REPORT ON RESEARCH
To the Black Rock Forest Consortium, Inc.

by David Grimaldi
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New York, New York

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Systematics and Life Histories of Fungus Gnats

CONTENTS

INTRODUCTION

The fauna of Diptera, or two-winged flies, that inhabit the fruiting bodies of mushrooms and other macrofungi in the forests of northeastern North America are among the most diverse components of these ecosystems. Julian Stark (Scientific Assistant at the AMNH) and I did field work at the Black Rock Forest in the summer and early Fall of 1990, intending to survey various aspects of the life histories of various mycophagous Diptera, principally the Mycetophilidae (or "fungus gnats"). From well over 130 mushrooms were reared flies belonging to 13 families, representative species of which are shown on the accompanying figures. Approximately 60 species of Diptera have been found, based on a simple examination of the specimens, but dissections are yet needed for more detailed separation of very similar species. The Drosophilidae and the Sciaridae were among the most common flies reared from the fungi. We had also reared numerous cohorts of adults from larvae found living beneath logs. These larvae were Mycetophilidae, which construct sheets of silken and mucous webs on which they rest and graze.

The research on the morphology of the immature stages of the many Mycetophilidae that were collected is still in progress. It is expected that several years yet will be required to complete the dissections, photography, illustrations, and descriptions. A more complete report to the BRF Consortium will follow.

Two detailed reports are included here, one on a project closely allied with the research that was proposed, and another that was unrelated. The first, on the "systematics and modes of reproductive isolation in the Holarctic *Drosophila testacea* species group," is a natural byproduct of the proposed research, since two of the (North American) species of this group are common in Black Rock Forest. One of the species, newly described as *Drosophila neotestacea*, in fact has the type locality for the species designated as Black Rock Forest (the species actually ranges from Maine and North Carolina to Alaska and California). Another, closely related species is found with *Drosophila neotestacea*, namely *D. putrida*.

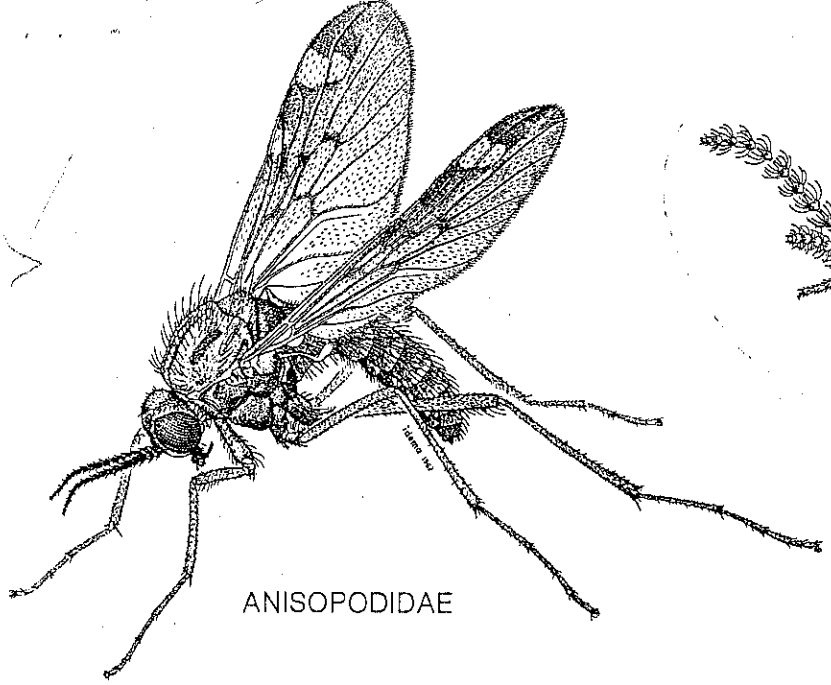
It is important to emphasize the implications of my work on the insect fauna of macrofungi. It has recently been documented in Europe that perhaps as much as 80% of the macrofungi of Europe have become endangered and even extinct (reviewed recently in Science, vol 254: 1458 [1992]). The culprit seems to be industrial pollution, due specifically to the effects of acid rain and a major disturbance of soil properties. No effort has been made in North America to make similar long-term comparisons of macrofungus populations, but it is very possible that the same trend is occuring here. Buxton (Ent. mon. mag., 1960) found 108 species of Diptera feeding on about 150 species of macrofungi in England. If the estimates in the decline of macrofungi are accurate, and given that about 90% of the insects found in macrofungi are fairly host specific, then macrofungal decline translates into a decline of the Diptera to about 30 species. This estimate does not take into account the numerous predators (e.g., staphylinid beetles) and parasitoids (e.g., microhymenoptera) that exploit Diptera larvae in macrofungi, as well as the beetles and other insects that thrive on macrofungi. The number of species of insects adversely affected by decline and extinction of British macrofungi could easily be about 100 species. If macrofungal decline is occuring here in the U.S., a comparable loss in diversity (and probably much more) could be expected. My data could provide a baseline for long term monitoring.

The other report is an excerpt from a large manuscript, "A Monograph on the Spittle Bug Parasites, Genus *Cladochaeta* (Diptera: Drosophilidae)" (for the Bull. AMNH). These fruit flies belong to the same family as the two species discussed above, but are very distinctive because of their habits. The larvae of the eastern North American species, *Cladochaeta inversa*, are always found attached to the abdomens of certain spittle bugs (Cercopidae: Homoptera). Work that I did at the BRF helped resolve the controversey as to whether the larvae are parasites or not. It appears that they feed on the host hemolymph and retard the nymphs' development.

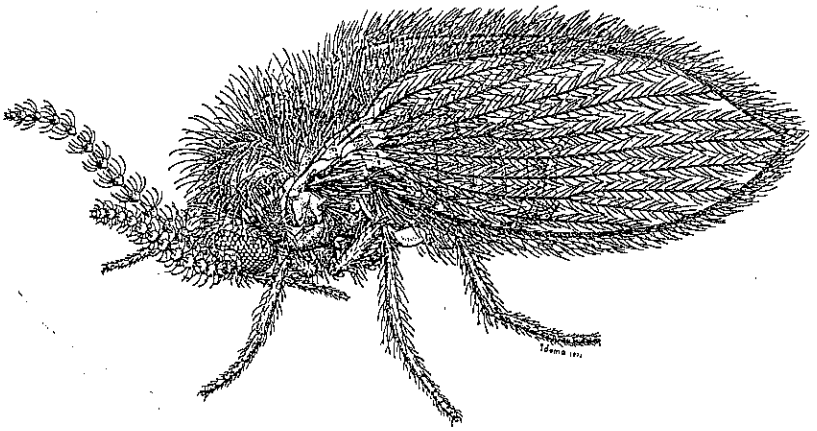
BUDGET

Tolls, Fuel	$132.20
Rearing & Collecting Supplies	408.66
Malaise Trap Materials	227.39
Illustration and Photo Supplies	909.71
Microscope supplies, bulbs	63.00
Microscope supplies, drawing tube extension	323.00
Specimen preparation supplies	210.02

TOTAL EXPENSES	$2273.98
GRANT	2265.00
	$ - 8.98



ANISOPODIDAE



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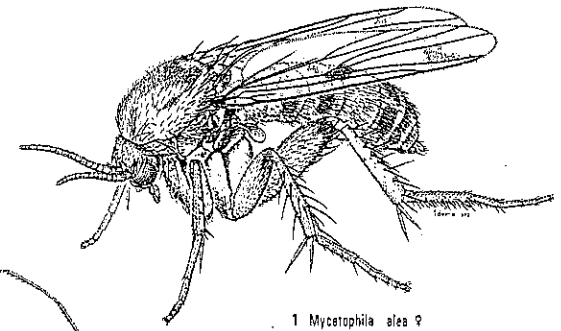
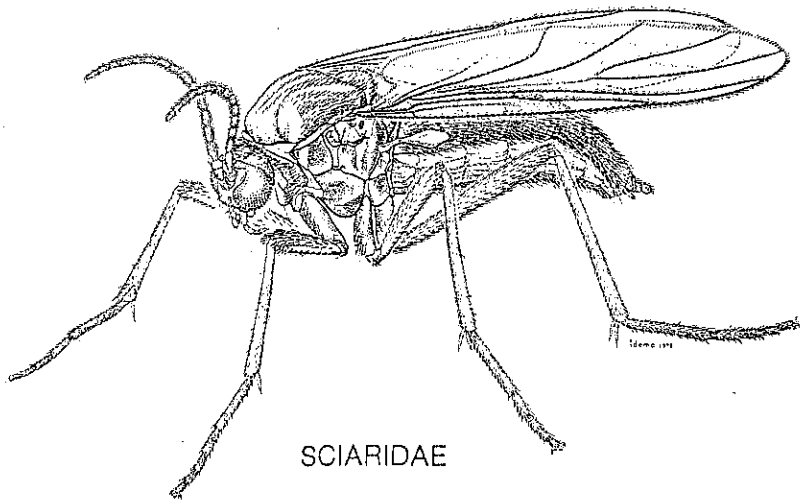


1 Mycetophila alea ♀



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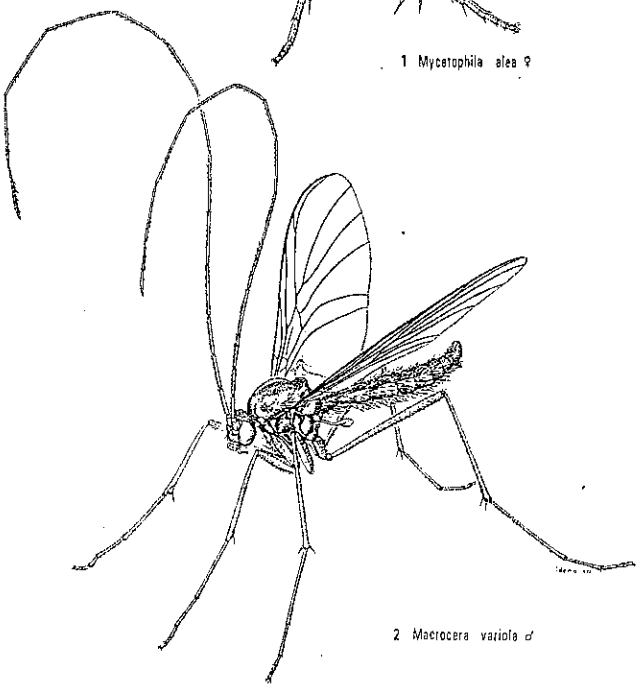
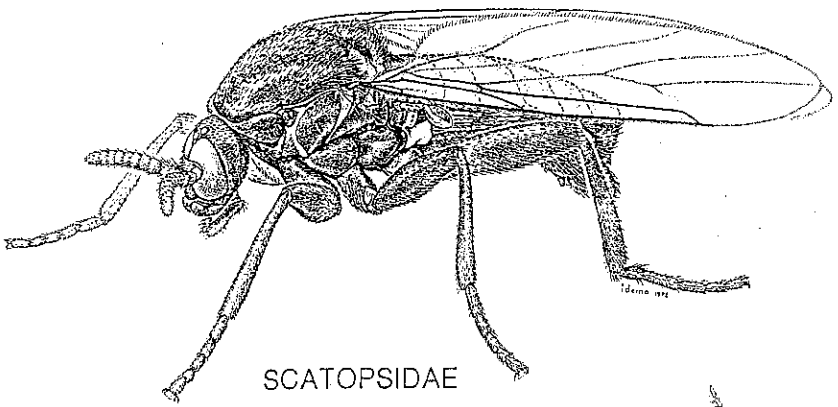


2 Macrocera variola ♂

MYCETOPHILIDAE



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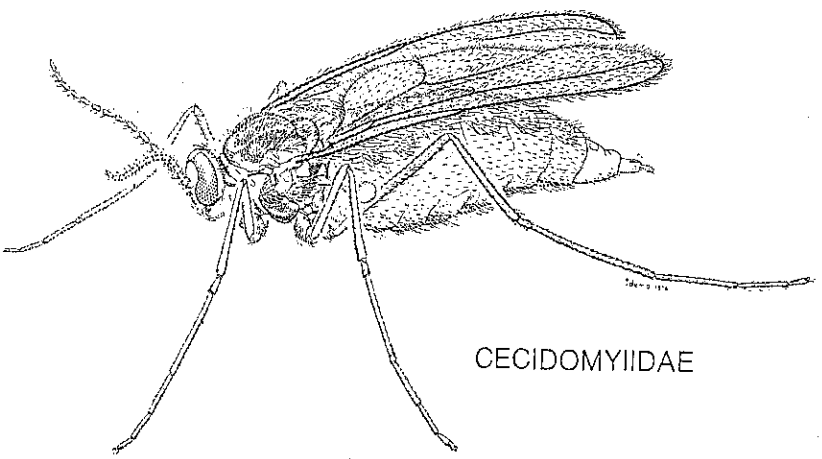

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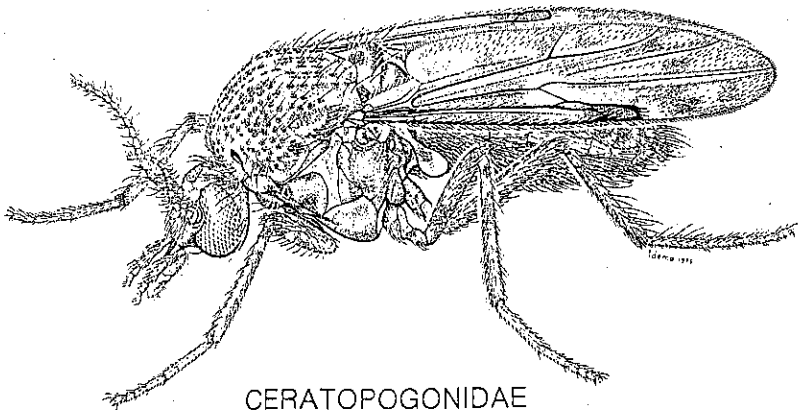
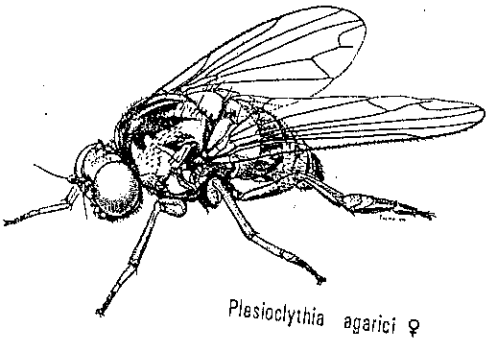
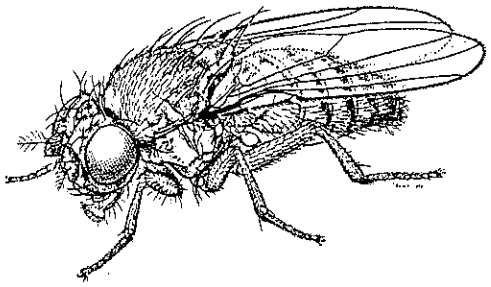


CERATOPOGONIDAE



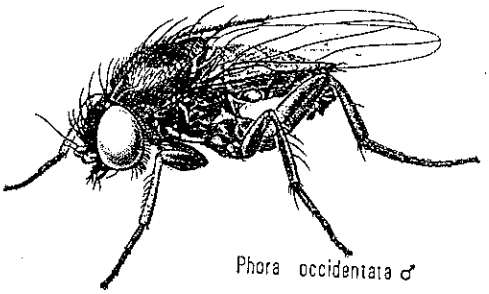
Plasioclythia agarici ♀

PLATYPEZIDAE



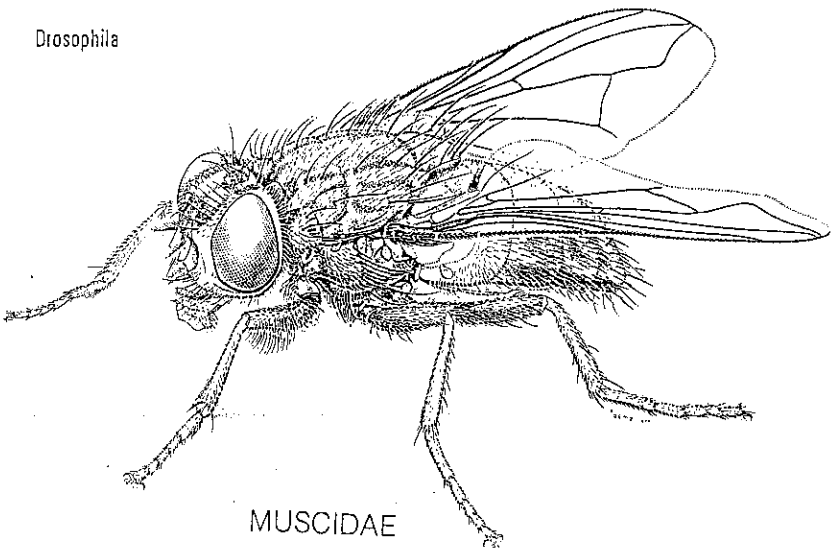
Drosophila

DROSOPHILIDAE

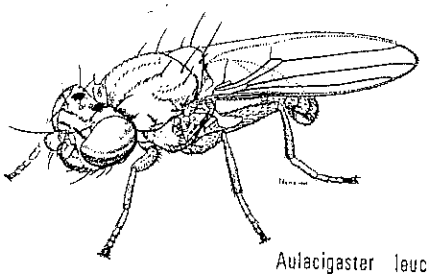


Phora occidentata ♂

PHORIDAE

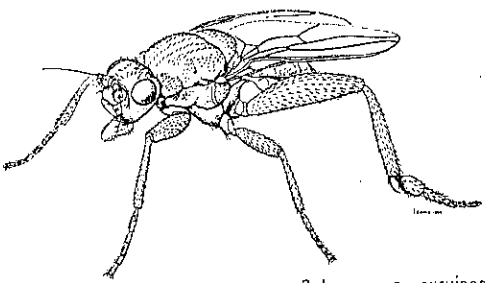


MUSCIDAE



Aulacigaster leucopeza ♂

AULACIGASTRIDAE



Sphaerocera curvipes ♂

SPHAEROCERIDAE

Systematics and Modes of Reproductive Isolation in the Holarctic *Drosophila testacea* Species Group (Diptera: Drosophilidae).

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ABSTRACT Reproductive isolation and morphological differences among allopatric populations of the Holarctic species *Drosophila testacea* v. Roser indicate that this taxon is actually a complex of three morphocryptic species: *D. testacea*, from Europe and continental Asia; *D. orientacea*, n.sp. from Japan; and *neotestacea*, n.sp. from North America. Diagnostically important morphological variation is presented, along with distributional data for these three species and *D. putrida*, the only other member of the *testacea* species group. Both pre- and post-mating barriers to reproduction were observed in various interspecific crosses. Pre-mating isolation is strongly asymmetric between *D. testacea* and *D. neotestacea*. Modes of post-mating isolation include lack of sperm transfer and failure of hybrid eggs to hatch. The revised taxonomy of this group should facilitate analyses of interesting aspects of the evolutionary ecology of these species.

KEY WORDS Drosophila, *testacea* group, systematics, reproductive isolation

Among the most common *Drosophila* inhabiting temperate and boreal forests around the world are mycophagous species, which feed, mate, oviposit, and develop as larvae in fresh and decaying mushrooms. Because of the facility with which these flies can be studied, both in the field and the laboratory, they and their communities are becoming model systems for studies in evolutionary ecology (Lacy, 1984a; Courtney et al., 1990; Hanski, 1988). The following are some general observations on the natural history of mycophagous species.

A major challenge to mycophagous *Drosophila* is being able to find and develop on patchy, ephemeral, chemically diverse resources. The spatial and temporal patchiness of their resources has forced these species to adopt a wide host range, breeding on many of the fleshy fungi they encounter (Bächli & Burla, 1967; Kimura et al., 1977; Lacy, 1984b). Despite this host generalism, they have evolved an extraordinary level of resistance to the mushroom toxin alpha-amanitin, which is produced in highly toxic quanitities by only a small fraction of the fungi which they utilize (Jaenike et al., 1983). This suggests that chemical barriers of their host fungi may be minor limitations on the fly population sizes.

A number of factors play an important role in the population ecology of these mycophagous species of *Drosophila*. Larval competition for limited food resources, which has been documented in natural populations of these species (Grimaldi and Jaenike, 1984), sets an upper bound to population size, but other factors may regulate populations at lower levels. One important such factor is parasitism by the nematode *Howardula aoronymphium*, which has been reported from Europe (Welch, 1959), North America (Montague and Jaenike, 1985), and Japan (Kimura and Toda, 1989). Parasitism by these nematodes greatly reduces the fertility of infected female flies (Jaenike, 1992), and rates of parasitism have been shown experimentally to increase at high densities of adult flies (Jaenike and Anderson, 1992).

There are approximately 27 species of mycophagous *Drosophila* in the Holarctic Region, and only one -- *D. testacea* -- is still considered to be Holarctic in its natural distribution (Wheeler, 1981). *Drosophila transversa* Fallén was formerly considered to be Holarctic, but it is now recognized that the North American populations comprise two species (*D. falleni* Wheeler and *D.*

recens Wheeler) distinct from the Palearctic one (Wheeler, 1960). The uniqueness of *D. testacea* in its Holarctic distribution raises the question of whether all populations currently classified as *D. testacea* are in fact conspecific. The present study was intended to clarify the relationships among North American, Eurasian, and Japanese populations of this taxon. Our studies include detailed morphological comparisons and laboratory investigations of pre- and post-mating reproductive isolation among these populations.

Methods and Materials

Morphology. Specimens were used and borrowed from the following institutions, with help from their respective curators: AMNH; California Academy of Sciences, San Francisco (P.H. Arnaud, Jr.); Canadian National Collection, BRC, Ottawa (J. Cumming); Hungarian Natural History Museum, Budapest (L. Papp); Institute of Pedology, Vladivostok, USSR (V. Sidorenko); Moscow State University, USSR (A. Ozerov); U.S. National Museum of Natural History, Smithsonian Institution (W. Mathis); Tokyo Science Museum (T. Okada); University of Guelph (S. Marshall); Snow Entomological Museum, Univ. of Kansas (G. Byers); University of Zürich Zoological Museum (G. Bächli); Utah State University (W. Hanson); Washington State University (W. Turner).

Measurements were done on dried point-mounted or minuten-pinned specimens at 64X using a digital stage micrometer (Boeckler Instr.). Each measurement was taken twice and averaged. Dissection techniques, morphological terminology, and standard measurements and ratios are as given in Grimaldi (1987) and McEvey (1990).

Reproductive Isolation. Modes of reproductive isolation were studied in crosses between strains of flies obtained from Rochester, New York in 1990; Regensburg, Germany in 1990; and Sapporo, Japan in 1991. Genetically heterogeneous strains were established with flies recently collected from natural populations.

Ethological studies were carred out on flies that had developed to sexual maturity at 22°C on our standard medium (Carolina instant Drosophila medium plus a piece of commercial

mushroom, *Agaricus bisporus*). The mating experiments themselves were conducted at room temperature (22-24°) on flies 5-7 days old. To study mating behavior, 5 males from one population and 5 virgin females from the same or a different population were placed in an 8-dram shell vial, containing instant medium and a piece of *A. bisporus*. All matings were scored for the next three hours. For each cross type, 5 replicate vials were set up simultaneously. For any given pair of populations, intra- and inter-population crosses were conducted at the same time. The data of primary interest are the total numbers of pairs that copulated during the 3 hour period, the time that elapsed until the first pair began copulation in each vial, and copulation duration of all mating pairs. Mean time until copulation for all mating pairs is not reported, because these means are substantially inflated by one or a few initially unreceptive females that mated late in the 3 hour period.

Following the mating experiments, 5 females known to have mated were dissected to determine if any sperm had been transferred. In the European x Japanese crosses, in which very few females mated, additional crosses were set up for analysis of sperm transfer. The remaining flies were left undisturbed in order to determine if they could produce any viable offspring. Finally, in those vials in which F1 offspring emerged, F1 males and females were crossed to each other and to both parental species to determine if they were fertile, as would be indicated by production of viable F2 and backcross progeny.

In those crosses that yielded inviable F1, parental strains were treated with antibiotics in order to test whether symbiotic microorganisms are involved in hybrid inviability, as they are in some other species of *Drosophila* (e.g., Hoffman et al., 1986; O'Neill and Karr, 1990). Larvae were reared at 22° for 4 generations on medium containing either tetracycline-HCl (Squibb) or rifampin (Ciba) at concentrations (0.03%) known to cure other *Drosophila* of such symbionts (Hoffman et al., 1986). Then, the parental strains were reciprocally crossed to determine if they could produce viable hybrid offspring. Cytological evidence of symbiotic microorganisms in the North American and European populations was sought using DAPI stain (Karr and Alberts, 1986; O'Neill and Karr, 1990).

RESULTS

Taxonomy

Drosophila testacea species group

Acrodrosophila Duda, 1924: 203 (as subgenus of *Drosophila*). Type species: *D. testacea.*

DESCRIPTION: Yellow to dark bodied *Drosophila* (intraspecifically variable), with a pair of dark brown spots or spots coalesced into incomplete band on each abdominal tergite (fig. 1). Defined as a monophyletic group by a pair of enlarged acrostichal setae ("presuturals") present on the mesonotum anterior to the transverse suture in or close to rows 2 and 5 (of the 6 rows between anterior dorsocentrals); lengths vary from about 3 to 5 times the length of other acrostichals. Presuturals erect and fine, or thicker and more decumbent (fig. 2). Face, cheeks, proboscis, and palps yellow. Pedicel yellow, flagellomere I slightly darker. Carina complete; slightly narrower than scape; with flat ridge. Two pairs of vibrissae. Clypeus very shallow. Eyes light red; with dense, stout interfacetal setulae. Posterior reclinate orbital seta slightly longer than proclinate. Anterior reclinate minute, barely larger than fronto-orbital setulae; length about one-quarter that of posterior reclinate, lying midway between ipsilateral proclinate and posterior reclinate. Base of inner vertical seta in line with ipsilateral orbitals. Verticals slightly longer than posterior reclinate. Ends of inner verticals nearly touching. Outer verticals divergent; slightly postero-lateral to inner verticals. Ocellar setae lying on edge of ocellar triangle. Arista with 4-5 dorsal, 2 ventral branches, in addition to small terminal fork (dorsal number intraspecifically variable). Mesonotum and pleura generally unicolorous yellow, sometimes to dark brown in colder latitudes, altitudes, and seasons. Anterior dorsocentrals slightly longer than one-half the length of posterior dorsocentrals. Distance between bases of ipsilateral dorsocentrals about one-half the length of

anterior dorsocentrals. Postpronotal (humeral) lobe with 2 large setae; 3 notopleural setae; 3 supra-alars, the posterior one largest. Katepisternum with 3 setae; ventral seta largest; middle seta smallest. 6 rows acrostichal setulae between anterior dorsocentrals. Legs unicolorous yellow; with dorso-preapical seta on each tibia. Longitudinal rows cuneiform setulae absent from tibiae/tarsi. Halter yellow; wing hyaline. Cerci (male) yellow to brown.

Sabath et al. (1973) found a direct correlation between body color and temperature of larval development in *Drosophila putrida*: larvae raised at cooler temperatures produced darker adults. This effect occurs in all species of the *testacea* group, since the darkest individuals have routinely been found during the cooler months and the most northerly parts of the distributions.

DISCUSSION: Research on North American *Drosophila* essentially began with Sturtevant (1916, 1921). He described many species and developed the species group and subgeneric classifications of *Drosophila*, including the *testacea* group. Sturtevant was apparently unaware of von Roser's (1840) obscure paper describing several mycophagous *Drosophila* from Europe. Duda (1935: 53) suggested that *putrida* Sturtevant was the same species as *testacea* v. Roser, basing his decision just on Sturtevant's description. Duda was very close to being right. The holotype specimen of *putrida*, in the American Museum of Natural History (examined by DG), is externally identical to European specimens of *testacea*. Most prominently the European *testacea* and Sturtevant's *putrida* type both have a pair of long, thin, erect, presutural acrostichal setae, which is unusual in *Drosophila* (so much so that Duda proposed a subgenus for the 2 species, *Acrodrosophila*, which is not recognized today). However, as is shown here, the male genitalia of Sturtevant's "*putrida*" are consistently, albeit subtly, different from *testacea*, as are other traits like mating, thus indicating their status as distinct species.

There would be no problem except that North American drosophilists have been calling *putrida* Sturtevant as *testacea* v. Roser for the past 50 years. This confusion apparently began at the University of Texas Drosophila Genetics Foundation, perhaps in the 1940's. J.T. Patterson and W.S. Stone recognized 2 North American species in the *testacea* group, not just the one that

Sturtevant had. They properly assumed (but were not exactly correct) that the individuals externally identical to European *testacea* were indeed that species; they erred in assuming that the species with short, stout, decumbent presutural setae was *D. putrida*. Up to now, all North Americans have been calling *Drosophila testacea* what we are describing here as *D. neotestacea*, and calling *putrida* what has actually been an undescribed species. Strict adherence to the use of types can be totally confusing in situations like this one, where recognition and agreement of species concepts in 50 years of literature would otherwise be abandoned. The ICZN accepted a proposal to suppress Sturtevant's holotype of *Drosophila putrida* and replace it with a neotype belonging to the current accepted species concept attached to that name (Grimaldi, 1992).

In the present study, only the three species in the *testacea* complex (*testacea*, *neotestacea*, *orientacea*) were measured and compared in detail, because they are so subtly different from each other but substantially divergent from *Drosophila putrida*. A series of specimens from widely separated areas of the distribution were chosen for measurements. The choice of structures to measure was made on the basis of ones traditionally useful to diagnose species (e.g., ratio of cheek depth/greatest depth of eye; wing ratios, such as Costal Index [C.I.] and 4-V Index), as well as structures which showed some variation.

The cheek depth/eye depth ratio, and 4-V Index were not significantly different among any of the 3 species ($p < .05$, pairwise ANOVA for unequal sample sizes). Likewise, ratio of the lengths of the mid-katepisternal seta/anterior katepisternal was not significantly different, because of the large variance for this character within each sample. Table 1 summarizes the measurement and meristic data; sets of statistically different values are denoted with asterisks. Diagnostically useful values are discussed under each species.

Drosophila testacea von Roser

Drosophila testacea von Roser, 1840: 62. Type locality: Würtemberg, Germany. Lectotype and paralectotypes (designated by E.B. Basden, 1957): Naturkunde Museum, Stuttgart (Bächli, 1990).
Drosophila nigrithorax Strobl, 1894: 132 (as variety of *Lordiphosa fenestrarum* Fallén, 1823), synonymy in Basden (1961: 184).
Drosophila setosa Villeneuve, 1921: 160.

DIAGNOSIS: Presutural setae long and fine, slightly curved, always erect (fig. 2). Costal Index c. 3.9. Length of aedeagus/length of aedeagal apodeme 0.99-1.41 (mean of 1.11). Distiphallus with flat apical margin and acute corners on margin (vs. rounded corners in *orientacea* and *neotestacea*) (fig. 3). Surstylus (clasper) with 4-5 (mean of 4.5) fine medial setae, 10-13 (mean of 11.5) pegs, and 3 (sometimes 2 or 4) fine lateral setae. Male genitalia are best separated from its most similar species, the Japanese *D. orientacea*, on basis of width of distiphallus shaft: ratio of greatest width of distiphallus/shaft width is 0.35-0.43 in *testacea*, 0.30-0.36 in *orientacea*. Also, the middle seta on katepisternum of *testacea* usually shorter than in *orientacea* (Table 2), but this is not an entirely consistent character.

Bächli (1990) provided a very detailed re-description of this species, based on the type series. Critical details of the distiphallus, however, were omitted, which are provided in the diagnosis above.

MATERIAL EXAMINED: **GERMANY**: *Bavaria*, Regensburg, VI/90, J. Jaenike. **HUNGARY**: *Börzsöny hg.*, Magyarkut, 21/VII/79, Bejza & Papp; Szokoya, 18/VII/81, L. Papp. *Kiskunsági N.P.*, Fülöpháza homokbuckás, VI/6/78. D. Draskovits; *Mäyagyüd*: hüvos, neowes volgy, 17/VII/76, L. Papp. *Mátra*: Mátrazentimre, 30/IX/79, Mihályi. Ujszentmargita, Hortobágy N.P., Margitai erdö, 29/VIII/75. *Visegrád*, 19/VIII/70, ex: *Lactarius piperatus* . **MONGOLIA**: *Bulgan aimak*, Namna ul Gebirge, 23 km NW von Somon, Chutag, 1150 m, 21/VII/68, Z. Kaszab (2 F). **SWITZERLAND**: *Heitersberg* AG, 20-29/VII/84, H. Jungen leg., G. Bächli. **USSR: Soviet Far East**: *Sikhote-Alin' Ra.*, *Ussuri*-region, Ussurian Preserve, near Vladivostok, 29/IX/86, Sidorenko (2 M); 15 km SW

Barabash-Lewada, 6/VII/86, Sidorenko; Kunashir I., 5 km. S Lagunnoe Labek, 15/VIII/89, Sidorenko; Komsomolsky Preserve, 50 km Upper mouth of Gorin Riv. 1/VIII/90, V. Sidorenko. *Primorsky kraj*, 40 km. SE Ussurijsk, "on rotten meat," A. Ozerov; Kedrovia Pad Nature Preserve, 18/VII/84, A. Shatalkin (1 M). **Izmaylovo:** *Moscow*, 18/V/68, V. Belyaeva.

OTHER LOCALITY INFORMATION: Bächli and Pité (1982) provided very extensive bibliographic information on this species, including records of its distribution. The species occurs throughout Europe, north to Norway, Sweden, and Finland; west to Great Britain and the Iberian Peninsula; south to Italy, Turkey, and Iran (specific locations within the latter two countries not specified, but presented by Bachli and Pite as new); east to South and North Korea. On the Asian continent *D. testacea* occurs throughout the USSR, extending north to at least Moscow and Lake Baykal. Bächli and Pité (1982) also mentioned but did not specify a location in India. They recorded all distribution records from Japan, which is a species described here as new. *Drosophila testacea* is unrecorded from China, Burma, Pakistan, and Afghanistan.

New records are presented under Material Examined (above) and records published since the bibliography of Bächli and Pité (1982) are the following. **BULGARIA:** Begovica Basin, Pirin Mtns., P. Lauterer, VII (1 F) (Máca, 1987). **GERMANY:** *Edersee* (Hesse), Nieder-Werbe, 12-17/VIII, Bächli (162 specimens) (Bächli et al., 1985). **NORWAY:** *Hardangervidda*, ZM Bergen, Hurum, 17/VI/85, Tofte; TRI: Malselv, Rosta (Bächli, 1986). **ROMANIA:** Navodari, VIII (1 F) (Maca, 1987). **USSR:** *Maritime Territory*: 15 km. SW Valentin, Glazkovka, 15-18/VIII/86, A. Ozerov (17 M, 19 F) (Gornostayev, 1989). *Ukraine*, Podolia Region, Manus, Kanev Nat. Reserve, "reared from *Russula* sp. and *Phallus impudicus*" collected VIII, M. Delikatnyj (13 M, 17 F) (Máca, 1987). **YUGOSLAVIA:** *Ohrid*, e. border Lake Ohrid, IX/6-8/79 (19 specimens); *Kupari*, near Dubrovnik, IX/16/79 (1 specimen); *Pôrec*, 24-27/VII/79 (1 specimen) (Baechli and Kekic, 1983).

A distribution map is provided in Fig. 4.

Drosophila neotestacea **new species**

Drosophila testacea of American authors (see text).

DIAGNOSIS: Presutural setae long, thin, slightly sinuate; usually erect, but sometimes barely so (Fig. 2). Costal index c. 3.6 (vs. 3.9-4.0 for Old World species). Postocellar setae cruciate for about one-third their length. Distinguished best from Palearctic *testacea* and Japanese *orientacea* by male genitalia: ratio of length of aedeagus/length of aedeagal apodeme less than in other 2 species (mean of 1.01, vs. 1.11); surstylus with more setae (mean of 5 fine laterals, vs. 2-4 in other species), and more pegs (mean of 13.7, vs. 9-13 in other 2 species)(see Table 2). Distiphallus with conspicuously rounded apical corners (Fig. 3).

HOLOTYPE: Male, point mounted. **NEW YORK**: *Orange Co.*, Cornwall, Black Rock Forest Preserve, 12/IX/90, Grimaldi & Stark, reared from fungus specimen no. 148 (*Ramaria* sp.). Paratypes: 13 females and 6 males with same label data as holotype. Holotype and paratypes in the AMNH.

ETYMOLOGY: *neo*, referring to new and nearctic.

MATERIAL EXAMINED: **CANADA: BRITISH COLUMBIA**: *Trinity Valley*, 22/VI/37, H. Leech (1 F). **MANITOBA**: Selkirk, VII/52, H.L. Carson (1 M). **NOVA SCOTIA**: *Cape Breton Highlands* N.P., Lone Shielding, 18-21/VII/83, flight intercept trap, D.&J. Bright (4 M, 3 F). **ONTARIO**: *Arthur*, 15-17/VII/83, S.A. Marshall, mushroom traps (2 M, 1 F); *Brockville*, 6/VIII/1903, W. Metcalfe (1 F); *Constance Bay*, 30/VIII/80, K. Barber (1 M); *Guelph*, Univ. Guelph Arboretum, VII-/10-13/83, "mushroom baited pitfall trap," B.V. Brown. *Maynooth*, 30/VIII/52, J.F. McAlpine, "from rotting fungi," (7 M, 3 F); *Ottawa*, 8/VIII/23, C.H. Curran (2 M), 19/X/51, J.F. McAlpine (1 M, 2 F); 27/X/56, J.R. Vockeroth (2 M); *Trenton*, 28/VIII/1902, 5/VIII/'08, Evans (1 M, 4 F). **QUEBEC**: *Bolton Pass*, *Knowlton*, 800 ft., 5/VI/63, J.R. Vockeroth (3 F); *Mt. Orford*, 1200 ft., 5/VI/63, J.R Vockeroth (1 M); *Norway Bay*, 26/VIII/38, G.E. Shewell (2 M, 2 F). **YUKON TERRITORY**: *Dawson City*, 13/VII/85, S.A. Marshall, on *Boletus*, aspen

hydrocut (3 M, 9 F); *Moose Cr.* Campground, aspen mushroom, 3/VII/85, S. Marshall (1 M, 1 F); *Deapster* Hwy., Tombstone Mtn. Camp 3/VII/85, S. Marshall (1 M).

UNITED STATES: ALASKA: *Anchorage*, D.D. Miller, 1957 (1 F). **ARKANSAS:** *Sawmill Cr.*, White Spruce Bog, 18 mi. S. Delta Jct., 15/VII/85, S. Marshall (1 M). **CALIFORNIA:** 5 mi. W. Willow Creek (county?), VII/51, M.R. Wheeler, W.B. Heed; near *Weott* (county?), VII/51, M.R. Wheeler, W.B. Heed (1 M). **IDAHO:** *Adams Co.*, New Meadows, VII/47, M.R. Wheeler (1 M, 3 F). **MAINE:** *Hancock Co.*, Mount Desert Island, 29/V-3/VI/82, J. Jaenike, VIII/48, M.R. Wheeler (1 M). **MASSACHUSETTS:** *Barnstable Co.*, Woods Hole, VIII/20/14, A.H. Sturtevant. **MICHIGAN:** *Crawford Co.*, V/3/59, R. & K. Dreisbach (1 F). *Ingham Co.*, East Lansing, 13/VI/71, D.D. Wilder (1 M). *Luce Co.*: VIII/30/52, R.R. Dreisbach (1 F). *Midland Co.*, IX/15/45, R.R. Dreisbach. **MINNESOTA:** *Beltrami Co.*, Bemidji, VII/47, M.R. Wheeler & F.D. Cowan, (1 M). Itasca St. Park, VII/52, H.L. Carson (1 M). **MONTANA:** *Glacier Co.*, Glacier National Park, VII/47, M.R. Wheeler (2 F). (county?) 1.5 mi. N. Fish Creek Camp, VII/47, M.R. Wheeler, F.A. Cowan. **NEW JERSEY:** *Bergen Co.*, Ridgewood, IV/87, "bred from *Symplocarpus spadice*," A. Soll (1 M). **NEW YORK:** *Broome Co.*, Chenango Valley State Park, 1-25/VIII/82, D. Grimaldi, "bred from *Russula* and *Cortinarius* mushrooms," (3 M, 4 F). *Tompkins Co.*, Trumansburg, VI/7/84, D. Grimaldi, "reared from *Pleurotus sapidus*," (1 M). **NORTH CAROLINA:** *Macon Co.*, Highlands, 22-26/IV/88, 3500 ft., D. Grimaldi; VIII/62, "bred, fungus" D.D. Miller. *Swain Co.*, Clingman's Dome, Great Smoky Mountains, VII/18-22/82, J. Jaenike. Smokemont Camp, VI/50, T.C. Hsu (5). **TENNESSEE:** *Great Smoky Mountains Nat. Park*, VII/11/53, H.D. Stalker (1); Alum Cave Bluffs VII/18-22/82, J. Jaenike. **VERMONT:** *Orleans Co.*, East Charleston, Mad Brook Farm, VII/15-25/82, D. Grimaldi.

OTHER LOCALITY INFORMATION: Patterson and Stone (1952) and Patterson and Wagner (1943) presented distribution maps of this species and *D. putrida*. They did not examine any Canadian portions of the distributions, but did discover differences in the distribution of the 2 species. A more complete distribution map is given in Fig. 5. *Drosophila neotestacea* is restricted to Canada, Alaska, and the northern half of the U.S. Its southernmost range is North Carolina,

where it occurs at higher altitudes in the Appalachians (especially during summer months). Patterson and Stone (1952) recorded specimens from western Nebraska, northwestern Montana, and northern Idaho. To the western portion of the distribution we add records here from northern California and British Columbia. It may occur at higher altitudes in the Rocky Mountains of Wyoming and Colorado. *Drosophila putrida* is more restricted to eastern North America and extends to more southerly latitudes (fig. 6).

Drosophila orientacea **new species**

Drosophila testacea of Japanese authors (see text).

DIAGNOSIS: Extremely similar to the true, European *testacea*, but differing in the following subtle characteristics. Male genitalia with usually 2 setae (rarely 3, as in *testacea*) on lateral surface of surstylus. Width of distiphallus shaft (relative to width of distiphallus) thinner than in *testacea* (fig. 3; Table 1). Presutural setae slightly thicker and more decumbent than in *testacea* (fig. 2). Length of mid-katepisternal seta (relative to anterior katepisternal seta) slightly longer than in *testacea* (Table 1).

HOLOTYPE: Male, from isofemale line lab cultured individuals derived from **JAPAN**: *Hokkaido*, Sapporo, collected July, 1990 by M.T. Kimura. Deposited in the Tokyo Science Museum. Paratypes (from same culture) in the American Museum of Natural History and the Tokyo Science Museum.

ETYMOLOGY: *orient*, referring to eastern and its Japanese distribution.

OTHER MATERIAL EXAMINED: **JAPAN**: *Hokkaido*, Sounkyo, Mt. Dolisetsu, 17/VIII/53, T. Okada (4M, 3F).

OTHER LOCALITY INFORMATION: Bächli and Pité (1982) listed 51 references on the distribution of *Drosophila testacea* in Japan. Although we examined specimens only from Hokkaido, which are here designated as the new species, *D. orientacea*, it is quite possible that all

the work on *D. "testacea"* in Japan are referable to *D. orientacea*. More extensive comparisons need to be made between individuals among various localities in Japan and with the proximal mainland.

Drosophila putrida Sturtevant

Drosophila putrida Sturtevant, 1916: 339. Neotype: Male, American Museum of Natural History (designated by D. Grimaldi [1992: .]). Type locality: Pompton Plains, New Jersey.

DIAGNOSIS: The most distinctive species in the *testacea* group. Easily and reliably separated from sympatric *neotestacea* on basis of thicker, shorter, decumbent presutural setae (fig. 2). Male genitalia distinctive: distiphallus is very flat; with deep median cleft producing 2 apically-pointed lobes, with slightly serrate lateral knob on each. Shape of median cleft is quite variable (fig. 3).

MATERIAL EXAMINED: **CANADA: ONTARIO**, *Guelph*, Univ. of Guelph Arboretum, VII/10-13/83, "in mushroom-baited pitfall traps," B.V. Brown (1M, F); *Wainfleet Bog*, 8 km. S. Welland, 22-28/IX/87, D28, pt2-1962, R. Sterling; *Oliver Bog*, 3 km. S. Salt, 19-25/VI/87, D. Blades; *Grand Bend*, Pinery P.P., 12/IX/81, K. Barber.

UNITED STATES: ALABAMA: *Kushla* (county?), IV/15, A.H. Sturtevant (1 F). **ARKANSAS**: *Johnson Co.*, Haw Creek, 8/VI/91, swept, J.E. Swann (1 F). *Logan Co.*, Ozark Nat. Forest, Magazine Mtn., mushroom-baited pans, 23/V-8/VI/91, J. Swann (3 M). **FLORIDA**: *Highlands Co.*, Lake Placid, Archbold Biol. Sta, 1/XI/89, "emerged from *Clitocybe* and *Boletus* spp," M. Deyrup. **GEORGIA**: *Liberty Co.*, St. Catherines Island, 11-20/IV/88, "banana-baited trap," D. Grimaldi (5 M, 4 F). **ILLINOIS**: *Flat Rock* (county?), 1915 (no month), "fungus", A.H. Sturtevant. **MAINE**: *Hancock Co.*, Mount Desert Island, 29/V-3/VI/82, J. Jaenike; VIII/48, M.R. Wheeler, T. Hsu. **MICHIGAN**: *Ingham Co.*, East Lansing, 13/VI/71, D.D. Wilder. **MISSISSIPPI**: *Warren Co.*, 2/VI/50, H.D. Stalker (1 M). **MISSOURI**: *Webster* Gr., IX/50, H.D. Stalker. **NEW JERSEY**: *Bergen Co.*, Ridgewood, IV/87, A. Soll, "bred from *Symplocarpus* [skunk cabbage] spadices". *Morris Co.*, Pompton Plains (type locality), X/19/80, D. Grimaldi, "caught in banana trap". **NEW YORK**:

North Greece (county?), I/V/36, A.W. Post. *Broome Co.*, Chenango Valley St. Pk., VIII/82, "reared from *Cortinarius* and *Russula* mushrooms", IV/15-26/82, D. Grimaldi; *Erie Co.*, Buffalo, VI/27/'08, Van Duzee (1 F); Gowanda, VI/7/12, Van Duzee (1 F); *Niagra Co.*, Niagra Falls, 7/26/14 (1 F), 10/15/11 (1 M), Van Duzee. *Tompkins Co.*, S. Lansing, 29/VII/86, W.L. Brown, Trumansburg, VI/7/84, "reared from mushroom, *Pleurotus sapidus*," D. Grimaldi. **NORTH CAROLINA**: Black Mountains (county?), VI (no year), A.H. Sturtevant; *Graham Co.*, Robbinsville, 9/VI/76, G.E. Bohart; *Jackson Co.*, Cherokee, 5/VI/76, G.E. Bohart; *Macon Co.*, Highlands, 22-26/IV/88, 3500 ft., D. Grimaldi; *Swain Co.*, Great Smoky Mountains, Metcalf, VII/16/82, "bred from *Amanita bisporigera*," (1 M). *Wake Co.*, Raleigh VIII/3/82, J. Jaenike, "reared from *Amanita* [*chlorinosoma*?]. **PENNSYLVANIA**: *Centre Co.*, Pine Grove Mills, 5/1/72, D.D. Wilder (1 M); State College, 5/VII/81, A.L. Norrbom, "at sap flow on *Quercus alba*" (1 F). **TENNESSEE**: *Elkmont* (County?), VII/20/82, "reared from mushroom, *Suillus pictus*," J. Jaenike (1 M); Alum Cave Bluffs VII/18-22/82, J. Jaenike. **TEXAS**: *Harris Co.*, Houston, White Oak Bayou, VI/20/83, J. Jaenike. *Travis Co.*, Austin, III/49, M.R. Wheeler. **VERMONT**: *Orleans Co.*, East Charleston, VII/15-25/82, D. Grimaldi.

OTHER LOCALITY INFORMATION: Patterson and Stone (1952) provided a distribution map of this species. Their records, and the ones given here indicate that the species ranges from southern Ontario and Quebec to the middle of Florida and Texas, and along the Gulf Coast. Patterson and Stone (1952) indicate westernmost records of *D. putrida* from eastern Nebraska and Kansas, throughout Oklahoma, and to western Texas. We did not examine specimens from these parts of the western edge of the range. Patterson and Stone (1952) suggested that the distribution of *D. putrida* closely matches that of the eastern deciduous forests. *Drosophila putrida* appears not to be as cold tolerant as other species of mycophagous *Drosophila*. In a comparison of larval development times at 15, 21, and 25°C, Grimaldi (1985) found *putrida* larval development to be shortest and larvae larger at 21C, but *D. recens* and *D. falleni* developed equally well at 15 and 21C (*testacea* was not studied). *Drosophila recens* and *D. falleni* have distributions that encompass *D. putrida*.

Reproductive Isolation

Flies mated readily, transferred sperm, and produced viable F1 in all intraspecific crosses (Table 2). The 3 species are isolated by a variety of pre- and post-mating mechanisms. For *D. testacea* and *D. orientacea*, very few flies mated in either reciprocal cross (Table 2), and those that mated did so much later than in intraspecific crosses (Table 3). This indicates strong, but incomplete ethological isolation. Those females that did mate were able to produce viable and fertile F1 hybrid progeny.

Strong (absolute in our tests) asymmetric ethological isolation was apparent in crosses between *D. testacea* and *D. neotestacea*: females of *D. testacea* readily accepted males of *D. neotestacea*, but none of the *D. neotestacea* females mated with males of *D. testacea* (Tables 2 and 3). In the former cross, sperm were transferred, but no F1 eggs hatched, indicative of complete post-mating isolation.

Little ethological isolation was seen in crosses between *D. neotestacea* and *D. orientacea*: nearly every female mated with a heterospecific male within the 3-hour observation period (Table 2), and times to first copulation in a vial were similar for crosses within and between species (Table 3). However, post-mating isolation was complete in these crosses. In crosses between *D. neotestacea* males and *D. orientacea* females, sperm were transferred, but no hybrid eggs hatched. In the reciprocal cross, no sperm were transferred.

Mean durations of copulation varied among species: matings within *D. neotestacea* and *D. orientacea* were similar in duration, but copulation duration was substantially longer in *D. testacea* (Table 3). In interspecific matings, copulation duration was similar to that of the male's species.

Antibiotic treatment failed to cure incompatibility between *D. testacea* and *D. neotestacea*. After 4 generations of treatment, no viable offspring were produced in crosses between these species. Furthermore, DAPI staining failed to reveal the presence of any cytoplasmic microorganisms.

DISCUSSION

Systematics. Some basic phylogenetic relationships in the *testacea* group are very obvious. On the basis of male genitalia *testacea* + *orientacea* + *neotestacea* form a monophyletic group, the *testacea* subgroup. The male genitalia of *Drosophila putrida* are strikingly different from those of the *testacea* subgroup and, in fact, bears close resemblance to some species in the *Drosophila quinaria* group. Because of the similar flattened, flanged distiphallus in *putrida* and some *quinaria* group species, it seems very possible that the *testacea* and *quinaria* groups are sister groups. The *quinaria* group is defined in part by three filaments on the egg; the *testacea* group (including *putrida*) retains the plesiomorphic state of 4 egg filaments. The erect, thin presutural setae in *neotestacea* and *testacea* indicates they would be sister species, but the male genitalia of *testacea* are most similar to those of *D. orientacea*. Furthermore, R1 elements (retroposons within ribosomal genes) are more similar in sequence between *D. testacea* and *orientacea* (T.H. Eickbush, pers. comm.). The 3 species in the *testacea* subgroup may have an unresolvable, trichotomous relationship.

Reproductive Isolation. Although the 3 species of the *Drosophila testacea* complex are extremely similar morphologically, they are reproductively very strongly isolated by a variety of mechanisms, including ethological barriers, lack of sperm transfer, and hybrid inviability.

Ethological isolation was found to be strong, but not complete, in both directions in crosses between *D. testacea* and *D. orientacea*. In contrast, *D. neotestacea* and *D. orientacea* mated as readily with each other as with members of their own species. Finally, ethological isolation was found to be strongly asymmetric between *D. testacea* and *neotestacea*; females of *D. testacea* readily accept males of *D. neotestacea*, but *D. neotestacea* females uniformly rejected *D. testacea* males. One possible cause of this asymmetry involves noticeably different courtship behavior by the males of these two species. Males of *D. neotestacea* exhibit a "normal" repertoire of courtship elements, including regular wing flicking (see Spieth, 1974). In contrast, males of *D. testacea* almost never employ discernable wing flicking while courting a female. Since many *Drosophila*,

including *D. neotestacea*, *D. orientacea*, and *D. putrida*, do employ wing-flicking, the lack of this courtship element is almost surely a derived state in *D. testacea*. If wing flicking is required to stimulate females of *D. neotestacea* but has no influence on *D. testacea* females, this could account for the asymmetrical ethological isolation between these species.

Another type of asymmetrical isolation concerned sperm transfer. Males of *D. neotestacea* successfully inseminated *D. orientacea* females with which they had mated, but in the reciprocal cross, *D. orientacea* males failed to transfer sperm to female *D. neotestacea*. Copulation was not interrupted in the latter crosses; in fact, these copulations lasted somewhat longer on average than any other crosses involving these two species (Table 3). Given the almost identical structures of the male genitalia of these species (Fig. 3), the lack of sperm transfer suggests that something other than genitalic fit must play a key role in copulation. Perhaps movement of the aedeagus and/or surstyli ("claspers") perform an internal or subtle courtship. The great variation in copulation duration in crosses between *D. orientacea* males and *D. neotestacea* females (Table 3), ranging from 1 to 63 minutes, suggests a breakdown of the normal process of copulation. In interspecific matings between all other species pairs, mating was always accompanied by successful sperm transfer.

The final mode of reproductive isolation was hybrid inviability, which was found in crosses between male *D. neotestacea* and females of either *D. testacea* or *D. orientacea*. It is possible in these cases that failure of sperm to fertilize heterospecific eggs, rather than hybrid inviability per se, resulted in the absence of F1 offspring. The species pair *D. testacea-D. orientacea* exhibited no post-mating isolation in either reciprocal cross, at least through the F1, which were both viable and fertile for both sexes.

It is interesting to note that pre- and post-mating isolation do not evolve in parallel in this set of species. Specifically, *D. neotestacea* and *D. orientacea* mate freely with each other, but they fail to produce any viable hybrid offspring. In contrast, *D. testacea* and *D. orientacea* are strongly isolated behaviorally, but in the few cases where they do mate they produce viable and fertile F1 of both sexes. These results support the conclusion of Coyne and Orr (1989) that neither pre- nor post-mating isolation consistently evolves faster in allopatric populations of *Drosophila*.

Post-mating isolation between *D. testacea* and *D. neotestacea* does not appear to be due to symbiotic microorganisms, as antibiotic treatment failed to eliminate incompatibility, and DAPI staining provided no cytological evidence for the presence of such microbes in these studies. Thus, post-mating isolation between them is most likely due to nuclear genes.

Elsewhere, we have reported that populations of the North American species *D. neotestacea* harbor at considerable frequencies an X-linked factor called "sex ratio" that causes male carriers to sire only daughters; this results in substantially female-biased sex ratios at the population level (James and Jaenike, 1990). Whether or not the "sex ratio" factor(s) occurs in *D. testacea* or *D. orientacea* remains to be determined, although we have not yet found it in our samples. Absence of "sex ratio" in these other species would suggest that this condition has arisen very recently in *D. neotestacea*. Clarification of phylogenetic relationships and of even more basic aspects such as species limits will allow evolutionary analyses of this and other patterns of interspecific variation.

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Table 1. Morphological differences among species of the *Drosophila testacea* complex. Entries are mean and (sample size) on first row and range below.

Character	Species		
	testacea	*neotestacea*	*orientacea*
presutural seta length (mm)	0.174 (25) 0.150-0.230	0.174 (18) 0.131-0.195	0.200* (17) 0.137-0.240
cheekdepth/eye depth	0.128 (25) 0.091-0.158	0.136 (18) 0.100-0.157	0.126 (17) 0.110-0.144
length: mid-katepisternal seta / anterior katepisternal	0.560 (25) 0.414-0.831	0.601 (18) 0.335-0.822	0.655 (17) 0.430-0.805
wing ratios: C. I.	3.89 (25) 3.34-5.12	3.63* (18) 3.15-4.06	3.98 (17) 3.48-4.65
4-V	1.59 (25) 1.47-1.74	1.57 (18) 1.35-1.74	1.48 (17) 1.37-1.75
length: aedeagus/aedeagal apodome	1.11 (4) 0.99-1.41	1.01* (9) 0.90-1.23	1.11 (10) 1.03-1.21
width: distiphalus/shaft	0.38 (11) 0.35-0.43	0.40 (8) 0.35-0.46	0.33* (10) 0.30-0.36
no. surstylar setae: medial	4.5* (6) 4-5	6.0* (12) 4-7	3.5* (9) 3-4
pegs	11.5 (13) 10-13	13.7* (13) 12-15	11.3 (11) 9-13
laterals	3.1* (13) 2-4	5.0* (13) 3-6	2.3* (11) 2-3

Asterisks denote means significantly different ($P < 0.05$) from all others within a row, as determined by ANOVA.

Table 2. Modes of reproductive isolation among species of the *Drosophila testacea* complex.

Cross type*		Pre-mating	Post-mating			
male	female	# pairs mating in 3 hours	sperm transfer	F1 egg hatch	F1 fertility males	F1 fertility females
Interspecific						
N	T	20/25	yes	no	-	-
T	N	0/25	-	-	-	-
T	O	1/25	yes	yes	fertile	fertile
O	T	3/25	yes	yes	fertile	fertile
N	O	23/25	yes	no	-	-
O	N	21/25	no	-	-	-
Intraspecific						
T	T	42/49	yes	yes	fertile	fertile
N	N	47/50	yes	yes	fertile	fertile
O	O	24/25	yes	yes	fertile	fertile

* N = *neotestacea*; O = *orientacea*; T = *testacea*

Table 3. Details of mating behavior within and among species of the *Drosophila testacea* complex.

Cross*		Time to copulation (min.) (first mating pair/vial)			Copulation duration (min.)		
male	female	mean	SD	N	mean	SD	N
Interspecific							
N	T	16.9	5.5	5	11.3	1.4	19
T	N	-			-		
T	O	48	-	1	20	-	1
O	T	33.5	21.9	2	12.6	7.4	3
N	O	2.2	0.8	5	10.9	3.6	23
O	N	3.2	0.4	5	20.3	12.3	20
Intraspecific							
T	T	7.8	4.7	10	21.2	4.2	37
N	N	2.2	1.2	10	12.5	3.7	46
O	O	3.0	2.9	5	14.8	3.4	21

* N = *neotestacea*; O = *orientacea*; T = *testacea*

FIGURES

Fig. 1. Dorsal habitus (excluding wings) of *Drosophila neotestacea*, n.sp., showing differences in coloration.

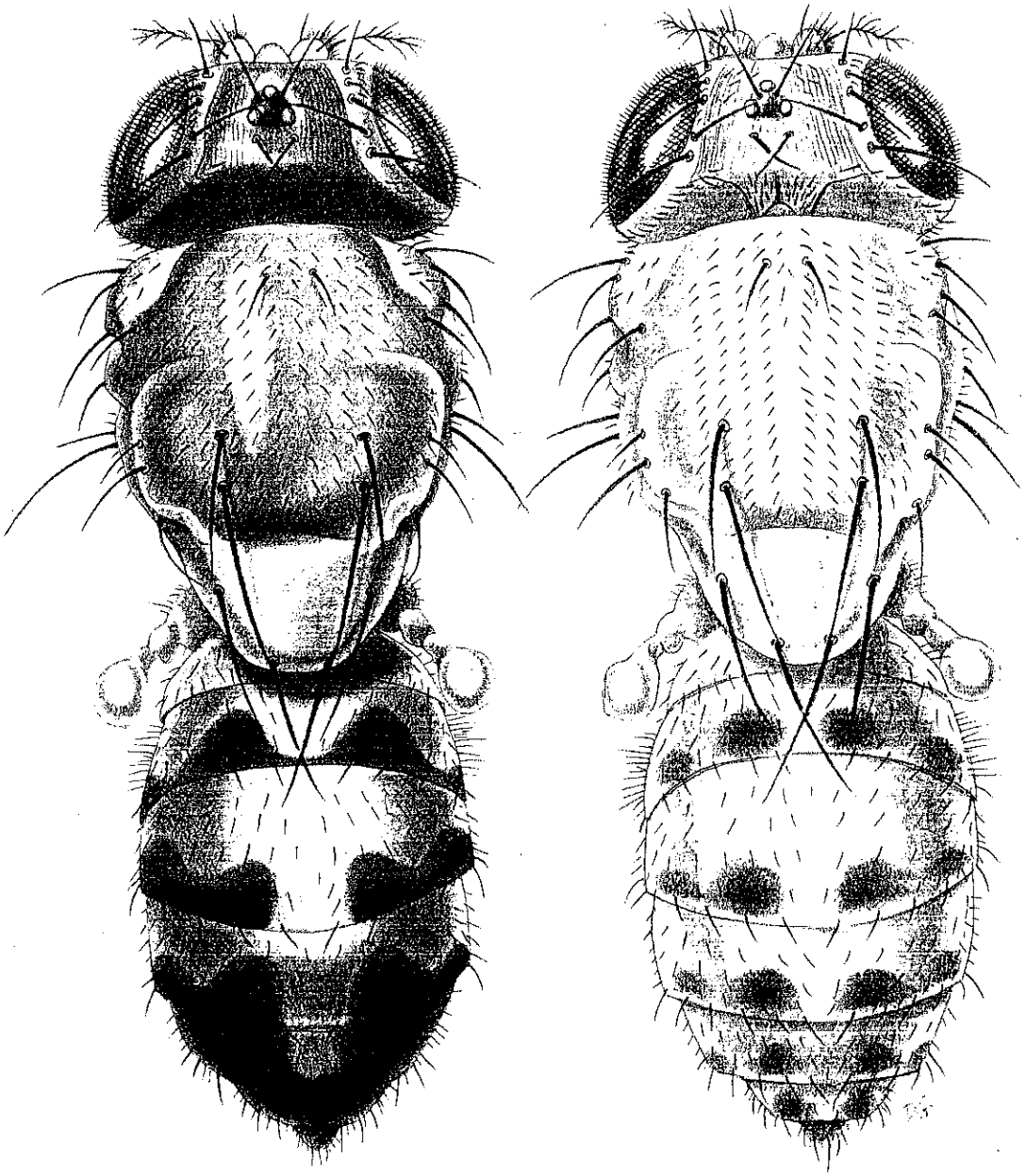
Fig. 2. Lateral view of mesonotum of *testacea* group species, showing diagnostic differences in presutural seta.

Fig. 3. Distiphallus of *testacea* group species. The entire aedeagus and aedeagal apodeme is shown for *Drosophila testacea*, and the entire aedeagus for *Drosophila putrida*.

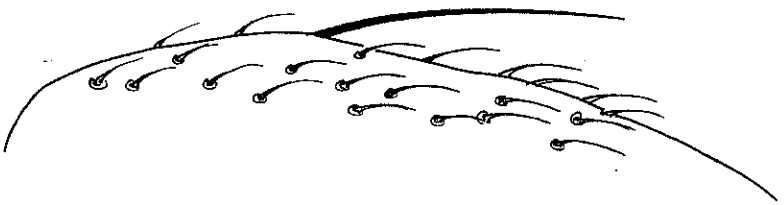
Fig. 4. Distribution map of Palearctic *testacea* group species.

Fig. 5. Distribution map of *Drosophila neotestacea*.

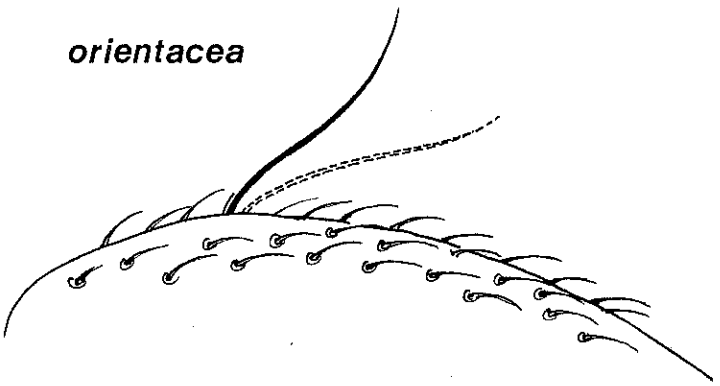
Fig. 6. Distribution map of *Drosophila putrida*.



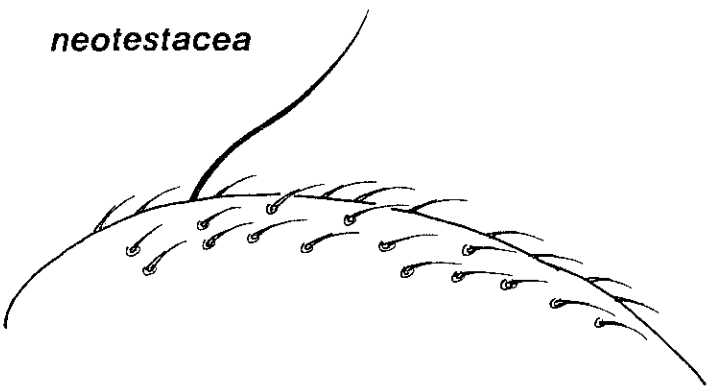
putrida



orientacea



neotestacea



testacea

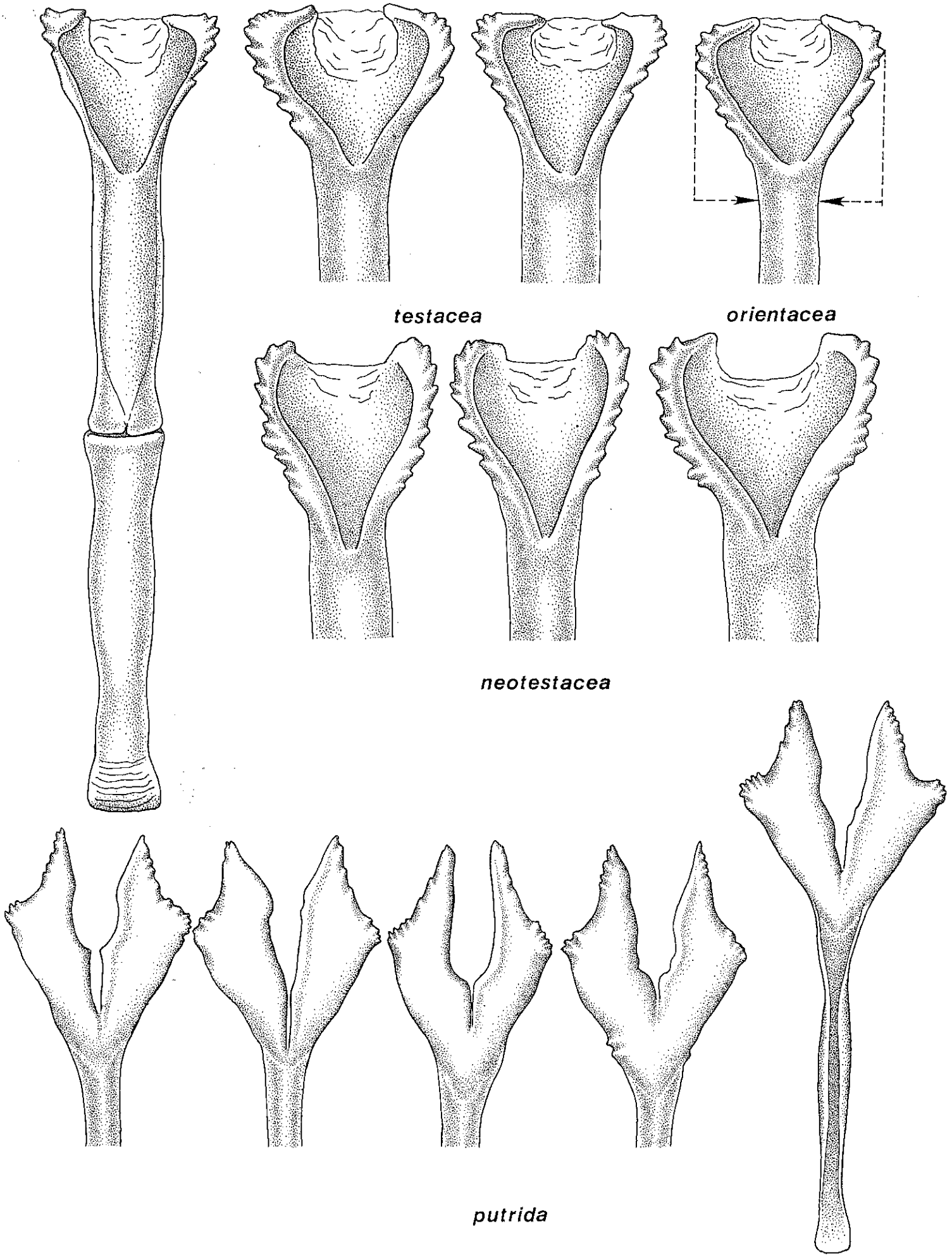
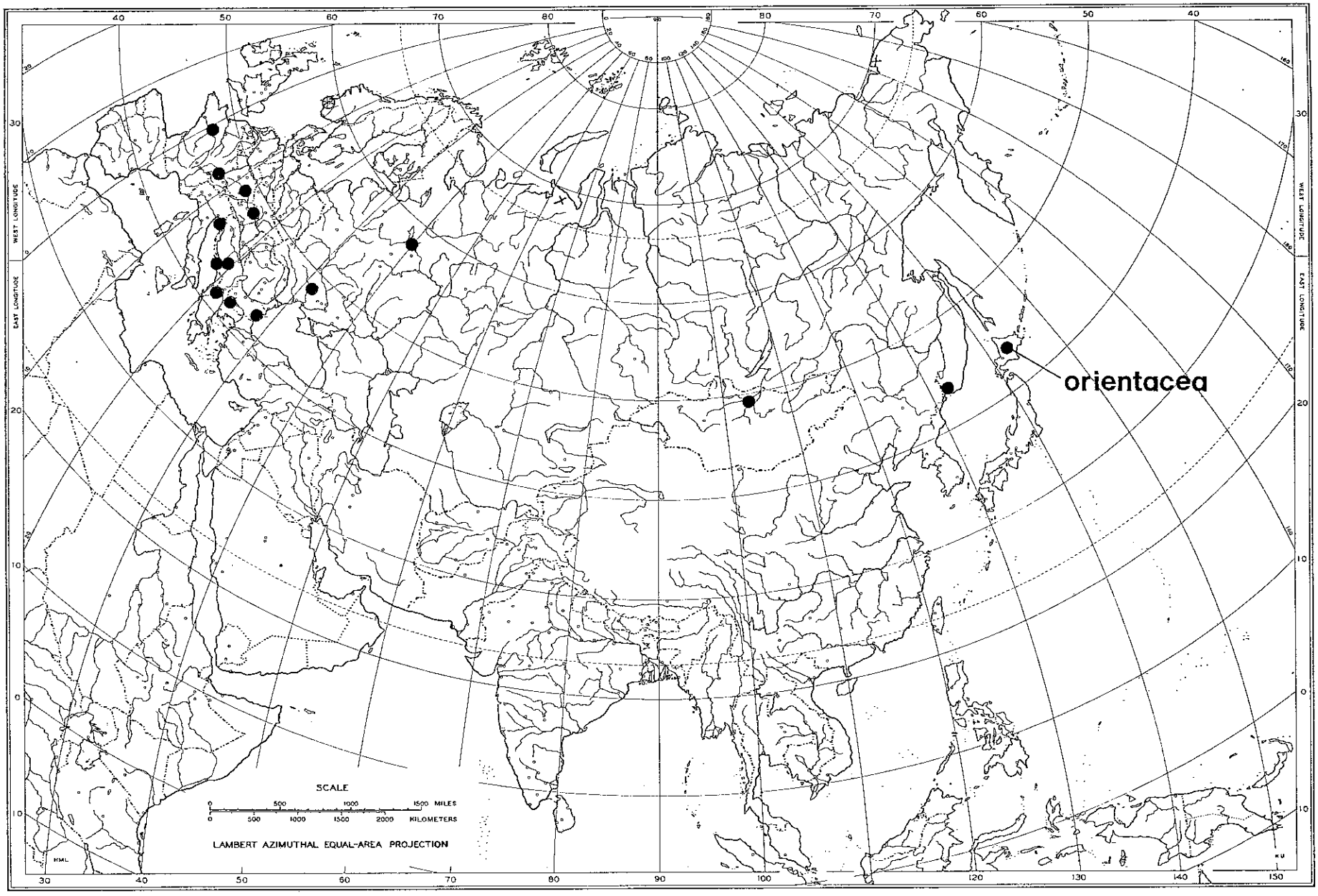

testacea
orientacea
neotestacea
putrida

Fig 4 -lestacea + orientacea

ASIA

No. 5



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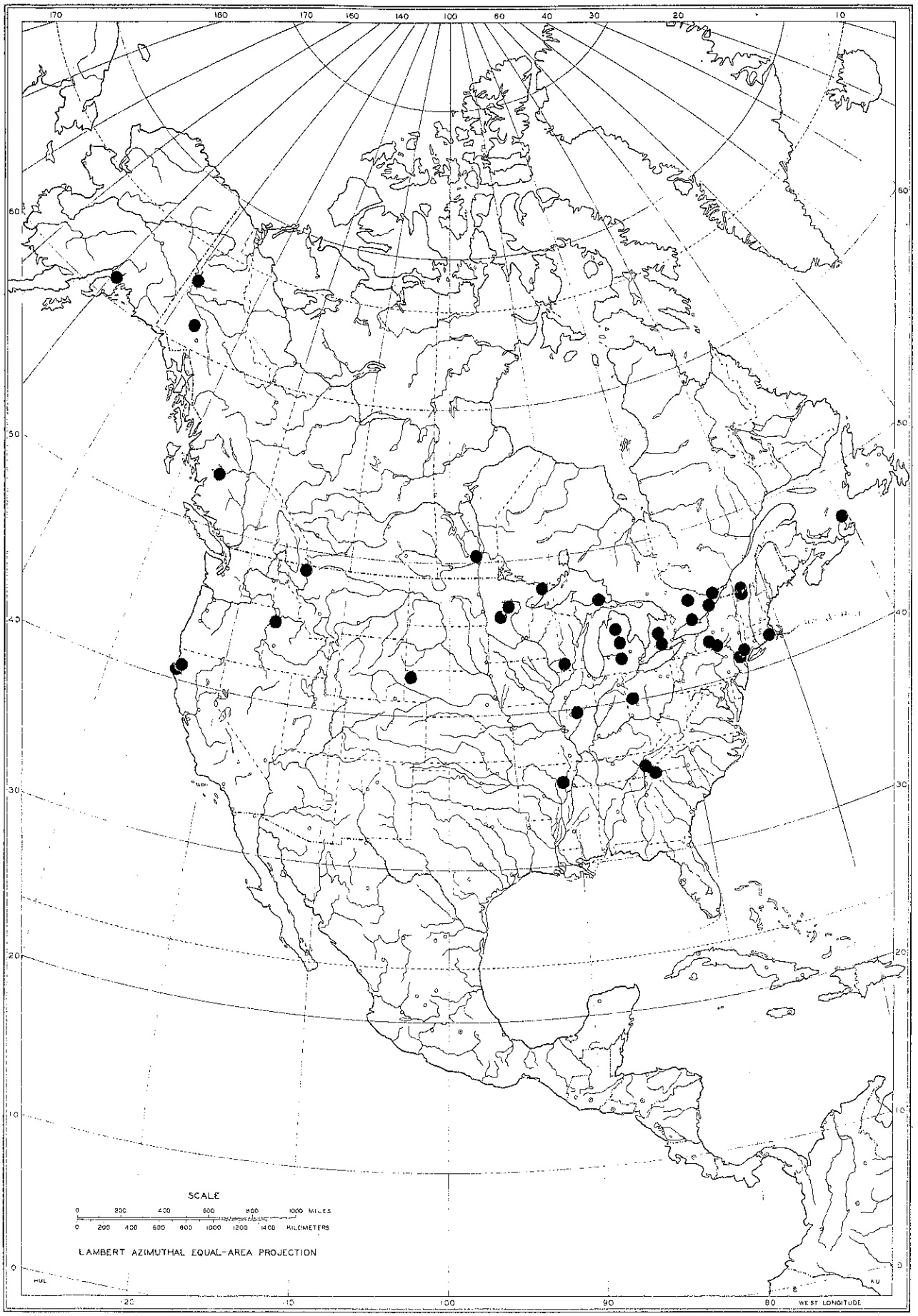
neotestacea Fig. 5



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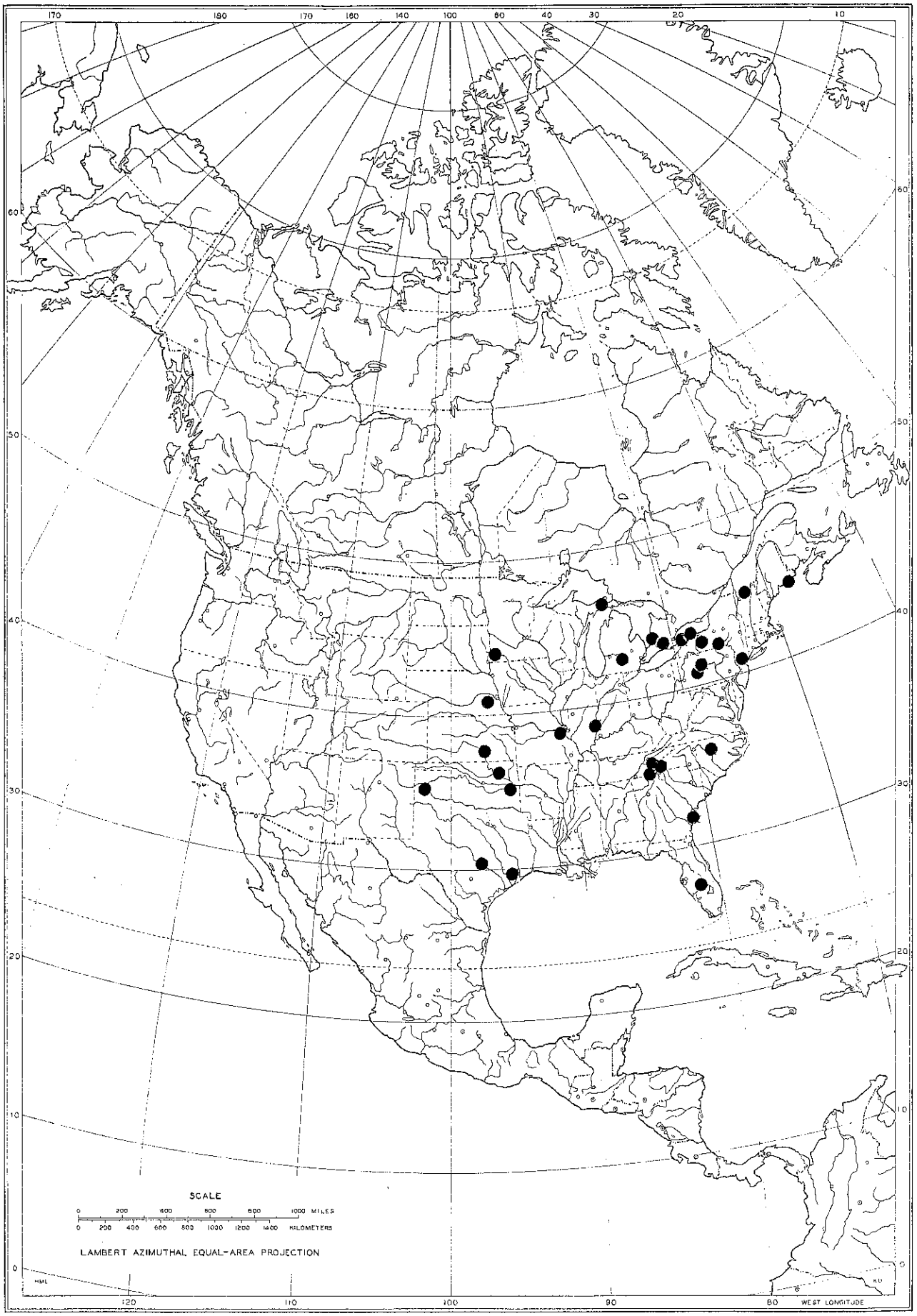
putrida Fig. 6



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IMMATURES

Viviparity

Only the egg of *C. dikra* was examined in detail, which came from pinned dissected specimens. The egg had 14 (7 pairs) of short filaments surrounding an oval operculum area. It was slightly asymmetrical bilaterally, with irregular longitudinal furrows (fig. xx). Fourteen filaments is the highest number yet recorded for the Drosophilidae, but a large number of filaments is a feature that is consistent with other "lower" drosophilines (sensu Grimaldi, 1990). For example, *Chymomyza* and *Scaptodrosophila* have between 6 and 10 (3-5 pairs) short to long filaments, which is distinct from the 2 pairs of filaments seen in the ground plan of most other drosophilines such as *Drosophila.*

Eggs were rarely seen, in fact, in the pinned specimens that were dissected for this study. In females of 5 species there were first instar larvae; these species were all collected in March, 1991 by Grimaldi at Las Alturas, Costa Rica: *antalba, fasciata, glapica, telescopica,* and *vivipara* (see records of specimens, above). It is known for *Drosophila melanogaster* that, if females with mature eggs are starved and not provided with an oviposition substrate, they apparently will hatch a first instar larva "in utero" (reference). Viviparity in *Cladochaeta*, however, was found in species belonging to 4, maybe 5, species groups (placement of *C. antalba* is uncertain without males), suggesting that the trait is rather widespread and not quite so facultative. Viviparity has not been found in other *Cladochaeta* species where a significant number of females has been dissected, such as *C. inversa* and *C. genuinus*. It is possible that viviparity is peculiar to the specimens collected at Las Alturas during that short period, because of factors related to seasonality and scarcity of hosts, although there is nothing to suggest this. Viviparity is known for *Amiota* (*Sinophthalmus*) *picta* Coquillett (facultatively) (Wheeler, 19xx), *Diathoneura cruciata* Duda (Vilela & Bchli, 1990), and in *Colocasiomyia* ------ (Yafuso & Okada, 19xx). It is a rare feature that occurs sporadically in the Drosophilidae, and seems certainly most common thus far in *Cladochaeta.*

Viviparity in *Diathoneura cruciata* is of particular interest, since this genus is the sister group to *Cladochaeta* (Grimaldi, 1990; and see previous discussions). Occurrence of the trait throughout

Diathoneura will be impossible to assess in lieu of numerous dissections and a revision of this speciose genus. The first instar larvae of *Cladochaeta* were always found with their anterior end pointed anteriad in the female abdomen (as reported for *D. cruciata* [Vilela & Bchli, 1990]) (figs. xx). There was only one larva per female, having a length approximately one-half the females' abdomen. The larvae had telescoping posterior spiracles (but were inverted). Larvae for 4 of the species were examined for details of the cephalopharyngeal skeleton (CS) and creeping welts. Two of the 4 species had simple, non-serrate mandibles (figs. xx). *C. glapica* (fig. x) had 4 rounded teeth posterior to the apical hook; *C. fasciata* had very small serrations. In comparison to the rest of the CS, the mandibles of these first instars were relatively larger than in third instars of *C. inversa* and *C. floridana* (described below). Only the mandibles and anterior portion of the CS were sclerotized (figs. xx). A few oral lamellae were found in *C. glapica*; the other 3 species examined had none (*glapica* appears plesiomorphic in this regard). Larvae of all species had 6 rows of creeping welts, as do mature instars of other species. Both *C. glapica* and *C. antalba* first instars had about 12 creeping welt spinules on a ventro-medial "proleg" posterior to creeping welt 6. All species had very minute, sparse spinules in creeping welt 1. In *C. antalba* and *C. vivipara*, creeping welts 2-6 were interrupted medially, with the spinules in each welt arranged in 2 ovals (figs. xx). Each oval had 2 anterior rows of spinules pointed anteriad, and 2 posterior rows pointed posteriad. The anterior and posterior rows each had a row of larger spinules towards the center of the oval. The medial separation of the creeping welts in *C. antalba* and *C. vivipara* are possibly an early developmental stage towards the extreme separation of the welts into prolegs with "crochets", as seen in mature larvae of *C. inversa* and *C. floridana* (described below). If hosts of *C. antalba* and *C. vivipara* are ever found, perhaps the third instar larvae will have these proleg structures. It would be extremely interesting to eventually obtain and study first instars of *C. inversa*, in order to trace the development of the creeping welts and the mandibles (e.g, are they serrate?).

Larval Morphology

Third instar larvae of 3 species were studied with SEM and compound light microscopy. They were *C. inversa* (from New York and Ohio), *C. floridana* (from Bermuda), and a species (perhaps *dracula*) from the Southwestern Research Station, Portal, Arizona, collected by Tom Eisner on cercopids feeding on the composit *Grindelia aphanictis* (see Host Table, below). Below is a description of common attributes, with differences peculiar to each species also noted.

Description: Amphipneustic, typical of Muscomorpha. Anterior spiracles with 3 filaments each (fig. xx). Head much smaller (relative to thoracic segment 1) than in saprophagous species (figs. xx, cf. figs.xxx). Antenna and maxillary palp complex typical of all drosophilids; apparently highly conserved (fig. xx). Labial lobe lost. Oral cavity small, round. Triangular lobe just lateral to maxillary palp, with small sensilla near apex (fig. xx). Oral lamellae ("oral ridges" sensu Teskey [1981]) reduced to 3 transverse rows with long, thin fringes anterior to oral cavity (figs. xxx)(not developed lateral to oral cavity, nor converged into oral cavity, as in saprophagous drosophilid larvae [figs. xxx]). Figs. xxx show 5 genera of drosophilid larvae, illustrating the more numerous oral lamellae and fringes in saprophagous larvae. Cephalopharyngeal skeleton reduced, with dorsal and ventral cornu, hypopharyngeal sclerite, parastomal bar, and dental sclerite (basal to mandible), all unsclerotized (figs. xxx). Pharyngeal ridges present, but without filter chambers. Mandible small, sharp, acutely hooked; with no teeth in *C. inversa* and Arizona sp. (figs. xx), with small basal-lateral tooth in *C. floridana* (fig. xx). Creeping welts on first 6 abdominal segments modified into prolegs, each with rosette of spinules forming a "crochet" (figs xxx). Each "crochet" with 20-25 flat, sharp, acutely hooked spinules; with 2 anterior rows pointed anteriad, posterior ones pointed posteriad (figs. xxx). Lateral to anus in *C. inversa* is pair of simple, bladder-like structures (fig. xx), not found in other two species examined. All 3 species with posterior spiracles on a telescoping trunk (figs. xx). Trunk c. twice the length of both everted spiracular tubes. Spiracle tubes separated, highly sclerotized, eversible (figs. xx). Spiracular plate with 3 spiracular openings to one side in mature larva (typical of Muscomorpha). Spiracular hairs reduced: not highly dissected and filamentous as in most drosophilines (figs. xxx); but short, scale-like, and

closely adpressed to rim of spiracular plate (figs. xxx). 3-4 ribs present under spiracular plate, giving it an "accordion" like appearance (fig. x).

In the course of searching for immatures of *Cladochaeta* at the Las Alturas field station near San Vito, Costa Rica, a larva was found associated with spittle bugs, belonging to a different family from Drosophilidae. The spittle bug is a large, black and red species, *Tomaspis inca*, common at the site on *Heliconia* and throughout Central America (figs. xx?). Adults were not reared from the larvae, but morphology strongly indicates the larvae to be a species of ephydrine ephydrid, possible even *Ephydra* (cf., Teskey, 1981; other refs). The larvae were never found attached to the spittlebug nymphs, but were creeping on the floor of the spittle mass. Four to 5 larvae were found in some spittle masses. Superficially, the larvae are very similar to *Cladochaeta*, suggesting preadaptation of the ephydrid larvae to living in spittle masses, and convergence of *Cladochaeta* larvae.

Similarities between the ephydrid larva and *Cladochaeta* are striking, in particular are the prolegs bearing crochets of 25 large spinules and several smaller ones (fig. xx). However, in the ephyrdrid there are 7 pairs of prolegs, and a medio-ventral one posterior to pair 7 (typical of *Ephydra*). In the ephydrid larva there is also a dorsomedial group of about 25 spinules at the base of the spiracular tubes (fig. xx), also typical of *Ephydra*. The spiracular tubes, like *Cladochaeta*, are long, bifurcate, eversible, and have a spiracular plate with flat, scale-like spiracular hairs (fig. xx). The head of the ephydrid larva is distinctive for the set of 9-10 transverse rows of lamellae; the anterior 4-5 rows bear elements resembling toothed scales (figs. xx).

In contrast to *Cladochaeta*, the ephydrid larva is definitely saprophagous. The lamellate oral ridges are well developed, with 12-13 horizontal rows feeding into the oral cavity, and 4 vertical rows flanking these (fig. xx). The lamellar filaments are dense, particularly above the oral cavity. These features indicate efficient filtering ability. The cephalopharyngeal skeleton is heavily sclerotized, including the cornuas (fig. xx). Mandibles are serrate, with a row of 3-4 teeth on both ventral edges of each mandible, and forming a scoop out of the ventro-apical surface of the

mandible (as in saprophagous forms) (figs. xx). Discovery of this larva and its habits allows important morphological comparisons with *Cladochaeta* and some inferences.

HOSTS AND PHENOLOGY

Hosts

Table 1 presents published and new, unpublished data on *Cladochaeta* hosts. Neotropical and Nearctic species are included. In all cases except one the hosts are known to be species of *Clastoptera*. For North America there are actually 9 other genera of cercopids, which are the following (numbers in parentheses are numbers of species): *Aphrophora* (), *Lepyronia* (), *Neophilaenus* (), *Paraphilaenus* (), *Philaenus* (), *Philaenarcys* (), *Philaronia* (), and *Prosapia* (). *Clastoptera* is by far the largest genus among the North American cercopids, with xx species. It is possible that the lack of host records with genera other than *Clastoptera* is due simply to the individual and species dominance of this one genus. There appears to be no difference in the variety and kinds of plant hosts used by *Clastoptera* and the other cercopid genera. Plant host use can be very restricted for some cercopid species (e.g., *Clastoptera arborina* Ball on *Juniperus virginiana*, and *Prosapia ignipectus* on roots of the little bluestem grass, ------ --------). Others are much less specific, but host use of the cercopids is restricted to plants of a particular growth form (e.g., perennial herbs, or shrubs, or grasses). Thus, the apparent restriction of *Cladochaeta* to *Clastoptera* does not seem to be mediated by association of *Clastoptera* with any particular kind or growth form of plant host, distinct from the other cercopids.

We believe that negative records encountered in the search of hosts (e.g, those cercopids on which *Cladochaeta* has not been found) are just as important to report as are the positive host records. The extent of negative records directly reflects on the confidence one has in the mono- or oligophagous nature of an insect. It is quite apparent that even though some cercopids are plentiful and readily available, they are not hosts to *Cladochaeta*. As well, there could be geographical and/or seasonal variation in host use, which is very difficult to document but suggested by some of the information given below.

Wheeler (1984) found no *Cladochaeta* on nymphs of *Clastoptera arborina* feeding on ornamental Juniper (*Juniperus chinensis*) in Pennsylvania. Howver, Kuenzi and Coppel (1985) reported finding larvae of a fly, "probably *Cladochaeta*" on nymphs of *Clastoptera arborina* feeding on red cedar (*Juniperus virginiana*) in Wisconsin. W.A. Palmer (personal comm. to DG) did not find *Cladochaeta* larvae in spittle masses of *Clastoptera xanthocephala* feeding on *Baccharis* in Texas (note, however, that Sturtevant did rear a male *Cladochaeta sturtevanti* from a cercopid on *Baccharis* in California, specimen cited above). Jorge Santiago-Blay (pers. comm. to DG) did not find any *Cladochaeta* larvae on nymphs of *Clastoptera lugubris* feeding on *Grindelia* in California, even though hundreds of spittle masses were examined (note, however, that Eisner found larvae on cercopid nymphs feeding on *Grindelia aphanactis* in southeastern Arizona; cited in Table 1). Baerg (1920) reported finding no *Cladochaeta inversa* larvae on nymphs of *Clastoptera proteus* (on dogwood), even though the larvae were common associates of *Clastoptera obtusa* (on alder)in the same immediate vicinity (note that Thompson et al. [1987] found *C. proteus* to be a host in Illinois -- Baerg did not indicate where the field work was done, presumably it was Ithaca, New York). Foote (unpubl.) found that even though spittle masses of the cercopid *Aphrophora cribrata* were common on white pine (*Pinus strobus*) in Kent, Ohio, no masses harbored fly larvae. Likewise, Bales and Furniss (1984) found no larvae of *Cladochaeta* associated with nymphs of the cone spittlebug, *Aphrophora canadensis* on Mugho Pine (*Pinus mugo*) in Idaho.

Ashburner (1981) cited Pipkin (1965, 1966) in saying that *Cladochaeta* may have a tropical niche that is much broader than just the use of spittle bug nymphs and/or their spittle. It is unclear if the larval breeding site of *Cladochaeta* are anything but inhabited spittle masses. Pipkin recorded that she occasionally captured Clastopteromyia adults while sweeping over fallen fruits and flowers. Her voucher collection at the AMNH and the Smithsonian Institution has been examined, and the only specimens to which she could have been referring are *Diathoneura* species (*albinota, opaca, superba* and relatives, and a few other species). The one anomalous record is that of Steyskal (1972), who reported breeding 5 individuals of *Cladochaeta nebulosa* out of 60 heads of *Bidens pilosa* (Compositae) collected in late November in Dundedin, south Florida. They

emerged approximately 2 weeks after the dried flower heads were collected. Re-examination of Steyskal's voucher specimens (in the Smithsonian Institution) shows that the species is actually [*C. floridana*]. *Cladochaeta nebulosa* is not known to occur in Florida. It is very unclear if the *Cladochaeta* larvae were feeding on the flower heads, as is assumed in the report, or if they emerged from puparia lodged in the flower heads. It is quite possible that *Cladochaeta* larvae, feeding on spittle bugs on the *Bidens* plants, crawled into the heads to pupate. *Cladochaeta inversa* pupae are always found cemented to the edge of a twig, near the apex, and are very cryptic.

Phenology

The most extensive phenological data taken thus far is by Foote, for *C. inversa* around the vicinity of Kent, Ohio. Table 2 shows the dates, numbers of nymphs examined and the number harboring larvae, and the number of larvae found. A range of 1-2 larvae were found per nymph, although 1 larva per nymph was most common (this was reported by Baerg [1920] for *C. inversa* larvae on *Clastoptera obtusa*, as well). Numbers of larvae of each instar per spittle mass were also recorded by Foote. There was apparently no indication that more first and second instar larvae occurred per spittle mass or per nymph compared to third instars; thus, an even larval distribution is due not to cannibalism, but probably to oviposition. Female *Cladochaeta* must selectively deposit and apportion their eggs among the available spittle masses (it is possible that resident larvae may cannibalize eggs placed in their spittle mass).

Collections of *C. inversa* in Ohio from the Fall always produced *Cladochaeta* larvae; Spring collections revealed none. It is possible that this is due to the smaller cercopid populations in the Spring (or just to the diapause pattern of the fly, presumably they would be cause and effect related). Even though many fewer spittle masses were censused in the Spring (total of 22) versus the Fall (166), it is unlikely that the absence of Spring larvae is due solely to sampling bias. For example, for any one Fall census the number of spittle masses was similar or much less than the Spring total (a range of 9-25), but a range of 8-21 larvae was found in all Fall censuses. Also, the

number of spittle masses harboring fly larvae almost always exceeded 50% during the Fall. Thompson et al. (1987) reported very low numbers of *Cladochaeta* larvae on *Clastoptera proteus* during late June and early July in Illinois and Michigan (about 3% of the nymphs with larvae--they stated, however, that "almost every *C. obtusa* spittle had associated *C. inversa* pupae", but did not indicate how many spittle masses were censused). It is most likely then that *Cladochaeta inversa* in Ohio and the other northern U.S. states has two generations per year: larvae breed in late Summer to early Fall ---> overwintering occurs as puparia or pharate adults, with probably considerable mortality ---> adults emerge in (mid/late?) Spring ---> mating and oviposition takes place, producing an early Summer larval generation ---> a second, much larger population of *Cladochaeta* larvae occurs in late Summer and early Fall, the offspring of the late Spring, overwintered adults.

Among 11 spittle masses of *Clastoptera* sp. (*undulata*?) sampled October 6, 1988 on *Casuarina* in Bermuda, 33 nymphs bore 8 *Cladochaeta floridana* immatures (larvae, sometimes puparia) (an infestation rate of about 25%). No nymph hosted more than 1 larva (D. Hilburn, pers. comm.). It is interesting to note that Bennett and Hughes (1963) found *Clastoptera undulata* populations on Bermuda from late August to early November to be higher than in the preceding months by a factor of almost 3-fold. If the larvae are indeed a mortality factor for the cercopids (say, even 10%) or simply impose stress (causing reduced size, fecundity, etc...), this association must then be a considerable selection pressure for the cercopids. The phenological data also indicates that an important source of host use variation can be seasonality.

INQUILINE OR PARASITE?

One question that immediately comes to mind with regard to the *Cladochaeta-Clastoptera* association is: Are *Cladochaeta* larvae just inquilines, are they parasites, or sometimes one and sometimes the other? Baerg (1920) found that for only 1 nymph out of about 100 was there any visible sign of injury from an attached nymph ("bruised" along the side of the abdomen). Baerg (p.

21) concluded that that the fly larva "apparently feeds on plant sap in the form of spittle produced by the *Clastoptera*." Williams (1922) came to the same conclusion, but Bennett (1965, p. 98)) stated about Williams' work that "later, when working with *Clastopteromyia paradoxa* in the froth of *C. taeniata* in Trinidad, [Williams] stated that this species 'undoubtedly kills some of the nymphs.'" Wheeler (1952) believed that *Cladochaeta inversa* was ectoparasitic and stated that "the larvae feed directly on the *Clastoptera* nymphs; in examining spittle masses for larvae they were invariably found lying on the abdominal dorsum of the nymph with the mouthparts inserted between two adjacent tergites, usually the third from the rear. The posterior spiracles are surrounded by a large bubble. We were never able to rear larvae to adults when the nymphs were removed from the spittle masses." Our observations agree with those by Wheeler. The larvae cling so tenaciously to their host, that often they could not be dislodged even when the nymph was immersed in ethanol for preservation.

Host Injury

Bennett (1965) reported finding no evidence of integument injury (e.g., holes or tears in the intersegmental membrane, or melanized spots) on *Clastoptera* nymphs hosting *Cladochaeta* larvae in Puerto Rico, Jamaica, and Trinidad. This contrasts with the situation we have found for *C. inversa* in New York. Nymphs of *Clastoptera obtusa* were examined on speckled alder at Black Rock Forest, Orange Co., New York, on 12 September, 1990. Nymphs with and without fly larvae were examined at 60x magnification for integumental injuries, and these injuries were even more closely examined under the scanning electron microscope. Very small melanized spots were found on nymphs hosting larvae, which were easily overlooked at low magnifications. Fig. xx shows the dorsal and ventral views of *Clastoptera obtusa*. Fig. xx is the dorsal surface of a *C. obtusa* abdomen, from which several spots were greatly magnified (figs. xxx). Injuries caused by the larvae are all holes, but varying in structure. Some were holes with irregular, diffuse edges, where the integument was seemingly abraded very thin. Other holes were surrounded by irregularly

shaped feeding tubes (fig. xx). Finely shredded regions of integument had caused other melanized spots (fig. xx). Mature nymphs with black spots on the dorsum (never on the ventral surface) of the abdomen were routinely found, occasionally they did not even have a larva attached to them. Table xx shows data on nymphal stage, size, and the number of black spots found per individual, for nymphs found with and without larvae. (A complicating factor is that, even though a nymph was found without a larva attached or even in the spittle mass, it is possible that at one time it hosted larvae). The data indicate that nymphs hosting *Cladochaeta* larvae incur more injuries [describe means and ranges briefly]. A few nymphs seemingly without larvae did have melanized spots; perhaps these nymphs at one time hosted larvae that had pupated on a concealed part of the branch. Injury by larvae is hardly conclusive evidence of parasitism, for it must be shown that injury has a deleterious effect on development, adult size, reproduction, and/or survivorship. One certainty does emerge, however: *Cladochaeta inversa* larvae make holes in the integument of their hosts, and embed their heads there. They probably feed on hemolymph.

Host Survivorship

In an attempt to experimentally prove if *Cladochaeta* is parasitic, Bennett (1965) took eggs and various instar *Cladochaeta* larvae and supplied them with fresh spittle but without nymphs. He found that older larvae readily pupated on just spittle, but that only 2 out of 10 eggs developed to the pupal stage. Numbers of tested larvae were not given but were apparently quite small, and the small experiment was not done in replicates nor with a control. This is a preliminary indication that at least older larvae may not require cercopid hemolymph in order to pupate. However, other measures of fly development are more meaningful to study for such an experiment, since death in the pupal and pharate adult stages is a very common form of inviability in *Drosophila*, and in fact third instar larvae of many *Drosophila* species will readily form a puparium (but will not metamorphose) if starved. It would be best to measure survivorship of larvae to the adult and size of the adult, compared between sets on *Clastoptera* nymphs with and without spittle, and a set with

just spittle (ideally, fertility would also be compared). Also, the fact that the older fly larvae can pupate with just spittle does not rule out deleterious effects that they have on the host nymph. Thus, development time, survivorship of nymphs to the adult, and adult size should be measured for cercopid nymphs with and without *Cladochaeta* larvae.

Two small studies were done by Foote to test whether *Cladochaeta inversa* larvae could develop solely on spittle and what effect larvae may have on the nymphs. In one experiment 10 second instar larvae were provided solely with spittle. Within 1 to 3 days they wandered from the spittle, even when it was supplied fresh, and all died within one week. Only one larva had formed an undersized and inviable puparium. These results suggest that larvae search for another nymph when displaced from their original one, only because they cannot subsist on spittle. In the other study, 10 nymphs each had a third instar larva attached to it and were placed in petri dishes (NB: significant nymphal mortality probably caused by removal from plant host; no control? e.g., nymphs without larvae, to see what the "baseline" nymphal mortality is in the lab). Within 1 to 2 days, 7 nymphs were dead with live larvae or puparia; the other 3 nymphs were still alive and 2 of them had a mature larva and a puparium. These results suggest, in lieu of an experimental control, that larvae can cause the mortality of *Clastoptera* nymphs.

Larval Diet

That cercopid spittle can be the major or even sole dietary component of *Cladochaeta* larvae is suggested by the comprehensive study of spittle composition by Wilson and Dorsey (1957). Cercopid spittle issues from the anus of the feeding nymph and is formed from excess sap acquired from the host plant. It reduces desiccation and possibly parasitism and predation. Wilson and Dorsey reported that Licent (1912) "found that cercopid spittle contained more water (99.4 vs. 94.57%), less organic material (0.14 vs. 3.83%) and less inorganic matter (0.38 vs. 1.60%) than the associated plant sap." Wilson and Dorsey reported that the spittle masses of the meadow spittlebug (species?) contained a few amino acids and some sugars, the pH is basically neutral,

having averaged 7.26 and ranged from 6.15 to 7.81 (n = 23). Marshall (1966) reported that not just sugars, but a mucopolysaccharide is secreted by the nymph (deriving from the malpighian tubules, the substance probably serving to reduce surface tension and stabilize the bubbles). Marshall reported that after hydrolysis of the polysaccharide, very small quantities of glucuronic acid, glucosamine, glucose, rhamnose, and proteins occured in the spittle. With a medium of such composition it is of little surprise that Wilson and Dorsey found bacteria thriving in it (at concentrations of 18.4 to 108.4 millions/ml of spittle, average of 59.4 millions; N = 12 samples). The bacteria were almost all gram negative rods, belonging to the genera *Flavobacterium* and *Achromobacter*. Bacteria and yeasts are among the foodstuffs ingested by saprophytic *Drosophila* larvae, from mycophagous to cactophilic and frugivorous species. The fact that spittle supports microorganismal growth and contains some basic ingredients for larval development is one reason why Ashburner (1981) concluded that it should "provide food for the development of a drosophilid." Perhaps the easiest way to test this conclusion is to examine the gut of *Cladochaeta* larvae: is it filled with hemolymph cells of its host?

If, as Licent (1912) reported, the spittle is not as rich in nutrients as the plant sap from which it is derived, then why would *Cladochaeta* evolve a use of the spittle? The spittle may simply be in such copious supply compared to sap flows, and the female *Cladochaeta* probably without any means to injure a plant to cause a sap flow. The problem with this explanation is that there are many acalyptrate flies that breed solely in the sap fluxes of trees (*Aulacigaster* [Aulacigastridae], *Stenomicra* [Anthomyzidae], *Chymomyza*, and various *Drosophila* species [Drosophilidae]), so an obligate, specialized sap flux guild has evolved, and *Cladochaeta* is not among them. The transition to a spittle-inhabiting larva need not have been via sap feeding, though, as suggested by anecdotal evidence from other drosophilids.

Wheeler (1991) reared *Scaptomyza pallida* (identification by DG) from spittle masses of the cercopid *Lepyronia coleoptrata* on the introduced weed legume, crown vetch (*Coronilla varia* L.) in Pennsylvania. Larvae were found inside the spittle masses and eggs were beneath the spittle, suggesting that females were actually ovipositing in the spittle. Larvae were not attached to the

nymphs but crawling through the spittle mass. The spittle mass was serving either as a refuge or larvae were feeding on debris and microorganisms in the spittle. *Lepyronia* is a widespread, abundant, polyphagous cercopid, feeding on various weeds including Canada thistle (*Cirsium arvense* L.). *Scaptomyza pallida* larvae were also found in spittles of *L. coleoptrata* on thistle. The intriguing aspect of these rearing records is that *S. pallida* is a leafminer, typical of the entire genus, albeit this species is extremely polyphagous and even is occasionally found in rotting vegetation. In many places in North America, particularly disturbed and weedy habitats, *S. pallida* can be by far the most abundant fly. High population densities should presumably lead to the use of all sorts of oviposition substrates in the immediate habitat, including, as in this case, spittle. Another example of larvae dwelling, presumably facultatively, in spittle masses is *Drosophila* (*Sophophora*) *azteca*. DG examined a series of 4 males and 3 females collected from California (San Francisco, Lobos Creek, VII/20/62, W.E. Kelson), which "emerged VII/24/62, reared from spittle bug." The labels gave no indication of the kind of spittle bug or its host plant. *Drosophila azteca* belongs to the *obscura* species group, which are very abundant flies in Holarctic forests of cooler climates and higher altitudes. Some species appear very polyphagous, but the main breeding sites of all species remain largely unknown. Lastly, Odhiambo (1957) reported having bred a species of *Leucophenga* (Drosophilidae: Steganinae) from *Ptyelus flavescens* F. (Cercopidae) on the legume tree *Millettia* in Uganda. Sguy (1932) originally reported this habit in a fly from Madagascar, found also in association with *Ptyelus*, and which he described as *Ptyelusimyia decaryi* (this genus has now been synonymized with *Leucophenga*; unfortunately, we cannot tell if Odhiambo's specimens are a different species than Sguy's -- even though Odhiambo mentioned that specimens were originally deposited in the Univ. Texas collection, they are not there and their identity can not be checked). A *Leucophenga* species, perhaps *L. decaryi*, has also been bred from *Ptyelus grossus* spittle masses in Nigeria (J. Deeming, M.C. Dike, pers. comm. to DG). These records, plus the record of the ephydrid larva discussed earlier, indicate at least 5 independent (some being facultative) invasions of spittle masses by various ephydroids, including *Cladochaeta*. The transition from being a saprophagous larva to an inquiline is apparently not a

major barrier.

Perhaps the best evidence for the parasitic nature of *Cladochaeta* larvae comes from comparative morphology. Several structures adapt *Drosophila* larvae to filter the microorganisms from their decaying, liquified host medium, which are lamellate oral ridges and a system of fine canals on the floor of the pharyngeal sclerite (Dowding, 1967). small, sharp mandibles of the *Cladochaeta* larvae can be construed as evidence mostly in favor of parasitism rather than inquilinism. They are obviously used for piercing the intersegmental membranes between abdominal tergites of the cercopid nymph. Apparently the 6 pairs of pseudopods with crochet-like hooks adapt the larva for attachment to its host, so the mandibles would not be all that useful for attachment. Also, mandible structure suggests the larva would be entirely ineffective in shoveling spittle into the oral cavity, wherein microorganisms would be strained out. The additional finding of an ephydrid species in Costa Rica (described above), with larvae that live in spittle masses, do not adhere to the host, and have a typical saprophagous oral morphology, indicates that saprophagy in spittle masses is behaviorally and morphologically distinct from what is seen in *Cladochaeta*.

CONCLUSIONS

The Drosophilidae are among the most ecologically diverse Diptera in terms of their breeding habits: there are species that breed in decaying fruits, in living leaves (as miners) and decaying leaves and stems, in highly toxic mushrooms, in rotting cacti, in living flowers, on the nephric exudates of land crabs, as predators of frog eggs, and as predators of sessile homopterans, blackfly larvae, and even spider eggs. The saprophytic habit is undoubtedly ancestral and by far most common in the family, which makes habits such as spittle-bug parasitism and even just inquilinism of such unusual interest. The breeding sites of very few *Cladochaeta* are known, and the independent development of spittle bug association in the Drosophilidae suggests that perhaps the habit may not be uniquely derived within the genus *Cladochaeta*. This can be tested with the distributions of other derived, morphological characters.

Are the cercopid-breeding species of Cladochaeta a monophyletic group? Relationships of the cercopid-breeding species of Cladochaeta, and the ancestral host of the group. Do we know enough about the non-cercopid-breeding species to infer an ancestral host? If we know enough, can we speculate on the steps in such a dramatic (assuming normal, fruit or flower breeding ancestry) host shift?

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TABLE 1
Host Records for Species of *Cladochaeta*

Host nymph (*Clastoptera* sp.)	Plant	Locality	Reference
	Cladochaeta paradoxa (Lamb)		
sp.	*Casuarina*?[1]	Trinidad	Lamb, 1918
taeniata	*Casuarina* sp.	Trinidad	Williams, 1922
	Cladochaeta floridana (Malloch)		
sp. (*undulata*?)	*Casuarina* sp.	Bermuda	Hilburn, here
sp. (*undulata*?)	*C. equisetifolia*	Florida	Wheeler, here
no cercopid mentioned	*Bidens pilosa*[2]	Florida	Steyskal, 19xx
	Cladochaeta inversa (Walker)		
achatina	*Carya glabra*[3]	Ohio	Foote, here
obtusa	*Alnus "americana"*[4]	New York?	Baerg, 1920
	Ostrya virginiana	Michigan	Thompson et al., 1987
	Alnus sp.	New York	Valley, here
	Alnus sp.	Pennsylv.	Valley, here
sp. (prob. *obtusa*)	*Alnus serrulata*	Ohio	Foote, here
proteus	*Cornus* spp.	Illinois	Thompson et al., 1987
sp.	*Corylus* sp.	Ohio	Foote, here
	Cladochaeta sturtevanti Wheeler and Takada		
lineaticollis	unknown	California	Sturtevant, here
spp.	*Artemisia* sp.	California	Sturtevant, here
	Baccharis sp.	California	Sturtevant, here
	Lepidospartium sp.	California	Sturtevnat, here
	Senecio douglasii	California	Sturtevant, here
	Cladochaeta spp. undetermined:		
	paradoxa?		
spp.	*Flacourtia indica*	Trinidad	Bennett, 1965
	Casuarina equisetif.	Trinidad	Bennett, 1965
	paradoxa/"nebulosa"?		
sp. nr. *diminuata*	*Coffea arabica*	Pto. Rico	Bennett, 1965
	Hibiscus rosa-sinensis	Pto. Rico	Bennett, 1965
flavidorsa	*Casuarina equisetif.*	Jamaica	Bennett, 1965
	probably *inversa*		
arborina	*Juniperus virginiana*[5]	Wisconsis	Kuenzi & Coppel, 1985

sp. (**dracula**?)

sp.	*Grindelia aphanactis*[4]	Arizona	Eisner, here

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[1] Lamb, 1918 indicates the cercopid plant hosts to have been cacao trees, but in an original reprint annotated by Lamb, cacao is crossed out and "*Casuarina*" is written in. Also, Bennett (1965) cited this record as being *Clastoptera theobromae*, but there is no mention in Lamb's paper to that species.

[2] This record actually refers to *Cladochaeta nebulosa* (Steyskal, 19xx), which does not occur in Florida. Examination of voucher specimens ------. Steyskal made no mention of spittle bugs among the insects he reared from the *Bidens* flower heads.

[3] This record is based on the presence of larvae and puparia in and near the spittle masses of this species, not on rearings of adults.

[4] The species name *Alnus* "*americana*" does not appear to be valid; as to what species this record actually represents is uncertain.

[5] Records based solely on larvae.

TABLE 2

Phenology of *Cladochaeta* Larvae on *Clastoptera* in Ohio

Date of Collection	Number of Nymphs	Number of Larvae	Infestation Rate (%)
VIII/25/83	23	9	39
VIII/26/83	21	12	57
VIII/30/83	25	15	60
IX/ 5/83	16	9	56
VIII/24/84	24	14	58
VIII/28/84	9	8	89
VI/21/85	2	0	0
VI/26/85	5	0	0
VI/27/85	5	0	0
VIII/ 8/85	13	8	62
VIII/17/85	25	21	84
VI/10/86	10	0	0

Figure Legends

Figs. xxx. Early immature stages of Cladochaeta, also showing viviparity. **A.** Egg of *Cladochaeta dikra*. **B.** First instar larva in abdomen of *C. vivipara*. **C.** First instar larva in abdomen of *C. telescopica*. **D-E.** Heads (lateral view) of first in-utero first instar larvae. **D.** *C. glapica*, also showing first two creeping welts. **E.** *C. vivipara*, with creeping welt 4. **F.** *C. antalba*.

Figs. xxx. 3rd instar larva of *C. inversa*. **A.** Larva, lateral view. **B.** Detail of head. **C.** Paranal sacs. **D.** Maxillary palp complex. **E.** Keilin's organ, thoracic segment 2.

Figs. xxx. Mouthparts of *Cladochaeta*. **A.** Head of 3rd instar *Cladochaeta floridana*, showing cephalopharyngeal skeleton and oral lamellae. **B.** 3rd instar mandibles of *C. floridana*, *C. inversa*, and a typical saprophagous species, xxxx, for comparison. **C.** *C. inversa* mandibles situated in oral cavity, with muscle attachment. **D.** Cephalopharyngeal skeleton of xxxx, a saprophagous species for comparison. **E.** Cephalopharyngeal skeleton of *C. inversa*.

Figs. xxx. Scanning electron micrographs of heads of 3rd instar larvae of representative saprophagous drosophilids. **A.** *Drosophila bromeliae*. **B.** *D. bromeliae* (ventral). **C.** *D.* (*Sophophora*) *melanogaster*. **D.** *D.* (*Drosophila*) *immigrans*. **E.** *Drosophila mimica*. **F.** *Hirtodrosophila pictiventris*. **G.** *Scaptodrosophila stonei*. **H.** *Zaprionus ghesquierei*. **I.** *Chymomyza procnemis*.

Fig. xx. Scanning electron micrograph of 3rd instar larva of *Cladochaeta floridana*, lateral view. Curled repose is typical of live larva, but integument is crumpled in this preserved specimen.

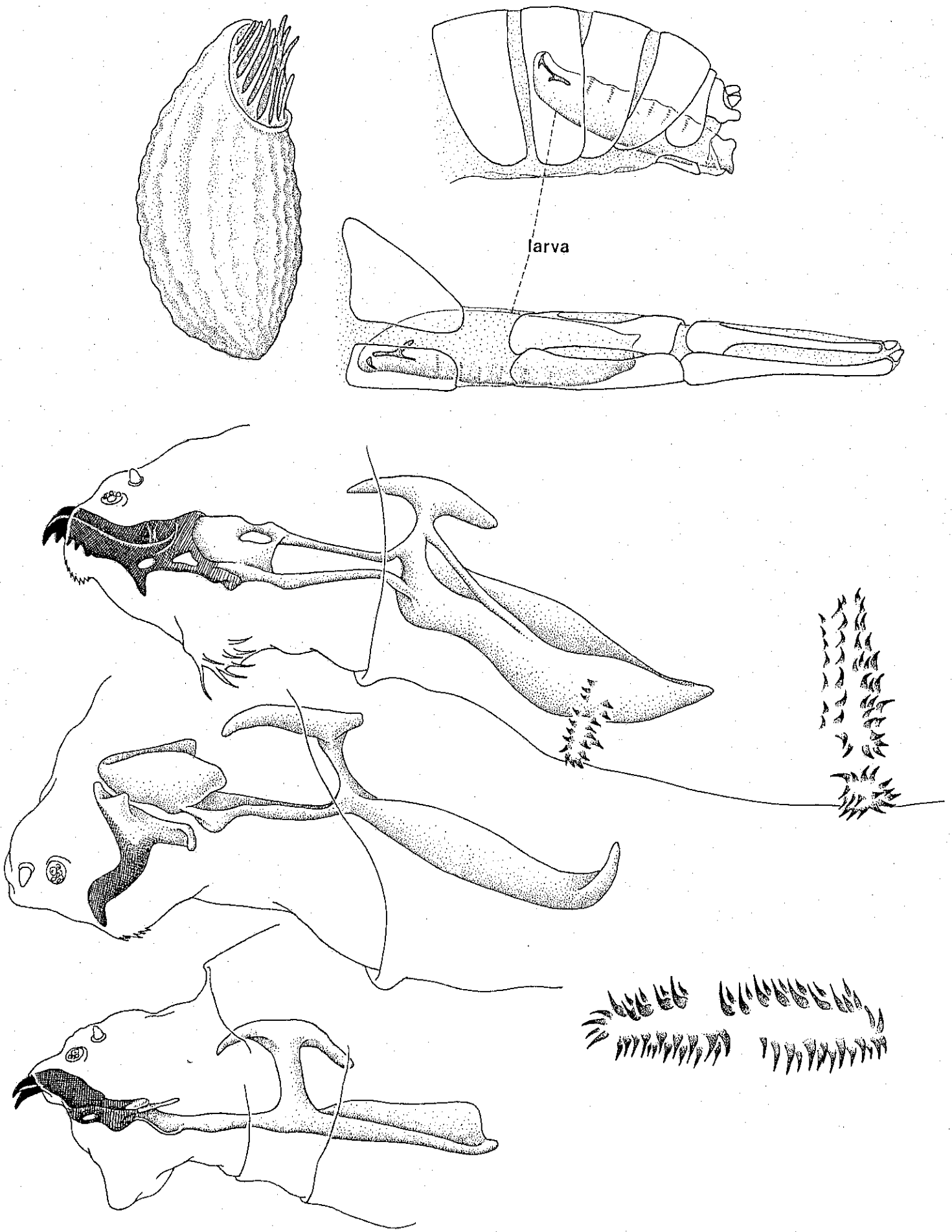
Fig. xxx. Scanning electron micrograph details of *C. floridana* 3rd instar. **A-C.** Various prolegs, showing "crochets" or rosettes of creeping welt spinules. **D.** Apex of posterior spiracle, showing spiracular disk, openings, and leaf-like spiracular "hairs."

Fig. xxx. Scanning electron micrographs of posterior spiracles of 3rd instar larvae of representative saprophagous drosophilids. **A.** *Drosophila* (*Dorsilopha*) *busckii*. **B.** *Drosophila bromeliae*. **C.** *D.* (*Sophophora*) *melanogaster*. **D.** *Zaprionus ghesquierei*. **E.** *Scaptodrosophila stonei*. **F.** *Drosophila mimica*. **G.** *Chymomyza procnemis*. **H.** *Hirtodrosophila pictiventris*. **I.** *Scaptomyza adusta*.

Fig. xxx. Scanning electron micrographs of ephydrid larva from Costa Rica, found in spittle masses of *Tomaspis inca*. **A.** head, oblique anterior view. **B.** Maxillary palp complex. **C.** Cephalic creeping welt spinules. **D.** 2nd proleg. **E.** Posteriormost ventro-median proleg. **F.** Posterior group of dorso-medial spinules. **G.** Posterior spiracles. **H.** Detail, apex of posterior spiracle.

Fig. xxx. Head, ventral view, of ephydrid larva from Costa Rica, found in spittle masses of *Tomaspis inca*, showing details of cephalopharyngeal skeleton and oral lamellae.

Fig. xxx. *Clastoptera* hosts and injuries. **A,B.** *Clastoptera obtusa*, dorsal and ventral. **C.** *C. obtusa*, tergites. Arrows point to melanized spots that have higher magnification SEM shots. **D.** detail of spot in C. **E.** detail of spot in C. **F.** detail of spot in C.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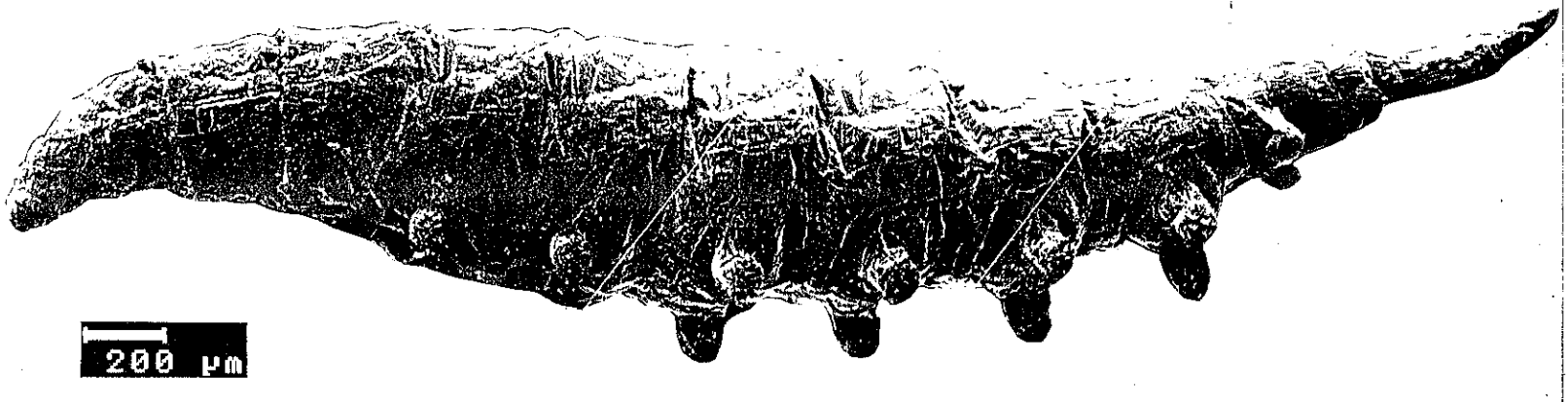
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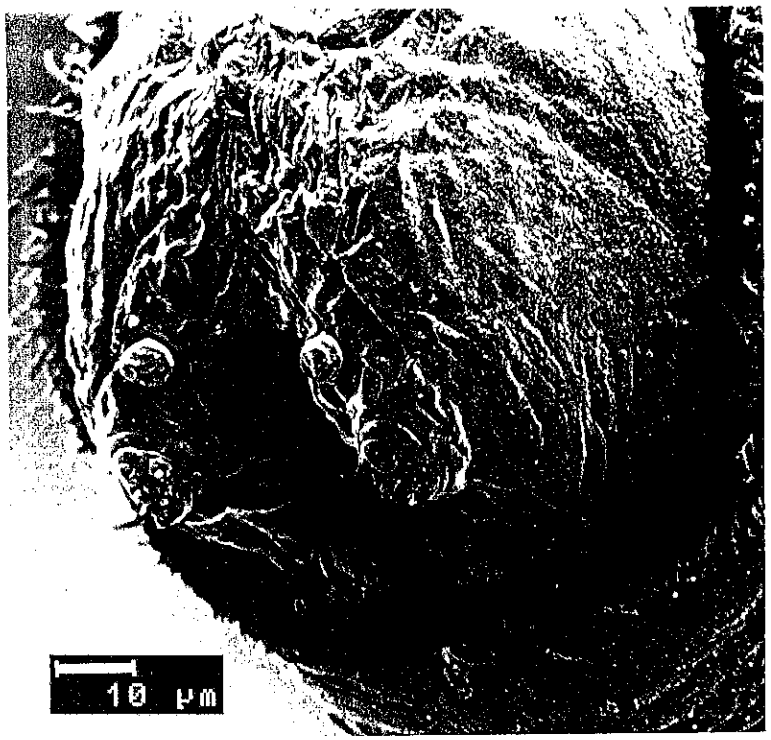
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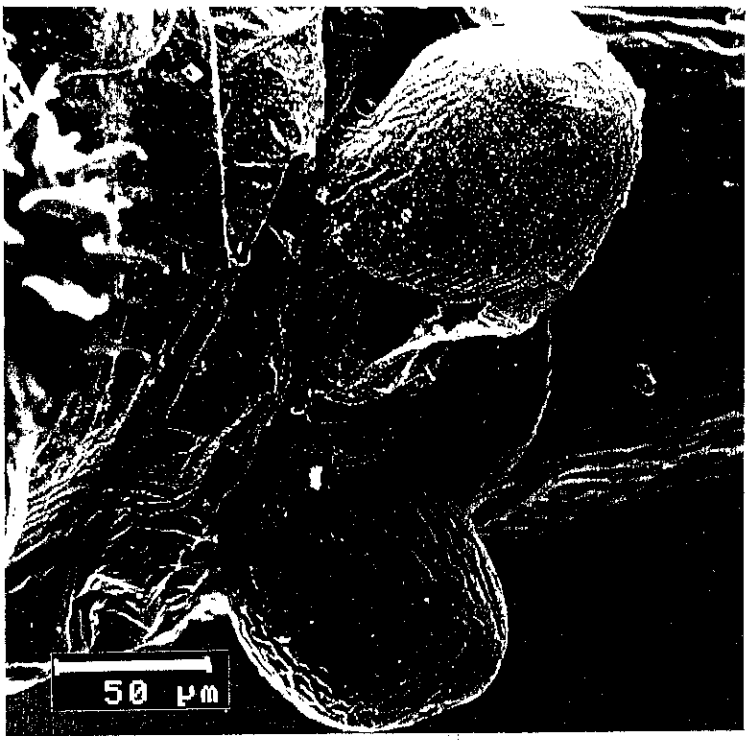
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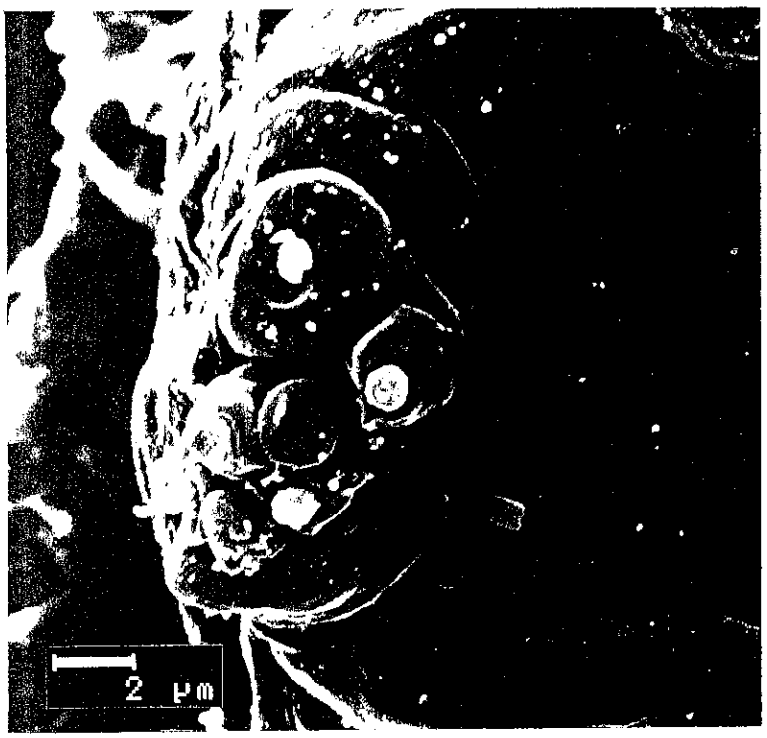
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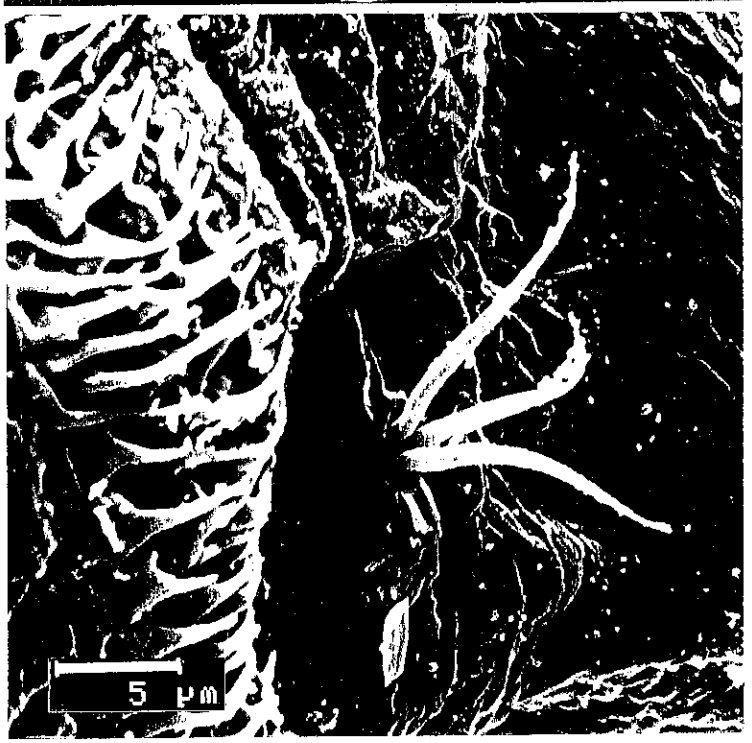
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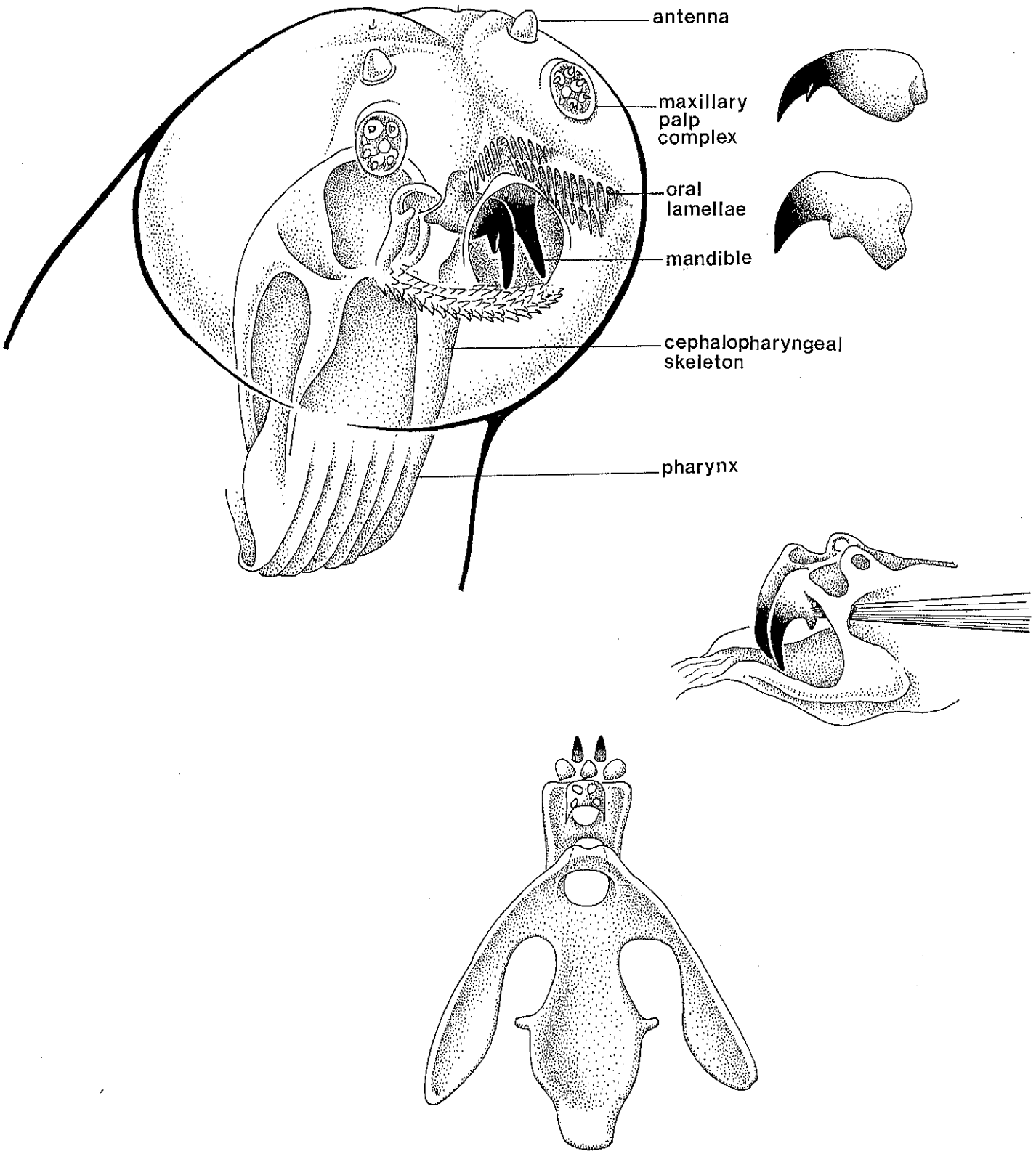
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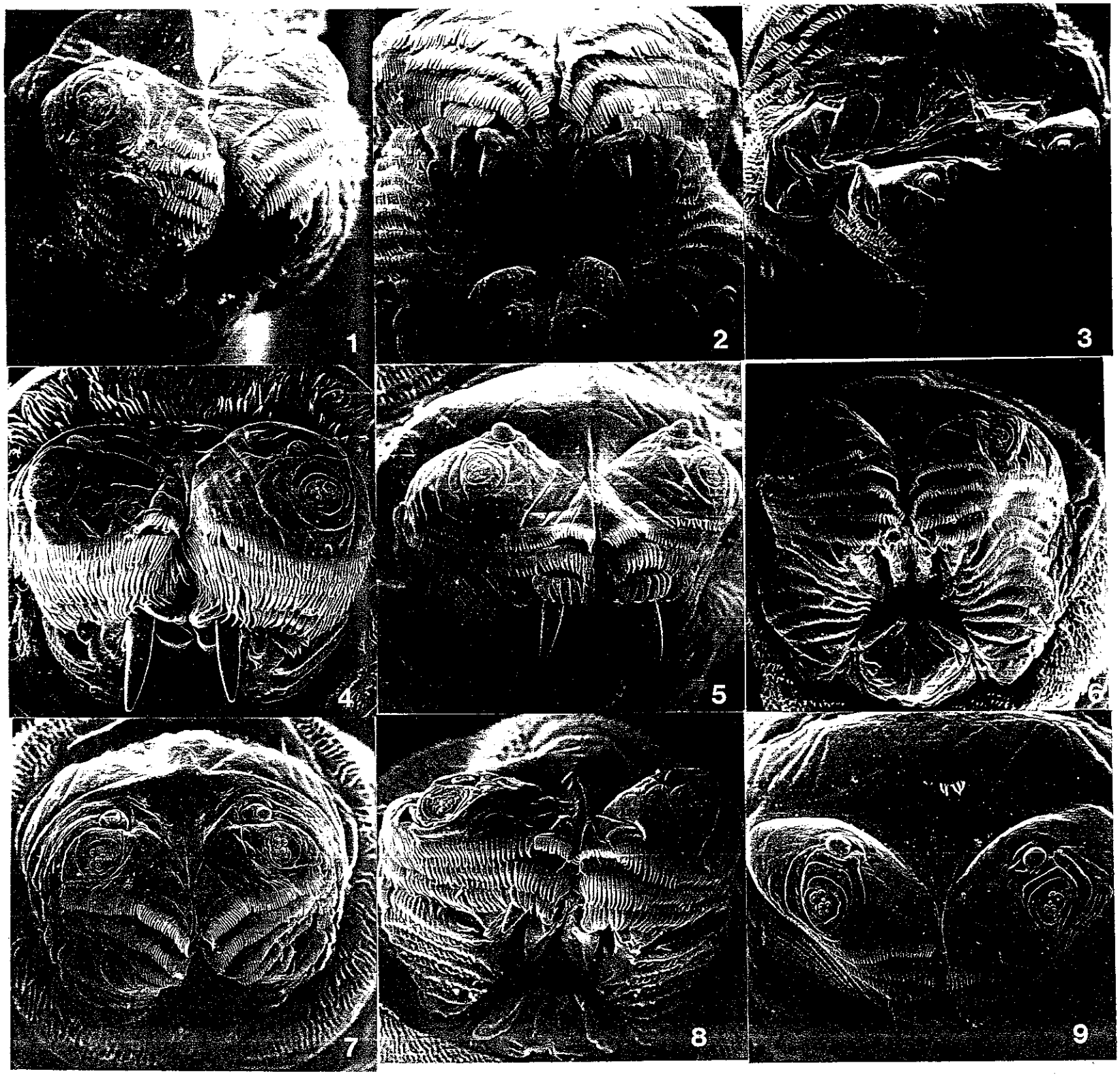
oral lamellae
mandible
cephalopharyngeal skeleton
pharynx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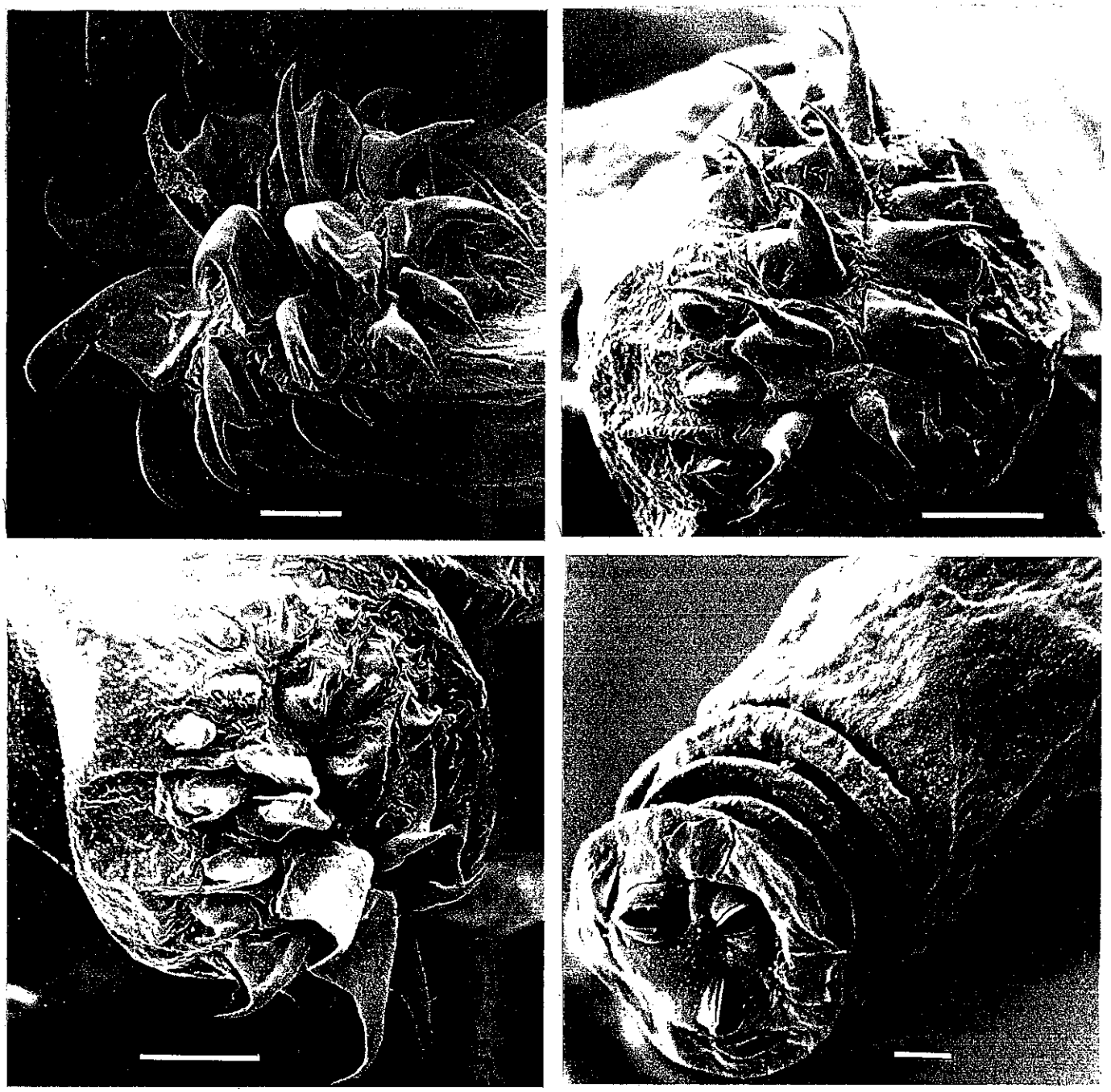
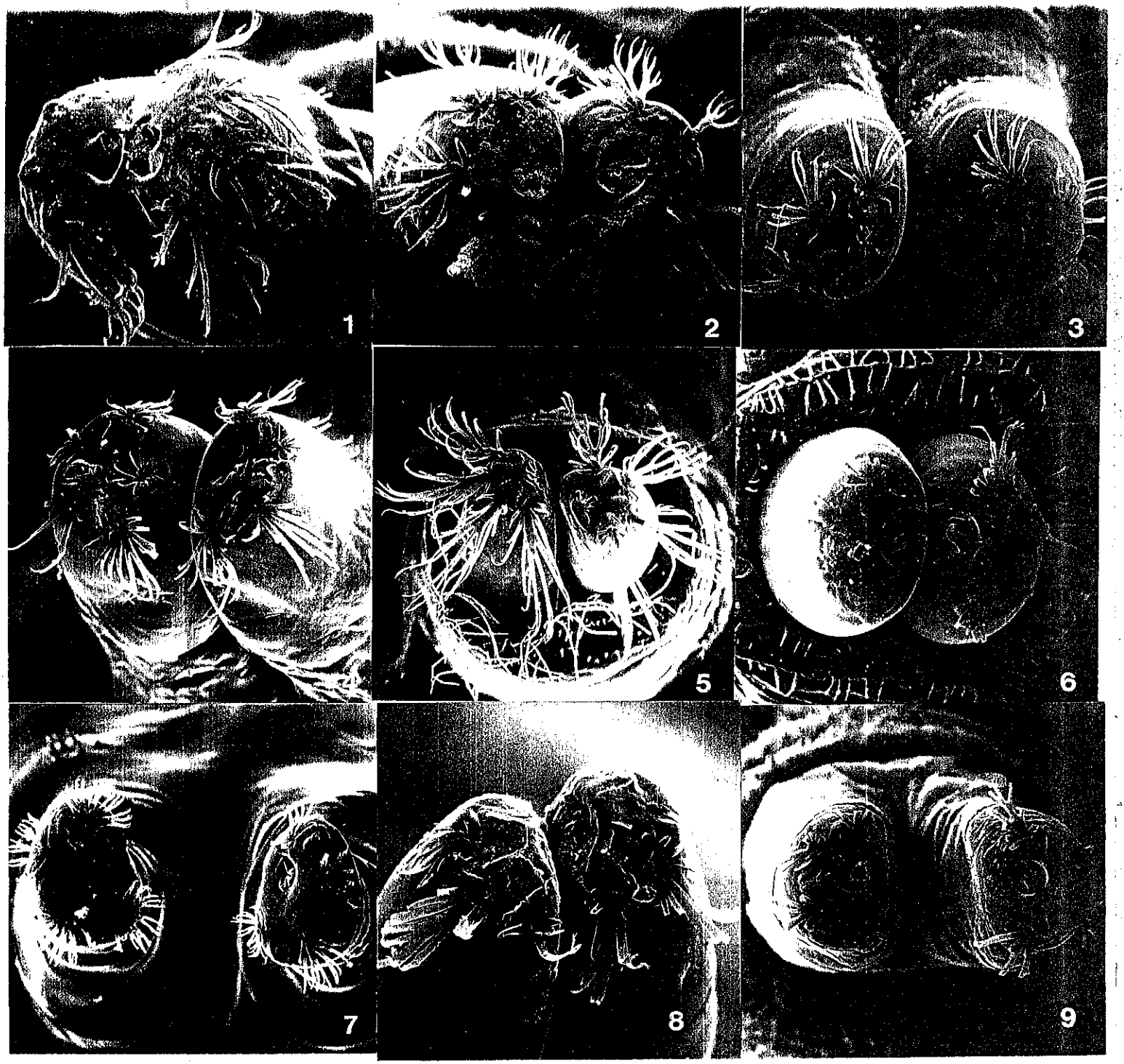
100 µm

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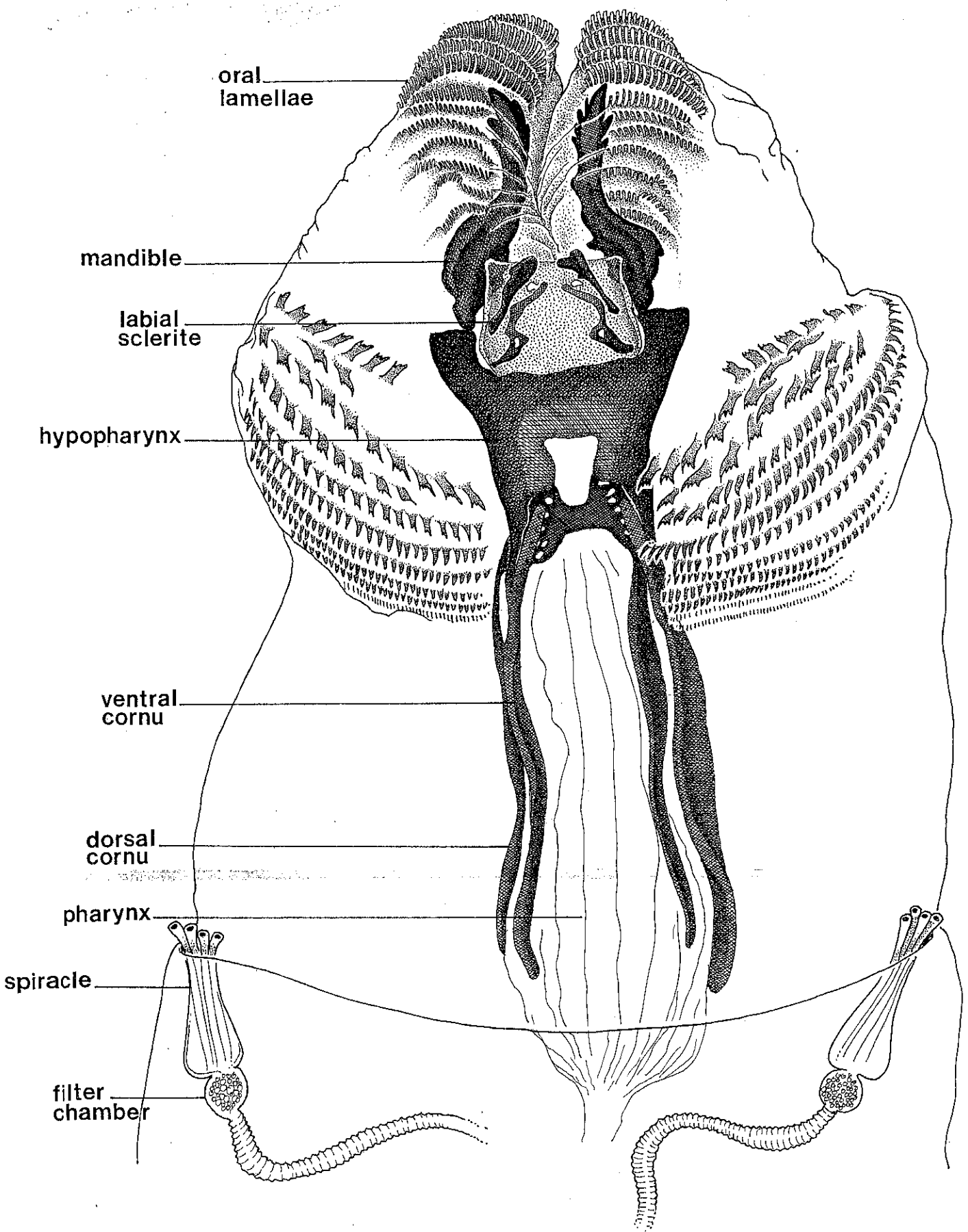
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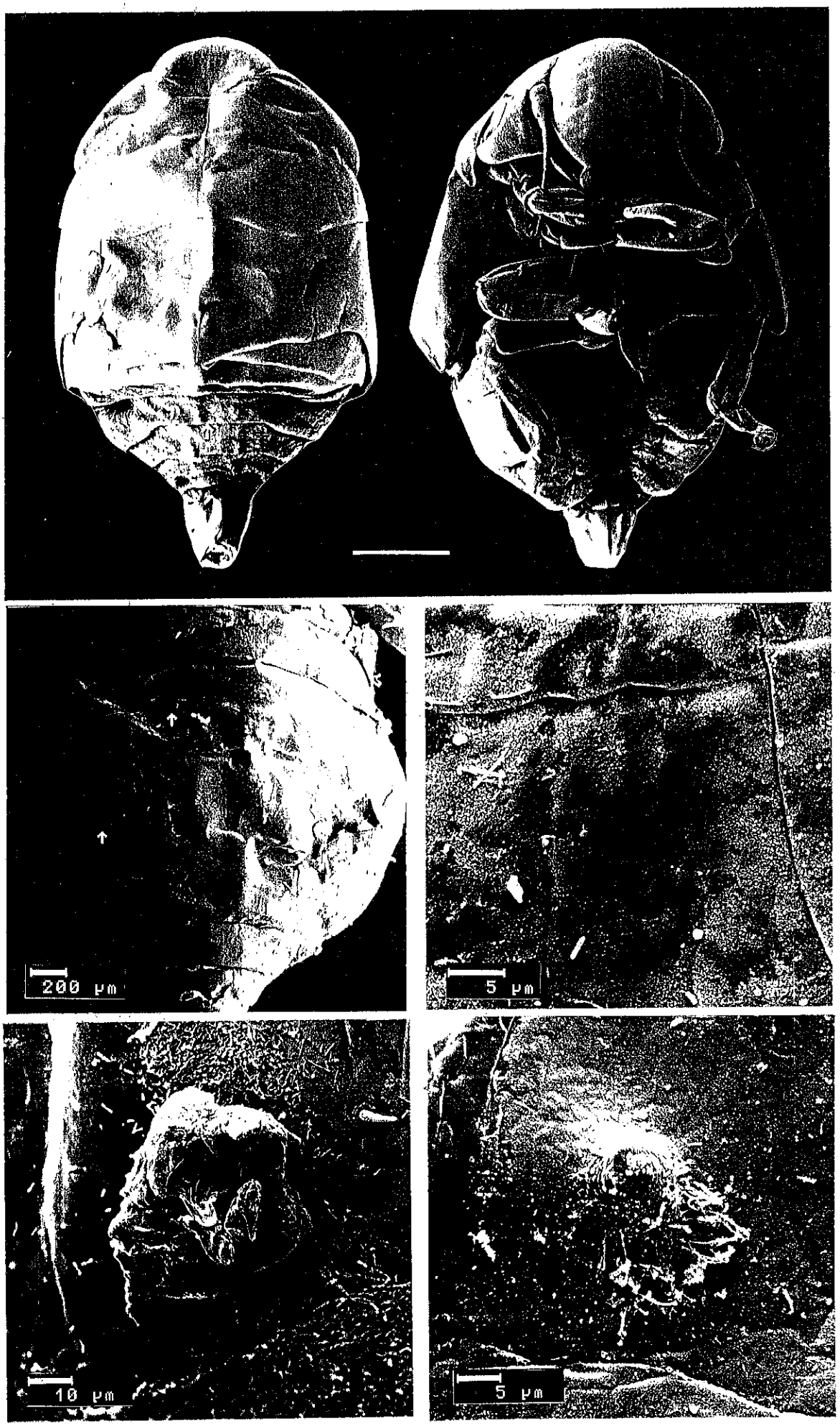
pharynx
spiracle
filter chamber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5 µm
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