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The functional morphology of species-specific clasping structures on the front legs of male *Archisepsis* and *Palaeosepsis* flies (Diptera, Sepsidae)

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Several possible explanations for the elaborate species-specific morphology of male front leg clasping organs were tested by comparing six species of *Archisepsis*, *Palaeosepsis* and *Microsepsis* flies. The only previously published hypothesis regarding these clasping organs was refuted by the finding that species-specific portions of the male femur and tibia consistently meshed tightly with prominent veins and folds in the female's wing, rather than meshing with each other. Female wing morphology in the region grasped by the male was relatively uniform and in general did not vary in ways that would prevent non-conspecific males from grasping them, arguing in all but one species against both simple lock-and-key and male–female conflict of interests hypotheses based on morphology. Interspecific differences in male front leg morphology generally represent alternative ways to accomplish the same basic mechanical function of holding tightly onto the relatively invariant female. Despite the fact that female resistance behaviour indicates that male–female conflict over male mounting is common, only one female wing structure in one species resembled an anti-clasper device, giving a second reason to doubt the morphological male–female conflict of interest hypothesis, at least for five of the six species. The positions of probable sensory structures on the wings of females were relatively similar in different species and did not correspond in any obvious way to species-specific features of male clasping structures. This, plus the intraspecific variation in both the positions of these sensilla and the exact site where the male grasped the female's wing, argued against simple 'sensory lock-and-key' ideas about male front leg function. By a process of elimination, it appears that generalized female receptors are able to sense species-specific differences in male front legs. This idea was supported by increased female rejection behaviour in cross-specific pairs.

ADDITIONAL KEY WORDS: genitalic evolution – sexual selection – cryptic female choice – lock-and-key – male–female conflict.

INTRODUCTION

Both genitalic and non-genitalic male body parts that are modified to contact females during courtship or copulation often evolve relatively rapidly and divergently when compared with other body parts (summary in Eberhard [1985] for a wide variety of animals). The question of why this pattern of evolution occurs in male genitalia is currently debated (Eberhard, 1985, 1996, 1997; Shapiro & Porter, 1989; Alexander, Marshall & Cooley, 1997; Arnqvist, 1998). One of the obstacles to resolving this controversy for genitalia is that it is usually difficult to observe male genitalia in action. They are generally hidden inside the female, and often contact soft, flexible female structures. These limitations are often less serious in non-genitalic contact structures, which thus offer useful cases for studies designed to test the competing theories.

Many male non-genitalic contact structures have elaborate species-specific forms and, just as genitalia, provide especially useful characters for distinguishing closely related species because of their elaborate species-specific forms. These include the male coxa I and dorsum of segment 16 in millipedes (Haacker, 1971, 1974), the male legs, chelicerae and cephalothorax in

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a variety of spiders (Exline & Levi, 1962; Millidge, 1980, 1981; Coyle, 1986; Schaible, Gack & Paulus, 1986; Dahlem, Gack & Martens, 1987; Huber, 1994; Huber & Eberhard, 1997), the male antennae in copepods and fairy shrimp (Pennak, 1978; Belk, 1984), various male legs, dorsal setae and hysterosoma in mites (Hartenstein, 1962; Santana, 1976; B. O'Connor, pers. comm.), the male antennae, front legs, and ventral abdominal spines and indentations in beetles (Fall, 1912; Selander & Mathieu, 1969; Eberhard, 1993a,b), the male front tibiae, abdominal sternites and hind tarsus in Hymenoptera (Ruttner, 1975; Bohart & Menke, 1976; Evans & Matthews, 1976; Richards, 1982; Toro & de la Hoz, 1976; Toro, 1985; Griswold, 1983), the male legs, antennae, abdominal sternites and lateral tergites in Hemiptera (Schuh & Slater, 1995), male legs and abdominal sternites in Diptera (Hennig, 1949; Pont, 1979; Schneider, 1993; McAlpine & Schneider, 1978; Dodson, 1997) and male abdominal tergites and wings in scorpionflies (Cooper, 1972; G. Byers, pers. comm.) (summary and additional references in Eberhard, 1985). The elaborate forms of such grasping structures often seem overly complex for their seemingly simple mechanical functions such as holding onto the female, suggesting either that grasping is mechanically more difficult than it would appear, or that the male structures have additional functions.

Although they have been little exploited in this context, such male grasping structures are especially useful for testing hypotheses concerning rapid divergent evolution. They consistently grasp the same portion of the female's body, so the mechanical significance of morphological variations is relatively easy to deduce. The potential roles of grasping structures in possible male–female conflicts are also relatively clear. In addition, the female structures that are grasped are often relatively rigid and are located on the external surface of her body, where their detailed morphology and their morphological mesh with the male structures are more easily studied. In some cases (including those of this study), it is also possible to determine the precise locations of female sense organs in the area grasped by the male.

HYPOTHESES

Several major hypotheses that were originally proposed in the context of genitalic evolution (and in some cases explicitly extended to the evolution of non-genitalic structures) could explain the evolutionary trend for non-genitalic contact structures of males to evolve rapidly and divergently.

(A) *Species isolation by lock-and-key mechanical fit.* The species-specific male structures fit only conspecific females and are mechanically incapable of effectively grasping females of other species. The function of interspecific differences in female morphology is to prevent grasping by cross-specific males (e.g. Fraser, 1943; Freitag, 1974; Toro, 1985; Toro and de la Hoz, 1976; see Shapiro & Porter, 1989, for data and arguments regarding male genitalia). The sole function of the male structure is to grasp the female; species-specific differences in female morphology prevent grasping by heterospecific males.

(B) *Male–female conflict.* Male grasping organs evolved as weapons in coevolutionary arms races with conspecific females. The females have in turn evolved defenses against being grasped, in order to maintain control over critical reproductive events and processes (Ward, Jemmi & Roosli, 1992, on sepsid flies; Arnquist & Rowe, 1995, on a water strider; see Lloyd, 1979, and Alexander *et al.*, 1997, for similar arguments regarding male genitalia, and Gowaty, 1997a, for further theoretical considerations). The sole function of the male structure is to grasp the female. The reason different male designs have evolved in different species is that female modifications to resist being grasped by conspecific males differ in different species, making the elaborate species-specific morphology of the male necessary. Female differences can be morphological or behavioral.

(C) *Stimulation.* Species-specific male organs serve both to grasp the female and to stimulate her so that she can distinguish (and favour with cooperative responses) particular males. Two functions for such discrimination by the female have been proposed: (C_1) to favour conspecific males over heterospecific males (species isolation) (Robertson & Paterson, 1982; Battin, 1993); (C_2) to favour those conspecific males with designs that are superior in eliciting favourable female responses (sexual selection by cryptic female choice) (Eberhard, 1985, 1996).

Male sepsid flies use modified areas on the ventral surfaces of the front femur and tibia to clamp the bases of the female's wings during the sometimes extended period during which the male rides on the female prior to intromission (Fig. 1 – also Šulc, 1928; Hennig, 1949; Parker, 1972). These surfaces are often more or less elaborately sculptured, and provided with spines and setae, and they are species-specific in form in the related genera *Archisepsis*, *Palaeosepsis* and *Microsepsis* (Silva, 1993; Ozerov, 1992, 1993) as well as in many other sepsids (e.g. Duda, 1925, 1926; Pont, 1979; Steyskal, 1987). The present study tests the hypotheses just presented by examining the comparative morphology of how the male front leg clamps the base of the female's wing in six species of sepsid flies in the genera *Archisepsis*, *Palaeosepsis* and *Microsepsis*.

There has been apparently only one published attempt, which does not fit with any of the more general

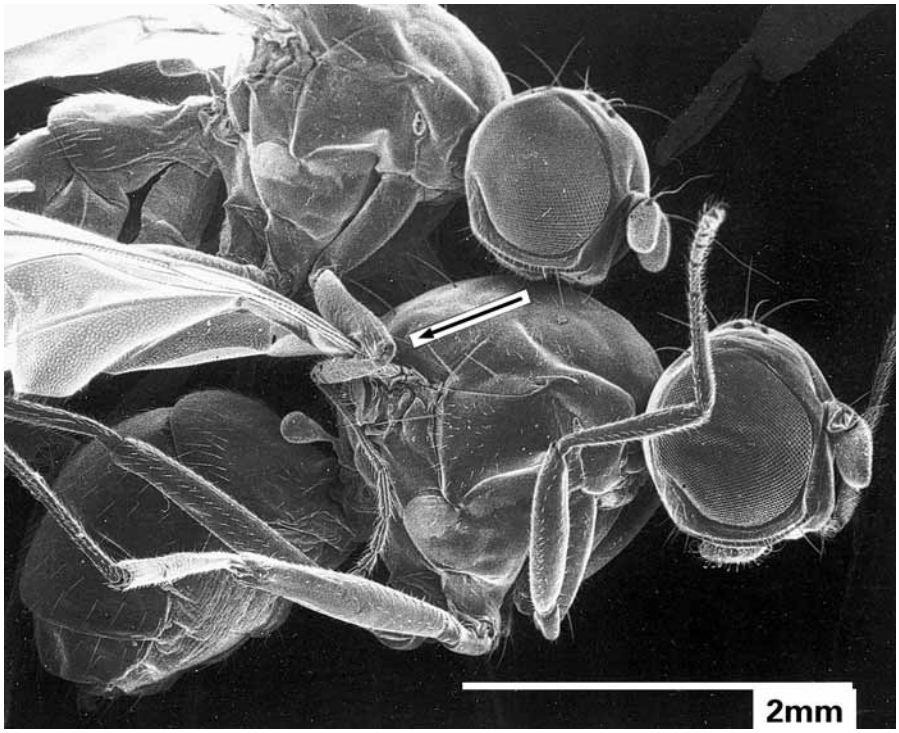

Figure 1. A male *A. diversiformis* holds onto the base of a female's wing by clamping it with his front femur and tibia (arrow).

hypotheses just described, to explain why the front legs of male sepsids are so elaborate. Šulc (1928) argued that the sculptured surfaces of the femur and the tibia fit tightly against each other just behind the rear margin of the female wing, forming a clamp that can be snapped shut. He proposed that this snapping action clamps the female's wing securely and is advantageous to the male because it allows him to expend less energy holding the female and to concentrate his attention on attempts to court and copulate. Šulc made no explicit attempt to explain why the morphology of closely related species should differ, nor did he cite specific structures that snapped shut. He did compare different genera and argued that the snap-tight design had been gradually perfected, starting from modifications that simply increased the friction between the wing and the male's leg, and that the designs of some groups are less specialized to lock than those of others.

To test these hypotheses, the present study employs morphological data regarding the mesh between male front legs and female wings, differences in female design, and a search for female rejection structures that males of *Archisepsis diversiformis* (Ozerov), *A. armata* (Schiner), *A. discolor* (Bigot), *A. pleuralis* (Coquillett), *Palaeosepsis pusio* (Schiner) and *Microsepsis armillata* Melander and Spuler must overcome. Some of these hypotheses have never been carefully tested for any non-genitalic male contact structure, a problem that makes some earlier studies difficult to interpret (Gowaty, 1997a,b).

METHODS

MORPHOLOGY

Pairs derived from flies collected near San Antonio de Escazu, San Jose Province, Costa Rica, were frozen while the male clasped the female's wings prior to copulation (the grip was relaxed soon after intromission occurred). Each pair was gently jarred from the Petri dish in which it had formed while it was held over a Dewar flask containing liquid N_2 or gently blown into liquid N_2 from the tip of an aspirator about 30 cm above the surface of the N_2. Pairs were then fixed without permitting the flies to thaw by placing the flask in a $-20\,^{\circ}C$ freezer, allowing the nitrogen to evaporate and then immersing the still frozen pair in $-20\,^{\circ}C$ absolute ethanol. Only after at least 7 days at $-20\,^{\circ}C$ were the specimens brought to room temperature. Specimens to be examined in the scanning electron microscope were dehydrated from glutaraldehyde and Karnovsky, dried by sublimation and coated with 20 nm of gold. Sample sizes of pairs ranged from 1 (*P. pusio*) to 20 (*A. diversiformis*). While this

method of preparing pairs guaranteed exact preservation of their positions at the moment of freezing, it is possible that the male's position shifted during the instant the pair was falling (or flying) into the flask containing liquid N_2. In only two or three pairs had the male begun to release the female's wings when they were frozen, however, so this was probably not a problem. Male structures whose outlines clearly corresponded to the outlines of female structures near which they were positioned were judged to contact the female structure even if there was a small space between them.

Slide preparations of female wings for light microscopy were made by carefully cutting away the side of the thorax before immersing the wing in mounting medium (euparol or Hoyer's). Numbers and positions of apparent campaniform sensilla (based on their external morphology) were measured in these preparations, and drawings were made using a camera lucida. Means are given followed by $\pm$ one standard deviation. The negatives of some photographs were turned over so that all images appear as left wings and legs to facilitate comparisons. Both male legs and female wings were bilaterally symmetrical.

BEHAVIOUR

Adult *A. armata* were raised from eggs laid by wild-caught females from a site about 10 km S of Horquetas (Limon Province, Costa Rica) (elevation about 1000 m) on fresh cow dung. They were separated by sexes less than 12 h after emerging as adults and kept with honey and fresh dung for 3–4 days before being tested. Males of *A. diversiformis* were collected in the field near San Antonio de Escazu less than 12 h before being tested and kept with honey and fresh dung. Field observations were made near freshly deposited cowpats near San Antonio de Escazu, except in the case of *A. ecalcarata*, which was observed near Federal, Entre Rios, Argentina.

Voucher specimens have been deposited in the US National Museum and in the Museo de Insectos of the Universidad de Costa Rica.

RESULTS

MORPHOLOGY

A. diversiformis

The morphology of the male and female structures of *A. diversiformis* will be presented in detail and then used as a basis for comparison with those of the other species.

Female wing base. Both the dorsal and the ventral surfaces of the base of the female wing of *A. diversiformis* showed great relief in the region grasped by the male. On the dorsal surface (Fig. 2), the most prominent dorsally projecting features, moving posteriorly from the wing's anterior margin, were the costal vein, the stem vein and the base of vein CuA1. The deepest indentation on the wing surface was just posterior to the stem vein. The basicostal cell, between the costal and stem veins, constituted a second, much less profound indentation. The distances between veins varied somewhat, depending on the degree of folding of the wing.

The pattern of relief on the ventral surface (Fig. 3) was quite different. The costal vein and the thinner subcostal vein were the most prominent features; the shallow basicostal cell between them was most deeply indented basally, near the junction of the two veins. The deepest indentation was directly posterior to the subcosta. Directly posterior to this indentation was the bulging br cell. The CuA1 vein protruded at the posterior margin of this cell, slanting diagonally rearward. Posterior to this vein was a relatively sharp invagination to the large, more or less flat area of the alula (Fig. 3).

Both wing surfaces were covered with a more or less evenly spaced array of fine microtrichia about 15–20 μm long and 1–2 μm in diameter at the base, which were direct outgrowths of the cuticle and did not have socketed bases (Fig. 4D,E). The microtrichia were smaller or absent in the dorsal area just posterior to the basal portion of the stem vein, part of the basal portion of the dorsal surface of the stem vein where the male grasped the wing with his femur and in the basal portion of the basicostal cell on the ventral side of the wing where the male's tibia grasped the wing (below) in both virgin and non-virgin females. Socketed setae occurred along the anterior margin of the costa, and also along the subcosta on the ventral surface of the wing (Figs 2, 3), in areas where the male's leg did not make contact with the female.

There was a short line of four closely spaced campaniform sensilla near the dorsal crest of the stem vein, and a pair of larger sensilla slightly posterior and basal to them (Figs 2, 4, 5) in the general area contacted by the male femur. On the ventral surface, there was a more widely spaced line of six or seven campaniform sensilla on the postero-ventral surface of the subcosta (Figs 3, 5) in the general area and distal to where the male tibia contacted the ventral surface of the wing. There were also campaniform sensilla on a fold just basal to the base of the subcosta (Fig. 3) and at many other sites on the wing. There was appreciable variation in the relative and absolute positions of female sensilla (Fig. 5, Table 1). Coefficients of variation for measured distances (Fig. 6) ranged from 7% to 57% for absolute values and from 9% to 40% for relative distances (standardized for the length of the stem vein) (Table 1). A 4+2 arrangement of

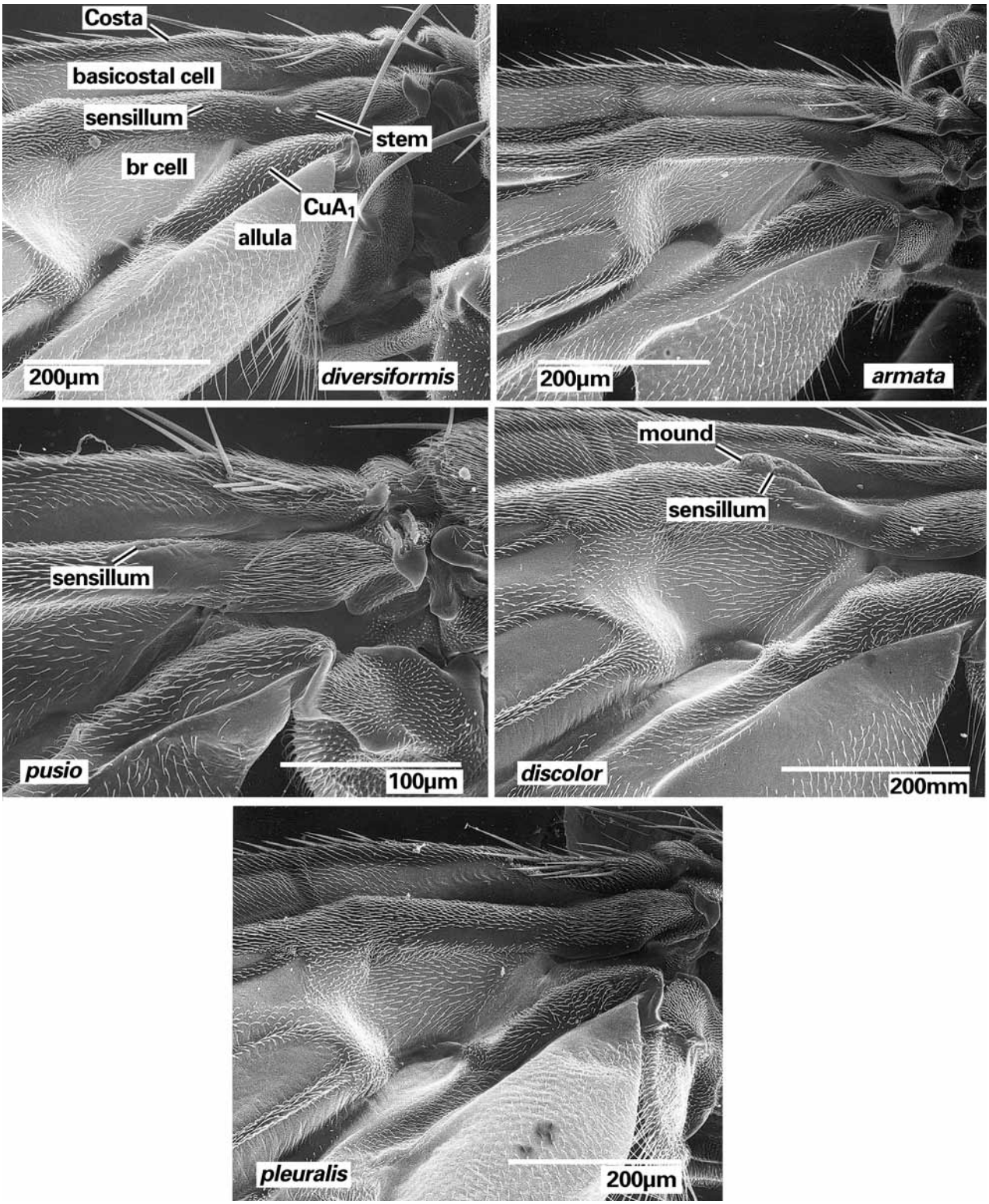

Figure 2. Dorsal views of the base of the female wing in four species of *Archisepsis* and *P. pusio*.

apparent campaniform sensilla on the dorsal surface of the stem vein in the area where the male femur contacted this vein was present in all but two of a sample of 44 females (the exceptions were 3+2 and 5+2).

Similar sensilla were present on the dorsal surface

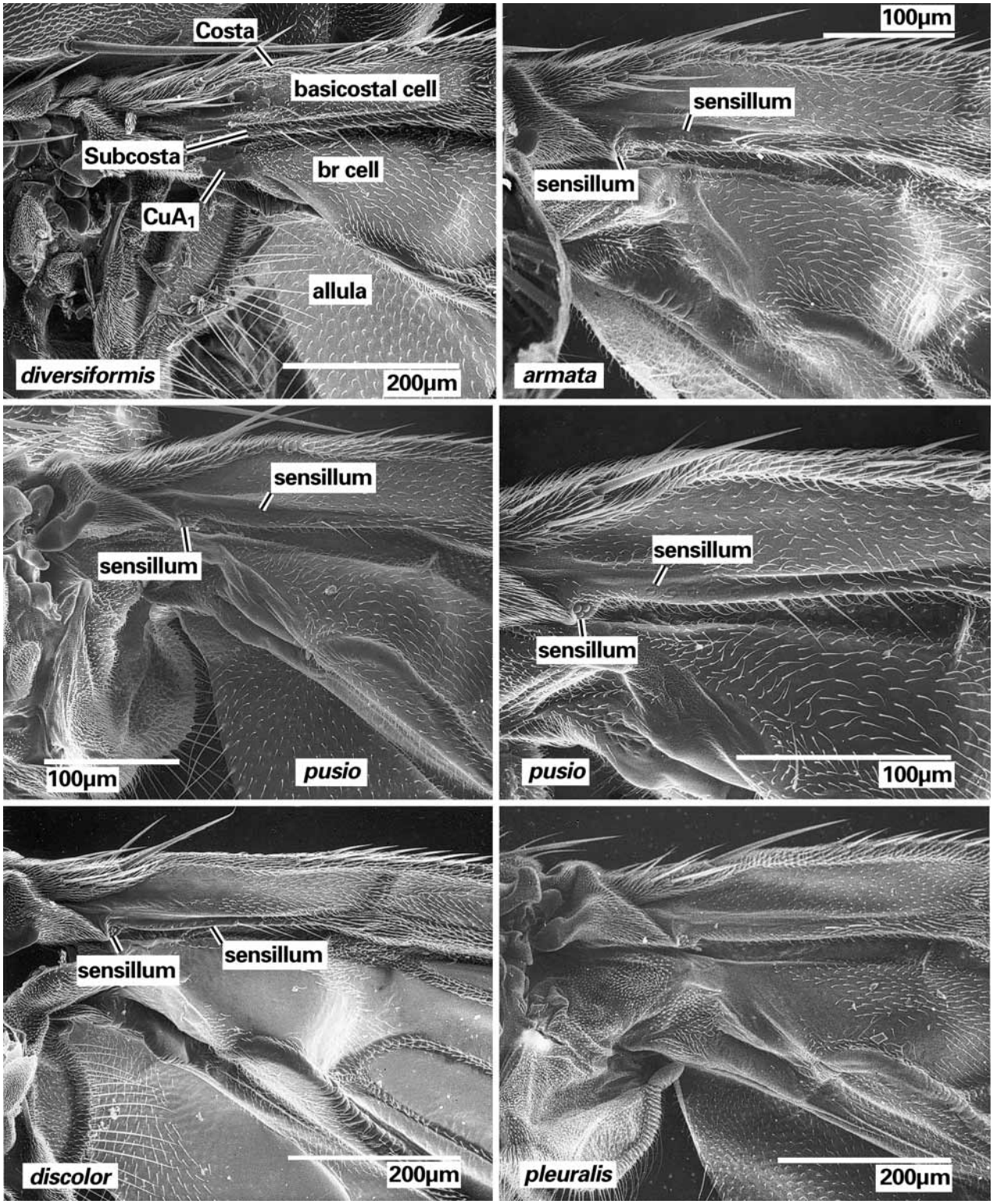

Figure 3. Ventral views of the base of the female wing in four species of *Archisepsis* and *P. pusio*.

of the stem vein of male wings, but differed somewhat in their relative positions (Fig. 5, Table 1). Male stem cells averaged just over 10% shorter than those of females. The line of four small sensilla was slightly longer (b in Fig. 6), the two larger sensilla were slightly farther from the base of the stem vein (d in Fig. 6)

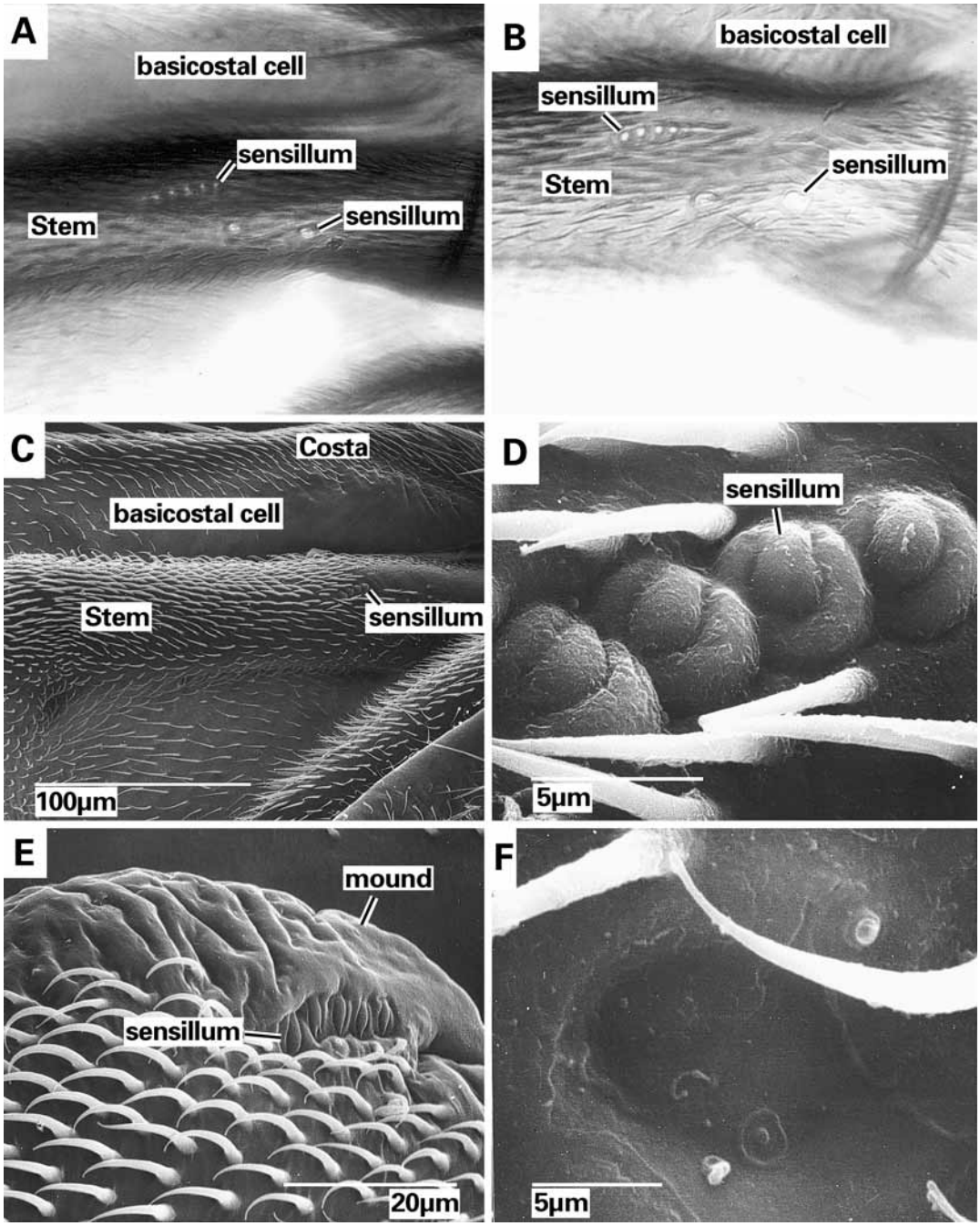

Figure 4. Campaniform sensilla and microtrichia on the dorsal and ventral surfaces of the wings of female *Archisepsis*. A, light micrograph of the stem vein of a female *A. diversiformis*. B, light micrograph of the stem vein of a male *A. diversiformis*. C, scanning electron microscopy image of the dorsal surface of the stem vein, showing the campaniform sensilla in a female *A. diversiformis*. D, close-up of line of four sensilla in C. E, close-up of mound on stem vein of a female *A. discolor*. F, close-up of one of the two larger sensilla below and to the right of the row of four on the stem vein in a female *A. diversiformis*.

and the line of four was slightly more basal with respect to the distal of the two larger sensilla (Table 1). Variation in positioning of male sensilla in males was similar to that in females (Fig. 5, Table 1). Coefficients of variation were greater than those for other body parts, such as head width, thorax length and femur length, which ranged from 3.6% to 7.8% (Eberhard *et al.*, 1998).

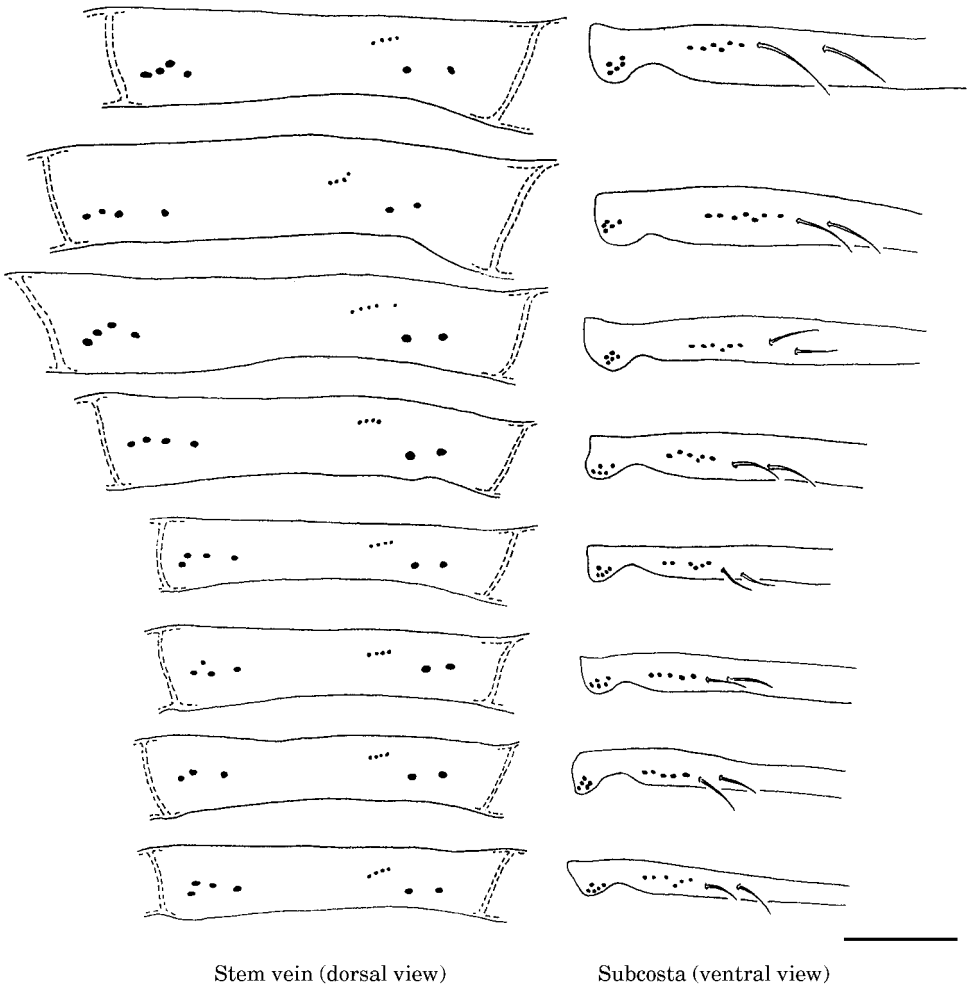

Figure 5. Intraspecific variation in the positions of campaniform sensilla (black dots) on the stem vein (left) and the subcostal vein (right) in eight females of *A. diversiformis* (scale = 0.1 mm). Some of the differences in the anterior–posterior positioning of sensilla may have resulted from differences in the degree of folding of the wing membrane, but the basal–distal positioning was presumably unaffected by folding.

Male front leg. On the femur, there was a single large, basal femoral seta on the postero-ventral surface (Figs 7, 8). Moving distally, there was a sloping prominence on the ventral surface that bore a thick curved black spine that projected ventrally and somewhat distally from a membranous base (Figs 8–10). A flattened, rigid extension of the antero-ventral surface (the 'antero-ventral thumb') originated nearby and also projected ventrally and distally (Figs 8–10). There was no projection on the postero-ventral surface of the femur. Distal to the sloping prominence, the ventral surface of the femur was flattened and slightly bowed, forming a small cavity whose surface was relatively smooth (Fig. 9). A pair of stout socketed 'postero-ventral setae' originated on small prominences near the basal edge of this surface (Figs 9, 10).

The ventral surface of the male front tibia (Figs 11, 12) bore a pair of strong setae near the base and a triangular 'basal prominence' just distal to them that had several small socketed setae on its antero-lateral surface (Figs 11, 12). Moving distally along the ventral surface, there was a small depression ('basal groove') just beyond the basal prominence and then a complexly sculptured medial prominence followed by a deep, abrupt transverse groove (the 'median groove') which was about 15 μm across and was approximately even with the first of a row of three strong bristles on the posterior margin of the tibia. The distal margin of this groove bore a second triangular distal prominence. More distally, the surface of the tibia was relatively round and smooth, bearing near its tip a patch of closely spaced, short and moderately stout setae.

Table 1. Relative positions of apparent campaniform sensilla on the dorsal surface of the stem vein in wings of different species of *Archisepsis* and *P. pusio*. All distances are expressed as fractions of the total length of the stem vein (f in Fig. 5) and given as mean ± one standard deviation. Pairs of values in the same column that are associated with the same letter differ with Mann–Whitney *U*-tests (a, $P<0.05$; b, $P<0.01$; c, $P<0.001$)

Species	Sex	a	b	d	e	g	f	*N*
Archisepsis								
diversiformis	F	0.330 ± 0.029 $b_1b_2c_1$	0.056 ± 0.010 a_1	0.107 ± 0.043	0.130 ± 0.018 a_2	0.023 ± 0.061 b_3c_2	116.5 ± 10.9 b_4	44
diversiformia	M	0.315 ± 0.053	0.062 ± 0.012 a_1	0.113 ± 0.039	0.144 ± 0.029 a_2	-0.011 ± 0.057 b_3	110.1 ± 8.5 b_4	39
armata	F	0.350 ± 0.27 a_3	0.055 ± 0.001	0.100 ± 0.025	0.148 ± 0.029	0.032 ± 0.059 b_5b_8		6
discolor	*F*	$a_3b_6b_7c_1$	*0.419 ± 0.044*	*0.056 ± 0.011*	*0.104 ± 0.020*	*0.141 ± 0.033* $b_8b_9b_{10}c_2$	*0.120 ± 0.023*	*9*
pleuralis	F	0.224 ± 0.037 b_1b_7	0.043 ± 0.010	0.065 ± 0.017	0.138 ± 0.013	0.026 ± 0.034 b_{10}		5
Palaeosepsis								
pusio	F	0.262 ± 0.028 b_2b_6	0.091 ± 0.015	0.119 ± 0.018	0.186 ± 0.034	-0.058 ± 0.040 b_9		5

Clamped pairs. A total of 19 pairs was frozen while the male gripped the female's wings (Figs 14–16). In all pairs the male's grasp on both wings was always somewhat transverse, so that his leg crossed the anterior margin of the wing closer to the wing base than at the posterior margin (e.g. Fig. 16). The distal portion of the femur and the basal portion of the tibia projected beyond the anterior margin of the wing (Figs 14–16). There was substantial variation in the exact position of the male's femur. The anterior margin of the femur crossed the anterior margin of the wing at about the basal fourth of the basicostal cell (mean of $x/(x+y+z)$ in Fig. $6 = 0.27 \pm 0.10$, range 0.17–0.67 in 15 measured pairs), while the posterior margin of the femur crossed just over half-way out (mean of $z/(x+y+z)$ in Fig. $6 = 0.38 \pm 0.10$, range 0–0.48). There was also variation in the same female from one wing to the other. The greatest difference in where the anterior margin crossed was 0.27 on one side and 0.67 on the other; the next greatest was 0.25 and 0.52.

Structures on the male leg consistently meshed in several places with particular structures of the female wing. The large black femoral spine was inserted into the deep indentation just posterior to the stem vein (Fig. 15). The male's antero-ventral thumb pressed against the dorsal and posterior surface of the female's stem vein (Fig. 14). Thus the smooth curved surface formed by the combined distal and prolateral surfaces of the black spine and the thumb pressed against the similarly rounded posterior and dorsal surface of the stem vein. The postero-ventral setae of the femur were in the indentation between the rear margin of the costa and the anterior margin of the stem vein (Fig. 15). The position of the basal femoral seta varied with respect to the female's wing. In eight pairs it apparently contacted the wing near the indentation at the anterior margin of the aula on at least one side of the female, but in nine other pairs it was out of contact with both of the female's wings.

On the ventral surface of the female's wing, the antero-ventral surface of the basal tibial prominence pressed against the basicostal cell in all but three cases (each in a different pair); the triangular tip of the prominence fit into the concave, basal end of this cell (Fig. 16). The small setae on the anterior surface of this prominence (Fig. 11) were thus pressed against the wing. The larger basal setae did not contact the wing (Figs 15, 16). More distally, in all but one case the line of stout setae on the postero-ventral margin of the tibia pressed against the bulging br cell of the wing (Fig. 16); in nine of these pairs, the stout setae caused a perceptible inward (dorsal) indentation in the wing membrane (Fig. 16).

Direct views of the details of the contact with the other complex tibial structures were not possible, but the locations of male and female structures permit the following deductions: the CuA1 vein was almost certainly inserted into the median groove of the tibia; the median tibial prominence probably pressed near the group of four probable sensilla at the base of the subcostal vein; the distal portion of the tibia generally did not contact the wing (Fig. 16).

The male tarsus was in all cases flexed dorsally and rested on the postero-lateral surface of the female's

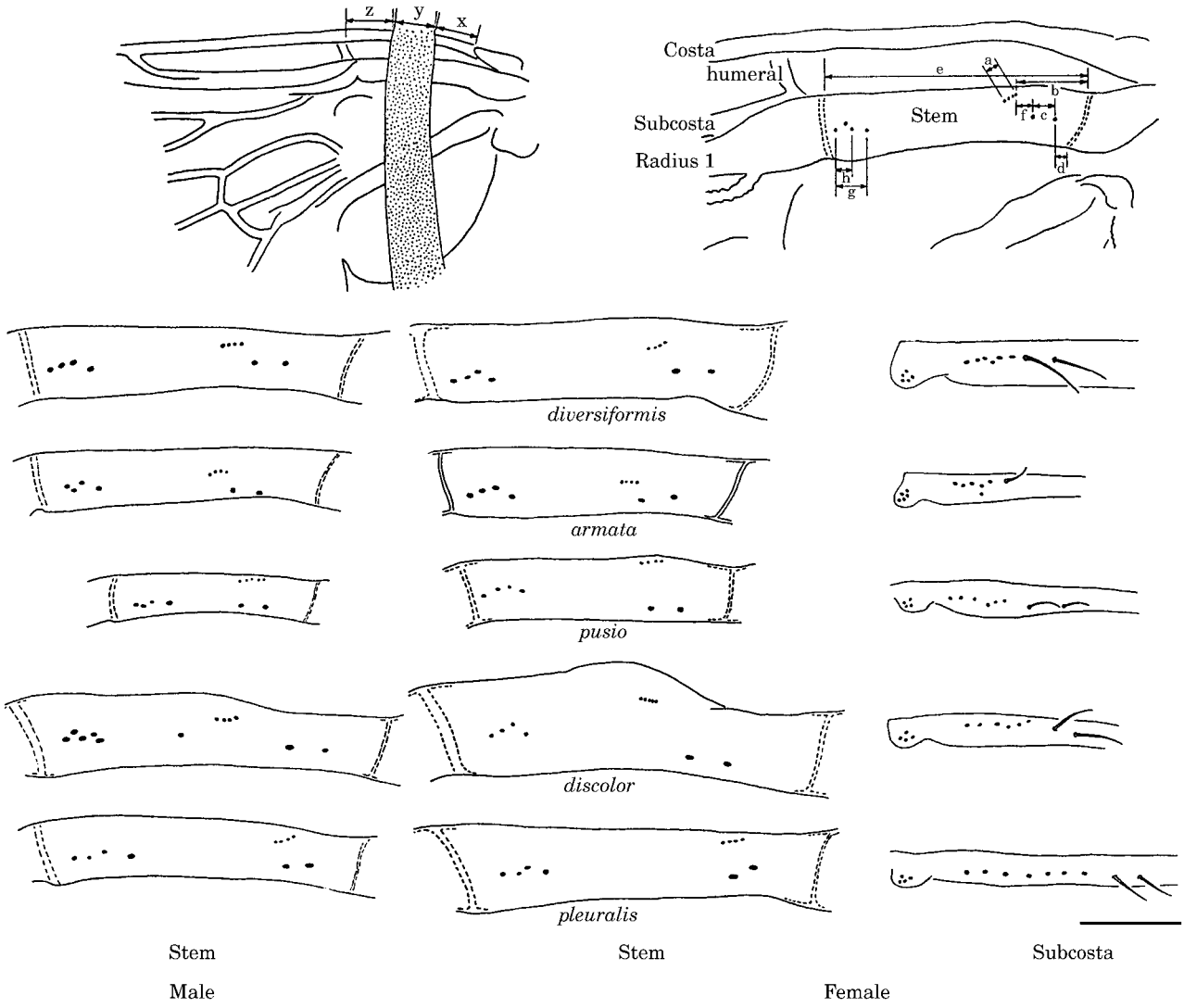

Figure 6. Measurements (x–z) to specify the position of the male femur (stippled) on the female wing, and the positions (a-l) of campaniform sensilla, and interspecific variation in the positions of the campaniform sensilla (black dots) on the dorsal surface of the stem vein (left) and the ventral surface of the basal portion of the subcosta (right) in females and males of four species of *Archisepsis* and *P. pusio* (scale = 0.1 mm). Measurements of the distance from the lateral margins of the femur to reference points on the wing (x, z) were standardized on the basis of the wing size; the angle of the femur with the wing was based on a scanning electron microscopy image of *A. pleuralis*, and the positions of the basal portions of the femur are based on this photograph rather than direct measurements (which were often difficult because of the position of the wing with respect to the female's thorax). Some of the differences in measurements of the anterior–posterior positioning of sensilla may have resulted from differences in the degree of folding of the wing membrane, but the basal–distal positioning was presumably unaffected.

thorax between the base of the wing and the base of the haltere. The exact site varied from about half-way between the two to next to the wing base.

A. armata

Female wing base. There was no clear overall difference in the general shapes and locations of the veins and cells from the wing of *A. diversiformis* (Figs 2, 3, 18). The positions of campaniform sensilla on the dorsal surface of the stem vein and the ventral surface of the subcosta varied as shown in Figure 6.

Male front leg. The ventral processes on the femur resembled those of *A. diversiformis* in shape and orientation except that they were set on a larger sloping

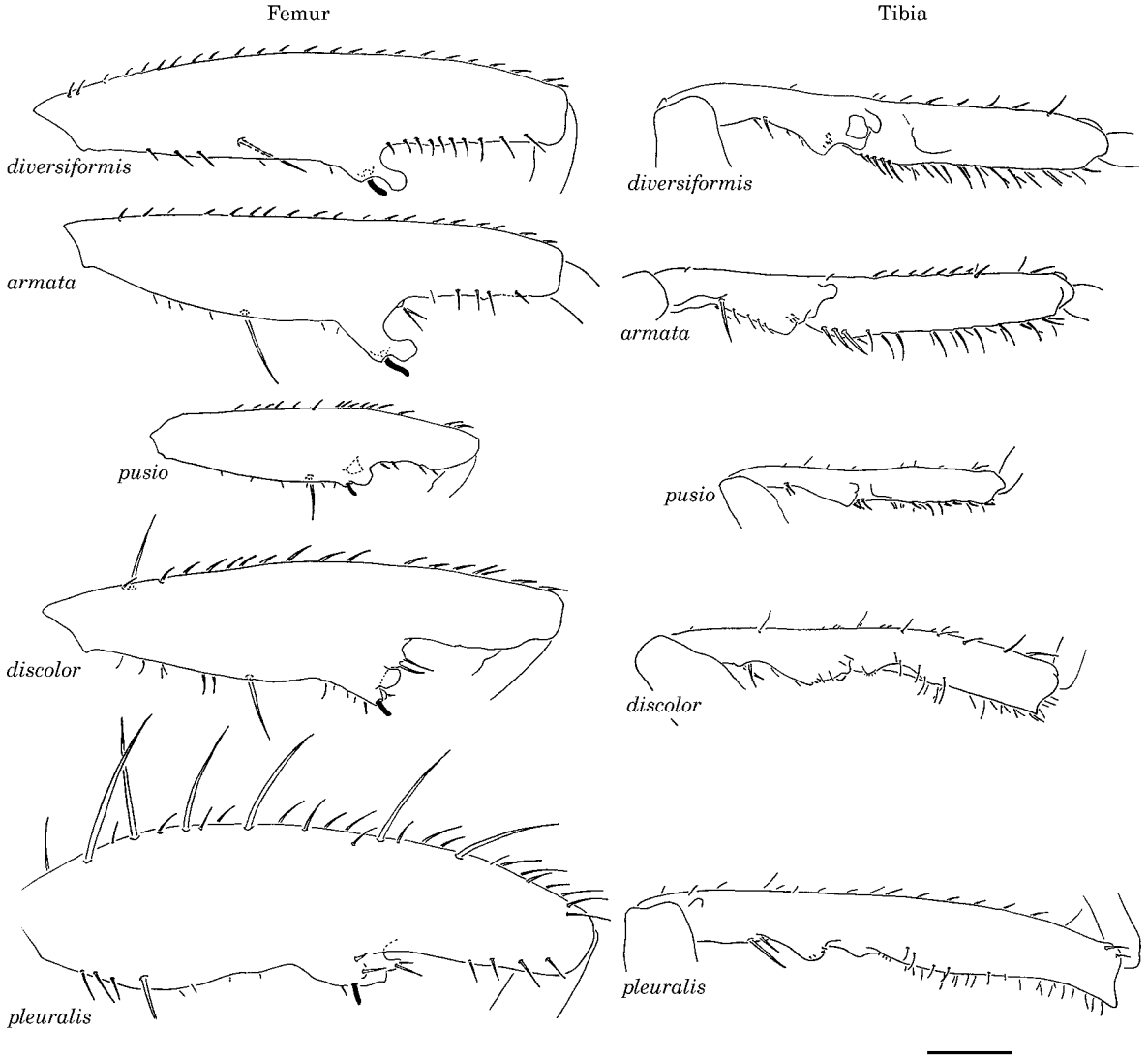

Figure 7. Front femur (left) and tibia (right) or males of four species of *Archisepsis* and *P. pusio* (scale = 0.1 mm). Note that these prolateral views do not give a perfect profile of the clamping structures.

prominence and the antero-ventral thumb was somewhat larger (Figs 8–10). The basal prominence on the tibia was slightly larger, the median prominence projected partly over the median groove (Figs 11, 12), this groove itself slanted so that its posterior end was more basal than its anterior end (Fig. 11), and the distal prominence was less pronounced. The basal setae were slightly larger (Figs 7, 11).

Clamped pairs. The three pairs of *A. armata* frozen while the male clamped the female's wings were similar to those of *A. diversiformis* with respect to the following (Figs 14–16): the male's leg crossed the female's wing in a similar slanting orientation and at about the same site; the male's front femur and tibia both pressed against the wing rather than against each other; the distal portion of the male's femur projected beyond the anterior margin of the female's wing; the large black femoral spine was inserted in the deep indentation posterior to the stem vein, and pressed against the posterior surface of this vein (Fig. 15); the flat surface of the antero-ventral thumb pressed on the dorsal surface of the stem vein, and thus the curved surface between them pressed against the curved dorsal-posterior surface of this vein (Fig. 14); the pointed basal tibial prominence pressed into the basal indentation of the female's basicostal cell (Fig. 16); and the stout setae on the retrolateral edge of the tibia pressed the br cell of the wing (Fig. 16). The CuA1 vein was almost certainly in the median groove of the tibia. Because of the relatively long sloping femoral prominence, the central portion of the femur was farther from the

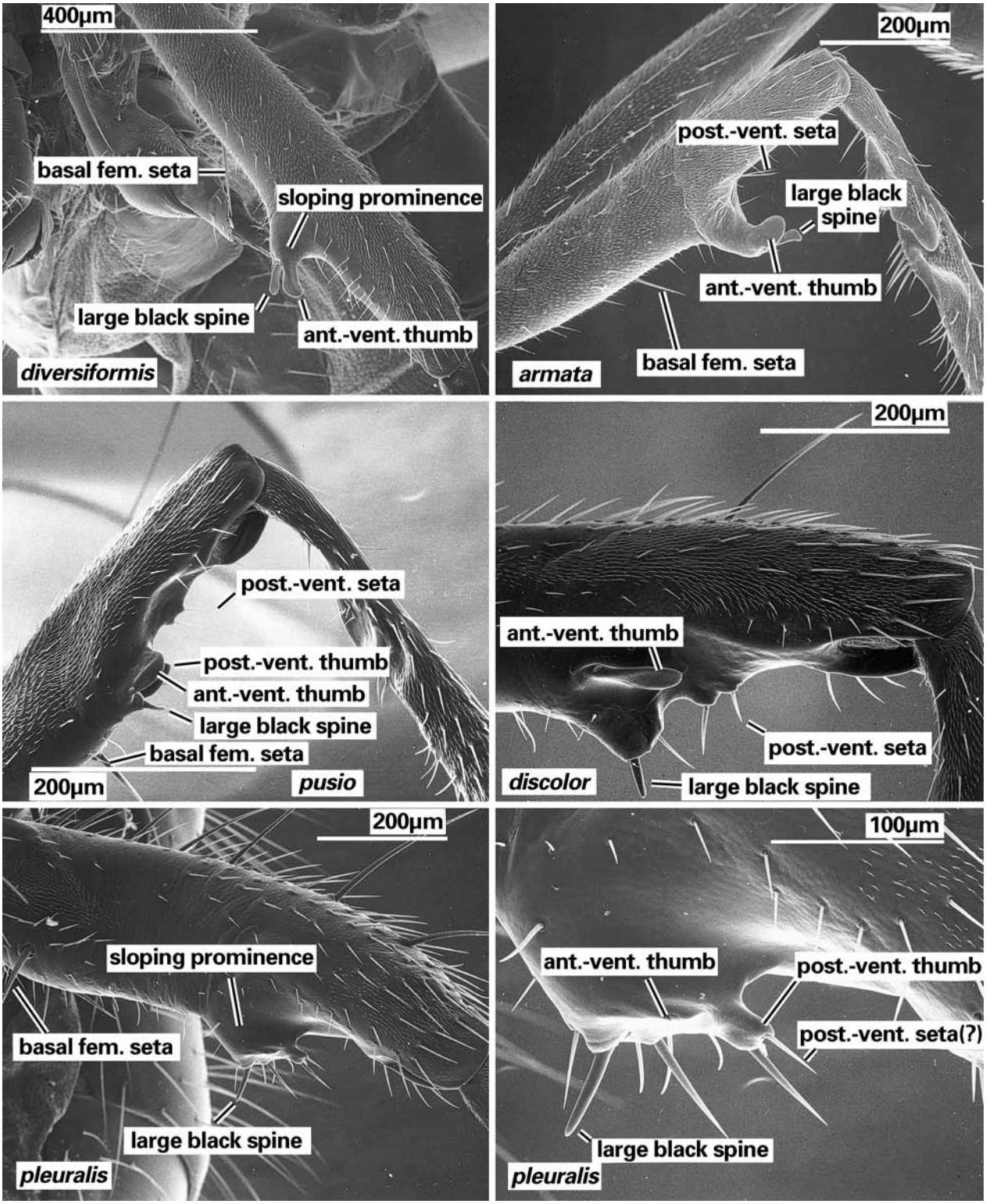

Figure 8. Anterior views of the front femora of males of four species of *Archisepsis* and *P. pusio*.

dorsal surface of the female's wing than in *A. diversiformis*, and the relatively short basal femoral seta was out of contact with the wing (e.g. Fig. 20) in all pairs.

A. discolor

Female wing base. The stem vein differed from that of all the other species in this study in having a dark, wrinkled mound on its dorso-anterior surface at about

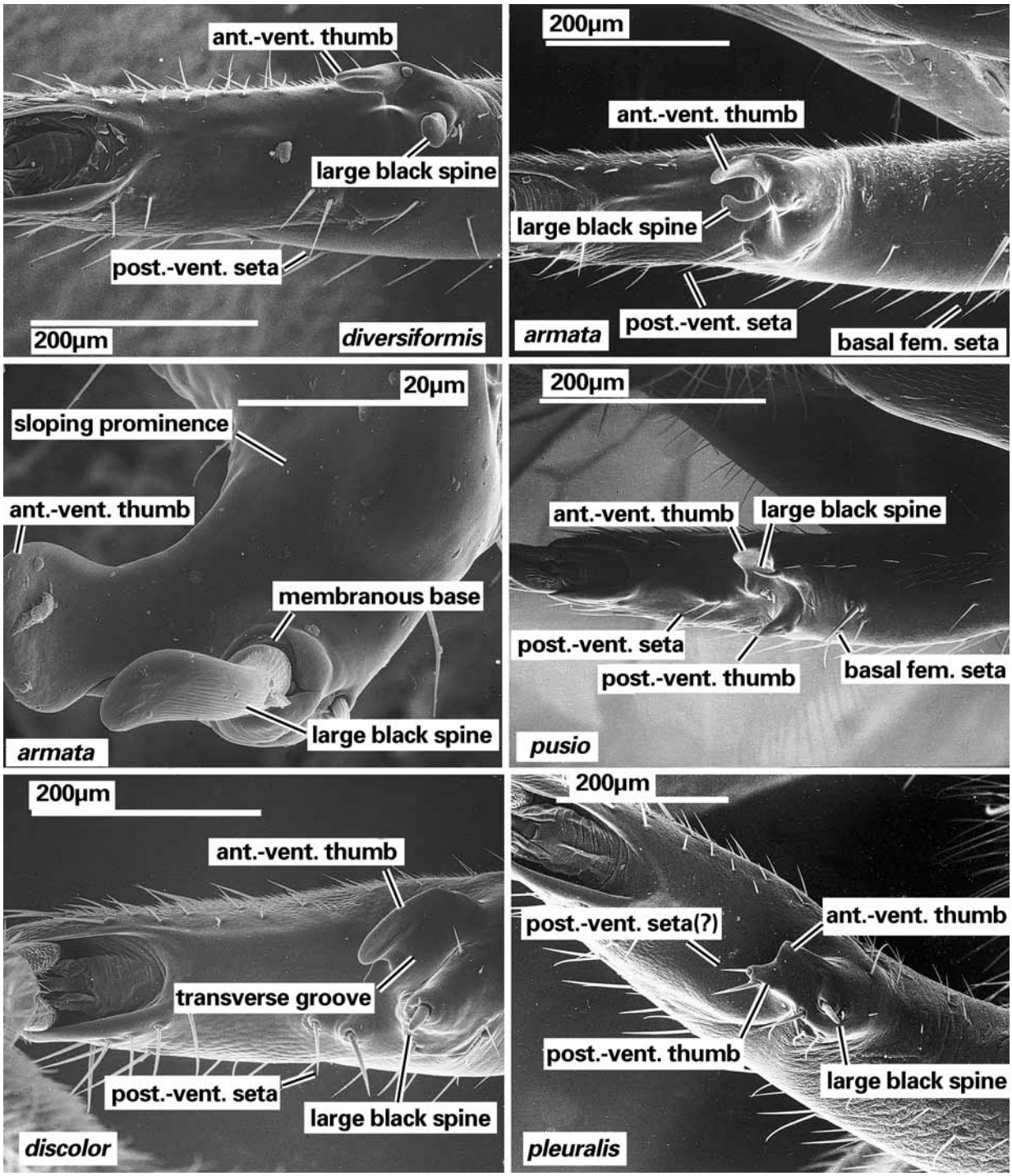

Figure 9. Ventral views of the front femora of males of four species of *Archisepsis* and *P. pusio*.

the middle of the basicostal cell (Figs 2, 4, 6) which was rigid when touched with an insect pin. Smaller mounds of this sort were also present on male wings. In both sexes there was a normal $4+2$ set of campaniform sensilla (Fig. 6, Table 1). The surface of the female's mound lacked the microsetae that covered adjacent regions and bore the row of four campaniform sensilla near its base (Fig. 4). These were positioned more distally than in other species, and the pair of stem vein sensilla posterior to these were more basal in

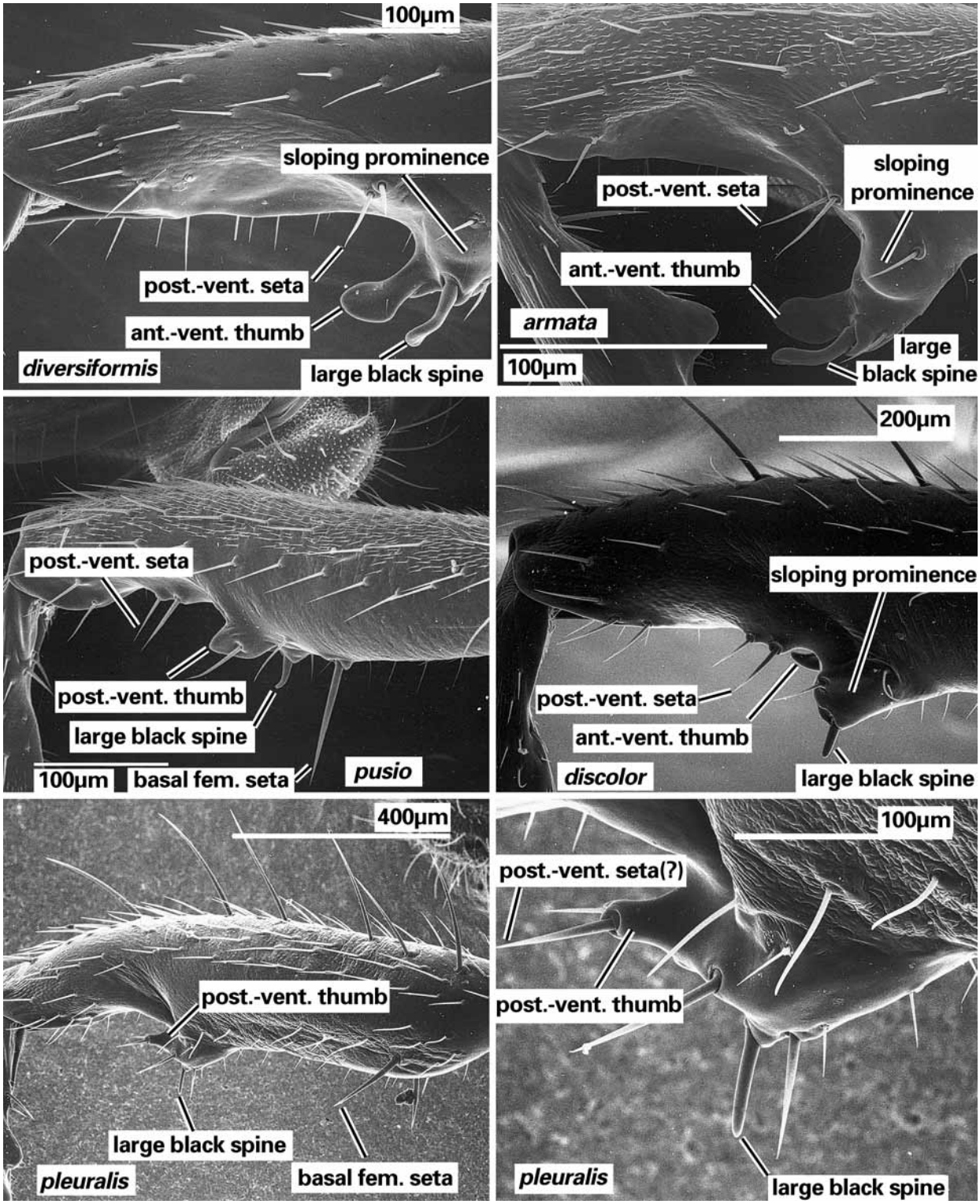

Figure 10. Posterior views of the front femora of males of four species of *Archisepsis* and *P. pusio*.

position (Table 1). Apart from these differences, the overall layout of the base of the female wing was similar to that of *A. diversiformis* (Figs 2, 3, 18).

Male front legs. The male front femur differed sharply from that of *A. diversiformis* in having the large black spine mounted at the tip of a large triangular ventral

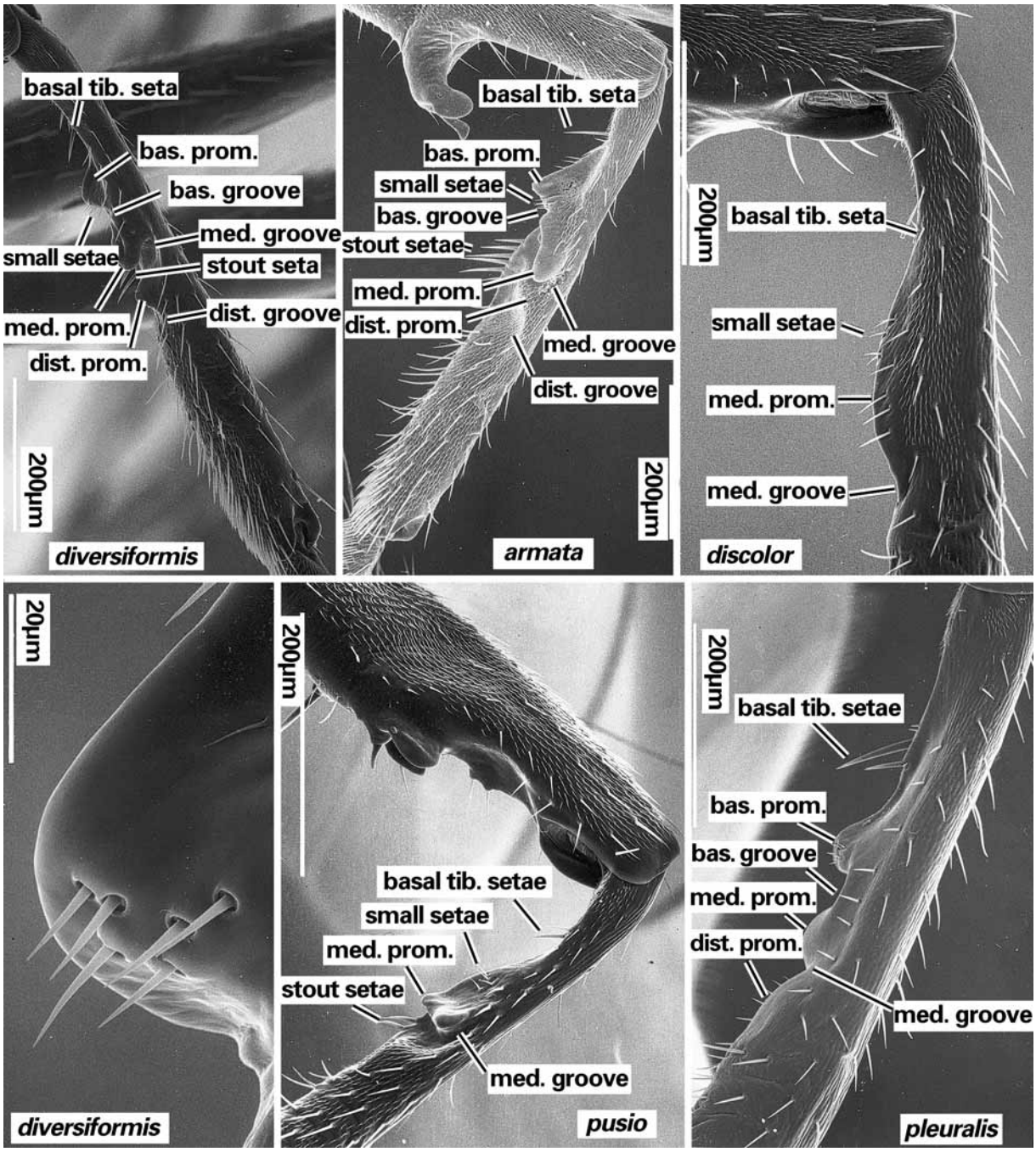

Figure 11. Anterior views of the front tibiae of males of four species of *Archisepsis* and *P. pusio*.

prominence (Fig. 8). The antero-ventral thumb had a transverse groove at its base (Fig. 9) and extended distally as a plane with a rounded thickened distal edge (Figs 8, 9). There was no postero-ventral thumb, but a ridge on the ventral surface just distal to the prominence with the black spine sloped sharply inward, forming a groove (Fig. 8) just basal to the postero-ventral setae, each of which was set on a prominence (Figs 8, 10). The basal seta was relatively small (Fig. 7).

The front tibia lacked a sharply defined basal prominence as in *A. diversiformis*. The small tibial spines that corresponded to the setae near the tip of the basal prominence of *A. diversiformis* were placed about halfway along a gradually sloping, rounded median prominence (Fig. 11). The median groove was relatively wide, with gently sloping sides; just distal were a small distal prominence and a weakly defined distal groove (Figs 12, 13). There were only moderately sized setae

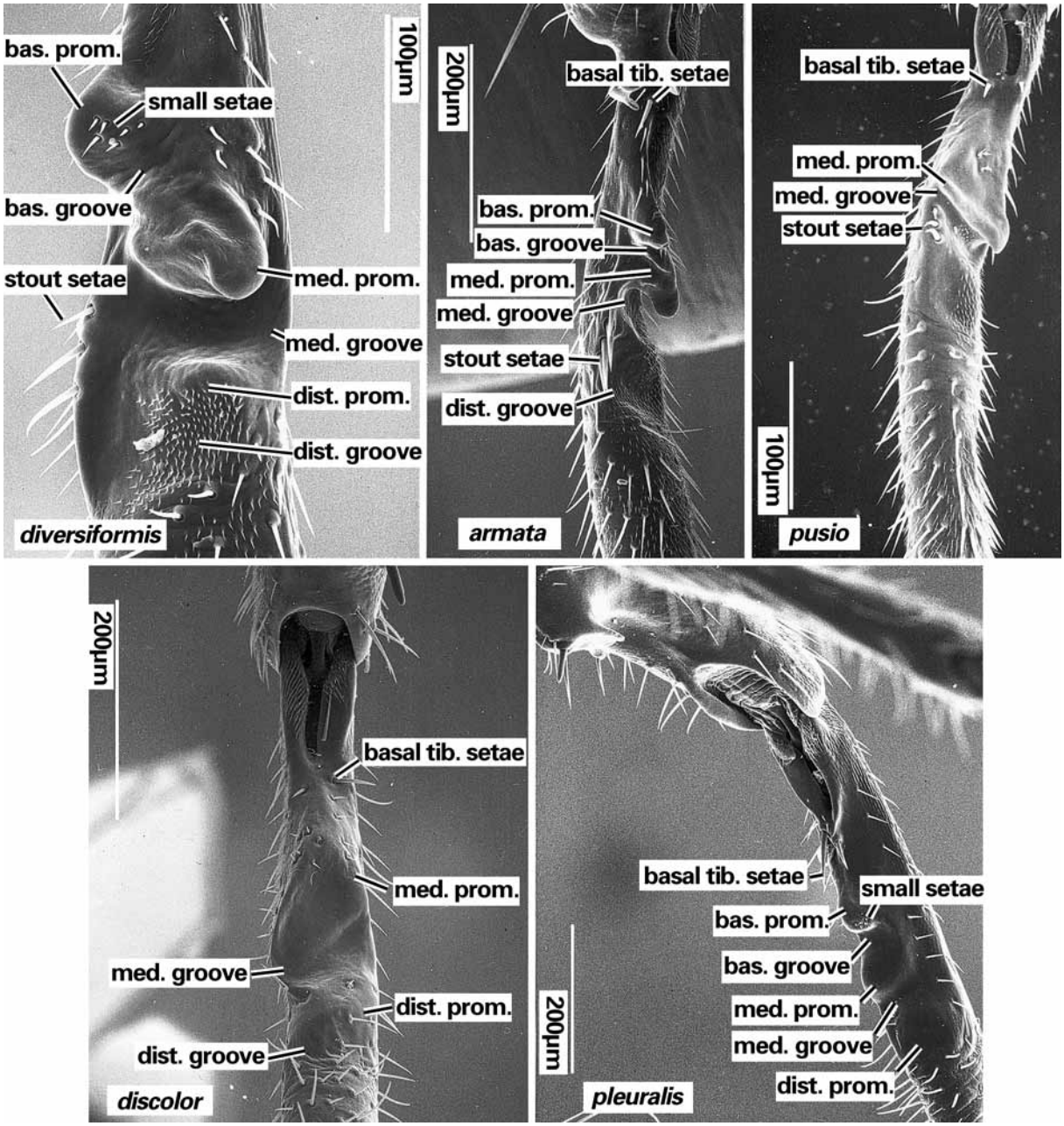

Figure 12. Ventral views of the front tibiae of males of four species of *Archisepsis* and *P. pusio*.

in the area of the tibia occupied by stout setae in other species (Figs 7, 12).

Clamped pairs. On the dorsal side of the female wing, the large black spine pressed near the rear margin of the stem vein (Fig. 19). The distal surface of the large ventral prominence of the femur pressed on the posterior surface of the stem vein, and the curved ridge between the prominence and the first of the two postero-ventral setae pressed on its dorsal surface (Fig. 18). Although the force with which the large black spine pressed against the wing caused the spine to be pushed part way into its socket, this pressure did not deform the wing at all (Fig. 19). The groove between the prominences bearing the postero-ventral setae was just dorsal to the costa and may have pressed against it (Fig. 20). The basal portion of the antero-ventral thumb appeared to press on the antero-dorsal surface of the stem vein, while the more distal portion was on the dorsal surface of the costa (Fig. 14).

On the ventral surface of the wing, the basal tibial prominence was lodged between the costa and the

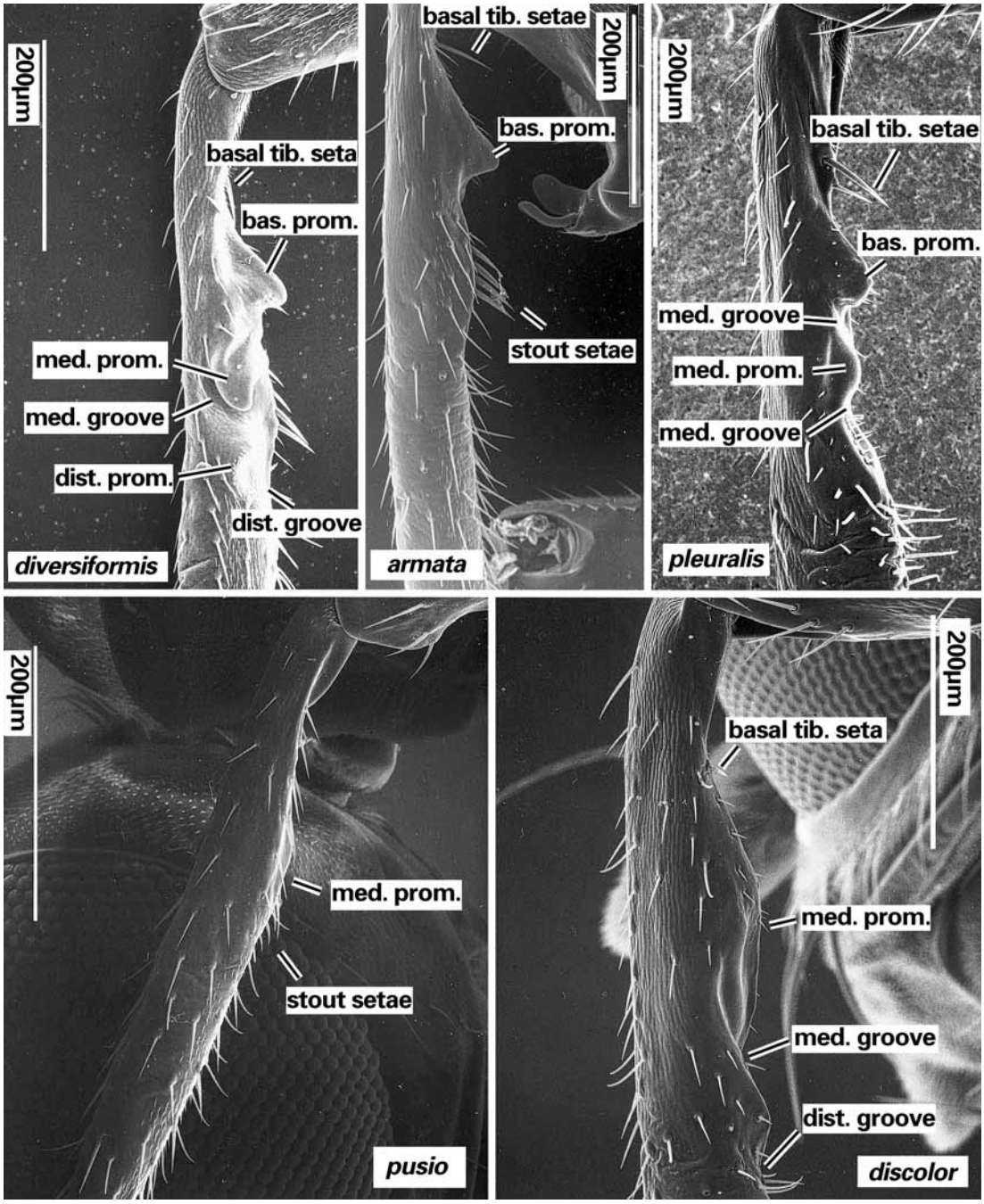

Figure 13. Posterior views of the front tibiae of males of four species of *Archisepsis* and *P. pusio*.

subcosta but did not penetrate deeply into the basicostal cell between them (Fig. 16). The sloping basal surface of the median groove pressed the antero-ventral surface of the subcosta.

A. pleuralis

Female wing base. There were no clear overall differences the positions and shapes of the veins and cells compared with the wing of *A. diversiformis* (Figs 2, 3, 18). The positions of the campaniform sensilla on the stem vein and the subcosta were also similar (Fig. 6).

Male front leg. The large black femoral spine was at the basal tip of a rounded ventral projection (Figs 8–10). This process extended distally with two projections which presumably corresponded to the antero-

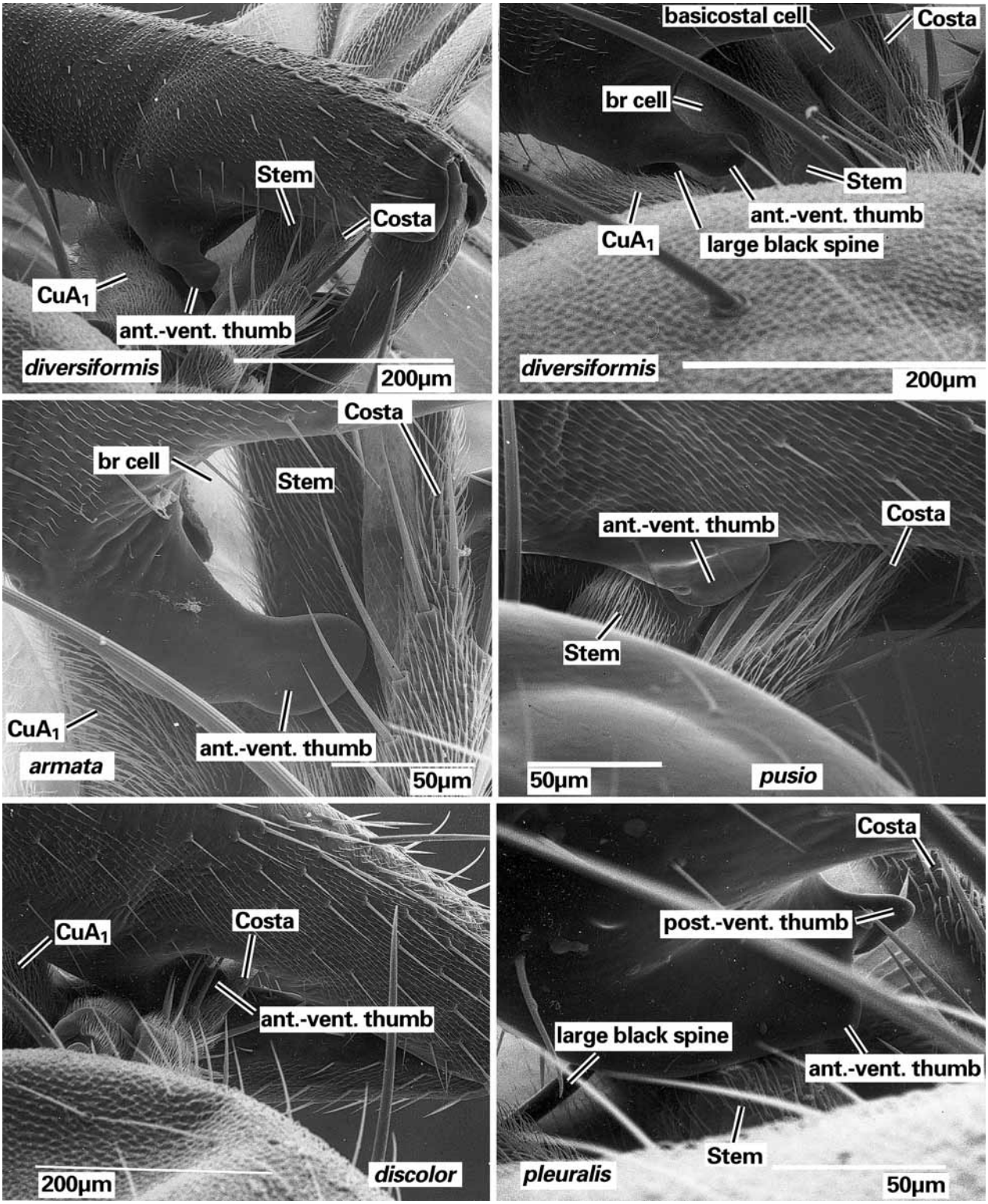

Figure 14. Anterior views of the front femora of males as they grasped the female wing (or, in the case of *A. diversiformis*, as it released the wing).

ventral and the postero-ventral thumbs of other species. The postero-ventral thumb bore a pair of setae that presumably corresponded to the two postero-ventral setae of the other species (Figs 8–10). The ventral surface of the antero-ventral thumb was flat, forming a relatively wide, curved surface (Fig. 9). The moderate-

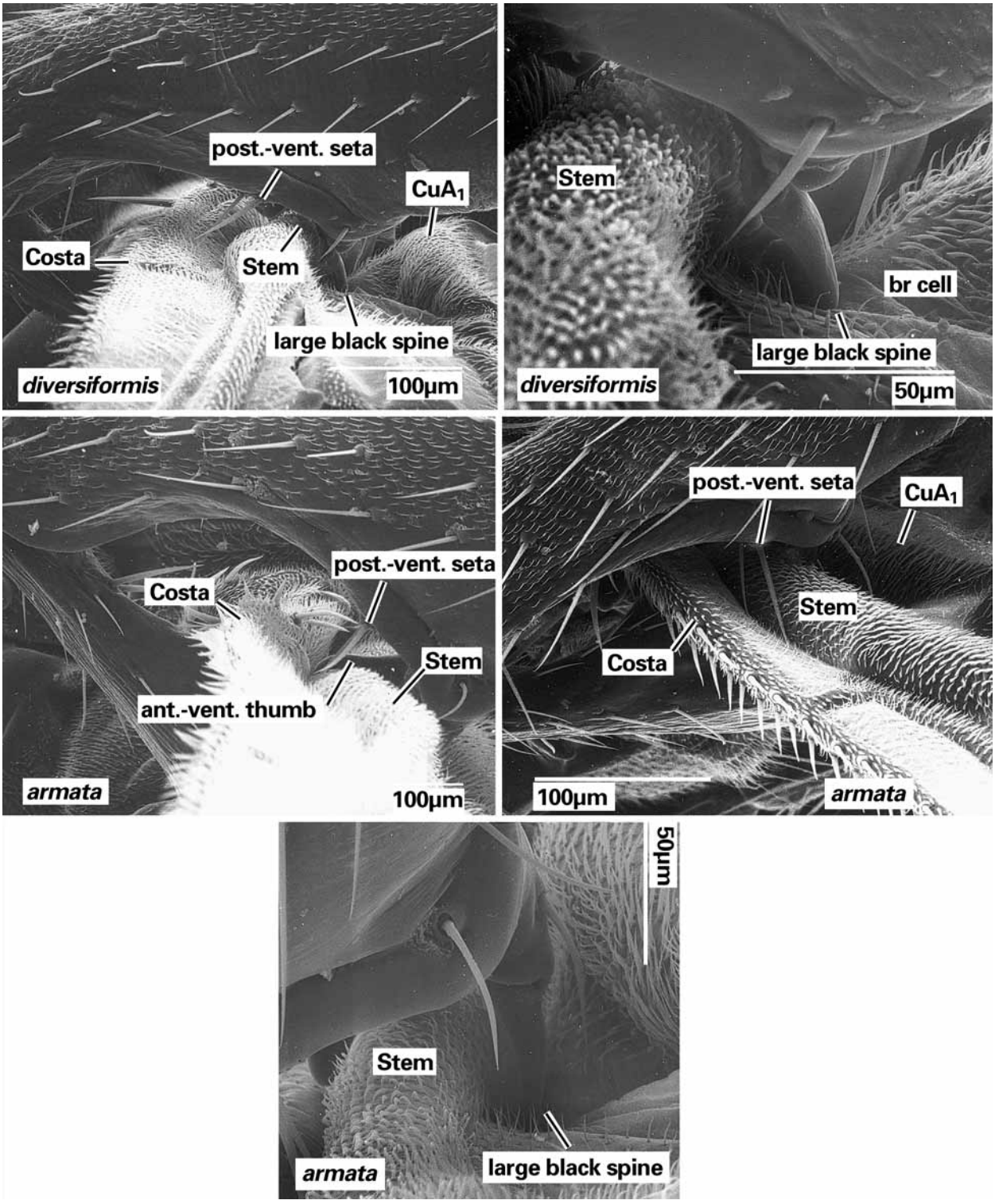

Figure 15. Posterior views of the front femora of males as they grasped the female wing in two species of *Archisepsis*.

sized basal femoral spine arose near the base of the femur. The dorsal surface of the femur bore a row of long setae which, along with similar setae on other parts of the male's body, may be associated with the frequent and intense male–male battles that occur in this species but not the others of this study (W. Eberhard, in prep.).

The basal tibial prominence was more rounded than

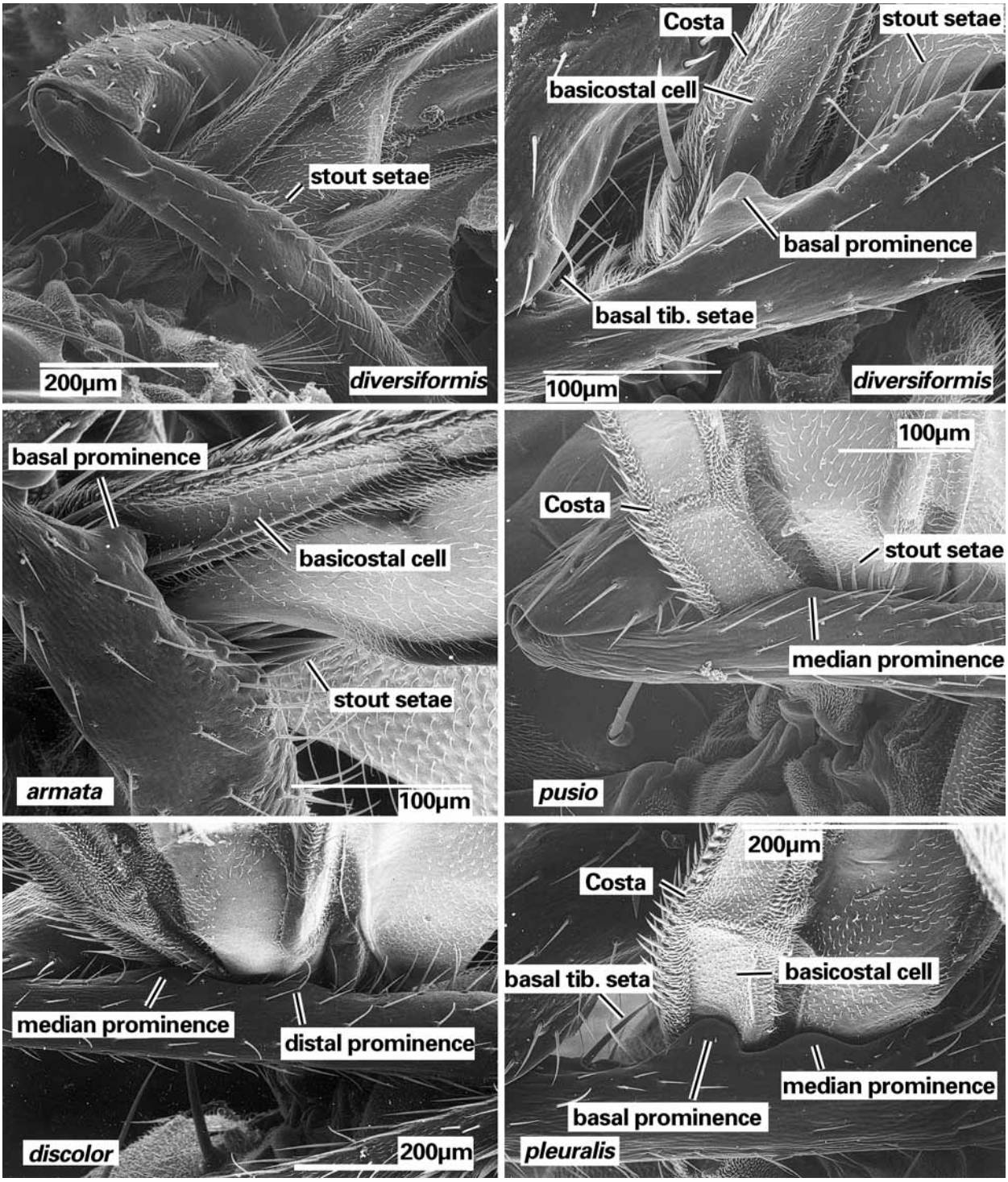

Figure 16. Posterior views of the front tibiae of males as they grasped the female wing in four species of *Archisepsis* and *P. pusio*.

in *A. diversiformis*, with about five short, socketed setae on its anterior surface (Figs 11, 12). Distal to this were a deep but rounded basal groove, a clear median prominence, a less well-marked median groove and a reduced distal prominence (Figs 11–13). There were no stout setae just distal to the median groove, as in *A. diversiformis* (Figs 7, 12).

Clamped pairs. In all but two of the clamps observed, the tip of the antero-ventral thumb was just posterior

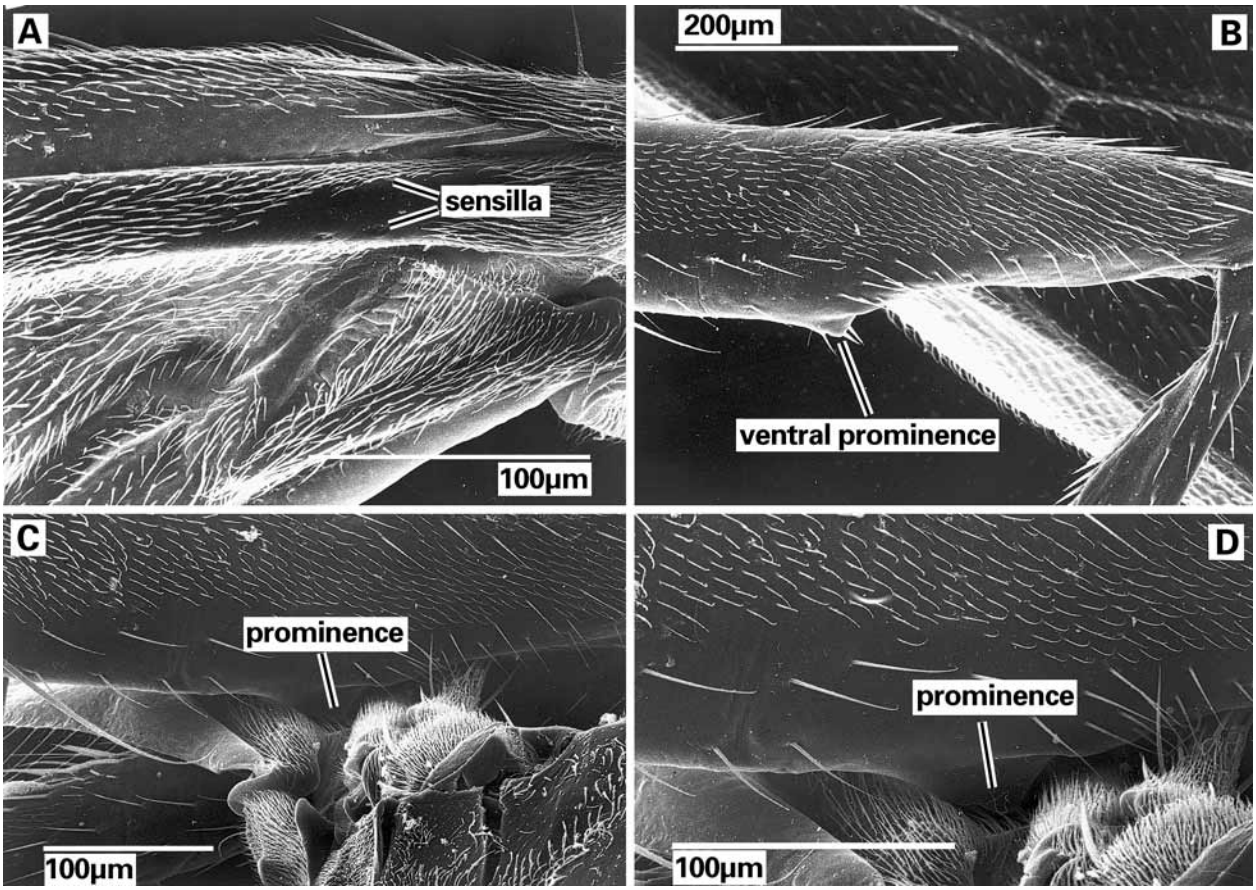

Figure 17. Male and female structures of *M. armillata*: A, dorsal view of base of the female wing; B, anterior view of male front femur; C and D, anterior view of male front femur as it grasps the female's wing.

to the costa but did touch it, and its wide, curved ventral surface pressed the dorsal surface of the stem vein (Fig. 14). The large black spine pressed against the posterior surface of the stem and on the br cell just posterior to it (Figs 14, 19).

In one case, the tip of the antero-ventral thumb pressed against the posterior surface of the stem vein, deflecting rearward at least one of the possible postero-ventral setae at its tip (the other seta was probably also deflected but could not be observed directly).

On the ventral surface of the female's wing, the rounded basal tibial prominence of the male pressed into the basicostal cell but in a somewhat less basal portion of the cell than in *A. diversiformis* (Fig. 16). The subcosta was apparently in the large basal groove of the tibia, and the rounded median prominence of the tibia pressed on the br cell (Fig. 16).

The site at which the male's femur crossed the anterior margin of the female's wing varied substantially. The mean site where the anterior margin of the femur crossed the costa ($x/(x+y+z)$ in Fig. 6) was 0.32 ± 0.07 (range 0.15–0.44) in 29 wings from 15 pairs. There was similar variation in the site of posterior crossing ($z/(x+y+z)$ in Fig. 6) (mean = 0.26 ± 0.08, range 0.11–0.40). As in *A. diversiformis*, asymmetric grasps of a given female's two wings occurred, although to a lesser extent; the most dramatic anterior asymmetry was 0.25 and 0.15, and the next most extreme was 0.21 and 0.34.

P. pusio

Female wing base. There were no clear differences in the general shapes and positions of veins and cells (Figs 2, 3, 18) with the wing of *A. diversiformis*. The four small capaniform sensilla on the dorsal surface of the stem vein were in a small groove (Fig. 2). The positions of the campaniform sensilla on the stem and subcostal veins varied as shown in Figure 6.

Male front leg. The ventral surface of the femur differed from that of *A. diversiformis* in several details (Figs 7–10). The basal femoral seta was relatively large and placed more distally. The black spine was relatively

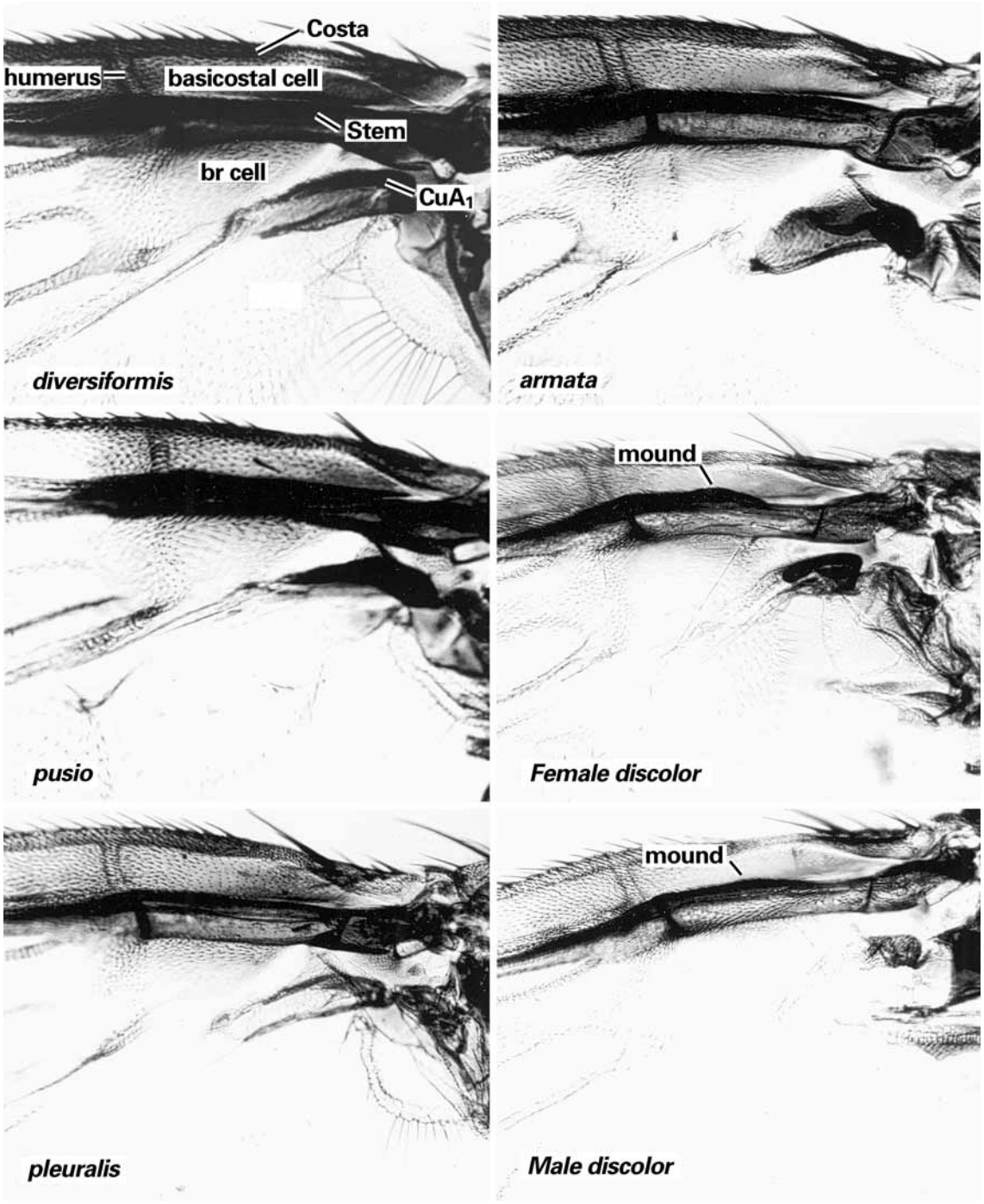

Figure 18. Light micrographs of wings of females of four species of *Archisepsis* and *P. pusio* and of a male of *A. discolor*.

small and was set on a smaller prominence (Figs 7, 8, 10). The antero-ventral thumb was also relatively reduced and distally formed a flattened plate (Figs 8, 9). The posteroventral edge of the femur bore a second flattened extension (the 'postero-ventral thumb') which was pointed on its distal edge (Figs 9, 10). The two postero-ventral setae were relatively large and were set on relatively large prominences (Figs 8, 10).

The ventral surface of the tibia also differed in several respects from that of *A. diversiformis*. It lacked a basal prominence and a basal groove, and the small setae that apparently correspond to the small setae

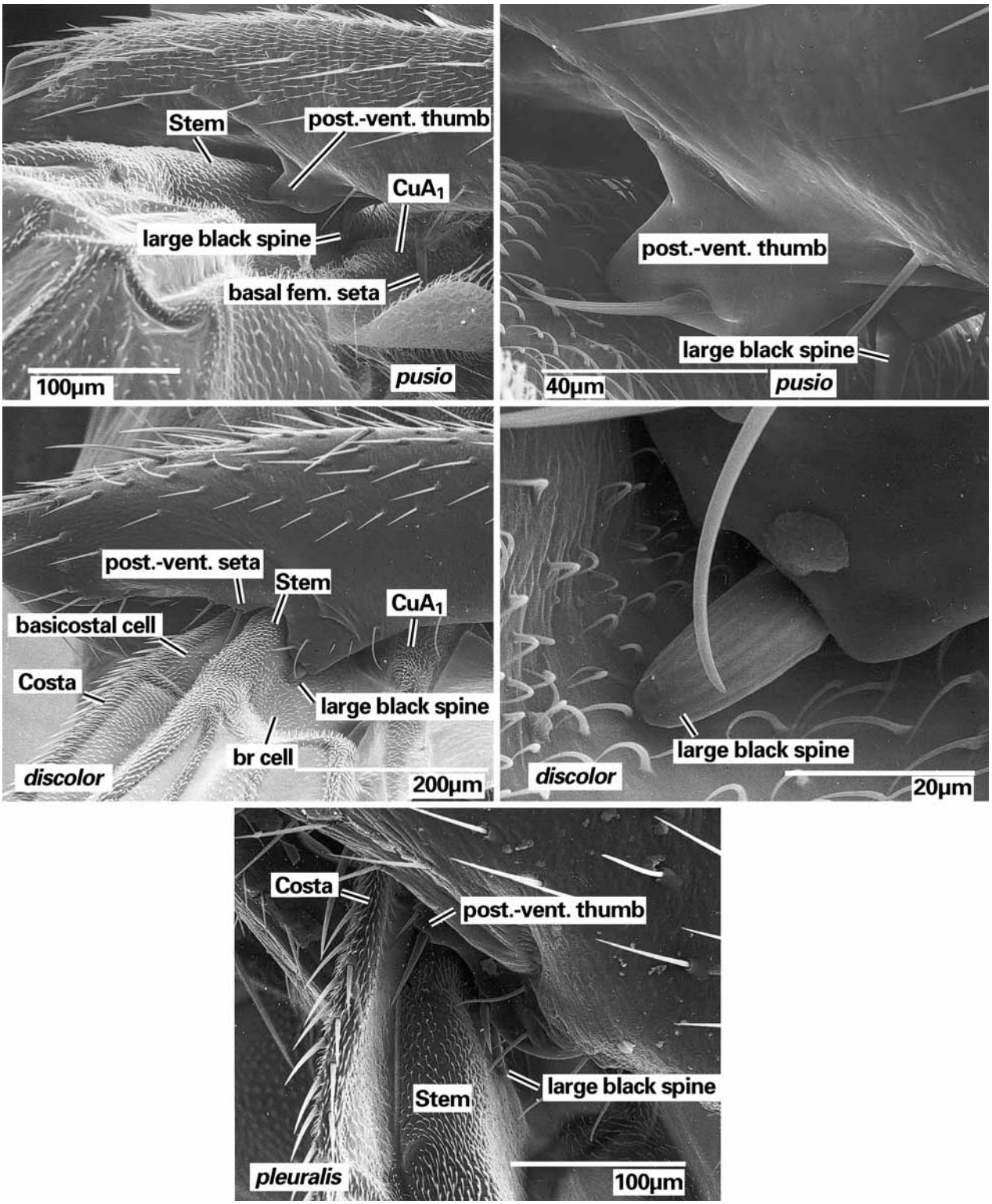

Figure 19. Posterior views of the front femora of males as they grasped the female wing in *P. pusio* and two species of *Archisepsis*.

on the anterior surface of this prominence in *A. diversiformis* were set part way along a low sloping ridge that ended in a small median prominence and the sharply demarcated and angled median groove (Fig. 12). A short row of stout setae began at the distal edge of this groove.

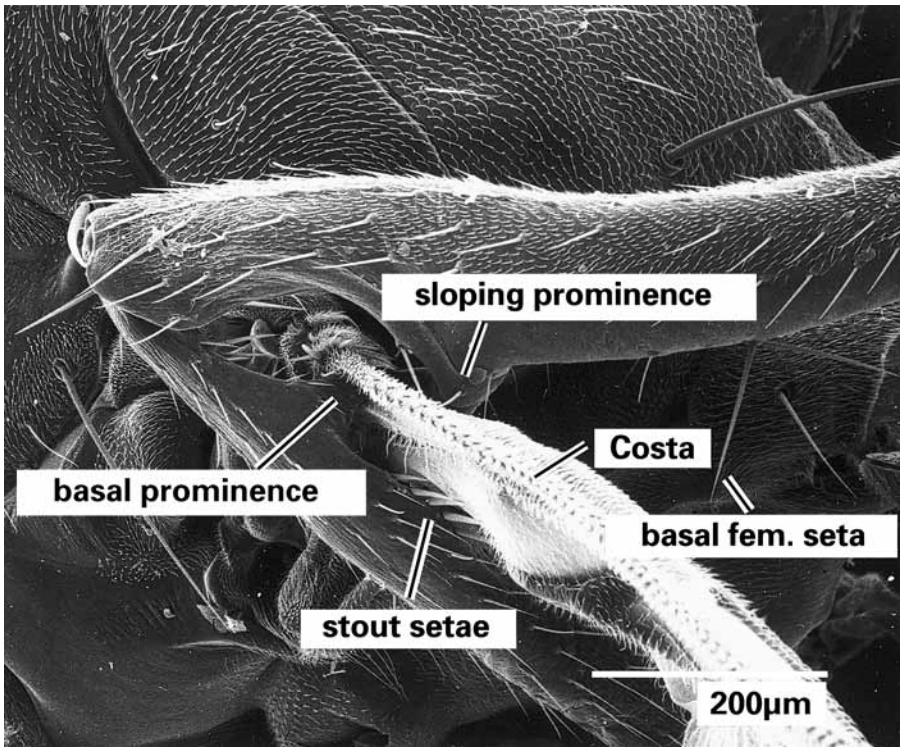

Figure 20. Posterior view of front femur and tibia of a male *A. armata* grasping the wing of a female. The portion of the ventral surface of the wing pressed by the basal prominence of the male's tibia was anterior on the female's wing to the portion on the dorsal surface pressed by the thumb and large black seta on his femur. The male's clamping must thus exert a twisting stress on the wing.

Clamped pairs. As in *A. diversiformis,* the large black femoral spine pressed against the posterior surface of the stem vein (Fig. 19), and the flattened ventral portion of the antero-ventral thumb rested on the dorsal surface of the stem vein (Fig. 14). In addition, the pointed tip of the postero-ventral thumb pressed against the posterior side of this vein (Fig. 19), and the distal surface of the strong basal seta of the femur pressed against the posterior surface of the CuA1 vein (Fig. 19). In contrast to several *Archisepsis* species, there was no basal prominence on the tibia to press on the basicostal cell, although the cell itself was similar (Fig. 3). Instead, the median prominence pressed just posterior to the subcosta (Fig. 16). The subcosta probably rested in the median groove of the tibia. As in *Archisepsis* species, the row of stout spines pressed against the membrane of the br cell (Fig. 16).

M. armillata

Brief observations were made of this species because the much simpler morphology of the male's front femur (Fig. 17B) offers a useful point of comparison. Although the male's simple femoral prominence meshed with the dorsal surface of the female's wing, pressing the dorso-posterior surface of the stem vein (Fig. 17C,D), there was no corresponding difference in the morphology of this area of the female's wing (compare Fig. 17A with Fig. 2).

BEHAVIOUR

Field observations

Field observations of the different species were uneven, owing to differences in abundance and in the difficulty of identification of some species in the field. Data on one additional species, *A. ecalcarata*, are included because the males of this species also have species-specific front leg morphology and use their front legs to grasp the base of the female's wings, whose forms are very similar to those of the females of the other species of this study.

Mounting in *Archisepsis* and *P. pusio* was a forceful rather than a 'luring' interaction (*sensu* Alexander *et al.*, 1997). Males of *A. diversiformis*, *A. ecalcarata*, *A. pleuralis* and *P. pusio* generally mounted females with few or no preliminaries, darting onto her from a distance of one or more body lengths after brief vibration of their wings or, more often, after no perceptible courtship. Once mounted, the male clamped the base of each of the female's wings with his front legs (Fig.

1) and then usually performed relatively elaborate courtship both before and during genitalic coupling and intromission (Eberhard, 2001a; M. Baena, in prep.). The majority of mounting attempts failed, and the male dismounted without having copulated. Of 22 mounting attempts by *A. diversiformis*, only 45% resulted in the male grasping the female's wings for even a second. None of 136 mounting attempts observed in *A. ecalcarata* was successful, nor were any of the 10 attempts observed in *A. pleuralis*.

Several female movements were associated with mounting failures in *A. diversiformis*, *A. ecalcarata* and *P. pusio*. In the least forceful, the female briefly lifted the tip of her abdomen, and the male only partially mounted her and then immediately moved away. Several types of more forceful responses occurred when the male remained mounted for longer (these will be termed 'resistance' behaviour; it is possible that some movements function as testing behaviour – see Eberhard, 2001a; M. Baena, in prep.): the female swayed rapidly and sharply from side to side ('wobbling'); she rocked rapidly forward and backward; she bent her abdomen ventrally so that her genitalia were out of reach of the male's genitalia; she ran or walked rapidly and jerkily; she kicked, pushed or fended off the male with her middle or hind legs (sometimes dislodging the male or prevented him from clamping her wings at the start of a mount); she walked jerkily away from the oviposition site and down into the leaf litter or grass nearby (only seen consistently in *A. diversiformis*, where it induced the male to dismount and walk away in each of the 17 cases in which it occurred; walking into the grass this way occurred less often and had no obvious effect on the male in *A. ecalcarata* and was not seen in *P. pusio*). Females of *A. ecalcarata* also sometimes fell or flew repeatedly short distances, colliding sharply with objects in the vicinity and apparently sometimes jarring the male loose. Female rejection movements were sometimes quite energetic and lasted up to 30–40 s (in *A. ecalcarata*). All rejection movements except walking into the grass and short flights were also seen in captivity in *A. diversiformis*, *A. armata*, *A. discolor*, *A. pleuralis* and *P. pusio* (for a detailed description of female rejection behaviour in *A. diversiformis*, see M. Baena, in prep.). Most female rejection movements were not forceful enough to physically force a mounted male to dismount. Videotaped dismounts in *A. diversiformis* in captivity almost always occurred between rather during bouts of resistance (M. Baena, in prep.).

Even after a male had mounted and clasped a female's wings, most of his attempts to intromit also failed. A failed attempt occurred when the male succeeded in touching his genitalia to those of the female, but failed to intromit, usually for <5 s (but for up to 100 s in *A. ecalcarata*). Since termination of even relatively shallow intromission involves repeated tugging by the male on his genitalia and partial eversion of the female's ovipositor (Eberhard & Huber, 1998), it was possible to deduce that intromission had not occurred when the male and female abdomens separated immediately and without any sign of pulling. Frequencies of failed intromission attempts in the field were 86% in *A. diversiformis* ($N=21$), 91% in *A. ecalcarata* ($N=34$) and 97% in *A. pleuralis* ($N=35$). Probably many, if not all, intromission failures resulted from a failure by the female to flex her proctiger dorsally and to expose her vulva to penetration by the male (Eberhard, 2001a, b).

Still another point at which mating attempts failed in the field were occasional 'pseudocopulations' (one of three in *A. diversiformis*, one of three in *A. ecalcarata* and none of one in *A. pleuralis*). After achieving intromission for only 40–90 s, the male climbed off the female, pulled his genitalia free from her and walked away. Since sperm transfer occurred only after more than 10 min in copulations with virgin females of *A. diversiformis* and *A. armata* (Eberhard & Huber, 1998), the short intromissions of pseudocopulations probably did not result in sperm transfer. The causes of pseudocopulations are not known.

These data are uneven in their coverage, and some of the rates probably vary in different contexts. For example, rejections may be more common on older dung, where females of at least some species appear less likely to oviposit. Nevertheless, they serve to show that females in the field reject many, and probably the large majority, of both male attempts to mount and clasp their wings and male attempts to copulate once they have succeeded in clasping the female's wings. Mounted males hold onto the female's wings using their modified front legs during most types of female resistance behaviour, and their hold is nearly always strong enough that the male is not displaced forcefully by the female. Instead, the male abandons her between bouts of resistance movements.

Direct male–male interactions were seldom important in determining copulation success of mounts in nature. Although males of *A. diversiformis*, *A. ecalcarata* and *P. pusio* sometimes briefly struck against other males that were mounted, they always left immediately and generally gave no further sign of aggression; they never caused a pair to break apart (numbers of interactions were >25, >50 and >50 respectively). Limited observations of *A. discolor* also suggested that male–male aggression was of little importance. In contrast, violent takeover attempts were common in *A. pleuralis*. Sexual selection on male ability to hold onto females to resist attacks by other males (e.g. Darwin, 1871) thus does not occur in *A. diversiformis*, *A. ecalcarata* or *P. pusio* and probably not in *A. discolor*. In none of these species do solitary

males generally interact aggressively other than to chase briefly or to strike each other. Videotapes of such aggressive behaviour in *A. diversiformis* showed that males did not use the modified portions of their front legs in aggressive interactions.

Experimental cross-pairings

Female responses to species-specific differences in male front legs was tested by creating cross-specific pairs in captivity between the two species with the most similar male front legs. Each of five virgin *A. armata* was first placed with a male *A. diversiformis* and then with a conspecific male. The differences in female responses were clear. The female immediately responded to each of 16 mounts by cross-specific males by wobbling, in 12 cases quite violently, and the male dismounted (in 15 of the 16 cases within 10 s). In five mounts direct observation confirmed that the male was never able to bring his genitalia into contact with those of the female; violent female movements precluded direct observation of this detail in the other mounts.

In contrast, only 1 of 10 mounts by a conspecific *A. armata* male of these same five females and of five other females from the same culture which had not been with any male previously resulted in female wobbling (and the behaviour was relatively weak in the exceptional case). In all 10 of these cases the male contacted the female's genitalia with his and succeeded in copulating.

Attempts to produce reciprocal cross-specific pairings failed, as *A. armata* males (which had been reared in captivity) either did not mount virgin female *A. diversiformis* that they encountered (45 of 57 encounters in nine pairs) or immediately dismounted before attempting to clamp their wings.

DISCUSSION

ŠULC'S HYPOTHESIS

The idea that a clamp is formed when modified areas of the male tibia snap tight against modified portions of the femur (Šulc, 1928) can be discarded confidently for all species of this study. The modified portions of the male femur and tibia never contacted each other while the male grasped the female's wings. Instead they pressed against the dorsal and ventral surfaces of the female's wing. The general complementarity in sepsids between indentations and prominences on the male's front femur and tibia, which may have motivated Šulc's interpretation, is probably due to a similar complementarity in the female's wing. Bulges on the dorsal surface are correlated with indentations on the ventral surface, and vice versa.

THE MALE FRONT LEG AS A GRASPING DEVICE

The results of this study support the long-held view that modifications of the front femur and tibia in male sepsids function to clamp the base of the female's wing (e.g. Hennig, 1949; Pont, 1979). Many morphological details of the front femur and tibia of males meshed precisely with structures on the female wing (Table 2) and thus make sense as devices that mechanically improve the male's grip on the female. For instance, in *A. diversiformis* the curved large black femoral spine was inserted in the deep indentation on the dorsal surface of the wing just posterior to the stem vein (Fig. 15); the flat antero-ventral thumb pressed against the curved postero-dorsal surface of the stem vein (Fig. 14); the sharp tip of the basal tibial prominence pressed into the indentation in the basal portion of the basicostal cell on the ventral surface of the wing (Fig. 16); the CuA1 vein fitted into the deep median groove in the tibia. Similar multiple fits occurred in the other species (Table 2). These details of mechanical complementarity do not explain, however, the more difficult question of why it is that the grasping devices on male front legs are different in different species (Fig. 7). This question will be discussed in following two sections.

Mechanical complementarity during clamping does not explain the setae on the ventral surfaces of the male femur and tibia. Some of these setae may have sensory functions while the male holds the female's wings (e.g. the small setae near the tip of the basal tibial prominence in *A. diversiformis* – Figs 11, 12 – and the postero-ventral setae near the tip of the postero-ventral thumb in *A. pleuralis* – Figs 8–10). Others are normally out of contact with the female when the wing is clasped (e.g. the basal ventral setae of the tibia – Figs 16, 20) but might function at the moment the male is attempting to seize the wing (e.g. by informing him if his leg is not located far enough forward on the female's wing). In only three cases did it seem that setae may have served to reinforce mechanically the male's grip on the female: the two postero-ventral femoral setae of *A. armata*, which were placed relatively transversely on the femur, both projected into the fold between the costal and stem veins (Fig. 15); the basal femoral seta in *P. pusio*, which was inserted into the fold just posterior to the base of the CuA1 vein (Fig. 19); the pair of setae near the tip of the femoral prominence in *M. armillata*, which fitted behind the stem vein (Fig. 17).

The benefits that the male derives from the tight mechanical coupling between his front legs and the female's wings are more subtle than might appear. Observations in nature showed that, except for *A. pleuralis*, males almost never make forceful attempts to dislodge other males that are mounted on females. Thus resisting other males is a very unlikely function

Table 2. Mechanical mesh between structures on the male front femur and the female wing in five species of *Archisepsis* flies and *P. pusio*. At those points of contact that are marked with '*', the male could presumably exert force; others may only represent contact (Sc = subcosta vein; CuA1 = base of CuA1 vein)

Species	Male structure: femur						
	Large black spine	Antero-ventral thumb	Curved surf. of ant.-vent. thumb and large black spine	Postero-ventral thumb	Basal femoral seta	Bases of post.-vent. setae	Post.-vent. setae
Archisepsis							
diversiformis	Insert in indentation at post. margin stem (Figs 14, 15)	Press on post.-dors. surf. of stem vein (Fig. 14)	Fit curved surf. of stem vein (Figs 14, 15)	None	No contact or touch post. surf. CuA1 (Fig. 14)	No contact (Fig. 15)	Both touch. post. surf. costa (Fig. 15)
armata	(Same as *divers.*) (Fig. 15)	(Same as *divers.*) (Figs 14, 15)	(Same as *divers.*) (Figs 14, 15)	None	No contact	No contact	Both in costa–stem groove (Fig. 15)
discolor	Push very base stem vein (Fig. 19)	Press dors. surf. costa vein (Fig. 14)	None (press with ridge betw. large black spine and retrolat. spines) (Fig. 19)	None	No contact? (Fig. 19)	Press both sides costa (Fig. 19)	Touch both sides costa (Fig. 19)
pleuralis	(Same as *divers.*) (Fig. 19)	(Same as *divers.* but large)[a] (Figs 14, 19)	None	None[b]	No contact	None[a]	Both touch post. surf. costa[a] (Fig. 19)
Palaeosepsis							
pusio	(Same as *divers.*) (Fig. 19)	(Same as *divers.* but small) (Fig. 14)	(Same as *divers.*) (Fig. 19)[c]	Press post. surf. stem vein (Fig. 19)	Press post. surf. CuA1 vein (Fig. 19)	Press dors. surf. costa? (Fig. 19)[c]	Touch both sides costa? (Fig. 19)[c]

Species	Femur	Tibia			
	Transverse femoral groove	Basal triang. prominence	Row strong setae	Basal groove	Median groove
Archisepsis					
diversiformis	None	Press into basal end of basicostal cell (Fig. 16)	Press surf. br cell (Fig. 16)	Loose grip subcosta (Fig. 16)[c]	Tight grip CuA1 (Fig. 16)[c]
armata	None	(Same as *divers.*) (Fig. 16)	(Same as *divers.*) (Fig. 16)	(Same as *divers.*) (Fig. 16)[c]	(Same as *divers.*) (Fig. 16)[c]
discolor	Press dors. surf. stem mound (Fig. 14)[c]	None (contact C and Sc but possib. not memb. in cell) (Fig. 16)	None	None	Press vent. surf Sc but not grip it (Fig. 16)[c]
pleuralis	None	Fill basicost. cell (Fig. 16)	None	Loose grip subcosta (Fig. 16)	Weak grip on CuA1 (Fig. 16)
Palaeosepsis					
pusio	None	None (weak prom. press post. subcosta (Fig. 16)	(Same as *divers.*) (Fig. 16)	None	Tight grip subcosta (Fig. 16)[c]

[a] Homologies uncertain (see text)
[b] Homologies uncertain; there was extensive contact between the ventral surface of the male femoral structure and the dorsal surface of the stem vein of the female
[c] Indirect deduction from the figure

for the male's tight grip on the female. In contrast, females often resist mounting relatively violently, and the male's grasp on the female is apparently so secure that only rarely can she physically force him off. A mounted male is not, however, able to force intromission (Eberhard, 2001a), and males of *A. diversiformis* whose femora were modified so they cannot mesh tightly with the female were nevertheless seldom thrown off forcefully (Eberhard, submitted). The possible pay-offs to males and females in these struggles are thus complex (next section).

EXPLAINING DIVERGENCE I: LOCK-AND-KEY AND CONFLICT OF INTEREST HYPOTHESES

Given that male front leg structures clearly meshed very tightly with the contours of the female's wing base, the remaining puzzle is to explain why the male structures should be different in different species. Both the lock-and-key and the conflict of interests hypotheses explain male divergence on the basis of differences among females. According to both lock-and-key and the morphological version of the conflict of interest, female morphology changes (under, respectively, selection to avoid cross-specific mating and selection to wrest control of events associated with copulation and insemination from the male), and male structures then evolve new forms to adjust appropriately. As shown in Table 3, however, there were few such species differences among the wings of females of the species of *Archisepsis* and *Palaeosepsis* that were correlated with the differences in the ways in which the male femur and tibia meshed with the wing. Similarly, more limited data from *M. armillata* showed that the dorsal surface of the female's wing base differed little, despite the much simpler structure of the male femur in this species (Fig. 17).

The lack of interspecific differences in female morphology corresponding to interspecific differences in males was particularly clear on the dorsal surface of the female wing. Nearly all male femur structures meshed with the female's stem vein, but the form of this vein was nearly invariant. For example, *P. pusio* was unique in having a pointed postero-ventral femoral thumb that pressed against the posterior surface of the stem vein (Fig. 19), but there was no corresponding modification of the female stem vein in this area (Figs 2, 18).

The situation was similar on the undersurface of the wing. In *A. diversiformis*, *A. armata* and *A. pleuralis* the large basal tibial prominence was pressed into the indentation at the base of the basicostal cell, while no male structure was pressed into this cell (which was nevertheless present in the same form) in the wings of female *P. pusio* and *A. discolor* (Figs 3, 16). Even the forms and patterns of microtrichia in the base of the female basicostal cell in the latter species were essentially identical to those of the first group of species (Fig. 3). Several other cases of lack of correlation between male and female structures are described in Table 3. The one clear exception in 12 different comparisons in Table 3 was the transverse femoral furrow and the mound on the dorsal surface of the female stem vein in *A. discolor*.

The predicted co-evolution of male leg and female wing morphology has thus generally not occurred (as is also true in the genitalia of a number of species of insects and millipedes, which have been studied in somewhat less detail – Eberhard, 1985). In fact, as illustrated by the intraspecific variation in the exact site where the male grasped the female's wing in *A. diversiformis* and *A. pleuralis*, and by the variation in the angle that the female's wing makes with her body (and thus the angle with the male's legs), extreme precision in the position of the male's leg was not necessary for the male to hold onto the female's wings. Another important source of variation in details of mechanical fit was the substantial differences in size among both males and females. Although front tibiae of small males of *A. diversiformis* were only about 70% as long as those of large males, small males were nevertheless able to hold the wings of large as well as small females successfully; riding times in mounts of virgin females that did not result in copulation averaged 270 s for small males (166 mounts) in captivity and 129 s for large males ($N = 261$ mounts) (M. Baena, in prep.). Thus, even intraspecifically, the male's legs must function to some extent as mechanically versatile, 'general-purpose' clamping structures. This versatility is striking in the light of the rigidity of most of the male leg structures other than the large black spine, which was sometimes deflected posteriorly (Fig. 15) or pushed deep into its socket (Fig. 19). The variability in the sites where the male's leg grasped the female wing echoes similar variation in the sites where another species-specific structure in *Archisepsis*, the male genitalic surstyli, grasp the ventral surface of the female's abdomen (Eberhard & Pereira, 1996).

A second reason to doubt the morphological conflict of interest hypothesis, at least for most of the species of this study, is that there is only one candidate for the predicted female rejection structures that would make it advantageous for males to evolve new counter-adaptations in their clasping devices (the dorsal mound on the stem vein of female *A. discolor*). In addition to the interspecific uniformity in female morphology, there was little sexual dimorphism in the basic contours of the morphology of the base of the wing in males and females (again with the exception of the dorsal mound in female *A. discolor*) (Fig. 18).

Rejection of the morphological conflict hypothesis for most species is particularly meaningful because

females of these and other sepsids often actively resist male attempts to mount and copulate, both in the laboratory and in nature. Male–female conflicts of interest would seem to occur as clearly in these flies as in any other group in which conflict is thought to occur (e.g. Alexander *et al.*, 1997), but the morphological traits predicted to be associated with conflict are generally lacking.

An alternative version of the conflict of interest hypothesis is that there are species-specific differences in female behaviour rather than in female morphology, and that it is these differences that have favoured differences in male morphology. The data of this study are not appropriate to test this possibility directly, but there are three indirect indications that it is unlikely. In the first place, it is not at all clear which differences in rejection behaviour could conceivably result in selection favouring the kinds of differences in curvature and size of male structures observed in different species. The same basic mechanical problem faces the males of all species – hold on tight to the base of the female's wing. Secondly, the same basic types of female rejection behaviour that could dislodge the male, including kicking at him and shaking him from side to side, occur in all of the species in this study. Finally, video analyses of *A. diversiformis* show that, in any case, female rejection behaviour almost never dislodges males by physical force (M. Baena, in prep.). Female resistance seems to act not to force the male off but to induce him to dismount on his own (see Linley & Adams, 1974, and Linley & Mook, 1975, for a similar situation in the ceratopogonid fly *Culicoides melleus*, and Belk & Serpa, 1992, for similar female inability to force male release in fairy shrimp). It will probably always be possible to suppose that behavioural observations are not sufficiently detailed and that finer details of female behaviour that are as yet unanalysed will prove to differ between species and differ in ways for which the species-specific designs of males are especially effective. However, there are currently no data to support this conjecture; the available observations suggest the opposite conclusion, that female rejection behaviour in different species is similar.

One further consideration bearing on the question of male–female conflict is that successfully mounted males are physically incapable of forcing intromission. The female can (and usually does) prevent intromission by simply bending her abdomen ventrally out of reach of the male or by failing to deflect her proctiger dorsally from its resting position, thus not exposing her vulva to the male's intromittent genitalia (Eberhard, 2001a, b). This means that one can safely ignore several of the possible pay-offs to females for resisting male mounting attempts that involve avoiding copulation itself (see Arnqvist, 1997; Alexander *et al.*, 1997). In addition, a female with a riding male can walk, feed and oviposit apparently unimpeded; she can also fly, although probably less agilely.

If females do derive naturally selected benefits from rejecting male mounting attempts, as proposed by the conflict of interest hypothesis, then the most likely candidate for such a benefit appears to be avoidance of predation (as, for instance, occurs in water striders – Arnqvist, 1997). However, I have only once seen a mounted female being attacked by a predator (a staphylinid beetle) in several hundred pair-hours of observation in the field; flies in all three genera are apparently chemically defended from some predators by odorous compounds produced by a large gland associated with the rectum, as with some other sepsids (Bristowe, 1979; Pont, 1979). Thus, while there are possible naturally selected pay-offs to females for controlling mounting, they are not clearly important.

Female *A. discolor* are possible exceptions in having a structure that may impede male mounting. The mound on the female stem vein could impede clasping by inappropriately designed male legs, since the ventral surface of the male's femur rested directly on the mound (Figs 2, 4, 19). The transverse groove on the ventral surface of the antero-ventral thumb of male *A. discolor*, a feature not seen in the other species, probably fits directly over the mound. The presence of similar mounds strategically located at sites on the female wing that meshed with species-specific male structures (e.g. the stem vein) would have constituted evidence in accord with lock-and-key and conflict of interest predictions. However, in fact they are entirely lacking from the wings of females of the other five species of this study, as well as from those of female *A. ecalcarata* and *A. polychaeta* (W. Eberhard, unpub.).

The mound's wrinkled cuticle (Fig. 4) suggests that it might be inflatable, and thus a possible facultative anti-grasping device, such as are expected to evolve under the conflict of interest hypothesis (see Arnqvist & Rowe, 1995, for a non-facultative device of this sort). This possibility was ruled out, however, by the fact that the mound is rigid. The stem vein of male *A. discolor* bore a similar but reduced mound (Fig. 18), suggesting that the functional significance of the mound is somehow related to sexual interactions.

In sum, the lock-and-key and mechanical conflict of interest hypotheses fail to explain male and female morphology of five of the six species. The mound on the stem vein of the female wing and the corresponding groove on the male femur of the sixth, *A. discolor*, are in accord with these hypotheses.

EXPLAINING DIVERGENCE II: STIMULATION HYPOTHESES

Males could presumably stimulate females when they grasp their wings with their front legs in at least two

Table 3. Comparisons of portions of female wings in which contact by male clasping structures differed among four species of *Archisepsis* and *P. pusio*. Species in which the contact with the male leg was similar were grouped (justifications in column at left) and then compared with respect to the particular areas of the female wing contacted by the male (column at right)

Area of female wing and type of contact with male leg			Comparisons of female morphology between groups of species	Correlation between species-specific traits in male and female?
DORSAL				
Pocket at anterior edge of br cell:				
Insertion of large black spine: *diversiformis*, *armata*, *pusio*, *pleuralis*	No insertion of large black spine: *discolor*		Not obviously smaller in *discolor*	No
Basal portion dorsum of stem vein:				
Wide dors.-post. contact w. a.-v. thumb: *diversiformis*, *armata*	Wide contact dors. only w. a.-v. thumb: *pusio*, *pleuralis*	Small contact a.-v. thumb: *discolor*	No obvious correlation with differences in orientation, shape, microtrichia or sensilla	No
More distal portion of stem vein:				
Press post. surf. w. p.-v. thumb: *pusio*	Press with ridge: *discolor*	No contact w. p.-v. thumb or ridge: *diversiformis*, *armata*, *pleuralis*	No obvious correlation with orientation, shape, microtrichia or sensilla	No
Dorsal surface of costa:				
Press with bases retrolat. spines: *discolor*	Press with thumb: *pleuralis*	No contact: *armata*, *diversiformis*, *pusio*	No obvious correlation with differences in orientation, shape or microtrichia	No
Anterior and posterior surfaces of costa:				
Touch both with retrolat. spines: *pusio*, *discolor*	Touch rear with one or both retrolat. spines: *diversiformis*, *armata*, *pleuralis*		No obvious correlation with differences in orientation, shape or microtrichia	No
Dorsal mound stem vein:				
Present, fits in transverse groove of femur: *discolor*	Mound and groove both absent: *diversiformis*, *armata*, *pusio*, *pleuralis*		Slant of male groove matches unique slant of female stem vein and mound, row of four sensilla displaced distally	Yes
Rear margin of CuA1 vein and furrow posterior to it:				
Press with basal femoral spine: *pusio* (sometimes in *diversiformis*)	Not press with basal femoral spine: *armata*, *discolor*, *pleuralis*		No obvious correlation with differences in shape of vein, depth of furrow	No

different ways: by directly stimulating touch receptors or by twisting, bending or otherwise stressing the wing cuticle, thus stimulating stress receptors. Direct tactile stimulation is apparently ruled out by the general absence of socketed bristles in the areas of the female wing that are grasped by the male. The possible importance of stress is supported, in contrast, by the presence of campaniform sensilla, which in other species of

Table 3 – *continued*

Area of female wing and type of contact with male leg			Comparisons of female morphology between groups of species	Correlation between species-specific traits in male and female?
VENTRAL				
Basal end of basicostal cell:				
Insert tip triangular prominence of tibia: *diversiformis*, *armata*, *pleuralis*[a]	Not insert prominence: *pusio*, *discolor*	No obvious correlation with differences in shape, depth, or pattern or microtrichia lengths		No
Basal portion of br cell:				
Press with stout setae: *diversiformis*, *armata*, *pusio*	Not press with stout setae: *discolor*, *pleuralis*	No obvious correlation with differences in depth or shape; lacks microtrichia in *discolor* and *pleuralis*		No?[b]
Costa:				
Post. surf. pressed by basal promin. tib.: *diversiformis*, *armata* (weaker in *pleuralis*)	Only vent. surf. pressed by tib.: *pusio*, *discolor*	No obvious correlation with differences in shape, setae		No
Subcosta:				
Weak mesh with tib. groove: *diversiformis*, *armata*, *pleuralis*, *discolor*	Tight mesh with tib. groove: *pusio*	Perhaps slight bulge in *pusio* but also present in *discolor*, *pleuralis*; no obvious correl. w. differences in sensilla		No
Base of CuA1 vein:				
Grasped tightly in tib. groove: *diversiformis*, *armata*	Grasped weakly in tib. groove: *discolor*, *pleuralis*	Not grasped with tib. groove: *pusio*	Perhaps more prominent in *diversiformis* and *armata* (with tight grip), but also in *pusio* (no grip)	No[c]

[a] Rounded rather than sharply pointed tip as in other two species

[b] It is not obvious that a lack of microtrichia implies a mechanical adaptation to the lack of contact with stout tibial spines

[c] Uncertain because folding of wing causes changes in this detail (see Fig. 3)

insects are sensitive to stress in the cuticle (Wigglesworth, 1965; Zill & Moran, 1981). The fact that the tibial structures pressed the ventral surface of the wing at different sites to those that were pressed by the femur on the dorsal surface (e.g. Fig. 20) indicates that the male's grip applies twisting or bending forces to the female's wing. Different stresses on the wings of different species may also result from variation in the depths and angles of the grooves on the male tibia (Fig. 12). The extensive shaking and wobbling movements of females that often occurred preceding copulation in successful mounts, and which were both ineffective in preventing mounted males from staying on the female and superfluous in preventing copulation (above), undoubtedly caused additional twists and strains on the wings. These female movements could represent testing of the mounted male's ability to produce particular stresses and strains.

Morphological evidence from this study argues against a simple version of the 'sensory lock-and-key' hypothesis that supposes that the form of the male clasping organs of each species contacts a similarly

species-specific distribution of female sense organs (as, for instance, is thought to occur in some damselflies – Robertson & Paterson, 1982; Battin, 1993). Leaving aside for the moment *A. discolor*, the positions of the apparent campaniform sensilla on the stem veins of female wings varied somewhat in different species (Fig. 6), but the differences did not correspond in any obvious ways to the forms of the male front femur (Table 3). For example, the rounded, extensive posterior and dorsal surface of contact of the antero-ventral femoral thumb and large black spine in *A. diversiformis* and *A. armata* with the dorsal and posterior surface of the stem vein (Figs 14, 15) was not associated with a particular array of sensilla in this region in females of these species (Fig. 6), nor was the extensive dorsal contact with the stem vein in *A. pleuralis* associated with a corresponding difference in the sites of sensilla in females (Fig. 6). There were no sensilla on or near the surface of the stem vein where the sharp point of the postero-ventral thumb of *P. pusio* pressed (Fig. 19). In addition, the patterns of sensilla on male wings of different species showed differences that were similar to those between females (Fig. 6). The pattern of sensilla more distal on the stem vein, where the male's femur never made contact, varied interspecifically (Fig. 6).

The differences in the patterns of sensilla on the ventral surface of the subcostal vein were relatively minor and again showed no clear relation to differences in the legs of males. For instance, the relatively gently slope of the sides of the median groove on the tibia of male *A. discolor* was not associated with any obvious corresponding differences in the sensilla on the ventral surface of the female subcosta (Fig. 6).

Another reason to doubt the sensory lock-and-key hypothesis was the substantial intraspecific variation in the sites at which the female sense organs occurred on the stem and the subcostal veins (Fig. 5). This, combined with the variation in the sites at which males clasped female wings, and the variation in both male and female size, suggests that consistent precise fits of the male structures to contact particular female receptors are unlikely to be selectively important. Variation in male positions is not unexpected, since the male generally grasps the female's wings more or less instantaneously when he mounts and probably does not have time to make fine adjustments of the positions of his legs. A female's wings are probably often moving at the moment the male mounts, as their wings often perform nearly continuous scissoring movements (e.g. Pont, 1979).

Versions of the stimulation hypothesis which do not suppose that there is a precise alignment between particular female sensory structures and male structures (e.g. Eberhard, 1985; Eberhard & Pereira, 1996), are compatible with the findings of this study. It is clear that there are some interspecific differences in the positions of the campaniform sensilla on the female wing (Table 1) which may represent differences in female response traits. Behavioural observations show that females frequently reject mounting attempts of conspecific males and that the females of one species are apparently capable of distinguishing and rejecting cross-specific males. The behaviour of cross-specific pairs suggested that these discriminations are made on the basis of species-specific structures on the male front leg, using the campaniform sensilla, but the possibility of other species-specific male cues (e.g. odours, behaviour) was not ruled out. Further data have shown that experimental alteration of male femur form results in increased female rejection of conspecific males but not in reduced male ability to hold onto the female's wing (W. Eberhard, submitted).

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