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Chorionic morphology of mayfly (Ephemeroptera) eggs of the Lower Rio Grande Valley

Maria D. De Leon
University of Texas-Pan American

CHORIONIC MORPHOLOGY OF MAYFLY (EPHEMEROPTERA) EGGS OF THE LOWER RIO GRANDE VALLEY

A Thesis

By

MARIA D. DE LEON

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Approved as to style and content by:

Brad C. Henry Jr.
Brad C. Henry Jr.
Chair of Committee

Anxiu Kuang
Anxiu Kuang
Committee Member

Kenneth R Summy
Kenneth Summy
Committee Member

May 2003

ABSTRACT

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External egg morphology of ten species of Lower Rio Grande Valley (LRGV) mayflies are compared using scanning electron microscopy (SEM) and a taxonomic key to the eggs is provided. The eggs of five species are described here for the first time. Preserved female adults or subadults were critical-point dried using liquid CO_2. The eggs were removed and placed on metal stubs for sputter coating with gold palladium. The coated eggs were then observed and photographed by SEM. The morphological features described were the chorionic sculpturing, polar caps, accessory attachment structures, and micropyles. This is the first mayfly egg comparison and key to the genera of mayfly eggs from the LRGV.

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Last, but certainly not least, I thank my husband Francisco Cadena Jr. for his support and tolerance of the long hours I spent in the SEM lab, and my parents Simon and Ismelda for their understanding and support.

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INTRODUCTION*

Mayflies (Ephemeroptera) live in all freshwater, including rivers, lakes, permanent and temporary ponds, canals and ditches. They are important components of freshwater ecosystems and are used extensively in monitoring water quality and environmental degradation (McCafferty 1983). Life span varies from a month to a year (Edmunds, Jensen & Berner 1976) and except for a day, this is spent underwater as nymphs or larvae (Leahy 1987). Larvae are known from all functional feeding groups (Merritt & Cummins 1996) but are primarily scrapers, gatherers, and filterers. Larval instar number varies between and within species. *Baetis* species are known to have up to 43 instars (Edmunds, Jensen & Berner 1976). The subimago or subadult stage emerges from the water; this stage is unique to mayflies and is an ancestral characteristic of the early lineages of insects (Edmunds, Jensen & Berner 1976). Most species will molt from the subadult to the adult stage within a day. There are a few species in which the females do not molt to the adult stage, but lay eggs as subadults (Edmunds, Jensen & Berner 1976). All mayfly adults and subadults have atrophied mouthparts and do not feed. Males form aerial swarms and mating is aerial. Oviposition occurs on the water surface and by aerial release close to the surface (McCafferty 1983). Oviposition may be of a single mass of eggs. Oviposition strategies are highly important to mayfly reproductive success (Butler 1984). Swarming, mating, and oviposition all occur within a few hours. Death soon follows (B. Henry, personal communication 2003).

* Format follows the **Entomological News.**

Mayfly taxonomy is based on morphological characters from all life stages. This includes the egg stage, but the eggs of only a few species are known. Elaborate chorionic structures and patterns are species specific and important in mayfly phylogenetic studies and classification (Mazzini and Gaino 1985). Chorionic egg data has been used to confirm the existence of a new species *Acentrella almhodes* of the family Baetidae, found in Spain (Alba-Tercedor & Almai 1999), and of *Rhithrogena grischuna* (Heptageniidae) found in eastern Switzerland (Sartori and Oswald 1988).

Bengtsson (1913), Morgan (1913) and Degrange (1960) provided the first useful information of mayfly eggs using compound microscopes. Koss (1968) and Koss & Edmunds (1974) egg descriptions were made using a phase contrast light microscope. They provided descriptions of mayfly egg morphology for the eggs of most mayfly families and many species. The terminology used by Koss & Edmunds (1974) to identify the morphological traits was used here. The morphological traits include the egg's chorionic sculpturing, polar caps, accessory attachment structures, and micropyles. Egg polar caps and accessory attachment structures both function as substrate attatchment devices and prevent excessive drift.

Scanning electron microscopy (SEM) has been used in egg morphology and studies beginning in 1982 (Malzacher 1982). Since then, other studies have identified eggs from all over the world using SEM (Malzacher 1982, Mazzini & Gaino 1985, Studdemann et al. 1995, and Gaino and Rebora 2001).

Polar Caps. Attachment structures that are located at either pole of the egg are called polar caps. Degrange (1960) previously referred to the polar caps as epithemata (singular *epithema*), and classified them into four groups. Koss (1968) further modified

these groups and established five categories, types I-V. Type I is a non-coiled cap formed by short compacted threads. Type II is made up of short, non-coiled, compacted threads arranged in several units. Type III is a coiled cap made up of multiple threads that coil together about the pole. Type IV polar caps are composed of 1-3 single threads coiled independently at the pole. The type V polar cap is made up of knob-terminated coiled threads (KCTs).

Accessory attachment structures. The attachment structures under this category are those that are located laterally on the egg, or that do not conform to the description of polar caps. Accessory attachment structures include knob-terminated coiled threads (KCTs) which are made up of coiled threads with a centrally located knob. Other smaller attachment structures are tubercles, peg-like structures and, sucker-like disks. Additionally, the presence of an adhesive layer covering the chorion aids the attachment structures or may occur instead of accessory attachment structures. The adhesive layer has been characterized here as thin and molded when some of the chorionic structures could be distinguished beneath; otherwise, it was described as thick. With the exception of *Callibaetis* eggs, most of the specimens studied here have adhesive layers.

Micropyles. These structures allow the sperm to enter the egg, and are characterized by a micropylar canal, micropylar opening and in some eggs a sperm guide (Koss 1968). A sperm guide is a depression in the chorion that helps channel the sperm towards the micropylar canal. Degrange (1960) and Koss & Edmunds (1974) separated the micropyles into three categories: tagenoform, funnelform, linear. Southwood (1956) described a fourth category known as a micropylar process. These studies categorized micropyles based on the position of the micropylar canal. However, because the SEM is

limited to observation of external morphology, some of the internal micropylar canals could not be seen. Therefore, terminology will be based on either the position of the micropylar opening or the visible micropylar canals. A tagenoform micropyle (from the Greek. Word *tagenon* meaning frying pan) is one in which the micropylar opening is located to one side of an oval sperm guide. A funnelform micropyle has the micropylar opening located at the center of the sperm guide, or if a sperm guide is lacking, the micropylar opening is relatively parallel to the chorion. A linear micropyle either follows an elongate sperm guide or, when a sperm guide is missing, the micropylar opening is approximately perpendicular to the chorion. A micropylar process is characterized by lack of sperm guide with the micropylar canal protruding from the chorion through a chorionic projection.

Most of the eggs of the Lower Rio Grande Valley (LRGV) mayfly species are unknown. SEM micrographs will allow identification of chorionic structures and patterns, and provide additional egg data for several mayfly species.

MATERIALS AND METHODS

Mayfly adults and subadults studied were collected from various sites in the Lower Rio Grande Valley (LRGV) and placed in vials containing 70-90% ethanol. Table 1 lists the LRGV species as identified by Dr. Brad C. Henry (University of Texas-Pan American in Edinburg, Texas.

Females of each species were isolated in separate vials containing 95% ethanol. These females were further dehydrated in a series of ethanol solutions of increasing concentrations (95% and 100%). Each ethanol concentration was replaced three times at 20 minute intervals. Following dehydration, eggs were critical-point dried with a SAMDRI 780B critical-point dryer using liquid CO_2. The female abdomen was then dissected with a clean razor blade and eggs were dusted onto numbered stubs containing carbon adhesive. Eggs were then sputter coated with gold-palladium using a Denton Vacuum Sputter Etch coating unit (Appendix A). The coated eggs were viewed and photographed using a LEO VP 435 scanning electron microscope. Adjusting the image to its maximum resolution was required for image analysis. Chorionic structures were observed and differences between genera and species noted.

The images of the eggs will be stored in a CD for future use in the aquatic entomology lab, and the stubs with dry specimens will be kept in a dry chamber at the electron microscopy lab located in the University of Texas-Pan American in Edinburg.

Table 1. Classification of mayflies known in the Lower Rio Grande Valley.*

Family	Species
Baetidae	*Fallceon quilleri*
	Callibaetis montanus
	Camelobaetidus mexicanus
Caenidae	*Caenis sp.*
Ephemeridae	*Hexagenia limbata*
Heptageniidae	*Stenonema femoratum*
Leptophlebiidae	*Thraulodes gonzalesi*
	*Leptophlebia bradleyi***
Polymitarcyidae	*Campsurus decoloratus*
Leptohyphidae	*Tricorythodes sp.*

*Specimens for list provided by Dr. B. Henry

** Species not known to occur in the LRGV

RESULTS & DISCUSSION

Baetidae Leach, 1815

The baetids are known as the small minnow mayflies because of their small streamlined bodies as nymphs. They inhabit both lentic and lotic waters and can live at extremely high altitudes (McCafferty 1983). As the largest family over 150 species in 23 genera are known in North America. Although a relatively widespread family, only four genera are known in the LRGV. The eggs of only a few of these have been studied.

***Fallceon quilleri* (Dodds), 1923**. Eggs are ovoid and elongate with a thick adhesive layer molded to their chorion (Fig. 1). In areas where this layer was removed a tuberculate netlike chorion could be seen (Fig. 2). Each tubercle appeared to be surrounded by tiny fibers interconnected forming a small circular net with a centrally located tubercle. This arrangement forms the raised ridges of that give the egg a golf ball appearance. There are no polar caps present. Only one tagenoform micropyle could be seen with a small sperm guide (Fig. 3).

Previous studies on the eggs of *Fallceon longifolius* by Kluge (1992) identified a porous chorionic sculpturing similar to that found in *Fallceon quilleri*. However, chorionic sculpturing of *Fallceon quilleri* is defined as netlike (not porous) according to the terminology defined by Koss & Edmunds (1974). Studies of *Baetis bicaudatus* and *Baetis sp.* by Koss & Edmunds (1974) revealed this netlike mesh, but instead of tubercles

found in *Fallceon quilleri* they found centrally located coiled threads. This is the first report of *Fallceon quilleri* egg morphology.

Specimens examined. TX: Hidalgo County (1989): 1 female imago.

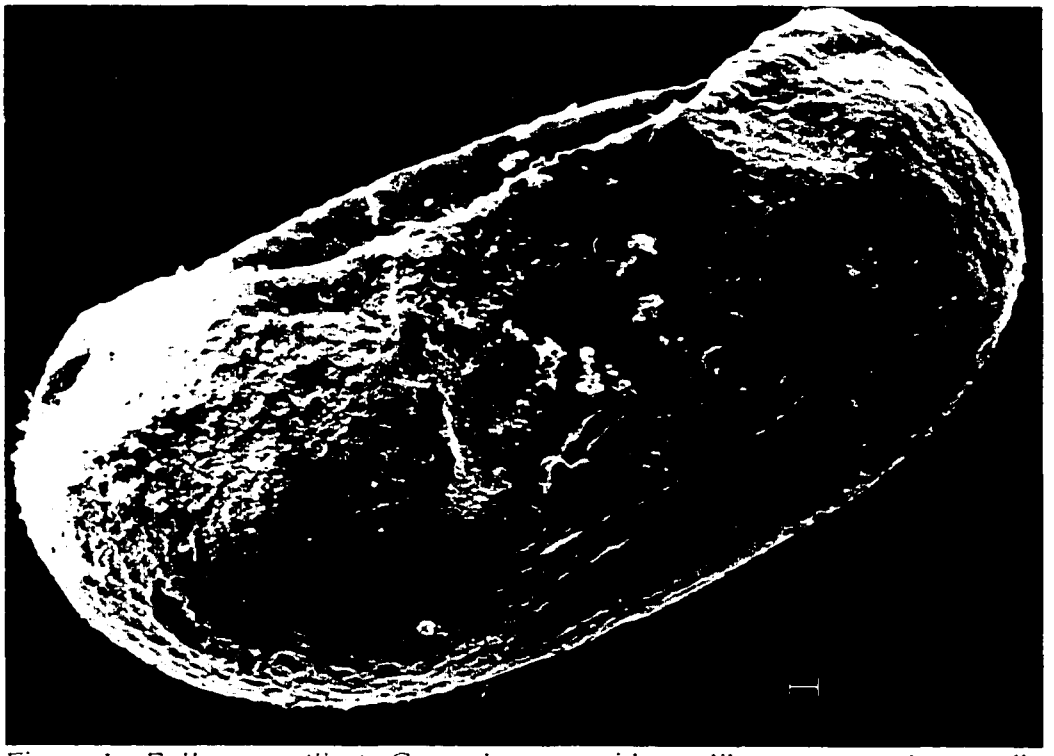

Figure 1. *Fallceon quilleri*. General aspect with net-like pattern under an adhesive layer. Bar represents 3µm.

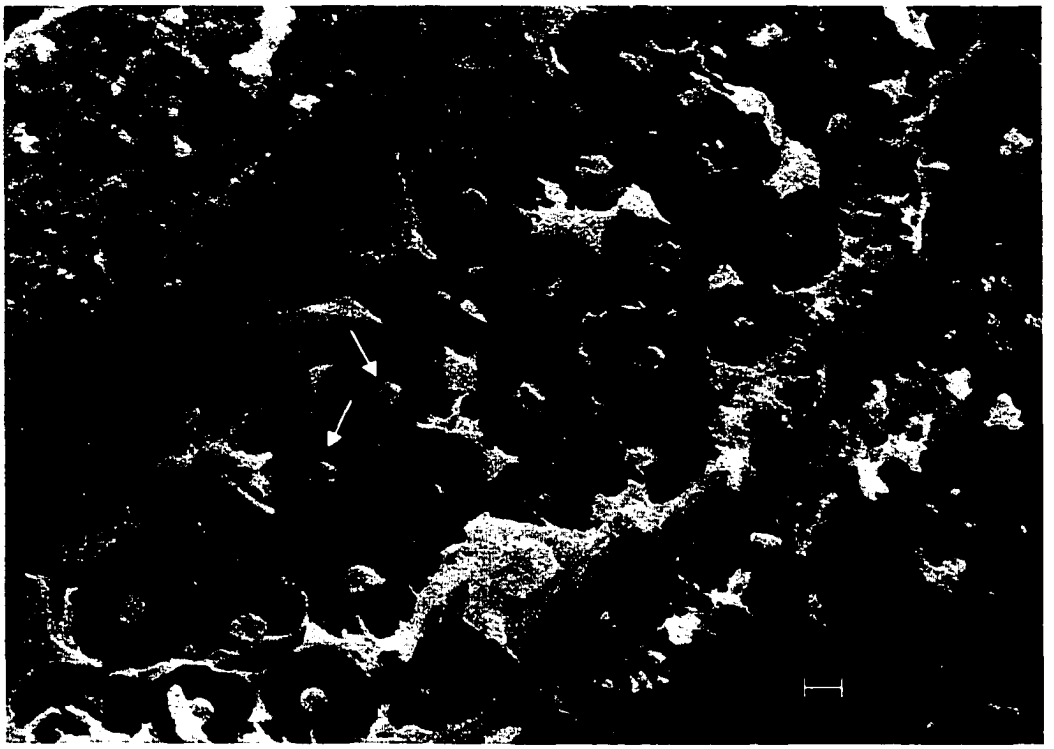

Figure 2. *Fallcon quilleri*. Net-like chorionic pattern with central tubercles (arrows) beneath the adhesive layer. Bar represents 1 µm.

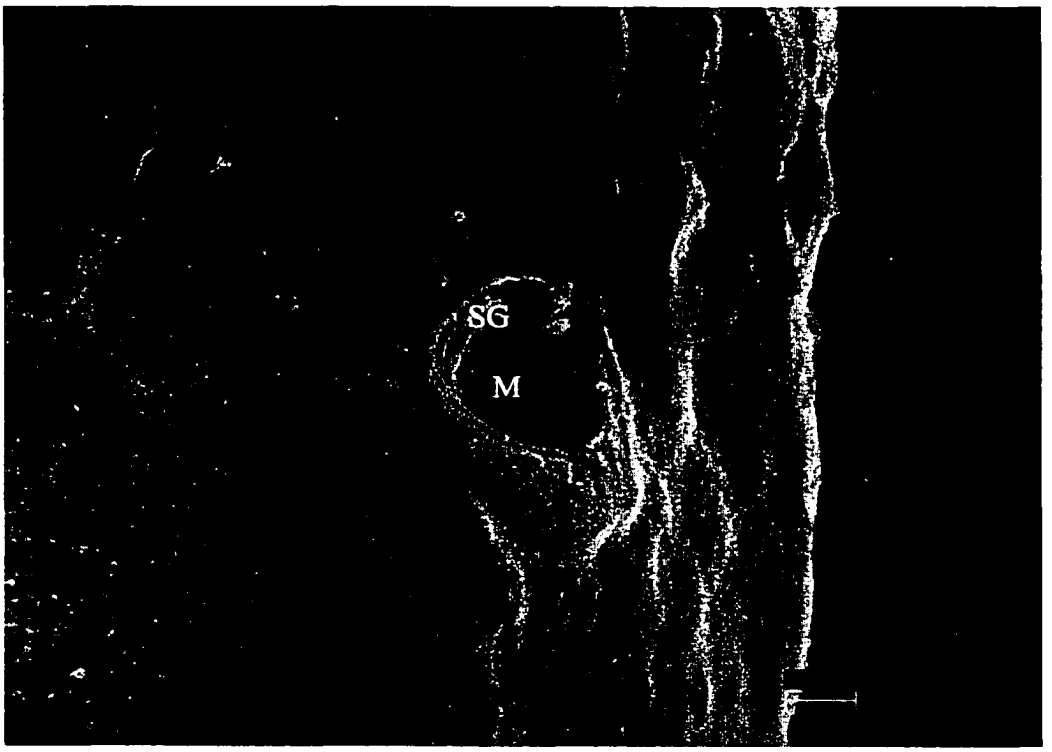

Figure 3. *Fallcon quilleri*. Tagenoform micropyle (M) with small slightly oval sperm guide (SG). Bar represents 1 µm.

***Callibaetis montanus* Eaton, 1885**. The chorion morphology of these eggs could not be identified because all of the eggs were in an advanced developmental stage (Fig. 4,5). Only a loose layer could be seen surrounding the fertilized egg (Fig. 4). It is not known whether this layer is an adhesive layer or the chorion itself. This genus is one of the few that are known to be ovoviviparous. No other studies have been done to study the chorion of this species.

Callibaetis montanus Eaton primarily inhabit lentic environments, and as such it was not expected that the egg chorion would have much sculpturing. They are one of two species of pond mayflies in the LRGV (B. Henry, personal communication 2003). They are known to inhabit a wide area extending from Arizona to Nicaragua (Lugo-Ortiz & McCafferty 1996). One reason for their wide distribution is the long female lifespan of approximately two weeks (B. Henry, personal communication 2003).

Specimens examined. TX: Hidalgo County (1990); 2 female imagos.

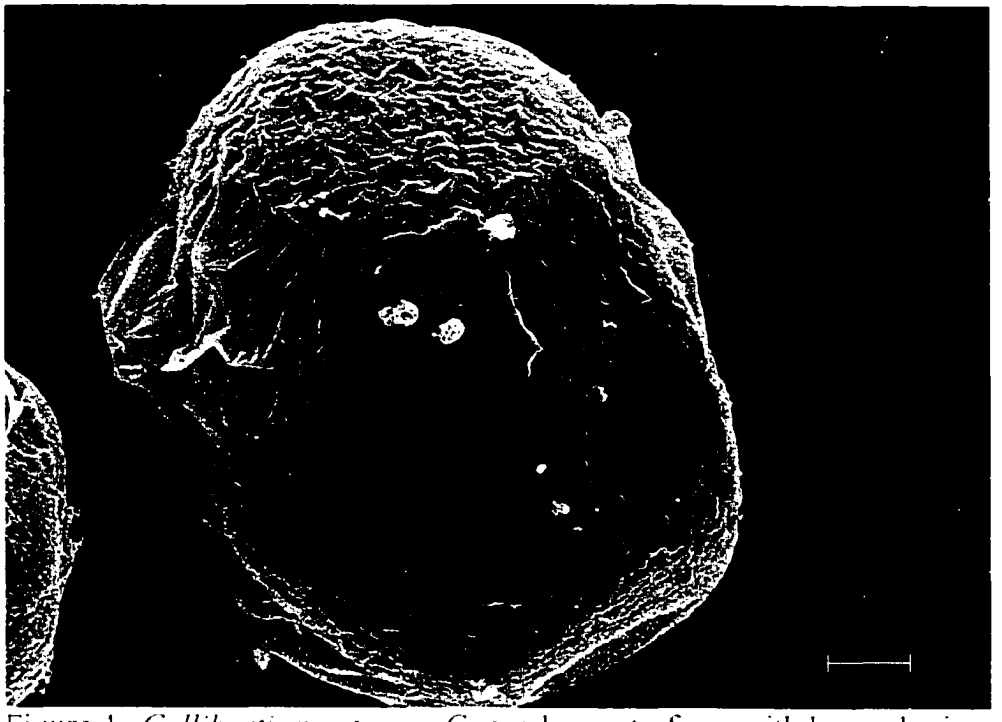

Figure 4. *Callibaetis montanus*. General aspect of egg with loose chorion. Bar represents 10 µm.

Figure 5. *Callibaetis montanus*. General aspect of almost fully developed larvae. Bar represents 10 µm.

***Camelobaetidius mexicanus* (Traver & Edmunds), 1968.** These eggs have a rectangular shape with a thin layer around the chorion. Althought the properties of this layer are unknown, it appears to have many sucker-like disks arranged at random. Removal of this layer reveals a peg-like chorion (Fig. 6). The pegs are formed by KCTs arranged in rows around the egg (Fig. 7). Each row is separated by thin fibers along the circumference. The spaces between KCTs are wrinkled giving the egg a fibrous appearance. No polar caps were seen. The eggs have one tagenoform micropyle with a well-defined sperm guide. This is the first report of egg morphology for a species of *Camelobaetidius* genus.

Specimens examined. TX: Hidalgo County (1987 & 2001); 2 female imagos.

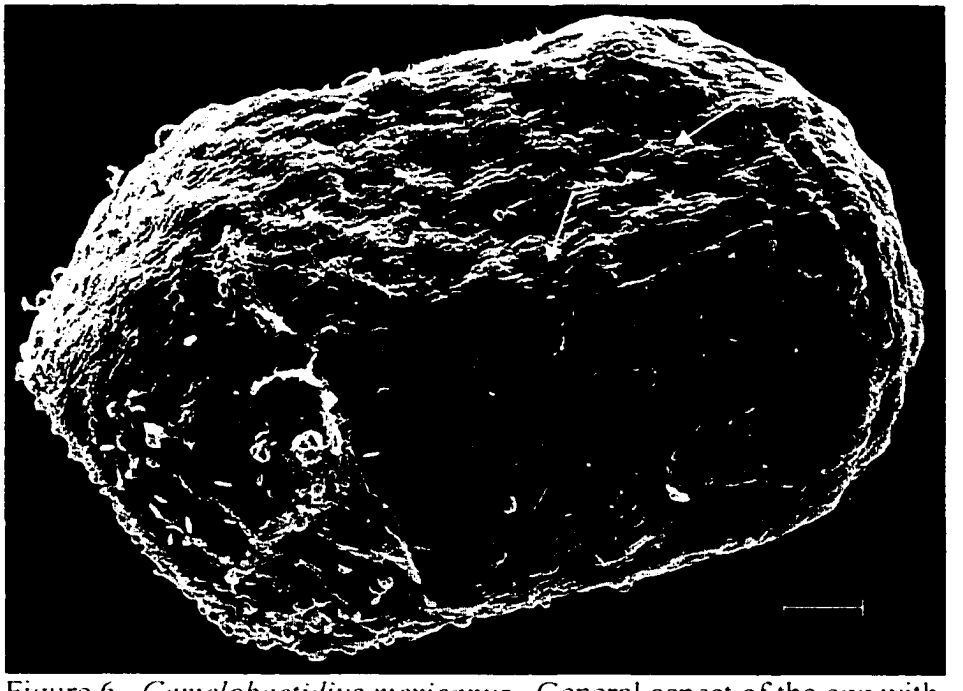

Figure 6. *Camelobaetidius mexicanus*. General aspect of the egg with KCTs (arrows). Bar represents 10 µm.

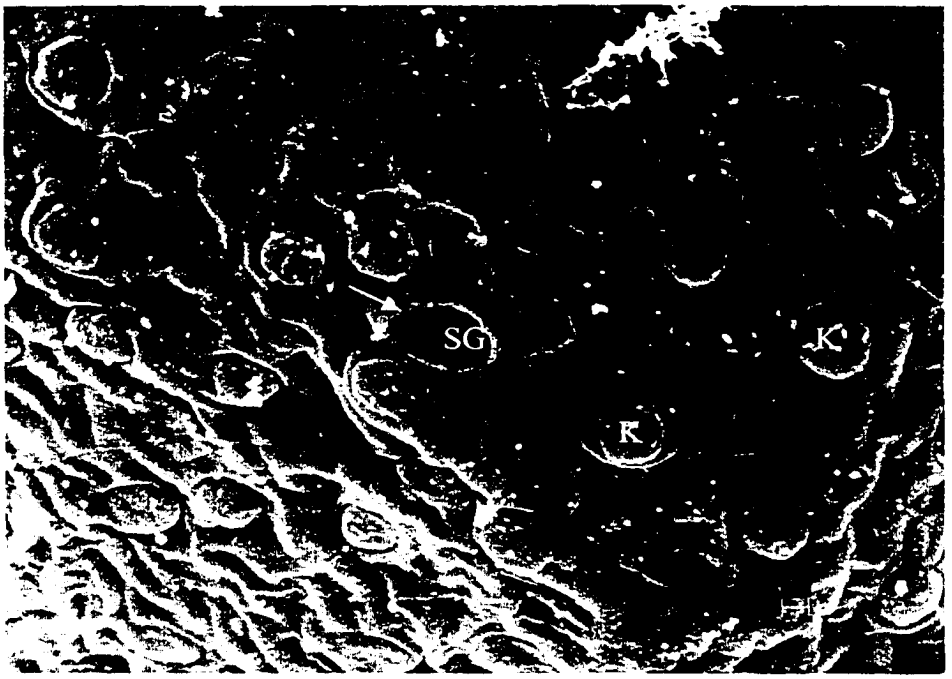

Figure 7. *Camelobaetidius mexicanus*. Tagenoform micropyle (arrow) with a small well defined sperm guide (SG) and KCTs (K). Bar represents 1 μm.

Caenidae Newman, 1853

Larvae of this family consists of the small square-gills mayflies with operculate gills that protect underlying filamentous gills from bottom silt and debris. They live in various waters with silt bottoms, or dense filamentous plants (McCafferty 1983). Four genera with 26 species are known from North America. Only one species is known in the LRGV.

***Caenis* Stephens, 1835**. The ovoid eggs have a thin slightly wrinkled adhesive layer covering them (fig.8). There is no chorionic sculpturing or accessory attachment structures beneath this layer. The eggs have one Type III polar cap with various size knobs at each pole (Fig. 8.9). The knobbed fibers seem to attach to any surface they come into contact with including each other (Fig. 10). One tagenoform micropyle could

be seen with a large well-defined sperm guide (Fig. 11). The micropylar canal can be seen through the exterior of the egg.

Smith (1935), Degrange (1960), and Koss (1968) studied various *Caenis* eggs. Their observations identified the polar caps formed by various coiled threads with terminal knobs. Although previously stated by Koss and Edmunds (1974) that the micropyle had no sperm guide, Malzacher (1983) corrected this misconception with his micrographs of European caenids. The adhesive layer surrounding the chorion was not identified until Malzacher's (1983) study of the European caenids. His was the first publication with mayfly egg micrographs. Through comparison, however, it is clear that the *Caenis* species studied here is not one of the species in the Malzacher article.

Specimens examined. TX: Hidalgo County (1987); 2 female imagos.

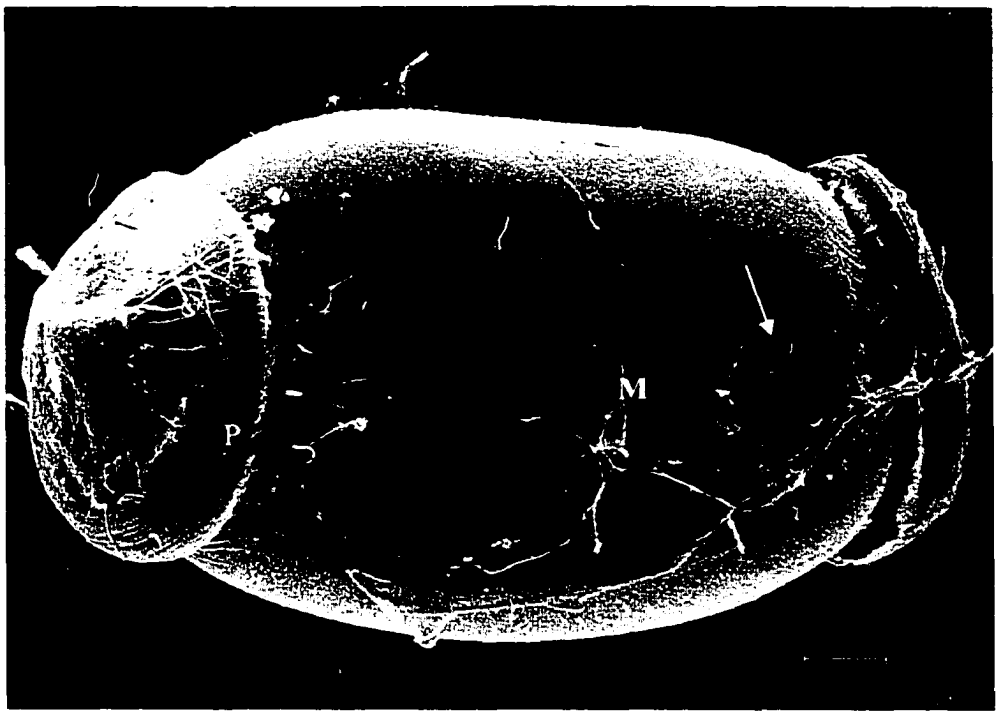

Figure 8. *Caenis* sp. General aspect of the egg showing 2 polar caps (P), a micropyle (M), and slight wrinkling of the adhesive layer (arrow). Fibers on the chorion are from its polar caps and from the polar caps of neighboring eggs. Bar represents 10μm.

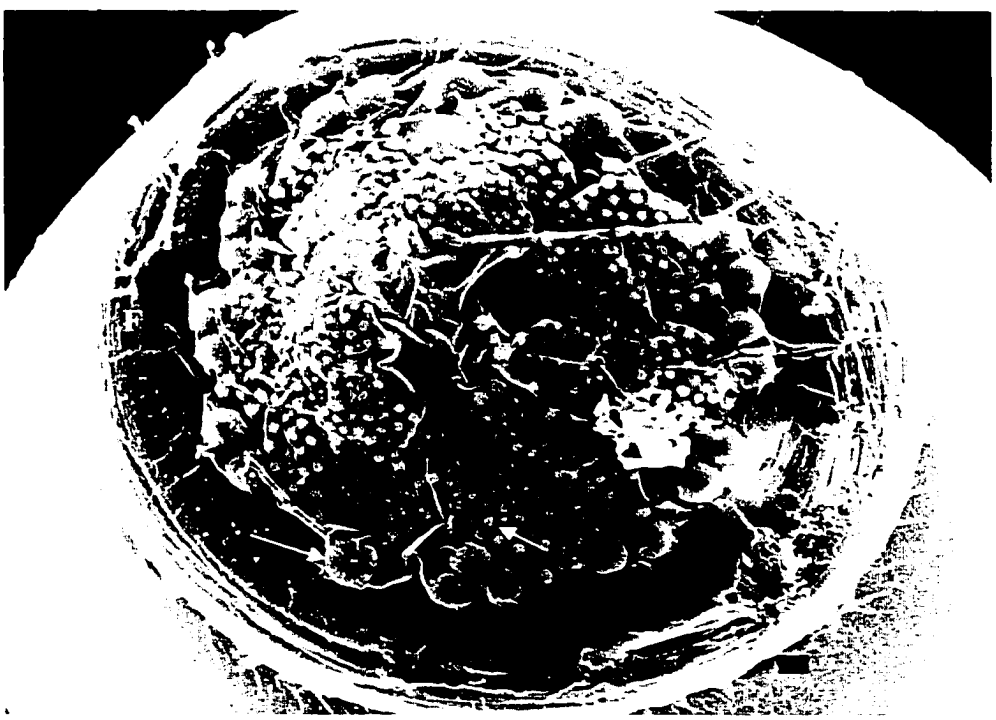

Figure 9. *Caenis* sp. Polar cap showing the fibers (F) coiled about the pole with various sized knobs (arrows). Bar represents 2μm.

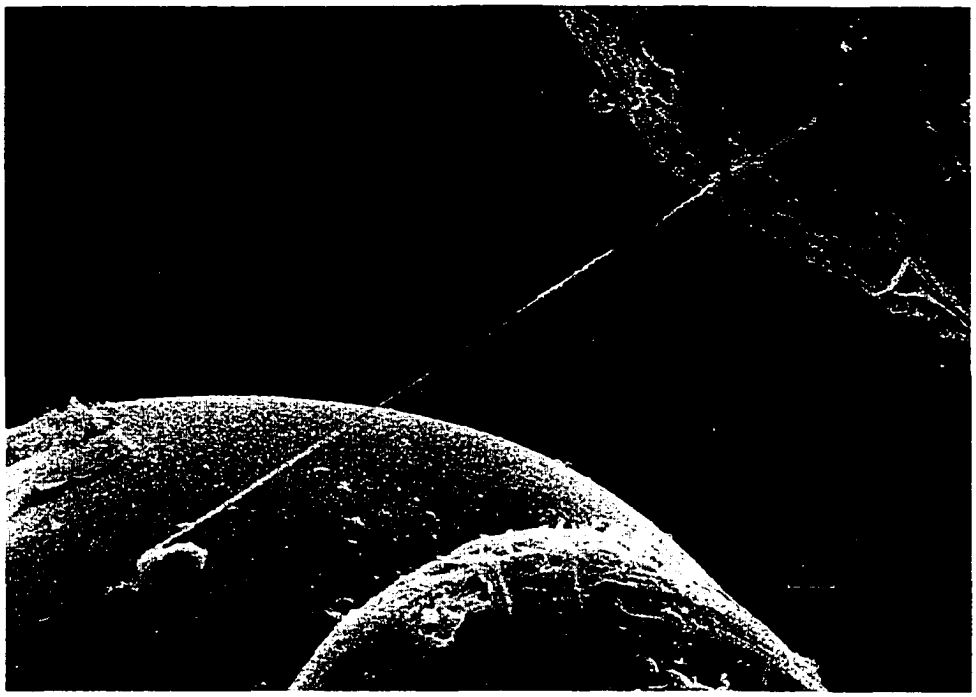

Figure 10. *Caenis sp.* Polar cap fiber with knob attached to nearby egg. Bar represents 3 μm.

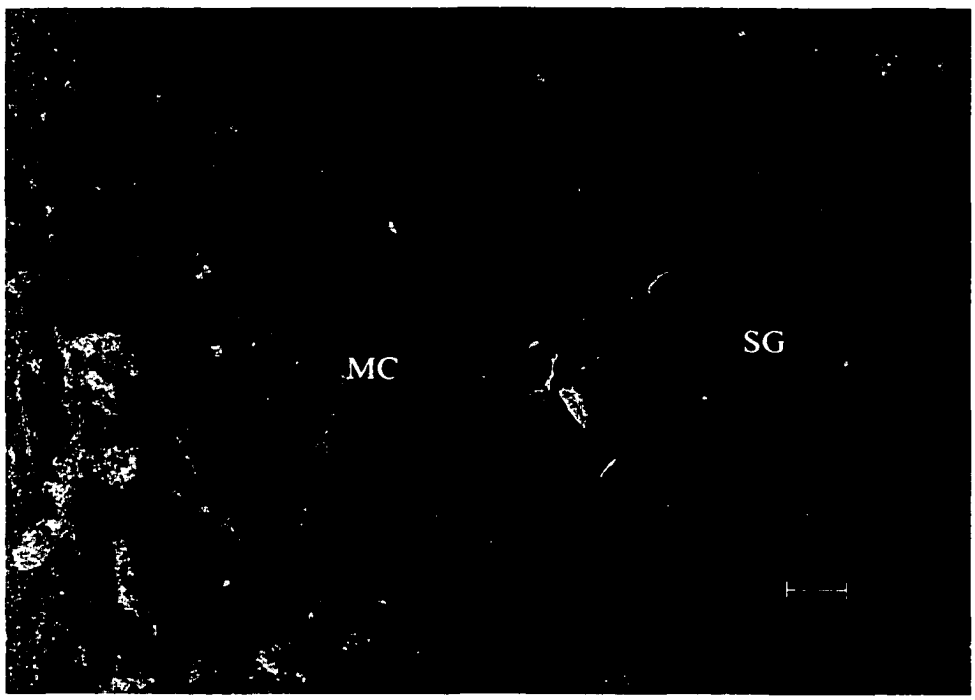

Figure 11. *Caenis sp.* Micropyle showing its large ovoid sperm guide (SG) and its conspicuous micropylar canal (MC). Bar represents 2μm.

Ephemeridae Latreille, 1810

The common burrowers, as they are known, are a widespread family. Their habitat consists of waters with silt, silt-marl, or silt-sand for burrowing (McCafferty 1983). Only 16 species from 4 genera are reported in North America. One species occurs in the LRGV.

***Hexagenia limbata* (Serville), 1829**. These eggs are oblong and relatively large. They have net-like chorionic sculpturing formed by reticulation. This reticulation is caused by raised ridges giving the egg a honeycomb appearance. The raised ridges are not straight but are sinuous. The entire egg is coated with a thin, inconspicuous adhesive layer that is molded to the chorion (Fig. 13). There are no polar caps or accessory attachment structures. They have 1-2 linear micropyles with elongate sperm guides and obvious micropylar canals. Sperm guides seem to be larger closest to the micropylar canal, and tapers off at the end. The tapered end forms a furrow in the middle of a raised ridge of the reticulation (Fig. 12).

Smith (1935) and Koss (1968) have described the egg morphology of *Hexagenia limbata* (Serville) 1829, and *H. rigida* McDunnough. Both agree that the raised ridges of the polygons are slightly sinuous in *H. limbata* (Serville). I have observed that some eggs have one micropyle with variable location, while some have two micropyles one located close to one of the poles and the other is located around the equator of the egg.

Specimens examined.—TX: College Station (1983): 1 female imago.

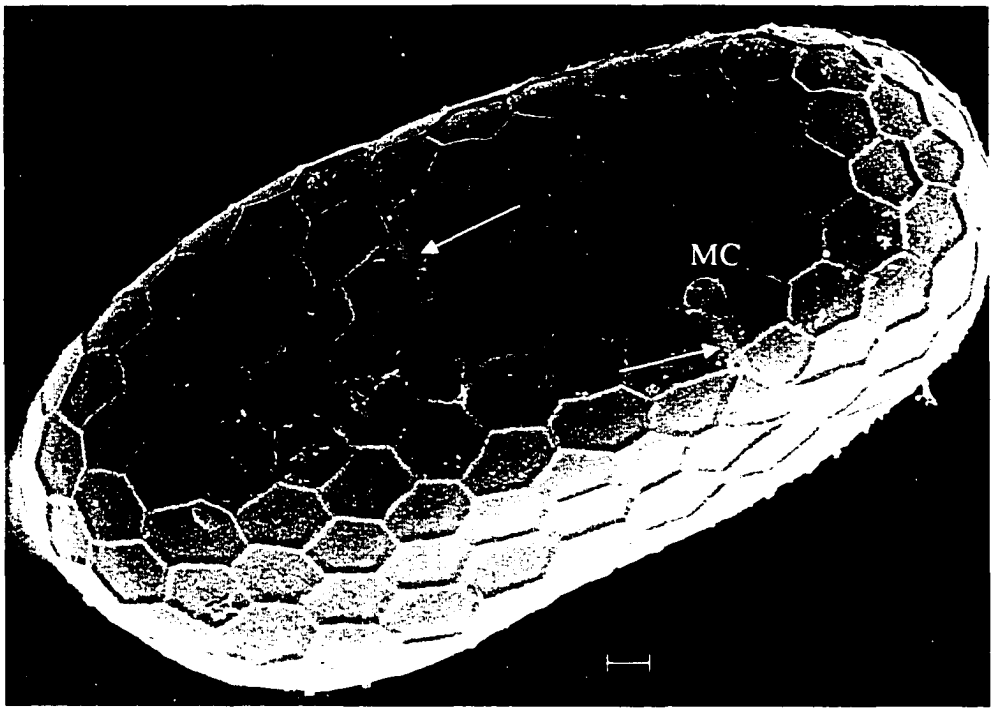

Figure 12. *Hexagenia limbata*. General aspect showing two partial micropyles with elongate sperm guide (arrows) and obvious micropylar canal (MC). Bar represents 10 μm.

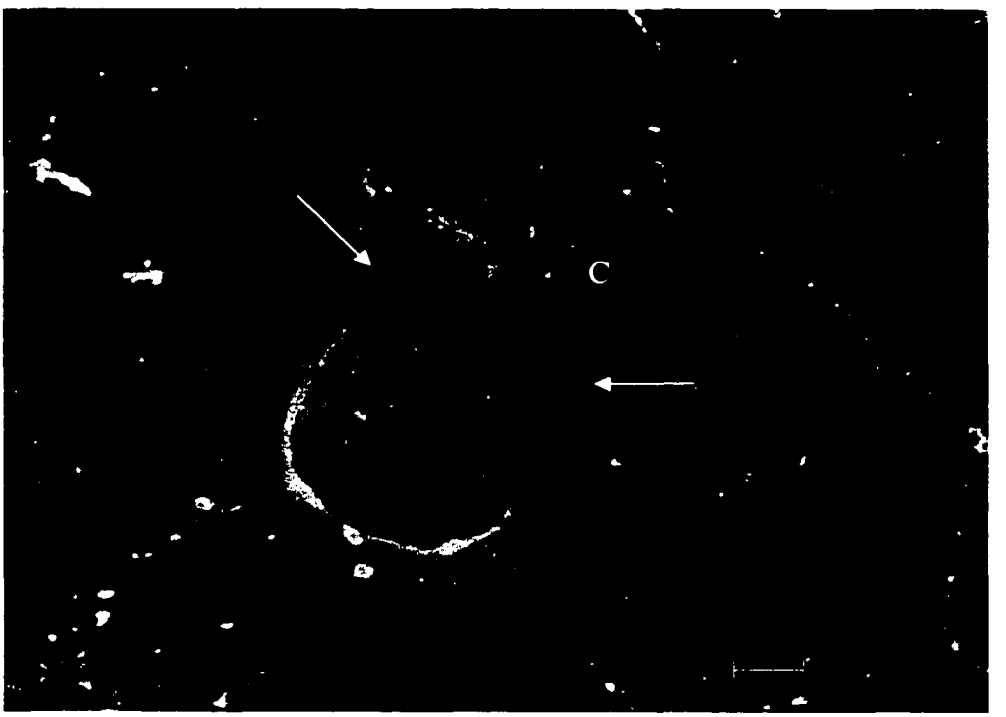

Figure 13. *Hexagenia limbata* micropyle showing the elongate sperm guide that looks like it was cut into the egg intersecting the chorion (C) and the adhesive layer (arrows). Bar represents 3 μm.

Heptageniidae Needham, 1901

This family is made up of the flatheaded mayflies because of their flattened bodies as larvae. They live in lotic habitats as well as shallow littoral areas of lakes. Larvae can be found under stones or among detritus (McCafferty 1983). The family consists of 132 species in 14 genera in North America. Only one of these is known to occur in the LRGV.

***Stenonema femoratum* (Say), 1823**. These somewhat round eggs are tuberculate due to the fibrous adhesive layer covered by tubercles (Fig. 14). The shape of the egg itself is not completely round because of the raised dome at one of the poles (Fig. 15) . This can be difficult to see with the adhesive layer covering the chorion. The only accessory attachment structures seen were tubercles that look like buttons that are in fact a part of the adhesive layer (Fig. 18). They appear to extend slightly when they come into contact with a substrate or another egg. The function of such structures could be to hold the eggs together in a mass when they are laid. No polar caps could be seen. A structure that looks like polar cap could be seen, but is actually a continuation of the fibrous adhesive layer, which is easier to remove at this section because of the indentation caused by the dome. The eggs have 2-3 linear micropyles with elongate sperm guides (Fig. 16, 17). Folds in the adhesive layer form the sperm guides, and as a result they appear "hooded" (Fig. 16).

Koss (1968) correctly identified the linear micropyle of *Stenonema femoratum* (Say) and identified the structure known as the "hood" which is formed by the folding of the fibrous adhesive layer and encloses the entry to the micropylar canal beneath. However, he believed that this folding caused an absence of adhesive material at the

distal portion of the sperm guide. Upon closer observation, it is now known that the adhesive layer is thick enough to cover the entire sperm guide, and its fibrous nature leads to the formation of channels on the sperm guide that might aid the sperm. The adhesive layer of the *S. femoratm* eggs has never been described as fibrous, but as gelatinous (Koss 1968).

Specimens examined. TX: Hidalgo County (1990); 2 female imagos & Starr County (1994); 1 female imago.

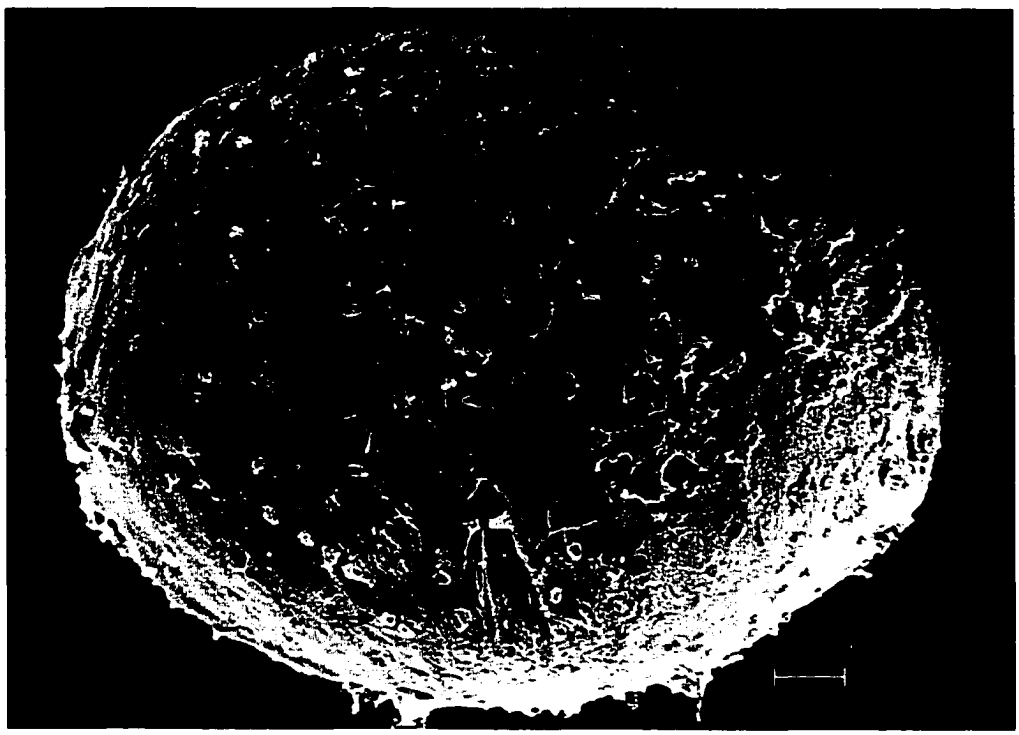

Figure 14. *Stenonema femoratum*. General aspect of egg with tubercles (arrows) that have been altered by the critical-point drying procedure. Bar represents 10μm.

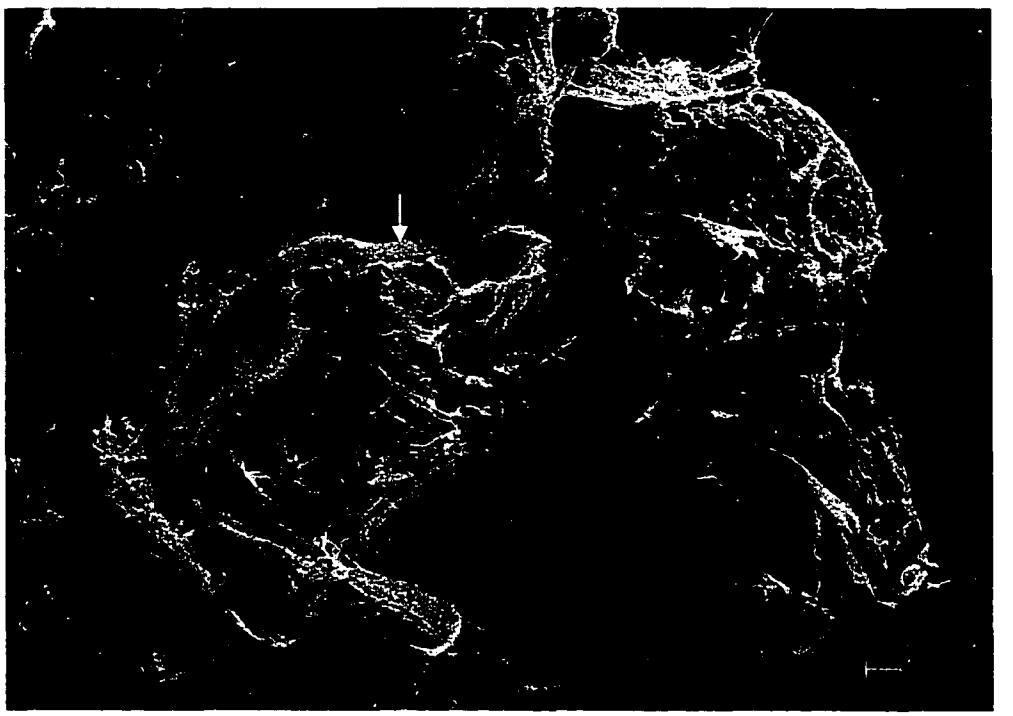

Figure 15. *Stenonema femoratum*. Eggs with fibrous adhesive layer partially removed. Arrow indicates the indentation formed by the dome at the pole. Bar represents 10 μm.

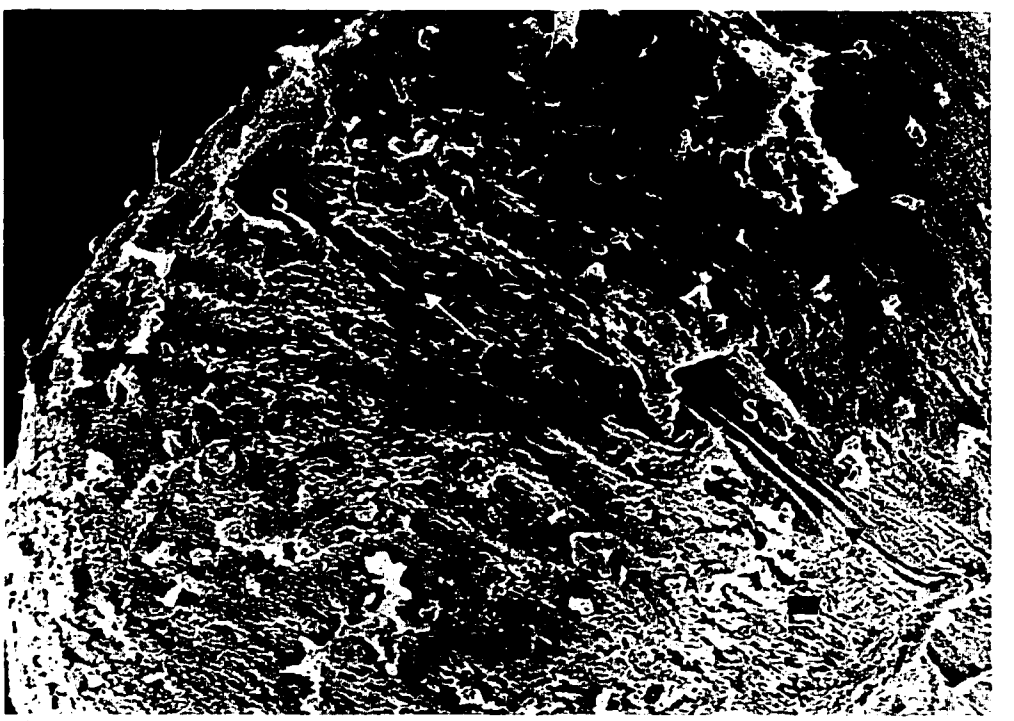

Figure 16. *Stenonema femoratum*. Sperm guides (S) on the adhesive layer formed by elongate folds. Note the channels (arrows) formed by the folding. Bar represents 3μm.

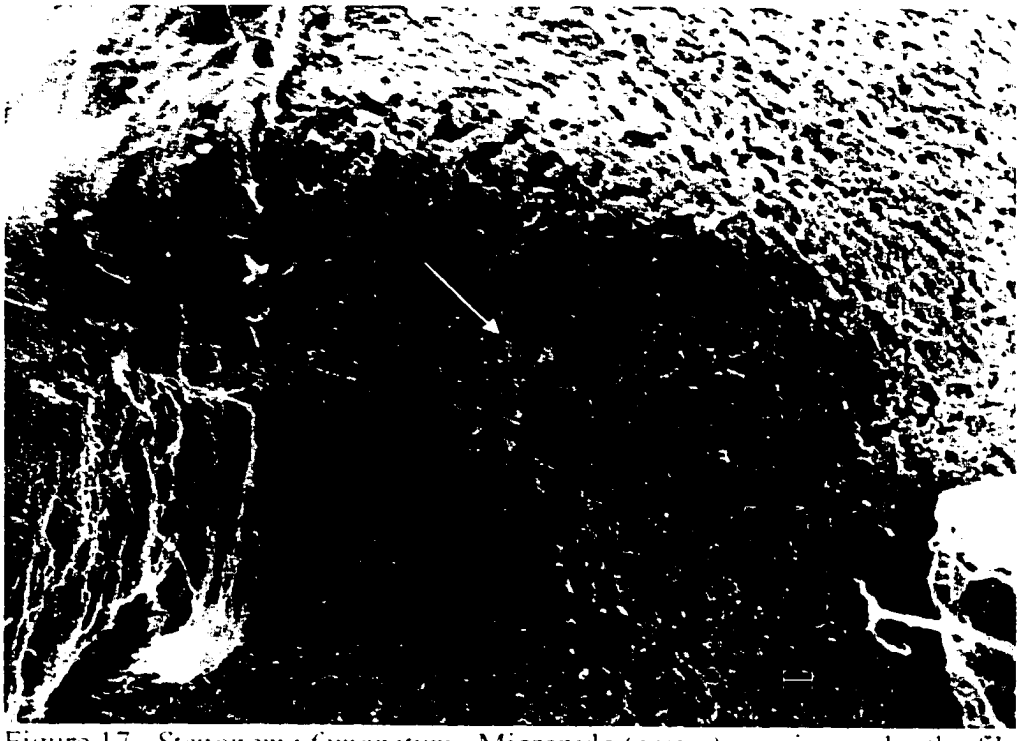

Figure 17. *Stenonema femoratum*. Micropyle (arrow) opening under the fibrous adhesive layer. Bar represents 2 μm.

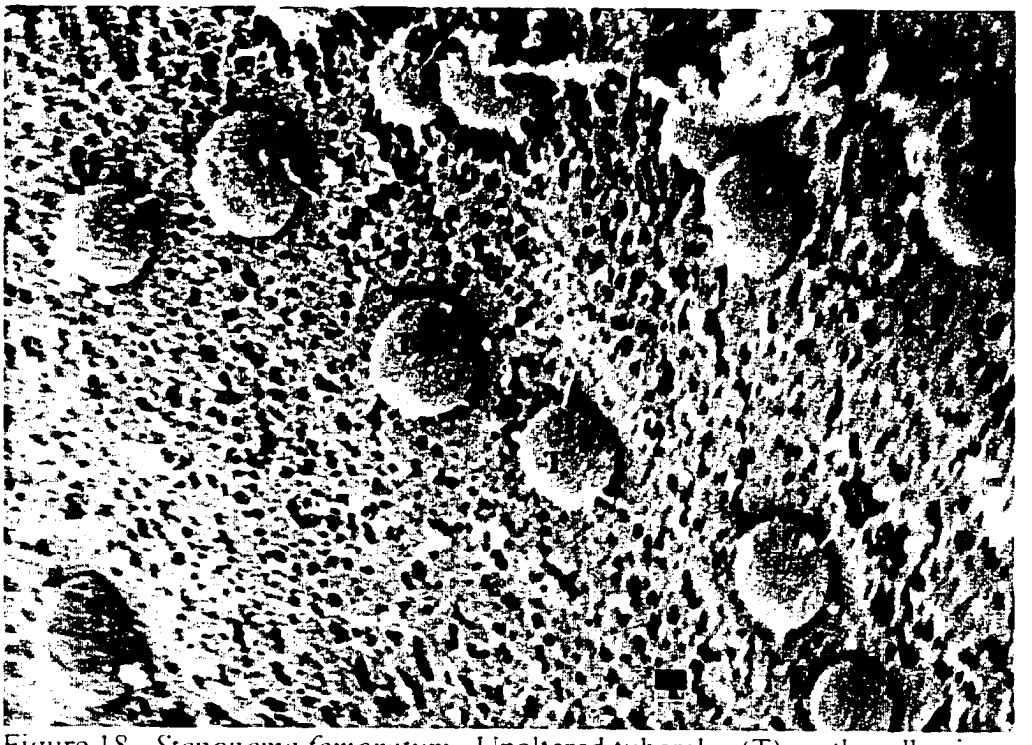

Figure 18. *Stenonema femoratum*. Unaltered tubercles (T) on the adhesive layer. Bar represents 1 μm.

Leptophlebiidae Banks, 1900

These mayflies are commonly known as the pronggills due to the forked gills at segments 2-6 on the larvae (McCafferty 1983). They are a widespread family with the maximum diversity on the Southern Hemisphere. In North America, there are 86 species in 10 genera classified. Only two species are known in the LRGV of which only one is described here.

***Thraulodes gonzalesi* Traver & Edmunds, 1967**. These eggs are ovoid and have a peg-like sculpturing of the chorion. They have an adhesive layer surrounding the chorion. The accessory attachment structures are KCTs arranged in rows surrounded by peg-like structures (Fig. 19, 20). When wet the adhesive layer looks web-like and the knobs of the KCTs appear fibrous (Fig. 21). They have no polar caps. Only one micropylar process could be seen protruding from the egg chorion without a sperm guide (Fig. 22).

Koss (1968) and Koss and Edmunds (1974) identified the chorionic morphology of *Thraulodes speciosus* Traver, *Thraulodes brunneus* Koss, and *Thraulodes sp.* Both agree that the micropyle is funnelform thoughout the genus based on the position of the micropylar canal. However, *T. gonzalesi* does not have a funnelform micropyle, but a protruding one or a micropylar process. One or more micropyles have been described from the previously mentioned species. These occur equatorially around the egg, whereas the *T. gonzalesi* micropyle is located at one of the poles. This is the first description of *Thraulodes gonzalesi* eggs.

Specimens examined.—TX: Hidalgo County; 1 female imago.

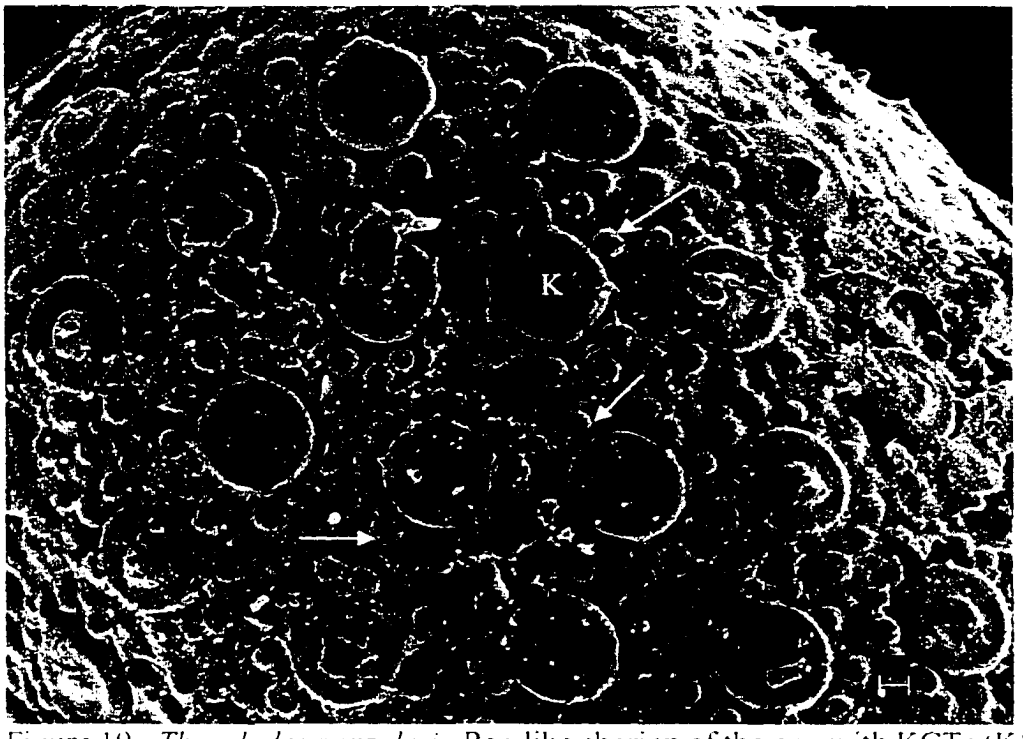

Figure 19. *Thraulodes gonzalesi*. Peg-like chorion of the egg with KCTs (K) surrounded by other peg-like structures (arrows). The bar represents 2 μm.

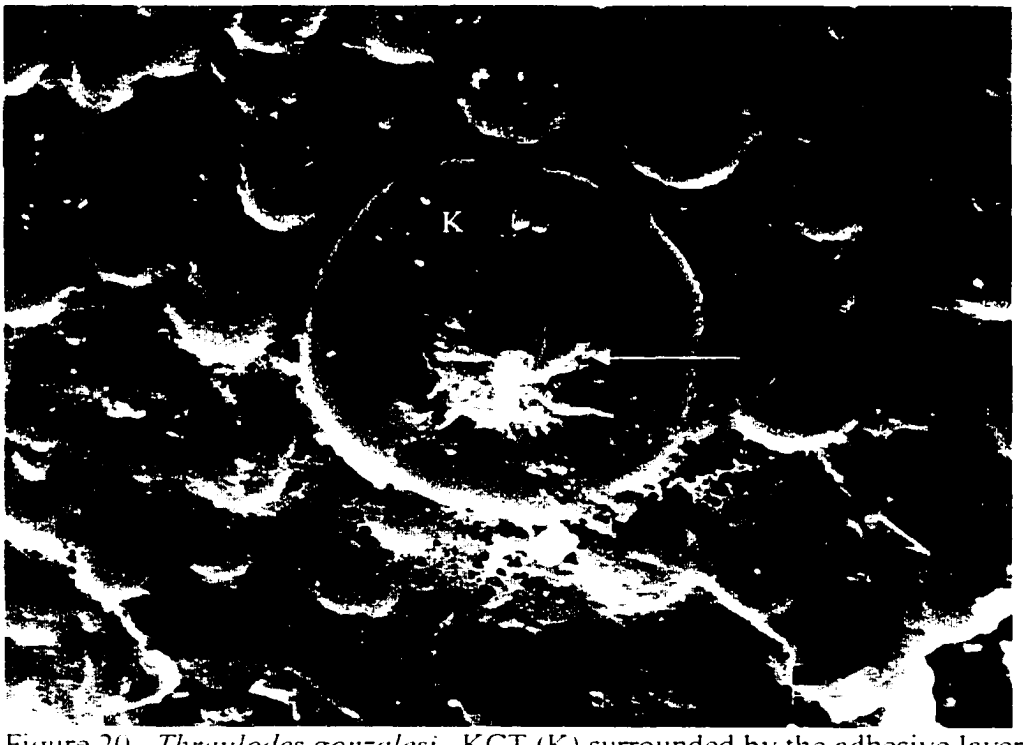

Figure 20. *Thraulodes gonzalesi*. KCT (K) surrounded by the adhesive layer. Note the wrinkling of the adhesive layer at the centered knob (arrow). The bar represents 1 μm.

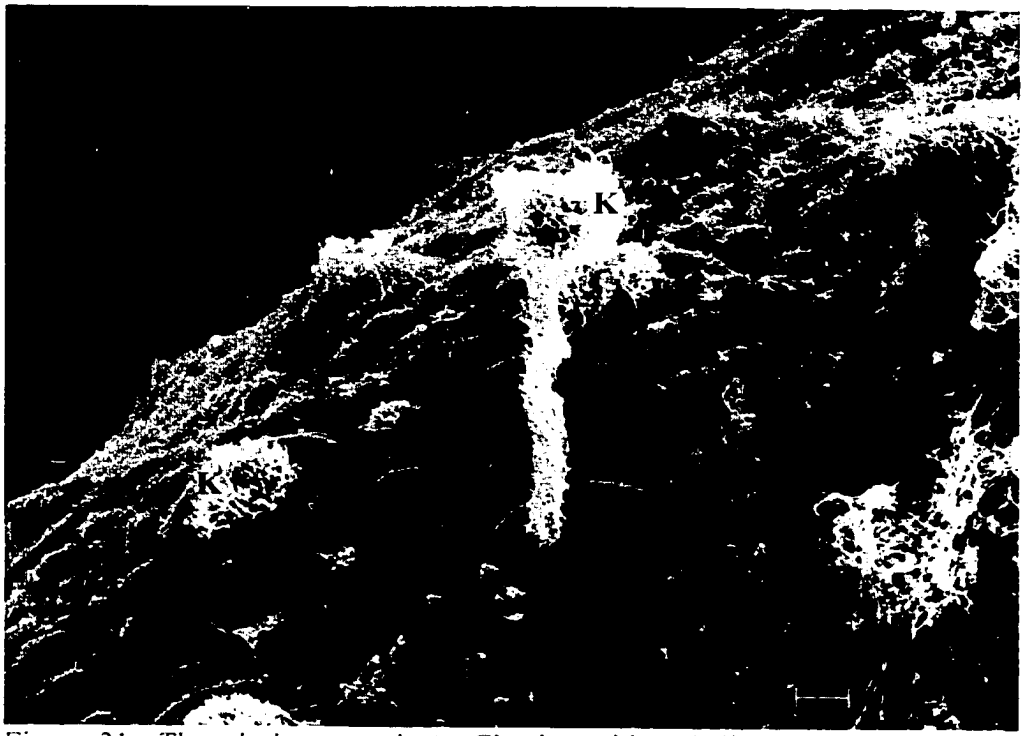

Figure 21. *Thraulodes gonzalesi.* Chorion with web-like adhesive layer. Activated KCT (K) with fibrous knob and stalk. The bar represents 2 µm.

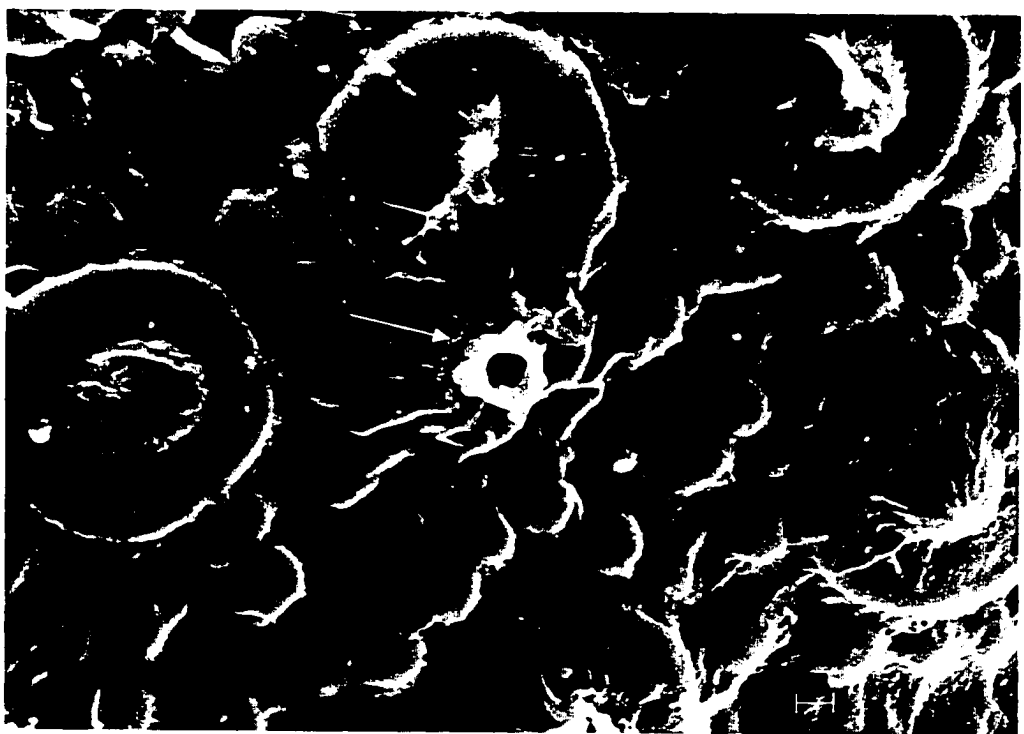

Figure 22. *Thraulodes gonzalesi.* Protruding micropyle (arrow). The bar represents 1 µm.

***Leptophlebia bradleyi* Needham, 1932**. Eggs are oval shaped and have a net-like chorion covered with an adhesive layer (Fig. 23). Beneath the adhesive layer are a variety of peg-like structures arranged very close together forming the raised ridge reticulation of the net. They have no polar caps, and they have a unique funnelform micropyle with suprachorionic flaps on either side of the sperm guide (Fig.24).

The reticulations of the chorion and the peg-like projections have been previously identified (Koss 1968) for *Leptophlebia sp.*, however the structure of the sperm guide is not described as flapped. This is the first observation of the egg chorion of *Leptophlebia bradleyi*, and it is not known to occur in the LRGV.

Specimens examined.—TX: Culberson County (1986); 1 female imago.

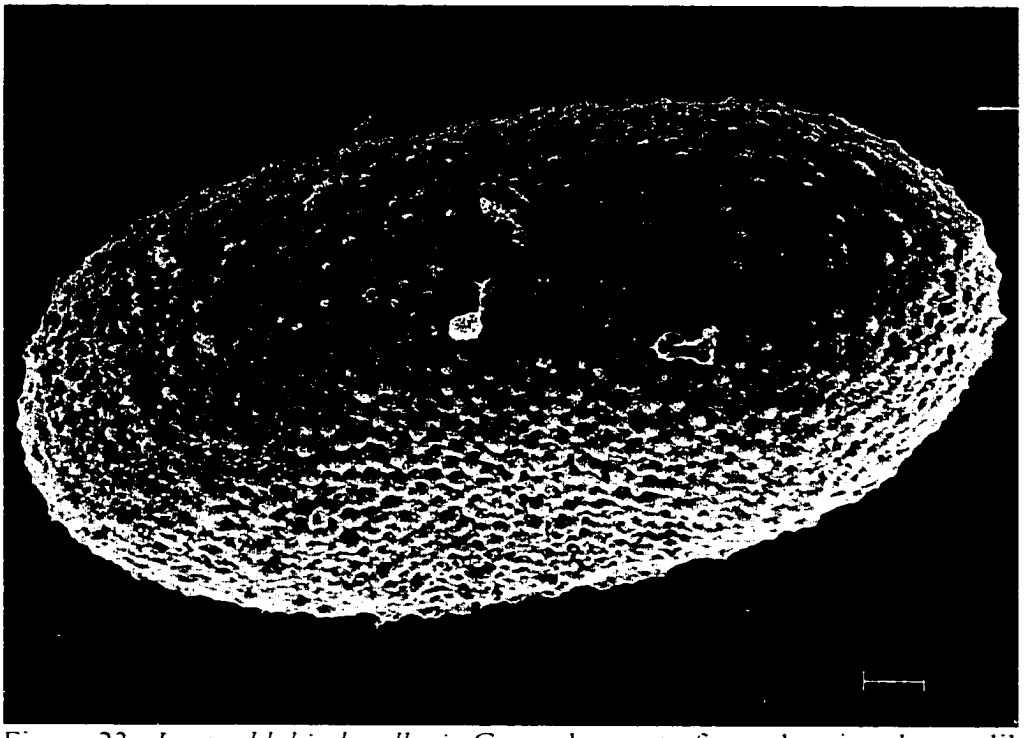

Figure 23. *Leptophlebia bradleyi*. General aspect of egg showing the net-like reticulation formed by the various peg-like projections. Bar represents 10µm.

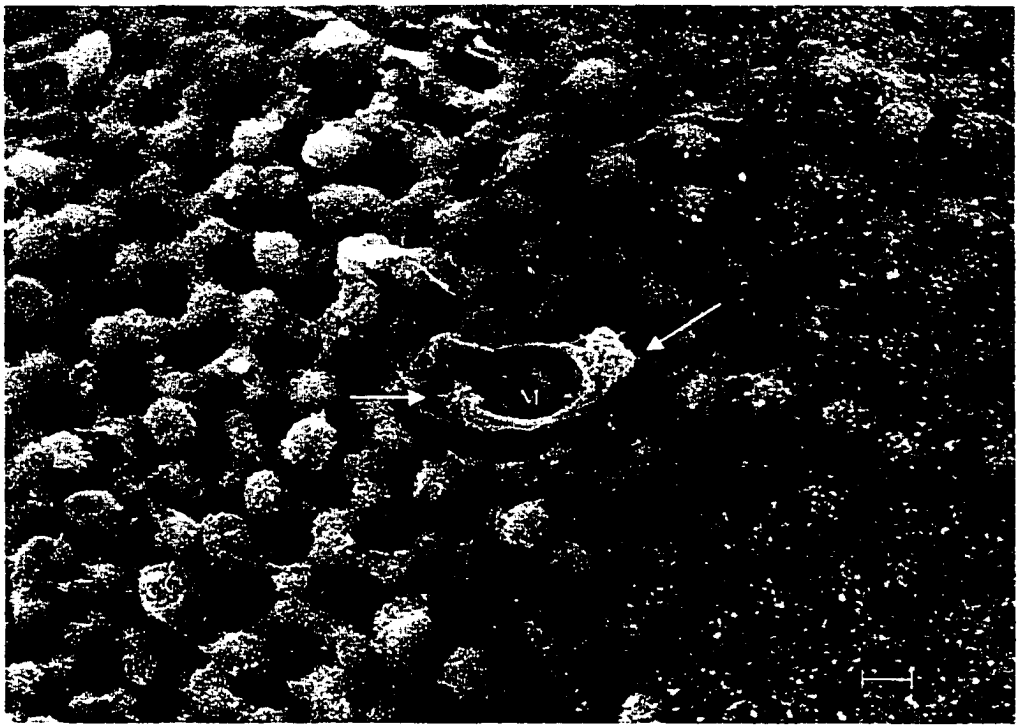

Figure 24. *Leptophlebia bradleyi*. Funnelform micropyle (M) with flapped sperm guide (arrows). Bar represents 2 μm.

Polymitarcyidae Banks,1900

The pale burrowers are identified by their light coloring. They live in silt, silt-gravel or clay bottoms as well as banks of waters (McCafferty 1983). They are widespread even though the family is made up of only 8 species in 4 genera . Only one of those occurs in the LRGV.

***Campsurus decoloratus* (Hagen), 1861**. These eggs have a unique C-shape with one concave side. A thin adhesive layer surrounds their chorion. This layer is slightly rugose covering a punctate chorion (Fig. 25). The eggs have one Type III polar cap (Fig. 26) and, no other attachment structures. Only one funnelform micropyle could be seen with an inconspicuous, punctate sperm guide (Fig. 27). The sperm guide appears as just a

slight depression of the chorion. It was difficult to see this micropyle because of its punctate chorion.

Previous observations of this species by Koss & Edmunds (1974) revealed some eggs with and without punctate sculpturing. This study has found that the chorion breaks apart easily revealing a smooth surface underneath. This could be the cause of the variation in the previous report (Koss & Edmunds 1974).

Specimens examined. TX: Hidalgo County (1987): 1 female imago & Live Oak County (2002): 1 female imago.

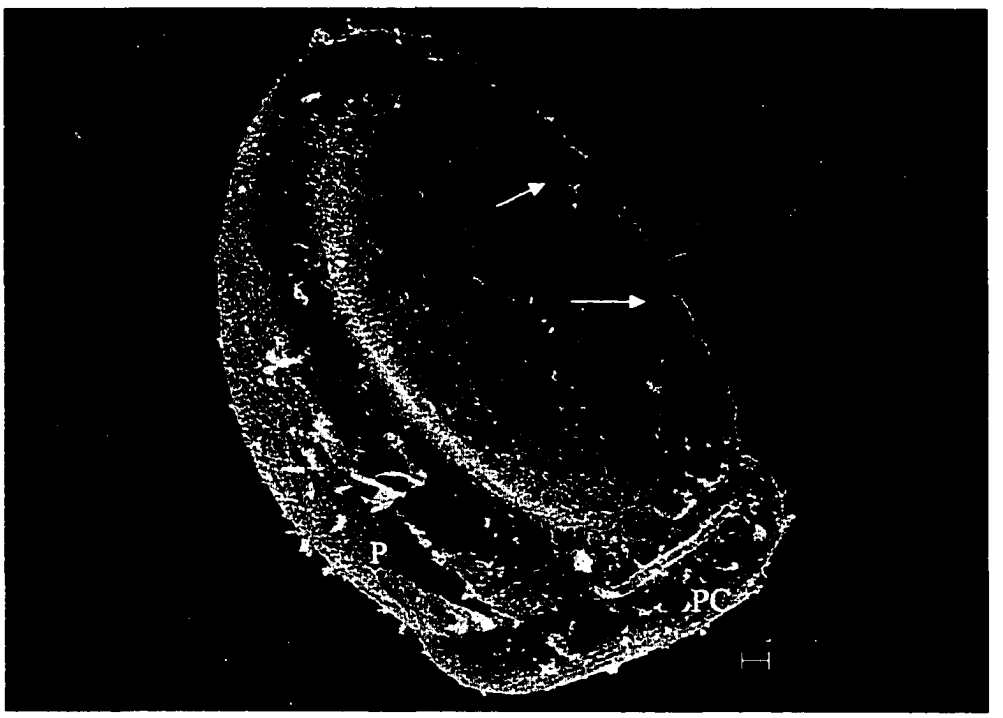

Figure 25. *Campsurus decoloratus*. General aspect of the egg showing the punctate sculpturing (P) beneath the rugose adhesive layer (arrows), and the Type III polar cap (PC). The bar represents 10 µm.

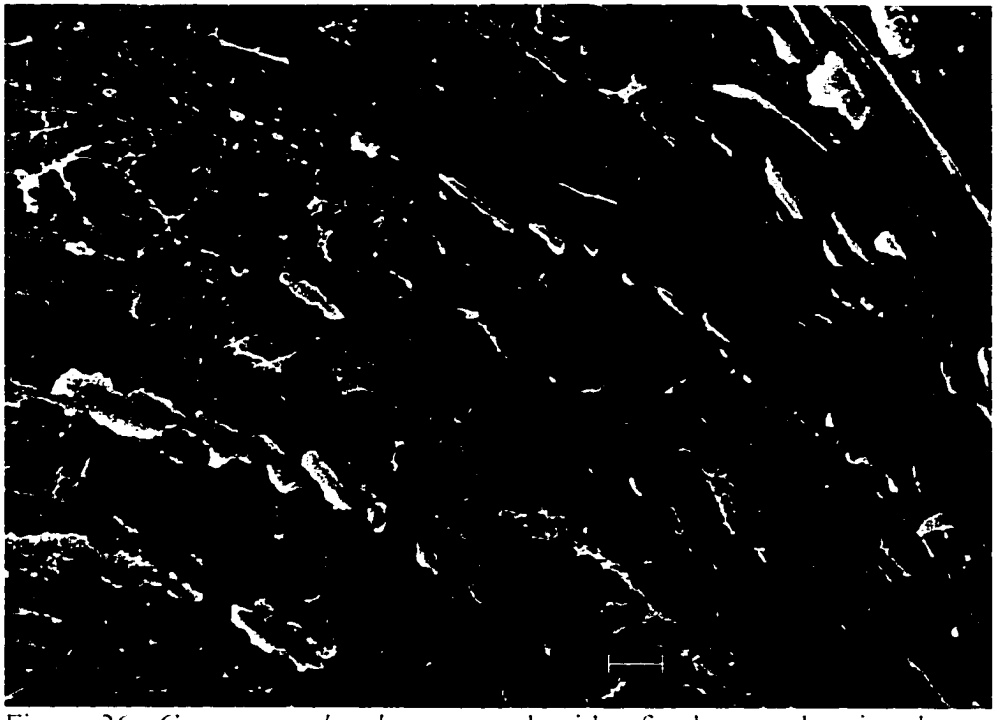

Figure 26. *Campsurus decoloratus* underside of polar cap showing the many coiled fibers that make up the threads of the polar cap. The bar represents 3μm.

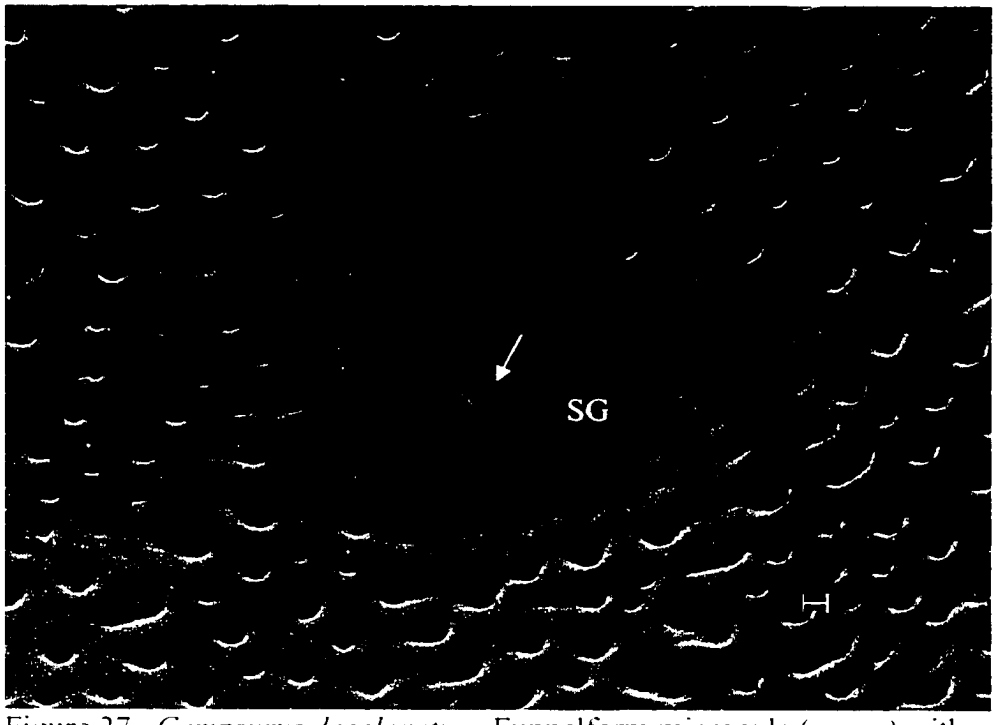

Figure 27. *Campsurus decoloratus*. Funnelform micropyle (arrow) with an inconspicuous, punctate sperm guide(SG). The bar represents 1 μm.

Leptohyphidae (Landa & Soldan), 1985

They are commonly known as the little stout crawlers because of their stout thorax in relation to their abdomen. They live among vegetation, detritus, or silty and gravely bottoms (McCafferty 1983). Although widespread, only 39 species in 8 genera occur in North America. Only two of these species is known in the LRGV, but only one species with females has been collected.

***Tricorythodes* Ulmer, 1920.** These eggs are ovoid with net-like sculpturing made up of polygonal plates with raised ridges (fig.28). The attachment sites of these plates form furrows, and at some sections the plates appear to overlap (fig. 28). They have a Type II polar cap (fig. 29), and attachment fibers that seem to originate from the furrows between plates or from the sperm guide of the micropyle (fig. 28, 30). A thin adhesive layer can be seen molded to the chorion of the egg. There is 1-2 tagenoform micropyles with large sperm guides (fig. 30).

Morgan (1913), Smith (1935), and Koss (1968) have studied *Tricorythodes allectus* egg morphology. Additionally, Koss (1968) studied *T. explicatus*, *T. minutus* (*T. fallax* syn.), and *T. stygiatus*, and found no difference between species. Previously believed to overlap completely (Morgan 1913 & Koss 1968), it can now be seen that the plates do not overlap exclusively. Some plates are flush against each other while some appear to overlap. Reticulation, however, does occur (Smith 1935) caused by raised ridges on the individual plates. It might be that the raised ridges house the fibers that seem to emerge from the furrows between the plates. Koss (1974) identified the polar cap as Type I, but it is now obvious that it is in fact a Type II polar cap because of the

arrangement of the fibers into distinct groups (fig. 29). Some eggs have been found to have two micropyles as opposed to just one (Koss & Edmunds 1974).

Specimens examined.—TX: Hidalgo County (1987, 1989 & 1993); 3 female imagos.

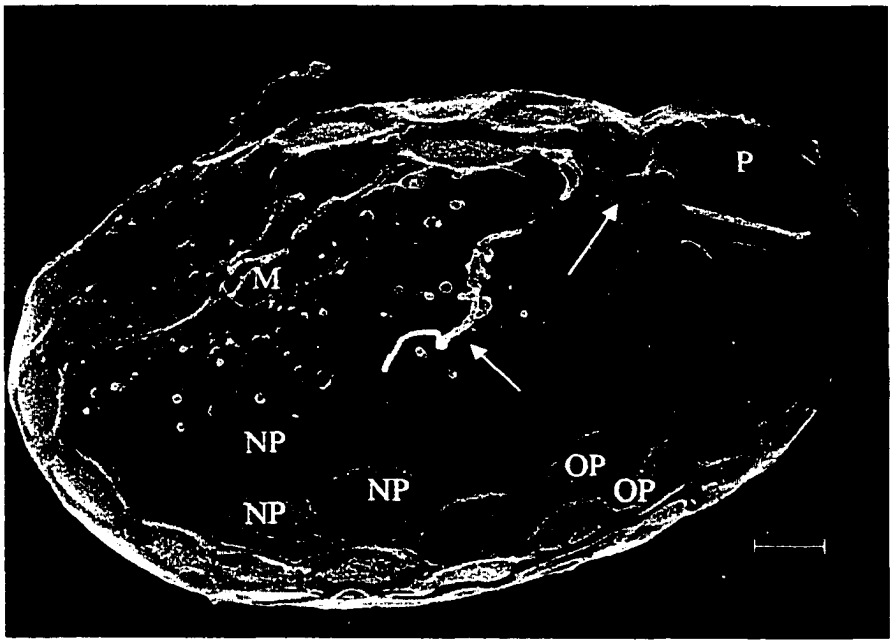

Figure 28. *Tricorythodes* sp. General aspect of the egg showing Type II polar cap (P). Note the attachment fibers (arrows), micropyle (M), overlapping polygonal plates (OP), and polygonal plates not overlapping (NP). Bar represents 10 μm.

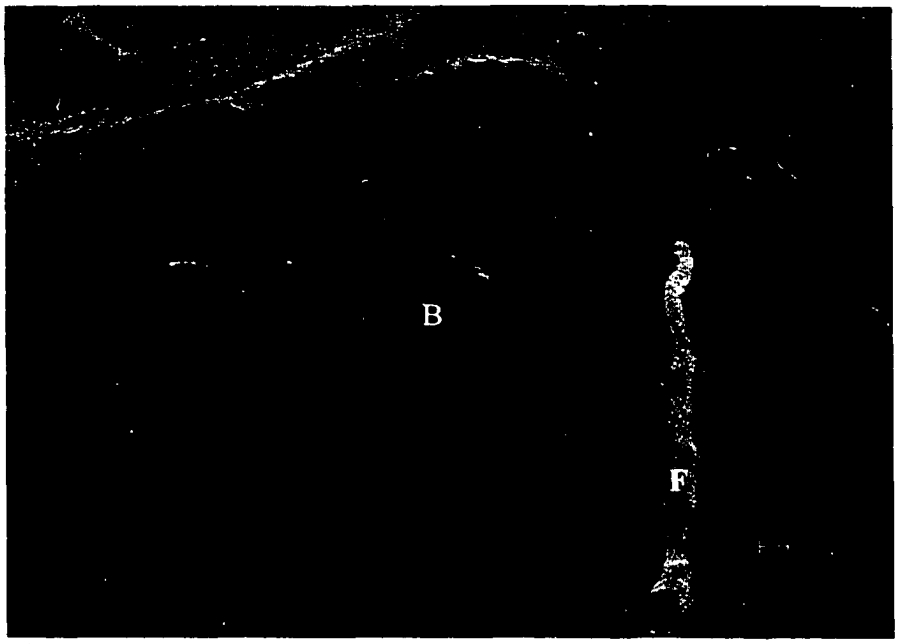

Figure 29. *Tricorythodes sp.* Polar cap with bundles of non-coiled fibers (B). Part of one of the accessory attachment fibers can also be seen (F). The bar represents 1 μm.

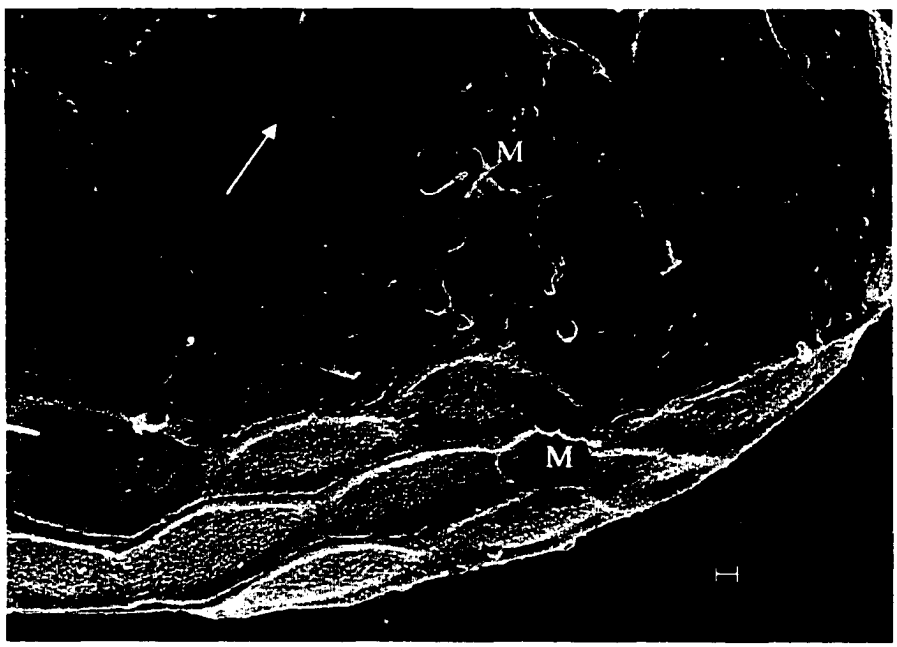

Figure 30. *Tricorythodes* sp. Two micropyles (M) can be seen here as well as one of the attachment fibers (arrow) within the furrows between the plates. Bar represents 2 μm.

Key to the Mayfly Eggs of the LRGV

The following key is applicable only to the species from the Lower Rio Grande Valley, and to *Leptophlebia bradleyi*.

1. Eggs with polar caps.....2

   Eggs without polar caps.....4

2. Eggs with 1 polar cap.....3

   Eggs with 2 polar caps (Fig. 8).....*Caenis sp.*

3. Type II polar cap (Fig. 28).....*Tricorythodes sp.*

   Type III polar cap (Fig. 25).....*Campsurus decoloratus*

4. Micropyle protruding (Fig. 22).....*Thraulodes gonzalesi*

   Micropyle not protruding.....5

5. Micropyle is funnelform (Fig. 24).....*Leptophlebia bradleyi*
   Micropyle is not funnelform.....6

6. Micropyle is linear.....7

   Micropyle is tagenoform.....8

7. Micropylar sperm guide is formed by folds in the adhesive layer (Fig. 16).....*Stenonema femoratum*

   Micropylar sperm guide is not formed by folds in the adhesive layer (Fig. 12).....*Hexagenia limbata*

8. Chorion has net-like sculpturing (Fig. 1).....*Fallceon quilleri*

   Chorion has peg-like sculpturing (Fig. 6).....*Camelobaetidius mexicanus*

Concluding Remarks

Although this study answered many questions about the egg morphology of LRGV mayflies, it raised many questions as well. All of the eggs studied here had chorions covered with an adhesive layer regardless of the presence or absence of other attachment structures as stated in Table 2. Could the adhesive layer be merely a covering around the egg that is lost when the egg is laid or is it indeed adhesive? I believe that this layer is adhesive and it gives the attachment structures their attachment properties. In most species with attachment structures the adhesive layer is thin and molded to the structures beneath. However, when these structures are absent the adhesive layer is thick and fibrous as in *Stenonema femoratum*.

On the other hand, the adhesive layers of most of the *Stenonema* species previously observed through light microscopy were described as gelatinous (Koss 1968) not fibrous. Consequently, the structures described here might take different properties once they are laid in water. Similarly, the polar caps and other attachment structures are compacted when inside the female imago, but once activated by water or substrate they swell, unwind, or extend. The *Caenis sp.* imagos identified here are known to have clusters of eggs attached to their abdomens, which they lay as a cluster (Henry personal communication 2003). Is this the function of the adhesive layer, or are the polar caps involved in this phenomenon as seen in Fig. 10? These are questions that should be answered by future research.

Table 2 Morphological features of the mayfly eggs from the LRGV

Genus	Sperm guide	Micropyle Type	Micropyle Number	Polar cap Number	Polar cap type	Accessory attachment structures	Adhesive Layer	Chorionic sculpturing
Fallceon quilleri	Small, oval	Tagenoform	1	0	NA	Tubercles	Thin, molded	Net-like
Callibaetis montanus	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Camelobaetidus mexicanus	Small, oval	Tagenoform	1	0	NA	Sucker-like discs and KCTs	Thin with sucker-like discs	Peg-like
Caenis sp	Large, oval	Tagenoform	1	2	Type III w/knobs	None	Thin, molded	Slightly Rugose
Hexagenia limbata	Long	Linear	1-2	0	NA	Reticulation	Thin, molded	Net-like
Stenonema femoratum	Medium	Linear	2-3	0	NA	Tubercles	Thick, fibrous	Tuberculate
Thraulodes gonzalesi	None	Protruding	1	0	NA	KCTs	Thin, molded	Peg-like
*Leptophlebia bradleyi**	Small, flapped	Funnel form	2-4	0	NA	Tubercles	Thin, molded	Tuberculate
Campsurus decoloratus	Indented	Funnel form	1	1	Type III	None	Thin, molded	Punctate
Tricorythodes sp	Large, round	Tagenoform	1-2	1	Type II	Fibers	Thin, molded	Net-like

NA: not applicable

* Species not found in the LRGV

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APPENDIX A

Critical point drying

1. Samples are placed in the sample holder with the appropriate number making sure that samples are always covered with 100% ethanol.
2. Switch the machine to "ON" and allow it to warm up for 5-7 minutes. (Make sure the valves are all closed.)
3. Place about 10-15 ml of 100% ethanol in the pressure chamber.
4. Transfer sample holder into the pressure chamber.
5. Place the chamber lid down over the chamber and tighten the three knobs with equal pressure by hand.
6. Open the CO_2 tank valve and open the Cool Valve on the chamber.
7. Wait for temperature to drop below 0°C, and then close the Cool Valve.
8. Open the Inlet Valve on chamber slowly until chamber is full. Make sure the temperature stays between 1° and 3°C by controlling the Inlet Valve. (When the chamber is full a bubble will travel across the viewing window.)
9. Open the Purge Valve slowly to let the ethanol out of the chamber. Use a beaker to collect the ethanol. (Note: the purge rat should be equal to or less than the liquid CO_2 inlet rate.) When all of the ethanol has been collected, use a paper towel to check the exhaust.

10. If paper towel is not wet and solid CO_2 comes out, then close the Purge valve and allow liquid CO_2 to refill the chamber.
11. When the chamber is full close the inlet valve and the CO_2 tank valve.
12. Turn the heat switch "ON" to raise the temperature to 31°C (critical temperature) and the pressure to 1070 psi (critical pressure).
13. Allow the chamber to remain in this condition for 4 minutes and, then open the Bleed valve slowly to decrease pressure to 250 psi at a rate of 100psi/minute. (This should take about 8-12 minutes.)
14. Open the Purge valve completely to decrease pressure to 0 psi.
15. Turn heat swith off and remove sample holder from chamber.

Sputter coating

1. Stubs with eggs will be placed onto the Sputter Etch unit and the power switch is turned on.
2. Chamber is pumped down to 30 mT and then the ETCH button is pressed.
3. After the system pumps down to 50 mT turn the argon gas tank valve on and adjust to 5 psi.
4. Adjust the gas flow control knob to 125-150 mT.
5. Cover the specimen with the shutter, and preset time to 30 seconds.
6. Press Start button (time) and bring current up to 5 milliamps.
7. When time runs out reduce current to 0 milliamps and, press Sputter button.
8. Pump down system pressure using the gas flow knob and stabilize at about 50 mT.
9. Open the shutter and preset time to 1 minute.

10. Press Start (time) button and bring current to 45 milliamps.

11. When time runs out reduce current to 0 milliamps.

12. Turn argon gas off and press off button.

13. Power down the machine and remove specimens.

APPENDIX B

Additional References

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VITA

Maria D. De Leon was born October 25, 1972 in Weslaco, Texas to Simon and Ismelda De Leon. She was a migrant worker until the age of 16, and graduated from Edcouch-Elsa High School in May 1991. She received a Bachelor of Science with a major in Biology and a minor in Chemistry from the University of Texas-Pan American in Edinburg, Texas in December 1996. After graduating, she obtained a position as a research aide at the Texas A&M Agricultural Experiment Station in Weslaco, Texas. In August 2000, she began graduate school at the University of Texas-Pan American in Edinburg, Texas. During her graduate studies she was employed as a teaching assistant for General Biology. She married Francisco Cadena Jr. in June 30, 2001.

Permanent address: P. O. Box 1238

La Blanca, TX 78558