To the Graduate Council:

I am submitting herewith a dissertation written by Amanda Jane Jacobson entitled "Phylogenetic analysis of the Nearctic *Blepharicera* Macquart (Diptera: Blephariceridae) with an emphasis on the eastern *Blepharicera tenuipes* group Hogue." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils and Insects.

John K. Moulton, Major Professor

We have read this dissertation
and recommend its acceptance:

Ernest C. Bernard

Juan Luis Jurat-Fuentes

Randall L. Small

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and Dean
of the Graduate School

(Original signatures are on file with official student records.)

Phylogenetic analysis of the Nearctic *Blepharicera* Macquart (Diptera: Blephariceridae) with an emphasis on the eastern *Blepharicera tenuipes* group Hogue

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I am indebted to the many individuals who had a part in making this research possible: Ken Kingsley at Zion National Park whose interest and many efforts provided DNA-quality specimens of *Blephariceru zionensis* for the molecular aspects of this paper. Dr. Peter Zwick took time out of his collecting schedule for attempts to locate *B. fasciata*. Dr. John K. Moulton collected *B. tenuipes* in Canada and located many ideal blephariceriḋ habitats in the southeast. Dr. John Dunlap at the Bioimaging and Electron Microscopy Facility at the University of Tennessee served as a mentor for acquiring the techniques associated with scanning electron microscopy. Drs. Randall L. Small, P. Brandon Matheny, and Brian O'Meara were helpful obtaining and learning the various phylogenetic programs.

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ABSTRACT

Abstract. The eastern Nearctic fauna of *Blephariceral* Macquart (Diptera: Blephariceridae) is revised to include twenty-three species, six of which are new to science. Descriptions of the larvae, pupae, and adults of *B. amnicula* n. sp., *B. conifera* n. sp., *B. crista* n. sp., *B. enoristera* n. sp., *B. hillabee* n. sp., and *B. opistera* n. sp. are presented. Keys to instar IV larvae, pupae, and adults of all eastern *Blepharicera* (except *B. caudata* Courtney) are provided. Phylogenetic studies were conducted to determine the relationships between eastern and western Nearctic *Blepharicera* and among species within these groups. Larvae, pupae, and adults were available for all known Nearctic species except *B. caudata* and *B. micheneri* Alexander. Molecular data acquired from two genes and morphological data for 44 characters were used to test previous phylogenetic hypotheses on the historical relationships and biogeography of Nearctic *Blepharicera*. Analyses using maximum parsimony, maximum likelihood, and Bayesian inference were conducted. Resulting phylogenies support monophyly of the *B. tenuipes* and *B. micheneri* groups and suggest that multiple species complexes may exist within the *B. tenuipes* group.

Keywords: net-winged midges, United States, new species, lotic, phylogeny, Big Zinc Finger 2, BZF2, NADH dehydrogenase 2, ND2

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CHAPTER 1. GENERAL INTRODUCTION

Blephariceridae, commonly known as the net-winged midges, is a unique highly adapted group of aquatic dipterans. Immature stages are found attached to smooth rocks or bedrock in swift flowing water. Habitats include cool, clear, well-oxygenated mountain streams that are flowing at velocities greater than 1 m/s. Adults usually are found close to the natal stream and have a variety of habits, often genus and sex-dependent, that include being short-lived, resting on the underside of vegetation or logs, and feeding on either insects (females of most species), nectar (males of many species), or non-feeding (Courtney 2000a). Blephariceridsrange in size from 3 mm to ≈15 mm in the larval, pupal and adult life stages.

The larvae of Blephariceridae are highly adapted to fast-flowing streams. The larval body is composed of the cephalic division, abdominal segments II–V, and an anal division. The cephalic division contains the fused head, thorax, and first abdominal segment. The anal division contains the fused abdominal segments VII–X (Craig 1967). This arrangement, however, is the primitive condition. More derived genera tend to have the sixth abdominal segment as part of the anal division. These divisions are equipped with hydraulic suckers for a total of six ventral suckers. The suckers are important for locomotion and provide a strong hold to smooth surfaces (Frutiger 1998, 2002). Blephariceridshave four larval instars, which are differentiated by cranial width and number of gill filaments. Cranial width, as defined here, is the distance between the two antennal bases. In many genera, including *Blepharicera*, larval instars I, II, III, and IV have 0, 1, 4, and 7 gill filaments, respectively.

Much like the larvae, pupae of Blephariceridae are highly adapted to fast-flowing streams. They are streamlined and somewhat dorsoventrally flattened. The pupae of most blepharicerid genera, including *Blepharicera*, have six adhesive discs, on abdominal segments IV–VI, that permanently adhere the pupa to the rock surface. The respiratory organs are located at the anterior end of the pupal case. The pupae of most genera have two respiratory organs, each consisting of four sclerotized lamellae. In most species, the outer lamellae are darker and more heavily sclerotized than the inner lamellae.

Pupae tend to orient in the same direction, with the posterior end upstream. Instead of being streamlined, as they would be with the anterior end upstream, they are acting more as a "bluff body." As water flows around the pupa, vortices form in front of and behind the respiratory organs, aiding the diffusion of oxygen into the plastron located between the respiratory organs or within the lamellae (Pommen and Craig 1995). Due to the spiracular structure of the respiratory organs, they are also able to function outside of the water (Arens 1995). This feature is advantageous in streams with high fluctuations in water level and makes rearing pupae in the lab less complicated (e.g., Courtney 1998).

Adult blephariceridss are delicate, long-legged flies that superficially resemble crane flies. Most species are identified by characteristics of the genitalia, but variation also can be seen in the wings, eyes, and mouthparts. The adult wings are distinctly folded, fully formed, and hardened in the pupal case, allowing the adult to fly upon emergence. At emergence, the folds leave a network of light colored creases on the wings, giving the appearance of secondary venation. This feature is the basis for the common name, net-winged midge.

Even though much work has been done on blephariceridd taxonomy, ecological studies have been lacking in most areas. More is known about blepharicerid larval feeding ecology than about that of the adults. Larvae are grazers (i.e., scrapers) whose main diet is diatoms (Alverson et al. 2001, Alverson and Courtney 2002). The mandibles are used to scrape periphyton off the surface of the rock. The feeding ecology of adults, on the other hand, is more complex. Most females have mandibles and are predaceous on other insects. The mandibles are used to lacerate the prey, from which the female then sucks up the body fluids. The feeding habits of females that lack mandibles are less certain, but they and males that lack mandibles are presumably either non-feeding or nectarivorous.

The fossil record for Blephariceridae is rather poor, likely due to their life history with immature stages being aquatic and adults not straying far from the stream. Two fossil specimens from the Magadan Region of northern Russia Far East and dating back to the Upper Cretaceous (≈94 mya), have been placed in the subfamily Blepharicerinae. These fossils resemble the Holarctic genera *Agathon* von Röder and *Bibiocephala* Osten Sacken (Lukashevich and Shcherbakov 1997). More recent fossil discoveries include two adult males in Burmese amber dating back to ≈100 mya (Grimaldi et al. 2002, Courtney unpublished).

Blephariceridae is a monophyletic family of Diptera defined primarily by larval characters (Zwick 1977; Wood and Borkent 1989; Courtney 1990, 1991; Oosterbroek and Courtney 1995). Larval characters supporting the monophyly of Blephariceridae include: cephalic division, six ventral suckers, cephalic apotome divided by the frontoclypeal suture, tracheal gills, and stalked Malpighian tubules. Adult males have a characteristic trifid aedeagus.

Blephariceridae is a cosmopolitan group, occurring on every continent except Antarctica. There are approximately 320 described species in 28 described genera. Four genera occur in the Nearctic region (Hogue 1987, Courtney 2000a). *Agathon* von Röder is found in western North America and central and eastern Asia; *Bibiocephala* Osten Sacken in western North America and Japan; *Blepharicera* Macquart in western and eastern North America and throughout Eurasia; and *Philorus* Kellogg in western North America and central and eastern Asia.

The only genus of Nearctic Blephariceridae found in both eastern and western North America is *Blepharicera*. There are currently five described species (*B. micheneri* group and *B. ostensackeni*) from western North America and seventeen described species and six undescribed species (*B. tenuipes* group) from eastern North America (Table 1.1) (Hogue 1987, Zwick 1990, Courtney 2000b, Moulton and Curler 2007, Jacobson and Courtney 2008). Distribution of the Nearctic fauna is limited to mostly mountainous areas from northwestern Canada to southern California and Arizona and in the east from the Great Lakes to southeastern Canada and throughout the Appalachian Mountains (Hogue 1987).

In this study, *Blepharicera* species are characterized on the basis of the morphological species concept. According to the morphological species concept, organisms are classified as the same species if intraspecific variation is non-overlapping with interspecific variation. Conspecifics are classified by the same suite of morphological characters while congenerics are distinguished by non-overlapping morphological variation between species. Reproductive isolation is difficult to confirm

Table 1.1. Location and species names for Nearctic *Blepharicera*.

Location	**Western North America**	**Eastern North America**
Genus	*Blepharicera*	*Blepharicera*
Species	*jordani* Kellogg 1903 *kalmiopsis* Jacobson & Courtney 2008 *micheneri* Alexander 1959 *ostensackeni* Kellogg 1903 *zionensis* Alexander 1953	*amnicula* n. sp. *appalachiae* Hogue & Georgian 1986 *capitata* Loew 1863 *caudata* Courtney 2000 *chattooga* Courtney 2000 *cherokea* Hogue 1978 *conifera* n. sp. *corniculata* Courtney 2000 *courtneyi* Curler & Moulton 2007 *coweetae* Hogue & Georgian 1986 *crista* n. sp. *diminutiva* Hogue 1978 *enoristera* n. sp. *gelida* Courtney 2000 *hillabee* n. sp. *hispida* Courtney 2000 *magna* Courtney 2000 *opistera* n. sp. *separata* Alexander 1963 *similans* Johannsen 1929 *tenuipes* Walker 1848 *tuberosa* Courtney 2000 *williamsae* Alexander 1953

in populations of *Blepharicera*, although it is implied based on consistent morphological characteristics. It is believed that much of the speciation occurring in the Nearctic *Blepharicera* is a result of allopatric speciation, but research on the *B. tenuipes* group shows high levels of sympatry in certain streams in the southern Appalachians (Courtney 2000b). In these streams, reproductive isolation of sympatric species is inferred by temporal and habitat separation, phenetic divergence, consistent morphological characteristics, and, to a lesser degree, by adult behaviors (e.g., swarming and resting locations) that serve to minimize interaction with congeneric species.

Hypothesized relationships and biogeography of the Nearctic *Blepharicera* were discussed by Hogue (1978), Zwick (1984), Hogue and Georgian (1986), and Jacobson (2006). Hogue (1978) believed the eastern Nearctic species arrived in North America via a transatlantic connection between Europe and Newfoundland. Support for this hypothesis was based on Hogue's contention that the eastern species show no close ties to the major western clade, the *B. micheneri* group. He suggested further that the western species arrived in North America via a connection between eastern Asia and Alaska, i.e. a Bering land bridge. There is a great divide in the Nearctic species across the central portion of North America.

Zwick (1984) suggested numerous similarities between Asian *Blepharicera* and western North American species but no similarities between Asian or European *Blepharicera* and eastern North American species. The latter differed from Hogue's hypothesis, which included a transatlantic dispersal route. Zwick constructed a phylogenetic tree that showed the *B. micheneri* group as sister group to the *B. tenuipes* group (Figure 1.1).

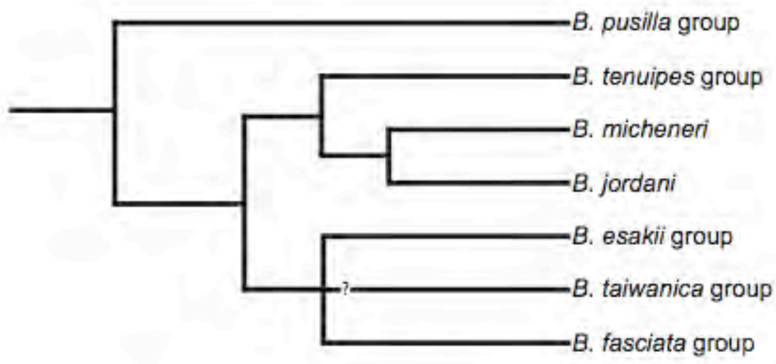

Figure 1.1. Phylogenetic relationships of *Blepharicera* based on Zwick (1984). (adapted from Zwick 1984).

In 1986, Hogue and Georgian concurred with Zwick, concluding that the European clade is too distinct to have given rise to the eastern North American clade. They also noted the likelihood that both the western and eastern groups derived from an Asian lineage since most *Blepharicera* species are from that area. Hogue and Georgian (1986) attempted to answer the question, "by what route did the *B. tenuipes* group reach eastern North America from the northwest? Also, what disruptive events forced the separation of the two groups?" They hypothesized a northern rather than southern route and suggested that the groups diverged due to arid conditions in the late Cenozoic.

Zwick (1984) was the first to discuss relationships among the Nearctic *Blephoricera* (Figure 1.1). Results of his analyses suggested a sister-group relationship between the *B. micheneri* group and *B. tenuipes* group. Hogue and Georgian (1986) decided not to evaluate the phylogenetic relationships within the *B. tenuipes* group due to numerous similarities throughout the group.

Results of morphological analyses conducted by Jacobson (2006) supported the monophyly of both the *B. micheneri* group and the *B. tenuipes* group, but a sister-group relationship between the *B. micheneri* group and *B. tenuipes* group was not supported. Rather, the two species groups were separated from each other by the European species (*B. fasciata*), an Asian clade (*B. esakii*), and *B. ostensackeni*. That study also suggested that colonization of the Nearctic was rather complex and may have involved two or three separate invasions resulting in the ancestors of the *B. micheneri* group, *B. tenuipes* group, and *B. ostensackeni* arriving at different times.

This dissertation includes descriptions of six new species and a detailed phylogenetic analysis of the Nearctic *Blephoricera*, with the exception of *B. micheneri* and the undescribed life stages of *B. caudata*. DNA-quality material of *B. micheneri* was unavailable for molecular analysis. Keys to instar IV larvae, pupae, and adults of eastern *Blephoricera* are revised to include all known species. Descriptions are provided for *B. amnicula* n. sp., *B. conifera* n. sp., *B. crista* n. sp., *B. enoristera* n. sp., *B. hillabee* n. sp., and *B. opistera* n. sp. Phylogenetic and biogeographic hypotheses of Nearctic *Blephoricera* are also discussed.

Dissertation organization

This dissertation is organized into four chapters. Chapter 1 summarizes the current knowledge of the family Blephariceridae and the Nearctic genus *Blephoricera*. Chapter 2 includes descriptions of six new species and revised keys to eastern Nearctic *Blephoricera*. Chapter 3 provides information on the phylogenetics of Nearctic *Blephoricera*. Chapter 4 is a general conclusion and discussion of future projects.

As per Article 8.2 of the International Code of Zoological Nomenclature (1999), this document is not issued for the permanent scientific record or for purposes of zoological nomenclature. Consequently, any species names contained herein should not be considered as published (*sensu* ICZN).

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CHAPTER 2. NEW SPECIES OF NET-WINGED MIDGES OF THE GENUS *BLEPHARICERA* MACQUART (DIPTERA: BLEPHARICERIDAE) FROM EASTERN NORTH AMERICA

Abstract. The eastern Nearctic fauna of *Blephariceera* (Diptera: Blephariceridae) is revised to include twenty-three species, six of which are described as new to science. Descriptions of the larvae, pupae, and adults of *B. amnicula* n. sp., *B. conifera* n. sp., *B. crista* n. sp., *B. enoristera* n. sp., *B. hillabee* n. sp., and *B. opistera* n. sp. are presented. Keys to instar IV larvae, pupae and adults of all eastern *Blephariceera*, except *B. caudata* for which pupae and adults remain unknown, are provided.

Introduction

Although Blephariceridae (net-winged midges) is considered a small dipteran family, with approximately 320 described species in 28 described genera, they can be an important component of stream ecosystems. At least some species of blephariceerids are known to be sensitive bioindicators. Habitats include cool, clear, well-oxygenated mountain streams that are flowing at velocities greater than 1 m/s. With densities sometimes greater than 1000/m^2, blephariceerids can be the prevailing insect in such a harsh environment (Georgian and Wallace 1983, Anderson 1992, Johns 1996). Immature stages are found attached to smooth rocks or bedrock in swift flowing water. Adults are usually found close to the natal stream resting on the underside of vegetation or logs (Courtney 2000b).

The larvae of Blephariceridae are highly adapted for life in fast-flowing streams. Adaptations include a cephalic division and six ventral suckers (Courtney 2000a). The cephalic division contains the fused head, thorax, first abdominal segment, and first ventral sucker. The suckers are important for locomotion and provide a strong hold to smooth surfaces (Frutiger 1998, 2002).

Much like the larvae, pupae of Blephariceridae are highly adapted to fast-flowing streams. They are streamlined and somewhat dorsoventrally flattened. The pupae of most blephariceerid genera, including *Blephariceera*, have six adhesive discs, on abdominal segments IV–VI, that permanently adhere the pupa to the rock surface. The respiratory organs are located at the anterior end of the pupal case. The pupae of most genera have two respiratory organs, each consisting of four sclerotized lamellae. In most species, the outer lamellae are darker and more heavily sclerotized than the inner lamellae.

Adult blephariceridas are delicate, long-legged flies that superficially resemble crane flies. The adult wings are distinctly folded, fully formed, and hardened in the pupal case, allowing the adult to fly upon emergence. At emergence, the folds leave a network of light colored creases on the wings, giving the appearance of secondary venation. This feature is the basis for the common name, net-winged midge.

Blephariceridae is a monophyletic family of Diptera defined primarily by larval characters (Zwick 1977; Wood and Borkent 1989; Courtney 1990, 1991; Oosterbroek and Courtney 1995). Larval characters supporting the monophyly of Blephariceridae include: cephalic division, six ventral suckers, cephalic apotome divided by the frontoclypeal suture, tracheal gills, and stalked Malpighian tubules. Adult males have a characteristic trifid aedeagus.

Blephariceridae is a cosmopolitan group, occurring on every continent except Antarctica. Numerous studies have been conducted on blepharicerid taxonomy, but ecological studies, including those pertaining to adult behavior and feeding habits, have been limited. Considerably more is known about larval feeding ecology. Larvae are grazers (i.e., scrapers) whose main diet is diatoms (Alverson et al. 2001, Alverson and Courtney 2002). The mandibles are used to scrape periphyton off the surface of the rock. The feeding ecology of adults, on the other hand, is more complex. Most females have mandibles and are predaceous on other insects. The mandibles are used to lacerate the prey, from which the female then sucks up the body fluids. The feeding habits of females that lack mandibles are less certain, but these and males that lack mandibles are presumably either non-feeding or nectarivorous.

Four genera of Blephariceridae occur in the Nearctic region (Hogue 1987, Courtney 2000a). *Agathon* von Röder is found in western North America and central and eastern Asia; *Bibiocephala* Osten Sacken in western North America and Japan; *Blepharicera* Macquart in western and eastern North America and throughout Eurasia; and *Philorus* Kellogg in western North America and central and eastern Asia. The only genus found in both eastern and western North America is *Blepharicera*. There are currently five species (*B. micheneri* group and *B. ostensackeni*) known from western North America and twenty-three species, including six undescribed species, (*B. tenuipes*

Table 2.1. Location and species names for Nearctic *Blepharicera*.

Location	**Western North America**	**Eastern North America**
Genus	*Blepharicera*	*Blepharicera*
Species	*jordani* Kellogg 1903 *kalmiopsis* Jacobson & Courtney 2008 *micheneri* Alexander 1959 *ostensackeni* Kellogg 1903 *zionensis* Alexander 1953	*amnicula* n. sp. *appalachiae* Hogue & Georgian 1986 *capitata* Loew 1863 *caudata* Courtney 2000 *chattooga* Courtney 2000 *cherokea* Hogue 1978 *conifera* n. sp. *corniculata* Courtney 2000 *courtneyi* Curler & Moulton 2007 *coweetae* Hogue & Georgian 1986 *crista* n. sp. *diminutiva* Hogue 1978 *enoristera* n. sp. *gelida* Courtney 2000 *hillabee* n. sp. *hispida* Courtney 2000 *magna* Courtney 2000 *opistera* n. sp. *separata* Alexander 1963 *similans* Johannsen 1929 *tenuipes* Walker 1848 *tuberosa* Courtney 2000 *williamsae* Alexander 1953

group) known from eastern North America (Table 2.1) (Hogue 1987, Zwick 1990, Courtney 2000b, Moulton and Curler 2007, Jacobson and Courtney 2008). Distribution of the Nearctic fauna is limited to mostly mountainous areas from northwestern Canada to southern California and Arizona and in the east from the Great Lakes to southeastern Canada and throughout the Appalachian Mountains (Hogue 1987) (Figure 2.1).

The objective of the current study was to search for and describe any new species of eastern Nearctic *Blepharicera*. Descriptions are given for *B. amnicula* n. sp., *B. conifera* n. sp., *B. crista* n. sp., *B. enoristera* n. sp., *B. hillabee* n. sp., and *B. opistera* n. sp. Keys are provided for all known life stages of all known species of eastern Nearctic *Blepharicera*.

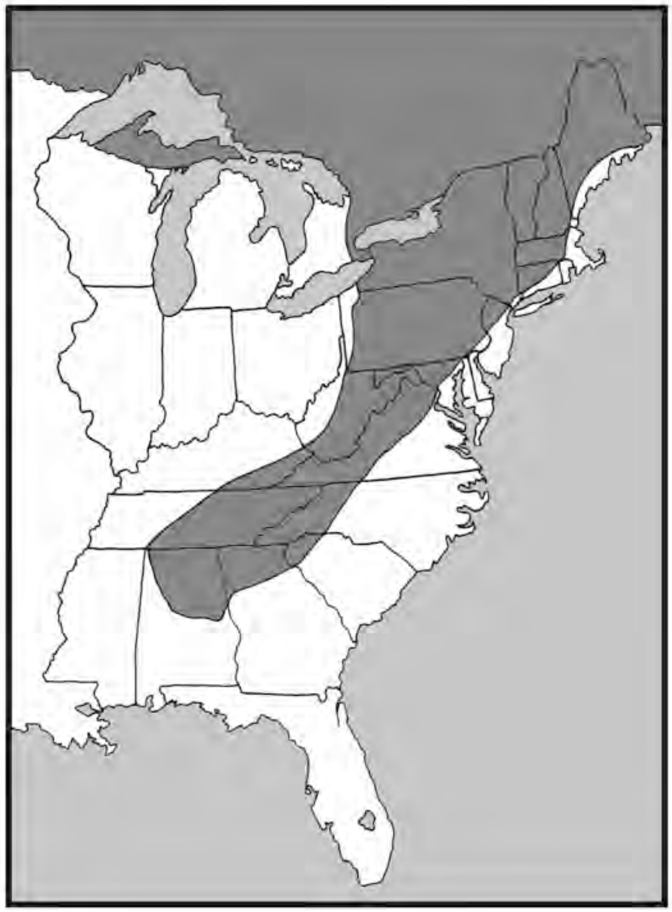

Figure 2.1. Distribution of the eastern Nearctic *Blepharicera*.

Methods

Material.—Most of the material was obtained during 2006 to 2010 by A.J. Jacobson, G.R. Curler, J.K. Moulton, and G.W. Courtney. Specimens examined were on loan from or are deposited with the following institutions (acronyms used throughout the text): CNC, Canadian National Insect Collection, Ottawa, Canada; ISIC, Iowa State Insect Collection, Iowa State University, Ames, Iowa; LACM, Natural History Museum of Los Angeles County, Los Angeles, California; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Specimen preparation.—Specimens were collected and preserved in 70 or 95% ethyl alcohol (EtOH). Morphological studies were based on whole-animal preparations, pupal dissections, slide mounts, and scanning electron microscopy (SEM). Slide-mounted material was cleared in cedarwood oil and mounted in Canada balsam (Courtney 1990). Additional material was prepared by removing soft tissues with dilute (approximately 10%) potassium hydroxide (KOH). Specimens were examined with a Meiji Techno RZ dissecting microscope and an Olympus BH-2 compound microscope. Drawings were rendered by G.R. Curler with the aid of a drawing tube on the Olympus system. Material for SEM examination was sonicated briefly (5–10 sec) in EtOH or an EtOH-KOH mixture and prepared by critical point drying or hexamethyldisilazane (HMDS) and gold-palladium coating in a sputter coater. Material was examined with a Leo 1525 SEM, which captured direct digital images.

Rearing.—All adult specimens examined for this study were reared following the protocol described by Courtney (1998).

Terminology.—Terms for structures are based on Courtney (2000a).

Descriptive format.—Measurements are given in millimeters (mm), as a mean followed by a range in parentheses. Descriptive format is based on, and values are recorded according to, procedures outlined in Courtney (2000b). Abbreviations for label and locality data are Co = County; coll = collected by; confl = confluence; ft = feet; Hwy = Highway; nr = near; Rt = Route; @ = at. Label data written as is for collection records. Abbreviations for life stages are L = larvae; P = pupae; Pex = pupal exuviae; A = adult.

Taxonomy of eastern *Blepharicera*

Blepharicera Macquart 1843

Blepharicera Macquart 1843: 61. Type species: *Blepharicera limbipennis* Macquart 1843: 63 (= *fasciata* (Westwood 1842)) [original designation]; Agassiz 1846a: 5 [catalog of Diptera genera]; Curran 1934: 62 [generic key]; Georgian & Wallace 1983: 1237 [feeding ecology]; Hogue 1987: 1 [review of Nearctic fauna]; Lenat 1993: 289 [as bioindicator]; Courtney 2000b: 1 [revision of eastern fauna]; Alverson et al. 2001: 564 [feeding ecology]; Alverson & Courtney 2002: 2087 [feeding ecology].

Asthenia Westwood 1842: 94. Type species: *Asthenia fasciata* Westwood 1842: 94. [junior homonym of *Asthenia* Hübner 1825 and *Asthenia* Westwood 1841].

Liponeura Loew 1844: 118 [description of genus].

Blepharocera Macquart: Agassiz 1846b: 47 [unjustified emendation of *Blepharicera*]; Loew 1858: 107 [unjustified emendation of *Blepharicera*]; Loew 1869: 85 [review of family, adult keys], 1877: 56 [review of family, adult keys]; Osten Sacken 1895: 148 [review of family]; Kellogg 1903: 188 [review of Nearctic fauna]; Aldrich 1905: 171 [catalog]; Curran 1923: 267 [generic key]; Johannsen 1934: 50 [review of immature stages, larval key]; Alexander 1953: 813 [review of family, generic key], 1963: 39 [review of family, generic key].

Blepharoptera Macquart: Loew 1863: 298 [*lapsus calami* for *Blepharocera*].

Ablepharocera Loew 1877: 56 [revision of Blephariceridae].

Parablepharocera Kitakami 1931: 97. Type species: *Blephariceru shirakii* Alexander 1922. [synonymy by Zwick 1990: 234].

Larva: Cranial sclerites with deep lateral incisions encompassing eyespots. Antennae two-segmented with enlarged membranous portion. Dorsal prolegs absent, some species with anterolateral and posterolateral appendages on lateral lobes of segments II–VI. Ventral gills as erect whorls, instar IV typically with 6–7 gill filaments.

Pupa: Body outline ovoid to ellipsoid. Scutum mostly glabrous. Cephalic, scutal, branchial, and alar sclerites typically glabrous. Cephalic sclerite rounded dorsally. Metathoracic and abdominal tergites usually papillose. Ventral sclerites not heavily sclerotized. Adhesive pads present on abdominal segments IV–VI. Respiratory organs erect, parallel, lobate, projected anteriorly nearly to plane of anterior margin, with four lamellae: outer lamellae sclerotized and dark brown; inner lamellae opaque and light brown.

Adult: Head normally dichoptic (male) to subholoptic (female); female eye typically with enlarged, dorsally flattened dorsal division and wide callis oculi; ommatidia in dorsal division larger in diameter than ommatidia in ventral division; antennae with 15 articles. Wing widest at middle or just beyond; membrane hyaline; wing venation as follows: radial sector two-branched, division of R_4 and R_5 asymmetrical, vein $R_{4\text{-}5}$ weak basally, crossvein r-m present, vein M_2 detached, and crossvein bm-cu absent. Legs slender, segments more or less straight, femora and tibiae slightly expanded distally, basitarsi moderately long; midtibial spurs usually absent, hind tibial spurs often present; mid coxa of female with setose median outgrowth; claws non-setate dorsally. *Male terminalia*: Epandrium simple, subquadrate, moderately sclerotized, and usually setose. Cerci prominent, two in number. Gonostylus and gonocoxite setose. Gonostylus simple or lobed. Gonocoxite subrectangular and fused ventrolaterally with hypandrium. Gonocoxal lobe typically simple, glabrous, extending from inner base of gonocoxite. Aedeagus composed of three rods; ventral parameres arising on either side of aedeagus, apices simple to complex, length variable in relation to aedeagal rods. *Female terminalia*: Sternite VIII typically bilobate, medial depression emarginate. Sternite IX (genital fork) Y- to T-shaped, sclerotized. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves variable. Spermathecae usually three in number; corpora shape variable, with simple or coiled necks.

***Blepharicera tenuipes* group Hogue 1978**

Blepharicera tenuipes group Hogue 1978: 6 [original designation], Hogue 1987: 102 [review of Nearctic fauna].

Included species.—*Blepharicera*:

amnicula, **new species**

appalachiae Hogue and Georgian 1986

capitata Loew 1863

caudata Courtney 2000

chattooga Courtney 2000

cherokea Hogue 1978

conifera, **new species**

corniculata Courtney 2000

courtneyi Curler and Moulton 2007

coweetae Hogue and Georgian 1986

crista, **new species**

diminutiva Hogue 1978

enoristera, **new species**

gelida Courtney 2000

hillabee, **new species**

hispida Courtney 2000

magna Courtney 2000

opistera, **new species**
separata Alexander 1963
similans Johannsen 1929
tenuipes Walker 1848
tuberosa Courtney 2000
williamsae Alexander 1953

Larva: Prolegs large, extended laterally, visible from above; dorsal prolegs absent, setate convexity present in place of dorsal prolegs. Anal division trilobate with rounded posterior margin; lateral lobes well developed and typically round, extended obliquely or posteriorly.

Pupa: Metathoracic and abdominal tergites papillose; papillae with spinules. Cuticle between papillae variable, glabrous to reticulate. Branchial sclerite glabrous in most species.

Adult: Head with ultimate antennal flagellomere elongate, approximately 1.2–1.6x longer than penultimate flagellomere. Ultimate palpal segment elongate. Parietal setae rarely present in males, few to numerous in females. *Male terminalia*: Cerci well developed, prominent, parallel; quadrate with various apical sublobes and marginal forms. Gonostylus simple. Sperm sac small, bilobate, without internal spines. Ventral parameres broad with complex apices. Aedeagus trifid with medial rod straight and lateral rods sinuous. *Female terminalia*: Individual valves of hypogynial plate short, broad; spermathecal duct short, extended approximately one-quarter of the way into the abdomen.

Keys to species of eastern Nearctic *Blepharicera*

Instar IV Larvae

1. Lateral processes on anal division pointed apically; body large (> 6 mm)………..…..2
- Lateral processes on anal division bluntly rounded apically; body usually small (< 6 mm)……………………………………………………………….…..…..3

2(1). Anal division acutely trilobed, medial lobe elongate and tapered apically………
…………………………….……………………..………...…*B. caudata* Courtney

- Anal division bluntly trilobed, medial lobe never elongate……….…*B. magna* Courtney

3(1). One or two rows of prominent dorsal tubercles……………………………………..4

- Dorsal tubercles absent………………………………………………………………..6

4(3). Dorsum with row of small, submedian tubercles on each side; tubercles consist of an area of elongate-ellipsoid sensilla, body coloration usually uniformly dark…………………………..…………...……*B. tuberosa* Courtney

- Dorsum with row of prominent, median tubercles, one per abdominal division; body coloration either piebald or uniformly pale………………..………………….5

5(4). Body coloration piebald; dorsal secondary sensilla sparse, mostly clavate………………...…………………………………….…*B. separata* Alexander

- Body coloration uniformly pale (yellow), except cranial sclerites (dark brown); dorsal secondary sensilla numerous, clavate and long setiform................
………………..*B. capitata* Loew

6(3). Dorsal secondary sensilla arranged in small distinct clusters on abdominal segments (Figs. 2.6 A–C)...*B. crista* n. sp.

- Dorsal secondary sensilla present over larger area of abdominal segments, not arranged in small distinct clusters…….………………………………..………….7

7(6). Clypeus with pair of prominent, anteriorly projected spines; presently known only from Chattooga River………………………..……...*B. corniculata* Courtney

- Clypeal spines absent………………………………………………………….........8

8(7). Dorsal secondary sensilla mostly setiform……………………………………….9

- Dorsal secondary sensilla mostly clavate to globose………………………………...10

9(8). Dorsal secondary sensilla short (shorter than first antennal segment)…………
……………………………………..……...*B. appalachiae* Hogue and Georgian

- Dorsal secondary sensilla elongate (longer than first antennal segment)…………..
…………………………………………………………….*B. gelida* Courtney

10(8). Dorsal secondary sensilla mostly globose…………………...*B. similans* Johannsen

- Dorsal secondary sensilla mostly clavate or digitiform……………………………...11

11(10). Body and cranial sclerites uniformly colored (light to dark brown)…………….12

- Body and cranial sclerites usually with contrasting color patterns………………..……19

12(11). Substernal sensilla (adjacent to first suctorial disc) dark; dorsal sensilla minute, about as long as broad; body small (instar IV < 5 mm)…………… …………………………………………………………….*B. diminutiva* Hogue

- Substernal sensilla (adjacent to first suctorial disc) light, pale to light brown; dorsal sensilla well developed, usually dense; body large (instar IV > 5 mm) ……………………………………………………………………………………13

13(12). Dorsal sensilla arranged in transverse anterior and posterior clusters (Fig. 2.8 A–B)…………………………………………………………………14

- Dorsal sensilla densely distributed over segments, not arranged in distinct transverse clusters…...…………………………………………………………...16

14(13). Dorsal sensilla arranged in two transverse clusters…………*B. tenuipes* (Walker)

- Dorsal sensilla arranged in four transverse clusters (Fig. 2.8 A–B)…...………………15

15(14). Anal division slightly concave; ecdysial stem line long……………………. ……………………………………………......*B. courtneyi* Curler and Moulton

-Anal division rounded; ecdysial stem line short (Fig. 2.8 D)…………*B. enoristera* n. sp.

16(13). Anal division broadly rounded (Fig. 2.8 D, 2.12 B)……………………………17

-Anal division truncate to slightly concave (Fig. 2.2 B, 2.4 B).…………………………18

17(16). Dorsal sensilla elongate-fustiform; setiforms mostly absent on abdominal segments and lateral lobes……………………………………*B. hispida* Courtney

- Dorsal sensilla fustiform; setiforms mostly absent medially, numerous on lateral lobes (Fig. 2.10 B–C)……………………………………………..*B. hillabee* n. sp.

18(16). Dorsal sensilla elongate-coniform and digitiform; presently known only from Cloudland Canyon State Park in northwest Georgia (Fig. 2.4 C)…… ………………………………………………………………*B. conifera* n. sp.

- Dorsal sensilla digitiform; presently known only from Little River Canyon National Preserve in northeast Alabama (Fig. 2.2 C)…………...*B. amnicula* n. sp.

19(11). Ecdysial stem line short, frontoclypeal apotome reaching or almost reaching posterior margin of head capsule; head capsule mottled or uniformly colored, with frontoclypeal apotome typically lighter than rest of head capsule; membranous region of antenna much shorter than apical sclerotized region…..………………………………………………………….20

- Ecdysial stem line long basally, frontoclypeal apotome separated from posterior margin of head capsule; head capsule mottled or uniformly colored, with frontoclypeal apotome typically darker than rest of head capsule; membranous region of antenna approximately the same length as apical sclerotized region…..………………………………………………………….21

20(19). Ecdysial line with little to no stem line, frontoclypeal apotome reaching posterior margin of head capsule; body with chevronlike or banded color pattern; anal division truncate to slightly concave………...*B. chattooga* Courtney

- Ecdysial line with short stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin; body with darkly pigmented medial band, margins of which extend onto lateral lobes; lateral lobes dark basally with pale crescent band at apex; anal division rounded (Fig. 2.12 A–B)…… ……………………………………………………………*B. opistera* n. sp.

21(19). Cephalothorax, trunk, and prolegs mostly yellow but sometimes with dark highlights; cranial sclerites usually yellow but with dark frontoclypeal apotome; antennae long, total length greater than length of frontoclypeal apotome…………………………………………………......*B. cherokea* Hogue

-Cephalothorax, trunk, and prolegs mostly dark but with light highlights; cranial sclerites either uniformly dark or dark with light highlights; antennae short, total length less than length of frontoclypeal apotome…….……….........22

22(21). Body coloration: dark medial band, with lateral margins extended onto dorsum of proleg; lateral muscle scars usually well inside the margins of these bands; pale crescentic band near apex of proleg; cranial sclerites usually with contrasting color pattern, with anterior half dark and posterior half light; if cranial sclerites with only small light bands, these extend perpendicular to frontoclypeal apotome; dorsal sensilla arranged in transverse anterior and posterior clusters………*B. coweetae* Hogue and Georgian

-Body coloration: uniformly dark or, if with dark medial band, the lateral margins not extended onto the dorsum of proleg; lateral muscle scars at margins of these bands; apex of proleg without pale crescentic band; if cranial sclerites with contrasting color pattern, light bands typically extend parallel to frontoclypeal apotome; dorsal sensilla arranged rather diffusely over segment, not in transverse anterior and posterior clusters…………………
……………………………….…………………………...*B. williamsae* Alexander

<u>Pupae</u>

(Unknown in *B. caudata*)

1. Middle lamellae of respiratory organ narrow at base, width approximately half width of outer lamellae.………………………………………………………...2
- Middle lamellae of respiratory organ broad at base, width greater than half width of outer lamellae………...……………………………………………...………5

2(1). Branchial sclerite papillose; cephalic sclerite with pair of ridges extended dorsoventrally for nearly half its height……………………....*B. diminutiva* Hogue
- Branchial sclerite glabrous; cephalic sclerite without submedian ridges….…………....3

3(2). Integument of abdominal tergites with minute, dark papillae bearing microscopic spinules apically; found early summer to fall………………….
………………………………...*B. chattooga* Courtney and *B. similans* Johannsen
- Integument of abdominal tergites without distinct papillae, but with minute, circular patches of spinules; these patches may give the appearance of light-colored papillae or papillae surrounded by light colored halo; found spring to early summer……………………………………………………………………4

4(3). Integument of abdominal tergites with micropunctures between circular patches, surface luster somewhat dull (less so than in *B. williamsae*); abdominal tergites often with double row of small, submedian tubercles (remnants of larval tubercles)…………...…………………...*B. tuberosa* Courtney
- Integument of abdominal tergites glabrous between circular patches, surface luster shiny, abdominal tergites without submedian tubercles…………….

……………………………………………………………….*B. cherokea* Hogue

5(1). Integument of abdominal tergites with fine, reticulate (areolate) pattern; surface luster of tergites and branchial sclerite very dull..………………… ……………………………………………………………*B. williamsae* Alexander

- Integument of abdominal tergites homogenous or otherwise sculpted, no reticulate pattern; surface luster shiny on at least branchial sclerite………………6

6(5). Integument of abdominal tergites without papillae, but with minute, circular patches of spinules; patches may give appearance of light-colored papillae or papillae surrounded by light-colored halo……............*B. corniculata* Courtney

- Integument of abdominal tergites with minute, dark or white papillae………………….7

7(6). Abdominal papillae appearing as white dots against a darker background…………8

- Abdominal papillae darker than background coloration………………………………...9

8(7). Abdominal papillae large (≥ 10 μm), adjacent papillae separated by distance that approximates papilla width; anterior lamella of respiratory organ thick, broadly rounded apically; large (> 6 mm)……….............*B. gelida* Courtney

- Abdominal papillae small (≤ 10 μm), adjacent papillae separated by distance that typically exceeds papilla width; anterior lamella of respiratory organ broadly pointed apically; relatively small (< 6 mm)……………………… …………………………….................*B. capitata* Loew and *B. separata* Alexander

9(7). Abdominal papillae in clusters of 2, 3, or more, arrangement most apparent lateral to muscle scars………………………..*B. appalachiae* Hogue and Georgian

- Abdominal papillae more or less evenly spaced, rarely forming clusters lateral to muscle scars...……………………………………………………………………10

10(9). Abdominal papillae large (≥ 10 μm), adjacent papillae separated by distance that approximates papilla width; large (> 6 mm)…......*B. magna* Courtney

- Abdominal papillae small (≤ 10 μm), adjacent papillae separated by distance that typically exceeds papilla width; relatively small (< 6 mm)…….…………...11

11(10). Integument of abdominal tergites with micropunctures or other pattern between papillae, surface luster somewhat dull (less so than in *B. williamsae*) …………………………………………………………………….12

- Integument of abdominal tergites mostly glabrous between papillae, surface luster shiny……………………………………………………………………….14

12(11). Integument of abdominal tergites with faint reticulation; anal tergite smooth, unwrinkled (Fig. 2.6 D–E)………………………………...*B. crista* n. sp.

- Integument of abdominal tergites with micropunctures between papillae; anal tergite wrinkled (Fig. 2.8 E–G)…………………………………………………13

13(12). Body outline roughly ellipsoid………………………………...*B. enoristera* n. sp.

- Body outline ovoid………….*B. coweetae* Hogue and Georgian and *B. hispida* Courtney

14(11). Anal tergite smooth, unwrinkled………………...*B. courtneyi* Curler and Moulton

- Anal tergite wrinkled…………………………………………………………………...15

15(14). Lamellae of respiratory organs broadly pointed apically………………………..16

- Lamellae of respiratory organs broadly rounded apically……………………………...17

16(15). Outer margins of respiratory lamellae curving medially, inner margins slightly curving medially; parallel in basal half, convergent apically; apices separated medially……………………………………...*B. opistera* n. sp.

- Outer margins of respiratory lamellae curving medially, inner margins straight; apices separated medially………………………………………*B. hillabee* n. sp.

17(15). Collection location around Great Lakes and in northern Appalachians, north of Tennessee..………………………………………*B. tenuipes* (Walker)

- Collection location in southern Appalachians, south of Tennessee; canyon habitat near waterfalls………..…………………*B. conifera* n. sp. and *B. amnicula* n. sp.

Adult Males
(Unknown in *B. caudata*)

1. Dorsal and ventral eye division subequal in size……………………………………….2

- Dorsal division of eye much smaller than ventral division……………………………...7

2(1). Inner margin of cercus convex or expanded……………………………………...3

- Inner margin of cercus straight, neither convex nor expanded…………………………5

3(2). Inner margin of cercus greatly expanded near base, reaching to or beyond midline; southern Appalachians………..………………..*B. williamsae* Alexander

- Inner margin of cercus convex or expanded broadly near apex; mostly central or northern Appalachians……………………………………………………………….4

4(3). Posterior margin of cercus with small process near inner edge and prominent, triangular lobe near outer margin; cercus and epandrium set with few, mostly short setae……………………………………*B. gelida* Courtney

- Posterior margin of cercus with elongate, pointed process near inner edge and more or less rectangular near outer margin; cercus and epandrium set with numerous, elongate setae……………………………………..*B. hispida* Courtney

5(2). Cercus with sparse setae, posterior margin relatively straight; dorsal paramere opaque…………………………………………*B. corniculata* Courtney

- Cercus densely set with long setae, posterior margin with pronounced lobe near inner edge; dorsal paramere with pigmented outer margin……………………….6

6(5). Posterior margin of cercus with elongate, medially directed hook near inner edge; dorsal paramere without medial dorsal carina…………..*B. magna* Courtney

- Posterior margin of cercus with blunt, ventrally projected lobe near inner edge; dorsal paramere with medial dorsal carina……..*B. coweetae* Hogue and Georgian

7(1). Inner margin of cercus expanded…………………………………………………8

- Inner margin of cercus straight, not expanded………...………………………………...9

8(7). Inner margin of cercus expanded evenly and broadly, apical margin slightly convex, making outer margin somewhat lobulate; median aedeagal filament of approximately same length as lateral filaments; apex of ventral parameres simple…………………………………………………...………..*B. capitata* Loew

- Inner margin of cercus shallowly convex and expanded slightly near base, apical margin straight and contributing to somewhat obtuse angulate outer margin; median aedeagal filament distinctly longer than lateral filaments; apex of ventral parameres incurved, asymmetrically bifurcate……..*B. similans* Johannsen

9(7). Cercal lobes narrow, elongate, and generally pointed apically…………………….10

- Cercal lobes relatively broad and generally truncated apically………………………...11

10(9). Apical margin of cercal lobe with acutely pointed lobe medially; apex of dorsal paramere deeply incised on either side of pronounced medial dorsal carina…………………………………………….............*B. tuberosa* Courtney

- Apical margin of cercal lobe with broadly rounded inner corner; apex of dorsal paramere entire on either side of weak, medial dorsal carina……………. ………………………………………………………...*B. separata* Alexander

11(9). Apex of dorsal paramere moderately to deeply incised on either side of pronounced medial dorsal carina; apex of ventral parameres complex, with stout, dorsally recurved hook or expanded inner wall; medium sized (wing length ≈5–6 mm)……………………………………………….….………..12

- Apex of dorsal paramere weakly incised or entire on either side of medial dorsal carina……………………………………………………………………..18

12(11). Posterior margin of cercus with indistinct lobe near inner margin, giving appearance of small posteromedian notch…...*B. appalachiae* Hogue and Georgian

- Posterior margin of cercus with pronounced, acute lobe apically……………………...13

13(12). Medial margin of cercus sinuous……………………………………………..14

- Medial margin of cercus straight……………………………………………………15

14(13). Lateral margin of cercus straight; aedeagal rods subequal in length (Fig. 2.7 B–C)……………………………………………...……..*B. crista* n. sp.

- Lateral margin of cercus concave arcuate; aedeagal rods equal in length (Fig. 2.3 B–C)………………………………………………...*B. amnicula* n. sp.

15(13). Ejaculatory apodeme short, extended approximately one-half distance to anterior margin of lateral parameral lobes (Fig. 2.9 C, 2.11 C)…………………16

- Ejaculatory apodeme elongate, extended approximately two-thirds distance to anterior margin of lateral parameral lobes (Fig. 2.3 C, 2.5 C)…………………..17

16(15). Posterior margin of cercus with prominent medial hook, posterolateral margin extended into rounded lobe; body coloration brown to dark brown…………………………………...........*B. courtneyi* Curler and Moulton

- Posterior margin of cercus largely undeveloped with median hook-like lobe, posterolateral margin undeveloped; body coloration light brown (Fig. 2.11 B)……………………………………………………*B. hillabee* n. sp.

17(15). Dorsal eye division smaller (0.30x) than ventral, with 12 rows of ommatidia along mid-meridian; known from locations in Virginia north to Canada and the Great Lakes Region ………………..........*B. tenuipes* (Walker)

- Dorsal eye division smaller (<0.25x) than ventral, with 10 rows of ommatidia along mid-meridian; presently known only from Cloudland Canyon State Park in northwest Georgia (Fig. 2.5 A).……………………*B. conifera* n. sp.

18(11). Posterior margin of cercus without conspicuous lobes or processes………. ……………………………………………..…………*B. diminutiva* Hogue

- Posterior margin of cercus with conspicuous lobes or processes……………..……….19

19(18). Posterior margin of cercus distinctly trilobate, posterolateral and posteromedial lobes developed, medial lobe hook-like………………………….20

- Posterior margin of cercus not trilobate, conspicuous lobe triangular…………………21

20(19). Dorsal eye division smaller (0.33x) than ventral; lateral and medial cercal margins convex; ejaculatory apodeme extended approximately one-half distance to anterior margin of lateral parameral lobes (Fig. 2.9 A–C)…… ……………………………………………………………*B. enoristera* n. sp.

- Dorsal eye division smaller (0.25x) than ventral; lateral and medial cercal margins sub parallel; ejaculatory apodeme subequal in length to lateral parameral lobes (Fig. 2.13 A–C)…………………………………*B. opistera* n. sp.

21(19). Posterior cercal lobe near medial margin……………………...*B. cherokea* Hogue

- Posterior cercal lobe equidistant between medial and outer margin……………… ………………………………………………………...*B. chattooga* Courtney

<u>Adult Females</u>

(Unknown in *B. caudata*)

1. Two spermathecae, medial spermatheca vestigial……....………*B. similans* Johannsen

- Three spermathecae………………………………………...………………………2

2(1). Ducts of lateral spermathecae sclerotized and pigmented near genital aperture………………………………………….………..*B. capitata* Loew

- Ducts of spermathecae membranous and unpigmented for entire length……..…………3

3(2). Dorsal and ventral eye divisions contiguous laterally, without callis oculi…………4

- Dorsal and ventral eye divisions separated by callis oculi……………………………………6

4(3). Distal palpomere long, length approximately equal to length of previous three palpomeres combined; number of parietal setae > 30; scutum and scutellum concolorous, except light rectangular patch just anterior to scutellum; scutellum with lateral sensilla distinctly clustered………..…. ……………………………………………………………*B. williamsae* Alexander

- Distal palpomere short, length approximately equal to length of previous two palpomeres combined; number of parietal setae usually five or fewer; scutellum distinctly lighter than scutum; scutellum with lateral sensilla arrangement diffuse……………………………………………………………….5

5(4). Scutum with short, setiform sensilla along prescutal suture and behind transverse suture……………………………………………….*B. magna* Courtney

- Scutum without short, setiform sensilla along prescutal suture and behind transverse suture……………………………………………..*B. gelida* Courtney

6(3). Callis oculi narrow at anterior margin of head (width approximately equal to diameter of dorsal ommatidium)…………………………………………………7

- Callis oculi broad at anterior margin of head (width equal to diameter of 2–3 dorsal ommatidia)……………………………………………………………..17

7(6). Distal palpomere long (> 3x length of 2nd palpomere)……………………………...8

- Distal palpomere short (< 3x length of 2nd palpomere)……………………………...14

8(7). Number of parietal sensilla > 20…………………………………………………….9

- Number of parietal sensilla < 20…………………………………………………….10

9(8). Spermathecae ovoid; accessory gland dilated anteriorly; number of parietal sensilla > 20……………………………………..*B. coweetae* Hogue and Georgian

- Spermathecae pyriform; accessory gland not wider than corpora of spermathecae; number of parietal sensilla 25–30 (Fig. 2.3 D)………………….*B. amnicula* n. sp.

10(8). Hypogynial valves subquadrate; number of parietal sensilla 12…………… …………………………………………………...*B. courtneyi* Curler and Moulton

- Hypogynial valves rounded; number of parietal sensilla variable……………………..11

11(10). Number of parietal sensilla 3; clypeal sensilla ≈10 (Fig. 2.13 D)…………

……………………………………………………………………*B. opistera* n. sp.

- Number of parietal sensilla ≥ 10; clypeal sensilla ≥ 15………………………………...12

12(11). Spermathecae spherical; number of parietal sensilla 15–17; antennal flagellomeres barrel-shaped (Fig. 2.5 D)…………………………*B. conifera* n. sp.

- Spermathecae pyriform; number of parietal sensilla ≥10; antennal flagellomeres cylindrical in shape………………………………………………13

13(12). Hypogynial valves rounded apicolaterally, pointed apicomesally, inner margin parallel and outer margin slightly convex, apices straight, not convergent or divergent, intervalvular area narrowly V-shaped (Fig. 2.9 E) ……………………………………………………………….*B. enoristera* n. sp.

- Hypogynial valves rounded at apex, inner margin strongly convex and outer margin convex, apices slightly convergent, intervalvular area broadly U-shaped (Fig. 2.7 D)………………………………………………….*B. crista* n. sp.

14(7). Number of parietal sensilla 20–25…………………………..*B. chattooga* Courtney

- Number of parietal sensilla < 20……………………………………………………15

15(14). Hypogynial valves rounded at apex; dorsal eye division with 11 rows of ommatidia along mid-meridian; number of parietal sensilla 12–18 (Fig. 2.11 D)……………………………………………………*B. hillabee* n. sp.

- Hypogynial valves quadrate to subquadrate; dorsal eye division with 15–20 rows of ommatidia along mid-meridian; number of parietal sensilla ≈10……….16

16(15). Hypogynial valves quadrate; dorsal eye division with 15 rows of ommatidia along mid-meridian……………………………..*B. separata* Alexander

- Hypogynial valves subquadrate; dorsal eye division with 18–20 rows of ommatidia along mid-meridian………………………………..*B. hispida* Courtney

17(6). Distal palpomere short (< 3x length of 2nd palpomere)…………………………...18

- Distal palpomere long (> 3x length of 2nd palpomere)…………………………………19

18(17). Number of parietal sensilla < 10…………….*B. appalachiae* Hogue and Georgian

- Number of parietal sensilla > 10……………………..…………..*B. tenuipes* (Walker)

19(17). Number of parietal sensilla < 15…………………………………………………...20

- Number of parietal sensilla > 20……………………………………………………21

20(19). Hypogynial valves subrectangular, inner margin parallel and outer margin slightly divergent; number of parietal sensilla 10–15…….*B. corniculata* Courtney

- Hypogynial valves subpentagonal, truncate apically, inner margin parallel basally and divergent apically, outer margin angulate; number of parietal sensilla < 10……………………………………………….*B. tuberosa* Courtney

21(19). Posterolateral margin of sternite VIII with approximately 10 setiform sensilla, medial depression U shaped; medium-sized species (wing length usually > 5 mm)……………………………………………..*B. cherokea* Hogue

- Posterolateral margin of sternite VIII with no more than three setiform sensilla, medial depression V shaped; very small species (wing length < 5 mm)……………………………………………………*B. diminutiva* Hogue

Species diagnoses and descriptions

Blephariceraamnicula Jacobson, new species (Figs. 2.2 A–F, 2.3 A–D)

Diagnosis.—A medium-sized *Blephariceral*. Larva: Dorsal secondary sensilla numerous, digitiform; anal division truncate to slightly concave, lateral processes rounded. Pupa: Body outline ovoid; papillae dark brown with minute spinules; cuticle glabrous; anal tergite wrinkled. Adult male: Dorsal eye division smaller (0.25x) than ventral; cerci quadrate, posterior margin trilobate with lateral lobe and median hook well developed, lateral margin concave arcuate, medial margin sinuous; dorsal paramere emarginate at apex. Adult female: Eyes divided; callis oculi present; hypogynial valves short, with lateral and medial margins slightly convex, inner margin narrowly separated, apices rounded; three pyriform spermathecae.

Description.—Larva (Figs. 2.2 A–C): Measurements, instar II (N = 3) total length 2.64 mm (2.33–2.86), cranial width 0.27 mm, antennal segments 0.11 mm, 0.02 mm, membrane 0.01 mm; instar III (N = 10) total length 3.68 mm (2.75–5.00), cranial width 0.46 mm (0.43–0.51), antennal segments 0.14 mm (0.12–0.16), 0.05 mm (0.04–0.05), membrane 0.03 mm (0.01–0.03); instar IV (N = 10) total length 6.35 mm (5.68–

7.03), cranial width 0.66 mm (0.61–0.71), antennal segments 0.19 mm (0.16–0.22), 0.09 mm (0.07–0.09), membrane 0.08 mm (0.07–0.09). Cranial sclerites brown; ecdysial line with stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division, abdomen, and lateral lobes uniformly brown. Anal division truncate to slightly concave, lateral processes rounded. Chaetotaxy: Cranial sclerites densely covered in digitiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, light brown, ≈45 in number; dorsal secondary sensilla numerous, digitiform; anal division with 6–7 prominent setiforms marginally.

Pupa (Figs. 2.2 D–F): Measurements, male (N = 7) length 5.11 mm (5.00–5.40), width 2.94 mm (2.80–3.15); female (N = 7) length 5.95 mm (5.68–6.29), width 3.18 mm (3.00–3.30). Body outline ovoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae dark brown with minute spinules. Cuticle between papillae glabrous and brown. Branchial sclerite without papillae. Anal tergite wrinkled. Respiratory lamellae wider at base and rounded apically; inner and outer margins curving medially; apices separated medially. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 6): Total length 4.96 mm (4.50–5.61), wing length 5.81 mm (5.54–6.29), width 1.75 mm (1.64–2.02).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.47 (3.35–3.75)	3.75 (3.60–4.00)	4.98 (4.70–5.50)
tibia		3.20 (3.00–3.40)	3.19 (3.03–3.26)	4.39(4.10–4.80)
tarsus	1	1.80 (1.71–1.93)	1.71 (1.63–1.82)	1.81 (1.64–1.90)
	2	0.91 (0.81–0.99)	0.87 (0.81–0.97)	0.64 (0.59–0.68)
	3	0.62 (0.58–0.68)	0.62 (0.61–0.68)	0.39 (0.34–0.41)
	4	0.32 (0.27–0.36)	0.33 (0.32–0.34)	0.27 (0.21–0.32)
	5	0.30 (0.27–0.32)	0.30 (0.27–0.34)	0.27 (0.27–0.28)

Head (Fig. 2.3 A): Structure: Eyes semidichoptic, interocular ridge present, interocular distance 0.11 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (0.25x) than ventral; dorsal ommatidia larger in diameter; dorsal

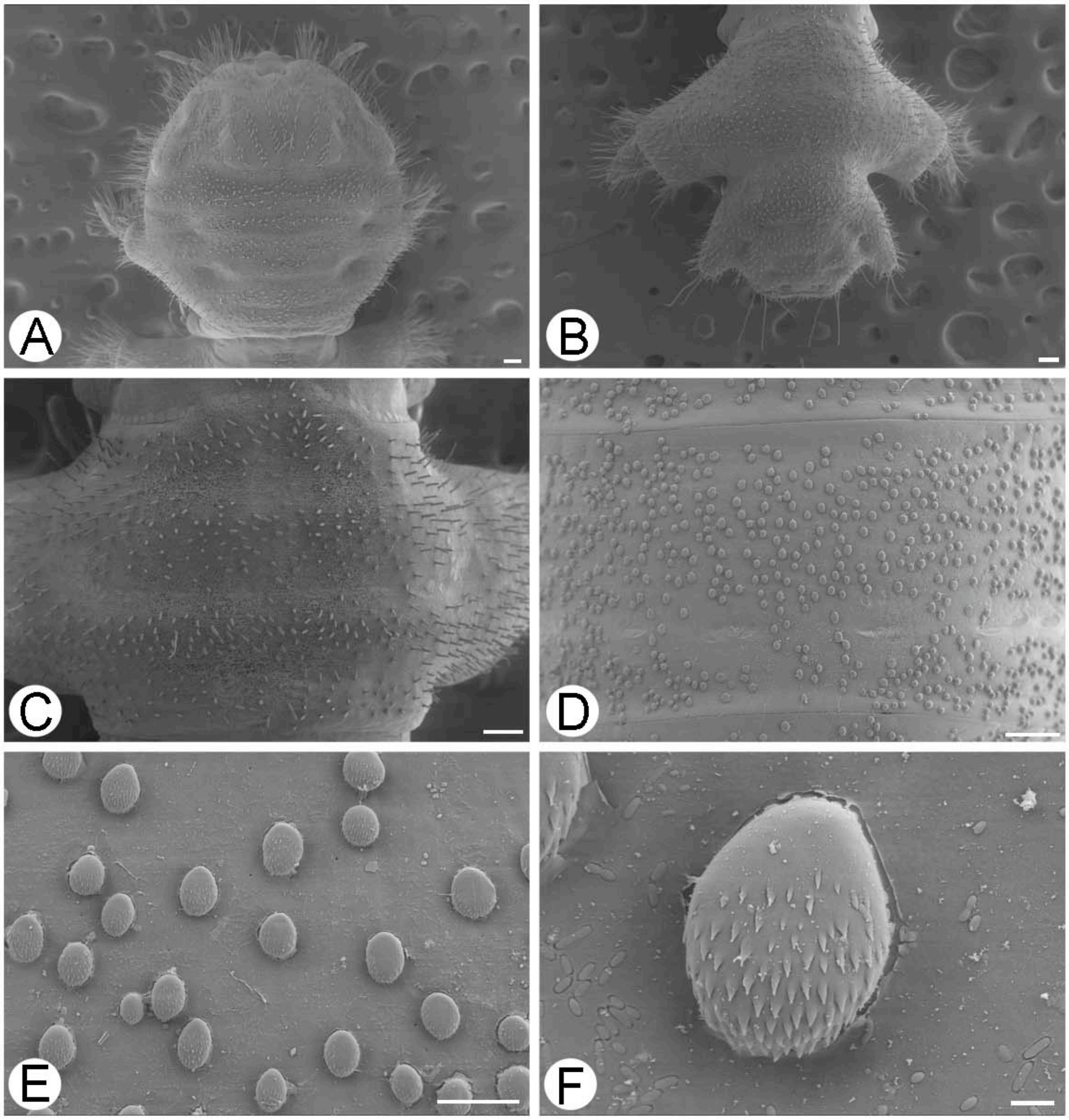

Figure 2.2. Scanning electron micrographs of larval and pupal *Blepharicera amnicula*. **A.** cephalic division, dorsal view. **B.** anal division, abdominal segments VI–X, dorsal view. **C.** larval dorsal sensilla. **D–E.** pupal abdominal tergite microsculpture. **F.** pupal abdominal papilla. Scale bars = 3 μm (F), 30 μm (E), 100 μm (A–D).

division with 12 rows of ommatidia along mid-meridian. Clypeus length/width = 2.0. Proboscis about 0.40x head width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.2, 1.6, 4.0. Antennal flagellomeres cylindrical; ultimate flagellomere 1.34x length of penultimate flagellomere; scape light brown and pedicel brown, both with prominent setiforms; flagellomere 1 brown, glabrous in basal half and setose apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈11), parietals (0), occipitals (≈30), postgenals (10–15).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.17 mm (0.14–0.20). Leg-segment proportions: foreleg—33:30:17:8:6:3:3, midleg—35:29:16:8:6:3:3, hindleg—39:35:14:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈20); coxae with prominent setae.

Coloration: Frons, clypeus, and face brown, pruinose. Thorax brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Figs. 2.3 B–C): Abdominal segment VIII greatly reduced. Epandrium bilobate, emarginated posteromedially, irregular V-shape medially; >35 prominent setae per side. Cerci quadrate, posterior margin trilobate with lateral lobe and median hook well developed. Cerci with lateral margin concave, arcuate; medial margin sinuous; ≈35 prominent setae per side; intercercal area narrowly U-shaped. Genital capsule slightly wider than long. Gonostylus and gonocoxite setose. Aedeagal rods equal in length; medial rod straight with slight thickening of membranous sheath apically, lateral rods sinuous. Ventral parameres longer than aedeagal rods, broad basally, tapered to complex apex with expanded inner wall. Dorsal paramere emarginate at apex, medial lobe ≈1.5x length of lateral lobes. Dorsal carina prominent. Gonocoxal apodeme and lateral parameral lobes well developed. Ejaculatory apodeme elongate, extended approximately two-thirds distance to anterior margin of lateral lobes.

<u>Adult female:</u> Size: medium. Measurements (N = 8): Total length 6.68 mm (6.42–7.23), wing length 7.03 mm (6.69–7.49), width 2.21 mm (1.90–2.28).

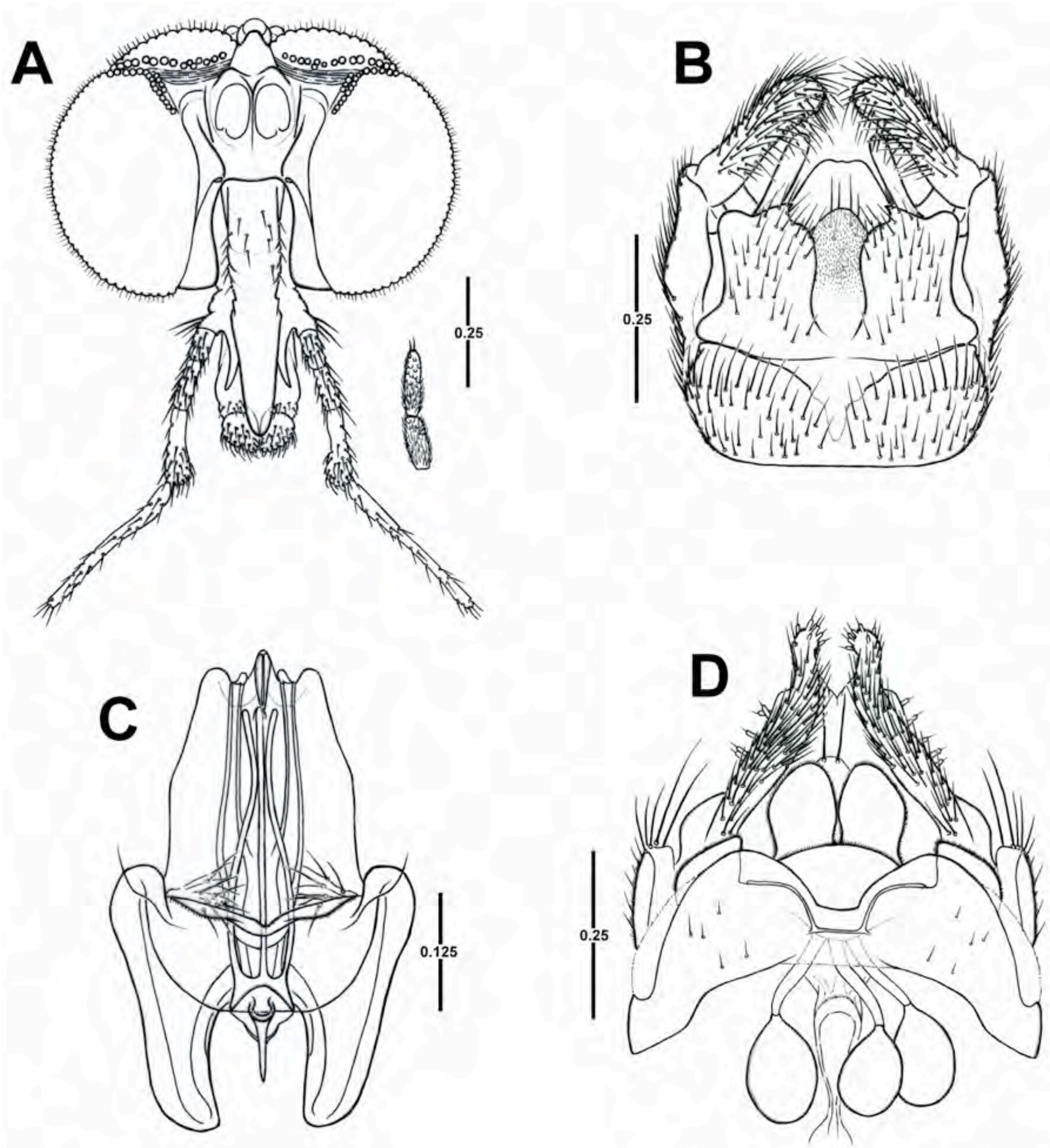

Figure 2.3. Adults of *Blepharicera amnicula*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female terminalia, ventral view.

Leg-segment lengths:		foreleg	midleg	hindleg
femur		4.19 (3.85–4.45)	4.14 (3.80–4.40)	5.77 (5.35–6.08)
tibia		3.51 (3.23–3.65)	3.38 (3.08–3.75)	5.17 (4.75–5.41)
tarsus	1	1.72 (1.56–1.83)	1.66 (1.52–1.85)	2.24 (2.00–2.61)
	2	0.93 (0.81–1.02)	0.92 (0.86–1.02)	0.78 (0.74–0.88)
	3	0.56 (0.43–0.61)	0.57 (0.54–0.61)	0.48 (0.45–0.51)
	4	0.34 (0.30–0.38)	0.34 (0.30–0.38)	0.34 (0.32–0.38)
	5	0.38 (0.32–0.41)	0.40 (0.36–0.41)	0.38 (0.34–0.41)

Head: Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.06 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 14 rows of ommatidia along mid-meridian. Clypeus length/width = 2.5. Proboscis about 0.46x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.2, 1.4, 3.5. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.60x length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere 1 light brown in basal half, setose and brown apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈20), parietals (25–30), occipitals (≈40), postgenals (≈10).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.20 mm) and short (0.07 mm). Leg-segment proportions: foreleg—36:30:15:8:5:3:3, midleg—36:30:15:8:5:3:3, hindleg—38:34:15:5:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈25); coxae with prominent setae.

Coloration: Frons and clypeus brown, pruinose. Thorax brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Fig. 2.3 D): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves short, with lateral and medial margins slightly convex, inner margin narrowly separated, apices rounded; intervalvular area narrowly U-shaped. Accessory gland not wider than corpora of spermathecae, not extending beyond anterior margin of spermathecae. Spermathecae three in number; corpora pyriform, with short necks; ducts short, unpigmented. Chaetotaxy: Sternite VIII with 6–8 setiforms laterally; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.—Holotype [adult male, reared]: UNITED STATES: ALABAMA: *Cherokee Co*: Little River Canyon National Preserve, Little River @ Canyon Mouth Park, 34°17'N 85°40'W, 9 April 2008, coll. A.J. Jacobson and G.R. Curler, emerged 13 April 2008. Specimen pinned, genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype, emerged 18 April 2008; pinned, genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [2 instar IV L (slides), 2 male and 2 female P (EtOH), 1 male and 1 female Pex (slides), 2 male and 1 female A (reared, pinned, head and genitalia slide mounted), 1 male and 2 female A (reared, pinned, genitalia in glycerin microvial)]; 12 March 2007 [9 instar IV L (EtOH)], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.—same locality as type material: 12 March 2007 [L], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton; 25 April 2007 [PPex], coll. A.J. Jacobson and G.R. Curler. Little River Canyon National Preserve, Little River above falls, 34°23'N 85°37'W, 12 March 2007 [L], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton; 25 April 2007 [P], coll. A.J. Jacobson and G.R. Curler.

Etymology.—From the Latin for "river-dwelling" (*amnicola*) plus the diminutive suffix (*-ula*) in reference to the type locality, Little River, AL.

Distribution.—*Blepharicera amnicula* has been collected only from Little River within Little River Canyon National Preserve in northeast Alabama. Little River is a unique fourth-order stream that forms and flows for most of its length on top of Lookout Mountain.

Bionomics.— Collection records indicate *Blepharicera amnicula* is likely a univoltine, spring species. Pupae were absent in mid-March collections but were collected on rocks for rearing in early and late April. Reared material is only available from Canyon Mouth Park site since river habitats above the falls consisted mostly of large boulders and bedrock.

Taxonomic comparison.—*Blephariccera amnicula* is the only species known to occur in Little River at the Little River Canyon National Preserve. All life stages are similar to *B. conifera* and *B. tenuipes*. The larvae are unique in the type and arrangement of the dorsal secondary sensilla. In *B. amnicula*, the sensilla are digitiform and evenly spaced on the dorsum. *Blephariccera tenuipes* sensilla are fusiform and arranged in two bands on the abdominal segments while *B. conifera* has coniform sensilla. Pupae of *B. amnicula* are very similar to other species in the group. Adult males of *B. amnicula* can be distinguished by characters of the genitalia and size of the dorsal eye division. The dorsal eye division in *B. amnicula* is larger than that of *B. conifera* and the inner margin of the cercus is sinuous in comparison to the straight inner margin of *B. tenuipes*. Adult females of *B. amnicula* have pyriform spermathecae and, like the males, have a slightly larger dorsal eye division than that of *B. conifera*.

Blephariccera conifera Jacobson, new species (Figs. 2.4 A–F, 2.5 A–D)

Diagnosis.—A medium-sized *Blephariccera*. <u>Larva:</u> Dorsal secondary sensilla numerous, elongate-coniform and digitiform; anal division truncate to slightly concave, lateral processes rounded. <u>Pupa:</u> Body outline ovoid; papillae brown with minute spinules; cuticle between papillae glabrous with a few micropunctures, brown; anal tergite wrinkled. <u>Adult male:</u> Dorsal eye division smaller (<0.25x) than ventral; cerci quadrate, posterior margin trilobate with lateral lobe and median hook, lateral margin concave angulate, medial margin straight; dorsal paramere emarginate at apex. <u>Adult female:</u> Eyes divided; callis oculi present; hypogynial valves short, lateral margins convex and medial margins slightly convex, inner margin narrowly separated, apices rounded; intervalvular area narrowly V-shaped; three spherical spermathecae.

Description.—<u>Larva</u> (Fig. 2.4 A–D): Measurements, instar II (N = 2) total length 2.41 mm (2.16–2.66), cranial width 0.27 mm, antennal segments 0.11 mm, 0.01 mm, membrane 0.03 mm; instar III (N = 15) total length 3.66 mm (2.91–4.53), cranial width 0.46 mm (0.43–0.49), antennal segments 0.15 mm (0.14–0.18), 0.05 mm (0.04–0.07), membrane 0.03 mm (0.03–0.04); instar IV (N = 15) total length 6.30 mm (5.81–7.10), cranial width 0.64 mm (0.57–0.69), antennal segments 0.20 mm (0.18–0.23), 0.10 mm (0.08–0.12), membrane 0.07 mm (0.05–0.11). Cranial sclerites brown; ecdysial line with stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division, abdomen, and lateral lobes uniformly light brown. Anal division truncate to slightly concave, lateral processes rounded. Chaetotaxy: Cranial sclerites densely covered in elongate-coniform and digitiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, light brown, approximately 30 in number; dorsal secondary sensilla numerous, elongate-coniform and digitiform; anal division with 6–8 prominent setiforms marginally.

Pupa (Fig. 2.4 E–F): Measurements, male (N = 15) length 4.06 mm (3.70–4.75), width 2.33 mm (2.05–2.75); female (N = 15) length 5.08 mm (4.45–6.15), width 2.88 mm (2.50–3.25). Body outline ovoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae brown with minute spinules. Cuticle between papillae glabrous with a few micropunctures, brown. Branchial sclerite without papillae. Anal tergite wrinkled. Respiratory lamellae wider at base and rounded apically; inner and outer margins curving medially; apices separated medially. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 6): Total length 5.03 mm (4.40–5.61), wing length 6.01 mm (5.79–6.29), width 1.78 mm (1.67–1.98).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.56 (3.40–3.70)	3.83 (3.69–4.06)	5.08 (4.80–5.34)
tibia		3.21 (3.08–3.40)	3.08 (3.05–3.15)	4.56 (4.25–4.85)
tarsus	1	1.85 (1.75–1.96)	1.67 (1.57–1.75)	1.96 (1.80–2.10)
	2	0.96 (0.86–1.08)	0.88 (0.82–0.95)	0.67 (0.61–0.77)
	3	0.64 (0.57–0.69)	0.64 (0.60–0.69)	0.42 (0.39–0.47)
	4	0.32 (0.30–0.34)	0.33 (0.29–0.35)	0.29 (0.27–0.34)
	5	0.30 (0.27–0.34)	0.30 (0.27–0.34)	0.30 (0.27–0.34)

Head (Fig. 2.5 A): Structure: Eyes semidichoptic, interocular distance 0.09 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (<0.25x) than ventral; dorsal ommatidia larger in diameter; dorsal division with 10 rows of

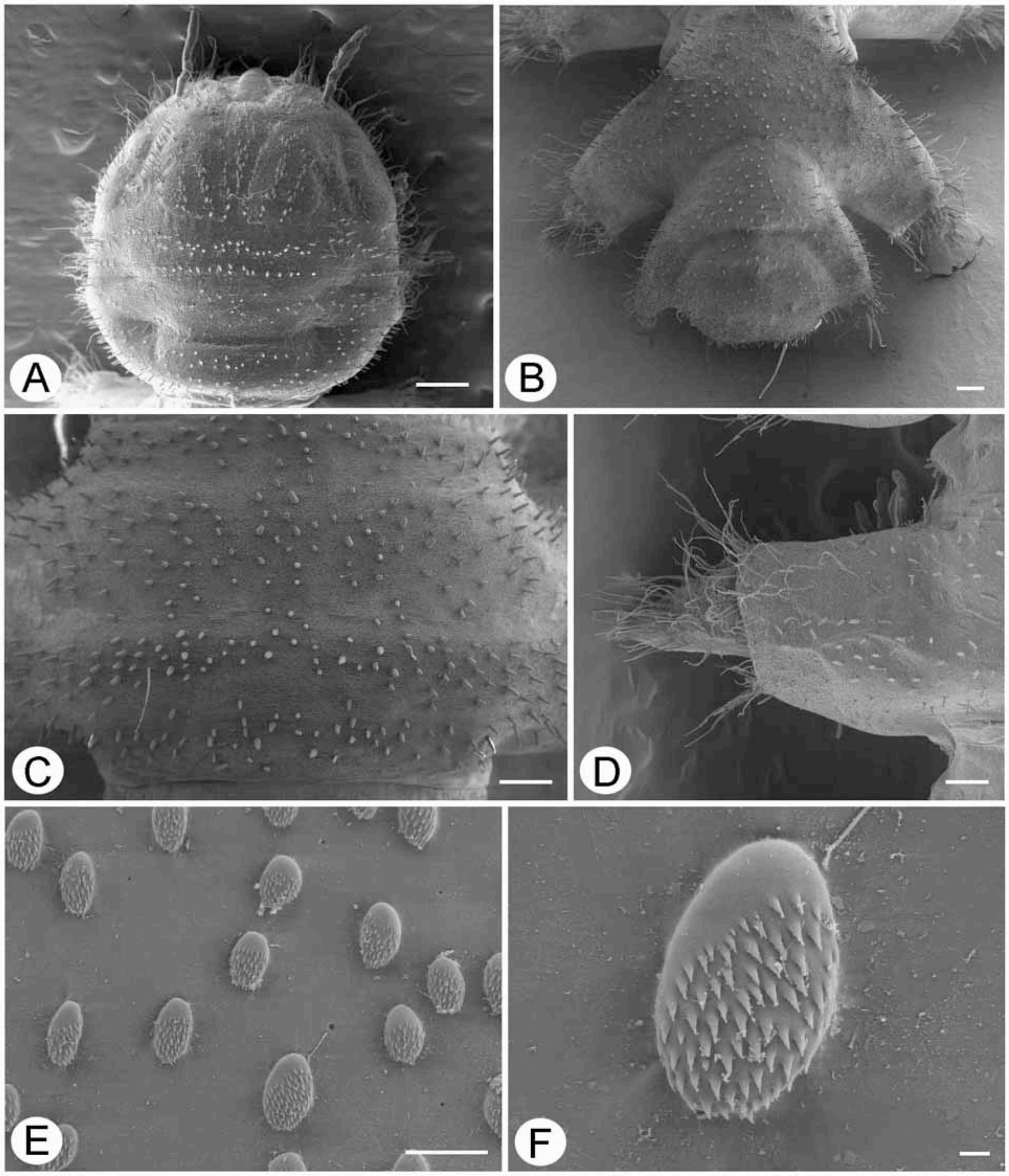

Figure 2.4. Scanning electron micrographs of larval and pupal *Blepharicera conifera*. **A.** cephalic division, dorsal view. **B.** anal division, abdominal segments VI–X, dorsal view. **C.** larval dorsal sensilla. **D.** proleg on abdominal segment III, dorsal view on left side. **E.** pupal abdominal tergite microsculpture. **F.** pupal abdominal papilla. Scale bars = 2 μm (F), 20 μm (E), 100 μm (B–D), 200 μm (A).

ommatidia along mid-meridian. Clypeus length/width = 2.0. Proboscis about 0.43x head width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.3, 1.5, 3.8. Antennal flagellomeres barrel-shaped; ultimate flagellomere 1.2x length of penultimate flagellomere; scape pale and pedicel brown, both with prominent setiforms; flagellomere 1 pale and glabrous in basal half but setose and brown apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈10), parietals (0), occipitals (>30), postgenals (10–20).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.15 mm (0.12–0.18). Leg-segment proportions: foreleg—33:29:17:9:6:3:3, midleg—36:29:15:8:6:3:3, hindleg—38:35:15:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈30); coxae with prominent setae.

Coloration: Frons, clypeus, and face light brown, pruinose. Thorax light brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites light brown, sternites pale.

Terminalia (Figs. 2.5 B–C): Abdominal segment VIII greatly reduced. Epandrium bilobate, emarginated posteromedially, broad U-shape medially; ≈45 prominent setae per side. Cerci quadrate, posterior margin trilobate with lateral lobe and median hook. Cerci with lateral margin concave, angulate; medial margin straight; >40 prominent setae per side; intercercal area narrowly U-shaped. Genital capsule slightly longer than wide. Gonostylus and gonocoxite setose. Aedeagal rods equal in length; medial rod straight with slight thickening of membranous sheath apically, lateral rods sinuous. Ventral parameres longer than aedeagal rods, broad basally, tapered to complex apex with expanded inner wall. Dorsal paramere emarginate at apex, medial lobe ≈1.5x length of lateral lobes. Dorsal carina prominent. Gonocoxal apodeme and lateral parameral lobes well developed. Ejaculatory apodeme elongate, extended approximately two-thirds distance to anterior margin of lateral lobes.

<u>Adult female:</u> Size: medium. Measurements (N = 7): Total length 6.62 mm (6.42–7.00), wing length 6.96 mm (6.56–7.40), width 2.09 mm (2.00–2.25).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		4.08 (3.92–4.25)	3.99 (3.90–4.00)	5.64 (5.35–5.81)
tibia		3.47 (3.40–3.57)	3.39 (3.23–3.60)	5.14 (4.80–5.41)
tarsus	1	1.68 (1.60–1.77)	1.65 (1.57–1.70)	2.19 (2.02–2.37)
	2	0.88 (0.78–0.95)	0.91 (0.80–0.95)	0.85 (0.81–0.88)
	3	0.54 (0.42–0.61)	0.59 (0.55–0.61)	0.50 (0.47–0.54)
	4	0.33 (0.30–0.34)	0.33 (0.31–0.36)	0.35 (0.32–0.38)
	5	0.36 (0.34–0.40)	0.36 (0.34–0.39)	0.38 (0.34–0.47)

Head: Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.04 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 12 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.46x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.3, 1.5, 3.8. Antennal flagellomeres barrel-shaped; ultimate flagellomere 1.65x length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere 1 light brown in basal half and setose and brown apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈25), parietals (15–17), occipitals (≈35), postgenals (≈20).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.21 mm) and short (0.08 mm). Leg-segment proportions: foreleg—36:30:15:8:5:3:3, midleg—36:30:15:8:5:3:3, hindleg—37:34:15:6:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈20); coxae with prominent setae.

Coloration: Frons and clypeus brown, pruinose. Thorax brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Fig. 2.5 D): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves short, with lateral margins convex and medial margins slightly convex, inner margin narrowly separated, apices rounded; intervalvular area narrowly V-shaped. Accessory gland not wider than corpora of spermathecae, not extending beyond anterior margin of spermathecae. Spermathecae three in number; corpora spherical, with short necks; ducts short, unpigmented.

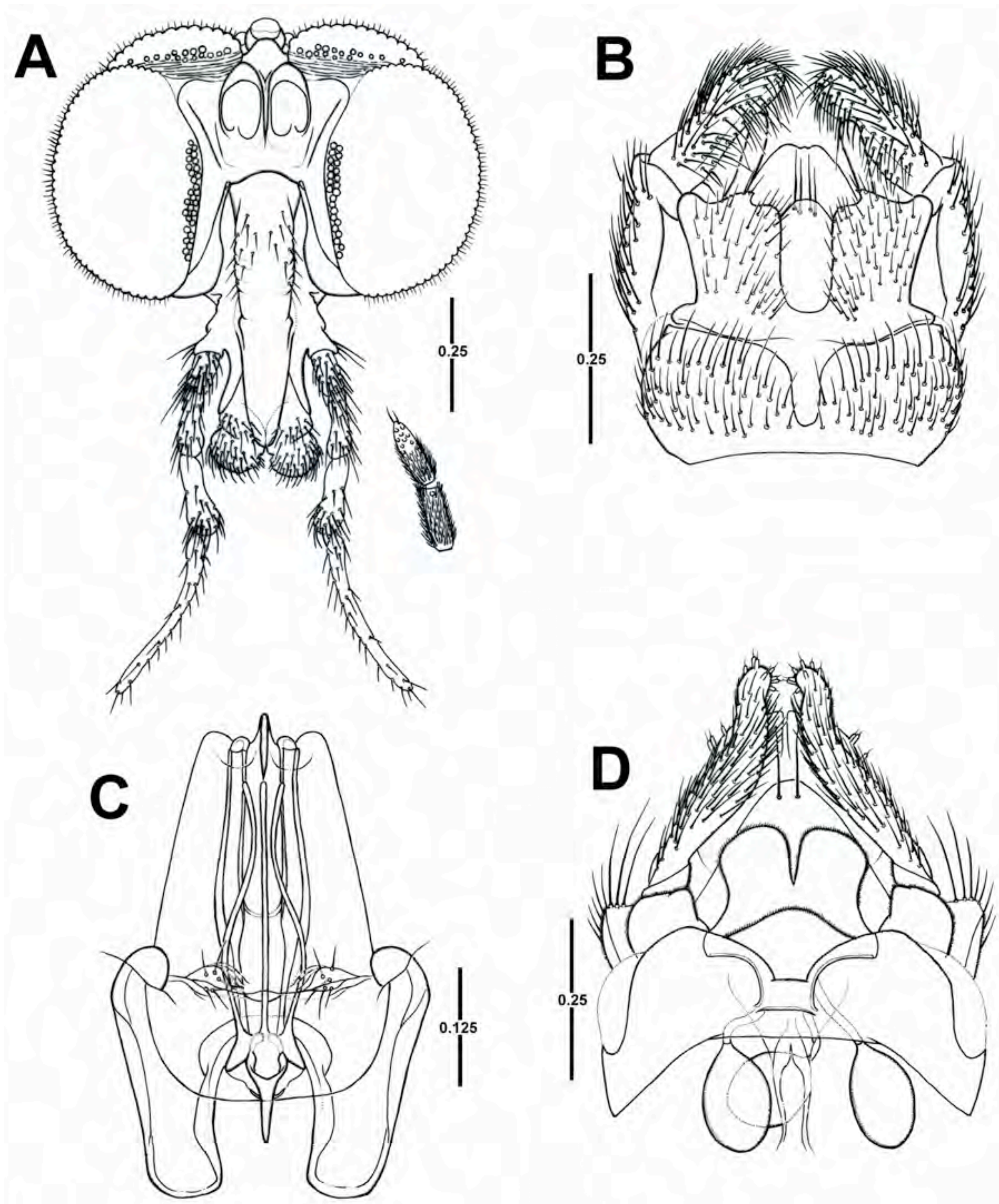

Figure 2.5. Adults of *Blepharicera conifera*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female terminalia, ventral view.

Chaetotaxy: Sternite VIII with 2–6 setiforms laterally; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.—Holotype [adult male, reared]: UNITED STATES: GEORGIA: *Dade Co*: Cloudland Canyon State Park, Daniel Creek below upper falls, 34°50'N 85°28'W, 9 April 2008, coll. A.J. Jacobson and G.R. Curler, emerged 15 April 2008. Specimen pinned, head and genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype, emerged 16 April 2008; pinned, head and genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [1 male and 1 female Pex (slides), 2 male A (reared, pinned, head and genitalia slide mounted), 2 male and 1 female A (reared, pinned, head and genitalia in glycerin microvial)]; 12 March 2007 [2 instar IV L (slides)], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton; 25 April 2007 [9 instar IV L (EtOH), 3 male and 3 female P (EtOH)], coll. A.J. Jacobson and G.R. Curler. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.— same locality as type material: 8 May 1952 [A], coll. G.S. Walley; 12 March 2007 [L], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton; 25 April 2007 [LPPex], coll. A.J. Jacobson and G.R. Curler.

Etymology.—From the Latin for "cone-bearing" (*conifera*) in reference to the unique elongate-coniform larval dorsal secondary sensilla.

Distribution.—*Blephariceraconifera* has only been collected from Daniel Creek within Cloudland Canyon State Park in northwest Georgia. Cloudland Canyon State Park is located on the Cumberland Plateau and Lookout Mountain.

Bionomics.— The first collection record of *Blepharicera conifera* was from 8 May 1952, coll. G.S. Walley (Courtney 2000b). A.J. Jacobson, G.R. Curler, and J.K. Moulton have collected additional material, including the unknown larval and pupal stages, in the last several years. Due to its unique, isolated habitat, *B. conifera* is likely an endemic, univoltine, spring species. As with other *Blepharicera* species locations, Daniel Creek near the upper falls is largely bedrock, making it difficult to locate rocks small enough to carry out of the canyon for rearing.

Taxonomic comparison.—*Blepharicera conifera* can be identified by collection location as it is the only species known to occur in Daniel Creek within Cloudland

Canyon State Park. Larvae are unique in the presence of elongate-coniform sensilla on the dorsal segments of the abdomen. The sensilla become more digitiform towards the lateral lobes but are clearly cone-shaped dorsally. Pupae of *B. conifera* are very similar to other species in the group. Adult males of *B. conifera* are most similar to *B. amnicula* and *B. tenuipes* but can be distinguished by differences in the shape of the cerci as well as differences in the aedeagus. *Blephariceera conifera* and *B. amnicula* are also much smaller in size when compared to *B. tenuipes* specimens from Canada. Adult females of *B. conifera* have spherical spermathecae and a slightly smaller dorsal eye division than that of *B. amnicula*.

Blephariceera crista Jacobson, new species (Figs. 2.6 A–F, 2.7 A–D)

Diagnosis.—A medium-sized *Blephariceera*. Larva: Dorsal secondary sensilla intermediate between digitiform and elongate-claviform, narrow basally, widened apically, arranged in six clusters on each abdominal segment; anal division truncate to slightly concave, with triangular lateral lobes. Pupa: Body outline ovoid; papillae brown with minute spinules; cuticle with faint reticulation; anal tergite smooth, unwrinkled. Adult male: Dorsal eye division smaller (<0.25x) than ventral; cerci quadrate, posterior margin trilobate with lateral lobes rounded, median hook well developed, and mesal lobes reduced and broadly rounded, lateral margin straight, medial margin sinuous; aedeagal rods subequal in length; dorsal paramere moderately incised at apex. Adult female: Eyes divided; callis oculi present; hypogynial valves short, inner margin strongly convex, outer margin convex, narrowing and rounded at apex, apices slightly convergent; accessory gland spade shaped; three pyriform spermathecae.

Description.—Larva (Figs. 2.6 A–C): Measurements, instar III (N = 3) total length 4.93 mm (4.46–5.34), cranial width 0.49 mm (0.46–0.51), antennal segments 0.16 mm (0.15–0.16), 0.07 mm (0.07–0.08), membrane 0.04 mm (0.03–0.04); instar IV (N = 10) total length 6.23 mm (5.75–6.69), cranial width 0.65 mm (0.62–0.68), antennal segments 0.19 mm (0.16–0.22), 0.09 mm (0.08–0.11), membrane 0.05 mm (0.03–0.07). Cranial sclerites dark brown; ecdysial line with stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division, abdomen, and lateral lobes uniformly brown. Anal division truncate to slightly concave, with triangular lateral lobes. Chaetotaxy: Cranial sclerites densely covered in fusiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, brown, ≈25 in number; dorsal secondary sensilla intermediate between digitiform and elongate-claviform, narrow basally, widened apically, arranged in six clusters on each abdominal segment; anal division with 4–6 prominent setiforms marginally.

Pupa (Figs. 2.6 D–F): Measurements, male (N = 10) length 4.42 mm (4.25–4.65), width 2.67 mm (2.55–2.85); female (N = 10) length 5.11 mm (4.75–5.30), width 3.12 mm (2.90–3.25). Body outline ovoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae brown with minute spinules. Cuticle between papillae brown, with faint reticulation. Branchial sclerite without papillae. Anal tergite smooth, unwrinkled. Respiratory lamellae wider at base and broadly pointed apically; inner and outer margins parallel. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 6): Total length 4.77 mm (4.50–5.07), wing length 5.54 mm (5.30–5.68), width 1.80 mm (1.70–1.89).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.19 (3.10–3.36)	3.42 (3.25–3.69)	4.48 (4.30–4.60)
tibia		2.84 (2.60–2.96)	2.72 (2.45–2.85)	4.26 (4.15–4.35)
tarsus	1	1.54 (1.45–1.63)	1.45 (1.39–1.52)	1.54 (1.45–1.65)
	2	0.84 (0.81–0.86)	0.73 (0.68–0.77)	0.55 (0.53–0.61)
	3	0.55 (0.51–0.59)	0.51 (0.43–0.54)	0.35 (0.34–0.37)
	4	0.30 (0.30–0.31)	0.28 (0.24–0.31)	0.24 (0.23–0.24)
	5	0.29 (0.28–0.30)	0.28 (0.24–0.31)	0.27 (0.24–0.31)

Head (Fig. 2.7 A): Structure: Eyes semidichoptic, interocular distance 0.12 mm; eye divided; callis oculi absent; dorsal division mostly contiguous with ventral, smaller (<0.25x) than ventral; dorsal ommatidia larger in diameter; dorsal division with 9 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.39x head

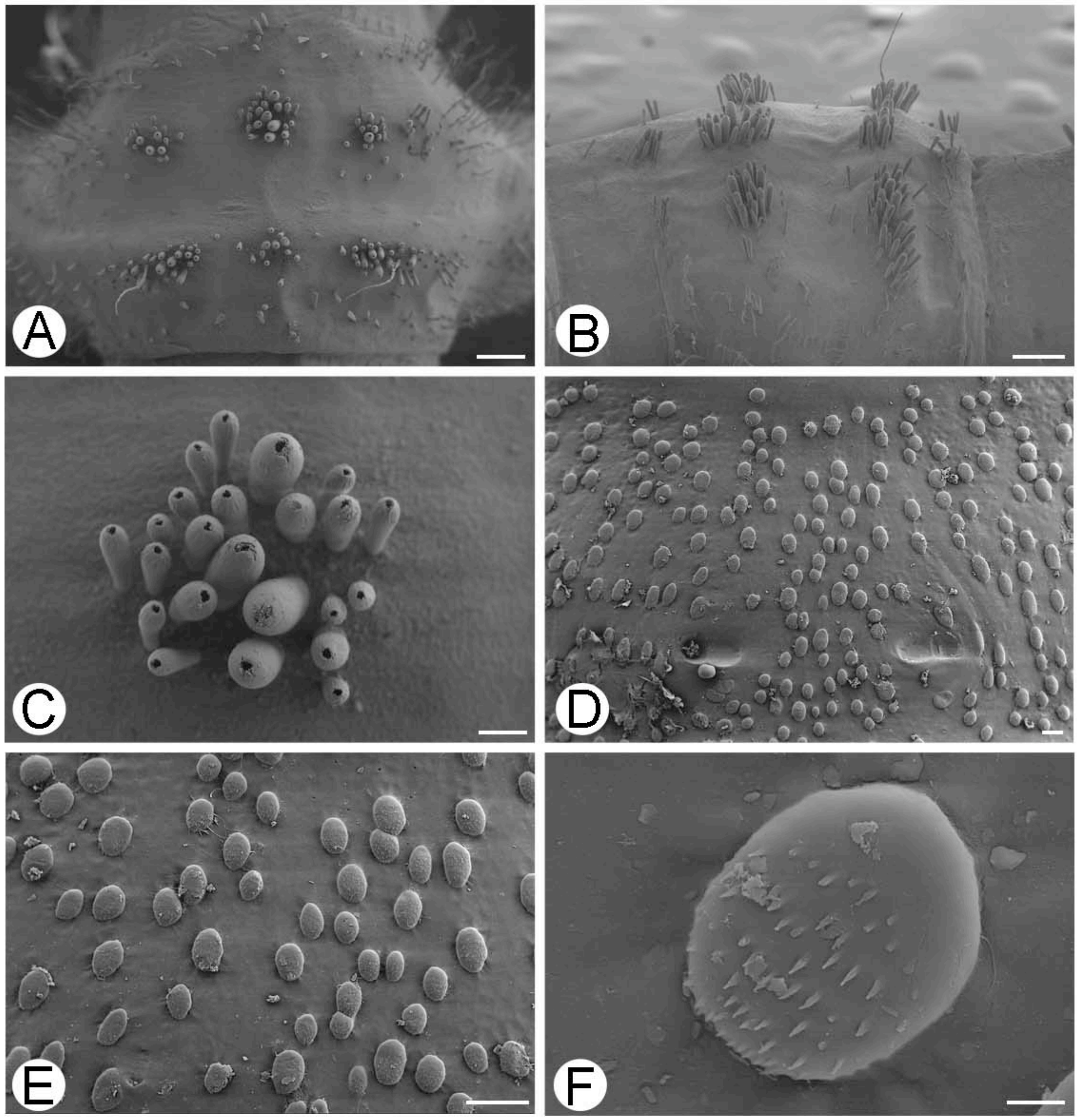

Figure 2.6. Scanning electron micrographs of larval and pupal *Blepharicera crista*. **A.** larval dorsal sensilla, dorsal view. **B.** larval dorsal sensilla, lateral view. **C.** larval dorsal sensilla cluster. **D–E.** pupal abdominal tergite microsculpture. **F.** pupal abdominal papilla. Scale bars = 3 μm (F), 20 μm (C–D), 30 μm (E), 100 μm (A–B).

width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.4, 1.6, 4.2. Antennal flagellomeres cylindrical; ultimate flagellomere 1.33x length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere 1 light brown and glabrous in basal half but setose and brown apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈10), parietals (0–1), occipitals (≈25), postgenals (10–20).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.17 mm (0.15–0.20). Leg-segment proportions: foreleg—33:30:16:9:6:3:3, midleg—36:29:16:8:5:3:3, hindleg—38:37:13:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons, clypeus, and face brown, pruinose. Thorax brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Figs. 2.7 B–C): Abdominal segment VIII greatly reduced. Epandrium bilobate, emarginated posteromedially, broad irregular V-shape medially; ≈40 prominent setae per side. Cerci quadrate; posterior margin trilobate with lateral lobes rounded, median hook well developed, and mesal lobes reduced and broadly rounded. Cerci with lateral margin straight, medial margin sinuous, ≈40 prominent setae per side, intercercal area U-shaped. Genital capsule about as long as wide. Gonostylus and gonocoxite setose. Aedeagal rods subequal in length; medial rod straight, with prominent thickening of membranous sheath near apex, with apical portion spearhead-like; lateral rods sinuous with slight thickening of membranous sheath apically. Ventral parameres longer than aedeagal rods, wider basally, tapering slightly to complex apex with expanded inner wall. Dorsal paramere moderately incised at apex, median lobe approximately 1.25x length of lateral lobes. Dorsal carina prominent. Gonocoxal apodeme and lateral parameral lobes well developed. Ejaculatory apodeme extended approximately one-half distance to anterior margin of lateral parameral lobes.

<u>Adult female:</u> Size: medium. Measurements (N = 8): Total length 5.80 mm (5.41–6.02), wing length 6.29 mm (6.00–6.79), width 2.07 mm (1.96–2.20).

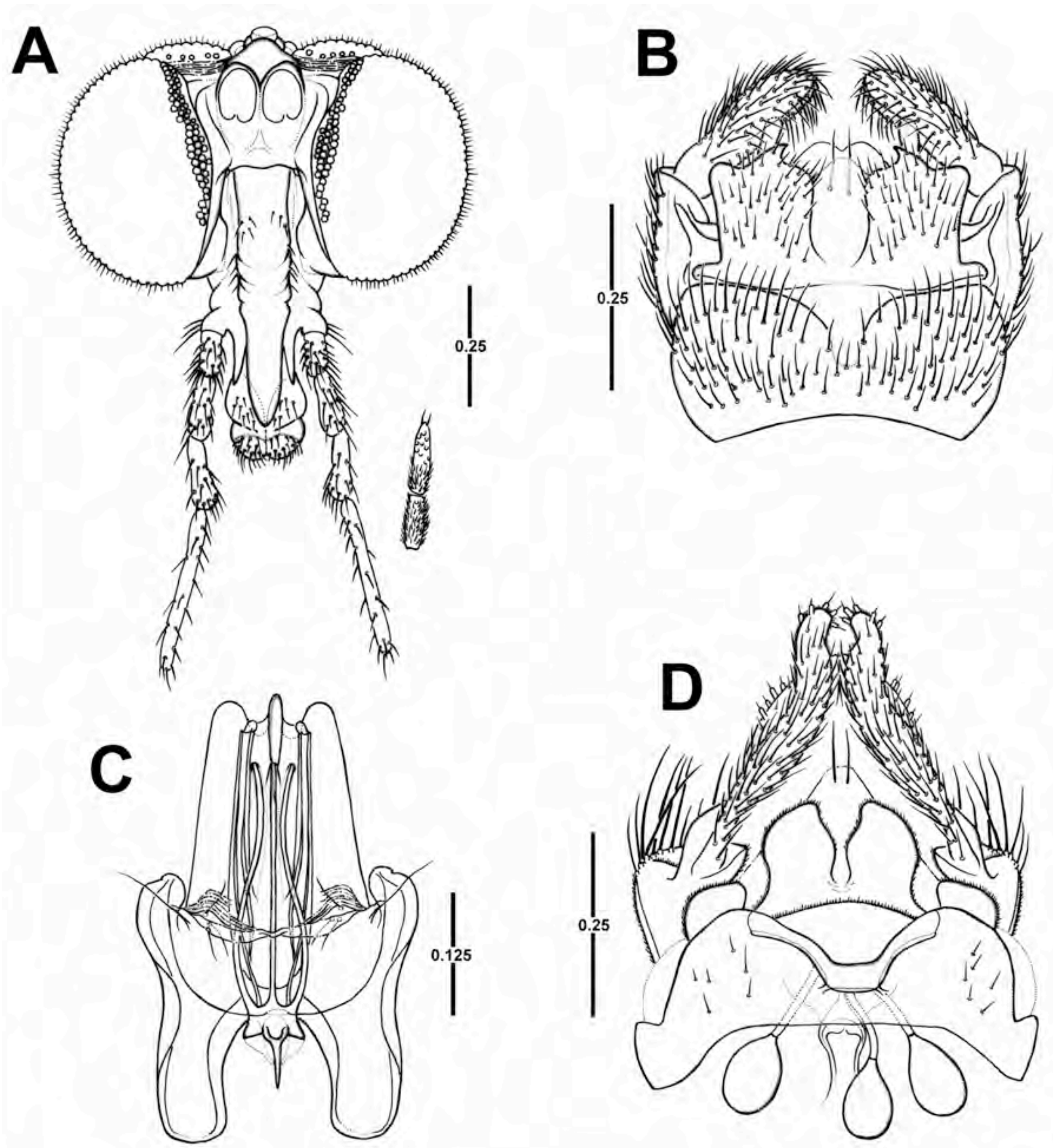

Figure 2.7. Adults of *Blepharicera crista*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female terminalia, ventral view.

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.63 (3.45–3.81)	3.46 (3.30–3.61)	4.71 (4.25–5.00)
tibia		3.02 (2.95–3.16)	2.83 (2.68–2.99)	4.22 (4.00–4.35)
tarsus	1	1.47 (1.35–1.67)	1.37 (1.28–1.44)	1.93 (1.80–2.11)
	2	0.72 (0.68–0.74)	0.69 (0.66–0.74)	0.65 (0.62–0.68)
	3	0.45 (0.41–0.48)	0.43 (0.41–0.45)	0.41 (0.41–0.43)
	4	0.29 (0.27–0.30)	0.28 (0.27–0.30)	0.28 (0.27–0.30)
	5	0.35 (0.34–0.36)	0.34 (0.34–0.36)	0.37 (0.36–0.38)

Head: Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.03 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 11 rows of ommatidia along mid-meridian. Clypeus length/width = 2.3. Proboscis about 0.45x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.3, 1.4, 3.2. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.67x length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere 1 light brown and glabrous in basal half, setose and brown apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈15), parietals (10–20), occipitals (≈40), postgenals (≈30).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.20 mm) and short (0.07 mm). Leg-segment proportions: foreleg—36:30:15:7:5:3:4, midleg—37:30:14:7:5:3:4, hindleg—38:34:15:5:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons and clypeus brown, pruinose. Thorax brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Fig. 2.7 D): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves short, inner margin strongly convex, outer margin convex, narrowing and rounded at apex, apices slightly convergent, intervalvular area broadly U-shaped. Accessory gland not wider than corpora of spermathecae, not extending to anterior margin of spermathecae, spade shaped. Spermathecae three in number; corpora pyriform, with short necks; ducts short, unpigmented. Chaetotaxy: Sternite VIII with 6–8 setiforms laterally; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.—Holotype [adult male, reared]: UNITED STATES: VIRGINIA: *Giles Co:* Little Walker Creek @ Hwy 100, 37^{o}12'N 80^{o}44'W, 1844ft, 30 April 2010, coll. A.J. Jacobson and G.R. Curler, emerged 4 May 2010. Specimen pinned, head and genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype, emerged 4 May 2010; pinned, head and genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [9 instar IV L (EtOH), 3 instar IV L (slides), 3 male and 3 female P (EtOH), 1 male and 1 female Pex (slides), 1 male and 1 female A (reared, pinned, head and genitalia slide mounted), 2 male and 2 female A (reared, pinned, head and genitalia in glycerin microvial)]. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.— UNITED STATES: VIRGINIA: *Giles Co:* Little Walker Creek @ Hwy 100, 37^{o}12'N 80^{o}44'W, 1844ft, 27 April 2009 [LP]; Walker Creek nr confl with Little Walker Creek, 37^{o}12'N 80^{o}44'W, 1796ft, 27 April 2009 [L]. All material coll. A.J. Jacobson and G.R. Curler.

Etymology.—From the Latin for "tufted" (*crista*), in reference to the arrangement of the larval dorsal secondary sensilla.

Distribution.—*Blephariccera crista* has been collected from only two locations in Giles County, Virginia. The locations are within 500 meters of each other.

Bionomics.— Collection records indicate *Blephariccera crista* is likely a univoltine, late spring species and is sympatric with at least *B. gelida*, *B. appalachiae*, and *B. separata* at some sites. Little Walker Creek and Walker Creek run through pasture bottomland before their confluence. The confluence and areas along Highway 100 have yielded the greatest numbers of *B. crista* larvae and pupae.

Taxonomic comparison.—*Blephariccera crista* larvae are unique in the arrangement of the dorsal secondary sensilla. The sensilla are arranged in six clusters on each abdominal segment. Pupae of *B. crista* are very similar to other species in the group except for the respiratory lamellae, which are not convergent, as in many *Blephariccera*, and the dorsal cuticle, which has a faintly reticulate pattern. Adult males of *B. crista* can be distinguished by features of the genitalia and small dorsal eye division. The posterior medial hook-like lobe of the quadrate cercus is longer and more prominent than in most species. Adult females of *B. crista* have slightly convergent hypogynial valves and a spade shaped accessory gland.

Blepharicera enoristera Jacobson, new species (Figs. 2.8 A–H, 2.9 A–E)

Diagnosis.—A medium-sized *Blepharicera*. <u>Larva:</u> Dorsal secondary sensilla numerous, digitiform; ecdysial line with short stem line. <u>Pupa:</u> Body outline roughly ellipsoid; papillae dark brown with minute spinules; cuticle with micropunctures; anal tergite wrinkled; distance between lamellae at base ≈2x basal width of individual lamella. <u>Adult male:</u> Dorsal eye division smaller (0.33x) than ventral; cerci quadrate; posterior margin trilobate, lateral and medial margins convex; dorsal paramere subquadrate at apex; ejaculatory apodeme extended approximately one-half distance to anterior margin of lateral parameral lobes. <u>Adult female:</u> Eyes divided; callis oculi present; hypogynial valves short, rounded apicolaterally, pointed apicomesally, inner margin parallel and outer margin slightly convex, apices straight, not convergent or divergent; intervalvular area narrowly V-shaped; three pyriform spermathecae.

Description.—<u>Larva</u> (Fig. 2.8 A–D): Measurements, instar II (N = 4) total length 2.25 mm (1.92–2.53), cranial width 0.27 mm (0.26–0.28), antennal segments 0.11 mm, 0.03 mm, membrane 0.03 mm; instar III (N = 10) total length 4.38 mm (3.75–5.00), cranial width 0.44 mm (0.42–0.47), antennal segments 0.14 mm (0.12–0.16), 0.07 mm (0.07–0.08), membrane 0.05 mm (0.04–0.07); instar IV (N = 10) total length 6.80 mm (6.29–7.39), cranial width 0.60 mm (0.57–0.62), antennal segments 0.19 mm (0.16–0.22), 0.14 mm (0.12–0.16), membrane 0.07 mm (0.05–0.09). Cranial sclerites dark brown; ecdysial line with short stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division, abdomen, and lateral lobes uniformly brown. Anal division rounded, lateral processes bluntly rounded. Chaetotaxy: Cranial sclerites densely covered in digitiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, pale, ≈15 in number; dorsal secondary sensilla numerous, digitiform; anal division with 4–6 prominent setiforms marginally.

Pupa (Figs. 2.8 E–H): Measurements, male (N = 10) length 5.08 mm (4.93–5.41), width 2.70 mm (2.57–2.84); female (N = 7) length 5.75 mm (5.68–5.95), width 3.13 mm (3.04–3.24). Body outline roughly ellipsoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae dark brown with minute spinules. Cuticle between papillae brown with micropunctures. Branchial sclerite without papillae. Anal tergite wrinkled. Respiratory lamellae wider at base and broadly pointed apically; outer margins curving medially, inner margins slightly curving medially; parallel in basal half, convergent apically; apices separated medially. Distance between lamellae at base ≈2x basal width of individual lamella. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 7): Total length 5.09 mm (4.75–5.54), wing length 5.76 mm (5.41–6.08), width 1.77 mm (1.62–1.90).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.68 (3.52–3.90)	3.84 (3.69–4.01)	5.01 (4.80–5.39)
tibia		3.31 (3.10–3.52)	3.19 (3.00–3.28)	4.43 (4.25–4.60)
tarsus	1	1.79 (1.72–1.90)	1.73 (1.63–1.90)	1.80 (1.73–1.85)
	2	0.93 (0.88–1.01)	0.92 (0.88–0.95)	0.67 (0.58–0.74)
	3	0.64 (0.62–0.69)	0.65 (0.63–0.68)	0.41 (0.38–0.46)
	4	0.34 (0.30–0.36)	0.35 (0.30–0.38)	0.28 (0.27–0.31)
	5	0.32 (0.27–0.36)	0.31 (0.27–0.34)	0.28 (0.27–0.30)

Head (Fig. 2.9 A): Structure: Eyes semidichoptic, interocular ridge present, interocular distance 0.05 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (0.33x) than ventral; dorsal ommatidia larger in diameter; dorsal division with 13 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.37x head width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.3, 1.4, 4.5. Antennal flagellomeres cylindrical; ultimate flagellomere 1.2x length of penultimate flagellomere; scape and pedicel brown with prominent setiforms; flagellomere 1 light brown and glabrous in basal half, setose and

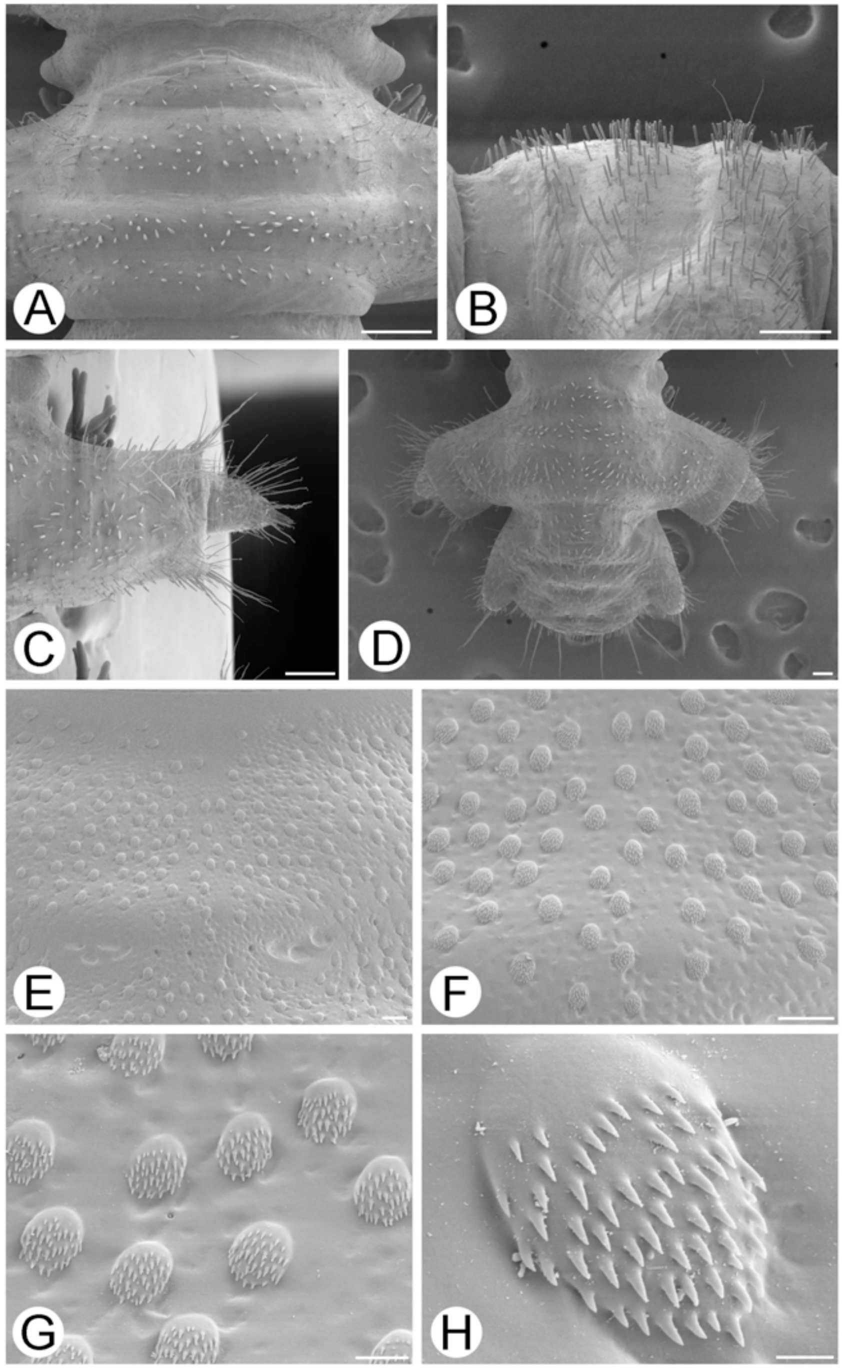

Figure 2.8. Scanning electron micrographs of larval and pupal *Blepharicera enoristera*. **A.** larval dorsal sensilla, dorsal view. **B.** larval dorsal sensilla, lateral view. **C.** proleg on abdominal segment III, dorsal view on right side. **D.** anal division, abdominal segments VI–X, dorsal view. **E–G.** pupal abdominal tergite microsculpture. **H.** pupal abdominal papilla. Scale bars = 3 μm (H), 10 μm (G), 30 μm (E–F), 100 μm (D), 200 μm (A–C).

brown apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈5), parietals (0–2), occipitals (≈30), postgenals (15–25).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.15 mm (0.14–0.17). Leg-segment proportions: foreleg—33:30:16:9:6:3:3, midleg—35:29:16:8:6:3:3, hindleg—39:35:14:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons, clypeus, and face brown, pruinose. Thorax brown, pruinose. Forecoxae brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Figs. 2.9 B–C): Abdominal segment VIII greatly reduced. Epandrium bilobate, emarginated posteromedially; ≈45 prominent setae per side. Cerci quadrate; posterior margin trilobate with median lobe prominent, hook-like; lateral lobes small, less than half length of median lobe, subequal in size. Cerci with lateral and medial margins convex; ≈40 prominent setae per side; intercercal area narrowly U-shaped. Genital capsule slightly wider than long. Gonostylus and gonocoxite setose. Aedeagal rods equal in length, with slight thickening of membranous sheath apically; medial rod straight, lateral rods sinuous. Ventral parameres longer than aedeagal rods, broad basally, tapered to complex apex with expanded inner wall. Dorsal paramere subquadrate at apex. Dorsal carina prominent, extending slightly beyond ventral parameres. Gonocoxal apodeme and lateral parameral lobes well developed. Ejaculatory apodeme short, extended approximately one-half distance to anterior margin of lateral parameral lobes.

<u>Adult female:</u> Size: medium. Measurements (N = 2): Total length 6.76 mm, wing length 6.67 mm (6.25–7.09), width 2.09 mm (1.90–2.28).

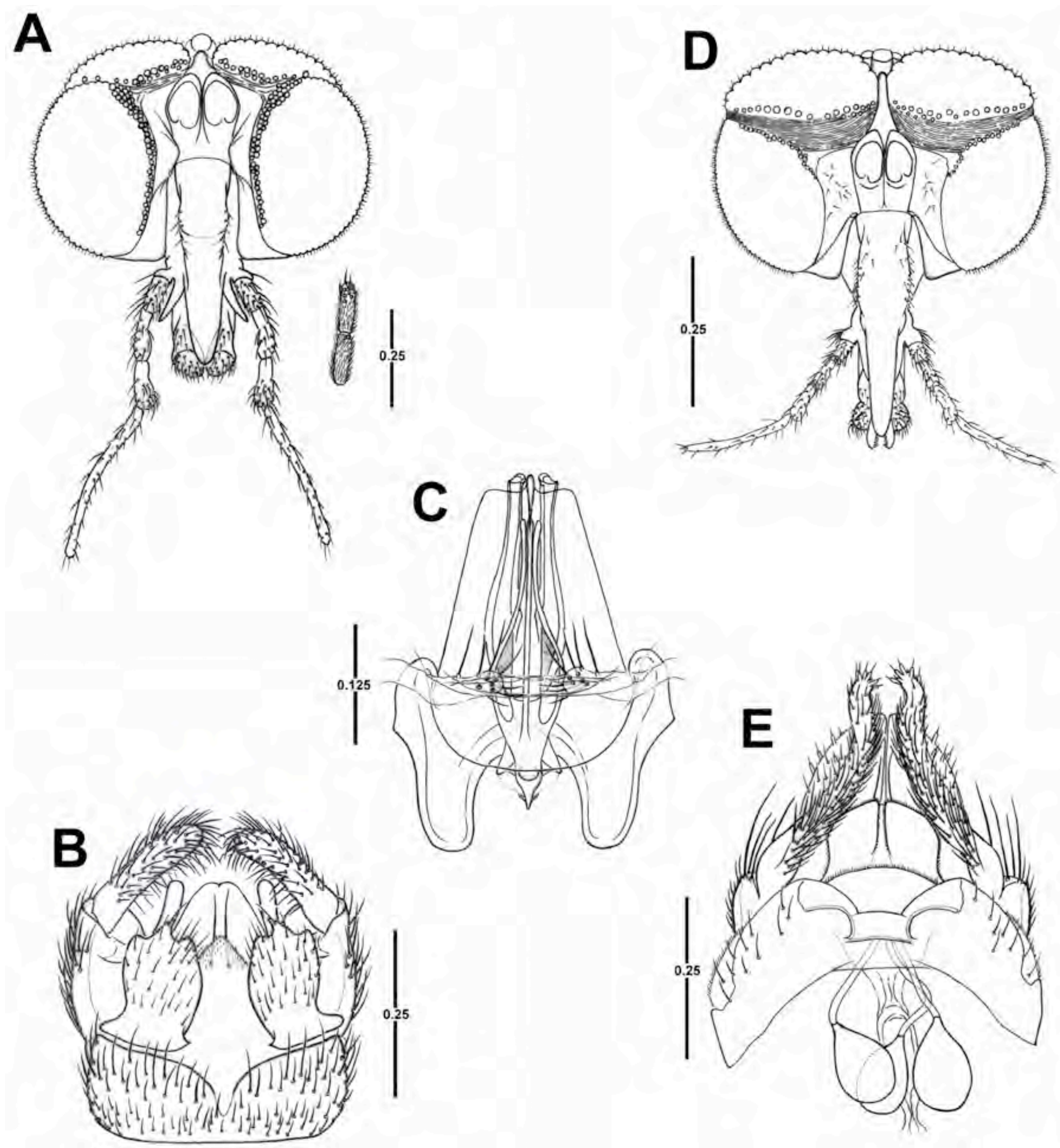

Figure 2.9. Adults of *Blephariceras enoristera*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female head, frontal view. **E.** female terminalia, ventral view.

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.95 (3.81–4.10)	3.86 (3.69–4.02)	5.61
tibia		3.37 (3.30–3.44)	3.33 (3.16–3.49)	5.10
tarsus	1	1.46 (1.40–1.52)	1.46 (1.45–1.47)	2.15
	2	0.81 (0.76–0.85)	0.78 (0.74–0.81)	0.77
	3	0.46 (0.45–0.47)	0.47	0.43
	4	0.34 (0.32–0.36)	0.35 (0.34–0.35)	0.34
	5	0.42 (0.41–0.43)	0.41	0.43

Head (Fig. 2.9 D): Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.04 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 12 rows of ommatidia along mid-meridian. Clypeus length/width = 2.3. Proboscis about 0.52x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.2, 1.4, 3.7. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.53x length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere 1 light brown and glabrous in basal half, setose and brown apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈15), parietals (≈10), occipitals (≈30), postgenals (15–20).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.16 mm) and short (0.08 mm). Leg-segment proportions: foreleg—37:31:14:7:4:3:4, midleg—36:31:14:7:5:3:4, hindleg—38:34:15:5:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons and clypeus brown, pruinose. Thorax brown, pruinose. Forecoxae brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Fig. 2.9 E): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves short, rounded apicolaterally, pointed apicomesally, inner margin parallel and outer margin slightly convex, apices straight, not convergent or divergent, intervalvular area narrowly V-shaped. Accessory gland narrow, elliptical. Spermathecae three in number; corpora pyriform, with short necks; ducts short, unpigmented. Chaetotaxy: Sternite VIII with 5–15 setiforms laterally and 15 setiforms medially; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.—Holotype [adult male, reared]: UNITED STATES: GEORGIA: *Murray Co*: Jacks River @ Cottonwood Patch campsites, 34°59’N 84°38’W, 26 March 2009, coll. A.J. Jacobson and G.R. Curler, emerged 5 April 2009. Specimen pinned, genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype, emerged 7 April 2009; pinned, genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [4 instar IV L (EtOH), 3 male and 4 female P (EtOH), 1 male and 1 female A (reared, pinned, head and genitalia slide mounted), 2 male A (reared, pinned, genitalia in glycerin microvial)]; 13 March 2007 [5 instar IV L (EtOH), 2 instar IV L (slides)], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.—UNITED STATES: TENNESSEE: *Polk Co:* Conasauga River @ TN/GA State Line, 34°59’N 84°38’W, 26 March 2009 [LP], coll. A.J. Jacobson and G.R. Curler.

Etymology.—From the Greek for “earlier in the season” (*enoristera)* in reference to the emergence period of the adults. This is in comparison to the emergence dates of *Blepharicera opistera*, another new species from Jacks River.

Distribution.—*Blepharicera enoristera* has been collected from the Jacks and Conasauga Rivers where they flow through the Cherokee National Forest of southeastern Tennessee and the Cohutta Wilderness of north central Georgia.

Bionomics.— *Blepharicera enoristera* is found in medium to large streams during the spring and is sympatric with *B. appalachiae* and *B. opistera*. Emergence of adults from the 26 March 2009 collection date yielded only males and females of *B. enoristera*. This suggests an approximately four-week divergence in emergence times for *B. enoristera* in comparison to the emergence times of *B. appalachiae* and *B. opistera* which were present in a collection made by J.K. Moulton on 29 April 2007.

Taxonomic comparison.—*Blepharicera enoristera* larvae have a short ecdysial stem line and are much darker in coloration than *B. appalachiae* and *B. opistera*. Pupae of *B. enoristera* are roughly ellipsoid in shape and can be differentiated from sympatric species by overall shape, background dorsal microsculpture, and the arrangement of the dorsal papillae. Adult males of *B. enoristera* are distinguished by features of the genitalia and the size of the upper eye division. The upper eye division in males of *B. enoristera* is larger than that of both *B. appalachiae* and *B. opistera*. The lateral and medial margins of the cerci are convex. The lateral margins of *B. appalachiae* are concave and divergent and the lateral and medial margins of *B. opistera* are subparallel. Adult females of *B. enoristera* have hypogynial valves that are rounded apicolaterally and pointed apicomesally. The hypogynial valves of *B. appalachiae* and *B. opistera* are rounded apically.

Blepharicera hillabee Jacobson, new species (Figs. 2.10 A–F, 2.11 A–D)

Diagnosis.—A medium-sized *Blepharicera*. Larva: Dorsal secondary sensilla numerous, fustiform; anal division broadly rounded, lateral processes bluntly rounded apically. Pupa: Body outline ovoid; papillae brown with minute spinules on posterior half; cuticle glabrous; anal tergite wrinkled. Adult male: Dorsal eye division smaller (0.25x) than ventral; cerci strongly quadrate, parallel, posterior margin with median hook-like lobe; dorsal paramere with posterior margin deeply incised; ejaculatory apodeme short, extended approximately one-half distance to anterior margin of lateral parameral lobes. Adult female: Eyes divided; callis oculi present; hypogynial valves short, inner margin narrowly separated, apices rounded; intervalvular area not deeply cleft, narrowly V-shaped; three pyriform spermathecae.

Description.—Larva (Figs. 2.10 A–C): Measurements, instar III (N = 10) total length 4.14 mm (3.65–5.25), cranial width 0.47 mm (0.45–0.49), antennal segments 0.14 mm (0.13–0.15), 0.06 mm (0.05–0.07), membrane 0.03 mm (0.02–0.04); instar IV (N = 10) total length 6.25 mm (5.27–7.03), cranial width 0.63 mm (0.57–0.66), antennal segments 0.19 mm (0.18–0.20), 0.09 mm (0.07–0.11), membrane 0.06 mm (0.03–0.07). Cranial sclerites light brown; ecdysial line with stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division, abdomen, and lateral lobes uniformly light brown. Anal division broadly rounded, lateral processes bluntly rounded apically. Chaetotaxy: Cranial sclerites densely covered in fustiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, light brown, ≈30 in number; dorsal secondary sensilla numerous, fustiform; setiforms mostly absent medially, numerous on lateral lobes; anal division with 6–7 prominent setiforms marginally.

Pupa (Figs. 2.10 D–F): Measurements, male (N = 10) length 5.14 mm (4.70–5.40), width 3.07 mm (3.00–3.16); female (N = 10) length 5.83 mm (5.68–5.95), width 3.25 mm (3.06–3.40). Body outline ovoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae brown with minute spinules on posterior half. Cuticle between papillae glabrous and brown. Branchial sclerite without papillae. Anal tergite wrinkled. Respiratory lamellae wider at base and broadly pointed apically; outer margins curving medially, inner margins straight; apices separated medially. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 5): Total length 5.43 mm (5.20–5.61), wing length 6.16 mm (6.08–6.35), width 1.92 mm (1.75–2.03).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.67 (3.50–3.90)	4.08 (4.00–4.15)	5.35 (5.27–5.41)
tibia		3.44 (3.25–3.69)	3.40 (3.30–3.56)	4.81 (4.40–5.00)
tarsus	1	1.83 (1.71–1.96)	1.86 (1.75–1.94)	2.00 (1.89–2.10)
	2	0.95 (0.92–0.99)	0.93 (0.87–0.98)	0.70 (0.66–0.74)
	3	0.68	0.70 (0.68–0.71)	0.47 (0.42–0.51)
	4	0.34 (0.34–0.35)	0.37 (0.34–0.41)	0.32 (0.30–0.34)
	5	0.32 (0.31–0.34)	0.33 (0.32–0.34)	0.30 (0.28–0.30)

Head (Fig. 2.11 A): Structure: Eyes semidichoptic, interocular distance 0.13 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (0.25x) than ventral; dorsal ommatidia larger in diameter; dorsal division with 10 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.37x head width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.1,

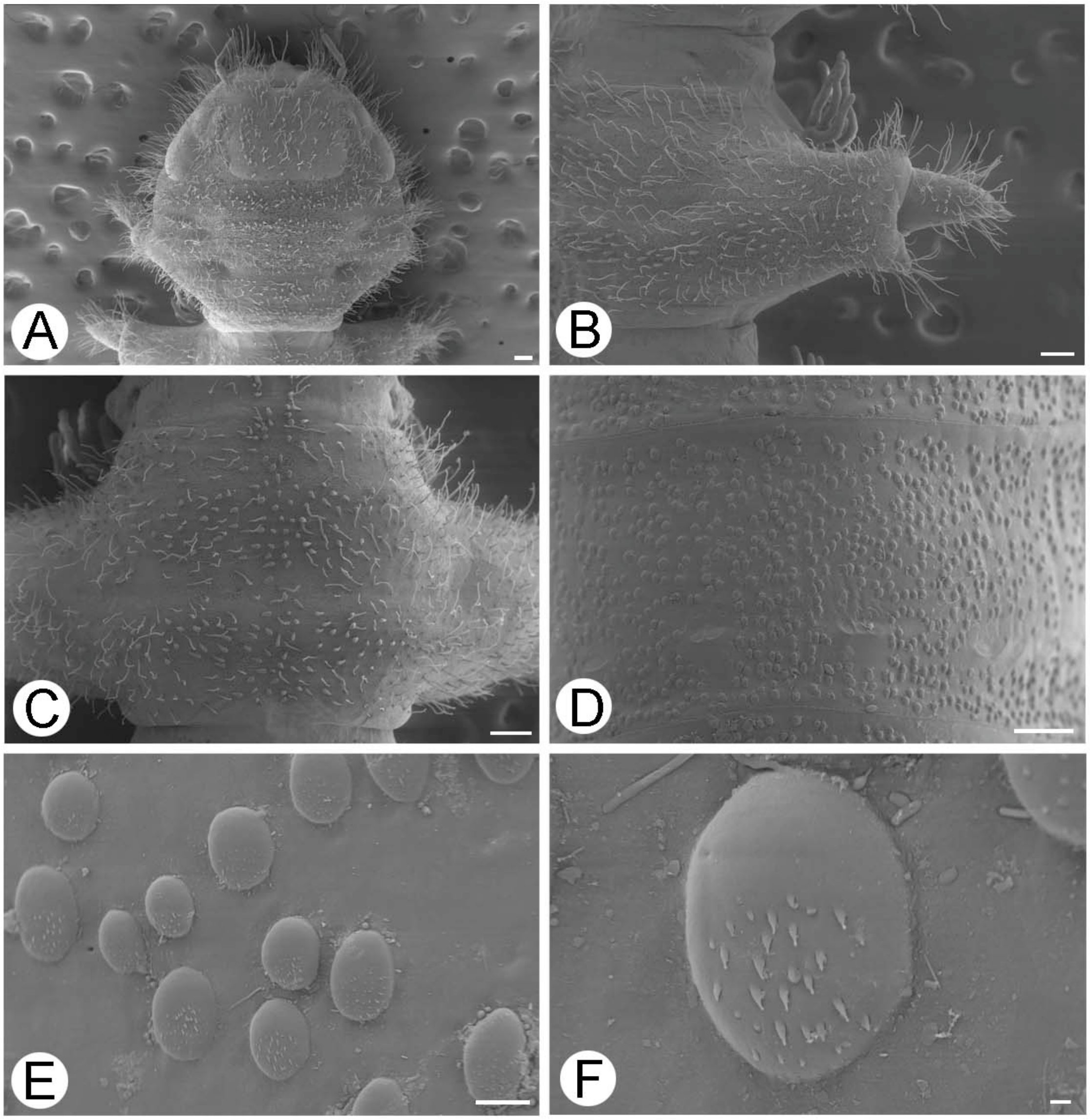

Figure 2.10. Scanning electron micrographs of larval and pupal *Blephariceга hillabee*. **A.** cephalic division, dorsal view. **B.** proleg on abdominal segment III, dorsal view on right side. **C.** larval dorsal sensilla. **D–E.** pupal abdominal tergite microsculpture. **F.** pupal abdominal papilla. Scale bars = 1 µm (F), 10 µm (E), 100 µm (A–D).

1.3, 3.5. Antennal flagellomeres cylindrical; ultimate flagellomere 1.5x length of penultimate flagellomere; scape pale and pedicel brown, both with prominent setiforms; flagellomere 1 pale and glabrous in basal half but setose and brown apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈12), parietals (0), occipitals (>30), postgenals (15–20).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.17 mm (0.16–0.18). Leg-segment proportions: foreleg—33:31:16:8:6:3:3, midleg—35:29:16:8:6:3:3, hindleg—39:35:14:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons, clypeus, and face light brown, pruinose. Thorax light brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites light brown, sternites pale.

Terminalia (Figs. 2.11 B–C): Abdominal segment VIII greatly reduced. Epandrium simple, slightly emarginate posteromedially; >50 prominent setae per side. Cerci strongly quadrate, parallel, posterior margin with median hook-like lobe, densely set with approximately 40 prominent setae; intercercal area consisting of a U-shaped notch. Genital capsule slightly wider than long. Gonostylus and gonocoxite setose. Aedeagal rods equal in length; medial rod straight, lateral rods sinuous. Ventral parameres longer than aedeagal rods, broad basally, tapered to complex apex with expanded inner wall. Dorsal paramere with posterior margin deeply incised, trilobed with median lobe extending slightly beyond lateral lobes. Dorsal carina prominent. Gonocoxal apodeme and lateral parameral lobes well developed. Ejaculatory apodeme short, extended approximately one-half distance to anterior margin of lateral parameral lobes.

<u>Adult female:</u> Size: medium. Measurements (N = 6): Total length 5.84 mm (5.61–6.08), wing length 6.73 mm (6.22–7.49), width 2.07 mm (1.90–2.40).

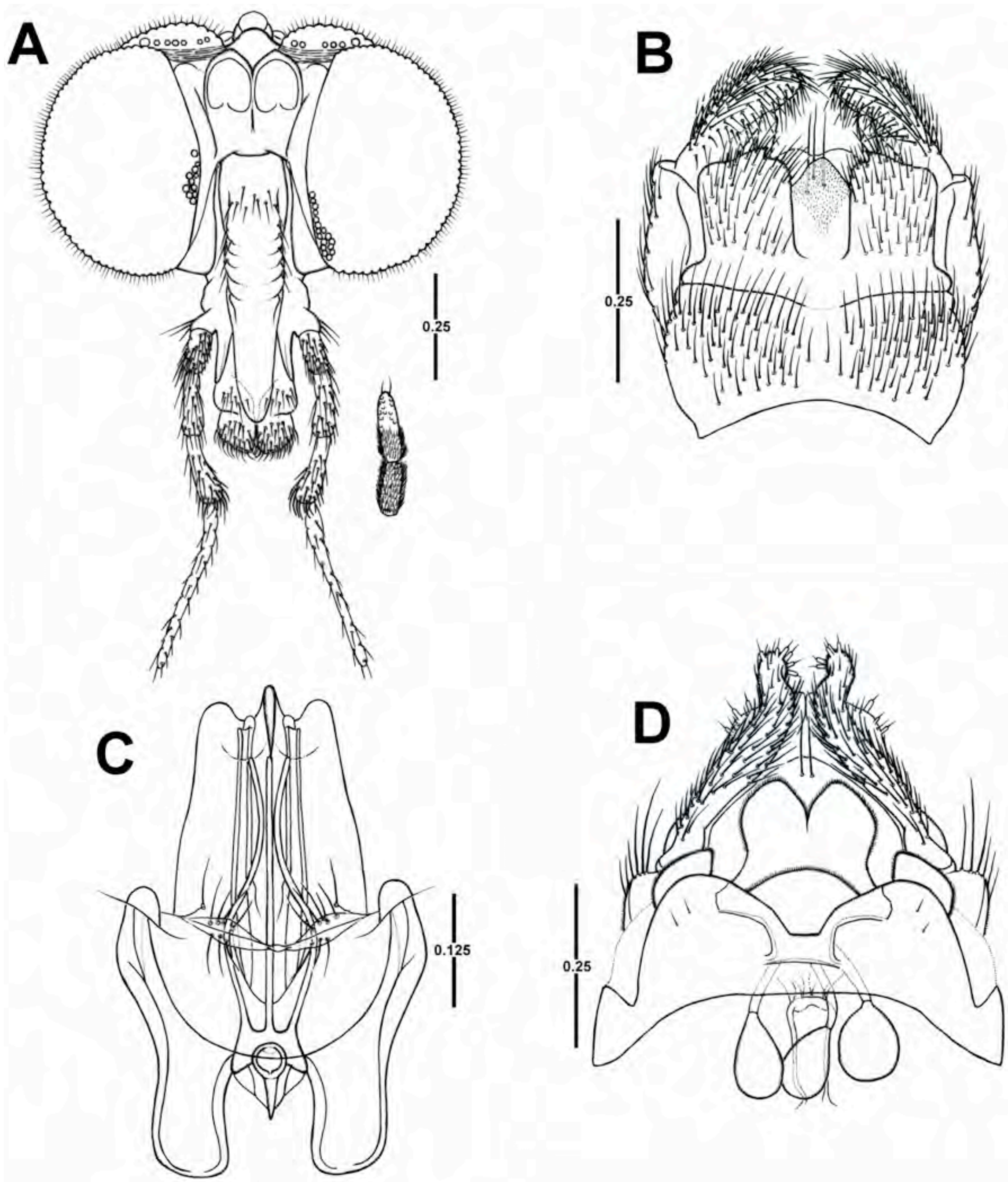

Figure 2.11. Adults of *Blepharicera hillabee*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female terminalia, ventral view.

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.88 (3.60–4.15)	3.97 (3.81–4.15)	5.56 (5.41–5.81)
tibia		3.30 (3.00–3.60)	3.39 (3.16–3.64)	5.02 (4.75–5.40)
tarsus	1	1.51 (1.40–1.63)	1.58 (1.50–1.64)	2.02 (1.90–2.28)
	2	0.79 (0.74–0.91)	0.81 (0.76–0.88)	0.79 (0.70–0.89)
	3	0.51 (0.44–0.61)	0.50 (0.45–0.57)	0.49 (0.45–0.56)
	4	0.32 (0.30–0.36)	0.32 (0.30–0.34)	0.34
	5	0.41 (0.37–0.47)	0.41 (0.37–0.45)	0.43 (0.41–0.48)

Head: Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.05 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 11 rows of ommatidia along mid-meridian. Clypeus length/width = 2.7. Proboscis about 0.47x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.1, 1.3, 2.8. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.97x length of penultimate flagellomere; scape and pedicel light brown with prominent setiforms; flagellomere 1 light brown in basal half and setose and brown apically, remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈30), parietals (12–18), occipitals (≈35), postgenals (≈20).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.20 mm) and short (0.06 mm). Leg-segment proportions: foreleg—36:31:14:7:5:3:4, midleg—36:31:14:7:5:3:4, hindleg—38:34:14:6:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈25); coxae with prominent setae.

Coloration: Frons and clypeus light brown, pruinose. Thorax light brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites light brown, sternites pale.

Terminalia (Fig. 2.11 D): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves; individual valves short, inner margin narrowly separated, apices rounded; intervalvular area not deeply cleft, narrowly V-shaped. Accessory gland not wider than corpora of spermathecae, not extending beyond anterior margin of spermathecae. Spermathecae three in number; corpora pyriform, with short necks; ducts short, unpigmented. Chaetotaxy: Sternite VIII with 2–4 setiforms laterally; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.—Holotype [adult male, reared]: UNITED STATES: ALABAMA: *Tallapoosa Co*: Hillabee Creek @ Rt 22 nr Alexander City, 32°59'N 85°51'W, 31 March 2007, coll. G.W. Courtney, emerged 5 April 2007. Specimen pinned, genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype, emerged 6–7 April 2007; pinned, genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [2 instar IV L (slides), 1 male and 1 female Pex (slides), 2 male and 2 female A (reared, pinned, head and genitalia slide mounted), 2 male and 2 female A (reared, pinned, genitalia in glycerin microvial)]; 25 April 2007 [9 instar IV L (EtOH), 3 male and 3 female P (EtOH)], coll. A.J. Jacobson and G.R. Curler. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.— same locality as type material: 23 March 2006 [LP], coll. A.J. Jacobson and G.R. Curler; 31 March 2007 [LPPexA], coll. G.W. Courtney; 25 April 2007 [LPPex], coll. A.J. Jacobson and G.R. Curler.

Etymology.—The species is named in reference to the type locality, Hillabee Creek. Hillabee is adapted from the Muskogee (Creek) word "helvpe," a tribal town.

Distribution.—*Blepharicera hillabee* is currently known from one location in Alabama. It was first discovered in 1989 in Hillabee Creek, a fourth-order stream, near Alexander City in east central Alabama. Subsequent collections have resulted in additional material and reared adults.

Bionomics.— Collection records indicate *Blepharicera hillabee* is likely a univoltine, spring species and is sympatric with *B. capitata* and *B. separata*. The collection location has been impacted significantly over the past several years. While populations of *Blepharicera* flies appear to be stable, that may not continue to be the case. This area, much like the type locality of *B. chattooga*, deserves special attention in order to preserve the habitat.

Taxonomic comparison.—*Blepharicera hillabee* larvae are most similar to *B. courtneyi*, however, *B. hillabee* larvae have a larger number of setiforms on the lateral prolegs. Pupae of *B. hillabee* are very similar to other species in the group. Adult males and females are larger than other sympatric species. Adult males of *B. hillabee* can be distinguished by the small dorsal eye division and characters of the genitalia. The cerci are strongly quadrate with only a hook-like medial lobe on the posterior margin. The dorsal paramere is deeply incised as in *B. tenuipes*. Adult females of *B. hillabee* have hypogynial valves that are separated by a short cleft, giving the appearance of a single valve basally. The hypogynial valves of *B. separata* are divergent along the entire length. The accessory gland of *B. separata* is ovoid while the gland in *B. hillabee* is narrow, ≈3x longer than wide.

Blepharicera opistera Jacobson, new species (Figs. 2.12 A–H, 2.13 A–E)

Diagnosis.—A medium-sized *Blepharicera*. Larva: Cranial sclerites patterned; cephalic division and abdomen with darkly pigmented medial band, margins of which extend onto lateral lobes; lateral lobes dark medially with pale crescent band at apex; dorsal secondary sensilla digitiform; ecdysial line with short stem line. Pupa: Body outline ovoid; papillae dark brown with minute spinules; cuticle glabrous; anal tergite wrinkled; distance between lamellae at base >1.5x basal width of individual lamella. Adult male: Dorsal eye division smaller (0.25x) than ventral; cerci quadrate; posterior margin trilobate; lateral and medial margins sub parallel; dorsal paramere subquadrate at apex; ejaculatory apodeme subequal in length to lateral parameral lobes. Adult female: Eyes divided; callis oculi present; hypogynial valves short, narrowing and rounded at apex, inner margin sinuous, outer margin convex, apices slightly divergent; intervalvular area narrowly U-shaped; three spherical spermathecae.

Description.—Larva (Figs. 2.12 A–E): Measurements, instar II (N = 2) total length 1.59 mm (1.49–1.69), cranial width 0.27 mm, antennal segments 0.13 mm (0.12–0.14), 0.03 mm, membrane 0.04 mm; instar III (N = 8) total length 3.81 mm (2.67–4.84), cranial width 0.43 mm (0.41–0.46), antennal segments 0.14 mm (0.12–0.16), 0.07 mm (0.05–0.07), membrane 0.05 mm (0.04–0.05); instar IV (N = 10) total length 6.35 mm (5.75–6.96), cranial width 0.58 mm (0.54–0.65), antennal segments 0.22 mm (0.20–0.24), 0.12 mm (0.11–0.12), membrane 0.07 mm (0.04–0.09). Cranial sclerites patterned, frontoclypeal apotome yellow, genae yellow with brown accents; ecdysial line with short stem line, posterior margin of frontoclypeal apotome not extended to posterior cranial margin. Cephalic division and abdomen with darkly pigmented medial band, margins of which extend onto lateral lobes; lateral lobes dark medially with pale crescent band at apex. Anal division rounded, lateral processes bluntly rounded. Chaetotaxy: Cranial sclerites densely covered in digitiform sensilla; numerous setiforms along frontal margin of cephalic division; substernal sensilla digitiform, pale, ≈25 in number; dorsal secondary sensilla numerous, digitiform; anal division with 4–6 prominent setiforms marginally.

Pupa (Figs. 2.12 F–H): Measurements, male (N = 10) length 4.62 mm (4.46–4.80), width 2.62 mm (2.57–2.70); female (N = 10) length 5.22 mm (5.07–5.41), width 2.95 mm (2.84–3.18). Body outline ovoid. Integument: Dorsal papillae uniformly distributed on abdominal segments; metatergite with papillae present medially, absent on lateral surface beyond abdominal segment I. Papillae dark brown with minute spinules. Cuticle between papillae glabrous and brown. Branchial sclerite without papillae. Anal tergite wrinkled. Respiratory lamellae wider at base and broadly pointed apically; outer margins curving medially, inner margins slightly curving medially; parallel in basal half, convergent apically; apices separated medially. Distance between lamellae at base >1.5x basal width of individual lamella. Middle lamellae less sclerotized; broad, width at midpoint greater than half width of outer lamellae.

Adult male: Size: medium. Measurements (N = 8): Total length 4.33 mm (4.06–4.70), wing length 5.06 mm (4.80–5.41), width 1.66 mm (1.57–1.77).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.02 (2.95–3.09)	3.17 (3.06–3.33)	4.16 (4.05–4.30)
tibia		2.86 (2.76–3.00)	2.66 (2.56–2.85)	3.89 (3.65–4.00)
tarsus	1	1.47 (1.43–1.55)	1.38 (1.33–1.50)	1.50 (1.35–1.60)
	2	0.80 (0.78–0.82)	0.76 (0.74–0.81)	0.52 (0.47–0.54)
	3	0.54	0.53 (0.48–0.54)	0.33 (0.31–0.36)
	4	0.29 (0.27–0.31)	0.29 (0.27–0.31)	0.21 (0.20–0.22)
	5	0.28 (0.27–0.30)	0.27 (0.26–0.28)	0.27

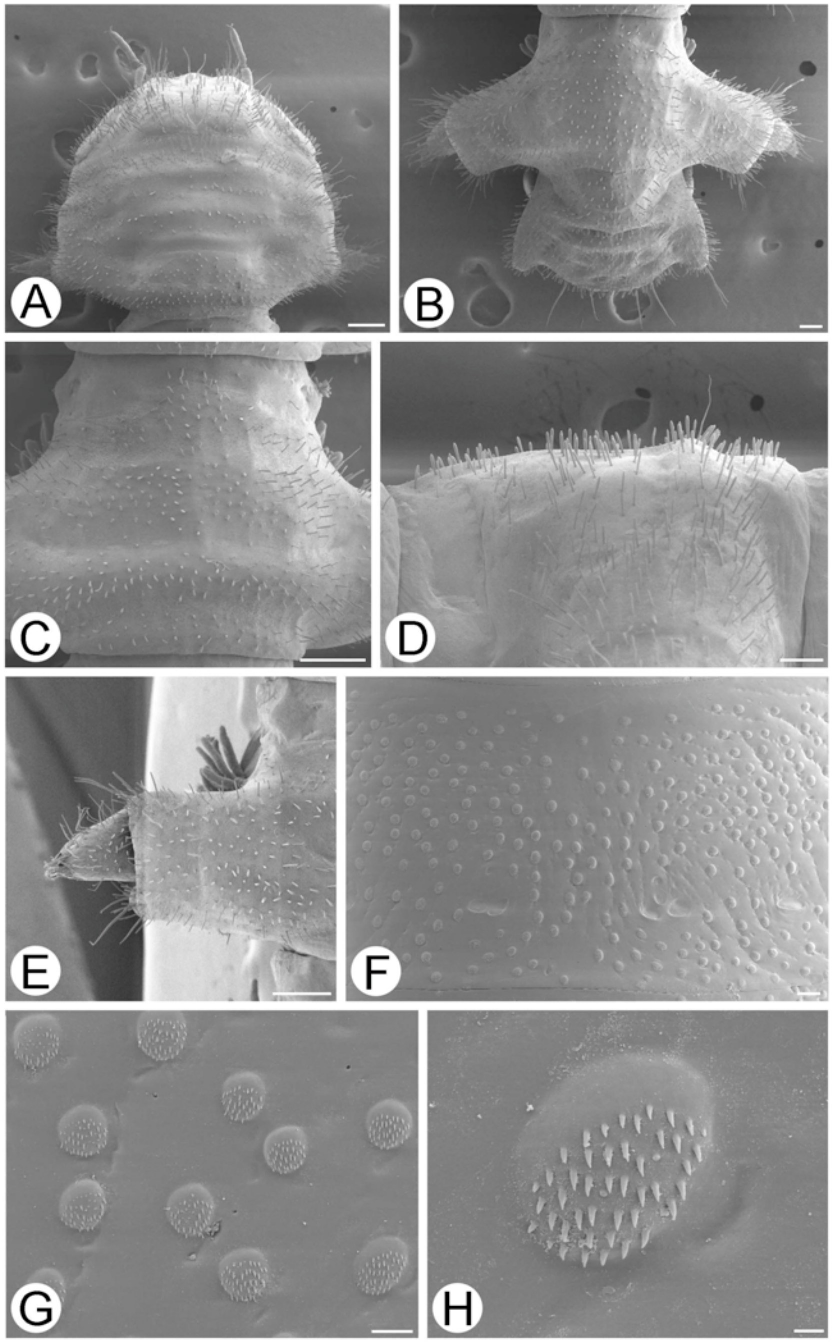

Figure 2.12. Scanning electron micrographs of larval and pupal *Blephariceras opistera*. **A.** cephalic division, dorsal view. **B.** anal division, abdominal segments VI–X, dorsal view. **C.** larval dorsal sensilla, dorsal view. **D.** larval dorsal sensilla, lateral view. **E.** proleg on abdominal segment III, dorsal view on left side. **F–G.** pupal abdominal tergite microsculpture. **H.** pupal abdominal papilla. Scale bars = 2 μm (H), 10 μm (G), 30 μm (F), 100 μm (B, D), 200 μm (A, C, E).

Head (Fig. 2.13 A): Structure: Eyes semidichoptic, interocular ridge present, interocular distance 0.06 mm; eye divided; callis oculi absent; dorsal division contiguous with ventral, smaller (0.25x) than ventral; dorsal ommatidia larger in diameter; dorsal division with 11 rows of ommatidia along mid-meridian. Clypeus length/width = 2.2. Proboscis about 0.36x head width; mandibles absent; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.2, 1.2, 3.9. Antennal flagellomeres cylindrical; ultimate flagellomere 1.1x length of penultimate flagellomere; scape and pedicel brown with prominent setiforms; flagellomere 1 brown and glabrous in basal half, setose apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈10), parietals (0–3), occipitals (≈30), postgenals (≈15).

Thorax and Appendages: Structure: Tibial spurs 0–0–1, length 0.12 mm (0.09–0.14). Leg-segment proportions: foreleg—32:31:16:9:6:3:3, midleg—35:29:15:9:6:3:3, hindleg—38:36:14:5:3:2:2. Chaetotaxy: Thorax glabrous except for few sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons, clypeus, and face light brown, pruinose. Thorax light brown, pruinose. Forecoxae light brown, other coxae pale. Abdominal tergites light brown, sternites pale.

Terminalia (Figs. 2.13 B–C): Abdominal segment VIII greatly reduced. Epandrium bilobate, emarginated posteromedially; ≈45 prominent setae per side. Cerci quadrate; posterior margin trilobate with median lobe only slightly longer and more acute than lateral lobes. Cerci with lateral and medial margins subparallel, ≈30 prominent setae per side, intercercal area U-shaped. Genital capsule slightly longer than wide. Gonostylus and gonocoxite setose. Aedeagal rods equal in length; medial rod straight, lateral rods sinuous. Ventral parameres longer than aedeagal rods, broad basally, tapered to complex apex with expanded inner wall. Dorsal paramere subquadrate at apex. Dorsal carina prominent, extending slightly beyond ventral parameres. Gonocoxal apodeme and lateral parameral lobes well developed. Lateral parameral lobes short, broad and convergent. Ejaculatory apodeme subequal in length to lateral parameral lobes.

Adult female: Size: medium. Measurements (N = 8): Total length 5.79 mm (5.27–6.08), wing length 5.99 mm (5.75–6.20), width 1.95 mm (1.80–2.10).

Leg-segment lengths:		foreleg	midleg	hindleg
femur		3.56 (3.36–3.70)	3.50 (3.30–3.69)	4.76 (4.35–5.00)
tibia		3.01 (2.87–3.10)	2.88 (2.75–3.05)	4.35 (4.15–4.55)
tarsus	1	1.33 (1.24–1.45)	1.38 (1.30–1.46)	1.76 (1.68–1.89)
	2	0.73 (0.71–0.76)	0.72 (0.70–0.74)	0.66 (0.61–0.71)
	3	0.43 (0.41–0.48)	0.46 (0.43–0.50)	0.40 (0.36–0.41)
	4	0.31 (0.30–0.32)	0.30 (0.27–0.33)	0.29 (0.27–0.31)
	5	0.36 (0.34–0.38)	0.35 (0.33–0.37)	0.36 (0.33–0.38)

Head (Fig. 2.13 D): Structure: Eyes subholoptic, interocular ridge present, interocular distance 0.03 mm; eye divided; callis oculi present; dorsal division separated from ventral, subequal in size; dorsal division with 12 rows of ommatidia along mid-meridian. Clypeus length/width = 2.4. Proboscis about 0.45x head width; palpi with 5 palpomeres, distal 4 segment proportions 1.0, 1.3, 1.3, 3.6. Antennal flagellomeres cylindrical in shape; ultimate flagellomere 1.67x length of penultimate flagellomere; scape light brown and pedicel brown with prominent setiforms; flagellomere 1 light brown and glabrous in basal half, setose and brown apically; remaining flagellomeres setose and brown. Chaetotaxy: Setiform groups as follows (number per side): clypeals (≈10), parietals (3), occipitals (≈35), postgenals (≈15).

Thorax and appendages: Tibial spurs 0–0–2; spurs asymmetrical, one each long (0.17 mm) and short (0.07 mm). Leg-segment proportions: foreleg—37:31:14:7:4:3:4, midleg—36:30:14:8:5:3:4, hindleg—38:35:14:5:3:2:3. Chaetotaxy: Thorax glabrous except for sparse setae; scutellum with numerous setae grouped at posterolateral corner (N≈15); coxae with prominent setae.

Coloration: Frons and clypeus brown, pruinose. Thorax brown, pruinose. Forecoxae brown, other coxae pale. Abdominal tergites brown, sternites pale.

Terminalia (Fig. 2.13 E): Sternite VIII bilobate, medial depression broadly U-shaped. Sternite IX (genital fork) broadly Y-shaped. Hypogynial plate broad basally, narrowed slightly to base of apical valves, individual valves short, narrowing and rounded at apex, inner margin sinuous, outer margin convex, apices slightly divergent,

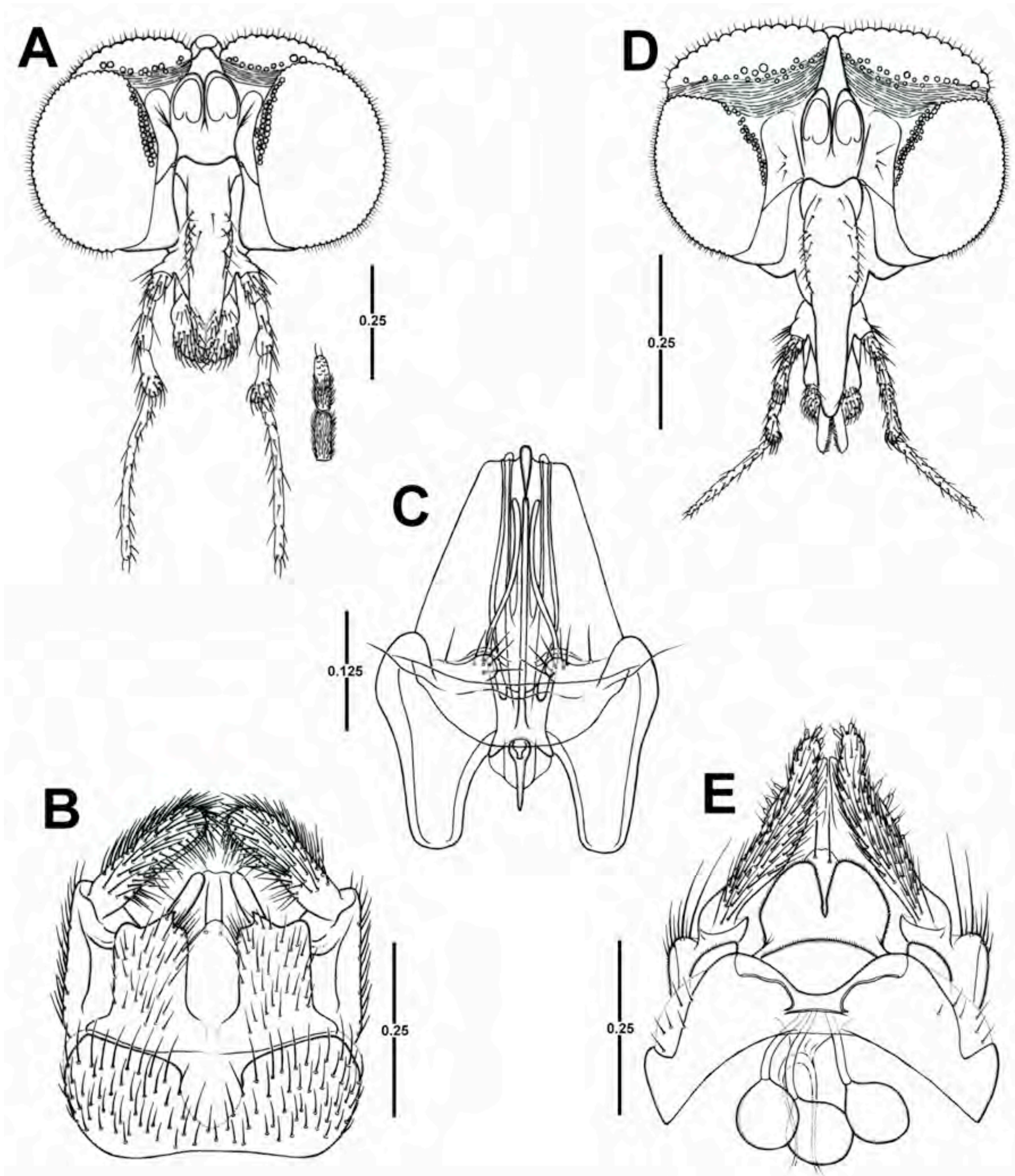

Figure 2.13. Adults of *Blepharicera opistera*. **A.** male head and antennal apex, frontal view. **B.** male terminalia, dorsal view. **C.** male terminalia (phallic structures), dorsal view. **D.** female head, frontal view. **E.** female terminalia, ventral view.

intervalvular area narrowly U-shaped. Accessory gland narrow, elliptical. Spermathecae three in number; corpora spherical, with short necks; ducts short, unpigmented. Chaetotaxy: Sternite VIII with 5 setiforms laterally; hypogynial plate with numerous small setae; epiproct with 2 prominent setiforms apically.

Type material.— Holotype [adult male, reared]: UNITED STATES: GEORGIA: *Murray Co*: Jacks River @ Cottonwood Patch campsites, 34°59'N 84°38'W, 29 April 2007, coll. J.K. Moulton. Specimen pinned, genitalia in glycerin microvial [USNM]. Allotype [adult female, reared]: same data as holotype; pinned, head and genitalia in glycerin microvial [USNM]. Paratypes: same data as holotype [1 female A (reared, pinned, head and genitalia slide mounted), 3 male and 1 female A (reared, pinned, head and genitalia in glycerin microvial), 3 male A (reared, pinned, genitalia in glycerin microvial)]; 13 March 2007 [6 instar IV L (EtOH), 2 instar IV L (slides)], coll. A.J. Jacobson, G.R. Curler, and J.K. Moulton; 26 March 2009 [6 instar IV L (EtOH), 2 male and 2 female P (EtOH)], coll. A.J. Jacobson and G.R. Curler. Paratypes deposited in CNC, LACM, and USNM.

Other material examined.— UNITED STATES: TENNESSEE: *Polk Co:* Conasauga River @ TN/GA State Line, 34°59'N 84°38'W, 26 March 2009 [LP], coll. A.J. Jacobson and G.R. Curler.

Etymology.— From the Greek for "later" (*opistera)* in reference to the emergence period of the adults. This is in comparison to the emergence dates of *Blepharicera enoristera*, another new species from Jacks River.

Distribution.— *Blepharicera opistera* has been collected from the Jacks and Conasauga Rivers as they flow through the Cherokee National Forest of southeastern Tennessee and the Cohutta Wilderness of north central Georgia.

Bionomics.— *Blepharicera opistera* is found in medium to large streams during the spring and is sympatric with *B. appalachiae* and *B. enoristera*. Emergence of adults from a mid-spring (29 April 2007) collection by J.K. Moulton yielded males and females of both *B. opistera* and *B. appalachiae*. This overlap suggests an approximately four-week divergence in emergence times for *B. opistera* and *B. appalachiae* from the emergence times of *B. enoristera*, which were present in a collection made on 26 March 2009.

Taxonomic comparison.—*Blepharicera opistera* larvae most closely resemble *B. coweetae* in overall coloration. Differences between the two include the arrangement and type of the dorsal secondary sensilla. *Blepharicera coweetae* sensilla are fusiform and arranged in two transverse bands medially while the sensilla of *B. opistera* larvae are digitiform and less constrained to two dorsal bands. Pupae of *B. opistera* are very similar to other species in the group. They can be differentiated from sympatric species by the arrangement of the dorsal papillae and overall shape. Adult males of *B. opistera* are distinguished by features of the genitalia and the size of the upper eye division. The upper eye division in males of *B. opistera* are significantly larger than the upper eye division of *B. appalachiae* but only slightly smaller than those of *B. enoristera*. The cerci are unique in form with subparallel lateral and medial margins. The lateral margins of both *B. appalachiae* and *B. coweetae* are concave and divergent. Adult females of *B. opistera* have spherical spermathecae and slightly divergent hypogynial valves. The spermathecae of *B. appalachiae* are ovoid while the hypogynial valves of *B. enoristera* are straight along the entire length.

Discussion

While the *Blepharicera tenuipes* group has been extensively studied over the past twenty years, new species are still being discovered. Hogue (1987) stated, "Because collecting in the last few years has turned up only two cryptic new species [in North America], the likelihood of major new discoveries seems small." However, Hogue also mentioned that basic taxonomic work on the *B. tenuipes* group was still needed. This may have been a foresight into the seven new species described by Courtney in 2000, the new species discovered by Moulton and Curler in 2007, and the six new species described here.

The *Blepharicera tenuipes* group is unique in its pattern of diversity. Dipteran diversity is typically greater in the western Nearctic in comparison to that of the eastern Nearctic (Courtney, personal communication). However, the eastern Nearctic

Blephariceera, in comparison to the western Nearctic *Blephariceera*, exhibits a higher level of diversity with twenty-three and five described species, respectively.

Extreme morphological similarities abound, particularly in immature stages, thus encumbering studies on the Nearctic fauna (Georgian and Wallace 1983, Lenat 1993, Courtney 2000b). Adult males remain the most reliable stage in species identification (Courtney 2000b). Consistent differences in the males of the six new species described here support this hypothesis, with the exception of *B. crista* which is also unique in both the larval (i.e., dorsal secondary sensilla clusters) and pupal (i.e., faint reticulation of dorsal cuticle) life stages. Adult females are increasingly difficult to separate, with only subtle differences evident among species. Most females in this study were associated through the ontogenetic method of dissecting pharate adults from pupae.

All six of the new eastern species appear to be spring species from the central to southern Appalachians and have moderately to strongly restricted distributions. *Blephariceera conifera* and *B. amnicula* are two highly secluded canyon species. They are currently known only from Cloudland Canyon State Park in northwestern Georgia and Little River Canyon National Preserve in northeast Alabama, respectively. *Blephariceera crista* appears to be restricted to two valley streams in western Virginia. Collections in streams in and around the area (i.e., Wolf Creek near Bastian, VA; Little River near Snowville, VA) did not yield specimens of *B. crista*. *Blephariceera enoristera* and *B. opistera* have been discovered only in two adjacent locations in the Cherokee National Forest and Cohutta Wilderness in southeastern Tennessee and north central Georgia. *Blephariceera hillabee* is restricted to a single location in east central Alabama. Even though the *B. tenuipes* group shows high levels of sympatry, the type localities for the six new species range from no sympatry to sympatric levels of only three to four species.

Walker and Little Walker Creek appeared initially to be ideal blephariceerid habitats, both incorporating drainages that are in valleys, separated from other sources. Upon further inspection of the sites, it was unclear whether blephariceerids would be present due to the high impact of agriculture in the area. Highly impacted areas typically do not yield blephariceerid midges. Surprisingly, there were up to four sympatric species at these locations, which indicates a higher level of tolerance to environmental impacts.

Phylogenetic studies have been performed on the Nearctic *Blephariccera* and are presented elsewhere. Analyses were conducted to determine the phylogenetic relationships between eastern and western Nearctic *Blepharicera* and within eastern and western Nearctic *Blepharicera* using morphological characteristics from larval, pupal, and adult life stages and molecular data from two genes: Big Zinc Finger 2 (BZF2), a nuclear protein-coding gene, and NADH dehydrogenase 2 (ND2), a mitochondrial protein-coding gene.

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CHAPTER 3. PHYLOGENETIC ANALYSIS OF NEARCTIC *BLEPHARICERA* MACQUART (DIPTERA: BLEPHARICERIDAE)

Abstract. Phylogenetic studies were conducted to determine the relationships between eastern and western Nearctic *Blephariceras* and among species within these groups. Larvae, pupae, and adults were available for all known Nearctic species, except *B. caudata* Courtney and *B. micheneri* Alexander, the latter of which DNA-quality material was not available. Morphological data from forty-four characters and DNA sequence for two genes, Big Zinc Finger 2 (BZF2), a nuclear protein-coding gene, and NADH dehydrogenase 2 (ND2), a mitochondrial protein-coding gene, were used to test previous phylogenetic hypotheses on the historical relationships and biogeography of Nearctic *Blephariceras*. Analyses using maximum parsimony (PAUP* 4.0b10 and TNT), maximum likelihood (RAxML 7.0.3), and Bayesian inference (MrBayes 3.1.2) methods were implemented. Resulting phylogenies support monophyly of the *B. tenuipes* and *B. micheneri* groups and suggest that multiple species complexes may exist within the *B. tenuipes* clade.

Introduction

Blephariceridae is a monophyletic family of Diptera defined primarily by larval characters (Zwick 1977; Wood and Borkent 1989; Courtney 1990, 1991; Oosterbroek and Courtney 1995). Larval characters supporting the monophyly of Blephariceridae include: cephalic division, six ventral suckers, cephalic apotome divided by the frontoclypeal suture, tracheal gills, and stalked Malpighian tubules. Adult males have a characteristic trifid aedeagus.

Blephariceridae is a cosmopolitan family, occurring on every continent except Antarctica. There are approximately 320 described species in 28 described genera. Four genera occur in the Nearctic region (Hogue 1987, Courtney 2000a). *Agathon* von Röder is found in western North America and central and eastern Asia; *Bibiocephala* Osten Sacken in western North America and Japan; *Blephariceras* Macquart in western and eastern North America and throughout Eurasia; and *Philorus* Kellogg in western North America and central and eastern Asia. The only genus of Nearctic Blephariceridae found in both eastern and western North America is *Blephariceras*. There are currently five

Table 3.1. Location and species names for Nearctic *Blepharicera*.

Location	**Western North America**	**Eastern North America**
Genus	*Blepharicera*	*Blepharicera*
Species	*jordani* Kellogg 1903	*amnicula* n. sp.
	kalmiopsis Jacobson & Courtney 2008	*appalachiae* Hogue & Georgian 1986
	micheneri Alexander 1959	*capitata* Loew 1863
	ostensackeni Kellogg 1903	*caudata* Courtney 2000
	zionensis Alexander 1953	*chattooga* Courtney 2000
		cherokea Hogue 1978
		conifera n. sp.
		corniculata Courtney 2000
		courtneyi Curler & Moulton 2007
		coweetae Hogue & Georgian 1986
		crista n. sp.
		diminutiva Hogue 1978
		enoristera n. sp.
		gelida Courtney 2000
		hillabee n. sp.
		hispida Courtney 2000
		magna Courtney 2000
		opistera n. sp.
		separata Alexander 1963
		similans Johannsen 1929
		tenuipes Walker 1848
		tuberosa Courtney 2000
		williamsae Alexander 1953

species (*B. micheneri* group and *B. ostensackeni* Kellogg) known from western North America and twenty-three species (*B. tenuipes* group) known from eastern North America (Table 3.1) (Hogue 1987, Zwick 1990, Courtney 2000b, Moulton and Curler 2007, Jacobson and Courtney 2008, Jacobson and Moulton unpublished). Distribution of the Nearctic fauna is limited to mostly mountainous areas from northwestern Canada to southern California and Arizona and in the east from the Great Lakes to southeastern Canada and throughout the Appalachian Mountains (Hogue 1987) (Figure 3.1).

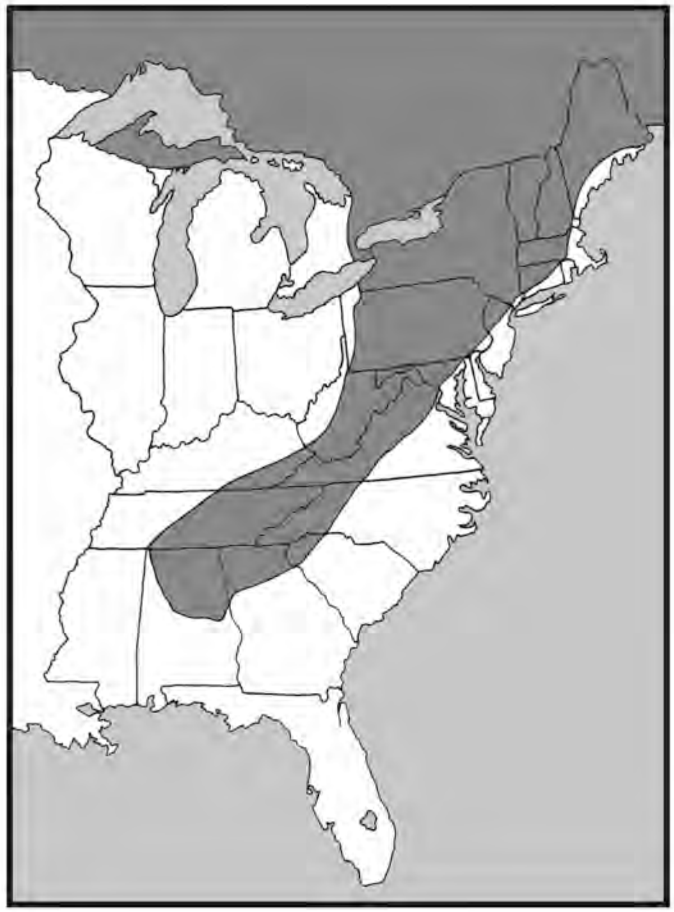

Figure 3.1. Distribution of the eastern Nearctic *Blepharicera*.

Hypothesized relationships and biogeography of the Nearctic *Blepharicera* were discussed by Hogue (1978), Zwick (1984), Hogue and Georgian (1986), and Jacobson (2006). Hogue (1978) believed the eastern Nearctic species arrived in North America via a transatlantic connection between Europe and Newfoundland. Support for this hypothesis was based on Hogue's contention that the eastern species show no close ties to the major western clade, the *B. micheneri* group. He suggested further that the western species arrived in North America via a connection between eastern Asia and Alaska, i.e. a Bering land bridge. There is a great divide in the Nearctic species across the central portion of North America.

Zwick (1984) suggested numerous similarities between Asian *Blepharicera* and western North American species but no similarities between Asian or European

Blepharicera and eastern North American species. The latter differed from Hogue's hypothesis, which involved a transatlantic dispersal route. Zwick constructed a phylogenetic tree that showed the *B. micheneri* group as sister group to the *B. tenuipes* group (Figure 3.2).

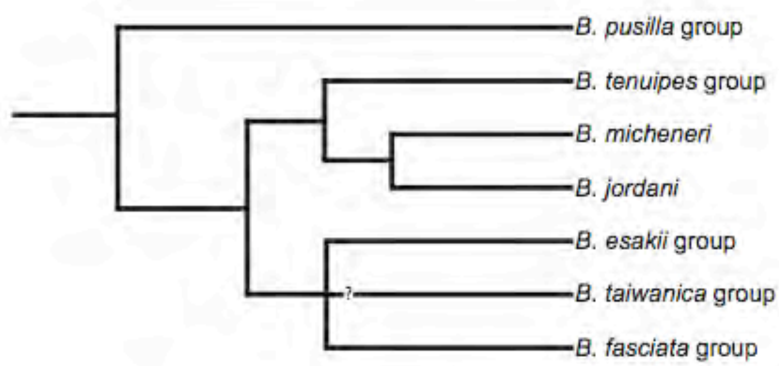

Figure 3.2. Phylogenetic relationships of *Blepharicera* based on Zwick (1984). (adapted from Zwick 1984).

In 1986, Hogue and Georgian concurred with Zwick, concluding that the European clade is too distinct to have given rise to the eastern North American clade. They also noted the likelihood that both the western and eastern groups derived from an Asian lineage since most *Blepharicera* species are from that area. Hogue and Georgian (1986) attempted to answer the question, "by what route did the *B. tenuipes* group reach eastern North America from the northwest? Also, what disruptive events forced the separation of the two groups?" They hypothesized a northern rather than a southern route and suggested that the groups diverged due to arid conditions in the late Cenozoic.

Zwick (1984) was the first to discuss relationships among the Nearctic *Blepharicera* (Figure 3.2). Results of his analyses suggested a sister-group relationship between the *B. micheneri* group and *B. tenuipes* group. Hogue and Georgian (1986) decided not to evaluate the phylogenetic relationships within the *B. tenuipes* group due to numerous similarities throughout the group.

Results of morphological analyses conducted by Jacobson (2006) supported the monophyly of both the *B. micheneri* group and the *B. tenuipes* group, but a sister-group relationship between the *B. micheneri* group and *B. tenuipes* group was not supported. Rather, the two species groups were separated from each other by the European species (*B. fasciata*), an Asian clade (*B. esakii*), and *B. ostensackeni*. That study also suggested that colonization of the Nearctic was rather complex and may have involved two or three separate invasions resulting in the ancestors of the *B. micheneri* group, *B. tenuipes* group, and *B. ostensackeni* arriving at different times.

All 28 described species of Nearctic *Blephariccera* are included in the current phylogenetic analysis.

Methods

Morphological Phylogenetic Analysis

Material.—Thirty-two species of *Blephariccera* were examined in this study. Of these, all but one species, *B. caudata*, were available as larvae, pupae, and adults (both male and female).

Terminology.—Terms for structures are based on Courtney (2000a).

Phylogenetic Analysis.—Cladistic methods (*sensu* Hennig (1966), as modified by Wiley (1981), Schuh (2000), etc.) based on synapomorphies, or shared, derived character states, were used to test the phylogenetic relationships among the Nearctic *Blephariccera*. Morphological characters were evaluated for 32 taxa, including four outgroup species. Outgroups were chosen based on relationships established in previous studies (Zwick 1984, 1990) and included the Asian species *B. pusilla* Zwick, *B. esakii* Alexander, and *B. acanthonota* Jacobson and Courtney and the single European *Blephariccera* species, *B. fasciata* Westwood. *Blephariccera pusilla* is presumed to belong to the basal-most clade of *Blephariccera,* with *B. esakii, B. fasciata,* and *B. acanthonota* chosen to serve as representatives of other species groups that are presumably closely related to the Nearctic fauna. Ingroup taxa included the *B. tenuipes* group, *B. micheneri* group, and *B. ostensackeni* Kellogg (Table 3.1). Character polarity (i.e., the direction of character-state evolution) was determined using outgroup methods (Watrous and Wheeler 1981, Maddison et al. 1984, Schuh 2000). A total of 44 characters, including 12 larval, 10 pupal, and 22 adult characters (Table 3.2), were designated for use in the phylogenetic analysis. Character states were specified for each character and given a numerical code (Table 3.3). All characters were equally weighted and unordered, with characters coded as “0”, “1”, “2”, “3”, etc. Missing data was coded as “?” for the unknown pupae and adults of *B. caudata*, for a character of the male genitalia not visible in *B. fasciata*, and for certain characters not applicable for *B. acanthonota*. Cladistic analyses using maximum parsimony were performed in PAUP* 4.0b10 (Swofford 2003) and TNT (Goloboff et. al 2008). Support at each node was assessed using bootstrap proportions (Felsenstein 1985). In PAUP* 4.0b10, a heuristic search with 500 random stepwise addition replicates was performed and bootstrap values were calculated for 1000 replicates. Character state transformations were viewed in MacCLADE 4.05 (Maddison and Maddison 2002). In TNT, a heuristic search with the default 10 random stepwise addition replicates was performed and bootstrap values were calculated for 10,000 replicates.

Table 3.2. Characters and alternate states used in phylogenetic analysis of Nearctic *Blephariceracera*.

Larva	
1. Prolegs	0. visible in dorsal view 1. obscured in dorsal view
2. Cranial width	0. 0.40–0.49 mm 1. 0.50–0.69 mm 2. 0.70–0.79 mm
3. Substernal setae color	0. pale 1. light brown 2. brown
4. Stem line	0. absent 1. short, posterior margin of frontoclypeal apotome almost reaching posterior cranial margin 2. long, posterior margin of frontoclypeal apotome well separated from posterior cranial margin
5. Dorsal tubercles	0. absent 1. present
6. Dorsal sensilla shape	0. fusiform 1. setiform 2. digitiform 3. claviform 4. globose 5. coniform 6. fustiform 7. setiform & digitiform
7. Dorsal sensilla length	0. short 1. long
8. Dorsal sensilla arrangement	0. clumped 1. uniform
9. Lateral lobe appendages	0. absent 1. present, smaller than proleg 2. present, subequal to proleg

Table 3.2. (continued)

10. Anal division posterior margin	0. rounded 1. truncate or concave 2. tapered to a point 3. tapered to two blunt apices
11. Anal division lateral lobes	0. rounded or blunt 1. triangular 2. pointed 3. indistinct, poorly developed
12. Anal division sensilla	0. 2 1. 4–10 2. > 10
Pupa	
13. Outline shape	0. ovoid 1. ellipsoid 2. broadly ovoid
14. Branchial sclerite	0. glabrous 1. papillose
15. Metatergite papillae	0. absent 1. present, few mediodorsally 2. present, covering most of tergite
16. Dorsal papillae	0. absent, “false” papillae 1. present
17. Dorsal papillae size	0. small 1. large
18. Dorsal papillae color	0. dark 1. light 2. light surrounded by halo
19. Dorsal tubercles	0. absent 1. present
20. Dorsal cuticle	0. glabrous 1. sculptured (punctures or reticulate)

Table 3.2. (continued)

21. Anal tergite	0. wrinkled 1. smooth, unwrinkled
22. Respiratory lamellae	0. pointed 1. broadly pointed 2. broadly rounded
Adult male	
23. Ultimate antennal flagellomere (X times penultimate flagellomere)	0. 1.0–1.5 1. 1.6–2.0 2. > 2.0
24. Tibial spurs	0. absent 1. present, 1
25. Maxillary palpus (ultimate palpomere in comparison to 2nd palpomere)	0. < 2x 1. > 2x
26. Dorsal eye division (in comparison to ventral eye division)	0. smaller 1. subequal or larger
27. Gonostylus	0. lobed 1. simple
28. Hypopygium	0. not rotated 1. rotated
29. Cercus	0. triangular 1. quadrate 2. rounded
30. Aedeagal rods	0. equal, parallel 1. equal, outer rods sinuous 2. subequal, outer rods sinuous
31. Ventral paramere apex	0. simple, apex rounded or pointed 1. complex, apex concave, lobed, or hooked
32. Ventral paramere length (in comparison to aedeagal rods)	0. longer 1. subequal 2. shorter

Table 3.2. (continued)

33. Ventral paramere canal	0. present 1. absent
34. Dorsal paramere	0. pointed 1. rounded 2. rectangular 3. trilobed
35. Dorsal paramere carina	0. inconspicuous 1. prominent
36. Ejaculatory apodeme (size in comparison to lateral parameral lobes)	0. elongate 1. subequal 2. short
Adult female	
37. Ultimate antennal flagellomere (X times penultimate flagellomere)	0. 1.0–1.4 1. 1.5–2.0 2. > 2.0
38. Tibial spurs	0. absent 1. present, 2
39. Maxillary palpus (ultimate palpomere in comparison to 2nd palpomere)	0. < 2x 1. > 2x
40. Dorsal eye division (in comparison to ventral eye division)	0. smaller 1. subequal or larger
41. Spermathecal duct	0. short, extending ≈1/4 into abdomen 1. long, extending ≈1/2 into abdomen 2. long with coiled or wide portion
42. Duct sclerotization	0. absent 1. apically 2. basally 3. apically and basally
43. Genital fork	0. broadly V-shaped 1. broadly U-shaped 2. broadly Y-shaped

Table 3.2. (continued)

44. Hypogynial valve	0. subquadrate 1. quadrate 2. rounded 3. other

Table 3.3. Matrix of characters and alternate states used in phylogenetic analysis of Nearctic *Blepharicera* (0, 1, 2, 3, 4, 5, 6, 7 = character states; ? = character state unknown).

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
B. pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. esakii	0	1	2	1	0	2	0	1	2	3	0	2	0	0	2	1	0
B. fasciata	0	1	1	1	0	4	0	1	0	0	0	1	0	0	2	1	0
B. acanthonota	0	1	0	0	?	1	?	1	0	3	2	?	0	0	2	1	0
B. appalachiae	0	1	0	2	0	1	0	1	0	1	1	1	0	0	1	1	0
B. capitata	0	0	0	1	1	7	1	0	0	0	3	1	0	0	1	1	0
B. caudata	0	2	1	2	0	2	0	1	0	2	2	1	?	?	?	?	?
B. chattooga	0	1	0	1	0	3	0	1	0	1	0	1	1	0	0	1	0
B. cherokea	0	1	0	2	0	3	0	1	0	0	0	1	1	0	1	0	0
B. corniculata	0	1	0	1	0	2	0	1	0	0	1	1	1	0	1	0	0
B. courtneyi	0	2	1	2	0	6	0	1	0	1	0	1	0	0	2	1	0
B. coweetae	0	1	0	1	0	3	0	1	0	0	1	1	0	0	2	1	0
B. diminutiva	0	1	2	1	0	0	0	1	0	1	1	1	1	1	2	1	1
B. gelida	0	1	2	1	0	1	1	1	0	1	3	2	2	0	2	1	1
B. hispida	0	1	1	2	0	6	0	1	0	0	0	1	0	0	2	1	0
B. magna	0	1	2	1	0	7	1	1	0	0	2	2	2	0	2	1	1
B. separata	0	0	0	1	1	2	0	0	0	0	3	1	0	0	1	1	0
B. similans	0	1	0	2	0	4	0	0	0	1	0	1	0	0	1	1	0
B. tenuipes	0	1	0	2	0	3	0	0	0	1	0	1	0	0	2	1	0
B. tuberosa	0	1	0	1	0	0	0	0	0	1	1	1	1	0	1	0	0
B. williamsae	0	2	1	2	0	3	0	1	0	0	1	1	0	1	2	1	0
B. crista	0	1	2	2	0	2	0	0	0	1	1	1	0	0	2	1	0
B. conifera	0	1	2	2	0	5	0	1	0	1	0	1	0	0	2	1	0
B. littleriver	0	1	1	2	0	2	0	1	0	1	0	1	0	0	2	1	0
B. hillabee	0	1	1	2	0	6	0	1	0	0	0	1	0	0	2	1	0
B. enoristera	0	1	0	1	0	2	0	1	0	0	0	1	1	0	2	1	0
B. opistera	0	1	0	1	0	2	0	1	0	0	0	1	0	0	1	1	0
B. ostensackeni	0	1	1	1	2	3	0	0	0	1	1	1	2	0	2	1	0
B. micheneri	1	2	1	1	0	3	0	1	1	0	3	1	1	0	2	1	0
B. jordani	1	1	1	2	0	3	0	1	2	0	0	2	0	0	2	1	0
B. zionensis	1	1	0	2	0	0	0	1	2	0	0	2	0	1	2	1	0
B. kalmiopsis	1	2	0	1	0	0	0	1	2	0	0	1	0	0	2	1	0

Table 3.3. (continued)

Taxon	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
B. pusilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. esakii	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1
B. fasciata	0	0	0	0	1	0	0	1	0	0	1	2	0	0	?
B. acanthonota	0	?	0	0	0	?	?	?	1	1	0	0	?	0	0
B. appalachiae	0	0	1	0	1	0	1	1	0	1	0	1	1	1	0
B. capitata	1	1	0	1	1	0	1	1	0	1	0	1	2	1	0
B. caudata	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
B. chattooga	0	0	0	1	1	0	1	1	0	1	0	1	2	1	0
B. cherokea	2	0	0	0	0	0	1	1	0	1	0	1	1	1	0
B. corniculata	2	0	1	0	2	0	1	1	1	1	0	1	1	1	0
B. courtneyi	0	0	0	1	2	0	1	1	0	1	0	1	2	1	0
B. coweetae	0	0	1	0	2	0	1	1	1	1	0	1	1	1	0
B. diminutiva	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0
B. gelida	1	0	1	1	2	0	1	1	1	1	0	1	1	1	0
B. hispida	0	0	1	0	1	0	1	1	1	1	0	1	1	1	0
B. magna	0	0	1	0	2	0	1	1	1	1	0	1	1	1	0
B. separata	1	1	0	1	1	0	1	1	0	1	0	1	1	1	0
B. similans	0	0	0	0	1	0	1	1	0	1	0	1	2	1	0
B. tenuipes	0	0	1	0	2	0	1	1	0	1	0	1	1	1	0
B. tuberosa	2	0	1	0	1	0	1	1	0	1	0	1	1	1	0
B. williamsae	0	0	1	0	1	0	1	1	0	1	0	1	1	1	0
B. crista	0	0	1	1	1	0	1	1	0	1	0	1	2	1	0
B. conifera	0	0	0	0	2	0	1	1	0	1	0	1	1	1	0
B. littleriver	0	0	0	0	2	0	1	1	0	1	0	1	1	1	0
B. hillabee	0	0	0	0	1	0	1	1	0	1	0	1	1	1	0
B. enoristera	0	0	0	0	1	0	1	1	0	1	0	1	1	1	0
B. opistera	0	0	0	0	1	0	1	1	0	1	0	1	1	1	0
B. ostensackeni	0	1	0	1	1	0	0	1	1	1	1	2	0	0	2
B. micheneri	0	0	0	1	1	2	1	1	0	0	1	0	0	0	0
B. jordani	0	0	0	1	1	2	1	1	0	0	1	0	0	0	0
B. zionensis	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1
B. kalmiopsis	0	0	0	0	1	2	1	1	0	0	1	0	1	0	1

Table 3.3. (continued)

Taxon	33	34	35	36	37	38	39	40	41	42	43	44
B. pusilla	0	0	0	0	0	0	0	0	0	0	0	0
B. esakii	1	1	1	1	1	1	0	1	2	1	0	1
B. fasciata	1	1	0	0	1	1	0	1	2	0	0	0
B. acanthonota	?	?	?	?	0	1	?	0	1	1	1	0
B. appalachiae	0	3	1	1	1	1	1	1	0	0	2	1
B. capitata	0	2	0	1	1	1	1	1	0	2	0	1
B. caudata	?	?	?	?	?	?	?	?	?	?	?	?
B. chattooga	0	2	0	2	1	1	1	1	0	0	0	2
B. cherokea	0	1	0	2	1	1	1	1	0	0	0	0
B. corniculata	0	2	0	2	1	1	1	1	0	0	1	0
B. courtneyi	0	3	1	2	1	1	1	1	0	0	2	0
B. coweetae	0	1	1	1	1	1	1	1	0	0	2	0
B. diminutiva	0	1	0	1	1	1	1	1	0	0	0	2
B. gelida	0	2	0	1	1	1	1	1	0	0	1	2
B. hispida	0	2	0	1	1	1	1	1	0	0	2	0
B. magna	0	1	0	0	1	1	0	1	0	0	2	2
B. separata	0	2	0	1	1	1	1	1	0	0	0	1
B. similans	0	1	0	0	1	1	1	1	0	0	0	0
B. tenuipes	0	3	1	2	1	1	1	1	0	0	0	1
B. tuberosa	0	3	1	2	1	1	1	1	0	0	1	3
B. williamsae	0	1	0	2	1	1	1	1	0	0	0	1
B. crista	0	3	1	2	1	1	1	1	0	0	2	3
B. conifera	0	3	1	2	1	1	1	1	0	0	2	2
B. littleriver	0	3	1	2	1	1	1	1	0	0	2	2
B. hillabee	0	3	1	2	1	1	1	1	0	0	2	2
B. enoristera	0	2	1	2	1	1	1	1	0	0	2	2
B. opistera	0	2	1	1	1	1	1	1	0	0	2	2
B. ostensackeni	1	0	1	1	1	0	0	0	2	3	1	2
B. micheneri	1	2	1	0	2	1	1	1	1	1	1	2
B. jordani	1	2	1	0	2	1	1	1	1	1	0	2
B. zionensis	1	2	1	0	2	1	1	1	1	1	1	2
B. kalmiopsis	1	2	1	0	2	1	1	1	1	3	2	2

Molecular Phylogenetic Analysis

Material.—Exemplars of 37 blephariceridtaxa were used in the molecular aspect of this study. When appropriate, several specimens from each taxon, representing as disparate populations as possible, were sequenced to infer molecular phylogenies. A total of 68 specimens representing 8 genera and 37 species were included (Table 3.4). Abbreviations for locality data are Ck = Creek; Co = County; CG = Campground; CR = County Road; E = East; Exper = Experimental; Fk = Fork; GSMNP = Great Smoky Mountains National Park; Hwy = Highway; M = Middle; N = North; NHP = National Historical Park; NP = National Park; nr = near; Pkwy = Parkway; R = River; Rd = Road; Rt = Route; SP = State Park; USFS = United States Forest Service; W = West; Xing = crossing/bridge; @ = at. Abbreviations for life stages are L = larva; P = pupa; A = adult.

DNA extraction, PCR amplification, Sequencing.—Whole animal specimens of each taxon were used to extract total DNA using a phenol-chloroform based method or the DNeasy Tissue Kit (Qiagen) per the manufacturer's suggested protocol. For the organic extraction, specimens were homogenized in a sodium dodecyl sulfate (SDS)-based lysis buffer including proteinase K. The homogenates were incubated at 55°C for several hours prior to organic extraction, once with a solution of phenol, chloroform and isoamyl alcohol. DNA salt was created by applying sodium acetate and was precipitated and pelleted by addition of cold (-20°C) absolute isopropanol and centrifugation. DNA was washed with 70% and 95% ethanol, air-dried and resuspended in 100 µL of 1X TE. Purified DNA samples were stored at -20°C (Moulton and Wiegmann 2004). Gene amplification was performed in MasterCycler (Eppendorf North America, Westbury, NY) thermal cyclers using 50-µl PCR reactions filled with Hotstart Ex Taq (TakaraMirus) kit components, template DNA, and custom primers. An approximately 1400 base pair segment of the mitochondrial ND2 gene was amplified and about 2100 bp of the nuclear BZF2 gene was also amplified (Table 3.5). Reactions were performed with 1–2µl of template DNA. The following PCR regime was employed: initial 90s denaturing step at 94˚C, then 4 cycles of 30s at 94˚C, 25s at 57˚C and 90+s at 72˚C, followed by 14 cycles of 30s at 94˚C, 20s at 53˚C and 90+s at 72˚C, 32 cycles of 30s at

94˚C, 15s at 47˚C and 90+s at 72˚C and ended at 72˚C for 7 min. PCR products were electrophoresed in 1% agarose gels at 110V for 30 minutes. Bands were excised from the gel, purified using QiaQuick Gel Extraction Kits and eluted in 35μl of elution buffer (10 mM Tris, pH 8.5). Purified PCR products served as templates for sequencing reactions using the same primers used in PCR reactions or internal primers.

Table 3.4. Geographic origins of blephariceridae specimens sequenced for molecular studies.

Taxon	Location	Coordinates	Life Stage
Agathon comstocki	OR: Benton Co: Woods Ck, mile 4	44°32'N 123°30'W	L
Agathon dismaleus	OR: Harney Co: spring, N side Big Indian Gorge	42°40'N 118°35'W	L
Apistomyia collini	AUSTRALIA: Queensland: Wooroonooran NP: Mt. Bartle-Frere, Josephine Falls	17°26'S 145°51'E	L
Blephariceria acanthonota	THAILAND: Prachinburi Province: Khao Yai NP: Namtok Than Rattana	14°14'N 101°23'E	L
B. amnicula	AL: Cherokee Co: Little River Canyon National Preserve @ Canyon Mouth Park	34°17'N 85°40'W	L
B. appalachiae	NC: Alleghany Co: Elk Ck @ Hwy 221 nr Stratford	36°30'N 81°13'W	L
B. capitata (Chattooga R)	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49'N 83°18'W	L
B. capitata (Hillabee Ck)	AL: Tallapoosa Co: Hillabee Ck @ Rt 22 nr Alexander City	32°59'N 85°51'W	L
B. caudata	NC: Haywood Co: GSMNP: Lost Bottom Ck	35°38'N 83°08'W	L
B. caudata 2	NC: Haywood Co: GSMNP: Rough Fork	35°35'N 83°08'W	L
B. chattooga larva	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49'N 83°18'W	L
B. chattooga pupa	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49'N 83°18'W	P
B. cherokea	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35°40'N 83°04'W	L
B. conifera	GA: Dade Co: Cloudland Canyon SP: Daniel Ck below upper falls	34°50'N 85°28'W	L
B. conifera 2	GA: Dade Co: Cloudland Canyon SP: Daniel Ck below upper falls	34°50'N 85°28'W	L
B. conifera 3	GA: Dade Co: Cloudland Canyon SP: Daniel Ck below upper falls	34°50'N 85°28'W	L
B. corniculata	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49'N 83°18'W	L
B. courtneyi	TN: Rhea Co: Piney R @ Shut-in Gap Rd	35°42'N 84°52'W	L
B. coweetae	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49'N 83°18'W	L
B. crista	VA: Giles Co: Little Walker Ck @ Hwy 100	37°12'N 80°44'W	L
B. diminutiva	NC: Haywood Co: W Fk Pigeon R @ falls: NC 215, N of Blue Ridge Pkwy	35°20'N 82°54'W	L
B. diminutiva Cosby	TN: Cocke Co: GSMNP: Hen Wallow Falls	35°45'N 83°13'W	L

Table 3.4. (continued)

Taxon	Location	Coordinates	Life Stage
B. enoristera	GA: Murray Co: Jacks R @ Cottonwood Patch campsites	34°59’N 84°38’W	L
B. enoristera 2	GA: Murray Co: Jacks R @ Cottonwood Patch campsites	34°59’N 84°38’W	L
B. gelida	VA: Bland Co: Wolf Ck nr Bastion	37°10’N 81°08’W	L
B. hillabee	AL: Tallapoosa Co: Hillabee Ck @ Rt 22 nr Alexander City	32°59’N 85°51’W	L
B. hispida	VA: Bland Co: Wolf Ck nr Bastion	37°10’N 81°08’W	L
B. jordani	CA: Del Norte Co: M Fk Smith R @ Panther Flat	41°50’N 123°55’W	L
B. jordani 2	OR: Curry Co: Pistol R nr Glade Ck	42°16’N 124°19’W	L
B. kalmiopsis	CA: Del Norte Co: M Fk Smith R @ Panther Flat	41°50’N 123°55’W	L
B. kalmiopsis (Pistol R)	OR: Curry Co: Pistol R nr Glade Ck	42°16’N 124°19’W	L
B. magna GA	GA: Gilmer Co: Mountaintown Ck @ Hwy 282 nr Ellijay	34°42’N 84°32’W	L
B. magna Kevin	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35°40’N 83°04’W	L
B. magna spring Cat	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35°40’N 83°04’W	L
B. magna spring Cat 2	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35°40’N 83°04’W	L
B. magna winter Cat	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35°40’N 83°04’W	L
B. opistera	GA: Murray Co: Jacks R @ Cottonwood Patch campsites	34°59’N 84°38’W	L
B. opistera 2	GA: Murray Co: Jacks R @ Cottonwood Patch campsites	34°59’N 84°38’W	L
B. ostensackeni	OR: Lane Co: H.J. Andrews Exper. Forest: Lookout Ck @ Gypsy Camp	44°13’N 122°14’W	L
B. ostensackeni (Smith R)	CA: Del Norte Co: M Fk Smith R @ Panther Flat	41°50’N 123°55’W	L
B. separata (Chattooga R)	SC: Oconee Co: Chattooga R @ Hwy 76 Xing	34°49’N 83°18’W	L
B. separata (Bluewater Ck)	AL: Lauderdale Co: Bluewater Ck @ CR71	34°54’N 87°26’W	L
B. separata (Hillabee Ck)	AL: Tallapoosa Co: Hillabee Ck @ Rt 22 nr Alexander City	32°59’N 85°51’W	L
B. separata (Little R)	TN: Blount Co: Little R @ Old Walland Hwy	35°45’N 83°51’W	L
B. similans	TN: Blount Co: GSMNP: Little R @ Little River Rd nr The Sinks	35°39’N 83°40’W	L

Table 3.4. (continued)

Taxon	Location	Coordinates	Life Stage
B. tenuipes	CANADA: Ontario: Ottawa: Bate Island: Remic Rapids	45^{o}24'N 75^{o}45'W	A
B. tuberosa	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35^{o}40'N 83^{o}04'W	L
B. williamsae	NC: Haywood Co: GSMNP: Cataloochee Ck @ lower Xing, Hydro Benchmark Station	35^{o}40'N 83^{o}04'W	L
B. zionensis	UT: Washington Co: Zion NP: Virgin R @ the Narrows	37^{o}18'N 112^{o}56'W	A
Edwardsina confusa	AUSTRALIA: New South Wales: Chichester State Forest: Allyn R @ White Rocks CG	32^{o}07'S 151^{o}28'E	L
Edwardsina sp. (Chile)	CHILE: Region V (Valparaiso): Ruta 60 above Guardia Vieja: small Ck ≈2km above Guardia Vieja	32^{o}54'S 70^{o}14'W	L
Liponeura sp.	IRAN: Azerbaijan: Soolik Waterfall	-	L
Neocurupira hudsoni	NEW ZEALAND: Westland: Jackson R Rd, south "Twin" Ck	44^{o}05'S 168^{o}37'E	L
Paulianina rivalis	MADAGASCAR: Antananarivo: RS d'Ambohitantely	18^{o}11'S 47^{o}16'E	L
Theischingeria rieki	AUSTRALIA: Queensland: Wooroonooran NP: Mt. Bartle-Frere, Josephine Falls	17^{o}26'S 145^{o}51'E	L
Abrams Ck, GSMNP	TN: Blount Co: GSMNP: Abrams Ck along Abrams Falls Trail	35^{o}35'N 83^{o}51'W	L
Buck Ck, KY (light)	KY: Pulaski Co: Buck Ck @ KY 192 nr Somerset	37^{o}03'N 84^{o}25'W	L
Buck Ck, KY (lightstripe)	KY: Pulaski Co: Buck Ck @ KY 192 nr Somerset	37^{o}03'N 84^{o}25'W	L
Buck Ck, KY (piebald)	KY: Pulaski Co: Buck Ck @ KY 192 nr Somerset	37^{o}03'N 84^{o}25'W	L
Cumberland Gap NHP, VA	VA: Lee Co: Cumberland Gap NHP: Gap Ck @ Iron Furnace	36^{o}36'N 83^{o}40'W	L
Dan R, VA	VA: Patrick Co: Dan R @ 631 (Squirrel Ck Rd)	36^{o}36'N 80^{o}27'W	L
Etowah R, GA	GA: Lumpkin Co: Etowah R @ USFS Rd 28-1	34^{o}36'N 84^{o}05'W	L
Little Walker Ck, VA	VA: Bland Co: Little Walker Ck @ State Rt 601 nr Volunteer Fire Dept	37^{o}04'N 80^{o}57'W	A
Mayo River State Park, NC	NC: Rockingham Co: Mayo River SP: Mayo R	36^{o}32'N 79^{o}59'W	L
Reed Ck, TN	TN: Blount Co: Reed Ck nr Walland @ E Millers Cove Rd	35^{o}44'N 83^{o}47'W	L
Sequatchie R, TN	TN: Cumberland Co: Sequatchie R @ Old Hwy 28 nr Burke	35^{o}46'N 85^{o}01'W	L

Table 3.4. (continued)

Taxon	Location	Coordinates	Life Stage
Warrior R, AL (dark)	AL: Blount Co: Locust Fk of Black Warrior R: Swann Bridge nr Cleveland	33°59'N 86°36'W	L
Warrior R, AL (light)	AL: Blount Co: Locust Fk of Black Warrior R: Swann Bridge nr Cleveland	33°59'N 86°36'W	L

Table 3.5. BZF2 and ND2 primers used in molecular phylogenetic analysis of Nearctic *Blepharicera*.

Gene	Primer	F/R	Sequence (5' → 3')*	Length	Position**
BZF2	94F	F	GARGARGTNATHACNGAYGAYTG	24-mer	280
	5E Cleanup	R	TYACKCKCGAATGSGATGC	19-mer	-
	Bleph Intron 1 3’	R	GANAGCAAYCGCATRTCYTCRTC	23-mer	-
	Bleph Intron 2 5’	F	CAYGCNAGYATGACNATYCAYAC	23-mer	-
	Bleph Intron 3 3’	R	TTRCTNGTYTGRTCNGGRTTRTG	23-mer	-
	Bleph P2 5’	F	GGYGTTAGTTTTGCACGAGAAAAR	26-mer	-
	OS Spec. P2 5’	F	AAACGAACTCAACCTCAACAYCA	23-mer	-
	OG P1 3’	R	TTGCTCGCCTGATCSGGRTGRTG	23-mer	-
	385F	F	CCNTTYGTNTGYCARCARTGYGG	23-mer	1018
	385Frpl	F	CCNTTYGTYTGYCARCATTGYGG	23-mer	1018
	385 BAD Rev	R	CGGGCRTGAAYTTCGGCRTG	20-mer	-
	Bl.BZF2P2.Fwd1	F	GGYGATTCRCCNTTCGAATGYGA	23-mer	-
	Bl.BZF2P2.Fwd2	F	GTGCAYGGTGGYGATTCGCC	20-mer	1093
	INGR P2 Mid Fwd	F	TGTTCTGGGATATCRGCCTTTTG	23-mer	-
	INGR P2 Mid Rev	R	ACAGATTTRTTRCGATGYGAMACC	26-mer	-
	392R	R	CCRCAYTGYTGRCANACRAA	20-mer	1040
	394R	R	ACNCCRCARTGYTGRCA	17-mer	1043
	Dixid 2.2Rrpl	R	AACATNTCNCCRCAYGTYTCRCA	23-mer	-
	765/777 BAD 5’	F	AAYCATTCGTGCTCGAARTGYGG	23-mer	-
	777 Bad 5’	F	GCYGAAGTTCAYGCMCGCTAYCA	23-mer	-
	sep/cap mid 5’	F	GAGTTTCAGTGTGTTTTTTGG	21-mer	-
	sep/cap mid 3’	R	GTTAAAGGAAACAATAATTGTG	22-mer	-
	coweetae P1 5’	F	CMCTARCATAAACGACGACC	20-mer	-
	coweetae P1 3’	R	TYCATATCGCCGGTGTCATC	20-mer	-
	Abrams P1 3’ conn	R	TCATCGAACTCCATATCGCC	20-mer	-
	appalachiae P2 5’	F	CACATTTGCCGAGGCACTGG	20-mer	-
	zion P1-2 5’	F	GACGATCATGTCGGAATGAC	20-mer	-
	zion P1-2 3’ conn	R	GTCATGCCGCACGTTTCACA	20-mer	-
	jordani P1 3’ conn	R	GAATTGTCATTCCGACATGATCG	23-mer	-
	jordani P2 5’ conn	F	TCTGAAATTGATACRAAATACGG	23-mer	-
	jordani P2 3’ conn	R	GTCACGACACAATTTTCCA	19-mer	-
	765R	R	CCRTCNGCRAANGCYTTCCARCA	23-mer	1973
	766R	R	CCRCCRTCNGCRAAYGCYTTCCA	23-mer	1976
	777R	R	CCNGTRTGDATNCGYTCRTGYTT	23-mer	2009

Table 3.5. (continued)

Gene	Primer	F/R	Sequence (5' → 3')*	Length	Position**
ND2	5’#1	F	CTAATTAAGCTANTGGGTTCATAC	24-mer	-
	5’#2	F	AAGCTAATYAAGCTANTGGGTTCA	24-mer	-
	5’#3	F	GCTANTRGGTTCATACCC	18-mer	-
	BlTenGrp 5’int	F	ATAGCNWAYTCHTCHATYAAYCA	23-mer	-
	BlTenGrp 3’int	R	TGRTTTAARTARTAVAGYTTRAA	23-mer	-
	clade 1 5’	F	CCYYTNACHCTYCTYTCYTATTC	23-mer	-
	clade 1 3’	R	AAAATRGAAAAWTTYAVARTTGT	26-mer	-
	clade 2 5’	F	CCYTTYCAYTTYTGATTYCC	20-mer	-
	clade 2 3’	R	GAAAAWADYTGRTTTAARTARTA	26-mer	-
	3’END Cleanup 5’	F	TTRATTTGAYTWATRGTYTTYATA	26-mer	-
	5’END Cleanup 3’	R	CCYTCYATYACATTYGGRAATCA	23-mer	-
	ost 3’END Cleanup 5’	F	TTAGGRTTTATYCCYAARTGA	21-mer	-
	3’	R	ARTGGCTGAAGTTTAGGCGATA	22-mer	-
	3’#2	R	TGAYATTAGACKGCAATTCTAAAG	25-mer	-

***** R=A/G; Y=C/T; S=C/G; W=A/T; K=G/T; M=A/C; H=A/C/T; D=A/G/T; V=A/C/G; N=A/T/G/C
****** Based on *Drosophila yakuba* mRNA sequence. GenBank Accession # XM002097814

Templates were sequenced in both directions with BigDye® v3.1 terminators (Applied Biosystems, Carlsbad, California) in 1/16th reactions utilizing BetterBuffer® (The Gel Company, San Francisco, CA). Sequencing reactions were cleaned using Centri-sep purification columns (Princeton Separations, Adelphia, New Jersey), electrophoresed through a 6% polyacrylamide gel using an MJ Research BaseStation-100 Automated DNA Sequencer (Bio-Rad, Hercules, California), and analyzed using Cartographer 1.2.7 software. Sequences from opposing strands were reconciled and verified for accuracy using Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, Michigan). Determination of exon-intron boundaries was accomplished in Sequencher employing the GT-AG rule (Rogers and Wall 1980) while maintaining a continuous open reading frame. Most sequences of BZF2 contained three to five introns. All introns were removed for analysis. All sequences will be deposited in GenBank and their accession numbers will be made available at a later date.

Phylogenetic Analysis.—Final sequences were exported into NEXUS-formatted files (Maddison et al. 1997) for subsequent alignment and phylogenetic analysis. Initial alignment of nucleotides and amino acids was performed using Clustal X 1.81 (Thompson et al. 1997) with optimization facilitated using the conceptual amino acid translation visualized in MacClade (Maddison and Maddison 2000). Data type (exon versus intron) and codon positions in the final nucleotide matrix were designated using MacClade. Modeltest 3.7 (Posada and Crandall 1998) was used to establish the optimal evolutionary model for both individual and combined molecular partitions.

Maximum parsimony analysis was performed using TNT (Goloboff et. al 2008). Ten random stepwise addition replicates were performed and bootstrap values were calculated for 1,000 replicates. Maximum likelihood was performed using RAxML 7.0.3 (Stamatakis 2006, Stamatakis et al. 2008). Data were partitioned into codons and analyzed using the evolutionary model GTRGAMMAI with 1,000 rapid bootstrap replicates. Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) using the optimal evolutionary model, which was general time reversible with invariant characters and rates following the gamma distribution (GTR+I+Γ). For Bayesian analyses, two runs (each with four chains) were started with an initial setting of

1,000,000 generations and a temperature setting of 0.02. Various statistics (Huelsenbeck et al. 2001) were used to determine if additional generations would be needed and generations were added as deemed necessary. Multiple starting points for each chain were used to reduce the probability of convergence on local optima. Uncorrected pairwise distances of various natural partitions of both molecular data sets were calculated using PAUP* (Swofford 2003) to determine the extent long-branch attraction, especially of introns and third codon positions, may have had on resultant inferred phylogenies.

Phylogenetic analyses were conducted on each of the genes individually and combined. The ND2 data set consists of 61 taxa with 1204 characters. The BZF2 data set consists of 60 taxa with 1724 characters. When concatenated, the combined data set, consisting of data from ND2 and BZF2, has 68 taxa with 2928 characters.

Outgroups were chosen as in the morphological methods above with *Blepharicera acanthonota* as the proximal outgroup representative. *Agathon comstocki* (Kellogg), *Agathon dismaleus* (Hogue), *Apistomyia collini* Bezzi, *Edwardsina confusa* Zwick, a Chilean representative of *Edwardsina* Alexander, *Liponeura* Loew, *Neocurupira hudsoni* Lamb, *Paulianina rivalis* Stuckenberg, and *Theischingeria rieki* Zwick served as distal outgroups. These outgroups were also based largely on the availability of molecularly viable representatives and material that would amplify with the given genes.

Results

Morphological Phylogenetic Analysis

Maximum Parsimony.—Strict parsimony analysis in PAUP* 4.0b10 resulted in 180 equally parsimonious trees of 211 steps while analysis in TNT resulted in 20 equally parsimonious trees of 211 steps. A 50% majority consensus of the trees generated in PAUP* 4.0b10 is presented. Bootstrap values showed little support for the consensus tree (Fig. 3.3). Character state transformations are plotted on the consensus tree (Fig 3.4).

These phylogenetic analyses supported the monophyly of both the *B. micheneri* and the *B. tenuipes* groups. Monophyly of the *B. micheneri* group was supported by the following synapomorphies: larval prolegs obscured in dorsal view, adult male ultimate antennal flagellomere >2x length of penultimate, adult male dorsal paramere rectangular, female ultimate antennal flagellomere >2x length of penultimate, and female spermathecal duct long extending ≈½ into abdomen. However, the western clade, as a whole, was not monophyletic. *Blepharicera esakii* is nested within the western clade indicating that *B. ostensackeni* is not the closest relative to the *B. micheneri* group. Characters that support *B. esakii* as sister to the *B. micheneri* group include: larval lateral lobe appendages are present, male ventral paramere length (in comparison to aedeagal rods) is subequal, and male cercus is triangular. Monophyly of the *B. tenuipes* group was supported by the following synapomorphies: male gonostylus simple, male cercus quadrate, and male ventral paramere apex complex. In the 50% majority consensus tree, there was good resolution within the *B. tenuipes* group. However, bootstrap values provided little support for this resolution with many nodes collapsing to form a bush-like polytomy, likely due to conflicting characters and high levels of homoplasy.

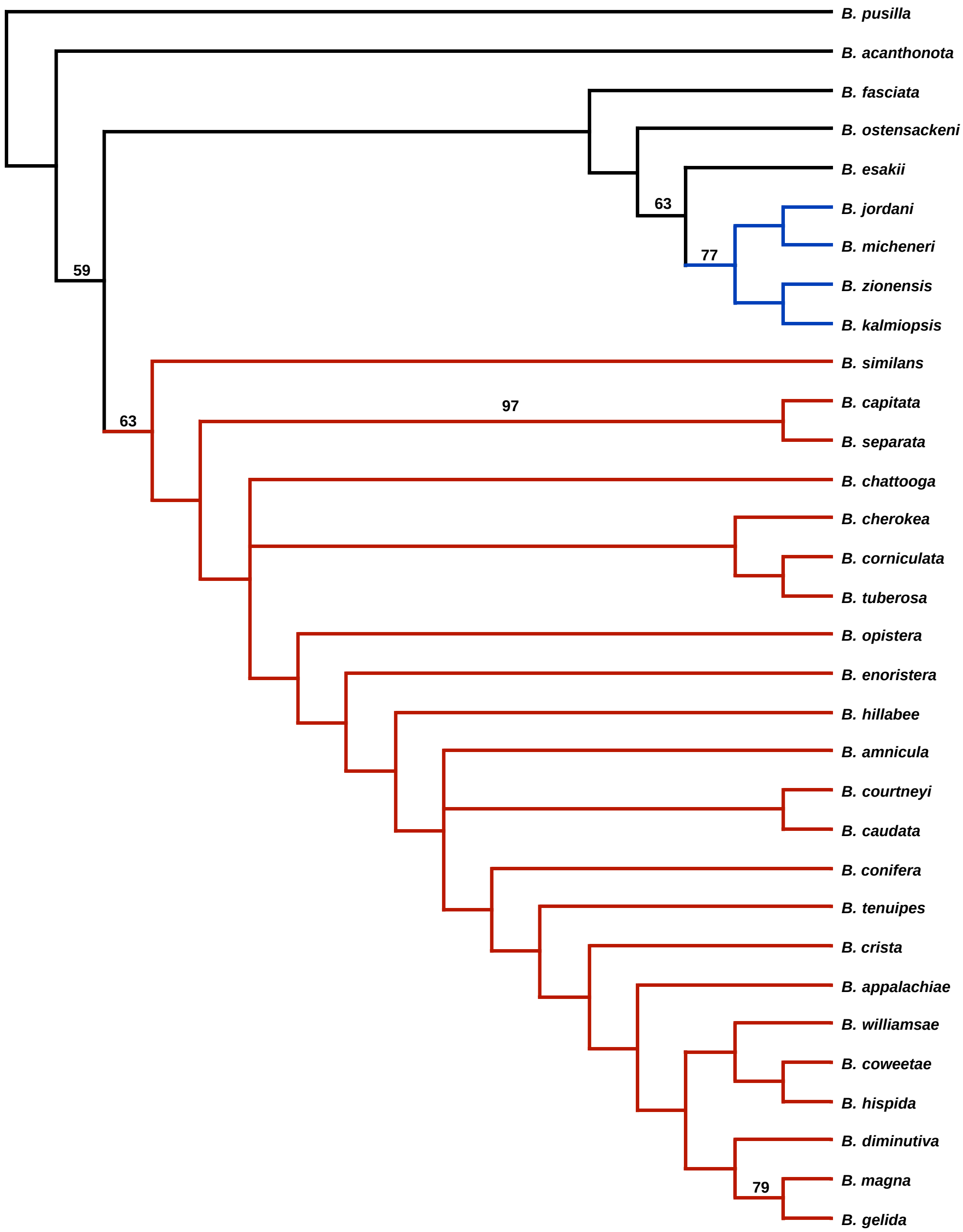

Figure 3.3. Hypothesized phylogenetic relationships of Nearctic *Blephariceras* based on maximum parsimony analysis of morphological data. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

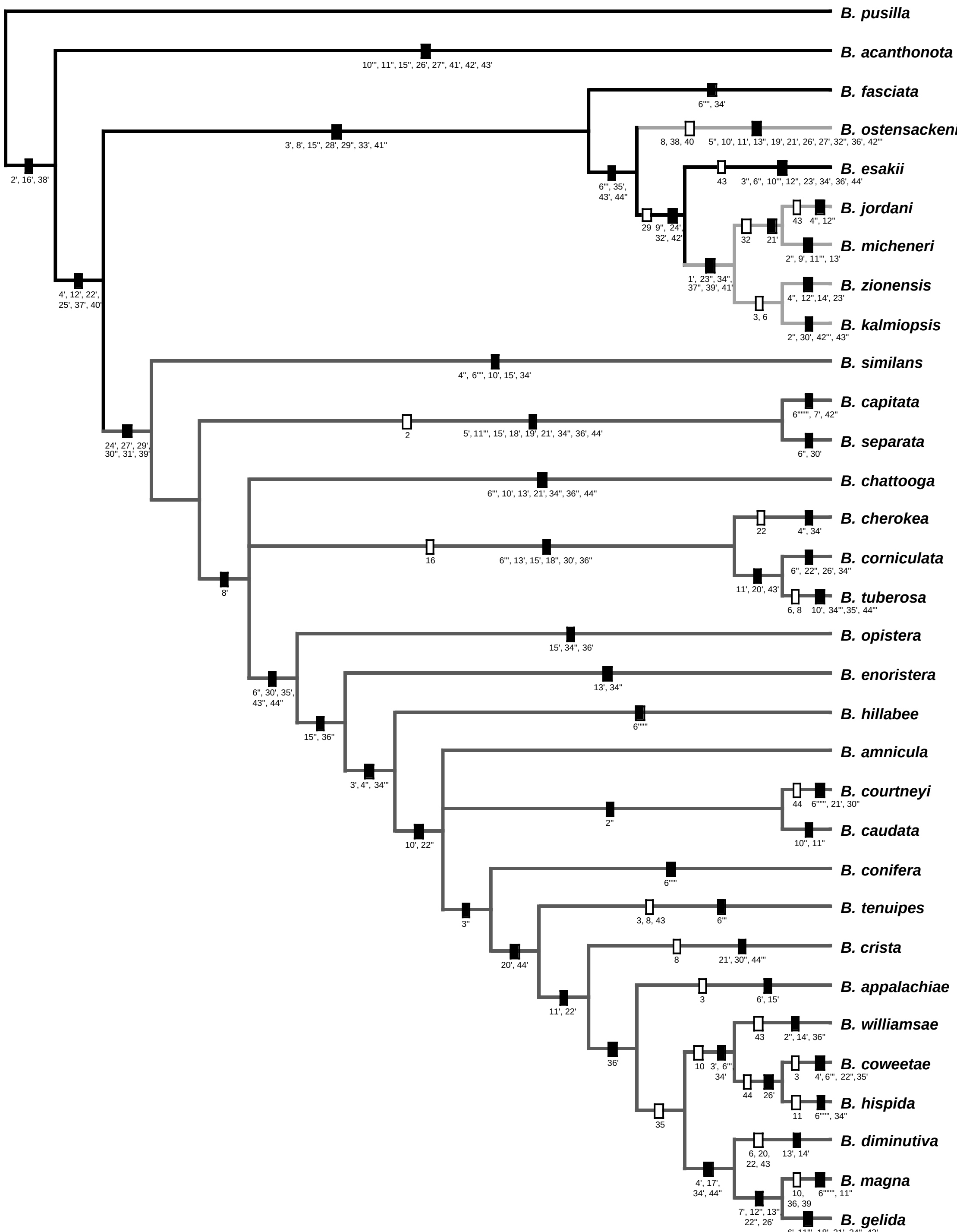

Figure 3.4. Character state distribution within hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum parsimony analysis of morphological data. Black boxes indicate derived character states and white boxes indicate character reversals. Numbers below boxes denote characters and accents denote character states: ’ = character state 1, ’’ = character state 2, etc. Black branches = outgroup taxa, dark gray branches = *B. tenuipes* group, light gray branches = *B. micheneri* group and *B. ostensackeni*.

Molecular Phylogenetic Analysis: NADH dehydrogenase 2 (ND2)

Maximum Parsimony.—Strict parsimony analysis in TNT resulted in 38 equally parsimonious trees of 2805 steps. A consensus of the trees is illustrated (Fig. 3.5).

Maximum Likelihood.—Best scoring maximum likelihood tree (Fig. 3.6).

Bayesian Inference.—1,000,000 generations with final chain swap values of 0.63–0.88. Standard deviation of split frequencies=0.009152 (Fig. 3.7).

As in the morphological analysis, ND2 analyses strongly supported the monophyly of both the *Blepharicera micheneri* group and the *B. tenuipes* group. Bootstrap values and posterior probabilities are at or above the 95^{th} percentile for the *B. micheneri* group (parsimony=96, likelihood=95 and Bayesian=99) and at 100% for the *B. tenuipes* group. *Blepharicera ostensackeni* consistently paired with *B. acanthonota*, the Asian *Blepharicera* representative, indicating a closer relationship to Asian *Blepharicera* than Nearctic species. Jacobson (2006) observed the same relationships in a morphological parsimony analysis of the Nearctic *Blepharicera*. Support for the *B. acanthonota* + *B. ostensackeni* clade is at or above the 97^{th} percentile. The *B. tenuipes* group showed relatively high levels of resolution in the phylogenetic trees. While trees inferred by parsimony, likelihood, and Bayesian analyses did not possess identical topologies, they did consistently recover several clades. The most inconsistent part of the tree was the basal part of the *B. tenuipes* group. Considerable discrepancy occurred in the positioning of the basal branches. *Blepharicera diminutiva*, *B. magna*, *B. similans*, *B. cherokea*, *B. williamsae*, *B. caudata*, and *B. capitata/separata* consistently were recovered at or near the base of the species group in all analyses. Consistently recovered clades within the *B. tenuipes* group included the *B. appalachiae* clade (*B. appalachiae* and *appalachiae*-like larval forms from Abrams Creek, Great Smoky Mountains National Park; Reed Creek, near Walland, TN; Etowah River, GA; Dan River, VA; Little Walker Creek, VA; Cumberland Gap National Historical Park, TN and VA; Mayo River State Park, NC) with 100 percent support, the *B. coweetae/hispida* clade (*B. coweetae, B. hispida, B. enoristera, B. opistera, B. tuberosa* and *B. corniculata*) with 99–100 percent support, the *B. tenuipes* clade (*B. tenuipes, B. courtneyi, B, crista, B. hillabee, B.*

amnicula, B. conifera, and Warrior River, AL) with 100 percent support, and the *B. capitata/separata* clade (*B. separata* from Chattooga River, SC, Hillabee Creek, AL, Little River, TN, and Blue Water Creek, AL; *B. capitata* from Chattooga River, SC and Hillabee Creek, AL; representatives from Sequatchie River, TN and Buck Creek, KY) with 100 percent support. While these clades consistently appeared in resulting phylogenies, relationships within the clades were variously resolved. The analyses also consistently recover two pairs of sister taxa (*B. williamsae* + *B. caudata, B. corniculata* + *B. tuberosa)* with high levels of support. *Blepharicera williamsae* + *B. caudata* has bootstrap support of 91 (likelihood) and a posterior probability of 100, and *B. corniculata* + *B. tuberosa* has bootstrap support of 100 (parsimony) and 78 (likelihood), and a posterior probability of 100.

Figure 3.5. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum parsimony analysis of mitochondrial protein-coding gene, ND2. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri group*.

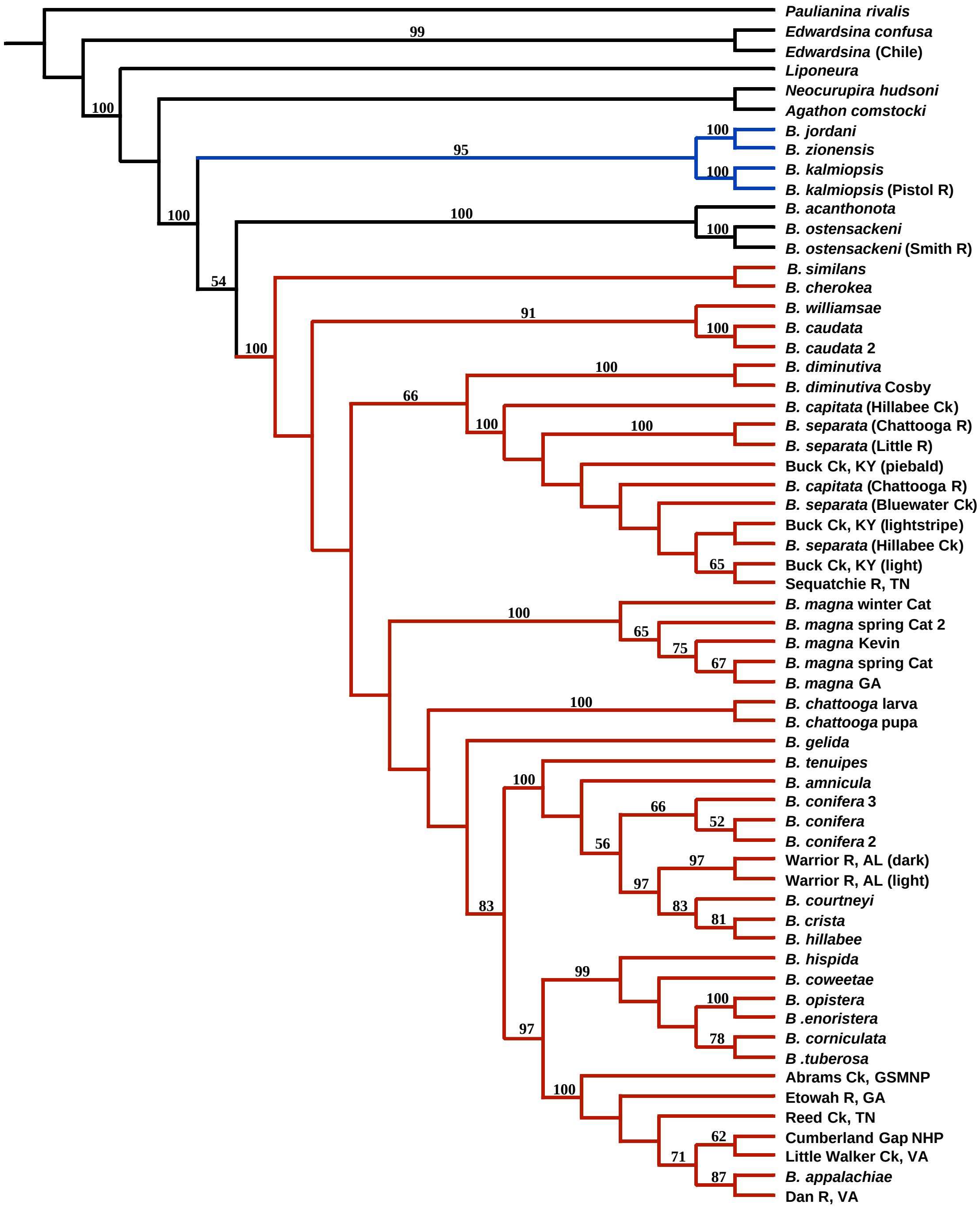

Figure 3.6. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum likelihood analysis of mitochondrial protein-coding gene, ND2. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri group*.

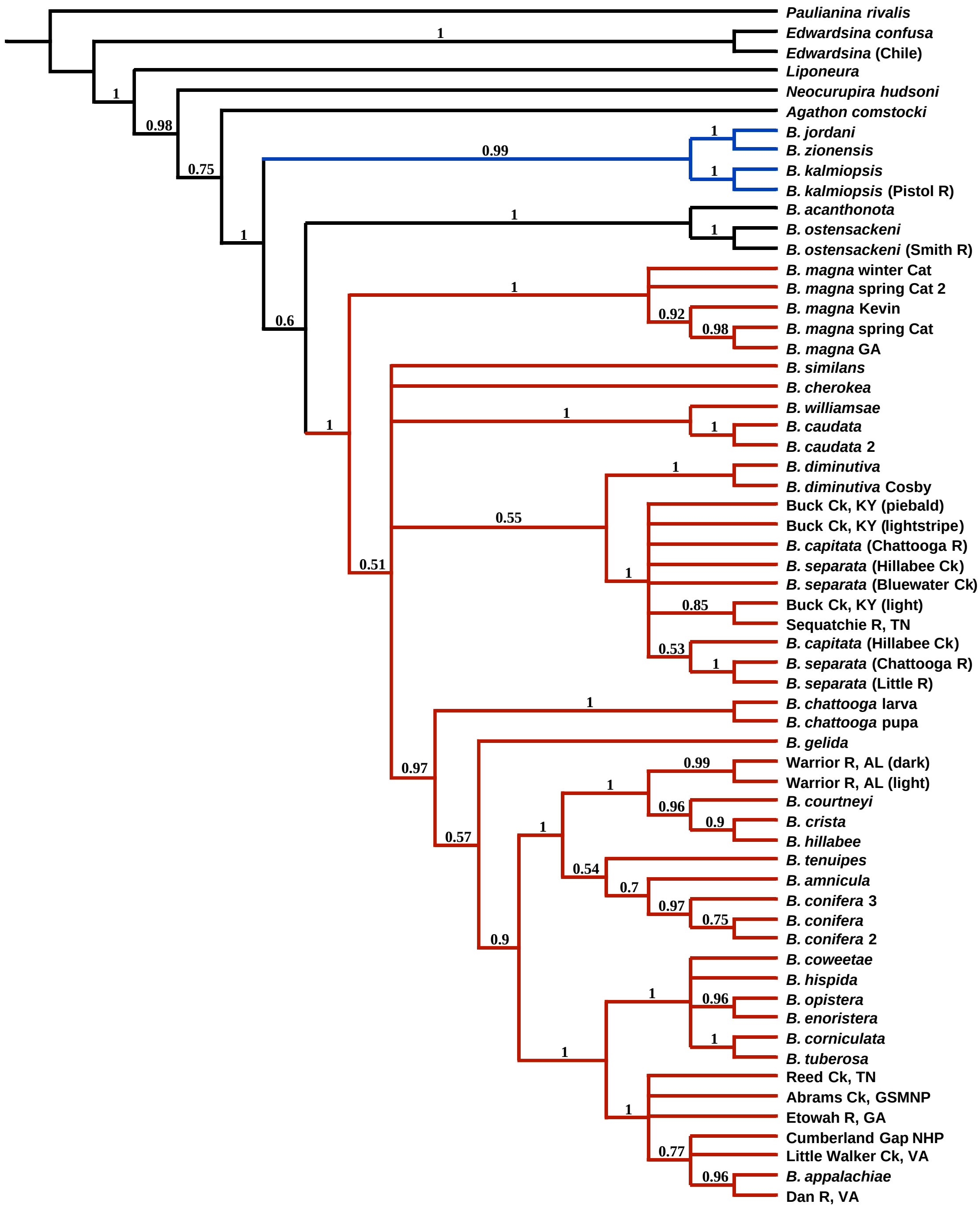

Figure 3.7. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on Bayesian inference of mitochondrial protein-coding gene, ND2. Values above branches denote posterior probabilities. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri group*.

Molecular Phylogenetic Analysis: Big Zinc Finger 2 (BZF2)

Maximum Parsimony.—Strict parsimony analysis in TNT resulted in 49 equally parsimonious trees of 2530 steps. A consensus of the trees is illustrated (Fig. 3.8).

Maximum Likelihood.—Best scoring maximum likelihood tree (Fig. 3.9).

Bayesian Inference.—1,000,000 generations with final chain swap values of 0.57–0.86. Standard deviation of split frequencies=0.007518 (Fig. 3.10).

As in the previous analyses, BZF2 analyses strongly supported the monophyly of both the *Blephariceera micheneri* group and the *B. tenuipes* group. Bootstrap values and posterior probabilities were at 100 percent for the *B. micheneri* and *B. tenuipes* groups. *Blephariceera ostensackeni* consistently paired with *B. acanthonota*, the Asian *Blephariceera* representative, indicating a closer relationship to Asian *Blephariceera* than Nearctic species. Support for the *B. acanthonota* + *B. ostensackeni* clade was at the 100th percentile. Once again, the most inconsistent part of the tree was the basal part of the *B. tenuipes* group. There was great discrepancy regarding the positioning of the basal branches. *Blephariceera diminutiva*, *B. magna*, *B. similans*, *B. capitata/separata*, and, to a lesser degree, *B. chattooga* were recovered at or near the base of the species group in all analyses. *Blephariceera chattooga* was basal only in the parsimony analysis, but significant support values (≥70% for bootstrap and ≥95% for posterior probabilities) were not present to validate the placement of the species in the likelihood or the Bayesian analyses. Trees inferred by parsimony, likelihood, and Bayesian analyses did not possess identical topologies, but certain clades were consistently recovered. Consistently recovered clades within the *B. tenuipes* group include the *B. appalachiae* clade with 91–100 percent support, the *B. coweetae/hispida* clade (without *B. tuberosa* and *B. corniculata*) with 74–99 percent support, the *B. tenuipes* clade with 92–100 percent support, and the *B. capitata/separata* clade with 99–100 percent support. While these clades consistently appeared in resulting phylogenies, relationships within the clades were variously resolved. Two other sister taxa, with relatively high levels of support, consistently pair. *Blephariceera williamsae* + *B. caudata* has bootstrap support of 85 (parsimony) and 100 (likelihood), and a posterior probability of 100. *Blephariceera* *corniculata* + *B. tuberosa* has bootstrap support of 75 (likelihood) and a posterior probability of 100.

Figure 3.8. Hypothesized phylogenetic relationships of Nearctic *Blephariceria* based on maximum parsimony analysis of nuclear protein-coding gene, BZF2. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

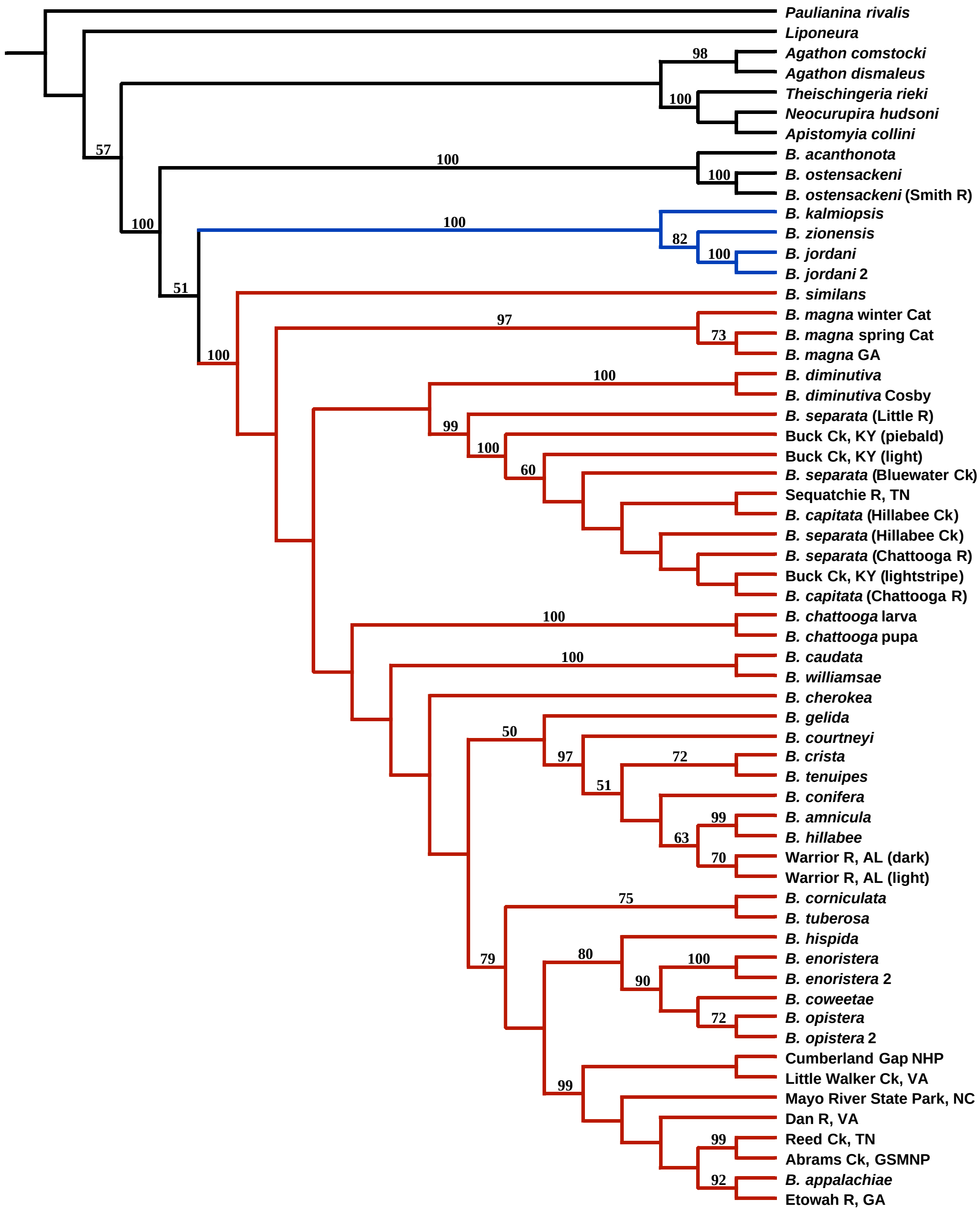

Figure 3.9. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum likelihood analysis of nuclear protein-coding gene, BZF2. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

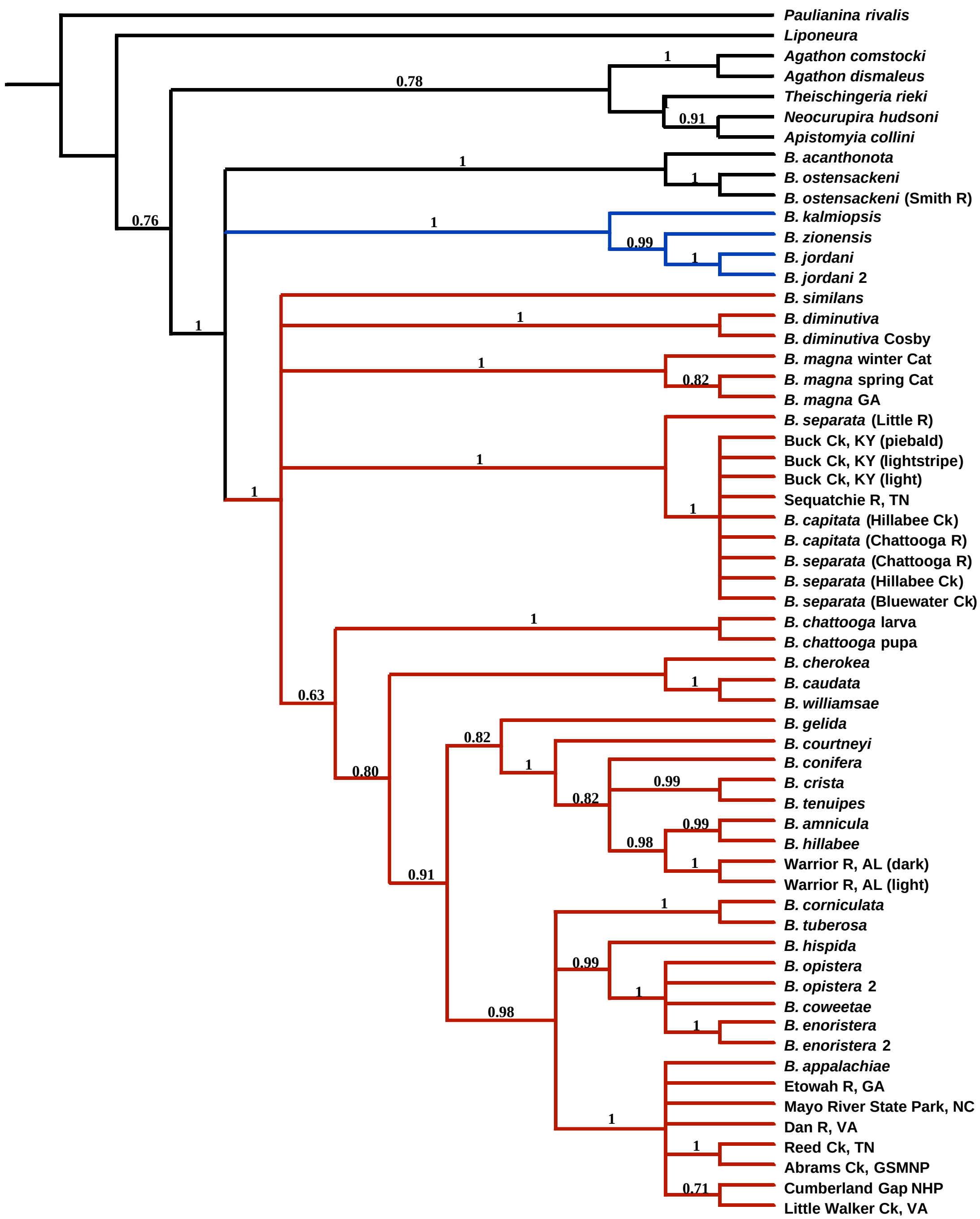

Figure 3.10. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on Bayesian inference of nuclear protein-coding gene, BZF2. Values above branches denote posterior probabilities. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

Molecular Phylogenetic Analysis: Combined ND2/BZF2 dataset

Maximum Parsimony.—Strict parsimony analysis in TNT resulted in 70 equally parsimonious trees of 5363 steps. A consensus of the trees is illustrated (Fig. 3.11).

Maximum Likelihood.—Best scoring maximum likelihood tree (Fig. 3.12).

Bayesian Inference.—1,000,000 generations with final chain swap values of 0.01–.85. Standard deviation of split frequencies=0.007453 (Fig. 3.13).

As in the previous analyses, the combined ND2/BZF2 analyses strongly supported the monophyly of both the *Blephariccera micheneri* group and the *B. tenuipes* group. Bootstrap values and posterior probabilities were at 100 percent for the *B. micheneri* and *B. tenuipes* groups. *Blephariccera ostensackeni* consistently paired with *B. acanthonota*, the Asian *Blephariccera* representative, indicating a closer relationship to Asian *Blephariccera* than Nearctic species. Support for the *B. acanthonota* + *B. ostensackeni* clade was at the 100th percentile. The placement of taxa in the basal part of the *B. tenuipes* group was also quite inconsistent. There was great discrepancy regarding the positioning of the basal branches. *Blephariccera diminutiva*, *B. magna*, *B. similans*, and *B. capitata/separata* were recovered at or near the base of the species group in all analyses. Trees inferred by parsimony, likelihood, and Bayesian analyses did not possess identical topologies, but certain clades are consistently recovered. Consistently recovered clades within the *B. tenuipes* group included the *B. appalachiae* clade with 99–100 percent support, the *B. coweetae/hispida* clade (with *B. tuberosa* and *B. corniculata*) with 96–100 percent support, the *B. tenuipes* clade with 100 percent support, and the *B. capitata/separata* clade with 100 percent support. While these clades consistently appeared in resulting phylogenies, relationships within the clades were variously resolved. Two other sister taxa, with relatively high levels of support, consistently paired. *Blephariccera williamsae* + *B. caudata* has bootstrap support of 71 (parsimony) and 100 (likelihood), and a posterior probability of 100. *Blephariccera corniculata* + *B. tuberosa* has bootstrap support of 99 (parsimony) and 100 (likelihood), and a posterior probability of 100.

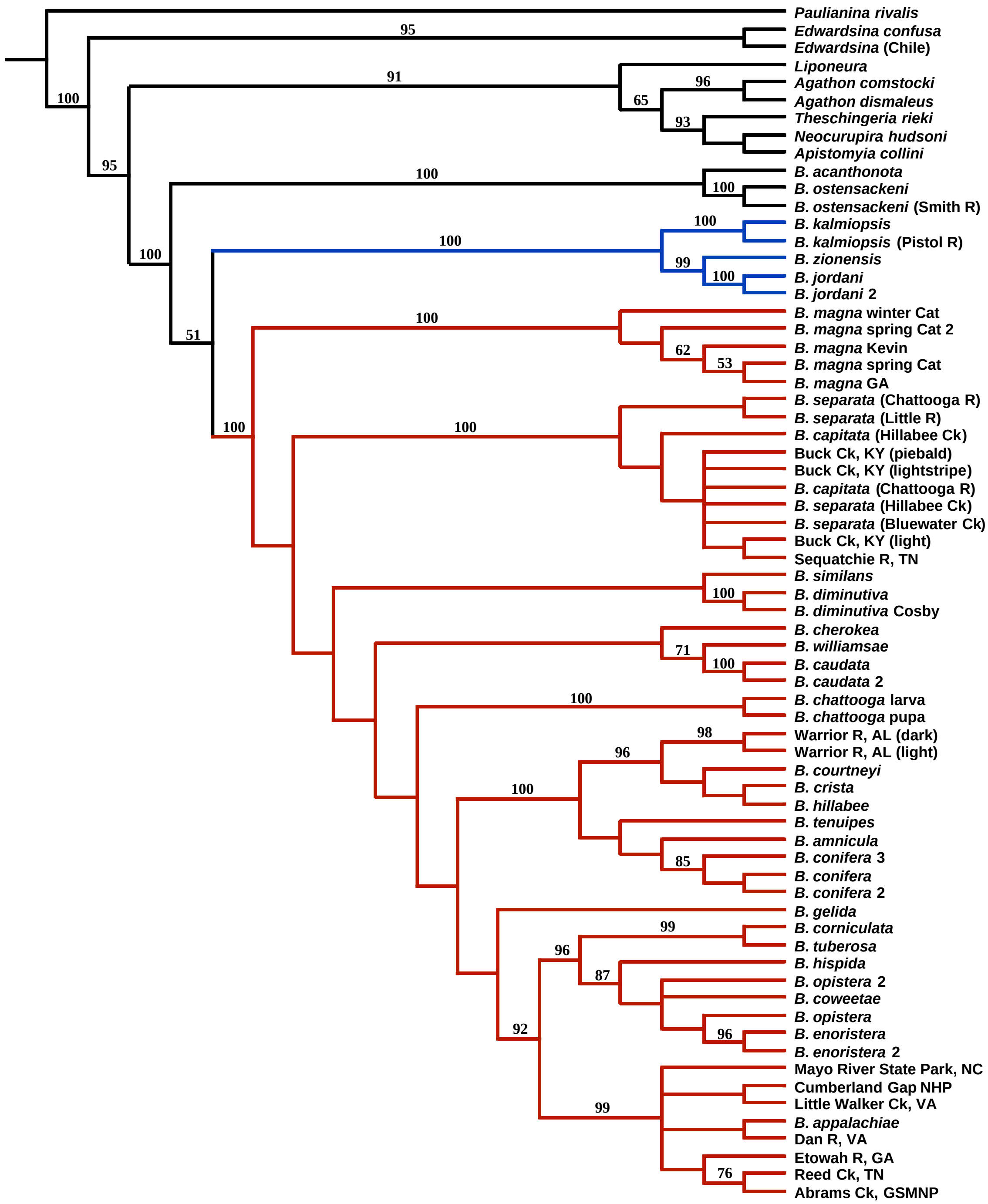

Figure 3.11. Hypothesized phylogenetic relationships of Nearctic *Blephariceral* based on maximum parsimony analysis of combined molecular data. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri group*.

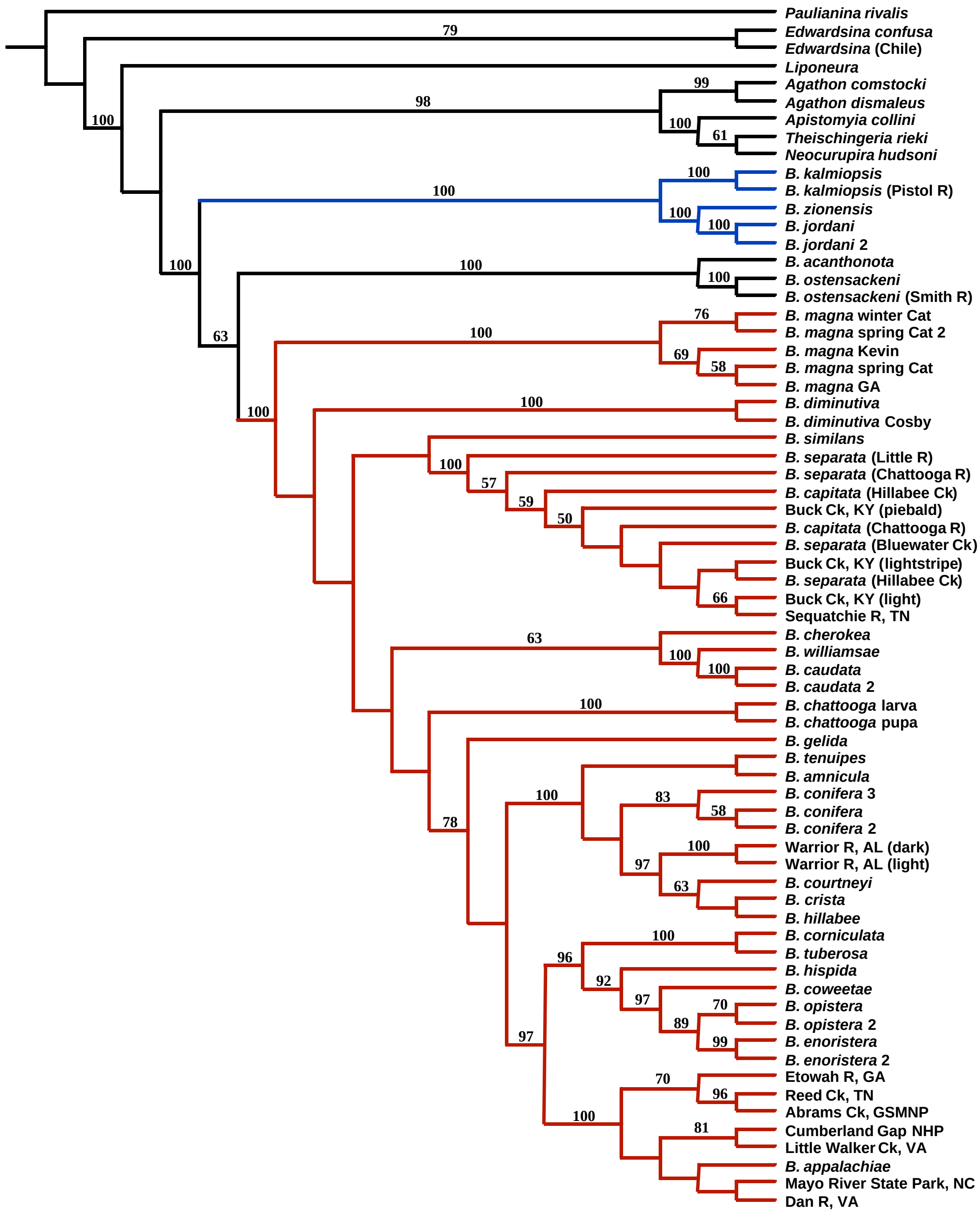

Figure 3.12. Hypothesized phylogenetic relationships of Nearctic *Blepharicera* based on maximum likelihood analysis of combined molecular data. Values above branches denote Bootstrap support. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

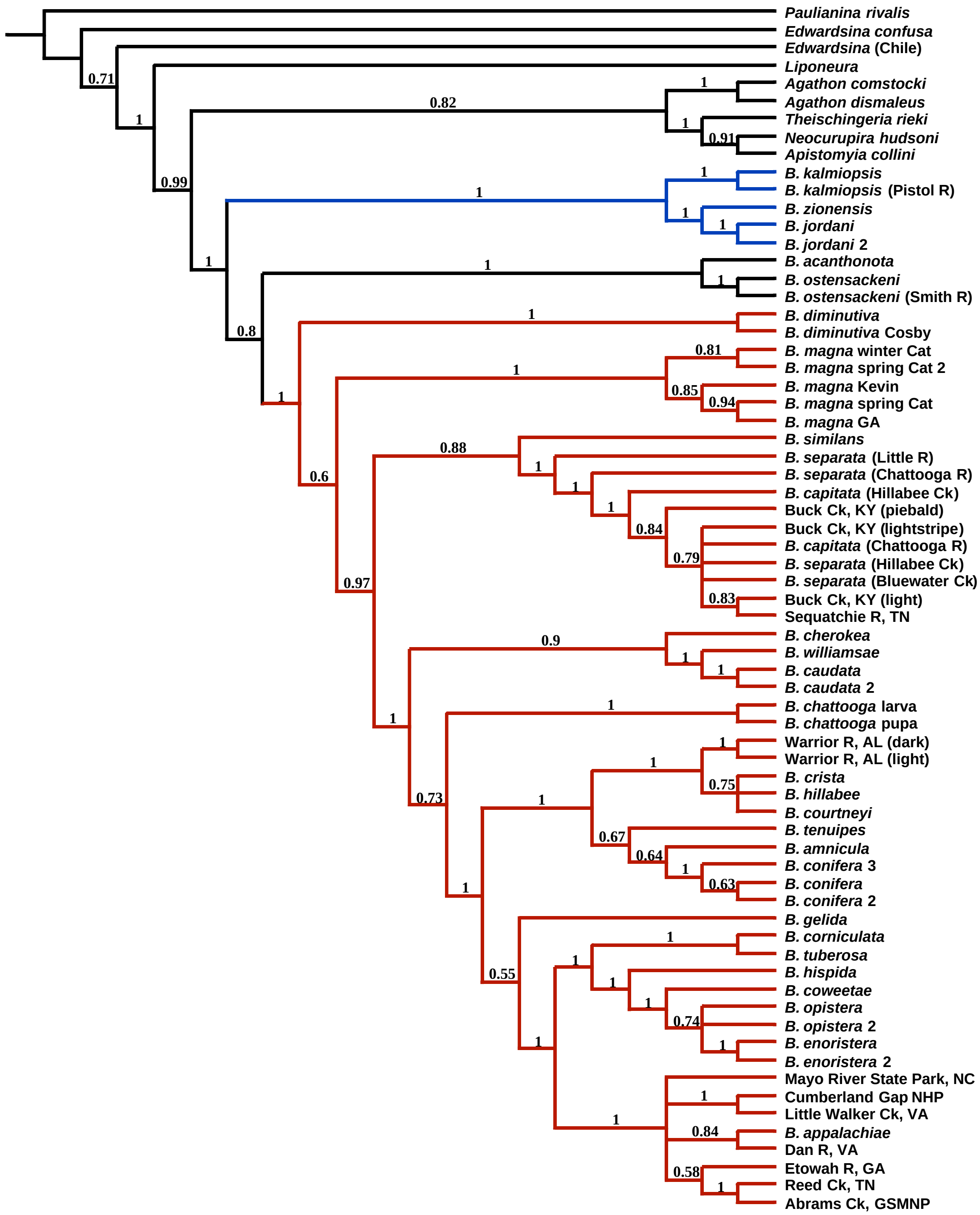

Figure 3.13. Hypothesized phylogenetic relationships of Nearctic *Blephariceera* based on Bayesian inference of combined molecular data. Values above branches denote posterior probabilities. Black branches = outgroup taxa, red branches = *B. tenuipes* group, blue branches = *B. micheneri* group.

Discussion

This study was conducted to determine the phylogenetic relationships between eastern and western Nearctic *Blepharicera* and among species in these groups using morphological characteristics from larval, pupal, and adult life stages and molecular characters from the DNA sequence of two genes, Big Zinc Finger 2 (BZF2), a nuclear protein-coding gene, and NADH dehydrogenase 2 (ND2), a mitochondrial protein-coding gene.

Jacobson (2006) proposed a phylogenetic hypothesis of the relationships within the *B. tenuipes* group based on morphology. That phylogeny was based on 44 morphological characters from 24 taxa. Eight taxa were added to that phylogeny and the resulting hypothesis, while largely similar, has some very distinct changes. *Blepharicera similans*, *B. chattooga*, *B. capitata*, and *B. separata* remain basal and relationships in the two clades including: *B. tuberosa*, *B. corniculata*, and *B. cherokea*; and *B. gelida*, *B. magna*, *B. diminutiva*, *B. coweetae*, and *B. hispida* are similar. However, the phylogenetic hypothesis introduced here no longer supports the sister taxa *B. williamsae* and *B. caudata*, instead, *B. caudata* is sister to *B. courtneyi*, a relationship that is not supported by any of the molecular analyses. The clades that are apparent in the molecular analyses are not mirrored in the morphological analysis. Like the earlier morphological phylogeny, bootstrap values provide little support for resolution within the tree and many nodes collapse in the consensus. This is likely due to conflicting characters and high levels of homoplasy.

According to Jacobson (2006), the western *Blepharicera* species, as a whole, are not monophyletic. That hypothesis is supported in the current study. Depending on the analysis, the Asian exemplar of *Blepharicera* either is nested within the western clade (*B. micheneri* group and *B. ostensackeni*) or forms a sister group (with *B. ostensackeni*) to the *B. micheneri* group.

Two representatives from the Locust Fork of the Warrior River in north central Alabama were included in the molecular studies. Initially it was believed that these specimens represented a new species. Molecularly they are distinct, with high levels of support, but morphologically they are nearly indistinguishable from *B. hillabee*. There is no doubt that the Warrior River specimens are closely related to described species within the *B. tenuipes* clade, but significant morphological characters for differentiating them from other species are lacking.

Courtney (2000b) revalidated *Blephariceras separata* (formerly synonymized with *B. capitata*), based on consistent morphological differences of the larvae and adult males, particularly in sympatric populations in the Chattooga River. Molecular analyses provide high levels of support (bootstrap values of 97–100 and posterior probabilities of 100) for the *B. capitata/separata* clade in the phylogenies. Within the clade there is no differentiation between the two species and there is little resolution. All representatives within the clade represent either *B. capitata* or *B. separata* from different localities or unique *capitata/separata*-like larval forms (i.e., dark body with red tubercles, piebald coloration, light body, dark body, dark body with light medial stripe). The *capitata/separata* material was collected from six different sites across the southeastern United States. We expected all the *B. capitata* to group together, all the *B. separata* to group together and for those two groups to be sister to one another. Instead, the representatives of the two species are intermingled within one clade. This observation does not support the revalidation of *B. separata* as a valid species as was previously determined based upon morphological differences.

As in the *Blephariceras capitata/separata* clade, support for the *B. appalachiae* clade is high, but relationships within the clade are variously resolved. Material for *B. appalachiae* and *appalachiae*-like larval forms was gathered over four states — Tennessee, Georgia, North Carolina and Virginia — and was compared using sequences from both the ND2 and BZF2 genes. Analysis of these widespread populations provides insight into the evolutionary significance of the morphological differences observed in the larval stages of the various populations. These morphological differences, in addition to minute molecular sequence differences, suggest the possibility of a *B. appalachiae* species complex.

It is likely that several species complexes exist within the Nearctic fauna. *Blephariceras ostensackeni*, with its many larval forms and indistinguishable adults, may be a complex in the western Nearctic (Courtney, personal communication) and at least *B. capitata* + *B. separata* and *B. appalachiae* may be complexes in the eastern Nearctic.

Our analyses represent the most up-to-date phylogenetic study of Nearctic *Blephariceera*. However, some life stages of *B. caudata* are unknown and thus could not be included in this analysis, and a lack of molecular data for *B. micheneri* leaves gaps in this Nearctic phylogeny. Future areas of study should emphasize addition of these life stages and molecular data for *B. micheneri*. The inclusion of more Asian *Blephariceera* species, in order to elucidate the origins of the Nearctic fauna, is necessary. Ideally, a genus-level study would help to clarify the relationships. The biggest obstacle to the genus-level study would be acquiring molecular-quality material of all known species. Finally, this analysis would have been more complete if material for the European species, *B. fasciata*, also had been available. The addition of the European species in the molecular analysis could have further clarified the origin and probable arrival means of the genus to the Nearctic.

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CHAPTER 4. GENERAL CONCLUSIONS

As stated elsewhere (Jacobson 2006) Blephariceridae is a fairly well studied group, taxonomically. Although its distribution is cosmopolitan, certain regions have been covered more intensely. The fauna is particularly well known in the Nearctic, western Palaearctic, and Australasian regions, but remains relatively unknown in the eastern Palaearctic region, Oriental region, and the southern hemisphere. Analyses on Palaearctic and Oriental *Blepharicera* could help to clarify the relationships of the Nearctic *Blepharicera*.

Analyses of morphological and molecular characteristics from representatives of all described Nearctic species were used to determine the phylogenetic relationships of eastern and western Nearctic *Blepharicera*. These analyses provided a test of the hypotheses of Hogue (1978), Zwick (1984), Hogue and Georgian (1986) and Jacobson (2006). The results concurred with those of Jacobson (2006), revealing that the Nearctic fauna is likely of Asian origin, and that both the *B. micheneri* group and the *B. tenuipes* group are monophyletic but not sister groups. It is probable that *Blepharicera* colonized the Nearctic region in three separate events with the *B. micheneri* group, *B. tenuipes* group, and *B. ostensackeni* arriving at different times.

Additional collections in the eastern United States would provide further information on the distribution of *Blepharicera* in the Nearctic region. Currently, the southernmost record for the *B. tenuipes* group is *B. hillabee* in east central Alabama. Further investigations into similar habitats in east central to south central Alabama could lead to additional records and possibly another southern extension to the distribution. Numerous attempts were made at locating the unknown life stages of the highly isolated species, *B. caudata*. Unfortunately, the stream is mostly bedrock, making it difficult to remove rocks for rearing, and *B. williamsae*, which is also more numerous, pupates at the same time.

Future molecular studies could clarify the composition of probable Nearctic species complexes (*Blepharicera ostensackeni*, *B. appalachiae*, *B. capitata,* and *B. separata*). Further investigations into family level molecular phylogenies would provide additional support to existing phylogenies. A family-level study by Courtney and Moulton (2006) used sequence from the gene encoding septin-tuftelin interacting protein

(STIP). Their analyses inferred phylogenetic trees in which the two subfamilies, Edwardsininae and Blephariceridae, are well supported and the three tribes within Blephariceridae, Blephariceridini, Apistomyiini, and “Paltostomatines”, are monophyletic with the exception of *Horaia*, which falls outside of the remaining sampled Apistomyiini. Future molecular research on the Blephariceridae with additional nuclear genes and increased taxon sampling will help to test phylogenetic hypotheses based on previous molecular research on the family.

Our analyses represent the most up-to-date phylogenetic study of Nearctic *Blepharicera*. However, there are unknown life stages of *B. caudata* that are not included in this analysis and a lack of molecular data available for *B. micheneri* leaves gaps in this Nearctic phylogeny. Future areas of study should emphasize addition of these life stages and molecular data for *B. micheneri*. The inclusion of more Asian *Blepharicera* species, in order to determine the origins of the Nearctic fauna, is necessary. Ideally a genus level study would help to clarify the relationships. The biggest obstacle to the genus level study would be acquiring molecular quality material of all known species. The addition of the European species, *B. fasciata*, in future molecular analyses may help to clarify the origin and probably arrival means of the genus to the Nearctic.

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VITA

Amanda Jacobson was born in Ames, Iowa, the first of two children, to Larry and Sandy Jacobson. She spent her childhood in rural central Iowa. Amanda attended school in the Collins-Maxwell School District from 1987 to 2000. After graduating as valedictorian she continued her education at Iowa State University in Ames, Iowa. Amanda started out with a general biology major with the aspirations of becoming a marine biologist. That plan came to a halt in the spring of 2002 when Amanda took an introductory entomology class with Dr. Ken Holscher. That class opened her eyes to the fascinating world of insects. Amanda finished her undergraduate work in 2004, receiving a double major in Entomology and Biology with a minor in Spanish. That fall, she started a Master's program with Dr. Gregory W. Courtney at Iowa State University working on the western Nearctic *Blephariceral*. In 2006, Amanda received her Master's degree with a co-major in Entomology and Ecology and Evolutionary Biology. She moved to Knoxville, TN in August 2006 to pursue a Doctorate degree with Dr. John K. Moulton working on the molecular and morphological systematics and phylogenetics of eastern Nearctic *Blephariceral*.