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A Comparative Study of the Egg, First-instar Larva, Puparium, Female Reproductive System and Natural History of *Curtonotum helvum* (Curtonotidae; Ephydroidea; Diptera)

RUDOLF MEIER,[1] MARION KOTRBA,[2] AND KEVIN BARBER[3]

ABSTRACT

Curtonotum helvum (Loew) (Curtonotidae, Diptera), the only Nearctic representative of the Curtonotidae was reared in the laboratory from a decaying grasshopper egg pod. *Curtonotum helvum* can be collected from mid-June to September throughout its wide Nearctic range. In a study of a population on the southern coast of Lake Huron, the species was found to be crepuscular and possibly even nocturnal. Copulations were only observed at dusk. In captivity the flies readily consumed coyote dung, sand cherries, and insect carcasses, but failed to oviposit on any of these substrates. The female reproductive system is characterized by two sclerotized, scimitar-shaped spermathecae and an anteroventral pouch arising from the vagina. The ventral receptacle is short and tubular with a sclerotized tip. The anteroventral pouch is also present in the remaining two genera of the family (*Axinota* Wulp, *Cyrtona* Séguy) and likely constitutes a new synapomorphy of the Curtonotidae whereas the two spermathecae are a groundplan feature of the Ephydroidea. First descriptions of curtonotid eggs are provided. The eggs of *Curtonotum helvum* and those of *Axinota uniformis* (Malloch) bear longitudinal ridges, have a cup-shaped micropyle, and aeropyles at the anterior pole. They are strikingly similar to the eggs of ephydrids and dissimilar to drosophilid eggs. The larva of *Curtonotum helvum*

[1] Curator and Associate Professor, Zoological Museum Copenhagen Ø, Universitetsparken 15, 2100 Copenhagen, DENMARK.
[2] Curator, Museum für Naturkunde, Humboldt Universität Berlin, Invalidenstraße 43, 10115 Berlin, GERMANY.
[3] Research Scientist, Canadian Forest Service, Sault Ste. Marie, P.O. Box 490, Sault Ste. Marie ON, P6A 5M7, CANADA.

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is more similar to the larvae of Drosophilidae than to those of Ephydridae. The puparium of *Curtonotum helvum* has all anal tubercles that also belong to the groundplan of the drosophilid puparium. At present, not enough comparable information is available from the other families of Ephydroidea for a satisfactory phylogenetic evaluation of this character information.

INTRODUCTION

Curtonotum helvum (Loew) is the only Nearctic representative of the small dipteran family Curtonotidae (Ephydroidea). Currently the family comprises three genera, and according to the most recent catalogs, there are at least 45 *Curtonotum* Macquart, nine *Axinota* Wulp, and four *Cyrtona* Séguy species (Evenhuis and Okada, 1989; McAlpine, 1987a; Papp, 1984; Wirth, 1975; 1977; Wirth and Tsacas, 1980). *Curtonotum* is cosmopolitan, *Axinota* is known from the Oriental Region and Madagascar, and *Cyrtona* is restricted to Africa. Pollock's (1996) recent collection of several undescribed species of *Cyrtona* in Zimbabwe reflects our poor knowledge of the Curtonotidae and confirms Wirth and Tsacas's (1980) suspicion that many African species remain undescribed.

Curtonotidae was not accorded family status until Duda (1934) raised the taxon to family rank and gathered the species that had previously been classified in at least six other families (McAlpine, 1987a). Curtonotidae is certainly monophyletic (McAlpine, 1989) but has yet to receive monographic treatment. Also, the phylogenetic relationships among the genera and species have not been studied. Most species are small to medium-sized (3–11 mm), dull gray to brownish, and hump-backed (Wirth, 1977; Wirth and Tsacas, 1980).

Among the seven families of the Ephydroidea, the Drosophilidae, and Ephydridae probably comprise more than 95% of the species and have received almost all of the attention. The remaining families, Camillidae, Campichoetidae, Curtonotidae, Diastatidae, and Risidae (which is controversial in rank and systematic placement) have few species and have been largely ignored. None of their eggs have been described, and, except for a short note on the third-instar larvae of an African *Curtonotum* species of uncertain identity, their larval morphology is unknown. In this paper, we will discuss the natural history and morphology of the only Nearctic curtonotid, *C. helvum*. The morphology of the female reproductive system, egg, first-instar larva, and puparium is described and compared with the limited information on other curtonotid species and the remaining families of the Ephydroidea.

ACKNOWLEDGMENTS

We thank J. Skevington, S. A. Marshall, and L. Kobayashi for facilitating collection of material and allowing access to the Pinery Provincial Park. We are grateful to J. N. Pollock for providing a copy of an unpublished manuscript. Peling Melville helped with taking the SEM micrographs at the American Museum of Natural History. D. A. Grimaldi, W. N. Mathis, and R. Bang read the manuscript and suggested improvements. R. M. acknowledges financial support by a Kalbfleisch Fellowship of the American Museum of Natural History. M. K. was supported by a postdoctoral fellowship by the National Museum of Natural History of the Smithsonian Institution.

MATERIALS AND METHODS

The adults, eggs, larvae, and the single known puparium of *Curtonotum helvum* were preserved in 70% alcohol. The immature stages were subsequently critical-point dried, sputter-coated with gold-palladium, and observed under a scanning electron microscope (Zeiss DSM 950). Figures were prepared from 9 × 9 cm Polaroid micrographs. The internal female reproductive system of *Curtonotum helvum* was dissected and transferred into lactic acid onto a depression slide. Micrographs were taken with Nomarski optics on an Olympus BX 50 compound scope. Pinned specimens of *Axinota dissimilis, A. uniformis,* and *Cyrtona* sp. from the collection of the United States National Museum (USNM) were studied after maceration in KOH and subsequent embedding in po-

lyvinyllactophenol with an admixture of chlorazol black E.

RESULTS

A. Natural History and Collecting

Curtonotum helvum has a wide distribution in the Nearctic Region. It appears that throughout the wide range of the species, the imagines are only active during three months of the year (mid-June through mid-September). The specimens in the collections of the AMNH (American Museum of Natural History) and the USNM were collected in mid-June (2 specimens), July (104 specimens), August (48 specimens), and September (6 specimens). Our studies were restricted to populations occurring in the dunes of the Great Lakes where adults can be common in late August/early September. *Curtonotum helvum* is abundant on the northern shore of Lake Ontario (Sandbanks Provincial Park, Prince Edward County west of Kingston, Ontario) and somewhat less common on the southern shore of Lake Huron (west of Ipperwash Provincial Park, Grand Bend, Ontario). In the Grand Bend area (Lake Huron), the flies were collected in secondary dunes shielded from the lakewinds by primary dunes along the lake shores. The flies were common in the vicinity of a temporary freshwater pool on small dunes sparsely vegetated with sand cherries (*Prunus pumila* L.). On only one occasion were flies seen in a secondary dune landscape lacking freshwater pools.

Curtonotum helvum appears to be largely crepuscular and/or possibly even nocturnal. Only a few specimens, presumably picked up while resting on plants, were found during the day by sweeping vegetation. However, in late afternoon and during sunset the flies were abundant on the shaded slopes of the dunes. Therefore, it proved more efficient to start collecting at dusk, when the flies appeared on the edges of the vegetation (mostly on the leaves of sand cherries). We recorded the exact collection time and number of specimens for two evenings (5 and 6 Sept., 1995; fig. 1). On both days, specimens were taken until darkness prevented further collecting. The number of flies increased toward sunset (fig. 1). The sex ratio was heavily skewed during one evening (24♀; 6♂) but approximately equal during the previous night (24♀; 21 ♂). Copulations were only observed toward the end of sunset in almost complete darkness (fig. 1A, B). Copulating pairs tended to cluster on the vegetation with single flies occasionally also found in such assemblages. The flies were very easily disturbed, and especially pairs in copula would readily fly up. They usually settle after one or two meters of a flight on the sand where they were well camouflaged by their light-brown coloration. On one morning only females were caught. With increasing daylight, the number of flies on the vegetation decreased and collecting was discontinued when no specimens were collected within 30 minutes (fig. 1C). Since the flies could be collected mainly during dusk and dawn and have been repeatedly taken at blacklights at Pinery Provincial Park (Jeff Skevington, personal commun.), *C. helvum* may also be active during the night.

A number of the females collected in Grand Bend during sunset were dissected to determine the ovarial status. They either had undeveloped ovaries or the oocytes were small to medium size. A single in-copula female was dissected, and it also had undeveloped ovaries (table 1).

Observations in captivity

In 1981 one of us (K. B.) successfully reared one specimen of *C. helvum.* The culture was established from flies that were caught on the northern shore of Lake Ontario and brought back to the laboratory, where they were kept in screened cages or a plastic box and supplied with dishes of moist sand into which grasshoppers had oviposited egg pods. After several days, 30–40 curtonotid eggs were laid at random into the sand (September 8). Four days later first-instar larvae were discovered, and a single puparium was found on September 29 (larval development time about 17 days). A female fly emerged on October 28 (pupation time: 29 days). A diapause therefore was absent in the single fly that was reared in the laboratory. However, in the field adults are not collected until June–August of the following year and it remains unclear which life history stage overwinters under natural conditions.

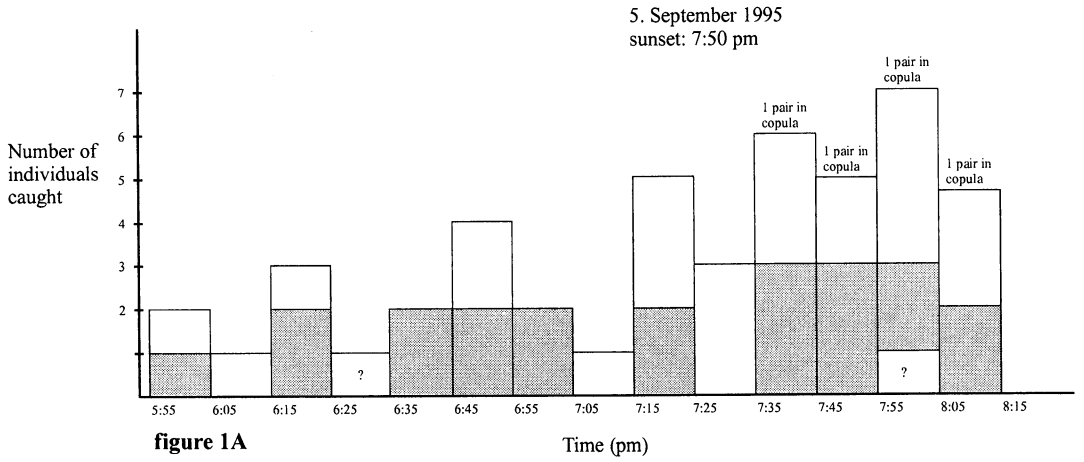


figure 1A

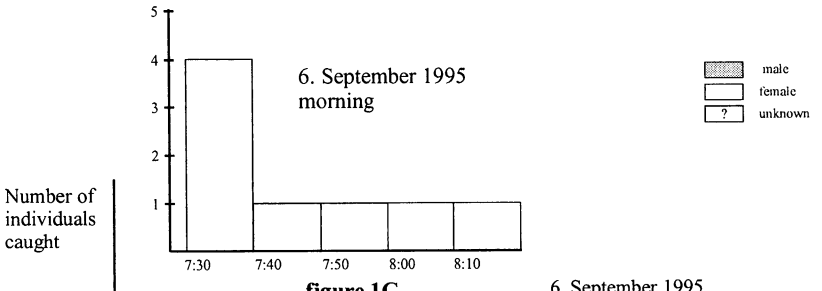


figure 1C

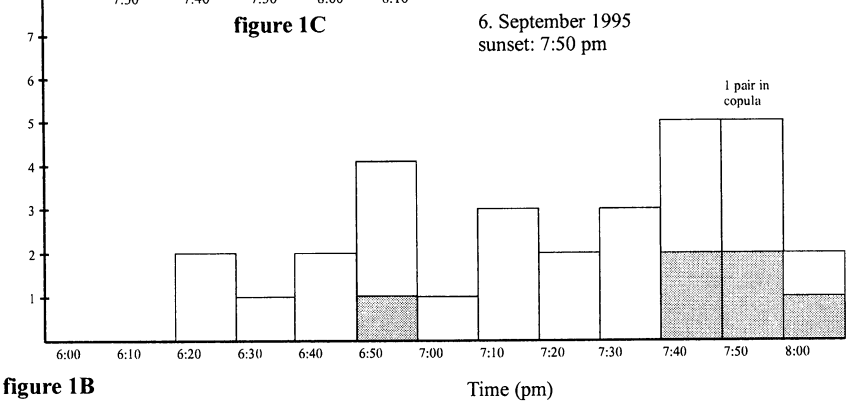


figure 1B

Fig. 1. Number, sex and temporal distribution of *Curtonotum helvum* caught during dusk on September 5 **(1A)** and 6 **(1B)** and the dawn of September 6 **(1C)** in Sandbanks Provincial Park, Ontario.

Most flies collected in the Grand Bend area in 1995 (by R. M.) were kept in a large glass aquarium. A few branches of sand cherries were provided as perches, and the flies were fed with sugar water and several substrates naturally occurring in their habitat, most notably coyote dung, overripe sand cherries and crushed insects (mayflies, grasshoppers). While the sugar water was largely ignored, all other food items were readily consumed. Copulations were observed frequently, but the females failed to oviposit into the different substrates. Since no gravid grasshoppers were found in the habitat of the flies, egg pods could not be provided. To determine ovarial status, several females that

TABLE 1
Ovarian Conditions of 21 Dissected *Curtonotum helvum* females

Female	Date and source of specimen	Oocyte size in ovaries
1	6 Sept.	Medium
2	6 Sept.	40 medium
3	6 Sept.	1 medium; remaining oocytes undeveloped
4	6 Sept.	7 and 18 medium in the two ovaries
5	5 Sept. 6:00 p.m.	Undeveloped
6	5 Sept. 6:18 p.m.	Undeveloped
7	5 Sept. 6:42 p.m.	Undeveloped
8	5 Sept. 6:49 p.m.	Undeveloped
9	5 Sept. 7:14 p.m.	Small to medium
10	5 Sept. 7:24 p.m.	Small to medium
11	5 Sept. 7:26 p.m.	Undeveloped
12	5 Sept. 7:36 p.m.	Medium
13	5 Sept. 7:46 p.m.	Undeveloped
14	5 Sept. 8:02 p.m.	Small to medium
15	5 Sept. 8:10 p.m.	Undeveloped
16	5 Sept. 8:11 p.m.	Small to medium
17	6 Sept. in copula	Undeveloped
18	From culture	15 and 14 mature ova in the two ovaries
19	From culture	1 mature ova
20	From culture	14 mature ova
21	From culture	18 mature ova

had died in the culture were dissected (table 1). In most of them, large eggs were found in the ovaries, and SEM study of the eggs revealed that the chorion was fully formed and the eggs indistinguishable from those naturally deposited by females. The lack of oviposition under laboratory conditions was therefore not due to undeveloped ovaries.

B. Female Reproductive System

The female reproductive system of curtonotids has not been extensively studied: we present here the first detailed description. The external female terminalia, including sternite 8 and the supra-, and subanal plates show some modification in *C. helvum.* The very short cerci bear three prominent, strongly sclerotized, upcurved thorns on their posterior margin and a number of smaller, likewise strongly sclerotized tubercules on the dorsal surface (see fig. 518 in Grimaldi, 1990; fig. 3 in McAlpine, 1987a).

The internal female reproductive tract comprises paired ovaries and lateral oviducts, a common oviduct, a tubular vagina, a small ventral receptacle, a large anteroventral pouch attached to the vagina, two dorsal spermathecae, and a pair of accessory glands (fig. 2A, B). The ovaries of the four dissected females contained between 1 and 29 mature eggs (table 1: females 18–21). No eggs were found in the oviducts or within the vagina. The tubular vagina is surrounded by a massive layer of circular musculature. Its anterior portion is internally structured by conspicuous, somewhat pigmented cuticular folds, while the narrower posterior portion is entirely membranous. The vulva opens behind sternite 8. From the dorsal rim of the vulva, a rod-shaped apodeme extends anteriorly within the muscle wall of the vagina.

The common oviduct enters the anterior portion of the vagina from the dorsal side. Ventrad of the oviduct, a large, roughly hemispherical, membranous pouch arises from the anterior end of the vagina (fig. 2A, B). Its wall is lined by a thin muscle layer. Embedded in the muscle tissue between the anteroventral pouch and the common oviduct lies a relatively small, tubular ventral receptacle (figs. 2, 9). It consists of a stout, anteriorly directed duct that terminates in a

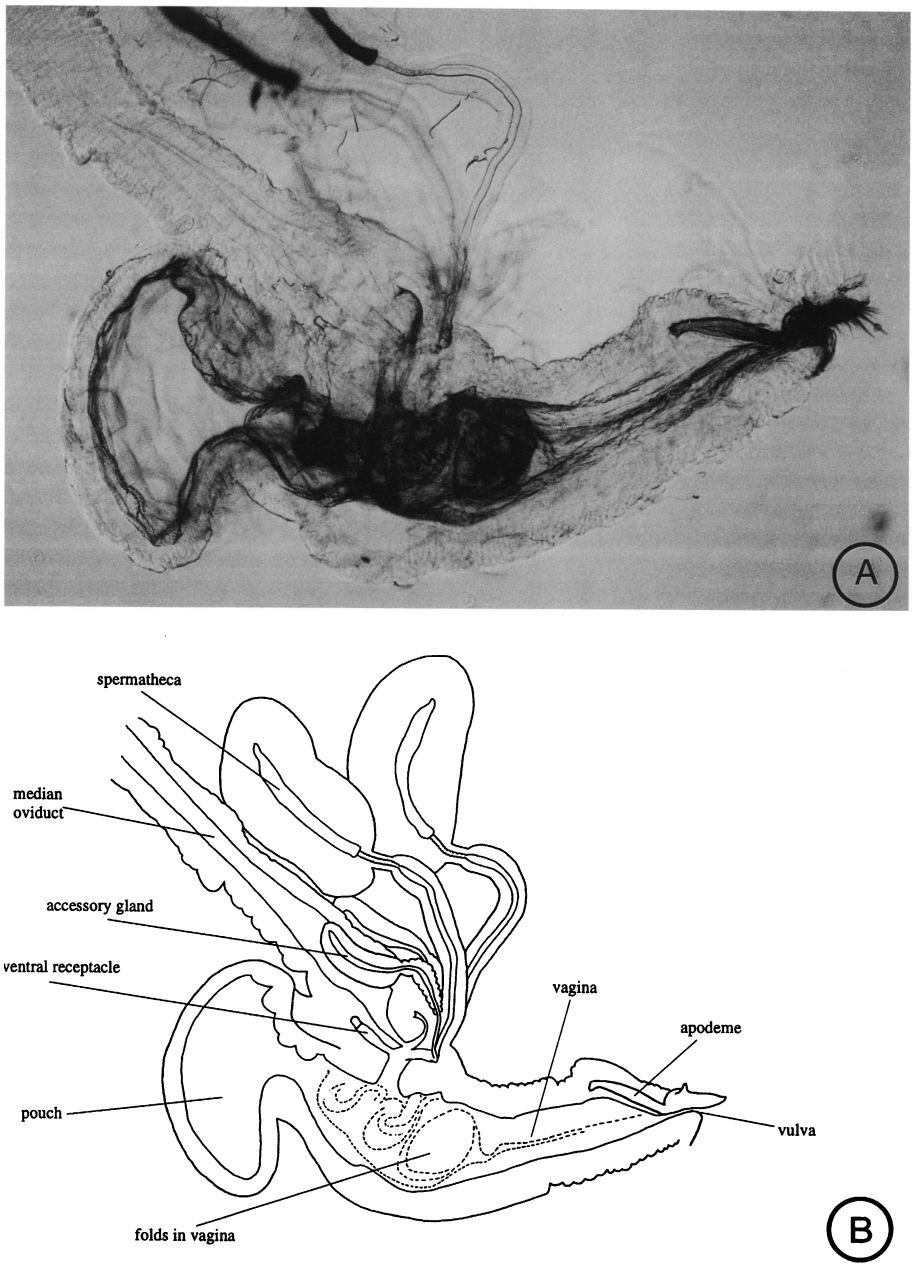


Fig. 2. Overview of the female reproductive system of *Curtonotum helvum.*

slightly sclerotized cylindrical cap (16 μm in diameter). Directly opposite the ventral receptacle, the two spermathecal ducts open into the dorsal wall of the vagina, and, close by, the two accessory glands are attached (figs. 2, 3, 8). The spermathecal ducts have relatively thick, unsclerotized walls with spiral ridges. Except for a short apical portion, they are surrounded by a sheath of longitudinal muscles. The heavily sclerotized spermathecae are elongate, flat, and slightly bent, like a scimitar, about 720 μm long and 50 μm wide. The spermathecal surface is densely studded with tiny protuberances, each

bearing a slender, colorless ductule that connects to the cuticular endapparatus of a spermathecal gland cell (fig. 7). The accessory glands are much shorter than the spermathecae (fig. 3). Their thin-walled ducts are lined by a muscle sheath. The membranous, sausage-shaped gland lumina receive the endapparatuses of numerous gland cells, that are very similar to those of the spermathecae (figs. 3, 8).

C. Egg Description

The following is the first description of curtonotid eggs. Only eggs laid under laboratory conditions were used. The egg is white and slender, 0.98—1.13 mm long ($\bar{x}$ = 1.08), and its greatest width is 0.26-0.32 mm ($\bar{x}$ = 0.29). The surface is covered with longitudinal ridges that lack aeropyles (figs. 12; 15). In cross section of the ridges, a thin, outer endochorion can be distinguished from a very delicate, unordered meshwork of pillars and struts underneath (fig. 16). In the grooves between the ribs, the plastron communicates freely with the surrounding air and the struts can be seen (figs. 12, 16). Since the egg tapers toward the anterior and posterior ends, the ridges would either have to be wider in the middle (as in some syrphids, Ferrar, 1987) or their number has to be increased. The latter is the case, with two new ridges originating via bifurcation of a single one (figs. 12, 15). At the anterior end, the ridges flatten to form a smooth cap from which the micropyle arises (figs. 12, 13). It is in a terminal position and consists of a single pore surrounded by a chorionic welt (fig. 13). The whole structure is cup-shaped. At the posterior end of the egg, the ridges are similarly fused, but the posterior cap is perforated by about 100 aeropyles connected to the underlying plastron (fig. 14). Each aeropyle is surrounded by a ring of thickened chorion, presumably strengthening the edge of the aeropyles. The diameter of the aeropyles is smaller toward the margin of the posterior cap and some are located at the ends of the furrows between the chorionic ridges (fig. 14).

D. Larval Morphology of First-instar

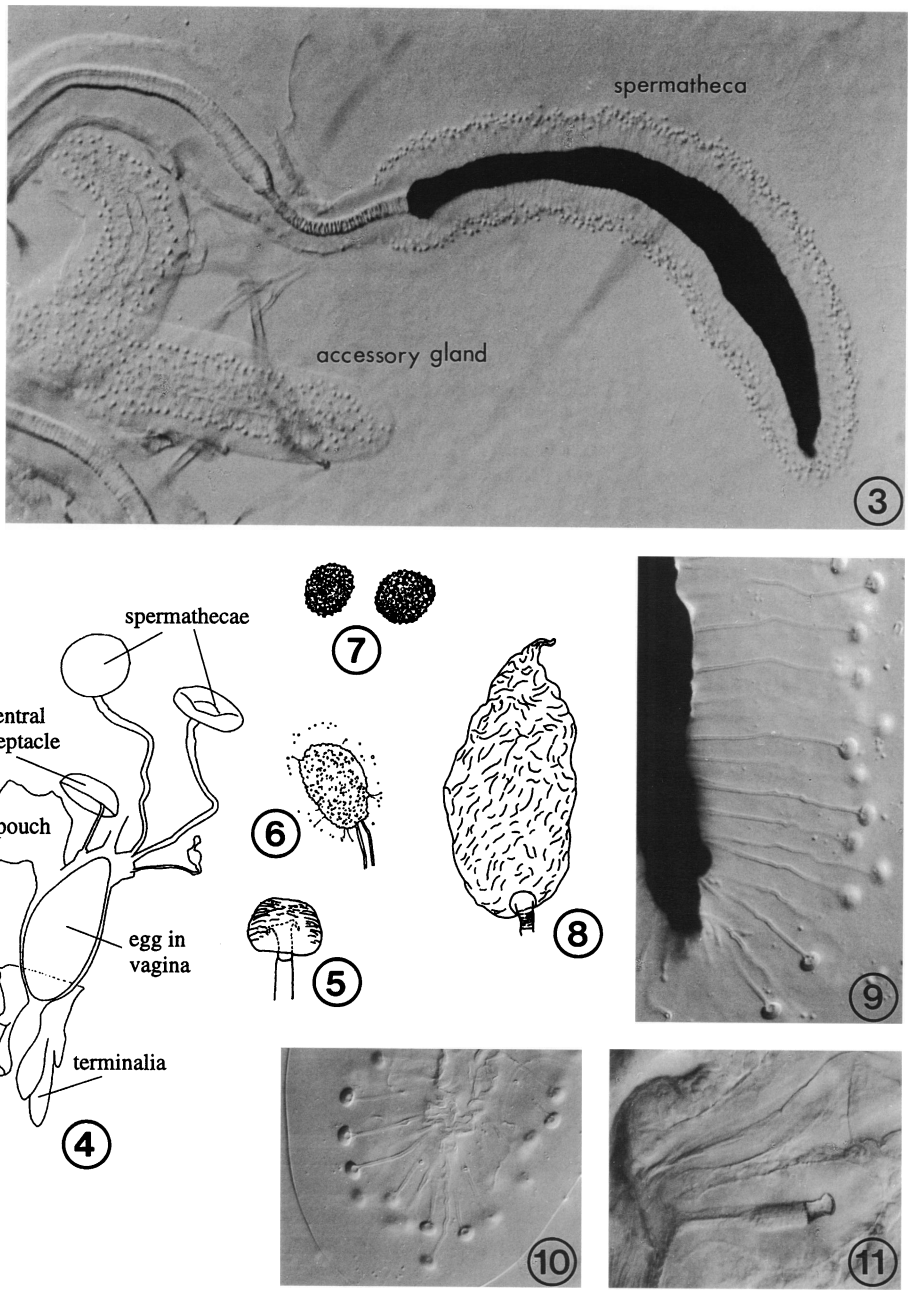
The first-instar larvae and puparium used in this study come from the rearing study of 1981. The cephalic region consisting of all larval head segments was poorly preserved in all specimens. However, the antennae, maxillary palp, and mouth opening could be identified (fig. 17). Surprisingly, no combs were found on the facial mask surrounding the mouth opening. Such facial combs are usually found in SEM studies of cyclorrhaphan first-instar larvae including drosophilids, sepsids, and various calyptrates (R.M., personal obs.). Since they were missing in several specimens of *C. helvum*, they may be lacking in this species. However, it cannot be ruled out that they had decayed before the larvae were preserved in alcohol.

The integument is ornamented with longitudinal ridges that span entire segments (fig. 18). The segments are strongly constricted at both ends, but this could be a preservation artifact. The creeping welts are located at the anterior border of each abdominal segment and consist of multiple (about 3–7) rows of tightly packed spinules (fig. 18). The entire creeping welt is located on a single segment and not spread across two segments as, for example, in most Sepsidae (Meier, 1996).

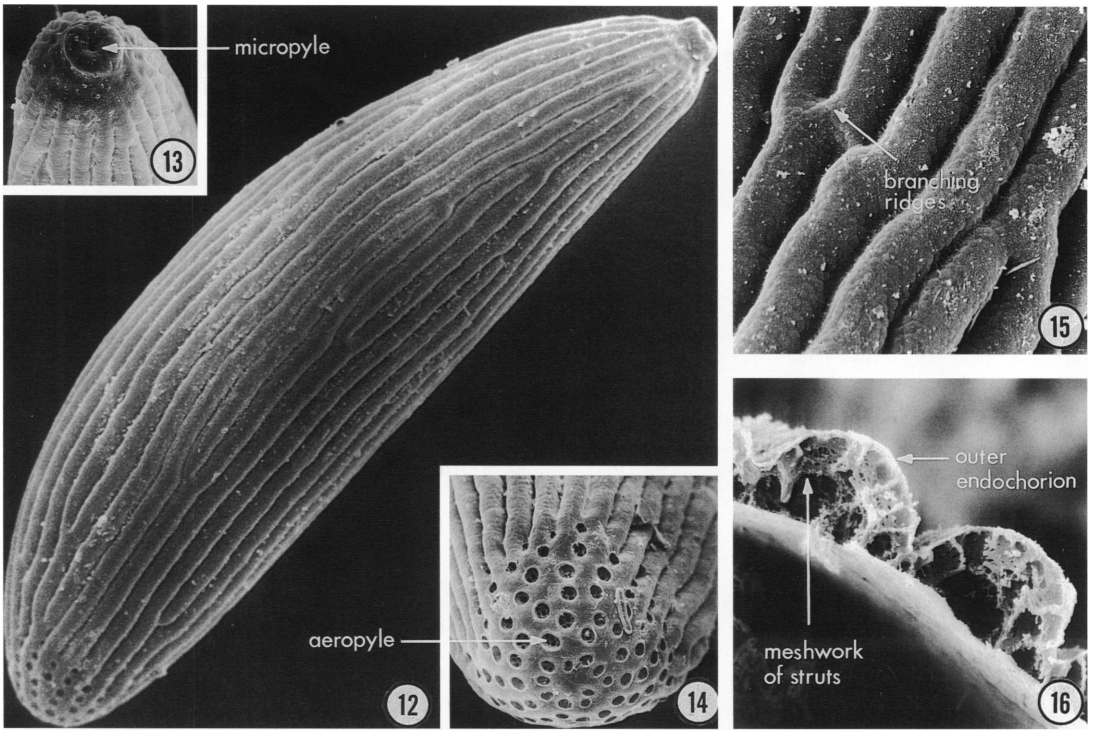
Because the larvae were preserved after dying in the culture, the posterior ends (fused abdominal segments 8–11) were not visible in all specimens, and the distribution of caudal tubercles could not be inferred. However, the posterior spiracles could be seen in two specimens (fig. 19). The very small spiracular plate is adorned with four bundles of spiracular hairs. Each bundle has two origins on the spiracular plate and branches into 3–5 hairs.

E. Puparium

Only the creeping welts and posterior end of the puparium could be studied. The posterior end of the larval skin, within which pupation takes place in Cyclorrhapha, is usually contracted during the formation of the pupa, so it conveys only an imperfect picture of the shape of the third-instar larva. The nomenclature for the tubercles follows as far as possible Okada's (1968) study of drosophilid larvae. Anterior spiracles were not found, presumably having been retracted into the puparium during pupation. Also, the protho-



Figs. 3–11. **(3)** Spermatheca and accessory gland of *Curtonotum helvum*; **(4)** female reproductive system of *Axinota uniformis* with egg in vagina; **(5)**: spermatheca of *Axinota* sp.; **(6)** spermathecae of *Cyrtona* sp.; **(7)** spermathecae of *Curtonotum angustipennis*; **(8)** spermatheca of *Curtonotum pauliani*; **(9)** detail of spermatheca of *Curtonotum helvum*; **(10)** detail of accessory gland of *Curtonotum helvum*; **(11)** ventral receptacle of *Curtonotum helvum*.

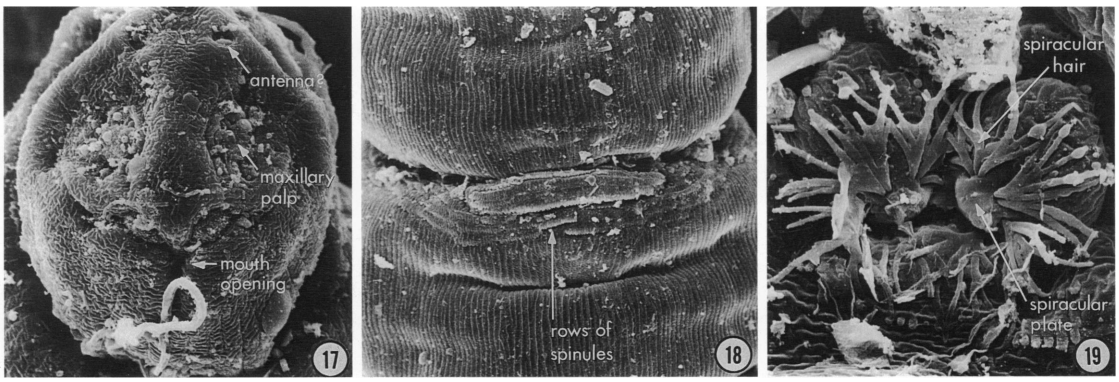


Figs. 12–16. Egg of *Curtonotum helvum*. **(12)** Whole egg; **(13)** micropyle; **(14)** aeropyles at anterior pole; **(15)** chorionic ridges; **(16)** chorionic ridges in cross section.

racic segment was torn. The caudal tubercles were shriveled, but their approximate, inflated size is shown in the drawings.

Creeping welts (fig. 22) are found on the first through sixth abdominal segment and consist of multiple short, transverse, sinuous rows of small spinules extending laterally on each segment but not reaching the dorsum. The rows bend posteriad on the ventral side of the puparium. Between the creeping welts, the integument is bare. A faint line anterior to the creeping welt apparently indicates the segmental borders.

The anal plate (Okada's "circumanalis," 1968), on either side of the anal opening, is wing-shaped with the inner lobe and outer



Figs. 17–19. First-instar larva of *Curtonotum helvum*. **(17)** Cephalic region; **(18)** creeping welt and segmental border; **(19)** posterior spiracles with spiracular hairs.

lobe being only weakly separated (fig. 20). The anal plate is large, stretching across approximately two-thirds the width of the fused last abdominal segments. The median postanal tubercle is weakly developed. Short rows of spinules form a meshlike pattern around the posterior spiracles. They reach dorsally to the dorsal tubercles (fig. 24) and laterally to the lateral and dorsolateral tubercles (fig. 23). Ventrally they extend in two bands between the anal and ventral tubercles and the lateral and dorsolateral tubercles downward to the area below the lateral tubercles (fig. 20). The spinules are small and arranged in short rows like the creeping welts. The remaining integument was shriveled, forming short cuticular ridges. The anal, lateral, dorsolateral, and dorsal tubercles are moderately large, and the ventral, subventral tubercles small. One pair of tubercles (medioventral) was found for which there is no homologous structure in drosophilids (fig. 20). These tubercles are very small and positioned far posteriad and ventrad of the posterior spiracles.

The posterior spiracles (fig. 21) are located on short horns with three straight openings radiating from the center of a flat, spiracular plate. The ecdysial scar is found on the spiracular plate. Four bundles of about five unbranched, very short spiracular hairs are found in the interspaces between the spiracular openings.

DISCUSSION

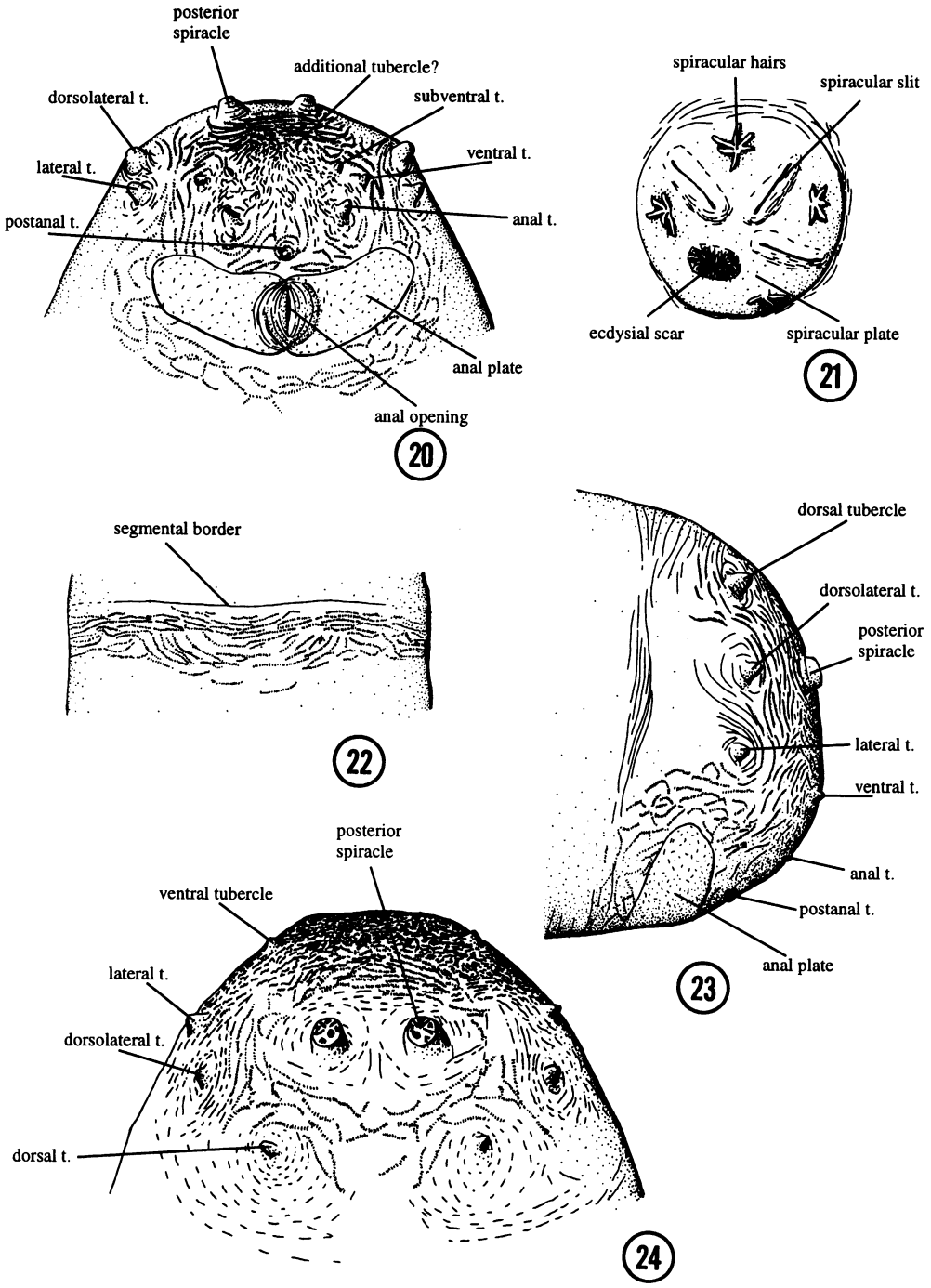
A. Natural History

1. Natural Habitat and Crepuscular Habits: There are numerous records of curtonotids in burrows of various mammals. In Africa, the burrows of warthogs harbor *C. cuthbertsoni* Duda, *C. quinquevittatum* Curran and *C. tigrinum* Séguy (Tsacas, 1977) with the association being particularly strong for *C. cuthbertsoni,* which Stuckenberg (Tsacas, 1977) found "seemed invariably to be present, resting on the walls a few feet down from the entrance." *Curtonotum quinquevittatum* is known to occur in anteater burrows and *C. sao* Tsacas in burrows of porcupines (Tsacas, 1977). Other observations of curtonotids in their natural habitat also indicate a preference for dimly lit environments. For example, Barraclough (in litt.) collected possibly yet another curtonotid species "at the base of a steep cliff . . . [that] are quite heavily shaded, even at mid-day." *Curtonotum saheliense* Tsacas has been collected in a hollow Baobab tree (Tsacas, 1977), and Grimaldi (personal commun.) found a Tanzanian species of *Curtonotum* on bushes that were shaded by Baobab and fig trees but not on similar bushes exposed to sunlight. He also collected four species of *Curtonotum* in Venezuela from shaded understory plants in an inundation rain forest.

From these observations, it appears likely that many curtonotid species either avoid well-lit environments or may only be active in the early morning or late afternoon and use mammal burrows or similarly dark places as midday shelters. However, the evidence is only circumstantial, and further confirmation is needed to generalize the behavior.

Many species of Curtonotidae also appear to be associated with sandy or muddy shores of rivers and lakes. Freidberg (in litt.) collected a series of *Curtonotum anus* (Meigen) "at the muddy shore of the Jordan River in May." Tsacas (1977) described an association of *Curtonotum cuthbertsoni* with river banks although the species has also been collected in a forest (Tsacas, 1977). The close association of *Curtonotum helvum* with a moist habitat in the vicinity of freshwater is, therefore, in agreement with the findings on several other species of curtonotids. However, the *Curtonotum* species that Grimaldi (personal commun.) observed in Tanzania was not close to any fresh water.

2. Feeding Substrate. Adult feeding has been observed for four species and all were feeding on dung. One record each is known from human feces (*Curtonotum albomacula* Curran; Cuthbertson, 1936) and from fresh elephant droppings (*Curtonotum tigrinum*; Tsacas, 1977). Grimaldi (personal commun.) found a Tanzanian *Curtonotum* species feeding baboon feces and Barraclough (in litt.) observed a South African curtonotid on hyrax dung. Coprophily is also suggested for *C. cuthbertsoni* since Greathead (1958) mentioned that the "flies were found on dung, dead locusts and sand wetted by camel urine" (see below for comment on species identity). The feeding behavior of *Curtono-*



Figs. 20–24. Puparium of *Curtonotum helvum*. **(20)** Ventral view of posterior segments; **(21)** disc of posterior spiracle; **(22)** creeping welt and segmental border; **(23)** lateral view of posterior segments; **(24)** dorsal view of posterior segments.

tum helvum under laboratory conditions on dung, dead insects, and berries therefore closely resembles the behavior of free-living curtonotid species.

3. Breeding Substrate: Very little is known about the natural history of other curtonotids. Greathead (1958) found the larvae of a *Curtonotum* species living on damaged egg pods of the swarming desert locust *Schistocerca gregaria* (Forskål). According to Greathead, the species was *Curtonotum cuthbertsoni*, but based on distribution, habitat, and some additional information, Tsacas (1977) regarded it more likely as *Curtonotum saheliense*. The flies were regularly associated with grasshopper eggs during three subsequent field seasons, but not as abundantly as other insect predators were found on locust egg pods. The proportion of infected egg pods was small (1.9—3.6 %), and often only on pods already damaged by other insect larvae. Greathead (1958) therefore considered the association between *Curtonotum* and the locust eggs as "merely incidental," although adult flies were seen specifically ovipositing into damaged egg pods. He proposed that the larvae are saprophagous rather than predatory, and up to ten immatures will develop within a single pod. Pupation either occurred in the substrate or in the surrounding soil. The larval development was very rapid (2–3 days) and adults emerged 23–25 days after pupation (Greathead, 1958).

The successful breeding of *Curtonotum helvum* solely on grasshopper egg pods confirms the suitability of this substrate. However, there is some indication that the success of this substrate may be merely incidental. First, the ovipositing female(s) randomly oviposited into the sand and not specifically onto the egg pods. Second, most first-instar larvae died after they were placed into pods with damaged eggs. Third, the development time of the larva in this substrate was extremely long (17 days) compared to Greathead's (1958) observations of the African *Curtonotum* species (supposedly 2–3 days), while the time between pupation and eclosion of the adult was similar [about 29 days in *C. helvum*; 23–25 days in *C. saheliense*(?)]. This longer development time may indicate a suboptimal substrate for larval development.

B. Female Reproductive System

Little is known about the morphology of the female reproductive system of other curtonotid species and information is usually restricted to the number and morphology of spermathecae. Three types of spermathecae are found in *Curtonotum*. Two slender, sclerotized spermathecae like the ones described here for *C. helvum* (fig. 3; 720 × 50 μm) are known from the South American *Curtonotum taeniatum* Hendel (Sturtevant, 1925; 1926 as *C. gibba*) and the species of Tsacas' (1977) African *striatifrons* group, where the tubular spermathecae measure from 412 × 47 μm to 529 × 53 μm. The spermathecae are densely studded with small protuberances.

The second type of spermatheca occurs in the species of Tsacas' (1977) African *cuthbertsoni* group and ten Madagascan species (fig. 8; Tsacas, 1974). They are obclavate (i.e., have a wide base tapering toward the tip) with a wrinkled surface (fig. 8) and are comparatively large, ranging from 176 × 130 μm to 600 × 233 μm. Curiously, Delfinado (1969) depicted two spermathecae for *C. ceylonese* Delfinado that have the same general shape, but the opposite orientation (obovate spermathecae: base pointed, tip wide). We suspect that the spermathecae may have been depicted in the incorrect orientation.

The third type of spermatheca is only known from *C. angustipennis* (De Meijere), for which Delfinado (1969) illustrated two, small, subspherical spermathecae that are studded with small protuberances, as in *Curtonotum helvum* (fig. 7).

The spermathecae of the second curtonotid genus, *Cyrtona,* are only known from a description by Pollock (1996) and our recent dissection of a specimen of an unidentified species from Kenya (fig. 6). According to Pollock, they are "tubular" in several undescribed Zimbabwean species, while in the Kenyan species they are small (56 × 100 μm), oval, and studded with protuberances, thus closely resembling those of *Curtonotum angustipennis*.

There is some confusion over the number of spermathecae in the third genus of Curtonotidae, *Axinota*. Both Delfinado (1969) and McAlpine (1987a) reported three sper-

mathecae, with one being unusually shaped. McAlpine (1989) later elaborated that the third is a "peculiarly formed spermatheca reminiscent of the abortive, mutant-type, third spermatheca that occurs on some species of *Drosophila* Fallén (see Sturtevant, 1925; 1926)." Our dissections (M. K.) of *Axinota dissimilis* (Malloch) and *A. uniformis* revealed that the third, sclerotized structure is not a spermatheca, but a fairly large and slightly sclerotized ventral receptacle (fig. 4; see below).

The two genuine spermathecae of *Axinota* are rounded to mushroom-shaped and sclerotized with a smooth surface (figs. 4; 5). In *Axinota dissimilis* and *A. uniformis,* the spermathecae are comparatively large, disc-shaped, and without a neck. In *A. hardyi* (Delfinado) each has a circular fold before the opening, and the neck is also missing while the spermathecae of *A. hyalipennis* (Hendel) are mushroom-shaped with a slender neck (Delfinado 1969). Tsacas (1974) illustrated the spermatheca of an undescribed *Axinota* species from Madagascar that is doliform (short cylindrical to round; fig. 5), with a deep basal and shallow, apical invagination. It is considerably smaller in diameter (83 μm) than the spermathecae of *A. uniformis* and *A. dissimilis* (~190μm). Since we were able to demonstrate that the "third" spermatheca of *Axinota* is, in fact, the ventral receptacle (fig. 4), we can now conclude that two spermathecae belong to the groundplan of the family.

Sturtevant (1926) and Pollock (1996) are the only authors who furnished information on the morphology of the ventral receptacle. Sturtevant (1926) found "no chitinized ventral receptacle appears" to be present in *Curtonotum taeniatum* (as *C. gibba*), while our study revealed that the tip of the tubular receptacle is sclerotized in *Curtonotum helvum* (fig. 11). According to one of our dissections, a similar ventral receptacle is also present in the Kenyan *Cyrtona,* a result that confirms Pollock's observation (1996) of "a small ventral receptacle attached to the common oviduct . . . about one third the length of the spermathecae, and about the same width."

The ventral receptacle of *Axinota* has been depicted by Delfinado (1969), who interpreted the structure as an abnormal third spermatheca (see also McAlpine, 1987a; 1989). Our dissection of a female of *Axinota uniformis* revealed beyond doubt that, based on the position of this abnormal "spermatheca" in the female reproductive tract (fig. 4), it is, in fact, a ventral receptacle (diameter 180 μm) that superficially resembles the spermathecae in shape and size.

According to Grimaldi (1990), the Drosophilidae are the sister group of the Curtonotidae. Since the Drosophilidae have a tubular ventral receptacle, the similar receptacle of *Curtonotum* and *Cyrtona* is probably plesiomorphic, and the large receptacle of *Axinota* is derived within the Curtonotidae.

Pollock (1996) also described a large, anteroventral pouch originating from the vagina in *Cyrtona* and used to incubate eggs. We identified a similar pouch in a dissection of the unidentified Kenyan *Cyrtona* species. A similar, but much smaller, pouch is present in *Axinota dissimilis, A. uniformis* (fig. 4), and *Curtonotum helvum* (fig. 2A, B). In *C. helvum* the function remains obscure, since the oviparous habit of the latter species has been confirmed under laboratory conditions, and none of the dissected females with mature eggs carried them in that pouch. One mature egg (no embryo visible) was found in the vagina but not in the pouch of the only female of *Axinota uniformis* that we dissected (fig. 4). Regardless of its function, the anterior pouch apparently belongs to the groundplan of the Curtonotidae and very likely constitutes a synapomorphy of the family. The only other occurrence of a superficially similar structure in the Ephydroidea is in the ephydrid genus *Discocerina* Macquart, where a membranous, ventral evagination arises from the posterior portion of the vagina (Sturtevant 1926; M. K. personal obs.). It is almost certainly not homologous to the pouch of curtonotids according to its position.

Implications for the relationships within the Curtonotidae: The phylogenetic relationships implied by the characters of the female reproductive system can be analyzed using the Drosophilidae as outgroup. Two sclerotized, small subspherical spermathecae belong to the groundplan of the Drosophilidae (Grimaldi, 1990). A basal invagination of the spermathecal capsule belongs to the ground-

plan of the Drosophilinae and is also known from at least one genus of Steganinae (Wheeler, 1987). If the introvert belonged to the groundplan of the Drosophilidae, it would follow that the doliform spermatheca with a deep, basal invagination found in the undescribed Madagascan *Axinota* species constitutes the plesiomorphic spermathecal shape of the Curtonotidae (fig. 5; Delfinado, 1969; Tsacas, 1974) and that *Axinota* is the sister group of the remaining Curtonotidae. However, spermathecae that strongly resemble the most common spermathecal form and size of the Drosophilidae are only found in this undescribed Madagascan species (Tsacas, 1974). Since *Axinota* is apparently monophyletic based on the absence of the proclinate orbital bristle, which is present in the groundplan of the Ephydroidea (Chandler, 1987), we would conclude that Tsacas' undescribed Madagascan *Axinota* species occupies a basal position within the genus and that the large, disc-shaped spermathecae of the Oriental *A. dissimilis* and *A. uniformis* are derived within the genus (fig. 4). Additional evidence for the basal position of *Axinota* in the Curtonotidae comes from spinules on the spermathecae that are shared by a putatively monophyletic clade composed of *Cyrtona* and some *Curtonotum* but lacking in *Axinota.* They only occur as a derived and, thus, convergent feature in the Drosophilidae, and have evolved independently within many schizophoran families.

Alternatively, one could argue that the small spermathecae of *Curtonotum angustipennis* and the *Cyrtona* species lacking an introvert are plesiomorphic. Such spermathecae are known from some campichoetid and camillid species. However, this conclusion would imply that the remarkably similar spermathecae of *Axinota* and the drosophilids are convergent structures.

We therefore conclude that in the groundplan the Curtonotidae have two sclerotized, subspherical spermathecae, probably with a basal introvert, an anteroventral evagination of the vagina, and a short, tubular, ventral receptacle. The obclavate (fig. 8) and scimitar-shaped spermathecae of most species (fig. 3) and possibly the elongated oval spermathecae of some *Cyrtona* species are clearly derived within *Curtonotum.* However, since our speculations about the phylogenetic relationships within the Curtonotidae are only based on a single character system, the suggestions are at best very tentative and await testing by additional characters.

Implications for the relationships within the Ephydroidea: Our dissections of *Axinota* refuted previous records of three spermathecae in this genus. We can now conclude that there is no ephydroid species with three spermathecae and that two spermathecae belong not only to the groundplan of the family but also the superfamily. The only questionable exception concerns some derived species within *Drosophila* which are allegedly variable with regard to the number of spermathecae (2 or 3; Miller, 1950; Sturtevant, 1925). Two spermathecae are now known from all studied Curtonotidae, Drosophilidae (Grimaldi, 1990; Sturtevant 1925, Throckmorton 1962), Campichoetidae (Chandler, 1987), and some Camillidae (McAlpine, 1987c: *Camilla glabra* Fallén). Two spermathecal ducts bearing rudimentary spermathecae are found in Ephydridae (Chandler 1987, Sturtevant 1926), Diastatidae (Chandler, 1987; McAlpine, 1987b) and the remaining Camillidae (Barraclough, 1992; 1993).

Large, sclerotized spermathecae in combination with a comparatively small, ventral receptacle have been described for *Curtonotum helvum* (fig. 2), *C. taeniatum* (Sturtevant, 1925 as *C. gibba*) and the unidentified Kenyan species of *Cyrtona.* They are also found in some Drosophilidae, where the ventral receptacle is typically a delicate, narrow tube that is not voluminous, although sometimes extremely long (Miller, 1950; Nonidez, 1920; Sturtevant, 1925). In the Ephydridae and within lineages of the Camillidae (e.g., *Afrocamillau* Barraclough) and Diastatidae (e.g. *Diastata* Meigen) an opposite situation to the conditions in *Curtonotum* and Drosophilidae is encountered. The spermathecal capsules are small, with sometimes only a few spermathecal gland cells remaining, while the ventral receptacle is enlarged and strongly sclerotized. It has taken over the function of sperm storage (Sturtevant, 1926; Chandler, 1987; McAlpine 1987b, c, 1989; Barraclough, 1992, 1993). However, since functional spermathecae belong to the

groundplan of the Camillidae and Diastatidae, the strongly sclerotized ventral receptacles functioning as sperm storage organs were probably acquired independently and are of little use in determining the phylogenetic relationships among the families of the Ephydroidea.

C. Comparison of the Eggs of Curtonotidae, Drosophilidae, and Ephydridae

During a dissection of the female reproductive system of *Axinota uniformis* and *A. dissimilis* mature eggs were found. Closely resembling the eggs of *C. helvum,* they were ridged, with some ridges branching toward the center of the egg. The micropyle was also cup-shaped, and the posterior pole of the egg was smooth with numerous aeropyles. The only noticeable difference was the smaller size of the eggs in *Axinota* (length 206 μm; width 80 μm). Since *Axinota* is probably the sister group of the remaining Curtonotidae, we can assume that ridged eggs with a cup-shaped micropyle and numerous pores on a smooth, posterior pole are plesiomorphic for the Curtonotidae.

The eggs of two other families in the Ephydroidea have been described. The eggs of the Curtonotidae are very dissimilar to the ones of the Drosophilidae and remarkably similar to those of some ephydrids. In both steganines and drosophilines, studies revealed a hexagonal pattern of imprints on the egg shells usually left by the chorion secreting cells (Hinton, 1981; Okada, 1968; Kambysellis, 1975; 1993; Klug et al., 1974; Margaritis et al., 1983). Only rarely, for example, in some derived Hawaiian drosophilids, are these imprints missing (Kambysellis, 1993; Margaritis et al., 1983). The egg shell in such cases superficially resembles that of curtonotids, especially when it is ridged as in *Idiomyia grimshawi* (Perkins) (Margaritis et al., 1983).

The eggs of steganine drosophilids carry two longitudinal vela while the eggs of almost all drosophilines have at least two egg filaments with respiratory function. Neither vela nor filaments are found in the eggs of *Axinota* and *Curtonotum helvum.* Similarly missing in *Curtonotum helvum* is the operculum, a field between the respiratory filaments, or vela, at the posterior pole of the egg usually consisting of particularly well-formed chorionic heaxagons. Such an operculum is known from steganines and drosophilines (Kambysellis, 1993; Okada, 1968). Last, but not least, the micropyle of the egg of *Axinota* and *Curtonotum* is short, stout, and cup-shaped while it is very long, slender, and turretlike in the Drosophilinae (Kambysellis, 1993; Margaritis et al., 1983).

Although the eggs of drosophilids and curtonotids are very dissimilar, there are striking similarities between the eggs of curtonotids and many ephydrids. Eggs with conspicuous, smooth ridges (as in *C. helvum*) are known from at least *Discocerina obscurella* (Fallén) (Wirth et al., 1987), *Leptopsilopa atrimana* (Loew) (Steinly and Runyan, 1979), *Lemnaphila* Cresson (Ferrar, 1987), *Lytogaster* Becker (Ferrar, 1987), *Notiphila caudata* Fallén (Ferrar, 1987), *Paracoenia bisetosa* (Coquillet) (Zack, 1983), *P. fumosa* Stenhammar (Dahl, 1959), *Hydrellia williamsi* Cresson (Williams, 1938), *H. hawaiiensis* Cresson (Williams, 1938), *H. bergi* Cresson (Wirth et al., 1987), *Ochthera mantis* (De Geer) (Simpson, 1975), *O. tuberculata* Loew (Simpson, 1975), *O. exculpta* Loew (Simpson, 1975), and possibly *Scatella stagnalis* Fallén (description unclear: Sen, 1931). The resemblance between the eggs of curtonotids and those of *Paracoenia bisetosa* is particularly striking, with the posterior pole of the eggs of both taxa perforated by a large number of round aeropyles. However, ridged eggs are not universally found in all ephydrid species. The same hexagonal reticulation pattern, widespread within the drosophilids, is also known from a number of ephydrid species (e.g., *Scatella bryani* Cresson, *S. hawaiiensis* Grims: Williams, 1938; *Pelina truncatula* Loew: Foote, 1981; *Zeros flavipes* Cresson: Scheiring and Connell, 1979; *Notiphila brunnipes* Robineau-Desvoidy: van der Velde and Brock, 1980).

Analysis of the distribution of ridged eggs across the Ephydridae, based on a recent phylogenetic hypothesis (Zatwarnicki 1992), indicates that ridged eggs might belong to the groundplan of the family. However, the ornamentation of the egg chorion varies considerably within this family. Species lacking

egg ridges are widespread and can occur even within the same genera along with species with ridged eggs (*Notiphila, Scatella*). Ridged eggs are also very widespread among other schizophoran families (e.g., Sphaeroceridae, Heleomyzidae, Anthomyiidae, Lauxaniidae, Chamaemyiidae, Chloropidae, Diopsidae, Psilidae, Sciomyzidae, Helosciomyzidae, and Opomyzidae: Ferrar 1987; Hinton 1981). The egg ornamentation, therefore, cannot be utilized in a phylogenetic analysis on or above family level unless the groundplan condition is established for each taxon.

D. Comparison of the Puparium of *Curtonotum helvum* with the Larval Description of *Curtonotum saheliense*(?)

The description of *Curtonotum saheliense*(?) was based on light-microscopic study of third-instar larvae. Since some of the caudal tubercles are very small, it cannot be ruled out that they may have been overlooked. Unfortunately, the specimens could not be restudied, since they could not be located and may have been lost (Greathead, in litt; Pitkin, in litt.).

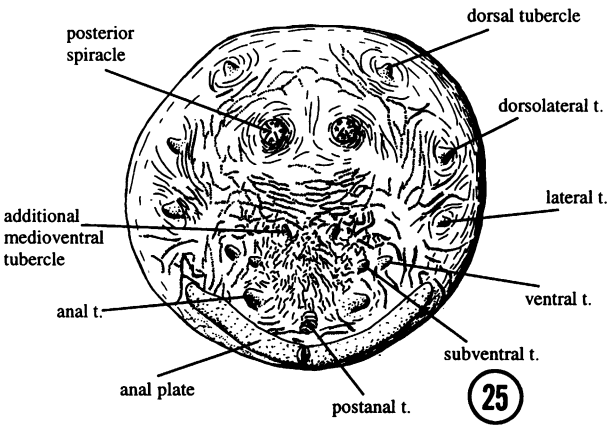
The posterior spiracles of the larvae of *Curtonotum saheliense* (?) are very similar to the ones of the puparium of *C. helvum.* Three straight slits radiate from the center of the spiracular plate and the ecdysial scar is positioned there (fig. 21). Whereas the hairs of *Curtonotum helvum* are very small, no spiracular hairs are depicted for *Curtonotum saheliense* (?) although they are normally shown in drawings based on light-microscopical study. Their absence in the drawing may indicate that they were overlooked, possibly due to a similarly small size as in *C. helvum.* The creeping welts of *Curtonotum saheliense*(?) are described in passing. They consist of "very minute recurved spines," which would be consistent with what we describe for *Curtonotum helvum* (fig. 22).

Greathead (1958) also depicted the posterior end of the larvae. He labeled and mentioned "a papilla bearing thorn-like spines" in his description and on his figure 6. Since the papilla is the only unpaired structure on the ventral side of the larvae, we assume that it is homologous to the postanal tubercle in *Curtonotum helvum.* The anal opening would then be located immediately below on the ventral side. The inferred position of the anal plate and the posterior spiracles drawn into Greathead's (1958) figure can serve as landmarks to homologize caudal tubercles. Greathead (1958) mentioned and depicted "a ring of short, conical, fleshy processes on the caudal end. There is a pair below the spiracles projecting posteriorly and two dorsal, two lateral, and two ventral projecting radially." This description implies seven pairs of tubercles, but only six are illustrated on the drawings of a lateral and a caudal view (figs. 27, 28). Only three pairs of tubercles can be homologized with confidence. These are the dorsal, dorsolateral, and lateral tubercles. For the remaining tubercles, no satisfactory homologies can be derived since the relative positions of the tubercles to each other are radically different from those found in *Curtonotum helvum.*

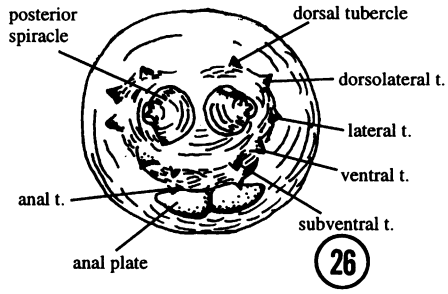
It is surprising that establishing homology among the caudal tubercles of the two *Curtonotum* species is so difficult (figs. 25, 27, 28), while the same comparison between the larvae of *Curtonotum helvum* and the drosophilids is straightforward (see below). We surmise that more detailed study of the larvae of *C. saheliense* would be needed to resolve the situation.

E. Comparison to Other Larvae of Ephydroidea

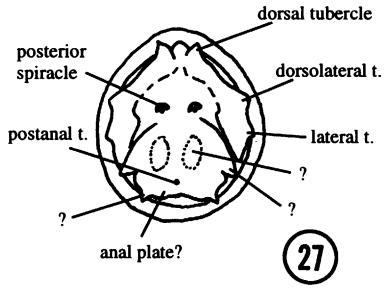
As with the eggs, information about the larval morphology is only available from two other families of Ephydroidea, the drosophilids and the ephydrids. With respect to the caudal tubercles, the puparium of *Curtonotum helvum* is remarkably similar to the larvae of most drosophilids (Okada, 1968). There is some variation with respect to the caudal tubercles in the Drosophilidae, but Okada (1968) described six pairs of tubercles for at least some species of steganines and drosophilines. Therefore, these tubercles are here assumed to belong to the groundplan of the Drosophilidae (fig. 26). They are the anal, ventral, subventral, lateral, dorsolateral, and dorsal tubercles. All six pairs can be ho-



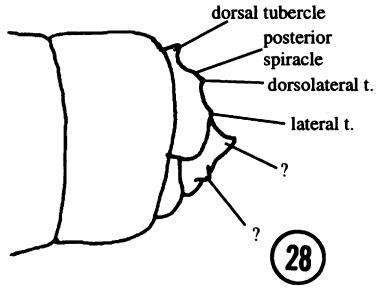
Curtonotum helvum
(puparium)



Leucophenga nigrinervis
(puparium)



Curtonotum saheliense (?)
(third instar larva)



Curtonotum saheliense (?)
(third instar larva)

Figs. 25–28. **(25)** Caudal view of *Curtonotum helvum*; **(26)** Caudal view of *Leucophenga nigrinervis* (Drosophilidae; after Okada, 1968); **(27, 28)** *Curtonotum saheliense*(?) caudal and lateral views of posterior segments (after Greathead, 1958) indicating putative homologies between caudal tubercles of *C. helvum* and *C. saheliense*(?); ? denotes tubercles of uncertain homology.

mologized with caudal tubercles that we found on the puparium of *Curtonotum helvum*, which is so straightforward that we refrain from discussing it in detail and instead refer the reader to figures 25 and 26.

A similar comparison with the larvae of Ephydridae is desirable, but the variation is more extensive, and it is again difficult to establish the groundplan. Also, in many descriptions little attention has been paid to the distribution of caudal tubercles. However, at least one tubercle potentially homologous with the lateral one in drosophilids and *Curtonotum helvum* is present in *Scatella hawaiiensis* (Williams, 1938), *Parydra quadrituberculata* Loew (Deonier and Regensburg, 1978), and *Pelina truncatula* Loew (Foote, 1981). Additional sensory organs on small protuberances are indicated for *Parydra quadrituberculata.* They may correspond to the anal and ventral tubercles. More detailed study at higher magnification is required to adequately compare the distribution of caudal tubercles. However, it seems unlikely that more detailed SEM study of ephydrid larvae will reveal many more caudal tubercles than have been described based on light microscopy, and it seems safe to conclude that with respect to the distribution of caudal tubercles, *Curtonotum helvum* is much more similar to the Drosophilidae than to the Ephydridae.

With respect to the arrangement of the spiracular slits of the posterior spiracles, *Curtonotum helvum* conforms with drosophilids and ephydrids although the spiracular hairs of *C. helvum* are unusually short and simple.

In all drosophilids and ephydrids, they extend well beyond the spiracular plate, and the spiracular hairs are more numerous and branched. The spiracular horns of *C. helvum* are also shorter than in most drosophilids and ephydrids, but this shortness may be related to the shrinking of the larval cuticle during pupation.

The creeping welts are very similar to the ones found throughout the drosophilids—except in Drosophilidae the lateral and dorsal sides of the abdominal segments bear spines whereas they are bare in *Curtonotum helvum*. A comparison with the creeping welts of Ephydridae is beyond the scope of this paper and would be difficult because of the extreme variability of this feature in ephydrids.

CONCLUSIONS

The larvae of *Curtonotum helvum* are very similar to the larvae of the Drosophilidae, although the eggs more closely resemble those of the Ephydridae. Unfortunately, these similarities could not be evaluated phylogenetically. The larvae of most ephydroid families are unknown, and the egg characters are so variable in the outgroup that polarity cannot be established. The female reproductive system of *Curtonotum helvum* and the family as a whole is unusual in several ways. The vagina bears an anteroventral pouch that is likely synapomorphic for the family. The diversity of spermathecal shapes is striking, and the size of the spermathecae as well as the ventral receptacle, is variable. The natural histories of *Curtonotum helvum* and the remaining species in the Curtonotidae remain largely undiscovered. Many species appear to avoid direct sunlight and may be crepuscular, and their natural breeding substrate remains mostly unknown.

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