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Morphology of the Immature Stages of *Culicoides sonorensis* Wirth and Jones (Diptera: Ceratopogonidae) With Observations on Their Biology

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Introduction

Whether they are called no-see-ums, biting midges, gnats, punkies, or one of many other local names, *Culicoides* Latreille, species often are serious biting pests (Mullen & Durden, 2009). This genus is a part of the family Ceratopogonidae (order Diptera), which has 125 genera containing 6180 extant species (Borkent, 2014). *Culicoides* is the main one of only four genera which include blood-feeding species; the others are *Leptoconops* Skuse, *Forcipomyia* Meigen, and *Austroconops* Wirth and Lee.

I. *Culicoides*-Associated Viruses in North America

Culicoides sonorensis Wirth and Jones is a key North American species and a primary vector of viruses (Tabachnick, 1996). It may be a pest of livestock, but it seldom bites people and is not currently known as a major vector of disease agents which affect humans. Other mammals, particularly ruminants, are not quite so lucky. In the United States alone there are two important arboviruses: Bluetongue Virus (BTV; family Reoviridae, genus *Orbivirus*) and Epizootic Hemorrhagic Disease Virus (EHDV; family Reoviridae, genus *Orbivirus*), which plague our domestic and wild ruminant populations. They are common in the western and southern USA wherever *C. sonorensis* thrives (Tabachnick, 1996; Holbrook *et al.*, 2000). These two viruses (especially BTV) have greatly impacted the agricultural community here in the United States, particularly trade with areas lacking these viruses (historically western Europe) (Tabachnick, 1996; MachLachlan & Osburn, 2006; Carpenter *et al.*, 2008; Carpenter *et al.*, 2009). This has spurred research into various control methods for the main vector, *C. sonorensis*.

Although EHDV can have a very high mortality rate in wild ruminants, it has not been as well studied as BTV. This is due to the fact that EDHV primarily affects deer, specifically the white-tailed deer, instead of farmers' livestock. Recent emphasis on breeding deer for hunting, however, is changing that (Nol *et al.,* 2008). Once a deer has contracted EHDV, it will usually exhibit a fever, anorexia, respiratory distress, weakness, swelling of the tongue and conjunctivae, and edema of the neck and head. In some cases the individuals will also show signs of extensive internal hemorrhages as well as excessive salivation, nasal discharge, and ulcers on the tongue (Spickler, 2006b). Despite the gruesome nature of EHDV and its ability to wipe out most of an entire population of deer with which *C. sonorensis* can be closely related (Tabachnick, 1996), *Culicoides sonorensis* is more famous for transmitting BTV to domestic livestock.

The primary reason that BTV is the focus for those interested in *C. sonorensis* is that BTV affects the agricultural industry. Bluetongue virus is widespread in the various wild and domesticated ruminants which it can infect globally. It mostly can be found between latitude 35°S and 40°N and as far at 50°N in parts of North America (Dulac *et al*., 1989; Mellor *et al.,* 2000). Recently even Northern Europe has experienced devastating and persistent BTV as far north as southern Sweden (Carpenter *et al.,* 2009; Lewerin *et al.,* 2010). The list of BTV-susceptible ruminants includes everything from sheep, goats, and cattle, to bison, elk, and deer (MacLachlan & Mayo, 2013). BTV was first described as Malarial Catarrhal Fever in 1902 when it was found in sheep in South Africa (Hutcheon, 1902). In the United States it was not found until 1952, first in Texas and then in California at which time it was termed "soremuzzle." The name was due to

the fact that sheep with BTV do appear to have a rather sore snout (Hardy & Price, 1952; McKercher *et al.,* 1953). Since *Culicoides sonorensis* is known to be the primary vector of BTV in the USA (Tabachnick, 1996), it follows that BTV is primarily found within *C. sonorensis*' range. The BTV serotypes 2, 10, 11, 13, and 17 are historically common throughout the contiguous western and southern United States; it is rare or absent in the northern and northeastern US (Barber, 1979; Tabachnick, 1996). Recently many new BT serotypes have been found in the USA, including Florida, and other vectors are suspect (Johnson, 2011; MacLachlan *et al.,* 2013).

When an unfortunate ruminant has been bitten by a BTV-infected female midge, the ruminant has a chance to contract the disease. Once infected, the more susceptible animals, such as certain species of sheep and deer, will begin to show characteristic clinical signs and symptoms. These include fever, depression, excessive salivation, nasal discharge, anemia, facial edema, ulceration of the oral mucosa, coronitis, muscle weakness, secondary pneumonia, and occasionally death (Spickler, 2006a). The mortality rate in sheep typically falls within the range of 0-30% but in highly susceptible breeds of sheep the mortality rate can be as high as 100%. However, less susceptible breeds may not exhibit any symptoms at all when infected: among infected cattle, only about 5% show any signs, while the rest are subclinical and deaths are a rarity (Spickler, 2006a). Cattle can carry BTV for up to about 60 days, and so they are regarded as a reservoir host (Singer *et al.,* 2001). BTV is still very detrimental to cattle producers, since trade restrictions are imposed on any ruminants coming from areas endemic for BTV.

II. Justification for Studies on *Culicoides* Immatures

Research into the four larval stages of *Culicoides* began over 200 years ago. In 1713 Reverend Derham, the Rector of Upminster in Essex, England, published the "Physico-Theology: or a demonstration of the being and attributes of God, from His Works of Creation" (Murphree & Mullen, 1991). Derham described and illustrated an unknown larval instar of what he called *Culex minimus nigricans maculates sanguisuga*, a species now known to be a member of the genus *Culicoides*. Unfortunately, the overall knowledge concerning the larvae of this genus is still sadly incomplete. Murphee and Mullen (1991) noted that larval descriptions exist for 51 of 144 North American species. The lack of detailed data is somewhat surprising due to the relatively large amount of research which has been done on the adults of *Culicoides*.

There are many factors which could cause this disparity, but three likely ones are 1) the small sizes of the immature stages, 2) the fact that they inhabit opaque substrates, and 3) relative ease and glamour in researching blood-feeding adults. There are few associations between the adult and immature stages (Provonsha & McCafferty, 1975; Resh & Unzicker, 1975). It is somewhat surprising that complete descriptions do not exist for *Culicoides sonorensis*, even though colonies have existed for over 50 years (Jones, 1961). However, these colonies were labeled as *C. variipennis* until 2000 when Holbrook *et al.* used both adult morphological and electrophoretic analysis to separate the species *C. variipennis* into three species including *C. occidentalis, C. sonorensis,* and *C. variipennis*. Since 2000 though, none of the three species larval morphologies have been reexamined (Table 1).

Ignoring the immatures is even more surprising when *Culicoides* are compared with other insect vectors. For example, when looking to control a mosquito population, researchers will often focus on the larval population for a solution but for *Culicoides*, managing immatures is not usually considered seriously. This difference is possibly partially due to the simple fact that serious human diseases are not associated with species of *Culicoides* (Holbrook, 1996). The midges are only considered to be pestiferous instead of dangerous. Despite this, *C. sonorensis* still affects the agricultural industry. The lack of knowledge of immatures is especially detrimental when one is attempting to understand and control a disease system such as BTV.

In 1977 Kettle eloquently explained the importance of studying *Culicoides* immatures when he noted that

> "Detailed knowledge of the larval biology is essential for a full understanding of bionomics. Without this information it is impossible to construct life tables or to identify key factors limiting pest populations. Such studies are dependent on sound taxonomy, and although the characters separating the immature stages at subfamily level have been known for nearly 50 years, the taxonomy of larvae and pupae, with the exception of Forcipomyinae, is very poorly understood."

III. Historical Progression of *Culicoides* Immature Studies

In 1809 the genus *Culicoides* was erected by Latreille who used *Culicoides punctatus* (Meigen) as the type species. After Derham's 1713 work (Murphree & Mullen, 1991), it was not until Pratt (1907) that any reference to the larval stages of *Culicoides* was addressed in scientific articles.

The earliest work on *Culicoides* immatures spanned from approximately 1907 until the late 1940's. In this period the work was quite sporadic and unorganized. Those

researchers who did treat the immatures at all did not do so in any great detail and instead focused primarily on the adult stages. There was no standardization of terminology. Their small size, the difficulty in rearing them, as well as the difficulty in finding the immatures probably also contributed to the apparent disinterest. During this time the majority of the descriptions included general morphological features of only the fourth (last) larval instar, occasionally the pupae, and typically included a couple of superficial measurements such as body length or markings.

The species, immature stage studied, whether it is a new description or a re-description, whether the description is basic or advanced, the country, and any special techniques used in the study can be seen in Table 1. During this time period only one research group, Carter *et al.* (1920), showed serious interest in the larvae. Although his observations were not as detailed as many of the more current articles, for the time Carter's work in Africa on five different species of *Culicoides* was groundbreaking. With no standard terminology or methods for descriptions, pre-1940 researchers decided to measure whatever they thought was relevant. It is also uncertain whether many studies were done using larvae which had been chemically cleared. It was possible to clear insects at this time, so perhaps they were (Carter *et al.*, 1920; Lutz, 1922). Chemically clearing specimen of debris greatly facilitates observation of features such as feeding structures inside of the head capsule. In addition to a lack of detailed methodology, many of the early papers did not mention larval biology or behavior beyond noting the larval habitat.

By the late 1940's there was a notable increase in the efforts to better understand both the immature biology and morphology for various *Culicoides* species. The first of the researchers to try to bring some sort of order to studies of the larval stages was Hill (1947). Although her research into the larval stages was elementary by modern standards, she did attempt to consolidate all of the previously published research into the immature stages and managed to develop a fairly comprehensive index for this.

Fortunately, several subsequent researchers began to build on that foundation. Some of the most notable efforts came from Lawson (1951) and Kettle and Lawson (1952). Lawson is probably one of the most influential workers on *Culicoides* immatures because he did not simply focus his research on the fourth larval instar (L4). Up until his 1951 article, the only stage which had been reported on in much detail usually was the L4. Lawson actually made advanced observations on all four larval stages as well as the pupae. Even to this day very few researchers have focused on anything other than the fourth larval instars, occasionally eggs, or pupae. Lawson's (1951) descriptions of the larval morphology of *C. nubeculosus* remain some of the most in-depth. Lawson addressed not only the basics of external morphology but also delved into the internal morphology of the head capsule and noted the differences among the four instars. Lawson noted that the pharynges became more complex and sclerotized as the instars progressed from one stage to another. In addition to this, Lawson observed that the first instar of *C. nubeculosus* had a pro-thoracic pseudopod and that, unlike many other Diptera which have a larval thoracic pseudopod, the pseudopod is lost after the first instar.

He proposed that this pseudopod was used in order to help with the movement of the first larval instar, particularly when they are attempting to extricate themselves from the egg.

In 1952 Kettle and Lawson published data for 28 of the *Culicoides* species found in Great Britain. By 1979 Kettle and Lawson had augmented this and published descriptions for a total of 46 species of *Culicoides* in Great Britain which included 88% of all known species found there (Knausenberger 1986, unpub. in Murphree & Mullen, 1991).

"Across the pond" in the United States, Jamnback (1965) published rudimentary descriptions for 23 North American *Culicoides* species, 17 of which came from New York and of that 17, 15 of the descriptions were entirely new. Jamnback (1965) not only developed a key to species for *Culicoides* from New York, but also developed keys for the fourth instar larvae as well as the pupae. Jamnback included observations concerning the larval habitats. However, his descriptions of the larvae were quite basic and typically contained only brief descriptions (head capsule shape and color) along with measurements for the frontoclypeus, the total epipharyngeal comb width, and number of teeth per comb. Despite a lack of in-depth exploration into the larval stages, Jamnback's key remains one of the better regional keys to the species level.

While Jamnback was working in New York, one of the most influential researchers in *Culicoides* morphology was working in the Soviet Union. In 1968 Glukhova began to publish the very first comparative morphological studies of *Culicoides* species. Glukhova began by publishing general morphological descriptions of 29 species of *Culicoides* found in the Palearctic, and one year later expanded upon the

descriptions for 8 of these species. By combining both the larval and adult characteristics found in the various *Culicoides* species, Glukhova (1977a) was able to attempt to redefine the subgenera classifications within *Culicoides*. Up to this point in time, subgeneric classifications within *Culicoides* relied solely on adult characteristics (Murphree & Mullen, 1991). By including the larval morphology as well, Glukhova was able to more accurately establish the classifications. Once she had done this, Glukhova (1977b) was then able to also redefine the relationships that *Culicoides* had with other genera within Ceratopogonidae by not only comparing the adults but also the larvae as well. Not only did Glukhova help to place *Culicoides* in the appropriate systematic location, she also helped to standardize morphological terminology of *Culicoides* larvae. Previously, many researchers looking into the larvae of *Culicoides* simply reported the measurements which they themselves thought were necessary or interesting. Some researchers such as Kettle and Lawson (1952) used some standards such as the head ratio (length:width) but it was not until Glukhova (1968a) that a standardized method of reporting was developed and widely accepted. The measurements chosen by Glukhova are still viewed as the standard today. After Glukhova developed the standards for *Culicoides* larvae, the research into the genus began to become more sophisticated and uniform. As can be seen in Table 1, from the 1970s until now, the quality of the descriptions began to be more consistently advanced even though there were still some more basic descriptions being produced. In 1978 Kettle and Elson published a larval key to 18 Australian *Culicoides* species, which was a step up from Jamnback since it included descriptions of both the third and fourth larval instars. One year later Blanton and Wirth

(1979) developed a key for 12 species using the larvae, but their key was focused on species found in Florida.

After Glukhova, the next big event in traditional morphology of *Culicoides* came from Murphree and Mullen (1991). Before Murphree and Mullen there were some select keys which were used, but the majority of these keys were very restricted to specific areas (e.g. New York state and Florida), did not include a large variety of species in each individual key, or did not have advanced descriptions for the larvae. Murphree and Mullen (1991) developed the first attempted comprehensive key for *Culicoides* in North America. In their work they described 12 new species, supplemented previously unpublished descriptions for 5 species, and re-described 35 species of *Culicoides*, totaling 52 species. While still short of the approximately 150 North American *Culicoides* species, this was a large improvement (Murphree & Mullen, 1991).

Not only did they have a large number of species described, Murphree and Mullen also had consistent and detailed descriptions of all of the species. What is probably most notable is the fact that they utilized scanning electron microscopy (SEM) on a few of the larvae in their studies which allowed them to describe various features with more detail. Prior to Murphree and Mullen SEM was not utilized in the study of the internal and external morphology of *Culicoides* larvae although it was used somewhat on adult insects (Rowley & Cornford, 1972; McKeever *et al.,* 1988). Since Murphree and Mullen began using SEM on the larvae of *Culicoides* species, the majority of researchers looking into various species began to follow suit. In Argentina, Ronderos *et al.* (2010) has been working primarily on the fourth larval instar and the pupa of Neotropical species, using

SEM as an aid. She actually described all four larval instars of *C. debilipalpis* as well as the pupae, making her only the second researcher to treat all larval instars in any depth. Lawson (1951) described all larval stages of *C. obsoletus* and *C. nubeculosus*.

Now that researchers have begun using more advanced techniques and are also better trained, the research into the morphology of immature stages of *Culicoides* can become more detailed. Day *et al.* (1997) and Breidenbaugh and Mullens (1999a, b) have used SEM to document the structures of the ansulae on eggs. There has been a surge in SEM use on larvae also, in order to better understand and visualize their structures (Murphree & Mullen, 1991; Huerta *et al.*, 2001). Most notable is the recent work on the pupae of various *Culicoides* species; this has primarily taken place in South America by several different researchers utilizing both SEM and transmission electron microscopy (TEM) to supplement their work (Ronderos, 2010; Marino *et al.*, 2013; Spinelli *et al.*, 2007). All of the new and growing advances in technology have helped in the study of all these various immature stages and hopefully will continue to do so.

IV. Historical Work on Subgenus *Monoculicoides* Immature Morphology

Within the genus *Culicoides*, *Culicoides sonorensis* joins *C. nubeculosus* Meigen, *C. occidentalis* Wirth and Jones, *C. parroti* Kieffer, *C. puncticollis* Becker, *C. riethi* Kieffer, *C. stigma* Meigen, *C. variipennis* Coquillett, and the recently added *C. grandensis* Grogan and Philips to make up the subgenus *Monoculicoides* Khalaf. Members of subgenus *Monoculicoides* differ from other members of *Culicoides* in terms of their size and anatomy, including their internal mouthpart structures of the larvae. As with every other species of *Culicoides*, the members of *Monoculicoides* subgenus have

had their immature stages largely ignored. According to Murphree and Mullen (1991), *Monoculicoides* subgenus species are known for having relatively very large larvae (among *Culicoides*) and large mandibles which have 2 subapical teeth. The subgenus *Monoculicoides* particularly differ in their uniquely massive larval epipharynx with 4 combs. A pictorial representation of the epipharynx of *Culicoides sonorensis* can be seen in Figure 1. Based on these structures as well as the overall morphology of the head capsule in which the head is both long and narrow with its mouthparts found anteriorly, the *Monoculicoides* subgenus is considered to be either carnivorous or omnivorous (Thomson, 1937). In addition to this, many researchers also believe that the epipharynges of the various species of the subgenus *Monoculicoides* are so heavily sclerotized because they have adapted in order to grind and crush their food (Kettle & Lawson, 1952; Jamnback, 1965; Kettle, 1984).

Initial research was conducted on the morphology of immature *Monoculicoides* in 1915, when Malloch studied *C. variipennis* he collected from the northeastern US. However, his descriptions were not up to modern standards primarily due to a lack of modern technology. In 1965 Jamnback recorded the larval morphology of what he called *C. variipennis australis* (as per the 5 "subspecies" described by Wirth and Jones in 1957). The larvae were collected from Missouri and could have been *C. variipennis variipennis*, or more likely, *C. sonorensis*. Jamnback did not describe in depth any of the features of the mouthparts of this species and only had two specimens, but he did note the width of the dorsal comb sclerite as well as the number of dorsal (Table 2). A far more complete and detailed description of *C. variipennis* came much later from Murphree and

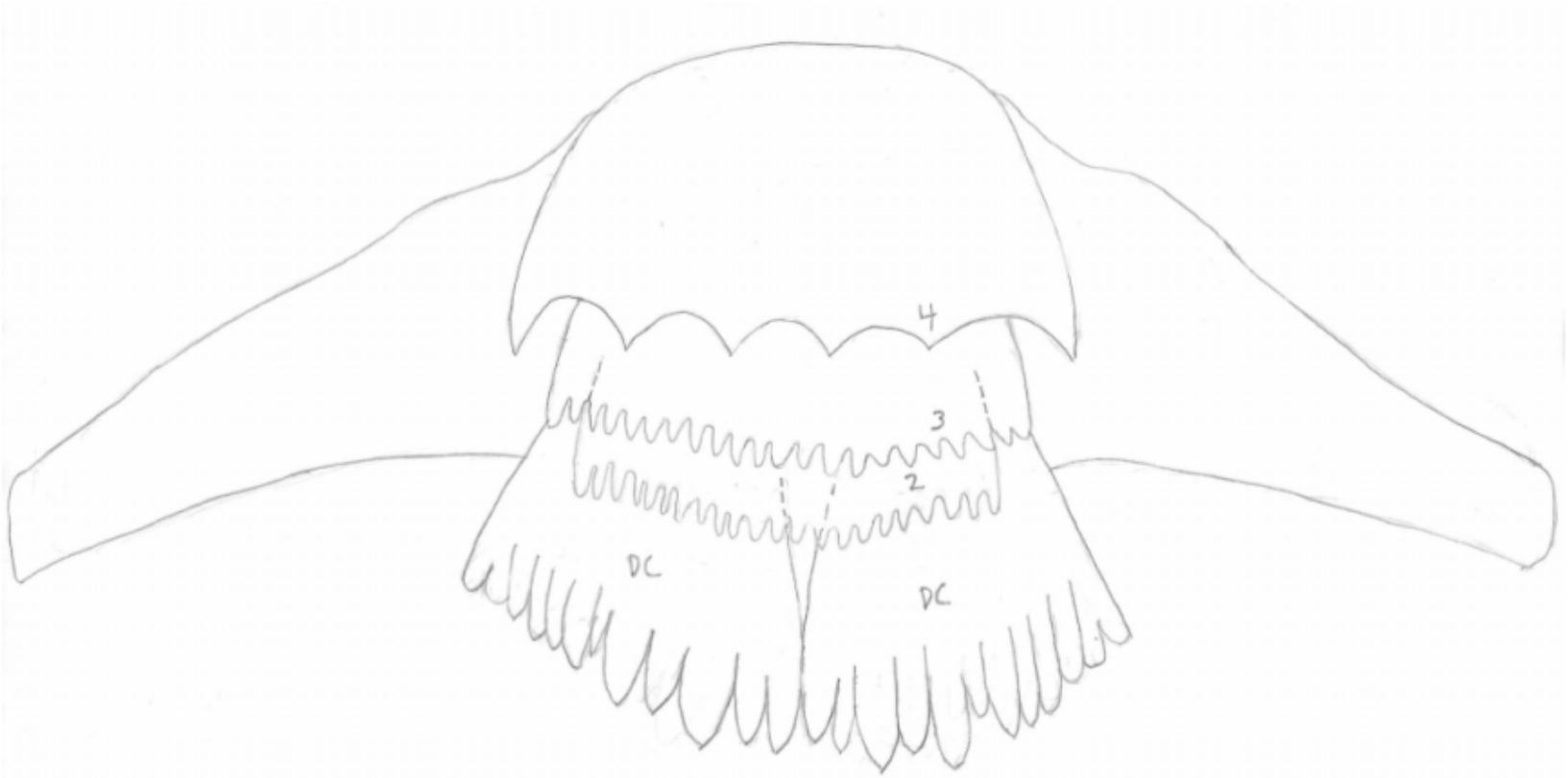

Figure 1. Epipharynx morphology of *C. sonorensis*. DC, dorsal-comb sclerite; 2-4, epipharangeal combs 2, 3, 4.

Mullen (1991). The specimens which were used in this study came from Missouri, California, and Texas and were presumably from *C. variipennis, C. sonorensis,* as well as *C. occidentalis*; at that point *C. sonorensis* and *C. occidentalis* were still considered to be subspecies of *C. variipennis*. The large variations in measurements could also indicate that 2 or more species were represented. They also determined that the mandibles and epipharynx were similarly large and that the epipharynx had 4 combs with the dorsal comb (comb 1) having 13 teeth per sclerite, comb 2 and 3 with many rounded tubercles, and a large comb 4 with 6-10 rounded teeth on each side (Table 2). This research into the larvae of *C. variipennis* is especially important for the research into *C. sonorensis* due to the fact that the two were only split into separate species very recently (Holbrook *et al.,* 2000) and the data on *C. variipennis* may give us insights into how *C. sonorensis* functions as well.

Although *C. variipennis* was described first, the first detailed description of the morphology of the larvae of a species of the subgenus *Monoculicoides* was for *C. nubeculosus*. In 1951 Lawson was one of the first researchers to study all four larval instars of a species of *Culicoides*. His measurements for the L4 can be seen in table 2 and for L1 he found HL = 72 µm and HW = 54 µm, for L2 he found HL = 122 µm and HW = 88 µm, and for L3 he found HL = 200 µm and HW = 122 µm. He also mentioned that the mandibles were heavily sclerotized and that although they could be moved either together or independently, they never met. One particularly interesting point that Lawson made is that *C. nubeculosus* had very similar mouthparts to species of *Tetraphora* (= *Dasyhelea*). When *Tetraphora* was fed algae or detritus, it made scratching movements with its

mandibles, detaching pieces and then pushed the pieces into the pre-oral cavity. He postulated that the similarities in their structures suggested that *C. nubeculosus* larvae might feed in a similar fashion. In 1952 Kettle and Lawson had similar findings and conclusions for *C. nubeculosus*.

Beyond these two important species, other members of the subgenus *Monoculicoides* have not been studied in depth. Kettle and Lawson (1952) documented the measurements for *C. riethi* as well as *C. stigma,* noting that the two species were very similar in size (Table 2). In addition to this, throughout her career, Glukhova supplemented data on some of the species as well as adding the description for *C. puncticollis*. Unfortunately there have been no studies on the larval stages of *C. parroti* or *C. grandensis*. Earlier work on *C. stigma* may also be suspect; it is very easy to confuse *C. stigma* with *C. parroti* adults.

Table 2: Historic morphological data for the species of the subgenus *Monoculicoides* larvae

Species	Researcher	TBL (mm)	HL (µm)	HW (µm)	SGW (µm)	ML (µm)	LAW (µm)	DCW (µm)	Teeth/ Comb
C. variipennis s.l.	Murphree and Mullen 1991		319 (291-367)	219 (190-271)	113 (94-156)	64 (45-86)	129 (98-159)	30 (25-39)	13 (10-15)
	Jamnback 1965							28-31	7-8
C. nubeculosus	Lawson 1951	7	311	207					8-12
C. riethi	Kettle and Lawson 1952		271	178				42	9-10
C. stigma	Kettle and Lawson 1952		268	166				34	8

V. Immature Biology and Behavior of *Culicoides* Immatures

In addition to a general lack of complete studies of *Culicoides* immature morphology, there is also an even greater lack of knowledge when it comes to their

behavior and biology. Some of the first studies into larval biology and behavior were published in the late 1920s and 1930s. Painter (1926) examined the larval habitats of *C. furens* Poey and *C. phlebotomus* Williston and considered control methods based on his observations. Myers (1932) studied the ecology of *C. furens* larvae in the Bahamas in order to help alleviate the economic problems (tourism impacts) caused by this pest. Hull *et al.* (1934) then delved into the seasonal prevalence and concentrations of *C. dovei* Hall larvae. Similarly to the studies on the immature morphology of *Culicoides*, the early studies on behavior and biology have been scattered and lack cohesion.

In 1966 Linley first studied the basic biology of larvae of *C. furens* and gave an account of their feeding habits as well as the activity within a substrate. Of particular interest is Linley and Adam's (1972) work in which he showed that in the field the pupae of *C. mellus* Coquillett were found to be either at or just below the high tide line and were capable of surviving flooding for 4 days. Linley continued his work with various *Culicoides* species into the 1980s and 1990s. In 1986 Linley studied how the swimming behavior of *C. variipennis* larvae varied due to temperature and viscosity and in 1994 he joined Aussel to determine what the larvae of *C. furens* were eating.

Linley examined several species, especially salt marsh biting pest species. Mullens focused his attention primarily on the main BTV vector, *C. sonorensis* (at the time known as *C. variipennis*) in southern California. Studies of *C. sonorensis* immature ecology included the type of dairy wastewater pond the immatures preferred and which factors determined where the highest densities of *C. sonorensis* were found (Mullens, 1989). Mullens also looked into how the immature stages of *C. sonorensis* responded to

experimental variations in the water levels, pond slope, water level in a pond, and manure pollution (Mullens & Rodriguez, 1988; 1989; 1992; Mullens, 1989). These studies showed that fluctuating the water level in a pond should cause the population of *C. sonorensis* to decrease by stranding larvae above the waterline. Larvae of *C. sonorensis* also avoid shade (Mullens & Rodriguez, 1985), steep slopes (Mullens, 1989), and are limited by low or high nutrient levels (Mullens & Rodriguez, 1988). Mullens and Lii (1987) monitored immature positions, similar to Linley's work on *C. mellus*, and determined where along the shoreline the different immature stages of *C. sonorensis* could be found. They determined that the eggs and the majority of the first larval instars could be found above the waterline, while the second, third, and fourth instars were found below the waterline. His work did not include the location of the pupae, however. Later on he elaborated on this work when he looked into the responses of the third and fourth larval instars of *Culicoides sonorensis* in drying mud habitats (Mullens & Rodriguez, 1992). Mullens *et al.* (2008) reviewed all available literature on the relationship between *Culicoides* species and their main natural enemy group, mermithid nematodes in the genus *Heleidomermis* Rubtsov.

Others have contributed valuable efforts towards understanding the ecology and behaviors of other *Culicoides* species. While not exhaustively treated here, they include Blackwell and King (1997) who determined the vertical distribution of *C. impunctatus* Goetghebuer larvae, Uslu and Dik (2006) who looked into the vertical distributions of various species of *Culicoides* immatures in Turkey, and the work on *C. denningi* Foote and Pratt by Fredeen (1969) to determine their vertical distribution as well. More recently,

Swanson (2012) completed his dissertation on the ecology and phylogeny of *Culicoides*, which includes the first use of molecular approaches to identify immatures in North America. The work on *Culicoides* immature behavior and ecology is still very incomplete.

The ability of *Culicoides* spp. to transmit devastating disease agents can cause economic problems, particularly in the western part of the United States. This makes it imperative that we learn as much as we can about *Culicoides sonorensis* ecology. The research which I have done is intended to expand upon the knowledge concerning *C. sonorensis*. I have specifically filled the gap in terms of the descriptions of larval morphology, especially regarding larval mouthparts of all four larval instars as well as the egg and pupae. In addition to this, I have added to the base of expanding knowledge concerning some aspects of the behavior and the ecology of the immature stages in the laboratory.

Table 1: Historical Studies of Morphology of *Culicoides* Immature Stages

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Derham	1713	*Culex minimus nigricans maculates*					X		New	B	Great Britain
Pratt	1907	*C. guttipennis*					X	X	New	B	C+N America
Patton	1913	*C. oxystoma* (described as *C. kiefferi*)					X	X	New	B	India
Lutz	1913	*C. bambusicola*					X		New	B	Brazil
		C. maruim	X					X	New	B	Brazil
		C. reticulatus					X	X	New	B	Brazil
Reith	1915	*C. pulicaris* (described as *C. setosinervis*)					X	X	New	B	Great Britain
		C. riethi					X	X	New	B	Great Britain
		C. salinarius					X	X	New	B	Great Britain
		C. stigmaticus					X	X	New	B	Great Britain
Malloch	1915	*C. variipennis*					X	X	New	B	C+N America
Goetghebuer	1919	*C. pulicaris*					X	X	Re-	B	Great Britain
Carter *et al*	1920	*C. accraensis*					X	X	New	B	Africa
		C. clarkei					X	X	New	B	Africa
		C. confusus					X	X	New	B	Africa
		C. eriodendroni					X	X	New	B	Africa
		C. inornatipennis					X	X	New	B	Africa
		C. nigripennis					X	X	New	B	Africa
		C. punctithorax					X	X	New	B	Africa
		C. schultzei					X	X	New	B	Africa
Ingram and Macfie	1921	*C. austeni*					X	X	New	B	West Africa
		C. distinctipennis					X	X	New	B	West Africa
		C. grahamii						X	New	B	West Africa
		C. lamborni						X	New	B	West Africa
		C. neavei						X	New	B	West Africa
		C. pycnostictus						X	New	B	West Africa
		C. rutilus						X	New	B	West Africa
		C. similis					X	X	New	B	West Africa
Kieffer	1923	*C. parroti*						X	New	B	Palearctic

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Kieffer (Cont.)	1923	*C. puncticollis* (described as *C. donatieni*)						X	New	B	Palearctic
Ingram and Macfie	1925	*C. lamborni*						X	Re-	B	Africa
Painter	1926	*C. furens*					X	X	New	B	C+N America
		C. phlebotomus					X	X	New	B	C+N America
Medwedewa	1927	*C. nubeculosus*					X	X	New	B	Great Britain
Thienemann	1928	*C. circumscriptus* (described as *C. algarum* and *C. salicola*)					X	X	New	B	Great Britain
		C. festivipennis						X	New	B	Great Britain
		C. newsteadi (described as *C. halophilus*)						X	New	B	Great Britain
		C. nubeculosus						X	Re-	B	Great Britain
		C. obsoletus					X	X	New	B	Great Britain
		C. pictipennis						X	New	B	Great Britain
		C. pulicaris					X	X	Re-	B	Great Britain
		C. riethi					X	X	Re-	B	Great Britain
		C. salinarius					X	X	Re-	B	Great Britain
		C. stigmaticus						X	Re-	B	Great Britain
Sasaki	1928	*C. arakawai*					X		New	B	Japan
Jobling	1929	*C. vexans*	X						New	B	Great Britain
Dove *et al*	1932	*C. furens* (described as *C. dovei*)	X				X	X	New	B	C+N America
Steward	1933	*C. nubeculosus*	X						New	B	C+N America
Mayer	1934	*C. accraensis*					X	X	Re-	B	Europe
		C. anophelis					X		New	B	Europe
		C. austeni					X	X	Re-	B	Europe
		C. circumscriptus					X	X	Re-	B	Europe
		C. clarkei					X	X	Re-	B	Europe
		C. distinctipennis						X	Re-	B	Europe
		C. eriodendroni					X	X	Re-	B	Europe
		C. fascipennis						X	New	B	Europe

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Mayer (Cont.)	1934	*C. festivipennis* (described as *C. winnertzi*)						X	Re-	B	Europe
		C. grahamii						X	Re-	B	Europe
		C. inornatipennis						X	Re-	B	Europe
		C. lamborni						X	Re-	B	Europe
		C. neavei						X	Re-	B	Europe
		C. newsteadi (described as *C. halophilus*)						X	Re-	B	Europe
		C. nigripennis					X	X	Re-	B	Europe
		C. nubeculosus					X	X	Re-	B	Europe
		C. obsoletus (described as *C. rivicola*)					X		Re-	B	Europe
		C. peregrinus						X	New	B	Europe
		C. pictipennis					X	X	New	B	Europe
		C. pulicaris					X	X	Re-	B	Europe
		C. punctithorax						X	Re-	B	Europe
		C. pycnostictus						X	Re-	B	Europe
		C. riethi					X	X	Re-	B	Europe
		C. salinarius					X	X	Re-	B	Europe
		C. schultzei					X	X	Re-	B	Europe
		C. similis					X	X	Re-	B	Europe
		C. stigmaticus						X	Re-	B	Europe
Lenz	1934	*C. circumscriptus* (described as *C. algarum* and *C. salicola*)						X	Re-	B	Palearctic
		C. festivipennis (described as *C. winnertzi*)						X	Re-	B	Palearctic
		C. meeserellus						X	New	B	Palearctic
		C. newsteadi (described as *C. halophilus*)						X	Re-	B	Palearctic
		C. nubeculosus						X	Re-	B	Palearctic
		C. obsoletus						X	Re-	B	Palearctic

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Lenz (Cont.)	1934	*C. pictipennis*						X	Re-	B	Palearctic
		C. pulicaris						X	Re-	B	Palearctic
		C. riethi (described as *C. crassiforceps*)						X	Re-	B	Palearctic
		C. salinarius						X	Re-	B	Palearctic
		C. stigmaticus						X	Re-	B	Palearctic
De Meillon	1936	*C. meeserellus*						X	Re-	B	Africa
		C. pycnostictus (described as *C. alexis*)						X	Re-	B	Africa
Tokunaga	1937	*C. circumscriptus*					X	X	Re-	B	Japan
De Meillon	1937	*C. cornutus*						X	New	B	Zululand
		C. engubandei						X	New	B	Zululand
		C. nivosus						X	New	B	Zululand
Thomsen	1937	*C. biguttatus*						X	New	B	C+N America
		C. crepuscularis						X	New	B	C+N America
		C. haematopotus						X	New	B	C+N America
		C. sanguisuga					X		New		C+N America
		C. variipennis						X	Re-	B	C+N America
		C. venustus						X	New	B	C+N America
Fox	1942	*C. arboricola*						X	New	B	Americas
		C. arubae (described as sp. 1)						X	New	B	Americas
		C. bambusicola					X		Re-	B	Americas
		C. crepuscularis						X	Re-	B	Americas
		C. furens						X	Re-	B	Americas
		C. guttipennis						X	Re-	B	Americas
		C. hylas (described as sp. 3)						X	New	B	Americas
		C. nanus						X	New	B	Americas
		C. pallidicornis (Described as *C. niger*)						X	New	B	Americas
		C. phlebotomus						X	Re-	B	Americas

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Fox (Cont.)	1942	*C. stellifer*						X	New	B	Americas
		C. variipennis						X	Re-	B	Americas
		C. villosipennis						X	New	B	Americas
Fujito	1942/ 1943	*C. miharai*	X	X	X	X	X	X	New	B	Japan
Hill	1947	*C. festivipennis* (described as *C. odibilis*)	X		X	X	X	X	New	B	Great Britain
		C. impunctatus	X	X	X	X	X	X	New	B	Great Britain
		C. kibunensis (described as *C. cubitalis*)			X	X	X	X	New	B	Great Britain
		C. nubeculosus			X	X	X		New	B	Great Britain
		C. obsoletus	X		X	X	X	X	New	B	Great Britain
		C. pallidicornis				X	X	X	New	B	Great Britain
Lane	1947	*C. bambusicola*					X	X	New	B	Brazil
Cameron	1947	*C. impunctatus*	X				X	X	Re-	B	Scotland
Parker	1950	*C. fascipennis*	X						New	B	Great Britain
		C. grisescens	X						New	B	Great Britain
		C. impunctatus	X						Re-	B	Great Britain
		C. newsteadi (described as *C. halophilus*)	X						New	B	Great Britain
		C. obsoletus	X						Re-	B	Great Britain
		C. pallidicornis	X						New	B	Great Britain
		C. pulicaris	X						New	B	Great Britain
Lawson	1951	*C. obsoletus*		X	X	X	X	X	New	A	Great Britain
		C. nubeculosus		X	X	X	X	X	New	A	Great Britain
Williams	1951	*C. furens*					X	X	Re-	B	C+N America
		C. mellus					X	X	New	B	C+N America
		C. tristriatulus	X				X	X	New	A	C+N America
		C. unicolor					X	X	New	B	C+N America
Kettle and Lawson	1952	*C. albicans*				X	X	X	New	B	UK
		C. chiopterus					X	X	New	B	UK
		C. circumscriptus					X	X	Re-	B	UK

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Kettle and Lawson (Cont.)	1952	*C. cunctans*					X	X	New	B	UK
		C. delta					X	X	New	B	UK
		C. deltus (described as *C. lupicaris*)				X	X	X	New	B	UK
		C. dewulfi (described as *C. pseudochiopterus*)					X	X	New	B	UK
		C. fascipennis				X	X	X	New	B	UK
		C. festivipennis (described as *C. odibilis*)					X	X	Re-	B	UK
		C. grisescens					X	X	New	B	UK
		C. heliophilus				X	X	X	New	B	UK
		C. impunctatus		X	X	X	X	X	Re-	B	UK
		C. kibunensis (described as *C. cubitalis*)				X	X	X	Re-	B	UK
		C. maritimus				X	X	X	New	B	UK
		C. newsteadi (described as *C. halophilus*)				X	X	X	New	B	UK
		C. nubeculosus				X	X	X	Re-	B	UK
		C. obsoletus				X	X	X	Re-	B	UK
		C. pallidicornis				X	X	X	Re-	B	UK
		C. parroti						X	Re-	B	UK
		C. pictipennis					X	X	Re-	B	UK
		C. pulicaris				X	X	X	New	B	UK
		C. punctatus				X	X	X	New	B	UK
		C. riethi					X	X	Re-	B	UK
		C. salinarius		X	X	X	X	X	New	B	UK
		C. scoticus					X	X	New	B	UK
		C. simulator					X	X	New	B	UK
		C. stigma				X	X	X	New	B	UK
		C. truncorum		X	X	X	X	X	New	B	UK
		C. vexans					X	X	New	B	UK

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Wirth	1952a	*C. luteovenus*					X	X	New	B	C+N America
		C. unicolor					X	X	Re-	B	C+N America
		C. variipennis					X	X	Re-	B	C+N America
Wirth	1952b	*C. furens*					X	X	Re-	B	C+N America
		C. melleus					X	X	New	B	C+N America
Barbosa	1952	*C. bambusicola*					X	X	Re-	B	Brazil
		C. debilipalpis						X	New	B	Brazil
		C. maruim						X	Re-	B	Brazil
Jobling	1953	*C. vexans*	X	X					New	B	Great Britain
Jones	1955	*C. arboricola*						X	Re-	B	C+N America
		C. crepuscularis					X	X	New	B	C+N America
		C. flukei						X	New	B	C+N America
		C. guttipennis					X	X	Re-	B	C+N America
		C. haematopotus						X	Re-	B	C+N America
		C. nanus					X	X	New	B	C+N America
		C. obsoletus					X	X	Re-	B	C+N America
		C. piliferus					X		New	B	C+N America
		C. spinosus						X	New	B	C+N America
		C. stellifer						X	Re-	B	C+N America
		C. travisi						X	New	B	C+N America
		C. unicolor						X	Re-	B	C+N America
		C. variipennis (possibly *C. sonorensis*)	X						New	B	C+N America
		C. venustus						X	Re-	B	C+N America
		C. villosipennis					X	X	New	B	C+N America
		C. wisconsinensis						X	New	B	C+N America
Kettle and Lawson	1955	*C. achrayi*					X	X	New	A	C+N America
		C. duddingstoni					X	X	New	A	C+N America
Forattini and Rabello	1956	*C. guyanensis*					X	X	New	B	Brazil
Forattini *et al*	1956	*C. insignis*					X	X	New	B	Brazil
Das Gupta and Ghosh	1956	*C. palpifer*					X		New	B	India
Forattini	1957	*C. arubae*						X	Re-	B	Brazil

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Forattini (Cont.)	1957	*C. bambusicola*						X	Re-	B	Brazil
		C. debilipalpis						X	Re-	B	Brazil
		C. furens					X	X	Re-	B	Brazil
		C. guyanensis					X	X	Re-	B	Brazil
		C. hylas						X	Re-	B	Brazil
		C. insignis					X	X	Re-	B	Brazil
		C. maruim						X	Re-	B	Brazil
		C. phlebotomus						X	Re-	B	Brazil
		C. stellifer						X	Re-	B	Brazil
Jamnback *et al*	1958	*C. hollensis*					X	X	New	B	C+N America
		C. melleus					X	X	Re-	B	C+N America
Bullock and Akiyama	1959	*C. dendrophilus*					X	X	New	B	Japan + Korea
Forattini *et al*	1960	*C. reticulatus*					X		Re-	B	Brazil
Becker	1960	*C. circumscriptus*	X	X	X	X	X	X	New	B	Scotland
Tokunaga *et al*	1961	*C. arakawai*					X	X	New	B	Japan
Jones	1961	*C. bergi* (Described as *C. baueri*)						X	New	B	C+N America
		C. footei						X	New	B	C+N America
		C. furens						X	Re-	B	C+N America
		C. haematopotus						X	Re-	B	C+N America
		C. hieroglyphicus						X	New	B	C+N America
		C. jamesi						X	New	B	C+N America
		C. melleus						X	Re-	B	C+N America
		C. multipunctatus						X	New	B	C+N America
		C. neopulicaris						X	New	B	C+N America
		C. obsoletus						X	Re-	B	C+N America
		C. stonei						X	New	B	C+N America
		C. tristriatulus						X	Re-	B	C+N America
		C. venustus						X	Re-	B	C+N America
Jamnback and Wirth	1963	*C. chiopterus*					X	X	Re-	B	New York
		C. obsoletus					X	X	Re-	B	New York
		C. sanguisuga					X	X	New	B	New York

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Linley and Kettle	1964	*C. furens*					X	X	Re-	A	Jamaica
		C. hoffmani					X	X	New	A	Jamaica
Dzhafarov	1961/ 1964	*C. chiopterus*					X		Re-	B	Eastern Europe
		C. circumscriptus						X	Re-	B	East Europe
		C. cunctans						X	Re-	B	East Europe
		C. duddingstoni						X	Re-	B	East Europe
		C. fagineus						X	New	B	East Europe
		C. festivipennis (described as *C. odibilis*)					X	X	Re-	B	East Europe
		C. firuzae						X	New	B	East Europe
		C. gejgelensis						X	New	B	East Europe
		C. kibunensis (described as *C. cubitalis*)						X	Re-	B	East Europe
		C. longipennis (described as *C. pseudosimilis*)						X	New	B	East Europe
		C. nubeculosus					X	X	Re-	B	East Europe
		C. obsoletus						X	Re-	B	East Europe
		C. odiatus (described as *C. kurekchaicus*)					X	X	New	B	East Europe
		C. pallidicornis						X	Re-	B	East Europe
		C. pictipennis (described as *C. achkamalicus*)						X	Re-	B	East Europe
		C. pulicaris						X	Re-	B	East Europe
		C. puncticollis						X	Re-	B	East Europe
		C. riethi	X				X	X	New	B	East Europe
		C. saevus						X	New	B	East Europe
		C. salinarius						X	Re-	B	East Europe
		C. scoticus					X		Re-	B	East Europe
		C. semimaculatus (described as *C. karajevi*)						X	New	B	East Europe

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Dzhafarov (Cont.)	1961/ 1964	*C. shaklawensis* (described as *C. caspius*)						X	New	B	East Europe
		C. stigma						X	Re-	B	East Europe
		C. subfasciipennis						X	New	B	East Europe
		C. truncorum						X	Re-	B	East Europe
Linley	1965	*C. barbosai*						X	New	A	Jamaica
		C. borinqueni						X	New	A	Jamaica
		C. insignis						X	Re-	A	Jamaica
Jamnback	1965	*C. alexanderi* (described as *C. pseudopiliferus*)						X	New	B	New York
		C. arboricola						X	Re-	B	New York
		C. bergi (Described as *C. baueri*)						X	Re-	B	New York
		C. bermudensis					X	X	New	B	New York
		C. bickleyi					X	X	New	B	New York
		C. biguttatus					X	X	New	B	New York
		C. chiopterus					X	X	Re-	B	New York
		C. crepuscularis					X	X	Re-	B	New York
		C. denticulatus					X	X	New	B	New York
		C. flukei					X	X	New	B	New York
		C. furens					X	X	Re-	B	New York
		C. guttipennis					X	X	Re-	B	New York
		C. haematopotus					X	X	New	B	New York
		C. hollensis					X	X	Re-	B	New York
		C. jamnbacki					X	X	New	B	New York
		C. loisae						X	New	B	New York
		C. melleus					X	X	Re-	B	New York
		C. obsoletus					X	X	Re-	B	New York
		C. pallidicornis (described as *C. niger*)						X	Re-	B	New York
		C. parapiliferus						X	New	B	New York
		C. piliferus					X	X	New	B	New York

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Jamnback (Cont.)	1965	*C. sanguisuga*					X	X	Re-	B	New York
		C. spinosus					X	X	New	B	New York
		C. stellifer					X	X	New	B	New York
		C. testudinalis						X	New	B	New York
		C. travisi					X	X	New	B	New York
		C. utowana					X	X	New	B	New York
		C. variipennis					X	X	Re-	B	New York
		C. venustus					X	X	New	B	New York
		C. villosipennis					X	X	Re-	B	New York
		C. wisconsinensis					X	X	New	B	New York
Matta	1967	*C. hayesi*					X	X	New	B	Honduras
Hogue and Wirth	1968	*C. cancer*					X	X	New	B	Costa Rica
Wirth and Blanton	1968	*C. heliconiae*						X	New	B	Neotropics
Glukhova	1969	*C. dendrophilus*					X		Re-	A	USSR
		C. dispersus					X		New	A	USSR
		C. fagineus					X		New	A	USSR
		C. manchuriensis					X		New	A	USSR
		C. saevus					X		New	A	USSR
		C. sinanoensis					X		New	A	USSR
		C. subfasciipennis					X		New	A	USSR
Nevill	1969	*C. bedfordi*					X	X	New	B	South Africa
		C. distinctipennis					X	X	Re-	B	South Africa
		C. enderleini (described as *C. schultzei*)					X	X	New	B	South Africa
		C. imicola (described as *C. pallidipennis*)					X	X	New	B	South Africa
		C. magnus					X		New	B	South Africa
		C. milnei					X	X	New	B	South Africa
		C. nivosus					X	X	New	B	South Africa
		C. pycnostictus					X	X	New	B	South Africa
		C. schultzei					X	X	Re-	B	South Africa
Linley	1970a	*C. arboricola*					X	X	New	A	Jamaica

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Linley	1970b	*C. floridensis*						X	New	A	Jamaica
Atchley	1970	*C. denningi*					X	X	New	B	C+N America
		C. brookmani						X	New	B	C+N America
		C. hieroglyphicus					X	X	New	B	C+N America
		C. jacksoni					X	X	New	B	C+N America
		C. jamesi					X	X	New	B	C+N America
		C. multipunctatus						X	Re-	B	C+N America
		C. tenuistylus					X	X	New	B	C+N America
Gutsevich and Glukhova	1970	*C. achrayi*					X		Re-	A	Palearctic
		C. albicans					X		Re-	A	Palearctic
		C. chiopterus					X		Re-	A	Palearctic
		C. circumscriptus					X		Re-	A	Palearctic
		C. deltus (described as *C. lupicaris*)					X		Re-	A	Palearctic
		C. dendrophilus					X		Re-	A	Palearctic
		C. dispersus					X		Re-	A	Palearctic
		C. fagineus					X		Re-	A	Palearctic
		C. fascipennis					X	X	Re-	A	Palearctic
		C. festivipennis (described as *C. odibilis*)					X		Re-	A	Palearctic
		C. grisescens					X		Re-	A	Palearctic
		C. impunctatus					X		Re-	A	Palearctic
		C. kibunensis (described as *C. cubitalis*)					X		Re-	A	Palearctic
		C. manchuriensis					X		Re-	A	Palearctic
		C. maritimus					X		Re-	A	Palearctic
		C. nubeculosus					X		Re-	A	Palearctic
		C. obsoletus	X				X		Re-	A	Palearctic
		C. pallidicornis					X		Re-	A	Palearctic
		C. pulicaris	X				X		Re-	A	Palearctic
		C. puncticollis					X		New	A	Palearctic
		C. saevus					X		Re-	A	Palearctic

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Gutsevich and Glukhova (Cont.)	1970	*C. salinarius*					X		Re-	A	Palearctic
		C. simulator					X		Re-	A	Palearctic
		C. sinanoensis					X		Re-	A	Palearctic
		C. stigma					X		Re-	A	Palearctic
		C. subfasciipennis					X	X	Re-	A	Palearctic
		C. truncorum					X		Re-	A	Palearctic
Damian-Georgescu and Spataru	1971	*C. riethi*					X	X	Re-	B	Great Britain
Linley and Davis	1971	*C. barbosai*	X						New		West Indies
		C. furens	X						Re-	B	West Indies
Spataru	1971	*C. stigma*					X	X	Re-		Europe
Atchley	1971	*C. denningi*						X	Re-	B	Australia
		C. hieroglyphicus						X	Re-	B	Australia
		C. jamesi						X	Re-	B	Australia
Gornostaeva and Gachegova	1972	*C. filicinus*	X				X	X	New	B	USSR
Kwan	1972	*C. obsoletus*					X		Re-	B	C+N America
		C. sanguisuga					X		Re-	B	C+N America
Glukhova	1973	*C. kirgizicus*					X	X	New	A	USSR
Kwan and Morrison	1974	*C. sanguisuga*	X				X		New	A (SEM)	C+N America
Cochrane	1973	*C. furensoides*					X	X	New	B	C+N America
Dubrovskaya	1973	*C. homochrous*					X		New	B	East Europe
		C. ustinovi					X		New	B	East Europe
Glukhova and Yasakova	1974	*C. arboreus*					X		New	B	East Europe
Cochrane	1974a	*C. baueri*					X		New	B	C+N America
		C. franclemonti					X		New	B	C+N America
		C. furensoides					X		Re-	B	C+N America
		C. spinosus					X		Re-	B	C+N America
		C. villosipennis					X		Re-	B	C+N America
Cochrane	1974b	*C. franclemonti*					X	X	New	B	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Jeu and Rong	1974	*C. homotomus* (described as *C. riethi*)	X				X	X	New	B	China
Campbell and Kettle	1975	*C. brevitarsis*	X						New	A (SEM)	Australia
Kettle and Elson	1975a	*C. interrogatus*			X	X	X	X	New	B	Australia
Kettle and Elson	1975b	*C. belkini*					X	X	New	B	Australia
Kettle and Elson	1975c	*C. austropalpalis*				X	X		New	B	Australia
Kettle *et al*	1976	*C. gladysae*			X	X	X	X	New	B	Australia
Kettle and Elson	1976	*C. austropalpalis*				X	X	X	New	B	Australia
		C. belkini					X	X	Re-	B	Australia
		C. brevitarsis				X	X	X	New	B	Australia
		C. gladysae					X	X	Re-	B	Australia
		C. interrogatus					X	X	Re-	B	Australia
		C. marksi				X	X	X	New	B	Australia
		C. marmoratus				X	X	X	New	B	Australia
		C. molestus				X	X	X	New	B	Australia
		C. narrabeenensis				X	X	X	New	B	Australia
		C. rabauli (described as *C. angularis*)				X	X	X	New	B	Australia
		C. subimmaculatus				X	X	X	New	B	Australia
		C. victoriae					X	X	New	B	Australia
Kettle and Elson	1978	*C. bundyensis*				X	X	X	New	B	Australia
		C. bunrooensis					X	X	New	B	Australia
		C. dycei					X	X	New	B	Australia
		C. fulbrighti					X	X	New	B	Australia
		C. histrio					X	X	New	B	Australia
		C. narrabeenensis					X		Re-	B	Australia
		C. parvimaculatus					X	X	New	B	Australia
Atchley and Wirth	1979	*C. defoliarti*						X	New	B	C+N America
		C. erikae						X	New	B	C+N America
		C. haematopotus					X	X	Re-	B	C+N America
Blanton and Wirth	1979	*C. arboricola*					X	X	Re-	B	C+N America
		C. barbosai						X	Re-	B	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Blanton and Wirth (Cont.)	1979	*C. bermudensis*					X	X	Re-	B	C+N America
		C. bickleyi					X	X	Re-	B	C+N America
		C. biguttatus					X	X	Re-	B	C+N America
		C. chiopterus					X	X	Re-	B	C+N America
		C. crepuscularis					X	X	Re-	B	C+N America
		C. edeni						X	New	B	C+N America
		C. floridensis						X	Re-	B	C+N America
		C. footei						X	Re-	B	C+N America
		C. furens					X	X	Re-	B	C+N America
		C. guttipennis					X	X	Re-	B	C+N America
		C. haematopotus					X	X	Re-	B	C+N America
		C. hollensis					X	X	Re-	B	C+N America
		C. insignis						X	Re-	B	C+N America
		C. loisae						X	Re-	B	C+N America
		C. melleus					X	X	Re-	B	C+N America
		C. nanus						X	Re-	B	C+N America
		C. pallidicornis (Described as *C. niger*)						X	Re-	B	C+N America
		C. parapiliferus						X	Re-	B	C+N America
		C. piliferus					X	X	Re-	B	C+N America
		C. scanloni						X	New	B	C+N America
		C. snowi						X	New	B	C+N America
		C. spinosus						X	Re-	B	C+N America
		C. stellifer					X	X	Re-	B	C+N America
		C. testudinalis						X	Re-	B	C+N America
		C. travisi					X	X	Re-	B	C+N America
		C. variipennis					X	X	Re-	B	C+N America
		C. venustus					X	X	Re-	B	C+N America
		C. villosipennis					X	X	Re-	B	C+N America
Cornet and Nevill	1979	*C. hildae*						X	New	B	South Africa
Glukhova	1979	*C. achrayi*					X		Re-	A	USSR

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Glukhova (Cont.)	1979	*C. alazanicus*					X		New	A	USSR
		C. albicans					X		Re-	A	USSR
		C. amossovae					X		New	A	USSR
		C. arboreus					X		Re-	A	USSR
		C. chiopterus					X		Re-	A	USSR
		C. circumscriptus					X		Re-	A	USSR
		C. deltus					X		Re-	A	USSR
		C. dendrophilus					X		Re-	A	USSR
		C. desertorum					X		New	A	USSR
		C. dispersus					X		Re-	A	USSR
		C. fagineus					X		Re-	A	USSR
		C. fascipennis					X		Re-	A	USSR
		C. festivipennis					X		Re-	A	USSR
		C. filicinus					X		Re-	A	USSR
		C. grisescens					X		Re-	A	USSR
		C. helveticus					X		New	A	USSR
		C. homochrous					X		Re-	A	USSR
		C. impunctatus					X		Re-	A	USSR
		C. kibunensis (described as *C. cubitalis*)					X		Re-	A	USSR
		C. kirgizicus					X		Re-	A	USSR
		C. longicollis					X		New	A	USSR
		C. manchuriensis					X		Re-	A	USSR
		C. maritimus					X		Re-	A	USSR
		C. nubeculosus					X		Re-	A	USSR
		C. obsoletus					X		Re-	A	USSR
		C. pallidicornis					X		Re-	A	USSR
		C. pictipennis					X		Re-	A	USSR
		C. pulicaris					X		Re-	A	USSR
		C. punctatus					X		Re-	A	USSR
		C. puncticollis					X		Re-	A	USSR
		C. reconditus					X		New	A	USSR

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Glukhova (Cont.)	1979	*C. riethi*					X		Re-	A	USSR
		C. saevus					X		Re-	A	USSR
		C. salinarius					X		Re-	A	USSR
		C. schultzei					X		Re-	A	USSR
		C. simulator					X		Re-	A	USSR
		C. sinanoensis					X		Re-	A	USSR
		C. stepicola					X		New	A	USSR
		C. stigma					X		Re-	A	USSR
		C. subfasciipennis					X		Re-	A	USSR
		C. trivittatus (described as *C. bulbostylus*)					X		New	A	USSR
		C. truncorum					X		Re-	A	USSR
		C. turanicus					X		New	A	USSR
		C. ustinovi					X		Re-	A	USSR
		C. vexans					X		Re-	A	USSR
Knoz	1980	*C. achrayi*					X		Re-	B	East Europe
		C. fascipennis					X		Re-	B	East Europe
		C. festivipennis (described as *C. odibilis*)					X		Re-	B	East Europe
		C. kibunensis (described as *C. cubitalis*)					X		Re-	B	East Europe
		C. manchuriensis					X		Re-	B	East Europe
		C. nubeculosus					X		Re-	B	East Europe
		C. obsoletus					X		Re-	B	East Europe
		C. pallidicornis					X		Re-	B	East Europe
		C. pulicaris					X		Re-	B	East Europe
		C. riethi					X		Re-	B	East Europe
		C. salinarius					X		Re-	B	East Europe
		C. simulator					X		Re-	B	East Europe
		C. stigma					X		Re-	B	East Europe
		C. subfasciipennis					X		Re-	B	East Europe
Kann	1980	*C. arboricola*					X	X	Re-	B	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Kann (Cont.)	1980	*C. californiensis*					X	X	New	B	C+N America
		C. flukei					X	X	Re-	B	C+N America
		C. guttipennis					X	X	Re-	B	C+N America
		C. oklahomensis					X	X	New	B	C+N America
		C. ousairani					X	X	New	B	C+N America
		C. villosipennis					X	X	Re-	B	C+N America
Kettle and Elson	1980	*C. marginalis*						X	New	A	Australia
		C. ornatus						X	New	A	Australia
		C. purus						X	New	A	Australia
		C. williwilli						X	New	A	Australia
Vitale *et al*	1981	*C. annuliductus*						X	New	B	Panama
		C. bayano						X	New	B	Panama
		C. filiductus						X	New	B	Panama
Jeu and Rong	1981	*C. homotomus*	X				X	X	Re-	B	China
Wirth and Soria	1981	*C. dicrourus*					X	X	New	B	Brazil
		C. macieli					X	X	New	B	Brazil
Chaker	1982/ 1983	*C. chiopterus*					X		Re-	B	France
		C. circumscriptus	X				X	X	Re-	B	France
		C. dewulfi					X		Re-	B	France
		C. fagineus					X		Re-	B	France
		C. fascipennis					X		Re-	B	France
		C. festivipennis (described as *C. odibilis*)					X		Re-	B	France
		C. kibunensis (described as *C. cubitalis*)					X		Re-	B	France
		C. nubeculosus					X		Re-	B	France
		C. obsoletus					X		Re-	B	France
		C. pictipennis					X		Re-	B	France
		C. puncticollis					X		Re-	B	France
		C. salinarius					X		Re-	B	France
		C. stigma					X		Re-	B	France

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Chaker	1984	*C. alazanicus* (described as *C. musilator*)					X		Re-	B	France
		C. clastrieri					X		New	B	France
		C. furcillatus					X		New	B	France
		C. semimaculatus					X		New	B	France
		C. truncorum (described as *C. sylvarum*)					X		Re-	B	France
Hagan	1984	*C. henryi*				X	X	X	New	B	Australia
Howarth	1985	*C. arakawai*						X	Re-	B	Laos
		C. arenicola						X	New	B	Laos
		C. calcaratus						X	New	B	Laos
		C. circumscritpus						X	Re-	B	Laos
		C. flavescens						X	New	B	Laos
		C. geminus						X	New	B	Laos
		C. guttifer						X	New	B	Laos
		C. hegneri						X	New	B	Laos
		C. huffi						X	New	B	Laos
		C. kamrupi						X	New	B	Laos
		C. kisangkini						X	New	B	Laos
		C. notatus						X	New	B	Laos
		C. okinawensis						X	New	B	Laos
		C. oxystoma						X	Re-	B	Laos
		C. palpisimilis						X	New	B	Laos
		C. peregrinus						X	Re-	B	Laos
		C. shortti						X	New	B	Laos
		C. similis						X	Re-	B	Laos
		C. tenuipalpis						X	New	B	Laos
Hagan and Reyes	1986	*C. longior*			X	X	X	X	New	B	Australia
		C. ornatus				X	X	X	New	B	Australia
Nunamaker *et al*	1987	*C. variipennis*	X						Re-	A (SEM)	C+N America
Ronderos and Spinelli	1989	*C. venezuelensis*						X	New	B	South America
Liu	1989	*C. circumscriptus*						X	Re-	B	China

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Liu (Cont.)	1989	*C. erairai*						X	New	B	China
		C. kibunensis						X	Re-	B	China
		C. pallidicornis						X	Re-	B	China
		C. pictimargo						X	New	B	China
		C. pulicaris						X	Re-	B	China
		C. punctatus						X	Re-	B	China
		C. simulator						X	Re-	B	China
		C. subfasciipennis						X	Re-	B	China
Pappas and Pappas	1989	*C. elemae*						X	New	B	C+N America
Kariya *et al*	1989	*C. actoni*	X						New	A (SEM)	Japan
		C. arakawai	X						New	A (SEM)	Japan
		C. brevitarsis	X						Re-	A (SEM)	Japan
		C. oxystoma	X						New	A (SEM)	Japan
		C. punctatus	X						New	A (SEM)	Japan
		C. sumatrae	X						New	A (SEM)	Japan
		C. tainanus (described as *C. maculates*)	X						New	A (SEM)	Japan
Glukhova	1989	*C. achrayi*					X	X	Re-	A	USSR
		C. alazanicus					X	X	New	A	USSR
		C. albicans					X	X	Re-	A	USSR
		C. arboreus					X	X	New	A	USSR
		C. chiopterus					X	X	Re-	A	USSR
		C. circumscriptus					X		Re-	A	USSR
		C. clintoni						X	New	A	USSR
		C. deltus					X	X	Re-	A	USSR
		C. dendrophilus					X	X	Re-	A	USSR
		C. desertorum					X	X	New	A	USSR
		C. dispersus					X		Re-	A	USSR
		C. fascipennis					X	X	Re-	A	USSR
		C. festivipennis					X	X	Re-	A	USSR
		C. filicinus						X	Re-	A	USSR
		C. gejgelensis						X	Re-	A	USSR

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Glukhova (Cont.)	1989	*C. grisescens*					X	X	Re-	A	USSR
		C. helveticus					X	X	New	A	USSR
		C. kibunensis						X	Re-	A	USSR
		C. kirgizicus					X	X	Re-	A	USSR
		C. langeroni						X	New	A	USSR
		C. longipennis						X	Re-	A	USSR
		C. manchuriensis					X	X	New	A	USSR
		C. nubeculosus					X	X	Re-	A	USSR
		C. obsoletus					X	X	Re-	A	USSR
		C. odiatus						X	Re-	A	USSR
		C. pallidicornis					X	X	Re-	A	USSR
		C. punctatus					X	X	Re-	A	USSR
		C. puncticollis					X	X	Re-	A	USSR
		C. reconditus					X	X	New	A	USSR
		C. riethi					X	X	Re-	A	USSR
		C. saevus					X	X	Re-	A	USSR
		C. salinarius					X	X	Re-	A	USSR
		C. simulator					X	X	Re-	A	USSR
		C. stepicola					X	X	New	A	USSR
		C. subsylvarum						X	New	A	USSR
		C. turanicus					X	X	New	A	USSR
		C. ustinovi						X	New	A	USSR
		C. vexans					X		Re-	A	USSR
Hribar and Mullen	1991	*C. arboricola*					X		Re-	A	C+N America
		C. chiopterus					X		Re-	A	C+N America
		C. furens					X		Re-	A	C+N America
		C. guttipennis					X		Re-	A	C+N America
		C. haematopotus					X		Re-	A	C+N America
		C. hollensis					X		Re-	A	C+N America
		C. melleus					X		Re-	A	C+N America
		C. obsoletus					X		Re-	A	C+N America
		C. piliferus					X		Re-	A	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Hribar and Mullen (Cont.)	1991	*C. sanguisuga*					X		Re-	A	C+N America
		C. travisi					X		Re-	A	C+N America
		C. variipennis					X		Re-	A	C+N America
		C. wisconsinensis					X		Re-	A	C+N America
Murphree and Mullen	1991	*C. arboricola*					X		Re-	A	C+N America
		C. arubae					X		New	A	C+N America
		C. barbosai					X		New	A	C+N America
		C. baueri					X		Re-	A	C+N America
		C. bermudensis					X		Re-	A	C+N America
		C. bickleyi					X		Re-	A	C+N America
		C. biguttatus					X		Re-	A	C+N America
		C. cacticola					X		New	A (SEM)	C+N America
		C. californiensis					X		Re-	A	C+N America
		C. cavaticus					X		New	A (SEM)	C+N America
		C. chiopterus					X		Re-	A	C+N America
		C. cochisensis					X		New	A	C+N America
		C. crepuscularis					X		Re-	A	C+N America
		C. denningi					X		Re-	A	C+N America
		C. denticulatus					X		Re-	A	C+N America
		C. flukei					X		Re-	A	C+N America
		C. footei					X		New	A	C+N America
		C. franclemonti					X		Re-	A	C+N America
		C. furens					X		Re-	A	C+N America
		C. furensoides					X		Re-	A	C+N America
		C. guttipennis					X		Re-	A (SEM)	C+N America
		C. haematopotus					X		Re-	A	C+N America
		C. hieroglypicus					X		Re-	A	C+N America
		C. hinmani					X		New	A (SEM)	C+N America
		C. hollensis					X		Re-	A	C+N America
		C. jacksoni					X		Re-	A (SEM)	C+N America
		C. jamesi					X		Re-	A	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Murphree and Mullen (Cont.)	1991	*C. jamnbacki*					X		Re-	A	C+N America
		C. jonesi					X		New	A (SEM)	C+N America
		C. melleus					X		Re-	A	C+N America
		C. mississippiensis					X		New	A	C+N America
		C. nanus					X		Re-	A (SEM)	C+N America
		C. neofagineus					X		New	A (SEM)	C+N America
		C. obsoletus					X		Re-	A	C+N America
		C. oklahomensis					X		Re-	A	C+N America
		C. ousairani					X		Re-	A	C+N America
		C. paraensis					X		New	A (SEM)	C+N America
		C. piliferus					X		Re-	A	C+N America
		C. sanguisuga					X		Re-	A	C+N America
		C. snowi					X		New	A (SEM)	C+N America
		C. spinosus					X		Re-	A	C+N America
		C. stellifer					X		Re-	A	C+N America
		C. tenuistylus					X		Re-	A	C+N America
		C. travisi					X		Re-	A (SEM)	C+N America
		C. tristriatulus					X		Re-	A	C+N America
		C. variipennis					X		Re-	A (SEM)	C+N America
		C. venustus					X		Re-	A	C+N America
		C. villosipennis					X		Re-	A	C+N America
		C. wisconsinensis					X		Re-	A	C+N America
Spinelli and Martinez	1991	*C. charua*						X	New	B	Uruguay
Narladkar	1991	*C. peregrinus*	X				X	X	New	B	Marathwada
		C. schultzei	X				X	X	New	B	Marathwada
Lamberson *et al*	1992	*C. arboricola*						X	Re-	B	C+N America
		C. beckae						X	New	B	C+N America
		C. elemae						X	Re-	B	C+N America
		C. flukei						X	Re-	B	C+N America
		C. footei						X	Re-	B	C+N America
		C. guttipennis						X	Re-	B	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Lamberson *et al* (Cont.)	1992	*C. hinmani*						X	New	B	C+N America
		C. lahillei						X	New	B	C+N America
		C. nanus						X	Re-	B	C+N America
		C. oklahomensis						X	Re-	B	C+N America
		C. ousairani						X	Re-	B	C+N America
		C. paraensis						X	New	B	C+N America
		C. snowi						X	Re-	B	C+N America
		C. villosipennis						X	Re-	B	C+N America
Elson-Harris and Murray	1992	*C. multimaculatus*						X	New	B	Australia
		C. oxystoma						X	Re-	B	Australia
		C. sigmoidus						X	New	B	Australia
		C. waringi					X	X	New	B	Australia
Spinelli *et al*	1993	*C. charruus*						X	New	B	Argentina
		C. insignis						X	Re-	B	Argentina
		C. maruim						X	Re-	B	Argentina
Nevill and Dyce	1994	*C. exspectator*						X	New	B	Afrotropics
		C. kobae						X	New	B	Afrotropics
		C. micheli						X	New	B	Afrotropics
		C. olyslageri						X	New	B	Afrotropics
		C. ravus						X	New	B	Afrotropics
		C. similis						X	Re-	B	Afrotropics
		C. tropicalis						X	New	B	Afrotropics
Day *et al*	1997	*C. circumscriptus*	X						Re-	A (SEM)	Israel
		C. gejgelensis	X						New	A (SEM)	Israel
		C. imicola	X						New	A (SEM)	Israel
Cribb and Chitra	1998	*C. molestus*	X						New	A (SEM & TEM)	Australia
Isaev	1999	*C. nubeculosus*	X						Re-	B	East Europe
		C. punctatus	X				X		Re-	B	East Europe
Breidenbaugh and Mullens	1999a	*C. kettlei*	X				X	X	New	A (SEM)	C+N America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Breidenbaugh and Mullens (Cont.)	1999a	*C. vetustus*	X				X	X	New	A (SEM)	C+N America
Breidenbaugh and Mullens	1999b	*C. boydi*	X				X	X	New	A (SEM)	C+N America
		C. brookmani	X				X		New	A (SEM)	C+N America
		C. cacticola	X				X	X	New	A (SEM)	C+N America
		C. freeborni	X				X	X	New	A (SEM)	C+N America
		C. lahontan	X				X	X	New	A (SEM)	C+N America
		C. utahensis	X						New	A (SEM)	C+N America
Ronderos and Spinelli	2000	*C. bambusicola*					X	X	Re-	A (SEM)	South America
Hureta *et al*	2001	*C. albomaculus*					X	X	New	A (SEM)	Mexico
Spinelli and Borkent	2004	*C. annettae*						X	New	A	Central America
		C. chaverrii					X	X	New	A	Central America
Diaz *et al*	2005	*C. venezuelensis*	X				X	X	New	A (SEM)	South America
Spinelli *et al*	2007	*C. felippenbauerae*					X	X	New	A	South America
Nevill *et al*	2007	*C. bolitinos*						X	New	B	South Africa
		C. imicola						X	Re-	B	South Africa
		C. kwagga (Then known as sp. # 107)						X	New	B	South Africa
		C. loxodontis						X	New	B	South Africa
		C. tutti-frutti						X	New	B	South Africa
Ronderos *et al*	2008	*C. charruus*					X	X	New	A (SEM)	South America
		C. venezuelensis	X						Re-	A (SEM)	South America
Nevill *et al*	2009	*C. sp. #54 d.f.*						X	New	B	South Africa
		C. sp # 54 p.f.						X	New	B	South Africa
Ronderos *et al*	2010	*C. debilipalpis*		X	X	X	X	X	New	A (SEM)	South America
Bellis and Dyce	2011	*C. zentae*						X	New	A	Australia
Borkent	2012	*C. haematopotus*						X	Re-	A	C+N America
		C. sonorensis						X	New	A	C+N America
Marino *et al*	2013	*C. bambusicola*					X	X	Re-	A (SEM)	South America

Table 1. Continued

Researcher	Date	Species	Stages*						New vs.** Redescription	Description Type***	Location
			E	L1	L2	L3	L4	P			
Marino *et al* (Cont.)	2013	*C. insignis*						X	Re-	A (SEM)	South America
Ronderos *et al*	2013	*C. crucifer*						X	New	A	South America
Slama *et al*	2013	*C. cataneii*					X		New	A	Tunisia
		C. sahariensis					X		New	A	Tunisia

Table 1. Historical Studies of Morphology of *Culicoides* Immature Stages. *L1, L2, L3, and L4 refer to the larval instars 1, 2, 3, and 4; P refers to pupa; E refers to egg. ** New descriptions refer to the first time that a particular life stage was described. If any of the descriptions are new then the description is labeled as new. *** "B" refers to basic in which the immature descriptions are cursory with few details. "A" refers to advanced in which the immature descriptions delve deeper into at least one of the immature stages. This table is not representative of all of the studies for *Culicoides* immatures. Due to the fact that many of the articles are quite obscure it is very possible that some species have been left out. The instar was not always stated and is presumed to be the fourth instar.

Materials, Methods, and Results

I. Experiments in Behavior of Immature *Culicoides sonorensis*

A few select behaviors of both the larvae and pupae of *C. sonorensis* were examined. The swimming speed of the larvae as well as their orientation on a mud slope were studied in the laboratory. The location of the pupae in both the field and laboratory, as well as their responses to fluctuations in water level in the laboratory, were also studied. Understanding the behaviors and biology of the immatures of *C. sonorensis* will help inform those seeking to utilize various control methods against them.

i. Larval Swimming Speed Analysis

Purpose:

Little is known about the movements of *Culicoides sonorensis* larvae in the wild. By determining the speed at which the larva is able to move we may be able to discover how well or quickly they respond to environmental changes and predators, as well as whether the various larval instars differ in that regard.

Methods:

Four 9 cm plastic petri dishes were filled half way (1 cm deep) with 1% water agar. Once the agar had solidified and cooled, 10 ml of deionized (DI) water was added and they were left alone until they reached room temperature. This provided a uniform water layer of about 1 mm depth for larval speed measurements. Concentric circles spaced 5 mm apart were drawn on a black surface under an illuminated magnifier (3x). The black background aided detection of larvae, and the evenly circular fluorescent light avoided directional light cues. The agar dishes were placed on this surface with the center

of the dish in the center of the circle. *Culicoides sonorensis* larvae of instars 1-4 collected from the Van Ryn colony were then carefully introduced individually in the center of a dish in about 50-100 µl of water using a pipette. When the swimming larvae reached the edge of one circle, timing began and the timing (nearest 0.01 sec with a stopwatch) was stopped when the larvae reached the edge of the next designated concentric circle. The times were recorded only if the larva swam steadily in a straight line perpendicular to the two lines, without stopping or slowing during its progress. The younger instars (1, 2) were timed over a smaller distance (2.5-10 mm) than the older instars (3, 4; 10-30 mm) in order to obtain more accurate readings. The younger instars were unlikely to travel long distances in a straight line, while the older larvae traveled too quickly for an accurate reading over anything less than a 10 mm distance. The body lengths/second were calculated using the average body length for each larval instar.

Results:

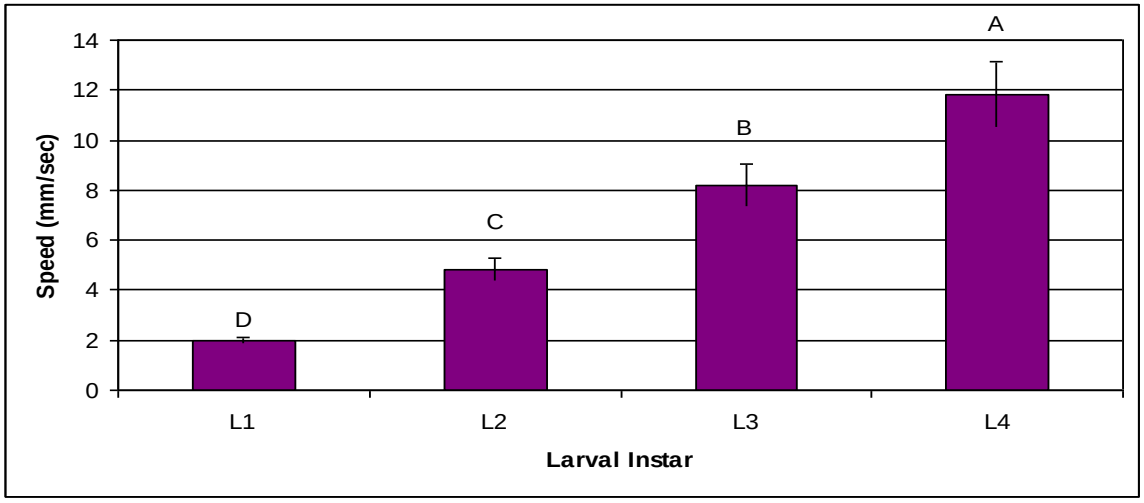

Figure 2. *Culicoides sonorensis* larval instar swimming speed comparison (mean + SE). The mean speed of each larval instar in mm/sec; n = 10 for L1, 24 for L2, 16 for L3, and 11 for L4. Note how the overall speed increases vs. age. Means with different letters are significantly different (Tukey's HSD, $p < 0.05$).

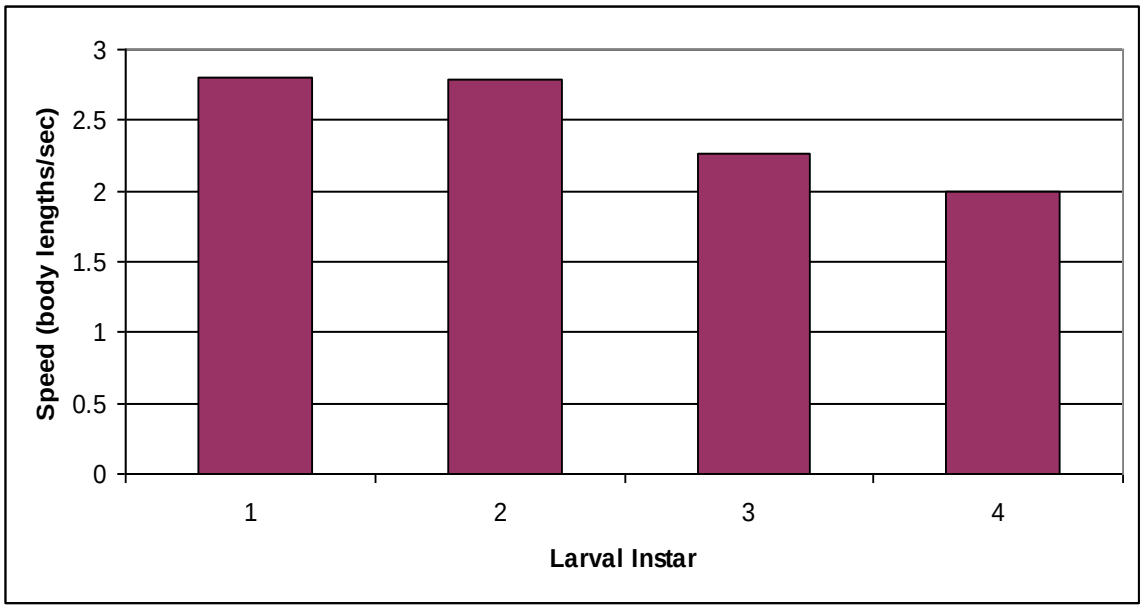

Figure 3. *Culicoides sonorensis* larval instar speed vs. average body length. The mean speed of each larval instar in body lengths/sec. Note how the relative speed decreases vs. age.

ii. Movement of Larvae on a Mud Habitat Slope

Purpose:

Little is known about the movements of *Culicoides sonorensis* larvae in the wild. Not only is it not known how the various instars orient themselves, it is also unclear as to whether or not the proleg is utilized in order to facilitate locomotion in the first larval instar. This experiment helped to determine how the larvae orient themselves on a slope with water. It also helped to elucidate the use of the proleg.

Materials and Methods:

Culicoides sonorensis larvae (instars 1-4) were collected from the Van Ryn colony and raised in 1% water agar dishes until they reached the desired instar (Mullens & Velten, 1994).

Natural *C. sonorensis* habitat mud was collected from the edge of a waste water pond at a dairy in San Jacinto, CA. It first was frozen (-20ºC for > 1 week) to kill preexisting arthropods. Mud then was placed into four-3.5 cm diameter dishes and arranged into slopes spanning the 3.5 cm, with a maximum height of 1cm. Approximately 2 ml of DI water was then added to each of the dishes, creating a small pool (0.25cm deep) of water at the base of each approximately 15º slope. The dishes were left for 2 hours to allow them to settle and reach room temperature. Live larvae were then individually placed in about 50-100 µl of water in the middle of each dish (above waterline). They had the choice to move up the slope, down the slope, or laterally. Each larva was observed under a dissecting microscope for 5 minutes or until they buried themselves completely in the mud, whichever came first. Their choice of direction was recorded. This procedure was repeated for five larvae of each instar.

In addition to this, the movement of the larvae was observed both on the soil as well as while under a coverslip with a minimal amount of water to determine how or if the L1 proleg was utilized.

Results:

All five specimens of each earlier instar (L1, L2, and L3) wandered in all directions (apparently randomly) during their time in the dishes. While wandering they appeared to try to enter into any small hole or mud irregularity they came across. Before the 5 minutes were up all of the specimens had buried themselves in the mud. The majority of the larvae managed to bury themselves above the waterline.

The five L4 specimens tended to go down the slope rather quickly. Only one of the specimens initially went upslope, before turning around and heading down the slope towards the water. Four of the five specimens began to search for a location to bury themselves once they reached the water and had successfully buried themselves below the waterline before the five minutes were up. The fifth specimen continued to travel through the water searching for a suitable site to bury itself but had not succeeded by minute 5.

All four instars moved in a sinusoidal manner when there was a sufficient amount of water to move easily. When there was a minimal amount or no free water, the L2-L4 continued to attempt to move in the same sinusoidal manner but they were not very successful. Their movements were very slow and they primarily thrashed around in the one location. The first instar larvae, on the other hand, used a very distinctive movement in which they repeatedly contracted their first few body segments and moved in a straight line on the mud surface. Under the Leitz compound microscope it could be seen that these body movements were causing the proleg to move as well. Although the L1 was not able to move as quickly as it did in free water, its ability to travel was not as impeded by a lack of water as were movements of the L2-L4.

iii. Lab Pupation Location Experiment

Purpose:

Although the field location of *C. sonorensis* larvae has been well documented by Mullens and Lii (1987), the location of the pupae in a substrate is unknown. By

determining where the pupae are found, various methods of control can perhaps be altered in order to better manage populations of *C. sonorensis.*

Materials and Methods:

Mud was collected from the edges of a waste water pond from a dairy in San Jacinto, CA and frozen at -20C (see above) in order to kill any living arthropods. The mud was then thawed and 150 ml placed into each of twenty plastic containers which were 8.5cm in diameter at the base. The mud was then molded to create a slope of approximately 15 degrees in order to mimic the conditions typically found in an actual waste water pond (McDermott and Mullens 2014). About 15 ml of DI water was then placed in each cup at a level of approximately 1cm from the base (depth) and allowed to settle over a 72-hour period. The water level was checked daily and consistently kept at a 1cm level throughout the experiment.

Twenty mature L4 larvae, collected from the Van Ryn colony, were placed in each of 15 containers (300 larvae total). The larvae were then left alone to pupate, and dishes were checked daily using a dissecting microscope. Once a larva had pupated, its position relative to the waterline as well as the position of its respiratory horns (which are found anteriorly on the pupae) in the mud were recorded on a "map" of the cup.

To ensure that pupae were not missed during the inspections, three of the dishes were selected at random after 7 days. Those three dishes were sectioned off into 1 cm sections relative to waterline (e.g. the 1 cm band below, 1 cm band above, etc.). The top layer of soil approximately 3 mm deep for each 1cm section was then carefully removed and placed into a dish filled with DI water. The water and mud were then agitated,

allowing the buoyant pupae to float to the surface. Pupae were then extracted using a pipette and the number of live pupae per section was recorded.

The experiment was then repeated with 10 dishes with 20 larvae in each with a gentler mud slope between 5-10 degrees. In this sandy soil type, it was hypothesized that the gentler slope might create a broader band of penetrable substrate.

Results:

Steeper (15º) slope:

With a 15º slope, 9 out of 13 dishes contained live pupae, and all were found only just below the waterline. Pupae were buried in the soil below water line with either only the top of their anterior end or just the respiratory horns exposed. The distal tip of the respiratory horn was the only structure in contact with the air (Fig. 4).

In the sectioned dishes, dish #7 had 3 pupae below the waterline, #11 had 2 pupae below the waterline, and #15 had 6 pupae below the waterline. No pupae were found above the waterline.

When disturbed while in their burrows, the pupae would quickly retreat deeper, submerging their respiratory horns. If displaced from their burrows mechanically, the pupae would bury themselves again immediately.

Gentler (5-10º) slope:

With a 5-10 degree slope, the pupae were all found in the same position as in the 15º slope. In 5 of the 10 dishes, live pupae were found below the waterline and, in 7 of the 10 dishes, the exuvia from the pupae could still be seen still partially in the burrows.

Two of the dishes were sectioned and in both dishes 2 live pupae were found below the waterline. No pupae were found above the waterline.

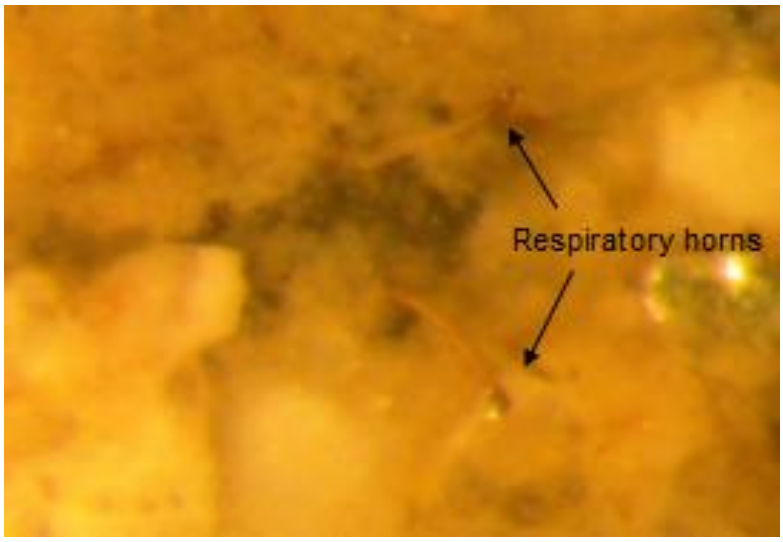

Figure 4. *Culicoides sonorensis* pupa in its burrow. Only the anterior end of the pupa can be seen, with the distal tips of the respiratory horns in contact with the air.

iv. Field Pupation Location

Purpose:

Although the locations of the larvae have been well documented by Mullens and Lii (1987), the location of the pupae in a substrate is unknown. By determining where the pupae are found, various methods of control can be altered in order to better manage populations of *C. sonorensis.*

Materials and Methods:

Mud was collected from a waste water pond at in San Jacinto, CA (same mud supplying mud for the lab studies). Sections of mud 2 cm wide x 10 cm long x 1 cm deep were collected from the shore of the wastewater pond, which had a slope of approximately 5-6%. These sections were taken parallel to each other and to the waterline.

Samples were centered at 5 cm and 1 cm below waterline as well as 1 cm, 5 cm, and 9 cm above waterline. This was repeated at six different sites along the shore. As sampling proceeded, added samples centered 13 cm and 17 cm above waterline were also collected from sites 5 and 6 (Fig. 5). This was due to the fact that while collecting pupae the wind ceased and the water line began receding, which could be seen by the "high tide" line, which was marked by a distinct line of chironomid pupae. Once a sample was collected it was placed into a white Styrofoam cup and clean tap water was added. The sample was allowed to sit for one minute with occasional light stirring. The buoyant pupae which floated to the surface within the minute timeframe were counted and collected. The pupae were placed into 3.5 cm plastic cups with wet cotton in the bottom and labeled with the site number and section.

The cups were kept at approximately 22^{o}C. Every 12 hours the cups were checked for emerged adults. These adults were counted and collected using an aspirator and kept in the freezer (Figs. 5 & 6). The adults were then sexed in order to determine if there was a difference in pupation location between males and females.

Results:

The pupae were consistently found above the waterline with the majority of the pupae either at or below the "chironomid line" as can be seen in Figure 6. Adult midges from sections above the "chironomid line" emerged in greater numbers sooner than those at or below the line (Fig. 7). The number of male vs. female in each section remained constant with more males than females. The only outlier which can be seen in Figure 8

occurred at 2 – 0 cm below the waterline where there were 20% males and 80% females with the rest of the sections averaging 65% males and 35% females.

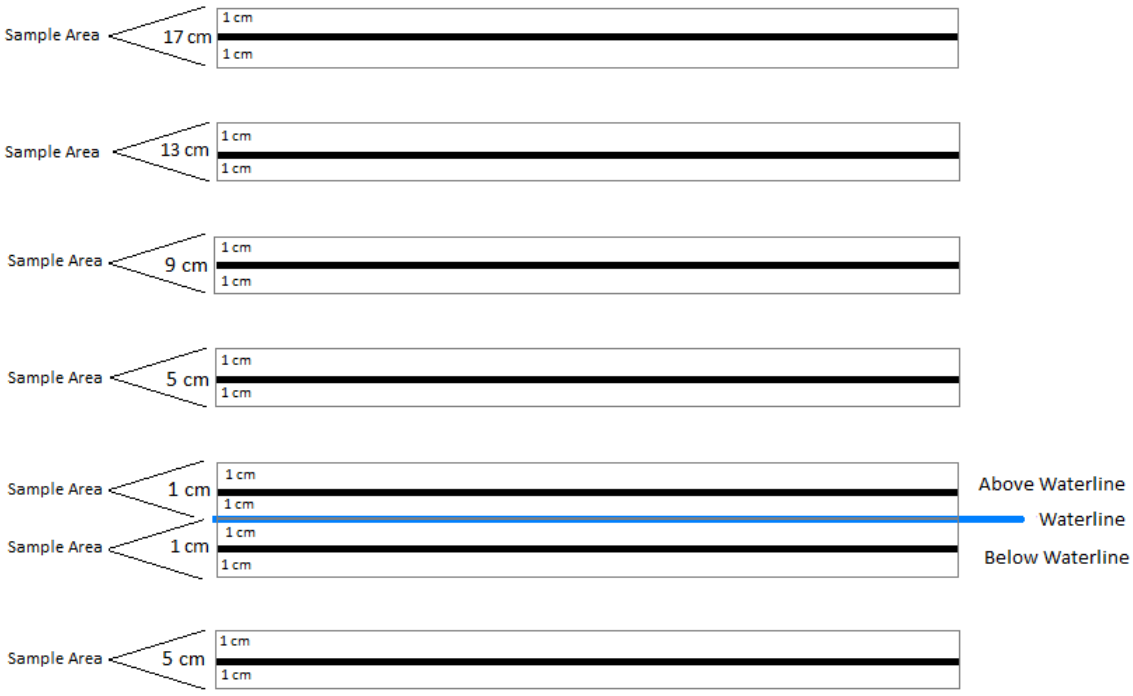

Figure 5. Locations of field mud samples collected for *Culicoides sonorensis* field pupation location study.

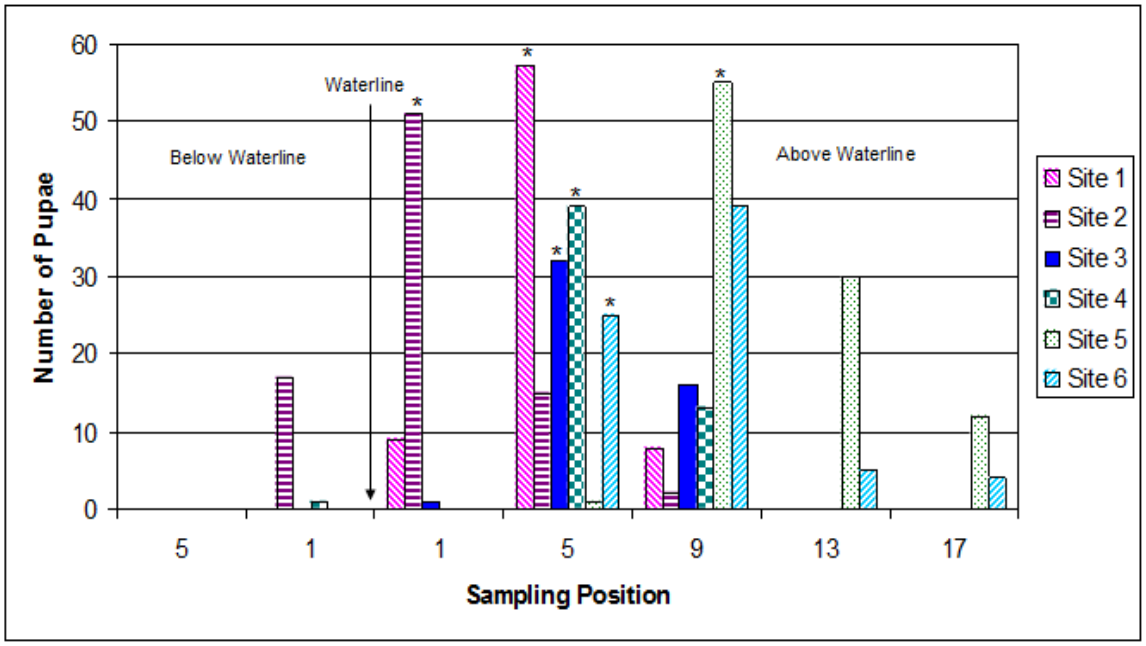

Figure 6. The locations of *Culicoides sonorensis* pupae along the shore of a dairy waste water pond in the field. The * above a section indicates the location of the "chironomid line." Note how the numbers of *Culicoides* pupae are highest when the location coincided with this line.

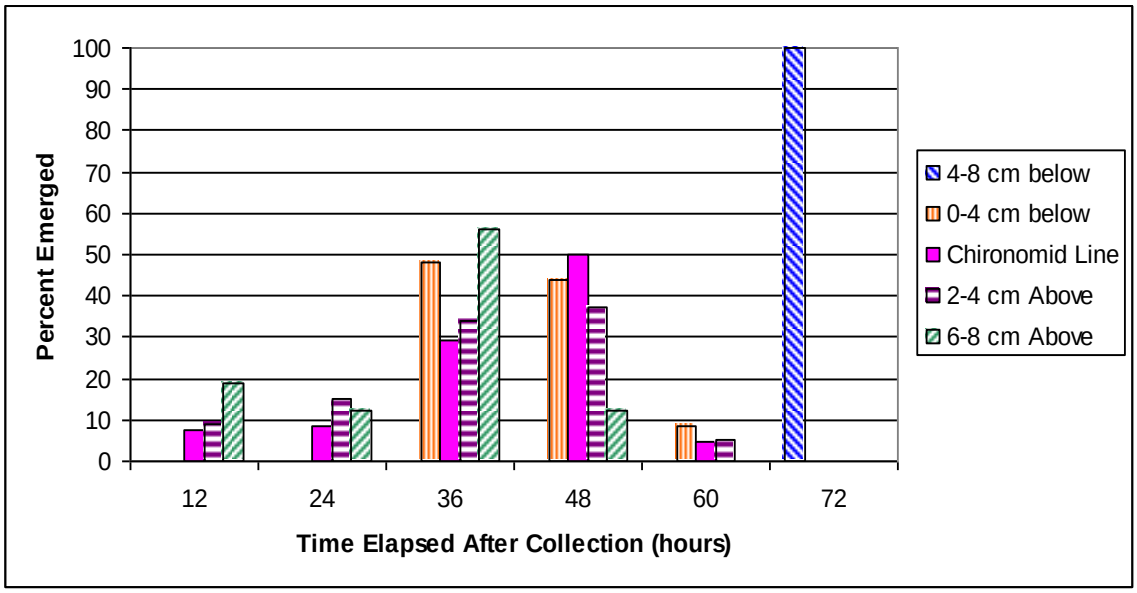

Figure 7. Adult *Culicoides sonorensis* emergence relative to "Chironomid line." Numbers emerging from all six sites have been combined relative to the "chironomid line" and the percentage of emergence for each section calculated. Note how the pupae from sections above the "chironomid line" emerged in greater numbers sooner than those at or below the line. Also note that only one pupa was found 4-8 cm below the line.

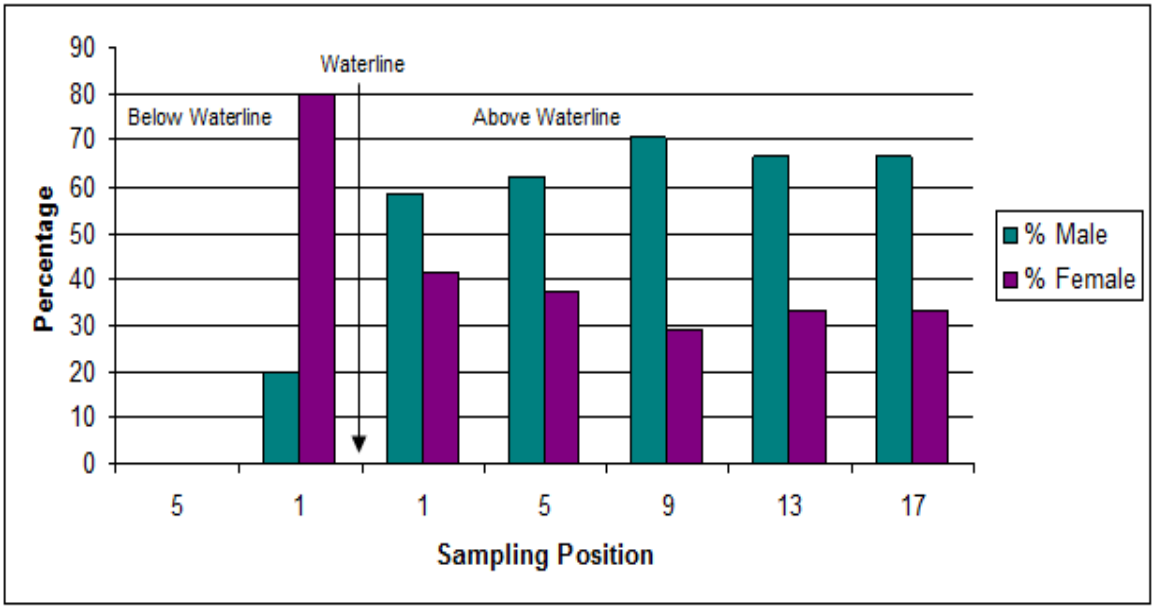

Figure 8. Male vs. female *Culicoides sonorensis* emergence based on the distance of the sample from the waterline.

v. Pupal Behavior

Purpose:

Little is known about the behaviors of the pupae of *C. sonorensis*. By determining how the pupae react to fluctuations in water level, various methods of control (i.e. flooding and draining of wastewater ponds) may be altered in order to better manage populations of *C. sonorensis.*

Methods and Materials:

Small plastic cups with a base diameter of 2 cm were filled 1cm deep with mud collected from the wastewater pond in San Jacinto, CA. Five pupae from the Van Ryn colony were then placed directly on the dry mud. Their reactions were recorded over a 10 minute period. A thin layer of DI water was then added using a plastic pipette so that the water barely covered the entirety of the pupae. More water was then added so that their

respiratory horns were submerged completely. More water was then added at so that it reached a depth of 1cm. The water was then partially removed so that there was only a thin layer. Finally all water was removed. After each change in the water, the reactions of the pupae were recorded for 10 minutes. This was repeated three more times (20 pupae total).

Step 1: Dry Mud

During the entire 10 minutes the pupae attempted to bury themselves unsuccessfully by continually moving their abdomens back and forth while sticking the caudal segment in the mud. They were unable to penetrate the sandy substrate.

Step 2: Thin layer of water

Once the water was added the pupae began to move much faster and were more active. By moving their abdomens back and forth they were able to quickly bury themselves in the mud with only the tips of their respiratory horns in contact with the surface of the water. All pupae were able to do this within 2 minutes and stayed in their positions for the remainder of the 10 minutes.

Step 3: Respiratory horns submerged

Within a minute of having their respiratory horns submerged, the pupae began to partially leave their burrows. As soon as the tips of their horns touched the surface of the water they stopped moving and remained in that position, partially in their burrows. They were able to remain connected to their burrows only if the entirety of their caudal segment was still in contact with the mud. When the water was disturbed they retreated back into their burrows underwater, submerging the respiratory horns completely, and

reemerged to make respiratory contact when the water was still again. They remained there for the rest of the time.

Step 4: 1 cm of water

Similarly to step 3, the pupae slowly emerged from their burrows in an attempt to make contact with the surface of the water. They were reluctant to leave their burrows entirely. While leaving their burrows, the pupae would swiftly return if the water was disturbed. Within 5 minutes all of the pupae had completely removed themselves from their burrows and floated to the top of the water, again with just their respiratory horns touching the surface. When the water was disturbed the pupae did not go back underwater but instead continued to float.

Step 5: Removal of water to a thin layer

As soon as the pupae were resting on the surface of the mud, they again quickly buried themselves as they did in step 2.

Step 6: Complete water removal

When the pupae lacked water they burrowed deeper into the mud and remained in that position for the entire time with their respiratory horns only slightly above the surface of the mud.

vi. Pupae Drowning

Purpose:

Determining how long pupae can survive underwater will help us to understand the behaviors they exhibit.

Methods:

Culicoides sonorensis pupae from the Van Ryn Colony were placed in each of ten small plastic cups with bases 3 cm in width. Eight of these cups were then filled with 15 ml of DI water, while the remaining two cups were used as controls, with a small piece of moist cotton in them on which the pupae rested. Ten identical plastic cups were prepared, with the bases cut off 1/2 cm from the base. Thin, closely knit organdy fabric, cut into 4x4" squares, was placed at on top of all of the cups containing the pupae except for one of the controls. The cut cups were then inserted into the cups containing the pupae, pushing the cloth down so that the pupae were forced underwater (Fig. 9). Any air

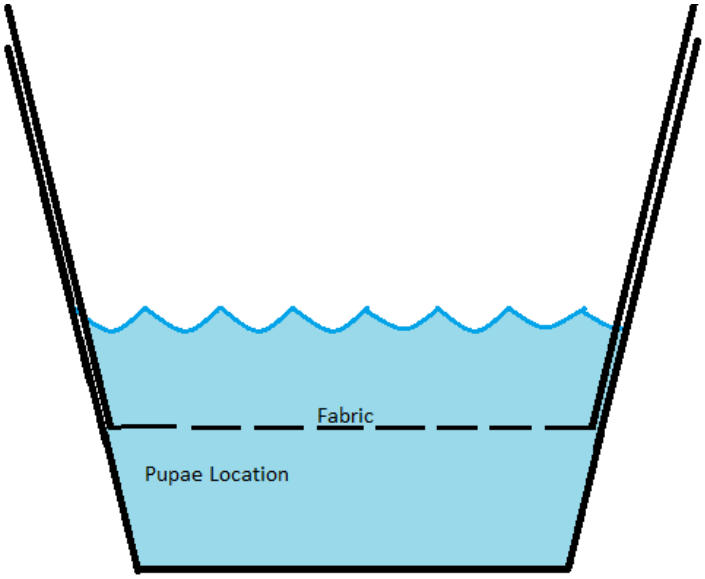

Figure 9. Experiment cup to test survival of submerged *Culicoides sonorensis* pupae.

Results:

remaining under the fabric was removed using a 10 gauge needle attached to a 1 ml syringe. The cut cup was then taped into place using masking tape around the outside of the cups. The pupae were kept at 22ºC. At 2, 4, 6, 8, 10, 12, 14, and 24 hours of pupal submersion, the cut cup and fabric was removed and the water drained from one of the

cups per time interval. The pupae were then placed onto a wet piece of cotton inside of the cup and left alone to emerge. The number of emerged adults was then recorded. This experiment was done once with 20 pupae per cup (run 3) 10 pupae per cup (run 1) and once with 6 pupae per cup (run 2) due to the limitations of the colony at the time. The survival rate of the *Culicoides sonorensis* pupae consistently dropped as the number of hours submerged under water increased up to 24 hours. For unknown reasons about 18% of control pupae failed to emerge. Approximately 50% of the pupae failed to emerge when subjected to between 8 – 10 hours of submersion, a 50% reduction in corrected emergence (versus control pupae) was seen after 10-12 h of submersion. After the total 24 hours only 13.9% of the pupae survived (Fig. 10), a relative pupal mortality (versus controls) of 83%.

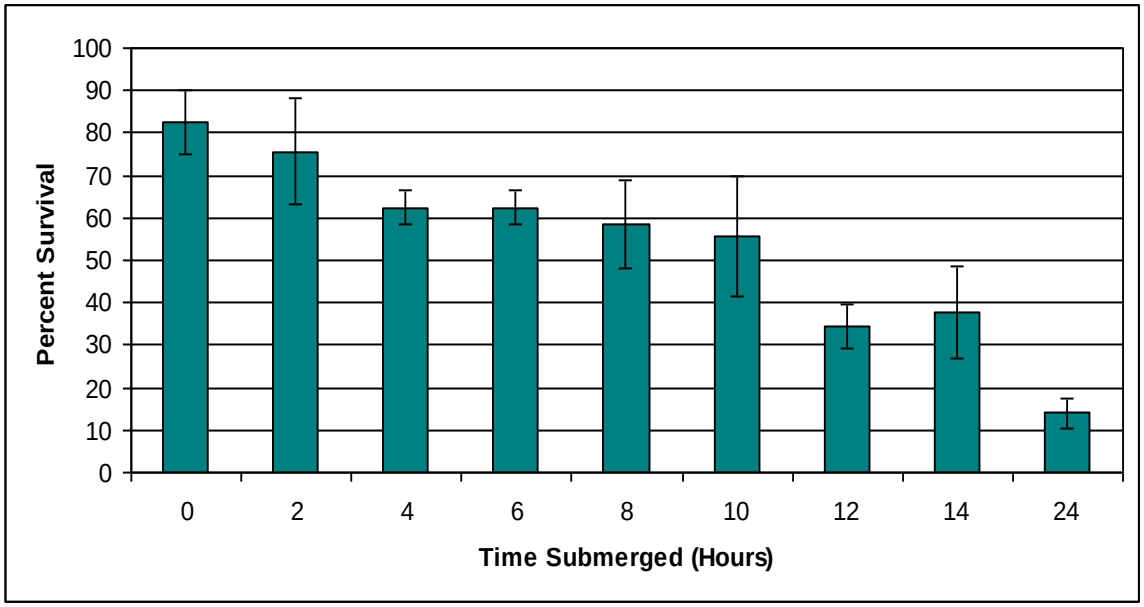

Figure 10. Survival rate of *Culicoides sonorensis* pupae submerged underwater.

The effect of submersion on mortality was described well (r^2 = 83.9%) by linear regression (y = 79.2–2.87), and the negative slope was highly significant (t=-11.42,

$p<0.001$). Using ANOVA and then Tukey's HSD to separate individual time means, submersion of pupae for 10 h or longer was required to result in significant differences from the control.

II. Morphology of *Culicoides sonorensis* immatures

Methods:

Culicoides sonorensis larvae were collected from multiple sources for these studies in order to determine whether there were differences between populations. Mud containing *C. sonorensis* larvae was collected from the edges of waste water ponds in summer and fall at 1) the S Dairy (San Jacinto, CA) or 2) F Dairy (Chino, CA), placed into 3.8 l plastic bags, and was transported back to the lab inside of an ice chest. The mud was then stirred and separated (30 ml aliquots) into small plastic cups which were partially filled with water (approximately 50 ml). Saturated MgSO4 was then added via pipette to the cups (approximately 50 ml) in order to aid in larval flotation. The cups were then observed under a dissecting microscope (12-25x), and the L4 larvae which appeared at the top were captured using pipettes and placed in DI water. They were washed three times using DI water and were then separated by larval instar (head width) in order to ensure only L4 larvae were measured. The instars were then separated into two different groups: Group 1 was destined for scanning electron microscopy (SEM) and Group 2 for measurements using a compound microscope (see below). Measurements of L4 structures were compared statistically for Van Ryn Colony vs. Wild, using t-tests and $\alpha = 0.05$.

Larvae from the Van Ryn Colony were raised from eggs in 1% water agar dishes (Mullens & Velten, 1994). After larvae reached the desired instar they were removed from the dish via pipette, washed three times using DI water, and separated in the same manner as the larvae collected from the field sites. Larvae of instars 1-4 were obtained in this manner.

Eggs and pupae were collected from the Van Ryn Colony and washed 3 times using DI water.

SEM Specimen Preparation:

The specimens which were used for SEM were treated using different concentrations of KOH for different amounts of time, based upon their stage as well as the structure which was to be viewed (Table 3). Those specimens which were to be used to view external structures were typically left in a lower concentration for KOH for a shorter amount of time. This prevented the internal tissue from dissolving so that the specimen would stay largely intact. Specimens which were to be used to view internal structures were left in a higher concentration of KOH for a longer amount of time. This ensured that the soft tissue would dissolve. It allowed the sclerotized internal structures to be easily removed from the head capsule with as little debris remaining as possible, without deforming the structures of interest.

After the specimens (all immature stages) were removed from the KOH solutions, they were again washed three times using DI water. They were then dehydrated using a standard ethanol series (15 minutes in each of 30, 50, 70, 80, 85, 90, 95%, and 3 consecutive periods in 100%) to prepare them for hexamethyldisilazane (HMDS)

Table 3. Exposure schedule of immature *Culicoides sonorensis* to KOH to achieve adequate clearing for microscopic examination

Instar	Internal vs. External Structure Examination	KOH (%)	Time (hours)
1	External	10	24
2	External	10	24
	Internal	10	48
3	External	10	48
	Internal	20	48
4	External	20	48
	Internal	20	72
Egg	External	20	6
Pupa	External	20	6

(Drodowitz *et al*, 1982). The specimens were then placed in HMDS for two 30-minute periods and left to air-dry overnight, with a change of HMDS after each period. Whole specimens were adhered to PELCO Tabs™ Carbon Conductive Tabs, Double Coated on standard SEM pin stub mounts. To view internal structures, the head capsules were examined under a dissecting microscope and were disrupted using minuten pins. The internal structures thereby were either simply exposed or removed (Fig. 11). If possible, any excess tissue was removed from the internal structures (which would interfere with SEM). For this a small amount of glue obtained from packing tape was adhered to the end of a minuten pin and used to carefully remove the tissue without disturbing the structures of interest.

The specimens were then sputter coated with platinum (Pt) for 60 seconds. The SEM images were obtained using an XL30-FEG Scanning Electron Microscope at the University of California, Riverside. The internal structures of first instars were not viewed due to an inability to remove the structures without excessive damage.

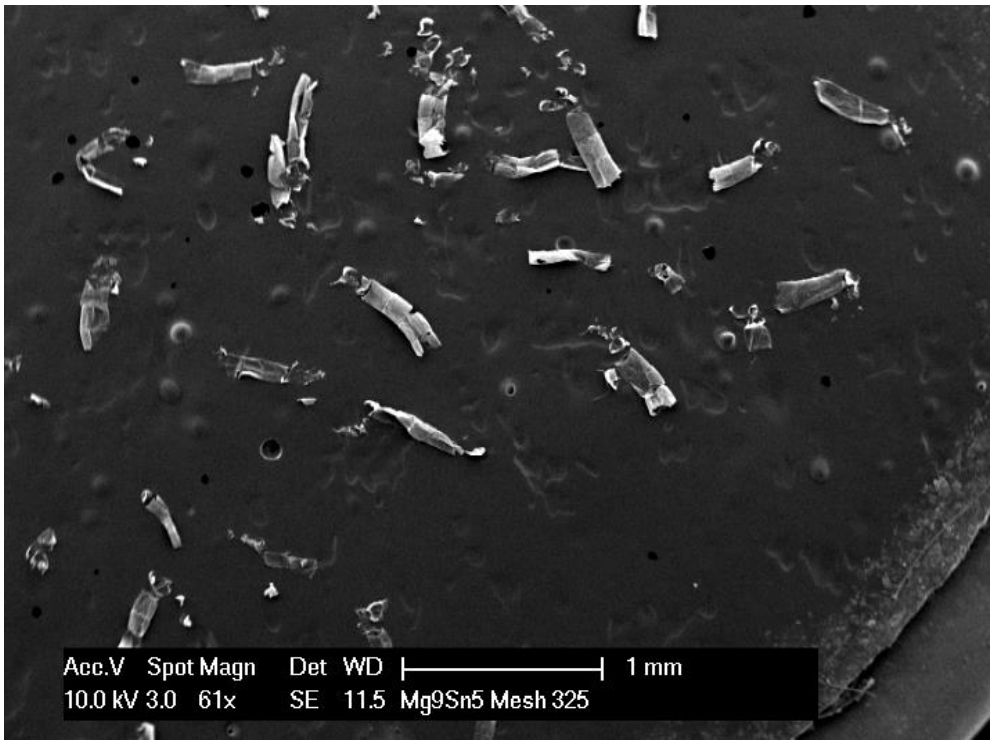

Figure 11. *Culicoides sonorensis* L2 larvae prepared for study of internal structures on SEM pin stub mount. Note distribution of larval head capsules via rough dissection.

Compound Microscope Preparation:

Specimens needed for external feature measurements were moved from the DI water to 30% EtOH in order to kill them with minimal structural collapse. After the larvae were dead, they were then placed in that fluid, gently secured on a glass microscope slide under a cover slip and structures were measured using an ocular micrometer in a Leitz Wetzlar compound microscope. Adequate fluid was maintained under the slip to prevent deforming of the specimen.

Specimens needed for internal feature measurements were run through the same KOH treatments as those needed for SEM. This dissolved the internal tissues so that the sclerotized structures were more visible. After the KOH treatment, the specimens were washed three times in DI water before being placed in 30% EtOH. They were then

measured using a Leitz Wetzlar microscope while on a glass slide under a cover slip. Sufficient fluid was maintained on the slide to avoid deforming the specimens via cover slip weight as above.

Diagrams of the body structures measured are shown in Figures 11 and 12, using standards terminology from Murphree and Mullen (1991).

The total body length (TL), head length (HL), head width (HW) at the widest point, subgenal width (SGW), mandible length (ML), epipharynx lateral arm width (LAW), epipharynx dorsal comb width (DCW), caudal segment length (CSL), and caudal segment width (CW) at the widest point were all measured. The head ratio (HR) was determined by dividing the head length by the head width. The subgenal ratio (SGR), also known as the head-width ratio (Glukhova, 1968a; 1979), was calculated by dividing the head width by the subgenal width. The caudal segment ratio (CSR) was determined by dividing the caudal segment length by the caudal segment width.

The height, base width, and the width of the tip of the ansulae found on the eggs of *C. sonorensis* were measured using the images obtained from SEM. The SEM images were also used in order to determine the arrangement of the ansulae on the surface of the eggs. Four eggs were imaged utilizing SEM and visually segregated into 5 μm^2 areas. On each egg 10% of the 5 μm^2 sections were randomly selected using a random number generator without replacement and the number of ansulae within each selected region counted and tested for randomness (variance = mean), versus either clumped (variance: mean >>1) or regular dispersion (variance: mean <<1) patterns (http://home.ubalt.edu/ntsbarsh/business-stat/otherapplets/Randomness.htm).

For the pupae, the focus of the SEM studies was on the respiratory horns due to their presumed importance for respiration.

Results:

Egg – Cigar-shaped. Average length = 453.15 (432-477) µm, width = 63 µm. Surface covered in ansulae (Fig. 12 a-d) and lacking secondary features. Each individual ansula is narrow along the stalk and widens apically, similar to a tree (Fig. 12a). Ansulae height = 1.29 (1.277-2.142) µm, base width = 0.647 (0.357-0.851) µm, and tip width = 1.057 (0.357-1.277) µm. The ansulae were arranged in no discernable pattern and were statistically random with an average of 1.98 (range 0-5) ansulae per 5 µm² of egg surface.

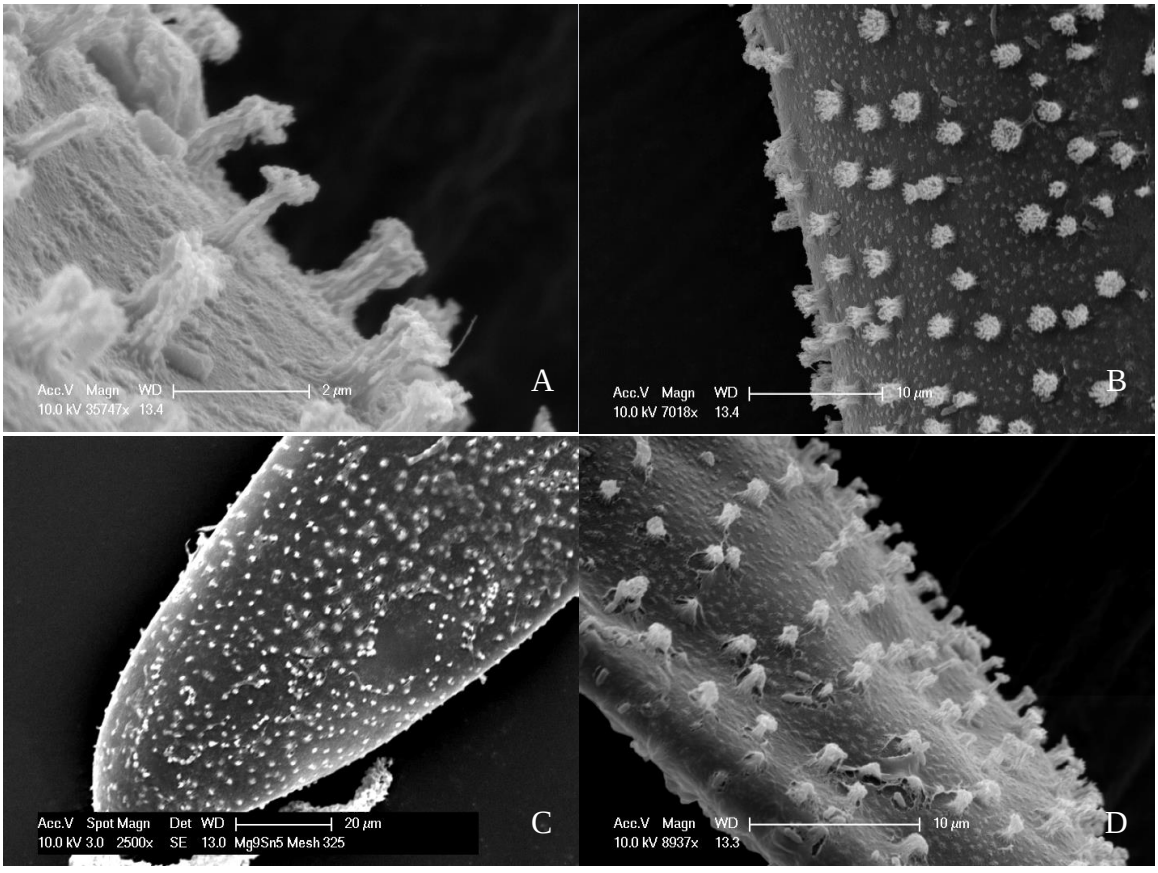

Figure 12. *Culicoides sonorensis* egg.

First larval instar – (Van Ryn colony) Total length (Fig. 13) = 716.57 (range 387-918) µm. Head capsule (Fig. 14A): medium yellowish-brown, small, heavily sclerotized. HL = 66.75 (64-70) µm, HW = 50 (42-60) µm, SGW = 32.67 (28-38) µm; long and narrow, HR = 1.342 (1.333-1.524); triangular, SGR = 1.525 (1.313-1.714). Mandible (Fig. 14C) large, ML = 20 (18-22) µm, curved, pointed apically, mandibular seta near base, 2 subapical teeth, inner tooth greatly reduced. Epipharynx (Fig. 14B): large, wide dorsal comb sclerite, DCW = 8.2 (8-9) µm; number of teeth/sclerite count not be counted accurately; teeth unequal in both width and length, with rounded tips. Other combs were not seen. LAW = 23.6 (22-28) µm. Thoracic segments (Fig. 13): the 3 thoracic and 9 abdominal segments all have a rather uniform, very light, yellowish-brown pigment. A pseudopod (Fig. 14D-E) can be found on the ventral side of the first thoracic segment. It is a complex structure which contains five rows of small setae which vary in their length.

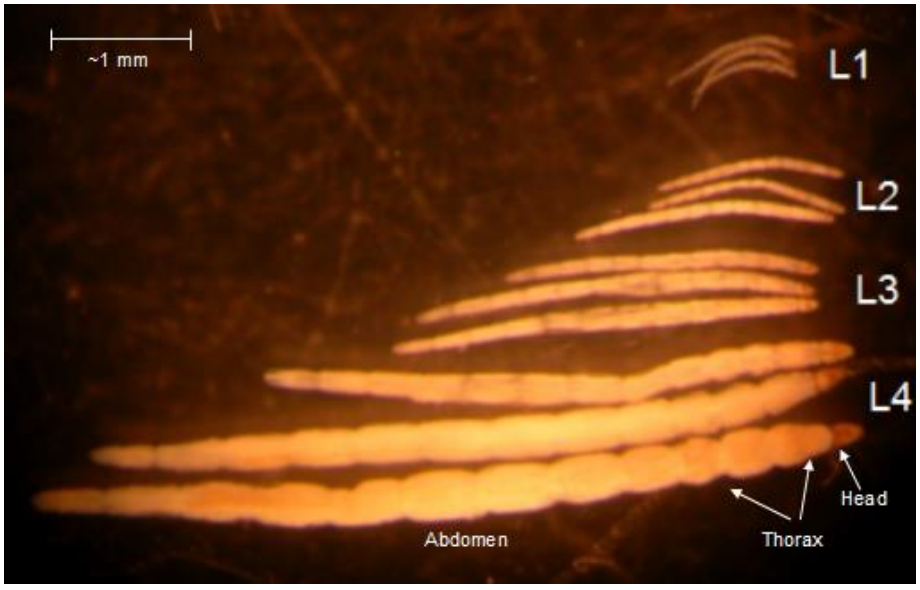

Figure 13. *Culicoides sonorensis* larval instars 1-4 (L1-L4). Note the continuity of body length, but consistency in dimensions of the head capsule within an instar (Dyar's Law). Photo by A. Diniz.

They are oriented in a triangular fashion. These are accompanied by approximately 8-10 long, strong setae which vary in size but are approximately 18 µm in length and are curved at the tips. This structure is only found on the first instar. Caudal Segment (Fig. 14F): long CSL = 97.67 (76-140) µm, and narrow CSW = 46.33 (40-64) µm, CSR = 2.118 (1.643-2.538). Four extensible fleshy anal papillae are usually held within the body. They are wide at the base and split into two segments approximately half way along the stalk and the two segments taper to points.

Second larval instar – (Van Ryn colony) Total length (Fig. 13) = 1757.41 (range 1334-2185) µm. Head capsule (Fig. 15A): medium yellowish-brown, small, heavily sclerotized. HL = 100.27 (94-110) µm, HW = 71.27 (62-82) µm, SGW = 44.55 (40-54) µm; long and narrow, HR = 1.416 (1.220-1.613); triangular, SGR = 1.603 (1.44-1.8). Mandible (Fig. 15B) large, ML = 26.4 (24-34) µm, curved, pointed apically, mandibular seta near base, 2 subapical teeth, inner tooth greatly reduced. Epipharynx (Fig. 15A, 15C): large, wide dorsal comb sclerite, DCW = 12.7 (10-16) µm; number of teeth/sclerite could not be accurately counted; teeth unequal in both width and length with rounded tips. Slightly more complex in structure with more defined teeth on the dorsal comb than that seen in the first instar. Other combs were not seen. LAW = 35.7 (32-40) µm. Thoracic segments (Fig. 13): overall body pigments the same as L1. Pseudopod not present. Caudal Segment: long CSL = 190.18 (144-244) µm, and narrow CSW = 78.36 (60-96) µm, CSR = 2.434 (1.895-2.778). Anal papillae are the same as found in L1.

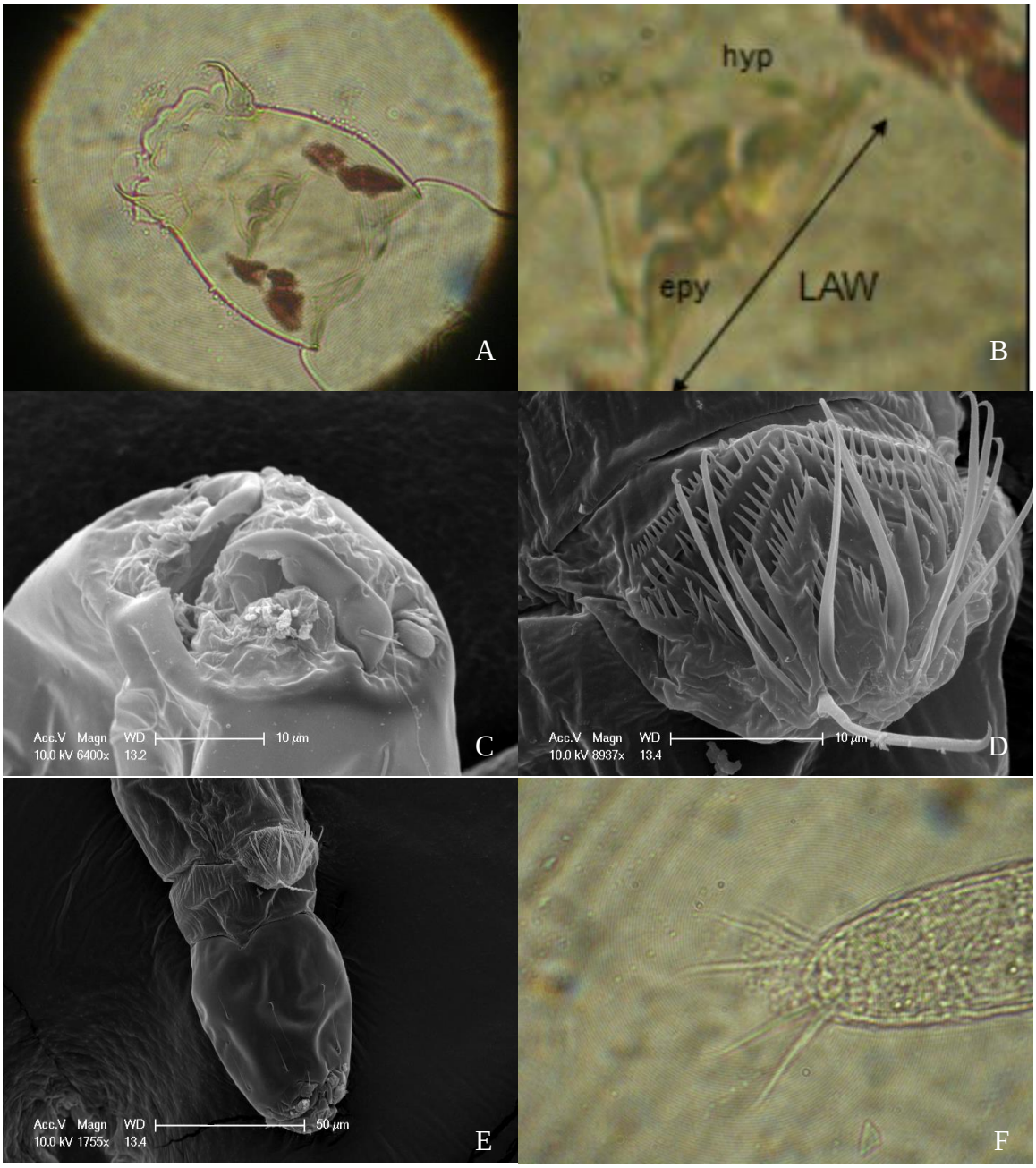

Figure 14. *Culicoides sonorensis* L1 larvae (Van Ryn Colony). A. Head capsule (dorsal view). B. Epipharynx. C. Overview of anterior head including mandible and antennae. D. Thoracic pseudopod. E. Overview of L1 including pseudopod. F. Caudal segment with anal papillae extended. epy, epipharynx; hyp, hypopharynx; LAW, lateral arm width.

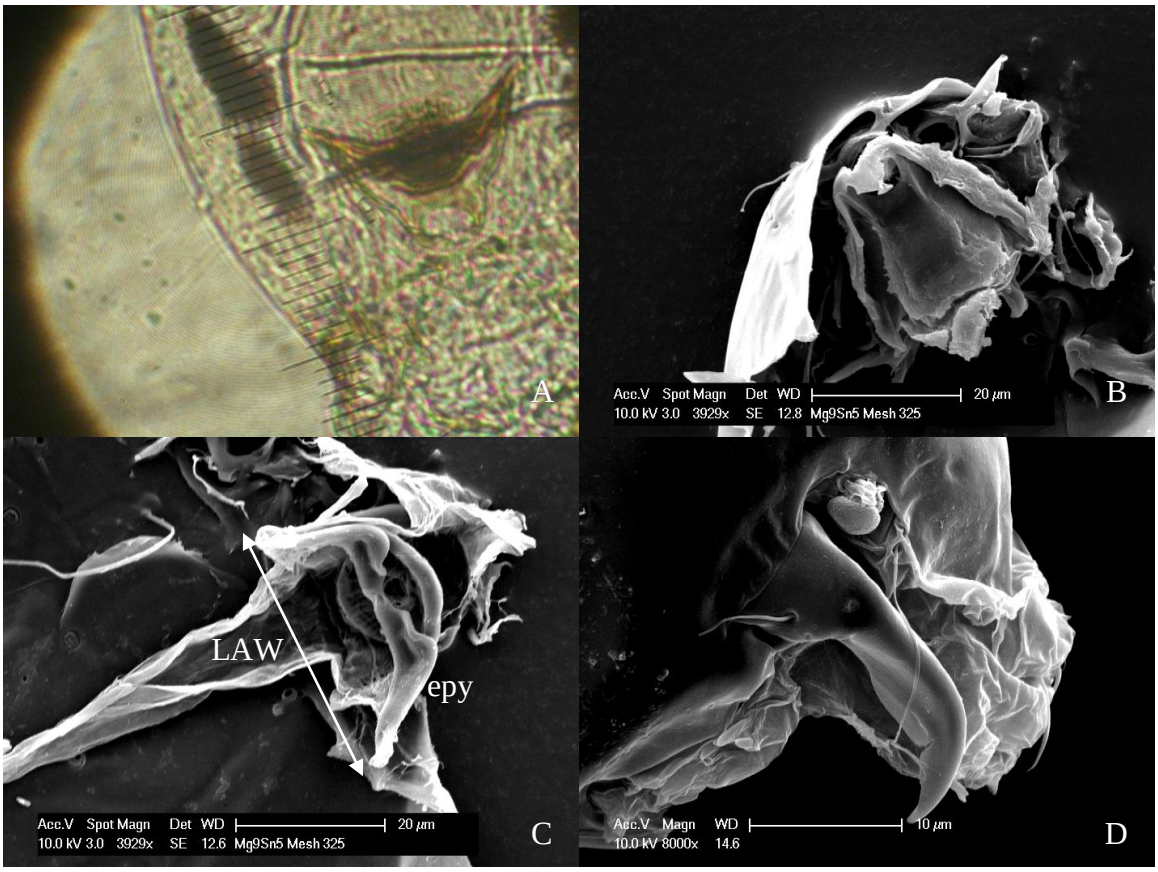

Figure 15. *Culicoides sonorensis* L2 larvae (Van Ryn Colony). A. Epipharynx (dorsal view). B. Hypopharynx C. Epipharynx. D. Overview of external mouthparts including mandible. epy, epipharynx; LAW, lateral arm width.

Third larval instar (Van Ryn Colony) - Total length (Fig. 13) = 3.616 (range 2.2-5) mm. Head capsule: medium yellowish-brown, small, heavily sclerotized. HL = 156.84 (144-168) µm, HW = 99.68 (92-110) µm, SGW = 64.16 (58-74) µm; long and narrow, HR = 1.575 (1.5-1.717); triangular, SGR = 1.581 (1.459-1.667). Mandible (Fig. 16B) large, ML = 39.8 (38-44) µm, curved, pointed apically, mandibular seta near base, 2 subapical teeth, inner tooth greatly reduced. Epipharynx (Fig. 16A, 16C, 16D): large, wide dorsal comb sclerite, DCW = 24.1 (20-28) µm; 11-13 teeth/sclerite (n=20); teeth unequal in both width and length with rounded tips. Slightly more complex in structure with even better

defined teeth on the dorsal comb sclerite than that seen in the first and second instars. Comb 2 contains only a small number of blunt tubercles. Comb 3 contains many blunted tubercles. A much larger comb 4 is present with a rounded medial process and few blunted tubercles. LAW = 61.8 (56-66) µm. Thoracic segments (Fig 14): overall body pigment is the same as L1 and L2. Pseudopod not present. Caudal Segment: long CSL = 314.78 (252-351) µm, and narrow CSW = 116.17 (90-171) µm, CSR = 2.771 (2-3.4). Anal papillae are the same as seen in L1 and L2.

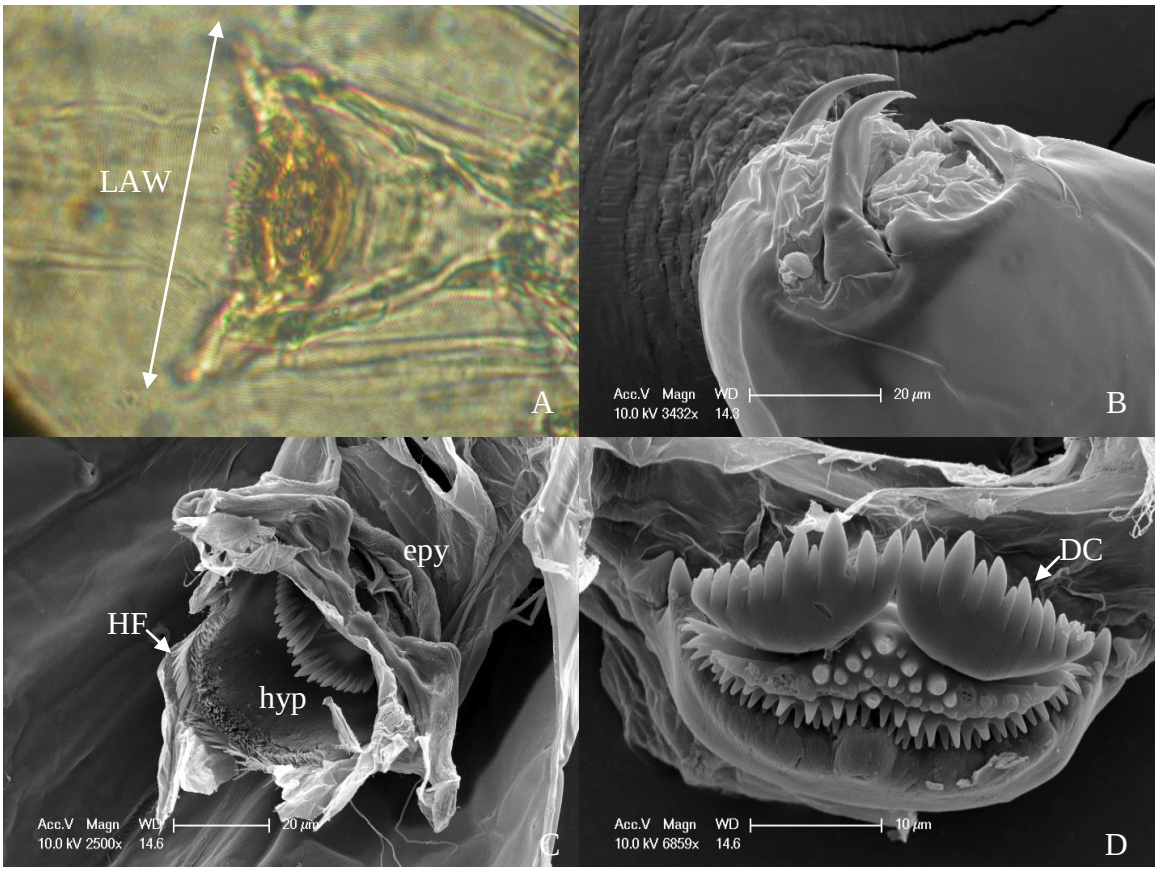

Figure 16. *Culicoides sonorensis* L3 larvae (Van Ryn Colony). A. Epipharynx (dorsal view). B. Overview of external mouthparts including mandibles and antenna. C. Epipharynx and hypopharynx. D. Dorsal combs. epy, epipharynx; hyp, hypopharynx; LAW, lateral arm width; HF, Hypopharyngeal fringe; DC, dorsal comb.

Fourth larval instar (Van Ryn Colony) – Total length (Fig. 13) = 5.938 (range 5-6.9) mm. Head capsule (Fig. 17A): medium yellowish-brown, small, heavily sclerotized. HL = 315.5 (300-324) µm, HW = 199.33 (188-212) µm, SGW = 121.83 (112-132) µm; long and narrow, HR = 1.584 (1.49-1.702); triangular, SGR = 1.638 (1.485-1.733. Mandible (Fig. 17B) large, ML = 72.4 (68-76) µm, curved, pointed apically, mandibular seta near base, 2 subapical teeth, inner tooth greatly reduced. Epipharynx (Fig. 17C, 17D, 17E): large, wide dorsal comb sclerite, DCW = 48.4 (44-52) µm; 11-13 teeth/sclerite (n=20); teeth unequal in both width and length with slightly more pointed tips. The structure is more complex with well defined teeth on the dorsal comb sclerite and deeper indentations on the epipharynx than seen in the L1-L3. Comb 2 contains only a few blunt tubercles. Comb 3 contains many blunted tubercles. A much larger comb 4 is present with a rounded medial process and few blunted tubercles. LAW = 125.6 (104-140) µm. Thoracic segments (Fig. 13): overall body pigment is the same as that seen in L1-L3. Pseudopod not present. Caudal Segment (Fig. 17F): long CSL = 591.38 (549-630) µm, and narrow CSW = 216.38 (189-252) µm, CSR = 2.749 (2.393-3.182). Anal papillae are the same as those seen in L1-L3. The wild-type L4 were significantly different for TL = 6.4 (6-7) mm, HL = 270.9 (252-297), HW = 169.2 (162-180), SGW = 107.1 (99-117), SGR = 1.57 (1.46-1.64), ML = 68.8 (64-74) µm, CSL = 538.2 (495-585), and CSR = 2.37 (1.71-2.61). They were not significantly different for HR = 1.61 (1.56-1.74), LAW 120.8 (116-128) µm, DCW 48 (44-52) µm, and CSW = 230.5 (180-189) (Figs. 19 & 20).

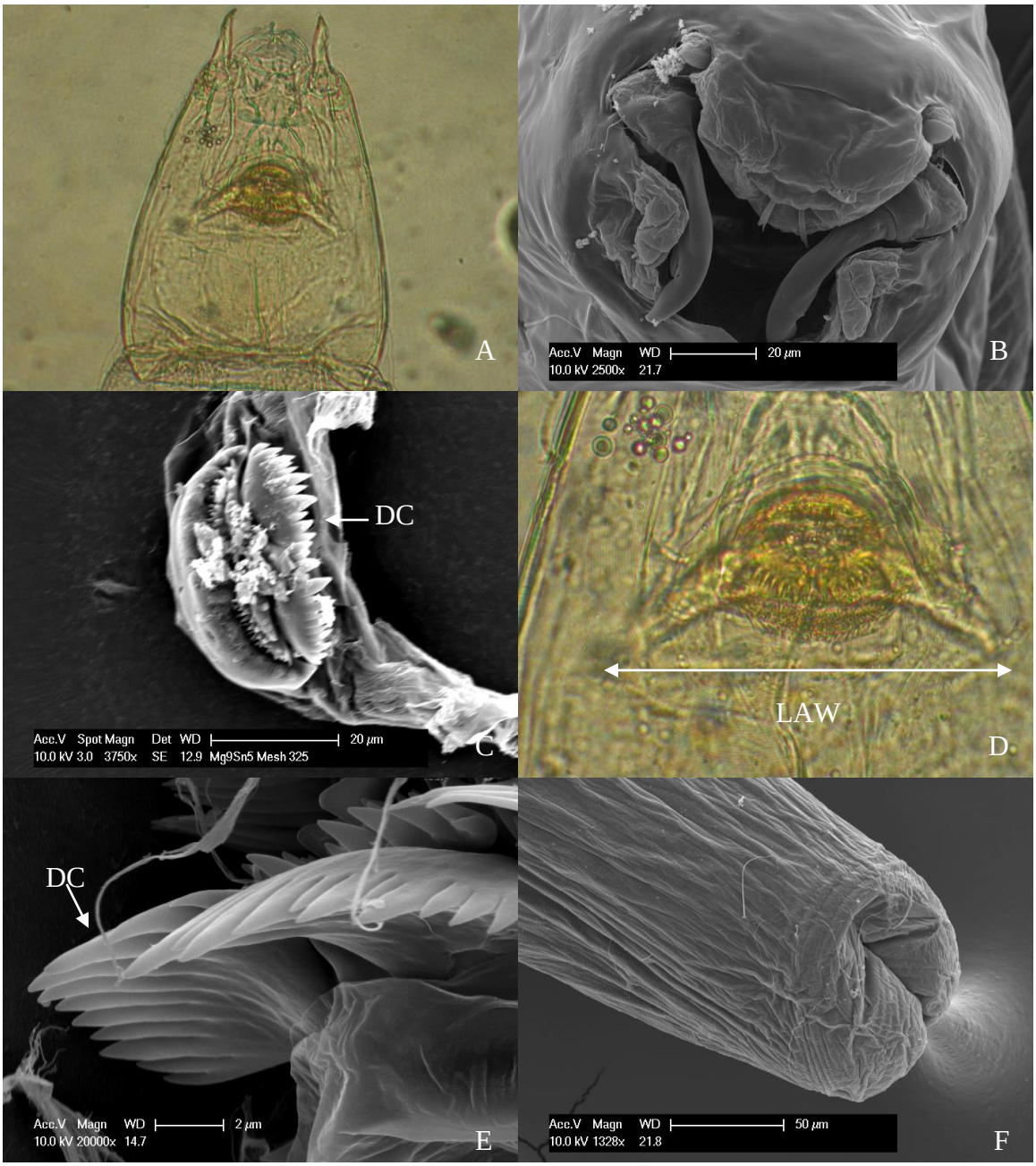

Figure 17. *Culicoides sonorensis* L4 larvae (Van Ryn Colony). A. Anterior overview of the head capsule (dorsal view). B. Overview of external mouthparts including antennae and mandibles. C. Epipharynx. D. Epipharynx (Wild). E. Dorsal combs of epipharynx. F. Caudal segment. epy, epipharynx; LAW, lateral arm width; DC, dorsal comb.

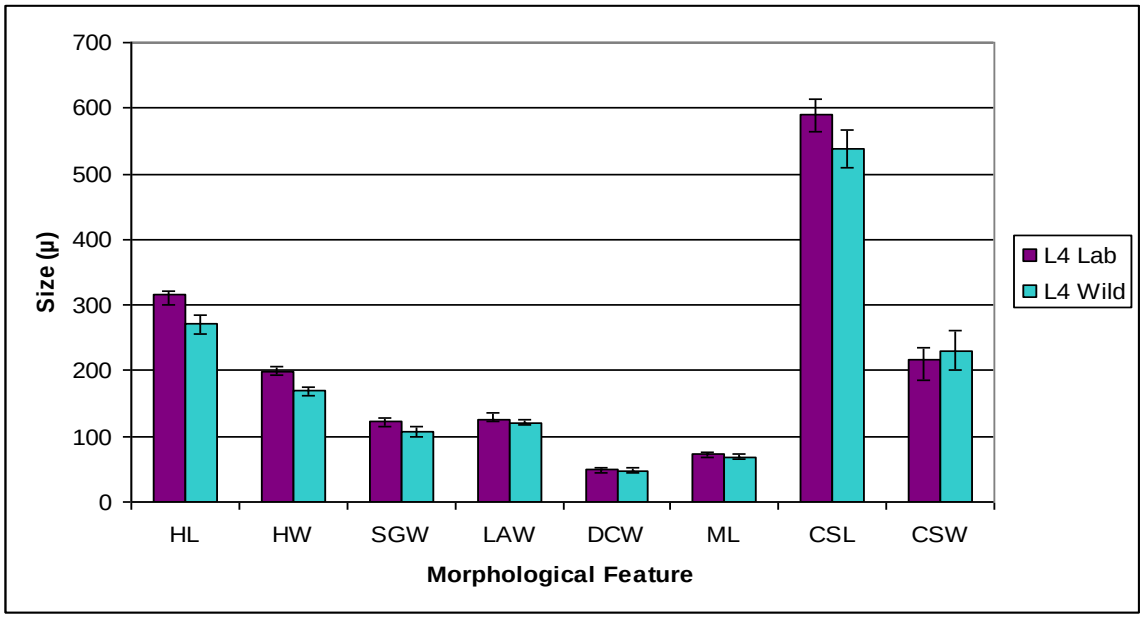

Figure 18. Size comparison of lab-reared (Van Ryn Colony) and field-collected (S and F Dairy) *C. sonorensis* fourth instar larvae (L4).

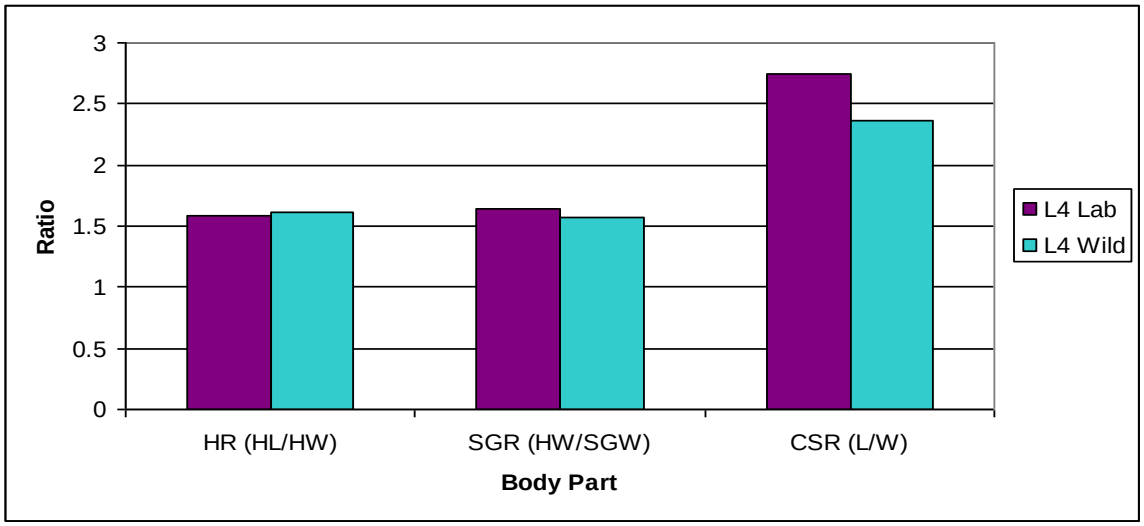

Figure 19. Ratio comparison of lab-reared (Van Ryn Colony) and field-collected (S Dairy) *C. sonorensis* fourth instar larvae (L4).

Pupa Respiratory Horns (Fig. 20) – Dark brown; lower 1/3 lightly crenulated, middle 1/3 strongly scaled, upper 1/3 smooth; 3 lateral protuberances which lack openings; 10-13 cross-shaped distal spiracular openings present; color darkens at apex.

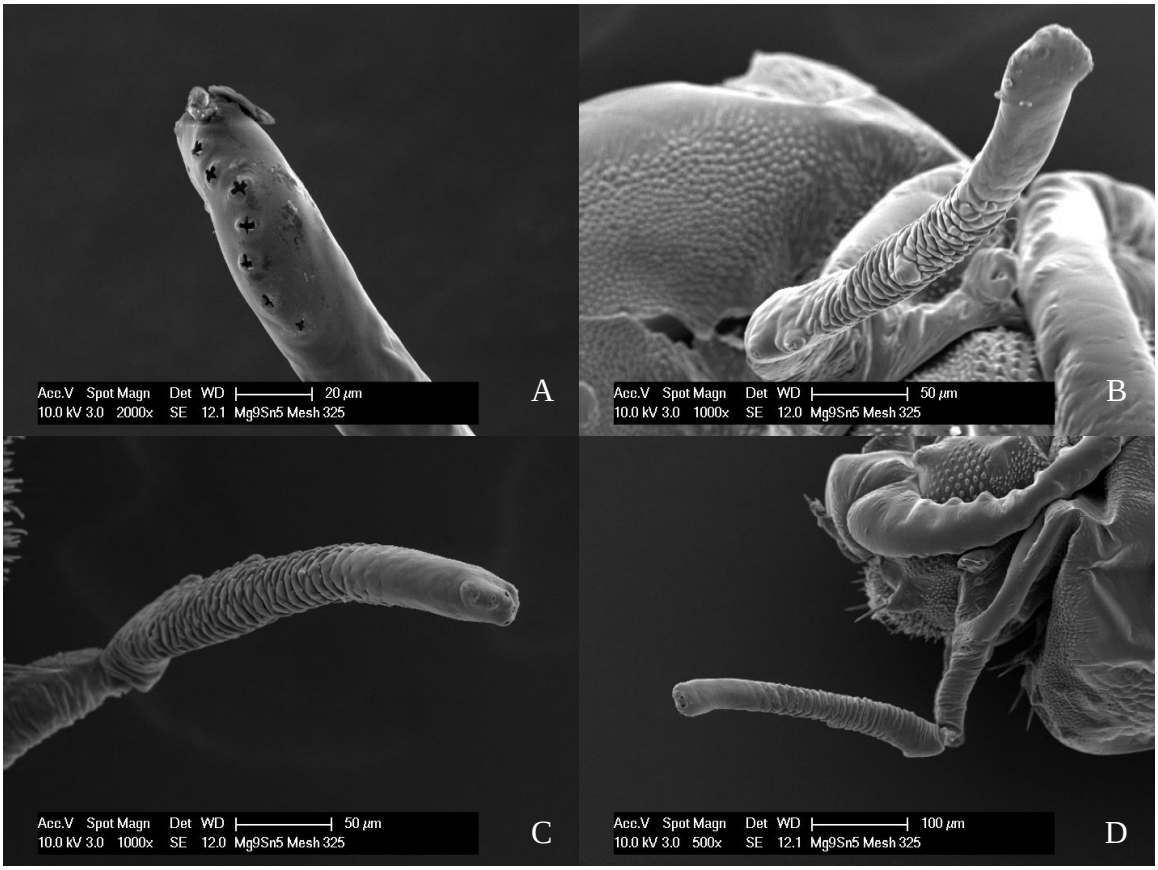

Figure 20. Respiratory horns of *Culicoides sonorensis* pupae (Van Ryn Colony). A. Close up of the distal spiracular openings. B. Overview of the respiratory horn including the lateral protuberances. C. Overview of the respiratory horn including the lateral protuberances. D. Overview of the respiratory horn.

Discussion

Egg Ansulae:

While the overall shape of the eggs of *Culicoides sonorensis* is very similar to many other species of *Culicoides*, the ansulae which are found on the surface of the egg are unique. Various other researchers have utilized SEM in order to help determine not only the structure and placement of the ansulae, but also to guess their purpose (Kwan & Morrison, 1974; Campbell & Kettle, 1975; Nunamaker et al., 1997; Day et al., 1997; Cribb & Chitra 1998; Breidenbaugh & Mullens 1999a; 1999b). In known Californian species, *C. freeborni* Wirth and Blanton, *C. lahontan* Wirth and Blanton, *C. boydi* Wirth and Mullens, *C. cacticola* Wirth and Hubert, *C. vetustus* Breidenbaugh and Mullen, *C. kettlei* Breidenbaugh and Mullen, and *C. utahensis* Fox, all have either *ansulae elongata* or *ansulae papillae* which are arranged in relatively regular formations, typically longitudinal rows, while *C. brookmani* Wirth appears to completely lack surface structures (Breidenbaugh & Mullens, 1999a; 1999b). Eggs of *C. sonorensis*, on the other hand, appear to have only *ansulae elongata* which are not in any discernible formation. While random features typically make it harder to identify a species, the randomness of the ansulae on *C. sonorensis* compared to the patterns of other species actually helps in identification. The tree-like structure of the ansulae, lack of secondary structures, as well as the size and color of the egg should always also be considered cautiously when identifying species using an egg. This is especially important since the majority of the species within *Culicoides* have yet to have their eggs described and so what is assumed to be a unique feature may not truly be unique.

The purpose of the ansulae is not yet known, but two predominant hypotheses have been proposed. The original idea was that the ansulae were used in order to facilitate attachment to a substrate via adhesive secretions (Becker, 1961; Cribb & Chitra, 1998). The second and more recent hypothesis is that the ansulae act as a plastron in order to help in respiration, especially on the concave area of the egg. The idea behind this is that the ansulae will allow for a layer of air to remain on the surface of the egg when it is in a moist environment (Campbell & Kettle, 1975). Throughout the years the plastron hypothesis has gained momentum and in 2013 a textbook, The Encyclopedia of Medical and Veterinary Entomology, ascribed this function to the ansulae (Russell *et al*, 2013). However, the lack of descriptions for ansulae as well as the environments where the eggs are found makes it impossible to say with any certainty what the function of ansulae is. When looking only at *Culicoides sonorensis* eggs, it would appear as though the adhesion hypothesis is plausible. The random nature of the ansulae could possibly make the plastron less effective, even in the concave area, due to the increased areas between many of the ansulae. In order to determine the true use of the ansulae, the environment the eggs are found in, along with all spectrums of ansulae, must be analyzed. This should include the random *C. sonorensis* ansulae, the patterned ansulae of species such as *C. freeborni*, as well as a lack of ansulae as in *C. brookmani*.

Finally, it is possible that the severe dehydration that is imposed on the eggs for SEM has created an artifact in ours and other researchers' images. In some SEM photos (e.g *C. utahensis* in Breidenbaugh & Mullen, 1999), vestiges of a membrane "blanket" on the ansulae appear. Perhaps such a membrane is common and involved in plastron

respiration (e.g. *C. molestus* Skuse; Cribb and Chitra 1998). Better methods of imaging the eggs are needed in order to better visualize their surface structures. However, this may prove rather difficult due not only to the delicate nature of the eggs, but also to the need to clear the eggs before imaging to eliminate extra debris. Current preparations may not only severely dehydrate the eggs but the clearing of the eggs as is described in Ronderos *et al.* (2008) using chemicals may also destroy essential structures.

Larval Swimming Speed:

The swimming speed of the larvae increased with each larval instar, going from 2.013 mm/sec for the first instar larvae, 4.803 mm/sec for second instar larvae, 8.184 mm/sec for third larval instar, and 11.816 mm/sec for the fourth larval instar. These observations agree with Linley (1986), who determined that within the fourth larval instar of *C. variipennis* the beat rate declined as the length of the larva decreased while the speed increased as the larvae increased in size. Although there was an increase in swimming speed overall, there was actually a relative decrease in the swimming speed when comparing the number of body lengths traveled per second. The increased beat rate that Linley (1986) noted is probably what contributes to the ability of the earlier, smaller instars to move further relative to their body length while the increased size in itself is what allows the later, larger instars to move at an overall faster rate.

It is possible that the earlier instars utilize more of their energy to move due to the fact that they are more at risk than the later instars are. Mullens and Lii (1987), found some eggs of *Culicoides sonorensis* about 6 cm above the waterline, while the first instar

is found primarily 2 cm above to 1 cm below the water line, the second instar is found 1 cm below the waterline, the third instar is found 1 to 4 cm below the waterline, and the fourth instar is found 4 cm below the waterline. This means that the first larval instar may need to travel a longer distance in order to reach the area which larvae are most often found after they hatch. A faster swimming speed would help with this. In addition to this, being above and at the waterline and having a thinner cuticle for both the first and second larval instars makes them more exposed to predators as well as the elements (e.g. desiccation).

This exposure could also contribute to the behaviors of the earlier instars on a dry slope. This research showed that all instars except for the fourth instar tended to attempt to bury themselves in mud above the waterline as a priority rather than attempting to locate the waterline. It may be more important for the first three instars to hide themselves in the mud than it is for the fourth instar. It could also be that the fourth instar's larger size makes it more difficult to bury themselves in slightly compacted mud and therefore they must seek the looser soil below the waterline before they are capable of burying themselves.

Pupation Location:

This research has indicated that *Culicoides sonorensis* prefer to pupate either at or directly below the "high tide" line both in the lab as well as in the wild. These findings were very similar to Linley and Adam (1972) who found that *C. melleus* Coquillett also preferred to pupate in this area.

In the lab, the larvae were allowed to choose a pupation position in conditions very similar to those found in their natural environment except for the lack of water level fluctuation. In these conditions the pupae were always found immediately below the waterline with their bodies completely submerged underwater with only the very tips of their respiratory horns in contact with the surface. This location was ideal for them since they maintained contact with the air, remained in a wet environment, and were almost completely protected in a burrow. By burying their bodies in the mud and only allowing their respiratory horns to show it was extremely difficult to find the pupae even when their location was already known.

In the wild at the dairy however, the conditions were not quite as consistent as in the lab and the pupae were forced to adapt to the changes. While the samples in the wild were being taken, the waterline was receding, leaving the pupae stranded above the waterline which made it difficult to quantify where the pupae were located. Because of this, the pupae were most often found between 0 – 10 cm above the waterline. However, it was fortunate that along the shore there was what we deemed the "chironomid line" where the exuviae of chironomids formed a distinct line which indicated where the water level had recently been highest. At each of the sites the majority of the *C. sonorensis* were found at this line.

The behavioral aspects of this study also indicate that the pupae prefer to be at or directly below the waterline. It is unlikely that the larvae would chose to pupate far above the waterline for two primary reasons. The first reason is that the fourth instar larvae are very inefficient when attempting to travel across soil which is not at least somewhat

submerged by water. It would be difficult for the larvae to reach an area above the waterline to pupate and would also risk exposing them to predators. Secondly, the pupae are unable to travel any distance in those same conditions and are also almost incapable of burying themselves without a thin layer of water on the ground. It is also very improbable that they would pupate too far below the waterline primarily due to their tendency to remove themselves from their burrows and float to the surface of the water when they are completely submerged and travel to the waterline.

The "high tide" line is a logical location of the pupae of *C. sonorensis* to be most common. In a rising water situation, flooded pupae float free and then rebury themselves at the waters edge. They are able to camouflage themselves by being buried and are also able to handle water level fluctuations efficiently from this location, although they apparently don't relocate when water levels drop. This location allows the pupae to remain hidden below the surface of the mud somewhat protected from drying and predators, while still in contact with the air. It also reduces the chance that the pupae will be submerged underwater.

Whereas Linley and Adams (1972) found that *C. mellus* was able to survive up to four days when submerged, the pupae of *C. sonorensis* do not survive well underwater. Only 50% of pupae are capable of surviving even 8-9 hours underwater. Due to their inability to survive underwater, the pupae extract themselves from their burrows within a minute of submersion and float to the top. This saves them from drowning but also probably exposes them to predators. It usually is far safer for the pupae to remain in a burrow which occasionally dries out rather than risk being submerged and exposed to

predators as they wriggle free. It is not known how deep the pupae may try to bury themselves as the soil dries, or exactly how well they can tolerate desiccation. Those might be areas of future study.

Proleg:

One of the most interesting features found on the larvae of *Culicoides sonorensis* is the pseudopod found on the first larval instar. This feature has not been previously described in depth for many species of *Culicoides* and its purpose is still debated. The pseudopod, when present, is on the ventral side of the first thoracic segment and is only found on the first larval instar. Very few *Culicoides* spp. L1 larvae have ever been observed, however. Although Lawson (1951) described the pseudopod of *C. obsoletus* Meigen as a simple protuberance, he was limited to light microscopy. The pseudopod of *C. sonorensis* appears to be quite complex. This structure exhibits bilateral symmetry with 4-5 strong setae on each side which are hooked at the ends along with 5 rows of setae which are placed in a triangular fashion. This arrangement is actually somewhat similar to descriptions of *C. obsoletus* as well as *C. nubeculosus* and *C. scoticus* Downes and Kettle. Lawson (1951) described *C. nubeculosus* as having 4 long setae on each side of the pseudopod. Later in Kettle and Lawson (1952), *C. obsoletus* is described as having 3 pairs of hooks, 4 median rows of teeth, and 2 pairs of lateral rows of teeth while *C. scoticus* has 3 pairs of hooks and 2 median rows of fine teeth. It is interesting to note, however, that *C. halophilus* Kieffer does not have a pseudopod (Kettle & Lawson, 1952).

These differences in the pseudopod in the various species could help to determine the purpose of this structure. There are currently two ideas on pseudopod function. The first hypothesis is that the larvae use the pseudopod in order to facilitate their escape from the egg (Lawson 1951). This argument is difficult to refute due to the fact that very few researchers have actually witnessed the initial escape of the larvae from the egg. It is true that while escaping from the egg *C. sonorensis* larvae repeatedly contract their first few body segments in a way that would utilize the hooks on the pseudopod to move forward. However, the lack of a pseudopod in *C. halophilus* brings into question the necessity of this structure for egg escape. It may be that the true purpose of this structure is to facilitate locomotion.

The hypothesis that the pseudopod is utilized in order to facilitate locomotion is reasonable. Looking at the backward-facing arrangement of rows of smaller spinelike structures, they should grip the substrate when the larva uses them to propel itself forward, and in turn should slide readily in the reverse direction. The longer setae also have a hook-like tip that might grip the substrate when moving forward. The research presented here has shown that the first larval instar is more capable than later stage larvae in traveling across dry soil. The L1 utilizes the pseudopod in order to pull itself along incrementally when it is incapable of swimming. In addition to this, as previously stated, the eggs of *C. sonorensis* are found above the waterline while the second through fourth instars are found below the waterline (Mullens & Lii, 1987). This indicates that the first instar larvae are required to travel over the land in order to reach the water. Since the

pseudopod is lost in the later instars it follows that the pseudopod may be necessary for the conditions which are only found in the first larval instar.

The Ceratopogonidae genera *Apelma* Kieffer and *Austroconops* possess a pseudopod in all four larval instars similar to that found in first instar of *Culicoides*; in both cases the pseudopod is described as being used in locomotion (Saunders, 1925; Borkent & Craig, 2004). It is important to determine if the pseudopod is truly used for this purpose. In order to do this, more research into the egg emergence mechanisms, their movements on various substrates, and the structure of the pseudopod on various species of *Culicoides* is needed. Of particular interest is *C. halophilus*, which lacks a pseudopod and is found in shallow, muddy brackish pools as well as salt flats (Kettle & Lawson, 1952). By comparing the structure of the pseudopod with the environment and location of the eggs, first larval instars, and second larval instars it would be possible to hypothesize what factors might influence the structure and presence of the pseudopod over evolutionary time. Unfortunately, there is a lack of data for all of these factors due to the small size of the eggs and the larvae; in many cases even the breeding locations of *Culicoides* species are unknown.

Larval Morphology:

The morphology of *Culicoides sonorensis* is relevant to studies of their behaviors and biology as well as control methods. Unfortunately, the feeding behaviors and the natural diet of *Culicoides* larvae are not well known. Due to their small size and the opaque environment in which they live, the majority of experimental research into the

feeding preferences of *Culicoides* has taken place in artificial environments, which may lead to inaccurate results (Mullen & Hribar, 1988). By knowing the details of their morphology it is possible to pose hypotheses on habitat and food sources.

There have been several attempts to place Ceratopogonidae species into categories based on their larval feeding habits. Mayer (1934), separated the Ceratopogonidae into two categories; the coarse detritus feeders, (those which feed on larger particulate matter), and the fine detritus feeders, (those which feed on smaller particulates). He placed *Culicoides* within the coarse detritus feeders. This was a crude system to separate genera, based primarily on what could be seen in the gut, and did not provide many dependable clues as to larval feeding preferences. Glukhova (1977) suggested placing the larvae into the ecological categories similar to what we use for many other organisms; predators, herbivores, and omnivores. This categorization is probably the easiest to understand but also is not very helpful in terms of the nature of what has been ingested (Mullen & Hribar, 1988). More recently, Teskey (1984) categorized them as scrapers, collector-gatherers, and engulfer-predators with Ceratopogoninae described as primarily engulfers or predators, but this categorization has not been widely used. Mullen and Hribar (1988) felt most ceratopogonids might be opportunistic feeders.

There are however a few key structures which have been widely agreed upon as the most important features for determining the feeding preferences of species. These features include the head-ratio, the mandibles (particularly the subapical teeth), the hypopharynx, and most importantly, the epipharynx (Kettle & Lawson, 1952; Neville,

1969; Glukhova, 1979; 1989; Kettle & Elson, 1976; 1978; Mullen & Hribar, 1988; Murphree & Mullen, 1991; Spinelli et al, 2005; Ronderos *et al*, 2008b; Ronderos *et al*, 2010). By comparing these features with other species whose preferences are better known, a researcher can make more accurate assumptions as to what the natural feeding habits may be.

Head-Ratio:

One of the features which is quite easy to measure, if done correctly, is also one of the most telling clues as to the feeding habits of *Culicoides*. This is the head-ratio, which is the total length of the head divided by the total width of the head at the widest point. If measurements are done improperly though, this ratio can be deceiving. Many researchers have a tendency to clear their specimen in either phenol or potassium hydroxide prior to slide-mounting the specimen. The problem with this procedure is that clearing may also dissolve some of the internal tissue, distorting the head capsule. That is why in these experiments the larvae were not cleared prior to taking these measurements. This method provided a more accurate measurement without any unnecessary alterations. In addition to this, since the head capsule is quite easy to crush and distort, fresh specimens were used for the measurements. This ensures that preservation methods such as storage in ethanol or slide mounting in Canada Balsam would not have an effect on the dimensions. It is important that all researchers interested in measuring the head capsules dimensions should avoid distortion, so that all comparisons are accurate.

The head ratio is important because of the correlation of head capsule size with feeding behaviors (Thomsen, 1937). Larvae with shorter heads tend to be more herbivorous while those with longer, narrower heads are more carnivorous. In that categorization, the first, second, third, and fourth larval instars all have head ratios which indicate that the members of *Culicoides sonorensis* are carnivorous with the head ratios averaging 1.342 (1.333-1.524), 1.416 (1.220-1.6113), 1.575 (1.5-1.717), and 1.584 (1.49-1.702) respectively. The increasing ratio may also indicate that the third and fourth instars are more likely to be predators than the first and second instars are. Colonies of *C. sonorensis* feed only on microorganisms, but later stage larvae can readily feed on nematodes (Mullens & Velten, 1994).

Mandibles:

The mandibles are one of the most commonly described but also one of the most useful in both species identification (as well as feeding preference determination based upon the number of subapical teeth they have). The mandibles are important for identification, since the size of their mandibles, the placement and number of setae on, the number of teeth, and the number and placement of sensory pits differ among *Culicoides* species (Murphree & Mullen, 1991). In the case of *Culicoides sonorensis*, this research has shown that the mandible length is fairly consistent. Each larval instar has a mandible which is curved and pointed apically, a single mandibular seta near the base of the mandible, two rounded subapical teeth with the inner tooth greatly reduced in size, and one sensory pit. The only difference between the mandibles of the larval instars is the

size. These measurements were 20 (18-22) µm, 26.4 (24-34) µm, 39.8 (38-44) µm, and 72.4 (68-76) µm respectively for the four larval instars.

These findings place the larvae of *Culicoides sonorensis* in between Thomsen's (1937) two categories of herbivorous and carnivorous ceratopogonid larvae. According to that, the more herbivorous larvae have mandibles with 3 teeth while the carnivorous larvae have only a single tooth. This system of classification is well known and supported by findings such as those concerning the well known species *C. guttipennis* Coquillett which was previously shown to feed on small mosquito larvae (Hair & Turner, 1966; Bay, 1974). *C. guttipennis* has been described as having either two subapical teeth, one of which is reduced (Murphree & Mullen, 1991), or one strong subapical tooth (Ronderos *et al*, 2010). The Ronderos study was South American, while the Murphree and Mullen study was North American, so there is some possibility they are dealing with two species. Since the second tooth found in *Culicoides sonorensis* is very reduced and can only truly be seen using SEM, it is likely that they have more carnivorous tendencies similar to *C. guttipennis* while still feeding on other materials, making them omnivorous. Without question older *C. sonorensis* larvae can prey on nematodes (Mullens and Velten, 1994).

Hypopharnyx:

The usefulness of the hypopharynx in identification of Ceratopogonidae larvae has been debated, with some authors choosing to include it in their descriptions while others do not. There are two primary reasons for why this structure is often omitted from descriptions of *Culicoides*. First this structure is not complex and has very little variation

between species, making it less useful, presumably, for species identification. It is simply a structure found ventrally, below the epipharynx, and is narrow at its anterior end and widens at the posterior end. Second, in many species this structure is almost completely membranous. As such it is difficult to observe; in order to see the finer details the specimen must be dissected and the epipharynx removed (Murphree & Mullen, 1991). However, it is important to note that this structure can be important in some groups. The members of the subgenus *Monoculicoides* have a rather unique hypopharynx, which is fairly well-sclerotized, such as was found in this research for *Culicoides sonorensis*. This feature could perhaps help in determining exactly what *C. sonorensis* are feeding upon in their natural environment. The heavier sclerotization suggests that *C. sonorensis* needs either a more resilient or a harder structure in order to be able to process whatever it is they are feeding on. A predator could require this more heavily sclerotized hypopharynx in order to both prevent damage from its prey as well as hold it in place so that that epipharynx could work properly.

Epipharynx:

The epipharynx has been universally recognized by researchers as the single most important morphological feature in identifying species of Ceratopogonidae currently. This is a complex structure which varies greatly among genera and often between species within a genus. The epipharynx is a heavily sclerotized structure which consists of between 2 and 4 combs with teeth that are directed posteriorly and are held dorsally above the hypopharynx by two lateral arms (Murphree & Mullen, 1991). Fortunately for

those who research species of *Culicoides*, the variation in almost all of the features of the epipharynx from the dorsal comb width, the number of teeth on the dorsal comb, the lateral arm width, and the number of combs, all make it far easier to identify a specimen to the species level. This is especially true for members of the subgenus *Monoculicoides* due to the fact that they have what can only be described as a "uniquely massive" epipharynx which has been well illustrated by Murphree and Mullen (1991).

In the case of *Culicoides sonorensis*, the epipharynx clearly marks this species as a member of the subgenus *Monoculicoides*. The structure is well sclerotized with a lateral arm width of 23.6 (22-28) µm, 35.7 (32-40) µm, 61.8 (56-66) µm, and 125.6 (104-140) µm for the first, second, third, and fourth larval instars respectively. There is also a total of four combs with a dorsal comb whose width also increased with each instar with the first instar measuring 8.2 (8-9) µm, the second measuring 12.7 (10-16) µm, the third measuring 24.1 (20-28) µm, and the fourth measuring 48.4 (44-52) µm. It is important to note that the measurements for the dorsal comb followed the method used by Murphree & Mullen (1991) in which both dorsal combs were measured together instead of separately. This increase in size between the instars was expected and follows the pattern of their overall growth. There were, however, differences between the dorsal combs of the instars, primarily in the number and complexity of the teeth. Although for the first and second instar the exact number of teeth was not determined with confidence, it was evident that the numbers of teeth on the first and second instars were fewer than found on the third and fourth instars. In addition to this, with each increase in instar, the complexity of the teeth also increased. One of the reasons that it was difficult to

determine the exact number of teeth in the youngest instars was that they were not clearly defined. As can be seen in Figures 16, 17, and 18, there are deeper indentations and better defined teeth as the instars progress. This trend was also found by Lawson 1951 for *C. nubeculosus*.

The morphology of this structure is also important in determining both what and how members of *Culicoides* feed. The massive epipharynx combined with a well-sclerotized hypopharynx is unique to the subgenus *Monoculicoides*. In the past it has been postulated that these two structures act together as a type of mortar and pestle between which their food is ground up and crushed (Murphree & Mullen, 1991; Kettle & Lawson 1952). This hypothesis stems partially from the movement which can be seen within the head capsule of a live specimen. As a larva feeds on materials, the epipharynx swings from front to back with the dorsal comb swinging across the trough of the hypopharynx. Naturally, any food caught between these two structures could be crushed.

However, I would like to present an alternative hypothesis. The larger epipharynx and more heavily sclerotized hypopharynx found in the *Monoculicoides* subgenus is also found to a lesser extent in other species known to be carnivorous such as *C. guttipennis* (Ronderos *et al*, 2010). From my observations, both while the larvae were alive as well as from SEM, it appears as though the two structures do not actually meet and are better suited for another purpose. It may be that instead of acting in a grinding and crushing fashion, the teeth which are found on the dorsal comb may instead be used to perforate the cuticle of their prey items to more easily digest them. This would explain the need for a more massive epipharynx with stronger teeth as well as a more heavily sclerotized

hypopharynx which would be needed in order to hold the prey item in place as well as protect the larvae. More research needs to be done on the actual mechanism in action in order to discover exactly how these structures are used to process food.

Species Comparison:

The measurements in this research are a close match to those seen in Murphree and Mullen (1991) for *C. variipennis* s.l. As previously stated, their research may have included various different species including *C. sonorensis*. The comparison of features can be seen in Table 4. The only measurement which does not fall within the range found by Murphree and Mullen (1991) is the DCW. This difference could be due to the difference in species or measuring techniques. All other features such as epipharynx and mandible structure are also similar.

Table 4. Comparison of larval feature of last instar *C. variipennis* s.l. and *C. sonorensis*

Species	HL	HW	SGW	ML	LAW	DCW	Teeth/Comb
C. variipennis	319	219	113	64	129	30	13
(Murphree &	(291-367)	(190-271)	(94-156)	(45-86)	(98-159)	(25-39)	(10-15)
Mullen, 1991)							
C. sonorensis	315.5	199.33	121.83	72.4	125.6	48.4	11-13
(this study)	(300-324)	(188-212)	(112-132)	(68-76)	(104-140)	(44-52)	

Pupal Respiratory Horns:

One of the most ecologically relevant features of *Culicoides* pupal morphology is the pair of respiratory horns. In almost every description of the pupae seen in Table 1, the respiratory horns are described. This is because the horns are not only a prominent feature but also because it is recognized that their morphology is some of the most varied as well as the easiest to recognize for the pupa. In addition to this, many keys such as Jamnback (1965) utilize the respiratory horns as one of the primary means to differentiate

species. The respiratory horns vary in pigmentation, scaling, lateral protrusions, number of spiracular openings, and as can be seen from this study, the shape of the spiracular openings.

In the case of *Culicoides sonorensis*, their respiratory horns are quite unique. The horns in this study were found to be dark brown in color throughout, with the color darkening at the apex, the lower third of the horn lightly crenulated, the middle third strongly scaled, and the upper third smooth. This is all as expected for the genus. However, there were two findings in this study which were quite surprising. First, although it is normal for the horns to have three lateral protuberances as they did, it is surprising that none of the protuberances had spiracular openings. As can be seen in many descriptions of *Culicoides*, the lateral protuberances are depicted with circular openings (Ronderos et al, 2010; Breidenbaugh & Mullens, 1999a; 1999b; Kettle & Lawson, 1952; Neville et al, 2007). This may however not be true for all of these described species. In the case of *C. sonorensis*, under a light microscope it appears as though there are internal respiratory horn tracheae with an opening (Fig. 21). However, when viewed using SEM, there are no apparent external openings. It may be that membranes cover the openings, allowing for some exchange of gases while effectively excluding water. The present research discovered that the preferred location for the pupae is at or directly below the waterline with only the tips of the respiratory horns in contact with the air, and it would follow that these structures spend a large amount of time below the surface of the water. In such a situation it would be beneficial not to have any openings on these submerged lateral protuberances. Without SEM it would be very

difficult to determine if there were in fact openings on these features. The older research such as Kettle and Lawson (1952) may have inaccurately reported the presence of openings on these lateral protuberances, as it appears so using a light microscope. This illustrates that new research into *Culicoides* species should include the use of SEM, in order to ensure that small but important differences such as these are properly recorded.

In addition to the lateral protuberances lacking actual openings, another surprise in the morphology of the respiratory horns was the presence of cross-shaped distal spiracular openings. Similar to the case of lateral protuberances, previous research on *Culicoides* has always depicted the horns spiracular openings as circular, which may in fact be the case. However, in the case of *C. sonorensis* it was immediately apparent when

Figure 21. Pupal respiratory horn lateral protuberance of *C. sonorensis* viewed under a light microscope.

utilizing SEM that the distal spiracular openings were cross-shaped. This unique structure could be an adaptation to the environment in which *C. sonorensis* pupae live. Since, as previously stated, the pupae prefer to be almost completely submerged with only the tips of the respiratory horns in contact with the air, it may follow that a circular opening

would not be ideal. The cross-shaped openings may in fact allow for better sustained contact with the air while also excluding water when the pupae are temporarily submerged. Since these openings are their only direct access to the air, they would need to function as optimally as possible in these conditions and the crosses may be an adaptation for this species. However, it is again important to note that this feature was only seen using SEM. Very few previous researchers have utilized SEM when examining the pupae and in many cases the pupae are described using only the exuviae of the pupa.

Conclusions

Since it is one of the most important vectors of both BTV and EHDV, *Culicoides sonorensis* needs to be studied in depth. The research however, cannot only include the adults as has been done in the past but must instead include all stages of life. This research has only delved into the morphology, biology, and behaviors of the immature stages but there is far more which can and should be explored.

The earliest stage, the egg, has been ignored in not only *C. sonorensis* but within the *Culicoides* genus in general. *C. sonorensis* has been shown to have eggs which are covered in randomly distributed ansulae whose purpose is still unknown. Although there are theories as to their use, there are no definitive conclusions. It is important that far more species eggs are examined utilizing SEM and that better techniques for imaging and clearing are developed so that all structures can be seen in as natural a state as possible. In addition, the location of the eggs needs to be determined for more species so that accurate comparisons can be made between the structures on the eggs and the environment they have adapted to.

Similar reseach is also needed for the larvae of more species of *Culicoides*. This work has shown that there are few differences in the morphology of the larvae throughout their stages but the differences that are seen are important. One of the most important differences is the proleg which is only found in the first larval instar. The proleg is likely used in order to assist the L1 in both escaping from the egg and in traveling across mud. Since very few species have had the L1 described in any detail, it is important that this instar is included in future morphology research to help determine why many species

have a proleg while others such as *C. halophilus* lack one (Kettle & Lawson, 1952). Again, it is also important to determine the environments that various species are found in so that accurate comparisons between structure and function can be made.

The observations of internal and external mouthparts of *C. sonorensis* have all fallen into the size and shape ranges of those seen in other members of the subgenus *Monoculicoides* (Murphree & Mullen, 1991). Everything from their massive epipharynx to their heavily sclerotized hypopharynx place them comfortably within this classification. The structure of their mandibles and their head ratio also mark them as carnivores according to Thomsen (1937). This research has also led me to develop an alternative function for the epipharynx and the hypopharynx. While previous research has indicated that these two structures act together as a mortar and pestle (Murphree & Mullen, 1991), I believe that it is more likely that they are instead used to disrupt the cuticle of their prey. More research into the mechanism in action as well as better research into the natural prey items of various species would help to clarify the true mechanism.

The last immature stage, the pupa, is also woefully underrepresented in research. This research has only explored the morphology of the respiratory horns of *C. sonorensis* but the anomalies found there are important. The lack of spiracular openings on the lateral protuberances and the cross-shaped distal spiracular openings are both new and exciting discoveries which emphasize the lack of information concerning pupae. Many researchers in the past have described species using only a single exuvium under a light microscope. These descriptions must be updated to include more details, reasonable representations of variation amoung individuals, and SEM is basically essential for some

features. The lack of lateral openings as well as the cross-shaped distal openings would have been overlooked if not for SEM. In addition to this, more research into the behaviors and environments of the pupae of various species must also be done. By discovering that the pupae appear to prefer to be immediately below the waterline, the features of the respiratory horns could be explained to some extent. By adding the data from additional species, these hypotheses can be better tested. A more comprehensive morphological and ecological research base should help those hoping to better understand not only *C. sonorensis* but all species of *Culicoides*.

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