Molecules, Morphology and Ecology:

An Analysis of Black Fly Taxonomy, Phylogeny, and Species Tolerance Values.

By

DIANA M. CARLE

A Dissertation submitted to the

Graduate School – New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Entomology

Written under the direction of

Professor Mark Gregory Robson

And approved by

____

____

____

____

New Brunswick, New Jersey

May 2015

ABSTRACT OF THE DISSERTATION

By DIANA M. CARLE

Dissertation Director:

Mark Gregory Robson, PhD, MPH

Comparative black fly taxonomy has largely been limited in scope to comparisons within a continent, or at most by hemisphere. This gap in black fly systematics has been aided by barriers of language, geography, and specimen availability. This thesis provides a morphological comparison of 19 of the 26 known world genera of black flies, along with a new genus, *Adlerella*, which is described herein. This genus is also supported by molecular data. A morphology based phylogeny was generated, providing evidence for a Holarctic origin for the family Simuliidae. The somewhat aberrant genus *Crozetia'*s basal relationship to the other Simuliini suggests a possible black fly relict.

This study includes the first combined molecular and morphological based phylogeny of the world Simuliidae genera from all of Wallace's biogeographic regions, minus seven of the known genera. The first analysis of paralogous genes in the Simuliidae is also performed. A new alignment for the 12S and 28S nucleotide sequences is proposed based on manual secondary structural alignment. Strong branch support and congruence between molecular and morphological data confirm the subfamiliy and tribe divisions of the Simuliidae. Due to particularly strong branch support for the tribe Prosimuliini, it is raised to the subfamily Prosimuliinae, and a new combination for the subfamily Simuliinae is proposed.

This thesis also includes a comprehensive analysis of simuliid species tolerance values based on chromosomal identifications. Pollution tolerance values are reported for the 39 species of black flies known in New Jersey. Species of the same genus exhibited high variation in tolerance values, questioning the accuracy of generic-level tolerance values for the Simuliidae. An index for predicting the occurrence and proliferation of the *Simulium jenningsi* group, which includes the major pest species of Simuliidae in the eastern United States, is proposed, with implications for control programs worldwide.

ACKNOWLEDGEMENTS

This is dedicated to those people who have most lovingly and devotedly supported me. I would like to thank my dad, Frank Carle, who taught me the importance of hard work, whether it be rebuilding an engine or writing a paper, and always encouraged me to pursue what I desired most. I would like to thank my sister, Kristina Carle, who as a child both encouraged my love of nature by excitedly exploring all types of fauna with me, and tested it by throwing cicadas at my hair! She has always grounded and protected me just like a good big sister should. And thank you to my best friend, Katherine Talalas, who always reminded me of the truth and beauty inherent in science, and also made certain that I spent some of my time shopping and going to the beach, instead of endlessly toiling over my research.

Special thanks to my major adviser, Dr. Robson, whose words at our first meeting, "Are you ready to do some good!" kept me centered on the most basic purpose of science, to make the world we live in a better place. His utterance, "Perfection is the enemy of progress," his constant encouragement, and his guidance to put boundaries on my seemingly ever expanding thesis was just the advising this student with a never ending well of ideas needed!

Thank you to my committee members, Dr. Fonseca, Dr, Hamilton, and Dr. Adler who took the time to review my progress and mentor me with their scientific knowledge. Special thanks to Dr. Hamilton who funded my Ph.D. through granting me the departmental teaching assistantship. Special thanks also to Dr. Adler who took the time to teach me Cytotaxonomy, and whose conversations have helped expand and refine this thesis.

TABLE OF CONTENTS

LIST OF TABLES

Introduction

Simuliidae, or black flies are holemetabolous members of the suborder "Nematocera" whose immature stages live in lotic aquatic habitats. Adults emerge through the pupal integument, usually in the morning hours (Davies 1950) in a pocket of air (Hannay & Bond 1971) with males typically exhibiting protandry. Female simuliid dispersal flight distances range from less than 15km for most species (Baldwin et al. 1975) to more than 500km for the African *Simulium damnosum* Theobald (Garms & Walsh 1988), though *Simulium jenningsi* Malloch females, a North American species, frequently fly more than 500km from the breeding sites (Amrine 1982). The females relentlessly pursue humans and livestock to obtain blood meals. In North America, black fly adult activity spikes from May to October, resulting in economic losses to tourism and recreation, livestock operations, as well as producing complaints from disgruntled taxpayers. Attempts have been made to quantify the negative economic impact of black flies, such as a paper company in Texas that lost $10,000 for every roll of paper that one *Cnephia pecuarum* Riley was mashed into (Guyette 1999). Golf courses are particularly affected by this pest, with the decreased attendance at an infested South Carolina course causing losses to the surrounding businesses of $27,000 in 1994 (Gray et al. 1996), while five courses along the Los Angeles River lost $34,000 a month spanning the 1994-1995 season (Shaw in O'Connor et al. 2001). A hindrance to identifying a simuliid problem in an area is the public's common misidentification of black flies as mosquitoes (Crans & McCuiston 1970).

Black flies are vectors of onchocerciasis, commonly known as river blindness, so named for the condition in advanced cases where the *Wolbachia* bacteria that are carried by the simuliid transmitted *Onchocera volvulous* nematodes cause an inflammatory response in the eye resulting in loss of sight. This disease is endemic in Africa and epidemic in South and Central America, including parts Mexico (Vachon 1993). In North America however cases of simuliid-borne pathogens of humans are undocumented. However, North American black flies do transmit livestock pathogens including, leucocytozoonosis, a malaria-like avian illness caused by a protozoan. *Leucocytozoon simondi* is transmitted mainly to waterfowl by *Simulium anatinum* Wood and *Simulium rugglesi* Nicholson & Mickel to, while *Leucocytozoon smithi* is principally transmitted to turkeys by *Simulium congareenarum* Dyar & Shannon, *Simulium meridionale* Riley, and *Simulium slossonae* Dyar & Shannon (Adler *et al.* 2004). *Onchocerca lienalis* is transmitted by *S. jenningsi* to cattle in North America, and though observable symptoms are usually absent, the parasite and vector interactions are used as a model to study the parasite that causes river blindness, *Onchocerca volvulus*, and its vector, the *S. damnosum* species complex (Lok *et al*. 1980, 1983).

A greater understanding of the ecology of the Simuliidae requires a firm knowledge of their phylogeny. A deep knowledge of how the different subfamilies, tribes and genera are related to one another provides the essential syntax of how to proceed with and communicate regarding simuliid scientific inquiry. Though simuliid taxonomy was initially mostly phenetic, in the early 20th century Diptera workers were already beginning to study the higher classifications of the order through a phylogenetic perspective. As early as 1926 Edwards observed that attempts at phylogenetic interpretation of the Diptera were fraught with problems because specious similarities were being used to group taxa. He noted three major sources of similarity: convergence of an acquired character, convergence through character loss and homologous characters. He then made the further distinction between homologous traits that are the result of “archaic” character states that arose far back in an organism’s lineage and those that are the result of “new” or novel character states. Hennig (1950, 1965, 1966) would later revolutionize the field with his ideas of grouping organisms based upon synapomorphies, shared derived characters as opposed to symplesiomorphies, shared ancestral characters.

Crampton’s (1924) phylogenetic proposals and morphological analysis of “Nematoceran” thoracic structures (Crampton 1925, 1926) have greatly influenced modern dipteran phylogenies, even though he failed to distinguish between synapomorphy and symplesiomorphy. Crampton defined the assemblage now known as the infraorder containing black flies, Culicomorpha, which was later adopted by Edwards and Hennig. Edwards (1926) however was hesitant to include the Thaumaleidae and Simuliidae in this group. Though not officially formalized, Crampton’s and Edwards’ work has exerted profound influence over “Nematoceran” systematics.

Hennig’s phylogenetic analysis (1969, 1973, 1981) technique has been essential in simuliid systematics, and “Nematoceran” systematics as a whole. Hennig (1973) suspected the possible paraphyly of the “Nematocera”, a question still engaging phylogeneticists today. Hennig’s work divided the infraorder Culicomorpha into the superfamilies Culicoidea and Chironomoidea, the latter of which contained Simuliidae, Thaumaleidae, Ceratopogonidae, and Chironomidae. In the same work, Henning recognized the Thaumaleidae as its own family group and placed the Simuliidae, Ceratopogonidae, and Chironomidae in the Chironomidea family group.

Black flies were first described in the literature by Linnaeus in *Systema Naturae* (1758) where he assigned them to the mosquito genus, *Culex*. Nearly half a century later Latreille (1802) recognized black flies as separate from the Culicidae and established the genus *Simulium* to include all related species recognized at that time. Newman's (1834) work would finally establish the family name "Simuliites" which would become the modern, Simuliidae. However, generic level distinctions would not be added until the early 20th century (Surcouf & Gonzalez-Rincones 1911). The years spanning 1914 to 1943 would see Gunther Enderlein propose 50 genera, and in his (1937) treatise he would recognize seven subfamilies and five tribes as follows; Ectemniinae, Cnesiinae, Nevermanniinae (Friesiini, Nevermanniini, and Wilhelmiini), Prosimuliinae, Stegopteriinae, and Simuliinae (Odagmiini and Simuliini). Enderlein's contemporary, Frederick Edwards, would however take a more conservative approach and in his (1931) paper did not acknowledge any supraspecific groupings, but instead recognized *Simulium* and *Parasimulium* as the only two black fly genera, and further recognized five subgenera within *Simulium*: *Austrosimulium*, *Cnephia*, *Gigantodax, Prosimulium*, and *Simulium*.

Smart (1945) reexamined Enderlein's and Edwards' classification and was the first to recognize *Parasimulium* as a subfamily within the Simuliidae, citing the widely separated radial sector vein. Consequently, Smart recognized the subfamily Simuliinae which included five genera**.** The discoveries of two new Nearctic genera, *Gymnopais* Stone (1949) and *Twinnia* Stone and Jamnback (I955) spurred new investigations into simuliid classification. Rubtzov (1955) considered the fanless larvae of *Gynopais* and *Twinnia* to be a distinct subfamily sister to the rest of Simuliidae based upon the author's presupposition that simuliids were derived from a fanless chironomid-like ancestor. With the use of cytological analysis Rothfels and Freeman (1966) revealed the transition between *Prosimulium, Helodon, Twinnia*, and *Gymnpais*. However, cytotaxonomy requires molecular or morphological characters to establish evolutionary polarity and root the tree; thus the authors were not able to determine the evolutionary direction of the sequences from *Prosimulium* to *Gymnpais*, or vice versa. Grenier and Rageau (1960) were the first to offer a classification including these two new genera, while organizing the Simuliidae into the three subfamilies: Parasimuliinae, Prosimuliinae, and Simuliinae**.** The authors subdivided the Prosimuliinae into the two tribes Prosimuliini, which consisted of Prosimulium s. str., and the Gymnopaidiini, which contained *Twinnia* and *Gymnopais*. Grenier and Rageau's Simuliinae contained three tribes; Cnephiini (*Cnephia* sensu Enderlein), Austrosimuliini (*Austrosimulium* and *Gigantodax*) and Simuiiini (*Simulium* s. 1.). Dumbleton (1963) proposed a comparable classification, with the modification of assigning *Gigantodax* and *Cnephia* to the Prosimuliinae instead of the Simuliinae. In his work on the Nearctic Simuliidae, Stone (1965) would condense Grenier and Rageau's (1960) taxa of the Parasimuliinae, Prosimuliini, and Gymnopaidini all into the subfamily Prosimuliinae, leaving the Simuliinae composed of only *Cnephia* and *Simulium* s. 1.

Davies (1965) established the genus *Crozetia* and in his (1974) paper performed a morphological comparison of it with other simuliids. Davies deemed the bristle-like labral fans of first instar *Gymnopais*, *Twinnia* and *Prosimulium* larvae to be ancestral and so considered *Crozetia* to be ancestral based upon the rake-like fans of its larvae**.** Davies proposed that simuliid labral fans evolved via two distinct evolutionary pathways: one being the complete loss of fans seen in *Gymnopais* and *Twinnia*, and the other being the rake-like fans of *Crozetia*, which he stated eventually developed into the filtering fans found in most simuliid larvae. This stand implied that *Gymnopais* and *Twinnia* were ancestors to all other simuliids. Wood (1978) however would assert that the labral fans were a paramount feature shared by all simuliid larvae, and even found in some closely related families (Culicidae, Dixidae, Ptychopteridae, and Tanyderidae). Wood also attributed the absence of fans in select Simuliidae and certain closely related families (Ceratopoginidae, Chaoboridae, Chironomidae, and Thaumaleidae) to evolutionary loss**.** Rubstov (1974) made one of the nascent attempts to elucidate simuliid phylogeny when he integrated the known data into a classification where he recognized 59 genera in the following four subfamilies: Parasimuliinae (*Parasimulium)*, Gymnopaidinae (*Gymnopais* and *Twinnia*), Prosimuliinae (*Gigantodax, Paracnephia, Procnephia, and Prosimulium* s. l.), and lastly the Simuliinae which comprised the remaining genera divided into five tribes: Austrosimuliini, Cnephiini, Eusimuliini, Simuliini, and Wilhelmiini. This is the predominant classification followed by Eastern European simuliid workers.

Wygodzinsky and Coscaron (1973) would be the first to propose potential synapomorphies for possible relationships among pairs of similar Neotropical genera, but it was not until the 1980's that the first rigorous efforts were made in constructing simuliid phylogenies based upon phylogenetic systematics (Hennig 1966). This renaissance in simuliid systematics was largely precipitated by the rediscovery of the genus *Parasimulium* (Wood & Borkent 1982, Borkent & Wood 1986, and Courtney 1986), a genus whose ancient status was long determined morphologically based upon plesiomorphic wing venation, head, and terminalia character states by multiple authors (Smart 1945, Shewell 1958, Grenier & Rageau 1960, Crosskey 1969, Rubstov 1974, Peterson 1977). Wood and Borkent (1982) stated that the *Parasimulium* was sister to the rest of Simuliidae, thus confirming previous phenetic based research. Currie (1988) would be the first to conduct a phylogenetic analysis using all simuliid life stages in the analysis he examined 81 morphological characters, including external and internal structures, polytene chromosomes morphology, and ecological data. Currie's findings supported the ancestral sister-group status of *Parasimulium* to all other known Simuliidae. Currie also provided characters for two monophyletic tribes recognized within the Simuliinae: Prosimuliini Enderlein and Simuliini Newman. Though there were no attempts made at resolving relationships within the Simuliini, Currie proposed the following relationships among the Prosimuliini: Prosimulium + Helodon s. 1. + (Urosimulium + (Levitinia + (Gvmnopais + Twinnia))). Yankovsky (1992) later sought to combine the classifications of Rubstov (1974) and Crosskey (1981, 1988) and proposed a phylogeny containing nine tribes and two subfamilies, Parasimuliinae and Simuliinae**.** Py-Danial (1990) also proposed a morphology based phylogeny which included the analysis of 20 New World black fly genera. He hypothesized a two-subfamily classification, with *Gymnopais* and *Twinnia* constituting the Gymnopaidinae, and the remaining 18 genera placed within the Simuliinae. The absence of labral fans in larvae of the Gymnopaidinae was cited as proof of the ancestral relationship of this subfamily relative to the rest of the Simuliidae. Though Py-Danial deemed the absence of larval labral fans as plesiomorphic, this character was considered the result of a secondary loss by Wood (1978) and Currie (1988). The predominant taxonomy currently followed by North American simuliid workers is that of Adler and Crosskey (2014), which recognizes two subfamilies: Parasimuliinae, comprising the single genus *Parasimulium* and Simuliinae, which includes all other simuliid genera.

Some of the greatest confusion in simuliid systematics has revolved around the Australian Simuliidae, specifically those species presently within the genus *Paracnephia*. This began with Tonnoir (1925) assigning the presently known *Paracnephia umbratora* and *Paracnephia aurantiaca* to the genus *Simulium*, and later Drummond (1931) assigning *Paracnephia tonnoiri* to the genus *Simulium*. Mackerras and Mackerras (1949) reassigned the before mentioned species to the genus *Cnephia*, and would later add to this genus the new species, *Cnephia strenua* (1950). Mackerras and Mackerras (1949) established the *aurantiacum* species group to contain *Cnephia aurantiacum, Cnephia tonnoiri tonnoiri, and Cnephia tonnoiri fuscoflava* followed by the addition of *Cnephia strenua* in their (1950) publication. The proper placement of *Cnephia umbratorum* however has proven a perplexing task as Mackerras and Mackerras (1948) assigned it the *terebans* species group based upon characters of the maxillary palpus, abdomen, and female genitalia. However, Mackerras and Mackerras (1950) would later amend their previous judgment and reassign *Cnephia umbratorum* to the *aurantiacum* species group, citing characters of wing veination and the female claw. The uncertainty revolving around *Cnephia umbratorum* serves to underscore this species' poor fit among its seemingly related taxa. Crosskey & Howard (1997) have reassigned the abovementioned *aurantiacum* and *terebans* group species to the genus *Paracnephia* Rubstov. Though the two subgenera *Paracnephia* and *Procnephia* have been recognized within the *Paracnephia* based upon analysis of the African fauna, all Australian *Paracnephia* species remain unplaced to subgenus (Adler & Crosskey 2014).

Another genus that has caused considerable confusion to simuliid systematists is *Greniera* Doby & David which was first described from France with the type species *Greniera fabri* (Doby & David 1959 a & b). There are presently 13 extant species worldwide within the genus, with six species that occur in North America, and seven that occur in Europe and the Middle East (Adler & Crosskey 2014). In 1962 Davies *et al*. described *Cnephia denaria* Peterson, which was later assigned to the genus *Greniera*. The larvae of all species presently recognized within *Greniera* share relatively large labral fans (as long as or longer than the head capsule) that support an abundance of primary fan rays, usually more than 60. The larvae also have in common patently elongate antennae that well surpass the length of the labral fan stalk. These are characters that can be associated with life in a slow lotic habitat as such modifications presumably increase the larvae's ability to filter. Taxa in the subgenus *Hellichiella* Rivosecchi & Cardinali within the genus *Simulium* have also evolved these characters, though their antennae never attain quite the length of *Greniera.* These similarities of *Greniera* to *Hellichiella* are however thought to be the result of convergence rather than synapomorphy (Adler *et al.* 2004).

In the 1990s the first attempts were made to recreate simuliid phylogenies using molecular techniques (Tang et al 1995, Pruess et al. 1992, Xiong & Kocher 1991), but they failed to answer the unresolved problems in simuliid phylogeny, and suffered from small data sets, poor gene selection, insufficient taxon sampling and questionable sequence alignments. More recently, Smith (2002) resolved relationships among species of the subgenus *Simulium* by using one nuclear and two mitochondrial genes. Moulton (1997, 2000) conducted a molecular analysis of 20 simuliid genera using four nuclear and two mitochondrial loci. Moulton's (1997) work confirmed many expected relationships, but purported some unanticipated groupings, such as the basal placement of *Austrosimulium* Tonnoir within the Simuliini. Though the most comprehensive molecular study of the Simuliidae to date, there were deficiencies in the analysis methods, specifically the 12S sequences were backwards, perhaps why the author restricted the analysis of this marker to a small subset of the taxa, the 12S and 28S were aligned by Clustal with no manual structural alignment, and there was no method for treating the paralogous regions of the EF-1alpha. The problem of paralogous genes has never been addressed in simuliid research, and may be the reason why the data that have been published do not match the morphology or other markers. Sequencing the taxa is the first step in a phylogenetic systematic approach, but the next just as essential and grueling process is analyzing that data, from editing and eliminating paralogous genes or regions in the EF1-alpha, to manually aligning and determining the secondary structure of the 28S and 12S. Thus the field of simuliid phylogenetic systematics is primed for a thorough analysis of the molecular data, and for a comprehensive examination of the molecular and morphological data combined. As keenly stated by Adler et al (2004), an accurate tree of the Simuliidae will require multiple independent data sources, not a single source of data. Establishing a classification based upon evolution through a phylogenetic systematic approach allows for a more complete understanding of what questions to ask concerning simuliid ecology and how to explain the data observed.

One such area of simuliid ecology primed for research is the study of species tolerance values. Because black flies are pests, vectors of disease, and have an aquatic immature stage, knowing their species tolerance values can be a valuable piece of information in their control. Though Hilsenhoff (1987) attempted to score the tolerance values of some simuliid species, knowledge of their tolerance values has largely been left at the generic level. This lack of information has led to presumptions such as associating the proliferation of pest species like the *jenningsi* group in the Northeast with The Clean Water Act of 1972, and a general decrease in farming, citing that black flies as a family are quite pollution intolerant.

Aquatic macroinvertebrates are widely surveyed to assess the ecological health of lotic ecosystems through the use of tolerance values. A tolerance value is s number assigned to a taxon on a scale, usually from 0-10 for best precision, though sometimes a scale of 0-5 is used. Those taxa that are most sensitive to pollution are assigned a relatively low number, and those that are tolerant of pollution are assigned a relatively high number on the scale. After determining a taxon's tolerance value the health of a waterway can be determined through analyzing whether taxa from pollution tolerant or intolerant groups are predominantly collected. One of the first appearances of tolerance values was a system that evolved from German biologist Lauterborn's (1901) concept of the saprobic zone in lotic systems, which he described as the, "zone of processes of decomposition." Soon after, the "new" saprobien system of Kolkwitz & Marsson (1902) sought to use indicator organisms to document differences in samples collected at increasing distances downstream of sewage outflows. Later, Kolkwitz & Marsson (1908 and 1909) published the first extensive list of indicator organisms associated with their four zones of contamination (polysaprobien, mesosaprobien, oligosaprobien, and pristine). The Saprobic system would usher in new research and investigations in pollution tolerance values, both in the USA and worldwide. Chutter (1972) would assign tolerance values to South African macroinvertebrates, citing best professional judgment as the criteria for establishing the values. Soon after, research was conducted in Great Britain by the Biological Monitoring Working Party (BMWP) (Armitage et al., 1983), which gave family level tolerance values to indigenous macroinvertebrate. Hilsenhoff (1977, 1987) however would pioneer the cost and time effective method of utilizing aquatic arthropods alone as indicator organisms. Hilsenhoff's still widely used set of tolerance values were developed with regards to a gradient of organic pollution. Lenat (1993) added to Hilsenhoff's method, and established tolerance values based upon a more expansive list of anthropogenic factors.

The New Jersey Department of Environmental Protection currently conducts biological assessments of NJ waterways through its Ambient Biomonitoring Network which utilizes macroinvertebrate assemblage data. The NJDEP regularly samples and determines the quality of NJ streams throughout all five of the state's physiographic provinces using macroinvertebrate data. This data is entered into an index that is adapted specifically for use in NJ, but based in the ideas of Chutter's Biotic Index (1972) and Hilsenhoff's Biotic Index (1987). Up until 2007, the NJ Impairment Score (NJIS) was used to asses steam quality, but in more recent years three different indices have been used to assess the biological health of NJ streams over different land use areas: High Gradient Macroinvertebrate Index (HGMI), Pinelands Macroinvertebrate Index (PMI), and the Coastal Plain Macroinvertebrate Index (CPMI) (Poretti *et al.* 2007). The latter is based upon a 30 point as opposed to a 100 point scale. The NJDEP also calculates the Family Biotic Index for each site.

Tolerance values should reflect the paramount aspects of the species relationship with its environment, consistently rank different species in terms of their tolerance for a specific stressor, and be functional in analyzing taxa occurrence data to infer the environmental conditions at a site. Because the relationships of taxa to their environments differ greatly in their functional forms, it is impractical to completely characterize the relationship with a singular value. However, discrete tolerance values have demonstrated utility as a tool in biological monitoring, especially where a more comprehensive representation of the species' relationship with its environment decrease the clarity of the data.

Weighted averaging, a direct method of tolerance value calculation, has a long history of use in ecology for estimating species level tolerance values (ter Braak and Looman, 1986, Hilsenhof 1987). In calculating tolerance values the goal is to compare different taxa relative to one another, thus the central tendency of the organism is of less interest than the relationship among taxa. All weighted average tolerance values within a known data set are calculated using the same set of environmental data, thus bias arising from a non-uniform distribution of data will be equivalent for all taxa. Weighted averaging and all its incarnations function using the same basic rules. The tolerance value, u_{wa}, for species j is computed by calculating the average of the ecological variable being used at the sites in which the species is detected as follows.

$$u_{wa\,j} = \sum_{i=1}^{N} Y_{ij} x_i \Big/ \sum_{i=1}^{N} Y_{ij}$$

In the above equation, N is the number of sites sampled and x_i is the value of the ecological variable of study at the site. In presence/absence analyses, Y_{ij} is equal to 0 when species j is absent, and 1 when species j is present.

One hurdle in establishing black fly pollution tolerance values has been the presence of cryptic species, also known as sibling species, within the family that cannot be identified by morphology and require species diagnosis through cytological analysis. This problem was noted by Hilesenhoff (1987) who stated, "…discovery that *Simulium vittatum* is really two genetically distinct species (Rothfels and Featherston 1981) with differing ecological requirements (Adler and Kim 1984) altered my recommendation for dealing with these sibling species." Thus, all work in establishing species level pollution tolerance values for the Simuliidae has been stymied by lack of cytological analysis to confirm species identity. Consequently, the generic level values of roughly 3 and 6 (Merritt *et al*. 2008) that most authors posit for two of the most abundant North American genera, *Prosimulium* and *Simulium* respectively, fail to illustrate the vast variation in pollution tolerance values of species within these genera. Another matter necessary to address in establishing pollution tolerance values for black flies is the collection process. The sampling method necessary for cytological analysis involves removing any excess water from the larvae and immediately plunging the larvae into an approximate one larva to one or more ml ratio of Carnoy's solution (3 parts 95%-100% ethanol: 1 part glacial acetic acid) to ensure the chromosomes are fixed (Adler *et al* 2004). Therefore, black flies need to be sampled by hand and not using a more quantitative stream sampler or kick net sample, thus making the calculation of the tolerance values best completed with a presence/absence analysis.

By calculating species level pollution tolerance values confirmed by cytological analysis with a molecular and morphological based phylogeny, questions can be answered about how the Simuliidae have evolved to fit different ecological niches. Furthermore, an understanding of the pollution tolerance values of the *jenningsi* group species may be useful in predicting their spread. Data such as these can have great utility in their application to black fly control.

Literature Cited

Adler, P.H., & Crosskey R. W. (2014). World Blackflies (Diptera: Simuliidae) A Comprehensive Revision of the Taxonomic and Geographical Inventory [2014]. Available at: http://www.clemson.edu/cafls/departments/esps/biomia/pdfs/blackflyinventory.pdf Accessed 16 March 2014.

Adler, P.H., Currie, D. C., & Wood D. M. (2004). The Black Flies (Simuliidae) of North America. Cornell University Press in Association with the Royal Ontario Museum.

Adler, P. H., and Kim, K. C. (1984). Ecological characterization of two sibling species, IIlL-l and IS-7, in the Simulium vittatum complex (Diptera: Simuliidae). *Canadian J. Zool.*, 62, 1308-1315.

Amrine, J. W. (1982). The New River connection to the black fly problem in southern West Virginia. West Virginia University Agricultural and Forestry Experiment Station Bulletin, 678, 1-30

Armitage, P.D., Moss, D., & Wright, J.F. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Res.*, 17, 333–347.

Baldwin, W. F., West, A. S., & Gomery, J. (1975). Dispersal patterns of black flies (Diptera: Simuliidae) tagged with P. Canadian Entomologist 107:113-118.

Borkent, A. and D. M. Wood (1986). The first and second larval instars and the egg of Parasimulium stonei Peterson. *Proc. Entomol. Soc. Wash*., 88, 287-296.

Chutter, F.M. (1972). An empirical biotic index of the quality of water in South African streams and rivers. *Water Res.*, 6, 19–30.

Courtney, G. W. (1986). Discovery of the immatures stages of Parasimulium crosskeyi

Peterson (Diptera: Simuliidae), with a discussion of a unique black fly habitat. Proc. *Entomol. Soc. Wash*., 88, 280-286.

Crampton, G. C. (1924). Remarks on the phylogeny and interrelationships of nematocerous Diptera. Psyche (Camb.) 31, 238-242.

Crampton, G. C. (1925). A phylogenetic study of the thoracic sclerites of the non-tipuloid nematocerous Diptera. *Ann. Ent. Soc. Am*. l8: 49-74.

Crampton, G. C. (1926). A phylogenetic study of the thoracic sclerites of the psychodoid Diptera, with remarks on the interrelationships of the Nematocera. *Ent. News,* 37, 33-38.

Crans, W. J., & McCuiston, L. G. (1970). The current status of black fly investigations in New Jersey. *Proceedings of the Annual Meeting of the New Jersey Mosquito Extermination Association*, 57, 103-106.

Crosskey, R. W. (1969). A re-classification of the Simuliidae (Diptera) of Africa and its islands. *Bull. Brit. Mus. Nat. Hist. (Entomology)*. Supplement 14. 195 pp.

Crosskey, R. W. (1981). Simuliid taxonomy - the contemporary scene.. 1n Laird, M. (Ed.), *Blackflies: the future for biological methods in integrated control* (pp. 3-18). Academic Press.

Crosskey, R. W. (1988). An annotated checklist of the world's black flies (Diptera: Simuliidae). In Kim, K. C., & R. W. Merrit (Eds.), Black flies: Ecology, Population Management and Annotated World List Penn (pp. 425-520). State Press.

Crosskey, R. W., & Howard, T.R. (1997). *A new taxonomic and geographical inventory of world blackflies (Diptera: Simuliidae).* Natural History Museum, London, 144 pp.

Currie, D. C. (1988). *Phylogeny of primitive Simuliidae (Insecta: Diptera: Culicomorpha)*. Ph.D. dissertation. University of Alberta, Edmonton, Canada.

Davies, D. M. (1950). A study of the black fly population of a stream in Algoquin Park, Ontario. *Transaction of the Royal Canadian Institute* 28:121-159.

Davies, D. M., Peterson, B. V., & Wood, D. M. (1962). The Black Flies (Diptera: Simuliidae) of Ontario. Part 1. Adult identification and distribution with descriptions of six new species. *Proceedings of the Entomological Society of Ontario,* 92, 70-154.

Davies, L. (1965). The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island black-fly. *Proc. Linn. Soc. Lond.,* 176, 159-180.

Davies, L. (1974). Evolution of larval head-fans in Simuliidae (Diptera) as inferred from the structure and biology from *Crozetia crozetensis* (Womersley) compared with other genera. *Zoological Journal of the Linnean Society of London* 55,193-224 + 2 plates.

Doby J. M., & David, F. (1959a). Description de Greniera fabri (Diptera-Nematocera Simuliidae) en Provence de l'ouest de la France (Atlantic Loire). Bulletin de la Société de pathologie exotique, 52, 669-78.

Doby J. M., & David, F. (1959b). Greniera, genre nouveau de Simuliidé (Diptères-Nématocères) Discussion de la position systématique. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, 249, 763-768.

Drummond, F.H.N. (1931). West Australian Simuliidae. *Journal of the Royal Society of Western Australia*, 18, 1–12.

Dumbleton, L. J. (1963). The classification and distribution of the Simuliidae (Diptera) with particular reference to the genus Austrosimulium. N. Z. J. Sci. 6, 320-357.

Edwards, F. W. (1926). The phylogeny of nematocerous Diptera: a critical review of some recent suggestions. Verh. III int. Kongr. Ent. (Zurich) 1, 111- 130.
Edwards, F. W. (1931). Simuliidae. |a Diptera of Patagonia and South Chile, Part 2, 121-154.
Enderlein, G. (1937). Uber bemerkenswerte Unkenntnisse von der Entwicklung zweier einheimischer Simuliiden Gattungen (Diptera). Markische Tierwelt 3,68-70.
Garms, R., & Walsh, J. F. (1988). The migration and dispersal of black flies: Simulium damnosum s. 1., the main vector of human onchocerciasis. Pp. 201-214. In K.C. Kim, & R. W. Merrit (Eds.), *Blackflies. Ecology, population management, and annotated world list* (pp. 201–214). Pennsylvania State University, University Park..
Gray, E. W., Adler, P. H., & Noblet, R. (1996). Economic impact of black flies (Diptera: Simuliidae) in South Carolina and development of a localized suppression program. *Journal of the American Mosquito Control Association*, 12, 676-678.
Grenier, P., & Rageau, J. (1960). Simulies (Dipt., Simuliidae) di Tahiti. Remarques sur la classification des Simuliidae. *Bull, de la. Soc. de Path. Exot*., 53, 727-742.
Guyette, J. (1999). Battling black flies for profit. *Pest Control*, 67, 55-56.
Hannay, C. L., & Bond, E. F. (1971). Blackfly wing surface. *Canadian Journal of Zoology*, 495, 543-549.
Hennig, W. (1950). *Die Larvenformen der Dipteren. Eine Ubersicht uber die bisher bekannten Jugendstadien der zweiflugeligen Insekten*. 2. Tell. Akademie-Verlag, Berlin.
Hennig, W. (1965). Phylogenetic systematics. *A. Rev. Ent*., 10, 97-116.
Hennig, W. (1966). Phylogenetic systematics. *Translated by* D. D. Davis and R. Zanger. University of Illinois Press, Urbana, I11.
Hennig, W. (1969). Die Stammesgeschichte der Insekten. Senckenbergischen Naturforschenden Gesellschaft. W. Kramer, Frankfurt am Main. Senkenberg-Buch 49.
Hennig, W. (1973). Ordnung Diptera (Zweiflugler). *Handb. Zool*., 4 (2), 1-337 .
Hennig, W. (1981). *Insect phylogeny*. Translated and edited by A. C. Pont (revisionary notes by D. Schlee). John Wiley & Sons, New York.
Hilsenhoff, W. L. (1977). Use of arthropods to evaluate water quality of streams. *Technical Bulletin Number 100*, Department of Natural Resources, Madison, WI.
Hilsenhoff, W. L. (1987). An improved biotic index of organic stream pollution. The *Great Lakes Entomologist* 20, 31–37.
Kolkwitz, R., & Marsson, M. (1902). Principles for the biological assessment of water after its flora and fauna. Communications of the royal testing laboratory for water supply and sanitation, 1, 33-72 (Berlin-Dahlem)
Kolkwitz, R., & Marsson, M. (1908). O¨kologie der pflanzlichen Saprobien. *Ber Deutsch Bot Ges*, 26a, 505—519
Kolkwitz, R., & Marsson, M. (1909). Ecology of animal Saprobien. Contributions to the theory of biological water evaluation. *In: International Review of Hydrobiology and Hydrography whole* , Volume 2, pp. 126-152. Lenat, DR. (1993) A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society, 12, 279–290.
Latreille, P. A. (1802). *Histoire naturelle, generale et particuliere des crustaces et des insects. 3. Familles naturelles des genres*. F. Dufart, Paris. 467 pp.
Lauterborn, R. (1901). Die sapropelische Lebewelt. *Zool Anz*, 24, 50—55.

Lenat, D. R. (1993). A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of the North American Benthological Society*, 12, 279–290.

Linnaeus, C. (1758). *Systema naturae per regna tria naturae*. Edition 10, Volume 1, Holmiae Stockholm. 824 pp.

Lok, J. B., Cupp, E. W., Bernardo, M. J., & Pollack, R. J. (1983). Further studies on the development of Onchocerca spp. (Nematoda: Filarioidea) in Nearctic black flies (Diptera: Simuliidae). *American Journal of Tropical Medicine and Hygiene*, 32, 1298-1305.

Mackerras, I. M., & Mackerras, M. J. (1949). Revisional notes on Australasian Simuliidae (Diptera). *Proceedings of the Linnaean Society of New South Wales*, 73, 372–405.

Mackerras, M. J., & Mackerras, I. M. (1950). Notes on Australasian Simuliidae (Diptera). II. *Proceedings of the Linean Society of New South Wales*, 75, 167-187.

Merritt, R. W., Cummins, K.W., & Berg, M.B. (2008). *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Publishing Company.

Moulton, J. K. (1997). *Molecular Systematics of the Simuliidae (Diptera: Culicomorpha)*. PhD Dissertation, University of Arizona, Tuscon, Arizona.

Moulton, J. K. (2000). Molecular sequence data resolves basal divergences within Simuliidae (Diptera). *Systematic Entomology*, 25, 95-113.

Moulton, J. K. (2002). Can the current molecular arsenal adequately track rapid divergence events within Simuliidae (Diptera)? *Molecular Phylogenetics and Evolution*, 27, 45-57.

Newman, E. (1834). Attempted division of British insects into natural orders. *Entomological Magazine*, 2, 379-431.

Oosterbroek, P., & Courtney, G. W. (1995). Phylogeny of the nematocerous families of Diptera O'connor, P. F., Hazelrigg, J. E., Shaw, M. W. & Madon, M. B. (2001). Black fly control program at Greater Los Angeles County Vector Control District. *Proceedings and Papers of the Annual Conference of the Mosquito and Vector Control Association of California*, 69, 26-31.

Peterson, B. V. (1977). A synopsis of the genus Parasimulium Malloch (Diptera: Simuiiidae), with descriptions of one new subgenus and two new species. Proc. Entomol. Soc. Wash. 79: 96-106.

Poretti, V. Bryson, D. Miller, T., & Honachefsky, W. (2007). Standard Operating Procedures; Ambient Biological Monitoring Using Benthic Macroinvertebrates, Field, Lab and Assessment Methods. Available at: http://www.state.nj.us/dep/wms/bfbm/download/AMNET_SOP.pdf Accessed on 8 April 2014.

Pruess, K. P., X. Zhu, & Powers, T. O. (1992). Mitochondrial transfer RNA genes in a black fly. *Simulium vittatum* (Diptera: Simuiiidae), indicate long divergence from mosquito (Diptera: Culicidae) and fruit fly (Diptera: Drosophilidae). *J. Med. Entomol.*, 29, 644-651.

Py-Daniel, V. (1990). *Revisao da Tribo Prosimuliini seg. Crosskey (Diptera, Culicomorpha) nas regioes Zoogeographicas Neartica E Neotropical (larvas and pupas).* Ph. D. Dissertation. Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil.

Rothfels, K. H., & Featherston, D. (1981). The population structure of Simulium vittatum (Zett.): the IIIL-l and IS-7 sibling species. *Canadian J. Zool*., 59, 1857-1883.

Rothfels, K. H., & Freeman, D. M. (1966). The salivary gland chromosomes of three North America species of *Twinnia* (Diptera: Simuliidae). *Canadian Journal of Zoology*, 44, 937-945.

Rubtzov, I. A. (1955). Peculiar new species of black flies (Diptera, Simuliidae) from Eastern Siberia. *Entomol. Obozr*, 34, 323-339.

Rubtzov, 1. A. (1974). *Evolution, phylogeny, and classification of the family Simuliidae (Diptera).* [translated from Russian]. British Library Lending Division, Wetherby. 83 pp.

Smart, J. (1945). The classification of the Simuliidae (Diptera). *Trans. Royal. Soc. London,* 95, 463-532.

Shewell, G. E. (1958). Classification and distribution of arctic and subarctic Simuliidae. *Proc. 10th Int. Cong. Entomol.* (Montreal, 1956), 1, 635-643.

Smith, M. L. (2002). *Molecular systematics of the Neartctic black fly subgenus Simulium s.s.(Diptera: Simuliidae: Simulium s.l.).* M.S. thesis. University of Toronto, Toronto. 172 pp.

Stone, A. (1949). A new genus of Simuliidae from Alaska (Diptera). Proceedings of the *Entomological Society of Washington*, 51, 260-267.

Stone, A. (1965). Family Simuliidae. In Stone et al. (Eds.), A catalogue of the Diptera of America north of Mexico. *Agriculture Handbook No. 276*. United States Department of Agriculture,

Stone, A., & Jamnback, H. (1955). The black flies of New York State (Diptera: Simuliidae). New York State Bulletin, 349, 1-144.

Surcouf. J. M. R. & Gonzalez-Rincones, R. (1911). *Essai sur les dipteres vulnerants du Venezuela: materiaux pour server a l'etude des dipteres piqueurs et suceurs de sang de l'Amerique intertropicale.* Part 1. A. Maloine, Paris, 320 pp.

Tang, J., L. Toe, Back, C., Zimmerman, P. A., Pruess, K., & Unnasch, T. R. (1995). The *Simulium damnosum* species complex: phylogenetic analysis and molecular identification based upon mitochondrially encoded gene sequences. *Ins. Mol. Biol*., 4, 79-88.

Ter Braak, C. J. F., & Looman, C. W. N. (1986). Weighted averaging, logistic regression and the Gaussian response model. Vegetatio 65:3–11.

Tonnoir, A. L. (1925). Australasian Simuliidae. Bulletin of Entomological Research, 15, 213–25

Vachon, M. (1993). *Onchocerciasis in Chiapas, Mexico*. Geographical Review, Vol. 83, No. 2 (Apr., 1993), pp. 141-149.

Wood, D. M. (1978). Taxonomy of the Nearctic species of Twinnia and Gvmnopais (Diptera: Simuliidae) and a discussion of the ancestry of the Simuliidae. *Can. Entomol*, 110, 1297-1337.

Wood, D. M., & Borkent, A. (1982). Description of the female of Parasimulium crosskeyi Peterson (Diptera: Simuliidae) and a discussion of the phylogenetic position of the genus. *Memoirs of the Entomological Society of Washington*, 10, 193-210.

Wygodzinsky, P. & Coscaron, S. (1973). A review of the Mesoamerican and South American black flies of the tribe Prosimuliini (Simuiiinae, Simuliidae). *Bull. Amer. Mus. Nat. Hist.*, 151, 129-200.

Xiong, B., & Kocher, T. D. (1991). Comparison of mitochondrial sequences of seven morphospecies of black flies (Diptera: Simuliidae). *Genome*, 34, 306-311.

Yankovsky, A. V. (1992). A review of the system of blackflies (Diptera, Simuliidae) on the suprageneric level. *Parazitologichescky Sbornik,* 37, 203-217 [in Russian, with English summary].

The Phylogeny of Holarctic Simuliidae (Diptera: Simuliidae), with the Description of *Adlerella* New Genus

ABSTRACT

A new genus of Simuliidae, *Adlerella* Carle, is established from morphological data. Diagnostic characters for the larval, pupal and adult stages are provided. A comparison with *Greniera* is given because convergent traits shared by both genera in the larval stage can cause confusion in their classification. Autapomorphies for *Greniera* are also provided. Phylogenetic placement of *Adlerella* within the Northern Hemisphere Simuliini is determined. *Adlerella*'s absence of parameral spines and of spiniform macrotrichia on the costal vein suggests a basal placement within the Simuliini.

Key words: Simuliidae, *Adlerella,* convergence, synapomorphy, taxonomy, phylogeny.

Introduction

Distinguishing whether morphological characters are the result of shared ancestry or convergent evolution is a dilemma in systematics, and the evolution and systematics of black flies (Diptera: Simuliidae) are no exception. As early as 1926, Edwards observed that attempts at phylogenetic interpretation of dipteran morphology were fraught with problems because specious similarities were being used to group taxa. In his treatise, Edwards (1926) cited three main sources of similarity: convergence of an acquired character, convergence through character loss, and homologous characters. The later would portend the modern concept of synapomorphy (Hennig 1969).

The genus *Greniera* Doby & David was first described from specimens collected in France, with the type species designated as *Greniera fabri* (Doby & David 1959). The genus *Greniera* consists of 13 extant species worldwide, with six species that occur in North America, and seven that occur in Europe and the Middle East (Adler & Crosskey 2014). In 1962 Davies *et al*. described *Cnephia denaria* Peterson, and it was later assigned to the genus *Greniera*. The larvae of these taxa all share a relatively large labral fan (as long as or longer than the head capsule) supporting many primary fan rays, typically numbering more than 60. They also share markedly elongate antennae that far surpass the length of the labral fan stalk. These characters are apparently associated with life in slow-flowing water, as it probably increases their ability to filter when feeding is not aided by a fast current carrying an ample food supply. Taxa in the subgenus *Hellichiella* Rivosecchi & Cardinali have also evolved these characteristics, though their antennae never are quite as long as those of *Greniera*. These similarities, however, are thought to be the result of convergence rather than representing a synapomorphy (Adler *et al.* 2004).

A stable classification requires a phylogenetic analysis to differentiate among synapomorphy, symplesiomorphy and homoplasy. Within Diptera, the work of Wood and Bokent (1989) was a notable step in the delineation of synapomorphies and determination of relationships of families within “Nematocera”. Currie (1988) was the first to conduct a phylogenetic analysis using all simuliid life stages, and in the analysis he examined 81 morphological characters, including external and internal structures, polytene chromosome morphology, and ecological data. Currie’s (1988) findings verified the ancestral sister-group status of *Parasimulium* to all other known Simuliidae, and provided characters for two monophyletic tribes recognized within the Simuliinae: Prosimuliini Enderlein and Simuliini Newman. Adler *et al.* (2004) expanded upon this work and conducted an extensive morphological analysis of North American Simuliidae. The genus *Greniera*, however, remained ambiguously defined based on homoplastic characters, with Adler *et al.* (2004) noting that, "The phylogenetic relationships of and within the genus require deeper study."

Herein I describe a new genus of Simuliidae from the Holarctic region as well as illustrate its phylogenetic relationship relative to the rest of the Holarctic Simuliidae. I also provide autapomorphies for the genus *Greniera*.

Materials and Methods

For morphological analysis a comprehensive review of the literature was performed for all specimens. Additionally, physical specimens of adult, larval and pupal *Simulium, Cnephia, Ectemnia, Prosimulium,* and *Stegopterna* were analyzed. New characters as well as select ones from Adler *et al.* (2004) were used. A morphology matrix of 127 apparently minimally correlated characters from four life stages, and select behavioral characters was created for the genera analyzed (Table 1). Particular attention was given to specimens from the genus *Greniera*, including a translation of the original French description of the genus. Outgroup selection was determined by both morphological (Hennig 1973, Wood and Borkent 1989, Oosterbroek and Courtney 1995) and molecular evidence (Pawlowski et al. 1996). Pawlowski et al. (1996) demonstrated the sister group relationship of Thaumaleidae to Simuliidae, using 28S rDNA. Nineteen taxa were analyzed including: 17 simuliida genera, and the two outgroups, *Thaumalea* and *Cardiocladius* (Figure 1). All specimens were from the Northern Hemisphere, except for *Crozetia* from the Crozet Islands in the Indian Ocean, which has been posited as a basal simuliid by Davies (1965b & 1974), but thought to be more derived by Wood (1978). Craig *et al.* (2003) however illustrated the sister group relationship of *Crozetia* to the extant Simuliini. A parsimony analysis was performed using PAUP version 4.0 (Swofford 1999). The bootstrap method with heuristic search was performed with 10,000 replicates. All morphological characters received equal weight. The bootstrap 50% majority-rule was utilized and the most likely consensus tree was then generated.

Results

Seventeen character states support the monophyly of the family Simuliidae, eight character states support the monophyly of the subfamily Parasimuliinae, and eleven character states support the monophyly of the subfamily Simuliinae (Table 1 and 2). The tribe Prosimuliini was supported by five character states: pupal abdominal segments IV and V bearing pleural plates, larval antennae with medial and basal articles unpigmented contrasting with blackish-brown apical article, larval hypostomal paralateral teeth absent, first-instar larval anteromedian palatal brush composed of scoop-shaped fringed hairs, and the first-instar larval maxillary papal sensilla positioned subapically in a linear pattern. The tribe Simuliini was supported by three character states: the vestigial fork of the radial sector vein, genital fork of the female bearing a dorsal apodeme, and the paramere of the male with a straplike connection between the paramere and the ventral plate that arises subapically on the basal arm of the ventral plate. Two often-overlooked apomorphies of the genus *Greniera* are the larval distal antennal article with characteristic dark cuticular striations, and the rudimentary pupal cocoon that consists of a few silk threads entwined in the grapnel setae of segment IX. The new genus *Adlerella* is supported by the apomorphies of the medial antennal article bearing minute spicules visible when slide mounted, and the antenna greatly surpassing the length of the labral fan stalk by about 2/3 the length of the medial article. A full list of the characters used in the morphology matrix and their distribution can be found in Tables 1 and 2.

As seen in the consensus tree, the genus *Greniera* appears paraphyletic, with the type *Greniera fabri* grouping more closely with the more derived Simuliini than with *Adlerella denaria,* (formerly *Greniera denaria* or *Cnephia denaria*). The group including *Greniera, Ectemnia, Cnephia, Metacnephia* and *Simulium* is supported by a bootstrap value of 69. *Crozetia, Stegopterna, Tlalocomyia,* and *Adlerella* conversely are basal to the higher Simuliini (Figure 1). A morphological comparison is provided (Table 3) to illustrate the relationships between *Adlerella* and other related genera. Genera in the table are arranged from left to right in phylogenetic order. *Adlerella* lacks dimorphic setae on the costal vein, similar to *Prosimulium*. However, *Adlerella* differs from *Prosimulium* in that it possesses a distally forked R vein instead of an R vein which forks more basally. In addition, *Adlerella* also possesses a pedisulcus and calcipala, unlike *Prosimulium*.

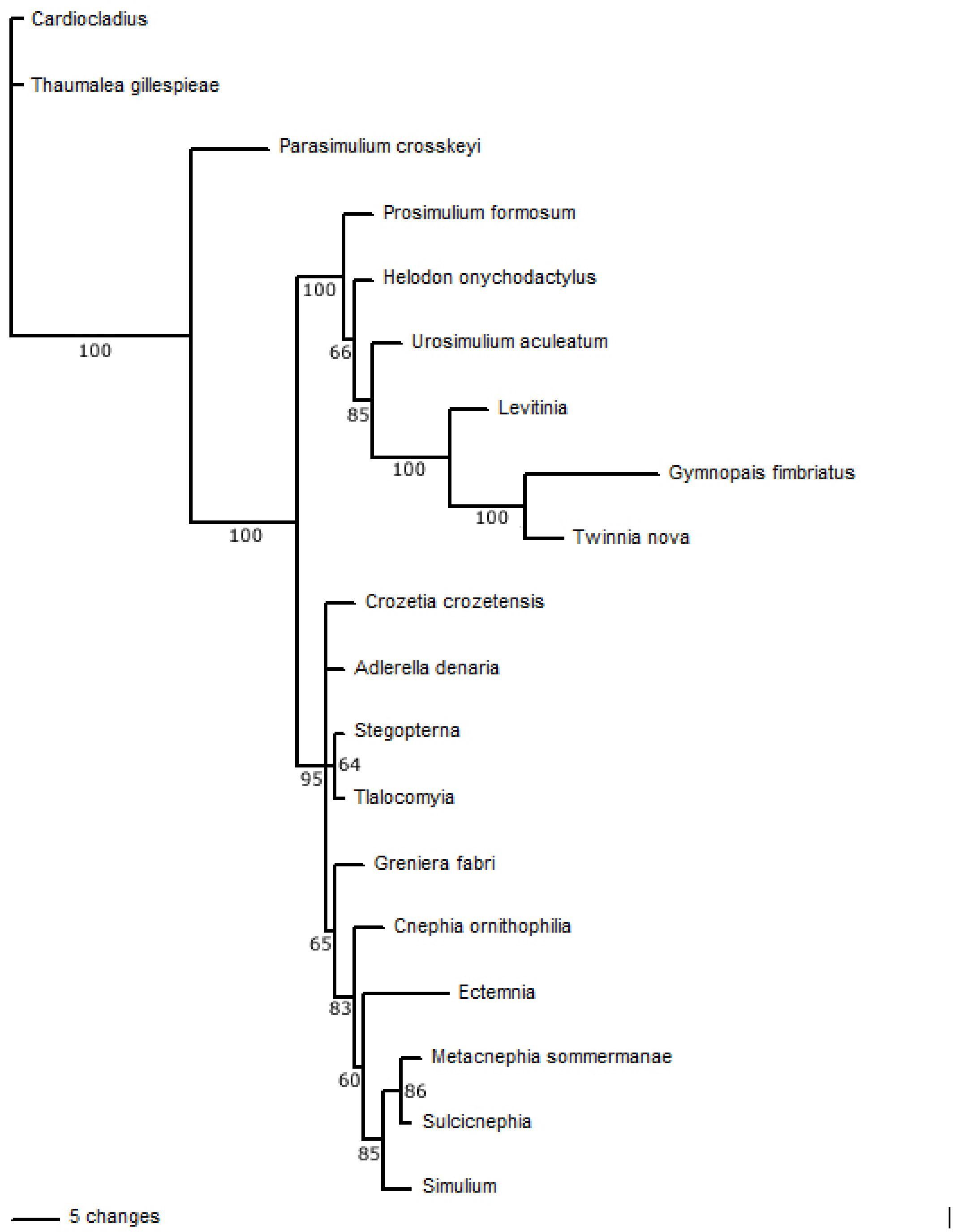

FIGURE 1. Phylogram showing bootstrap values for Holarctic simuliid genera and *Crozetia.*

Adlerella Carle D.M. new genus

Type-species: *Cnephia denaria* Peterson.

Possible included species: *G. brachiata* Rubstov and *G. ivanovae* Ivashchenko

Etymology. *Adlerella* - In recognition of Dr. Peter H. Adler's contributions to the knowledge of the Simuliidae. Much as the genus *Greniera* was named after the prominent European Simuliidae researcher Paul Grenier it seems appropriate to name this new genus after the renowned North American Simuliidae researcher Peter Adler.

Female. General color of body dark. Length of body, 3.0 mm. Length of wing, 3.5 mm.

Head with ventral and posterior surfaces covered in dense long hair, medial and lateral borders of eyes bearing a row of coarse, upright, dark hair. Frons widening above, with narrowest part approximately half as wide as long, reasonably covered with hair laying flat against body. Clypeal width greater than length, moderately coated in both quasi-erect and erect hair. Antennae of 10 flagellomeres, pubescent, and covered in coarse hair; first flagellomere longest and all distal flagellomeres gently tapered. Palpus with third segment enlarged and heavily cloaked in hairs. Edges of mandible bearing many serrations; galea with large, recurved teeth.

Pronotum and prescutum with somewhat long hair. Scutum covered in dense, fine, short, recurved, hair; lateral margins bearing longer hairs; posterior area with coarse, long, hair intermixed among pale hair. Scutellum clothed in dense mixture of pale shorter hair and long, coarse hair. Postscutellum glabrous and shiny. Wing basal cell present; costa with spiniform macrotrichia only, not intermingled with dark spinules; subcosta glabrous dorsally, but hirsute ventrally; radial sector except for a small apical section bare dorsally, and ventral surface strongly hirsute, radial sector fork short and indistinct, CuA2 distinctly sigmoidal. Legs clothed in short hair mixed with a few longer coarse hairs covering hind tibial margins. Calcipala diminutive; pedisulcus absent or represented by weak wrinkles; hind basitarsus roughly five times as long as wide; claw thin and elongate, gently curved, bearing a sub-basal, thumb-like projection that is approximately half as long as claw, and a smaller basal projection, giving claw a trifid appearance.

Dorsum of abdominal tergites all somewhat covered in short hair, last two segments with a few long coarse hairs on posterior margins; remainder of abdomen thinly clothed with short hair, sternite 1 small with rounded posterolateral margins, and posterior and anterior margins straight; sternites 7 and 8 highly sclerotized. Basal scale fringe long laterally, but short dorsally. Anal lobe sparsely setose, L-shaped restricted dorsally, widening ventrally near level of ventral border of cercus, with a small anteroventral protuberance, posteroventral region produced only a small distance beneath cercus. Cercus broad, roughly twice as wide as long, and heavily hirsute. Flaps of ovipositor situated near each other, short, with hind and medial margins straight. Genital fork Y-shaped, with a thickening at bifurcation of arms; stem slender and highly sclerotized; arms broader, but less sclerotized, and apical region of each arm rather curved with a short uneven internal sclerotized crest.

Male. Darker blackish-brown overall body color compared with the female.

Posterior and ventral surfaces of head, frons and clypeus thickly clothed in long to intermediately long hair. Antenna covered in hair; first flagellomere thin, notably more elongate than other flagellomeres. Palpus covered in hair.

Thorax: anterior one-third of scutum with fairly long fine quasi-upright to upright hair; central one-third bearing short recurved, hair; posterior one-third of scutum along with scutellum compactly covered in long bristly upright hair; postscutellum glabrous, and glossy, bearing a pleural tuft. Wing vein hair: subcosta dorsum and ventral surfaces without hair. Legs with hair; posterior borders of femora and tibiae bearing elongate hair; length of hind basitarsus approximately four times width.

Dorsum of abdominal segments somewhat burnished, strongly covered in somewhat long hair. Fringe of basal scale with long lateral hairs, but short dorsal ones. Gonocoxite large, conical, quadrate, distal half lightly hirsute; attached to inner ventral margin a thin triad plate with a long thin strongly sclerotized, dull-tipped bar that projects posteroventrally and ends near arms of median sclerite. Gonstylus four-fifths length of gonocoxite, appearing triangular in cross-section, basally broad, and at roughly distal one-third of its length rapidly narrowed distally into an elongate thin tip supporting a lone apical spine. Paramere and aedeagal membrane without spines. Ventral plate bearing a short, sharp, ventrally recurved lip; basal arms short and bluntly pointed. Median sclerite in shape of Y, with stem somewhat broad, and with short arms, each supporting an apically sclerotized crest.

Pupa. Length approximately 4.0 mm. Respiratory organ comprised of three major trunks; filaments radiating in all directions resulting in a bush-like appearance. Tergites 2-5 with delicate, pale hooks lining their posterior margins; tergites 5-8 sometimes with an anterior row of fine, faint hooks on some segments. Terminal spines distinct, only weakly recurved. Cocoon an amorphous slackly woven sac that partially covers pupal body.

Larva. Fusiform**,** abdomen gradually expanding at segment 5. Postgenal cleft small narrow and rounded, shaped like an inverted V. Antenna long, exceeding length of labral fan by approximately 2/3 the length of the medial article, medial article bearing minute spicules, apical article dark and shorter than basal 2 articles combined. First posterolateral head spot pale or indistinct, second posterolateral head spot strongly curved and anteriorly directed. Lateral hypostomal teeth minute and arising from large parallel-sided lateral lobes which are one half or more times as long as width at base and much longer than median tooth.

Discussion

The elongated antennae of *Adlerella* are most likely correlated with the enlargement of the labral fans which are adapted for filter feeding in low-flow environments. Though the larvae of both genera share these character states, each has characters unique to both the larval and adult life stages. In the original French description Doby and David (1959) described the costal vein of *Greniera* as having a mixture of the classic setiform macrotrichia and smaller bristles that are not quite as well developed as the spines found in most other higher Simuliini, whereas *Adlerella* has only setiform macrotrichia.

Adults of *Adlerella* are also characterized by a lack of parameral spines, a likely plesiomorphic condition shared with the Prosimuliini and *Tlalocomyia*, whereas *Greniera* possesses a few weakly sclerotized spines, as stated by Doby & David (1959) "Paramères avec 5 à 7 crochets tres peu chitinisés." The latter state is perhaps an intermediate form between Prosimuliini and lower Simuliini as compared to the well-developed spines found in the higher Simuliini. The gonostylus of *Adlerella* is also broad and slightly curved, whereas that of *Greniera* is thin and almost straight. In addition, these characters can be used to distinguish these genera: *Greniera* possesses a large gonocoxite which is more than twice the widest width of the gonostylus, whereas the gonocoxite of *Adlerella* is comparably smaller, less than twice the widest width of the gonostylus, similar to that of *Prosimulium.*

The pupal lateral abdominal hooks on segment 9 of *Adlerella* are rather simple, whereas those of *Greniera* are anchor or grapnel like, similar to those of *Metacnephia*. The pupa of *Adlerella* also possesses a definite cocoon that loosely covers the body, similar to that of *Tlalocomyia* and other lower Simuliini, whereas *Greniera* appears to lack a typical simuliid cocoon, as indicated by Doby & David (1959), "absence totale de cocoon." However Doby and David (1959) also state concerning *Greniera*:

> "La nymphe est fixée au support par quelques fils pris dans les crochets latéraux contournés du 9th segment abdominal, parfois par quelques fils en écharpe en travers du thorax. Elle est accrochée toujours dans des endroits pratiquement sans courant (fentes dans les pierres ouface inférieure de celles-ci, entre les couches de feuilles mortes, etc.)."

Which translates to: the pupa is affixed to the substrate by silk threads held in the grapnel shaped lateral hooks of segment IX, sometimes by some strands that form a sling around the thorax. They are always found in areas with practically no current (cracks in the underside of rocks, packs of dead leafs, etc.).

This rudimentary pupal cocoon appears a convergent character common to black flies living in slow currents. This is evident in *Gymnopais* whose pupa was thought to lack a cocoon until Wood (1978) discovered the cocoon was a delicate covering spun by the larva that quickly disintegrates, except for a small ventral pad of silk that the pupa uses to attach to the substrate. The cocoon of *Greniera* may be similar to that of *Gymnopais* in that it might start out covering more of the body, but soon the delicate case breaks down. However, pupation would have to be observed to know for certain. It does appear that *Greniera* has a rudimentary cocoon consisting of silk strands entangled in the anchor-like setae of abdominal segment IX, and sometimes with strands wrapped around the thorax with the aid of the fishhook-shaped dorsal hooks. *Adlerella* however has a distinct cocoon, though it is very reduced and never covers the thorax or all of the abdomen. The reduction of the pupal cocoon is likely an adaptation to low flow habitats because the larvae do not need to invest energy into constructing such sturdy mechanisms of attachment, like simuliids in fast currents require. Functionally, *Greniera*, *Gymnopais*, and *Adlerella* use their meager cocoons to attach to the substrate, however, the way in which the three genera achieve this is unique to each genus and is reflected in their distinct morphology.

The larva of *Adlerella* also bears antennae of 3 articles with the third article smooth and not longer than the basal articles. Conversely, *Greniera*'s third antennal article bears characteristic ring-like cuticular striations and its length accounts for almost half the length of the entire antenna as Doby & David (1959) stated, "…le dernier, très long représente à lui seul presque la moitié de la longueur totale (striation chitineuse caractéristique)…" These cuticular striations on the third article add numerous points of flexibility possibly utilized to optimize cleaning of *Greniera*'s large fans. *Adlerella* is further distinguished by possessing minute spicules on the medial antennal article. This compilation of characters establishes the uniqueness of *Adlerella.*

The basal branching of *Adlerella* within the Simuliini is quite unexpected, and the significance of its position further validates the distinctiveness of this genus compared to the more derived *Greniera.* The characters of the markedly elongate larval antennae and the pupal gill consisting of numerous flimsy filaments that previously defined the genus *Greniera* appear homoplastic, and were noted as so by Adler *et al.* (2004). The character of the greatly elongate antennae has evolved multiple times in the Simuliidae, but the length of antennal articles in relation to the antennal length that surpasses the labral fan, as well as the sculpturing of the antennae are more accurate characters for a phylogenetic based taxonomy. *G. brachiata* and *G. ivanovae* may be included in the genus *Adlerella.* However, further study is needed to truly understand the systematic placement of species currently placed in *Greniera*, with special attention paid to distinguish characters indicative of synapomorphies from similarities based upon convergence.

TABLE 1. List of apomorphies for Holarctic Simuliidae
(1) indicates the apomorphic state; (0) indicates the plesiomorphic state

1. Mouthparts in almost all adults: (1) non-functional; (0) functional. (Azar *et al.* 2008)
2. Setae of flagellomeres arranged: (1) irregularly; (0) in circular whorls of setae. (Ooesterbroek & Courtney 1995)
3. Costa: (1) encircles the hind margin of the wing to varying degree; (0) not attaining hind margin of wing leaving it membranous. (Wood & Borkent 1989)
4. Pedicel of antenna: (1) not markedly enlarged, male flagellum not greatly plumose or distinct from that of the female; (0) enlarged with male flagellum conspicuously plumose. (Wood & Borkent 1982)
5. Male eye: (1) with line of dissimilarity dividing large upper facets and small lower facets; (0) no line of discontinuity present. (Wood & Borkent 1982)
6. Wing: (1) significantly broadened basally; (0) without marked broadening at the base. (Hennig 1973)
7. Basal medial cell: (1) very small; (0) large. (Adler *et al.* 2004)
8. Male claw: (1) with dorsal surface bearing cuticular grooves basally; (0) simple, without such grooves. (Wood & Borkent 1982)
9. Hind basitarsus: (1) ventrally keeled and laterally compressed; (0) cylindrical. (Wood & Borkent 1982)
10. Adult abdominal tergite 1: (1) bearing posterior fringe of long hairs; (0) without basal fringe. (Wood & Borkent 1982)
11. Anal sclerite of abdominal segment IX (1) present; (0) absent. (Adler *et al.* 2004)
12. Blood-feeding: (1) diurnal and prolonged; (0) Nocturnal and more rapid. (Adler *et al.* 2004)
13. Pupal gill: (1) with basal lateral fenestra; (0) without basal lateral fenestra. (Adler *et al.* 2004)
14. Pupal spiracle: (1) with regulatory apparatus operated by pharate adult; (0) without such a regulatory apparatus. (Adler *et al.* 2004)
15. Pupal abdominal tergites & sternites III or IV-VIII: (1) broadly divided by pleural membrane; (0) fused. (Adler *et al.* 2004)
16. Pupa: (1) pharate, capable of feeding and spinning its cocoon; (0) larva spins cocoon and feeding halts after larval to pupal apolysis. (Adler *et al.* 2004)
17. Silk glands: (1) exceptionally large and folded; (0) comparatively reduced. (Adler *et al.* 2004)
18. Larvae: (1) with bacteria-covered multiporous sensillum ventral to the membranous antennal base; (0) without any such organ. (Adler *et al.* 2004)
19. Rectal papillae: (1) with three lobes, simple or compound; (0) with even number (2 or 4) of lobes. (Adler *et al.* 2004)
20. Hooks of prothoracic and posterior prologs: (1) arranged in longitudinal rows; (0) irregularly arranged. (Adler *et al.* 2004)
21. Egg: (1) subtriangular, with a dorsal transverse prominence; (0) ovoid. (Borkent & Wood 1986)
22. Katepisternum: (1) distinctly reduced; (0) markedly convex. (Wood & Borkent 1982)
23. Mesepimeral tuft: (1) lost; (0) present. (Wood & Borkent 1982)

TABLE 1. Continued

24. Basal radial cell length as measured from base of cell: (1) markedly less than half the wing length; (0) equal to about half the length of the wing. (Adler *et al.* 2004)
25. Mating behavior: (1) females rest still the leaf undersides while males search underneath for them; (0) mating via aerial swarms. (Adler *et al.* 2004)
26. Maxillary palpus of first-instar larvae with sensilla positioned: (1) subapically; (0) apically. (Adler *et al.* 2004)
27. Larval abdominal segment IX: (1) with single, large, conical, ventral lobe; (0) simple venter without noticeable cuticular projections. (Adler *et al.* 2004)
28. Habitat: (1) hypogean; (0) surface-flowing streams. (Adler *et al.* 2004)
29. Larval pigment: (1) absent; (0) present. (Adler *et al.* 2004)
30. Radial sector vein branches: (1) positioned near to one another; (0) widely separated. (Wood & Borkent 1982)
31. False vein: (1) forked apically; (0) simple or not divided. (Adler *et al.* 2004)
32. Katepisternum sulcus: (1) present; (0) absent. (Adler *et al.* 2004)
33. Female sternum X medially: (1) completely divided; (0) incompletely divided. (Adler *et al.* 2004)
34. Pupal felt chamber of gill: (1) lost; (0) present. (Adler *et al.* 2004)
35. Pupal spiracle corresponding: (1) with the plastron lumen; (0) directly with the plastron. (Adler *et al.* 2004)
36. Pupal sternites VI & VII, & sometimes more: (1) medially divided by a semimembraneous longitudinal striate region; (0) not divided. (Adler *et al.* 2004)
37. Pupal abdominal tergites V to IX: (1) each bearing an anterior row of spine combs; (0) without such spine combs. (Adler *et al.* 2004)
38. Larval antennae: (1) consisting of 3 articles; (0) 1 article. (Adler *et al.* 2004)
39. Larva with a postgenal cleft: (1) present; (0) absent. (Adler *et al.* 2004)
40. Lateral sclerite of prothoracic proleg: (1) present; (0) absent. (Adler *et al.* 2004)
41. Pupal abdominal segments IV and V: (1) bearing large pleural plates; (0) without any such plates. (Adler *et al.* 2004)
42. Larval antennae with basal two segments: (1) unpigmented, contrasting with black apical segment; (0) pigmented. (Adler *et al.* 2004)
43. Larval paralateral teeth of hypostom: (1) absent; (0) present. (Adler *et al.* 2004)
44. Anteromedian palatal brush of first-instar larva: (1) with fringed hairs arranged in scoop shape; (0) with simple hairs. (Adler *et al.* 2004)
45. Maxillary papal sensilla of first-instar larva: (1) positioned subapically and in a linear pattern; (0) apically positioned and in circular pattern. (Adler *et al.* 2004)
46. Hypogynial valves: (1) elongate, with anteromedial corner bearing a nipple-like projection; (0) markedly shorter and truncate. (Adler *et al.* 2004)
47. Spermatheca: (1) with a large differentiated area at the connection with the spermathecal duct; (0) without a differentiated area or with the differentiated area reduced at the connection with the spermathecal duct. (Adler *et al.* 2004)
48. Lateral sclerite of prothoracic proleg: (1) wide with vertical section distinct; (0) narrow without a vertical extension. (Adler *et al.* 2004)
49. Paramere: (1) distinct, but reduced; (0) generally larger.

TABLE 1. Continued

50. Chromosomes: (1) with fixed inversions in IIIL (IIIL-1) and IIS; (0) without fixed inversions in IIIL (IIIL-1) and IIS. (Adler *et al.* 2004)
51. Larval mandible bearing apical brush consisting of: (1) bristle-like setae; (0) slender hairlike setae. (Adler *et al.* 2004)
52. Anal sclerite: (1) with a short posteromedially pointed projection between posteroventral arms; (0) without such a projection between posteroventral arms. (Adler *et al.* 2004)
53. Female cercus: (1) long, and apically narrowing to a fine point; (0) broader than long and subrectangular. (Adler *et al.* 2004)
54. Gonostylus base; (1) bearing a lateral accessory lobe; (0) simple, without such a lobe. (Adler *et al.* 2004)
55. Larval basal antennal two articles: (1) reversed to pigmented state; (0) non-pigmented among the Prosimuliini. (Adler *et al.* 2004)
56. Pupal abdominal tergites IV and V with anterior line of spine combs: (1) absent; (0) present. (Adler *et al.* 2004)
57. Larva with labral fans in 2^{nd} through final instar: (1) absent; (0) present. (Adler *et al.* 2004)
58. Larval head with posterior frontolabral muscles: (1) separated into two fascicles; (0) of one fascicle. (Adler *et al.* 2004)
59. Larval mandible apically: (1) wide, with compressed, equally sized apical teeth; (0) slender, with teeth more conical and of unequal lengths. (Adler *et al.* 2004)
60. Larval mandible with large apical brush: (1) extensive, and composed of many rows of comblike scales; (0) with comblike reduced in size and number. (Adler *et al.* 2004)
61. Larval mandible with covering brush of first external brush, and second external brush: (1) reduced or lost; (0) not reduced and present. (Adler *et al.* 2004)
62. Larval abdomen with segments I-IV: (1) thin and corrugated ventrally, expanded abruptly at segment V, and then narrowed posteriorly to a slight posterior proleg; (0) not corrugate and more gently tapered. (Adler *et al.* 2004)
63. Adult antenna composed of: (1) 7 flagellomeres; (0) 9 flagellomeres. (Adler *et al.* 2004)
64. Stematic bulla located adjacent to posterior margin of compound eye: (1) present; (0) absent. (Adler *et al.* 2004)
65. Female tarsal claw with basal or subbasal tooth (1) absent; (0) present. (Adler *et al.* 2004)
66. Pupal abdominal segment III: (1) ringlike, with the tergite and sternite merged laterally; (0) divided laterally by pleural membrane. (Adler *et al.* 2004)
67. Pupal abdominal tergites III and IV with recurved hooks: (1) lost or reduced in quantity or lost, and positioned between the middle of tergite and its posterior margin; (0) present. (Adler *et al.* 2004)
68. Pupal abdominal tergites V-IX with anterior row of spine combs: (1) absent; (0) present. (Adler *et al.* 2004)
69. Larval anal sclerite with posteroventral arms: (1) rudimentary or absent; (0) present. (Adler *et al.* 2004)

TABLE 1. Continued

70. Larval anal sclerite: (1) with anterodorsal arms arising from long common stalk, creating a Y-shaped formation; (0) subrectangular and giving rise to a pair of both anterodorsal and posteroventral arms. (Adler *et al.* 2004)
71. Female tergite IX: (1) extended like shield tapered to a point over the circus; (0) not extended shieldlike over circus and subtriangular in shape, not as pointed. (Adler *et al.* 2004)
72. Female lateral plate of genital fork (1) not connected directly to tergite IX; (0) connected via sclerotized structures directly to tergite IX. (Adler *et al.* 2004)
73. Male ventral plate of aedeagus: (1) with short basal arms compared to length of ventral plate; (0) with much longer basal arms. (Adler *et al.* 2004)
74. Larval hypostoma with lateral serrations in form of: (1) spiniform setae; (0) short, rounded denticles. (Adler *et al.* 2004)
75. Anal sclerite: (1) with prominent supernumerary arm extended posteromedially, forming an inverted the shape of a star; (0) X shaped. (Adler *et al.* 2004)
76. Ventral plate of aedeagus near base of anterolateral apodeme: (1) strongly notched laterally; (0) not strongly notched laterally. (Adler *et al.* 2004)
77. Gonostylus: (1) with 1 apical spinule; (0) with 2 spinules. (Adler *et al.* 2004)
78. Larval mandible: (1) not distinctly curved medially near apex; (0) medially curved distally. (Adler *et al.* 2004)
79. Pupal cocoon: (1) a clear gelatinous casing that envelopes entire pupa, retaining larval exuvia; (0) woven silk sac that does not retain the larval exuvia. (Adler *et al.* 2004)
80. Adult vestiture: (1) meager, petite, and erect, not dense fine recumbent setae; (0) primarily soft, with few coarse upright setae. (Adler *et al.* 2004)
81. Adult clypeus: (1) nearly devoid of setae, none medially; (0) entirely clothed in setae. (Adler *et al.* 2004)
82. Female mandible and lacinia with teeth: (1) absent; (0) present. (Adler *et al.* 2004)
83. Adult thorax: (1) weakly arched; (0) more strongly arched. (Adler *et al.* 2004)
84. Adult Postnotum: (1) relatively small and markedly arched, with a variously developed median longitudinal ridge; (0) larger and less severely arched. (Adler *et al.* 2004)
85. Adult mesepimeron with vestiture: (1) limited to the dorsal region of the sclerite above the level of the metathoracic spiracle; (0) more extensive in other Prosimuliini. (Adler *et al.* 2004)
86. Wing membrane: (1) smoky colored and slightly furrowed; (0) hyaline and less wrinkled in other Prosimuliini. (Adler *et al.* 2004)
87. Petiole of M1+2 vein: (1) long, approximately half the length of the petiole of the radial sector; (0) one fifth the length of the petiole of the radial sector in other Prosimuliini. (Adler *et al.* 2004)
88. Spermatheca base: (1) with short to long sclerotized collar where the spermathecal duct is joined; (0) connected to the duct via a membranous ring in other Prosimuliini. (Adler *et al.* 2004)
89. Female anal lobe and cercus: (1) merged into one solid sclerite; (0) separated by membrane. (Adler *et al.* 2004)

TABLE 1. Continued

90. Male gonostylus: (1) thin apically, with a minute apical spinule; (0) relatively broad throughout with a more rounded apex in other Prosimuliini. (Adler *et al.* 2004)
91. Pupal abdominal Sternites IV-VII: (1) each bearing up to 5 pairs of recurved hooks; (0) each with less than 5 hooks in other Prosimuliini and most Simuliidae. (Adler *et al.* 2004)
92. Pupal abdominal pleurite IV: (1) barely distinguishable from adjoining tergite and sternite; (0) with distinguishable pleurite on segments IV and V in other Prosimuliini. (Adler *et al.* 2004)
93. Larval labrum and labropalatum: (1) distinctively longer than wide, bearing clear tubercle on each side, epipharyngeal mechanism anteriorly bearing V-shaped brush; (0) without such an arrangement. (Adler *et al.* 2004)
94. Radial sector: (1) unbranched or extremely small and apically forked; (0) more definitively branched and not positioned apically. (Adler *et al.* 2004)
95. Male straplike link joining the paramere and ventral plate arising subapically on anterolateral apodeme of ventral plate: (1) present; (0) absent. (Adler *et al.* 2004)
96. Female genital fork with lateral plate with a variously formed dorsal apodeme: (1) present; (0) absent. (Adler *et al.* 2004)
97. Gonocoxite: (1) massive, sub-trapezoidal; (0) less enlarged and angulate.
98. Larval rake-like fans modified for scraping: (1) present; (0) absent.
99. Larval medial antennal article with minute spicules: (1) present; (0) absent.
100. Larval antenna: (1) greatly surpassing the length of the labral fan stalk by about 2/3 the length of the medial article; (0) either not surpassing the length of the labral fan stalk, or not by about 2/3 the length of the medial article.
101. Form of pupal: (1) gill diverse, and differentiated from all other simuliids; (0) less variation in pupal gill diversity. (Adler *et al.* 2004)
102. Larval abdominal segment VIII with transverse midventral bulge: (1) present; (0) absent. (Adler *et al.* 2004)
103. Tibial spurs of hind leg: (1) distinctly long and with a hyaline apex; (0) subequal in length or shorter than the tibia without hyaline apex. (Adler *et al.* 2004)
104. Male paramere with apical spines: (1) present; (0) absent. (Adler *et al.* 2004)
105. Larval apical antennal segment with extremely characteristic dark cuticular striations: (1) present; (0) absent.
106. Pupal cocoon: (1) rudimentary, consisting of a few silk threads entwined in the grapnel setae of segment IX; (0) well developed, or not reduced in such a way.
107. Larval postgenal cleft extended anteriorly about: (1) 1/3 or more distance to hypostomal groove; (0) 1/4 or less the distance to the hypostomal groove. (Adler *et al.* 2004)
108. Larval hypostomal teeth: (1) small, and not organized on prominent lobes; (0) larger and organized on prominent lobes. (Adler *et al.* 2004)
109. Pupal gill filaments radiating from bulbous central protuberance: (1) present; (0) absent. (Adler *et al.* 2004)
110. Larval labral-fan rays with distinctively elongate microtrichia intermingled with the normal-sized microtrichia: (1) present; (0) absent. (Adler *et al.* 2004)

TABLE 1. Continued

111. Pupal cocoon: (1) of definite form and complete ventrally; (0) an ill formed sac or if well-formed than not as complete ventrally.
112. Gill filaments: (1) convergent apically; (0) not convergent apically. (Moulton & Adler 1997)
113. Gill filaments with surface: (1) shingle-like; (0) smooth or furrowed. (Moulton & Adler 1997)
114. Pupal terminal abdominal spines: (1) inflated and hollow; (0) thin and solid. (Moulton & Adler 1997)
115. Larval mandible with inner subapical margin: (1) smooth; (0) dentate. (Moulton & Adler 1997)
116. Larval lateral abdominal segments V-VIII: (1) drastically expanded ventrally and laterally; (0) expand more gently. (Moulton & Adler 1997)
117. Anal sclerite: (1) absent; (0) present. (Moulton & Adler 1997)
118. Larval construction of a long silk pedicel to serve as a feeding and pupation substrate: (1) present; (0) absent. (Moulton & Adler 1997)
119. Katepisternal sulcus: (1) narrow and deep its entire length; (0) wide, shallow and indistinct anteriorly. (Adler *et al.* 2004)
120. Pupal abdomen: (1) weakly sclerotized, often losing its form after emergence; (0) strongly sclerotized. (Adler *et al.* 2004)
121. Pupal cocoon: (1) boot shaped and spun of delicate silk; (0) slipper shaped or amorphous of coarser silk. (Adler *et al.* 2004)
122. Larval postgenal cleft: (1) wide and deep, extended anteriorly to or beyond hypostomal groove; (0) shorter and narrower, not extended to the hypostomal groove. (Adler *et al.* 2004)
123. Larval antenna with basal and medial articles: (1) unpigmented, contrasting with dark apical article; (0) pigmented in other Simuliini. (Adler *et al.* 2004)
124. Chromosomes with whole-arm interchange between chromosomes I and II: (1) present; (0) absent. (Adler *et al.* 2004)
125. Gonostylus subtruncate, with internal triangular lobe bearing one apical spinule, without very large laterally directed parameral spine: (1) present; (0) absent.Modified from (Adler *et al.* 2004)
126. Basal radial cell length compared to the distance from the base of radial sector to wing apex: (1) less than 1/3; (0) greater than 1/3. (Adler *et al.* 2004)
127. Larval rectal papillae: (1) mostly compound; (0) simple. (Adler *et al.* 2004)

TABLE 2. Sorted character state matrix for Holarctic Simuliidae. X=Apomorphic character state, CA=*Cardiocladius*, TH=*Thaumalea*, PA=*Parasimulium*, PR=*Prosimulium*, HE=*Helodon*, UR=*Urosimulium*, LE= *Levitinia*, TW=*Twinnia*, GY=*Gymnopais*, CR=*Crozetia*, AD=*Adlerella*, TL= *Tlalocomyia*, ST=*Stegopterna*, GR=*Greniera*, CN=*Cnephia*, ET=*Ectemnia*, ME=*Metacnephia*, SU=*Sulcicnephia*, SM=*Simulium.*
Numbers in first column correspond to character numbers in Table 1.

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM
1.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
35.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM
40.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
42.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
43.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
44.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
45.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
46.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
50.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51.	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0
52.	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0
53.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
54.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
55.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
56.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
57.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
58.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
59.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
60.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
61.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
62.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
63.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
64.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
65.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
66.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
67.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
68.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
69.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
70.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
71.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
72.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
73.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
74.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
75.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
76.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
77.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
78.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
79.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
80.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
81.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
82.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
83.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
84.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
85.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM
86.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
87.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
88.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
89.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
90.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
91.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
92.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
93.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
94.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
95.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
96.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1
97.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
98.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
99.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
100.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
101.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
102.	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
103.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
104.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
105.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
106.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
107.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
108.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
109.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
110.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
111.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
112.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
113.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
114.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
115.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
116.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
117.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
118.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
119.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
120.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
121.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
122.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
123.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
124.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
125.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
126.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
127.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

TABLE 3. Morphological comparison of *Adlerella* to more ancestral and more derived genera

Character	*Prosimulium*	*Adlerella*	*Tlalocomyia*	*Greniera*	*Ectemnia*	*Metacnephia*
Dimorphic costal setae- mixture of hair-like and spinoform macrotrichia	No	No	Yes	No spines, but some shorter more strongly chitinous spines	Yes	Yes
Fork of R vein	Distinct, though with arms more closely approximated than in *Parasimuliinae*	Very distal	Very distal, some species south of U.S.A. unbranched	Very distal and indistinct	Indistinct	Indistinct
Basal medial cell	Present	Present	Present	Present	Present	Present
Parameral spines	Absent	Absent	Absent	Present, but few	Short numerous	Short numerous
Gonocoxite compared to gonostylus in ventral aspect	Large, less than twice the basal width of gonostylus	Large- less than twice the basal width of gonostylus	Large- less than twice the basal width of gonostylus	Very large- more than twice the basal width of gonostylus	Small- less than twice the basal width of gonostylus	Large- about twice the basal width of gonostylus
Gonostylus	In inner lateral view conical, broad and curved	Not strongly tapered, slightly curved and broad	Not strongly tapered, 2 rarely 1 apical spine	Styles thin and tapered, almost straight	Curved, not strongly tapered, somewhat small	Tapered , slightly curved, of various diameters
Pedisulcus	absent	Absent, or represented by weak wrinkles	Absent, or represented by weak wrinkles	Extremely rudimentary	Represented at most by shallow wrinkles	Represented at most by shallow wrinkles
Calcipala	Absent	Small	Large and usually lamellate	Small	Small	Minute or absent
Pupal Cocoon	Ill formed sac	Ill formed sac poorly covers body	Ill formed sac poorly covers body	Rudimentary, made of few silk threads	Slipper shaped	Typically boot shaped
Pupal lateral abdominal hooks	Segment 9 without anchorlike hooks	Segment 9 without anchorlike hooks	Segment 9 without anchorlike hooks	Segment 9 hooks anchorlike- triramous and recurved	Segment 9 without anchorlike hooks	Segment 9 hooks anchorlike- triramous and recurved
Larval labral fan	Of moderate size	Very enlarged, as long or longer than head capsule	Of moderate size	Very enlarged. As long or longer than head capsule	Of moderate size	Of moderate size
Larval antennae	Antenna not extended beyond length of labral fan stalk	Antenna extended far beyond stalk of labral fan	Antenna not extended far beyond length of labral fan stalk	Antenna extended far beyond stalk of labral fan	Antenna not extended far beyond length of labral fan stalk	Antenna not extended far beyond length of labral fan stalk
3rd antennal segment with cuticular striations	Absent	Absent	Absent	Present	Absent	Absent
3rd antennal length	Less than the combined length of segments 1 and 2	Less than the combined length of segments 1 and 2	Less than the combined length of segments 1 and 2	Almost as long as combined length of segments 1 and 2	Less than the combined length of segments 1 and 2	Less than the combined length of segments 1 and 2
Larval anal sclerite	x	x	x	x	Absent	x

Acknowledgements

This research was supported by the Hatch Multistate Research Fund and the New Jersey Agricultural Experiment Station at Rutgers University, New Brunswick, New Jersey, Project: Evaluating the Physical and Biological Availability of Pesticides and Contaminants in Agricultural Ecosystems Project 224316 and NIEHS Center Grant ES020522.

Literature cited

Adler P. H., Currie D. C., & Wood D. M. (2004). *The black flies (Simuliidae) of North America*. Cornell University Press Ithaca, New York, 941 pp.

Adler, P. H., & Crosskey, R. W. (2013). World Blackflies (Diptera: Simuliidae): a Comprehensive Revision of the Taxonomic and Geographical Inventory 2013, 120 pp. Available from: http://www.clemson.edu/cafls/departments/esps/biomia/pdfs/blackflyinventory1.pdf (Accessed 9 August 2013).

Azer, D., Veltz, I., & Nel, A. (2008). Mandibulate chironomids: primitive or derived? (Diptera: Chironomidae). *Systematic Entomology*, 33, 688-699.

Borkent, A., & Wood, D. M. (1986). The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). *Proceedings of the Entomological Society of Washington*, 88, 287-296.

Collins, K. P., & Wiegmann B. M. (2002). Phylogenetic relationships and placement of the Empidoidea (Diptera: Brachycera) based on 28S rDNA and EF-1alpha sequences. *Insect Systematics and Evolution*, 33, 421-444.

Craig, D. A., Currie D. C. & Vernon P. (2003). *Crozetia* Davies (Diptera: Simuliidae): redescription of *Cr. crozetensis*, *Cr. seguyi*, number of larval instars, phylogenetic relationships and historical biogeography. *Zootaxa* 259, 1-39.

Currie, D. C. (1988). *Phylogeny of primitive Simuliidae (Insecta: Diptera: Culicomorpha).* Ph.D. dissertation. University of Alberta, Edmonton, Canada.

Davies, D. M., Peterson, B. V., & Wood D. M. (1962). The Black Flies (Diptera: Simuliidae) of Ontario. Part 1. Adult identification and distribution with descriptions of six new species. *Proceedings of the Entomological Society of Ontario,* 92, 70-154.

Davies, L. (1965). The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island black-fly. *Proceedings of the Linnean Society of London*, 176, 159-180.

Davies, L. (1974). Evolution of larval head-fans in Simuliidae (Diptera) as inferred from the structure and biology *Crozetia crozetensis* (Wormsley) compared with other genera. *Zoological Journal of the Linnean Society of London,* 55, 193-224 + 2 plates.

Doby J. M., & David, F. (1959a). Description de *Greniera fabri* (Diptera-Nematocera Simuliidae) en Provence de l'ouest de la France (Atlantic Loire). *Bulletin de la Société de pathologie exotique*, 52, 669-78.

Doby J. M., & David, F. (1959b). Greniera, genre nouveau de Simuliidé (Diptères-Nématocères) Discussion de la position systématique. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, 249, 763-768.

Edwards, F. W. (1926). The phylogeny of nematocerous Diptera: a critical review of some recent suggestions. *Verhandlungen des III. Internationalen Entomologen-Kongresses* 1, 111-130.

Hennig, W. (1969). Die Stammesgeschichte der Insekten. Frankfurt A.M, Kramer, 436 pp.

Hennig, W. (1973). Diptera (Zweiflügler). *In*: Helmcke, J. G., Starck, D. & Wermuth, H. (Eds), *Handbook of Zology*. Walter d Gruyter Berlin, 1-337 pp.

Moulton, J.K. & Adler, P.H. 1(997). The genus *Ectemnia* (Diptera: Simuliidae): taxonomy, polytene chromosomes, new species, and phylogeny. *Canadian Journal of Zoology*, 121, 1-57.

Oosterbroek, P., & Courtney, G. W. (1995). Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, 115, 267-311.

Pawlowski, J., R. Szadziewski, D. Kmieciak, J. Fahmi, & Bittar, G. (1996). Phylogeny of the infraorder Culicomorpha (Diptera) based upon 28S RNA gene sequences. *Systematic Entomology*, 21, 167-178.

Swofford, K. L. (1999). *PAUP 4.0: phylogenetic analysis with parsimony (and other methods), version 4.0 b2a*. Sunderland, Massachusetts Sinauer Associates Inc.

Wood, D. M. (1978). Taxonomy of the Nearctic species of *Twinnia* and *Gymnopais* (Diptera: Simuliidae) and a discussion of the ancestry of the Simuliidae. *Canadian Entomologist*, 110, 1297-1337.

Wood, D. M., & Borkent, A. (1982). Description of the female of Parasimulium crosskeyi Peterson (Diptera: Simuliidae) and a discussion of the phylogenetic position of the genus. Memoirs of the Entomological Society of Washington 10, 193-210.

Wood, D. M., & Borkent, A. (1989). *Nearctic Diptera Manual, Vol. 3. Phylogeny and classification of the Nematocera.* Agriculture Canada Monograph 32, 1333-1370.

A phylogeny of black flies (Diptera: Simuliidae), with descriptions of two new taxonomic groups from the southern hemisphere

ABSTRACT

A phylogenetic analysis shows *Paracnephia* to be polyphyletic. Two groups of Simuliidae, Taxon A and Taxon B are described from morphological data, and are likely new genera that have been mistakenly included in the genus *Paracnephia*. Diagnostic characters for the larval, pupal and adult stages of Taxon A, and characters for the adult female and proposed larva of Taxon B are provided. A morphological comparison of these new taxonomic groups to the African *Paracnephia* is also provided. The separation of Taxon A and Taxon B from the African *Paracnephia* follows the biogeography of these taxa. A phylogenetic analysis of Taxon B reveals a close relationship with *Austrosimulium* and *Paraustrosimulium.*

Key words: Simuliidae, *Paracnephia,* Taxon A*,* Taxon B*,* biogeography, phylogenetic, apomorphy.

Introduction

Previous to Tonnoir's (1925) work on 14 Australian black fly species only three species were known from the continent. In his study, Tonnoir described 10 new species placing four in the genus *Simulium*, and the other six in the newly established genus *Austrosimulium*, which comprised black flies looking similar to *Simulium* adults from New Zealand and Australia, but whose antennae bear eight or sometimes seven flagellomeres. The knowledge of Australian Simuliidae would soon be augmented by Taylor's (1927) further description of *Austrosimulium bancrofti* and discovery of a new *Simulium* species, and by Drummond's (1931) survey of five species from Western

Australia: *S. ornatipes, A. bancrofti, S. tonnoiri* n. sp., and two unidentified species. The papers of Mackerras and Mackerras (1948, 1949, 1950, 1952, 1955) would prove the most comprehensive work on Australian black fly taxonomy as they analyzed freshly collected specimens and Tonnoir's specimens to write keys for all life stages except the egg stage, synonomize names, reevaluate the characters of the Australian genera, and describe 16 new species.

Dumbleton's (1962, 1964) work would reinvigorate Australian investigations into simuliid taxonomy that had waned for nearly 10 years as he focused on the characters of the immature stages. Dumbleton's (1963) treatment of the Simuliidae of the southern hemisphere would mark some of the first attempts at investigating the Australian taxa through a phylogenetic framework. Dumbleton (1972) would later publish a comprehensive revision of *Austrosimulium,* complete with keys to larvae, pupae, and adults. The work of Colbo (1976) would add four new *Simulium* (*Morops* Enderlein) species to the Australian fauna. Later, in 1988 Davies and Gyorkos described *Austrosimulium colboi* from Australian Capital Territory and *Cnephia pilfreyi* from Victoria, and added speculations as to their phylogenetic relationships to other simuliids. The most recently described Australian simuliid species, *Paracnephia gladiator* (Moulton *et al.* 2004), belongs to the same genus that currently comprises the species of the former Australian *Cnephia* Enderlein.

Some of the greatest confusion in Australian Simullid systematics has revolved around the species presently within the genus *Paracnephia*. This began with the assignment of the presently known *Paracnephia umbratora* and *Paracnephia aurantiaca* to the genus *Simulium* by Tonnoir (1925), and *Paracnephia tonnoiri* to the genus

Simulium by Drummond (1931). Mackerras and Mackerras (1949) would later assign all of the above mentioned species to the genus *Cnephia*, along with the new species *Cnephia strenua* (1950). Mackerras and Mackerras (1949) soon established the *aurantiacum* species group and placed *Paracnephia aurantiaca, Paracnephia tonnoiri, and Paracnephia fuscoflava* to it followed by the addition of *Paracnephia strenua* in their (1950) publication. The proper placement of *Paracnephia umbratora* however had perplexed Mackerras and Mackerras, in 1948 they assigned it to the *terabans* species group based upon characters of the maxillary palpus, abdomen and female genitalia. Mackerras and Mackerras (1950) would revise their previous finding and assign *Paracnephia umbratora* to the *aurantiacum* species group based upon wing veination and the claw of the female. The uncertainty about *Paracnephia umbratora*'s placement within these species groups highlights its ill fit among these taxa.

Crosskey & Howard (1997) reassigned the abovementioned *aurantiacum* and *terebrans* group species to the genus *Paracnephia* Rubstov. Though Crosskey (1969) stated regarding this genus, "…Paracnephia, a segregate rather weakly defined by Rubstov (1962) that he proposed for the inclusion of all the primitive southern African black-flies previously placed by Freeman & de Meillon (1953) in the genus Cnephia." Both *Prosimulium* and *Cnephia* are currently recognized as exclusively Holarctic genera, and African taxa previously placed in those genera have been reassigned (Adler & Crosskey 2014). Though two subgenera, *Paracnephia* and *Procnephia*, have been recognized within the *Paracnephia* based on the African fauna, all Australian *Paracnephia* remain unplaced to subgenus (Adler & Crosskey 2014). The phylogeny of the southern hemisphere Simuliidae proposed by Gil-Azevedo & Maia-Herzog (2007) showed Paracnephia to be paraphyletic.

Based on a phylogenetic study of 140 morphological characters two new taxonomic groups of Australian Simuliidae are described and a new phylogeny is presented.

Materials and Methods

Specimens of *Prosimulium, Gymnopais, Twinnia, Ectemnia, Stegopterna, Cnephia, and Simulium* were analyzed and a thorough review of the literature was conducted for the remaining genera. New characters as well as ones from the literature were used (Table 1). A morphology matrix of 140 apparently minimally correlated characters from four life stages, and select behavioral characters (Adler *et al.* 2004) was created for the genera analyzed (Table 1). Particular attention was given to specimens from the genus *Paracnephia*. Outgroup selection was determined by both morphological (Hennig 1973, Wood and Borkent 1989, Oosterbroek and Courtney 1995) and molecular evidence (Pawlowski *et al*. 1996, Bertone *et al.* 2008).

Twenty nine taxa were analyzed including: 21 simuliid genera, and the two outgroups *Thaumalea* and *Cardiocladius* (Figure 1). Heuristic parsimony analysis was performed using PAUP version 4.0 (Swofford 1999). Bootstrap branch support was calculated from 1,000 replicates. All morphological characters received equal weight. The bootstrap 50% majority-rule was utilized and the most likely consensus tree was then generated.

Results

Seventeen character states support the monophyly of the family Simuliidae, eight character states support the monophyly of the subfamily Parasimuliinae, and eleven character states support the monophyly of the subfamily Simuliinae (Table 1 and 2). The tribe Prosimuliini is supported by five character states: pupal abdominal segments IV and V bearing pleural plates, larval antennae with medial and basal article unpigmented contrasting with blackish-brown apical article, larval hypostomal paralateral teeth absent, first-instar larval anteromedian palatal brush composed of scoop-shaped fringed hairs, and the first-instar larval maxillary papal sensilla positioned subapically in a linear pattern. The tribe Simuliini was supported by three character states: the vestigial fork of the radial sector vein, the genital fork of the female bearing a dorsal apodeme, and the paramere of the male with a straplike connection between the paramere and the ventral plate that arises subapically on the basal arm of the ventral plate. Taxon group A is supported by the apomorphy of the strongly arched thorax of the adult. Taxon group B is defined by the distinctive subasal claw of the female which is markedly curved with a laterally directed basal tooth, and the larva with a T-shaped anal sclerite. Though the male for Taxon B is still unknown, genital characters are inferred from congruent female morphology. A full list of the character states used in the morphology matrix and their distribution are provided (Table 1 and 2).

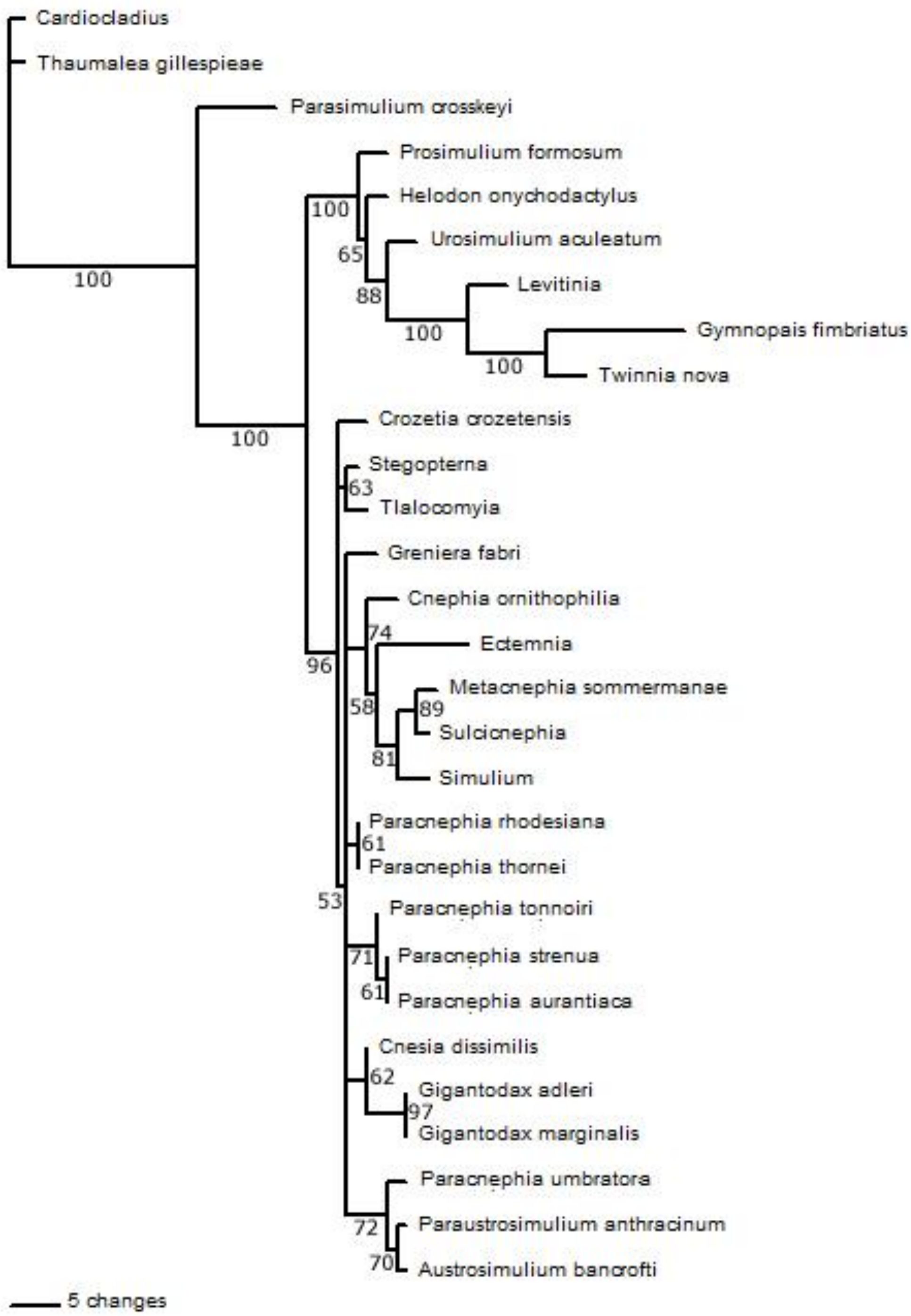

FIGURE 1. Phylogram showing bootstraps for simuliid genera analyzed.

The current assemblage of species within the genus *Paracnephia* appears polyphyletic forming multiple monophyletic groups throughout the tree (Figure 1). The African *Paracnephia* forms a monophyletic group with *P. rhodesiana* and *P. thornei*. The species *Paracnephia aurantiaca, Paracnephia strenua,* and *Paracnephia tonnoiri* form a monophyletic group distinct from the African *Paracnephia*. Based upon morphological data, Taxon A is established to include the species *Paracnephia aurantiaca, Paracnephia strenua,* and *Paracnephia tonnoiri.* The species *Paracnephia umbratora* is basal to *Austrosimulium* and *Paraustrosimulium.* Based on morphology Taxon B is established to include *Paracnephia umbratora*. The monophyly of the African *Paracnephia* is supported by a bootstrap value of 61, the monophyly of Taxon A is supported by a bootstrap value of 71, and the placement of Taxon B as sister to the *Austrosimulium* and *Paraustrosimulium* is supported by a bootstrap value of 72 (Figure 1).

A morphological comparison chart is provided to illustrate the relationships between Taxon A and B and other closely related genera (Table 3). The species of Taxon A share morphological characters including: the slight curvature of the CuA2 vein, the elongate terminal adult palpal segment that is as large as the other two combined, the strongly arched thorax of the adult, and the female genatalia with long paraprocts, and cerci that are concavely excavate on the inner margin. Taxon B, though known only from the female and larva can be distinguished by a CuA2 vein that is nearly straight, a female claw with a well developed outwardly pointing basal tooth, and a genital fork with short broad arms and a short stem.

There is a morphological and biogeographical division between the African *Paracnephia* and the Australian Taxon A and Taxon B (Figure 2). This follows the divide between the Ethiopian and Australian biogeographical regions proposed by Wallace (1876). Thus far the African *Paracnephia* are known from: Uganda, Nambia, Zimbabwe, and the Western Cape of South Africa. Taxon A is known from: New South Wales, Queensland, Tasmania, Victoria, and Western Australia. Taxon B is known from: Queensland, Victoria, and Western Australia. Morphologically, Taxon B and Taxon A share a large conspicuous calcipala and a shallow but distinct pedisulcus, while the African *Paracnephia* lack these characters.

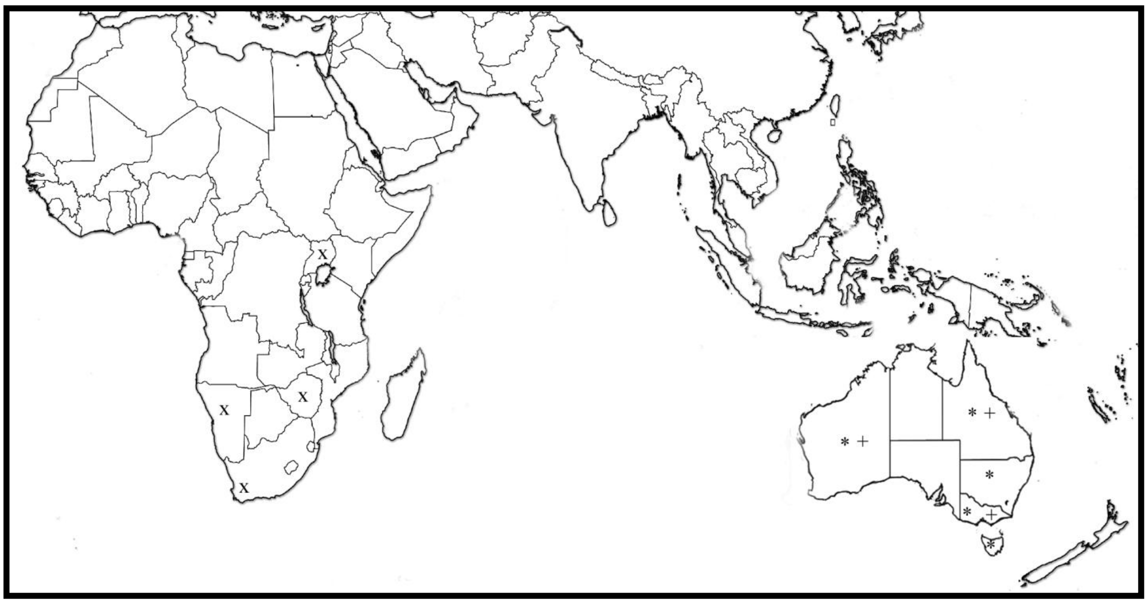

FIGURE 2. Country and state distribution of: **x**-African *Paracnephia,* *- Taxon A, and **+-** Taxon B.

Taxon A

Included species: *Paracnephia aurantiaca, Paracnephia Tonnoiri,* and *Paracnephia strenua*

Female. Ochraceous in color, with strongly humped thorax. Antennae with nine flagellomeres, pubescence short and fine; maxillary palpi with terminal segment as long as previous two combined. Scutum strongly humped and covered in short fine appressed setae; pronotum and upper mesepimeron clothed in hairs; propleural hairs present or absent. Costal vein of wing covered in setiform and weakly to well developed spiniform micotrichiae; R vein with weak distal fork, R1 vein clothed in hair dorsally, but without spinules. Basal section of radius clothed in hair dorsally; Rs vein clothed in hair ventrally throughout length, but with dorsal pubescence restricted to distal section. Underside of Sc vein clothed in hairs; CuA2 gently sinuous; Small basal cell present. Hind legs with large, conspicuous calcipala, pedisulcus shallow but distinct; claws of female with strong basal tooth. Abdomen strongly hirsute. Paraprocts relatively long, and closely approximated basally; cerci concavely excavate on inner sides. Genital fork with relatively long and slender stem and arms. Spermatheca smooth to strongly sculptured.

Male. Gonostylus with 2 apical spines; gonocoxite longer and wider than gonostylus. Anterior area of phallosome with flat, extremely delicately setulose ventral membrane, strongly chitinized distal edge, and chitinized dorsal surface; posterior area with pair of medial hooks, and areas of irregular denticles medial to distal end of apodeme; without apparent median process.

Pupa. Bearing a multiply branched respiratory organ. Abdomen with row of flat, triangular, backwardly directed, sub-basal spines on dorsal surface of segments 5-9; sub-apical dorsal and ventral hooks also present on various segments; terminal spines well developed and forwardly directed. Cocoon loosely woven, of indefinite shape, often containing foreign material.

Larva. Postgenal cleft shallow. Abdomen gradually expands backwards from segment 1, maximum width at segment 7, with posterior end appearing truncate. Anal gills simple; without ventral papillae; anal sclerite x-shaped; anterior arms of anal sclerite without backwardly directed struts.

Taxon B

Included species. *Paracnephia umbratora* Tonnoir

Female. Small and brown; thorax not strongly arched. Head with short pubescence interspersed with longer hairs mainly on vertex and occiput; antennae with 9 flagellomeres, pubescence short and fine; maxillary palpi terminal segment little shorter than penultimate. Frons ¼ width of head. Scutellum and notum clothed in short hairs; thorax with no propleural hairs. Costal vein of wing covered in setiform and weakly to well developed spiniform micotrichiae; R vein with weak distal fork, R1 clothed in hair dorsally, but without spinules. Basal section of radius clothed in hair dorsally; Rs vein clothed in hair ventrally throughout its length, but with dorsal pubescence restricted to its distal section. Underside of Sc vein clothed in hairs; CuA2 almost straight; small basal cell present. Hind legs with large, calcipala with a distinct notch at base, pedisulcus shallow but distinct; claws of female with strong outwardly pointed basal tooth. Abdomen brown, relatively bare, with pubescence on dorsum greatly reduced, though still fairly conspicuous at sides. Relatively short broad paraprocts and cerci not concavely excavated on inner sides. Relatively short stem and broad arms of genital fork; Spermatheca not strongly sculptured.

Larva. Based on the proposed larva of *C. umbratora* from Mackerras and Mackerras (1952). Length 6 mm. Fusiform body shape. Tip of abdomen narrowed and ventrally curved. Head capsule pale with faint head spots with medial ones nearly contiguous. Antennae pale and markedly short, slightly extended beyond midlength of labral fan stalk. Postgenal cleft wide and deep. Hypostoma with concave appearance bearing seventeen teeth; fifth most lateral tooth from each side is very enlarged, widened and slightly curved medially; level row of seven relatively uniform small trifid teeth rests between prominent lateral teeth. Gill histoblast when dissected out consisting of 10 filaments. Posterior circlet of spines quite narrow, consisting of approximately 60 widely separated rows, with up to seven spines per row. Anal sclerite in shape of inverted T, lacking lateral expansions or struts. Ventral papillae absent, and rectal gills not visible.

Discussion

The polyphyletic arrangement of the African and Australian "*Paracnephia"* supports the establishment of two new taxon groups for the Australian species examined, which may in time be established as two new genera. Taxon A and Taxon B both share a morphological affinity with other Australian genera such as the possession of a conspicuous calcipala, mirroring the lines of biogeographical divide proposed by Wallace. Though generalists like *Simulium* have a rather cosmopolitan distribution, most simuliid genera are more restricted in their biogeography (Adler & Crosskey 2014).

The perplexing mixture of species presently within *Paracnephia*: including *P. rhodesiana*, *P. thornei*, *P. aurantiaca*, *P. strenua*, *P. tonnoiri*, and *P. umbratora* is the result of grouping taxa without synapomorphies. As Crosskey (1969) said, "…Paracnephia, a segregate rather weakly defined by Rubzov (1962) that he proposed for the inclusion of all the primitive southern African black-flies previously placed by Freeman & de Meillon (1953) in the genus Cnephia."

Taxon B, appears closely related to *Austrosimulium* and *Paraustrosimulium.* Taxon B, *Austrosimulium,* and *Paraustrosimulium* share a thickening of the arms of the genital fork as compared to their stem, which may be homoplasious. The arms of the genital fork of Taxon B, *Austrosimulium,* and *Paraustrosimulium* share a shortened stem, and the later two genera are further modified by possessing a widening of the stem.

The African *Paracnephia* and species of Taxon A share a mixture of homoplasious traits such as the well developed basal tooth of the female claw and 9 antennal flagellomeres which may have speciously made them appear related. However, species of Taxon A exhibit synapomorphies differentiating them from the African *Paracnephia,* namely a strongly arched thorax, the CuA2 vein with a slightly sinuous curve, the exceptionally hirsute female abdomen, the Cerci concavely excavate on inner margin, and long paraprocts. In contrast, the African *Paracnephia* possesses relatively short, broad paraprocts, and short cerci with relatively straight inner margins.

The lack of autapomorphies for "*Paracnephia"* coupled with a definition based on symplesiomorphy have caused much taxonomic confusion regarding this genus, and is likely why taxa as autapomorphic as Taxon A and Taxon B were placed in it. Reliable autapomorphies for *Paracnephia* need to be confirmed before any future assignment of taxa to the genus. Consequently, the taxonomic status of the Australian "*Paracnephia"* should be evaluated. Undoubtedly a better understanding of Australian simuliid systematics will require additional collections of different life stages for morphological, cytotaxonomic, and molecular analyses.

TABLE 1. List of apomorphies for southern hemisphere Simuliidae: 0) indicates the plesiomorphic state; 1) indicates the apomorphic state.

---

1. Mouthparts in almost all adults (Azar *et al.* 2008): 0) functional; 1) non-functional.
2. Setae of flagellomeres arranged: 0) in circular whorls; 1) irregularly. (Ooesterbroek & Courtney 1995)
3. Costa: 0) not attaining hind margin of wing leaving it membranous; 1) encircles the hind margin of the wing to varying degree. (Wood & Borkent 1989)
4. Pedicel of antenna: 0) enlarged with male flagellum conspicuously plumose; 1) not markedly enlarged, male flagellum not greatly plumose or distinct from that of the female. (Wood & Borkent 1982)
5. Male eye: 0) without line of dissimilarity; 1) with line of dividing large upper facets and small lower facets. (Wood & Borkent 1982)
6. Wing: 0) without marked broadening at basally; 1) significantly broadened basally. (Hennig 1973)
7. Basal medial cell: 0) large; 1) very small. (Adler *et al.* 2004)
8. Male claw: 0) simple, without cuticular grooves basally; 1) with dorsal surface bearing cuticular grooves basally. (Wood & Borkent 1982)
9. Hind basitarsus: 0) cylindrical; 1) ventrally keeled and laterally compressed. (Wood & Borkent 1982)
10. Adult abdominal tergite one: 0) without basal fringe of long hairs; 1) bearing posterior fringe of long hairs. (Wood & Borkent 1982)
11. Anal sclerite of abdominal segment IX: 0) absent; 1) present. (Adler *et al.* 2004)
12. Blood-feeding: 0) nocturnal and rapid; 1) diurnal and prolonged. (Adler *et al.* 2004)
13. Pupal gill: 0) without basal lateral fenestra; 1) with basal lateral fenestra. (Adler *et al.* 2004)
14. Pupal spiracle: 0) without regulatory apparatus operated by pharate adult; 1) with regulatory apparatus operated by pharate adult. (Adler *et al.* 2004)
15. Pupal abdominal tergites & sternites III or IV-VIII: 0) fused; 1) broadly divided by pleural membrane. (Adler *et al.* 2004)
16. Pupa: 0) larva spins cocoon and feeding halts after larval to pupal apolysis; 1) pharate, capable of feeding and spinning its cocoon. (Adler *et al.* 2004)
17. Silk glands: 0) comparatively reduced; 1) exceptionally large and folded. (Adler *et al.* 2004)
18. Larvae: 0) without any such organ; 1) with bacteria-covered multiporous sensillum ventral to membranous antennal base. (Adler *et al.* 2004)
19. Rectal papillae: 0) with 2 or 4 lobes; 1) with three lobes, simple or compound. (Adler *et al.* 2004)
20. Hooks of prothoracic and posterior prologs: 0) irregularly arranged; 1) arranged in longitudinal rows. (Adler *et al.* 2004)
21. Egg: 0) ovoid; 1) subtriangular, with a dorsal transverse prominence. (Borkent & Wood 1986)
22. Katepisternum: 0) markedly convex; 1) distinctly reduced. (Wood & Borkent 1982)
23. Mesepimeral tuft: 0) present; 1) absent. (Wood & Borkent 1982)

TABLE 1. Continued

24. Basal radial cell length measured from base of cell: 0) equal to about half the length of wing; 1) markedly less than half wing length. (Adler *et al.* 2004)
25. Mating behavior: 0) mating via aerial swarms; 1) females rest on leaf undersides while males search underneath for them. (Adler *et al.* 2004)
26. Maxillary palpus of first-instar larvae with sensilla positioned: 0) apically; 1) subapically. (Adler *et al.* 2004)
27. Larval abdominal segment IX: 0) venter simple without noticeable cuticular projections; 1) venter with single, large, conical, ventral lobe. (Adler *et al.* 2004)
28. Habitat: 0) surface-flowing streams; 1) hypogean. (Adler *et al.* 2004)
29. Larval pigment: 0) present; 1) absent. (Adler *et al.* 2004)
30. Branches of radial sector: 0) widely separated; 1) positioned near to one another. (Wood & Borkent 1982)
31. False vein: 0) simple or not divided; 1) forked apically. (Adler *et al.* 2004)
32. Katepisternum sulcus: 0) absent; 1) present. (Adler *et al.* 2004)
33. Female sternum X medially: 0) incompletely divided; 1) completely divided. (Adler *et al.* 2004)
34. Pupal felt chamber of gill: 0) present; 1) absent. (Adler *et al.* 2004)
35. Pupal spiracle corresponding: 0) directly with plastron; 1) with lumen of plastron. (Adler *et al.* 2004)
36. Pupal sternites VI & VII, & sometimes more: 0) not divided; 1) medially divided by a semimembraneous longitudional striate region. (Adler *et al.* 2004)
37. Pupal abdominal tergites V to IX: 0) without such spine combs; 1) each bearing an anterior row of spine combs. (Adler *et al.* 2004)
38. Larval antennae: 0) consisting of 1 article; 1) consisting of 3 articles. (Adler *et al.* 2004)
39. Larva with a postgenal cleft: 0) absent; 1) present. (Adler *et al.* 2004)
40. Lateral sclerite of prothoracic proleg: 0) absent; 1) present. (Adler *et al.* 2004)
41. Pupal abdominal segments IV and V: 0) without any such plates; 1) bearing large pleural plates. (Adler *et al.* 2004)
42. Larval antennae with basal two segments: 0) pigmented; 1) unpigmented, contrasting with black apical segment. (Adler *et al.* 2004)
43. Larval paralateral teeth of hypostom: 0) present; 1) absent. (Adler *et al.* 2004)
44. Anteromedian palatal brush of first-instar larva: 0) with simple hairs; 1) with fringed hairs arranged in scoop shape. (Adler *et al.* 2004)
45. Maxillary papal sensilla of first-instar larva: 0) apically positioned and in circular pattern; 1) positioned subapically and in a linear pattern. (Adler *et al.* 2004)
46. Hypogynial valves: 0) markedly shorter and truncate; 1) elongate, with anteromedial corner bearing a nipple-like projection. (Adler *et al.* 2004)
47. Spermatheca: 0) without a differentiated area or with the differentiated area reduced at connection with spermathecal duct, 1) with a large differentiated area at connection with spermathecal duct. (Adler *et al.* 2004)
48. Lateral sclerite of prothoracic proleg: 0) narrow without a vertical extension; 1) wide with vertical section distinct. (Adler *et al.* 2004)
49. Paramere: 0) generally larger; 1) distinct, but reduced.

TABLE 1. Continued

50. Chromosomes: 0) without fixed inversions in IIIL (IIIL-1) and IIS; 1) with fixed inversions in IIIL (IIIL-1) and IIS. (Adler *et al.* 2004)
51. Larval mandible bearing apical brush consisting of: 0) slender hairlike setae; 1) bristle-like setae. (Adler *et al.* 2004)
52. Anal sclerite: 0) without such a projection between posteroventral arms; 1) with a short posteromedially pointed projection between posteroventral arms. (Adler *et al.* 2004)
53. Female cercus: 0) broader than long and subrectangular; 1) long, and apically narrowing to a fine point. (Adler *et al.* 2004)
54. Gonostylus base: 0) simple, without such a lobe; 1) bearing a lateral accessory lobe. (Adler *et al.* 2004)
55. Larval basal antennal two articles: 0) non-pigmented among the Prosimuliini; 1) reversed to pigmented state. (Adler *et al.* 2004)
56. Pupal abdominal tergites IV and V with anterior line of spine combs: 0) present; 1) absent. (Adler *et al.* 2004)
57. Larva with labral fans in 2^{nd} through final instar: 0) present; 1) absent. (Adler *et al.* 2004)
58. Larval head with posterior frontolabral muscles: 0) of one fascicle; 1) separated into two fascicles. (Adler *et al.* 2004)
59. Larval mandible apically: 0) slender, with teeth more conical and of unequal lengths; 1) wide, with compressed, equally sized apical teeth. (Adler *et al.* 2004)
60. Larval mandible with large apical brush: 0) with comblike reduced in size and number; 1) extensive, and composed of many rows of comblike scales. (Adler *et al.* 2004)
61. Larval mandible with covering brush of first external brush, and second external brush: 0) present and not reduced, 1) reduced or lost. (Adler *et al.* 2004)
62. Larval abdomen with segments I-IV: 0) not corrugate and more gently tapered; 1) thin and corrugated ventrally, expanded abruptly at segment V, and then narrowed posteriorly to a slight posterior proleg. (Adler *et al.* 2004)
63. Female tergite IX: 0) not extended shieldlike over each circus and subtriangular in shape, not as pointed; 1) extended like shield tapered to a point over each circus. (Adler *et al.* 2004)
64. Female lateral plate of genital fork: 0) connected via sclerotized structures directly to tergite IX; 1) not connected directly to tergite IX. (Adler *et al.* 2004)
65. Male ventral plate of aedeagus: 0) with much longer basal arms; 1) with short basal arms compared to length of ventral plate. (Adler *et al.* 2004)
66. Larval hypostoma with lateral serrations in form of: 0) short, rounded denticles; 1) spiniform setae. (Adler *et al.* 2004)
67. Anal sclerite: 0) X-shaped; 1) with prominent supernumerary arm extended posteromedially, forming an inverted shape of an star. (Adler *et al.* 2004)
68. Antenna composed of: 0) 9 flagellomeres; 1) 7 flagellomeres. (Adler *et al.* 2004)
69. Stematic bull alocated adjacent to posterior margin of compound eye: 0) absent; 1) present. (Adler *et al.* 2004)
70. Female tarsal claw with basal or subbasal tooth: 0) present; 1) absent. (Adler *et al.* 2004)

TABLE 1. Continued

71. Pupal abdominal segment III: 0) divided laterally by plueral membrane; 1) ringlike, with tergite and sternite merged laterally. (Adler *et al.* 2004)
72. Pupal abdominal tergites III and IV with recurved hooks: 0) present; 1) lost or reduced in quantity or lost, and positioned between middle of tergite and its posterior margin. (Adler *et al.* 2004)
73. Pupal abdominal tergites V-IX with anterior row of spine combs: 0) present; 1) absent. (Adler *et al.* 2004)
74. Larval anal sclerite with posteroventral arms: 0) present; 1) rudimentary or absent. (Adler *et al.* 2004)
75. Larval anal sclerite: 0) subrectangular and giving rise to a pair of both anterodorsal and posteroventral arms; 1) with anterodorsal arms arising from long common stalk, creating a Y-shaped formation. (Adler *et al.* 2004)
76. Ventral plate of aedeagus near base of anterolateral apodeme: 0) not strongly notched laterally; 1) strongly notched laterally. (Adler *et al.* 2004)
77. Gonostylus: 0) with 2 spinules; 1) with 1 apical spinule. (Adler *et al.* 2004)
78. Larval mandible: 0) medially curved distally; 1) not distinctly curved medially near apex. (Adler *et al.* 2004)
79. Pupal cocoon: 0) woven silk sac that does not retain the larval exuvia; 1) a clear gelatinous casing that envelopes entire pupa, retaining larval exuvia. (Adler *et al.* 2004)
80. Adult vestiture: 0) primarily soft, with few coarse upright setae; 1) meager, petite, and erect, not dense fine recumbent setae. (Adler *et al.* 2004)
81. Adult clypeus: 0) entirely clothed in setae; 1) nearly devoid of setae, none medially. (Adler *et al.* 2004)
82. Female mandible and lacinia with teeth: 0) present; 1) absent. (Adler *et al.* 2004)
83. Adult thorax : 0) more strongly arched; 1) weakly arched. (Adler *et al.* 2004)
84. Adult Postnotum: 0) large and not severely arched; 1) relatively small and markedly arched, with a variously developed median longitudinal ridge. (Adler *et al.* 2004)
85. Adult mesepimeron with vestiture: 0) more extensive in other Prosimuliini; 1) limited to dorsal region of the sclerite above the level of the metathoracic spiracle. (Adler *et al.* 2004)
86. Wing membrane: 0) hyaline and less wrinkled in other Prosimuliini; 1) smoky colored and slightly furrowed. (Adler *et al.* 2004)
87. Petiole of M_{1+2}: 0) one fifth length of petiole of radial sector in other Prosimuliini; 1) long, approximately half length of petiole of radial sector. (Adler *et al.* 2004)
88. Spermatheca base: 0) connected to duct via a membranous ring in other Prosimuliini; 1) with short or long sclerotized collar where spermathecal duct is joined. (Adler *et al.* 2004)
89. Female anal lobe and cercus: 0) separated by membrane; 1) merged into one solid sclerite. (Adler *et al.* 2004)
90. Male gonostylus: (0) relatively broad throughout with a more rounded apex in other Prosimuliini; 1) thin apically, with a minute apical spinule. (Adler *et al.* 2004)
91. Pupal abdominal sternites IV-VII: 0) each with less than 5 hooks in other Prosimuliini and most Simuliidae; 1) each bearing up to 5 pairs of recurved hooks. (Adler *et al.* 2004)

TABLE 1. Continued

92. Pupal abdominal pleurite IV: 0) with distinguishable pleurite on segments IV and V in other Prosimuliini; 1) barely distinguishable from adjoining tergite and sternite. (Adler *et al.* 2004)
93. Larval labrum and labropalatum: 0) less drastically narrowed; 1) distinctively longer than wide. (Adler *et al.* 2004)
94. Radial sector: 0) more definitively branched and not positioned apically; 1) unbranched or extremely small and apically forked. (Adler *et al.* 2004)
95. Male straplike link joining the paramere and ventral plate arising subapically on anterolateral apodeme of ventral plate: 0) absent; 1) present. (Adler *et al.* 2004)
96. Female genital fork with lateral plate with a variously formed dorsal apodeme: 0) absent; 1) present. (Adler *et al.* 2004)
97. Gonocoxite: 0) less enlarged and angulate; 1) massive, sub-trapezoidal.
98. Larval rake-like fans modified for scraping: 0) absent; 1) present.
99. Larval abdominal segment VIII with transverse midventral bulge: 0) absent; 1) present. (Adler *et al.* 2004)
100. Form of pupal: 0) less variation in pupal gill diversity; 1) gill diverse, and differentiated from all other simuliids. (Adler *et al.* 2004)
101. Tibial spurs of hind leg: 0) subequal in length or shorter than the tibia without hyaline apex; 1) distinctly long and with a hyaline apex. (Adler *et al.* 2004)
102. Male paramere with apical spines: 0) absent; 1) present. (Adler *et al.* 2004)
103. Larval apical antennal segment with extremely characteristic dark cuticular striations: 0) absent; 1) present.
104. Pupal cocoon: 0) well developed, or not reduced in such a way, 1) rudimentary, consisting of a few silk threads entwined in grapnel setae of segment IX.
105. Larval postgenal cleft extended anteriorly about: 0) 1/4 or less distance to hypostomal groove; 1) 1/3 or more distance to hypostomal groove. (Adler *et al.* 2004)
106. Larval hypostomal teeth: 0) larger and organized on prominent lobes; 1) small, and not organized on prominent lobes. (Adler *et al.* 2004)
107. Pupal gill filaments radiating from bulbous central protuberance: 0) absent; 1) present. (Adler *et al.* 2004)
108. Larval labral-fan rays with distinctively elongate microtrichia intermingled with the normal-sized microtrichia: 0) absent; 1) present. (Adler *et al.* 2004)
109. Pupal cocoon: 0) an ill formed sac or if well formed than not as complete ventrally; 1) of definite form and complete ventrally.
110. Gill filaments: 0) not convergent apically; 1) convergent apically. (Moulton & Adler 1997)
111. Gill filaments with surface: 0) smooth or furrowed; 1) shingle-like. (Moulton & Adler 1997)
112. Pupal terminal abdominal spines: 0) thin and solid; 1) inflated and hollow. (Moulton & Adler 1997)
113. Larval mandible with inner subapical margin: 0) dentate; 1) smooth. (Moulton & Adler 1997)
114. Larval lateral abdominal segments V-VIII: 0) expand more gently; 1) drastically expanded ventrally and laterally. (Moulton & Adler 1997)

TABLE 1. Continued

115. Anal sclerite: 0) present; 1) absent. (Moulton & Adler 1997)
116. Larval construction of a long silk pedicel to serve as a feeding and pupation substrate: 0) absent; 1) present. (Moulton & Adler 1997)
117. Katepisternal sulcus: 0) wide, shallow and indistinct anteriorly; 1) narrow and deep its entire length. (Adler *et al.* 2004)
118. Pupal abdomen: 0) strongly sclerotized; 1) weakly sclerotized, often losing its form after emergence. (Adler *et al.* 2004)
119. Basal radial cell length compared to the distance from the base of radial sector to wing apex: 0) greater than 1/3; 1) less than 1/3. (Adler *et al.* 2004)
120. Rectal papillae: 0) simple; 1) mostly compound. (Adler *et al.* 2004)
121. Pupal cocoon: 0) slipper shaped or amorphous of coarser silk; 1) boot shaped and spun of delicate silk. (Adler *et al.* 2004)
122. Larval postgenal cleft: 0) shorter and more narrow, not extended to the hypostomal groove; 1) wide and deep, extended anteriorly to or beyond hypostomal groove. (Adler *et al.* 2004)
123. Larval antenna with basal and medial articles: 0) pigmented in other Simuliini; 1) unpigmented, contrasting with dark apical article. (Adler *et al.* 2004)
124. Chromosomes with whole-arm interchange between chromosomes I and II: 0) absent; 1) present. (Adler *et al.* 2004)
125. Gonostylus subtruncate, with internal triangular lobe bearing one apical spinule, without very large laterally directed parameral spine: 0) absent; 1) present. (Adler *et al.* 2004)
126. Larval Hypostoma: 0) without such arrangement; 1) with of 6-7 pairs of teeth plus median tooth arranged on three prominent lobes, and paralateral teeth present.
127. Vein CuA_2: 0) strongly sigmoidal; 1) not strongly sigmoidal. (Adler *et al.* 2004)
128. Adult thorax: 0) not so strongly arched; 1) extremely strongly arched.
129. Male paramere: 0) large to small, but always distinct; 1) apparently represented by only apical spines. (Adler *et al.* 2004)
130. Female genital fork with anteriorly directed apodemes: 0) more reduced; 1) extremely elongate. (Adler *et al.* 2004)
131. Vein A_1: 0) curved; 1) straight. Modified from (Adler *et al.* 2004)
132. Larval mandible bearing: (0) two or four outer teeth; (1) three outer teeth. (Wygodzinsky & Coscarón 1973)
133. Anal sclerite with posterior arms expanded ventrally around abdomen, creating a complete ring encircling the base of posterior circlet of hooks: (1) present; (0) absent. (Adler *et al.* 2004)
134. Genital fork: (1) with a short stem anterior arms broad; (0) without such a combination.
135. Larval anal sclerite T-shaped; (1) present; (0) absent.
136. Female claws with markedly curved laterally directed basal tooth: (1) present; (0) absent.
137. Larval mandible serration and sensillum: (1) complex; (0) more simple. (Gil-Azevedo & Maia-Herzog 2007)

TABLE 1. Continued

138. Genital fork: (1) nearly unpigmented with short and wide stem; (0) pigmented and not shaped as such. (Coscarón & Coscarón 2007)
139. Male paramere: (1) absent; (0) present. (Craig 2012)
*140. Anal sclerite with median notch closed posteriorly to create a circle: 0) absent; 1) present.

*Indicates synapomorphy of *P. strenua* and *P. aurantiaca* used in the constructing the phylogenetic tree in figure 1, but not in the generic level sorted data matrix of characters in table 2.

TABLE 2. Sorted character state matrix for southern hemisphere Simuliidae. 1=Apomorphic character state, 0= Plesiomorphic character state, CA=*Cardiocladius*, TH=*Thaumalea*, PA=*Parasimulium*, PR=*Prosimulium*, HE=*Helodon*, UR=*Urosimulium*, LE= *Levitinia*, TW=*Twinnia*, GY=*Gymnopais*, CR=*Crozetia*, TL= *Tlalocomyia*, ST=*Stegopterna*, GR=*Greniera*, CN=*Cnephia*, ET=*Ectemnia*, SM=*Simulium*, ME=*Metacnephia*, SU=*Sulcicnephia* PC=*Paracnephia*, PS=Taxon A, CS=*Cnesia*, GX=*Gigantodax*, EO=Taxon B. PU=*Paraustrosimulium*, AU=*Austrosimulium.*

Numbers in first column correspond to character numbers in Table 1.

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	TL	ST	GR	CN	ET	SM	ME	SU	PC	PS	CS	GX	EO	PU	AU
1.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	TL	ST	GR	CN	ET	SM	ME	SU	PC	PS	CS	GX	EO	PU	AU
21.	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
22.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
31.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
32.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
33.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
34.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
35.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
36.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
37.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
38.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
39.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
40.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
41.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45.	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46.	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	TL	ST	GR	CN	ET	SM	ME	SU	PC	PS	CS	GX	EO	PU	AU
47.	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48.	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.	0	0	0	0	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50.	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51.	0	0	0	0	0	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52.	0	0	0	0	0	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53.	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54.	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55.	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62.	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63.	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64.	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65.	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66.	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67.	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	TL	ST	GR	CN	ET	SM	ME	SU	PC	PS	CS	GX	EO	PU	AU
73.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75.	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76.	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77.	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78.	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79.	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93.	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94.	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
95.	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
96.	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
97.	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98.	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	TL	ST	GR	CN	ET	SM	ME	SU	PC	PS	CS	GX	EO	PU	AU
99.	0	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
100.	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101.	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
102.	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
103.	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
104.	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
105.	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0
106.	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0
107.	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
108.	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
109.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	0	0	0	0	0	0	0
110.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
111.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
112.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
113.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
114.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
115.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
116.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
117.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0
118.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	0	0	0	0	0	0	0
119.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
120.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
121.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0
122.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	0	0	0	0	0
123.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0
124.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0

125.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0
126.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0
127.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**	**1**	0	0
128.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0
129.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	0
130.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	0	0	0
131.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0
132.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0
133.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0
134.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**	**1**
135.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0
136.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0
137.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**1**
138.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0
139.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**

Table 3. Morphological Comparison of Taxon A and Taxon B to Related Genera

Character	***Austrosimulium***	***Paraustrosimulium***	**Taxon B**	**Taxon A**	***Paracnephia* (from Africa)**
Female claw with strong basal tooth	Sometimes	Medium-sized	X	X	X
Outwardly pointed basal claw			X		
Larval ventral papillae absent	X	X	X	X	X
CuA2 vein	Strongly sinuous	Strongly sinuous	Nearly straight	Gently sinuous	strongly sinuous
Large conspicuous calicpala	X	X	X	X	
Shallow, but distinct pedisulcus	Distinct	large and distinct	X	X	
Palpal terminal segment same length as other two combined				X	
Female abdomen strongly hirsute				X	
Gonostylus apical spines	2 to 4	2 to 4	???????	2	4
Thorax strongly arched				X	
Genital fork with short and broad arms and stem	X	X			
Genital fork with short arms and stem and broad arms only			X		
Cerci concavely excavate on inner margin, and paraprocts long				X	
Larval hypstoma of 3 prominent lobes					X
Anal sclerite	X-shaped with backward directed struts	X-shaped with backward directed struts	T-shaped	X-shaped	X-shaped

Acknowledgements

This research was supported by the Hatch Multistate Research Fund and the New Jersey Agricultural Experiment Station at Rutgers University, New Brunswick, New Jersey, Project: Evaluating the Physical and Biological Availability of Pesticides and Contaminants in Agricultural Ecosystems Project 224316 and NIEHS Center Grant ES020522.

Literature cited

Adler, P. H., Currie, D. C., & Wood, D. M. (2004). *The black flies (Simuliidae) of North America*. Cornell University Press Ithaca, New York, 941 pp.

Adler, P. H., & Crosskey, R.W. (2014). World Blackflies (Diptera: Simuliidae): a Comprehensive Revision of the Taxonomic and Geographical Inventory 2014, 120 pp. Available from: http://www.clemson.edu/cafls/departments/esps/biomia/pdfs/blackflyinventory1.pdf (Accessed 9 January 2014).

Azer, D., Veltz, I., & Nel, A. (2008). Mandibulate chironomids: primitive or derived? (Diptera: Chironomidae). *Systematic Entomology*, 33, 688-699.

Bertone, M. A, Courtney, G. W., & Wiegmann, B.M. (2008). Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes *Systematic Entomology*, 33, 668-687

Borkent, A., & Wood, D. M. (1986). The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). *Proceedings of the Entomological Society of Washington*, 88, 287-296.

Coscaron, S., & Coscaron Arias C. L. (2007). *Neotropical Simuliidae (Diptera: Insecta). Aquatic Biodiversity in Latin America Vol 3. Neotropical Simuliidae.* Pensoft Publishers Bulgaria, 685 pp.

Craig, D. A., Craig, R. E. G., & Crosby, T. K. (2012). Simuliidae (Insecta: Diptera). *Fauna of New Zealand*, 68, 336 pp.

Crosskey, R.W. (1969). A re-classification of the Simuliidae (Diptera) of Africa and its islands. *Bulletin of the British Museum (Natural History), Entomology*, Supplement 14, 1–195.

Crosskey, R. W., & Howard, T. R. (1997). *A new taxonomic and geographical inventory of world blackflies (Diptera: Simuliidae).* Natural History Museum, London, 144 pp.

Drummond, F.H.N. (1931). West Australian Simuliidae. *Journal of the Royal Society of Western Australia,* 18, 1–12.

Dumbleton, L. J. (1962). Taxonomic characters in the pre-adult stages of Simuliidae. *New Zealand Journal of Science,* 5, 496–506.

Dumbleton, L. J. (1963). The classification and distribution of the Simuliidae (Diptera) with particular reference to the genus *Austrosimulium*. *New Zealand Journal of Science,* 6, 320–357.

Dumbleton, L. J. (1964). The first instar larva in the genus *Austrosimulium* (Diptera: Simuliidae).*New Zealand Journal of Science,* 7, 32–37.

Freeman, P., & de Meillon, B. (1953). *Simuliidae of the Ethiopian region.* British Museum Natural History, London, 224 pp.

Gil-Azevedo, L. H., & Maia-Herzog, M. (2007). Preliminary considerations on phylogeny of Simuliidae Genera from Southern Hemisphere (Insecta, Diptera). *Zootaxa*, 1643, 39-68.

Hennig, W. (1973). Diptera (Zweiflügler). *In*: Helmcke, J. G., Starck, D., & Wermuth, H. (Eds), *Handbook of Zology*. Walter d Gruyter Berlin, 337 pp.

Mackerras, M. J., & Mackerras, I. M. (1948). Simuliidae (Diptera) from Queensland. *Australian Journal of Scientific Research* (B), 1, 231–270.

Mackerras, I. M., & Mackerras, M. J. (1949). Revisional notes on Australasian Simuliidae (Diptera). *Proceedings of the Linnaean Society of New South Wales*, 73, 372–405.

Mackerras, M. J., & Mackerras, I. M. (1950). Notes on Australasian Simuliidae (Diptera). II. *Proceedings of the Linnaean Society of New South Wales,* 75, 167–187.

Mackerras, I. M., & Mackerras, M. J. (1952). Notes on Australasian Simuliidae (Diptera). III. *Proceedings of the Linnaean Society of New South Wales,* 77, 104–113.

Mackerras, M. J., & Mackerras, I. M. (1955). Notes on Australasian Simuliidae (Diptera). IV. *Proceedings of the Linnaean Society of New South Wales,* 80, 105–112.

Moulton, J. K., & Adler, P. H. 1(997). The genus *Ectemnia* (Diptera: Simuliidae): taxonomy, polytene chromosomes, new species, and phylogeny. *Canadian Journal of Zoology,* 121, 1-57.

Moulton, J. K., Adler, P. H. & Prince, J. (2004). An unusual new species of *Paracnephia* Rubtsov (Diptera: Simuliidae) from Western Australia. *Zootaxa*, 409, 1–12

Oosterbroek, P., & Courtney, G.W. (1995). Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, 115, 267-311.

Pawlowski, J., Szadziewski, R., Kmieciak, D., Fahmi, J., & Bittar, G. (1996). Phylogeny of the infraorder Culicomorpha (Diptera) based upon 28S RNA gene sequences. *Systematic Entomology*, 21, 167-178.

Rubtsov, I. A. (1962). Genera of buffalo-gnats (Fam. Simuliidae) in the fauna of the Ethiopian Region. *Zoologichesky Zhurnal*, 41, 10, 1488–1502.

Swofford, K. L. (1999). *PAUP 4.0: phylogenetic analysis with parsimony (and other methods), version 4.0 b2a*. Sunderland, Massachusetts Sinauer Associates Inc.

Taylor, F. H. (1927). A note on *Simulium bancrofti* Taylor, with the description of a new species of Simulium (Dipt.). *Bulletin of Entomological Research,* 18, 70–72.

Tonnoir, A. L. (1925). Australasian Simuliidae. *Bulletin of Entomological Research*, 15, 213–255.

Wallace, A. R. (1876). *The geographical distribution of animals; with a study of the relations of living and extinct faunas as elucidating the past changes of the Earth's surface*. New York: Harper & Brothers. Volume 1, 503 pp.

Wood, D. M., & Borkent, A. (1982). Description of the female of Parasimulium crosskeyi Peterson (Diptera: Simuliidae) and a discussion of the phylogenetic position of the genus. Memoirs of the Entomological Society of Washington, 10, 193-210.

Wood, D. M., & Borkent, A. (1989). *Nearctic Diptera Manual, Vol. 3. Phylogeny and classification of the Nematocera.* Agriculture Canada, Monograph 32, 37 pp.

Wygodzinsky, P., & Coscarón, S. (1973). A review of the Mesoamerican and South American black flies of the tribe Prosimuliini (Simuiiinae, Simuliidae). *Bull. Amer. Mus. Nat. Hist*., 151, 129-200.

The Higher Level Molecular and Morphological Phylogenetics of the Simuliidae

ABSTRACT

A higher level phylogeny of the Simuliidae is proposed with 20 of the known world genera. This study includes the first combined molecular and morphological based phylogeny of all but seven of the Simuliidae genera from the northern and southern hemispheres. The first analysis of paralogy in the simuliid EF-1α is performed. A new alignment for simuliid 12S and 28S nucleotide sequences is proposed based on manual secondary structural alignment. Molecular analysis illustrates the long founded morphological relationship of *Parasimulium* as the immediate ancestor of all other Simuliidae by using two non simuliid outgroups. Congruence between morphological and molecular data reveals Simuliidae as ancestrally Holarctic, and *Crozetia* as a relict of an early simuliid invasion into the southern hemisphere. Strong branch support and congruence between molecular and morphological data confirm the subfamiliy and tribe divisions of the Simuliidae. Due to particularly strong branch support for the tribe Prosimuliini, it is raised to the subfamily Prosimuliinae, and a new combination for the subfamily Simuliinae is proposed.

Molecular evidence reveals a dichotomy between the higher Simuliini in the northern and southern hemispheres. Molecular and morphological analysis also recovers a derived placement for the genus *Austrosimulium.*

Key words: Simuliidae, Prosimuliinae, manual secondary structural alignment, paralogous genes, phylogenetic systematics.

Introduction

Not until the early 20th century did generic level distinctions arise in the Simuliidae (Roubaud 1906, Surcouf & Gonzalez-Rincones 1911). Spanning 1914 to 1943, Gunther Enderlain proposed 50 genera, and in his (1937) work he would propose 7 subfamilies and five tribes; Ectemniinae, Cnesiinae, Nevermanniinae (Friesiini, Nevermanniini, and Wilhelmiini), Prosimuliinae, Stegopteriinae, and Simuliinae (Odagmiini and Simuliini). Enderlain's contemporary, Frederick Edwards (1931) meanwhile decided to not recognize any supraspecific groupings, purported *Simulium* and *Parasimulium* Malloch to be the only two black fly genera, and recognized five subgenera within *Simulium*: *Austrosimulium* Tonnoir, *Cnephia*, Enderlein, *Gigantodax* Enderlein, *Prosimulium* Roubaud, and *Simulium* Latrielle.

Smart (1945) reviewed the classifications of Enderlein and Edwards, and was the first to raise *Parasimulium* to subfamily ranking on the basis of the widely separated radial sector vein. Smart also recognized the subfamily Simuliinae which included five genera. The discoveries of two new genera, *Gymnopais* Stone (1949) and *Twinnia* Stone and Jamnback (1955) offered new diversity to simuliid classification. Rubtzov (1955), who presumed simuliids to be derived from a fanless chironomid-like ancestor, considered the fanless larvae of these two genera to be a separate subfamily sister to the rest of Simuliidae. In 1966 Rothfels and Freeman using cytology were able to show the transition between *Prosimulium, Helodon*, *Twinnia*, and *Gymnpais*, but in this study they were not able to determine the polarity of the sequences from *Prosimulium* to *Gymnpais*, or vice versa. Grenier and Rageau (1960) were the first to construct a classification including *Gymnopais* and *Twinnia*. They organized the Simuliidae into the three subfamilies: Parasimuliinae, Prosimuliinae, and Simuliinae. They subdivided the Prosimuliinae into the tribes Prosimuliini, which consisted of Prosimulium s. str., and the Gymnopaidini, which contained *Twinnia* and *Gymnopais*. Grenier and Rageau's Simuliinae was comprised of three tribes; Cnephiini (*Cnephia* sensu Enderlein), Austrosimuliini (*Austrosimulium* and *Gigantodax*) and Simuiiini (*Simulium* s. 1.). Dumbleton (1963) devised a similar classification, but moved *Gigantodax* and *Cnephia* from the Simuliinae to the Prosimuliinae. Stone's (1965) work on the Nearctic would condense Grenier and Rageau's (1960) Parasimuliinae, Prosimuliini, and Gymnopaidini into the subfamily Prosimuliinae, leaving the Simuliinae composed of *Cnephia* Enderlein and *Simulium* s. 1.

Davies (1965, 1974) described the genus *Crozetia* and conducted a morphological comparison of it with other simuliids. Davies considered the rake-like fans of *Crozetia* to be primitive as he deemed the bristle-like labral fans of first instar *Gymnopais*, *Twinnia* and *Prosimulium* larvae to be primitive. Davies posited that evolution in simuliid labral fans followed two courses: one, the complete loss of fans as seen in *Gymnopais* and *Twinnia*, and two, the rakelike fans of *Crozetia* which he presumed eventenually gave rise to the filtering fans of typical simuliid larvae. Thus implying that *Gymnopais* and *Twinnia* were the sister group to all other simuliids. Wood (1978) assumed a different position, contending that fans were a paramount shared character of all simuliid larvae and some closely related families (Culicidae, Dixidae, Ptychopteridae, and Tanyderidae). Thus Wood considered the absence of fans in select Simuliidae and certain closely related families (Ceratopoginidae, Chaoboridae, Chironomidae, and Thaulmaleiadea) to be the result of a loss. Rubstov (1974) made one of the nascent attempts to elucidate simuliid phylogeny and integrate that data into a classification. Rubstov recognized 59 genera in the following four subfamilies: Parasimuliinae (*Parasimulium)*, Gynopaidinae (*Gymnopais* and *Twinnia*), Prosimuliinae (*Gigantodax, Paracnephia, Procnephia, and Prosimulium* s. l.), and Simuliina,e which was comprised of the remaining genera subdivided among the five tribes: Austrosimuliini, Cnephiini, Eusimuliini, Simuliini, and Wilhelmiini. This is classification followed by most Eastern European simuliid workers.

Though Wygodzinsky and Coscaron (1973) proposed potential synapomorphies for possible relationships among pairs of similar Neotropical genera, the 1980's would see the first concerted efforts at constructing simuliid phylogenies based on systematic phylogenetics (Hennig 1966), which was precipitated by the rediscovery of the genus *Parasimulium.* The ancient standing of *Parasimulium* has been determined morphologically by plesiomorphic wing venation, head, and terminalia character states (Smart 1945, Shewell 1958, Grenier & Rageau 1960, Crosskey 1969, Rubstov 1974, Peterson 1977). Wood and Borkent (1982) proposed the monophyletic group *Parasimulium* as sister to the rest of simuliidae, supporting previous research based on a phenetic approach. Currie (1988) conducted the first phylogenetic analysis using all simuliid life stages which consisted of 81 characters including: external and internal structures, polytene chromosomes, and ecological data. Currie's analysis confirmed the sister-group affiliation of *Parasimulium* (Parasimuliinae Smart) to the rest of Simuliidae (Simuliinae Newman), and provided characters for two monophyletic tribes within the Simuliinae: Prosimuliini Enderlein and Simuliini Newman. Currie determined the relationships within the Prosimuliini as follows: Prosimulium + Helodon s. 1. + (Urosimulium + (Levitinia + (Gvmnopais + Twinnia))) though there were no attempts made at resolving relationships within the Simuliini. Yankovsky (1992) attempted to compromise between the classifications of Rubstov (1974 a) and Crosskey (1981, 1988) and constructed a phylogeny with nine tribes and two subfamilies, Parasimuliinae and Simuliinae. The only other phylogenetic analysis of simuliids based on morphology was that of Py-Danial (1990), whose study of 20 New World black fly genera led to a two-subfamily classification, with *Gymnopais* Stone and *Twinnia* Stone & Jamnback constituting the Gymnopaidinae, and the remaining 18 genera placed within the Simuliinae. Gymnopaidinae was proposed as the sister taxa to Simuliinae based upon the argument that the absence of labral fans in these larvae was plesiomorphic within Simuliidae, but was considered a secondary loss by Wood (1978) and Currie (1988).

In the 1990's the first attempts were made to recreate simuliid phylogenies using molecular techniques (Tang et al 1995, Pruess et al. 1992, Xiong & Kocher 1991), but they failed to resolve the problems in simuliid phylogenetics, and suffered from small data sets, poor gene selection, insufficient taxon sampling, and questionable sequence alignments. More recently, Smith (2002) resolved relationships among species of the subgenus *Simulium* by using one nuclear and two mitochondrial genes. Moulton (1997, 2000, & 2002) conducted a comprehensive molecular analysis of 20 simuliid genera using four nuclear and two mitochondrial loci. Moulton's (1997) work confirmed many expected relationships, and revealed some unexpected groupings such as the basal placement of *Austrosimulium* Tonnoir within the Simuliini. According to Adler *et al.* (2004), an accurate tree of the Simuliidae is unlikely from a single source of data, and will require multiple independent data sources. The predominant taxonomy followed by North American simuliid workers today is that of Crosskey and Howard (1997), which recognizes two subfamilies: Parasimuliinae, comprising the single genus *Parasimulium* and Simuliinae, which includes all other simuliid genera.

This study conducts a morphological and molecular analysis of higher level simuliid phylogeny, confirming many relationships posited by morphologists, and offering new insights.

Materials and Methods

Twenty of the world genera of Simuliidae were analyzed both morphologically and molecularly along with the two outgroups: *Thaumalea* and *Cardiocladius* (Figure 1). Genera where there was no molecular evidence available coupled with a lack of specimens to analyze were excluded from the phylogeny: *Araucnephia, Araucnephioides, Cnesiamima, Lutzsimulium,* and *Pedrowygomyia.*

The D1-D3 and D9-D10 regions of the 28S nuclear large ribosomal subunit were selected because of their blend of conserved and variable regions, the latter applying especially to the D2 and D10, as well as their utility in reconstructing both shallow (Vossbrink 1989, Pelandakis & Solignac 1993) and deep (Perasso & Baroin Tourancheau 1993, Hoc *et al.* 1993) internodes. The protein coding nuclear gene EF-1α was chosen to resolve shallow internodes because of its relatively low substitution rates (Jordal 2002). The third domain of the 3' end of the 12S mitochondrial small ribosomal subunit was selected for its proven utility in phylogenetic studies of arthropods (Ballard et al. 1992) and cytospecies of black flies (Ballard 1994), and its relatively conserved evolutionary rate.

A search of GenBank (www.ncbi.nih.gov) for Simuliidae and outgroup 28S, EF-1α, and 12S sequences was conducted using, Entrez, National Center for Biotechnology Information's integrated search system. Outgroup selection was determined by both morphological (Hennig 1973, Wood and Borkent 1989, Oosterbroek and Courtney 1995) and molecular evidence (Pawlowski *et al*. 1996). Pawlowski *et al*. (1996) demonstrated the sister group relationship of Thaumaleidae to Simuliidae using 28S rDNA. A second outgroup to the Simuliidae was added with the chironomid genus *Cardiocladius* to increase the definition of the tree. A representative sample of 29 Simuliidae sequences (D1-D3 and D9-D10) were selected from 20 genera, along with outgroup examples from Thaumaleidae and Chironomidae with accession numbers from AF007295 to AF007318, AF007321 to AF007332, AF007343 to AF007344, AF007347 to AF007348, AF007351 to AF007364, and AF007371-AF007374 (Moulton 1997) (Table 1 and 2). A representative sample of 15 12S Simuliidae sequences were selected from 15 genera, along with outgroup examples of Thaumalidae and Charonomidae with accession numbers from AF49470 to AF49483, AF49485 (Moulton 1997), JQ793809(Conflitti, *et al.* 2012), and L02390 (Ballard *et al.* 1992) (Table 3). A representative sample of 29 Simuliidae EF1-alpha sequences were selected from 20 genera, along with outgroup examples of Thaumalidae and Charonomidae with accession numbers from AAF31050 to AAF31061, AAF31063 to AFF31076, AAF31079 to AAF31080, and AAF31087 (Moulton 1997) (Table 4). For morphological the analysis a thorough review of the literature was conducted for all specimens. In addition, physical specimens of adults, larvae, and pupae were analyzed for: *Simulium, Cnephia, Ectemnia, Prosimulium,* and *Stegopterna*. New characters as well as ones from the literature were used. A morphology matrix of 142 apparently non-correlated characters, from all life stages and select behavioral characters was also constructed (Table 5 and 6).

The 28S and 12S sequences were manually aligned by secondary structure. Manual secondary structural alignments are widely used and have demonstrated repeatable and reliable results (Kjer *et al.* 2007). The work of Gillespie *et al.* (2006) demonstrated the accuracy with which secondary and even some tertiary structures can be reconstructed with manual alignment utilizing the discoveries in crystal structures of the ribosome and chemical probing, and comparative analysis with published secondary structural alignment models. The 28S was manually aligned using the dragonfly model of *Ophiopetalia diana* (Carle unpublished) and the *Apis melifera* model of Gillespie *et al.* (2006) (Table 7 and 8). The 12S was aligned using the dragonfly model of *Ophiopetalia diana* (Carle unpublished) and *Drosophila virilis* (Roderic 2000) (Table 9). To make alignment possible all 12S sequences, except *Simulium murmanum* JQ793809 (Conflitti, *et al.* 2012) and *Austrosimulium bancrofti* L02390 (Ballard *et al.* 1992) had to be reverse complemented prior to alignment to visualize the data in accordance with 5' to 3' denotation of DNA. Regions of the 28S and 12S where nucleotide homologies could not be unambiguously aligned were eliminated prior to phylogenetic analysis.

The presence of paralogous genes in the insect EF-1α is a known complication to phylogenetic analyses using this marker (Danforth & Ji 1998, Djernaes & Damgaard 2006) and two paralogous copies of the gene have been identified in Diptera (Hovemann *et al.* 1988). The Simuliidae EF-1α sequences were compared to the *Drosophila melanogaster F1 and F2 sequences* of Hovemann *et al.* (1988) to determine what copy of the Simuliid, Thaumalid, and Chironomid EF-1α sequences were entered into genbank, and at what point those sequences were a chimera of the F1 and F2 copies of the gene (Table 10). As a result, 513 base pairs of each taxa's EF-1α F2 sequence were used for analysis. The EF-1α single gene tree was the only tree run with one outgroup, Thaumalea instead of *Cardiocladius*, to alleviate the problem of long branch attraction.

The 12S, which had less available sequences, was analyzed with a subset of 17 of the same taxa from the 28S and EF-1α. A combined 28S, 12S, and EF-1α analysis was also conducted with 28 taxa, and question marks were added for the missing 12S sequences.

The morphology trees were generated using a parsimony analysis in PAUP version 4.0 (Swofford 1999). The bootstrap method with heuristic search was performed with 1,000 replicates. All morphological characters received equal weight. The bootstrap 50% majority-rule was utilized and the most likely consensus tree was then generated. For comparison to the higher level molecular phylogenetics of Moulton (2002), two PAUP analyses were performed; one for the entire EF-1α, including chimeric F1/F2 sequences, and one for the 28S using a Clustal (Thompson *et al.* 1996) alignment.

Bayesian trees were generated by mrbayes v.3.2.2 (Ronquist and Huelsenbeck 2003). The rRNA (12S, 28S) partitions were run under a GTR+I+G substitution model (nst=6, rates=invgamma). The protein-coding (EF-1α) sequence data was partitioned into first, second, and third codon positions. The first and second codon positions were run under an HKY+G substitution model (nst=2 rates=gamma), and third positions under a GTR+G model (nst=6 rates=gamma). The morphology partitions used the Markov k model (Lewis 2001) with gamma rate parameter. The gamma shape, proportion of invariant sites, state frequencies, GTR substitution rates and rate prior were unlinked for all multi-partition analyses. Trees were sampled every 1,000 generations. The first 25% of the sample was discarded as burn-in.

Results

Seventeen character states support the monophyly of the family Simuliidae, and eight character states support the monophyly of the subfamily Parasimuliinae.

The 28S, 31 taxa tree was run on Mr. Bayes for 50,000,000 generations with a convergence diagnostic of 0.002841. The EF-1α, 30 taxa tree with no Cardiocladius was run for 80,000,000 with a convergence diagnostic of 0.001368. The combined 12S, 28S, EF-1α, and morphology 17 taxa tree was run for 130,000,000 generations with a convergence diagnostic of 0.007847. The DNA (12S, 28S, EF-1α) only 17 taxa tree was run for 80,000,000 generations with a convergence diagnostic of 0.00106. The combined 28S, EF-1α, morphology 31 taxa tree was run for 80,000,000 generations with a convergence diagnostic of 0.001019. The DNA (12S, 28S, EF-1α) only 28 taxa tree was run for 80,000,000 generations with a convergence diagnostic of 0.001751.

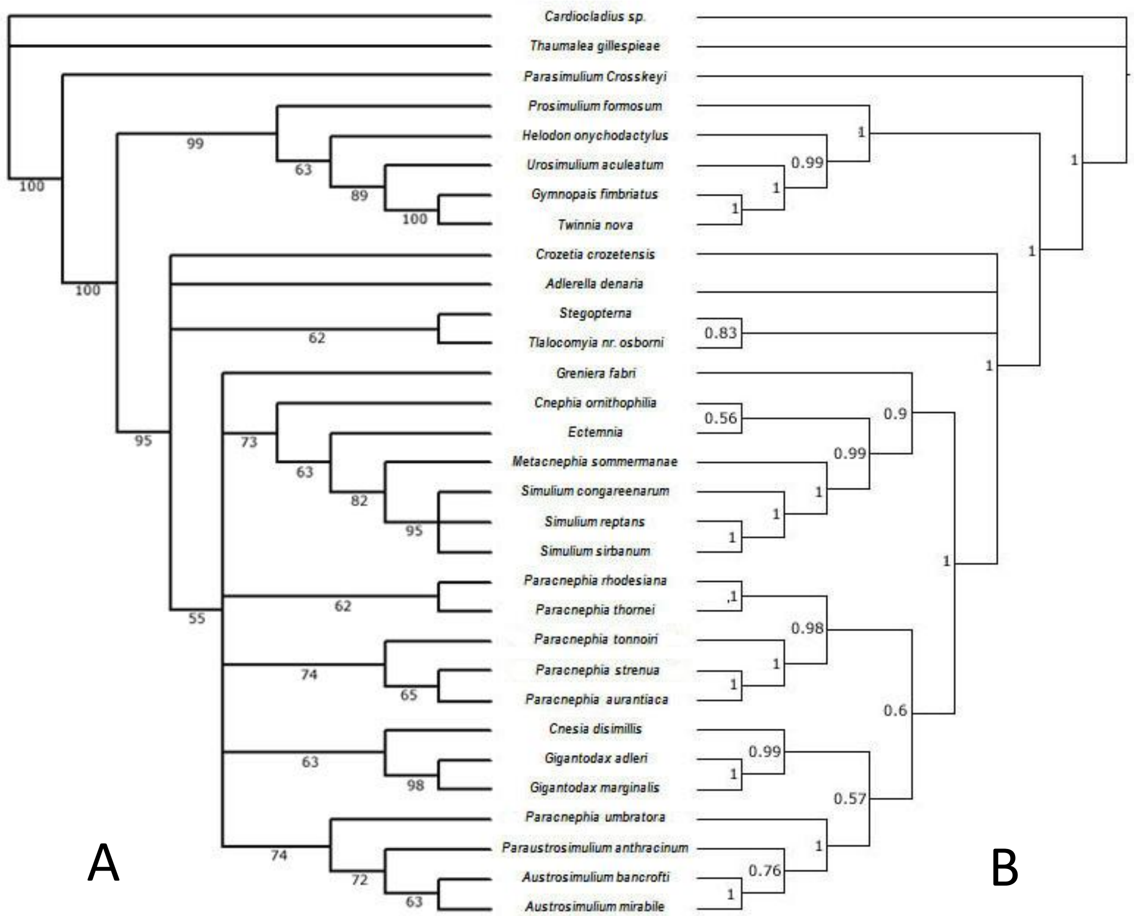

FIGURE 1. Congruence between morphology and combined molecular and morphology trees. **A**, PAUP morphology consensus tree with bootstrap values. **B**, MrBayes morphology, 28S, and EF-1α combined tree with posterior probabilities.

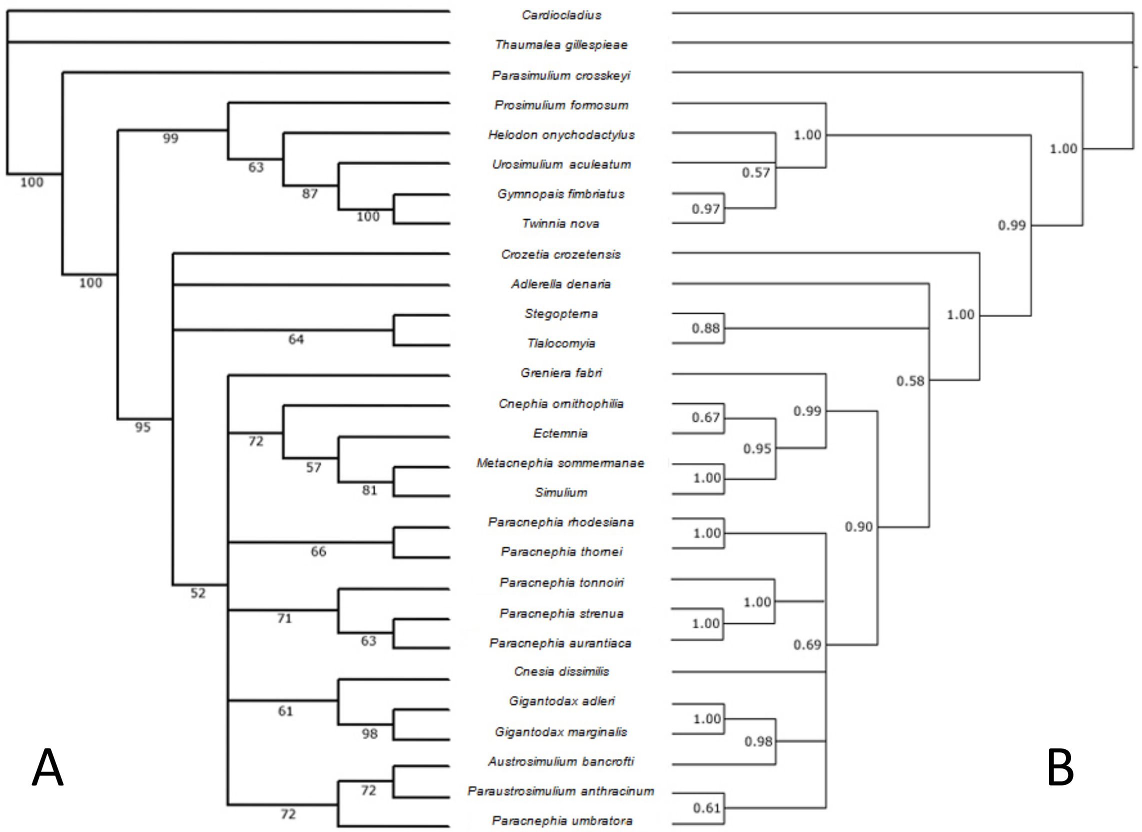

FIGURE 2. Congruence between morphology and molecular trees. **A**, PAUP morphology consensus tree with bootstrap values. **B**, MrBayes combined 28S, 12S and EF-1α DNA only tree with posterior probabilities.

All PAUP trees were run for 1,000 replicates. The CI for the 31 taxa morphology tree was 0.949. The CI for the 17 taxa morphology tree was .993. The CI for the 28 taxa morphology tree was .949. The two PAUP analyses for the entire EF-1α, including chimeric F1/F2 sequences, and for the 28S using a Clustal alignment, both resulted in the same problematic placement of *Austrosimulium* as basal to all other Simuliini as demonstrated by Moulton (2002).

All molecular and morphological trees recover the grouping of *Parasimulium* as sister to all higher Simuliidae, as morphologically posited by Wood and Borkent (1982) and confirmed by Currie (1988). The monophyletic subfamily Simuliinae, and tribes Prosimuliini and Simuliini were also recovered by all molecular and morphological trees in agreement with Moulton (2000). All molecular and morphological trees place *Crozetia* as basal within the Simuliinae in agreement with Craig *et al.* (2003) (Figure 1-6).

All morphological, combined DNA, and combined morphology plus DNA trees recover a derived placement for *Austrosimulium.* The single gene phylograms for the 28S and EF-1α reveal that *Austrosimulium* has the longest internode and among the longest branches of all other Simuliidae, making long branch attraction a problem (Figure 3 and 4). All morphological, combined DNA, and combine morphology plus DNA trees show *Crozetia, Adlerella, Stegopterna,* and *Tlalocomyia* as the immediate ancestors of the higher Simuliini: *Greniera, Cnephia, Ectemnia, Metacnephia, Simulium, Paracnephia, Cnesia, Gigantodax, Austrosimulium,* and *Paraustrosimulium* (Figure 1, 2, 3 and 4).

The base of the Simuliidae, Parasimuliinae, Prosiumuliini, and the basal four Simuliini are all genera of the Holarctic region. Both the 31 taxa combined 28S, EF-1α, plus morphology tree, and the 28 taxa 12S, 28S, EF-1α tree reveal a division between the higher Simuliini of the northern hemisphere and those of the southern hemisphere (Figure 1 and 2). The tree topologies of the 17 taxa set, for which all three genes, 12S, 28S, EF-1α were available for all taxa, were completely congruent with the morphological trees (Figure 5 and 6).

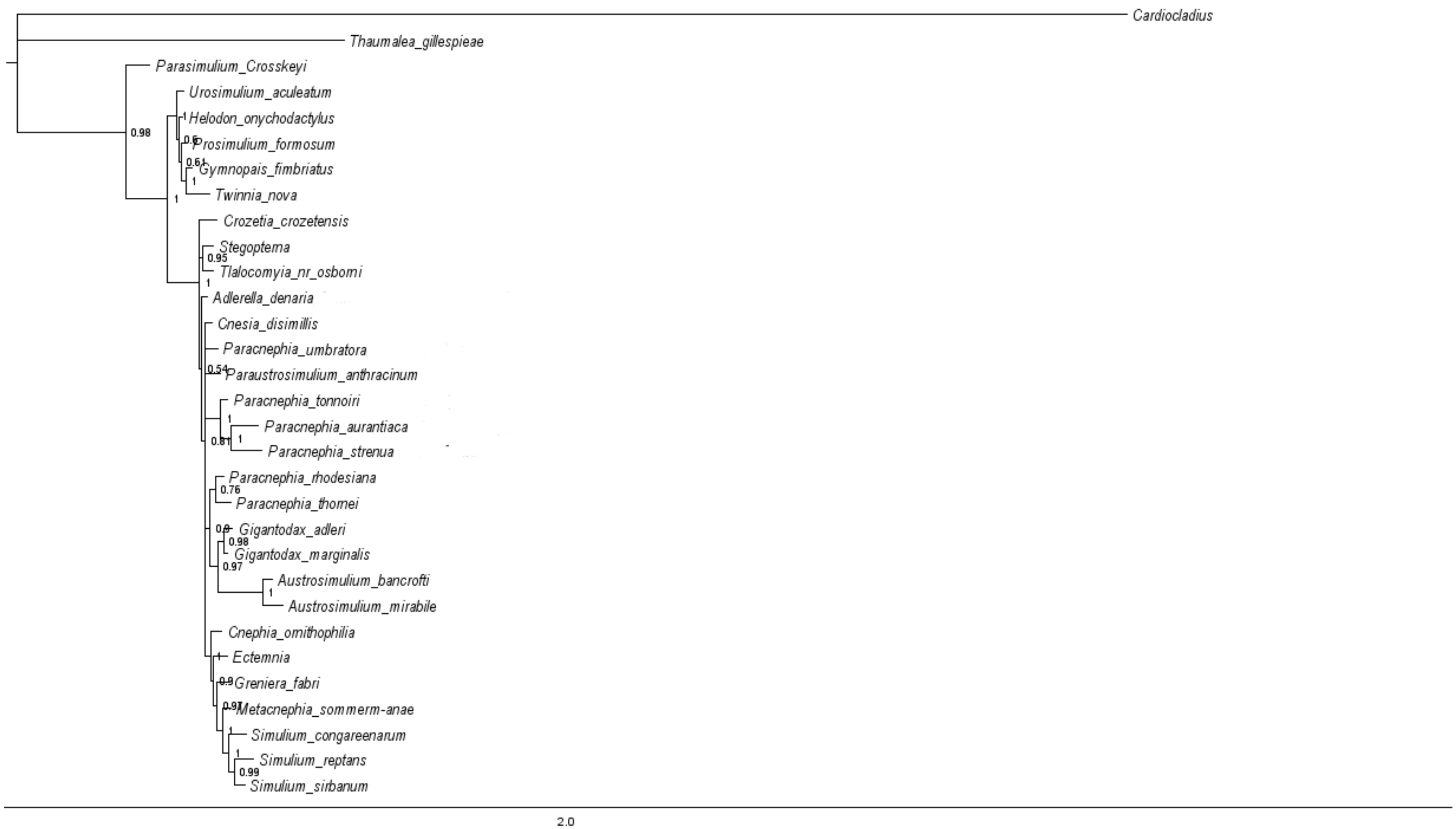

FIGURE 3. Phylogram of the 28S.

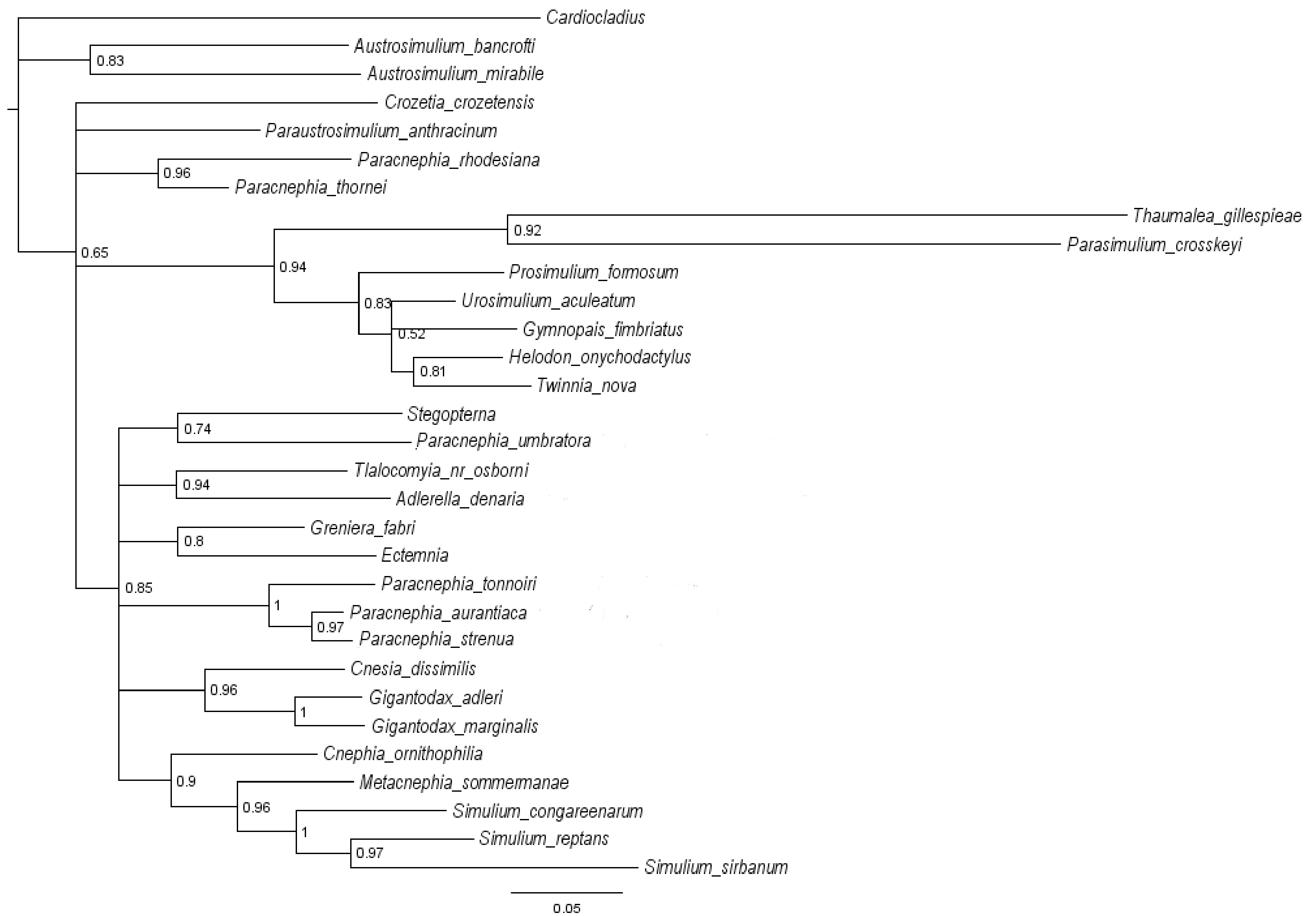

FIGURE 4. Phylogram of the EF-1α.

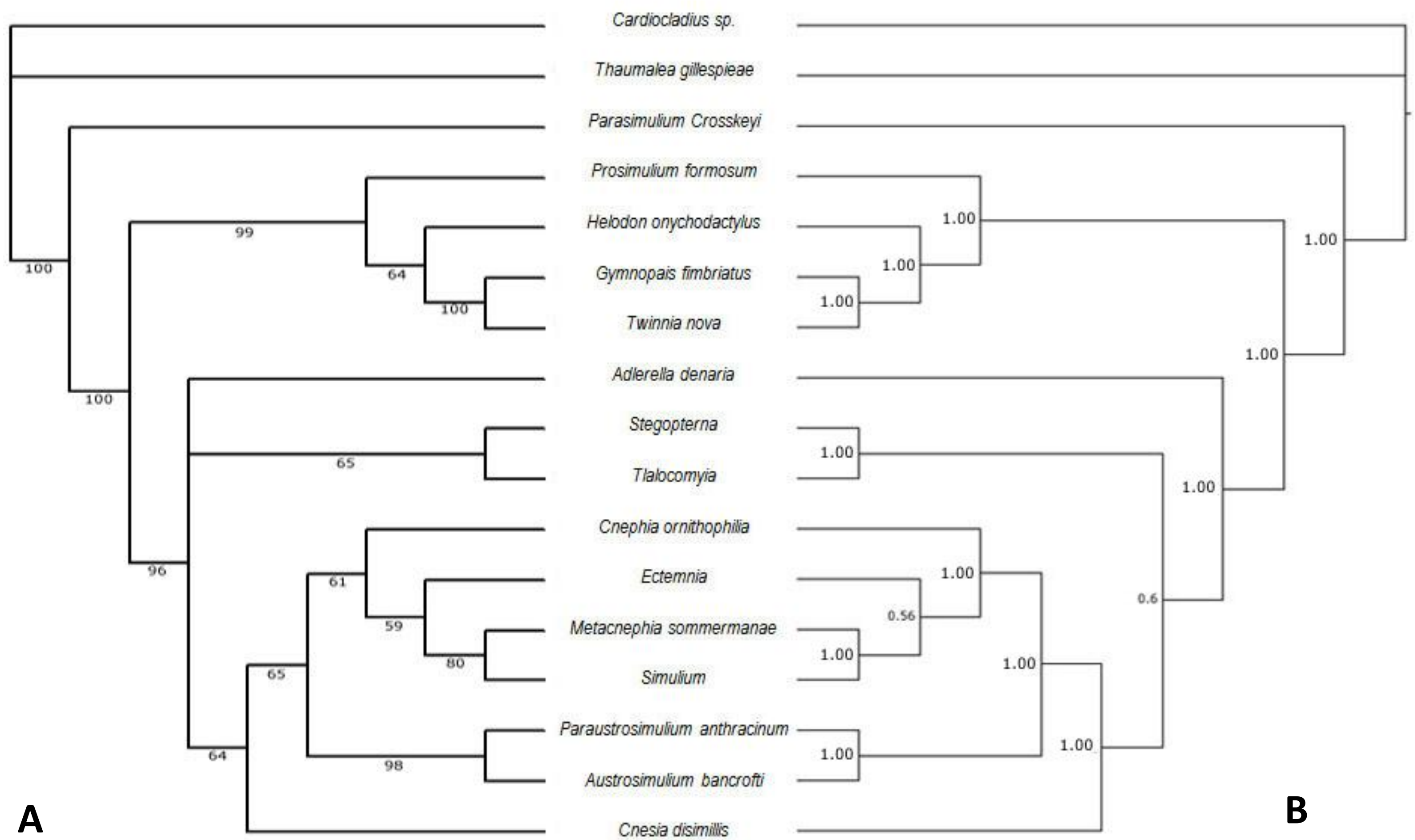

FIGURE 5. Congruence between morphology and morphology plus molecular data. **A**, PAUP morphology consensus tree with bootstrap values. **B**, MrBayes morphology, 28S, 12S and EF-1α combined tree with posterior probabilities.

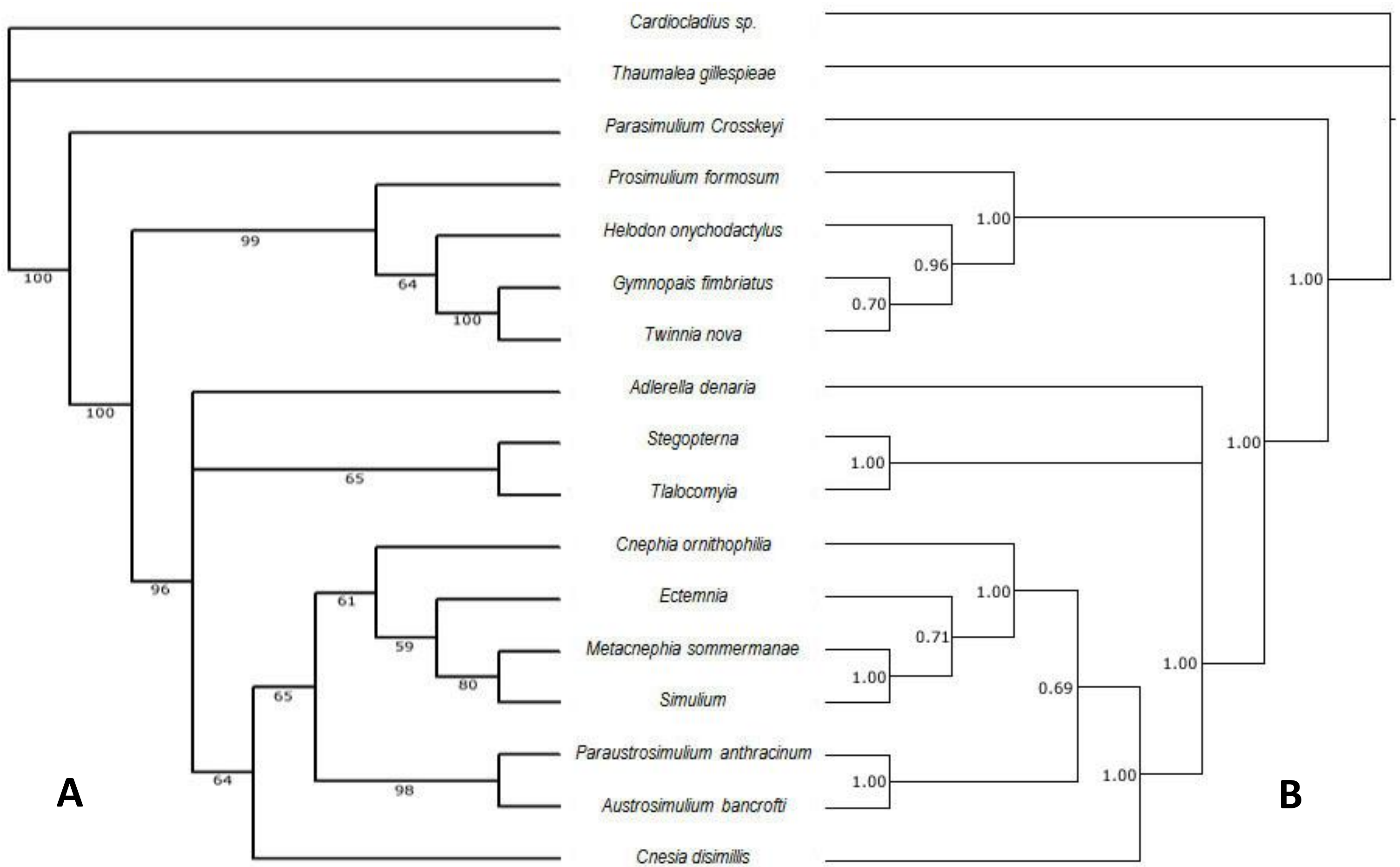

FIGURE 6. Congruence between morphological and molecular trees. **A**, PAUP morphology consensus tree with bootstrap values. **B**, MrBayes combined 28S, 12S, and EF-1α molecular tree with posterior probabilities.

Due to the high level of congruence between molecular and morphological data and branch support for the monophyletic group comprised of the genera *Prosimulium, Helodon, Urosimulium, Gymnopais,* and *Twinnia,* this new combination is established as the subfamily Prosimuliinae. *Levitinia* is also included in the new combination for Prosimuliinae due to the morphological synapomorphies. Also due to the high level of congruence and branch support in morphological and molecular data, the genera *Crozetia, Adlerella, Stegopterna, Tlalocomyia, Greniera, Cnephia, Ectemnia, Metacnephia, Simulium, Paracnephia, Cnesia, Gigantodax, Austrosimulium,* and *Paraustrosimulium* comprise the new combination for the subfamily Simuliinae. Other genera included in the new combination of the Simuliinae based on morphological synapomorphies are: *Sulcicnephia, Mayacnephia, Cnesiamima, Kempfsimulium, Lutzsimulium, Pedrowygomyia,* and *Araucnephioides.*

Prosimuliinae, sensu novum

Type genus: *Prosimulium* Roubaud

The Prosimuliinae is defined by five synapomorphies: pupal abdominal segments IV and V bearing pleural plates, larval antennae with medial and basal articles unpigmented contrasting with blackish-brown apical article, larval hypostomal paralateral teeth absent, first-instar larval anteromedian palatal brush composed of scoop-shaped fringed hairs, and the first-instar larval maxillary papal sensilla positioned subapically in a linear pattern.

Simuliinae, sensu novum

Type genus: *Simulium* Latrielle

The Simuliinae is defined by three synapomorphies: the vestigial fork of the radial sector vein, the genital fork of the female bearing a dorsal apodeme, and the paramere of the male with a straplike connection between the paramere and the ventral plate that arises subapically on the basal arm of the ventral plate.

Discussion

The current commonly used primers for the Simuliidae EF-1α reliably amplify the first 513 base pairs of the F2 copy of the gene with introns removed, but beyond that point is the region of primer overlap, and a chimera of the F1 and F2 sequences is amplified. However, by using the amino acid sequences of Hovemann *et al.* (1988) in editing Simuliidae EF-1α electrophoretograms the F1 and F2 copies of the gene can be distinguished from one another. In cases where only the edited sequences and not the electrophoretograms are available, the amino acid sequences of Hovemann *et al.* (1988) can be used to determine which base pairs are pure representations of a single gene copy of the EF-1α, and which copy that is.

The same way great care is needed to determine homology in morphological characters, so too is careful analysis required to determine the secondary structural homologies between sequences of the 28S and 12S using manual alignment. Utilizing manual secondary structural alignment along with exclusion of chimeric EF-1α sequences resulted in molecular tree topologies highly congruent with morphology. This was most clearly demonstrated in the 17 taxa trees, in which all 3 genes studied were used, and tree topologies between the morphology, molecular, and morphology plus molecular trees were identical (Figure 5 and 6). This is because the more genes with different rates of evolution that there are included in the analysis, the more levels of divergence that can be reconstructed, and the more constrained the data, which all results in a more accurate picture of phylogenetic relationships.

Simuliid taxa of the southern hemisphere are part of the new combination of the subfamily Simuliinae, while the two basal subfamilies, Parasimuliinae and Prosimuliinae are Holarctic in origin, showing Simuliidae to be an ancestrally northern hemisphere group. This mirrors the origin of the sister group of Simuliidae, Thaumaleidae, which is predominantly a Holarctic family. *Crozetia* appears to be a surviving relict of an initial invasion of a basal Simuliinae group (*e.g. Adlerella*, *Tlalocomyia*, and *Stegopterna*) possibly between 180 and 160 mya via a temperate montane path connecting North America to South America to Antarctica (Carle 1995). *Crozetia* could have made the jump to the Crozet Islands from Antarctica as late as 130 mya. Further study of the ocean floor may reveal Jurassic ages for extinct islands in the Crozet chain currently under sea level. This would make the true age of *Crozetia*'s inhabitance of this area older than the current age estimate for the Crozet Plateau of approximately 60-63 mya (Craig 2003).

All other southern hemisphere simuliids are most likely the evolutionary outshoot of a second invasion into the southern hemisphere. The global cooling period between the Jurassic and Cretaceous about 145 mya (Scotese 1999) was likely the catalyst for a second invasion of Simuliidae into the southern hemisphere, giving rise to a rapid ancient radiation. The particularly fast rates of molecular evolution within the southern hemisphere simuliids analyzed also support a sudden adaptive radiation of these taxa. The genus *Austrosimulium* in particular has the longest internode in all of the Simuliidae, which perhaps explains its questionable relationship to *Gigantodax* in the combined molecular tree (Figure 2) and the 28S phylogram (Figure 3). Without the conserved 12S data that groups *Paraustrosimulium* and *Austrosimulium* together in the 17 taxa trees (Figure 5 and 6), *Austrosimulium* can attach easily to unrelated genera due to long-branch attraction resulting from homoplasy. To obtain better resolution of the relationships among the southern hemisphere Simuliidae the 12S for all taxa should eventually be sampled.

The relationships of the Holarctic Simuliidae are particularly stable, especially as evidenced in the more basal taxa. This is reflected in the established morphological characters that support these topologies (Figure 1, 2, 5, and 6). The division of the higher Simuliinae into northern and southern hemisphere groups indicates that closer analysis of morphology should also reveal the characters that separate these taxa.

TABLE 1. GenBank EF1-alpha sequence information

Taxa	**Accession Version**	**GI**	**Authors**	**Year**
Catocala ultronia	AAC47593.1	2190639	Mitchell,A., Cho,S., Regier,J.C., Mitter,C., Poole,R.W. and Matthews,M.	1997
Drosophila melanogaster F2	P05303.2	45644942	Hovemann,B., Richter,S., Walldorf,U. and Cziepluch,C.	1988
Drosophila melanogaster F1	AAA28526.1	157401	Walldorf,U., Hovemann,B. and Bautz,E.K.	1985
Cardiocladius sp.	AAF31051.1	6901507	Moulton,J.K.	1997
Thaumalea gillespieae	AAF31050.1	6901505	Moulton,J.K.	1997
Parasimulium crosskeyi	AAF31052.1	6901509	Moulton,J.K.	1997
Twinnia nova	AAF31056.1	6901517	Moulton,J.K.	1997
Urosimulium aculeatum	AAF31054.1	6901513	Moulton,J.K.	1997
Helodon onychodactylus	AAF31055.1	6901515	Moulton,J.K.	1997
Prosimulium formosum	AAF31053.1	6901511	Moulton,J.K.	1997
Gymnopais fimbriatus	AAF31057.1	6901519	Moulton,J.K.	1997
Greniera fabri	AAF31072.1	901549	Moulton,J.K.	1997
Greniera denaria	AAF31071.1	6901547	Moulton,J.K.	1997
Mayacnephia sp. nr. osbornei	AAF31070.1	6901545	Moulton,J.K.	1997
Cnephia umbratorum	AAF31073.1	6901551	Moulton,J.K.	1997
Cnephia tonnoiri	AAF31063.1	6901531	Moulton,J.K.	1997
Cnephia aurantiacum	AAF31061.1	6901527	Moulton,J.K.	1997
Cnephia ornithophilia	AAF31075.1	6901555	Moulton,J.K.	1997
Ectemnia sp.	AAF31087.1	6901579	Moulton,J.K.	1997
Metacnephia sommermanae	AAF31076.1	6901557	Moulton,J.K.	1997
Simulium sirbanum	AAF31079.1	6901563	Moulton,J.K.	1997
Simulium reptans	AAF31080.1	6901565	Moulton,J.K.	1997
Simulium venustum	ABB92742.1	82880387	Minhas,M.S. and Currie,D.C.	2005
Gigantodax adleri	AAF31064.1	6901533	Moulton,J.K.	1997
Gigantodax marginale	AAF31065.1	6901535	Moulton,J.K.	1997
Stegopterna mutata	AAF31069.1	6901543	Moulton,J.K.	1997
Paracnephia rhodesiense	AAF31068.1	6901541	Moulton,J.K.	1997
Paracnephia thornei	AAF31067.1	6901539	Moulton,J.K.	1997
Crozetia crozetensis	AAF31060.1	6901525	Moulton,J.K.	1997
Paraustrosimulium anthracinum	AAF31074.1	6901553	Moulton,J.K.	1997
Austrosimulium bancrofti	AAF31058.1	6901521	Moulton,J.K.	1997
Austrosimulium mirabile	AAF31059.1	6901523	Moulton,J.K.	1997
Cnesia dissimilis	AAF31066.1	6901537	Moulton,J.K.	1997

TABLE 2. GenBank 28S D1-D3 sequence information				
Taxa	**Accession Version**	**GI**	**Authors**	**Year**
Cardiocladius sp.	AF007295.1	6940300	Moulton,J.K.	1997
Thaumalea gillespieae	AF007297.1	6940320	Moulton,J.K.	1997
Parasimulium crosskeyi	AF007299.1	6940331	Moulton,J.K.	1997
Twinnia nova	AF007307.1	6940362	Moulton,J.K.	1997
Urosimulium aculeatum	AF007303.1	6940342	Moulton,J.K.	1997
Helodon onychodactylus	AF007305.1	6940348	Moulton,J.K.	1997
Prosimulium formosum	AF007301.1	6901580	Moulton,J.K.	1997
Gymnopais fimbriatus	AF007309.1	6957468	Moulton,J.K.	1997
Greniera fabri	AF007357.1	6958141	Moulton,J.K.	1997
Greniera denaria	AF007355.1	6958139	Moulton,J.K.	1997
Mayacnephia sp. nr. osbornei	AF007353.1	6958137	Moulton,J.K.	1997
Cnephia umbratorum	AF007343.1	6958127	Moulton,J.K.	1997
Cnephia tonnoiri	AF007321.1	6940403	Moulton,J.K.	1997
Cnephia aurantiacum	AF007317.1	6957470	Moulton,J.K.	1997
Cnephia ornithophilia	AF007359.1	6958143	Moulton,J.K.	1997
Ectemnia sp.	AF007361.1	6958145	Moulton,J.K.	1997
Metacnephia sommermanae	AF007363.1	6958147	Moulton,J.K.	1997
Simulium sirbanum	AF007371.1	6958155	Moulton,J.K.	1997
Simulium reptans	AF007373.1	6958157	Moulton,J.K.	1997
Gigantodax adleri	AF007327.1	6940408	Moulton,J.K.	1997
Gigantodax marginale	AF007329.1	6863109	Moulton,J.K.	1997
Stegopterna sp.	AF007351.1	6958135	Moulton,J.K.	1997
Paracnephia rhodesiense	AF007325.1	6958113	Moulton,J.K.	1997
Paracnephia thornei	AF007323.1	6855496	Moulton,J.K.	1997
Crozetia crozetensis	AF007315.1	6901582	Moulton,J.K.	1997
Paraustrosimulium anthracinum	AF007347.1	6958131	Moulton,J.K.	1997
Austrosimulium bancrofti	AF007311.1	6940380	Moulton,J.K.	1997
Austrosimulium mirabile	AF007313.1	6940386	Moulton,J.K.	1997
Cnesia dissimilis	AF007331.1	6958115	Moulton,J.K.	1997

TABLE 3. GenBank 28S D9-D10 sequence information				
Taxa	**Accession Version**	**GI**	**Authors**	**Year**
Cardiocladius sp.	AF007296.1	6940316	Moulton,J.K.	1997
Thaumalea gillespieae	AF007298.1	6940326	Moulton,J.K.	1997
Parasimulium crosskeyi	AF007300.1	6957465	Moulton,J.K.	1997
Twinnia nova	AF007308.1	6957467	Moulton,J.K.	1997
Urosimulium aculeatum	AF007304.1	6940345	Moulton,J.K.	1997
Helodon onychodactylus	AF007306.1	6957466	Moulton,J.K.	1997
Prosimulium formosum	AF007302.1	6901581	Moulton,J.K.	1997
Gymnopais fimbriatus	AF007310.1	6940377	Moulton,J.K.	1997
Greniera fabri	AF007358.1	6958142	Moulton,J.K.	1997
Greniera denaria	AF007356.1	6958140	Moulton,J.K.	1997
Mayacnephia sp. nr. osbornei	AF007354.1	6958138	Moulton,J.K.	1997
Cnephia umbratorum	AF007344.1	6958128	Moulton,J.K.	1997
Cnephia tonnoiri	AF007322.1	6940405	Moulton,J.K.	1997
Cnephia aurantiacum	AF007318.1	6940394	Moulton,J.K.	1997
Cnephia ornithophilia	AF007360.1	6958144	Moulton,J.K.	1997
Ectemnia sp.	AF007362.1	6958146	Moulton,J.K.	1997
Metacnephia sommermanae	AF007364.1	6958148	Moulton,J.K.	1997
Simulium sirbanum	AF007372.1	6958156	Moulton,J.K.	1997
Simulium reptans	AF007374.1	6958158	Moulton,J.K.	1997
Gigantodax adleri	AF007328.1	6940411	Moulton,J.K.	1997
Gigantodax marginale	AF007330.1	6863110	Moulton,J.K.	1997
Stegopterna sp.	AF007352.1	6958136	Moulton,J.K.	1997
Paracnephia rhodesiense	AF007326.1	6958114	Moulton,J.K.	1997
Paracnephia thornei	AF007324.1	6855497	Moulton,J.K.	1997
Crozetia crozetensis	AF007316.1	6901583	Moulton,J.K.	1997
Paraustrosimulium anthracinum	AF007348.1	6958132	Moulton,J.K.	1997
Austrosimulium bancrofti	AF007312.1	6957469	Moulton,J.K.	1997
Austrosimulium mirabile	AF007314.1	6940390	Moulton,J.K.	1997
Cnesia dissimilis	AF007332.1	6958116	Moulton,J.K.	1997

TABLE 4. GenBank 12S Sequence Information				
Taxa	**Accession Version**	**GI**	**Authors**	**Year**
Cardiocladius sp.	AF049470.1	6958185	Moulton,J.K.	1997
Thaumalea gillespieae	AF049471.1	6958186	Moulton,J.K.	1997
Parasimulium crosskeyi	AF049472.1	6958187	Moulton,J.K.	1997
Twinnia Nova	AF049476.1	6958191	Moulton,J.K.	1997
Helodon onychodactylus	AF049474.1	6958189	Moulton,J.K.	1997
Prosimulium formosum	AF049475.1	6958190	Moulton,J.K.	1997
Gymnopais fimbriatus	AF049473.1	6958188	Moulton,J.K.	1997
Greniera denaria	AF049480.1	6958195	Moulton,J.K.	1997
Cnephia ornithophilia	AF049477.1	6958192	Moulton,J.K.	1997
Ectemnia reclusa	AF049479.1	6958194	Moulton,J.K.	1997
Metacnephia sommermanae	AF049482.1	6958197	Moulton,J.K.	1997
Simulium murmanum	JQ793809.1	384096006	Conflitti,I.M., Spironello,M. and Currie,D.C.	2012
Stegopterna mutata	AF049485.1	6958200	Moulton,J.K.	1997
Mayacnephia sp.	AF049481.1	6958196	Moulton,J.K.	1997
Cnesia dissimilis	AF049478.1	6958193	Moulton,J.K.	1997
Paraustrosimulium anthracinum	AF049483.1	6958198	Moulton,J.K.	1997
Austrosimulium bancrofti	L02390.1	173773	Ballard,J.W., Olsen,G.J., Faith, D.P., Odgers,W.A., Rowell,D.M. & Atkinson,P.W.	1992

TABLE 5. List of apomorphies
(1) indicates the apomorphic state; (0) indicates the plesiomorphic state

1. Mouthparts in almost all adults (Azar *et al.* 2008): (1) non-functional; (0) functional.
2. Setae of flagellomeres arranged: (1) irregularly; (0) in circular whorls of setae. (Ooesterbroek & Courtney 1995)
3. Costa: (1) encircles the hind margin of the wing to varying degree; (0) not attaining hind margin of wing leaving it membranous. (Wood & Borkent 1989)
4. Pedicel of antenna: (1) not markedly enlarged, male flagellum not greatly plumose or distinct from that of the female; (0) enlarged with male flagellum conspicuously plumose. (Wood & Borkent 1982)
5. Male eye: (1) with line of dissimilarity dividing large upper facets and small lower facets; (0) no line of discontinuity present. (Wood & Borkent 1982)
6. Wing: (1) significantly broadened basally; (0) without marked broadening at the base. (Hennig 1973)
7. Basal medial cell: (1) very small; (0) large. (Adler *et al.* 2004)
8. Male claw: (1) with dorsal surface bearing cuticular grooves basally; (0) simple, without such grooves. (Wood & Borkent 1982)
9. Hind basitarsus: (1) ventrally keeled and laterally compressed; (0) cylindrical. (Wood & Borkent 1982)
10. Adult abdominal tergite 1: (1) bearing posterior fringe of long hairs; (0) without basal fringe. (Wood & Borkent 1982)
11. Anal sclerite of abdominal segment IX (1) present; (0) absent. (Adler *et al.* 2004)
12. Blood-feeding: (1) diurnal and prolonged; (0) Nocturnal and more rapid. (Adler *et al.* 2004)
13. Pupal gill: (1) with basal lateral fenestra; (0) without basal lateral fenestra. (Adler *et al.* 2004)
14. Pupal spiracle: (1) with regulatory apparatus operated by pharate adult; (0) without such a regulatory apparatus. (Adler *et al.* 2004)
15. Pupal abdominal tergites & sternites III or IV-VIII: (1) broadly divided by pleural membrane; (0) fused. (Adler *et al.* 2004)
16. Pupa: (1) pharate, capable of feeding and spinning its cocoon; (0) larva spins cocoon and feeding halts after larval to pupal apolysis. (Adler *et al.* 2004)
17. Silk glands: (1) exceptionally large and folded; (0) comparatively reduced. (Adler *et al.* 2004)
18. Larvae: (1) with bacteria-covered multiporous sensillum ventral to the membranous antennal base; (0) without any such organ. (Adler *et al.* 2004)
19. Rectal papillae: (1) with three lobes, simple or compound; (0) with even number (2 or 4) of lobes. (Adler *et al.* 2004)
20. Hooks of prothoracic and posterior prologs: (1) arranged in longitudinal rows; (0) irregularly arranged. (Adler *et al.* 2004)
21. Egg: (1) subtriangular, with a dorsal transverse prominence; (0) ovoid. (Borkent & Wood 1986)
22. Katepisternum: (1) distinctly reduced; (0) markedly convex. (Wood & Borkent 1982)
23. Mesepimeral tuft: (1) lost; (0) present. (Wood & Borkent 1982)

TABLE 5. Continued

24. Basal radial cell length as measured from base of cell: (1) markedly less than half the wing length; (0) equal to about half the length of the wing. (Adler *et al.* 2004)
25. Mating behavior: (1) females rest still on the leaf undersides while males search underneath for them; (0) mating via aerial swarms. (Adler *et al.* 2004)
26. Maxillary palpus of first-instar larvae with sensilla positioned: (1) subapically; (0) apically. (Adler *et al.* 2004)
27. Larval abdominal segment IX: (1) with single, large, conical, ventral lobe; (0) simple venter without noticeable cuticular projections. (Adler *et al.* 2004)
28. Habitat: (1) hypogean; (0) surface-flowing streams. (Adler *et al.* 2004)
29. Larval pigment: (1) absent; (0) present. (Adler *et al.* 2004)
30. Radial sector vein branches: (1) positioned near to one another; (0) widely separated. (Wood & Borkent 1982)
31. False vein: (1) forked apically; (0) simple or not divided. (Adler *et al.* 2004)
32. Katepisternum sulcus: (1) present; (0) absent. (Adler *et al.* 2004)
33. Female sternum X medially: (1) completely divided; (0) incompletely divided. (Adler *et al.* 2004)
34. Pupal felt chamber of gill: (1) lost; (0) present. (Adler *et al.* 2004)
35. Pupal spiracle corresponding: (1) with the plastron lumen; (0) directly with the plastron. (Adler *et al.* 2004)
36. Pupal sternites VI & VII, & sometimes more: (1) medially divided by a semimembraneous longitudinal striate region; (0) not divided. (Adler *et al.* 2004)
37. Pupal abdominal tergites V to IX: (1) each bearing an anterior row of spine combs; (0) without such spine combs. (Adler *et al.* 2004)
38. Larval antennae: (1) consisting of 3 articles; (0) 1 article. (Adler *et al.* 2004)
39. Larva with a postgenal cleft: (1) present; (0) absent. (Adler *et al.* 2004)
40. Lateral sclerite of prothoracic proleg: (1) present; (0) absent. (Adler *et al.* 2004)
41. Pupal abdominal segments IV and V: (1) bearing large pleural plates; (0) without any such plates. (Adler *et al.* 2004)
42. Larval antennae with basal two segments: (1) unpigmented, contrasting with black apical segment; (0) pigmented. (Adler *et al.* 2004)
43. Larval paralateral teeth of hypostom: (1) absent; (0) present. (Adler *et al.* 2004)
44. Anteromedian palatal brush of first-instar larva: (1) with fringed hairs arranged in scoop shape; (0) with simple hairs. (Adler *et al.* 2004)
45. Maxillary papal sensilla of first-instar larva: (1) positioned subapically and in a linear pattern; (0) apically positioned and in circular pattern. (Adler *et al.* 2004)
46. Hypogynial valves: (1) elongate, with anteromedial corner bearing a nipple-like projection; (0) markedly shorter and truncate. (Adler *et al.* 2004)
47. Spermatheca: (1) with a large differentiated area at the connection with the spermathecal duct; (0) without a differentiated area or with the differentiated area reduced at the connection with the spermathecal duct. (Adler *et al.* 2004)
48. Lateral sclerite of prothoracic proleg: (1) wide with vertical section distinct; (0) narrow without a vertical extension. (Adler *et al.* 2004)
49. Paramere: (1) distinct, but reduced; (0) generally larger.

TABLE 5. Continued

50. Chromosomes: (1) with fixed inversions in IIIL (IIIL-1) and IIS; (0) without fixed inversions in IIIL (IIIL-1) and IIS. (Adler *et al.* 2004)
51. Larval mandible bearing apical brush consisting of: (1) bristle-like setae; (0) slender hairlike setae. (Adler *et al.* 2004)
52. Anal sclerite: (1) with a short posteromedially pointed projection between posteroventral arms; (0) without such a projection between posteroventral arms. (Adler *et al.* 2004)
53. Female cercus: (1) long, and apically narrowing to a fine point; (0) broader than long and subrectangular. (Adler *et al.* 2004)
54. Gonostylus base; (1) bearing a lateral accessory lobe; (0) simple, without such a lobe. (Adler *et al.* 2004)
55. Larval basal antennal two articles: (1) reversed to pigmented state; (0) non-pigmented among the Prosimuliini. (Adler *et al.* 2004)
56. Pupal abdominal tergites IV and V with anterior line of spine combs: (1) absent; (0) present. (Adler *et al.* 2004)
57. Larva with labral fans in 2^{nd} through final instar: (1) absent; (0) present. (Adler *et al.* 2004)
58. Larval head with posterior frontolabral muscles: (1) separated into two fascicles; (0) of one fascicle. (Adler *et al.* 2004)
59. Larval mandible apically: (1) wide, with compressed, equally sized apical teeth; (0) slender, with teeth more conical and of unequal lengths. (Adler *et al.* 2004)
60. Larval mandible with large apical brush: (1) extensive, and composed of many rows of comblike scales; (0) with comblike reduced in size and number. (Adler *et al.* 2004)
61. Larval mandible with covering brush of first external brush, and second external brush: (1) reduced or lost; (0) not reduced and present. (Adler *et al.* 2004)
62. Larval abdomen with segments I-IV: (1) thin and corrugated ventrally, expanded abruptly at segment V, and then narrowed posteriorly to a slight posterior proleg; (0) not corrugate and more gently tapered. (Adler *et al.* 2004)
63. Adult antenna composed of: (1) 7 flagellomeres; (0) 9 flagellomeres. (Adler *et al.* 2004)
64. Stematic bulla located adjacent to posterior margin of compound eye: (1) present; (0) absent. (Adler *et al.* 2004)
65. Female tarsal claw with basal or subbasal tooth (1) absent; (0) present. (Adler *et al.* 2004)
66. Pupal abdominal segment III: (1) ringlike, with the tergite and sternite merged laterally; (0) divided laterally by pleural membrane. (Adler *et al.* 2004)
67. Pupal abdominal tergites III and IV with recurved hooks: (1) lost or reduced in quantity or lost, and positioned between the middle of tergite and its posterior margin; (0) present. (Adler *et al.* 2004)
68. Pupal abdominal tergites V-IX with anterior row of spine combs: (1) absent; (0) present. (Adler *et al.* 2004)
69. Larval anal sclerite with posteroventral arms: (1) rudimentary or absent; (0) present. (Adler *et al.* 2004)

TABLE 5. Continued

70. Larval anal sclerite: (1) with anterodorsal arms arising from long common stalk, creating a Y-shaped formation; (0) subrectangular and giving rise to a pair of both anterodorsal and posteroventral arms. (Adler *et al.* 2004)
71. Female tergite IX: (1) extended like shield tapered to a point over the circus; (0) not extended shieldlike over circus and subtriangular in shape, not as pointed. (Adler *et al.* 2004)
72. Female lateral plate of genital fork (1) not connected directly to tergite IX; (0) connected via sclerotized structures directly to tergite IX. (Adler *et al.* 2004)
73. Male ventral plate of aedeagus: (1) with short basal arms compared to length of ventral plate; (0) with much longer basal arms. (Adler *et al.* 2004)
74. Larval hypostoma with lateral serrations in form of: (1) spiniform setae; (0) short, rounded denticles. (Adler *et al.* 2004)
75. Anal sclerite: (1) with prominent supernumerary arm extended posteromedially, forming an inverted the shape of a star; (0) X shaped. (Adler *et al.* 2004)
76. Ventral plate of aedeagus near base of anterolateral apodeme: (1) strongly notched laterally; (0) not strongly notched laterally. (Adler *et al.* 2004)
77. Gonostylus: (1) with 1 apical spinule; (0) with 2 spinules. (Adler *et al.* 2004)
78. Larval mandible: (1) not distinctly curved medially near apex; (0) medially curved distally. (Adler *et al.* 2004)
79. Pupal cocoon: (1) a clear gelatinous casing that envelopes entire pupa, retaining larval exuvia; (0) woven silk sac that does not retain the larval exuvia. (Adler *et al.* 2004)
80. Adult vestiture: (1) meager, petite, and erect, not dense fine recumbent setae; (0) primarily soft, with few coarse upright setae. (Adler *et al.* 2004)
81. Adult clypeus: (1) nearly devoid of setae, none medially; (0) entirely clothed in setae. (Adler *et al.* 2004)
82. Female mandible and lacinia with teeth: (1) absent; (0) present. (Adler *et al.* 2004)
83. Adult thorax: (1) weakly arched; (0) more strongly arched. (Adler *et al.* 2004)
84. Adult Postnotum: (1) relatively small and markedly arched, with a variously developed median longitudinal ridge; (0) larger and less severely arched. (Adler *et al.* 2004)
85. Adult mesepimeron with vestiture: (1) limited to the dorsal region of the sclerite above the level of the metathoracic spiracle; (0) more extensive in other Prosimuliini. (Adler *et al.* 2004)
86. Wing membrane: (1) smoky colored and slightly furrowed; (0) hyaline and less wrinkled in other Prosimuliini. (Adler *et al.* 2004)
87. Petiole of M1+2 vein: (1) long, approximately half the length of the petiole of the radial sector; (0) one fifth the length of the petiole of the radial sector in other Prosimuliini. (Adler *et al.* 2004)
88. Spermatheca base: (1) with short to long sclerotized collar where the spermathecal duct is joined; (0) connected to the duct via a membranous ring in other Prosimuliini. (Adler *et al.* 2004)
89. Female anal lobe and cercus: (1) merged into one solid sclerite; (0) separated by membrane. (Adler *et al.* 2004)

TABLE 5. Continued

90. Male gonostylus: (1) thin apically, with a minute apical spinule; (0) relatively broad throughout with a more rounded apex in other Prosimuliini. (Adler *et al.* 2004)
91. Pupal abdominal Sternites IV-VII: (1) each bearing up to 5 pairs of recurved hooks; (0) each with less than 5 hooks in other Prosimuliini and most Simuliidae. (Adler *et al.* 2004)
92. Pupal abdominal pleurite IV: (1) barely distinguishable from adjoining tergite and sternite; (0) with distinguishable pleurite on segments IV and V in other Prosimuliini. (Adler *et al.* 2004)
93. Larval labrum and labropalatum: (1) distinctively longer than wide, bearing clear tubercle on each side, epipharyngeal mechanism anteriorly bearing V-shaped brush; (0) without such an arrangement. (Adler *et al.* 2004)
94. Radial sector: (1) unbranched or extremely small and apically forked; (0) more definitively branched and not positioned apically. (Adler *et al.* 2004)
95. Male straplike link joining the paramere and ventral plate arising subapically on anterolateral apodeme of ventral plate: (1) present; (0) absent. (Adler *et al.* 2004)
96. Female genital fork with lateral plate with a variously formed dorsal apodeme: (1) present; (0) absent. (Adler *et al.* 2004)
97. Gonocoxite: (1) massive, sub-trapezoidal; (0) less enlarged and angulate.
98. Larval rake-like fans modified for scraping: (1) present; (0) absent.
99. Larval medial antennal article with minute spicules: (1) present; (0) absent.
100. Larval antenna: (1) greatly surpassing the length of the labral fan stalk by about 2/3 the length of the medial article; (0) either not surpassing the length of the labral fan stalk, or not by about 2/3 the length of the medial article.
101. Form of pupal gill: (1) diverse, and differentiated from all other simuliids; (0) less variation in pupal gill diversity. (Adler *et al.* 2004)
102. Larval abdominal segment VIII with transverse midventral bulge: (1) present; (0) absent. (Adler *et al.* 2004)
103. Tibial spurs of hind leg: (1) distinctly long and with a hyaline apex; (0) subequal in length or shorter than the tibia without hyaline apex. (Adler *et al.* 2004)
104. Male paramere with apical spines: (1) present; (0) absent. (Adler *et al.* 2004)
105. Larval apical antennal segment with extremely characteristic dark cuticular striations: (1) present; (0) absent.
106. Pupal cocoon: (1) rudimentary, consisting of a few silk threads entwined in the grapnel setae of segment IX; (0) well developed, or not reduced in such a way.
107. Larval postgenal cleft extended anteriorly about: (1) 1/3 or more distance to hypostomal groove; (0) 1/4 or less the distance to the hypostomal groove. (Adler *et al.* 2004)
108. Larval hypostomal teeth: (1) small, and not organized on prominent lobes; (0) larger and organized on prominent lobes. (Adler *et al.* 2004)
109. Pupal gill filaments radiating from bulbous central protuberance: (1) present; (0) absent. (Adler *et al.* 2004)
110. Larval labral-fan rays with distinctively elongate microtrichia intermingled with the normal-sized microtrichia: (1) present; (0) absent. (Adler *et al.* 2004)

TABLE 5. Continued

---

111. Pupal cocoon: (1) of definite form and complete ventrally; (0) an ill formed sac or if well-formed than not as complete ventrally.
112. Gill filaments: (1) convergent apically; (0) not convergent apically. (Moulton & Adler 1997)
113. Gill filaments with surface: (1) shingle-like; (0) smooth or furrowed. (Moulton & Adler 1997)
114. Pupal terminal abdominal spines: (1) inflated and hollow; (0) thin and solid. (Moulton & Adler 1997)
115. Larval mandible with inner subapical margin: (1) smooth; (0) dentate. (Moulton & Adler 1997)
116. Larval lateral abdominal segments V-VIII: (1) drastically expanded ventrally and laterally; (0) expand more gently. (Moulton & Adler 1997)
117. Anal sclerite: (1) absent; (0) present. (Moulton & Adler 1997)
118. Larval construction of a long silk pedicel to serve as a feeding and pupation substrate: (1) present; (0) absent. (Moulton & Adler 1997)
119. Katepisternal sulcus: (1) narrow and deep its entire length; (0) wide, shallow and indistinct anteriorly. (Adler *et al.* 2004)
120. Pupal abdomen: (1) weakly sclerotized, often losing its form after emergence; (0) strongly sclerotized. (Adler *et al.* 2004)
121. Pupal cocoon: (1) boot shaped and spun of delicate silk; (0) slipper shaped or amorphous of coarser silk. (Adler *et al.* 2004)
122. Larval postgenal cleft: (1) wide and deep, extended anteriorly to or beyond hypostomal groove; (0) shorter and narrower, not extended to the hypostomal groove. (Adler *et al.* 2004)
123. Larval antenna with basal and medial articles: (1) unpigmented, contrasting with dark apical article; (0) pigmented in other Simuliini. (Adler *et al.* 2004)
124. Chromosomes with whole-arm interchange between chromosomes I and II: (1) present; (0) absent. (Adler *et al.* 2004)
125. Gonostylus subtruncate, with internal triangular lobe bearing one apical spinule, without very large laterally directed parameral spine: (1) present; (0) absent. Modified from (Adler *et al.* 2004)
126. Basal radial cell length compared to the distance from the base of radial sector to wing apex: (1) less than 1/3; (0) greater than 1/3. (Adler *et al.* 2004)
127. Larval rectal papillae: (1) mostly compound; (0) simple. (Adler *et al.* 2004)
128. Larval hypostoma: (1) with of 6-7 pairs of teeth plus the median tooth arranged on three prominent lobes, and paralateral teeth present; (0) without such arrangement.
129. Adult thorax: (1) extremely strongly arched; (0) not so strongly arched.
130. Vein CuA2: (1) not strongly sigmoidal; (0) strongly sigmoidal. (Adler *et al.* 2004)
131. Male paramere: (1) apparently represented by only apical spines; (0) large to small, but always distinct. (Adler *et al.* 2004)
132. Female genital fork with anteriorly directed apodemes: (1) extremely elongate; (0) more reduced. (Adler *et al.* 2004)
133. Vein A1: (1) straight; (0) curved. Modified from (Adler *et al.* 2004)

TABLE 5. Continued

134. Larval mandible bearing: (1) three outer teeth; (0) two or four outer teeth (Wygodzinsky & Coscarón 1973)
135. Anal sclerite with posterior arms expanded ventrally around abdomen, creating a complete ring encircling the base of posterior circlet of hooks: (1) present; (0) absent. (Adler *et al.* 2004)
136. Genital fork: (1) with a short stem anterior arms broad; (0) without such a combination.
137. Larval anal sclerite T-shaped; (1) present; (0) absent.
138. Female claws with markedly curved laterally directed basal tooth: (1) present; (0) absent.
139. Larval mandible serration and sensillum: (1) complex; (0) more simple. (Gil-Azevedo & Maia-Herzog 2007)
140. Genital fork: (1) nearly unpigmented with short and wide stem; (0) pigmented and not shaped as such. (Coscarón & Coscarón 2007)
141. Male paramere: (1) absent; (0) present. (Craig 2012)
*142. Anal sclerite with median notch closed posteriorly to create a circle: (1) present; (0) absent.

*Indicates synapomorphy of *P. strenua* and *P. aurantiaca* used in the constructing the phylogenetic tree in figure 1, but not in the generic level sorted data matrix of characters in table 2.

TABLE 6. Sorted character state matrix. X=Apomorphic character state, CA=*Cardiocladius*, TH=*Thaumalea*, PA=*Parasimulium*, PR=*Prosimulium*, HE=*Helodon*, UR=*Urosimulium*, LE= *Levitinia*, TW=*Twinnia*, GY=*Gymnopais*, CR=*Crozetia*, AD=*Adlerella*, TL= *Tlalocomyia*, ST=*Stegopterna*, GR=*Greniera*, CN=*Cnephia*, ET=*Ectemnia*, ME=*Metacnephia*, SU=*Sulcicnephia*, SM=*Simulium*, PC=*Paracnephia*, PS=Taxon A, CS=*Cnesia*, GX=*Gigantodax*, EO=Taxon B. PU=*Paraustrosimulium*, AU=*Austrosimulium*.. Numbers in first column correspond to character numbers in Table 1.

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
1.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

TABLE 6. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
22.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
32.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
34.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
35.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
36.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
37.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40.	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45.	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
48.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49.	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51.	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52.	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
56.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
58.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62.	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70.	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
74.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
95.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
96.	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
97.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
100.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102.	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
103.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
104.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
105.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
106.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
107.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
108.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
109.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
110.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
111.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0
112.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
113.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
114.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
115.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
116.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
117.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
118.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
119.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
120.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
121.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
122.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
123.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
124.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
125.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

TABLE 6. Continued

Char. #	CA	TH	PA	PR	HE	UR	LE	TW	GY	CR	AD	TL	ST	GR	CN	ET	ME	SU	SM	PC	PS	CS	GX	EO	PU	AU
126.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
127.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
128.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
129.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
130.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
131.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
132.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
133.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
134.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
135.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
136.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
137.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
138.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
139.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
140.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
141.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

TABLE 7. 28S-D1-3 Region Secondary Structural Alignment

D1-18 D1-19

2.Cardiocladius_sp.	UAG) GAU (UCCUUGAGUAGUUGCGAACGAAAAAGGA) AA [AGUU] -CA (GCACGUAG (GGUUGUAGCGCUUUAUUGCUUAUUGACC) GGUUCCGUGU)
3.Thaumalea_gillespieae	AAG) AAU (UCCUUGAGUAGCUGCGAGCGAAAUAGGA) AA [AGCC] -CA (GCACGUAG (GCUUCCUAUUG-UUG-UGAUAGGUAAGC) GAUGCCGUGU)
4.Parasimulium_Crosskeyi	UAG) GAU (UCCUU-AGUAGCGGCGAGCGAAAAGGGA) UA [AGUC] -CA (GCACGUAG (ACUUGCAAUUG-UUA-UGAUUGUUAAGU) GAUGCCGUGU)
5.Urosimulium_aculeatum	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (ACUUGCAACUG-UUA-UGGUUGUUAAGU) GAUGCCGUGU)
6.Helodon_onychodactylus	UAG) GAU (UCCUU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (ACUUGCAACUG-UUA-UGGUUGUUAAGU) GAUGCCGUGU)
7.Prosimulium_formosum	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (ACUUGCAACUG-UUA-UGGUUGUUAAGU) GAUGCCGUGU)
8.Gymnopais_fimbriatus	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (ACUUGCAAUUG-UUA-UGAUUGUUAAGU) GAUGCCGUGU)
9.Twinnia_nova	UAG) GAU (UCCUU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (ACUUGCAACUG-UUA-UGGUUGUUAAGU) GAUGCCGUGU)
10.Crozetia_crozetensis	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) UA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
11.Stegopterna_sp.	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
12.Tlalocomyia_nr_osbornei	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
13.Adlerella_denaria	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
14.Greniera_fabri	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
15.Cnephia_ornithophilia	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
16.Ectemnia_sp.	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
17.Metacnephia_sommermanae	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
18.Simulium_congareenarum	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
19.Simulium_reptans	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAAUUG-UUA-UGAUUGUUGAUU) GAUGCCGUGU)
20.Simulium_sirbanum	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAAUUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
21.Paracnephia_rhodesiana	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
22.Paracnephia_thornei	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUA-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
23.Paracnephia_tonnoiri	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAAUUG-UUA-UGAUUGUUAAUU) GAUGCCGUGU)
24.Paracnephia_aurantiaca	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAAUUG-UUA-UGAUUGUUAAUU) GAUGCCGUGU)
25.Paracnephia_strenua	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAAUUG-UUA-UAAUUGUUAAUU) GAUGCCGUGU)
26.Gigantodax_adleri	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
27.Gigantodax_marginalis	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
28.Cnesia_disimillis	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
29.Paracnephia_umbratora	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUUGCAACUG-UUA-UGGUUGUUAAUU) GAUGCCGUGU)
30.Paraustrosimulium_anthracinum	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAACUG-UUA-UGGUUGUUGAUU) GAUGCCGUGU)
31.Austrosimulium_bancrofti	UAG) GAU (UCCCU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAAUUG-UUA-UGAUUGUUGAUU) GAUGCCGUGU)
32.Austrosimulium_mirabile	UAG) GAU (UCCUU-AGUAGCGGCGAGCGAAAAGGGA) AA [AGUC] -CA (GCACGUAG (AAUCGCAAUUG-UUA-UGAUUGUUGAUU) GAUGCCGUGU)

TABLE 7. Continued

	D1-19A D1-20 D1-18′
2.Cardiocladius_sp.	AA[AGGA-GGUCCAUUAUCUUGCGCAUAUAAGCAUCA-UAU]UCAA(GUCCAGCUUGAAUGCGGC)UCAAUAUA[CC] (CAUUG)GA(GGGUGCCA
3.Thaumalea_gillespieae	AC[AGGUGUGUCCAUUAUCUAUUAUAUAC-AAUAGCAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--CAUUUA[CC] (CAUAG)-A(GGGUGCCA
4.Parasimulium_Crosskeyi	AA[AGGAGUGUCCAUUAUCUUAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
5.Urosimulium_aculeatum	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
6.Helodon_onychodactylus	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
7.Prosimulium_formosum	AC[AGGUUCGGCCGUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
8.Gymnopais_fimbriatus	AC[AGGUUCGGCCGUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
9.Twinnia_nova	AC[AGGUUCGGCCGUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
10.Crozetia_crozetensis	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
11.Stegopterna_sp.	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
12.Tlalocomyia_nr_osbornei	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
13.Adlerella_denaria	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
14.Greniera_fabri	AC[AGGUUUGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
15.Cnephia_ornithophilia	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
16.Ectemnia_sp.	AC[AGGUUUGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
17.Metacnephia_sommermanae	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
18.Simulium_congareenarum	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
19.Simulium_reptans	AC[AGGUGCGGCCAUUAACUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
20.Simulium_sirbanum	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
21.Paracnephia_rhodesiana	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
22.Paracnephia_thornei	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
23.Paracnephia_tonnoiri	AC[AGGUUUGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
24.Paracnephia_aurantiaca	AC[AUGUUCGGCCAUUAUCUCAUAU-UACAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
25.Paracnephia_strenua	AU[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
26.Gigantodax_adleri	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
27.Gigantodax_marginalis	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
28.Cnesia_disimillis	AC[AGGUUCGGCCGUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
29.Paracnephia_umbratora	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
30.Paraustrosimulium_anthracinum	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CACAG)-A(GGGUGAUA
31.Austrosimulium_bancrofti	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA
32.Austrosimulium_mirabile	AC[AGGUUCGGCCAUUAUCUCAUAU-UAUAAUUAGUAUGUU]-CAA(GUCCUUCUUGAAUGAGGC)--AAUAUA[CC] (CAUAG)-A(GGGUGAUA

TABLE 7. Continued

	D1-21 D1-22 D1-14′ D1-3′ D1-23 D1-2′
2.Cardiocladius_sp.	[GG]CCC)GUUUAU[GUAUGAUGUU-UGUGUGAGAGGGCUACA-ACUCCU]AAGAGUC(AUGUUGCUUGAUAGUGCAGCAU)AAAGUG
3.Thaumalea_gillespieae	[GG]CCC)GUUU--[AACUGUUAUU-CGUAAUAGAGGAUAUU----ACUU]AAGAGUC(GUAUUGCUUGAUAAUGCAAUAC)AAAGUU
4.Parasimulium_Crosskeyi	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUAAGAGGAUAUU---CUCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGUG
5.Urosimulium_aculeatum	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
6.Helodon_onychodactylus	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
7.Prosimulium_formosum	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
8.Gymnopais_fimbriatus	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
9.Twinnia_nova	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
10.Crozetia_crozetensis	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
11.Stegopterna_sp.	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
12.Tlalocomyia_nr_osbornei	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
13.Adlerella_denaria	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
14.Greniera_fabri	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUAUA----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
15.Cnephia_ornithophilia	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
16.Ectemnia_sp.	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUAUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
17.Metacnephia_sommermanae	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
18.Simulium_congareenarum	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGUG
19.Simulium_reptans	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGUGGUUGUU----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
20.Simulium_sirbanum	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
21.Paracnephia_rhodesiana	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
22.Paracnephia_thornei	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
23.Paracnephia_tonnoiri	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUAUU----ACCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
24.Paracnephia_aurantiaca	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AAGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
25.Paracnephia_strenua	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----GCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
26.Gigantodax_adleri	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
27.Gigantodax_marginalis	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
28.Cnesia_disimillis	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGUG
29.Paracnephia_umbratora	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGUG
30.Paraustrosimulium_anthracinum	[GG]CCC)GUAU--[AGCUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
31.Austrosimulium_bancrofti	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG
32.Austrosimulium_mirabile	[GG]CCC)GUAU--[AACUGCUAUGAUAUAUGAGAGGUUGUU----UCCU]AGGAGUC(GUGUUGCUUGAAAGUGCAGCAC)UAAGCG

TABLE 7. Continued

	D1-23 D1-2′ D1-24 D1-2′ D1-1′ D2-1
2.Cardiocladius_sp.	(GGAGGUAAACUCCUUC)UAA[AGCU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
3.Thaumalea_gillespieae	(GGAG-UAAACUCCUUC)UAA[GGCU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
4.Parasimulium_Crosskeyi	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
5.Urosimulium_aculeatum	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
6.Helodon_onychodactylus	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
7.Prosimulium_formosum	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
8.Gymnopais_fimbriatus	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
9.Twinnia_nova	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
10.Crozetia_crozetensis	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
11.Stegopterna_sp.	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
12.Tlalocomyia_nr_osbornei	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
13.Adlerella_denaria	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
14.Greniera_fabri	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
15.Cnephia_ornithophilia	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
16.Ectemnia_sp.	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
17.Metacnephia_sommermanae	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
18.Simulium_congareenarum	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
19.Simulium_reptans	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUG[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
20.Simulium_sirbanum	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
21.Paracnephia_rhodesiana	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
22.Paracnephia_thornei	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
23.Paracnephia_tonnoiri	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
24.Paracnephia_aurantiaca	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
25.Paracnephia_strenua	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
26.Gigantodax_adleri	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
27.Gigantodax_marginalis	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
28.Cnesia_disimillis	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
29.Paracnephia_umbratora	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
30.Paraustrosimulium_anthracinum	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
31.Austrosimulium_bancrofti	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA
32.Austrosimulium_mirabile	(GGAGGUAAACUCCUUC)UAA[GACU]AAAUAUUA[CCAUGAGACC]GAUAGCGAACAAGUA(CCGUGAGG)GAAA[GU]UGAAAAGCA

TABLE 7. Continued

D2-2

2.Cardiocladius_sp.	(CUCUGAAUAGAG) AGUCAAAUAGUAC [G] UGAAA [CUGUCUAGGG] GUUCAAA-- [CCUAAAGAACCCAAAU] AUCUAAAC--------
3.Thaumalea_gillespieae	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CCAUUCAGAG] UUAUAAAAG [CCAGAAGUAGCUAAAU] AUCUGAAC--------
4.Parasimulium_Crosskeyi	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUAUCUAAAU] AUCUGAAU--------
5.Urosimulium_aculeatum	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
6.Helodon_onychodactylus	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
7.Prosimulium_formosum	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
8.Gymnopais_fimbriatus	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
9.Twinnia_nova	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGA] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
10.Crozetia_crozetensis	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
11.Stegopterna_sp.	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
12.Tlalocomyia_nr_osbornei	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUUUAAAU] AUCUGAAU--------
13.Adlerella_denaria	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
14.Greniera_fabri	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
15.Cnephia_ornithophilia	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
16.Ectemnia_sp.	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
17.Metacnephia_sommermanae	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
18.Simulium_congareenarum	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CAUGAAGUGUCUAAAU] AUCUGAAU--------
19.Simulium_reptans	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CAUGAAGUGUCUAAAU] AUCUGAAU--------
20.Simulium_sirbanum	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAGAAAG [CAUGAAGUGUCUAAAU] AUCUGAAU--------
21.Paracnephia_rhodesiana	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
22.Paracnephia_thornei	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
23.Paracnephia_tonnoiri	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
24.Paracnephia_aurantiaca	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
25.Paracnephia_strenua	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
26.Gigantodax_adleri	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
27.Gigantodax_marginalis	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
28.Cnesia_disimillis	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
29.Paracnephia_umbratora	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
30.Paraustrosimulium_anthracinum	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------
31.Austrosimulium_bancrofti	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAUGAAGGAAU
32.Austrosimulium_mirabile	(CUCUGAAUAGAG) AGUCAAAAAGUAC [G] UGAAA [CUGUCUAGGG] UUAAAAAAG [CCUGAAGUGUCUAAAU] AUCUGAAU--------

TABLE 7. Continued

	D2-3
2.Cardiocladius_sp.	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAAUAUU--UAUUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
3.Thaumalea_gillespieae	(AGAGAAAUUCAU (UAUCAUAUUGUUGUA (GCAUUAUU---AAUGC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
4.Parasimulium_Crosskeyi	(AGAGAAAUUCAU (UAUCAUAUUGUUGUA (AUAUUAUUAUUUAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
5.Urosimulium_aculeatum	(GGAGAAAUUCAU (UAUCAUAUUGUGUUA (GUAUUAUU---AAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
6.Helodon_onychodactylus	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
7.Prosimulium_formosum	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAAUAUU---AUUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
8.Gymnopais_fimbriatus	(GGAGAAAUUCAU (UAUCAUAUUGUGUUA (GUAUUAUU---AAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
9.Twinnia_nova	(GGAGAAAUUCAA (UUCUACUU------- (AUUAU-UUAAAAUAAU) -----GGGUAGUA) UGCACUUUUCUCA) A-AGUAAG
10.Crozetia_crozetensis	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCU) U-UGUAAG
11.Stegopterna_sp.	(GGAGAAAUUCAG (UAUCAUAUUGUUGUA (GUAAUAUUA--UUUAC) GAAGCAUAUGGUG) UGCACUUUUCUCC) U-UGUAAG
12.Tlalocomyia_nr_osbornei	(GGAGAAAUUCAU (UAUCAUAUUGUGUUA (GUAUUAUU---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
13.Adlerella_denaria	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU--UAAUAU) GAAGCAUAUGGUA) UGCACUUUUCUUC) U-UGUAAG
14.Greniera_fabri	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
15.Cnephia_ornithophilia	(GGAGAAAUUCAU (UAUCAUAUUGUUCUA (GUAU------------) --AGCAUAUGGUA) UGCACUUUUCUCU) U-UGUAAG
16.Ectemnia_sp.	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU--AUAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
17.Metacnephia_sommermanae	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUC---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
18.Simulium_congareenarum	(GGAGAAAUUCAU (UAUCAUAUUGUGUUA (GUAUUAUU---AAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
19.Simulium_reptans	(GGAGAAAUUCAU (UAUCAUAUUGUU--- (GUAUUAU-----AUAU) AUAGCAUAUGGUA) UGCACUUUUCUUC) U-UGUAAG
20.Simulium_sirbanum	(GGAGAAAUUCAG (UAUCAUAUUGUUGUA (GUAAAAUU---UUUGC) GAAGCAUAUGGUG) UGCACUUUUCUCC) U-UGUAAG
21.Paracnephia_rhodesiana	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU-UUAAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
22.Paracnephia_thornei	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU--UAAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
23.Paracnephia_tonnoiri	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU---AAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
24.Paracnephia_aurantiaca	(AGAGAGAUUCAA (UAUU-----GCUG-- (------UUAA------) -AGGC----AAUG) UGCACUUCUCUCU) U-AGUAAG
25.Paracnephia_strenua	(AGAGAAAUUCAU (UAUCAUAUUGUUCUA (GUAUUAUC--UAAUAU) GAAGCAUAUGGUA) UGCACUUUUCUCU) U-UGUAAG
26.Gigantodax_adleri	(GAAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUUA--UAUAC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
27.Gigantodax_marginalis	(GAAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU--UAAUAU) GAAGCAUAUGGUA) UGCACUUUUCUUC) U-UGUAAG
28.Cnesia_disimillis	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GAAUUAUU---AAUUC) GAAGCAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
29.Paracnephia_umbratora	(GGAGAAAUUCAU (UAUCAUAUUGUUGUA (GUAUUAUU--UAAUAU) GAAACAUAUGGUA) UGCACUUUUCUCC) U-UGUAAG
30.Paraustrosimulium_anthracinum	(GGAGAAAUUCAU (UACUAUAUUGUAUUA (AUUUUAUU-UUAAAAU) GAAACAUAUGGUA) UGCACUUUUCUCU) U-UGUAAG
31.Austrosimulium_bancrofti	(GAAGAAAUUCAU (CAUCAUAUUGUU--- (AGCU-AUA----AGUU) --GGCAUAUGGUG) UGCACUUUUCUUC) UAUGUAAG
32.Austrosimulium_mirabile	(AGAGAAAUUCAU (UAUCAUAUU------ (GUUG-ACU--UGUAAC) -----AUAUGGUA) UGCACUUUUCUCU) UAUGUAAG

TABLE 7. Continued

	D2-4
2.Cardiocladius_sp.	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUCAAUGAU---CAUU)-(UGGC-------(UAUCAA-
3.Thaumalea_gillespieae	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(AUUAUUUUGUU--GAAU)U(UGUUAUUG-UU(UGUCAAU
4.Parasimulium_Crosskeyi	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(AUUACCAUGUUGUGAAU)-(UAUUA-UA-UC(UAUCAA-
5.Urosimulium_aculeatum	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACAACCAUGUUGUGUGU)-(UAUUA-UA-UC(UAUCAA-
6.Helodon_onychodactylus	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACAACCAUGUUGUGUGU)-(UAUUA-UA-UC(UAUCAA-
7.Prosimulium_formosum	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACAACCAUGUUGUGUGU)-(UAUUA-UA-UC(UAUCAA-
8.Gymnopais_fimbriatus	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACAACCAUGUUGUGUGU)-(UAUUA-UA-UC(UAUCAA-
9.Twinnia_nova	[ACAUUGC]GAUCCAUUAGA(AUCGAUUCU(AUACAA(ACAACCAUGUUGUGUGU)-(UAUUA-UA-UC(UAUCAA-
10.Crozetia_crozetensis	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGCGU)-(UAUUA-UA-UC(UAUCAA-
11.Stegopterna_sp.	[ACACUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAAGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
12.Tlalocomyia_nr_osbornei	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
13.Adlerella_denaria	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGAUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
14.Greniera_fabri	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
15.Cnephia_ornithophilia	[ACAUUGC]GAUCUAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGAUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
16.Ectemnia_sp.	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAACA-(ACUACCAUGAUGUGAGU)-(UGUUA-UA-UC(UGUCAA-
17.Metacnephia_sommermanae	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(CAUUA-UA-UC(UAUCAA-
18.Simulium_congareenarum	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UGUUA-UA-UC(UAUCAA-
19.Simulium_reptans	[ACACUGC]GAUCCAUUAGA(ACCUUGUGU(UAACAA(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
20.Simulium_sirbanum	[ACACUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
21.Paracnephia_rhodesiana	[ACACUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUG-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UGUCAA-
22.Paracnephia_thornei	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UGUCAA-
23.Paracnephia_tonnoiri	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
24.Paracnephia_aurantiaca	[ACGCCGU]GAUCCAUUAGA(AA-UUG-GA(UCAU--(-CUACCAUGAUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
25.Paracnephia_strenua	[ACAUUGC]GAUCUAUUAGAACC-UUGUGU(UAAUA-(AUUACCAUGAUGUGAAU)-(UAUUA-UA-UC(UAUCAA-
26.Gigantodax_adleri	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAACA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
27.Gigantodax_marginalis	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
28.Cnesia_disimillis	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGAUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
29.Paracnephia_umbratora	[ACAUUGC]GAUCUAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
30.Paraustrosimulium_anthracinum	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGAUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
31.Austrosimulium_bancrofti	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(ACUACCAUGUUGUGAGU)-(UAUUA-UA-UC(UAUCAA-
32.Austrosimulium_mirabile	[ACAUUGC]GAUCCAUUAGA(ACCUUGUGU(UAAUA-(AUUACCAUGUUGUGAAU)-(UAUUA-UA-UC(UAUCAUU

TABLE 7. Continued

	D2-5 D2-6a
2.Cardiocladius_sp.	(UAGGCCAGCACGAAG-UUCAU) CAAUA) ---UAAAUAACUCG) AUCU-UUAAUG) GCUGGGU) ---AUAUUGUA-----UGGUGAGC
3.Thaumalea_gillespieae	(UAGGCCUUGUUAUGGAGCUUA) C-AUU) --UAUAUGUUAGUA) CAA--UAUUUU) CUAAGGU) GUAAUAUUGGAAAGCAGUGUAAAC
4.Parasimulium_Crosskeyi	(UAGGCC-UACUAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) UUAUAUAUUCG) UUAAGGU) GAUAUAUAAGAAAACAAUGUAAAC
5.Urosimulium_aculeatum	(UGGGCC-CGCUGUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAUCAAUGUAAAC
6.Helodon_onychodactylus	(UGGGCC-UGCUGUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
7.Prosimulium_formosum	(UGGGCC-UGCUGUGGAGCUUA) C-AUA) -UUUUAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
8.Gymnopais_fimbriatus	(UGGGCC-UGCUGUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
9.Twinnia_nova	(UGGGCC-UGCUGUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAUCAAUGUAAAC
10.Crozetia_crozetensis	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUUAUGUUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUAUAGGAAAACAAUGUAAAC
11.Stegopterna_sp.	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUGUAUAUU-G) UUAAGGU) GAUAUCCAGGAAAACAAUGUAAAC
12.Tlalocomyia_nr_osbornei	(UGGGCC-CGGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUGUAUAUU-G) UUAAGGU) GAUAUCCAGGAAAACAAUGUAAAC
13.Adlerella_denaria	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUUAUGUUAGUA) CUGUAUAUU-G) UUAAGGU) GAUAUGCAGGAAAUCAAUGUAAAC
14.Greniera_fabri	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUGAAUAUU-G) UUAAGGU) GAUAUCCAGGAAAUCAAUGUAAAC
15.Cnephia_ornithophilia	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGCAAGUA) CUGGAUAUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
16.Ectemnia_sp.	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUGGAUAUU-G) UUAAGGU) GAUAUACAGGAAAUCAAUGUAAAC
17.Metacnephia_sommermanae	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUUAUGUUAGUA) CUGGUUAUU-G) UUAAGGU) GAUAUCCAGGAAAUCAAUGUAAAC
18.Simulium_congareenarum	(UGGGCC-UGACAUGGAGCUUA) C-AUA) -AUUUAUGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUAAAGGAAAGCAAUGUAAAC
19.Simulium_reptans	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAACGUUAGUA) CUAUAUAUU-G) UUAAGGU) GAUAUACAGGAAAGCGAUGUAAAC
20.Simulium_sirbanum	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUUAUGUUAGUA) ACAUAUAUU-G) UUAAGGU) GAUAUAUGAGAAAGCAAUGUAAAC
21.Paracnephia_rhodesiana	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
22.Paracnephia_thornei	(UGGGCC-CGGCAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
23.Paracnephia_tonnoiri	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
24.Paracnephia_aurantiaca	(UGGGCC-UAACAUGGAGCUUA) C-AUA) -UUUAAUGGUAGUA) CUAUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
25.Paracnephia_strenua	(UGGGCC-CGACAUGGAGCUUA) C-AUA) =UUUAAUGCUAGUA) CUAUAUGUU-G) UUAAGGU) GAUAUAUAGGAAAACAAUGUAAAC
26.Gigantodax_adleri	(UGGGCCCUGGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUAUAGGAAAACAAUGUAAAC
27.Gigantodax_marginalis	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUAUAGGAAAACAAUGUAAAC
28.Cnesia_disimillis	(UGGGCCC-GGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUAUAUGCU-G) UUAAGGU) GGUAUACAGGAAAACAAUGUAAAC
29.Paracnephia_umbratora	(UGGGCC-UGGCAUGGAGCUUA) C-AUA) -UUUAAUGCUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC
30.Paraustrosimulium_anthracinum	(UGGGCC-UGGCAUGGAGCUUA) C-UUA) -UUUAAUGCUAGUA) CUGUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAGGUAAAC
31.Austrosimulium_bancrofti	(UGGGCC-CGAAAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUGUU-G) UUAAGGU) GAUAUACAGGAAAGCAAUGUAAAC
32.Austrosimulium_mirabile	(UGGACC-CGAAAUGGAGCUUA) C-AUA) -UUUAAUGUUAGUA) CUAUAUGUU-G) UUAAGGU) GAUAUACAGGAAAACAAUGUAAAC

TABLE 7. Continued

```
                                  D2-6b                                            D2-3'                D2-1'
2.Cardiocladius_sp.               GU-GUUG(UAACCUGCUUA-AUAGCUGUCUGAUG)GAUUU----UA(AAGGCCAUAUUAU--UG---(AUG----------AUUAU)
3.Thaumalea_gillespieae           AUAGUUG(AGUGUCCU-UGUACAAAUAUCUGAUG)GUUGUGUUAUA(UAC--GAUCACACUAAAUUU(AGAGGU--AAAU-UCUCU)
4.Parasimulium_Crosskeyi          -UAGUGG(UGACUUGUAUAUAUAGAUGUCUGAUG)GAUUU----AA(AAC--GAUCAUAAUAUG---(ACAUAU--UUAUAUAUGU)
5.Urosimulium_aculeatum           -CAGUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCAUAAUAUG---(ACAUCU---UAU-GAUGU)
6.Helodon_onychodactylus          -CAGUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCAUAAUAUG---(ACAUCU---UAU-GAUGU)
7.Prosimulium_formosum            -CAGUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCAUAAUAUG---(ACAUCU---UAU-GAUGU)
8.Gymnopais_fimbriatus            -CAGUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCAUAAUAUG---(ACAUCU---UAU-GAUGU)
9.Twinnia_nova                    -CAGUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCAUAAUAUG---(ACAUCU---UAU-GAUGU)
10.Crozetia_crozetensis           -UGUUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----AA(UAU--GAUCACAAUAUG---(ACAUCU---UUGUGAUGU)
11.Stegopterna_sp.                -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUCU---UC--GAUGU)
12.Tlalocomyia_nr_osbornei        -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUCU---UC--GAUGU)
13.Adlerella_denaria              -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUCU---UC--GAUGU)
14.Greniera_fabri                 -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UGC--GAUCACAAUAUG---(ACACCU---UU--GGUGU)
15.Cnephia_ornithophilia          -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----AA(UAC--GAUCACAAUAUG---(ACAUCU---UC--GAUGU)
16.Ectemnia_sp.                   -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(AGC--GAUCACAAUGCG---(ACAUCC---UC-GGAUGU)
17.Metacnephia_sommermanae        -UGCUGG(UGUCUCGU----AUAGAUGUCUAAUG)GAUUU----UA(GGC--GAUCACAAUAUG---(ACAUCC--UU---GAUGU)
18.Simulium_congareenarum         -UGUUGG(UGACUCGU----AUAGAUGUCUGAUG)GAUUU---UAA(UGC--GAUCACAAUAUG---(ACAUUU------AUUUGU)
19.Simulium_reptans               -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(AGC--GUUCACAAUAUG---(ACACCU----C--GGUGU)
20.Simulium_sirbanum              -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU---UAA(UGC--GAUCACAAUAUG---(ACACCU---UUU-GGUGU)
21.Paracnephia_rhodesiana         -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUC----AA(UAU--GAUCACAAUAUG---(ACAUUU-----UUAAUGU)
22.Paracnephia_thornei            -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAU--GAUCACAAUAUG---(ACAUUU--UAAUUAAUGU)
23.Paracnephia_tonnoiri           -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUA)GAUUU--UUUA(UAU--GAUCACAAUAUG---(ACAUCC---UUGUGAUGU)
24.Paracnephia_aurantiaca         -UGUUGG(UGUCUCGU----AUAGAUGUCUGAUA)GAU-------A(UAU--GAUCACAAUAUG---(ACAUUUUUUUUGUAAUGU)
25.Paracnephia_strenua            -UGUUGG(UGACUCGU----AUAGAUGUCUGAUA)GAUUU--UUUA(UAU--GAUUACAAUAUG---(ACAUUA------AUUUGU)
26.Gigantodax_adleri              -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GUUUU---UUA(UAC--GAUCACAAUAUG---(ACAUUU------AUUUGU)
27.Gigantodax_marginalis          -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUUU------AUUUGU)
28.Cnesia_disimillis              -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUCU---UC--GAUGU)
29.Paracnephia_umbratora          -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UAC--GAUCACAAUAUG---(ACAUCU--CUC--GAUGU)
30.Paraustrosimulium_anthracinum  -UGCUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU----UA(UGC--GAUCACAAUAUG---(ACAUCU-----UUGAUGU)
31.Austrosimulium_bancrofti       -UUUUGG(UGUCUCGU----AUAGAUGUCUGAUG)GAUUU---AAA(UAU--GA---CUAUAAGC--(ACAUUA------AUUUGU)
32.Austrosimulium_mirabile        -UUUUGG(UGUUUCGU----AUAGAUGUCUGAUG)GAUUU---AAA(UAU--GAUUAUAAUA-----(ACAUUA------AUUUGU)
```

TABLE 7. Continued

```
                                                  D3-6    D3->     D3-1                       D3-2                         D3-3
2.Cardiocladius_sp.                 (CAUCACUC-----AUUCGUG)--AGCUUAGAUAUGUGCCCG-)AUCA[ACGGCGG]-UCAA[AUUUGGGAUACCUUUUGG]ACCCGUCUUG
3.Thaumalea_gillespieae             (CUAUUGCAGCAUUAUAUGUG)UAUGCAUAGUGUUUUGCCGUA)AUCA[GCAAUGU]GACCA[GUUUAGUA-AUCUUCCGG]ACCCGUCUUG
4.Parasimulium_Crosskeyi            (AAUCAGAA------AUGAUU)--UUUAUUAUAUUAUGUUGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
5.Urosimulium_aculeatum             (AAUCAGAA------AUGAUU)----UAUUGUAUUCUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
6.Helodon_onychodactylus            (AAUCAGAA------AUGAUU)----UAUUGUAUUCUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
7.Prosimulium_formosum              (AAUCAGAA------AUGAUU)----UAUUGUAUUCUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
8.Gymnopais_fimbriatus              (AAUCAGAA------AUGAUU)----UAUUGUAUUCUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
9.Twinnia_nova                      (AAUCAGAA------AUGAUU)----UAUUGUAUUCUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
10.Crozetia_crozetensis             (AACCAGUA------AUGGCU)----UAUUAUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
11.Stegopterna_sp.                  (AACCAGCA------AUGGUU)----UAUUGUAUUGUGCCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
12.Tlalocomyia_nr_osbornei          (AACCAGAA------AUGGUU)----UAUUGUAUUGUGCCGUG)AUCA[GCAAUGU]-AUUU[GUUUAAUA-AUCUUCAGG]ACCCGUCUUG
13.Adlerella_denaria                (AACCAGAA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
14.Greniera_fabri                   (AACCAGUA------AUGGUU)----UUUUGUAUUGUGCCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
15.Cnephia_ornithophilia            (AACCAGAA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
16.Ectemnia_sp.                     (AACCAGUA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
17.Metacnephia_sommermanae          (AACCAGUA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAGUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
18.Simulium_congareenarum           (AACCAGAA------AUGGUU)----UAUUAUAUUGUGUCGUG)AUCA[GCAGUGU]-AUUU[GUUUAGUA-AUCUUCAUG]ACCCGUCUUG
19.Simulium_reptans                 (AACCAGUA------AUGGUU)----UCUUGUAUUGUGUCGUG)AUCA[GCAGUGU]-AUUU[GUUUAGUA-AUCUUCAUG]ACCCGUCUUG
20.Simulium_sirbanum                (AACCAGUA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAGUGU]-AUUU[GUUUAGUA-AUCUUCAUG]ACCCGUCUUG
21.Paracnephia_rhodesiana           (AACCAGAA------AUGGUU)----UUUUAUAUUGUGUCAUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
22.Paracnephia_thornei              (AACCAGAA------AUGGUU)--UUUAUUGUAUUGUGUCAUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
23.Paracnephia_tonnoiri             (AACCAGUA------AUGGUU)----UAUUAUAUUGUGCCAUG)AUCG[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
24.Paracnephia_aurantiaca           (AACCAGUA------AUGGUU)----UAUUAUAUUGUGUCAUG)AUCG[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
25.Paracnephia_strenua              (AACCAGUA------AUGGUU)--UUUUUAUAUUUGUAUCAUG)AUCG[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
26.Gigantodax_adleri                (AAUCAGAA------AUGAUU)---UUCUUAUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
27.Gigantodax_marginalis            (AAUCAGAA------AUGAUU)----UUUAAUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
28.Cnesia_disimillis                (AACCAGUA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
29.Paracnephia_umbratora             (AACCAGUA------AUGGUU)----UAUUGUAUUGUGUCGUG)AUCG[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
30.Paraustrosimulium_anthracinum    (AACCAGUA------AUGGUU)----UAUUGUAUUGUGCCGUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
31.Austrosimulium_bancrofti         (AAUCAGCA------AUGGUU)----UUUUAUAU---GUCAUG)AUCG[GCAAUGU]-AUUU[GUUUAGUA-AUCUUCAGG]ACCCGUCUUG
32.Austrosimulium_mirabile          (AACCAGAA------AUGGUU)------UUUUAU-AUAUCAUG)AUCA[GCAAUGU]-AUUU[GUUUAGUA-AUCUCCAGG]ACCCGUCUUG
```

TABLE 7. Continued

	D3-4
2.Cardiocladius_sp.	AAACACGG[ACCAAG]GAGUC[UAUCUAGUGUGC]AA(GUAAAUGAG-----CAUAU-----GAAACUCAUUUGC)AAA(AGAAACUU)
3.Thaumalea_gillespieae	AAACACGG[ACCAAG]AAGUU[UAACUUAUGCGC]AA(GUCAAUGAG----UUUAU---UUAAAGCUUAUAGGC)GAU(AUUAACGU)
4.Parasimulium_Crosskeyi	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
5.Urosimulium_aculeatum	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
6.Helodon_onychodactylus	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
7.Prosimulium_formosum	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
8.Gymnopais_fimbriatus	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
9.Twinnia_nova	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
10.Crozetia_crozetensis	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
11.Stegopterna_sp.	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
12.Tlalocomyia_nr_osbornei	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUUAUAGGC)GAA(ACUAACAU)
13.Adlerella_denaria	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
14.Greniera_fabri	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
15.Cnephia_ornithophilia	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
16.Ectemnia_sp.	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
17.Metacnephia_sommermanae	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
18.Simulium_congareenarum	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
19.Simulium_reptans	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GUA(AUUAACAU)
20.Simulium_sirbanum	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GGA(AUUAACAU)
21.Paracnephia_rhodesiana	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GGA(AUUAACAU)
22.Paracnephia_thornei	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA--UAUUUAU-----AAACCUCAGAGGC)GUA(AUUAACAU)
23.Paracnephia_tonnoiri	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
24.Paracnephia_aurantiaca	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUAAGA----UUUAU-----AAACCUUAGAGGC)GAA(AUUAACAU)
25.Paracnephia_strenua	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUAAGA----UUUAU-----AAACCUUAGAGGC)GAA(AUUAACAU)
26.Gigantodax_adleri	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
27.Gigantodax_marginalis	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
28.Cnesia_disimillis	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
29.Paracnephia_umbratora	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUCAGAGGC)GAA(AUUAACAU)
30.Paraustrosimulium_anthracinum	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUUAGAGGC)GAA(AUUAACAU)
31.Austrosimulium_bancrofti	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUUAGAGGC)GAA(AUUAACAU)
32.Austrosimulium_mirabile	AAACACGG[ACCAAG]AAGUC[UAUUUUAUGCGC]GA(GUCAAUGAGA----UUUAU-----AAACCUUAGAGGC)GAA(AUUAACAU)

TABLE 7. Continued

		D3-5		D3-1′
2.Cardiocladius_sp.	GACUGUU--------UAU	(GGGACUACGAGUAGCAUUUUA-UAUG-UUGCUCCUCAAUCCC)	G	(GGGUAUUU-UUA--UCAA---------
3.Thaumalea_gillespieae	AACUGUUAUUAUAAAUAU	(GGAAUUACGGUGUCUGUAAUAUUGUG-GACUUCCUUCUUUCC)	C	(AGGUAUCUGUUAAUCUAAGAUUUUACU
4.Parasimulium_Crosskeyi	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUUUCCAUCCC)	U	(GGGUAUCUAUUAAUCU-----------
5.Urosimulium_aculeatum	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
6.Helodon_onychodactylus	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
7.Prosimulium_formosum	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUUUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
8.Gymnopais_fimbriatus	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
9.Twinnia_nova	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
10.Crozetia_crozetensis	AACUGUU-UUAUAC--UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
11.Stegopterna_sp.	AACUGUU---------UU	(GGGAUUACGAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCUAUUAAUCU-----------
12.Tlalocomyia_nr_osbornei	AACUGUU---------UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
13.Adlerella_denaria	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
14.Greniera_fabri	AACUGUU---------UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCGUUAGUCU-----------
15.Cnephia_ornithophilia	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	G	(GGGUAUCCAUUAGUCU-----------
16.Ectemnia_sp.	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
17.Metacnephia_sommermanae	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
18.Simulium_congareenarum	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
19.Simulium_reptans	AACUAUU-UUGUU---U-	(GGGAUUACGAAUUCUGUAUUAAUAGA-GAAUUCCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
20.Simulium_sirbanum	AACUAUU-UUGUU---UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
21.Paracnephia_rhodesiana	AACUGUU--UAUU---UU	(GGGAUUACAAAUUCUGUUUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
22.Paracnephia_thornei	AACUGUU--UA-----UU	(GGGAUUACAUAUUCUGUAUUAAUAGA-GAAUAUUUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
23.Paracnephia_tonnoiri	AACUGUU-UUGUU-----	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
24.Paracnephia_aurantiaca	AACUGUU--UGUU-----	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
25.Paracnephia_strenua	AACUAUA-UAUUU-AAUU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCUAUUAAUCU-----------
26.Gigantodax_adleri	AACUGUU---------UU	(GGGAUUACAAAUUAUGUAUUAAUAGA-UAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
27.Gigantodax_marginalis	AACUGUU---------UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
28.Cnesia_disimillis	AACUGUU---------UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
29.Paracnephia_umbratora	AACUGUU---------UU	(GGGAUUACGAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------
30.Paraustrosimulium_anthracinum	AACUGUU---------UU	(GGGAUUACAAAUUCUGUAUUAAUAGA-GAAUUUCUCCAUCCC)	U	(GGGUAUCCAUUAGUCU-----------
31.Austrosimulium_bancrofti	AACUGUU---------AU	(GGGAUUACAAAUUCAUUUUU----GAUGAAUUUUUCCAUCCC)	U	(GGGUAUCUUUUAAUCU-----------
32.Austrosimulium_mirabile	AACUGUU-UU------AU	(GGGAUUACAAAUUCAUUUUU----GAUGAAUUUUUCCAUCCC)	U	(GGGUAUCCAUUAAUCU-----------

TABLE 7. Continued

2.Cardiocladius_sp.	CAUAUGUAGCAA---UGCAU-AUG-------GA--AAAUAUACC)CAUGA[GCAUACUGGAUG]--A[CC]C
3.Thaumalea_gillespieae	UGAUAUAUUUUA--UGUAUU-UUGUAUGCUUGGUAUGAUAUACU)-GUGA[GCGCAUAAGAUA]-GA[CC]C
4.Parasimulium_Crosskeyi	UAAUCUAUUUAACCGAUAGUAUUA-------AGUAAGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
5.Urosimulium_aculeatum	UGUUCUGUUUAACCGACAGUAGUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
6.Helodon_onychodactylus	UGUUCUGUUUAACCGACAGUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
7.Prosimulium_formosum	UGUUCUGUUUAACCGACAGUAAUG-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
8.Gymnopais_fimbriatus	UGUUCUGUUUUA-CGACAGUAAUG-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
9.Twinnia_nova	UGUUCUGUUUUA-CGACAGUAAUG-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
10.Crozetia_crozetensis	UGUUUUGUUUUA-CGACAAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
11.Stegopterna_sp.	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GUGCAUAAAAUG]UGA[CC]C
12.Tlalocomyia_nr_osbornei	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
13.Adlerella_denaria	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
14.Greniera_fabri	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
15.Cnephia_ornithophilia	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
16.Ectemnia_sp.	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
17.Metacnephia_sommermanae	UGUUUCGUCUUAACAACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
18.Simulium_congareenarum	UGUUUCGUUUUA-CGACGAUAAUA-------AGUUGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
19.Simulium_reptans	UGCUUCGUUUUA-CGACGAUAGUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
20.Simulium_sirbanum	UGCUUCGUUUUA-CGACGAUAGUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
21.Paracnephia_rhodesiana	UGUUUCGUUUUA-CGACGAUAAUA-------AGUGGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
22.Paracnephia_thornei	UGUUUCGUUUUA-CGACGAUAAUA-------AGUGGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
23.Paracnephia_tonnoiri	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
24.Paracnephia_aurantiaca	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
25.Paracnephia_strenua	UGUUUCGUUUUA-CGACGAUAAUA-------AGUUGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
26.Gigantodax_adleri	UAUUUCGUUUUA-CGACGAUAAUG-------UGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
27.Gigantodax_marginalis	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
28.Cnesia_disimillis	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
29.Paracnephia_umbratora	UGUUUUGUUUUA-CGACAAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
30.Paraustrosimulium_anthracinum	UGUUUCGUUUUA-CGACGAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
31.Austrosimulium_bancrofti	UGUUUUGUUUUA-CGACAAUAAUA-------AGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C
32.Austrosimulium_mirabile	UAAUUUGUUUUA-CGACAAUAUUA-------UGUAGGAUAUACC)-AUGA[GCGCAUAAAAUG]UGA[CC]C

TABLE 8. 28S-D9-10 Region Secondary Structural Alignment

	70	66′	63′ 53′		72	
1.Cardiocladius_sp.	AAUUAGUGACG)	CGCAUGAAUGGA)	UUAACGAGAUUCCCACUGUCCCUAUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	AAAC
2.Thaumalea_gillespieae	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUUUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	AAAC
3.Parasimulium_Crosskeyi	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	AAAC
4.Urosimulium_aculeatum	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CAAAGGGAACGGGCUUUG)	AAAC
5.Helodon_onychodactylus	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CAAAGGGAACGGGCUUUG)	AAAC
6.Prosimulium_formosum	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CAAAGGGAACGGGCUUUG)	AAAC
7.Gymnopais_fimbriatus	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CAAAGGGAACGGGCUUUG)	AAAC
8.Twinnia_nova	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CAAAGGGAACGGGCUUUG)	AAAC
9.Crozetia_crozetensis	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
10.Stegopterna_sp.	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
11.Tlalocomyia_nr_osbornei	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
12.Adlerella_denaria	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
13.Greniera_fabri	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
14.Cnephia_ornithophilia	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
15.Ectemnia_sp.	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
16.Metacnephia_sommermanae	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
17.Simulium_congareenarum	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
18.Simulium_reptans	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
29.Simulium_sirbanum	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
20.Paracnephia_rhodesiana	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
21.Paracnephia_thornei	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
22.Paracnephia_tonnoiri	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
23.Paracnephia_aurantiaca	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
24.Paracnephia_strenua	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
25.Gigantodax_adleri	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
26.Gigantodax_marginalis	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
27.Cnesia_disimillis	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
28.Paracnephia_umbratora	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
29.Paraustrosimulium_anthracinum	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
30.Austrosimulium_bancrofti	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC
31.Austrosimulium_mirabile	AAUUAGUGACG)	UGCAUGAAUGGA)	UUAACGUGAUUCCCUCUGUCCUUGUCUACUAUCUA)	GCGAAACCACAG	(CCAAGGGAACGGGCUUGG)	GAAC

TABLE 8. Continued

	73	74	75	76	77	
1.Cardiocladius_sp.	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUGGCAUU	(GUAAGGCGGUAUAAAAGGUGCAGCAUA	(GGUGGGA	(GAUGG
2.Thaumalea_gillespieae	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GAUUA
3.Parasimulium_Crosskeyi	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GAUAU
4.Urosimulium_aculeatum	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
5.Helodon_onychodactylus	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
6.Prosimulium_formosum	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
7.Gymnopais_fimbriatus	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
8.Twinnia_nova	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
9.Crozetia_crozetensis	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
10.Stegopterna_sp.	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
11.Tlalocomyia_nr_osbornei	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
12.Adlerella_denaria	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
13.Greniera_fabri	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
14.Cnephia_ornithophilia	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
15.Ectemnia_sp.	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
16.Metacnephia_sommermanae	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
17.Simulium_congareenarum	(ACUAGCGGGGAAAGAAGA	(CCCUGCUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
18.Simulium_reptans	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
29.Simulium_sirbanum	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
20.Paracnephia_rhodesiana	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
21.Paracnephia_thornei	(ACUAGCGGGGAAAGAAAA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
22.Paracnephia_tonnoiri	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
23.Paracnephia_aurantiaca	(ACUAGCGGGGAAAGAAGA	(ACCUGUUGAGCUUG	(ACUCUAAUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
24.Paracnephia_strenua	(ACUAGCGGGGAAAGAAGA	(CCCUUUUGAGCUUG	(ACUCUAAUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GAUAU
25.Gigantodax_adleri	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
26.Gigantodax_marginalis	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
27.Cnesia_disimillis	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
28.Paracnephia_umbratora	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
29.Paraustrosimulium_anthracinum	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
30.Austrosimulium_bancrofti	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU
31.Austrosimulium_mirabile	(ACUAGCGGGGAAAGAAGA	(CCCUGUUGAGCUUG	(ACUCUAGUCUAGCAUU	(GUAAAGCGAUAUAAGAGGUGCAGUAUA	(GGUGGGA	(GGUAU

TABLE 8. Continued

Taxon	78=D9			77′	76′	79	
1.Cardiocladius_sp.	[C-----------UUCG--------]	------	[--]	--------GUUAUC)	AACAAUGAGAUACCACC)	ACUCUUACUGCUGCCUUAC)	UUA
2.Thaumalea_gillespieae	[GCUAAA-UUUU-ACAA--UCUUUA]	AUGAGC	[GA]	AUAAAGUGUUAAUC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
3.Parasimulium_Crosskeyi	[UUUAAACUUUU-AAUCUUGUCAAA]	UUGAGC	[GA]	AUAAAUGAUUUAUC)	ACCAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
4.Urosimulium_aculeatum	[GAUAAACUUCU-GAUC--UCGCAA]	GGGGGA	[GG]	AUAAUUUUUAUAUC)	GCCAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
5.Helodon_onychodactylus	[GAUAAACUUCU-GAAC--UCGCAA]	GGGGGA	[AG]	AUAAUUUUUAUAUC)	GCCAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
6.Prosimulium_formosum	[GAUAAACUUUU-GAUC--UCGCAA]	GGGGGA	[GA]	AUAAUUUUUAUACC)	GCCAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
7.Gymnopais_fimbriatus	[AAUAAACUUCU-GAUC--CCUUAU]	GGGGGA	[GG]	AUAAUUUUUAUAUC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
8.Twinnia_nova	[AAUAAACUUUU-GAUC--CCGCAA]	GGGGGA	[UG]	AUAAUUUUUAUAUC)	GACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
9.Crozetia_crozetensis	[AAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
10.Stegopterna_sp.	[GAUAAACUUUG-GAUC--UCGCAA]	GGGCUA	[UG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
11.Tlalocomyia_nr_osbornei	[GAUAAACUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUCAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
12.Adlerella_denaria	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
13.Greniera_fabri	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	GACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
14.Cnephia_ornithophilia	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUCAUACC)	AAAAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
15.Ectemnia_sp.	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
16.Metacnephia_sommermanae	[GAUAAACUUUC-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
17.Simulium_congareenarum	[GAUAAACUUUU-GAUC--UCGCAA]	GGGCUA	[CG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
18.Simulium_reptans	[GAUAAACUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AAAAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
29.Simulium_sirbanum	[GAUAAACUUUC-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
20.Paracnephia_rhodesiana	[GAUAAACUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
21.Paracnephia_thornei	[GAUAAACUUCU-GAAC--UCGUAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAAAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
22.Paracnephia_tonnoiri	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
23.Paracnephia_aurantiaca	[AAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
24.Paracnephia_strenua	{ AAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUUAUAUC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
25.Gigantodax_adleri	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
26.Gigantodax_marginalis	[GAUAAGCUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
27.Cnesia_disimillis	[GAUAAACUUUU-GAAC--UCGUAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
28.Paracnephia_umbratora	[GAUAAACUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
29.Paraustrosimulium_anthracinum	[GAUAAACUUCU-GAAC--UCGCAA]	GGGCUA	[GG]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
30.Austrosimulium_bancrofti	[GAUAAACUUUC-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA
31.Austrosimulium_mirabile	[GAUAAACUUUU-GAAC--UCGCAA]	GGGCUA	[GA]	AUAAUUUUUAUACC)	AACAAUGAGAUACCACC)	ACUCUUACUGUUGCUUUAC)	UUA

TABLE 8. Continued

```
                                   80        D10a                    D10a'                                D10b
1.Cardiocladius_sp.                  (UUCGAUUAGG(UGGAACAA(GUAUGCUAU-----UUUG---AGCGUCGGCGC)GCAAGUGUCGGCCUCUAUAGUAUGCA-
2.Thaumalea_gillespieae              (CCUGAUAAAG(UGGAACAA(AUGUCUAUAUGCU-UAAU--UGCUUAUUGGAU)AUCCGGUUUCUUGUUUAUAGUAUUU--
3.Parasimulium_Crosskeyi             (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCG-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
4.Urosimulium_aculeatum              (CCUGAUUAGG(UGGAACAA(GUAUUAGAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
5.Helodon_onychodactylus             (CCUGAUUAGG(UGGAACAA(GUAUUGAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
6.Prosimulium_formosum               (CCUGAUUAGG(UGGAACAA(GUAUUAGAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
7.Gymnopais_fimbriatus               (CCUGAUUAGG(UGGAACAA(GUAUUGAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
8.Twinnia_nova                       (CCUGAUUAGG(UGGAACAA(GUAUUGAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAUAU--
9.Crozetia_crozetensis               (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
10.Stegopterna_sp.                   (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
11.Tlalocomyia_nr_osbornei           (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAGA---
12.Adlerella_denaria                 (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
13.Greniera_fabri                    (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
14.Cnephia_ornithophilia             (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
15.Ectemnia_sp.                      (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
16.Metacnephia_sommermanae           (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
17.Simulium_congareenarum            (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
18.Simulium_reptans                  (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
29.Simulium_sirbanum                 (CCUGAUUAGG(UGGAACAA(GUAUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
20.Paracnephia_rhodesiana            (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
21.Paracnephia_thornei               (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
22.Paracnephia_tonnoiri              (CUUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
23.Paracnephia_aurantiaca            (CUUGGUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUUAACGUAAA---
24.Paracnephia_strenua               (CUUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
25.Gigantodax_adleri                 (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
26.Gigantodax_marginalis             (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
27.Cnesia_disimillis                 (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
28.Paracnephia_umbratora             (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
29.Paraustrosimulium_anthracinum     (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
30.Austrosimulium_bancrofti          (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
31.Austrosimulium_mirabile           (CCUGAUUAGG(UGGAACAA(GUGUUAAAUAUCU-UAAU--UGAUUUUAAUAU)AU--GGUUUCUUGUUCAACGUAAA---
```

TABLE 8. Continued

	D10c D10d
1.Cardiocladius_sp.	(CGUAUU (CUUGUUCAACGUAU-AGAUAGUCGUCAUGCACAAUU (ACUGUGA [UGU--GUGCGCU---------------------UGUCGAGU
2.Thaumalea_gillespieae	(GAGGUC (GUUAUUCAACAAUA--AGGUGA--GAUUU--AGAUG (GUGAUGA [-----GUGUGGA-----------------------------
3.Parasimulium_Crosskeyi	(GAAGUC (GUUAUACAAUAAGAU-AGAUGACCGAUUUCUAAAUU (UAUAUGA [AA---AAUUGGAA---------------------------A
4.Urosimulium_aculeatum	(GAAGUC (GUUAUACAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
5.Helodon_onychodactylus	(GAAGUC (GUUAUACAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
6.Prosimulium_formosum	(GAAGUC (GUUAUACAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
7.Gymnopais_fimbriatus	(GAAGUC (GUUAUACAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
8.Twinnia_nova	(GAAGUC (GUUAUACAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
9.Crozetia_crozetensis	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---AAUUGGAA----------------------------
10.Stegopterna_sp.	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
11.Tlalocomyia_nr_osbornei	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
12.Adlerella_denaria	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
13.Greniera_fabri	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
14.Cnephia_ornithophilia	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUAGGAA----------------------------
15.Ectemnia_sp.	(AAAGUC (GUUAAUCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
16.Metacnephia_sommermanae	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUACGA [AA---GGUUGGAA----------------------------
17.Simulium_congareenarum	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUACGA [AA---GGUUGGAA----------------------------
18.Simulium_reptans	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUGCGA [AA---GGUUGGAA----------------------------
29.Simulium_sirbanum	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUGCGA [AA---GGUUGGAA----------------------------
20.Paracnephia_rhodesiana	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
21.Paracnephia_thornei	(AAAGUC (GUUAUUCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---AAUUGGAA----------------------------
22.Paracnephia_tonnoiri	(AAAGUC (GUUAUCUAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAUUUU----------------------GGA
23.Paracnephia_aurantiaca	(AAAGUC (GUUAUCUAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---AAUUGGAUU-------------------GGGGAAUU
24.Paracnephia_strenua	(AAAGUC (GUUAUCUAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [-AA---GAUUGGAUAAU-------------AAAAUUAUUUUA
25.Gigantodax_adleri	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAAUGGAA---------------------------A
26.Gigantodax_marginalis	(AAAGUC (GUUAUACAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAAUGGAA---------------------------A
27.Cnesia_disimillis	(AAAGUC (GUUAUCCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUUGGAA----------------------------
28.Paracnephia_umbratora	(AAAGUC (GUUAUUCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUAUGA [AA---GAUCGGA-----------------------------
29.Paraustrosimulium_anthracinum	(AAAGUC (GUUAUUCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAUGUGA [AA---GAUUGGAA----------------------------
30.Austrosimulium_bancrofti	(AAAGUC (GUUAUUCAACAAGAUUAGAUGACCGAUAUCUAAAUU (UAGGUGA [AA---GAAUGGAAAUU-------------------------
31.Austrosimulium_mirabile	(AAAGUC (GUUAUUCAAUAAGAUUAGAUGACCGAUAUCUAAAUU (UAGUUGA [AA---GAAUGGAAAUU-------------------------

TABLE 8. Continued

1.Cardiocladius_sp.	GAAUGGAUGUUAUCGCAAGGUGACG--------------------UCUGUUUGUCUUUGAUAUGG]U) ----------GCGCCAUUCGGUAGCGUUC
2.Thaumalea_gillespieae	UAUAUUUAUUUAUAUA-----------------------------------CACACGA-CUUGUG]U) ----------CUGUCUCUCUGUA-CGUAU
3.Parasimulium_Crosskeyi	AAAAUCUUACGGUUUUU-------------------------------AGAAAAUUGAGUAAUAU]A) UAAAUUGAUAUCAACUAUCAUGAUCGUAC
4.Urosimulium_aculeatum	AAAGCUUUCGGGCUUU--------------------------------AGAAAAUCAAGUAAUAU]A) UAAAUUGAUAUCAACUAUCACGAUCGUAC
5.Helodon_onychodactylus	AAAACUUUCGGGUUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGCUAUCAACUAUCACGAUCGUAC
6.Prosimulium_formosum	AAAACUUUCGGGUUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGCUAUCAACUAUCACGAUCGUAC
7.Gymnopais_fimbriatus	AAAACUUUCGGGUUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGCUAUCAACUAUCACGAUCGUAC
8.Twinnia_nova	GAAACUUUCGGGUUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGCUAUCAACUAUCACGAUCGUAC
9.Crozetia_crozetensis	AAGGCUUUCGGGCUUU--------------------------------CGAUAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
10.Stegopterna_sp.	AAGGCUUUCGGGCUUU--------------------------------AGAUAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUUGUAC
11.Tlalocomyia_nr_osbornei	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGAUAUCAACUAUCACGAUCGUAC
12.Adlerella_denaria	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
13.Greniera_fabri	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
14.Cnephia_ornithophilia	AAGGCUUUCGGGCUUU--------------------------------AGAAUAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
15.Ectemnia_sp.	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUGU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
16.Metacnephia_sommermanae	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAGUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
17.Simulium_congareenarum	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAGUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
18.Simulium_reptans	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAGUAU]A) UAAAUUGGUAUCAACUAUCAUGAUCGUAC
29.Simulium_sirbanum	AAGGCUUUCGGGCUUU--------------------------------AAAAAAUCGAGUAGUGU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
20.Paracnephia_rhodesiana	AAAGCUUUCGGGUUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
21.Paracnephia_thornei	AAGGCUCUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
22.Paracnephia_tonnoiri	AUUGCUUUCGGGUGAUUUC--------------------------UUAUGAUAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
23.Paracnephia_aurantiaca	UUGGCUUUCGGGUUGGAAUUUUUC----------------------UUUGAUAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCAUGAUCGUAC
24.Paracnephia_strenua	UUUGCUUUCGGGUAGAUAAAAUUUUUUA------------------UUUGAUUAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCAUGAUCGUAC
25.Gigantodax_adleri	AAGGCUUUCGGGUUUUU-------------------------------AAAUAUUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
26.Gigantodax_marginalis	AAGGCUUUCGGGUUUUU-------------------------------AGAUAUUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
27.Cnesia_disimillis	AAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
28.Paracnephia_umbratora	AAAGCUUUCGGGCUUU--------------------------------AGAUGAUCGAGUAAUAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
29.Paraustrosimulium_anthracinum	GAGGCUUUCGGGCUUU--------------------------------AGAAAAUCGAGUAACAU]A) UAAAUUGGUAUCAACUAUCACGAUCGUAC
30.Austrosimulium_bancrofti	-AAUUCGCAAGAGUU------------------------------UUUUUAAAUUCGAGUAAUCU]A) UAAAUUGGUAUCAACUAUCAUGAUCGUAC
31.Austrosimulium_mirabile	-AAUUCGCAAGAGUU------------------------------UUUUUAAAUUCGAGUAUUCU]A) UAAAUUGGUAUCAACUAUCAUGAUCGUAC

TABLE 8. Continued

	D10b′ 80′ 79′ 75′
1.Cardiocladius_sp.	GUAUGAC)---AUUU-AAGGCUUC)UAUACACUCCA)CGUC-GAA)GACAUUGCCAAGUGCGGAGU)
2.Thaumalea_gillespieae	GAAUAAC)---AUUAAAAGGCUUC)UUUACGCUCCA)AGUCCAGG)GACAGUGUUAAGUGCGGAGU)
3.Parasimulium_Crosskeyi	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCCA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
4.Urosimulium_aculeatum	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCUA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
5.Helodon_onychodactylus	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCUA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
6.Prosimulium_formosum	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCUA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
7.Gymnopais_fimbriatus	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCUA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
8.Twinnia_nova	GUAUAAC)---AUUAAAAGGCUUC)UAUACACUCUA)UGUC-AGG)GACAGUGCUAGGUGCGGAGU)
9.Crozetia_crozetensis	GUGUAAC)---AUUAAAAGGCUUU)UCUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
10.Stegopterna_sp.	GUGUAAC)---AUUAAAAGGCUUU)UCUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
11.Tlalocomyia_nr_osbornei	GUGUAAC)---AUUAAAAGGUUUU)UCUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
12.Adlerella_denaria	GGAUAAC)---AUUAAAAGGCUUU)UCUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
13.Greniera_fabri	GGAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
14.Cnephia_ornithophilia	GGGUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
15.Ectemnia_sp.	GAUUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
16.Metacnephia_sommermanae	GGAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
17.Simulium_congareenarum	GGAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
18.Simulium_reptans	GGAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
29.Simulium_sirbanum	GGAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
20.Paracnephia_rhodesiana	GGAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
21.Paracnephia_thornei	AAAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
22.Paracnephia_tonnoiri	GGAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
23.Paracnephia_aurantiaca	GGAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
24.Paracnephia_strenua	GGAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGA
25.Gigantodax_adleri	GUGUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
26.Gigantodax_marginalis	GUGUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
27.Cnesia_disimillis	GGGUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
28.Paracnephia_umbratora	GAAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
29.Paraustrosimulium_anthracinum	GGAUAAC)---AUUAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
30.Austrosimulium_bancrofti	GAAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)
31.Austrosimulium_mirabile	AAAUAAC)---AUCAAAAGGCUUU)CUUACACUCCA)UGUU-AGG)GACAGUGCUAGGUGCGGAGU)

TABLE 9. 12S Secondary Structural Alignment

	22 577 673
Cardiocladius	CAAAUAA (UGUAAGUAAAUAUUAUU (AGUA--C (UAGUUAAAUUGA [UAUAUUUAUUAAGAUG---ACUAUU] UUA-UU (UUUUAGGUGAAAUUUUAAA)
Thaumalea_gillespieae	CAAAUAA (UGCAAAUAAAAUUUUUU (AGUG--U (UAGUUAAAUGUG [AUAAAUAAAAAAAAAA--UUAAAUU] UAA-UU (UAUUAGGUAAAAUUUUAAA)
Parasimulium_crosskeyi	CAAAUAA (UAUAAAUAAAGUUAUUU (AGUU--U (UAGUUAAAUGUA [CCAUUUUUUUUUAUAAAAAUAAUU] UAUAUU (UUUUAGGUGAAAUUUUAAA)
Twinnia_nova	CAAAUAA (UGCAAAUAAAAUUGAUC (AGUA--A (UAGUUAAUUUUA [AUAAGU---UGAUUAAUUAUAUAUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Helodon_onychodactylus	CAAAUAA (UGCGAUUAAGAUUGAUU (AGUAAUA (UAGUUAGUUUGA [UUUAAA----UUUUAAUAUUAAAUU] ACU-UU (UUUUAGGUGAAAUUUUAAG)
Prosimulium_formosum	CAGAUAA (UGCAAAUAAGAUUGUUU (AGUA--A (UAGUUAAUUUGA [UUUAAU---UAUAUAAUGUUGUUUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Gymnopais_fimbriatus	CAAAUAA (UGCAAAUAAGAUUUAUU (AGUA-GU (AAGUUAAUUUGG [UUUAAU--UAAAUAAUUAUUAUAUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Adlerella_denaria	CAAAUAA (UACAAAUAAAAUUUAUU (AGUA--A (UAGUUAAUUUGU [AAUUUA---UAAAUAAAAUUAAAUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Cnephia_ornithophilia	CAAAUAA (UGCAAAUAAGAUUAGUU (AGUA--A (UAGUUAGUUUGU [AAUUUU---UCAAUAAAAUUAAUUU] AAAUUU (UUUUAAGUGAAAUUUUAAA)
Ectemnia	CAAAUAA (UGCAAAUAAGAUUGAUU (AGUA--A (UAGUUAGUUUGU [AGAAUA-----AUUAAAAUUAAUUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Metacnephia_sommermanae	CAAAUAA (UCCAAAUAAGAUUUAUU (AGUA--A (UAGUUAAUUUGU [AAUGUU----AAAUAAAAUUAAUUU] AA--UU (UUUUAAGUGAAAUUUUAAA)
Simulium	CAAAUAA (UACAAAUAAGAUCUAUU (AGUA--A (UAGUUAAUUUGU [U-AUAA-----AAUAAAAUUAAUUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Stegopterna	CAAAUAA (UGCAAAUAAAAUUUAUU (AGUA--A (UAGUUAAUUUGU [UAUAAU-UUUAAAUAAGAAUAAUUU] AAA-UU (UUUUAAGUGAAAUUUUAAG)
Tlalocomyia	CAAAUAA (UACAAAUAAAAUUUAUU (AGUA--A (UAGUUAGUUUGU [UAUUAU-UUAUUAUGAUAUUAAUUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Cnesia_dissimilis	CAAAUAA (UACAAAUAAGAUUGAUU (AGUA--A (UAGUUAAUUUGU [UAAUAA-----UUUAAAAUUAAUUU] AAA-UU (UUUUAAGUGAAAUUUUAAA)
Paraustrosimulium_anthracinum	CAAAUAA (UGCAAAUAAGAUUGAUU (AGUA--U (UAGUUAAUUUGU [UAUAGA--UACAGUAAAAUUAAAUU] AAA-UU (UUUUAGGUGAAAUUUUAAA)
Austrosimulium_bancrofti	

TABLE 9. Continued

	577 3′	27			22 3′	
Cardiocladius	[------AUAAAGUUUUAAUUUUAAUAUGAUUAAUU]	UAAGCUA)	UA-AAA	(GUUUAUUUAAAAAC	(UAGG-AUUAGAUACCCUA)	UUAU-UAAAA)
Thaumalea_gillespieae	[--UUUUUUCCAGUUUUUAG-AAUUAAAUUAAU--U]	UAAACUA)	UA-AAA	(CUUUAUUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAG)
Parasimulium_crosskeyi	[UAUAAUAAAUUUUAUAUUAU--UAAAAAUUAU--U]	GAAACUA)	UAAAAA	(UUUUAGUUUUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAUA)
Twinnia_nova	[---UUUUAUUAUUAUUAUA--AGUAAAUUUAA--U]	GAGGCUA)	UA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Helodon_onychodactylus	[---AGUAAUUAUUAUUAUAUAAGUAA-GUUAA--U]	GAAGCUA)	UA-AAA	(UUUUAACUAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Prosimulium_formosum	[---UUUAGUUAAUAUUAUA-AUAAGAGGUUAA--U]	GAAGCUA)	UA-AAA	(UUUUAAUUAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Gymnopais_fimbriatus	[---UUUAAUUAAUAUUAUA-AUAUAAAGUUAA-UU]	GAAGCUA)	UA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Adlerella_denaria	[---UUUAAUUAAUUUUAUA--UUUGAAUUUAA--U]	GAAGCUA)	GA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Cnephia_ornithophilia	[---UUUAAUUAGUUUUAUA--UUUUAAGUUAA--U]	GAAGCUA)	GA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Ectemnia	[---UUUAAUUAAUUUUAUA--AUAUUUAUUAA-UU]	GAAGCUA)	GG-AAA	(UUUUAAUUAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAG)
Metacnephia_sommermanae	[---UUUAAUUAAUUUUAUAAUUAAAAGGUUAA-UU]	GAAGCUA)	GA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Simulium	[---UUUAAUUAAUUUUAUA--CAUCAAGAUUUAAU]	GAAGCUA)	GG-AAA	(UUUUAAUUAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Stegopterna	[---UUUAAUUAAUCUUAUUUAUAUUUUUUAAA-UU]	GAAGCUA)	AA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Tlalocomyia	[---UUUAAUUAAUCCUAAUUAUUUUUAUUAAA-UU]	GAAGCUA)	GA-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Cnesia_dissimilis	[---UUUAAUUAGUUUUAUA--UAUAAUUAUUUGAU]	GAAGCUA)	GA-AAA	(UUUUAAUGAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAA)
Paraustrosimulium_anthracinum	[---UUUAUUUAAUUUUAUA---UUUUAUUUGA-UU]	GAAGCUA)	GG-AAA	(UUUUAAUAAUAAAC	(UAGG-AUUAGAUACCCUA)	UUAUUUAAAU)
Austrosimulium_bancrofti						

	31			32	33		34	35	
Cardiocladius	UGUAACAAUUUGAAUGCU)	A	(GAGUAGUAU	(UAGUUAUAAUCUU)	GAAACUU)	AAAAAAUU	[UGGCGGUGUUUUAGUCUAU	[UCAGA	(GGAACCUGUUCU
Thaumalea_gillespieae	UGU-AAAAUUUAAAUGCU)	U	(GAGUAGUAA	(UAGUUAAUUUCUU)	GAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAAUCUGUUCU
Parasimulium_crosskeyi	UUU-----UUUUUUUACU)	A	(AAGUAGUAA	(UAGUUAUGAUCUU)	UAAACUU)	AAAGAAUU	[UGGCGGUAUUUUAAUUUAU	[UUAGA	(GGAAUUUGUUUU
Twinnia_nova	UGU--AAAUUUAAGUGCU)	U	(GAGUAGUAA	(UAGGUAUGGACUU)	UAAACUU)	AAAUAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Helodon_onychodactylus	UGU--GAUGCUUAAUACU)	U	(GAGUAGUAG	(UAGAUAAGAUCUU)	UAAACUU)	AAAGAAUU	[UGGCGGCGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Prosimulium_formosum	UGU--AGGCCUUUAUACU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Gymnopais_fimbriatus	UGU--AAAUUUAAAUACU)	U	(GAGUAGUAA	(UAGUUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Adlerella_denaria	UGU--AAAUUUAAAUACU)	U	(GAGUAGUAA	(UAGAUAUAAUCUU)	CAAACUU)	AAAAAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Cnephia_ornithophilia	UGU--AAAUUUAUAUACU)	A	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Ectemnia	UGU--AAAGUUAAAUACU)	U	(GAGUAGUAA	(UAGAUAUGGUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACUUGUCCU
Metacnephia_sommermanae	UGU--AAAUCUAAAUACU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Simulium	UGU--AAUUUUUAAUACU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	(UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Stegopterna	UGU--AAAUUUAUUUACU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Tlalocomyia	UGU--AAAUUUAUAUACU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Cnesia_dissimilis	AGU--AAUAUUUAAAGCU)	U	(GAGUAGUAA	(UAGAUAUGAUCUU)	CAAACUU)	AAAGAAUU	(UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Paraustrosimulium_anthracinum	UGU--AAUUUUAAAUACU)	U	(GAGUAGUAA	(UAGUUAUAAUCUU)	CAAACUU)	AAAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU
Austrosimulium_bancrofti						AAGAAUU	[UGGCGGUGUUUUAGUCUAU	[UUAGA	(GGAACCUGUCCU

TABLE 9. Continued

		36	38 **	*	39	40	39 3′		42
Cardiocladius	(AUA	(AUCGAUAAUCCACGUU)	AUACCUUA)	(CUUAAUU	(UUGU-UACA)	(AUUUGUAUAUC	GCCGUCAUCA	(GAAUAU	(CUUAAAAGAG)
Thaumalea_gillespieae	(GUA	(AUCGAUAGUCCACGUU)	GAACCUUU)	(CUUAAUU	(-----UAAA)	(GUUUGUAUACC	GCCGUUGUCA	(GAAUAU	(CUUAUUAGGG)
Parasimulium_crosskeyi	(UUA	(AUCGAUAAUCCACGUA)	UA-CCUUA)	(CUAAAAU	(--UAAUAUA)	(AUUUGUAUAUC	GCCGUUAUUG	(AAUAUU	(CUAAAAGGAA)
Twinnia_nova	(UUA	(AUCGAUAAUCCACGUU)	GGACCUCA)	(CUAAUAU	(UUGUUUUCA)	(ACUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Helodon_onychodactylus	(UUA	(AUCGAUAGCCCACGUU)	GGACCUUA)	(CUAAAAU	(UAGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Prosimulium_formosum	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAAU	(UUGUUUACA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Gymnopais_fimbriatus	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAGAU	(UUGUUUUCA)	(ACUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Adlerella_denaria	(AUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAGAU	(UUGUUUACA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Cnephia_ornithophilia	(AUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAAU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Ectemnia	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Metacnephia_sommermanae	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Simulium	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Stegopterna	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAGGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Tlalocomyia	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAAU	(UUGUUUACA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(UUUAAAAGAG)
Cnesia_dissimilis	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CUAAAGU	(UUGUUUUCA)	(ACUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Paraustrosimulium_anthracinum	(GUA	(AUCGAUAAUCCCCGUU)	GGACCUUA)	(CUAAGGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)
Austrosimulium_bancrofti	(GUA	(AUCGAUAAUCCACGUU)	GGACCUUA)	(CGAAGGU	(UUGUUUUCA)	(AUUUGUAUAUC	GCCGUUAUCA	(GAAUAU	(CUUAAAAGAG)

	* 38 3′**	36 3′	34 3′		45			
Cardiocladius	A-AAAAUUUUC)	UU-AAAUUUUUAUUAAUAAUU-AUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AAUUAAG)	U	AGAAAUGGGUUAC)	AAU
Thaumalea_gillespieae	U----UAUUUUC)	UU-UAUUAAUUAAUAUUAAUU-ACAUCAGGUCAAGG	UGCAGU)	(UUAU)	AAUUAAG)	U	AGAAAUGGAUUAC)	AAU
Parasimulium_crosskeyi	A--AAAAAUUUC)	UU-AAUUUAAAAAAUAUAGUUAAUGUCAGGUCAAGA	UGCAGU)	(UUAU)	AUUUUAG)	U	AAAAAUGAGUUAC)	AAU
Twinnia_nova	U-AAUAAUUUUC)	UU-AAUCUAUUAUU-AUAGAUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Helodon_onychodactylus	U-AAUAAUUUUC)	UU-AAUUUAUUAUU-AUAAAUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Prosimulium_formosum	AAAAAAAUUUUC)	UU-AACUUAUAAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Gymnopais_fimbriatus	U-AAUAAUUUUC)	UU-AAUUUAUUAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Adlerella_denaria	G-AAUAAUUUUC)	UU-AAUCUAUAAUU-AUAGUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Cnephia_ornithophilia	A-AAUAAUUUUC)	UU-AAUUUAUUAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Ectemnia	G-AAUAAUUUUC)	UU-AAUUUAUUAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	UUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Metacnephia_sommermanae	G-AAUAAUUUUC)	UU-AAUCUAUAAUU-AUAGUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Simulium	G-AAUAAUUUUC)	UU-AAUUUAUAAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Stegopterna	G-AAUAAUUUUC)	UU-AAUUUAUAAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Tlalocomyia	U-AAUAAUUUUC)	UU-AAUUUAUAAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Cnesia_dissimilis	G-AAUAAUUUUC)	UU-AAUUUAUAAUU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	GCUUUAG)	U	AGAGAUGGGUUAC)	AAU
Paraustrosimulium_anthracinum	G-AAUAAUUUUC)	UU-AACUUAAAAAU-AUAAUUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU
Austrosimulium_bancrofti	G-AAUAAUUUUC)	UA-AAUUUAAUAAU-AUAAAUGAUGUCAGGUCAAGG	UGCAGU)	(UUAU)	AUUUUAG)	U	AGAGAUGGGUUAC)	AAU

TABLE 9. Continued

	47			33 3′		48		32′
Cardiocladius	(AA-AAUUUAUU)	UAGA-	(U-GAA	(UUAUUUUAUAAAAAGAAG--UUU)	GAAG)	GUGGAUUUGA]	UAGUA	(AAUUUAUUUAAUUAAAAUUAAUU)
Thaumalea_gillespieae	(AA-GGCAUAGU)	UAUA-	(U-GGU	(AAAUUAUGUGAAAUUUAUAAUUU)	UAAA)	GUGGAUUUAA]	UAGUA	(AAAUAAUAAAGAA-UAAUUAUUU)
Parasimulium_crosskeyi	(AAAUUUUUAUU)	UAUUA	(CAGUU	(-AAUUAAUUGAAAUAUUA-AUUU)	UAAG)	GUGAAUUUAA]	UUGUA	(AAAUAAUUAAUAA-AAUUUAUUU)
Twinnia_nova	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAGUUAUUUGAAAUAGUA-AUUU)	UAAG)	GUGGAUUUAA]	UAGUA	(AAAUAAUGUAUAU-AAUUUAUUU)
Helodon_onychodactylus	(AA--AAAUAUU)	UAUA-	(C-GGU	(AAUUUGUUGGAAAUAAUA-AAUU)	CAAG)	GCGGAUUUAA]	UAGUA	(AAACAAUGAAUAU-AGUUUAUUU)
Prosimulium_formosum	(AA--GUUUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAAUAGUA-AUUU)	UAAG)	GUGGAUUUAA]	UAGUA	(AAGUAAUUAAUAU-AGUUUACUU)
Gymnopais_fimbriatus	(AA--AAAUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAACAAUA-AUUU)	UAAG)	GUGGAUUUAA]	UAGUA	(AAAUAAAGGAUAG-AGUUUAUUU)
Adlerella_denaria	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAAUAAUA-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAGUAAUUAAUAA-AAUUUACUU)
Cnephia_ornithophilia	(AA--AAUUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAAUAAUA-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUGUAUAU-UACUUAUUU)
Ectemnia	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAAUAAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUCAUCAAUAU-UAUUUAUUU)
Metacnephia_sommermanae	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAAUUGUUUGAAAUAAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUGAUGGAUAU-AAUUCAUUU)
Simulium	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAGUUGUUUGAAAUAAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUAGAUAU-UAUUUAUUU)
Stegopterna	(AA--AAUUAUU)	UAUA-	(C-AUU	(AAAUUAUUUGAAAUAAUA-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUUAAUAU-AAUUUAUUU)
Tlalocomyia	(AA--GUUUAUU)	UAUA-	(C-GUU	(AAAUUGUUUGAAAUAAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUAGAUAU-AAUUUAUUU)
Cnesia_dissimilis	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAAUUAUUUGAAAUAAUA-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUUAAUUA-UUUUUAUUU)
Paraustrosimulium_anthracinum	(AA--AUUUAUU)	UAUA-	(C-AUU	(AAAUUGUCUGAAAUGAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUGAAUAU-AGUUUAUUU)
Austrosimulium_bancrofti	(AA--AUUUAUU)	UAUA-	(C-GUU	(AAAUUGUUUGCAAUAAUG-AUUU)	UAAG)	GUGGAUUUAA]	AAGUA	(AAAUAAUUAAUAA-AAUUUAUUU)

Cardiocladius	GAUUUUAGCUCU]
Thaumalea_gillespieae	GGUUAUAGCUCU]
Parasimulium_crosskeyi	GAUUUUAUUUUU]
Twinnia_nova	GAUUAUAGCUCU]
Helodon_onychodactylus	GAUUAUAGCUCU]
Prosimulium_formosum	GAUUAUAGCUCU]
Gymnopais_fimbriatus	GAUUAUAGCUCU]
Adlerella_denaria	GAUUUUAGCUCU]
Cnephia_ornithophilia	GAUUAUAGCUCU]
Ectemnia	GAUUAUAGCUCU]
Metacnephia_sommermanae	GAUUAUAGCUCU]
Simulium	GAUUAUAGCUCU]
Stegopterna	GAUUAUAGCUCU]
Tlalocomyia	GAUUAUAGCUCU]
Cnesia_dissimilis	GAUUAUAGCUCU]
Paraustrosimulium_anthracinum	GAUUAUAGCUCU]
Austrosimulium_bancrofti	GAUUAUAGCUCUAAAAC]

TABLE 10. Elongation Factor-1 Alpha Amino Acid Sequences

SGI-*Gymnopias fimbriatus*
SCD-*Cnesia dissimilis*
SAM-*Austrosimulium miribale*
SAB-*Austrosimulium bancrofti*
SPA-*Paraustrosimulium anthracinum*
SCC-*Crozetia crozetensis*
SPU-*Paracnephia thornei*
SPR-*Paracnephia rhodesiense*
SSM-*Stegopterna mutate*
SGM-*Gigantodax marginalis*
SGA-*Gigantodax adleri*
SMP-*Tlalocomyia nr osborni*
SSS-*Simulium sirbanum*
SSR-*Simulium reptans*
SSC-*Simulium congareenarum*
SMS-*Metacnephia sommermanae*
SES- *Ectemnia sp.*
SCO-*Cnephia ornithophilia*
SCA-*Paracnephia aurantiaca*
SCT-*Paracnephia tonnoiri*
SCS-*Paracnephia strenua*
SCU-*Paracnephia umbratora*
SGF-*Greniera fabri*
SGD-*Adlerella denaria*
SPF-*Prosimulium formosum*
SHO-*Helodon onychodactylus*
SUA-*Urosimulium aculeatum*
STN-*Twinnia Nova*
SPC-*Parasimulium crosskeyi*
TTG-*Thaumalea gillespieae*
CCS-*Cardiocladius sp.*
SSV-*Simulium venustum* from Minhas & Currie 2005
DF1- *Drosophila melanogaster* from Hovemann *et al.* 1988
DF2- *Drosophila melanogaster* from Hovemann *et al.* 1988
Indicates congruent novel amino acids between the simuliid taxa analyzed and the F2 copy of *Drosophila melanogaster*
Indicates congruent novel amino acids between the simuliid taxa analyzed and the F1 copy of *Drosophila melanogaster*

TABLE 10. Continued

CCS	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWALDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
TTG	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SPC	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMAKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SUA	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SHO	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMIPGTSQADCAVLIVAAGTGEFEAGI
SPF	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SGI	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYCVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
STN	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETGKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCC	HVHTGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SSM	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SMP	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SGD	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SGF	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCO	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SES	HVDSGKSTTTGHLIYKCGGIDKRSIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SMS	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SSC	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SSR	HVHSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SSS	HVHSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SPR	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SPU	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCT	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCA	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCS	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SGA	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SGM	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCD	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SCU	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SPA	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITTDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SAB	HVNSGKSTTTGHLIYKCGGIDKRTIEKFEKEAREMGRGSFKYAWVLDKLKAERERGITIDIALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SAM	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
SSV	KSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI
DF1	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETAKYYVTIIDAPGHRDFIKNMITGTSQADCAVQIDAAGTGEFEAGI
DF2	HVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDIALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGI

TABLE 10. Continued

CCS	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPYNEARFEEIKKEVSSYIKKIGYNPATVAFVPISGWHGDNMLEASSKMPWFKGWAVERKEGKADGKCLIEALDAILPPSRP
TTG	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFAETRFEEIKKEVSSYIKKIGYNPAAVAFVPISGWHGDNMLEVSAKMPWFKGWKVERKDGNAEGKCLIEALDAILPPSRP
SPC	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSETRFEEIKKEVSSYIKKIGYNPLCVAFVPISGWHGDNMLEISSKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
SUA	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNMLEVSAKMPWFKGWKVERKEGNADGKCLIEALDAILPPSRP
SHO	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSEPRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNMLEVSAKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
SPF	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPARVAFVPISGWHGDNMLEVSAKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
SGI	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNMLEISEKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
STN	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNKLEVSVKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
SCC	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPTFSEPRFEEIKKEVSSYIKKIGYNPACVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SSM	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKDGNADGKCLIESLDAILPPSRP
SMP	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVCCYIKKIGYNPMCVPFVPISGWHGDNMLEISAKMPWYKGWKVERKEGNADGKCLIESLDAILPPSRP
SGD	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPACVPFVPISGWHGDNMLEVSSNMGWFKGWKVERKEGNADGKCLIEALDAILPPSRP
SGF	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNMLEVSAKMPWFKGWKVERKDGNADGKCLIEALDAILPPSRP
SCO	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SES	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPACVAFVPISGWHGDNMLEVSAKMPWYKGWKVERKDGNASGTCLIEALDAILPPSRP
SMS	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPICVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNAEGKCLIEALDAILPPSRP
SSC	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SSR	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFCESRFEEIKKEVSSYIQKIGYNPAAVSFVPISGWHGDNLLDVSAKMPWYKGWKVERKDGNADGKCLIEALDAILPPSRP
SSS	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPAAVAFVPISGWHGDNMLEVSSKMPWYKGWKVERKEGNVDGKCLIEALDAILPPSRP
SPR	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSEARFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSSKMPWYKGWKVERKEGNADGKCLIEALDAILPPTRP
SPU	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SCT	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SCA	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPACVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SCS	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSEARFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SGA	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSEARFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMGWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SGM	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMGWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SCD	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPACVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKEGNADGKCLIESLDAILPPSRP
SCU	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTDPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSAKMPWYKGWKVERKDGNADGKCLIEALDAILPPSRP
SPA	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEISAKMPWYKGWKVERKDGNADGKCLIEALDAILPPSRP
SAB	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSEARFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSSKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SAM	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPVCVPFVPISGWHGDNMLEVSVKMPWYKGWKVERKEGNADGKCLIEALDAILPPSRP
SSV	SKNGQTXEHALLAFTLGVKQLIVGVNKMDSTEPPFSESRFEEIKKEVSSYIKKIGYNPAAVAFVPISGWHGDNMLEVSAKMPWYKGWKVERKDGNADGKCLIEALDAILPPSRP
DF1	SKNDQTREHALLAFTLGVKQLIVGVNKMDSSEPPYSEARYEEIKKEVSSYIKKVGYNPAAVAFVPISGWHGDNMLEPSTNMPWFKGWEVGRKEGNADGKTLVDALDAILPPARP
DF2	SKNGQTREHALLAFTLGVKQLIVGVNKMDSTEPPYSEARYEEIKKEVSSYIKKIGYNPASVAFVPISGWHGDNMLEPSEKMPWFKGWSVERKEGKAEGKCLIDALDAILPPQRP

TABLE 10. Continued

CCS	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVAFAPVNLTTEVKSVEMHHEALQEAVPGDNVGFNVKNVSVKELRRGYVAGDSKNNPPKGAADFTAQVIVLNHPGQISN
TTG	TGKPLRLPLQDVYKIGGIGTVPVGRVETGILKPGMLVIFAPVNLTTEVKSVEMHHEALQEAVPGDNVGFNVKNVSVKDLRRGFVAGDSKVSPPRGAADFTAQVIVLNHPGQISN
SPC	TDKPLRLPLQDVYKIGGIGTVPVGRVETGLLKPGMVVVFAPVNLTTEVKSVEMHHEALVEAVPGDNVGFNVKNVSVKELRRGYVAGDTKNNPPRGAENFTAQVIVLNHPGQISN
SUA	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVIVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SHO	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVIVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SPF	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVIVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNASVKELRRGYVAGDSKSNPPKGADNFTAQVIVLNHPGQISN
SGI	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVIVFAPVNLTTEVKSVEMHHEALVEAGPGDNVGFNVKNVSVKELRRGYVAGDTKSNPPKGAENFTAQVIVLNHPGQISN
STN	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVIVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDTKSNPPKGAESFTAQVIVLNHPGQISN
SCC	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALPEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SSM	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SMP	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SGD	TDKALRLPLQDVYKIGGIGTVPVGRVETGIIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SGF	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SCO	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SES	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGGLFLGNLEMFFFSTFHFLLVLLRYGRRVRPRQFDH
SMS	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQKAGPGDNVGFNVKNVAVKDLRRGYVAGDSKSNPPKGADNFTAQVIVLNHPGQISN
SSC	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKNNPPKGAENFTAQVIVLNHPGQISN
SSR	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SSS	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SPR	TDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSSPPKGAENFTAQVIVLNHPGQISN
SPU	TDKALRLPLQDVYKIGGIGTEPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAEDFTAQVIVLNHPGQISN
SCT	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSSPPKGAENFTAQVIVLNHPGQISN
SCA	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDTKSNPPKGAENFTAQVIVLNHPGQISN
SCS	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SGA	TDKALRLPLQDVYKIGGIGTVHVGRVETGIIKPGMVVVFAPVNLTTEVKSVEMHHEALQEASPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SGM	TDKALRLPLQDVYKIGGIGTVPVGRVETGIIKPGMVVVFAPVNLTTEVKSVEMHHEALQEASPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SCD	TDKALRLPLQDVYKIGGIGTVPVGRVETGIIKPGMVVVFAPVNLTTEVKSVEMHHEALQEASPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SCU	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALAEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSSPPRGAENFTAQVIVLNHPGQISN
SPA	TDKALRLPLQDVYKIGGIGTVPVGRVETGVINPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SAB	TDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEASPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SAM	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
SSV	TDKALRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVVFAPVNLTTEVKSVEMHHEALQEAGPGDNVGFNVKNVSVKELRRGYVAGDSKSNPPKGAENFTAQVIVLNHPGQISN
DF1	TDKALRLPLQDVYKIGGIGTVPVGRVETGVLKPGTVVVFAPANITTEVKSVEMHHEALQEAVPGDNVGFNVKNVSVKELRRGYVAGDSKANPPKGAADFTAQVIVLNHPGQIAN
DF2	TDKPLRLPLQDVYKIGGIGTVPVGRVETGLLKPGMVVNFAPVNLVTEVKSVEMHHEALTEAMPGDNVGFNVKNVSVKELRRGYVAGDSKNNPPRGAADFTAQVIVLNHPGQIAN

TABLE 10. Continued

CCS	GYTPVLDCHTAHIACKFGEIKEKVDRRSGKSVEDNPKSIKSGDAAMVVLVPSKPLCVESFSDFPPLGRFAVR
TTG	GYTPVLDCHTAHIACKFSEIKEKVDRRSGKSTEDNPKSIKSGDAAIVILAPSKPLCVESFSEFPPLGRFAVR
SPC	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SUA	GYTPVLDCHTAHIACKLSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SHO	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SPF	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SGI	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLRVESFSEFPPLGRFAVR
STN	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCC	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SSM	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEYPPLGRFAVR
SMP	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SGD	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVVLVPSKPLCVESFSEFPPLGRFAVR
SGF	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCO	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SES	
SMS	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SSC	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SSR	GYTPVLDCHTAHIACKFAEIKEKVDRRSGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SSS	GYTPVLDCHTAHIACKFSEIKEKVDRRSGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SPR	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTENDPKAIKSGDAAIVILVPSKPLCVEFFSEFPPLGRFAVR
SPU	GYTPVLDCHTAHIACKFPEIKEKVDRRTGKSTEDNPKAIKSGDAAMVILVPSKPLCVEFFSEFPPLGRFAVR
SCT	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCA	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCS	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SGA	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SGM	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCD	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SCU	GYTPVLDCHTAHIACKFAEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SPA	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SAB	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SAM	GYTPVLDCHTAHIACKFSEIKEKVDRRTGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGRFAVR
SSV	GYTPVLDCHTAHIACKFAEIKEKVDRRSGKSTEDNPKAIKSGDAAIVILVPSKPLCVESFSEFPPLGR
DF1	GYTPVLDCHTAHIACKFAEILEKVDRRSGKTTEENPKFIKSGDAAIVNLVPSKPLCVEAFQEFPPLGRFAVRDMRQTVAVGVIKAVNFKDASGGKVTKAAEKATKGKK
DF2	GYTPVLDCHTAHIACKFSEIKEKCDRRTGKTTETEPKAIKSGDAAIIVLVPSKPLCVESFQEFPPLGRFAVRDMRQTVAVGVIKSVNFKETTSGKVTKAAEKAQKKK

Acknowledgements

This research was supported by the Hatch Multistate Research Fund and the New Jersey Agricultural Experiment Station at Rutgers University, New Brunswick, New Jersey, Project: Evaluating the Physical and Biological Availability of Pesticides and Contaminants in Agricultural Ecosystems Project 224316 and NIEHS Center Grant ES020522.

Literature Cited

Adler P. H., Currie D. C., & Wood D. M. (2004). The black flies (Simuliidae) of North America. Ithaca, New York: Cornell University Press. 941.

Azer, D., Veltz, I., & Nel, A. (2008). Mandibulate chironomids: primitive or derived? (Diptera: Chironomidae). *Systematic Entomology*, 33, 688-699.

Ballard, J. W. O. (1994). Sequence data resolves a morphological conundrum in Austrosimulium (Diptera: Simuliidae). *J. Aust. Entomol. Soc.*, 33, 131-135.

Ballard, J. W., Olsen, G. J. , Faith, D. P. , Odgers, W. A., Rowell, D. M., & Atkinson, W. P. (1992). Evidence from 12S ribosomal RNA sequences suggest that onycophorans are modified arthropods. *Science*, 258, 1345-1348.

Borkent, A., & Wood, D. M. (1986). The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). *Proceedings of the Entomological Society of Washington*, 88, 287-296.

Carle, F. L. (1995). Evolution, Taxonomy and Biogeography of ancient gondwanian libelluloids, with comments on anisopteroid evolution and phylogenetic systematics. *Odonatologica*, 24, 383-424

Coscaron, S., & Coscaron Arias C. L. (2007). *Neotropical Simuliidae (Diptera: Insecta). Aquatic Biodiversity in Latin America Vol 3.* Neotropical Simuliidae. Pensoft Publishers Bulgaria, 685 pp.

Craig, D. A. (2003). Deconstructing Gondwana - words of warning from the Crozet Island Simuliidae. *Cimbebasia*., 19, 157-164.

Craig, D. A., Currie D. C., & Vernon P. (2003). *Crozetia* Davies (Diptera: Simuliidae): redescription of *Cr. crozetensis, Cr. seguyi*, number of larval instars, phylogenetic relationships and historical biogeography. *Zootaxa*, 259, 1-39.

Craig, D. A., Craig, R. E. G., & Crosby, T. K. (2012). Simuliidae (Insecta: Diptera). *Fauna of New Zealand*, 68, 336 pp.

Crosskey, R. W. (1969). A re-classification of the Simuliidae (Diptera) of Africa and its Islands. *Bull. Brit. Mus. Nat.* Hist. (Entomology)., Supplement 14, 195 pp.

Crosskey, R. W. (1981). Simuliid taxonomy - the contemporary scene. Pp. 3-18. 1n Laird, M. (Ed.), *Blackflies: the future for biological methods in integrated control.* Academic Press.
Crosskey, R. W. (1988). An annotated checklist of the world's black flies (Diptera: Simuliidae). In Kim, K. C. and R. W. Merrit (Eds.), *Black flies: Ecology, Population Management and Annotated World List* (pp. 425-520). Penn. State Press.
Crosskey, R. W., & Howard, T. M. (1997). *A new taxonomic and geographical inventory of world blackflies (Diptera: Simuliidae).* London: The Natural History Museum, Cambridge University Press.
Currie, D. C. (1988). *Phylogeny of primitive Simuliidae (Insecta: Diptera: Culicomorpha).* Ph.D. dissertation. University of Alberta, Edmonton, Canada.
Danforth, B. N., & Ji, S. (1998). Elongation factor-1 alpha occurs as two copies in bees: implications for phylogenetic analysis of EF-1 alpha sequences in insects. *Molecular Biology and Evolution.*, 15, 225-235
Davies, L. (1965). The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island black-fly. *Proc. Linn. Soc. Lond.,* 176, 159-180.
Davies, L. (1974). Evolution of larval head-fans in Simuliidae (Diptera) as inferred from the structure and biology from *Crozetia crozetensis* (Womersley) compared with other genera. *Zoological Journal of the Linnean Society of London,* 55, 193-224.
Djernaes, M., & Damgaard, J. (2006). Exon-Intron Structure, Paralogy and Sequenced Regions of Elongation Factor-1 alpha in Hexapoda. *Arthropod Systematics & Phylogeny*., 64, 45-52.
Dumbleton, L. J. (1963). The classification and distribution of the Simuliidae (Diptera) with particular reference to the genus Austrosimulium. *N. Z. J. Sci*, 6, 320-357.
Edwards, F. W. (1931). Simuliidae. In *Diptera of Patagonia and South Chile, Part 2* (pp. 121-154).
Enderlein, G. (1937). Uber bemerkenswerte Unkenntnisse von der Entwicklung zweier einheimischer Simuliiden Gattungen (Diptera). *Markische Tierwelt,* 3, 68-70.
Gil-Azevedo, L. H. & Maia-Herzog, M. (2007) Preliminary considerations on phylogeny of Simuliidae Genera from Southern Hemisphere (Insecta, Diptera). *Zootaxa*, 1643, 39-68.
Gillespie, J. J., Johnston, J. S., Cannone, J. J., & Gutell, R. R. (2006). Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of *Apis mellifera* (Insecta: Hymenoptera): structure, organization, and retrotransposable elements. *Insect Molecular Biology* 15, 657–686
Grenier, P., & Rageau, J. (I960). Simulies (Dipt., Simuliidae) di Tahiti. Remarques sur la classification des Simuliidae. *Bull, de la. Soc. de Path. Exot*., 53, 727-742.
Hennig, W. (1966). Phylogenetic systematics. *Translated by* D. D. Davis and R. Zangerl. University of Illinois Press, Urbana, I11.
Hennig, W. (1973). Diptera (Zweiflügler). *In*: Helmcke, J. G., Starck, D., & Wermuth, H. (Eds), *Handbook of Zology*. Walter d Gruyter Berlin, 1-337 pp.
Hoc, L.V. L., Lecointre, G., & Perasso, R. (1993) A 28S rRNA-based Phylogeny of the Gnathostomes: first steps in the analysis of conflict and congruence with the morphologically bases cladograms. *Molecular Phylogenetics and Evolution*, 2, 31-51.

Hovemann, B., Richter, S., Walldorf, U., & Cziepluch, C. (1988). Two genes encode related cytoplasmic elongation factors 1 (EF-1) in Drosophila melanogaster with continuous and stage specific expression. *Nucleic Acids Research*, 16, 3175--3194

Jordal, B. H. (2002). Elongation Factor 1α resolves the monophyly of the haplodiploid ambrosia beetles Xyleborini (Coleoptera: Curculionidae). *Insect Molecular Biology*, 11, 453–465.

Kjer, K. M., Gillespie, J. J., & Ober, K. A. (2007). Opinions on multiple sequence alignment, and an empirical comparison of repeatability and accuracy between poy and structural alignment. *Systematic Biology*, 56, 133–146.

Moulton, J. K. (1997). *Molecular Systematics of the Simuliidae (Diptera: Culicomorpha).* PhD Dissertation, University of Arizona, Tucson, Arizona.

Moulton, J. K. (2000). Molecular sequence data resolves basal divergences within Simuliidae (Diptera). *Systematic Entomology*, 25, 95-113.

Moulton, J. K. (2002). Can the current molecular arsenal adequately track rapid divergence events within Simuliidae (Diptera)? Molecular Phylogenetics and Evolution. 27, 45-57.

Moulton, J. K., & Adler, P. H. (1997). The genus *Ectemnia* (Diptera: Simuliidae): taxonomy, polytene chromosomes, new species, and phylogeny. *Canadian Journal of Zoology*, 121, 1-57.

Oosterbroek, P. & Courtney, G. W. (1995). Phylogeny of the nematocerous families of Diptera (Insecta). Zool. J. Linn. Soc. 115, 267-311.

Pawlowski, J., Szadziewski, R., Kmieciak, D., Fahmi, J., & Bittar, G. (1996). Phylogeny of the infraorder Culicomorpha (Diptera) based upon 28S RNA gene sequences. *Syst. Entomol.*, 21, 167-178.

Pelandakis, M., & Solignac, M. (1993). Molecular phylogeny of Drosophila based on ribosomal RNA sequences. *J. Mol. Evol.*, 37, 525-543.

Perasso, R., & Tourancheau Baroin, A. (1993) The eukaryogenesis A model derived from rRNA phylogenies. *Comptes Rendus des Seances de la Societe de Biologie et de Ses Hliales*, 186, 656-665.

Peterson, B. V. (1977). A synopsis of the genus Parasimulium Malloch (Diptera: Simuiiidae), with descriptions of one new subgenus and two new species. Proc. Entomol. Soc. Wash. 79, 96-106.

Pruess, K. P., Zhu, X., & Powers, T. O. (1992). Mitochondrial transfer RNA genes in a black fly. *Simulium vittatum* (Diptera: Simuiiidae), indicate long divergence from mosquito (Diptera: Culicidae) and fruit fly (Diptera: Drosophilidae). *J. Med. Entomol.*, 29, 644-651.

Py-Daniel, V. (1990). *Revisao da Tribo Prosimuliini seg. Crosskey (Diptera, Culicomorpha) nas regioes Zoogeographicas Neartica E Neotropical (larvas and pupas).* Ph. D. Dissertation. Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil.

Roderic, D. M. (2000). Comparative analysis of secondary structure of insect mitochondrial small subunit ribosomal RNA using maximum weighted matching. *Nucleic Acids Research*, 28, 3839-3845.

Ronquist, F., & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572-1574.
Rothfels, K. H., & Freeman, D. M. (1966). The salivary gland chromosomes of three North America species of *Twinnia* (Diptera: Simuliidae). *Canadian Journal of Zoology*, 44, 937-945.
Roubaud, E. (1906). Apercus nouveaux, morphologiques et biologiques, sur les Dipteres piqueurs du groupe des Simulies. *Complies Rendus Hebdomadaires des Seances de I'Academie des Sciences*, Paris, 143, 519-521.
Rubtzov, I. A. (1955). Peculiar new species of black flies (Diptera, Simuliidae) from Eastern Siberia. *Entomol. Obozr.*, 34, 323-339.
Rubtzov, 1. A. (1974). Evolution, phylogeny, and classification of the family Simuliidae (Diptera). [Translated from Russian]. British Library Lending Division, Wetherby. 83 pp.
Scotese, C. R. Boucot, A.J., & McKerrow, W.S., (1999). Gondwanan paleogeography and paleoclimatology, *Journal of African Earth Sciences*, 28, 99-114
Shewell, G. E. (1958). Classification and distribution of arctic and subarctic Simuliidae. *Proc. 10th Int. Cong. Entomol*. (Montreal, 1956), 1, 635-643.
Smart, J. (1945). The classification of the Simuliidae (Diptera). *Trans. Royal. Soc. London*, 95, 463-532.
Smith, M. L. (2002). *Molecular systematics of the Neartctic black fly subgenus Simulium s.s.(Diptera: Simuliidae: Simulium s.l.).* M.S. thesis. University of Toronto, Toronto. 172 pp.
Stone, A. (1949). A new genus of Simuliidae from Alaska (Diptera). *Proceedings of the Entomological Society of Washington*, 51, 260-267.
Stone, A. (1965). *Family Simuliidae. In Stone et al. (Eds.), A catalogue of the Diptera of America north of Mexico*. Agriculture Handbook No. 276. United States Department of Agriculture,
Stone, A. & Jamnback, H. (1955). The black flies of New York State (Diptera: Simuliidae). *New York State Bulletin*, 349, 1-144.
Surcouf, J. M. R., & Gonzalez-Rincones, R. (1911). *Essai sur les dipteres vulnerants du Venezuela: materiaux pour server a l'etude des dipteres piqueurs et suceurs de sang de l'Amerique intertropicale. Part 1*. A. Maloine, Paris, 320 pp.
Swofford, K. L. (1999). *PAUP 4.0: phylogenetic analysis with parsimony (and other methods), version 4.0 b2a*. Sunderland, Massachusetts Sinauer Associates Inc.
Tang, J., Toe, L. , Back, C. , Zimmerman, P. A., Pruess, K., & Unnasch, T. R. (1995). The *Simulium damnosum* species complex: phylogenetic analysis and molecular identification based upon mitochondrially encoded gene sequences. *Ins. Mol. Biol.*, 4, 79-88.
Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882.
Vossbrinck, C. R. (1989). A 28S ribosomal RNA phylogeny of certain cyclorrhaphous

Diptera based upon a hypervariable region. *Syst Entomol*., 14, 417-431.
Wood, D. M. (1978). Taxonomy of the Nearctic species of Twinnia and Gvmnopais (Diptera: Simuliidae) and a discussion of the ancestry of the Simuliidae. *Can. Entomol.* 110: 1297-1337.
Wood, D. M., & Borkent, A. (1982). Description of the female of Parasimulium crosskeyi Peterson (Diptera: Simuliidae) and a discussion of the phylogenetic position of the genus. *Memoirs of the Entomological Society of Washington*, 10, 193-210.
Wood, D. M., & Borkent, A. (1989). Nearctic Diptera Manual, Vol. 3. Phylogeny and classification of the Nematocera. *Agriculture Canada Monograph*, 32, 1333-1370.
Wygodzinsky, P., & Coscaron, S. (1973). A review of the Mesoamerican and South American black flies of the tribe Prosimuliini (Simuiiinae, Simuliidae). *Bull. Amer. Mus. Nat. Hist*., 151, 129-200.
Xiong, B., & Kocher, T. D. (1991). Comparison of mitochondrial sequences of seven morphospecies of black flies (Diptera: Simuliidae). *Genome*, 34, 306-311.
Yankovsky, A. V. (1992). A review of the system of blackflies (Diptera, Simuliidae) on the suprageneric level. *Parazitologichescky Sbornik*, 37, 203-217.

Pollution Tolerance Values for Predicting the Spread of Black Flies (Diptera: Simuliidae) in New Jersey, with Implications for Control

Abstract

Pollution tolerance values are reported for the 39 species of black flies known in New Jersey. Morphologically similar species were identified chromosomally to ensure accurate identification. Species of the same genus varied markedly in tolerance values, questioning the accuracy of generic-level tolerance values for the Simuliidae. An index for predicting the colonization and spread of the *Simulium jenningsi* group, which includes the major pest species of black flies in the eastern United States, is proposed, with implications for control programs worldwide.

Key words: Tolerance values, Cryptic species, *Simulium jenningsi* group, Black fly control, Pollution.

Introduction

Black flies are vectors of the agent of human Onchocerciasis (river blindness) in tropical Africa and Latin America (Crump *et al.* 2012). In North America, they are major pests of humans and domesticated animals, and are vectors of the agents of livestock and poultry diseases (Barnett 1977, Bennett *et al.* 1993, Schnitzlein & Reichmann 1985, Mead *et al.* 2004). As pests and vectors, black flies have long been the subjects of control programs (Davis *et al.* 1957, Laird 1981, Crump *et al.* 2012).

The *Simulium jenningsi* group consists of seven species in New Jersey, including several species that are major pests (Table 1) (Carle 2010, Adler *et al.* 2004). Their proliferation in the eastern United States has been attributed to trends in river cleanup (Adler *et al.* 2004). Adler *et al.* (2004) stated that, "*Simulium jenningsi* in historical times might have been unbearable", citing the name of the Pennsylvania town named by the Native Americans who once lived there—"Punxsutawney", which means "land of gnats." The first reported outbreaks of the *S. jenningsi* group in the Northeast came in the late 1970s (Adler *et al.* 2004). Pest problems arising from the Susquehanna River in Pennsylvania caused the citizens to form "NAG", Neighbors Against Gnats, which pressured legislators to fund a black fly suppression program. As a result, a small two-river treatment program was initiated in 1983 (Arbegast 1994), and by 1999 had grown to a $5 million program treating rivers in 33 counties with *Bacillus thuringiensis israelensis* (*Bti*) (Adler *et al.* 2004).

The *S. jenningsi* group was documented as a pest problem in northwestern New Jersey as early as the mid-1990s and prompted the taxpayers of Hunterdon County to form a NAG group to treat the South Branch of the Raritan River, which putatively was colonized by black flies from the Delaware River around 1991 (Cole 1996). Subsequently, NJ sporadically funded a program to treat the Delaware River and nearby streams, and to conduct exploratory surveillance of streams farther inland. The *S. jenningsi* group is typically associated with large, warm rivers, and is multivoltine, adding to its pest status (Adler *et al.* 2004). Species in the group are livestock and poultry pests, taking blood from horses, mules, cattle and turkeys (Underhill 1944, Anderson & DeFoliart 1961). The incessant swarming by females that often enter the ears, eyes, nose, and mouth can prohibit people from going outdoors in the summer. Allergic reactions to the bites, including allergic asthma have been reported, particularly among children

(Adler *et al.* 2004). About 63 cases of allergic reactions from the bites of *S. jenningsi* were recorded in Raleigh County, West Virginia, in 1983 (Adler *et al.* 2004).

Although Hilsenhoff (1987) established pollution tolerance values for some Wisconsin simuliid species, tolerance values for black flies largely have remained at the generic level. An obstacle in establishing pollution tolerance values for black flies has been the presence of structurally similar or identical (cryptic or sibling) species that typically require chromosomal analysis for identification. This problem was noted by Hilsenhoff (1987) who stated, "…discovery that *Simulium vittatum* is really two genetically distinct species (Rothfels and Featherston 1981) with differing ecological requirements (Adler and Kim 1984) altered my recommendation for dealing with these sibling species." Establishing species-level tolerance values for simuliids has been stymied by a lack of chromosomal analysis to confirm species identity. Consequently, the generic level values of roughly three and six that most authors (Merritt *et al*. 2008) recognize for two of the most abundant North American genera, *Prosimulium* and *Simulium*, respectively, do not illustrate the variation in pollution tolerance values at the species level.

Discrete tolerance values can be a valuable tool for determining waterway health (Hilsenhoff 1977, Lenat 1993). However, when addressing the conditions necessary for species establishment in new habitats, the range of pollution tolerance values of a species becomes critical. The New Jersey Department of Environmental Protection (NJDEP) currently conducts biological assessments of NJ waterways through its Ambient Biomonitoring Network (AMNET). This program determines the quality of NJ streams throughout all five of the state's physiographic provinces, using macroinvertebrate data.

The Family Biotic Index for each stream is calculated, along with indices adapted specifically for use in NJ, which are based on the ideas of Chutter's Biotic Index (1972) and Hilsenhoff's Biotic Index (1987), providing a database to analyze the factors necessary for simuliid colonization of streams and predict possible sites of future black fly invasion.

This study seeks to determine if the occurrence of the *S. jenningsi* group can be predicted from their pollution tolerance values.

Materials and Methods

Black fly larvae were collected in acetic ethanol (1: 3) from 55 sites spanning all five physiographic provinces of NJ from 2009 to 2012 (Table 2 and 3), following the procedures of Adler *et al.* (2004). Collection sites were stratified across streams deemed by the NJDEP as excellent, good, fair, and impaired. Larval black flies were identified morphologically, but for members of species complexes identification was done chromosomally using the Feulgen technique (Rothfels & Dunbar 1953) to stain the polytene chromosomes in the silk glands of last-instar larvae and their gonads, allowing for gender identification in situ from gonad shape. Reference specimens were deposited in the Rutgers University Insect Museum.

A database of the Family Biotic Indices (FBI) for the streams where black flies were collected was compiled from the NJDEP's AMNET surveys (NJDEP 2009, 2010, & 2012a-c). The weighted average, which is useful for determining an organism's tolerance value (ter Braak and Looman, 1986, Hilsenhoff 1987), of each of the simuliid species sampled was calculated using the FBI of each stream as the ecological variable. A presence/absence weighted average was used. The average, range, standard deviation, and range for one standard deviation of each species' pollution tolerance value were recorded. For specimens found in only one of the sampled streams, the singular number was recorded.

The tolerance value and standard deviation were calculated for the *S. jenningsi* group as a whole. These data, combined with habitat data for widths of streams inhabited by the *S. jenningsi* group were used to calculate a *jenningsi* index for NJ to predict streams of future proliferation, and streams where species in the group could occur but not proliferate. In this study, occurrence is defined as the presence of the *S. jenningsi* group in a stream. Proliferation is defined as streams colonized by the *S. jenningsi* group where they propagate in numbers large enough to result in a pest problem, which in Pennsylvania is defined as 5 flies captured in 10 overhead sweeps with a standard insect net on a golf course, and 10 flies captured in 10 overhead sweeps with a standard insect net for the general public (Gray *et al.* 1996). NJ has also adopted this nuisance threshold for its control programs (Scott Crans, personal Communication 2004). Consequently, streams suitable for occurrence of the *S. jenningsi* group, but not proliferation are streams with very low, sometimes imperceptible populations, not high enough to cause a pest problem. Historical data taken from the NJ Black Fly surveillance program from 1996 to 1998 were compared with collection data from 2004-2008 (Carle 2010) and correlated with NJDEP AMNET data (NJDEP 2000a-c) to test the validity of the hypothesis that NJJGI can be used to accurately predict what streams the *S. jenningsi* group will colonize or proliferate in.

Results

Four genera and 39 species of black flies in New Jersey were identified (Table 1). Species in the *S. jenningsi* group were collected from 26 of the 53 sites. The NJ species of black flies have tolerance values from 3.01 to 5.71 (Table 1), or excellent to fair, according to the Hilsenhoff Biotic Index rating scale (Table 4). However, the range of tolerance values spans 2.32 to 7.85, meaning the family occurs in excellent to very poor quality streams. The NJ black fly fauna exhibits a high degree of tolerance value disparity at the species level in each genus. Within the genus *Prosimulium*, tolerance values range from 3.32 to 5.24. Tolerance values in the *P. hirtipes* species group exhibit a large range from 3.75 to 5.24. The genus *Cnephia* has a relatively narrow range of 5.18 to 5.71, whereas the genus *Stegopterna* has a broad range spanning 3.85 to 5.23. The genus *Simulium* also has a wide range of tolerance values, from 3.01 to 5.49, and the subgenus *Simulium* ranges from 3.44 to 5.49. A similar pattern is seen with the range plus or minus one standard deviation (Table 1).

The tolerance values of species in the *S. jenningsi* group range from 3.83 to 5.08. The species typically colonize streams with an FBI rating of very good to fair. *S. lakei* has the highest threshold for pollution of the known NJ species in this group, whereas *S. anchistinum* and *S. notiale* are the most pollution-sensitive species in this group. The range of tolerance values for individual species of the *S. jenningsi* group plus or minus one standard deviation is 2.78 to 6.58, which is the range of the most pollution sensitive *S. jenningsi* group species minus one of its standard deviations, and the most pollution tolerant *S. jenningsi* group species plus one of its standard deviations. The value of 6.58

is set by *S. lakei*, while the value of 2.78 is set by *S. jenningsi*. This range of values is indicative of streams with an FBI rating of excellent to very poor.

The *S. jenningsi* group was analyzed as a whole and its mean was calculated as 4.29, with a standard deviation of 1.34, making the range plus or minus one standard deviation 2.95 to 5.63. The group proliferated in streams 8 m or wider, with an FBI ranging from 2.96 to 5.63. However, the group colonized streams 8 m or wider, with an FBI of 2.78 to 6.58. These findings led to the development of the NJ Jenningsi Group Index (NJJGI) (Figure 1).

The NJ Jenningsi Group Index
Stream width $\geq$ 8 meters and 2.95 $\leq$ Stream FBI $\leq$ 5.63 = Stream suitable for *jenningsi* group proliferation
Stream width $\geq$ 8 meters and 2.78 $\leq$ Stream FBI $\leq$ 6.58 = Stream suitable for *jenningsi* group occurrence

FIGURE 1. How to use the NJ Jenningsi Group Index to predict the occurrence and proliferation of the *S. jenningsi* group in NJ streams.

The historical data on the distribution of the *S. jenningsi* group in NJ from 1996 to 1998 came from 16 sites: four from the Musconetcong River, nine from the South Branch of the Raritan River, and three from the Lamington River (Figure 2). During this time, the *S. jenningsi* group was prolific in the Musconetcong River at Point Mountain and Changewater Road. The group was also prolific at Lower Landsdown Road, Route 523, and Woodfern Road in the South Branch of the Raritan River. However, no species of the group were found in the Lamington River from 1996 to 1998, even though, according to the NJJGI, the conditions were appropriate for them to expand into and proliferate in that river.

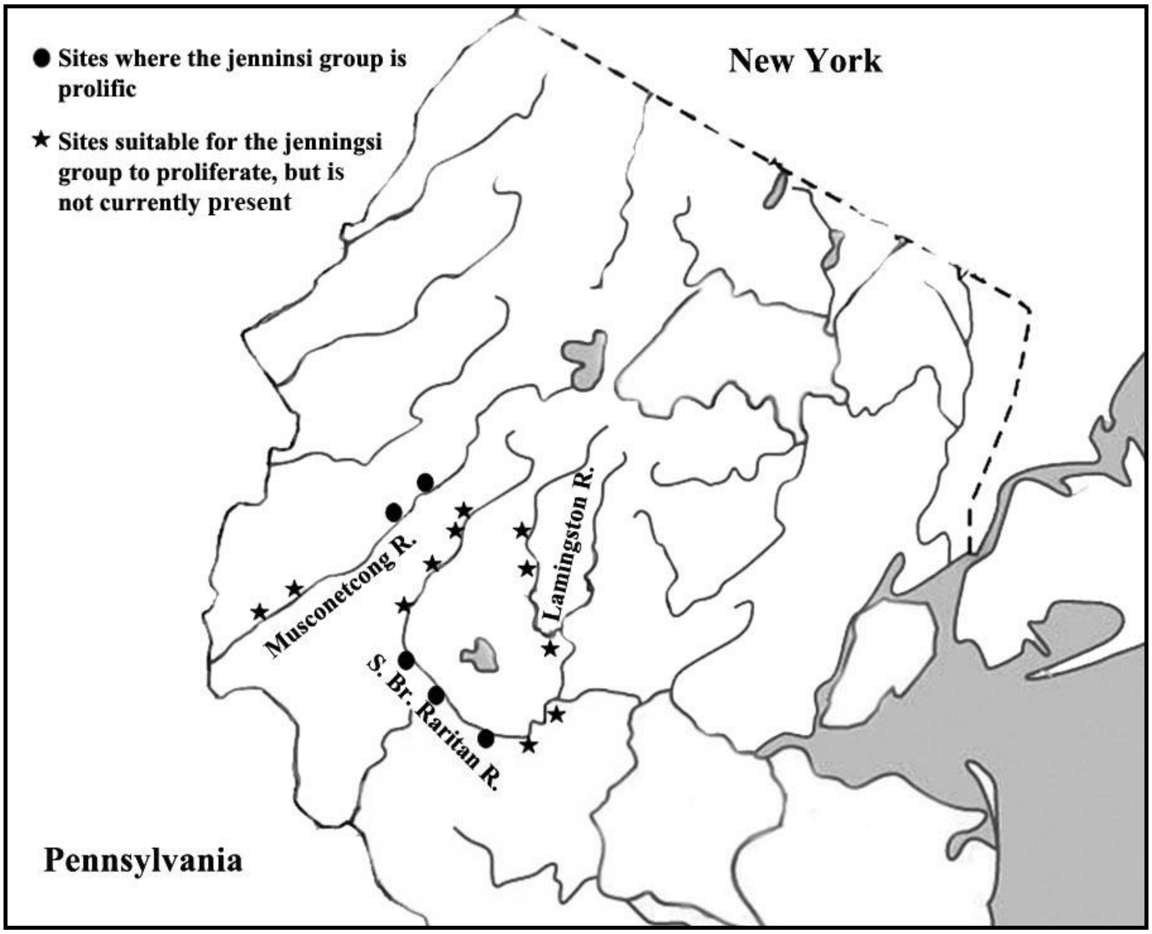

FIGURE 2. Distribution of the *S. jenningsi* group and predicted sites of proliferation in northern New Jersey in 1996-1998.

From 1996-1998 to 2004-2008 the *S. jenningsi* group proliferated in all of the sites predicted by the NJJGI (Figure 3). The group proliferated in the Musconetcong River at Bloomsbury and at the Musky Fish Hatchery. The group also spread to and propagated in the upper South Branch of the Raritan River at High Bridge, the Ken Lockwood Gorge, Califon Road, and Vernoy Road, and in the southern stretches of the river at Neshanic Station and Studdiford Road. Lastly, The *S. jenningsi* group expanded into and multiplied in all three sites sampled on the Lamington River: Black River Road, Pottersville, and Burnt Mills Road.

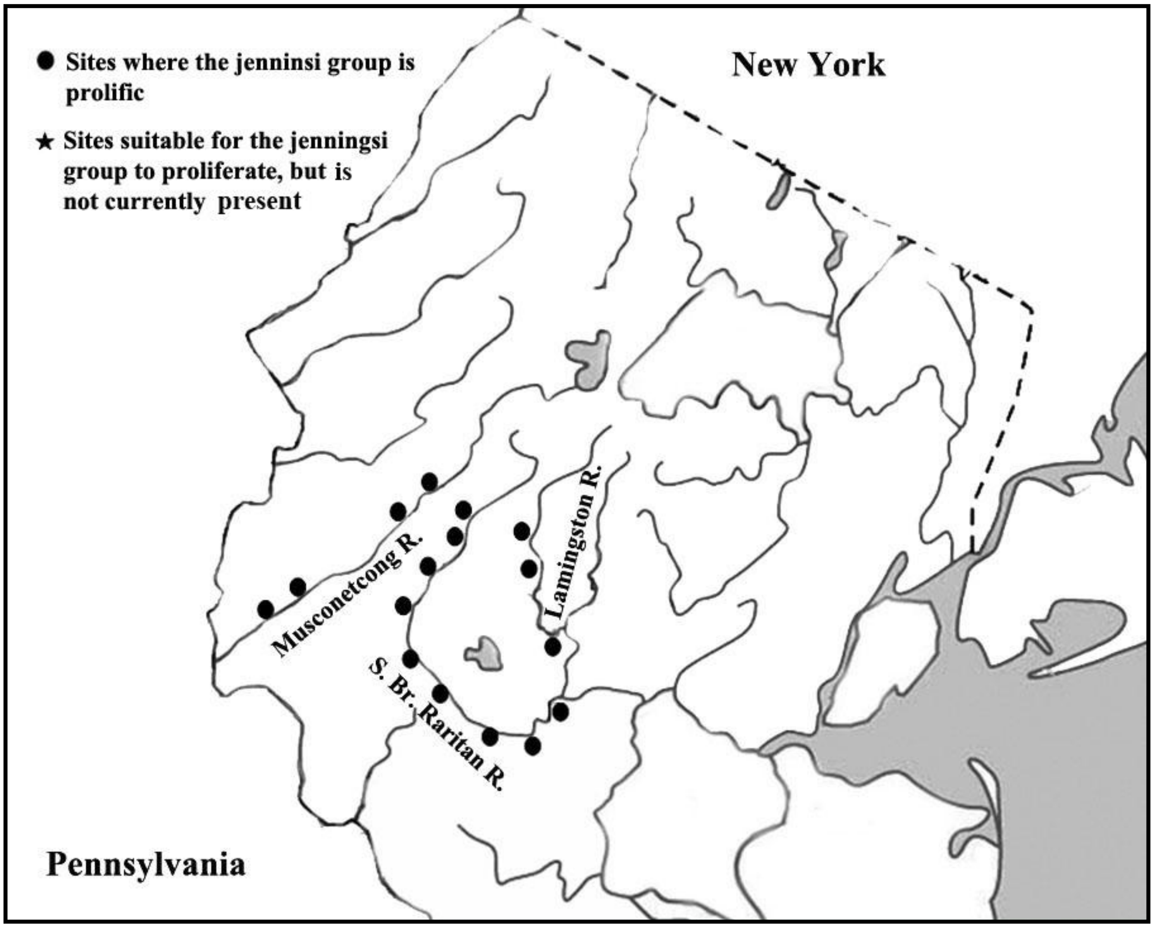

FIGURE 3. Distribution of the *S. jenningsi* group and predicted sites of proliferation in northern New Jersey in 2004-2008.

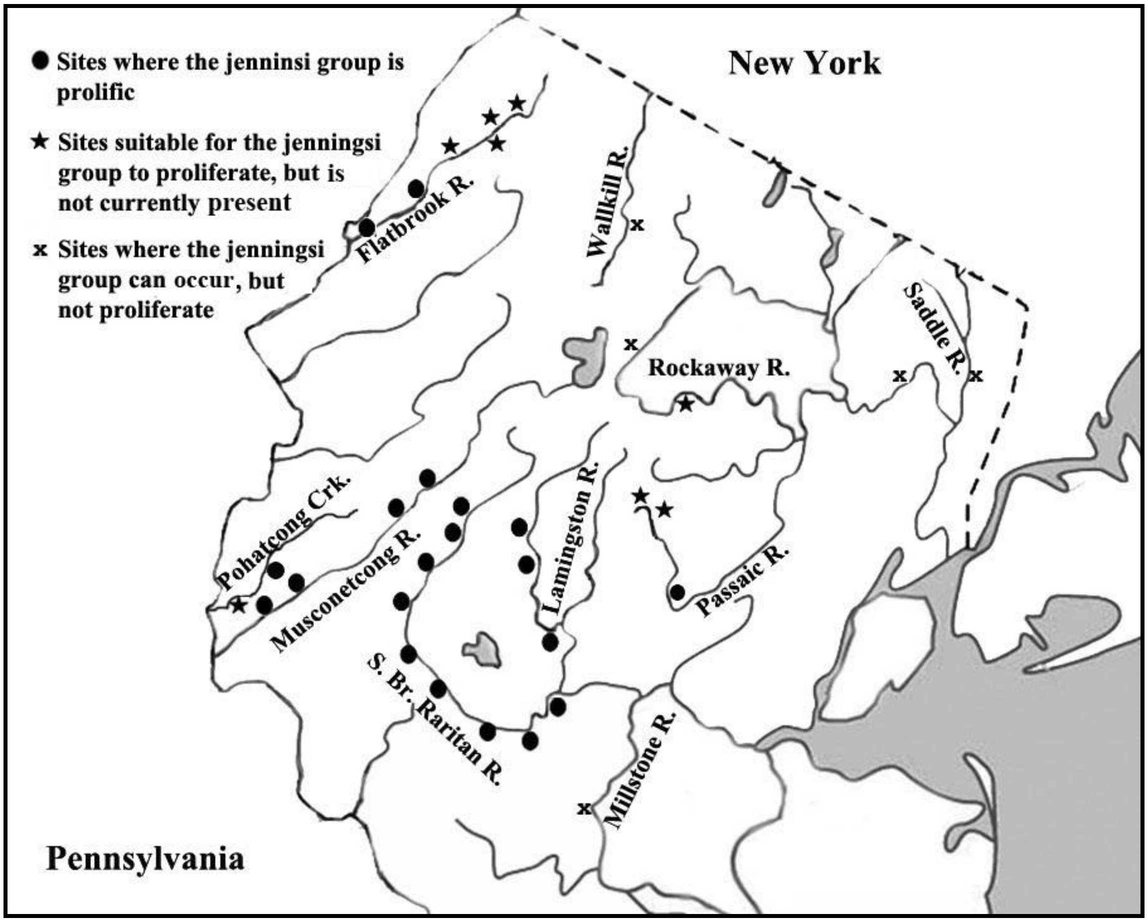

FIGURE 4. Current distribution of the *S. jenningsi* group and predicted sites of occurrence and proliferation in northern New Jersey.

A survey was conducted at 33 sites in 10 northern NJ streams, with NJJGI indicating that conditions were suitable for proliferation or occurrence (Figure 4). The Flatbrook River from Coursen Road into High Point State Park is suitable for the S. *jenningsi* group to proliferate, but the group currently occurs only as far upstream as Walpack Center. The Wallkill River below Franklin Pond is suitable for occurrence, but not proliferation. The Rockaway River is suitable for proliferation at Boonton, but farther north at Berkshire Valley Road, the river is only adequate for occurrence by the group. The Saddle River at Railroad Road is currently a prime location for occurrence but not proliferation. The Passaic River harbors multiple habitats suitable for proliferation in the western stretches of the river from Morristown to Sterling, but farther east into Totowa downstream of the Paterson Falls the conditions are appropriate for the group to occur but not proliferate. The Millstone River at Rocky Hill is another site where the group is likely to occur but not proliferate. Pohatcong Creek at the Route 78 Bridge supports prolific populations of the group; however, farther downstream at Still Valley Road, the conditions are suitable for the group to proliferate; though only one individual was found at the site.

Discussion

The use of generic-level pollution tolerance values for black flies does not reflect the range at the species level. Identification to the subgeneric or species-group level also lacks resolution; high variation in tolerance values exists among species in these taxonomic categories. Future stream analysis with the Simuliidae should use species-level identifications for best precision. This variation in pollution tolerance values can be useful in forecasting where species of interest will colonize and propagate. Though the tolerance values do not all align with expected numbers based on previous work (Hilsenhoff 1987), this may be due to the sampling methods or the technique of not identifying specimens to the species level used by the NJDEP to collect their AMNET data. However, the purpose is to correlate Simuliidae species tolerance values to the methodologies used by the NJDEP so that they can be used to predict the occurrence of black flies using the ongoing and regularly collected data of the NJDEP.

Unlike other pests, such as mosquitoes, whose immatures inhabit temporary habitats, such as tree holes and tires (Wilton 1968, Reiter & Sprenger 1987), black flies require more stable running-water habitats (Adler *et al.*); as a result, their proliferation is more predictable. The time required for the *S. jenningsi* group to propagate once it is present in a stream suitable for proliferation is unknown. The single larva from the *S. jenningsi* group found at Pohatcong Creek downstream of the Still Valley Road is likely the beginning stages of the group's propagation there since it is suitable for proliferation of the group.

As a relatively pollution intolerant group of species, the *S. jenningsi* group can be expected to become an increasing nuisance, especially in heavily populated areas throughout NJ as stream health improves. The main areas affected by the group in NJ are in the northwest of the state, possibly moving in from Pennsylvania via the Delaware River. This region constitutes some of the least densely populated areas of the state (New Jersey State Data Center 2010). However, as development continues in northwestern NJ and more farms are replaced with houses, the black fly nuisance to humans will likely increase. *S. lakei* is the principal species of the *S. jenningsi* group present in the coastal plain of southern NJ. Its tolerance value and upper range plus one standard deviation is similar to that of *S. jenningsi,* which is the most abundant species of the group in the northern region of the state. As stream health improves and the biotic indices of streams in the southern and northeastern regions of the state decrease, the *S. jenningsi* group could also become a more prolific pest in those regions.

The NJJGI has possible applications for areas where river blindness is epidemic and endemic. The technique for predicting occurrence of the *S. jenningsi* group in NJ by using pollution tolerance values and stream width could be applied to species such as those in the *S. damnosum* complex, which includes the principal African vectors of river blindness. African countries in the Nile River Basin and the Volta River Basin gather metrics for water-quality evaluation (Council of Ministers of Water Affairs of the Nile

Basin States 2001Malzbender & Earle 2008) and these data could be used in control programs to construct an index of their own for occurrence and proliferation of vector species. Therefore, there is definite utility in employing stream data that is recurrently collected for environmental monitoring in the predicting and planning measures of black fly control.

TABLE 1. NJ simuliid species tolerance values, and their standard deviation, range, and range ± one standard deviation.

Subgenus	Species group	Species	Tolerance value± standard deviation	Tolerance value range	Tolerance value range ±1 standard deviation
Prosimulium	*hirtipes*	*P. arvum*	4.86±1.54	3.69-7.03	3.32-6.40
		P. fuscum	4.30±1.02	3.32-5.71	3.28-5.32
		P. mixtum	3.77±0.80	2.97-5.45	2.97-4.57
		P. rhizophorum	3.75±0.02	3.73-3.76	3.73-3.77
		P. clandestinum	5.24±2.61	2.97-8.9	2.63-7.85
	magnum	*P. magnum*	3.86	3.86	3.86
		P. canutum	3.32	3.32	3.32
		P. albionense	3.33	3.33	3.33
Cnephia	NA	*C. dacotensis*	5.71	5.71	5.71
		C. ornithophillia	5.18±0.50	4.31-5.71	4.68-5.68
Stegopterna	NA	*S. diplomutata*	5.23±0.24	4.95-5.48	4.99-5.47
		S. mutata	3.85±1.21	2.32-5.71	2.64-5.06
Eusimulium	*aureum*	*S. bracteatum*	3.01±0.69	2.32-3.69	2.32-3.70
Hellichiella	*congareenarum*	*S. congareenarum*	5.34±0.36	4.95-6.01	4.98-5.70
Boreosimulium	*annulus*	*S. emarginatum*	3.53±0.56	2.97-4.29	2.97-4.09
Nevermannia	*vernum*	*S. Silvestre*	4.14±0.64	3.69-5.05	3.50-4.78
		S. croxtoni	4.70±1.01	3.69-5.71	3.69-5.71
		S. fionae	4.70±0.66	3.69-5.71	4.04-5.36
		S. gouldingi	4.83±0.90	3.69-6.23	3.93-5.73
		S. impar	3.33	3.33	3.33
		S. quebecense	4.08±0.61	3.32-5.2	3.47-4.69
Psilozia	NA	*S. tribulatum*	4.79±1.57	2.32-7.03	3.22-6.36
		S. vittatum	4.28	4.28	4.28
Simulium	*noelleri*	*S. decorum*	5.30±1.37	3.69-7.15	3.93-6.67
	jenningsi	*S. anchistinum*	3.83±0.98	2.97-5.2	2.85-4.81
		S. definitum	4.50±0.58	3.92-5.08	3.92-5.08
		S. fibrinflatum	4.55±1.72	2.97-8.4	2.83-6.27
		S. jenningsi	4.26±1.48	2.97-8.4	2.78-5.74
		S. lakei	5.08±1.91	3.92-7.03	4.54-6.58
		S. luggeri	3.99±0.07	3.92-4.05	3.92-4.06
		S. *notiale*	3.83±0.98	2.97-5.02	2.85-4.81
	parnassum	*S. parnassum*	5.49±2.14	3.33-8.4	3.35-7.63
	tuberosum	*S. tuberosum*	4.05	4.05	4.05
		S. appalachiense	4.14±.0.58	3.32-5.2	3.56-4.72
		S. perissum	4.14	4.14	4.14
		S. ubiquitum	5.12±0.44	3.99-6.23	4.68-5.56
		S. vandalicum	3.44±0.58	2.32-4.14	2.86-4.02
	venustum	*S. venustum*	4.35±1.01	2.32-5.71	3.34-5.36
		S. verecundum	5.17±1.45	2.32-7.03	3.72-6.62

TABLE 2. Black Fly Occurrence By Site

Site	*P. arvum*	*P. fuscum*	*P. mixtum*	*P. rhizophorum*	*P. clandestinum*	*P. magnum*	*P. canutum*	*P. albionense*	*C. dacotensis*	*C. ornithophilia*	*S. diplomutata*	*S. mutata*	*S. bracteatum*	*S. congareenarum*	*S. emarginatum*	*S. Silvestre*	*S. croxtoni*	*S. fionae*	*S. gouldingi*	*S. impar*	*S. quebecense*	*S. tribulatum*	*S. vittatum*	*S. decorum*	*S. anchistinum*	*S. definitum*	*S. fibrinflatum*	*S. jenningsi*	*S. lakei*	*S. luggeri*	*S. notiale*	*S. parnassum*	*S. tuberosum*	*S. appalachiense*	*S. perissum*	*S. ubiquitum*	*S. vandalicum*	*S. venustum*	*S. verecundum*
A																								X															
B																													X							X			
C											X			X					X																	X		X	
D			X							X	X																		X									X	X
E																																				X			X
F														X															X							X			
G																																						X	
H										X	X			X																									
I														X					X																	X		X	
J										X				X																						X		X	X
K																			X																	X			X

TABLE 2. Black Fly Occurrence By Site

Site	*P. arvum*	*P. fuscum*	*P. mixtum*	*P. rhizophorum*	*P. clandestinum*	*P. magnum*	*P. canutum*	*P. albionense*	*C. dacotensis*	*C. ornithophillia*	*S. diplomutata*	*S. mutata*	*S. bracteatum*	*S. congareenarum*	*S. emarginatum*	*S. Silvestre*	*S. croxtoni*	*S. fionae*	*S. gouldingi*	*S. impar*	*S. quebecense*	*S. tribulatum*	*S. vittatum*	*S. decorum*	*S. anchistinum*	*S. definitum*	*S. fibrinflatum*	*S. jenningsi*	*S. lakei*	*S. luggeri*	*S. notiale*	*S. parnassum*	*S. tuberosum*	*S. appalachiense*	*S. perissum*	*S. ubiquitum*	*S. vandalicum*	*S. venustum*	*S. verecundum*
L																													X							X			
M										X																													
N																													X							X			X
O														X																									
P																								X															X
Q																																		X	X		X		
R																											X	X											
S																											X	X				X							
T																					X																		
U				X																																			
V																										X													
W																					X							X											

TABLE 2. Black Fly Occurrence By Site

Site	*P. arvum*	*P. fuscum*	*P. mixtum*	*P. rhizophorum*	*P. clandestinum*	*P. magnum*	*P. canutum*	*P. albionense*	*C. dacotensis*	*C. ornithophillia*	*S. diplomutata*	*S. mutata*	*S. bracteatum*	*S. congareenarum*	*S. emarginatum*	*S. Silvestre*	*S. croxtoni*	*S. fionae*	*S. gouldingi*	*S. impar*	*S. quebecense*	*S. tribulatum*	*S. vittatum*	*S. decorum*	*S. anchistinum*	*S. definitum*	*S. fibrinflatum*	*S. jenningsi*	*S. lakei*	*S. luggeri*	*S. notiale*	*S. parnassum*	*S. tuberosum*	*S. appalachiense*	*S. perissum*	*S. ubiquitum*	*S. vandalicum*	*S. venustum*	*S. verecundum*
X																								X															
Y																						X		X															
Z																												X											
AA																						X		X															X
AB																						X							X										X
AC																												X						X					
AD																												X											
AE																					X																		
AF				X																																			
AG																						X				X		X					X	X					
AH																												X		X									
AI					X																																		

TABLE 2. Black Fly Occurrence By Site

Site	*P. arvum*	*P. fuscum*	*P. mixtum*	*P. rhizophorum*	*P. clandestinum*	*P. magnum*	*P. canutum*	*P. albionense*	*C. dacotensis*	*C. ornithophillia*	*S. diplomutata*	*S. mutata*	*S. bracteatum*	*S. congareenarum*	*S. emarginatum*	*S. Silvestre*	*S. croxtoni*	*S. fionae*	*S. gouldingi*	*S. impar*
AJ																				
AK	X		X									X								
AL																				
AM		X	X				X								X					
AN															X					
AO	X	X	X		X	X														
AP												X								
AQ											X					X			X	
AR		X							X	X		X					X	X		
AS													X							
AT													X			X			X	
AU												X				X	X	X	X	

Site	*S. quebecense*	*S. tribulatum*	*S. vittatum*	*S. decorum*	*S. anchistinum*	*S. definitum*	*S. fibrinflatum*	*S. jenningsi*	*S. lakei*	*S. luggeri*	*S. notiale*	*S. parnassum*	*S. tuberosum*	*S. appalachiense*	*S. perissum*	*S. ubiquitum*	*S. vandalicum*	*S. venustum*	*S. verecundum*
AJ		X				X		X	X	X									
AK				X													X	X	X
AL	X				X			X			X			X				X	
AM	X	X						X						X			X	X	
AN																			
AO	X							X						X			X	X	
AP																		X	
AQ																			
AR		X		X														X	X
AS		X															X		X
AT																		X	X
AU		X		X														X	

TABLE 2. Black Fly Occurrence By Site

Site	*P. arvum*	*P. fuscum*	*P. mixtum*	*P. rhizophorum*	*P. clandestinum*	*P. magnum*	*P. canutum*	*P. albionense*	*C. dacotensis*	*C. ornithophillia*	*S. diplomutata*	*S. mutata*	*S. bracteatum*	*S. congareenarum*	*S. emarginatum*	*S. Silvestre*	*S. croxtoni*	*S. fionae*	*S. gouldingi*	*S. impar*	*S. quebecense*	*S. tribulatum*	*S. vittatum*	*S. decorum*	*S. anchistinum*	*S. definitum*	*S. fibrinflatum*	*S. jenningsi*	*S. lakei*	*S. luggeri*	*S. notiale*	*S. parnassum*	*S. tuberosum*	*S. appalachiense*	*S. perissum*	*S. ubiquitum*	*S. vandalicum*	*S. venustum*	*S. verecundum*
AV																						X						X				X		X					
AW																					X				X		X	X			X								
AX																											X	X											
AY			X		X										X										X		X	X			X								
AZ			X					X												X												X					X	X	
BA																											X												
BB																											X												
BC																						X	X											X					

TABLE 3. Key to Site Abbreviations Used In Table 3		
County	**Site**	**Site Code**
Atlantic	Stevens Creek Estellville	A
Burlington	Batsto upstream Batsto lake	B
Burlington	Bisphams mill creek	C
Burlington	Mullica river upstream rt. 542	D
Burlington	Oswego River upstream Oswego Lake	E
Burlington	Pappoose Branch, Oswego river downstream Jenkis rd.	F
Burlington	Pappoose Branch, Oswego river upstream Jenkis rd.	G
Burlington	Skit branch, Batsto river downstream Hampton Gate Rd.	H
Burlington	Tommy's Branch Bass River upstream lake Absegami	I
Burlington	Trib. Bisphams mill creek downstream 4 mile rd.	J
Burlington	Tulpehocken Creek west of Hawkins rd.	K
Burlington	Wading River downstream Oswego river	L
Cape May	East Creek, downstream pine swamp rd.	M
Cumberland	Maurice river upstream rt. 540	N
Cumberland	Trib. Buckshutem creek upstream rt. 555	O
Gloucester	Reed Branch, Maurice River at Garrison Lake outlet rt. 553/611	P
Hunterdon	Capoolong creek upstream lower landsdown road	Q
Hunterdon	Delaware at Lynn Island	R
Hunterdon	Musconetcong at Changewater	S
Hunterdon	Musconetcong at rt. 643	T
Hunterdon	South Branch at Califon	U
Hunterdon	South Branch at Deer Path	V
Hunterdon	South Branch upstream rt. 523	W
Hunterdon	Small Stream at Glen Gardner	X
Morris	South Branch downstream Budd Lake	Y
Morris	South Branch at Middle Valley	Z
Salem	Muddy run at Elmer Lake outlet near Elmer	AA
Salem	Muddy run upstream Parvin Lake	AB
Somerset	Lamington downstream Black River rd.	AC
Somerset	Lamington downstream rt. 641	AD
Somerset	Lamington river at Pottersville	AE
Somerset	North Branch at Burnt Mills Rd.	AF

TABLE 3. Key to Abbreviations Used In Table 3		
County	**Site**	**County**
Somerset	North Branch downstream rt.512	AG
Somerset	North Branch upstream rt. 202	AH
Somerset	Passaic River at Davies Bridge	AI
Somerset	South Branch upstream Woodfern road	AJ
Sussex	Big Flat Brook upstream Deckertown turnpike	AK
Sussex	Delaware at Quicks Island	AL
Sussex	Flat Brook upstream old mine rd.	AM
Sussex	Flat Brook Walpack Center	AN
Sussex	Little Flat Brook upstream of confluence with Big Flat Brook	AO
Sussex	Outlet of Beaver Swamp upstream Blue Mountain Lake	AP
Sussex	Outlet Cedar Swamp at Hhigh Point	AQ
Sussex	Outlet of Steeny Kill lake	AR
Sussex	Outlet stream Blue Mountain Lake	AS
Sussex	Trib. Big Flat Broom upstream of rt. 23	AT
Sussex	Trib. Big Flat Brook downstream Sawmill rd.	AU
Warren	Musconetcong River at Penwell	AV
Warren	Delaware River at Arrow Island	AW
Warren	Delaware River at Martins creek	AX
Warren	Delaware River at Poxono Island	AY
Warren	Dunnfield Creek	AZ
Warren	Paulinskill River at East Chrisman rd.	BA
Warren	PaulinsKill River at Hainesburg	BB
Warren	Pequest Fish Hatchery	BC

TABLE 4. Hilsenhoff Biotic Index Rating Scale		
Family Biotic Index	**Water Quality**	**Degree of Organic Pollution**
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very poor	Severe organic pollution likely

Acknowledgements

This research was supported by the Hatch Multistate Research Fund and the New Jersey Agricultural Experiment Station at Rutgers University, New Brunswick, New Jersey, Project: Evaluating the Physical and Biological Availability of Pesticides and Contaminants in Agricultural Ecosystems Project 224316 and NIEHS Center Grant ES020522.

Literature Cited

Adler, P. H., Currie, D. C., & Wood, D. M. (2004). *The Black Flies (Simuliidae) of North America*. Cornell University Press, Ithaca, NY, 941 pp.

Adler, P. H., & Kim, K. C. (1984). Ecological characterization of two sibling species, IIlL-l and IS-7, in the *Simulium vittatum* complex (Diptera: Simuliidae). *Canadian Journal of Zoology*, 62, 1308-1315.

Anderson, J. R., & DeFoliart, G. R. (1961). Feeding behavior and host preference of the black flies (Diptera; Simuliidae) in Wisconsin. *Annals of the Entomological Society of America*, 54,716-729.

Arbegast, D. H. (1994). Blackfly suppression, the Pennsylvania experience. *Proceedings of the Annual Meeting of the New Jersey Mosquito Control Association*, 81, 107-113

Barnett, B. D. (1977) Leucocytozoon Disease of Turkeys. *World's Poultry Science Journal*, 33, 76-87.

Bennett, G. F. Peirce, M. A., & Ashford, R. W. (1993). Avian Haematozoa: mortality and pathogenicity. *Journal of natural history*, 5, 993-1001

Carle, D. M. (2010). The black flies (Diptera; Simuliidae) of New Jersey, U.S.A. *Entomological News*, 121, 6-22.

Chutter, F. M. (1972). An empirical biotic index of the quality of water in South African streams and rivers. *Water Res*, 6, 19–30.

Cole, J. (1996). Neighbors against gnats. Proceedings *of the Annual Mosquito Control Association*, 83, 72-74

Council of Ministers of Water Affairs of the Nile Basin States (2001). Nile Transboundary Environmental Action Project Draft Gef Project Brief 97 pp. Available from: http://iwlearn.net/iw-projects/1094/reports/nile-transboundary-environmental-action-111p-996k.pdf

Crump, A., Morel, C. M., & Omura, S. (2012). The onchocerciasis chronicle: from the beginning to the end? *Trends in Parasitology*, 28, 208-88.

Davis, A. N., Gahan, J. B., Fluno, J. A. & Anthony, D. W. (1957). Larvicide tests against blackflies in slow-moving streams. *Mosquito News*, 17, 261-265.

Gray, E. W., Adler, P. H., & Noblet, R. (1996). Economic impact of black flies (Diptera: Simuliidae) in South Carolina and development of a localized suppression program. *Journal of the American Mosquito Control Association*, 12, 676-678.

Hilsenhoff, W. L. (1977). Use of arthropods to evaluate water quality of streams. *Technical Bulletin Number 100*, Department of Natural Resources, Box 7921, Madison, WI 53707.

Hilsenhoff ,W. L. (1987). An improved biotic index of organic stream pollution. *The Great Lakes Entomologist*, 20, 31–37.

Laird, M. (1981). *Blackflies: The Future for Biological Methods in Integrated Control.* Academic Press, London, England, 399 pp.

Lenat, D. R. (1993) A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of the North American Benthological Society*, 12, 279–290.

Malzbender, D., & Earle, A. (2008). Detailed review of the preliminary TDA, 37pp. Available at: file:///C:/Users/Diana/Downloads/REVIEW%20TDA%20FINAL.pdf (Accessed 26 June 2014).

Mead, D. G., Gray, E. W., Noblet, R., Murphy, M. D., Howerth, E. W., & Stallknecht, D. E. (2004). Biological transmission of vesicular stomatitis virus (New Jersey serotype) by Simulium vittatum (Diptera: Simuliidae) to domestic swine (Sus scrofa). *Journal of Medical Entomology*, 41, 78-82.

Merritt, R.W., Cummins, K.W. & Berg, M.B. (2008). *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Publishing Company 1158 pp.

NJDEP (2000a). Ambient Biomonitoring Network; Water Management Areas 7, 8, 9 and 10 Raritan Regions. Available at: http://www.state.nj.us/dep/wms/bfbm/download/rar99.pdf. Accessed 8 March 2014.

NJDEP (2000b). Ambient Biomonitoring Network; Water Management Areas 3, 4, 5, and 6 Passaic Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/neb98.pdf. Accessed 8 March 2014.

NJDEP (2000c). Ambient Biomonitoring Network; Water Management Areas 1, 2 and 11 Upper Delaware Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/neb98.pdf. Accessed 8 March 2014.

NJDEP (2009). Ambient Biomonitoring Network; Raritan Water Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/rarRnd4.pdf. Accessed 8 March 2014.

NJDEP (2010). Ambient Biomonitoring Network; Lower Delaware Water Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/ldlRnd3.pdf. Accessed 8 March 2014.

NJDEP (2012a). Ambient Biomonitoring Network; Upper Delaware Water Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/udelRnd4.pdf. Accessed 8 March 2014.

NJDEP (2012b). Ambient Biomonitoring Network; Northeast Water Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/nebRnd4.pdf. Accessed 8 March 2014.

NJDEP (2012c). Ambient Biomonitoring Network; Atlantic Water Region. Available at: http://www.state.nj.us/dep/wms/bfbm/download/atlRnd4.pdf. Accessed 8 March 2014.

New Jersey State Data Center (2010). Demographic Profile for New Jersey-2010 Census. Available at: http://lwd.dol.state.nj.us/labor/lpa/census/2010/gdp_index.html (Accessed 26 June 2014).

Reiter, P., & Sprenger, D. (1987). The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *Journal of the American Mosquito Control Association*, 3, 494-501.

Rothfels, K. H. & Dunbar R. W. (1953). The salivary gland chromosomes of the black fly Simulium vittatum Zett. *Canadian Journal of Zoology,* 31, 226-241.

Rothfels, K. H., & Featherston, D. (1981). The population structure of *Simulium vittatum* (Zett.): the IIIL-l and IS-7 sibling species. Canadian Journal of Zoology, 59, 1857-1883.

Schnitzlein, W. M., & Reichmann, M. E. (1985). Characterization of New Jersey vesicular stomatitis virus isolates from horses and black flies during the 1982 outbreak in Colorado. *Virology* 142, 426-31.

Ter Braak, C. J. F., & Looman, C. W. N. (1986). Weighted averaging, logistic regression and the Gaussian response model. *Vegetatio*, 65, 3–11.

Underhill, G. W. (1944). Blackflies found feeding on turkeys in Virginia, (*Simulium nigroparvum* Twinn and *Simulium slossonae* Dyar and Shannon). *Virginia Agricultural Experiment Station Technical Bulletin,* 94, 1-32.

Wilton, D. P. (1968). Oviposition site selection by the tree-hole mosquito, *Aedes triseriatus* (say). *Journal of Medical Entomology,* 5, 189-194.