one representative in the analysis), clades in normal type. *Drosophila melanogaster* is a non-Hawaiian outgroup. (d) After Bonacum, 2001, large dataset, with tarsal features of each group illustrated on the branches. The relationships and monophyly of the bristle tarsus and ciliated tarsus groups are uncertain.

TABLE 1. *Taxa studied*

Group	Subgroup	Species	Locality
<i>modified tarsus</i>	<i>bristle tarsus</i>	<i>D. basimacula</i> Hardy	Kaua'i: Pihea Trail, Na Pali, Kona forest reserve
	<i>bristle tarsus</i>	<i>D. petalopeza</i> Hardy	Maui: Waikamoi preserve
	<i>bristle tarsus</i>	<i>D. quasiexpansa</i> Hardy	Maui: Waikamoi preserve
	<i>split tarsus</i>	<i>D. proceriseta</i> Hardy	Moloka'i: Pu'u Kolelele, Kamakou preserve
	<i>split tarsus</i>	<i>D. variabilis</i> Hardy	Moloka'i: Z68
	<i>spoon tarsus</i>	<i>D. atroscutellata</i> Hardy	Kaua'i: Kokee (in University of Manoa Insect Museum Collection)
<i>antopocerus</i>	<i>spoon tarsus</i>	<i>D. dasyncnemia</i> Hardy	Hawai'i: Solid Waste Transfer Station, Volcano
	<i>spoon tarsus</i>	<i>D. waddingtoni</i> Hardy	Maui: Heed Trail, East Maui Irrigation Co. land
	<i>adunca</i>	<i>D. adunca</i> Hardy	Maui: Waikamoi Preserve
	<i>diamphidiopoda</i>	<i>D. tanythrix</i> Hardy	Hawai'i: O'laa Forest, Telephone Pole #44, Wright Road, Volcano
<i>modified mouthparts</i>	<i>villosa</i>	<i>D. villosa</i> Hardy	Maui: Heed Trail, East Maui Irrigation Co. land
	<i>freycinetiae</i>	<i>D. comatifemora</i>	
<i>picture wings</i>	<i>mitchelli</i>	<i>D. hystricosa</i>	
	<i>punalua</i>	<i>D. punalua</i>	

species of the AMC clade. The goal of this article is to survey the diversity of male foreleg morphology in the Hawaiian *Drosophilidae* using scanning electron microscopy. Specific attention is paid to the high diversity within the AMC clade species and how these modifications may differ from other groups of Hawaiian *Drosophila*. Such detailed analysis will facilitate future cladistic studies on this remarkable group of Diptera.

MATERIALS AND METHODS

Collections and Taxon Sampling

Specimens were field collected by either general sweeping or bait trapping with fermented banana or mushroom bait and maintained in sugar-vials until they could be identified. The specimens were subsequently preserved in 70% ethanol. The species under study, and the locality information, are summarized in Table 1. An attempt was made to include species representing each subgroup within the *modified tarsus* and *antopocerus* groups. The *antopocerus* (Hardy, 1977) and *split tarsus* (Hardy and Kaneshiro, 1979) subgroups have already been proposed in the literature. Others are to be proposed in future work. *Drosophila basimacula* is representative of *bristle tarsus* flies possessing a dorsal brush and anterior comb of strong bristles. *Drosophila quasiexpansa* is included as a further representative of this group. *Drosophila petalopeza* is included because of its unique modification, among the *bristle tarsus* group flies, of the mesothoracic tarsi. *Drosophila proceriseta*, of the *split tarsus* subgroup, is included because it is a representative of the species complex with short lower reclinate bristles. *Drosophila variabilis* is a representative of the species complex with long lower reclinate bristles. *Drosophila atroscutellata* represents both the *spoon tarsus* species complex possessing four strong anteroventral setae on the basitarsus and also possesses a unique, and perhaps plesiomorphic, shallow and nonpilose configuration of the spoon. *Drosophila dasyncnemia* and *Drosophila waddingtoni* both belong to the *spoon tarsus* complex of species with a strong porrect seta on the dorsal apex of the spoon. The *antopocerus* group is represented in our study by *Drosophila adunca*, of the *adunca* subgroup, *Drosophila tanythrix*, of the *diamphidiopoda* subgroup, and *Drosophila villosa*, of the *villosa* subgroup, the study thus examining one species from each of the subgroups proposed by Hardy. *Drosophila comatifemora* and *Drosophila hystricosa* of the *modified mouthparts* group (*D. hystricosa* of the *mitchelli* subgroup, *hystricosa* complex), as well as *Drosophila punalua* of the *picture wings* group, *punalua* subgroup, are included for comparison of the disposition of ventral tarsal setal rows among the Hawaiian *Drosophila*.

Scanning Electron Microscopy. Before critical point drying, the specimens were dehydrated in an ethanol series of 80, 90, and 100%. The specimens were critical point dried in a Balzers CPD 030 critical point dryer and mounted on SEM stubs with the aid of a Nikon SMZ-2T stereoscopic microscope. The specimens were Au-Pd coated with a Denton Desk II Sputter Coater and observed on a Hitachi S-4700 scanning electron microscope.

Morphological Terminology

The leg is subdivided into the femora, tibia, and tarsi (Fig. 2a). The terms used to describe the morphological characteristics of legs can be divided into several categories: 1) shape of the leg segments and various segment structures, 2) chaetotaxy and sculpturing, and 3) spatial orientation of leg segments, setae, and cilia.

Legs and leg segments are characterized by having a variety of shapes. Leg segments can be narrowed or broadened relative to other leg segments, typically at the base or apex of the segments or along a variety of axes (e.g., posteriorly).

Chaetotaxy in the Hawaiian *Drosophilidae* is very diverse. Setae are sclerotized, hair-like processes (Torre-Bueno, 1989). Tarsal pegs are very stout, specialized setae, located on the ventral surface of the leg segments (Grimaldi, 1990). Grimaldi (1990) restricted his definition of these setae to the second and third legs, but this is extended here to describe the ventral setae fitting the description on the foretarsi. Within the AMC Clade, the preponderance of tarsal modifications are seen in the first and second tarsomeres. Within this clade, and the other examined Hawaiian *Drosophila*, the terminal three tarsomeres bear two ventral rows of pegs, whereas the number of ventral rows on the first and second segment is greater than two. Cilia are also hair-like, but tend to be thinner and more elongate than setae. Scaliform processes are present on the proximal side of many setal sockets, cuticular rings (Peterson, 1948, 1951) into which the setae are inserted (Fig. 2b). Hairbeds (Chapman, 1998) are groupings of tiny filaments, probably tonic receptors (Fig. 2c). Setae and cilia typically form more or less regular rows, although they can be found inserted individually. Some Hawaiian *Drosophila* have stout setae arranged in combs ("peculiar comb"; Hardy, 1965) or brushes (Fig. 3b, inset). Combs are defined by arrangement in a series in which the contours of the strongly bent setae are parallel. Brushes are defined by the arrangement in a series of straight or somewhat curved setae that approximate or meet at their apices. Sculpturing (Torre-Bueno, 1989) can occur on setae as well as on the cuticle. The surface of the setae may be smooth, or in the case of those with thicker diameter, striated, or twisted. *Drosophila petalopeza*, treated here, has a unique (for Hawaiian

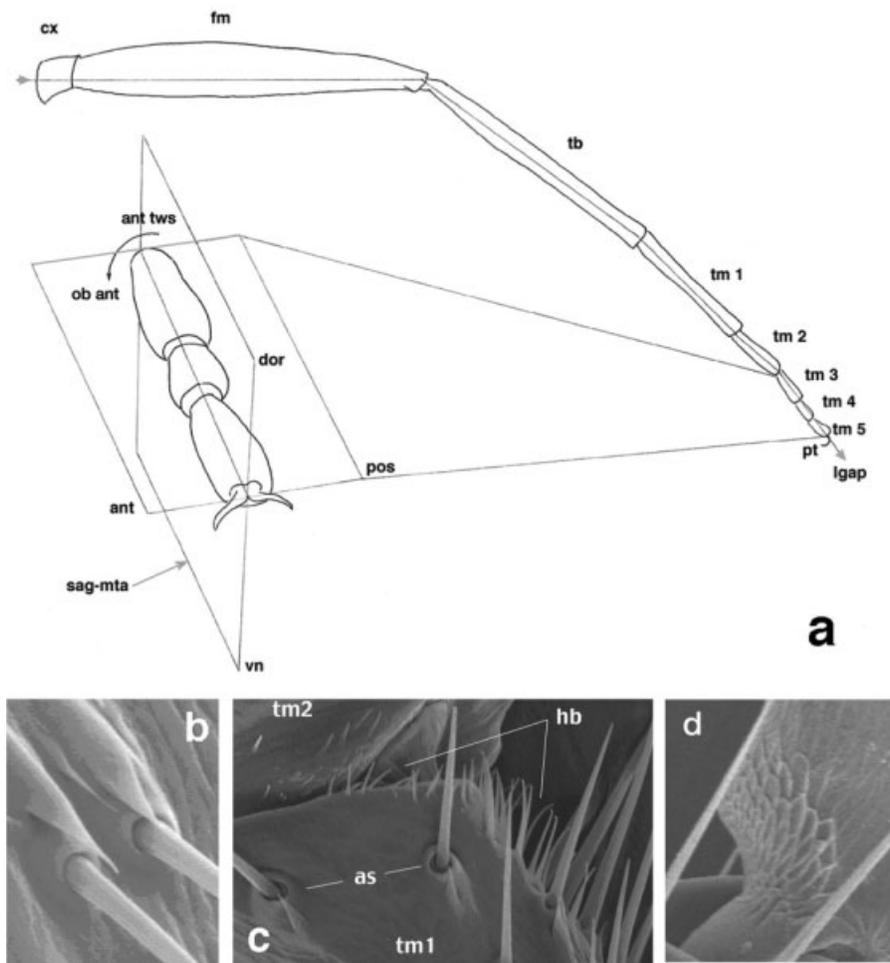


Fig. 2. Modified Tarsus Fly leg orientation and features. (a) Leg orientation. A labeled side view of a fly leg with detail of the apical three tarsomeres is provided as a reference for the text and figures. ant, anterior; ant tws, anterior twist; cx, coxa; dor, dorsal; fm, femur; lgap, leg apex; ob ant, obliquely anterior; pt, pretarsus (used throughout the figures); sag-mta, sagittal plane/main tarsal axis; tb, tibia; tm 1,2,3,4,5, tarsomeres 1 (also known as the basitarsus) to 5 (these abbreviations used throughout the figures); vn, ventral. (b) Setal socket scaliform processes (from *Drosophila dasycnemia*). (c) Hairbeds (from *Drosophila waddingtoni*). as, apical setae; hb, hairbeds (cf. Fig. 4a). (d) Sculpturing on the proximal anterior basitarsus (from *Drosophila petalopeza*).

Drosophila), truncated setal type characterized by the unequal length of the anterior and posterior sides of the seta, producing a flattened “cut-off” end (Fig. 3e, inset). The pronounced twisted striations on tarsal pegs have been referred to as “cuneiform” (de Castro, 1953).

Several terms are used to describe chaetotaxy. Inclined setae are those whose angle of insertion is less than 60° to their inserted surface. Semiporrect setae are those whose angle of insertion equals or exceeds 60° to their inserted surface. Porrect setae are those which are perpendicular, or nearly so, to their inserted surface. Curved setae are those bent from the perpendicular. Sinuous setae are those in the form of a sine-wave. Wavy setae are those which have more than two opposing curves along their length. Strong setae are those which have a basal diameter at least twice that of the thinnest setae on the segment. The setae may be inserted in a linear row or they may be alternately staggered along a line passing between the points of insertion.

In describing the spatial orientation of leg segments, setae, and cilia, we use standard terminology such as is found in Hardy (1965) in which the orientation of the leg is given as if the leg was fully extended to the side of the body, such that anterior faces front, dorsal is upward, etc. The setae and cilia are

treated in a similar fashion with respect to the leg on which they are situated. It is important to remember, however, that in life, the forelegs especially are held in an orientation such that anterior is virtually medial with respect to the body and posterior thus virtually lateral. Hardy himself recognized this, using terms such as “posterolateral” in his 1966 “Descriptions and notes on Hawaiian *Drosophilidae*” to refer to foretarsal morphology. To avoid confusion, we remain with the earlier terminology. The tarsomeres have a great range of rotation about the axis of the leg so that these orientations are hardly fixed in the living fly. These considerations become paramount in the courtship function of the tarsomeres as described by Spieth (1966, 1968). In numbering parallel rows of setae (e.g., setal row 1, setal row 2), the dorsal most row receives the number 1.

RESULTS

The modified tarsus Group

The configuration of the brush and comb setae, the specially modified, anteriorly concave spoon on the second tarsal segment, and the orientation of

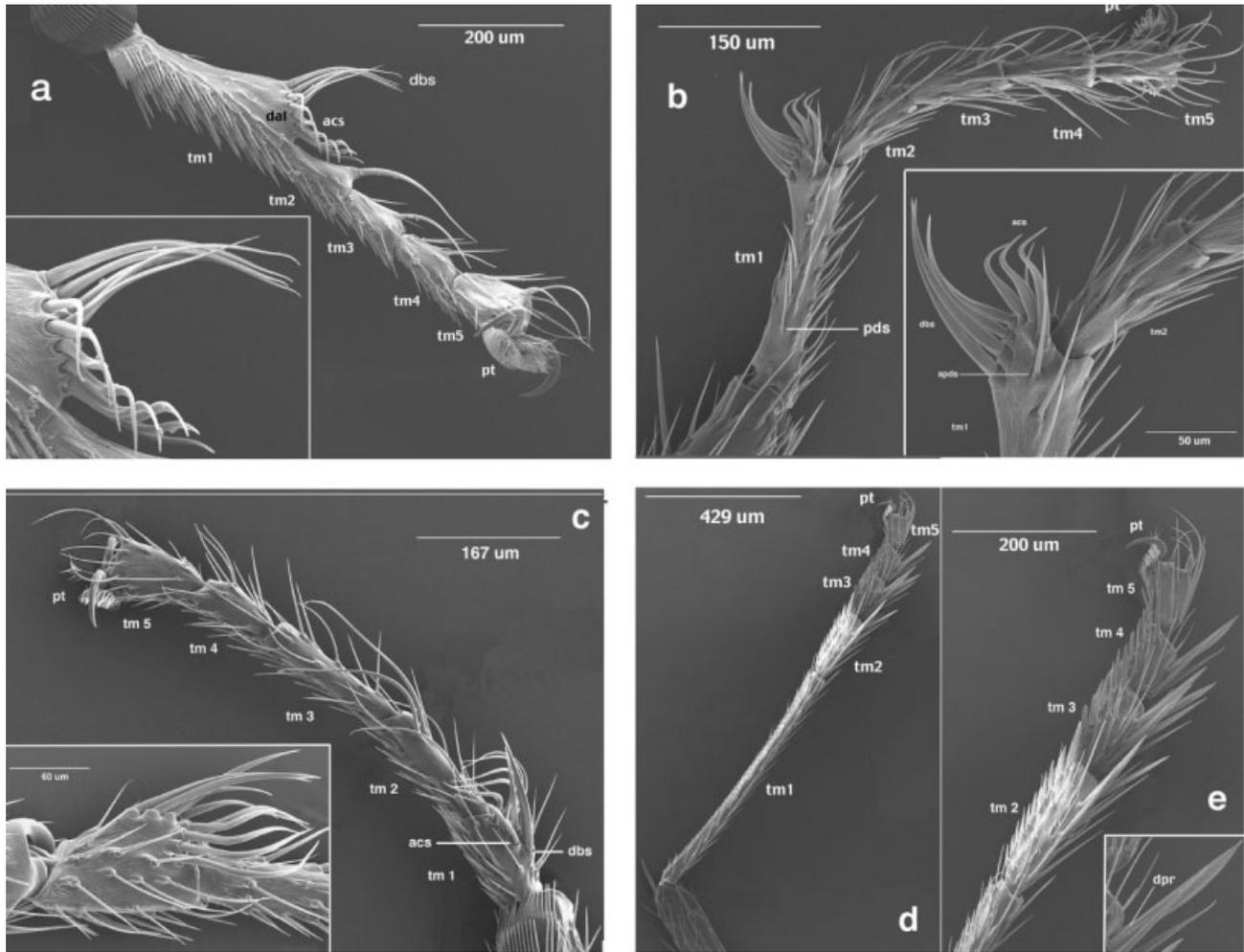


Fig. 3. *Bristle tarsus* group tarsi. (a) *Drosophila basimacula*, left foretarsus, male, anterior view; inset, setal brush and comb. (b) *Drosophila petalopeza*, right foretarsus, male, posterodorsal view; inset, setal brush and comb, second tarsomere. (c) *Drosophila quasiexpansa*, right foretarsus, male, anterior view; inset: setal brush and comb, posterior view. (d) *Drosophila petalopeza*, left mid-tarsus, male, anterior view. (e) *Drosophila petalopeza*, midtarsus, ultimate four tarsomeres; inset, fourth tarsomere; large subapical truncate seta. acs, anterior comb setae: an anteroapical row of setae, whose contours parallel each other; apds, apicodorsal seta; dal, dorsoapical lobe; dbs, dorsal brush setae: a dorsoapical group of setae which meet at a point; dpr, depression on ventral side of modified truncate setae; pds, posterodorsal setae.

tarsomeres and lack of a fifth tarsal segment are all characters which define the three subgroups (*bristle*, *spoon*, and *split*) of the *modified tarsus* species group. These physical features of the foretarsi are unique to Hawaiian *Drosophila*. The *ciliated tarsus* flies lack such a decisive synapomorphy and recent evidence suggests their close relationship to the *bristle tarsus* subgroup (Bonacum, 2001). Examination of the *ciliated tarsus* flies by the criteria established here and elsewhere (Hardy, 1965), specifically the presence of strong dorsal brush and/or anterior comb setae, indicate that *D. clavitibia* and *D. fusticula* are more appropriately placed in the *bristle tarsus* subgroup. Scanning electron microscopy is used below to describe some of these unique structures in detail.

The *bristle tarsus* Subgroup

There are 17 species in this group: *D. apicisetae*, *D. apodasta*, *D. basimacula*, *D. bicondyla*, *D. brevitarisus*, *D. brunneisetae*, *D. expansa*, *D. gubleri*, *D. lemniscata*, *D. perissopoda*, *D. petalopeza*, *D. prodita*, *D. quasiexpansa*, *D. redunca*, *D. seclusa*, *D. torula*, and *D. trichaetosa*. Throckmorton (1966) and Kaneshiro (1976), based on internal morphology and male genitalic characters, considered these taxa to belong in the *modified tarsus* species group, the sister taxon of the *antopocerus* group. Recently, Bonacum (2001) has suggested that the *bristle tarsus* subgroup may not be monophyletic with respect to the *ciliated tarsus* species group and the other subgroups within the *modified tarsus* group. More detailed morphological and molec-

ular systematic analysis of these taxa will be necessary to fully resolve this issue, but based on detailed examination some changes are proposed later.

All the species of the *bristle tarsus* subgroup are characterized by the setal ornamentation of the dorsoapical portion of the basitarsus. Most species (*D. apicisetae*, *D. apodasta*, *D. basimacula*, *D. expansa*, *D. perissopoda*, *D. petalopeza*, *D. quasiexpansa*, *D. redunca*, and *D. seclusa*) have two groups of strong setae in this region of the basitarsus, one porrect and another inclined. Other taxa (*D. bicondyla*, *D. brevitarsus*, *D. brunneisetae*, *D. gubleri*, *D. lemniscata*, *D. prodita*, and *D. torula*) possess only a dorsoapical group of well-developed setae, although these are not arranged in a brush as those species mentioned earlier. There is also frequent dorsoventral expansion of the apex of the basitarsus and second tarsomere, as in *D. lemniscata* and *D. torula*. Variation among the species in this group (Hardy, 1965, 1966) involves the number and disposition of the dorsoapical setal group of the basitarsus, the relative length of the basitarsus, dorsal setation of the succeeding tarsal segments, and dorsoventral expansion of the apex of the basitarsus and of the second tarsomere. The foretarsi of *D. basimacula* and *D. quasiexpansa* and the fore- and mid-tarsi of *D. petalopeza* are examined. All are members of the complex possessing two groups of strong setae on the dorsoapex of the basitarsus, although *D. basimacula* differs from *D. petalopeza* and *D. quasiexpansa* in that the anterior comb bristles curve first posteriorly and then anteriorly, the opposite of the other two. This may indicate the need for further subdivision of the complex into species clusters, but the orientation of the tarsomeres of all species in the complex must first be examined. *D. petalopeza* is particularly interesting in that it is one of the only Hawaiian Drosophilidae with extensive modifications present on the mesothoracic set of legs. This species was, as a result of this extensive modification and that of the orbital bristles, originally placed in its own subgenus, *Trichotobregma* (Hardy, 1965).

Drosophila basimacula. The basitarsus of *D. basimacula* is expanded apically, starting roughly in the middle of the segment (Fig. 3a). The widest point of this lobe is roughly twice the width of the remainder of this segment. The dorsal brush and anterior comb setae are well-developed and show prominent striation along their length (Fig. 3a, inset). The dorsal brush consists of five curvate setae. The anterior comb, consisting of eight sinuous setae, is inserted ventral to the brush, the setae oriented gradually from the anterior at its proximal end to apically at its most distal. The two series introgress at the apical end of the brush and the proximal end of the comb. The most distal seta in the comb closely approximates the outline of the second tarsomere, which lies ventral to it.

Drosophila petalopeza. On the mesothoracic leg of *D. petalopeza* is a remarkable brush on the tarsomeres. Starting with the second tarsomere, there are 12 rows of setae, size-graduated from proximal to distal, the distal setae slightly more than three times the length and twice the basal width of the proximals. The striations on these setae, as on several other setae on the remaining tarsomeres, show the unusual feature of appearing cut-off: instead of all ending at the apex of the seta, most of the striations end before the apex at a slanted edge (Fig. 3e). Some of the larger setae have a depression on their anterior side, where the striations converge (Fig. 3e, inset). This cut-off setal feature is responsible for the brush-like gestalt of tarsomeres two, three, and four (Fig. 3e). The second tarsomere on the mid leg is a little more than one-quarter the length of the basitarsus and tapered toward its proximal end (Fig. 3d). In addition to the scaliform processes usually present on the setal sockets, thin supernumerary processes also adorn many of the sockets.

The forebasitarsus of *D. petalopeza* is two-thirds the length of the remaining segments combined and 40% narrower at its midpoint than at its ends, expanding gradually toward each end (Fig. 3b). The dorsal brush setae are inserted on an apical expansion of the segment (Fig. 3b, inset). There are four of these very strong setae with prominent striations, the largest 2.5 times wider than the largest setae of the anterior (other side, not visible) and posterior (pds) rows 1 and 2 and almost half the length of the basitarsus, inserted semiporrectly to porrectly in a descending-size series, the apicalmost and smallest just short of the apex, and all curved toward the leg apex. The dorsal brush series is met, at its apical end, by a series of three similar setae, inserted along the apical dorsal edge of the segment. These apical comb setae are curved first anteriorly, then bend sharply, roughly perpendicularly (the ventralmost seta is bent exactly perpendicularly in this specimen), toward the leg apex. The apical comb setae are enclosed by the apical setae of the anterior and posterior (Fig. 3b, inset, apds) rows. Apart from the chaetotaxy, there is some unusual scale-like sculpturing at the proximal end of the basitarsus (Fig. 2d).

Drosophila quasiexpansa. The basitarsus of *D. quasiexpansa* is cylindrical, slightly longer on its ventral side (Fig. 3c). The three dorsal brush setae are inserted on an expansion of the dorsal surface of the segment in parallel with the sagittal plane (Fig. 2a) and are the strongest setae on the segment, 50% wider at their bases than the largest apical comb setae (Fig. 3c, inset). There are seven apical comb setae, arrayed at an approximate right angle on the segment, the ventralmost aligned with the apical setae of posterior setal row 1. The middle seta of the apical comb is anterior to the apical seta of the dorsal brush and the remaining

three setae are anterior and parallel to the dorsal brush setae.

Summary

The apical swelling on the basitarsus of *D. basimacula* is much more prominent, doubling the width of the segment, than it is in *petalopeza* or *quasiexpansa*. The number of dorsal brush and apical comb setae is variable in the three species, with five and eight in *basimacula*, four and three in *petalopeza*, and three and seven in *quasiexpansa*, but their configuration is similar in all. The sculpturing seen at the proximodorsal end of the *petalopeza* basitarsus is also found in the other two species, but in different configurations.

The setae of the remaining tarsomeres are much more developed in *petalopeza* than in the other two species, although the apical dorsal setae of *basimacula* are particularly well-developed. The pretarsal claws of *basimacula* are relatively smooth, with a few extra teeth along their dorsal mid-point, whereas the dorsum of those of *petalopeza* are toothed from their proximal end to past the mid-point.

Some behavioral observations give us clues to the possible function of these characters. Spieth (1966) observed *D. basimacula* striking the female's genitalia. *Drosophila petalopeza*, in contrast, extends its forelegs and grasps the female's genitalia with the porrect setae from below after signaling the female with the ornamented tarsi of the extended mesothoracic legs.

The *split tarsus* Subgroup

There are currently 24 described species in the *split tarsus* subgroup: *D. ancyla*, *D. attenuata*, *D. basisetosa*, *D. capitata*, *D. chaetocephala*, *D. clavata*, *D. cnecopleura*, *D. cornutitarsus*, *D. cracens*, *D. dicropeza*, *D. dorsigera*, *D. enoplotarsus*, *D. forficata*, *D. fundita*, *D. furcatarsus*, *D. kokeensis*, *D. paracracens*, *D. paucitarsus*, *D. pectinitarsus*, *D. proceriseta*, *D. propiofacies*, *D. spiethi*, *D. systemopeza*, and *D. variabilis*. One outstanding synapomorphy of this subgroup is the presence of only four tarsal segments, rather than the typical five segments in the remainder of the order Diptera. The second tarsomere is attached to the ventral surface of the basitarsus. As a result, a significant portion of the basitarsus extends beyond this insertion point. Hardy and Kaneshiro (1979) informally, but not explicitly, proposed species complexes based on the disposition of the orbital bristles and the coloration of the notum. *Split tarsus* species with lower reclinates less than twice the length of proclimates: *ancyla* complex, *ancyla* cluster. *Split tarsus* species with lower reclinates less than twice the length of proclimates and having a yellow mesonotum and dark scutellum: *ancyla*

complex, *clavata* cluster. *Split tarsus* species with lower reclinates at least twice the length of proclimates: *basisetosa* complex. Many species in the *split tarsus* subgroup are also characterized by a brush of setae on the apex of the first tarsomere and variations between the species involve primarily differences in the chaetotaxy and shape of the basitarsus. The tarsus of *D. proceriseta*, a *basisetosa* complex species found on Molokai, is examined. As a representative of the *ancyla* complex, *D. variabilis* is examined.

Drosophila proceriseta. The second tarsomere of *D. proceriseta* is inserted into a strongly expanded socket located at about 2/5 of the distance of the basitarsus from the junction of the tibia and basitarsus (Fig. 4a). With the exception of a hair bed, there are no ventral setae on the basitarsus distal to the insertion of the second tarsomere. This hair bed is on the proximal half of the basitarsal extension (Fig. 4a). The basitarsus bends posteriorly distal to the insertion of the second tarsomere. There is coarse sculpturing of the cuticle on the surface of the basitarsus. Finer sculpturing is present at the proximal end of this segment, both above and below the articulation with the tibia. A brush of large, anteriorly curved setae is inserted on the dorsal surface of the basitarsus, beginning at about 2/3 the length of this segment. Several wavy setae are also present at the apex, with the largest being more than twice the length of the others. There are also three straight anteriorly directed setae, of similar strength as the curved setae, inserted perpendicular to the apical setae on the anterior side of the basitarsus. The second tarsomere lacks setae on its basal half.

Drosophila variabilis. *Drosophila variabilis* has the second tarsomere inserted on the ventral surface of the basitarsus, about 1/3 the length from its distal end (Fig. 4b). The segment is posteriorly curved distal to the insertion of the second tarsomere, such that this portion of the segment is concave. Apart from a ventral depression adjacent to the insertion of the second tarsomere, the diameter of the distal portion is subequal to that of the proximal portion. There is coarse sculpturing along the ventral, posterior, and anterior surfaces of the tarsomere. The basitarsus is truncated apically, with a brush of posteriorly directed curved setae apicodorsally, and four (one is missing in our specimen) wavy setae at the apex, one more than twice the length of the others. There are two strong and four less-developed distally directed setae inserted perpendicularly to the other apical setae on the anterior side of the segment.

Summary

The relative length of the basitarsus to the remaining segments is about two-thirds in *D. proceriseta* and just over one-half in *variabilis*. The

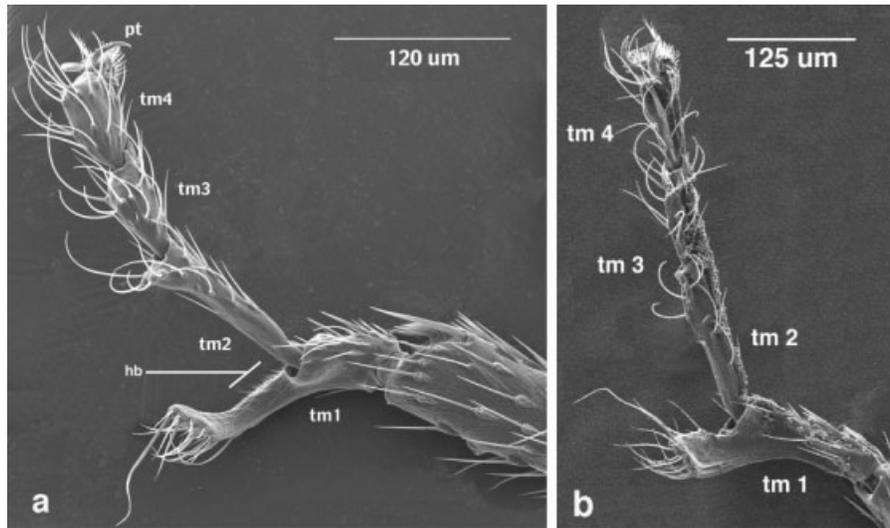


Fig. 4. *Split tarsus* group tarsi. (a) *Drosophila proceriseta*, right foretarsus, male, posterodorsal view. (b) *Drosophila variabilis*, right foretarsus, male, posterodorsal view. hb, hairbeds.

point of insertion of the remaining segments also differs, being a little more than a third of the length from the proximal end of the basitarsus in *proceriseta*, and one-third the length from the distal end in *variabilis*. The change in the apparent insertion point of the distal segments may also be due to the elongation of the first tarsomere distal to the insertion in *proceriseta* as opposed to *variabilis*. The socket receiving the second tarsomere is broadly flared and rounded in *proceriseta*, but not especially modified in *variabilis*. The distal portion of the basitarsus is tubular in *proceriseta*, but flattened in *variabilis*, and lacks a hair bed in *variabilis*, perhaps because the arrangement of the distally directed setae on the smaller elongation of the basitarsus would allow tonic reception of the second tarsomere, which would not be the case in *proceriseta*. The apex of the basitarsus in *variabilis* is also broader and contains more inserted setae. The remaining tarsal segments are relatively thicker and more tubular in *proceriseta*, and are adorned with more setae. The mainly strongly curved setae are arranged in whorls in *proceriseta*, especially subapically on the third segment, and are somewhat more irregularly arranged in *variabilis*. Spieth (1966) observed the courtship of three species in this group, *pectinitarsus*, *proceriseta*, and *spiethi*. In each case, during one phase of courtship, the males were observed to extend the forelegs under the abdomen of the female such that the basitarsal extension, with its brush of setae, engaged the female genitalia. The tarsi are then rapidly vibrated in short bursts and cleaned with the proboscis in between these bursts.

The spoon tarsus Subgroup

Lapoint et al. (2009) recently reviewed the spoon tarsus group, recording a total of 12 species: *D.*

atroscutellata, *D. conformis*, *D. contorta*, *D. dasyncnemia*, *D. kikalaeleele*, *D. fastigata*, *D. incognita*, *D. mimiconformis*, *D. neutralis*, *D. percnosoma*, *D. sordidapex*, and *D. waddingtoni*. The subgroup is characterized by having the distal end of the basitarsus expanded dorsally, accommodating the bizarrely modified anterodorsally concave second tarsomere or spoon. The substructure of the spoon is difficult to homologize with an unmodified second tarsomere: it possesses a radically different conformation. Apparently this “spoon” is filled with a dense hair pile, except in *atroscutellata* from Kauai (*D. fastigata* has recently been added to the spoon tarsus subgroup by Lapoint and the disposition of its spoon has not as yet been investigated by SEM). The spoon also bears one or more strong porrect dorsoapical setae in *D. dasyncnemia*, *D. mimiconformis*, *D. neutralis*, *D. septuosa*, *D. sordidapex*, *D. waddingtoni*; *D. atroscutellata*, *D. conformis*, and *D. incognita* have a series of four long parallel setae posterodorsally on the basitarsus. In contrast to any of the other species, *D. contorta* has a distinctly bent basitarsus.

Hardy's (1965) description of the variation in foreleg morphology among these species involved mainly differences in the character of the dorsal and ventral setation of the tibia and basitarsus. The foretarsus of *D. atroscutellata* is examined here. This species, along with *D. fastigata*, are the only species in this group found west of Molokai. Both display what may be a plesiomorphic state of the spoon. *D. dasyncnemia* and *D. waddingtoni* are also examined. *D. waddingtoni* is the only species of this subgroup found on more than one island: it is found on all the islands except for Oahu and Kauai.

***Drosophila atroscutellata*.** The second tarsomere or spoon is quadrate, antero-posteriorly flat-

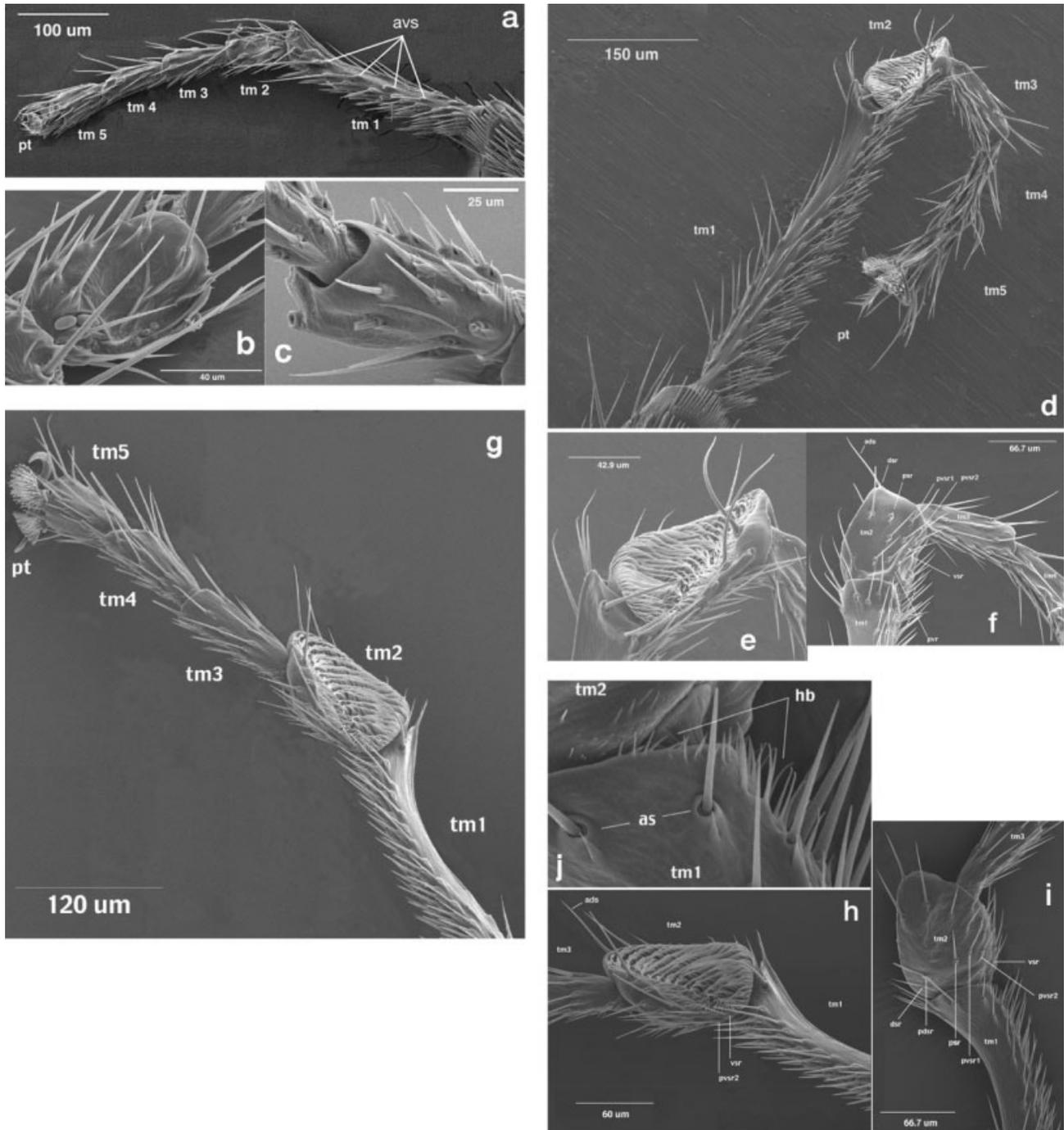


Fig. 5. Spoon tarsus group tarsi. (a) *Drosophila atroscutellata*, right foretarsus, male, anterior view. (b) *Drosophila atroscutellata*, second tarsomere, anterior view, reverse proximal-distal orientation. (c) *Drosophila atroscutellata*, second tarsomere, anteroventral view. (d) *Drosophila dasyncnemia*, left foretarsus, male, anteroventral view. (e) *Drosophila dasyncnemia*, second tarsomere, anteroventral view. (f) *Drosophila dasyncnemia*, second tarsomere, posterior view. (g) *Drosophila waddingtoni*, right foretarsus, male, anteroventral view. (h) *Drosophila waddingtoni*, second tarsomere, anteroventral view. (i) *Drosophila waddingtoni*, second tarsomere, posterior view. (j) *Drosophila waddingtoni*, second tarsomere, posterior view, detail of hairbeds. ads, apicodorsal setae; as, apical setae; avs, anteroventral setae; dsr, dorsal setal row; hb, hairbeds; pdsr, posterodorsal setal row; psr, posterior setal row; pvsr1, posteroventral setal row 1; pvsr2, posteroventral setal row 2; vsr, ventral setal row.

tened, the anterior edge rounded, and ventral edge straight, symmetrical in the anterior–posterior dimension, and sunken anterodorsally (Fig. 5b).

Unlike any of the other known species in this subgroup, it lacks pilosity in the concave dorsal surface. Two large posteroapical setae are inserted in

protuberances similar in character and position to those on the basitarsus. The posterior surface is somewhat flattened in comparison to the posterior, with a long sculptured lobe, not apparent from the anterior side, supporting a very strong dorsoapical seta (Fig. 5c; unfortunately missing in this specimen).

The basitarsus of *D. atroscutellata* is almost the length of the other four segments combined (Fig. 5a). Two large anterodorsal apical setae are borne in knob-like protuberances. The posteroventral setae distal to mid-forebasitarsus are strong, parallel, and striated.

Drosophila dasyncnemica. The anterior face of the second tarsomere is oriented obliquely anterior to the main tarsal axis and possesses fine hairs arranged in diagonal rows in the concavity on its anterodorsal side (Fig. 5e). The shorter of these hairs appear to be on the ventral edge of the spoon. The spoon is somewhat cylindrical-rectangular when viewed from its ventral aspect and appears twisted anteriorly when viewed from above. It is less than one-third the length of the basitarsus and barely longer than wide. The posteroventral setal row 2 is inserted in a ridge roughly the shape of a railroad rail profile, its base extended along the proximal border of the spoon (Fig. 5f). The ventral setal row extends on to the anterior surface, its apical seta inserted posteriorly.

The basitarsus of *D. dasyncnemica* is slightly longer than the other four tarsomeres combined (Fig. 5d). There are sometimes supernumerary scaliform processes, usually much smaller than those adjacent to setae. Also present at the ends of the basitarsus, at its articulations with the tibia and second tarsomere, both dorsally and ventrally, are small "hairs" arising socketless from the cuticle. These may be tonic receptors, sensu the hair beds of Chapman (1998; Fig. 2c). These surface features are also to be found on the other tarsomeres.

Drosophila waddingtoni. The second tarsomere is oriented obliquely anterior to the main tarsal axis (Fig. 5g). Its anterodorsal surface (the spoon) is concave and filled with fine hairs arranged in diagonal rows, as in *D. dasyncnemica*. The shorter of these hairs are to the ventral side. It is shaped like the head of a spoon, the posterior side strongly convex, at least 25% longer than wide, and is about one-third the length of the basitarsus (Fig. 5i).

The basitarsus of *D. waddingtoni* is shorter than the length of the other tarsomeres combined (Fig. 5g). The apical setae bear small proximal processes arising from the sockets and there are hair beds at the proximal and distal ends (Fig. 5j).

Summary

The spoon shares an oblique orientation in each species, but the second tarsomere is antero-posteriorly flattened in *D. atroscutellata*, unlike the other

two species. Its overall shape is distinctly different: quadrate in *D. atroscutellata*, cylindrical-rectangular and anteriorly twisted in *D. dasyncnemica*, and spoon-shaped and distally tapered in *D. waddingtoni*. In the latter two species, the fine hairs forming the oblique rows on the dorsal concave side have the shorter of these hairs ventrally. *D. dasyncnemica* has a posteroventral ridge on the posterior side of the spoon and also has the ventral setal row migrating to the anterior surface apically. *D. waddingtoni* possesses neither of these characters.

In *D. dasyncnemica*, the length of the basitarsus exceeds that of the remaining tarsal segments, whereas in *D. atroscutellata* and *D. waddingtoni*, the basitarsus is shorter than the length of the remaining segments. In *D. dasyncnemica*, the second tarsomere is roughly equal in length to each of the remaining segments, in *D. atroscutellata* it is shorter, and in *D. waddingtoni*, it is distinctly longer than the remaining segments. The number of ventral setae in rows decreases steadily from seven to one in *D. atroscutellata*, from five to three distal to mid-basitarsus in *D. dasyncnemica*, but remains at five in *D. waddingtoni*.

The males have been observed to stroke the females with the spoon during courtship (Heed, personal communication). Spieth (1966) observed the males of *D. dasyncnemica*, *D. waddingtoni*, and *D. sordidapex* using the spoon to lift the abdomen of the female by the vaginal plates and draw the tip of her abdomen toward his extended (*D. dasyncnemica*, *D. waddingtoni*) or nonextended (*D. sordidapex*) proboscis. Interestingly, *D. atroscutellata*, which lacks pilosity in the spoon, was not observed by Spieth to use the foreleg to directly stimulate the female's genitalia.

The *Antopocerus* Group

Grimshaw (1901) described *cognata* and *longisetata* as members of the genus *Drosophila*. Hardy (1965) erected the genus *Antopocerus*, placing these two species within it, and described an additional seven species. Kaneshiro (1976) sank *Antopocerus* into *Drosophila* suggesting that as a genus, it was defined by sexually selected characters of the head and front tarsus not shared by the females, which aside from their large size (comparable with the picture-wing flies) display no readily identifiable characteristics that might warrant their inclusion in a genus separate from the rest of the Hawaiian *Drosophila*. Hardy (1977) followed with a review of the group in which he described six new species: *D. kaneshiroi*, *D. yooni*, *D. curvata*, *D. nigricolor*, *D. apicalis*, and *D. stigma*, bringing the total to 15. He suggested three species subgroups and a complex:

1. The *adunca* subgroup, with the front tibia lacking long curled hairs, including *D. adunca* and *D. longisetata*,

2. The *diamphidiopoda* subgroup, with a straight (lacking curvature compared with the other subgroups) costal wing margin, and the front and hind margins nearly parallel, including *D. diamphidiopoda*, *D. cognata*, *D. kaneshiroi*, *D. orthoptera*, *D. tanythrix*, and *D. yooni*,
3. The *villosa* subgroup, with ventral hairs extending most of the length of the tibia, including *D. villosa*, *D. entrichocnema*, *D. curvata*, *D. nigricolor*, and *D. arcuata*,
4. The *apicalis* complex of the *villosa* subgroup, with apical wing spots, including *D. apicalis* and *D. stigma*.

The *antopocerus* species are recognized primarily by the male antenna having a long, porrect scape and numerous fine dorsal rays on the arista, decreasing in length distally, as well as the anterior surface of the male tibia being flattened and lacking setae; in other aspects, there can be extensive setation. The subgroups suggested by Hardy characterize well the variation in the forelegs of the males of the various species. In most species, the basitarsus is longer than the rest of the tarsomeres combined; in *D. curvata*, it is three times their length. The second tarsomere is by far the smallest segment in all species except *D. curvata* and the *adunca* subgroup.

The foretarsi of one species from each of the subgroups proposed by Hardy, *D. adunca*, *D. tanythrix*, and *D. villosa*, are examined.

Drosophila villosa. Unlike the foretibia of *D. adunca* and *D. tanythrix*, which have unremarkable chaetotaxy, the foretibia of *D. villosa* has a dense anterodorsal and anteroventral row of curved setae, extending the entire length of the segment (Fig. 6a). The anterodorsal row consists of a single file of setae, whereas the anteroventral row is several files thick, the additional rows ventral to posterior. The largest setae are inserted proximally and the setae of the proximal half of the segment are wavy at their ends. The anterior of the tibia is totally devoid of setation except for a single subapical seta, which is inserted on a callus. It is also clearly flattened.

The basitarsus is almost three times the length of the other tarsomeres combined (Figs. 6b). It has a dense brush of about 16 distally directed dorsal curved setae on its proximoanterior third (Figs. 6b,c). These setae are wavy at their ends and are subequal to the length of the segment. They are arranged in transverse rows of four. An outstanding feature of these setae is that they become flattened beginning about one quarter of their length and continuing until the wavy tips, the flattened and broadened striations giving the appearance of several laterally fused setae (Fig. 6c, inset). The rows continue distally as staggered pairs of small setulae, inserted anterodorsally, for about another third of the segment (Fig. 6b). Posteroventrally,

the rows continue distally as staggered pair of widely spaced, strong anterodistally curved setae, wavy at their apex like the brush setae but not similarly flattened (Fig. 6c). This row extends to the apex of the segment, and the apical three setae are mirrored by three similar though oppositely curved setae inserted anterodorsally.

Drosophila adunca. The foretibia of *D. adunca* is about equal in length to the basitarsus and does not possess unusual setation, other than the lack of a dorsal setal row (Fig. 6d). The basitarsus is about 2.5 times the length of the remaining tarsal segments. It is bare of setae on its basal half. Distal to mid-tarsus is a staggered, anterodorsally directed row of long curved setae beginning about mid-tarsus and extending past three-quarters its length, their length several times the diameter of the basitarsus. There is one larger wavy seta, about twice the length of the longest of the aforementioned row, inserted proximally on the basitarsus and curling around it ventrally in the examined specimen (Fig. 6e). Tarsomeres 2, 3, and 4 each bear two large curved to wavy setae dorsally (Fig. 6d).

Drosophila tanythrix. The basitarsus of *D. tanythrix* is about two-thirds the length of the tibia (Fig. 6f). The basitarsus is slightly longer than the length of the second and third tarsomeres combined. The rows of tarsal pegs on the basitarsus as well as on the succeeding tarsomeres are noticeably less dense than those of *D. adunca*. It is bare of dorsal setae except one present subapically. There is an antero- and posterodorsal row of setae, with only two small setae in each row on the basal two-thirds of the segment. There are numerous setae on the apical third of the basitarsus, curved proximally and very strong wavy setae subapically, weakening at the apex. These setae are oppositely curved such that they meet each other dorsally. One of the posterodorsal setae is much longer and more strongly developed than any of the others on the segment, being twice the length and diameter of the next largest, and half again as long as the basitarsus itself. The larger posterodorsal setae are inserted on a swelling of the basitarsal surface. The larger anterodorsal setae are inserted on a projection that extends posteriorly about one-third the diameter of the basitarsus at that point, its thickness about twice that of the large wavy bristle. The second tarsomere is extremely short and bears two setae similar to those of the subapex of the basitarsus. The third and fourth tarsomeres are concave and flattened on their antero- and posterodorsal aspects, appearing roof-like. The third tarsomere has a comb of five setae on its apical anteroventral third.

Summary

D. adunca and *D. tanythrix* have a rather unadorned foretibia, unlike most of the flies in this

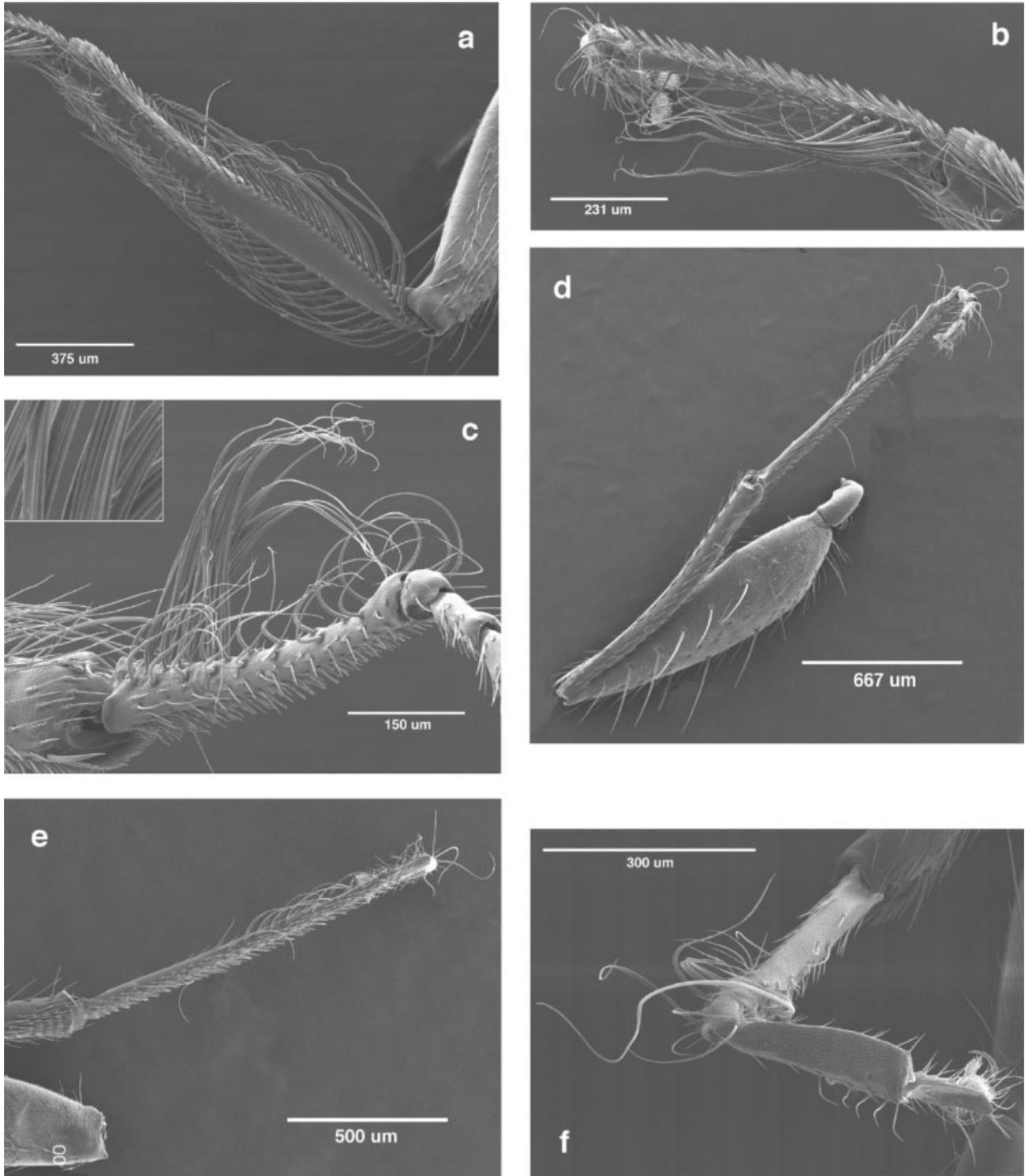


Fig. 6. *Antopocerus* group tarsi. (a) *Drosophila villosa*, foretibia, male, anterior view. (b) *Drosophila villosa*, foretarsus, anterior view. (c) *Drosophila villosa*, basitarsus, posteroventral view; inset, flattened dorsal brush setae. (d) *Drosophila adunca*, foretibia and tarsus, male, posterodorsal view. (e) *Drosophila adunca*, foretarsus, anteroventral view. (f) *Drosophila tanythrix*, foretarsus, male anterodorsal to posterodorsal view. Note: tarsomeres not labeled in this figure due to the shortness of those distal to the basitarsus.

group. *D. villosa* has the dense anterodorsal and anteroventral tibial setation also seen in *D. nigricolor*. *D. villosa* also has a total lack of setation

on, and a much more pronounced flattening of the anterior surface of the tibia than the other two species. *D. adunca* and *D. villosa* share the charac-

teristic of having the basitarsus much longer than the other tarsal segments combined, whereas *D. tanythrix* has a much shorter basitarsus. The tarsal peg setae of *tanythrix* are noticeably weaker and less dense, both in terms of the number of setae in each row and the number of rows than in *D. adunca* and *D. villosa*. *D. adunca* only has an anterodorsally directed row of setae on the basitarsus, whereas *D. tanythrix* and *D. villosa* possess both anterior and posterior rows of much more strongly developed setae. These setae are curved in *D. adunca*, but both curved and wavy in *D. tanythrix* and *D. villosa*. They are more apical in *D. adunca* and *D. tanythrix*, but more basal in *D. villosa*. The more terminal tarsomeres of these flies are also adorned with elongated setae, curved in *D. adunca* and *D. villosa*, and *D. tanythrix* has the interesting modifications of having a comb on its apical anterodorsal third and the roof-like structure of the third and fourth tarsomeres.

Spieth (1966) described the courtship behavior of *D. adunca*, *D. longiseta*, *D. orthoptera*, *D. tanythrix*, and *D. villosa*, and Spieth (1968) included these descriptions and added observations on *D. diamphidiopoda* and *D. entrichonema*. In the 1966 article, *Drosophila adunca* and *D. longiseta*, both species with some wing infuscation, were observed to drum the substrate in a preliminary courtship element. *Drosophila adunca* was observed to grasp the dorsum of the female's abdomen and *D. longiseta* to drum on the venter of the female's abdomen, with the setae on the basitarsus and second segment striking the genitalia. In the 1968 article, he classified both foreleg behaviors as culminating with the stimulation of the venter of the female's abdomen, presumably also of her genitalia. He referred to this as "Type I" behavior; the descriptions of the behaviors also include those of gross body wing, antennal, and proboscis movement. The other three clear-winged species, were not observed to use the forelegs in courtship, despite, as noted by Spieth, especially for *D. villosa*, having similar if not more pronounced development of the group-characteristic foreleg features. Spieth attributed this to a failure in observing a behavior experimentally that the morphology, both of the other *antopocerus* species and considering observations in other Hawaiian species groups, suggested should occur. He was able to shed further light on the question with his 1968 article. *Drosophila diamphidiopoda* has a similar foreleg to *D. orthoptera* and *D. tanythrix* [and the other members of the *diamphidiopoda* species group, as defined in Hardy (1977); Spieth also includes *D. arcuata* with these species], the most common element being the group of long setae on the dorsoapex of the basitarsus/dorsum of the short second tarsomere. He therefore proposed that the behaviors of *D. orthoptera* and *D. tanythrix* involving the use of the forelegs were likely similar to those of *D. diamphidio-*

poda that had been observed. Specifically, the forelegs are advanced such that the setal ornamentation of the first and second tarsomeres contacts the female's genitalia when the forelegs are vibrated. This pattern is defined as Type II. Type III behavior is exhibited by *D. entrichonema* and *D. villosa*. *Drosophila nigricolor*, at the time undescribed or undiscovered, has very similar chaetotaxy. These species, especially *D. villosa* as described earlier, have prominent rows of setae on the lateral and medial sides of the tibia, which stimulate the female's genitalia in concert with the apical three tarsomeres.

DISCUSSION

Truncated Inverse Conic Setae

This work is only the beginning of a nearly limitless journey into the fine structure of the Hawaiian *Drosophila*. An exciting possibility is the correlation of the fine structural features with their function in the organism. We see several examples of this here. Spieth (1966) noted the brush-like mesotarsomeres of *Drosophila petalopeza* of the *bristle tarsus* group, but was not able to observe their unique truncated inverse conic shape. Such setae are not unknown elsewhere in the Diptera, for example, the orbitals on the head in the genus *Strauzia* (Tephritidae) have a similar, if not even more exaggerated gestalt. The determination as to whether the character of these setae, and those of *D. petalopeza*, is similar, awaits further examination, as does the determination as to whether the developmental regime of these setae in the two groups is similar.

Which Tarsomere is Missing?

Without peer in the Diptera are the four-segmented tarsi of the *split tarsus* subgroup. The ground plan number of tarsomeres in the Endopterygota is five (Romoser, 1994). Some Coleoptera and a small number of taxa from other orders are exceptions to this. In all described Diptera besides the *split tarsus* subgroup there are five tarsomeres. Not only are the foretarsi of the *split tarsi* aberrant with respect to the number of tarsomeres, the insertion of the ultimate three tarsomeres basally on the first is also unknown in other Diptera, if not the rest of Insecta. This unique tarsal arrangement cries out for a microdissection (by microtome) in the pupal stage to attempt a determination of the provenance of the basitarsus and second tarsomere with respect to the five-segment groundplan. A developmental study could answer the question of whether there has been a fusion of these two segments due to a turning off of the joint formation program. However, our SEM examination reveals no trace of this.

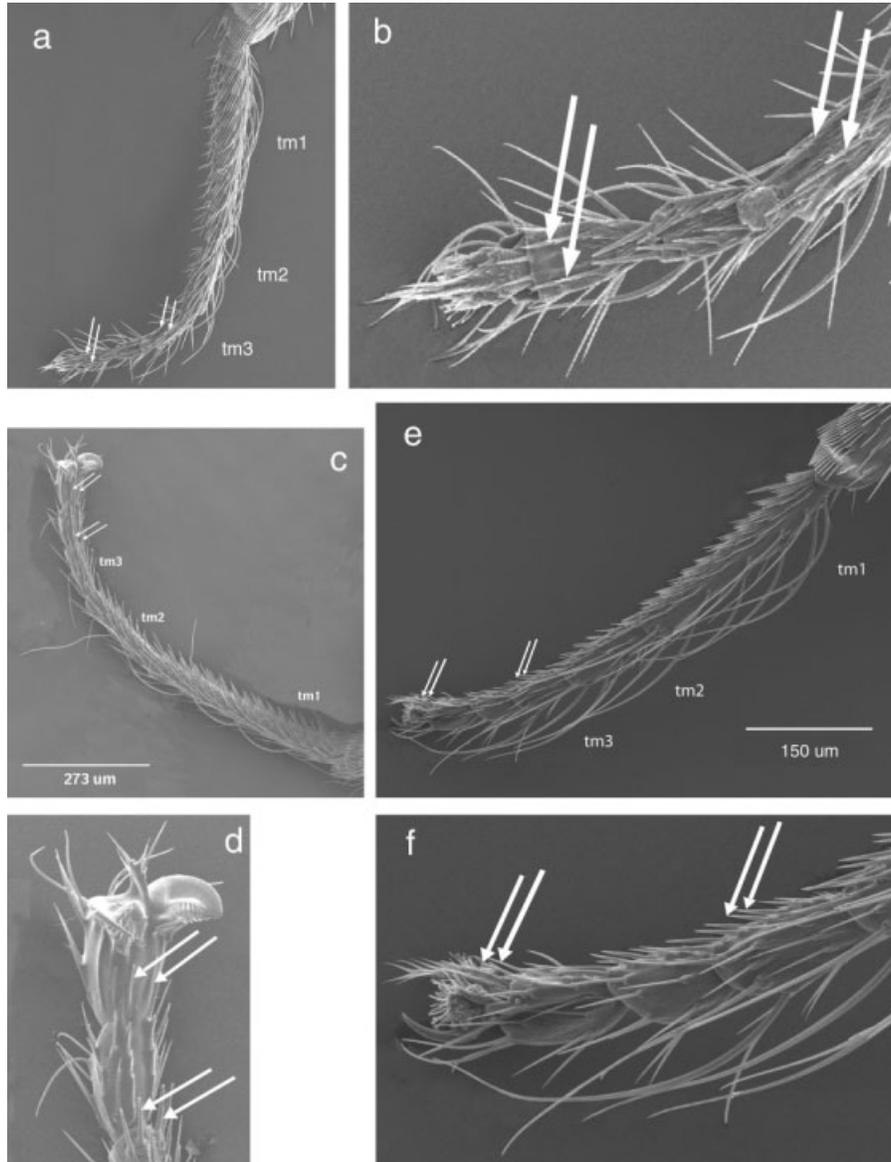


Fig. 7. Ventral setation of terminal three tarsomeres in non-AMC Clade species. Arrows show ventral setal rows on tarsomeres 3 and 5. (a) *Drosophila comatifemora*, modified mouthparts group, tarsomeres, male, ventral view. (b) Close-up of terminal three tarsomeres. (c) *Drosophila punalua*, picture wing group, punalua subgroup, tarsomeres, male, anteroventral view. (d) Close-up of terminal three tarsomeres. (e) *Drosophila hystricosa*, modified moutparts group, mitchelli subgroup, hystricosa complex, tarsomeres, male, anteroventral view. (f) Close-up of terminal three tarsomeres.

Although we find no hard evidence as to which is the missing segment, examination of the ventral setation of the foretarsi in the Hawaiian *Drosophila*, as well as the knowledge of which segments are typically modified within the AMC Clade, suggest its identity. Figure 7 shows the disposition of the ventral setation in the foretarsi of three non-AMC Clade species. In all examined species, both those included in this study and otherwise, the terminal three tarsomeres possess only two rows of ventral setae. In contrast, the second tarsomere and basitarsus possess more than two ventral rows of setae. Figure 8a,d shows that in the split tarsus group species *Drosophila proceriseta*, the

terminal three tarsomeres possess two rows of ventral setae, whereas the basitarsus has more than two rows of setae. This suggests that the missing segment is either the basitarsus (in the five-segment groundplan) or the second tarsomere. If we then examine the pattern of tarsal modification within the AMC Clade, excluding the *split tarsus* subgroup, both in Figure 8 and throughout this article, several patterns emerge:

1. The basitarsus is by far the most commonly and radically modified segment. This is true of the *bristle tarsus* subgroup and the *antopocerus* group, and while not true of the *spoon tarsus*

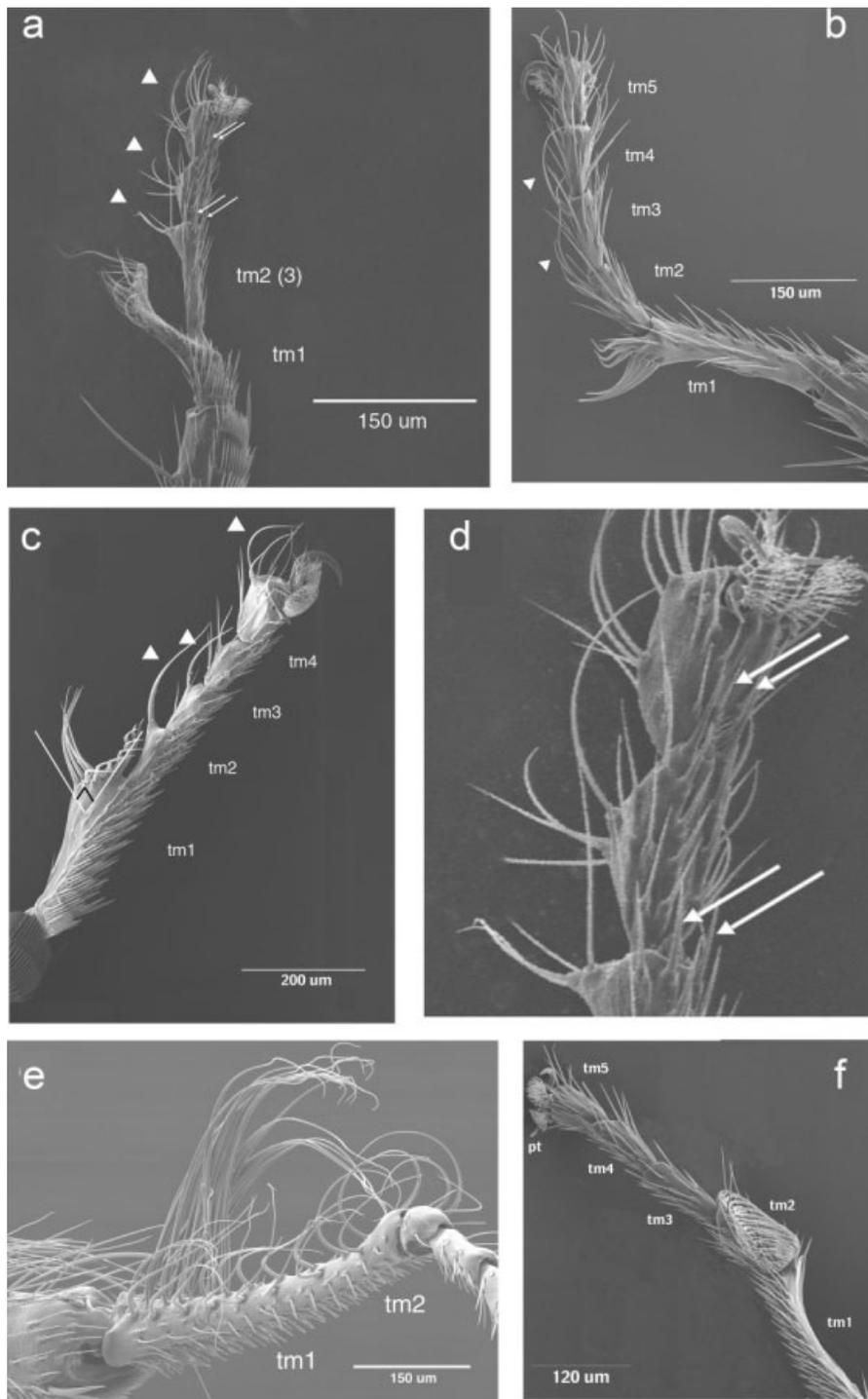


Fig. 8. Comparison of *split tarsus* tarsomeres with other AMC Clade subgroups. Arrowheads show elongate anterodorsally directed cilia or setae on tarsomeres distal to the basitarsus. (a) *Drosophila proceriseta*, foretarsus, male, anterodorsal view. Abbreviation: tm 2 (3) refers to the suggestion that the second tarsomere is actually the third. (b) *Drosophila petalopeza*, foretarsus, male, posterodorsal view. (c) *Drosophila basimacula*, foretarsus, anterior view. The rough isometric right angle represents the perpendicular orientation between the brush and comb setae (cf. *Morphological Terminology*), an orientation between major setal groups common to all AMC Clade subgroups except the *spoon tarsi*. (d) *Drosophila proceriseta*, close-up of distal tarsomeres of (a) arrows show ventral setal rows on tarsomeres 3 and 5. (e) *Drosophila villosa*, first and second tarsomere, posteroventral view. Shows the perpendicular orientation between the dorsal and medial setal groups of the basitarsus, and the extreme reduction of the second tarsomere. (f) *Drosophila waddingtoni*, foretarsus, anterodorsal view. The anterior twist and thus obliquely anterior orientation of the dorsal surface of second tarsomere accommodates both endpoints of the perpendicular.

- subgroup, it is also modified there with respect to the remaining tarsomeres. In the *split tarsus* subgroup, the first tarsomere is strikingly modified as previously described.
2. In the *bristle tarsus* subgroup and to a lesser extent the *antopocerus* group species, there is a perpendicular arrangement of different setal groups (Fig. 8c) on the basitarsus. This arrangement consists of anteriorly and dorsally arranged setae, and undoubtedly has a functional relationship to the stimulation of the female genitalia, as noted by Spieth (1966). The dorsal setae frequently continue on the more distal segments (Figs. 3, 4, 6d, 8a,b,c). In the *split tarsus* subgroup, we see a preservation of this perpendicular arrangement, the anterior element on the basitarsus and the dorsal elements on the ultimate three tarsomeres (Fig. 4a,b). Interestingly, the perpendicular arrangement is retained in the *spoon tarsus* subgroup species through the oblique arrangement of the spoon from the posterior side (dorsal element) to the anterior side (Fig. 5d,g, 8f).
 3. The *spoon tarsus* subgroup shows a radical modification of the second tarsomere, to the point where the homology of most of the surfaces of this segment and its dorsal pilosity is totally unclear. The loss of the second tarsal segment in the split tarsus flies could also be considered a radical modification.
 4. The second tarsomere is extremely reduced in most of the *antopocerus* group species, perhaps indicating another trend toward its loss within the AMC Clade [interestingly, Bonacum (2001), the large dataset, recovers the *split tarsus* subgroup as the most basal clade within the AMC Clade, with a clade containing the *antopocerus* group and the rest of the AMC Clade species sister to it].

Considering the ventral setation pattern common to all examined Hawaiian *Drosophila* as well as the four patterns mentioned earlier, we suggest that it is the second tarsomere that has been lost in the split tarsus group.

Also of interest in our examination of the *split tarsus* subgroup is the position or lack of a sensory hairbed on the ventral surface of the basitarsus. Spieth's (1966) analysis of courtship behavior in this subgroup revealed a complicated and perhaps the most complex of the courtship behaviors in the AMC Clade. The lack of the hairbed in *Drosophila variabilis* and its presence in *D. proceriseta*, perhaps for mechanoreception, may indicate a subtle difference in the suite of behavioral elements between the two species that could possibly be elucidated by a microscopic video examination. Also noteworthy in this respect is Spieth's observation that the *split tarsus* flies draw their foretarsi through the labellar lobes repeatedly during court-

ship, which he attributed to the deposition of a pheromone-like substance on the tarsi from the proboscis. The hairbed might conceivably play a role in this behavior, if the supposition is correct.

Drosophila atroscutellata was noted by Spieth not to use its forelegs during courtship, whereas *D. dasyncnemia*, *D. sordidapex*, and *D. waddingtoni* were all observed to both stimulate the female genitalia and pull the female abdomen toward their proboscis. There is an interesting similarity in the configuration of the basitarsus and second tarsomere between *D. atroscutellata* and *D. fastigata*, assigned to the *spoon tarsus* subgroup, and *D. gubleri* of the *bristle tarsus* subgroup: all share the characteristics of a shortened but not very spoon-like second tarsomere and a slightly flared basitarsus with an apical seta. This is similar to *D. unicola* and *D. trichaetosa* of the *bristle tarsus* subgroup, suggesting that all these species could perhaps be the sister clade to a clade containing the bristle + spoon species. Assuming that *D. atroscutellata* belongs with the other *spoon tarsus* flies (there has been no cladistic analysis of this subgroup as a whole), it may be a pleisomorphic representative of the subgroup: the apical flaring of the basitarsus accommodating the spoon tarsomere is reminiscent of the species in this group, and the spoon itself shows a similar broadening and is concave on its anterior surface. It lacks the dense pilosity within the concavity seen in the other two species examined here. Although this may be an artifact of preparation for SEM, the hairs in the concavity always appear as if they had been wetted (Fig. 5e,h): as opposed to other fine trichia observed on the insect cuticle, they may be liquid absorbent rather than repellent. Spieth observed repeated cleaning of the forelegs by *D. waddingtoni* during courtship as he did in the flies of the *split tarsus* group. However, he was not aware of the pilosity in the spoon. Had he known, he might have suggested a similar transfer of a pheromone-like substance.

Spieth also observed the use of the forelegs in courtship in the *antopocerus* group species *Drosophila adunca* and *D. longiseta*, *diamphidiopoda* group member *D. diamphidiopoda*, and *villosa* group member *D. entrichocnema*, as well as a drawing of the foretarsi across the labellum in *D. diamphidiopoda* and *D. longiseta*. He was not able to observe their use in the other species of the group he examined: *D. orthoptera*, *D. tanythrix*, and *D. villosa*. There is a wide variation in the foretarsal characteristics in the *antopocerus* group, from minimal to the intensely dense population of setae seen in *D. villosa*. *D. adunca* and *D. longiseta* both were assigned to their own *adunca* species group by Hardy (1977), based primarily on the nonelaborate nature of their tarsal ornamentation with respect to the other species, and their infuscated wings. Spieth, however, observed very differ-

ent use of the foretarsi in courtship in his 1966 article: *D. adunca* stimulating the female's abdomen dorsally, whereas *D. longiseta* stimulated the venter. As hinted at by Spieth, the wide variety of foretarsal morphology in this group would predict an equally broad range of behaviors, if the *adunca* subgroup species are any guide.

Future Cladistic Studies

This study clearly illustrates the need for a far more extensive cladistic morphological examination of the Hawaiian *Drosophila*, going hand in hand with results obtained by molecular analyses. Very unfortunately, as nothing is better than an analysis obtained from independent sources, many specimens may no longer be obtainable for molecular studies. Some specimens are singletons, known only from types, which have not been collected for decades, and may now be extinct. This makes the contribution from morphological analyses all the more important. Regardless of the great contributions to the phylogenetic hypothesis of the relationships between groups of the Hawaiian *Drosophila* made by Hardy (1965, 1966, 1977), Hardy and Kaneshiro (1979), Hardy et al. (2001), Throckmorton (1966, 1975), Kaneshiro (1976), and Carson (1983, 1987, 1992), none of these are explicitly cladistic analysis. It is only recently that Baker and DeSalle (1997), and to a much greater extent Bonacum (2001) and O'Grady and Zilversmit (2004; morphology also used here), have used cladistic methods on molecular data to arrive at phylogenetic hypotheses that are explicit and unambiguous in their presentation. Although the fruit of the earlier works is a division of the fauna into species groups, subgroups, complexes, and clusters, which for the most part have been corroborated to the extent possible by the sampling of the later molecular work, these groups were, in general, erected by Throckmorton (1966) on the basis of his personal knowledge and implicit methodology and on their "major diagnostic character" (Hardy et al., 2001). That is, *picture wings*, because of the wing patterns, *modified mouthparts*, because of the labellar modification, *fungus feeders*, because of their trophic association, etc. [these have also been called "white-tipped scutellum" and "rimmed-labellum"; neither of these diagnoses, as well as the fungus-feeding habit, have been shown to be exclusive (Hardy et al., 2001)]. As far as the modified tarsus character is concerned, a perusal of Hardy (1965) will reveal modified tarsi in many of the species not placed in the *modified tarsus* group. Many of these are *modified mouthparts* group flies, already possessing elaborate specializations of the labellum. Many of the others are *not* assigned to the *modified tarsus* group. An ambitious, overall cladistic analysis of the fauna, with a large amount of character data

is needed to really define which species these groups, subgroups, complexes, and clusters contain. It is quite likely, in the context of such an analysis, that all of them will be shown to be paraphyletic to a greater or lesser degree. This may already be the case for the *bristle* and *ciliated tarsus* subgroups as shown by the study of Bonacum (2001). A detailed SEM analysis along the lines of the one presented here, and the wealth of characters it can reveal, will be an essential part of a combined analysis with molecular and other data sources, and the best hope for elucidating the relationships for those taxa where molecular analysis is not feasible.

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LITERATURE CITED

- Baker RH, DeSalle, R. 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. *System Biol* 46:654-673.
- Bonacum J. 2001. Molecular Systematics of the Hawaiian Drosophilidae [Dissertation]. New Haven, CT: Yale. p 129.
- Carson HL. 1971. Speciation and the Founder Principle. *Stadler Symposia*. Columbia, MO: University of Missouri. pp 51-70.
- Carson HL. 1983. Chromosomal sequences and interisland colonizations in the Hawaiian *Drosophila*. *Genetics* 103:465-482.
- Carson HL. 1987. Tracing ancestry with chromosomal sequences. *Trends Ecol Evolut* 2:203-207.
- Carson HL. 1992. Inversions in Hawaiian *Drosophila*. In: Krimbas CB, Powell JR, editors. *Drosophila* Inversion Polymorphism. Ann Arbor, MI: CRC Press. pp 407-439.
- Chapman RF. 1998. *The Insects: Structure and Function*. Cambridge: Cambridge University Press. p 770.
- de Castro LE. 1953. Estudo comparativo das cerdas cuneiformes das pernas dos cuneiformes das pernas dos drosophilídeos (Diptera). *Rev Brasil Biol* 13:363-368.
- Grimaldi DA. 1990. A Phylogenetic, Revised Classification of Genera in the Drosophilidae (Diptera). *Bull Am Museum Natural History* 197:139.
- Grimshaw PH. 1901. *Diptera. Fauna Hawaiiensis* 3:68-69.
- Hardy DM. 1965. *Insects of Hawaii: Diptera: Cyclorrhapha II, Series Schizophora, Section Acalypterae I, Family Drosophilidae*. In: Zimmerman EC, editor. Honolulu: University of Hawaii Press. p 814.
- Hardy DM. 1966. Descriptions and notes on Hawaiian Drosophilidae (Diptera). *Studies in genetics, III. Morgan Centennial Issue* 6615:195-244.
- Hardy DM. 1977. Review of the Hawaiian *Drosophila* (Antopocerus) Hardy. *Proceedings of the Entomological Society of Washington* 79 (January 1) Washington, DC. pp 82-95.
- Hardy DM, Kaneshiro KY. 1979. A Review of the Modified Tarsus Species Group of Hawaiian *Drosophila* (Drosophilidae: Diptera). I. The "Split-Tarsus" Subgroup. *Proceedings of the Hawaiian Entomological Society*. Honolulu, HI. pp 71-90.
- Hardy DE, Kaneshiro KY, Val FC, O'Grady PM. 2001. Review of the haleakalae species group of Hawaiian *Drosophila* (Dip-

- tera: Drosophilidae). Bishop Museum Bulletin in Entomology Vol. 9. Bishop Museum Press. pp 1–88.
- Heed WB. 1968. Ecology of the Hawaiian Drosophilidae. Studies in Genetics, IV. Research Reports. University of Texas Publications 6818:387–419.
- Heed WB. 1971. Host plant specificity and speciation in Hawaiian *Drosophila*. Taxon 20:115–121.
- Kambysellis MP, Craddock EM. 1997. Ecological and reproductive shifts in the adaptive radiation of the endemic Hawaiian *Drosophila*. In: Givnish TJ, Sytsma KJ, editors. Molecular Evolution and Adaptive Radiation. Cambridge: Cambridge University Press. pp 475–509.
- Kaneshiro KY. 1976. A revision of generic concepts in the biosystematics of Hawaiian Drosophilidae. Proceedings of the Hawaiian Entomological Society. Honolulu, HI. pp 255–278.
- Kaneshiro KY. 1997. R.C.L. Perkins' legacy to evolutionary research on Hawaiian Drosophilidae (Diptera). Pacific Sci 51:450–461.
- Kaneshiro KY, Boake CRB. 1987. Sexual selection and speciation: Issues raised by Hawaiian *Drosophila*. Trends Ecol Evol Biol 2:207–212.
- Lapoint RT, Magnacca KN, O'Grady PM. 2009. Review of the *spoon tarsus* subgroup of Hawaiian *Drosophila* (Drosophilidae: Diptera), with a description of one new species. Zootaxa 2003:53–68.
- Magnacca K, Foote D, O'Grady PM. 2008. A review of the endemic Hawaiian Drosophilidae and their host plants. Zootaxa 1728:1–58.
- McAlpine JF. 1981. Morphology and terminology—Adults. In: McAlpine JF, editor. Manual of Nearctic Diptera. Ottawa: Research Branch, Agriculture Canada. pp 9–64.
- Montgomery SL. 1975. Comparative breeding site ecology and the adaptive radiation of picture-winged *Drosophila* (Diptera: Drosophilidae) in Hawaii. Proc Haw Ent Soc 22:65–103.
- O'Grady P, Zilversmit M. 2004. Phylogenetic relationships within the haleakalae species group inferred by molecular and morphological characters (Diptera: Drosophilidae). Bishop Museum Bull Entomol 10:117–134.
- Peterson A. 1948. Larvae of Insects. I. Lepidoptera and Hymenoptera. Ann Arbor: Edwards Brothers. p 315.
- Peterson A. 1951. Larvae of Insects. II. Coleoptera, Diptera, Neuroptera, Siphonaptera, Mecoptera, Trichoptera. Ann Arbor: Edwards Brothers. p 416.
- Romoser WS, Stoffolano JG. 1994. The Science of Entomology, 3rd edition, Dubuque, IA: Wm. C. Brown Publishers. xiv+532 pp.
- Russo CAM, Takezaki N, Nei M. 1995. Molecular phylogeny and divergence times of Drosophilid species. Mol Biol Evol 12:391–404.
- Spieth HT. 1966. Courtship behavior of endemic Hawaiian *Drosophila*. Studies in genetics, III. Morgan Centennial Issue. University of Texas Publications 6615:335–396.
- Spieth HT. 1968. Evolutionary implications of the mating behavior of the species of *Antopocerus* (Drosophilidae) in Hawaii. Studies in genetics, IV. Morgan Centennial Issue Research Reports. University of Texas Publications 6818:319–334.
- Throckmorton LH. 1966. The relationships of the endemic Hawaiian Drosophilidae. Studies in Genetics, III. Morgan Centennial Issue. University of Texas Publications 6615:335–396.
- Throckmorton LH. 1975. The phylogeny, ecology, and geography of *Drosophila*. In: King RC, editor. Handbook of Genetics. New York: Plenum Press. pp 421–470.
- Torre-Bueno JR, Tulloch GS. 1989. The Torre-Bueno Glossary of Entomology, including Supplement A. In: Nichols SW, Schuh RT, editors. New York: The New York Entomological Society. p 840.