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MORPHOLOGY OF THE SPERMATHECA OF SEVERAL SPECIES OF *DYSMACHUS* (DIPTERA: ASILIDAE) FROM TURKEY: A SCANNING ELECTRON MICROSCOPE STUDY, SECOND PART¹

Mahmut Erbey,² Selami Candan,³ and Abdullah Hasbenli³

ABSTRACT: The spermathecae of four species of the asilid genus *Dysmachus* (*D. cephalenus*, *D. obtusus*, *D. safranboluticus*, and *D. tricuspis*) were studied with both light and scanning electron microscopy (SEM). There are three spermathecae the species examined. The spermatheca consists of receptaculum seminis (reservoir), ductus receptaculum (spermathecal ducts), ejection apparatus, apodemes of furca and furca. Receptaculum seminis of *D. cephalenus*, *D. obtusus*, *D. safranboluticus* and *D. tricuspis* have a swollen structure at the base but it extends apically like a duct. Sperm is kept in receptaculum seminis. In all species, the spermathecal ducts are covered by chitin layer. Spermathecal ducts are not connected with a common duct, but open separately to the bursa copulatrix. The ejection apparatus controls the passage of spermatozoa. The ejection apparatus are covered by strong muscles that regulate its opening and closing. Furca in *D. cephalenus* and *D. tricuspis* divided, with a triangular apodeme, lateral arms broad; Y-shaped in *D. obtusus* and *D. safranboluticus*.

KEY WORDS: spermatheca, *Dysmachus*, Asilidae, Diptera, scanning electron microscopy (SEM)

The spermatheca is an accessory female reproductive organ that occurs in all orders of insects except for Protura and Collembola (Matsuda, 1976). It is a complex organ and it varies greatly in shape and histology between insect groups (Pendergrast, 1957). The spermatheca plays a significant role in sperm storage, copulation, fertilization, and oviposition, among others (Gschwentner and Tadler, 2000).

In general, insect spermatozoa are transferred during copulation and stored by the female in the spermatheca where they remain viable until they are used to fertilize eggs (Davey, 1965). The period of storage ranges in different insects, from hours to months, and in exceptional cases such as the honey bee, sperm may be stored in the spermatheca for years.

In insects, the spermatheca has different structures depending on the insect group (Lay et al., 1999). The spermatheca opens to the vagina through spermathecal ducts (Dallai et al., 1993). The spermatheca usually consists of a receptaculum seminis (receptacle) and a spermathecal duct (ductus receptaculum), which is covered by muscle fibers. While the receptacle stores and nourishes the spermatozoa, the spermathecal duct transports spermatozoa in opposite directions, first to the receptacle at mating and then to the vagina at fertilization. The structural organization of the spermatheca reflects these two functions (Dallai et

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al., 1993). The ejection apparatus is generally located at the middle of the spermathecal ducts, and it controls the passage of spermatozoa by contractions of muscle fibers (Theodor, 1976; Rodriguez, 1994; Pabalan et al., 1996; Yuval et al., 1996; Bloch Qazi et al., 1998; Gschwentner and Tadler, 2000; Fritz et al., 2002).

In most species of Asilidae, the spermatheca consists of the following parts: reservoir; spermathecal duct, which may be short or very long, it may show differentiations in its various parts; a funnel-shaped valve which opens into a differentiated part of the duct (= the furca); and two accessory glands (Theodor, 1976).

The literature contains little information on the spermathecae of the Asilidae although it has been extensively studied by light microscopy. The spermathecae of *Laphria flava* have been described by Reichardt (1929). Osley (1946), Artigas and Papavero (1988a, b), Artigas et al. (1988), and Artigas and Papavero (1991) described with egg as well as the spermathecae of several American species of asilids and gave information on the glands and musculature but did not describe other structures in more detail.

Sixteen species of *Dysmachus* are known from Turkey (Hasbenli and Geller-Grimm, 1999; Geller-Grimm, 2006). The structure of the spermatheca in four species of *Dysmachus* (*D. fuscipennis*, *D. picipes*, *D. praemorsus*, and *D. transcaucasicus*) from Turkey was examined by Candan and Erbey (2006). Also Hasbenli et al. (2006) described a new species (*Leptogaster suleymani*) from Turkey and described the with egg as well as the spermathecal structure. In this study, the spermatheca structure of four additional species of *Dysmachus* has been examined in detail by light and SEM microscopes for the first time.

METHODS

In this study, specimens of *Dysmachus cephalenus*, *D. obtusus*, *D. Safranboliticus*, and *D. tricuspis* were obtained from dried museum material (Zoological Museum of Gazi University). To prepare the spermatheca, abdomens were first soaked in the 10% KOH (potassium hydroxide) for about 24 hours at 30°C to soften by hydrolysis the surrounding tissues. Then, the tissues were removed manually and the spermatheca were exposed and cleaned by fine dissection. Six cleaned spermatheca of each species were placed in glycerine. Observations and measurement were made using stereomicroscope (Olympus SZX12) at a magnification of 40x.

For examination in the scanning electron microscope (SEM), the samples were fixed with 2.5 % glutaraldehyde in sodium phosphate buffer (pH 7.2) for 2 hours. Thereafter, they were washed with same buffer (pH 7.2) for 10 min., and then gradually dehydrated in an increasingly concentrated ethanol series (10 min each in 50-100%). Spermathecae were cleaned, dried, and mounted using a double-sided sticky tape on SEM stubs, coated with gold in a Polaron SC 502 Sputter Coater, and examined with a JOEL JSM 5600 Scanning Electron microscope at accelerating voltage of 20 kV. Spermathecal nomenclature follows Theodor (1976).

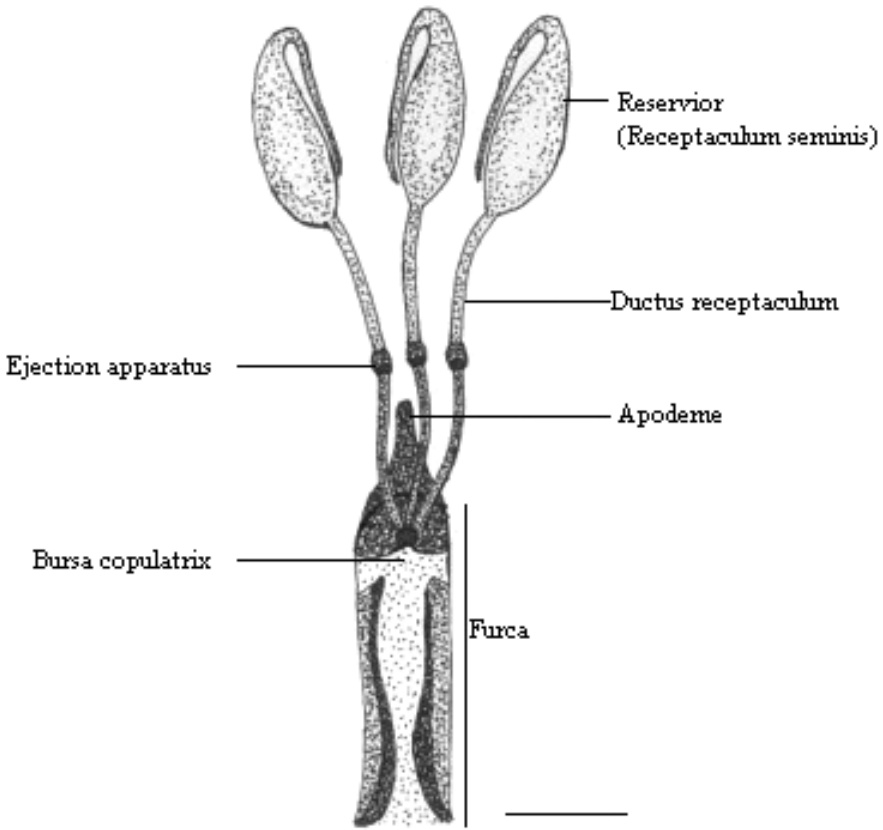


Fig.1. Spermatheca in *Dismachus* (*D. cephalenus*) Scale bar: 0.5 mm.

SYSTEMATIC ENTOMOLOGY

Dismachus cephalenus Loew, 1871

(Figs. 2 a- d)

The receptacle is very wide at the base and extends cylindrically towards the anterior. In anterior, spines are towards the receptacle (Fig. 2a). The length of receptaculum is 1.7 mm; its diameter, 0.3mm. Posteriorly, the receptacle is connected to spermathecal duct. The surface of the receptacle is covered by layer of chitin. The spermathecal ducts do not connect, instead they open separately into bursa copulatrix. The ducts are covered by chitin in a curly structure (Fig. 2b). The length of ducts between receptacle and ejection apparatus is 0.65 mm; its diameter, 0.03mm. The ejection apparatus is located at the middle of spermathecal duct. The ejection apparatus is a wider, swollen structure than the spermathecal duct (Figs. 2a, c). The spermathecal ducts are connecting to the anterior furca (Fig. 2d). The length of the ducts between the ejection apparatus and furca is 1.15mm; its diameter is 0.02mm. The length of furca is 1.55 mm; its diameter

0.28mm. In the posterior portion of the furca, the lateral signs are clearly evident. The bursa copulatrix extends anteriorly. The spermathecal ducts open to between the extension of bursa copulatrix and the apodeme (Fig. 2d). The furca is strongly chitinized and divided, bearing a triangular apodeme; the lateral arms are broad. Apodeme is chitinized and triangular shaped. The length of apodeme is 0.8mm. The portion of the apodeme that is connected to the furca is wide while the rest is thin (Fig. 2d).

***Dysmachus obtusus* Becker, 1923**

(Figs. 3 a- d)

The receptacle is very wide at the base and extends cylindrically. In the anterior, spines are towards receptacle (Fig. 3a). The length of receptaculum is 0.85mm; its diameter, 0.14 mm. The surface of receptacle is covered by layer of chitin. The spermathecal ducts are not connected but separate from the bursa copulatrix. The ducts are covered by chitin in curly structure. Groove-like structures are present on the surface of ducts, extending parallel to the longitudinal axis of duct (Fig. 3b). The length of duct between receptacle and ejection apparatus is 0.3mm; its diameter, 0.02mm. The ejection apparatus is wider, more swollen than the spermathecal duct. The ejection apparatus is nearer the receptacle than to the furca (Figs. 3a, c). The length of the ejection apparatus is 0.05mm; its diameter 0.03mm. The ejection apparatus is strongly chitinized and has overlapping layers of chitin (Fig. 3c). The length of duct between ejection apparatus and furca is 1.3mm; its diameter 0.01mm. The furca is strongly chitinized. The length of furca is 1.15mm; its diameter 0.3mm. The furca are wide anteriorly, narrow posteriorly and V shaped (Figs. 3a, d). The portion of apodeme that is connected to the furca is narrow and the rest of apodeme is wide. The apodeme is strongly chitinized and triangular shaped (Fig. 3d). The length of the apodeme is 0.45mm.

***Dysmachus safranboluticus* Hasbenli and Geller-Grimm, 1999**

(Figs. 4 a- d)

The receptacle is a little wide but it becomes narrow anteriorly. Anteriorly, the spines point toward the receptacle (Fig. 4a). The length of receptaculum is 1.1mm; its diameter 0.14mm. The surface of the receptacle is covered by a layer of chitin. The length of the spermathecal duct between the receptacle and the ejection apparatus is 0.3mm, its diameter 0.05mm. The ducts are covered by chitin forming a curly structure (Fig. 4b). The ejection apparatus is wider and more swollen than the spermathecal duct (Figs. 4a, c). The length of ejection apparatus is 0.05mm, its diameter 0.03mm. The ejection apparatus is strongly chitinized with overlapping layers of chitin. (Fig. 4c). The length of duct between the ejection apparatus and the furca is 1.5mm, its diameter 0.01mm. The furca is strongly chitinized (Fig. 4a). The length of furca is 1.4mm, its diameter 0.5mm. It is wide anteriorly, narrow posteriorly and V shaped. The portion of the apodeme connected to the furca is narrow and other part of apodeme is wide (Figs. 4a, d). The apodeme is strongly chitinized and triangular-shaped (Fig. 4d). The length of apodeme is 0.46mm.

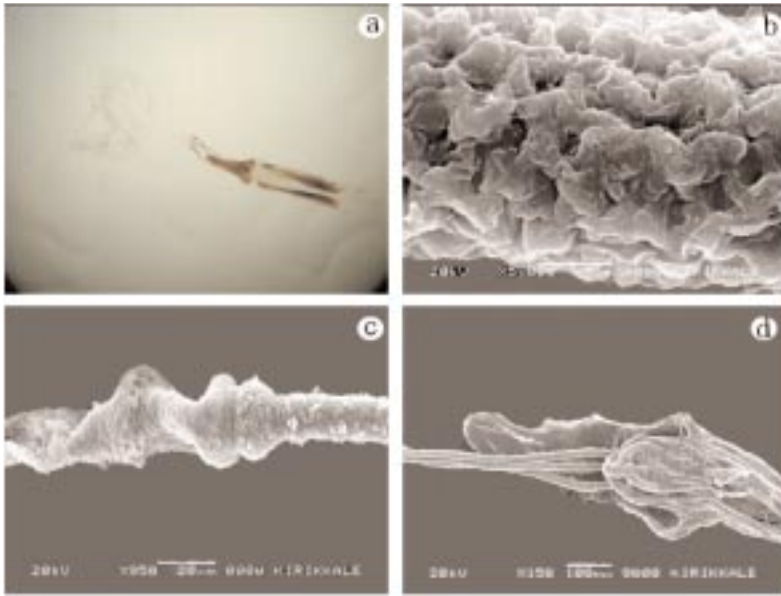


Figure 2 a-d. Light and SEM micrographs of spermatheca of *D. cephalenus*. a. Spermatheca general view (Light microscope, scala bar: 0.5mm). b. Spermathecal ducts. c. Ejection apparatus. d. Apodeme and anterior of furca.

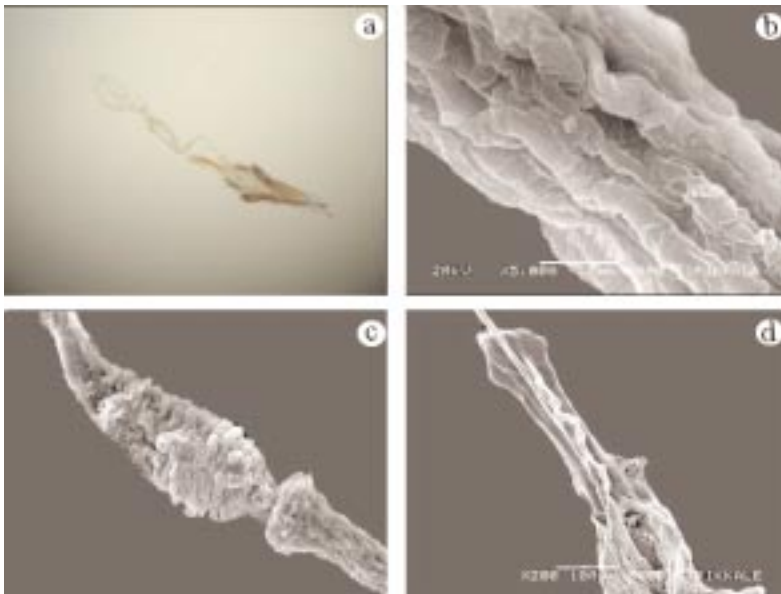
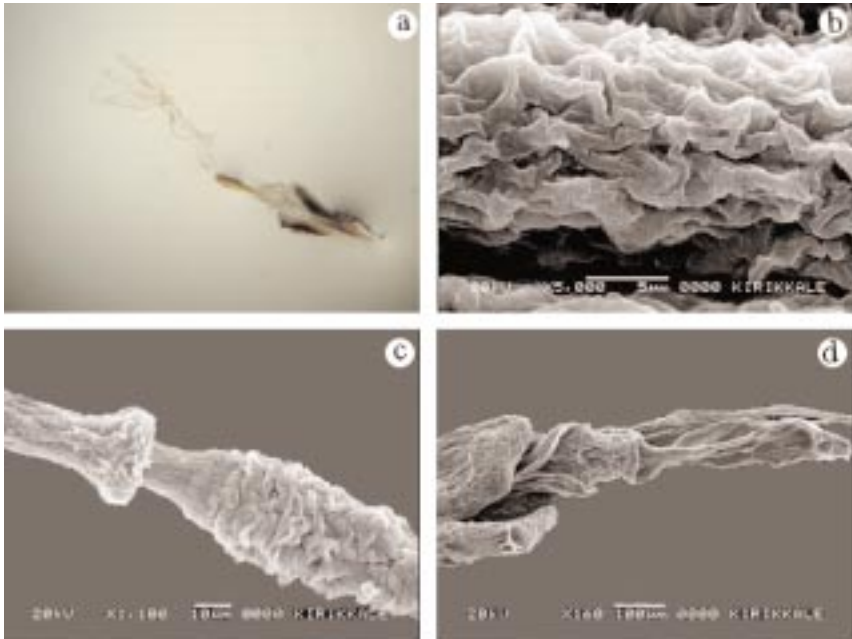


Figure 3 a-d. Light and SEM micrographs of spermatheca of *D. obtusus*. a. Spermatheca general view (Light microscope, scala bar: 0.5mm). b. Spermathecal ducts. c. Ejection apparatus. d. Apodeme and anterior of furca.



Figures 4 a-d. Light and SEM micrographs of spermatheca of *D. safranboluticus*. a. Spermatheca general view (Light microscope, scala bar: 0.5mm). b. Spermathecal ducts. c. Ejection apparatus. d. Apodeme and anterior of furca.

Dysmachus tricuspis (Loew, 1848)

(Figs. 5 a-d)

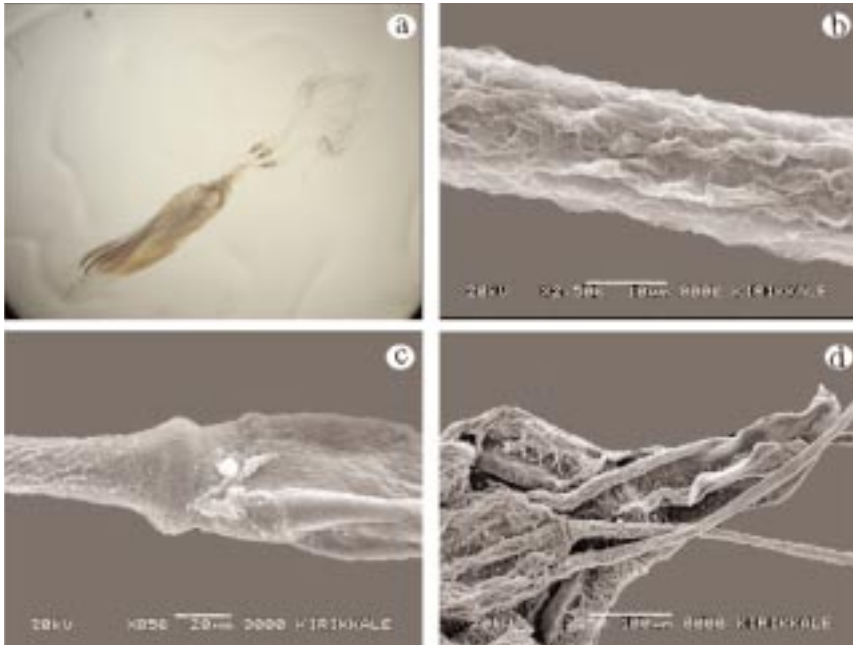
The receptacle is very wide at the base and extends cylindrically anteriorly. Anteriorly, the spines point towards the receptacle (Fig. 5a). The length of receptaculum is 2.4mm; its diameter 0.3 mm. Posteriorly, the receptacle is connected to the spermathecal duct. The surface of receptacle is covered by a layer of chitin. The spermathecal ducts connect separately to the bursa copulatrix. The surroundings of ducts are covered by chitin in curly structure (Fig. 5b). The length of duct between the receptacle and ejection apparatus is 0.65mm; its diameter 0.05mm. The ejection apparatus is located at the middle of spermathecal duct. The ejection apparatus is wider and more swollen than the spermathecal duct (Figs. 5a, c). The length of ejection apparatus is 0.16mm; its diameter 0.08mm. The spermathecal ducts are connected to the anterior of furca. The length of duct between the ejection apparatus and furca is 0.8mm; its diameter 0.02mm. The furca is strongly chitinated and divided, and the lateral arms are broad (Fig. 5d). The length of furca is 2.4mm; its diameter 0.62mm. The apodeme is chitinated and triangular-shaped. The length of apodeme [0.4mm] is short and wide (Fig. 5d).

DISCUSSION

Spermathecae are ectodermal glands opening into the anterior tract of the common oviduct of female insects. It plays a significant role in many functions such as sperm storage, copulation, fertilization and oviposition (Gschwantner and Tadler, 2000). In the vast majority of insects, spermatozoa are transferred to the genital area of female during copulation. Thereafter, spermatozoa are stored in the spermatheca where they remain until they are used for fertilization (Davey and Webster, 1967; Villavaso, 1975).

In insects, accessory glands of the spermatheca produce secretions that flow into the lumen of the spermathecal duct or to the receptacle, contributing to the protection of the spermatozoa (Villavaso, 1975; Dallai, 1975). Fertilization occurs on the apical part of the bursa copulatrix. The eggs are inseminated by spermatozoa and fertilized eggs are oviposited.

All insects, except the orders Protura and Collembola, have spermatheca (Matsuda, 1976). Some groups of Diptera have one (e.g. Drosophilidae), two (e.g. Tephritidae, a few members of the Asilidae and Muscidae) or three spermathecae (e.g. some of Muscidae, most members of Asilidae and Bombyliidae) (Couri 2004; Theodor 1976, 1983, Hasbenli et al., 2006). Three spermathecae are common in flies. Theodor states "There are three spermathecae in all the Asilidae examined except *Proctacanthus*, *Eccritosia*, and *Myaptex*," in which there are



Figures 5 a-d. Light and SEM micrographs of spermatheca of *D. tricuspis*. a. Spermatheca general view (Light microscope, scala bar: 0.5 mm). b. Spermathecal ducts. c. Ejection apparatus. d. Apodeme and anterior of furca.

only two. In this study, we observed that the examined species of *Dysmachus* have three spermatheca (Figs. 2a, 3a, 4a, 5a). All three spermathecae are usually of the same form, but the median spermatheca is of slightly different form in some species (*Polyphonies*). The only exceptions so far found are *Leptogaster* and *Euscelidae* in which the median spermatheca is markedly different from the lateral spermatheca in some species (Theodor, 1976; Hasbenli et al., 2006).

In most examined *Dysmachus* species, the spermatheca is basally swollen but apically is extended and narrowed, like a duct (Figs. 2a, 3a, 4a, 5a). According to Theodor (1976), there are two different types of receptacle-reservoir in *Dysmachus*, one type is a cylindrical or tubular; the second type is very wide. In this study, examined species of *Dysmachus* (*D. cephalenus*, *D. obtusus*, *D. safranboluticus* and *D. tricuspis*) have wide types. In all species, the structure of receptacle seems to be similar throughout, but the length of receptacles varies between species (Figs. 2a, 3a, 4a, 5a). Candan and Erbey (2006) stated that there are two different types of receptacles in some *Dysmachus* species. Cylindrical, as in *D. fuscipennis* and rather wide as in *D. praemorsus*, *D. picipes*, and *D. transcaucasicus*.

In different insect groups, the surface of the receptacle and of the spermathecal duct is covered by a layer of chitin, especially in *Ceratitis capitata* and *Dacus oleae* (Tehpirtidae: Diptera), *Locusta migratoria* (Acrididae: Orthoptera), and *Aedes aegypti* (Culicidae: Diptera) (Clements and Potter, 1967; Dallai et al., 1993; Lay et al., 1999). In *Dysmachus*, we determined that the surface of the receptacle and of the spermathecal ducts is also covered by a curly chitin structure. However, the character of the surface is different among species (Figs. 2b, 3b, 4b, 5b). The ejection apparatus differs in the four investigated species. In *D. obtusus* and *D. safranboluticus*, it is strongly chitinized or there are overlapping layers of chitin (Fig. 3c, 4c). Chitin is accumulated in middle part of ejection apparatus, but in *D. cephalenus* and *D. tricuspis*, the ejection apparatus is located at the middle of the spermathecal duct. The ejection apparatus is very wide and more swollen than the spermathecal duct (Figs. 2a, 5a).

In this study, we observed that the spermathecal duct is connected to the anterior portion of the furca. The bursa copulatrix occurs in the anterior portion of the furca. Spermathecal ducts are not connected with a common duct but open separately to the bursa copulatrix. In all species, spermathecal duct connections to bursa copulatrix are different from each other (Figs. 2d, 3d, 4d, 5d). In *D. obtusus* and *D. safranboluticus* spermathecal ducts are similar structurally (Figs. 3b, 4b).

Theodor (1976) noted that the furca has apodeme in some group of Diptera, but he does not provide information on the functional significance of apodeme. Members of the Laphriinae and the Dasypogoninae (Asilidae) have a short and wide apodeme but members of Asilinae have a long apical apodeme (Theodor, 1976). In all species, we observed that apodeme of furca has a different structure (Figs. 2d, 3d, 4d, 5d).

In *D. cephalenus* and *D. tricuspis*, the apodeme is wide basally, but apically it is narrow and triangular. A swollen pillow-like structure is present at the base of the apodeme. This structure probably supports the spermathecal ducts (Figs. 2d, 5d). In *D. obtusus* and *D. safranboluticus*, the apodeme is narrow basally and triangular (Figs. 3d, 4d).

The furca is a modified sternite 9. It consists of a 'frame' with a membrane between the lateral arms in which the ducts, or the common duct, opens. It varies widely in form. It is more or less U or V shaped in the Laphriinae and Dasypogoninae; it consists of only lateral arms connected by a membrane in *Leptogaster* and *Dioctia*. The furca of the Asilinae differs markedly. It consists of a usually long, slender frame, with a distal apodeme of varying form and length and a posterior median sclerite between the posterior ends of the lateral arms (Theodor 1976). In this study, the furca of *D. cephalenus* and *D. tricuspis* is divided and the lateral arms are broad (Figs. 2a, 5a), but it is Y shaped in *D. obtusus* and *D. safranboluticus* (Figs. 3a, 4a).

The structure of the spermathecae has important systematic characters in some groups of insect. It has been used successfully in the systematics of fleas, and may be useful to elucidate taxonomic and phylogenetic relationships among asilids.

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ERRATA

The recently published paper, entitled “New state record of the mayfly *Baetisca laurentina* ...” by Tarter et al. [2007 *Entomological News* 118(4), pp. 407–416] was published with several mistakes in the correspondence between the figures and the legends. Herein, these mistakes are corrected.

Figure 1. County distribution records of *Baetisca berneri* in West Virginia.

Figure 2. County distribution records of *Baetisca carolina* in West Virginia.

Figure 3. County distribution records of *Baetisca gibbera* in West Virginia.

Figure 4. County distribution records of *Baetisca lacustris* in West Virginia.

Figure 5. County distribution records of *Baetisca laurentina* in West Virginia.

Figure 6. County distribution records of *Baetisca rubescens* in West Virginia. Figure 6 was printed twice.