

## MORPHOLOGY OF THE MALE REPRODUCTIVE SYSTEM OF *CORETHRELLA* (DIPTERA: CHAOBORIDAE)

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**Abstract.** Morphology of *Corethrella appendiculata*, *C. brakeleyi*, and *C. wirthi* was studied by light and scanning electron microscopy. Testes are located in the 4th and 5th abdominal segments; they have no apical filament or fat jacket. Pyriform testes of *C. appendiculata* are shorter and wider than fusiform testes of *C. wirthi*. Heads constitute approximately 8% of the total length of spermatozoa, compared to 20% in *Aedes*. Spermatozoa are present in the testes and vasa deferentia, but are massed in the seminal vesicles that are located on the anteromedial surface of separated spherical to oval accessory glands. A narrow, irregular lumen, bounded by tall columnar cells, runs the full length of each accessory gland; it is confluent with the vas deferens-ejaculatory duct junction. The ejaculatory duct is branched anteriorly; 5 or 6 layers of circumferential smooth muscles form the thick wall of the unbranched portion. Shape and arrangement of the vasa deferentia, seminal vesicles, accessory glands, and ejaculatory duct are similar to that of *Anopheles*. Sclerotized penis valves, which support the penis, and lateral claspers, each of which consists of a large proximal gonocoxite and a long, thin gonostyle, are appendages of the 9th abdominal segment. Both segments of the claspers bear setae, which have a characteristic size, shape, and arrangement for each species.

The family Chaoboridae consists of 7 genera, of which 4 (*Corethrella* Coquillett, *Chaoborus* Lichtenstein, *Mochlonyx* Loew, and *Eucorethra* Underwood) occur in the Nearctic (Cook 1956, Freeman 1962). Members of this family have short mouthparts and were not known to bite prior to the report by Williams & Edman (1968) of the presence of mammalian and avian blood in the digestive tract of 2 species of *Corethrella*. Hence, the Chaoboridae were long classified as a subfamily of the Culicidae and were known as nonbiting mosquitoes.

Members of the genus *Corethrella* are the smallest of the Nearctic Chaoboridae; adults range from 1.25-2.5 mm in length. They attain greatest diversity in the tropics (Cook 1956) but are represented in the United States by 4 species, *C. appendiculata* Grabham, *C. brakeleyi* Coquillett, and *C. wirthi* Stone in the east, and *C. laneana* Vargas from California (Stone 1969).

Male external genitalia of *Corethrella* provide taxonomic characters; accordingly, these structures were described and illustrated by Cook (1956) for the then 3 known North American species. Male external genitalia of *C. wirthi* were described by Stone (1969) when he proposed it as a new species; those of *C. laneana* were described by Belkin & McDonald (1955) when they redescribed the species. Cook (1956) also described and illustrated male external genitalia of members of the other 3 genera of Nearctic Chaoboridae.

Although male external genitalia of Chaoboridae have been described in detail,

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to my knowledge there has been no study of the internal reproductive system of any species of the family. Several studies have been made of the entire male reproductive system of the closely related Culicidae; among the most recent are those of Christopher (1960) and Hodapp & Jones (1961) on *Aedes aegypti* L. The latter study also compared 7 other species of culicids from 4 genera with *Ae. aegypti*. Lum (1961a) studied the male reproductive tract of 10 species from 7 genera of Culicidae from Florida. This report presents a description of the male reproductive system of *Corethrella*, based upon specimens of the 3 species from the eastern United States. The internal morphology is compared with that reported for Culicidae.

### MATERIALS AND METHODS

Adult male *C. appendiculata* were reared in a colony established from larvae obtained from Vero Beach, Florida. Males of *C. brakeleyi* and *C. wirthi* were reared from eggs obtained from females that were collected near Statesboro, Georgia while they were feeding, or immediately after feeding, on Gray Treefrogs, *Hyla versicolor*. Some males were sectioned for histological study and others were prepared as whole mounts. Those used for histological study were killed in 50 °C Bouin's fixative, sectioned at 5  $\mu$ m, and stained with either Masson's trichrome or PAS and Orange G.

Specimens to be used for whole mounts were etherized, decapitated, placed in physiological saline, dissected with tungsten needles that had been sharpened in boiling potassium nitrate, fixed in 2% glutaraldehyde in 0.067 M sodium cacodylate, and rinsed in phosphate buffer (2.26%  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ). Some specimens were then mounted in acid fuchsin-CMC® (Turtox—Chicago); others were stained with aceto-orcein, briefly destained in 70% acid alcohol, dehydrated, cleared in clove oil, and mounted in Clearmount® (Carolina Biological Supply). Specimens mounted in CMC were measured with an ocular micrometer. Average values,  $\pm 2$  standard errors, are given in micrometres for all measurements.

Sperm were prepared for examination with a scanning electron microscope (SEM) by removing the spermatheca from a freshly killed female and crushing it between acid-cleaned cover glasses. The extruded sperm adhered to the cover glasses; they were then fixed with glutaraldehyde, washed with phosphate buffer, dehydrated in a graduated ethanol series, transferred to acetone, air dried, and gold coated.

### OBSERVATIONS

Structures considered in this report consist of the paired testes, vasa deferentia, seminal vesicles, and accessory glands; the ejaculatory duct and penis; paired penis valves, gonocoxites and gonostyles; and sperm.

Testes are located laterally in the 5th abdominal segment and may extend into the posterior  $\frac{1}{4}$  of the 4th segment. Within the abdomen they are of various shapes, from ovoid or conical to elongated fusiform, as a result of pressure from other abdominal structures. When dissected free, testes of *C. appendiculata* are pyriform (Fig. 1), while those of *C. wirthi* are fusiform (Fig. 2, 3). One testis is usually con-

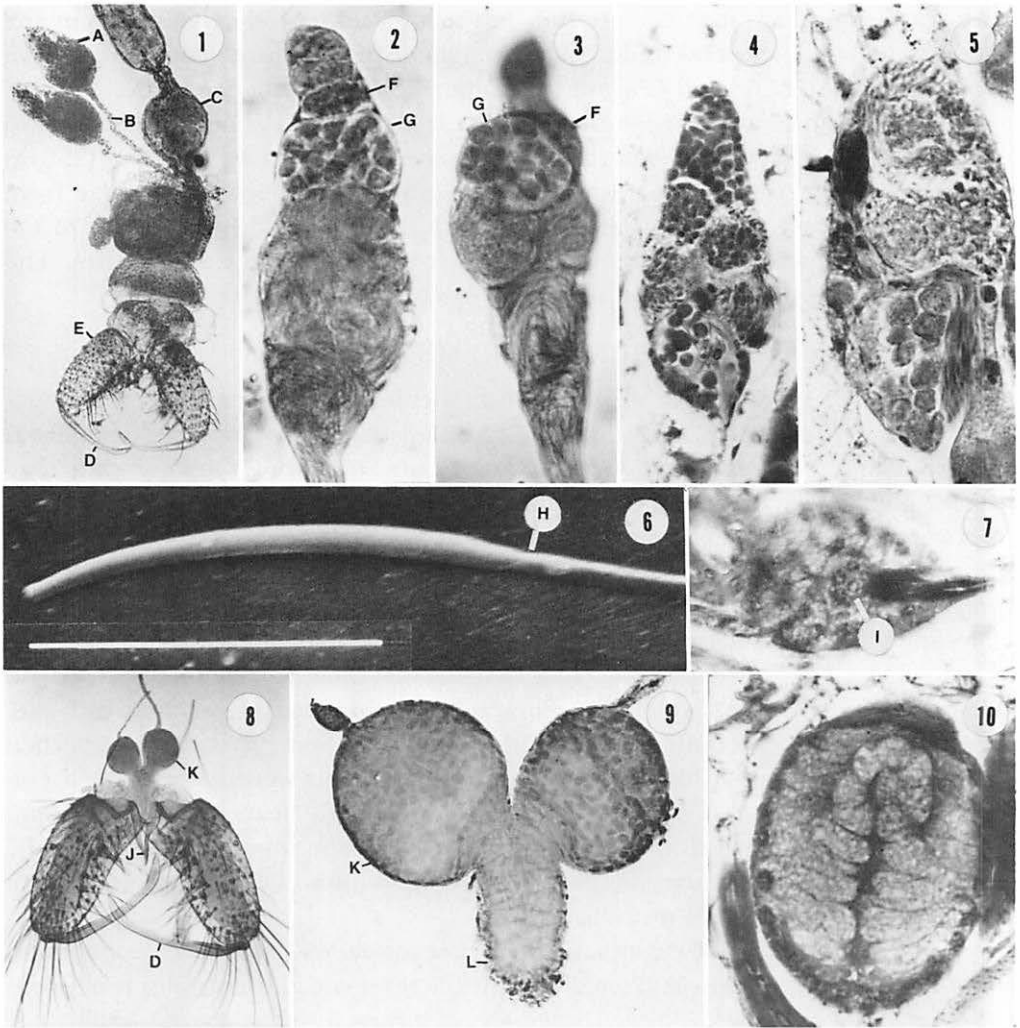


FIG. 1-10. Photomicrographs of reproductive structures of  $\delta$  *Corethrella* from whole mounts and histological sections. 1, whole mount of *C. appendiculata*. 2, 3, whole mounts of testes of *C. wirthi*. 4, longitudinal section of testis of *C. appendiculata*. 5, longitudinal section of testis with sperm tract leading anteriorly (top) past secondary spermatocytes. 6, head of spermatozoan, bar = 5  $\mu$ m. 7, seminal vesicle with terminal valve on surface of accessory gland. 8, accessory glands and external genitalia of *C. appendiculata*. 9, accessory glands and ejaculatory duct. 10, longitudinal section of accessory gland. Structures: A, testis; B, vas deferens; C, intestine; D, gonostyle; E, gonocoxite; F, primary spermatocytes in cyst cell; G, secondary spermatocytes in cyst cell; H, junction of head and flagellum of spermatozoan; I, valve at posterior end of seminal vesicle; J, penis valves; K, accessory gland; L, ejaculatory duct.

spicuously smaller than the other. Eighteen free testes of *C. appendiculata* averaged  $106.2 \pm 0.9$  in length and  $60.3 \pm 1.9$  in width; transition from testis to vas deferens was distinct. Twelve free testes of *C. wirthi* averaged  $133.3 \pm 14.1$  in length and  $37.8 \pm 3.2$  in width. High variability in testis size of this species results from the fact that transition from testis to vas deferens is indistinct, so the posterior limit of the testis is difficult to determine; also, the posterior portion of some testes was elongated, which resulted in a corresponding reduction in maximum width. No free testes of *C. brakeleyi* were obtained.

Each testis consists of a single undivided sac, the equivalent of a follicle in insects with multifollicular testes. The sac is formed by a very thin nucleated epithelial membrane. Internally, the testis is organized anteroposteriorly into zones, beginning with the spermatogonia in the germarium and proceeding through the zones of growth, maturation and reduction, and transformation or spermiogenesis.

Spermatogonia become enclosed in cyst cells and undergo mitotic division; cells so produced in a given cyst remain synchronized during subsequent stages of development of spermatozoa (Fig. 2–4). Cysts move progressively caudally as new ones are formed; at the posterior end of the testis, cyst cells rupture to release fully formed spermatozoa. The spermatozoa migrate anteriorly in a spiral path at the periphery of the testis; in many whole mounts and sections, fully formed spermatozoa, released from their cyst, were observed anterior to spermatids and some secondary spermatocytes (Fig. 5). When the adult emerges, all stages of development, from spermatogonia to spermatozoa, are present in the testes; spermatozoa are also present in the vasa deferentia.

Spermatozoa have long, thin heads that are straight or gently curved (Fig. 6). Examination by SEM shows that the head is only slightly larger in diameter than the adjacent tail. Four specimens of *C. wirthi* spermatozoa, for which measurements were obtained from SEM photographs, had an average head length of  $7.64 \pm 0.98$  and an average total length of  $85.63 \pm 13.64$ .

Vasa deferentia are tubes composed of simple squamous epithelium that is continuous with and histologically identical to the testis sac. They have neither muscle cells nor a chitinous lining. The tubes are uniform throughout their length and contain spermatozoa. They pass through the 6th and 7th abdominal segments; in the 8th segment each unites with an accessory gland (Fig. 11). At this point the vasa deferentia may be termed seminal vesicles for, while not dilated, they are distinguished by the presence of accumulated sperm. Seminal vesicles extend along approximately  $\frac{1}{2}$  the length of the medial surface of the accessory glands. Each terminates in a valve; at this point, densely packed masses of distally-directed spermatozoa heads form a rounded cone (Fig. 7). Posterior to the valve the vasa deferentia continue along the accessory gland and open into the forked ejaculatory duct; sperm are absent from this section.

Paired accessory glands open at the vasa deferentia–ejaculatory duct junction. They are spherical to ovoid structures (Fig. 8, 9), which, in 6 specimens of *C. appendiculata*, averaged  $43.7 \pm 6.5$  in length and  $40.0 \pm 6.8$  in width. In *C. wirthi*, 26 specimens

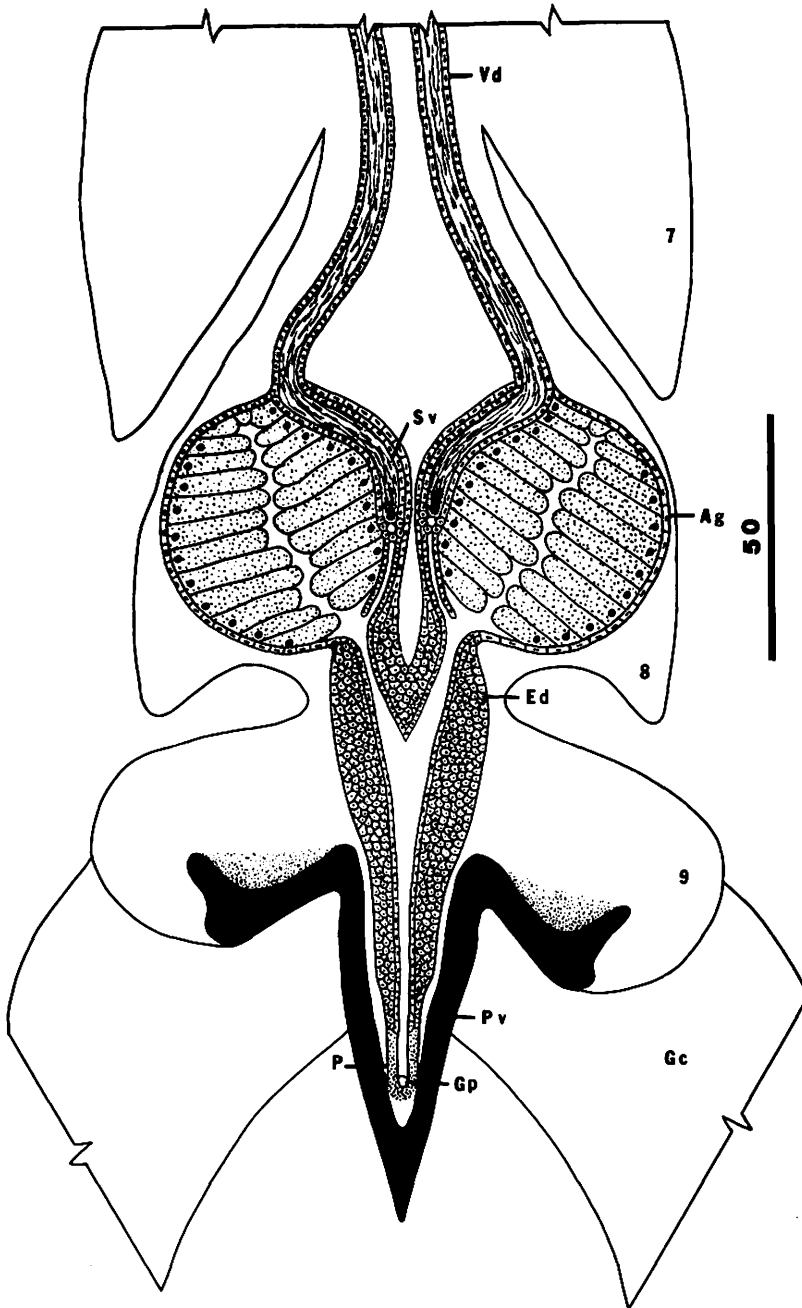


FIG. 11. Diagram of longitudinal section of reproductive structures of male *Corethrella* from whole mounts and histological sections. Scale value is in  $\mu\text{m}$ . Structures: Ag, accessory gland; Ed, ejaculatory duct; Gc, gonocoxite; Gp, gonopore; P, penis; Pv, penis valve; Sv, seminal vesicle; Vd, vas deferens; 7-9, abdominal segments.

averaged  $63.3 \pm 2.5$  in length and  $40.0 \pm 6.8$  in width. They consist of an outer squamous epithelium and inner tall columnar glandular cells with large spherical basal nuclei. A narrow, irregular lumen runs the length of each gland (Fig. 10) and is confluent with the vas deferens–ejaculatory duct junction. The vas deferens–seminal vesicle passes between the glandular and surface epithelium of each accessory gland.

Vasa deferentia open into 2 short branches of the ejaculatory duct. The thick wall of this duct consists of 5 or 6 layers of circumferential smooth muscles and a lining of squamous epithelium. Distally, the lining is continuous with a membranous tube, the penis, which opens by the gonopore.

As in Culicidae, the posterior body of male Chaoboridae, beginning with the 8th abdominal segment, rotates  $180^\circ$  soon after the adult emerges from the pupa. External genitalia arise as appendages of the 9th abdominal segment. They consist of the lateral claspers, which are formed from a large proximal gonocoxite and a long, thin sclerotized gonostyle; and of the sclerotized penis valves, which are morphologically mesoventral processes that arise from a basal plate located at the anterior margins of the gonocoxites. The valves are separated basally but fused at the tip to form a single spine (Fig. 8, 11). A membrane attaches the distal lateral surface of the penis to the valves anterior to their point of fusion; the gonopore is located there. Collectively, the ejaculatory duct, penis, and valves form the aedeagus.

Paired proximal gonocoxites and distal gonostyles, posterolateral extensions of the 9th abdominal segment, constitute the outer claspers or gonoforceps. Numerous setae are present on the gonocoxite; 5 strong ones occur in a row on the topographical middorsal surface, and a stout one is present ventral to the proximal end of the row. *Corethrella wirthi* has a flattened stout spine. Lengths of these 6 setae and their distribution on the gonocoxite are illustrated in Fig. 13.

The terminal gonostyle is heavily sclerotized (Fig. 8). In all 3 species from the eastern United States, a single median seta occurs approximately  $\frac{1}{5}$  the distance from base to tip of the gonostyle. Rarely, 2 setae occur at this site in *C. appendiculata*; and Cook (1956) illustrates 2 setae in *C. laneana*. Short setae are present in a medial depression and on lateral surfaces of the tip of the gonostyle. Distribution and length of the basal seta and of the setae that occur at the tip of the gonostyle are illustrated in Fig. 12. The longest seta at the tip of the gonostyle of *C. wirthi* is branched.

## DISCUSSION

The morphology of internal male reproductive structures of *Corethrella* will be compared to that of the Culicidae as presented by Christopher (1960), Hodapp & Jones (1961), and Lum (1961a). External morphology will be discussed principally in relation to the presentations of Belkin & McDonald (1955), Cook (1956), and Stone (1969) concerning *Corethrella*, and of Hodapp & Jones (1961) concerning Culicidae. Terminology relating to external morphology follows that of Michener (1944) and Cook (1956).

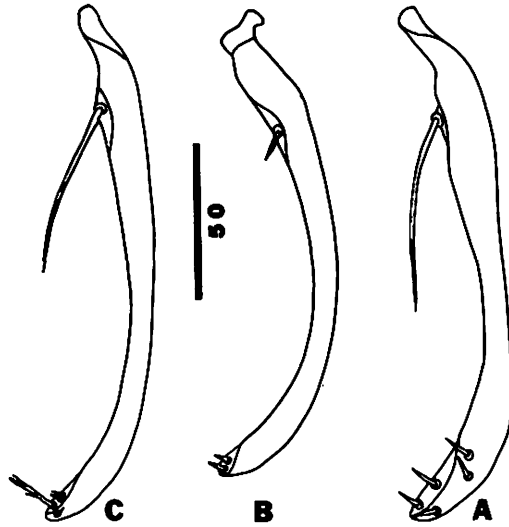


FIG. 12. Gonostyles: A, *C. appendiculata*; B, *C. brakeleyi*; C, *C. wirthi*.

Testes of *Corethrella* differ from those of Culicidae in being located in the 4th and 5th rather than the 6th abdominal segment and in lacking both an apical filament and a thick jacket of fat as described for *Anopheles* Meigen by Hodapp & Jones (1961). They are similar to Culicidae in being composed of a single tube surrounded by a thin nucleated membrane and in being organized internally by cyst cells that surround synchronized groups of sex cells in various stages of development, from primary spermatocytes anteriorly to spermatozoa posteriorly (Snodgrass 1935). This is the pattern of development of most insects (Romoser 1973), although Wensler & Rempel (1962) reported that spermatogenesis in *Chironomus plumosus* L. was completed in the last larval stage, and only mature spermatozoa occurred in adult testes.

*Corethrella appendiculata* has pyriform testes that are shorter and wider than the fusiform testes of *C. wirthi*. In both species, spermatozoa were frequently observed anterior to spermatids and secondary spermatocytes as they make a peripheral circuit of the testes prior to entering the testicular tube; this phenomenon has been described for other insects by Chapman (1969).

Spermatozoa of *Corethrella*, like those of Culicidae, have a gently curved head that is only slightly larger in diameter than the flagellum; there is no external evidence of a middle piece. The head constitutes approximately 8% of the total length, compared to approximately 20% in *Aedes* Meigen.

The terminology of Matsuda (1976) and Christopher (1960) has been followed in describing the tubes that lead from the testes to the ejaculatory duct as vasa deferentia. Matsuda states that the vas deferens leads from the testis in Diptera and that the vas efferens is not differentiated. Hodapp & Jones (1961) consider the term vas deferens to be incorrect because, in Culicidae and all other Diptera, each testis is the equivalent

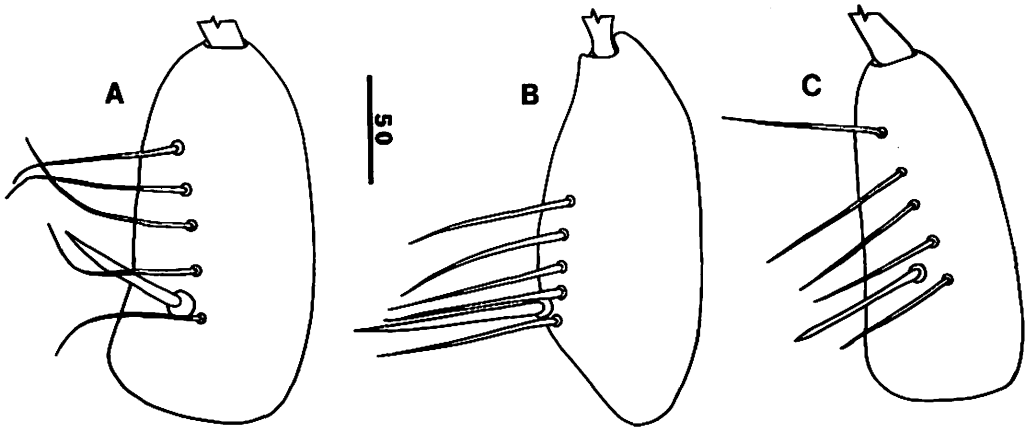


FIG. 13. Gonocoxites: A, *C. appendiculata*; B, *C. brakeleyi*; C, *C. wirthi*.

of a single sperm tube or follicle, and its duct is of mesodermal origin. Therefore, they maintain that the anterior duct is a vas efferens that joins the larger posterior vas deferens of ectodermal origin; their junction is marked precisely by attachment of the accessory gland filament. Contrastingly, Wensler & Rempel (1962) refer to the duct that leaves the testis in Chironomidae as the testicular duct. They state that, since this duct is of mesodermal origin, it may represent either the primary mesodermal vas deferens or the vas efferens.

Each vas deferens may be enlarged into a sperm reservoir or vesicula seminalis (Snodgrass 1935). As described by Hodapp & Jones (1961), the vas deferens of Culicidae are short and muscular; they unite into a common duct that divides into 2 seminal vesicles in the 8th abdominal segment. The seminal vesicles may be separated or they may be externally fused. The seminal vesicles and 2 large lateral accessory glands open into a single short median ejaculatory duct that is surrounded by a thin layer of muscles in some Culicidae and by a thick layer in others.

In *Corethrella* the vasa deferentia are uniform in diameter throughout their length, including the portion designated as the seminal vesicles. The latter occur between the glandular and surface epithelium of the anteromedial surfaces of the separated accessory glands. Although spermatozoa are present in the testes and throughout the vasa deferentia, they are stored principally as a densely packed mass in the seminal vesicles. Hodapp & Jones (1961) described the seminal vesicles of *Anopheles* as that portion of the efferent duct that is attached along its entire dorsolateral aspect to the spherical accessory gland. Lum (1961a) referred to the seminal vesicles as the region of the vas deferens where spermatozoa collect. His photograph of the seminal vesicles, accessory glands, and ejaculatory duct indicates that the structure and relationship of these structures in *Anopheles* is similar to that observed in *Corethrella*.

Accessory glands of *Corethrella*, as in those of *Anopheles*, are separated throughout



their length. They have a central lumen that extends the length of the gland as it does in *Anopheles* (Lum 1961b). There is no filament extending from the tip of the gland to the vas deferens. Lum (1961a) found no filament in *Anopheles*, but Hodapp & Jones (1961) reported the presence of accessory gland filaments in 8 species of culicids belonging to 5 genera, including 2 species of *Anopheles*.

The ejaculatory duct is forked, as occurs frequently in other insects (Snodgrass 1935). Its squamous epithelial lining is surrounded by a heavy layer of circumferential smooth muscles that rapidly decreases in thickness toward the end of the duct. Of 5 genera of culicids examined by Hodapp & Jones (1961) only one, *Anopheles*, was found to possess a heavy layer of muscles on the ejaculatory duct. In *Corethrella* the distal ejaculatory duct and penis are supported by lateral chitinized penis valves; collectively these structures constitute the male copulatory organ or aedeagus (Tuxen 1970).

Spines occur on the gonocoxite and gonostyle; the size, shape, and arrangement of these spines is distinctive for each species of *Corethrella*. As Cook (1956) indicated, the stout spine on the proximal middorsal surface of the gonocoxite extends almost to the distal end of the gonocoxite in *C. brakeleyi* and is shorter and stouter in *C. appendiculata*. In *C. wirthi* this spine is intermediate in length and diameter to that of the other 2 species. Although Cook indicated that no setae are present on the gonostyle of *C. brakeleyi*, a median basal setae, which is approximately  $\frac{1}{6}$  the length of the equivalent structure in *C. appendiculata* and *C. wirthi*, is present. Also, both *C. brakeleyi* and *C. appendiculata* have 2 small setae of approximately equal length in a medial depression at the apex of the gonostyle; these are not mentioned by Cook, but Belkin & McDonald (1955) indicate the presence of 1 basal and 1 apical seta in *C. brakeleyi*. Cook described and illustrated the conspicuous apical setae of *C. laneana*; he indicated that it could be used to distinguish this species from *C. brakeleyi* and *C. appendiculata*. Stone (1969), in his description of *C. wirthi*, mentioned the presence of a very indistinct spine on the tip of the gonostyle. In specimens of *C. wirthi* examined during this study, the seta is quite conspicuous and is branched; 2 small setae occur anterolateral to it. Small setae also occur on the lateral surface of the tip of the gonostyle of *C. appendiculata*.

*Acknowledgments.* This work was supported by a fellowship from the Georgia Southern College Foundation, by an equipment grant from the National Science Foundation, and by a grant from the Georgia Southern College Faculty Research Committee. Dr L.P. Lounibos, Florida Medical Entomology Laboratory, Vero Beach, supplied larvae of *C. appendiculata*.

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